

Abstract of the thesis entitled “Structural and functional characterisation of small Heat Shock Proteins from *Mycobacterium marinum* M”.

Small Heat Shock Proteins (sHSP) are ATP-independent molecular chaperones that exhibit diversity in structure, function and mode of action. They are present ubiquitously in all kingdoms of life. They function mainly by preventing the aggregation of non-native and destabilised proteins during stress as well as normal conditions. Their subunit molecular weight ranges from 12 kDa to 42 kDa and exist as higher order oligomers in their resting or native state. Structurally, these proteins have a tripartite domain organization with a structured α -crystallin domain (ACD) at the centre flanked by variable and flexible N-terminal domain and C-terminus. The protomers associate into dimers, which further assemble into higher order structures. The N-termini is longer than the C-termini, harbour sites for post-translational modifications, usually found buried within the oligomer and are required for substrate recognition and binding. The C-termini on the otherhand, harbour a conserved motif, called the I-X-I motif which facilitate formation of higher order structures. The thesis reports the structural and functional characterisation of three sHSPs (M1, M2 and M3) and their deletion constructs from *Mycobacterium marinum* M. In *mycobacteria*, these proteins are immunodominant antigens and are also used as biomarkers for disease identification. All the three proteins formed oligomers of different stoichiometry as determined through Size exclusion chromatography-Multi angle light scattering (SEC-MALS) experiments. Lysozyme aggregation assay was performed for assessing the ATP-independent molecular chaperone activity of these proteins. M1 and M3 were observed to be active while M2 was inactive. From the three sHSPs, one of the proteins, M3, crystallised and hence was taken up for structural investigations. The protein crystallised in different conditions and the structure was determined using data from a Se-Met derivative (Se-SAD) and molecular replacement (MR) phasing in space groups I23 (at 2.8 Å and 2.0 Å resolution, respectively) and C222₁ (3.75 Å). The structure was a dodecamer with a cage-like architecture, exhibiting a 23 symmetry. The dodecameric assembly was observed to be hydrophobic inside and hydrophilic outside. The I-X-I mode of interaction, as observed in other sHSP structures, formed the dimer-dimer association and stabilized the cage. Electron micrographs collected for M3 protein, further confirmed that the structure reported was a dodecamer in solution. Further, on comparison of the high resolution

sHSP structure of M3 with other reported structures, a similar arrangement of trimers was observed. This is the first report of a high resolution sHSP crystal structure from *mycobacteria*.