

PRIME 2011

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

Proposed Research

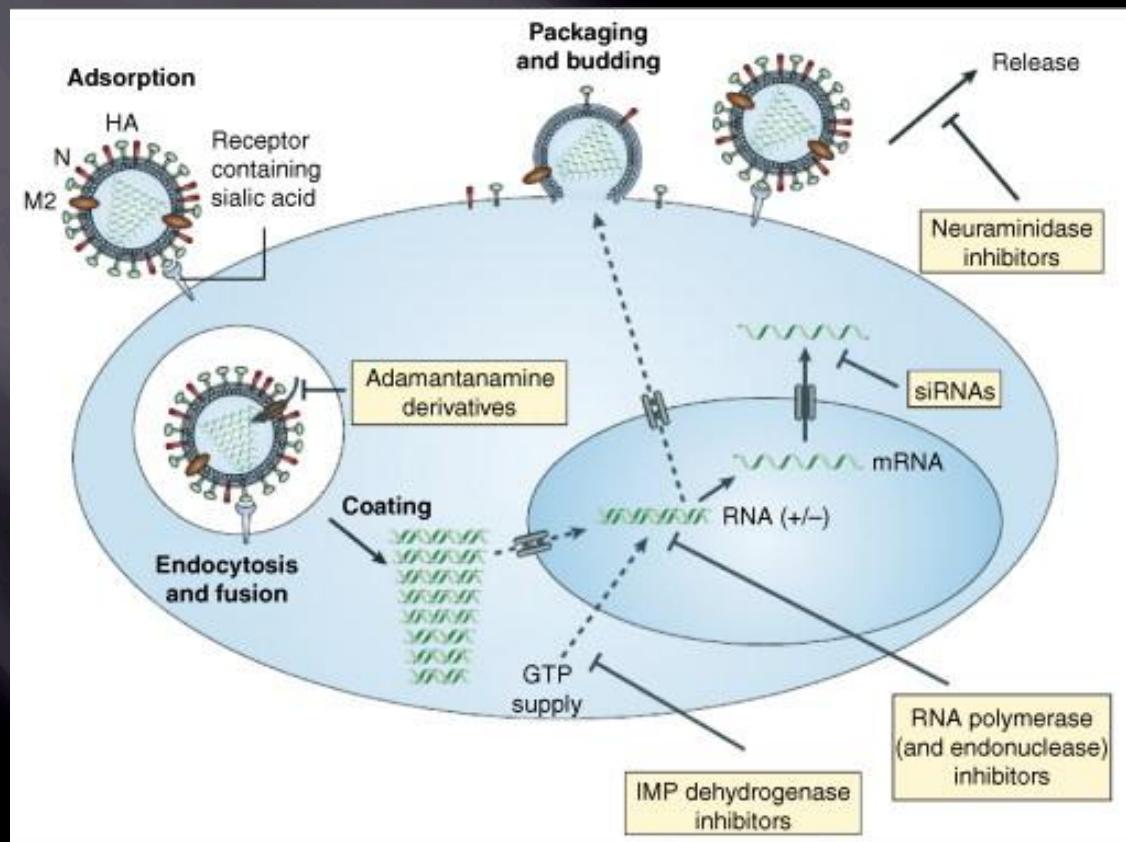
Aims:

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

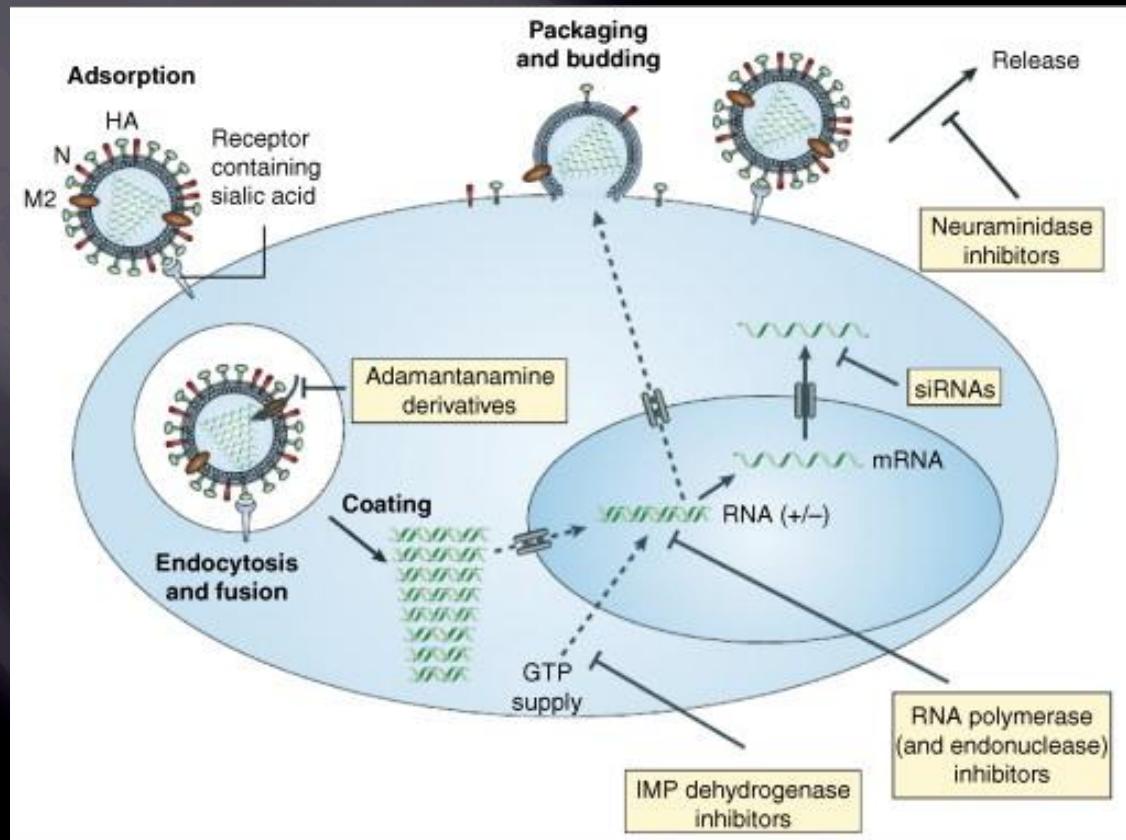
- Annual epidemics (an influenza virus that is localized)
- Sparse pandemics (an influenza virus that affects multiple regions)
- Potential threat of new lethal pandemic strain
 - Through antigenetic shift or genome re-assortment
- Highly Pathogenic Avian Influenza (HPAI) A
 - Has been transmitted between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

