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Phycobiliproteins: Molecular Structure, Production, Applications, and Prospects

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Abstract: Phycobiliproteins (PBPs) are the main component of light-harvesting complexes in cyanobacteria and red algae. In addition to their important role in photosynthesis, PBPs have many potential applications in foods, cosmetics, medical diagnosis and treatment of diseases. However, basic researches and technological innovations are urgently needed for exploring those potentials, such as structure and function, their biosynthesis as well as downstream purification. For medical use and application, mechanisms underlying their therapeutic effects must be elucidated. Focusing on these issues, this article gives a critical review on the current status on PBPs, including their structures and functions, preparation processes and applications. In addition, key technical challenges and possible solutions are prospected.

Keywords: Phycobiliprotein; Structure and function; Biosynthesis; Purification; Oxidative stress; Anti-tumor effect

Contents

1. Introduction

Oxygenic photosynthesis is an ancient and important biochemical process. Solar energy at wavelengths of 400–700 nm (photosynthetically active radiation) is captured by photosynthetic organisms and converted into chemical energy that can be used directly by living cells. Different from higher plants and green algae, major light-harvesting antennae in cyanobacteria and red algae are a large multi-subunit protein complex called phycobilisome (PBS), which was discovered in the 1960s (Gantt and Conti, 1965, 1966). PBSs, which harvest solar energy and transfer it into photosystems with extremely high efficiency, can be classified into three morphological types: hemi-ellipsoidal, hemi-discoidal, and bundle shaped (Gantt and Lipschultz, 1972; Guglielmi et al., 1981; Elmorjani et al., 1986; Glauser et al., 1992). All three types of PBSs are composed of water-soluble phycobiliproteins (PBPs) and hydrophobic linker peptides (Liu, 2016).

PBPs are a group of disk-shaped macromolecular proteins with covalently attached open-chain tetrapyrroles known as phycobilins (Apt et al., 1995). The structure, function, and application of PBPs have been studied intensively since their discovery (Guan et al., 2013). Recently, a PBS with molecular mass of 16.8 MDa was collected from the red alga *Griffithsia pacifica*, and its structure was deciphered by single-particle cryo-electron microscopy (Zhang et al., 2017). This is the first high-resolution molecular structure of PBS with a resolution of 3.5 Å, through which how PBSs are assembled from PBPs and linker peptides was elucidated, and several putative energy transfer pathways were speculated.

In addition to be used as natural pigments in food, cosmetics, dyes and so on, PBPs have been proven to have anti-oxidative, anti-viral, anti-tumor, immunity enhancing, and anti-inflammatory effects (Romay et al., 2003; Eriksen, 2008; Kuddus et al., 2013; Manirafasha et al., 2016). Therefore, PBPs, as physiologically active substances, have potential medicinal applications. Besides, PBPs have been also widely used as fluorescent labeling probes or photosensitizers in anti-tumor research (Fernandez-Rojas et al., 2014). However, extending the application of PBPs depends on a comprehensive understanding of their structures and spectral properties.

Current research on PBPs mainly focuses on the following aspects: 1) their structures, functions and biosynthesis, 2) mechanism underlying of the energy transfer in PBPs, 3) methods for their preparation, and 4) their potential applications, which are critically reviewed in this article.

2. PBPs

2.1. Types of PBPs

PBPs can be classified into four types according to their spectral properties: phycoerythrin (PE), phycocyanin (PC), allophycocyanin (APC), and phycoerythrocyanin (PEC) (Apt et al., 1995; MacColl, 1998). In early research, prefixes to PC and PE were used to distinguish their taxonomic origins. For example, R- was derived from red algae, while C- denoted those derived from cyanobacteria.

With improved understanding of PBPs, researchers found that PBPs from different sources could share similar spectral properties. For example, the spectral properties of PC from some red algae are similar to those from cyanobacteria. Therefore, nowadays prefixes are used to denote spectral properties rather than taxonomic sources (Table 1). Based on the absorption and fluorescence spectra, PE can be divided into R-PE, B-PE, b-PE and C-PE; PC can be divided into R-PC and C-PC. According to differences in PBPs with specific light spectra, R-PE can be further divided into four sub-types: R-PE I to IV; C-PE can be divided into C-PE I and II; and R-PC can be divided into R-PC I and II, and other types (Kursar et al., 1983; Tandeau, 2003).

Table 1

2.2. Structures and functions

2.2.1. Structures

In the past decades, crystal structures of PBPs from various sources have been resolved. The basic building block of PBPs is a monomer comprising α and β subunits, each with a molecular mass from 15 to 20 kDa for 160 - 165 amino acids.

The monomers oligomerize into $(\alpha\beta)_3$ trimer in a face-to-face to form with C_3 symmetry. Two $(\alpha\beta)_3$ form an $(\alpha\beta)_6$ hexamer structure with three-fold symmetry. In PE, an additional γ subunit generally binds to one $(\alpha\beta)_6$ moiety, resulting in a more stable PBP. In cyanobacteria and red algae, PBP trimers or hexamers assemble into PBS with the assistance of linker peptides. However, in Cryptophyta, PBPs exist as $(\alpha\beta)_2$ unit, and do not form into higher aggregation state (Anwer et al., 2015).

The aggregation state of isolated PBP is related to its concentration, pH value and ions in solution (Anwer et al., 2015). A dynamic equilibrium between different aggregated states of PBPs is present in solution. Dissociation of $(\alpha\beta)_3$ and $(\alpha\beta)_6$ units during the purification process usually lead to a blue-shift of the fluorescent peak (Kupka and Scheer, 2008). When genes encoding α and β subunits of PBP in *Synechocystis* sp. PCC 6803 were expressed in *E. coli*, the subunits were found to self-assemble into trimmers (Liu et al., 2010). One prominent spectroscopic characteristic of recombinant APC is its strong red-shift in the absorption and emission maxima when the monomers are assembled into a trimer, which was the first report of the assembly of recombinant ApcA and ApcB into a trimer with the native structure (Adir et al., 2006). Because PBPs have a higher molar extinction coefficient, they are widely used in fluorescence microscopy and immunoassays. However, the spectral characteristics of purified PBPs usually depend on their aggregation states. If PBPs lose their higher order protein structures, their absorption and fluorescence properties may be diminished, even completely lost.

The absorption properties of PBPs are attributed to the presence of open-chain tetrapyrrole chromophores called phycobilins, which include phycocyanobilin (PCB, Amax = 640 nm), phycoerythrobilin (PEB, Amax = 550 nm), phycouroblilin (PUB, Amax = 490 nm), and phycoviolobilin (PXB, Amax = 590 nm). Two bilins are linked to conserved cysteine residues at position 84 to the α and β subunits, while other pigments (if present) are bound at additional cysteine sites (α75, α140, β50/61, β155, etc) (Glazer, 1994; Apt et al., 1995; MacColl, 1998; MacColl et al., 1999; Sonani et al., 2015).

2.2.2. Optical properties

Water presents a different light environment. The reason for this is that the preferential absorption of long wavelength photons with low energy such as red light mainly determines the spectral distribution of light attenuation, but short wavelength blue photons with high energy can penetrate the depth. As a result, deep water is full of blue-green light. Many small cyanobacteria and red algae can survive in the light environment less than 1% surface irradiance, and some much smaller microalgae may require light irradiance as low as 0.1%, even less, because PBPs can efficiently capture and transmit light energy in deep-water areas, in particular, the blue-green light that can penetrated into deep water. Interestingly, cyanobacteria exhibit a form of photomorphogenesis termed chromatic acclimation (CA), and one of the characteristics of CA is to regulate the pigment composition of PBP to optimize light absorption for photosynthesis, thus adapting to the special light environment in water (Montgomery, 2017).

The four PBPs can be divided into three types according to energy levels associated with light absorption: high energy (PE and PEC), medium energy (PC) and low energy (APC). The

absorption and fluorescence emission spectra of PBPs with different energy levels have a strong overlap (Fig. 1), an denergy is transferred in the PBS progress from PE, PC and APC to the photosynthetic reaction center: light energy absorbed by phycobilins is transmitted first between the subunits, then between different PBPs and finally to the reaction center located in the thylakoid membrane. The efficiency of this energy transfer is higher than 95% (Sidler, 1994; Onishi et al., 2015; Zhang et al., 2015). PBPs have the advantages of high water solubility, non-toxicity, high fluorescence quantum yield, Stokes shift and slowness in fluorescence quenching. In addition, the spectral properties and quantum yield are generally maintained after cross-linking with other proteins. Therefore, in the 1980s, Glazer first proposed that PBPs could be used as fluorescent probes (Oi et al., 1982), leading to applications of PBPs in clinical diagnostic chemistry (Glazer and Stryer, 1984).

Figure 1

3. Production of PBPs

Methods for purifying PBPs vary depending on cyanobacteria or algal source. In general, two steps are involved: extraction of crude protein and PBP purification. Different methods have significantimpact on the purity and activity of PBPs. The purity of PBPs is usually expressed by the ratio A_{max}/A_{280}.

3.1. Cell disruption and crude PBP extraction

PBPs are water-soluble intracellular proteins. The first step for preparing PBPs is to choose technique to release PBPs from cells, and in the meantime maintains their structures and functions unaffected significantly. In general, the higher the proportion of broken algal cells is, the higher the yield of PBPs will be. However, violent disruption may have negative effect on the structures and functions of the PBPs. Common methods for cell disruption include mechanical (grinding, high-pressure homogenization, ultrasonication, etc) and non-mechanical ones (repeated freezing and thawing, lysozyme treatment, osmotic shock, etc) (Sekar and Chandramohan, 2007).

Freezing-thawing: Freezing-thawing treatment is among the most widely used methods for extracting PBPs, which is very effective for most cyanobacteria and some red algae. After treatment with freezing and thawing, the permeation barrier of cells is damaged and internal materials are released (Calcott and MacLeod, 1975). Generally, cyanobacteria or red algae are frozen at about –20 °C for several hours, and then thawed at 4°C or room temperature. For a better extracting efficiency, several rounds of freezing-thawing are usually used.

Sonication: Sonication is a time-saving method for destructing cell structures by creating violent blast pressure in solutions (Le Guillard et al., 2015; Mittal et al., 2017), which is widely used in laboratory for disrupting cyanobacteria or single-celled red algae with high efficiency. However, this method is not considered to be suitable for the large scale extraction of PBPs, because it is difficult to transmit sufficient energy to large volumes of cell suspension (Balasundaram et al., 2009). Moreover, the energy of ultrasound waves could be transformed into heat, which may influence the stability of the protein structure.

Osmotic shock: Fresh cyanobacteria or red algae could be mixed with distilled water or extracting buffers and kept in dark for hours. This would induce cell lysis by hypotonicity. In some reports, cyanobacteria or red algae were freeze-dried before mixing with distilled water or extracting buffers, which would enhance productivity for the crude extract (Kissoudi et al., 2018).

Selection of proper extraction methods depends on cyanobacterial or red algal species. A comparison for extracting C-PC from *S. platensis* showed that extraction efficiency with the methods of repeated freezing-thawing, lysozyme treatment, and bacteria (*Klebsiella*

pneumoniae) treatment was comparable, but glass bead grinding and sonication were not efficient (Zhu et al., 2007). Another work compared different methods for extracting PBPs from the red alga *Porphyridium cruentum*, and the authors considered that buffer extraction from lyophilized alga was better than other methods such as repeated freezing-thawing and sonication (Bermejo et al., 2003). To enhance the extraction, a combination of several methods is usually used in laboratory.

3.2. Purification of PBPs

The concentration of PBPs in crude extract is relatively low. Therefore, further work must be carried out to improve the purity of the PBPs. The procedure of purification often involves several steps. The most commonly used methods include ammonium sulfate fractionation, chromatographic separation and two-phase aqueous extraction.

Ammonium sulfate fractionation is widely used in PBP purification before further treatment. The colloidal stability of the protein surface could be disrupted by increasing ammonium concentration in the solution (Burgess, 2009). Usually, ammonium sulfate fractionation involves two steps: the first step is to precipitate impurities with 20-30% (w/v) ammonium sulfate, which can be removed by centrifugation, and the second step is to precipitate most PBPs from the supernatant by further increasing ammonium sulfate to 60-70% (w/v). Ammonium sulfate fractionation treatment could help to remove large amounts of impurities. Therefore, this treatment is recommended for PBP purification.

Various chromatographic technologies have been developed for purifying PBPs, including gel filtration chromatography, ion exchange chromatography, hydroxyapatite chromatography, expanded bed adsorption chromatography, hydrophobic interaction chromatography, etc. Gel filtration chromatography, also known as size-exclusion chromatography, separates proteins mainly based on their sizes. Ion exchange chromatography separates proteins mainly based on their affinity to ions. These two chromatographic methods are most commonly used when purifying PBPs in laboratory. Several reports showed that APC could only be separated with high efficiency from other PBPs by ion exchange chromatography (Yan et al., 2011; Sorensen et al., 2013). Most ion exchange chromatographic separation works through a gradient of ionic strength when purifying PBPs. However, advances indicate that an elution method with a gradient of pH is quite efficient for separating PBPs (Liu et al., 2005; Su et al., 2010; Yan et al., 2011; Kumar et al., 2014). Expanded bed adsorption chromatography allows separating proteins directly from the crude extract, bypassing initial treatment such as centrifugation and ammonium sulfate precipitation. Therefore, this method reduced the steps used in the purification of PBPs, which is a time saving technique compared with other methods.

Aqueous two-phase extraction has been widely used for separating biomolecules, which has been the subject of extensive studies in the purification of PBPs (Table 2). This process is easily to be scaled up, but less efficient compared to other separation processes such as chromatographic separation and purification. As a result, it is an alternative method for industries to obtain products at large scale with low purification cost, which may be not suitable for the separation of PBPs when they are developed as value-added products to be produced in relatively small volume.

Table 2

3.3. Production of recombinant PBPs

In 1985, the APC gene of *Cyanophora paradoxa* and the PC gene of *Synechococcus* sp. PCC7002 were expressed in *E. coli*, showing the successful heterologous expression of PBPs using genetic engineering techniques (Bryant et al., 1985). Two processes are involved in the

biosynthesis of PBPs: 1) the synthesis of apoproteins and phycobilins and 2) the binding of phycobilins to apoproteins by enzymatic catalysis. When bacteria are engineered to produce recombinant PBPs, special molecular tags have often been used, which facilitate the purification of the recombinant PBPs (Gambetta and Lagarias, 2001; Kohchi et al., 2001; Zhao et al., 2005; Li et al., 2017).

