YidC as a potential antibiotic target

Ross E. Dalbey[1*], Sharbani Kaushik[1] and Andreas Kuhn[2*]

[1]Dept. of Chemistry and Biochemistry, The Ohio State University, Columbus, Ohio 43210

[2]Institute of Biology, University of Hohenheim, Stuttgart, Germany, 70599

*For correspondence: Ross E. Dalbey, dalbey.1@osu.edu; Andreas Kuhn, Andreas.Kuhn@uni-hohenheim.de

Keywords (YidC; antibacterial target; structure; function; mechanism)

Abstract

The membrane insertase YidC, is an essential bacterial component and functions in the folding and insertion of many membrane proteins during their biogenesis. It is a multispanning protein in the inner (cytoplasmic) membrane of *Escherichia coli* that binds its substrates in the “greasy slide” through hydrophobic interaction. The hydrophilic part of the substrate transiently localizes in the groove of YidC before it is translocated into the periplasm. The groove, which is flanked by the greasy slide, is within the center of the membrane, and provides a promising target for inhibitors that would block the insertase function of YidC. In addition, since the greasy slide is available for the binding of various substrates, it could also provide a binding site for inhibitory molecules. In this review we discuss in detail the structure and the stepwise mechanism of how YidC interacts not only with its substrates, but also with its partner proteins, the SecYEG translocase and the SRP signal recognition particle. Insight into the substrate binding to the YidC catalytic groove is presented. We wind up the review with the idea that the hydrophilic groove would be a potential site for drug binding and the feasibility of YidC-targeted drug development.

1. Introduction

To cope with the upcoming challenge of antibiotic resistant bacterial infections, it is essential for health research and developmental programs within pharmaceutical companies and academic institutions to discover new antibiotic targets. Identification of drugs targeted towards unexplored pathways/proteins can offer a significant advancement in this regard. In particular, membrane proteins offer a wide source of possible new targets since many are essential factors for bacterial life [1]. An important advantage of bacterial integral membrane proteins is that they are exposed to the cell surface making it possible for many drugs to reach their target without being imported into the cytosol. In addition, hydrophobic drugs can easily reach into the transmembrane parts of these proteins which often constitute the functional core of membrane proteins. In this review, we focus on the structure and function of the membrane insertase YidC, and the potential of YidC as an antibacterial target.

1.1 YidC discovery and the Oxa1 superfamily

The YidC homolog Oxa1 was discovered in the 1990s to play a prominent role for inserting mitochondrial inner membrane proteins, that are mostly encoded by the mitochondrial DNA [2-7]. These proteins are expressed in the matrix and subsequently inserted into the inner membrane. Interestingly, homologs of Oxa1 called YidC and Alb3 were found to exist in bacteria and plant chloroplasts, respectively [2, 8]. The first evidence of YidC as a functional component of the membrane insertion machinery was provided by Scotti et al. in 2000 [9]. The authors found that the *Escherichia coli* YidC copurified with SecYEG and could be crosslinked to FtsQ, a type II membrane protein involved in cell division [9]. In the same year, YidC was discovered to be essential for the growth of *E. coli* when the function of YidC was inhibited in the cell using a promoter-controlled YidC depletion strain. The insertion and processing of the Sec-independent M13 procoat protein was blocked [10]. Other studies revealed that YidC is essential for cell growth, stability of secretory proteins in some cases, and environmental stress tolerance in other bacterial strains as well [11, 12]. However, for bacteria containing two YidC paralogs, both had to be inactivated in order to make it appreciably toxic/lethal [13, 14].

In addition to members of this YidC/Oxa1/Alb3 family being found in bacteria, mitochondria and plant chloroplasts [15], homologs were discovered in archaea [16] and very recently in the ER [17, 18]. The eukaryotic homologs show a low level of sequence identity [17] and are found as subunits of large membrane protein complexes [19-24].

1.2 Function and insertion of proteins

YidC plays a crucial role in the biogenesis of respiratory complexes [25]. Depletion of YidC results in defects in the assembly of these complexes that lead to an impaired proton motive force (pmf) and ATP levels [25, 26]. As expected, by a pmf perturbation, there is a dramatic induction of the phage shock response [25]. The PspA protein as well as other phage shock response proteins are highly overexpressed when the function of YidC is impaired [27]. It is well known that the phage shock response is switched on in case of membrane damage, or pmf disruption [28].

