Cyanobacteriochromes : photoreceptors covering the entire UV-to-visible spectrum

Cyanobacteriochromes: Photoreceptors covering the entire UV-to-visible spectrum

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Highlights

- Combination of four color-tuning mechanisms results in diverse spectral properties.
- Structural information uncovers general and specific photoconversion mechanisms.
- Protein engineering studies for optogenetic tools are now proceeding.

Abstract

Cyanobacteriochrome photoreceptors are linear tetrapyrrole-binding photoreceptors that are distantly related to the canonical phytochrome photoreceptors. The chromophore-binding region of the cyanobacteriochromes consists of only a cGMP-phosphodiesterase/adenylate cyclase/FhlA (GAF) domain, while that of the phytochromes consists of three domains, including the GAF domain. Most of the canonical phytochromes homogenously show red/far-red reversible photoconversion. Conversely, the cyanobacteriochrome photoreceptors are highly diverse in the colors of light they sense. Since the discovery of the first cyanobacteriochrome photoreceptor around 15 years ago, physiological, biochemical, and biophysical studies on cyanobacteriochromes have been extensively performed to date. In this review, we focus on color-tuning mechanisms of diverse cyanobacteriochromes.

Introduction

The first appearance of the word “cyanobacteriochrome” was in 2004 in a paper by Dr. Ikeuchi’s group [1]. This paper revealed that a putative photoreceptor SyPixJ1 covalently binds a linear tetrapyrrole chromophore and shows reversible photoconversion between a blue-absorbing form (Pb) and a green-absorbing form (Pg). This protein possesses a cGMP-phosphodiesterase/adenylate cyclase/FhlA (GAF) domain similar to those of the phytochromes and many homologous domains are identified from cyanobacterial signal transduction proteins (Fig. 1a). Ikeuchi and his collaborators named proteins possessing these GAF domains “cyanobacteriochromes” (CBCRs). Since then, many physiological, biochemical, and biophysical studies have been performed for various CBCRs and their GAF domains. These GAF domains covalently bind a linear tetrapyrrole chromophore and show reversible photoconversion, which is provoked by *Z*/*E* isomerization that occurs around the C15=C16 double bond and is the primary photochemical event for plant and bacterial phytochromes as well (Fig. 2a) [2]. Now, CBCRs are known to act as the main photoreceptors in cyanobacteria that sense various light qualities covering UV-to-visible spectra [3–15] and regulate phototactic motility, chromatic acclimation and light-dependent cell aggregation [16–25]. In this review, we focus on the photochemical and structural aspects of the CBCRs.

Domain architecture and sequence diversity

Only the GAF domain is needed for chromophore incorporation and proper photoconversion of the CBCRs, while three domains, including the GAF domain are needed for those of the canonical phytochromes [2,26,27]. Most CBCRs have tandem GAF domains at the N-terminus and signal output domains at the C-terminus (Fig. 1a). His kinase (HisKA + HATPase_c), Methyl-accepting (MA), GGDEF, and EAL domains are frequently detected as signal output domains.

The CBCR GAF domains are highly diversified and categorized into many lineages; e.g. expanded red/green (XRG) and Asp-Xaa-Cys-Ile-Pro (DXCIP) lineages (Fig. 1b). All CBCR GAF domains except the DXCIP lineage possess a conserved canonical Cys residue to covalently attach to the A ring of a linear tetrapyrrole chromophore and to stabilize chromophore incorporation.

Color-tuning mechanism

To date, four distinctive color-tuning mechanisms have been revealed for these CBCR GAF domains, and the combination of these four mechanisms results in highly diversified spectral properties. Hence, we describe details of these mechanisms individually (Fig. 2a, Fig. 3).

a. Chromophore variation

To date, four kinds of linear tetrapyrrole chromophores, biliverdin (BV), phytochromobilin (PΦB), phycocyanobilin (PCB), and phycoviolobilin (PVB), have been identified to attach to the CBCR GAF domains (Fig. 2a) [3,5,8,28,29]. The lengths of the conjugated systems of these chromophores are in the order of BV > PΦB > PCB

> PVB from longer to shorter systems. A longer conjugated system results in longer wavelength light absorption. Thus, typical CBCR GAF domains binding BV, PΦB, PCB, and PVB line up in this order from longer to shorter wavelengths absorption. Fig. 2b shows the different absorbance spectra of the XRG lineage CBCR GAF domains that bind BV, PΦB, PCB and PVB [3,13,29]. In the case of PVB-bound domains, PCB is initially incorporated into the GAF domain followed by isomerization from PCB to PVB [7,30]. Because no biosynthetic pathway to produce PΦB has been found in the cyanobacterial genomes to date, PΦB is unlikely to function as a native chromophore for the CBCR GAF domains *in vivo*.

