[5] Lagouge M, Argmann C, Gerhart-Hines Z, Lerin **C**, Daussin **F**, Messadeq N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J (2006) *Cell* **127:** 1109-1122.
[6] Rolo AP, Palmeira CM (2006) *Toxicol. Appl. Pharmacol.* **212:** 167-178.

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9P.3 A novel drug for uncomplicated malaria: Targeted high throughput screening (HTS) against the type II NADH:ubiquinone oxidoreductase (PfNDH2) of *Plasmodium falciparum*

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The respiratory chain of the human malaria parasite *Plasmodium falciparum* lacks a canonical protonmotive NADH:ubiquinone oxidoreductase (Complex I), containing instead a single-subunit, non-protonmotive NDH2, similar to that found in plant mitochondria, fungi and some bacteria [1,2]. As such, the *P. falciparum* NDH2 (PfNDH2) presents itself as an attractive anti-malarial chemotherapeutic target, and we have developed a heterologous expression system for this enzyme in the *E. coli* NADH dehydrogenase knockout strain ANN0222 (generously provided by Prof. Thorsten Friedrich, Freiburg) to facilitate its physicochemical and enzymological characterisation [3]. PfNDH2 represents a metabolic choke point in the respiratory chain of *P. falciparum* mitochondria and is the focus of a drug discovery programme towards the development of a novel therapy for uncomplicated malaria. Here we describe a miniaturised assay for recombinant PfNDH2 with robust assay performance measures that has been utilised for the high throughput screening (HTS) of small molecule inhibitors. The objectives of the HTS were to (i) increase the number of selective PfNDH2 inhibitors and (ii) to expand the number of inhibitor chemotypes. At the time of screening, only one proof of concept molecule, 1-hydroxy-2-dodecyl-4-(1H)quinolone (HDQ), was known to have PfNDH2 inhibitory activity ($IC_{50} = 70$ nM) [3,4]. This molecule was used to initiate a primary similarity-based screen of 1000 compounds from a compound collection of 750 000 compounds (curated by Biofocus-DPI). A range of chemoinformatics methods and filters were applied to the hits from this initial phase in order to perform a hit expansion screen on a further about 16 000 compounds. The chemoinformatic strategy allowed us to cover about 16% diversity whilst screening just about 2% of the compound collection. The HTS resulted in a hit rate of 0.29% and 150 compounds were progressed for potency against PfNDH2. Of these compounds, 50 were considered active with IC_{50}s ranging from 100 nM to 40 µM. Currently, seven distinct chemotypes are being progressed from hit to lead using traditional synthetic medicinal chemistry strategies.

References

[1] Biagini GA *et al.* (2006) *Antimicrob. Agents Chemother.* **50:** 1841-1851.
[2] Fisher N *et al.* (2007) *Trends. Parasitol.* **23:** 305-310.
[3] Fisher N. *et al.* (2009) *Meth. Enzymol.* **456:** 303-320.
[4] Saleh A *et al.* (2007) *Antimicrob. Agents Chemother.* **51:** 1217-1222.

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9P.4 Mitochondrial function and idebenone: A good therapy for Leber's hereditary optic neuropathy?

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Idebenone [2,3-dimethoxy-5-methyl-6(10-hydroxidecyl)-1,4-benzoquinone] is a synthetic analogue of coenzyme Q10 (CoQ10), an essential constituent of the mitochondrial electron transport chain and a powerful antioxidant. Idebenone is also a good electron carrier in the mitochondrial respiratory chain. Quinones (including idebenone) have also been shown to affect the mitochondrial permeability transition (PT) pore (PTP) a high-conductance inner membrane channel modulated by the proton electrochemical gradient and by many signaling molecules. PTP links oxidative stress to cell death and seems to be involved in Leber's hereditary optic neuropathy (LHON) and other pathologies of neurological interest. Given these complex effects of idebenone on cellular bioenergetics we have investigated its effects on bioenergetics and PTP modulation in intact cells. Our preliminary results indicate that: (i) idebenone modulates the PTP *in situ* through an interaction with NEM-sensitive thiols, with an effect that can be inhibited by Cyclosporin A (CsA); (ii) DTT prevents the PTP-inducing effects of idebenone, and promotes electron transfer from idebenone to complex III of the respiratory chain bypassing the lack of complex I activity; (iii) in the presence of DTT, idebenone considerably increases antimycin A-sensitive respiration both in normal and in RJ206 cells (harboring the 3460/ND1 LHON mutation) and XTC.UC1 thyroid oncocytoma cells (bearing a disruptive frameshift mutation in the MT-ND1 gene which impairs complex I assembly). The key question of whether idebenone-supported respiration is used for ATP synthesis is being addressed.

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9P.5 Dietary supplementation with docosahexaenoic acid, but not eicosapentanoic acid, remodels cardiac mitochondrial phospholipid fatty acid composition and prevents permeability transition

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Treatment with the ω-3 polyunsaturated fatty acids (PUFAs) docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) exerts cardioprotective effects in patients, and suppresses Ca^{2+}-induced opening of the mitochondrial permeability transition pore (MPTP) *in vitro*. These effects are associated with increased DHA and EPA and lower arachidonic acid (ARA) in cardiac phospholipids. ARA is implicated in inflammation and induction of MPTP opening. While clinical studies suggest the triglyceride lowering effects of DHA and EPA are equivalent, there is growing evidence that DHA may be superior at remodeling mitochondrial phospholipids and preventing MPTP. Therefore we compared the effects of dietary supplementation with the ω-3 PUFAs DHA and EPA on cardiac mitochondrial phospholipid fatty acid composition and Ca^{2+}-induced MPTP opening. Rats were fed either a control (CTRL) low-fat chow, or a similar diet supplemented with either DHA or EPA only at 2.5% of energy intake for 8 weeks. These doses of DHA and EPA are comparable to about 5 g/day in humans. Cardiac mitochondria were isolated and analyzed for Ca^{2+}-