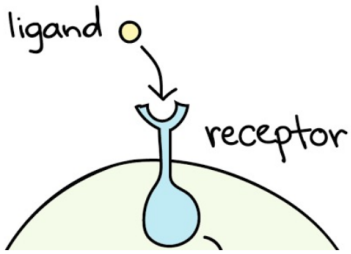
1. During the lecture you heard about the ligands and receptors.
   a) Please draw a cartoon to show how the ligand-receptor binding looks like on the cell membrane, and list 3 types of ligands and receptors.
   Answer:

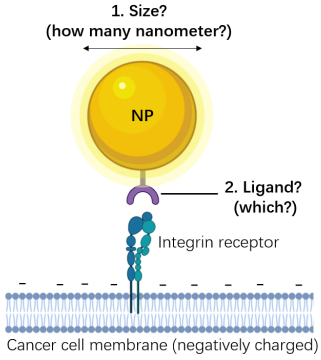


   Ligand: antibodies, proteins/peptides, DNA/aptamer, small molecules, polymers…
   Receptors: Integrin receptors, receptors for endocytosis (clathrin), enzyme…

   b) In previous lectures you learned about the Arg-Gly-Asp (RGD) motif, which serves as a ligand for integrin receptors on the surface of cells to mediate cell adhesion. What is the binding mode between RGD-integrin? What are the other binding modes between ligands and receptors?
   Answer: conformational isometric.
   Other binding modes: lock and key, induce-fit.

   c) To maximize the treatment effects biomaterials and avoid their side effects on normal organs/tissues, therapeutic biomaterials (i.e, nanoparticles) need to recognize diseased cells rather than healthy cells. This is typically achieved by either one or both means of targeting: passive or active. What are the differences between passive and active targeting? Which targeting strategy do you think would work better?
   Answer: The main difference between passive and active targeting is its mechanism of action. Passive targeting relies on the physical properties of the material to arrive at the destination. In contrast, active targeting relies on the biding between ligands and certain receptors on the cell. In this manner, passive targeting is non-specific and nanoparticles could go everywhere in our body and result in a wide biodistribution. Since receptor expression on disease cell and healthy cell could be different, active targeting is more specific and works better.

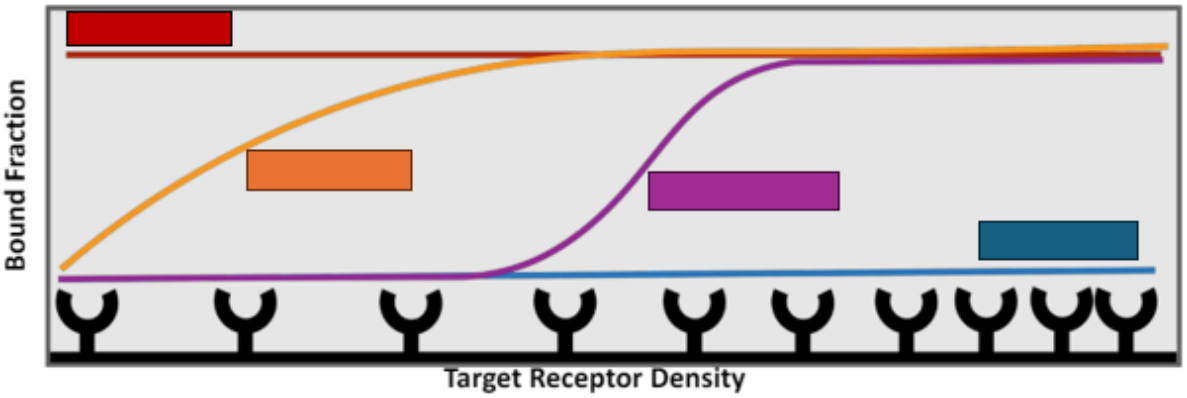
   d) Cancer cells overexpress integrin receptors on their surface. Below you can see a nanoparticle (NP) core, that you would like to make into a cancer-targeting nanoparticle. Please supplement the design parameters that you think may improve the targeting efficiency.



