ESRF	**Experiment title:** Trehalose-induced elimination of x-ray damage in the bacterial photosynthetic reaction center	**Experiment number**: SC-2211
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Report:

Trehalose, a disaccharide of glucose, is well known to be particularly effective in biopreservation from adverse environmental conditions such as high temperatures and dehydration (Crowe et al.1996); it is also known to hinder the conformational dynamics of different types of proteins even at room temperature, as shown by spectroscopic studies and molecular dynamics simulations (reviewed in Cordone et al., 2005; Giachini et al., 2007).
A preliminary study performed in the framework of experiment SC-1976 let us suppose that incorporation of the membrane-pigment protein complex photosynthetic reaction center (RC) in strongly dehidrated trehalose matrices could eliminate the x-ray damage affecting the same protein incorporated in a weakly interacting medium (PVA, polyvinil alcohol).

The Fe^{2+} site of photosynthetic RC is an interesting case of study because of its structural role in the photosynthetic process (Stowell et al.,1997); a few XAFS studies provide accurate information about its local structure (Bunker at al, 1982), while several XRD stuctures of the whole RC are available (Koepke et al, 2007).

In order to determine the behavior of the Fe^{2+} site as a function of the absorbed dose, we measured fluorescence-detected XANES spectra as a function of the X-ray flux, both in PVA films and dry trehalose matrices at room temperature and in the dark. Different fluxes were obtained by appropriately inserting metallic foil filters in the impinging x-ray beam, from a minimum of about 10^8 ph/s up to a maximum of ~10^{11} ph/s (see Figure 1).

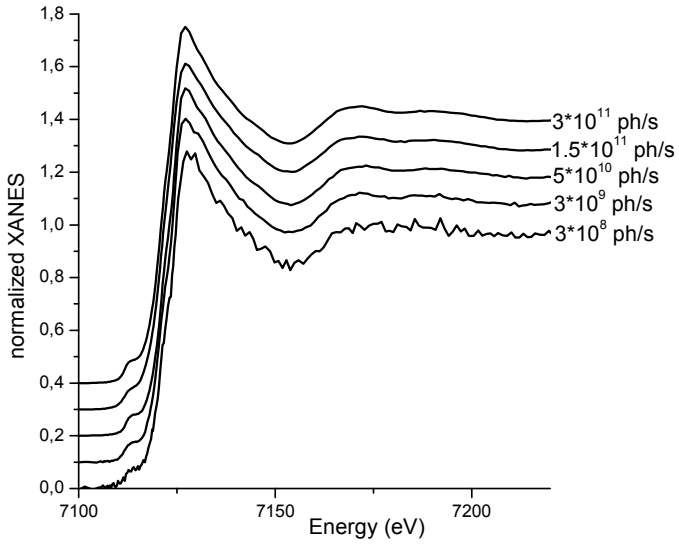

Fig.1: XANES spectra of photosyntetic RC in PVA films as a function of the incoming flux.

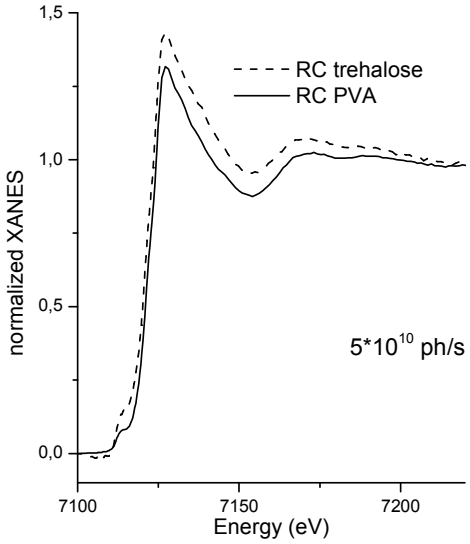

Fig.2: Comparison between XANES spectra of RC in PVA and in trehalose at equal fluxes

Despite the high quality of the collected data, no changes were observed in the XANES spectra of RC in PVA as a function of the absorbed dose, to the contrary of what was expected on the basis of preliminary measurements and published studies (Yano et al., 2002).

From a comparison between spectra of RC in PVA and trehalose matrices irradiated with the same x-ray flux (see Fig.2), a small difference in the white line intensity emerges. The absence of an x-ray induced damage in the PVA sample strongly suggests that this is due to a consequence of a different arrangement in the first shell Fe ligands rather than to the preservation from radiation damage as previously expected. This was confirmed by a detailed data analysis which allowed us to determine with a high resolution the local structure around Fe for the RC embedded in both in trehalose in the PVA matrix (Veronesi et al., 2008).

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