Glycans in Bacterial and Viral Infections

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Objectives

- Explain how sugars are involved in:
  - Bacterial infections
    - Structure of bacteria
    - Interactions with human hosts
    - Pathogenesis of disease
    - Virulence
    - Antibiotic resistance
  - Viral infections
    - Viral life cycle
    - Antiviral treatments (drugs and antibodies)

Microbes

- Always there
  - Living tissues
  - Non-living surfaces
- More bacteria present than total number of cells in our body
- Very small fraction are pathogenic
  - Recognition
  - Invasion
  - Evasion of immune response
  - Proliferation
- Glycans are involved in ALL these processes

Glycans in bacterial infections

Peptidoglycan

- **A.k.a. muropeptide**
- Forms the cell wall

- Cross-linked mesh-like framework
- Critical for survival of bacterium
- Maintains structure
  - Avoids cell destruction due to osmotic pressure changes

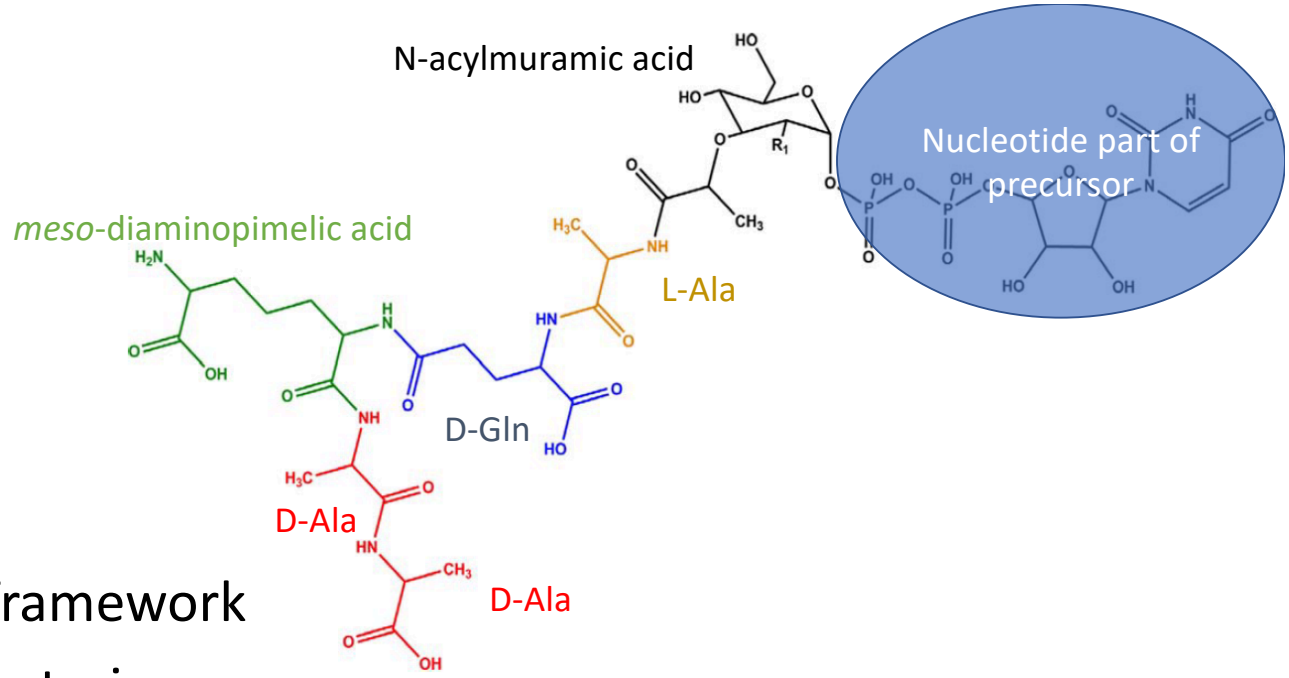
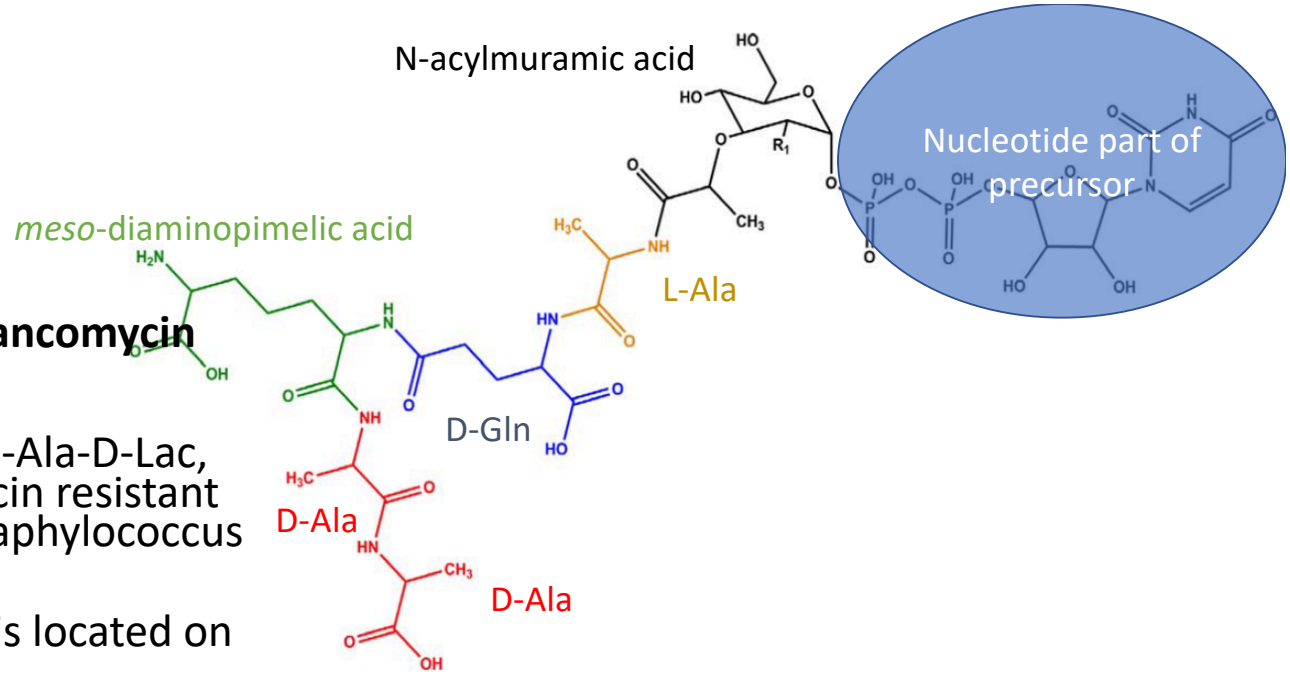


FIGURE 1 PG nucleotide precursor (Park's nucleotide). Basic structure of the PG monomer precursor with the muropeptide L-alanyl-D-glutaminyl-*meso*-DAP-D-alanyl-D-alanine. R_1 denotes the presence of either an *N*-acetyl or *N*-glycolyl modification of the muramic acid moiety. L-Ala, D-Glu, *meso*-DAP, and D-Ala are depicted in gold, blue, green, and red, respectively. doi:10.1128/microbiolspec.MGM2-0034-2013.f1

Peptidoglycan

- **D-Ala-D-Ala is the target of vancomycin**

- If D-Ala-D-Ala is modified to D-Ala-D-Lac, bacterium becomes vancomycin resistant (e.g., vancomycin-resistant Staphylococcus aureus or VRSA)
- The gene responsible for this is located on the ***van*** operon
  - Transferred from vancomycin-resistant Enterococci to MRSA during a co-infestation on the foot ulcer of a diabetic patient
- D-Ala-D-Ala is not a saccharide, but tangentially related and of SUPREME importance



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Microbiology Spectrum
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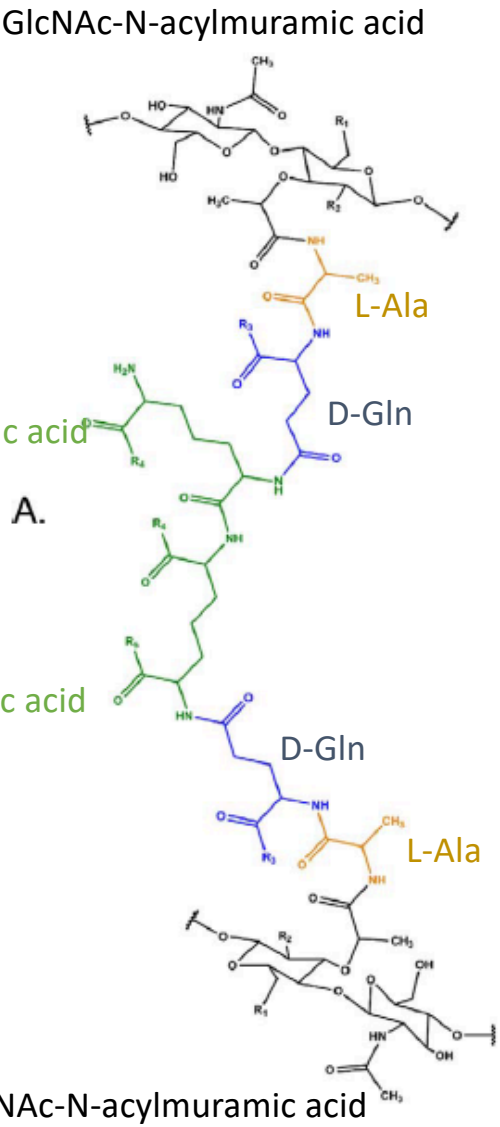
Genetics of Peptidoglycan Biosynthesis

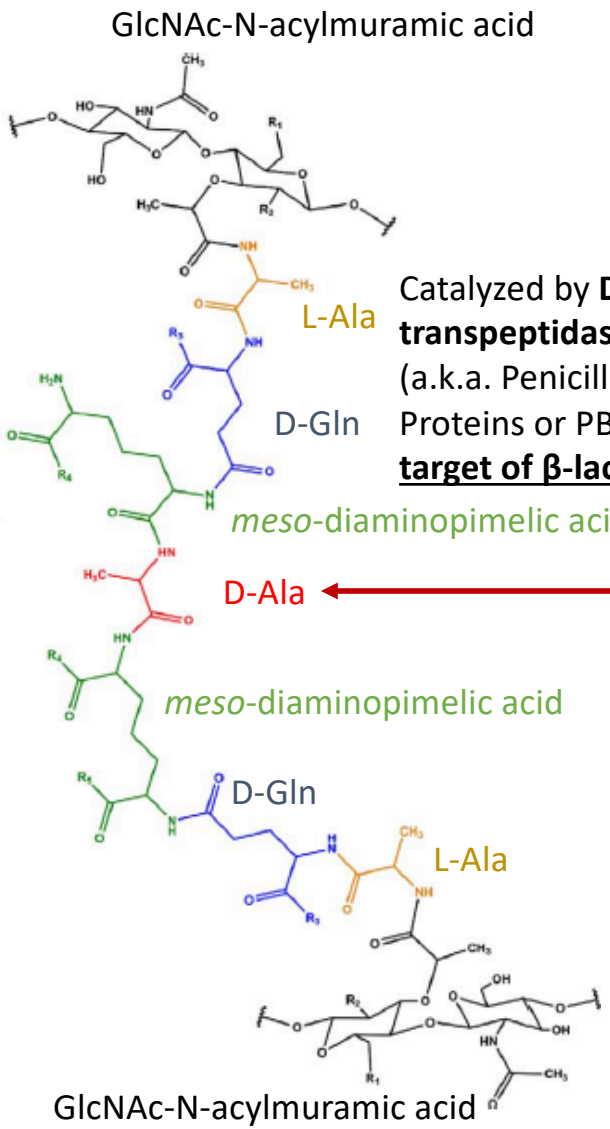
MARTIN S. PAVELKA, Jr.,[1] SEBABRATA MAHAPATRA,[2] and DEAN C. CRICK[2]

[1]University of Rochester Medical Center, Department of Microbiology and Immunology, Rochester, NY 14642; [2]Mycobacteria Research Laboratories, Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO 80523

Peptidoglycan



Catalyzed by concerted action of D,D-carboxypeptidases and **L,D-transpeptidases (resistant to β-lactams except carbapenems)**



Catalyzed by **D,D-transpeptidases** (a.k.a. Penicillin-Binding Proteins or PBPs, the **target of β-lactams**)

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Genetics of Peptidoglycan Biosynthesis

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Discuss

- What therapies would you use for a bacterium that contains a point mutation in the D,D-transpeptidases the active site?