The YidC only pathway is used by proteins that insert in the absence of SecYEG and SecA and include the bacteriophage M13 procoat, Pf3 coat, subunit c (FoATPase), MscL, and TssL (also called SciP) [10, 29-38] (Figure 1A). The commonality of these proteins is that they have one or two transmembrane (TM) segments with a short, translocated domain, suggesting that YidC has a limited translocase function. Indeed, when the translocated region of the M13 procoat-lep and Pf3-lep proteins were extended or were made more hydrophilic, their insertion required both YidC and SecYEG for translocation [39-42]. Above a certain hydrophilicity threshold, the translocation of the periplasmic region was inhibited and did not occur even by the YidC/SecYEG pathway [42].

Other proteins require the combined action of YidC and SecYEG for membrane protein biogenesis (Figure 1B). For example, the minor coat protein G3p of bacteriophage M13 is inserted with both YidC and SecYEG [43]. Also, the subunit a (FoATPase) is inserted by the Sec/YidC pathway [34, 35]. Depletion of either YidC or SecE blocked the insertion of subunit a. A variation of this is seen with CyoA, a subunit of the ubiquinol reductase complex, where the translocation of the amino-terminal domain occurs by the YidC-only pathway while the large carboxyl-terminal domain is inserted by the SecYEG pathway [44, 45]. Finally, Sec-dependent LacY and MalF do not require YidC for insertion but for the correct folding of the protein [39, 46, 47].

2. The structure of YidC

Strikingly, YidC does not possess a membrane-spanning hydrophilic pore structure like the SecYEG complex [48-51] that can also translocate proteins into the bacterial periplasm. Rather, YidC has a hydrophobic slide and a membrane embedded hydrophilic groove that allows proteins to penetrate the membrane partway (see below, Figure 2). The groove is open to the cytosol and the membrane interior but closed to the periplasmic face. The center of the groove contains a conserved positively charged arginine. The function of this residue is to maintain the hydrophilic environment of the groove that most likely accommodates the hydrophilic region of a substrate prior to its translocation [52, 53].

Another remarkable feature is the helical coiled-coil region (Figure 2) found in the first cytoplasmic loop (C1) that is present in all YidC/Alb3/Oxa1 family of proteins, and in the ER homologs Get1, TCO1, and EMC3 [19]. The binding sites within the C1 loop and the C-tail of YidC have been shown to be important for interaction with the targeting components SRP and FtsY, and SecY [54].The cytoplasmic C2 loop along with the C-tail form a composite site for ribosome binding [55]. The C1 loop, in particular the coiled-coil region has been implicated in substrate binding, most likely facilitated by the dynamic nature of this region [56].

The TM3 that is a part of the greasy slide (Figure 2) is predicted to be flexible as well, since it contains several prolines and glycine residues at its boundary to the C1 loop. Along with TM5, TM3 has been shown to contact YidC substrates during the membrane insertion process [57-59].

The large periplasmic domain P1 of YidC has an extensive β-sandwich structure and a potential binding pocket for a protein chain [60, 61]. A deletion of a major part of P1 did not show any effect on the bacterial growth. In contrast, residues 215-265 within the P1 domain is required for the interaction of YidC with SecF [62]. This interaction is important because SecDFYajC helps link YidC to SecYEG complex [63].

2.1 YidC and SecY can form a complex and interacts dynamically

A major unanswered question in the field is the structure of the YidC/SecYEG complex that plays a crucial role in the membrane insertion, folding and assembly of many membrane proteins [64]. While there is a low-resolution structure of the YidC/SecYEG/SecDF holocomplex [65], the details of the interfacial region between SecYEG and YidC is not yet clear. Having an atomic structure of this region would provide a major step in understanding how Sec and YidC cooperate in membrane protein insertion. Several studies have verified their dynamic interaction: YidC and SecYEG can form a heterotetrameric channel [66], YidC occupies the SecY lateral gate [67], and YidC greasy slide contacts the SecY lateral gate [68].