BV-bound CBCR GAF domains have been identified from the unique cyanobacterium *Acaryochloris marina* that possesses chlorophyll *d* as a main photosynthetic pigment instead of chlorophyll *a* [29,31]. These domains belong to the XRG lineage and show reversible photoconversion between a far-red-absorbing form (Pfr) and an orange-absorbing form (Po) upon BV-binding [29,31]. Because chlorophyll *d* absorbs red-shifted far-red light, these CBCR GAF domains may have physiologically important roles in *A. marina*. Before these reports, BV has been known to bind to only bacteriophytochromes via a conserved Cys residue located at the N-terminus. In this case, the Cys residue at the N-terminus binds to the $C3^2$ position of BV [32]. On the other hand, the canonical Cys residues within the GAF domains of the other phytochromes and CBCRs bind to the $C3^1$ position of PCB and PΦB. Although these facts had suggested that there was a correlation between chromophore species and the binding position of the Cys residue, the presence of BV-bound CBCRs disproves this correlation. In this context, it is of interest whether covalent bond sites of the BV-bound CBCR GAF domains are $C3^1$ or $C3^2$.

b. Reversible or stable second Cys adduct formation

Reversible Cys adduct formation has repeatedly emerged during the evolutionary process in various CBCR lineages (Fig. 1b). A Cys residue distinct from the canonical Cys, denoted the "second Cys", reversibly attaches to C10 of the chromophore, a methine carbon between the B and C rings, which results in the shortening of the chromophore conjugation and a large blue-shift (Fig. 3a)[6,15,30,33–35]. Because only the C and D rings are conjugated in the Cys-adducted form, the absorbing region is restricted to the UV-to-blue region irrespective of the binding chromophore species. On the other hand, the absorbing region of the Cys-free form is largely affected by the binding chromophore species. In fact, AM1_1186g2 covalently binds PCB and shows reversible photoconversion between a Cys-free red-absorbing form (Pr) and a Cys-adducted Pb form [15], while TePixJg covalently binds PVB and shows reversible photoconversion between a Cys-free Pg form and a Cys-adducted Pb form [28,30].

Some two-Cys type CBCR GAF domains showed stable attachment of both canonical and second Cys residues to the chromophore [6,36]. In this case, both forms absorb the short wavelength UV-to-blue region. Since these second Cys residues have been repeatedly and independently acquired by several CBCR lineages, these second Cys residues map differently on the known structure (Fig. 4a). Domains that have second Cys residues within the highly conserved Asp-Xaa-Cys-Phe (DXCF) motif are most abundant among the two-Cys type CBCR GAF domains. The structures of both Cys-free and Cys-adducted forms have been elucidated for TePixJg (Fig. 4b). The reversible attachment of Cys494 has been structurally elucidated. Since these domains with conserved DXCF motifs are detected from many diverse lineages (Fig. 1a), the

origin of the photocycle using the DXCF Cys residue should be positioned at the initial phase of CBCR evolution.

c. Reversible protonation

It has been revealed that GAF domains of a green/red lineage containing RcaC and CcaS domains exhibited green/red reversible photoconversion with a protochromic photocycle (Fig. 3b) [5,37]. Namely, green-absorbing Pg forms bind deprotonated PCB, whereas red-absorbing Pr forms bind protonated PCB [37]. Protonation should stabilize the conjugated system, resulting in the red-shifted absorbance. Site-directed mutagenesis revealed that the protonation state of the chromophore is modulated by three key amino acid residues (Glu217, Leu249, and Lys261 of RcaE) that are predicted to be located near the chromophore and specifically conserved in this lineage. Among these residues, Glu is likely to act as a proton donor/acceptor during the protochromic photocycle.

d. Trapped-twist model

The trapped-twist model in which the A and/or D rings are placed with twisted geometry relative to the B-C rings plane was proposed for several lineages to sense shorter wavelength light (Fig. 3c). Typical domains applying to this model are DXCF-type CBCR GAF domains [7,9,12,14,38]. Although typical DXCF-type CBCR GAF domains showed blue/green reversible photoconversion via reversible Cys adduct formation, some atypical ones showed blue/teal reversible photoconversion. In this photocycle, the Cys-adducted Pb form is almost identical to those of the blue/green reversible CBCR GAF domains, but the Cys-free teal-absorbing form (Pt) is

blue-shifted in comparison with the typical Pg forms. This blue-shift of the Pt form is derived from the twisted geometry of the D ring, and the conserved Phe residue contributes to this ring D twist [38,39]. In fact, replacement of this Phe residue resulted in the conversion of the Pt form into the typical red-shifted Pg form [39].

