Modeling Glycan Diversity to Better Understand H1N1 Receptor Binding Specificity

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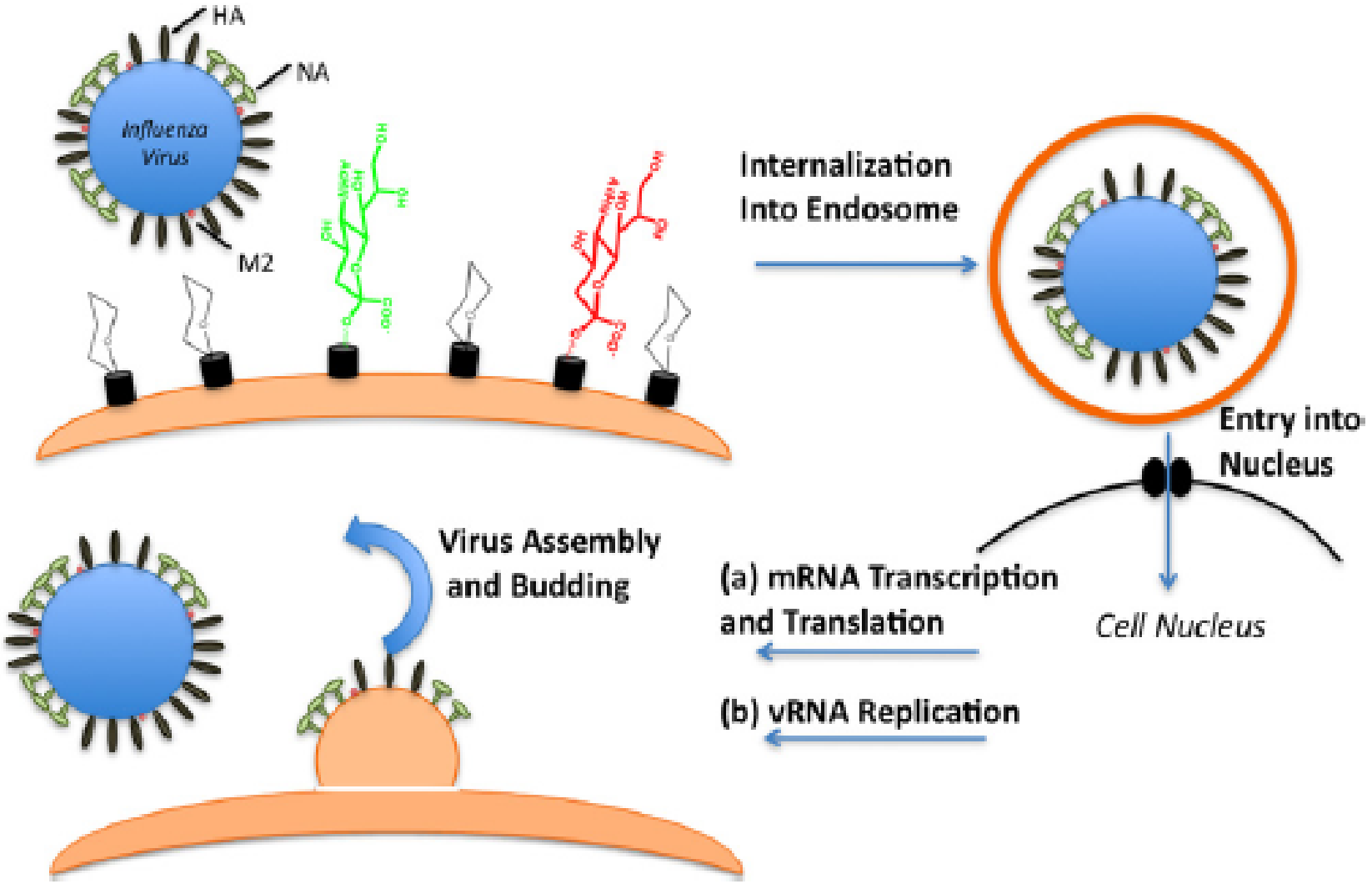
PRIME 2010

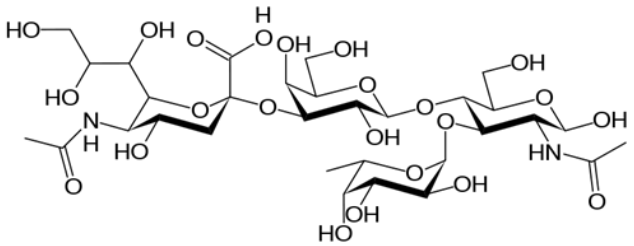
Influenza Virus Life Cycle



- Image via Z. Shriver, R. Raman, K. Viswanathan, R. Sasisekharan. (2009) Context-Specific Target Definition in Influenza A Virus Hemagglutinin-Glycan Receptor Interactions. *Chemistry and Biology,* v. 16, p. 803-814

Specific Aims

- *Model glycan diversity*
  - sulfation and fucosylation of tetrasaccharides as found in Sialyl Lewis X, a blood antigen



