PRIME 2011

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Hierarchical Screening of Inhibitors against Hemagglutinin and Identifying Possible Binding Pockets of the Trimeric Interface
Aims:

1. Hierarchical Screening of Inhibitors against Hemagglutinin (HA)
   • Looking for molecule inhibitors that may have high affinity for HA RBD of influenza virus

2. Identifying Possible Binding Pockets of the Trimeric Interface
   • Scan for high affinity binding pockets
   • Then find compounds that could potentially block formation of trimeric HA
Influenza virus

- Annual epidemics (an influenza virus that is localized)
- Sparse pandemics (an influenza virus that affects multiple regions)
- Potential threat of new lethal pandemic strain
  - Through antigenetic shift or genome re-assortment
- Highly Pathogenic Avian Influenza (HPAI) A
  - Has been transmitted between species from birds to humans
Influenza Life Cycle

1) HA on membrane surface of the virus binds to sialic acid (sia) receptors on the host cell
2) Virions are taken in by receptor-mediated endocytosis
3) Lysosome fusion, lowers pH, changes HA conformation to fuse to lysosomal membrane

De Clercq, Erik. Nature Reviews Drug Discovery 5, 1015-1025 (December 2006)
Influenza Life Cycle

4) Genetic materials are released into the host cell
5) RNA replication and virus assembly
6) Neuraminidase (NA) cleaves virus from infected cell

De Clercq, Erik. Nature Reviews Drug Discovery 5, 1015-1025 (December 2006)
Fusion peptide needed to begin infection
- Composed of a trimer of subunits
- Contains a domain that binds to the sia receptors called the receptor binding domain (RBD)

· α 2,3-linked lactoseries tetrasaccharide a (LSTa)
  · avian glycan receptor analogue
· α 2,6-linked lactoseries tetrasaccharide c (LSTc)
  · human glycan receptor analogue

Established conditions

- The initial screening process:
  - Virtual screening for high affinity of small molecule inhibitors for HA RBD
    - Using Vina and AutoDock4
  - Initial screening conditions were established by Michael Siy, Kevin Wu, and Wendy Fong
Aims:

1. Hierarchical Screening of Inhibitors against Hemagglutinin (HA)
   • Perform hierarchical screening using a larger library Drug Bank from ZINC
   • Using established initial screening and conditions

2. Identifying Possible Binding Pockets of the Trimeric Interface
   • Use AutoLigand to identify possible binding pockets of the trimeric interface
   • Identification of potential trimeric interface inhibitors
Tools

- **AutoDock** - predicts how small molecules bind to a receptor of known 3D structure
- **AutoDockVina** - a newer program for docking and virtual screening and is much faster compared to AutoDock
- **ZINC** - database of commercially-available compounds for virtual screening
- **AutoLigand** - scans for high affinity binding pockets and reports the best volume, shape, and atom types that would for the binding pocket
Tools

- Chimera - a molecular graphics program used to visualize PDB structures and molecules
- CADD (Computer Aided Drug Discovery) - runs workflows on Virtual Screening and prepares receptors and ligands for docking
- vRocs/Vida - a shape comparison program used to visualize and group molecules
- AutoDockTools - a set of docking tools that predicts how ligands will bind to a receptor and used to visualize binding interactions
Screen on a larger library of known drugs
  - Identifies more potential inhibitors for the HA RBD

Look for compounds in known drugs that have high affinity for HA RBD

- Identify possible binding pockets of the trimeric interface using AutoLigand
- Look for compounds which could potentially block formation of the trimeric HA
Obtained protein structures from Protein Data Base

- **H3 (1MQL)**
  - Contains a total of 6 chains:
    - HA1 is composed of chains A, D, and G
    - HA2 is composed of chains B, E, and H
  - Length (Å):
    - a = 147.68
    - b = 147.10
    - c = 251.99

- **H5 (3EYM)**
  - Contains a total of 6 chains:
    - HA1 is composed of chains A, D, and G
    - HA2 is composed of chains B, E, and H
  - Length(Å):
    - a = 160.46
    - b = 160.46
    - c = 176.55
Key Residues

- Highlighted key residues from research papers for binding on H3 and H5
- Key residues matched with the analysis of sialic acid and top molecules with binding site
Standardized HA

- Isolated chain A
  - Contains HA RBD
- Removed all waters and nonstandard residues
- Ran Prepare Receptor workflow on CADD to prepare HA RBD for docking
Alignment allows for the use of the same gridbox and docking molecules would be in the same area.
Prepared for Virtual Screening

- Made GPF (grid perimeter file) by setting a gridbox to binding region on AutoDockTools
- Made DPF (docking perimeter file) by setting number of GA runs and number of evaluations with AutoDockTools
Virtual Screening was run on a CADD workflow

- Required a receptor (HA), GPF, and DPF in an input folder, an output folder, and a Ligand Database
Public Virtual Screening had to be preformed for each databank

Local Virtual Screening had to be preformed for Drug-like filter
Analysis

- Converted summarized data from each Virtual Screen into excel files for analysis
- Noted similarities between databases and highlighted non drug-like molecules
- Checked all molecules on ZINC database
- Noting retired molecules and their replacements
- Downloaded SDF to use on vRocs to group molecules by shape
vRocs/Vida

- Sorted and grouped top molecules from Virtual Screening by grouping molecules using vRocs and Vida
  - Noting similar structures
Interactions

- Noted interactions of sialic acid and top 5 molecules
  - Interactions matched the Key Residues from readings on the HA RBD
## Results: H3

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Significance

- Potential small molecule inhibitors of HA RBD
  - Vaccine development
- Understanding the differences of the receptor binding domain in different Hemagglutinins
- Checking similar compounds in top molecules of the different HAs to look for potential inhibitors that would inhibit both receptor binding domains
Future Directions

- Run Virtual Screening on H5 and analyze top molecules similar to the analysis of H3
- Identify possible binding pockets of the trimeric interface
- Contact experimental collaborator for assays of top molecule inhibitors
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