Gates of Enzymes

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1. INTRODUCTION

Enzymes are very efficient catalysts that are essential for the functioning of living organisms. The low efficiency of biocatalysts produced de novo relative to those that have evolved naturally demonstrates that our understanding of enzymatic catalysis is still incomplete.1−4 The dynamic motion of enzymes during catalytic events is one of the many aspects of protein chemistry that are currently insufficiently well understood.5−9 On one hand, proteins need to have well-defined and organized structures in order to maintain stable functionality in the intracellular environment. On the other hand, some degree of flexibility is often required for catalytic activity. Molecular dynamics simulations have provided key insights into the importance of protein dynamics in catalysis, such as the observation of substrate access and product exit pathways that cannot be identified by inspecting crystal structures.10 Csermely et al. recently reported that mutations in regions that affect protein dynamics, such as hinge regions that are important in substrate binding, can have dramatic effects on catalytic activity.11 In this review, we highlight the role of protein gates as another class of highly dynamic structures that play key roles in protein function.

Given the importance of gates for enzymatic catalysis, the number of studies that have examined them systematically is surprisingly small. Conformational gating in proteins was first described by McCammon and co-workers in 1981, but there have been relatively few systematic studies in this area since then.12−14 Moreover, much of the available data on gates in macromolecular systems is hidden or otherwise dispersed within the scientific literature, partly because there is currently no consensus regarding what defines a gate. Some authors describe all residues that affect the ligand’s access to a target area as gating residues, whereas others apply the term exclusively to structural features that undergo large movements during the gating event. In this review, we define a gate as a dynamic system consisting of individual residues, loops, secondary structure elements, or domains that can reversibly switch between open and closed conformations and thereby control the passage of small molecules—substrates, products, ions, and solvent molecules—into and out of the protein structure. Under this definition, the anchoring residues that stabilize the open or closed conformations of a gate are not themselves gating residues. However, because of their various interactions with the gating residues, they can control the size and properties of the ligands that pass through the gate as well as the frequency of the exchange events.

Gates can be found in various systems, including enzymes, ion channels, protein–protein complexes, and protein–nucleic acid complexes.14,15 In this work, we focus specifically on gates in enzymes. We attempt to answer three basic questions—why, how, where—by describing the molecular function, structural
basis, and location of gates within protein structures. We discuss 71 illustrative examples of enzymes that together contain 129 different molecular gates and propose a system for their classification. Reviewed enzymes were chosen based on a literature search with a set of keywords corresponding to gates and conformational changes in enzymes. A preliminary set of
2. MOLECULAR FUNCTION OF GATES

Analyses of protein dynamics have identified a number of enzymes with gates, suggesting that these structures are rather common. What is the molecular function of the gates? It seems that in enzymes they facilitate precise control over processes that are directly linked to catalysis. Enzyme gates can (i) contribute to enzyme selectivity by controlling substrate access to the active site, (ii) prevent solvent access to specific regions of the protein, and (iii) synchronize processes occurring in distant parts of the protein (Figure 2). The proper function of even the simplest gates can potentially be essential for catalysis, and the gating event may even represent the rate-limiting step of the catalytic cycle. Interestingly, different gating residues within a single protein molecule may be responsible for restricting the access of specific substrates. High variability of the gating residues within an enzyme scaffold can lead to the evolution of enzyme families whose members are selective for specific substrate types. The best known example of such specialization within a single enzyme family is provided by the cytochromes P450.16

2.1. Control of Substrate Access

Enzyme selectivity has been traditionally explained by the “lock and key” model,17 which was subsequently complemented by the “induced fit” or “hand in glove”,18 “selected fit”19, and “keyhole, lock, and key”20 models. In many cases, these models provide an adequate description of enzyme selectivity based on adjustable complementarity between the active site and the cognate substrates. However, research conducted over the past decade has shown that regions located further from the active site can also affect enzyme selectivity. Substrate access pathways, which often incorporate molecular gates, impose additional constraints on ligand binding to the active site.20 The ability of ligands to traverse these access pathways can be controlled by (i) size discrimination at the narrowest point along the pathway forming a bottleneck, (ii) geometrical constraints, e.g., the curvature of the pathway, and (iii) specific molecular interactions such as hydrogen bonds, electrostatic interactions, and hydrophobic interactions with the residues comprising the access pathway. Protein gates can be regarded as molecular filters that discriminate between molecules as similar as molecular oxygen and carbon monoxide in NiFe hydrogenases21,22 or water and hydroxyperoxide in catalases23,24 Gates act as filters in a wide range of enzymes, controlling the range of substrates that can be accepted by broad-specificity cytochromes P450,25 the stereospecificity of epoxide hydrolases,26 and product length in undecaprenyl-pyrophosphate synthetases.27

One of the first systematic descriptions of the influence of the gating process on substrate binding was reported by Szabo et al., who assumed that the switching between the open and the closed conformations of the gate was a stochastic process.28 This model was successfully used to demonstrate that despite conformational gating13 acetylcholinesterase can bind acetylcholine with a rate constant of $10^9 \text{M}^{-1} \text{s}^{-1}$ and predict the rate of formation of the enzyme—substrate complex in choline oxidase.31 Since gates create a barrier on the substrate access pathway, the kinetic rate constant for passage over the barrier can be obtained using Kramers’ reaction rate theory or its later modifications.32–34 This methodology was used to compare the results of computational and experimental studies on the passage of the tetramethylammonium cation through acetylcholinesterase35 and migration of ammonia through carbamoyl phosphate synthetase.36

2.2. Control of Solvent Access

Spatial localization of the hydrophobic and hydrophilic regions within the structure of a protein is important in maintaining its proper fold and can also be crucial for catalytic function. The various steps of an enzymatic reaction may require different environments. These distinct environments can be generated by having the individual steps occur in spatially separate regions of the protein, but this does not eliminate the problem of transporting the substrate between these sites. There are important problems to be addressed, including transporting polar molecules from a polar environment to a nonpolar one and separating hydrophilic compartments from hydrophobic ones within the structure of a single protein.

In some proteins, these problems are addressed by the presence of selective barriers that permit passage of solutes but not water molecules. Crystallographic and NMR data can be used to identify cavities within a protein structure accessible to water molecules. Exclusion of water from some parts of the cavity, such as the active site or a specific tunnel, is essential for functioning of numerous enzymes. In simple cases, the gates may prevent the entrance of water molecules into the cavity when a substrate or a cofactor is not present, as occurs in rabbit 20a-hydroxysteroid dehydrogenase.37 In more complex cases, the gates may permit access only to a specific part of the cavity, as occurs in carbamoyl phosphate synthetase38 and imidazole glycerol phosphate synthase.39 In the cytochromes P450, a “water channel” controls hydration of the substrate in the active site, which is extremely important for cytochrome activity.16
The potential importance of gates that act as solvent barriers is further illustrated by the example of enzymes with ammonia tunnels. In these proteins, gates prevent water from entering the channel and protonating the ammonia, which is essential for maintaining its nucleophilic character.39 Control of water access can be seen as a special case of the function described in section 2.1. However, when discussing water exclusion, the main emphasis is on the water permeability of the gates and their ability to distinguish water molecules from other ligands. The gate can simultaneously act as a barrier to passage of water molecules while acting as a selective filter for other molecules, allowing them to access the active site. Gates of this type resemble semiselective membranes that can distinguish between species such as water and ammonia, allowing only the second to pass. It is worth noting that passage of ‘permitted’ species can be facilitated by rearrangement of an individual gating residue, such as K99 in imidazole glycerol phosphate synthase. Conversely, passage of water molecules through gates of this type would often require significant conformational changes in all of the residues that comprise the gate.38 This makes gates in enzymes far more sophisticated than semipermeable membranes.

2.3. Control and Synchronization of Reactions

Another function of gates becomes apparent when considering enzymes with two or more active sites. Many protein structures contain tunnels to facilitate efficient migration of intermediates and gates to synchronize chemical reactions. Such arrangements can be compared to a pair of workers on an assembly line. The second worker has to be ready before he can receive a product from the first one. Moreover, the products generated by the first worker must satisfy certain standards. The control gates located between the workers regulate the exchange of products over a well-defined period of time. Gates of this kind are common in ammonia-transfering enzymes, suggesting that they are old in evolutionary terms and functionally important.40–43 The need to efficiently transport ammonia within the interior of the protein may be related to its high cellular toxicity. We speculate that gates of this kind may be present in many enzymes that have multiple active sites connected by internal tunnels for the transport of intermediates. Many such enzymes have been studied in some detail, including carbamoyl phosphate synthetase, which has tunnels for ammonia and carbamate transportation;44 asparagine synthetase,44 glucosamine 6-phosphate synthase,45 and glutamate synthase,46 all of which have tunnels for ammonia transportation; tryptophan synthase for indole47 and carbon monoxide dehydrogenase/acetyl coenzyme A synthase for carbon monoxide transportation.48

Systematic analysis of the functions of the known gates in the 71 proteins discussed in this article revealed the following distribution of gate types: 40% of the studied gates control substrate access, 19% control solvent access, 15% control and synchronize catalytic events, and 26% have other function (Figure 3).

3. STRUCTURAL BASIS OF GATES

Gates are dynamic systems that can make reversible transitions between open and closed states. They vary in size and complexity, from individual amino acid residues to loops, secondary structure elements, and even domains. The simplest gates consist of only one amino acid side chain that can close or open an access pathway by rotating. Opening and closing of more complicated systems can involve the synchronized movement of two or more residues, and the largest systems involve rearrangements of secondary elements or even entire domains (Table 1). For larger systems, movement of the gate may cause formation of a tunnel or enclosed cavity in addition to permitting or denying access to selected species.49,50 The following parameters can be useful for describing and discriminating between gates: (i) their constituent residues, (ii) their anchoring residues, (iii) the hinge region, i.e., the amino acids that make the structure flexible and allow it to move, (iv) the gate’s position, (v) the gate’s bottleneck diameter in the open and closed states, (vi) changes in the bottleneck’s size over time, (vii) the energy required to switch the gate from one state to the other, and (viii) the energy required for passage of specific molecules through the gate.

3.1. Residue Motion: Wings

The energetic barriers for residue rotation are quite small, 1–16 kcal/mol.51 While generally low, such barriers can nevertheless be large enough to significantly affect the probability that a given species will be able to pass through the gate or the rate at which they do so. Depending on the particular amino acid and its surroundings, one or both states of the gate may be stabilized by interactions with anchoring residues, e.g., hydrophobic interactions, H bonds, ionic interactions, salt bridges, and $\pi-\pi$ interactions. The strongest effect on the control of the passage is achieved when a large gating residue is located in the bottleneck of the pathway. The most common residues in this role are those whose side chains contain aromatic rings, i.e., W, F, and Y (Figure 4). Wing-type gates are common and can be found in enzymes such as imidazole...
Table 1. Classifying Enzyme Gates According to Their Structural Basis

|                | 1                  | 2                  | 3                  |
|----------------|--------------------|--------------------|--------------------|
| **Symbol**     | —                  | — —                |                    |
| **Scheme of closed state** | ![Image](image1) | ![Image](image2) | ![Image](image3) |
| **Illustration of closed state** | ![Image](image4) | ![Image](image5) | ![Image](image6) |
| **Scheme of open state** | ![Image](image7) | ![Image](image8) | ![Image](image9) |
| **Illustration of open state** | ![Image](image10) | ![Image](image11) | ![Image](image12) |
| **Metaphor**   | wing               | swinging door      | aperture           |
| **Mechanism and moving part** | side chain rotation | side chain rotation | backbone motion |
| **Structural basis** | 1 residue          | 2 residues         | 2-4 residues       |
| **Amplitude of motion** | < 2Å               | < 3Å               | < 3Å               |
| **Time scale**  | ps – µs            | ps – µs            | ns – µs            |
| **Other features** | anchoring residue  | anchoring residues | hinge and anchoring free |
glycerol phosphate synthase, cytidine triphosphate synthase, methane monooxygenase hydroxylase, FabZ β-hydroxyacyl-acyl carrier protein dehydratase, and cytochrome P450. Even small gates of this type may require an activating agent to open. For example, the gate in the water channel of human monooxygenase CYP3A4 is created by the
interaction of the conserved residue R375 with the heme, which opens upon cytochrome P450 reductase binding to the enzyme.58

3.2. Residue Motion: Swinging Doors

A more complex type of gate consists of two residues that can rotate but are stabilized in the closed conformation by a mutual interaction. Lario et al. introduced the phrase “swinging door” to describe gates of this type that were identified in cholesterol oxidase type I.57 Some swinging door gates open by having both residues rotate in the same direction, while in others the two residues rotate in opposite directions. Common stabilizing interactions in swinging door gates include π stacking as occurs in the F−F pair of cytochrome P450cam,58,59 and acetylcholinesterase,60 ionic interactions as in toluene-4-monooxygenase61 and cytochrome P450cam, P450BM3, and P450eryF,25,55 aliphatic hydrophobic interactions such as those between the F−I, the F−V, and the F−L pairs of cytochrome P450cam58,59 and hydrogen bonds such as that between the R−L and the L−I pairs of cytochrome P450cam.58 The open conformations of one or both of the gate residues may also be anchored, depending on the amino acids surrounding the gate. In comparison to wing gates, gates consisting of two residues can control wider tunnels and channels. It is worth mentioning that the individual residues that comprise a swinging door gate may simultaneously be components of another gate, as occurs in cytochrome P450cam.58 Literature data indicate that most gates of this type consist of F−F pairs, and one way to screen for potential gates is to search for phenylalanine sandwiches.

3.3. Residue Motion: Apertures

Proteins undergo low-frequency breathing motions that may involve synchronized movements of bottleneck residues. In contrast to the previously described gates, the residues that form aperture type gates do not need to rotate and can maintain a rigid conformation. Their movements occur as a result of the synchronized relocalization of the enzyme backbone during its breathing motions. The ability of a given species to pass through gates of this type depends on the length of time the gate remains in the open state, which is determined by the enzyme’s rigidity (especially in terms of the compartments housing the gating amino acids) and the strength of the interactions between the gating residues. Gates of this kind can therefore switch between states at different frequencies, which can be adjusted by mutating the gating residues. Aperture-type gates have been identified in several enzymes including carbamoyl phosphate synthetase,66 choline oxidase,51 glutamate synthase64, extradiol dioxygenases-homoprotocatechuate 2,3-dioxygenase,63 cytochrome P450eryF25, and acetylcholinesterase.64

3.4. Motions of Loops and Secondary Structure Elements: Drawbridges and Double Drawbridges

The movements of loops and secondary structure elements can provide an energetically favorable method of controlling access for larger ligands. The gates described above consist of individual residues and would not provide sufficient control for enzymes that have large substrates and correspondingly large active site cavities. In many cases, the loops involved in access control also contribute to formation of substrate/cofactor binding cavities. Alternatively, in enzymes with complex systems of internal tunnels such as the members of the cytochrome P450 family, the dynamic motion of the protein structure, especially the flexible B−C and F−G loops in the cytochromes P450, plays a vital role in the opening and closing of the tunnels.16 Protein motions of this type can also merge different tunnels, creating a wider opening. Here, gating elements control the access of large substrates by merging and dividing the space shared by the tunnels.16 However, in such cases the movements of the loops can cause formation of smaller and more selective gates such as the swinging doors described in the preceding sections.56,58

Movements of loops and secondary structure elements can change the solvation of a cavity or the gate itself. The equilibrium between the open and the closed conformations depends on the anchoring residues and the flexibility of the hinge region. All of these elements play important roles in the movements of large gates. The conserved GxG motif found in most cytochrome P450 family members provides a good example.65 Depending on cytochrome isoform, the motif flanks either one or both ends of the B-C loop. It increases the

Figure 4. Relative occurrences of specific amino acid residues in wing and swinging door gates; 71 proteins with 129 gates were analyzed, and 154 residues that form wings or swinging doors were identified. Detailed description of the analyzed proteins is provided in Table 3. Values were normalized against the frequency with which each amino acid appears in all of the protein structures of the UniProtKB/Swiss-Prot database (2012_07).
Figure 5. Frequencies of different gate types based on analysis of 71 proteins with 129 gates. Detailed description of the analyzed proteins is provided in Table 3.

4. LOCATIONS OF GATES

The roles of gates in the enzymatic catalysis discussed above suggest that these structures are natural hot spots for modifying enzyme properties. Identification of structural components of natural gates would therefore be very useful to protein designers. This raises a question: how and where should one look for the gates? Gates in proteins can be identified experimentally by protein crystallography and NMR spectroscopy and computationally by molecular dynamics simulations and normal-mode analysis.

