Functional dynamics of photosynthetic cells useful for biosensor development

Daniela Russo [a,b], Maya Dimova Lambreva [c], Gaetano Campi [c], Alessio De Francesco [a], Christiane Alba Simionesco [d], Pierre Sebban [e], Michael Haertlein [f], Martine Moulin [f], Giuseppina Rea [c]

[a] Istituto Officina Materiali, CNR, c/o Institut Laue Langevin, Grenoble, 38042, France
[b] Institut Lumière Matière, Université Lyon 1 and CNRS, Villeurbanne CEDEX, 69622, France
[c] Istituto di Cristallografia, CNR, Rome, 00015, Italy
[d] Laboratoire Leon Brillouin (CEA/CNRS), CEA Saclay, 91191, France
[e] Laboratoire de Chimie Phisique, University Paris Sud, Orsay, 914000, France
f Life Sciences Group, Institut Laue-Langevin, Grenoble 38042, France

e-mail: giuseppina.rea@ic.cnr.it

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Photosynthesis is receiving renewed interest due to the possibility to integrate whole plant cells or their photosynthetic active sub-components into optoelectronic devices such as biosensors and organic semiconductors. Photosynthetic assemblies use light energy to power electron transfer and charge separation across a charge-impermeable lipid membrane. One of the main working photosynthetic units is the pigment/protein complex photosystem II (PSII) which hosts, among others, the D1/D2 reaction center (RC) proteins, where most of the photosynthetic redox active components are located. Photonic energy is captured by the antenna systems and sequentially transferred towards the RC at the primary acceptor plastoquinone Q_A, a single-electron carrier tightly bound to the D2 protein. Thereafter, electrons are transferred from Q_A to the secondary plastoquinone Q_B, bound to the D1 subunit, via a non-heme iron. Q_B is a two-electron carrier and after full reduction and protonation to Q_{BH2}, it is displaced by another molecule of the plastoquinone pool [1]. Some classes of environmental pollutants are competitive inhibitors of Q_B binding and can interrupt the photosynthetic electron transport chain. This activity can be easily monitored and it is the working principle underlying the functioning of PSII-based biosensors for the detection of toxic compounds [2-4]. The primary goal of our studies is to determine how structural, dynamics and functional proprieties of natural and mutated photosynthetic D1 RC proteins in green algae influence the sequential electron transfer reactions leading to efficient photochemical energy conversion. A better understanding of these factors will help to identify the parameters underlying an increased performance in terms of protein stability and functional reliability for biosensoristic purposes and to design molecular systems mimicking the high efficiency of solar energy conversion in natural photosynthesis. While the structure and the function of those systems are known, the protein dynamics and its relationship with the activity is still a focus of interest. The understanding and the ability to modulate the existing relations between structure-dynamics-functionality through selective genetic mutation, is extremely important for the biotechnological applications and fundamental research studies.

Recently, we measured the flexibility and diffusive dynamics of *Chlamydomonas* cells and thylakoids carrying both native and single point mutated D1 proteins using neutron spectroscopy. In particular, we analyzed a set of mutants having improved or reduced affinity for specific classes of herbicides, and increased tolerance to ionizing radiation [5-7] (Figure 1A). By combining elastic and quasi-elastic neutron scattering data with chlorophyll fluorescence measurements, we revealed that single aminoacid replacements in the D1- plastoquinone binding niche impair electron transfer efficiency, and notably affect the temperature dependence of the overall protein dynamics, inferring increased flexibility to the host membranes, expanding to the entire cells [8] Furthermore, to get a more detailed dynamical picture and to better understand the relation with the activity of the photosynthetic RC, we completed the single particle dynamics experiment with a collective dynamics study by neutron Brillouin spectroscopy. Results indicated that single point mutations affect collective density fluctuations in hydrating water providing important insight on the transmission of information possibly correlated to biological functionality.

(Figure 1B). Furthermore, to get a more detailed dynamical picture and to better understand the relation with the activity of the photosynthetic RC, we completed the single particle dynamics experiment with a collective dynamics study by neutron Brillouin spectroscopy [9]. Results indicated that single point mutations affect collective density fluctuations in hydrating water providing important insight on the transmission of information possibly correlated to biological functionality (Figure 2).

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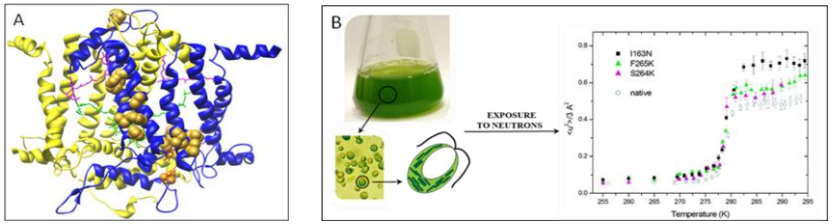

Figure 1. A. Ribbon-diagram overview of the *C. reinhardtii* PSII reaction center D1-D2 heterodimer. B. Algal mutants hosting amino acids replacement in the photosynthetic RC core proteins acquire more flexibility in term of mean square fluctuations.

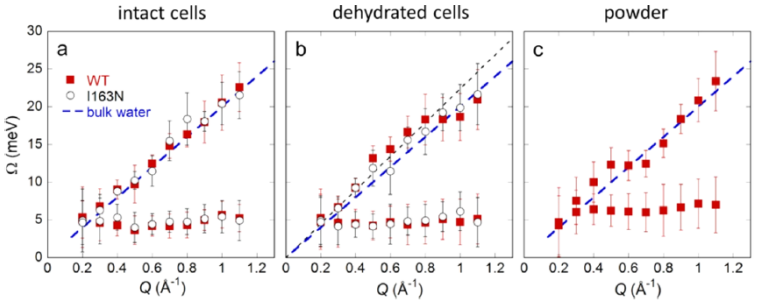

Figure 2. Experimental dispersion curves of THz collective modes in hydration water of (a) hydrated wild-type and I163 mutant cells, (b) 60% dehydrated wild-type and I163 mutant cells, and (c) highly hydrated powders.