In nature as in biotechnology, light-oxygen-voltage photoreceptors perceive blue light to elicit spatiotemporally defined cellular responses. Photon absorption drives thioadduct formation between a conserved cysteine and the flavin chromophore. An equally conserved, proximal glutamine processes the resultant flavin protonation into downstream hydrogen-bond rearrangements. Here, we report that this glutamine, long deemed essential, is generally dispensable. In its absence, several light-oxygen-voltage receptors invariably retained productive, if often attenuated, signaling responses. Structures of a light-oxygen-voltage paradigm at around 1 Å resolution revealed highly similar light-induced conformational changes, irrespective of whether the glutamine is present. Naturally occurring, glutamine-deficient light-oxygen-voltage receptors likely serve as bona fide photoreceptors, as we showcase for a diguanylate cyclase. We propose that without the glutamine, water molecules transiently approach the chromophore and thus propagate flavin protonation downstream. Signaling without glutamine appears intrinsic to light-oxygen-voltage receptors, which pertains to biotechnological applications and suggests evolutionary descendance from redox-active flavoproteins.
Light-oxygen-voltage (LOV) proteins form a sensory photoreceptor class that elicit a wide palette of physiological responses to blue light across archaea, bacteria, protists, fungi, and plants\textsuperscript{1-3}. Complementing their eminent role in nature, LOV receptors also serve as genetically encoded actuators in optogenetics\textsuperscript{4} for the spatiotemporally precise control by light of cellular state and processes\textsuperscript{5}. At the heart of these responses lies the flavin-binding LOV photosensor module which belongs to the Per-ARNT-Sim superfamily\textsuperscript{6} and comprises several a-helices (denoted Ca, Da, Ea, and Fa) arranged around a five-stranded antiparallel \textbeta-sheet (strands A\textbeta, B\textbeta, G\textbeta, H\textbeta, and I\textbeta)\textsuperscript{7,8} (Suppl. Fig. 1). Light absorption by the flavin triggers a well-studied photocycle\textsuperscript{9,10,11}, as part of which an initial electronically singlet state (S\textsubscript{1}) decays within nanoseconds to a triplet state (T\textsubscript{1}) (Fig. 1a). Likely via a radical-pair mechanism\textsuperscript{12,T}, T\textsubscript{1} reacts within microseconds to the signaling state, characterized by a covalent thioadduct between the flavin C4a atom and a conserved cysteine residue in the LOV photosensor and the C4a atom of the flavin isoxazolone ring system. Once illumination ceases, the signaling state passively reverts to the base-catalyzed dark-recovery reaction\textsuperscript{13}. Thioadduct formation entails a hybridization change of the flavin C4a atom from sp\textsuperscript{2} to sp\textsuperscript{3} and concomitant protonation of the adjacent N5 atom. The resultant conversion of the N5 position from a hydrogen bond acceptor to a donor serves as instrumental in reading out the flavin N5 position and elicits the downstream transitions. Supported by spectroscopy, structural and functional data, chemical reasoning, and molecular simulations\textsuperscript{8,18-24}, the glutamine residue is strongly conserved across LOV receptors\textsuperscript{6,24} and has been identified as instrumental in reading out the flavin N5 position and eliciting blue-light responses\textsuperscript{21,30}. By contrast, reports on other LOV receptors considered the glutamine residue non-essential in several model LOV receptors (Fig. 1b and Suppl. Fig. 1). Unexpectedly, the glutamine residue, which is considered essential in LOV signaling as productive blue-light responses were generally maintained even in its absence. Almost all other amino acids could functionally substitute for the conserved glutamine, with notable exceptions. High-resolution crystal structures of the paradigm Avena sativa phototropin 1 LOV2 (AsLOV2) domain revealed that after glutamine substitution by leucine, closely similar structural changes are evoked by light as in the wild type. Based on structural data, chemical reasoning, and molecular simulations, we propose that in the absence of the glutamine, water molecules relay hydrogen-bonding signals from the flavin N5 position to the LOV \textbeta-sheet. The ability to transduce light signals without the glutamine appears to be an inherent, general trait of LOV receptors and may reflect their evolutionary origin. This notion finds support in the

![Fig. 1](https://example.com/fig1.png)

**Fig. 1** Photochemistry of light-oxygen-voltage (LOV) receptors and sequences of proteins under study. a) Photocycle of light-oxygen-voltage (LOV) receptors. Absorption of blue light by the dark-adapted state (D\textsubscript{450}) prompts the LOV receptor to traverse short-lived excited singlet (S\textsubscript{1}) and triplet (T\textsubscript{1}) states before assuming the light-adapted state (S\textsubscript{390}), which is characterized by a thioadduct between the flavin atom C4a and the sidechain of a conserved cysteine. Auctord formation goes along with protonation of the N5 atom which entails changes in hydrogen bonding within the LOV receptor, particularly of a glutamine residue situated in strand I\textbeta which coordinates the pteridin portion of the flavin. Indications and to provide further insight into signal transduction, here we systematically investigated the role of the conserved glutamine in several model LOV receptors (Fig. 1b and Suppl. Fig. 1). Unexpectedly, the glutamine residue is not essential in LOV signaling as productive blue-light responses were generally maintained even in its absence. Almost all other amino acids could functionally substitute for the conserved glutamine, with notable exceptions. High-resolution crystal structures of the paradigm Avena sativa phototropin 1 LOV2 (AsLOV2) domain revealed that after glutamine substitution by leucine, closely similar structural changes are evoked by light as in the wild type. Based on structural data, chemical reasoning, and molecular simulations, we propose that in the absence of the glutamine, water molecules relay hydrogen-bonding signals from the flavin N5 position to the LOV \textbeta-sheet. The ability to transduce light signals without the glutamine appears to be an inherent, general trait of LOV receptors and may reflect their evolutionary origin. This notion finds support in the
existence in nature of numerous LOV receptors that lack the conserved glutamine and presumably serve as blue-light receptors, as we confirm for a glutamine-deficient, proteobacterial LOV-diguanulate cyclase.

**Results**

**Signal transduction in LOV receptors lacking the active-site glutamine.** To evaluate if and how LOV photosensors can transduce light signals to associated effector units in the absence of the conserved glutamine, we initially resorted to the histidine kinase YF1, as it allows the efficient assessment of signaling responses. Together with the response regulator BfFixJ, the engineered LOV receptor YF1 forms a light-sensitive two-component system (TCS) (Fig. 2a). E. coli cultures harboring the pDsRed plasmid, which encodes the YF1/BfFixJ TCS, exhibited strong expression of the red-fluorescent reporter DsRed as YF1 acts as a net kinase in darkness. Blue light converts YF1 to a net phosphatase, and accordingly, the DsRed fluorescence decreased by around 12-fold (Fig. 2b).

To probe the role of the active-site glutamine (position Q123) in signal transduction, we substituted this residue for all 19 other canonical amino acids. Strikingly, most of the resultant glutamine-deficient YF1 variants prompted a blue-light-induced reduction of reporter gene fluorescence, similar to the original YF1 and almost regardless of which residue replaced the glutamine. These data clearly indicate that at least in the pDsRed setup, the majority of residue substitutions, including alanine, cysteine, glutamic acid and leucine, leave light-dependent signal transduction largely unimpaired. Merely, the substitution by proline and the bulky aromatic amino acids His, Trp, and Tyr abolished responsiveness and resulted in high reporter expression independently of light. Similarly, the Q123R variant did not react to light but exhibited constitutively low reporter fluorescence.

The Q123A and Q123N exchanges were previously assessed in *Bacillus subtilis* YtvA from which YF1 derives. As probed by photocalorimetry and in vivo analysis, the Q123A substitution slightly impaired signal transduction, but Q123N completely abolished any light responsiveness. Whereas the Q123A findings are consistent with the present data, YF1 Q123N retained attenuated light responses. The divergent observations for the Q123N exchange might be tied to the different effector modules in *B*YtvA and YF1. We note that asparagine in this position can principally support LOV signal transduction, as indicated by partial preservation of light responsiveness in the corresponding Q513N variant of AsLOV2.