Crystallographic analyses can provide information on alternative configurations of particular amino acids in a protein structure. The presence of residues that can adopt both open- and closed-type conformations along the access or release pathways of ligands, ions, and solvents may suggest the presence of a gate. However, in order for a gate to be detected by crystallography, it is necessary for both the open and the closed conformations to be sufficiently represented. Despite this restriction, crystallographic analyses have identified gates in tryptophan synthase,71 haloalkane dehalogenase LinB,72 L-lysine oxidase,73 and toluene-o-xylene monoxygenase. In some cases, only one conformation will be present in the solved crystal structure, which may create a somewhat distorted picture, suggesting the absence of a gate when the open conformation is stabilized or the absence of a pathway when the closed conformation is stabilized.31,61,75

The limitations of crystallographic analysis can be overcome by advanced NMR spectroscopy, which makes it possible to study multiple protein conformations simultaneously, over time scales ranging from picoseconds to milliseconds.7,76 Such analyses provide information on both the open and the closed states as well as the population of each state and rate of their interconversion. NMR techniques have been used to measure the rate of exchange between the open and the closed conformations of triosephosphate isomerase,77,78 HIV-1 protease,79 and dihydrofolate reductase.80,81 Overall, the utility of NMR for studying distant effects of mutations on protein dynamics suggests that it has great potential for investigating gating mechanisms.7

Some of the most useful tools for identifying gates are the computer programs developed for detecting tunnels, channels, and cavities in protein structures.82 The outputs of CAVER,83 MOLE,84 and MOLAXIS85 can be analyzed to detect bottleneck residues that form a potential gate or identify the best position for introduction of a new gate. Mutations at these “hot spots” can provide enzymes with new selectivities or
Table 2. List of Enzymes Possessing Gates Described in the Scientific Literature with Indication of Their Function, Structural Basis, and Location

| No. | Enzyme name                                      | Function | Structural basis | Location |
|-----|--------------------------------------------------|----------|------------------|----------|
| 01  | 2-Amino-2-Desoxyochoisinate Synthase PhzE         | +        | +                | +        |
| 02  | 3-Hydroxybenzoate Hydroxylase MBBH               | +        | +                | +        |
| 03  | 4-Hydroxy-2-Ketovaleraldehyde Aldolase DmpG / Acetylating Acetaledehyde Dehydrogenase DmpF | +          | +                | +        |
| 04  | 4-Hydroxybenzoate Hydroxylase PHBH               | +        | +                | +        |
| 05  | Acetylcholinesterase AChE                        | +        | +                | +        |
| 06  | Acetylaminoacyl Peptidase                        | +        | +                | +        |
| 07  | n-Amylase TK1436                                  | +        | +                | +        |
| 08  | Asparagin Synthetase                             | +        | +                | +        |
| 09  | ATP-Dependent Proteases HalIVU                   | +        | +                | +        |
| 10  | Carboxymol Phosphate Synthetase CPS – type II    | +        | +                | +        |
| 11  | Carbon Monoxide Dehydrogenase / Acetyl Coenzyme  | +        | +                | +        |
| 12  | α Synthase                                       | +        | +                | +        |
| 13  | Carbonic Anhydrase β – type I                    | +        | +                | +        |
| 14  | Carbonic Anhydrase β – type II                   | +        | +                | +        |
| 15  | Carboxylesterase pnbcCE                          | +        | +                | +        |
| 16  | Catalase CAT-1 and CAT-3                         | +        | +                | +        |
| 17  | Cellulohydrolase CEL 7A                          | +        | +                | +        |
| 18  | Cellulose Phosphatase                            | +        | +                | +        |
| 19  | Chalcone Synthase                                | +        | +                | +        |
| 20  | Chloramphenicol Halogenase CmlS                  | +        | +                | +        |
| 21  | Cholesterol Oxidase – type I SCHOX               | +        | +                | +        |
| 22  | Cholesterol Oxidase – type II BoChTx            | +        | +                | +        |
| 23  | Choline Oxidase                                  | +        | +                | +        |
| 24  | Chondroitin AC Lyase                             | +        | +                | +        |
| 25  | Copper-Containing Amine Oxidase                  | +        | +                | +        |
| 26  | Cytochrome P450 CPR3A4                           | +        | +                | +        |
| 27  | Dihydroxilatedeductase                           | +        | +                | +        |
| 28  | Digeranylglycerolipidiphospholipid Reductase      | +        | +                | +        |
| 29  | Epoxide Hydrolase H37Rv                          | +        | +                | +        |
| 30  | Epoxide Hydrolase M200                           | +        | +                | +        |
| 31  | FabF β-Hydroxyl-Carrier Peptide Dehydratase      | +        | +                | +        |
| 32  | Formiminitransferase-Cycloamidase FTCD           | +        | +                | +        |
| 33  | Glucosamine 6-Phosphate Synthase GlmS            | +        | +                | +        |
| 34  | Glutamate Synthases GltS                        | +        | +                | +        |
| 35  | Glutamine Phosphoribosylpyrophosphate            | +        | +                | +        |
| 36  | Amidotransfase                                   | +        | +                | +        |
| 37  | Halolactone Dehalogenase DhaA                    | +        | +                | +        |
| 38  | Halolactone Dehalogenase LinB                    | +        | +                | +        |
| 39  | Histone Deacetylase HDAC1 and HDAC2              | +        | +                | +        |
| 40  | Histone Deacetylase HDAC8                        | +        | +                | +        |
| 41  | HIV-1 Protease                                   | +        | +                | +        |
| 42  | Homoproateatechutan 2,3-Dioxygenase              | +        | +                | +        |
| 43  | Hydrogenase FeF                                  | +        | +                | +        |
| 44  | Hydrogenase NiFe                                | +        | +                | +        |
| 45  | Imidazole Glycerol Phosphate Synthase 1GPS       | +        | +                | +        |
| 46  | Isocitrate 5- Monophosphate Dehydrogenase        | +        | +                | +        |
| 47  | Kinetocyl Synthase KS                           | +        | +                | +        |
| 48  | L-Amino Acid Oxidase                             | +        | +                | +        |
| 49  | Lipase B                                         | +        | +                | +        |
| 50  | Lon Protease                                     | +        | +                | +        |
| 51  | Mannitol 2-Dehydrogenase                         | +        | +                | +        |
| 52  | Methane Monoxygenase Hydroxylase MMOH            | +        | +                | +        |
| 53  | Monoamine Oxidase A                              | +        | +                | +        |
| 54  | Monoamine Oxidase B                              | +        | +                | +        |
| 55  | Monoxygenase ArpVA06                             | +        | +                | +        |
| 56  | NADH Oxidase                                     | +        | +                | +        |
| 57  | O-Acetylserine Sulphhydrolase Cysteine Synthase  | +        | +                | +        |
| 58  | OXidosqualene Cyclase SceOSC                     | +        | +                | +        |
| 59  | Phenol Hydroxylase PHHY                         | +        | +                | +        |
| 60  | Phospholipase A2                                 | +        | +                | +        |
| 61  | Phosphatidylinositol-Specific Phospholipase C     | +        | +                | +        |
| 62  | Quercetin 1,3-Dioxygenase                        | +        | +                | +        |
Table 2. continued

| 62 | Rabbit 20α-Hydroxysteroid Dehydrogenase | + | + | + | + | + | + |
| 63 | Raucaffrinic O-β-Dglucosidase | + | + | + | + | + | + |
| 64 | RNA-Dependent RNA Polymerase | + | + | + | + | + | + |
| 65 | RNA Polymerase | + | + | + | + | + | + |
| 66 | Toluene-Monoxygenase TAMO | + | + | + | + | + | + |
| 67 | Toluene-Ortho-Xylen Monoxygenase ToMO | + | + | + | + | + | + |
| 68 | Triosephosphate Isomerase | + | + | + | + | + | + |
| 69 | rRNA-Dependent Amidotransferase GatDE and GatCAB | + | + | + | + | + | + |
| 70 | Tryptophan Synthase | + | + | + | + | + | + |
| 71 | Undecaprenyl-Pyrophosphate Synthase | + | + | + | + | + | + |

1 Indicates gates interacting with cofactor. 2 Cofactor assisted gating. 3 Classification uncertain.

![Diagram of gate locations within a protein](image)

Wing → Swinging door → Aperture → Drawbridge → Double drawbridge → Shell

Activities.86 Zawaira et al.59 used the CAVER software together with the Protein Interaction Calculator87 for identifying gating residues within the cytochrome P450 family.

MD simulations are well suited for identification and analysis of gates and their behavior over time. Detailed descriptions of MD methods and their applications in simulating ligand migration can be found in recent reviews.9,88 Movements of large protein fragments on microsecond time scales can be investigated using Brownian dynamics,9,89 while Random Expulsion Molecular Dynamics and Steered Molecular Dynamics can be used to study pathways dedicated to transport of specific ligands.95,95 Some proteins have multiple pathways, each of which accommodates a different ligand or ligand class. This may in fact be a lot more common than is currently realized and can dramatically increase the complexity of gating systems arising from protein movement and the difficulty of identifying the true gating residues. For example, different residues control the ability of inhibitors E2020 and Huperzine A to access the active site of Torpedo californica acetylcholinesterase.96 Similarly, in cytochrome P450, different residues in the same tunnel control access of temazepam and testosterone-6OH.88 The importance of a gating residue identified by computational methods can be confirmed experimentally by site-directed mutagenesis and kinetic experiments.

Studies using the experimental and theoretical approaches for gate identification discussed above have demonstrated that their locations within the protein can vary widely. Gates have been observed (i) at the entry to the active site or even directly inside the active site, (ii) at the entry or in the bottleneck of the protein tunnel connecting the buried active site to the protein surface or connecting two active site cavities, and (iii) at the interface of the cofactor and active site cavities (Figure 6).

4.1. Active Site Entrance and Active Site

The entrance to the active site cavity is a suitable location for a gate, and gates situated here can have strong effects on enzyme activity. In some cases, the gating residues may even be a part of the active site.91 The simplest gates serve as filters that discriminate between potential substrates and thus play an important role in controlling enzyme selectivity. More advanced systems can prevent substrate entry when the active site residues are not properly oriented, e.g., in enzymes that require conformational changes before substrate binding. Many enzymes have gates at the entrance to their active sites, including acetylcholinesterase,92 imidazole glycerol phosphate synthase,92 glutamate synthase,93 toluene-α-xylene monooxylene-

4.2. Tunnel Entrance and Tunnel Bottleneck

The ability of ligands and solvent molecules to move from the media surrounding the protein to the active site can be controlled by gates located at any point along the tunnel. Gating residues may be situated at the tunnel entrance. However, it is more common to find them at the tunnel bottleneck. The tunnel entrance refers to the first shell of residues that define the tunnel and have contact with the bulk solvent. The tunnel bottleneck refers to the narrowest part that can be positioned anywhere along the tunnel (Figure 6). Even a single large residue whose side chain can project into the interior of the tunnel can exert efficient control over the access pathway. One might speculate that it might be favorable to have gates located inside tunnels because this allows their position to be more tightly controlled; their movements are restricted by the surrounding residues, and both the open and the closed conformations can be stabilized via interactions with neighboring amino acids. In contrast, residues located on the surface of the protein possess more degrees of freedom, and it is rare for both the open and the closed conformations to be stabilized. Examples of such gates inside the tunnels can be found in cholesterol oxidase type 1,75 toluene-4-monoxygenase,61 undecaprenyl-pyrophosphate synthase,27 homoprotocatechuate 2,3-dioxygenase,63 4-hydroxy-2-ketovalerate aldolase/acylating acetdehyde dehydrogenase,96 epoxide hydrolase from Aspergillus niger M200,26 and FabZ β-hydroxacyl-acyl carrier protein dehydratase.44 Similarly, gates can be situated in the bottlenecks of tunnels connecting two active sites. Gates in such positions are essential for enzymes that catalyze two reactions requiring different environments, such as glucosamine 6 phosphate synthase,45 imidazole glycerol phosphate synthase,38 cytidine triphosphate synthetase,52 carbamoyl phosphate synthetase,36 and glutamate synthases.46

4.3. Cofactor Cavity

Gates can be positioned at the interface of the active site and the cofactor cavity, allowing for more fine-grained control during the reaction. In NADH oxidase, the W47 residue acts as a gate that controls the accessibility of the FAD flavin ring and thus plays a crucial role during the catalytic cycle. The closed conformation is stabilized by hydrogen bonds between the cofactor and the peptide backbone, whereas stabilization of the open form may be advantageous during the initial steps of
Table 3. Detailed Description of Enzymes Possessing Gates Presented in the Scientific Literature

| No. | Enzyme name | EC number | Enzyme function | Enzyme function |
|-----|-------------|-----------|-----------------|-----------------|
| Gate function | Information about gate function | | Small picture of whole enzyme with detected tunnels and gates | |
| Gate location | Information about gate location | | | |
| Gate structural basis | | | protein – light blue surface | Large picture – close-up on gate residues |
| | Schematic drawing of gate class | | tunnel – dark blue wire | protein – light blue cartoon |
| | * GATE1 - indicates important residues used for gates engineering | | gates residues – red ball and stick | tunnel profile – grey spheres |
| | GATE1 - font colour corresponds to the colour of residues on the picture | | active sites residues (if shown) – green ball and stick | |
| | * GATE1 - * indicates important residues used for gates engineering | | gates residues (elements) – ball and stick | |
| | Information about residues, open and closed conformation and mechanism of the changes of gate states | | representation colour correspond to | |
| | ? in front of a picture indicates a lack of data – classification has been made based on residues localisation | | colour of gate name | |
| Gate engineering | Information about mutants that change gate state or create new gates, including information about mutants closing and opening tunnels | | cofactor – yellow ball and stick | |
| PDB: | from the literature | | substrate – yellow ball and stick | |
| | Wild-type (WT) or mutants available in Protein Data Bank database | | | |
| References: | All references used for table preparation | | | |

| 01 | 2-Amino-2-Deoxyisochorismate Synthase PhzE | 4.1.3.27 |
|----|--------------------------------------------|---------|
| Gate function | GATE1 – Controls access of ammonia, synchronizes active sites | Enzyme function | Utilizes chorismate and glutamine to synthesize 2-amino-2-deoxyisochorismate in the first step of phenazine biosynthesis |
| Gate location | GATE1 – Between the MST (menaquinone, siderophore, tryptophan) domain and GATase1 active site | | |
| Gate structural basis | GATE1 E251 | | | |
| | forms two hydrogen bonds with N149 in closed conformation | | PDB ID: 3R74 | |
| | interacts with K254 in open conformation | | Tunnel | U-shaped |
| | | | Length | 25 |
| PDB: | H7T – 3R74 (open), 3R75 (closed) | Bottlecneck | | |
| References: | T11 | Role | Ammonia transport | |

5881
dx.doi.org/10.1021/cr300384w | Chem. Rev. 2013, 113, 5871–5923
Table 3. continued

| 02 | 3-Hydroxybenzoate Hydroxylase MBBH | 1,14,13,23 |
|----|-----------------------------------|------------|
| Gate function | **GATE 1** – Controls the contact of NADPH with the isoolxazidine ring, protects FAD from the solvent | Enzyme function | Conversion of 3-hydroxybenzoate to 3,4-dihydroxybenzoate |
| Gate location | **GATE 1** – Between substrate tunnel and the FAD binding pocket | | |
| Gate structural basis | **GATE 1** Y317 | The residues create parallel π-π stacking interaction with FAD | Opening – reorientation of both FAD and Y317 |
| Gate engineering | | | |

![Image](image-url)

PDB ID: 2DKH

| Tunnel | E1 | E2 (not shown) |
|--------|----|---------------|
| Length | 20-22 | 32 |