The biosynthesis of phycobilins is derived from the metabolism of heme. Heme is split into biliverdin by heme oxygenase (HO), and then biliverdin can be reduced by the phycobiferous reductase family and be further reduced to other types of phycobilin. For example, PCB is reduced by the reductase PcyA (Tooley et al., 2001), while the synthesis of PEB is catalyzed by two enzymes (PebA and Peb) (Kohchi et al., 2001). Because the exogenous phycocyanin-related ferredoxin oxidoreductase gene can be expressed in *E. coli*, PBPs can be produced on a large scale by biosynthesis in *E. coli*. (Gambetta and Lagarias, 2001).

After being synthesized in *E. coli*, phycobilins need to bind to the correct site of the apoprotein. This process can be catalyzed by either autocatalysis or lytic enzymes. As an example of the autocatalytic process, studies have shown that PCB can spontaneously form a pigment–protein structure in vitro with similar spectral properties to the native protein in vivo (Zhao et al., 2005). However, in recombinant PBPs, the linkage between a phycobilin and apoprotein is formed with higher efficiency and correctness when catalyzed by a lyase. Therefore, the use of PBP lyases is the key to synthesizing recombinant PBPs efficiently in vitro. Some PBP lyases are shown in Table 3.

Table 3

Zhao's group has performed considerable research on recombinant PBPs (Wang et al., 2011; Zhou et al., 2014; Dong et al., 2016), which discovered three PBP lyases and created a molecular design method for switching light-activated red fluorescent PBPs. Recently, this group reported the trimeric crystal structure of recombinant AP-B derived from *Synechocystis* sp. PCC6803, with a resolution of 1.75 Å. Both AP-B and APC are located in the core of the PBS, and AP-B passes the captured light energy to the photoreaction center. Because it is difficult to purify AP-B from natural sources, recombinant AP-B provides an effective approach for studying the structure, energy transfer function and applications of PBSs (Peng et al., 2014).

Based on genetic engineering techniques, large-scale production of low-cost recombinant PBPs can be achieved. The genes of APC and PC subunits have been successfully expressed in *E. coli* or *Pichia pastoris* (Qin et al., 2004). Recombinant PBP subunits usually contain either a His tag or a maltose binding protein tag at the terminus. Therefore, the recombinant PBPs could be purified by affinity chromatography. Research indicated that the recombinant PBP subunit exhibited similar spectral characteristics as the native PBP subunit (Guan et al., 2007; Ge et al., 2009; Li et al., 2017). By molecular design and recombinant synthesis, various types of PBPs with improved functions could be obtained for future applications.

4. Applications of PBPs

Although PBPs have been produced at large scale in China from *Spirulina* with a total production capacity of ~40 tons / year, few companies sell relative products in large quantities. This is because high value-added products based on PBPs are lacking in the market and current research mainly focuses on their applications in pharmaceutical and optical products (Fig. 2).

Figure 2

4.1. Pharmacological applications

Research on the physiological activity of PBPs has been carried out for more than 20 years. PBPs have been found to have a strong antioxidant effect through eliminating excess reactive oxygen species (ROS) and increasing the amount of anti-oxidative enzymes (Wu et al., 2016). Therefore, PBPs have potentials for treating a variety of diseases caused by oxidative stress. Since the antioxidant effect of PBPs was demonstrated, these proteins have been investigated for treating several diseases *in vivo* and *in vitro* (Fernandez-Rojas et al., 2014). Many studies *in vitro* models have also shown that PBPs have anti-inflammatory, anti-viral, anti-tumor, and enhanced immunity functions (Table 4).

Table 4

4.1.1. Antioxidant effects

4.1.1.1. In vitro studies of antioxidant properties

Romay et al. (1998) first reported that PBPs showed anti-oxidative properties both *in vitro* and *in vivo*. PC could effectively eliminate hydroxyl radicals (•OH) and alkoxy radicals (RO•) and inhibit lipid peroxidation. The anti-oxidative mechanism of PC was found to be similar to that of commonly used antioxidants such as to copherol and ascorbic acid. PC can also inhibit oxidative-induced hemolysis of red blood cells (Romay and Gonzalez, 2000).

It has been proposed that both components of PBPs, the apoprotein (α and β subunits) and phycobilins, are involved in antioxidant effect through mechanism of stabilizing systems for the detoxification of ROS (Pleonsil et al., 2013). When PC was hydrolyzed with trypsin, it was found that the apoprotein portion of the molecule showed partial antioxidant properties (Zhou et al., 2005). Proteins can lose activity in the presence of sodium lauryl sulfate, urea, or under alkaline conditions, which may lose the ability to produce hydroxyl radicals, but the ability to scavenge hydroxyl radicals increases. This indicates that PCB in PBPs may play an important role in clearing hydroxyl radicals. Hirata et al. (2000) studied the anti-oxidative effect of PC using a hydrophobic system with phosphatidylcholine liposomes and showed that PCB had a higher antioxidant activity than tocopherol. The PCB of PC is easily oxidized. Micromolar concentrations of C-PC can halve the steady-state concentration of hydrogen peroxide radicals, and PCB might be the main target for free-radical to attack for antioxidant activity similar to catechin (Lissi et al., 2000).

Light impacts the antioxidant activity of PBPs, which can produce free radicals under light conditions, but those free radicals can be removed under dark conditions. C-PC produced from *Spirulina* was exposed to blue and white light, and the antioxidant properties of C-PC under different light conditions were examined, which indicated that C-PC under blue light had higher oxidation resistance than under normal light. In addition, amino acid sequence studies of the C-PC subunits showed that blue light can result in a rearrangement of the positions of amino groups in the beta chain indicating a modification of the polypeptide with higher numbers of cysteine residues (Madhyastha et al., 2009). Huang et al. (2007) obtained selenium-containing PCs (Se-PCs) from selenium-rich *S. platensis*, and studied their antioxidant activities such as the ability to scavenge the free radicals of superoxide, hydrogen peroxide, and 2,2-diphenyl-1-picryl-hydrazyl (DPPH). The antioxidant activity of Se-PC toward different free radicals was different, and the ability of the Se-PCs to scavenge superoxide and hydrogen peroxide radicals was positively correlated with the Se content. When PC reacted with antioxidants, the maximum absorption peak at 620 nm disappeared, and in the mean time the blue color of PC gradually disappeared and the PC oxidation resistance decreased rapidly (Benedetti et al., 2004).

Ge et al. (2006) expressed apo-APC and its subunits from *Cyanobacteria* in *E. coli.* and

used a deoxyribose assay to evaluate its antioxidant properties. The recombinant APC fused with a MBP tag and a 6-His tag scavenged hydroxyl radicals successfully as the α and β subunits could, but the α and β subunits had a higher inhibitory effect on hydroxyl radicals when tested separately than they were combined together, and the effect on the radical clearance by the subunits was improved as their concentrations of the subunits increased. These results showed that apo-APC was involved in the antioxidant and free-radical scavenging activity of PC, and indicated that antioxidant activity might be partly responsible for the anti-tumor effect of APC .The α subunit and the chromophore of APC in *E. coli* can be catalyzed to become holo-ApcA, which can inhibit hydroxyl and hydrogen peroxide radicals more strongly than ApcA and native APC (Zhang et al., 2009). Guan et al. (2009) expressed and produced a fluorescent antioxidant holo-alpha-PC from *S. platensis* with a His-tag (rHHPC: recombinant holo-alpha-phycocyanin of S. platensis) biosynthesized in E. coli BL21. The combined biosynthesis of rHHPC was successful and rHHPC was conveniently purified by Ni^{2+} affinity column chromatography. rHHPC was demonstrated to be effective at clearing hydroxyl and hydrogen peroxide radicals. The IC_{50} values of rHHPC were 277.5 ± 25.8 and 20.8 ±2.2 μg/ mL for hydroxyl and peroxyl radicals, respectively.

These studies indicate that both natural PBPs and recombinant PBPs are promising antioxidant with potential use in the food and pharmaceutical industries.

4.1.1.2. Antioxidant effects in human and animal diseases

Atherosclerosis: Oxidative stress involved in the development of atherosclerosis (AS) is a complex process. ROS and other pathogenic factors exert a synergistic effect resulting in microvascular damage. In addition, ROS has toxic effect on the vascular cell walls. It has been shown that low density lipoproteins (LDLs) and similar compounds in blood are easily attacked and affected by ROS when free radicals and lipid peroxidation products are increased in the blood of model animals and patients (Vogiatzi et al., 2009). Many enzymes contribute to the development of AS, including NAD oxidase, endothelial nitric oxide synthase, xanthine oxidase, and myeloperoxidase. In blood vessels, the main enzyme generating ROS is NAD oxidase. Riss et al (2007) reported that PC from *Spirulina* can increase the level of antioxidant enzymes in the body, and consequently inhibit the production of ROS, which eventually leads to the enhancement of plasma antioxidant capacity. PC can also reduce the expression of NAD oxidase to decrease ROS production, and thus ameliorate atherosclerosis caused by oxidative stress (Riss et al., 2007). Strasky et al. (2013) reported that PC could activate the expression of encoding heme oxygenase-1 to increase the enzyme's content in the atherosclerotic lesion of mice with apolipoprotein E gene deletion to reduce the severity of the disease. This is because heme oxygenase-1 can catabolize heme to produce bilirubin, which has potent antioxidant capacity. PC can also regulate oxidative-stress and endothelial cell-dysfunction marker proteins such as endothelial nitric oxide synthase and NAD oxidase, and reduce atherosclerotic lesions. These studies indicate that PC can potentially treat atherosclerosis by attenuating the oxidative stress.

Liver disease: Oxidative stress may induce the development of liver diseases such as fatty liver, viral hepatitis, and hepatic fibrosis. Research shows that increased ROS can deplete intracellular ATP, impair the oxidative capacity of mitochondria, and affect the oxidation of acetaldehyde for its accumulation in the liver. Therefore, if oxidative stress can be reduced, liver will be protected from the damage. (Day and James, 1998; Zhu et al., 2012). Pak et al. (2012) studied the effect of PC on non-alcoholic fatty liver disease which showed that PC has anti-oxidative and anti-inflammatory effects and can prevent the progression of non-alcoholic fatty liver disease. In a mouse model of non-alcoholic fatty liver, the levels of mitochondrial ROS and inflammatory cytokines were significantly increased, but ROS in mice treated with PC did not change significantly compared to that in the control, indicating

that PC can reduce oxidative stress, inhibit inflammation, and thus treat non-alcoholic fatty liver disease. Xia et al. (2016) studied the protective effects of PC on alcoholic fatty liver , and found that PC reduced the serum levels of alanine aminotransferase, aspartate aminotransferase, triglyceride, total cholesterol and low-density lipoprotein, and increased the content of SOD and malondialdehyde (MDA) in the liver, thereby reducing oxidative stress. Ou et al. (2010) demonstrated that PC could reduce CCl4-induced liver damage by scavenging ROS and enhancing the activity of SOD and glutathione peroxidase (GSH-Px).

Cataracts: The formation of cataracts is one of the most common eye diseases (Alfawaz et al., 2014; Miric et al., 2014). In recent years, some researchers have found that there is a correlation between occurrence in cataracts and the oxidation index. An imbalance between the oxidation and antioxidant systems in eyes can produce an oxidative stress response, which may denature the lens protein, and cause cataracts (Kaur et al., 2012). As an antioxidant, PC can play a role in the treatment of cataracts. Kothadia and Shabaraya (2011) found that PC could reduce galactose-induced cataracts by increasing the expression of glutathione (GSH) and eliminating free radicals. Kumari et al. (2013) used sodium selenite to induce cataracts in rats for PC treatment, and their results showed that PC could regulate the expression of antioxidant enzymes, increase the activity of antioxidant enzymes, and reduce the oxidative stress response. These results suggest that PBPs as antioxidant might provide an effective treatment for cataracts.

Neuropathy: When oxidative stress occurs, high levels of ROS can cause lipid peroxidation in neuronal cell membranes, and increase their permeability. Neuronal cells are more likely to develop toxic edema, leading to neuronal damage (Buonocore et al., 2001). Min et al. (2015) demonstrated that PC protects the brain from oxidative damage. Kainic acid can produce a large number of oxygen free radicals, leading to epilepsy in rats. Rimbau et al. (1999) used kainic acid to induce epilepsy in rats and found that treating the rats with PC eliminated the free radicals produced by the kainic acid and protected neurons. Iron can cause oxidative stress in SH-SY5Y neurons. Bermejo-Bescós et al. (2008) found that PC protected SH-SY5Y nerve cells from oxidative stress by activating antioxidant enzymes, such as SOD, catalase, and glutathione peroxidase (GSH-Px), and the same study also indicated that PC may play a protective role in nerve injury caused by free radicals with potentials for treating Alzheimer's and Parkinson's diseases. Therefore, patients with neurological diseases might be treated with anti-oxidative therapy based on PBPs to achieve a better therapeutic effect.

Kidney disease: Overproduction of ROS can damage kidneys and cause diabetes. After 10 consecutive weeks of an oral administration of PC in mice with type-2 diabetes, the expression of NADPH oxidase, an oxidative stress marker, levels of proteinuria in the kidneys, and mesangial expansion were reduced, which showed that PC could prevent the incidence of diabetes in mice by reducing oxidative stress. ROS can increase the permeability of glomerulus as its concentration gradually increases, resulting in the deposition of plasma proteins in the basement membrane and causeing renal arteriosclerosis (Zheng et al., 2013). In canine kidney cells, PC can reduce oxalic acid-induced ROS and lipid peroxidation reactions, thereby preventing cells from the damage (Farooq et al., 2014). ROS also creates deposits in the extracellular matrix, causing expansion of mesangial area and damaging the kidneys. PC could prevent the cisplatin-induced decrease in glutathione reductase, decrease the levels of hydrogen peroxide, maintain the blood urea nitrogen at normal levels, and inhibit cisplatin-induced nephrotoxicity (Fernandez-Rojas et al., 2014).

