Hydrogen sulfide (H_2S) is a new addition to the family of gaseous signaling molecules, earlier consisting of Nitric oxide (NO) and Carbon monoxide (CO). Multiple studies have led to the acceptance of H_2S as a cytoprotective molecule involved in neurotransmission, vasodilation, antioxidant signaling, inflammation, etc. Endogenous H_2S has also recently been reported to play an essential role in life span extension and other benefits of dietary restriction. The relevance of the H_2S in cellular physiology with particular emphasis on autophagy has been summarized in **Chapter 1**.

Importantly, many published studies suggest both positive and negative regulation of autophagy by H_2S. Therefore in **Chapter 2** of the thesis, we have explored the role of H_2S in the modulation of Autophagy. Herein, using multiple lines of evidence, we demonstrate that H_2S positively regulates autophagic flux in different cell types of human and mice origin. These evidence included western blot analysis of LC3, confocal microscopy using LC3-GFP probe and analysis of autophagy flux using the tandem-RFP-EGFP-LC3.

As autophagy has been known to act as a defense system against invading pathogens, in **Chapter 3**, we studied the effect of H_2S induced autophagy on the trafficking and survival of intracellular. *tuberculosis*. We observed that H_2S also enhanced trafficking of *Mycobacterium tuberculosis* to the lysosomes and decreased its survival in macrophages. These findings suggest that modulation of cellular H_2S could help in clearance of mycobacterial infections.

Interestingly, there is a parallel correlation between the cellular effects of H_2S and upregulation of Sirt1 expression/activity. However, the effects of H_2S on autophagy and Sirt1 are poorly understood and the molecular mechanism regulating H_2S induced autophagy remains elusive. In **Chapter 4** of the thesis, we dissected the molecular

events that guide the H_2S mediated induction of autophagy flux. Interestingly, the H_2S induced autophagy was dependent on the Sirt1 activity. siRNA mediated knockdown of Sirt1 or Inhibition of Sirt1 using a specific inhibitor led to abrogation of H_2S induced autophagy. Furthermore, the trafficking of *M. tuberculosis* to the lysosomes in response to H_2S was also dependent on the Sirt1 activity. Moreover, H_2S enhanced the interaction of Sirt1 with autophagic proteins, LC3, ATG5, and ATG7. We also demonstrated that upon exposure to H_2S, cytosolic GAPDH translocates to the nucleus. The nuclear-translocated GAPDH interacts with the Sirt1 repressor, deleted in breast cancer 1 (DBC-1). This interaction between GAPDH with DBC-1 leads to autophagy induction due to the derepression of Sirt1. Furthermore, we also show that H_2S enhanced Sirt1 mediated deacetylation of LC3 which is critical for upregulating autophagy as suggested by various other studies. Additionally, the DBC1 was sulfhydrated in response to H_2S exposure. Sulfhydration of proteins, a type of post translational modification of cysteine, has been reported to modulate the protein function and activity. Thus, sulfhydration of DBC1 may be critical for its GAPDH mediated Sirt1 derepression. Although, there was no significant difference in ATP levels upon H_2S treatment as measured by using the PercevalHR probe. We found an induction of the AMPK pathway upon H_2S exposure. However, unlike starvation induced autophagy, the H_2S induced autophagy was not dependent on AMPK. This suggests that AMPK activation could be regulated by the upstream Sirt1 pathway. We believe that these findings improve the current understanding of the regulation of autophagy by H_2S.