Influenza Life Cycle



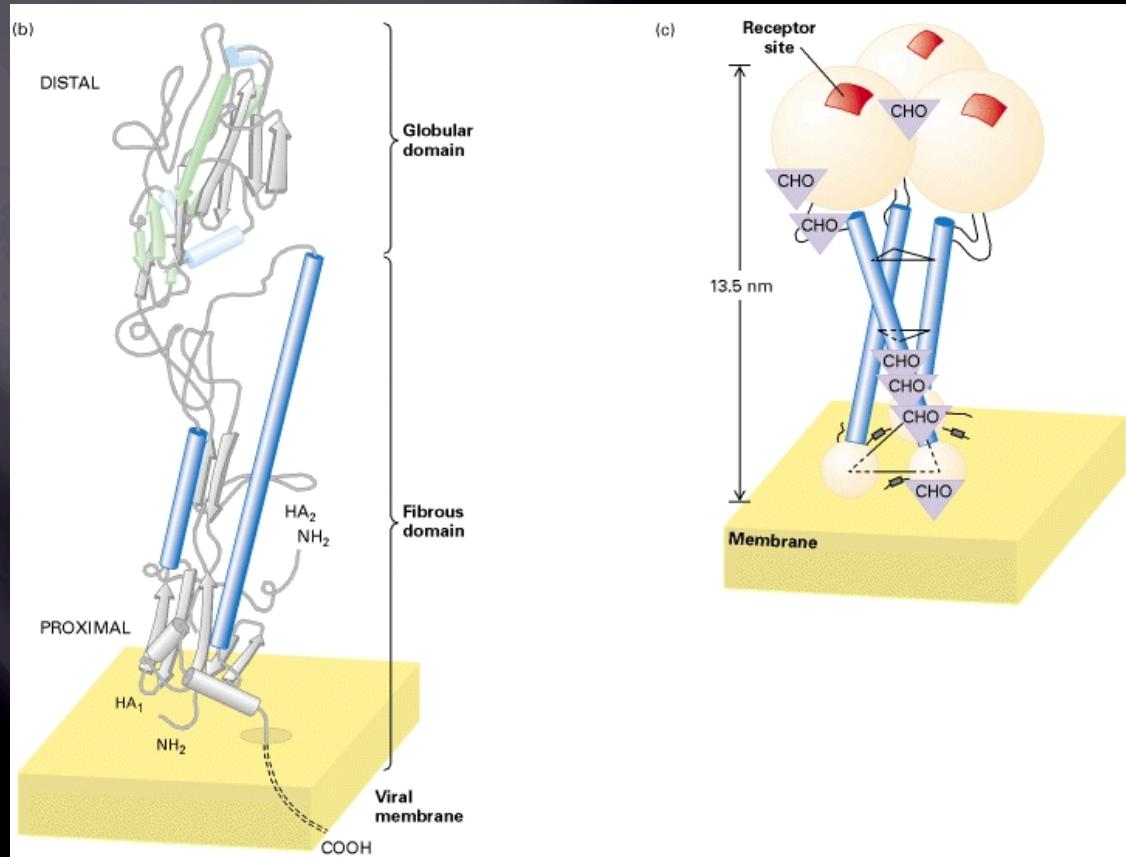
- 1) HA on membrane surface of the virus binds to sialic acid (sia) receptors on the host cell
- 2) Virions are taken in by receptor-mediated endocytosis
- 3) Lysosome fusion, lowers pH, changes HA conformation to fuse to lysosomal membrane

Influenza Life Cycle



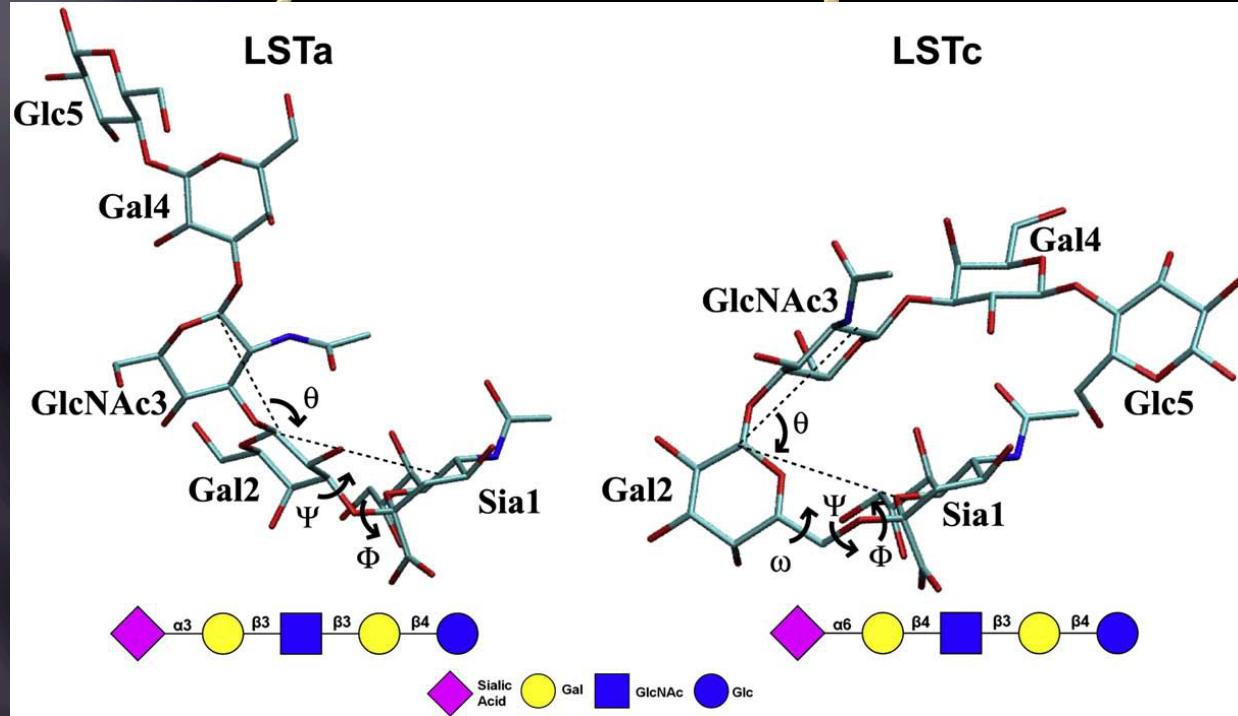
- 4) Genetic materials are released into the host cell
- 5) RNA replication and virus assembly
- 6) Neuraminidase (NA) cleaves virus from infected cell

