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Identification of novel inhibitors of the ABC transporter BmrA

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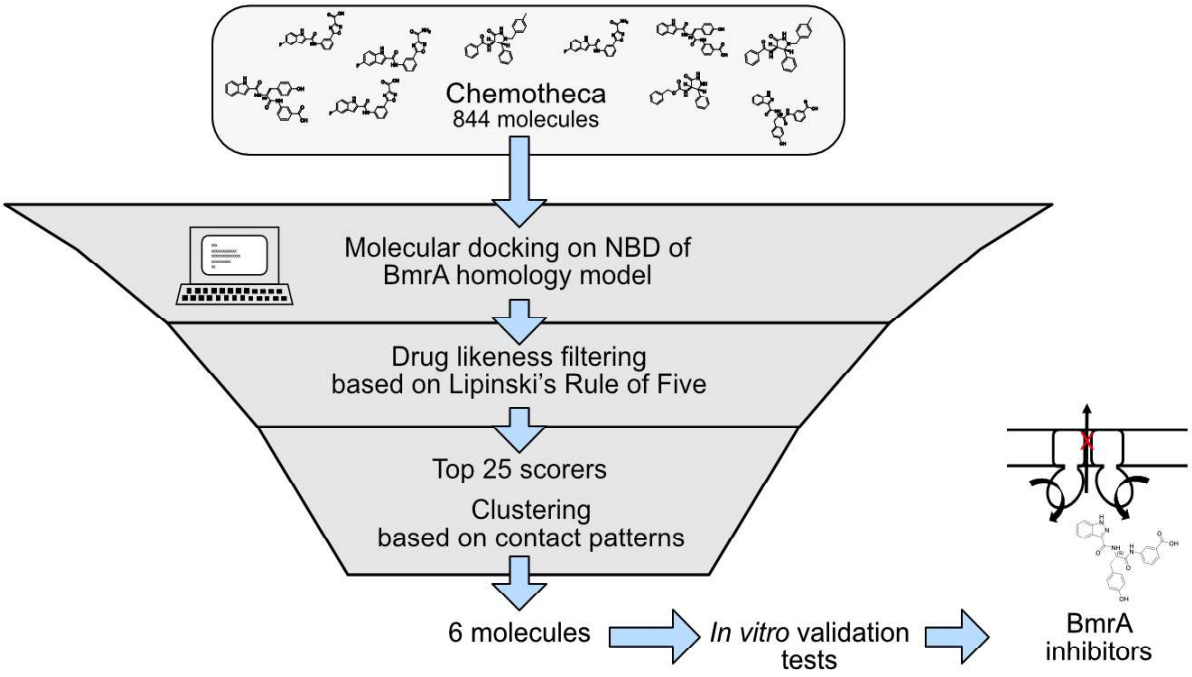
Graphical Abstract



Abstract

The resistance of microbes to commonly used antibiotics has become a worldwide health problem. A major underlying mechanism of microbial antibiotic resistance is the export of drugs from bacterial cells. Drug efflux is mediated through the action of multidrug resistance efflux pumps located in the bacterial cell membranes. The critical role of bacterial efflux pumps in antibiotic resistance has directed research efforts to the identification of novel efflux pump inhibitors that can be used alongside antibiotics in clinical settings. Here, we aimed to find potential inhibitors of the archetypical ATP-binding cassette (ABC) efflux pump BmrA of *Bacillus subtilis* via virtual screening of the Mu.Ta.Lig. Chemotheca small molecule library. Molecular docking calculations targeting the nucleotide-binding domain of BmrA were performed using AutoDock Vina. Following a further drug-likeness filtering step based on Lipinski's Rule of Five, top 25 scorers were identified. These ligands were then clustered into separate groups based on their contact patterns with the BmrA nucleotide-binding domain. Six ligands with distinct contact patterns were used for further *in vitro* inhibition assays based on intracellular ethidium bromide accumulation. Using this methodology, we identified two novel inhibitors of BmrA from the Chemotheca small molecule library.

Keywords: BmrA, efflux pump inhibitors, antibiotic resistance, nucleotide-binding domain, molecular docking, virtual screening, Chemotheca

1. Introduction

All types of cells possess proteinaceous transporters, termed as efflux pumps, that are involved in the extrusion of all sorts of molecules from the cells. The fundamental role of these pumps is to help cells regulate their internal environment by the extrusion of metabolites and toxic substances such as antibiotics and different chemicals [1–3]. On the other hand, these transporters are also major contributors of intrinsic and acquired drug resistance for a wide range of diseases, and therefore constitute a major global threat [4,5]. A deep understanding of mechanisms involved in the development of resistance and the discovery of new drug exporter inhibitors are of utmost importance to bring back the drugs with lost efficacy into the clinic.

Based on the number of their membrane-spanning helices, energy sources, substrates, and sequence similarities, bacterial efflux pumps are currently categorized into six families; ATP-binding cassette (ABC) superfamily, the major facilitator superfamily (MFS), the multidrug and toxin extrusion (MATE) family, the small multidrug resistance (SMR) family, the resistance-nodulation-cell division (RND) superfamily, and the proteobacterial antimicrobial compound efflux (PACE) family. While ABC superfamily utilizes ATP as the energy source for transportation, the other five groups use electrochemical energy generated by the transmembrane ion gradients [6].

Within these classes, ABC transporters represent one of the largest and most abundant superfamilies of proteins found in every form of life [7]. An ABC transporter minimally has four core domains; two transmembrane domains (TMDs) that include substrate-binding pockets and two nucleotide-binding domains (NBDs) that bind and hydrolyze ATP to drive the transport cycle. These four domains can be individually synthesized or can be fused in all combinations to form heterodimers, homodimers or a transporter made from a single polypeptide [8]. The NBDs possess several functional signatures; the three main ones being the P-loop or Walker A motif (GX_4GKT/S), the Walker B motif (hy_4D, where hy is a hydrophobic residue), and the ABC signature motif (S signature) starting with LSGGQ sequence [9–11]. During the transport cycle, multidrug ABC transporters generally switch between inward- and outward-facing conformational states [12,13]. ATP binding or hydrolysis by the two NBDs provides the necessary trigger for this switch [6,14].

Among the substrates of ABC transporters are found a large number of hydrophobic compounds and metabolites in addition to sugars, amino acids, and peptides. Unfortunately, the presence of ABC transporters also has a significant role in resistance development to drugs. Overexpression of three main ABC transporters in cancer cell lines and tumors results in resistance to unrelated drugs over a period of time. This is a major contributor to failure of chemotherapy. Similarly, bacterial cells, express different families of ABC transporters that play a role in resistance to a variety of antibiotics [8,15].