   Answer:

Size: 20~150 nm. If particle size > 150 nm, NP will get entrapped within the liver and spleen. If NP is too small, (<5 nm) it will be filtered out by the kidneys and excreted through urine. Generally, after entering the tumor region, NP with a large size is capable of being well retained in the tumor surroundings but it is hard for them to penetrate deeply in the dense tumor matrix. While it's more difficult for small-sized ones to retain in the tumor tissue, they are more sufficient in penetrating in the tumor.
Ligand: RGD. RGD is an efficient ligand for integrin receptor and therefore has been widely used to develop active tumor-targeting nanoparticles.

e) Now considering that all cells overexpress integrin receptors and we want to minimize off-target effects, we aim to design nanoparticles that will only interact with cells expressing integrin receptors above a certain threshold. First, name the different binding types represented on this curve and secondly select the binding type that is suited best for designing this type of nanoparticle.

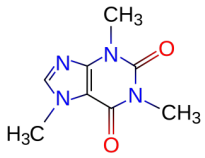


Red: Too strong
Orange: Multivalent
Purple: Super-selective
Blue: Too weak

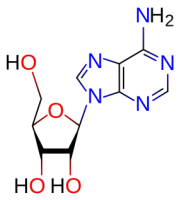
The binding type we are aiming for a super-selective. This means that the nanoparticle will not interact with cells that are expressing integrin receptors below a given density threshold, only when the cancer cell overexpresses the receptors will the nanoparticle interact strongly with the cell, reducing the off-target effects significantly compared to a multivalent binding type.

2. Ligands can be classified into agonists or antagonists according to their efficacy upon their binding to receptors.
   a) What are agonists and antagonists?
   Answer: Agonists are able to activate the receptor upon binding and result in a strong biological response, while antagonists bind to receptors but do not activate the receptor.

   b) During the lecture you heard about adenosine, a natural molecule in our body. Naturally, it binds to adenosine-receptors and makes us feel tired. Often, we drink coffee to feel more awake and alert. Caffeine, a component of coffee, is a ligand for the adenosine-receptor. Based on the structure below, why do you think both adenosine and caffeine can bind to the same receptor?



Caféine

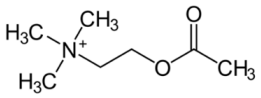


Adénosine

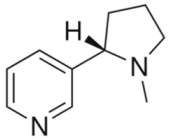
Answer: Both caffeine and adenosine have a similar chemical core structure showing a 5 and 6 membered ring (purine) that can interact with the receptor.

c) Based on the effect, is caffeine an agonist or antagonist for the adenosine receptor? Explain why?
Answer: An Antagonist. Since naturally, when the receptor is active, the result is that we feel tired. With Caffeine bound to the receptor, we do not feel tired, so the natural pathway is blocked. This is the function of an antagonist.

d) Both Nicotine and Acetylcholine act as agonists for the nicotinic acetylcholine receptor (nAChRs). The binding between these two molecules and nAChRs could make us feel excited, however Nicotine is also known an addictive drug in tobacco products. Based on the dissociation constant (Kd) shown below, could you explain why tobacco is so addictive?
- Kd of Nicotine-nAChRs is: 1 nM to 1 pM
- Kd of Acetylcholine-nAChRs is: 140 µM to 0.1 mM



Acetylcholine



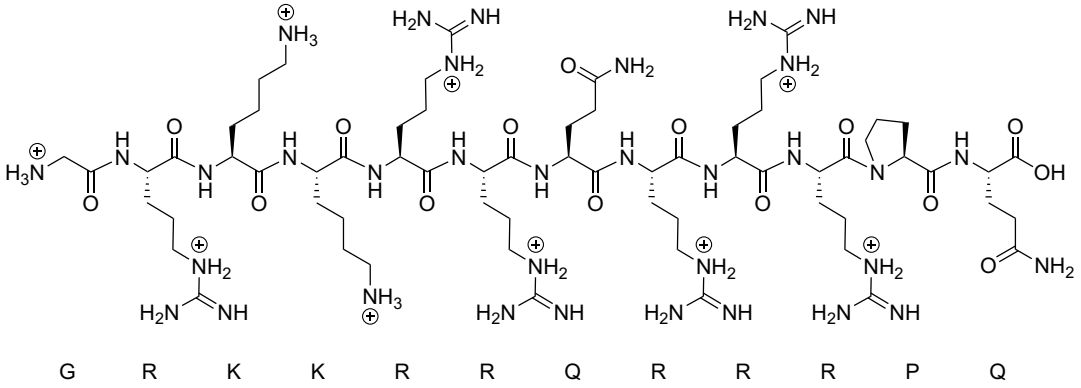
Nicotine

Answer: One measure of how well a molecule fits a receptor is its binding affinity, which is inversely related to the dissociation constant Kd. The higher the affinity of a ligand, the higher its tendency to bind to the receptor. In this case, the Kd of Nicotine is much higher than that of Acetylcholine, which means the binding between Nicotine-nAChRs is much stronger than that of Acetylcholine-nAChRs. Therefore, nicotine can easily be addictive.

