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Discussion

Comment on "Another look at the molecular mechanism of the resistance of H5N1 influenza A virus neuraminidase (NA) to oseltamivir (OTV)"

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Using the molecular docking method, detailed information of the oseltamivir-resistance in the avian influenza H5N1 virus caused by different substitutions in the neuraminidase subtype N1 residue H274 were revealed in a recent paper in this journal [1]. The ligand, oseltamivir, was docked into the active site of the wild-type strain and mutant N1 models in which the residue H274 of the wild-type crystal structure was replaced by various smaller (Gly, Ser, Asn and Gln) and larger (Phe and Tyr) residues. Although, the calculated free energies of oseltamivir binding to the wild-type and mutants derived in this study were consistent with the experimentally observed K_i values [2], the orientations of the docked oseltamivir were found to lie incorrectly in the active site of the enzyme and consequently its intermolecular interactions with the key binding residues were lost. This oversight is likely to simply be due to the use of improper crystal structure to create the six mutant complexes, *i.e.,* an open conformation found in wild-type N1 (Fig. 1, yellow), where the 150-loop was crystallographically found to form a large cavity (see 150-cavity shaded by grey in Fig. 1) adjacent to the active site, was used instead of the closed conformation of wild-type (cyan) [3]. In addition, the two recent crystal structures (see Fig. 1) of H274Y (pink) and N294S (green) mutants with oseltamivir bound are shown in the closed conformation [4].

Discrepancy was critically observed for the H274Y modeled system, where the orientation of oseltamivir in the initial (X-ray, open form) and final (docking) structures are totally different, *i.e.,* relative to the X-ray complex, the docked conformation of oseltamivir was rotated by almost 90° and flipped in horizontal axis (Figs. 4a and 4b of the paper [1]). This leads to the disappearance of the intermolecular interactions with its binding residues. Whilst those docking results were properly presented, whether the crystal structure of the H274Y N1 mutant (closed form displayed by pink in Fig. 1) [4] was served as the target receptor (Fig. 5 of the paper [1]). The use of inappropriate (open) conformation is a clear reason why particular interactions of oseltamivir with the neighboring residues were completely lost.

One of the other obvious examples is the loss of interaction between oseltamivir and R152 in the wild-type system, but instead it strongly interacted to R156 (Fig. 1a of the paper [1]). Note that R152 is a key residue, known to bind to the –NHAc group of substrate and inhibitors in all neuraminidase subtypes [3,5].

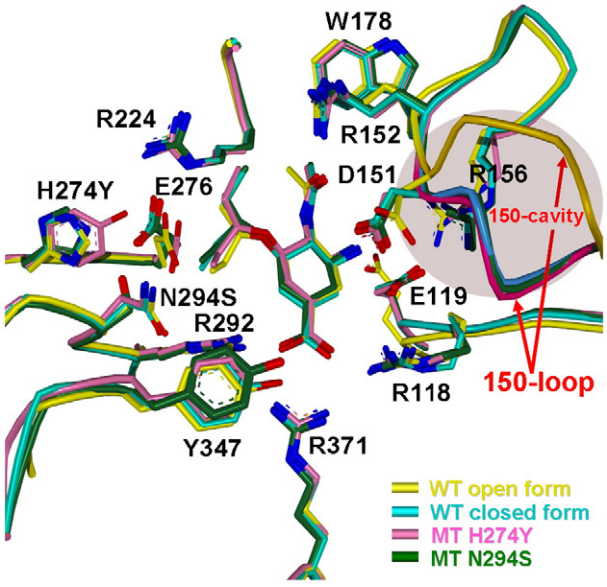

Fig. 1. Superimposition of the four co-crystallographic structures of the N1-oseltamivir complexes [3,4]: two wild-types in open (PDB code: 2HU0, yellow) and closed (2HU4, cyan) conformations; and two mutants, H274Y (3CL0, pink) and N294S (3CL2, green) where the active site residues of N1, the 150-loop (darker colored tubes) and its cavity were displayed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Taking into account all of the comments given above, the docking data reported by Mihajlovic and Mitrasinovic cannot be used to explain the mutation effect at the H274 position in neuraminidase due to the use of an improper initial structure. The closed conformation of the co-crystal structure of N1-oseltamivir complex, which is more likely to be presented in the inhibited enzyme, is the more appropriate conformation to be used for such studies.

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