PDB: **WT** 2DKH, 2DK1

References: \(^{98}\)

| 03 | 4-Hydroxy-2-Ketovalerate Aldolase DmpG / Acylating Acetaldehyde Dehydrogenase DmpF | 4.1.3.9 / 1.2.1.10 |
|----|-------------------------------------|------------------|
| Gate function | **GATE1** – Controls access of the substrate to the DmpG active site | Enzyme function | Catalyses final two steps in degradation of toxic aromatic intermediates in the meta-cleavage pathway of catechol aldehydes |
| **GATE2** – Proton transfer, synchronizing two active sites by controlling the passage of the acetaldehyde | | |
| **GATE3** – Controls intermediate entry to the DmpF active site; facilitates interaction between N171 and NAD | | |
| Gate location | **GATE1** – Tunnel entrance in aldolase subunit | | |
| **GATE2** – Tunnel entrance in aldolase subunit | | |
| **GATE3** – Tunnel exit in dehydrogenase subunit | | |
| Gate structural basis | **GATE1** H21 | Opening – reorientation of H21 |
| | **GATE2** Y291 | Opening – reorientation of Y291 |
| | **GATE3** I172 + I196 and M198 | I172, I196 and M198 block the tunnel exit in the dehydrogenase subunit |
| | Opening – in the structure with bound NAD⁺ – an interaction of N171 with NAD induce unique orientation of I172, additionally I196 and M198 adopt open conformations |
| Gate engineering | H21A – acetaldehyde and propionylaldehyde channelling reduced by more than 70% | | |
| Y291F – reduced channelling efficiencies by >30% | | |
| I196L, I196F – no significant changes in the channelling efficiency | | |

![Image](image-url)

PDB ID: 1NVM

| Tunnel between active sites | Length | 29 |
|---------------------------|--------|----|

PDB: **WT** 1NVM

References: \(^{96,112}\)

Role Acetaldehyde intermediate transport

References: dx.doi.org/10.1021/cr300384w Chem. Rev. 2013, 113, 5871–5923
### Table 3. continued

| 04 | 4-Hydroxybenzoate Hydroxylase PHBH | 1.14.13.2 |
|----|-----------------------------------|-----------|
| **Gate function** | GATE1 – Controls access of the solvent | **Enzyme function** |
| | GATE2 – Controls access of the solvent | Monooxygenation of 3-hydroxybenzoate (3-OHB) to 3,4-dihydroxybenzoate |
| **Gate location** | GATE1 – Close to the FAD cofactor | |
| | GATE2 – One domain of the protein | |
| **Gate structural basis** | **GATE1** |
| | R220 and FAD | |
| | R220 modulates the dynamics of flavin movements | |
| | Opening – reorientation of R220; out – solvent exposed; in – solvent excluded | |
| | **GATE2** |
| | Large domain movement | |
| | As substrate (p-OHB) moves forward in the tunnel and reaches its high-affinity site, the βββ (1-180) and the sheet domains (180-270) are expected to rotate and close the active site onto the substrate | |
| **Gate engineering** | R220Q – keeps enzyme in the open conformation – loss of selectivity and decrease of activity (100-fold) | |
| | PDB: WT – 1IUW, Mutant R220Q – 1K0I, 1K0J, 1K0L | |
| **References:** | | 111 |
| **Role** | Substrate access |

| 05 | Acetylcholinesterase ACHE | 3.1.1.7 |
|----|----------------------------|-------|
| **Gate function** | GATE1 – Controls access of the substrate to the active site | **Enzyme function** |
| | GATE2 – Controls escape of the acetic acid and/or the water molecule | Hydrolysis of acetylcholine |
| **Gate location** | GATE1 – Main tunnel – 12 Å from the bottom of the gorge – entrance to the active site | |
| | GATE2 – Back door tunnel – on the C67–C94 wall | |
| **Gate structural basis** | **GATE1** |
| | F330 | |
| | F330 controls the entrance of the natural substrate | |
| | Second most important residue – Y121, followed by W84, F288, F290, F331, Y334 | |
| | Cationic substrate (Huperzine A): movement of F330, Y121 and D72 generate an electrostatic field affecting the substrates | |
| | Aromatic substrate (E2020): residues grouped in 3 groups acting as “sender” and “receiver”, compose a “conveyor belt” via π-π stacking interactions with benzene ring of E2020 | |
| | Group I contains W84, F330, and F331 | |
| | Group II consists of F288, F290, and Y334 | |
| | Group III includes Y70, Y121, and W279 | |
| | In mACHE F338–Y124 | |
| | **GATE2** |
| | W84, 441, and Y442 (TeAChE); W86, 444, and Y449 (mAChE) | |
| | Opening - movement of the W84 indole ring, almost 90° rotation to a position where it interacts with Y442 | |
| | Alternative propositions | |
| | ?GATE2b – E32, P76, and G77 (with some small movement of D72, E73, and N85) | |
| | ?GATE2c – Between V71, N85, P96, and M90 | |
| | ?GATE2d – 90° rotation of the F78 and the displacement of V431 and W432 | |
| | ?GATE2e – W84, V129, and G441 | |
| | ?GATE2f – 180° rotation of the loop with W48 could undergo a flap-like transition | |
| | ?GATE2g – Facial rearrangement of the loop between W279 and S291 | |
| **Gate engineering** | V129W – 4-fold increase of $K_m$ | |
| | V431C – 2-fold increase of $K_m$ | |
| | PDB: WT – 1W75, 1ACJ, 1ACL, 1EAS, 2ACE, 1MAH, 1QTI, 1DX6, 1EVE, 1OCE, 2X4H (open back door) | |
| **References:** | | 111 |
| **Role** | Acetylcholine |
| **Substrate** | Small molecules |

| 06 | 4-Hydroxybenzoate Hydroxylase PHBH | 1.14.13.2 |
|----|-----------------------------------|-----------|
| **Gate function** | GATE1 – Controls access of the solvent | **Enzyme function** |
| | GATE2 – Controls access of the solvent | Monooxygenation of 3-hydroxybenzoate (3-OHB) to 3,4-dihydroxybenzoate |
| **Gate location** | GATE1 – Close to the FAD cofactor | |
| | GATE2 – One domain of the protein | |
| **Gate structural basis** | **GATE1** |
| | R220 and FAD | |
| | R220 modulates the dynamics of flavin movements | |
| | Opening – reorientation of R220; out – solvent exposed; in – solvent excluded | |
| | **GATE2** |
| | Large domain movement | |
| | As substrate (p-OHB) moves forward in the tunnel and reaches its high-affinity site, the βββ (1-180) and the sheet domains (180-270) are expected to rotate and close the active site onto the substrate | |
| **Gate engineering** | R220Q – keeps enzyme in the open conformation – loss of selectivity and decrease of activity (100-fold) | |
| | PDB: WT – 1IUW, Mutant R220Q – 1K0I, 1K0J, 1K0L | |
| **References:** | | 111 |
| **Role** | Substrate access |

| 07 | 4-Hydroxybenzoate Hydroxylase PHBH | 1.14.13.2 |
|----|-----------------------------------|-----------|
| **Gate function** | GATE1 – Controls access of the solvent | **Enzyme function** |
| | GATE2 – Controls access of the solvent | Monooxygenation of 3-hydroxybenzoate (3-OHB) to 3,4-dihydroxybenzoate |
| **Gate location** | GATE1 – Close to the FAD cofactor | |
| | GATE2 – One domain of the protein | |
| **Gate structural basis** | **GATE1** |
| | R220 and FAD | |
| | R220 modulates the dynamics of flavin movements | |
| | Opening – reorientation of R220; out – solvent exposed; in – solvent excluded | |
| | **GATE2** |
| | Large domain movement | |
| | As substrate (p-OHB) moves forward in the tunnel and reaches its high-affinity site, the βββ (1-180) and the sheet domains (180-270) are expected to rotate and close the active site onto the substrate | |
| **Gate engineering** | R220Q – keeps enzyme in the open conformation – loss of selectivity and decrease of activity (100-fold) | |
| | PDB: WT – 1IUW, Mutant R220Q – 1K0I, 1K0J, 1K0L | |
| **References:** | | 111 |
| **Role** | Substrate access |
### Table 3. continued

| 06 | Acylaminoacyl Peptidase |
|----|--------------------------|
| **Gate function** | **Enzyme function** |
| GATE1 – Controls enzyme activity – only closed form is active | Removes acylated amino acid residues from the N terminus of oligopeptides |
| **Gate location** | |
| GATE1 – Two domains of an enzyme monomer | |
| **Gate structural basis** | | ![Open and closed structures](image1) |
| GATE1 Two domains may move away to form an opening of about 30°, with D376 being the hinge | Open - accept substrate |
| | Closed - rearrange active site |
| **Gate engineering** | |
| | PDB ID: 3O4G |
| | Tunnel |
| | Length |
| PDB: 3T – 3O4G | Bottleneck |
| References: 124, 125 | Role |

| 07 | α-Amylase TK1436 |
|----|------------------|
| **Gate function** | **Enzyme function** |
| GATE1 – Controls access of the substrate to the active site, regulates transglycosylation | Formation of branch points in glycogen and amylopectin by cleavage of α-1,4 glycosidic bonds and subsequent transfer to a new α-1,6 position |
| **Gate location** | |
| GATE1 – At the entrance of the active site | |
| **Gate structural basis** | |
| GATE1 W270 | The residue displays different conformations depending on the presence or absence of ligands in the active-site pocket |
| | Other possible gate-keepers W28, W407, W416 |
| **Gate engineering** | | ![PDB ID: 3N8T](image2) |
| | Tunnel |
| | Length |
| PDB: 3T – 3N8T, 3N92, 3N98 | Bottleneck |
| References: 128 | Role |
| | Substrate recognition and binding |
Table 3. continued

| Gate function | Asparagine Synthetase | Enzyme function |
|---------------|-----------------------|-----------------|
| **Gate function** | | ATP dependent synthesis of L-asparagine from L-aspartic acid |
| ? GATE1 – Synchronizing active sites, establish the intramolecular tunnel for ammonia passage | | |
| ? GATE2 | | |
| **Gate location** | | |
| ? GATE1 – C-terminal end of the ammonia tunnel linking the active site near to the ATP moiety | | |
| ? GATE2 – C-terminal domain | | |
| **Gate structural basis** | | |
| ![Diagram](image1.png) | | |
| | | |
| ? GATE1 | | |
| E348 | | |
| ? GATE2 | | |
| N389 | | |

**Gate engineering**

E348D – impairs acyl-adenylate formation, tunnel is more solvent exposed leading to loss of the ammonia because of an impaired rate of [γAspAMP] formation.

| PDB: WT – 1CT9 | | |
| References: 42,48,127,128 | | |

| ATP-Dependent Protease HslVU | | |
|-------------------------------|-----------------|
| **Gate function** | | Degradation of the majority of proteins in a cell |
| **Gate location** | | |
| GATE1 – Controls access of the substrate to the active site located inside the chamber | | |
| GATE1 – Translocation tunnel | | |
| **Gate structural basis** | | |
| ![Diagram](image2.png) | | |
| | | |
| GATE1 | | |
| Y91 | | |
| “Twist-and-open” mechanism - conformational changes induced by ATP hydrolysis are propagated to the gating sequence | | |
| Y91 can move (180° rotation) from inside HslU toward HslV through the pore – closed pore has diameter 4.4Å open pore has diameter of 19.3Å | | |
| HslVU works as a hexamer therefore pore diameter depends on the number of Y91 pointed toward HslV | | |

**Gate engineering**

Y91F, V92I, V92A, and V92S – decrease in protein degradation activity
G90P, G93P, G90A, G93P, Y91I, Y91E, Y91S, Y91A, V92F, V92C – are not capable to support the proteolytic activity of HslV

| PDB: WT – 1G4A, 1G4B | | |
| References: 103,103 | | |

| Tunnel | Length | PDB ID: 1G4A | Bottleneck | Closed - 4.4; open up to 19.3 |
| Role | Ammonia transport | - | - | - |
| Role | - | - | - | - |

dx.doi.org/10.1021/cr300384w | Chem. Rev. 2013, 113, 5871–5923
### Table 3. continued

| Gate function | Carbamoyl Phosphate Synthetase CPS – type II | Enzyme function |
|---------------|--------------------------------------------|----------------|
| **Gate function** | **Enzyme function** |
| **GATE1** – Desolvation of ammonia | **Synthesis of carbamoyl phosphate** |
| **GATE2** – Controls entry of carbamate into the tunnel prior to phosphorylation to carbamoyl phosphate | |
| **GATE3** – Controls entry of carbamate into the tunnel prior to phosphorylation to carbamoyl phosphate | |

| Gate location | Gate structural basis |
|---------------|-----------------------|
| **GATE1** – Large subunit/ammonia tunnel | **GATE1** C232, A251, A314 (barrier 7.2 kcal/mol) Two more triad that may act as gate switches for an ammonia passage are: E254-T249-L310 E217-T244-S307 |
| **GATE2** – Carbamate tunnel near the carboxylic phosphate binding site | **GATE2** R306, E25, ?E383, ?E604 R306 – ion pair with E25 |
| **GATE3** – Carbamate tunnel near the site for the synthesis of carbamoyl phosphate | **GATE3** R848, E577 and ?E916 R848 – ion pair with E577 |

| Gate engineering | Chemical Reviews dx.doi.org/10.1021/cr300384w Chem. Rev. 2013, 113, 5871−5923 |
|-------------------|----------------------------------------------------------------------------------|
| C232V/A251V/A314V – closing ammonia tunnel - unable to synthesize carbamoyl phosphate using glutamine as a nitrogen source | G359F, G359Y – decoupling separate chemical reactions via creation an escape route for the ammonia intermediate |
| aP360A/a1361A/b1265A – unable to utilize glutamine for the synthesis of carbamoyl phosphate via creation of an escape route for the ammonia intermediate; full catalytic activity with external ammonia source | F913Q – 10-fold decrease in the rate of carbamoyl phosphate synthesis |
| E25Q/E383Q – carbamoyl phosphate synthetase activity was diminished 50-fold | E25Q/E383Q/E604Q – glutaminase activity is decreased about 5-fold, and the bicarbonate-dependent ATPase activity is diminished at least 20-fold |
| E377Q – decrease of carbamoyl phosphate synthesis (100-fold) | |

| PDB ID: | JDB |
|---------|-----|
| Tunnel | Whole | I part | II part |
| Length | >100 | 45 |
| PDB: | | |
| | WT – IBXR, JDB | | |
| Role | Connects all 3 active sites | Ammonia transport | Carbamate transport |

| 11 | Carbon Monoxide Dehydrogenase / Acetyl Coenzyme A Synthase | 1.2.7.4 / 1.2.99.2 / 2.3.1.169 |
| Gate function | Controls the reaction, protects from CO leakage and controls CO access to N4–N4–[Fe–S4] cluster (A cluster – acetyl-CoA synthase active site) |

| Gate location | Gate structural basis |
|---------------|-----------------------|
| **GATE1** – 20 Å from the A cluster – on the A and C cluster border | **GATE1** Large conformation change of subunit a, gating residues – residues of the a subunit N-terminal domain 143-148 Open conformation |
| – accessible active site | Closed tunnel |
| – F312 moves to a position within 4 Å of both N4 and N4 blocking putative ligand binding to the axial coordination site of N4 Close conformation | – closed active site |
| – open tunnel | – F229 blocks axial ligand binding to N4 but not to the Zn ion |

| Gate engineering | Chemical Reviews dx.doi.org/10.1021/cr300384w Chem. Rev. 2013, 113, 5871−5923 |
|-------------------|----------------------------------------------------------------------------------|
| A110C, A222L, A265M – block the tunnel between the A and C-clusters; A222L complete blocking A378C, L215F, A219F – block the tunnel between the C clusters | F70W, N101Q – block a region at the ββ subunit interface that might dynamically connect the tunnel with a newly discovered water tunnel |

| PDB ID: | 1OAO |
|---------|-------|
| Only one from two symmetrical tunnels is shown on a picture. |

| Tunnel | |
|--------|---|
| Length | 130 between A clusters, additional two tunnels connecting C clusters 37 + additional water channel |

| PDB: | WT (M. thermoautotrophicum) – 1OAO (open-closed form), WT (C. hydrogenoformans) – 1RU3 (closed form) |
| PDB ID: | 1OAO |
| Role | CO transport between two active sites |