Sialyl Lewis X,

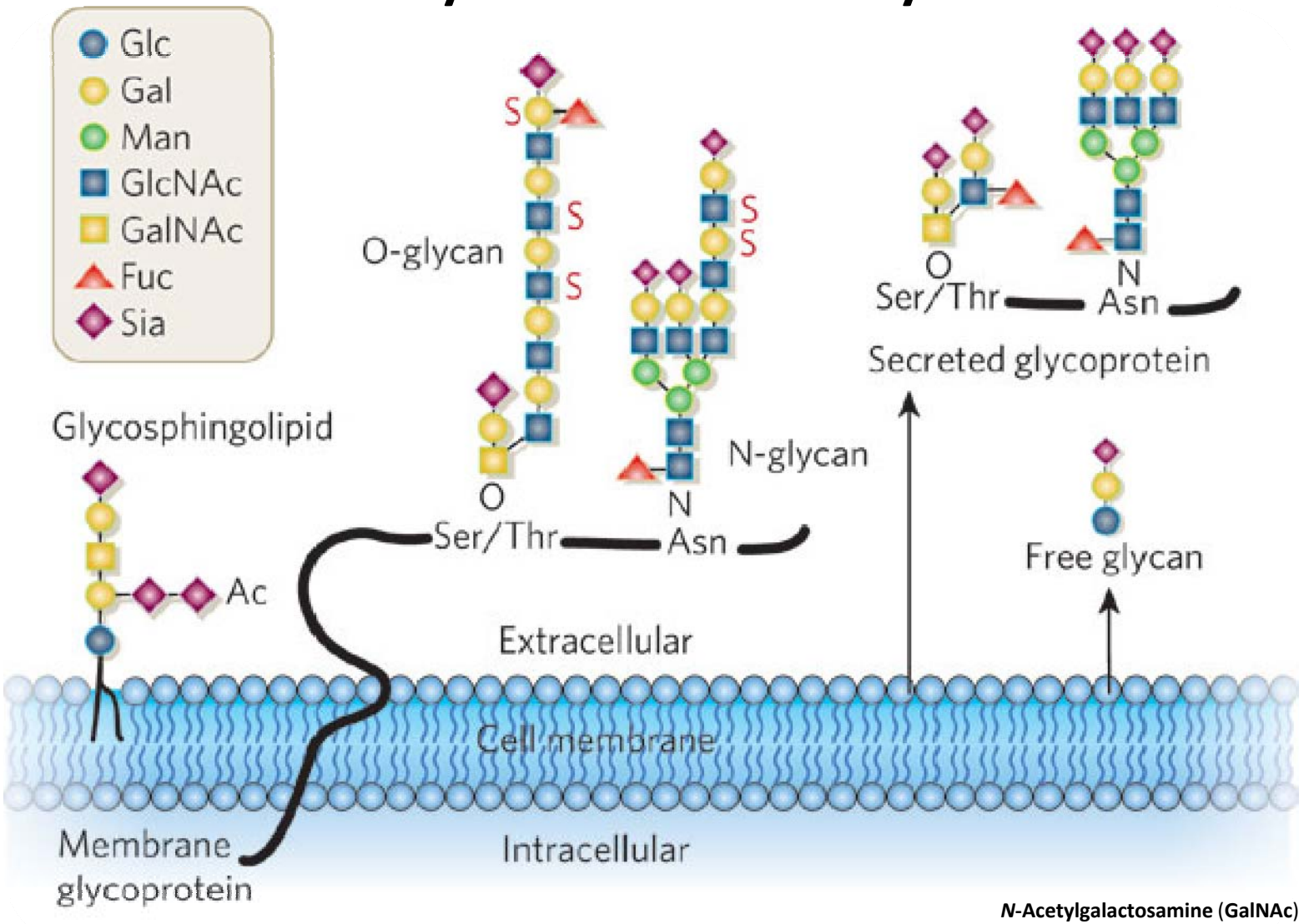
  - for study in H1N1 HA and NA receptor binding specificity
- *Use a docking program to measure relative binding affinities of different glycans to 2009 H1*

Project Goal

- To find whether the sulfate groups will create increased interactions with the H1 binding site
- Lead to drug analogue design that will prefer the sulfated glycan over human glycans that lack sulfate groups

- Ultimately, help prevent the spread of H1 viral disease

Glycan Diversity



***N*-Acetylgalactosamine (GalNAc)**

N.M.Varki, A.Varki. (2007) *Pathobiology in Focus.* Diversity in Sialic Acid Presentations: implications for biology and disease. *Laboratory Investigation*, p. 1-7

Methodology

- Collect PDB's of H1's that have >70% similarity→10 PDB's
- Compare binding sites of H1's
  - Multiple Sequence Analysis
  - Superimposition, only 3 H1's have ligands
  - Derive reference for docking results
- Review docking software functions
  - Why Vina chosen over AD4
- Sulfate Sialyl Lewis X for docking from 2D structures
  - see Gambaryan paper
- Selection of Receptor PDB for Docking
  - Why 3LZG selected to dock→ resolution, relevance
- Docking SuSLex using Vina
  - Shattered results→ docking can only vary torsion, orientation, translation
  - Needed to optimize structure
  - Results don't look like reference structure
- Modify parameters
- Continue modifying parameters to achieve better results

That's how nature does it.

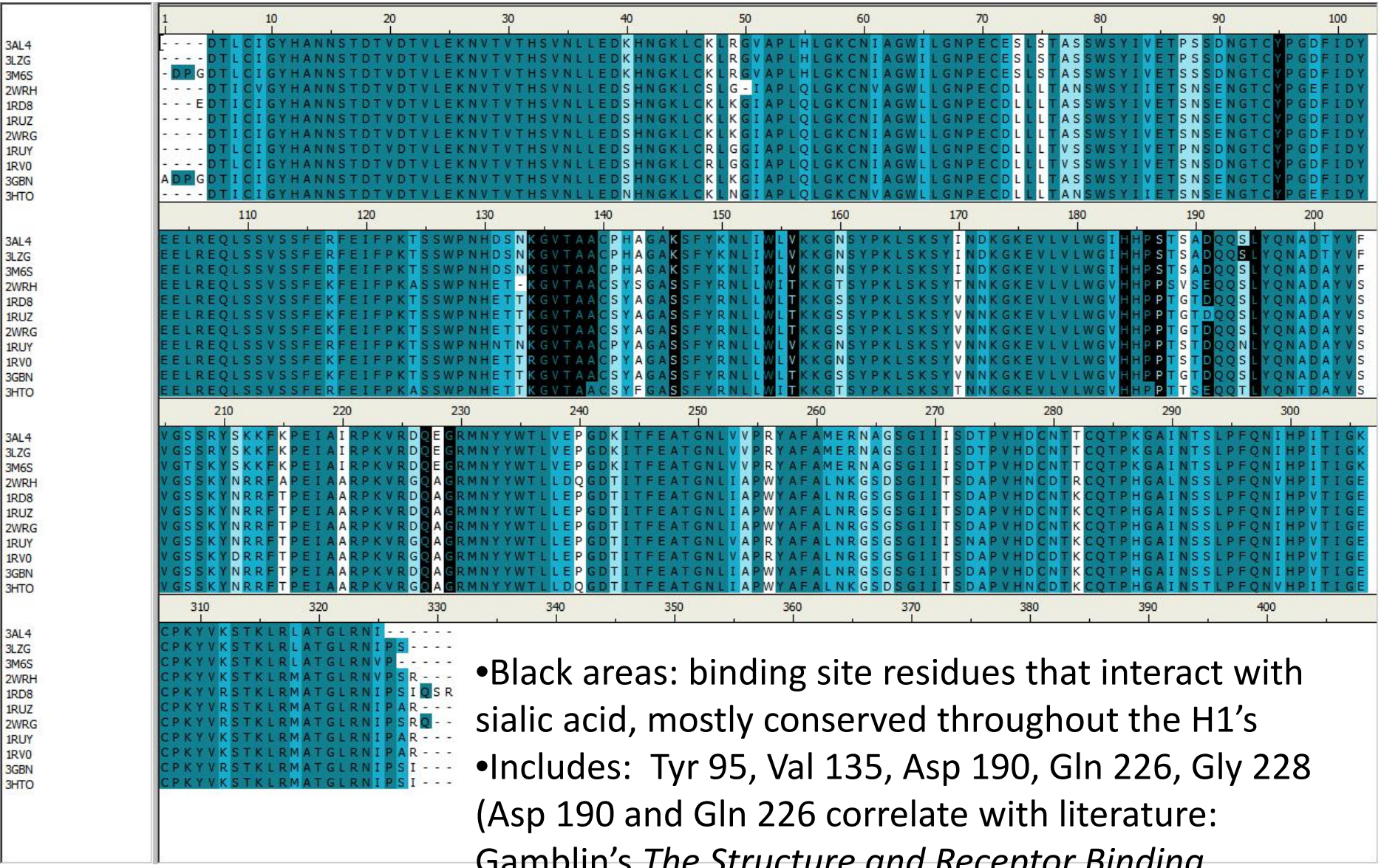
To find the natural binding site behavior of H1 with ligand:

