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An introduction to color-changing systems from the cephalopod protein reflectin

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Abstract

Cephalopods possess unrivaled camouflage and signaling abilities that are enabled by their sophisticated skin, wherein multiple layers contain chromatophore pigment cells (as part of larger chromatophore organs) and different types of reflective cells called iridocytes and leucophores. The optical functionality of these cells (and thus cephalopod skin) critically relies upon subcellular structures partially composed of unusual structural proteins known as reflectins. Herein, we highlight studies that have investigated reflectins as materials within the context of color-changing coatings. We in turn discuss these proteins' multi-faceted properties, associated challenges, and future potential. Through our presentation of selected case studies, we hope to stimulate additional dialogue and spur further research on photonic technologies based on and inspired by reflectins.

Introduction

Due to their stunning camouflage and signaling abilities, cephalopods (e.g. squid, octopuses, and cuttlefish) have fascinated scientists since antiquity [1–6], with some of the earliest observations of their behavior recorded by the Greek philosopher Aristotle [7]. Indeed, cephalopods like the cuttlefish in figure 1(A) readily alter the appearance of their skin to emulate various objects or to blend into their surroundings [1–6]. For cuttlefish, such displays are enabled by the generalized skin morphology shown in figure 1(B), wherein multiple layers contain chromatophore pigment cells (as part of larger chromatophore organs) and reflective cells called iridocytes and leucophores [2, 5]. These cephalopod cells perform distinct optical functions but work in tandem to modulate the skin's overall coloration, with the chromatophore pigment cells acting as spectral filters and the iridocytes and leucophores acting as different types of reflectors [2, 5, 8–15]. In addition, cuttlefish (and other cephalopods) readily alter the texture and shape of their skin via an underlying musculature, affording an additional level of control over their appearance [2, 5, 16]. Due to the unique hierarchical architecture of their skin, cephalopods effectively behave like living 3D bioelectronic displays,

with capabilities that are enviable from the viewpoint of analogous artificial systems [5, 6, 17].

The properties of cephalopod skin cells' constituent subcellular structures are particularly fascinating. For instance, chromatophore pigment cells, like the one shown within the chromatophore organ in figure 1(C), encompass internalized sacculi containing networks of pigment- and protein-based granules [8–11]. These sacculi, which are actively controlled through the action of radial muscle cells in all cephalopods, effectively serve as spectral filters with distinct absorbances/reflectances but variable sizes in their punctate and expanded states, thereby enabling the central yellow, red, or brown chromatophore pigment cells to absorb, reflect, and transmit light of specific wavelengths [8–11]. In addition, iridocytes, like the one shown in figure 1(D), feature membranes that fold to form periodic, alternating arrangements of proteinaceous platelets and extracellular space [8, 11–13]. These arrangements, which are actively controlled in some squid but passively controlled in most other cephalopods, effectively function as biological Bragg stacks with geometry-dependent narrowband reflectances, thereby allowing the iridocytes to reflect light of variable wavelengths across the visible spectrum [8, 11–13]. Finally, leucophores, like the one shown in figure 1(E), possess disordered arrays
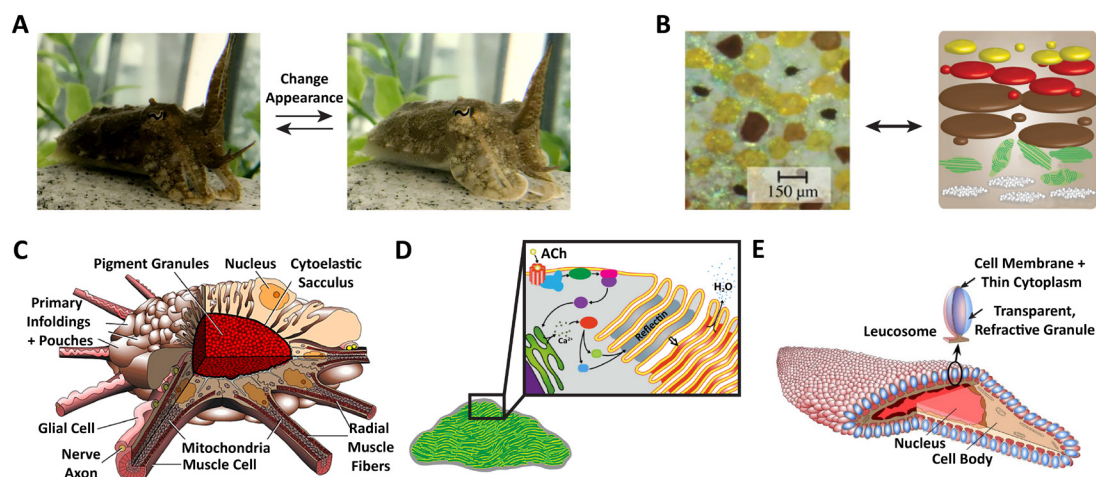
Figure 1. (A) An image of a cuttlefish changing its coloration from dark brown to light brown (B) Left: an optical image of cuttlefish skin, wherein the chromatophores are yellow, red, and brown; the iridocytes are iridescent (green); and the leucophores are white. Right: a diagram of the arrangement of optically-active components in cuttlefish skin, wherein the chromatophores (yellow, red, and brown) lie above the iridocytes (green) and leucophores (white). (C) An illustration of a cephalopod chromatophore organ with a chromatophore pigment cell in its center, which encompasses an internalized sacculus containing pigment- and protein-based granules. The sacculus modulates the absorption and reflection of light of specific wavelengths. (D) An illustration of a cephalopod iridocyte, which features a folded membrane that forms a periodic alternating arrangement of proteinaceous platelets and extracellular space. The arrangement reflects variable-wavelength light. (E) An illustration of a cephalopod leucophore, which possesses a disordered array of proteinaceous particles. The array diffusely reflects white light. Part A was reproduced with permission from Christine Bedore. Part B (left) was reproduced with permission from [5]. Copyright 2013, The Royal Society. Part B (right) was reproduced with permission from [9]. Copyright 2014, The Royal Society. Part C was adapted with permission from [5]. Copyright 2013, The Royal Society. Part D was adapted with permission from [13]. Copyright 2013, The Company of Biologists. Part E was adapted with permission from [8]. Copyright 1983, The Royal Society.

of typically spheroidal proteinaceous particles within their membranes [8, 14, 15, 18]. These arrays, which are passively controlled in all but a few cephalopod species, effectively behave like Lambertian surfaces with broadband reflectances, thereby making it possible for leucophores to diffusely reflect white light (note that complementary iridocyte localization may augment leucophore brightness in some instances) [8, 14, 15, 18]. Given their functionality, the subcellular structures found both in chromatophore pigment cells and in iridocyte and leucophore reflective cells have emerged as exciting paradigms for the development of biomimetic photonic technologies [5, 6, 17].

