Lecture 4:

Immunology and Immunotherapies

- Review of Antibody Response
- Antibody based therapies for cancer (Final presentations)
- Cell-based Response
- Cell-based cancer therapy (Final presentations)
- Vaccines & Vaccine development

Enzymes & Enzyme Inhibitors

- Review of steady-state enzyme kinetics
- Suicide inhibitors
- Competitive inhibitors
- Allosteric inhibitors
- HIV drug therapy

B-Cell Biology - From Stem Cells to Pathogen Killing.

1. Generation of high diversity of chains during development of stem cells to B-cells in bone marrow.

- DNA rearrangements to generate functional exons for variable segments of both light and heavy chain.

2. Molecular & cellular biology of membrane bound antibodies on cell surface = B-cell receptor (BCR)

- Transcriptional enhancers, mRNA splicing
- Light chain and heavy chain exported to surface of B-cells.

3. Self tolerance test to prevent autoimmune diseases, autoreactive B-cells eliminated.

4. Encounter and capture of antigen in lymph nodes

5. Activation of B-cells by T_H cells

- Peptides from pathogen presented on major histocompatibility proteins (MHC II).
- T-cell activation by tyrosine kinase receptors (T-cell Receptor, TCR), secretion of signaling molecules.

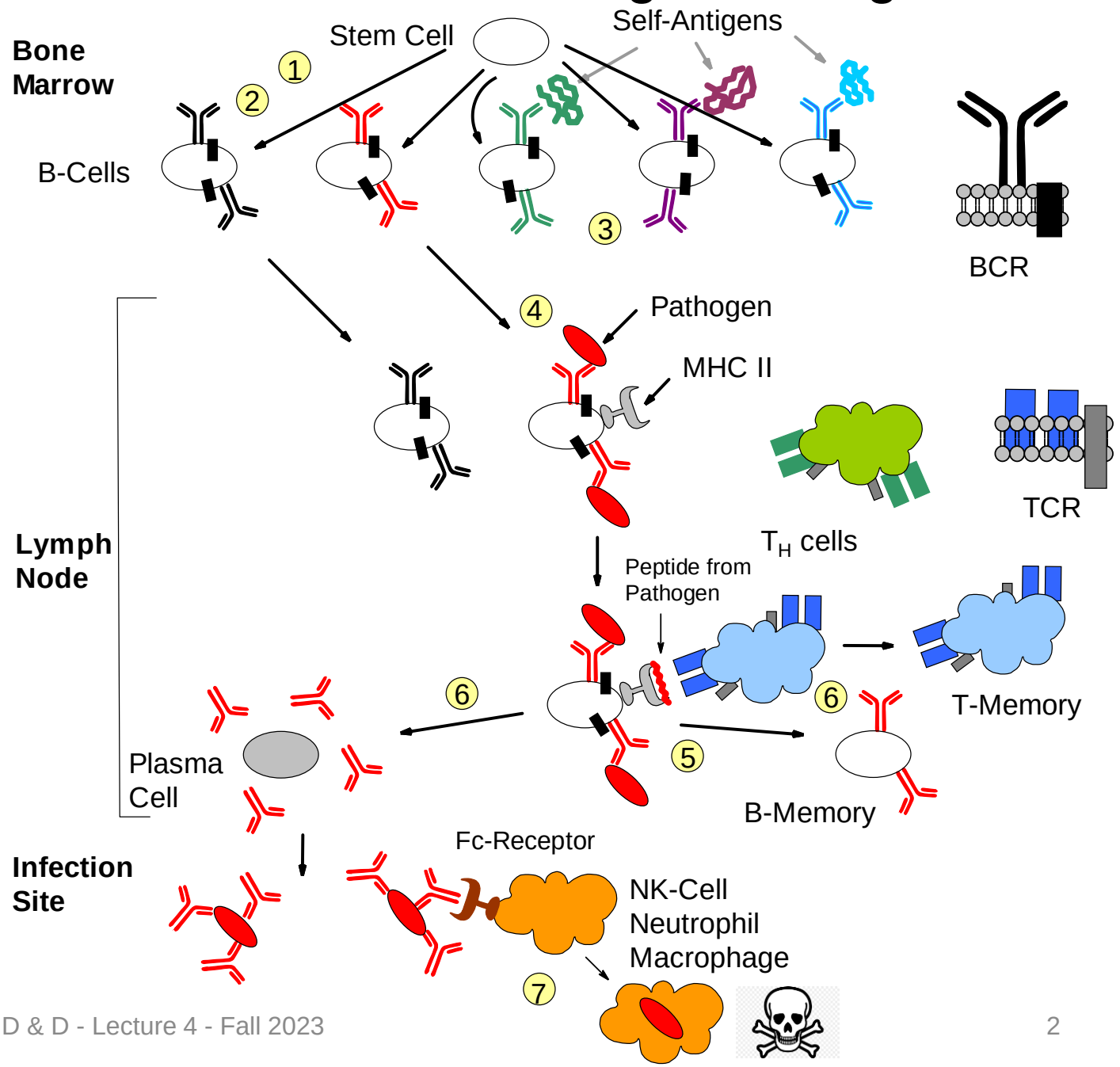
6. Development of

- Plasma cells - Production of soluble antibodies of the same specificity as the parent B-cell.
- B-memory cells (basis of immunity)
- T-memory cells (basis of immunity)

7. Destruction of Pathogens

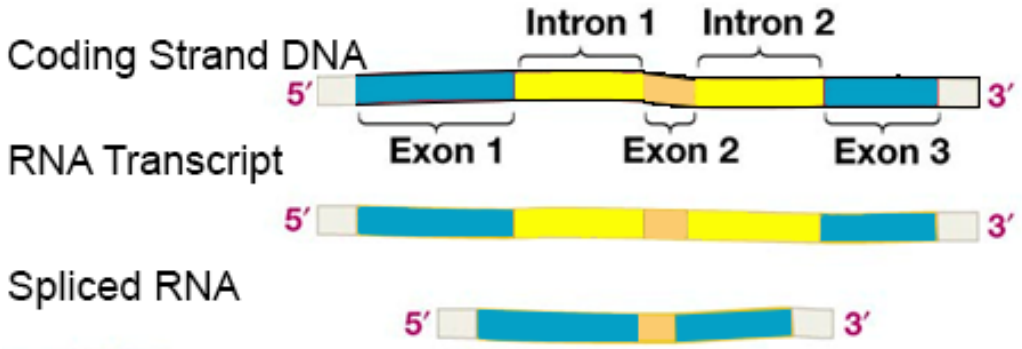
- Fc region of antibody binds to Fc Receptor on NK cells, neutrophiles, macrophages
- Pathogen internalized and destroyed.

BCR – B-cell receptor = antibody + signaling chains.
TCR – T cell receptor = MHC-peptide recognition + signaling.

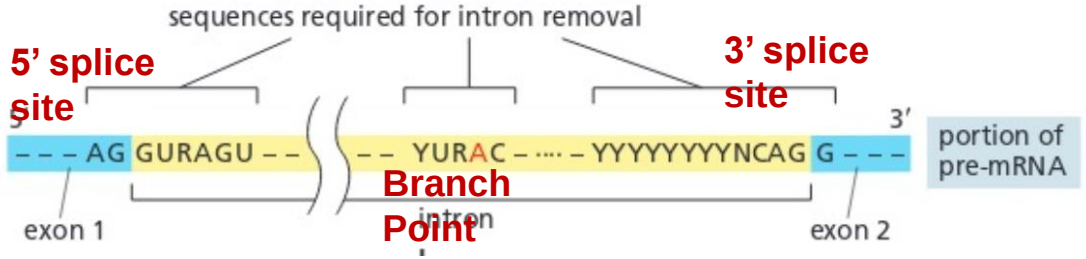


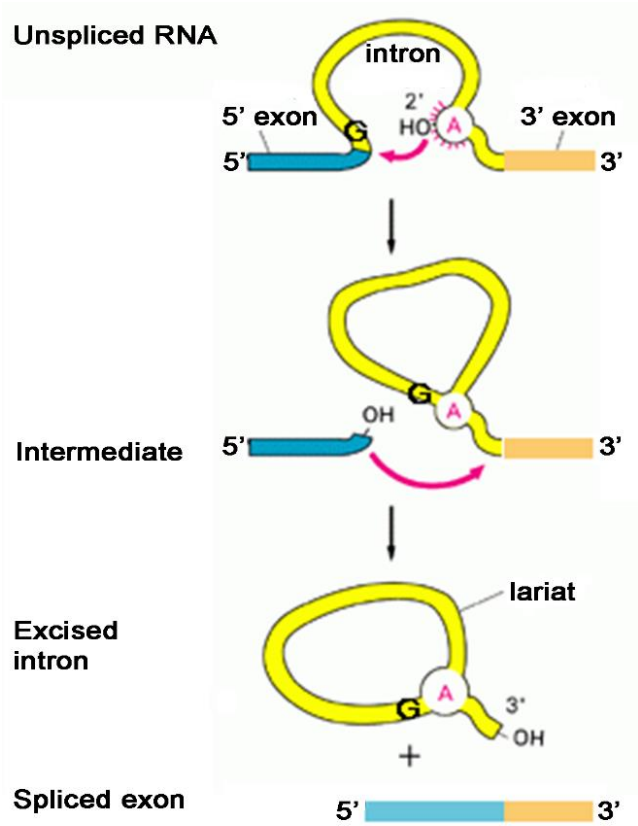
Antibody Genes are Modular – mRNA Splicing Required to Produce mRNA

- When DNA sequences were aligned to RNA sequences, it was found that segments were deleted in the final RNA.
- This suggested that the gene encoding a protein was coded by segments of the DNA:
  - Those to be in the final mRNA were called **exons**.
  - Those sections not in the mRNA were called introns (intervening sequences).



Splice sites are recognized due to specific sequences at the exon-intron boundaries. *Sequences in both the exon and intron are recognized.*





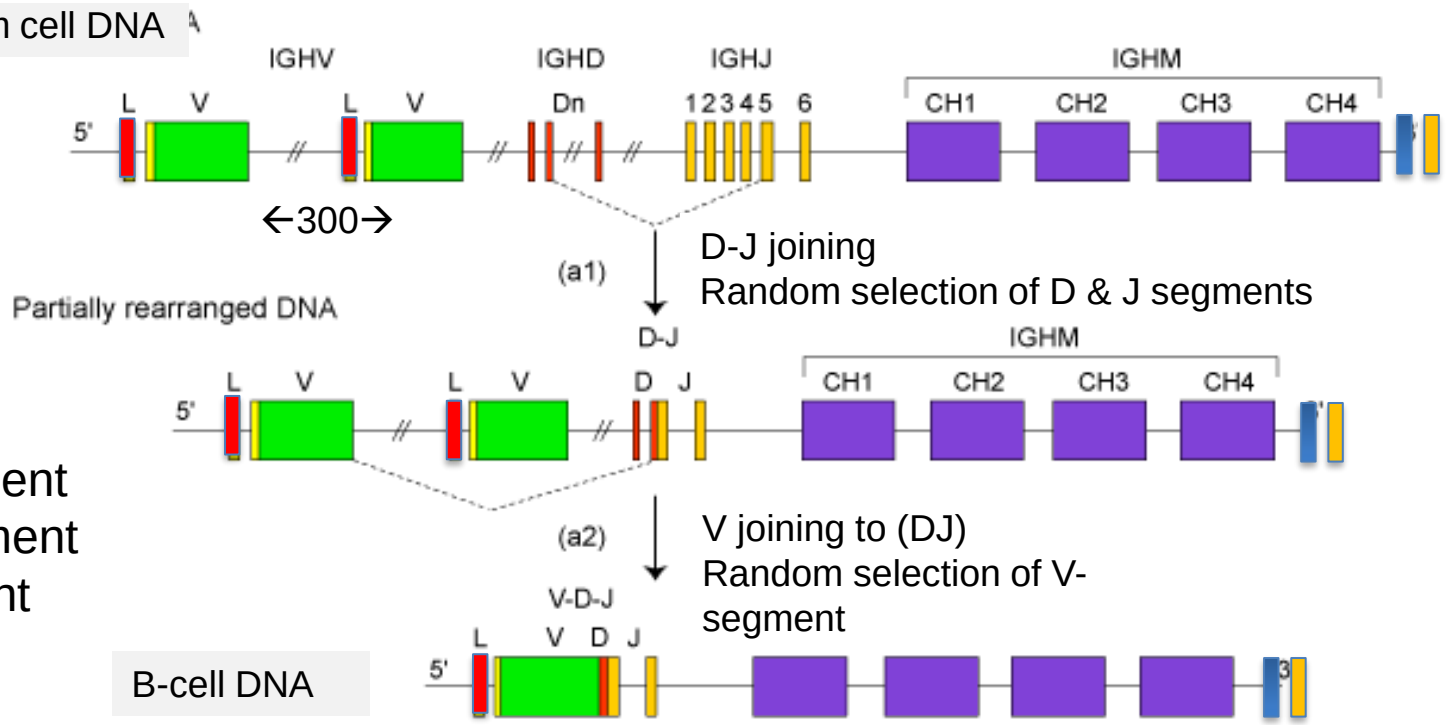
1. There is a 5’ splice site with a conserved sequence: (A/C)AG|GU(A/G)AGU
2. There is a 3’ splice site with a conserved sequence: CAG|G
3. There is an A in the intron (branch point) required for splicing.

Steps:

1. The branch A breaks at the 5’ splice site, forming a lariat.
2. The 5’-OH is joined to the 5’ end of the downstream 3’ exon.

Antibody genes are assembled from DNA segments, giving many different sequences.

Production of Heavy Chain Gene:
V_H exon = V+D+J segment (selected at random)

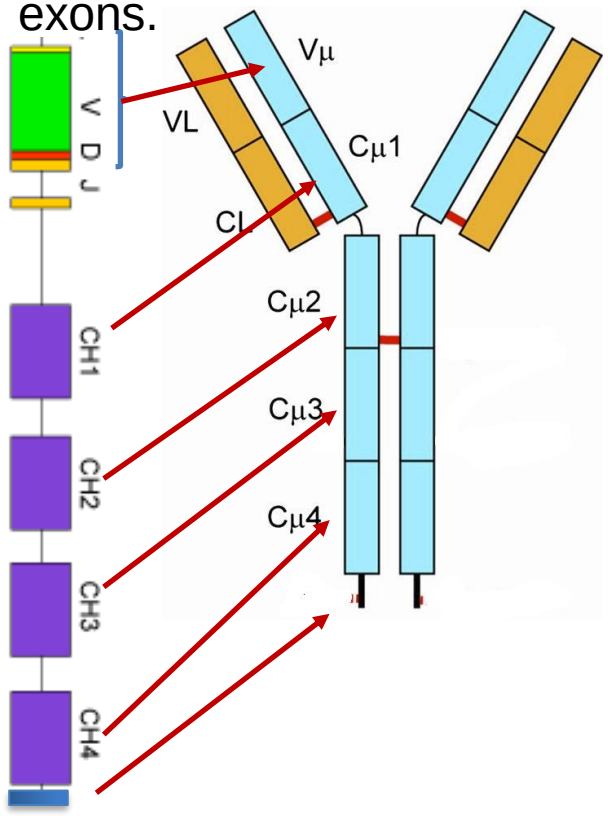


V_H exon differs from one cell to the next.

Heavy chains have the same constant sequences in all B-cells.

L = leader (signal)
V = variable segment
D = diversity segment
J = joining segment

The mRNA coding for antibodies contains 5 exons.



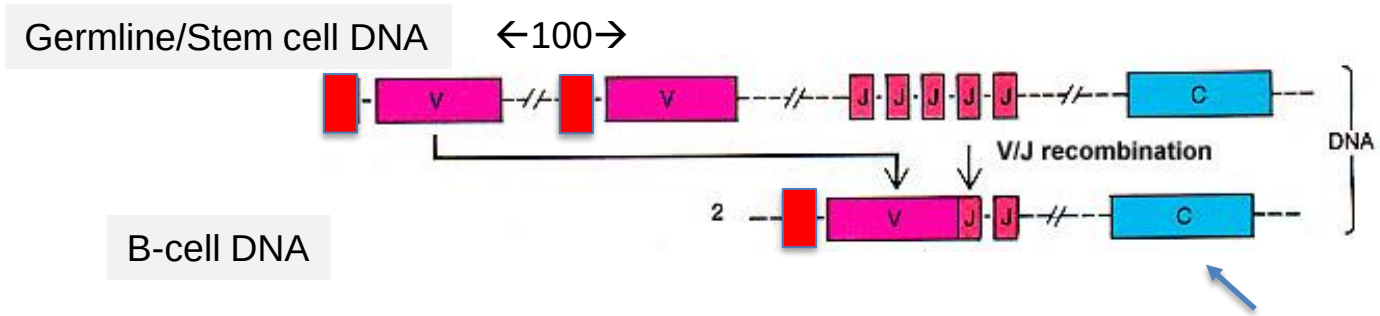
Stem cells -> B-cells

- The exon that codes for the variable region of the heavy chain is generated by the random joining of a V, D, and J DNA segments.
- Each B-cell will generate a unique sequence for its heavy and light chain DNA.
- This is a permanent change to the DNA (***genome)*** of the B-cell.

1. If there are 300 possible V-heavy segments, 10 possible D segments, and 6 possible J segments, how many different heavy chains can be made?

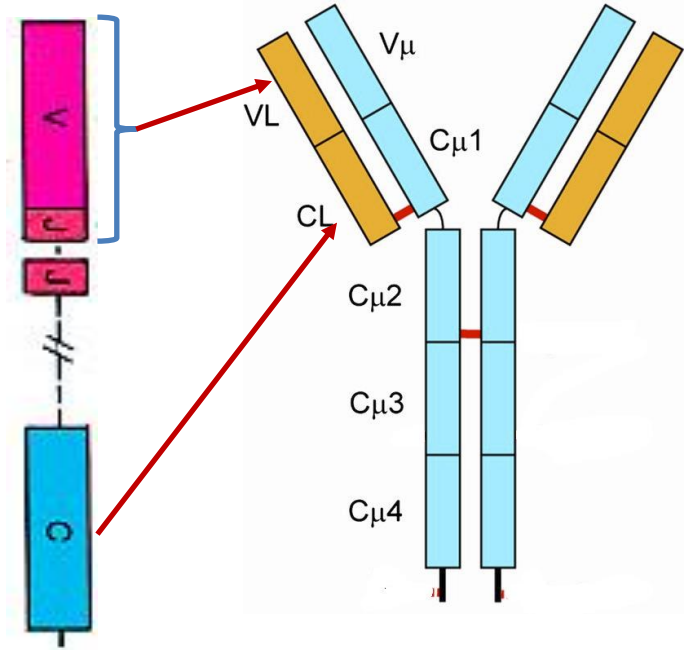
Light-chain genes are assembled from DNA segments, giving many different sequences.

Production of Light Chain Gene



Light chains have different V-sequences in different B-cells.

Light chains have the same constant sequences in all B-cells.



Antibody Diversity

1. If there are 100 possible V-heavy segments and 5 possible J segments, how many different light chains can be made?

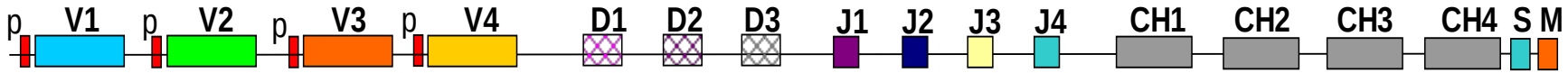
2. If any possible heavy chain can pair with any possible light chain, how many different antibodies can be generated, assuming there are 10,000 possible heavy chains and 500 different light chains?

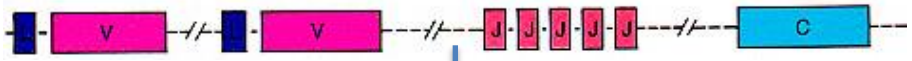
Stem cells -> B-cells

- In the case of the light chain, the variable region is generated by VJ joining.
- Each B-cell will generate a unique sequence for its heavy and light chain DNA.
- This is a permanent change to the DNA (***genome)*** of the B-cell.

Antibody Production – From Stem Cells to B-Cells

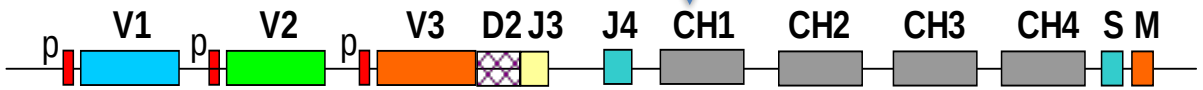
Stem Cell



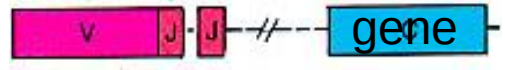


B-Cell

Rearranged heavy chain gene



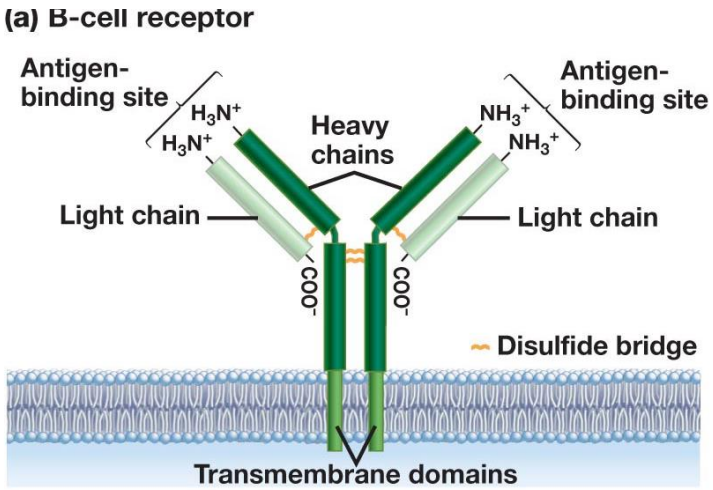
Rearranged light chain gene



Transcription

mRNA Splicing

Translation

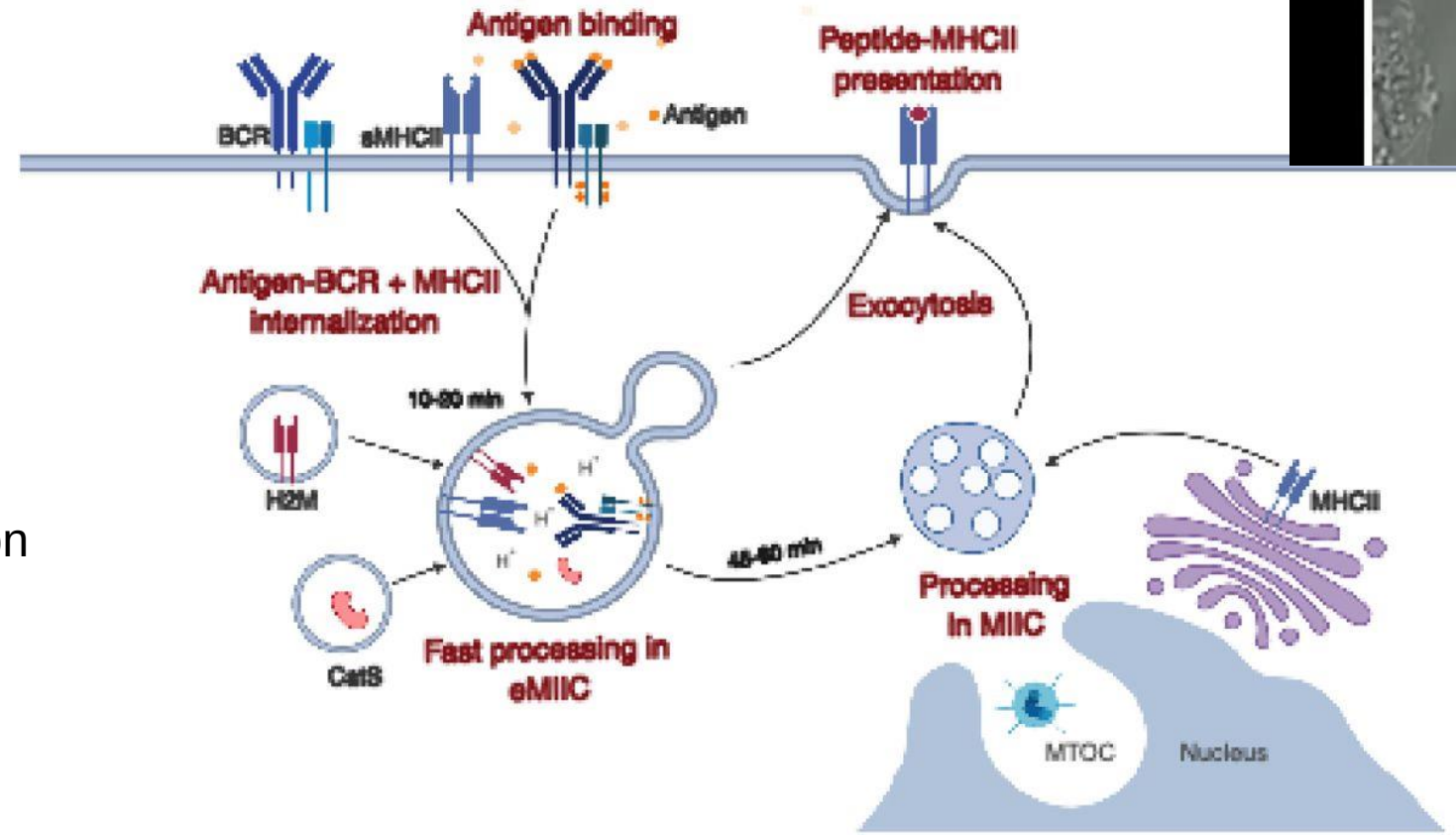


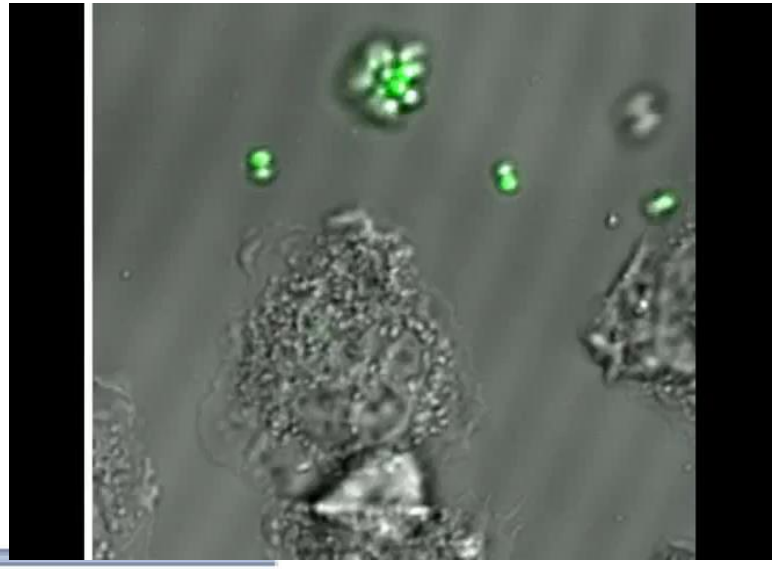
Export to cell surface (ER -> golgi -> Membrane)

Antigen Capture by B-Cells - Endocytic Pathways

1. Antigen binds to variable domains of antibody on the BCR (B-cell receptor)
2. Antigen is internalized and digested into peptides
3. Peptides are loaded on to class II MHC for presentation to T-cells

Endocytosis of bacteria by a B-cell



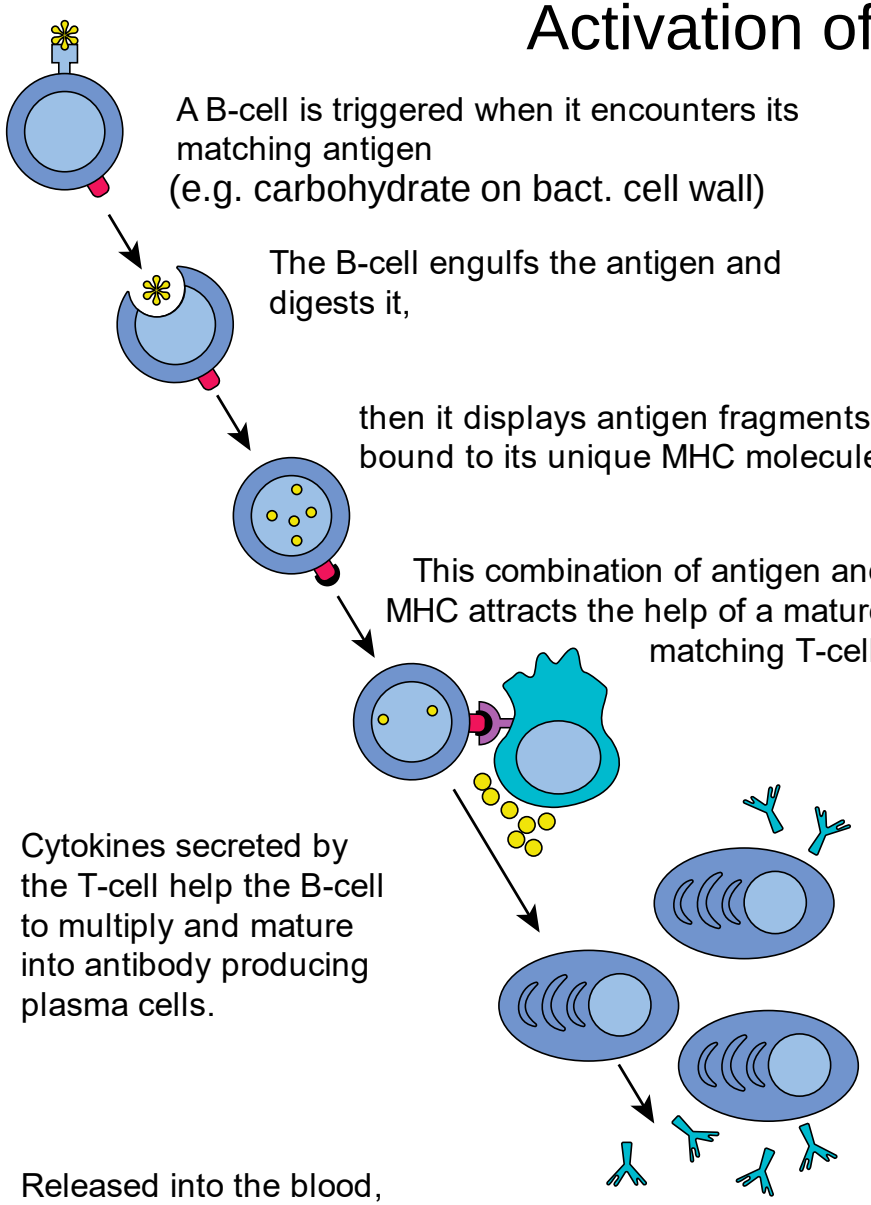


Bacteria labeled with Green fluorescent protein.

1. Capture of the bacteria
2. Internalization (endocytosis)
3. Degradation of the bacterial proteins, producing peptides.

Journal of Cell Science doi: [10.1242/jcs.235192](https://doi.org/10.1242/jcs.235192)

Activation of B cells by Antigen - Lymph Node



A B-cell is triggered when it encounters its matching antigen
(e.g. carbohydrate on bact. cell wall)

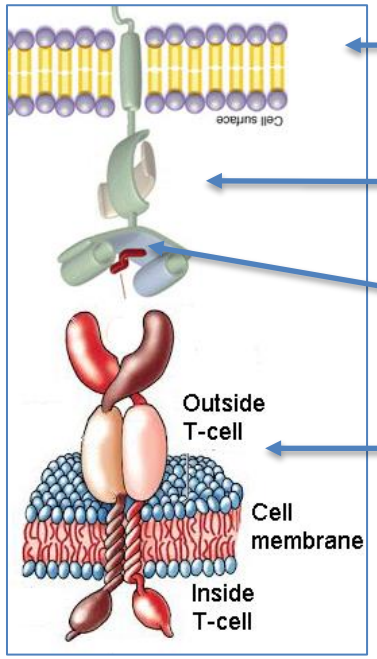
The B-cell engulfs the antigen and digests it,

then it displays antigen fragments bound to its unique MHC molecules

This combination of antigen and MHC attracts the help of a mature matching T-cell.

Cytokines secreted by the T-cell help the B-cell to multiply and mature into antibody producing plasma cells.

Released into the blood, antibodies lock onto matching antigens. The antigen-antibody complexes are then cleared by the complement cascade or by the liver and spleen.



