Chapter 1

General introduction and outline
Before the 19th century it was generally believed that the molecules of life could only be produced by organisms. The synthesis of urea in 1828 by Friedrich Wöhler was a major breakthrough showing the possibility of artificial production of organic compounds. Moreover, this finding was a milestone on the road to the belief that, just like non-life, also life is subject to the laws of science. Many years later, life has been defined as the outcome of an elaborate organization based on trivial ingredients and ordinary forces.

Living systems are set aside from the rest of matter by some unique properties. They duplicate, transform energy, metabolize compounds and control their exchanges with the surrounding environment. Moreover, they are capable to maintain their internal equilibrium when changes occur in the outer world: a process commonly known as homeostasis. Various unrelated types of disturbance can be discriminated, yet all change the optimal functioning conditions of the organism and will be opposed by appropriate action.

A well known cellular stress factor is a raised temperature (or heat shock) which causes denaturation of proteins. The adaptation consists of up-regulation of chaperone and heat shock proteins (Hsps). Hsps are present in all organisms assisting the efficient folding of newly synthesized proteins and maintaining proteins in a stable conformation, preventing their aggregation under stress conditions. In chapter 2 the recycling of ribosomal 50S-nc-tRNA complexes by Hsp15 is described. This small heat shock

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‡ Living matter consists of relatively few common chemical elements (C, H, N, O, P and S supplemented with some trace elements)
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Protein is extremely up-regulated during thermal stress and has a much higher affinity for blocked 50S-nc-tRNA ribosomal subunits compared to empty 50S subunits. Heat shock causes translating ribosomes to dissociate prematurely, resulting in 50S subunits that carry tRNA covalently attached to the nascent chain of an incomplete protein (50S-nc-tRNA). The 50S-nc-tRNA subunits cannot re-initiate protein synthesis, so translational reactivation of a heat shock aborted 50S-nc-tRNA complex requires removal of the nc-tRNA by severing of the aminoacylester bond between these moieties. Cryo-EM reconstructions and functional assays show that Hsp15 reversibly translocates the tRNA moiety from the A- to the P-site of stalled 50S subunits. By stabilising the tRNA in the P-site, Hsp15 indirectly frees up the A-site, allowing a release factor to land there and cleave off the tRNA.

Another stress factor for an organism is a viral, bacterial or parasitical attack. The host organism will attempt to defend itself by raising an immune response against the foreign material. Chapter 3 describes the monoclonal antibody 54-5C10-A, which is used to diagnose the parasitic disease schistosomiasis in humans. The parasitic nematode Schistosoma mansoni expresses oligomers of Lewis X trisaccharides, a carbohydrate that normally functions, in its monomeric form, as human cell-cell interaction mediator. Our structural and biochemical studies indicate a radically different mode of binding compared to Fab 291-2G3-A, an antibody specific for monomeric Lewis X, thus providing a structural explanation of the diagnostic success of 54-5C10-A. Chapter 4 and 5 present the bacterial detoxification enzyme chlorite dismutase. Metabolising strong oxidizing agents like perchlorate
(ClO$_4^-$) and chlorate (ClO$_3^-$) as electron acceptors, the bacterium *Azospira oryzae* forms the toxic chlorite (ClO$_2^-$) as a byproduct. To prevent *Azospira* from poisoning itself, this chlorite waste is converted very efficiently into chloride and molecular oxygen. The X-ray structure of the chlorite dismutase gives insight into the active site of the first described haem enzyme performing O-O bond formation as its primary task. Furthermore, native mass spectrometry data demonstrate that the oligomeric organization of chlorite dismutase is different than the hitherto supposed tetramer.

In **chapter 6** analytical ultracentrifugation, gelfiltration and small-angle X-ray solution scattering (SAXS) experiments have been used to obtain the low resolution structure of full length EB1. The end-binding protein 1 (EB1) is a highly conserved group of proteins that uses its localization at the plus-ends of microtubules to regulate microtubule dynamics and chromosome segregation. Our results show that the distance between the centers of gravity of the two globular microtubule binding domains of EB1 is about 80 to 90 Å. This is close to the distance between adjacent tubulin subunits along a microtubule protofilament. Binding of EB1 could stabilize the GTP or GDP-Pi conformation of tubulin and promote the lateral interactions of protofilaments when they assemble into the microtubule lattice.

**References**