Lung disease: Two main factors cause oxidative stress in lung diseases. The first is exogenous factors such as smoke, environmental pollutants and chemicals. These compounds contain a large number of free radicals, which can directly stimulate the respiratory tract and lungs, causing cell and organ damage. Another is endogenous factors. Neutrophils in the pulmonary microcirculation can be activated, resulting in the release of large amounts of ROS

and causing cell and tissue damage. ROS can activate the signal pathway of NK-κB, exacerbate the inflammatory response, and directly stimulate the proliferation of fibroblasts to cause pulmonary fibrosis through factors such as TNF-α and ET-1 (Villegas et al., 2014). The herbicide Paraquat (PQ) is a highly toxic compound that causes pulmonary fibrosis. PC could inhibit the PQ-induced lipid peroxidation reaction, including increasing SOD activity and decreasing the MDA content to diminish its damage to cells and tissue in lungs, and improving the pathological damage induced by PQ (Sun et al., 2011). PC was also found to alleviate the TGF-β1 expression in lung tissue. Compared with the control group, the production of NF-κB p65 and TNF-α in the lungs was inhibited in the PQ-treated group. It has been suggested that PC possesses an anti-pulmonary fibrosis effect (Sun et al., 2012). Subsequently, the protective effect of PC on pulmonary fibrosis was found to involve protecting alveolar epithelial type I cells, alleviating fibroblast proliferation, and inhibiting the epithelial-mesenchymal transition (EMT) and oxidative stress. The PC-induced inhibition of the TLR2-MyD88-NF-κB signaling pathway in the early stages was very important for protection against pulmonary fibrosis (Li et al., 2017).

Intestinal disease: Inflammatory bowel disease (IBD) is a chronic inflammatory disorder. Although the etiology of IBD is unknown, currently there is consensus that ROS are involved in the induction and development of the disease. PC was used to treat acetic acid-induced ulcerative colitis in rats: Neutrophil infiltration was significantly reduced in rats with colitis after PC treatment. PC reduced the myeloperoxidase activity, inhibited inflammatory cell infiltration, and reduced colonic injury to some extent (Gonzalez et al., 1999).

4.1.2. Anti-tumor properties

Tumors result from the abnormal proliferation and differentiation of tissue cells when the body's normal regulation of cell growth is disrupted. Much research has focused on developing anti-tumor drugs. However, most of existing synthetic anticancer drugs also have very strong toxic side-effect on normal cells of the human body. Studies have found that PBPs have inhibitory effect on a variety of tumors.

4.1.2.1. In vitro studies of anti-tumor properties

Thangam et al (2013) investigated the cytostatic effect of C-PC on cancer cells, and found C-PC inhibited the growth of HT-29 (colon cancer) and A549 (lung cancer) cells, as detected by fluorescence and phase contrast microscopies, arresting DNA replication in tumor cells. Recombinant APC has been found to be able to treat tumors. For example, recombinant APC inhibited H22 hepatoma cells significantly, and the inhibition rates ranged from 36% to 62% when the dose of PC was 6.25 to 50 mg/kg/day (Ge et al., 2005). The PE gene was successfully expressed in *E. coli* BL21, and tumor cytotoxicity assays showed that recombinant PE could inhibit the growth of HeLa cells, and the inhibition rate increased from 37.3 to 63.26% as the protein concentration increased (Ruobing et al., 2007). These studies indicate that both native PBP and recombinant PBP have potential medical value in anti-tumor applications.

4.1.2.2. In vivo studies of anti-tumor properties

The therapeutic effect of C-PC on mouse skin tumors has been studied. Tumors were developed in mouse skin using 12-O-tetradecanoylphorbol-13-acetate (TPA), and tumor-specific factors, including ornithine decarboxylase, cyclooxygenase-2, and interleukin 6, as well as phosphorylation signal transducers and activators of transcription 3, were detected in the mice with TPA-induced tumors, which were inhibited by C-PC in a dose-dependent manner (Gupta and Gupta, 2012). PBP not only reduces side-effects of anticancer drugs, but also increases their effectiveness. The drug topotecan used in

conjunction with PC was found to be more effective than topotecan treated alone at regular doses through activating a large number of caspase-9 and caspase-3 enzymes, and increased the effectiveness of topotecan treatment (Gantar et al., 2012). Although PBPs have an inhibitory effect on a variety of in vitro animal tumor models, their mechanism of action in inhibiting tumors is complex, and further research is needed.

4.2. Optical applications

PBPs can be used in photodynamic therapy and other fields, because PBPs can emit strong fluorescence after being irradiated with a laser (Table 5).

Table 5

4.2.1. Photodynamic therapy

Photodynamic therapy is a new type of oncology therapy based on enriching a lesion area with photosensitizers, which cause oxidative damage to tumor tissue by generating free radicals and active oxygen species upon illumination. The selection of photosensitizers with high efficiency, low toxicity and good selectivity is the core of effective photodynamic therapy.

As early in the 1980s, researchers found that PC could act as a cytotoxic photosensitizer. Examination of arterial sections by fluorescence microscopy revealed that PC can bind to human atherosclerotic plaques, enabling the plaque to be observed by fluorescence of PC (Morcos et al., 1988), which suggested a potential use of PC as a guide for photodynamic therapy. PC from *Microcystis* (MC-PC) has been studied for its photosensitization effect on HepG2 cells. After cancer cells were incubated with MC-PC, followed by laser irradiation, the cell viability was measured by the MTT method, and it was found that MC-PC at a dose of 200 μg/mL effectively inhibited the growth of HepG2 under laser irradiation and induced apoptosis after 24 h, which identified a new source of PC from *Microcystis* as a safe and effective photosensitizer (Wang et al., 2012). C-PC was incubated with breast cancer cells MDA-MB-231, and the results showed that C-PC showed no photo-toxicity without laser irradiation, but when irradiated with a 625 nm laser, the cells were able to produce oxygen free radicals and ROS, leading to apoptosis and killing of MDA-MB-231 breast cancer cells (Bharathiraja et al., 2016). APC has a similar pigment structure to PC, and laser pulsed radiation technology has been used to characterize APC photochemical and photophysical transient intermediates. Under laser irradiation, APC is capable of generating triplet states and free radical cations, indicating that APC can perform photoexcitation and photoionization simultaneously, and can also be used as type I and type II photosensitizers (Suping et al., 2001).

PBPs have a stronger affinity for tumor cells than for normal cells, but the mechanism underlying this phenomenon is still unclear. In addition, because PBPs can also be used as a health food supplement to enhance immunity (Levi et al., 2018), some researchers believe that PBPs may have the effect of inhibiting tumor growth through a variety of synergistic effects.

4.2.2. Fluorescence probes

Much attention has been paid to the development of fluorescent probes based on PBPs as markers in immunohistochemistry, immunocytochemistry, flow cytometry, confocal laser microscopy, fluorescence-activated cell sorting, single-molecule detection, and other fluorescent immunoassays (Siiman et al., 1999; Guan et al., 2007). Companies such as Boehringer Ingelheim in Germany and Sigma and Molecular Probes in the USA have developed PBP-related probe products.

APC is commonly used as a fluorescent probe to detect apoptosis (Tang et al., 2017; Li

et al., 2018). However, because of the stabilizing effect of its γ subunits, PE is the ideal fluorescent probe, and is more commonly used than other PBPs (Leney et al., 2018). Detection of IgG antibodies to Hendra virus in serum is important to help monitor outbreaks of the virus. The commonly used enzyme-linked immunosorbent assays and fluorescence-based Luminex assays typically consist of three steps, and take at least a few hours to complete the process. R-PE was used as a fluorescent label to bind directly to IgG protein, and because of the large specific surface area of the magnetic nanoparticles, it was possible to reduce each step in the detection process to 20 minutes (Gao et al., 2015). Using genetic engineering techniques and large-scale fermentation, recombinant PBPs can be produced at lower cost with improved fluorescence characteristics. Wu et al. (2017) co-expressed streptomycin and a fusion protein (SLA) from the APC α subunit of *Thermosynechococcus elongatus* BP-1, together with PEB synthase (Ho1 and PebS) or PCB synthetases (Ho1 and PcyA) in *E. coli*, and two recombinant PBPs (SLA-PEB and SLA-PCB) capable of binding biotin were obtained. The detection limits of these fusion proteins in tumor marker alpha-fetoprotein assays were 0.11 and 0.35 ng/mL, respectively.

The extraction and purification of PBPs is difficult, and there is no mature process for industrial production, resulting in expensive products, and making it difficult to develop and apply PBP-based fluorescent probes. However, the characteristic fluorescence peak of some PBPs containing PCB is at 660 nm, which lies within visual imaging window for living tissue at 650~1100 nm (Shcherbo et al., 2007). Therefore, PBPs might be used as a development of near-infrared fluorescent probes. Higher organisms cannot biosynthesize PCB of PBPs, which greatly limits the application of the fluorescent proteins in mammalian cells, but mammalian cells are rich in heme, which can synthesize biliverdin (BV) under the catalysis of heme enzymes. The development of BV-based PBPs as near-infrared fluorescent probes has become a trend.

5. Perspectives

5.1. Assessment of structure and function of PBPs

Since the last century, there has been much research on PBSs and PBPs and many high-resolution crystal structures of PBPs have been obtained. PBPs have a more complex structure and a more flexible type of assembly than other protein structures involved in photosynthesis. In the past decade, research on the energy transfer process of PBPs has progressed slowly because of the complexities of dynamic changes in the chromophore conformations and the intricate interaction between the chromophores and the microenvironment. With increasing knowledge of PBP structure and chromophore binding, it should be possible in the future to simulate the dynamic changes. PBSs usually contain hundreds of chromophores, and the energy delivery pathway is more complicated. Zhang et al (2017) solved the problem of the poor stability and orientation of PBSs during sample preparation and for the first time, reported the three-dimensional structure at a near-atomic resolution of an intact PBS which provides a basis for revealing the PBS assembly and light energy-transfer processes.

More in-depth studies on the structure and energy transfer of PBPs, with techniques across physical and life sciences, including circular dichroism spectroscopy, transient spectroscopy, three-dimensional reconstruction technology using cryogenic electron microscopy, scanning tunneling microscope manipulation, and local electric field resonance enhancement regulation will enable the potential application of PBPs in many fields. Detailed studies of PBPs structure will help not only to understand the process of PBP self-assembly, but also to explore the relationship between the microenvironment and the spectroscopic properties of the phycobilin binding region. Studies of the structure of PBPs will also help to deepen the understanding of their anti-oxidative and other pharmaceutical activities.

5.2. Purification of PBPs on large scales at low cost

The oceans are rich in seaweed resources, which provide a wealth of raw materials as a source for PBPs. However, the separation and purification processes are complicated and time-consuming with low recovery. At present, no efficient separation and purification methods have been found to yield high purity PBPs, which has greatly restricted their applications. How to develop fast and efficient extraction and purification technology to make the products with high purity and high recovery yield will remain the focus of future research. The preparation of natural PBPs from algae to study their light energy transfer mechanism for drug development is complicated, since natural PBPs often exist in the form of complexes. When used as drugs, their effects are often unpredictable. In the future, if high-density fermentation and high-level expression of recombinant PBPs can be achieved, not only will recombinant PBPs be produced on large scales, but also with improved quality.

5.3. Mechanism underlying the pharmaceutical use of PBPs

PBPs have potential pharmacological and biological uses, and may play an important therapeutic role in treating a variety of diseases such as atherosclerosis, hepatitis, pneumonia, and cataracts. Because PBPs can elicit allergic reactions with immune systems, they cannot be injected intravenously, and instead are orally administered. However, when PBPs enter the digestive system, they are broken down into amino acids, small peptides and phycobilins. Although the therapeutic effects of PBPs have been demonstrated, it is not clear how PBPs or their metabolites affect their targets and metabolic pathways, and mechanistic studies on the pharmacological effect of PBPs and their metabolites are thus needed, but little work has been done so far in this regard. Recently, we have observed the regulation of PC on mouse intestinal flora, and found that PC intervention promoted the colonization of beneficial bacteria and reduced intestinal permeability. Therefore, to evaluate the pharmacokinetic properties properly, it is necessary to detect PBPs and their main metabolites in blood and intestine. In addition, if the structure of the PBPs could be modified with improved activities, smaller and simpler mutant molecules may be obtained. In the future, PBP mutants are expected to help solve some current problems with the applications of PBPs.

5.4. Applications of PBPs in optics

With the completion of the whole genome sequencing of some algal species, more and more PBPs and chromophore lyases will be identified. Through construction of genetically engineered bacteria, production of recombinant PBPs with added molecular tags is possible, which not only solves problems with the preparation of fluorescent proteins, but also broadens the scope of their applications. For example, the use of solar energy in the bionic field might be expanded by establishing artificially directed evolutionary technology for PBPs and constructing efficient light-trapping devices for use under low light. Research into genetically recombinant PBPs has also laid material and technical foundations for the construction of PBP-based artificial solar energy capture devices.

6. Conclusions

PBPs have been studied for more than 50 years since Gantt and Conti first discovered them. Through the interplay of multiple disciplines, including structural biology, biochemistry, genetic engineering and computational biology, the molecular structures of PBPs have become increasingly clear while large-scale preparation techniques have also become more mature. In the future, commercial applications of high value-added products based on PBPs will be more promising with potentials for economic benefits.

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References

Alfawaz A, Alrashidi S, Kalantan H, Al-Mezaine H, Abu AM. Cataract surgery under systemic infliximab therapy in patients with refractory uveitis associated with behcet disease. Ann Saudi Med 2014;34:328-333.

Anwer K, Sonani R, Madamwar D, Singh P, Khan F, Bisetty K, et al. Role of N-terminal residues on folding and stability of C-phycoerythrin: simulation and urea-induced denaturation studies. J Biomol Struct Dyn 2015;33:121-133.

Apt KE, Collier JL, Grossman AR. Evolution of the phycobiliproteins. J Mol Biol 1995;248:79-96.

Balasundaram B, Harrison S, Bracewell DG. Advances in product release strategies and impact on bioprocess design. Trends Microbiol 2009;477-85.

Benavides J, Rito-Palomares M. Simplified two-stage method to B-phycoerythrin recovery from *Porphyridium cruentum*. J Chromatogr B Analyt Technol Biomed Life Sci 2006;844:39-44.

