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Site, trigger, quenching mechanism and recovery of non-photochemical quenching in cyanobacteria: Recent updates

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Abstract

Cyanobacteria exhibit a novel form of non-photochemical quenching (NPQ) at the level of the phycobilisome. NPQ is a process that protects photosystem II (PSII) from possible highlight-induced photo-damage. Although significant advancement has been made in understanding the NPQ, there are still some missing details. This critical review focuses on how the orange carotenoid protein (OCP) and its partner fluorescence recovery protein (FRP) control the extent of quenching. What is and what is not known about the NPQ is discussed under four subtitles; where does exactly the site of quenching lie? (*site*), how is the quenching being triggered? (*trigger*), molecular mechanism of quenching (*quenching*) and *recovery* from quenching. Finally, a recent working model of NPQ, consistent with recent findings, is been described.

Keywords:

Cyanobacteria; Non-photochemical quenching; Orange carotenoid protein; Phycobilisome; Fluorescence recovery protein

1. Introduction

Most aerobic photosynthetic organisms frequently encounter situations where the intensity of the incident solar radiation is more than saturating for photosynthesis (Franck and Gaffron 1941; Melis 2009). This situation is potentially damaging, especially for photosystem II (Melis 1999). One of the key defence mechanisms that have evolved to mitigate this problem is non-photochemical quenching (NPQ). In NPQ, the efficiency of the light harvesting system is reduced so that the excess solar energy is dissipated as heat rather than being transferred to the reaction centre (RC) where the major potential site of damage is located (Müller et al. 2001). NPQ in plants occurs in the light harvesting complex II (LHCII) antenna and is triggered by changes in the thylakoid luminal-pH (Ruban et al. 2007; Ruban 2016). Recently, a novel form of NPQ regulating the light harvesting efficiency of the phycobilisome (PB) has been described in cyanobacteria (El Bissati et al. 2000; Wilson et al. 2006). The PB is devoid of *chlorophyll* and is located proximal to the reaction centre at the outer surface of thylakoid membrane. The PB contains linear tetrapyrrol chromophores covalently attached to its major constituent proteins called phycobiliproteins (PBPs) (Adir 2008; Singh et al. 2015). Typically the PB is composed of two major domains; the *core*, made up of the low energy absorbing PBP allophycocyanin (APC; λ_{max}, 650-655 nm) that is directly associated with the RCs on thylakoid membrane and the *rods*, made up of the higher energy absorbing PBP phycocyanin (PC; λ_{max}, 600-620 nm) and/or phycoerythrin (PE; λ_{max}, 540-565 nm) that are arranged perpendicular to the *core* as mini-rods (Fig. 1A) (Adir 2005). This arrangement of PBPs in the PB creates an energy gradient from PE → PC → APC → RC, which enables the absorbed-light energy to be 'funnelled' from *rods* to RC via the *core* (Fig. 1B) (Gantt and Lipschultz 1973; Adir 2008). NPQ in cyanobacteria occurs at the level of the PB-*core* and involves a water-soluble protein called the orange carotenoid protein (OCP) (Kirilovsky 2007). This critical review provides an up to date account of what is, and what is not, known

about the *site*, *trigger*, *quenching mechanism* and the *recovery process* of NPQ in cyanobacteria.

2. *Site*: APC_{660} trimer of the PB-core

Simultaneous reduction in both maximal ($F_{m'}$) and minimal ($F_{0'}$) fluorescence observed in NPQ is the characteristic hallmark of an antenna based quenching mechanism (El Bissati et al. 2000). Interestingly, this mechanism exists in the strains without *Chl* antennae CP43, CP47 and RCII (Rakhimberdieva et al. 2004; Scott et al. 2005; Wilson et al. 2006). With these facts, the non-*Chl* antenna PB was suggested as a possible *site* of NPQ. This has been confirmed by inability of PB-lacking cyanobacteria to perform NPQ under highlight (Wilson et al. 2006). Furthermore, a mutant whose PB is made up of only PC *rods* (lacking APC *core*) was unable to show the quenching, whereas another mutant whose PB is made up of only APC *cores* (lacking PC *rods*) does show quenching (Wilson et al. 2006). The room temperature fluorescence decay kinetics during NPQ indicate that the enhanced decay component originates from the APC PB-*core*, whereas those components that originate from PC remain unaltered (Scott et al. 2006). These results imply collectively that the presumed '*quencher*' of NPQ is 'the interaction of OCP with PB-*core*'. This was later experimentally confirmed by *in vitro* reconstitution of the cyanobacterial NPQ (Gwizdala et al. 2011). Generally, the PB-*core* is made up of three APC cylinders; two, attached to the thylakoid membrane and one, positioned on the top of these two. Each of them contains four discs, mainly made up of APC that have their fluorescence emission centred at 660 nm (APC_{660}) (Fig. 2). APC cylinders, attached to thylakoid membrane also contain some other proteins called terminal emitters (ApcD, ApcF and ApcE); that along with APC in these discs have their fluorescence emission centred at 680 nm (APC_{680}) (Glazer 1984; MacColl 1998; Adir 2005; Singh et al. 2015) (Fig. 2). APC_{680} functions as an energy transfer-bridge coupling the PB APC_{660} to the RCs.

It is clear that OCP^r binds to PB-*core* very specifically since only one OCP^r molecule is required to quench the fluorescence of one PB (Gwizdala et al. 2011). The binding of OCP^r to the PB has been proposed to be with the two basal cylinders of PB-*core* because the presence of upper cylinder is noticed to be non-essential for NPQ (Jallet et al. 2012). As discussed above (Fig. 2), in basal cylinders of PB-*core*, two possible candidates APC_{660} and $APC_{680,}$ have been proposed to be the OCP-binding site. Time resolved fluorescence measurements of NPQ have shown that the fluorescence originating from APC_{660} is quenched (Tian et al. 2011; Tian et al. 2012) and these make the hypothesis 'OCP^r binds to APC_{660}' reasonable. The observation of cross-linked OCP-PB in the quenched state has provided evidence of closer proximity of OCP^r with APC_{680} during NPQ (Zhang et al. 2013). Moreover, the observation of quenching of APC_{680} fluorescence (Kuzminov et al. 2012) combined with a docking study suggesting a role of the PB-loop of APC_{680} in the OCP-APC interaction (Stadnichuk et al. 2013) opened up the possibility of OCP^r-APC_{680} interaction in NPQ. However, NPQ persists in a *Synechocystis* ApcE (APC_{680})-mutant (containing chromophore-less ApcE). This suggests that the APC_{680} is not a site of quenching but it rather may be a part of the OCP-binding site.

3. *Trigger*: Photo-conversion of OCP^o to OCP^r

The OCP, a unique carotenoid-containing water soluble protein, is now widely accepted as the '*trigger*' molecule for NPQ in cyanobacteria. It was first discovered in three cyanobacterial genera by Holt and Krogmann (1981). Its function remained unclear for almost two decades until NPQ in cyanobacteria was discovered. El Bissati et al. (2000) noticed highlight-induced reversible fluorescence quenching in *Synechocystis*, which is the first evidence of NPQ in cyanobacteria. This quenching was found to recover very quickly even in the presence of protein synthesis inhibitors, which does not happen in the other well-known response to highlight induced damage, the D1 repair cycle (Melis 1999). The

involvement of a carotenoid pigment in NPQ was first proposed by Rakhimberdieva et al. (2004) based on the '*quenched-action spectra*' showing typical carotenoid absorption (500, 470 and 430 nm) bands. However, the first report that orange carotenoid protein (OCP) (defined by Holt and Krogmann (1981)) is involved in NPQ was from Wilson et al. (2006). These authors observed the increased vulnerability of an OCP-lacking mutant to highlight. This was further confirmed by the same group when they noticed increased-NPQ activity in an OCP-over-expressing mutant (Wilson et al. 2008).