For cephalopods, many of the diverse optical components found in their tissues and cells (including the granule networks of chromatophores, platelet arrangements of iridocytes, and particle arrays of leucophores) are 'built' in part from unusual structural proteins known as reflectins [9, 13, 15, 17, 19]. As one example, these proteins form the reflective platelets found in the light organ of the *Euprymna scolopes* squid, as illustrated in figure 2(A), from which they were first isolated in 2004 [19]. The *E. scolopes* reflectins were immediately benchmarked as unusual because they contained high percentages of charged and aromatic amino acids, were deficient in several common amino acids (i.e. A, I, L, and K), and consisted of variable linker regions separated by repeating motifs of the general form $(M/F - D - X_5)$ $(M - D - X_5)_n(M - D - X_{3/4})$, as illustrated for the sequence of the reflectin 1a isoform in figure 2(B) [19]. Subsequently, several reflectins were isolated from other cephalopods, including *Loligo forbesii*, *Doryteuthis*

(Loligo) pealeii, *Doryteuthis opalescens*, and *Sepia* officinalis. These isoforms featured additional variability in their sequences and different numbers of motifs further subcategorized by their positions with respect to the N- and C-termini (see [17] for an excellent review). For these reflectin variants, the realization that they form a diverse array of micro-/nano-structures *in vivo* spurred the investigation of their self-assembly *in vitro*, leading to the generally-accepted model of reflectin monomers aggregating into nanoparticles that then form higher order ensembles, as illustrated in figure 2(C) and (D) [20–25]. Simultaneously, such efforts contributed to exciting applications-oriented discoveries *in vitro*, with reflectin films demonstrating functionality as proton-transporting media with electrical figures of merit rivaling those of artificial materials [26–29], biocompatible substrates that support the proliferation and differentiation of neural stem cells [30], and reconfigurable camouflage coatings that respond to various exogenous stimuli [20, 21, 31–34]. In general, reflectins' interesting combination of properties pointed at the potential of these proteins for applications in stimuli-responsive color-changing platforms.

In this targeted perspective, we describe studies that have focused on exploring reflectins as materials within the context of color-changing coatings and devices. First, we describe the seminal experiments for films not only from *E. scolopes* reflectins 1a and 1b but also from designer recombinant variants of these proteins. Next, we detail experiments with coatings from *D. pealeii* reflectin A1, wherein chemical, mechanical, and electrical stimuli all induce changes in coloration.
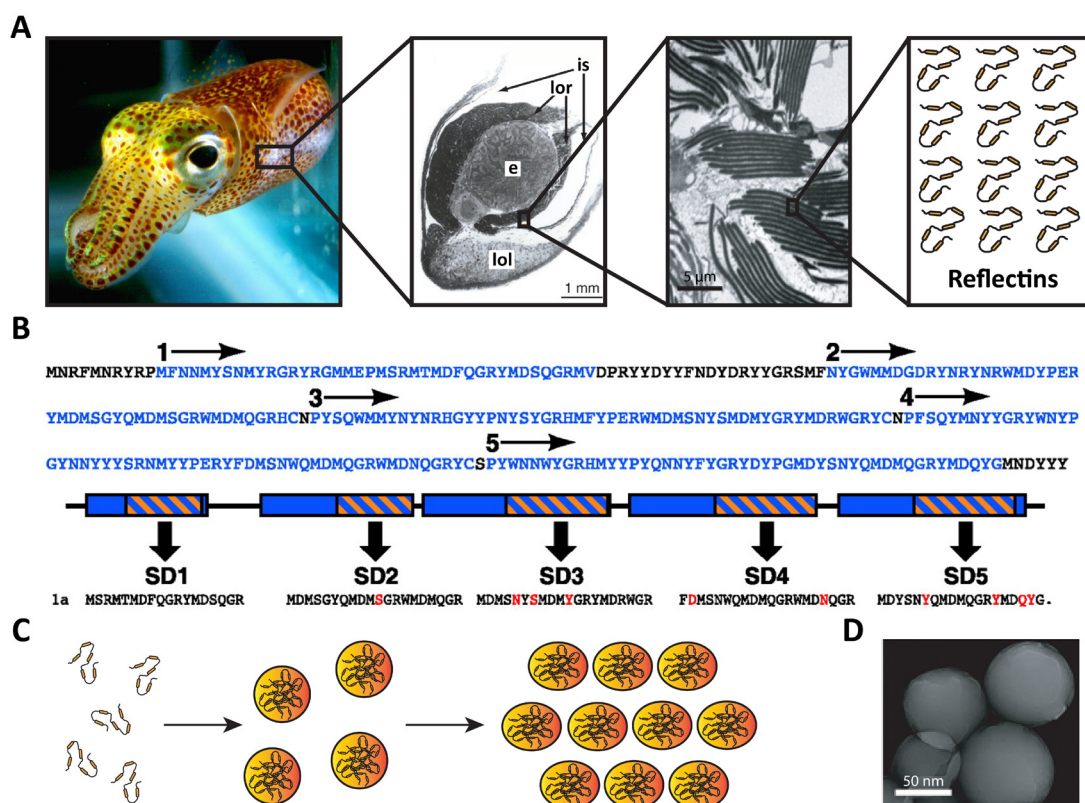
Figure 2. (A) Far left: a picture of the Hawaiian bobtail squid *E. scolopes*. Middle left: a light micrograph of an *E. scolopes* light organ cross section, where the ink sack diverticula (is) surrounds the reflector (lor), which in turn surrounds the central epithelium (e). The lens tissue (lol) on the ventral surface of the light organ is also shown. Middle right: a cross-sectional transmission electron microscopy image of reflectin platelet stacks within the light organ of *E. scolopes*. Far right: an illustration of the reflectin proteins contained within the platelets. (B) Top: the amino acid sequence of *E. scolopes* reflectin 1a. Bottom: an illustration of the conserved motifs for *E. scolopes* reflectin 1a (hatched boxes) along with their corresponding amino acid sequences. (C) An illustration of the assembly of reflectins into nanoparticles, followed by aggregation of the nanoparticles into larger multimeric ensembles. (D) A transmission electron microscopy image of aggregated nanoparticles from *E. scolopes* reflectin 1a. Part A (left and middle left) were adapted with permission from [19]. Copyright 2004, American Association for the Advancement of Science. Part A (middle right) was adapted with permission from [20]. Copyright 2007, Nature Publishing Group. Part B was adapted with permission from [19]. Copyright 2004, American Association for the Advancement of Science. Part D was reproduced with permission from [20]. Copyright 2007, Nature Publishing Group.

