Exploring the bacterial ClpAP protein complex as a target for antibiotics

There is an acute need for novel antibiotics, since resistance to all clinically used antibiotics continues to increase and there are very few new compounds coming to market. One of the challenges in new antibiotic discovery has been the lack of new cellular targets and lead molecules with *in vivo* effect. Recently, identification of the bacterial protease ClpP as the target for a new class of antibiotics, Acyldepsipeptides (ADEPs), has been heralded as a major advance in the search for new drug leads.

Our laboratory has recently elucidated the mechanism of activation of the ClpP protease by this new class of antibiotics. In bacteria, ClpP forms a barrel-shaped particle that encloses a central hollow chamber containing the proteolytic sites. The ClpA ATPase forms rings that bind to one or both faces of the proteolytic component ClpP and translocate substrate proteins into the degradation chamber of ClpP for degradation. Studies suggest, that regulatory caps ClpA communicate with the proteolytic core ClpP via an interface region, which forms a peptide loop. ADEP-mediated activation of ClpP occurs, because binding of ADEP mimics the interaction of ClpP with its regulatory ATPase ClpA. However, these interactions are not yet understood in detail, since to date, no native structure of the ClpAP holocomplex exists.

My proposal aims to determine the structure of the ClpAP complex using cryo-electron microscopy and a high-throughput system that allows us to collect thousands of images in a fully automated fashion using a high-end electron microscope. This structure is essential in the understanding how the two components of the system (ClpA and ClpP) communicate, and in turn, to identify new mechanisms of activation of ClpP using antimicrobials. This information will be useful in the development of novel ADEPs that are able to act as more efficient drugs and to assess structure activity relationship patterns in lead optimization.

Cryo-electron microscopy is a valuable tool for structural and cellular biology; it enables scientists to gain insights into the structure and function of cells and cellular components. Electron microscopy of protein complexes can visualize these protein complexes in all their native conformations and thus a working cycle can be deducted.

However, to reconstruct a protein complex at molecular resolution, a large number of 2D projections (up to millions) of the 3D complexes are needed. This in turn requires a large number of micrographs to be acquired and processed, which is very demanding and a challenging endeavor. To acquire micrographs in an efficient and reliable way, automated control algorithms to enable acquisition in an unsupervised fashion are necessary.

During my PhD studies at the Max Planck Institute of Biochemistry (Martinsried, Germany), I have developed an acquisition software called TOM^2, that is optimized to efficiently acquire the data required for this study. During the course of this project, I will establish a collaboration between the Max-Planck Institute of Biochemistry and McMaster University (Hamilton, ON, Canada) to further develop TOM^2 and guarantee its maintenance.