To glean additional insight, we expressed and purified the variants Q123H, Q123L, Q123P, and Q123R alongside YF1. Absorbance spectroscopy revealed flavin incorporation, as indicated by a three-pronged peak around 450 nm, for all variants but Q123R which failed to incorporate the chromophore and was prone to aggregation (Fig. 2c and Suppl. Fig. 2). As indicated by circular dichroism (CD) spectroscopy, the variants Q123H, Q123L, and Q123P were folded and adopted secondary structure similar to YF1 (Suppl. Fig. 2). Upon blue-light exposure, YF1 and its variants Q123L and Q123P underwent the canonical LOV photocycle and adopted the thioadduct state with a characteristic absorption maximum near 390 nm (Fig. 2c and Suppl. Fig. 2). By contrast, the Q123H variant failed to form the adduct state despite incorporating the flavin cofactor, in line with earlier reports on AsLOV2. Only at high blue-light doses, the flavin absorption band slightly decreased in intensity but no band at 390 nm was formed. As reported earlier, replacement of the glutamine residue incurred a hypsochromic shift by around 8 nm of the flavin absorbance peak in both the dark-adapted and light-adapted
states. This spectral shift can tentatively be attributed to the breaking of hydrogen bonding to the flavin O4 atom (see Fig. 1a) and is reminiscent of a bathochromic shift of similar magnitude during the photocycle of the so-called “sensors of blue light using flavin adenine dinucleotide” (BLUF)37,38. Taken together, the absorbance data account for the absent light responses in the pDusk context (see Fig. 2b) of the Q123H (no photocycle) and Q123R variants (no chromophore).

We next recorded the dark recovery after blue-light exposure and found the return to the dark-adapted state 10-fold decelerated in Q123L relative to YF1 (Suppl. Fig. 2). The Q123P variant exhibited even slower kinetics that was not completed even after several days. Given that the Q123L variant principally retained the capability of transducing signals (see Fig. 2b), we reasoned that modification of the active-site glutamine provides an additional, little-tapped means of altering recovery kinetics39 and thus modulating photosensitivity at photostationary state40. To explore this effect, we assessed the response of YF1 Q123L to pulsatile blue-light illumination41 in the pDawn system that derives from pDusk but exhibits an inverted response to blue light31. The Q123L variant was toggled by much lower light doses than YF1, fully consistent with its retarded dark recovery (Suppl. Fig. 3). Compared to the V28I substitution, which also decelerates dark recovery by around 10-fold39,41,42, the Q123L exchange was somewhat less sensitive to blue light. Combining the substitutions V28I and Q123L did not provide a further gain but slightly reduced the effective light sensitivity.

As the pDusk system only indirectly reports on the molecular activity of the receptors, we probed the catalytic activity and response to light of purified YF1 and its variants in a coupled fluorescence anisotropy assay (Fig. 2d). In darkness and in the presence of ATP, YF1 phosphorylates its cognate response regulator BfFixJ, thus prompting its homodimerization and binding of the FixK2 DNA operator sequence14,43. Phosphorylation-induced binding of BfFixJ to a short, double-stranded DNA molecule slows its rotational diffusion and causes an increase in fluorescence anisotropy of a 5′-attached tetramethylrhodamine (TAMRA) moiety. As noted above, blue light converts YF1 into a net phosphatase, thus promoting BfFixJ dephosphorylation, DNA dissociation, and a decrease in fluorescence anisotropy. Upon ATP addition, the dark-adapted YF1 and the Q123H, Q123L, and Q123P variants all exhibited increasing fluorescence anisotropy, albeit with somewhat differing kinetics and amplitude. Whereas Q123L showed a similar response as YF1, the Q123H and Q123P variants reached higher anisotropy values which likely reflects a higher degree of BfFixJ phosphorylation than the roughly 50% achieved for YF115. The intrinsic equilibrium between the elementary histidine kinase and phosphatase activities of the TCS thus appears tilted towards the kinase state for Q123H and Q123P compared to YF1 and Q123L44,45. Upon blue-light application, the Q123L variant responded with a rapid fluorescence-anisotropy decay of around half the amplitude seen for YF1, indicating that light signals are transduced by this variant but less efficiently (Fig. 2e). Consistent with the pDusk reporter assay (see Fig. 2b), neither the Q123H nor the Q123P variant showed any response in their catalytic activities to blue light. In the case of Q123H, these observations are readily explained by its inability to undergo light-induced adduct formation and flavin N5 protonation. By contrast, the absorbance measurements unequivocally showed that Q123P can progress through the canonical LOV photocycle (see Suppl. Fig. 2). As the LOV photophysics hence remains intact, signal transduction in the Q123P variant must be interrupted further downstream.

LOV signal transduction can generally occur in the absence of the active-site glutamine. We next addressed whether the striking ability to transduce light signals without the conserved glutamine residue is specific for YF1 or more widely shared across LOV receptors. To this end, we examined light-dependent signaling responses in Nakamuraella multipartita PAL46, as a naturally occurring LOV receptor, and the A. sativa phototropin 1 LOV2 domain, as the arguably best-studied and optogenetically most widely used LOV module18,22,35,47,48. Namely, NmPAL differs from YF1 by an unusual C-terminal arrangement of its LOV photosensor and binds a small RNA aptamer sequence-specifically and in a light-activated manner46. By embedding this aptamer directly upstream of the Shine-Dalgarno sequence in an mRNA encoding the fluorescent DsRed protein, NmPAL activity and response to light can be assessed in a bacterial reporter assay (Fig. 3a). In its dark-adapted state, wild-type NmPAL has little affinity for the aptamer, and DsRed is readily expressed. Light-induced binding by NmPAL interferes with expression, presumably at the translational level, and reporter fluorescence is diminished by 10-fold (Fig. 3b). Using this assay, we tested the effect of replacing the active-site glutamine (residue Q347 in NmPAL) with histidine, leucine, or proline. Consistent with the findings for YF1, the resultant Q347H and Q347P variants no longer exhibited light-induced changes in reporter fluorescence. As in the YF1 case, the proline variant had constitutive activity similar to the dark-adapted parental wild-type NmPAL. Conversely, for Q347H we observed constitutively low fluorescence values, indicative of RNA binding and thus corresponding to light-adapted wild-type NmPAL. This contrasts with YF1 where the corresponding histidine variant functionally corresponded to the dark-adapted state of the parental receptor. The Q347L variant exhibited a light-induced decrease of DsRed fluorescence by around 17-fold, thus even surpassing the value for wild-type NmPAL. Taken together, the results from the NmPAL reporter assay are broadly consistent with the findings for YF1 in that the leucine substitution supported light responses to a significant extent whereas the histidine and proline substitutions incurred a loss of light-dependent signal transduction.

We next tested whether the ability of NmPAL Q347L to transduce light signals extends to applications in eukaryotic cells. To this end, we harnessed an approach based on the translational repression of a luciferase reporter in HeLa cells46 (Fig. 3c). Under blue light, wild-type NmPAL can bind to an aptamer sequence embedded in the 5′-untranslated region of an mRNA and thereby represses luciferase expression by 10-fold relative to darkness (Fig. 3d). Upon introduction of the Q347L substitution into NmPAL, blue-light-induced downregulation of reporter expression was maintained, albeit at reduced, fourfold efficiency.

