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Biomimetic Approaches to Understanding the Mechanism of Haemozoin Formation

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1. Introduction

Haemozoin, also known as malaria pigment has been attracting increasing attention over the last twenty years. Interest spans subjects as diverse as immunology, medicinal chemistry, bioinorganic chemistry and biophysics and has mainly been concerned with elucidation of its structure, investigation of its role as a drug target, possible role as a diagnostic marker and immunological effects of haemozoin on the host (Hänscheidt et al., 2007). This chapter is restricted to a review of the state of the art in the physico-chemical aspects of haemozoin structure and formation.

1.1 A brief history

The discovery of haemozoin predates knowledge of the malaria parasite and indeed of the existence of infectious microorganisms. It was first described in a book by Giovanni Maria Lancisi published in 1717, who observed a black discolouration in the brains of cadavers of malaria victims during a fever outbreak in Rome (Lancisi, 1717). The pigment was rediscovered in 1847 by a German physician, H. Meckel and subsequently conclusively linked to malaria by the pathologist Rudolf Virchow in 1849 (Virchow, 1849). Originally believed to be melanin, it finally played a role in the discovery both of the malaria parasite by Laveran in 1880, whose original microscope drawings show clear evidence of pigment granules (Laveran, 1880), and of the role of mosquitoes in transmitting the parasite by Ronald Ross (Ross, 1897). Later, T. Carbone and W. H. Brown independently demonstrated that it is not melanin, but contains haem, which is the chromophore responsible for its brown-black colour (Carbone, 1891; Brown, 1911). For eight decades following Brown's paper, haemozoin was largely ignored. Indeed, as late as 1990, one school of thought was that it merely consisted of partially degraded haemoglobin (Hb) fragments within the parasite digestive vacuole (Goldie et al., 1990), while others suggested that it was a specific haemoprotein (Ashong et al., 1989). However, in 1987, Fitch and Kanjananggulpan demonstrated that the intact pigment could be freed of protein using detergents and proteases and that it consisted entirely of iron(III)protoporphyrin IX (Fe(III)PPIX; Fitch & Kanjananggulpan, 1987). Finally, in 1991 Slater et al. elucidated the chemical composition of haemozoin (Slater et al., 1991). Our current understanding of this substance traces back to this paper. Indeed, a search of the ISI Web of Science database reveals that over 95% of the literature dealing with haemozoin has been published since 1991.

1.2 Biological occurrence and origin

Haemozoin has been shown to be present in the trophozoite stage of several species of malaria parasite, including all of the species that infect humans, namely *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi* (Noland et al., 2003). The same study also demonstrated the presence of haemozoin in *P. brasilianum*, *P. yoelii* and *P. gallinaceum*, parasites that infect New World monkeys, rodents and birds respectively. The last decade has also seen the discovery of haemozoin in another protozoan, the bird-infecting parasite *Haemoproteus columbae* (Chen et al., 2001), as well as a number of other blood-feeding organisms from unrelated phyla. These include the helminth worms *Schistosoma mansoni* and *Echinostoma trivolvis* (Oliveira et al., 2000; Chen et al., 2001) and the blood-sucking insect *Rhodnius prolixus* (Oliveira et al., 1999).

A common feature of these organisms is that haemozoin formation occurs within an acidic environment. In *R. prolixus* haemozoin forms within perimicrovillar membrane-derived vesicles (PMVM) within the acidic insect midgut, while in the *S. mansoni* gut it is associated with lipid droplet-like structures (Oliveira et al., 2005). It was previously assumed that haemozoin formation in *Plasmodium* occurs in the aqueous lumen of the acidic digestive vacuole. However, recently it has been shown in *P. falciparum* that haemozoin formation is intimately associated with small lipid droplet-like structures dubbed “lipid nanospheres” (Coppens & Vielemayer, 2005; Pisciotta et al., 2007). Isolation of purified haemozoin with associated lipids by sucrose cushion centrifugation allowed the lipids to be characterized by thin layer chromatography and mass spectrometry. These investigations showed that the lipid component is predominantly a 4:2:1:1:1 mixture of monostearoylglycerol (MSG), monopalmitoylglycerol (MPG), 1,3-dipalmitoylglycerol (DPG), 1,3-dioleoylglycerol (DOG) and 1,3-dilinoleoylglycerol (DLG) respectively (Pisciotta et al., 2007). No proteins could be detected associated with haemozoin.

The processes leading up to haemozoin formation have been most closely studied in *P. falciparum*. During its asexual blood stage the parasite goes through a 48 h cycle consisting of merozoite, ring, trophozoite and schizont stages. The parasite lives within the host red blood cell throughout all but the first of these stages. During the late ring stage the organism begins to form a digestive vacuole (DV) which becomes prominent during the trophozoite stage (Hanssen et al., 2010). There is some controversy over the initial stages of DV assembly, with a recent report that its formation begins with a macropinocytotic event dubbed a “big gulp” (Elliot et al., 2008), while others find no support for this hypothesis (Abu Barkar et al., 2010). There is consensus among these studies that red cell cytoplasm is taken up via an endocytosis process involving a structure called a cytostome later in the trophozoite stage. Regardless of these details, there is clear evidence that by the late trophozoite stage (36 h into the cycle) parasites have already digested about 60% of the red cell Hb (Egan et al., 2002). Within the DV, Hb is hydrolysed to peptides by a battery of enzymes consisting of aspartic proteases (plasmepsins I, II and IV and histo-aspartic protease or HAP), cysteine proteases (falcipains 1, 2 and 3) and a zinc metalloprotease (falcilysin) (Banerjee et al., 2002; Rosenthal et al., 2002; Eggleston, 1999). The peptide fragments are subsequently hydrolysed to amino acids in the parasite cytoplasm by an aminopeptidase enzyme (Stack et al., 2007), and a relatively small fraction are utilized for protein synthesis (Krugliak et al., 2002). During this digestive process, the haem released from Hb is oxidised and at least 95% is converted to haemozoin (Egan et al., 2002). When merozoites are released at the end of the 48 h cycle, haemozoin is deposited into the

circulation of the host and is phagocytosed by monocytes and neutrophils (Day et al., 1996). The final fate of haemozoin in the host is largely unexplored, but in mice it can persist in both the circulation and spleen for at least 270 days (Levesque et al., 1999). From the point of view of the parasite, this is a detoxification process, because the potentially toxic haem molecule is first sequestered as a solid within the DV and then discarded into the host.

Much less is known about these processes in other organisms. A recent study in the insect *R. Prolixus* has shown that haemozoin formation is also highly efficient and that haemozoin is the only detectable iron species in the midgut following Hb digestion. The process is promoted by PMVM, with the lipids playing a major role (Stiebler et al., 2010a). A protein, α -glucosidase may also play a role in haemozoin formation in this organism (Mury et al., 2009).

1.3 Haemozoin formation *in vivo*

Following the discovery that haemozoin consists solely of Fe(III)PPIX, it was initially proposed to be a coordination polymer (Slater et al., 1991). Subsequently Slater and Cerami showed that parasite extracts support conversion of Fe(III)PPIX to haemozoin under acidic conditions (pH 5.5) and proposed that an enzyme (haem polymerase) is responsible for catalysing its formation (Slater & Cerami, 1992). Although no such enzyme was ever isolated, subsequent studies revealed that histidine rich protein II (HRP II), a protein produced in large quantities by the parasite, can support the process (Sullivan et al., 1996). Later however, it was shown that that most HRP II is not localised in the DV and that the efficiency of HRP in converting Fe(III)PPIX to the synthetic counterpart of haemozoin (β -haematin) is in any case low (Papalexis et al., 2001; Pandey et al., 2003). More recently, a *P. falciparum* clone lacking genes for both HRP II and HRP III was found to form haemozoin normally (Sullivan, 2002; Noland et al., 2003). Furthermore, the genomes of *P. vivax* and *P. beghei* lack HRP homologues, but also form haemozoin (Sullivan, 2002). Recently, another protein, dubbed haem detoxification protein (HDP), has been proposed to be involved in haemozoin formation (Jani et al., 2008). This protein was shown to be able to bind two to three equivalents of Fe(III)PPIX and to convert about 75% of the Fe(III)PPIX present to haemozoin within 20 min at 5 μ M concentration. Given direct evidence that haemozoin formation occurs in lipid nanospheres (Pisciotta et al. 2007), the precise role of this protein in haemozoin formation remains to be elucidated, but it has been suggested that it may work in conjunction with lipids, possibly acting as a chaperone (Jani et al., 2008).

Even long before the recent discoveries of the relationship between haemozoin and lipids, there had been a number of studies suggesting that lipids mediate its formation. The proposal was first made by Bendrat et al. (1995) and subsequently supported by Dorn et al. who showed that an acetonitrile extract of *P. falciparum* trophozoites supports haemozoin formation (Dorn et al. 1998). Fitch and co-workers also demonstrated that chloroform extracts of infected and uninfected red blood cells, monooleoylglycerol (MOG) and DOG as well as certain fatty acids and detergents efficiently support its formation (Fitch et al. 1999).

Two other early suggestions for the mechanism of haemozoin formation *in vivo* include spontaneous formation and autocatalysis (Egan et al., 1994; Dorn et al., 1995). Current evidence strongly points to a major role of lipids in a process of self-assembly. Much of what we know of this process has been derived from biomimetic mechanistic studies, as discussed below.

2. Structure, physical properties and spectroscopy

2.1 Structure

When Fitch and Kanjananggulpan first isolated purified haemozoin in 1987 (Fitch & Kanjananggulpan, 1987), they suggested that it was identical to a synthetic Fe(III)PPIX precipitate obtained from acidic solutions known since the 1930s as β -haematin (Hamsik, 1936). However, at the time little was known about the structure of either. In 1991 Slater et al. demonstrated that β -haematin prepared by heating haemin (Cl-Fe(III)PPIX) in 4.5 M acetic acid at pH 4.5 and 70 °C is identical to haemozoin with respect to its infrared spectrum, X-ray powder diffraction pattern and solubility in DMSO and alkaline aqueous solution. Using extended X-ray absorption fine structure (EXAFS) spectroscopy they demonstrated that the Fe(III)PPIX molecules are linked via coordination of the haem-propionate group of one Fe(III)PPIX to the Fe(III) centre of another (Slater et al, 1991). While this model of the immediate bonding environment around the Fe(III) centre has proved to be correct, their suggestion that haemozoin is polymeric (Figure 1) was later found to be wrong. Nonetheless, this is a sentinel study, because it opened the way to routinely prepare synthetic haemozoin (β -haematin) on which much of our subsequent understanding is based.