5886 dx.doi.org/10.1021/cr300384w Chem. Rev. 2013, 113, 5871−5923
### Table 3. continued

| Gate function | Chemical Anhydrase β – type I | 4.2.1.1 |
|---------------|--------------------------------|---------|
| GATE1 – Protects zinc cation site | | |

| Enzyme function | | |
| Caters a reversible reaction to form bicarbonate from carbon dioxide and water | | |

| Gate location | | |
| Vicinity of the active site | | |

| Gate structural basis | | |
| D162 donates a hydrogen bond to Q151 | | |
| Acts as a gatekeeper residue by excluding anions from the zinc ligand environment that cannot donate a hydrogen bond at this position | | |

| Gate engineering | | |
| | | |

| PDB: WT – 1EKJ | | |
| | | |

| References: | | |
| | | |

| Table 3. continued |
|---------------------|

| Gate function | Chemical Anhydrase β – type II | 4.2.1.1 |
|---------------|--------------------------------|---------|
| GATE1 – Controls access of the bicarbonate ion | | |
| GATE2 – Controls access of the solvent, protects active site, controls transport of bicarbonate ion from the active site or from solution to the allosteric pocket | | |

| Enzyme function | | |
| Caters a reversible reaction to form bicarbonate from carbon dioxide and water | | |

| Gate location | | |
| GATE1 – Middle of the tunnel | | |
| GATE2 – Vicinity of the active site in a narrow hydrophobic active site cleft that lies along the dimer or pseudo-dimer interface | | |

| Gate structural basis | | |
| GATE1 | | |
| R64 – HICA | | |
| Rotation of the guanidinium group allows migration of bicarbonate ion into the allosteric site | | |

| Gate engineering | | |
| | | |

| PDB: WT (HICA) – 2A8D, WT (PPCA) – 1DDZ, WT (Rv3588) – 1YM3, WT (Nce103) – 3EYX | | |

| References: | | |
| | | |

| PDB ID: 2A8D | | |
| | | |

| Tunnel Length | | |
| | | |

| Role | Acetate ion, CO₂ transport | | |
| | | |

| References: | | |
| | | |

| PDB ID: | | |
| | | |

| Length | | |
| | | |

| References: | | |
| | | |

5887 dx.doi.org/10.1021/cr300384w | Chem. Rev. 2013, 113, 5871−5923
Table 3. continued

| 14 | Carboxylesterase pubCE | 3.1.1 |
|----|------------------------|------|
| **Gate function** | **Enzyme function** | Detoxification of xenobiotics |
| GATE1 – Controls exit of hydrolysis products by a side door | | |
| GATE2 – Controls access to the active site gorge | | |
| GATE3 – Controls access to the active site gorge | | |
| **Gate location** | | |
| GATE1 – Between active site and side door | | |
| GATE2 – Entrance to active site gorge | | |
| GATE3 – Entrance to active site gorge | | |
| **Gate structural basis** | | |
| GATE1 | | |
| L362 | | |
| Rotates 180° around its C-C bond and adopts two distinct conformations | | |
| GATE2 | | |
| Loop coil_5 (residues 61-82) and coil_21 (residues 408-422) | | |
| Move away from their equilibration conformation, the active site opens and can accommodate incoming substrate | | |
| GATE3 | | |
| Loop coil_17(residues 308-323) and coil_21 | | |
| Close for substrate hydrolysis, move away to release products | | |
| **Gate engineering** | | |
| Δ coil_5 – kcat 4.5-fold smaller | | |
| Δ coil_21– kcat 3-fold smaller | | |
| PDB: IRT – IQE3 | | |
| Tunnel Length | | |
| Bottleneck | | |
| References: 178, 140 | | |

| 15 | Catalase CAT-1 and CAT-3 | 1.11.1.6 |
|----|--------------------------|---------|
| **Gate function** | **Enzyme function** | Decomposition of hydrogen peroxide to water and O2 |
| GATE1 – Blocks access to the heme, controls solvation | | |
| GATE2 – ? | | |
| **Gate location** | | |
| GATE1 – Entrance to the active site cavity from main tunnel | | |
| GATE2 – Side tunnel | | |
| **Gate structural basis** | | |
| GATE1 | | |
| H54, V95, and F132 | | |
| Barrier to reach the active site: | | |
| H2O2 | < 4.8 kcal/mol | | |
| O2 | ~2.2 kcal/mol | | |
| H2O | ~ 3.6 kcal/mol | | |
| Opening of the gate may be regulated according to the H2O2 concentration in the small cavity before the gate; H2O2 in the cavity would change the net of hydrogen bonds and trigger opening of the gate; water molecules interacting with amino acid residues in the cavity would determine closure of the gate | | |
| GATE2 (not shown on picture) | | |
| CAT-1 – hydroxyl group of S198 blocks tunnel | | |
| CAT-3 – hydroxyl group of T208 blocks tunnel | | |
| **Gate engineering** | | |
| PDB: IRT – NM0 | | |
| Tunnel Main Back | | |
| Bottleneck | | |
| References: 23, 24, 88 | | |
| Role O2 and H2O2 transport | | |
### Table 3. continued

| 16 | Cellubiohydrolase CEL7A | 3.2.1.91 |
|----|------------------------|----------|
| **Gate function** | **Enzyme function** |
| *GATEI* – Facilitates processing of crystalline cellulose degradation | Hydrolysis of amorphous and crystalline cellulose |
| **Gate location** | |
| *GATEI* – Exo loop |
| **Gate structural basis** | |
| Exo loop + Y247 |
| Y247 in closed conformations interacts with Y371 from the short loop – acting like a button – it creates tunnel, loop covers the active site during reaction time |
| **Gate engineering** | |
| Y247F – removes hydrogen bond between Y247 and substrate - small effect on cellulase hydrolysis |
| D241C/D249C – reduces mobility of the loop, disulphide bridge enhanced the activity on both amorphous and crystalline cellulose |
| Deletion G245-Y252 – increases activity on amorphous cellulose, and half of the activity on crystalline cellulose |

![Image](image1.png)

- PDB ID: 1CEL
- Tunnel
  - Length: 50
- References: [GATEI](#)

| 17 | Cellobiose Phosphorylase | 2.4.1.20 |
|----|-------------------------|----------|
| **Gate function** | **Enzyme function** |
| *GATEI* – Controls access of the substrate | Phosphorylation of cellubiose into α-D-glucose 1-phosphate (G1P) and D-glucose |
| **Gate location** | |
| *GATEI* – Flexible loop |
| **Gate structural basis** | |
| Flexible loop (495–513) |
| Loop undergoes conformational changes during substrate binding and release |
| **Gate engineering** | |
| T508I – changes substrate specificity |
| N156D and N163D – increase of the activity |
| N156D/N163D/T508I/E649G/N667A – improves activity towards a whole range of β-glucosidic acceptors |

![Image](image2.png)

- PDB: HT – 2CQT, 1VTX
- Tunnel
  - Length
- References: [GATEI](#)
Table 3. continued

| 18 | Chalcone Synthase CHS | 2.3.1.74 |
|----|----------------------|---------|
| **Gate function** | **Enzyme function** | |
| GATE1 – Controls orientation of the substrate | Decarboxylative condensations of malonyl-CoA with a CoA-linked starter |
| **Gate location** | | |
| GATE1 – Between the active-site cavity and the CoA binding tunnel | | |
| **Gate structural basis** | | |
| GATE1 Medicago sativa CHS | | |
| F215 and F265 | | |
| Block the lower portion of the opening between cavities and help with folding and the internal orientation of the tetramer intermediate during the cyclization reaction; F215 facilitates the decarboxylation of malonyl-CoA by maintaining the orientations of substrates and intermediates during the sequential condensation reactions | | |
| **Gate engineering** | | |
| M. sativa CHS | | |
| F215S – changes the substrate specificity via opening a space at the cavity entrance | | |
| A. arborescens PCS | | |
| *GATE2 | | |
| M207G – opens a gate to two novel hidden pockets behind the active site of the enzyme - residues 207 controls the number of condensations of malonyl-CoA | | |
| F80A/Y82A/M207G – provide further elongation of products | | |

References: [12,13,6,14]

| 19 | Chloramphenicol Halogenase CmlS | Not determined |
|----|----------------------------------|---------------|
| **Gate function** | **Enzyme function** | |
| GATE1 – Blocks access to the active site | Formation of the dichloroacetetyl group |
| **Gate location** | | |
| GATE1 – Flexible loop (the C-terminal lobe of CmlS) | | |
| **Gate structural basis** | | |
| GATE1 | Flexible loop (the C-terminal lobe of CmlS) + F562 acting as a plug | | |
| **Gate engineering** | | |

References: [15]

PDB: WT (M. sativa CHS) – 1CGK, WT (A. arborescens PCS) – 3ALE

References: [12,13,6,14]
### Table 3. continued

| Gate function | Enzyme function |
|---------------|----------------|
| **Gate function** | Catalysts the first step in the pathway of cholesterol degradation |
| **Gate location** | **Gate structural basis** |
| GATE1 – Controls access of the solvent | GATE1 – Frames the entrance to the tunnel |
| GATE2 – Controls access of O₂ to the flavin and assures that isomerization occurs before the oxidative half of the reaction, tuning the redox state of the cofactor | GATE2 – Gate is in the tunnel leading to the isoalloxazine ring of flavin |

#### Gate structural basis

- **GATE1**
  - F359
  - F359 adopts two distinct alternate conformations separated by a 65° rotation of the benzene group
  - Closed gate - maximizing hydrophobic packing interactions with V189, V124 and G347
  - Open conformation - the tunnel becomes solvent accessible
  - Control of F359 gate appears to be dependent on the adopted conformation of N485

- **GATE2**
  - N485, E361 and M122
  - Switch between their side-chain conformations, controls the access of O₂ to flavin
  - 1. Binding of steroid → rotation of the methyl group of M122 → pushes N485 sealing the tunnel and creating an ideal environment for oxidation
  - 2. After substrate oxidation, a strong hydrogen bond forms when N485 moves to conformation near the flavin, forcing M122 to a conformation that destabilizes the binding of the oxidized product.
  - 3. When the substrate is oxidized and the FAD cofactor is reduced, the side chain of N485 rotates toward the cofactor → tunnel opening - regulates access of oxygen to the active site

#### Gate engineering

| F359W – rate of catalysed reaction decreases 13-fold |
| G347N – could not be saturated with oxygen |
| N485D – could not be saturated with oxygen |

**PDB ID:** 1MXT

- **Tunnel**
  - Length 32

- **PDB:** WT – 1MXT, Mutant F359W – 3CNJ, Mutant N485D – 3GYI

**References:**

| Role | O₂ and H₂O₂ transport |

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| Gate function | Enzyme function |
|---------------|----------------|
| **Gate function** | Catalysts the first step in the pathway of cholesterol degradation |
| **Gate location** | **Gate structural basis** |
| GATE1 – Modulates access/reactivity of dioxygen | GATE1 – Bottleneck of the tunnel |

#### Gate structural basis

- **GATE1**
  - E311, I423, E475 and R477
  - Open conformation of R477 is stabilized by a salt bridge with E311
  - E311 tunes the E475—R477 pair

**Gate engineering**

| E311DQQL – cause a switch in the basic kinetic mechanism of the reoxidation with dioxygen, while BsChOx wild type and most mutants show saturation behaviour with increasing O₂ |
| For E311 – a linear dependence was found that would reflect a second-order process |
| R477A – limits both oxidation and isomerization activities |
| A204C, G309A, G309C, I423L, I423V, E475D, E475Q, R477K – no significant changes |

**PDB ID:** 1119

- **Tunnel**
  - Length 23

- **PDB:** WT – 1119

**References:**

| Role | Oxygen transport |
### Table 3. continued

| 22 | Choline Oxidase | 1.1.3.17 |
|----|-----------------|----------|
| **Gate function** | | |
| 7GATE1 – Controls access of the positively charged substrate | | |
| GATE2 – Controls access of the positively charged substrate | | |
| **Gate location** | | |
| 7GATE1 – Loop adjacent to the active site of choline oxidase - residues 74-85 | | |
| GATE2 – Located in the tunnel above active site | | |
| **Gate structural basis** | | |
| 7GATE1 Movement of the loop can open active site | | |
| GATE2 M62, L65, V355, F357, and M359 Breathing motion of M62, L65, V355, F357, and M359 The weakly hydrophobic interactions between the gating residues ensure that the positively charged substrate can easily slip to the highly electronegative active site The distribution of the residues just outside the five gating residues contains a considerable amount of negatively charged amino acids, which include E65, E66, E358, and E370, which may attract and guide the positively charged choline substrate to the active site | | |
| **Gate engineering** | | |

PDB ID: 2JBV

Tunnel Length

References: 31

| 23 | Chondroitin AC Lyase | 4.2.2.5 |
|----|----------------------|---------|
| **Gate function** | | |
| GATE1 – Creates tunnel and the active site, controls access of the solvent | | |
| **Gate location** | | |
| GATE1 – N-terminal domain and C-terminal domain | | |
| **Gate structural basis** | | |
| GATE1 Two loops Loops are flexible and open periodically allowing the glycosaminoglycan chain to slide in; Gating involves the movement of the tips of one or two loops only, D71-W76 of the N-terminal domain and G373-K375 of the C-terminal domain | | |
| **Gate engineering** | | |

PDB ID: 1CB8

Tunnel Length

References: 31

|  |  |  |
|---|---|---|
| PDB: 1CB8 | Bottleneck | Role | Active site cavity |
Table 3. continued

| 24 | Copper–Containing Amine Oxidase | 1.4.3.21 |
|----|---------------------------------|----------|
| Gate function | GATE1 – Blocks the back side of the active site from solvent access | Enzyme function | Oxidation of primary amines to aldehydes reducing molecular oxygen to hydrogen peroxide |

Gate location
GATE1 – Amine tunnel

Gate structural basis

- **GATE1**
  - Y381 in amine oxidase ECAO
  - W156 in amine oxidase HCAO
  - Y296 in amine oxidase AGAO
  - F298 in amine oxidase PSAO

Gating residues can form a π/π ring stacking interaction with the pyridine ring of the cofactor (TPQ)

Gate engineering

| PDB: WT (ECAO) – 1OAC, WT (HCAO) – 20QE, 200V, WT (AGAO) – 1RJO, 1RKY, WT (PSAO) – 1WZZ | BottleNeck | Role |

References: 98,152/134

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| 25 | Cytidine Triphosphate Synthetase CTPS | 6.3.4.2 |
|----|----------------------------------------|----------|
| Gate function | GATE1 – Creates tunnel, protects tunnel from access of the solvent | Enzyme function | Catalyses the formation of CTP from UTP, ATP and glutamine |
| GATE2 – Controls passage of ammonia to the amidotransferase active site | GATE3 – Controls passage of ammonia to the amidotransferase active site |

Gate location
GATE1 – Loop of the glutaminase domain
GATE2 – The domain interface, interacts with the amide group of the bound glutamine
GATE3 – Between UTP site and the tunnel exit

Gate structural basis

- **GATE1** (PDB ID: 1VCM)
  - Loop
  - The synthetase active site is exposed to the solvent and the binding pocket for the allosteric effector GTP is not properly formed
  - After ATP and acceptor binding loop covers the entrance of the glutaminase site shielding glutamine and creates the tunnel

- **GATE2** (PDB ID: 1VCM)
  - Y64
  - Y64 might act as a door to the ammonia tunnel leading to the synthetase site

- **GATE3** (PDB ID: 2ADP)
  - H57
  - 1. Binding of the substrate UTP induces the rotation of H57
  - 2. Rotation of H57 opens the tunnel for ammonia passage
  - The ligand-induced change is postulated to regulate the timing for the translocation of ammonia

Gate engineering

| PDB: WT – 1VCM, 1VCN, 1VCO, 2AD5, 1S1M | BottleNeck | Role |

References: 85,165/192
### Table 3. continued

| Gate function | Cytochrome P450 CYP3A4 | Enzyme function |
|---------------|------------------------|-----------------|
| Controls access to the active site, controls selectivity and specificity of the enzyme | Catalyses mono-oxygenation reactions such as hydroxylation and epoxidation, major drug metabolizing enzyme in humans |

#### Gate location
Depends on the P450 isoform, tunnel and the gate

#### Gate structural basis
Common gating mechanisms in cytochrome P450 family are:
(i) associated with F-G-helix-loop-helix or B-C loop movement (tunnels 2a, 2ae, 2b, 2c, 2e);
(ii) associated with swinging door type;
(iii) associated with wing type;
(iv) cofactor assisted (CPR)
For details of other cytochrome P450 gates see references 16, 58, 96

### CYP3A4

| Residue | Position | Mechanism of gates opening: |
|---------|----------|-----------------------------|
| 2a      | F57      | (i) breaking F-F z-stacking  |
| 2b      | F108     | (ii) breaking aliphatic contacts between R212 and L482 - S tunnel |
| 2c      | F108     | (iii) breaking the H bond, as observed during the exit of TST-OH through tunnel 2c between R105 and S119 |
| 2e      | I120     | (iv) opening of water tunnel when the FMN domain of CPR binds to CYP3A4 via R1375 rotation. |

