Structure-based design of novel selective cell wall biosynthesis inhibitors in Penicillin-resistant bacteria

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Introduction

The spreading of antibiotic resistance by bacterial pathogens accounts for a main drawback in the therapeutic treatment of infections. β-lactam antibiotics mimic the natural peptidoglycan substrate of Penicillin-binding proteins (PBPs), which confer stability to the bacterial cell wall and allow for cell viability and growth. To struggle for pathogen's survival, specific hydrolysis of β-lactam rings by widely-spread β-lactamases and key mutations in the active site of PBPs have emerged as main resistance strategies.[1,2]
Herein, we have performed a multiple-target high-throughput virtual screening experiment on several specific PBPs and β-lactamases expressed in *Staphylococcus aureus* (MRSA), a dangerous Penicillin- and Methicillin-resistant bacterial strain.[3] The analysis of the docking profile allows for the identification of chemical scaffolds that are not vulnerable to the β-lactamases and have a high affinity to the target PBPs.

Methods

The structures of PBP2, PBP2a, PBP4, and a class B (Zinc-dependent) and class C serine cephalosporin β-lactamases from the MRSA252 strain were derived from homology modeling using wild-type crystals of the enzymes.[4] These structures were subjected to a multiple-target high-throughput docking experiment using the docking software Glide 5.6, Schrödinger Inc. Around 9000 β-lactam containing compounds were screened with the XP Glide scoring level (H-bonds between the catalytic serine or the zinc cation and the carbonylic oxygen in the β-lactam ring were set as constraints).

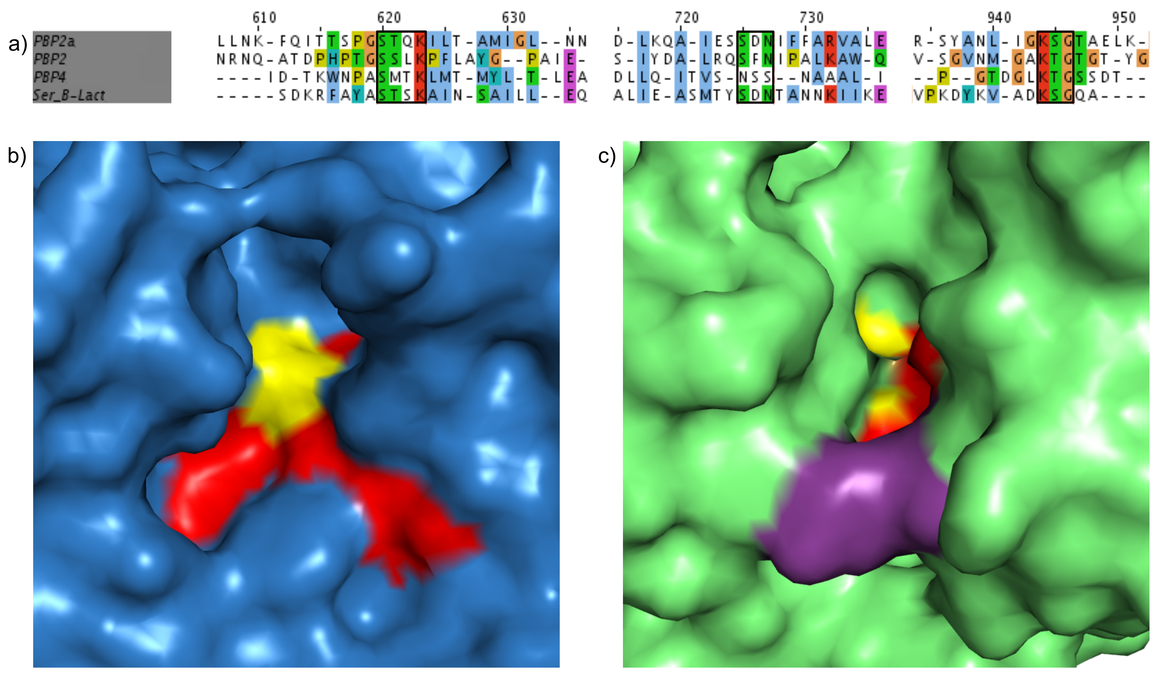
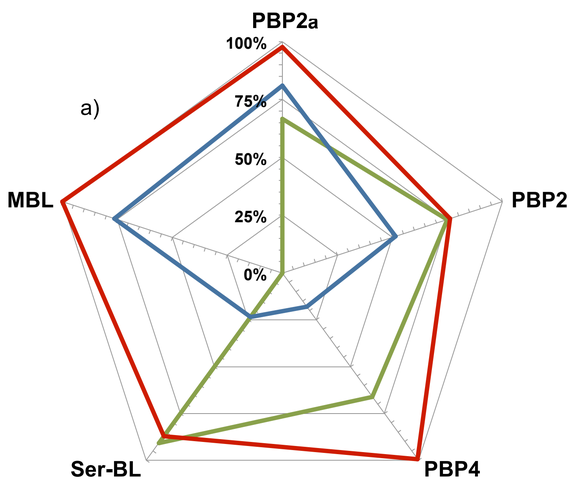


