Exploiting Chromophore–Protein Interactions through Linker Engineering To Tune Photoinduced Dynamics in a Biomimetic Light-Harvesting Platform

Milan Delor,†,§‡ Jing Dai,†,§‡ Trevor D. Roberts,†,§ Julia R. Rogers,†,§ Samia M. Hamed,† Jeffrey B. Neaton,†,§‡ Phillip L. Geissler,†,§⊥ Matthew B. Francis,†,§ and Naomi S. Ginsberg†

†Department of Chemistry and ‡Department of Physics, University of California Berkeley, Berkeley, California 94720, United States
§Kavli Energy NanoSciences Institute, Berkeley, California 94720, United States
∥Department of Physics, University of California Berkeley, Berkeley, California 94720, United States
‡Kavli Institute for Theoretical NanoSciences, Berkeley, California 94720, United States
†Department of Chemical Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, United States
§Kavli Energy NanoSciences Institute and Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, California 94720, United States

Supporting Information

ABSTRACT: Creating artificial systems that mimic and surpass those found in nature is one of the great challenges of modern science. In the context of photosynthetic light harvesting, the difficulty lies in attaining utmost control over the energetics, positions and relative orientations of chromophores in densely packed arrays to transfer electronic excitation energy to desired locations with high efficiency. Toward achieving this goal, we use a highly versatile biomimetic protein scaffold from the tobacco mosaic virus coat protein on which chromophores can be attached at precise locations via linkers of differing lengths and rigidities. We show that minor linker modifications, including switching chiral configurations and alkyl chain shortening, lead to significant lengthening of the ultrafast excited state dynamics of the system as the linkers are shortened and rigidified. Molecular dynamics simulations provide molecular-level detail over how the chromophore attachment orientations, positions, and distances from the protein surface lead to the observed trends in system dynamics. In particular, we find that short and rigid linkers are able to sandwich water molecules between chromophore and protein, leading to chromophore–water–protein supracomplexes with intricately coupled dynamics that are highly dependent on their local protein environment. In addition, cyclohexyl-based linkers are identified as ideal candidates to retain rotational correlations over several nanoseconds and thus lock relative chromophore orientations throughout the lifetime of an exciton. Combining linker engineering with judicious placement of chromophores on the hydrated protein scaffold to exploit different chromophore–bath couplings provides a clear and effective path to producing highly controllable artificial light-harvesting systems that can increasingly mimic their natural counterparts, thus aiding to elucidate natural photosynthetic mechanisms.

INTRODUCTION

Electronic energy transfer to redox reaction centers in photosynthetic organisms can approach near-perfect quantum efficiencies by virtue of highly evolved chromophore–protein structures with delicately tuned and optimized spatioenergetic landscapes.1–15 Much has yet to be learned by systematically elucidating the factors governing their exceptional properties; unfortunately, perturbing the intricate configurations of photosynthetic organisms by removing chromophores or mutating protein residues often destabilizes the overall molecular architecture or alters multiple variables at a time.6–15 In practice, however, the energy collection and transfer efficiencies over nanometer-to-micron length-scales achieved in nature are extremely difficult to reproduce in artificial systems. This challenge is largely due to the requirement that synthetic platforms both accommodate large chromophore densities and establish the precise positioning and energetic properties that protect fragile electronic excitations and optimize excitation energy transfer.16–21

Although much progress has been made on biomimetic light-harvesting scaffolds, control of the specific configuration of chromophores relative to one another and relative to the protein itself has been lacking and has differed substantially from natural light-harvesting complexes, where bound

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chromophores often fit tightly into protein pockets.22−25 Most biomimetic attempts employ dyes used in fluorescent bioimaging, where long and flexible tethers connecting the chromophore to the protein scaffold are common.10,26,27 Such bioconjugation schemes often preclude precise control over positioning and orientation, as the chromophores can sample large and random conformational volumes. A clear need therefore exists for crafting chromophore−protein linkers that can establish and maintain interchromophore orientations and separations through conformational restrictions.

Here we address the need for well-defined chromophore configurations by comparing the ultrafast transient absorption (TA) time scales and results from molecular dynamics (MD) simulations of chromophores bound to supramolecular protein assemblies derived from the tobacco mosaic virus (TMV) coat protein via a series of increasingly constricting linkers. Self-assembled structures based on TMV coat proteins have been shown to be exceptionally versatile platforms for biomimetic light-harvesting assemblies.9−12,28,29 In this work, we show that small changes in linker length, rigidity, and chiral configuration can significantly extend the excited state dynamics of the system. By finely tuning the position and range of motion of chromophores, we demonstrate an unprecedented level of control over chromophore−protein−solvent interactions and identify with great precision the mechanisms responsible for the linker-mediated dynamic slow-down of excited state processes. We identify the most rigid linkers as ideal for biomimetic light harvesting, as they both retard chromophore energy-loss processes and retain orientational correlations throughout the excited state lifetime of the chromophore. Our results highlight the crucial but typically overlooked importance of using purpose-specific linkers to emulate the dynamic tuning of spatioenergetic landscapes in naturally occurring light-harvesting assemblies. They suggest a simple but powerful design principle to balance chromophore−bath and interchromophore couplings to achieve specific light-induced functions.