Hemagglutinin



- 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)

Glycan Receptors



- 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

Proposed Research

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 fit 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

Experimental Design

- 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

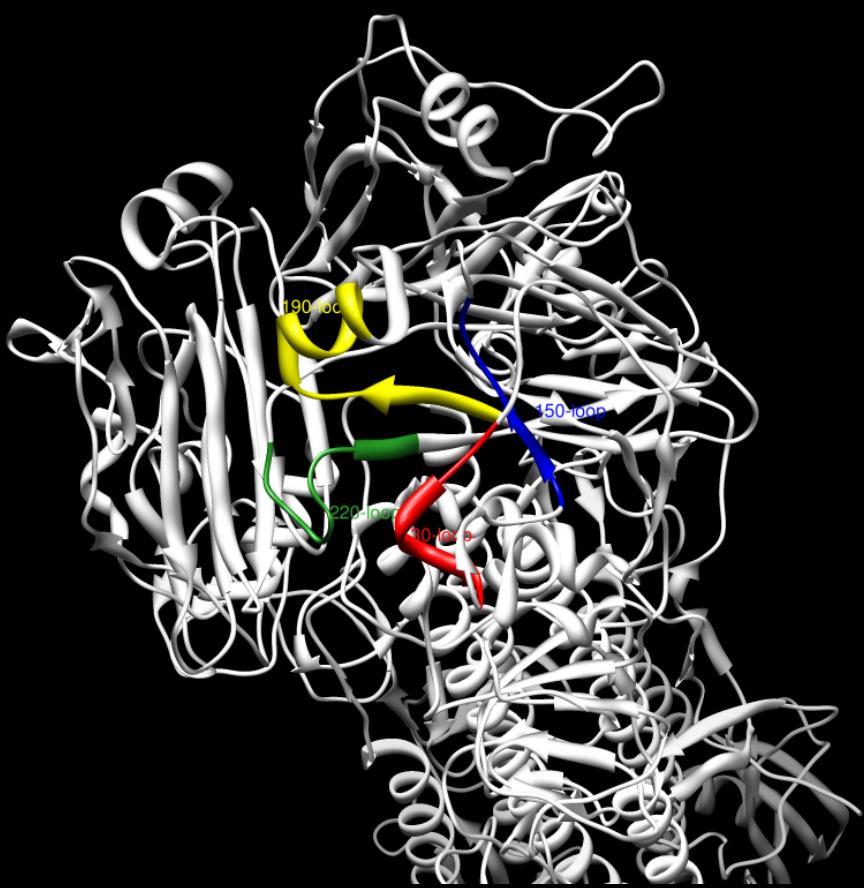
- Identify possible binding pockets of the trimeric interface using AutoLigand
- Look for compounds which could potentially block formation of the trimeric HA

Hemagglutinin Receptors

Obtained protein structures from Protein Data Base

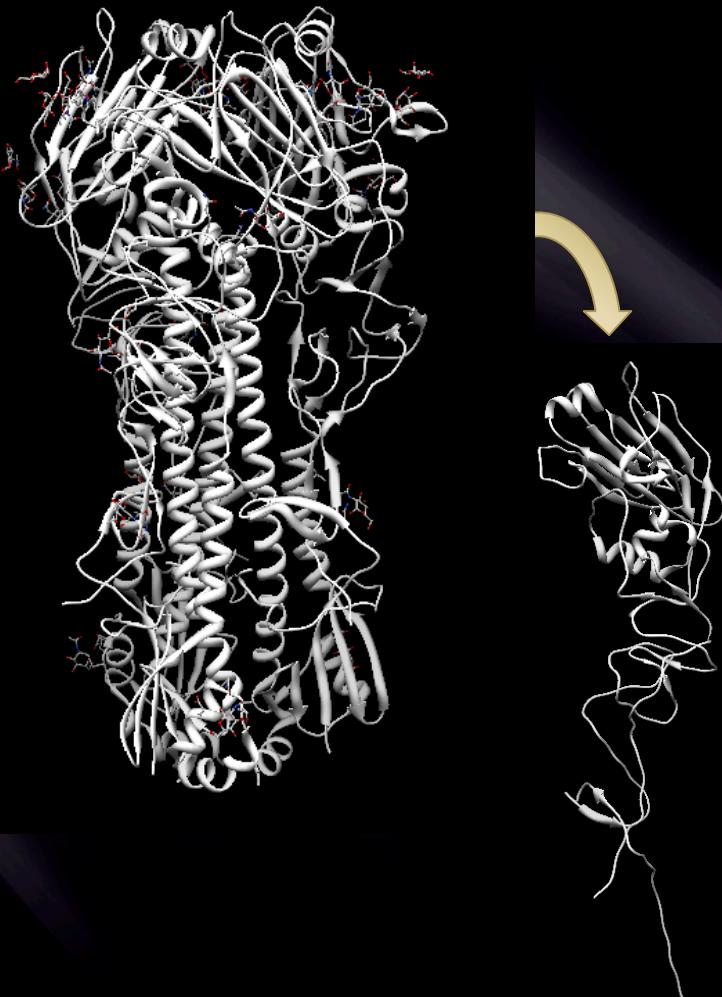
- H3 (1MQL)
 - Contains a total of 6 chains:
 - HA1 is composed of chains A, D, and G
 - HA2 is composed of chains B, E, and H
 - Length (Å):
 - a = 147.68
 - b = 147.10
 - c = 251.99
- H5 (3EYM)
 - Contains a total of 6 chains:
 - HA1 is composed of chains A, D, and G
 - HA2 is composed of chains B, 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

