Piecing Together the Phycobilisome

Ailie Marx, Liron David and Noam Adir*

Schulich Faculty of Chemistry, Technion, Technion City, Haifa Israel 32000.

*To whom correspondence should be addressed.

E-mail:nadir@tx.technion.ac.il

Abstract

Photosynthesis is driven by the absorption of light by arrays of pigments bound within protein complexes called antennas, followed by the efficient transfer of energy to the photochemical reaction centers. Cyanobacteria, red algae and cyanelles contain phycobilisomes (PBS) as their major antenna complex. The PBS is an extremely large (3–7MDa), multi-layered complex bound to the stromal side of the photosynthetic membrane. In this review, we will describe the important structural and functional characteristics of the phycobilisome complex experimentally obtained over the past 40 years, especially in relation to the phycobilisomes unique absorption characteristics and its ability to self-assemble and disassemble.

1. The Phycobilisome antenna – An enormous pigment-protein complex

Photosynthesis is the essential life supporting process which converts light energy into chemical energy, and in oxygenic species, the

concomitant splitting of water to produce molecular oxygen. Organisms that have evolved in different environmental surroundings have different molecular strategies on how to perform the first step in this process, light harvesting. The efficient capture of the ambient light followed by transfer of the absorbed energy into the photosynthetic reaction centers (RCs) are performed by pigment-protein complexes called antennas or light-harvesting complexes (LHC). The variety of architectures in the LHCs is more varied than that of the RCs, establishing both the need to preserve the internal functional details required for photochemistry in the RCs and the evolutionary liberty to find different solutions for actual light absorption (Neilson and Durnford 2010; Cogdell et al. 2004; Blankenship et al. 1995; Adir 2005; Adir 2008; Adir et al. 2006; Grossman et al. 1995). Among the different LHCs which have evolved to carry out this task, the phycobilisome (PBS) is unique in that it is a truly enormous pigment-protein complex found outside the membrane. The PBS anchored to the stromal side of the thylakoid membrane in cyanobacteria and red algae can reach molecular weights of 3-7MDa and dimensions of over 50-75nm in size – an order of magnitude larger

than the photosystem II (PSII) complex to which it primarily funnels light energy.

Early structural characterization of isolated PBSs was carried out on the level of the entire complex and the earliest EM pictures revealed an aesthetic structure made up of a core surrounded by rods (Gantt and Conti 1966a, b; Gantt and Lipschultz 1972; Teale and Dale 1970; Edwards and Gantt 1971; Bryant et al. 1976; Tandeau de Marsac and Cohen-Bazire 1977; Bryant and Cohen-Bazire 1981; Glazer 1989). A precise characterization of the macrostructure of the PBS has however proved not so trivial since original models of radiating rods would appear at odds with what is now known about the dimensions of the phycobiliprotein (PBP) disks which compose the core and rods. Not visualized in the EM pictures were the unpigmented protein components of the PBS, assumed to be buried within the PBS complex occupying the hollow cavities within the rods and core (Liu et al. 2005). Their presumed position, and the overall architecture of the PBS led to the proposal that these proteins play a role in complex assembly and stability and were thus termed linker proteins (LPs)

Since these early glimpses were obtained, more advanced techniques have provided vastly improved images of the subunits, but little more specific detail on the architecture of the entire PBS. Various sub-assemblies of the basic PBS units have been studied extensively and numerous high resolution structures of individual PBP components have been determined by X ray crystallography (Schirmer et al. 1986; Adir 2005). These structures have simultaneously revealed both the structural basis for functional details and also raised questions as to the manner by which the complex undergoes assembly. The wealth of information obtained from the structures has been combined with spectroscopic and biochemical techniques to reveal facets of energy absorption and transfer (Sauer and Scheer 1988; Beck and Sauer 1992), complex stability, self-assembly properties and possible mechanisms of disassembly (MacColl 1983; MacColl et al. 2003; McGregor et al. 2008). Significant questions which remain are the arrangement of the rods around the core, what form of rod-rod interactions exist and what is the mode of attachment of the rods to the core. The fashion of attachment has been attributed to the presence of LPs, however one of the well known properties of the PBS, is that the complex disintegrates into trimeric

rings (and lose their association with the LPs) when isolated in the absence of high concentrations of buffered phosphate (Gantt and Lipschultz 1972). It appears that the LPs are only weakly bound to the PBPs, and thus their role in stabilization of the entire PBS structure cannot be easily rationalized. This observation is puzzling, but may be due to the physiological phenomenon of PBS disassembly under nutrient stress (Collier and Grossman 1992; Grossman et al. 1993). The loss of the linkers in isolated PBS sub-assemblies has led to only limited structural information, however as we will describe below, partial LPs structures have recently become available. In this review we will describe the basic facets of PBS subunit structure and function based on various experimental evidence and attempt to build a picture of the entire complex consistent with these experiments.

2. Building blocks – Crystal structures of individual components

The PBS is made up of phycobiliproteins (PBPs), the pigmented component of the complex, and five types of mostly unpigmented proteins commonly referred to collectively as linker proteins (LPs). Two PBPs are always present in PBS complexes – allophycocyanin

(APC), which forms the basic building block for the core, and phycocyanin (PC), which is the major component of rods that is always proximal to the core. Some species contain additional PBP rod components, phycoerythrin (PE) or phycoerthyrocyanin (PEC), which occupy positions more distal than PC in relation to the core (MacColl 1998). Each of the different PBPs have highly similar three dimensional structures and in each case the higher order aggregates are assembled analogously from a basic building block, the monomer (Fig. 1). The monomer in actuality is made up of two similar but not identical polypeptide chains (always denoted as the α and β subunits). The association of three monomers forms a trimeric ring, and two such rings can further associate through contacts between the α subunits, into hexamers (in PC, PE and PEC). Four APC trimers associate into a cylinder and 2-5 cylinders, depending on the species, bundle together to form the core. Both the cylinders and hexamers form structures which resemble a tube with ample empty space to accommodate the LPs. The rods are composed of hexamers which extend by stacking face to face one onto the other through mostly β subunit interactions. In those species which contain them, the hexamers of PE or PEC seamlessly extend out from

the PC elongating the rods further. The specific placement of PBPs within the overall PBS structure is functionally crucial as it forms the basis for the creation of an energy funnel (Fig. 2) whereby the higher energy absorbing components ((PE (λmax = 560nm), PEC (λmax = 575nm) and PC (λmax = 620nm) are more distal to the core which is comprised of the lowest energy absorbing PBP species, APC (λmax = 650nm). The core also contains variants of APC subunits (α^B, β^{16} and L_{CM}) which have red-shifted absorbing pigments (MacColl 2004; Lundell et al. 1981; Glazer and Bryant 1975; Ducret et al. 1998) that closely overlap the absorption of chlorophyll *a*, the primary pigment found in cyanobacterial photosystems to which the energy is ultimately transferred.