The most powerful strategy to circumvent drug efflux through these pumps is through inhibition of their action by small molecules, known as efflux pump inhibitors (EPIs) [1]. A large number of EPIs have been identified against different efflux pump families in the past. However, poor pharmacological properties and adverse toxicity profiles at concentrations required for inhibitory activity prevented these inhibitors from reaching the clinical development stages. Novel and more potent inhibitors against these efflux pumps are therefore urgently needed. In order to facilitate and speed up the discovery of novel EPIs, *in silico* virtual screening studies based on available crystal structures of efflux pumps or homology models can be incorporated into the drug discovery pipelines. Moreover, small molecules that were investigated as drug candidates for other biological targets may be given priority in such screens in a drug repurposing context [16,17]. For this purpose, small molecule libraries developed specifically for drug repurposing may be used. The Chemotheca small molecule library, developed as part of the EU Cost Action "Multi-target paradigm for innovative ligand identification in the drug discovery process" (Mu.Ta.Lig), is one such database [18].

Here, we aimed to find new EPIs for the homodimeric multidrug ABC transporter BmrA from *Bacillus subtilis* via virtual screening of the Chemotheca library. *B. subtilis* is a widely used model organism to study multidrug ABC transporters [19]. The significance of this transporter is its high homology to the mammalian P-glycoprotein, which transports a wide range of drugs and toxins out of cells [20]. Therefore, potent inhibitors of BmrA may have the potential to reverse drug resistance mediated through efflux by P-glycoprotein. The obtained results will also help the design of new inhibitor molecules with improved properties.

2. Material and Methods

2.1. Homology modeling

Homology modeling of the BmrA homodimer with each chain possessing 589 amino acids (Accession: O06967.1, GI: 81814872) was performed using Modeller v9.19 [21]. As a template, the structure of the efflux pump MsbA (EcMsbA) from *Escherichia coli* was used (PDB code: 3B5W, full coordinates kindly provided by Geoffrey Chang). The pairwise sequence alignment between the template and the BmrA amino acid sequences are given in Supplementary Figure S1. The quality of the resulting model was evaluated using the Protein Structure Analysis web service (ProSA-web) [22].

2.2. Virtual screening

Small molecules from the Chemotheca library from Mu.Ta.Lig COST Action project (CA15135) [18], which consisted of 844 ligands by that time, were docked onto the NBD of the modeled BmrA structure using AutoDock Vina 1.1.2 [23]. For macromolecule preparation, all water molecules were deleted and all polar hydrogens were added to the structure. Docking of each ligand was performed five times, and each

repeated simulation generated twenty poses, yielding a total of 100 poses for each ligand. From these, the top scoring pose (in terms of binding free energy as estimated by AutoDock Vina) was selected for further analysis. The whole NBD surface was chosen as a possible ligand-binding site. The exhaustiveness was set to 10 for all docking calculations.

2.3 Evaluation of virtual screening results

The different ligands that were predicted to target the NBDs were first ranked according to their respective docking scores, (in terms of binding free energy as estimated by Autodock Vina) in increasing order. Then, a further refinement was performed based on Lipinski's Rule of Five (Ro5) as calculated by the Drug-Likeness Tool (DruLiTo) (http://www.niper.gov.in/pi_dev_tools/DruLiToWeb/DruLiTo_index.html). The top 25 ligands in this final list were selected for further analysis. Interactions between the top 25 scorers and the NBD were identified using the Protein Ligand Interaction Profiler (PLIP) tool [24]. The interaction profiles of the selected 25 ligands were then clustered into three separate groups, based on the sites they bind. At least one binder molecule was selected from each group for *in vitro* tests. Hydrophobic interactions reported by PLIP were excluded from the clustering analysis.

2.4 Bacterial strains used

Wild-type *B. subtilis* 168 (DSM 402) and *B. subtilis* Δ*bmrA* [20] were used for testing the EPI properties of selected compounds. The mutant cell has been constructed by Steinfels et al. [20] by knocking out the *yvcC* gene that encodes BmrA in *B. subtilis*.

2.5 Candidate inhibitor molecules tested in vitro experiments

Among the best 25 binders, we were able to get only six of them to be tested experimentally. Compounds CM263 and CM299 were gifts from Črtomir Podlipnik. Compounds CM311, CM13 [25], CM616, and CM48 [26], have been synthesized as reported previously. All EPI candidates were dissolved in dimethylsulfoxide (DMSO).

2.6 Monitoring ethidium bromide accumulation

The potential of these inhibitors was assayed monitoring ethidium bromide (EtBr) accumulation on 96-well plates using a Synergy HTX Multi-Mode Reader (BioTek Instruments, Inc., Winooski, VT, USA) equipped with filters of 540 and 590 nm for excitation and emission, respectively. Relative fluorescent intensities of the accumulated EtBr in control and EPI treated cells were compared for both wild-type and mutant cells using a modified version of the previously reported method [20,27].

Overnight grown wild-type *B. subtilis* 168 or *B. subtilis* Δ*bmrA* [20] cells were inoculated into 5 mL fresh Nutrient Broth (NB, Merck, Germany). Cell growth was achieved at 37ºC and 180 rpm and growth was

monitored spectrophotometrically. As OD_{600} reached 0.5, cells were centrifuged at 2000g and 4ºC for 4 minutes. Cell pellets were suspended in 2 mL of 0.35 M NaCl. 180 µL of the cell suspension was mixed with 50 mM KP_i, 5 mM $MgSO_4$, and 25 mM glucose. Immediately after the addition of glucose, 10 µM of EtBr (Invitrogen, California, USA) was added to the mixture and fluorescence intensity was recorded for 20 minutes. To see the effect of the EPI candidates, they were added to the cell suspension to a final concentration of 50 $\mu g \cdot mL^{-1}$ before the addition of glucose. Control cell suspensions contained an equal amount of DMSO.

Fluorescence of these cells was then measured as a function of time. The higher fluorescence measured indicates the accumulation of ethidium bromide in the cells.

2.7 Determination of minimum inhibitory concentration

Minimum inhibitory concentration (MIC) of the compounds for *B. subtilis* was determined using broth microdilution method (Amsterdam, 1996). Two-fold serial dilutions of the compounds starting from 1000 $\mu g \cdot mL^{-1}$were prepared with nutrient broth (NB) broth in sterile 96-well U-bottom plates. A single column of the test plate was prepared with serial dilutions of solvent DMSO as control. The test plates were inoculated with a bacterial inoculum of 1×10^6 $CFU \cdot mL^{-1}$ and incubated for 24 h at 37°C. MIC was determined using 2,3,5-triphenyltetrazolium chloride (TTC) (Sigma, Germany) dye reduction test. At the end of a 24-h incubation, TTC solution was added into each well as a visualization agent at a final concentration of 0.5% (w/v). Test plates were further incubated 1 h at 37°C. The development of red color indicated the presence of viable cells in the wells. The lowest concentration showing no color change was determined as the MIC value.