Generally, OCP is found in two interchangeable conformational isoforms; orange (OCP^o) and red (OCP^r) (Wilson et al. 2008). OCP^r is the active form and has been found to accumulate during NPQ in whole cells (Wilson et al. 2008). *In vitro* binding studies show that only OCP^r binds to the PB resulting in the quenching of its fluorescence (Gwizdala et al. 2011). Both isoforms contain a single non-covalently bound carotenoid, 3-hydroxyechinenone (hECN) that has a hydroxyl group at one end and keto-group at the other (Kerfeld et al. 2003; Leverenz et al. 2015). Absorption of high intensity light by this carotenoid induces the conversion of OCP^o to OCP^r and this then '*triggers*' NPQ in cyanobacteria. However, the key question - 'How exactly does this interconversion occur at molecular level?' is still unanswered.

Most of the detailed information on the changes upon the conversion of OCP^o to OCP^r has come from structural studies. In the crystal, OCP^o is found in the dimeric form, bears an antiparallel association of two OCP^o-monomers stabilized by both hydrophobic and polar interactions between several conserved or conservatively substituted residues (Kerfeld et al. 2003). In another works, OCP was found as a dimer in solution (Zhang et al. 2013; Bao et al. 2017a). However, the exact oligomeric form of OCP *in vivo* has not been established unequivocally due to the following contradictory results. The OCP was determined to be in the monomeric form through size exclusion chromatography (SEC), Native-PAGE and Small

Angle X-ray Scattering (SAXS) analysis (Leverenz et al. 2014; Gupta et al. 2015; Maksimov et al. 2017a; Moldenhauer et al. 2017a). Furthermore, the light-trigged conversion of OCP-dimer into monomer has been described (Zhang et al. 2013). These contradictory results raised the doubt that the OCP-dimer is might be an artefact of the high concentration of protein used for crystallization. Moreover, at higher protein concentration the presence of dimeric form of OCP is as compared to monomeric form (Lu et al. 2016). What is the real functionally relevant state of OCP is, therefore, still an open question?

Structurally, the OCP^{o}-monomer comprises of 13 α helices and 6 β strands, arranged in two domains, an all helical C-terminal domain (CTD) and the α/β N-terminal domain (NTD). The OCP contains a single carotenoid, which is buried and shared between these two main domains (Fig. 3A). The NTD consists of two clearly distinguished sub-domains, each made up of four α helices, sub-domain 1 and sub-domain 2 (Fig. 3A). The cleft between these sub-domains provides the binding pocket for carotenoid's hydroxyl end group. The fold of the CTD belongs to the nuclear transport factor 2 (NTF2) superfamily, and its β-strands are bent to form the hydrophobic binding pocket for the carotenoid's keto end (Fig. 3A). Strong H-bonds between the carotenoid's keto end with conserved Trp290 and Tyr203 (Trp288 and Tyr201 in some cases) residues hold carotenoid more firmly at this keto end (Fig. 3A). These H-bonds have been proposed to have a major role in maintaining the stability of OCP^{o} as the other end (hydroxyl end) of carotenoid does not form any direct hydrogen bond with protein, rather only with water molecule. The importance of these H-bonds in the stability of OCP^{o} has been confirmed by removal of either of the amino acids (Tyr201/Trp288 or Carbonyl group of carotenoids) participating in this H-bond. Mutation of Tyr201 and Trp288 has been noticed to decrease the stability of OCP^{o} form (Wilson et al. 2011; Sluchanko et al. 2017a). Similarly, the importance of the carbonyl group (of carotenoid) participation in this crucial H-bonds has also been confirmed by the dramatic decrease in stability of OCP^{o}, when

carotenoids (like zeaxanthin) that do not contain a keto-group are incorporated (Punginelli et al. 2009; Wilson et al. 2011). The carotenoid is ~24 Å long in OCP and adopts a trans-configuration with an average deviation of 16^{o} from planarity between its two end rings (Kerfeld et al. 2003). This deviation from planarity is thought to be the major tuner of OCP's spectral properties (Kerfeld et al. 2003). The conformation of the carotenoid is due to interactions with the protein. A slight twist (with 28 Å radius of curvature) is imposed on carotenoid along its length due to the protein-carotenoid interactions (Kerfeld et al. 2003). It has been suggested that this twisting stabilizes an intramolecular charge transfer state in carotenoid, which makes it a more effective energy dissipater (Polívka et al. 2005; Kirilovsky and Kerfeld 2012). The charged and polarizable residues in OCP have been proposed to play a major role in the large bathochromic shift observed between the free and the protein bound-carotenoid.

Unfortunately, the structure of OCP^{r} is not available. Leverenz et al. (2015) and Gupta et al. (2015) have suggested that the OCP^{o} is converted to OCP^{r} under highlight by adopting substantial changes in its conformation. The OCP, after conversion, seems to be more elongated based on low resolution SAXS data (Gupta et al. 2015). Therefore, the conversion of OCP^{o}→OCP^{r} is believed to possibly involve the dissociation of the NTD and CTD domains of OCP^{o} (Leverenz et al. 2015). The 'domain separation hypothesis' for OCP photo-conversion is also supported by increased hydrodynamic radius of activated-OCP (Moldenhauer et al. 2017b). Further, the fact 'OCP^{o} is converted into OCP^{r} by chaotropes without light'(King et al. 2014) also suggests that the OCP^{o} needs to unfold for its conversion in to OCP^{r}. Recently, structures of OCP NTD (binding canthaxanthin) (PDB IDs: 4xb4, 4xb5), which are presumed to be equivalent to the dissociated NTD of OCP^{r}, have been solved and studied (Leverenz et al. 2015). These structures show that the carotenoid moves 12 Å further into the NTD (as compared to OCP^{o}), in a way that the polyene chain becomes

matrix and the β-ionone rings are solvent-exposed (Fig. 3B). The network of protein-carotenoid contacts reconfigures drastically in these structures (Leverenz et al. 2015) and this substantially alters the carotenoid's conformation. The polyene chain, which is now entirely within NTD, adopts a planner conformation as compared to that in OCP^o. The out of plane torsion of both β-ionone rings (in OCP^o) is reduced significantly (Leverenz et al. 2015). The reduced torsions between the β-ionone rings and the increased polyene planarity in OCP^r have been confirmed by Raman-spectroscopy (Wilson et al. 2008; Leverenz et al. 2014; Kish et al. 2015). However, the ultimate cause for this change has yet to be definitively confirmed. Several causes like all-cis to all-trans isomerization, relaxation of the degree of bending (Kish et al. 2015) and dipole moment-induced conformation change (Mori 2016) are the most feasible to explain the photoconversion of OCP^o to OCP^r. Upon photoactivation when the carotenoid is encased in NTD due to dramatic rearrangement of carotenoid-proteins H-bonding network, its effective conjugation length is increased giving red colour to the OCP^r (Niedzwiedzki et al. 2014; Liu et al. 2016). These authors (Niedzwiedzki et al. 2014) also suggest that the photoactivation of OCP is associated with its monomerization that was already described by Zhang et al. (2013).