3. Mechanism of membrane protein insertion

To elucidate the mechanism by which YidC catalyzes membrane protein insertion independently of SecYEG, the stepwise movement of the single spanning Pf3 coat protein was tracked using thiol crosslinking [69]. Different sized Pf3 coat derivatives were generated with a translational arrest peptide that stops protein synthesis to mimic the different stages of the membrane insertion process of Pf3 coat. The Pf3 coat proteins with the introduced cysteines were studied using YidC mutants with cysteines located at different positions in the TM3 and TM5 greasy slide region spanning from the cytoplasmic to the periplasmic side of the slide.

The disulfide crosslinking results showed that the Pf3 coat protein moves towards the periplasm up the slide from the cytoplasmic surface of the membrane. Crosslinking was observed between 24C in the TM segment of Pf3 and different greasy slide residues along the YidC TM3 and TM5. The results revealed that the hydrophobic segment of Pf3 moves up the slide during membrane insertion, as an α-helix (Figure 3).

In addition, the contacts between the N-tail region of Pf3 coat with the hydrophilic groove of YidC was investigated by incorporating cysteines in the YidC hydrophilic groove and the Pf3 hydrophilic N-tail region [69]. The thiol crosslinking results show that the C-terminal region of the hydrophilic N-tail is transiently incorporated into the hydrophilic groove. However, no crosslinking was observed between the YidC groove cys mutants and the N-terminal region containing cysteine substitutions of the N-tail suggesting that the N-terminus was still in the cytoplasm. Taken together, this study shows that the Pf3 coat inserts as a hairpin structure during its movement through YidC.

Recently, the role of the cytoplasmic helical coiled-coil domain of YidC was investigated in detail [56]. The coiled-coil domain consists of two α-helical regions (CH1 and CH2) and functions in the binding of the substrate protein. A fluorescently labelled Pf3 protein was inserted into the YidC proteoliposomes

whereas a YidC mutant with a deletion of CH2 in the coiled-coil domain was severely affected for the binding to YidC. Similarly, a mutation of the conserved arginine in the groove with a negatively charged residue inhibited the insertion of Pf3 coat into the proteoliposomes. To gain further insight into the substrate protein binding mechanism, the C-terminus of the Pf3 proteins was linked to a cantilever of an AFM and tested for binding to YidC that had been reconstituted into proteoliposomes. Using single molecule force spectroscopy, it was observed that Pf3 interacted with YidC within 2 ms with the help of the cytoplasmic α-helical hairpin of YidC. Although the substrate strengthens its binding to wildtype YidC until saturation (52 ms), the two YidC mutants (with CH2 deletion and R366E) did not, suggesting that the electrostatic binding to the helical hairpin and groove is involved in the second substrate binding step.

Molecular dynamic simulations were then employed to gain further insight into the conformational variability and kinetic stability of the inserting Pf3 protein with the helical hairpin and the hydrophilic groove of YidC [56]. These data suggest that the negatively charged residues in the N-terminal region of Pf3 are interacting electrostatically with the helical hairpin and are then guided to the hydrophilic groove prior to their membrane translocation.

How does YidC insert and fold proteins into the membrane? The answer depends on the protein that is being inserted. Some proteins require YidC for membrane insertion [35], others for the folding of the protein [46, 47, 70] and some only for the assembly of the protein into an oligomeric state [71, 72]. CyoA, a subunit of ubiquinol oxidoreductase, is a protein that spans the membrane two times, with a short N-tail and a large C-terminal domain facing the periplasmic space (Fig.1). CyoA is a lipoprotein and is initially made in a precursor form with a signal peptide that is processed by lipoprotein signal peptidase [73]. The amino-terminal domain with a short periplasmic region is inserted by the YidC only pathway while the large C-terminal domain is translocated by the SecA motor protein and SecYEG [44, 45]. Interestingly, it is necessary for the amino terminal domain to insert in order for the SecA/SecYEG translocase to translocate the large C-terminal domain. The mechanism by which the YidC insertase and Sec components cooperate in this dynamic process is yet to be determined.

YidC is required for the folding of lactose permease (LacY) that spans the membrane 12 times with the N and C-termini located in the cytoplasm [74] (Fig. 1). LacY is targeted to the membrane by SRP, which then binds to the SRP receptor at the membrane [75, 76]. The membrane embedded Sec translocase is required for the membrane insertion of LacY [77] while YidC is crucial for LacY to obtain its correct 3D conformation as the binding of two monoclonal antibodies that recognize the folded epitopes was perturbed [46]. A detailed investigation showed that each of the 6 periplasmic loops are inserted by SecYEG and do not require YidC for their translocation [70]. However, YidC contacts the LacY TM segments during insertion and assists in the folding of the protein [70]. In this chaperone capacity, YidC directs the proper helix-helix interactions of LacY. It also has this chaperone function for mannitol permease, where YidC may act as an assembly site for the folding of α-helical bundles in membrane proteins [78]. Furthermore, it has been

suggested to play a role in the lateral transfer of a membrane protein from the SecYEG complex into the lipid bilayer [79].