B-cell

MHC II - Major histocompatibility complex

Peptide from pathogen (foreign peptide)

T-cell Receptor (TCR)

T_H cell

Events:

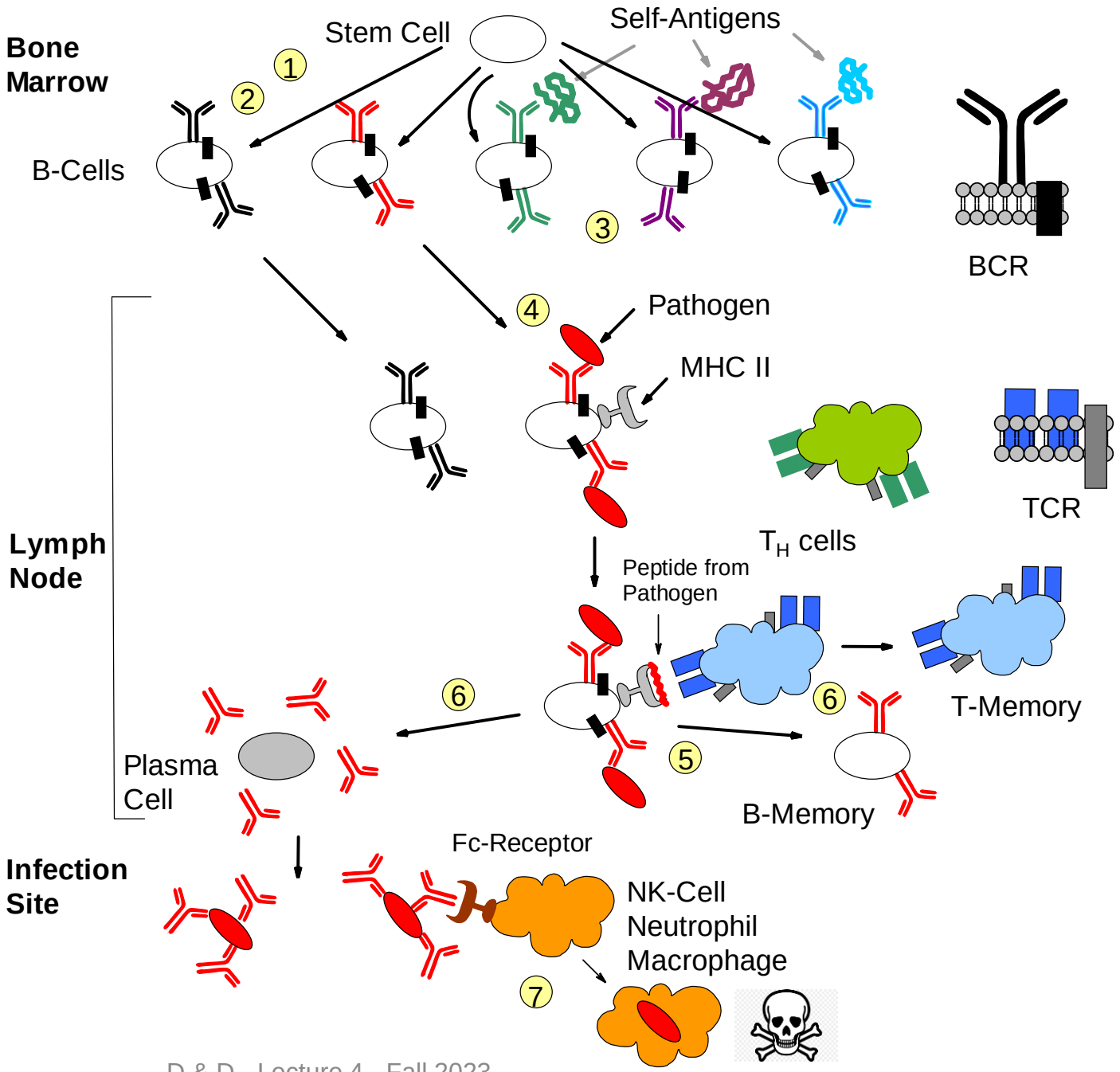
1. Recognition of MHC II-peptide by TCR
2. Tyrosine kinase signaling in T_H cell
3. Cytokines (protein messengers) produced.
4. Cytokines activate B-cells.

- B-cells develop into antibody secreting *plasma cells*.
- B and T-helper cells develop into **memory** cells, that are long-lived and are quickly activated by the same pathogen. ***This is the basis of vaccination.***

Soluble antibody from plasma cells has the same light and heavy chains as the original B-cell.
Membrane anchors are missing, so antibody is secreted outside the cell.

Can you:

- Describe how the genes for the heavy and light chain are generated, and how this give rise to many different antibodies?
- Do you understand the process of B-cell activation, including presentation of foreign peptides on MHC II and the role of the T-helper cell.
- Describe how antibodies inactivate pathogens?



Cell Based Immunology

Key Questions:

1. How does your immune system fight viruses?
2. How does your immune system detect and destroy cancer cells?
3. How can the immune response be engineered to fight cancer?

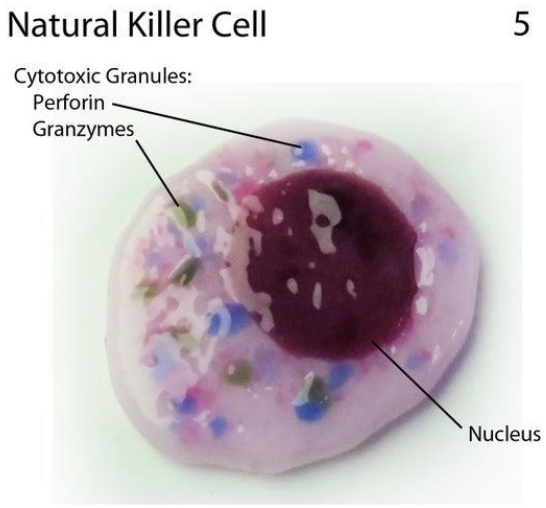
Cell Types:

Innate

- Natural Killer (NK) cell

Acquired

- T_H
- T_C, T_{CTL}

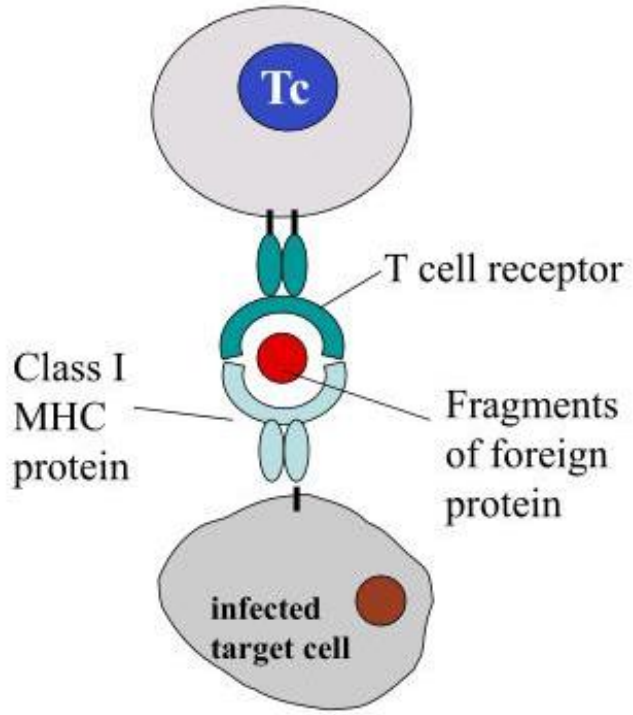


NK: Innate

- Kill virally infected cells
- Kill cancer cells

T_C → T_{CTL}

T_C Memory



Activation of Tc cells requires:

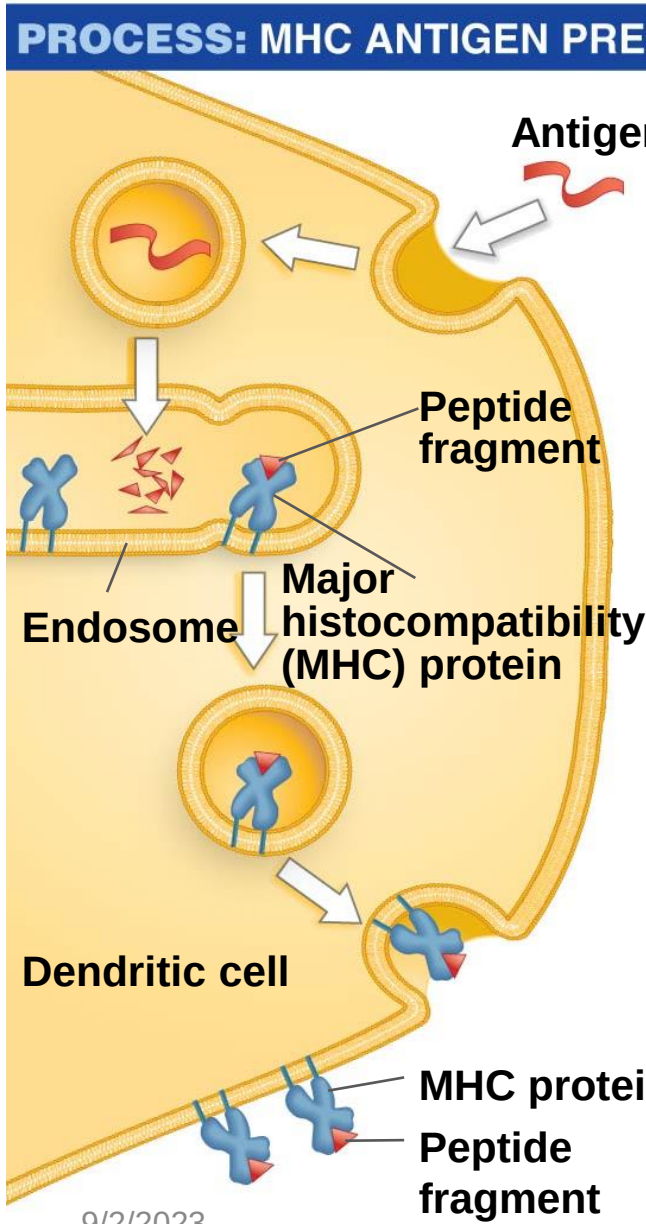
1. Recognition of ***foreign*** peptide on MHC I.
2. Assistance from T-helper cells.

Activated Tc cell becomes a cytotoxic T-lymphocyte T_{CTL}

T_{CTL}

- Kill virally infected cells
- Kill cancer cells

Dendritic cells acquire antigen from viruses and cancerous cells, activating T-cells



1. Dendritic cell ingests antigen via ***phagocytosis*** (intact virus, cell debris from cancer cell).

2. Enzymes break antigen proteins into peptide fragments.

3. Peptide fragments are loaded onto both class I and class II MHC proteins in endosomes.

4. MHC I & II –peptide complex is transported to cell surface.

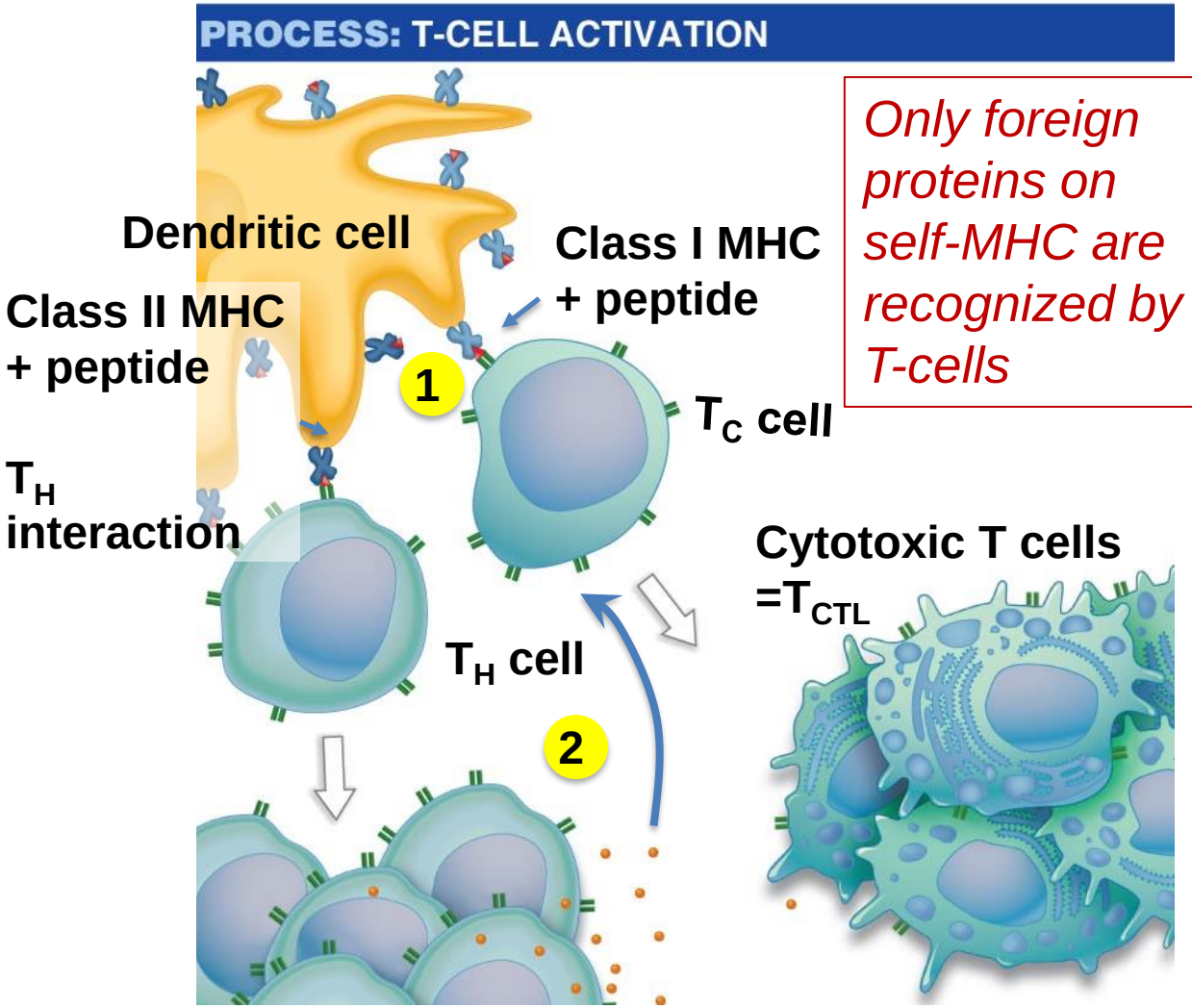
5. MHC protein presents peptide fragment on cell surface.

Dendritic cells acquire antigen from viruses and cancerous cells, activating T-cells

Activation of Tc cells requires:

1. Recognition of foreign peptide on MHC I by TCR on Tc cell

2. Assistance from T-helper cells via secreted messengers (small proteins called cytokines)

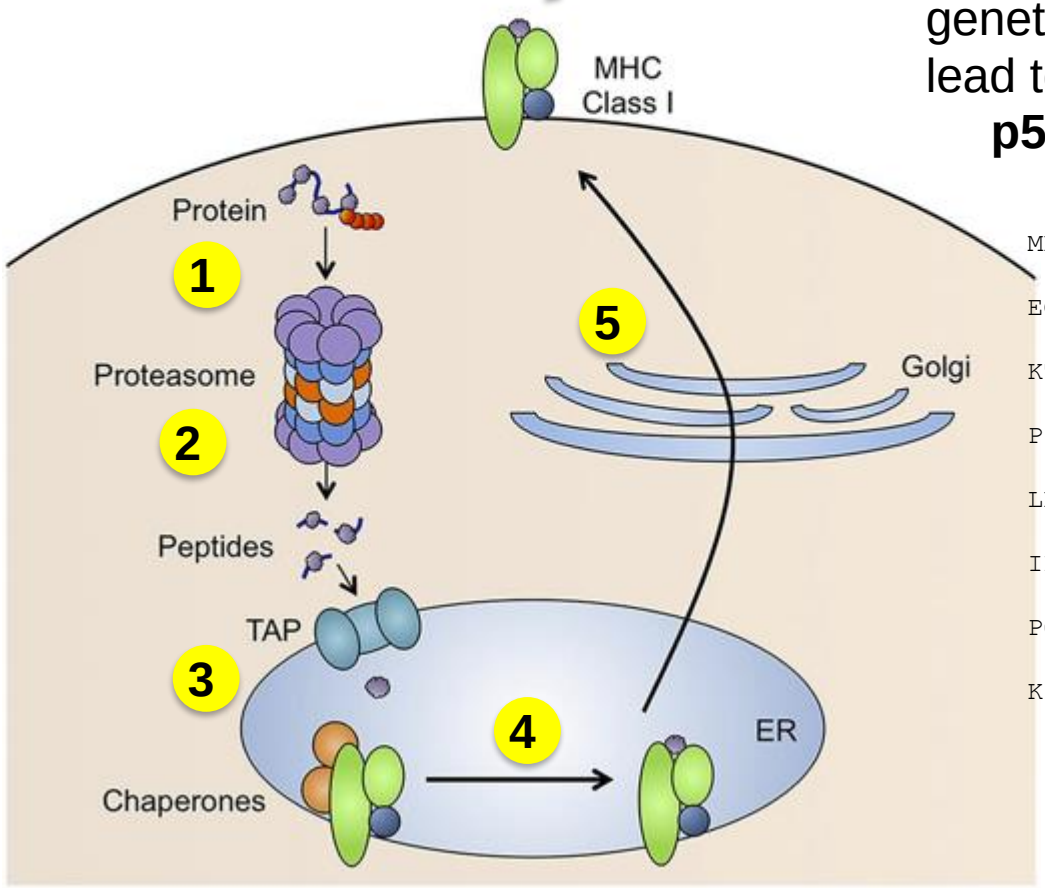


Only foreign proteins on self-MHC are recognized by T-cells

Acquired Cellular Immunity - Role of MHC I in Presentation of Peptides

- MHC I present peptides
- Peptides are generated from of ***all*** of the proteins that are made in the cell.
- Steps:
  1. protein targeted for degradation by ubiquitin
  2. Protein digested by proteasome
  3. Peptides transported into ER
  4. Peptides loaded on to MHC I
  5. Peptide/MHC complex transported to cell membrane.

Only foreign peptides activate T-cells



Foreign Peptide Source:

1. From replication of viruses in the cell
2. From replication of intracellular bacteria (e.g. TB)
3. New coding sequences in cancer cells due to genetic changes (e.g. mutations in p53 may lead to novel sequences).

p53 Protein Sequence

Zn Fingers (DNA binding

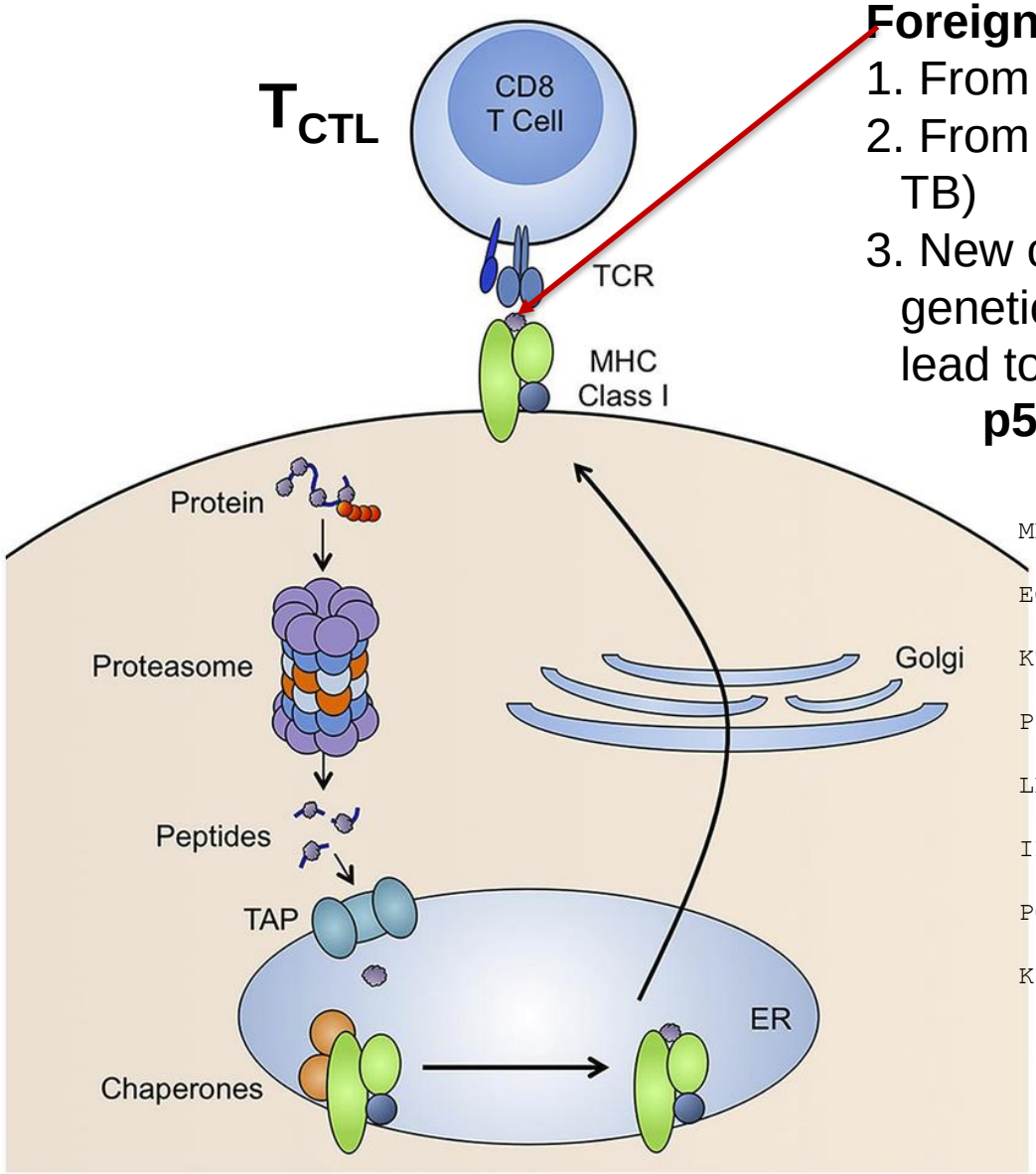
```
        10         20         30         40         50
MEEPQSDPSV EPPLSQETFS DLWKLLPENN VLSPLPSQAM DDLMLSPDDI
        60         70         80         90        100
EQWFTEDPGP DEAPRMPEAA PPVAPAPAAP TPAAPAPAPS WPLSSSVPSQ
       110        120        130        140        150
KTYQGSYGFR LGFLHSGTAK SVTCTYSPAL NKMFCQLAKT CPVQLWVDST
       160        170        180        190        200
PPPGTRVRAM AIYKQSQHMT EVVRRCPHHE RCSDSDGLAP PQHLIRVEGN
       210        220        230        240        250
LRVEYLDDRN TFRHSVVVPY EPPEVGSDCT TIHYNYMCNS SCMGGMNRRP
       260        270        280        290        300
ILTIITLEDS SGNLLGRNSF EVRVCACPGR DRRTEEENLR KKGEPHHELP
       310        320        330        340        350
PGSTKRALPN NTSSSPQPKK KPLDGEYFTL QIRGRERFEM FRELNEALEL
       360        370        380        390
KDAQAGKEPG GSRAHSSHLK SKKGQSTSRH KKLMFKTEGP DSD
```

EVVRRCPHHE — Normal seq., ***ignored*** by TCR

EVVGGCPHHE — Mutant seq. in cancer, ***detected*** by TCR

Acquired Cellular Immunity – Recognition of MHC I + Peptide by Tc Cells

- Tc cells will be activated ***only*** by ***foreign peptides*** on MHC class I.
- Tc cells develop into:
  - Tc memory cells
  - cytotoxic T-lymphocytes (T_{CTL})
- T_{CTL} cells kill the infected/cancer cell presenting the same peptide that activated it.



