Photobiologically Directed Assembly of Gold Nanoparticles

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In nature, photoreceptor proteins undergo molecular responses to light, that exhibit supreme fidelity in time and space and generally occur under mild reaction conditions. To unlock these traits for material science, the light-induced homodimerization of light-oxygen-voltage (LOV) photoreceptors is leveraged to control the assembly of gold nanoparticles. Conjugated to genetically encodable LOV proteins, the nanoparticles are monodispersed in darkness but rapidly assemble into large aggregates upon blue-light exposure. The work establishes a new modality for reaction control in macromolecular chemistry and thus augurs enhanced precision in space and time in diverse applications of gold nanoparticles.

Adaptations to light abound in biology across wide time and length scales. At the molecular level, these adaptations rely on sensory photoreceptor proteins that absorb photons and initiate photochemical and biochemical reaction cascades with exquisite resolution in time and space. Often, these reactions entail the formation or dissolution of non-covalent interactions among proteins and other biomolecules. Notably, photoreceptors are genetically encoded and generally operate in aqueous milieu under mild reaction conditions. These core aspects are exemplified by the light-oxygen-voltage (LOV) photoreceptor class which respond to blue light via flavin nucleotide chromophores (Figure 1A).[3] Certain LOV receptors undergo light-controlled homo- or heterodimerization reactions (Figure 1B), which have been harnessed to optogenetically control a cohort of cellular processes.[2]

Here, we extend the concept of photobiological reaction control from biology to materials science. We develop the on-demand assembly of gold nanoparticles (AuNPs) as model nanoparticles via photobiological direction by associating LOV receptors (Figure 1C). Nanoparticles in general are of fundamental interest for a variety of industrial processes,[3] AuNPs in specific underpin diverse applications in, e.g., biological sensing and imaging,[5–7] drug delivery,[8] and intracellular gene regulation.[9] Moreover, AuNPs of various shapes and sizes were ordered and arranged in defined architectures.[5–12] Nanoparticle assembly was for instance controlled by various ligands,[13–15] but also by external stimuli, such as magnetic fields or light.[16–24] These advances notwithstanding, the controlled higher-order assembly in response to external cues remains challenging; but worthwhile to explore because it could significantly extend the application scope of AuNPs. In particular, the interfacing of the inorganic nanoparticles with genetically encodable, adaptable, light-responsive proteins augurs innovative use cases. As a cue signal, light appears ideal as it can be applied non-invasively and supports high spatial and temporal precision, which is not least evidenced by ample applications in biology and biotechnology.[6]

To achieve LOV-directed AuNP assembly, we envisioned a direct link between the organic photoreceptor proteins and the inorganic particles. Initial attempts to covalently couple the light-responsive proteins to citrate-capped AuNPs via gold sulfide bonds failed, because unspecific interactions (owing to cysteine residues within the protein and to the presence of salt ions, required for protein stability) invariably triggered nanoparticle aggregation and precipitation.[23,26] Therefore, we selected for the non-covalent attachment of the LOV receptors via nickel coordination chemistry. This strategy necessitates that the photoreceptors be equipped with hexa-histidine (His6) tags and the AuNPs be functionalized with Ni2+–nitrilotriacetic acid (NTA) ligands (Figure 1C).[10,21,27] We selected two well-characterized LOV domains from Phaeodactylum tricornutum aureochrome 1a (Ptaur) and from Neurospora crassa Vivid (NcVVD), both previously shown to undergo light-induced homodimerization[28,29] and used to bestow light sensitivity on cellular processes.[2] Size-exclusion chromatography (SEC) confirmed that in their unmodified forms both LOV receptors adopt homogenous states in either darkness or blue light (Figure S1, Supporting Information); the apparent molecular size under blue light was higher, indicative of light-induced dimerization. Next, we introduced His6 tags at either the N or C termini of the LOV domains, separated by short glycine-serine linkers. N-terminal modification of Ptaur led to constitutive dimerization independent of light and accompanied by aggregation, whereas C-terminal modification incurred protein insolubility. The sensitivity of the Ptaur N- and C-terminal segments likely
owes to them contributing to the light-driven homodimerization.\cite{30,31} Similarly, introduction of the His\textsubscript{6} tag at the N terminus of NcVVD caused protein insolubility, consistent with the crucial role of this protein segment in dimerization.\cite{32} By contrast, appendage to the C terminus (NcVVD-His\textsubscript{6}) was tolerated, and light-induced dimerization of NcVVD was preserved as assessed by SEC (Figure 1B).