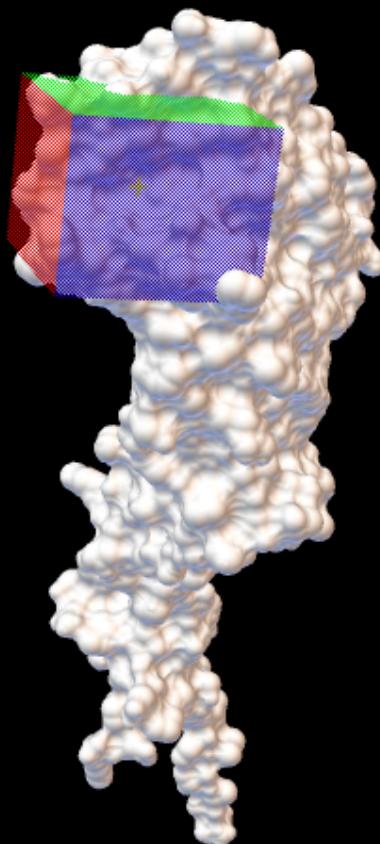
Alignment

- Alignment allows for the use of the same gridbox and docking molecules would be in the same area

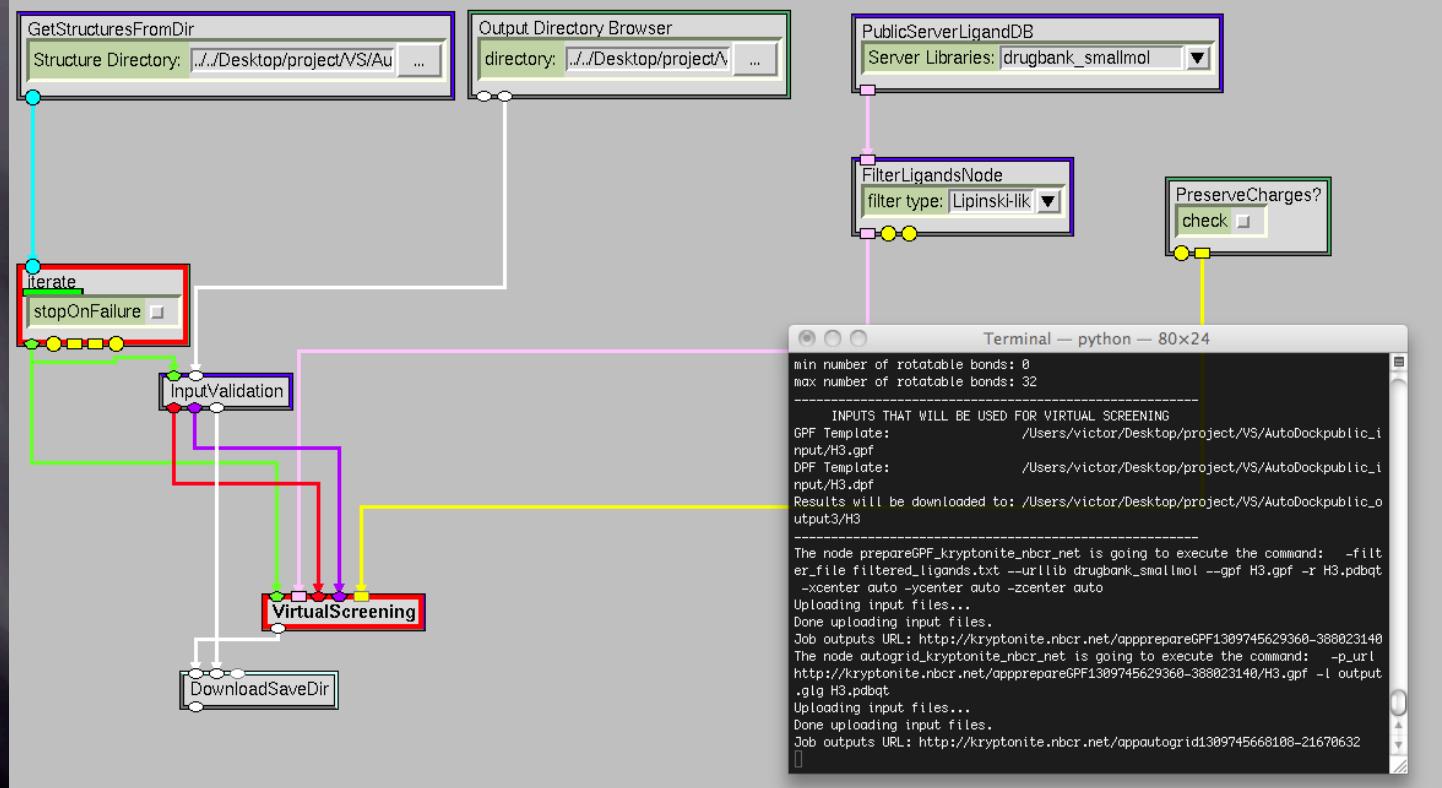


Prepared for Virtual Screening

- 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



