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Mimicking light-sensing chromophore in visual pigments and determination isomerization site

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ABSTRACT

Three retinal derivatives are designed and synthesized under the inspiration of natural visual pigments. The retinal derivative **V3** (retinal-phenylenediamine) is able to respond sensitively to visual light in the absence of a protein environment through isomerization and deprotonation. The response process is applied to verification of information security.

1. Introduction

At present, there are many classical systems of photochromism, such as azobenzenes, stilbenes, spiropyrans, diarylethenes, thiophenefulgides, hemithioindigos [1–4]. They have been widely used in the fields of light-controlled release, photo-controlled biological activity, chemical probes, high resolution optical imaging, etc [5]. The light acts as a trigger for molecular switching and it can be manipulated in situ with high spatial and temporal resolution, non-invasive characteristic [6]. However, the above photochromic systems inevitably use ultraviolet light as a trigger. As known, light in ultraviolet region shows poor penetration and greater phototoxicity [7,8]. Therefore, it is meaningful to develop a visible light-regulated photochromic systems.

The visible light-sensing chromophore retinal derivative in visual pigments is an ideal candidate for developing visible light-regulated photochromic system [9]. There are more than 300 photochemically active proteins in the living system with retinal as their photochromic chromophore [10]. Visual molecular biology studies have shown that retinal derivatives can be regulated by different protein environments to achieve response to different wavelengths of visible light [11]. Therefore, the retinal derivative is able to respond sensitively to visual light, which relies heavily on the protein environment [12].

In 2013, Berbasova et al. achieved pH colorimetric sensing by synthesizing more than ten proteins and interacting with retinal derivatives to display different colors at the same pH [13]. Since the retinal derivative absorption spectrum can be regulated in the human visual range, its color could be easily recognized by the human eye. However, the implementation of this process also shows the complexity of the application of retinal derivative. Retinal derivative has multiple sites for cis-trans isomerism due to its conjugated polyene structure (Fig. 1). The natural selection of 11-cis-retinal as the light-sensing chromophore in visual pigments was proved by Sekharan using a hybrid quantum mechanics/molecular mechanics method [14]. The electrostatic interaction between retinal and opsin dominates the natural selection of 11-cis-retinal over other cis isomers in the dark state.

Exploring the interaction between protein and retinal requires revealing the retinal's photoisomerization properties. But with the deepening of research, the application of retinal cannot be separated from the protein environment, which has great limitations. Both the molecular structure and environmental control of intramolecular energy flow can effectively regulate the photochemical properties of molecules [15,16]. Therefore, it is also theoretically feasible to regulate retinal's photochemistry and photophysical properties through modifying the molecular framework without protein environment. Sovdat and Bassolino et al. greatly improved the quantum efficiency of photoisomerization, which shortened the response time and regulated the position of photoisomerization by modifying the retinal skeleton [15,17].

In this study, different electron withdrawing and donor groups malononitrile, diaminomaleonitrile, and o-phenylenediamine are introduced at the end of retinal. These groups will take the place of proteins to regulate the charge distribution in retinal molecules. Next,

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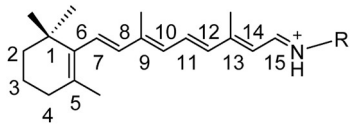


Fig. 1. Chemical structure of retinal derivatives.

the charge distribution effect on the photosensitivity of retinal will be investigated. The chemical modification and protein environment effects on cis-trans isomerization sites of retinal are quite different. Here copper ion coordination will be used for the detection of isomerization site.

