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Review

The Radiant World of Cyanobacterial Phycobiliproteins: Examining Their Structure, Functions, and Biomedical Potentials

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Abstract: Phycobiliproteins (PBPs) are accessory light-harvesting pigment complexes found in cyanobacteria, red algae, and certain types of cryptophytes. The unique spectral features (strong absorbance and fluorescence), proteinaceous nature, and some imperative properties such as the anti-oxidative, hepato-protective, anti-inflammatory, and anti-aging activity of PBPs allow their use in biomedical industries. However, basic research and technological innovations are required to explore their potential in biomedical applications. The techniques responsible for therapeutic effects need to be standardized for medical application purposes. This review focuses on the current status of PBPs, their structure, functions, methods of preparation, and applications. Additionally, the stability, bioavailability, and safety issues of PBPs, along with their use in therapeutics, are discussed.

Keywords: bioavailability; biomedical; cyanobacteria; phycobiliprotein

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1. Introduction

The photosynthetic apparatus of cyanobacteria is composed of three primary light-harvesting systems: two photosystems common to other photosynthetic organisms and a phycobilisome (PBS). Phycobilisomes (PBSs) are large antenna complexes found in cyanobacteria that are crucial for photosynthesis. They capture light energy in the range of 450–650 nm, transfer it within its structure in a unidirectional way, and deliver it to the chlorophyll molecule of photosystems [1]. Although the PBSs of cyanobacteria are mainly composed of phycobiliproteins (PBPs) and linker proteins, their composition may vary from individual organism to different species. PBPs are in charge of absorbing and transmitting light energy, whereas linkers enable proper PBS assembly and control energy transmission [2]. Cyanobacterial PBPs are large, water-soluble supramolecular protein aggregates that are important accessory pigments during photosynthesis. Based on their spectrum features, PBPs can be generically classified into three classes: phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC). PBPs are commercially produced from *Spirulina platensis*, *Anabaena* sp., and *Galdieria sulphuraria* and have been extracted and purified from *Spirulina* sp., *Synechococcus* sp., *Oscillatoria* sp., etc. [3]. The structure, amino acid composition, abundance, and types of PBPs of cyanobacteria and algae vary depending on the species and the habitat in which they grow. The spectral properties of PBPs result largely from the environment of the chromophore conferred by the apoprotein rather than the structural properties of the chromophore itself. PBPs in cyanobacteria account for 50% of total cellular protein [4].

Each PBS has two distinct substructures: the rods and the core [5,6]. The core is made up of APC. Several rods made up of PE and PC extend from the core in an outward orientation, which further functions as the light-harvesting antennae. Certain cyanobacteria species, including *Spirulina platensis*, have rods that solely contain PC and protein linkers that are nonetheless connected to the PBS core [7]. APC and linker proteins combine to create the core. This core transfers the energy captured by the rods to the chlorophyll present in the thylakoid membrane [8].

A growing interest in the study of microorganisms like cyanobacteria has resulted from the search for bioactive compounds in recent decades. This is because these organisms may have commercial applications in a variety of fields, including nutrition, animal and human health, wastewater treatment, energy production, and the chemical and pharmaceutical industries [9]. More specifically, cyanobacterial PBPs have widespread biotechnological applications due to their potent biological and pharmaceutical properties. PBPs, especially PC, have commercial and industrial applications due to their primary functions, including nutraceutical and therapeutic values (due to their pharmacology and biological activities, including anticarcinogenic, antioxidative, and anti-inflammatory activities, as well as a protective effect against various conditions), over-the-top fluorescent properties (high quantum yield, high Stokes shift, and an essential insensitivity to quenching), and natural colorants [10]. PBPs are useful fluorescent tags that find wide-ranging uses in immunoassay, reactive oxygen species (ROS) detection, flow cytometry, and fluorescence-activated cell sorting. These uses take advantage of PBP's special spectroscopic and physical characteristics. They have a broad range of biomedical applications, such as in food coloring, biotechnology, medicines, cosmetics, and nutraceuticals [11]. The review aims to highlight the spectral and structural features of cyanobacterial PBPs along with their biotechnological and biomedical applications.

2. Spectroscopic and Structural Characteristics of Cyanobacterial Phycobiliproteins

Based on their absorption, PBPs have been divided into three major categories: APC (λ_{max} = 650–660 nm), PC (λ_{max} = 610–625), and PE (λ_{max} = 490–570) [12]. The most diverse chromophores among PBP are found in PE, which has a vibrant pink color. PE can be found in the PBS rods' distal region. At 542 nm, C-PE exhibits a single absorption maximum. At a maximum emission value of 575 nm, the fluorescence emission of the various types of PE does not demonstrate significant variations [13]. To date, Phycoerythrocyanin (PEC) is exclusively reported in the photosynthetic membrane of *Mastigocladus laminosus*. It is a PBP with a purple-blue tint. One of the primary features of PEC is that during growth, its production is highly dependent on the kind and intensity of light. A significant increase in abundance was seen under low light conditions with green light [14]. The proximal portion of PBS rods contains the bright blue protein known as PC. There is just a single absorption maximum at 615 nm (Figure 1) [15]. APC, which is only present in the PBS core, is a bright turquoise color with a maximum absorption of 650 nm [8]. A highly effective energy transfer in the PBS is made possible by these spectroscopic features of PBP absorption and emission.

2.1. Subunits and Heterodimers ($\alpha\beta$)

The basic component of PBPs is its subunits, specifically α and β subunits, which stabilize each other to form an $\alpha\beta$ heterodimer. The self-assembly of all PBPs appears when two subunits, α and β, dock together [16,17]. This α and β heterodimer is known as the fundamental component of PBPs' monomeric structure. It subsequently comes together to form trimeric discs $(\alpha\beta)_3$. Monomer subunits are primarily distinguished from native trimeric units by dissociated subunits, which are usually less intense in color. In various PBPs, native trimeric and hexameric units have different molecular weights. When it comes to capturing solar light energy, the hexamer assembly is more functional and stable. On the other hand, the trimeric form of the majority of PCs has demonstrated an intriguing rod structure assembly without a requirement for linker polypeptides [18]. Four trimers of APC biliproteins come together to form core cylinders when they are assembled. A full functional structure is then formed by the subsequent assembly of two to five core cylinders into the core substructure with the help of linker polypeptides, as in Figure 2.

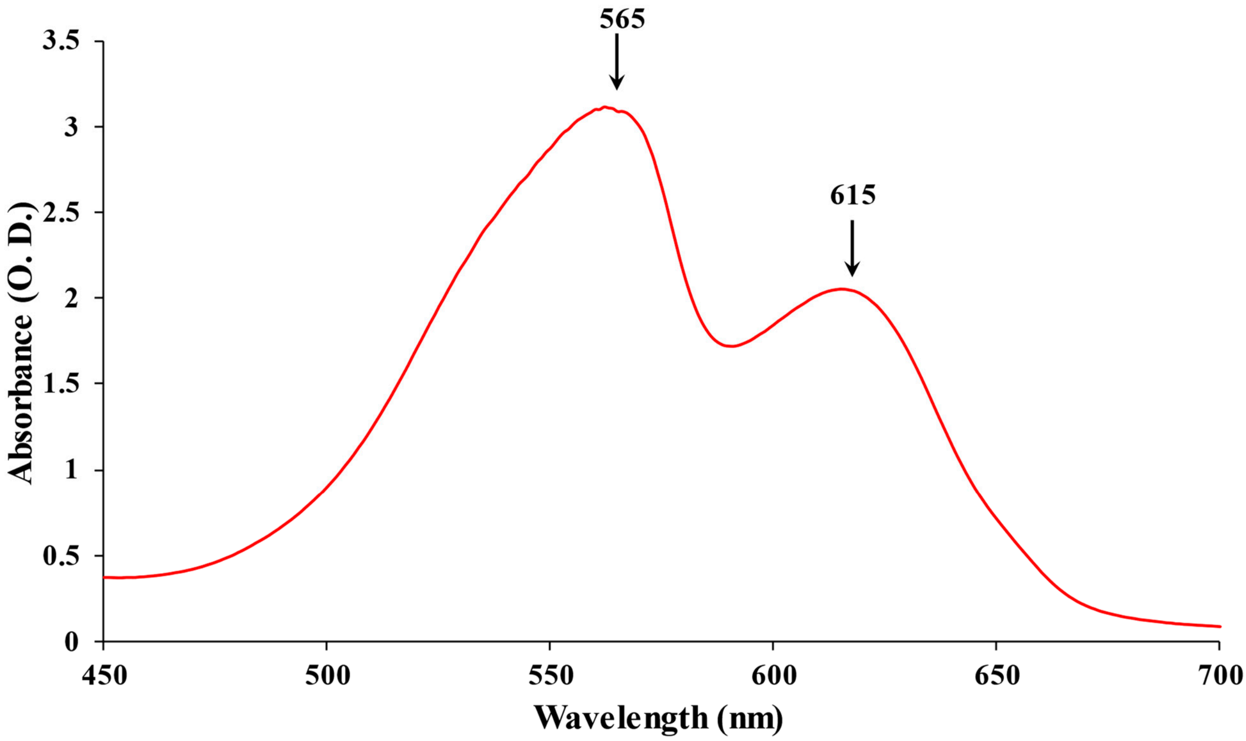


Figure 1. The absorption spectrum of PBPs: PE maximum at 565 nm and PC maximum at 615 nm.

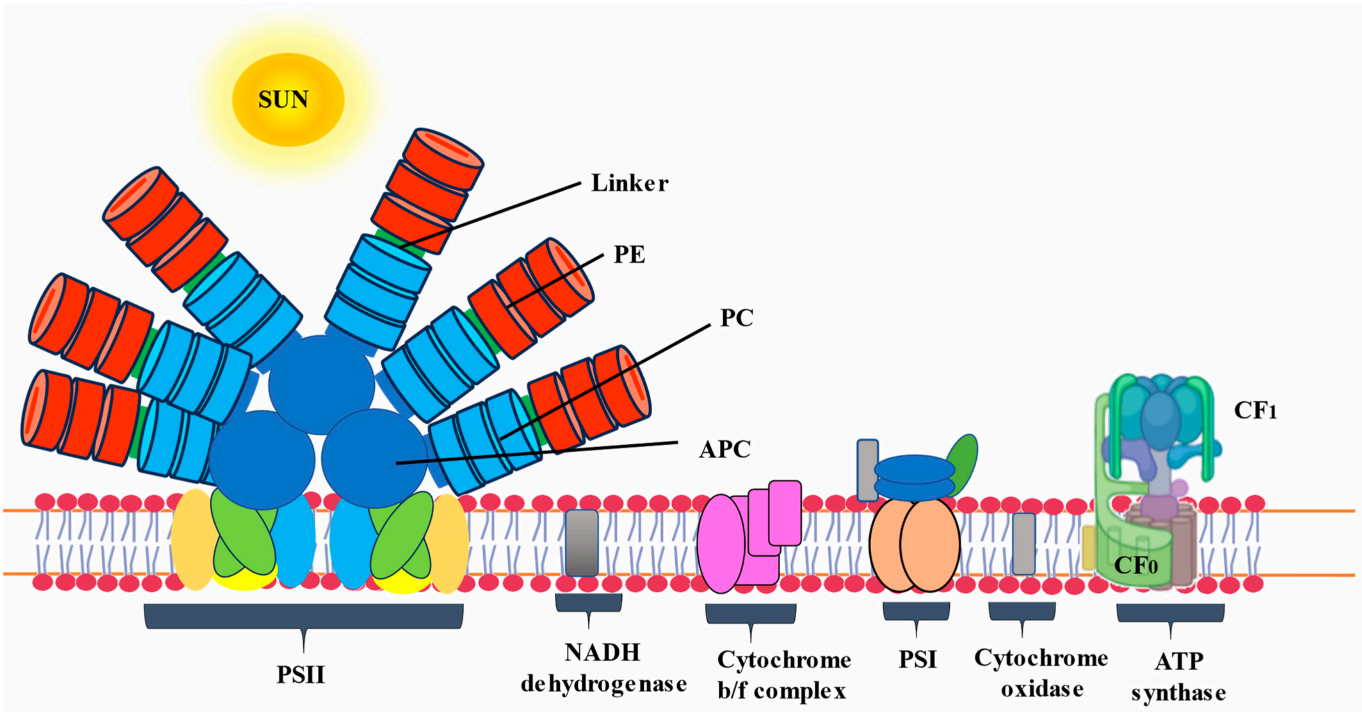


Figure 2. Schematic diagram of phycobilisome located on the thylakoid membrane, modified from [3].