All PBS pigments are linear tetrapyrolle bilins (Glazer 1989; Adir 2008), connected covalently to the PBPs through thioether bonds to conserved cysteine residues (Fig. 3). APC and PC contain a common pigment, phycocyanobilin (PCB), bound to analogous positions on the α and β subunits. PC binds an additional third PCB on a short loop, inserted towards the end of the C-terminal of the β chain, positioned on the outer disk surface. Other bilin types can be found bound to PE and PEC and these differ from PCB in the number and

arrangement of conjugated double bonds. That APC and PC bind chemically identical bilins and yet possess distinctive absorption properties highlights the role of the protein scaffold in influencing the structure and chemical environment which tunes the spectroscopic characteristics of these pigments (McGregor et al. 2008).

X-ray crystal structures of each of the PBPs have revealed a highly conserved fold highlighting the subtlety with which they are able to tune absorption properties (Fig. 3). This allows the PBPs to act as highly similar "building blocks" which are capable of assembling analogously and yet structural subtleties (further detailed in following sections) allow for specificity in binding partners with no intermixing between the various kinds of PBPs and specific tuning of the cofactor environment to create different absorption and fluorescence properties necessary to funnel energy down the complex. Indeed numerous crystal structures for each of the individual PBPs are now available in the Protein Data Bank (www.pdb.org, reviewed in Adir, 2005). The structures of the different PBP types are highly similar, which is a direct result of the relatively high homology within the primary sequences. In each case the structure forms an eight α-helical, globin-like structure. Consideration of the most conserved

areas of any structure is useful in elucidating functionally significant areas. Given that the homology among the PBPs of the same type from different species and also between the different PBP types is quite high, it is interesting to observe that the areas of particular structural conservation in the different PBPs is subtlety variant. The ConSurf Server (Ashkenazy et al. 2010) (http://consurf.tau.ac.il/) was used to map evolutionary conservation as determined from sequence alignment onto the three dimensional structure of PC and APC from *T. vulcanus* (Fig. 4).

In the process of forming trimers, the PBPs present two different faces, one made up primarily of residues of the β subunits of each of the three monomers and the other primarily made up residues from the α subunits. In the formation of hexamers, the face made up of α subunit residues (Fig. 4A) associate face to face (which can be notated as: [βα][αβ]) while contacts between the β PC faces (Fig. 4B) allow for the formation of rods by assembly of hexamers ([βα][αβ][βα][αβ]). In APC the trimers form similar faces (Fig. 4C and D, respectively) however on the basis of EM and X-ray crystallographic studies, the cylinder formed by four APC trimers is not thought to be composed of two hexamers, due to looser interactions

between trimers, and perhaps also due the influence of the L_{CM} linker that serves to provide contacts for core formation and to bind the entire PBS to PSII (Capuano et al. 1991). This may be the reason for differences in sequence conservation between PC and APC. In PC, the α subunits face of the PC trimer are more conserved than the β subunits face. This suggests that the hexameric level of assembly ([βα][αβ]) cannot tolerate extensive changes, without harming other functionalities. On the other hand, in APC the β subunits face of the trimers are more conserved than that of the α subunits face, strengthening the suggestion that APC cylinders form by association of four trimers in a different order: [αβ][βα][αβ][βα]. This order enables the positioning of the L_C linker (Reuter et al. 1999; Ducret et al. 1998; Ducret et al. 1996) to terminate the core cylinder on both sides. For both PC and APC the inner cavity is far more conserved that the outer circumference and this may allude to the importance of specific interaction (or perhaps the need to avoid strong interactions) between PBPs and LPs.

One of the interesting aspects of the different crystal structures is the lattice packing (Fig. 5). In 16 of the 17 PC structures available to date, hexamers are formed *in crystal*. The single structure which

does not form hexamers is one of several structures of PC isolated from *Thermosynechococcus vulcanus* (Adir and Lerner 2003). This particular structure (PDB code 1ON7) originated from a unique PC fraction which was shown to be a blue shifted (PC_{612}) form with an unmethylated Asnβ72 residue (Klotz et al. 1986; Swanson and Glazer 1990) and was proposed to be a minor PBS component, a trimeric form of PC which may form contacts between rods and cores. The importance of considering the crystal packing in the PBP structures stems from the resemblance between *in crystal* aggregates formed by symmetry related molecules and the assumed physiological form of hexamers and rods. The hexamers from different structures pack within the crystal in a number of different ways, among them with the formation of infinitely long rods.

In those structures where rods are formed *in crystal*, a tantalizing question arises as to what extent these crystal rods resemble biological rods, particularly in terms of the stacking orientation and the lateral contacts formed by adjacent rods both factors which could significantly affect energy transfer pathways within the complex and perhaps hint at possible rod arrangements around the core in complex. Whilst initial PBS models suggested a radiating arrangement

of rods (Glauser et al. 1992; Yamanaka et al. 1982) subsequent models have suggested the possibility of close lateral contact with for example the formation of rod pairs (Glazer 1989; Adir 2005). The biological relevance of *in crystal* rod formation is not certain, since numerous alternative forms of crystal packing have been observed in the structures. This may be a result of the crystallization process or indicate that several hexamer interactions are possible, or be the result of the lack of LPs. A rather unique result has been recently reported, whereby several essentially identical structures from the same PC source have been obtained from crystallization and cryoprotection under vastly different conditions (David et al. 2011). This appears to suggest that the contacts formed may be indicative of the forces governing the self assembly of the PBS complex and not a mere artefact of crystallization. Indeed a recent structure of a functional PC rod and PC trimer from the same species (David et al. 2011) has further provided evidence to support the biological relevance of interactions between symmetry related molecules in the crystal structures of isolated components to subcomplexes of the PBS (see below).

