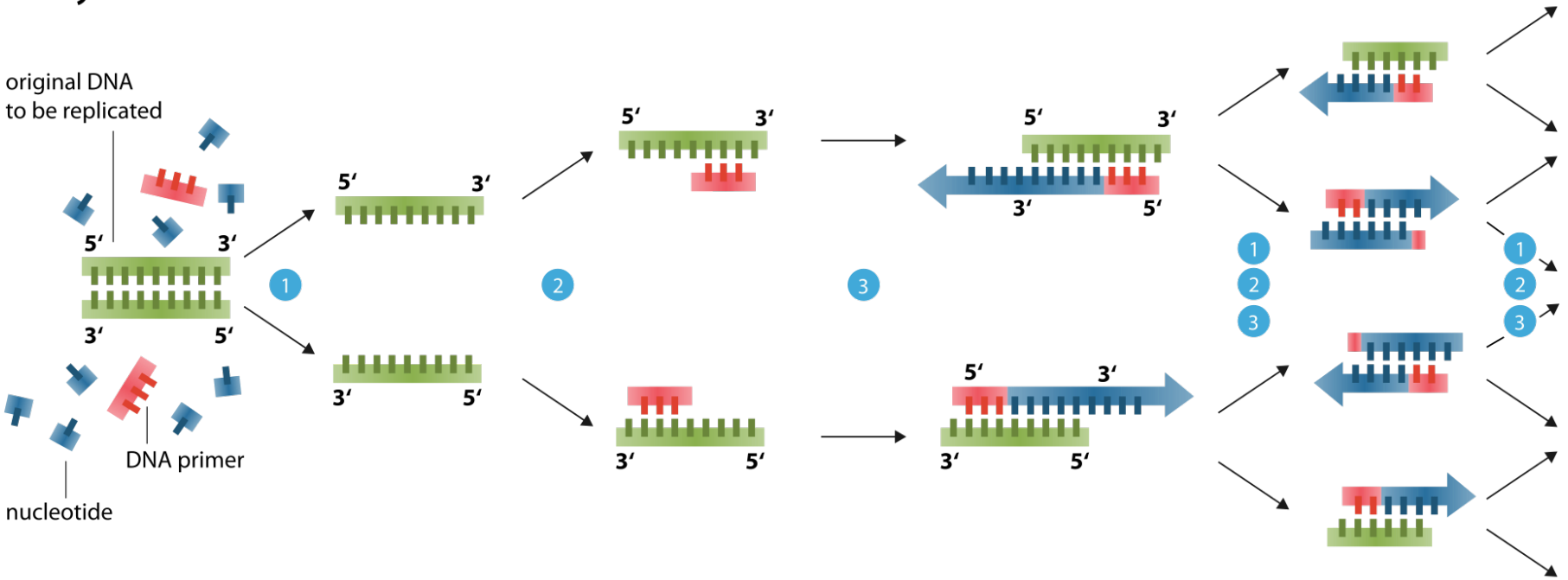
REVIEW OF LAST LECTURE

PCR

Polymerase chain reaction - PCR



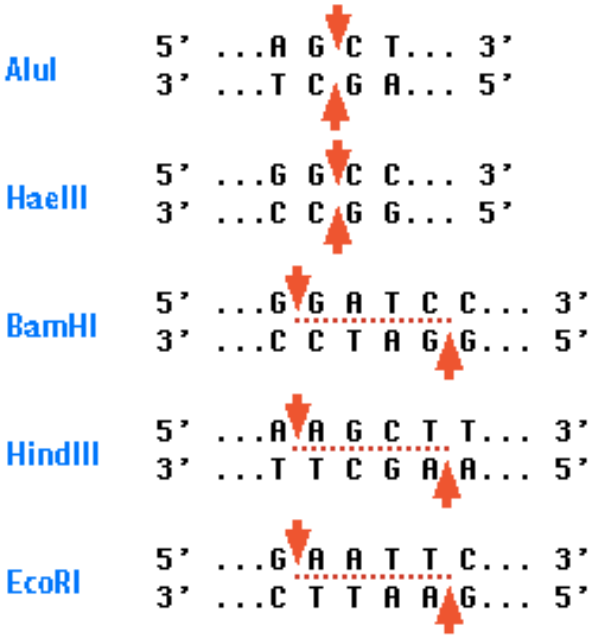
1. **Denaturation** at 94-96°C
2. **Annealing** at ~68°C
3. **Elongation** at ca. 72 °C

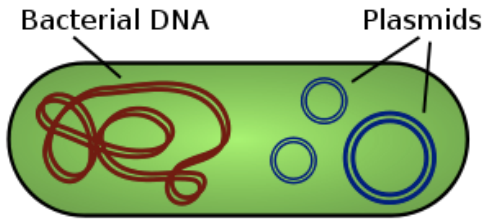
Molecular Cloning

- **Restriction enzymes** – enzymes (mostly from bacteria) that make cuts in DNA at specific sequences (palindromic site); these are "Type II"

- **Plasmids** – small DNA separate from chromosomal DNA, and can replicate separately; commonly found in bacteria

- **Origin of Replication (ORI)** – DNA sequence which allows initiation of replication within a plasmid by recruiting transcriptional machinery proteins



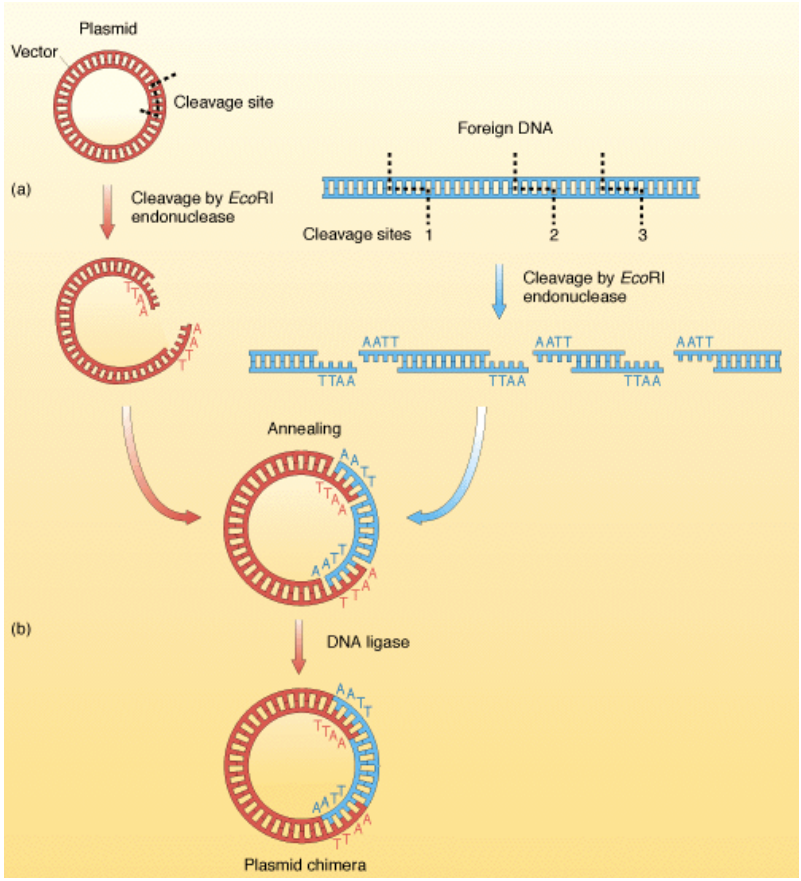


https://en.wikipedia.org/wiki/Recombinant_DNA

http://www.biology-pages.info/R/RestrictionEnzymes.gif
By User:Spaully on English wikipedia (Own work) [CC BY-SA 2.5 (http://creativecommons.org/licenses/by-sa/2.5)], via Wikimedia Commons

Molecular cloning

- **Vector -** DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated/expressed
- Cut the plasmid vector; Cut the insert sequence using the same restriction enzyme
- Join/"Ligate" the two together

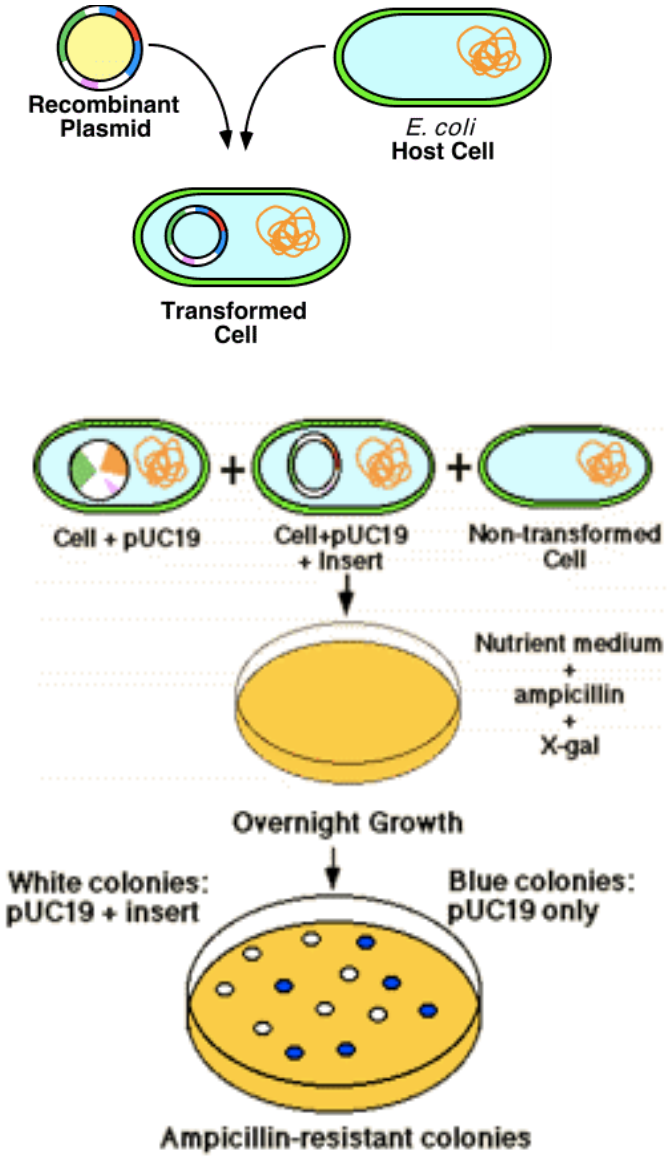


https://en.wikipedia.org/wiki/Recombinant_DNA
http://www.bio.miami.edu/dana/pix/chimericDNA.gif

Molecular cloning

- **Transformation** – genetic alteration of a cell resulting from direct uptake and incorporation of exogenous DNA through the cell membrane; typically achieved by heat shock, electroporation, or chemical treatment of cells (DNA precipitation)

- **Selection** – use of a selectable marker or antibiotic resistance gene to distinguish cells that did not take up plasmid, or did not insert the gene in the right place, or took up empty plasmid

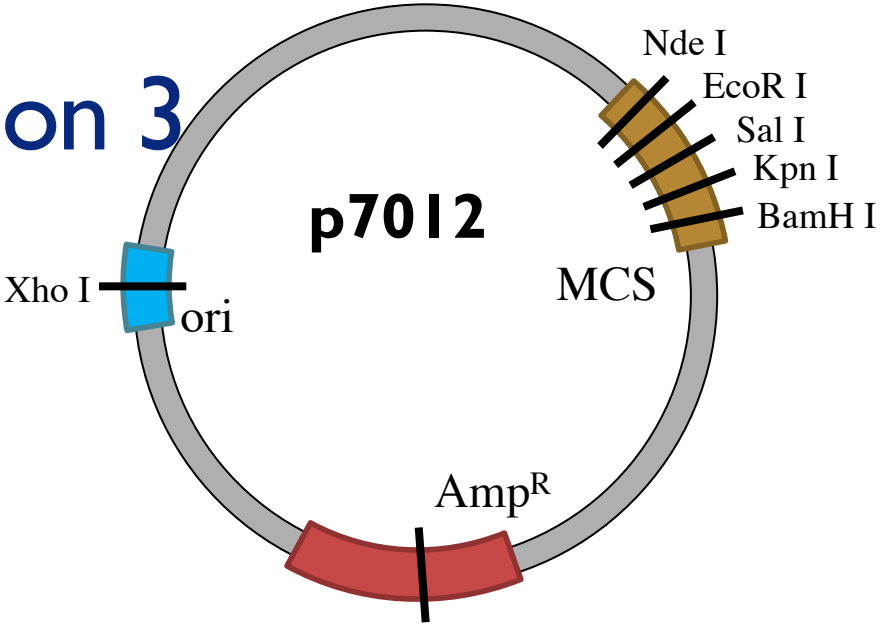


"Homework" Question 3

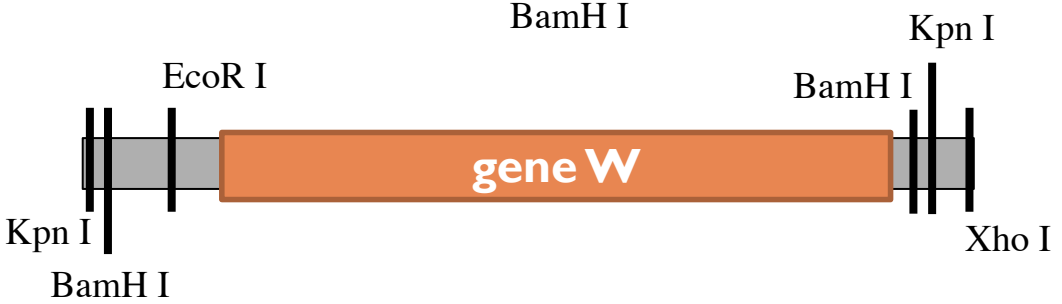
- Here is vector p7012:
- Here are the restriction enzymes:

Nde I:	Sal I:	Kpn I:
5' CATATG	5' GTCGAC	5' GGTACC
3' GTATAC	3' CAGCTG	3' CCATGG

EcoR I:	BamH I:	Xho I:
5' GAATTC	5' GGATCC	5' CTCGAG
3' CTTAAG	3' CCTAGG	3' GAGCTC



- Here is gene W:



a) *What are 3 strategies for cloning gene W into p7012?*

Answers will be posted online next week

b) *In which strategies would gene W be inserted into the vector in only one direction?*

c) *After cloning, you transform and plate bacterial cells using your cloned plasmid. Onto what type of growth medium will you plate your cells in order to distinguish between bacterial cells that obtained the plasmid and those that did not?*

Sample question adapted from MIT OpenCourseware: 7-01sc-fundamentals-of-biology-fall-2011

"Homework" Question 3 – Solution

a) *What are 3 strategies for cloning gene W into p7012?*

ANSWER:

1) KpnI to cut both
2) EcoRI + SalI to cut p7012; EcoRI + XhoI to cut gene W
3) EcoRI + KpnI to cut both

b) In which strategies would gene W be inserted into the vector in only one direction?

