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P24 AI and AutoDock to Study a Novel Halogenase JDH3

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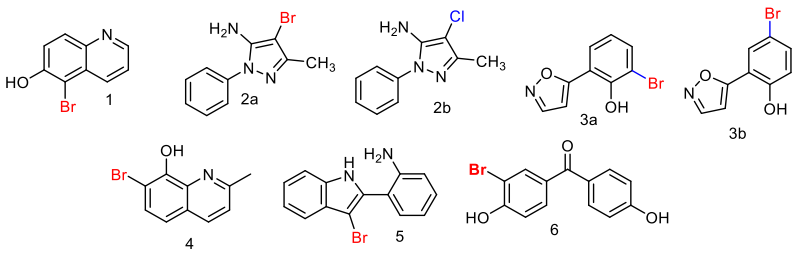
Many organic compounds with pharmaceutical applications or intermediates for their synthesis are halogenated with chlorine gas. In this case, a mixture of halogenation products is usually formed. VirX1 and other FAD-dependent Halogenases catalyse regioselective halogenation reactions under more favourable conditions compared to chlorine gas, which has a positive effect not only on the directionality and yield reaction, but also on the pricing policy of the final product and the environmental safety of the modern synthesis.

The structure of the VirX1 enzyme and its mechanism of action was studied by members of the Goss Lab.[1–3] However, there is a large variety of halogenases of which the spatial structure, their substrate and regioselectivity have not been discovered yet. That opens up a lot of opportunities for studying possible biotransformations.

JDH3 structure was predicted with AlphaFold and Phyre2. Comparison of the two models revealed differences in the structure of the internal pocket. The Phyre2 model demonstrated a wider and shorter pocket, then AlphaFold (size 62 × 71 × 85; volume 374170 Å^3 and size 60 × 72 × 94; volume 4060808 Å^3; relatively). The last model was chosen for the next research.

Active site with catalytic LYS was recognized, a map of the possible binding site was created using AutoSite v1.0. Presumably the binding site is located in the depth of the cavity containing the second WxWxIP and third FxxPxxSxG motifs. Flexible docking with grid box with size 22 × 22 × 24 was carried out with AutoDock (total number of torsions: 28).



Flexible docking allowed more accurate predict of the regioselectivity. So, for compounds **1**–**2** and **4**–**6** the only possible product of halogenation was predicted, while for structure 3 two possible regioisomers were predicted. In **3a** and **3b** cases, binding energy was –5.5 kcal/mol with distance to catalytic LYS 8.3 Å. This result was confirmed experimentally.

In all cases, the substrates were located into the depth of the inner cavity, relative potential binding site and interacted with it mainly hydrophobically with amino acid residues TYR and TRP (second motif), as well as PHE, PRO (third motif). In most cases, ILE (second motif) also interacted hydrophobically with the substrates. Hydroxyl group of the compounds **1**,**3**,**4** and **6** was stabilized by hydrogen bonds with SER of the third motif, which played a key role in compound orientation relative catalytic residue. Overall, second and third motifs promoted the specific orientation of the substrate relative to the catalytic residue. It can be stated with some certainty that the JDH3 binding site consists of the above listed residues.

To sum up, all docking results were confirmed experimentally, which indicates the correctness of the working model and the possibility of in-depth study of the binding site structure, the substrate and regioselectivity.

[1] D. S. Gkotsi, H. Ludewig, S. V. Sharma, J. A. Connolly, J. Dhaliwal, Y. Wang, W. P. Unsworth, R. J. K. Taylor, M. M. W. McLachlan, S. Shanahan, J. H. Naismith and R. J. M. Goss, *Nat. Chem.*, **2019**, *11*, 1091–1097.
[2] H. Ludewig, S. Molyneux, S. Ferrinho, K. Guo, R. Lynch, D. S. Gkotsi and R. J. M. Goss, *Curr. Opin. Struct. Biol.*, **2020**, *65*, 51–60.
[3] C. Crowe, S. Molyneux, S. V. Sharma, Y. Zhang, D. S. Gkotsi, H. Connaris and R. J. M. Goss, *Chem. Soc. Rev.*, **2021**, *50*, 9443–9481.