Subsequently, we present an overview of reflectins' multi-faceted properties, associated challenges, and future potential. Here, we note that this perspective does not constitute an exhaustive survey of the literature but rather presents personal viewpoints on a limited number of 'key' literature excerpts. Through the presentation of selected case studies, we hope to stimulate additional dialogue and spur further research on reflectin-based and reflectin-inspired photonic technologies.

Summary of prior studies

In a seminal *in vitro* study, Kramer and co-workers first reported the use of reflectin as an optical material [20]. In their work, the authors drew inspiration both from the reflective platelets found in the *E. scolopes* light organ and from the multilayer reflectors found in *Lolliguncula brevis* iridocytes. Initially, Kramer and co-workers developed protocols for the expression and purification of *E. scolopes* reflectin 1a from *E. coli*, obviating the need for extraction of the protein directly from cephalopod specimens. Next, they flow coated reflectin films onto solid substrates, as illustrated in figure 3(A), thereby demonstrating that the protein could be processed as a material for the first time. The fabrication method furnished films like the one shown in figure 3(B), which featured an unusually high refractive index of ~1.6 and thin film interference-dictated coloration. The authors subjected the reflectin films to a water vapor stimulus, which changed the films' thickness and reversibly shifted their reflectance across the visible spectrum, as illustrated in figure 3(C). The experiments demonstrated that the coloration of reflectin-based materials could be modulated with chemical stimuli for the first time. In additional proof-of-principle experiments, the authors used reflectin precipitates to draw variable-sized fibers, as illustrated in figure 3(D), thereby emphasizing the protein's processability. Overall, this work established an essential conceptual and technical foundation for the subsequent design and preparation of reflectin-based color-changing devices.

Qin and co-workers followed up on the previous seminal report and explored the properties of recombinant reflectin-like peptides [21]. In their work,
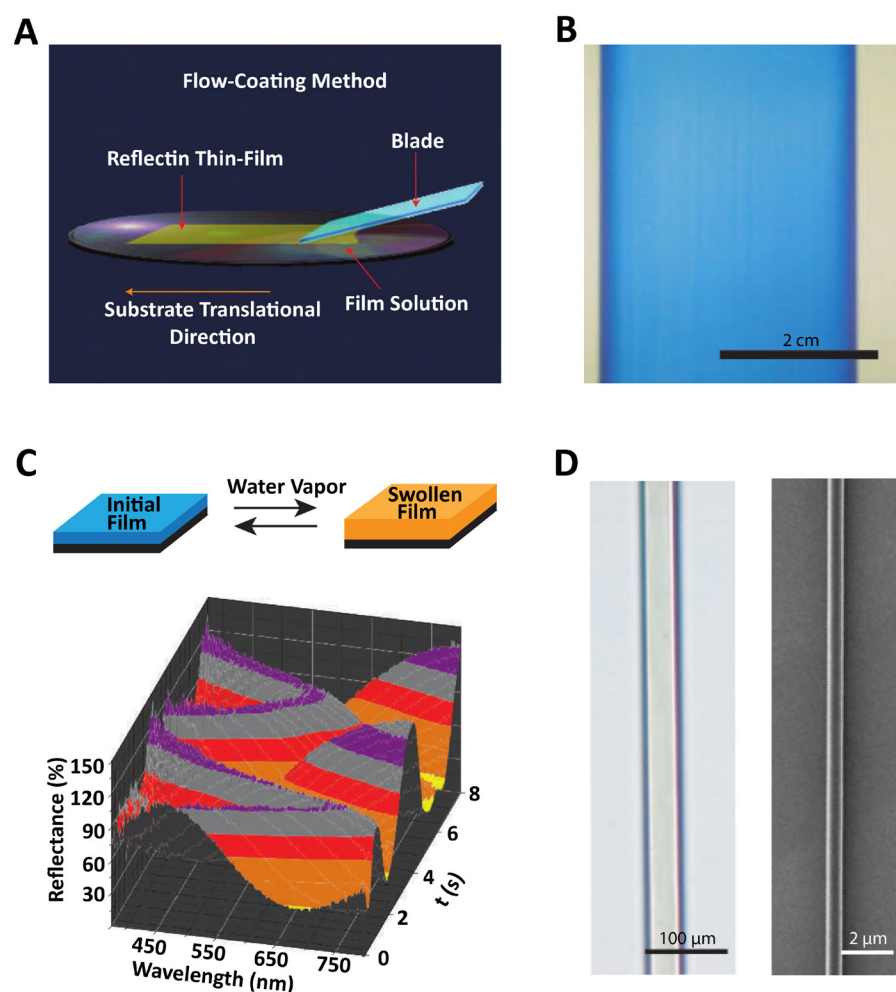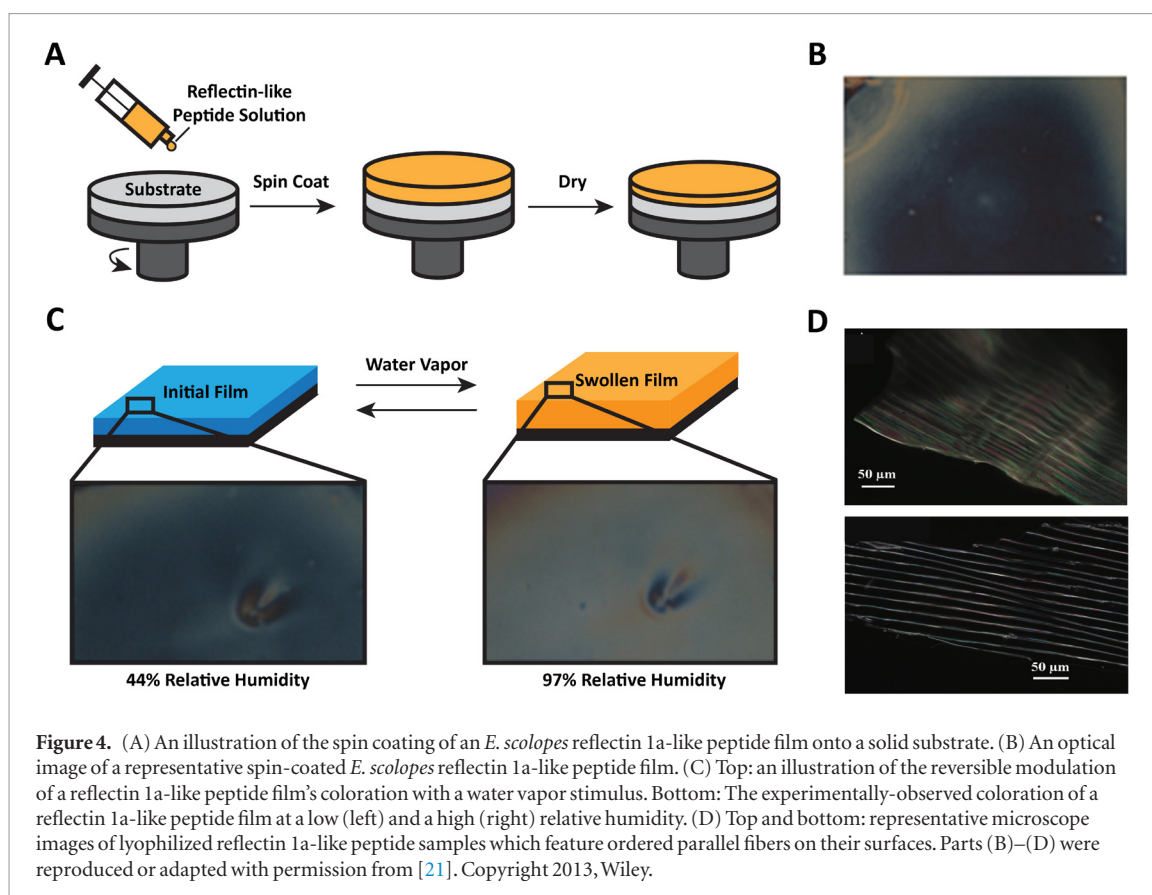
Figure 3. (A) An illustration of the flow coating of an *E. scolopes* reflectin 1a film onto a solid substrate. (B) An optical image of a representative flow-coated *E. scolopes* reflectin 1a film. (C) Top: an illustration of the reversible modulation of a reflectin film's coloration with a water vapor stimulus. The water vapor leads to an increase in film thickness and a red shift in the coloration. Bottom: the experimentally-observed reversible time-dependent change in the reflectance spectrum of a reflectin 1a film due to the application and removal of a water vapor stimulus. (D) Left: representative optical image of a large diameter fiber drawn from precipitated reflectin 1a. Right: representative scanning electron microscope image of a small diameter fiber drawn from precipitated reflectin 1a. Parts (A)–(D) were reproduced or adapted with permission from [20]. Copyright 2007, Nature Publishing Group.