4. YidC as an antibiotic target

The bacterial YidC provides a potential target for novel antibiotics. YidC is a promising candidate primarily because YidC is essential for bacterial cell growth [10-14]. YidC is evolutionarily conserved among bacteria sharing rather high sequence homology [80]. The hydrophilic groove contains some of the most strongly conserved residues of the protein. Therefore, inhibitors against YidC should act as broad-spectrum antibiotics.

An encouraging study, showing that YidC could be an antibacterial target, employed antisense-mediated gene silencing to lower the expression of YidC within *E. coli* [81]. By down regulation of YidC, the authors determined that the cells become sensitive to the antibacterial oils, eugenol and carvacrol (Figure 4), which are found in clove and oregano, respectively [82]. Previously, these oils were shown to inhibit the membrane embedded ATPase in *E. coli* [83]. However, it cannot be excluded that this observed inhibition is a secondary effect of YidC inactivation or loss of the membrane potential.

A second study [12] reported that YidC2 was the target of specific compounds (celecoxib derivatives Cpds) that had previously been shown to kill methicillin-resistant *Staphylococcus aureus* (MRSA) [84]. The authors demonstrated this by isolating and studying *S. aureus* mutants that were resistant to the celecoxib derivatives. By sequencing the whole genomes, they identified 7 mutations in Cpd9-resistant and Cpd36-resistant *S. aureus*. Both isolates had missense mutations (P139L or P73L) within the *yidC2* genes, that resulted in substitutions in the transmembrane region of the YidC2 protein. They confirmed that overproduction of the wild-type YidC2 and the YidC2 resistant mutants were less vulnerable to Cpd36 and Cpd46. Also, as expected based on work with *Streptococcal mutans* showing that YidC2 plays a crucial role for growth under acid and salt stress conditions [14], the addition of Cpd36 and Cpd46 impaired growth even more under acidic and high NaCl stress conditions.

Quite promising, the compounds Cpd36 and Cpd46 effectively killed a wide variety of *Staphylococci* species even if they contained two YidC paralogs. Interestingly, both Cpd compounds were quite effective against vancomycin-induced persisters and Cdp46 had activity in killing *S. aureus* in biofilms. They were also potent against other Gram-positive bacteria such as *Bacillus subtilis, Enterococcus faecium*, and *Streptococcus pyogenes* to name a few. However, they did not kill the tested Gram-negative bacteria such as *E. coli* or *Salmonella typhimurium* suggesting their ineffectiveness as YidC inhibitors may be due to the failure to pass the bacterial outer membrane or due to efflux pumps that prevent the compounds from accumulating in the cell.

To provide further evidence that the Cpd compounds target YidC2, the authors confirmed that the membrane insertion of Foc (subunit c of the F1Fo ATP synthase) was inhibited by showing that the amount of membrane localized Foc was markedly reduced upon addition of Cpd46. Additionally, the intracellular ATP levels were strongly affected by this compound. These compounds target the *S. aureus* YidC2 by directly binding to the

protein as the thermal stability of YidC in cell lysates was increased by over 10 ^{o}C after the addition of Cpd36 or Cpd46. In order to identify potential residues important for binding of Cpd36 to *S. aureus* YidC2, first a homology model was built based on the solved structure of the *Bacillus halodurans* YidC2. Not surprisingly, many of the substrate contact sites found with the *E. coli* YidC [57-59, 69] were localized to the hydrophilic groove and the greasy slide region of the *S. aureus* YidC2 (Figure 5). Cpd36 most likely contacts the hydrophilic groove as mutations in this region had a large effect on the binding affinity of Cpd36 to YidC2. For example, mutation of the hydrophobic residues (Y188A and L240A) affected binding of YidC2 to Cpd36 or completely blocked binding (P73L, P139L, and Y79A). This work, combined with the antisense study with the antibacterial oils, point to YidC being a promising target for novel antibacterial compounds. Notably, both carvacrol and celecoxib are well known inhibitors of cyclooxygenase-2 (COX2), which are used as anti-inflammatory drugs [85, 86].