- Use Sequence Search tool in PDB
  - found 10 H1's with ≥70% similarity to my H1 of interest (3LZG)
  - 3M6S, 3AL4, 2WRG, 1RD8, 3GBN, 2WRH, 1RUZ, 1RUY, 3HTO, 1RV0
- Superimpose H1's using DS using Multiple Sequence Analysis

Table of H1 PDB's

PDB Code	Isolated from	Year of Strain	Resolution
3LZG	Human	2009	2.60
3M6S	Human	2010	2.80
3AL4	Human	2010	2.87
2WRG	Human	1918	3.00
1RD8	Human	1918	3.00
3GBN	Human	1918	2.20
2WRH	Duck	1957	3.00
1RUZ	Human	1918	2.90
1RUY	Swine	1930	3.00
3HTO	Avian	2009	2.90
1RV0	Swine	1930	2.50

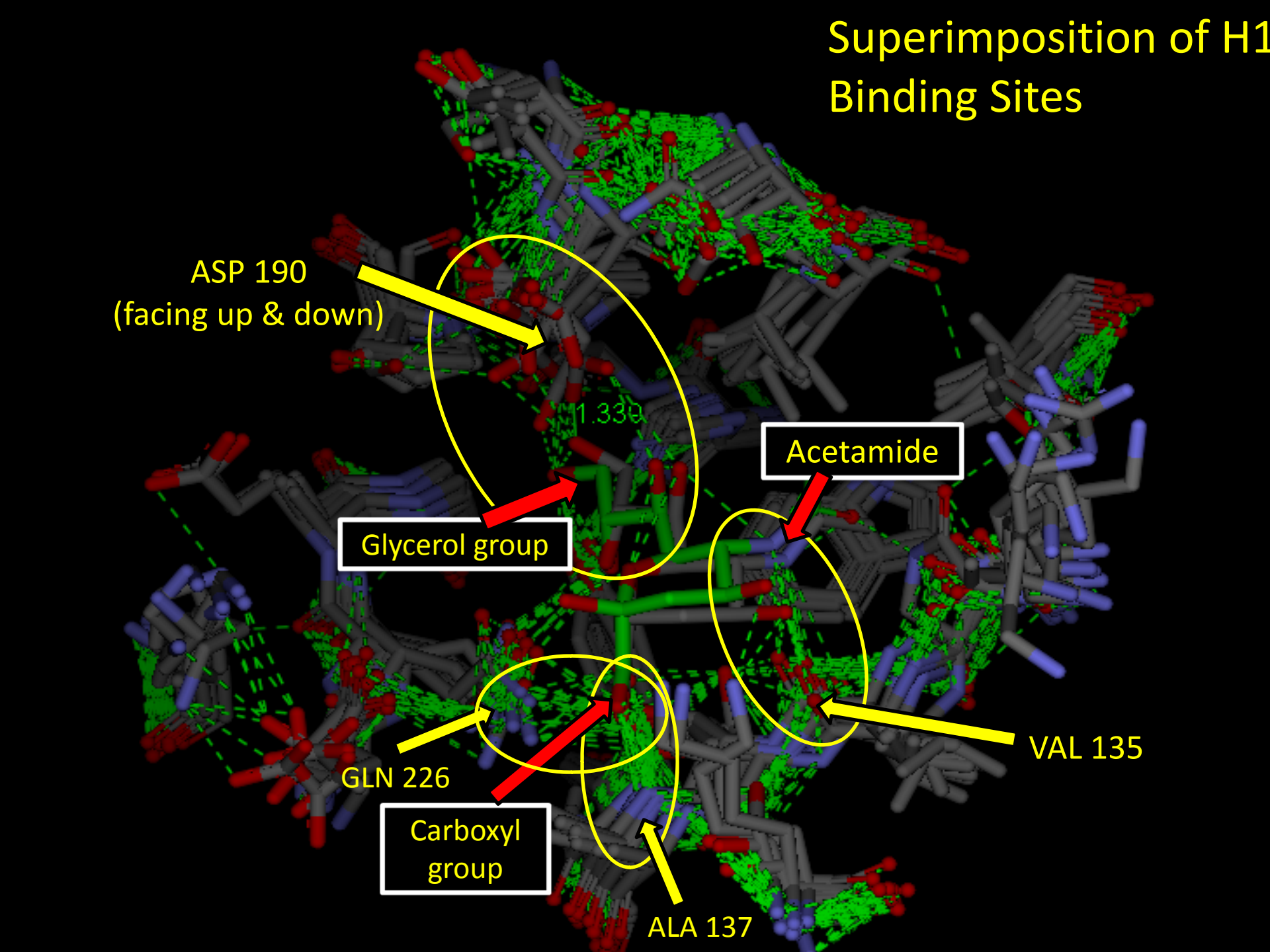
Multiple Sequence Alignment



- Black areas: binding site residues that interact with sialic acid, mostly conserved throughout the H1's
- Includes: Tyr 95, Val 135, Asp 190, Gln 226, Gly 228 (Asp 190 and Gln 226 correlate with literature: Gamblin's *The Structure and Receptor Binding Properties of 1918 Hemagglutinin*)