#### Gate engineering
The CYP3A4 tunnel structure is shown for clarity only gate of tunnel 2a and tunnel S are shown

| PDB ID: 1TNQ - for clarity only gate of tunnel 2a and tunnel S are shown |
|--------------------------|--------------------------|
| Tunnel                  | 2a | 2b | 2c | 2e | S | W |
| Length                  | 19 | 18 | 17 | 12 | 17 | 13 |

#### References:
10, 16, 23, 55, 68, 98, 99, 118, 119

### Dihydrofolatereductase

| Gate function | Enzyme function |
|---------------|-----------------|
| GATE1 - Controls the activity | Catalyses the stereospecific reduction of dihydrofolate to tetrahydrofolate |

#### Gate location
GATE1 – Entrance to the cofactor cavity

#### Gate structural basis
GATE1

M20 loop (residues 9 to 24)
In closed conformation the loop packs tightly against the nicotinamide ring of the cofactor
In occluded conformation the loop projects into the active site and sterically blocks the cofactor cavity

#### Gate engineering
N233P – kcat decreased 5-fold
S148A – kcat decreased 2-fold
N233PS148A – kcat decreased 6-fold

#### References:
80, 90, 160–162

| PDB ID: 1RX2 |
|--------------|
| Tunnel      |
| Bottleneck  |
| Role        |

| PDB: 1RX2, Mutants – 3QL0, 3QL3 | Bottleneck |
|--------------------------------|------------|
| References: 80, 90, 160–162 | Role |
### Table 3. continued

| Gate function | Enzyme function |
|---------------|-----------------|
| GATE1 – Opens tunnel, controls entry of the substrate, controls reduction of FAD | Converts 2,3-di-O-geranylglycerol phosphate to 2,3-di-O-phytylglycerol phosphate (archaetidic acid) |
| GATE2 – Controls substrate binding/release | |

**Gate location**

- **GATE1** – Vicinity of the cofactor binding site (FAD)
- **GATE2** – C-terminal helical subdomain

**Gate structural basis**

- **GATE1**
  - Glycine-rich α–β loop (residues 289–298)
  - Cofactor FAD has two conformation IN (not accessible by solvent) and OUT (exposes the soulphosphate ring allowing it to be reduced by NADH or NADPH). In IN conformation tunnel A is blocked, to open it has to turn into OUT conformation. The conformational changes in the glycine-rich α–β loop disrupt interaction between Y209 and T299 allowing change of the FAD position. Conserved RxxxFD and LxGD motifs may play a role in FADs IN/OUT conformational switch.

- **GATE2**
  - Two regions, the β6–β7 loop (residues 87–94) and the C-terminal helices (residues 370–396)
  - The conformation changes of the C-terminal helical subdomain may be involved in substrate binding/release

**Gate engineering**

| PDB ID: 3OZ2 |
|--------------|
| Tunnel | A | B |
| Length | |
| References: 10B | Bottleneck | Role |

### Epoxydase Hydrolase H37Rv

| Gate function | Enzyme function |
|---------------|-----------------|
| GATE1 – Regulates access to the active site | Hydrolysis of epoxides |

**Gate location**

- **GATE1** – Cap domain

**Gate structural basis**

- **GATE1 (Mycobacterium tuberculosis H37Rv)**
  - Movement of the cap domain regulates access to the active site

**Gate engineering**

| PDB ID: 2F3J |
|--------------|
| Tunnel | |
| Length | |
| PDB: WT (Aspergillus niger M200) – 1QO7 | Bottleneck | Role |

References: 16
### Table 3. continued

| Gate function | Epoxide Hydrolase M200 | 3.3.2.3 |
|---------------|------------------------|---------|
| 7GATE1 – Controls enantioselectivity and activity | | |
| 7GATE2 – Controls enantioselectivity and activity | | |

| Enzyme function |
|----------------|
| Hydrolysis of epoxides |

#### Gate location

- **7GATE1** – Tunnel entrance
- **7GATE2** – Middle of the tunnel

#### Gate structural basis

- **7GATE1** (*Aspergillus niger* M200)
  - R219
- **7GATE2** (*Aspergillus niger* M200)
  - A217

#### Gate engineering

*Aspergillus niger* M200

A217C, A217E, A217G, A217L, A217P, A217Q, A217R, A217T, A217V – at the entrance to the tunnel result in different enantioselectivity and activity; e.g.: A217G mutation results in a 33-fold decrease of activity A217V 6.6-fold increase of activity with no changes in the products enantioselectivity for the reaction with allyl glycicyl

**PDB ID:** 1Q07

- **Tunnel:** C
- **N terminal clefl:** (not shown)
- **Length:** 25
- **N terminal clefl:** 15

**Bottleneck**

**Role:** Substrate binding cavity sEH

**References:** 26,143

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| Gate function | FabZ β-Hydroxacyl-Acyl Carrier Protein Dehydratase | 4.2.1.59 |
|---------------|--------------------------------------------------|---------|
| GATE1 – Controls access of the substrate, prohibits binding of small hydrophobic molecules to the unliganded enzyme | | |
| GATE2 – Controls length of the substrate | | |

| Enzyme function |
|----------------|
| Elongation cycles of both saturated and unsaturated fatty acids biosyntheses in the type II fatty acid biosynthesis system (FAS II) pathway |

#### Gate location

- **GATE1** – Entrance to the tunnel
- **GATE2** – Exit (back door) of the tunnel

#### Gate structural basis

- **GATE1**
  - Y100 (Y98 in PaFabZ and L170 in PfFabZ) adopts either an open or closed conformation in the crystal structure
  - In closed conformation - Y100 is stabilized by the Van der Waals interactions with M102, M154, and P112; in open one it flips ~120° and is stabilized by the Van der Waals interactions with M154, K62, and I64

- **GATE2**
  - F83
  - Refined in two additional alternative conformations, leading the tunnel to form a L-shape or an U-shape, in closed conformation - F83 points toward R88, in open position it rotates ~120° points toward H3, exposing the exit to the bulk solvent
  - T85 or F74 play a similar role in PaFabZ

#### Gate engineering

Y100A – drops the activity of the mutant to less than 50% of the enzymatic activity of the wild type, the new entrance has ~15 Å in width, completely exposes the active site to the bulk solvent – as a result ACP binds to the mutant more strongly than to the wild-type. In particular, the dissociation step of ACP from the HpFabZ mutant is extremely slow.

**PDB ID:** 2GLL

- **Tunnel:** Slow
- **Length:** 20

**Bottleneck**

**Role:** Substrate binding tunnel, ACP binding groove

**References:** 54,104,165

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5896  
[dx.doi.org/10.1021/cr300384w](dx.doi.org/10.1021/cr300384w) | Chem. Rev. 2013, 113, 5871–5923
Table 3. continued

| 32 | Formiminotransferase-Cyclodeaminase FTCD | 2.1.2.5 |
|----|----------------------------------------|---------|
| **Gate function** | | |
| GATE1 – Controls position of H82 in proper orientation for reaction | | |
| **Gate location** | | |
| GATE1 – Blocks entrance from a short tunnel into active site | | |
| **Gate structural basis** | | |
| GATE1 | Loop rearrangement including a change in the histidine H82 side-chain position | | |
| **Gate engineering** | | |

![Diagram of GATE1 and GATE2](image)

| PDB ID: 1QO1 |
|--------------|
| Tunnel Main Second |
| Length 38 9 |
| Bottleneck 8 4 |
| Role Intermediate transport Formiminoglutarate substrate entry, glutamate product exit |

References: """"44

| 33 | Glucosamine 6 Phosphate Synthase GlmS | 2.6.1.16 |
|----|-------------------------------------|---------|
| **Gate function** | | |
| GATE1 – Protects Fru 6P site from the solvent, creates the ammonia tunnel | | |
| GATE2 – Protects glutaminase active site from the solvent | | |
| GATE3 – Opens ammonia tunnel, ammonia enters the sugar site, acts as a solvent barrier | | |
| GATE4 – Opens ammonia tunnel | | |
| **Gate location** | | |
| GATE1 – Part of the active site cavity | | |
| GATE2 – Part of the active site cavity | | |
| GATE3 – Tunnel between two active sites | | |
| GATE4 – Bottleneck of the tunnel between active sites | | |
| **Gate structural basis** | | |
| GATE1 | C-terminal loop (residues 600–608) Open state – relaxed loop Closed state – anchored by Y28 and W74 with Q loop | | |
| GATE2 | Q-loop (residues 73–81) Open state - anchored by R539* Closed state - after Q enters the active site anchored by Y28 and W74 with C-terminal loop | | |
| GATE3 | W74 x1 torsion angle of W74 changes by 75° | | |
| GATE4 | A602 and V605 A shift of the side-chain of the A602 residue is concomitant to a re-orientation of its backbone carbonyl group, which can then form a strong H-bond with the hydroxyl group of the Y28 | | |
| **Gate engineering** | | |
| W74A, W74L, W74F – inefficient ammonia transfer A602L, V605L – efficiency of ammonia transfer decreased 2-fold | | |

![Diagram of GlcM GlmS structure](image)

| PDB ID: 2BPL |
|--------------|
| Tunnel |
| Length 18 |
| Bottleneck |
| Role Ammonia transport |

References: """"59
### Table 3. continued

| Gate function | Glutamate Synthases GltS | Enzyme function |
|---------------|--------------------------|-----------------|
| **Gate function** | **Glutamate Synthases GltS** | **Enzyme function** |
| GATE1 – Controls correct conformation of active site for L-glutamine binding and hydrolysis, crucial for glutaminase activation and coupling of the glutaminase and synthase sites, creates ammonia tunnel | | Formation of L-glutamate from L-glutamine and 2-oxoglutarate |
| GATE2 – Shields substrate from bulk solvent | | |
| GATE3 & GATE4 – Controls transport of ammonia | | |
| **Gate location** | **Glutamate Synthases GltS** | **Enzyme function** |
| GATE1 – Loop protecting Fru 6P site from solvent | | |
| GATE2 – Loop protecting glutaminase site from solvent | | |
| GATE3 – Entrance to the interdomain tunnel | | |
| GATE4 – Entrance to the synthase site (end of the tunnel) | | |
| **Gate structural basis** | **Glutamate Synthases GltS** | **Enzyme function** |
| GATE1 | Loop 4 (residues 933–978) | |
| | Shifts to the active conformation – C-terminal residue E1013 forms a hydrogen bond with C1 and keeps correct conformation of C1 and loop 29–34 for L-glutamine binding and hydrolysis. E1013 side chain may also play a role in the precise geometry of the tunnel entry point | | |
| GATE2 | Loop 206–214 | | |
| | Closing of loop 206–214 after L-glutamine binding | | |
| GATE3 | T503, N504, S1011 and I1012 | | |
| | Small conformation changes of T503, N504 of the central domain, S1011 and I1012 of loop 4 | | |
| GATE4 | E903 and K966 | | |
| | Small conformation changes of E903 and K966. Additionally residues T507 and N508 and S976 and R977 may function as a gates for signalling between active sites | | |
| **Gate engineering** | **Glutamate Synthases GltS** | **Enzyme function** |
| E1013D – 100-fold decrease of activity, a sigmoid dependence of initial velocity on L-glutamine concentration | | |
| E1013N – 100-fold decrease of activity, exhibited hyperbolic kinetics | | |
| E1013A – 1000-fold decrease of activity | | |
| **PDB ID**: 1OFD – GATE3 closed | | |
| **Tunnel** | **Length** | **33** |
| **PDB**: 1F7T – 1OFD, 1OFE, 1EA0 | **Botulienck** | | |
| **References**: 46,63,155,168 | | | |

| Gate function | Glutamine Phosphoribosylpyrophosphate Amidotransferase | Enzyme function |
|---------------|---------------------------------------------------|-----------------|
| **Gate function** | **Glutamine Phosphoribosylpyrophosphate Amidotransferase** | **Enzyme function** |
| GATE1 – Closes synthase site and creates a narrow, solvent-inaccessible tunnel between active sites | | Catalyses the initial reaction in de novo purine nucleotide biosynthesis |
| GATE2 – Covers glutaminase site | | |
| GATE3 – Protects tunnel from the solvent access and avoids wasteful release of ammonia into solution | | |
| **Gate location** | **Glutamine Phosphoribosylpyrophosphate Amidotransferase** | **Enzyme function** |
| GATE1 – Loop near the synthase active site | | |
| GATE2 – Loop near the glutaminase active site | | |
| GATE3 – On Q-loop | | |
| **Gate structural basis** | **Glutamine Phosphoribosylpyrophosphate Amidotransferase** | **Enzyme function** |
| GATE1 | Loop (residues 326–350) | | |
| | The active site cavity is created by closure of the loop (residues 326–350) | | |
| | Closing of the active site creates a narrow, solvent-inaccessible tunnel between active sites I335 interacts with Y74 | | |
| GATE2 | Q-loop (residues 73–84) | | |
| | A key residue in coupling the glutamine and acceptor sites upon acceptor binding may play a similar gate role as W74 in GlnS | | |
| **Gate engineering** | **Glutamine Phosphoribosylpyrophosphate Amidotransferase** | **Enzyme function** |
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### Table 3. continued

| Gate function | 3.8.1.5 |
|---------------|---------|
| GATE1, GATE2, GATE3, GATE4, GATE5 – Controls access to the tunnel |  |

#### Enzyme function
Hydrolytic dehalogenation of various halogenated aliphatic hydrocarbons

#### Gate location
- GATE1 – Tunnel p1
- GATE2 – Tunnel p2a, p2b
- GATE3 – Tunnel p2b, p2c
- GATE4 – Tunnel p2c
- GATE5 – Tunnel p3

#### Gate structural basis
- **GATE1**: F144 and F149
- **GATE2**: I135
- **GATE3**: V245, W141
- **GATE4**: β-bridge interaction between P210 and A212 of the CC loop and I135 of the NC loop
- **GATE5**: W138 blocks tunnel p3

#### Gate engineering
C176Y, V245F, A172F, A145F – limiting access of water to the active site

| PDB ID: 1CQW |  |
|--------------|---------|
| Tunnel | p1 | p2a, p2b, p2c | p3 | Length | 20 | 22 | 24 |
| Role | Product | Product | Product |
| References: | 106110 |

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### Table 3. continued

| Gate function | 3.8.1.5 |
|---------------|---------|
| GATE1 – Controls access to the active site |  |
| GATE2 – Controls selectivity |  |

#### Enzyme function
Hydrolytic dehalogenation of various halogenated aliphatic hydrocarbons

#### Gate location
- GATE1 – Tunnel bottleneck
- GATE2 – Tunnel bottleneck

#### Gate structural basis
- **GATE1**: D147
  - Motion of this residues is necessary for entry of large substrates
- **GATE2**: L177
  - Partially blocks the entrance of the main tunnel

#### Gate engineering
L177A, L177C, L177G, L177F, L177K, L177T, L177W, L177D, L177H, L177M, L177Q, L177R, L177S, L177V, L177Y influence the substrate specificity and activity

| PDB ID: 1CV2 |  |
|--------------|---------|
| Tunnel | p1 | Length | 12 |
| Role | Product |
| References: | 7219071 |

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dx.doi.org/10.1021/cr300384w | Chem. Rev. 2013, 113, 5871–5923
Table 3. continued

| Enzyme function | Chemical Reviews | dx.doi.org/10.1021/cr300384w | Chem. Rev. 2013, 113, 5871−5923 | 5900 |
|-----------------|------------------|--------------------------|--------------------------|---|

| Gate function | Histone Deacetylase HDAC1 and HDAC2 | 3.5.1.98 |
|---------------|--------------------------------------|---------|
| GATE1 – Controls access to the active site through tunnel A | | |
| GATE2 – Controls access to the active site through tunnel B1 | | |
| GATE3 – Controls access to the active site through tunnel B2 (only HDAC1) | | |

**Gate location**

- GATE1 – Bottleneck of tunnel A
- GATE2 – Bottleneck of tunnel B1
- GATE3 – Bottleneck of tunnel B2 (only HDAC1)

**Gate structural basis**

- **GATE1**
  - F155 (F150)
  - Side chain of F155 has to rotate ~ 180°

- **GATE2**
  - F114 + Y27 and Y29 (F109 + Y22 and Y24)
  - F114 residue is located between two tyrosine rings in closed conformation
  - Opening requires unzipping of the gate