Any lingering doubts that the structure of haemozoin may be different from that of β -haematin, or that β -haematin might be produced only during the extraction procedure was removed when Bohle et al. showed that the high resolution X-ray powder diffraction pattern of freeze-dried whole parasitized red blood cells obtained using synchrotron radiation is identical to that of β -haematin prepared by dehydrohalogenation of haemin with 2,6-lutidine in rigorously dry methanol (Bohle et al. 1997). In this study the β -haematin crystal was found to belong to the centrosymmetric space group P-1 and the structure was proposed to consist of two anti-parallel polymer chains linked by hydrogen bonds (Figure 1). Finally, in 2000 the same group solved the structure of β -haematin from the powder diffraction pattern by Rietveld refinement (Pagola et al. 2000). This showed that it is not in fact a polymer, but rather a crystal formed from cyclic dimers of Fe(III)PPIX each linked via coordination of the propionate group of one porphyrin to the Fe(III) centre of the other and vice versa. These dimers are linked to neighbouring dimers by hydrogen bonds between the remaining uncoordinated and protonated propionic acid groups (Figure 1). The structure demonstrates that β -haematin is really a haematin anhydride and indeed this change in nomenclature has recently been proposed (Bellemare et al., 2009; Walczak et al., 2010 and 2011).

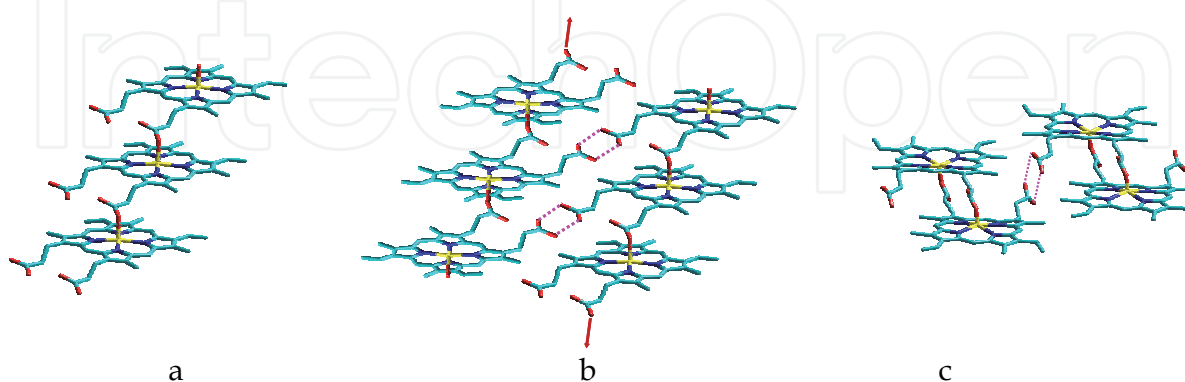


Fig. 1. Knowledge of the structure of haemozoin has progressed over time. (a) A coordination polymer proposed by Slater et al. in 1991, (b) antiparallel coordination polymer chains proposed by Bohle et al. in 1997 and (c) the structure determined by Rietveld refinement of the X-ray powder pattern (Pagola et al., 2000).

Structures of natural haemozoin have only been reported fairly recently (Figure 2). The unit cell dimensions of haemozoin from *S. mansoni* and *R. prolixus* were determined in 2005 and structures determined by Rietveld fitting of experimental parameters obtained from the X-ray powder diffraction pattern collected with synchrotron radiation (Oliveira et al., 2005). In this study, the atomic coordinates reported for the original β -haematin structure were used and only the unit cell and profile parameters were refined. Thereafter, the structures were subjected to simulated annealing to find minimum energy structures and the *R. prolixus* structure was found by Rietveld refinement with constrained inter-atomic distances. The *S. mansoni* structure was fitted to the *R. prolixus* structure. This study unequivocally confirmed that the crystals formed by these organisms are essentially the same as the malaria pigment. Unit cell parameters and the Fe(III)–O bond length were found to be almost the same as those of β -haematin (Table 1), with subtle differences accounted for by differences in crystallization conditions. Hydrogen bonding distances between the propionic acid groups were also very similar to β -haematin. More recently, the structure of haemozoin from *P. falciparum* has also been solved from the X-ray powder diffraction pattern (Klonis et al., 2010). This study used a somewhat different method to solve the structure (the so-called maximum entropy-based pattern fitting approach). Nonetheless, the structure originally reported by Pagola et al. (2000) was essentially confirmed (Figure 2). The unit cell parameters and Fe(III)–O bond length were again found to be very similar (Table 1). The only significant difference in this structure was evidence of greater disorder in the Fe(III)–O bonds than in the original β -haematin structure, which may be related to lower occupancy of the O site in the structure as had previously been suggested based on EXAFS studies (Walczac et al., 2005). Somewhat controversially, Klonis et al. (2010) suggest that haemozoin should be viewed as an assembly of π - π dimers which are linked by μ -propionate bonds and co-planar, laterally displaced (so-called P-type) interactions (Figure 2) rather than μ -propionate (μ -Pr) dimers linked by π - π interactions and hydrogen bonds. Interestingly, until very recently there has been no evidence that the μ -Pr dimer was stable or could be isolated which tended to support this view, although it should be noted that in the crystal all of these interactions occur together, so this is merely a matter of how one views the crystal and has no real physical meaning. It is however pertinent to the question of how the crystal assembles.

	unit cell dimensions						bond length
	a (Å)	b (Å)	c (Å)	α (°)	β (°)	γ (°)	Fe–O (Å)
β -haematin	12.196(2)	14.684(2)	8.040(1)	90.22(1)	96.80(1)	97.92(1)	1.898(4)
<i>Rhodnius</i>	12.206(12)	14.776(8)	8.028(5)	90.48(9)	97.09(7)	97.38(7)	1.82
<i>Schistosoma</i>	12.21(2)	14.784(15)	8.034(9)	90.54(15)	97.10(12)	97.23(12)	–
<i>Plasmodium</i>	12.187(2)	14.692(2)	8.030(1)	90.94(1)	96.99(1)	96.81(1)	1.91(8)

Table 1. Key structural comparisons between β -haematin (Pagola et al., 2000) and haemozoin from *Rhodnius prolixus*, *Schistosoma mansoni* (Oliveira et al., 2005) and *Plasmodium falciparum* (Klonis et al., 2010).

Very recently Walczak et al. have reported two haemozoin analogues based on Fe(III)meso- and deuteroporphyrin that are slightly soluble in DMSO (Walczak et al., 2010; Walczak et al., 2011). Using EXAFS, the authors demonstrated that the anhydrides of these two iron porphyrins have similar local order to haemozoin and that this persists in DMSO at least in the case of Fe(III)mesoporphyrin. This is the first evidence of a non-Fe(III)PPIX analogue of haemozoin and the first example of a stable, soluble μ -Pr dimer. It is likely that these compounds will significantly aid our understanding of haemozoin in the future.

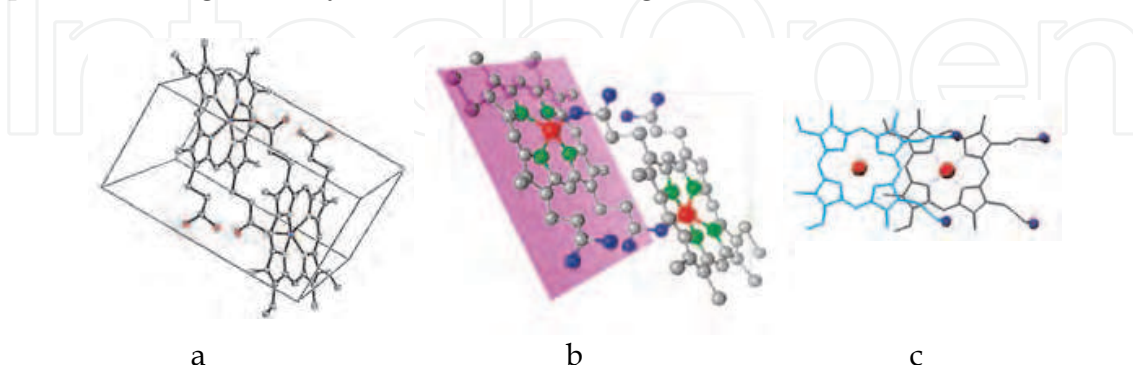


Fig. 2. The structures reported for natural haemozoin. (a) The unit cell structure of haemozoin from *R. prolixus* and *S. mansoni* proposed by Oliveira et al. (2005), (b) haemozoin from *P. falciparum* (Klonis et al., 2010) and (c) P-type interactions between porphyrin rings in haemozoin (from Klonis et al., 2010). (a) Reprinted from: M.F. Oliveira, S. Kycia, A. Gonzales, A.D. Kosar, D.S. Bohle, E. Hempelmann, D. Menezes, M. Vannier-Santos, P.L. Oliveira, S.T. Ferreira, Structural and morphological characterization of hemozoin produced by *Schistosoma mansoni* and *Rhodnius prolixus*, FEBS Lett. 579, 6010– 6016. ©Federation of European Biochemical Societies (2005), with permission from Elsevier (<http://www.sciencedirect.com/science/journal/00145793>). (b) and (c) Reprinted with permission from: N. Klonis, R. Dilanian, E. Hanssen, C. Darmanin, V. Streltsov, S. Deed, H. Quiney, L.Tilley, Hematin-hematin self-association states involved in the formation and reactivity of the malaria parasite pigment, hemozoin. *Biochemistry* 49 (2010) 6804–6811. © The American Chemical Society (2010).

2.2 Physical properties and spectroscopy

The ability to synthesize haemozoin in the laboratory has opened the way to most of what we know about the physical and spectroscopic properties of this material. Despite the fact that haemozoin and β -haematin are structurally identical at the molecular level, X-ray powder diffraction and scanning electron microscopy studies have demonstrated that the crystal size, crystallinity and uniformity of β -haematin prepared in different ways varies. Thus, preparation by abstraction of HCl from haemin in dry methanol produces larger, less disordered and more uniformly crystalline material than does preparation from 4.5 M aqueous acetate at pH 4.5 and 70 °C (Bohle et al. 2002). On the other hand, the material produced via the non-aqueous route is more absorptive to water than is the less ordered material obtained from aqueous medium. The former can reversibly absorb up to 14% by mass H₂O, while the latter product absorbs a smaller quantity (Bohle et al. 2003). The determination of the relationship between the external macroscopic morphology of the crystal (crystal habit) and the unit cell structure by Buller et al. (2002) (Figure 3) has been important in later work that has attempted to understand the molecular mechanism of haemozoin formation.