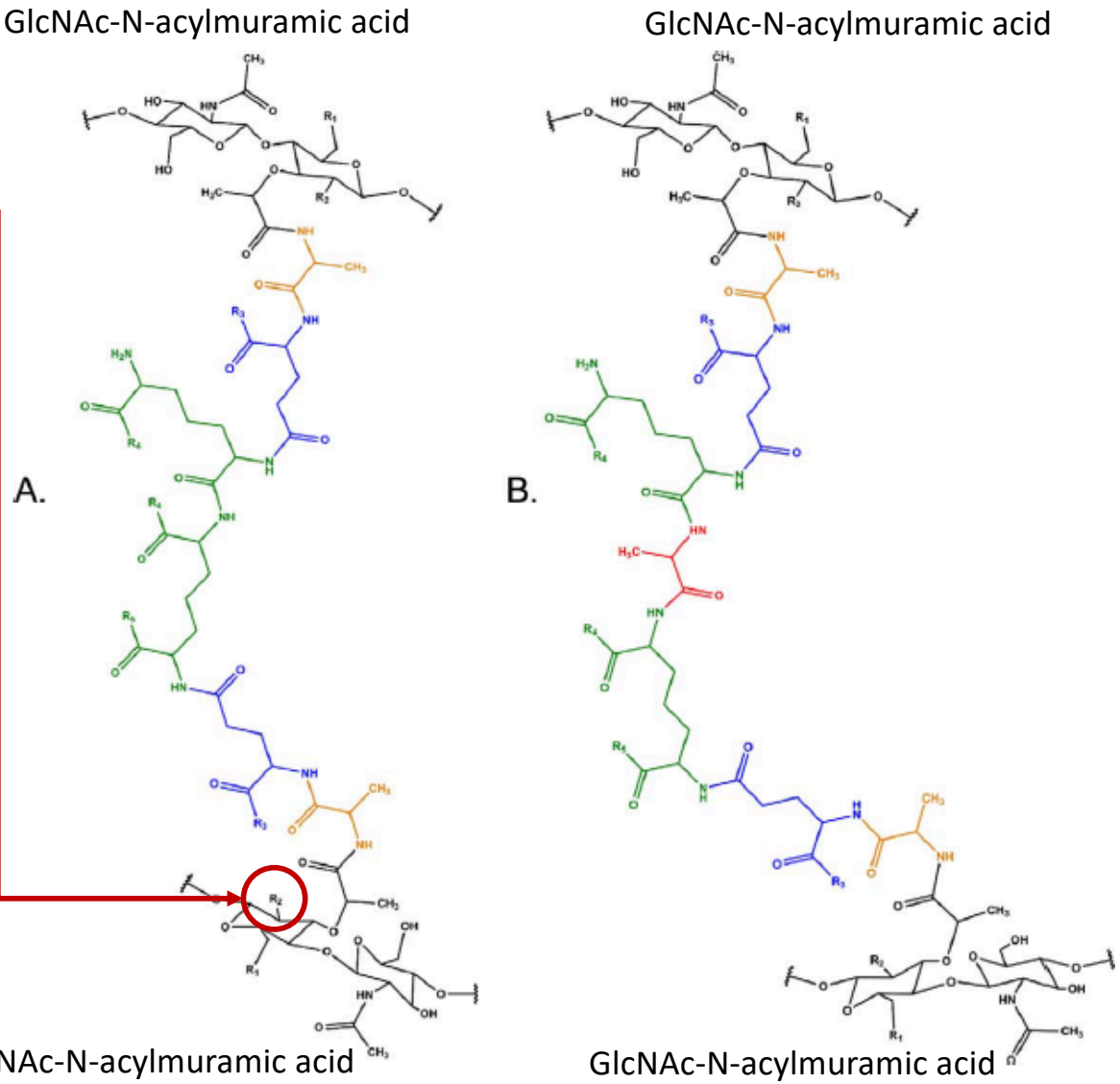
- What therapies would you use for a bacterium that expresses a β-lactamase?

Peptidoglycan

R_2 could be acetyl or glycolyl

If **R_2 = glycolyl,** bacterium is **mycobacterium** like *M. tuberculosis*
Resistant to lysozyme that typically would have killed the bacterium with IgA

Nod2 receptor is the pattern-recognition receptor (PRR) that recognizes peptidoglycan in mycobacteria (**pathogen-associated molecular pattern, or PAMP**)



Peptidoglycan

- Case: Vancomycin intermediate-resistant Staphylococcus aureus (**VISA**)

- Increases the thickness of its peptidoglycan layer
- Blocks access of vancomycin to the target, which lies far below the surface
- Also increases number of un-linked D-Ala-D-Ala chains at surface to minimize vancomycin penetration

Journal of Antimicrobial Chemotherapy (1998) **42**, 199–209

JAC

Activated cell-wall synthesis is associated with vancomycin resistance in methicillin-resistant *Staphylococcus aureus* clinical strains Mu3 and Mu50

H. Hanaki[a], K. Kuwahara-Arai[a], S. Boyle-Vavra[b], R. S. Daum[b], H. Labischinski[c] and K. Hiramatsu[a]*

[a]*Department of Bacteriology, Juntendo University, 2-1-1 Hongo, Bunkyo-ku, Tokyo, Japan 113-8421;* [b]*The University of Chicago Children's Hospital, Chicago, IL, USA;* [c]*Bayer AG, PH-Research Antiinfectives, Wuppertal, Germany*

Vancomycin intermediate-resistant strain with **higher levels of resistance**

Control strain

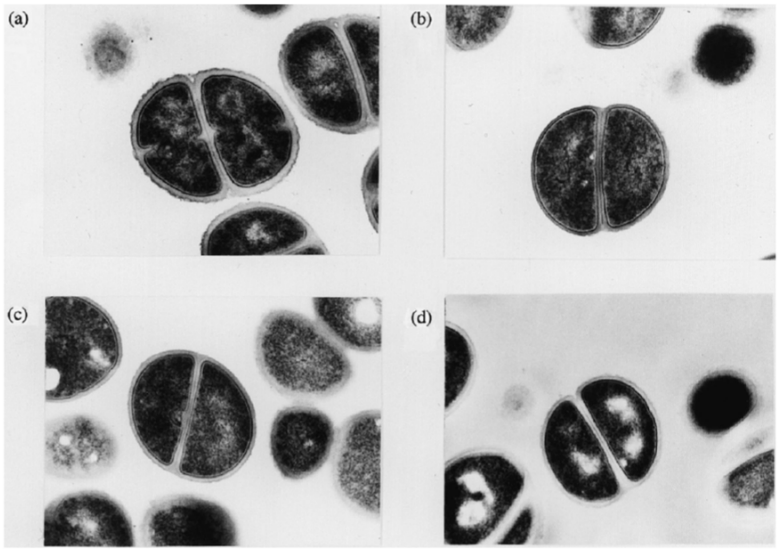


Figure 7. Transmission electron microscopy of (a) Mu50, (b) FDA209P, (c) H1 and (d) Mu3.

Control strain

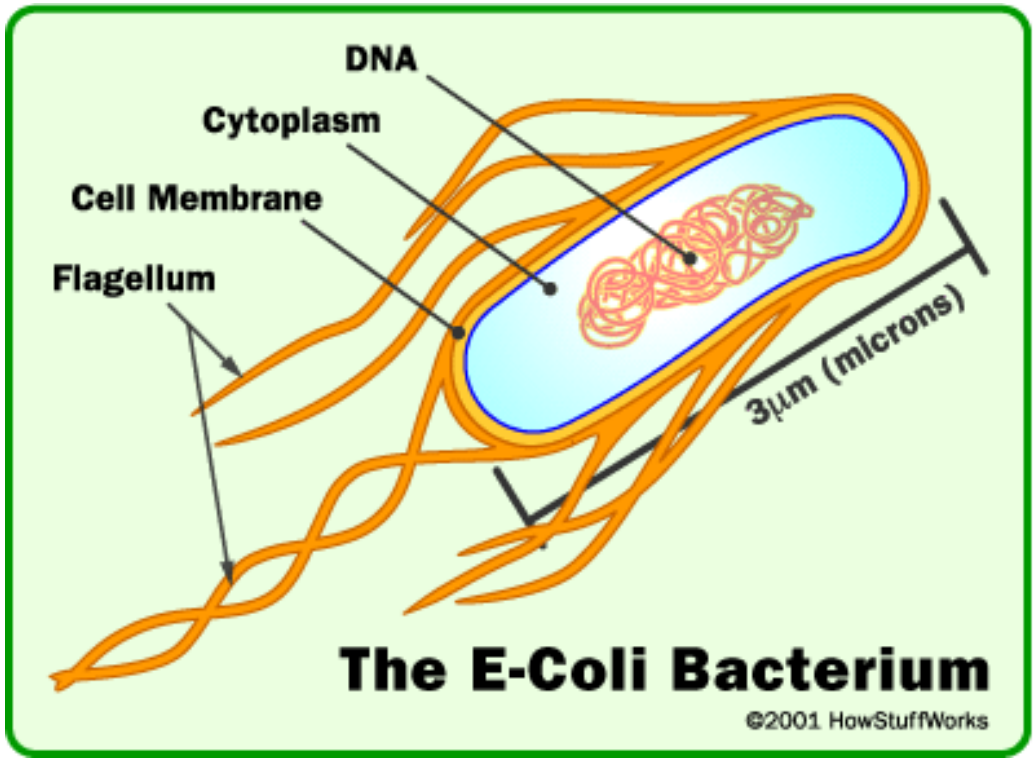
Vancomycin intermediate-resistant strain with **lower levels of resistance**

Discuss

- Clearly, VISA produces a thicker cell wall to keep out vancomycin. Would the activity of other antibiotics be affected by this?

- How about VRSA?

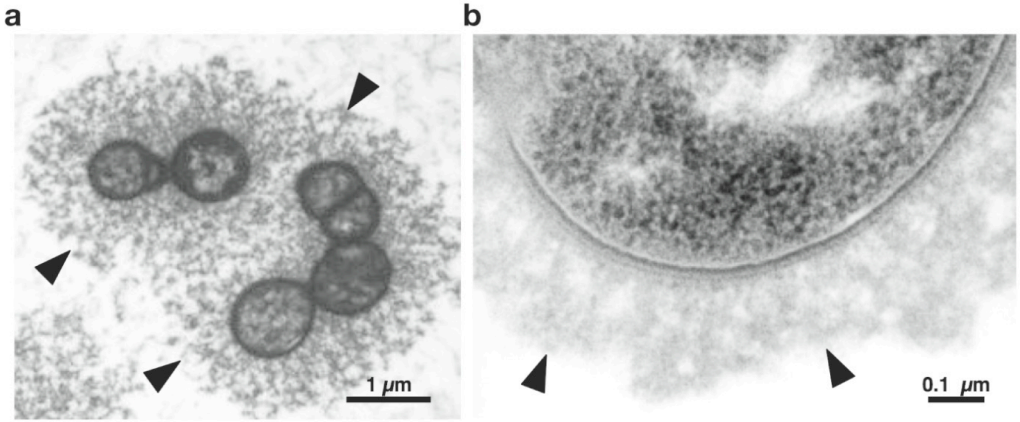
Mythical bacterium



Picture from: http://science.howstuffworks.com/life/cellular-microscopic/cell1.htm, accessed April 14, 2014

The bacterial capsule

- Outermost layer of bacterial cell
- Functions:
  - Hydration… prevents desiccation
  - Mediate adhesion
  - Important for colonization
  - Important for virulence

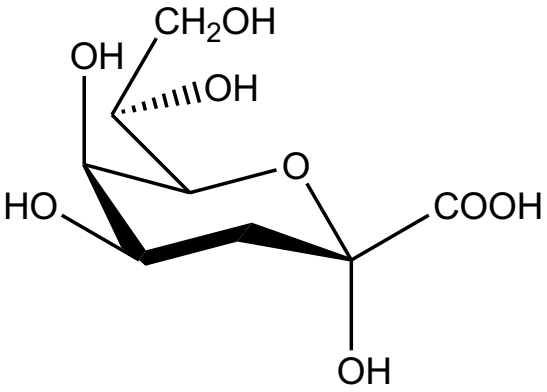


- Capsular polysaccharide is an important component

'Essentials of Glycobiology' 2nd Edition (Varki, A.; et al. Editors), Cold Spring Harbor, New York, (2009) pg. 226