Multiple Sequence Alignment

- The darker the color, the more similar/identical the sequences
- Sequence Similarity: 86.7%
- Sequence Identity: 76.3%
- The high sequence similarity/identity within the binding site indicates that the ligand will bind quite similarly across the H1's
- Black areas highlight the highly conserved binding site


Superimposition of H1
Binding Sites
ASP 190
(facing up & down)
1.33Å
Acetamide
Glycerol group
VAL 135
GLN 226
Carboxyl
group
ALA 137

Stabilizing Interactions between SA and H1 receptors

- **Glycerol group**
  - H bonds with TYR 95
  - Holo form (ligand bound): ASP 190 flips above glycerol, permitting a less sterically hindered interaction
  - Apo form (ligand free): ASP 190 and glycerol are sterically hindered→ affects glycerol group interaction
- **Carboxyl group**
  - Forms H bonds with ALA 137, GLN 226, THR 136

Stabilizing Interactions between SA and H1 receptor

- **Acetamide group**
  - N forms H bonds with VAL 135

Experimental Evidence suggesting High BA of H1 for Su-SLex

Higher values: lower binding affinity

Red: maximal binding
Yellow: good binding
Cyan: weak binding
Blue: no detectable binding

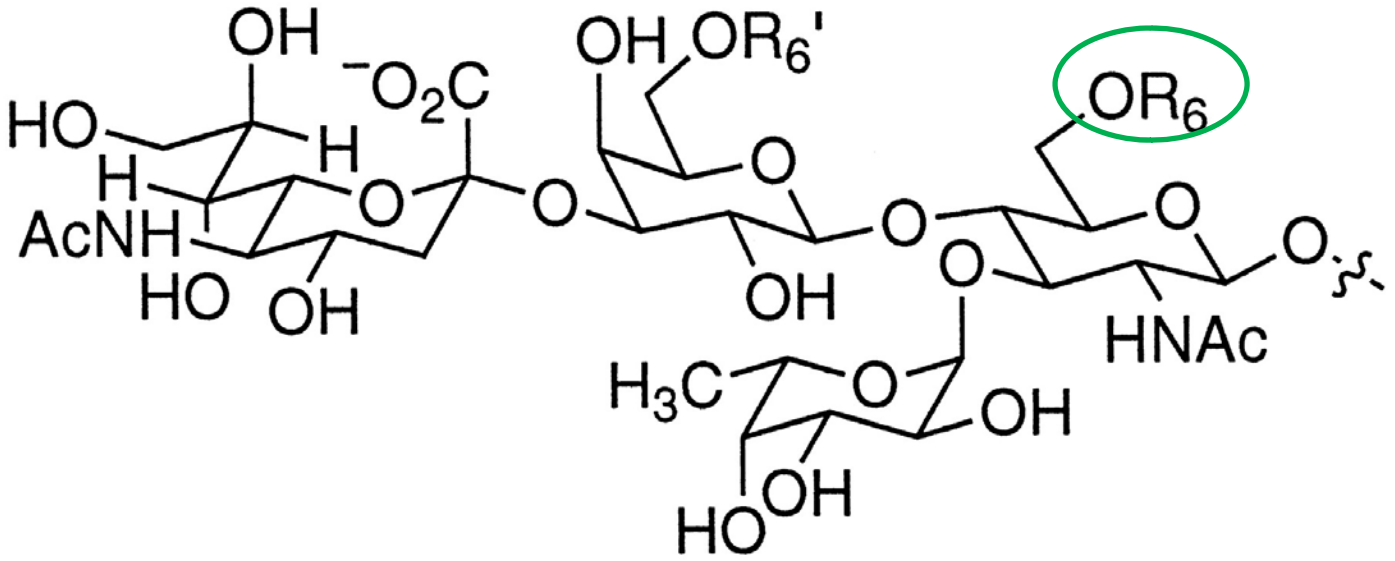
from Gambaryan's paper, *6-sulfo sialyl Lewis X is the common receptor determinant recognized by H5, H6, H7 and H9 influenza viruses of terrestrial poultry*

Virus		Sialylglycoconjugate					
		3`SLN	Su-3`SLN	SLe^x	Su-SLe^x	SLe^c	6`SLN
Duck viruses							
Duck /Hong Kong/278/78	H2N9	20	10	>50	>50	10	>5000
Duck/Nanchang/2-0485/00	H2N9	20	10	>50	>50	10	>5000
Duck/Buryatia/652/88*	H3N8	10	10	50	50	5	>5000
Mallard/New York/670/78	H4N6	4	8	100	>200	2	>5000
Duck /Buryatiya/1905/00*	H4N6	20	20	100	100	10	>5000
Duck/Primorie/3628/02*	H9N2	10	8	100	30	6	>5000
Mallard/Netherlands/02/00	H10N4	20	7	>50	30	5	>5000
H6							
Turkey/Massachusetts/65	H6N2	20	30	20	30	10	>5000
Shearwater/Australia/1/72	H6N5	20	30	10	30	15	>5000
Teal/Hong Kong/W312/97	H6N1	50	>50	20	>50	20	>5000
Chicken/New York/13237/98	H6N8	7	10	7	20	5	>5000
Gull/Moscow/3100/06	H6N2	5	5	2	2	3	>5000
H7							
Turkey/Virginia/4529/02	H7N2	2	1	20	4	10	200
Avian/New York/273874/03	H7N2	10	2	30	8	20	500
Chicken /NJ/294598-12/04	H7N2	5	1	20	5	5	200
Chicken /Delaware/296763/04	H7N2	15	5	30	15	20	200
New York/107/03	H7N2	10	2	>100	15	20	200
Netherlands/219/03	H7N7	4	1	5	0.5	5	500
Netherlands/230/03	H7N7	4	1	5	0.5	5	500
Netherlands/231/03	H7N7	4	1	5	0.5	5	500
H9N2							
Goose/Minnesota/5773/80	H9N2	20	10	50	20	10	>5000
Turkey/Wisconsin/1/66	H9N2	5	5	20	30	2	>5000
Turkey/Minnesota/38391-6/95	H9N2	10	10	10	20	5	>5000
Pheasant/Wisconsin/1780/88	H9N2	10	3	2	1	10	>5000
Chicken/New Jersey/12220/97	H9N2	5	5	0.5	0.5	5	>5000
Chicken/Korea/96323/96	H9N2	10	3	30	10	10	>5000
Hong Kong/1073/99	H9N2	>100	>100	>100	>100	>100	20
Quail/Hong Kong/G1/97	H9N2	>100	>100	20	20	>100	20
Chicken/Hong Kong/FY20/99	H9N2	>100	3	>100	5	>100	50
Duck/Hong Kong/Y280/97	H9N2	>100	0.3	>100	0.1	>100	10
Chicken/Hong Kong/G9/97	H9N2	>100	5	>100	10	>100	50
Chicken/Hong Kong /SF3/99	H9N2	>100	3	>100	5	>100	50
Hong Kong/2108/03	H9N2	>100	10	>100	10	>100	50
Swine							
Swine/Hong Kong/9/98	H9N2	>100	1	>100	2	>100	50
Swine/Finistere/2899/82*	H1N1	30	30	20	5	30	100
Swine/France/80*	H1N1	30	20	40	10	40	100
Swine/Kazakhstan/48/82*	H3N6	10	3	40	3	15	1000
Equine							
Equine/Kentucky/5/02	H3N8	3	1	10	1	4	>5000
Equine/Ohio/1/03	H3N8	3	0.5	5	0.4	4	>5000
Canine/Florida/43/04	H3N8	3	0.5	5	0.4	4	>5000
H5N1							
Chicken/ Hong Kong /220/97	H5N1	3	0.5	80	20	10	>5000
Chicken/Vietnam/NCVD11/03	H5N1	1	0.3	20	1	2	>5000
Human pandemic viruses							
USSR/039/68	H3N2	>200	>200	>200	>200	>200	4
Canada/228/68	H3N2	>200	>200	>200	>200	>200	2

