Tuberculosis (TB) remains a major global health problem, despite so much effort has been put into combating this disease. In particular, the emergence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) as well as co-infection with HIV poses a serious challenge. Moreover, the flexibility of *M. tuberculosis* metabolism and heterogeneity of bacterial populations makes the treatment of TB more complicated. Therefore, new drugs with novel working mechanism are desperately needed to adequately kill the heterogeneous population of bacteria and to counter MDR and XDR tuberculosis. In recent years, energy metabolism has emerged as a new target for development of antimycobacterial drugs and the identification of new candidate drugs targeting energy metabolism illustrates the therapeutic potential of blocking mycobacterial energy conversion. In this thesis, we expanded the knowledge of energy metabolism in mycobacteria as drug target. We investigated the mechanism of a presently used front-line drug, tested new drug combinations, described a recently found target and explored a new potential target. The knowledge acquired in this thesis may contribute to the identification of putative important factors in mycobacterial energy metabolism, which might lead to the discovery of new antimycobacterial drug targets. The aim of the work described in this thesis was to characterize key components of oxidative phosphorylation in mycobacteria and to explore their suitability as target of (new) drugs. We also investigated the mechanism of drugs acting on energy metabolism and evaluated their usage in drug combinations, which may be suitable for combating tuberculosis.
The ATP synthase is known as key enzyme in oxidative phosphorylation of mycobacteria and as target of diarylquinolines. In chapter 2, an overview of current knowledge on mycobacterial ATP synthase is given, idiosyncratic features are described and the potential implications for utilization as drug target are discussed. In chapter 3, the working mechanism of pyrazinamide is deliberated. Pyrazinamide (PZA), an important first-line drug employed in tuberculosis chemotherapy, played a key role in shortening the duration of tuberculosis treatment from 9 months to 6 months. Despite the importance of PZA, its mechanism of action is probably the least understood among all first- and second-line anti-tuberculosis drugs. We here tested and extended the hypothesis that PZA via its active entity pyrazinoic acid acts as an uncoupler and interferes with mycobacterial bioenergetics. A better understanding of PZA action may help in development of new drugs to further shorten tuberculosis treatment. Chapter 4 builds up on results from chapter 3, investigating the combination of PZA and BDQ in an in vitro model. This drug combination appears highly promising for further shortening TB treatment. In this chapter, we used static M. bovis BCG culture to understand the combination use of these two drugs in vitro. Multidrug resistant TB (MDR-TB) is become a major obstacle in controlling tuberculosis. MDR mycobacterial strains can express efflux pumps to extrude the anti-tubercular drugs. BDQ, an inhibitor of ATP synthesis, which depletes cellular ATP reserves, may concomitantly indirectly inactivate ATP-dependent efflux pumps. The study described in chapter 5 tests this hypothesis. Chapter 6 deals
with the cytochrome bd, one of the two terminal oxidases in the
mycobacterial respiratory chain. Using mutants in which one of the
two branches of the mycobacterial respiratory chain was inactivated,
we show that cytochrome bd plays an important role in protection
against peroxide stress and antibacterials. Cytochrome bd might be a
future target in TB drug development.

The work presented in this thesis has provided insight in
exploiting the new concept of energy metabolism as drug target in
anti-TB drug development. We started out with characterizing the
mechanism of a long-known front line anti-TB drug. Subsequently,
we investigated new drug combinations acting on energy metabolism,
analyzed the features of ATP synthase as a recently found target and
explored cytochrome bd as a new potential target. Results obtained
from these experiments might help us pinpoint components of energy
metabolism as Achilles’ heel of mycobacterial metabolism.
Altogether, these studies provide more information on using energy
metabolism as drug target, which may aid in an effective drug
discovery and to a better TB treatment.