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Structure of a light-activated LOV protein dimer that regulates transcription in *Neurospora crassa*

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Abstract

Light oxygen or voltage (LOV) domains are widely represented signaling modules in bacteria, archaea, protists, plants and fungi. The *Neurospora crassa* LOV protein VIVID (VVD) allows adaptation to constant or increasing light levels and proper entrainment of circadian rhythms. The crystal structure of the fully light-adapted VVD dimer reveals the mechanism by which light driven conformational change alters oligomeric state. Photo-induced formation of a cysteinyl-flavin adduct generates a new hydrogen bond network that releases the N-terminus from the protein core and restructures an acceptor pocket for its binding on the opposite subunit. Substitution of residues key to the monomer/dimer switch have profound effects on light adaptation in *Neurospora*. The VVD dimerization mechanism provides the molecular details for how a large family of photoreceptors converts light responses to alterations in protein interactions.

INTRODUCTION

Per-ARNT-Sim (PAS) domains (~120 residues) respond to environmental change through chemical reactions of bound cofactors (e.g., flavin, heme, Fe-S cluster, etc.), by the binding of exogenous ligands, and/or by alteration of oligomeric state (1–3). The light oxygen or voltage (LOV) family of PAS proteins binds flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN) and senses blue-light through photochemically driven formation of a cysteinyl-flavin adduct (4–6). Two such LOV domain-containing proteins manifest light-induced resetting and phasing of the *Neurospora crassa* circadian clock (7–10) and are the founding members of a principal class of photoreceptors conserved among all fungi (11). The first, White collar-1 (WC-1), is a large multi-domain transcription factor (12, 13) that along with White Collar-2 (WC-2) comprises the White Collar Complex (WCC), the primary photoreceptor for the *Neurospora* circadian system (8, 9, 14, 15). In response to blue light, the WCC induces transcription of most light-responsive genes (16), including many clock-controlled genes (CCGs) as well as the gene encoding the central circadian oscillator protein FREQUENCY (FRQ) (8, 9, 11–13). The second light sensor, VIVID (VVD), strongly induced by the WCC in response to light, antagonizes the action of the WCC by interacting directly with it, and in doing so adapts the organism to constant and increasing light levels (7, 1–21). VVD is almost entirely composed of a LOV domain that has high homology to the LOV domain of WC-1 (7, 18). Thus, in WC-1 and VVD, two similar light sensory modules exhibit very different biological activities in the same signaling circuit. Currently there are several examples of LOV protein crystal structures;

however, none are characterized in both “on” and “off” conformations that have been verified as biologically relevant signaling states (22–24). Elegant solution NMR studies on the *Avena sativa* phototropin1 LOV2 domain reveal how a C-terminal α -helix displaces from the core LOV domain upon light excitation (25), but a detailed structural description for how the remodeled LOV domain affects enzymatic activity in the full-length protein is currently lacking for this system (26) and others (27). Light induces VVD homodimerization, thereby providing a mechanism for how photon absorption can be converted to the remodeling of protein interactions important for signaling (28).

Previously, we reported a light-state structure of VVD that was determined by exposing dark state crystals to white light (22). These crystals contain a majority population of the cysteinyl-flavin adduct. The structure shows a set of structural rearrangements that result from protonation of flavin atom N5 and propagate through a conserved hinge to the N-terminal cap (N-cap) region of the PAS domain. One of the most variable regions in PAS domains, the N-cap, packs against the β -sheet of the PAS core and in VVD is composed of an α helix ($\alpha\alpha$), a short β -strand ($b\beta$) and an N-terminal “latch” (residues 37–44) that wraps around the domain, toward the surface-exposed adenosine moiety of FAD. The largest structural changes in the light-exposed crystals involve a ~ 4 Å shift of the $b\beta$ strand (22). However, in solution, adduct formation drives greater conformational change at the N-terminus and subsequent dimerization (22, 28). The N-cap connects to the first β -strand of the PAS core ($A\beta$) through a hinge region that contains Cys71. Substitution of the hinge residue Cys71Ser, which changes from a buried to an exposed position in the irradiated crystals, prevents conformational change at the N-terminus, inhibits dimerization *in vitro*, and curtails VVD function *in vivo* (20–22, 24, 29). In contrast, the Cys71Val variant, predisposes the hinge to the light-state conformation, releases the N-terminus and generates some dimer, even in the dark (24, 28, 30). Thus, the light-exposed dark-state crystal represents an intermediate conversion to the fully light-adapted state: conformational changes within the protein have taken place, but the crystal lattice has prevented large-amplitude rearrangements and subunit association. Herein, we describe the crystallographic structure of the VVD fully activated light-state dimer. The structure contrasts with the monomeric form of the VVD dark-state and represents an activated LOV protein in complex with its immediate target, in this case, another subunit of the same light sensor. The design of VVD variants based on the structure perturb *Neurospora* photosensory transduction in predictable ways.