Nonetheless, the question "how is the $OCP^o \rightarrow OCP^r$ conversion being triggered in response to highlight?" remains to be answered. Some plausible ideas for the molecular basis of this '*trigger*' reaction have been proposed. The light activated carotenoid could drive the $OCP^o \rightarrow OCP^r$ conversion by adopting a less-constrained conformation in OCP^r as discussed above (Fig. 4A). The αA- helix (2-18 residues) of OCP^o that appears to play a major role in bridging the NTD and the CTD could then act as the primary site of conformation changes. Light-induced structural changes in this region would result in loss of the important bridging-interaction of this helix with the CTD (Fig. 4B). This idea has been proposed based on two lines of evidence. This helix showed the largest conformational movement during NPQ and

its fold bears similarities with that seen in the *Per-Arnt-Sim* photo-sensor family (Gupta et al. 2015). A change in pH, clearly potentiates the conversion of $OCP^{o} \rightarrow OCP^{r}$. This conversion process becomes 8 fold faster at pH 12 than at pH 8 (Wilson et al. 2011). At higher pH, Tyr203 (to which the carotenoid is H-bonded) is suggested to be de-protonated and this could result in the detachment of carotenoid, thereby facilitating the dissociation of NTD from CTD (Fig. 4C) (Wilson et al. 2011). It also has been suggested recently that light-mediated excitation of the conjugated carbonyl group in ionone ring of carotenoid could disrupt its hydrogen bond linkage with Trp290 and Tyr203, which in turns could then release the strain of carotenoid, unlocking the αA- helix of NTD from CTD and so initiate domain separation (Bandara et al. 2017). This most recent model, obtained through dynamic crystallography (Bandara et al. 2017), is plausible as it explains the key question 'how is the light being sensed and operated in photo-activation of OCP?' It is also supported by other evidence, such as increased 'Trp' fluorescence in OCP^{r} (Maksimov et al. 2015). Recently, spectroscopic interrogation of the OCP photocycle on a faster time scale has revealed the existence of a very short-lived OCP^{oI} intermediate (having partially joined NTD and CTD domains) during the reversion of OCP^{r} in to OCP^{o} (Maksimov et al. 2017c). However, even if one of these ideas is true it is still unclear how these changes are induced by carotenoid photochemistry, especially when this reaction has such a low quantum yield.

4. *Quenching mechanism*: Structural change in the PB-*core or* direct bilin to carotenoid energy transfer

The exact molecular mechanism of how OCP^{r} induces NPQ in the PB is still obscure. Since, the strong correlation between OCP-PB binding and fluorescence quenching is clear (Gwizdala et al. 2011), two important and obvious questions arise, 'where does the OCP bind on PB-*core*?' (Described in section 2) and 'how does the OCP-PB interaction translate into quenching?'

Two major theories have been proposed for how OCP-mediated NPQ take place. First, OCP^r introduces a structural change in PB-*core* upon binding. This change then results in the bilin pigment being converted into a form that has much faster fluorescence decay. Second, the carotenoid of OCP may directly interact with the bilin pigment and quench excess energy through three different possible mechanisms, excitation energy transfer (Berera et al. 2013), charge transfer (Holt et al. 2005; Tian et al. 2011) or excitonic interactions (Bode et al. 2009). At present, either one or indeed both of these mechanisms seem possible. These mechanisms neither have been ruled out nor have been unequivocally proven. The spectral properties of $APC_{660,}$ believed to be the major site of quenching, appears to be highly susceptible to even minor conformational changes because PCB is embedded in an unique hydrophobic pocket within the APC structure (McGregor et al. 2008). This could favour the PB-*core* structural change theory. However, it has been shown that singlet-singlet annihilation rate in PB during NPQ does not change very much. This suggests that any conformational changes in the PB-*core* must be quite subtle rather than very extensive (Kuzminov et al. 2012). Docking studies of OCP with APC complex suggested that the distance between carotenoid and closest PCB, in case of $APC_{680,}$ is ~24.7 Å and in case of APC_{660} is ~21 Å (Stadnichuk et al. 2013; Stadnichuk et al. 2015; Zlenko et al. 2016). With these distances, assuming the carotenoid in this conformation has a stable intramolecular charge-transfer (ICT) and a lower energy S1-excited states in OCP^r, direct energy transfer from PCB to the carotenoid seems possible (Berera et al. 2012; Berera et al. 2013). Nevertheless, one cannot ignore the apparently unfavourable distance between carotenoid and PCB. Recently, recognising that the carotenoid moves significantly within NTD of OCP during photo-conversion, the possibility of further change in OCP^r structure (after binding to PB-*core*) that may place carotenoid closer for direct energy transfer has been proposed (Leverenz et al. 2015). This idea would also require that the PB-*core* undergo a conformational change to allow the carotenoid to pierce PB-core,

and move closer to PCB. Recently, Harris et al. (2016) has provided a clearer picture about interaction of OCP^r and PB. The LC/MS-MS analysis of cross-linked OCP-PB complex suggested that the NTD of OCP^r burrows into PB-*core* leaving CTD outside and ApcC stabilizes this interaction (Harris et al. 2016). This then could move the carotenoid close enough for quenching via energy transfer.

5. *Recovery*: Fluorescence recovery protein (FRP)

The quenching of PB fluorescence recovers rapidly when highlight goes away. Boulay et al. (2010) discovered that fluorescence recovery protein (FRP), a non-chromophoric ~15 kDa protein, plays an important role in the fluorescence recovery process. The specific occurrence of FRP encoding gene, *slr1964*, in all OCP-containing cyanobacteria strongly supports its role in the reversal of NPQ. Interestingly, though the genes *slr1964* and *slr1963* (the OCP gene) are found on same mRNA, the relationship between their expression is still unclear (Boulay et al. 2010). Later, the proposal by Gwizdala et al. (2011) that there are two distinct promoters for these two genes provides an explanation for the separate regulation of OCP and FRP transcription. By interacting with OCP^r, FRP promotes fluorescence recovery by detaching OCP^r from the PB and converting the free OCP^r to OCP^o.

Three different models have been proposed for FRP-OCP interactions. The first model of FRP-OCP interaction was proposed by the Kirilovsky-Kerfeld group (Sutter et al. 2013). These authors found two different forms of FRP, a dimer and a tetramer in its crystal structure (Sutter et al. 2013). Sutter et al. (2013) and Thurotte et al. (2017) also demonstrated the strong interaction of a patch of conserved residues (W50, D54, H53, and R60) of FRP with the CTD domain of OCP through docking simulations and co-immunoprecipitation. As these residues are buried inside in the FRP-tetramer, this oligomeric form of FRP is as an inactive form. This was confirmed later by the recently described *Fremyella* FRP crystal

structure (PDB ID: 5tz0) that only showed a dimeric form of FRP (Bao et al. 2017b). Furthermore, the inability of FRP in binding to the OCP NTD-analogue affirmed that the FRP binding does not involve NTD. According to this model, the FRP dimer is the biologically active form.

The Blankenship group (Lu et al. 2016; Lu et al. 2017) suggests that dimeric-FRP first binds with the CTD of OCP^r and induces conformational changes in dimeric-FRP that enabls its binding to the NTD domain. This, in turn, induces monomerization of dimeric-FRP, bridging and pulling of the CTD and NTD together and thereby promoting the conversionl of OCP^r in to OCP^o. The rejoining of the NTD and the CTD results in the detachment of monomeric-FRP and its subsequent dimerization. This model describes a concerted interaction between FRP and OCP in which the binding of FRP to OCP^r destabilizes each other and where detachment of FRP from OCP^o stabilizes each other.

