Summary

Protein molecules play an important role in the machinery of life, participating in every process within cells. Knowledge of their three-dimensional structure can provide insight into the way they function and thus into the way life functions. X-ray diffraction (XRD) is the main route to determine the three-dimensional molecular structure of proteins. The success of this method depends on the availability of high-quality protein single crystals. Due to the wide variety of protein molecules a single recipe for the growth of XRD-quality crystals does not exist. To find the right crystallisation conditions for a given protein, one needs to perform an extensive screening of solution conditions. On the one hand, technology speeds up the process of finding the proper conditions using large-scale (automated) trial-and-error screening methods. On the other hand, understanding the fundamentals of the crystal growth processes can help in reducing the parameter space.

Crystal growth from solution, like protein crystal growth, can be regarded as a combination of two successive processes; mass transport of growth units toward the crystal, and incorporation of those growth units into the crystal, i.e. the surface kinetics. The growth rate of the crystal is determined by the slowest of these two processes. This thesis focuses on the balance between mass transport and surface kinetics in protein crystal growth, and aims to contribute to the understanding of these processes. Hen egg-white lysozyme (HEWL) is an often used test case for protein crystal growth experiments, and as a result literature on HEWL is widely available. To take advantage of this large base of knowledge, lysozyme is used in the studies on protein crystal growth in this thesis.

In chapter 2 and 3 the surface kinetics during growth of lysozyme crystals are investigated by using optical and atomic force microscopy. Atomic force microscopy reveals rounded, highly anisotropic spirals on the (001) surface of triclinic lysozyme. The sticking fraction for HEWL molecules to become attached to a kink site at a step on the (001) surface is significantly different from that of the orthorhombic lysozyme polymorph. The difference between lysozyme polymorphs is further investigated by optical microscopy in chapter 3. Series of time-lapse microscope images show different growth kinetics for different polymorphs. Depending on the symmetry of the polymorph the growth of one of a pair of opposite crystal faces is blocked. This effect may be attributed to the surface phenomenon of self-poisoning; molecules can metastably attach to the surface in the wrong orientation with respect to the crystal structure. Such an effect might explain why some proteins are notoriously difficult to crystallise.

Moving toward the subject of mass transport, in chapter 4 spherulitic growth of lysozyme crystals is investigated. Spherulites consist of a bundle of crystalline needles, which often are found in protein crystallisation processes and are usually discarded as they are useless for XRD structure determination. With respect to surface kinetics, a tip splitting mechanism is proposed for the formation of the characteristic sheave-like structure of the lysozyme spherulites. For the mass transport mechanism, growth kinetics indicate that for a single needle surface kinetics determine the overall growth process, but all needles together deplete the solution faster than mass diffusion can replenish.

Besides the separation into a diluted solution phase and a crystalline phase, a lysozyme solution has a third phase consisting of a dense liquid phase. The formation of this phase is called liquid-liquid phase separation. The dense liquid phase is dense to such an extent that a gel network is created. This gel prohibits the formation of crystals in the dense phase, thus leaving the nucleation to the dilute phase. When crystals have formed, a three-phase system exists consisting of the dilute liquid phase, the dense gelled phase, and the crystalline phase. The presence of the dense liquid phase offers a means to investigate mass transport, as explained in chapter 5. Optical micrographs

and comparison with a computer simulation indicates that the presence of a third phase influences the kinetics of the growth process by changing the supply of growth units.

Convection is considered an important mass transport process in protein crystallisation, because it is efficient in supplying impurities to the crystal surface. When present at the crystal surface, these impurities may be incorporated into the crystal and break down the crystal quality. Natural convection is a result of solution density differences created by the growing crystal combined with gravity. When gravity is cancelled, these density differences do not give rise to convection, and the much slower process of mass diffusion remains. To damp convection, various methods have been and are being developed, including experiments in microgravity. Normally, microgravity for a prolonged time as needed for protein crystal growth can only be achieved in outer space. Another way to counteract the gravitational forces is to use a magnetic force, albeit at present not long enough for a typical protein growth experiment. Chapter 6 shows the utilisation of this technique for the paramagnetic salt nickel sulphate hexahydrate, for which an inhomogeneous field of around 1.6 Tesla is required. In these experiments, the concentration profiles are visualised by schlieren microscopy. Proteins typically are diamagnetic and thus require a significantly larger magnetic field, 27 Tesla for lysozyme, to damp convection. Chapter 7 describes this experiment in which shadowgraphy is used to visualise the disappearance of natural convection during lysozyme crystal growth.

Whether convection occurs or not depends not only on gravity but also on (vertical) system size and viscosity. In chapter 8 a study is presented into the influence of polyethylene-glycol on the morphological stability of tetragonal lysozyme. In the almost two-dimensional system of a droplet of solution placed in between two glass microscope slides, the viscosity of the solution controls the rate of mass diffusion. If mass transport limits the growth rate, a crystal looses its faceted shape because its corners protrude, increasing locally the supply of growth units. In the experiments, lysozyme crystal growth is followed from start to depletion of the closed system, i.e. equilibrium between solution

and crystal, showing the whole spectrum of different balances between mass transport and surface kinetics.

The experiments described in this thesis show that the way a protein crystal grows depends on the balance between mass transport and surface kinetics. One can improve a protein crystallisation by in-situ observations of the growth process. By observing the protein crystal growth process, one can explain unexpected end results based on the crystals early life, and even act upon these observation to improve crystallisation conditions, for instance with respect to the balance between mass transport and surface kinetics.