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       110        120        130        140        150
KTYQGSYGFR LGFLHSGTAK SVTCTYSPAL NKMFCQLAKT CPVQLWVDST
       160        170        180        190        200
PPPGTRVRAM AIYKQSQHMT EVVRRCPHHE RCSDSDGLAP PQHLIRVEGN
       210        220        230        240        250
LRVEYLDDRN TFRHSVVVPY EPPEVGSDCT TIHYNYMCNS SCMGGMNRRP
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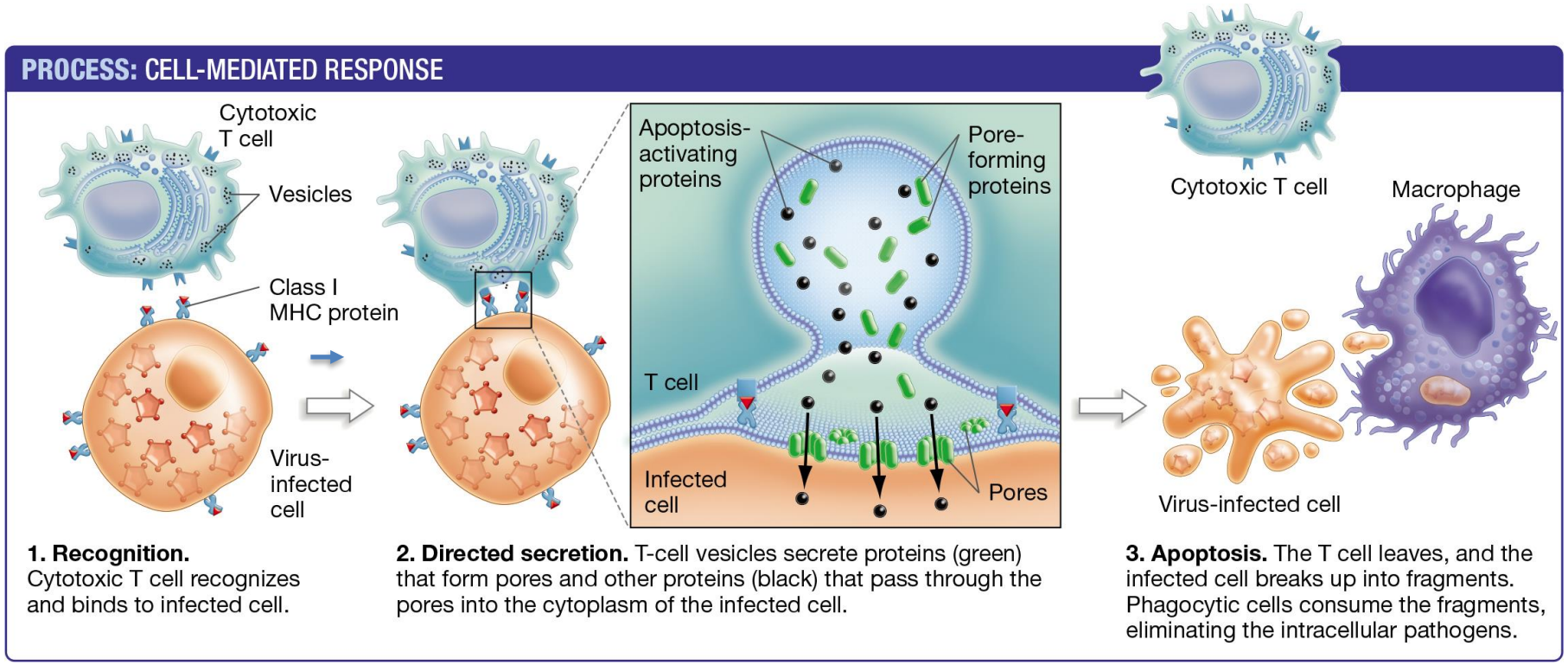
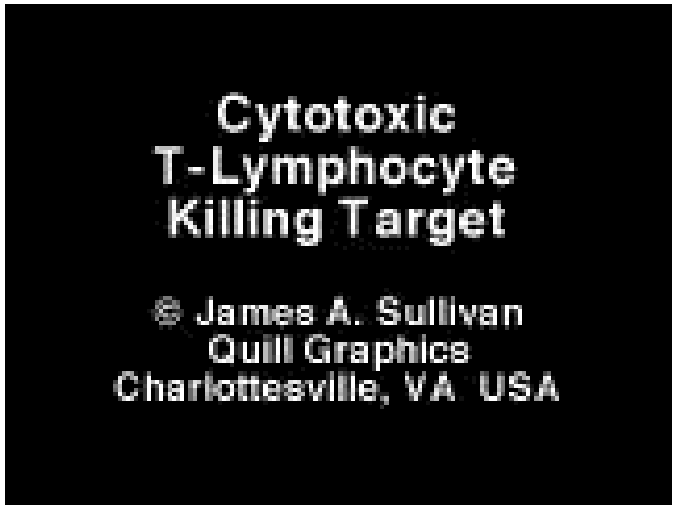
ILTIITLEDS SGNLLGRNSF EVRVCACPGR DRRTEEENLR KKGEPHHELP
       310        320        330        340        350
PGSTKRALPN NTSSSPQPKK KPLDGEYFTL QIRGRERFEM FRELNEALEL
       360        370        380        390
KDAQAGKEPG GSRAHSSHLK SKKGQSTSRH KKLMFKTEGP DSD
```

EVVRRCPHHE Normal seq., ***ignored*** by TCR

EVV**GG**CPHHE Mutant seq. in cancer, ***detected*** by TCR

Detection and Killing of Virally Infected or Cancer Cells:





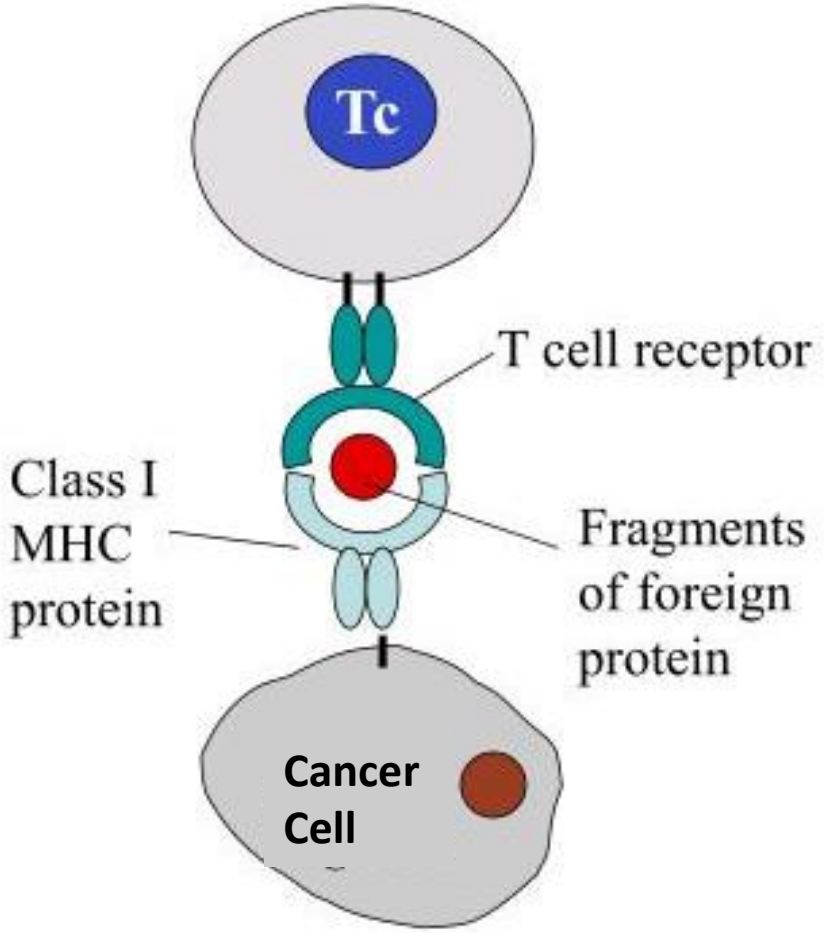
Cancer cell or Infected cell

- Granzymes enter through perforin pore and cause cell undergo programmed cell death (apoptosis)

Cancer Evasion Mechanism I - Loss of MHC I on Tumor Cell

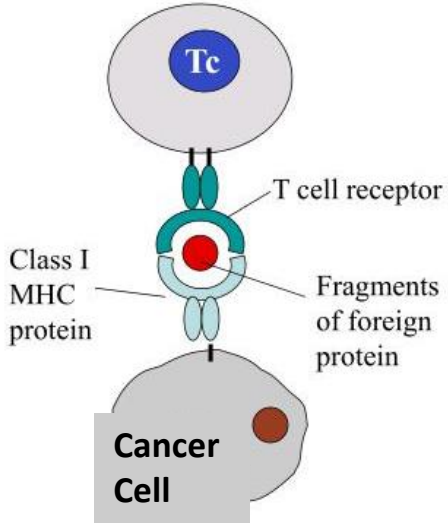
Loss of MHC I expression means that T_{CTL} cells can no longer recognize and kill cancer cells because T-cell activation requires recognition of the MHC-peptide complex.

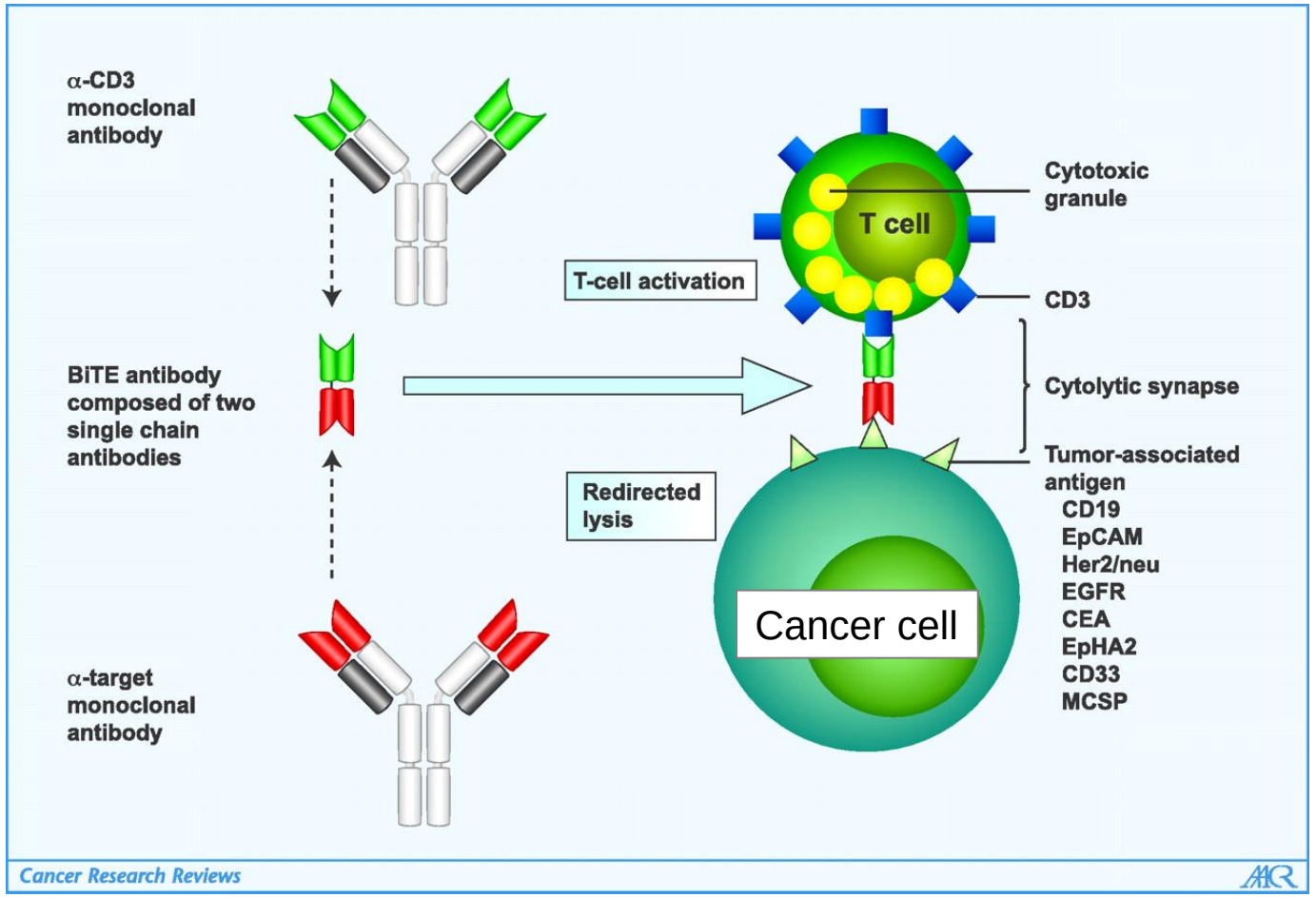
How to re-establish T_C contact with tumor cell and activation of the T-cell so that the cancer cell is killed?



Cancer Treatment with Antibodies - 1. Cancer Evasion - Loss of MHC I on Tumor Cell

Loss of MHC I expression means that T_{CTL} cells can no longer recognize and kill cancer cells because T-cell activation requires recognition of the MHC-peptide complex.



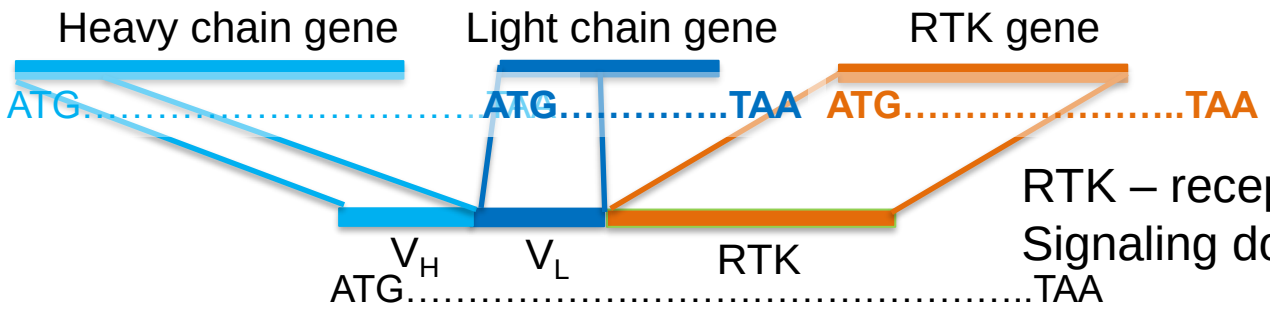


How to re-establish T_C contact with tumor cell and activation of the T-cell so that the cancer cell is killed?

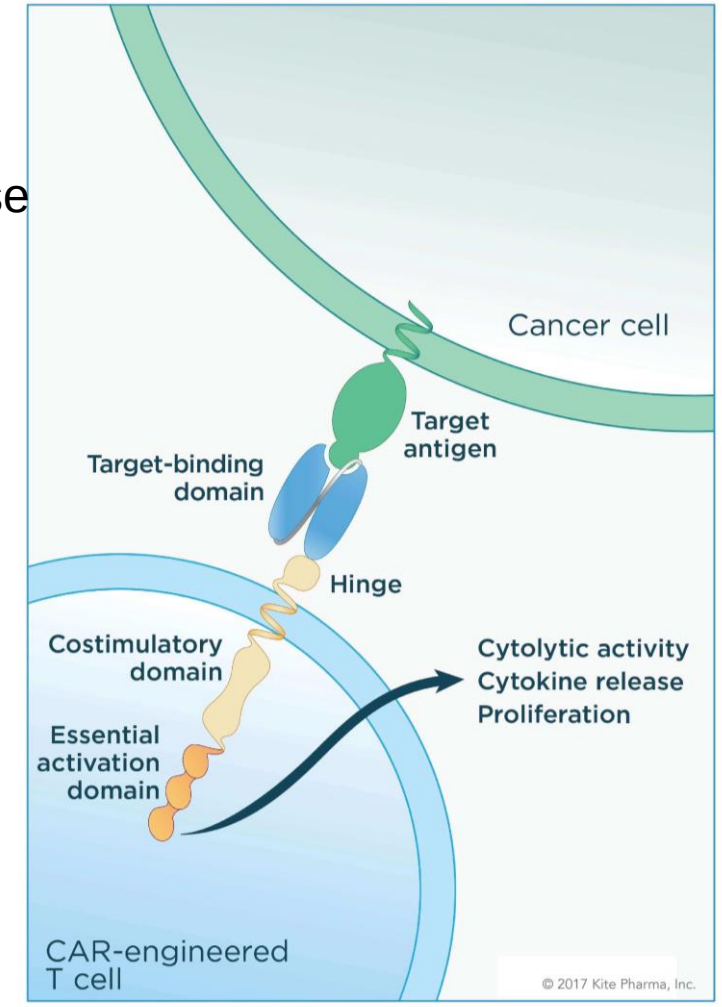
- Bispecific antibodies are generated from two separate antibodies:
  - One recognizes CD3, which is part of the T-cell receptor (TCR)
  - Other recognizes a tumor antigen.
- The two variable regions from each antibody are linked into a single polypeptide chair
- The dual binding event mimics the original MHC-I TCR interaction.,

Chimeric Antigen Receptor T-cells = CAR T-Cells

A. Obtain antibodies against cancer antigen, isolate genes that code for light and heavy chains for those antibodies.
B. Fuse coding region for variable light and heavy domains to coding region for RTK on T-cells = single CAR-T gene.

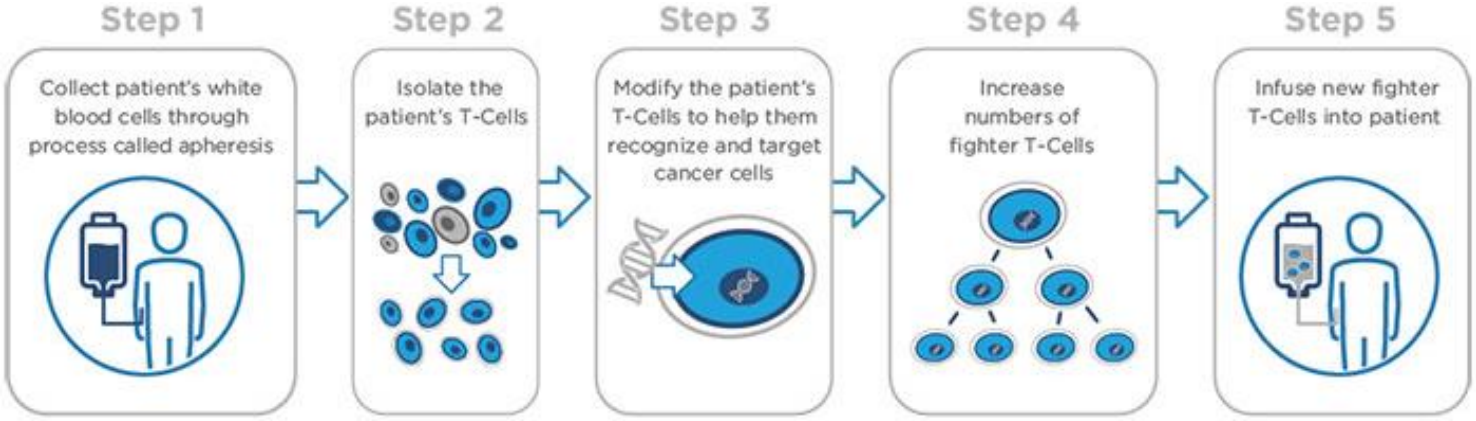


RTK – receptor tyrosine kinase
Signaling domain on T-cells



C. Introduce gene for CAR-T cell into Patient
1. Obtain white blood cells from patient
2. Isolate T-cells
3. Introduce DNA into T-cells
4. Obtain large amounts of T-cells by cell culture
5. Inject CAR-T cells into cancer patient.

D. What happens when cancer cell is encountered by CarT cell?



Cancer Evasion Mechanism II – Downregulation/killing of Tc cells.

The Nobel Prize in Physiology or Medicine 2018

James P. Allison
Prize share: 1/2

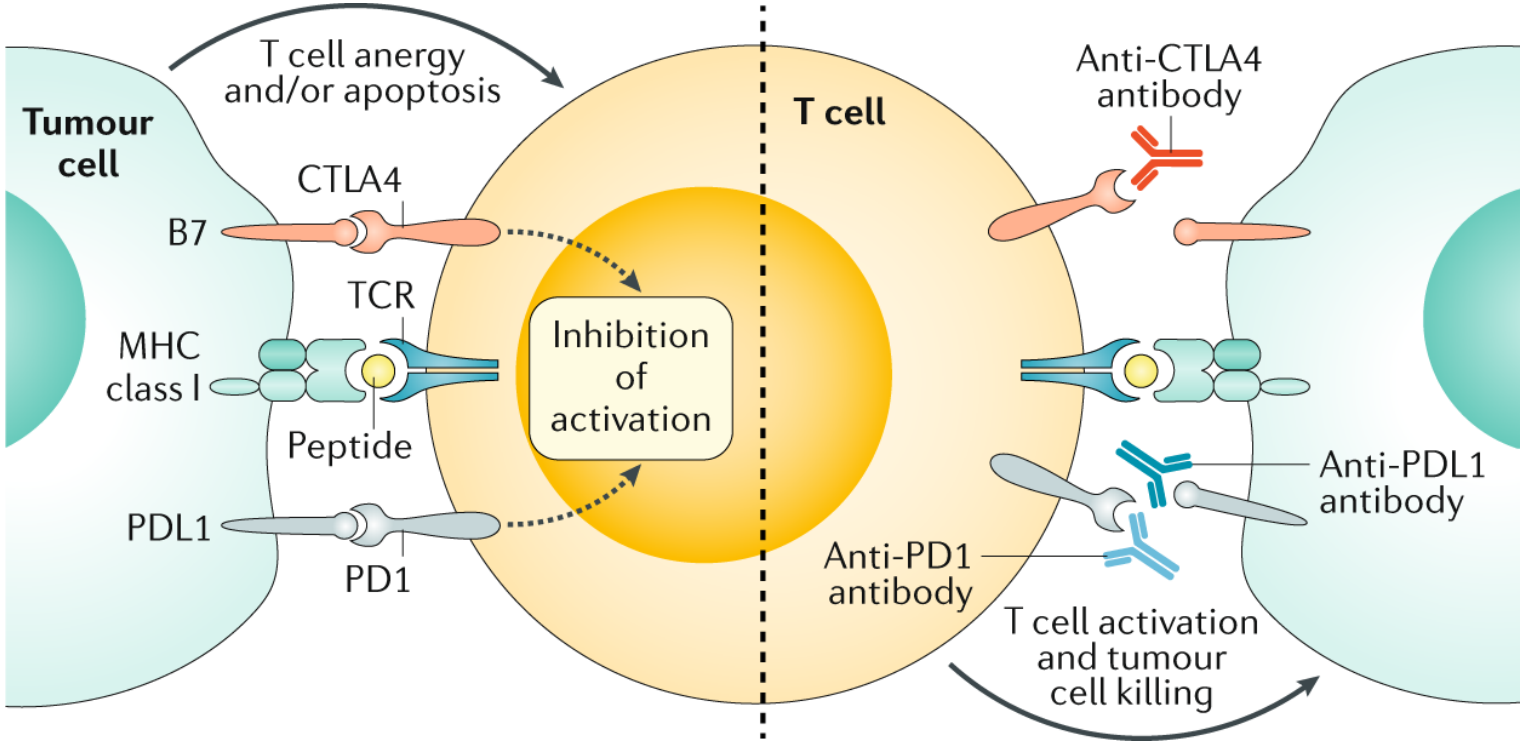
Tasuku Honjo
Prize share: 1/2

The Nobel Prize in Physiology or Medicine 2018 was awarded jointly to James P. Allison and Tasuku Honjo "for their discovery of cancer therapy by inhibition of negative immune regulation."

1. **Cancer cells overproduce b7.** Binding of b7 to CTLA4 receptor on the surface of the Tc cell deactivates the Tc cell - immunosuppressive reaction called anergy.

2. **Cancer cells overproduce PDL1.** PDL1 binds to PD1 on T-cells. Activation of signaling causes Tc cell to enter apoptosis (programmed cell death).

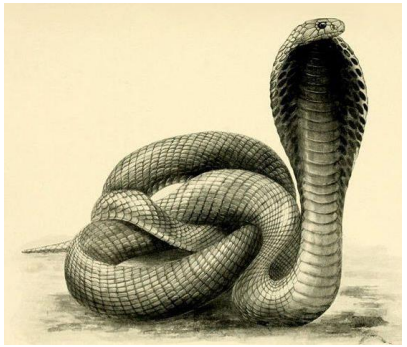
How to block this signaling?

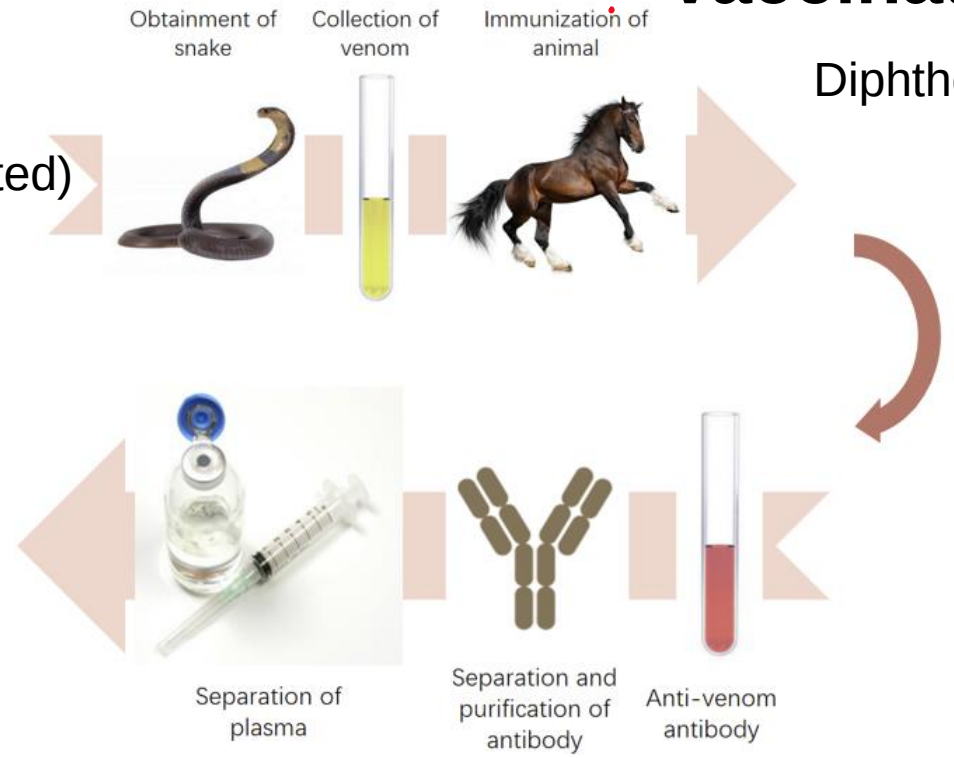


Vaccination

Types of vaccines:

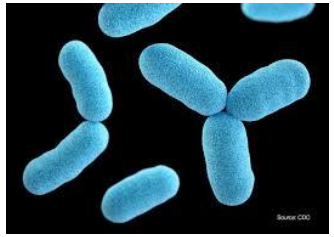
1. Passive (Ab injected)





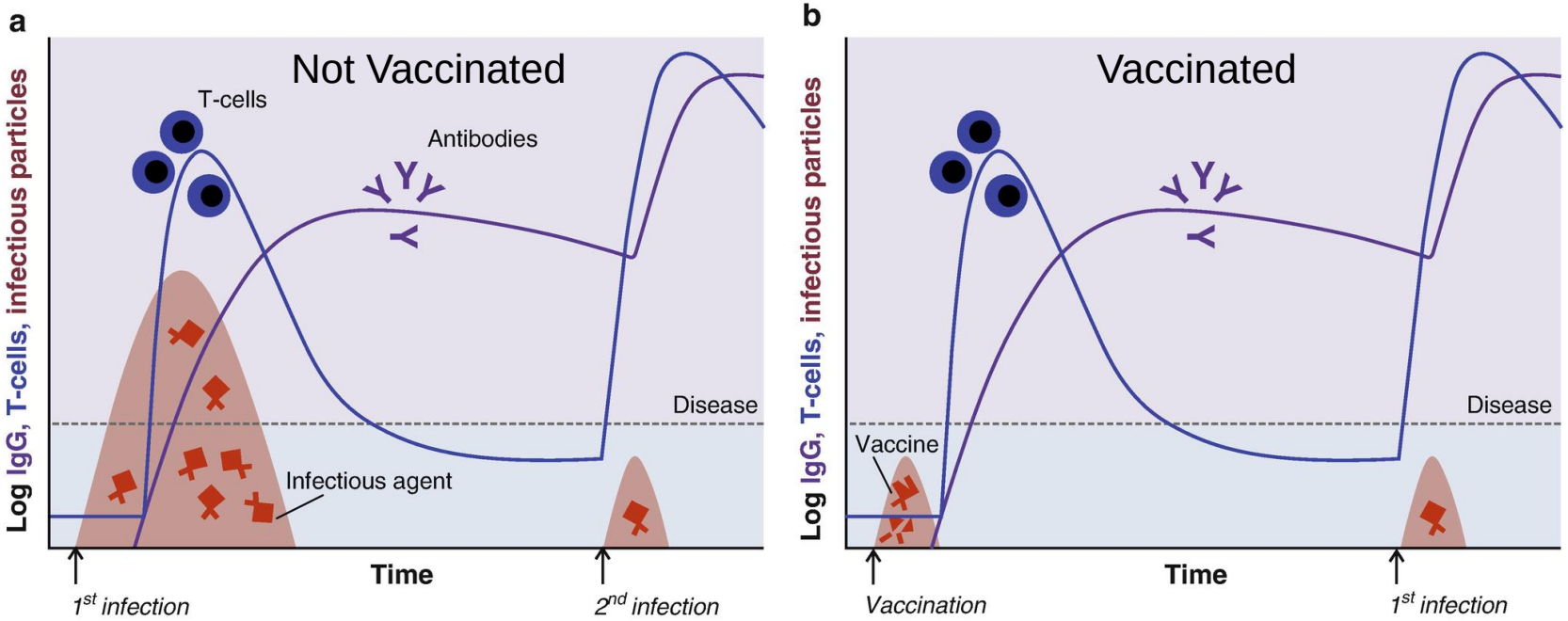
2. Active (Antigen Provided)

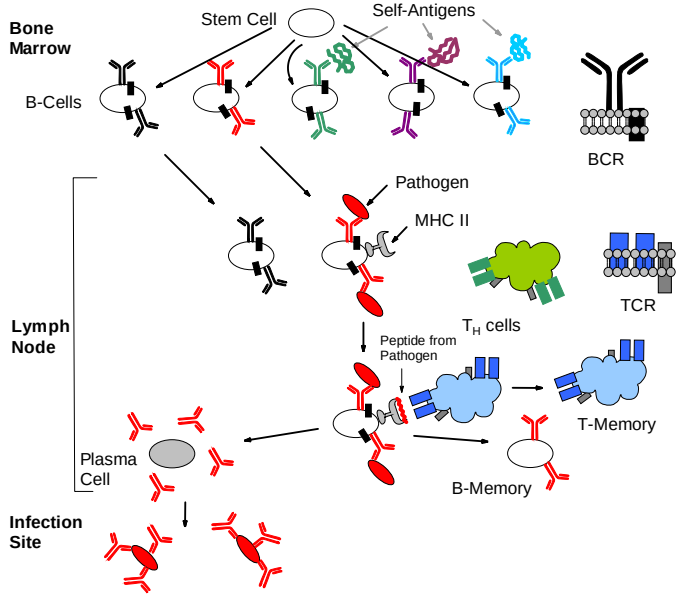
Diphtheria

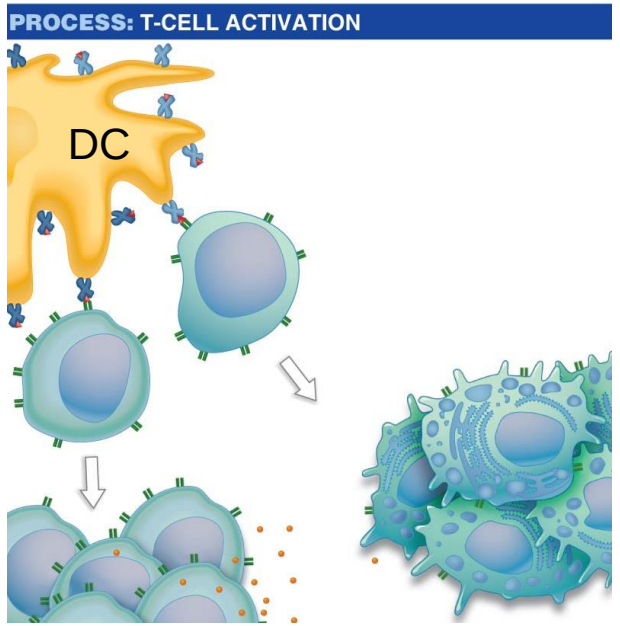


Breast feeding provides IgA to the newborn.

Vaccine: a vehicle containing a **form of an antigen** that is administered to induce memory B and T cells specific for that antigen.







T_H-Memory T_C-Memory

Large number of pathogens during first (primary) infection causes disease symptoms

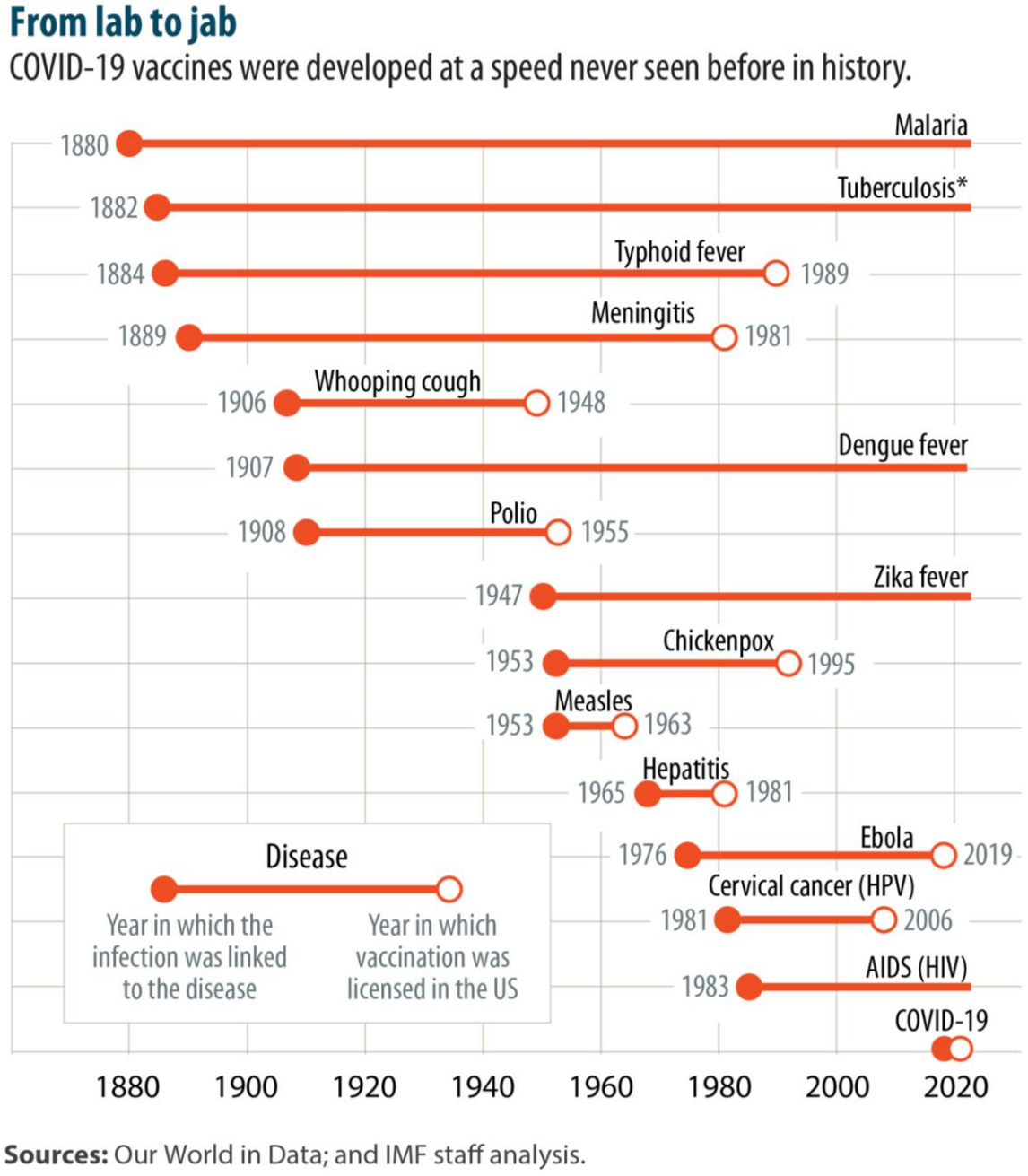
More rapid secondary response prevents extensive pathogen growth – no symptoms.

Jiskoot W., Kersten G.F.A., Mastrobattista E., Slütter B. (2019) Vaccines. In: Crommelin D., Sindelar R., Meibohm B. (eds) Pharmaceutical Biotechnology. Springer, Cham. https://doi.org/10.1007/978-3-030-00710-2_14

Vaccine History

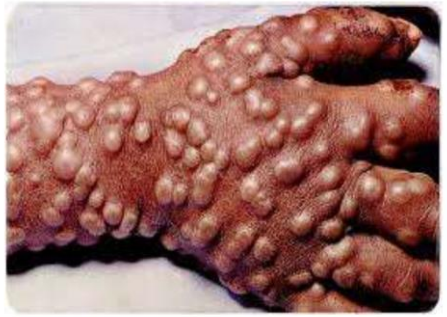
- Some diseases still do not have vaccines
- Other diseases have been eliminated by vaccination

https://www.imf.org/en/Publications/fandd/issues/2021/12/Journey-covid-19-vaccine-Stanley



Smallpox - A Success Story for Vaccination

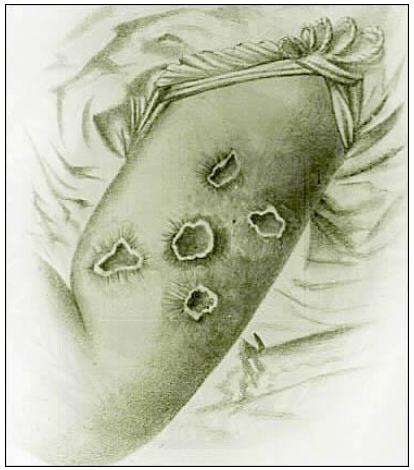
Vaccination – to introduce immunity prior to infection by pathogen

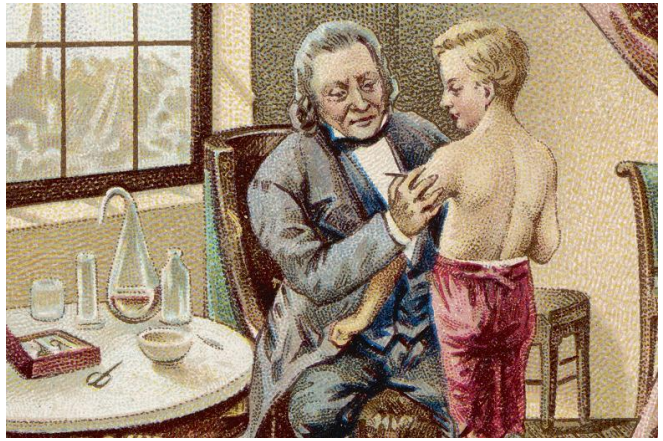


Variolation (1800) provided protection by exposing people to small amounts of smallpox virus (obtained from blisters on infected people).

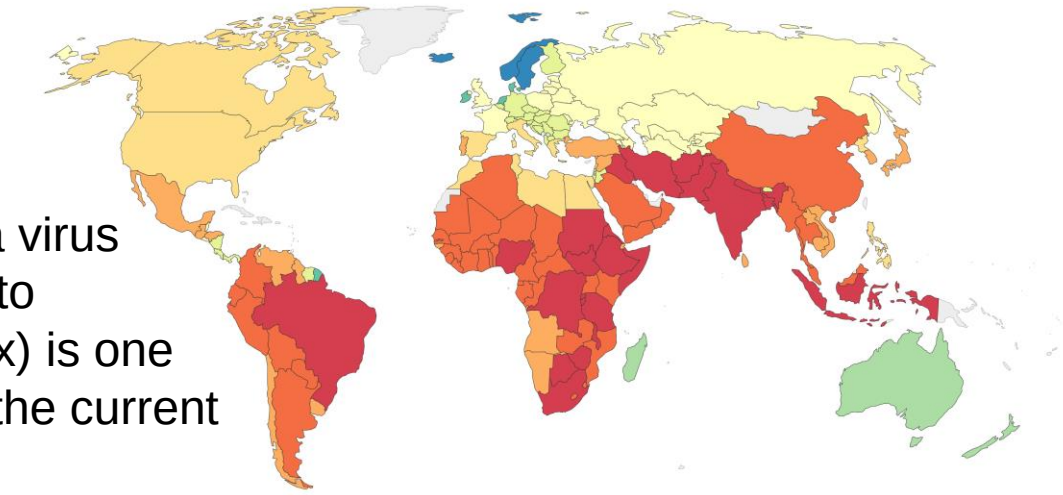
Risky because smallpox was used to vaccinate

Smallpox - 30% lethality





Vaccinia virus (similar to smallpox) is one form of the current vaccine.



Cowpox virus causes production of antibodies against smallpox
Jenner was the first to use cowpox to vaccinate against smallpox

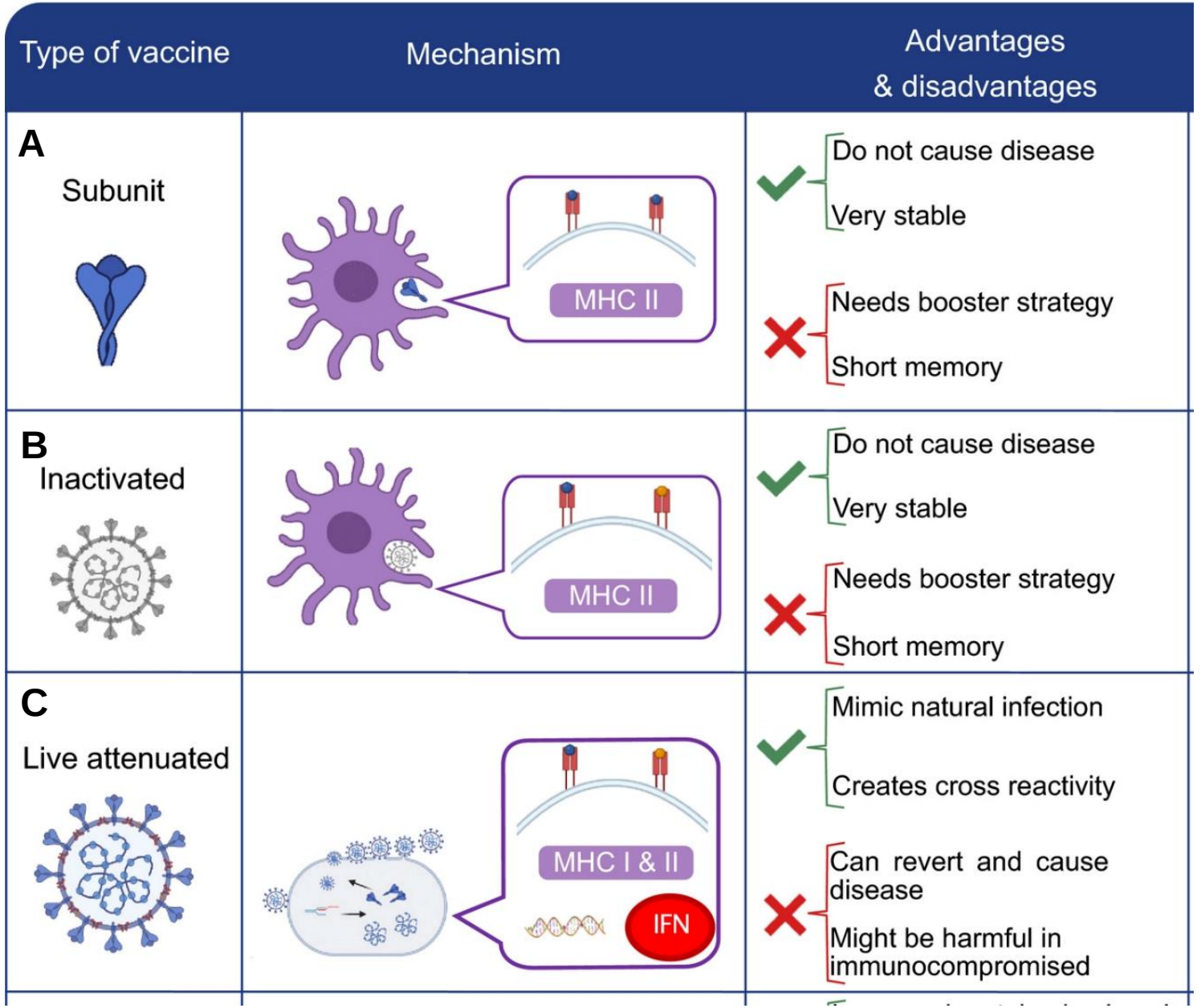
Types of Vaccines

A. Subunit Vaccine:
A protein from the pathogen is used to induce memory cells, e.g. spike protein from the virus.

B. Inactivated Virus
The virus is chemicall inactivated before administration.

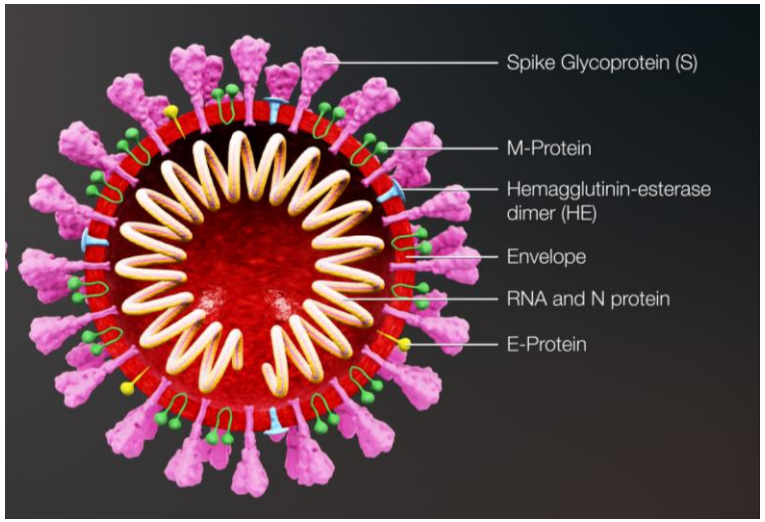
C. Live Attenuated
The virus is grown under conditions that select for mutant viruses that:

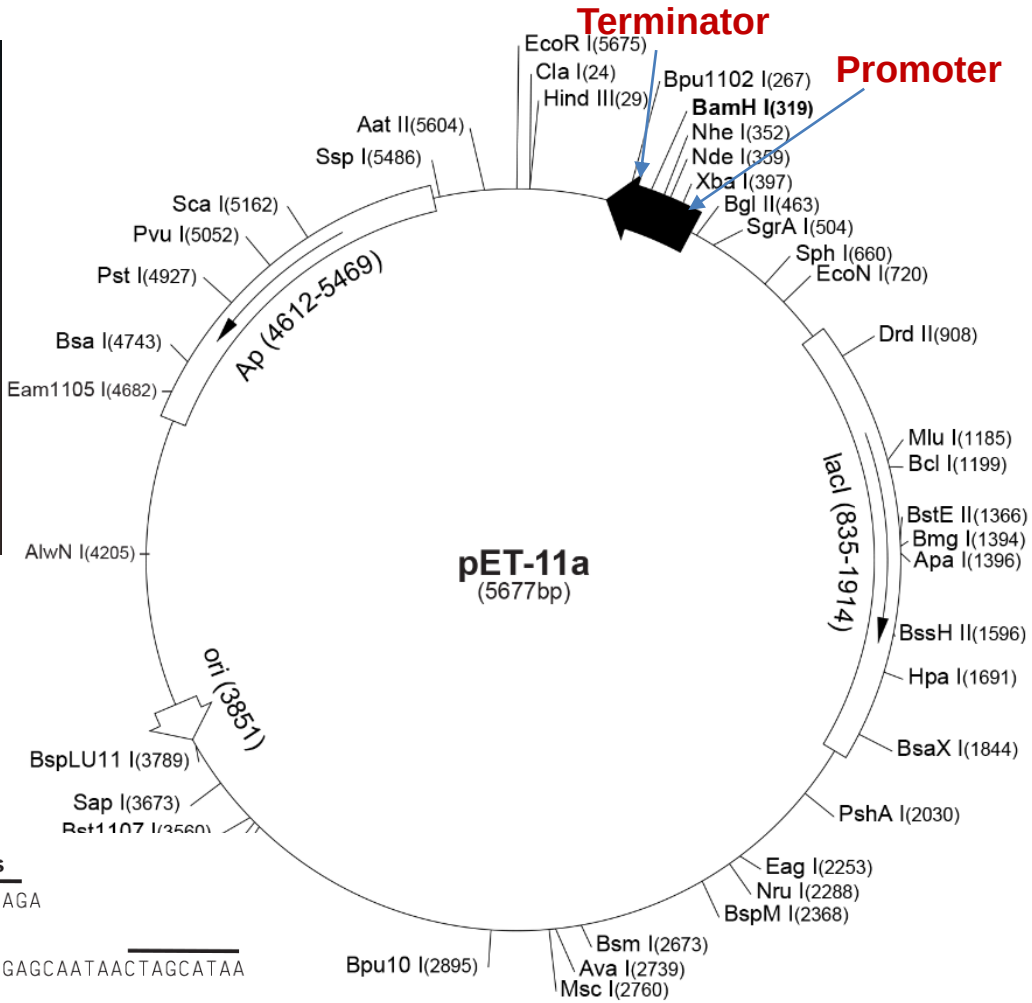
i) Induce memory cells in humans
ii) Do not cause disease symptoms

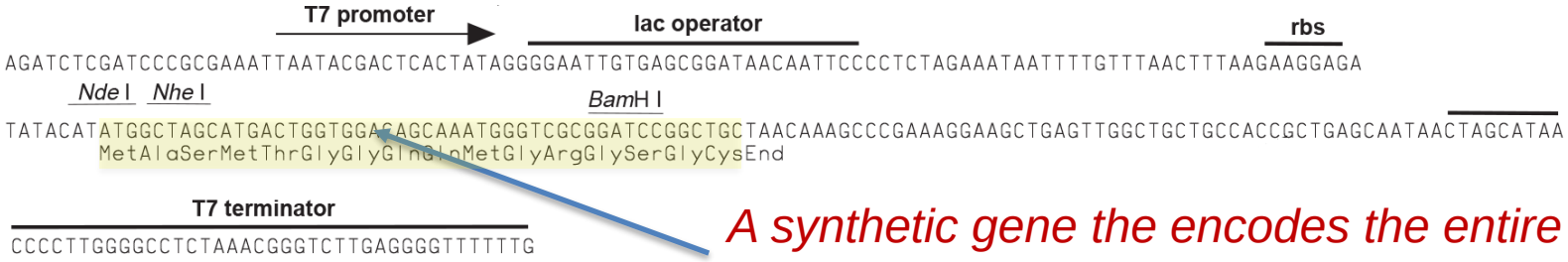


A. Subunit Vaccine - Producing Covid-19 Spike Protein in Bacteria

- *We will incorporate the gene for the spike protein into a plasmid.*
- *Plasmid is a circular DNA molecule that is replicated by the bacteria.*
