ScFv barcoding – technology development towards MIcroArrays in Solution

Imagine your doctor being able to give an accurate diagnosis and treatment based only on a tiny drop of your blood. Handy, isn't it? The project called MIcroArrays in Solution is about to make this idea become a reality. The key part of this technology is to find the right molecules in the mixture of many, which are present in our blood. Based on these molecules, it will be possible to find molecular differences between healthy and sick patients.

Before the whole diagnostic part can be performed in practice, the detection of desired molecules in complex samples, such as blood, has to be improved. A lot. The mentioned desired molecules are often proteins, called protein markers, which are present in a different quantity than normally if a patient is sick. But the methods which are currently used in detecting those protein markers are not good enough for that purpose. For instance, antibody microarrays use antibodies as »search tools« for the desired proteins, since they are capable of very specific binding. That often works fine, but in antibody miroarrays, antibodies can be attached to a surface in different directions. This is problematic because the site of the antibody recognizing and binding the protein can be damaged or unable to function properly. Another technique, called two-dimensional gel electrophoresis, which is used together with mass spectrometry, has disadvantage as well. In this case, the complex sample has to be pre-treated, which means that proteins present in the sample in large amounts can be detected. But this is not what we want. We are also interested in detecting those present in small amounts. Therefore MIcroArrays in Solution (MIAS) are being developed. The idea of MIAS is to keep antibodies freely in a solution, which means that antibodies will be able to detect the »right« proteins without being damaged due to attachment. In addition, proteins present in lower amounts could be detected as well. However, before we know if there are any proteins present in the sample, we have to detect them. Special flags, called DNA barcodes, will thus be attached to antibodies. The DNA barcodes are short parts of DNA with a unique sequence. Every antibody, specific for a certain protein, will be equipped with this unique DNA barcode which is later detected by reading its sequence using modern sequencing technology.

In this thesis, attachment of DNA barcodes to antibodies was the main focus. Two methods were used and evaluated. First one uses a reagent that acts as a connector between an antibody and the DNA barcode. The second method uses a specially engineered antibody with added unnatural amino acid. These amino acids cannot be synthesized in nature and are mostly used for their special properties. In our case photoreactive unnatural amino acid *p*Bpa was used, which has the ability to, under certain UV light, couple to a molecule called β-CD, to which the DNA barcode is attached. In our experiments we mostly used β-CD without the DNA barcode in order to test how they bind to each other. And what we managed to achieve? Our results showed that the first method using the connector, performed poorly and has to be optimized before it can be of any use in MIAS. On the other hand, the second method was easy to perform and it seems to be quite promising approach for potential MIAS technology. This thesis addressed some basic technological aspects of the larger MIAS project and its findings brought us one step closer to development of the new technology.