Photoactivation dynamics in photosynthetic and signal transduction proteins studied by ultra-fast time-resolved spectroscopy

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door

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geboren te Bari, Italië

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a Giada e Bruno

When words become unclear, I shall focus
with photographs. When images become
inadequate, I shall be content with silence.

(Ansel E. Adams, 1902-1984)

Introduction: To see the light

Life on Earth would not be possible without the presence of our closest star: the Sun. This is true not only from a cosmological point of view (Sun formation leads to the formation of its planets), but also in terms of the energy source that the Sun provides for (almost) all biological processes. The daily experience that we have of the Sun is mediated by our highly refined light detectors, the eyes, together with the most sophisticated signal processing system present in nature, the human brain. With these tools we can see and interpret the surrounding environment, using the light coming from the Sun and reflected by objects or people around us. However, human-like vision is not the only kind of vision existing in nature; there are free-moving cells (like photosynthetic bacteria) or more complex organisms that once illuminated by light react to it by directed migration (Figure 1A). This phenomenon is called *phototaxis*. A *taxis* (from Greek, *tactics*, arrangement) is an innate behavioral response by an organism to a directional stimulus, the process is called phototaxis when the stimulus comes from a light source. Photosynthetic bacteria use light to produce the chemical energy needed to survive, so for them it is absolutely necessary to sense the environment where they live so they can swim towards the green, yellow or red light maximizing the exposure to 'photosynthetic' light, or escape from the potentially harmful blue/UV light. This response to a light stimulus is found even in organisms not capable of directed physical movement; in plants (or other eukaryotic organisms like fungi, Figure 1B) *Tropism* (from Greek, *tropos*, to turn) is a biological phenomenon, indicating growth or turning movement in response to an environmental stimulus, *phototropism* is a reaction to light. A commonly known phototropism phenomenon comes from the sunflowers, which turn their heads during the day towards the sun (a phenomenon also called *heliotropism* from Greek – *Helios*, Sun). Other more complex biochemical mechanisms, allow photosynthetic algae to live deep in the sea, plants to live happily either under direct sun light illumination or under a dense canopy or, pathogenic bacteria like *Brucella melitensis* to "see" whether or not they are inside a host and then turn-on or – off their virulency [1]. All these responses to an external stimuli from different organisms imply a sensing of the surrounding environment.

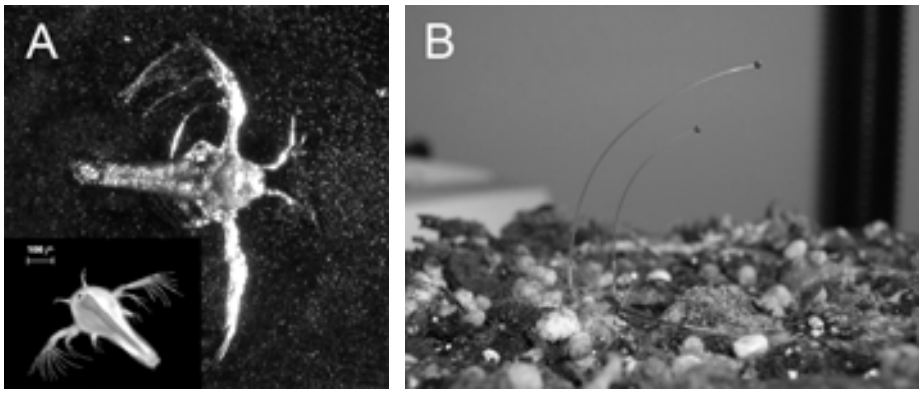

Figure 1: **A**, Brine shrimps are a species of aquatic crustaceans of the genus *Artemia*. Artemia belongs to the *Arthropoda* phylum, *Branchiata* sub-phylum, *Crustace*a class, *Branchiopoda* sub-class, *Anostraca* order, *Arterniidae* family and *Artemia* genus. *Artemia*, the only genus in the family *Artemiidae*, have evolved little since the Triassic period. First discovered in Lymington, England, in 1755, *Artemia* are found worldwide in inland saltwater lakes, but not in oceans. Their color varies from greenish to bright red depending on food and oxygen concentrations within the water. The adults swim away from light, and the larval (*napulii*) forms swim toward it. The shrimp always orient themselves so that their ventral surface faces the light. **B**, *Phycomyces* is a genus of fungi in the *Zygomycota* family. They are known for their strong phototropic response.

All the above-mentioned processes are based on very different biochemical mechanisms but they have the same starting point: a light stimulus. Once a photon (elementary particle of light) is absorbed by the organism, a photochemical reaction starts and evolves in a direction determined by the organism under observation. Each organism has developed during its evolutionary path the best strategy to collect light. The functional part that is involved in the first photoactivation step is, in general, a protein called photoactive protein. If such a protein has only light collection and transferring functions it is called a *Light Harvesting Complex* (LHC), mainly found in photosynthetic organisms. Instead, if the protein has the function of light detection and generation of a signal is called a *photoreceptor*.

This Thesis

In this thesis I report studies performed on a selected number of photoactive proteins and chromophores involved in light harvesting (PCP from marine alga *Amphidinium carterae*, and Chlorophyll-*a* and Peridinin) and in signal transduction (OCP from the cyanobacterium *Arthrospira maxima* and Slr1694 from *Synechocystis PCC 6803*), trying to understand which are the primary reactions that take place upon the photo-excitation and lead to energy transfer or signalling. The thesis is divided in two parts, chapters two and three will investigate the energy dissipation that takes place upon light excitation of Peridinin-Chlorophyll-a-Protein (PCP – Chapter 2) and between Chlorophyll-a and Peridinin (Chapter 3), the LHC and isolated chromophores involved in the light harvesting function in some photosynthetic organisms. Chapters five and six, will explore the photochemistry of Slr1694 in its wild type form (Chapter 5) and in some of its mutants (Chapter 6). Chapter 4 is a chapter that links the photosynthetic and photoreceptor blocks where I report the primary photophysics of the orange carotenoid protein (OCP) containing a carotenoid chromophore typical for photosynthetic organisms (3' -hydroxy-echinenone), which play the role of a photoreceptor. Chapter 1 is an introduction to the arguments presented in the thesis, giving more detailed information on experimental systems, measurement and analysis techniques used for the data collection and interpretation.

Reference:

1. Swartz TE, Tseng TS, Frederickson MA, Paris G, Comerci DJ, Rajashekara G, Kim JG, Mudgett MB, Splitter GA, Ugalde RA *et al*: **Blue-light-activated histidine kinases: Two-component sensors in bacteria**. *Science* 2008, **317**(5841):1090-1093.