- *The plasmid will also provide a promotor and mRNA termination sites so that the bacteria will make mRNA and then the mRNA will be translated into the spike protein.*

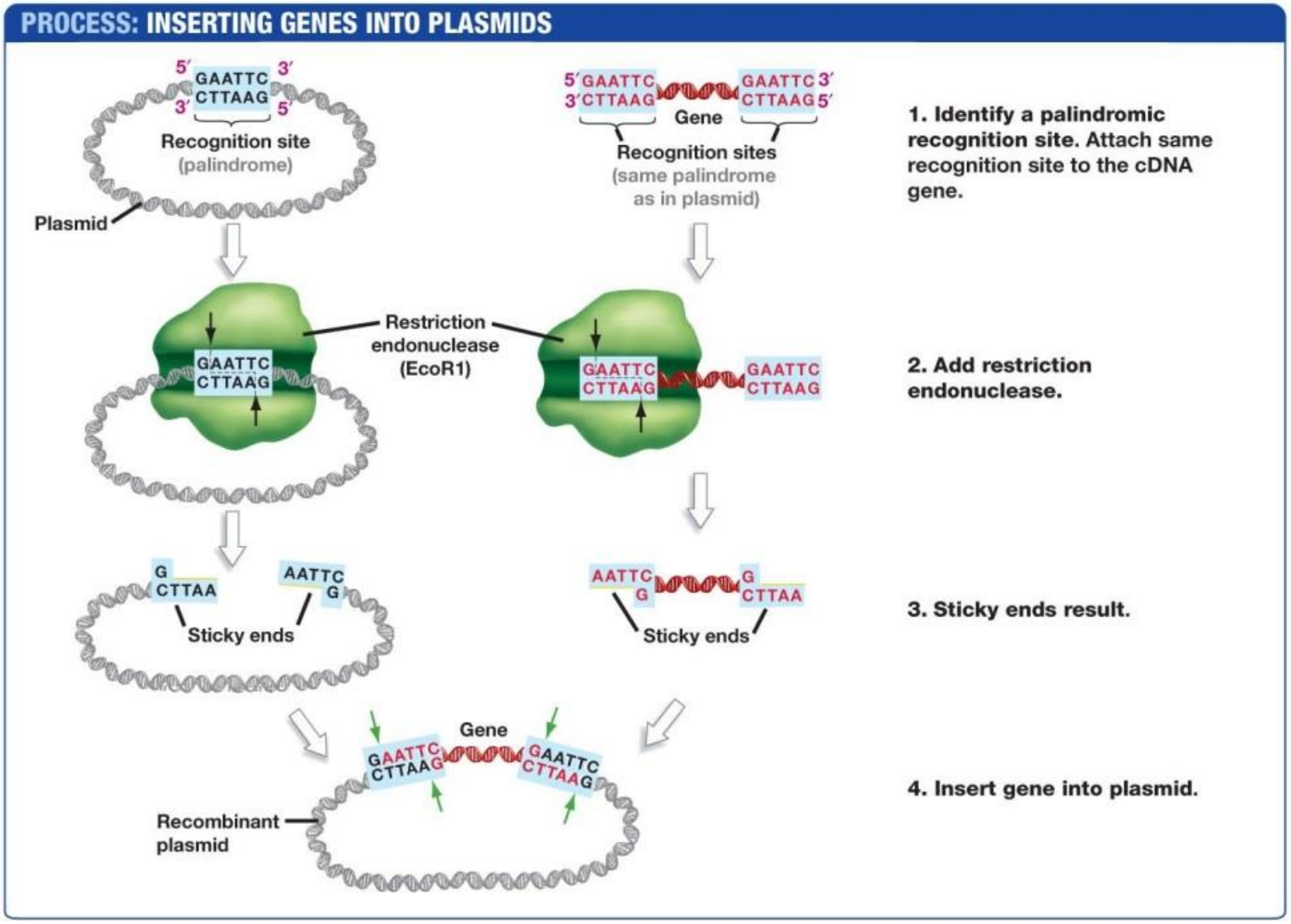






A synthetic gene the encodes the entire spike protein will be inserted downstream from the promoter using restriction enzymes.

How to insert a DNA fragment into a Plasmid – Restriction enzymes



EcoR1

GAATTC → G AATTC
CTTAAG → CTTAA G

NdeI

CATATG → CA TATG
GTATAC → GTAT AC

BamHI

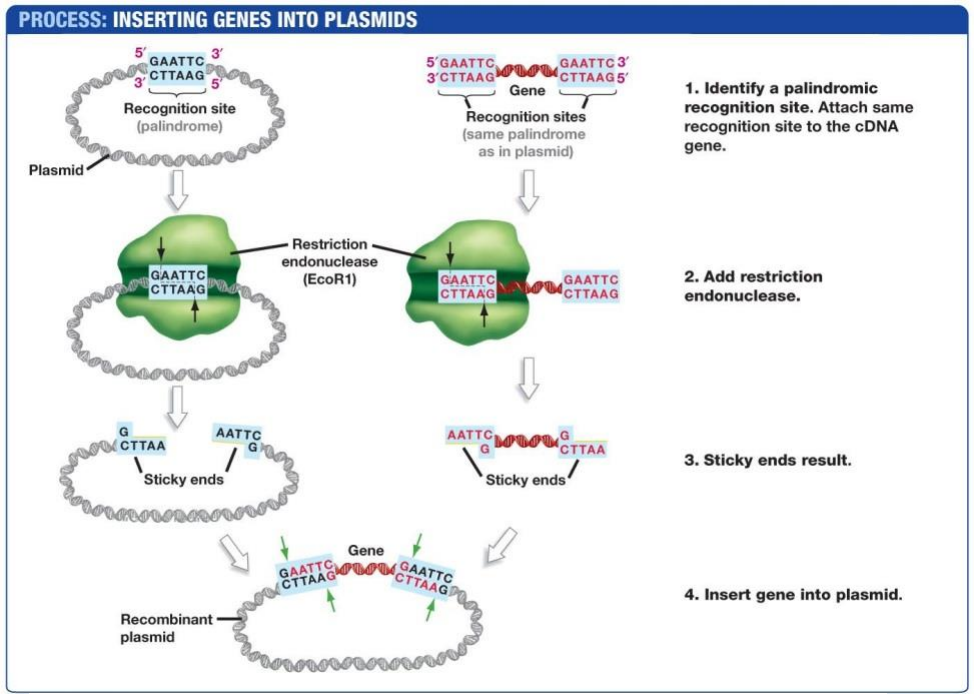
GGATCC → G GATCC
CCTAGG → CCTAG G

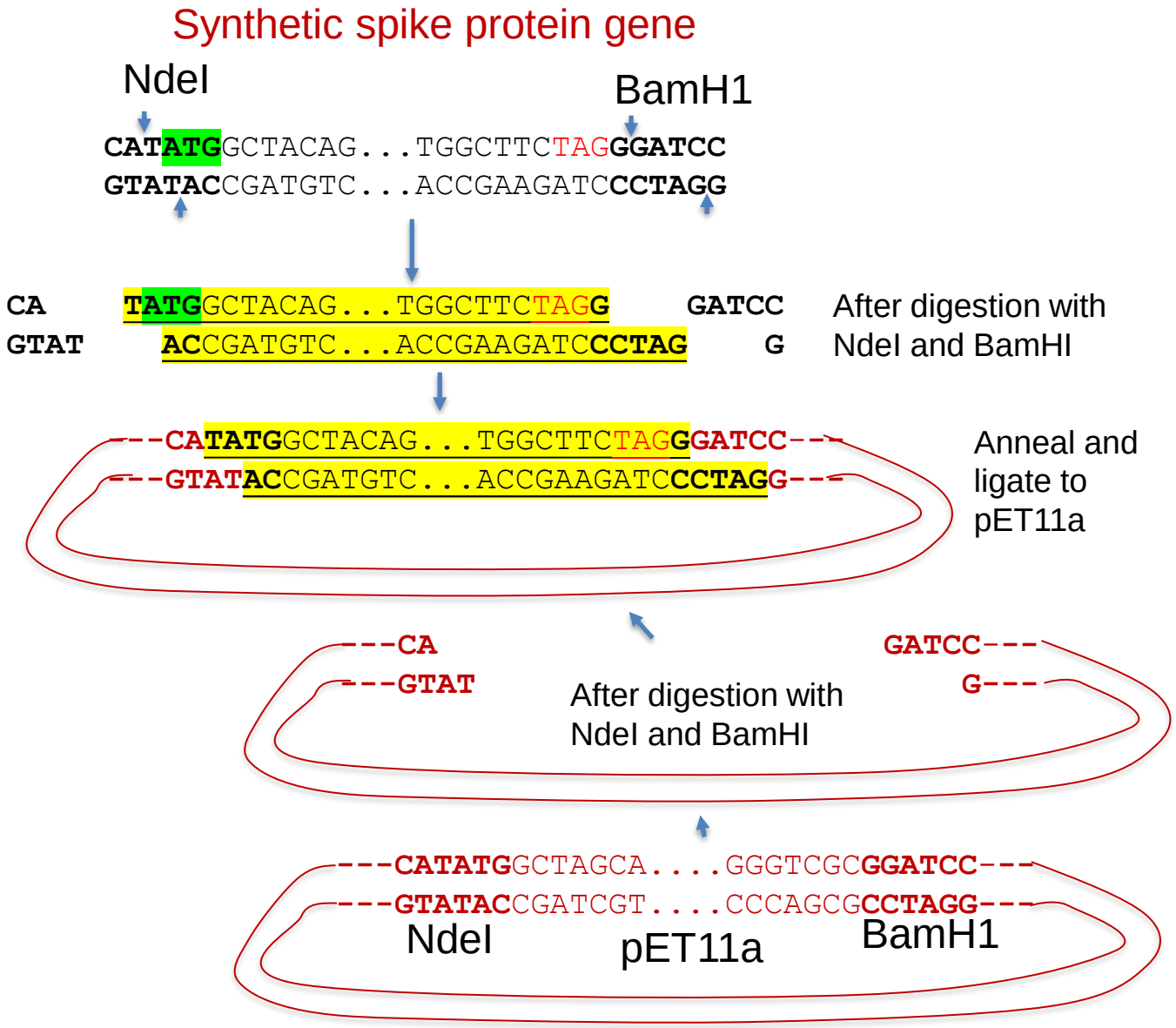
Cuts both strands, generating single-stranded DNA (sticky ends).

Complementary sticky ends can bind to each other.

DNA can be joined by DNA ligase.

How to insert a DNA fragment into a Plasmid



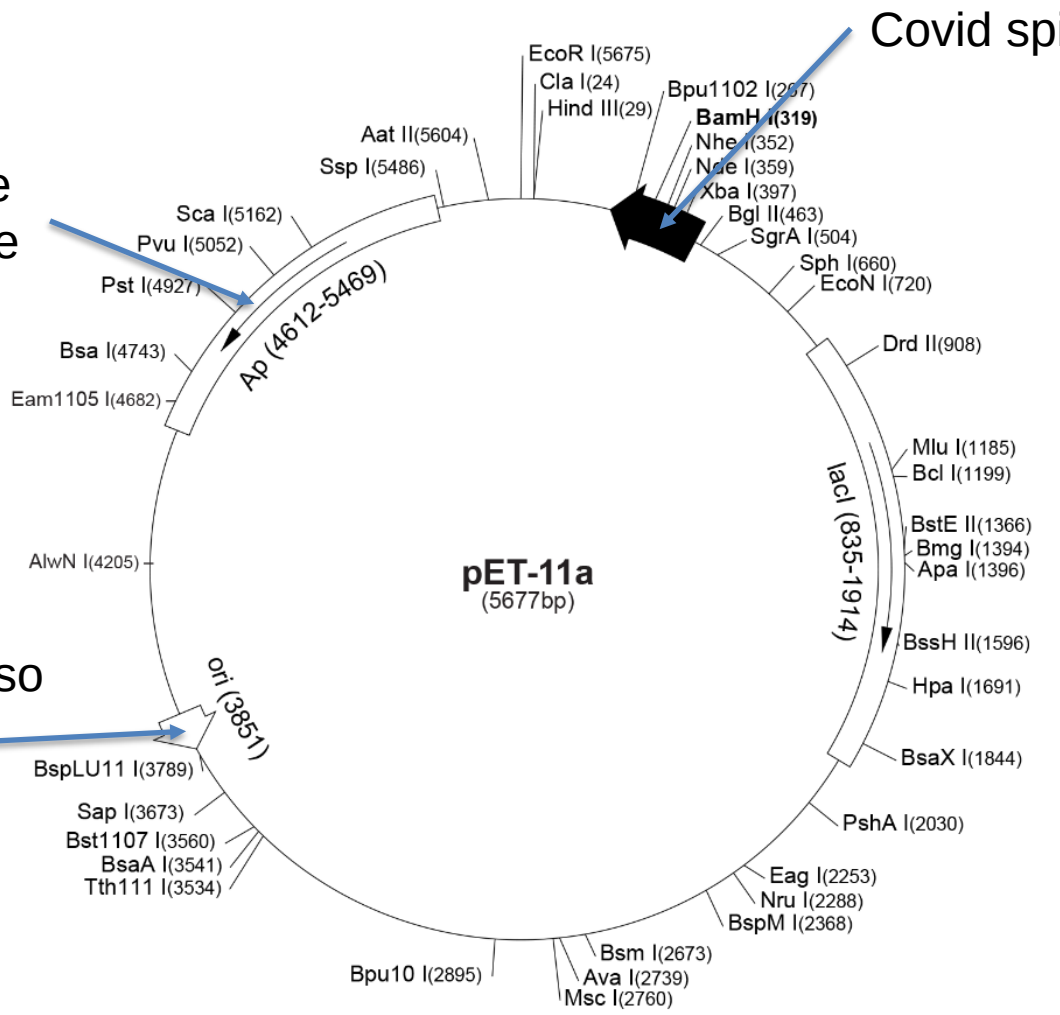


- Cut plasmid and synthetic gene with both NdeI and BamHI enzymes to make sticky ends.
- Cool to allow sticky ends to anneal TA from NdeI will anneal, GATC from BamH1 will anneal.
- DNA ligase to join fragments.

Final Product – Covid-19 Spike protein codons in plasmid

Gene that codes for a protein that makes the bacteria containing the plasmid resistant to penicillin.

Origin of replication so that the plasmid is copied when the bacteria divide.



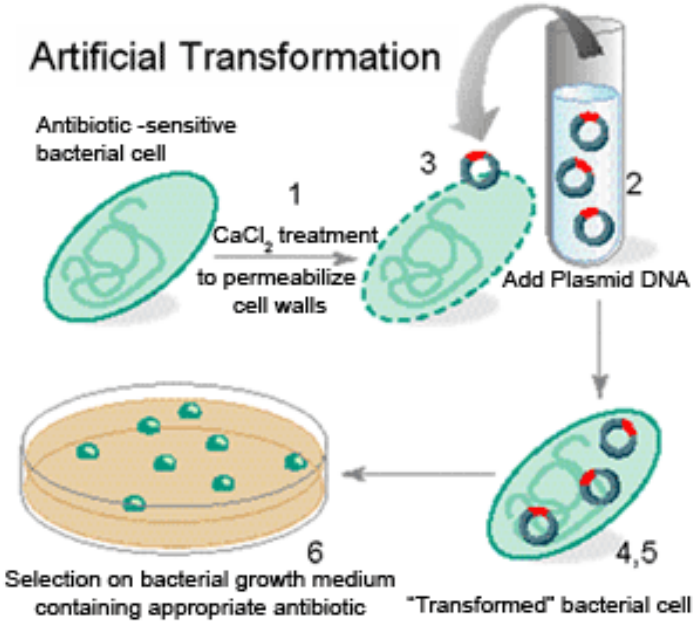
Inserting the Plasmid into Bacterial Cells

- Cells can take up DNA from the environment and incorporate it into their genomes in a process called transformation.

- To transform bacterial cells with a plasmid, researchers increase the permeability of the cell's membrane using a chemical treatment (calcium chloride).

- Cell that contain the plasmid are selected by growth on ampicillin.

- Only those cells with plasmid can grow.

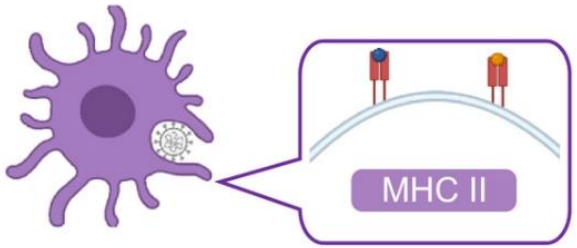


- The plasmid with the cDNA produces the spike protein in bacteria.

- Large amounts of E.coli that produce the vaccine can be grown in a fermenter.

- The spike protein can be used as a vaccine

B. Inactivated

Inactivated 		✓ Do not cause disease Very stable ✗ Needs booster strategy Short memory

THE TAMPA DAILY TIMES NIGHT FINAL

SIXTY-THIRD YEAR—No. 56 TAMPA, FLORIDA, TUESDAY, APRIL 12, 1955 PRICE FIVE CENTS

POLIO VACCINE 'SUCCESSFUL'

End of Quick Divorce Law Gains Favor

Senate Committee Clears Bill for 6 Months' Residence

Top Prize Story In Today's News

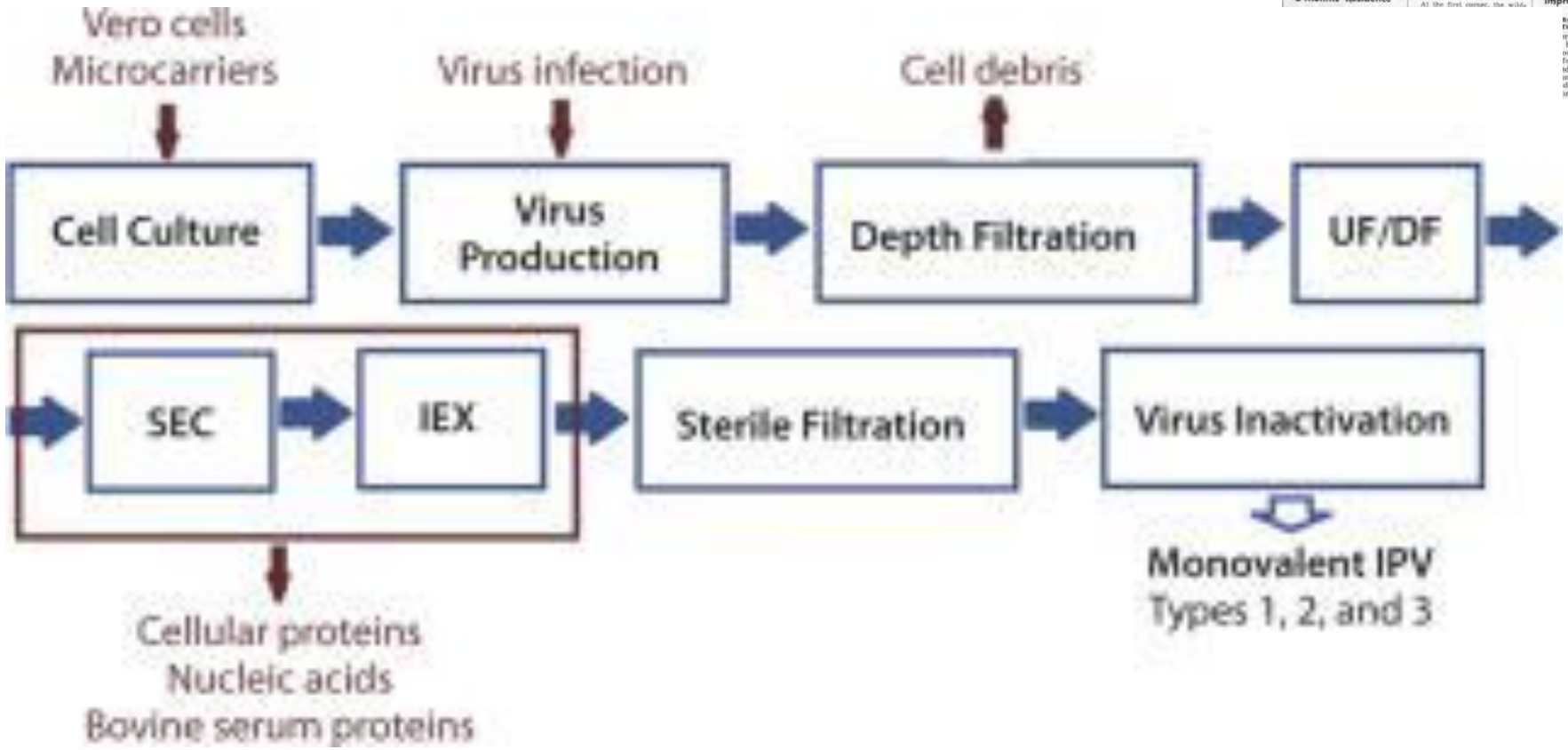
Auditorium, Bridge Bonds Get Okay

Consultants Give Go Ahead on Tampa Improvement Projects

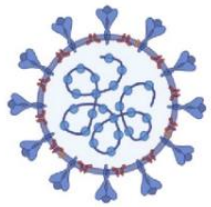
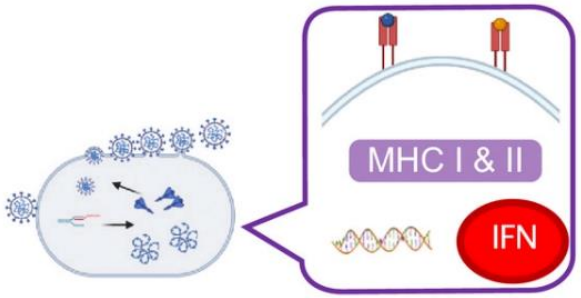
Paralysis Prevention Proved in Mass Tests

'Effective, Safe' Verdict Reported After Long Study of Innoculations

Dr. Salk Says 'Too Busy Now For Comment'



C. Attenuated

Live attenuated 		✓ Mimic natural infection Creates cross reactivity ✗ Can revert and cause disease Might be harmful in immunocompromised

THE CINCINNATI ENQUIRER

122nd YEAR NO. 76—DAILY SUNDAY MORNING, JUNE 24, 1962 FINAL EDITION—PRICE 25 CENTS

'Sabin Sundaes' Ready For Area Polio Drive Beginning At 11 A.M.

You can start getting rid of polio in Metropolitan Cincinnati today.

Take a few minutes and go to a vaccine station in a school near you and get your cube of sugar enriched with Sabin vaccine.

This campaign is for everyone over six weeks of age — everyone, babies to grandmothers, who has never had Sabin vaccine.

There are a million doses waiting—there's plenty for everybody. Most stations in this five-county area will be open from 11 a. m. to 5 p. m.

Crushing polio is a big job. Today you can take a giant first step against Type III polio. The other two types will be dealt with later, using Sabin vaccine, a swallow at a time.

On page 3-A is a list of places, most of them schools, where the vaccine is being given. Near the list is a coupon for the head of the household to sign to give consent for the giving of the vaccine.

Donations, of 25 cents a dose, will be accepted at the distribution stations to help pay for the vaccine. You may give more if you

suburbs were doing the same.

Business and industry have risen to the challenge giving time and materials and men.

Firemen will help keep order, giving up their off days. So will the doctors, nurses, druggists and Boy Scouts you see today when you get your vaccine.

Dr. Kenneth Macleod, Cincinnati Health Commissioner, with Dr. J. Edwin Reed, Hamilton County Health Commissioner, have thrown their weight behind the campaign. With Dr. Sabin they make up the top planners of the operation.

Dr. Macleod said if half the population got vaccine it would be a "good" effort considering the short time of preparation. Sixty per cent would be "superlative." Over 70% would be a miracle, he said.

Any bets on a miracle?

(Related Photo, Page 6A)

Where To Go

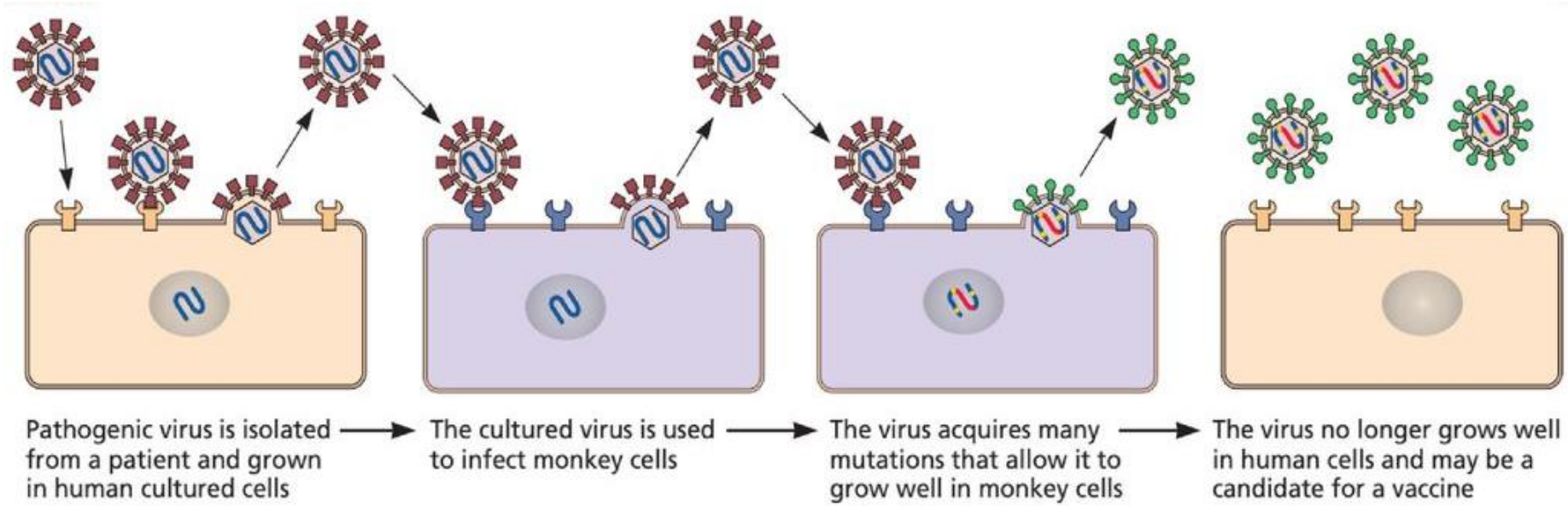
A list of places where you can get the polio vaccine and a form for you to sign to give your consent for minors are on page 3A.

On Page 6-A is a picture showing volunteers at work getting the vaccine ready to dispense today.

Don't forget—this is the vaccine you eat. There are not shots.

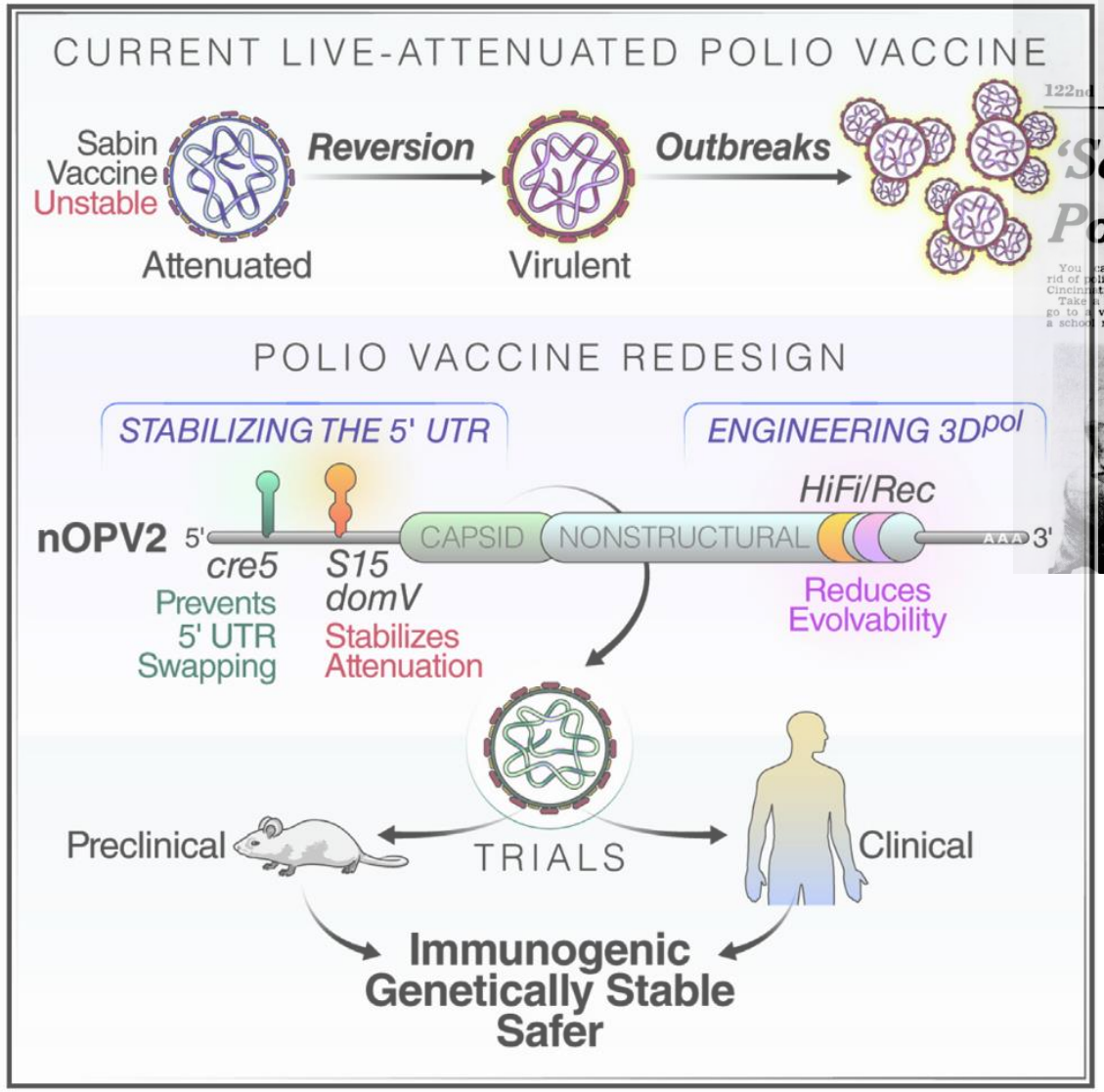
'First' Family Gets Sabin Vaccine On Sugar

. . . Dr Sabin oversees feeding to Tom and Marilyn Bross and grandmother, Mrs. Ruby Johnson



Pathogenic virus is isolated from a patient and grown in human cultured cells → The cultured virus is used to infect monkey cells → The virus acquires many mutations that allow it to grow well in monkey cells → The virus no longer grows well in human cells and may be a candidate for a vaccine

C. Attenuated Viruses



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... Dr Sabin oversees feeding to Tom and Marilyn Bross and grandmother, Mrs. Ruby Johnson

—Enquirer (Bob Free) Photo

Cell Host & Microbe

CellPress

Volume 27, Issue 5, 13 May 2020, Pages 736-751.e8

Article

Engineering the Live-Attenuated Polio Vaccine to Prevent Reversion to Virulence

Ming Te Yeh [1], Erika Bujaki [2], Patrick T. Dolan [1], Matthew Smith [2], Rahnuma Wahid [3], John Konz [3], Amy J. Weiner [4], Ananda S. Bandyopadhyay [4], Pierre Van Damme [5], Ilse De Coster [5], Hilde Revets [5], Andrew Macadam [2], Raul Andino [1 6]

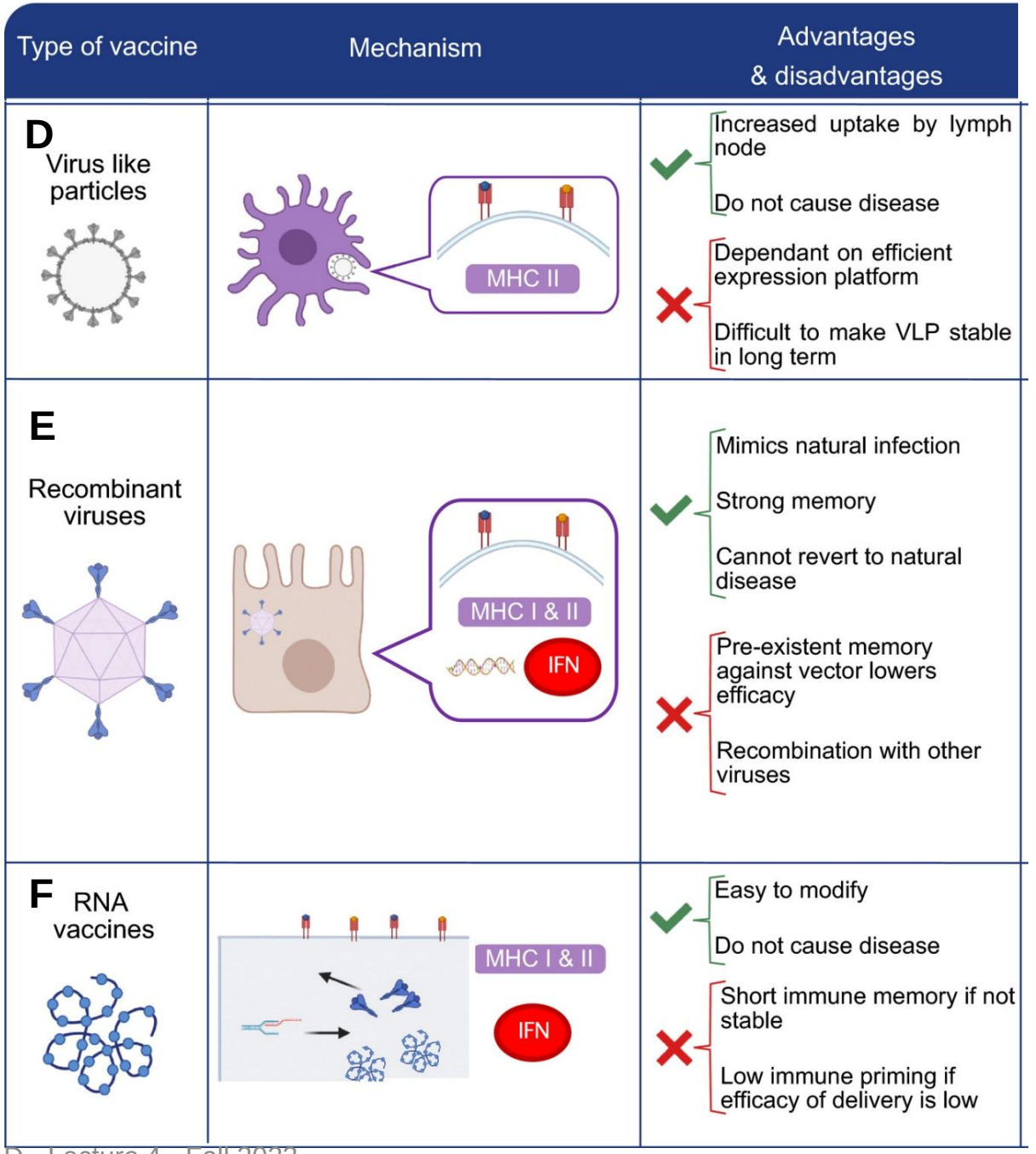
D – Virus Like Particles:
Proteins isolated from the virus form virus-like-particles, *without* the genetic material of the virus

E. Recombinant Virus:
A “safe virus” is used (e.g. cold virus)