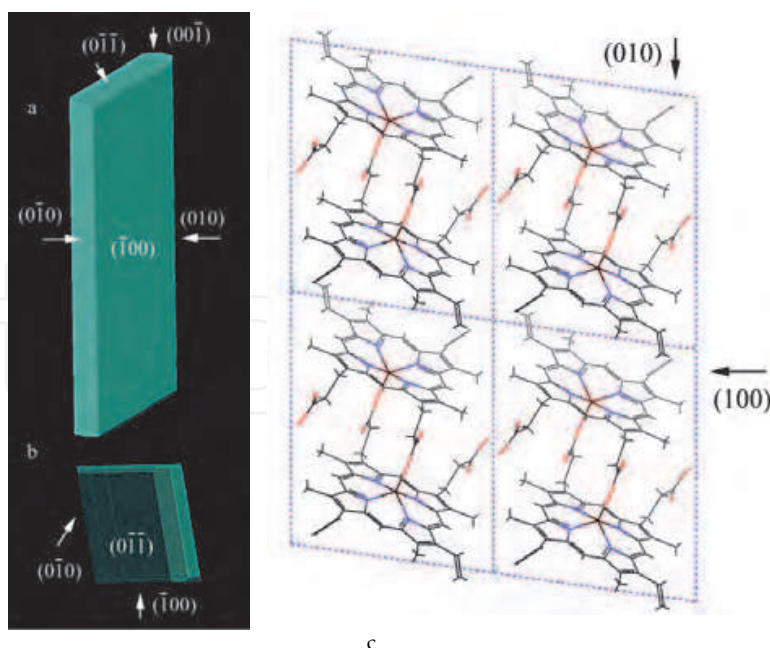


Fig. 3. The proposed relationship between crystal habit (external morphology) of haemozoin and its unit cell structure proposed by Buller et al. (2002). (a and b) The predicted crystal faces. (c) The corresponding unit cell structure looking down the c axis of the crystal.

Reprinted with permission from: R. Buller, M.L. Peterson, Ö. Almarsson, L. Leiserowitz, Quinoline binding site on malaria pigment crystal: a rational pathway for antimalaria drug design. *Cryst. Growth Des.* 2 (2002) 553–562. ©The American Chemical Society (2002).

Magnetic properties have been investigated using X-band electron paramagnetic resonance (EPR) spectroscopy and magnetic Mössbauer spectroscopy and have unequivocally demonstrated that the Fe(III) centre exists in a high spin $S = 5/2$ state (Bohle et al. 1998) with very weak to negligible magnetic exchange between Fe(III) centres later confirmed by multi-frequency high field EPR (Sienkiewicz et al. 2006). The uv-visible spectrum of β -haematin has also been determined and its luminescence properties investigated (Bellemare et al., 2009). These authors have shown that autofluorescence of haemozoin and β -haematin arises from excitation of the Q_0 band of the porphyrin at 555 nm which corresponds to the lowest energy $\pi \rightarrow \pi^*$ transition in the molecule. Fluorescence at 577 nm is only observed in the crystalline product, either very dry or fully hydrated, while partially hydrated material is non-fluorescent as is poorly crystalline product. This strongly indicates an exciton recombination mechanism and the authors ascribe the fluorescence to a Frenkel-type exciton process based on its fluorescence lifetime. The observed fluorescence is likely to prove useful in characterizing the crystallinity of synthetic haemozoin produced under different conditions, but has not been exploited so far.

Finally, vibrational spectra of haemozoin and β -haematin have been investigated in some depth. Fourier-transform infrared (FTIR) spectroscopy has been widely used to characterise β -haematin and to demonstrate that the synthetic product is the same or similar to natural haemozoin. Indeed, the infrared spectra are essentially identical. Bands at 1660 and 1210 cm^{-1} have been assigned to stretching of the carboxylate double and single bonds respectively in the Fe(III)-coordinated group (Slater et al., 1991). Resonance Raman (rR) spectra of β -haematin and haemozoin have recently generated considerable interest. Again, spectra of the synthetic and natural products are virtually identical. A particularly

noteworthy feature is the observation that many bands are dramatically enhanced when the substance is excited with long wavelength radiation. In particular, band enhancements when β -haematin or haemozoin is excited at 780 nm, which does not correspond to an absorbance band, has been ascribed to an exciton process in the crystal lattice (Wood et al., 2003). This phenomenon permits haemozoin to be readily distinguished from Hb and hence has permitted fluorescence imaging of haemozoin in infected erythrocytes. It has further been adapted to acoustic levitation micro-Raman spectroscopy, which has been suggested to be the basis for a possible hand held diagnostic device (Puskar et al., 2007).

3. Mechanistic studies on haemozoin formation

Although there has been a recent report in which the growth of haemozoin crystals in individual parasites has been observed through the blood-cycle using spinning-disk confocal microscopy (Gligorijevic et al., 2006), the growth of haemozoin in the organism does not provide direct insight into mechanism. This is because the rate of formation in the parasite will reflect rates of Hb uptake from the red blood cell cytoplasm, rate of Hb proteolysis as well as rate of haemozoin formation. Given that the concentration of non-haemozoin haem in mature trophozoites cannot be detected by Mössbauer spectroscopy, thus representing less than 5% of iron present in the parasite (Egan et al., 2002), it would seem likely that uptake and digestion of haemoglobin are probably rate limiting. As a result, what we know of the kinetics and mechanism of haemozoin formation arises from biomimetic studies of β -haematin formation.

3.1 β -Haematin formation in homogeneous solvent systems

The first kinetic studies of β -haematin formation were carried out in 4.5 M acetate/acetic acid at pH 4.5 and at elevated temperatures (typically 60 °C). The earliest such study attempted to follow the process using Mössbauer spectroscopy (Adams et al., 1996). Apart from being very expensive in instrument time (>40 h of data collection), signal to noise ratio proved inadequate, resulting in the incorrect conclusion that the process is zero-order, occurring at a constant rate until completion. A later study using infrared absorbance showed sigmoidal kinetics that can be modelled by the Avrami equation (equation 1), a semi-theoretical equation that is often used to model solid state processes (Egan et al., 2001).

$$m/m_0 = \exp(-zt^n) \quad (1)$$

where m is the mass of unreacted starting material (haematin or $\text{H}_2\text{O-Fe(III)PPIX}$), m_0 is the initial mass of haematin, z is the rate constant, t is the reaction time and n is a constant known as the Avrami constant. For this type of system, n is expected to be an integer between 1 and 4 (Sharples, 1966). Under most conditions, in the acetate system n was found to be 4, a value which indicates sporadic (i.e. continuous and random) nucleation throughout the reaction, with three dimensional growth (spreading spheres) from the nucleation sites (Egan et al., 2001). A later study in 0.05 M benzoic acid/benzoate at pH 4.5 and 60 °C which used differential solubilisation in the presence of 5% pyridine to measure the process colourimetrically gave essentially the same result (Egan & Tshivhase, 2006). It was concluded from these studies that a key role of the carboxylic acid is to solubilise Fe(III)PPIX slightly at low pH, since haematin itself has low solubility at pH 4.5, requiring dissolution and redeposition. In the case of benzoic acid, electron withdrawing substituents

were found to enhance the rate, suggesting that π - π interactions between the aromatic ring and haematin may play a role in dissolving or disaggregating the amorphous haematin precipitate and facilitating conversion to the even less soluble, but presumably thermodynamically more stable β -haematin, with extrusion of water from the crystal (Egan & Tshivhase, 2006).

In a later study, Huy et al. (2007a) showed that water-miscible alcohols also promote β -haematin formation. The effectiveness of different alcohols (methanol, ethanol, *n*-propanol and *n*-butanol) was compared. It was found that the more hydrophobic the alcohol, the more it solubilised haematin and the faster the reaction. Furthermore, the alcohols were found to reduce surface tension of water in the same order. The energy barrier to crystal nucleus formation is known to depend on surface tension (Myerson, 1993), so the authors proposed that alcohols also play a role in reducing the energy barrier to crystallisation in this way.

A recent study on β -haematin formation in aqueous DMSO and solutions of several polyethylene glycols (PEGs) has provided direct support for the solubilisation hypothesis (Stiebler et al., 2010b). In this investigation it was demonstrated spectrophotometrically that Fe(III)PPIX solubility at pH 4.8 in 0.5 M acetate buffer increases linearly with increasing DMSO concentration and that the yield of β -haematin is directly proportional to the solubility of Fe(III)PPIX in this medium. The process was shown to follow Avrami kinetics with $n = 4$, at least in the presence of the highest concentration of DMSO used (27.7%). Apart from the low molecular weight PEG 300 which was found to both decrease the solubility of Fe(III)PPIX and decrease the rate of β -haematin formation relative to the already very slow control (spontaneous formation), PEG 3350, PEG 6000, PEG 8000 and PEG 22 000 were also shown to support β -haematin formation according to Avrami kinetics, with $n = 4$ for the first of these, and $n = 2$ for the remaining three. As in the case of DMSO, these PEGs were found to increase Fe(III)PPIX solubility, with yields of β -haematin being directly proportional to the extent of solubilisation. It was also demonstrated that, at least in aqueous DMSO, both extent of solubilisation and yield are proportional to water activity, with a decrease in water activity resulting in increased solubilisation and yield. This observation seems to suggest that the organic component may also play a role in dehydrating H₂O-Fe(III)PPIX and hence driving β -haematin formation.

Detergents have also been shown to support β -haematin formation. A pioneering study by Fitch et al. (1999) showed that the neutral detergents *n*-octylglucopyranoside and Tween-80 promote β -haematin formation with yields around 25% at pH 5 and 37 °C after 2 h. The anionic detergent SDS was found to have negligible activity at pH 5, with yields rising to about 25% at pH 4. In these studies *n*-octylglucopyranoside (20 mM) was slightly below its critical micellar concentration (cmc) of 23 mM, while Tween 80 (0.1%) and SDS (2.5%) were well above their respective cmc concentrations of 0.002 and 0.23%. In a more recent study, Huy et al. (2007b) have shown that the detergent Tween 20 gives a maximal yield of β -haematin at 37 °C and pH 4.8 (1 M acetate buffer) at a concentration close to 0.001 %, below the cmc of 0.006%. This suggests that detergents are most efficient at mediating β -haematin formation below their cmc values. Indeed, Carter et al. (2010) have investigated a series of detergents as mediators of β -haematin with yields ranging between 7 and 74% all well below their cmc values. NP 40, Tween 20 and Tween 80 were found to give the highest yields, ranging between 69 and 74%, while SDS, Triton X-100 and Chaps were found to be inefficient with yields between 7 and 10%. While it has been pointed out in both these recent publications that detergents are considered mimics of lipid membranes, it is noteworthy that the detergents appear to be most efficient under conditions where there are no micelles. At

these concentrations the detergents may be more akin to solvents such as alcohols and DMSO, or solutes such as PEGs. Possibly their action partly involves solubilisation and partly a decrease in surface tension, hence lowering the activation energy of crystallisation.

