Characterization of proteasome precursor complexes

The ubiquitin-proteasome system (UPS) of eukaryotic cells provides an essential proteolytic control system, malfunctions of which are linked to various human diseases. A central component of the UPS is the 26S proteasome, a 2.5~MDa multimeric protease complex composed of the 20S catalytic core particle (CP) and one or two attached 19S regulatory particles (RP). Proteasome assembly is a conserved ordered process involving several dedicated chaperones, which was thought to be initiated by the formation of rings of seven distinct α subunits. A critical intermediate in CP assembly is the 15S precursor complex (PC) containing the chaperones Ump1 and Pba1-Pba2, as well as thirteen of the fourteen α and β subunits. Ultimately, the dimerization of two such complexes is triggered by incorporation of β7. Although the crystal structure of the mature 20S CP and of the Pba1-Pba2-20S CP is already known, the structural roles of Ump1 and Pba1-Pba2 chaperones during early and late steps of the 20S assembly are still unclear. By using an integrate approach employing biochemical methods, electron microscopy, cross-linking and mass spectrometry analysis, we characterized the structure of the 15S PC and compared it with late precursor complexes Pba1-Pba2-20S from the *pre1-1* mutant, containing unprocessed β subunits and Ump1, as well as with the reconstituted Pba1-Pba2-20S CP (Stadtmueller et al., 2012). This project was done in collaboration with the laboratories of Dr. Petra Wendler and Dr. Franz Herzog (Gene Center Munich). We also biochemically analyzed a complex in cells lacking functional Pba1-Pba2, which appears to be an early precursor intermediate *en route* to the 15S PC.

This thesis reveals significant conformational changes within the α and β rings of 15S PCs, with the Pba1-Pba2 heterodimer changing from a partially embedded position in the center of the α ring to a more elevated position in the late intermediate complex Pba1-Pba2-20S *pre1-1*. It is shown that after maturation of the 20S CP, the Pba1-Pba2 chaperone is expelled from the α ring and recycled for a new round of proteasome assembly. In the 15S PC, Ump1 is mostly unstructured, lining the inner chamber of the complex and interacting with multiple proteasomal α and β subunits. The N-terminus of Ump1 is exposed at the α ring pore, while the C-terminus is buried inside the complex. These data suggest that the Ump1 N-terminus might have a sensory function during the dimerization of two 15S PCs.