


An Approach for Zika Virus Inhibition Using Homology Structure of the Envelope Protein

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Abstract To find an effective drug for Zika virus, it is important to understand how numerous proteins which are critical for the virus' structure and function interact with their counterparts. One approach to inhibiting the flavivirus is to deter its ability to bind onto glycoproteins; however, the crystal structures of envelope proteins of the ever-evolving viral strains that decipher glycosidic or drug-molecular interactions are not always available. To fill this gap, we are reporting a holistic, simulation-based approach to predict compounds that will inhibit ligand binding onto a structurally unresolved protein, in this case the Zika virus envelope protein (ZVEP), by developing a three-dimensional general structure and analyzing sites at which ligands and small drug-like molecules interact. By examining how glycan molecules and small-molecule probes interact with a freshly resolved ZVEP homology model, we report the susceptibility of ZVEP to inhibition via two small molecules, ZINC33683341 and ZINC49605556—by preferentially binding onto the primary receptor responsible for the virus' virulence. Antiviral activity was confirmed when ZINC33683341 was tested in cell culture. We

anticipate the results to be a starting point for drug discovery targeting Zika virus and other emerging pathogens.

Keywords Zika virus · Homology model · Druggability · Drug discovery

Significance

There are no drugs available yet to treat many viral infections including Zika virus. In order to find drugs, the three-dimensional structures of the protein(s) responsible for virus' virulence are essential; and in many cases, these structures are not available. However, advances in biotechnology have enabled rapid availability of nucleotide sequence data. This work presents a method to use gene sequence data to develop an accurate three-dimensional representation of a virulent protein and use this model to assess the druggability of a *structurally unresolved protein* using protein-specific ligands and probe molecules via molecular dynamic simulations. The methods are applicable to many proteins of not only viruses, but also emerging pathogens of which structural data are not available.

The World Health Organization has declared a global emergency due to the potential of Zika disease, an infection caused by Zika virus, to reach epidemiological levels [1, 2]. Current estimates are that three to four million infections will occur over the next 12-month period. An infection with the virus causes a mild form of fever termed Zika Fever [3], and although experiments are ongoing on numerous fronts [4, 5], to date, no vaccines or drugs are available to prevent or treat the infection. However, the virus' possible links to microcephaly in newborn babies of infected mothers [1] and neurological conditions in

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infected adults, including cases of the Guillain–Barré syndrome [6], make Zika disease potentially destructive.

One of the first steps in drug discovery is the assessment of whether the target protein is druggable. Druggability assessment requires the protein's crystal structural data. Unfortunately, in the case of Zika virus, the conformational structural information is not available. However, to circumvent this gap, we used gene sequence data to develop a ZVEP homology model for deciphering protein–ligand intermolecular interactions. The amino acid sequence was resolved initially using Zika virus' 10,794-nucleotide National Center for Biotechnology Information (NCBI) NC_012532.1 [7] via Basic Local Alignment Search Tool (BLAST) [8]. Then, to isolate the key domains responsible for glycosidic interactions, a profile/function search was performed using Interpro [9] embedded in Swiss-model [10] protein modeling tool. This exercise resulted in several initial templates, with 500-amino-acid segment of Dengue virus (PDB ID: 3j27) showing the best fit with 55.2 % identity to ZVEP (Fig. 1a). The sensitivity and performance of profile-based template search methods can often be improved when the template search is performed on individual domains, which often reflect evolutionary relationships and may correspond to units of molecular function, rather than the whole target sequence [10]. The profile/function search helped isolate the domains responsible for glycosidic interactions (Fig. 1b), and further refinement suggested domain III of the West Nile Virus Envelope Protein (WNEP), Strain 385-99 (PDB ID: 1S6N [11]), to be the best template to base the development of the final ZVEP homology model. Figure 1c shows the alignment of a 115-amino-acid section of ZVEP that was used to build the homology model using WNEP (1s6n.1.A). The resulting ZVEP homology structure (yellow) when overlaid with WNEP (blue) shows >50 % structural identity preserved (red), indicating the model's utility as a proxy for studying ZVEP behavior.