H1 Binding Affinity for Su-SLex

Swine	3'SLN	Su-3'SLN	SLex	Su-SLex	Su-Slex	SLec	6'SLN
Swine/Hong Kong/9/98	H9N2	>100	1	>100	2	>100	50
Swine/Finistere/2899/82*	H1N1	30	30	20	5	30	100
Swine/France/80*	H1N1	30	20	40	10	40	100
Swine/Kazakhstan/48/82*	H3N6	10	3	40	3	15	1000

2D Structure of Su-SLex



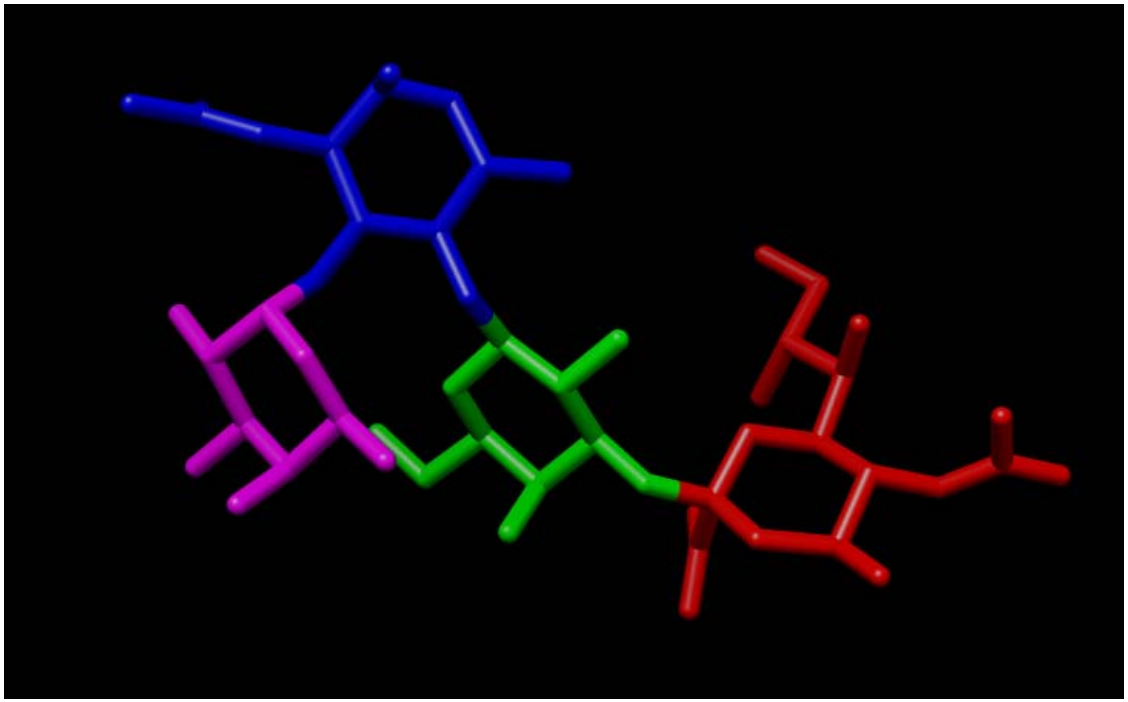
1 $R_{6'} = R_6 = H$ sialyl Lewis x

2 $R_{6'} = H$, $R_6 = SO_3^-$ 6-sulfo sialyl Lewis x

3 $R_{6'} = SO_3^-$, $R_6 = H$ 6'-sulfo sialyl Lewis x

Image from jbc.org

Sialyl Lewis X



SIA=O-sialic acid
NDG=2-(acetylamino)-2-deoxy-a-d-glucopyranose
GAL=Beta-d-galactose
FUC=Alpha-l-fucose