- **GATE3**
  - Y303
  - Observed only in HDAC1 - Y303 residue may rotate opening access through tunnel – anchoring residue in closed position M30

**Gate engineering**

- PDB ID: 3MAX
- PDB: WT (HDAC2) – 3MAX
- References: 172

| Tunnel | A | B1 | B2 |
|--------|---|----|----|
| Length | 11 | 14 | 16 |

**Histone Deacetylase HDAC8**

| Enzyme function | 3.5.1.98 |
|-----------------|---------|
| Deacetylation of the ε-amino group of specific lysine residues within histones and other proteins |

| Gate function | Histone Deacetylase HDAC8 |
|---------------|---------------------------|
| GATE1 – Regulates water or product (acetate) transit from the active site through the internal tunnel |

**Gate location**

- GATE1 – Centre of the internal tunnel

**Gate structural basis**

- **GATE1**
  - R37
  - A structural reorientation of R37 and the loop is required for opening the access to the active site via the 14 Å “internal” tunnel. R37 forms multiple hydrogen bond interactions with the backbone carbonyl oxygen atoms of conserved G303 and G305 positioned in a loop between the β8and α10-helix
  - Gating interaction between G139 and G303

**Gate engineering**

- R37A – the values for $k_{on}/k_{off}$ decrease 530-fold

**PDB ID: 2V5W**

| Tunnel | |
|--------|---|
| Length | 11 | 14 |

**PDB: WT – 2V5W**

| Bottle | |
|--------|---|
| Role | Access to active site |

**References:** 173
Table 3. continued

| 40 | HIV-1 Protease | 3.4.23.16 |
|----|----------------|-----------|
| **Gate function** | **Enzyme function** | |
| GATE1 – Controls access to the active site | Central role in processing HIV-1 viral polypeptide precursors | |
| **Gate location** | | |
| GATE1 – β-turn flaps | | |
| **Gate structural basis** | | |
| GATE1 | β-turn flaps (residues 43–58) |
| | Movement of two β-turn flaps controls access to the active site |

Gate engineering

WT – open 14% of time (Brownian dynamics simulations)
G48V/V82A – open 2% of time (Brownian dynamics simulations)
184V/190M – open 2% of time (Brownian dynamics simulations)
L90M, G48V – open 14% of time (Brownian dynamics simulations)
F53L – unstabilised semi open conformation due to lack of F53-150 interaction

PDB ID: 1HVR

| Tunnel | Length |
|--------|--------|
|        |        |

PDB: WT – 1HHP, 1HVR

Bottleneck

References: 75,83,124–127

Role: Active site gorge

| 41 | Homoprotocatechuate 2,3-Dioxygenase | 1.13.11.15 |
|----|-----------------------------------|-----------|
| **Gate function** | **Enzyme function** | |
| GATE1 – Controls O2 diffusion pathway | Degradation of catechol and its derivatives by cleavage of aromatic rings | |
| GATE2 – Controls alternative O2 diffusion pathway | | |
| **Gate location** | | |
| GATE1 – Just below the protein surface | | |
| GATE2 – Below the protein surface | | |
| **Gate structural basis** | | |
| GATE1 | T205, H213, and W304 |
| | Breathing motion of protein causes synchronizing movement of residues |
| GATE2 | R293, H213 |
| | The fluctuation of R293 along with H213 could result in opening of the pathway |

Gate engineering

PDB ID: 2G9

| Tunnel | Length |
|--------|--------|
|        |        |

PDB: WT – 2G9A, 2G9

Bottleneck

References: 63

Role: O2 transport
Table 3. continued

| Hydrogenase FeFe | 1.12.7.2 |
|------------------|---------|
| **Gate function** | Controls access of oxygen and hydrogen to the active site |
| **Gate location** | Not specified, between dynamic cavities |
| **Gate structural basis** | (Image of molecular structure) |
| No permanent tunnel – O₂ moves from cavity to cavity as the cavities fluctuate inside the protein |
| **Gate engineering** | | |
| | | |

| Hydrogenase NiFe | 1.12.2.1 |
|------------------|---------|
| **Gate function** | Protects the active site against O₂ |
| **Gate location** | Vicinity of NiFe cluster, bottleneck close to the active site |
| **Gate structural basis** | (Image of molecular structure) |
| ? | GATE1-E25, V74 and L122 |
| ? | The size of the amino acids at positions 122 and/or 74 may determine the accessibility of the active site and therefore the resistance to O₂ |
| **Gate engineering** | V74Q, V74M, V74E, V74N, V74W, V74F, V74D, L122M/V74M, L122F/V74L, L122A/V74M – two contributions, size and polarity, are independent and have different effects to H₂, CO, O₂ molecules, different influences on reaction rate and inhibition effect |
| | | |

Table:

| Hydrogenase FeFe | Hydrogenase NiFe |
|------------------|------------------|
| **Gate function** | Protects the active site against O₂ |
| **Gate location** | Vicinity of NiFe cluster, bottleneck close to the active site |
| **Gate structural basis** | (Image of molecular structure) |
| | GATE1-E25, V74 and L122 |
| | The size of the amino acids at positions 122 and/or 74 may determine the accessibility of the active site and therefore the resistance to O₂ |
| **Gate engineering** | V74Q, V74M, V74E, V74N, V74W, V74F, V74D, L122M/V74M, L122F/V74L, L122A/V74M – two contributions, size and polarity, are independent and have different effects to H₂, CO, O₂ molecules, different influences on reaction rate and inhibition effect |
| | | |

The table shows the comparison of gate functions and structural basis between Hydrogenase FeFe and Hydrogenase NiFe. The gate functions and gate locations are described, along with the gate structural basis and the gate engineering. The PDB IDs and references are also provided for each entry.
Table 3. continued

| Gate function | Enzyme function |
|---------------|-----------------|
| **GATE1** – Prevents penetration of bulk water molecules into chambers I and II | Catalysts the closure of the imidazole ring within histidine biosynthesis and provides 5-aminimidazole-4-carboxamide ribotide (AICAR) for use in the de novo synthesis of purines |
| **GATE2** – Controls ammonia transfer, discriminates between ammonia and water | |

**Gate location**

| **GATE1** – Near the entrance of the (βα)₈ barrel of hisF |
| **GATE2** – Between chamber I and chamber II |

**Gate structural basis**

| **GATE1** for water | **GATE2** for ammonia |
|---------------------|----------------------|
| R5(R239), E46(E293), K99(K360), and E167(E465) numbers from yeast (Thermoanaerobacter tengcongensis) | K99(K360) and E46(E293) |
| Four strictly conserved gate residues act as the wall barrier for water molecules | Conformational change of all residues – high barrier 25 kcal/mol for ammonia transfer |
| K99(K360) side chain rotation barrier 10 kcal/mol Both K99 and R5 – fully open gate barrier 2 kcal/mol | Ammonia enters to chamber II through a side-opening between residues K99 and E46, the only requisite is the slight bending of the side chain of K99(K360) - eliminates the need for an energetically costly gate-opening mechanism |

**Gate engineering**

Y138F – experimentally no change of kinetics and stoichiometry, in simulations Y138 is not a gate but prevents bulk water from entering the interface during a reaction, keeping ammonia sequestered within the tunnel R5A(R239A) – loss of ammonia through new hole and results in a 10² decrease in ΔGΔ values for the cyclase reaction, in simulations possibly because the mutation allowed water molecules to access chamber II; these additional water molecules increased the energetic barrier to ammonia entry and passage through the tunnel K99A(K360A) – experimentally 3-fold decrease in the overall reaction stoichiometry, in simulations larger opening between chamber I and chamber II, and simultaneously depletes E167 and E46 of a salt-bridge partner T78A – allowed a rapid and unhindered conduction of ammonia through the tunnel T78F, P76F – block the ammonia conduction Any mutation K181(K196), D98(D359), and Q123(Q387) – decouples the two reactions

**PDB ID:** 1JVN

Gate in closed position blocking the tunnel

**References:** 38,43,155,181–186

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| Gate function | Enzyme function |
|---------------|-----------------|
| **GATE1** – Conformational change converts the enzyme from a dehydrogenase into hydrolase | Transformation of inosine 5′-monophosphate into xanthosine 5′-monophosphate |

**Gate location**

| **GATE1** – Loop covering active site cavity |

**Gate structural basis**

[Diagram of gate structural basis]

**Gate engineering**

[Diagram of gate engineering]

**PDB ID:** 1ME9

Tunnel

**References:** 187

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**Inosine 5′- Monophosphate Dehydrogenase**

| Length | 22 |
|--------|----|
| Bottleneck | 3.8 |

**Role:** Ammonia transport

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**Imidazole Glycerol Phosphate Synthase IGPS**

| 2.4.2 |

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**References:** 5903

dx.doi.org/10.1021/cr300384w | Chem. Rev. 2013, 113, 5871–5923
Table 3. continued

| Gate function | Keisacyl Synthase KS | Enzyme function |
|---------------|----------------------|-----------------|
| GATE1 – Shields the active site | | Elongates an ACP- or CoA-associated acyl chain by adding C2 units through a ping-pong decarboxylating condensation mechanism |
| GATE2 – Controls access of the substrate | | |

| Gate location | GATE1 – Entrance to the active site | | GATE2 – Centre of the acyl-binding tunnel |
|---------------|-----------------------------------|-----------------|

| Gate structural basis | GATE1 | |
|-----------------------|-------|-----------------|
| F1646 | Shields the active site, flips and allows access to the nucleophilic cysteine |

| Gate structural basis | GATE2 | |
|-----------------------|-------|-----------------|
| M1251 | Rotates and unlocks the inner part of the fatty acid binding cavity |

**Gate engineering**

![Image of GATE1 and GATE2 structural basis]

PDB ID: 2VKZ

Tunnel

Length

PDB: 2RK – 2VKZ

References: 188

| L-Amino Acid Oxidase | 1.4.3.2 |
|----------------------|---------|

| Gate function | Controls access to the oxygen tunnel, binds the substrate initially |
|---------------|---------------------------------------------------------------|

| Gate location | GATE1 – 15 Å from surface in main funnel |
|---------------|-----------------------------------------|

| Gate structural basis | GATE1 |
|-----------------------|-------|
| H223 | Has two conformations and can act as a gate and binds the substrate initially |
| H223 | Conformation A closing oxygen tunnel → substrate entry and deprotonation of the zwitterion → substrate into Michaelis position → H223 turn into conformation B → opening of oxygen tunnel |

**Gate engineering**

![Image of L-Amino Acid Oxidase structural basis]

PDB ID: 1F8S

Tunnel

Main funnel

Y shape tunnel

Length 25 9

PDB: 1F8R, 1F8S

References: 73,189

| Role | Access to active site |
|------|-----------------------|
| H2O2 release |

![Image of L-Amino Acid Oxidase gate engineering]

PDB ID: 1F8S

Tunnel

Main funnel

Y shape tunnel

Length 25 9

PDB: 1F8R, 1F8S

References: 73,189

| Role | Access to active site |
|------|-----------------------|
| H2O2 release |

![Image of L-Amino Acid Oxidase gate engineering]

PDB ID: 1F8S

Tunnel

Main funnel

Y shape tunnel

Length 25 9

PDB: 1F8R, 1F8S

References: 73,189

| Role | Access to active site |
|------|-----------------------|
| H2O2 release |
### Table 3. continued

| 48 | Lipase B | 3.1.1.3 |
|----|----------|---------|
| **Gate function** (lipase from *Candida antarctica*) | **Enzyme function** | Hydrolyse triacylglycerols and a broad range of other substrates, important for asymmetric synthesis |
| GATE1 – Controls regiospecificity, controls length of the substrate | |
| **Gate location** | GATE1 – Bottom of the substrate tunnel | |
| **Gate structural basis** | | |
| ![Image](image1.png) | ![Image](image2.png) | |
| GATE1 | W104 | |
| The stereospecificity pocket is defined by T42, S47 and W104 |
| Pocket is buried under a surface helix and delimited by the side chain of W104 |
| *R* enantiomer of butanoic ester fits well into the active site pocket after a small movement of the side chain of W104 |
| **Gate engineering** | W104A, W104Q – change in substrate specificity | |
| **Gate creation in other lipases** | | |
| Lipase from *Burkholderia cepacia* – mutation in bottleneck for increased enantioselectivity – the best double mutant L178/L287I - 15-fold increased activity and a tenfold enhanced enantioselectivity |
| *Candida rugosa* lipase 1 – mutation of amino acids in different position inside the tunnel – P246F, L413F, L410W, L410F/S300E, L410F/S365L – different chain length selectivity |
| PDB: **WT (Candida antarctica)** – 1LBS, 3HCV, 3HCW, **WT (Burkholderia cepacia)** – 3LIP, **WT (Candida rugosa)** – 1LPO |
| **References:** 80,130–132 |

| 49 | Lon Protease | 3.4.21.53 |
|----|--------------|-----------|
| **Gate function** | **Enzyme function** | ATP-dependent proteolysis |
| GATE1 – Controls the access of the substrate | |
| **Gate location** | GATE1 – At the entrance to an internal unfolding and degradation chamber | |
| **Gate structural basis** | | |
| ![Image](image3.png) | ![Image](image4.png) | |
| GATE1 | F216 loop | |
| GATE2 | M275 loop | |
| **Gate engineering** | F216A – lost almost all of the ATP-dependent proteolytic activity against a casein and the aromatic peptide | |
| **Gate creation in other lipases** | | |
| | | |
| **PDB:** | **3K1J** | |
| **References:** 197 | | |

**PDB ID:** 1LBS, 3HCV, 3HCW, **WT (Burkholderia cepacia)** – 3LIP, **WT (Candida rugosa)** – 1LPO

**References:** 80,130–132

**PDB ID:** 3K1J

**References:** 197
### Table 3. continued

| Gate function | Mannitol 2-Dehydrogenase | Enzyme function | 1.1.1.67 |
|---------------|--------------------------|-----------------|----------|
| GATE1         | E292 functions as a gate in water chain mechanism of proton translocation | Dehydrogenation of mannitol |          |

**Gate location**

- **GATE1** – Bottle neck of the tunnel connecting active site with protein surface

**Gate structural basis**

- **GATE1** E292 may adopt two conformation open and closed

**Gate engineering**

- E292A – 120-fold decrease in a rate of microscopic steps preceding catalytic oxidation of mannitol

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| Parameters | Value |
|-----------|-------|
| Tunnel Length |       |
| PDB ID: | 1M2W (open), 1LJ8 (closed) |

**References:** T04

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| Gate function | Methane Monoxygenase Hydroxylase MMOH | Enzyme function | 1.14.13.25 |
|---------------|--------------------------------------|-----------------|------------|
| GATE1         | Controls transport of the substrate between cavities 1 and 2 | Converts hydrocarbon substrates either to alcohols or epoxides |          |
| GATE2         | Controls entrance of small substrates such as O₂, H₂O, CH₄ |                        |          |

**Gate location**

- **GATE1** – Separates cavities 1 and 2
- **GATE3** – Separates cavities 2 and 3
- **GATE3** – One of the residue from active site is positioned at the interface between the cavity and the surface above the iron-coordinating residue

**Gate structural basis**

- **GATE1** L110, F188
  - Conformation changes of L110 and F188 opens the access between the cavities 1 and 2, other residue involved T21

- **GATE2** L209
  - Moves to allow the substrate analogues to traverse between the cavities 2 and 3, other residues creating the bottleneck F109, V285 and Y291

- **GATE3** N214
  - Conformation changes – movement of N214 forms a deep crevice in the four-helix bundle

**Gate engineering**

- PDB: 1HT – 1MHY, 1XVG, 1XVF, 1XVE, 1XVB, 1XVC, 1XU5, 1XVD, 1XU3

**References:** T03, X08

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**PDB ID:** 1MHY

**Length**

**PDB ID:** 1MHY

**Role**

**References:** T03, X08
Table 3. continued

| S2 | Monoamine Oxidase A | 1.4.3.4 |
|----|---------------------|---------|
| Gate function | **GATE1** – Controls the access of the substrate | **Enzyme function** Oxidative deamination of biogenic amines and amine neurotransmitters (serotonin, dopamine and epinephrine) |
| Gate location | **GATE1** – At the entrance to the active site cavity | |
| Gate structural basis | | |
| ![Diagram](image1) | Loop 99-104 The loop movement is regulating access to the active site cavity and the loop flexibility is critical for opening the entry for substrates/inhibitors | PDB ID: 2Z5X |
| Gate engineering | G110A – increase of $K_m$ of 5-fold | Tunnel |
| | G110P – increase of $K_m$ of 19-fold | Length |
| References: | Botleneck | |