Gene for a protein from a pathogen is inserted into the DNA of the virus.

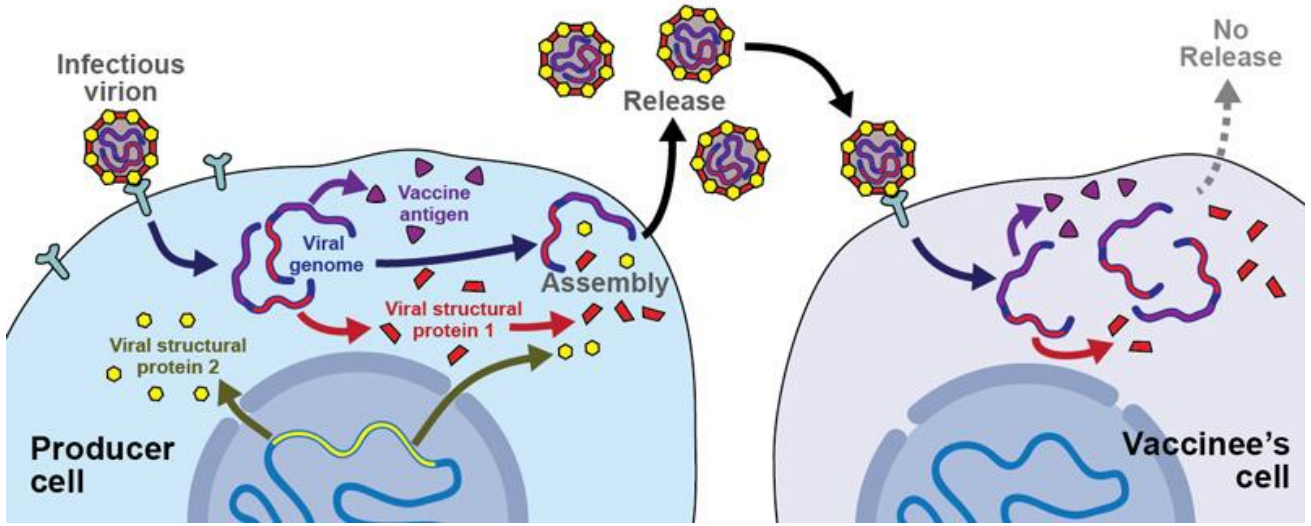
- When virus grows it produces the protein from the pathogen generating immunity.

Also includes vaccines that are a mixture of genetic material from human and animal viruses (reassortment viruses)

F. RNA Vaccines (Pfizer Covid Vaccines)
RNA coding for a viral protein is introduced into cells. The RNA is used by the cell to make viral proteins, inducing an immune response.



E. Viral Vectors for Antigen Delivery AstraZeneca Covid

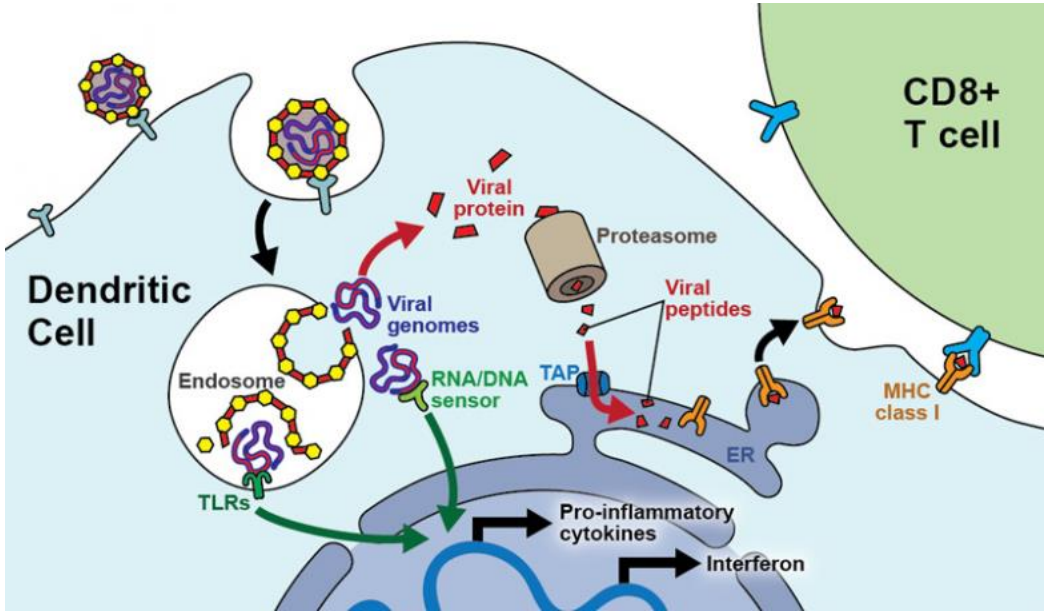


2. Action of vaccine

a) Virus infects host cell in vaccinated person.
b) Viral genome is used to make viral proteins, including proteins from the pathogen.
c) Activate T_C cells to become T_C – memory cells (can be re-activated by MHC I + Peptide).
d) B-cell response can occur due to antigens that are sent to the surface of the cell, generating B- and T_H-memory cells.

1. Production of vaccine

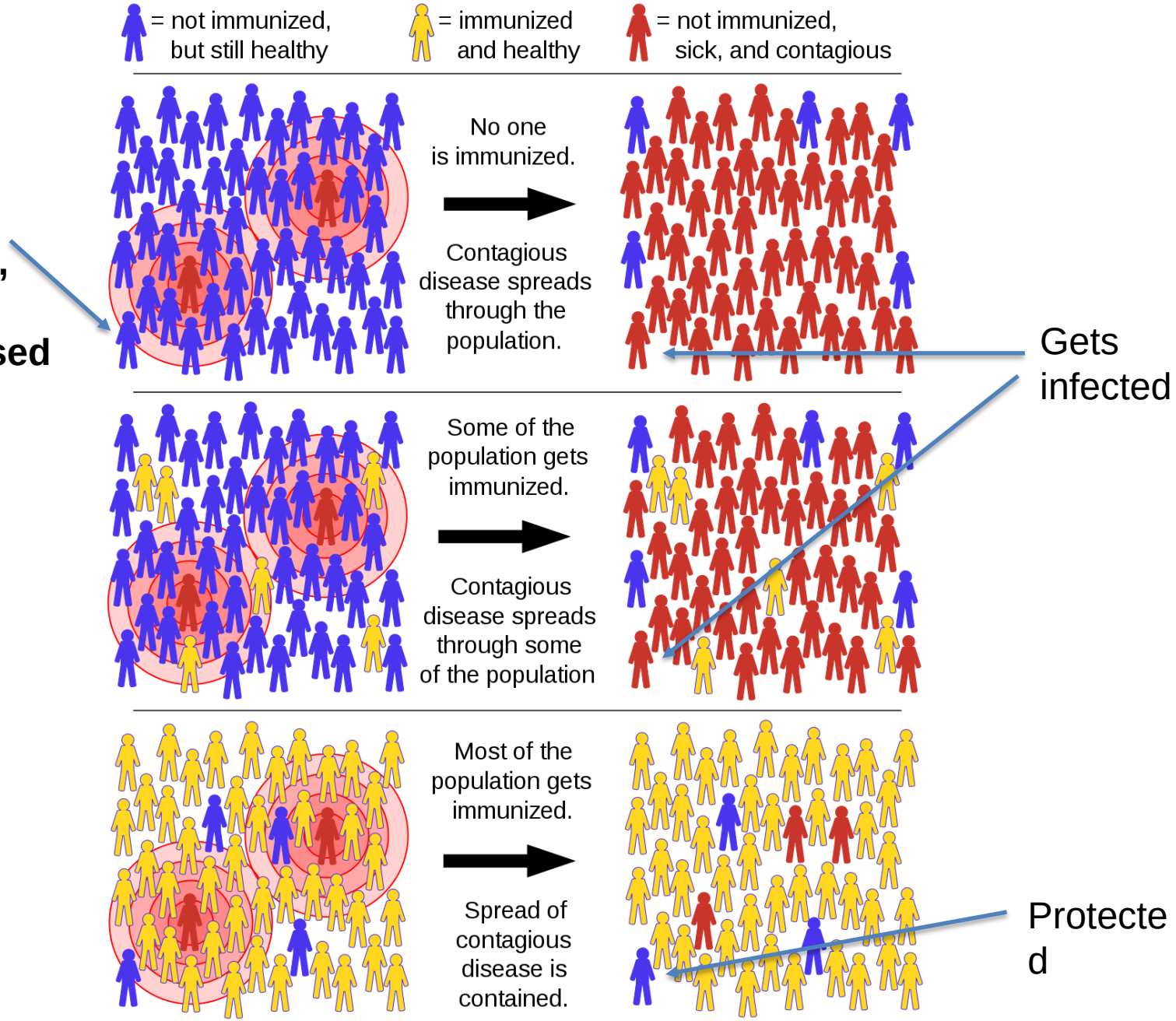
I. Genes from the pathogenic virus are added to an adenovirus (common cold)
II. Adenovirus is defective and cannot replicate without key structural proteins that are provided in the producer cell.
III. No viral particles are released in the vaccinee's cells because they lack the key structural proteins.



DOI:
https://doi.org/10.4414/smw.2017.14465
Publication Date: 08.08.2017
Swiss Med Wkly. 2017;147:w14465

Herd Immunity:

- Vaccinated individuals prevent disease from spreading from sick to unvaccinated.
- At sufficient levels, the “herd” is immune because the virus cannot spread.



Herd Immunity

How Many People need to be vaccinated to achieve herd immunity?

10% ?

20% ?

50% ?

90% ?

100% ?

It depends on the how infectious the virus is

Our Experimental Viruses:

Ebola: Low infectivity
Polio: Moderate infectivity
Measles: High infectivity

Simulation to Determine Infectivity Versus Vaccination Level (Pset)

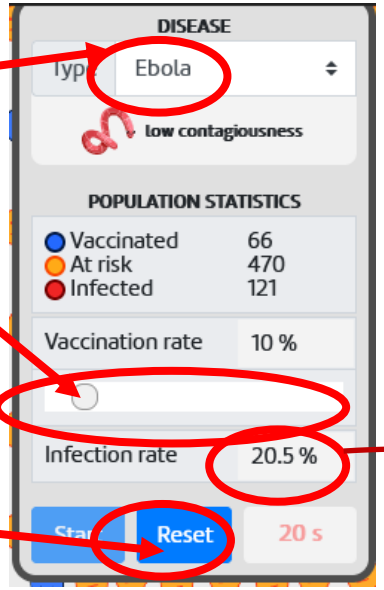
1. Go to the following web site and open **both** links: http://www.andrew.cmu.edu/~rule/stayin-alive
2. Copy the googlesheet.

3. On the **Infection Simulator link**, scroll down (2/3 page) to the image of the plane, and click on it.

A. Select the virus.

B. Use the slider to select the different vaccination levels. For each of the vaccination levels do ***three*** simulations.

C. Enter the value for the ***% Infection rate at 20s*** into the appropriate cell of the google sheet. Your data will be automatically averaged and plotted.

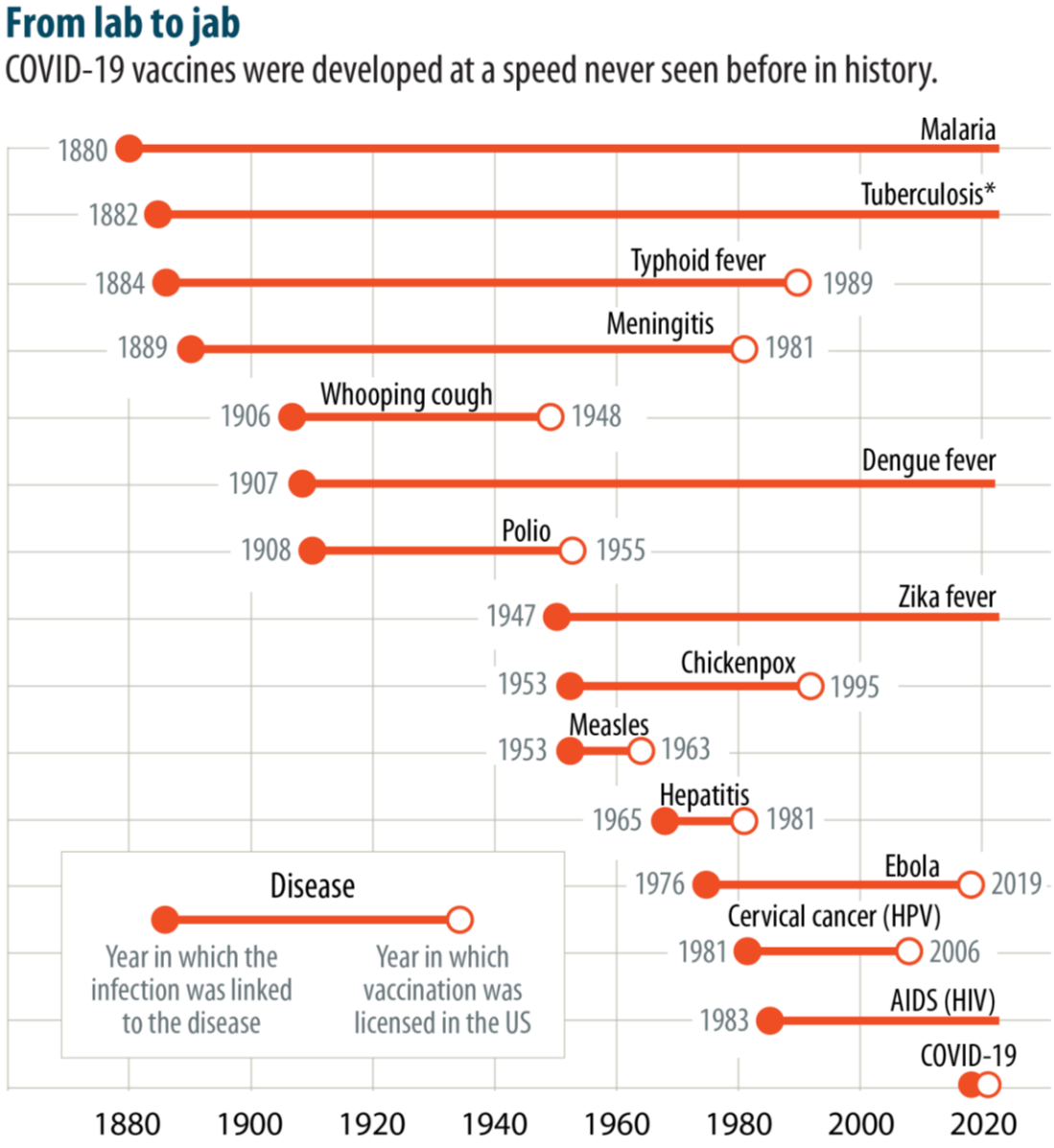


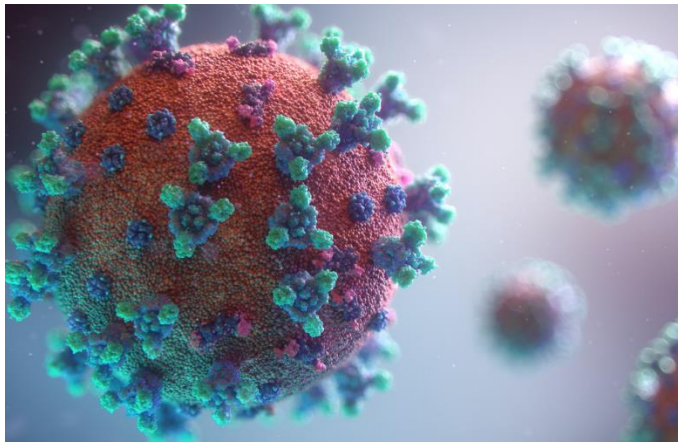
Group Number	Virus	% Vaccination
1	Ebola	10, 20, 40, 50
2	Ebola	70, 80, 90
2	Polio	10, 20, 40, 50
3	Polio	70, 80, 90
4	Measles	10, 20, 40, 50
5	Measles	70, 80, 90

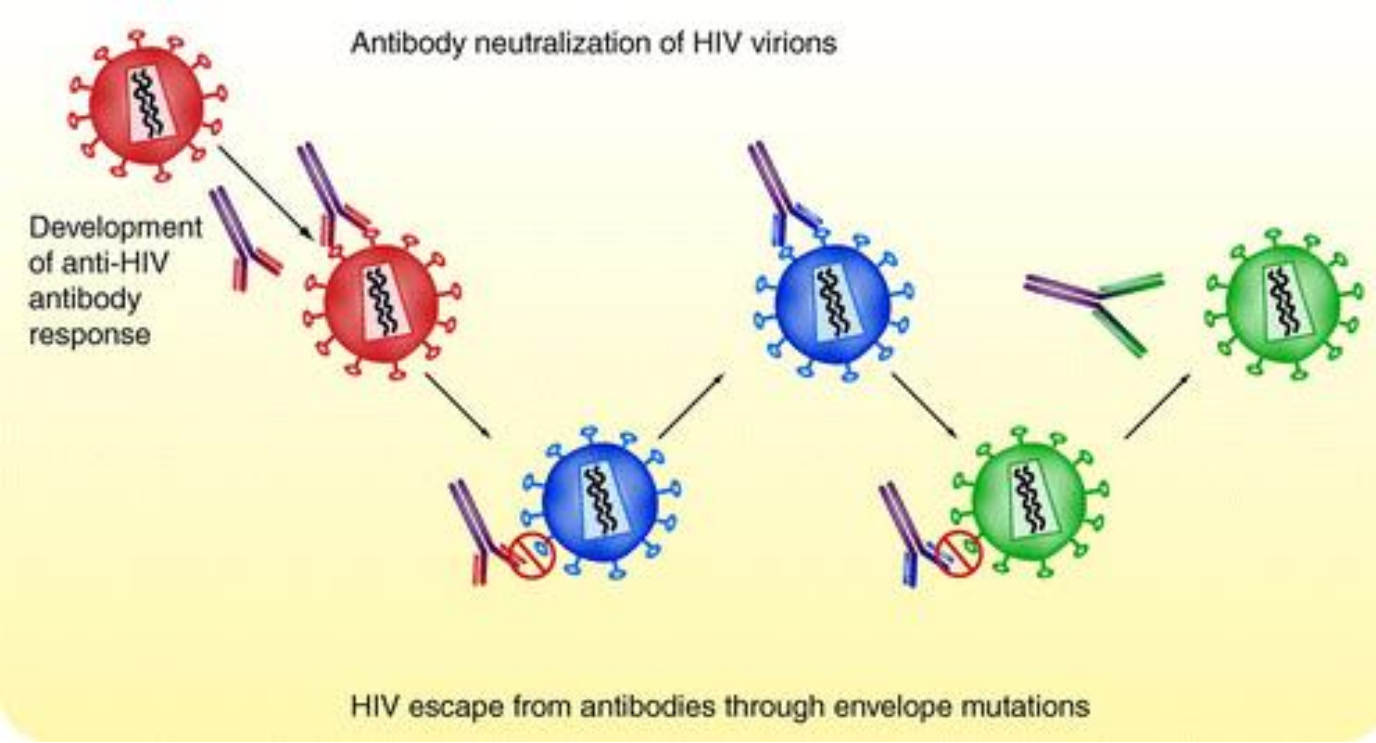
3 Simulation Runs at 10%

	Ebola (Ave)	Polio (Ave)	Measles (Ave)	Ebola			Polio			Measles		
%Vaccinated				#1	#2	#3	#1	#2	#3	#1	#2	#3
10	#DIV/0!	#DIV/0!	#DIV/0!									
20	#DIV/0!	#DIV/0!	#DIV/0!									
40	#DIV/0!	#DIV/0!	#DIV/0!									
50	#DIV/0!	#DIV/0!	#DIV/0!									
70	#DIV/0!	#DIV/0!	#DIV/0!									
80	#DIV/0!	#DIV/0!	#DIV/0!									
90	#DIV/0!	#DIV/0!	#DIV/0!									

Why Are There No Vaccines for HIV?







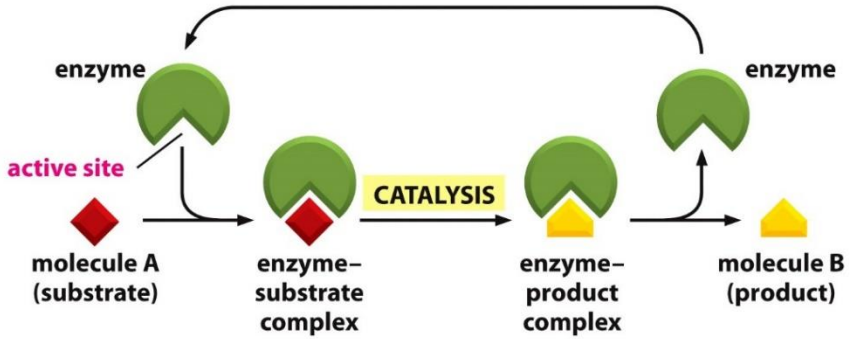
Summary Questions for Immunology:

1. What are the two major branches of the immune system? Why are both important?
2. What are the roles of different cell types in each system, e.g. what would happen if T_H-cells disappeared?
3. What is the quaternary structure of an antibody? Can you sketch an antibody and indicate where the antigen binds?
4. What defines the specificity of antibodies?
5. What are the steps in the production of antibody genes, at the molecular level:
   a) How do DNA rearrangements produce functional heavy and light chain genes
   b) How are is the mature mRNA generated in B-cells and Plasma cells.
   c) What is the difference between the heavy chain export process for B-cells and plasma cells.
6. Can you describe how antibodies kill/inactivate pathogens
7. How are virally infected cells and tumor cells recognized by Tc cells?
8. How does the Tc cell kill those cells?
9. What evasion mechanisms are used by cancer cells and how have these been addressed by antibody therapy?

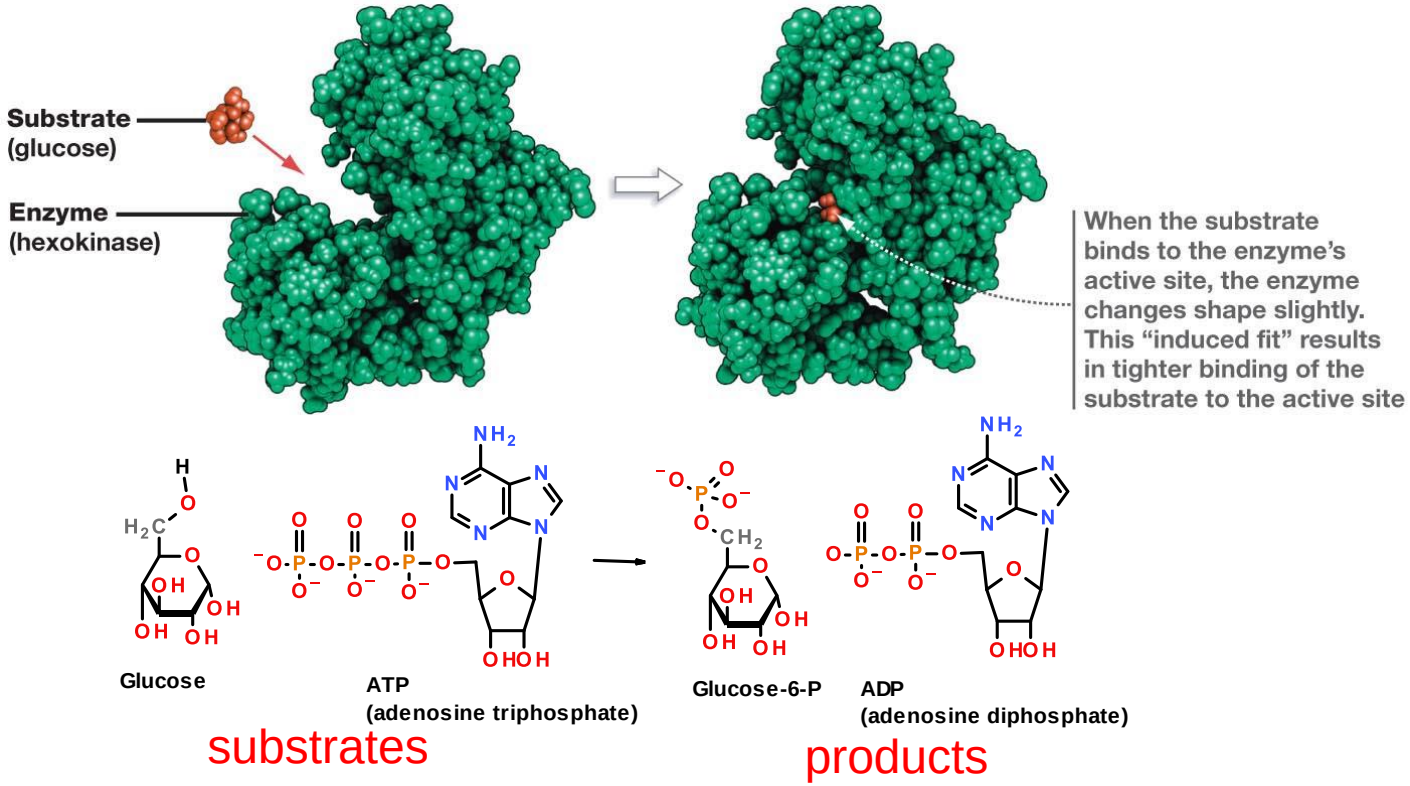
10. What was the origin of the idea for vaccination?
11. What was one of the first “safe” vaccines? What disease has now been eradicated due to this vaccine?
12. Why is it important to be vaccinated (Herd immunity)
13. Can you describe one way to generate a vaccine for a pathogen? Do you know the pros and cons for that method?

Enzymes

- **Enzymes** are protein or RNA catalysts. They increase the rate of the reaction.
- They bind “substrates” and convert them to “products”. Usually, the substrate undergoes a chemical reaction and is changed in its structure.
- Substrates bind specifically to the enzyme’s **active site,** interacting with amino acid side chains.
- The chemical change caused by the enzyme is catalyzed by additional functional groups in the active site.
- Many enzymes undergo a conformational change when the substrates are bound to the active site; this change is called an **induced fit**.
- **The rate (or velocity) is the number of products produce/unit time.**

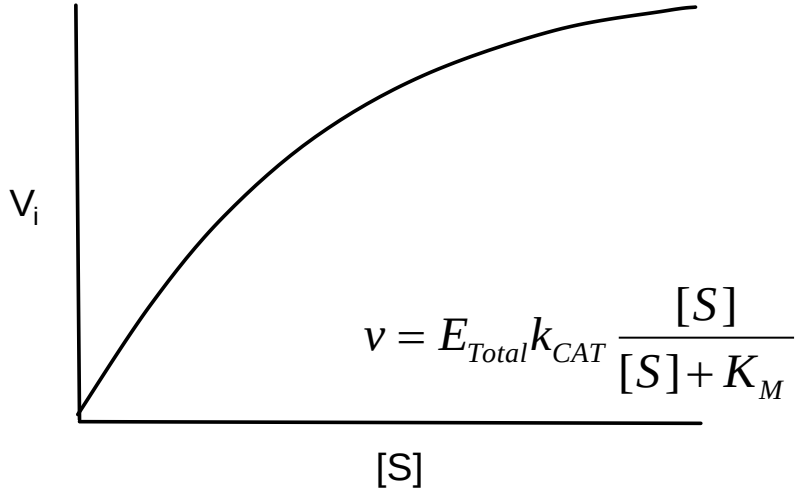


$$E + S \rightarrow (ES) \rightarrow (EP) \rightarrow E + P$$



Summary of Kinetic Parameters

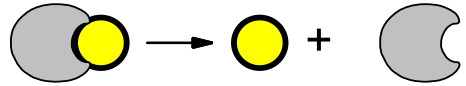
$$E + S \rightarrow (ES) \rightarrow (EP) \rightarrow E + P$$



i) The K_M or Michaelis constant: This is *almost* the same as the K_D (= k_{off}/k_{on}), the dissociation constant, except for the presence of the k_{CAT} term. Therefore, it is related to the affinity of a substrate to an enzyme. *It is a constant for any particular enzyme-substrate pair. Substrates with slow off-rates (k_{off}) bind more tightly, and possess a smaller K_M.*

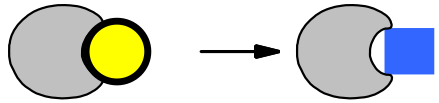
When [S]=K_M the enzyme is ½ saturated with substrate: v = ½ V_{Max}

$$K_M = \frac{k_{off} + k_{CAT}}{k_{on}}$$



ii) V_{MAX} = $k_{CAT}[E_T]$: This is the highest rate of product production possible. It is obtained at high substrate levels ([S]>>K_M). Under these conditions *all* of the enzyme is in the [ES] form (i.e. [ES]=[E_T]), the enzyme is ***saturated*** with substrate. k_{CAT} is obtained from V_{MAX} since the total amount of enzyme is known: $k_{CAT}=V_{MAX}/[E_T]$.

