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# Elucidating efflux inhibition and avoidance in Pseudomonas aeruginosa

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**Summary.** — Antibiotic resistance is a major threat to public health. Gram-negative pathogens, such as *Pseudomonas aeruginosa*, are of particular concern. One of the first bacterial defence mechanisms is the active export of drugs out of the cell, mainly mediated by resistance-nodulation-division (RND) efflux pumps. The major RND efflux pump of *P. aeruginosa* is MexAB-OprM, in which the inner membrane transporter MexB is responsible for the recognition and binding of compounds. Due to the difficulty of producing co-crystals, computational methods are crucial to gaining insights into the interactions between compounds and MexB. We exploited multi-copy molecular dynamics simulations to investigate the binding of peptidomimetics compounds to MexB. Based on microbiology studies, this series was shown to include efflux substrates, inhibitors, and avoiders. The detailed analysis of protein-ligand interactions (both direct and water-mediated) revealed characteristic patterns for each class of compounds, highlighting significant differences. Our results outline molecular-level information that could help the rational design of new inhibitors and new antibiotics less susceptible to the efflux mechanism.

## 1. – Introduction

*Pseudomonas aeruginosa* is an opportunistic Gram-negative pathogen responsible for infections associated with high morbidity and mortality rates [1]. The lack of effective antimicrobials against this bacterium arises from different resistance mechanisms, among which the action of efflux pumps represents one of the major contributors [2, 3]. The tripartite system MexAB-OprM, belonging to the Resistance Nodulation cell Division (RND) superfamily, plays the leading role in translocating a plethora of different antimicrobial compounds outside the cell (*i.e.*, substrates) [4]. The homotrimer protein MexB

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is the RND transporter responsible for binding, recognition and the first step of extrusion of substrates [5]. The protomers can cyclically assume three different and subsequent states, namely Loose (L), Tight (T) and Open (O). Based on the available structural data of these transporters, two main binding pockets named Access Pocket ($AP_L$) and Distal Pocket ($DP_T$) have been identified in the L and T states, respectively [6]. Consistent efforts have been made to identify new chemical classes of molecules able to either avoid efflux (*i.e.*, avoiders) or inhibit the RND transporter (*i.e.*, inhibitor), leading to effective accumulation of antibiotics inside the cell [7]. In a previous work [8], 260 peptidomimetics (Rempex compounds) were characterized through biochemical analyses and classified into three groups: substrates (SUBs, 136), inhibitors (EPIs, 24), and avoiders (AVDs, 66). Predictors of efflux avoidance and inhibition were identified through machine-learning models fed by physical-chemical properties of the compounds and descriptors of interaction with MexB extracted from molecular docking. However, a fully atomistic, dynamical view of the binding of Rempex compounds to MexB, including the role played by water molecules in mediating protein-ligand interactions [9], was not directly considered. Here, to complement this previous investigation, we performed multi-copy molecular dynamics (MD) simulations of three representatives Rempex compounds belonging to different classes, that were selected according to biochemical and microbiology studies. The detailed analysis of the simulations allowed us to identify predictive patterns of recognition and avoidance, and specific MexB residues/regions important for inhibition or translocation. Furthermore, the role of solvent water molecules in binding and inhibition processes was explicitly investigated.

## 2. – Results and discussion

**2˙1.** *Substrates and inhibitors engage more MexB residues than efflux avoiders.* – Starting from the binding modes predicted at both $AP_L$ and $DP_T$ through molecular docking, we performed all-atom MD simulations for the representative Rempex compounds (*i.e.*, SUB58, EPI18, and AVD108). For each ligand-protein complex we performed ten MD replicas of 100 ns (1 $\mu$s total sampling for each pose). Our primary aim was to infer any potential term of diversification between the three Rempex classes. Note that, consistently with its nature, the avoider AVD108 was simulated only in the $AP_L$. We investigated the persistence of direct interactions of each compound with MexB residues, by monitoring the contacts along the MD trajectories (cutoff distance 3.0 Å). The average persistence of direct interactions is reported in fig. 1. Overall, we found that SUB58 interacted with the largest amount of protein residues (especially at the $AP_L$) as compared to the other compounds. Conversely, as expected, AVD108 showed the lowest values of persistence. Interestingly, some of the residues appear to interact with all compounds (although to a different extent), while others seem unique for each class.

Figure 1(A) shows that all compounds interact with D566 with percentages 100%, 46%, and 47%, for SUB58, EPI18, and AVD108, respectively. Noteworthy, the interaction between D566 and SUB58 is constantly maintained for all the trajectories. Differently, unique interactions for SUB58 were found for instance with E567, D568 and S868, for EPI18 with F563 and E865. Similarly, as shown in fig. 1(B), at the $DP_T$ all compounds interacted with E81, D681 and E825, while unique interactions were found for SUB58 with T91 and L682.