The model, proposed by the Maksimov group (Sluchanko et al. 2017a; Sluchanko et al. 2017b) also proposed that the FRP-monomerization is crucial for recovery from NPQ. Their most recent conclusion regarding the FRP-active form is '*The dimer form of FRP may be of importance for binding with OCP; after binding, the FRP is converted in to monomer, an active form*'. This model suggests that the FRP monomer contains the site for CTD and NTD binding, which is hidden at FRP dimer interface. The binding of dimeric-FRP at the same site where the αA- helix of NTD binds induces its monomerization, and opens the hidden OCP-binding region that has affinity for both CTD and NTD (Sluchanko et al. 2017b). Unlike the Blankenship group's model, this model is only based on the monomerization of FRP, no conformation change (except monomerization) in FRP structure is suggested. The MS analysis of cross-linked FPR and OCP and the analysis of the flash-induced absorption transients of FRP and ΔNTE OCP (NTD-deleted OCP) suggested that FRP binds to the CTD

of OCP and brings the CTD and the NTD together inducing recovery (Sluchanko et al. 2017a; Sluchanko et al. 2017b; Maksimov et al. 2017b). This model also explains the lower affinity of FRP for OCP^o because the FRP binding site is hidden in the compact state of OCP^o (Sluchanko et al. 2017a). Clearly this area needs further detailed study to get to an accepted mechanism of FRP's mode of action.

It should also be noted that the fluorescence recovery depends on the FRP/OCP ratio. Fluorescence recovery is achieved rapidly or fluorescence quenching is completely inhibited if this ratio is high (Boulay et al. 2010; Gwizdala et al. 2013). The amplitude of the NPQ depends on the concentration of OCP^r. As FRP decreases the concentration of OCP^r, it is clear that the amplitude of NPQ depends on OCP^r concentration and the FRP/OCP ratio. (Gwizdala et al. 2013; Boulay et al. 2010).

6. Working model of cyanobacterial NPQ

The recent model of NPQ in cyanobacteria based on the available facts is shown in Fig. 5. In minimal light or darkness, the NTD and CTD of OCP^o interact and carotenoid molecule is encased between them. Here, the oligomeric state of OCP is still uncertain. Orange OCP has a low affinity for the PB-core. Therefore, OCP in its orange form does not interact with the PB-core and transfer of excitation energy to reaction centre occurs with high efficiency. Under highlight, the NTD and CTD of OCP^r dissociate from each other and there is a dramatic reordering of the carotenoid-protein H-bonding network induced by excitation of the carotenoid. It remains uncertain how strong light, absorbed by the carotenoid in OCP^o, sensitises the triggering process that induces this major conformational event. The dissociation results in a substantial movement of the carotenoid so that it is now buried inside the NTD of OCP^r. The separation of NTD and CTD make the OCP structure more elongated resulting in a form that is able to burrow into the space between basal core cylinders of PB.

The NTD of OCP^r interacts with PB-*core* in such a way that the bilin-carotenoid distance becomes shorter and may, or may not, allow direct participation of the carotenoid in the quenching process. The exact details of interaction between the NTD-OCP^r and the PB-core remain to be determined. The energy transfer efficiency of the PB is restored by FRP, which performs two major functions, detachment of OCP^r from PB-*core* and back-transformation of OCP^r into OCP^o. The recovery process starts with binding of FRP dimer to OCP^r, which in turn results in OCP^r detachment from the PB-*core*. However, the site of FRP-OCP interaction is still an open question although it is clear that the position of the N-terminal helix in OCP^o hinders this interaction. It is thought to be coupled with the monomerization of FRP and release of a FRP monomer from the OCP^r-FRP dimer complex. The other OCP^r-attached FRP monomer is then proposed to bring NTD and CTD closer together, relocating the carotenoid and transforming OCP^r back into OCP^o. It will be interesting to re-evaluate this general model when more detailed mechanistic information becomes available.

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9. References

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

Adir N (2008) Structure of the phycobilisome antennae in cyanobacteria and red algae. Photosynthetic Protein Complexes: A Structural Approach:243-274

Bandara S, Ren Z, Lu L, Zeng X, Shin H, Zhao K-H, Yang X (2017) Photoactivation mechanism of a carotenoid-based photoreceptor. Proceedings of the National Academy of Sciences 114 (24):6286-6291

Bao H, Melnicki MR, Kerfeld CA (2017a) Structure and functions of Orange Carotenoid Protein homologs in cyanobacteria. Current opinion in plant biology 37:1-9

Bao H, Melnicki MR, Pawlowski EG, Sutter M, Agostoni M, Lechno-Yossef S, Cai F, Montgomery BL, Kerfeld CA (2017b) Additional families of orange carotenoid proteins in the photoprotective system of cyanobacteria. Nature plants 3 (8):17089

Berera R, Gwizdala M, van Stokkum IH, Kirilovsky D, van Grondelle R (2013) Excited states of the inactive and active forms of the orange carotenoid protein. The Journal of Physical Chemistry B 117 (31):9121-9128

Berera R, van Stokkum IH, Gwizdala M, Wilson Al, Kirilovsky D, van Grondelle R (2012) The photophysics of the orange carotenoid protein, a light-powered molecular switch. The Journal of Physical Chemistry B 116 (8):2568-2574

Bode S, Quentmeier CC, Liao P-N, Hafi N, Barros T, Wilk L, Bittner F, Walla PJ (2009) On the regulation of photosynthesis by excitonic interactions between carotenoids and chlorophylls. Proceedings of the National Academy of Sciences 106 (30):12311-12316

Boulay C, Wilson A, D'Haene S, Kirilovsky D (2010) Identification of a protein required for recovery of full antenna capacity in OCP-related photoprotective mechanism in

cyanobacteria. Proceedings of the National Academy of Sciences 107 (25):11620-11625

El Bissati K, Delphin E, Murata N, Etienne A-L, Kirilovsky D (2000) Photosystem II fluorescence quenching in the cyanobacterium Synechocystis PCC 6803: involvement of two different mechanisms. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1457 (3):229-242

Franck J, Gaffron H (1941) Photosynthesis, facts and interpretations. Advances in Enzymology and Related Areas of Molecular Biology, Volume 1:199-262

Gantt E, Lipschultz C (1973) Energy transfer in phycobilisomes from phycoerythrin to allophycocyanin. Biochimica et Biophysica Acta (BBA)-Bioenergetics 292 (3):858-861

Glazer AN (1984) Phycobilisome a macromolecular complex optimized for light energy transfer. Biochimica et Biophysica Acta (BBA)-Reviews on Bioenergetics 768 (1):29-51

Gupta S, Guttman M, Leverenz RL, Zhumadilova K, Pawlowski EG, Petzold CJ, Lee KK, Ralston CY, Kerfeld CA (2015) Local and global structural drivers for the photoactivation of the orange carotenoid protein. Proceedings of the National Academy of Sciences 112 (41):E5567-E5574

Gwizdala M, Wilson A, Kirilovsky D (2011) In vitro reconstitution of the cyanobacterial photoprotective mechanism mediated by the Orange Carotenoid Protein in Synechocystis PCC 6803. The Plant Cell 23 (7):2631-2643

Gwizdala M, Wilson A, Omairi-Nasser A, Kirilovsky D (2013) Characterization of the Synechocystis PCC 6803 fluorescence recovery protein involved in photoprotection. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1827 (3):348-354

