Summary

A large of studies have been conducted on proteins to determine their structures. For that, many research groups are used to growing protein crystals, but less are interested in understanding the physical factors which affect this process. This thesis focuses on the these factors and how they can improve the crystal final quality. Because protein crystals are grown from solution, the rate limiting step can be the transport of the solute to the crystal surface (diffusion control) or the addition of solute to the crystal surface (interface control). In unstirred stagnant solution, diffusion control dominates and crystals can grow relatively large and perfect. Therefore, sources of convection should be avoided. A number of attempts were made in order to accomplish convection-free crystallisation conditions, yet the application of these methods is often quite complex. In this thesis, I have studied the possibility of growing protein crystals using an efficient setup based on an up-side down configuration, in which the protein crystals nucleate and grow downwards from the *ceiling* of a growth cell overfilled with crystallisation solution. This allows for exploiting the gravitational force to effectuate diffusion-limited crystal growth, which is far simpler than, for instance, using microgravity conditions.

This approach, dubbed the ceiling crystallisation method, is described in chapter two. Ceiling crystallisation results in a significant improvement of X-ray diffraction resolution for a number of model protein crystals. Their resolution exceeded the current world records realised using other growth methods. Also, the ceiling crystals are larger, purer and resulted in higher resolution compared to their batch counterparts grown at the bottom of the same growth cells.

The time evolution of the solute concentration in the vicinity of the crystals is reported in chapter three. This is studied using a combination of microscopic and interferometric experiments and numerical simulations. This synergy provides a comprehensive view of the direct effects of solution movement and depletion and how this can directly affect the ceiling and batch crystal growth. While the microscopic inspections provide details about the external morphology of the crystals, the Mach-Zehnder interferometer provides a tool to monitor the spatial changes in solute concentration and flows in the crystallisation solution. Combining and processing a number of consecutive interferograms, phase-shifted with

respect to each other, allow the determination of the spatial changes in the refractive index. Using the relation between solute concentration and refractive index, the concentration profiles are estimated with high precision. The concentration profile for crystal growth and dissolution at the ceiling and the bottom of growth cells were validated by numerical simulation using FLEX software suite. The results show the progression of the depletion zone during ceiling crystal growth, while convection plumes are rising from the batch crystals. The development of the depletion zone depends on growth cell dimensions, supersaturation and diffusion coefficient of the crystallising solutes.

In chapter four, a systematic study is presented on the influence of impurities on protein crystallisation in the presence and absence of convection currents, by comparing ceiling and batch crystals growing simultaneously in the same vials. The incorporation of impurities is determined by both the so-called segregation coefficients of impurities and the mass transport. For most studied impurities, the difference between ceiling and batch crystals follows the expected behaviour. Ceiling crystallisation is especially favourable for impurities that are preferentially incorporated. I have found, in contrast to previous reports, that the low growth rate during ceiling crystallisation results in very much reduced incorporation of large heterogeneous impurities. In addition, ceiling crystals beat others grown under convection currents in their structural resolution in all the studied cases.

By exploiting fluorescent labelled proteins as additives during protein growth, chapter five reports the possibility of monitoring the crystal growth history and symmetry through studying the optical properties of protein crystals by polarisation and laser confocal fluorescence microscopy. I present this new microscopic approach perspective to protein crystallographers which not only allows distinguishing protein crystals from salt crystals, but also shows their detailed growth behaviour. It is found that the optical properties, incorporation and distribution of the fluorescently labelled proteins in the various protein crystals differ significantly for the different additive-host systems. This shows that impurity segregation is indeed a complex issue.

Finally, I report on our ceiling crystallisation kit in chapter six. This technical development is meant to facilitate the application of the ceiling method. The set-up prevents solvent evaporation and allows the crystals to grow large enough for optimal diffraction experiments.

Gravity is often blamed for hindering the growth of large and high quality crystals, because it drives convection currents. Through experiments and simulations, this thesis demonstrates the positive effect of gravity on protein crystal growth, provided that the correct configuration of ceiling growth is chosen. I show here that the convection current dilemma can be overcome, if we use the ceiling method and have an "opposite" perspective to the gravitational force. If I would define a key phrase for the concept elaborated in this thesis, it would be "keep it simple!".