$$v = V_{MAX} \frac{[S]}{[S] + K_M} = E_{TOT} k_{CAT} \frac{[S]}{[S] + K_M}$$



iii) k_{CAT} is also called the turn-over number – *how many products are produced/sec by a **single** enzyme molecule.*

Enzyme Inhibitors

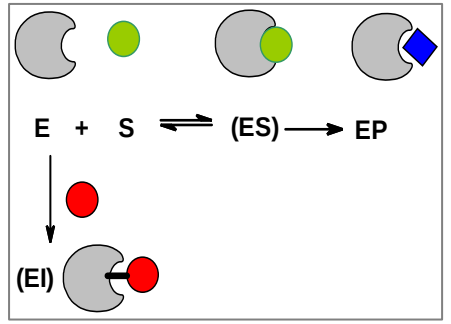
Studies on Inhibitors are useful for:

1. Mechanistic studies to learn about how enzymes interact with their substrates.
2. Understanding the role of inhibitors in enzyme regulation.
3. Drugs if they inhibit aberrant biochemical reactions:
   - penicillin, ampicillin, etc. interfere with the synthesis of bacterial cell walls, acting as suicide inhibitors.
4. Understanding the role of biological toxins.
   - Amino acid analogs - useful herbicides (i.e. roundup)
   - Insecticides - chemicals targeted for insect nervous system.

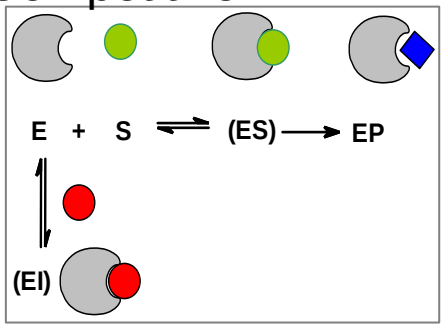
Types of Inhibitors:

1. Covalent – inhibitor *covalently* modifies enzyme, usually in active site, these are generally *irreversible* – the enzyme is dead! *Example – Sarin gas (Tokyo subway 1995)*
2. Competitive – inhibitor blocks substrate, binds *reversibly to active site with a* $K_D = K_I$. Enzyme activity returns when drug is removed.
3. Allosteric (mixed type) – inhibitor causes allosteric change. Binds *reversibly to a different location, with two different* K_D*s:* K_I *and* K_I*'. Enzyme activity returns when drug is removed.*

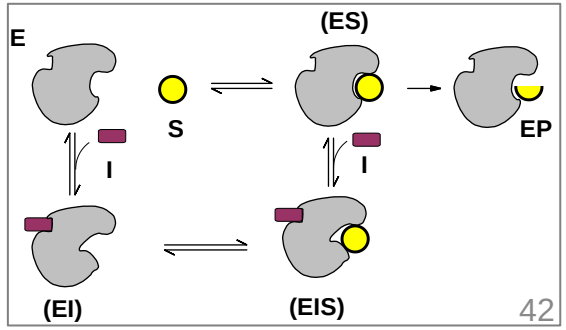
Covalent



Competitive



Allosteric (Mixed type)



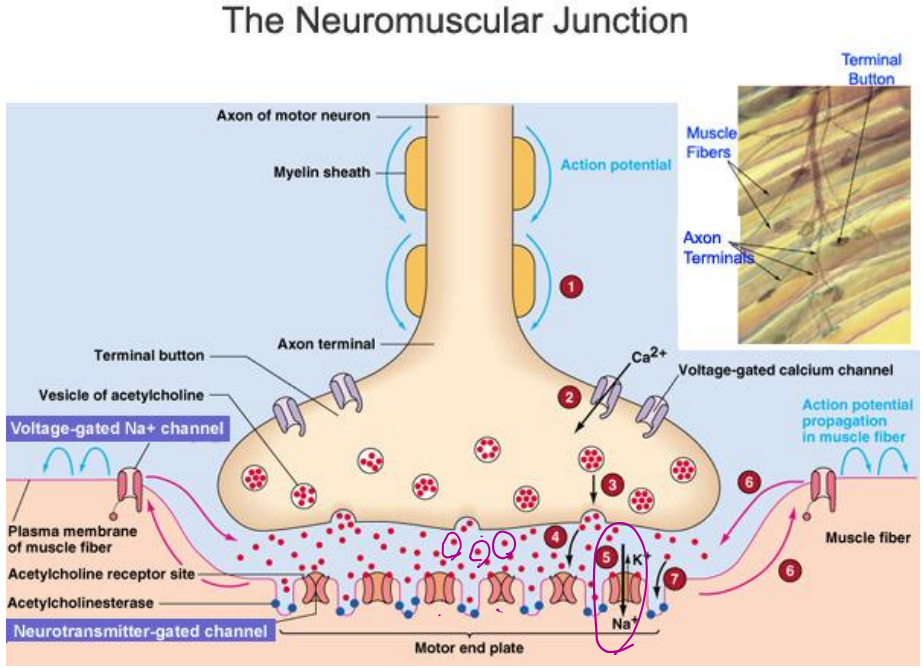
1. Suicide Inhibitors:

Inhibitor binds in the active site. This type of inhibitor binds *irreversibly*.

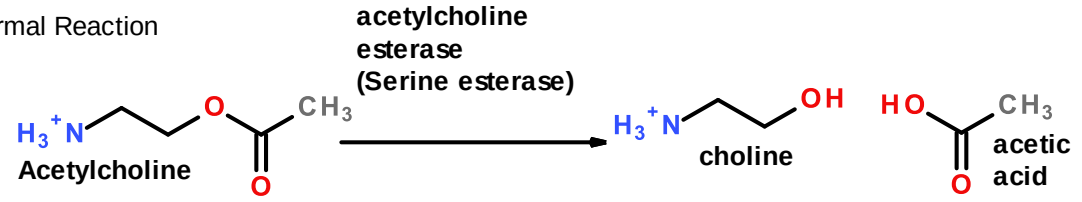
- Inhibitor becomes a reactive transition state that forms a covalent bond with the enzyme, irreversibly inactivating it.

Example: Sarin nerve gas

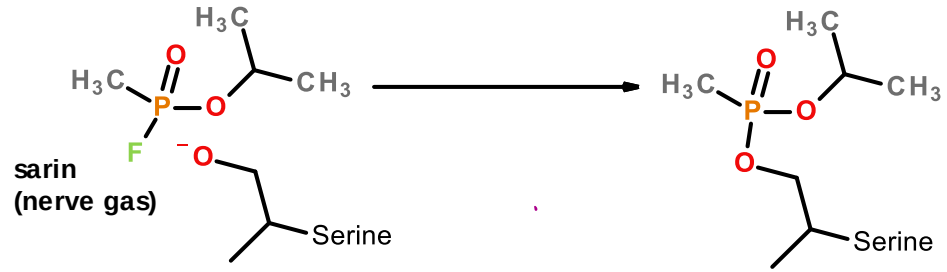
- Acetylcholine esterase is required to breakdown the neural transmitter acetylcholine in neuro-muscular junctions so that the muscle will relax.
- The esterase has an active site Serine that is activated in a similar manner as serine proteases.
- Sarin modifies the active site serine in the esterase by forming a stable covalent bond with the serine that cannot be easily hydrolyzed.
- Inhibition of acetylcholine esterase results in suffocation since the diaphragm muscles no longer function properly.
- The enzyme has committed suicide by trying to perform the chemical step on sarin.



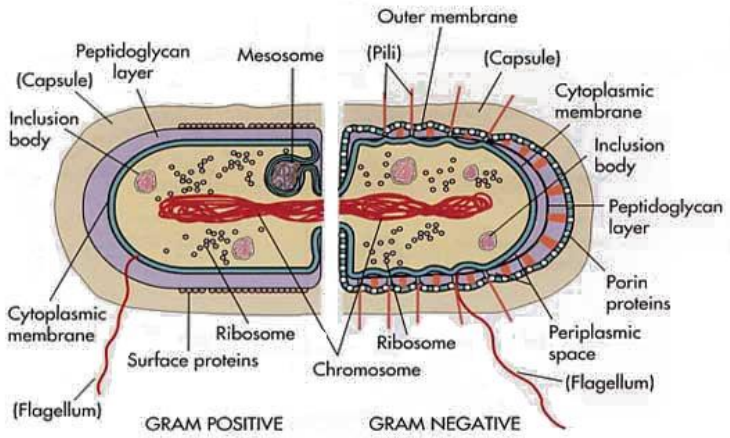
Normal Reaction



Suicide Inhibitor (Sarin)



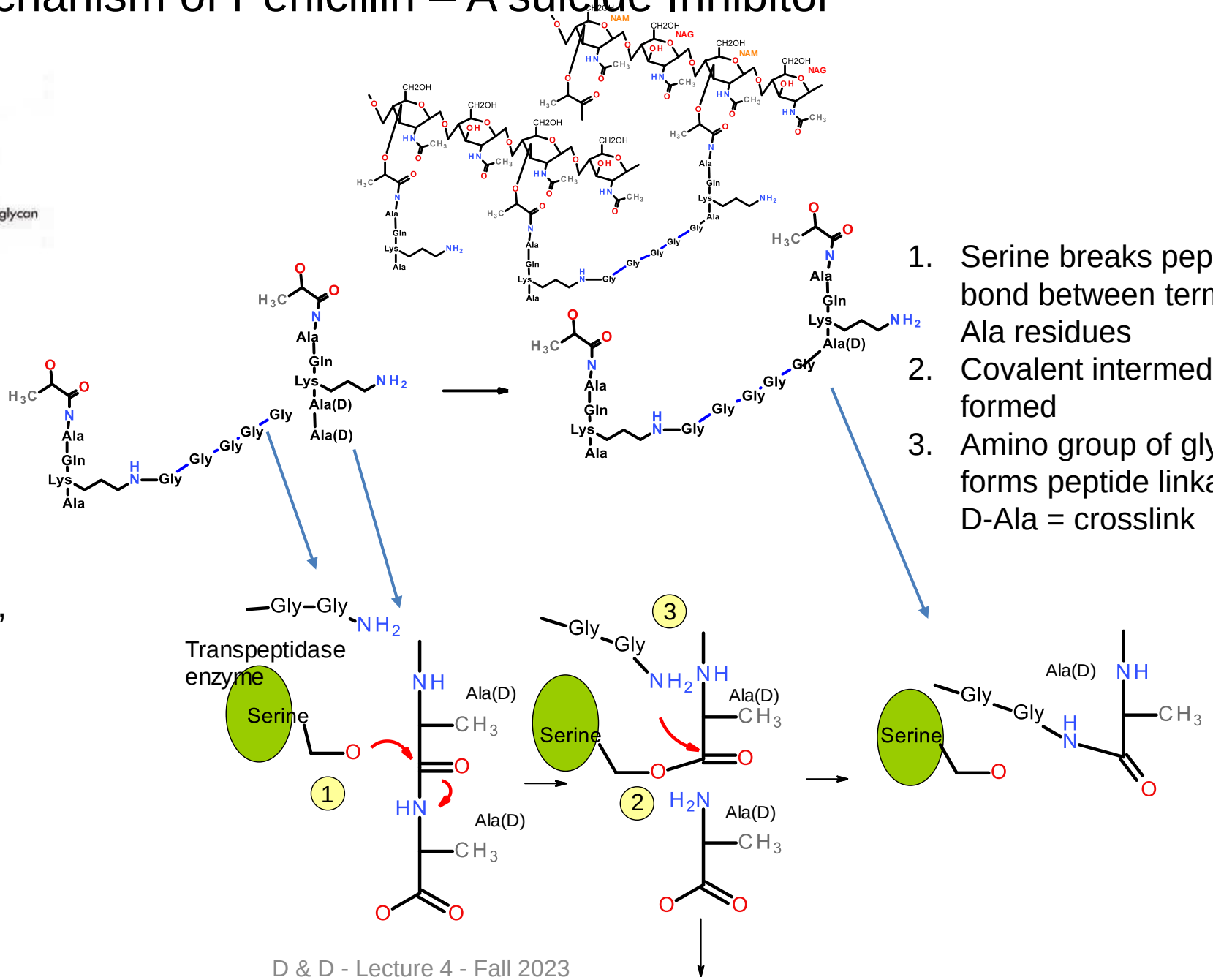
Bacterial Cell Wall



Bacterial cell wall:

- Linear polymers of alternating NAM (N-acetylmuramic acid) and NAG (N-acetylglucosamine), beta(1-4) linkage
- NAM units on adjacent strands are linked via a peptide linker.
- Crosslinking catalyzed by serine-containing ***transpeptidase.***

Mechanism of Penicillin – A suicide Inhibitor

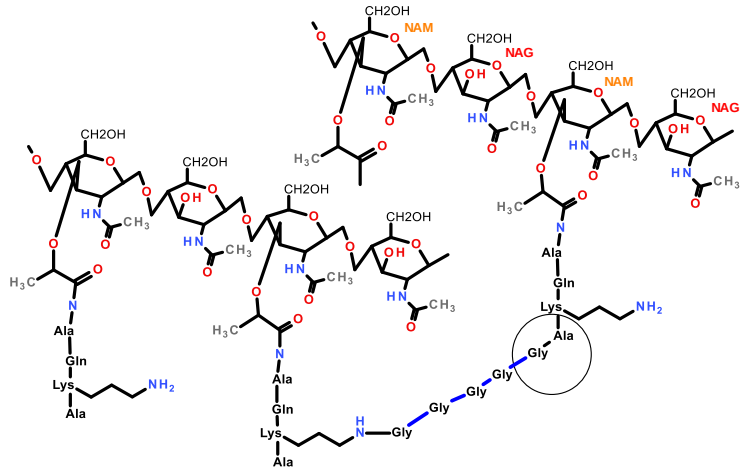


1. Serine breaks peptide bond between terminal D-Ala residues
2. Covalent intermediate formed
3. Amino group of glycine forms peptide linkage with D-Ala = crosslink

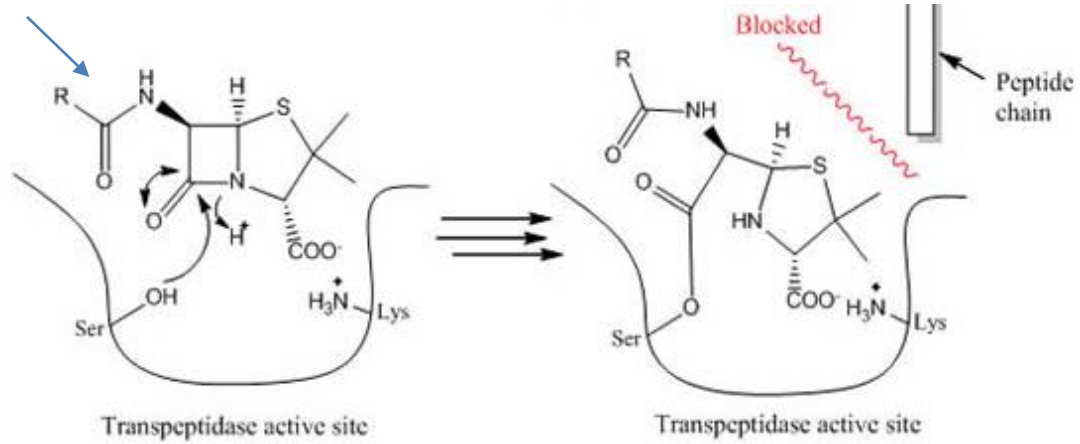
Mechanism of Penicillin

Mechanism of Action of Penicillin:

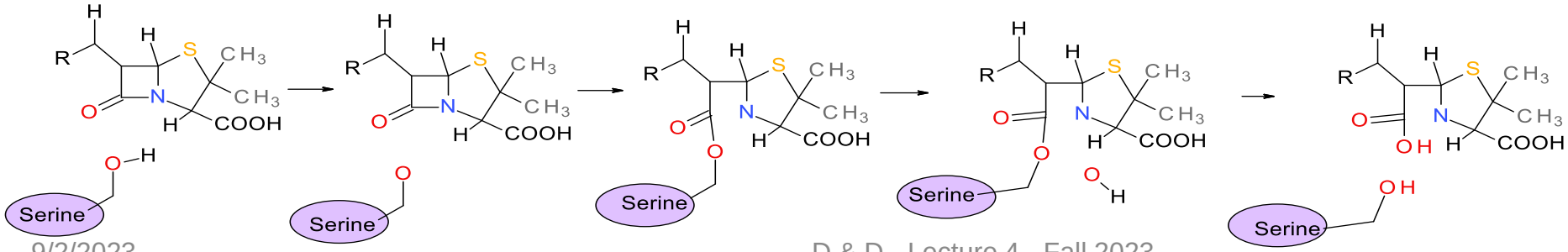
- Penicillin inhibits enzymes that are responsible for crosslinking the Gly_5 chain to alanine (circled on diagram).
- The crosslinking of the cell wall is broken, making the bacteria fragile to breakage.
- Inhibition is by formation of a chemical bond between penicillin and the enzyme (covalent inhibitor). This type of inhibition is not the same as competitive inhibition because: i) the penicillin forms a covalent bond with the enzyme, ii) penicillin is modified by the reaction, iii) the reaction is essentially irreversible.



Penicillin



Penicillin Resistance: Bacteria produce a protein that degrades penicillin (β-lactamase). This is a common antibiotic resistance gene that is used on plasmids. The transformed bacterial are resistant to penicillin.



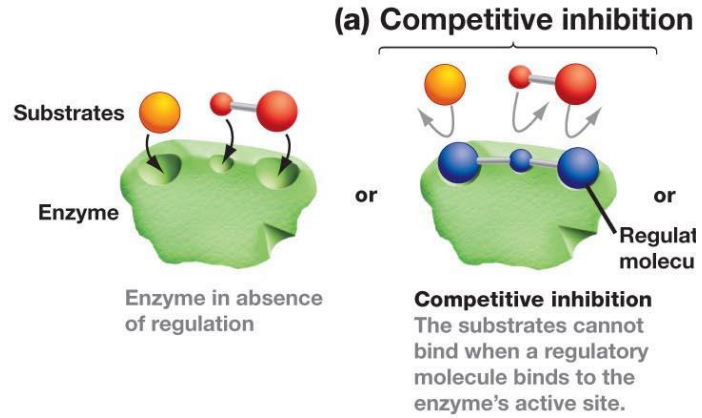
Lactamase can regenerate the serine, regenerating the enzyme.

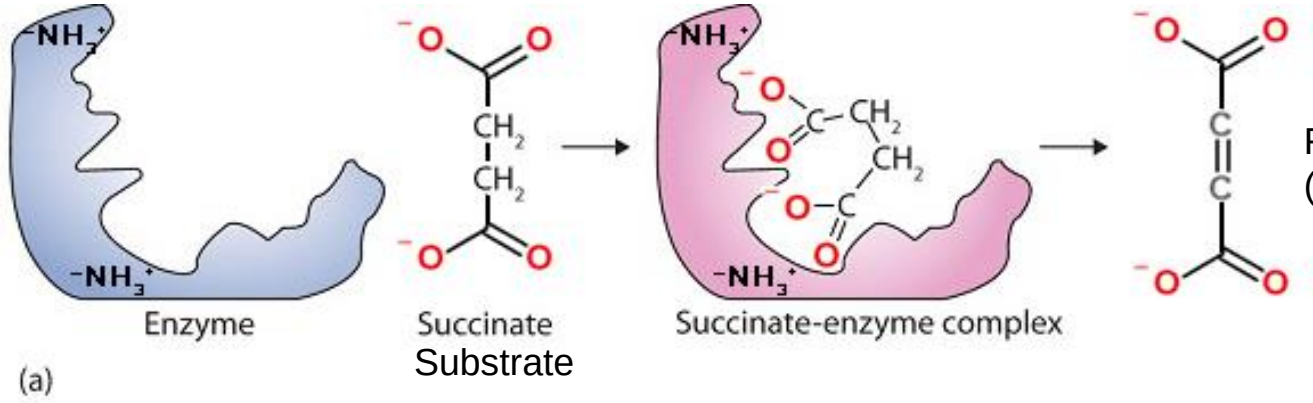
Competitive Inhibitors

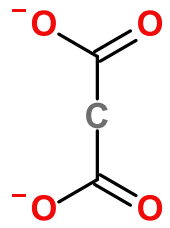
Succinate dehydrogenase converts succinate to fumarate by removal of two hydrogens.

Malonate is a **competitive inhibitor**, because:

- It is similar to the substrate – binds in active site – substrate cannot bind at the same time.
- Cannot undergo the chemical reaction – it is not possible to remove two hydrogens without leaving carbon with too few bonds.

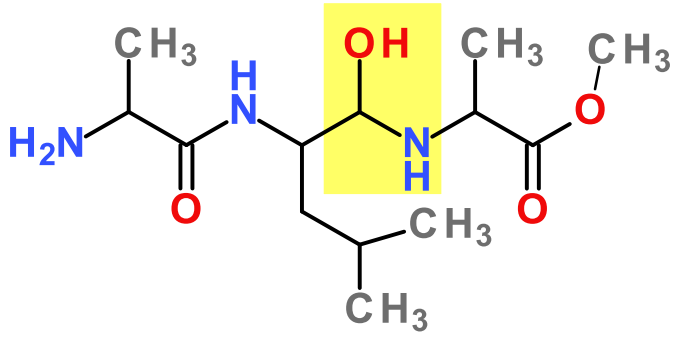




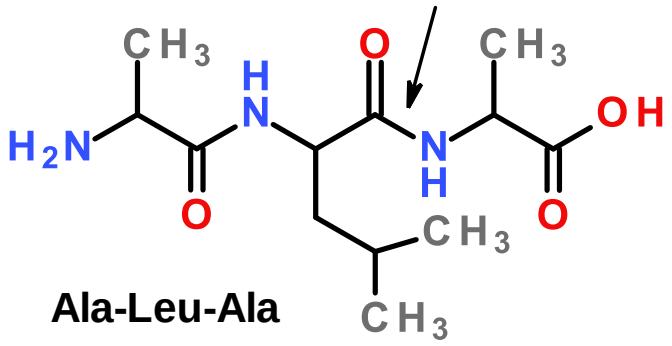


Impossible product

Example: A competitive inhibitor of elastase is shown below.

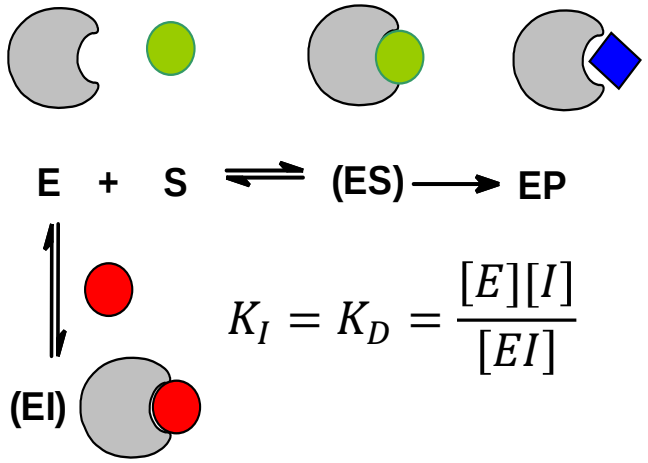


Why is this compound a competitive inhibitor? Recall the ***two*** key properties of competitive inhibitors.
(A substrate for elastase is shown below)



Ala-Leu-Ala

Quantification of Inhibitor Binding



Y=(EI)/[(EI)+(E)]

[I]

Effect of Competitive Inhibitor on Steady-State Kinetics:

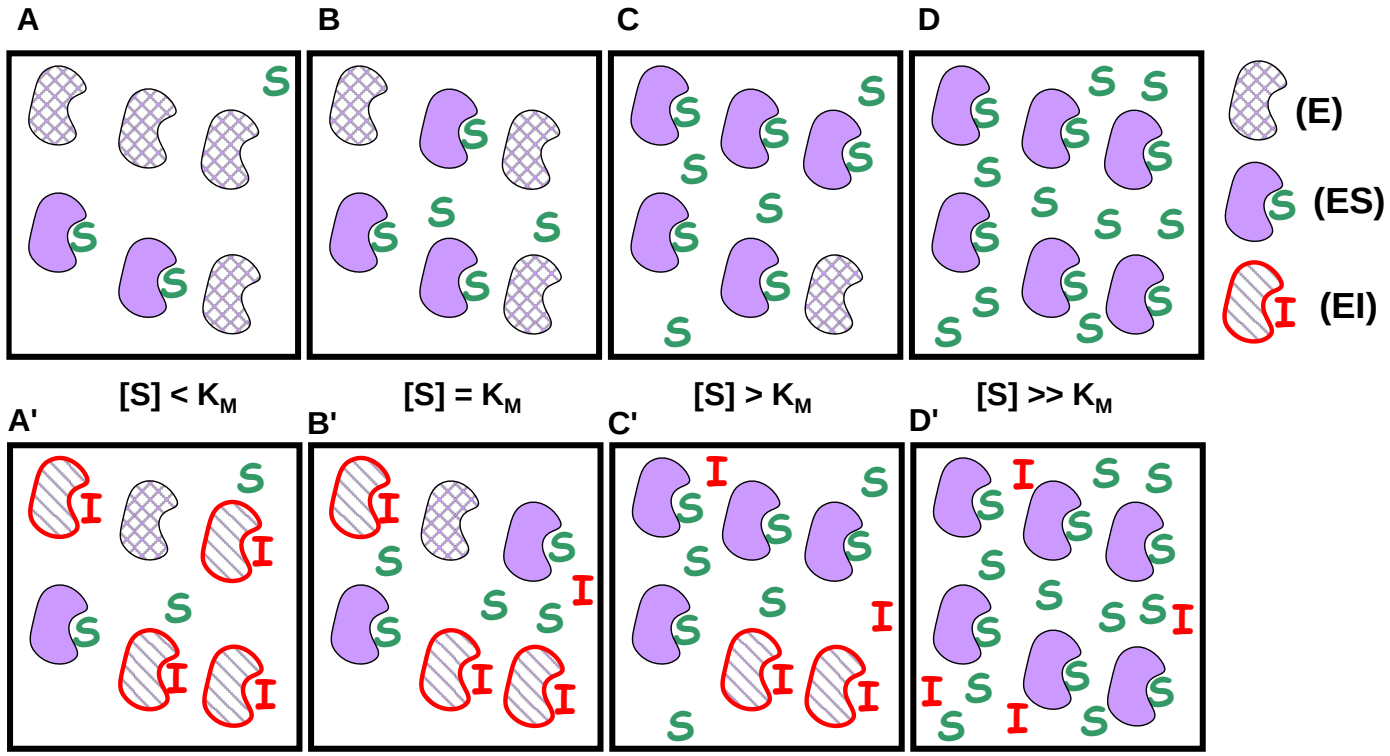
- A competitive inhibitor reduces the amount of [E] by the formation of [EI] complex.
- The inhibitor cannot affect the [ES] complex since the inhibitor can no longer bind.

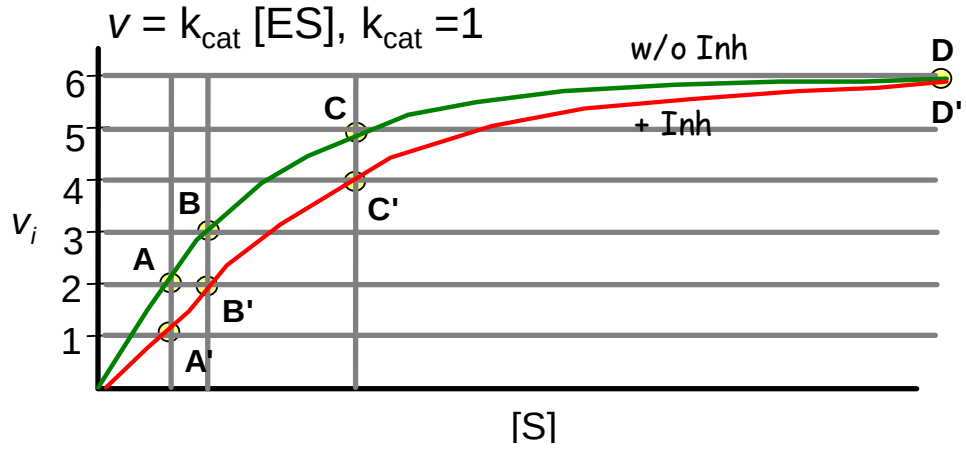
There are two consequences of a competitive inhibitor binding on the kinetics of the enzyme:

1. **V_{MAX} is unchanged**: At high levels of substrate all of the inhibitor is displaced by substrate, so [ES]=E_{TOTAL}, and v_{MAX} = $k_{CAT}[E_{TOT}]$.
2. **The *observed* K_M is increased:** It requires more substrate to reach 1/2 maximal velocity because some of the enzyme is complexed with inhibitor.

$$K_M^{OBS} = \alpha K_M$$

The change in K_M can be used to determine how well the inhibitor binds to the free enzyme, *if we know how α is related to K_I.*





No inhibitor

$$v = V_{MAX}\frac{[S]}{K_M + [S]}$$

Comp inhibitor

$$v = V_{MAX}\frac{[S]}{\alpha K_M + [S]}$$

$$= E_{Tot}k_{CAT}\frac{[S]}{K_M+[S]}$$

$$= E_{Tot}k_{CAT} \times Y_{(ES)}$$

$$=E_{Tot}k_{CAT}\frac{[ES]}{[E]+[ES]+[EI]}$$

$$= E_{Tot}k_{CAT}\frac{\frac{[S][E]}{K_M}}{[E]+\frac{[S][E]}{K_M}+\frac{[I][E]}{K_I}}$$

$$= E_{Tot}k_{CAT}\frac{\frac{[S]}{K_M}}{1+\frac{[S]}{K_M}+\frac{[I]}{K_I}}$$

$$= E_{Tot}k_{CAT}\frac{\frac{[S]}{K_M}}{1+\frac{[S]}{K_M}+\frac{[I]}{K_I}} \times \frac{\frac{K_M}{1}}{\frac{K_M}{1}}$$

