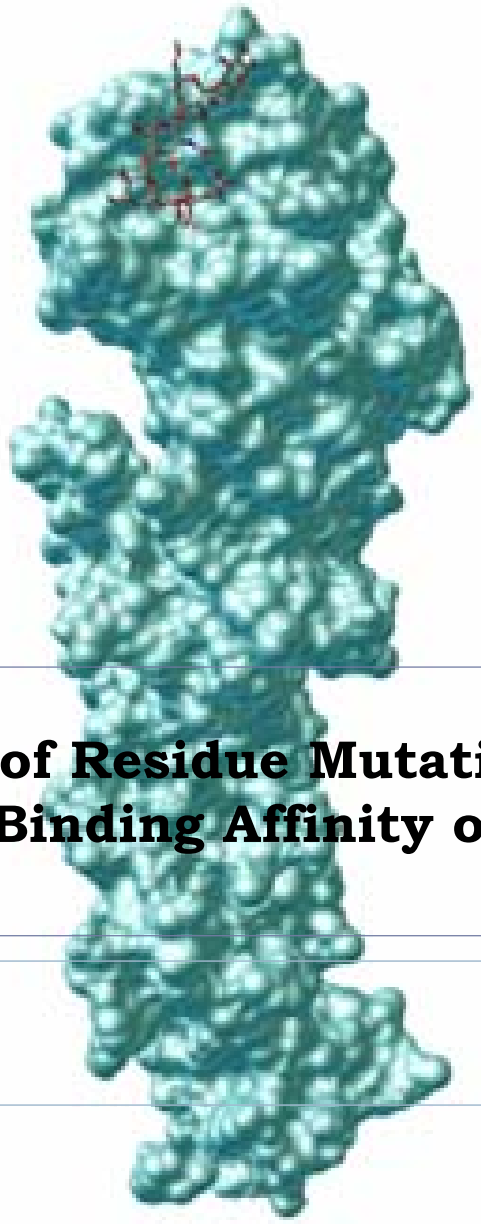

Final Report

Identification of Residue Mutations that Increase the Binding Affinity of LSTc to HA RBD

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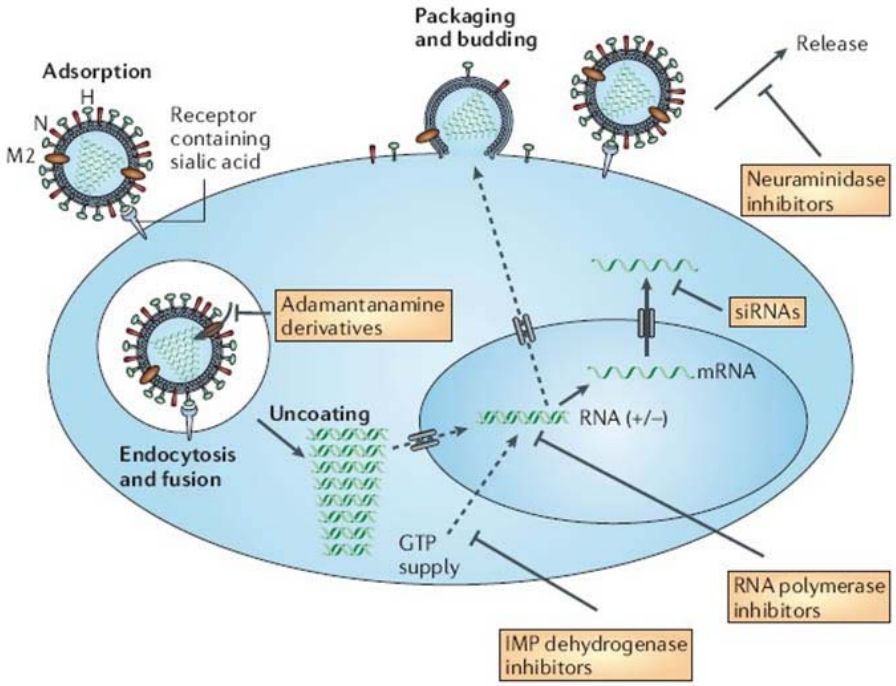
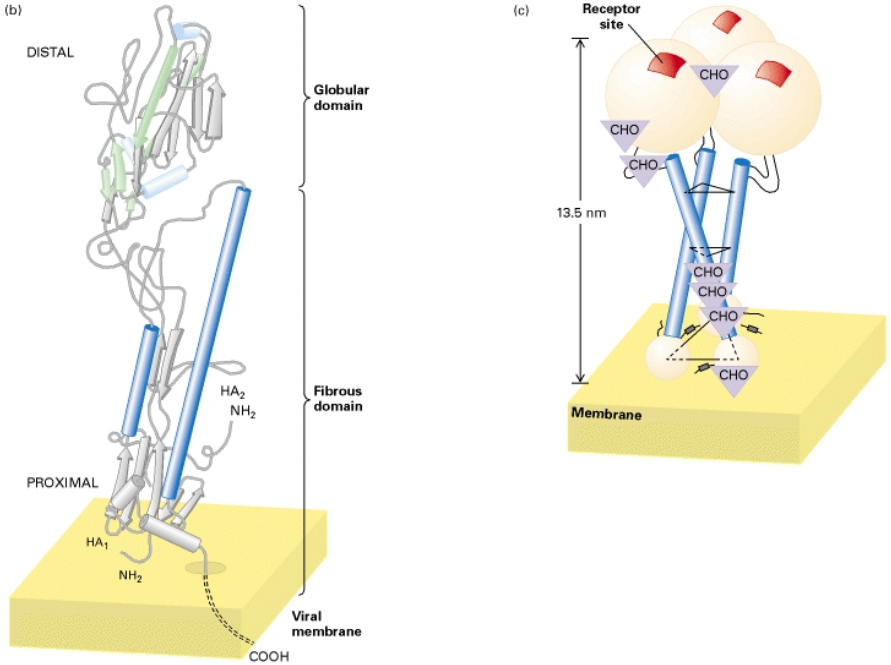
Influenza's Viral Life Cycle