Harris D, Tal O, Jallet D, Wilson A, Kirilovsky D, Adir N (2016) Orange carotenoid protein burrows into the phycobilisome to provide photoprotection. Proceedings of the National Academy of Sciences 113 (12):E1655-E1662

Holt NE, Zigmantas D, Valkunas L, Li X-P, Niyogi KK, Fleming GR (2005) Carotenoid cation formation and the regulation of photosynthetic light harvesting. Science 307 (5708):433-436

Holt TK, Krogmann DW (1981) A carotenoid-protein from cyanobacteria. Biochimica et Biophysica Acta (BBA)-Bioenergetics 637 (3):408-414

Jallet D, Gwizdala M, Kirilovsky D (2012) ApcD, ApcF and ApcE are not required for the Orange Carotenoid Protein related phycobilisome fluorescence quenching in the cyanobacterium Synechocystis PCC 6803. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1817 (8):1418-1427

Kerfeld CA, Sawaya MR, Brahmandam V, Cascio D, Ho KK, Trevithick-Sutton CC, Krogmann DW, Yeates TO (2003) The crystal structure of a cyanobacterial water-soluble carotenoid binding protein. Structure 11 (1):55-65

King JD, Liu H, He G, Orf GS, Blankenship RE (2014) Chemical activation of the cyanobacterial orange carotenoid protein. FEBS letters 588 (24):4561-4565

Kirilovsky D (2007) Photoprotection in cyanobacteria: the orange carotenoid protein (OCP)-related non-photochemical-quenching mechanism. Photosynthesis Research 93 (1-3):7

Kirilovsky D, Kerfeld CA (2012) The orange carotenoid protein in photoprotection of photosystem II in cyanobacteria. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1817 (1):158-166

Kish E, Pinto MMM, Kirilovsky D, Spezia R, Robert B (2015) Echinenone vibrational properties: from solvents to the orange carotenoid protein. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1847 (10):1044-1054

Kuzminov F, Karapetyan N, Rakhimberdieva M, Elanskaya I, Gorbunov M, Fadeev V (2012) Investigation of OCP-triggered dissipation of excitation energy in PSI/PSII-less Synechocystis sp. PCC 6803 mutant using non-linear laser fluorimetry. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1817 (7):1012-1021

Leverenz RL, Jallet D, Mathies RA, Kirilovsky D, Kerfeld CA (2014) Structural and functional modularity of the orange carotenoid protein: distinct roles for the N-and C-terminal domains in cyanobacterial photoprotection. The Plant Cell 26 (1):426-437

Leverenz RL, Sutter M, Wilson A, Gupta S, Thurotte A, de Carbon CB, Petzold CJ, Ralston C, Perreau F, Kirilovsky D (2015) A 12 Å carotenoid translocation in a photoswitch associated with cyanobacterial photoprotection. Science 348 (6242):1463-1466

Liu H, Zhang H, Orf GS, Lu Y, Jiang J, King JD, Wolf NR, Gross ML, Blankenship RE (2016) Dramatic domain rearrangements of the cyanobacterial orange carotenoid protein upon photoactivation. Biochemistry 55 (7):1003-1009

Lu Y, Liu H, Saer R, Li VL, Zhang H, Shi L, Goodson C, Gross ML, Blankenship RE (2017) A molecular mechanism for nonphotochemical quenching in cyanobacteria. Biochemistry 56 (22):2812-2823

Lu Y, Liu H, Saer RG, Zhang H, Meyer CM, Li VL, Shi L, King JD, Gross ML, Blankenship RE (2016) Native mass spectrometry analysis of oligomerization states of fluorescence recovery protein and orange carotenoid protein: two proteins involved in the cyanobacterial photoprotection cycle. Biochemistry 56 (1):160-166

MacColl R (1998) Cyanobacterial phycobilisomes. Journal of structural biology 124 (2):311-334

Maksimov EG, Shirshin EA, Sluchanko NN, Zlenko DV, Parshina EY, Tsoraev GV, Klementiev KE, Budylin GS, Schmitt F-J, Friedrich T (2015) The signaling state of orange carotenoid protein. Biophysical journal 109 (3):595-607

Maksimov EG, Sluchanko NN, Slonimskiy YB, Mironov KS, Klementiev KE, Moldenhauer M, Friedrich T, Los DA, Paschenko VZ, Rubin AB (2017a) The unique protein-to-protein carotenoid transfer mechanism. Biophysical Journal 113 (2):402-414

Maksimov EG, Sluchanko NN, Slonimskiy YB, Slutskaya E, Stepanov A, Argentova-Stevens A, Shirshin E, Tsoraev G, Klementiev K, Slatinskaya O (2017b) The photocycle of orange carotenoid protein conceals distinct intermediates and asynchronous changes in the carotenoid and protein components. Scientific Reports 7 (1):15548

Maksimov EG, Sluchanko NN, Slonimskiy YB, Stepanov AV, Shirshin EA, Tsoraev GV, Klementiev KE, Slatinskaya OV, Lukashev EP, Friedrich T (2017c) The photocycle of orange carotenoid protein conceals distinct intermediates and asynchronous changes in the carotenoid and protein components. bioRxiv:167478

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

Melis A (1999) Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage in vivo? Trends in plant science 4 (4):130-135

Melis A (2009) Solar energy conversion efficiencies in photosynthesis: minimizing the chlorophyll antennae to maximize efficiency. Plant science 177 (4):272-280

Moldenhauer M, Sluchanko NN, Buhrke D, Zlenko DV, Tavraz NN, Schmitt F-J, Hildebrandt P, Maksimov EG, Friedrich T (2017a) Assembly of photoactive orange carotenoid protein from its domains unravels a carotenoid shuttle mechanism. Photosynthesis research 133 (1-3):327-341

Moldenhauer M, Sluchanko NN, Tavraz NN, Junghans C, Buhrke D, Willoweit M, Chiappisi L, Schmitt F-J, Vukojević V, Shirshin EA (2017b) Interaction of the signaling state analog and the apoprotein form of the orange carotenoid protein with the fluorescence recovery protein. Photosynthesis Research:1-15

Mori Y (2016) Computational study on the color change of 3′-hydroxyechinenone in the orange carotenoid protein. Chemical Physics Letters 652:184-189

Müller P, Li X-P, Niyogi KK (2001) Non-photochemical quenching. A response to excess light energy. Plant physiology 125 (4):1558-1566

Niedzwiedzki DM, Liu H, Blankenship RE (2014) Excited state properties of 3′-hydroxyechinenone in solvents and in the orange carotenoid protein from Synechocystis sp. PCC 6803. The Journal of Physical Chemistry B 118 (23):6141-6149

Polívka T, Kerfeld CA, Pascher T, Sundström V (2005) Spectroscopic properties of the carotenoid 3 ‘-hydroxyechinenone in the orange carotenoid protein from the cyanobacterium Arthrospira maxima. Biochemistry 44 (10):3994-4003

Punginelli C, Wilson A, Routaboul J-M, Kirilovsky D (2009) Influence of zeaxanthin and echinenone binding on the activity of the orange carotenoid protein. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1787 (4):280-288

Rakhimberdieva MG, Stadnichuk IN, Elanskaya IV, Karapetyan NV (2004) Carotenoid-induced quenching of the phycobilisome fluorescence in photosystem II-deficient mutant of Synechocystis sp. FEBS letters 574 (1-3):85-88