Table 3. continued

| S3 | Monoamine Oxidase B | 1.4.3.4 |
|----|---------------------|---------|
| Gate function | **GATE1** – Controls the access of the substrate | **Enzyme function** Oxidative deamination of biogenic amines and amine neurotransmitters (serotonin, dopamine and epinephrine) |
| Gate location | **GATE1** – At the entrance to the active site cavity | |
| | **GATE2** – Separates two internal cavities | |
| Gate structural basis | | |
| ![Diagram](image2) | Loop 99-104 F103 side chain conformation movement is synchronized with the conformation changes of I199 residues (I199 open $\rightarrow$ F103 closed) | PDB ID: 2VSZ |
| Gate engineering | GATE1 | Tunnel |
| | I199, Y326 Movement of side chain residues separate or merge the entrance cavity and the substrate cavity | Length |
| References: | Botleneck | |

PDB: WT – 2Z5X, Mutant G110A – 2Z5Y
References: [19,198]

References: [19,198]
### Table 3. continued

| Gate function | Monoxygenase ActVAOrf6 | Enzyme function |
|---------------|------------------------|-----------------|
| GATE1 – Controls access of the substrate, hydrogen bond donor and acceptor/proton gate | Oxidation of a phenolic compound 6-deoxydehydrolafungin at the C-6 position into the corresponding quinone dihydrodehydrolafungin |
| GATE2 – Controls opening of the narrow tunnel, can also control exit for H₂O, O₂, H₂O | |

#### Gate location
- **GATE1** – Entrance to the active site
- **GATE2** – Opposite side of the bound substrate

#### Gate structural basis

| Gate | Structure |
|------|-----------|
| GATE1 | Y72 |
|       | The residue possess two possible conformation that can act as a gate and act as hydrogen bond donor and acceptor |
| GATE2 | I110 + loop (residues 34-38) |
|       | Conformation changes close and open the narrow tunnel; gate can also control an exit path for H₂O |

#### Gate engineering

| PDB ID: | 1LQ9 |
|---------|------|
| Tunnel  | Length |

| References: | 11,92 |
|-------------|------|
| Bottleneck  | Role Proton transfer Oxygen/water transport |

| Gate function | NADH Oxidase | Enzyme function |
|---------------|--------------|-----------------|
| GATE1 – Controls accessibility of the flavin ring and plays a crucial role during the catalytic cycle | Hydride transfer from NADH to the intrinsic flavin cofactor |

#### Gate location
- **GATE1** – Above the active site

#### Gate structural basis

| Gate | Structure |
|------|-----------|
| GATE1 | W47 and cofactor FAD |
|       | Cofactor-assisted gating mechanism, W47 moves from the original position toward the pyrimidine section |
|       | Closed conformation is stabilized by the hydrogen bonds between cofactor and peptide backbone |
|       | Stabilization of the open form may have advantages during the initial steps of the substrate binding, it may slow down the product dissociation |

#### Gate engineering

| PDB ID: | 1NOX |
|---------|------|
| Tunnel  | Length |

| References: | 95,109 |
|-------------|------|
| Bottleneck  | Role |
### Table 3. continued

| Gate function | O-Acetylerine Sulphohydrolase Cysteine Synthase | Enzyme function |
|---------------|-----------------------------------------------|-----------------|
| GATE1 – Controls access to the active site | GATE2 – Controls access to the active site | Production of cysteine from the O-acetyl-serine intermediate |
| Gate location | **GATE1** – Bottleneck of tunnel 12–20 Å from the active site cavity | |
|               | **GATE2** – Bottleneck of tunnel 8–12 Å from the active site cavity | |
| Gate structural basis | **GATE1** Motion of side chains in addition to backbone movements M101 or S100 on the loop of the upper domain | |
|               | **GATE2** Motion of side chains in addition to backbone movements S75 on the Asn-loop for the inner gate | |

**Gate engineering**



| PDB ID: 1Z7Y | Tunnel Length | ~ 28 |
|--------------|---------------|------|
| Bottleneck Role | Access to the active site |

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| Gate function | Oxidosqualene Cyclase SccOSC | Enzyme function |
|---------------|--------------------------------|-----------------|
| GATE1 – Controls access of the substrate | GATE2 – Controls access of the substrate | Cyclization of the 2,3-oxidosqualene into lanosterol |
| Gate location | **GATE1** – Bottleneck between tunnel and active site cavity | |
|               | **GATE2** – Bottleneck between tunnel and active site cavity | |
| Gate structural basis | **GATE1** Y239 (Y237 in HsaOSC) A rotation of the side chain stabilized by a hydrogen bond bridge with P228 could open the channel and enable the substrate to enter the active site | |
|               | **GATE2** T235 (C233 in HsaOSC) | |
| Gate engineering | Y239F – 5-fold decrease in enzyme activity, mutation keeps the channel in a closed conformation | |
|               | Y239A – 2-fold decrease in enzyme activity, mutation keeps the channel in an open conformation | |

**Gate engineering**



| PDB ID: 1W6K | Tunnel Length | |
|--------------|---------------|------|
| Bottleneck Role | Connects active site cavity with bulk solvent |

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### Table 3. continued

| S8 | Phenol Hydroxylase PII | 1.14.13.7 |
|----|------------------------|----------|
| **Gate function** | **Enzyme function** | Hydroxylates aromatic compounds |
| GATE1 – Controls access to the active site | | |
| GATE2 – Closes cavity 2 | | |
| GATE3 – Closes cavity 2 | | |

| **Gate location** | | |
| GATE1 – Entrance to the tunnel | | |
| GATE2 – Between active site pocket and cavity 2 | | |
| GATE3 – Between cavity 3 and cavity 2 | | |

| **Gate structural basis** | | |
| GATE1 | | |
| N204 | | |
| The residue shift is redox-dependent | | |
| Helix E orients N204 away from the active site and opens the tunnel | | |
| N204 in closed conformation forms a hydrogen bond with S72 | | |

| GATE2 | | |
| L107 and L272 | | |

| GATE3 | | |
| L399 and V455 | | |

| **Gate engineering** | | |
| | | |

| **PDB ID:** 2INP | | |
| Tunnel | | |
| Length | | |

| References: | | |
| 74,195 | | |

| **Table 3. continued** |
|-------------------------|

| 59 | Phospholipase A2 | 3.1.1.4 |
|----|------------------|----------|
| **Gate function** | **Enzyme function** | Catalyses the hydrolysis of acyl bonds in sn-3-phospholipids |
| GATE1 – Controls access to the interface and active sites | | |

| **Gate location** | | |
| GATE1 – Position of monomers | | |

| **Gate structural basis** | | |
| GATE1 | | |
| The dimer interface might act as a hinge | | |
| The homodimer can be observed in open and closed conformations formed by different angle between monomers | | |

| **Gate engineering** | | |
| | | |

| **PDB ID:** 1CLP – open | | |
| Tunnel | | |
| Length | | |

| **PDB:** 1CLP (open), 1PP2 (closed) | | |
| Bottleneck | | |

| References: | | |
| 80,202,205 | | |
### Table 3. continued

| Gate function | Enzyme function |
|---------------|-----------------|
| GATE1 – Controls access to the active site, controls product exit | Catalyses the cleavage of glycan-phosphatidylinositol anchored proteins |

#### Gate location

**GATE1** – Entrance to the active site

#### Chemical Reviews

| Phosphatidylinositol-Specific Phospholipase C | 4.6.1.13 |
|---------------------------------------------|---------|

#### Gate structural basis

**GATE1**
- Rim loop (residues 241-252) and W45
- Rim loop undergoes the pH-dependent movement with a maximum backbone displacement of 9.4 Å between the acidic and basic forms
- H258 serves as an anchoring residue holding F249 residue from a loop by a-ionic interaction
- W45 closes the product exit pathway when the loop is in extended position

#### Gate engineering

H258Y – keeps the rim mobile loop in extended conformation

#### References: 204

| PDB: *WT* – 3V16, 3V18, *Mutants H258Y* – 3V11H |
|-----------------------------------------------|

#### Quercetin 2,3-Dioxygenase

| Quercetin 2,3-Dioxygenase | 1.13.11.24 |
|----------------------------|-----------|

#### Gate function

**GATE1** – Controls access of small molecules into the active site

**GATE2** – Controls access of small molecules into the active site

#### Enzyme function

Degradation pathway of flavonoids

#### Gate location

**GATE1** – Tunnel connecting the bulk solvent and the active site cavity

**GATE2** – Tunnel connecting the bulk solvent and the active site cavity

#### Gate structural basis

**GATE1**
- F175 Side-chain of F175 switches from the enzyme surface to the bulk and opens access to the cavity
- This tunnel opening occurs as soon as the cavity is inflated by a sufficient number of water molecules (16–18)

**GATE2**
- F132 and L135 Side-chains of F132 and L135 are structurally mobile enough to provide access for the entry of small molecules into the enzyme active site

#### Gate engineering

| PDB: *WT* – 1GQG, 1JUH, 1H1L |
|--------------------------------|

#### References: 75

| Tunnel | Length |
|--------|--------|
| 19     |        |

| PDB ID: 1GQG |

| Product | Role |
|---------|------|
| O$_2$ transport | 1.6 |

PDB: *WT* – 1GQG, 1JUH, 1H1L

| PDB ID: 3V16 – blue |
| PDB ID: 3V18 – light green |

| PDB ID: 1GQG |

| Tunnel Length |
|---------------|
| 19 |
### Rabbit 28a-Hydroxysteroid Dehydrogenase

| Gate function | Enzyme function |
|---------------|-----------------|
| **GATE1** – Protects cavity from the solvent in the absence of a steroid | Dehydrogenation of hydroxy steroid |

**Gate location**
- **GATE1** – Loops making up the active site cavity

**Gate structural basis**
- **GATE1**
  - Flexible loop B (residues 223–230)
  - Movement of the loop partly controlled by the nature of Q230
  - Loops A and C mostly contribute in the pocket creation changing depth and size of the cavity

**Gate engineering**
- E230P – changes in the selectivity by reduced loop flexibility
- K274R – increases flexibility of loop by and eliminating K274-E227 contact, high $k_{cat}$ for NADPH-dependent reduction of xylene

**PDB:** 1Q13
- **WT** – 1Q55, 1Q13, **WT (ALK5H1)** – 2WZT 2WZM small loops

**Role**
- Substrate steroid binding and orienting towards cofactor NADPH

**References:** 37, 205-214

### Raucaffricine O-β-D-glucosidase/glucoisidase

| Gate function | Enzyme function |
|---------------|-----------------|
| **GATE1** – Controls the access of the substrate | Hydrolyses alkaloid raucaffricine to aglycone and aminolene |

**Gate location**
- **GATE1** – At the entrance to the active site cavity

**Gate structural basis**
- **GATE1**
  - W392
  - Conformation of W392 is controlled by S390

**Gate engineering**
- S390G – leads to more flexible conformation of W392
- F485W – results in more fixed conformation of W392

**PDB:** 3J5U
- **WT** – 4A3Y, **Mutants E186Q** – 3J5U, 3J57, 3J5Y

**Role**
- Substrate steroid binding and orienting towards cofactor NADPH

**References:** 36
Table 3. continued

| 64 | RNA-dependent RNA polymerase | 2.7.7.48 |
|---|---|---|
| **Gate function** | **Enzyme function** | |
| GATE1 – Controls enzyme activity | RNA replication | |

**Gate location**
GATE1 – Surface loop

**Gate structural basis**

[Image of GATE1 structure]

GATE1
A1-loop + L30
A1-loop is involved in keeping the enzyme in a closed conformation, makes interaction with L30 (closed conformation is active, open is inactive)

**Gate engineering**
L30R and L30S – open conformation
K222E – exhibited 2-fold reduction in activity, positively charged residue inside the tunnel
K151E – activity was 7–10-fold higher at 21 °C, and 2–3-fold higher at 37 °C, positively charged residues near the tunnel entrance

[Image of GATE1 structure with annotations]

PDB: 2XWH

**Tunnel**
Length: 19

References: 216

| 85 | RNA Polymerase | 2.7.7.6 |
|---|---|---|
| **Gate function** | **Enzyme function** | |
| GATE1 – Controls the access to the DNA-binding tunnel | RNA synthesis | |

**Gate location**
GATE1 – Entrance to the DNA binding tunnel

**Gate structural basis**

[Image of GATE1 structure]

GATE1
Clamp domain may open to permit entry of promoter DNA during initiation, close to establish the tight grip on DNA during elongation and then open again to allow release of DNA during termination

**Gate engineering**

[Image of GATE1 structure with annotations]

PDB ID: 116H

**Tunnel**
Length

PDB: 205J, 166H, 116V

References: 166, 211

Role: NTP transport

Role: RNA

Role: Clamp domain
### Table 3. continued

| Toluene-4-Monoxygenase T4MO | 1.14,13 |
|----------------------------|---------|
| **Gate function** | **Enzyme function** |
| GATE1 – Controls access to the active site, controls selectivity | Hydroxylates toluene primarily at the para position to cresol |
| GATE2 – Controls access to the tunnel | |
| GATE3 – Protects cavity from access of the solvent | |
| **Gate location** | |
| GATE1 – Boundary between the active site pocket and the tunnel | |
| GATE2 – Tunnel entrance | |
| GATE3 – Cavity entrance | |
| **Gate structural basis** | |
| GATE1 | I100 Side chain rotation |
| GATE2 | D285 Steric blocking by negatively charged residues D285 |
| GATE3 | R60, E64, E75, R69 Interactions between E64 and R60 and between E75 and R69, ionic gate for the cavity |
| **Gate engineering** | |
| D285L, D285Q – improves oxidation of bulky substrates (11-fold) | |
| D285S – improves oxidation of styrene (1.7-fold) | |
| I100A – improves oxidation of bulky substrates (35-fold) | |
| I100G – improves oxidation of methyl-p-tolyl sulphide (11-fold) | |
| PDB: WT – 3DIHG, WT (T4moC) – 1VM9, WT (T4moD) – 1G10 | Bottleneck |
| References: | Substrate binding cavity |
| SI,LS4 |

### Table 3. continued

| Toluene-Ortho-Xylene Monoxygenase ToMO | 1.14 |
|----------------------------|---------|
| **Gate function** | **Enzyme function** |
| GATE1 – Controls access to the active site, controls selectivity | Catalyses hydroxylation of aromatics, oxidize benzene to phenol, catechol and trihydroxybenezene |
| GATE2 – Open pore allows access to the diron centre for substrate (maybe also dioxygen or protons) | |
| **Gate location** | |
| GATE1 – Border of the pocket near Fe atoms – entrance to active site | |
| GATE2 – Entrance to the tunnel | |
| **Gate structural basis** | |
| GATE1 | I100 Residues I96, I100, T201, F205, and F196 border the pocket – I100 is an analogue of L110 (gate) in MMOH. I100 contributes to defining the hypothetical para site, but it is also at the boundary between the active site pocket and the tunnel which connects the pocket to the surface of the protein |
| GATE2 | N202 The conserved tunnel is gated by N202. The residue shifts in a redox-dependent manner. Its side chain is oriented away from the active site in the oxidized form and points inward in the reduced or Mn(II) reconstituted forms of the hydroxylase. This motion correlates with the carboxylate shift that occurs upon reduction of the dinuclear centre. |
| **Gate engineering** | |
| I100A, I100W, F205L, A107V, A107I – decreased or removes activity | |
| Creation of new GATE3 | |
| E214 – hot spot for new gate creation localized on helix E at the entrance to the tunnel 23 Å from the active site, mutation of those residue regulates access to the tunnel (E214A, E214C, E214V, E214W, E214F, E214Q, E214P) | |
| E214G – 15-fold improvement for p-nitrophenol oxidation | |
| PDB: WT – 2INC, 1T9Q | Bottleneck |
| References: | Substrate and product transport |
| SI,LS4,LS5 | Role |

**PDB ID:** 3DIHG, 1VM9, 1G10, 2INC, 1T9Q | 1.14,13 | Bottleneck |
| Length | 30-35 |
| References: | Substrate binding cavity |
| SI,LS4,LS5 | Role |