$$=E_{Tot}k_{CAT}\frac{[S]}{K_M+[S]+\frac{[I]}{K_I}K_M}$$

$$=E_{Tot}k_{CAT}\frac{[S]}{(1+\frac{[I]}{K_I})K_M+[S]}$$

$$=E_{Tot}k_{CAT}\frac{[S]}{\alpha K_M+[S]}$$

No-you don't need to know this derivation

Steady-State Analysis of Competitive Inhibitors

$$K_I = \frac{[I][E]}{[EI]} \qquad K_M \approx \frac{[S][E]}{[ES]}$$

(EI)

$$[EI] = \frac{[I][E]}{K_I} \qquad [ES] = \frac{[S][E]}{K_M}$$

α = degree of inhibition $\quad \alpha = 1 + \frac{[I]}{K_I}$

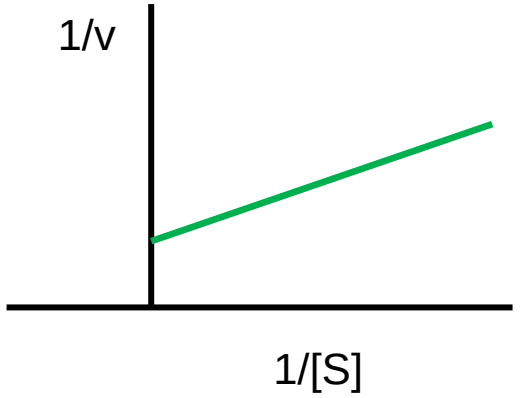
A double reciprocal plot can be used to obtain α:

No Inhibitor Present → Double Reciprocal Equation

$$v = V_{MAX}\frac{[S]}{K_M+[S]} \qquad \frac{1}{v} = \frac{K_M}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}}$$

Inhibitor Present → Double Reciprocal Equation

$$v = V_{MAX}\frac{[S]}{\alpha K_M+[S]} \qquad \frac{1}{v} = \frac{\alpha K_M}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}}$$



A competitive inhibitor changes the slope of a double recip. plot!
The change in slope can be used to obtain α and then K_I.

Experimental Determination of Inhibitor-Enzyme Affinity (K_I)

A. Data Collection

i) Measure initial velocity for different [S], in the *absence* of inhibitor.

ii) Measure initial velocity for different [S], in the *presence* of a fixed concentration of inhibitor (i.e. only [S] is varied, not [I]).

B: Obtaining K_I

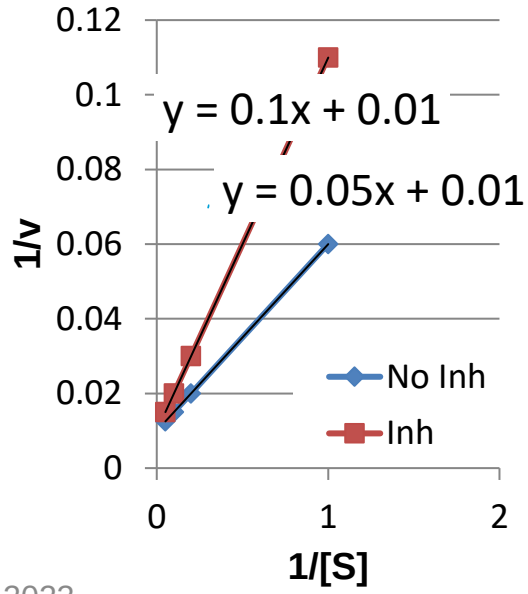
Linearization of Data using Double-Reciprocal Plot:

i) Both data sets are plotted on a double reciprocal plot.

ii) Ratio of the slopes gives α (degree of inhibition).

iii) **K_I = [I]/(α-1).**

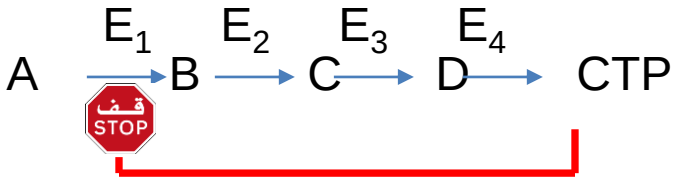
[S] mM	v_i [I]=0	v_i I=10μM	1/[S]	1/v [I]=0	1/v I=10μM
1	16.7	9.1	1.0	0.060	0.110
5	50.0	33.3	0.2	0.020	0.030
10	66.7	50.0	0.1	0.015	0.020



α=

K_I =

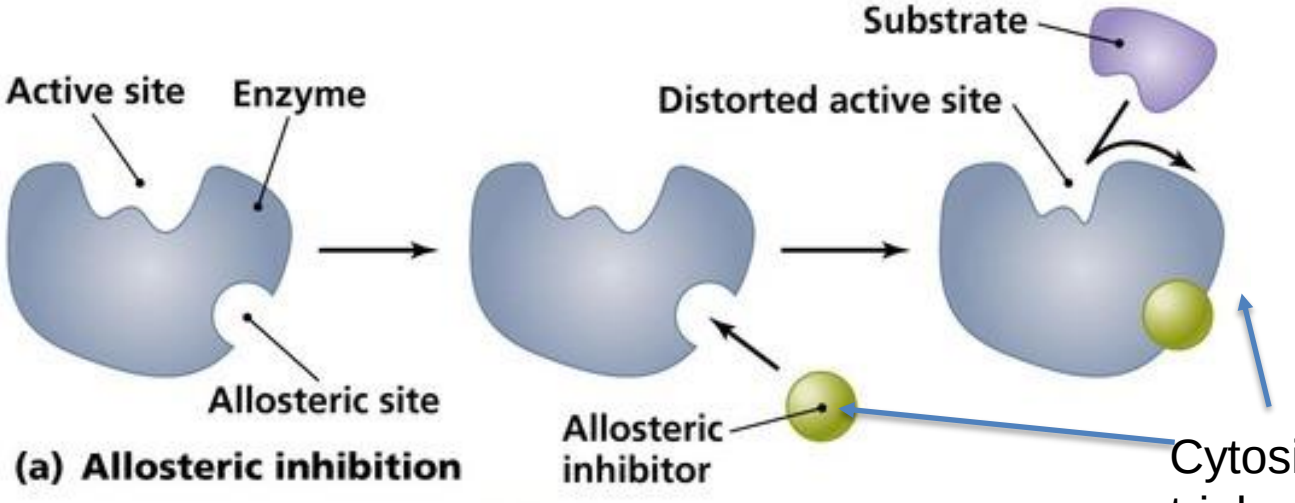
Allosteric Inhibition of an Enzyme

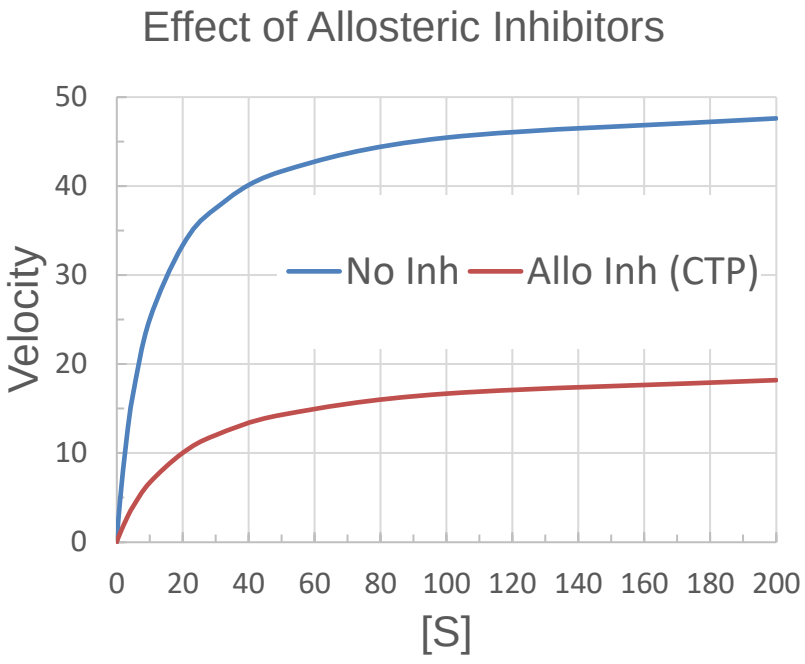


- Inhibitor does not bind in the active site.
- Inhibitor can bind to both the free enzyme (E) and the (ES) complex

Feedback Regulation:
Product of pathway prevents production by inhibition of enzyme earlier in the pathway.

Example - Allosteric regulation of CTP production



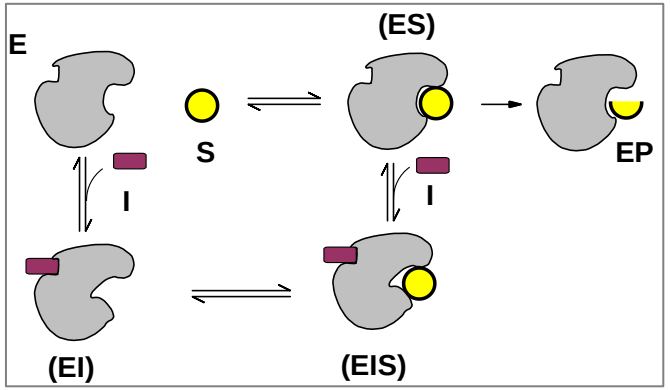


- CTP (a component of RNA) is generated by a series of enzymatic steps – a metabolic pathway.
- The first enzyme in the pathway is inhibited by the final product of the pathway, CTP (cytosine triphosphate)
- The CTP binds at an allosteric site, cause a conformational change in the active site, decreasing V_{MAX}, ***shutting off its own production.***

Allosteric (Mixed Type) Inhibitors

The binding site of the inhibitor is ***not*** at the active site. The inhibitor does not look like the substrate.

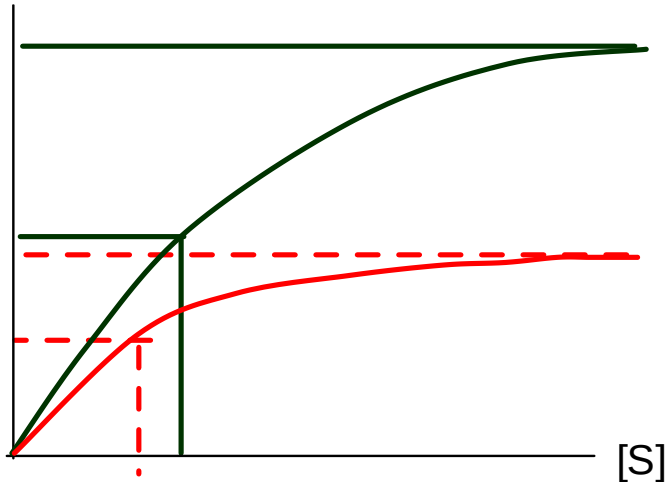
- The binding is reversible.
- The inhibitor can bind to both [E] and [ES].
- The inhibitor binding causes a change in the conformation of the protein that can affect substrate binding (K_M) or the chemical step (k_{CAT}) or both!



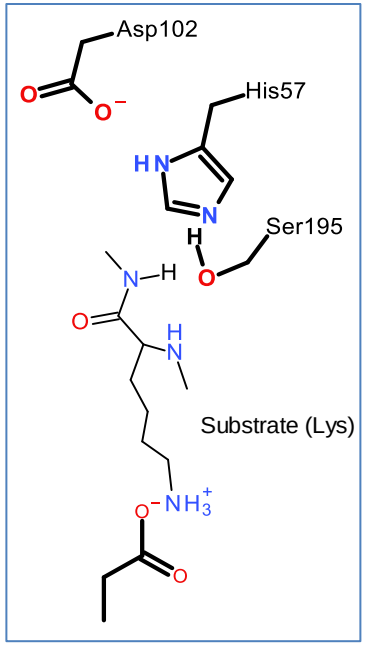
Nomenclature: Uncompetitive & noncompetitive are unrealistic simplifications of mixed type inhibitors.

	Binds to (E)	Binds to (ES)
Mixed type	yes	yes
Uncompetitive	no	yes
Noncompetitive	Same affinity	Same affinity

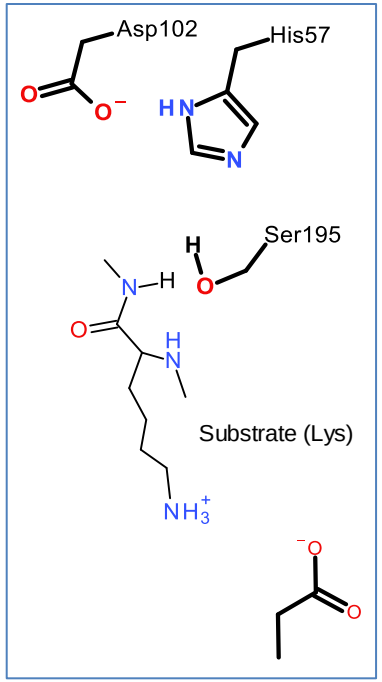
- Both V_{MAX} and K_M can be altered by mixed inhibitors since the precise geometry of the active site is altered when the inhibitor is bound, potentially affecting binding and catalysis.
- The change in V_{MAX} can be used to find K_I': $\mathbf{V_{MAX}^{OBS} = V_{MAX}/\alpha'}$
- The change in K_m can be used to find K_I. $\mathbf{K_M^{OBS} = (\alpha/\alpha')\, K_M}$



Active Site W/O Inh

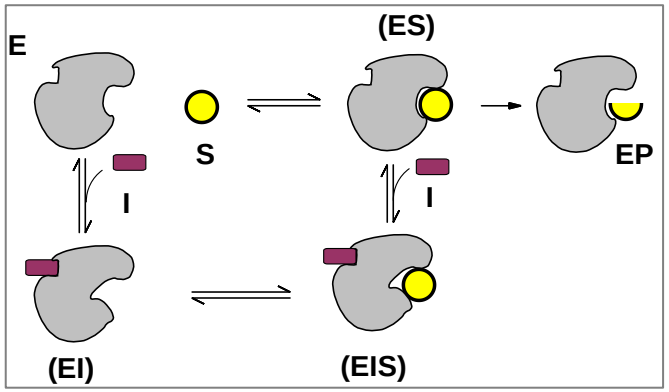


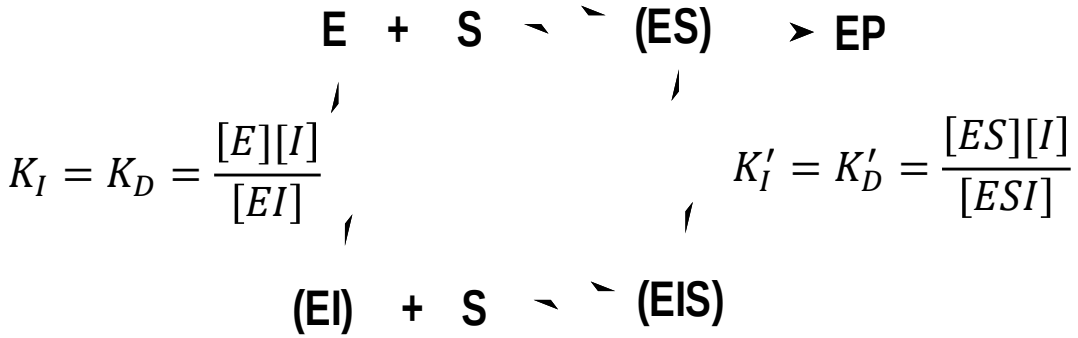
Active Site with Inh



2. Allosteric (Mixed Type) Inhibitors

There are ***two*** K_D values that describe the binding.

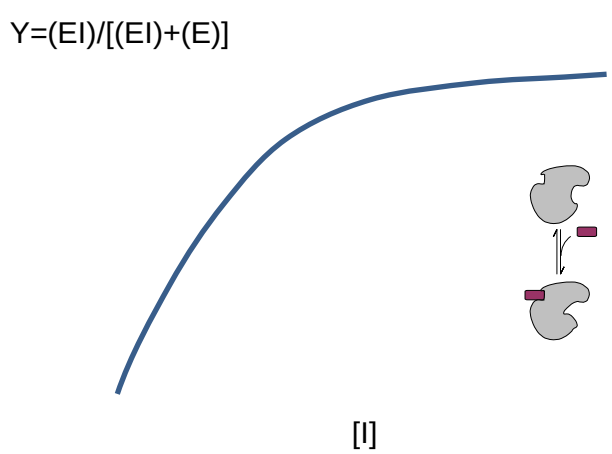




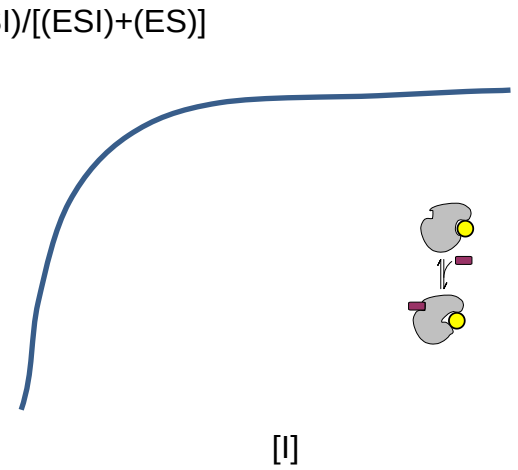
$$K_I = K_D = \frac{[E][I]}{[EI]}$$

$$K_I' = K_D' = \frac{[ES][I]}{[ESI]}$$

$$K_I = \frac{[E][I]}{[EI]}$$



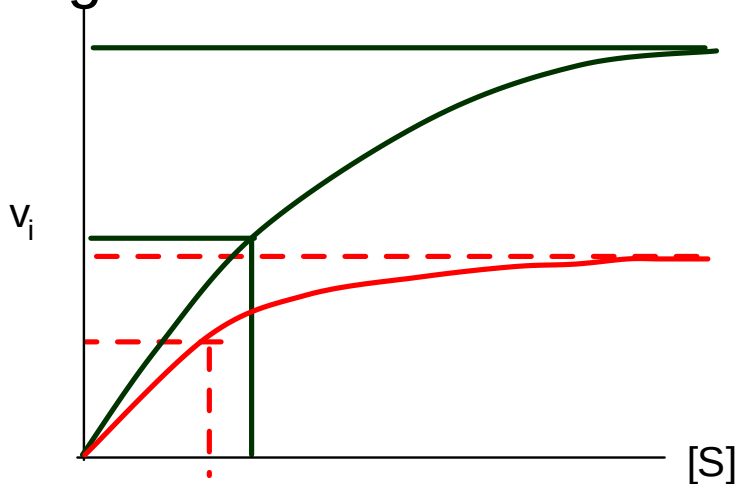
$$K_I' = \frac{[ES][I]}{[ESI]}$$



Determining Binding of Allosteric Inhibitors:

$$\alpha = 1 + \frac{[I]}{K_I} \qquad \alpha' = 1 + \frac{[I]}{K_I'}$$

- The change in V_{MAX} can be used to find K_I': **$V_{MAX}^{OBS} = V_{MAX}/\alpha'$**
- The change in K_m can be used to find K_I. **$K_M^{OBS} = (\alpha/\alpha')\, K_M$**



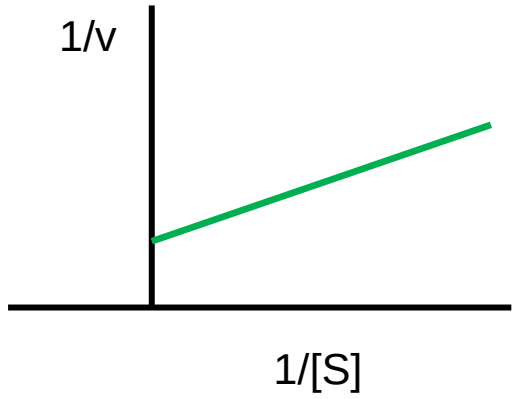
Obtaining K_is from Double Reciprocal plots:

α = ratio of slopes (+Inh/no inh)

K_I = [I]/(α -1)

α' = ratio of y-intercept (+Inh/no inh)

K_I' = [I]/(α'-1)



No Inhibitor

$$v = V_{MAX}\frac{[S]}{K_M + [S]}$$

$$\frac{1}{v} = \frac{K_M}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}}$$

Allosteric Inhibitor

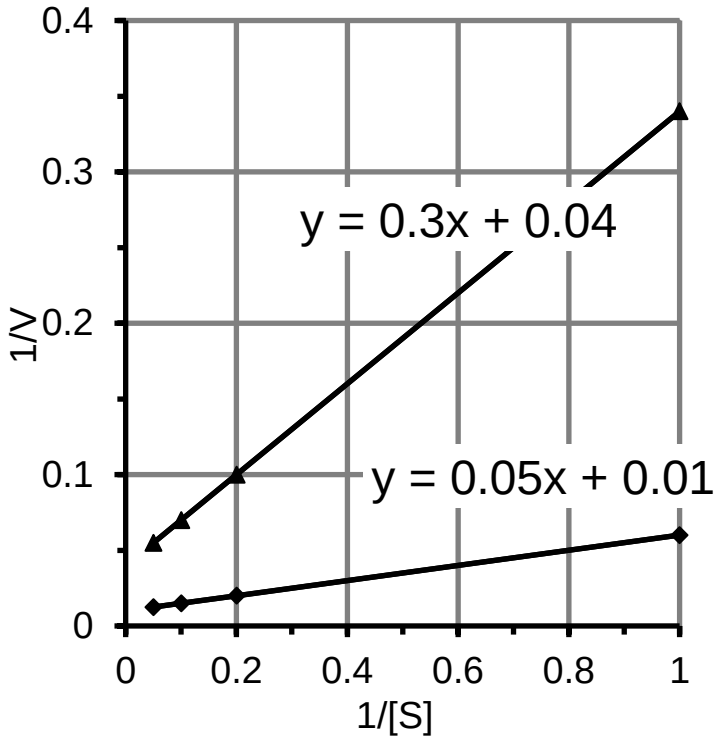
$$v = \frac{\frac{V_{MAX}}{\alpha'}[S]}{\frac{\alpha}{\alpha'}K_M + [S]}$$

$$\frac{1}{v} = \frac{\alpha K_M}{V_{MAX}}\frac{1}{[S]} + \frac{\alpha'}{V_{MAX}}$$

Example:

Allosteric (mixed type) Inhibitor

[S] mM	v ([I]=0)	v I=10μM	1/S	1/V ([I]=0)	1/V I=10μM
1	16.7	2.9	1.0	0.060	0.340
5	50.0	10.0	0.2	0.020	0.100
10	66.7	14.3	0.1	0.015	0.070
20	80.0	18.2	0.05	0.0125	0.055



K_I - Obtain α - ratio of slopes.

$$K_I = \frac{[I]}{(\alpha - 1)}$$

Slope ([I]=0): Slope ([I]>0):

α =slope([I]>0)/slope([I]=0): K_I:

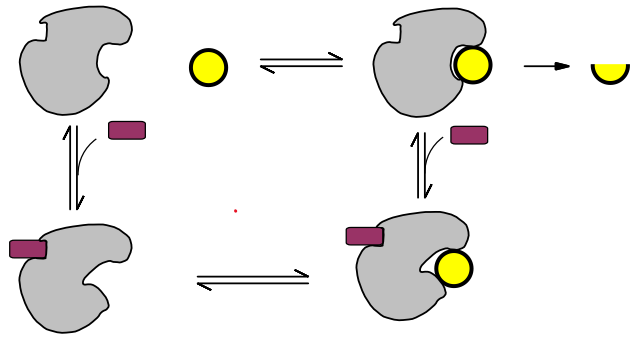
K_I' Obtain α' – ratio of y-intercepts:

$$K_I' = \frac{[I]}{(\alpha' - 1)}$$

y-int([I]=0): y-int([I]>0):

α' = y-int([I]>0)/y-int([I]=0): K'_I:

Which form of the enzyme, (E) or (ES), binds the inhibitor with higher affinity?



HIV Drug Therapy

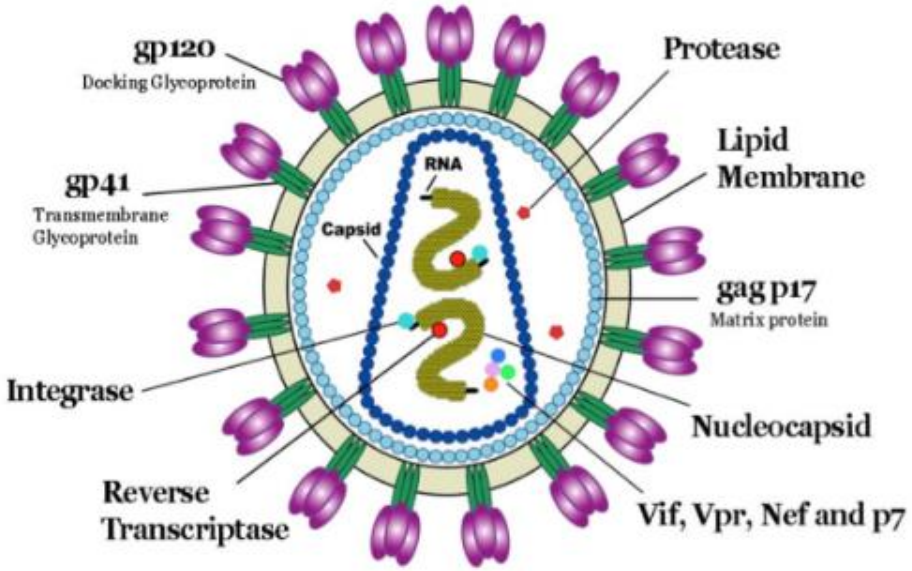
Retroviruses & Inhibitors - HIV Protease.

Goals

- Identify potential drug targets, based on viral life cycle.
- Mechanism of aspartyl proteases
- Measure inhibitor binding to characterize drug efficiency.

Human Immunodeficiency Virus (HIV)

- Retrovirus, genetic material is RNA that is converted to DNA first, and then the copy in the DNA is used to make new copies of the viral RNA (as well as viral proteins)
- Infects specialized cells in the immune system – *T-helper cells* (T_H) cells, killing them.
- T_H cells are required for activation of the immune response to all pathogens (bacteria, virus)
- Killing of T_H cells by the HIV virus causes AIDS (**A**cquired **I**mmuno**d**eficiencie**s**), making the individual susceptible to serious infection by many otherwise harmless bacteria, as well as developing rare cancers.