To allow conjugation of the NcVVD-His\textsubscript{6} protein to the particles, we synthesized citrate-capped AuNPs according to Frens\cite{33,34} and subsequently equipped their surface with Ni\textsuperscript{2+}-NTA groups via ligand exchange and Ni\textsuperscript{2+} complexation (Figure S2, Supporting Information). Owing to the sensitivity of the surface plasmon resonance (SPR) signal to surface properties,\cite{35} the functionalization with the Ni\textsuperscript{2+}-NTA groups induced a shift of the SPR absorption band of the AuNPs, that could be followed spectroscopically (Figure S3, Supporting Information). Transmission electron microscopy (TEM) revealed that prior to and after Ni\textsuperscript{2+}-NTA functionalization, the AuNPs did not associate and were essentially monodisperse with a mean diameter of (17.7 ± 1.6) nm (Figure S4, Supporting Information). Dynamic light scattering (DLS) showed similar average sizes of (21.8 ± 3.6) and (30.6 ± 3.4) nm for the citrate-capped and the Ni\textsuperscript{2+}-NTA-ligated AuNPs, respectively (Figure S5, Supporting Information). Based on the functionalization of AuNPs with similar ligands,\cite{36} we estimate that around 4000 NTA ligands are bound to the surface of each AuNP.

Next, we assessed the interaction of NcVVD-His\textsubscript{6} and the Ni\textsuperscript{2+}-NTA functionalized AuNPs. As light scattering scales with the sixth power of particle dimensions, the DLS signal is dominated by larger particles present in solution. To obtain a more accurate view of particle sizes and distributions, we hence switched to asymmetric flow field-flow fractionation (AF4) (Figures S6 and S7, Supporting Information). While the citrate-capped and the Ni\textsuperscript{2+}-NTA-functionalized AuNPs eluted with apparent radii of (11 ± 2) nm and (12 ± 2) nm, respectively, for the main particle fraction, addition of a fiftyfold molar excess of NcVVD-His\textsubscript{6} induced a shift to (18 ± 2) nm. Given a protein diameter of ≈4 nm, these results indicate the association of LOV proteins to the AuNPs. When exposed to 450-nm light, the

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**Figure 1.** Photobiologically directed assembly of gold nanoparticles (AuNPs) by light-oxygen-voltage (LOV) photoreceptors. A) Simplified LOV photocycle, showing the oxidized quinone state of flavin nucleotide non-covalently bound within the LOV domain in the dark, and the light-induced formation of a covalent thioadduct between a conserved cysteine residue (Cys) and the C4a atom of the flavin molecule. The adduct form spontaneously recovers to restore the dark-adapted state. The photocycle can be observed spectroscopically by absorption of the dark-adapted (black) and light-adapted states (blue). B) Size-exclusion chromatography of NcVVD-His\textsubscript{6} in darkness (black traces) and following blue-light exposure (blue traces). C) To achieve light-induced AuNP assembly, associating LOV domains are immobilized on the particle surface by Ni\textsuperscript{2+}-NTA coordination chemistry. Exposure to blue light triggers the dimerization of the LOV domain, which consequently drives the assembly of AuNPs.
solution of NcVVD-conjugated AuNPs rapidly changed color from light red to purple, indicative of altered plasmon resonance and nanoparticle assembly. The formation of such blue light-induced particle clusters was qualitatively confirmed by AF4 measurements during which a purple precipitate of high molecular weight formed on the analysis membrane (Figure S7, Supporting Information).

To better characterize the light-induced association reaction, we followed its kinetics by UV-vis absorption spectroscopy (Figure 2). In the absence of the LOV protein, the Ni²⁺-NTA-AuNPs at $0.9 \times 10^{-9}$ M concentration exhibited broad absorption overlain by a narrower SPR band peaking at 524 nm, irrespective of whether the samples were kept in darkness or illuminated with blue light. Addition of $25 \times 10^{-9}$ or $50 \times 10^{-9}$ M NcVVD-His₆ did not induce significant spectral changes provided the samples were kept in the dark. When exposed to 450-nm light, over the course of 90 min, the SPR band successively broadened, and its maximum shifted to longer wavelengths up to 630 nm. The substantial spectral shift of more than 100 nm reflects the formation of heterogeneous, large-scale nanoparticle assemblies. The kinetics of this process were faster for $50 \times 10^{-9}$ M NcVVD-His₆ than for $25 \times 10^{-9}$ M. By approximating the shape of the NcVVD-His₆ protein as a sphere of 4 nm diameter, we calculate that at maximum around 30 protein molecules can be accommodated on the surface of each particle. Notably, the NcVVD-His₆ concentrations of $25 \times 10^{-9}$ and $50 \times 10^{-9}$ M correspond to molar ratios of 28 and 56 proteins per nanoparticle, respectively. Reduction of the protein amount severely slowed down the assembly process to the extent that at $6.25 \times 10^{-9}$ M NcVVD-His₆ almost no spectral changes could be detected. Imidazole competes with the His₆ tag for NTA binding and thus prevents light-induced particle assembly.

