Deciphering the resistance-counteracting functions of ferroquine in *Plasmodium falciparum*-infected erythrocytes

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SUPPLEMENTARY INFORMATION

Chemistry. Ferroquine was synthesized as previously described.[1] Chloroquine was purchased from Sigma Aldrich.

Culturing of Parasites. *Plasmodium falciparum* clones W2 were routinely maintained in cultures in complete RPMI 1640 medium (25 mM HEPES and 300 mg/l l-glutamine; Invitrogen), enriched with 10% decomplemented human serum (AB+), and 6% human red blood cells (O+) at 37°C under controlled atmosphere (O_2 5%/CO_2 5%/N_2 90%; Air Liquide, Paris, France). Serum and red blood cells were supplied by Etablissement Francais du Sang, Lille, France. Cultures were controlled by thin smears stained with Giemsa (Merck, Darmstadt, Germany). Parasitemia were monitored on 1000 red blood cells.

The assays were carried out on *P. falciparum* cultures with ~10% parasitemia containing old trophozoites and at 3% hematocrit. Ferroquine and chloroquine were added at a final concentration of 40 nM. After 30 min of incubation, the treated and untreated (DMSO alone) were deposited on 500-nm thick silicon nitride windows (Silson Pty Ltd), fixed in methanol and washed with distilled water. Normal RBC morphology was retained throughout the treatment and fixation procedures as evidenced in the optical micrographs.

Synchrotron X-ray Fluorescence (XRF) Studies. Synchrotron X-ray fluorescence experiments were carried out at the nanoimaging end-station ID22NI of the European Synchrotron Radiation Facility (ESRF; Grenoble, France). Dynamically bent graded multilayers set in the Kirkpatrick–Baez geometry were used to focus the x-ray beam from an undulator source to a spot size of approximately 80 nm^2 on

the sample. Regions of interest were chosen using an online optical microscope, which also allowed control of the beam position onto the sample. The sample, mounted in air at room temperature on a nanopositioner stage, was raster scanned with a step size of 100 nm through the focal plane, while the spectrum of the emitted fluorescence was recorded with an energy dispersive silicon drift diode collimated detector (SII Nanotechnology Vortex, 50 mm^2 sensitive area) placed in the horizontal plane at 75° from the incident beam.[3] The integration time per scan point was 1s and normalization against variations in the synchrotron incident beam intensity was achieved using a downstream Si PIN-diode detector. Cell images were obtained for each of the conditions described. This allowed the generation of a pixel-by-pixel spectral image providing topographical elemental maps by fitting the full fluorescence spectrum at every single point using the PyMCA software.[4] Quantification was performed using fundamental parameter method revised and expanded implemented within the PyMCA software. NIST standards reference materials SRM1832 (thin film standard) and SRM1577b (bovine liver) were used to calibrate experimental parameters.

References

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