In addition to the advantages mentioned in the introduction of YidC as antibacterial target, YidC is easy to purify and to test its activity both *in vivo* [87] and *in vitro* [88]. The different YidCs in various pathogenic strains can therefore be adapted to the optimal inhibitory action using drug discovery approaches. Very helpful for discovery of novel antibiotic inhibitors is that a structure of YidC is available making it also possible to determine the structural details of YidC in complex with an antibacterial compound. A possible location for compounds to bind is the hydrophilic groove which is essential for the hydrophilic regions to be located prior to their translocation across the membrane. Alternatively, hydrophobic inhibitors could directly bind to the YidC TM3 and TM5 greasy slide which the TM segments of YidC substrate utilize to cross the membrane.

Another approach is to use a fluorescent-based substrate binding assay to YidC that is suitable for high throughput screening to identify compounds in a chemical library that disrupt the interaction of substrate with YidC. After completing the screening, the compounds will be used in cell-based studies.

4.1 Feasibility of YidC antibiotics

In order for YidC inhibitors to be used safely in humans they should be selectively toxic to bacterial cells, with no or low toxicity for the patient. Although human mitochondria contain a YidC homolog (Oxa1), it is located in the mitochondria, and the inhibitor binding site would most likely face the matrix. Thus, an inhibitor must traverse three membranes (cytoplasmic membrane and mitochondrial outer and inner membranes) before it can bind to Oxa1. In addition to a mitochondrial homolog there are three endoplasmic reticulum (ER) homologs that are Oxa1-like conserved proteins. They all have a 3TM core (corresponding to TM2, TM3 and TM6 of the *E. coli* YidC) and possess a hydrophilic groove within the structure of the multi-subunit membrane protein complex (Get complex, EMC, and TMC01, respectively). However, they all possess low homology to the bacterial YidC. Therefore, it is unlikely that a bacterial YidC inhibitor would bind and affect membrane protein insertion into the ER.

Acknowledgement

This work was supported by a National Science Foundation Grant MCB-1814936 to R. E. D. and by the Deutsche Forschungsgemeinschaft DFG Grant Ku749/6-2 to A. K.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Figure Legends

Fig. 1. YidC Substrates. Proteins that inserted by the YidC only (**A**) and YidC/Sec YEG pathway (**B**). Some of the substrates are made in a precursor form and the signal peptide processing site is shown by a red arrow.

Fig. 2. The insertase YidC. The crystal structure of the *E. coli* YidC showing the large periplasmic domain, the conserved membrane embedded 5 TM region and the C1 region with the coiled-coil domain (**A**)[adapted from [49] , PDB:3WVF] is indicated. The greasy slide region (**B**) comprised of TM3 and TM5 is indicated with the residues that have been shown to contact the YidC substrates indicated. A closeup view of the hydrophilic groove (**C**) showing some of the groove residues. Note the periplasmic domain P1 is not shown in B and C.

Fig. 3. The model of membrane insertion of Pf3 coat protein by YidC. A. The substrate approaches the membrane (**A**), and binds to the cytoplasmic helical hairpin region of YidC (**B**). The hydrophobic region of Pf3 moves up the greasy slide and the hydrophilic region of the N-tail is transiently incorporated in the hydrophilic groove (**C**). After translocation of the hydrophilic region across the membrane Pf3 coat dissociates from YidC (**D**). The transmembrane segment of Pf3 is orange while the hydrophilic regions are blue. The greasy slide TM3 and TM5 region of YidC are depicted in purple and red. The blue arrow indicates the pathway by which Pf3 coat dissociates from the greasy slide region of YidC.

Fig. 4. Chemical structure of Carvacrol and Eugenol, and the Celecoxib compounds Cpd9, Cpd36 and Cpd46. YidC depletion by antisense RNA expression results in sensitization to the antibacterial oils Carvacrol (**A**) and Eugenol (**B**). Celecoxib derivatives (**C-E**) target YidC and eradicate antibiotic-resistant *Staphylococcus aureus*.