1. HA on virus binds to sialic acid receptors on the host cell.
2. Virus enters via endocytosis.
3. Change of pH causes fusion peptides to extend and draw the viral and endosomal membranes together.
4. Viral contents enter the host cell.
5. RNA replication and viral assembly.
6. NA cleaves sialic acid as new viral particles are released.

http://camd.yonsei.ac.kr/AvianInfluenza

Hemagglutinin



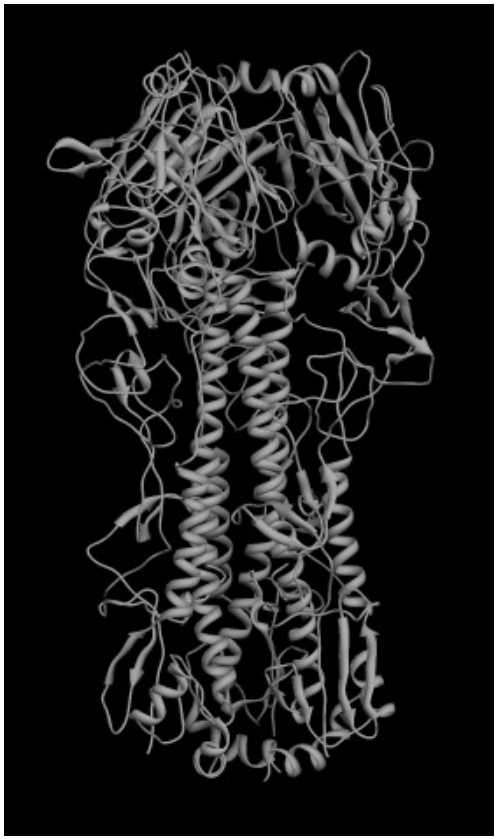
- Synthesized as single polypeptide
- Cleavage by protease makes it active
- Forms a trimer
  - Coiled-coil stem
  - Fusion at low pH
- RBD at top of HA

http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=mcb&part=A524&rendertype=figure&id=A524

Proposed Project

- Mutate residue sets in the hemagglutinin (HA) receptor binding domain (RBD) to artificially increase the binding affinity of a human glycan receptor analogue to the HA RBD.
  - Compare the sequence to that of seasonal subtypes to correlate with the virulence (pathogenicity and transmissibility) of the virus.
- Use the mutant models to identify small molecule inhibitors that could block the binding of the influenza virus to human receptors through virtual screening experiments.
  - Experimentally validate it using a hemagglutination inhibition (HI) assay.