The bacterial capsule

- Different types of polysaccharide
  - Group Ia hexuronic acid + neutral sugar
  - Group Ib hexuronic acid + N-acetylhexosamine
  - Group II hexuronic acid, Kdo or sialic acid + neutral or amino sugar
- Examples
  - *Hyaluronan* *→4) GlcA (β1→3) GlcNAc (α1→*
  - *K5* *→4) GlcA (β1→4) GlcNAc (α1→*
  - *K1* *→8) Neu5Ac (α1→8) Neu5Ac (α1→*



Kdo or 3-deoxy-D-manno-oct-2-ulosonic acid

Decides serotype of bacterium

Prevnar 7, 10 and 13 are capsular polysaccharides from pneumococci used as vaccines

'Essentials of Glycobiology' 2nd Edition (Varki, A.; et al. Editors), Cold Spring Harbor, New York, (2009) pg. 226

The bacterial capsule: evasion of immune response

- Prevents host immune response (**molecular mimicry**)
- *Neisseria meningitidis* has two types of capsules
- Group C (α2-9-linked) and group B (α2-8-linked polysialic acid)
- Group B is non-immunogenic, but group C is immunogenic

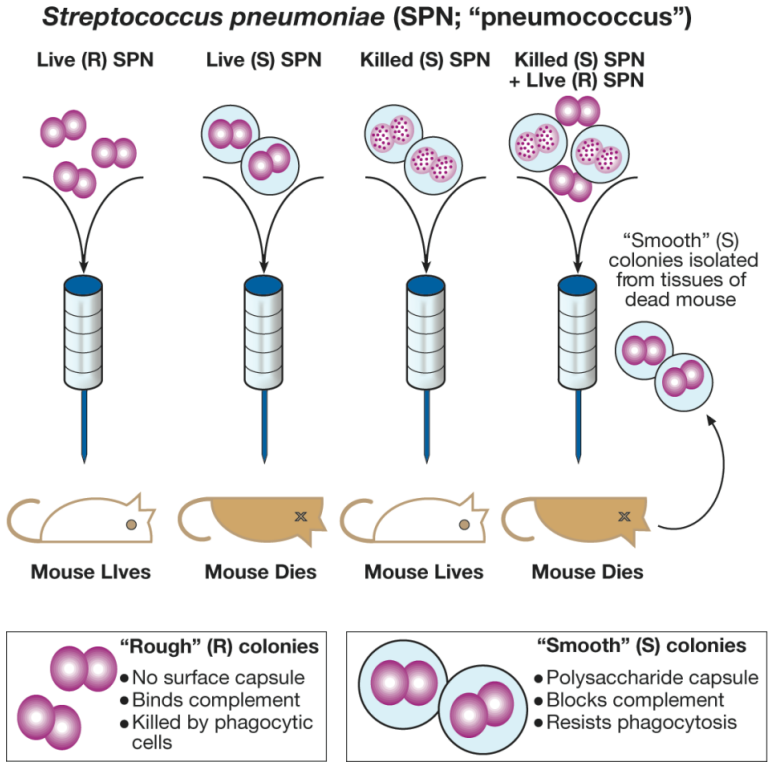
The bacterial capsule: evasion of immune response

- Streptococcus A **produces hyaluronan identical to human hyaluronan**

- Important in virulence
- Human immune response to capsule is lowest at extremes of age
- Infants and older people are most susceptible to infection
- Virulence genes may be passed from bacterium to bacterium
  - Horizontal gene transfer

Bacterial capsule is critical for virulence

- **Horizontal gene transfer** from killed smooth *Streptococcus pneumoniae* to the rough strain is clearly demonstrated
- Allows the rough strain to start building a polysaccharide capsule that protects it from the mouse's immune system
- The genetically modified rough strain can now infect the mouse



'Essentials of Glycobiology' 2nd Edition (Varki, A.; et al. Editors), Cold Spring Harbor, New York, (2009) pg. 540

Bacterial serotypes and biosynthesis

- **<u>Many different serotypes for each species of bacterium may exist</u>**
  - Purely due to diversity in saccharide composition

- 5 major serotypes of Meningococcus (causes meningitis and brain abscess)
- 6 serotypes of *H. influenzae* (causes acute exacerbation of chronic bronchitis)
- 9 serotypes of Group B Streptococcus (GBS or *Streptococcus agalactae*)
- **>90 serotypes of Streptococcus pneumoniae!**
  - pneumonia, sepsis and meningitis

- Different types of bacteria synthesize the capsule differently …

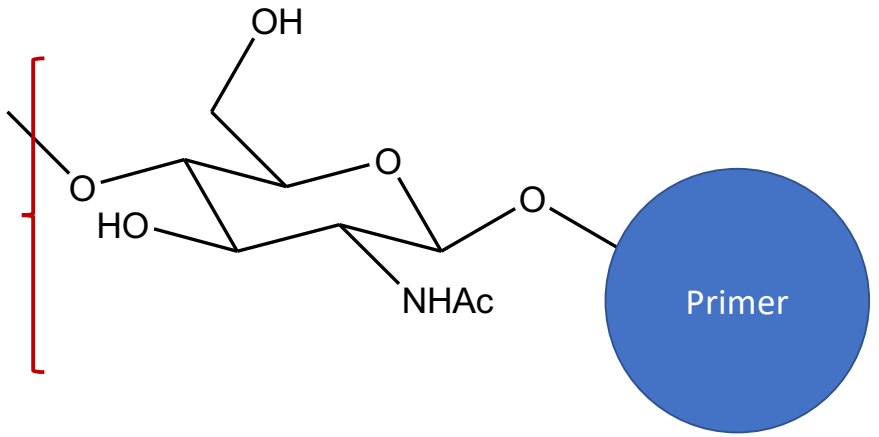
Discuss

- What happens if a different bacterium produces the same polysaccharide? Will it be recognized by the immune system if vaccinated using Prevnar 13?

- What happens if a different strain of Pneumococcus produces a polysaccharide that is structurally different from Prevnar 13?

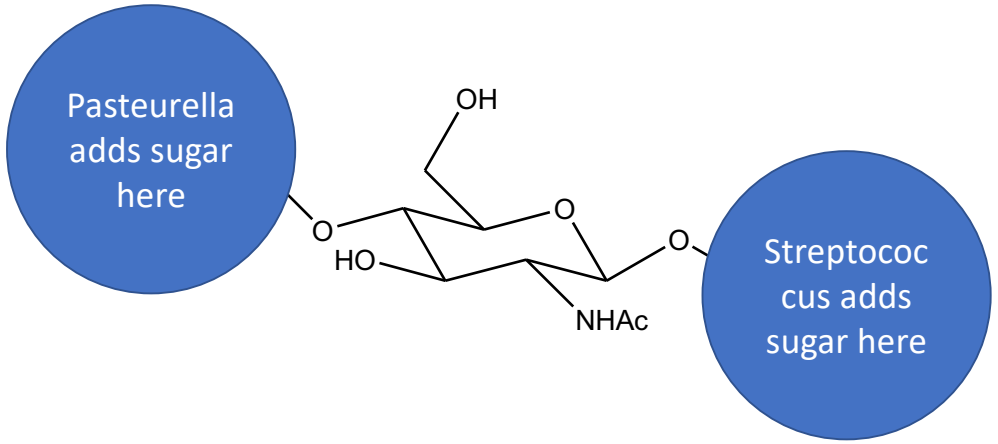
Bacterial serotypes and biosynthesis of capsular polysaccharide

- Biosynthetic differences may exist
  - Primers on which glycans are formed may vary
  - e.g., Phosphatidic acid-Kdo conjugates
  - e.g., Lipid A
  - Primers are always at the reducing end of capsular oligosaccharides



Bacterial serotypes and biosynthesis of capsular polysaccharide

- Biosynthetic transferases may vary significantly from bacterium to bacterium
  - Pasteurella synthesizes hyaluronan by adding sugars to the non-reducing end, but Streptococcus adds sugars to the reducing end
  - Pasteurella uses an enzyme that is completely unrelated to Streptococcus or ANY vertebrate



Discuss

- How likely is it that a drug that targets the Pasteurella transferase will also target Streptococcus?
- Why is this relevant to drug discovery?
- Is there any reason why you might want to invest in such a drug?

Bacterial serotypes and biosynthesis of capsular polysaccharides

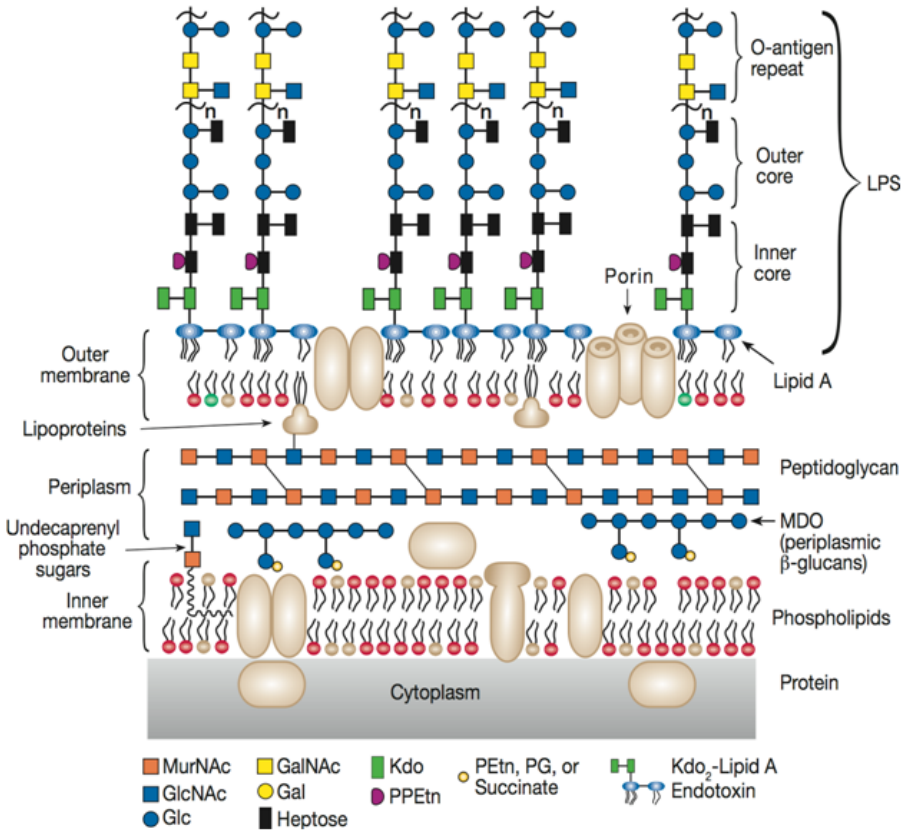
- Pasteurella uses a unique biosynthetic enzyme with two glycosyltransferase domains:
  - Aminoglycosyltransferase, uses UDP-GlcNAc
  - Glucuronyltransferase, uses UDP-GlcA

- Found in a few other microorganisms
  - *E. coli* (major pathogen in UTIs)
  - *Klebsiella pneumoniae* (major nosocomial Gram –ve pathogen)

- No equivalent human enzyme!