ANSWER: Options 2 and 3

c) *After cloning, you transform and plate bacterial cells using your cloned plasmid. Onto what type of growth medium will you plate your cells in order to distinguish between bacterial cells that obtained the plasmid and those that did not?*

ANSWER: Media plates that contain ampicilin

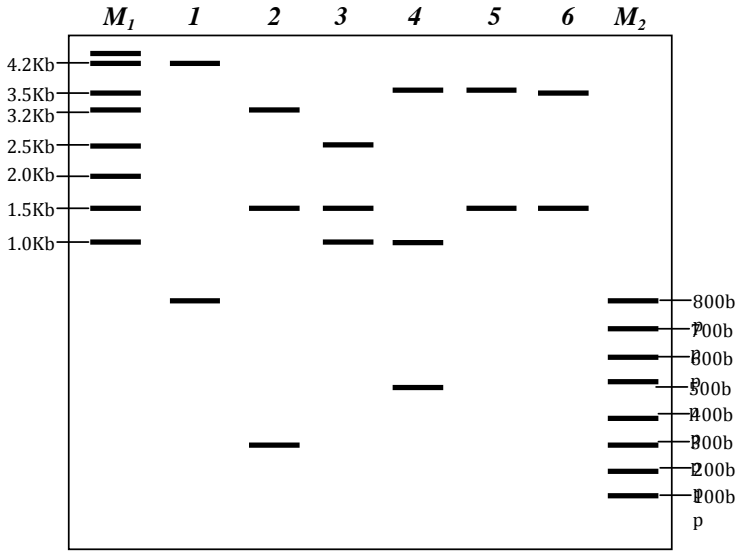
Sample question adapted from MIT OpenCourseware: 7-01sc-fundamentals-of-biology-fall-2011

"Homework" Question 4

You are given a plasmid. In order to map this plasmid you set up a series of restriction digests and obtain the following results using agarose gel electrophoresis.

a) *What is the approximate size of the plasmid?*

b) *Add the SmaI, KpnI, BglII sites to plasmid map. On your map give the distances between each of the restriction sites.*



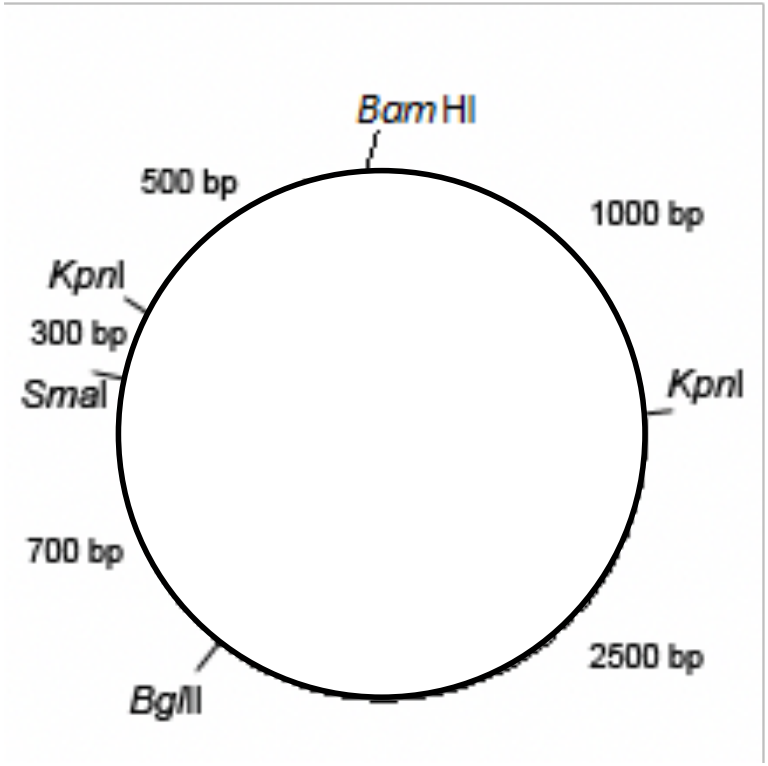
***M1 and M2 are DNA markers.**

Lane	*Digest*	*Size of fragments in bp*
1	Bam*HI* and Sma*I*	*4200, 800*
2	Sma*I* and Kpn*I*	*3200, 1500, 300*
3	Kpn*I* and Bgl*II*	*2500, 1500, 1000*
4	Bam*HI* and Kpn*I*	*3500, 1000, 500*
5	Kpn*I*	*3500, 1500*
6	Bgl*II* and Bam*HI*	*3500, 1500*

Sample question taken from MIT OpenCourseware: 7-01sc-fundamentals-of-biology-fall-2011

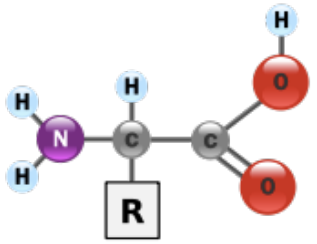
Homework Question 4 - solution

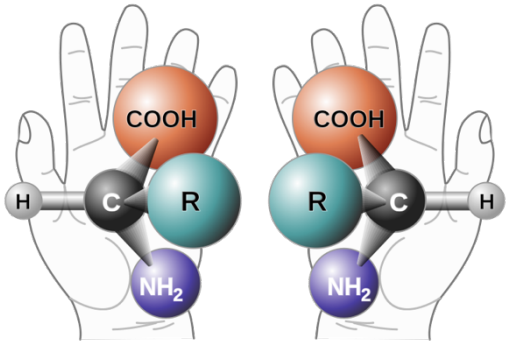
- Total length ~5kb



Amino acids

- Basic component of proteins
  - Amine group
  - Carboxylic acid group
  - Side chain (R)
- They are chiral (handed-ness)
  - The body only uses the L-amino acid

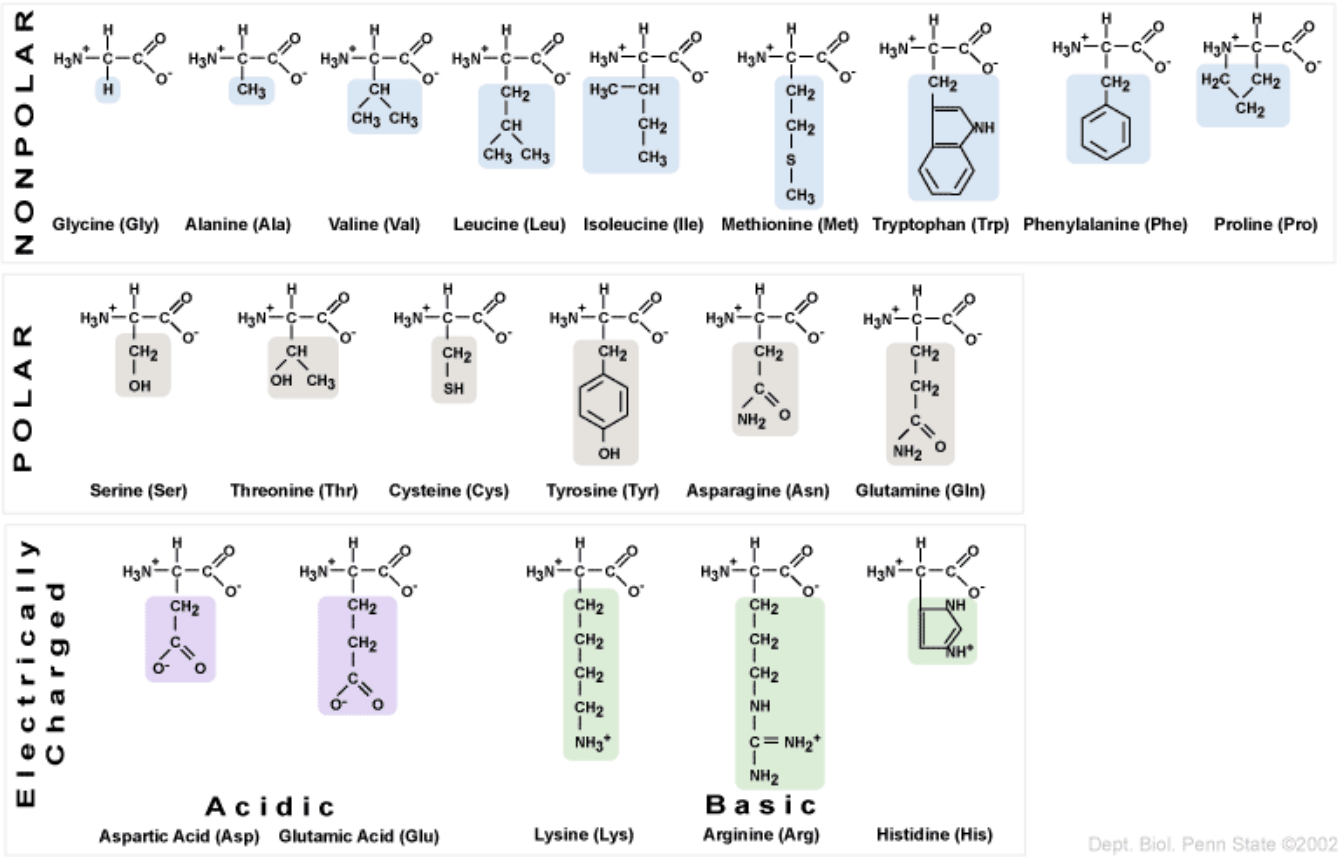




https://upload.wikimedia.org/wikipedia/commons/thumb/e/e8/Chirality_with_hands.svg/765px-Chirality_with_hands.svg.png

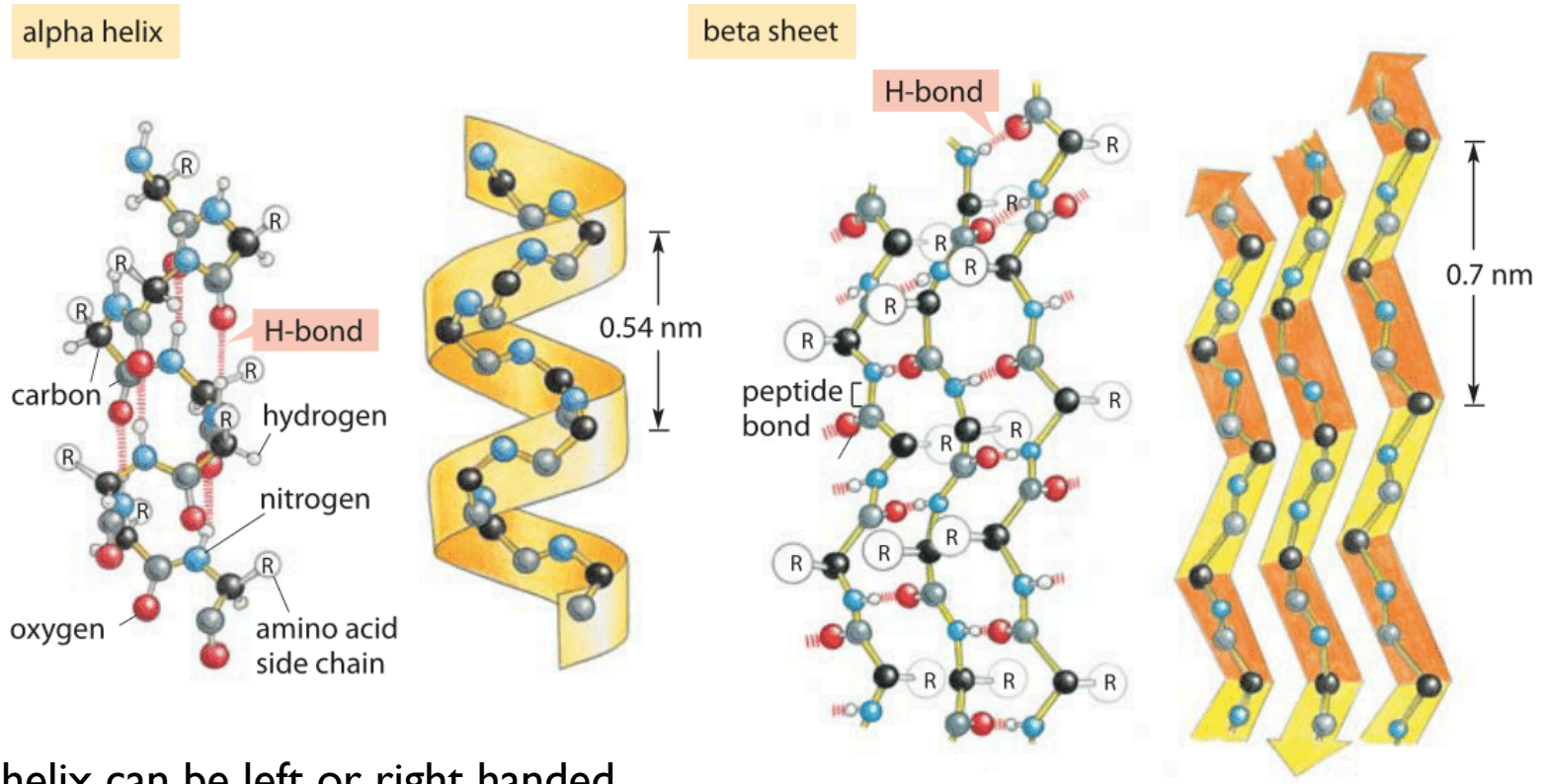
Amino acids

- There are 20 different side-chains



http://www.personal.psu.edu/staff/m/b/mbt102/bisci4online/chemistry/chemistry8.htm

Protein folding – Secondary structures/motifs



- Alpha helix can be left or right handed
- Common in DNA-binding/recognition domains
- Common for lipid-membrane spanning domains
- Common when structure requires elasticity

Chromatography

Type of Chromatography	Separates Proteins By	Bind With	Elute With
Affinity	A specific interaction	No competing ligand	Competing ligand (specific); conditions that disrupt protein/protein interactions (non-specific)
Ion Exchange	Net surface charge	Low ionic strength	High ionic strength; Increased (cation exchange) or decreased (anion exchange) pH
Hydrophobic Interaction	Hydrophobicity	High ionic strength	Low ionic strength
Size Exclusion	Hydrodynamic radius		

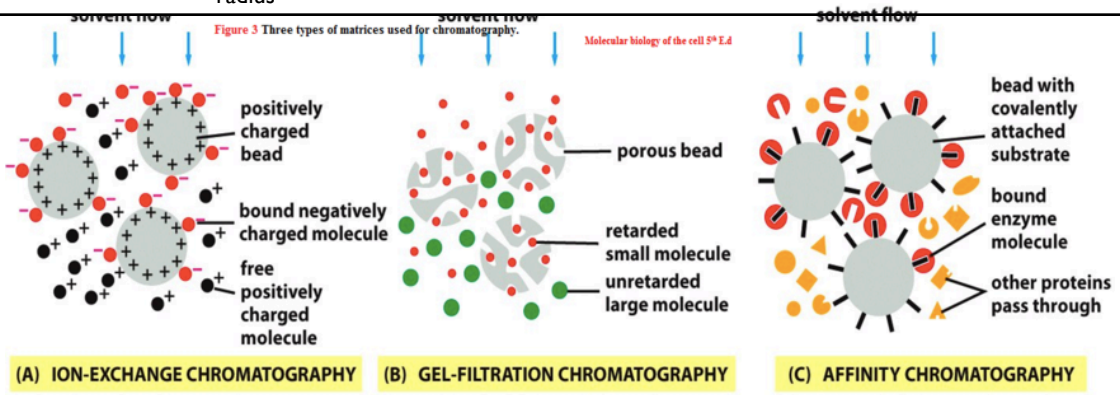
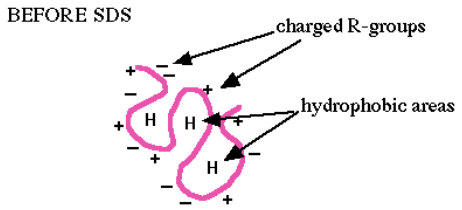


Figure 3 Three types of matrices used for chromatography. Molecular biology of the cell 5th Ed

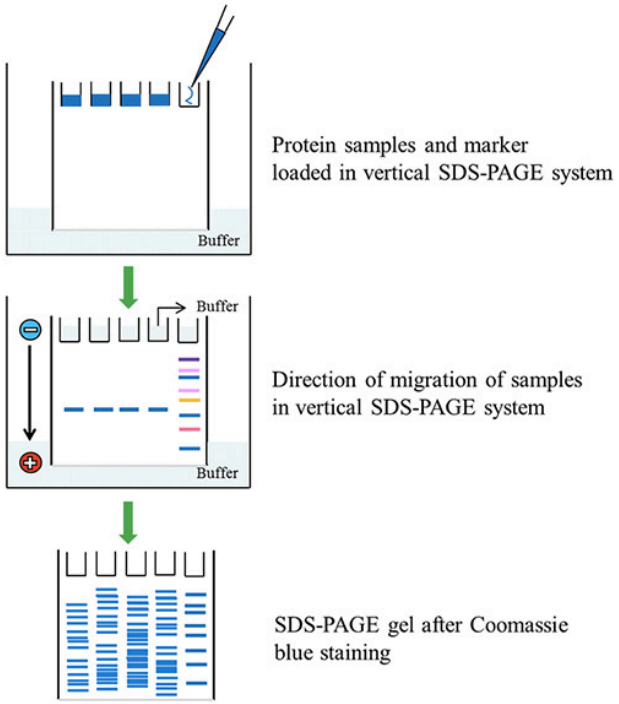
Alberts et al., Molecular Biology of the Cell, 5th Ed.

Protein separation



- **SDS-PAGE**
  - SDS – **S**odium **D**odecyl **S**ulfate
  - PAGE – **P**oly**A**crylamide **G**el **E**lectrophoresis
- SDS unravels the protein into its peptide chain (linearize/denature)
- PAGE separates the proteins based on their mobility in the gel
  - Mobility is determined by size, charge, conformation
  - SDS removes/minimizes charge and conformation contribution, allowing separation by only size
- Idea and setup is similar to DNA gel electrophoresis

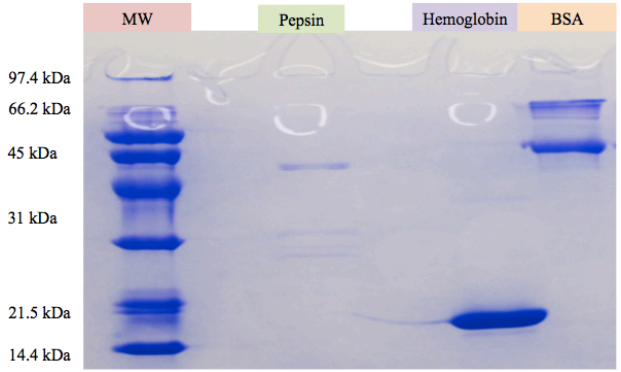
Protein separation



Protein samples and marker loaded in vertical SDS-PAGE system

Direction of migration of samples in vertical SDS-PAGE system

SDS-PAGE gel after Coomassie blue staining

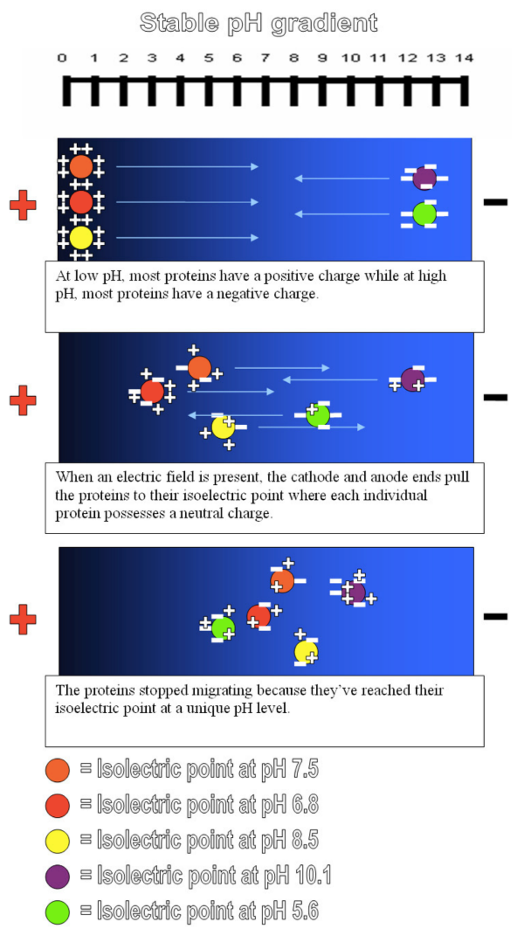


http://www.sigmaaldrich.com/technical-documents/articles/biology/sds-page.html

Protein separation

- **IEF,** or **electrofocusing**
  - **I**so**E**lectric **F**ocusing
- **Isoelectric point (pI)** is the pH at which a particular molecular (i.e. amino acid or protein) carries NO CHARGE
- Different from the SDS-PAGE that just has a charge gradient, IEF requires a pH gradient as well

https://youtu.be/9jW8n1Allc



VIRUSES, VIRAL VECTORS, AND GENE TRANSFER

What are viruses? How do they cause infection and disease? How do we harness them in biology?

