Summary

Typical 2-Cys peroxiredoxin (Prx) enzymes reduce H_2O_2 and various organic hydroperoxides to less reactive products. These enzymes are characterised by interacting active site peroxidatic Cys (C_P) and resolving Cys (C_R) residues located on separate subunits. C_P in its reduced/thiol state can become hyperoxidised by high concentrations of H_2O_2 and thus the hyperoxidised form of the enzyme loses its peroxidase activity which can be restored by sulfiredoxin proteins. There are "sensitive" (mostly eukaryote) and "robust" (mostly prokaryote) types of typical 2-Cys Prx enzymes. The category ("sensitive" or "robust") is not known for parasitic or non-parasitic alveolates. Thus, here we have used the well-studied *Tetrahymena thermophila* (a non-parasitic alveolate) to investigate this. This thesis focuses on the study of the typical 2-Cys Prx proteins in selected organisms of the clade of the Alveolata. It also makes comparisons with typical 2-Cys Prx proteins from humans and investigates the possible utility of garlic and tea tree oils, and their active constituents, as potential novel anti-malarial drugs. *T. thermophila*, as a representative for the alveolates, was highly resistant to the anti-proliferative effects of treatment with H_2O_2 and cumene hydroperoxide (CMHP) but it was susceptible to the anti-proliferative effects of treatment with garlic oil and its major constituents diallyl disulphide (DADS) and diallyl trisulphide (DATS) as well as tea tree oil and its major constituent terpinen-4-ol. This suggested that *T. thermophila* should have a highly active complement of antioxidant enzymes capable of removing peroxides from the cell. Indeed, *T. thermophila* had genes encoding four typical 2-Cys Prx proteins and these proteins are known, from other studies, to be highly efficient at using H_2O_2 and CMHP as substrates. We predicted that *T. thermophila Prx1a* and *Prx1b* are located in the cytosol or nucleus,

Prx1m in the mitochondria and *Prx1c* is likely to be secreted. The *T. thermophila* Prx deduced amino acid sequences contained C_P and C_R residues that are highly conserved among typical 2-Cys Prx proteins. They also contained the GGLG and Y*X* (where *X* is F or W) motifs that are highly conserved among the "sensitive" type eukaryotic typical 2-Cys Prx proteins, including those from humans. However, surprisingly, the *T. thermophila Prx1m* protein was insensitive to hyperoxidation despite containing the GGLG and Y*X* motifs. Thus it belongs to the "robust" type of typical 2-Cys Prx proteins. This indicates that the GGLG and YF (or similar) motifs might not be always associated with susceptibility to hyperoxidation. *T. thermophila* lacked any sulfiredoxin proteins, normally found only in organisms that have "sensitive" typical 2-Cys Prx proteins. Thus, the resistance of the *T. thermophila Prx1m* protein to hyperoxidation was associated with the absence of sulfiredoxin proteins. This thesis reports for the first time on the capacity of garlic oil and tea tree oil and their dominant constituents to disrupt the redox state of the typical 2-Cys Prx proteins in *T. thermophila* cells and Jurkat T-lymphocytes (human cancer cells). Inhibition of thioredoxin reductase activity by allyl sulphides (found in garlic oil) but not by terpinen-4-ol (found in tea tree oil) could, at least in part, explain the oxidation/inactivation of the Prx proteins in these cells. Expression analysis of the four *T. thermophila* Prx genes revealed that the *Prx1a* and *Prx1m* genes were the most highly expressed and also the most responsive to pro-oxidants and therefore the most important in protecting the organism against oxidative stress. Furthermore, this thesis reports that garlic oil and tea tree oil disrupt the redox state of the erythrocyte Prx2 protein and make treated erythrocytes less susceptible to infection by *P. falciparum*, suggesting that these oils could potentially be used as antimalarial treatments.