PBP structures have of course not only provided insight into PBS assembly but have also provided high resolution detail on structural fine points. The geometries and orientations of the different bilins within the protein scaffold have been well described by high resolution X-ray crystallography (Fig. 6), and the role of structurally essential water molecules has also been addressed. Increasingly higher resolution structures (better that 1.0Å) will hopefully facilitate the description of protonation states allowing for an accurate description of energy states.

In addition to the PBPs, around 15% of the PBS is made up of LPs, dubbed as such after one of their two proposed functions, to guide the association of the PBPs into larger subcomplexes (Liu et al. 2005). Their second function has been clearly shown as performing modification or tuning of the spectroscopic properties of the PBPs within the complex. The LPs are generally subgrouped according to their assumed position within the complex; the core-membrane linker (L_{CM}), core linkers (L_C), rod-core linkers (L_{RC}) and rod linkers (L_R). The LPs are much less conserved across species than the PBPs and indeed the number and type of LPs varies from species to species. A recent report on the interaction between

linkers and a homologue of a HSP90 chaperone (Sato et al. 2010) would indicate that linkers cannot associate with PBPs spontaneously. Whether the higher order oligomers of PBPs assemble around LPs is not known, however reconstitution studies (Ducret et al. 1998) do not indicate this to be the case.

In stark contrast to the PBPs, structures of the LPs have remained elusive. The highly hydrophobic nature of these proteins makes the isolation of the homogenous, soluble and concentrated solutions of LPs that are required for crystallization or NMR difficult. Indeed of the LP structures available to date, only one was actually visualized in actual association with a PBP (the APC structure, 1B33). Very recently the Northeast Structural Genomics Consortium in the USA have solved several structures of domains of the $L_{CM,}$ using NMR techniques (PDB codes 2L06 and 2KY4) and X-ray crystallography (PDB codes 3OSJ and 3OHW) as well as a fragment of the L_R also using NMR (PDB code 2L3W) and X-ray crystallography (PDB code 3PRU, Fig. 7). The structures of the L_R represent the N terminal half of the protein from two different species and the four structures of L_{CM} domains are homologous to this portion of the L_R meaning that these six new structures have a certain degree of struc-

tural redundancy (root mean square deviation of the aligned α-carbon positions are between 1 – 1.5Å). Elucidation of the complete structure of these LPs, along with their exact positioning within the complex, seems again to be a problem that can only be solved by total structural characterization of the PBS complex or subcomplexes. Even a low to medium resolution structure could provide a wealth of information particularly considering that it may be merged with the high resolution detail now available for the PBPs.

3. A problem of symmetry – Crystallizing the complex and subcomplexes

The PBS complex was first visualized by EM experiments as a central core surrounded by rods and led to the first PBS model which suggested an arrangement whereby the rods are arranged in radial fashion around the core. A second model, already mentioned above, suggests a parallel rod pair arrangement in which the rods are arranged as two rod doublets that are parallel to the thylakoid membrane and one rod doublet which is perpendicular to the membrane. Cryo-EM micrographs (Yi et al. 2005) of PBSs have supported the parallel pair model which is also consistent with the crystal structures of PC (Adir 2005). Atomic force microscopy studies per-

formed on isolated PBSs and native thylakoid membranes from red algae (Arteni et al. 2008) visualized the isolated PBS as a dimer in opposition to previous TEM micrographs that have only ever shown the isolated PBS as a monomeric unit. The native architecture of PBS attached to the thylakoid membrane and their crowding distribution under different light intensities was also shown by AFM (Liu et al. 2008). Under low light intensity the PBSs are organized in ordered rows whereas under medium light the PBS shows a different pattern of organization, namely a random arrangement with the tendency to form clusters (Arteni et al. 2008; Arteni et al. 2009). These studies have demonstrated that the PBS can form a kind of crystalline lattice and suggest perhaps that there is a natural tendency of the PBS organize in an ordered fashion, a feature conducive to crystallizing the entire complex despite its enormity.

The use of X-ray crystallography to characterize the structure of subcomplexes of the PBS to higher resolutions has been hindered by the same symmetry and self assembly properties that facilitates the crystallization of PBPs. Most of the PBPs characterized to date have crystallized in space groups with three fold symmetry. Since the LPs traverse the internal cavity of the PBP complexes along the symme-

try axis, the result is a super position of at least six different LP orientations, there by cancelling out the contribution of the LPs to the calculated electron density. The anomalous nature of the crystal packing in the 1B33 structure (Reuter et al. 1999), in which two full trimers occupy the asymmetric unit at almost right angle, overcomes this problem and so this APC-L_C complex is the only complete PBP-LP complex structure available to date. The LP in this structure is composed of both beta sheets and alpha helices and is asymmetrically positioned within the cavity, interacting with two out of the three monomers within the trimer. The LPs presence induced a flattening effect on the overall trimer structure of APC, within the crystal lattice.