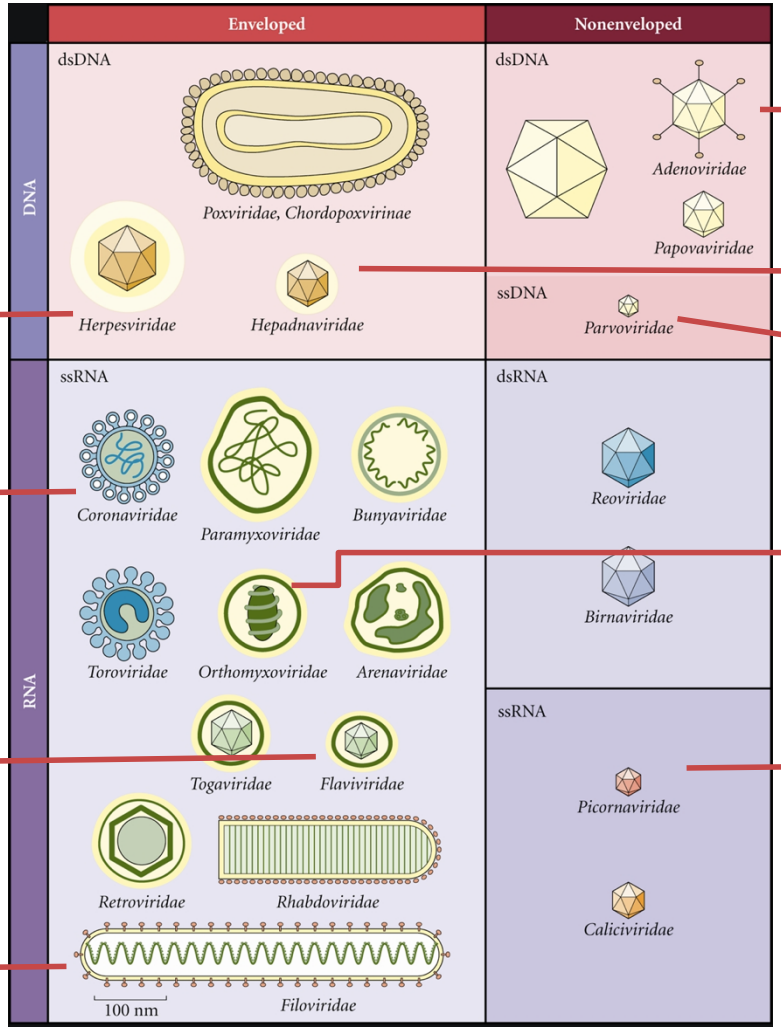
Viruses are extremely diverse

Oral/genital herpes ("cold sores"); Burkitt's Lymphoma; Mononucleosis (mono/"kissing disease")

SARS, MERS

Zika virus

Ebola (ebolavirus)



Cold-like symptoms; pneumonia; bronchitis

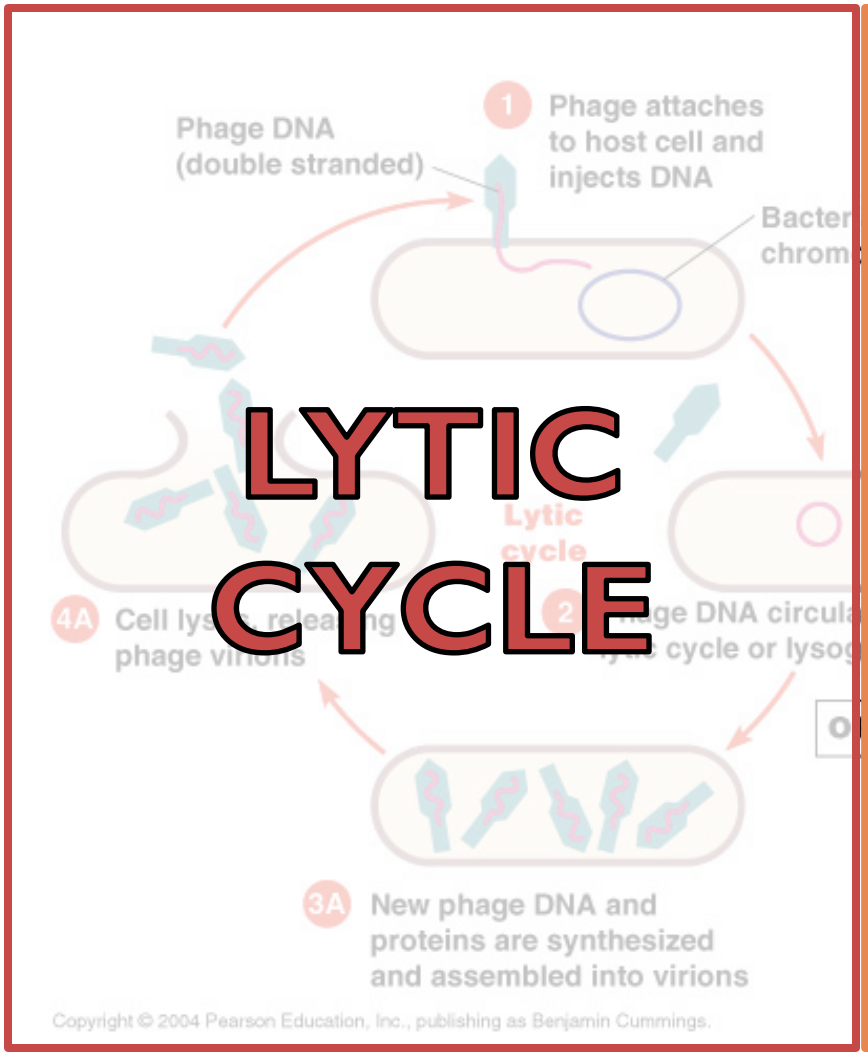
Hepatitis B

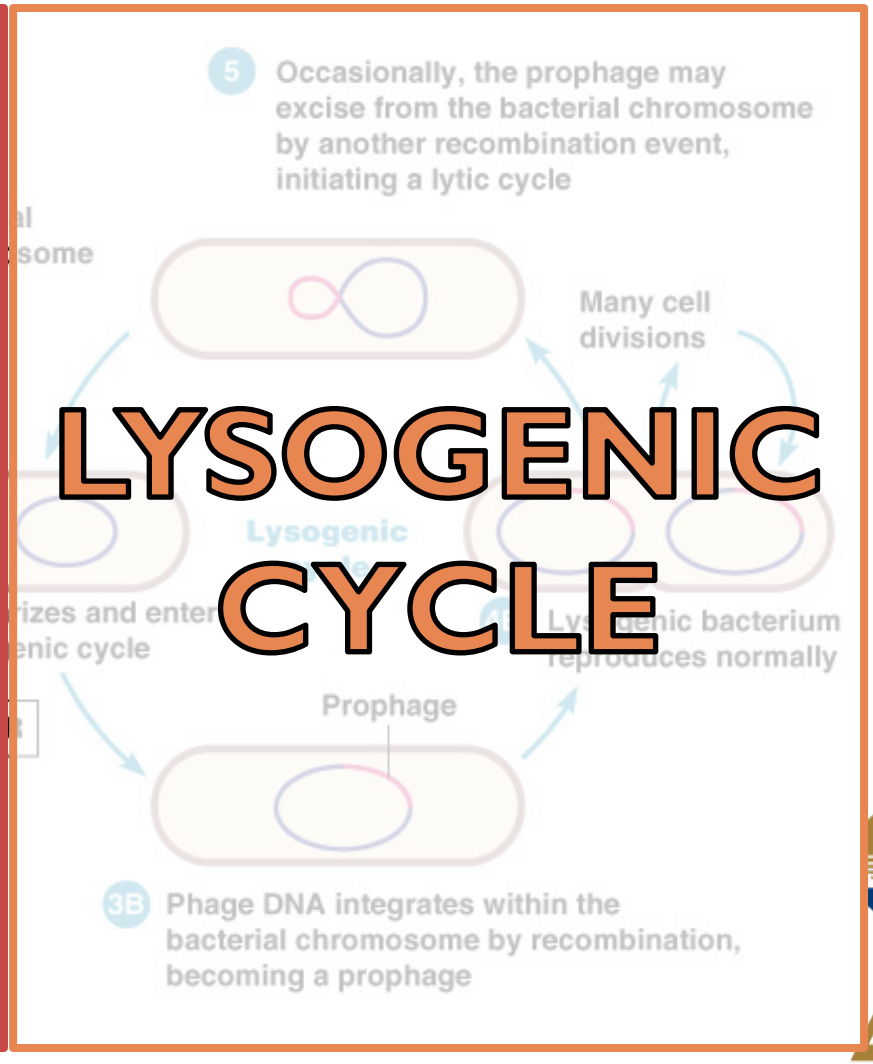
Very deadly for dogs

Influenza (the flu), including swine flu, chicken flu

Common cold (Rhinovirus)

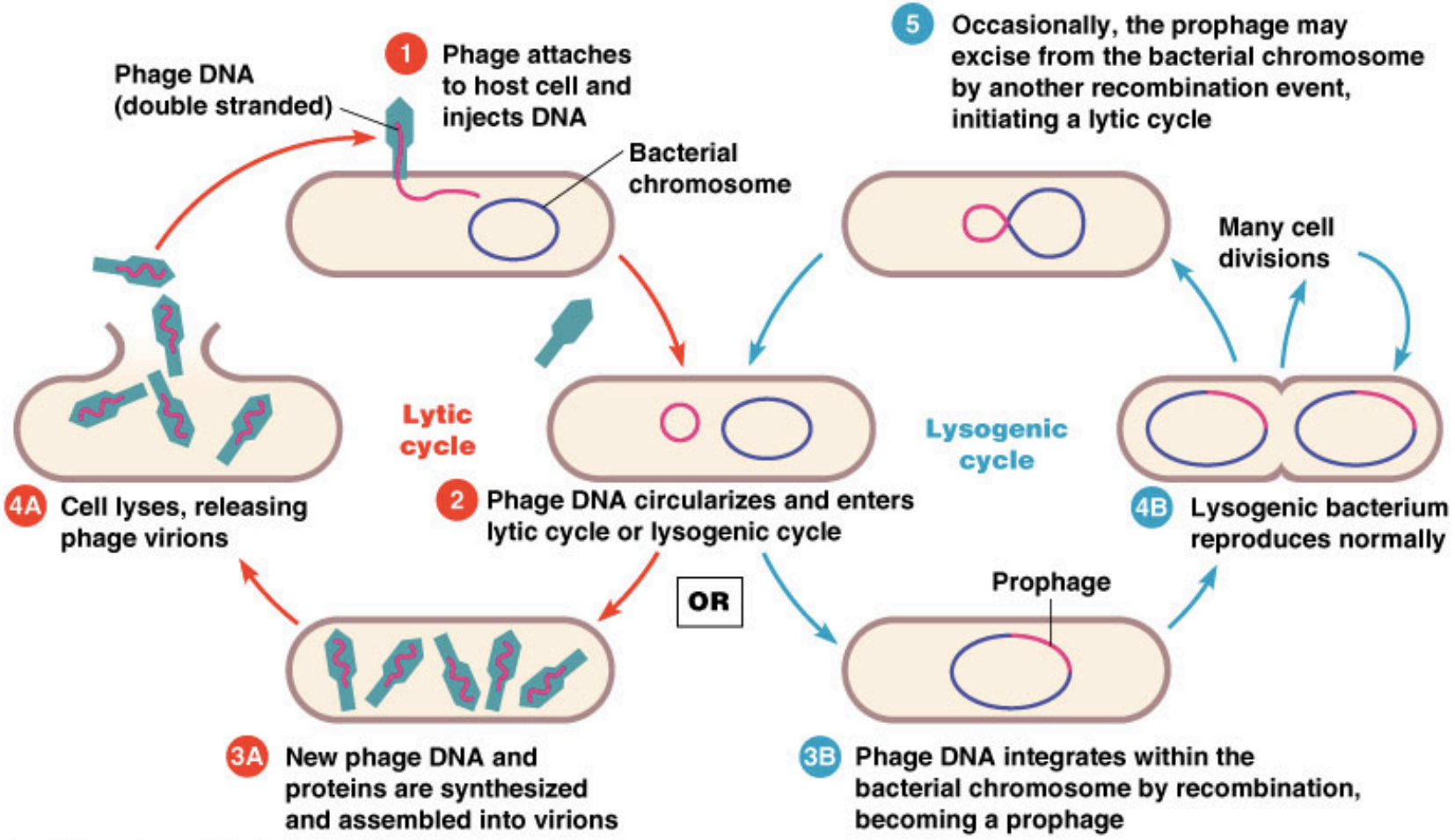
The life cycle of viruses



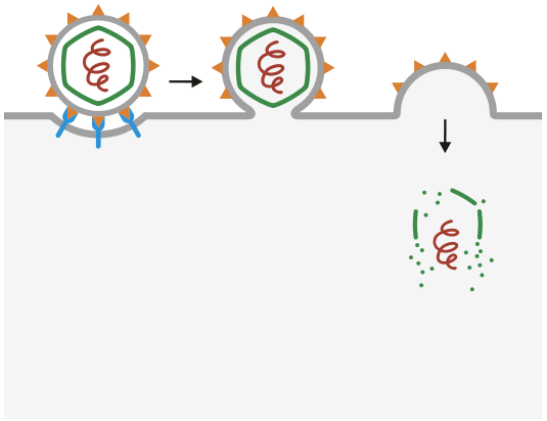


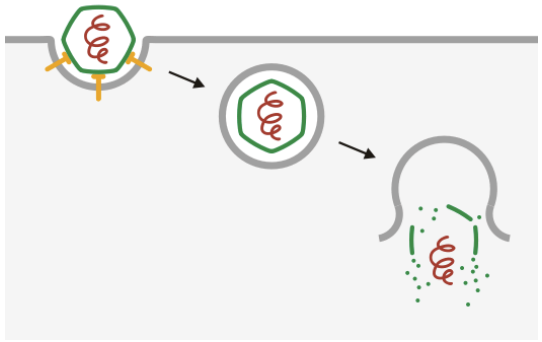
NOTE: this uses bacteria and bacteriophage (virus that infects bacteria) as example