■ RESULTS AND DISCUSSION

To explore our ability to control chromophore−protein interactions for biomimetic light-harvesting, we developed a new library of linkers of varying lengths and rigidities to tether a chromophore to one of two specific positions on the TMV protein with differing degrees of confinement. In this study, we use the circular permutant of TMV (cpTMV), which self-assembles into an 18 nm-wide C2 symmetric double-ring structure. By introducing a uniquely reactive cysteine residue on the pH and ionic strength conditions. The assembly consists of 34 monomers (17 per ring) with a hydrated cavity extending radially outward from the central pore (Figure 1a,b).23,24 We combine this versatile platform with chromophore−protein linker engineering to conduct a systematic study of the effects of conformational constriction and attachment orientation on chromophore photophysics. Because a major aim is to mimic environmentally protected photosynthetic chromophores, we study systems where only one chromophore is present per cpTMV assembly, thus allowing detailed characterization of the chromophore−protein interactions, free of complicating factors such as interchromophore interactions. We use ultrafast TA to characterize the excited state dynamics of each system, focusing on solvation and chromophore intramolecular structural reorganization occurring within picoseconds of light absorption. MD simulations with explicit inclusion of the protein scaffold and residues, the surrounding water, and the chromophore then allow us to rationalize the trends in excited state dynamics.

Figure 1. Biomimetic light-harvesting assembly comprised of the cpTMV scaffold labeled by a single chromophore via different linkers studied here. (a) A side-view cross-section of the cpTMV assembly, displaying C2 symmetry with a hydrated cavity at the center of the assembly. (b) A top view of the full assembly, with a single monomer highlighted as well as the mutated S23C residue (yellow) to which a chromophore can be conjugated. (c) Top-bottom monomer pairs, illustrating the S23C and Q101C labeling positions (yellow and pale blue, respectively), with a chromophore conjugated for scale. (d) Sulforhodamine B (SRB) is linked to a maleimide for bioconjugation to cysteine residues via the different linkers shown in (e). The red, green, orange, and blue color scheme for linkers are maintained throughout the manuscript.
extracted from TA in terms of specific interactions between chromophore and protein bath, depending on the linker used.

The attachment positions, chromophore and series of linkers used to study these protein−chromophore interactions are displayed in Figure 1c−e. As shown in Figure 1c, two distinct environments are studied: the protein−water interface (chromophore labeling site S23C) and the nanoscale hydrated protein cavity (site Q101C). The chromophore in question is Sulforhodamine B (SRB, Figure 1d), chosen for its high visible light absorption cross-section (ε_{570 nm} ≈ 83 000 M^{-1} cm^{-1} in water) and relative rigidity due to few rotational degrees of freedom in the electronically active moieties (see below). Figure 1e shows the series of linkers used to control the distance of the chromophores from the protein surface and their conformational volume. In all cases, these linkers are shorter and more rigid than in typical bioimaging labels, which use pentyl or longer alkyl chain linkers. In our series, changing from a 4-carbon butyl (Bu) to a 2-carbon ethyl (Et) moiety straightforwardly shortens the linker. Alkyl 1,3-strain when using cyclohexyl (Cyc) moieties further reduces conformational freedom compared to alkyl chains. The chiral center of these linkers provides an additional degree of control, as the two enantiomers (Cyc-SS and Cyc-RR) are expected to lead to different attachment orientations on the protein surface. Altogether 12 systems are investigated: SRB attached to two separate positions (S23C and Q101C, Figure 1c) on cpTMV via four different linkers (Bu, Et, Cyc-SS, Cyc-RR, Figure 1e), along with control experiments using free maleimide-functionalized chromophores after reaction with the sodium salt of 2-mercaptoethanesulfonate in buffer. All experiments are performed in solution using a sodium phosphate buffer. These 12 systems form a series with systematically varied chromophore−bath couplings, enabling extraction of detailed information about how the protein environment affects the nuclear-electronic dynamics of each assembly.