the authors drew inspiration not only from the reflective platelets of *E. scolopes,* but also from the adaptive Bragg stack-like structures in *D. pealeii* iridocytes. Initially, Qin and co-workers designed peptides containing key conserved sequence motifs from *E. scolopes* reflectin 1a and then demonstrated the recombinant expression of these peptides, for which enhanced solubility greatly facilitated purification. Next, they perfected spin coating of the peptides onto various substrates, as illustrated in figure 4(A), significantly simplifying film fabrication. This procedure yielded films, like the one shown in figure 4(B), whose coloration was governed by standard thin film interference theory. The authors exposed the peptide films to water vapor, which increased their thickness and altered their coloration, as illustrated in figure 4(C). These experiments showed that recombinant reflectin-like peptides with greatly reduced complexity could capture some of the properties of their naturally occurring parent protein(s). Furthermore, the authors noted that

ordered parallel fibers formed on the surfaces of lyophilized peptide samples as illustrated in figure 4(D), showcasing the peptides' diverse self-assembly properties. Altogether, the observations validated a path for the high-throughput production and processing of reflectin-like materials and further highlighted their potential for adaptive color-changing systems.

Dennis and co-workers extended their efforts to the investigation of both another reflectin isoform and more advanced recombinant reflectin-based peptides [31]. In their work, the authors again drew inspiration both from *E. scolopes'* static reflective platelets and from Loliginids' nanostructured tunable reflective platelets. Initially, Dennis and co-workers showcased the utility of their established protocols by producing both full length and truncated variants of *E. scolopes* reflectin 1b, as well as various rationally-designed peptides comprised of multiple minimal repeats selected from the reflectin 1b sequence (called concatemers). Next, they iteratively flow coated either

Figure 4. (A) An illustration of the spin coating of an *E. scolopes* reflectin 1a-like peptide film onto a solid substrate. (B) An optical image of a representative spin-coated *E. scolopes* reflectin 1a-like peptide film. (C) Top: an illustration of the reversible modulation of a reflectin 1a-like peptide film's coloration with a water vapor stimulus. Bottom: The experimentally-observed coloration of a reflectin 1a-like peptide film at a low (left) and a high (right) relative humidity. (D) Top and bottom: representative microscope images of lyophilized reflectin 1a-like peptide samples which feature ordered parallel fibers on their surfaces. Parts (B)–(D) were reproduced or adapted with permission from [21]. Copyright 2013, Wiley.

reflectin variants or concatemers onto glass or silicon substrates, as illustrated in figure 5(A), thereby generating films with consistent thicknesses for comparative purposes. This fabrication approach resulted in reflectin or concatemer films like the ones shown in figure 5(B), which qualitatively possessed a second-order, typically yellow specular reflectance (due to thin-film interference). Here, the authors exposed the full length reflectin films to repeated vapor pulses, which irreversibly altered their microscale porosity and/or nanoscale structure, qualitatively enhancing the scattered reflectance, as illustrated in figure 5(C). The experiments revealed that altering the architecture of reflectin films across multiple length scales could provide nuanced control over their optical properties. Moreover, the authors observed analogous vapor pulsing-induced changes in the architectures and scattered reflectances of reflectin-type concatemer films, as illustrated in figure 5(D), underscoring the concatemers' ability to recapitulate the properties of the full length reflectins. In their totality, the findings suggested that reflectin-like biopolymers might ultimately inform and guide the design of artificial synthetic polymers with analogous stimuli-responsive optical functionalities.