Fig. 5. Comparison of the Cpd36-contacting residues of YidC2 in *S. aureus* (magenta) and *E. coli* (navy blue). The YidC2 mutations identified in the drug resistant *S. aureus* isolates are shown in green. The homology model of *S. aureus* YidC2 was built based on X-ray structures of *B. halodurans* YidC2 (PDB code: 3WO6 and 3WO7) [Adapted from [12]]

Graphical abstract**.** Adapted from ref [12].

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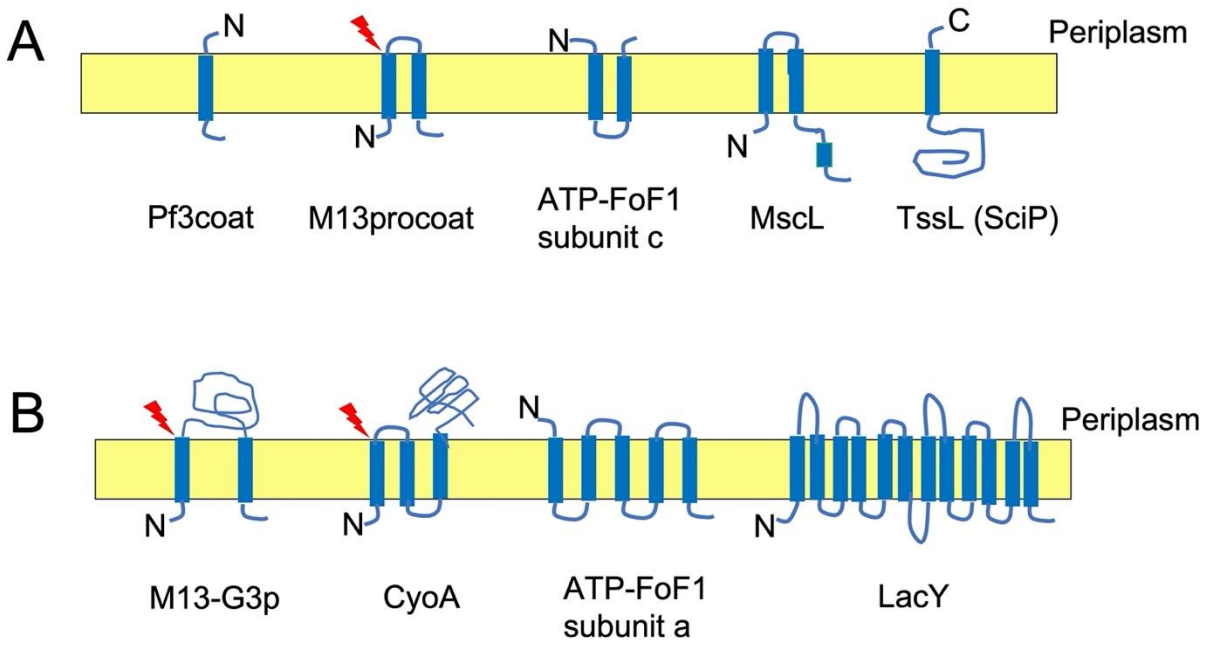
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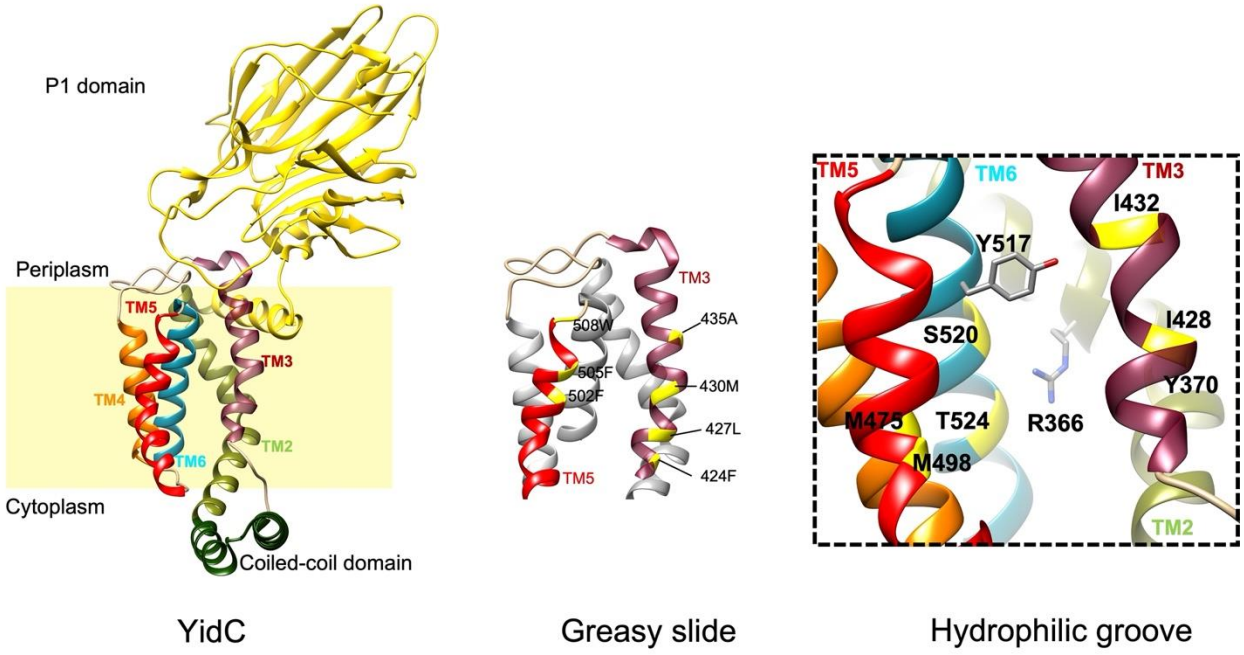
Fig. 1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Hydrophilic groove