A docking simulation was performed in the presence of N-linked glycan fragment in the adhesion domain of human T lymphocyte glycoprotein CD2 (PDB ID: 1GYA) to identify any pockets that have a high affinity to glycosidic interactions. The simulation revealed a sub-domain where several sites that bind to glycan with high affinity were congregated forming the primary receptor site. The sub-domain carried sites with binding free energies ranging from -3.92 to -4.89 kcal/mol. Another domain that hosted sites that had a high affinity to glycan was discovered to the opposite of the primary glycan binding domain. The arrangement of the sites indicates the remarkable adaptability of the virus to be susceptible to glycosidic attack to create an opening for accessing material inside the capsid.

Once the ligandable sites were uncovered, druggability [12] of these sites was resolved by running NAMD [13] molecular dynamic simulations in the presence of small organic molecular probes that consisted of 60 % isopropanol and 10 % each of isobutene, acetamide, acetate, and isopropylamine. These small molecules are the most commonly used probes for druggability analyses [12]. The druggability assessment was performed with the intention of unraveling any clusters of “hot spots” that indicate the existence of druggable receptors. The druggability analysis revealed 56 small-molecule binding hotspots ranging from a minimum ΔG of -2.14 kcal/mol to a maximum of -1.00 kcal/mol. A high enrichment of isopropanol was observed throughout the protein surface with 34 binding hotspots and lowest binding free energy of -2.08 kcal/mol while isobutene (four hotspots, -1.73 kcal/mol), isopropylamine (four hotspots; -1.60 kcal/mol), acetamide (four hotspots, -1.71 kcal/mol), and acetate (12 hotspots, -2.14 kcal/mol) enrichment were more isolated. Information on all hotspots is provided under supplementary information.

The analysis predicted the presence of the two druggable domains, one that coincided with the exterior glycan-binding domain and the other coinciding with the interior. The exterior site, which in fact is the only one accessible to drugs, had a lowest drug-like binding free energy of -10.92 kcal/mol, highest drug-like affinity of 10.991 nM, and a volume of 488.73 \AA^3 . The occupancy grid distribution of the probes across the ZVEP surface and at the vicinity of the receptor—suggesting active site compositions of a potential drug candidate—are shown in Fig. 2a and b, respectively. The analysis suggests that the primary active probe for the receptor is isopropanol, while the secondary is isobutene.

Armed with potential compositions suitable for the receptor site, a pharmacophore analysis was performed. Interestingly, of ~ 22.7 million available compounds, only five were qualified as receptor-compatible candidates. Of these, excluding organochlorides, only two, i.e., ZINC49605556 (Fig. 2c) and ZINC33683341 (Fig. 2d), would qualify as small molecules (i.e., <900 Daltons). Various confirmations of the two compounds bound on ZVEP are shown in Fig. 2c and d, respectively. The significantly lower binding free energy values of the two molecules as compared to glycan indicate the ability of both molecules to preferentially bind to the receptor deterring any glycan binding which in turn dissuades the formation of glycosidic linkages between the glycan and ZVEP. An analysis on how the two compounds interact with N-linked glycan fragment in the adhesion domain of human CD2 revealed that both have higher affinities, i.e., higher binding free energy values, to ZVEP as compared to the adhesion domain of CD2 (Fig. 3), indicating that the molecules would preferentially bind to ZVEP.

