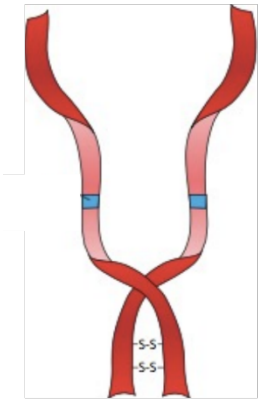
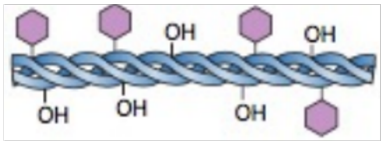
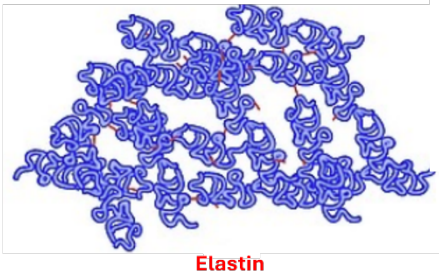
1. During the lecture you heard about the extracellular matrix (ECM), that is surrounding cells.
   a) Why is it so important?
   Answer: The extracellular matrix (ECM) is a crucial part of our tissues that maintain the cell structure and regulate cell functions. It provides cells with adhesion points and signals for cell proliferation and differentiation. It confers stiffness and elasticity to tissues and therefore defines very specific properties of the given structure. For example, brain tissue does require a completely different stiffness than muscle tissue. But these physical parameters are not only important for the resistance towards external stimuli, but also have an impact on gene expression and therefore also cellular states.
   b) Can you name these major structural proteins of the ECM?

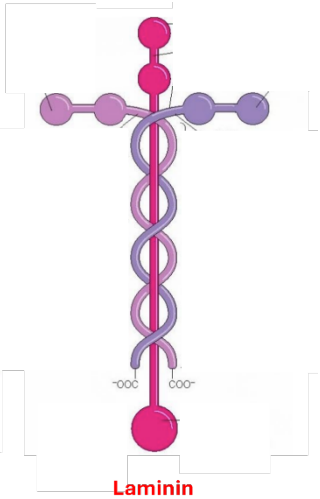


Fibronectin



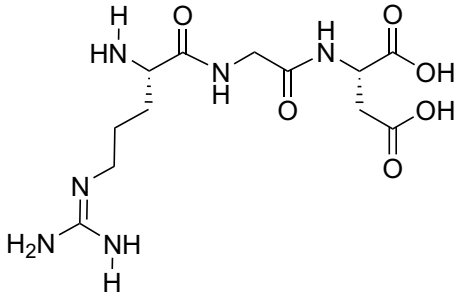
Collagen


Elastin



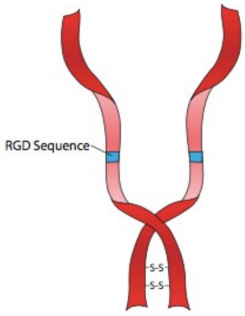
Laminin

   c) Please draw the structure of the RGD motif and name the structural ECM protein it is present in? You can use the codon-table from last time.



R G D

   Answer: The RGD-motif is present in Fibronectin, where it serves as anchor for integrin receptors on the surface of cells.



   d) How would you engineer an ECM-mimicking biomaterial to take over the function of the ECM?
   Answer: When designing an ECM-mimicking biomaterial, the design factors you should

take into account are: polymer type, crosslinking density, stiffness, degradability, adhesive motif, and growth factors. The first thing is to make a network structure of the ECM-mimicking biomaterial. By defining an appropriate type of polymer (natural or synthetic or both) and suitable polymerization condition, one can engineer the crosslinking density of the polymer to adjust the stiffness to match the mechanical properties of the biological ECM). Then you could add RGD peptide as adhesive motif to allow for focal adhesion formation. Often degradation is included to allow for dynamic changes over time. Factors that affect biomaterial degradation are pH, temperature, enzyme, hydrolysis, etc. Therefore, you can endow you ECM-mimicking biomaterial with bioresponsive property by incorporating hydrolytically or cell-mediated enzymatic degradable moieties. Last but not least, the ability of the ECM to present or release growth factors confers the ECM with a controlling role in the cell development.