RESULTS

Although the adduct lifetime of VVD (~ 5 hrs) is long compared to many LOV proteins, it is not long enough to crystallize the light-state dimer. We thus turned to a Met135Ile:Met165Ile variant (VVD-II) that was designed to increase adduct lifetime by stabilizing the reduced form of the flavin ring (31). Adduct lifetime increases 10 fold in VVD-II and the recombinant protein can be purified from *E. coli* in a partial neutral semiquinone state, which is indicative of a higher flavin redox potential (Fig. S1). After white light exposure, this protein grew crystals overnight that were clear and diffracted to 2.7 Å resolution. To limit further reduction and cleavage of the adduct state in the synchrotron X-ray beam, diffraction data were collected from 10 different positions on a single crystal under conditions that limited radiation exposure.

As expected, light-activated VVD-II crystallizes as a dimer in a new crystal lattice, with one dimer per asymmetric unit (Table S1). The fully formed adduct has strong electron density between the Cys108 thiol group and flavin C4a and noticeable puckering of the isoalloxazine ring at C4a (Fig. 1A). Overlays of the structures for the light-state dimer (LSD), the intermediate-state monomer (ISM) and the dark-state monomer (DSM) reveal

that the LSD not only undergoes many of the internal structural rearrangements found in the ISM structure, but also shows much larger changes in the N-cap region where the N-terminal latch has been released from the subunit core (Fig. 1B, C, D). In the ISM structure (at 1.7 Å resolution), protonation of flavin N5 causes Gln182 to flip and alter hydrogen bonding interactions with the backbone of Ala72 and the hinge region to the N-cap. In the LSD, at 2.7 Å resolution, the Gln182 side chain amide orientation cannot be assigned unambiguously based on electron density, but changes in its position and that of the Ala72 carbonyl compared to the DSM reflect the expected flip (Fig. 1C).

The hinge region (residues 65 to 72) plays a major role in the light activated conformational changes in VVD. In both the ISM, and the LSD, formation of the new hydrogen bond between the Ala72 carbonyl and Gln182 couples to rearrangement of the hinge region. In both structures, Cys71 is released from its buried position in the DSM and no longer hydrogen bonds to the backbone of Asp68 (Fig. 1C). In the LSD, however, the Cys71 thiol group orients toward the Ala72 carbonyl and not the Asp68 peptide nitrogen, as found in the ISM structure (Fig. 1C). Similar to the ISM structure, b β shifts toward the PAS β -sheet with Pro66 moving by ~3.5 Å. Although the hinge loop containing Cys71 has a similar conformation in both subunits of the LSD, it displaces further over b β in one subunit than the other (Fig. 1C, inset). The N-terminal helix shifts along its axis to a greater extent than that observed in the ISM, where it moves very little (Fig. 1D). The helical shifts are within the range that do not require repacking of side chains (32) and appear to couple directly to repositioning of b β and the Cys71-containing hinge loop, against which $\alpha\alpha$ packs. Movements of $\alpha\alpha$ and b β destabilize contacts of the N-terminal latch, which assumes an entirely new conformation compared to that of the DSM and the ISM by the backbone rotation of Gly44 (Fig. 1B, D). We cannot rule out the possibility that other long-range effects such as changes in dipole and charge distributions associated with the adduct state contribute to latch release, but given the distance of separation and requirement of residues in the connecting loop for propagating the signal, the influence of through-space factors is likely less than conformational changes propagated through b β and $\alpha\alpha$.

