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Engineering chimeric light harvesting proteins to determine the physical basis for the emergence of quantum coherence.

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14. ABSTRACT
The strange phenomena of quantum mechanics were not expected to play a direct role in life, however, it appears that quantum effects may be important in the efficient capture of sunlight for photosynthesis. The conditions for the emergence of quantum phenomena appear to be set by the structures of proteins. The aim of the project is to relate protein structure to the emergence of quantum effects in the light harvesting proteins of marine algae. We have discovered two different structural forms of the same type of light harvesting protein where one supports quantum phenomena while the other does not appear to do so. Our aim is to determine the structural features of these proteins that result in the emergence of quantum coherence. To do this, we will engineer chimeric proteins that have been built by separating the two components of natural light harvesting proteins and then recombining them to produce novel pair of matched chimeric proteins. We will use x-ray methods to determine the threedimensional structures of these chimeric proteins so as to confirm our bioengineering. We will then characterize the chimeric protein pairs using ultrafast laser spectroscopy so as to detect quantum coherence. In this manner we aim to determine the precise nature of the link between protein structure and the presence or absence of quantum phenomena. Understanding the link between structure and quantum effects will improve our knowledge of how nature achieves its remarkable efficiency in utilizing the energy from the sun. This is likely to foster new technologies that improve the efficiency of solar energy systems.

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Accomplishments

Research objectives

Our aim is to test whether the quaternary structure of the light harvesting protein is directly correlated to the observation of quantum beats. To do this, we will create a matched pair of chimeric proteins formed by switching the subunits from two parent proteins, one open and the other closed form. We will determine whether the nature of the α subunit determines the quaternary structure of the chimera via x-ray crystallography. We will determine which of the two chimeras produces quantum beats.

Details of accomplishments

Formation of Hybrid Proteins

To create synthetic hybrid phycobiliproteins, subunits of different open and closed form phycobiliproteins were refolded after the subunits are purified by HPLC. Once the subunits are mixed, they are subsequently dialysed into 4:1 (v/v) CH_3CN in 0.1 M $HCl_{(aq)}$ at pH 2, and refolding requires an additional 20 fold dilution out the organic solvent to reform the native proteins.

We studied these proteins spectroscopically using steady state absorbance and fluorescence. The hybrids showed that the quaternary structure (open versus closed) altered the spectroscopic properties.

Although our aim was to verify the quaternary structure of the synthetic hybrid proteins via x-ray crystallography, we did not succeed in obtaining crystals of sufficient quality to allow structure determination. Given this, we turned to naturally occurring alternatives: proteins from a single organism that generate both closed and open quaternary structure light harvesting proteins with near identical chromophores and protein subunits.

Structural studies of closed and open state quaternary structures from a single organism

At the outset of this work, the research community believed that each cryptophyte only expressed a single type of light harvesting protein. Such as statement appears in the introduction

of many research papers, including our own. In working with the cryptophyte *Hemiselmis andersenii*, we have discovered that we can separate at multiple, distinct colored, soluble light harvesting proteins (Fig. 1).