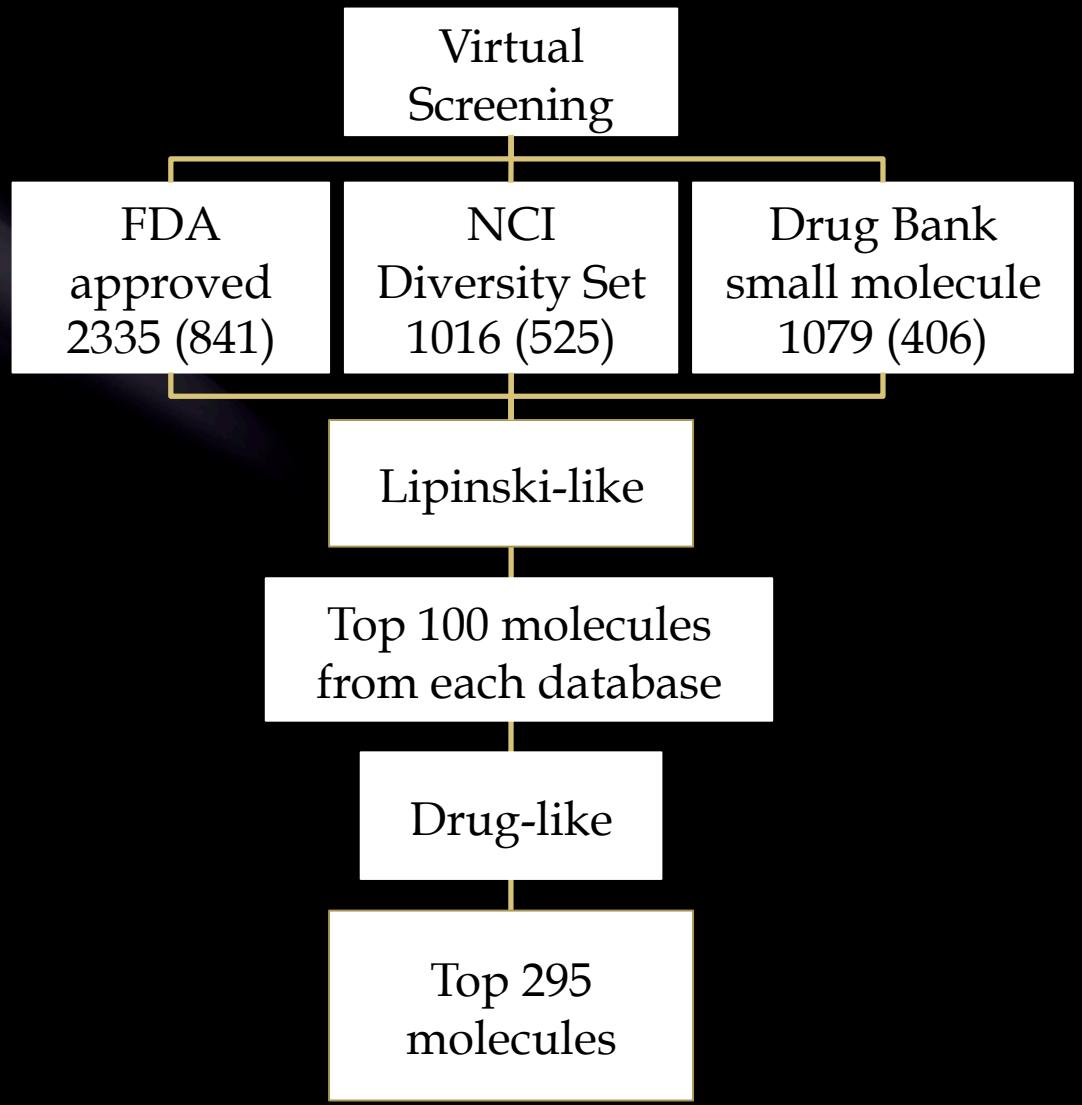
Virtual Screening



- 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

Virtual Screening

- Public Virtual Screening had to be preformed for each databank
- Local Virtual Screening had to be preformed for Drug-like filter