Figure 2. Blue-light exposure drives the assembly of Ni²⁺-NTA functionalized AuNPs coupled to NcVVD-His₆. Time series of UV–vis spectra of Ni²⁺-NTA functionalized AuNPs in the absence and presence of $25 \times 10^{-9}$ or $50 \times 10^{-9}$ M NcVVD-His₆. Spectra were acquired at 2, 5, 10, 15, 20, 30, 40, 60, and 90 min after protein addition (from top to bottom). Ni²⁺-NTA functionalized AuNPs show a strong absorption with a maximum at 524 nm independent of illumination. In presence of NcVVD-His₆, blue light induces a broadening of the plasmon peak and a shift toward greater wavelengths, indicating the assembly of AuNPs. The velocity of light-directed assembly can be set by the LOV protein concentration. As control, Ni²⁺-NTA functionalized AuNPs were incubated in the presence of imidazole without or with different concentrations of NcVVD-His₆ (right panels). After 90 min of incubation in either darkness (black traces) or under constant blue light (blue traces) no significant spectral changes could be detected. Imidazole competes with the His₆ tag for NTA binding and thus prevents light-induced particle assembly.
Our results showcase AuNP assembly at will through photo-biological direction by LOV receptors. By welding inorganic and proteinaceous building blocks, we synthesize a composite system with emergent properties and thereby establish a new paradigm for reaction control in macromolecular chemistry. We thus lay the foundation for future applications at the interface of colloidal chemistry and biochemistry, as the principal strategy generally extends to diverse nanoparticles of various shape, composition and properties. The use of light triggers stands to permit the controlled nanoparticle assembly with superior spatial resolution, for example by lithography. Although not exploited here, we note that owing to genetic encoding and thereby identity, sensory photoreceptors can be readily attached to other proteins and biomolecules, or displayed on the surface of cells.[27] Doing so enables the defined incorporation of nanoparticles into complex biological circuits and reaction cascades. Of advantage, biological systems are generally malleable and can be adapted in their properties to meet specific application demands. As a case in point, the lifetime of the light-adapted state of LOV receptors, and therefore their effective light sensitivity at photostationary state, can be routinely varied across several orders of magnitude.[18,19] The combination of suitably modified, light-sensitive biological systems with nanoparticles thus augurs novel applications in biology and materials science alike. For instance, the light-triggered assembly of AuNPs, presently configured to be irreversible, may be harnessed for security purposes, such as monitoring the light exposure of sensitive materials.

**Experimental Section**

**Expression and Purification of LOV Proteins:** The genes of the N-terminally truncated short-LOV protein *Neurospora crassa* Vivid (NcVVD) (residues 37–186, including the mutations CTIV and N56K)[29,32] and the A′α-lovatryptophan module of *Phaeodactylum tricornutum* Aureob (Patuor) (residues 235–378) were obtained by gene synthesis (GeneArt, Regensburg, Germany) and cloned by Gibson assembly[40] into expression vectors. For the preparation of untagged protein and variants with N- or C-terminal His6 tags, the vectors pET-19b-SUMO, pET-28c, and pET-41a were used, respectively. Where applicable, a (GS)5-linker was inserted between the LOV core protein and the His6 tags.