3. Results

3.1 Homology modeling of the BmrA structure

An experimentally-determined structure of the BmrA is currently unavailable. Therefore, we generated a homology model using the inward-facing state of the EcMsbA as a template (Fig. 1A). BmrA and EcMsbA primary sequences display 30% identity and 26% strong similarity. A quality assessment by the ProSA-web server [22] yielded a Z-score of -6.01 for the BmrA monomer which was only narrowly included within the Z-score range of experimentally-determined protein chains of the same size (Fig. 1B). The quality of the homology model was highest in the NBD (right part of the panel) as compared to the TMD moiety (left part of the panel), as evidenced by the energy plot of individual amino acids (Fig. 1D). The latter is an important feature of the homology model, as high quality of the model in the NBD is crucial for reliable molecular docking calculations and hence prioritization of compounds for biological evaluation by the use of virtual screening.

3.2 Virtual screening of Chemotheca library for BmrA inhibitors

BmrA homology model included two identical NBD domains. This NBD domain functions as an ATP-binding subunit and features several conserved motifs including the Walker A/B motifs and Q, H, D, and X loops (Fig. 1C) [8]. Moreover, coupling helices (CHs) from the TMDs of both monomers of the BmrA structure play a critical role in transmitting conformational changes from the NBDs to enable the transition between the outward- and inward-facing state. Since all of these residue groups are functionally important, small molecules that disrupt the interactions of any of these sites with the ATP-binding site or their surrounding residues may have the potential to inhibit BmrA activity.

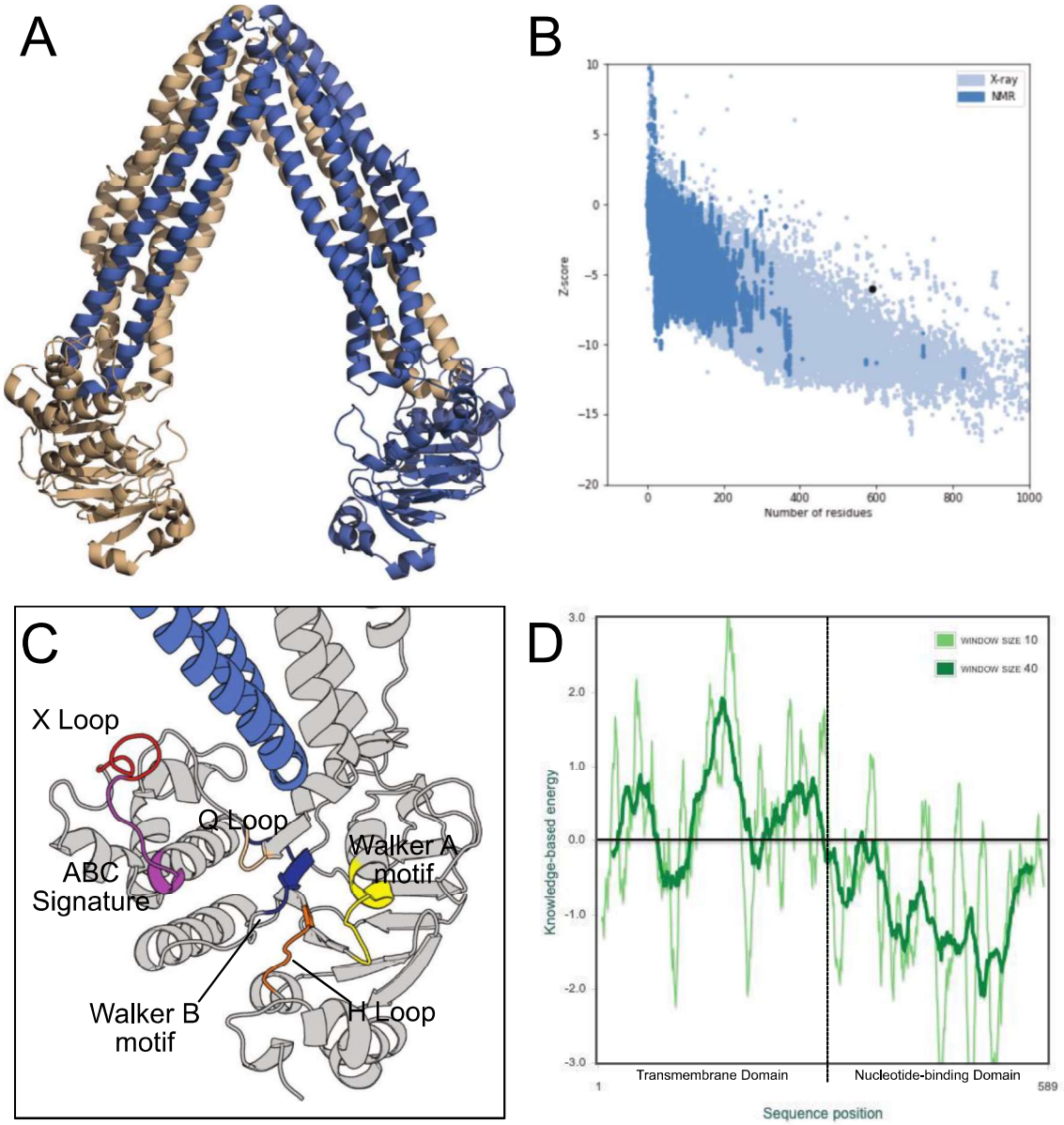


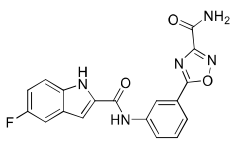
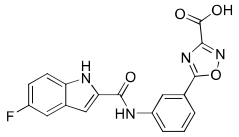
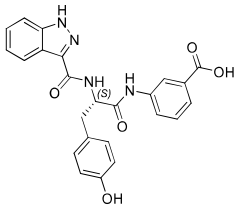
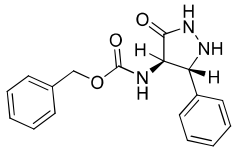
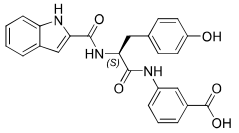
Figure 1. (A) BmrA homology model. The two monomers are colored differently. (B) ProSA-web Z-score of the generated homology model with respect to reference distributions. Plot as generated by the ProSA-web service. (C) Functional sites in the BmrA NBD. (D) Position-specific energy levels as reported by the ProSA-web service.

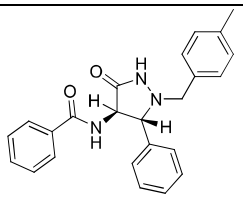
We first performed molecular docking simulations of the Chemotheca small molecule library (844 molecules) by targeting the whole NBD surface. Then, we applied the Lipinski's Ro5 as a drug-likeness filter to include only those molecules with favorable drug-like properties. Docking scores of the resulting top 25 binders satisfying Lipinski's Ro5 are given in Table 1. We then computed interaction profiles of these ligands using PLIP tool [24] and performed a hierarchical clustering analysis to identify ligands with similar interaction profiles (please refer to Methods section for details). As indicated by the contact clustering analysis, the top scorers were found to prefer three different binding sites on the BmrA NBD surface (Fig. 2A). Within this list, we were able to get access to a total of six molecules (CM13, CM311, CM299, CM48, CM263, and CM616) that were predicted to bind to three different sites on the NBD surface. While CM48 and CM616 were found in the first binding site, CM263 was found in the second binding site, and CM299, CM13, and CM311 were found in the third binding site. These molecules were further tested for their *in vitro* activities. Detailed molecular interactions between these molecules and the BmrA NBD are listed in Table 2 and shown in Fig. 2B-C. In the first binding site, CM48 and CM616 made contacts with a pocket formed by P352, L355, G349, S392, R389, V329, T328, D327, E326, Y391 of the NBD and V112' of the CH of the opposite NBD. In the second binding site, CM263 contacted E325, V329, E411, E415 of the NBD and K217'and N220' of the CH of the opposite NBD. Finally, in the last binding site, CM299 contacted T381 of the Walker A motif and K385' and R214' of the CH of the opposite NBD. On the other hand, CM13 and CM311 additionally contacted D116' of the CH as well as the catalytic glutamate E504.