Analysis

F	G	H	I	J	K	L	M	Styles	Spacing	Lists
lowestEnergy_dgfn	#runs	#cl	#LEC	LE	rmsd_	LE	largestCI_dgfn			
ZINCO1561925	50	20	4	-9.08	124.8832	ZINCO1561925	ZINCU4582957/ZINCU4582997, 50, 4, 31, -7,-USOU, 120.9853,, ZINCU4582957/ZINCU4582997, 50, 4, 31, -7,-			
ZINCO4430013	50	3	48	-8.75	121.4471	ZINCO4430013	ZINCO1743643	ZINCO1743643	ZINCO1743643/ZINCO1743643, 50, 15, 16, -6,	
ZINCO1588813	4	30	-	-8.24	125.3222	ZINCO1588813	ZINCO3954451/ZINCO3954451, 50, 9, 22, -6, -			
ZINCO1588816	4	32	-	-8.24	126.3113	ZINCO1588816	ZINCO4683870/ZINCO4683870, 50, 3, 36, -6, -			
ZINCO374077	3	48	-	-8.21	126.3092	ZINCO374077	ZINCO1760323/ZINCO1760323, 50, 14, 21, -6, -			
ZINCO4792016	5	33	-	-8.15	124.1617	ZINCO4792016	ZINCO1657023/ZINCO1657023, 50, 15, 16, -6, -			
ZINCO1724549	7	24	-	-8.08	120.2561	ZINCO1724549	ZINCO1760323/ZINCO1760323, 50, 2, 46, -6, -			
ZINCO1588814	3	48	-	-8.07	124.8239	ZINCO1588814	ZINCO3954030/ZINCO3954030, 50, 6, 16, -6, -			
ZINCO1588230	4	17	-	-7.86	119.0178	ZINCO1588230	ZINCO5766249/ZINCO5766249, 50, 9, 21, -6, -			
ZINCO5648459	10	24	-	-7.84	126.213	ZINCO5648459	ZINCO3918156/ZINCO3918156, 50, 12, 25, -6, -			
ZINCO1588815	7	34	-	-7.82	124.4792	ZINCO1588815	ZINCO4416487/ZINCO4416487, 50, 1, 50, -6, -			
ZINCO4080423	9	26	-	-7.8	123.579	ZINCO4080423	ZINCO1657023/ZINCO1657023, 50, 1, 50, -6, -			
ZINCO1671616	1	50	-	-7.73	122.1852	ZINCO1671616	ZINCO1667461/ZINCO1667461, 50, 3, 43, -6, -			
ZINCO3953952	16	8	-	-7.72	124.7326	ZINCO3953952	ZINCO4416489/ZINCO4416489, 50, 1, 50, -6, -			
ZINCO1532935	14	18	-	-7.7	123.0342	ZINCO1532935	ZINCO1721006/ZINCO1721006, 50, 2, 43, -6, -			
ZINCO3953811	14	3	-	-7.83	121.7227	ZINCO3953811	ZINCO1657023/ZINCO1657023, 50, 11, 20, -6, -			
ZINCO1573832	7	7	-	-7.75	128.1809	ZINCO1573832	ZINCO3918156/ZINCO3918156, 50, 1, 50, -6, -			
ZINCO1588461	5	43	-	-7.64	122.7558	ZINCO1588461	ZINCO4416487/ZINCO4416487, 50, 1, 50, -6, -			
ZINCO3954590	19	3	-	-8.06	120.2939	ZINCO3954590	ZINCO1693097/ZINCO1693097, 50, 3, 35, -6, -			
ZINCO1757978	7	20	-	-7.59	123.4415	ZINCO1757978	ZINCO1706827/ZINCO1706827, 50, 2, 48, -6, -			
ZINCO1607465	17	9	-	-7.56	125.2247	ZINCO1607465	ZINCO1657023/ZINCO1657023, 50, 9, 39, -6, -			
ZINCO5648460	14	17	-	-7.55	127.0493	ZINCO5648460	ZINCO1657023/ZINCO1657023, 50, 9, 39, -6, -			
ZINCO1570771	4	19	-	-7.54	117.3385	ZINCO1570771	ZINCO1646281/ZINCO1646281, 50, 9, 39, -6, -			
ZINCO1532529	21	8	-	-7.52	126.5791	ZINCO1532529	ZINCO1722261/ZINCO1722261, 50, 1, 50, -6, -			
ZINCO163576	19	14	-	-7.48	125.549	ZINCO163576	ZINCO1657421/ZINCO1657421, 50, 1, 50, -6, -			
ZINCO1704721	11	6	-	-7.54	125.2218	ZINCO1704721	ZINCO1657023/ZINCO1657023, 50, 1, 50, -6, -			
ZINCO3954520	4	15	-	-7.45	126.9528	ZINCO3954520	ZINCO4416489/ZINCO4416489, 50, 9, 37, -6, -			
ZINCO1723557	3	13	-	-7.5	125.1928	ZINCO1723557	ZINCO1657023/ZINCO1657023, 50, 1, 50, -6, -			
ZINCO3876078	15	2	-	-7.69	121.2651	ZINCO3876078	ZINCO1581022/ZINCO1581022, 50, 5, 35, -6, -			
ZINCO0831029	22	9	-	-7.37	116.6874	ZINCO0831029	ZINCO1581022/ZINCO1581022, 50, 5, 35, -6, -			
ZINCO3953843	15	14	-	-7.37	121.2411	ZINCO3953843	ZINCO0351092/ZINCO0351092, 50, 6, 26, -6, -			
ZINCO3954358	9	14	-	-7.29	125.4601	ZINCO3954358	ZINCO1578482/ZINCO1578482, 50, 1, 50, -6, -			
ZINCO1578482	28	8	-	-7.25	123.7586	ZINCO1578482	ZINCO1646483/ZINCO1646483, 50, 9, 39, -6, -			
ZINCO3954311	7	19	-	-7.25	124.7401	ZINCO3954311	ZINCO1692120/ZINCO1692120, 50, 15, 26, -6, -			
ZINCO1761349	3	48	-	-7.24	120.7504	ZINCO1761349	ZINCO1769075/ZINCO1769075, 50, 2, 38, -6, -			
ZINCO1717025	2	47	-	-7.23	126.1574	ZINCO1717025	ZINCO180912/ZINCO180912, 50, 8, 15, -6, -			
ZINCO05351088	4	46	-	-7.21	120.7485	ZINCO05351088	ZINCO1657023/ZINCO1657023, 50, 10, 23, -6, -			
ZINCO4538409	19	3	-	-7.76	124.7936	ZINCO4538409	ZINCO1657023/ZINCO1657023, 50, 10, 23, -6, -			
ZINCO1701547	2	38	-	-7.19	126.2799	ZINCO1701547	ZINCO1657023/ZINCO1657023, 50, 10, 23, -6, -			
ZINCO1668172	10	9	-	-7.36	122.8605	ZINCO1668172	ZINCO1668253/ZINCO1668253, 50, 8, 12, -6, -			
ZINCO1691364	5	18	-	-7.25	123.7628	ZINCO1691364	ZINCO1769083/ZINCO1769083, 50, 4, 23, -6, -			
ZINCO1711063	6	17	-	-7.27	122.5094	ZINCO1711063	ZINCO1668253/ZINCO1668253, 50, 12, 23, -6, -			
ZINCO1677764	1	50	-	-7.11	128.6468	ZINCO1677764	ZINCO1668253/ZINCO1668253, 50, 8, 15, -6, -			
ZINCO1588236	6	3	-	-7.3	128.5651	ZINCO1588236	ZINCO1619033/ZINCO1619033, 50, 13, 9, -6, -			
ZINCO1572081	9	27	-	-7.07	123.1751	ZINCO1572081	ZINCO3953867/ZINCO3953867, 50, 3, 11, -6, -			
ZINCO1657016	23	7	-	-7.36	124.476	ZINCO1657016	ZINCO3953867/ZINCO3953867, 50, 13, 10, -6, -			
ZINCO3953903	7	16	-	-7.15	128.1	ZINCO3953903	ZINCO1653697/ZINCO1653697, 50, 24, 10, -6, -			
ZINCO4428805	13	22	-	-7.07	124.482967	ZINCO4428805	ZINCO180912/ZINCO180912, 50, 7, 28, 4			
ZINCO4468297	3	32	-	-7.06	127.482967	ZINCO4468297	ZINCO1657023/ZINCO1657023, 50, 30, 4			
ZINCO4376856	7	5	-	-7.21	128.856	ZINCO4376856	ZINCO1657023/ZINCO1657023, 50, 3, 27, 3			
ZINCO3954451	10	16	-	-6.98	124.	ZINCO3954451	ZINCO1657023/ZINCO1657023, 50, 3, 27, 3			
ZINCO4683870	2	36	-	-6.96	121.9	ZINCO4683870	ZINCO1657023/ZINCO1657023, 50, 21, 24, 2			