Fig. 1: a) Structure-based multiple-sequence alignment for the serine-dependent enzymes analyzed in this project. Three conserved motifs present in the active site (SXXK, SXN and KTG) are shown.[2] Overall sequence identity between the enzymes is below 20%.
b) and c) Surface representation of the binding pocket of PBP4 and PBP2a, in blue and lime. The location of the conserved motifs from a) and the catalytic serine residue are colored in red and yellow, respectively. In PBP2a, the serine is located deeper in the pocket and Tyr446 (in purple) encloses the cavity, thus reducing its affinity to some known antibiotics such as Methicillin.

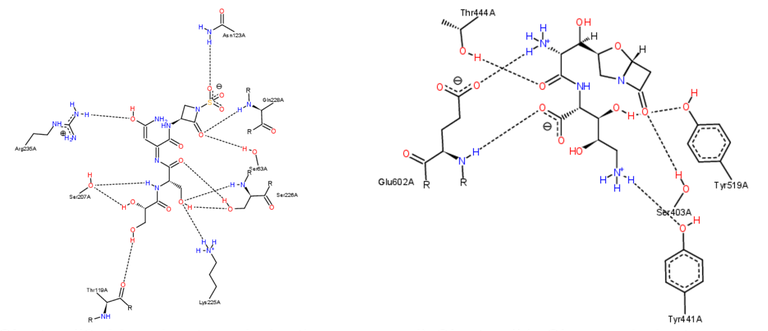
Results

Top-ranked compounds obtained from the HTVS experiment were selected and clustered by diversity. For each cluster, a binding profile was derived by comparison of its absolute ranking among the five enzymes.



b)

	PBP2a	PBP2	PBP4	Ser-BL	MBL	Mean
Methicillin	0.00	0.62	0.41	0.88	0.00	0.38
#1	0.00	0.69	1.00	0.98	0.82	0.70
#2	0.00	1.00	0.97	0.93	0.00	0.58
#3	0.00	0.98	0.98	1.00	1.00	0.79
#4	0.98	0.76	0.99	0.87	1.00	0.92
#5	0.69	0.58	0.28	0.40	0.75	0.54
#6	0.67	0.75	0.66	0.91	0.00	0.60
#7	0.90	0.91	0.87	0.68	0.92	0.86
#8	0.64	0.20	0.43	0.51	0.56	0.47
#9	0.81	0.51	0.18	0.23	0.76	0.50
#10	0.76	0.74	0.22	0.37	0.58	0.53
#11	0.79	0.57	0.51	0.83	0.67	0.67



c) Cluster #2: Monobactam derivatives
d) Cluster #4: Clavam derivatives

The resistance of MRSA arises from the expression of PBP2a that has a low affinity to Methicillin and many other tested scaffolds indicated by red colored squares in the heat map. Methicillin is also an average binder to PBP2 and a weak binder to PBP4. Furthermore, the green square of Ser-BL indicates a strong interaction between Methicillin and this metabolizing β-lactamase. From the specific binding profiles diverse additional selectivity patterns can be derived.

Fig. 2: a) Radar representation of the binding profile of 3 different chemical scaffolds obtained by clustering of the HTVS results (#4, #6, and #9 in red, green, and blue). *Binding profile* defined as the ratio between the rank of the best-scored member of a cluster and the total number of compounds kept after docking for the each receptor. Diverse selectivity patterns are observed.
b) Heat map representation of the binding profile of methicillin and 11 cluster-representatives. The mean value is shown in the most-to-right column. Top- to bottom-ranked best-scored member of each cluster colored by a green to red gradient.
c) and d) Poseview [5] representation of the binding mode of a monobactam derivative (cluster #2) and clavamycin (cluster #4) in the active site of PBP2a and the class C β-lactamase, respectively. The hydrogen-bond acceptor interaction of the carbonylic oxygen of the β-lactam ring and the catalytic serine was set as docking constraint.

Conclusions

The multiple-enzyme docking experiment in MRSA yielded chemical scaffolds selective for the different targets. We have been able to reproduce the resistance to Methicillin by the model, and identify diverse selectivity patterns for the receptors studied herein. The resulting predictions will be validated *in vitro* to assess the capability of the docking protocol to predict selectivity of β-lactam containing compounds among proteins targeted by antibiotics.

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