**2˙2.** *Water-mediated hydrogen bonding remarkably contributes to the interaction with MexB.* – To quantify the role of the solvent and pinpoint specific protein residues

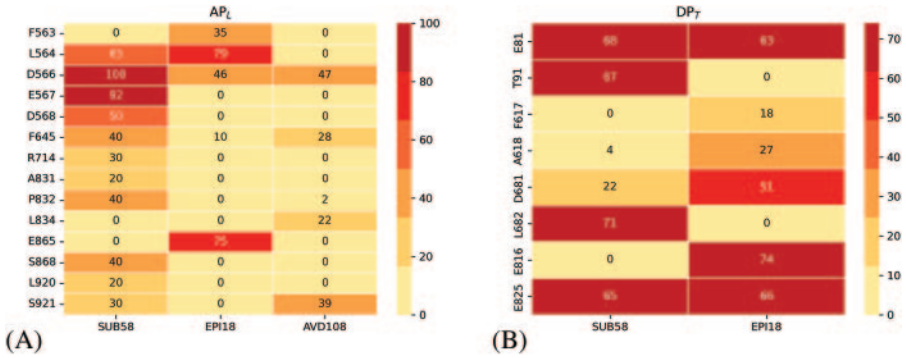


Fig. 1. – Mean persistence of interaction (%) between MexB residues and the Rempex compounds, recorded in the (A) $AP_L$ and (B) $DP_T$. We report only residues for which percentages greater than 12% were registered for at least one compound.

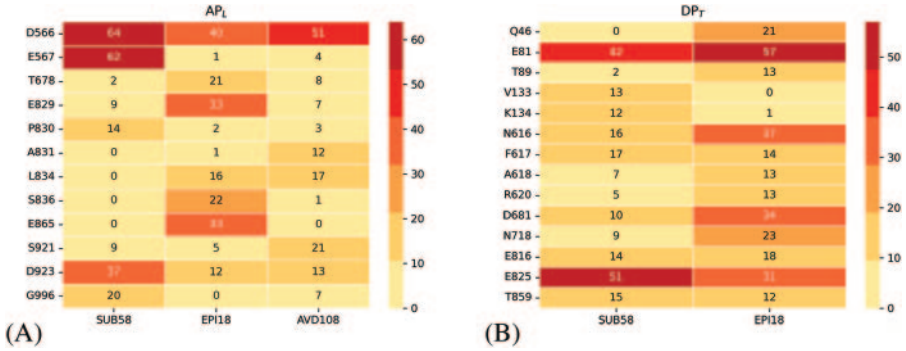


Fig. 2. – Mean values (%) of the persistence of hydrogen bond bridges mediated by water molecules during the MD trajectories, between Rempex compounds and MexB residues, at the (A) $AP_L$ and (B) $DP_T$. We report only residues for which percentages greater than 12% were registered for at least one compound.

interacting indirectly with compounds, we computed the persistence of hydrogen bond bridges mediated by water molecules during the MD runs. As shown in fig. 2 H-bridges mediated by water molecules appear to remarkably contribute to the interaction with MexB.

Overall, by combining the results from the analysis of contacts and water-mediated H-bridges, we identified MexB residues interacting with the compounds both directly and indirectly.

## 3. – Conclusions

The detailed analysis of MD trajectories revealed profound differences. SUB58 and AVD108 engage the highest and the lowest number of interactions with MexB residues, respectively, consistently with experimental data [8]. Furthermore, we found specific protein residues that appear to interact with all compounds and others that are specific for the different Rempex classes. The detailed analysis of water-mediated interactions

suggests a prominent role of the solvent in all compounds but AVD108. Taken together, the computational results suggest that the $AP_L$ and $DP_T$ of MexB feature multiple hot spots important for the binding of the different Rempex classes. Work is underway to correlate the above findings with experimental biochemical data.

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REFERENCES

[1] Murray C. J. L. *et al.*, *Lancet*, **399** (2022) 629.
[2] Alav I., Kobylka J., Kuth M. S., Pos K. M., Picard M., Blair J. M. A. and Bavro V. N., *Chem. Rev.*, **121** (2021) 5479.
[3] Klenotic P. A., Moseng M. A., Morgan C. E. and Yu E. W., *Chem. Rev.*, **121** (2021) 5378.
[4] Zgurskaya H. I., Malloci G., Chandar B., Vargiu A. V. and Ruggerone P., *Curr. Opin. Microbiol.*, **61** (2021) 115.
[5] Sakurai K., Yamasaki S., Nakao K., Nishino K., Yamaguchi A. and Nakashima R., *Sci. Rep.*, **9** (2019) 4359.
[6] Glavier M., Puvanendran D., Salvador D., Decossas M., Phan G., Garnier C., Frezza E., Cece Q., Schoehn G., Picard M., Taveau J. C., Daury L., Broutin I. and Lambert O., *Nat. Commun.*, **11** (2020) 4948.
[7] Zgurskaya H. I., Walker J. K., Parks J. M. and Rybenkov V. V., *Acc. Chem. Res.*, **54** (2021) 930.
[8] Mehla J., Malloci G., Mansbach R., López C. A., Tsivkovski R., Haynes K., Leus I. V., Grindstaff S. B., Cascella R. H., D'Cunha N., Herndon L., Hengartner N. W., Margiotta E., Atzori A., Vargiu A. V., Manrique P. D., Walker J. K., Lomovskaya O., Ruggerone P., Gnanakaran S., Rybenkov V. V. and Zgurskaya H. I., *MBio*, **12** (2021) e02785.
[9] Vargiu A. V., Ramaswamy V. K., Malvacio I., Malloci G., Kleinekathöfer U. and Ruggerone P., *Biochim. Biophys. Acta - Gen. Subj.*, **1862** (2018) 836.