Fig. 1

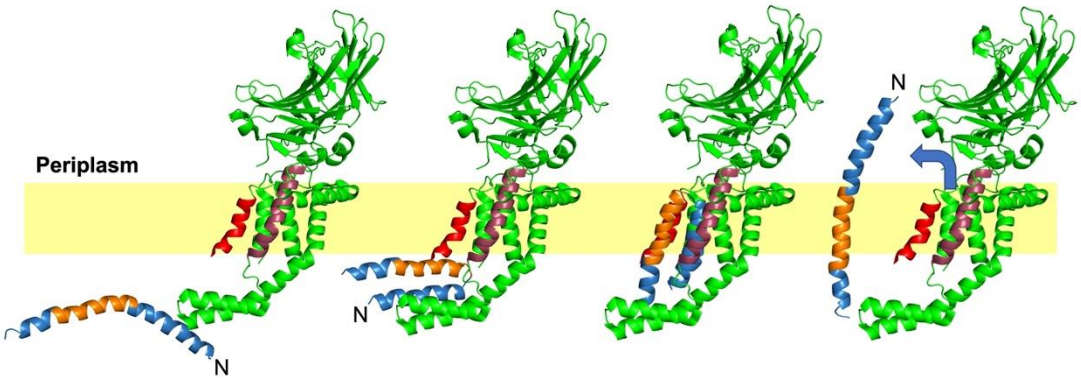

Fig. 3

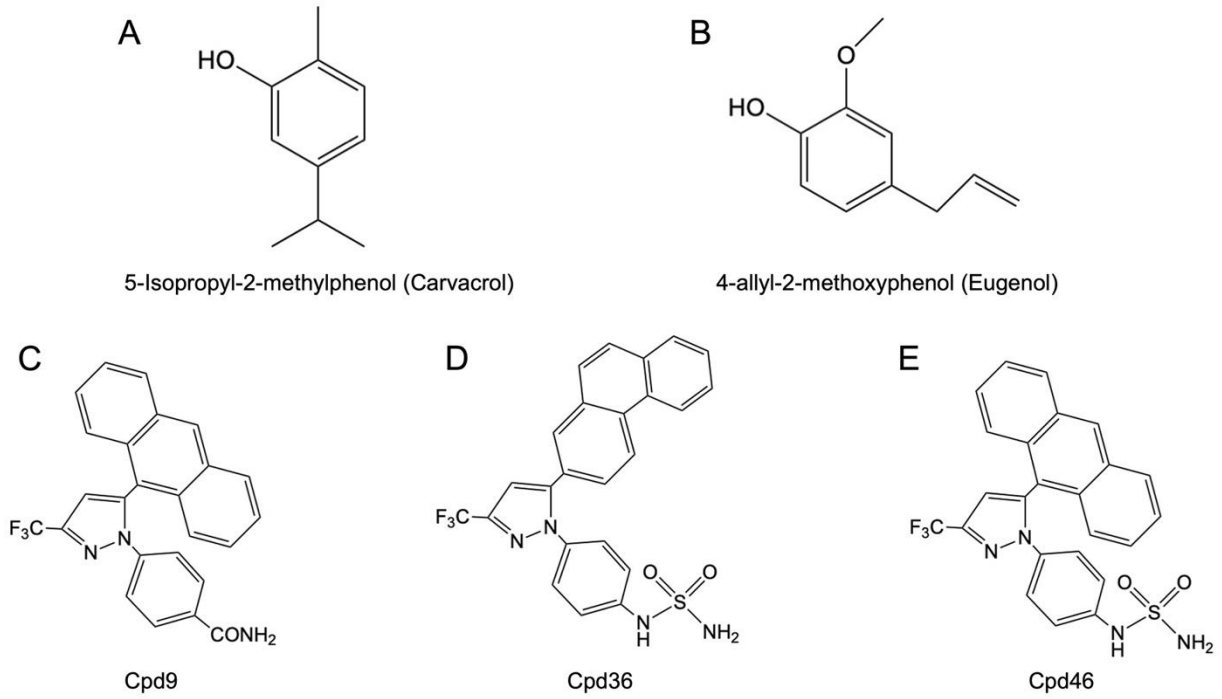
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Fig. 4