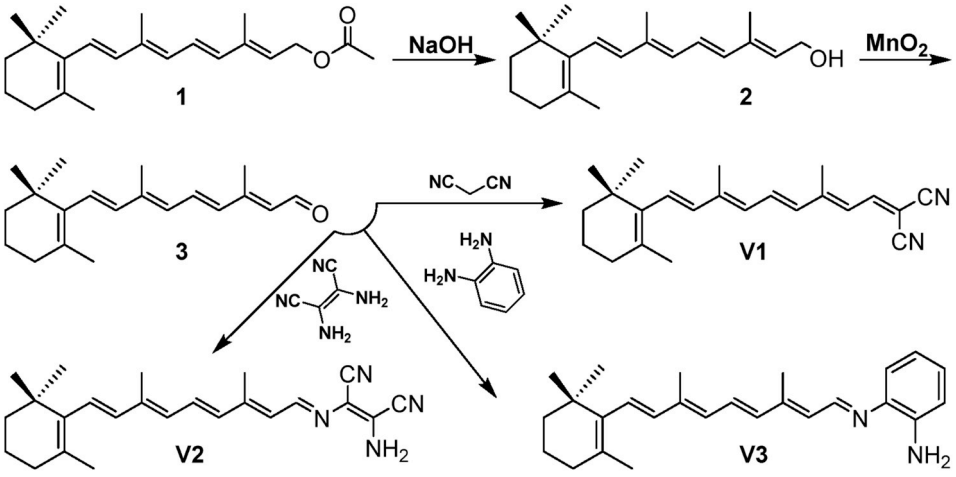
2. Experimental part

2.1. Reagents

Retinol acetate, MnO_2, o-Phenylenediamine were purchased from J&K Chemical Ltd. Tetrahydrofuran, ethyl acetate, acetonitrile, metal ions purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai). Other chemicals were provided from Sigma-Aldrich. All commercially purchased chemical reagent materials were used without further purification.

2.2. General method

^{1}H NMR (400 MHZ) and ^{13}C NMR (100 MHZ) spectra data were recorded on a Bruker UltrashiedTM 400 MHZ Plus nuclear magnetic resonance spectrometer using DMSO-d_6/$CDCl_3$ as solvent and tetramethylsilane as an internal standard. High-resolution mass spectra were carried out on a Bruker Micro TOF II 10257 instrument. Fourier transform infrared (FT-IR) spectra were performed by using NICOLET NEXUS 470 spectrometer (KBr discs) in the 4000-400 cm^{-1} region. UV–visible spectra were recorded on a Shimadzu UV-2600 spectrometer. Steady-state fluorescence spectra were recorded on Hitachi F-4600 spectrophotometers. The visible irradiations were carried out on a CHF-



Scheme 1. The synthesis route for **V1**, **V2**, **V3**.

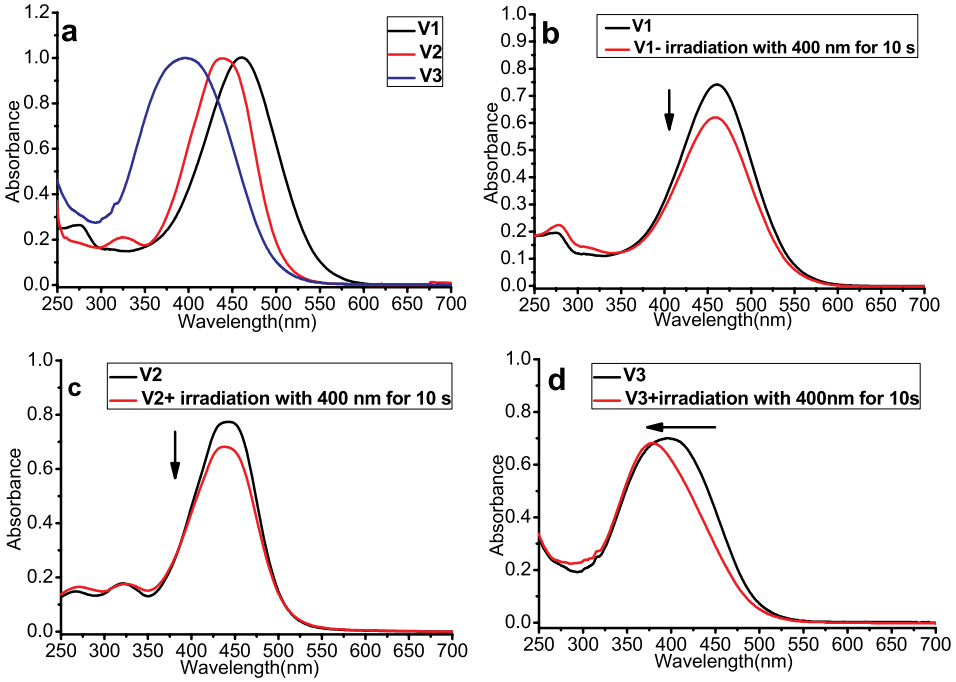


Fig. 2. Absorption spectra of **V1**, **V2**, **V3** (10 μmol/L) in acetonitrile.