Benchekroun M, Romero A, Egea J, Leon R, Michalska P, Buendia I, et al. The antioxidant additive approach for Alzheimer's Disease Therapy: new ferulic (Lipoic) acid plus melatonin modified tacrines as cholinesterases inhibitors, direct antioxidants, and nuclear factor (Erythroid-Derived 2)-Like 2 activators. J Med Chem 2016;59:9967-9973.

Benedetti S, Benvenuti F, Pagliarani S, Francogli S, Scoglio S, Canestrari F. Antioxidant properties of a novel phycocyanin extract from the blue-green alga *Aphanizomenon flos-aquae*. Life Sci 2004;75:2353-2362.

Benedetti S, Rinalducci S, Benvenuti F, Francogli S, Pagliarani S, Giorgi L, et al. Purification and characterization of phycocyanin from the blue-green alga *Aphanizomenon* flos-aquae. J Chromatogr B Analyt Technol Biomed Life Sci 2006;833:12-18.

Bermejo R, Acien FG, Ibanez MJ, Fernandez JM, Molina E, Alvarez-Pez JM. Preparative purification of B-phycoerythrin from the microalga *Porphyridium cruentum* by expanded-bed adsorption chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 2003;790:317-325.

Bermejo RR, Alvarez-Pez JM, Acien FF, Molina GE. Recovery of pure B-phycoerythrin from the microalga *Porphyridium cruentum*. J Biotechnol 2002;93:73-85.

Bermejo-Bescos P, Pinero-Estrada E, Villar DFA. Neuroprotection by *Spirulina platensis* protean extract and phycocyanin against iron-induced toxicity in SH-SY5Y neuroblastoma cells. Toxicol in Vitro 2008;22:1496-1502.

Bharathiraja S, Seo H, Manivasagan P, Santha MM, Park S, Oh J. In vitro photodynamic effect of phycocyanin against breast cancer cells. Molecules 2016;21.

Brejc K, Ficner R, Huber R, Steinbacher S. Isolation, crystallization, crystal structure analysis and refinement of allophycocyanin from the cyanobacterium *Spirulina platensis* at 2.3 Å resolution. J Mol Biol 1995;249:424-440.

Bryant DA, de Lorimier R, Lambert DH, Dubbs JM, Stirewalt VL, Stevens SJ, et al. Molecular cloning and nucleotide sequence of the alpha and beta subunits of allophycocyanin from the cyanelle genome of *Cyanophora paradoxa*. Proc Natl Acad Sci USA 1985;82:3242-3246.

Buonocore G, Perrone S, Bracci R. Free radicals and brain damage in the newborn. Biol

Neonate 2001;79:180-186.
Burgess RR. Protein precipitation techniques. Methods Enzymol 2009:331-342.
Calcott PH, MacLeod RA. The survival of Escherichia coli from freeze-thaw damage : the relative importance of wall and membrane damage. Can J Microbiol 1975:1960-1968.
Carmona-Aparicio L, Zavala-Tecuapetla C, Gonzalez-Trujano ME, Sampieri AI, Montesinos-Correa H, Granados-Rojas L, et al. Status epilepticus: Using antioxidant agents as alternative therapies. Exp Ther Med 2016;12:1957-1962.
Castangia I, Manca ML, Caddeo C, Bacchetta G, Pons R. Santosomes as natural and efficient carriers for the improvement of phycocyanin reepithelising ability in vitro and in vivo. Eur J Pharm Biopharm 2016:149-158.
Chang C, Yang Y, Liang Y, Chiu C, Chu K, Chou H, et al. A Novel Phycobiliprotein Alleviates Allergic Airway Inflammation by Modulating Immune Responses. Am J Resp Crit Care 2011;183:15-25.
Chang YK, Show PL, Lan JC, Tsai JC, Huang CR. Isolation of C-phycocyanin from *Spirulina platensis* microalga using Ionic liquid based aqueous two-phase system. Bioresour Technol 2018;270:320-327.
Chen H, Yang T, Chen M, Chang Y, Wang EIC, Ho C, et al. Purification and immunomodulating activity of C-phycocyanin from *Spirulina platen*sis cultured using power plant flue gas. Process Biochem 2014;49:1337-1344.
Chen T, Wong YS, Zheng W. Purification and characterization of selenium-containing phycocyanin from selenium-enriched *Spirulina platensis*. Phytochemistry 2006;67:2424-2430.
Chen Y, Jiang P, Liu S, Zhao H, Cui Y, Qin S. Purification of 6xHis-tagged phycobiliprotein using zinc-decorated silica-coated magnetic nanoparticles. J Chromatogr B Analyt Technol Biomed Life Sci 2011;879:993-997.
Chethana S, Nayak CA, Madhusudhan MC, Raghavarao KS. Single step aqueous two-phase extraction for downstream processing of C-phycocyanin from *Spirulina platensis*. J Food Sci Technol 2015;52:2415-2421.
Choi WY, Lee HY. Effect of ultrasonic extraction on production and structural changes of C-Phycocyanin from marine *Spirulina maxima*. Int J Mol Sci 2018;19.
Chueh C. Method of allophycocyanin inhibition of enterovirus and influenza virus reproduction resulting in cytopathic effect. US 2002.
Cui J, Chen X, Zhai X, Shi D, Zhang R, Zhi X, et al. Inhalation of water electrolysis-derived hydrogen ameliorates cerebral ischemia-reperfusion injury in rats - A possible new hydrogen resource for clinical use. Neuroscience 2016;335:232-241.
Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology 1998;114:842-845.
Del Pilar Sanchez-Saavedra M, Castro-Ochoa FY, Margarita Nava-Ruiz V, Ruiz-Guereca DA, Laura Villagomez-Aranda A, Siqueiros-Vargas F, et al. Effects of nitrogen source and irradiance on *Porphyridium cruentum*. J Appl Phycol 2018;30:783-792.
Denis C, Masse A, Fleurence J, Jaouen P. Concentration and pre-purification with ultrafiltration of a R-phycoerythrin solution extracted from macro-algae *Grateloupia turuturu*: Process definition and up-scaling. Sep Purif Technol 2009;69:37-42.
Deniz I, Ozen MO, Yesil-Celiktas O. Supercritical fluid extraction of phycocyanin and investigation of cytotoxicity on human lung cancer cells. The Journal of Supercritical Fluids 2016:13-18.
Ding W, Miao D, Hou Y, Jiang S, Zhao B, Zhou M, et al. Small monomeric and highly stable near-infrared fluorescent markers derived from the thermophilic phycobiliprotein, ApcF2. Bba-Mol Cell Res 2017;1864:1877-1886.
Dong L, Li Q, Wu D, Sun Y, Zhou M, Zhao K. A novel periplasmic protein (Slr0280) tunes photomixotrophic growth of the cyanobacterium, *Synechocystis sp* PCC 6803. Gene

2016;575:313-320.
Duerring M, Huber R, Bode W, Ruembeli R, Zuber H. Refined three-dimensional structure of phycoerythrocyanin from the cyanobacterium *Mastigocladus laminosus* at 2.7 Å. J Mol Biol 1990;211:633-644.
Echenique-Subiabre I, Dalle C, Duval C, Heath MW, Coute A, Wood SA, et al. Application of a spectrofluorimetric tool (bbe BenthoTorch) for monitoring potentially toxic benthic cyanobacteria in rivers. Water Res 2016;101:341-350.
Elmorjani K, Thomas JC, Sebban P. Phycobilisomes of wild type and pigment mutants of the cyanobacterium *Synechocystis* PCC 6803. Arch Microbiol 1986;146:186-191.
Eriksen NT. Production of phycocyanin—a pigment with applications in biology, biotechnology, foods and medicine. Appl Microbiol Biot 2008;80:1-14.
Fairchild CD, Zhao JD, Zhou JH, Colson SE, Bryant DA, Glazer AN. Phycocyanin alpha-subunit phycocyanobilin. P Natl Acad Sci Usa 1992;89:7017-7021.
Fan C, Jiang J, Yin X, Wong K, Zheng W, Chen T. Purification of selenium-containing allophycocyanin from selenium-enriched *Spirulina platensis* and its hepatoprotective effect against t-BOOH-induced apoptosis. Food Chem 2012;134:253-261.
Farooq SM, Boppana NB, Devarajan A, Sekaran SD, Shankar EM, Li C, et al. C-phycocyanin confers protection against oxalate-mediated oxidative stress and mitochondrial dysfunctions in MDCK cells. Plos One 2014;9:e93056.
Fernandez-Rojas B, Hernandez-Juarez J, Pedraza-Chaverri J. Nutraceutical properties of phycocyanin. J Funct Foods 2014;11:375-392.
Ficner R, Lobeck K, Schmidt G, Huber R. Isolation, crystallization, crystal structure analysis and refinement of B-phycoerythrin from the red alga *Porphyridium sordidum* at 2.2 Å resolution. J Mol Biol 1992;228:935-950.
Fisher RG, Woods NE, Fuchs HE, Sweet RM. Three-dimensional structures of C-phycocyanin and B-phycoerythrin at 5-Å resolution. J Biol Chem 1980;255:5082-5089.
Galland-Irmouli AV, Pons L, Lucon M, Villaume C, Mrabet NT, Gueant JL, et al. One-step purification of R-phycoerythrin from the red macroalga *Palmaria palmata* using preparative polyacrylamide gel electrophoresis. J Chromatogr B Biomed Sci Appl 2000;739:117-123.
Gambetta GA, Lagarias JC. Genetic engineering of phytochrome biosynthesis in bacteria. Proc Natl Acad Sci USA 2001;98:10566-10571.
Gantar M, Dhandayuthapani S, Rathinavelu A. Phycocyanin induces apoptosis and enhances the effect of topotecan on prostate cell line LNCaP. J Med Food 2012;15:1091-1095.
Gantar M, Simovic D, Djilas S, Gonzalez WW, Miksovska J. Isolation, characterization and antioxidative activity of C-phycocyanin from *Limnothrix sp* strain 37-2-1. J Biotechnol 2012;159:21-26.
Gantar M, Simovic D, Djilas S, Gonzalez WW, Miksovska J. Isolation, characterization and antioxidative activity of C-phycocyanin from *Limnothrix* sp. strain 37-2-1. J Biotechnol 2012;159:21-26.
Gantt E, Conti SF. The ultrastructure of Porphyridium cruentum. J Cell Biol 1965;26:365-381.
Gantt E, Conti SF. Granules associated with the chloroplast lamellae of Porphyridium cruentum. J Cell Biol 1966;29:423-434.
Gantt E, Lipschultz CA. Phycobilisomes of *Porphyridium cruentum*. I. Isolation. J Cell Biol 1972;54:313-324.
Gao Y, Pallister J, Lapierre F, Crameri G, Wang L, Zhu Y. A rapid assay for Hendra virus IgG antibody detection and its titre estimation using magnetic nanoparticles and phycoerythrin. J Virol Methods 2015;222:170-177.
Ge B, Lin X, Chen Y, Wang X, Chen H, Jiang P, et al. Combinational biosynthesis of

dual-functional streptavidin-phycobiliproteins for high-throughput-compatible immunoassay. Process Biochem 2017;58:306-312.

Ge B, Qin S, Han L, Lin F, Ren Y. Antioxidant properties of recombinant allophycocyanin expressed in *Escherichia coli*. J Photoch Photobio B 2006;84:175-180.

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

Ge B, Tang Z, Lin L, Ren Y, Yang Y, Qin S. Pilot-scale fermentation and purification of the recombinant allophycocyanin over-expressed in *Escherichia coli*. Biotechnol Lett 2005;27:783-787.

Ge B, Tang Z, Zhao F, Ren Y, Yang Y. Scale-up of fermentation and purification of recombinant allophycocyanin over-expressed in *Escherichia coli*. Process Biochem 2005;40:3190-3196.

Glauser M, Bryant DA, Frank G, Wehrli E, Rusconi SS, Sidler W, et al. Phycobilisome structure in the cyanobacteria *Mastigocladus laminosus* and *Anabaena* sp. PCC 7120. Eur J Biochem 1992;205:907-915.

Glazer AN, Stryer L. Phycofluor Probes. Trends Biochem SCI 1984;9:423-427.

Gonzalez R, Rodriguez S, Romay C, Ancheta O, Gonzalez A, Armesto J, et al. Anti-inflammatory activity of phycocyanin extract in acetic acid-induced colitis in rats. Pharmacol Res 1999;39:55-59.

Gorham T, Jia Y, Shum CK, Lee J. Ten-year survey of cyanobacterial blooms in Ohio's waterbodies using satellite remote sensing. Harmful Algae 2017:13-19.

Gu D, Lazo-Portugal R, Fang C, Wang Z, Ma Y, Knight M, et al. Purification of R-phycoerythrin from *Gracilaria lemaneiformis* by centrifugal precipitation chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 2018;1087-1088:138-141.

Guan X, Qin S, Su Z, Zhao F, Ge B, Li F, et al. Combinational biosynthesis of a fluorescent cyanobacterial holo-alpha-phycocyanin in Escherichia coli by using one expression vector. Appl Biochem Biotechnol 2007;142:52-59.

Guan X, Wang J, Zhu J, Yao C, Liu J, Qin S, et al. Photosystem II photochemistry and phycobiliprotein of the red algae Kappaphycus alvarezii and their implications for light adaptation. Biomed Res Int 2013;2013:256549.

Guan XY, Zhang WJ, Zhang XW, Li YX, Wang JF, Lin HZ, et al. A potent anti-oxidant property: fluorescent recombinant alpha-phycocyanin of *Spirulina*. J App Microbiol 2009;106:1093-1100.

Guglielmi G, Cohen-Bazire G, Bryant DA. The structure of *Gloeobacter violaceus* and its phycobilisomes. Arch Microbiol 1981;129:181-189.

Gupta NK, Gupta KP. Effects of C-Phycocyanin on the representative genes of tumor development in mouse skin exposed to 12-O-tetradecanoyl-phorbol-13-acetate. Environ Toxicol Pharmacol 2012;34:941-948.

Han X, Lv L, Yu D, Wu X, Li C. Coordination induced supramolecular assembly of fluorescent C-Phycocyanin for biologic discrimination of metal ions. Mater Lett 2018;215:238-241.