The N-terminal latch, freed from its association with the PAS core, docks into the hinge region of the adjacent subunit (Fig 2). Thus, by restructuring of the hinge, photo-induced adduct formation not only facilitates release of the N-terminus on one subunit, but also provides a recognition pocket to accept the latch from the opposite subunit. Indeed, previous time-resolved SAXS data of VVD variants with altered dimerization properties indicate the release of the N-terminus is necessary but not sufficient for dimer formation (30). At the center of the extensive dimer interface (1342 Å² surface area per subunit; Predicted free energy of formation (ΔG_i) = -26.8 kcal mol⁻¹; Hydrophobic specificity factor (ΔG_p) = 0.009 (33)). Tyr40 inserts into the hinge pocket created by b β and the C-terminal β -strand, E β , stacks between Phe162 and Phe181, and hydrogen bonds with both the side-chain and main-chain of E β and the Cys71 thiol (Fig. 2). Although the hinge loops of the two LSD subunits differ in position by ~4 Å, their conformations and interactions with Tyr40 are similar. The N-terminal helix $\alpha\alpha$ also contributes to the interface through the symmetric contacts of an exposed hydrophobic face involving Met48 and Ile52 (Fig. 2). The molecular envelope of the transient dimer species observed in time-resolved SAXS experiments (30) agrees well with the overall dimensions of the LSD structure and much better than those of non-physiological VVD dimers found in crystal lattices (Fig. S2).

The behavior of many previously studied VVD variants are explained by the LSD structure, including the requirement of the latch residues (37–44) for high-affinity dimerization (20). Truncations beyond Gly44, will not dimerize under any conditions (28). Substitutions within the hinge are expected to have complex effects on dimerization depending on their propensity to release the latch and/or restructure the recognition pocket. Cys71Ser prevents

dimerization by short-circuiting latch release and preventing latch binding (22), while Cys71Val produces an extended monomer with the latch released and some dimerization in the dark state (28). Substitution of contacts along the interaction surface of $\alpha\alpha$ can prevent dimerization (e.g. Met55Arg) (22) whereas changes on the internal surface of the helix (Tyr50Trp) promote light-induced dimerization by presumably destabilizing $\alpha\alpha$ packing and facilitating latch release (28). Chemical modifications at Cys183, which lies at the end of E β and contacts the N-terminal latch, prevents dimerization (22). Light-dependent disulfide cross-linking of Glu171Cys is much better explained by the LSD structure than a crystallographic dimer formed by the DSM (28).

To further confirm that the LSD forms in solution and create variants for use *in vivo*, we examined other residue substitutions that disrupt the hydrogen bonds at the dimer interface. As expected, mutations at Tyr40 drastically reduced light-triggered association, with Tyr40Lys completely abrogating dimerization (Fig. 3). The Tyr40Phe variant reduces the LSD population, but also promotes some dark-state dimer formation, which suggests that it decouples subunit association from the conformational signals initiated by the adduct state (Fig. 3). In the extreme, Thr69Trp dimerizes in both the dark and the light, presumably because it facilitates intersubunit contacts that overcome light promoted conformational switching (Fig. 3). We also tested interface positions for enhanced cross-linking and found that Ile52Cys spontaneously cross-links in the light when purified from *E. coli*, whereas other positions removed from the dimer interface, do not (Fig. S3).

Armed with VVD variants that both enhance and abrogate light-induced dimer formation without perturbing VVD photochemistry, we tested whether such alternations could affect *Neurospora* light sensing when expressed from a corresponding allele in a Δvvd strain (Fig. S4). Consistent with previous studies (21), the Cys71Ser and Cys108Ala show a partial loss-of-function phenotype as assayed by coloration in constant light (Fig. 4A). Carotenoid production, which turns cells orange, is activated by WC-1, but inhibited by VVD. The Cys71Val, Thr69Trp and Ile52Cys mutant alleles appear to have a normal coloration phenotype (Fig. 4A). The Tyr40Lys mutant allele, which is incapable of light induced dimerization, displayed high levels of carotenoid accumulation similar to a Δvvd strain (Fig. 4A, B, Fig. S4A). We observed no difference when strains were kept in constant darkness (DD, Fig. 4A), indicating that the coloration phenotypes are light-dependent.