Lipopolysaccharide

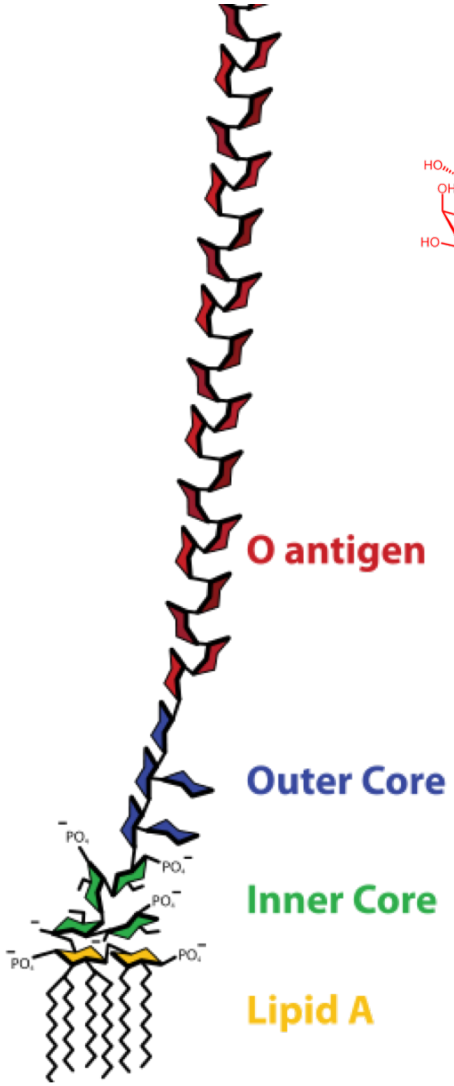
- Gram –ve bacteria produce lipopolysaccharide
- Highly immunogenic
  - Severe reactions are observed (septic shock)

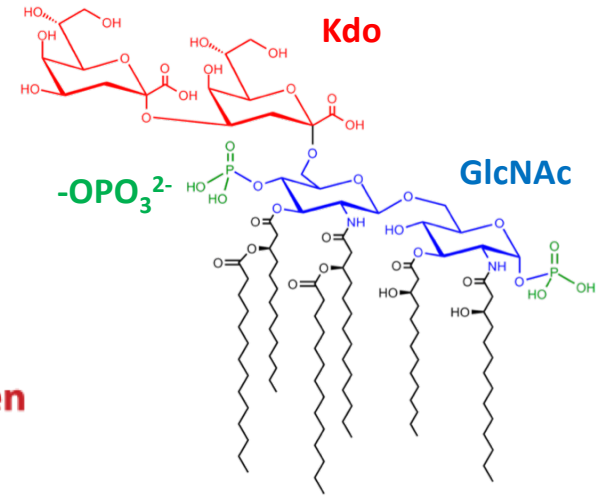


'Essentials of Glycobiology' 2nd Edition (Varki, A.; et al. Editors), Cold Spring Harbor, New York, (2009) pg. 294

Lipopolysaccharide

- **3 major regions**
- Lipid A
  - Membrane-anchoring fatty acid chains and phosphorylated GlcNAc dimer (generally conserved)
- Core polysaccharide
  - Connected to C6 of GlcNAc
  - Oligosaccharide: Kdo-Kdo-Hep-Hep-Glu-Gal-Glu-GluNAc
  - Kdo is invariable
  - Rest of the core is somewhat variable
- O polysaccharide/antigen
  - Up to 40 repeat units
  - Hydrophilic and major antigenic determinant
  - >20 different sugars, many unique
  - Terminal sugars confer immunological specificity





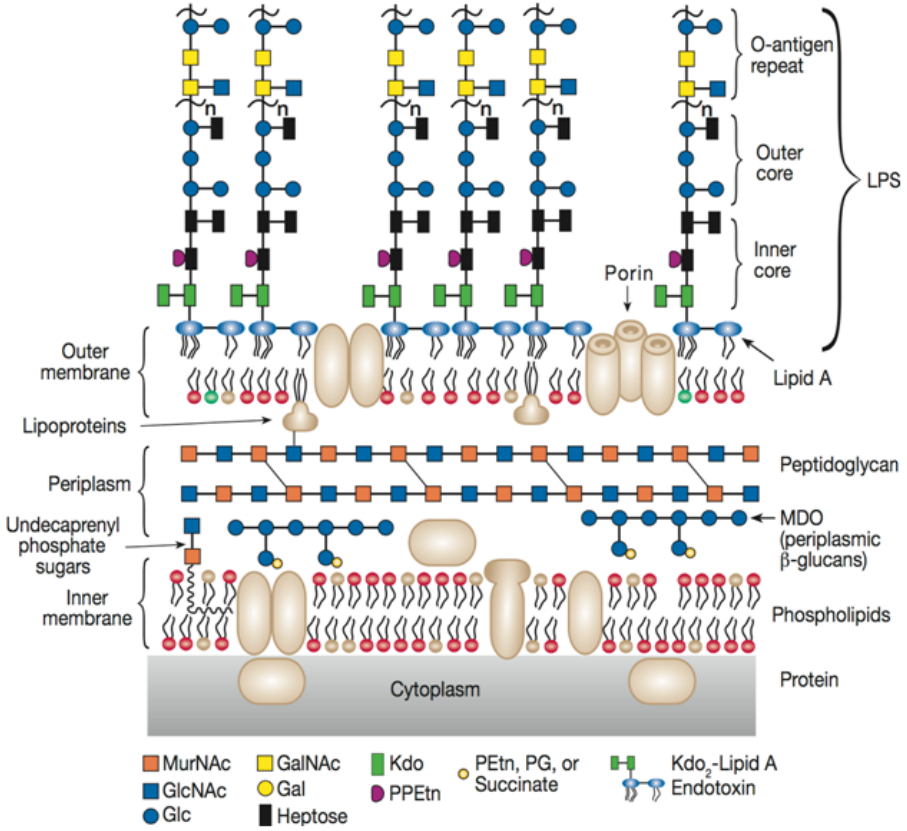
β-hydroxymyristic acid

Image credit: Mike Jones

Lipopolysaccharide

- **Critical functions in pathogens**

- Loss of the core makes Gram –ves <u>susceptible</u> to antibiotics, detergents, bile salts, and mutagens
- Highly charged nature ensures <u>only select agents are able to penetrate via diffusion</u>
- Lipid A assembly is ESSENTIAL; if k/o, the Gram –ve <u>CANNOT survive</u>!



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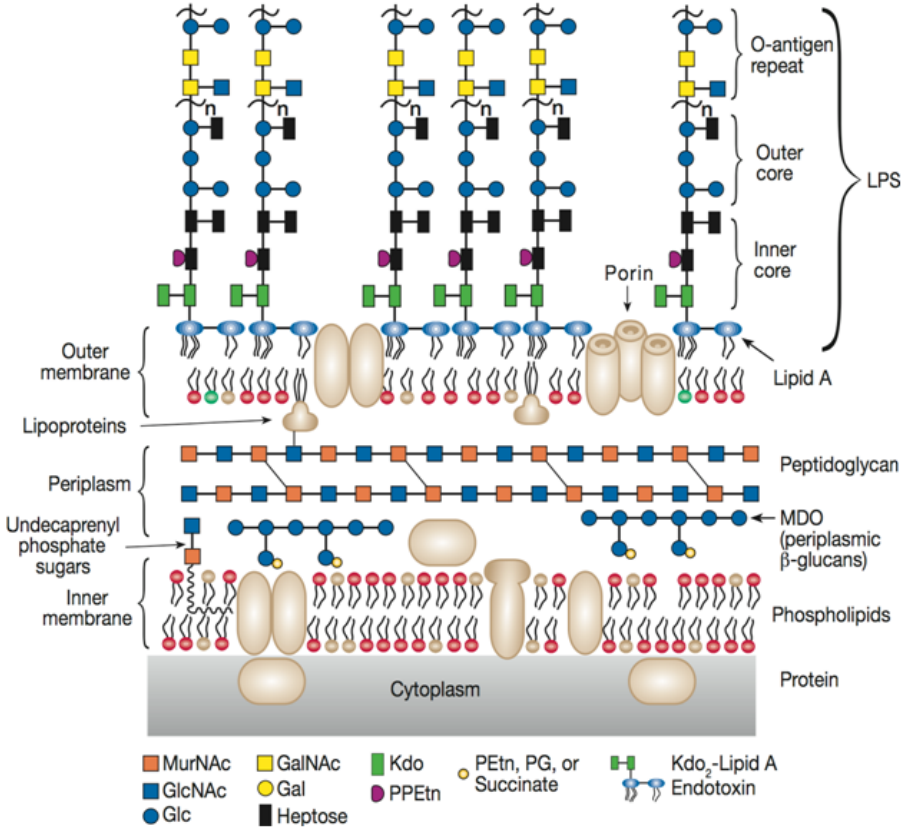
Lipopolysaccharide

- **Critical functions in pathogens**

- O antigen
  - Small changes = large changes in pathogenicity
    - Immune response modulation
    - Molecular mimicry
- Lipid A
  - Heat-stable endotoxin; immune response is strong against it
  - Killing Gram –ves releases Lipid A into blood



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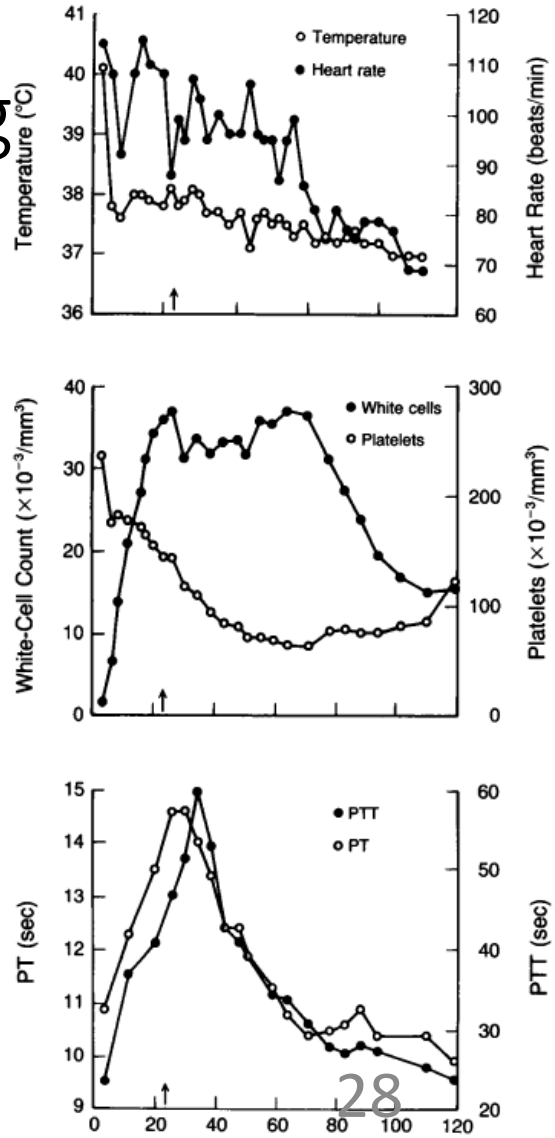
An interesting case report regarding lipopolysaccharide

Case Report

A middle-aged laboratory worker was brought to the emergency department because of malaise, headaches, nausea, and vomiting. The patient was awake but listless with a pulse of 114 per minute, a blood pressure of 42/20 mm Hg, and an oral temperature of 40°C. The patient was treated with intravenous fluids, and a dopamine infusion was started at a dose of 5 μg per kilogram per minute. Blood cultures were obtained, and vancomycin and gentamicin were administered intravenously. The results of a urinalysis, chest roentgenography, and electrocardiography were normal. The patient was admitted to the medical intensive care unit with a presumptive diagnosis of septic shock.

da Silva, et al. *N. Engl. J. Med.* **1993**; *328*, 1457-1460



What do you think caused this problem?

