ESRF	**Experiment title: Unveiling interactions of 4-aminoquinoline antimalarials with heme in solution**	**Experiment number**: CH4370
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Shifts: 9	**Local contact(s)**: Alessandro Longo	*Received at ESRF:*

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Report:

We investigated the interaction of the antimarial drug chloroquine (CQ) with heme in acidic 1:1 H_2O:DMSO solutions at room temperature by EXAFS spectroscopy. The chemical composition of the solutions was set in order to reproduce as closely as possible the conditions within the DV of the malaria parasite. Spectra were recorded across the Fe Kα absorption edge (7.11 keV), at room temperature, in the 6.9-7.7 keV energy range. This strategy allowed a practical maximum resolution of 10.0 $Å^{-1}$ in k space. Glass capillaries (∅ 1.5 – 2.0 mm, ≈ 3/4 filled) were employed to host solutions. Fluorescence mode was selected to maximize the signal intensity. Great care was taken to avoid degradation of the solutions. All the specimens were either stored in the dark at T ≤ 4°C prior being sent to the experimental station, or measured as freshly prepared. To limit radiation damage, each recorded spectrum was obtained upon averaging a total of 6 scans, obtained from 2-3 repeated scans on a sequence of 2-3 capillaries filled with the same solution.

Spectroscopic results were complemented by high-grade DFT simulations. We found evidence that the first coordination shell of the iron ion is closely involved in the drug:substrate recognition process. This implies that a complex where the quinoline nitrogen of the drug is coordinated to the iron center (Fig. 1) might coexist with other possible (e.g. π···π stacked) adduct geometries. *It is the first time that a direct Fe-N bond, formerly hypothesized on the basis of solid-state NMR findings, is observed in solution.*

The single-protonated form of the drug, which is the active one in producing the Fe-N complex, is nevertheless minoritarian in the acidic parasite DV. Our quantum mechanical

simulations demonstrated that the proposed adduct might form directly from the fully protonated form of the drug, following a bimolecular ligand-exchange reaction between hematin and CQ.CAHB interactions among the protonated tertiary amine of the drug and the free propionate chain of the heme system were also found to be determinant in stabilizing the adduct. In computational works, the putative effectiveness of CQ-like drugs is often judged based on their heme-binding strength.

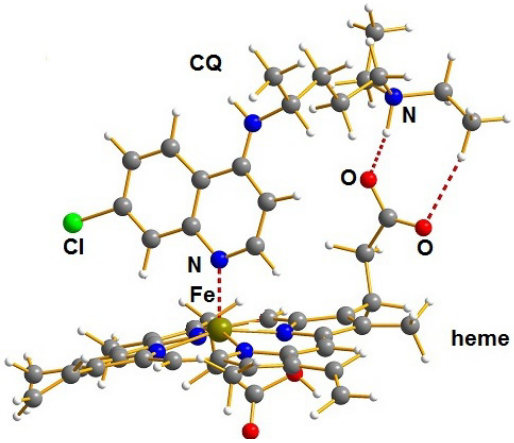

Figure 1. Structure of the proposed heme:CQ complex, as derived from EXAFS experiments and high-grade DFT calculations

Anyhow, their ability in slowing down heme self-recognition and self-assembly could be as much important. In this respect, CAHBs might be even more crucial than direct Fe-N or π···π stacking interactions.

The following paper was published based on the results obtained from the present research. Please refer to the following reference for more datails on EXAFS results and data treatment.

Bibliographic reference: Giovanni Macetti, Silvia Rizzato, Fabio Beghi, Lucia Silvestrini and Leonardo Lo Presti*, 2016 *Phys. Scr.* **91** 023001. doi:10.1088/0031-8949/91/2/023001

Title: On the molecular basis of the activity of the antimalarial drug chloroquine: EXAFS-assisted DFT evidence of a direct Fe–N bond with free heme in solution

Abstract: 4-aminoquinoline antiplasmodials interfere with the biocrystallization of the malaria pigment, a key step of the malaria parasite metabolism. It is commonly believed that these drugs set stacking π···π interactions with the Fe-protoporphyrin scaffold of the free heme, even though the details of the heme:drug recognition process remain elusive. In this work, the local coordination of Fe(III) ions in acidic solutions of hematin at room temperature was investigated by extended x-ray absorption fine structure (EXAFS) spectroscopy in the 4.0–5.5 pH range, both in the presence and in the absence of the antimalarial drug chloroquine. EXAFS results were complemented by DFT simulations in polarizable continuum media to model solvent effects. We found evidence that a complex where the drug quinoline nitrogen is coordinated with the iron center might coexist with formerly proposed adduct geometries, based on stacking interactions. Charge-assisted hydrogen bonds among lateral chains of the two molecules play a crucial role in stabilizing this complex, whose formation is favored by the presence of lipid micelles. The direct Fe–N bond could reversibly block the axial position in the Fe 1st coordination shell in free heme, acting as an inhibitor for the crystallization of the malaria pigment without permanently hampering the catalytic activity of the redox center. These findings are discussed in the light of possible implications on the engineering of drugs able to thwart the adaptability of the malaria parasite against classical aminoquinoline-based therapies.