Figure 2a displays the TA spectra of SRB-Cyc-SS attached at the outside S23C site of cpTMV at representative delay times following light excitation at 570 nm, ~40 fs laser light excitation. (a) TA spectra at representative delay times between 1 ps and 1.2 ns for SRB-Cyc-SS attached at the S23C (outside) position of cpTMV. The normalized linear absorption and emission spectra are plotted for comparison. (b) Decay associated spectra (DAS) for the two time components needed to fit the data in (a). The shorter, 9.9 ps, component (red) displays two dispersive differential profiles characteristic of (1) redshifting ESA (positive/negative) and (2) redshifting SE (negative/positive) around 450 and 600 nm, respectively assigned to intramolecular structural reorganization and solvation of the chromophore’s excited state. The inset shows the overlaid optimized ground and excited state structures as calculated from DFT, displaying minor intramolecular structural rearrangement. (c) TD-DFT calculations at the PBE0/6-31+g* level including solvation effects from the IEF-PCM have yielded vertical transition energies from the ground state to the bright S1 state and dark S2 state as a function of phenyl-xanthene rotation angle. The region of the excited state surface that is effectively inaccessible at room temperature (RT) is shaded light gray. Although an excited state crossing exists around a phenyl-xanthene rotation angle of 55 degrees, this configuration is inaccessible at RT. For comparison, the ground state energies are plotted in gray, with energies that rapidly increase past a rotation of 10 degrees from an orthogonal geometry. (d) Short time-component TA lifetimes corresponding to the red DAS in (b) for all chromophore-linker compounds in buffer and at their respective attachment sites on cpTMV.
kinetics across the visible spectrum are well fit by a biexponential function, with a short component on the order of a few picoseconds and a longer component on the order of a few nanoseconds. Although the spectral evolution is equivalent across systems, indicating the protein environment does not change the nature of the populated excited states, the time scales for these processes are vastly different, providing a useful handle on the influence of the biomolecular environment on chromophore-bath dynamics.

Before comparing results across the 12 different compounds, we provide a brief interpretation of the observed chromophore dynamics themselves, using time-dependent density functional theory (TD-DFT) to support our excited state assignments. Although the excited state dynamics of rhodamine dyes are well-studied, the excited state processes can depend on the specific molecular details of both the phenyl and xanthene moieties.\(^\text{30−35}\) Solvent and intramolecular structural reorganization are expected to occur within the first few picoseconds after photoexcitation. Intramolecular reorganization in rhodamines is not yet fully characterized, but involves some combination of the following: rotation of the dialkylamino groups and/or C–N bond order reduction, rotation of the initially orthogonal phenyl ring with respect to the xanthene core, and/or buckling of the xanthene moiety.\(^\text{30,33,36}\) This reorganization can facilitate electron delocalization in the excited state. Previous TD-DFT calculations on Rhodamine B, a close analogue to SRB with a carboxylate moiety replacing the \(\text{SO}_3^-\) group on the phenyl ring, identified a dark, low-lying charge transfer (CT) state that may be responsible for partial quenching of the lowest-lying bright state.\(^\text{37}\) Our TD-DFT calculations corroborate the existence of a lower-lying CT state when the phenyl-xanthene dihedral approaches a planar configuration in SRB. Accessing this CT state would, however, necessitate rotation of the phenyl ring with respect to the xanthene core towards a quasi-planar geometry, representing an energetic barrier of \(\sim 2 \text{ eV}\) on the excited state potential energy surface from the initially orthogonal geometry (Figure 2c). This barrier arises due to severe steric constraints imposed by the \(\text{SO}_3^-\) group, rendering this quenching pathway unfeasible at room temperature without large excess energy in the excitation pulse. Instead, the only major intramolecular nuclear relaxation pathway involves subtle buckling of the xanthene core and slight rotation of the dialkylamino groups (Figure 2b, inset). From these TD-DFT calculations we deduce that the short lifetime component in the TA of SRB is primarily a result of nuclear relaxation along the intramolecular and solvent reaction coordinates, rather than internal conversion between different excited states. The spectral changes accompanying these processes are a redshift of the stimulated emission between 580 and 610 nm and a slight redshift of the excited state absorption (ESA) at 450 nm, as seen more clearly in the decay-associated spectra (i.e., the spectral amplitudes of the exponential components of the global kinetic fits, Figure 2b). The population in the \(S_1\) excited state then decays back to the ground state on nanosecond time scales.