Viral particle contains enzymes required for the replication of the virus:

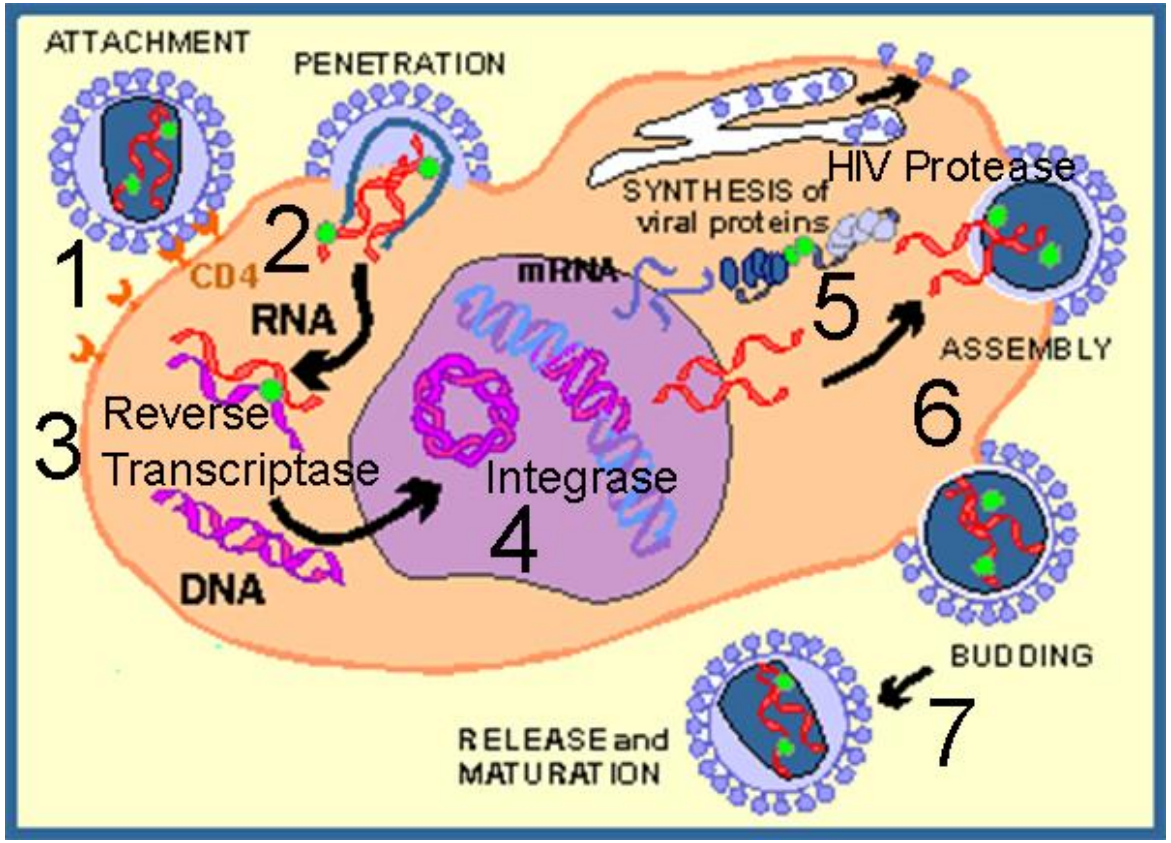
Reverse Transcriptase: Copies viral RNA to DNA

Integrase: Integrates viral DNA into host chromosome.

HIV Protease: Cleaves immature viral protein to produce smaller mature proteins.

HIV Viral Infection of T-Helper Cells:

1. Viruses bind to molecules displayed on the T_H cell surface.
2. The virus then fuses with the cell membrane and releases its RNA genome from its lipid envelope.
3. The HIV enzyme **reverse transcriptase** first makes a double-stranded DNA copy of the viral RNA molecule. This process is error prone, leading to mutations in the virus. *These mutations cause drug resistant strains of the virus to arise.*
4. The DNA is integrated into the host cell's DNA by an enzyme called **integrase,** also from the HIV virus.
5. Integrated DNA produces vRNA, the genetic material for new virus particles. mRNA is also made from this DNA, to produce proteins for new particles.
6. **HIV protease,** coded for by the virus, is required for maturation of viral proteins, by cleaving them into smaller proteins that form the mature virus.
7. Mature virus buds out of cell.

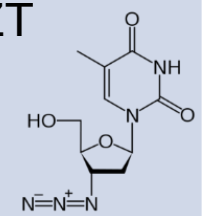
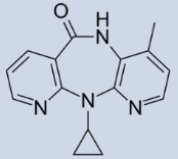


Drug Targets to Combat the HIV Virus –

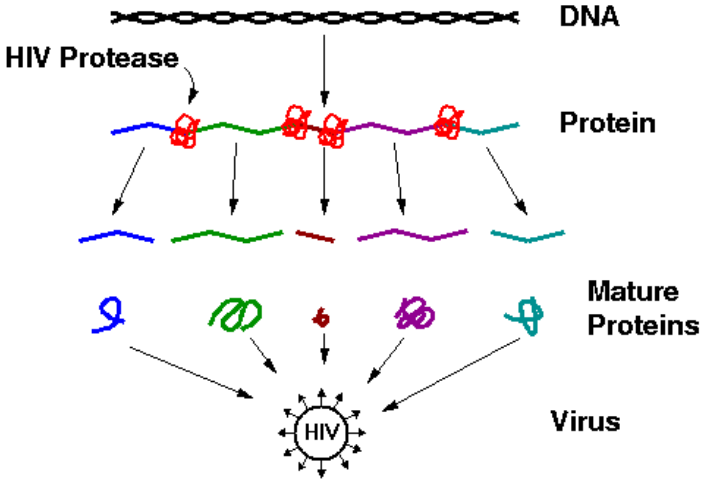
a) Viral fusion
b) Reverse transcriptase
c) Integrase
d) HIV Protease

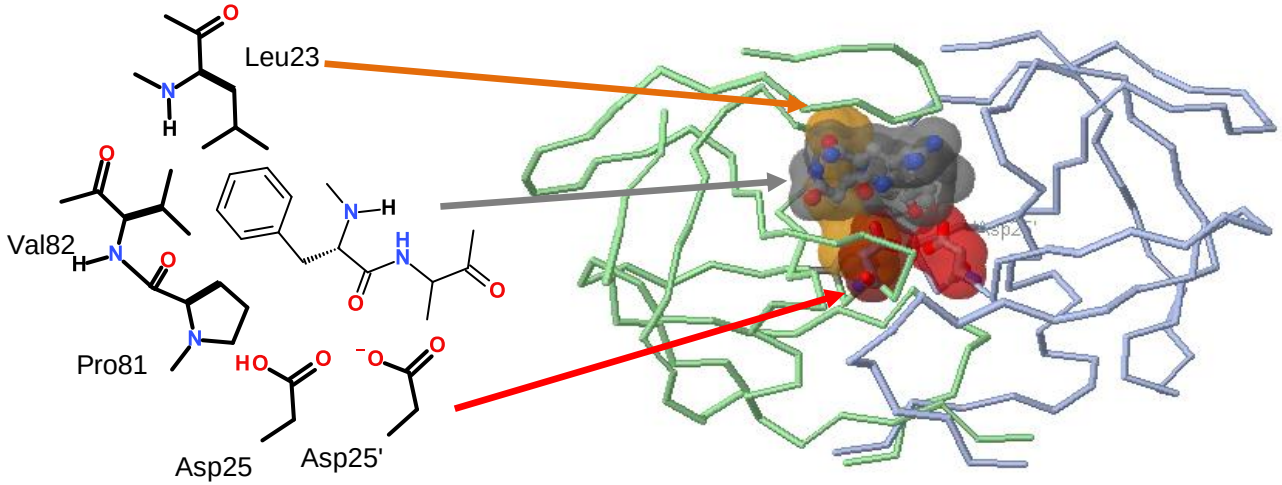
Why are these good targets for inhibitors that are anti-viral drugs?

HIV Anti-viral Drugs

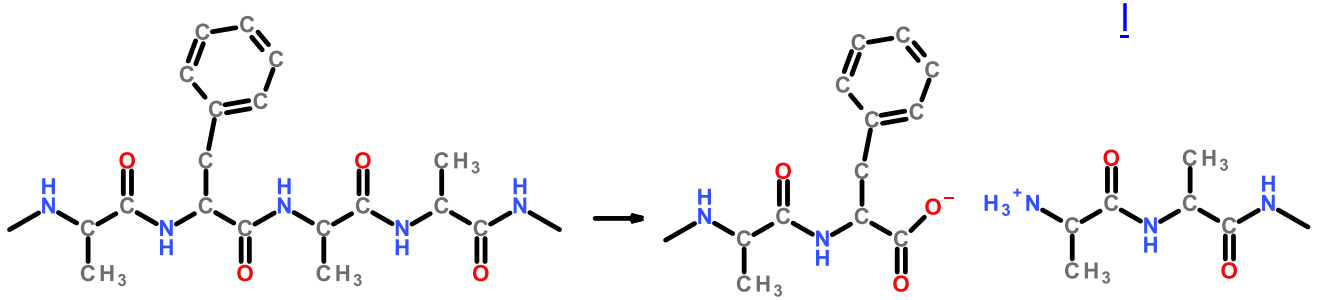
1980-84 HIV virus characterized	Fusion Inhibitors	RevTrans Inhibitors – NRTI (competitive)	RevTrans Inhibitors – NNRTI (allosteric)	Integrase Inhibitors	Protease Inhibitors (competitive)
1985-89		AZT 			
1990-94		Didanoisine, Zalcitabine, Stavudine			
1995-99		Lavidudine	Nevirapine, Delavirdine, Efavirenz 		Saquinavir, Ritonovair, Indinavir, Nelfinavir
2000-04	Enfuvirtide	Didanosine Emtricitabine			Atazanavir
2005-09	Maraviroc		Etravirine	Raltegravir	Darunavir, Tipranavir
2010-14			Nevirapine XR, Rilpivirine	Dolutegravir, Evitegravir	

HIV Protease (Aspartyl protease)





https://www.andrew.cmu.edu/user/rule/jsmol/hiv_prot.html

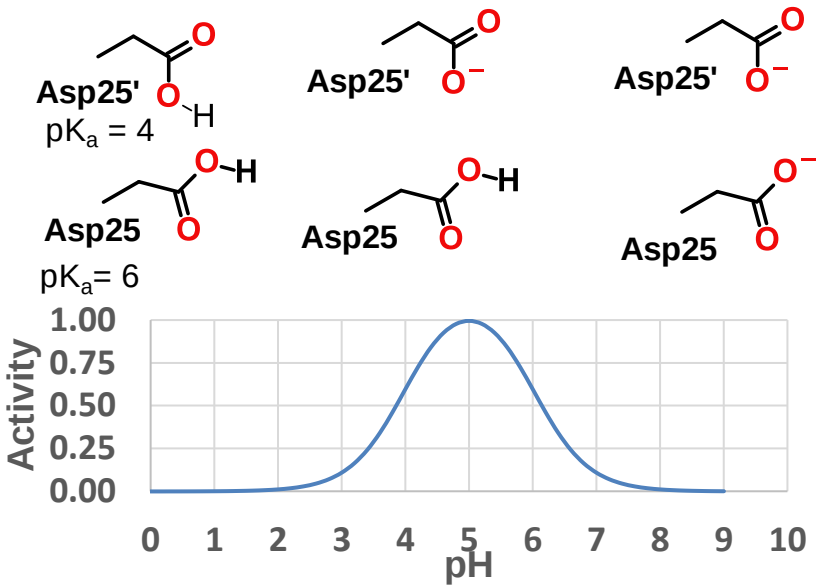


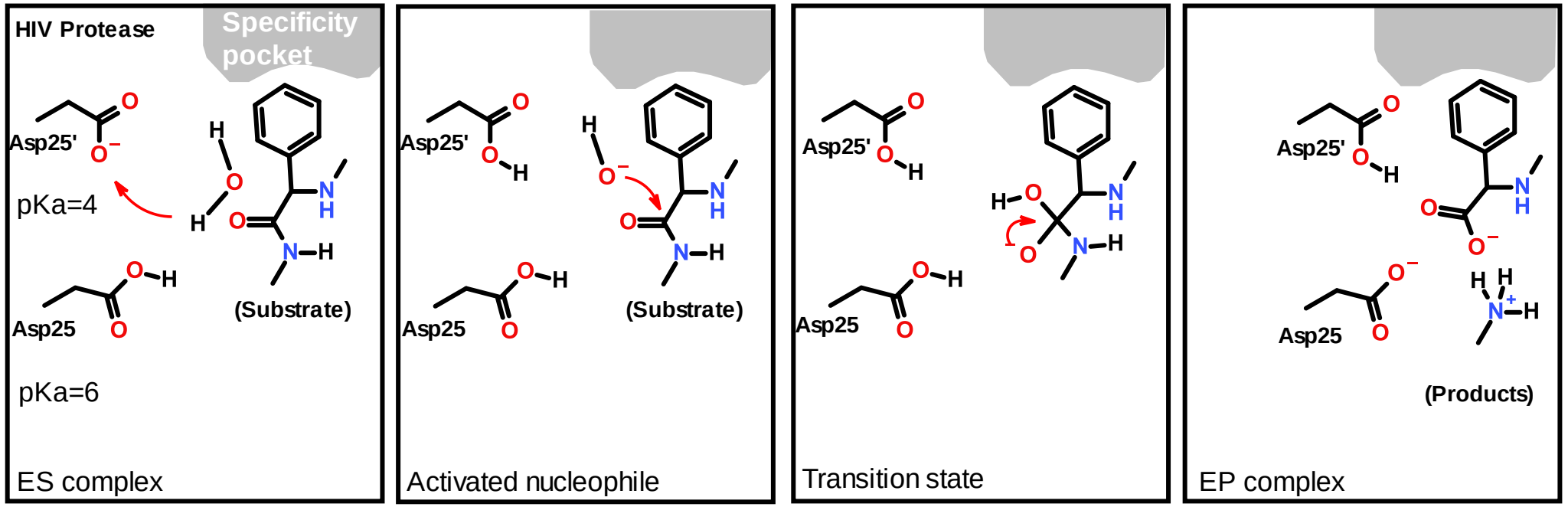
1. An essential enzyme in the maturation of HIV viral proteins. If inhibited, the virus cannot replicate.
2. A homo-dimeric protein, containing two catalytic Asp residues, Asp25 and Asp25', the same residue on each chain. The pK_a values of these two differ widely, one is about 4.0 and the other about 6.0. One of the Asp residues must be protonated the other must be deprotonated for full activity.
4. Prefers hydrophobic substrates due to Leu23, Pro81, and Val82, in its specificity pocket.

Mechanism – Single Transition State – No Acyl Intermediate.

1. Activation of H_2O by Asp25'
2. Nucleophilic attack on C=O of substrate.
3. Tetrahedral transition state, no oxyanion hole.
4. Peptide bond cleavage
5. Protonation of new NH2 terminal by Asp25

pH Dependence of Activity





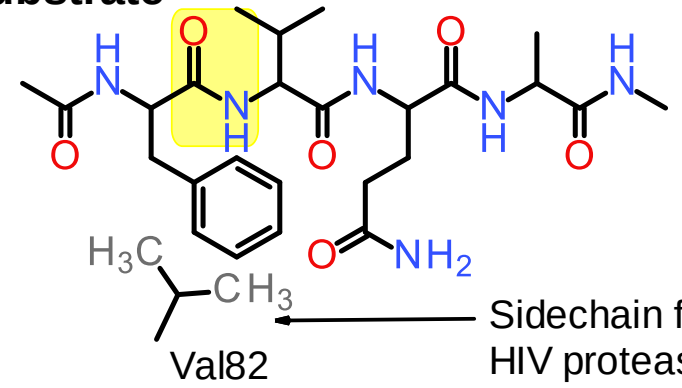
Inhibition of HIV Protease (HIV Drugs):

- Most drugs are small peptide-like analogs with non-cleavable bonds (highlighted).

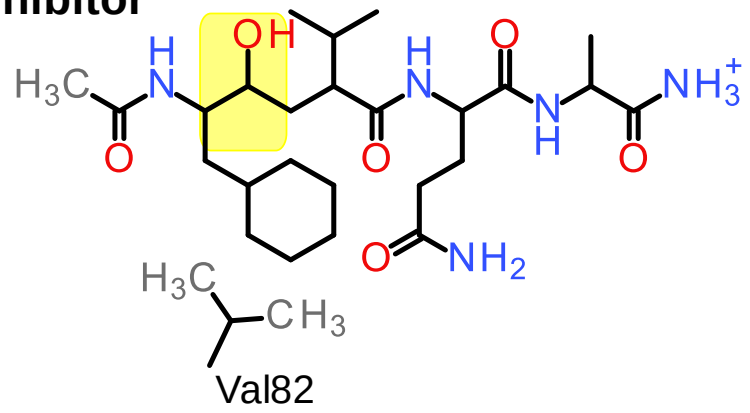
Where will they bind on the enzyme?

What will happen to them after they bind?

Substrate



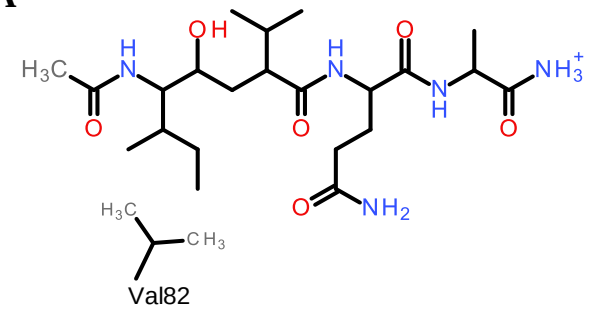
Inhibitor



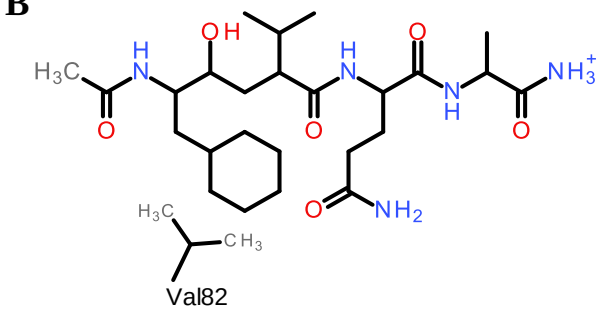
Drug Design and Analysis:

Question: Compounds A (Isobutyl) and B (cyclohexane) are candidates for HIV protease inhibitors. Which of the two drugs will be more effective at inhibiting the wild-type protease?

A



B



Answer: We will assume that these are competitive inhibitors. Therefore**,** we need to compare the K_I values for each inhibitor binding to the protease, using the following steps:

Measuring K_I for both Drugs:

a) Acquire v versus (S), no inhibitor. Use substrate that generates colored product (nitrophenylate ion).

b) Acquire v versus (S), fixed inhibitor.

c) Analysis – Graphical (Double Recp Plot)

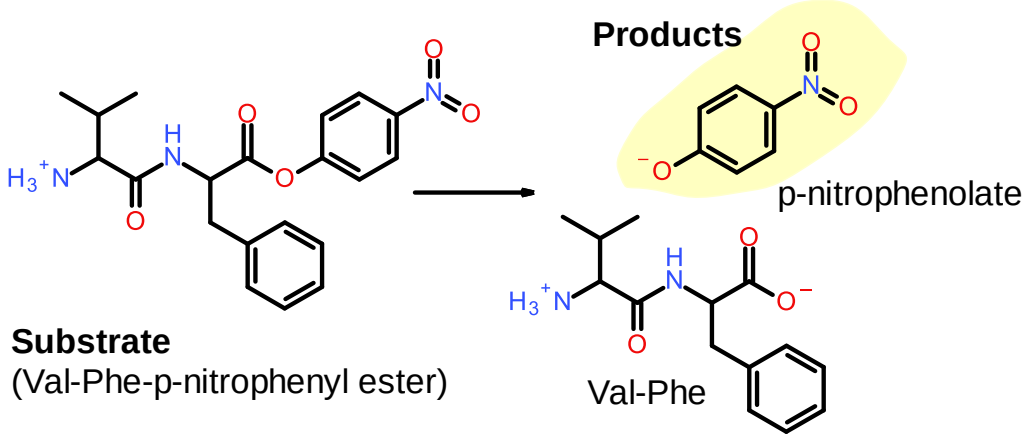
i) Plot double reciprocal plots.

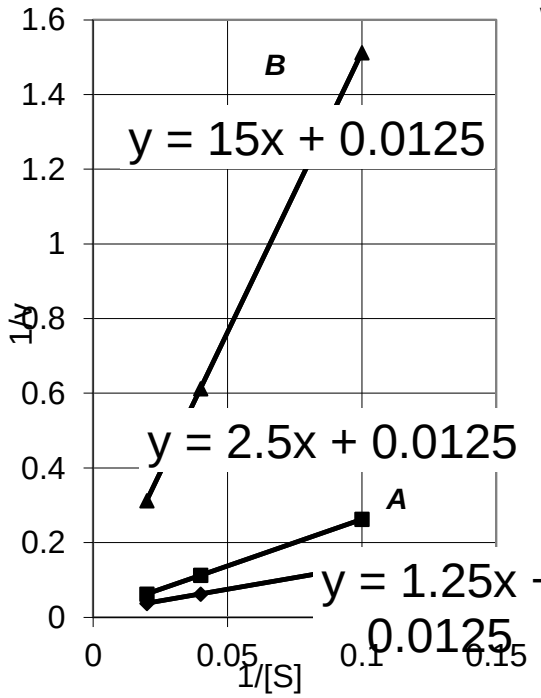
ii) Obtain α from ratio of slopes

iii) Once the α values are found, we can calculate the K_I for each inhibitor using the formula: **K_I=[I]/(α-1).**

[S] μM	v (I=0)	v ([A]=10 nM)	v ([B]=10 nM)
10	7.3	3.8	0.7
25	16	8.9	1.6
50	26.7	16.0	3.2
1/[S]	**1/V (I=0)**	**1/V ([A]=10nM)**	**1/V ([B]=10nM)**
0.10	0.138	0.263	1.513
0.04	0.063	0.113	0.613
0.02	0.038	0.063	0.313

The units of velocity are μmoles product/sec.



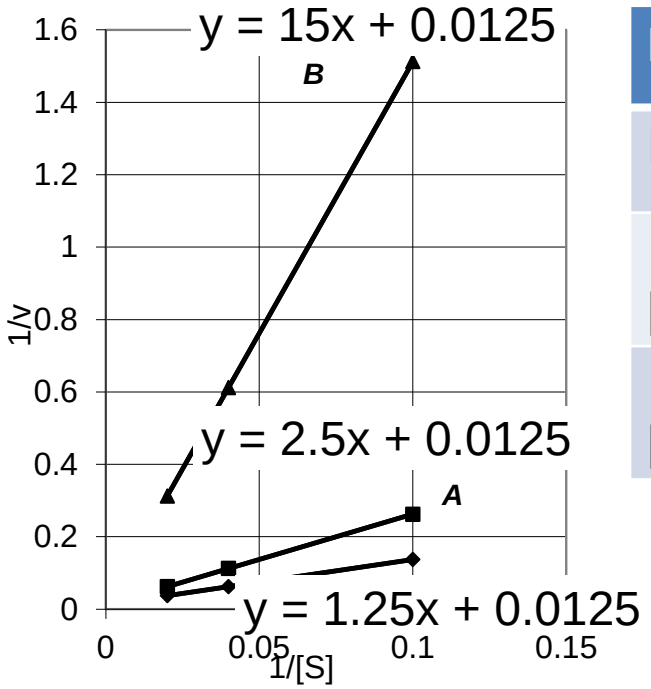


With Inhibitor

$$\frac{1}{v} = \frac{\alpha K_M}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}}$$

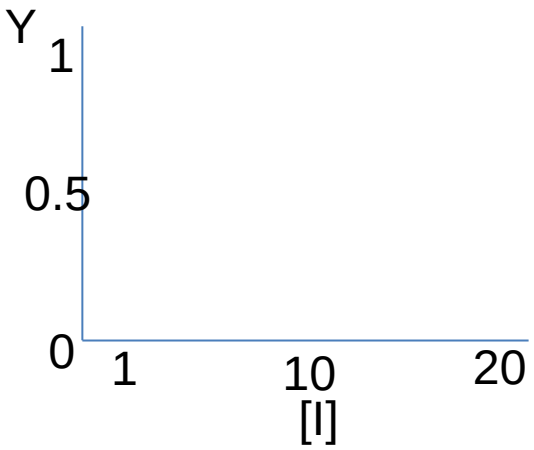
No Inhibitor

$$\frac{1}{v} = \frac{K_M}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}}$$

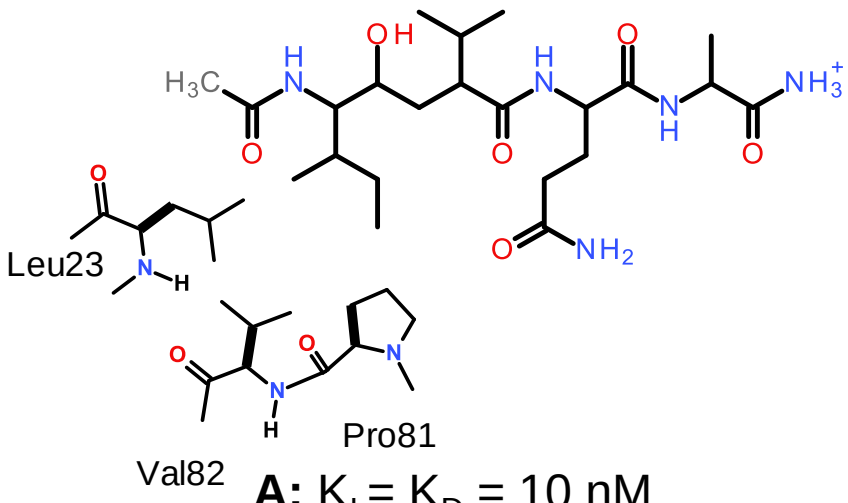


Data	Slope	α	$K_I = [I]/(\alpha-1)$
No Inhibitor	***1.25***		
Inhibitor A [I] = 10 nM	***2.5***	***(2.5/1.25)=2***	***10 nM/(2-1) = 10 nM***
Inhibitor B [I] = 10 nM	***15***	***(15/1.25) =12***	***10 nM/(12-1) = 0.9 nM***

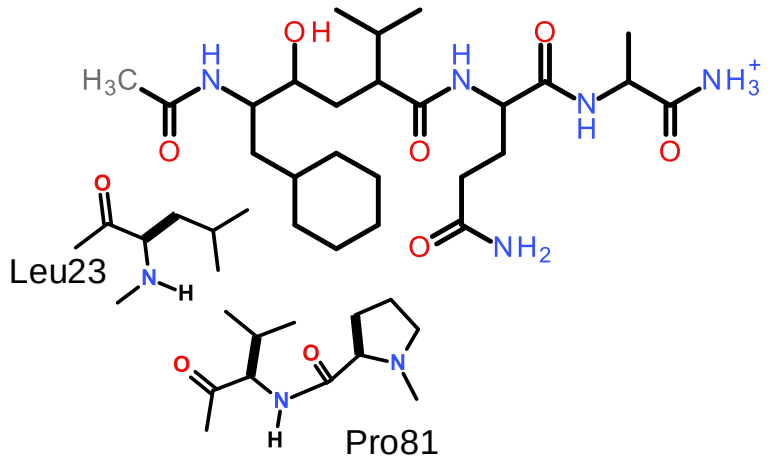
1. Which is the more effective inhibitor? A or B?



2. Why does B have a lower K_I?



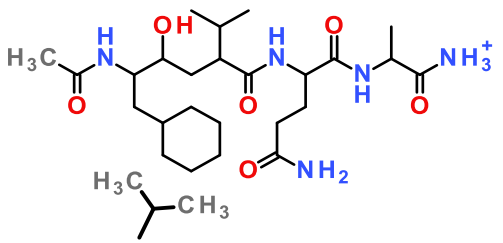
A: $K_I = K_D = 10$ nM

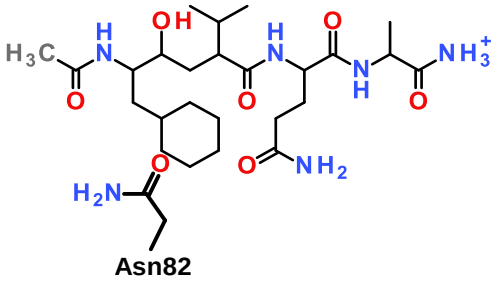


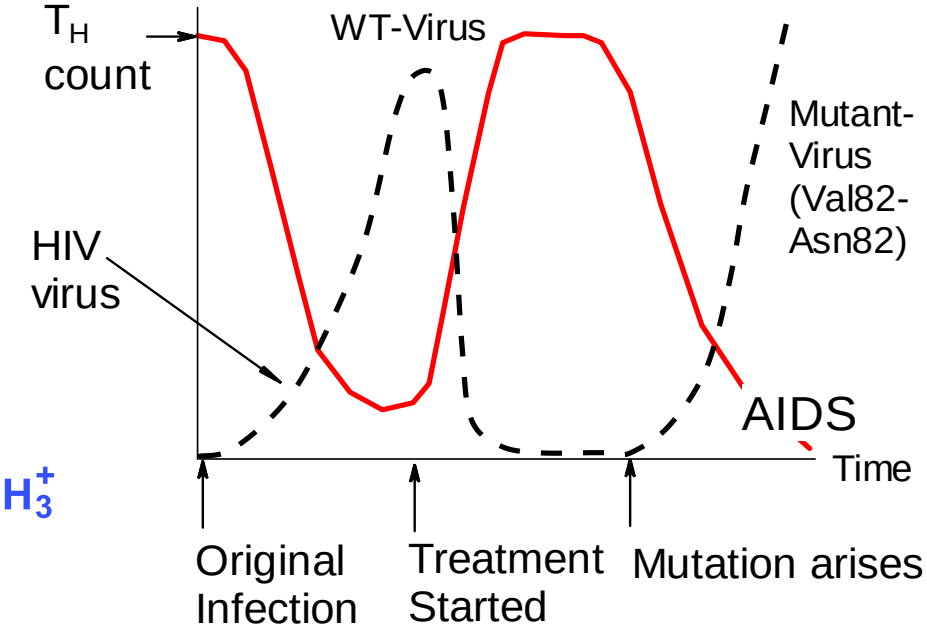
B: $K_I = K_D = 0.9$ nM

Drug resistance & Rational Drug Design:

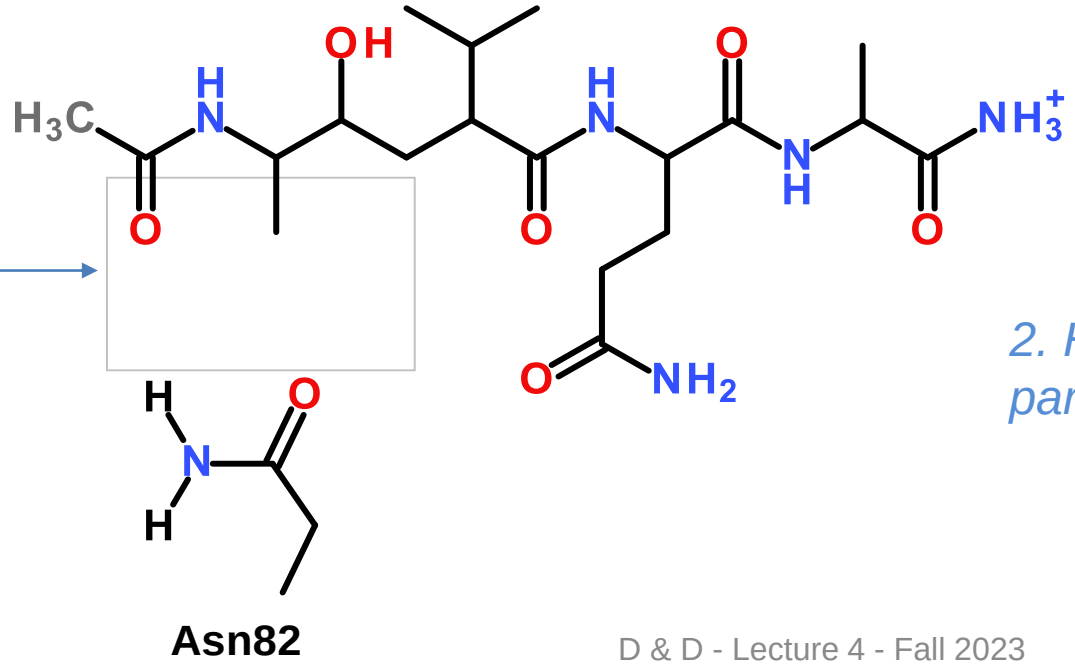
- Error prone copying of vRNA to DNA introduces changes in the sequence of the viral RNA (mutations), leading to altered amino acids in the viral proteins.
- Changes in the residues that are involved in drug binding may reduce binding.
- The mutant virus is no longer inhibited and will quickly overgrow the wild-type virus.
- A common mutation that arises in many HIV patients is changing Val82→Asn82 in HIV protease.
- The altered HIV protease can be inhibited with modified protease inhibitors (personalized medicine).







1. How might you alter the existing inhibitor to be effective at binding to HIV protease with the Asn82 mutation?



2. How would you test your new drug? What parameter would you measure?

K_M or k_{CAT} or K_I