To investigate photochemistry and RNA binding in detail, we expressed and purified NmPAL wild-type and Q347L. In line with the reporter assays (see Fig. 3b–d), the Q347L variant retained flavin chromophore binding and underwent canonical LOV photochemistry upon blue-light exposure (Suppl. Fig. 4). As in YF1, replacement of the glutamine entailed a hypsochromic shift of the flavin absorption. Recovery kinetics after blue-light illumination were however only slowed down by 1.2-fold in the Q347L variant, rather than the 10-fold slowdown in YF1. Far-UV CD spectroscopy showed that NmPAL and its Q347L variant adopt closely similar secondary structures (Suppl. Fig. 4c). We next assessed the binding of NmPAL wild-type and Q347L to a TAMRA-labeled RNA aptamer by fluorescence anisotropy46 (Fig. 3e, f). Wild-type NmPAL bound the RNA with an affinity of (45.4 ± 5.4) nM in its dark-adapted state but showed much-reduced interaction in darkness [(1200 ± 93) nM]. Under the same conditions, NmPAL Q347L interacted with the aptamer somewhat less strongly under blue light [(202.5 ± 8.5) nM] but exhibited more pronounced residual binding in darkness with an affinity of around (930 ± 70) nM. Thus, light-dependent signal
transduction is principally retained in NmPAL Q347L but is impaired compared to the wild-type receptor, similar to the observations on YF1.

We next turned to the LOV2 domain from A. sativa phototropin 1 (AsLOV2) as a widely studied paradigm that underpins manifold applications in optogenetics. Whereas AsLOV2 wild-type, Q513H, and Q513L could all be produced with good yield and purity, the Q513P variant suffered from poor expression and severe aggregation, thus precluding its further analysis. The Q513H and Q513L variants incorporated flavin cofactors and exhibited a hypsochromically shifted absorbance spectrum compared to wild-type AsLOV2 (Suppl. Fig. 5), as seen for YF1 and NmPAL. Under blue light, the Q513L variant populated the thioadduct state which recovered to the resting state in darkness with kinetics around 22-fold slower than those of the wild-type domain (Suppl. Fig. 5). By contrast, the Q513H variant failed to undergo the canonical LOV photochemistry, consistent with the YF1 and NmPAL scenarios. The dark-adapted wild-type, Q513H, and Q513L proteins showed closely similar far-UV CD spectra, characterized by two minima of the molar ellipticity per residue, \([\Theta]_{\text{MRW}}\), at ~208 nm and 220 nm, and consistent with the mixed \(\alpha\beta\) fold of AsLOV2 (Fig. 4 and Suppl. Fig. 5). Exposure to blue light diminished the amplitude of the minima by ~30–35% for both AsLOV2 wild-type and Q513L, reflecting the unfolding of the N-terminal A'\(\alpha\) and the C-terminal J\(\alpha\) helices. However, given the relatively fast recovery of AsLOV2 wild-type (see Suppl. Fig. 5), a significant return to the dark-adapted state is expected during the spectral scan (taking ~1 min). We hence monitored the \(\alpha\)-helical CD signal at (208 ± 5) nm immediately after the withdrawal of blue
Molecular bases of LOV signal transduction without the active-site glutamine. The above findings compellingly show that several LOV receptors transduce light signals in the absence of the active-site glutamine, long considered essential. To arrive at a molecular understanding, we solved the crystal structures of AsLOV2 wild-type and Q513L in the dark-adapted states to resolutions of 1.00 Å and 0.90 Å, respectively. Notably, both AsLOV2 variants formed crystals at the previously published solution conditions and adopted the same space group with closely similar cell dimensions (Suppl. Tables 1 and 2). To additionally acquire information on the light-adapted state, we pursued a freeze-trapping strategy. Dark-grown crystals were exposed to blue light and rapidly cryo-cooled, X-ray diffraction was recorded, and structures were refined to resolutions of 1.09 Å (wild type) and 0.98 Å (Q513L) (Suppl. Tables 1 and 2). Although the crystal lattice stands to influence any structural rearrangements, in the past light-induced conformational transitions could thus be resolved for several LOV receptors, if likely at well-defined and tractable experimental setup. Replacement of the active-site cysteine (residue 450) in wild-type AsLOV2 by alanine abolished canonical photochemistry but the NSQ yield was poor, even at prolonged illumination and in the presence of the reductant TCEP (Suppl. Fig. 5). Nor did the additional introduction of the Q513L exchange significantly enhance NSQ formation. We thus capitalized on the recent finding that replacement of the active-site glutamine by aspartate in an Arabidopsis thaliana phototropin LOV domain greatly promoted photoreduction to the NSQ. Given that the corresponding Q123D substitution in YF1 retained signaling capability (see Fig. 2b), we generated AsLOV2 Q513D and the doubly substituted C450A:Q513D variant. Absorbance spectroscopy revealed that the Q513D variant underwent the canonical LOV photochemistry and formed the thioadduct state (Suppl. Fig. 5). CD spectroscopy showed a light-induced 25% loss of [θ]MRW, indicating that the Q513D variant can indeed transduce blue-light signals (Suppl. Fig. 5). In the case of AsLOV2 C450A:Q513D, blue light drove rapid conversion to the NSQ state even without the addition of reductants, as evidenced by absorbance spectroscopy (Suppl. Fig. 5). Analysis by CD spectroscopy identified an ~10% loss in α-helical content upon blue-light exposure (Fig. 4e). Notably, the underlying conformational change was reversible, and upon slow reoxidation of the NSQ to the quinone state, the CD signal recovered over time. To ascertain that the change in helical content truly involves the Aα and Jα helices as in wild-type AsLOV2, we generated the AsLOV2 C450A:Q513D ΔAα ΔJα derivative with these helices truncated. Consistent with the removal of Aα and Jα, this variant exhibited a 20% reduction in [θ]MRW at 220 nm (Fig. 4f). Rather than a decrease, blue light elicited a small signal gain at ~208 nm whose molecular origin is unclear. Given that both the quinone and NSQ states strongly absorb in the far-UV region, we tentatively ascribe this signal to flavin photoreduction. As we observed no loss in α-helical structure, the light-induced structural changes in AsLOV2 C450A:Q513D are likely caused by the partial unfolding of the terminal Aα and Jα helices. Although the amplitude of the structural response is greatly reduced compared to wild type, it is striking that light-induced responses can be elicited in the absence of two highly conserved active-site residues.

Fig. 4 Light response of AsLOV2 variants. a Far-UV circular dichroism (CD) spectra of AsLOV2 in its dark-adapted (black) and light-adapted states (blue), and after dark recovery (gray dotted). b Recovery reaction of AsLOV2 following blue-light exposure, as monitored by the CD signal at (220 ± 5) nm. Data were fitted to a single-exponential decay (red line), yielding a recovery rate constant k−1 of (1.43 ± 0.05) × 10−2 s−1. c As a but for AsLOV2 Q513L. d As panel b but for AsLOV2 Q513L, with k−1 amounting to (6.61 ± 0.15) × 10−4 s−1. e As a but for AsLOV2 C450A:Q513D. f As a but for AsLOV2 C450A:Q513D ΔAα ΔJα. Experiments were repeated at least twice with similar results.

light (Fig. 4b, d). The kinetic measurements revealed that the initial amplitude of the light-induced CD change in AsLOV2 Q513L was only half that in the wild-type protein. For both variants, the CD spectra fully recovered to their original states (Fig. 4) with kinetics matching those of the photochemical recovery probed by absorbance measurements (see above and Suppl. Fig. 5). In agreement with our findings, an earlier study reported light-induced CD changes for the Q513L variant but at much-reduced amplitude compared to wild type, it is unclear. Given that both the quinone and NSQ states strongly absorb in the far-UV region, we tentatively ascribe this signal to flavin photoreduction. As we observed no loss in α-helical structure, the light-induced structural changes in AsLOV2 C450A:Q513D are likely caused by the partial unfolding of the terminal Aα and Jα helices. Although the amplitude of the structural response is greatly reduced compared to wild type, it is striking that light-induced responses can be elicited in the absence of two highly conserved active-site residues.
chromophore-binding pocket and its surroundings (Fig. 5, Suppl. Fig. 7). Notably, these differences were consistent across several crystals, implying that they are genuinely tied to the Q513L exchange and illumination, respectively.