We observe several revealing trends in the rate of excited state evolution that clearly demonstrate the importance of chromophore–protein linker configuration and positioning in biomimetic light-harvesting systems. Figure 2d and Table 1 display the lifetimes associated with the picosecond solvation and intramolecular reorganization process for all systems. The nanosecond relaxation component lifetimes are shown in Table 1 and Figure S5 and follow approximately similar trends, albeit less prominently. The most dominant features immediately observed from Figure 2d are the up to 5-fold slowing of solvation and structural rearrangement when the chromophores are attached to \(\text{cpTMV}\) at outer surface site S23C, and up to 14-fold slowing when attached at intracavity site Q101C, when compared to chromophores in buffer. The fact that dynamic retardation is clearly present even at the outside S23C site for the more rigid linkers (all except butyl) suggests that the chromophores are close enough to the protein–solvent interface to be directly affected by it. Interestingly, however, SRB-Bu, which has the longest and most flexible linker, does not exhibit dynamic retardation at the outside position, instead displaying the same time scales as for the free chromophore in solution. This result indicates that even if the chromophore is covalently attached to the protein, provided the linker is long and flexible enough, it remains unaffected by the latter and only samples the bulk solvent environment far away from the protein surface. Inside the protein cavity, SRB-Bu is slowed down by an amount similar to other linkers. At this attachment site, confinement by the top and bottom protein surfaces, separated by \(\sim 2 \text{ nm}\)\(^\text{35}\), precludes the presence of a bulk-like environment, leading to much more constrained dynamics irrespective of the linker used.

Another surprising feature in Figure 2d is that the greatest retardation is experienced by SRB-Cyc-SS both inside and outside of the cavity. This difference is especially salient when comparing the enantiomers Cyc-SS and Cyc-RR, with the former slowing by a factor greater than the latter by 1.7 times at the outside position and by 2.5 times inside the cavity. Thus, a chiral modification of the chromophore linker translates into major changes in the excited state dynamics of the system. This finding suggests that enantiomers can be selected for purposes of generating slow excited state dynamics that approximate the environmental protection of chromophore electronic excitations in natural photosynthesis. Overall, in line with our previous findings using generic linkers,\(^\text{12}\) the interior protein cavity can be used to constrain both chromophores and water molecules and, in this way, substantially slow relaxation along the electronic excited state potential energy surface. Yet, going beyond our previous findings relating to confinement, we also learn here that purpose-specific linkers enable dynamic retardation at the protein surface (which would be absent using the long tethers typical for commercial bioimaging dyes), and provide far better control over the extent to which chromophore–protein interactions tune the excited state dynamics both inside and outside the protein cavity. These compounds therefore facilitate a much more systematic and

| Table 1. Short and Long Lifetime Components, along with Dynamic Retardation Ratios Resulting from Chromophore Attachment to the Protein at Different Sites, Extracted from Global Analysis of TA Data for All Measured Systems |
|---|---|---|---|---|---|
| linker | component | buffer | S23C | Q101C | S23C/ buffer | Q101C/ buffer |
| Butyl | short (ps) | 1.7 | 1.7 | 18 | 1.0 | 11 |
| | long (ns) | 1.7 | 1.9 | 2.8 | 1.1 | 1.6 |
| Ethyl | short (ps) | 2.2 | 7.3 | 16 | 3.3 | 7.3 |
| | long (ns) | 1.5 | 2.1 | 2.4 | 1.4 | 1.6 |
| Cyc-SS | short (ps) | 1.8 | 9.9 | 26 | 5.5 | 14 |
| | long (ns) | 1.4 | 2.1 | 2.3 | 1.5 | 1.6 |
| Cyc-RR | short (ps) | 1.9 | 6.1 | 11 | 3.2 | 5.8 |
| | long (ns) | 1.9 | 1.9 | 3.1 | 1.0 | 1.6 |

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precise way to study the role of chromophore–protein–solvent interactions in light-harvesting systems, as will be shown in detail below.

To obtain a molecular-level understanding of the trends in excited state dynamics observed for the four chromophore–linker systems attached to the surface site of cpTMV, we investigate how each linker alters the orientation and positioning of the chromophore on the protein surface using MD simulations. For computational tractability, a reduced system consisting of surface helices 1 and 2 of a cpTMV monomer, along with helix 2 of an adjacent monomer is simulated. The chromophore is attached to S23C on helix 1. A close analogue of SRB, AlexaFluor 488, is used since accurate force field parameters for AlexaFluor 488 have been developed to reproduce experimental data, whereas parameters have not yet been developed for SRB. The MD simulations not only confirm an intuitive picture of cyclohexyl-linked chromophores being constrained closer to the protein surface, but also reveal a detailed and subtle picture of chromophore–protein–water interactions that successfully explains the observed trends in excited state dynamics.

