ESRF	**Experiment title: Isoprenoid biosynthesis via the MEP pathway: Substrate and inhibitor interaction of an unusual 4Fe-4S center of the LytB protein studied by nuclear inelastic scattering**	**Experiment number**: SC-3085
Beamline: ID-18	**Date of experiment**: from: 24/11/2010 to: 27/11/2010	**Date of report**: 01/09/2011
Shifts: 9	**Local contact(s)**: Aleksandr Chumakov	*Received at ESRF:*

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Report:

Disease-causing microbes have became rapidly resistant to antibiotic drug therapies and diseases that were thought to be eradicated, are re-emerging. Tuberculosis for example is reappearing even in the developed world causing 1.1 million deaths worldwide. The methylerythritol phosphate pathway (MEP) is used for the biosynthesis of essential terpenoids in most pathogenic bacteria (including *Mycobacterium tuberculosis*) and in plant plastids. This pathway does not exist in humans and is therefore a promising target for the development of new specific antibacterial and antiparasitic drugs. The iron sulfur enzyme LytB, also called IspH, is involved in the last steps of the MEP pathway and we have found that it has a catalytically competent unusual 4Fe-4S center [1], the structure of which has been a matter of debate [2,3]. Based on our Mössbauer spectroscopic studies [1] a crystal structure of the substrate bound LytB has been published recently [4]. A structure of the substrate-free LytB however could not yet be obtained.

In order to study the interaction of LytB with substrates and enzyme inhibitors we proposed to use nuclear inelastic scattering (NIS) in order to detect directly iron-ligand vibrations. During the experiment SC-3085 we have obtained NIS data sets of two LytB samples: One data set was obtained from the substrate-free LytB and another one from the substrate-free LytB after the addition of a potential inhibitor complex (see Fig. 1 a and b). The NIS spectra were taken in a continuous flow cryostat at T=30 K on frozen isotopically enriched ^{57}Fe-LytB solutions (^{57}Fe-concentration 4 mM, sample volume ~ 50µl). Already from the inspection of the raw NIS-data displayed in Fig. 1a and b one can see an influence on inhibitor binding on the three main

bands between (i) 15 to 20 meV, (ii) 30-35 meV and (ii) 40 to 50 meV. The changes are even more evident in the partial density of phonon states (Fig. 1c).

The so obtained NIS data are currently being calculated by means of combined quantum chemical and molecular mechanics (QM/MM) calculations assuming model structures of the active site/inhibitor complexes. Within these calculations the 4Fe-4S active site and its ligands are treated by density functional theory (DFT) and the rest of the protein by molecular mechanics.

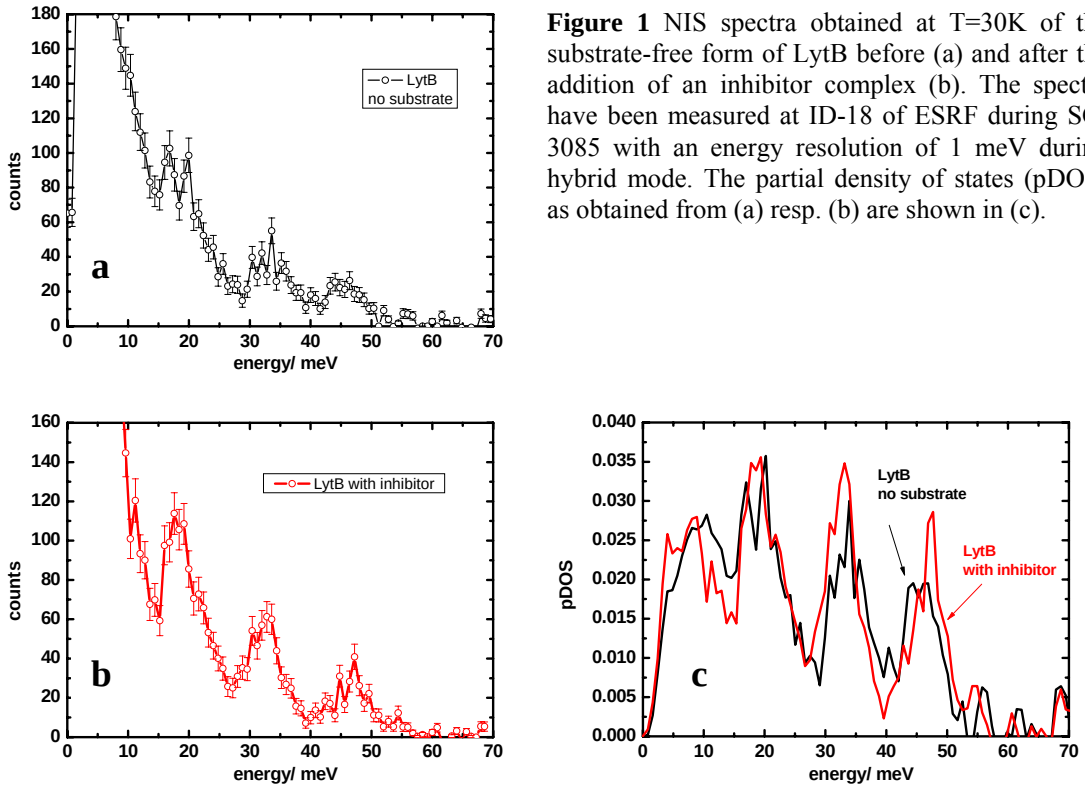

Figure 1 NIS spectra obtained at T=30K of the substrate-free form of LytB before (a) and after the addition of an inhibitor complex (b). The spectra have been measured at ID-18 of ESRF during SC-3085 with an energy resolution of 1 meV during hybrid mode. The partial density of states (pDOS) as obtained from (a) resp. (b) are shown in (c).

Unfortunately during the experiment CH-3085 a sample of the LytB-Protein with its natural substrate HMBPP could not be measured because of problems with the sample preparation. These measurements are important reference measurements because only for the substrate bound form of LytB a crystal structure is available which serves as input for our currently undertaken QM/MM calculations. In order to publish our results in a high-ranked journal so far not obtained NIS measurements of the substrate bound form of LytB are absolutely nessecary.

Therefore we would like to apply for one more beamtime in order to collect NIS spectra on more concentrated frozen LytB solutions for better spectral quality (^{57}Fe conc. ≥6 mM, ~ 50µl). We want to measure isotopically enriched ^{57}Fe-LytB after the addition of the substrate HMBPP and also after the addition of one more new potential inhibitors recently developed by our collaborator Prof. C. Dale Poulter, University of Utah.

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