Recently the X-ray crystal structure of an intact and functional PBS rod was solved (David et al. 2011). The presence of all rod components (α and β PC subunits as well as three rod LPs, the rod capping $L_R^{8.7}$, L_R and one L_{RC} (the *cpcG4* gene product) was confirmed by SDS-PAGE and mass spectrometry and the functional integrity of the rod was demonstrated using spectroscopy on both solutions and crystals, which confirmed the characteristic red shift in absorption and fluorescence emission as compared to trimeric PC

(Fig. 8). The crystal structure of the rod was identical to that of trimeric PC– a monomer in the asymmetric unit cell which extends in the crystal lattice to form hexamers which extend into infinite rods. While the biochemical evidence from the crystal analysis left little doubt as to the presence of LPs in the crystal the absence of clear electron density prevented the building of an ordered structure. None the less the PC rod structure, within which the linker is enclosed, showed subtle hints of the linkers' presence. Comparison of the isolated PC and rod PC structures led to the identification of several amino acids located on the wall of the inner cavity, αGlu7, αArg15, βLys7, and βGln113, with improved electron density in the rod structure. This improved electron density indicates a stabilizing force, presumably interaction with the LPs, and indeed homology modeling to the 1B33 APC-L_C structure shows that these residues are in analogous positions to those APC residues which interact with the L_C C-terminal. Further comparison of crystallographic B factors between the isolated PC and PC rod structures further hinted at the stabilizing influence of the linkers' presence. Whilst on average the B factors were similar, comparison between individual atoms showed that there were fewer cases of significantly higher B factors

in the rod structure. Several side chains distributed throughout the PC monomer were identified as having reduced B factors in the rod structure and among them αGlu109 was noted as significant due to its positioning on the inner side of the rod and its possible interaction with a LP based again on homology modelling to the 1B33 structure.

This PC rod structure is not the first PBP-linker complex to be crystallized. Aside from the APC-Lc structure mentioned above, two structures from crystals of PE containing the γ linker subunits exist, and again in these cases, the electron density from the linker was also averaged out during structure refinement and so its particular structure remained largely unassigned (Ficner et al. 1992; Ritter et al. 1999).

Elucidating the complete LP structures and locating their position within the rods and core, challenges plagued by solubility and symmetry problems, may require the use of more indirect methods or a combination of techniques. Using the available structures for L_C (which is homologous to both the C terminal portion of L_R and L_{RC} as well as the rod capping linker, $L_R^{8.7}$) and the N terminal portion of L_R (which is also homologous to the N terminal section of L_{RC})

models may be created to aid in understanding the nature of the LP structures and their general interactions with the PBPs. Such models were created for the LPs present, but not visualized, in the above mention PC rod structure. Calculations for the hexamer cavity volume and the modeled LP volume demonstrated that there is ample space available within the central channel of the rod to accommodate the linkers without protruding from either end of the rod, or indeed without actually linking adjacent hexamers. It was suggested that since the major role of the linkers may be in fine-tuning the spectral properties of the PBPs, they might be renamed “tuning proteins”(David et al. 2011).

4. Essential functionalities revealed by structural subtleties

One of the elegant aspects of the PBS structure is the way in which the various PBPs are similar enough to use the same principles of association and yet dissimilar enough to maintain specificity. The α and β subunits of each PBP type associate to form structurally similar monomers followed by the formation of similarly shaped trimers. There have been no reports of isolation of mixed PBP monomers or trimers *in vivo* (or in mutants lacking oneof the subunits) indicating a tightly controlled assembly mechan-

ism(Anderson and Toole 1998). Heterologus expression of single PBPs in *E. coli* enables the formation of some homodimeric species (Tooley et al. 2001; Ge et al. 2009), but when both subunits are expressed, then the natural monomer assembles (Arciero et al. 1988; Biswas et al. 2010). In addition, APC and PC bind the same cofactor and yet have significantly different absorption and fluorescence properties. These are both functionally critical properties since the correct association of PBPs and the differing absorption properties create the energy funnel which facilitates the efficient collection and transfer of light into the photosystems.

In order to understand the mechanisms of energy capture and transfer in the complex it would be sage to question the origin of the different absorption properties of the PBPs. PC and APC bind chemically identical chromophores, (the PCB), and form highly similar three dimensional structures. The monomeric forms of these two PCBs have nearly identical absorption and fluorescence properties (although PC has an additional PCB). However, during trimer assembly, the α84 PCB of one monomer comes into proximity with the β84 PCB of an adjacent monomer radically changing the electronic environment of both PBPs (Fig. 9). While the geometries and

orientations of these proximal PCBs are nearly identical, the absorption maxima of the trimeric form of PC is 620nm while trimeric APC absorbs at 652nm (with a shoulder at 620nm) (MacColl 2004; MacColl et al. 2003) .

The significant red shift upon the trimerization in APC had long been rationalized in general terms as a result of conformational changes in the PCB, a change in the chemical environment of the PCB or a delocalization of excited states resulting from the nearing of two cofactors on adjacent monomers. Recent experiments however showed that only a slight loosening of the trimer (as opposed to complete monomerization) is enough to revert the APC absorption spectra to one resembling that of PC or monomeric APC (McGregor et al. 2008). This means that the simple proximity between two cofactors on adjacent monomers is not sufficient to afford the change alone. A careful structural analysis and comparison between PC and APC and the chemical environment surrounding the β84 PCB, which is positioned at the trimer interface has provided a more complete and detailed explanation for the absorption shift (Fig. 10). In the monomeric form of the PBP the β84 PCB is half embedded in protein and half solvent exposed. Upon trimerization the solvent

exposed portion becomes enclosed by a protein pocket provided by the adjacent monomer and the chemical nature this pocket in PC and APC is significantly different in both shape and chemical character. The PC pocket is more shallow and polar than the deeper APC pocket which is created by aromatic and hydrophobic residues.

Surrounding the cofactor with a pocket of hydrophobic character would ordinarily induce a small blue shift in its absorption, and not the observed strong (30nm) red shift. It was proposed that the source of the red shift could only be the result of a strong coupling between the two PCBs, although 20Å separate their centers. The strong coupling was suggested to be a result of a unique structural environment (with respect to PBPs) that surrounds the hydrophobic pocket with a second shell of charged amino acids and it is this combination that induces coupling. This would explain why even slight loosening of the trimer interaction domain (without actual disassembly), would allow a certain amount of solvent exposure, resulting in the loss of a hydrophobic environment, and loss of coupling.