Table 1. Top 25 molecules from the Chemotheca Library in terms of their binding affinity of BmrA NBD as predicted by Autodock Vina (Molecules tested are marked in bold).

Molecule ID	**Docking Score (kcal/mol)**	**Molecule ID**	**Docking Score (kcal/mol)**
CM48	**-10.26**	CM62	-10.06
CM552	-10	CM612	-9.98
CM59	-9.96	CM67	-9.9
CM610	-9.6	**CM616**	**-9.54**
CM302	-9.26	CM178	-9.2
CM311	**-9.1**	CM206	-9
CM613	-9	**CM299**	**-8.94**
CM355	-8.94	CM573	-8.94
CM697	-8.94	CM476	-8.92
CM581	-8.88	**CM13**	**-8.86**
CM503	-8.86	**CM263**	**-8.8**
CM322	-8.8	CM419	-8.8
CM312	-8.78		

Table 2. Detailed interactions with selected molecules with BmrA NBD and CHs.

Molecule ID	Structure	Interactions with BmrA NBD*	
CM48		Hydrogen bonds	D327, T328, V329, G349, R389, F390, S392
		π-π stacking	Y391
		Hydrophobic interaction	V112*, E362, P352, L355
CM616		Hydrogen bonds	D327, T328, G349, S392
		π-π stacking	Y391
		Salt bridge	R389
		Hydrophobic interaction	V112*, E326, P352, L355
CM311		Hydrogen bonds	D116*, T381, K385*, E504, R214*
		π-π stacking	Y350
		π-cation interaction	K385*
		Hydrophobic interaction	T381, K385*, Y419, R214*
CM299		Hydrogen bonds	T381, D503, R214*
		π-π stacking	Y419
		π-cation interaction	K385*
		Hydrophobic interaction	K385*, I533
CM13		Hydrogen bonds	D116*, T381, E504, R214*
		π-cation interaction	K385*
		Salt bridge	K385*
		Hydrophobic interaction	D116*, Y350, K385*, F390, Y419
CM263		Hydrogen bonds	K217*, E325
		Hydrophobic interaction	N220*, E325, V329, L410, E411, E415

*Interactions marked with * denote interaction with the CH of the opposite monomer

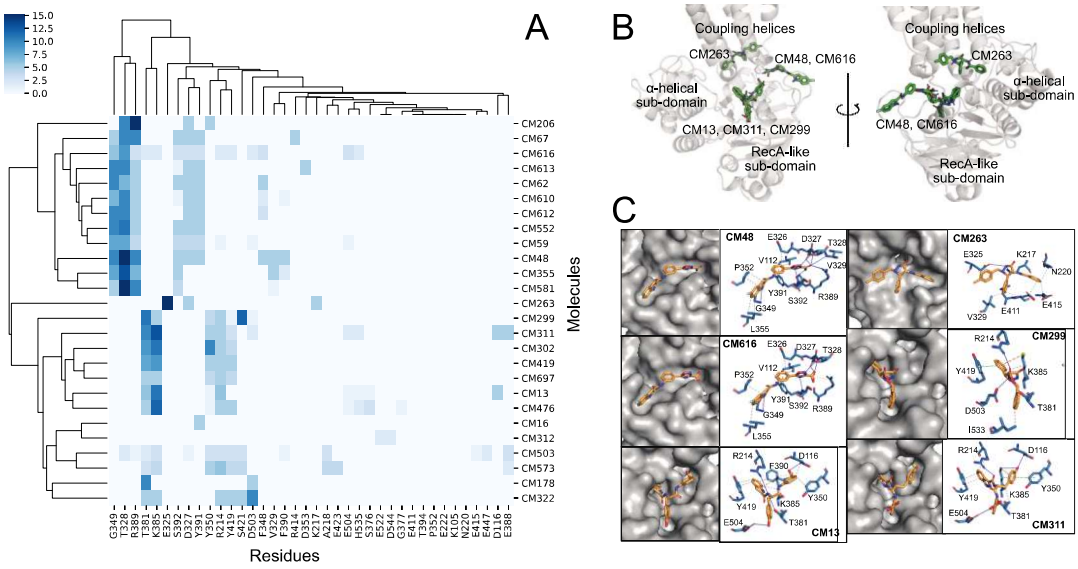


Figure 2. (A) Contact clustering heatmap of docking results. Color intensity according to the number of contacts found between each ligand and BmrA in the top scoring pose of five docking simulations for each ligand. (B) An overview of the binding sites of selected six ligands (C) Detailed molecular interactions between selected six ligands and BmrA.

3.3 In vitro tests with selected molecules

We have tested the EPI potential of six of the compounds found in our final list of BmrA inhibitors. Among these, solubility issues were encountered with CM263; it barely dissolved in DMSO and formed precipitates in the reaction mixture. Therefore, the results obtained with this molecule were not consistent. Of the tested molecules, CM299 and CM616 exhibited no inhibition at all. CM48 showed inhibition only at very high concentrations (125 µg·mL^{-1}), which made us eliminate it from further discussion since it would likely reveal

some toxic effects. The results obtained with CM13 and CM311 indicated that both could be considered as EPIs (Fig. 3).

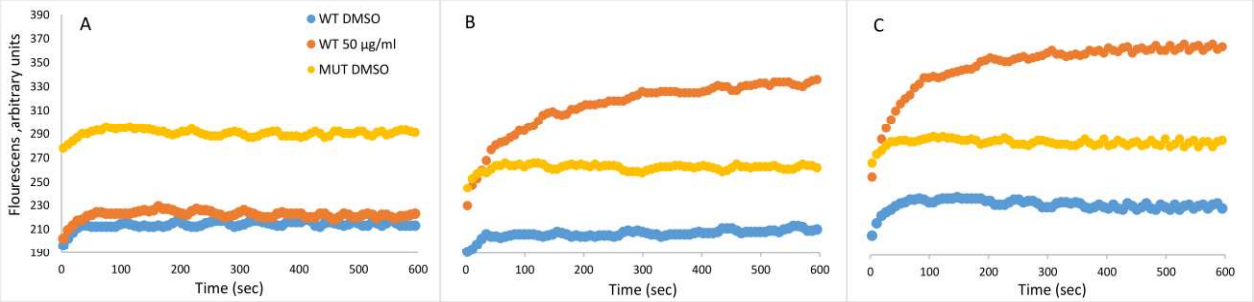


Figure 3. Fluorometric measurements for recording EtBr accumulation in *B. subtilis* cells (WT: Wild-type *B. subtilis* 168, MUT: *B. subtilis* Δ*bmrA*) in the presence of the molecules CM299(A), CM13(B), and CM311(C).