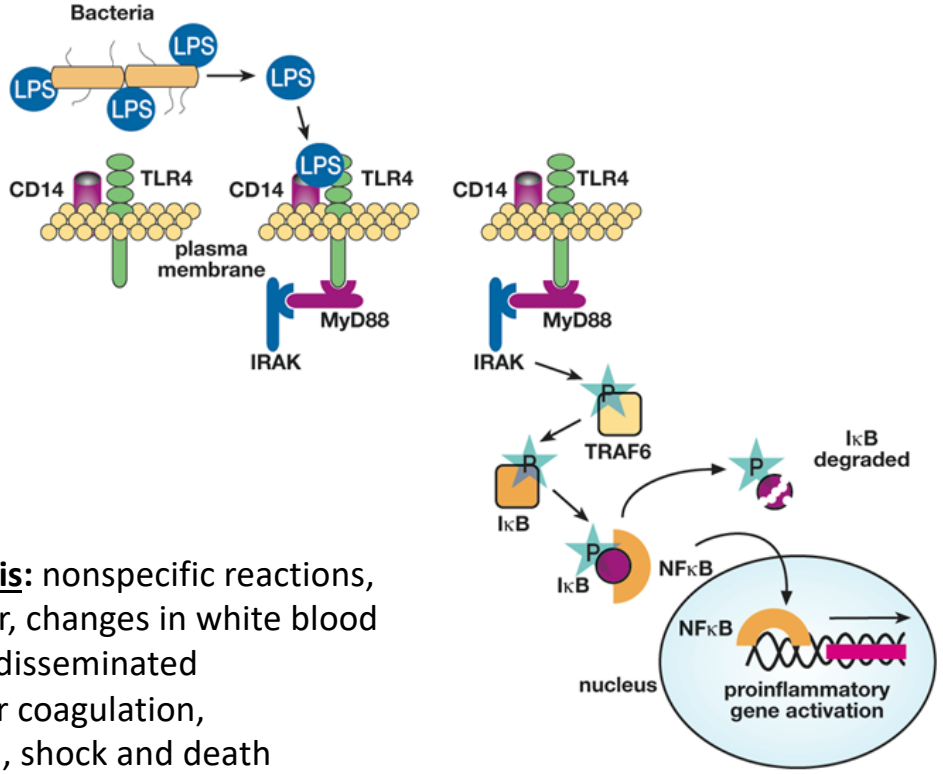
An interesting case report regarding lipopolysaccharide

- Patient had taken 1 mg of pure *Salmonella minnesota* endotoxin
  - Self-administered to cure a recently diagnosed tumor

- This dose is ~4,000-fold higher than what is given to patients during controlled clinical trials

- The patient survived when appropriate antibody was administered

Inflammatory response to lipopolysaccharide



Causes <u>sepsis</u>: nonspecific reactions, such as fever, changes in white blood cell counts, disseminated intravascular coagulation, hypotension, shock and death

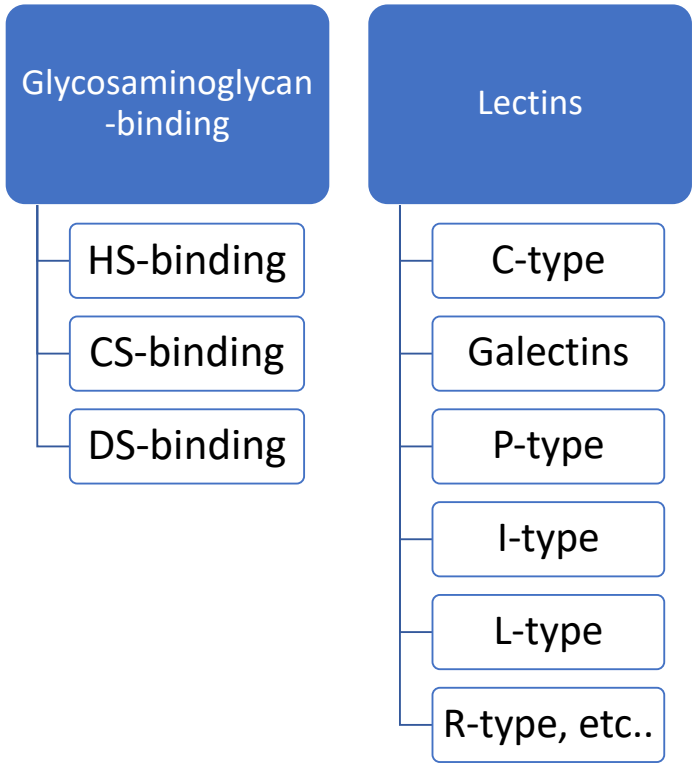
'Essentials of Glycobiology' 2nd Edition (Varki, A.; et al. Editors), Cold Spring Harbor, New York, (2009) pg. 541

Modifications of lipopolysaccharide

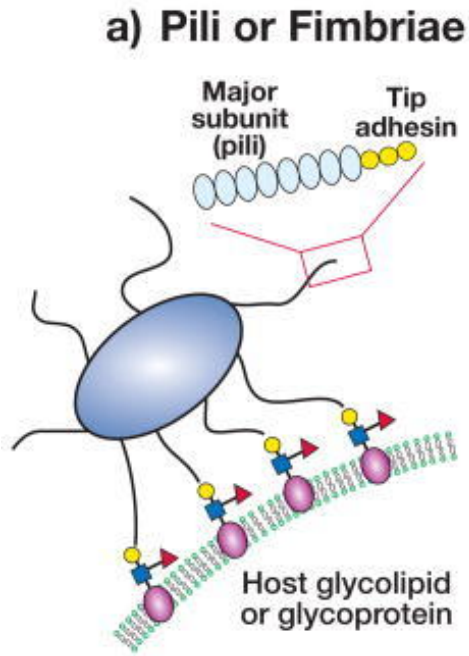
- Salmonella enterica
  - 4-aminoarabinose added to phosphate to LPS core (less negative charge)
  - Protects from positively charged antimicrobial peptides
- Pseudomonas aeruginosa
  - Hexa-acetylated lipid A containing palmitate and 4-aminoarabinose (more hydrophobic)
  - Again, repels cationic antimicrobial peptides
- Yersinia pestis
  - At 21 ^{o}C: produces more hexa-acetylated LPS (more immunogenic)
  - At 35 ^{o}C: produces more tetra-acetylated LPS (less immunogenic)

Adhesion and colonization by bacteria

- Adhesion: first step towards infection
  - *Adhesins:* Factors that promote "sticking" to host cell or tissue surface
    - Lectins: glycan-binding factors
    - Glycosaminoglycan-binding factors
- Tissue tropism: selectivity of microbes towards certain tissues
  - Decided by adhesins
  - Fimbriae
  - Afimbrial adhesion

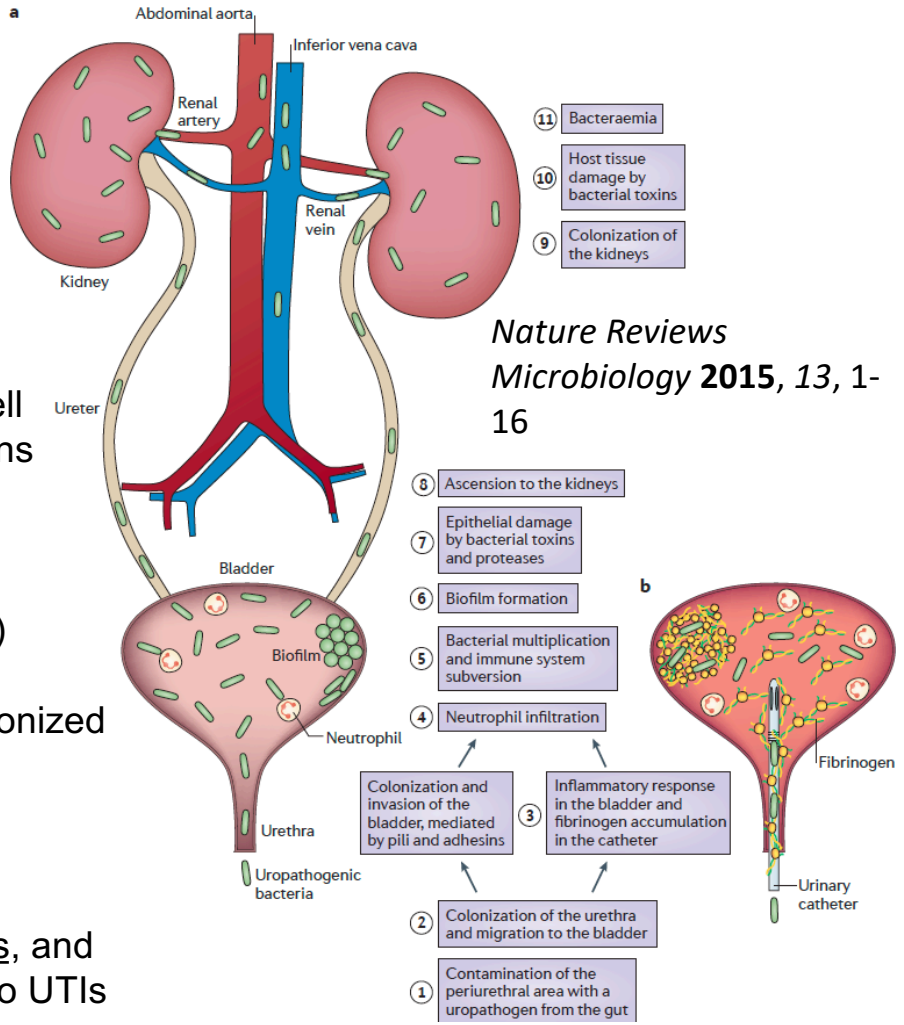


Fimbriae and pili



Velcro-like attachment to host cell
- Multiple low-affinity interactions
- High resultant avidity

e.g. Urinary tract infections (UTI)
- caused by *E. coli*
- feces and perineal region colonized first
- then ascend the urethra
- Typical patients of UTIs are sexually active women
- Women have shorter urethras, and so are 14-times more prone to UTIs than men



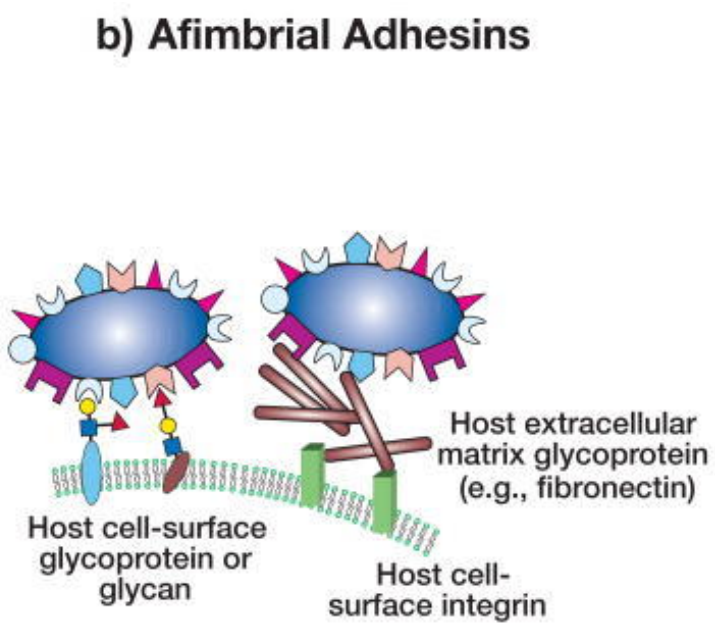
Nature Reviews Microbiology **2015**, *13*, 1-16

'Essentials of Glycobiology' 2nd Edition (Varki, A.; et al. Editors), Cold Spring Harbor, New York, (2009) pg. 543

Afimbrial adhesion

<u>Bordetella pertussis</u>

- Uses sialic acid-containing glycans on host lung cell surface
- “Whooping cough”



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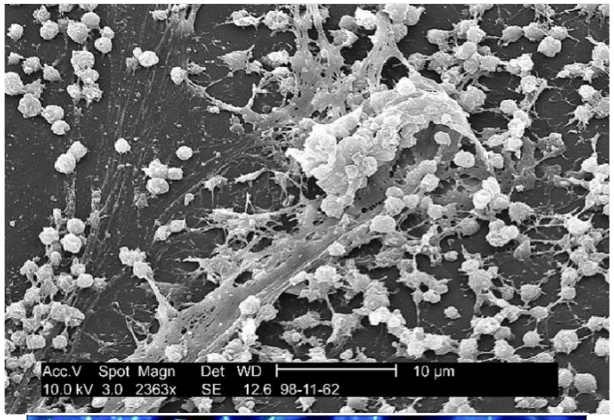
Biofilm



The CDC's public health health image library (phil.cdc.gov)

