Chapter 1

Aim & Outline of the thesis
The origin of the research conducted in this thesis, lies in the finding that the molecular chaperone DNAJB6 is a superior suppressor of amyloidogenic protein aggregation. This irreversible type of aggregation arises when normally soluble proteins adopt a highly ordered, energetically favorable, amyloid conformation, and is a hallmark of several diseases including Huntington’s, Parkinson’s, and Alzheimer’s disease. In recent years, protein aggregation and amyloid formation have gained increasing attention as more details are being uncovered on the process driving these phenomena. Moreover, the view on protein interactions within the cellular environment in general, has been subject to a major paradigm shift with the emergence of studies on membraneless compartments that are formed through liquid-liquid phase separation or condensation. Through understanding of the underlying process of the formation of protein condensates in relation to aggregation, we also might get a better understanding of the mechanism through which molecular chaperones – the guardians of the proteome –, and specifically DNAJB6, could maintain amyloidogenic substrates.

Chapter 2 provides an overview of what is so far known about the chaperone DNAJB6, from its discovery until the recent structural data. The features of DNAJB6 are discussed, starting from its N-terminal end where the J-domain is located with which DNAJB6 binds to and regulates HSP70. As a co-chaperone of HSP70, DNAJB6 requires to work in the context of the entire HSP70 machinery for its full anti-aggregation potential. Mutations in the G/F-domain, a stretch following J-domain, cause a dominant form of Limb Girdle Muscular Dystrophy (LGMD), something that we discuss briefly in Chapter 3 and in the general discussion (Chapter 6). The mechanism by which these mutants cause disease is yet incompletely understood. Lastly, we describe the insights obtained from various studies from our lab that show that the unique C-terminal domain of DNAJB6, containing the S/T-domain, provides it with its ability to prevent amyloid formation of several substrates.

The finding that DNAJB6 can prevent aggregation of amyloidogenic substrates led us to investigate if DNAJB6 also has the capability to prevent the aggregation of other proteins that form more amorphous types of aggregates. In Chapter 3, we therefore make use of the parkin C289G mutant, which is responsible for causing juvenile Parkinsonism. This mutant forms non-amyloid like aggregates in cells and we found that DNAJB6 is able to also suppress this aggregation. Surprisingly, the S/T-domain, which is crucial for the anti-aggregation activity of DNAJB6 on amyloidogenic substrates, is dispensable for preventing aggregation of parkin C289G. DNAJB6 fully relies on its interaction with HSP70, which is less the case for amyloidogenic substrates. Interestingly, all other cytoplasmic J-domain proteins (JDPs) tested were also effective against parkin C289G aggregation in a HSP70-dependent manner, suggesting that for more amorphous aggregates, multiple JDPs can interact with the substrate for processing in the HSP70 cycle and provide protection against aggregation.
The classical model proteins that are often used for amyloidogenic aggregation, and on which DNAJB6 has exceptional anti-aggregation activity compared to other JDPs, include proteins containing an extended polyglutamine (polyQ) stretch. DNAJB6 can directly act on the polyQ sequences, unlike most chaperones which require the presence of polyQ-flanking sequences to (mildly) counteract aggregation. PolyQ stretches in at least nine unrelated proteins lead to inherited neuronal dysfunction and degeneration, including Huntington's disease and spinocerebellar ataxias. Interestingly, the length of the expansion generally correlates with the age at onset, but there are still large differences between patients with the same Q-length. In Chapter 4, we discuss several factors that could potentially account for this variability, including the flanking regions of the polyQ stretch, post-translational modifications, and availability and activity of chaperones.

Research until this point was mainly focused on the anti-aggregation activity of DNAJB6 on (mutant) disease causing proteins, and on the LGMD mutations of DNAJB6 itself. The latter studies indicated a crucial role for DNAJB6 in skeletal muscle. However, the physiological role of DNAJB6 remained unclear. During attempts to gain more in-depth knowledge on the activity of DNAJB6 on polyQ protein, a fortunate stroke of serendipity led us to discover that DNAJB6 is involved in nuclear pore complex (NPC) biogenesis (Chapter 5). We found that DNAJB6 is a resident of herniations at the nuclear envelope which arise when NPC biogenesis is stalled. In the absence of DNAJB6, membrane stacks containing partly assembled NPC (annulate lamellae) accumulate in the cytoplasm and nucleocytoplasmic transport is partially impaired. We show that DNAJB6 has FG-Nucleoporins (FG-Nups) as its native substrates. The FG-Nups have large disordered regions with FG motifs, which they need for their functional state to make up the barrier between the cytoplasm and the nucleus. The disordered FG-regions are prone to aggregate and DNAJB6 can delay the aggregation of several nucleoporins by controlling their behaviour when they form condensates. These results are the first to show that a molecular chaperone is key to NPC assembly.

The general discussion, Chapter 6, integrates all our data we gathered on DNAJB6, not only from this thesis, but also previously published and as yet unpublished work, into a new vision on how the unique functionality of DNAJB6 fits within the cellular environment, and which processes it could control. There are many proteins that contain intrinsically disordered regions that need their unstructured regions for their functioning. Whereas most chaperones are thought to guide the folding of structured proteins, our research identifies DNAJB6 as a chaperone for natively disordered proteins that are at risk to aggregate. Thus far, it was not known if intrinsically disordered proteins required chaperones to maintain their functional state. The scope of substrates and cellular processes of the chaperone network has now expanded into a range of research areas with these truly exciting new findings.