More specifically, we consider how each linker determines the extent to which the chromophore is localized at the protein surface. The increased length and flexibility of the butyl and ethyl linkers allow the chromophore to sample many different configurations that reach far from the protein surface. In contrast, the Cyc-RR and Cyc-SS constrain the chromophore in specific configurations relative to the protein surface. In Figure 3a, the distribution of the minimum distance between any carbon atom of the chromophore’s xanthene core and a heavy protein atom is plotted. For all four systems, the chromophore can specifically interact with protein side chains, resulting in the peak at ∼0.35 nm. The distributions for the butyl and ethyl systems exhibit a broad second peak centered at ∼1.2 nm and ∼1 nm, respectively, whereas the distributions for the cyclohexyl systems both exhibit a second peak centered at ∼0.7 nm. The 3D density plots of the xanthene heavy atoms of the chromophore (Figure 3b), averaged over the last 500 ns of the MD simulations, provide further insight into common chromophore conformations on the protein surface. Plots at a low density isovalue (0.005 g/Å^3) show all possible locations where the chromophore can be found during the simulations. Consistent with the distance distributions, the chromophore...
samples the largest expanse of space when attached via butyl and ethyl linkers, as compared to Cyc-RR or Cyc-SS. Plots at a more stringent isovalue (0.06 g/Å³) help to identify the most common conformations of the chromophore on the protein surface. Since the butyl and ethyl linkers are quite flexible, the butyl- and ethyl-linked chromophores adopt many different conformations and reside in multiple regions on the protein surface with densities less than 0.06 g/Å³. As a result, large, significant regions at this density isovalue are not observed. In contrast, the Cyc-RR- and Cyc-SS-linked chromophores adopt only a few, very specific conformations on the protein such that significant regions of density are observed at the more stringent isovalue. These results support the conclusion that chromophores attached via either butyl or ethyl sample more regions of space farther from the protein surface than those attached via the cyclohexyl groups, which remain within the biomolecular hydration shells.39

An additional consequence of restricting chromophore conformational sampling via cyclohexyl linkers is the ability to preserve orientational correlations for longer. In Figure 3c, the transition dipole moment orientational correlation function of the chromophore is plotted for all systems, showing that rotational reorientation is considerably slower for Cyc-SS- and Cyc-RR-linked chromophores compared to ethyl- and butyl-linked complexes. The cyclohexyl linkers retain orientational correlations over several nanoseconds, i.e., over the full excited state lifetimes of the chromophores. In light-harvesting assemblies, the ability to retain orientational correlations between adjacent chromophores throughout the exciton lifetime allows for direct optimization of nonradiative energy transfer. Such control is necessary to reproduce the directional and efficient energy transport over long distances that is achieved in natural systems. The cyclohexyl linkers are therefore ideal chromophore–protein linkers for biomimetic light-harvesting assemblies.

To explain the differences between the excited state dynamics of Cyc-RR and Cyc-SS-linked chromophores, we investigated the dynamics of water molecules around the chromophore and the protein surface. The molecular interpretation of chromophore dynamic retardation due to slowed water molecules near biomolecular surfaces has been studied in depth by a wide range of experimental and computational techniques and remains somewhat controversial.39–44 Mounting evidence suggests that water dynamics near protein surfaces are not affected as much as previously thought, typically only slowing by a factor of 2–3 over the first hydration shell, with the effect rapidly dropping off radially toward bulk water.45,50 As a result, the commonly observed order-of-magnitude retardation in the excited state dynamics of chromophores located in the first few hydration shells is often attributed to slow biomolecular motion affecting both water and chromophore dynamics in its heterogeneous dielectric environment.30–33 Figure 3d displays the rotational time correlation function of the water dipole moments for molecules in the bulk (far away from the protein surface) compared to those within 5 Å of any of the chromophores’ xanthene heavy atoms in our cpTMV system. These simulations show that the water dynamics near the chromophores are slowed by a factor of ~2, corroborating the aforementioned studies, but with the largest retardation occurring around the Cyc-SS linker (see also Figure S11 and Table S1). The difference in water dynamics around Cyc-RR- and Cyc-SS-linked chromophores is a priori surprising given that they reside a similar distance away from the protein surface. The dissimilarity suggests that a mechanism based on specific chromophore–protein–solvent interactions is responsible for the observed dynamic differences, as discussed below.