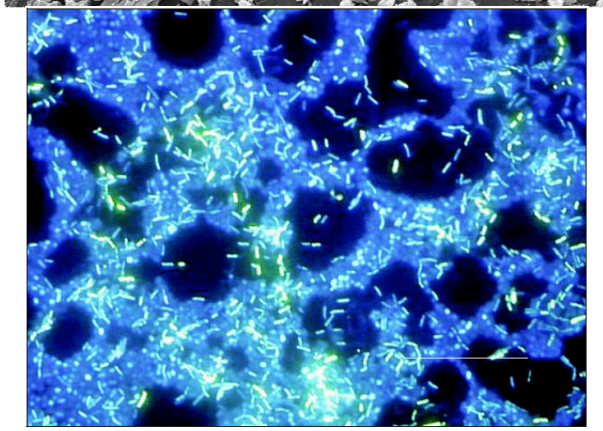
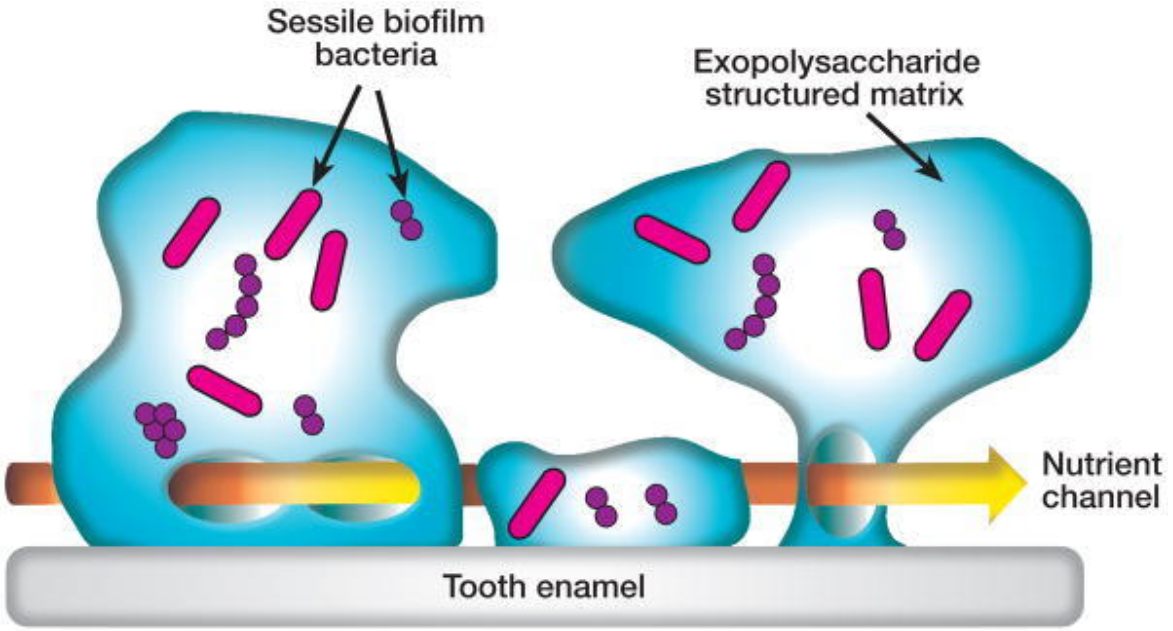


Figure 3. Polymicrobic biofilm grown on a stainless steel surface in a laboratory potable water biofilm reactor for 14 days, then stained with 4,6-diamidino-2-phenylindole (DAPI) and examined by epifluorescence microscopy. Bar, 20 µ.



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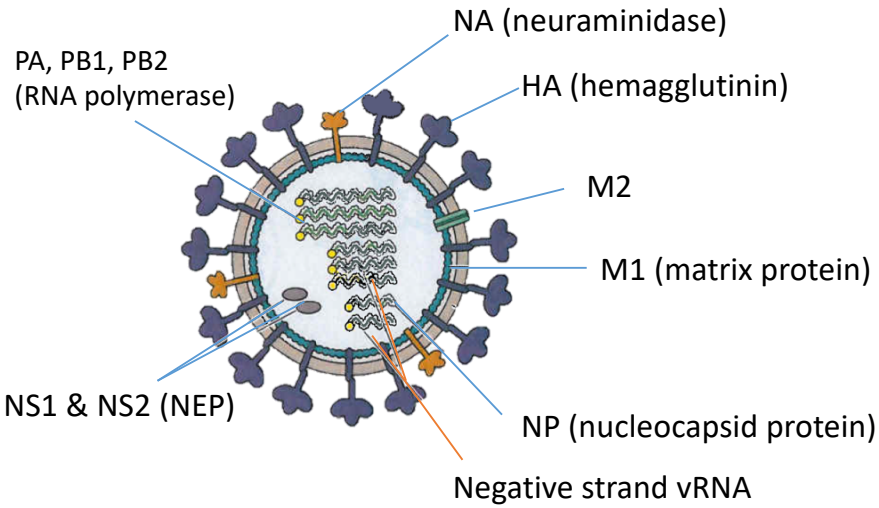
Alginate: the polysaccharide that comprises a majority of the biofilm bulk. Also a major component of ice cream.

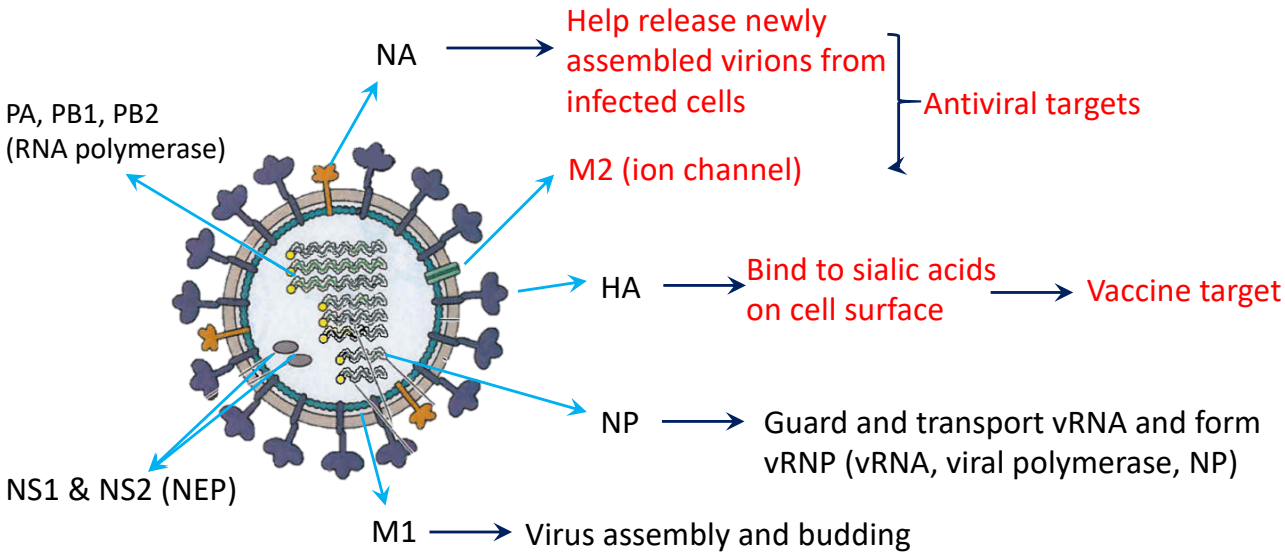
Dispersal of biofilm is being viewed as one of the next options for antimicrobial therapy

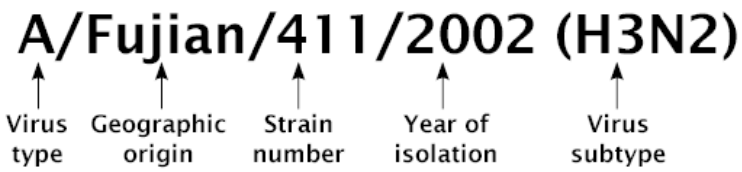
Glycans in viral infections

Influenza virus

- Influenza is a negative stranded RNA virus
- Encodes for 11 viral proteins of which hemagglutinin (HA) and neuraminidase (NA) are surface proteins
- 4 types of influenzas – A, B, C, and D
- Only type A and B infect humans, of which only type A has caused pandemics so far




NA
Help release newly assembled virions from infected cells
Antiviral targets
PA, PB1, PB2 (RNA polymerase)
M2 (ion channel)
HA
Bind to sialic acids on cell surface
Vaccine target
NP
Guard and transport vRNA and form vRNP (vRNA, viral polymerase, NP)
NS1 & NS2 (NEP)
M1
Virus assembly and budding

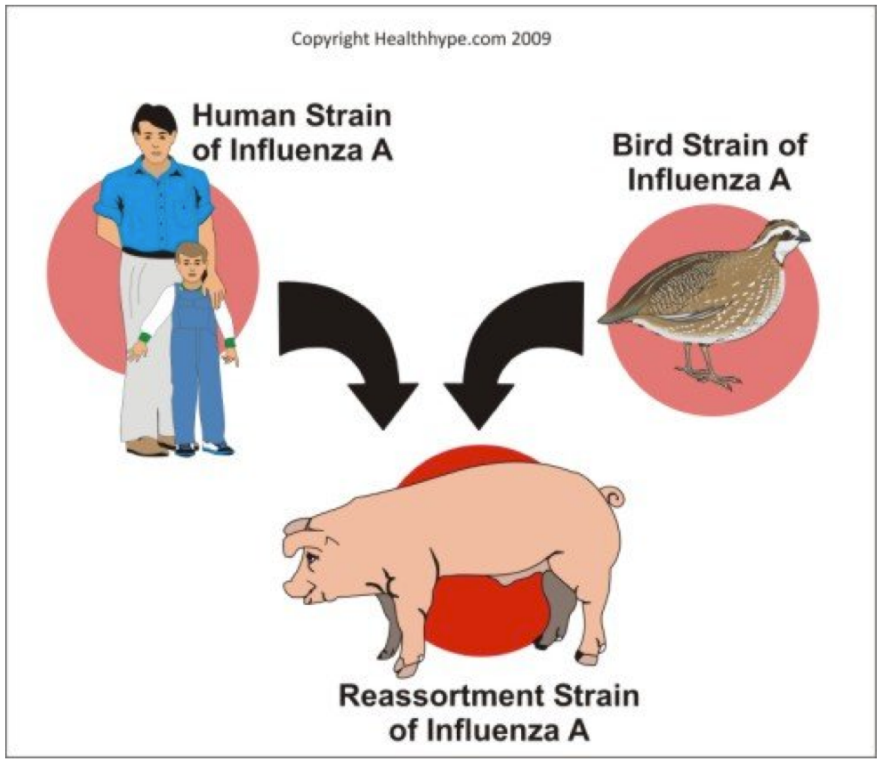

A/Fujian/411/2002 (H3N2)
Virus type
Geographic origin
Strain number
Year of isolation
Virus subtype