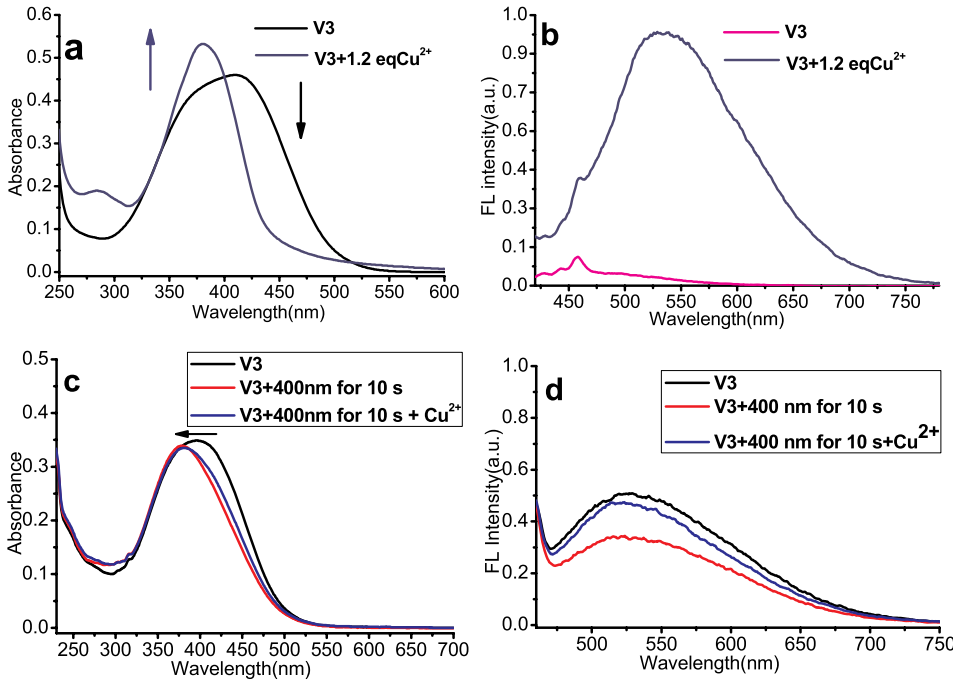


Fig. 3. Absorption spectra (a), and fluorescence spectra (b) of **V3** (10 μmol/L) with the addition of increasing concentrations of Cu^{2+} (0–12 μmol/L); Absorption spectra (c) and fluorescence spectra (d) of **V3** (10 μmol/L) with 400 nm light irradiation, on addition Cu^{2+} (10 μmol/L) in acetonitrile (Ex = 400 nm).

XM500W power system by using suitable band-pass filter.

3. Results and discussion

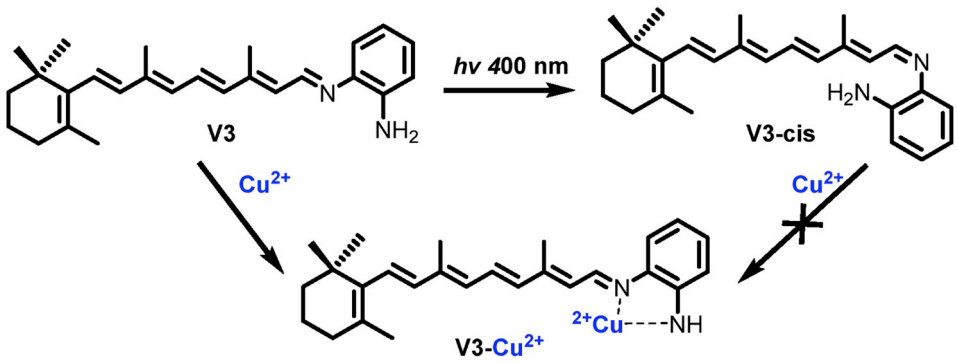
The synthetic route for retinal derivatives **V1** (retinal-malononitrile), **V2** (retinal-diaminomaleonitrile), **V3** (retinal-phenylenediamine) is outlined in Scheme 1. The commercially available retinol acetate **1** was hydrolyzed to produce retinol **2** according to the reported procedure [18]. Next, retinal **3** was prepared through oxidation of retinol **2** using MnO_2 as oxidant. The preparation of **V1** followed the Knoevenagel condensation reaction of retinal and malononitrile [19]. **V2**, **V3** were prepared though the condensation reaction of retinal **3** and diaminomaleonitrile, o-phenylenediamine, forming Schiff base [20]. The detail synthesis procedure andcharacterization information was provided in supporting information.

