BIOCHEMISTRY: FROM PROTEIN COMPOSITION TO ENZYME STRUCTURE AND FUNCTION

Proteins are the main functional components of life, with functions including storage of nitrogen, structural rigidity, transport, signaling, binding, and enzymatic catalysis. The projects offered in my lab span the broad area of protein biochemistry including: proteomic analysis of cereals, enzyme functional analysis, structural studies of molecular machines, regulation of pigment biosynthesis and protein-protein interaction studies.

BIOSYNTHESIS OF CHLOROPHYLLS

Chlorophyll is a dangerous molecule as it is a potent photosensitizer and producer of reactive oxygen species. The organisms which make chlorophyll go to great lengths to ensure that it or its coloured precursors don’t accumulate to reek oxidative devastation on the cell. The projects on offer aim to understand the mechanism by which chlorophyll is made by enzymes in the pathway and identify the regulatory mechanisms for chlorophyll synthesis.

PURIFICATION OF A REGULATORY KINASE

Characterisation of a kinase that phosphorylates the GENOMES UNCOUPLED 4 protein in plants or algae. This project builds on our recent paper in FEBS Letters and our recently published crystal structure which both show the algal and plant GUN4 differs significantly from the cyanobacterial GUN4.

MAGNESIUM CHELATASE COMPLEXES (with ANSTO)

We have all five magnesium chelatase subunits expressed and are able to reconstitute the large protein complexes in vitro. The stoichiometry of the subunits is variable as some subunits act as substrates with corresponding substrate like properties. X-ray and neutron scattering at ANSTO and the Australian Synchrotron allow us to probe the mechanism of assembly of these complexes as shown in the figure.

We also use synthetic biology techniques to examine the assembly of the complex in vivo by controlling expression of each subunit and monitor the magnesium protoporphyrin formation.

SYNTHETIC BIOLOGY: CHLOROPHYLL SYNTHESIS IN E. COLI

The 2013-2015 iGEM teams have cloned all of the known genes involved in chlorophyll biosynthesis from a eukaryotic organism, Chlamydomonas reinhardtii, into E. coli. Some of these genes have been assembled into operons. This project will assemble the different operons into super operons to test the effectiveness of chlorophyll biosynthesis in an organism that normally does not make chlorophyll. Optimisation of expression and testing of individual operons will allow for evaluation of how chlorophyll synthesis may be regulated in the absence of other potentially competing regulatory factors.
ANALYSIS OF HALOMICRONEMA: A CHLOROPHYLL F CONTAINING ORGANISM ISOLATED FROM STROMATOLITES

We have a complete genome sequence of this organism and have extensive proteomic data which indicate light dependent changes in protein expression.

There are numerous projects available on this novel organism, ranging from genomic analysis to light quality regulation of photosynthetic genes and structural studies of phycobilisomes (see articles 2, 7, 9, 11). One particularly interesting project is in identifying the light regulatory mechanisms for gene synthesis in this organism.

PROTEOMICS OF CEREALS PROTEOMICS OF CEREALS

The cereal research is associated with Grain Growers Pty Ltd looking at proteomics of barley and wheat grains. We are interested in determining the extent to which environmental and genetic variation determines protein composition in wheat, rice and barley. The protein profile between varieties grown at the same location and varieties grown at different locations is being determined. Initial trial scale analyses have found numerous differences between the varieties of barley grain grown at the same time and location. The reason for these variations may shed light on how cereals cope with environmental factors such as nutrient availability, drought, salinity and temperature as well as biological stresses. In addition, many cereal proteins have distinct impacts on quality; taste and performance in baking and malting are key examples. Understanding these factors are very important for optimizing quality in the food industry.

Selected publications