Phan and co-workers leveraged the foundation established by the previous studies and used reflectins from other cephalopod species as camouflage materials [32]. For their work, the authors drew inspiration from the subcellular structures found within the tunable, color-changing iridocytes of some Loliginid species.

First, the authors established new protocols for the expression and purification of histidine-tagged *D. pealeii* reflectin A1 in *E. coli*, wherein the protein yield was improved by over an order of magnitude with regard to the previous reports. In turn, they optimized doctor blading of reflectin onto substrates treated with a graphene oxide adhesion layer, as illustrated in figure 6(A), producing coatings with excellent uniformity. The fabrication method furnished films like the one shown in figure 6(B), which featured a refractive index of ~1.5 and coloration that was again determined by the thickness. The authors subjected the films to an acetic acid vapor stimulus, which dramatically increased their thickness and reversibly modulated their reflectance from the visible to the near infrared regions of the electromagnetic spectrum, as illustrated in figure 6(C). The experiments demonstrated that single-layer reflectin films could be induced to both emulate and even expand upon the optical functionality of the far more structurally-sophisticated natural systems. In additional experiments, the authors used acetic acid vapor to switch the appearance of reflectin-coated substrates from visible to invisible under infrared imaging, as illustrated in figure 6(D), thereby emphasizing the concealment capabilities of reflectin-based materials. Overall, this work established a strategy for the production of reflectins in high yield and showcased the protein's utility for novel near-infrared adaptive camouflage devices.