Hirata T, Tanaka M, Ooike M, Tsunomura T, Sakaguchi M. Antioxidant activities of phycocyanobilin prepared from *Spirulina platensis*. J Appl Phycol 2000.

Ho S, Liao J, Chen C, Chang J. Combining light strategies with recycled medium to enhance the economic feasibility of phycocyanin production with *Spirulina platensis*. Bioresource Technol 2018;247:669-675.

Huang B, Wang GC, Zeng CK, Li ZG. The experimental research of R-phycoerythrin subunits on cancer treatment: a new photosensitizer in PDT. Cancer Biother Radio 2002;17:35-42.

Huang Z, Guo BJ, Wong RNS, Jiang Y. Characterization and antioxidant activity of selenium-containing phycocyanin isolated from *Spirulina platensis*. Food Chem 2007;100:1137-1143.

Hussein MMA, Ali HA, Ahmed MM. Ameliorative effects of phycocyanin against gibberellic acid induced hepatotoxicity. Pestic Biochem Phys 2015;119:28-32.

Jiang T, Zhang J, Liang D. Structure and function of chromophores in R-Phycoerythrin at 1.9 Å resolution. Proteins 1999;34:224-231.

Jiang T, Zhang JP, Chang WR, Liang DC. Crystal structure of R-phycocyanin and possible energy transfer pathways in the phycobilisome. Biophys J 2001;81:1171-1179.

Kahn K, Mazel D, Houmard J, Tandeau DMN, Schaefer MR. A role for cpeYZ in cyanobacterial phycoerythrin biosynthesis. J Bacteriol 1997;179:998-1006.

Kathiravan A, Renganathan R. Photosensitization of colloidal TiO_2 nanoparticles with phycocyanin pigment. J Colloid Interf Sci 2009;335:196-202.

Kaur J, Kukreja S, Kaur A, Malhotra N, Kaur R. The oxidative stress in cataract patients. J Clin Diagn Res 2012;6:1629-1632.

Khatoon H, Leong LK, Rahman NA, Mian S, Begum H, Banerjee S, et al. Effects of different light source and media on growth and production of phycobiliprotein from freshwater cyanobacteria. Bioresource Technol 2018;249:652-658.

Kissoudi M, Sarakatsianos I, Samanidou V. Isolation and purification of food-grade C-phycocyanin from *Arthrospira platensis* and its determination in confectionery by HPLC with diode array detection. J Sep Sci 2018;41:975-981.

Kohchi T, Mukougawa K, Frankenberg N, Masuda M, Yokota A, Lagarias JC. The Arabidopsis HY2 gene encodes phytochromobilin synthase, a ferredoxin-dependent biliverdin reductase. Plant Cell 2001;13:425-436.

Kothadia AD, Shabaraya SAM. Evaluation of cataract preventive action of phycocyanin. J Pharm Sci-Us 2011.

Kuddus M, Singh P, Thomas G, Al-Hazimi A. Recent developments in production and biotechnological applications of C-phycocyanin. Biomed Res Int 2013;2013:742859.

Kumar D, Dhar DW, Pabbi S, Kumar N, Walia S. Extraction and purification of C-phycocyanin from *Spirulina platensis* (CCC540). Indian J Plant Physiol 2014;19:184-188.

Kumari RP, Sivakumar J, Thankappan B, Anbarasu K. C-phycocyanin modulates selenite-induced cataractogenesis in rats. Biol Trace Elem Res 2013;151:59-67.

Kupka M, Scheer H. Unfolding of C-phycocyanin followed by loss of non-covalent chromophore-protein interactions - 1. Equilibrium experiments. Bba-Bioenergetics 2008;1777:94-103.

Kursar TA, van der Meer J, Alberte RS. Light-Harvesting System of the Red Alga Gracilaria tikvahiae: II. Phycobilisome Characteristics of Pigment Mutants. Plant Physiol 1983;73:361-369.

Le Guillard C, Dumay J, Donnay-Moreno C, Bruzac S, Ragon J, Fleurence J, et al. Ultrasound-assisted extraction of R-phycoerythrin from *Grateloupia turuturu* with and without enzyme addition. Algal Res 2015;12:522-528.

Leney AC, Tschanz A, Heck A. Connecting color with assembly in the fluorescent B-phycoerythrin protein complex. Febs J 2018;285:178-187.

Levi M, Sendersky E, Schwarz R. Decomposition of cyanobacterial light harvesting complexes: NblA-dependent role of the bilin lyase homolog NblB. Plant J 2018;94:813-821.

Li B, Gao M, Chu X, Teng L, Lv C, Yang P, et al. The synergistic antitumor effects of all-trans retinoic acid and C-phycocyanin on the lung cancer A549 cells in vitro and in vivo. Eur J Pharmacol 2015;749:107-114.

Li B, Zhang Z, Qi J, Zhou N, Qin S, Choo J, et al. Quantum dot-based molecularly Imprinted polymers on three-dimensional origami paper microfluidic chip for fluorescence detection of phycocyanin. ACS Sens 2017;2:243-250.
Li C, Yu Y, Li W, Liu B, Jiao X, Song X, et al. Phycocyanin attenuates pulmonary fibrosis via the TLR2-MyD88-NF-kappaB signaling pathway. Sci Rep 2017;7:5843.
Li L, Li L, Shi K, Li Z, Song K. A semi-analytical algorithm for remote estimation of phycocyanin in inland waters. SCI Total Environ 2012;435:141-150.
Li L, Li L, Song K. Remote sensing of freshwater cyanobacteria: An extended IOP inversion model of inland waters (IIMIW) for partitioning absorption coefficient and estimating phycocyanin. Rrmote Sens Environ 2015;157:9-23.
Li R, Zhang H, Zheng X. MiR-34c induces apoptosis and inhibits the viability of M4e cells by targeting BCL2. Oncol Lett 2018;15:3357-3361.
Li W, Pu Y, Gao N, Tang Z, Song L. Efficient purification protocol for bioengineering allophycocyanin trimer with N-terminus histag. Saudi J Biol Sci 2017;24:451-458.
Lissi EA, Pizarro M, Aspee A, Romay C. Kinetics of phycocyanine bilin groups destruction by peroxyl radicals. Free Radic Biol Med 2000;28:1051-1055.
Liu LN. Distribution and dynamics of electron transport complexes in cyanobacterial thylakoid membranes. Biochim Biophys Acta 2016;1857:256-265.
Liu LN, Chen XL, Zhang XY, Zhang YZ, Zhou BC. One-step chromatography method for efficient separation and purification of R-phycoerythrin from *Polysiphonia urceolata*. J Biotechnol 2005;116:91-100.
Liu Q, Wang Y, Cao M, Pan T, Yang Y, Mao H, et al. Anti-allergic activity of R-phycocyanin from *Porphyra haitan*ensis in antigen-sensitized mice and mast cells. Int Immunopharmacol 2015;25:465-473.
Liu S, Chen Y, Lu Y, Chen H, Li F, Qin S. Biosynthesis of fluorescent cyanobacterial allophycocyanin trimer in *Escherichia coli*. Photosynth Res 2010;105:135-142.
Lyu H, Wang Q, Wu C, Zhu L, Yin B. Retrieval of phycocyanin concentration from remote-sensing reflectance using a semi-analytic model in eutrophic lakes. Ecol Inform 2013:178-187.
MacColl R. Cyanobacterial phycobilisomes. J Struct Biol 1998;124:311-334.
Macedo D, Bertolin TE, Oro T, Backes LTH, Brás IC. Phycocyanin protects against Alpha-Synuclein toxicity in yeast. J Funct Foods 2017:553-560.
Madhyastha HK, Sivashankari S, Vatsala TM. C-phycocyanin from *Spirulina* fussiformis exposed to blue light demonstrates higher efficacy of in vitro antioxidant activity. Biochem Eng J 2009;43:221-224.
Manirafasha E, Ndikubwimana T, Zeng X, Lu Y, Jing K. Phycobiliprotein: Potential microalgae derived pharmaceutical and biological reagent. Biochem Eng J 2016;109:282-296.
Michotte LJ, Van BP, Wauters M. Remote detection of cyanobacteria through phycocyanin for water supply source using three-band model. Ecol Inform 2013:22-33.
Miller CA, Leonard HS, Pinsky IG, Turner BM, Williams SR, Harrison LJ, et al. Biogenesis of phycobiliproteins. III. CpcM is the asparagine methyltransferase for phycobiliprotein beta-subunits in cyanobacteria. J Biol Chem 2008;283:19293-19300.
Min SK, Park JS, Luo L, Kwon YS, Lee HC, Shim HJ, et al. Assessment of C-phycocyanin effect on astrocytes-mediated neuroprotection against oxidative brain injury using 2D and 3D astrocyte tissue model. Sci Rep 2015;5:14418.
Minic SL, Stanic-Vucinic D, Mihailovic J, Krstic M, Nikolic MR, Velickovic TC. Digestion by pepsin releases biologically active chromopeptides from C-phycocyanin, a blue-colored biliprotein of microalga *Spirulina*. J Proteomics 2016;147:132-139.
Minkova K, Tchorbadjieva M, Tchernov A, Stojanova M, Gigova L, Busheva M. Improved

procedure for separation and purification of *Arthronema africanum* phycobiliproteins. Biotechnol Lett 2007;29:647-651.

Minkova KM, Tchernov AA, Tchorbadjieva MI, Fournadjieva ST, Antova RE, Busheva M. Purification of C-phycocyanin from *Spirulina (Arthrospira) fusiformis*. J Biotechnol 2003;102:55-59.

Miric DJ, Kisic BM, Zoric LD, Miric BM, Mirkovic M, Mitic R. Influence of cataract maturity on aqueous humor lipid peroxidation markers and antioxidant enzymes. Eye 2014;28:72-77.

Mishra SK, Shrivastav A, Mishra S. Preparation of highly purified C-phycoerythrin from marine cyanobacterium *Pseudanabaena* sp. Protein Expr Purif 2011;80:234-238.

Mitra S, Siddiqui WA, Khandelwal S. C-Phycocyanin protects against acute tributyltin chloride neurotoxicity by modulating glial cell activity along with its anti-oxidant and anti-inflammatory property: A comparative efficacy evaluation with N-acetyl cysteine in adult rat brain. Chem-Biol Interact 2015;238:138-150.

Mittal R, Tavanandi HA, Mantri VA, Raghavarao KSMS. Ultrasound assisted methods for enhanced extraction of phycobiliproteins from marine macro-algae, *Gelidium pusillum* (Rhodophyta). Ultrason Sonochem 2017;38:92-103.

Montgomery BL. Seeing new light: recent insights into the occurrence and regulation of chromatic acclimation in cyanobacteria. Curr Opin Plant Biol 2017;37:18-23.

Moraes CC, Kalil SJ. Strategy for a protein purification design using C-phycocyanin extract. BIioresource Technol 2009;100:5312-5317.

Morcos NC, Berns M, Henry WL. Phycocyanin: laser activation, cytotoxic effects, and uptake in human atherosclerotic plaque. Lasers Surg Med 1988;8:10-17.

Munier M, Morancais M, Dumay J, Jaouen P, Fleurence J. One-step purification of R-phycoerythrin from the red edible seaweed *Grateloupia turuturu*. J Chromatogr B Analyt Technol Biomed Life Sci 2015;992:23-29.

Nagaraj S, Arulmurugan P, Rajaram MG, Karuppasamy K, Jayappriyan KR, Sundararaj R, et al. Hepatoprotective and antioxidative effects of C-phycocyanin from *Arthrospira maxima* SAG 25780 in CCl4-induced hepatic damage rats. Biomedicine & Preventive Nutrition 2012;2:81-85.

Nakagawa K, Ritcharoen W, Sri-Uam P, Pavasant P, Adachi S. Antioxidant properties of convective-air-dried *Spirulina max*ima: Evaluation of phycocyanin retention by a simple mathematical model of air-drying. Food Bioprod Process 2016;100:292-302.

Nies M, Wehrmeyer W. Isolation and biliprotein characterization of phycobilisomes from the thermophilic cyanobacterium Mastigocladus laminosus Cohn. Planta 1980;150:330-337.

Niu J, Wang G, Tseng C. Method for large-scale isolation and purification of R-phycoerythrin from red alga *Polysiphonia urceolata Grev*. Protein Expres Puri 2006;49:23-31.

Niu JF, Wang GC, Lin XZ, Zhou BC. Large-scale recovery of C-phycocyanin from *Spirulina platensis* using expanded bed adsorption chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 2007;850:267-276.

Niu JF, Wang GC, Tseng CK. Method for large-scale isolation and purification of R-phycoerythrin from red alga *Polysiphonia urceolata Grev*. Protein Expr Purif 2006;49:23-31.

Niu JF, Wang GC, Zhou BC, Lin XZ, Chen CS. Purification of R-phycoerythrin from *Porphyra haitanensis* (Bangiales, Rhodophyta) using expanded-bed absorption1. J Phycol 2007;6:1339-1347.

Oi VT, Glazer AN, Stryer L. Fluorescent phycobiliprotein conjugates for analyses of cells and molecules. J Cell Biol 1982;93:981-986.