For expression, the plasmid constructs were transformed into *Escherichia coli* BL21(DE3) CmpX13 cells.[41] 800 mL of lysogeny broth (LB) medium, supplemented with 50 μg mL⁻¹ kanamycin, were inoculated with single clones and incubated at 37 °C and 200 rpm until an optical density at 600 nm of 0.6–0.8 was reached. At this point, expression was induced with 1 × 10⁻³ M isopropyl β-D-1-thiogalactopyranoside (IPTG), the temperature was lowered to 16 °C, and the incubation continued for ~18 h under constant blue-light illumination (450 nm, 100 μW cm⁻²). The light intensity was determined with a power meter (model 842-PE, Newport) and a silicon photodetector (model 918D-UV-OD3, Newport). Cells were harvested by centrifugation and lysed by ultrasound. The cell lysate was cleared by centrifugation and purified by Ni:NTA affinity chromatography under exclusion of blue light. Fractions eluted by imidazole from the affinity column were analyzed by denaturing polyacrylamide gel electrophoresis (PAGE) and pooled. Proteins with terminal His6 tags expressed from the pET-28c and pET-41a vectors were directly dialyzed into storage buffer (50 × 10⁻³ M Tris, 300 × 10⁻³ M NaCl, 50% (w/v) glycerol, pH 8). In case of the SUMO-tagged LOV domains, the N-terminal His6-SUMO tags were removed by Spen2 cleavage, followed by a reverse Ni:NTA affinity chromatography. The resulting, untagged LOV protein was dialyzed into storage buffer as above. At all times during purification, proteins were handled under dim red light, and

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**Figure 3.** Light-induced assembly of nanoparticles. Transmission electron microscopy images of Ni²⁺-NTA functionalized AuNPs in the presence of NcVVD-His6 in darkness (left) or following blue-light exposure (right). In the absence of light, LOV-coupled AuNPs are evenly distributed and do not interact, whereas light-programmed association of the LOV receptors directs the assembly of the particles, culminating in the formation of extended networks with up to micrometer dimensions.

Changes could be observed upon illumination (Figure S8, Supporting Information). Addition of imidazole, which competes for binding to the Ni²⁺-NTA group, abrogated any light-induced spectral changes (Figure 2).

We next analyzed by transmission electron microscopy (TEM) the size distribution of Ni²⁺-NTA-AuNPs conjugated with NcVVD-His6 in darkness and under blue light (Figure 3). Prior to illumination, the AuNPs appeared monodisperse and evenly distributed on the TEM grid, with a minor population of particles in smaller groups but without forming immediate contact. By contrast, after exposure to blue light, most particles were in direct contact and formed stacked, amorphous assemblies of up to 1.5 μm in size. Notably, the assemblies formed irreversibly as neither prolonged incubation in darkness nor the addition of imidazole could dissolve them. We ascribe this observation to fusion of the nanoparticles once they have been brought into spatial proximity.[26] The AuNPs thus resisted attempts at dispersion.
NcVVD constructs were kept in buffers containing 10% (v/v) glycerol to aid stability. Protein purity was assayed by denaturing PAGE analyses, and protein concentration was determined by UV-vis spectroscopy using an absorption coefficient of the flavin mononucleotide cofactor at 450 nm of 12 500 m−1 cm−1.

**Synthesis of AuNPs**: Gold chloride trihydrate (>99%), nickel (II) chloride (anhydrous, 98%), and N,N′-dicyclohexylcarbodiimide (DCC, 99%) were purchased from Alfa Aesar. Trisodium salt dihydrate (99%) and hydrazine acetate (97%) were bought from Acros Organics. 11-mercaptoundecanoic acid, N,N′-bis(carboxymethyl)-L-lysine, 1,2-dimethoxyethane (DME, 99%), N-hydroxysuccinimide (98%), and zinc (pure, powder) were purchased from Sigma-Aldrich. Sodium sulphate, sodium hydrogen carbonate, chloroform, and dimethylformamide (DMF) were purchased from Fisher Chemical. Sodium sulphate, tris(hydroxymethyl)-aminomethan (Tris), sodium chloride, glycerol and imidazole were purchased from Carl Roth. Hydrochloric acid (37%) and acetic acid were purchased from VWR. Milli-Q water was used throughout the experiments. To avoid unwanted nucleation during the synthesis and aggregation of gold colloidal solutions, all glassware and magnetic stir bars used in the syntheses were thoroughly cleaned in aqua regia (HCl/HNO3 3:1), rinsed in distilled water and then dried prior to use. 400 mL of an aqueous solution of HAuCl4 (2.5 × 10−4 M) was heated to boiling, ensuing the addition of 10.4 mL trisodium citrate solution [1% (w/w)] under continuous stirring. Within 5 min of boiling, the solution gradually changed color from yellow to white, grey, faint blue, violet, and finally red, indicating the formation of AuNPs. The reaction mixture was boiled for another 30 min and then cooled down to ambient temperature under mild stirring. The solution of AuNPs was stable and stored at 4°C.***