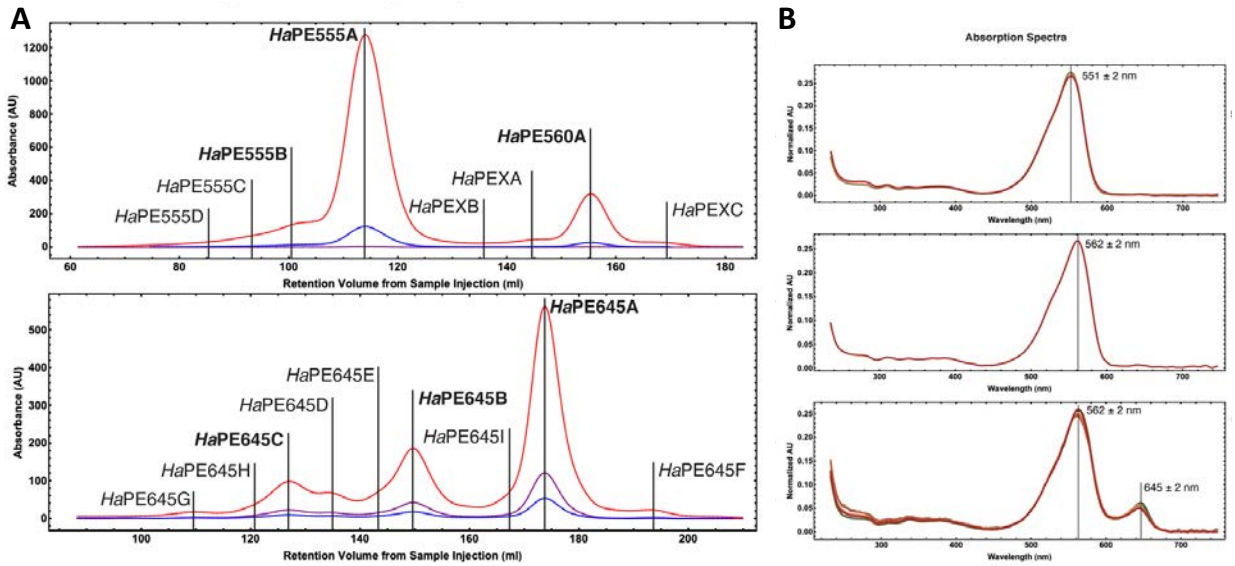

Fig. 1 Separation of three distinct spectrotypes from the cryptophyte H. andersenii. ***A***. *two fractions were separated by ion exchange chromatography, and then further fractionated (upper panel shows the "pink" fraction while the lower is the "blue" fraction). Each of these two protein fractions were further sub-fractionated into subspecies by anion exchange chromatography.* ***B***. *the absorption spectra of the three most abundant species: HaPE555 (top) HaPE560 (middle) and HaPE645 (bottom).*

We were able to purify the three most abundant proteins that represented the three distinct spectrotypes: HaPE555, HaPE560 and HaPE645 (Fig 1B) where HaPE555 has an absorbance maximum at 551 nm, Ha560 as a maximum at 562 nm and HaPE645 is unusual with a maximum at 562nm with a minor peak at 645 nm. We determined the crystal structure of each of these proteins (Fig. 2).

HaPE555 showed a normal open form light harvesting protein (Fig. 2A). However, it formed fibers in the crystal (Fig. 2A, lower). We crystallized this protein under four different conditions and each time, the crystal was composed of bundles of fibers, however, we noticed that these fibers differed in the monomer interface – with three morphologies: tight interface, loose interface and alternating tight-loose interfaces.

HaPE560 showed a new open form light harvesting protein (Fig. 2B). Here, a new insertion in the α subunit sequence altered the interface between αβ protomers (Fig. 2B, lower). increasing the interaction between the two αβ protomers. We did not observe any fibers.