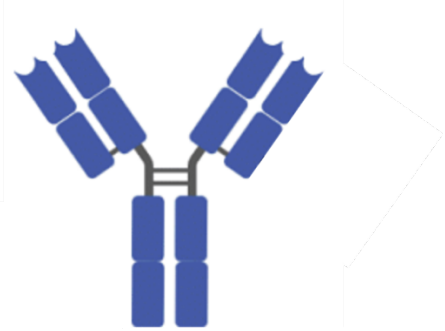
2. Could you think of a cellular system, that would allow for the screening of different drug candidates and their effect on physiology?
   Answer: Organoids
   a) What features would such a system require to be relevant for biomolecular research?
      Answer: Optimally this system would be a 3D-culture, as this is more representative to actual tissues in a living body in regards of its organization. Furthermore, it should always consist out of various cell types, that also make up the organ one tries to model in reality. This is especially important, as in a real tissue different cell types take up different roles and an organoid model does only recapitulate actual physiology well, if all these interactions are preserved. Additionally, it is also favourable, that the model/organoid is based on the organism of interest (e.g. human cells to do human organoids for testing of medications for humans).
   b) What environment would such a system require to thrive in?
      Answer: Such a system needs a matrix to grow in, that provides mechanical support and furthermore also the right set of growth factors and other morphogens required for tissue formation. The currently most commonly used matrix is called Matrigel and is derived from the natural source of Engelbreth-Holm-Swarm mouse sarcoma cells.
   c) Could you tell the major drawback of the currently available natural product used for the culturing of these systems?
      Answer: As it is a cancer-derived natural product, it is not very well defined and thus prone to be subject of batch-to-batch variations. This can have an impact on the reproducibility of the experiments conducted with the organoids. The cancerous origin is of course also very problematic.
   d) Name two major advantages of synthetic alternatives to the currently used natural product used for culturing such systems.
      Answer: The composition is chemically defined and therefore easier to control. Synthetic hydrogels can produced in a factory and not by test animals.

3. During the lecture you got a small introduction to the immune system.
   a) What are the main two branches of the immune system and what are their differences?
      Answer:
      Innate immunity: The innate immunity is an unspecific line of defence against common pathogens (bacteria, parasites, etc.). There are specific cells, like macrophages or dendritic cells, that can detect viruses and pathogens based on components that are generally absent in us humans. For example, components of the bacterial cell wall. Generally, innate immune reactions are fast, but unspecific and thus sometimes not sufficient as some pathogens achieve to hide from this layer of defence.

Adaptive immunity: The adaptive immunity provides very specific and strong immune responses towards pathogens, that cannot overcome the innate immunity. However, it relies on certain aspects of the innate immunity like the capability of some of the cells of the innate immunity to present them with pathogenic material they ingested. Furthermore, the adaptive immunity can lead to the formation of long-lasting immunity against certain pathogens through the formation of an immunological memory. Unfortunately, an adaptive immune reaction is relatively slow (takes a few days to kick in), but very targeted and thus efficient once triggered.

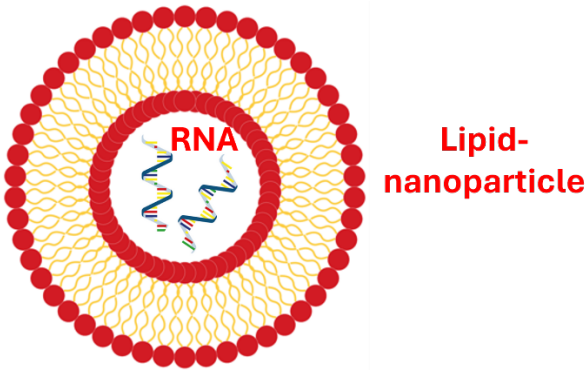
b) Can you name 2 major cell types of each of the immune system classes.
Answers:
Innate: e.g. Macrophages, Dendritic cells;
Adaptive: e.g. B-Cells, T-cells

c) What is this, which cell type does produce it and explain its role in immunological responses.



Answer: This is an antibody, that is produced by B-Plasma cells. It is important for blocking interaction and marking antigens/pathogens for degradation. Upon binding phagocytes can detect the marked antigen and degrade it. Furthermore, if it binds to an important part of a receptor or a ligand, that normally engages during interactions, then it can sterically hinder this interaction and thus change cellular communication. This makes it a therapeutically extremely attractive platform as it can be used for a wide variety of applications ranging from the treatment of Alzheimer's disease to cancer.