Ultimately, a complete PBS is formed by the association of both core cylinders and rods (Figure 3). When it comes to high-resolution views of proteins, X-ray crystallography has emerged as an extremely valuable instrument. Initially, the entire structure of PBS was explored at low resolution through electron microscopy. PBS crystal structures have also significantly improved our potential to analyze the tertiary, or hexameric, structure of proteins as well as their stability and functionalities in a range of environmental circumstances [19].

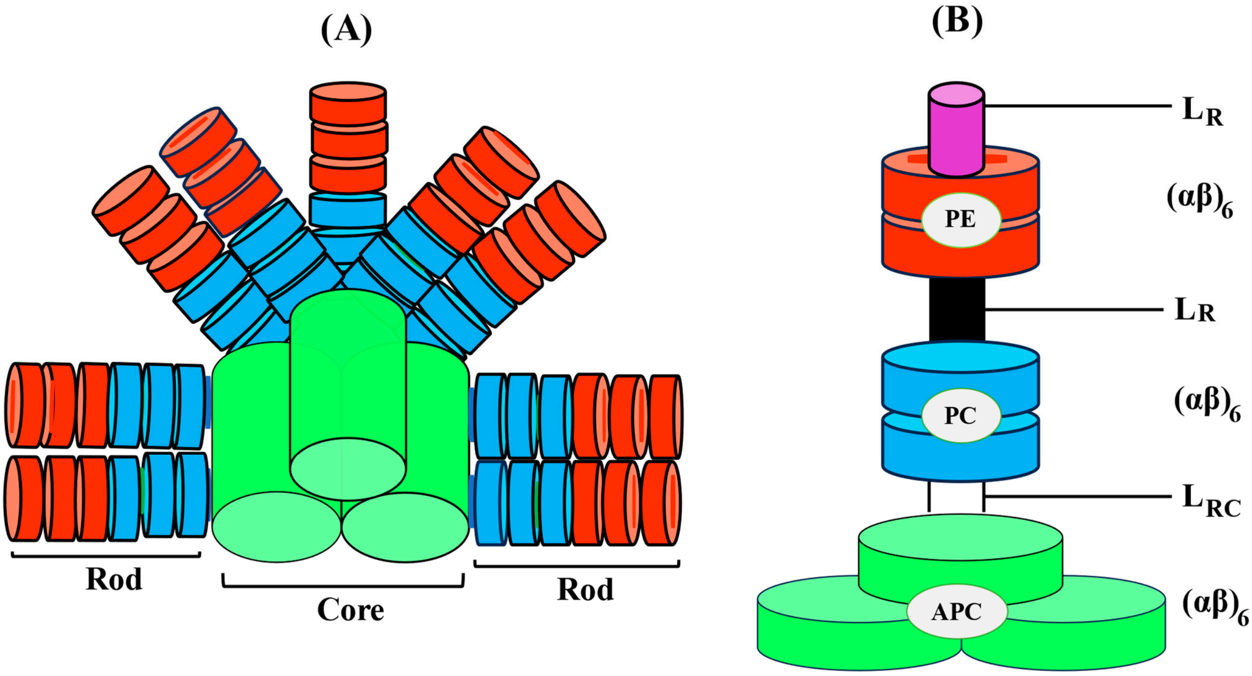
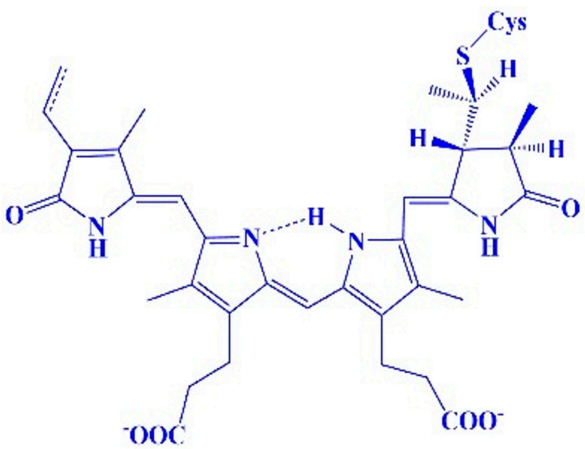


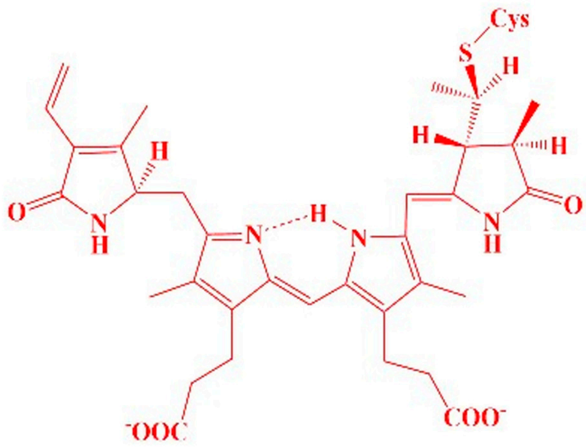
Figure 3. Diagrammatic illustration of the three-dimensional intact PBS with the PC/PE rod organized in a parallel orientation around the APC core (**A**), Dimension of PBP and associated linker polypeptides and, (**B**) schematic arrangement of cylindrical core (APC), modified from [3,20].

2.2. PBPs Chromophore

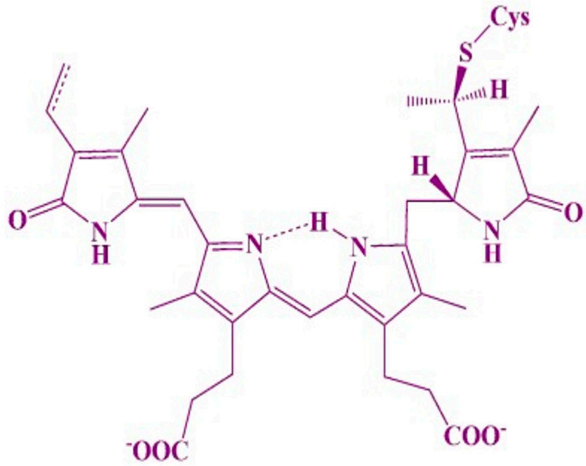
Chromophores are open-chain tetrapyrroles covalently linked to cysteine via thioether bonds. PBPs can vary in the number of chromophores they contain. Based on their structural characteristics, the chromophores can be categorized into the following categories: phycocyanobilin (PCB), phycoerythrobilin (PEB), phycoviolobilin (PVB), or phycourobilin (PUB) (Figure 4) [21]. Each biliprotein consists of the α and β subunits that are linked through covalent bonds to the apoprotein's cysteines through a thioether bond to C-3 on ring A, and occasionally through an extra thioether bond to C-18 on ring D. The chromophore that is developed from heme is joined to the apoproteins of bilin by an ethylidene bond, which adds a thiol group [22]. A single cysteinyl thioester linkage via the vinyl substituents on the pyrrole ring A of the tetrapyrrole, or periodically two cysteine linkages through the vinyl substituents on both the A and D pyrrole rings, are typically responsible for binding chromophores to the polypeptide chain at the conserved position. The assembly of chromophores within the clearly identified geometries of proteins' cores determines the properties of light-harvesting PBPs in photosynthetic cyanobacteria [23]. The electronic state of chromophores and related apoproteins plays a major role in the establishment of the stable chemical structure of proteins. Consequently, the chromophore serves as a report on the integrity of the protein structure; the protein possesses a stunning dark blue color when it is in its native form, but this color disappears when it is denatured [24]. PBPs have been studied using fluorescence techniques because, when detached from reaction centers, they show strong fluorescence. The fluorescence quantum yield of the tetrapyrrole chromophore in C-PC is roughly 60%, with a maximum fluorescence of 650 nm when it is maintained in a linear alignment by the protein scaffolding [25].



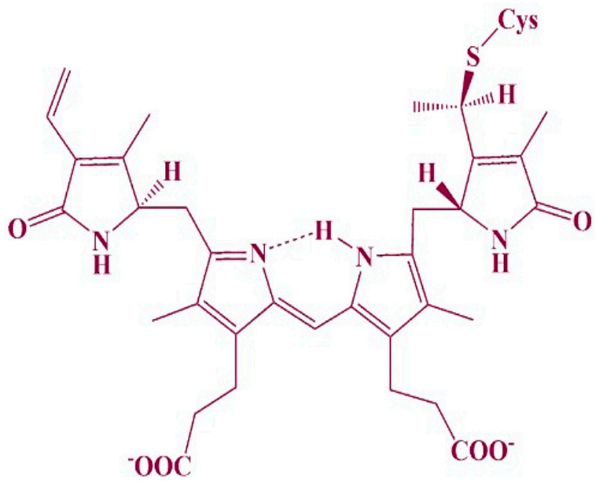
Phycocyanobilin (PCB)



Phycoerythrobilin (PEB)



Phycoviolobilin(PVB)



Phycourobilin (PUB)

Figure 4. A molecular structure illustrating the distinguished colors of the cysteine-linked chromophores in PBP complexes.

The heterodimeric (αβ) protomer of C-phycocyanin (C-PC) contains three PCB chromophores at cysteines α-84, β-84, and β-155. This residue is also conserved in the β subunit of the C-PC chromophore, where it is classified as Asp39 in the β-155 chromophore and Asp87 in β-84. It is present in both C-APC and C-PE. The chromophore's electronic state is established by an important amino acid called the Asp87 residue [26]. Position Cys84 on the α subunit contains a single PVB chromophore, while positions Cys82 and Cys153 on the β subunit contain two PVB chromophores. PEB comprises a set of two to three covalently linked tetrapyrrole chromophores integrated with the α (164 amino acid) and β (177 amino acid) subunits of C-PE, respectively [27].