3.2 β -Haematin formation at solvent/water and lipid/water interfaces

In 2006 it was reported that β -haematin forms with extraordinary speed and efficiency at the interface of aqueous solution buffered at pH 4.8 and water immiscible alcohols, pentanol and octanol at 37 °C (Egan et al., 2006). These alcohols were chosen because octanol in particular is commonly used in medicinal chemistry as a mimic of lipid membranes to assess partitioning between aqueous medium and membranes. FTIR, XRD, rR and scanning electron microscopy (SEM) all clearly showed formation of β -haematin, with substantial formation occurring within 5 min using the aqueous/pentanol interface. Several factors motivated the investigation of its formation at aqueous/organic interfaces. Firstly, it had already been observed in *S. mansoni* that haemozoin crystals appeared to form at the lipid/water interface of lipid droplets (Oliveira et al., 2005). Secondly, a molecular dynamics study that showed that in vacuum a π - π dimer of H_2O -Fe(III)PPIX in which the axial water ligands are directed outwards spontaneously forms a kind of precursor of the β -haematin μ -Pr dimer in which the propionate group of one Fe(III)PPIX is attracted to the iron centre of the other. It was recognised that displacement of water from the opposite face is all that is needed for such a dimer to convert to the μ -Pr dimer. However, when modelled in water, no such precursor is formed because the propionate groups preferentially hydrogen bond to competing solvent molecules (Figure 4).

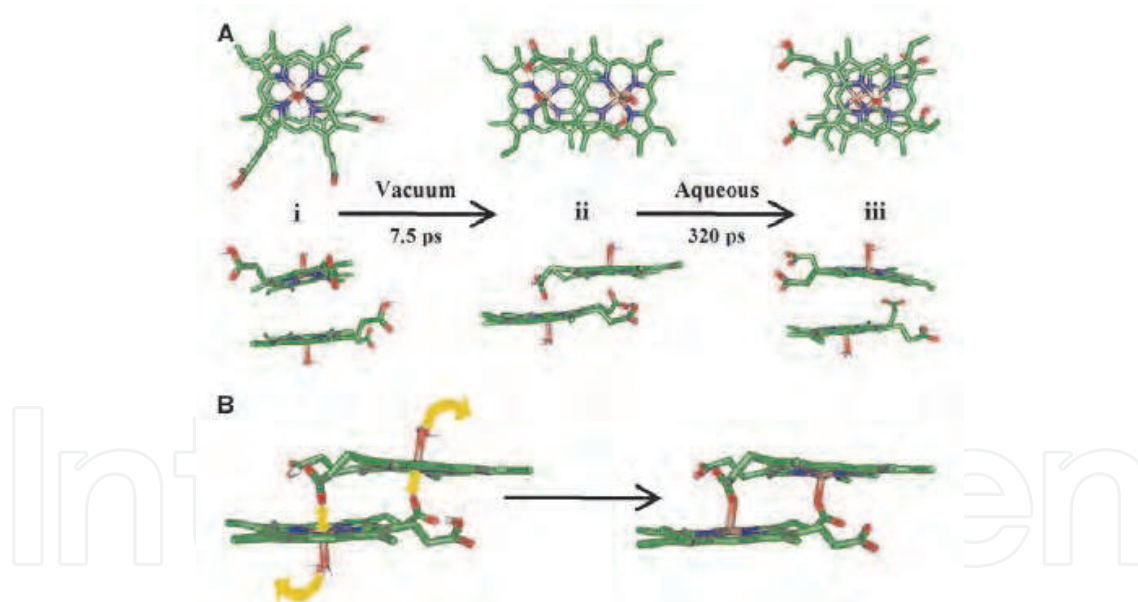


Fig. 4. A molecular dynamics simulation demonstrating (A) that two interacting haem molecules (i) form a haemozoin precursor rapidly in vacuum (ii), but that this is not stable in water because of competition for hydrogen bonding interactions with the solvent (iii). In (B) is the proposed process to convert the precursor to the μ -Pr dimer of haemozoin (Egan et al., 2006). Reprinted from: T.J. Egan, J. Y.-J. Chen, K.A. De Villiers, T.E. Mabotha, K.J. Naidoo, K.K. Ncokazi, S.J. Langford, D. Mcnaughton, S. Pandiancherri, B.R. Wood (2006).

Haemozoin (β -haematin) biomineralization occurs by self-assembly near the lipid/water interface, FEBS Lett. 580, 5105– 5110. ©Federation of European Biochemical Societies (2006), with permission from Elsevier (<http://www.sciencedirect.com/science/journal/00145793>).

Since Fe(III)PPIX can be expected to monomerise in purely organic media with low dielectric constants, it was surmised that the interface between aqueous and organic environments might facilitate β -haematin formation. Thirdly, haematin can be introduced as a solution directly to the interface using a syringe, thus avoiding precipitation of amorphous haematin and obviating the need to re-dissolve haematin, the apparent slow step in processes discussed above. In keeping with this prediction, it was confirmed that the interface is essential for the rapid conversion to β -haematin, since neither aqueous medium alone nor pentanol alone produced any product over the time scale of the study.

Recently a few additional solvents were investigated for their ability to mediate interfacial β -haematin formation (Hoang et al., 2010a). In this study, the long chain ester methyl laurate and the aromatic solvent toluene were found to be much less efficient at producing β -haematin (with yields of 40 and 42% respectively in 30 min) compared with octanol (83%) and pentanol (95%). The long chain alkane docosane was found not to mediate β -haematin formation at all. These findings suggest that the functional groups present at the interface play a major role in the process.

In addition to showing the efficacy of organic/aqueous interfaces in the formation of β -haematin, the 2006 paper also demonstrated that a solution of the neutral monoglyceride lipid 1-monomyristoyl glycerol (MMG) dissolved in acetone/methanol (1:10) and spread on the surface of the aqueous medium also efficiently promoted β -haematin formation (Egan et al., 2006). The initial assumption was that since acetone and methanol are fully miscible with water and only a very small volume was used relative to the aqueous medium, the lipid would be left spread over the surface. However, when grazing incidence X-ray diffraction (GIXD) and specular X-ray reflection (XR) were used to examine the surface (Figure 5), only a monolayer of lipid was seen on the surface in the absence of Fe(III)PPIX (de Villiers et al., 2009). When Fe(III)PPIX was present, this layer was evidently disturbed to the extent that no diffraction was seen, or was absent altogether. If sufficient time was allowed for β -haematin formation to occur, weak GIXD diffraction peaks corresponding to the (001), (020), (011), (031) and (131) planes of the crystal, but not the (100) planes were observed (Figure 5). Together with XR measurements showing clear evidence of the (100) Bragg peak (Figure 5), these data indicated that the β -haematin crystals were aligned with their {100} faces parallel to the surface. Mosaic size was however, found to be very small, suggesting poorly formed crystals.

Subsequent to these initial studies, a detailed investigation of the organisation of the lipid and its relationship to β -haematin formation has been conducted (Hoang et al., 2010a). Transmission electron microscopy (TEM) and dynamic light scattering experiments demonstrated that the neutral monoglyceride MPG spread on the aqueous surface as a solution in acetone/methanol forms an emulsion consisting of two populations of lipid-droplet like particles (dubbed synthetic neutral lipid droplets, SNLDs) with diameters centred around 100 nm and 5 μ m. Nile red (NR) labelling shows that these are non-hollow (since confocal microscopy shows NR fluorescence originating throughout the interior of these lipid particles).

Some TEM images provided striking evidence of β -haematin crystals parallel to the surface of the SNLDs that closely resemble crystals formed on lipid droplets in the *S. mansoni* gut (Figure 6). Kinetic studies indicated little difference between various neutral acylglycerols with respect to reaction rate or yield, but cholesterol was found not to mediate β -haematin formation. The activation energy for the reaction supported by MSG was found to be similar