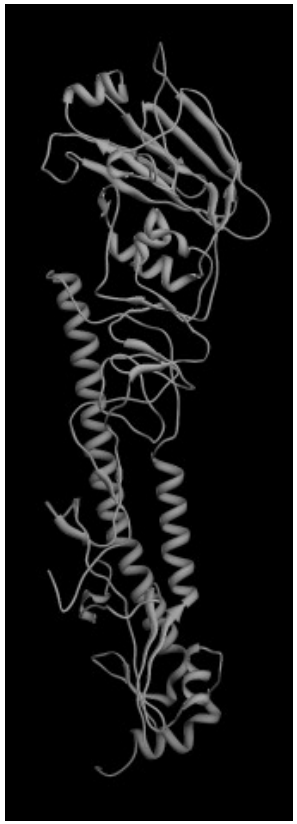
Receptors



H3

- PDB ID: 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

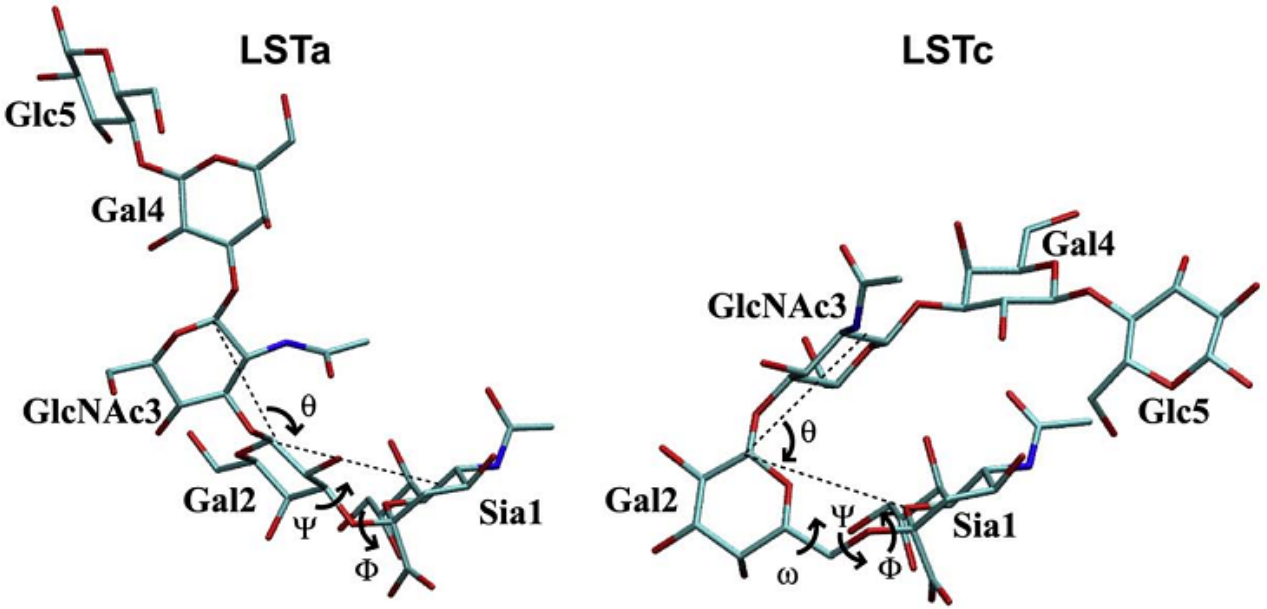
Receptors Cont.



