Report on the StellenCoA 2018 conference (28 October – 1 November)

I have attended the StellenCoA conference which was held for the third time and focuses on the Coenzyme A (CoA) pathway. This year the conference was organised at STIAS (Stellenbosch Institute for Advanced Study), Stellenbosch in South Africa.

The themes that were discussed were the following:

- Understanding CoA-related metabolism and biology
- Exploiting CoA biology for anti-infective discovery
- CoA and Ac(et)yl-CoA: Cross-talk, interplay and regulation
- CoA, Neurodegeneration and Aging
- CoA and Cancer: probing the links

Topics of the generation of the precursor of CoA, vitamin B5, to production of CoA, to utilization of products made from CoA in different organisms and diseases were discussed by top scientists in the CoA field. This was particularly interesting for my PhD project since I am studying the mode of action of a novel class of antimalarials, pantothenamides, that target the CoA pathway (see abstract below). This conference would give me ample opportunity to share and discuss our recent data and network with leading scientists in this field.

Although this was not specifically a parasite-related conference, there were six talks about parasites, including *Plasmodium*, *Toxoplasm* and *Entamoeba histolytica*. The talks that were particularly interesting to me were from Kevin Saliba's group, Australian National University, Canberra, Australia. Saliba's group is investigating vitamin utilisation pathways. One line of his research focuses on the enzyme pantothenate kinase (PanK) which is the first enzyme in the CoA pathway. They have performed pull-down experiments on a GFP-tagged PanK and are studying the function of this enzyme. Another line of research was presented by Christina Spry who is studying the mode of action of enamide-stabilised pantothenamides. These compounds are very similar to the compounds that we have generated, except that they modified the compounds differently in order to stabilize them. Spry showed metabolomics data of pantothenamide-treated parasites that was consistent with our data. In addition, she gave insights into a chemical rescue experiment for CoA targeting compounds. Another interesting talk was presented by Dominique Soldati-Favre. She showed that *Plasmodium* rely on scavenging vitamin B5 from the host cell, while *Toxoplasma* parasites can scavenge, but can also generate vitamin B5 by *de novo* synthesis.

This conference was not only an incredible opportunity to expand my knowledge on a fundamental aspect of my PhD project, it was also great opportunity for me to network with the leading scientists in the CoA field and discuss our recent data. I am very thankful to the Dutch Society for Parasitology (NVP) for awarding me a travel grant to attend this meeting.

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Unravelling the mode of action of a novel class of antimalarials using experimental genetics

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Due to emerging resistance against all front-line antimalarials, there is an urgent need for the development of new drugs that target novel pathways and affect multiple stages of the parasite. Malaria parasites strongly rely on the extracellular supply of pantothenate (vitamin B5) for CoA biosynthesis, and it was found that pantothenamides, experimental drugs based on the pantothenate scaffold, are active against *Plasmodium falciparum* asexual and sexual blood stages at nanomolar concentrations. Metabolomic analysis suggests that pantothenamides are processed into drug-CoA analogues that could inhibit the formation of acetyl-CoA (see the abstract by Patrick Jansen). Whole-genome sequencing of two independent, pantothenamide-induced, resistant parasite lines revealed mutations in the binding pockets of two CoA-binding enzymes. Both lines revealed the same mutation in acetyl-CoA synthetase (ACS) and a second mutation in acyl-CoA synthetase 11 (ACS11) though on different amino acids. Using CRISPR-Cas9, we first introduced the conserved point mutation in ACS in NF54 parasites. Subsequently, we introduced either of the ACS11 mutations. In comparison to wild-type parasites, IC50 values were 15 fold higher for the engineered ACS single mutant and at least 94 fold higher for all four double mutants, both genetically modified and drug-selected. Furthermore, all lines were cross-resistant to at least four different pantothenamides, suggesting a similar mechanism of action for these compounds. Conversely, reversion of the all single point mutations back to wild-type sequences in the drug-selected parasites, restored drug sensitivity similar to the single ACS mutation. Currently, we are exploring the resistance phenotype and mechanism of action of pantothenamides during transmission by generating the single and double mutants in a GFP-Luc reporter line. In conclusion, our data contribute to elucidation of the mechanism of action of a new class of potent antimalarials that target a novel pathway.