The structure of dark-adapted AsLOV2 wild-type (Fig. 5a) well agreed with a previous determination at 1.4 Å (PDB entry 2v0u, mainchain rmsd 0.13 Å)⁴⁷. As observed before, the active-site cysteine 450 adopted a major (80%) conformation a, pointing away from the flavin C4a atom, and a minor (20%) one b, oriented towards C4a (Suppl. Figs. 7 and 8). The flavin pteridin moiety was coordinated by the asparagines N482 and N492, and the flavin O4 atom hydrogen-bonded to the amide NeH₂ group of

Fig. 5 Structural analyses of AsLOV2 variants. a Chromophore-binding pocket of wild-type AsLOV2 in its dark-adapted state as revealed by a 1.00 Å crystal structure. b Chromophore-binding pocket of wild-type AsLOV2 in its light-adapted state as revealed by a 1.09 Å crystal structure. c Chromophore-binding pocket of AsLOV2 Q513L in its dark-adapted state as revealed by a 0.90 Å crystal structure. d Chromophore-binding pocket of AsLOV2 Q513L in its light-adapted state as revealed by a 0.98 Å crystal structure. For clarity, helices Cα and Dα are not shown in panels a-d. The Jα helix is drawn in orange, and the flavin- mononucleotide cofactor and key amino acids are highlighted in stick representation. Minor conformations of residues and the flavin nucleotide are drawn in narrower diameter. The active-site cysteine 450 adopts two principal orientations, denoted 'a' and 'b'. In the structures of dark-adapted AsLOV2 wild-type, dark-adapted Q513L, and light-adapted Q513L, orientation 'b' splits into two subpopulations with slightly different χ₁ angles. Dashed lines denote hydrogen bonds. e Water density in the interior of dark-adapted AsLOV2 Q513L derived from a 300 ns classical molecular dynamics simulation. The red mesh denotes a density level of 0.3 water molecules per Å³. f As e but for light-adapted AsLOV2 Q513L. Corresponding simulations for AsLOV2 wild-type are provided in Suppl. Fig. 13a, b.
the conserved Q513. Via its NδH₂ group, N414 at the start of strand αβ entered hydrogen bonds with the backbone carbonyl oxygen of Q513 and the carboxylate group of D515, situated at the tip of strand ββ and part of the conserved PAS DIT motif⁶. At the present high resolution, an alternate conformation could be resolved for the terminal turn of the Jα helix (residues 543–546), possibly reflecting the inherent equilibrium between folded and unfolded helical states⁵,36.

The light-adapted state of AsLOV2 wild type (Fig. 5b) exhibited a series of conformational differences consistent with a previous report at 1.7 Å resolution (PDB entry 2v0w; mainchain rmsd 0.21 Å)⁷. Given the higher resolution achieved presently, additional structural transitions could be pinpointed as summarized below. The sidechain of C450 reoriented towards the flavin C4a, thus shifting the ratio of the conformations a and b to 40%:60% (Suppl. Fig. 7). As in other structures of photoactivated LOV receptors⁸–⁴⁷, little electron density for the cysteinyl-flavin thioadduct was observed, likely owing to X-ray radiolysis of the metastable thioether bond. Beyond the altered conformation of C450, the population of the light-adapted state was indicated by a ~6.9° tilt of the isoalloxazine plane towards the cysteine (Suppl. Fig. 9)⁸,¹⁹. Based on earlier reports⁸,¹⁹,⁴⁷,⁵⁵,⁵⁷, chemical reasoning, and spectroscopic evidence¹⁸,²⁰, the sidechain of the conserved glutamine Q513 was modeled to undergo a 180° flip in response to enable hydrogen bonding between the amide Oδ group and the newly protonated flavin N5 position. Upon reorientation, the Q513 amide NH₂ group hydrogen-bonded with the backbone carbonyl O of N414. The asparagine 414 in turn rotated, thus breaking contact to D515 and enabling a new hydrogen bond between its amide Oδ group and NH₂ of Q513 (Suppl. Fig. 7).

Notably, the dark-state conformations of both Q513 and N414 were retained as a minor population (20%) in the light-adapted state, potentially due to incomplete photoactivation in the crystal. The reorientation of N414 correlated with a 0.4 Å shift of its Ca atom, thereby prompting the entire Aα segment to dislodge and move away from Q513 (Suppl. Fig. 10). Crucially, the Aα helix is interlocked with the C-terminal part of Jα via the hydrophobic residues L408, I411, I539, A542, and L546. The displacement of Aα thus went along with an outward movement of the last 1.5 helical turns of Jα, which could potentially promote its unfolding¹⁵. Support for this notion derives from the well-documented detrimental effect of the I539E substitution at the Aα-Jα interface⁸⁸, from molecular dynamics (MD) simulations that revealed correlated motions of the Aα and Jα helices⁴⁴, and from a recent study on circularly permuted AsLOV2 which pinpointed the Ja C-terminus as pivotal for light-dependent signaling, whereas the N-terminal part could be dispensable with⁴⁸. In addition to the above differences, the light-adapted state also exhibited enhanced flexibility of the Aβ-Bβ and Gβ-Hβ loops, consistent with a global gain of mobility upon light absorption in AsLOV2 and other LOV domains¹⁵,⁵⁸.

In dark-adapted AsLOV2 Q513L (Fig. 5c), the flavin plane was displaced by ~0.4 Å relative to the wild-type protein, arguably due to steric interactions between the flavin O4 and the C62 methyl group of L513. Notably, no ordered water molecules entered the space vacated by the glutamine removal. The resultant loss of hydrogen bonds at the flavin O4 atom may account for the hyperschismic absorbance shift evidenced above across the different LOV receptors with replaced glutamate. C450 adopted the orientations a and b, pointed away and towards the flavin C4a atom, respectively, at a ratio of 70%:30% (Suppl. Fig. 7). The Q513L replacement notwithstanding, the crucial N414 residue assumed the conformation seen in darkness for the wild type, i.e., engaged in hydrogen bonds with D515 and the backbone carbonyl O of residue 513. Interestingly, the Q513L dark state showed alternate conformations for the Aβ-Bβ and Gβ-Hβ loops, in the case of the wild-type receptor only seen upon light exposure. Despite lacking the conserved glutamine, the AsLOV2 Q513L variant displayed structural responses in its light-state structure remarkably similar to the wild type, in line with the above functional assays that invariably demonstrated qualitatively intact light responses after leucine introduction. Specifically, C450 adopted the conformations a and b at a 40%:60% ratio, and the flavin ring plane tilted towards the cysteine by ~4.6°. Strikingly, L513 did not exhibit any dark-light differences, implying that its sidechain is inert and not actively participating in the signal relay. This notion is supported by the observation that most of the canonical amino acids with diverse sidechains supported productive light responses in the YF1 receptor (see Fig. 2b).

Intriguingly, the crucial N414 assumed the light-adapted conformation to 40% extent; signals were evidently transduced from the flavin to this site even in the absence of the intermediary glutamine, if at reduced efficiency compared to wild-type AsLOV2. Rotation of the asparagine sidechain was accompanied by the same structural transitions evidenced in the wild-type receptor, most importantly an outward shift of the N414 Ca atom and the complete Aα segment (Suppl. Fig. 10).