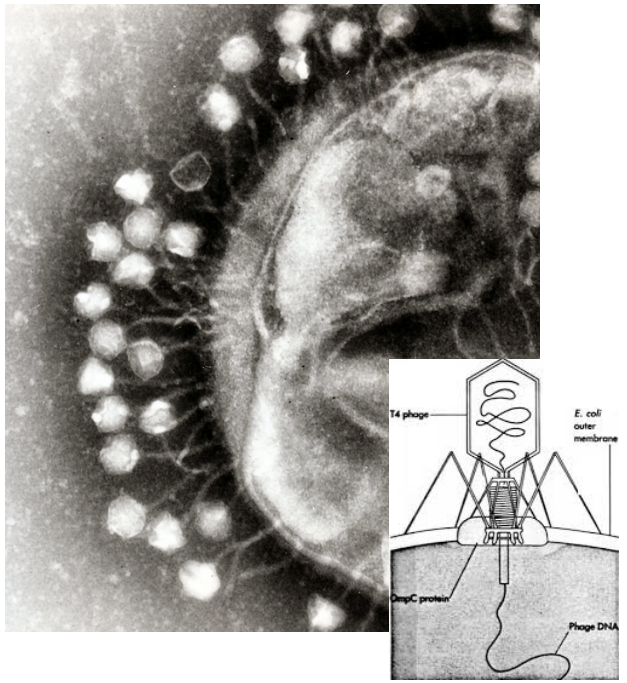
The life cycle of viruses



Attachment and entry







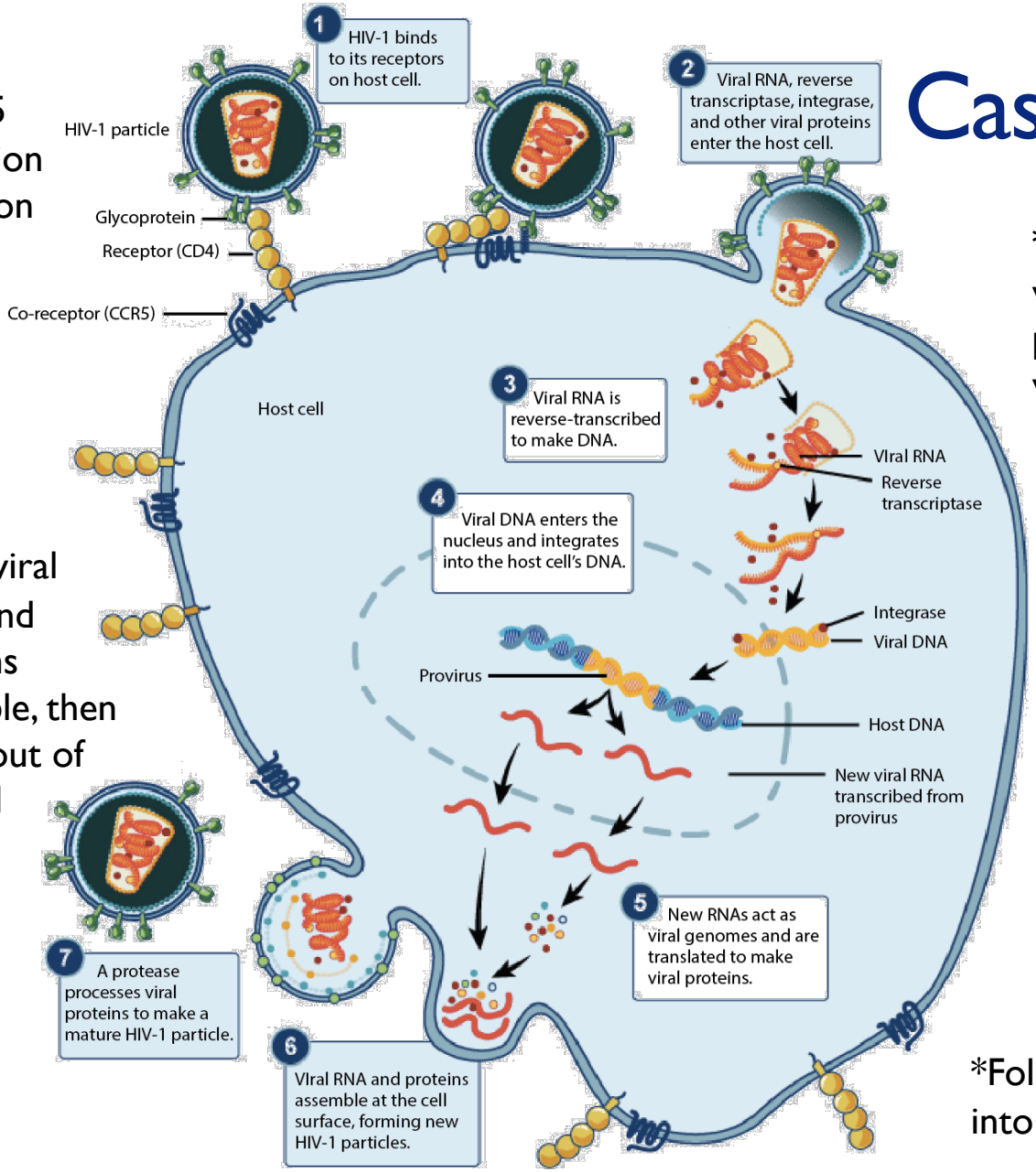
Membrane Fusion
- Viruses with envelope, infecting cells with a lipid bilayer membrane
- Bilayer membrane of virus is same as cell
- Needs receptors

Endocytosis
- Must have the right surface receptors
- Virus carried in by vesicle/endosome to the nucleus

Genetic Injection
- Bacteriophage infecting a bacteria
- Genetic material gets pooped into the bacteria
- Very high speed of injection!

Case study: HIV

*CCR5 reception mutation



*New viral RNA and proteins assemble, then break out of the cell

*The genetic material of the virus encodes for many proteins necessary for the virus to survive and replicate

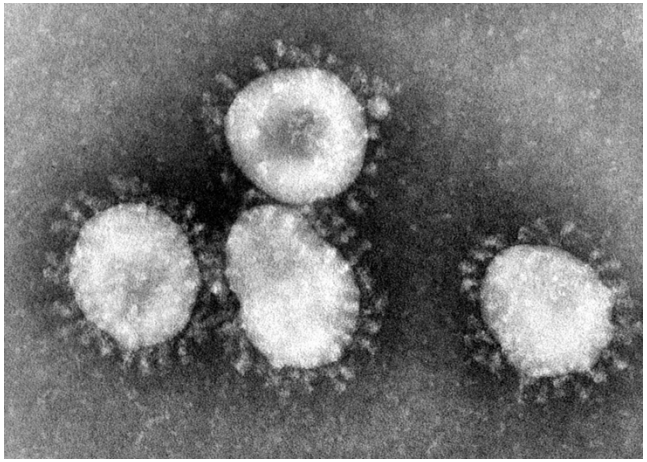
*HIV has reverse transcriptase

*Not all viruses integrate into the genome – integrase needed

*New viral RNA is transcribed from the provirus, by host polymerases!

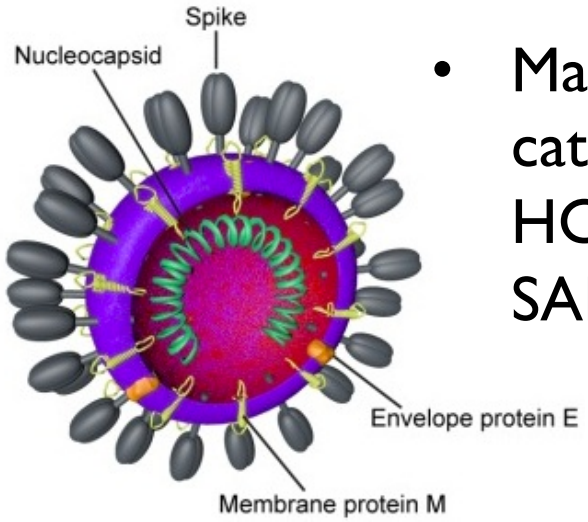
*Followed by translation into viral proteins

Case study: Coronavirus



EM image; By CDC/Dr. Fred Murphy

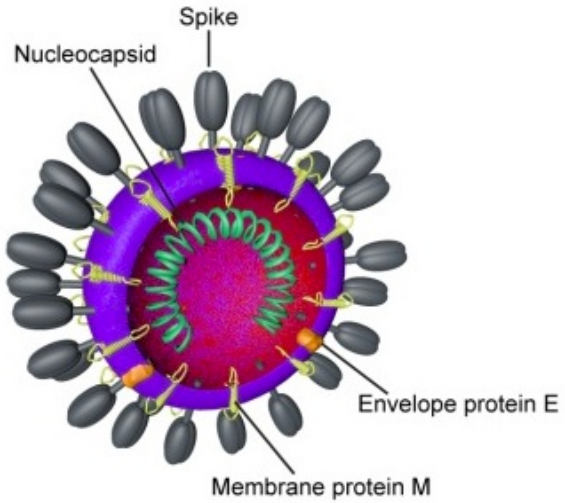
- **Enveloped, +sense, ssRNA**
- The viral genome is 26–32 kb
- Surface has large (~20 nm) projections ("peplomers"/"spikes")
- Generally infect humans and birds (avian)



Schematic; By Belouzard, et al - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3397359

- Many viruses fall under this category – common cold-causing HCoV-229E; SARS-CoV; MERS; SARS-CoV-2

Case study: Coronavirus

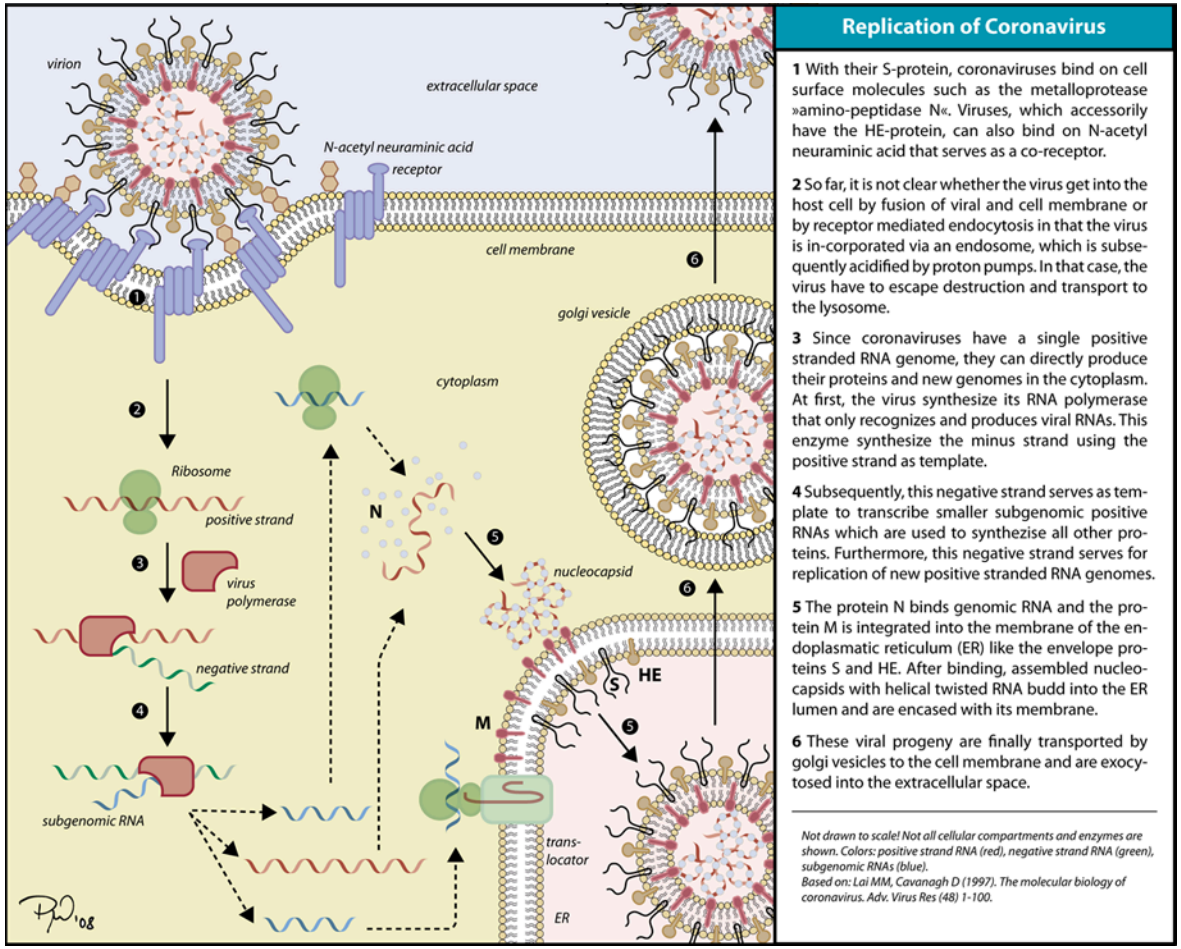


Schematic; By Belouzard, et al - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3397359

- FIVE key proteins are made:
  - S – spike
  - E – small envelope
  - M – membrane
  - N – nucleocapsid
  - *HE – hemagglutinin-esterase (only some subtypes have; it is a spike-like protein)
- The genome also makes some non-structural proteins, e.g.
  - RdRp – RNA-dependent RNA polymerase

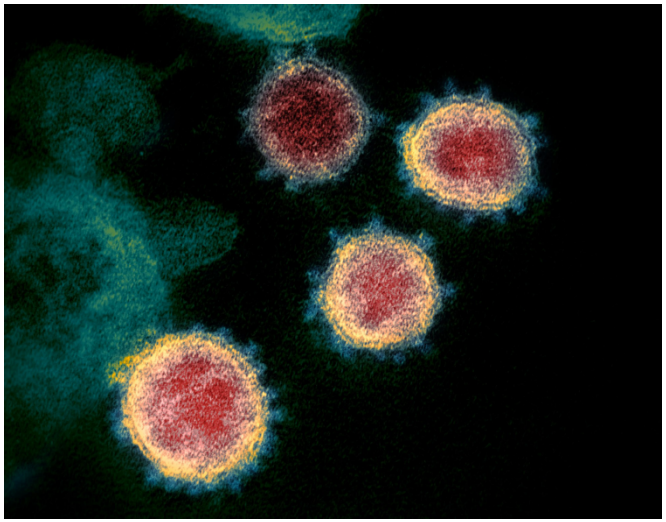
S protein has a domain that facilitates cellular entry by binding with cellular receptors

Case study: Coronavirus



By Crenim at English Wikipedia, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=26529404

Case study: SARS-CoV and SARS-CoV-2



SARS-nCoV-2 EM image; By NIAID Rocky Mountain Laboratories (RML), U.S. NIH

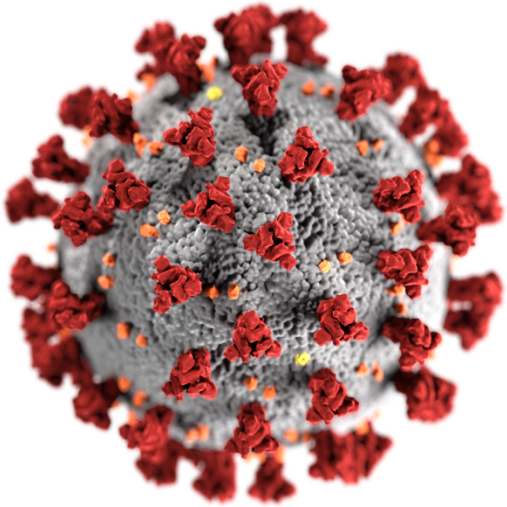
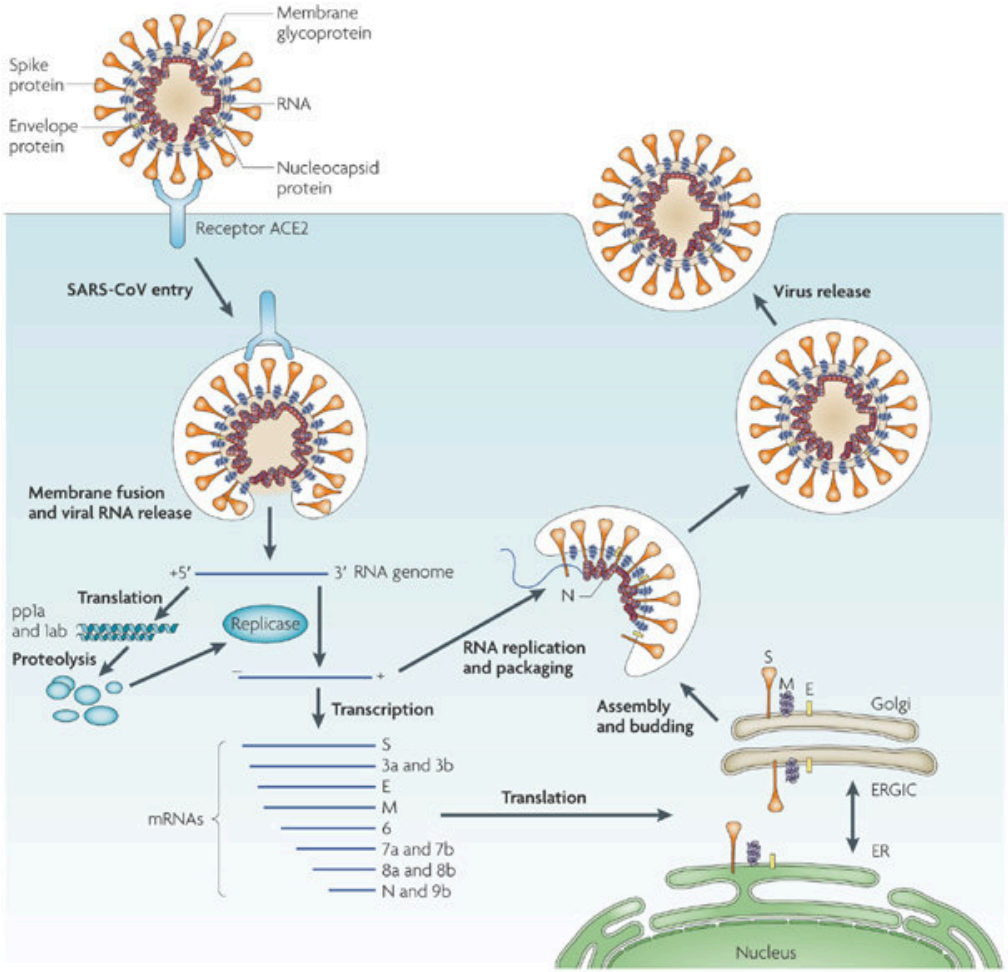