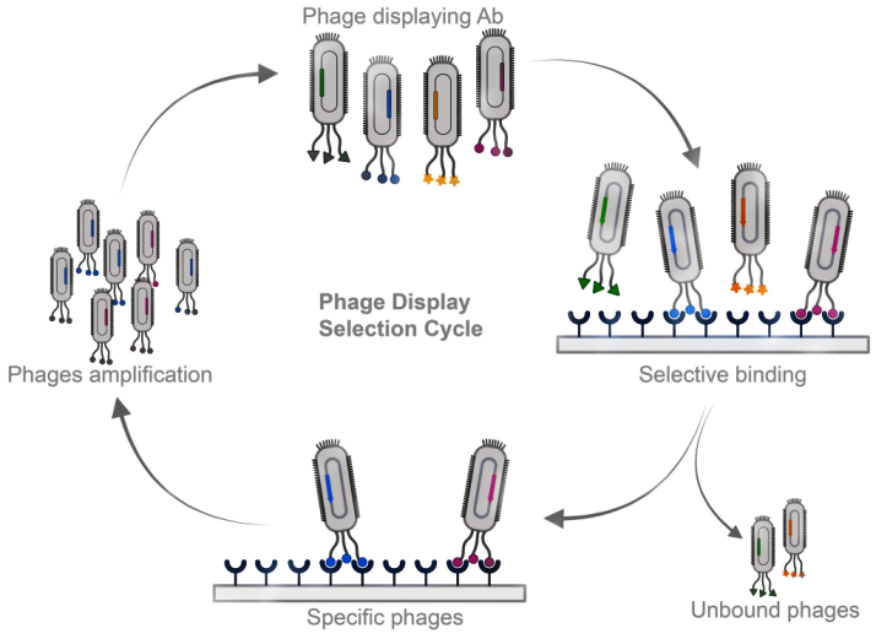
d) You are trying to do a vaccine and below you can see a nanoparticle, that you would start with. Please draw other important components, annotate the existing ones and explain how they contribute to the establishment of an immunological memory against the disease of interest.



Answer: Here we will mainly talk about mRNA-vaccines and how they create an immunological memory. The commonly used mRNA-vaccines like the Covid-19 vaccines from BioNTech or Moderna are generally based on two important things. Firstly, the mRNA that will code for the Spike protein of SARS-CoV 2 and the lipid nanoparticle, that will be formed around the mRNA to one hand protect it from external things and also allow for its delivery into the cytoplasm of cells. There the mRNA is translated to Spike-proteins, that

will be presented on the surface of the infected cells. Antigen-presenting cells like dendritic cells will detect these proteins, take them up, travel to lymph nodes and present them there further to cells of the adaptive immune system (For example T-cells). These T-cells will induce the activation of B-cells, that will produce antibodies and also induce the formation of cytotoxic T-cells, that will be able to attack infected cells. At the same time memory B- and T-cells specific for the Spike-protein introduced with our vaccine will be created, that will stay alive within a host for years. Once we come in contact with the real virus these memory cells will be activated and immediately induce the production of antibodies and effector cells, that will fight the disease. Therefore, we will have a very fast and specific response upon infection and thus be able to circumvent getting sick at all resp. have only very mild symptoms.

4. I would like to select a strong binding ligand with phage display for a specific receptor expressed in a cancer cell line.
   a) How would I go about selecting this ligand, starting from a large library of potential ligands. You can use a cartoon to explain the method.