H3h (Human X-31)

- PDB file obtained from original cluster representations
- Contains a total of 2 chains – A and B

Ligands

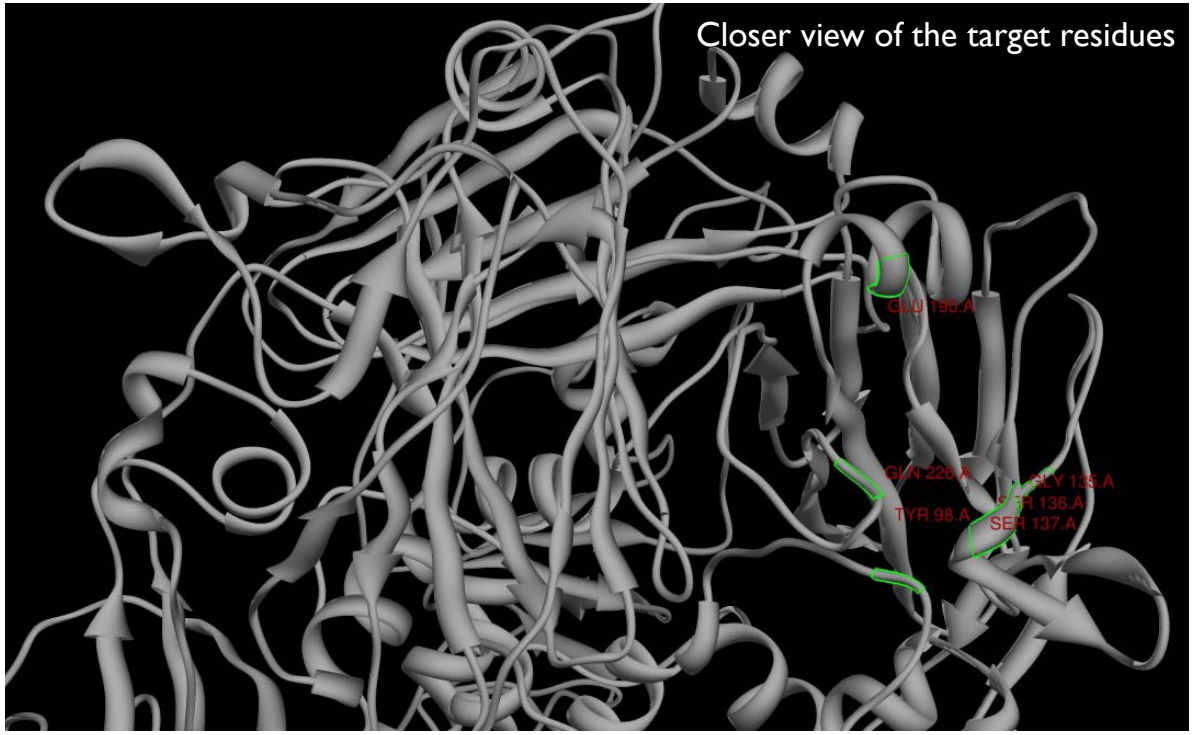


- LSTa = α-2,3-linked lactoseries tetrasaccharide a
  - Avian glycan receptor analogue
- LST c = α-2,6-linked lactoseries tetrasaccharide c
  - Human glycan receptor analogue

D. Xu, E. Newhouse, R. Amaro, H. Pao, L. Cheng, P. Markwick, J.A. McCammon, W. Li, P. Arzberger. (2009)Distinct Glycan Topology for Avian and Human Sialopentasaccharide Receptor Analogues upon Binding Different Hemagglutinins: A Molecular Dynamics Perspective. *J. Mol. Biol.*

Target Residues

Target residues in H3's RBD



Tools

- **AutoDock Tools (ADT)** – AutoDock Tools is a set of docking tools that predicts how ligands will bind to a receptor. It is the interface between calculated grids and docking. For the purpose of this project, ADT was used to prepare receptors and ligands for docking as well as generating grid parameter files (GPF).

- **AutoDock Vina** – Vina is a newer program for docking and virtual screening. It is not only faster and more accurate than AutoDock 4, but also suitable for more flexible ligands.

- **AutoDock2MMGBSA (A2M)** – A2M is a drug design tool that refines docking results through implicit solvent Generalized Born (GB) energy minimization and molecular dynamics (MD) simulations. It also rescores predicted binding free energies using molecular mechanics-Generalized Born surface area as a model for calculating free energies of binding.