Glycoproteins of influenza & spread

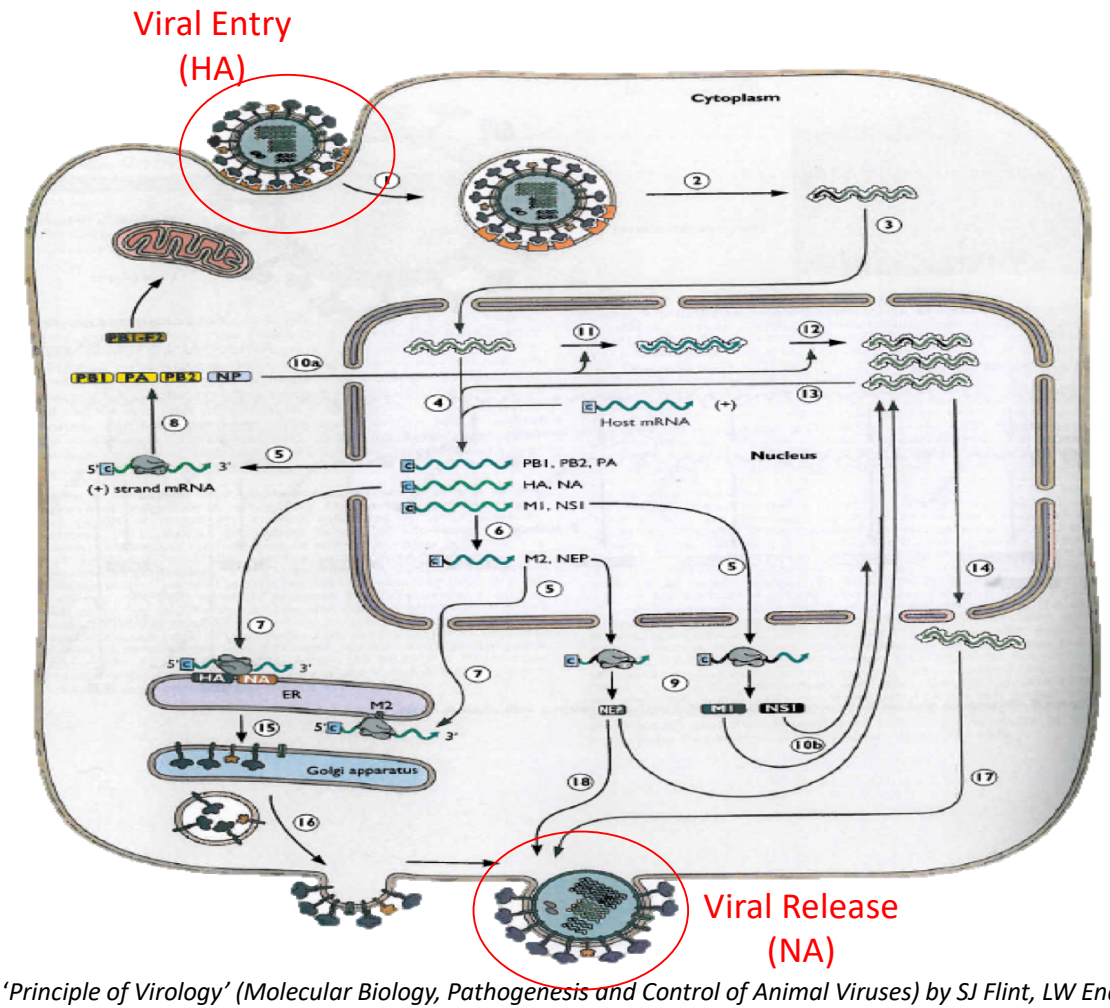
- Hemagglutinin (HA)
  - Recognizes sialic acids on cell surface for entry
  - Great plasticity …. 16 subtypes (H1, H2, H3, ……, H16)
- Neuraminidase (NA)
  - Possesses enzymatic activity to help release of newly assembled virions
  - Great plasticity …. 9 subtypes (N1, N2, N3, ……N9)

- Influenza virus may therefore be of H1N1, H7N9, etc.

- Pigs are a "mixing bowl" for human and avian flu
- Human: α2-6-linked sialic acid present, recognized by HA(Leu226)
- Bird: α2-3-linked sialic acid present, recognized by HA(Gln226)
- Pig: both, α2-3 and α2-6-linked present

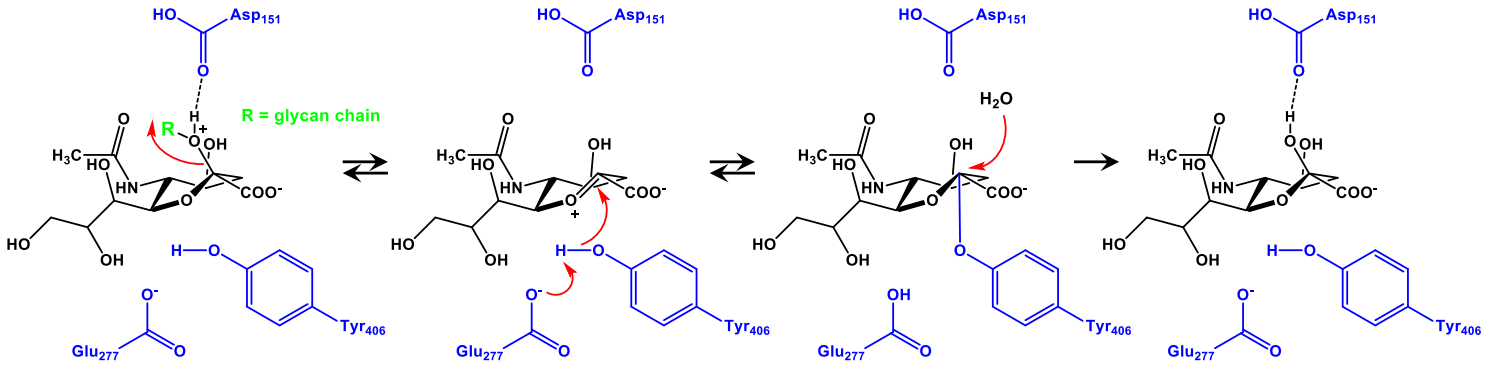


Viral life cycle



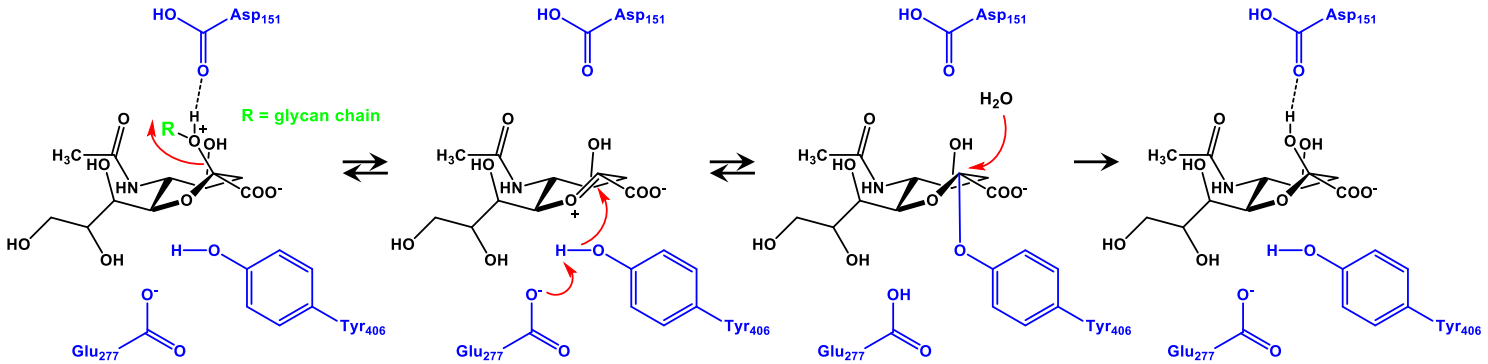
Taken from '*Principle of Virology' (Molecular Biology, Pathogenesis and Control of Animal Viruses) by SJ Flint, LW Enquist and VR Racaniello, 2nd Ed.*

Neuraminidase catalytic mechanism

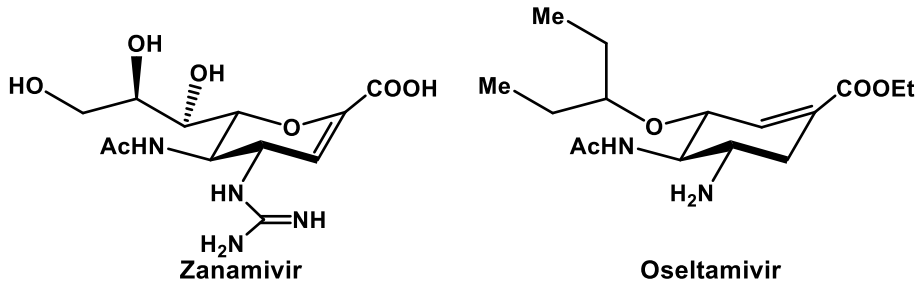


Your strategy for potent inhibitors?

Neuraminidase catalytic mechanism

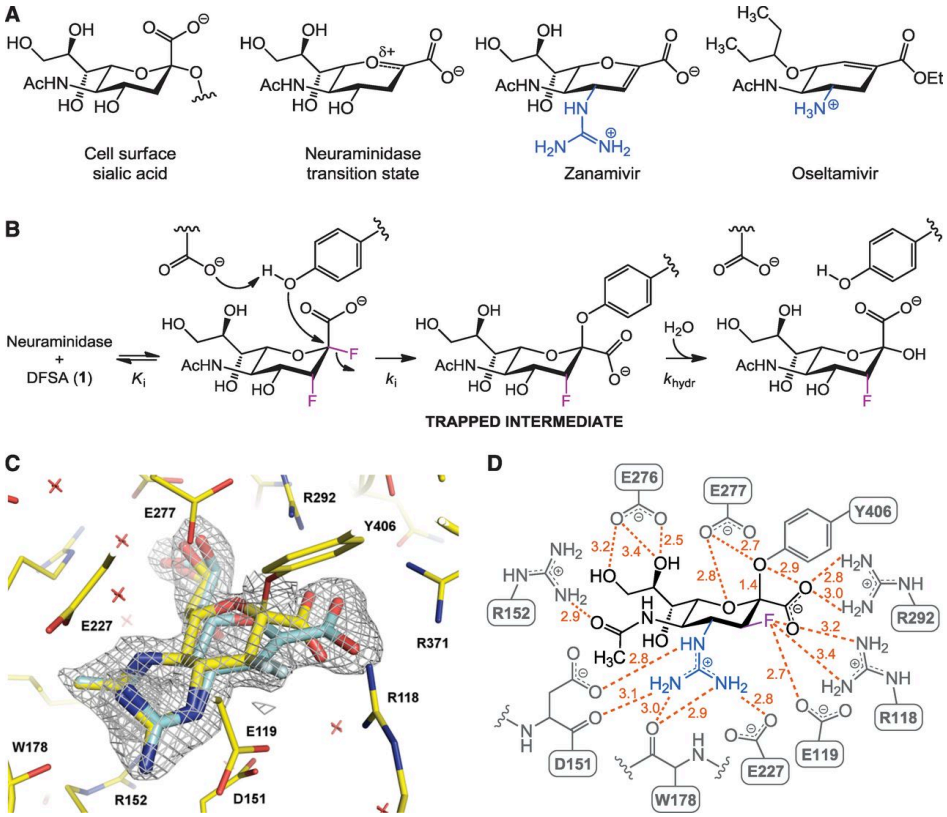


- Double bond at strategic position
- Affinities are µM and nM
- Both are approved drugs
- Zanamivir is inhaled, but oseltamivir is oral



Science **2013,** 340, 71-75

Mechanism-based covalent sialidase inhibitors



Science **2013,** 340, 71-75

Herpes simplex virus

- Herpesvirus family consists of over 100 viruses
- *Only 8 herpesviruses commonly infect humans*

- HSV-1 and HSV-2 viruses belong to the α-herpesvirus subfamily
  - Cause cold sores of the mouth
  - keratitis in the eyes (HSV-1)
  - genital lesions (HSV-2)
  - life-threatening in immunocompromised individuals (newborns, HIV patients, immunosuppressive treatment patients)
- Virus remains latent in neurons for years …. become reactivated by environmental triggers, e.g., stress
- Contagious during latency also, if shed asymptomatically
- *Nearly 80% of human population carries HSV-1 … 40% HSV-2*

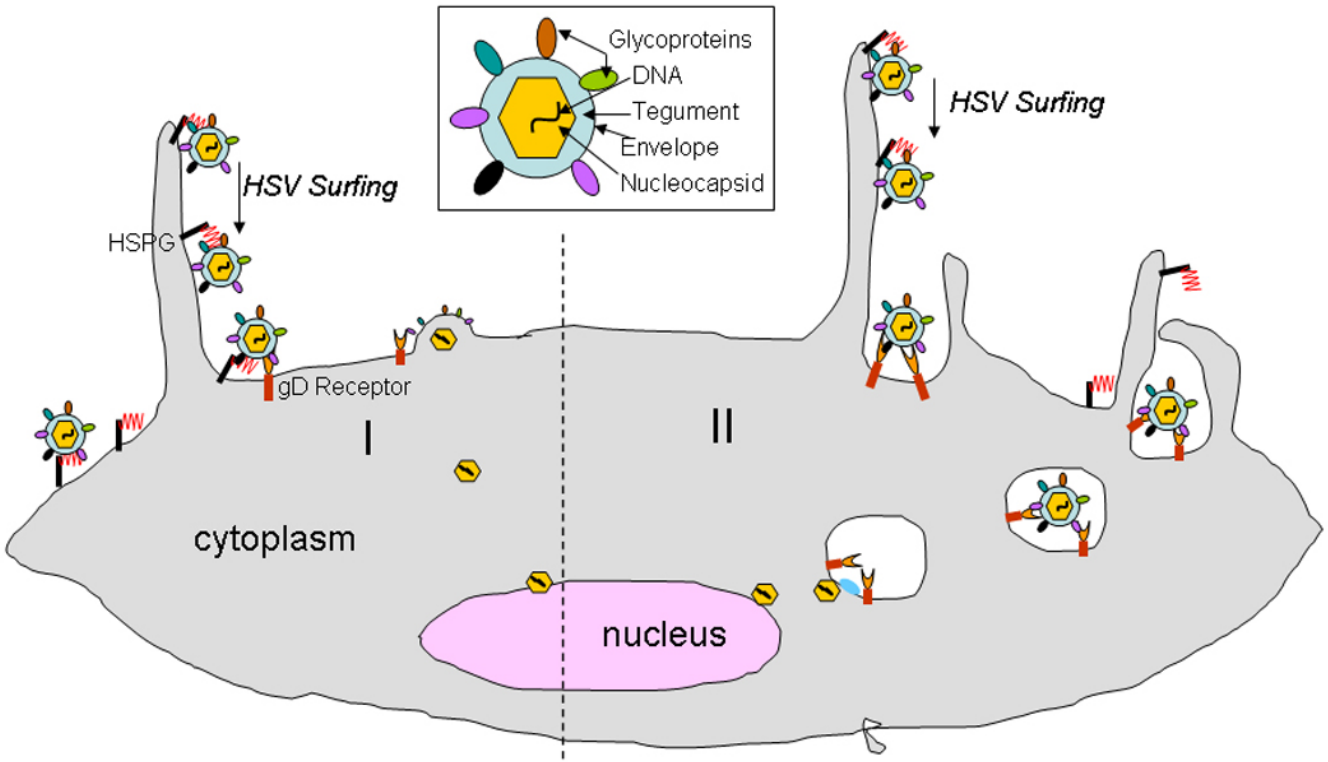
- Virus exploits the host cell machinery to replicate, spread and establish latency
- A good anti-viral strategy is to inhibit viral entry into target cells

Glycans and Herpes simplex virus

Two major modes of entry into cells.