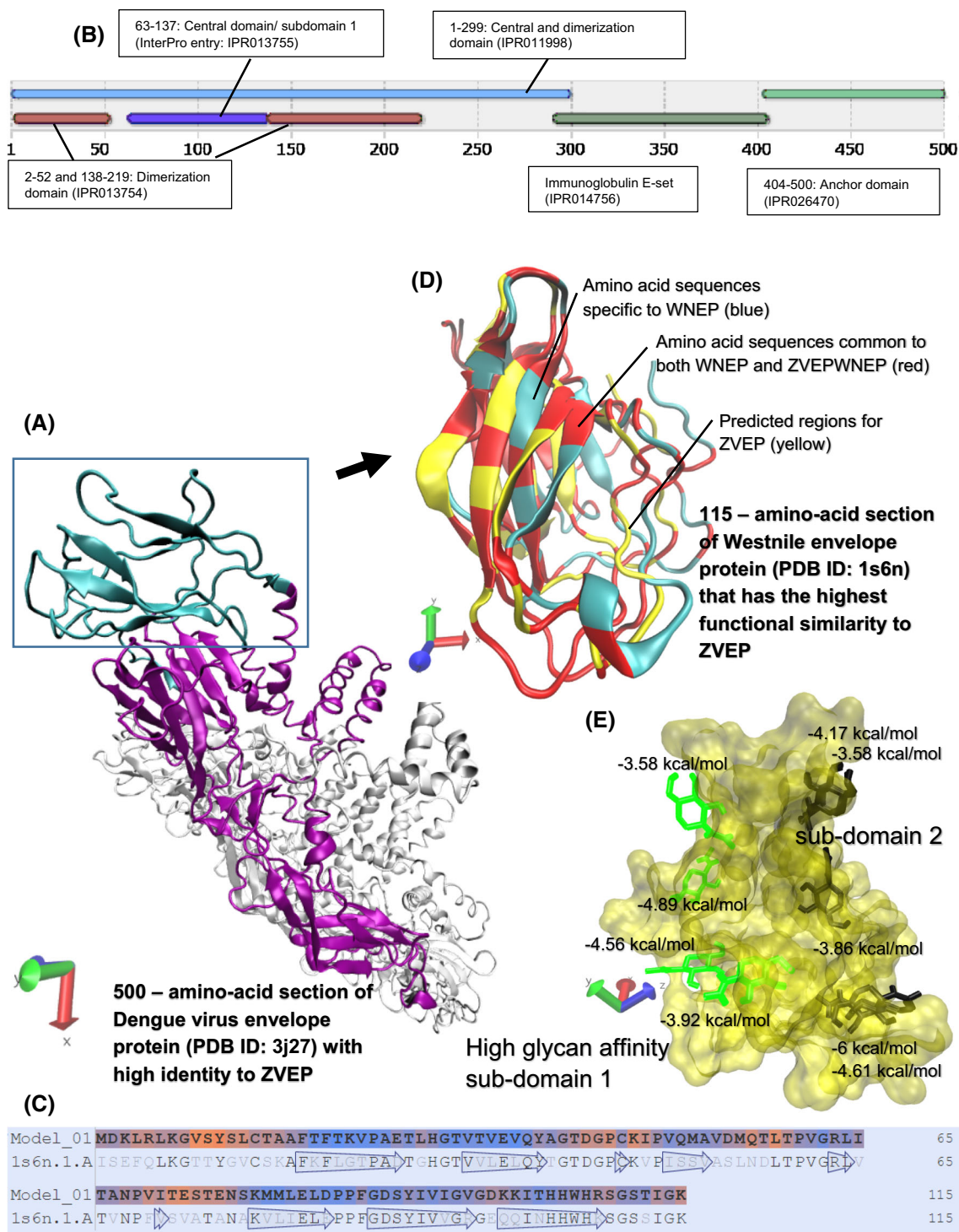


Fig. 1 **a** 500-Amino-acid segment of Dengue virus (PDB ID: 3j27) that resulted from the initial BLAST search as the best identity-fit for ZVEP; **b** Isolated domains representing profile-function relationships of Dengue virus; **c** The alignment of a 115-amino-acid section of WNEP (1s6n.1.A) and ZVEP that was the best fit for developing the homology model after considering profile-function relationships;

d An overlay of the WNEP and resulting ZVEP homology structures; **e** The glycan-binding domains of ZVEP showing the exterior domain that had several sites with high affinity to glycan molecules (green) and interior domain (black). The binding free energy values are depicted for each confirmation (Color figure online)