2.3. Linker Polypeptides

The PBP structure involves the non-pigmented linker polypeptides [28]. The polypeptides have molecular masses varying between 8 and 120 kDa. Two possible functions of linker polypeptides in the PBS have been proposed: (1) maintain the PBS structures and establish structural links between the nearest PBPs, and (2) adjust both the properties of absorption and fluorescence to support or directly take part in the energy transfer from the rod to the core and ultimately to the thylakoid membrane of the photosynthetic cells, which contain chlorophyll [29]. The two basic subunits (α and β) of PBPs along with its heterodimer, i.e., $\alpha\beta$ are represented in Figure 5.

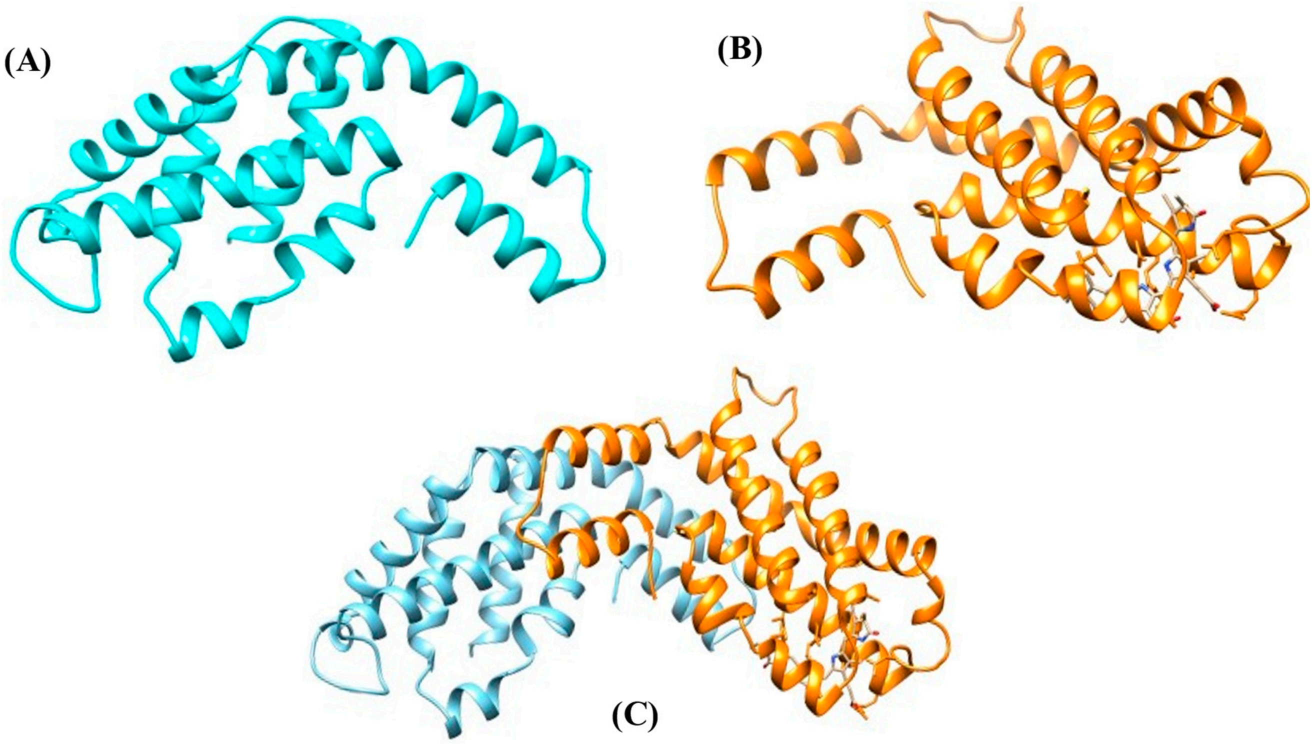


Figure 5. Protein model of PBPs. (**A**) α subunit, (**B**) β subunit, and (**C**) $\alpha\beta$ heterodimer.

The standard acronym for linker polypeptides is $L_X{}^Y$, where X represents the location and Y represents the molecular mass of the linker polypeptide (L) PBS complex. In addition, X can be assigned either to the letter R (rod) or C (core) for the main chain, while RC (rod-core) and CM (core-membrane) are used to represent junctions. In class I and II of R-PE, the γ-subunits and the core-membrane linker (LCM) PCB-ApcE are two of the linker polypeptides that contain covalently attached chromophores, while the majority of them are colorless [30]. Based on their location and functional characteristics, the linker polypeptides are divided into four groups. PBS: Group I consists of L_R polypeptides (27–35 kDa) involved in the peripheral rod assembly, together with a small number of 10 kDa rod linker polypeptides (LR^{10}, LR^{33}, and LR^{35}, for example, which connect trimeric or hexameric PC/PE structural components into rod segments); Group II L_{RC} polypeptides, which are between 25 and 27 kDa, take part in establishing the peripheral rods to the core subunits; LC polypeptides (8 kDa) belonging to Group III are essential for joining core components and ensuring their functional properties; and the main terminal energy emitter for PSII is Group IV, LCM (70–120 kDa), with a greater molecular weight of polypeptides that connects PBS to the photosynthetic membrane [31]. Six conserved domains (N-terminus) in rod linker polypeptides have been recognized by structural motif analysis as being crucial for the packing and assembly of rod discs into hexamers (Figure 3). There are connections between PBPs and linker polypeptides because the surfaces of linker polypeptides possess a positive charge, while the majority of globular proteins are probably hydrophobic [29].

3. Biomedical Potential of PBPs

In addition to their role in nature, PBPs have several biomedical applications. In Japan, China, India, and other European and Asian nations, PBPs are regarded as one of the most promising food products that people are using effectively as additives and marketing as food and cosmetic colorants [32]. In addition to PBPs' nutritional value, they also possess antiviral, antioxidant, hepatoprotective, anti-inflammatory, and immune-stimulating characteristics (Figure 6). Here are some biomedical applications of PBPs.

3.1. Antioxidant Properties

PC may prohibit the peroxidation of lipids by constraining the oxidative activity of several kinds of radicals, including peroxyl, hydroxyl, and superoxide. It is possible that the metabolic response to oxidative stress may have an impact on cellular elements such as proteins, nucleic acids, and the cellular membrane itself, which can lead to a variety of illnesses, including cardiovascular disease, diabetes, cancer, inflammation, degenerative diseases, ischemia, and anemia (Figure 6) [33]. Several phytochemicals, such as α-tocopherol, caffeic acid, zeaxanthin, etc., are currently being utilized in the prevention and treatment of these kinds of diseases. Additionally, since PCB is as effective as other phytochemicals, Hirata et al. [34] suggest that it has a high potential for antioxidant properties. The organisms produce anti-oxidant compounds as a defense mechanism to protect themselves against oxidative effects. ROS accumulation is the main triggering factor of oxidative stress. Both enzyme-based and non-enzyme antioxidative processes deal with the neutralization of ROS. In order for compounds like PC to function as non-enzymatic antioxidants, they typically scavenge free radicals from ROS, neutralize reactive molecules, and reduce the level of oxidation. Particularly, the effects of oxidative stress can destroy DNA and result in apoptosis or mutagenesis. Both PC and PCB have been demonstrated to be effective at scavenging peroxynitrite and prohibiting damage to DNA [35]. According to Strasky et al. [36], phycocyanobilins have structural similarities with the human bile pigments biliverdin and bilirubin, the former of which serves as the most effective endogenous antioxidant substrate. However, due to the insolubility of water, the scarcity of natural sources, and the challenges of synthesizing it, bilirubin is not appropriate for oral administration. It is interesting that PCB has recently been proposed as a potential mimic molecule with atheroprotective properties [36].

Additionally, according to the Oxygen Radical Absorbance Capacity (ORAC) method developed by Benedetti et al. [37], PCB (as well as PC) has a strong antioxidant capability, which suggests an effective antioxidant profile for particular biological samples. A number of research studies have additionally revealed that PBPs possess the ability to scavenge free radicals [38]. Additionally, PC has been reported by Datla [39] as having strong antioxidant, immune boosting, and anti-inflammatory properties. In rodent models of autoimmune encephalomyelitis, Cervantes-Llanos et al. [40] demonstrated an application of PC as a neuroprotector, whereby PC decreased the level of oxidative stress and the response of the immune system. According to research by Gdara et al. [41], PC reduces liver damage by inhibiting the activity of oxidative stress-activated enzymes like alkaline phosphatase and liver transaminases. The liver enzymes P450, aminopyrine-N-demethylase, and glucose-6-phosphatase can all be protected by PC, which can significantly decrease liver toxicity. To preserve the liver enzymes, PC is therefore essential as a hepatoprotective. Moreover, by inducing thioacetamide, PC's antioxidant property reduces hepatic brain injury [42]. Additionally, PC is already accepted for use as a food colorant and is utilized in cosmetic products as well.

3.2. Anticancer Properties

In addition to being one of the leading causes of death around the world, cancer is one of the most problematic diseases. To lessen the issue caused by cancer, several initiatives have been developed using various strategies. Over the past few decades, research has focused on natural products in the form of extracts and pure compounds from plants, algae, fungi, and cyanobacteria, with extremely positive outcomes [43]. Phycocyanin, in particular, is one of the cyanobacterial pigments that has been shown in the last few decades to have anticancer properties in a variety of cancer cell types such as liver, leukemia, melanoma, breast, etc., using both in vitro and in vivo assays [44]. Proliferation, deregulated apoptosis, and hyperplasia are the three fundamental features of cancer cells that can be used to describe the mechanisms of action of an anticancer compound. Thus, the anticancer medication may prevent the growth of new cells and/or trigger the death of tumor cells. By modifying the growth of different tumor cell lines at different levels of their molecular mechanisms, PC exhibits the highest level of activity. According to recent theories by McCarty and associates, phycocyanobilin inhibits ROS production in the mitochondria, which prevents pancreatic cancer, and PBP can prevent cancer cachexia by reducing TLR4 signaling in skeletal muscles [45]. Furthermore, by lowering RIPK1/NF-jB and TIRAP/NF-jB activity, C-PC inhibits the in vitro proliferation and migration of non-small-cell lung cancer cells [46]. Normal tissue cells exhibited virtually no or very little proliferative effects from PC, and normal cell viability is unaffected by high concentrations of PC. Ying et al. in 2021 reported that PEs are also crucial as a potential treatment for human ovarian cancer by inhibiting growth and inducing apoptosis in SKOV-3 cells. The ROS/JNK/Bcl-2 signaling pathway, upregulation of JNK, GADD45A, RAD23, and downregulation of XBP1 and OS9 are critical in PE-induced apoptosis in this cell [47].

