To: CONSIGLIO NAZIONALE DELLE RICERCHE
Accordi e Relazioni Internazionali
Piazzale Aldo Moro, 7, 00185 - ROMA

Scientific Report on the research activity of Dr. Vitaly Borisov carried out within the framework of the CNR Short Term Mobility program

The work was performed by Dr. Vitaly Borisov during a research stay of 10 days (June 4-14, 2011) in the group of Dr. Alessandro Giuffrè at Istituto di Biologia e Patologia Molecolari (IBPM), Consiglio Nazionale delle Ricerche (Piazzale Aldo Moro 5, I-00185 Rome) within the framework of the Short Term Mobility Program - 2011. According to the proposed research project, the study aimed at gaining insight into the reactivity of the *bd*-type terminal respiratory oxidase from *Escherichia coli* towards hydrogen peroxide.

Bacterial cells (*E. coli* strain GO 105/pTK1) were grown in a 30 L fermentor or in 5 L flasks at 37 °C. To obtain the bacterial membranes, the cells were passed through a French press. Intact and partially broken cells were removed by centrifugation at 14,500×g for 10 - 15 min at 4 °C. The membranes were sedimented from the supernatant (48,000×g, 60 min, 4 °C). For isolation of cytochrome *bd* complex, the membranes were solubilized with the use of 2% *sucrose monolaurate* detergent. Following centrifugation, the cytochrome *bd*-containing supernatant was loaded on a DEAE-sepharose fast flow column, and the enzyme was eluted with a 25 - 350 mM KCl gradient. The purified enzyme was stored at -80 °C.

Oxygraphic assays were carried out using a high-resolution respirometer (Oxygraph-2k, Oroboros Instruments). Static absorption spectra were recorded with a double beam UV/VIS spectrophotometer (Jasco V-550) with a light path of 1 cm. Cytochrome *bd* concentration was determined from the reduced *minus* 'as prepared' difference absorption spectra using $\Delta\varepsilon_{628-607}$ = 10.8 $mM^{-1}cm^{-1}$. The assays were performed in 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 0.05% N-lauroyl-sarcosine. Data analysis was carried out using the software Origin 7 (OriginLab Corporation) or MATLAB (MathWorks).

The experiments carried out during the research stay focused on the reaction of hydrogen peroxide with the cytochrome *bd* oxidase purified from *E. coli*. Based on these preliminary experiments, clear-cut evidence was provided for a remarkably different reactivity of the enzyme, depending on its redox/ligation state. Reactions were studied under a large variety of experimental conditions in the presence or absence of specific hemeprotein inhibitors. The explored experimental parameters included, among others, substrate and enzyme concentration, O_2 concentration (aerobic vs anaerobic conditions), presence/absence of typical heme ligands or reductants. The results achieved have successfully unveiled novel reaction pathways whose impact on bacterial physiology and pathogenicity will be assessed in future studies. A detailed plan of the future activities that will be collaboratively carried out by the two partners was agreed. These activities will allow to confirm and expand the very interesting observations made at IBPM in Rome.

Date: June 22, 2011
Dr. Vitaly BORISOV