When fluorescence intensity due to EtBr accumulation was recorded in wild-type *B. subtilis* 168 and *B. subtilis* Δ*bmrA* cells, following an initial increase for about one minute, the intensity of fluorescence remained constant for the following 9 minutes (Fig. 3A). Yet, the values recorded for the mutant cells were approximately 80 arbitrary units higher, which suggested that EtBr accumulation was higher in these cells, essentially because of the absence of the BmrA pump. The presence of 50 $\mu g \cdot mL^{-1}$ CM299 has made no change in EtBr accumulation (Fig. 3A, orange line); thus, this molecule did not display any EPI property. On the other hand, when wild-type cells were treated with CM13 or CM311, even though the intensity of the initial fluorescence was pretty close to that recorded for the mutant cells, it gradually increased with time (Fig. 3B and 3C, orange lines). Theoretically, we were expecting similar curves for wild-type cells treated with EPIs (orange lines) and for mutant cells without EPI (yellow lines) treatment; while BmrA pumps of the former cells would be inhibited, the latter cells lacked the BmrA pump. Recorded intensities clearly revealed that initial EtBr accumulation to be comparable in these cells. However, with time, wild-type cells accumulated more EtBr, which strongly suggested that CM13 and CM311 inhibited not only

BmrA but also other efflux pumps slowing down EtBr discharge from the cells. We have further determined the MIC values as > 1000 μg/ml for both CM13 and CM311.

Fig. 4 summarizes the theoretical and the observed cases with these two molecules.

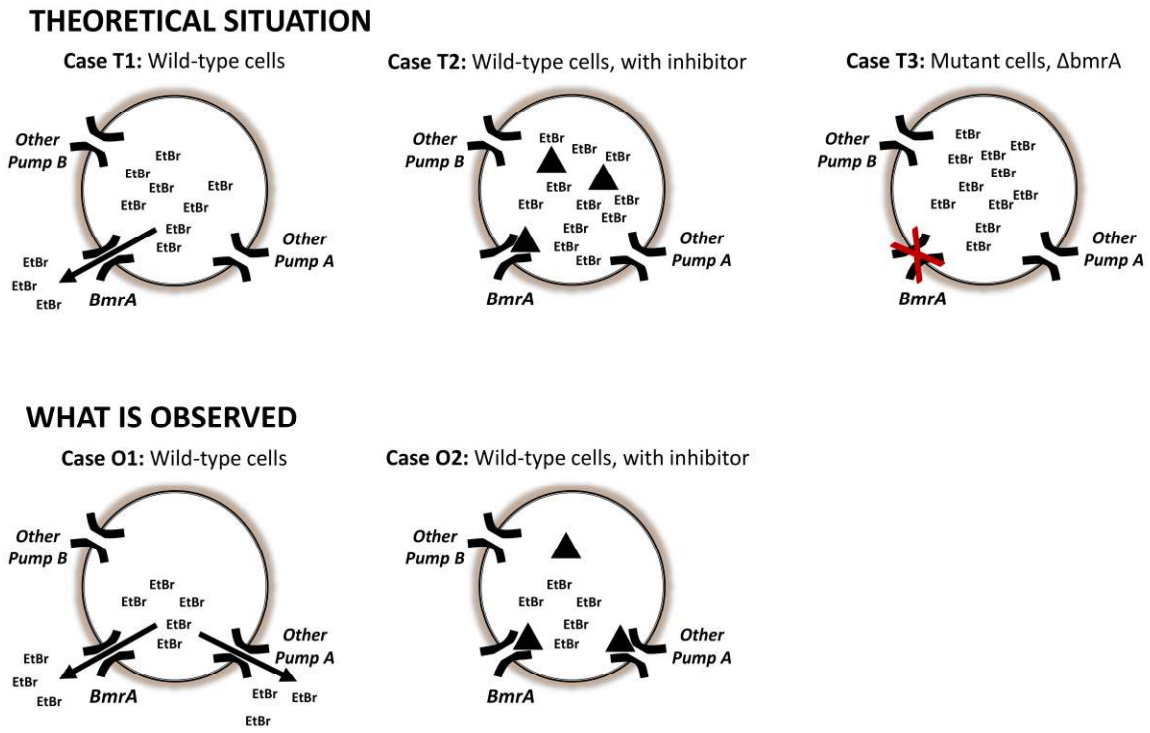


Figure 4. Summary of BmrA inhibition by CM13 and CM311. EtBr is effluxed through BmrA (Case T1). In the presence of the inhibitor (black triangle), EtBr accumulates in the cells (Case T2). Theoretically, this is identical to the mutant cells with impaired BmrA (Case T3). However, the experimental difference between Case T2 and Case T3 indicates that EtBr is effluxed by multiple pumps (Case O1) and the EPI not only binds BmrA but also other pumps that efflux EtBr.

4. Discussion

The critical role of bacterial efflux pumps in antibiotic resistance has directed research efforts into identifying novel EPIs that can be used alongside antibiotics in clinical settings [28,29]. EPIs have been identified against the multidrug resistance (MDR) efflux pumps of most of the clinically relevant and most

extensively studied pathogens. Reserpine and verapamil were among the first EPIs to be identified, yet too weak to be useful in clinical practice [30]. Later studies were focused on finding more potent and less toxic EPIs, and resulted in the identification of several EPIs targeting mostly MFS (NorA in *Staphylococcus aureus*) and RND transporters [31–38]. Nevertheless, none of the identified EPIs to date has reached the clinical development stage, either due to high toxicity at required concentrations, poor pharmacokinetics or low *in vivo* efficacy (for an overview of the current status of EPIs against clinically relevant pathogens, please refer to a recent review by Lamut *et al*. [39]).

The availability of crystal structures of MDR efflux pumps is crucial to obtain detailed information on the structural aspects of MDR efflux pump action and for the rational design and identification of more effective and potent inhibitors [40]. In the absence of crystal structures, homology modeling can also be used to obtain structural models [41]. Once a crystal structure or a homology model of the efflux pump of interest is available, computational biophysics methods including molecular docking and dynamics (MD) simulations can be used to identify novel inhibitors via virtual screening of compound databases in a similar fashion to our methodology here, and further investigate the inhibition mechanisms concerning conformational changes in the efflux pumps [31,42–51]. Most of the studies employing such methods to identify novel inhibitors have been conducted on RND and ABC transporters [52,53]. Among ABC transporters, the eukaryotic P-glycoprotein (a homolog of bacterial ABC transporters LmrA, and BmrA) was the focus of interest with respect to EPI screening due to its important role in cancer multidrug resistance [50,54–63].