Another example is found in the XRG lineage. The typical XRG lineage CBCR GAF domains show red/green reversible photoconversion. Although the mechanism by which the Pg form absorbs shorter wavelengths has been long discussed [3,33,39–45], the recently reported structure of the Pg form shows clearly that the twisted geometry of the A and D rings causes a blue-shift of the Pg form (Fig. 4c) [46].

Dark reversion

Both of the two absorbing forms of the CBCR GAF domains are mostly stable under dark conditions, and so these CBCR GAF domains can sense the ratio of two wavelengths. However, recent studies revealed that some CBCR GAF domains showed unidirectional photoconversion and rapid dark reversion. In this case, these domains can sense the intensity of certain light colors, and the first examples were reported in 2012 by Dr. Lagarias' group [8]. Some XRG CBCR GAF domains show unidirectional Pr-to-Pg photoconversion and rapid Pg-to-Pr dark reversion under dark conditions whose half lives are 4-25 seconds [8]. Since the photoconversion is dependent on light power, these GAF domains can sense red light intensity. Our group also identified a XRG CBCR GAF domain, AnPixJg4, that shows rapid Pg-to-Pr dark reversion [47]. Interestingly, we could not detect residues specifically conserved among domains showing rapid dark reversion, indicating that the dark reversion abilities are independently acquired during the evolutionary process.

A green light power sensor, cce_4193g1, has been identified in 2016 and is a member of the DXCIP CBCR lineage, which possesses a second Cys residue in a conserved DXCIP motif but not a canonical Cys residue [10]. The second Cys residue stably attaches to a linear tetrapyrrole chromophore, although the chromophore species is unknown at this moment. It is predicted that the second Cys residue ligates to a unique position, not the $C3^1$ nor $C3^2$ position, which enables cce_4193g1 to produce an unknown chromophore.

A blue light power sensor, AM1_1870g4, has also been identified in 2018 and is a member of DXCF-type CBCR GAF domains, but possesses a slightly rearranged GDCF motif [12]. This domain covalently binds PVB and showed a blue/blue photocycle, in which there is no color shift, but a slight absorption increase in response to blue light illumination.

The dark reversion kinetics of these GAF domains are highly dependent on temperature [10,12,47]. Higher temperature results in faster dark reversion. In this context, these GAF domains can integrate light and temperature signals. These characteristics may be physiologically relevant to sense light intensity for efficient photosynthesis, because photosynthesis is severely inhibited under lower temperatures even under the same light intensity.

Structural aspects

To date, structures of both the dark states and photoproducts from two lineages have been revealed for the CBCR GAF domains [33,34,46,48], which show red/green (NpR6012g4, AnPixJg2) and blue/green (TePixJg) reversible photoconversions (Fig. 4b, c). Because we have already described structural features important for color-tuning of

individual lineages, we address structural features common among the two lineages in this section. The “flip and rotate” model has been originally proposed for the phytochromes, in which structural changes of the bound chromophore sequentially occur; isomerization of the double bond between the rings C and D (flip) is followed by rotation of the whole chromophore configuration (rotate) (Fig. 4b, c) [49]. In the case of the cyanobacterichromes, the “flip and rotate” model is also applicable to the two CBCR lineages (Fig. 4b, c).

Although Asp residues near the chromophore (Asp492 for TePixJg and Asp657 for NpR6012g4) are highly conserved among the diverse CBCRs, structural changes of these Asp residues are somehow distinctive from each other. In these two dark-state structures, the side chains of the Asp residues are similarly arranged to form hydrogen bonds with the nitrogen atoms of the A, B, and C rings for both lineage GAF domains. On the other hand, in the photoproduct structures, the interaction networks differ from each other. In the case of TePixJg, the side chain of Asp492 directly interacts with the D ring nitrogen but not the A, B, or C rings. On the other hand, in the case of NpR6012g4, although the corresponding Asp residue, Asp657, shows large structural changes, the side chain of Asp657 still directly interacts with the nitrogen atoms of the B and C rings as well as the ground state. Instead, the main chain of the Asp residue can interact with the D ring nitrogen in the photoproduct. These significant structural changes may reflect the diversity of the resulting color-tuning events. In this context, it is of note that GAF domains from the green/red lineage exceptionally lack this Asp residue, indicative of a unique structural arrangement [5,37].