Ruban AV (2016) Nonphotochemical chlorophyll fluorescence quenching: mechanism and effectiveness in protecting plants from photodamage. Plant physiology 170 (4):1903-1916

Ruban AV, Berera R, Ilioaia C, Van Stokkum IH, Kennis JT, Pascal AA, Van Amerongen H, Robert B, Horton P, Van Grondelle R (2007) Identification of a mechanism of photoprotective energy dissipation in higher plants. Nature 450 (7169):575-578

Scott M, McCollum C, Vasil'ev S, Crozier C, Espie GS, Krol M, Huner NP, Bruce D (2006) Mechanism of the down regulation of photosynthesis by blue light in the cyanobacterium Synechocystis sp. PCC 6803. Biochemistry 45 (29):8952-8958

Scott M, Vasil'ev S, McCollum C, Crozier C, Espie G, Bruce D (2005) Blue light induced fluorescence quenching in a PSII-less mutant of Synechocystis PCC 6803. Photosynthesis: Fundamental Aspects to Global Perspectives, A van der Est and D Bruce, eds (Amsterdam: Elsevier Science Publishers):577-579

Singh NK, Sonani RR, Rastogi RP, Madamwar D (2015) The phycobilisomes: an early requisite for efficient photosynthesis in cyanobacteria. EXCLI journal 14:268

Sluchanko NN, Klementiev KE, Shirshin EA, Tsoraev GV, Friedrich T, Maksimov EG (2017a) The purple Trp288Ala mutant of Synechocystis OCP persistently quenches phycobilisome fluorescence and tightly interacts with FRP. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1858 (1):1-11

Sluchanko NN, Slonimskiy YB, Moldenhauer M, Friedrich T, Maksimov EG (2017b) Deletion of the short N-terminal extension in OCP reveals the main site for FRP binding. FEBS letters

Stadnichuk IN, Krasilnikov PM, Zlenko DV, Freidzon AY, Yanyushin MF, Rubin AB (2015) Electronic coupling of the phycobilisome with the orange carotenoid protein and fluorescence quenching. Photosynthesis research 124 (3):315-335

Stadnichuk IN, Yanyushin MF, Bernát G, Zlenko DV, Krasilnikov PM, Lukashev EP, Maksimov EG, Paschenko VZ (2013) Fluorescence quenching of the phycobilisome terminal emitter L CM from the cyanobacterium Synechocystis sp. PCC 6803

detected in vivo and in vitro. Journal of Photochemistry and Photobiology B: Biology 125:137-145

Sutter M, Wilson A, Leverenz RL, Lopez-Igual R, Thurotte A, Salmeen AE, Kirilovsky D, Kerfeld CA (2013) Crystal structure of the FRP and identification of the active site for modulation of OCP-mediated photoprotection in cyanobacteria. Proceedings of the National Academy of Sciences 110 (24):10022-10027

Thurotte A, de Carbon CB, Wilson A, Talbot L, Cot S, López-Igual R, Kirilovsky D (2017) The cyanobacterial Fluorescence Recovery Protein has two distinct activities: Orange Carotenoid Protein amino acids involved in FRP interaction. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1858 (4):308-317

Tian L, Gwizdala M, van Stokkum IH, Koehorst RB, Kirilovsky D, van Amerongen H (2012) Picosecond kinetics of light harvesting and photoprotective quenching in wild-type and mutant phycobilisomes isolated from the cyanobacterium Synechocystis PCC 6803. Biophysical journal 102 (7):1692-1700

Tian L, van Stokkum IH, Koehorst RB, Jongerius A, Kirilovsky D, van Amerongen H (2011) Site, rate, and mechanism of photoprotective quenching in cyanobacteria. Journal of the American Chemical Society 133 (45):18304-18311

Wilson A, Ajlani G, Verbavatz J-M, Vass I, Kerfeld CA, Kirilovsky D (2006) A soluble carotenoid protein involved in phycobilisome-related energy dissipation in cyanobacteria. The Plant Cell 18 (4):992-1007

Wilson A, Punginelli C, Couturier M, Perreau F, Kirilovsky D (2011) Essential role of two tyrosines and two tryptophans on the photoprotection activity of the Orange Carotenoid Protein. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1807 (3):293-301

Wilson A, Punginelli C, Gall A, Bonetti C, Alexandre M, Routaboul J-M, Kerfeld CA, Van Grondelle R, Robert B, Kennis JT (2008) A photoactive carotenoid protein acting as light intensity sensor. Proceedings of the National Academy of Sciences 105 (33):12075-12080

Zhang H, Liu H, Niedzwiedzki DM, Prado M, Jiang J, Gross ML, Blankenship RE (2013) Molecular mechanism of photoactivation and structural location of the cyanobacterial orange carotenoid protein. Biochemistry 53 (1):13-19

Zlenko DV, Krasilnikov PM, Stadnichuk IN (2016) Role of inter-domain cavity in the attachment of the orange carotenoid protein to the phycobilisome core and to the fluorescence recovery protein. Journal of Biomolecular Structure and Dynamics 34 (3):486-496

Figure captions

Figure 1: Schematic cartoon representation of phycobilisome (A) and the unique downhill energy gradient (B) within it, created by overlapping spectral characteristics of its constituent phycobiliproteins. Light energy (Yellow arrows), absorbed by outer most high energy absorbing phycoerythrin (PE) is successively transferred to reaction centre (RC)-chlorophyll (*chl*) via comparatively intermediate and low energy absorbing phycocyanin (PC) and allophycocyanin (APC), respectively.

Figure 2: Schematic view of phycobilisome core (PB-core). APC discs absorbing at 660nm and 680nm are represented by dark and light blue colour, respectively. APC_{660} disc is made up of only APC, whereas APC_{680} contains ApcD or ApcF and ApcE along with APC. 'A' denotes the heterogeneous APC-cylinders made up of two APC_{660} and two APC_{680} discs. 'B' denotes the homogeneous APC-cylinders made up of only APC_{660} discs.

Figure 3: Cartoon representation of OCP structure (A) and truncated-OCP structure containing only NTD (B). The NTD and CTD are represented by violet and cyan colour, respectively. Pigments and its H-bonded residues (Tyr203 and Trp290) are represented by red coloured stick model. Figure is prepared using protein data bank-retrieved OCP structures coordinates (PDB ID: 5ul2, 4xb4).

Figure 4: Schematic of $OCP^o \rightarrow OCP^r$ photo-conversion. The Yellow and red bars between NTD and CTD represents the constrained and non-constrained carotenoid in OCP^o and OCP^r, respectively. Yellow arrows represent the highlight. (**A**) The more constrained-carotenoid of OCP^o senses the highlight and transform into less-constrained conformation which causes dissociation of CTD and NTD in OCP^r. (**B**) The αA-helix (2-18 aminoacid) of NTD senses the highlight and undergoes structural rearrangement and loses interaction with CTD results in dissociation of CTD and NTD in OCP^r. (**C**) The H-bonds between carotenoids and protein

(Tyr203) is broken due to highlight induced pH-alteration. The loss of this H-bond causes detachment of CTD from carotenoid and thus from NTD.