Since the integrity of the higher order complexes is important to the functioning of the PBS it is interesting to consider the mechanisms by which different species maintain complex assembly in chal-

lenging environmental niches such as extreme cold or heat, high salinity and deep within the water column where light exposure is limited. Each of these challenges requires special survival mechanisms and often this is found at the level of the protein (Inoue et al. 2000). Among the numerous PBP structures are several isolated from thermophiles and it has been shown that indeed the isolated protein itself is resistant to heat denaturation. However a comparison of the primary sequences and structures originating from mesophiles and thermophiles shows very little variation and it is only structural nuances which can be attributed to affording thermostability. *T. vulcanus* is a cyanobacterium which grows optimally at 55-60°C and both APC and PC structures from protein isolated from this species have been determined. Comparison of these structures together with those of other PC and APC from thermophiles and mesophiles deposited in the PDB has enabled the identification of possible sources of the thermostability. In the case of PC, studies have indicated that general characteristics such as cavities, hydrogen bonding, ion pairs, secondary structure surface polarity and amino acid composition are not consistent with conferring thermostability. A single conserved amino acid change, αPhe28 to aspartic acid, was

suggested as providing an additional polar contact at both the monomer and hexamer interaction interfaces and that this is perhaps enough to provide the extra stability required to prevent denaturation at higher temperatures (Adir et al. 2001; Adir et al. 2002). In the case of APC, it was suggested that the presence of an extra charged residue in the second shell surrounding the PCB binding pocket at the trimer interface (described above) may provide extra stability (McGregor et al. 2008). Under certain circumstances however the ordered disassembly of the PBS is necessary and here too structural studies have helped elucidate the mechanisms by which this occurs.

5. Disassembly of the Phycobilisome – A David and Goliath battle at the molecular level

The ability to survive in harsh conditions is not the only environmental challenge faced by cyanobacteria and red alga. Adaptability to changes in environmental conditions, particularly the availability of essential nutrients, is necessary for the survival of any organism. PBSs are both abundant in the cell and enormous in size. In fact they compose around 50% of the dry weight of the cell meaning that these protein complexes comprise a substantial energy reserve. Dur-

ing times of nutrient starvation cells are capable of disassembling the PBS in order to access this energy store (Collier and Grossman 1992, 1994) and probably also as a mechanism important for protecting against overexcitation of the photosystems. Cyanobacteria, formerly known as blue-green alga, owe their distinctive color to the presence of both the PBSs and chlorophyll-containing complexes and the disassembly of the PBS results in a bleached phenotype whereby the cells take on a yellow-green color(Grossman et al. 1993). An ensemble of genes, including *nblA, nblB, nblC, nblR, nblS* and *ald* have been shown to be involved in initiating and carrying out this disassembly. However, a single small protein (only 6-7kDa) appears to be the active player in the actual disassembly mechanism(Collier and Grossman 1994)(Fig. 11). Expression of the gene encoding for this protein, the NblA, is increased 50 fold under conditions of nitrogen and in some cases sulfur starvation (Collier and Grossman 1994). Despite this, the NblA itself has not been shown to possess any proteolytic activity and presumably further proteins are required to carry out the actual degradation.

The exact means by which the disassembly occurs has yet to be conclusively established however mutational and structural studies

have offered different mechanistic proposals. Two studies in recent years have provided the NblA protein structures from three different organisms. The first structure of NblA to be solved was from *Anabaena* sp. PCC 7120 and revealed an open four helical bundle formed by the dimerization of two monomers with a helix-loop-helix motif (Bienert et al. 2006). The subsequent crystal structures of nblA from *T. vulcanus* and *Synechococcus elongatus* sp. PCC 7942 (Dines et al. 2008) demonstrated that whilst the sequence homology between different species is low the structural motif is similar (RMS of 1.0-1.7Å). These structures also enabled the development of hypothesis as to possible mechanisms by which NblA might interact with the phycobilisome and perform disassembly (Dines et al. 2008; Bienert et al. 2006; Karradt et al. 2008). In vitro binding assays of GST tagged NblA from *Anabaena sp. PCC 7120* demonstrated that NblA binds PC and PE. Mutation experiments on NblA originally proposed that this association in *Anabaena sp. PCC 7120* occurred through interaction with the C termini of the NblA protein however a more generally applicable model was later proposed. This alternative model takes into account the fact that NblA proteins have low homology (<30%) in contrast to their PBP binding partners (80-

90%). Indeed an NblA protein from one species can compliment function in a deletion strain from another species despite this relatively low sequence homology. Given the similarity between the three NblA structures it appears that the structural motif is important for function and yet an analysis of electrostatics showed variance between both NblA and PBP proteins that could not support a consistent model. The nature of the NblA helix-loop-helix motif is reminiscent of the PBPs themselves which are in essence bundles of helix-loop-helix motifs (Fig. 12). Comparison of these motifs with either the NblA monomer or the non-covalently bonded helix-loop-helix formed by dimerization, clearly demonstrates pairs (NblA and PBP) with similarity in terms of helices' length and separation angle. Based on this observation it has been suggested that a clever trick of structural mimicry may be the key to the NblA disassembly action. The first actions of disassembly may be actively coupled to proteolysis by specific proteases as has been suggested (Karradt et al. 2008), or by the battery of less-specific proteases in the cell.

The phycobilisome, enchanting researchers with its brilliant colors, symmetry and molecular enormity is slowly being pieced together by studies on both the individual components and the larger

subcomplexes, as well as investigations into the proteins by which assembly and disassembly are afforded. A continued multifaceted approach will simultaneously answer important questions on the macrostructure as well as fill in fine details, especially those pertaining to the exact mechanisms of energy transfer, in order to give a detailed description of light harvestings molecular giant.

6. Acknowledgements

This work was supported by the Israel Science Foundation founded by the Israel Academy of Sciences and Humanities (1045/06) and US-Israel Binational Science Foundation (2009406). We gratefully thank the staff of the European Synchrotron Radiation Facility (beamlines ID-14-1, ID23-1) for provision of synchrotron radiation facilities and assistance.

7. References

Adir N (2005) Elucidation of the molecular structures of components of the phycobilisome: reconstructing a giant. Photosynth Res 85 (1):15-32.