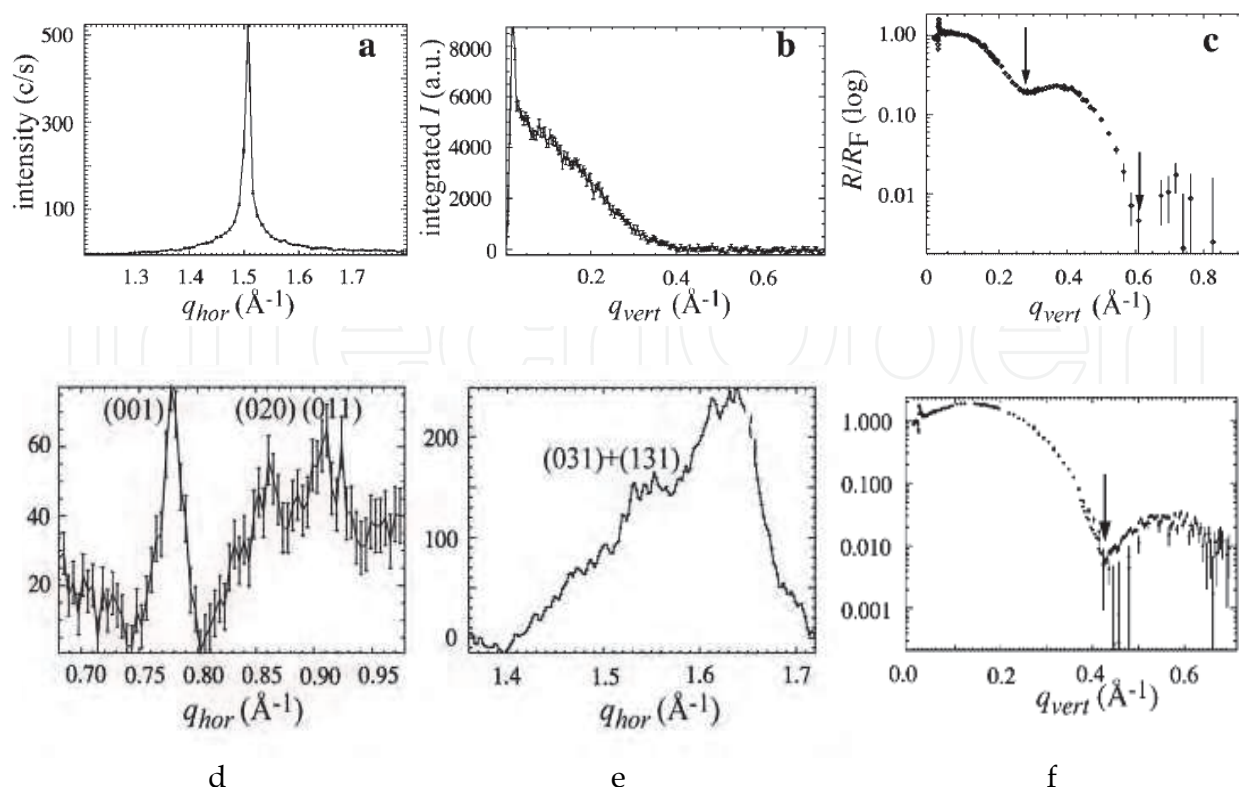


Fig. 5. GIXD pattern (a) and (b) Bragg rod profile of MMG on an aqueous surface and (c) the XR trace. The GIXD pattern and Bragg rod are diagnostic of hexagonal packing of the lipid on the surface and the XR trace indicates the presence of a lipid thickness corresponding to a monolayer. GIXD pattern (d) and (e) of β -haematin nucleated with MMG and (f) XR trace demonstrating the {100} face is aligned with the surface (de Villiers et al., 2009). Reprinted with permission from: K.A. de Villiers, M. Osipova, T.E. Mabothe, I. Solomonov, Y. Feldman, K. Kjaer, I. Weissbuch, T.J. Egan, L. Leiserowitz, L. Oriented nucleation of β -haematin crystals induced at various interfaces: relevance to hemozoin formation. *Cryst. Growth Des.* 9 (2009) 626–632. ©The American Chemical Society (2009).

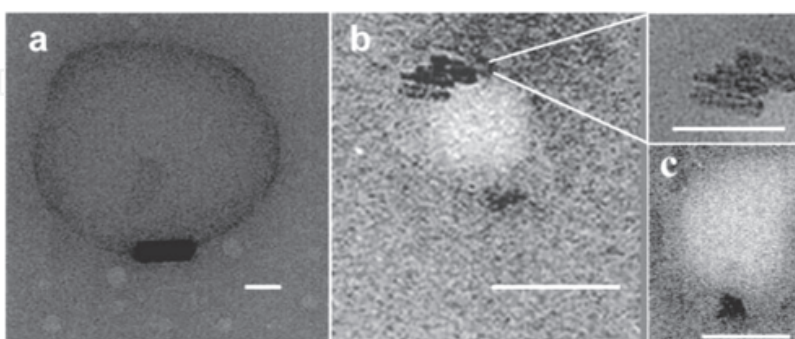


Fig. 6. TEM images of β -haematin crystals nucleated at the surface of MSG synthetic lipid droplets. Scale bars = 50 nm (Hoang et al., 2010a). Reprinted with permission from: A.N. Hoang, K.K. Ncokazi, K.A. De Villiers, D.W. Wright, T.J. Egan, Crystallization of synthetic haemozoin (β -haematin) nucleated at the surface of lipid particles. *Dalton Trans.* 39 (2010) 1235–1244. ©The Royal Society of Chemistry (2010). (<http://pubs.rsc.org/en/content/articlelanding/2010/dt/b914359a>)

to that reported in acetate or benzoate, illustrating that the massive increase in rate is a result of an increase in the pre-exponential term in the Arrhenius equation. This strongly suggests that the lipid surface has the role of pre-organising Fe(III)PPIX for conversion to β -haematin.

A further study concentrating on the blend observed in *P. falciparum* (4:2:1:1:1 MSG/MPG/DPG/DOG/DLG) demonstrated using NR fluorescence quenching that Fe(III)PPIX partitions rapidly into SNLDs and that the conversion to β -haematin occurs with an extraordinarily low activation energy (Hoang et al., 2010b). The partitioning of Fe(III)PPIX into SNLDs is pH dependent, with increased partitioning at lower pH where haematin is likely to exist as a neutral species. The unsaturated lipids, namely DOG and DLG exhibited activation energies for β -haematin formation almost as low as the blend. These low activation energies could be further correlated with low melting points of the lipid. Thus, the decrease in activation energy seems to relate to increased fluidity of the lipid. Interestingly, the rates of reaction are not much faster than those of the saturated high melting point lipids. Evidently the pre-exponential term in the Arrhenius equation decreases, suggesting that the Fe(III)PPIX molecules are less well pre-organised in the case of the fluid lipids. Presumably, this disadvantage is overcome by more rapid rearrangement of the Fe(III)PPIX molecules when they convert to β -haematin at such surfaces.

These studies on β -haematin formation in emulsions of neutral lipids have greatly expanded understanding of the process of haemozoin formation by closely mimicking the biological milieu. Nonetheless, a full mechanistic understanding of the process remains elusive. The early studies on lipid-mediated β -haematin formation suggested that the lipid merely solubilises Fe(III)PPIX (Fitch et al., 1999). With the discovery of the role of the aqueous-organic interface in the process it was suggested that the lipid environment would encourage dehydration of H_2O -Fe(III)PPIX to form β -haematin because of the strong propensity of water to partition out of the lipid environment and because hydrogen-bonding and electrostatic interactions are likely to be stronger in the low dielectric medium where competition for hydrogen bonding is absent (Egan et al., 2006; Pisciotta & Sullivan, 2008). More recently, it has been proposed that haemozoin is nucleated by lattice epitaxy at the lipid surface (Solomonov et al., 2007; Weissbuch & Leiserowitz, 2008; de Villiers et al., 2009). Although definitive evidence remains lacking, the idea is very appealing since it provides a logical explanation for the role of the interface and is consistent with the observation of crystals aligned with the SNLD surface.

3.3 β -Haematin formation on self-assembled monolayers (SAMs)

Recently there have been two attempts to grow β -haematin on SAMs. SAMs on Si(111) wafers coated with 10 nm of Cr and 90 nm of Au and prepared using various functionalised alkanethiols of the type $\text{HS}(\text{CH}_2)_{11}\text{X}$ (where $\text{X} = \text{OH}$, CH_3 or COOH as well as a 4:1 or 1:1 mixture of OH and CH_3) were used to investigate oriented nucleation at the surface (de Villiers et al., 2009). The β -haematin was formed from haemin (Cl-Fe(III)PPIX) dissolved in 0.5 ml of dry 2,6-lutidine to which 75 ml of a 1:1:1 mixture of methanol, DMSO and chloroform was added. Under these conditions, with the wafers dipped vertically into the solution, all of the SAMs nucleated β -haematin to a similar extent (Figure 7). Interestingly, however, specular XR experiments revealed that different SAMs induced β -haematin formation from different crystallographic faces (Figure 7). When $\text{X} = \text{OH}$, the nucleation was from the {100} face, when $\text{X} = \text{CH}_3$ nucleation from both the {100} and {010} faces was

observed, but the latter was more dominant, while $X = \text{COOH}$ nucleated the process from both faces. Where mixtures of functional groups were used ($X = \text{OH}$ and $X = \text{CH}_3$) there was a systematic increase in the number of crystals nucleated from the $\{010\}$ face as the proportion of CH_3 functional groups was increased. It was proposed that the OH groups would be more likely to interact with the carboxylate and CH groups exposed on the $\{100\}$ face, while CH_3 groups would interact more strongly with the porphyrin ring planes exposed on the $\{010\}$ face. This is also consistent with the idea that the OH groups at the surface of neutral lipid droplets would nucleate haemozoin formation from the $\{100\}$ face. Nonetheless, this study is not entirely definitive in this regard, since both in biomimetic systems involving SNLDs and in the biological environment itself the process would occur at an aqueous/organic interface rather than an interface with the relatively low dielectric constant of the solvent mixture used in the investigation involving SAMs.

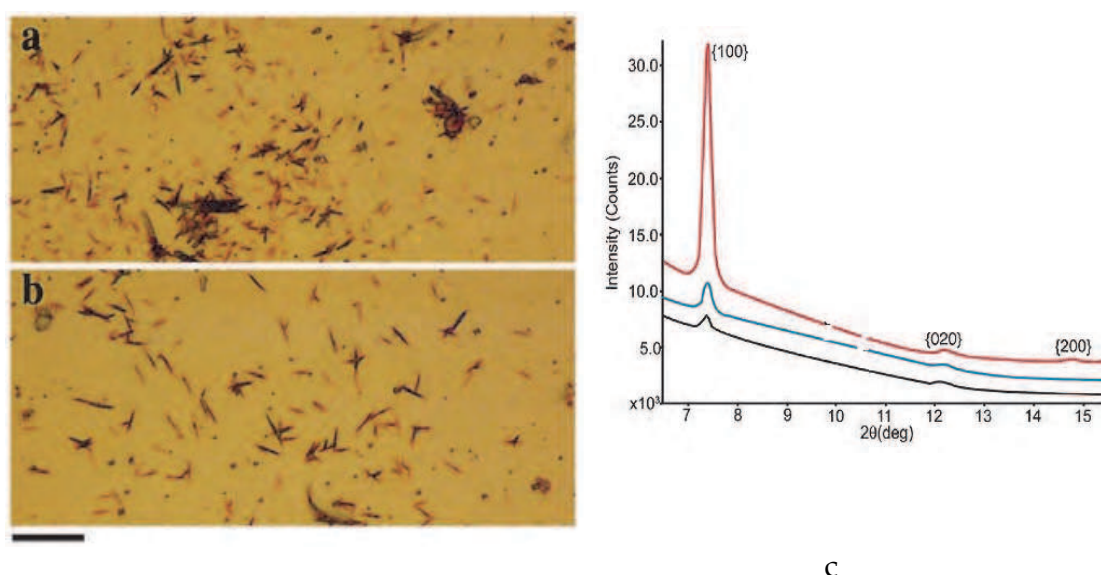


Fig. 7. Optical micrographs of β -haematin nucleated on SAMs with (a) OH and (b) CH_3 terminal functional groups illustrating similar extents of formation (scale bar = 5 μm). The XR patterns in (c) for OH (red), 1:1 OH/CH_3 (blue) and CH_3 (black) terminal groups illustrate the predominance of crystals nucleated from the $\{100\}$ face in the case of $X = \text{OH}$ which decreases as CH_3 groups increase (de Villiers et al., 2009). Reprinted with permission from: K.A. de Villiers, M. Osipova, T.E. Mabotha, I. Solomonov, Y. Feldman, K. Kjaer, I. Weissbuch, T.J. Egan, L. Leiserowitz, L. Oriented nucleation of β -hematin crystals induced at various interfaces: relevance to hemozoin formation. *Cryst. Growth Des.* 9 (2009) 626–632. ©The American Chemical Society (2009).

