To: CONSIGLIO NAZIONALE DELLE RICERCHE
Ufficio Accordi e Relazioni Internazionali
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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 (May 5-16, 2014) in the group of Dr. Alessandro Giuffrè at Istituto di Biologia, Medicina Molecolare e Nanobiotecnologie (IBMN), Consiglio Nazionale delle Ricerche (Piazzale Aldo Moro 5, I-00185 Rome) within the framework of the Short Term Mobility Program - 2014. According to the proposed research project, the study aimed at gaining insight into the interaction of cytochrome *bd* terminal oxidase from *Escherichia coli* with peroxynitrite.

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. Stopped-flow (DX.17MV, Applied Photophysics, Leatherhead, UK) experiments were performed in single-wavelength mode at millisecond time resolution. 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 100 mM sodium phosphate buffer (pH 7.0) containing 20 µM diethylenetriaminepentaacetic acid and 0.05% *N*-lauroyl-sarcosine. Data analysis was carried out using the software Origin (OriginLab Corporation) and MATLAB (The Mathworks, South Natick, MA).

The experiments carried out during the research stay focused on the reaction of peroxynitrite with the cytochrome *bd* oxidase purified from *E. coli*. Based on these experiments, clear-cut evidence was provided for a remarkably different reactivity of the bacterial cytochrome *bd* terminal oxidase as compared to mitochondrial cytochrome *c* oxidase. Reactions were studied under a large variety of experimental conditions in the presence or absence of specific reductants. In view of the information that peroxynitrite is produced by host immune cells to kill invading microbes, causing severe oxidative and nitrosative stresses in bacteria via DNA damage, lipid oxidation, and protein modification, the results achieved have successfully unveiled novel biomedically important functional properties of the cytochrome *bd* terminal oxidase whose impact on bacterial physiology and pathogenicity will be assessed in future studies. The obtained data allow us to understand how cytochrome *bd* can enhance bacterial resistance to nitrosative and oxidative stress. We anticipate that these data may pave the way to novel strategies to combat infectious diseases based on the targeting of cytochrome *bd* terminal oxidase in bacterial pathogens. A detailed plan of the future activities that will be jointly carried out by the two partners was agreed. These activities will allow to confirm and expand the very interesting observations made at IBMN in Rome. On the basis of the results obtained during the research activity, a manuscript is under preparation to be submitted for publication.

Date: May 22, 2014 Dr. Vitaly BORISOV