3. In the lecture you heard about the concepts of endo- and exocytosis.
   a) Please describe the general differences between the two processes.
   Answer: In exocytosis molecules get released from cells, whereas in endocytosis molecules get taken up into cells.
   b) Please give an example for both processes within our body.
   Answer: Phagocytosis of particles/bacteria/viruses by immune cells as example of endocytosis and release of cytokines from immune cells as example of exocytosis.
   c) Imagine you would like to deliver a nanoparticle like the one in 1d) into the cytoplasm of a specific cell how could this be induced and what features would the nanoparticle need to have to end up at the desired location?
   Answer: The best option is receptor mediated endocytosis, where your nanoparticle is decorated with ligands for receptors that are present on the target cell. These receptors on the target cells will trigger assembly of clathrin on the inside of the cell, which induces the endocytosis of the particle. Within the endosome stability at lower pH would be important to withstand the acidification occurring during the progression from early to late endosome. Additionally, the nanoparticle would need to be functionalized with motifs inducing endosomal escape like the TAT-peptide.

d) For what applications could such a nanoparticle inside cells be useful for?
Answer: Cancer therapy, gene expression modulators (miRNAs), etc.

4. During the lecture you heard about endosomal escape and potentially you talked already about it in 3c), but we would like to focus in a bit more detail on the aspect of endosomal escape.
   a) Why is it important, that a cargo, that would need to enter a cell escapes the endosome rather early than late?
   Answer: Because of the acidification, that is happening during the progression from early to late endosome and further to the lysosome in which also enzymes become active, that will degrade the cargo and the cargo will not have any effect to the cell.
   b) Please name at least two different ways of how a particle can escape the endosome.
   Answer: Break/destabilize the vesicle wall, membrane fusion, proton sponge leading to osmotic lysis, particle swelling inducing rupture of the membrane
   c) Please draw the chemical structure of this peptide: GRKKRRQRRRPQ
   Answer:



TAT-peptide

   d) What is the charge of this peptide and what is it used for?
   Answer: It has 9 positive charges and it can be used to deliver cargos into cytoplasm of cells.

5. Some questions about Kd calculations:
a) At a ligand concentration that equals the **Kd**, what percentage of receptors on a cell would be bound by the ligand?
At a ligand concentration that equals the Kd, 50% of receptors on a cell would be bound by the ligand. This is because at the Kd, half of the available receptors are occupied by the ligand, representing the equilibrium point where the rate of association equals the rate of dissociation.

b) Calculate the concentration of free receptors **[R]** at equilibrium when we know that the total concentration of receptors **[Rt]** and ligands **[Lt]** is 200pM and 300pM respectively and the concentration of ligand-receptor **[LR]** complex is 120pM at equilibrium.
We know that at equilibrium, [LR] = 120 pM, and [R] + [LR] = 200 pM.
Calculating [R]:
[R] = [Rt] - [LR]
[R] = 200 pM - 120 pM
[R] = 80 pM

c) Use the concentrations of **[R], [L]** (concentration of the ligand), and **[LR]** to calculate the dissociation constant (**Kd**) for the ligand-receptor interaction.
We already know the [R] and [LR] so we need to calculate (just like above) the ligand concentration free at equilibrium [L].
[L] + [LR] = [Lt]
[L] + 120 pM = 300 pM
[L] = 300 pM - 120 pM
[L] = 180 pM

Now, we can calculate Kd:
Kd = ([L] * [R]) / [LR]
Kd = (180 pM * 80 pM) / 120 pM
Kd = (14400 pM^2) / 120 pM
Kd = 120 pM

d) Does the dissociation constant calculated indicate a strong or weak interaction between the ligand and receptor? Does this mean that the receptor is a good target for nanoparticles taking advantage of super-selective binding?
A K_d of 120 pM is considered a very strong binding interaction between a ligand and a receptor. Such a low K_d suggests a very high affinity, meaning that the ligand binds tightly to the receptor even at very low concentrations. Therefore, this would not be a good candidate for a super-selective binding target, given that regardless of the target density the interaction would be very strong.