Phan and co-workers further extended and improved upon their study of reflectin-based camouflage materials [33]. For this work, the authors
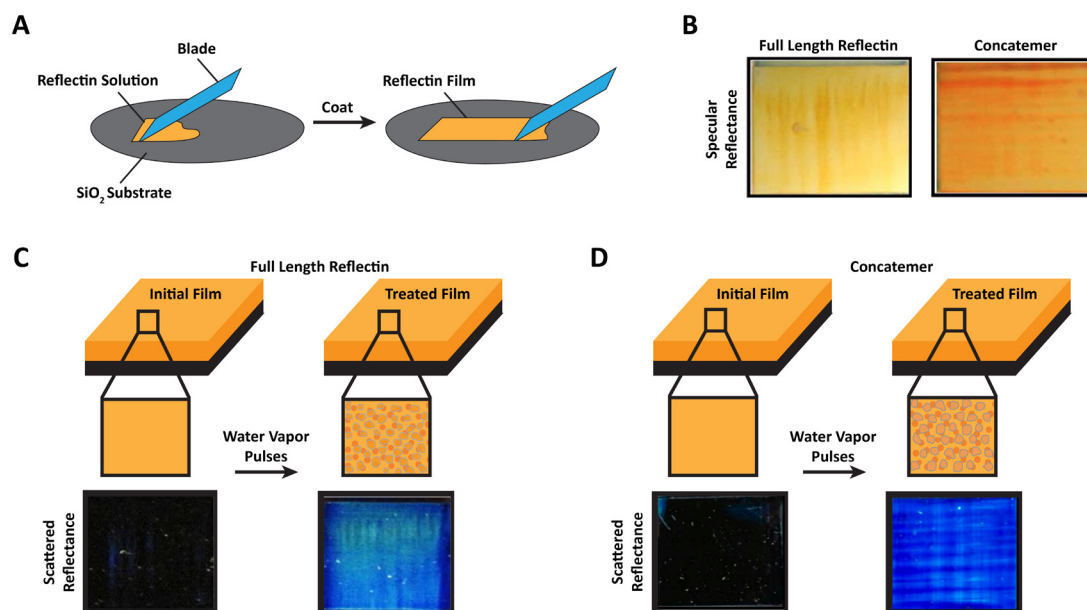
Figure 5. (A) An illustration of the flow coating of an *E. scolopes* reflectin 1b film onto a solid substrate. (B) Left: an optical image of a representative flow-coated *E. scolopes* reflectin 1b film. Right: an optical image of a representative flow-coated *E. scolopes* reflectin 1b-like concatemer film. (C) Top: an illustration of the irreversible modulation of a reflectin 1b film's microscale porosity and/or nanoscale structure with water vapor pulses. Bottom: the experimentally-observed scattered reflectance of a reflectin 1b film before (left) and after (right) the application of water vapor pulses. (D) Top: an illustration of the irreversible modulation of a reflectin 1b-like concatemer film's microscale porosity and/or nanoscale structure with water vapor pulses. Bottom: the experimentally-observed scattered reflectance of a reflectin 1b-like concatemer film before (left) and after (right) the application of water vapor pulses. Parts (B)–(D) were reproduced or adapted with permission from [31]. Copyright 2017, AIP Publishing, used in accordance with the Creative Commons Attribution (CC BY 4.0) license.
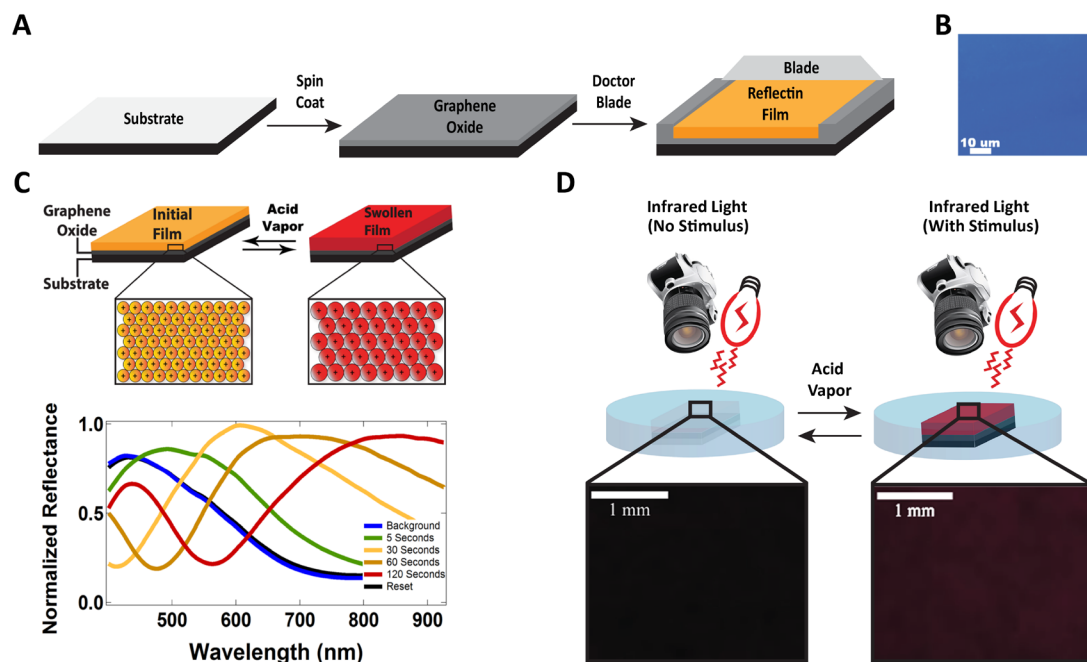
Figure 6. (A) An illustration of the doctor blading of a *D. pealeii* reflectin A1 film onto a graphene oxide-coated substrate. (B) An optical image of a representative doctor-bladed *D. pealeii* reflectin A1 film. (C) Top: an illustration of the reversible modulation of a reflectin film's coloration with an acetic acid stimulus. The acid vapor leads to an increase in the film thickness and a red shift in the coloration. Bottom: the experimentally-observed reversible time-dependent change in the reflectance spectrum of a reflectin A1 film due to the application and removal of an acetic acid vapor stimulus. (D) Top: an illustration of the reversible modulation of the infrared reflectance of a reflectin A1-coated substrate with acetic acid vapor under infrared imaging. Bottom: infrared images of a reflectin A1-coated substrate in the absence (left) and presence (right) of an acetic acid vapor stimulus. Parts (A)–(D) were reproduced or adapted with permission from [32]. Copyright 2013, Wiley.
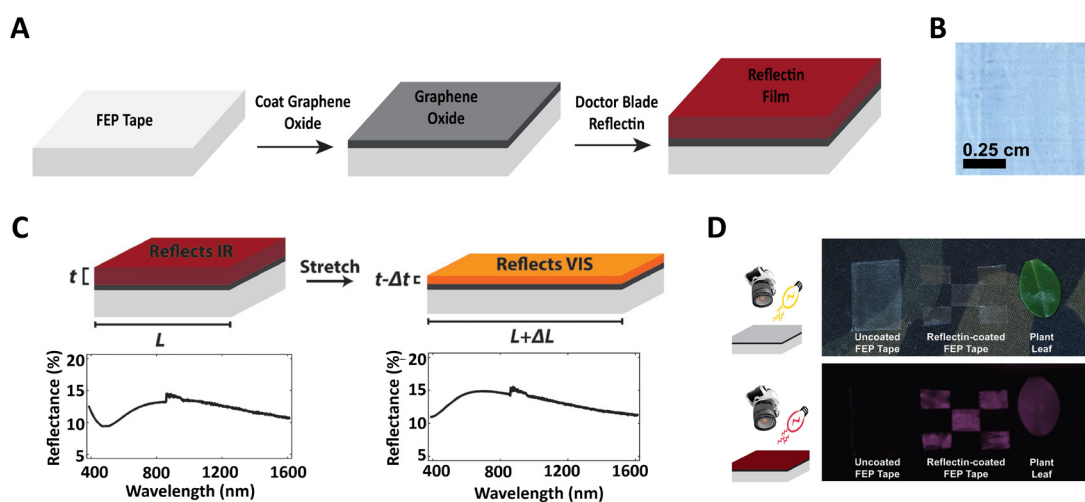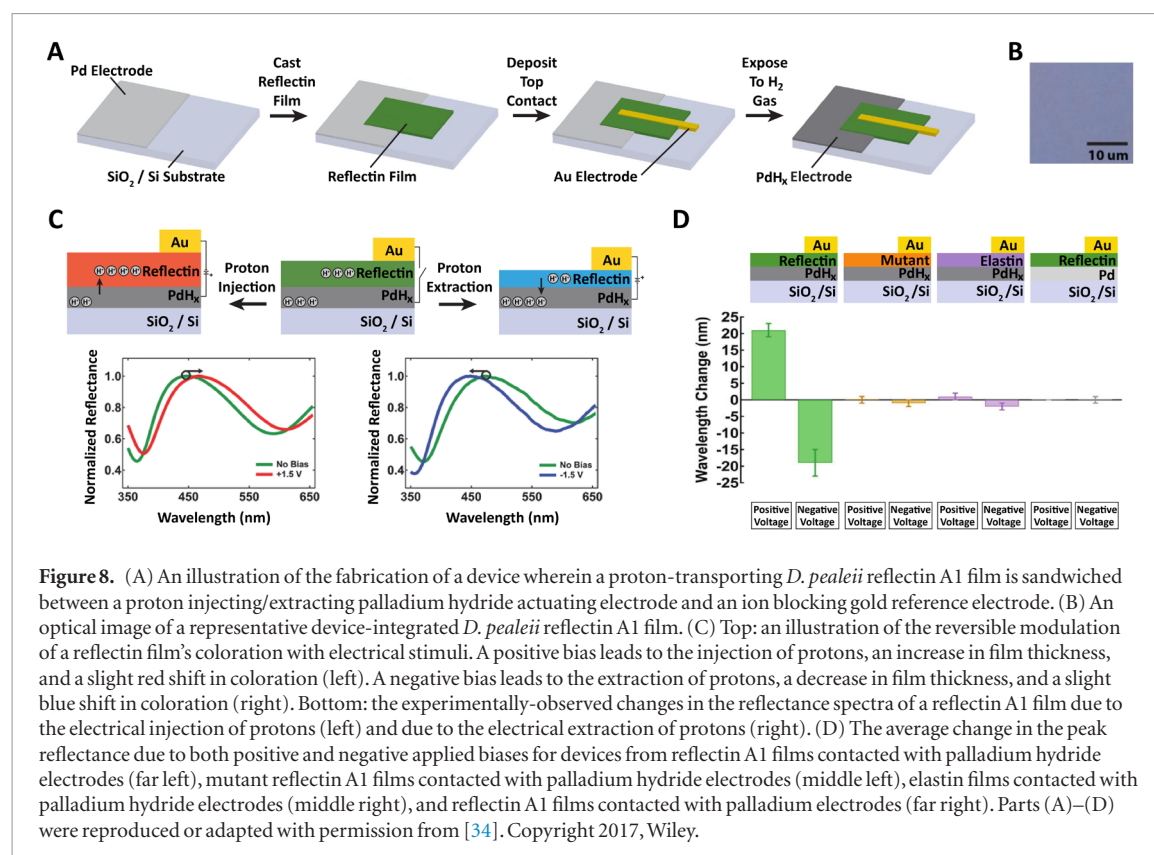
Figure 7. (A) An illustration of the doctor blading of a *D. pealeii* reflectin A1 film onto a graphene oxide-coated flexible fluorinated ethylene propylene (FEP) substrate. (B) An optical image of a representative doctor-bladed *D. pealeii* reflectin A1 film. (C) Top: an illustration of the reversible modulation of a reflectin film's coloration with a mechanical stimulus. An applied strain leads to a decrease in the film thickness and a blue shift in the coloration. Bottom: The experimentally-observed reflectance spectrum of a reflectin A1 film before (left) and after (right) the application of a uniaxial strain. (D) Top: an illustration of the optical imaging of a reflectin-coated substrate (left) and a corresponding image of a camouflage fatigue modified with an uncoated FEP tape rectangle, reflectin-coated FEP tape squares, and a plant leaf (right). Bottom: an illustration of the infrared imaging of a reflectin-coated substrate (left) and a corresponding image of a camouflage fatigue modified with an uncoated FEP tape rectangle, reflectin-coated FEP tape squares, and a plant leaf (right). Note that only the reflectin-coated tape and leaf are visible under infrared imaging. Parts (A)–(D) were reproduced or adapted with permission from [33]. Copyright 2015, Royal Society of Chemistry.