Adir N (2008) Structure of the phycobilisome antennae in cyanobacteria and red algae. In: Fromme P (ed) Photosynthetic Protein Complexes: A Structural Approach. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, pp 243-274

Adir N, Dines M, Klartag M, McGregor A, Melamed-Frank M (2006) Assembly and Disassembly of Phycobilisomes. In: Shively JM (ed) Microbiology

Monographs: Inclusions in Prokaryotes, vol 2. Springer Berlin / Heidelberg, pp 47-77

Adir N, Dobrovetsky Y, Lerner N (2001) Structure of C-Phycocyanin from the Thermophilic Cyanobacterium Synechococcus vulcanus at 2.5 A: Structural Implications for Thermal Stability in Phycobilisome Assembly. J Mol Biol 313 (1):71-81.

Adir N, Lerner N (2003) The crystal structure of a novel unmethylated form of C-phycocyanin, a possible connector between cores and rods in pycobilisomes. J Biol Chem 278 (28):25926-25932.

Adir N, Vainer R, Lerner N (2002) Refined structure of C-phycocyanin from the cyanobacterium Synechococcus vulcanus at 1.6 A: insights into the role of solvent molecules in thermal stability and co-factor structure. Biochim Biophys Acta 1556 (2-3):168-174.

Anderson LK, Toole CM (1998) A model for early events in the assembly pathway of cyanobacterial phycobilisomes. Mol Microbiol 30 (3):467-474.

Arciero DM, Bryant DA, Glazer AN (1988) In vitro attachment of bilins to apophycocyanin. I. Specific covalent adduct formation at cysteinyl residues involved in phycocyanobilin binding in C-phycocyanin. J Biol Chem 263 (34):18343-18349.

Arteni AA, Ajlani G, Boekema EJ (2009) Structural organisation of phycobilisomes from Synechocystis sp. strain PCC6803 and their interaction with the membrane. Biochimica et Biophysica Acta 1787 (4):272-279

Arteni AA, Liu LN, Aartsma TJ, Zhang YZ, Zhou BC, Boekema EJ (2008) Structure and organization of phycobilisomes on membranes of the red alga Porphyridium cruentum. Photosynthesis Research 95 (2-3):169-174

Ashkenazy H, Erez E, Martz E, Pupko T, Ben-Tal N (2010) ConSurf 2010: calculating evolutionary conservation in sequence and structure of proteins and nucleic acids. Nucleic Acids Research 38 Suppl:W529-533

Beck WF, Sauer K (1992) Energy-Transfer and Exciton-State Relaxation Processes in Allophycocyanin. J Phys Chem 96:4658-4666

Bienert R, Baier K, Volkmer R, Lockau W, Heinemann U (2006) Crystal structure of NblA from Anabaena sp. PCC 7120, a small protein playing a key role in phycobilisome degradation. J Biol Chem 281 (8):5216-5223

Biswas A, Vasquez YM, Dragomani TM, Kronfel ML, Williams SR, Alvey RM, Bryant DA, Schluchter WM (2010) Biosynthesis of cyanobacterial phycobiliproteins in Escherichia coli: chromophorylation efficiency and specificity of all bilin lyases from Synechococcus sp. strain PCC 7002. Appl Environ Microbiol 76 (9):2729-2739

Blankenship RE, Olson JM, Miller M (1995) Antenna complexes from green photosynthetic bacteria. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic Photosynthetic Bacteria. Kluwer Academic Publishers, Dordrect, The Netherlands, pp 399-435

Bryant DA, Cohen-Bazire G (1981) Effects of chromatic illumination on cyanobacterial phycobilisomes. Evidence for the specific induction of a second

pair of phycocyanin subunits in Pseudanabaena 7409 grown in red light. Eur J Biochem 119 (2):415-424.

Bryant DA, Glazer AN, Eiserling FA (1976) Characterization and structural properties of the major biliproteins of Anabaena sp. Arch Microbiol 110 (1):61-75.

Capuano V, Braux AS, Tandeau de Marsac N, Houmard J (1991) The "anchor polypeptide" of cyanobacterial phycobilisomes. Molecular characterization of the Synechococcus sp. PCC 6301 apce gene. J Biol Chem 266 (11):7239-7247.

Cogdell RJ, Gardiner AT, Roszak AW, Law CJ, Southall J, Isaacs NW (2004) Rings, ellipses and horseshoes: how purple bacteria harvest solar energy. Photosynth Res 81 (3):207-214.

Collier JL, Grossman AR (1992) Chlorosis induced by nutrient deprivation in Synechococcus sp. strain PCC 7942: not all bleaching is the same. J Bacteriol 174 (14):4718-4726

Collier JL, Grossman AR (1994) A small polypeptide triggers complete degradation of light-harvesting phycobiliproteins in nutrient-deprived cyanobacteria. Embo J 13 (5):1039-1047.

David L, Marx A, Adir N (2011) High-Resolution Crystal Structures of Trimeric and Rod Phycocyanin. J Mol Biol 405 (1):201-213

Dines M, Sendersky E, David L, Schwarz R, Adir N (2008) Structural, functional, and mutational analysis of the NblA protein provides insight into possible modes of interaction with the phycobilisome. Journal of Biological Chemistry 283 (44):30330-30340

Ducret A, Muller SA, Goldie KN, Hefti A, Sidler WA, Zuber H, Engel A (1998) Reconstitution, characterization and mass analysis of the pentacylindrical allophycocyanin core complex from the cyanobacterium Anabaena sp. PCC 7120. J Mol Biol 278 (2):369-388

Ducret A, Sidler W, Wehrli E, Frank G, Zuber H (1996) Isolation, characterization and electron microscopy analysis of a hemidiscoidal phycobilisome type from the cyanobacterium Anabaena sp. PCC 7120. Eur J Biochem 236 (3):1010-1024

Edwards MR, Gantt E (1971) Phycobilisomes of the thermophilic blue-green alga Synechococcus lividus. J Cell Biol 50 (3):896-900

Ficner R, Lobeck K, Schmidt G, Huber R (1992) Isolation, crystallization, crystal structure analysis and refinement of B-phycoerythrin from the red alga Porphyridium sordidum at 2.2 A resolution. J Mol Biol 228 (3):935-950

Gantt E, Conti SF (1966a) Granules associated with the chloroplast lamellae of Porphyridium cruentum. J Cell Biol 29 (3):423-434.