Collectively, the data reveal at high resolution how light stimuli propagate from the flavin to the LOV β-sheet interface and the terminal Aα and Ja helices, structural elements generally associated with downstream signal transduction across LOV domains¹⁵,³²,⁴⁶,⁵⁹–⁶¹. Strikingly, the Q513L variant underwent the same qualitative responses as the wild type which raises the question of how signal relay to N414 and beyond can be rationalized in the absence of the glutamine? As candidate mechanisms, we principally considered electrostatic interactions through space and water-mediated rearrangement of hydrogen-bonding networks. To assess the validity of these proposals, we resorted to molecular simulations. First, we evaluated how cysteinyl-flavin thioadduct formation and accompanying N5 protonation impact the electrostatic potential of wild-type AsLOV2 in the absence of other hydrogen-bonding changes. Electrostatics calculations revealed that changes in the potential were small in magnitude, largely confined to the immediate chromophore surroundings, and not extending far in space (Suppl. Fig. 11). Highly similar electrostatic potentials resulted for the corresponding AsLOV2 Q513L structures, and we thus deem signal transduction through space via altered electrostatics unlikely. Although the light-state structures of AsLOV2 wild-type and Q513L did not exhibit ordered water molecules in the immediate vicinity of position 513, we hypothesized that water might transiently enter the chromophore-binding pocket and thus relay the N5 protonation change in the light-adapted state. This notion finds support in classical MD simulations that indicate water penetration into the flavin binding pocket upon light exposure (Fig. 5e, f and Suppl. Fig. 12). Whereas in the simulations of dark-adapted AsLOV2 Q513L only two significant water clusters were observed inside the protein, the light-adapted state seemed to “soak” up water from the bulk solvent and displayed nine clusters in the protein interior. Closely similar results were obtained in simulations on AsLOV2 wild-type (Suppl. Fig. 13a, b). This striking phenomenon can be rationalized by reduced rigidity of the protein backbone upon formation of the cysteinyl adduct (Suppl. Fig. 13c, d). The pairwise root mean square deviation between snapshots from the MD trajectory was below 1.8 Å for dark-adapted AsLOV2 Q513L but lay in the region of 2.4 Å and higher for the light-adapted state. These findings concur with the above-mentioned increase in general protein mobility evidenced in LOV receptors upon thioadduct formation¹⁵,⁵⁸. An overall increase in protein dynamics of the light-adapted state was also observed in earlier simulations⁴⁴. As in the present study, the Aα:β-sheet interface
and residues N414 and Q513 were identified as crucial conduits for signal propagation. Consistent with our simulations, water was found to transiently enter the chromophore-binding pocket upon flavin thioadduct formation and N5 protonation. By contrast, the study reported reduced water influx and attenuated signal propagation in the case of the Q513L variant.

**Signal transduction in natural glutamine-deficient LOV receptors.** Given that LOV signal transduction evidently does not strictly depend on the conserved glutamine, we wondered whether LOV-like receptors exist in nature that lack this residue. To address this question, we conducted sequence searches and identified around 350 putative LOV receptors, denoted LOV\(^{ΔQ}\) in the following, that possess several residues highly conserved across LOV domains\(^{62}\) but lack the active-site glutamine (Fig. 6a and Suppl. Data 3). Interestingly, these receptors featured a range of other amino acids in lieu of the active-site glutamine, predominantly the hydrophobic amino acids leucine and isoleucine, but also polar residues such as serine or threonine, and even histidine and cysteine. By contrast, large aromatic residues (phenylalanine, tyrosine, and tryptophan) were largely absent, as were proline and charged amino acids (Suppl. Fig. 14).

The sheer existence of LOV\(^{ΔQ}\) proteins in nature raises the tantalizing prospect that they can truly serve as blue-light receptors. To principally address this possibility, we selected for further analysis a LOV\(^{ΔQ}\)-GGDEF receptor from the proteobacterium *Mesorhizobium loti* which features a methionine at position 140 instead of the conserved glutamine (Genbank entry WP_140774521.1, see Fig. 6a). GGDEF and EAL domains antagonistically synthesize and degrade, respectively, the ubiquitous bacterial second messenger cyclic-di-(3\(^-\),5\(^-\))-guanosine monophosphate (c-di-GMP)\(^{63}\). To assess potential light responses, we expressed the C-terminally truncated LOV\(^{ΔQ}\) GGDEF receptor in the *E. coli* reporter strain KN78 which lacks the major diguanylate cyclase DgcE and carries a translational fusion between the c-di-GMP-controlled csgB locus and β-galactosidase\(^{64}\). As a positive control, a strain expressing DgcE revealed high light responses, whereas a strain expressing the M140Q variant exhibited constitutively high activity of around 450 Miller units (M.u.), irrespective of illumination (Fig. 6c). The KN78 strain yielded activity levels and light responses similar to those of the wild-type protein. Taken together, the results suggest that the *M. loti* LOV\(^{ΔQ}\)-GGDEF protein acts as a blue-light-repressed
diguanilate cyclase despite lacking the conserved glutamine residue.

**Discussion**

**Mechanism of signal transduction sans glutamine.** Following the description of LOV receptors as blue-light-receptive flavoproteins, optical and nuclear magnetic resonance spectroscopy identified the formation of the cysteinyl-flavin adduct in the signaling state. Owing to a hybridization change of the flavin C4a atom from $sp^2$ to $sp^3$ in the adduct, the adjacent N5 atom is protonated and thus converted from a hydrogen-bond acceptor in the dark-adapted state to a donor in the signaling state (Suppl. Fig. 15). N5 protonation is an essential step in signal transduction as not least evidenced by the reconstitution of LOV receptors with 5-deaza-FMN. Despite retaining the ability to form the thiaodduct under blue light, these receptors are incapable of downstream signaling responses, arguably due to a lack of hydrogen bonding at the C5 position. Support for the pivotal role of N5 protonation derives from cysteine-deficient LOV receptors that undergo photooxidation to the NSQ state which is protonated at N5 and thus elicits intact signaling responses. Three-dimensional structures of phototropin LOV domains early on pinpointed the conserved glutamine residue close-by the flavin chromophore and in hydrogen-bonding distance to the O4 and N5 atoms. Supported by spectroscopic evidence, the glutamine is generally held to rotate its sidechain upon N5 protonation to satisfy hydrogen bonding.

Possibly, this rotation is aided by transient rearrangements of two conserved asparagines (residues N482 and N492 in AsLOV2, see Fig. 5) that coordinate the flavin-nucleotide chromophore. Reorientation of the glutamine residue in turn provokes a cascade of hydrogen-bonding and structural changes, as for instance revealed in the past and present structures of light-adapted AsLOV2 (see Fig. 5). Photochemical reactions within the flavin chromophore, i.e., thiaodduct formation or reduction to the NSQ state, are thus coupled to the protein scaffold, in particular, the LOV $\beta$-sheet and elements contacting it, e.g., N- and C-terminal extensions to the core domain. In AsLOV2 specifically, asparagine 414 responds with a sidechain flip, accompanied by a shift of the protein backbone. Signals are thus channeled to the A’α and Jα helices and likely drive their light-dependent unfolding.

Irrespective of the strong conservation of the glutamine and its central involvement in canonical LOV signal transduction, its removal unexpectedly does not abolish light-dependent signaling responses. Intriguingly, this effect spans LOV receptors of distant phylogenetic origin and with disparate associated output modules (see Figs. 2–5), that invariably retained intact responses upon replacement of the glutamine, if to different and often reduced quantitative extent. In line with these observations, two recent reports revealed that the LOV domains from 

Vaucheria frigida
aureochrome 1 and 

A. thaliana
ZTL also elicited intact downstream responses after replacement of the glutamine by leucine or other residues. Taken together, we propose that the conserved glutamine, long considered essential for LOV signal transduction, is in fact generally dispensable. This view is corroborated by the existence of hundreds of glutamine-deficient LOV$^{\text{2Q}}$ proteins in nature (see Fig. 6a, Suppl. Data 3 and 26), which presumably serve as blue-light receptors, as we presently demonstrate for a proteobacterial LOV$^{\text{AQ}}$-GDFDE protein (see Fig. 6b).