drew inspiration from mechanically-actuated chromatophore pigment cells that have been extensively characterized for many cephalopod species, including Loliginids. First, the authors produced histidine-tagged *D. pealeii* reflectin A1 in exceptional yields according to previously validated protocols, facilitating the throughput of the ensuing experiments. In turn, they used a modified doctor blading technique to deposit reflectin onto graphene oxide-modified sticky fluorinated ethylene propylene substrates (i.e. FEP tape), as illustrated in figure 7(A), readily producing uniform coatings over large areas. This procedure yielded films, like the one shown in figure 7(B), for which the coloration could be explained by thin film interference theory typically employed for oil films on water. The authors applied tension to the reflectin-coated tape, which decreased the thickness of the films and shifted their reflectance from the near infrared to the visible regions of the electromagnetic spectrum, as illustrated in figure 7(C). These experiments showed that the optical properties of reflectin-coated substrates could be controlled with straightforward mechanical stimuli. Furthermore, the authors adhered the reflectin-coated substrates onto fabrics, demonstrating that the materials changed the fabrics' appearance under infrared imaging, as illustrated in figure 7(D), and showcasing their deployment flexibility. Altogether, the observations indicated that reflectin-derived and reflectin-inspired materials were suitable for applications in fabric-integrated, mechanically-actuated, infrared camouflage systems.

Ordinario and co-workers built upon the literature precedent and developed more advanced color-chang-ing devices by harnessing the intrinsic protonic conductivity of reflectin films [34]. For their work, the authors drew inspiration from the electrical actuation of chromatophores and iridocytes within skin excised from some Loliginid species. First, the authors produced not only histidine-tagged *D. pealeii* reflectin A1, but also mutant reflectin A1 with a scrambled sequence in high yields. In turn, they prepared devices wherein a proton-conducting reflectin film was sandwiched between a proton injecting/extracting palladium hydride actuating electrode and an ion blocking gold reference electrode, as illustrated in figure 8(A). This fabrication methodology furnished device-integrated films, like the one shown in figure 8(B), for which the reflectance and coloration were dictated by the thickness. Here, the authors applied biases of different polarities to the devices, with positive voltages inducing the injection of protons and slightly red shifting the reflectance (due to an increase in thickness), and negative voltages inducing the extraction of protons and slightly blue shifting the reflectance (due to a decrease in film thickness), as illustrated in figure 8(C). The experiments revealed that the optical properties of reflectin-based devices could be modulated with electrical stimuli, albeit over a narrow wavelength range. Moreover, the authors observed negligible coloration changes for devices from proteins that did not effectively transport protons (e.g. mutant reflectin A1 and elastin) and for devices that lacked proton-injecting electrodes, as illustrated in figure 8(D), thereby underscoring the importance of the proton-transporting properties of reflectin films. In their totality, the findings suggested that reflectin-like materials could constitute a starting point for the devel-

Figure 8. (A) An illustration of the fabrication of a device wherein a proton-transporting *D. pealeii* reflectin A1 film is sandwiched between a proton injecting/extracting palladium hydride actuating electrode and an ion blocking gold reference electrode. (B) An optical image of a representative device-integrated *D. pealeii* reflectin A1 film. (C) Top: an illustration of the reversible modulation of a reflectin film's coloration with electrical stimuli. A positive bias leads to the injection of protons, an increase in film thickness, and a slight red shift in coloration (left). A negative bias leads to the extraction of protons, a decrease in film thickness, and a slight blue shift in coloration (right). Bottom: the experimentally-observed changes in the reflectance spectra of a reflectin A1 film due to the electrical injection of protons (left) and due to the electrical extraction of protons (right). (D) The average change in the peak reflectance due to both positive and negative applied biases for devices from reflectin A1 films contacted with palladium hydride electrodes (far left), mutant reflectin A1 films contacted with palladium hydride electrodes (middle left), elastin films contacted with palladium hydride electrodes (middle right), and reflectin A1 films contacted with palladium electrodes (far right). Parts (A)–(D) were reproduced or adapted with permission from [34]. Copyright 2017, Wiley.

opment of electrically-reconfigurable camouflage technologies that leverage proton conduction.

Discussion and future directions

To date, reflectins' diverse roles within the optically-active components of cephalopod skin *in vivo* have seemingly translated to a multi-faceted combination of properties *in vitro*. First, reflectins withstand fabrication techniques that would prove challenging for many other proteins, including spin coating, patterning, and direct metal depositions [20, 21, 26–29, 31–34]. Second, reflectins can be coaxed to self-assemble into a variety of nano-/micro-structures (e.g. nanoparticles, hexagonal plates, fibers, and gratings) when exposed to different environments or subjected to distinct stimuli [20–25, 31]. Third, squid reflectins (either alone in films or as part of larger hierarchical structures) feature refractive indices that are simultaneously high and tunable, and the coloration of various reflectin-based films/structures is readily modulated by chemical, mechanical, and electrical means [13, 20–25, 31–36]. Fourth, some squid reflectins possess electrical properties that are nearly on par with those of state-of-the-art artificial materials, enabling their application in unconventional bioelectronic devices like light-responsive protonic transistors [26–29]. Finally, certain reflectin isoforms appear compatible with other biological systems, as demonstrated by their ability to support human neural stem/progenitor cell attachment, proliferation, and differentiation [30]. When considered together, the above experiments underscore reflectins' unique

combination of properties and highlight the potential of these proteins for applications in a variety of advanced biomimetic technologies.