Figure 5: Working model of cyanobacterial NPQ. **(1)** Upon highlight exposure, OCP adopts more extended conformation (OCP^r), in which, NTD and CTD dissociates from each other and the hECN translocate to be buried in NTD protein matrix. This structural conformation increases the OCP-affinity towards PB-core; OCP^r interacts with PB-core causing decreased-excitation energy transfer from PB-core to photosystem (represented by thin red arrow as compared to thick arrow under normal condition). **(2-4)** The quenching is recovered under normal light with the help of soluble protein call fluorescence recovery protein (FRP). **(2)** FRP dimer interacts with OCP^r and cause dissociation of OCP^r from PB-*core* by forming OCP^r-FRP dimer complex. Simultaneous to this phenomenon, **(3)** monomerization of FRP occurs and one monomer from OCP^r-FRP dimer complex is released. **(4)** Remaining FRP-monomer brings NTD and CTD domains of OCP together, relocates carotenoid and restores the orange conformation of OCP. After formation of OCP^o, FRP-monomer detaches form OCP^o.

Figure 1A

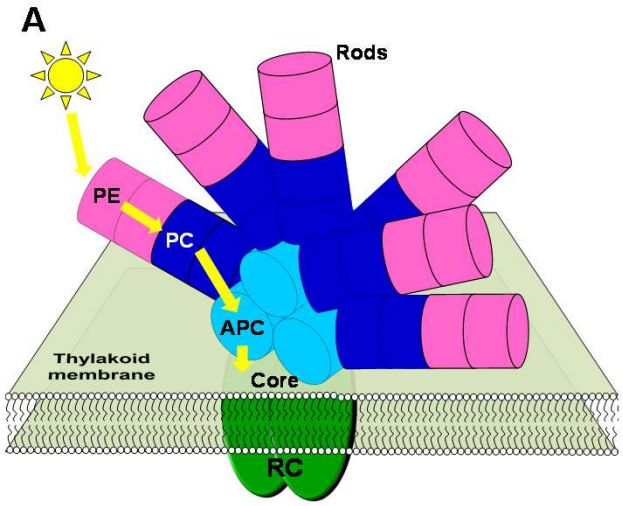


Figure 1B

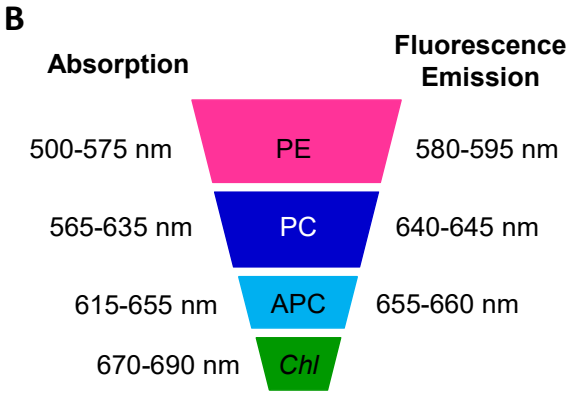

B
Absorption
Fluorescence Emission
500-575 nm
PE
580-595 nm
565-635 nm
PC
640-645 nm
615-655 nm
APC
655-660 nm
670-690 nm
Chl