Our functional and structural data suggest a potential mechanism for signal transduction in glutamine-deficient LOV receptors. The observation that most amino acids can stand in for the glutamine and support intact signal transduction (see Figs. 2 and 5) immediately argues against a direct involvement of the sidechain of these residues. Strikingly, the crystal structures of AsLOV2 wild-type and Q513L revealed highly similar light-induced conformational changes that culminated in reorientation and altered hydrogen bonding of N414 and translocation of the A’α segment. The problem of signal transduction in glutamine-deficient LOV receptors thus reduces to the question of how signals are relayed across 10 Å from the newly protonated N5 atom to the LOV $\beta$-sheet, and specifically to N414 in AsLOV2. In the following, we principally consider and discuss in turn as potential mechanisms i. steric rearrangements near the chromophore; ii. altered electrostatics in the thiaodduct state; and iii. water-mediated hydrogen-bonding changes.

First, as recently proposed for 

A. thaliana
ZTL, steric rearrangements upon adduct formation, i.e., bond strain, $sp^3 \rightarrow sp^2$ hybridization change of the C4a atom, and tilting of the isoalloxazine heterocyclic system, might underpin signal propagation. However, the light-state Q513L structure did not reveal substantial conformational changes of residues immediately next to the flavin. Moreover, as previously demonstrated, cysteine-deficient LOV receptors can elicit canonical signaling responses when photooxidized to their NSQ state which is protonated at the N5 position like the thiaodduct but experiences different steric constraints. Taken together, we thus regard steric effects as an unlikely general mechanism for signal propagation in glutamine-deficient receptors but note that for specific LOV proteins they plausibly play a crucial role. Second, the formation of the thiaodduct evidently modifies the electronic structure of the flavin and gives rise to an altered electrostatic potential. However, molecular simulations revealed (see Fig. 5) that such changes in electrostatics are comparatively small and of short reach. We hence deem it unlikely that electrostatic interactions transmitted through space are causative for signal transduction. Rather, we favor the third option of water-mediated hydrogen-bonding rearrangements, as illustrated in Fig. 7.

We propose that water molecules transiently enter the flavin-binding pocket, occupy the space vacated by glutamine removal, and form hydrogen bonds to the protonated flavin N5 and N414. Water would thus substitute for the glutamine side chain of canonical LOV receptors and relay hydrogen-bonding changes originating at the chromophore to the LOV $\beta$-sheet, and N414 in the case of AsLOV2. We note that neither the dark-adapted nor the light-adapted structures of AsLOV2 Q513L revealed direct evidence for ordered water molecules near the flavin N5 atom. However, support for our model derives from MD simulations suggesting that water dynamically enters this region of the light-adapted receptor. Moreover, the model would explain why, as one of only a few amino acids, proline cannot functionally substitute for glutamine, despite leaving chromophore binding and LOV photochemistry intact. In the imino acid proline, the Cγ and Cδ methylene groups of the sidechain loop back onto the amide nitrogen atom, thus sterically interfering with the proposed water-mediated hydrogen bonding. Alternatively, we cannot however rule out that proline fails to convey light signals because of its restricted conformational freedom or its lack of an amide proton. Lastly, the proposed mechanism would rationalize the near-identical conformational changes elicited by light in both AsLOV2 wild-type and Q513L. Regardless of the presence of the glutamine, light signals would initially be converted into altered flavin N5 protonation and a subsequent hydrogen-bonding cascade that propagates to N414 at the LOV $\beta$-sheet interface. Concomitant with the formation of new hydrogen bonds, N414 would break or weaken the hydrogen bonds formed in darkness between its backbone oxygen and the amide proton of residue 513, and between its NδH2 amide group and the sidechain of D515, respectively. The resultant weakening of the LOV $\beta$-sheet would then transmit to the A’α and Jα helices that interact with the outer face of the sheet.
Fig. 7 Signal transduction in light-oxygen-voltage (LOV) receptors lacking the conserved glutamine, exemplified for the A. sativa phototropin 1 LOV2 domain. **a** Lewis formulae show the flavin- nucleotide chromophore and surrounding residues of the glutamine-deficient leucine variant in the dark-adapted (left) and light-adapted states (right). As revealed by X-ray crystallography (see Fig. 5), qualitatively similar structural responses to light-induced N5 protonation (see Fig. 1) are observed in both the absence of the conserved glutamine Q513 and in its presence (see Suppl. Fig. 15). Without the glutamine, water molecules might transiently enter the chromophore-binding pocket, thereby stand-in for the glutamine, and relay the signal as changes in hydrogen bonding to the Iβ and Aβ strands of the central β pleated sheet (involving residues N414 and 513). Notably, signals are thus also propagated to the LOV C terminus (DS515) which is frequently engaged in signal transduction, and often exhibits a conserved DIT motif8. **b** The observation that LOV receptors can transduce signals without either or both of their strongly conserved cysteine and glutamine residues suggests a potential origin from redox-active flavoproteins14. LOV signal transduction in a primordial LOV ancestor lacking the Cys and Gln residues would have relied on flavin photoreduction to the NSQ state which is protonated at the Avin N5 atom14. We show LOV passes the QC. Our data demonstrate that LOV receptors can evidently transduce light signals without the conserved glutamine. As qualitatively intact light responses are evoked upon glutamine replacement across all systems tested, we consider signaling in the absence of the glutamine a general and inherent, yet dormant trait of LOV receptors. This view is borne out by the existence of numerous glutamine-deficient LOVΔG receptors in nature that could potentially serve as bona fide blue-light receptors. In a similar vein, we previously showed that LOVΔC receptors devoid of the conserved cysteine exist in nature and can elicit productive light responses owing to photoreduction to the NSQ state which is protonated at the flavin N5 atom14. We show presently that the paradigm AsLOV2 domain perplexingly retains signaling capability, if at greatly attenuated efficiency, even when both the conserved cysteine and glutamine are replaced. Building on our earlier proposal14, these observations jointly raise the prospect that LOV receptors arose during evolution from originally light-inert flavoproteins, e.g., enzymes involved in redox processes (Fig. 7b). The question then begs, if signal transduction can take place in the absence of cysteine and glutamine, why are these residues so prevalent in recent LOV receptors? Our data provide clues as to the potential driving forces underlying the strong glutamine conservation. First, the introduction of

Although residue N414 is not strictly conserved (see, e.g., Fig. 1 and Suppl. Fig. 1), the proposed model of signal transmission principally extends to other LOV receptors. Even in the absence of a polar residue at the position equivalent to N414, hydrogen-bond rearrangements could still be relayed to the β-sheet and beyond, as for instance evidenced in Neurospora crassa Vivid22,57. Across several LOV receptors, the outer β-sheet face and the adjacent DIT motif6 recurring take center stage in signal transduction15,33,46,59,61,67. Once relayed there, signals are then channeled into disparate structural responses in individual LOV receptors, including order-disorder transitions, association reactions, and quaternary structural transitions5. It is worth noting that our experimental data, simulations, and mechanistic proposal are not in contradiction with common models advanced for signal transition in the presence of glutamine. For instance, a recent study suggests that two conserved asparagine residues aid light-triggered glutamine reorientation23, which would be compatible with our model (see Fig. 5 and Suppl. Fig. 15). By principally rationalizing how signal transduction occurs in the absence of glutamine, our model reinforces the central roles of N5 protonation and hydrogen bonding in LOV signal transduction. These core aspects likely apply to receptors with intact glutamine as well, as also suggested by earlier molecular simulations23,24.
glutamine generally enhances the fidelity and degree of the light response. Second, glutamine induces a bathochromic absorbance shift of ~10 nm, thus expanding light sensitivity to longer wavelengths. Third, glutamine accelerates the base-catalyzed dark recovery reaction66, thus enhancing temporal resolution of light-dependent physiological responses. Similarly, the cytochrome may have prevailed as its introduction minimizes side reactions (fluorescence and photosensitizing), desensitizes the light-adapted signaling state against environmental influences (e.g., partial oxygen pressure and redox conditions), and enhances the fidelity of the signaling response14.

