



	Experiment title: Reactive intermediate of cytochrome P450 prepared by the freeze-quench method and investigated by nuclear forward scattering	Experiment number: LS-883 LS-1022
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Report:

It has been shown with the aid of rapid scan absorption spectroscopy that the low-spin form of cytochrome P450_{cam} reacts with m-chloroperbenzoic acid (m = CPBA) to yield transient intermediates, obtained in the time range of 20-200 ms after mixing. This method, however, does not allow their characterization. The aim of the present investigation was to contribute to the characterization of these intermediates by nuclear resonant forward scattering (NFS) of synchrotron radiation. Mixing ⁵⁷Fe-enriched cytochrome (⁵⁷Fe-P450) or porphyrin, i. e. tetramesitylporphyrin (⁵⁷Fe-TMP) and tetraphenylporphyrin (⁵⁷Fe-TPP), with m-chloroperbenzoic acid (m - CPBA) as oxidizing agent provides transient intermediates by the reaction mixture being freeze-quenched to -78°.

The following samples have been prepared:

- ferric low-spin form of cytochrome ⁵⁷Fe-P450 as starting material, 1 mM in ⁵⁷Fe
- ferric low-spin form of ⁵⁷Fe-TPP as biomimetic model for P450, 10 mM in ⁵⁷Fe
- transient intermediate (10 ms, 200 ms) of P450, 1 mM in ⁵⁷Fe
- transient intermediate of ⁵⁷Fe-TMP (5 mM in ⁵⁷Fe) as biomimetic model for compound I of P450

All samples had a volume of ~ 50 µl and were packed in plastic cylinders of 10 mm length providing an effective thickness t_{eff} ~ 1 for ⁵⁷Fe-P450, ~ 10 for ⁵⁷Fe-TPP, and ~ 5 for ⁵⁷Fe-

TMP. With this variation in effective thickness we were able to investigate the response of coherent resonant forward scattering to different concentrations in ^{57}Fe , at 4.2 K, in loosely packed ice crystals (estimated packing factor is $\frac{1}{2}$ - $\frac{1}{4}$ compared to homogeneously frozen solution).

Figure 1 shows the NFS spectra, recorded at 4.2 K, of ferric low-spin ^{57}Fe -TPP (a) in applied field of 610 G $\perp \gamma$ and $\parallel E$ (closed squares) and 610 G $\parallel \gamma$ and $\perp E$ (open squares) and of the compound I analog ^{57}Fe -TMP (b) in an applied field 150 G $\perp \gamma$ and $\parallel E$. Measuring time in 16 bunch mode was ~ 12 h/7 h (closed squares/open squares) for ^{57}Fe -TPP and ~ 2 h for ^{57}Fe -TMP. The solid curves are simulations using the SYNFOSS program package, which we have developed [1] to analyze the electronic structure of iron in the spin-Hamiltonian approximation. Measurements on ^{57}Fe -P450 in ferric low-spin form and as transient intermediate (10 ms) were recorded at 4.2 K in an applied field of 610 G $\perp \gamma$ and $\parallel E$. Measuring time was in both cases 15 h. The poor quality of the obtained spectra (not shown) does not allow to perform an unequivocal spin-Hamiltonian simulation of the electronic state of the heme iron. At present we are preparing new ^{57}Fe -P450 samples with higher packing factor, i. e. for each reaction time we have to perform several freeze-quench reactions under same conditions in order to collect enough material providing effective thickness close to 5.

[1] M. Haas, E. Realo, H. Winkler, W. Meyer-Klaube, A. X. Trautwein, O. Leupold and H. D. Rüter, Phys. Rev. B 56, 14082 (1997)

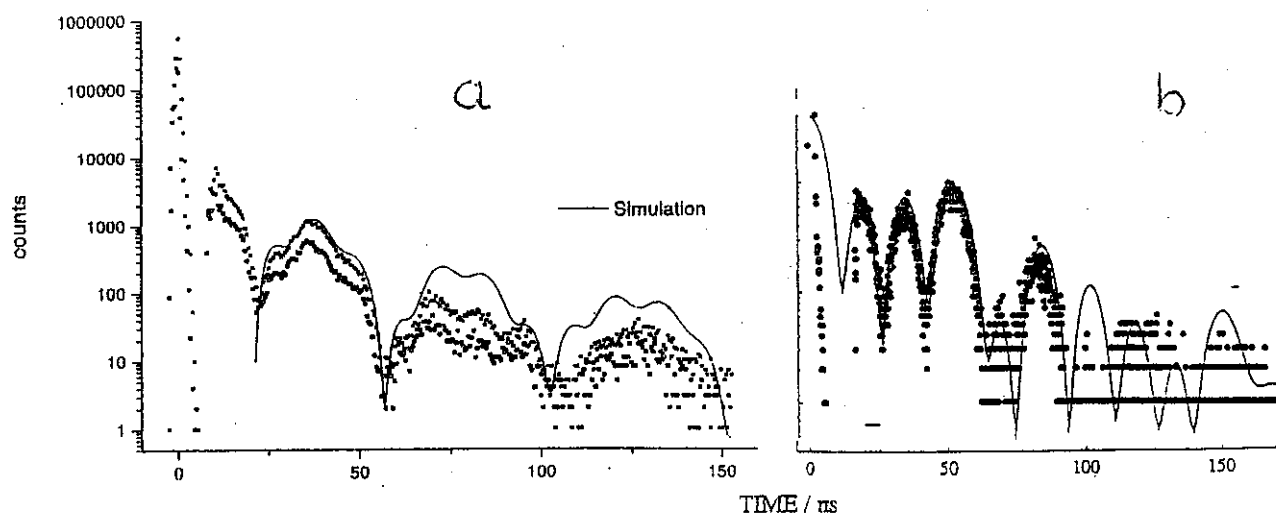


Figure 1: Nuclear forward scattering of synchrotron radiation (a) from ^{57}Fe -TPP, at 4.2 K, in an applied field of 610 G $\perp \gamma$ and $\parallel E$ (closed squares) and $\parallel \gamma$ and $\perp E$ (open squares) and (b) from ^{57}Fe -TMP, at 4.2 K, 150 G $\perp \gamma$ and $\parallel E$. Solid lines are simulations using the SYNFOSS program [1], representing (a) ferric low-spin iron and (b) an exchange coupled iron ($S=1$)-porphyrin cation radical ($S'=1/2$) pair.