ABC transporters function by using the energy deposited, namely ATP, to perform a cycle of transitions between inward- and outward-facing conformations [12,14,64–68]. This is termed the alternating access model. Here, the TMDs and NBDs are primarily responsible for substrate (i.e. ligands to be transported) binding and ATP hydrolysis, respectively. Both TMDs and NBDs of P-glycoprotein were targeted in previous virtual screening studies. Inhibitors may thus block substrate export by competing with the substrate for binding to the same site on the TMD or by binding to other site(s). They may also interfere with ATP hydrolysis by blocking ATP binding itself or preventing the occurrence of the hydrolysis reaction. On the other hand, some inhibitors were also predicted to bind to the TMD, yet prevent conformational transition by disrupting the signaling between NBDs and TMDs [69].

The spatial arrangement of the BmrA dimer determines the angle between the TMDs and this cavity angle is supposed to be important for the selection of transported drugs [70]. If the cavity is larger than that present in the actual structure, the selectivity would be very low in the selection of inhibitors. In that case, the cavity will accommodate a plethora of molecules that are not binders. Alternatively, if the cavity is too narrow, then only small molecules would fit into the cavity between the TMDs. In either case, it would be unlikely to get true hits. Since the BmrA structure was not available and that we had to model it, we suspected that the angle prediction between the TMDs could be inaccurate, therefore, we targeted here only the NBD to

identify inhibitors from the Chemotheca database. Nevertheless, the CHs that are responsible for transferring conformational changes from the NBDs to TMDs, were also included in the molecular docking search space. The two BmrA inhibitors identified here, CM13 and CM311, were predicted to dock into a pocket located at the interface between the NBD and CHs, and form non-bonded contacts with both functionally-important residues of the NBD (T381 of the Walker A motif and the catalytic glutamate E504) [71] as well as the CH residues A121. The inhibition mechanisms previously proposed for P-glycoprotein inhibitors described above may thus be valid for inhibition of BmrA by CM13 and CM311 as well. Accordingly, contacts with CH residues may directly prevent the transition from the inward- to outward-facing conformation by disrupting the connection between the NBDs and TMDs. Contacts with Walker A motif residues and the catalytic E504 may also exert effects similar to those of mutations introduced into these regions/positions of BmrA as previously shown [8,20,65,67,71–73], and prevent ATP hydrolysis.

Our *in vitro* experiments included monitoring of EtBr accumulation inside *B. subtilis* cells as an indicator of inhibition of the BmrA pump in the presence and absence of putative inhibitors. *B. subtilis* expresses a huge array of well-characterized efflux pumps [74]. Of these BmrA belongs to ABC, Bmr, Blt, Bmr3, Lmr3, and MdtP belong to MFS, and EbrAB belongs to SMR superfamilies. Despite the differences in their structures and mechanisms, they are usually not specific for a single substrate and share common substrates [75], e.g. EtBr has been reported to be a substrate for BmrA, Blt, Bmr, and EbrAB; thus, they presumably all contribute to its efflux [20,76–78]. The results obtained with EtBr accumulation assays support the fact that the identified molecules not only inhibit BmrA, but also one or more of those other pumps that are involved in EtBr efflux. This is not unexpected since it is a common feature of many inhibitor molecules. An example of this is encountered for *B. subtilis* with reserpine, a natural product that inhibits Bmr [79], Blt [77], and BmrA [20].

Furthermore, the experimental method used here is sufficient to demonstrate the inhibition, yet insufficient to provide details on the exact molecular mechanisms involved. The exclusion of the TMD from our virtual screening methodology does not also rule out an inhibition mechanism via the binding of these inhibitors to the TMD. To this end, further studies are necessary using experimental techniques with mutants of functionally-important NBD residues of BmrA and/or the characterization of BmrA in different conformational states.

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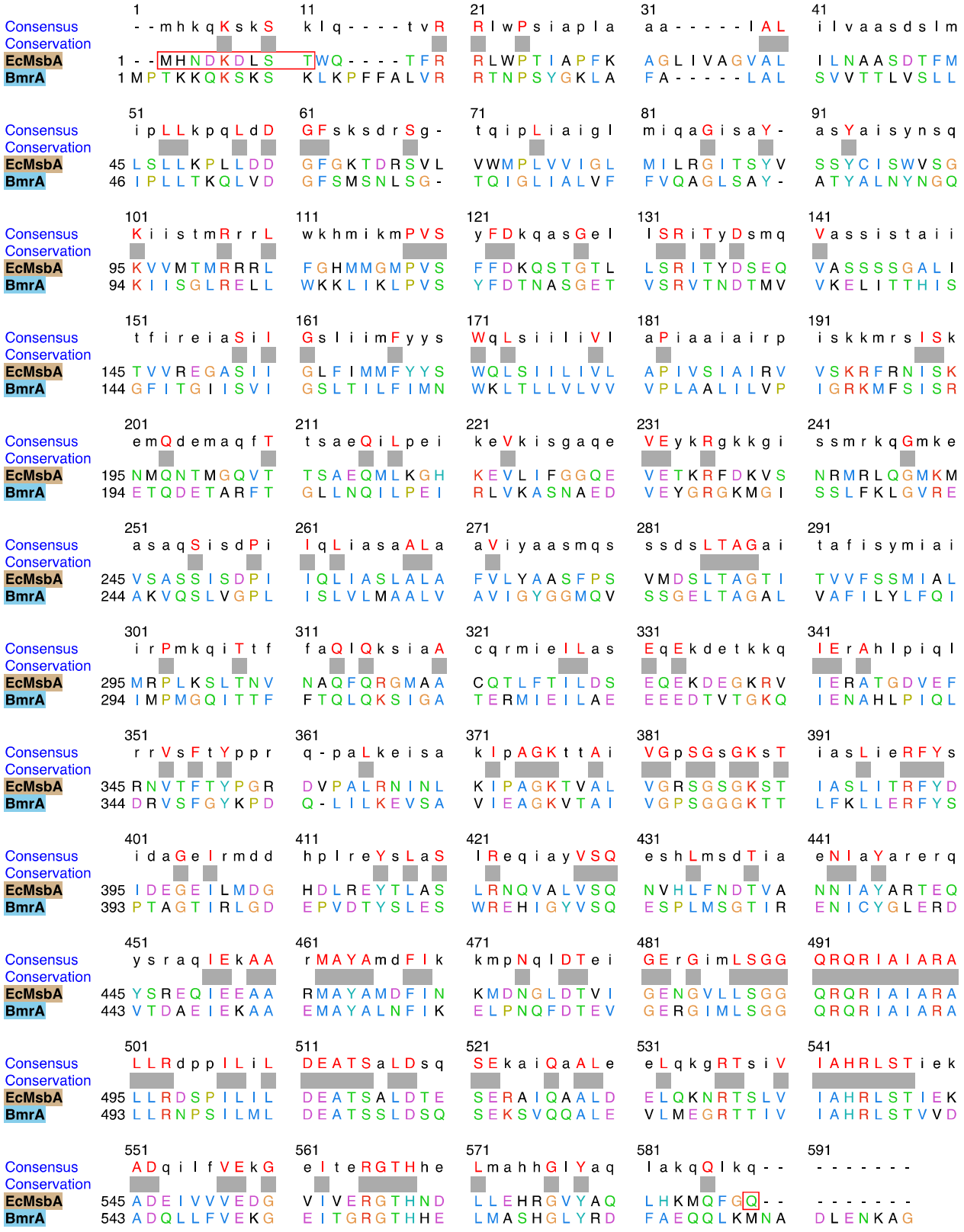

