EUROPEAN SYNCHROTRON RADIATION FACILITY
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ESRF

ESRF	**Experiment title: Progress in the understanding of the mode of action of 4-aminoquinoline antimalarials: a low-T study of the heme-binding ability of piperaquine and chloroquine in solution**	**Experiment number**: CH-4861
Beamline: BM26A	**Date of experiment**: from: 08 December, 2016 to: 11 December, 2016	**Date of report**: 27/02/2017
Shifts: 9	**Local contact(s)**: Alessandro Longo (email: alessandro.longo@esrf.fr)	*Received at ESRF:*

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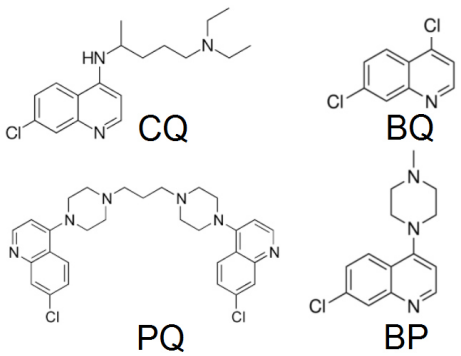
Report:

This experiment was performed in the same context of former room-temperature EXAFS measurements we carried out a couple of years ago at the BM26A beamstation on heme:chloroquine aqueous:DMSO solutions (see experiment CH4370). The goal was now to perform more accurate X-ray absorption measurements on heme-containing solutions of chloroquine (CQ) and piperaquine (PQ) 4-aminoquinoline (4-AQ) antimalarial drugs (Scheme 1). To this end, spectra were recorded at 155–157 K on solutions previously flash-frozen at the liquid nitrogen temperature. The idea was to reduce thermal motion and possible photolysis phenomena, hampering at the same time the formation of interfering well-ordered nanocrystals of the heme molecules. Relative orientations and coordination environments of the drug and heme in the frozen glassy state should indeed mimic the preferred interaction modes in the fluid state. We also collected EXAFS spectra on heme solutions in the presence of simpler chemical models of both CQ and PQ, namely 4,7-dichloroquinoline (BQ) and 7-chloro-4-(4-methyl-1-piperazinyl)quinoline (BP, Scheme 1), which lack long hydrocarbon chains and cannot exploit the presence of an alkylic nitrogen far from the quinoline nucleus to interact with the



Scheme 1

Fe centre.[1,2] These simplified systems should allow us to check our hypothesis on the importance of a long hydrocarbon chain to the effectiveness of the pharmacophore, avoiding at the same time complications due to possible multidentate coordination of PQ with multiple heme molecules.

All the experiments were performed on buffered solutions at pH = 5, using water, DMSO, $CHCl_3$ and MeOH as solvents. The presence of surfactants (SDS) was also considered. After various attempts, we found that a steel arm can be used as a suitable sample holder. Sample solutions were poured in kapton® capillaries (∅ 2 mm, ~ ¾ filled). Open extremities were closed by a piece of kapton® adhesive tape, and held in position by strips of lead adhesive tape (Figure 1). Then, the holder was put in a dewar vassel filled with liquid nitrogen and carried to the experimental hutch. Finally, it was rapidly mounted on the cryostat head at the spectrometer station. Fluorescence mode was selected to maximize the signal intensity. The energy scan performed across the Fe Kα edge at ~ 7.1 keV. All the specimens were measured as freshly prepared to avoid degradation of the solutions. To limit radiation damage, each recorded spectrum was obtained upon averaging a total of 4-8 scans, obtained from 2-4 repeated scans on a sequence of 2-3 capillaries filled with the same solution.

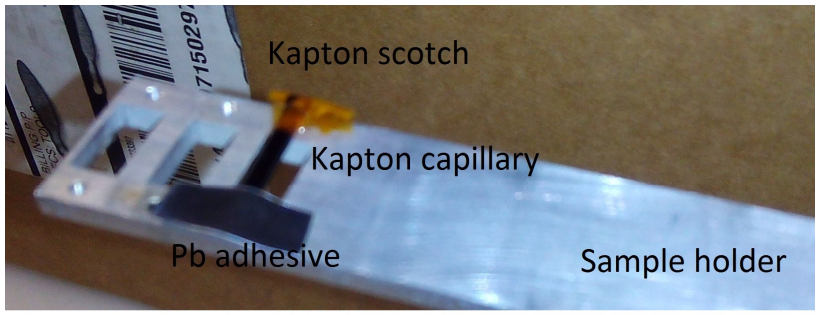


Figure 1. Example of sample mounting on the holder

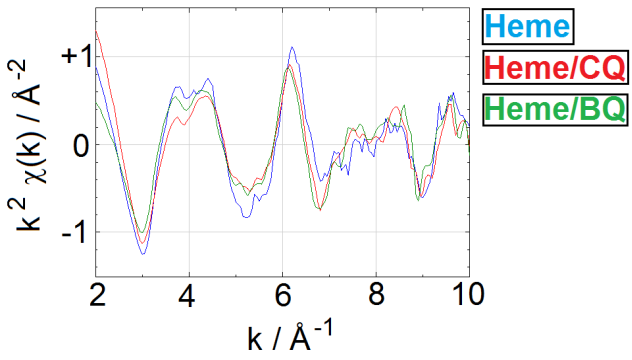


Figure 2. k-squared weighted fine structure function for 3 representative heme-containing solutions

Figure 2 shows representative results obtained for solutions containing either just heme (blue curve), or heme + compund CQ or BQ (Scheme 1, red and green lines). Due to the common square planar environment of Fe within the protoporphyrin ring, all the signals are quite similar, but when 4-AQ compounds are present some peaks (~ 6–8 $Å^{-1}$) appear slightly but neatly shifted toward lower k values. At the same time, also the relative oscillation amplitudes are somehow different, implying that both chloroquine and its model system somehow influence the coordination mode of the iron ion. Interestingly, CQ and BQ-containing solutions bear almost identical signals, implying that the interaction mode of the quinoline moiety with the substrate should be the same, irrespective of the presence of a tertiary amine group (Scheme 1). Fitting of the signals with backscattering paths developed on suitable structural models are currently being performed. The latter are mandatory to understand how the chemical nature of the pharmacophore and the composition of the chemical enviroment influence the recognition of the substrate at the molecular level.

References

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[2] G. Macetti, L. Loconte, S. Rizzato, C. Gatti, L. Lo Presti, *Crystal Growth & Design* **2016**, 16(10):6043–6054