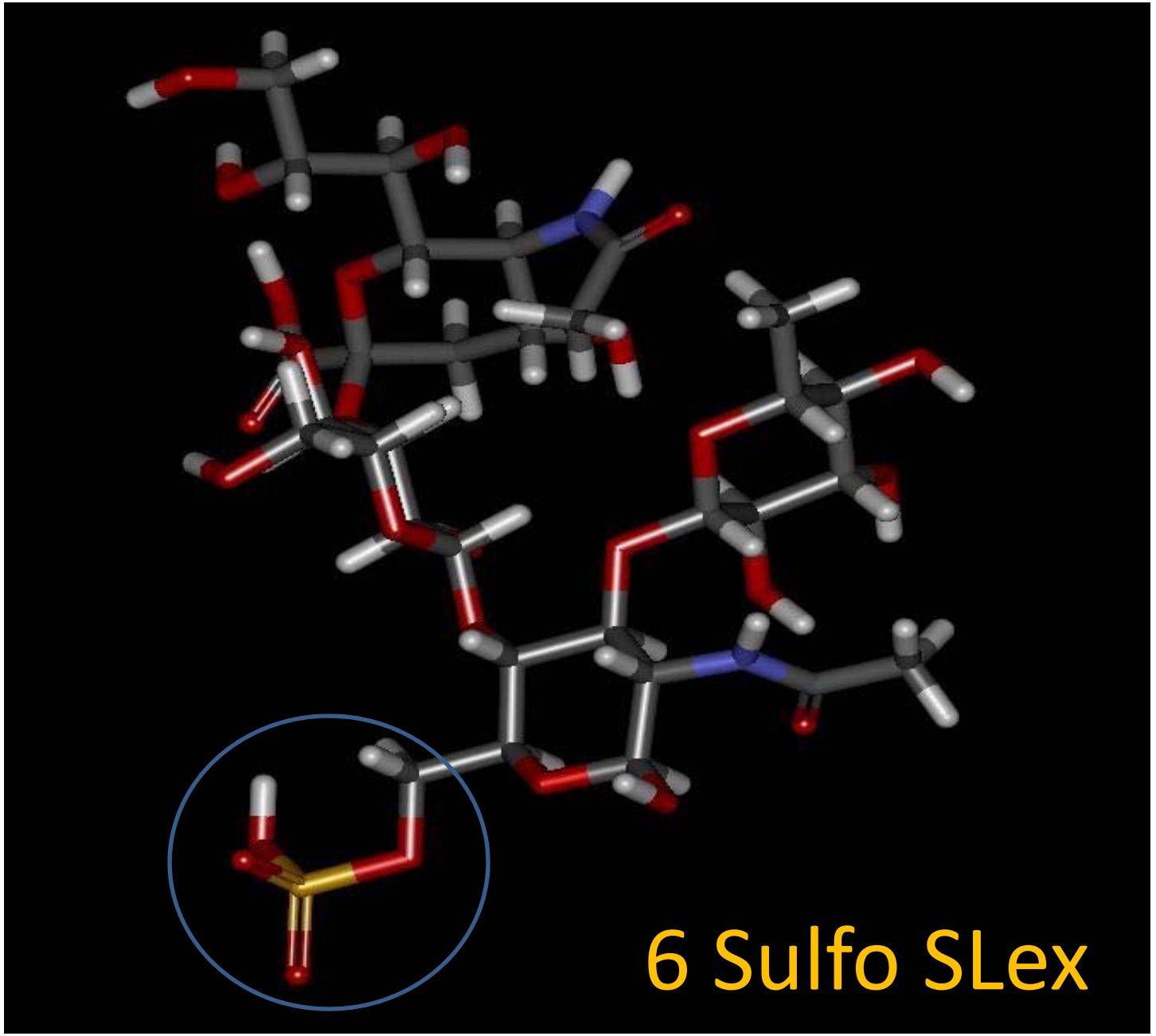

6 Sulfo SLex

Table of torsions and evals

Number of Torsions	ga_num_evals	ga_num_generations
0	25 000 to 250 000	27 000
1-10	250 000 to 25 000 000	27 000
>10	>25 000 000	27 000

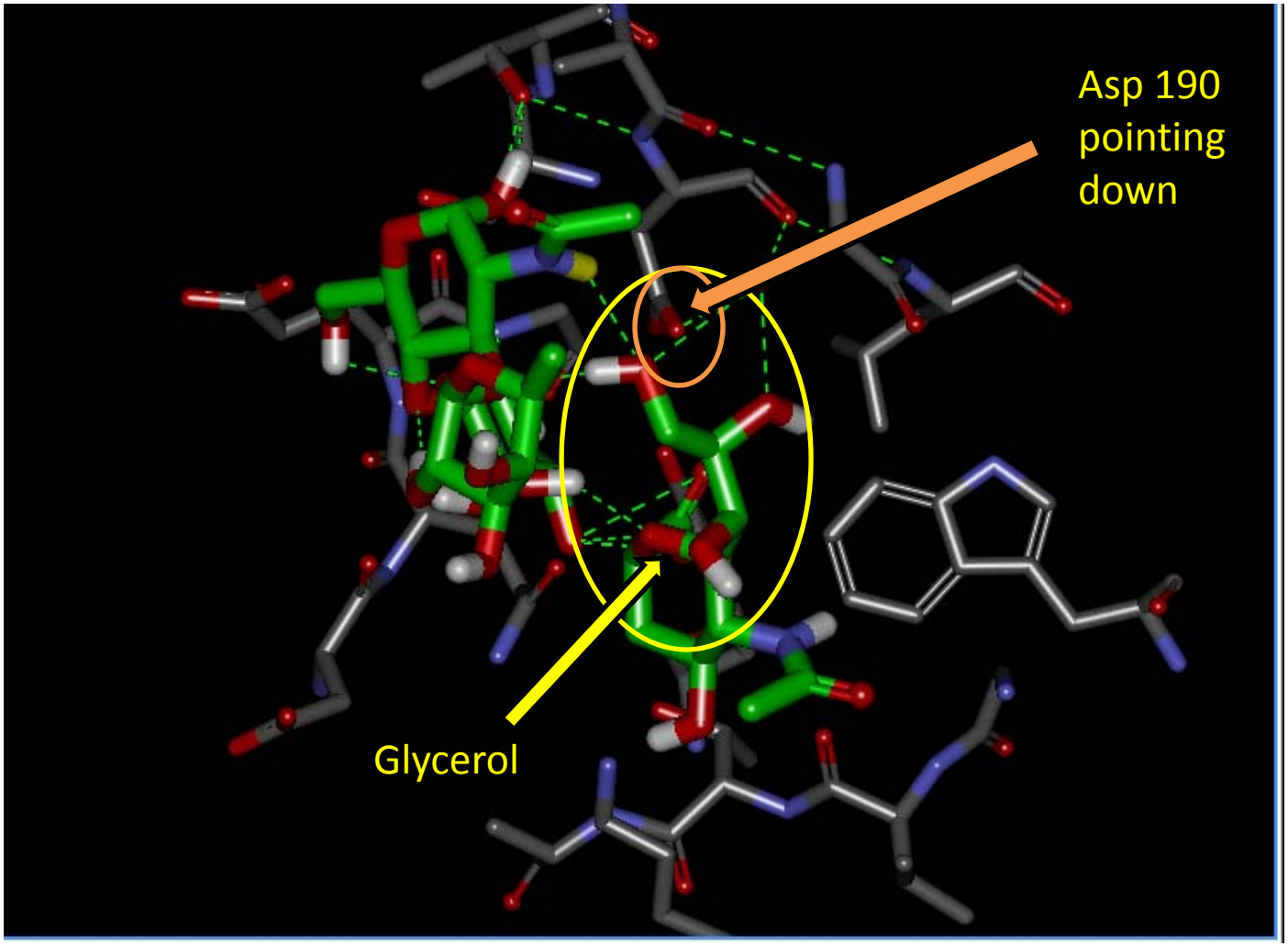
Since my ligand has too many torsions (26 torsions), would need more than 25 million ga_num_evals (energy runs)