- **BLAST** – Basic Local Alignment Search Tool is an algorithm used to compare biological sequences ranging from nucleotides to amino acids. The query sequence is searched against a database of sequences.

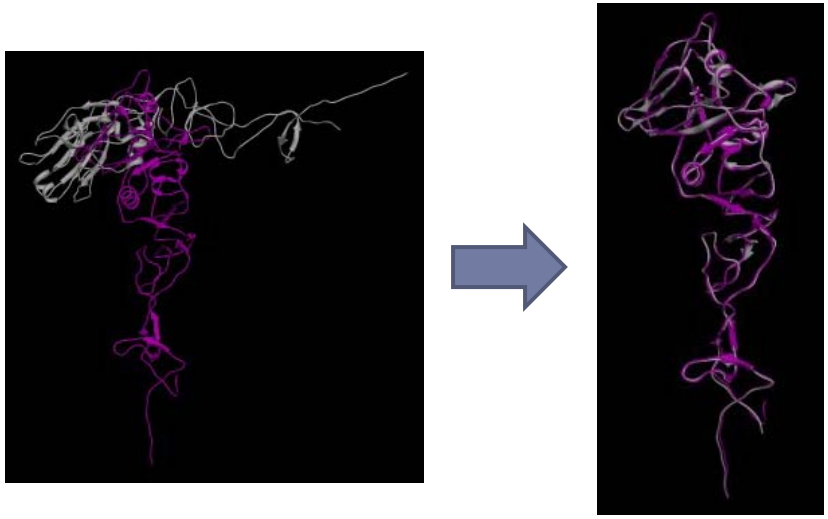
- **Chimera** – Chimera is a molecular graphics program used to visualize and analyze PDB structures.

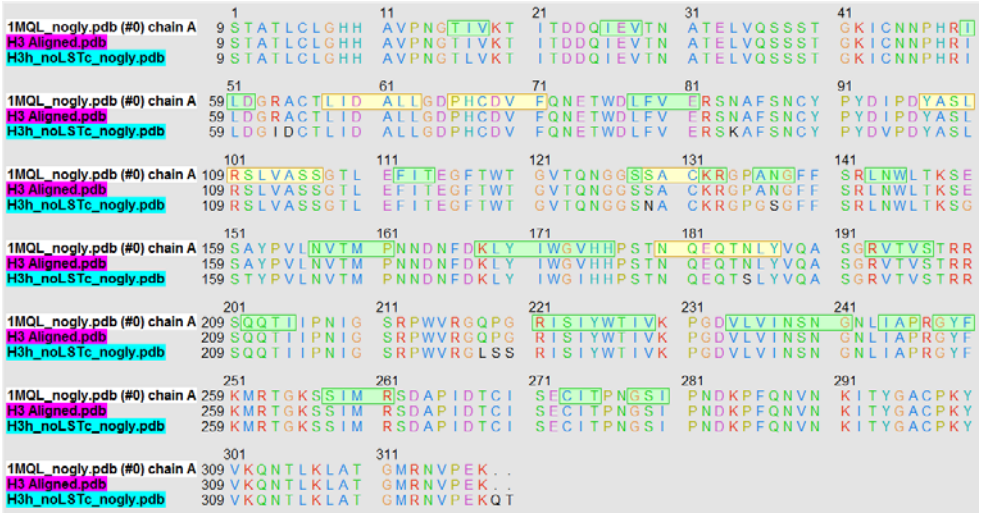
- **VMD** – Visual Molecular Dynamics is another molecular graphics program used to render and analyze PDB structures.

Approach

- Obtain ligands and receptors
  - Protein Data Bank (PDB)
  - Stripped ligand from receptor
- Mutate residue sets using VMD
  - Including single to multiple mutations
- Prepare ligands and receptors for docking using ADT and write config files
- Run docking job in Vina with subsequent analysis to determine which mode to rescore with A2M
- Analysis and compilation of results