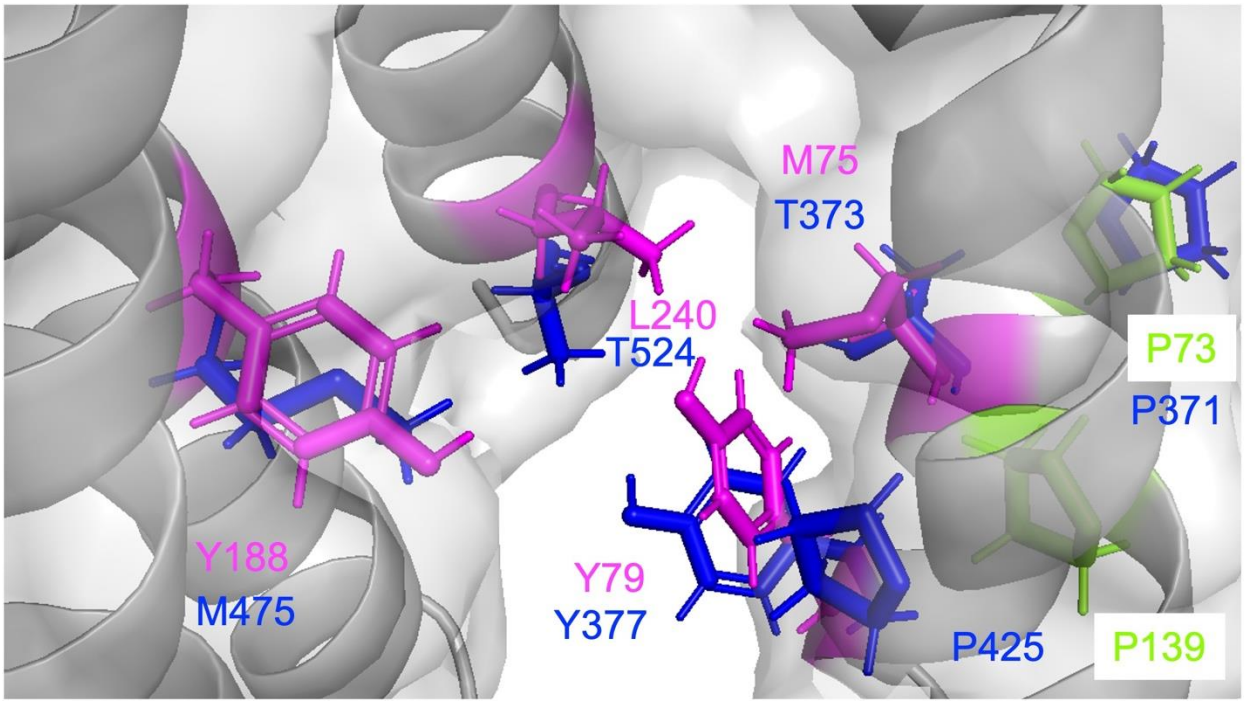

Fig. 5