To quantify the photoadaptation defects at the molecular level, we compared the level of *al-3* repression among various *vvd* mutant strains held for 60 minutes in constant light (LL60) by RT-QPCR analysis. The expression of *al-3* is strongly induced by light (Fig. S4B) and unambiguously displays photoadaptation in constant light in that, within an hour, levels of *al-3* expression were reduced to approximately the pre-induction level (21). Repression efficacy (Fig. 4C) was calculated based on the level of *al-3* repression between strains with either no *vvd* expression (0%, no repression) or a *vvd* wild-type allele (100%, maximal repression). Consistent with the coloration phenotype and high levels of carotenoid accumulation, the Tyr40Lys mutant allele displayed the most severe defect in repressing light responses (Fig. 4C), retaining merely 25% of the wild-type VVD activity. Unexpectedly, the Thr69Trp mutant allele showed a slight but significant reduction in activity. Meanwhile, the extent of early light activation of *al-3* and *vvd* RNA at LL15 were more or less unaffected in various *vvd* mutants (Supplemental Fig. 2B and 2C), and VVD proteins were induced to comparable levels *in vivo* at LL60 (Fig. S5). These data suggests that the photoadaptation defects are the result of mutations that interfere with the ability of VVD to undergo the crystallographically observed light-induced conformational changes that lead to dimerization.

DISCUSSION

Residues important for the light driven dimerization mechanism of VVD are conserved by a large number of fungal LOV domain containing light sensors such as ENVOY, WC-1 and other GATA-type transcription factors (Table S2). In particular, the WC-1 LOV domain contains critical residues necessary for a VVD-like light-induced dimerization mechanism (Fig. S6, Table S2). Light activation of WC-1 is also associated with an increase in oligomeric state (13, 19, 34) and swapping the LOV domain core of VVD (residues 71–186) for that of WC-1 maintains several primary light responses in *Neurospora* (18). The current model for the inhibition of the WCC by VVD requires direct interaction and involves competitive heterodimerization between the VVD LOV and the WC-1 LOV (19–21). Sequence conservation (Fig. S6) strongly suggests that the VVD:WC-1 heterodimer will most likely resemble the VVD light state dimer structure determined here. In support of this notion, the Thr69Trp mutant, which causes a constitutive VVD dimer in solution (Fig. 3), shows attenuated inhibition of the WCC in cells (Fig. 4), perhaps because the variant subunits do not exchange effectively with the WC-1 LOV domain.

In other LOV-containing proteins, such as the plant and algal phototropin kinases, and bacterial sensors such as YtvA, the residues corresponding to Cys108 and Gln182 are strictly conserved, whereas those in the hinge region and N-cap differ (Table S2). The light-excited intermediate structure of *Avena sativa* phototropin1 LOV2 (As phot1 LOV2) (24) reveals that the conserved Gln side chain also flips in response to Cys-flavin adduct formation. As in VVD, the Gln rearrangement alters hydrogen bonding to peripheral elements, in this case the LOV2 Asn414, which, like Ala72 in VVD, initiates the hinge to the N-cap (Fig. S7.). The N-caps of LOV2 and VVD are different, but changes in both structures correlate with light-induced hydrogen bonding rearrangements within the hinge region (24). In YtvA (23), the conserved Gln also flips upon adduct formation, but the peripheral hydrogen bonding network affected is again different from that of either phot1 LOV2 or VVD. In these systems, as with VVD, crystal packing constraints likely limit complete conversion to light-adapted conformations when dark-state crystals are irradiated. Nevertheless, we can conclude that core chemical mechanisms of the flavin and responses of immediately surrounding residues are conserved across the broad LOV family but the response of peripheral regions vary as sequences and structures diverge.

MATERIALS AND METHODS

(A) Biochemistry

Protein expression and purification—Protein expression, purification and size exclusion chromatography of VVD-36II was carried out as previously described (22, 28).

Mutagenesis—The Tyr40Lys, Tyr40Glu, Tyr40Phe, Tyr40Ile, Thr69Trp and Ile52Cys variants were prepared by the QuickChange protocol (Stratagene) using the following primers. Tyr40Phe 5′-AGC CAT ATG CAT ACG CTC TTC GCT CCC GGC GGT-3′, 5′-GTC ATA ACC GCC GGG AGC GAA GAG CGT ATG CAT-3′. Tyr40Lys 5′-AGC CAT ATG CAT ACG CTC AAG GCT CCC GGC GGT-3′, 5′-GTC ATA ACC GCC GGG AGC CTT GAG CGT ATG CAT-3′. Tyr40Glu 5′-AGC AGC CAT ATG CAT ACG CTC GAG GCT CCC GGC GGT-3′, 5′-GTC ATA ACC GCC GGG AGC CTC GAG CGT ATG CAT-3′. Tyr40Ile 5′-AGC CAT ATG CAT ACG CTC ATC GCT CCC GGC GGT-3′, 5′-GTC ATA ACC GCC GGG AGC GAT GAG CGT ATG CAT-3′. Thr69Trp 5′-GAA CTG GGA CCT GTT GAC TGG TCA TGC GCT CTG-3′, 5′-CAG AAT CAG AGC GCA TGA CCA GTC AAC AGG TCC-3′. Ile52Cys 5′-ATG GGC TAT CTG TGT CAG ATT ATG AAC AGG CCA-3′, 5′-GTT CAT AAT CTG ACA CAG ATA GCC CAT AAT GTC-3′, Thr69Cys 5′-GGA CCT GTT GAC TGC TCA TGC GCT CTG ATT CTG-3′, 5′-CAG