Onishi A, Aikawa S, Kondo A, Akimoto S. Energy transfer in Anabaena variabilis filaments under nitrogen depletion, studied by time-resolved fluorescence. Photosynth Res

2015;125:191-199.
Ou Y, Ren Z, Wang J, Yang X. Phycocyanin ameliorates alloxan-induced diabetes mellitus in mice: Involved in insulin signaling pathway and GK expression. Chem-Biol Interact 2016;247:49-54.
Ou Y, Zheng S, Lin L, Jiang Q, Yang X. Protective effect of C-phycocyanin against carbon tetrachloride-induced hepatocyte damage in vitro and in vivo. Chem Biol Interact 2010;185:94-100.
Padyana AK, Bhat VB, Madyastha KM, Rajashankar KR, Ramakumar S. Crystal structure of a light-harvesting protein C-phycocyanin from *Spirulina platensis*. Biochem Biophys Res Commun 2001;282:893-898.
Pak W, Takayama F, Mine M, Nakamoto K, Kodo Y, Mankura M, et al. Anti-oxidative and anti-inflammatory effects of spirulina on rat model of non-alcoholic steatohepatitis. J Clin Biochem Nutr 2012;51:227-234.
Pan R, Lu R, Zhang Y, Zhu M, Zhu W, Yang R, et al. *Spirulina* phycocyanin induces differential protein expression and apoptosis in SKOV-3 cells. Int J Biol Macromol 2015;81:951-959.
Park Y, Pyo J, Kwon YS, Cha Y, Lee H, Kang T, et al. Evaluating physico-chemical influences on cyanobacterial blooms using hyperspectral images in inland water, Korea. Water Res 2017;126:319-328.
Parmar A, Singh NK, Kaushal A, Sonawala S, Madamwar D. Purification, characterization and comparison of phycoerythrins from three different marine cyanobacterial cultures. Bioresour Technol 2011;102:1795-1802.
Patel A, Mishra S, Pawar R, Ghosh PK. Purification and characterization of C-Phycocyanin from cyanobacterial species of marine and freshwater habitat. Protein Expr Purif 2005;40:248-255.
Patil G, Chethana S, Sridevi AS, Raghavarao KS. Method to obtain C-phycocyanin of high purity. J Chromatogr a 2006;1127:76-81.
Pattarayan D, Rajarajan D, Ayyanar S, Palanichamy R, Subbiah R. C-phycocyanin suppresses transforming growth factor-β1-induced epithelial mesenchymal transition in human epithelial cells. Pharmacological Rep 2017;69:426-431.
Peng PP, Dong LL, Sun YF, Zeng XL, Ding WL, Scheer H, et al. The structure of allophycocyanin B from *Synechocystis* PCC 6803 reveals the structural basis for the extreme redshift of the terminal emitter in phycobilisomes. Acta Crystallogr D Biol Crystallogr 2014;70:2558-2569.
Pinto Rodrigues RD, de Castro FC, de Santiago-Aguiar RS, Ponte Rocha MV. Ultrasound-assisted extraction of phycobiliproteins from *Spirulina (Arthrospira) plat*ensis using protic ionic liquids as solvent. Algal Res 2018;31:454-462.
Pleonsil P, Soogarun S, Suwanwong Y. Anti-oxidant activity of holo- and apo-c-phycocyanin and their protective effects on human erythrocytes. Int J Biol Macromol 2013;60:393-398.
Pu Y, Zhu G, Ge B, Yu D, Wang Y, Qin S. Photocurrent generation by recombinant allophycocyanin trimer multilayer on TiO_2 electrode. Chinese Chem Lett 2013;24:163-166.
Qin S, Tang Z, Lin F, Sung LA, Tseng CK. Genomic cloning, expression and recombinant protein purification of α and β subunits of the allophycocyanin gene (apc) from the cyanobacterium *Anacystis nid*ulans UTEX 625. J Appl Phycol 2004;16:483-487.
Qin S, Zhao FQ, Tseng CK. Evidence for positive selection in phycoerythrin genes of red algae and cyanobacteria *Prochloroc*occus and *Synechococcus*. Photosynthetica 2005.
Ramos A, Acien FG, Fernandez-Sevilla JM, Gonzalez CV, Bermejo R. Development of a process for large-scale purification of C-phycocyanin from *Synechocystis aquatilis* using expanded bed adsorption chromatography. J Chromatogr B Analyt Technol Biomed Life

Sci 2011;879:511-519.
Rapsomanikis A, Sygkridou D, Voutsinas E, Stathatos E. Transparent quasi-solid state dye-sensitized solar cells sensitized with naturally derived pigment extracted from red seaweed. Curr Appl Phys 2016;16:651-657.
Ren Y, Ge B, Jin H, Tang Z, Guo C. Effect of Lanthanum on expression of recombinant allophycocyanin gene in *Pichia Pastoris*. J Rare Earth 2005.
Rimbau V, Camins A, Romay C, Gonzalez R, Pallas M. Protective effects of C-phycocyanin against kainic acid-induced neuronal damage in rat hippocampus. Neurosci Lett 1999;276:75-78.
Riss J, Decorde K, Sutra T, Delage M, Baccou JC, Jouy N, et al. Phycobiliprotein C-phycocyanin from *Spirulina platensis* is powerfully responsible for reducing oxidative stress and NADPH oxidase expression induced by an atherogenic diet in hamsters. J Agric Food Chem 2007;55:7962-7967.
Rito Palomares M, Nuñez L, Amador D. Practical application of aqueous two- phase systems for the development of a prototype process for c- phycocyanin recovery from *Spirulina maxima*. Journal of Chemical Technology & Biotechnology 2001;76:1273-1280.
Romay C, Armesto J, Remirez D, Gonzalez R, Ledon N, Garcia I. Antioxidant and anti-inflammatory properties of C-phycocyanin from blue-green algae. Inflamm Res 1998;47:36-41.
Romay C, Gonzalez R. Phycocyanin is an antioxidant protector of human erythrocytes against lysis by peroxyl radicals. J Pharm Pharmacol 2000;52:367-368.
Romay C, Gonzalez R, Ledon N, Remirez D, Rimbau V. C-phycocyanin: A biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. Curr Protein Pept Sc 2003;4:207-216.
Romay C, Ledon N, Gonzalez R. Further studies on anti-inflammatory activity of phycocyanin in some animal models of inflammation. Inflamm Res 1998;47:334-338.
Rumbeli R, Schirmer T, Bode W, Sidler W, Zuber H. Crystallization of phycoerythrocyanin from the cyanobacterium *Mastigocladus laminosus* and preliminary characterization of two crystal forms. J Mol Biol 1985;186:197-200.
Ruobing W, Zhenghong S, Xuecheng Z, Shuang Z, Song Q. Expression of the phycoerythrin gene of *Gracilaria lemaneiformis (Rhodophyta)* in *E. coli* and evaluation of the bioactivity of recombinant PE. J Ocean U China 2007;6:373-377.
Saini MK, Sanyal SN. Piroxicam and c-phycocyanin prevent colon carcinogenesis by inhibition of membrane fluidity and canonical Wnt/β-catenin signaling while up-regulating ligand dependent transcription factor PPARγ. Biomed Pharmacother 2014;68:537-550.
Saini MK, Vaiphei K, Sanyal SN. Chemoprevention of DMH-induced rat colon carcinoma initiation by combination administration of piroxicam and C-phycocyanin. Mol Cell Biochem 2012;361:217-228.
Santiago-Santos MC, Ponce-Noyola T, Olvera-Ramirez R, Ortega-Lopez J, Canizares-Villanueva RO. Extraction and purification of phycocyanin from *Calothrix sp*. Process Biochem 2004;39:2047-2052.
Saunee NA, Williams SR, Bryant DA, Schluchter WM. Biogenesis of phycobiliproteins: II. CpcS-I and CpcU comprise the heterodimeric bilin lyase that attaches phycocyanobilin to CYS-82 OF beta-phycocyanin and CYS-81 of allophycocyanin subunits in *Synechococcus sp*. PCC 7002. J Biol Chem 2008;283:7513-7522.
Schrantz K, Wyss PP, Ihssen J, Toth R, Bora DK. Hematite photoanode co-functionalized with self-assembling melanin and C-phycocyanin for solar water splitting at neutral pH. Catal Today 2017;284:44-51.
Sekar S, Chandramohan M. Phycobiliproteins as a commodity: trends in applied research, patents and commercialization. J Appl Phycol 2007:113-136.

Senthilkumar N, Kurinjimalar C, Thangam R, Suresh V, Kavitha G, Gunasekaran P, et al. Further studies and biological activities of macromolecular protein R-Phycoerythrin from *Portieria homemannii*. Int J Biol Macromol 2013;62:107-116.

Senthilkumar N, Suresh V, Thangam R, Kurinjimalar C, Kavitha G, Murugan P, et al. Isolation and characterization of macromolecular protein R-Phycoerythrin from *Portieria hornemannii*. Int J Biol Macromol 2013;55:150-160.

Setyaningsih I, Bintang M, Madina N. Potentially antihyperglycemic from biomass and phycocyanin of *Spirulina fusiformis Voronikhin* by in Vivo Test; in Setyobudi RH, Scheer H, Limantara L, Shioi Y, Fiedor L, Brotosudarmo T, Prihastyanti M, (eds): Procedia Chemistry. Procedia Chemistry, 2015, vol 14, pp. 211-215.

Shcherbo D, Merzlyak EM, Chepurnykh TV, Fradkov AF, Ermakova GV, Solovieva EA, et al. Bright far-red fluorescent protein for whole-body imaging. Nat Methods 2007;4:741-746.

Shen G, Saunee NA, Williams SR, Gallo EF, Schluchter WM, Bryant DA. Identification and characterization of a new class of bilin lyase: the cpcT gene encodes a bilin lyase responsible for attachment of phycocyanobilin to Cys-153 on the beta-subunit of phycocyanin in *Synechococcus sp*. PCC 7002. J Biol Chem 2006;281:17768-17778.

Shen G, Schluchter WM, Bryant DA. Biogenesis of phycobiliproteins: I. cpcS-I and cpcU mutants of the cyanobacterium *Synechococcus sp*. PCC 7002 define a heterodimeric phyococyanobilin lyase specific for beta-phycocyanin and allophycocyanin subunits. J Biol Chem 2008;283:7503-7512.

Sheu M, Hsieh Y, Lai C, Chang C, Wu C. Antihyperlipidemic and antioxidant effects of C-phycocyanin in Golden Syrian Hamsters Fed with a hypercholesterolemic diet. Journal of traditional and complementary medicine 2013;3:41-47.

Shih SR, Tsai KN, Li YS, Chueh CC, Chan EC. Inhibition of enterovirus 71-induced apoptosis by allophycocyanin isolated from a blue-green alga *Spirulina platensis*. J Med Virol 2003;70:119-125.

Sidler. Phycobilisome and phycobiliprotein structures, Springer Netherlands, 1994.

Siiman O, Wilkinson J, Burshteyn A, Roth P, Ledis S. Fluorescent neoglycoproteins: antibody-aminodextran-phycobiliprotein conjugates. Bioconjug Chem 1999;10:1090-1106.

Silveira ST, Quines LK, Burkert CA, Kalil SJ. Separation of phycocyanin from *Spirulina platensis* using ion exchange chromatography. Bioprocess Biosyst Eng 2008;31:477-482.

Sonani RR, Patel S, Bhastana B, Jakharia K, Chaubey MG, Singh NK, et al. Purification and antioxidant activity of phycocyanin from *Synechococcus sp* R42DM isolated from industrially polluted site. Bioresource Technol 2017;245:325-331.

Sonani RR, Singh NK, Kumar J, Thakar D, Madamwar D. Concurrent purification and antioxidant activity of phycobiliproteins from *Lyngbya sp* A09DM: An antioxidant and anti-aging potential of phycoerythrin in Caenorhabditis elegans. Process Biochem 2014;49:1757-1766.

Soni B, Kalavadia B, Trivedi U, Madamwar D. Extraction, purification and characterization of phycocyanin from *Oscillatoria quadripunctulata* - Isolated from the rocky shores of Bet-Dwarka, Gujarat, India. Process Biochem 2006;41:2017-2023.

Soni B, Trivedi U, Madamwar D. A novel method of single step hydrophobic interaction chromatography for the purification of phycocyanin from *Phormidium fragile* and its characterization for antioxidant property. Bioresour Technol 2008;99:188-194.

Sorensen L, Hantke A, Eriksen NT. Purification of the photosynthetic pigment C-phycocyanin from heterotrophic *Galdieria sulphuraria*. J Sci Food Agric 2013;93:2933-2938.

Spillert CR, Pelosi MJ, Parmer LP, Lazaro EJ. A peroxide-induced inflammation model for drug testing. Agents Actions 1987;21:297-298.

Storf M, Parbel A, Meyer M, Strohmann B, Scheer H, Deng MG, et al. Chromophore attachment to biliproteins: specificity of PecE/PecF, a lyase-isomerase for the photoactive 3(1)-cys-alpha 84-phycoviolobilin chromophore of phycoerythrocyanin. Biochemistry-Us 2001;40:12444-12456.

Strasky Z, Zemankova L, Nemeckova I, Rathouska J, Wong RJ, Muchova L, et al. *Spirulina platensis* and phycocyanobilin activate atheroprotective heme oxygenase-1: a possible implication for atherogenesis. Food Funct 2013;4:1586-1594.

Stumpf RP, Davis TW, Wynne TT, Graham JL, Loftin KA, Johengen TH, et al. Challenges for mapping cyanotoxin patterns from remote sensing of cyanobacteria. Harmful Algae 2016;54:160-173.

Su H, Xie B, Chen X, Wang J, Zhang X, Zhou B, et al. Efficient separation and purification of allophycocyanin from *Spirulina (Arthrospira) platensis*. J Appl Phycol 2010;22:65-70.

Sun Y, Zhang J, Yan Y, Chi M, Chen W, Sun P, et al. The protective effect of C-phycocyanin on paraquat-induced acute lung injury in rats. Environ Toxicol Pharmacol 2011;32:168-174.

Sun YX, Zhang J, Yu GC, Yan YJ, Chen WW, Chi MF, et al. Experimental study on the therapeutic effect of C-phycocyanin against pulmonary fibrosis induced by paraquat in rats. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi 2012;30:650-655.

Suping Z, Jingxi P, Zhenhui H, Jingquan Z, Side Y, Lijin J. Generation and identification of the transient intermediates of allophycocyanin by laser photolytic and pulse radiolytic techniques. Int J Radiat Biol 2001;77:637-642.

Tandeau DMN. Phycobiliproteins and phycobilisomes: the early observations. Photosynth Res 2003;76:193-205.

Tang Y, Xie M, Jiang N, Huang F, Zhang X, Li R, et al. Icarisid II inhibits the proliferation of human osteosarcoma cells by inducing apoptosis and cell cycle arrest. Tumour Biol 2017;39:1393383919.

Tang Z, Jilu Z, Ju B, Li W, Wen S, Pu Y, et al. One-step chromatographic procedure for purification of B-phycoerythrin from *Porphyridium cruentum*. Protein Expr Purif 2016;123:70-74.

Thangam R, Suresh V, Asenath PW, Rajkumar M, Senthilkumar N, Gunasekaran P, et al. C-Phycocyanin from *Oscillatoria tenuis* exhibited an antioxidant and in vitro antiproliferative activity through induction of apoptosis and G0/G1 cell cycle arrest. Food Chem 2013;140:262-272.