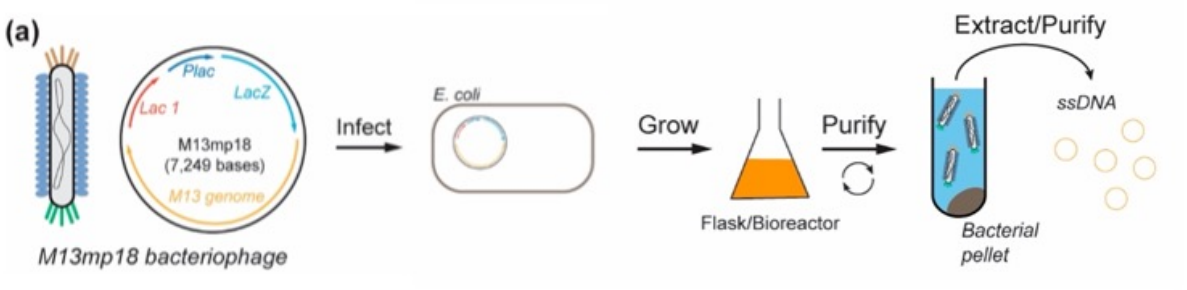


To select a strong binding ligand with phage display you would follow these steps:

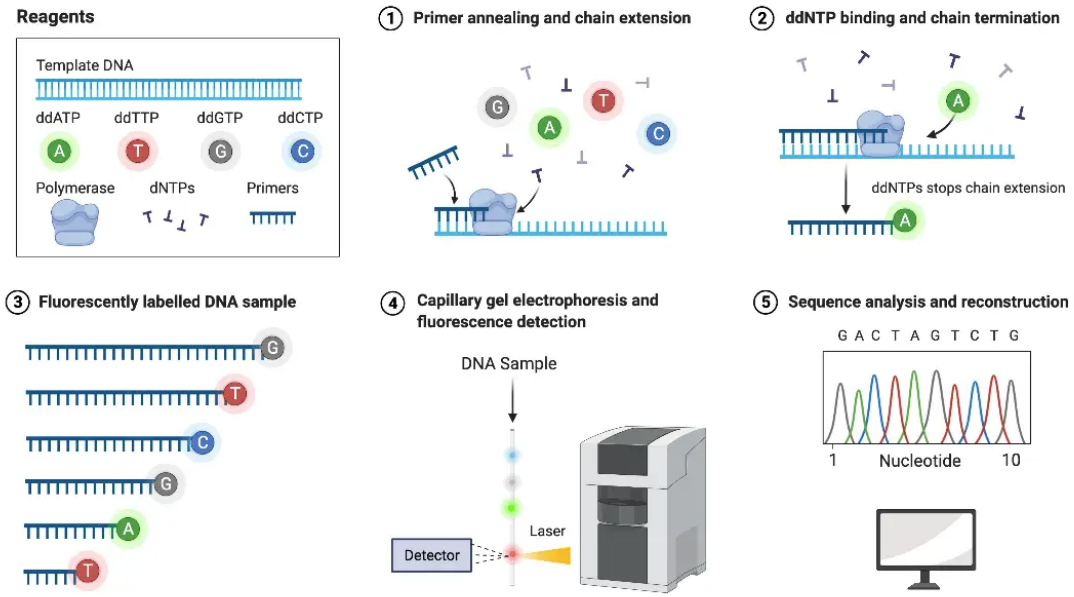
1. Generate a large library of potential ligands displayed on the surface of phage particles. You can achieve this by incorporating different peptide sequences into the recombinant phage plasmid.
2. Introduce the recombinant phagemid into bacterial cells for expression of the phages.
3. Incubate the newly created phage library with the specific target receptor. Select phage particles displaying ligands that bind to the receptor.
4. Wash away unbound phage particles and elute the bound phage particles containing ligands that bind strongly to the receptor.
5. Amplify the eluted phage particles by amplifying them again in bacterial cells to increase the population of high-affinity binders.
6. Conduct additional selection rounds to further enrich for strong binding ligands. Increase stringency in subsequent rounds to isolate the highest affinity binders.
7. Evaluate the selected phage clones to confirm their binding specificity and affinity for the receptor.

b) How do we determine the sequence of the selected ligand?

We need to purify out the DNA plasmid out of the phage and perform DNA sequencing



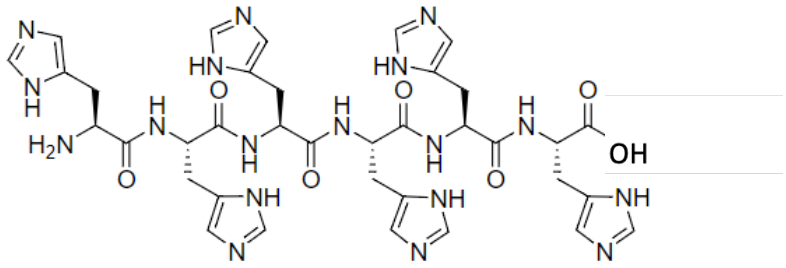
Not necessary to know the mechanism, but for you information: DNA sequencing can be done with the Sanger mechanism, as depicted bellow.