Illustration of SARS-nCoV-2 virion; By CDC/ Alissa Eckert, MS; Dan Higgins, MAM

Side note: COVID-19 is the name of the disease caused by the SARS-CoV-2 virus

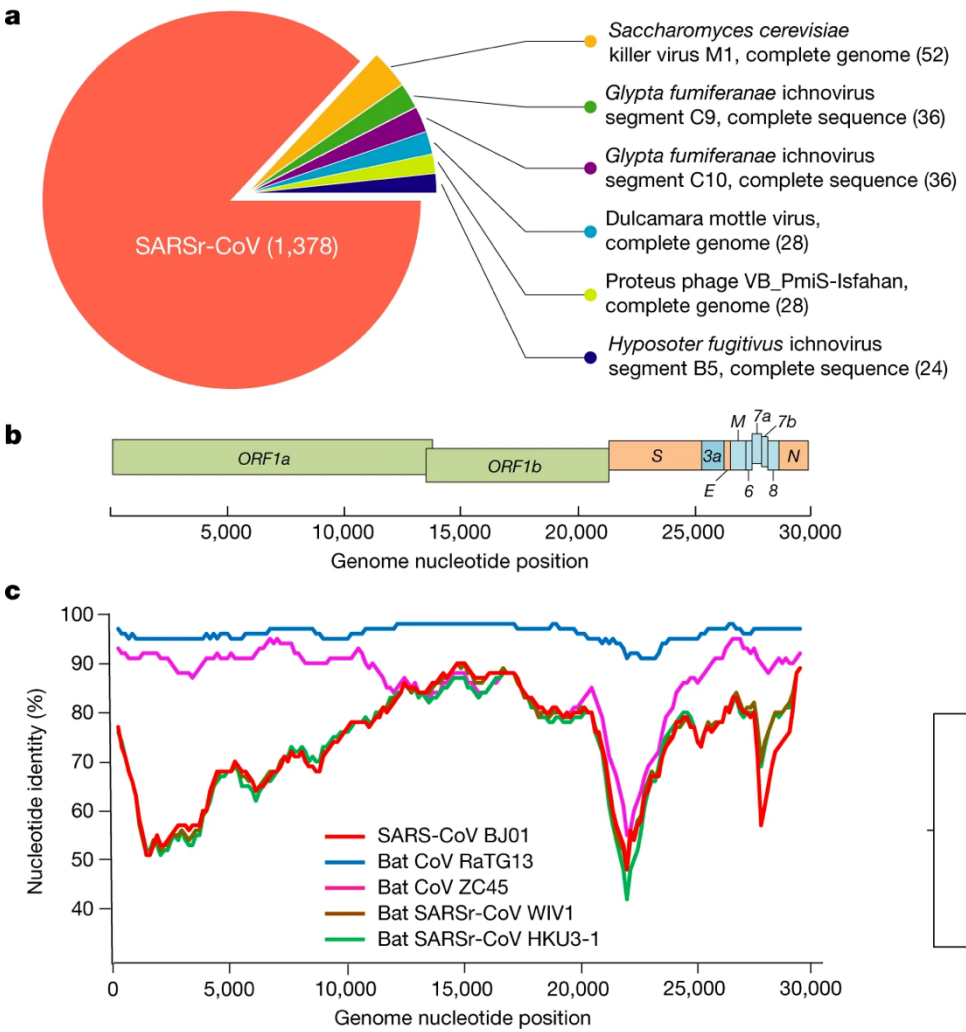
- CoV-2 has 96% sequence similarity to a bat coronavirus; widely suspected to originate from bats
- Primary receptor for both SARS-CoV and SARS-CoV-2 is angiotensin-converting enzyme 2 (ACE2)
- ACE2 is found in: lung, gastrointestinal tract, heart, kidneys

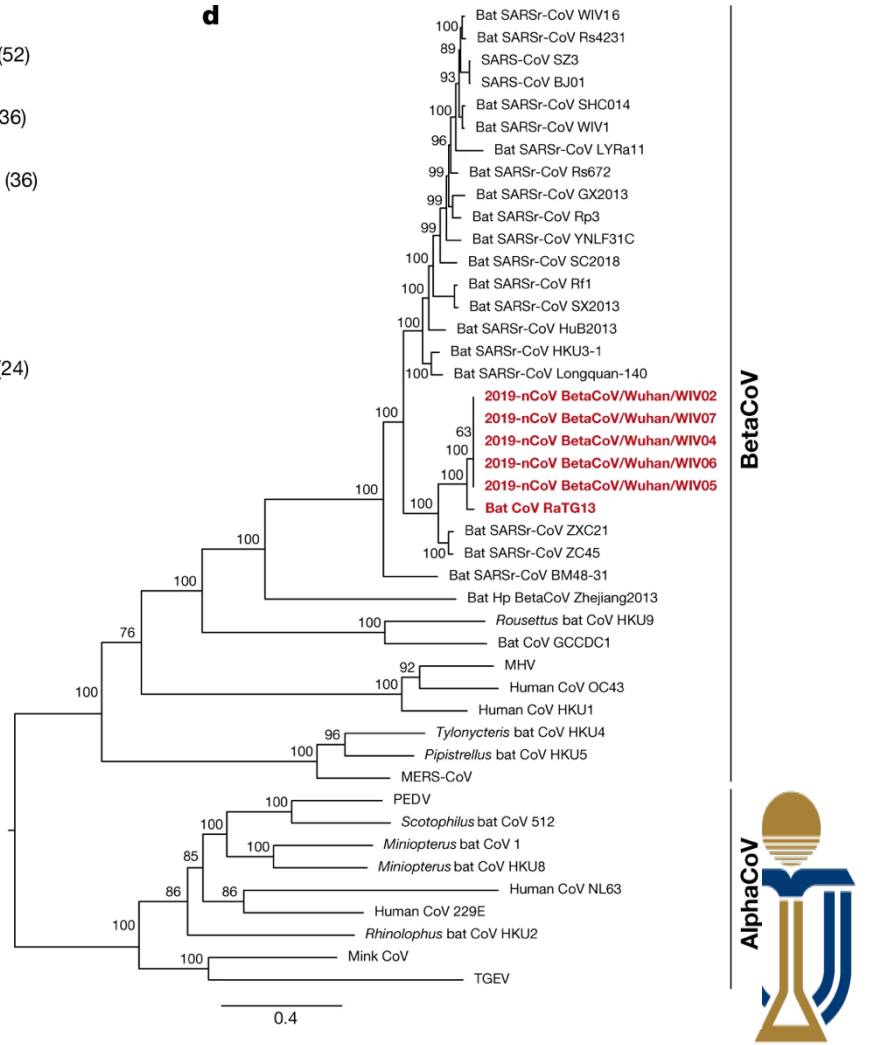
Case study: SARS-CoV and SARS-CoV-2



Du et al., Nature reviews. Microbiology. 7. 226-36. 10.1038/nrmicro2090.

Case study: SARS-CoV and SARS-CoV-2





Further reading

- A discussion forum for analysis and interpretation of virus molecular evolution and epidemiology (COVID19 tag): http://virological.org/c/novel-2019-coronavirus/ncov-2019-evolutionary-history/35
- *SARS-Coronavirus* ancestor's foot-prints in South-East Asian bat colonies and the refuge theory: https://www.sciencedirect.com/science/article/pii/S1567134811002346?via%3Dihub
- Evolutionary Relationships between Bat Coronaviruses and Their Hosts: https://wwwnc.cdc.gov/eid/article/13/10/07-0448_article
- Good article for lay-person: Bats Carry Many Viruses. So Why Don't They Get Sick? https://www.npr.org/sections/goatsandsoda/2020/02/09/803543244/bats-carry-many-viruses-so-why-dont-they-get-sick
- SARS-CoV-2 sequence similarity to bat coronavirus: https://www.nature.com/articles/s41586-020-2012-7
- Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding: https://www.sciencedirect.com/science/article/pii/S0140673620302518?via%3Dihub
- Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses: https://www.nature.com/articles/s41564-020-0688-y
- Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation: https://science.sciencemag.org/content/early/2020/02/19/science.abb2507

Treatments

- Anti-virals
  - Target before entry
  - Target replication/transcription
  - Target viral particle assembly
- Stimulate the immune system
  - Interferons – they call over immune cells to attack, eat, and kill virus infected cells
  - Antibodies – neutralize the viral particles that get released, so that it doesn't infect more cells, and maybe helps with being less contagious, and also signals for immune cells to destroy the viruses (by eating it)
- Resistance to treatment
  - Rate of viral evolution: some viruses get one or more points mutations per genome per round of replication!

Discussion

- What conditions favor the inactivation of this virus? Think about what you can do to prevent infection – why do they work?

- Lopinavir/ritonavir is the current preferred treatment for HIV/AIDS. It is a nucleoside analog.
- Remdesivir is a drug being developed and tested for Ebola and Marburg virus (Gilead). It is also a nucleotide analog.
- Both of these are currently being explored as promising treatments for COVID-19. **Why might these drugs be effective?**

- What are the challenges of diagnosing/detecting this virus?
  - Think about the samples that can be taken…

There will now be a short intermission…

http://stories.barkpost.com/wp-content/uploads/2013/05/sleepingpuppy4.jpg

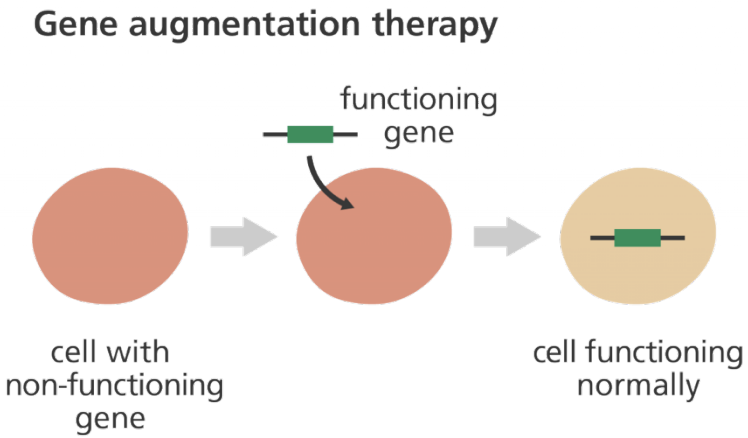
What is gene therapy?

1. Introduce a plasmid into the cell nucleus to replace missing or defective gene (GAT – gene augmentation therapy)
2. Introduce a plasmid into the cell nucleus to provide a new, beneficial protein (e.g. cancer-specific antibodies)
3. Inactivate or knock down a mutated gene by RNA interference
4. Replace the defective gene by genome editing

Remember that modifications to the genome can be either:
HERITABLE, if changes are made to the germline cells (sperm/egg); or
NOT INHERITABLE, if changes are made only to somatic cells

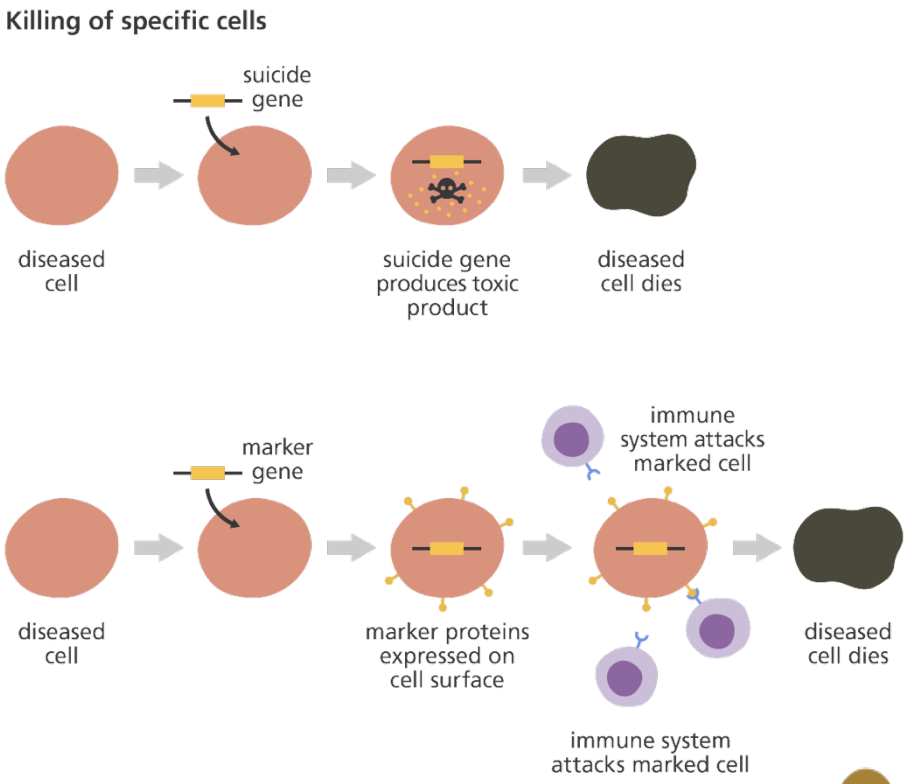
Gene augmentation: Fixing a defective gene

- We discussed how plasmids can be introduced into a cell
- Plasmid → mRNA → protein
- The goal is to have the new gene consistently being expressed, so:
  - Plasmid has to make it to the nucleus
  - Plasmid must contain necessary components to transcribe into mRNA
  - Plasmid needs to be replicated when the cell divides! (origin of replication)



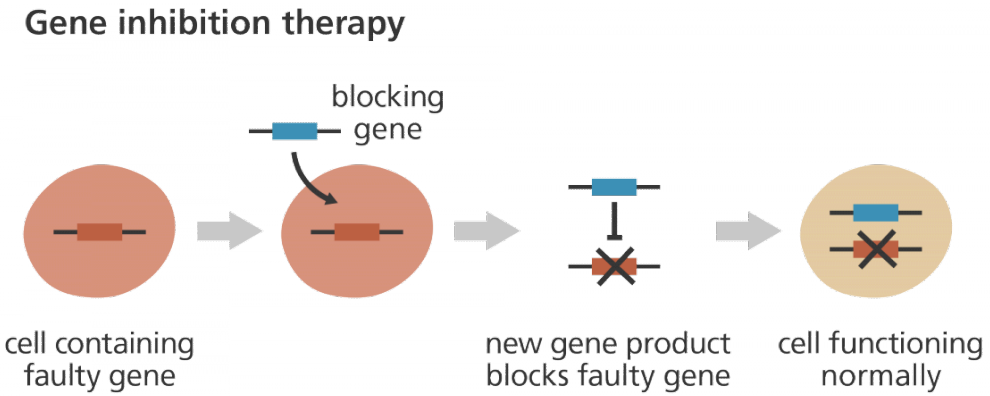
Gene augmentation: Targeted cell killing

- Adding genes that encode for toxins or "suicide" protein
- Adding genes that make the expressing cell more sensitive to a specific drug
- Adding genes that get expressed at the cell surface and induce immune response to kill the target cell