Would take over two days to dock using Genetic Algorithm on AD4

Not efficient as trying another method to dock→ use Vina over AD4

Table taken from Hetenyi, C. and van der Spoel, D. (2002) Efficient docking of peptides to proteins without prior knowledge of the binding site. *Protein Science*, 11(7): 1729-1737.

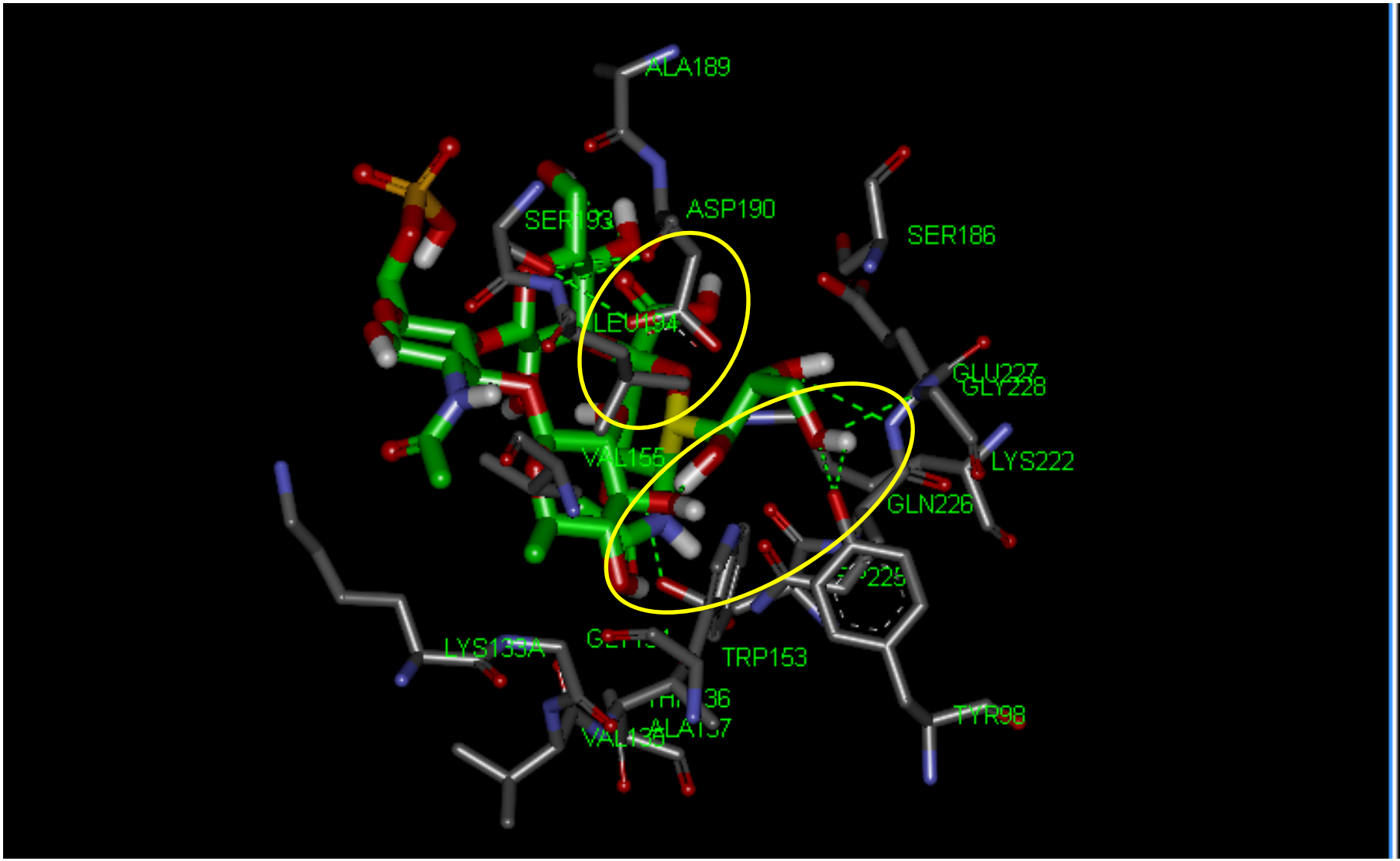
3LZG with SLex Docking Result (-5.7 kcal/mol)



-3LZG is not ideal structure

-ASP 190 is not the right position, facing down from glycerol rather than away from it.