**Synthesis of Thiolated Nitrilotriacetic Acid (NTA) Ligand—Synthesis of N-[NcNcNcNcNcNc]-bis(carboxymethyl)-L-lysine (11-[acetylthio]dodecanamide)**: A solution of 11-(acetylthio)undecanoic acid (347 mg, 1.59 mmol) was added to a solution of N-[NcNcNcNcNcNc]-bis(carboxymethyl)-L-lysine (11-acetylthio)dodecanamide (compound 3, 53 mg, 0.105 mmol) in dimethylformamide (6.5 mL) and afterward bubbled with argon for 20 min at room temperature. The solution was then degassed and again stirred for 20 h under argon. The solvent was removed under reduced pressure, followed by the treatment with 0.05 N HCl (20 mL). The resulting colloidal suspension was centrifuged (4000 rpm, 25 °C, 20 min) to give crude product 4, denoted NTA hereafter. The supernatant was removed, and the residue was then washed with water by repeating resuspension / centrifugation twice. The product was lyophilized to give 36 mg (74%) of compound 4 as a pale white solid. [1H NMR (300 MHz, DMSO-d4) δ 3.45 (d, 4H), 3.31 (t, J = 7.3 Hz, 2H), 2.98 (br, 2H), 2.81 (t, J = 7.2 Hz, 2H), 2.31 (s, 3H), 2.01 (s, J = 7.4 Hz, 2H), 1.64–1.11 (m, 22H).]

**Synthesis of Thiolated Nitrilotriacetic Acid (NTA) Ligand—Synthesis of N-[NcNcNcNcNcNc]-bis(carboxymethyl)-L-lysine (11-Mercaptododecanamide)**: Hydrazine acetate (147 mg, 1.59 mmol) was added to a solution of N-[NcNcNcNcNcNc]-bis(carboxymethyl)-L-lysine (11-acetylthio)dodecanamide (compound 3, 53 mg, 0.105 mmol) in dimethylformamide (6.5 mL) and afterward bubbled with argon for 20 min at room temperature. The solution was then degassed and again stirred for 20 h under argon. The solvent was removed under reduced pressure, followed by the treatment with 0.05 N HCl (20 mL). The resulting colloidal suspension was centrifuged (4000 rpm, 25 °C, 20 min) to give crude product 4, denoted NTA hereafter. The supernatant was removed, and the residue was then washed with water by repeating resuspension / centrifugation twice. The product was lyophilized to give 36 mg (74%) of compound 4 as a pale white solid. [1H NMR (300 MHz, DMSO-d4) δ 3.47 (d, 4H), 3.31 (t, J = 7.3 Hz, 1H), 2.98 (br, 2H), 2.44 (t, J = 7.2 Hz, 2H), 2.01 (t, J = 7.4 Hz, 2H), 1.66–1.10 (m, 22H).] 13C NMR (75 MHz, DMSO-d4) δ 174.03, 173.30, 171.92, 64.34, 53.43, 38.26, 35.46, 34.36, 29.37, 29.84, 28.81, 28.34, 25.70, 23.61, 23.15] Mass spectrometry: calculated (M + 1)H = 463.247, observed (M + 1)H = 463.2469.

**Analytical Methods—Size-Exclusion Chromatography**: Size-exclusion chromatography was performed on an AKTA pure system and a Superdex 200 Increase 10/300 analytical column at 4 °C. The column was equilibrated with buffer containing 50 × 10−3 m Tris/HCl, 100 × 10−3 m NaCl and 5 × 10−3 m β-mercaptoethanol at pH 8.0, and operated at a flow rate of 0.05 mL min−1. Samples were centrifuged at 13 200 × g and injected in 30 µL aliquots at a concentration of 50 × 10−6 m. Light-adapted samples were generated by irradiation with blue light (450 nm, 30 mW cm−2, 2 min) immediately prior to injection. The run of the light-adapted sample was performed under constant blue light, while the run of the dark-state sample was conducted under exclusion of light. The elution of the samples was followed by absorption measurements at 280 nm.