Engineering

Recently, engineering of several CBCRs has been reported to modify color-tuning and output activity. Two types of modifications have been successful in color-tuning. Upon the introduction of the second Cys residue and other residues, PCB-binding red/green reversible CBCR GAF domains have converted to show blue/green reversible photoconversion, in which PCB is isomerized to PVB and the second Cys residue reversibly attaches to the chromophore [13,50]. Cancellation of the twisted geometry of the D ring has also been successful [39]. In the case of output activity modification, several groups have focused on the regulation of adenylate cyclase [47,51,52]. Dr. Zhao and our groups independently succeeded in regulating adenylate cyclase activity to generate chimeric proteins composed of a red/green reversible CBCR GAF domain and adenylate cyclase catalytic domain from non-CBCR proteins [47,51]. On the other hand, Dr. Lagarias group discovered the first natural protein possessing a blue/green reversible CBCR GAF domain and an adenylate cyclase catalytic domain, which showed blue light-inducible adenylate cyclase activity [52]. Furthermore, they have produced chimeric adenylate cyclases responding to various light colors by replacing the native GAF domain with the other lineage domains. Dr. Sode's group succeeded in switching light responsiveness of the green/red lineage CcaS to modify the length of linker region between the CBCR GAF domain and the output HisKA domain [53].

Conclusions

Because about fifteen years have passed since the discovery of the CBCR superfamily, we can now understand the diversity of the CBCR proteins regarding their molecular, photochemical, and structural aspects. Investigation of many more uncharacterized CBCR proteins derived from emerging genomic information will further expand our

understanding, which would contribute to the development of various applications such as optogenetics and bio-imaging.

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••: Refs 1, 33, 34, 35, 46

Ref. 1: This is the first report to identify the CBCR superfamily. SyPixJ1 covalently bound a linear tetrapyrrole chromophore and showed blue/green reversible photoconversion.

Ref. 6: This paper identified novel examples of two-Cys photocycles from both the phytochrome and CBCR proteins. The second Cys residues showed stable or transient covalent bond formation during their photocycles.

Ref. 10: cce_4193g1 showed a green/green photocycle, in which there was no color shift, but a slight absorption decrease in response to green light illumination. The dark state and photoproduct are *Z*- and *E*-isomers of the unknown chromophore, respectively.

Ref. 11: This paper discovered far-red sensing CBCR GAF domains upon PCB binding. These domains are distantly related to the green/red lineage. The detailed mechanism by which far-red light is absorbed remains unknown.

Ref. 12: Both the dark state and photoproduct of AM1_1870g3 contained the *Z*- and *E*-isomers of PVB to the same extent, indicating that *Z*/*E* isomerization does not occur during the photocycle. Because replacement of the GDCF motif with the canonical DGCF motif resulted in reversible photoconversion with *Z*/*E* isomerization, the unique GDCF motif enabled such a photocycle without *Z*/*E* isomerization.

Ref. 28: This is the first report to show that the DXCF-type CBCR binds PVB, not PCB, as a chromophore.

Ref. 33: This paper reported the crystal structures of the *Z*-isomer of red/green reversible AnPixJg2 and the *E*-isomer of blue/green reversible TePixJg.

Ref. 34: This paper reported the crystal structure of the *Z*-isomer of blue/green reversible TePixJg.

Ref. 35: This is the first report to propose that the DXCF-type CBCR shows reversible Cys adduct formation during its photocycle.

Ref. 37: This paper demonstrated that the green/red lineage GAF domains showed

protochromic photocycles.

Ref. 46: This paper reported the crystal structures of the *Z*- and *E*- isomers of red/green reversible NpR6012g4. Further, the authors identified structural heterogeneity in the *Z*-isomers, which is consistent with spectroscopic observations.

Ref. 48: This paper reported the NMR structures of the *Z*- and *E*-isomers of blue/green reversible TePixJg, which is somehow different from those obtained by X-ray crystallography.

Ref. 50: Sequence comparison of AnPixJg4 and AnPixJg2, a close homolog of AnPixJg4, highlighted six residues distinctive from each other near the chromophore. Replacement of these six residues in AnPixJg2 succeeded in giving dark reversion ability.