AGC GCA TGA GCA GTC AAC AGG TCC CAG TTC-3'. Val86Cys 5'-GAC ACG CCA ATT TGC TAC GCC TCG GAA GCT TTT-3', 5'-TTC CGA GGC GTA GCA AAT TGG CGT GTC TTT TTG-3'. Ala101Cys 5'-GGA TAC AGC AAT TGC GAG GTC TTG GGG AGA AAC-3', 5'-CCC CAA GAC CTC GCA ATT GCT GTA TCC TGT CAT-3'. All the mutated genes were sequenced at the Biotechnology Resource Center of Cornell University.

(B) Crystallography

Crystallization—VVD light-state crystals were grown overnight at 17 °C from VVD-36 II (1) under by vapor diffusion from a 5 µl drop containing 3 µl of protein at 3.5 mg/ml, including 5mM DTT and 2 µl of reservoir solution. The protein was dissolved in a buffer containing 50mM HEPES pH 8.0, 150 mM NaCl and 10% glycerol and the reservoir solution contained 11–15% PEG 6000, 0.1M HEPES (pH=7.5 – 8.5), 5% MPD. The protein was exposed to white-light prior to crystallization and conversion to the light-adapted state was verified by UV-vis absorption spectroscopy. Crystal trays were exposed to white light once a day to maintain the protein in the light-adapted state. The largest crystals appeared as single plates (300 µm × 150 µm × 10 µm) that diffracted between 1.7 – 2.7 Å resolutions. Crystals were soaked in a cryoprotectant consisting of 80/20 percent volume reservoir solution with glycerol 30 sec prior to flash cooling in liquid N₂. Monoclinic crystals of space group P2₁ were obtained containing two molecules per asymmetric unit.

Structure determination and refinement—Diffraction data was collected at 100 K with synchrotron radiation on the 24-ID-E beamline at the Advanced Photon Source (APS) at the Argonne National Laboratory, Chicago. The data was reduced and scaled with HKL2000 (35) (Table S1). Initial phases for light-state VVD were obtained by molecular replacement by *Phaser* (36) using the dark-state structure (PDB ID: 2PD7) as a search model. Subsequent models were built in Xfit (37) and refined with CNS (38). The flavin was left out of the molecular replacement probe and added at the later stages of refinement. The quality of the flavin electron density was used as a metric for improvement of the model. It was determined that the X-ray beam reduced the covalent adduct formed in light-adapted protein, therefore two strategies were employed to keep the adduct intact, variations of which have been applied previously to VVD (22) and other LOV proteins (39). To reduce radiation exposure, each frame was collected over a 5° oscillation per second (X-ray flux of 2.33×10^{11} photons sec⁻¹ per 20×20 µm² beam at 0.979 Å) and secondly, data were collected from ten different spots on a single crystal and then merged to generate the complete dataset.

(C) VVD sequence analysis

Vivid sequence alignment and family analysis was performed using Pipealign (<http://bips.u-strasbg.fr/PipeAlign/>) (40). Once a protein sequence is submitted, the software conducts a BLAST search and aligns the sequences, followed by refinement, validation and subfamily classification. In case of VVD, the resulting 170 sequences were classified under 10 groups, which contained various WC-1, GATA factors, Phototropins, Histidine kinases and putative blue-light receptors.

(D) Phenotype analysis

Construction of plasmids—Point mutations were introduced into the plasmid pCHC01-gfp-vvd-v5 as previously described and confirmed by sequencing (Fig. S1A).

Strains—The wild-type strain used here is OR74A. Knockout strains came from the *Neurospora* knockout project (41). To establish that the *csr-1* knock-in strains used in this study were homokaryotic and carried only the transgene, we examined each strain by PCR

analysis (30 cycles) to confirm the absence of the *csr-1* ORF in the genome (21, 42) (Fig. S1B).