3LZG with Su-SLex



ASP 190 still facing down, sterically hindering the glycerol group

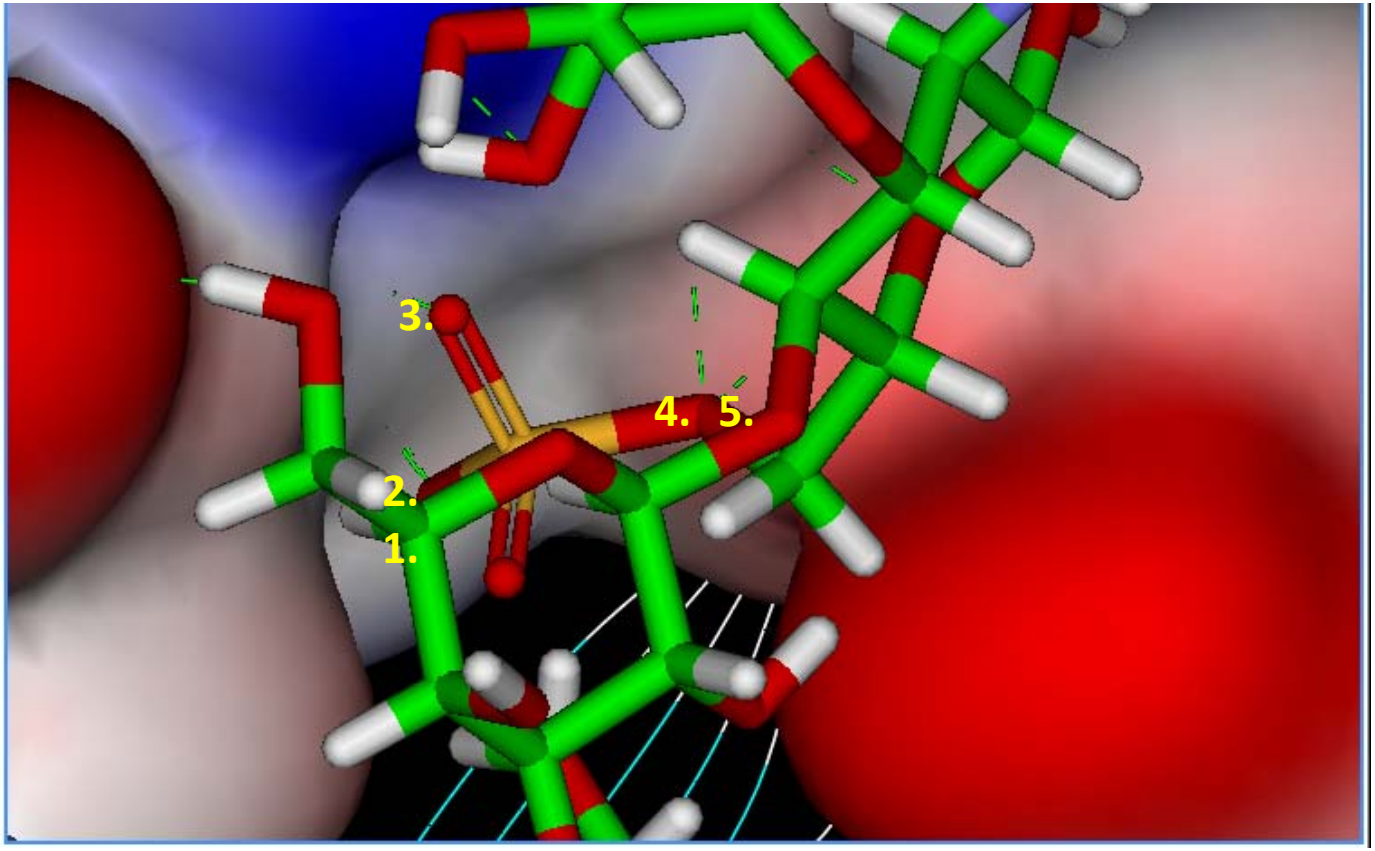
Initial Docked Results

- Su-Slex conformations do not resemble the reference structure
- Further mod necessary to achieve better results→ exhaustiveness, torsions
- Results still not like ref→ glycerol always pointing up over ASP 190→
- Using 3AL4 instead of 3LZG b/c in the crystallized 3lzg the ASP 190 pointing down→ sterically hinders glycerol
- 3AL4 then selected because ASP 190 pointing up, which gives space for glycerol (even tho lower resolution, 2.87 angstroms > 2.60 angstroms)
  - ASP behavior found in holo forms of H1

Dockings with 3AL4

- From the best (lowest energy) result, found that SA orientation is similar to reference
  - Acetamide: H bonds with carbonyl of Val 135
  - Carboxylate: stabilized by H bonds with Ala 137, Gln 226, Thr 136
  - Glycerol: has space under Asp 190, and has high prob stabilized by H bond with Gln 229
  - Sulfate group makes five H bonds with Glu 230 (Glu 230 later added by Yusuf), Gly 231, Ser 189

3AL4 with Su-SLex



Sulfate group creates **5** more H bonds with H1 receptor, thus stabilizing the H1 receptor more effectively than without the sulfate group.

Dockings Summary

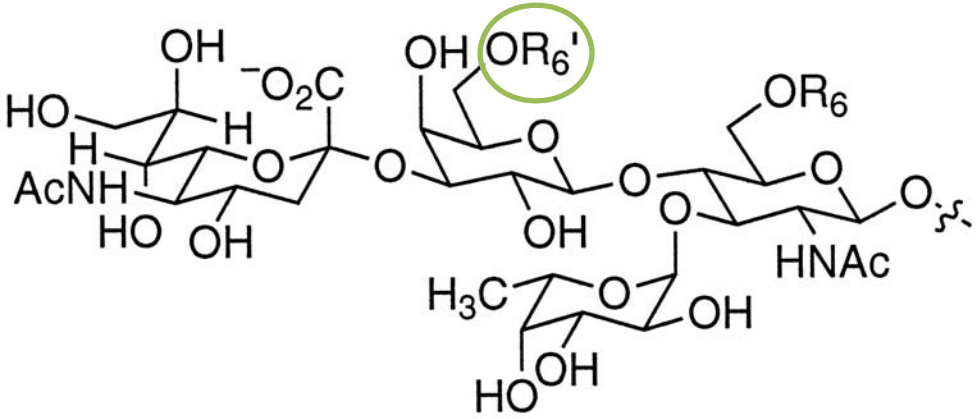
- Lowest docked energy found with SLex and 3LZG: -5.7 kcal/mol
- Lowest docked energy found with Su-SLex and 3AL4: -6.9 kcal/mol
- Hence, by adding sulfate and using a more appropriate receptor, a higher binding affinity is achieved
- Why?
  - Receptor with Asp 190 facing up concurs with holo form of H1 structures
  - Sulfate group creates increased H bond interactions between the receptor and ligand

Conclusion

- Sulfation of SLex does increase the binding affinity of H1 to SLex
- Possible to create Su-SLex as drug analogues that will compete with human glycans
- Fight spread of virus!

Future Directions

- examine the effect of sulfation on the H1 binding affinity by placing the sulfate at a different position



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Acknowledgements

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