In the second investigation, β -haematin was nucleated from an aqueous milieu containing propionic acid over periods ranging from 30 min to 1 week (Wang et al., 2010). Under these conditions findings differed somewhat compared to the non-aqueous results from the first study. Firstly, yields (in the form of surface coverage) varied, with a SAM prepared from $\text{HS}(\text{CH}_2)_{11}\text{OH}$ being the least effective, followed by $\text{HS}(\text{CH}_2)_{15}\text{CH}_3$, while both $\text{HS}(\text{CH}_2)_{11}\text{NH}_2$ and $\text{HS}(\text{CH}_2)_{15}\text{COOH}$ gave 100% coverage at 2 h and 48 h respectively. Once again, the faces from which the crystals were nucleated were investigated using specular XR. Here it was found that both SAMs terminating in COOH and CH_3 groups nucleate

crystals from the {100} face, while too little material was formed on the OH terminated SAM to detect an XR peak and the NH_2 terminated SAM was suggested to nucleate crystals from a poorly diffracting face, since no XR peak could be detected despite the observation of what appears to be crystals by atomic force microscopy.

In the case of the COOH terminated SAM it was suggested that π - π dimers of haematin present in aqueous solution interact with the carboxylate groups of the SAM via their propionic acid groups. These dimers can then convert over to μ -Pr dimers nucleating β -haematin crystals (Figure 8). This would then provide a mechanism for haemozoin formation that would also be pertinent at the surface of lipid droplets.

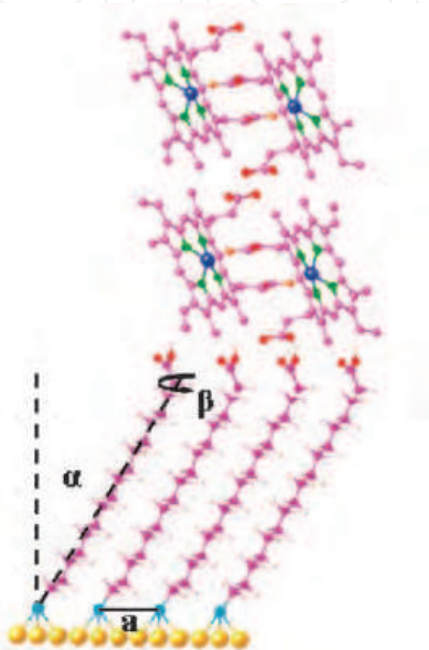


Fig. 8. The proposed geometric relationship between the surface of a SAM (with COOH functional end groups) and β -haematin proposed by Wang et al. (2010). Reprinted with permission from: X. Wang, E. Ingall, B. Lai, A.G. Stack, Self-assembled monolayers as templates for heme crystallization. *Cryst. Growth Des.* 10 (2010) 798–805. ©The American Chemical Society (2010).

Collectively, these studies strongly indicate that surfaces made up of OH, COOH, CH_3 and NH_2 groups at the termini of long alkyl chains are capable of nucleating β -haematin through specific intermolecular interactions giving rise to lattice epitaxial growth. However, the specific interactions remain to be conclusively demonstrated. Furthermore, it remains to be unequivocally shown that these interactions are the same as those that occur at the surface of lipid droplets present in vivo where haemozoin formation takes place.

4. Conclusion

As recently as 1997 haemozoin was referred to as “a seemingly intractable black solid” (Bohle et al., 1997). While there was considerable controversy over whether its formation was mediated by proteins or lipids, or was spontaneous or autocatalytic, little

was actually known about the milieu in which it was formed in vivo, or about its mechanism of formation until 2005. The last six years have seen major strides towards answering both of these questions. We now know that haemozoin formation is intimately associated with lipid-droplet like structures in both *Plasmodium* and *Schistosoma*, and with lipid vesicle bilayers in *Rhodnius*. At least in *Plasmodium*, these are now known to be neutral mono- and diacylglycerols. Biomimetic investigations using solvent interfaces and SNLDs have shown that interfaces between aqueous and non-aqueous media are extraordinarily active in promoting formation of the synthetic counterpart of haemozoin, β -haematin. In terms of reaction rates, they far exceed any other known process of β -haematin formation. Surface-sensitive measurements, specifically using GIXD and XR of β -haematin formed at such surfaces, and even more convincingly XR of crystals nucleated on SAMs strongly point to lattice epitaxy being the mechanism by which these surfaces mediate the process. These techniques provide a platform for further investigation. For example, what effects do various constituents of the digestive vacuole of the malaria parasite, such as ions and globin fragments have on the reaction rate? What role do proteins play in the process, either in chaperoning haem to the lipid surface and preventing it from precipitating as amorphous haematin, or in directly accelerating the process of haemozoin formation in conjunction with lipids? How do drugs such as chloroquine inhibit haemozoin formation? It is likely that with the methods and techniques now available, many of these questions will be able to be answered.

5. Acknowledgements

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6. References

- Abu Barkar, N. A., Klonis, N., Hanssen, E., Chan, C. & Tilley, L. (2010). Digestive-vacuole genesis and endocytotic processes in the early intraerythrocytic stages of *Plasmodium falciparum*. *Journal of Cell Science*, Vol.123, No.3, (February 2010), pp. 441-450, ISSN: 0021-9533
- Adams, P. A., Egan, T. J., Ross, D. C., Silver, J. & Marsh, P. J. (1996). The chemical mechanism of β -haematin formation studied by Mössbauer spectroscopy. *Biochemical Journal*, Vol.318, No.1, (August 1996), pp. 25-27, ISSN: 0264-6021
- Ashong, J. O., Blench, I. P. & Warhurst, D. C. (1989). The composition of haemozoin from *Plasmodium falciparum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, Vol.83, No.2, (March-April 1989), pp. 167-172, ISSN: 0035-9203
- Banerjee, R., Liu, J., Beatty, W., Pelosof, L., Klemba, M. & Goldberg, D. E. (2002). Four plasmepsins are active in the *Plasmodium falciparum* food vacuole, including a protease with an active-site histidine. *Proceedings of the National Academy of Sciences, USA*, Vol.99, No.2, (January 2002), pp. 990-995, ISSN: 0027-8424
- Bellemare, M.-J., Bohle, D. S., Brosseau, C.-N., Georges, E., Godbout, M., Kelly, J., Leimanis, M. L., Leonelli, R., Olivier, M. & Smilkstein, M. (2009). *Journal of Physical Chemistry B*, Vol.113, No.24, (May 2009), pp. 8391-8401, ISSN 1089-5647

- Bendrat, K., Berger, B. J. & Cerami, A. (1995). Haem polymerization in malaria. *Nature*, Vol.378, Issue 6519, (March 1995), pp. 138-139, ISSN: 0028-0836
- Bohle, D. S., Dinnebier, R. E., Madsen, S. K. & Stephens, P. W. (1997). Characterization of the products of the heme detoxification pathway in malarial late trophozoites by x-ray diffraction. *Journal of Biological Chemistry*, Vol.272, No.2, (January 1997), pp. 713-716, ISSN: 0021-9258
- Bohle, D. S., Debrunner, P., Jordan, P. A., Madsen, S. K. & Schulz, C. E. (1998). Aggregated haem detoxification byproducts in malarial trophozoites: β -haematin and malaria pigment have a single $S = 5/2$ iron environment in the bulk phase as determined by EPR and magnetic Mössbauer spectroscopy. *Journal of the American Chemical Society*, Vol.120, No.32, (July 1998), pp. 8255-8256, ISSN 0002-7863
- Bohle, D. S., Kosar, A. D. & Stephens, P. W. (2002). Phase homogeneity and crystal morphology of the malaria pigment β -hematin. *Acta Crystallographica*, Vol.D58, Part 10, No.1, (October 2002), pp. 1752-1756, ISSN: 0907-4449
- Bohle, D. S., Kosar, A. D. & Stephens, P. W. (2003). The reversible hydration of the malaria pigment β -hematin. *Canadian Journal of Chemistry*, Vol.81, No.11, (November 2003), pp. 1285-1291, ISSN: 1480-3291
- Brown, W. (1911). Malarial pigment (so-called melanin): its nature and mode of production. *Journal of Experimental Medicine*, Vol.13, No.2, (February 1911), pp. 290-299, ISSN: 0022-1007
- Buller, R., Peterson, M. L., Almarsson, Ö. & Leiserowitz, L. (2002). Quinoline binding site on malaria pigment crystal: a rational pathway for antimalaria drug design. *Crystal Growth and Design*, Vol.2, (August 2002), No.6, pp. 553-562, ISSN: 1528-7483
- Carbone, T. (1891). About the chemical nature of malaria pigment. *Giornale della Regia Accademia di Medicina di Torino*, Vol.39, pp. 901-906.
- Carter, M. D., Phelan, V. V., Sandlin, R. D., Bachmann, B. O. & Wright, D. W. (2010). Lipophilic mediated assays for β -hematin inhibitors. *Combinatorial Chemistry and High Throughput Screening*, Vol.13, No.3, (March 2010), pp. 285-292, ISSN 1386-2073
- Chen, M. M., Shi, L. & Sullivan, D. J. (2001). *Haemoproteus* and *Schistosoma* synthesize heme polymers similar to *Plasmodium* hemozoin and β -hematin. *Molecular and Biochemical Parasitology*, Vol.113, No.1, (March 2001), pp. 1-8, ISSN: 0166-6851
- Coppens, I. & Vielemeyer, O. (2005). Insights into unique physiological features of neutral lipids in Apicomplexa: from storage to potential mediation in parasite metabolic activities. *International Journal for Parasitology*, Vol.35, No.6, (May 2005), pp. 597-615, ISSN: 0020-7519
- Day, N. P., Pham, T. D., Phan, T. L., Dinh, X. S., Pham, P. L., Ly, V. C., Tran, T. H., Nguyen, T. H., Bethell, D. B., Nguyen, H. P., Tran, T. H. & White, N. J. (1996). Clearance kinetics of parasites and pigment-containing leukocytes in severe malaria. *Blood*, Vol.88, No.12, (December 1996), pp. 4694-4700, ISSN: 0006-4971
- de Villiers, K. A., Osipova, M., Mabotha, T. E., Solomonov, I., Feldman, Y., Kjaer, K., Weissbuch, I., Egan, T. J. & Leiserowitz, L. (2009). Oriented nucleation of β -hematin crystals induced at various interfaces: relevance to hemozoin formation. *Crystal Growth and Design*, Vol.9, No. 1, (January 2009), pp. 626-632, ISSN: 1528-7483
- Dorn, A., Stoffel, R., Matile, H., Bubendorf, A. & Ridley, R. G. (1995). Malarial haemozoin/ β -haematin supports haem polymerization in the absence of protein. *Nature*, Vol.374, Issue 6519, (March 1995), pp. 269-271, ISSN: 0028-0836

