Modeling Arenavirus Nucleocapsid and Z Protein Structures


Departments of Biophysics and Chemistry, University of Michigan, Ann Arbor, MI

Abstract:
The Arenaviridae family of viruses, responsible for neurological disease and hemorrhagic fever, is transmitted to humans via rodents. Over 20 different strains have been identified and phylogenetically classified since the first outbreak of the virus in 1933 and new strains are continuing to develop. It is known that Arenaviridae are enveloped and spherical, meaning that they are made up of a single protein copied numerous times, and contain two segments of single stranded RNA. There is no structural information about the major nucleocapsid protein (NP) and other proteins associated with the virus capsid structure. This lack of structural data has hindered the identification of potential drug targets and the development of effective drugs. Currently there are no vaccines or FDA approved drugs for Arenavirus infections.

The zinc-finger-like protein (Z) is known to interact with NP to induce budding, the process of viral proliferation. In order to better understand how NPs interact in the context of the spherical virus shells as well as with Z, structures of these two proteins were predicted using homology modeling methods. Amino acid sequences for the Old World Lympohocytic Choriomeningitis Virus (LCMV) and the New World Tacaribe Virus, two strains commonly studied in experimental labs, were used for prediction of tertiary structure. Models for Z were constructed from templates obtained through PSI-Blast and compared to the tertiary structure predictions from web servers. In addition, tertiary structures of all known virus capsids described in Viper DB [2] were used as potential templates for homology modeling of NP. Having constructed models of NP and Z, we identified putative protein-protein interaction sites, which may represent a better antiviral drug target.

Objectives/Methods:
What?
- Create models for NP and Z proteins
Why?
- No structural information
- No viral templates
- No FDA approved drugs
- Potential to design new drugs
- Z protein is major drug target
How?
- Eight virus families were identified as potential templates
- Built models using multiple structure alignment from same family
- Monomer energies were evaluated using CHARMM minimization with implicit solvent model
- Predicted secondary structure was compared to the secondary structure in the model
- I-Tasser was used to predict strongest RNA binding site on Reoviridae template and model
- RNA binding sites were found on the inside of the capsid in a reasonable location

Z Protein: Sequence Only Approach
- Zinc-finger motif in sequence
- PSI-Blast provided 10 templates
- E-value: Putative protein target
- Phylogenetic tree (presented)
- Model criteria: correct zinc binding geometry
- Model allows for incorporation of ligand geometry
- Evaluated CHARMM energies
- Models converged to a favorable fold
- All models were within 1.5-2.4 Å RMSD

Z Protein Model
- CHARMM monomer energy
- CHARMM protein energy
- CHARMM total energy
- I-Tasser models broke down into separate domains
- DALI Z scores indicate that these domains occur in other protein structures
- Domains are candidates if virus space hypothesis is incorrect

Comparison of Z and NP Modeling
Z Protein (90 residues)
- 10 templates from PSI-Blast
- Multiple methods converged on similar structure
- LCMV and Tacaribe shared best template (DOKL_B)
- High confidence in prediction

NP (570 residues)
- No suitable templates from PSI-Blast
- No consensus between web server predictions and Modeler
- Restricted search templates to virus structures
- LCMV and Tacaribe shared best virus template (Reoviridae)
- Moderate confidence in prediction

Interaction of Z and NP
NP-NP interaction
- Z protein was matched to putative helix-binding site

Conclusions/Future Directions
- High confidence in Z protein modeling
- Models based on Reoviridae templates are better than other virus-based models based on evaluation criteria
- Continue working on NP-NP interactions to build full capsid
- Improve upon models of Z binding to NP
- Further work could focus on drug development to inhibit Z and NP interaction to inhibit budding
- Possibility of using I-Tasser domains to build novel Arenavirus fold