A closer look at the different chromophore orientations for the Cyc-SS and Cyc-RR complexes in Figures 3b,e reveals that the chromophore attached via Cyc-SS resides primarily above the two helices of the same monomer, whereas a high density of hydrophobic amino acid residues are located, whereas the chromophore attached via Cyc-RR resides above a less dense region between adjacent monomers (Figure 3e). To determine a more detailed picture of slowed water dynamics, and whether it can be attributed to the protein residues alone or to both the protein and chromophore, the dynamics of waters within 5 Å of each protein side chain were analyzed for the model cpTMV system with and without a chromophore (Figures 3e and S12). The water diffusion coefficients are plotted in false color scale around the protein surface, with blue representing the greatest retardation in water dynamics. Without the chromophore present (left), it is already apparent that water dynamics are slowed around densely packed areas of the protein, such as the area between the helices of a cpTMV monomer, and within deeper pockets or grooves along the protein surface. Strikingly, when the chromophore is present, the water dynamics around specific residues are further slowed, suggesting that the chromophore also impacts the solvent dynamics. This retardation is particularly noticeable for the Cyc-SS complex, which is constrained just above a dense region of the protein surface. The same trends are observed when the dipole rotational correlation functions of waters around each residue are compared (Figure S13). These observations suggest that water molecules are effectively sandwiched between the chromophore and the protein surface, leading to retardation near the surface that is a factor of ~1.5 greater than in the absence of the chromophore. For the ethyl complex, which mainly resides in a similar but more distant region, water molecules near the surface are slightly slowed by a factor of ~1.1 compared to without the chromophore. Finally, the Cyc-RR complex, which primarily resides above the more recessed and sparser groove between adjacent monomers, has negligible effect on water dynamics in the already fairly dynamically constrained groove compared to the system without the chromophore.

The above differences in water dynamics correlate with the retardation trends observed experimentally between Ethyl-, Cyc-SS- and Cyc-RR-linked complexes at S23C (yellow bars in Figure 2d) remarkably well. In the TA, the intramolecular and solvent dynamics for Et-, Cyc-SS, and Cyc-RR-linked chromophores are slowed by factors of 3.3, 5.5, and 3.2, respectively (Table 1). In the simulations, the compounded retardation of water molecules between the protein and chromophore are by factors of ~4.8, 6.4, and 4.6, respectively. These results show that explicit inclusion of the chromophore in simulations is paramount to accurately determine the system dynamics, and that in certain cases, the strong interaction between chromophore, protein and solvent leads to a supracomplex with intricately coupled dynamics. We therefore believe that a combination of slowed hydration dynamics and protein motion, the latter being particularly effective at intracavity labeling sites where chromophores are surrounded by protein residues, are responsible for the up to 14-fold retardation (for Cyc-SS linked to inner cavity site Q101C) in solvent-chromophore dynamics in our experiments. By
engineering the distance from the protein surface and the volumes sampled by chromophores in relation to specific protein residues, solvent–solute interactions can be finely and predictively tuned. The complex interplay between protein, water, and chromophore motions and dielectric interactions thus provides an exceptionally rich and system-specific platform to control the excited state properties of biomimetic assemblies.

## CONCLUSIONS

Toward developing biomimetic light harvesting assemblies, we have combined a highly tunable protein scaffold based on the tobacco mosaic virus coat protein with a library of chromophore–protein linkers. This combination enabled us to systematically study the molecular dynamics of chromophore–protein–solvent interactions and their effect on the ultrafast excited state dynamics of the chromophores. Small modifications to the position, mobility, and attachment orientation of the chromophores on the protein surface, achieved through alkyl chain shortening or switching the chiral configurations of the linkers, suffice to control the nuclear relaxation dynamics of the system considerably. These results therefore highlight the often-overlooked importance of purpose-specific linker engineering in combination with judicious attachment positioning to tune the properties of artificial light-harvesting systems.

On the basis of these findings, we describe below how our assemblies enable a modular approach to tune the balance between chromophore–protein and interchromophore couplings, and how such fine-tuning will permit further understanding of how nature has optimized its complex photosynthetic environment for light harvesting. First, at the level of chromophore–protein interactions, we found that minor modifications to chromophore–protein linker length and rigidity transform into pronounced differences in the chromophore properties when bound to the protein assembly. For example, shortening the butyl linker by just two bonds to ethyl reins in the chromophore closer to the protein surface and into a region prone to chromophore–protein interactions. This shortening changes the chromophore environment from being completely bulk-like to the more heterogeneous protein–solvent interface. Consequently, a greater than 4-fold slowing of the Stokes shift time scale occurs at the outside labeling position between butyl-linked and ethyl-linked chromophores. Further rigidifying the chromophore to cyclohexyl-based linkers enhances the retardation up to 6-fold. This strategy to tune the optical properties of chromophores and preserve electronic excitations in the system by coupling the chromophore and protein may be a crucial photosynthetic design principle, where chromophores are tightly bound within protein pockets and thus necessarily interact with their biomolecular environment.

