“Quarternary Structure Imaging with Nano-Magnetic Resonance Imaging (325866 Nano-MRI)”

Romana Schirhagl, ETH-Zurich, Physics Department, Degen group

Final Report

Determining a protein structure very often is the first step to understand biological or medical problems e.g. a biological function or disease. Thus, it is not surprising that structural biology is a tremendously large field employing thousands of scientists. This includes several Nobel laureates which have been awarded for their groundbreaking achievements in this field. Currently the main limitation which occupies the vast majority of scientists in structural biology is sample preparation. This is due to a fundamental limitation of currently available methods, the need of sufficient amount (at least several thousand identical copies) of high purity protein. However, this not only poses a problem in preparing the protein samples but also makes it impossible investigate heterogeneous or rare samples where only a few protein particles have the interesting properties. These properties can e.g. be the resistance to a certain drug.

Arising from this need our ultimate goal is to determine a protein structure from a single protein. As a first test sample we chose virus particles due to their medical relevance and for practical reasons (they self-assemble under certain conditions and are thus relatively easy to synthesize). To determine the virus structure we were intending to use a new method called magnetic resonance force microscopy (MRFM). In an MRFM experiment the virus is mounted at the end of a floppy arm which moves relative to a small magnet on a microwave stripline. The motion of this arm, the cantilever, can be detected so precisely that the small forces between the magnet and the atoms in the sample can be detected. By measuring the force at different positions, the element specific 3D structure of the sample can be reconstructed. Currently, the spatial resolution for a single virus particle that can be achieved with this technique is around 5 nm (the virus is visible but not its fine structure). However, to obtain an atomic structure the resolution has to be further increased.

One strategy to achieve this goal is to „color“ certain parts of the virus which enables to differentiate different substructures and thus improves resolution. Since the method is element specific one way of „coloring“ virus parts is to introduce e.g. deuterium at a certain part and hydrogen at another.

The main achievement during our project was to reliably synthesize tobacco mosaic virus particles (a model virus which affects tobacco plants) with such alternating areas of different composition. The successful strategy is shown in Figure 1.

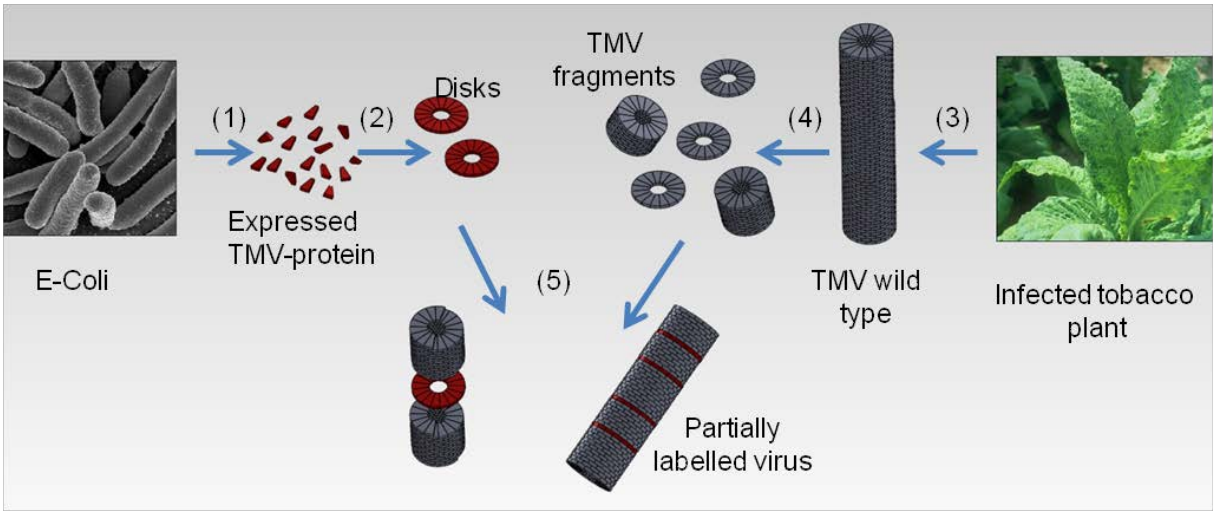

Figure 1: Schematic representation of the performed virus synthesis: To generate a contrast for our element specific measuring method virus (tobacco mosaic virus, TMV) parts were synthesized from two different sources. The natural virus (containing hydrogen) was produced via the natural route in tobacco plants. The deuterium containing parts were generated under special conditions in bacteria. Finally, different virus parts are assembled in a way that leads to striped virus particles.

To achieve the desired striped viruses hydrogen containing parts and deuterium containing parts have to be synthesized separately. Hydrogen containing viruses can be produced via the natural route in plants. Deuterium containing virus parts on the other hand have to be synthesized in bacteria that are fed with deuterium containing nutrients. Finally, we found conditions were the virus parts from different sources assemble. Furthermore, we investigated methods to attach the virus particles to the cantilever while maintaining its quality.

Since the project was terminated early we were not able to perform the final MRFM measurements. However, the samples are very promising and the work is currently continued in the Degen group. Although the final goal of determining a protein structure - which would have a huge impact on structural biology further consequences on related biomedical fields - has not yet been achieved, we believe that the experiments performed during the course of the Marie Curie project are a first milestone towards this goal.