9.3: Examples and applications

1) Now that you have an understanding of the mechanisms behind the phage display system, let's have a look at how it's used in research.
    i) Phage display has a broad spectrum of applications. This can go from improvement of enzymes, to proteome analysis or drug and target discovery but is certainly not restricted to these.
2) The first thing I want to focus on is the optimization of enzymes. A large variety of enzymes are being used today in different fields of industry and biotech. These enzymes are originally made by organisms for a specific function in a specific niche or environment, like inside the cell cytoplasm. Therefore, they often have undesirable properties for the function or environment we want to use them in. They might not work fast enough, be unstable in the conditions they are used in or do not work on the desired substrate. Phage display can aid in improving these enzymes by selecting for example for better thermostability, activity or enhanced substrate binding.
    i) Let's start with stability. Take for example an enzyme that is normally present In a cell at cytoplasm at 37°C. However when we use it in an industrial process where we need 50°C, the enzyme becomes unstable and denatures. In phage display we can make a library of mutagenized enzyme and display them on, in this case, the gene 3 protein of the phage. In the binding step of the biopanning most phages will not be able to bind the enzyme substrate at 50°C as they are denatured. Only phages who display an enzyme which is able to fold correctly at this temperature will be retained and amplified in subsequent steps of biopanning. These phages can then be sequenced to obtain the sequence for the stable protein.
    ii) Resistance against proteolysis can also be acquired through phage display. In this example, a library of mutagenized enzymes is expressed as a fusion protein with the enzyme of interest in the center of g3p. After digestion, only phages carrying a proteolysis resistant enzyme have the N-terminal tail of g3p which is required to interact with the F- pilus. Hence only these phages are amplified in the biopanning. The DNA sequence obtained from these phages will again encode this resistant enzyme and can be used for production of the modified enzyme.
3) It is also possible to enhance catalytic activity of enzymes. In this example I will discuss a way to do this for metalloenzymes. These are enzymes that require a metal atom for their activity. In this case a β-lactamase was used, an enzyme that is able to innactivate β-lactam antibiotics.
    i) A library of mutagenized β-lactamase was prepared fused to the gene 3 protein of M13. In a first step the phages are treated with a chelating agent, removing all metal atoms. This way the metalloenzyme is inactivated. However it is still able to bind its substrate. Hence, a first selection is made for enzymes that are still able to bind their substrate. After washing, metal is added again so that enzymes which are able to function are activated. More active enzymes will

cleave the substrate better and hence elute better. In this way a positive selection is made for enzymes that are catalytically more active.

ii) After 2 rounds of biopanning, the researchers acquired an enzyme that has a 60-fold increase in activity. The technique can also be extended towards other metalloenzymes.

4) Phage display can also be used in proteomics research. Many proteins effect their function by interaction with other proteins. However, in many cases this interaction partner is unknown. To find these protein interaction partners through phage display a cDNA or ORF library is made from the proteome of interest. These libraries encode practically all possible proteins that can be expressed in a certain cell. Hence, the different phages in the library will express and display all these possible proteins on their surface, in this case Gene 8 protein is used. Panning is then done by using the protein of interest as a bait. After a couple of rounds of panning, the remaining phages will be enriched in proteins that interact with the bait protein. Sequencing them will generate a list of potential targets which can then be verified by other techniques.
5) A last example I will show you is the search for a peptide that can pass the blood brain barrier. In drug development it is hard to develop drugs that are capable of passing into the brain. This is because the brains has a so called 'blood brain barrier'. This barrier is only present around blood vessels in the brain and not the rest of the body. The adjacent endothelial cells of these blood vessels are connected by tight junctions that form a continuous and hard to pass barrier between the blood vessel and the brain. This forms a problem in, for example development of drugs against Alzheimer disease. However, the barrier is not impossible to pass. Some proteins and nutrients are able to pass through this barrier. One way to allow drugs to pass would be to link the drug to a small peptide that is able to pass. Hence researchers set up a phage display system to find such a peptide.
    i) To this end a peptide library of random 12-mers was constructed to be displayed on the phage surface. Rather than doing *in vitro* biopanning, an *in vivo* panning approach is used to find peptides. In this panning technique, the phages are injected in the tail vain of mice. The phage is allowed to circulate for minutes to hours to allow binding to receptors. The mice are then euthanized and the phages are extracted from the brain.
6) After four rounds of *in vivo* panning, 20 peptides were sequenced. 12 of them had the same consensus sequence.
    i) This consensus peptide was fused to a nanoparticle and labeled with a fluorescent tag and injected in the mice. In vivo imaging was then used to look at the localization of the nanoparticles. The first 2 mice are control mice, the 3rd and 4th have the fused particles in different ratios. A clear localization to the brain region of the mice is visible. Also when you look at the localization in the organs separately on the right figure. You can clearly see a strong accumulation of the nanoparticles in the brain, which is not the case for the control mice. Hence the peptide, discovered by phage display could target nanoparticles or drugs to the brain.

These examples clearly show the versatility of the phage display system and how they can be applied in practice.

7) More information on the phage display system and the examples I discussed can be found in the following articles.