	Experiment title: Isoprenoid biosynthesis in pathogenic bacteria: Substrate and inhibitor interaction of the LytB protein investigated by nuclear inelastic scattering	**Experiment number**: SC-3567
Beamline: ID-18	**Date of experiment**: from: 19/09/2012 to: 25/09/2012	**Date of report**: 01/03/2013
Shifts: 9(18)	**Local contact(s)**: Aleksandr Chumakov	*Received at ESRF:*

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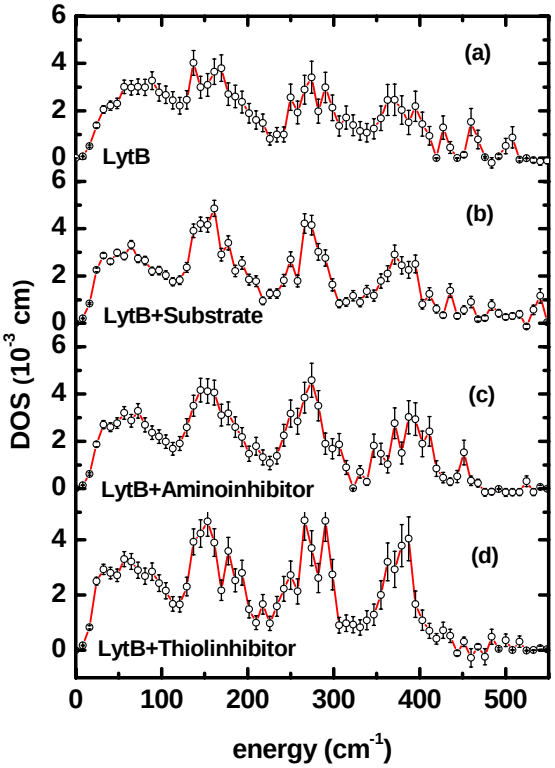
Report:

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 amino inhibitor complex (see Fig. 1a and c). During this beamtime SC-3567 we have completed the data sets and measured also the LytB-Protein with its natural substrate HMBPP (Fig. 1b) and a second inhibitor bound form namely a LytB thiolinhibitor complex (Fig. 1d) developed by our collaborator Prof. C. Dale Poulter, University of Utah. The NIS spectra were taken in a continuous flow cryostat at T=20 K on frozen isotopically enriched ^{57}Fe-LytB

solutions (^{57}Fe-concentration now 5 mM, sample volume ~ 50µl). In the meantime after finishing the experiment the so obtained NIS data have been calculated by means of combined quantum chemical and molecular mechanics (QM/MM) calculations assuming model structures of the active site/inhibitor complexes (see Fig. 2). 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.



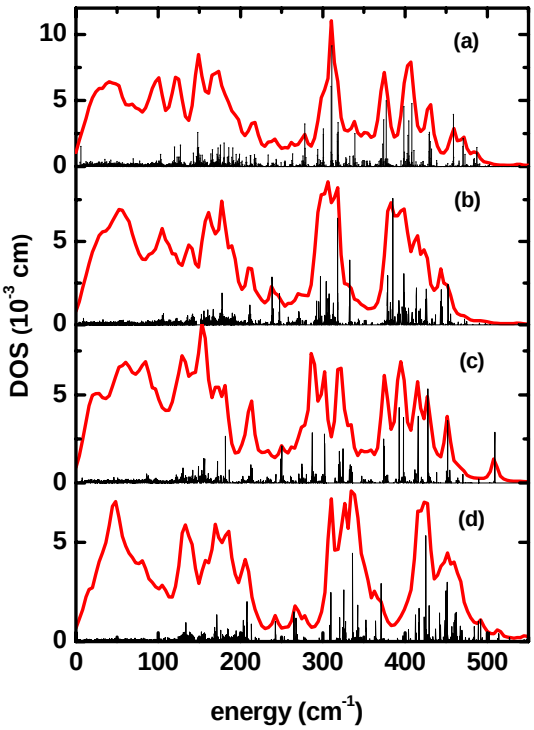


Figure 1: (left) Partial density of states (pDOS) obtained from NIS data of the substrate-free form of LytB (a) the substrate bound LytB (b) as well as after the addition of an amino-inhibitor (c) and a thiol-inhibitor complex (d). The data shown in (b) and (d) were taken during SC 3567 in 4-bunch mode. The data shown in (a) and (c) have already been measured at ID-18 of ESRF during SC-3085 with an energy resolution of 1 meV during hybrid mode.
Figure 2: (right) pDOS simulations obtained via combined quantum chemical and molecular mechanics (QM/MM) calculations assuming model structures of the active site/inhibitor complexes: (a) substrate-free form of LytB; (b) substrate bound LytB; (c) amino-inhibitor bound LytB; (d) thiol-inhibitor bound LytB.

In addition we could measure first data sets of the reduced form of the substrate bound LytB and an amino-inhibitor form (data not shown), also here QM/MM calculations are presently performed in our laboratory. Moreover due to the excellent stability of beamline ID-18 we could aquire a first NIS data set of an other very important 4Fe-4S protein (NadA) that is involved in nicotinamide adenine dinucleotide (NAD) biosynthesis (in cooperation with Dr. Sandrine Ollagnier de Choudens from CNRS-CEA Grenoble) and which is like LytB also a target for new antibiotics. The so obtained data is shown in a new proposal for beamtime submitted by March 1st 2013.

1. Seemann, M.; Janthawornpong, K.; Schweizer, J.; Böttger, L.H.; Janoschka, A.; Ahrens-Botzong, A.; Tambou, E.N.; Rotthaus,O.; Trautwein, A.X.; Rohmer, M.; Schünemann, V. *J. Am. Chem. Soc.* **2009** 131(37): 13184
2. Rekittke, I.; Wiesner, J.; Röhrich, R.; Demmer, U.; Warkentin, E.; Xu, W.; Troschke, K.; Hintz, M.; No, J. H.; Duin, E.C.; Oldfield, E.; Jomaa, H.; Ermler, U. *J. Am. Chem. Soc.* **2008** 130: 17206
3. Gräwert, T.; Rohdich, F.; Span, I.; Bacher, A.: Eisenreich, W.; Eppinger, J.; Groll, M. *Angew. Chem. Int. Ed.* **2009** 48: 5756
4. Grawert T, Span I, Eisenreich W, Rohdich, F. Eppinger, J.; Bacher, A.: Groll, M.et al. *Proc. Natl. Acad. Sci. U.S.A.* **2010** 107**(**3): 1077