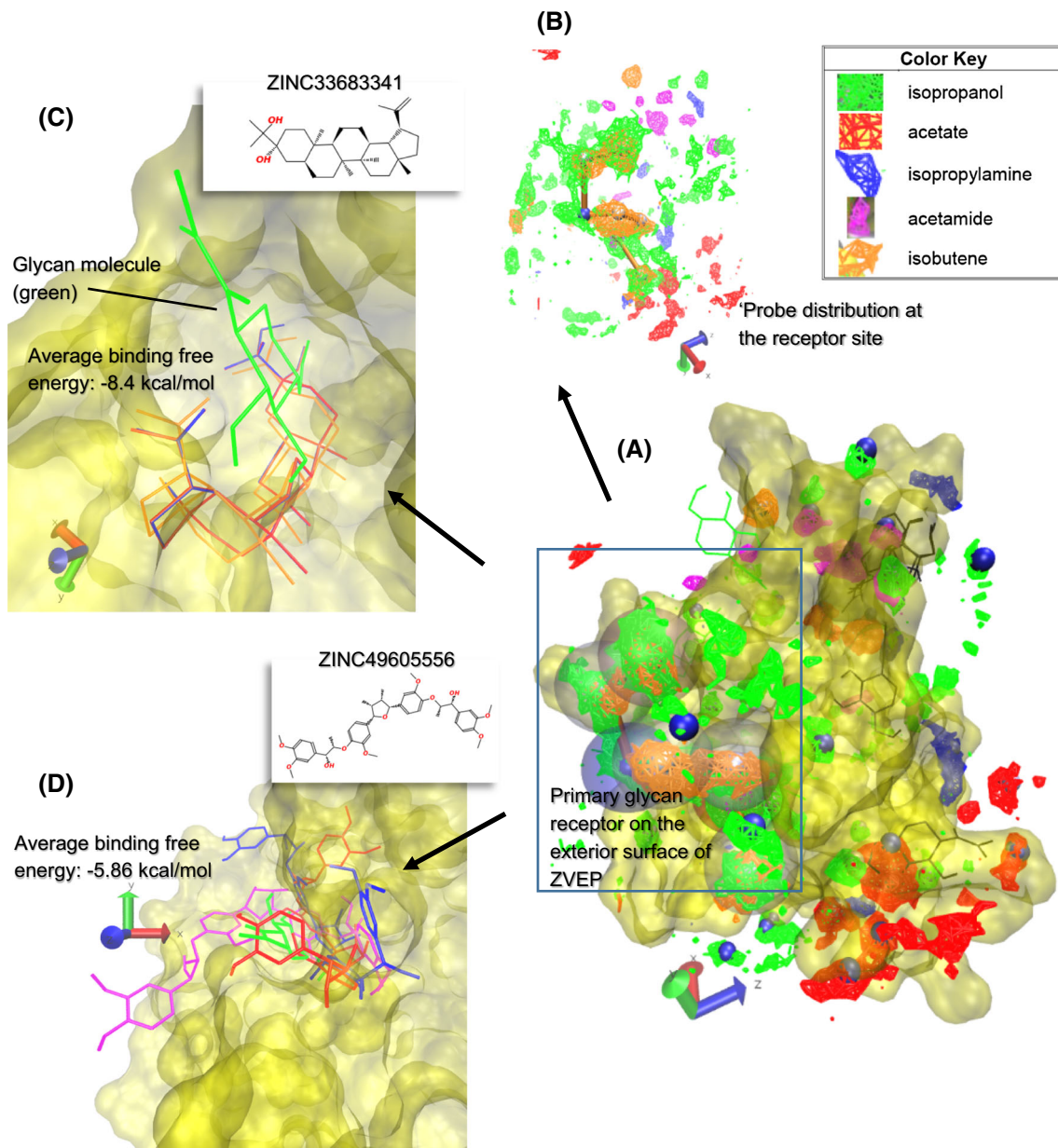


Fig. 2 **a** The occupancy grid distribution of the probes and hotspots across the ZVEP surface. **b** The occupancy grid distribution of the probes at the receptor site suggesting the composition at each locale. **c** Top binding confirmations of ZINC33683341, a potential drug

compound compatible with the receptor identified from the pharmacophore analysis. **d** Binding confirmations of ZINC49605556 another potential drug compound. Glycan molecules are depicted in green (Color figure online)

Antiviral activity of ZINC33683341 was subsequently analyzed using in vitro assay as described previously [5]. Briefly, monolayers of Vero cells were treated with ZINC33683341 (Sigma-Aldrich) at concentrations of 50 or 100 μM , respectively. When the compounds were added, the cells were infected with Zika virus (strain MR766) at a multiplicity of infection of 0.1. As a negative control, DMSO was added to virus- and mock-infected cells at a final concentration of 0.5 % (v/v). Culture supernatants were collected at 48 h after infection

and analyzed for Zika virus titers by plaque assay. No antiviral activity was seen at the concentration of 50 μM , but the compound at the concentration of 100 μM reduced virus titer for more than 2 \log_{10} pfu/ml ($p < 0.001$; Student's t test). Cytotoxicity of the compound was investigated in Vero cells using Dojindo's highly water-soluble tetrazolium salt test. No cytotoxicity was observed when the cells were treated with ZINC33683341 at concentrations of 50 or 100 μM , respectively (data not shown) (Fig. 4).