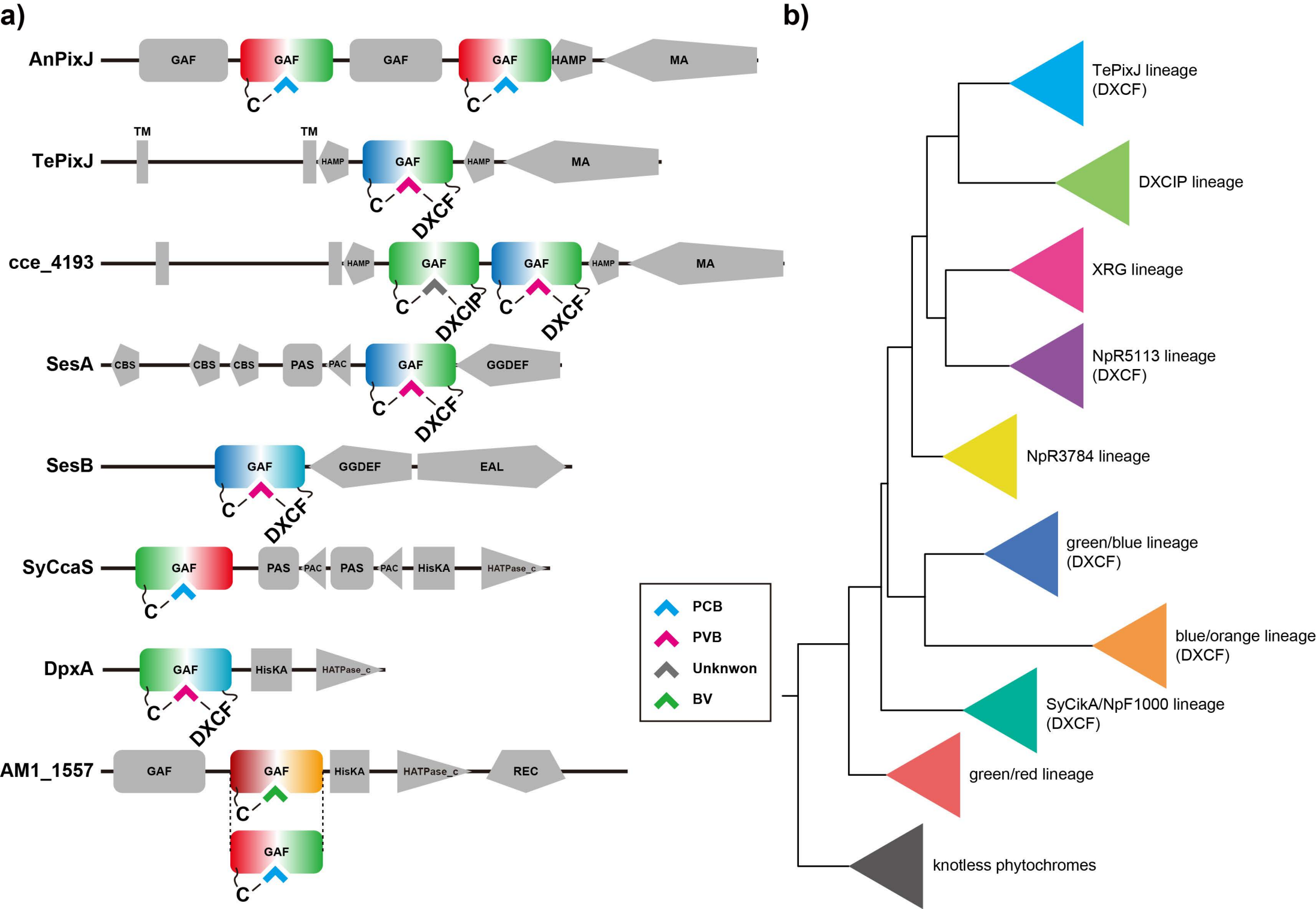
Figure legends

Figure 1. Diverse CBCR GAF domains in various proteins. (a) Domain architectures of the representative CBCR proteins. GAF: GAF domain, HAMP: linker domain present in His kinases, adenylate cyclases, methyl-accepting proteins and phosphatases, MA: methyl-accepting chemotaxis-like domain, TM: transmembrane region, CBS: domain in cystathionine beta-synthase and other proteins, PAS: Per-Arnt-Sim domain, PAC: PAS C-terminal domain, GGDEF: diguanylate cyclase domain with highly conserved GGDEF motif, EAL: diguanylate phosphodiesterase domain with highly conserved EAL motif, HisKA: His kinase A (phosphoacceptor) domain, ATPase_c: His kinase-like ATPase domain, REC: cheY-homologous receiver domain. The domain architectures are based on the SMART program (http://smart.embl-heidelberg.de/). (b) Schematic illustration of phylogeny of the diverse CBCR GAF domains, which is based on the phylogenetic tree shown in the previous paper [10]. *: Lineages including domains with reversible Cys adduct formation.