**Analytical Methods—Transmission Electron Microscopy (TEM)**: TEM micrographs were obtained on a ZEISS EM922 Omega microscope at an acceleration voltage of 200 kV. Functionalized AuNPs (0.9 × 10−9 m) were incubated with 50 × 10−8 m NcVVD-His6 either in the absence or presence of blue light irradiation (450 nm, 30 mW cm−2) for 10 min at 4 °C. Then, individual samples were prepared by drying the protein-AuNPs solution on a carbon-covered copper grid and drying at room temperature overnight. The
images were evaluated with ImageJ. For each sample at least 100 AuNPs were counted.

Analytical Methods—Asymmetric Flow Field-Flow Fractionation (AF4): AF4 measurements were carried out using an AF2000 system with a Smart Stream Splitter from Postnova Analytics (Landsberg am Lech, Germany). The channel had a length and width of 295 and 30 mm, respectively, and was equipped with a spacer height of 350 μm. The separation was conducted on a NovaRC 10 kDa regenerated cellulose membrane. Detection was performed with a UV detector at a wavelength of 270 nm. In addition, the samples were analyzed inline by a multiple angle light scattering (MALS) detector (Postnova Analytics), with 21 observation angles and operated with linearly polarized laser light at 532 nm. Individual runs were conducted as stated in Table S1 in the Supporting Information using 50 × 10⁻³ m Tris/HCl, 300 × 10⁻³ m NaCl and 10% (w/v) glycerol at pH 8.0 as eluent. The temperature was kept at 8 °C during the measurement, and the samples were prepared as described in the TEM section. The run of the light-adapted samples was performed under constant illumination, whereas the run of the dark-adapted sample was conducted under exclusion of light. Data from the MALS detector was processed using the Postnova AF2000 control software. A spherical model was used for obtaining the radius of gyration based on the angular dependence of scattered light recorded by the MALS detector.

Analytical Methods—Inductively Coupled Plasma Optical Emission Spectrometry: Measurements were performed on an Avio 200 instrument (Perkin Elmer) in radial viewed plasma with purged polychromator configurations. The measurement was calibrated with four standard solutions at concentrations of 0.1, 0.5, 1, and 10 mg L⁻¹.

Analytical Methods—Nuclear Magnetic Resonance (NMR) Spectroscopy: ¹H NMR and ¹³C NMR spectroscopy was carried out on a Bruker Ultra Shield 300 using deuterated chloroform and DMSO as solvents at 300 MHz. Spectra were calibrated by the signal of the residual protons of the deuterated solvents. Spectra were evaluated with the MestReNova software.

Analytical Methods—Mass Spectrometry (MS): Mass spectrometry was performed using direct infusion from an Ultimate 3000 UPLC system ( Dionex, Sunnyvale, CA, USA) to a Hybrid Quadrupole Orbitrap system with electrospray ionization (ESI, Thermo Fisher Scientific, Waltham, MA, USA). For the MS analysis, ESI was operated in positive mode. Full scan was applied with a mass range of 80–1200 amu. The measurement was calibrated with four standard configurations. The run of the light-adapted samples was conducted under exclusion of light. Data from the MALS detector was processed using the Postnova AF2000 control software. A spherical model was used for obtaining the radius of gyration based on the angular dependence of scattered light recorded by the MALS detector.

Supporting Information
Supporting Information is available from the Wiley Online Library or Supporting Information using 50 × 10⁻³ m Tris/HCl, 300 × 10⁻³ m NaCl and 10% (w/v) glycerol at pH 8.0 as eluent. The temperature was kept at 8 °C during the measurement, and the samples were prepared as described in the TEM section. The run of the light-adapted samples was performed under constant illumination, whereas the run of the dark-adapted sample was conducted under exclusion of light. Data from the MALS detector was processed using the Postnova AF2000 control software. A spherical model was used for obtaining the radius of gyration based on the angular dependence of scattered light recorded by the MALS detector.

Conflict of Interest
The authors declare no conflict of interest.

Author Contributions
J.D. and C.L. contributed equally to this work. J.D. and C.L. contributed equally to the results of the manuscript. J.D. and S.F. cloned, expressed, purified, and analyzed LOV proteins; C.L. synthesized, derivatized, and analyzed AuNP. J.D., C.L., and S.F. assessed the light-dependent interplay of protein and nanoparticles and evaluated data. A-K.M., J.D., and C.L. performed and analyzed the AF4 measurements. J.D. and C.L. prepared figures. A.M. and A.G. conceived and supervised the project. J.D., C.L., A.M., and A.G. wrote the manuscript with input from all authors.

Data Availability Statement
All data underlying the main text and the supplementary materials are available as a supplementary zip archive.

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