Figure 2

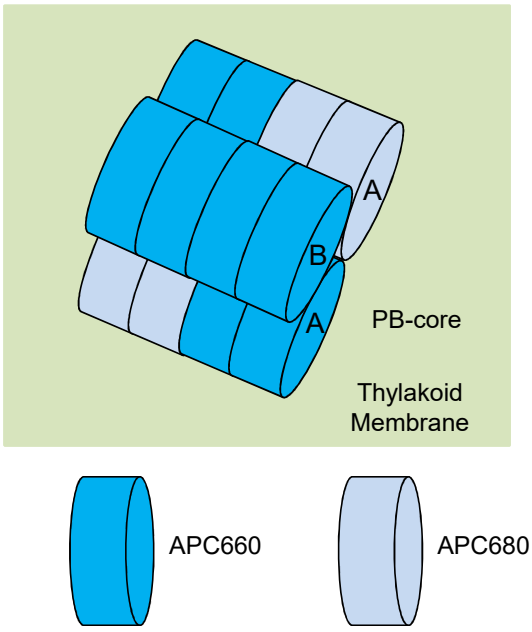


Figure 3A

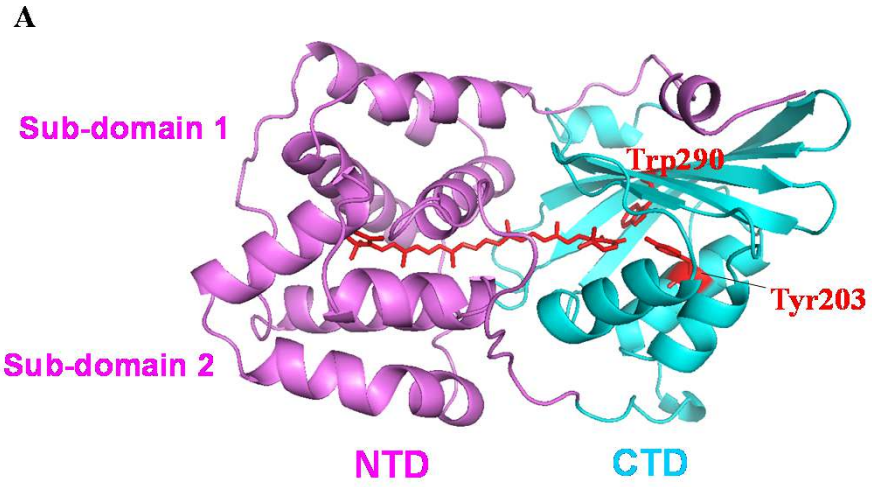


Figure 3B

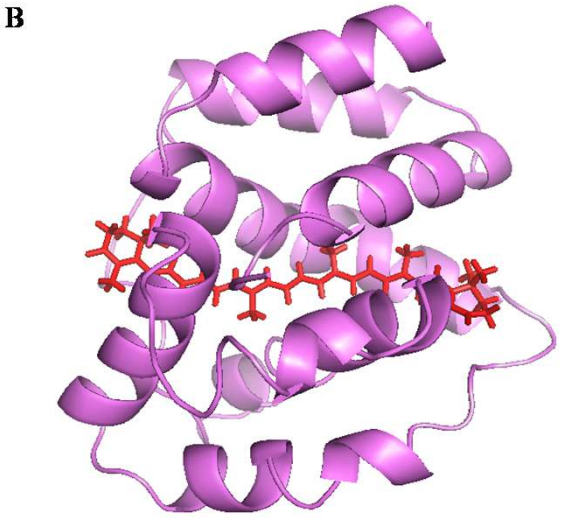


Figure 4A

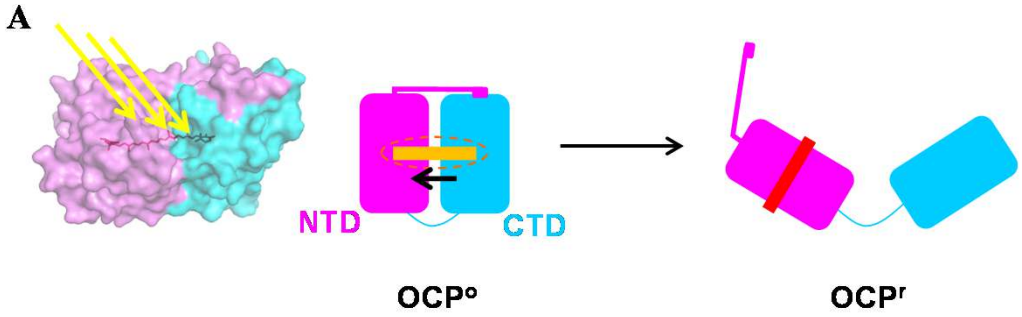


Figure 4B

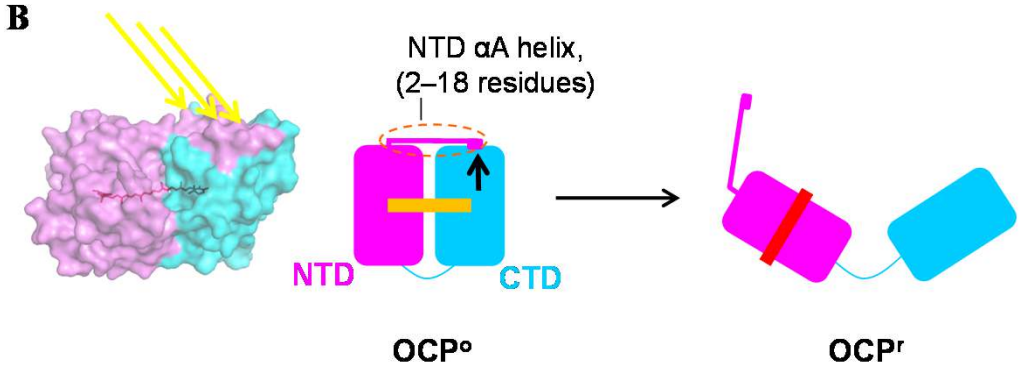


Figure 4C

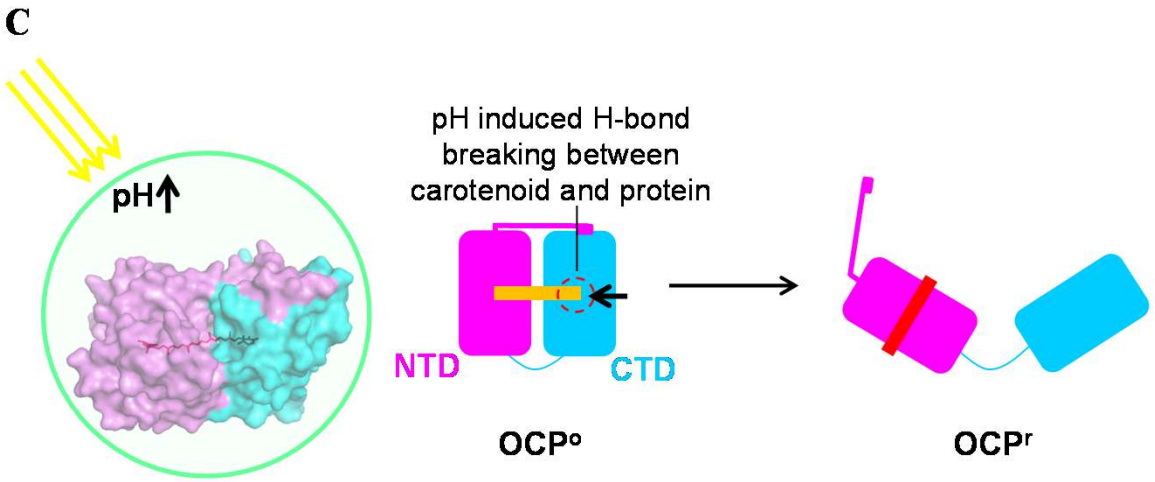
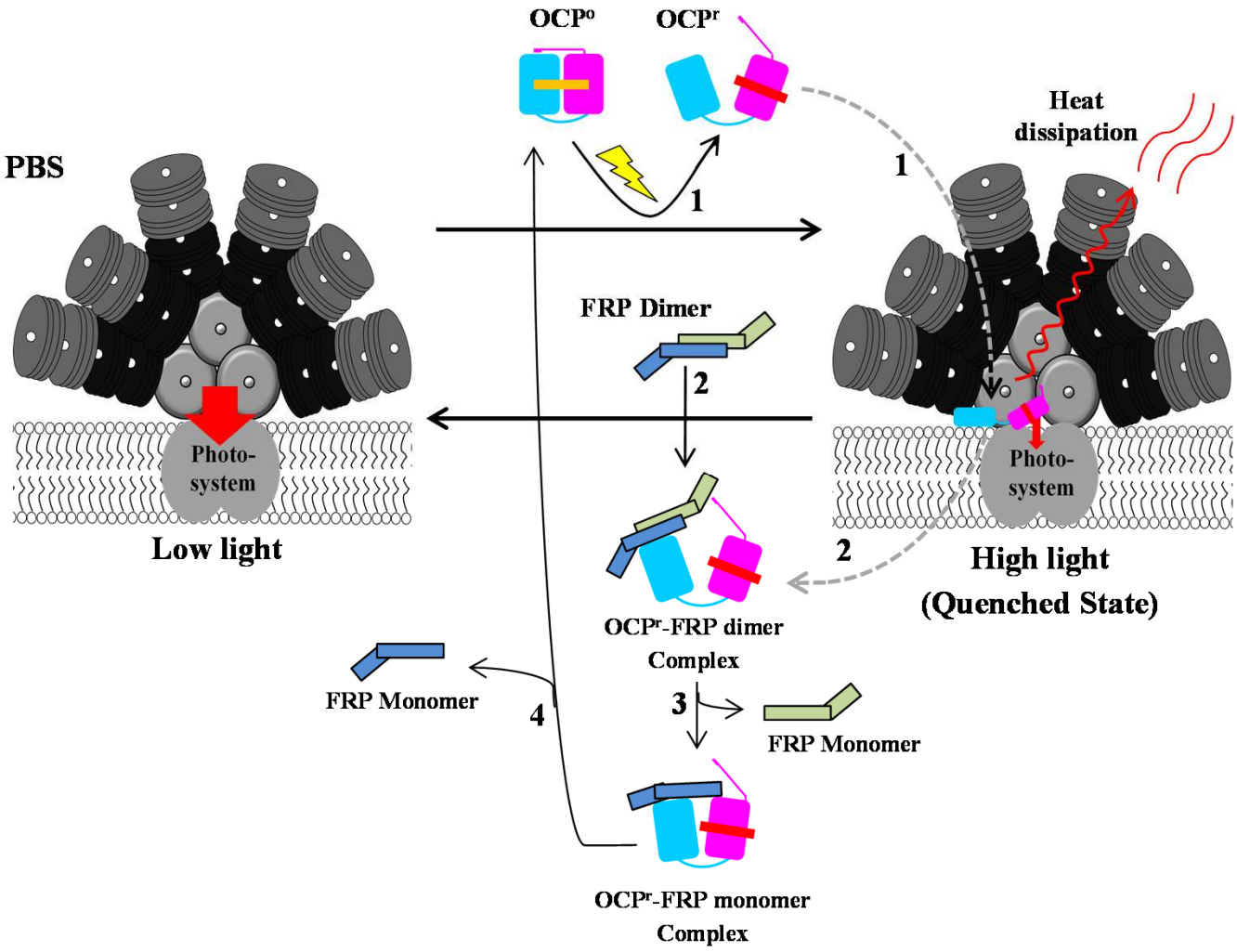

C
pH↑
pH induced H-bond
breaking between
carotenoid and protein
NTD
CTD
OCPo
OCPr

Figure 5


OCP^o
OCP^r
Heat
dissipation
PBS
1
1
FRP Dimer
2
Photo-
system
Photo-
system
Low light
2
High light
(Quenched State)
OCP^r-FRP dimer
Complex
FRP Monomer
4
3
FRP Monomer
OCP^r-FRP monomer
Complex