Raman microscopy and imaging in malaria research

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Strategies for drug design in the treatment of malaria aim at producing effective, non-toxic and inexpensive therapeutic agents for use in Africa, Asia and South America where the combined mortality rate is over one million per year with some 300-500 million people afflicted with the disease.[1] Most deaths occur in children under 5 years of age and even in hospitals with good facilities child mortality from cerebral malaria is ~20%. Only 3 of the 1223 new drugs developed during 1975-1996 were antimalarials.[2] The need for potent low-cost drugs is therefore ever increasing, yet effective ways to screen for new drugs remains elusive.

During the intra-erythrocytic phase of the malaria parasite's life cycle, the parasite can degrade up to 75 % of an infected cell's haemoglobin. While haemoglobin proteolysis yields requisite amino acids, it also releases toxic free haem (Fe(III)PPIX), which can kill the parasite by disrupting cell membranes. To balance the metabolic requirements for amino acids against the toxic effects of free haem, malaria parasites have evolved a detoxification method, which involves the formation of a crystallised haem aggregate known as haemozoin. Quinoline blood schizonticides such as chloroquine and quinine are thought to inhibit haem aggregation by binding to monomeric or dimeric haematin.[3] This results in a higher concentration of free haem in the food vacuole that is capable of inducing membrane lysis in the parasite, especially when complexed to chloroquine.[4]

Given that quinoline drugs such as chloroquine and quinidine concentrate to millimolar levels in the food vacuole a spectroscopic approach to screening such drugs in living cells would seem a logical alternative to chemically based and morphological methods. We have developed methodology that allows for Raman spectroscopy and imaging of haemozoin in living cells, using laser excitation frequencies in the near-IR that we have discovered give rise to enhanced signals.[5] We have demonstrated that these enhanced signals result from an Aggregated Enhanced Raman Scattering (AERS) mechanism that involves energy in the form of an exciton migrating throughout the porphyrin network.[6] Initial resonance Raman studies by our group have shown that drugs including chloroquine, quinidine and arteminisins inhibit β-haematin (synthetic haemozoin) formation. The Raman spectra (to be presented) clearly show evidence of the haematin pre-cursor following incubation for 2 hours whereas the controls (2 hour incubation without antimalarial) do not. The technique shows enormous promise as an *in vivo* drug screening tool and provides further armory in the fight against malaria.

References

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