3.3. Anti-Inflammatory Effects

Whenever there is an internal or external injury or infection, the immune system responds dynamically with inflammation [48]. PBPs, particularly PC, have been demonstrated to exhibit a number of anti-inflammatory properties. In general, an inflammatory response is an essential aspect of everyday life for people. This process may happen with minor symptoms like discomfort in the muscles or more serious conditions like acute lung injury, which is identified by injury to the epithelial and endothelial cells in the pulmonary system and can be lethal for patients in intensive care units (ICUs) [49]. Inflammatory disorders typically have macrophage induction as their primary source of pro-inflammatory signals. The primary mechanism by which PBPs exert their anti-inflammatory effects is through the stimulation and expression of enzymes, the modulation of macrophage function, and the suppression of pro-inflammatory signals. Depending on the type of infection, different target tissues and pro-inflammatory signals are produced. Additionally, the inhibition of two key signaling pathways, nuclear transcription factor-κB and mitogen-activated protein kinases (MAPKs), which play a significant role in the synthesis of numerous pro-inflammatory mediators, is known to be the cause of the anti-inflammatory activity of biologically active extracts and natural substances [33]. There are various ways that PBPs, and especially C-PC, can function. Since cyclooxygenase (COXs) (1 and 2) play a role in the production of significant biological mediators, known as prostanoids, which are directly connected to the inflammatory process, anaphylactic reactions, and vasoconstriction, they can provide relief from the symptoms of inflammation and pain by reducing the activity of COX-2 [50]. Additionally, C-PC was suggested as a radiosensitizing agent to lessen patient radiation therapy countermeasures because colon cancer radiation therapy hinders COX-2 expression (Figure 6) [51]. Furthermore, the PBPs inhibit the synthesis of hydrogen peroxide and hypochlorous acid, which oxidatively damage the host tissue, by inhibiting the performance of the myeloperoxidase enzyme (MPO) [2]. By inhibiting cofactors like NF-κB, PC can regulate apoptosis and inflammatory reactions. NF-κB is an important factor in controlling the immune system's reaction to infection, but its abnormal activity has been connected to autoimmune, inflammatory, and cancerous conditions. PC is useful in treatments and as a prophylactic, since it inhibits a portion of the cofactor [52]. Furthermore, Kim and colleagues [53] demonstrated the application of PC to inhibit UV-induced epithelial apoptosis. The process is induced by a protective cascade and is under the control of protein kinase C (PKC).

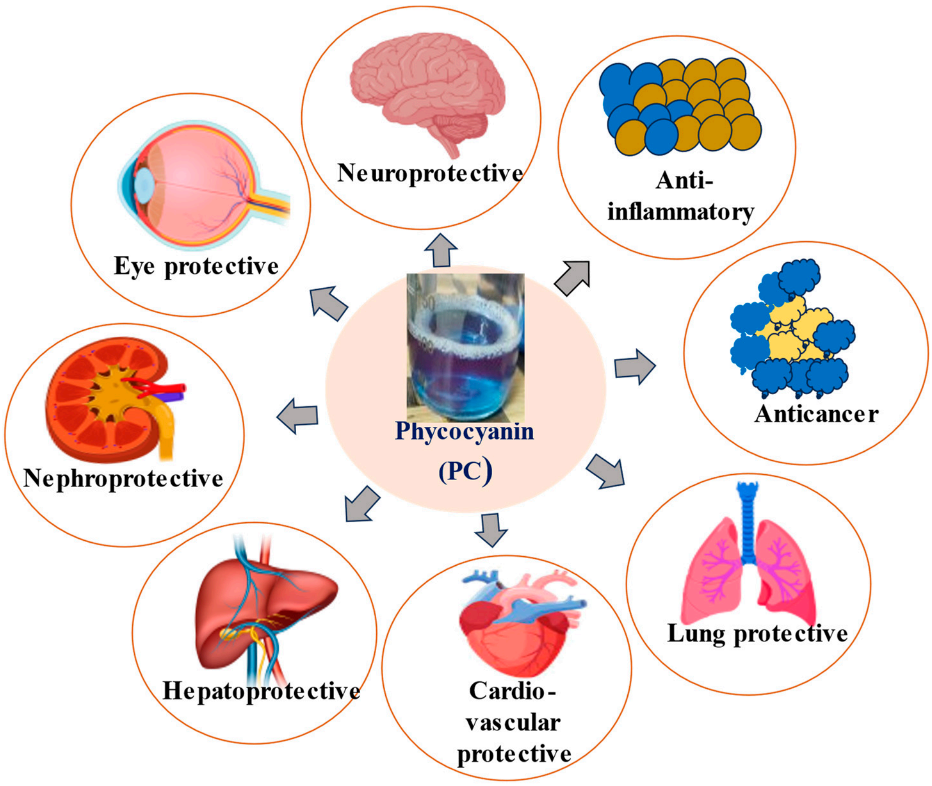


Figure 6. Antioxidative and biomedical potentials of PC, modified from [54].

PC has recently been used to determine anti-inflammatory properties, including the in vitro inhibition of albumin denaturation, anti-proteinase, hypotonicity-induced hemolysis, and anti-lipoxygenase activities [55]. However, in an immunosuppressive model induced by hydrocortisone (HC), R-PE was found to have immunomodulatory activity in both the innate and adaptive immune systems through TLR4/NF-κB-dependent immunocyte differentiation [56].

Additionally, Gonzalez et al. [57] suggested that PC plays an important role in treating several diseases and alleviating the symptoms of a variety of inflammatory processes.

3.4. Potential as Diagnostic Tools

PBPs are distinctive and beautifully colored proteinaceous photosynthetic pigments. In both medical and non-medical diagnostics, PBPs are frequently utilized as fluorescence-detecting systems. Primarily, its use was limited to the diagnosis of human infectious illnesses, metabolic abnormalities, malignancies, and immunological disorders. Later on, their use was expanded further to include the prognosis of a few human illnesses. PBPs are important in the innovation of human diagnostics, either as fluorescence-detecting elements or as both diagnostic systems and therapeutic molecules. They have been heavily employed in the identification of cell lineages, cell components, and cell subsets, as well as other biological and environmental probes.

Protein conjugates, molecular tags, antibody conjugates, fusion proteins, and DNA probes are only a few of the detecting probes that have been the subject of substantial patented research. Additionally, PBPs are incorporated into microbeads, magnetic beads, and biochips. Around the world, several businesses sell diagnostic PBPs in the form of preparations like streptavidin conjugates, activated PBPs, FITC-crosslinked forms, purified and activated forms, and multi-color detection systems with custom designs. Among all, PE is the most widely used PBP, followed by APC and PC.

PBPs continue to be extensively used for the detection and diagnosis of several metabolic diseases and syndromes in humans. For instance, the expression of integrin IIb-3 can be used to determine the functional capacity of platelets in thrombosis and hemostasis.

On resting platelets, its expression was not strong. The monoclonal antibody (mAb) against Integrin IIb-3 was conjugated to R-phycoerythrin (R-PE) in this diagnostic method [58]. PBPs are now applicable to environmental problems as well. The detection of diarrhetic shellfish poisoning toxin, Okadaic acid (OA), produced by toxigenic dinoflagellates, was performed using magnetic beads coated with streptavidin. It prevents hazards to public health [59]. The detection of Cu^{2+} ions based on the quenching effect between R-PE-AgNPs and Cu^{2+} ions, in conjunction with the fluorimetric approach, was made using the R-PE-AgNPs (silver nanoparticle) construct [60].

PE, as a fluorochrome, was used to detect the amyloid protein (A), which diagnoses Alzheimer's disease [61]. Similar to this, hemophagocytic lymphohistiocytosis showed down-regulated CD5 expression on activated CD8+ T cells utilizing an immunofluorescence kit including the appropriate antibody-coupled PE and other colors [62]. PBPs might be used as a component of detection in a range of human malignancies after the diagnosis of human pathogenic and non-pathogenic disorders. The imaging of cancer development and therapy effectiveness using PC-attached nano horns was successful in vivo. It is noteworthy that PC was previously shown to mediate the formation of ROS photodynamically to destroy cancer cells [63].

In one such implementation, CD5-FITC/CD19-PE immunostaining was used to identify chronic lymphocytic leukemia [64], where FITC was a cross-linker. The recombinant fusion protein streptavidin-phycobiliproteins (SA-PBPs) in Sandwich ELISA was used to identify liver cancer using the biomarkers -fetoprotein and carcinoembryonic antigen. According to Ge et al. [65], the protein streptavidin is derived from *Streptomyces avidinii.* Epithelial cell adhesion molecule (EpCAM), which is present in the cell membrane, was detected in live breast cancer cell lines using multicolor staining with 4,6-diamidino-2-phenylindole (DAPI) for the nucleus and an APC-labeled anti EpCAM antibody (Table 1) [66].

Streptavidin-phycobiliprotein conjugates a recombinant fusion protein, which was made in *E. coli* via combinational biosynthesis. SA-PCA-PEB (streptavidin-phycocyanin subunit phycoerythrobilin) and SA-PCA-PCB (streptavidin-phycocyanin subunit phycocyanbilin) were two recombinant fusion proteins. In *E. coli*, a dual plasmid system was used to biosynthesize another recombinant fusion protein called holo-ApcA, which contained streptavidin and the APC-subunit. An in vitro chromophore attachment reaction system including PCB and lyase gene cpcS providing the robust signal for detection was used to improve the rate of chromophorylation of the fusion protein with the prosthetic group PCB [67]. It has been conducted using a similar system of fusion protein with tandem repetitions of APC holo-subunits to increase fluorescence brightness [68].

Table 1. Patents on the diagnosis of human metabolic disorders/syndromes, infectious diseases, and immune disorders employing PBPs as fluorochromes (Modified from Vinothkanna and Sekar [69]).

