Summary

The goal of the research described in this thesis was to find a suitable substrate for protein crystallization in order to improve crystal size, the number of crystals, crystal quality, and nucleation speed. There are approximately 20.000 different protein molecules in the human body alone, all with a different function. Only a part of these have been crystallized so far. Protein crystals can be used to derive the structure using X-ray diffraction if the crystals are of sufficient size and quality. The structure can be used to derive the function of the protein, and provides insight into the working of the human body at the molecular level. This understanding could be of use in the development of drugs to treat diseases such as Alzheimer.

A substrate can be of use to crystallize proteins that would not crystallize otherwise. Muscovite mica was used as the basis of such a substrate because it is flat over large areas (chapter 2). The first approach to create a network of molecules on muscovite mica to create an ordered template with lattice sizes approaching protein crystal lattice size dimensions was using crown-ethers (chapter 4). These molecules can specifically bind to metal ions on the muscovite mica surface, thereby stabilizing the layer. Unfortunately this molecular layer was not stable under protein crystallization conditions, and therefore unsuitable in view of this application.

The surface of muscovite mica can be functionalized in other ways. The roughness can be tuned by evaporating a molecule onto the surface (chapter 6), and the surface metal ions can be exchanged (chapter 3).

Furthermore, the chemical functionality can be tuned by applying a thin molecular layer of organothiols (chapter 5), and the periodicity can be tuned by applying a thin polymer layer (chapter 9). These thin layers were investigated using surface sensitive techniques (AFM, SXRD, and XPS). The influence of functionalization on the crystallization behavior of proteins was investigated (chapters 6-9).

The substrates with varying degrees of roughness can have an effect on the protein crystallization outcome. A rough surface is more difficult to clean, because dirt can adhere better to the surface. In a similar way, protein molecules can attach to a rougher surface in a way that influences the crystallization. This was found to be the case. The protein lysozyme (from the chicken) formed fewer crystals on the rougher surfaces, while insulin (from the cow) formed more crystals on this surface (chapter 6).

The substrate was also functionalized with different chemical compounds, to provide a varying chemical interactions with protein molecules, which can have an effect on protein crystallization. This situation can be compared to a drop of water on a greasy and clean table surface. The shape of the drop is different on both surfaces, due to the chemical interactions between the surface and water. The chemistry of surfaces can influence the outcome of protein crystallization experiments in a similar way. The chemical functionalization of muscovite mica can be achieved by applying a thin layer of thiols on the surface (chapter 5). The chemical functionality of the surface was found to be of influence on protein crystallization. The protein albumin (from the cow) preferred to crystallize on surfaces containing an alcohol group, while it did not crystallize on well on surfaces containing a carboxylic acid group. The protein lysozyme crystallized well on the surfaces containing a carboxylic acid group, and slower on surfaces containing an alcohol group (chapter 7).

A third parameter that was investigated was the symmetry and periodicity of the substrate. Proteins are large molecules, so a surface with periodicity over similar distances could be beneficial to order molecules and ease crystallization. This situation can be compared to Lego blocks, which can be easily put on a surface which has the same distance between the pins on the Lego. Furthermore, the Lego blocks are confined to a few fitting orientations. Thus, the surface forces the Lego blocks in to assemble is a certain way and protein molecules can be ordered in a similar way using a substrate. This was investigated using a network consisting of a polymer (chapter 8), and with natural crystals (zeolites, chapter 9), both with a relatively large periodicity (nanometers). A couple of nice examples were found where the substrate had an effect on the protein crystallization, for example the protein talin crystallized well on heulandite (a zeolite, chapter 9) giving much larger crystals than usual. The most interesting result were ordered insulin crystals on the polymer network (chapter 8).

The three investigated parameters (roughness, chemical functionality, and

periodicity) also provided many examples where a change in the investigated parameter did not significantly change the crystallization outcome. The proteins talin and albumin crystallized equally well on surfaces with varying roughness, the chemical functionality of a surface did not influence the crystallization of talin, and in many cases zeolites did also not affect the protein crystallization outcome.

There were a few diversions during this research period, and in two cases this has led to a chapter. The growth of gallium nitride on muscovite mica was investigated (chapter 11). Papers reported the ordered

(epitaxial) crystal growth of this material on muscovite mica. If a method could be found to grow large epitaxial crystals on muscovite mica, this would probably lead to applications, as gallium nitride is used in many devices. Unfortunately no optimal growth conditions were found, further study is required.

A different diversion led to chapter 10, which is about the reaction of noble metals with organothiol molecules. Originally, thiol molecules were intended to functionalize noble metal surfaces to study the effect of these layers on protein crystallization. When the noble metal surface reacted with the thiols, this was investigated further and resulted in a chapter 10.

The summary given above leads to the conclusion that most likely no universal template for protein crystallization exists. Therefore, in the attempt to crystallize a new protein molecule, surfaces with varying roughness, chemical functionality, symmetry, and periodicity should be used.