Beyond implications for the potential origin of LOV receptors, our data directly pertain to applications in optogenetics and biotechnology. First, replacement of the conserved glutamine residue generally decelerated the dark-recovery kinetics but preserved signaling responses to a substantial extent. Targeted modification of the glutamine residue thus provides a so-far little-explored avenue towards modulating these kinetics and thus the effective light sensitivity at photostationary state (see Suppl. Figs. S39–40). In a similar vein, glutamine substitution may serve to deliberately weaken the light signal, which is desirable by application. Second, substitutions of either the conserved cysteine or glutamine residues have often been used as presumably light-insensitive, unresponsive negative controls. Our data however illustrate that even when these residues are replaced, LOV receptors can principally transduce light signals, although likely with reduced amplitude. These considerations transcend the optogenetic deployment of LOV receptors and also concern the widespread applications of cysteine-deficient (and often additionally glutamine-deficient)68 LOV modules as fluorescent proteins69,70 and photosensitizers for molecular oxygen53.

Methods biological. YF1 variants with residue Q123 replaced were constructed in the background of the pDusk-DiRed and pDawn-DiRed reporter plasmids81, or the expression plasmid pET-41a-YF132 by PCR amplification and blunt-end ligation. The gene of the cognate response regulator BFixI from B. subtilis dazd1, formerly designated B. japonicum, was amplified from an earlier expression construct15,47 and cloned onto the pET-19b vector (Novagen), and thus furnished with an N-terminal His6-SUMO tag. Substitutions of residue Q534 in the NmpN1 pal protein were performed according to the QuikChange protocol (Agilent Technologies) in either the pCD-PALoPt reporter plasmid or the pET-28c-PALoPt expression plasmid82. For the expression of AsLOV2, a gene encoding residues 404–546 of A. sativa parsley (Accession no. O94903) was synthesized with an N-terminal GFP extension15,54 and codon usage adapted to E. coli (GeneArt, ThermoFisher), and was cloned into the pET-19b vector. AsLOV2 was thus equipped with an N-terminal His6-SUMO tag and its expression was put under the control of a T7-promoter. Replicas of the active-site residues Q534 and C540 were generated by QuikChange. Deletions of the N- and C-terminal Aβ and Ja helices were prepared by PCR amplification and blunt-end ligation of the vector; the resultant truncated AsLOV2 variant comprised residues 411–517. The gene encoding residues 1–526 of the glutamine-deficient LOV-GGEF receptor (ANSS8620.1/WP_140774521.1) was amplified by PCR from genomic DNA of the proteobacterium Mesorhizobium loti NZZP037 (purchased from Deutsche Sammlung für Mikroorganismen und Zellkulturen, DSMZ no. 2627) and confirmed by DNA sequencing. A version of the gene with codons optimized for E. coli (GeneArt) was cloned into the pQE-30 vector (Qagen) via Gibson cloning71. Residue replacements were prepared by PCR amplification and blunt-end ligation. All oligonucleotide primers (Suppl. Table 3) were purchased from Integrated DNA Technologies. All constructs were verified by Sanger sequencing (Microsynth AG, Göttingen).

Protein expression and purification. Protein expression and purification were carried out as previously described for YF132 and NmpN148. To express and purify the response regulator BFixI, the above pET-19b BFixI expression plasmid was transformed into E. coli BL21 CmpX13 cells18. Bacteria were grown at 37°C in Luria broth (LB) medium to an optical density at 600 nm (OD600) of around 0.6–0.8, at which point the temperature was lowered to 16°C and expression induced by the addition of 1 mM isopropyl β-D-thiogalactopyranoside (IPTG). Following incubation overnight at 16°C, cells were lysed by sonication, and the supernatant was cleared by centrifugation and purified by Ni2+ immobilized metal affinity chromatography (His-Trap). The His-SUMO tag was cleaved off by SUMO-Specific protease Suv3, followed by a second IMAC purification. BFixI protein was dialyzed into storage buffer [20 mM tris(hydroxymethyl)methylammonium (Tris)/HCl pH 8.0, 250 mM NaCl, 10% (v/v) glycerol], and the concentration was determined using an extinction coefficient of 4860 M−1 cm−1 at 280 nm19.

The production of AsLOV2 variants, the pET-19b expression plasmid (see above) was transformed into E. coli BL21 CmpX13 or LOBSTR cells35. Protein expression was induced by the addition of 1 mM IPTG and conducted at 16°C overnight. When using the CmpX13 strain, the medium was supplemented with 50 µM riboflavin. The cleared bacterial cell lysate was purified by Co2+ IMAC, Spen2 cleavage of the His-,SUMO tagged and a second IMAC step for 37°C BFixI. Depending on purity, AsLOV2 variants were further purified by anion-exchange chromatography. Purified protein was dialyzed into storage buffer [20 mM Tris/HCl pH 7.4, 20 mM NaCl, 20% (v/v) glycerol], and its concentration was determined spectrophotometrically using an extinction coefficient of 13,800 M−1 cm−1 for the flavin absorption maximum around 447 nm44.

Spectroscopic analyses. UV/vis absorbance spectra were recorded on an Agilent 8435 diode-array spectrophotometer at 22°C, as controlled by an Agilent 89090 A Peltier thermostat. Absorbance spectra were acquired for the dark-adapted LOV receptors and after saturating illumination with a 455-nm light-emitting diode (LED) (30 mW cm−2) for the light-adapted states. Throughout the study, all light measurements were determined with a power meter (model 842-PE, Newport) and a silicon photodetector (model 918D-UV-OD3, Newport). The recovery to the dark-adapted state was monitored by recording spectra over time. The resultant kinetics were corrected for baseline drift and evaluated by nonlinear least-squares fitting to exponential functions using the Fit-o-mat software3. Absorbance spectroscopy on the flavin was conducted in 20 mM Tris/HCl pH 8.0, 250 mM NaCl, to accelerate the recovery in the Q123L variant, up to 1 M imidazole was added35, and the resulting rate constants for dark recovery were extrapolated to 0 M imidazole.

UV/vis-spectroscopic analysis of NmpN1 was performed in 12 mM 4-(2-hydroxyethyl)–1-piperazineethanesulfonic acid (HEPES)/HCl pH 7.7, 135 mM KCl, 10 mM MgCl2, 1 mM MgGlc68, AsLOV2 was analyzed in 10 mM sodium phosphate pH 7.5, 10 mM NaCl; to aid solubility, for the Q513D variant 20% (v/v) glycerol was added. To promote photoinduction in the cytochrome-derive AsLOV2 C40A variant, 1 mM tris(2-carboxyethyl)phosphine (TCEP) was added.

A secondary structure and light-induced changes were assessed by CD spectroscopy on a Jasco J710 spectrophotometer equipped with a PTC-348W1 Peltier element. CD spectra were recorded at 22°C in a 1-mm cuvette for the dark-adapted state and following saturating blue-light illumination for the light-adapted state. All spectra were corrected by blank spectra and represent the average of at least three independent scans of the case of the dark and of the light-illuminated sample. The spectra were assessed at 100 µM protein concentration. CD data were divided by OD600 and normalized to the value for YF1 under dark conditions. Data represent the mean ± s.d. of three biologically independent samples. The response to trains of blue-light pulses was assessed for pDawn-DiRed systems harboring different YF1 variants as previously described31. Briefly, bacteria were grown in standard Luria broth (LB) medium and incubated at 37°C and 700 rpm in either darkness or under constant blue light (470 nm, 100 µW cm−2). Following incubation, the fluorescence of the DiRed Express reporter26 was measured with a Tecan Infinite M200 PRO plate reader (Tecan Group Ltd, Männedorf, Switzerland). For the fluorescence measurements, the excitation wavelength was (554 ± 9) nm and that of the emission (591 ± 20) nm. Fluorescence data were divided by OD600 and normalized to the value for YF1 under dark conditions. Data represent the mean ± s.d. of three biologically independent samples. The response to trains of blue-light pulses was assessed for pDawn-DiRed systems harboring different YF1 variants as previously described31. Briefly, bacteria were grown in standard Luria broth (LB) medium and incubated at 37°C and 700 rpm in either darkness or under constant blue light (470 nm, 100 µW cm−2). Following incubation, OD600 and fluorescence were measured.