- Converted summarized data from each Virtual Screen into excel files for analysis
- Noted similarities between databases and highlighted non drug-like molecules

ZINC Database

UCSF University of California, San Francisco | About UCSF | Search UCSF | UCSF Medical Center Shoichet Laboratory docking.org

A free database for virtual screening

ZINC
is not commercial

Search Results

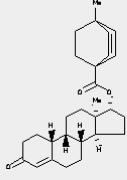
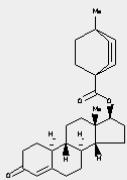
Home Subsets HELP! Mailing Lists

Performing the following mappings of retired ZINC codes... 3881612 -> 3881613 8552417 -> 17146534 Initial search took 0 second(s). Beginning : #1 Hits: 97 Display: per page: 100

Next Page Download Table Purchasing Info

Download: SMILES MOL2 SDF Flexibase Show Detail?

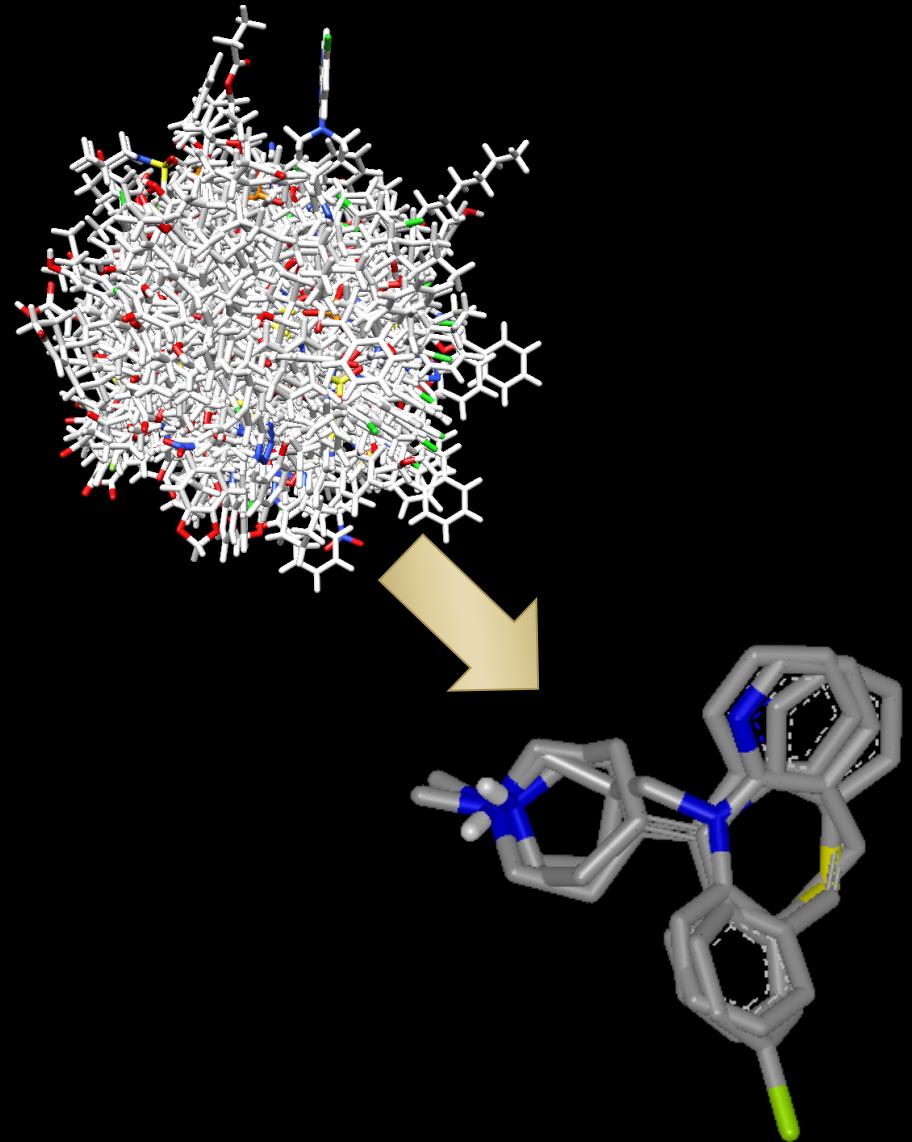
Done

ZINC ID	Supplier information; Representations Properties: xLogP, ap & p desolvation, HBD,HBA,Charge,Mwt,NRB Annotations; Similarity	Structure Click for quick 3D display
11681511 #1  	FDA-approved drugs (via DSSTOX); 812 ref: mol2, SDF, SMILES, flexibase 5.50,3.29,-10.07,0,3,0, 422.609, 3 Similar to: 39580768 , 39580767 , 4217038 . SEA predictions: 3-beta-hydroxysteroid dehydrogenase/delta 5->4-isomerase type I pValue:17 maxTC:28 (annotated) Estrogen receptor alpha pValue:12 maxTC:48 (annotated) Estrogen receptor beta pValue:12 maxTC:48 (annotated) Steroid 5-alpha-reductase 2 pValue:18 maxTC:32 (annotated) Progesterone receptor pValue:16 maxTC:48 (annotated) ... 10 in total.	