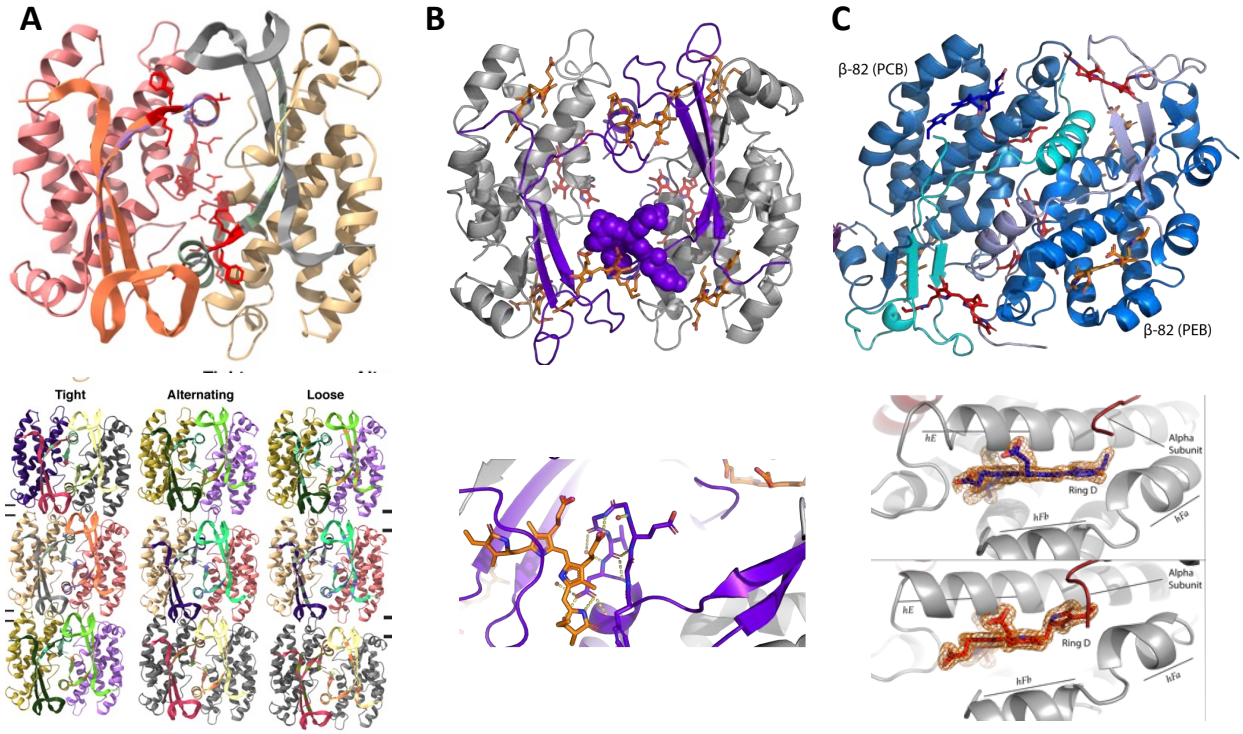

Figure 2. The crystal structures of: A. HaPE555, B. HaPE560 and C. HaPE645*. **A.** the open form HaPE555 shows a propensity to form fibers in all crystal forms (lower panel). **B**. the "super open" form HaPE560 shows an insertion in the α subunit that stabilizes the open quaternary structure (lower panel). **C**. the closed form HaPE645 structure shows a unique asymmetry, where the β82 chromophore is PCB on one chain (middle panel) and PEB on the other chain (bottom panel).*

The structure of HaPE645 was a complete surprise. The protein adopted the closed quaternary structure (Fig. 2C), where this is the first observation of a closed form being expressed in an organism that was previously only thought to produce open form proteins. However, we observed that there was an asymmetry in the chromophores attached to the β subunit: the chromophore on β82 was the expected PEB on one β subunit, however, it was replaced by PCB on the other subunit (Fig. 2C bottom). This is the first observation of an asymmetric chromophore distribution on the otherwise symmetric β subunit.

This asymmetry can be explained by the asymmetry in the α subunit, where in all closed forms one α subunit is longer than the other. However, in this case, it is a sequence difference in the N-terminus of the α subunits that appears to facilitate the loading of different chromophores. We are unaware of any possible mechanism that would achieve this, bar some yet to be discovered protein that alters the chemical nature of the chromophore on the behest of the distinguishing α subunit.

This set of different light harvesting proteins is the closest we have come to having a matched pair so as to test the origin of the observed quantum beats. Each of the three proteins have identical β subunits, as the organism only has a single gene expressing this subunit. We have also achieved having near identical proteins with both open and closed form quaternary structures. However, we now have the complication that one of the chromophores has been altered by an unexpected mechanism. Apart from this difference, all three proteins would have identical chromophore in identical sites (i.e. α19 chromophore is PEB, β50/61 is DBV, β158 is PEB, and β82 is PEB in all bar one subunit in HaPE645).