Figure 2. Color-tuning based on the binding chromophore species. (a) Four kinds of linear tetrapyrrole chromophores: phycoviolobilin (PVB), phycocyanobilin (PCB), phytochromobilin (PΦB), and biliverdin (BV). Conjugated regions are highlighted by colors, which correspond to the colors of the chromophore. Side chains at the C3 position are covalently bound to a conserved canonical Cys residue within the CBCR GAF domains except DXCIP lineage domains (b) Dark-state photoproduct. Difference spectra of various XRG lineage CBCR GAF domains bound PVB (Anacy_3174g6), PCB (AnPixJg2), PΦB (AnPixJg2) and BV (AM1_1557g2).

Figure 3. Three representative color-tuning mechanisms. Conjugated regions are highlighted by colors, which corresponds to absorbing light color. Left: *Z*-isomers. Right: *E*-isomers (a) Reversible Cys adduct formation model shown by representative blue/green reversible photoconversion. (b) Reversible protonation model shown by representative green/red reversible photoconversion. (c) Trapped-twist model shown by representative green/teal reversible photoconversion.

Figure 4. Structural aspects of the CBCR GAF domains. (a) Overall structure of the CBCR GAF domain. Upper: Protein and chromophore are shown as ribbon and stick models, respectively. Lower: Two-dimensional schematic illustration. Pink triangles represent positions of the second Cys residues. (b) Structures of the *Z*-isomer Pb (upper, 4GLQ) and *E*-isomer Pg (lower, 3VV4) forms of TePixJg shown as stick models. Hydrogen bonds between Asp492 and a chromophore are shown as dashed lines. (c) Structures of the *Z*-isomer Pr (upper, 6BHN) and *E*-isomer Pg (lower, 6BHO) forms of NpR6012g4 shown as stick models. Hydrogen bonds between Asp657 and chromophores are shown as dashed lines.