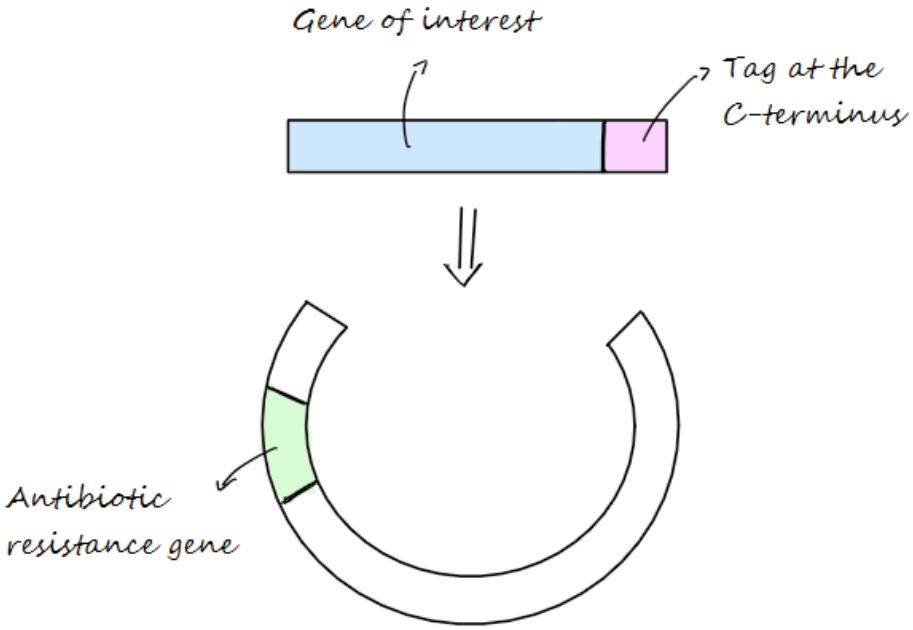
1. **Primer annealing and chain extension:** DNA template is mixed with a primer and DNA polymerase. The primer binds to the template at a specific site, and the polymerase extends the DNA chain by adding nucleotides complementary to the template.
2. **ddNTP binding and chain termination:** A mixture of regular nucleotides (dNTPs) and small amounts of fluorescently labeled dideoxyribonucleotides (ddNTPs) are added. When a ddNTP is incorporated by the polymerase, it terminates the DNA chain because it lacks a 3'-OH group needed for further extension.
3. **Fluorescently labelled DNA sample:** Each ddNTP is labeled with a different fluorescent dye, allowing the identification of which base was incorporated and thus the termination point in the sequence.
4. **Capillary gel electrophoresis and fluorescence detection:** The terminated DNA fragments are separated by size using capillary gel electrophoresis. As they pass through a gel matrix in a capillary tube, they are excited by a laser causing the fluorescent labels to emit light, which is detected and recorded.
5. **Sequence analysis and reconstruction:** The fluorescent signals are analyzed to determine the order of the bases in the DNA sequence. By examining the peaks corresponding to each dye color, the sequence of the DNA template can be reconstructed.

These short, high affinity peptides are often used as “tags” to couple to other proteins or nanoparticles, or even surfaces.

a) What important tag corresponds to this sequence and draw the chemical structure: CAT-CAC-CAT-CAT-CAC-CAC



b) How can we include a “tag” in an engineered protein? Can you schematically draw the plasmid?

