

**Experiment title:**

Long range interactions in mutated bacterial reaction centers in the vicinity of non-heme iron.

Experiment number:

SC 3566

Beamline:

ID18

Date of experiment:

from: 04/09/2012 to: 09/09/2012

Date of report:

15/02/2015

Shifts:

15

Local contact(s):

Dr Aleksandr Chumakov

*Received at ESRF:***Names and affiliations of applicants (* indicates experimentalists):**K.Burda^{1*}, J.Korecki², T.Ślęzak^{2*}, A.Hałas^{1*}¹*Department of Bio- and Medical Physics, Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, al. Mickiewicza 30, PL-30059Krakow, Poland*²*Department of Solid State Physics, Physics & Applied Computer Science, AGH University of Science and Technology, al. Mickiewicza 30, PL-30059Krakow, Poland***Report:**

Our aim was to investigate long range regulatory mechanisms controlling the activity of the photosynthetic bacterial reaction centers (BRCs) of type II. Especially, we concentrated on the role of the non-heme iron (NHFe) in these processes. Given the present knowledge on charge and energy transfer in the bacterial reaction centers, one believes that evolutionary conserved residues can be crucial to keep RCs working in a highly efficient way determining the paths of electron and proton transfer. For this purpose we used *Rb. sphaeroides* mutants. This project was a continuation of a previous proposal SC3334 when we studied vibrational state density originated from the collective motions of the protein matrix in the vicinity of NHFe in high purity His-tagged wild type and mutated BRCs [1-3]. We observed that the exchange of the following residues L212Glu, L213Asp and M249Ala in the vicinity of the Q_A and Q_B sites leading to the modification of the hydrogen network on the outer side of the reaction center influenced the collective vibrations crucial for an efficient functioning of the acceptor side of BRCs [3].

Within this project we investigated a double mutant in which the most rigid part of the RC protein core was influenced. The residues L187Phe and M216Leu located only about 7 and 9 Å from NHFe were exchanged into a smaller non-polar residue, alanine (L187Phe to Ala and M216Leu to Ala), see Fig.1.

Data obtained by use of Mössbauer absorption spectroscopy (MAS) showed that in this mutant, referred to as dFer, the reduced NHFe was mainly in a low spin (LS) state. Nuclear forward spectroscopy (NFS) with applied external magnetic field allowed us to characterize the other spin states of NHFe detected in the dFer RCs.

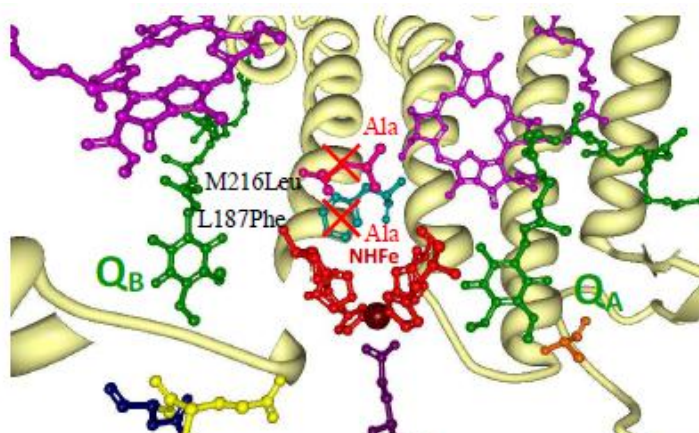


Fig. 1. The „dFer” mutant has two point mutations in the inner part of BRC, near the non heme iron ligands (in the second coordination sphere). Leucine and phenylalanine were replaced by neutral alanines (L187Phe/M216Leu → Ala/Ala).

In the case of the dFer mutant we could distinguish two paramagnetic high spin (HS) NHFe^{2+} states. One of them was strongly coupled to the semiquinone form Q_A^{\bullet} whereas the other one was decoupled from the semiquinone form or was in BRC's, in which the ubiquinone bound in the Q_A site is in its oxidized form. Besides two additional LS NHFe^{2+} (diamagnetic) states a new paramagnetic NHFe^{2+} was observed in this mutant. Its interaction with the external magnetic field proved that it was a ferrous NHFe in the intermediate spin (IS) state [3]. Nuclear inelastic scattering (NIS) providing unique information on the density of vibrational states (DOS) of NHFe in the quinone-iron complex of BRCs confirmed the presence of the intermediate spin state of iron in the dFer mutant. The experimental NIS data of WT and mutated dFer BRCs isolated from *Rb. sphaeroides* are presented in Fig 2a, b and their differential spectrum in Fig.2c.