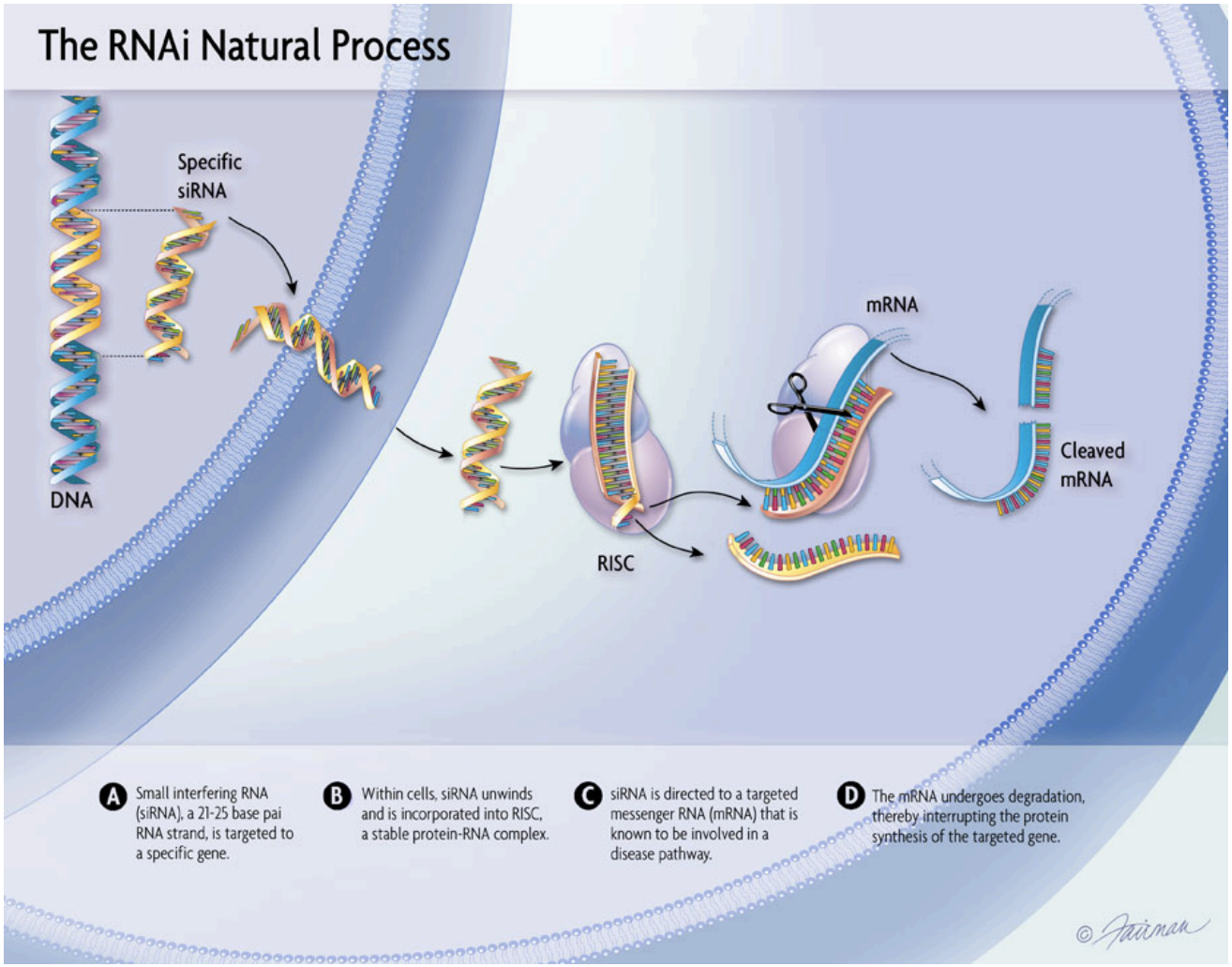


Inhibition of gene expression

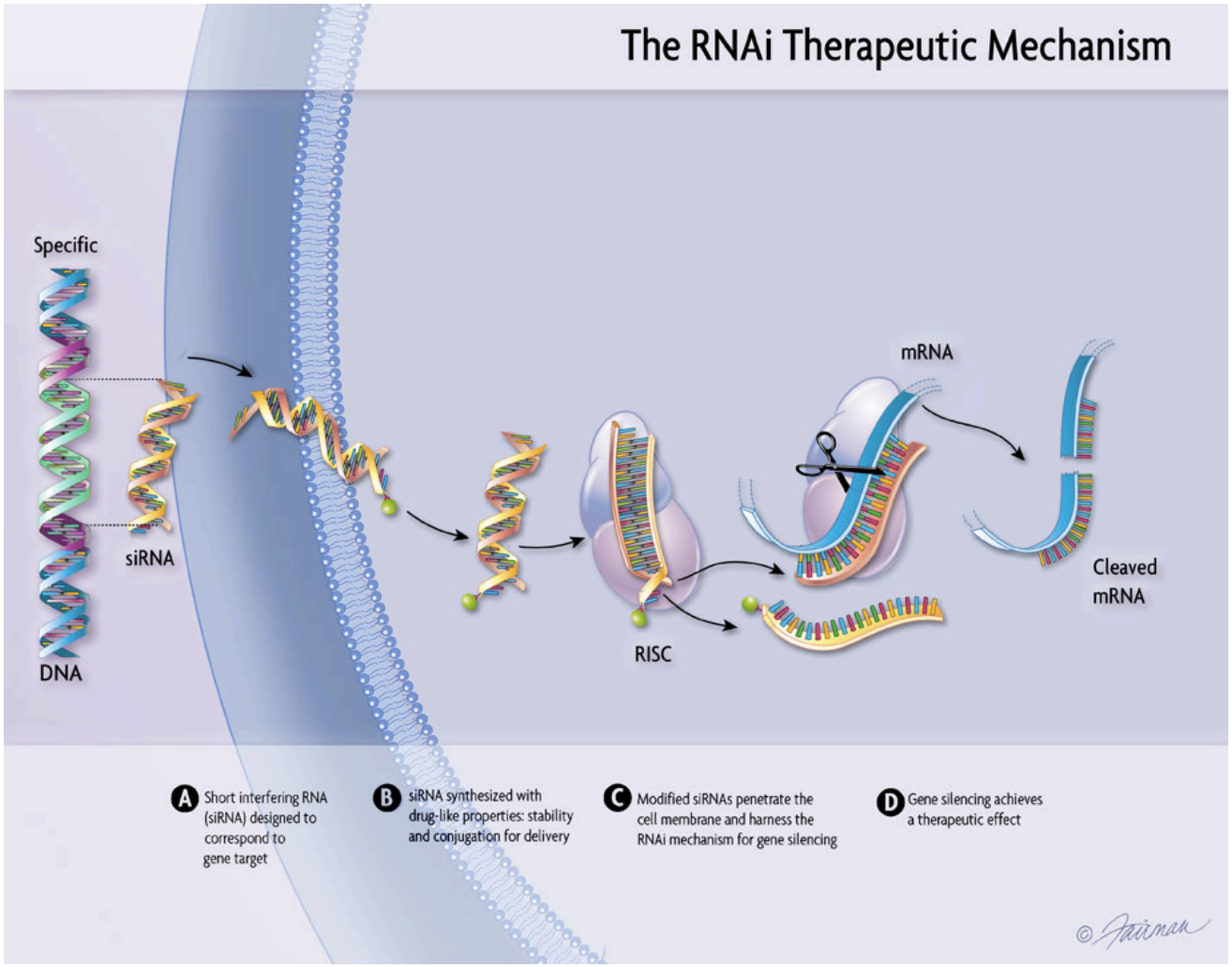
- New gene makes a protein that blocks/inhibits, or breaks down the faulty gene
- New gene makes small interfering RNA (siRNA) that causes the target mRNA to be degraded (RNA interference, or RNAi)



RNA interference (RNAi)

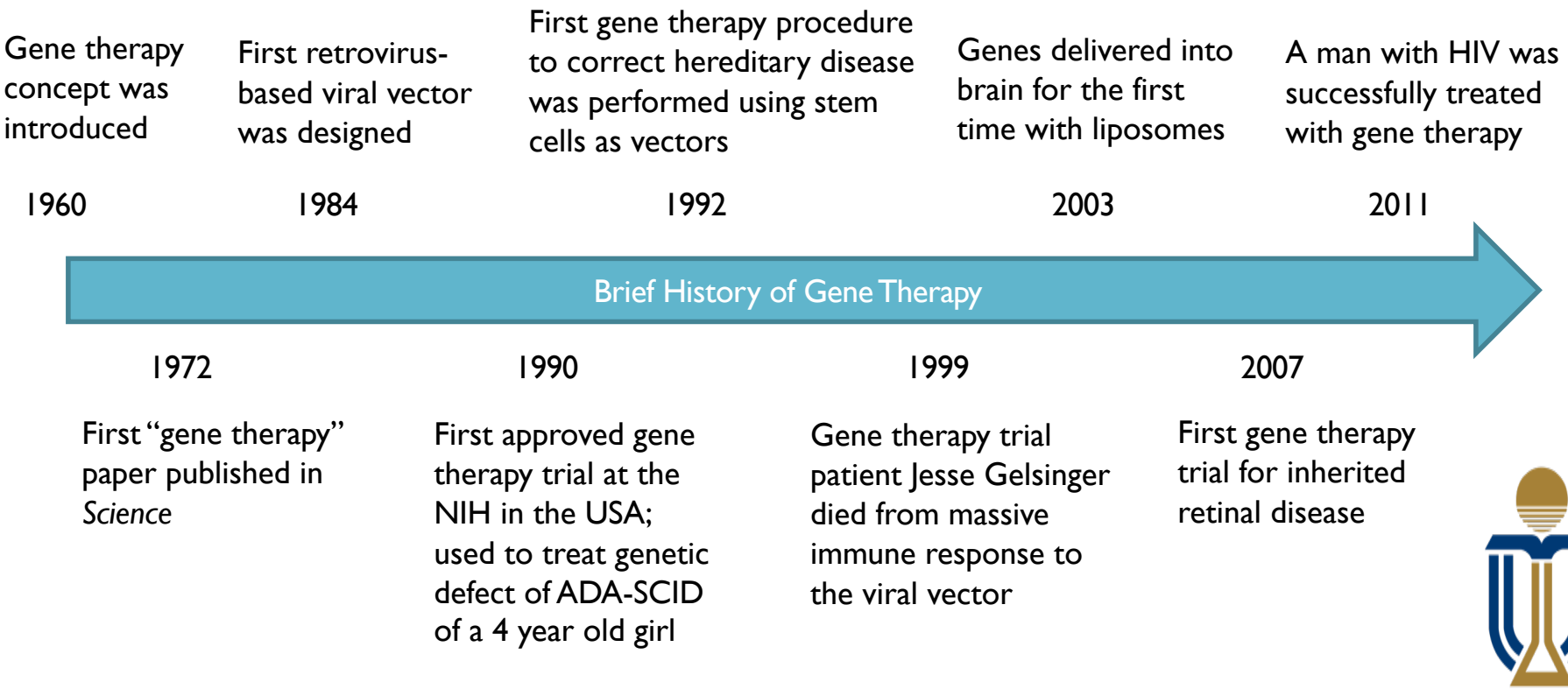


RNA interference (RNAi)



A brief history of gene therapy

- If gene therapy is so promising, and we have molecular biology tools to apply it, why is it not more prominently used today?



The bubble girl (1990)

This is David Vetter, who also had SCID. He wore a special 'spacesuit' to protect him from infections. Image credit: NASA Johnson Space Center

- Ashanthi, a 4 y.o. girl, with ADA-SCID
  - A form of severe combined immuno-deficiency caused by lack of adenosine deaminase (ADA) enzyme
  - Body cannot make any white cells
- A good target for gene therapy:
  - Effects of the disease are reversible
  - Disease results from loss of function of a single gene
  - ADA levels vary widely in the normal population so tight control of the introduced gene is not important
  - *ADA* gene is very small and easy to manipulate
  - Target cells are lymphocytes which are accessible, easy to grow and easy to put back into the body of a patient
  - Alternative treatments hazardous/non-existent (no marrow donor)

The bubble girl (1990)

- Again using viral vector as delivery
- Ex-vivo procedure
- Gene therapy on Ashanthi was initially successful:
  - Within six months her white blood cell count had risen to normal levels, and over the next two years she continued to improve
  - During trial, she continued receiving ADA supplement to ensure safety, which diminished significance of gene therapy result
  - When ADA supplement was discontinued briefly, her symptoms returned
- Since 2002, new methodology for performing this same treatment was developed and trial patients have seen success
  - Introduced procedure to partially ablate patient's own marrow

Jesse Gelsinger (1999)

- Gelsinger suffered from ornithine transcarbamylase deficiency (OTCD), a genetic disease of the liver
  - Liver cannot metabolize ammonia (byproduct of protein breakdown)
  - Usually fatal at birth, but Gelsinger had a less severe version – some of his cells were normal, enabling him to survive on a restricted diet and special medications
- The gene therapy was delivered using adenoviral vector (AAV), directly injected (in-vivo)
- He died 4 days later from multiple organ failure and brain death, as a massive immune response was triggered by the AAV

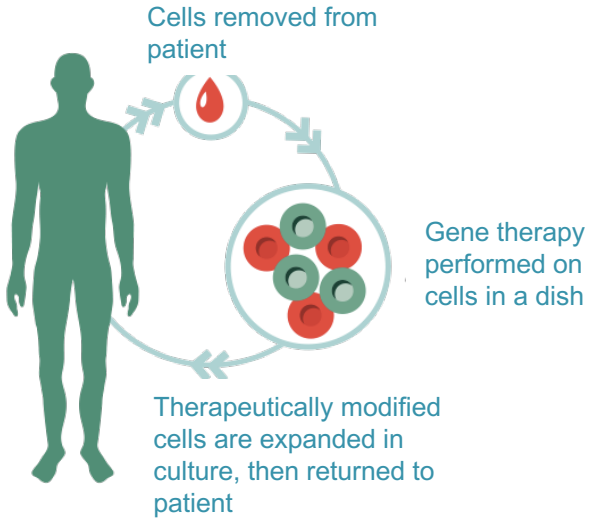
Delivery of therapeutic genes

- https://youtu.be/Ez560GnkSrE
- What?
  - What are the things that need to be delivered? (Single plasmid? Multiple plasmids? Viral vector? RNA? Protein?)
  - What are the cell type(s) it needs to target?
- Where?
  - Where in the body should it be targeted?
  - Where should it absolutely NOT go?
  - Where should the procedure take place, inside or outside the body?
- How?
  - How to deliver the payload? Viral? Non-viral?
  - How to introduce the vector? Injection? Cream? Incubation?

Delivery approaches

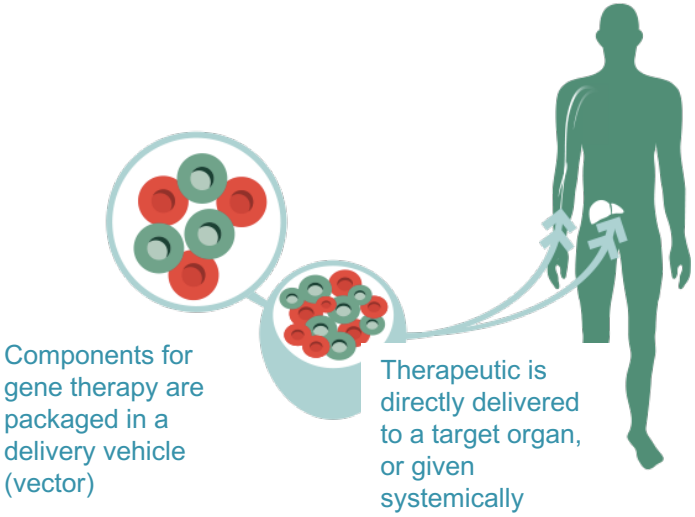
Ex-vivo delivery

- Target cells treated **outside** body
- Reduces safety risk; can screen for tumorigenic cells before giving to patient; but cannot be applied for many cell types



In-vivo delivery

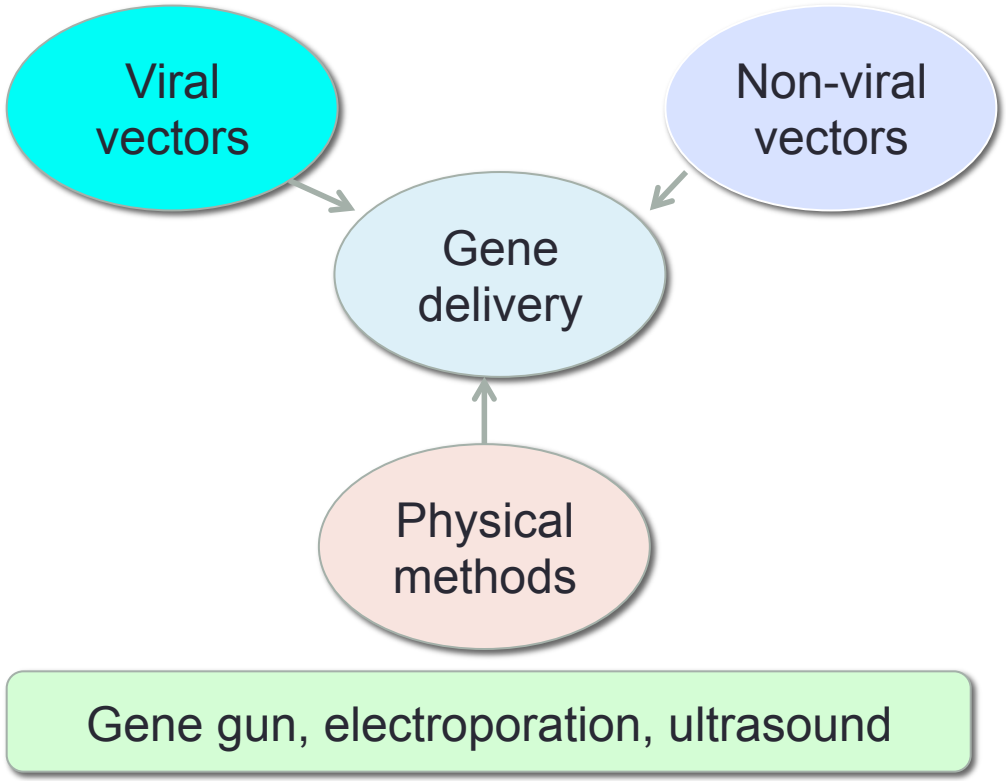
- Target cells treated **inside** body
- Useful if target cells are hard or impossible to culture (e.g. brain); but cell-specific targeting is hard



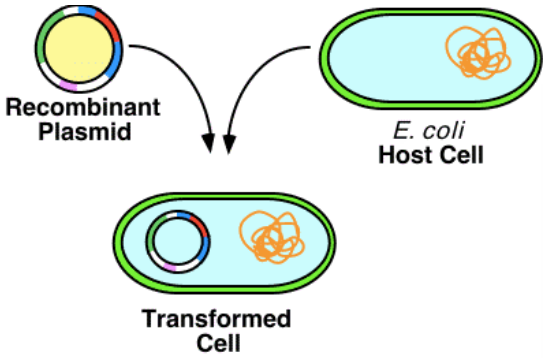
Case study – Leber congenital amaurosis trial, first in-vivo CRISPR treatment

- "AGN-151587 (EDIT-101) is an experimental medicine delivered via sub-retinal injection under development for the treatment of Leber congenital amaurosis 10 (LCA10), an inherited form of blindness caused by mutations in the centrosomal protein 290 (CEP290) gene. The BRILLIANCE clinical trial is a Phase 1/2 study to evaluate AGN-151587 for the treatment of patients diagnosed with LCA10 and is the world's first human study of an in vivo, or inside the body, CRISPR genome editing medicine. The trial will assess the safety, tolerability, and efficacy of AGN-151587 in approximately 18 patients with LCA10."
- "LCA10, is a monogenic disorder caused by mutations in the CEP290 gene and is the cause of disease in approximately 20-30 percent of all LCA patients."
- Uses AAV – adeno-associated virus
- Uses Cas9

