Investigation of the photosynthetic protein Light-Harvesting Complex II: protection against photo-damage and artificial light harvesting

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Introduction

Light-Harvesting Complex II (LHCII) is a membrane protein with embedded chromophores (chlorophylls and carotenoids) that absorb solar photons and transfer energy as electronic excited states towards Photosystem I and II. When environmental conditions lead to relatively high light intensities, LHCII can adopt a photoprotective state in which excitation energy is safely dissipated as heat, a process known as Non-Photochemical Quenching (NPQ) of chlorophyll fluorescence. *In vivo*, NPQ is triggered by a combination of factors including low pH and the action of specific enzymes leading to a dramatic shortening of LHCII fluorescence lifetime. *In vitro*, purified LHCII suspended in detergent or reconstituted in liposomes can reversibly adopt a quenched NPQ-like state. Previous spectroscopy of these *in vitro* models revealed changes in exciton dynamics and protein-pigment conformation that accompany quenching. However, the LHCII-LHCII interactions are poorly characterized.

Results and Discussion

We have performed correlated fluorescence lifetime imaging microscopy (FLIM) and atomic force microscopy (AFM) of LHCII complexes bound to mica and manipulated their environment to show varying degrees of quenching. AFM showed that LHCII self-assembles onto mica substrates forming 2D-domains of 100s nanometres in width. FLIM showed that LHCII trimers in these aggregates are in a quenched state, with a much lower fluorescence lifetime (<0.3 ns) compared to free trimers in solution (2 to 4 ns). In contrast, LHCII-LHCII interactions were disrupted by the presence of thylakoid lipids or common phospholipids and led to an intermediate length fluorescent lifetime (approx. 0.8 ns). To our knowledge, this is the first *in vitro* correlation of nanoscale membrane imaging to the state of LHCII quenching of a minimal number of complexes. The results suggest that lipids could play a key role in modulating the extent of LHCII-LHCII interactions within the thylakoid membrane and so alter the propensity for the complex to activate NPQ.

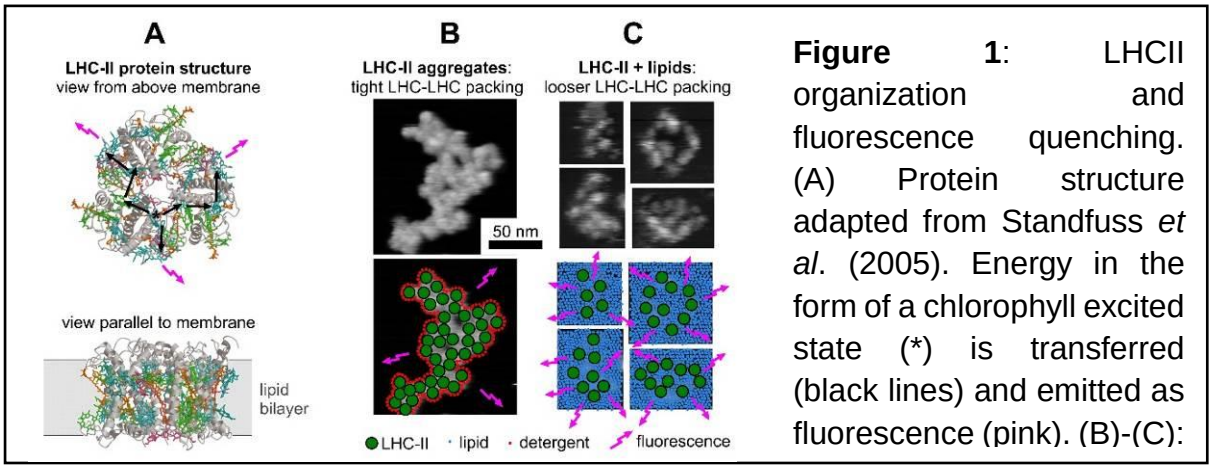

Figure 1: LHCII organization and fluorescence quenching. (A) Protein structure adapted from Standfuss *et al*. (2005). Energy in the form of a chlorophyll excited state (*) is transferred (black lines) and emitted as fluorescence (pink). (B)-(C):

In ongoing work, we are developing protein-lipid vesicles (proteoliposomes) comprised of thylakoid lipids and a variety of concentrations of LHCII. Preliminary data shows that quenching increases with LHCII/lipid ratio as shown by cuvette-based fluorescence spectroscopy and fluorescence microscopy. Furthermore, we are able to introduce non-native chromophores into the lipid bilayer which show evidence of Förster resonance energy transfer (FRET) to the LHCII. This could be a method to increase the effective absorption cross-section of LHCII, which would allow a greater portion of the solar spectrum to be utilized.

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Collaborators
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