

# CONTENTS

<b>ACKNOWLEDGEMENT</b>	<i>i - ii</i>
<b>ABBREVIATIONS</b>	<i>iii- iv</i>
<b>CHAPTER-1: INTRODUCTION</b>	
1.1 RD regions of <i>Mycobacterium tuberculosis</i>	2
1.1.1 Region of Deletion 1	4
1.2 Secretion system in bacteria	10
1.3 Other RD regions	14
1.4 <i>M. tuberculosis</i> specific RD encoded antigens	14
1.5 ESAT-6/ CFP-10 family proteins	16
1.5.1 Structural and biophysical characterization of ESAT-6/ CFP-10 family proteins	16
1.5.2 ESAT-6 mediated cell lysis	21
1.5.3 Survival of mycobacterium inside phagosome depends on stability of ESAT-6- CFP-10 complex	22
1.5.4 Immunological characterization of ESAT-6/ CFP-10 family proteins	23
1.6 Rv3619c and Rv3620c	27
1.7 Protein –Protein interaction by Isothermal Titration Calorimetry	28
1.8 Rationale	29
<b>CHAPTER-2: MATERIALS AND METHODS</b>	
2.1 Material	31
2.2 Methods	31
2.2.1 Cloning of genes from Rv3619c and Rv3620c from <i>M. tuberculosis</i> H37Rv in <i>E. coli</i> expression vector	31
2.2.1.1 Preparation of genomic DNA of <i>M. tuberculosis</i> H37Rv	

2.2.1.2 Isolation and amplification of the genes	32
2.2.1.3 Restriction digestion and Ligation of PCR products:	33
2.2.1.4 Confirmation of the clones	34
2.2.2 Over-expression and Purification of Rv3619c and Rv3620c proteins	34
2.2.3 Determination of protein concentration	35
2.2.4 Isothermal Titration Calorimetry	35
2.2.5 Recording CD spectra	36
2.2.5.1 Study of thermal unfolding/refolding using CD	37
2.2.5.2 Stability of complex under variable conditions	38
2.2.5.3 Recording the CD spectra in presence of membrane mimetic conditions	38
2.2.6 Fluorescence Spectroscopy	38
<b>CHAPTER-3: RESULTS</b>	
3.1 Cloning of Rv3619c and Rv3620c gene for over-expression	40
3.2 Over-expression and purification of Rv3619c and Rv3620c proteins	40
3.3 Analysis of complex formation between Rv3619c and Rv3620c	40
3.3.1 Thermodynamics of Rv3619c - Rv3620c complex formation	46
3.3.2 Comparison with other members of the family	46
3.3.3 Rv3619c-Rv3620c complex formation is associated with conformational changes	47
3.3.4 Modeling and Docking	47
3.4 Stability of Rv3619c, Rv3620c and 1:1 complex	55
3.4.1 Thermal Unfolding of proteins	55
3.4.2 Complex formation provides stability against GdmCl denaturation	58
3.4.3 Effect of pH on complex	59
3.5 Membrane interaction studies	64

3.5.1 Rv3619c possesses solvent exposed hydrophobic surface	64
3.5.2 Effect of TFE and DPC micelles on the conformation of Rv3619c and Rv3620c	65
3.6 Comparative sequence analysis	65
3.7 Mutational Studies	72
3.7.1 Mutational analysis of Rv3619c	72
3.7.2 Mutational analysis of Rv3620c	72
3.8 Complex formation between non- genomic partners	72
3.9 Analysis of immune response of Rv3619c	72
<b>CHAPTER-4: DISCUSSION</b>	
4.1 Biophysical characterization of Rv3619c and Rv3620c	81
4.2 Unfolding studies of Rv3619c and Rv3620c proteins and their 1:1 complex	84
4.3 Membrane interaction of Rv3619c and Rv3620c	86
4.4 Immunological characterization of Rv3619c	88
<b>BIBILOGRAPHY</b>	I - XIII