Thangam R, Suresh V, Princy WA, Rajkumar M, SenthilKumar N, Gunasekaran P, et al. C-Phycocyanin from *Oscillatoria tenuis* exhibited an antioxidant and in vitro antiproliferative activity through induction of apoptosis and G(0)/G(1) cell cycle arrest. Food Chem 2013;140:262-272.

Tooley AJ, Cai YA, Glazer AN. Biosynthesis of a fluorescent cyanobacterial C-phycocyanin holo-alpha subunit in a heterologous host. Proc Natl Acad Sci USA 2001;98:10560-10565.

Varunan T, Shanmugam P. An optical tool for quantitative assessment of phycocyanin pigment concentration in cyanobacterial blooms within inland and marine environments. J Great Lakes Res 2017;43:32-49.

Villegas L, Stidham T, Nozik-Grayck E. Oxidative stress and therapeutic development in lung diseases. J Pulm Respir Med 2014;4.

Vogiatzi G, Tousoulis D, Stefanadis C. The role of oxidative stress in atherosclerosis. Hellenic J Cardiol 2009;50:402-409.

Wang CY, Wang X, Wang Y, Zhou T, Bai Y, Li YC, et al. Photosensitization of phycocyanin extracted from *Microcystis* in human hepatocellular carcinoma cells: implication of mitochondria-dependent apoptosis. J Photochem Photobiol B 2012;117:70-79.

Wang L, Qu Y, Fu X, Zhao M, Wang S, Sun L. Isolation, purification and properties of an

R-phycocyanin from the phycobilisomes of a marine red macroalga Polysiphonia urceolata. Plos One 2014;9:e101724.

Wang X, Dong L, Zhang C, Zhu K, Zhao J, Zhao K, et al. Sll1466, a glycosyl transferase homolog involved in global cellular regulation and high-light tolerance of *Synechocystis* PCC 6803. Biochem Bioph Res Co 2011;408:674-679.

Wang X, Yu J, Kang Q, Shen D, Li J. Molecular imprinting ratiometric fluorescence sensor for highly selective and sensitive detection of phycocyanin. Biosens Bioelectron 2016;77:624-630.

Wang X, Yu J, Li J, Kang Q, Shen D, Chen L. Quantum dots based imprinting fluorescent nanosensor for the selective and sensitive detection of phycocyanin: A general imprinting strategy toward proteins. Sensors and Actuators B: Chemical 2018;255:268-274.

Wang XQ, Li LN, Chang WR, Zhang JP, Gui LL, Guo BJ, et al. Structure of C-phycocyanin from *Spirulina platensis* at 2.2 Å resolution: a novel monoclinic crystal form for phycobiliproteins in phycobilisomes. Acta Crystallogr D Biol Crystallogr 2001;57:784-792.

Wu J, Chen H, Jiang P. Chromophore attachment to fusion protein of streptavidin and recombinant allophycocyanin alpha subunit. Bioengineered 2018;9:108-115.

Wu J, Chen H, Zhao J, Jiang P. Fusion proteins of streptavidin and allophycocyanin alpha subunit for immunofluorescence assay. Biochem Eng J 2017;125:97-103.

Wu Q, Liu L, Miron A, Klimova B, Wan D, Kuca K. The antioxidant, immunomodulatory, and anti-inflammatory activities of *Spirulina*: an overview. Arch Toxicol 2016;90:1817-1840.

Xia D, Liu B, Luan X, Sun J, Liu N, Qin S, et al. Protective effects of C-phycocyanin on alcohol-induced acute liver injury in mice. Chin J Oceanol Limnol 2016.

Yan S, Zhu L, Su H, Zhang X, Chen X, Zhou B, et al. Single-step chromatography for simultaneous purification of C-phycocyanin and allophycocyanin with high purity and recovery from *Spirulina (Arthrospira) platensis*. J Appl Phycol 2011;23:1-6.

Yang Y, Ge B, Guan X, Zhang W, Qin S. Combinational biosynthesis of a fluorescent cyanobacterial holo-alpha-allophycocyanin in *Escherichia coli*. Biotechnol Lett 2008;30:1001-1004.

Yin L, Xu L, Yu K, Zhen Y, Han X, Xu Y, et al. Orthogonal test design for optimization of suitable conditions to separate C-phycocyanin from *Spirulina platensis* by high-speed counter-current chromatography using reverse micelle solvent system. J Sep Sci 2011;34:1253-1260.

Ying J, Wang J, Ji H, Lin C, Pan R, Zhou L, et al. Transcriptome analysis of phycocyanin inhibitory effects on SKOV-3 cell proliferation. Gene 2016;585:58-64.

Young I, Chuang S, Hsu C, Sun Y, Lin F. C-phycocyanin alleviates osteoarthritic injury in chondrocytes stimulated with H_2O_2 and compressive stress. Int J Biol Macromol 2016;93:852-859.

Zhang J, Ma J, Liu D, Qin S, Sun S, Zhao J, et al. Structure of phycobilisome from the red alga *Griffithsia pacifica*. Nature 2017.

Zhang W, Guan X, Yang Y, Ge B, Chen H, Li F, et al. Biosynthesis of fluorescent allophycocyanin alpha-subunits by autocatalysis in *Escherichia coli*. Biotechnol Appl Biochem 2009;52:135-140.

Zhang X, Zhao F, Qin S, Yan B. Cloning, expression and characterization of phycoerythrin gene from *Ceramium boydenn*. DNA Seq 2006;17:129-135.

Zhang YM, Chen F. A simple method for efficient separation and purification of c-phycocyanin and allophycocyanin from *Spirulina platensis*. Biotechnology Techniques 1999;13:601-603.

Zhang Z, Lambrev PH, Wells KL, Garab G, Tan HS. Direct observation of multistep energy

transfer in LHCII with fifth-order 3D electronic spectroscopy. Nat Commun 2015;6:7914.
Zhang Z, Li J, Fu L, Liu D, Chen L. Magnetic molecularly imprinted microsensor for selective recognition and transport of fluorescent phycocyanin in seawater. J Mater Chem a 2015.
Zhang Z, Li J, Wang X, Shen D, Chen L. Quantum dots based mesoporous structured imprinting microspheres for the sensitive fluorescent detection of phycocyanin. ACS Appl Mater Interfaces 2015;7:9118-9127.
Zhao F, Qin S. Evolutionary analysis of phycobiliproteins: implications for their structural and functional relationships. J Mol Evol 2006;63:330-340.
Zhao F, Qin S. Comparative molecular population genetics of phycoerythrin locus in *Prochlorococcus*. Genetica 2007;129:291-299.
Zhao KH, Su P, Bohm S, Song B, Zhou M, Bubenzer C, et al. Reconstitution of phycobilisome core-membrane linker, LCM, by autocatalytic chromophore binding to ApcE. Biochim Biophys Acta 2005;1706:81-87.
Zhao KH, Su P, Tu JM, Wang X, Liu H, Ploscher M, et al. Phycobilin:cystein-84 biliprotein lyase, a near-universal lyase for cysteine-84-binding sites in cyanobacterial phycobiliproteins. Proc Natl Acad Sci USA 2007;104:14300-14305.
Zhao KH, Wu D, Wang L, Zhou M, Storf M, Bubenzer C, et al. Characterization of phycoviolobilin phycoerythrocyanin-alpha 84-cystein-lyase-(isomerizing) from *Mastigocladus laminosus*. Eur J Biochem 2002;269:4542-4550.
Zheng J, Inoguchi T, Sasaki S, Maeda Y, McCarty MF, Fujii M, et al. Phycocyanin and phycocyanobilin from *Spirulina platensis* protect against diabetic nephropathy by inhibiting oxidative stress. Am J Physiol Regul Integr Comp Physiol 2013;304:R110-R120.
Zhou W, Ding W, Zeng X, Dong L, Zhao B, Zhou M, et al. Structure and mechanism of the phycobiliprotein lyase CpcT. J Biol Chem 2014;289:26677-26689.
Zhou ZP, Liu LN, Chen XL, Wang JX, Chen M, Zhang YZ, et al. Factors that effect antioxidant activity of C-phycocyanins from *Spirulina platensis*. J Food Biochem 2005;29:313-322.
Zhu Y, Chen XB, Wang KB, Li YX, Bai KZ, Kuang TY, et al. A simple method for extracting C-phycocyanin from *Spirulina platensis* using Klebsiella pneumoniae. Appl Microbiol Biotechnol 2007;74:244-248.

Table legends

Table 1. Aggregation states (in solution), spectral properties, and phycobilin composition of different PBPs.

Table 2. Production of PBPs.

Table 3. Some PBP lyases.

Table 4. Pharmacological and biological properties of PBPs.

Table 5. Optical applications of PBPs.

Table 1 Aggregation states (in solution), spectral properties, and phycobilin composition of different PBPs.

PBP	Aggregation state	Absorption peak and shoulder* (nm)	Fluorescence peak(nm)	Phycobilins	References
B-PE	$(\alpha\beta)_6\gamma$	545, 563*, 498*	575	12 α PEB, 18 β PEB, 2γ PUB， 2γ PEB	(Fisher et al., 1980; Ficner et al., 1992; Tang et al., 2016)
R-PE	$(\alpha\beta)_6\gamma$	498, 538, 567	578	12 α PEB, 12 β PEB, 6 β PUB, 2γ PUB， 1γ PEB	(Jiang et al., 1999; Liu et al., 2005)
C-PC	$(\alpha\beta)_3$	616	643	3 α PCB, 6 β PCB	(Padyana et al., 2001; Wang et al., 2001; Kumar et al., 2014)
R-PC	$(\alpha\beta)_3$	549*, 617	636	3 α PCB, 3 β PEB, 3 β PCB	(Jiang et al., 2001; Wang et al., 2014)
PEC	$(\alpha\beta)_3$	530*, 575, 595*	625	3 α PXB, 6 β PCB	(Nies and Wehrmeyer, 1980; Rumbeli et al., 1985; Duerring et al., 1990)
APC	$(\alpha\beta)_3$	650, 618*	663	3 α PCB, 6 β PCB	(Brejc et al., 1995; Chethana et al., 2015)

Table 2 Production of PBPs.

PBP	Source	Cell disruption and crude PBP extraction	Purification of PBPs	Purity	Yield	References
PC	*Spirulina platensis* (dry)	Sonication	Aqueous two-phase extraction	-	-	(Chang et al., 2018)
PC	*Arthrospira platensis* (dry)	Sonication Rreezing and thawing	Ammonium sulfate fractionation (25-70) Ion exchange chromatography	2.5	-	(Kissoudi et al., 2018)
PC	*Synechococcus* sp. R42DM	Triton-X 100 treatment Sonication	Ammonium sulfate Fractionation20-70 Ion-exchange chromatography	4.03	-	(Sonani et al., 2017)
PC	*Spirulina platensis*	Homogenized	Aqueous two-phase extraction	4.32	79%	(Chethana et al., 2015)
PC	*Spirulina platensis*	Freezing and thawing	Ammonium sulfate fractionation Anion exchange chromatography	4.5	14%	(Kumar et al., 2014)
PC	*Limnotrhrix sp*	Freeze–thawed	Activated carbon and chitosan treatment Ammonium sulfate fractionation tangential flow filtration	4.3	8%	(Gantar et al., 2012)
PC	*Spirulina platensis*	Freeze–thawed	Ammonium sulfate fractionation High-speed counter-current chromatography	4.25	-	(Yin et al., 2011)
PC	*Synechocystis aquatilis*	Osmotic shock	Expanded bed chromatography Anion-exchange chromatography	4.0	69%	(Ramos et al., 2011)
PC	*Spirulina platensis*	Homogenized	Anion-exchange chromatography	1.82	77.3 %	(Silveira et al., 2008)
PC	*Phormidium fragile*	Grinding under liquid nitrogen	Ammonium sulfate fractionation Hydrophobic interaction Chromatography	4.52	-	(Soni et al., 2008b)
PC	*Spirulina platensis*	Ammonium sulfate treatment	Expanded bed adsorption Chromatography Anion-exchange chromatography Hydroxyapatite chromatography	3.2	-	(Niu et al., 2007b)
PC and APC	*Arthronema africanum*	Freeze–thawed	Rivanol treatment	4.52 and 2.41	55% and 35%	(Minkova et al., 2007)
PC	*Spirulina platensis*	Homogenized by press	Aqueous two-phase extraction	4.05	85%	(Patil et al., 2006)
PC	*Spirulina platensis*	Klebsiella neumoniae treatment	-	1.09	91%	(Zhu et al., 2007)
PC	*Spirulina platensis*	Freezing and thawing Sonication	Ammonium sulfate fractionation Ion-exchange chromatography	5.12	-	(Chen et al., 2006)

			Gel filtration chromatography			
PC	*Spirulina platensis*	Homogenized at a pressure	Aqueous two phase extraction Ion-exchange chromatography	6.69	-	(Patil et al., 2006)
PC	*Aphanizomenon flos-aquae*	-	Ammonium sulfate fractionation Hydroxyapatite chromatography	4.78	-	(Benedetti et al., 2006)
PC	*Spirulina*	Freezing and thawing Sonication	Ion-exchange chromatography	4.42	45.6 %	(Patel et al., 2005b)
PC	*Phormidium* sp.	Freezing and thawing Sonication	Ion-exchange chromatography	4.43	35.2 %	(Patel et al., 2005b)
PC	*Lyngbya* sp.	Freezing and thawing Sonication	Ion-exchange chromatography	4.59	36.8 %	(Patel et al., 2005b)
PC	*Calothrix* sp.	EDTA and Lysozyme treatment	Anion chromatography Hydrophobic interaction Chromatography	3.5	80%	(Santiago-Santos et al., 2004)
PC	*Spirulina fusiformis*	Freezing and thawing	Rivanol treatment Gel filtration chromatography	4.3	46%	(Minkova et al., 2003)
PC	*Spirulina maxima*	Glass beads milling	Aqueous two phase extraction Ultrafiltration Precipitation	3.8	29.5	(Rito Palomares et al., 2001)
PC and APC	*Spirulina platensis*	Freezing and thawing Sonication	Ammonium sulfate fractionation Ion exchange chromatography Gel filtration chromatography	5.06 and 5.34	-	(Zhang and Chen, 1999)
APC	*Spirulina platensis*	Freezing and thawing	Ammonium sulfate fractionation Hydroxylapatite extraction Anion-exchange chromatography	5.0	43%	(Su et al., 2010)
C-PE	*Pseudanabaena* sp.	Freezing and thawing	Ammonium sulfate fractionation Gel filtration Ion exchange chromatography	6.86	47%	(Mishra et al., 2011)
C-PC and APC	*Spirulina platensis*	Frozen and thawed	Ammonium sulfate fractionation Ion exchange chromatography	5.59 and 5.19	67% and 80%	(Yan et al., 2011)
B-PE and R-PC	*Porphyridium cruentum*	Homogenized Sonicated	Ammonium sulfate fractionation Ion exchange chromatography	>4 and >3	32% and 12%	(Bermejo et al., 2002)
B-PE	*Porphyridium cruentum*	Buffer extraction from lyophilized alga	Expanded-bed adsorption Chromatography Ion-exchange chromatography	4.6	66%	(Bermejo et al., 2003)