Gantt E, Conti SF (1966b) Phycobiliprotein localization in algae. Brookhaven Symp Biol 19:393-405.

Gantt E, Lipschultz CA (1972) Phycobilisomes of Porphyridium cruentum. I. Isolation. J Cell Biol 54:313-324

Ge B, Sun H, Feng Y, Yang J, Qin S (2009) Functional biosynthesis of an allophycocyan beta subunit in Escherichia coli. J Biosci Bioeng 107 (3):246-249

Glauser M, Bryant DA, Frank G, Wehrli E, Rusconi SS, Sidler W, Zuber H (1992) Phycobilisome structure in the cyanobacteria Mastigocladus laminosus and Anabaena sp. PCC 7120. Eur J Biochem 205 (3):907-915.

Glazer AN (1989) Light guides. Directional energy transfer in a photosynthetic antenna. J Biol Chem 264 (1):1-4.

Glazer AN, Bryant DA (1975) Allophycocyanin B (lambdamax 671, 618 nm): a new cyanobacterial phycobiliprotein. Arch Microbiol 104 (1):15-22.

Grossman AR, Bhaya D, Apt KE, Kehoe DM (1995) Light-harvesting complexes in oxygenic photosynthesis: diversity, control, and evolution. Annu Rev Genet 29:231-288

Grossman AR, Schaefer MR, Chiang GG, Collier JL (1993) The phycobilisome, a light-harvesting complex responsive to environmental conditions. Microbiol Rev 57 (3):725-749.

Inoue N, Emi T, Yamane Y, Kashino Y, Koike H, Satoh K (2000) Effects of high-temperature treatments on a thermophilic cyanobacterium Synechococcus vulcanus. Plant Cell Physiol 41 (4):515-522.

Karradt A, Sobanski J, Mattow J, Lockau W, Baier K (2008) NblA, a key protein of phycobilisome degradation, interacts with ClpC, a HSP100 chaperone partner of a cyanobacterial Clp protease. Journal of Biological Chemistry 283 (47):32394-32403

Klotz AV, Leary JA, Glazer AN (1986) Post-translational methylation of asparaginyl residues. Identification of beta-71 gamma-N-methylasparagine in allophycocyanin. J Biol Chem 261 (34):15891-15894.

Liu LN, Aartsma TJ, Thomas JC, Lamers GE, Zhou BC, Zhang YZ (2008) Watching the native supramolecular architecture of photosynthetic membrane in red algae: topography of phycobilisomes and their crowding, diverse distribution patterns. J Biol Chem 283 (50):34946-34953

Liu LN, Chen XL, Zhang YZ, Zhou BC (2005) Characterization, structure and function of linker polypeptides in phycobilisomes of cyanobacteria and red algae: an overview. Biochim Biophys Acta 1708 (2):133-142.

Lundell DJ, Yamanaka G, Glazer AN (1981) A terminal energy acceptor of the phycobilisome: the 75,000-dalton polypeptide of Synechococcus 6301 phycobilisomes--a new biliprotein. J Cell Biol 91 (1):315-319

MacColl R (1983) Stability of allophycocyanin's quaternary structure. Arch Biochem Biophys 223 (1):24-32

MacColl R (1998) Cyanobacterial phycobilisomes. J Struct Biol 124 (2-3):311-334.

MacColl R (2004) Allophycocyanin and energy transfer. Biochim Biophys Acta 1657 (2-3):73-81.

MacColl R, Eisele LE, Menikh A (2003) Allophycocyanin: trimers, monomers, subunits, and homodimers. Biopolymers 72 (5):352-365.

McGregor A, Klartag M, David L, Adir N (2008) Allophycocyanin trimer stability and functionality are primarily due to polar enhanced hydrophobicity of the phycocyanobilin binding pocket. Journal of Molecular Biology 384 (2):406-421

Neilson JA, Durnford DG (2010) Evolutionary distribution of light-harvesting complex-like proteins in photosynthetic eukaryotes. Genome 53 (1):68-78

Reuter W, Wiegand G, Huber R, Than ME (1999) Structural analysis at 2.2 A of orthorhombic crystals presents the asymmetry of the allophycocyanin-linker complex, AP.LC7.8, from phycobilisomes of Mastigocladus laminosus. Proc Natl Acad Sci U S A 96 (4):1363-1368

Ritter S, Hiller RG, Wrench PM, Welte W, Diederichs K (1999) Crystal structure of a phycourobilin-containing phycoerythrin at 1.90-A resolution. J Struct Biol 126 (2):86-97

Sato T, Minagawa S, Kojima E, Okamoto N, Nakamoto H (2010) HtpG, the prokaryotic homologue of Hsp90, stabilizes a phycobilisome protein in the cyanobacterium Synechococcus elongatus PCC 7942. Mol Microbiol

Sauer K, Scheer H (1988) Exitation transfer in C-phycocyanin. Forster transfer rate and exciton calculations based on new crystal structure data for C-phycocyanins from *Agmenellum quadruplaticum* and *Mastigocladus laminosus*. Biochim Biophys Acta 936:157-170

Schirmer T, Huber R, Schneider M, Bode W, Miller M, Hackert ML (1986) Crystal structure analysis and refinement at 2.5 A of hexameric C-phycocyanin from the cyanobacterium Agmenellum quadruplicatum. The molecular model and its implications for light-harvesting. J Mol Biol 188 (4):651-676

Swanson RV, Glazer AN (1990) Phycobiliprotein methylation. Effect of the gamma-N-methylasparagine residue on energy transfer in phycocyanin and the phycobilisome. J Mol Biol 214 (3):787-796.

Tandeau de Marsac N, Cohen-Bazire G (1977) Molecular composition of cyanobacterial phycobilisomes. Proc Natl Acad Sci USA 74:1635-1639

Teale FW, Dale RE (1970) Isolation and spectral characterization of phycobiliproteins. Biochem J 116 (2):161-169.