The electrostatic interaction between retinal and opsin dominates the charge distribution of retinal molecules in physiological environment. In absence of protein environment, different charge donor and receptor groups would dominate charge distribution of retinal molecules. As shown in Fig. 2a, the maximum absorption peaks of **V1**, **V2**, **V3** in acetonitrile shift to short wavelength in order, corresponding to their different electronic environment. From strong electron-withdrawing group malononitrile, to medium electron-electron-donor group diaminomaleonitrile, and strong electron-donor group o-phenylenediamine. The substituents vary led to retinal electron density shifting, polyene chain molecular orbitals energy transition. Therefore, the absorption spectra of **V1**, **V2**, **V3** showed regular changes. Among three molecules, three charge distribution states, which one is more suitable for photoisomerization?

The preliminary photochemical reaction experiment of **V1**, **V2** and **V3** was carried out in acetonitrile under ≥400 nm light irradiation. The absorption spectra were recorded before and after irradiation as shown in Fig. 2a, b, c. Upon irradiation with 400 nm light, the characteristic absorption peaks, **V1** and **V2** decreased, **V3** blue shifted. The chemical shifts of **V1** and **V2** didn't show regular changes, but new fragment peaks appeared in NMR spectrum (Fig. S5). Generally, photo-bleaching causes degradation of dye molecules leading to reduced absorption, dye molecules photoisomerization lead to absorption spectra shift [21]. The blue shift of ultraviolet absorption spectra is a typical feature of the transition from trans-structure to cis-structure [22]. Therefore, preliminary speculation is that only **V3** can respond to 400 nm light and isomerize. The retinal protonated Schiff bases with aromatic Schiff bases showing no isomerization of the retinal skeleton has been reported [17]. Based on above conclusion, immine isomerization was taken as a concern point. The NMR technique usually could provide required information, but the chemical shift is not significant enough in **V3** (Fig. S1). This is attributed to the fact that there is only one proton at both ends of imine C=N. And it is difficult to characterize the isomeric change with the change of the coupling constant [22].

In order to solve above problem, the unique metal ion coordination strategy was adopted to determine the photoisomerization reaction and site. The diaminomaleonitrile and o-phenylenediamine in **V2** and **V3** are classical coordination groups of copper ion, which were used as recognition groups in copper ion chemical probe [20,23]. As shown in Fig. 3a-3b, as the quantitative Cu^{2+} was added to V3 solution, the absorption peak regularly blue shift to short wavelength area; The fluorescence emission peak gradually increased. The isosbestic point at 400 nm indicated the existence of an equilibrium between **V3** and **V3**–Cu^{2+} complex. To further explore the binding mechanism in detail, the infrared spectrum of **V3** and **V3**–Cu^{2+} were overlaid and compared (Fig. S2). Firstly, two N–H stretching vibration peaks of the N–H_2 group at 3369 cm^{-1} and 3474 cm^{-1} appeared in the free **V3** molecule. Secondly, after the N–H_2 group on phenylene diamino binded with Cu^{2+}, the two stretching vibration peaks of N–H_2 group disappeared. Instead, the single stretching vibration of the N–H at 3428 cm^{-1} appeared in the coordination complex **V3**–Cu^{2+}.

The same experiments were carried out after 400 nm light irradiation. As shown in Fig. 3c, d, the absorption peak of **V3** exhibited minimal shift when Cu^{2+} was added. Similarly, the fluorescence emission peak of **V3** also didn't enhance, indicating that the photoisomerization reaction of **V3** occurred in the coordination unit. The isomerized coordination group can no longer coordinate with copper ions. Another convincing proof is that the **V2** still could respond to Cu^{2+} after 400 nm light irradiation. As shown in Fig. S3, The absorption and fluorescence spectra



Scheme. 2. The photoisomerization and coordination routes for **V3**.