R-PE	*Polysiphonia urceolata*	Freezing and thawing	Ammonium sulfate fractionation Anion-exchange Chromatography	5.6	67.3 %	(Liu et al., 2005)
R-PE	*Polysiphonia urceolata*	Osmotic shock	Expanded-bed adsorption Chromatography Ion exchange chromatography or hydroxyapatite Chromatography	3.26	-	(Niu et al., 2006)
R-PE	*Porphyra haitanensis*	Freeze-thaw	Expanded-bed columns Anion-exchange	3.2	-	(Niu et al., 2007)
R-PE	*Portieria hornemannii*	Freezing and thawing	Ammonium sulfate fractionation Anionic-exchange	5.2	64.8 %	(Senthilkumar et al., 2013)
R-PE	*Grateloupia turuturu*	Liquid nitrogen grinding	Anion-exchange chromatography	2.89	27%	(Munier et al., 2015)
B-PE	*Porphyridium cruentum*	Osmotic shock	Ultrafiltration Anion-exchange chromatography	5.1	68.5 %	(Tang et al., 2016b)
R-PE	*Gracilaria lemaneiformis*	Osmotic shock refined	Ammonium sulfate fractionation Centrifugal precipitation Chromatography	6.5	-	(Gu et al., 2018)
PC	*Galdieria sulphuraria*	Homogenized	Ammonium sulfate fractionation Aqueous two-phase extraction Anion exchange Chromatography	4.5	39%	(Sorensen et al., 2013)

Table 3 Some PBP lyases.

Lys gene	Gene source	Catalytic site	Reference
cpcE/F	*Synechococcus* PCC 7002	PCB→PC α-Cys-84	(Fairchild et al., 1992)
	Synechocystis sp. PCC 6803	PCB→CPC α	(Tooley et al., 2001)
	Synechocystis sp. PCC 6803	PCB→APC α	(Yang et al., 2008)
pecE/F	*Nostoc* PCC 7120, *Mastigocladus laminosus*	PVB→PEC α-Cys-84	(Storf et al., 2001; Zhao et al., 2002)
cpeY/Z	Algae containing PE	PEB→PE α（PEsI）	(Kahn et al., 1997)
cpcS/U	*Synechococcus* PCC 7002	-	(Miller et al., 2008; Saunee et al., 2008; Shen et al., 2008)
cpeS	*Anabaena sp.* PCC 7120	APC β	(Ge et al., 2009)
cpeS1	*Anabaena* PCC 7120	PCB→Apophycophytin	(Zhao et al., 2007)
cpeS2	*Nostoc* PCC 7120	CPC β-Cys-84	(Zhao et al., 2007)
cpcT	*Synechocystis sp.* PCC 7002	CPC β-Cys-153	(Shen et al., 2006)
MpeV/U	Algae containing PE	PEB→PE α（PEsII）	(Zhao et al., 2007)

Table 4 Pharmacological and biological properties of PBPs.

Protein or gene source	PBP	Pharmacological and biological properties	References
Arthospira maxima	PC	Antioxidant	(Romay et al., 1998)
Arthospira maxima	C-PC	Antioxidant, Anti-inflammatory ability	(Romay et al., 1998)
AfaMax	PC	Antioxidant, Anti-inflammatory ability	(Castangia et al., 2016)
-	C- PC	Antioxidant, Reduce osteoarthritis	(Young et al., 2016)
-	C- PC	Antioxidant, Antihyperlipidemic	(Sheu et al., 2013)
Spirulina maxima (Dried marine micro alga)	C- PC	Antioxidant, Anti-inflammation activities	(Choi and Lee, 2018)
Portieria homemannii	R-PE	Antioxidant, Vitro anticancer	(Senthilkumar et al., 2013)
Synechococcus sp	PC	Antioxidant, Radical-scavenging activity	(Sonani et al., 2017)
Spirulina platensis	C- PC	Anti-inflammatory ability	(Chen et al., 2014)
Limnothrix sp strain 37-2-1	C- PC	Antioxidant	(Gantar et al., 2012)
Lyngbya sp. A09DM	PE	Antioxidant	(Sonani et al., 2014)
Spirulina	PC	Antioxidant	(Romay and Gonzalez, 2000)
Spirulina platensis	Apo-c-PC β subunit and C-PC	Antioxidant	(Pleonsil et al., 2013)
Spyrulina species	C- PC	Antioxidant	(Lissi et al., 2000)
Spirulina fussiformis	C- PC	Antioxidant	(Madhyastha et al., 2009)
Spirulina platensis	Selenium-containing PC	Antioxidant	(Huang et al., 2007)
Aphanizomenon flos-aquae	PC	Antioxidant	(Benedetti et al., 2004)
MaterialsS. maxima	PC	Antioxidant	(Nakagawa et al., 2016)
Spirulina platensis	Se-APC	Antioxidant, Inhibition of cancer cells	(Fan et al., 2012)
Anacystis nidulans UTEX 625	Recombinant APC	Antioxidant	(Ge et al., 2006)
Spirulina platensis	Recombinant α-PC	Antioxidant	(Guan et al., 2009)
Porphyra haitanensis	R- PC	Anti-allergy, Anti-inflammation activities	(Liu et al., 2015)
Spirulina platensis	C- PC	Decrease the blood glucose level	(Setyaningsih et al., 2015; Ou et al., 2016)
Spirulina platensis	C- PC	Anti-atherosclerosis	(Riss et al., 2007; Strasky et al., 2013)
Spirulina maxima	C- PC	Reduce liver damage	(Ou et al., 2010)
Spirulina platensis	C- PC	Reduce liver damage	(Pak et al., 2012; Hussein et al., 2015; Xia et al., 2016)
Arthlpira maxima SAG 25780	C- PC	Reduce liver damage	(Nagaraj et al., 2012)
Spirulina platensis	C- PC	Cataract treatment	(Kothadia and Shabaraya, 2011; Kumari et al., 2013)
Spirulina (Arthospira) species	C- PC	Reduce nerve damage	(Rimbau et al., 1999)

Spirulina platensis	C- PC	Reduce nerve damage	(Bermejo-Bescos et al., 2008; Min et al., 2015; Mitra et al., 2015)
-	PC	Reduce neurodegenerative diseases associated with proteotoxicity,	(Macedo et al., 2017)
Spirulina platensis	PC and PCB	Protect kidneys	(Zheng et al., 2013)
Spirulina platensis	C- PC	Protect kidneys	(Farooq et al., 2014; Fernandez-Rojas et al., 2014)
Spirulina platensis	C- PC	Reduces pulmonary fibrosis	(Sun et al., 2011; Sun et al., 2012; Li et al., 2017)
Bangioatropurpurea	R-PC	Alleviates allergic airway inflammation	(Chang et al., 2011)
Spirulina platensis	C- PC	Antifibrotic	(Pattarayan et al., 2017)
Arthospira maxima	C- PC	Reduce intestinal inflammation	(Gonzalez et al., 1999)
Spirulina platensis	APC	Reduce intestinal damage	(Chueh, 2002; Shih et al., 2003)
Spirulina (Dry powder)	C- PC	Anti-tumor	(Gupta and Gupta, 2012)
Spirulina platensis	C- PC	Anti-tumor	(Gantar et al., 2012; Saini et al., 2012; Saini and Sanyal, 2014; Li et al., 2015; Deniz et al., 2016)
Oscillatoria tenuis	C- PC	Anti-tumor	(Thangam et al., 2013)
Anacystis nidulans UTEX625	Recombinant APC	Inhibition of cancer cells	(Ge et al., 2005; Ge et al., 2005)
Gracilaria lemaneiformis	PE	Inhibition of cancer cells	(Ruobing et al., 2007)
Spirulina platensis	C- PC	Inhibition of cancer cells	(Pan et al., 2015; Ying et al., 2016)
Spirulina powder	Digestion by pepsin releases biologically active chromopeptides from C-PC	Antioxidant, Inhibition of cancer cells	(Minic et al., 2016)
Oscillatoria tenuis	C- PC	Antioxidant, Inhibition of cancer cells	(Thangam et al., 2013)

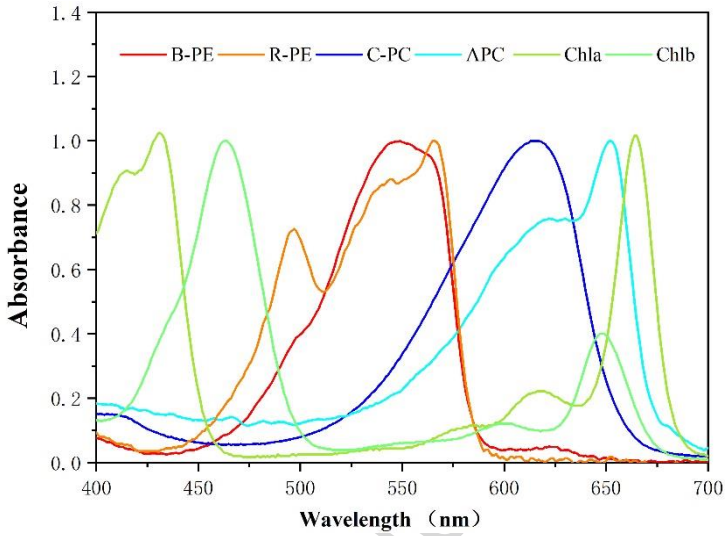
Table 5 Optical applications of PBPs.

Protein or gene source	PBP	Application	References
-	APC	Photodynamic therapy	(Suping et al., 2001)
Microcystis	PC	Photodynamic therapy	(Wang et al., 2012)
Spirulina platensis	C- PC	Photodynamic therapy	(Bharathiraja et al., 2016)
-	R-PE	Photodynamic therapy	(Huang et al., 2002)
Spirulina	C- PC	High efficient fluorescence sensors	(Wang et al., 2016)
-	C- PC	Fluorescence probe	(Han et al., 2018)
-	PBP	Fluorescence probe	(Siiman et al., 1999)
Gastroclonium coulteri (Rhodymeniales)	R-PE	Fluorescence probe	(Oi et al., 1982)
-	R-PE	Fluorescence probe	(Gao et al., 2015)
-	APC	Fluorescence probe	(Tang et al., 2017; Li et al., 2018)
Streptomyces avidinii	Recombinant fusion PBP (SLA-PEB and SLA-PCB)	Fluorescence probe	(Wu et al., 2017)
Synechococcus sp pcc 6803	Streptavidin-PBPs (SA-PBPs)	Immunoassay technologies	(Ge et al., 2017)
Chroococcidiopsis thermalis	ApcF2 (The phycobilisome core subunit)	Fluorescent markers	(Ding et al., 2017)

Figure captions

Fig. 1. Absorbance spectra of B-PE, R-PE, C-PC, APC, Chla, and Chlb.

Fig. 2. Applications of PBPs (for details see in Tables 4 and 5).



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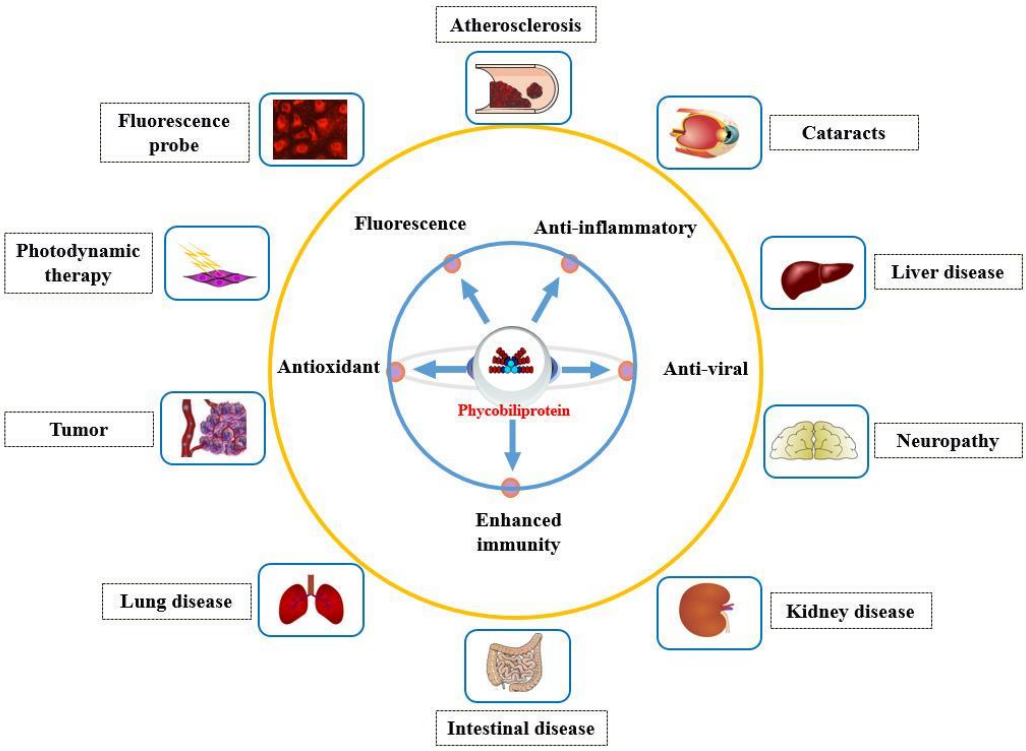

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