These considerations invite a more explicit comparison of our biomimetic light harvesting platform with the natural ones that it seeks to emulate. In light-harvesting systems where interchromophore energy transfer between identical chromophores is present, retarding Stokes shift dynamics could enable a larger number of resonant energy hops to proceed well out-of-equilibrium within the lifetime of an excitation. These energy hops can be more efficient than those initiated from structurally relaxed chromophores due to the larger spectral overlap between adjacent chromophores prior to nuclear relaxation, thus aiding long-range energy transport. Furthermore, the preservation of excess energy within the system can help overcome unwanted trapping in low-energy states that are inevitable in disordered environments. While individual chromophore dynamics in natural light-harvesting systems are known due to our inability to study them in the absence of interchromophore coupling, their degree of confinement, range of motion and distance from protein surfaces in complexes such as the LH2 of purple bacteria or the FMO of green sulfur bacteria are on the same order as for our cyclohexyl-based complexes.2,56 Chromophores in these natural systems are thus likely subject to similar or greater dynamic retardation of their individual Stokes shift dynamics. Our highly tunable scaffold will facilitate a bottom-up approach to test our hypotheses on the role of relaxation retardation on light-harvesting and provide direct insight into whether these mechanisms are also likely to be operative in photosynthetic organisms.

Second, looking toward the control of interchromophore interactions, we have shown that further constraints on chromophore environments can be readily applied by locking the chromophore into specific positions on the protein surface. To that end, we found that linker chirality can be exploited: despite no major difference in length or rigidity, changing the linker from (R,R) to (S,S)-cyclohexyl leads to considerably slower excited state dynamics due to different attachment positions. These different configurations lead to distinct chromophore–protein bath interactions, including sandwiching water molecules in a tight space between chromophore and protein. Furthermore, both cyclohexyl-linked chromophores exhibit much longer orientational decorrelation times than the more flexible alkyl linkers. Over the full course of the chromophores’ excited state lifetimes, this constraint enables greater control of the relative orientations of the chromophores with respect to each other and the protein surface—a crucial feature to achieve long-range energy transport. The ability to manipulate the relative position and orientation of adjacent chromophores in easily configurable artificial light harvesting constructs presents the further possibility to mimic and test the characteristics of specific interchromophore motifs found in nature.

Having refined chromophore–protein interactions through our studies performed in the absence of interchromophore interactions, we are now poised to reintroduce these interactions via saturating labeling densities to form complete biomimetic light harvesters. A key challenge will be to obtain the right balance between interchromophore and chromophore–protein coupling for a range of energy transport regimes found in nature, from long-range dipole–dipole to short-range excitonic processes, while maintaining systems free of contact quenching. The degree of control over chromophore–protein interactions that we have demonstrated in our bottom-up approach, and the flexibility over attachment positioning and hence interchromophore distance afforded by our synthetic scaffold, will prove essential in finding the tuning range over which energy transfer proceeds efficiently for different transport regimes. Examining the role of chromophore–solvent–protein interactions over the entirety of this tuning range will enable testing and refining our current models seeking to explain the high quantum efficiencies and vast diversity of photosynthetic organisms. To mimic nature even further, the construction of extended arrays of cPTMV rings, analogous to the arrays of natural light harvesting complexes packed into lipid membranes, could allow the exploration of longer range biomimetic energy transfer using recently developed spatially resolved approaches.57
Ultimately, the fine level of control over linker properties achievable through well-known synthetic procedures, along with high-throughput screening of candidate linkers using computational modeling, represents a highly effective strategy to design purpose-specific biomimetic tools to more easily test hypotheses regarding the molecular mechanisms of natural photosynthetic light harvesting. In particular, since nuclear-electronic coupling often dictates the fate of molecular excited states following light absorption, harnessing chromophore−bath couplings to affect the structural motion of electronically coupled arrays of chromophores\textsuperscript{58}−\textsuperscript{61} could prove paramount to achieving long-range energy transport as efficiently as in natural photosynthetic systems. Such an achievement would help elucidate the molecular and intermolecular origin of the unparalleled efficiency of photosynthetic light harvesting.

**METHODS**

**Synthesis.** Extensive details of the synthetic procedures, mutant generation, protein expression and bioconjugation and purity analyses are provided in the Supporting Information. The collection of maleimide functionalized sulforhodamine B dyes spaced by various linkers were synthesized starting from commercially available alcoholamines. The Boc-protection was first selectively carried out on the amine group, followed by Mitsunobu displacement of the free alcohol with maleimide. After release by deprotection, the free amine was able to couple the sulforhodamine B acid chloride to yield the respective sulforhodamine B maleimides.\textsuperscript{62}−\textsuperscript{65} The statistically single labeling of the double disk protein assembly was achieved by adjusting the equivalents of the functionalized dyes and the cysteine containing protein monomers as low as 0.01. The labeling yields of the protein bioconjugates were analyzed by a Time-of-Flight (TOF) mass spectrometer with a dual electrospray source connected in-line with high-performance liquid chromatography (ESI-TOF LC−MS). Analytical size exclusion chromatography was performed for assessment of purity and validation of assembly state.

