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# Differences between synthetic β-haematin and native hemozoin crystals

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*Plasmodium falciparum* causes the most severe/lethal form of malaria, a parasitic infection that affects 500 million people worldwide and leads to the death of nearly one million/year, about 91% being due to *P. falciparum* [1]. The malaria pigment or hemozoin crystals (HZ) are formed in an enzyme-independent polymerization of heme released during haemoglobin digestion by the parasite during the intra-erythrocytic life cycle [2]. The quinoline and artemisinin based antimalarials (the only available treatment options) appear to act by disrupting the formation of HZ. Therefore, the development of antimalarial agents based on the physicochemical process of heme crystallization appears a good option to drug design.

We have produced β-haematin following an assay adapted from the method described by Basilico et al, [3] with modifications. Briefly, hemin (ferriprotoporphyrin IX chloride; Sigma-Aldrich) was dissolved NaOH and polymerized by the addition of acetic acid and incubation at 37°C. Resulting pellet washed with dimethyl sulfoxide (DMSO) to remove unpolymerized hemin and resuspended in $H_2O$. To extract native HZ (nHZ) from *P. falciparum* (clone Dd2)-infected erythrocytes, when parasitemia reached >10%, parasites were harvested by saponin lysis, washed with phosphate-buffered saline (PBS) and sonicated in 2% sodium dodecyl sulfate (SDS), further washed in 2% SDS and pellet was resuspended (10 mM Tris-HCl (pH 8.0); 0.5% SDS; 2mg/ml of proteinase K) and incubated at 37°C overnight. The nHZ pellet was washed in 2% SDS and in distilled $H_2O$, then resuspended in distilled $H_2O$ water and sonicated again prior to use to minimize aggregation and maintain the nHZ in suspension. β-haematin and native nHZ crystals were analysed by Scanning Electron Microscopy (JEOL 7001FSEM) (Fig.1; A, B.1 and C.1) and Transmission Electron Microscopy (Hitachi H8100) (Fig.1; B.2 and C.2).

Here we report the crystal morphology of nHZ produced by the *Plasmodium falciparum* clone Dd2 and of β-haematin produced with the above protocol. Chemically, HZ is a stable dimer of iron(III)(protoporphyrin[PP]-IX), also known as β-hematin and consists of heme units dimerized through reciprocal iron-carboxylate bonds [4]. Even though the parasite nHZ and synthetic β-hematin are isostructural [4], there are large differences in the crystal morphologies of nHZ (Dd2) and β-hematin as evidenced by SEM and TEM images (Fig.1). nHZ crystals are remarkably uniform in size and shape, adopting a parallelipede morphology with high aspect ratio of about 800x200x200 nm (Fig. 1 C1 and C2). Different protocols to prepare β-haematin yield diverse material, which can be poorly crystalline and heterogeneous within the same sample [5]. The present results show that β-haematin is rather homogeneous and constituted by needle-like particles of sizes similar to those presented by nHZ ones (compare B1 with C1). Higher resolution observations evidence differences between the β-haematin and nHZ materials, since the latter seems constituted by uniform flat layers and the former shows flaked ones (Fig. B2).

Morphology seems to be of biological relevance, since structure-function studies revealed that the size and shape of the synthetic crystals influence their ability to activate, for instance, the inflammatory responses *in vitro* and *in vivo* [6]. Several protocols have been developed to measure drug inhibition of heme crystallization *in vitro* [6] although it has been difficult to standardize these assays. In the present study, we report a new method for β-haematin preparation that renders homogenous crystals, both in shape and size, favourable for reproducible drug inhibition of heme crystallization assays.

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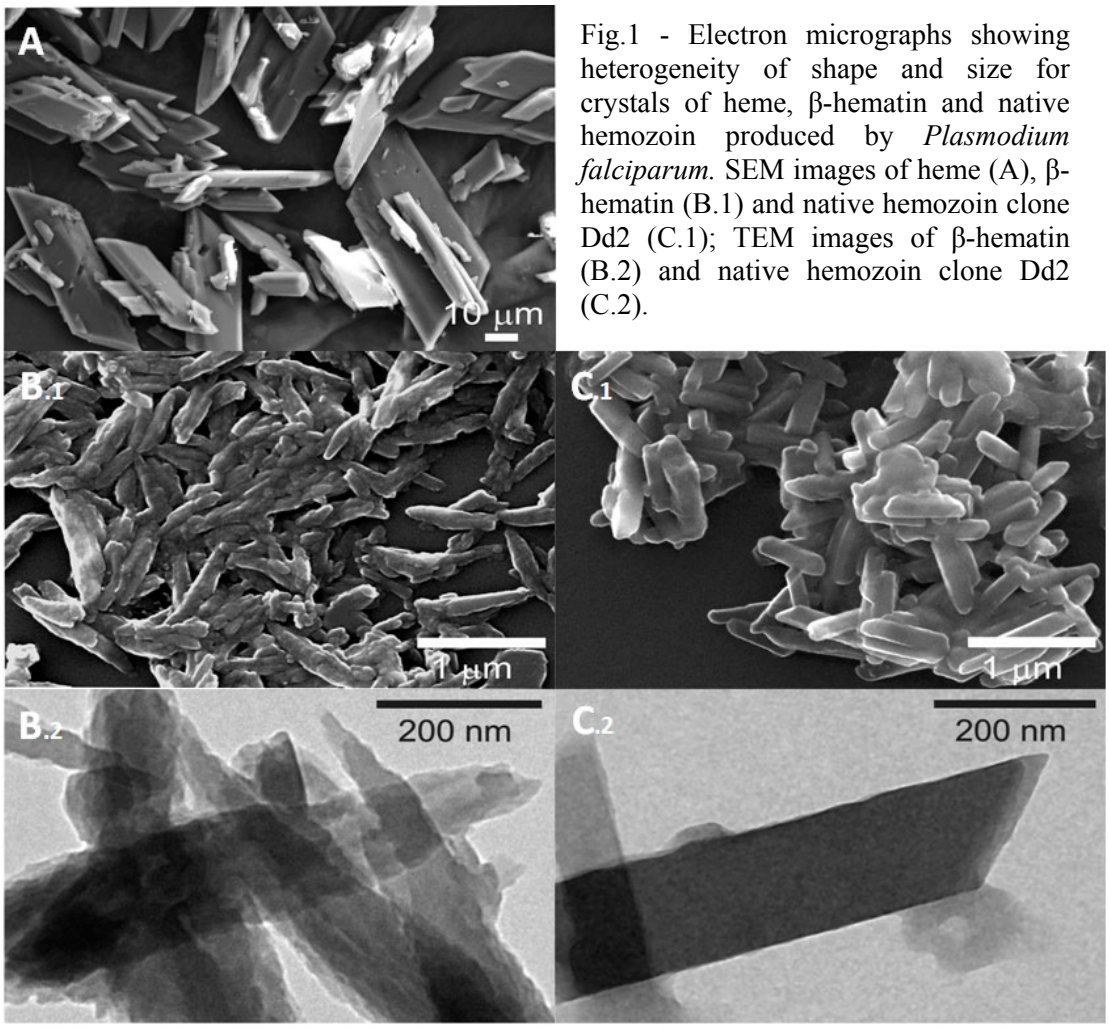


Fig.1 - Electron micrographs showing heterogeneity of shape and size for crystals of heme, β-hematin and native hemozoin produced by *Plasmodium falciparum.* SEM images of heme (A), β-hematin (B.1) and native hemozoin clone Dd2 (C.1); TEM images of β-hematin (B.2) and native hemozoin clone Dd2 (C.2).

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