Tooley AJ, Cai YA, Glazer AN (2001) Biosynthesis of a fluorescent cyanobacterial C-phycocyanin holo-alpha subunit in a heterologous host. Proc Natl Acad Sci U S A 98 (19):10560-10565

Yamanaka G, Lundell DJ, Glazer AN (1982) Molecular architecture of a light-harvesting antenna. Isolation and characterization of phycobilisome subassembly particles. J Biol Chem 257 (8):4077-4086.

Yi ZW, Huang H, Kuang TY, Sui SF (2005) Three-dimensional architecture of phycobilisomes from Nostoc flagelliforme revealed by single particle electron microscopy. FEBS Lett 579 (17):3569-3573.

Figure Legends

Fig. 1. Schematic representation of the ordered association of PBPs and LPs to form the PBS complex. The arrows indicate the direction of assmbly. All PBP components form trimers, with the PE, PEC or PC components forming hexamers that then further assemble into rods. Four APC trimers (containing both major and minor species) form cylinders, and 2-5 such cylinders form the PBS core. The six rod sub-structuctures bind to the core with the terminal ring flush with the outer cylinder circumference. In this depiction the rods are slightly displaced to reveal their inner cavity, proposed to house the rod-core linker proteins.

Fig. 2. Schematic description of the positioning of the PBPs within the complex to form an energy funnel. The rods of all species contain either PE or PEC (but not both) and (or only) PC. Cores contain only APC, however the number of cylinders is species dependent. A. A model of the PBS energy funnel. The size and color of the rings in

this model represent the drop in energy content as the maximal wavelength absorption of each species shifts further to the red, while all rings are physically of equal diameter. B. A model of the entire PBS showing the relative positions of each component. The length of the rods, determined by the number of hexamers, and their PBP content is species dependent.

Fig. 3. Structures of the different PBPs in monomeric form. The position of attachment of the different bilins are shown and the color of the structure approximately represents the color of the isolated protein. APC, allophycocyanin; PC, phycocyanin, PCB, phycocyanobilin cofactor; PEB, phycoerythrobilin cofactor; PEC, phycoerythrocyanin; PE, phycoerythrin; PUB, phycourobilin cofactor; PVB, phycobiliviolin cofactor.

Fig. 4. Evolutionary conservation mapped onto trimeric PC and APC structures. Conservation of residues on (i) the disk face composed of primarily α subunits of PC (A) and APC (E); (ii) residues on the disk face composed of primarily β subunits of PC (B) and APC (F); (iii) the outer circumference of the PC (C) and APC (G) disk and

(iv) the inner surface of the PC(D) and APC (H) disk. Red signifies a high level of conservation, blue no conservation and yellow not enough data to determine the level of conservation. The analysis indicates that the interaction interfaces (between trimers, between hexamers or between linkers and trimers/hexamers) are more highly conserved than the outer circumference of the discs and that APC is more highly conserved than PC.

Fig. 5. Schematic representation of the different types of crystal packing for each of the PC structures available in the PDB to date. In all structures the minimal physical unit is a trimer with the α subunits shown in yellow and the β subunits in blue. In all structures except 1ON7, the trimers associate into hexamers, and in some cases into infinite rods.

Fig. 6. 2Fo−Fc omit electron density maps contoured at 1.5 σ around each of the three phycyanobilin cofactors from the 3O18 PC structure determined to 1.35Å.

Fig. 7. X-ray crystal structure of the N terminal domain of the rod linker isolated from *Synechocystis sp. PCC 6803* (PDB code 3PRU). The protein is depicted in colors of the spectrum running from the N-terminal (blue) to the carboxyl terminal (red). The portion of the protein crystallized includes residues 14-158 out of the total 291 residues in the full length protein.

Fig. 8. Analysis of rod crystals. A. Rod crystals have a similar morphology to that of trimeric PC and X-ray crystallography shows that PC in rod form is nearly identical to that of trimeric PC (not shown). B. SDS-PAGE of the isolated rod shows the presence of three linker proteins (L_R, L_{RC} and $L_R^{8.7}$) in addition to the α and β subunits of PC, however core components are still present. C. Solubilized crystals were analysed by mass spectrometry showing that only a single rod type, containing CpcG4 crystallized. %Pep, percentage of possible peptides obtained by mass spectrometry. D. Confocal fluorescence microscopy (at 1 μm^3 resolution) analysis of rod crystals (full line) and trimeric PC crystals (dashed line) demonstrates the expected red shift in emission from PC in rod assembly. The same re-

sults were obtained from all depths of the crystal showing that the linkers were present throughout the crystal.

Fig. 9. Absorption characteristics of monomers and trimers of the phycocyanobilin containing PC and APC subunits. The monomeric form of both PC and APC as well as the trimeric form of PC have an absorption spectrum with λ_{max} = 620nm (broken line) whilst the trimeric form of APC has a blue shifted absorption spectrum with λ_{max} = 650nm and a shoulder at 620nm (line).

Fig. 10. Monomeric APC showing the solvent exposed position of the α84 cofactor (panel A), which becomes enclosed during trimerization (panel B). The nature of the pocket around α84 created by the adjacent monomer during trimerization is shown for APC (panel C) and PC (panel D). All proteins were depicted with their molecular surfaces colored by the calculated electrostatic potential of the surface. Red and blue patches indicate negative and positive potentials, respectively.

Fig. 11. The PBS complex is disassembled by interaction with the NblA protein. The relative sizes of the NblA protein (green, and shown enlarged in the inset) and a model of the PBS complex (blue) are shown.

Fig. 12. Source of NblA protein mimicry of phycobiliproteins lies in the presence of similar helix-turn helix motifs. Each motif of the PC subunit is shown in a different color on both the monomeric structure (bottom) and also shown carved out of the structure with corresponding colors (middle). The NblA is shown at the top in both monomeric (red) or dimer (green) form. Thin arrows show elements of the PC subunit with angles similar to those found in the two NblA assemblies.