a)
AnPixJ
GAF
GAF
GAF
GAF
HAMP
MA
C
C
TM
TM
TePixJ
HAMP
GAF
HAMP
MA
C
DXCF
cce_4193
HAMP
GAF
GAF
HAMP
MA
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DXCF
SesA
CBS
CBS
CBS
PAS
PAC
GAF
GGDEF
C
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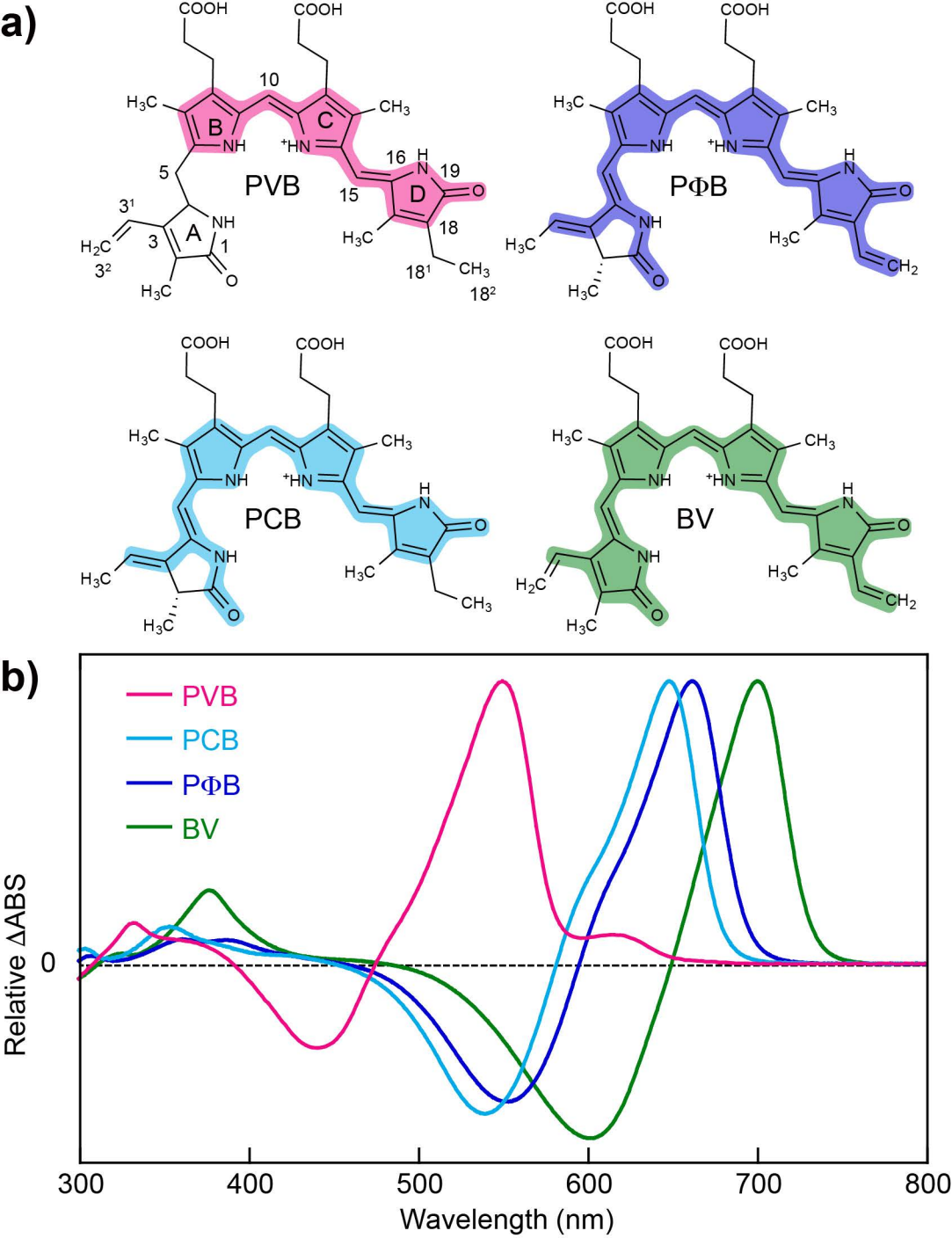
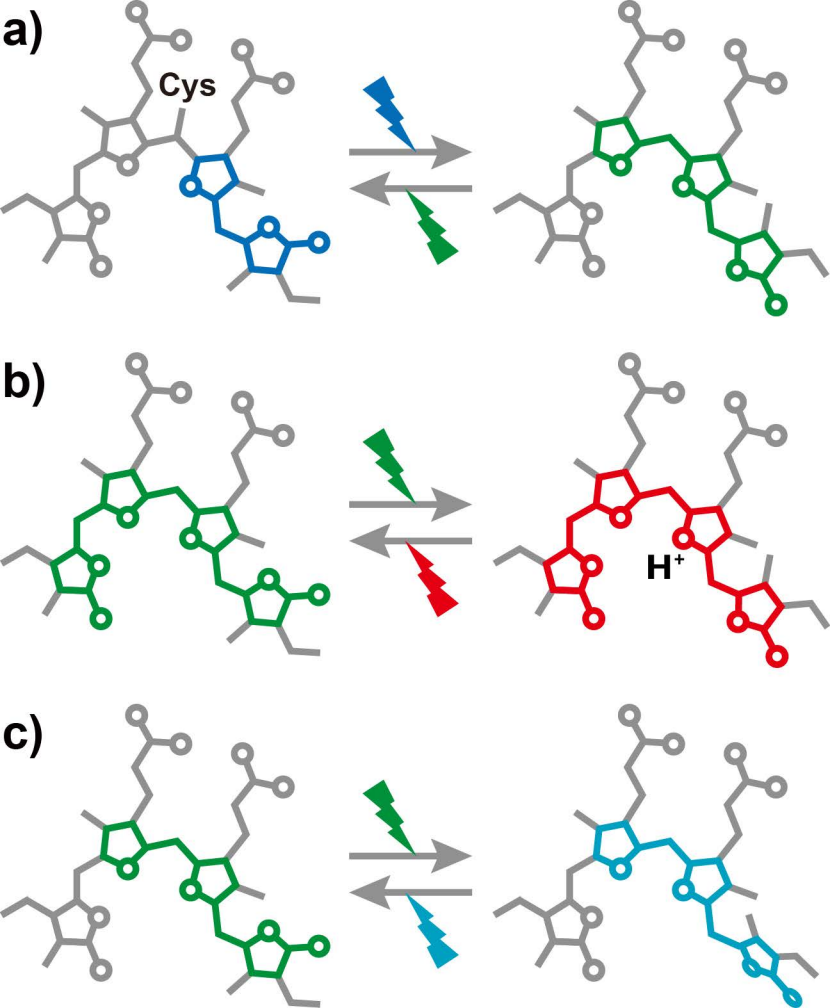
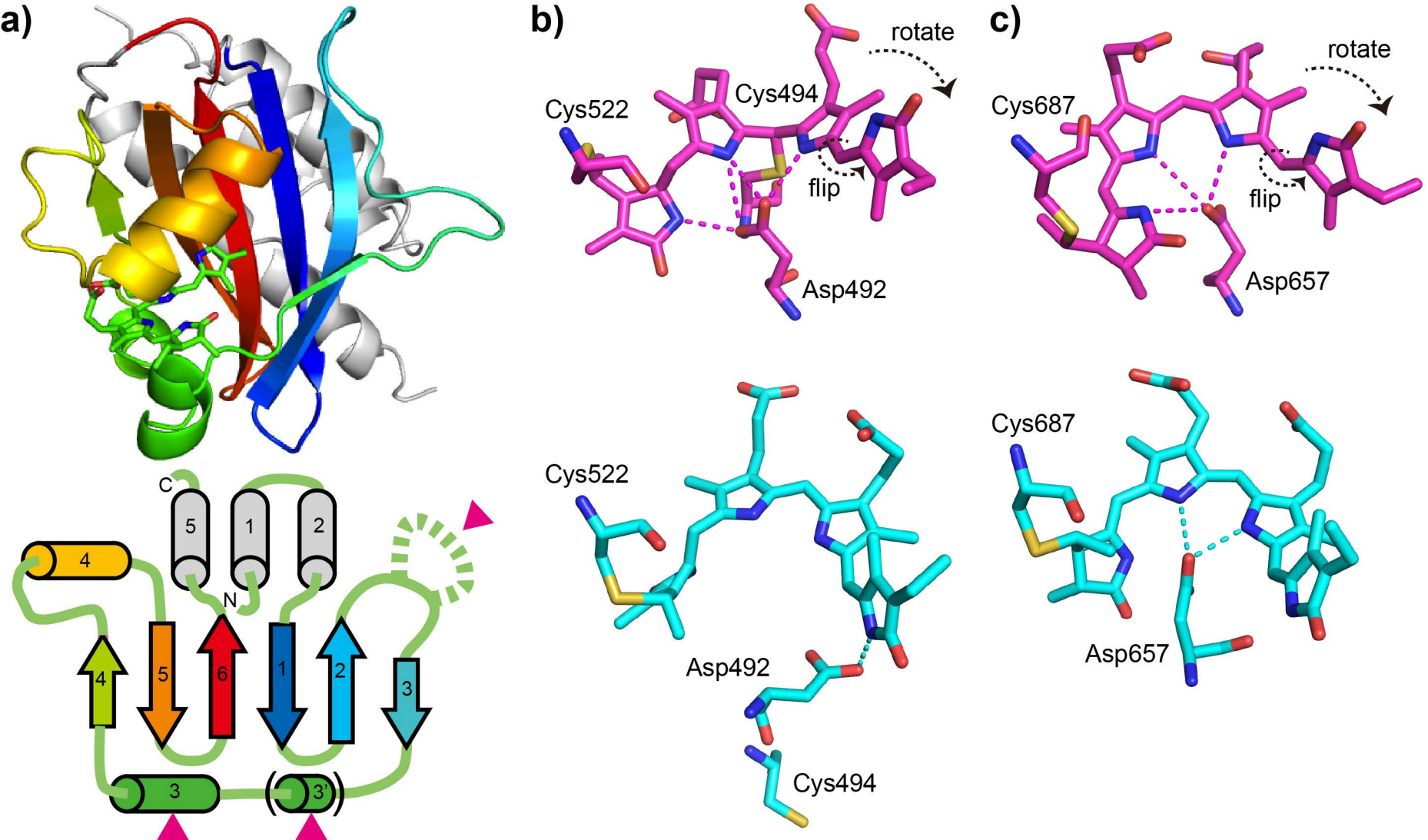
HisKA
HATPase_c
C
DXCF
AM1_1557
GAF
GAF
HisKA
HATPase_c
REC
C
GAF
C
PCB
PVB
Unknwon
BV
b)
TePixJ lineage
(DXCF)
DXCIP lineage
XRG lineage
NpR5113 lineage
(DXCF)
NpR3784 lineage
green/blue lineage
(DXCF)
blue/orange lineage
(DXCF)
SyCikA/NpF1000 lineage
(DXCF)
green/red lineage
knotless phytochromes


a)
PVB
PΦB
PCB
BV
b)
PVB
PCB
PΦB
BV
Relative ΔABS
0
300
400
500
600
700
800
Wavelength (nm)


a)
Cys
b)
H+
c)


a)
C
5
1
2
4
N
4
5
6
1
2
3
3
3'
b)
rotate
Cys522
Cys494
flip
Asp492
Cys522
Asp492
Cys494
c)
rotate
Cys687
flip
Asp657
Cys687
Asp657