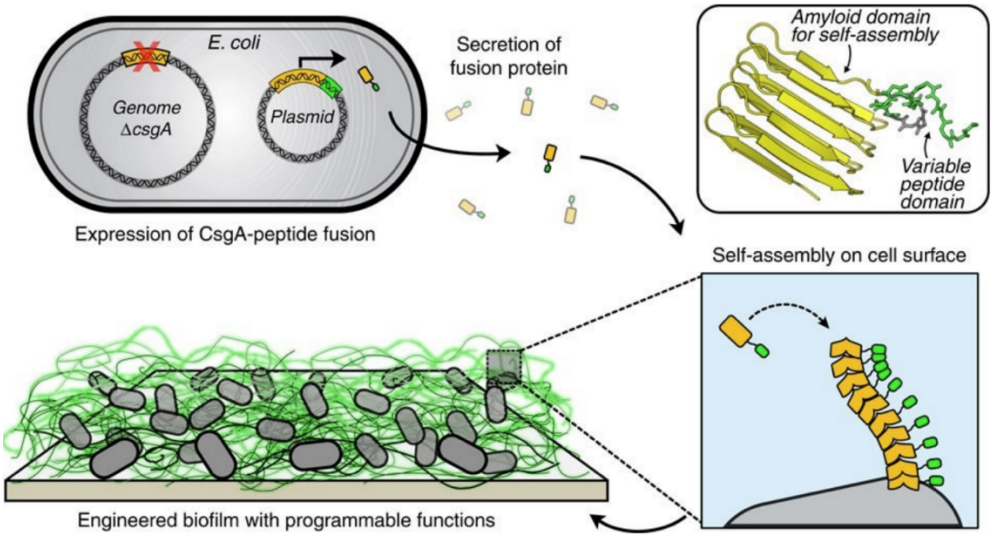


c) Why could it be interesting for scientists to find a strong binding ligand for a specific cancer cell line?

Finding a strong binding ligand for a specific cancer cell line is crucial for several reasons:

- Targeted Therapy: Strong binding ligands can be utilized to deliver therapeutic agents specifically to cancer cells, minimizing off-target effects and improving treatment efficacy.
- Diagnostic Imaging: Ligands can serve as probes for imaging techniques, aiding in cancer diagnosis, staging, and monitoring treatment response.
- Biomarker Discovery: Ligand-receptor interactions provide insights into the molecular characteristics and heterogeneity of cancer cells, leading to the discovery of novel biomarkers for diagnosis and prognosis.
- Basic Research: Understanding ligand-receptor interactions facilitates research into cancer biology, helping uncover underlying mechanisms and potential therapeutic targets.

5. Viruses and bacteria both are very common microorganisms on Earth. While the great majority are harmless to humans, pathogenic virus and bacteria can cause infectious diseases and even endanger our lives and health.
   a) Name 3 positive functions of bacteria for humans?
   Answer: Without our bacterial symbionts we cannot exist. They play a crucial role by colonizing many places on and in our bodies, where they can protect us from pathogens, produce vital micronutrients like vitamins in our gut or even produce signalling molecules, that can have positive impacts on our mental health (gut-brain axis). In the gut they furthermore contribute the to the digestion of fibres and also there fight off infections with pathogenic bacteria.
   b) What are 2 similarities and 2 differences between bacteria and viruses (think about their biological structures)?
   Answer:
   Similarities: They both have a genome (However the genome of bacteria is always out of DNA, whereas viruses can also have RNA-genomes), this genome is protected inside a capsid / membrane structure .
   Differences: Bacteria can reproduce by themselves. Viruses need to infect the host to be able to multiply. Viruses are generally way smaller than bacteria. This is possible, as they generally lack the whole machinery for their life cycle and produce only upon the infection of a host. Bacteria can have a cell wall and a membrane, whereas some viruses only have a capsid consisting out of proteins.
   c) If you would like to engineer a living material using bacteria, what components need to be introduced into the bacteria and how would you do this? Please draw a schematic below.
   Answer: For this one could introduce a plasmid carrying an expression construct for the curli-system, that has a tag fused to it which can allow it to bind to certain surfaces. This plasmid would require an antibiotic resistance gene for the selection of the bacteria, that received the construct. One can introduce such a construct using heat-shock.



   d) If you want to functionalize your living biomaterial with other proteins like the GFP to visualize the material, what would you need to add to the plasmid of these GFP proteins?
   Answer: One would need to add a tag either on the N- or the C-terminus that can bind to a complementary tag on the protein of interest or to the protein itself. Afterwards one would again need to introduce these constructs into bacteria using heat-shock or electroporation and then select for them using antibiotics. The bacteria will then express our new protein and we need to finally purify the proteins from the rest of the bacterial components.