Figure S1. Pairwise sequence alignment between the template and the BmrA amino acid sequences

Figure1

Click here to access/download;Figure;Fig1.tiff

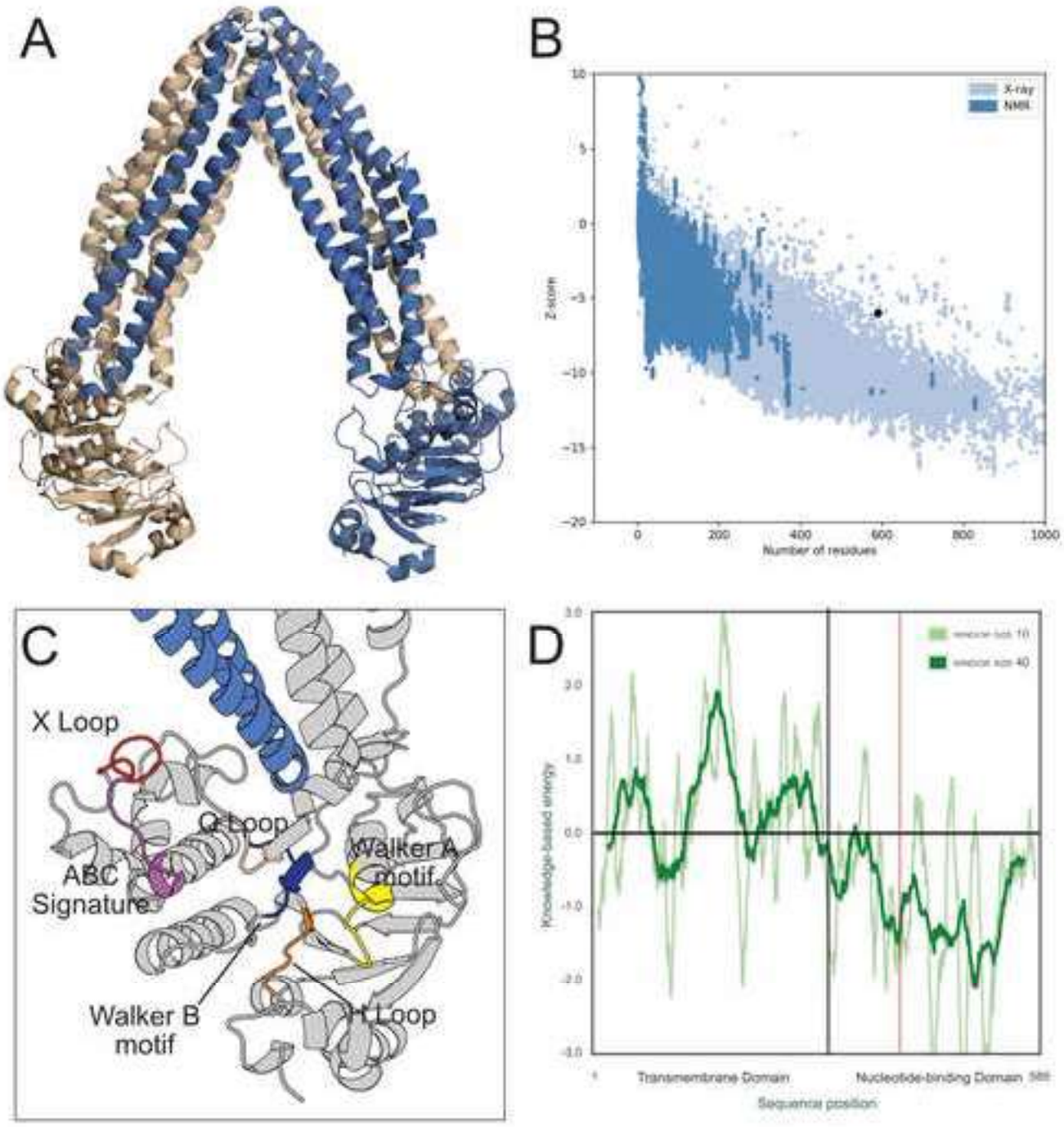

Figure2

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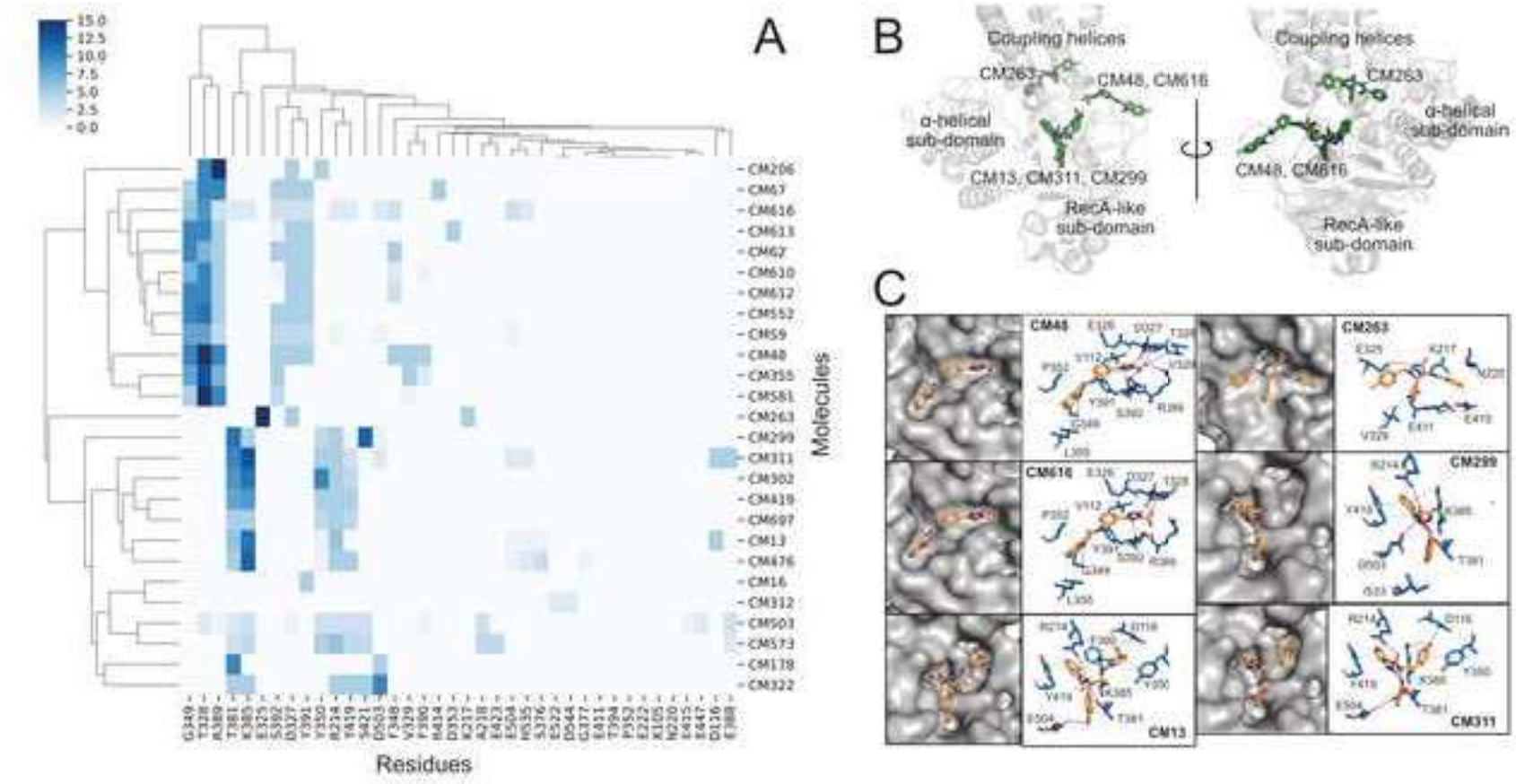

