Simulated Docking of Zanamivir with the 2009 Pandemic Strain Influenza A/H1N1 Neuraminidase Active Site

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Abstract

Influenza neuraminidases are glycoproteins that facilitate the transmission of the influenza virus from cell to cell. Zanamivir is a widely used neuraminidase inhibitor. Here I provide a computational docking analysis of zanamivir with the active site of the neuraminidase of the 2009 Influenza A/H1N1 strain. The computed inhibitor/receptor binding energy suggests that zanamivir would be only marginally effective against that strain.

Keywords: Influenza, H1N1, neuraminidase, zanamivir

1.0 Introduction

Influenza neuraminidases are glycoproteins that facilitate the transmission of the influenza virus from cell to cell. Zanamivir (5-(acetylamino)-2,6-anhydro-3,4,5-trideoxy-4-[(diaminomethylidene)amino]-D-glycero-D-galacto-non-2-enonic acid (4S,5R,6R)-5-acetamido-4-(diaminomethylideneamino)-6-[(1R,2R)-1,2,3-trihydroxypropyl]-5,6-dihydro-4H-pyran-2-carboxylic acid; [10]) is a widely used influenza therapeutic.

In the World Health Organization serotype-based influenza taxonomy, influenza type A has nine neuraminidase-related sero-subtypes, and these subtypes correspond at least roughly to differences in the active-site structures of the flu neuraminidases. The subtypes fall into two groups ([3]): group-1 contains the subtypes N1, N4, N5 and N8; group-2 contains the subtypes N2, N3, N6, N7 and N9. Zanamivir was designed to target the group-2 neuraminidases.

The available crystal structures of the group-1 N1, N4 and N8 neuraminidases ([1]) reveal that the active sites of these enzymes have a very different three-dimensional structure from that of group-2 enzymes. The differences lie in a loop of amino acids known as the "150-loop", which in the group-1 neuraminidases has a conformation that opens a cavity not present in the group-2 neuraminidases. The 150-loop contains an amino acid designated Asp 151; the side chain of this amino acid has a carboxylic acid that, in group-1 enzymes, points away from the active site as a
result of the 'open' conformation of the 150-loop. The side chain of another active-site amino acid, Glu 119, also has a different conformation in group-1 enzymes compared with the group-2 neuraminidases (8)).

The Asp 151 and Glu 119 amino-acid side chains form critical interactions with neuraminidase inhibitors. For neuraminidase subtypes with the “open conformation” 150-loop, the side chains of these amino acids might not have the precise alignment required to bind inhibitors tightly ([8]). The active site of the 1918 H1N1 strain has the 150-loop configuration.

The difference in the active-site conformations of the two groups of neuraminidases may also be caused by differences in amino acids that lie outside the active site. This means that an enzyme inhibitor for one target will not necessarily have the same activity against another with the same active-site amino acids and the same overall three-dimensional structure.

Crystallized Influenza A/California/04/2009(H1N1) neuraminidase is an atypical group 1 NA with some group 2-like features in its active site (lack of a 150-cavity) ([4]).

2.0 Method

The general objective of this study is straightforward: to computationally assess the binding energy of the active site of crystallized A/California/04/2009(H1N1) neuraminidase with zanamivir. Unless otherwise noted, all processing described in this section was performed on a Dell Inspiron 545 with an Intel Core2 Quad CPU Q8200 (clocked @ 2.33 GHz) and 8.00 GB RAM, running under the Windows Vista Home Premium (SP2) operating environment.

Protein Data Bank (PDB) 3TI3 ([6]) is a structural description of most of the crystallized neuraminidase of Influenza A/H1N1 3TI3 consists of two identical chains, designated Chain A and Chain B.

3TI3 was downloaded from PDB on 22 February 2011. A PDB description of zanamivir was extracted from PDB 3B7E ([10]) using AutoDock Tools v 4.2 (ADT, [9]). ADT was then used to perform the docking of zanamivir to the receptor. More specifically, in ADT, approximately following the rubric documented in [12]
-- Chain B, and the water in Chain A, of 3TI3 were deleted

-- Chain A's active-site was extracted. (3TI3 identifies the active site of Chain A as 15 amides: ARG118, GLU119, ASP151, ARG152, ARG156, TRP178, ARG224, GLU227, SER246, GLU276, GLU277, ARG292, ASN294, ARG371, and TYR406.)

-- the hydrogens, charges, and torsions in the ligand and active site were adjusted using the ADT-recommended defaults

and finally, the ligand, assumed to be flexible wherever that assumption is physically possible, was auto-docked to the active site, assumed to be rigid, using the Lamarckian genetic algorithm implemented in ADT. The best-fit (lowest-energy) configuration from the analysis was saved, and the distances between the receptor and ligand in 3TI3, and those computed here, were compared.