Despite the significant reported progress, the study of reflectins presents several challenges associated with their production and processing. For instance, during production, reflectins are typically heterologously expressed in bacteria, extracted via multiple steps, and purified with chromatography [20, 22, 23, 26, 31, 32]. However, due to reflectins' propensity for aggregation and/or precipitation (even under denaturing conditions) [24], the required protocols can often become painstaking and time-consuming. Furthermore, during processing, reflectins are usually solubilized in fluorinated solvents [20, 31], deionized water [22, 26, 32], or minimal buffers [23]. The polydispersity and long-term stability of these solutions can prove difficult to predict and control because many reflectin isoforms are exquisitely sensitive to changes in ionic strength, pH, or concentration [24]. In general, the continued optimization of improved protocols for the isolation of difficult-to-handle reflectins [29] and/or strategies for the stabilization of reflectin solutions with additives (e.g. surfactants) [25] will remain important for enabling many of the envisioned applications-oriented efforts.

The incomplete understanding of the relationship between reflectins' secondary/tertiary structures and self-assembly characteristics has also impeded their continued development as materials (although a number of studies have provided valuable insight in this regard). First, Kramer and co-workers have used x-ray diffraction to study fibers assembled from

E. scolopes reflectin 1a and have observed peaks consistent with the presence of some secondary structure (either alpha helices or beta sheets) for the fibers' constituent proteins [20]. In turn, Tao and co-workers have employed wide-angle x-ray scattering to investigate salt-aggregated nanoparticles from *D. pealeii* reflectin A1, finding peaks consistent with secondary structure (beta sheets) for the aggregated proteins [22]. Next, Levenson and co-workers have turned to theoretical modeling and fluorescence measurements for the interrogation of the assembly/disassembly of nanoparticles from *D. opalescens* reflectin A1 and have noted the emergence of reconfigurable secondary structure (transient alpha helices and/or beta sheets) within the proteins' conserved motifs during self-assembly [23]. Subsequently, Naughton and co-workers have leveraged deuterium exchange mass spectrometry and grazing-incidence wide-angle x-ray scattering to study solution-borne *D. pealeii* reflectin A1 nanoparticles and films, respectively, and they have discovered evidence for solvent protection of the conserved motifs along with signals characteristic of beta sheet character, supporting a connection between the motifs and the emergence of secondary structure for the proteins [24]. Finally, Guan and co-workers have leveraged cryo-electron microscopy and circular dichroism to study the assembly of various nanostructures from *S. officinalis* reflectin 2, with their measurements indicating a high proportion of beta sheets (but also some disorder) for the proteins and corroborating many of the previous observations [25]. Taken together, the above studies by multiple groups have laid important groundwork for further structural characterization of reflectins and have indicated that there exists a dynamic interplay between order and disorder for this class of proteins. Ultimately, the systematic elucidation of reflectins' structure-function relationships will likely prove critical for unlocking their full potential as materials for adaptive color-changing devices.

In the short term, reflectins' exciting *in vivo* roles and *in vitro* functionality have provided strong motivation for the continued investigation of this class of proteins. To date, a total of forty two reflectin sequences from various squid, octopus, and cuttlefish species (e.g. *E. scolopes*, *D. pealeii*, *D. opalescens*, *L. forbesii*, *O. bimaculoides*, and *S. officinalis*) have been deposited into the UniProt database [17, 37]. However, only some of these reflectins (e.g. *D. pealeii* reflectins A1 and A2; *D. opalescens* reflectins A1, A2, B1, and C; *E. scolopes* reflectins 1a and 1b; and *S. officinalis* reflectin 2) have been explored from the perspective of their materials properties [19–34]. Consequently, the study of the other reported reflectins, in tandem with the discovery of new variants from various cephalopod species, represents an exciting scientific opportunity for both newcomers and experts in the area. Importantly, such work would likely generate the knowledge base necessary for the continued rational design of unique reflectin-like biopolymers with customizable properties. In general, the systematic investigation of additional reflectins could not only provide additional insight into these proteins' biological roles but also expand the scope of their potential applications.

In the long term, the studies of rudimentary stimuli-responsive devices from reflectins have established a foundation for the engineering of more sophisticated hierarchical cephalopod-inspired technologies. For example, the periodic alternating arrangements of proteinaceous platelets and extracellular space found in some Loliginids' iridocytes exhibit dynamic Bragg-stack like functionality, wherein changes in the spacing, thickness, and refractive index of the reflectin-based platelets modulate the wavelength (and intensity) of the reflected light [11–13, 35, 36]. By drawing inspiration from these cells, it should be possible to design Bragg stacks in which every layer's physical properties are varied independently, thereby allowing for exquisitely-precise spatiotemporal control of the stacks' narrowband reflectances. Furthermore, the nanoparticle arrays embedded within the membranes of female *D. opalescens'* leucophores exhibit dynamic Lambertian surface-like behavior, wherein changes in the geometry and refractive index of the reflectin-based nanoparticles modulate the intensity of the diffusely reflected light [14, 15]. By looking to these cells, one could envision designing Lambertian surfaces for which all of the constituent arrayed nanoparticles are addressed individually and multiplexed, providing nanoscale control over the surfaces' broadband reflectances. These experiments represent just two of many possibilities and emphasize the value of reflectin-based systems for bridging the gap between basic and applied research in biophotonics.

In conclusion, we have provided an introduction to reconfigurable materials and devices from the reflectin family of proteins. We first described cephalopods' unique skin architecture and the optical functionality of cephalopod skin cells, which critically relies on reflectin-based subcellular structures. We then detailed and evaluated the case studies that explored reflectins as stimuli-responsive color-changing materials. We next summarized reflectins' multi-faceted and unique combination of properties. We in turn noted some remaining practical and scientific challenges associated with these proteins, as well as discussed potential opportunities with regard to reflectin-inspired materials and devices. Altogether, the future remains bright for reflectins as they light the way for unprecedented biologically-inspired adaptive optical technologies.

Acknowledgments

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