Water-soluble chlorophyll-binding proteins (WSCPs) as well-defined systems by which to probe and modify chlorophyll-chlorophyll and chlorophyll-protein interactions

E. Krausz,[1] J.L. Hughes,[1] A. Oakley,[1] B. Conlan[2] and T. Wydrzynski,[2] [1]Research School of Chemistry, Australian National University, Canberra, ACT 0200, Australia and [2]Research School of Biological Sciences, Australian National University, Canberra, ACT 0200, Australia.

Natural photo-systems are the most efficient, flexible and adaptable photovoltaics known. Chlorophyll(Chl) pigments are utilised as stable light harvesting components, and yet the same Chls, when bound at specific protein sites, spontaneously charge separate upon optical excitation to perform the primary photochemistry of photosynthesis. The properties of Chls that are important for light absorption, energy transfer and charge separation are strongly influenced and controlled byChl-Chl and Chl-protein interactions. Understanding these processes is important for artificial photosynthesis.

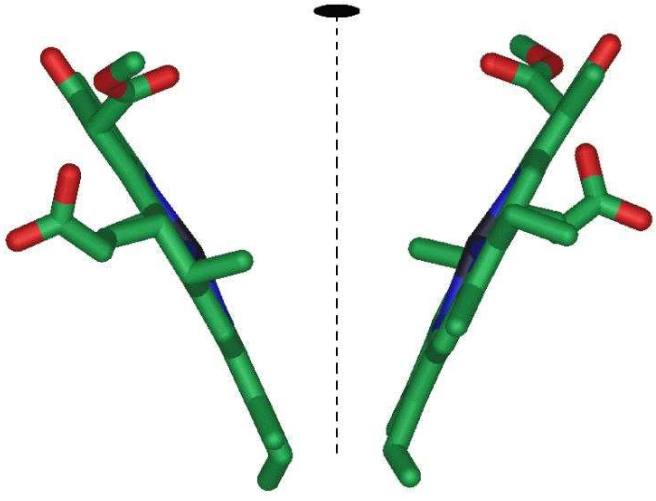
We report laser-based spectroscopic studies of a natively reconstituted cauliflower WSCP (see Schmidt *et al.*, 2003 and Hughes *et al.*, 2006). In the presence of Chl, this protein forms tetrameric units. During this process there are just *two* Chls bound per protein tetramer. The natural function of WSCPs is not fully understood, but may act as Chl transport proteins during Chl biosynthesis and/or catabolic pathways, and possibly as aChl scavenger when the plant is under stress. Chl bound to WSCP is protected against photo-induced singlet-oxygen formation (Schmidt *et al.*, 2003) and, with the absence of any carotenes in WSCP, the protection mechanism is not known.

Our previous circular dichroism (CD) and magnetic circular dichroism (MCD) studies (Hughes *et al.*, 2006) have established that in natively reconstituted Chl-WSCP, the two Chls are in a single and remarkably well defined protein environment. Furthermore, the two Chls are in a 'sandwich' configuration (see Figure), leading to a relatively strong exciton coupling between the pigments. One consequence of this arrangement is a *weak* lowest energy optical excitation of the system.

We report laser-induced spectral hole-burning measurements of this system. Hole-burning measurements allow further characterisation of the exciton-coupling in Chl-WSCP as well providing excited state lifetimes. The width of spectral holes provide lifetimes, without the need for time-domain experiments. These measurements also potentially identify the mechanism for the protection of Chl against photo-degradation due to singlet oxygen. We also use hole-burning to study the electron-phonon coupling of Chl-WSCP and Chl excited-state vibrational frequencies. We investigate ourprevious suggestion (Hughes *et al.*, 2006) of the presence of a unique high-frequency phonon mode (~90 cm^{-1}) for Chl-WSCP. Excited-state vibrational frequencies can be used as indicators of Chl-protein interactions such as the Mg^{2+} ligation state and hydrogen bonding of the protein to the Chl peripheral groups. Such interactions may play critical roles in the Chl-binding and WSCP tetramerisation processes as well as in the photo-protection mechanism.

Schmidt K, Fufezan C, Krieger-Liszkay A, Satoh H & Paulsen H (2003). *Biochemistry*, **42:** 7427-33.

Hughes JL, Razeghifard R, Logue M, Oakley A, Wydrzynski T & Krausz E. (2006) *Journal of the American Chemical Society*, **128:** 3649-58.



'Sandwich' geometry of Chls in WSCP