- *pH-independent fusion of viral envelope with the plasma membrane (I), or*
- *endocytic pathway (phagocytosis-like, II).*

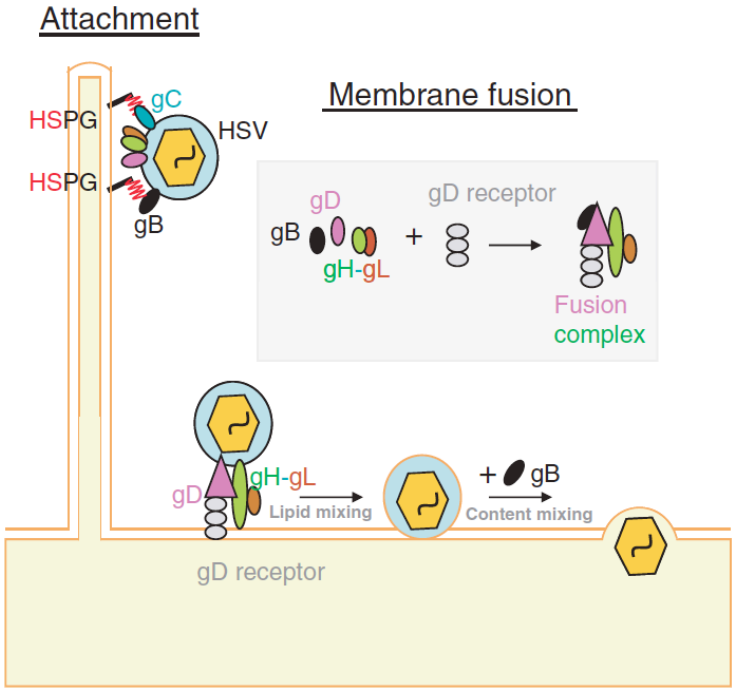
- *In both pathways, HSV particles may initially associate with filopodia-like membrane protrusions via HSPG.*



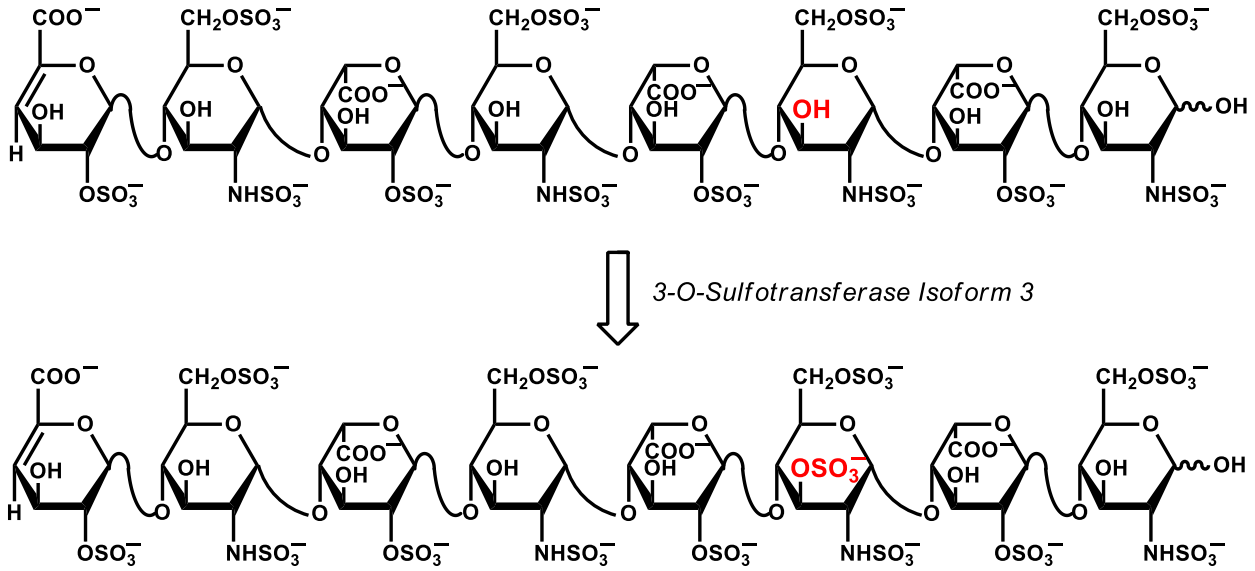
Glycans and Herpes simplex virus

- Various glycoproteins play an important part
- gB, gC, gD, gH & gL

- Initial attachment of gB and/or gC to HSPG on host cell
- gB, gD, gH-gL, and a host gD receptor form the membrane fusion complex
- gB interaction with its receptor (PILF-α) is important for lipid mixing during the fusion process



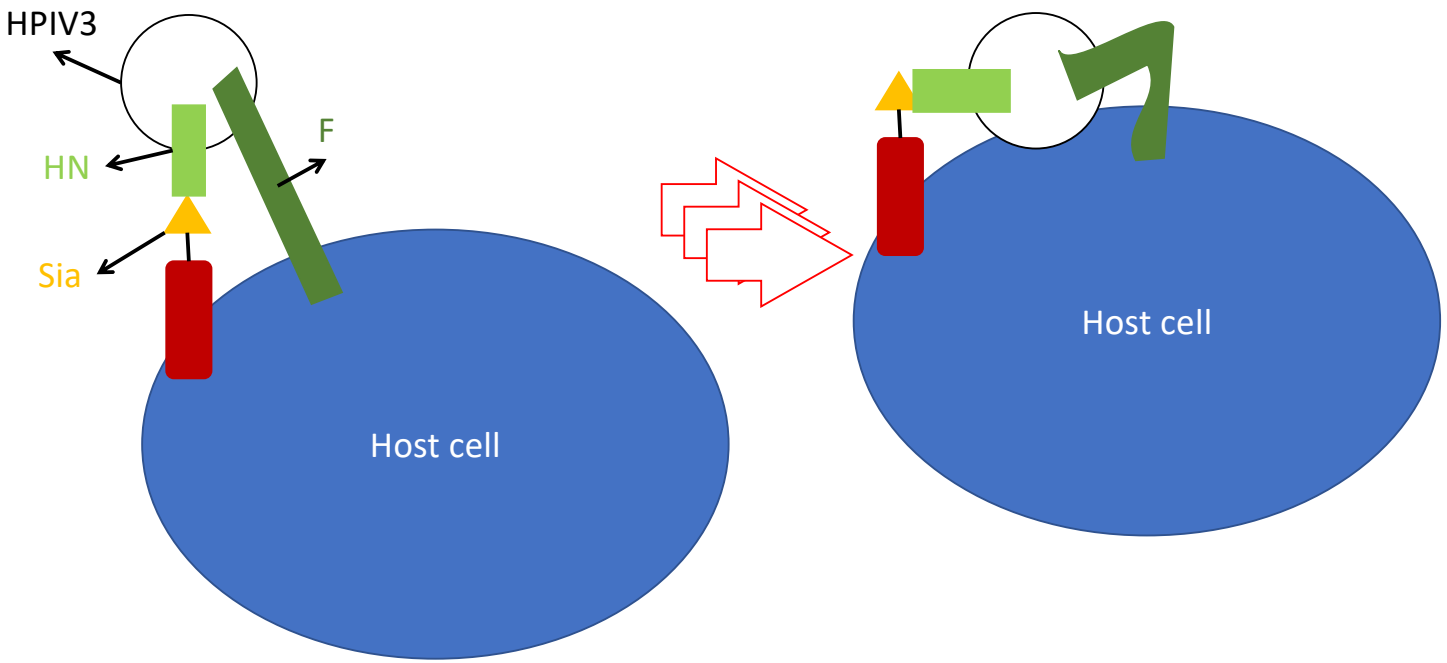
3-sulfate important for HSPG binding by HSV



Structure of HS octasaccharide that binds gD of HSV and inhibits infection

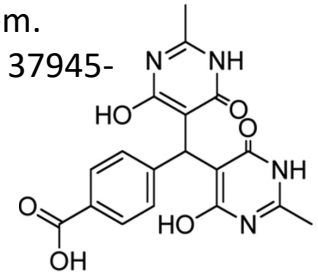
Biochemistry **2008**, *47*, 5774-5783

Hemagglutinin-neuraminidase in human parainfluenza virus type III

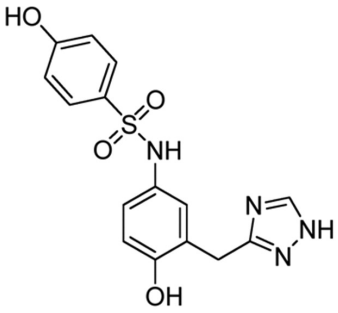


Hemagglutinin-neuraminidase in human parainfluenza virus type III

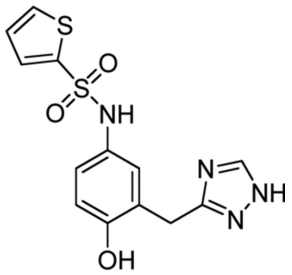
Site II inhibitors
J. Biol. Chem.
2011, 286, 37945-37954



CSC5

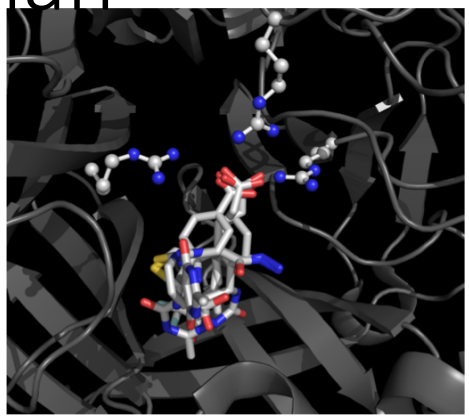


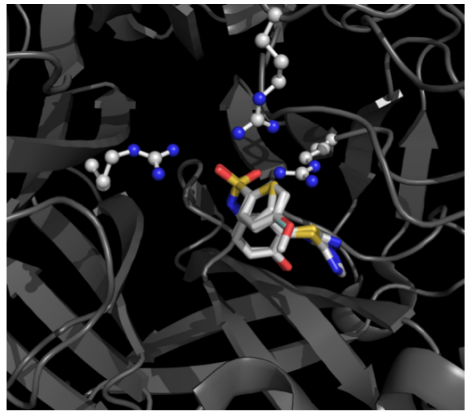
CSC7



CSC11

- Site I inhibitors: blocked viral recognition of host cell
- Site II inhibitors: elicited premature activation of the fusion mechanism; viral particles permanently inactivated


Site I inhibitors


Site II inhibitors