3831189 #2  	FDA-approved drugs (via DSSTOX); 812 ref: mol2, SDF, SMILES, flexibase 5.50,3.32,-8.4,0,3,0, 422.609, 3 Similar to: 4217038 , 1318408 . SEA predictions: Estrogen receptor alpha pValue:12 maxTC:48 (annotated) Progesterone receptor pValue:16 maxTC:48 (annotated) Progesterone receptor pValue:11 maxTC:31 (annotated) 3-beta-hydroxysteroid dehydrogenase/delta 5->4-isomerase type I pValue:17 maxTC:28 (annotated) Androgen Receptor pValue:11 maxTC:42 (annotated) ... 10 in total.	

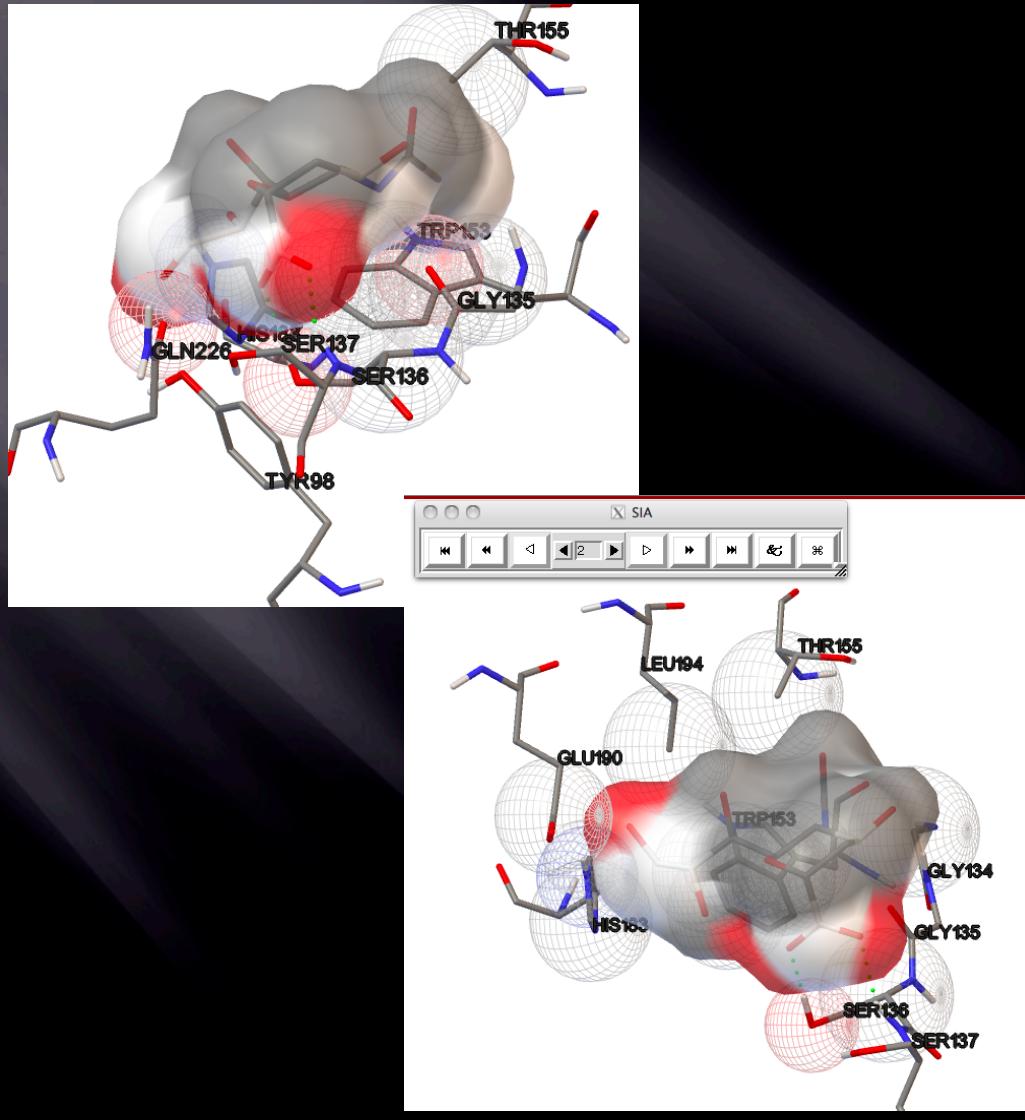
- Checked all molecules on ZINC database
 - Noting retired molecules and their replacements
 - Downloaded SDF to use on vRocs to group molecules by shape

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

Molecule	#runs	#cl	#LEC	LE	rmsd_LE	#cl	#LC	#LE_LC	#rmsd_LC	#ats	#tors	#lig_eff	Data Base
ZINC01561925	50	20	4	-9.08	124.8832	20	12	-8.89	126.3897	28	4	-0.3243	NCIDS
ZINC11681511	50	4	28	-8.60	126.5994	4	28	-8.60	126.5994	31	3	-0.2774	FDA
ZINC12153177	50	8	24	-8.51	120.8112	8	24	-8.51	120.8112	31	7	-0.2837	FDA
ZINC11682116	50	16	16	-8.26	127.5816	16	16	-8.26	127.5816	36	10	-0.2360	DBSM
ZINC13463906	50	4	30	-8.24	125.3222	4	30	-8.24	125.3222	30	5	-0.2747	NCIDS

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

Acknowledgements

- UCSD PRIME

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