Diagnostic Parameters	Disease	Patents Number	Patents Source/Country	Reference Link
Cardiac ailments	Cardiac arrhythmia	IN 4322/DELNP/2009	InPASS database, India	http://ipindia.gov.in/ (15 November 2023)
	Diastolic heart failure	IN 421/DELNP/2009	InPASS database, India	http://ipindia.gov.in/ (15 November 2023)
	Acute coronary syndrome	IN 1278/KOLNP/2009	InPASS database, India	http://ipindia.gov.in/ (15 November 2023)
Brain and other central nervous system-related ailments/assessments	Alzheimer's disease (β-peptide formation and cognitive impairment)	US 6770448; IN 148/KOLNP/2010; IN 6811/CHENP/2013	USPTO database, United States of America; InPASS database, India	http://patft.uspto.gov/ (15 November 2023); http://ipindia.gov.in/ (15 November 2023)
	Amyloid associated diseases (Due to Type II Diabetes mellitus, prions, etc.)	IN 380/CHENP/2006	InPASS database, India	http://ipindia.gov.in/ (15 November 2023)
	Multiple neurodegenerative disorders	IN 201717043447; IN 201717043449	InPASS database, India	http://ipindia.gov.in/ (15 November 2023)
Multi-organ ailments	Pulmonary hypertension and cardiac dysfunction	IN 201617043331	InPASS database, India	http://ipindia.gov.in/ (15 November 2023)
	Cardiac and kidney diseases using signal peptides	IN 201818036486	InPASS database, India	http://ipindia.gov.in/ (15 November 2023)
	Kidney disease	CN 109154621	CNIPA database, China	http://english.sipo.gov.cn/ (15 November 2023)
	Liver fibrosis (necrosis of hepatocytes)	IN 2894/DELNP/2004	InPASS database, India	http://ipindia.gov.in/ (15 November 2023)
Infectious Diseases	Microbial infections	US 6649356; JP3336406 (E)	USPTO database, United States of America; J-Plat Pat database, Japan	http://patft.uspto.gov/ (15 November 2023); https://www.jpo.go.jp/ (15 November 2023)
	Tick borne pathogens	CN 103173567	CNIPA database, China	http://english.sipo.gov.cn/ (15 November 2023)
	Mycobacterial infection	JP 2003-534810	J-Plat Pat database, Japan	https://www.jpo.go.jp/ (15 November 2023)

Table 1. *Cont.*

Diagnostic Parameters	Disease	Patents Number	Patents Source/Country	Reference Link
Infectious Diseases	Human Immunodeficiency Virus (HIV) infection	US 5108904; US 5156951; US 5597688	USPTO database, United States of America	http://patft.uspto.gov/ (15 November 2023)
	Japanese encephalitis virus	US 9783596	USPTO database, United States of America	http://patft.uspto.gov/ (15 November 2023)
	Hepatitis B Virus (HBV) genotyping	US 9927439; US 10067135; KR 1020040002964; CN101144815 (E)	USPTO database, United States of America; KIPRIS database, Korea; CNIPA database, China	http://patft.uspto.gov/ (15 November 2023); http://english.sipo.gov.cn/ (15 November 2023)
	Hepatitis C Virus (HCV) genotyping	KR 20040083940 (E); IN 9851/DELNP/2008	KIPRIS database, Korea; InPASS database, India	http://eng.kipris.or.kr/ (15 November 2023); http://ipindia.gov.in/ (15 November 2023)
Organ-specific cancers	Pancreatic cancer	US 7052859; US 7282567; US 8795662; IN 4773/CHENP/2012	USPTO database, United States of America; InPASS database, India	http://patft.uspto.gov/ (15 November 2023); http://ipindia.gov.in/ (15 November 2023)
	Pancreatic adenocarcinoma	US 9238081; IN 4612/DELNP/2012	USPTO database, United States of America; InPASS database, India	http://patft.uspto.gov/ (15 November 2023); http://ipindia.gov.in/ (15 November 2023)
	Lung cancer	CN103383395 (E); CN103439511 (E)	CNIPA database, China	http://english.sipo.gov.cn/ (15 November 2023)
	Breast carcinoma	US 8329875; CN 1012013570; IN 201641017874	USPTO database, United States of America; CNIPA database, China; InPASS database, India	http://patft.uspto.gov/ (15 November 2023); http://english.sipo.gov.cn/ (15 November 2023); http://ipindia.gov.in/ (15 November 2023)
	Cervical cancer	US 7067268; US 7901883; US 8389217; IN 2414/CHENP/2007; IN 3382/DELNP/2015	USPTO database, United States of America; InPASS database, India	http://patft.uspto.gov/ (15 November 2023); http://ipindia.gov.in/ (15 November 2023)

Table 1. *Cont.*

Diagnostic Parameters	Disease	Patents Number	Patents Source/Country	Reference Link
General immune disorders	Autoimmune diseases	US 6596501; US 7674632; US 7771932; CN1866013 (E); IN 4492/DELNP/2015; IN 5860/DELNP/2009	USPTO database, United States of America; CNIPA database, China; InPASS database, India	http://patft.uspto.gov/ (15 November 2023); http://english.sipo.gov.cn/ (15 November 2023); http://ipindia.gov.in/ (15 November 2023)
	Rheumatoid arthritis (autoimmune attack of joints)	JP 2005-527813; KR 1020080036215; IN 2179/MUMNP/2013	J-Plat Pat database, Japan; KIPRIS database, Korea; InPASS database, India	https://www.jpo.go.jp/ (15 November 2023); http://eng.kipris.or.kr/ (15 November 2023); http://ipindia.gov.in/ (15 November 2023)
	Antinuclear antibody	CN 108802375 (E)	CNIPA database, China	http://english.sipo.gov.cn/ (15 November 2023)
	Antihistone antibodies	US 8987421; US 10040848	USPTO database, United States of America	http://patft.uspto.gov/ (15 November 2023)
	Autoantibodies to drugs	IN 7324/DELNP/2013	InPASS database, India	http://ipindia.gov.in/ (15 November 2023)

Abbreviations: IN—InPASS database, India; US—USPTO database, United States of America; CN—CNIPA database, China; (E)—Espacenet database, European Patent Office; JP—J-Plat Pat database, Japan; KR—KIPRIS database, Korea. Patent numbers represent application numbers, except numbers with the prefix "CN" and suffix "(E)" that denote publication numbers. Except for Espacenet, numbers alone (without prefix alphabets) have to be entered in the suitable search field of the corresponding country's database. Such prefix alphabets are meant for identifying the database. In Espacenet alone (tagged with "(E)" as a suffix of numbers), given alphabetic prefixes along with the publication numbers must be fed to access patent details.

3.5. Application in Photodynamic Therapy (PDT)

PDT is a method that causes cancer cells to die by producing free radicals by strong sun radiation and photosensitizers [70]. According to Bharathiraja et al. [71], PDT is a non-invasive, cutting-edge approach to treating infectious illnesses caused by bacteria and viruses, psoriasis, and other terrible, incurable conditions. Compared to localized surgery and traditional chemotherapy and overcoming drug resistance mechanisms such as antibiotics, this treatment offers several benefits. Numerous photosensitizers, including protoporphyrin IX produced from chlorophyll and chlorine 6, have been identified; however, because of their hydrophobic nature, they clump readily in physiological solutions, which lessens the effectiveness of the photodynamic therapy mechanism [72].

Due to its high water solubility, nontoxicity, and immune-modulating qualities, PC offers several benefits over photosensitizers generated from chlorophyll [73–76]. PC might be utilized in PDT for the elimination of cancer without causing any harm to normal cells since it is easily metabolized in normal cells as opposed to diseased cells [71]. Low-level laser treatment (LLLT), a nonthermal irradiation, has recently been created to reduce pain and inflammation during the PDT process (Figure 7). According to Avci et al. [77], this cutting-edge technology has been utilized to treat a wide range of illnesses, including traumatic brain disorders, myocardial infarction, tumor removal, and other cancer-related conditions. Following PC-based PDT treatment, cancer cell lines such as HeLa tumor and MCF-7 breast cancer cells have demonstrated tumor cell death and immunological enhancement [78,79].

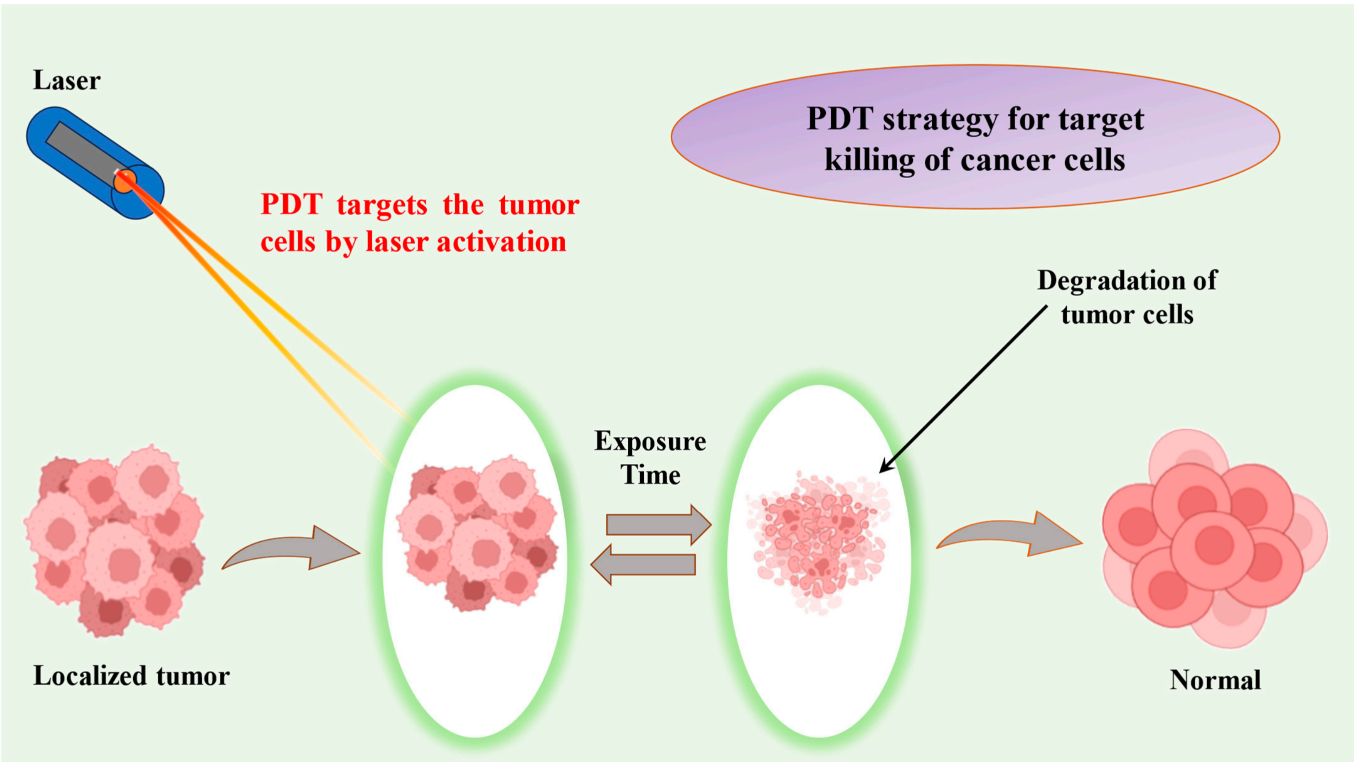


Figure 7. A schematic model for photodynamic therapy treatment, modified from [3].

4. Phycobiliproteins as Bioluminescent Markers

The disintegration or production of tetrapyrrole macrocycles produces the open chain tetrapyrroles, also known as bilins. These days, we know that bilins are involved in many different biological processes, including signaling, photomorphogenesis, redox chemistry, and light-harvesting (in PBSs). Depending on the degree of conjugation, bilins' absorption spectra cover the visible, ultraviolet, and near-infrared (NIR) areas, resulting in a broad spectrum of colors ranging from red/orange to blue/green. Since bilins frequently have very low fluorescence intensities, there are fewer accessible spectra. However, structural rigidification can significantly improve fluorescence, as shown by the widespread usage of biliproteins as fluorescent markers. Much work has gone into developing biliproteins as fluorescent fusion tags because they can absorb light wavelengths that reach the infrared.