Activity and light response of purified YF1 variants were characterized in a coupled assay that reports on the phosphorylation-induced binding of BFixI to a fluorescently labeled, double-stranded DNA (dsDNA). To this end, a dsDNA substrate with the sequence 5′-GAG CGA TAT CCT ATT AAG GGG GTC GTT CCA AGT ACA C-3′ and labeled at its 5′ end with (9-amino-6-carboxyfluorescein)carboxytetramethylrhodamine (TAMRA) was prepared as described before44. The underlined portion of the sequence corresponds to the BFixK2 operator site that BFixI binds to45. To assess light-dependent catalytic activity, 2.5 µM of each YF1 variant in its dark-adapted state were incubated at 25°C with 1.25 µM BFixK2 dsDNA substrate and 25 µM BFixI in buffer containing 10 mM HEPES/HCl pH 7.4, 80 mM KCl, 2.5 mM MgCl2, 0.1 µM ethylenediaminetetraacetic acid (EDTA), 1 µM bovine serum albumin (BSA), 10% (v/v) glycerol, 4% (v/v) ethylene glycol and 20 mM TCEP. The solution was transferred to a black 96-well microtiter
plate (FluoroNunc). Upon starting the reaction with the addition of 1 mM ATP, the kinetics were followed by measuring TAMRA fluorescence anisotropy with a multi-mode microplate reader (CLARIOstar, BMG Labtech) every 30 min. Fluorescence was recorded at excitation and emission wavelengths of (540 ± 10) nm and (590 ± 10) nm, respectively, and using a 566-nm long-pass beam splitter. After 30 min, the microtitre plate was ejected, the samples were illuminated for 30 s with the multi-mode microplate reader (CLARIOstar, BMG Labtech) over 30 min. As in a previous structural study47. Crystallization was conducted by sitting-drop vapor diffusion at solvent conditions adapted from the previous report47. The light-dependent binding of DgcE was conducted in Refmac85. Occupancies of residues with multiple conformations were calculated with phase information of the respective dark-adapted state. Corresponding MTZ files are provided as Supplementary Data. In case of LOV2 Q513L, the best-resolved dark and light datasets, used for structure refinement (see Table 2), differed in the orientation of the sugar moiety by 1.5 Å. We, therefore, selected a different light dataset at a resolution of 1.24 Å in which this difference only amounted to 0.65 Å.

**Electrostatics and molecular simulations.** The crystal structures of AsLOV2 wild-type and Q513L determined in this work (PDB 7pgx and 7pgz) served as starting points for the simulation of the dark-adapted states. These structures were then modified to mimic the light-adapted state by forming a covalent bond between the Sy atom of C450 and the C4a atom of the flavin residue. Further, a proton was transferred from the thiol group to the N5 atom of flavin. To specifically probe the effect of adduct formation and N5 protonation on electrostatics, the conformation of other residues was left unchanged. Subsequently, the protein structures were relaxed using a QM/MM geometry optimization. The electrostatic potential acting on a selected residue (e.g., N414) was calculated using the APBS 3.0 software68. The dielectric constants for inside/outside of the molecular regions were chosen to be 1.0 and 78.54, respectively. The solvent radius was set to 1.4 Å, and the temperature had the standard value of 298.15 K. The partial charges for all atoms were taken from the ff14SB force field of AMBER89. For each structure, four calculations were carried out to determine the electrostatic potential exerted by the protein environment on residues N414, N492, N499, and Q513. The electrostatic potential maps were plotted with UCSF chimera90 and are shown in Suppl. Fig. 11.

MD simulations were performed using the crystal structures obtained in this work. Missing hydrogen atoms were added to the initial structures using the leap protocol of AMBER89. The protonation states of all titratable residues were considered at a pH of 7.0. The protein was solvated in a truncated octahedral box of TIP3P water molecules with a distance of at least 15 Å between the protein atoms and the boundaries of the box. The system was neutralized by adding K+ and Cl- ions. The SHAKE algorithm91 was used to constrain the bonds involving hydrogen atoms, thus allowing a time step to be 2 fs. A Langevin thermostat with a collision frequency of 1 ps−1 was used for temperature control in all simulations. The VMD plugin VolMap served to analyze the water density inside the protein. The MM parameters for FMN and the FMN-Cys adduct were obtained from92. Initially, the solvent was minimized in 100,000 steps with restraints of 100 kcal mol−1 Å−1 on all atoms and FMN. The system was heated from 100 K to 300 K within 50 ns with restraints on the protein and FMN in NVT ensemble. The density of the solvent was then gradually equilibrated for another 20 ns under NPT conditions. The equilibration was extended for another 20 ns with weak restraints of 10 kcal mol−1 Å−1. Then, MD of 20 ns each was conducted with weakened restraints of 1 kcal mol−1 Å−1 and 0.1 kcal mol−1 Å−1, respectively, on the protein backbone. Finally, an unrestrained 3D production run of 300 ns was carried out.

Sequence analysis of LOV receptors lacking the active-site glutamine. As in a previous analysis18, a BLAST search was performed with Bacillus subtilis Yva (BBG11, residues 1-127) as query and with E-value cutoff of 10. Using a custom Python script (available from https://github.com/TheAngulon/LOVfilterQ), the results were filtered for entries that possess at least eight out of nine residues (residue positions Gly59, Asn61, Cy62, Arg63, Phe84, Leu85, Gin66, Asn94 and Asn104 in ByYva), which are highly conserved across LOV receptors83, but lack the active-site glutamine (position Gin123 in ByYva). Corresponding entries were aligned to the sequences of ByYva and AsLOV2 using ClustalX93. A sequence logo was generated with WebLogo version 3.796.
Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Atom coordinates and structure-factor amplitudes have been deposited in the Protein Data Bank under accession codes 7ppz (AlfLOV2 wild-type, dark), 7pwy (wild-type, light), 7ppg (Q513L, dark), and 7pdb (Q513L, light). MTZ files underpinning the light-dark difference electron density maps (see Suppl. Fig. 6) and a multiple sequence alignment of LOV2 receptors are provided as Supplementary Data. Other data generated in this study have been deposited in the Zenodo database under accession code 6341885.

Code availability
A Python script for sequence analysis of glutenine-deficient LOV receptors is available from https://github.com/TheAngiolot/LOVFilterQ.

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Author contributions
J.D., R.G., and J.K. contributed equally. J.D. performed all experiments on the YF1 and AsLOV2 domain and refined crystal structures. J.K. analyzed NmpAL in bacterial reporter assays and by absorbance spectroscopy, and she studied its RNA interaction by fluorescence anisotropy. V.B. and I.S. conducted and evaluated molecular simulations. C.R., S.P., and G.M. did experiments on NmpAL in eukaryotic cells. A.T.R. performed spectroscopy on NmpAL and analyzed RNA binding. A.G.F. analyzed ALOV2 variants by CD spectroscopy. T.G. and R.P.D. developed the fluorescence anisotropy assay for YF1. M.W. advised on crystallization and structure refinement. A.M. conducted sequence analyses, refined crystal structures, and conceived and coordinated the research. J.D. and A.M. wrote the manuscript with input from all authors.

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Competing interests
The authors declare no competing interests.