**Transient Absorption.** Ultrafast transient absorption spectroscopy was performed using a Ti:sapphire regenerative amplifier delivering 5 W, 5 kHz, 40 fs pulses centered at 800 nm. Pump pulses centered at 570 nm with 40 fs pulsewidth were generated in a home-build noncollinear parametric amplifier followed by a prism-pair compressor. The probe was a white light supercontinuum generated by focusing 800 nm light in a CaF\textsubscript{2} crystal. Pump and probe are set at magic angle (54.7°) polarization to each other. The pump power density used was ∼300 \(\mu\)J/cm\(^2\), well within a linear excitation regime. Optical densities at the excitation wavelength were at ∼0.1. All experiments are performed at room temperature. The samples were continuously rastered during data acquisition, and UV−vis spectra taken before and after every run verified that no sample decomposition occurred.

**DFT.** Vertical excitation energies, oscillator strengths, and natural transition orbitals (NTOs) with ground (\(S_0\)) and excited state (\(S_1\)) after every run measured with 40 fs pulsewidth were generated in a home-build high-performance liquid chromatography (ESI-TOF LC−MS). The pump power density used was ∼300 \(\mu\)J/cm\(^2\), well within a linear excitation regime. Optical densities at the excitation wavelength were at ∼0.1. All experiments are performed at room temperature. The samples were continuously rastered during data acquisition, and UV−vis spectra taken before and after every run verified that no sample decomposition occurred.

**ASSOCIATED CONTENT**

* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.7b13598.

**MD Simulations.** Simulation methods are briefly described below, and complete details are given in the Supporting Information. To investigate the protein environment around each chromophore, we performed MD simulations of a system composed of three surface exposed \(\alpha\)-helices of cpTMV from the crystal structure of cpTMV (PDB: 3KML), in which the chromophore was linked to S23C on the central \(\alpha\)-helix via each organic linker. Because force field parameters for sulforhodamine B have not been published, a similar rhodamine dye, AlexaFluor 488, was simulated. We used the Amber03s force field \textsuperscript{83} and TIP4P/2005s water model \textsuperscript{82} since the parameters of AlexaFluor 488 have been optimized to reproduce experimental data (this combination of force field and water model. \textsuperscript{84} To efficiently sample configurations of the chromophore on the protein surface, 1 \(\mu\)s bias-exchange metadynamics (BE-META) simulations \textsuperscript{75} were performed using GROMACS 4.6 \textsuperscript{66} with the PLUMED 2 patch. \textsuperscript{77} For all four systems with a chromophore, a bias was applied on the S23C dihedrals \(\chi_1 (N-Ca-C\beta-Sy), \chi_2 (Ca-C\beta-Sy-C_{mal}\), \(C_{mal}\) is the carbon of the maleimide group attached to Cys). Additional biases were applied to enhance sampling of all rotatable bonds of the two aliphatic linkers: For butyl, three additional biases were applied on the dihedrals \(N_{mal}-C_1-C_2-C_3, C_1-C_2-C_3-C_4, \) and \(C_2-C_3-C_4-C_{mal}\), where \(N_{mal}\) is the nitrogen of the maleimide group, \(C_1\), \(C_2\), \(C_3\), and \(C_4\) are the carbons of the butyl linker, and \(N_{male}\) is the nitrogen of the amide bond connecting the linker to the chromophore. For ethyl, one additional bias was applied on the dihedral \(N_{male}-C_1-C_2-N_{male}\), where \(C_1\) and \(C_2\) are the carbons of the ethyl linker. For all systems, one unbiased replica was also included, and the last 500 ns of this replica was used for accurate analysis of the unbiased ensemble. To investigate the dynamics of the chromophore and solvent, configurations obtained from the last 500 ns of the BE-META simulations were used to initialize ten short MD simulations for each system. For comparison, short MD simulations of a system without a chromophore were also performed.

**AUTHOR INFORMATION**

**Corresponding Author**

*nginsberg@berkeley.edu

**ORCID**

Milan Delor: 0000-0002-9480-9235

Matthew B. Francis: 0000-0003-2837-2538

Naomi S. Ginsberg: 0000-0002-5660-3586

**Author Contributions**

M.D., J.D., T.D.R., and J.R.R. contributed equally to this work.

**Notes**

The authors declare no competing financial interest.

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