Culture conditions and light treatment—Culture procedures were performed as previously described (21). After 24 h of culturing with constant shaking (125 rpm) in darkness (DD) at 25°C, the flasks were moved to a shaker at 25°C with continuous white light (LL), covering a wide-range of the spectrum from 400 nm to 700 nm (cool white fluorescent light bulb, General Electric F20T12-CW, 20 $\mu\text{mol}/\text{m}^2/\text{s}$). Mycelia were harvested before and after white light treatment.

Analysis of photoadaptation defects on slants—On day 0, conidia were inoculated onto a minimal slant and placed in an incubator with either constant white light (20 $\mu\text{mol}/\text{m}^2/\text{s}$) or constant darkness (DD) at 25 °C. Photographs were taken on day 4.

Analysis of carotenoid induction and RT-QPCR—These methods were performed as previously described (21).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Light-state structure of VVD and the mechanism of light-induced signal transduction. (A) Electron density corresponding to the light-induced adduct between the sulfur of Cys108 and the C4a carbon of the FAD ($F_o - F_c$ omit maps, blue contours at 2.5σ). (B, C) Adduct formation protonates flavin N5 and in response, Gln182 flips to form two new hydrogen bonds – one with the N5 proton and the other with Ala72 carbonyl oxygen. Ala72 shifts and the hydrogen bond between Cys71 thiol breaks as the thiol rotates the Asp68 carbonyl pivots away from the turn. A new hydrogen bond forms between Asp68 and the main chain nitrogen of Ser70. These systematic changes in the hydrogen bonding pattern of the hinge region, result in a 3.5 Å movement of Pro66. Shifts of $\alpha\alpha$, destabilize the N-terminal latch against the protein core, and lead to its release, which is further facilitated by the flexibility of Gly44. Inset: Adduct formation in one subunit (yellow) leads to smaller changes in the hinge loop position than the other (cyan), which moves forward over Pro66 despite maintaining a similar internal conformation. (D) In the light state structure, the N-cap (represented in darker shades of grey and yellow) restructure to release the N-terminal latch while the other regions of the protein do not change appreciably compared to the dark-state structure.

Fig. 2. The light state dimer of VVD. (A) The protein crystallizes as a dimer with the N-cap (represented in darker shades of yellow and cyan) composing the dimer interface. Met48 and Ile52 in α make important hydrophobic interactions within the interface. (B) Interactions within the dimer interface: Tyr40 (cyan) hydrogen bonds with the side chain of Thr164 and the main chains of Phe162 and Phe181 of the other subunit. The main chain carbonyl of Pro66, which shifts by 3.5Å, hydrogen bonds with the main chain nitrogen of Ala41 of the other subunit. Cys71, whose side chain flips in the light state, hydrogen bonds with the main chain of Tyr40 in the opposite subunit. (C) Within the dimer interface Tyr87 hydrogen bonds with the side chain and the backbone of Thr69, whereas Met48 and Asp46 hydrogen bond with the backbone of Val67.

Fig. 3.

Ability of VVD variants to undergo dimerization. The % monomer is calculated by areas of respective light and dark state elution peaks on size exclusion chromatography (28). The Y40K, Y40E, Y40I and Y40F variants disrupt important hydrogen bonds at the interface and lose the ability to dimerize in the presence of light. The T69W variant is a dimer in both the dark and the light, presumably because it facilitates intersubunit contacts that overcome light-promoted conformational switching. (* from reference 22, ** from reference 26)

Fig. 4.

Dimerization of VVD is essential for the biological functions of VVD *in vivo*. (A) Carotenoid accumulation as a measure of photoadaptation defects in various *vvd* mutants. LL indicates strains were exposed to constant white light stimulus with photon flux of $20\mu\text{mol}/\text{m}^2/\text{sec}$ for four days; DD indicates strains were kept in constant darkness. WT (74A) represents a wild-type strain without photoadaptation defects. $\Delta wc-1$ & $\Delta wc-2$ represents a “blind” strain. (B) Photoadaptation defects quantified by the amount of carotenoid extracted from mycelia at LL240 ($n=5$, biological replicates). Horizontal lines denote means. (C) Repression efficacy of various *vvd* mutant alleles as determined by the repression of *al-3* expression at LL60 with RT-QPCR analysis ($n=3$, mean values $\pm$ standard

error). Asterisks indicate statistical significance when compared to the wild-type allele of *vvd* as determined by *unpaired t-test*, *** $p < 0.001$, * $p < 0.05$.