Therefore, open-chain tetrapyrroles have applications in many different fields. These include chromophores in genetically modified fluorescence proteins [80], natural food colorants [81], cosmetic patches [82], photosensitizers for photodynamic therapy [83], fluorescent markers [84], absorbing elements in dye-sensitized solar cells [85], and absorbing elements in genetically modified solar cells [83], among many other fields. APCs are the building blocks of PBSs, which are massive light-harvesting antenna complexes found in cyanobacteria and red algae [86]. The α- and β-subunits of APC, ApcA, and ApcB unite to form heterodimers, which autonomously assemble into disk-shaped (αβ)3 trimers. ApcA/ApcB trimers are found in the central cavity of PBSs. The linker proteins ApcE (also known as LCM), ApcD, and ApcF are included in the PBS core. Because APC trimers are naturally luminous, they are frequently utilized as labels in immunofluorescent methods [87]. The conserved Cys residue and the C31 atom of pyrrole ring A are connected via a thioether bond formed by APCs using a PCB chromophore. ApcE binds PCB through an autocatalytic thioether interaction with Cys196. However, bilin lyases are the enzymes that ApcA, -B, -D, and -F require in order to appropriately attach to PCBs to establish a thioether bond with conserved Cys81 residues [88]. Though less effective, the later APCs can bind chromophores without lyases [89]. All APCs have comparable structures: an APC-like domain has seven α-helices that adopt a globin-like fold and an N-terminal extension mostly used in oligomerization [12,90]. In *E. coli*, APCs are generated as red FPs by the co-expression of PCB synthesizing enzymes and the proper bilin lyases [91]. Notably, APCs' spectral characteristics may differ based on where they come from. For instance, "ApcD from *Nostoc* sp. displays a fluorescence peak at 663 nm, but recombinant ApcD from *Synechocystis* sp. exhibits fluorescence with an emission peak at 642 nm" [88]. With an APC-like domain at its N-terminus, ApcE is a massive membrane-associated protein. Its APC-like domain has a 15% quantum yield and a maximum fluorescence at 672 nm when produced as FP in *E. coli*. ApcE-based monomeric soluble fluorescent proteins (FP) were produced with a quantum yield of 6% with emission at 663 nm by truncating the hydrophobic loop and N-terminal residues [92]. *Trichodesmium erythraeum* ApcA was modified to create the far-red FP known as smURFP. TeApcA could bind tetrapyrrole biliverdin (BV) without the need for lyases, thanks to amino acid changes identified through guided mutagenesis. Despite its fluorescence in mammalian cells treated with abundant exogenous BV, smURFP pales compared to BphP-derived NIR fluorescent proteins, including miRFPs [80]. APCs are strongly fluorescent and naturally evolved to maximize fluorescence resonance energy transmission. If everything is considered, APCs encourage molecular templates to create additional red-shifted NIR FPs.

5. Challenges and Future Perspectives in Biomedical Applications

PBPs derived from cyanobacteria possess various biomedical applications, including anti-oxidant, anti-inflammatory, anti-metabolic diseases, anti-cancer, anti-neurodegenerative, anti-pathogenic, etc. [24]. Despite being an important exploitation source in biomedical applications, the major challenge is the production of PBPs on a commercial scale. Extraction and purification at a laboratory scale are possible, but some bottlenecks, including poor selectivity, high energetic costs, and high investment in equipment such as chromatographic techniques, make it difficult to commercialize the production of cyanobacteria PBPs [93]. These drawbacks can be solved through linkages between laboratory researchers and industrial technologists. Although cyanobacteria sustain minimalist nutrients such as light, water, CO_2, and other nutrients, the challenge is to design the industrial bioreactor based on the light exposure and intensity required. A bioreactor should be designed so that the light intensity is uniformly distributed. An adequate amount of light is required for cyanobacteria growth, whereas too high an intensity of light can lead to photoinhibition or overheating [94]. To utilize biomedical applications and deliver services to a broad population, researchers need to focus on developing various approaches directed toward low-cost production and harvesting technologies. There are few cyanobacteria where the production techniques for PBPs have been developed. It is of utmost importance to develop sustainable production technologies for the mass culture of a broader range of cyanobacteria species. Recently, there has been significant progress in the growth of some cyanobacteria through the use of genetic tools and multiomics approaches. There is a great deal of potential to be further investigated in the near future, thanks to sophisticated genome editing techniques.

6. Conclusions

The review delves into the fascinating realm of cyanobacterial PBPs, shedding light on their intricate structures, diverse functions, and promising biomedical potential. Through a thorough exploration of the existing literature, it becomes evident that these pigmented proteins play a pivotal role in the photosynthetic machinery of cyanobacteria. Their unique spectral properties enable them to efficiently capture light energy across a wide range of wavelengths, facilitating photosynthesis in various environments.

Additionally, their antioxidative properties and potential anti-inflammatory effects suggest their utility in biomedical research and drug development. The biomedical potential of PBPs extends to areas such as cancer therapy, where their ability to selectively target cancer cells through photodynamic therapy holds promise. Moreover, their antioxidant properties could have implications for combating oxidative stress-related diseases. The present work underscores the significance of cyanobacterial PBPs in the natural world and highlights their multifaceted roles and biomedical applications. As our understanding of these remarkable molecules continues to grow, so does the potential for innovative applications in various scientific and medical fields, paving the way for a brighter and more sustainable future.

In conclusion, the radiant world of cyanobacterial PBPs presents a captivating area of research with immense scientific, biotechnological, and biomedical potential. Continued exploration of their structures, functions, and applications not only deepens our understanding of fundamental biological processes but also opens doors to innovative technologies and medical treatments.

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References

1. Masojídek, J.; Torzillo, G.; Koblížek, M. Photosynthesis in microalgae. In *Handbook of Microalgal Culture: Applied Phycology and Biotechnology*; Wiley: Hoboken, NJ, USA, 2013; pp. 21–36. [CrossRef]
2. Pagels, F.; Guedes, A.C.; Amaro, H.M.; Kijjoa, A.; Vasconcelos, V. Phycobiliproteins from cyanobacteria: Chemistry and biotechnological applications. *Biotechnol. Adv.* **2019**, *37*, 422–443. [CrossRef] [PubMed]
3. Kannaujiya, V.K.; Sundaram, S.; Sinha, R.P. Structural and functional significance of phycobiliproteins. In *Phycobiliproteins: Recent Developments and Future Applications*; Springer: Berlin/Heidelberg, Germany, 2017; pp. 21–44.
4. Bryant, D.A.; Guglielmi, G.; de Marsac, N.T.; Castets, A.M.; Cohen-Bazire, G. The structure of cyanobacterial phycobilisomes: A model. *Arch. Microbiol.* **1979**, *123*, 113–127. [CrossRef]
5. Arteni, A.A.; Ajlani, G.; Boekema, E.J. Structural organisation of phycobilisomes from *Synechocystis* sp. strain PCC 6803 and their interaction with the membrane. *Biochim. Biophys. Acta Bioenerg.* **2009**, *1787*, 272–279. [CrossRef] [PubMed]
6. Figueroa, M.; Martínez-Oyanedel, J.; Matamala, A.R.; Dagnino-Leone, J.; Mella, C.; Fritz, R.; Bunster, M. In silico model of an antenna of a phycobilisome and energy transfer rates determination by theoretical Förster approach. *Protein Sci.* **2012**, *21*, 1921–1928. [CrossRef]
7. Su, H.N.; Xie, B.B.; Chen, X.L.; Wang, J.X.; Zhang, X.Y.; Zhou, B.C.; Zhang, Y.Z. Efficient separation and purification of allophycocyanin from *Spirulina* (*Arthrospira*) *platensis*. *J. Appl. Phycol.* **2010**, *22*, 65–70. [CrossRef]
8. Dagnino-Leone, J.; Figueroa, M.; Uribe, E.; Hinrichs, M.V.; Ortiz-López, D.; Martínez-Oyanedel, J.; Bunster, M. Biosynthesis and characterization of a recombinant eukaryotic allophycocyanin using prokaryotic accessory enzymes. *Microbiology* **2020**, *9*, e989. [CrossRef]
9. Singh, V.K.; Jha, S.; Rana, P.; Mishra, S.; Kumari, N.; Singh, S.C.; Sinha, R.P. Resilience and mitigation strategies of cyanobacteria under ultraviolet radiation stress. *Int. J. Mol. Sci.* **2023**, *24*, 12381. [CrossRef] [PubMed]
10. Manirafasha, E.; Guo, L.; Jing, K. Nutraceutical and pharmaceutical applications of phycobiliproteins. In *Pigments from Microalgae Handbook*; Springer: Berlin/Heidelberg, Germany, 2020; pp. 575–584.
11. Gupta, A.; Singh, A.P.; Singh, V.K.; Singh, P.R.; Jaiswal, J.; Kumari, N.; Sinha, R.P. Natural sun-screening compounds and DNA-repair enzymes: Photoprotection and photoaging. *Catalysts* **2023**, *13*, 745. [CrossRef]
12. Scheer, H.; Zhao, K.H. Biliprotein maturation: The chromophore attachment. *Mol. Microbiol.* **2008**, *68*, 263–276. [CrossRef]
13. Ma, J.; You, X.; Sun, S.; Wang, X.; Qin, S.; Sui, S.F. Structural basis of energy transfer in *Porphyridium purpureum* phycobilisome. *Nature* **2020**, *579*, 146–151. [CrossRef]
14. Hirose, Y.; Chihong, S.; Watanabe, M.; Yonekawa, C.; Murata, K.; Ikeuchi, M.; Eki, T. Diverse chromatic acclimation processes regulating phycoerythrocyanin and rod-shaped phycobilisome in cyanobacteria. *Mol. Plant* **2019**, *12*, 715–725. [CrossRef] [PubMed]
15. Alvey, R.M.; Biswas, A.; Schluchter, W.M.; Bryant, D.A. Attachment of noncognate chromophores to CpcA of *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7002 by heterologous expression in *Escherichia coli*. *Biochemistry* **2011**, *50*, 4890–4902. [CrossRef] [PubMed]
16. McGregor, A.; Klartag, M.; David, L.; Adir, N. Allophycocyanin trimer stability and functionality are primarily due to polar enhanced hydrophobicity of the phycocyanobilin binding pocket. *J. Mol. Biol.* **2008**, *384*, 406–421. [CrossRef] [PubMed]
17. Li, W.; Su, H.N.; Pu, Y.; Chen, J.; Liu, L.N.; Liu, Q.; Qin, S. Phycobiliproteins: Molecular structure, production, applications, and prospects. *Biotechnol. Adv.* **2019**, *37*, 340–353. [CrossRef] [PubMed]
18. Marx, A.; Adir, N. Allophycocyanin and phycocyanin crystal structures reveal facets of phycobilisome assembly. *Biochim. Biophys. Acta Bioenerg.* **2013**, *1827*, 311–318. [CrossRef] [PubMed]
19. Zhang, J.; Ma, J.; Liu, D.; Qin, S.; Sun, S.; Zhao, J.; Sui, S.F. Structure of phycobilisome from the red alga *Griffithsia pacifica*. *Nature* **2017**, *551*, 57–63. [CrossRef]
20. Singh, N.K.; Sonani, R.R.; Rastogi, R.P.; Madamwar, D. The phycobilisomes: An early requisite for efficient photosynthesis in cyanobacteria. *EXCLI J.* **2015**, *14*, 268. [CrossRef]
21. Sui, S.F. Structure of phycobilisomes. *Annu. Rev. Biophys.* **2021**, *50*, 53–72. [CrossRef]
22. Dagnino-Leone, J.; Figueroa, C.P.; Castañeda, M.L.; Youlton, A.D.; Vallejos-Almirall, A.; Agurto-Muñoz, A.; Agurto-Muñoz, C. Phycobiliproteins: Structural aspects, functional characteristics, and biotechnological perspectives. *Comput. Struct. Biotechnol. J.* **2022**, *20*, 1506–1527. [CrossRef]
23. Stadnichuk, I.N.; Krasilnikov, P.M.; Zlenko, D.V. Cyanobacterial phycobilisomes and phycobiliproteins. *Microbiology* **2015**, *84*, 101–111. [CrossRef]
24. Chen, H.; Qi, H.; Xiong, P. Phycobiliproteins-A family of algae-derived biliproteins: Productions, characterization and pharmaceutical potentials. *Mar. Drugs* **2022**, *20*, 450. [CrossRef] [PubMed]
25. Zehetmayer, P.; Hellerer, T.; Parbel, A.; Scheer, H.; Zumbusch, A. Spectroscopy of single phycoerythrocyanin monomers: Dark state identification and observation of energy transfer heterogeneities. *Biophys. J.* **2002**, *83*, 407–415. [CrossRef] [PubMed]
26. Kikuchi, H. Redshifting and blueshifting of β82 chromophores in the phycocyanin hexamer of *Porphyridium purpureum* phycobilisomes due to linker proteins. *Life* **2022**, *12*, 1833. [CrossRef] [PubMed]
27. MacColl, R.; Eisele, L.E.; Malak, H.; Endres, R.L.; Williams, E.C.; Bowser, S.S. Studies on R-phycoerythrins from two Antarctic marine red algae and a mesophilic red alga. *Polar Biol.* **1999**, *22*, 384–388. [CrossRef]