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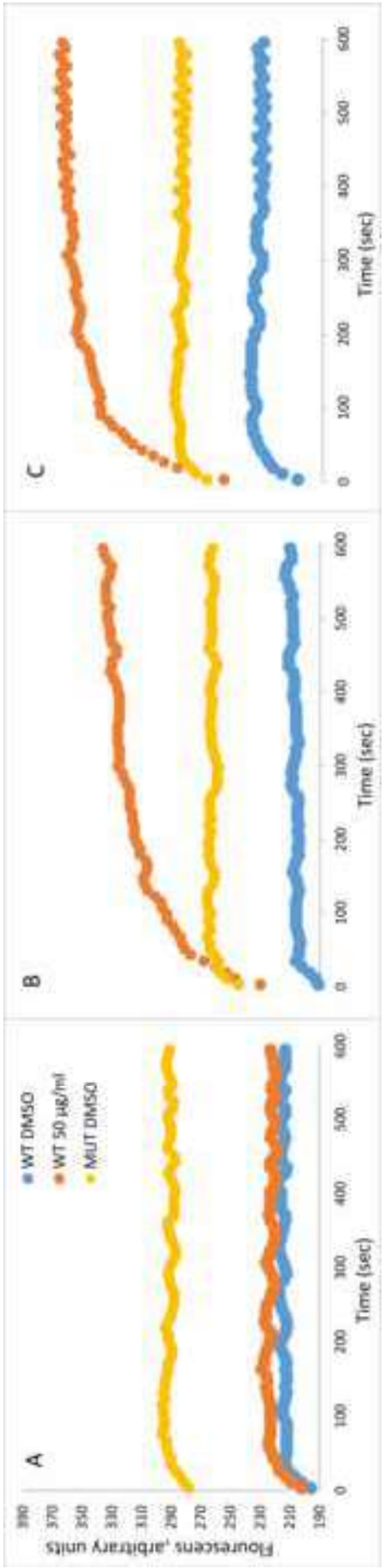

Figure3

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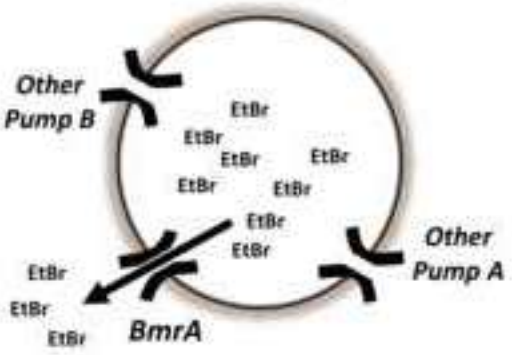
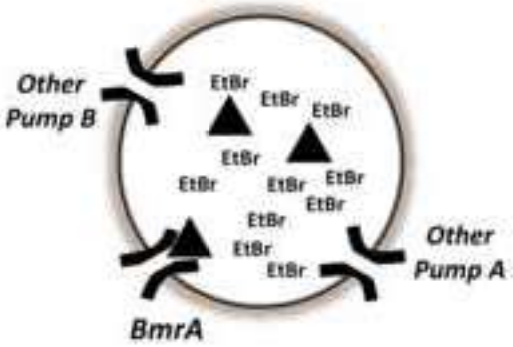
THEORETICAL SITUATION

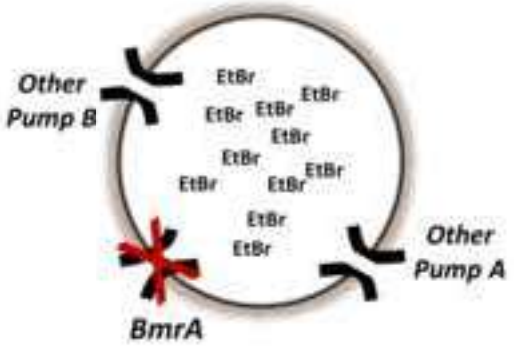
Case T1: Wild-type cells



Case T2: Wild-type cells, with inhibitor

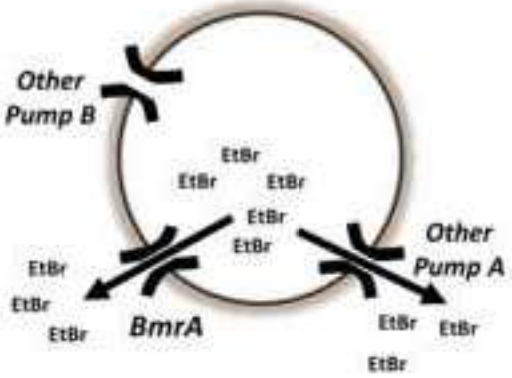


Case T3: Mutant cells, ΔbmrA

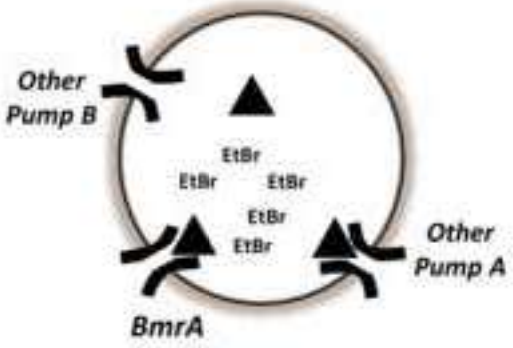


WHAT IS OBSERVED

Case O1: Wild-type cells



Case O2: Wild-type cells, with inhibitor



Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

FigureS1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Supplementary Material
SuppFig1.eps