Delivery approaches



Delivery vectors

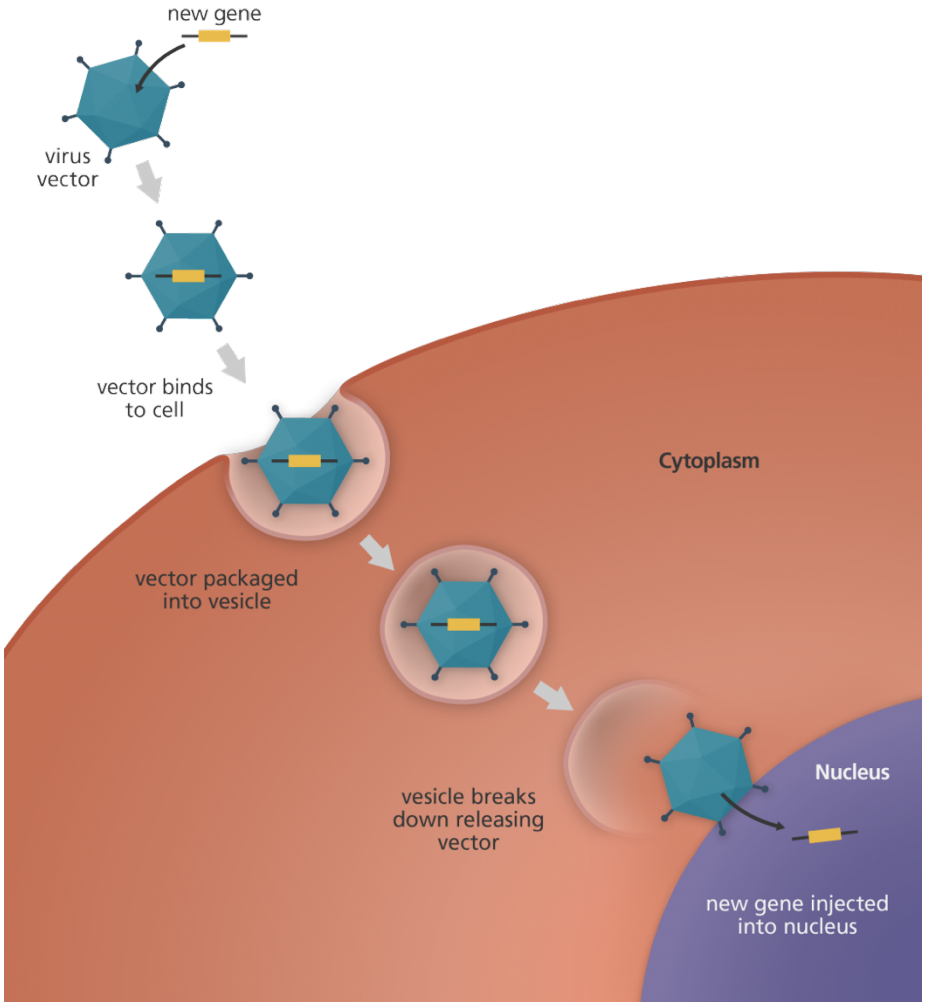


- Criteria of delivery vectors:
  - Target the right cells
  - Able to transfer and integrate genes into cells
  - Minimal harmful side effects
- Examples of types of vectors:

Adenovirus
Retrovirus
Vaccinia virus
Poxvirus
Adeno-associated virus
Herpes simplex virus
Lentivirus

Naked/plasmid DNA (gene gun)
Lipid complex
Liposomes
Peptides/proteins
Polymers
Other non-viral vehicles

Viral vectors



Choosing a viral vector

Table 1 | **The main groups of viral vectors**

Vector	Genetic material	Packaging capacity	Tropism	Inflammatory potential	Vector genome forms	Main limitations	Main advantages
Enveloped							
Retrovirus	RNA	8 kb	Dividing cells only	Low	Integrated	Only transduces dividing cells; integration might induce oncogenesis in some applications	Persistent gene transfer in dividing cells
Lentivirus	RNA	8 kb	Broad	Low	Integrated	Integration might induce oncogenesis in some applications	Persistent gene transfer in most tissues
HSV-1	dsDNA	40 kb* 150 kb‡	Strong for neurons	High	Episomal	Inflammatory; transient transgene expression in cells other than neurons	Large packaging capacity; strong tropism for neurons
Non-enveloped							
AAV	ssDNA	<5 kb	Broad, with the possible exception of haematopoietic cells	Low	Episomal (>90%) Integrated (<10%)	Small packaging capacity	Non-inflammatory; non-pathogenic
Adenovirus	dsDNA	8 kb* 30 kb§	Broad	High	Episomal	Capsid mediates a potent inflammatory response	Extremely efficient transduction of most tissues

*Replication defective. ‡Amplicon. §Helper dependent. AAV, adeno-associated viral vector; dsDNA, double-stranded DNA; HSV-1, herpes simplex virus-1; ssDNA, single-stranded DNA.

Thomas, Ehrhardt & Kay, Nature Reviews Genetics, 2003

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Main application: short term gene expression, for proof of concept studies

Thomas, Ehrhardt & Kay, Nature Reviews Genetics, 2003

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*Replication defective. ‡Amplicon. §Helper dependent. AAV, adeno-asso stranded DNA.

Main application: long term expression of small genes

Note: AAV is not known to cause disease in humans, therefore lower immune risk

Thomas, Ehrhardt & Kay, Nature Reviews Genetics, 2003

Choosing a viral vector

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*Replication defective. ‡Amplicon. §Helper dependent. AAV, adeno-associated viral vector; dsDNA, double-stranded DNA; HSV-1, herpes simplex virus-1; ssDNA, single-stranded DNA.

Main application: long term expression of small and large genes; ex-vivo applications

Note: lentivirus vs retrovirus – dividing cells

Thomas, Ehrhardt & Kay, Nature Reviews Genetics, 2003

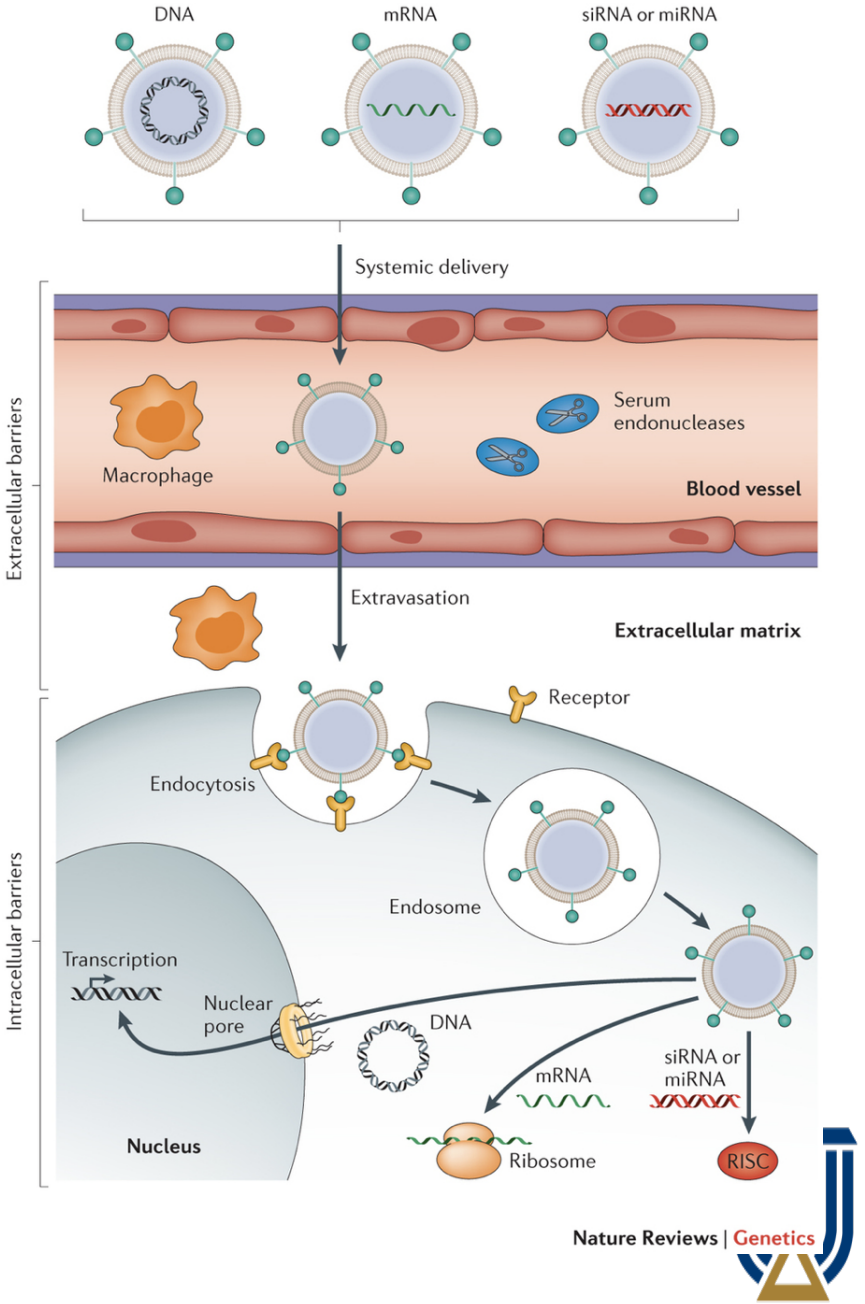
Choosing a viral vector

		Adenovirus	Adeno-associated virus	Alphavirus	Herpesvirus	Retrovirus / Lentivirus	Vaccinia virus
Particle characteristics	Genome	dsDNA	ssDNA	ssRNA (+)	dsDNA	ssRNA (+)	dsDNA
	Capsid	Icosahedral	Icosahedral	Icosahedral	Icosahedral	Icosahedral	Complex
	Coat	Naked	Naked	Enveloped	Enveloped	Enveloped	Enveloped
	Virion polymerase	Negative	Negative	Negative	Negative	Positive	Positive
	Virion diameter	70 - 90 nm	18 - 26 nm	60 - 70 nm	150 - 200nm	80 - 130 nm	170 - 200 X 300 - 450nm
	Genome size	39 - 38 kb	5 kb	12 kb	120 - 200 kb	3 - 9 kb	130 - 280 kb
Gene Therapy Net .com							
	Family	*Adenoviridae*	*Parvoviridae*	*Togaviridae*	*Herpesviridae*	*Retroviridae*	*Poxviridae*
Gene Therapy Properties	Infection / tropism	Dividing and non-diving cells	Dividing and non-diving cells	Dividing and non-diving cells	Dividing and non-diving cells	Dividing cells*	Dividing and non-diving cells
	Host genome interaction	Non-integrating	Non-integrating*	Non-integrating	Non-integrating	Integrating	Non-integrating
	Transgene expression	Transient	Potential long lasting	Transient	Potential long lasting	Long lasting	Transient
	Packaging capacity	7.5 kb	4.5 kb	7.5 kb	> 30 kb	8 kb	25 kb

http://sgugenetics.pbworks.com/f/1301871554/virus%20classification.jpg

Non-viral vectors

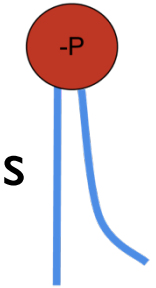
- Non-viral vectors can be used to deliver DNA, mRNA and short double-stranded RNA
  - siRNA and miRNA mimics must be loaded into the RNA-induced silencing complex (RISC)
  - mRNA must bind to the translational machinery
  - DNA has to be further transported to the nucleus to exert its activity



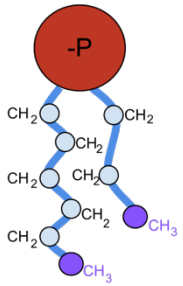
Yin et al., "Non-viral vectors for gene-based delivery", Nature Review Genetics, 2014

Creation of non-viral vectors

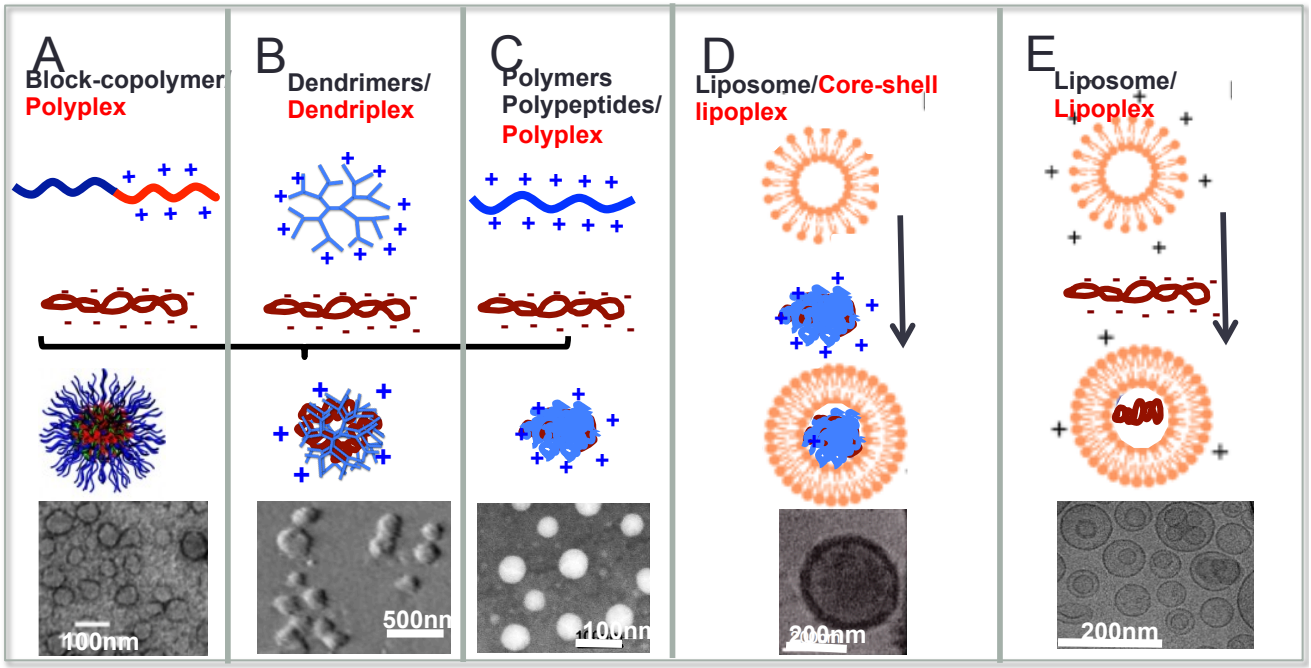
- Non-viral vectors form due to charge interactions
- https://youtu.be/RBjWwlnq3cA?t=10s
- https://youtu.be/04SP8Tw3htE?t=2m10s



A phospholipid with a hydrophilic head and a hydrophobic tail



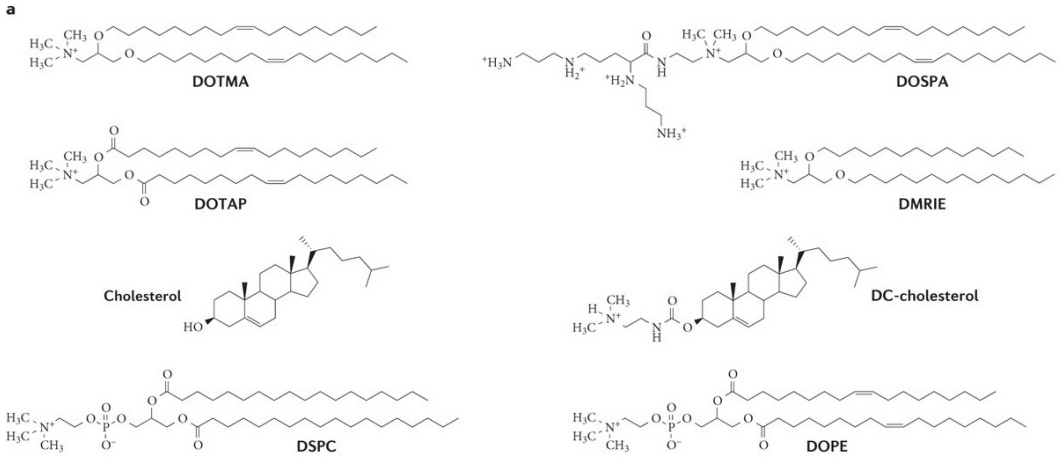
Chemical makeup of a single phospholipid

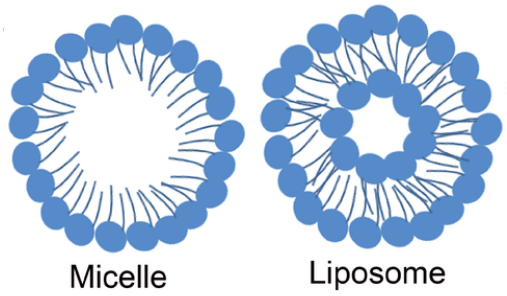


Lipoplex; liposome; lipid nanoparticle; micelle

Lipid-based vectors

- Lipid-based vectors are among the most widely used non-viral gene carriers.
- Limitations of cationic lipids include low efficacy (poor stability and rapid clearance), and tendency to generate inflammatory or anti-inflammatory responses





Yin et al., "Non-viral vectors for gene-based delivery", Nature Review Genetics, 2014
Wang et al, "Lipid Nanoparticles for Ocular Gene Delivery", J. Funct. Biomater. 2015

Polymeric vectors

- Cationic polymers are attractive due to their immense chemical diversity and potential for functionalization