28. Stadnichuk, I.N.; Tropin, I.V. Phycobiliproteins: Structure, functions and biotechnological applications. *Appl. Biochem. Microbiol.* **2017**, *53*, 1–10. [CrossRef]
29. Liu, L.N.; Chen, X.L.; Zhang, Y.Z.; Zhou, B.C. Characterization, structure and function of linker polypeptides in phycobilisomes of cyanobacteria and red algae: An overview. *Biochim. Biophys. Acta Bioenerg.* **2005**, *1708*, 133–142. [CrossRef] [PubMed]
30. Stadnichuk, I.N.; Kusnetsov, V.V. Phycobilisomes and phycobiliproteins in the pigment apparatus of oxygenic photosynthetics: From cyanobacteria to tertiary endosymbiosis. *Int. J. Mol. Sci.* **2023**, *24*, 2290. [CrossRef]
31. Nganou, C.; David, L.; Adir, N.; Mkandawire, M. Linker proteins enable ultrafast excitation energy transfer in the phycobilisome antenna system of *Thermosynechococcus vulcanus*. *Photochem. Photobiol. Sci.* **2016**, *15*, 31–44. [CrossRef]
32. Richa, K.V.; Kesheri, M.; Singh, G.; Sinha, R.P. Biotechnological potentials of phycobiliproteins. *Int. J. Pharma Bio Sci.* **2011**, *2*, 446–454.
33. Arulselvan, P.; Fard, M.T.; Tan, W.S.; Gothai, S.; Fakurazi, S.; Norhaizan, M.E.; Kumar, S.S. Role of antioxidants and natural products in inflammation. *Oxid. Med. Cell. Longev.* **2016**, *2016*, 5276130. [CrossRef]
34. Hirata, T.; Tanaka, M.; Ooike, M.; Tsunomura, T.; Sakaguchi, M. Antioxidant activities of phycocyanobilin prepared from *Spirulina platensis*. *J. Appl. Phycol.* **2000**, *12*, 435–439. [CrossRef]
35. Singh, P.R.; Gupta, A.; Pathak, J.; Sinha, R.P. Phylogenetic distribution, structural analysis and interaction of nucleotide excision repair proteins in cyanobacteria. *DNA Repair* **2023**, *126*, 103487. [CrossRef] [PubMed]
36. Strasky, Z.; Zemankova, L.; Nemeckova, I.; Rathouska, J.; Wong, R.J.; Muchova, L.; Nachtigal, P. *Spirulina platensis* and phycocyanobilin activate atheroprotective heme oxygenase-1: A possible implication for atherogenesis. *Food Funct.* **2013**, *4*, 1586–1594. [CrossRef] [PubMed]
37. Benedetti, S.; Benvenuti, F.; Scoglio, S.; Canestrari, F. Oxygen radical absorbance capacity of phycocyanin and phycocyanobilin from the food supplement *Aphanizomenon flos-aquae*. *J. Med. Food* **2010**, *13*, 223–227. [CrossRef] [PubMed]
38. Patel, S.N.; Sonani, R.R.; Jakharia, K.; Bhastana, B.; Patel, H.M.; Chaubey, M.G.; Madamwar, D. Antioxidant activity and associated structural attributes of *Halomicronema* phycoerythrin. *Int. J. Biol. Macromol.* **2018**, *111*, 359–369. [CrossRef] [PubMed]
39. Datla, P. The wonder molecule called phycocyanin. In *Parry Nutraceuticals*; Division of EID Parry (India) Ltd.: Chennai, India, 2011.
40. Cervantes-Llanos, M.; Lagumersindez-Denis, N.; Marín-Prida, J.; Pavón-Fuentes, N.; Falcon-Cama, V.; Piniella-Matamoros, B.; Pentón-Rol, G. Beneficial effects of oral administration of C-phycocyanin and phycocyanobilin in rodent models of experimental autoimmune encephalomyelitis. *Life Sci.* **2018**, *194*, 130–138. [CrossRef]
41. Gdara, N.B.; Belgacem, A.; Khemiri, I.; Mannai, S.; Bitri, L. Protective effects of phycocyanin on ischemia/reperfusion liver injuries. *Biomed. Pharmacother.* **2018**, *102*, 196–202. [CrossRef] [PubMed]
42. Liu, Q.; Huang, Y.; Zhang, R.; Cai, T.; Cai, Y. Medical application of *Spirulina platensis* derived C-phycocyanin. *Evid. Based Complement. Altern. Med.* **2016**, *2016*, 7803846. [CrossRef]
43. Simmons, T.L.; Andrianasolo, E.; McPhail, K.; Flatt, P.; Gerwick, W.H. Marine natural products as anticancer drugs. *Mol. Cancer Ther.* **2005**, *4*, 333–342. [CrossRef]
44. Ravi, M.; Tentu, S.; Baskar, G.; Rohan Prasad, S.; Raghavan, S.; Jayaprakash, P.; Venkatraman, G. Molecular mechanism of anti-cancer activity of phycocyanin in triple-negative breast cancer cells. *BMC Cancer* **2015**, *15*, 768. [CrossRef]
45. McCarty, M.F.; Iloki-Assanga, S.; Lujany, L.M.L. Nutraceutical targeting of TLR4 signaling has potential for prevention of cancer cachexia. *Med. Hypotheses* **2019**, *132*, 109326. [CrossRef] [PubMed]
46. Hao, S.; Li, S.; Wang, J.; Yan, Y.; Ai, X.; Zhang, J.; Wang, C. Phycocyanin exerts anti-proliferative effects through down-regulating TIRAP/NF-κB activity in human non-small cell lung cancer cells. *Cells* **2019**, *8*, 588. [CrossRef] [PubMed]
47. Ying, J.; Tang, Z.; Zhao, G.; Li, X.; Pan, R.; Lin, S.; Yan, C. Transcriptionomic Study on Apoptosis of SKOV-3 Cells Induced by Phycoerythrin from Gracilaria lemaneiformis. *Anti-Cancer Agents Med. Chem.* **2021**, *21*, 1240–1249. [CrossRef] [PubMed]
48. Nathan, C. Points of Control in Inflammation. *Nature* **2002**, *420*, 846–852. [CrossRef]
49. Piniella-Matamoros, B.; Marin-Prida, J.; Penton-Rol, G. Nutraceutical and therapeutic potential of phycocyanobilin for treating Alzheimer's disease. *J. Biosci.* **2021**, *46*, 42. [CrossRef]
50. Leung, P.O.; Lee, H.H.; Kung, Y.C.; Tsai, M.F.; Chou, T.C. Therapeutic effect of C-phycocyanin extracted from blue green algae in a rat model of acute lung injury induced by lipopolysaccharide. *Evid.-Based Complement. Altern. Med.* **2013**, *2013*, 916590. [CrossRef]
51. Kefayat, A.; Ghahremani, F.; Safavi, A.; Hajiaghababa, A.; Moshtaghian, J. C-phycocyanin: A natural product with radiosensitizing property for enhancement of colon cancer radiation therapy efficacy through inhibition of COX-2 expression. *Sci. Rep.* **2019**, *9*, 19161. [CrossRef]
52. Bannu, S.M.; Lomada, D.; Gulla, S.; Chandrasekhar, T.; Reddanna, P.; Reddy, M.C. Potential therapeutic applications of C-phycocyanin. *Curr. Drug Metab.* **2019**, *20*, 967–976. [CrossRef]
53. Kim, K.M.; Lee, J.Y.; Im, A.R.; Chae, S. Phycocyanin protects against UVB-induced apoptosis through the PKC α/βII-Nrf-2/HO-1 dependent pathway in human primary skin cells. *Molecules* **2018**, *23*, 478. [CrossRef]
54. Fernández-Rojas, B.; Hernández-Juárez, J.; Pedraza-Chaverri, J. Nutraceutical properties of phycocyanin. *J. Funct. Foods* **2014**, *11*, 375–392. [CrossRef]
55. Prabakaran, G.; Sampathkumar, P.; Kavisri, M.; Moovendhan, M. Extraction and characterization of phycocyanin from *Spirulina platensis* and evaluation of its anticancer, antidiabetic and anti-inflammatory effect. *Int. J. Biol. Macromol.* **2020**, *153*, 256–263. [CrossRef] [PubMed]