The ADT parameters for the docking are shown in Figure 1. Most values are, or are a consequence of, ADT defaults.

```
autodock_parameter_version 4.2       # used by autodock to validate parameter set
outlev 1                             # diagnostic output level
intelec                              # calculate internal electrostatics
seed pid time                        # seeds for random generator
ligand_types C HD OA N               # atoms types in ligand
fld 3TI3_active.maps.fld             # grid_data_file
map 3TI3_active.C.map
map 3TI3_active.HD.map
map 3TI3_active.OA.map
map 3TI3_active.N.map
elecmap 3TI3_active.e.map
desolvmap 3TI3_active.d.map
move zanamivir.pdbqt                 # small molecule
about -29.5772 12.7517 -20.6465      # small molecule center
tran0 random                         # initial coordinates/A or random
axisangle0 random                    # initial orientation
dihe0 random                         # initial dihedrals (relative) or random
tstep 2.0                            # translation step/A
qstep 50.0                           # quaternion step/deg
dstep 50.0                           # torsion step/deg
torsdof 9                            # torsional degrees of freedom
rmstol 2.0                           # cluster_tolerance/A
extrng 1000.0                        # external grid energy
e0max 0.0 10000                      # max initial energy; max number of retries
ga_pop_size 150                      # number of individuals in population
ga_num_evals 2500000                 # maximum number of energy evaluations
ga_num_generations 27000            # maximum number of generations
ga_elitism 1                         # number of top individuals to survive to next
generation
```
3.0 Results

The interactive problem setup, which assumes familiarity with the general neuraminidase "landscape", took about 20 minutes in ADT; the docking proper, about 28 minutes on the platform described in Section 2.0 The platform's performance monitor suggested that the calculation was more or less uniformly distributed across the four processors at ~25% of peak per processor (with occasional bursts to 40% of peak), and required a constant 2.9 GB of memory.

Figure 2 shows the best-fit zanamivir/receptor energy and position summary produced by ADT under the setup shown in Figure 1. The estimated free energy of binding under these conditions is ~ -8.7 kcal/mol; the estimated inhibition constant, ~408 nanoMolar at 298 K.
Table 1: ADT's zanamivir energy and position predictions.

Figure 2. ADT's zanamivir energy and position predictions.

Figure 3 is a rendering of the active-site/inhibitor configuration computed in this study.
Figure 3. Rendering of zanamivir computationally docked with the active site of PDB 3TI3. The molecular surface of the receptor is shown in white; the inhibitor, in stick form in green. Only the interior, inhibitor-containing region of the molecular surface of the active site can be compared to in situ data: the surface distal to the interior is a computational artifact, generated by the assumption that active site is detached from the rest of the receptor.

The distances between ligand and receptor atoms in 3TI3, and the corresponding distances in the present computation were within 10% of each other.

4.0 Discussion

The method described in Section 2.0 and the results of Section 3.0 motivate several observations:

1. The inhibition constant computed in this study (~408 nanoMolar at ~298 K) is comparable inhibition constant of neuraminidase inhibitors that are not clinically effective ([10], [11], [13], [14], [15]) against several H1N1 genotypes. This suggests that zanamivir would be only marginally effective against Influenza A/California/04/2009(H1N1)). It would, however, be more effective than oseltamivir (Tamiflu®) against that strain.
2. The docking study reported here assumes that the receptor is rigid. This assumption is appropriate for the binding energy computation for PDB 3TI3 per se. However, the calculation does not reflect what receptor "flexing" could contribute to the interaction of the ligand with native unliganded receptor.

3. The analysis described in Sections 2.0 and 3.0 assumes receptor is in a crystallized form. In situ, at physiologically normal temperatures (~310 K), the receptor is not in crystallized form. The ligand/receptor conformation in situ, therefore, may not be identical to their conformation in the crystallized form.

4. Minimum-energy search algorithms other than the Lamarckian genetic algorithm used in this work could be applied to this docking problem. Future work will use Monte Carlo/simulated annealing algorithms.

5. A variety of torsion and charge models could be applied to this problem, and future work will do so.

6. 3TI3 has two chains, each with its own active site. The work described in this paper was performed on Chain A only. Chain B appears to have an active site highly similar to the Chain A active site. Future work will assess the ligand/receptor binding energies of Chains B.

5.0 Acknowledgements

This work benefited from discussions with Tony Pawlicki. For any problems that remain, I am solely responsible.

6.0 References.


[13] Cheng Y and Prusoff WH. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_50) of an enzymatic reaction. *Biochemical Pharmacology* 22 (December 1973), 3099–3108. doi:10.1016/0006-2952(73)90196-2.