- Dorn, A., Vippagunta, S. R., Matile, H., Bubendorf, A., Vennerstrom, J. L. & Ridley, R. G. (1998). A comparison and analysis of several ways to promote haematin (haem) polymerisation and an assessment of its initiation in vitro. *Biochemical Pharmacology*, Vol.55, No.6, (March 1998), pp. 737-747, ISSN: 0006-2952
- Egan, T. J., Ross, D. C. & Adams, P. A. (1994). Quinoline anti-malarial drugs inhibit spontaneous formation of β -haematin (malaria pigment). *FEBS Letters*, Vol.352, No.1, (September 1994), pp. 54-57, ISSN: 0014-5793
- Egan, T. J., Mavuso, W. W. & Ncokazi, K. K. (2001). The mechanism of β -hematin formation in acetate solution. Parallels between hemozoin formation and biomineralization processes. *Biochemistry*, Vol.40, No.1, (January 2001), pp. 204-213, ISSN: 0006-2960
- Egan, T. J., Combrinck, J. M., Egan, J., Hearne, G. R., Marques, H. M., Ntentei, S., Sewell, B. T., Smith, P. J., Taylor, D., Van Schalkwyk, D. A. & Walden, J. C. (2002). Fate of haem iron in the malaria parasite *Plasmodium falciparum*. *Biochemical Journal*, Vol.365, No.2, (July 2002), pp. 343-347, ISSN: 0264-6021
- Egan, T. J. & Tshivhase, M. G. (2006). Kinetics of β -haematin formation from suspensions in aqueous benzoic acid. *Dalton Transactions*, No.42, (November 2006), pp. 5024-5032, ISSN 1477-9226
- Egan, T. J., Chen, J. Y.-J., De Villiers, K. A., Mabotha, T. E., Naidoo, K. J., Ncokazi, K. K., Langford, S. J., Mcnaughton, D., Pandiancherri, S. & Wood, B. R. (2006). Hemozoin (β -haematin) biomineralization occurs by self-assembly near the lipid/water interface. *FEBS Letters*, Vol.580, No.21, (September 2006), pp. 5105-5110, ISSN: 0014-5793
- Eggleson, K. K., Duffin, K. L. & Goldberg, D. E. (1999). Identification and characterization of falcilysin, a metallopeptidase involved in haemoglobin catabolism within the malaria parasite *Plasmodium falciparum*. *Journal of Biological Chemistry*, Vol.274, No.45, (November 1999), pp. 32411-32417, ISSN: 0021-9258
- Elliot, D. A., McIntosh, M. T., Hosgood Iii, H. D., Chen, S., Zhang, G., Baevova, P. & Joiner, K. (2008). Four distinct pathways of hemoglobin uptake in the malaria parasite *Plasmodium falciparum*. *Proceedings of the National Academy of Sciences, USA*, Vol.105, No.7, (February 2008), pp. 2463-2468, ISSN: 0027-8424
- Fitch, C. D. & Kanjanangulpan, P. (1987). The state of ferriprotoporphyrin IX in malaria pigment. *Journal of Biological Chemistry*, Vol.262, No.32, (November 1987), pp.15552-15555, ISSN: 0021-9258
- Fitch, C. D., Cai, G.-Z., Chen, Y.-F. & Shoemaker, J. D. (1999). Involvement of lipids in ferriprotoporphyrin IX polymerization in malaria. *Biochimica et Biophysica Acta*, Vol.1454, No.1, (May 1999), pp. 31-37, ISSN: 0006-3002
- Gligorijevic, B., Mcallister, R., Urbach, J. S. & Roepe, P. D. (2006). Spinning disk confocal microscopy of live, intraerythrocytic malarial parasites. 1. Quantification of hemozoin development for drug sensitive versus resistant malaria. *Biochemistry*, Vol.45, No.41, (October 2006), pp. 12400-12410, ISSN: 0006-2960
- Goldie, P., Roth, E. F., Oppenheim, J. & Vanderberg, J. P. (1990). Biochemical characterization of *Plasmodium falciparum* hemozoin. *American Journal of Tropical Medicine and Hygiene*, Vol.43, No.6, (December 1990), pp. 584-596, ISSN: 0002-9637
- Hamsik, A. (1936). Preparation of some blood pigment derivatives. *Zeitschrift für Physiologische Chemie*, Vol.190, pp. 199-215.

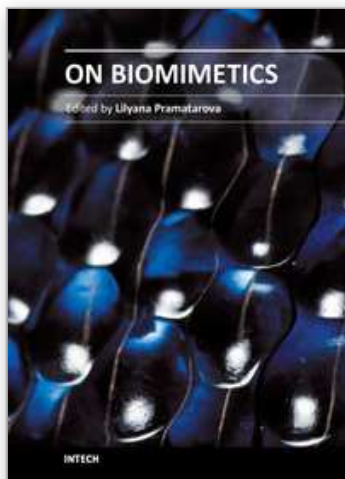
- Hänscheid, T.; Egan, T. J. & Grobusch, M. P. (2007). Haemozoin: from melatonin pigment to drug target, diagnostic tool, and immune modulator. *The Lancet Infectious Diseases*, Vol.7, No.10, (October 2007), pp. 675-685, ISSN 1473-3099
- Hanssen, E., Mcmillan, P. J. & Tilley, L. (2010). Cellular architecture of *Plasmodium falciparum*-infected erythrocytes. *International Journal for Parasitology*, Vol.40, No.10, (August 2010), pp. 1127-1135, ISSN: 0020-7519
- Hoang, A. N., Ncokazi, K. K., De Villiers, K. A., Wright, D. W. & Egan, T. J. (2010a). Crystallization of synthetic haemozoin (β -haematin) nucleated at the surface of lipid particles. *Dalton Transactions*, Vol.39, No.5, (February 2010), pp. 1235-1244, ISSN 1477-9226
- Hoang, A. N., Sandlin, R. D., Omar, A., Egan, T. J. & Wright, D. W. (2010b). The neutral lipid composition present in the digestive vacuole of *Plasmodium falciparum* concentrates heme and mediates β -hematin formation with an unusually low activation energy. *Biochemistry*, Vol.49, No.47, (November 2010), pp. 10107-10116, ISSN: 0006-2960
- Huy, N. T., Maeda, A., Uyen, D. T., Trang, D. T. X., Sasai, M., Shiono, T., Oida, T., Harada, S. & Kamei, K. (2007a). Alcohols induce beta-hematin formation via the dissociation of aggregated heme and reduction in interfacial tension of the solution. *Acta Tropica*, Vol.101, No.2, (February 2007), pp. 130-138, ISSN: 0001-706X
- Huy, N. T., Uyen, D. T., Maeda, A., Trang, D. T. X., Oida, T., Harada, S. & Kamei, K. (2007b). Simple colorimetric inhibition assay of heme crystallization for high throughput screening of antimalarial compounds. *Antimicrobial Agents and Chemotherapy*, Vol.51, No.1, (January 2007), pp. 350-353, ISSN: 0066-4804
- Jani, D., Nagarkatti, R., Beatty, W., Angel, R., Slebodnick, C., Andersen, J., Kumar, S. & Rathore, D. (2008). HDP - A novel heme detoxification protein from the malaria parasite. *PLOS Pathogens*, Vol.4, No.4, (April 2008), e1000053, ISSN 1553-7366
- Klonis, N., Dilanian, R., Hanssen, E., Darmanin, C., Streltsov, V., Deed, S., Quiney, H. & Tilley, L. (2010). Hematin-hematin self-association states involved in the formation and reactivity of the malaria parasite pigment, hemozoin. *Biochemistry*, Vol.49, No.31, (August 2010), pp. 6804-6811, ISSN: 0006-2960
- Krugliak, M., Zhang, F. & Ginsburg, H. (2002). Intraerythrocytic *Plasmodium falciparum* utilizes only a fraction of the amino acids derived from the digestion of host cell cytosol for the biosynthesis of its proteins. *Molecular and Biochemical Parasitology*, Vol.119, No.2, (February 2002), pp. 249-256, ISSN: 0166-6851
- Lancisi, G. M. (1717). *De noxiis paludum effluviis eorumque remediis*, J. M. Salvioni, Rome.
- Laveran, C. L. A. (1880). *A newly discovered parasite in the blood of patients suffering from malaria. Parasitic etiology of attacks of malaria*. Translated from the French and reprinted (1978), Ithaca, New York, Cornell University Press.
- Levesque, M. A., Sullivan, A. D. & Meshnick, S. R. (1999). Splenic and hepatic haemozoin in mice after malaria parasite clearance. *Journal of Parasitology*, Vol.85, No.3, (June 1999), pp. 570-573, ISSN: 0022-3395
- Mury, F. B., Da Silva, J. R., Ferreira, L. S., Dos Santos Ferreira, B., De Souza-Filho, G. A., De Souza-Neto, J. A., Ribolla, P. E., Silva, C. P., Do Nascimento, V. V., Machado, O. L., Berbert-Molina, M. A. & Dansa-Petretski, M. (2009). Alpha-glucosidase promotes hemozoin formation in a blood-sucking bug: an evolutionary history. *PloS ONE*, Vol.4, No.9, (September 2009), e6966, ISSN:1932-6203