56. Wang, C.; Shen, Z.; Li, L.; Li, Y.; Zhao, H.; Jiang, X. Immunomodulatory activity of R-phycoerythrin from *Porphyra haitanensis* via TLR4/NF-κB-dependent immunocyte differentiation. *Food Funct.* **2020**, *11*, 2173–2185. [CrossRef] [PubMed]
57. Gonzalez, R.; Rodriguez, S.; Romay, C.H.E.Y.L.A.; Gonzalez, A.; Armesto, J.; Remirez, D.; Merino, N. Anti-inflammatory activity of phycocyanin extract in acetic acid-induced colitis in rats. *Pharmacol. Res.* **1999**, *39*, 55–59. [CrossRef] [PubMed]
58. Bergmeier, W.; Schulte, V.; Brockhoff, G.; Bier, U.; Zirngibl, H.; Nieswandt, B. Flow cytometric detection of activated mouse integrin αIIbβ3 with a novel monoclonal antibody. *Cytometry* **2002**, *48*, 80–86. [CrossRef] [PubMed]
59. Pan, Y.; Wei, X.; Liang, T.; Zhou, J.; Wan, H.; Hu, N.; Wang, P. A magnetic beads-based portable flow cytometry immunosensor for in-situ detection of marine biotoxin. *Biomed. Microdevices* **2018**, *20*, 60. [CrossRef]
60. Xu, Y.; Hou, Y.; Wang, Y.; Wang, Y.; Li, T.; Song, C.; Wei, N.; Wang, Q. Sensitive and selective detection of Cu^{2+} ions based on fluorescent Ag nanoparticles synthesized by R-phycoerythrin from marine algae *Porphyra yezoensis*. *Ecotoxicol. Environ. Saf.* **2019**, *168*, 356–362. [CrossRef] [PubMed]
61. Patel, D.; Good, T. A rapid method to measure beta-amyloid induced neurotoxicity in vitro. *J. Neurosci. Methods.* **2007**, *161*, 1–10. [CrossRef]
62. Wada, T.; Sakakibara, Y.; Nishimura, R.; Toma, T.; Ueno, Y.; Horita, S.; Tanaka, T.; Nishi, M.; Kato, K.; Yasumi, T.; et al. Down-regulation of CD5 expression on activated $CD8^{+}$ T cells in familial hemophagocytic lymphohistiocytosis with perforin gene mutations. *Human Immunol.* **2013**, *74*, 1579–1585. [CrossRef]
63. Lin, Z.; Jiang, B.P.; Liang, J.; Wen, C.; Shen, X.C. Phycocyanin functionalized single-walled carbon nanohorns hybrid for near-infrared light-mediated cancer phototheranostics. *Carbon* **2019**, *143*, 814–827. [CrossRef]
64. Gupta, R.; Jain, P.; Deo, S.V.S.; Sharma, A. Flow cytometric analysis of $CD5^{+}$ B cells: A frame of reference for minimal residual disease analysis in chronic lymphocytic leukemia. *Am. J. Clin. Pathol.* **2004**, *121*, 368–372. [CrossRef]
65. Ge, B.; Lin, X.; Chen, Y.; Wang, X.; Chen, H.; Jiang, P.; Huang, F. Combinational biosynthesis of dual-functional streptavidin-phycobiliproteins for high-throughput-compatible immunoassay. *Process Biochem.* **2017**, *58*, 306–312. [CrossRef]
66. Tawa, K.; Yamamura, S.; Sasakawa, C.; Shibata, I.; Kataoka, M. Sensitive detection of cell surface membrane proteins in living breast cancer cells using multicolor fluorescence microscopy with a plasmonic chip. *ACS Appl. Mater. Interfaces* **2016**, *8*, 29893–29898. [CrossRef] [PubMed]
67. Wu, J.; Chen, H.; Jiang, P. Chromophore attachment to fusion protein of streptavidin and recombinant allophycocyanin α subunit. *Bioengineered* **2018**, *9*, 108–115. [CrossRef] [PubMed]
68. Chen, H.; Jiang, P. Combinational biosynthesis and characterization of fusion proteins with tandem repeats of allophycocyanin holo-α subunits, and their application as bright fluorescent labels for immunofluorescence assay. *J. Biosci. Bioeng.* **2018**, *126*, 778–782. [CrossRef] [PubMed]
69. Vinothkanna, A.; Sekar, S. Diagnostic applications of phycobiliproteins. In *Pigments from Microalgae Handbook*; Springer: Berlin/Heidelberg, Germany, 2020; pp. 585–610.
70. Wainwright, M. Photodynamic therapy-from dyestuffs to high-tech clinical practice. *Rev. Prog. Color Relat. Top.* **2004**, *34*, 95–109. [CrossRef]
71. Bharathiraja, S.; Seo, H.; Manivasagan, P.; Santha Moorthy, M.; Park, S.; Oh, J. In vitro photodynamic effect of phycocyanin against breast cancer cells. *Molecules* **2016**, *21*, 1470. [CrossRef] [PubMed]
72. Ormond, A.B.; Freeman, H.S. Dye sensitizers for photodynamic therapy. *Materials* **2013**, *6*, 817–840. [CrossRef]
73. Muthulakshmi, M.; Saranya, A.; Sudha, M.; Selvakumar, G. Extraction, partial purification, and antibacterial activity of phycocyanin from *Spirulina* isolated from fresh water body against various human pathogens. *J. Algal Biomass Util.* **2012**, *3*, 7–11.
74. Chen, J.C.; Liu, K.S.; Yang, T.J.; Hwang, J.H.; Chan, Y.C.; Lee, I.T. *Spirulina* and C-phycocyanin reduce cytotoxicity and inflammation-related genes expression of microglial cells. *Nutr. Neurosci.* **2012**, *15*, 252–256. [CrossRef]
75. Chen, H.W.; Yang, T.S.; Chen, M.J.; Chang, Y.C.; Eugene, I.; Wang, C.; Ho, C.L.; Lai, Y.J.; Yu, C.C.; Chou, J.C.; et al. Purification and immunomodulating activity of C-phycocyanin from *Spirulina platensis* cultured using power plant flue gas. *Process Biochem.* **2014**, *49*, 1337–1344. [CrossRef]
76. Chen, E.P.; Markosyan, N.; Connolly, E.; Lawson, J.A.; Li, X.; Grant, G.R.; Grosser, T.; FitzGerald, G.A.; Smyth, E.M. Myeloid Cell COX-2 deletion reduces mammary tumor growth through enhanced cytotoxic T-lymphocyte function. *Carcinogenesis* **2014**, *35*, 1788–1797. [CrossRef] [PubMed]
77. Avci, P.; Gupta, A.; Sadasivam, M.; Vecchio, D.; Pam, Z.; Pam, N.; Hamblin, M.R. Low-level laser (light) therapy (LLLT) in skin: Stimulating, healing, restoring. *Semin Cutan. Med. Surg.* **2013**, *32*, 41–52. [PubMed]
78. Li, B.; Chu, X.; Gao, M.; Li, W. Apoptotic mechanism of MCF-7 breast cells in vivo and in vitro induced by photodynamic therapy with C-phycocyanin. *Acta Biochim. Biophys. Sin.* **2010**, *42*, 80–89. [CrossRef] [PubMed]
79. Li, B.; Chu, X.M.; Gao, M.H. Treatment of HeLa tumor in mice with c-phycocyanin mediated photodynamic therapy and its immune mechanism underlying apoptosis. *Chin. J. Laser Med. Surg.* **2011**, *20*, 1–6.
80. Rodriguez, E.A.; Tran, G.N.; Gross, L.A.; Crisp, J.L.; Shu, X.; Lin, J.Y.; Tsien, R.Y. A far-red fluorescent protein evolved from a cyanobacterial phycobiliprotein. *Nat. Methods* **2016**, *13*, 763–769. [CrossRef]
81. Jespersen, L.; Strømdahl, L.D.; Olsen, K.; Skibsted, L.H. Heat and light stability of three natural blue colorants for use in confectionery and beverages. *Eur. Food Res. Technol.* **2005**, *220*, 261–266. [CrossRef]
82. Adli, S.A.; Ali, F.; Azmi, A.S.; Anuar, H.; Nasir, N.A.M.; Hasham, R.; Idris, M.K.H. Development of biodegradable cosmetic patch using a polylactic acid/phycocyanin–alginate composite. *Polymer* **2020**, *12*, 1669. [CrossRef]

83. Zhang, Y.; Liu, H.; Dai, X.; Li, H.; Zhou, X.; Chen, S.; Li, Z. Cyanobacteria-based near-infrared light-excited self-supplying oxygen system for enhanced photodynamic therapy of hypoxic tumors. *Nano Res.* **2021**, *14*, 667–673. [CrossRef]
84. Sekar, S.; Chandramohan, M. Phycobiliproteins as a commodity: Trends in applied research, patents and commercialization. *J. Appl. Phycol.* **2008**, *20*, 113–136. [CrossRef]
85. Xing, F.L.; Zhang, Z.H.; Yang, C.L.; Wang, M.S.; Ma, X.G. Phycoerythrobilin/phycourobilin as efficient sensitizers of dye-sensitized solar cell. *Soil Energy* **2022**, *243*, 494–499. [CrossRef]
86. Grossman, A.R.; Schaefer, M.R.; Chiang, G.G.; Collier, J. The phycobilisome, a light-harvesting complex responsive to environmental conditions. *Microbiol. Rev.* **1993**, *57*, 725–749. [CrossRef] [PubMed]
87. Loos, D.; Cotlet, M.; De Schryver, F.; Habuchi, S.; Hofkens, J. Single-molecule spectroscopy selectively probes donor and acceptor chromophores in the phycobiliprotein allophycocyanin. *Biophys. J.* **2004**, *87*, 2598–2608. [CrossRef] [PubMed]
88. Peng, P.P.; Dong, L.L.; Sun, Y.F.; Zeng, X.L.; Ding, W.L.; Scheer, H.; Zhao, K.H. 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. [CrossRef] [PubMed]
89. Xu, Q.Z.; Han, J.X.; Tang, Q.Y.; Ding, W.L.; Miao, D.; Zhou, M.; Zhao, K.H. Far-red light photoacclimation: Chromophorylation of FR induced α-and β-subunits of allophycocyanin from *Chroococcidiopsis thermalis* sp. PCC7203. *Biochim. Biophys. Acta Bioenerg.* **2016**, *1857*, 1607–1616. [CrossRef]
90. Sonani, R.R.; Gupta, G.D.; Madamwar, D.; Kumar, V. Crystal structure of allophycocyanin from marine cyanobacterium *Phormidium* sp. A09DM. *PLoS ONE* **2015**, *10*, e0124580. [CrossRef]
91. Biswas, A.; Vasquez, Y.M.; Dragomani, T.M.; Kronfel, M.L.; Williams, S.R.; Alvey, R.M.; Schluchter, W.M. Biosynthesis of cyanobacterial phycobiliproteins in *Escherichia coli*: Chromophorylation efficiency and specificity of all bilin lyases from *Synechococcus* sp. strain PCC 7002. *Appl. Environ. Microbiol.* **2010**, *76*, 2729–2739. [CrossRef]
92. Tang, K.; Ding, W.L.; Höppner, A.; Zhao, C.; Zhang, L.; Hontani, Y.; Zhao, K.H. The terminal phycobilisome emitter, LCM: A light-harvesting pigment with a phytochrome chromophore. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 15880–15885. [CrossRef]
93. Kovaleski, G.; Kholany, M.; Dias, L.; Correia, S.F.; Ferreira, R.A.; Coutinho, J.A.; Ventura, S.P. Extraction and purification of phycobiliproteins from algae and their applications. *Front. Chem.* **2022**, *10*, 1065355. [CrossRef]
94. Zahra, Z.; Choo, D.H.; Lee, H.; Parveen, A. Cyanobacteria: Review of current potentials and applications. *Environments* **2020**, *7*, 13. [CrossRef]