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dx.doi.org/10.1021/cr300384w | Chem. Rev. 2013, 113, 5871–5923
### Table 3. continued

| Gate function | Enzyme function |
|---------------|-----------------|
| **GATE1** – Controls access of the water to the active site | Catalyses isomerisation D-glyceraldehyde 3-phosphate to dihydroxyacetone phosphate |

### Gate location

**GATE1** – Entrance to active site

### Gate structural basis

**GATE1**

Loop 6 (residues 166 to 176). Residues 169 – 173 move as a rigid body, which position is controlled by flexible three residues N-terminal and C-terminal hinge regions. In closed conformation loop sequesters the enzyme reaction from solvent. Closed conformation is stabilised by conserved anchoring residues from loop 7

### Gate engineering

P(166)VW-AIGTG-KTA to P(166)GG-AIGTG-GGG mutant – $k_{cat}$ decreases 2500-fold

$Y208F$ – $k_{cat}$ decreases 2400-fold

$Y208T$ – $k_{cat}$ decreases 200-fold

**PDB ID:** 1TIM – blue  
**PDB ID:** 1TPH – light green  

### Tunnel

**Length**

**References:** 37,824; 2-16

### tRNA-Dependent Amidotransferase GatDE and GatCAB

| Gate function | Enzyme function |
|---------------|-----------------|
| **GATE1** – Protects active site from access of the solvent, avoids wasteful release of ammonia into solution  
**GATE2** – Controls ammonia transport | Conversion of Gln-tRNA$^{Gln}$ into Gln-tRNA$^{Glu}$ or Asp-tRNA$^{Asp}$ into Asp-tRNA$^{Asp}$ |

### Gate location

**GATE1** – β hairpin loop surrounding asparaginase active site (in GatDE)  
**GATE2** – Inside ammonia tunnel (in GatCAB)

### Gate structural basis

**GATE1** GatDE  
β hairpin loop D7-D8, residues 100–118 in GatD. G180, the first glycine in the strictly conserved GGT motif, active conformation substrate in contact with T102

**GATE2** GatCAB in *A. aeolicus* (S. avermex)  
E128 (E125), K90 (K88)  
E128 (E125) side chain can block the tunnel via a salt bridge with K90 (K88)

### Gate engineering

**PDB:** *WT (GatDE)* – 1ZQ1, *WT (GatCAB)* – 3H0L, 3H0M, 3H0R  

**PDB ID:** 1ZQ1 GatDE  
**PDB ID:** 3H0L, GatCAB

**Tunnel**  
**Length** 35

**References:** 135,217–119

**Role** Ammonia transport
substrate binding since it is believed to slow down product dissociation.\textsuperscript{22,97} Other gates of this type have been reported in 3-hydroxybenzoate hydroxylase,\textsuperscript{98} 4-hydroxy-2-ketovalerate aldolase/acylating acetaldehyde dehydrogenase,\textsuperscript{96} and cholesterol oxidase type I\textsuperscript{57} and type II.\textsuperscript{99} Moreover, cofactors themselves can also function as gates. The FAD cofactor of digeranylgeranylglycerophospholipid reductase has two different conformations, referred to as the “in” and “out”

| Gate function | Tryptophan Synthase | Enzyme function |
|---------------|---------------------|-----------------|
| GATE1 – Creates the tunnel and protects substrate binding cavity from the access of the solvent | Synthesis of L-tryptophan; subunit α cleavage of 3-indole-D-glycerol 3-phosphate; subunit β pyridoxal phosphate dependent condensation of indole with L-serine |
| GATE2 – Controls transport of the indol | |
| GATE3 – Controls access to the subunit β and activates the β active site | |

| Gate location | |
|---------------|---------------|
| GATE1 – Surface loop from subunit u at the border between the subunits β and α | |
| GATE2 – Tunnel wall in subunit β | |
| GATE3 – Subunit β | |

| Gate structural basis | |
|-----------------------|---------------|
| **GATE1** | Loop L177 - A190 |
| A disordered surface loop in the subunit u after substrate binding becomes ordered and clamps down over the active site, isolating this region from solvent |
| **GATE2** | F280, Y279 |
| The residue F280 can adopt alternative conformations in closed interaction with C170 in open one it adopts Y279 position, Y279 moves toward the subunit u and interacts with part of the flexible loop-2 residues (54-61) of the subunit u |
| **GATE3** | D305 |
| In the closed conformation D305 creates the H-bonded salt bridge with R141 with the associated H-bonding network involving S197 and S199 |

Conformation changes are initiated by binding IGF - the allosteric communication between the two sites that results in full coupling of the reaction at the subunits α and β.

**Gate engineering**

- E49F, G51L, D60Y – decreases activity (2-fold)
- F280C, F280S – increases transport
- C170W, C170F – chemical modification of C170 (C170-NEM, C170-MMTS), obstructs the tunnel and accumulates indole intermediate

PDB: IF7 – closed 3CEP, open conformations: 1BKS, 1KFK, 1KFL, 1ITP, 2CLI, 2CLM, 2CLO

Bottleneck

| Role | Indol transport between two active sites |

| 71 | Undecaprenyl-Pyrophosphate Synthase | 2.5.1.31 |
|----------------|--------------------------|----------------|
| Gate function | GATE1 – Controls length of the final product | Enzyme function |
| | | Condensation reactions of isopentenyl pyrophosphate with allylic pyrophosphate to generate linear isoprenyl polymers |

**Gate location**

GATE1 – Loop closing the entrance to the tunnel

**Gate structural basis**

- **GATE1**
  - 71 – 83 loop
  - The helix a3 in open tunnel conformation is kinked by ~30° at E96, and the helix a3 in closed tunnel conformation is kinked by ~45° at A92.
  - Reaction starts with closed conformation → when the chain length reaches C55, the dimethyl end is stepped at the end of the tunnel → a3 helix moves away from the closed position into the open position (71 – 83 loop) → fully synthesized C55 long product can exit easily through the open gate

- **LAT17A** – removes the floor of the tunnel and allows formation of a longer chain length products (normal length C55), bulky side chain of L137 serves to block further elongation of undecaprenyl-pyrophosphate

- A69L – results in long lived accumulation of a short chain intermediate C30 final product C55
  - A143V – similar to wild type, rate 5-fold lower

- S71A, N74A, or R77A – decreases in kₐ values (25–200-fold)
- W75A – increases in Kₐ for farnesyl pyrophosphate (8-fold)
- E81A, S71A – decreases in Kₐ for isopentenyl pyrophosphate (22–33-fold)

PDB: 1JP3

| Tunnel | Length |
|--------|--------|
| 30     |        |

PDB: WT – 1JP3

References: 37

Bottleneck

Role

Active site cavity
conformations. In the “in” conformation, the tunnel is blocked by FAD. To open the tunnel, FAD has to adopt the “out” conformation.

Gates are most commonly located at the tunnel entry and the tunnel bottleneck (51%). This is to be expected because the bottleneck represents the narrowest point of the tunnel, and its diameter often dictates the tunnel’s permeability. Another common location is the entrance to the active site cavity (28%). Gates at the entrance of the cofactor cavity are less common (5%), which is not surprising since not all enzymes have a cofactor cavity. In 16% of the cases studied, the gate was not located within any of these functional regions (Figure 7).

5. ENGINEERING OF GATES

The average rate of evolution of the gating residues in the cytochrome P450 family is significantly greater than that for the protein sequence as a whole. The high rate of evolution at the gating residues suggests that gate engineering may be an attractive alternative to other rational enzyme design strategies. This idea is supported by a few observations: (i) the gates are often spatially separated from the active site, and so mutations at the gating residues should not be deleterious to protein function, (ii) the opening and closing of the access pathways can affect ligand exchange and thus enzyme activity and selectivity, and (iii) gate modification can modulate the solvent’s ability to access the active site, which in turn affects solvation and stabilization of the transition state and also product release. In the following section, we describe selected cases in which an enzyme’s catalytic properties have been successfully altered by modifying its gates.

5.1. Gate Modification

Gates can be modified by substitution of the gating residues, hinge residues, or anchoring residues depending on the nature of the gate in question. Modification of the gating residues has been shown to change the selectivity and activity of toluene-oxylene monoxygenase, with the E214G mutation improving oxidation of p-nitrophenol by a factor of 15. A similar improvement in overall activity was achieved in a lipase from Burkholderia cepacia by the mutations L17S + L287I. This double mutant also exhibited a 10-fold increase in enantioselectivity compared to the wild-type enzyme. The T78F or P76F mutations in imidazole glycerol phosphate synthase override some of the control exerted by the wild-type gate and block the passage of ammonia through the tunnel. In NiFe hydrogenases, mutations of the V74 and V74 + L122 residues changes the rates of transport for H2, CO, and O2, thereby modulating the overall rate of reaction.

Gates that incorporate secondary structure elements are dependent on hinge and anchoring regions. It has been demonstrated that modification of the hinge region can change an enzyme’s activity and selectivity. Notably, the Q230P mutation in rabbit 20A-hydroxysteroid dehydrogenase decreases the flexibility of a key loop and thereby changes its selectivity. Similarly, access to the active site in the HIV-1 protease is controlled by two β-turn flaps. Results from Brownian dynamics simulations suggest that the G48V + V82A or I84V + L90M mutations in this enzyme reduce the likelihood that the active site will be exposed at any given point in time from 14% in the wild type to 2% in the mutants. The importance of the anchoring residues in the HIV-1 protease was demonstrated by the F53L mutation, which generates a semiopen conformation due to removal of the stabilizing F53−I50 interaction.

5.2. Gate Removal

Removing gates typically increases substrate and product exchange rates but also allows more extensive access of water molecules to protein tunnels and cavities. The overall effect of gate removal is therefore equal to the combined effects of these two processes. Gate deletion has been shown to increase the rate of substrate binding to tryptophan synthase. The F280C and F280S mutations both increased the rate of indole binding
by a factor of 2. Similarly, the T78A mutation allowed ammonia to rapidly pass through the tunnel in imidazole glycerol phosphate synthase.\textsuperscript{38} The R239A mutation in the cyclase caused a 1000-fold decrease in the enzyme’s \(k_{\text{cat}}/K_m\) value and decoupling of the reaction.\textsuperscript{38} This dramatic change in enzyme catalytic efficiency was attributed to creation of a new route for ammonia release. A similar leakage of ammonia was caused by the G359F and G359Y mutations in carbamoyl phosphate synthetase.\textsuperscript{103,104} Negative consequences of gate removal were also observed for the FabZ-\(\beta\)-hydroxacyl-acyl carrier protein dehydratase (HpFabZ),\textsuperscript{54} in which the Y100A mutation leaves the active site completely exposed to the bulk solvent. As a result, the acyl carrier protein binds to the HpFabZ Y100A mutant much more strongly than to the wild-type HpFabZ, decreasing the mutant enzyme’s activity by more than 50% due to the very slow dissociation of the acyl carrier protein.

In some cases, gate removal enables bulky substrates to access the active site cavity. Mutations D285I and D285Q in toluene-4-monooxygenase improved its ability to oxidize the large and bulky substrates 2-phenylethanol and methyl \(p\)-tolyl sulfide by factors of 8 and 11, respectively, while the D285S mutation improved the rate of styrene oxidation by 7-fold.\textsuperscript{105} The L137A mutation in undecaprenyl-pyrophosphate synthase removed the bottom of the tunnel in this enzyme, allowing formation of products with longer chain lengths.\textsuperscript{32} A similar situation was encountered in type III polyketide synthases from \textit{Aloe arborescens} PCS, in which the M207 residue controls the number of condensations of malonyl-CoA.\textsuperscript{106} The M207G mutation opened a connection between the tunnel and two hidden pockets located behind the active site, resulting in formation of extended products. Further product elongation was achieved with the triple mutant F80A + Y82A + M207G.

5.3. Gate Insertion

To best of our knowledge, there have been no reports of an intentional introduction of a new gate into an enzyme structure. However, there have been studies in which an access tunnel was systematically modified with multiple substitutions, and it is reasonable to expect that some of these mutations might have created new gates. More research will clearly be needed to confirm this expectation. To verify successful intentional insertion of a new gate into a protein structure, it would be necessary to confirm the existence of both the open and the closed conformations at a position where previously only a single conformation could be adopted.

Site-directed mutagenesis targeting specific residues at various positions along the access tunnel of \textit{Candida rugosa} lipase has been used to alter the acceptable substrate chain length for this enzyme. The mutants for which this was observed were P246F, L413F, L410W, L410F + S300E, and L410F + S365L.\textsuperscript{107} We note that the aromatic residues F and W, which are common in wing and swinging door gates, were introduced in each of these variants.

In another study, the residue L177 that is located near the entrance to the access tunnel of the haloalkane dehalogenase LinB from \textit{Sphingobium japonicum} UT26 was substituted with all of the natural amino acids, yielding 19 mutants with significantly altered substrate specificity and activity.\textsuperscript{108} Preliminary computational analyses of these variants using molecular dynamics revealed that the two residues possessing a single aromatic ring (F and Y) exhibited large fluctuations, as might be expected for gating.

Residue A217 is located at the entrance to the tunnel in the epoxide hydrolase EH from \textit{Aspergillus niger} M200. This residue was substituted with C, E, G, L, P, Q, R, T, and V, and the effect of each mutation on the enzyme’s activity and enantioselectivity was studied.\textsuperscript{26} The mutants exhibited different enantioselectivity and activity relative to the wild type. For instance, the activity of the A217G mutation toward allyl glycidyl ether was lower than that of the wild type by a factor of 33, whereas the A217V mutation increased activity toward this substrate 6.6-fold.

Residues I135, W141, C176, V245, L246, and Y273 are positioned close to the entrance to the main and side tunnels of the haloalkane dehalogenase DhaA from \textit{Rhodococcus rhodochrous} NCIMB 13064. These residues were simultaneously permuted in an attempt to improve this enzyme’s activity against 1,2,3-trichloropropane. The most successful mutant, which featured the I135F, C176Y, V245F, L246I, and Y273F substitutions, showed 26-fold greater activity toward the target substrate than the wild type.\textsuperscript{109} In this mutant, three aromatic residues were introduced in place of aliphatic ones in the vicinity of the tunnels. Computational analysis of product release from the mutant suggests that substitutions introduced a transient rather than permanent structural feature and gating residues prevented access of water to the active site.\textsuperscript{110} Crystallographic analysis of the mutant revealed two distinct conformations for the Y176 side chain.\textsuperscript{110}

6. CONCLUSIONS

This review highlights the importance of gates in enzymes. Gates play vital roles in controlling the catalytic activity and selectivity of enzymes and are more common in protein structures than is generally thought. In particular, gates control substrate access to the active site and product release, prevent or restrict solvent access to specific regions of the protein, and can synchronize processes occurring in distinct parts of the enzyme. Our literature survey of 129 gates in 71 enzymes revealed a large variety of systems with sophisticated structures. We presented a rigorous definition of gates and established a new scheme for their classification. The large number of inspected cases allowed us to build a catalogue of gates assigned to six distinct classes—wings, swinging doors, apertures, drawbridges, double drawbridges, and shells—with three different functions and three distinguishable locations. We also presented summary statistics that give a preliminary overview of the propensity of specific amino acid residues to occur in particular gate classes. The proposed classification scheme can be easily extended and updated but even in its present form can provide guidance for analysis and engineering of gates in biomolecular systems.

The biochemical relevance and specific location of gates within protein structures make them attractive targets for protein engineering. Attempts to rationally redesign gates typically involve computer-assisted gate identification followed by modification using focused directed evolution. This approach is compatible with a recent trend in protein engineering that stresses construction of small and smart libraries. Gate modification and deletion have been demonstrated in numerous cases, but the intentional insertion of new gates remains a challenge. Convenient methods for identifying gates in protein structures are essential prerequisites for their engineering. In silico, this can be achieved by coupling the software tools developed for describing pathways to tools developed for study of protein dynamics. Of the available
experimental techniques, NMR spectroscopy is particularly suitable for analysis of highly dynamic protein structures and can be expected to play an indispensable role in the study of gate dynamics at the atomic level. The field would also benefit from development of new experimental techniques for monitoring the passage of ligands through the protein pathways. One day it will be possible to control the catalytic properties of enzymes by rational engineering of their gates. To achieve this goal, we have to learn how gates evolved, how they interact with the other parts of the protein structure as well as with the ligand and solvent molecules, and how they fulfill their biological functions.

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