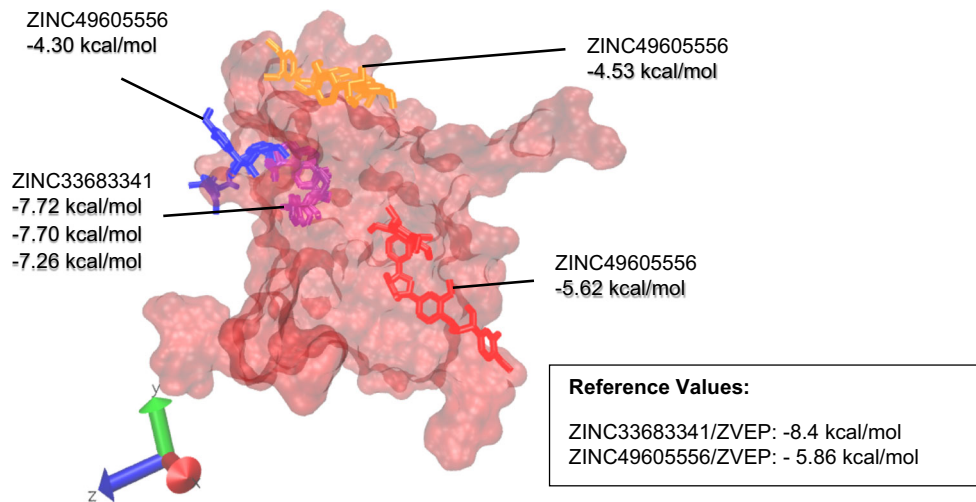


Fig. 3 ZINC33683341 and ZINC49605556 binding affinity (given in terms of binding free energy) to N-linked glycan fragment in the adhesion domain of human CD2. It can be seen that ZINC33683341 binds to one primary site with a relatively higher affinity while ZINC49605556 binds to several sites scattered across the surface, but

with a lower affinity. All these affinity values are lower than the values when the compounds bind to ZVEP suggesting that the compounds will preferentially bind to ZVEP in the presence of human CD2

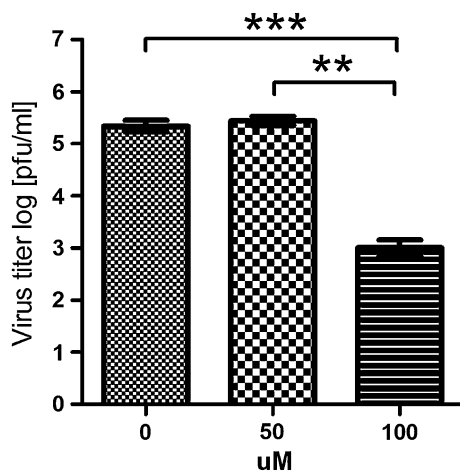


Fig. 4 ZINC33683341 inhibits Zika virus replication in cell culture. Vero cells were treated with different concentrations of ZINC33683341 and at the same time infected with Zika virus. Culture supernatants were collected at 48 h after infection and analyzed for Zika virus titers by plaque assay. ** $p < 0.01$; *** $p < 0.001$

Conclusions

Armed with only nucleotide sequence data, it was possible to study how ligands and small-molecule drug-like probes interact with ZVEP using a three-dimensional homology model developed with experimentally determined crystal structures of proteins that belonged to the same family. The small molecular probe-based druggability study revealed a druggable site that coincided with the primary site that was identified as responsible for the virus' virulence, and also two compounds that would preferentially bind onto the

glycan-binding domain. We believe that identification of these compounds that have a high affinity to the glycan receptor is a decent starting point for drug discovery targeting ZVEP. Indeed, antiviral activity of one of the identified compounds was confirmed using in vitro assay. Results have broader implications for biological surface science and specifically for drug discovery research since the methods developed, once verified when crystal structures of ZVEP of the same strain become available, would provide an additional set of tools to confront Zika virus, not to mention other emerging pathogens that thus far have been overlooked.

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