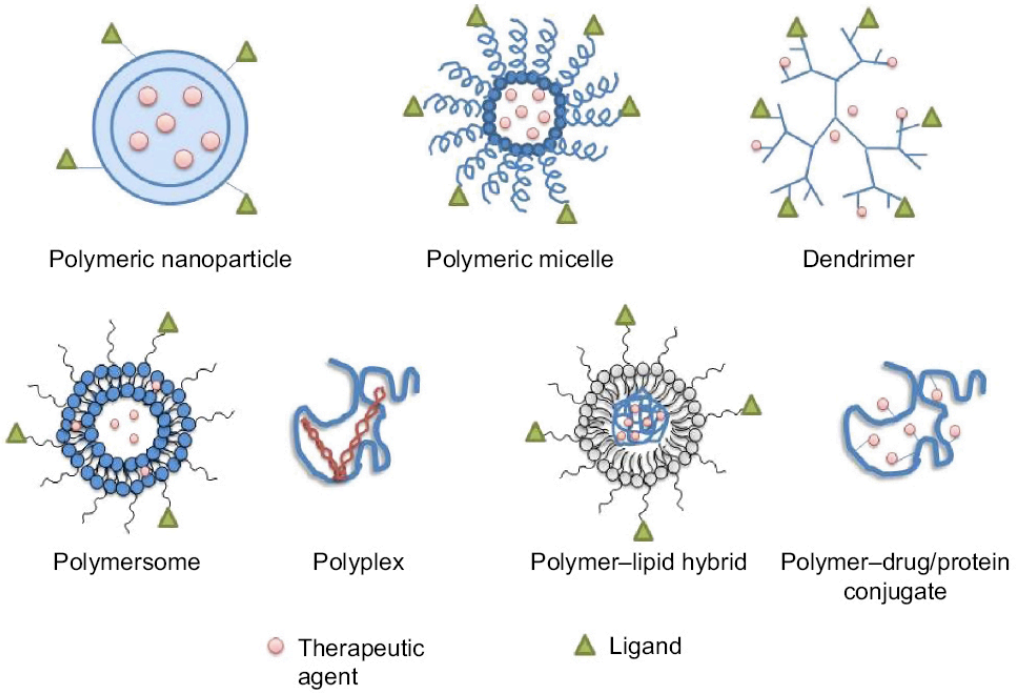
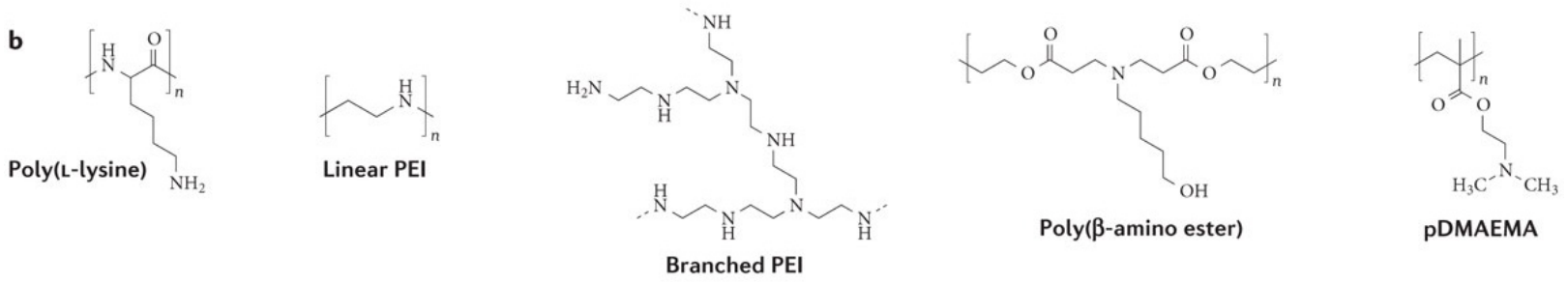


Figure 1 Schematic illustration of polymeric nanoparticle platforms.
Note: Blue color represents the polymeric platform.

Prabhu et al. "Polymeric nanoparticles for targeted treatment in oncology: current insights", Intl J Nanomedicine, 2014

Polymeric vectors

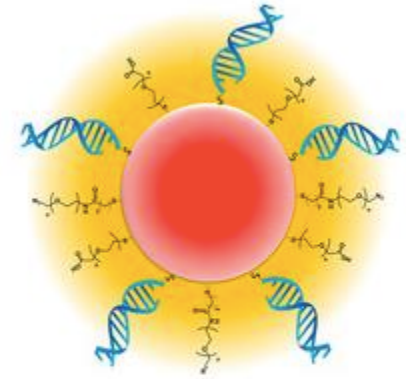
- Early examples of polymeric vectors: poly(L-lysine) (PLL) and polyethylenimine (PEI) – PEI and its variants are among the most studied polymeric materials for gene delivery
- A nitrogen atom at every third position along the polymer means PEI has a high charge density at reduced pH, which seems to aid in condensation of DNA and endosomal escape
- PEI can actually induce cytotoxic, so requires chemical modifications to improve biocompatibility and biostability



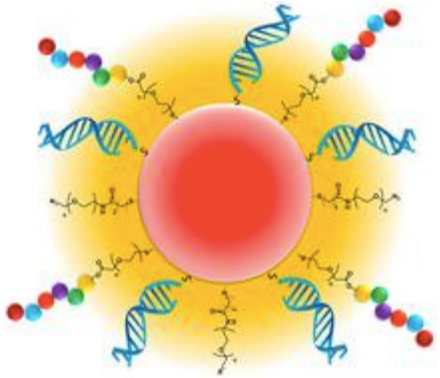
Yin et al., "Non-viral vectors for gene-based delivery", Nature Review Genetics, 2014

Inorganic and mechanical delivery

- Gold nanoparticles/nanoshells
  - Au-S bond covalently linked nucleic acids - cargo can be released from the particle by light-inducible mechanisms (e.g. pulse laser)
- Direct injection of naked DNA plasmid into the cell/tissue
- Electroporation
  - Uses short pulses of high voltage to temporarily form pores in the cell membrane so DNA can pass through



Au-siRNA



Au-Tat-siRNA

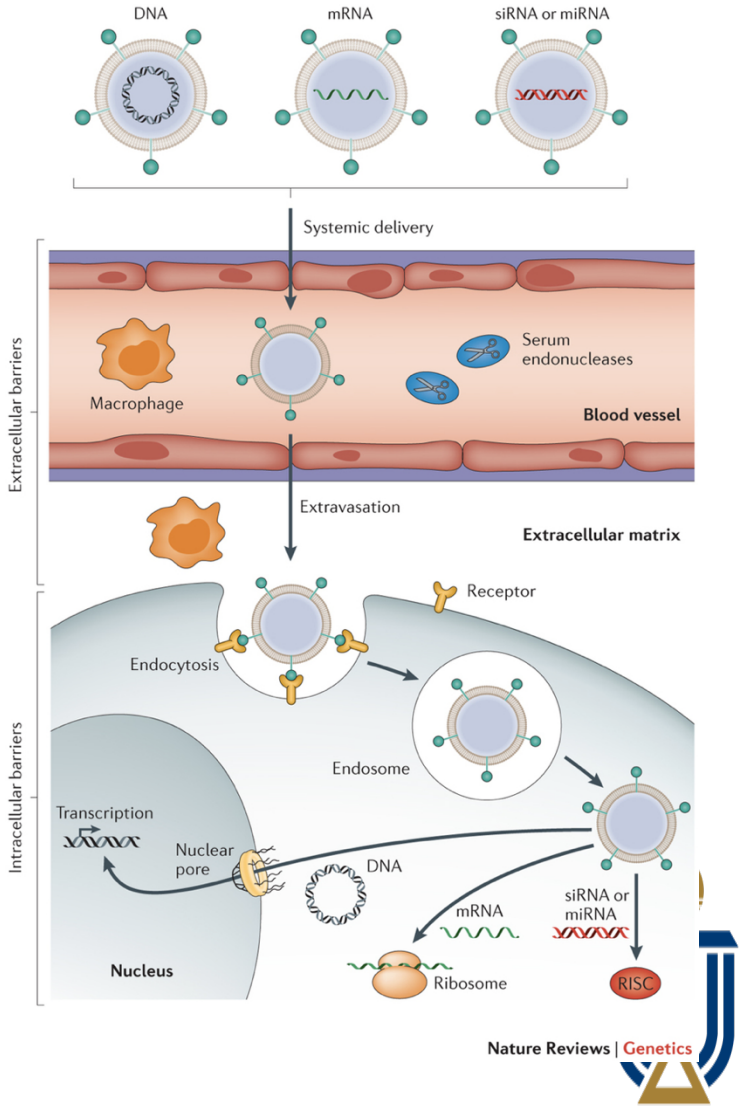
Child et al."Gold Nanoparticle-siRNA Mediated Oncogene Knockdown at RNA and Protein level, with associated Gene effects", Nanomedicine (Lond.), 2015

Inorganic and mechanical delivery

- Gene gun
  - DNA is coated onto gold particles and loaded into a device which generates a force to achieve penetration of the DNA into the cells
- Sonoporation
  - Uses ultrasound to deliver DNA into cells. The process of acoustic cavitation is thought to disrupt the cell membrane and allow DNA to move into cells
- Hydrodynamic delivery
  - Rapid injection of a high volume of a solution containing DNA/RNA into vasculature; elevated hydrostatic pressure helps molecules enter the cell

Designing non-viral vectors

- To survive from outside to cell target, non-viral vectors need to:
  - Avoid degradation by serum endonucleases and evade immune detection, e.g. by chemical modifications of nucleic acids/encapsulation of vectors
  - Avoid renal clearance from the blood and prevent nonspecific interactions, e.g. using polyethylene glycol (PEG) or through specific characteristics of particles
  - Extravasate from bloodstream to target tissues, e.g. by using certain characteristics of particles and specific ligands
  - Mediate cell entry and endosomal escape, e.g. by specific ligands and key components of carriers



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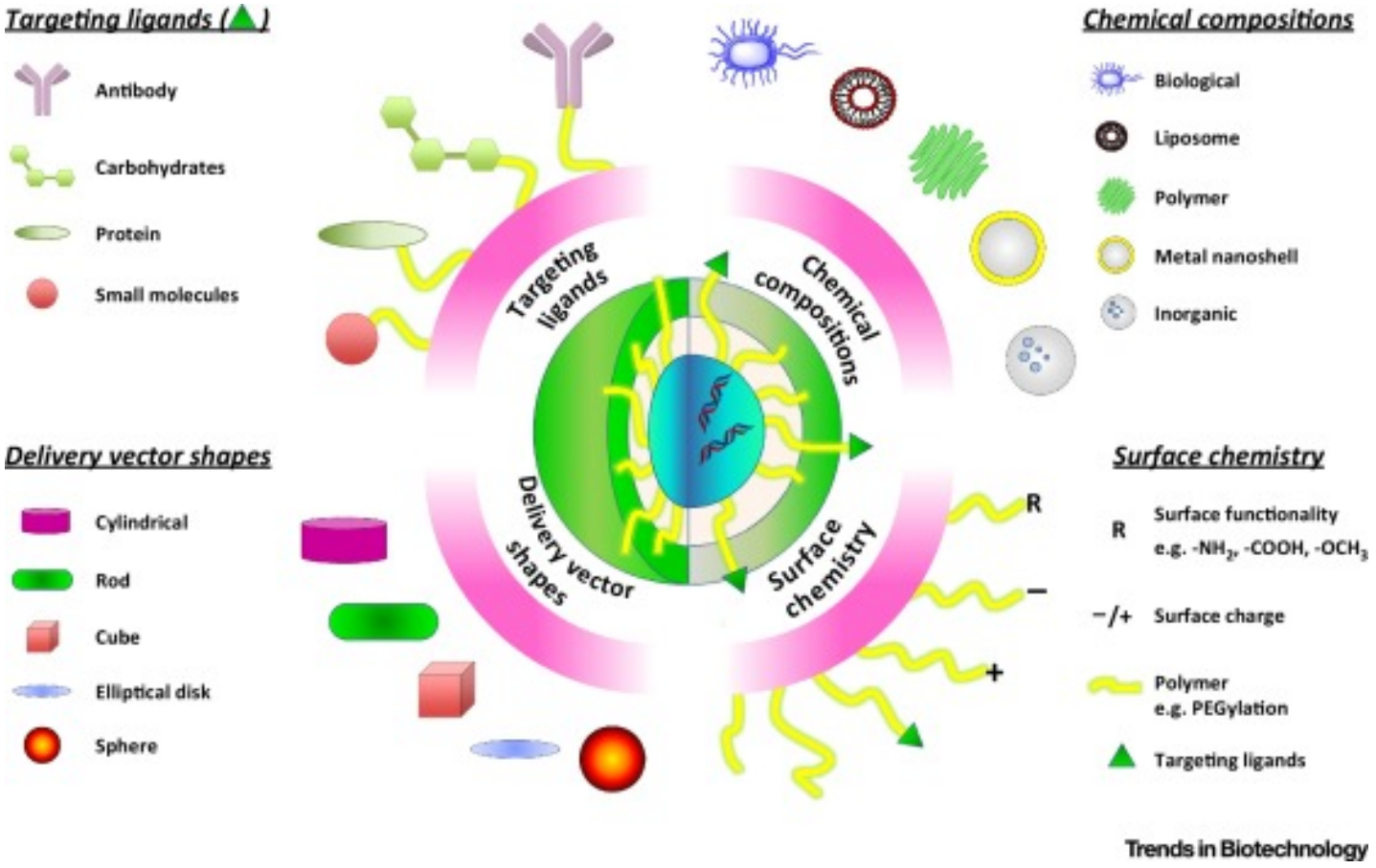
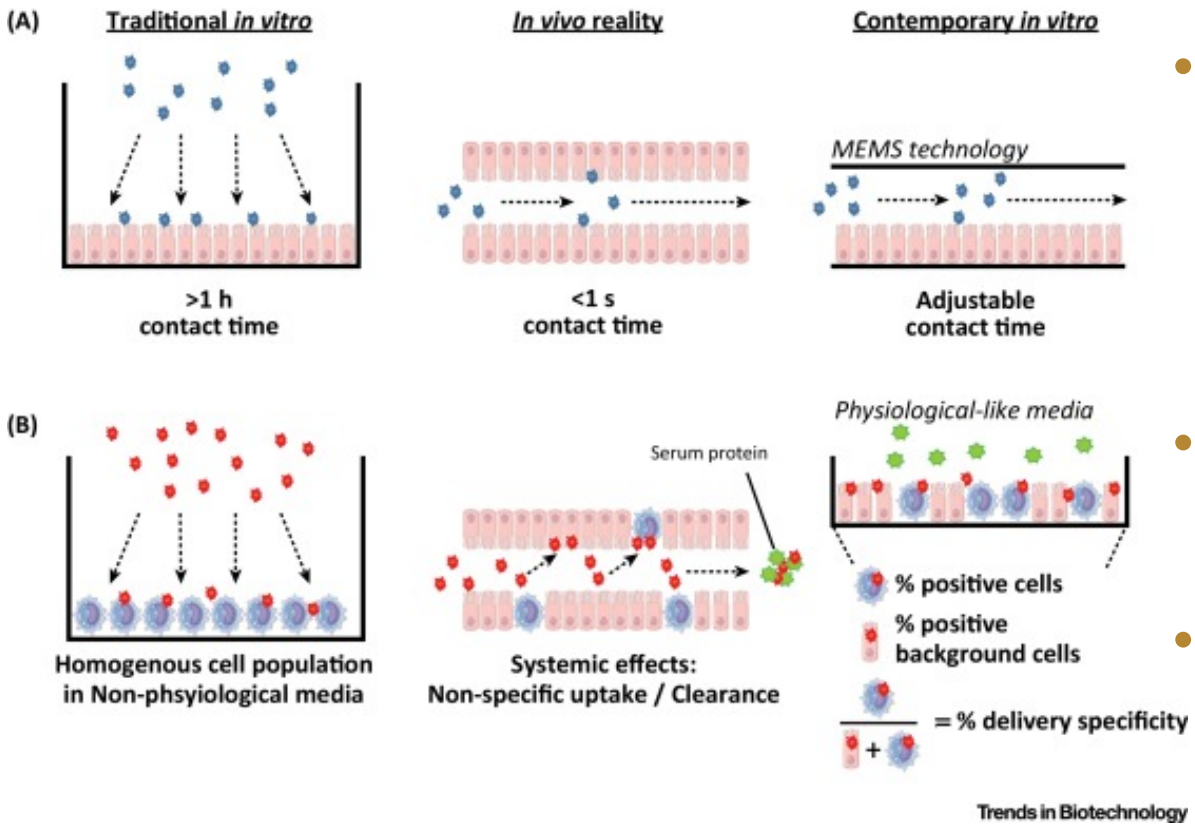
Designing non-viral vectors



Hill et al. "Overcoming Gene-Delivery Hurdles: Physiological Considerations for Nonviral Vectors", Trends in Biotechnology, 2015

Challenges in designing non-viral vectors



- Balancing protecting vs. releasing the cargo
- Endosome escape
- Nuclear entry (DNA)

Hill et al. "Overcoming Gene-Delivery Hurdles: Physiological Considerations for Nonviral Vectors", Trends in Biotechnology, 2015

Pros and cons of viral vs. non-viral vectors

Viral vectors

Pros

1. They are very efficient, and the rate of successful gene expression is very high
2. Naturally, we can select viruses to target specific cell-types

Cons

1. Size of cargo is restricted
2. They can cause immune response in patients which reduce treatment effectiveness, or worst case case death
3. Integration mechanism could cause mutations/cancer

Non-viral vectors

Pros

1. Low immune risk
2. No cargo size limitation
3. Can design intelligently according to needs
4. More cost-effective and available because they are easier to make

Cons

1. Efficiency is much lower than viral systems
2. Difficult to design parameters precisely/accurately and difficult to model in-vivo dynamics