Comparing NHFe vibrational modes between dFer and WT BRCs in the acoustic mode, the dFer mutant showed a slightly increased flexibility of the whole systems. However, the most striking differences in the DOS spectra were observed at higher energies. Between 21 meV and 31 meV a strong decrease in vibrational modes and simultaneously a significant increase in vibrations from 39 meV to 55 meV were detected for the mutant. These changes in DOS modes are directly related to the decreased contribution of the HS NHFe^{2+} state and an increased contribution of its LS state as well as an occurrence of the IS state. This is in accordance with the modifications of DOS spectra found for the AATyr mutant at our earlier investigations [3, report SC3334], although the changes are less pronounced comparing to the dFer mutant because of the lower contribution of NHFe diamagnetic states in the case of AATyr BRCs [2,3].

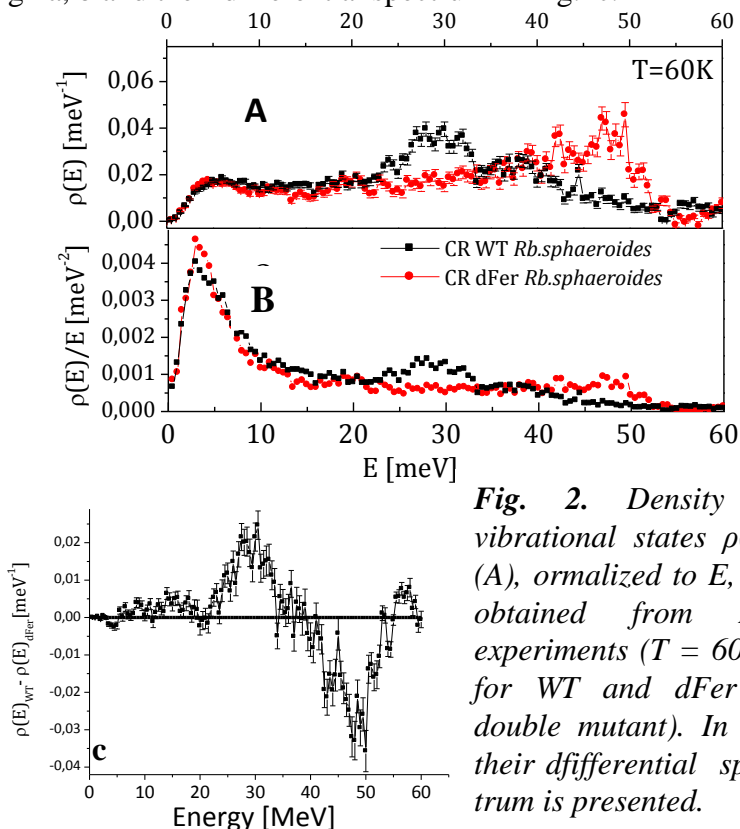


Fig. 2. Density of vibrational states $\rho(E)$, (A), ormalized to E , (B) obtained from NIS experiments ($T = 60\text{ K}$) for WT and dFer (a double mutant). In (C) their *df*ifferential spectrum is presented.

Recently, we have also checked by simulations using density functional theory (DFT) (*Q-SPECTOR Python program*) that one has to take into account an intermediate spin state of NHFe in order to get a good agreement with the experimental DOS spectrum of the dFer mutant.

The NFS and NIS experiments performed within this project allowed us to elucidate how mutations of the conserved residua from the most rigid part of the BRC core change a partial phonon density of NHFe states in comparison to a wild type of BRC and understand their role in regulation of coupling between the Q_A and Q_B quinone.

All the obtained results are included in the PhD thesis of Agnieszka Hałas. Two publications are under preparation. The experimental data was presented at the ICAME 2013 in Opatija (Croatia).

[1] A. Hałas, V.Derrien, P.Sebban, K. Matlak, J.Korecki, J. Kruk, K. Burda, “*Chemical properties of the iron-quinone complex in mutated reaction centers of Rb. sphaeroides*” *Hyperfine Interactions* (2011) DOI 10.1007/s10751-011-0451-0

[2] A. Hałas, A. Orzechowska, V. Derrien, A.,I. Chumakov, P. Sebban, J. Fiedor, M. Lipińska, M. Zając, T. Ślęzak, K. Strzałka, K. Matlak, J. Korecki, L. Fiedor, K. Burda (2012) The dynamics of the non-heme iron in bacterial reaction centers from *Rhodobacter sphaeroides* *Biochim. Biophys. Acta.* 1817: 2095–2102

[3] A.Hałas (2013) “Dynamics of nanostructural organization and activity of photosynthetic systems of type II.”, PhD thesis, Faculty of Physics and Applied Computer Science, AGH-University of Science and Technology, Krakow, Poland