- Myerson, A. S. (1993). *Handbook of industrial crystallization*. Butterworth-Heinemann series in Chemical Engineering, ISBN-10: 0750691557, USA, Butterworth-Heinemann.
- Noland, G. S., Briones, N. & Sullivan, D. J. (2003). The shape and size of hemozoin crystals distinguishes diverse *Plasmodium* species. *Molecular and Biochemical Parasitology*, Vol.130, No.2, (August 2003), pp. 91-99, ISSN: 0166-6851
- Oliveira, M. F., Silva, J. R., Dansa-Petretski, M., De Souza, W., Lins, U., Braga, C. M. S., Masuda, H. & Oliveira, P. L. (1999). Haem detoxification by an insect. *Nature*, Vol.400, Issue 6744, (August 1999), pp. 517-518, ISSN: 0028-0836
- Oliveira, M., D'avila, J. C. P., Torres, C. R., Oliveira, P. L., Tempone, A. J., Rumjanek, F. D., Braga, C. M. S., Silva, J. R., Dansa-Petretski, M., Oliveira, M. A., De Souza, W. & Ferreira, S. T. (2000). Haemozoin in *Schistosoma mansoni*. *Molecular and Biochemical Parasitology*, Vol.111, No.1, (November 2000), pp. 217-221, ISSN: 0166-6851
- Oliveira, M. F., Kycia, S., Gonzales, A., Kosar, A. D., Bohle, D. S., Hempelmann, E., Menezes, D., Vannier-Santos, M., Oliveira, P. L. & Ferreira, S. T. (2005). Structural and morphological characterization of hemozoin produced by *Schistosoma mansoni* and *Rhodnius prolixus*. *FEBS Letters*, Vol.579, No.27, (November 2005), pp. 6010-6016, ISSN: 0014-5793
- Pagola, S., Stephens, P. W., Bohle, D. S., Kosar, A. D. & Madsen, S. K. (2000). The structure of malaria pigment (β -haematin). *Nature*, Vol.404, Issue 6775, (March 2000), pp. 307-310, ISSN: 0028-0836
- Pandey, A. V., Babbarwal, V. K., Okoyeh, J. N., Joshi, R. M., Puri, S. K., Singh, R. L. & Chauhan, V. S. (2003). Hemozoin formation in malaria: a two-step process involving histidine-rich proteins and lipids. *Biochemical and Biophysical Research Communications*, Vol.308, No. 4, (September 2003), pp. 736-743, ISSN: 0006-291X
- Papalexis, V., Siomos, M.-A., Campanale, N., Guo, X.-G., Kocak, G., Foley, M. & Tilley, L. (2001). Histidine-rich protein 2 of the malaria parasite, *Plasmodium falciparum*, is involved in detoxification of the by-products of haemoglobin degradation. *Molecular and Biochemical Parasitology*, Vol.115, No. 1, (June 2001), pp. 77-86, ISSN: 0166-6851
- Pisciotta, J. M., Coppens, I., Tripathi, A. K., Scholl, P. F., Shuman, J., Bajad, S., Shulaev, V. & Sullivan, D. J. (2007). The role of neutral lipid nanospheres in *Plasmodium falciparum* haem crystallization. *Biochemical Journal*, Vol.402, No.1, (February 2007), pp. 197-204, ISSN: 0264-6021
- Pisciotta, J. M. & Sullivan, D. J. (2008). Hemozoin: oil versus water. *Parasitology International*, Vol.57, No.2, (June 2008), pp. 89-96, ISSN: 1383-5769
- Puskar, L., Tuckermann, R., Frosch, T., Popp, J., Ly, V., Mcnaughton, D. & Wood, B. R. (2007). Raman acoustic levitation spectroscopy of red blood cells and *Plasmodium falciparum* trophozoites. *Lab on a Chip*, Vol.7, No.9, (September 2007), pp. 1125-1131, ISSN: 1473-0197
- Rosenthal, P. J., Sijwali, P. S., Singh, A. & Shenai, B. R. (2002). Cysteine proteases of malaria parasites: targets for chemotherapy. *Current Pharmaceutical Design*, 8, No.18, (August 2002), 1659-1672, ISSN: 1381-6128
- Ross, R. (1897). On some peculiar pigmented cells found in two mosquitoes fed on malarial blood. *British Medical Journal*, Vol.18, No.2, (December 1897), pp. 1736-1788, ISSN: 0007-1447
- Sharples, A. (1966). *Introduction to polymer crystallization*, London, Edward Arnold.

- Sienkiewicz, A., Krzystek, J., Vilen, B., Chatain, G., Kosar, A. J., Bohle, D. S. & Forró, L. (2006). Multi-frequency high-field EPR study of iron centers in malarial pigments. *Journal of the American Chemical Society*, Vol.128, No.14, (April 2006), pp. 4534-4535, ISSN 0002-7863
- Slater, A. F. G., Swiggard, W. J., Orton, B. R., Flitter, W. D., Goldberg, D. E., Cerami, A. & Henderson, G. B. (1991). An iron-carboxylate bond links the heme units of malaria pigment. *Proceedings of the National Academy of Sciences of the USA*, Vol.88, No.2, (January 1991), pp. 325-329, ISSN: 0027-8424
- Slater, A. F. G. & Cerami, A. (1992). Inhibition by chloroquine of a novel haem polymerase enzyme activity in malaria trophozoites. *Nature*, Vol.355, Issue 6356, (January 1992), pp. 167-169, ISSN: 0028-0836
- Solomonov, I., Osipova, M., Feldman, Y., Baehtz, C., Kjaer, K., Robinson, I. K., Webster, G. T., McNaughton, D., Wood, B. R., Weissbuch, I. & Leiserowitz, L. (2007). Crystal nucleation, growth, and morphology of the synthetic malaria pigment β -hematin and the effect thereon by quinoline additives: the malaria pigment as a target of various antimalarial drugs. *Journal of the American Chemical Society*, Vol.129, No.9, (March 2007), pp. 2615-2627, ISSN 0002-7863
- Stack, C. M., Lowther, J., Cunningham, E., Donnelly, S., Gardiner, D. L., Trenholme, K. R., Skinner-Adams, T. S., Teuscher, F., Grembecka, J., Mucha, A., Kafarski, P., Lua, L., Bell, A. & Dalton, J. P. (2007). Characterization of the *Plasmodium falciparum* M17 leucyl aminopeptidase. *Journal of Biological Chemistry*, Vol.282, No.3, (January 2007), pp. 2069-2080, ISSN: 0021-9258
- Stiebler, R., Timm, B. L., Oliveira, P. L., Hearne, G. R., Egan, T. J. & Oliveira, M. F. (2010a). On the physico-chemical and physiological requirements of hemozoin formation promoted by perimicrovillar membranes in *Rhodnius prolixus* midgut. *Insect Biochemistry and Molecular Biology*, Vol.40, No.3, (March 2010), pp. 284-292, ISSN: 0965-1748
- Stiebler, R., Hoang, A. N., Egan, T. J., Wright, D. W. & Oliveira, M. F. (2010b). Increase on the initial soluble heme levels in acidic conditions is an important mechanism for spontaneous heme crystallization *in vitro*. *PloS ONE*, Vol.5, No.9, (September 2010), e12694, ISSN:1932-6203
- Sullivan, D. J., Gluzman, I. Y. & Goldberg, D. E. (1996). Plasmodium hemozoin formation mediated by histidine-rich proteins. *Science*, Vol.271, Issue 5246, (January 1996), pp. 219-222, ISSN: 0036-8075
- Sullivan, D. J. (2002). Theories on malarial pigment formation and quinoline action. *International Journal for Parasitology*, Vol.32, No.13, (December 2002), pp. 1645-1653, ISSN: 0020-7519
- Virchow, R. (1849). About the pathophysiology of blood. *Archiv für Pathologische Anatomie*, Vol.2, pp. 587-598.
- Walczak, M., Lawniczak-Janlonska, K., Sienkiewicz, A., Demchenko, I. N., Piskorska, E., Chatain, G. & Bohle, D. S. (2005). Local environment of iron in malarial pigment and its substitute β -hematin. *Nuclear Instruments and Methods in Physics Research B*, Vol.238, No.1-4, (August 2005), pp. 32-38, ISSN: 0969-8051
- Walczak, M. S., Lawniczak-Jablonska, K., Sienkiewicz, A., Klepka, M. T., Suarez, L., Kosar, A. J., Bellemare, M.-J. & Bohle, D. S. (2010). XAFS studies of the synthetic

- substitutes of hemozoin. *Journal of Non-crystalline Solids*, Vol.356, No.37-40, (August 2010), pp. 1908-1913, ISSN: 0022-3093
- Walczak, M. S., Lawniczak-Jablonska, K., Wolska, A., Sienkiewicz, A., Suárez, L., Kosar, A. J. & Bohle, D. S. (2011). Understanding chloroquine action at the molecular level in antimalarial therapy: X-ray absorption studies in dimethyl sulfoxide solution. *Journal of Physical Chemistry B*, Vol.115, No.5, (February 2011), pp. 1145-1150, ISSN 1089-5647
- Wang, X., Ingall, E., Lai, B. & Stack, A. G. (2010). Self-assembled monolayers as templates for heme crystallization. *Crystal Growth and Design*, Vol.10, No.2, (January 2010), pp. 798-805, ISSN: 1528-7483
- Weissbuch, I. & Leiserowitz, L. (2008). Interplay between malaria, crystalline hemozoin formation, and antimalarial drug action and design. *Chemical Reviews*, Vol.108, No.11, (November 2008), pp. 4899-4914, ISSN: 0009-2665
- Wood, B. R., Langford, S. J., Cooke, B. M., Glenister, F. K., Lim, J. & Mcnaughton, D. (2003). Raman imaging of hemozoin within the food vacuole of *Plasmodium falciparum* trophozoites. *FEBS Letters*, Vol.554, No.3, (November 2003), pp. 247-252, ISSN: 0014-5793

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Bio-mimicry is fundamental idea – How to mimic the Nature™ by various methodologies as well as new ideas or suggestions on the creation of novel materials and functions. This book comprises seven sections on various perspectives of bio-mimicry in our life; Section 1 gives an overview of modeling of biomimetic materials; Section 2 presents a processing and design of biomaterials; Section 3 presents various aspects of design and application of biomimetic polymers and composites are discussed; Section 4 presents a general characterization of biomaterials; Section 5 proposes new examples for biomimetic systems; Section 6 summarizes chapters, concerning cells behavior through mimicry; Section 7 presents various applications of biomimetic materials are presented. Aimed at physicists, chemists and biologists interested in biomineralization, biochemistry, kinetics, solution chemistry. This book is also relevant to engineers and doctors interested in research and construction of biomimetic systems.

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