Dissemination to communities of interest

We have published three papers arising from this work. We have a fourth manuscript ready for submission and a fifth that is in an early draft form.

Rathbone, H.W., Davis, J.A., Michie, K.A., Goodchild, S.C., Robertson, N.O., and Curmi, P.M.G. (2018a) Coherent phenomena in photosynthetic light harvesting: part one-theory and spectroscopy. *Biophys Rev* 10: 1427-1441.

Rathbone, H.W., Davis, J.A., Michie, K.A., Goodchild, S.C., Robertson, N.O., and Curmi, P.M.G. (2018b) Coherent phenomena in photosynthetic light harvesting: part two-observations in biological systems. *Biophys Rev* 10: 1443-1463.

Rathbone, H. W., Michie, K. A., Landsberg, M. J., Green, B. R., and Curmi, P. M. G. (2021) Scaffolding proteins guide the evolution of algal light harvesting antennas. Nat Commun 12, 1890

Manuscripts in preparation:
Michie, KA, Harrop, SJ, Rathbone, HW, Wilk, KE, Teng CY, Hoef-Emden, K, Hiller, RG, Green BR and Curmi PM, Molecular structures reveal the origin of spectral variation in cryptophyte light harvesting antenna proteins

Rathbone, HW, Michie, KA, Laos, AJ, Thordarson, P and Curmi PM, Structure of the antenna from *Hemiselmis andersenii* shows both open and closed form phycobiliproteins

Impacts

Development of the principal discipline(s) of the project
Understanding how photosynthetic organisms achieve their remarkable photon efficiency is a major scientific problem, whose solution is likely to impact the development of efficient photon

capture technology. Photosynthetic organisms employ antennas constructed from light harvesting proteins that capture solar photons so that nearly every photon impinging on the organism is sent to the photosynthetic reaction center for generating energy. Our work focusses on the cryptophyte (single celled algae) light harvesting antenna. Our work aims to discover the physical origin of quantum beats that are observed in these antenna proteins. Our current result come closest to discovering and characterizing a near identical set of proteins where they only differ in their quaternary structure. These new protein structures are likely to spawn new spectroscopic experiments to probe the nature of energy transfer within these antenna structures.

Our discoveries also change paradigms established in the algal light harvesting field. We have shown that a single organism can have two distinct types of antenna protein: one with an open quaternary structure and the other with a closed form. Additionally, we have observed an asymmetry in chromophores that are attached to chemically identical sites. This implies that there exist some yet to be discovered protein that can alter the chemical nature of the chromophore after it is sited in a fully assembled and folded antenna structures.

Other disciplines:
Ultimately, understanding how photosynthetic organisms attain near perfect quantum efficiency should provide a blueprint for designing optical devices with such efficiency. We are not currently at this point, however, our discoveries make progress towards this goal. With such a design principle, it should be possible to create instruments that effectively capture every available photon, which would have an impact beyond scientific research.

Describe the impact in this reporting period on the development of human resources
This research has provided training for a postdoctoral fellow who has since gone on to establish an independent career is science.

Describe the impact on teaching and educational experiences
Not applicable.

Describe the impact in this reporting period on physical, institutional, and information resources that form infrastructure.
Not applicable.

Impact on society beyond science and technology:
As stated above, the major long term goal is to discover the physical and architectural principles by which photosynthetic organisms achieve near perfect quantum efficiency. If we had these principles, they would be employed to create optical technologies that are sensitive to low light at the quantum limit.

Changes

We have kept to the goals of this project. The only difference is that we have shifted our focus from synthetic matched open-closed form proteins to discovering naturally occurring pairs from a single organism. The analysis of our newly discovered proteins via ultrafast spectroscopy to analyze the existence of quantum beats is still ongoing.

Technical Updates

None.

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