Alignment





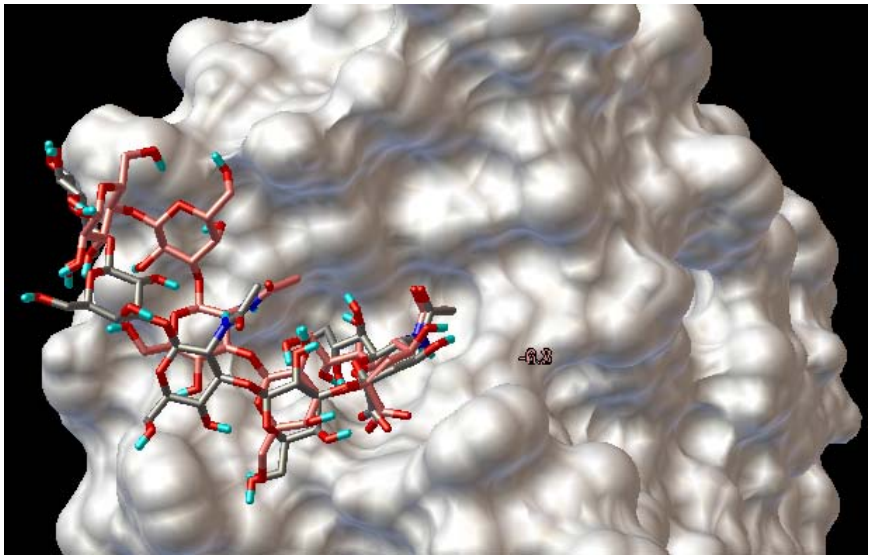
- H3 (silver) had to be aligned with H3h (purple) because the original ligand could not be compared to the docked ligand otherwise
  - Only chain A, where the RBD is located, was aligned
- To ensure that H3 had truly been aligned, a sequence alignment was performed against H3h and the unaligned H3
- Alignment allowed for the same grid box to be used on their respective ligands regardless of the receptor

Docking and Analysis

- Docking was performed on NBCR's Opal2 server
  - Webservices are offered for a number of applications
- Analysis through comparison of ligands
  - Primarily compared the sialic acid portion of the ligand
  - Looked specifically at the carboxylate

Submission results for AutodockVina

Date and time :	**Sunday, August 22, 2010 8:13:16 PM**
JobId :	appAutodockVina12825331619621797719367
Status code:	2
Message:	Execution in progress
Output Base URL:	http://kryptonite.nbcr.net/appAutodockVina12825331619621797719367



Results

	Mutations	Free Energy of Binding (kcal/mol) when docked with LSTa	Free Energy of Binding (kcal/mol) when docked with LSTc
Single	Y98S	-16.77	-39.16
	G135C	-15.17	-34.46
	E190D	-12.89	-30.01
Double	Y98N, G135C	-8.27	-33.44
	Y98N, Q226T	-15.17	-30.01
	S136T, E190D	-14.60	-45.38
	S136T, E190N	-19.39	-48.33
	S137N, E190L	-16.92	-30.13
Triple	Y98N, S136T, E190N	-14.22	-29.93
	Y98S, G135C, E190D	-10.76	-23.93
	Y98S, S137N, E190D	-5.76	-19.96
	G135C, S137N, E190D	-14.16	-31.98
Quadruple	Y98S, G135C, S137N, E190D	-12.74	-33.77
	Y98S, S136T, S137N, E190N	-8.96	-28.32
	Y98S, S136T, E190D, Q226T	-13.86	-24.51
Quintuple	Y98S, G135C, S136T, S137N, E190D	-10.53	-23.53
	Y98S, G135C, S136T, S137N, E190N	-12.25	-21.53
	Y98S, S136T, S137N, E190D, Q226T	-5.86	-28.76

Key Findings

- Preferential LSTc-binding with three types of mutations:
  1. Block LSTa-binding
  2. Preserve or disrupt hydrogen bonds
  3. Known
  - Same residue positions were mutated for both types of mutations
    - G135 was the exception
- Free energy of binding for LSTc seemed to increase with more mutations
  - Less mutations may be more favorable
  - RBD less stable
- None of the mutations have occurred in existing strains

Significance

- Better understanding of influenza virus
  - Glycan binding
  - Species specificity switch
  - Pandemicity
- Prevent cross-species infection
- Potential small molecule inhibitors
  - Vaccine development
- Prepare for future emergence

Acknowledgements

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