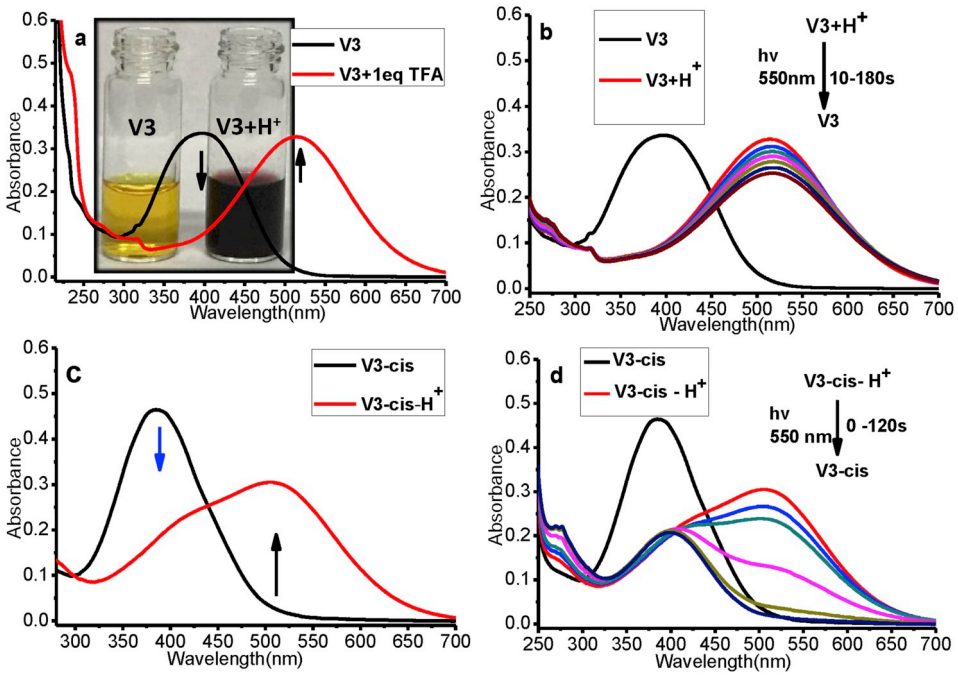
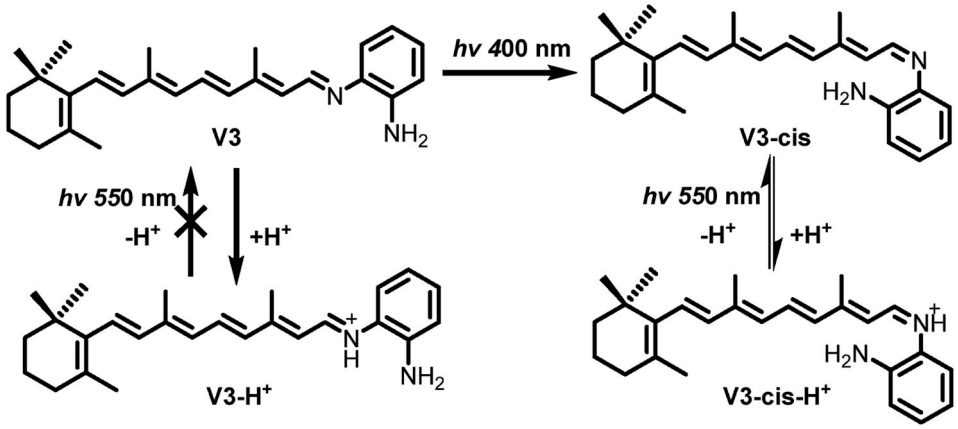


Fig. 4. Absorption spectra of **V3**, protonated **V3** (a) and **V3-cis**, protonated **V3-cis** (c); Absorption spectra of protonated **V3** (c) and protonated **V3-cis** (d) under ≥550 nm light irradiation.

of **V2** maintained the same response trend to copper ions before and after 400 nm irradiation. It means that the coordination group of **V2** kept stable without photoisomerization. The detailed coordination and isomerization process of **V3** is shown in Scheme 2.

The absorption spectra range of **V3** in neutral state is 300–500 nm (Fig. 2d) without completely covering the visible range. In protein



Scheme. 3. Photoisomerization, protonation and deprotonation routs for **V3**.

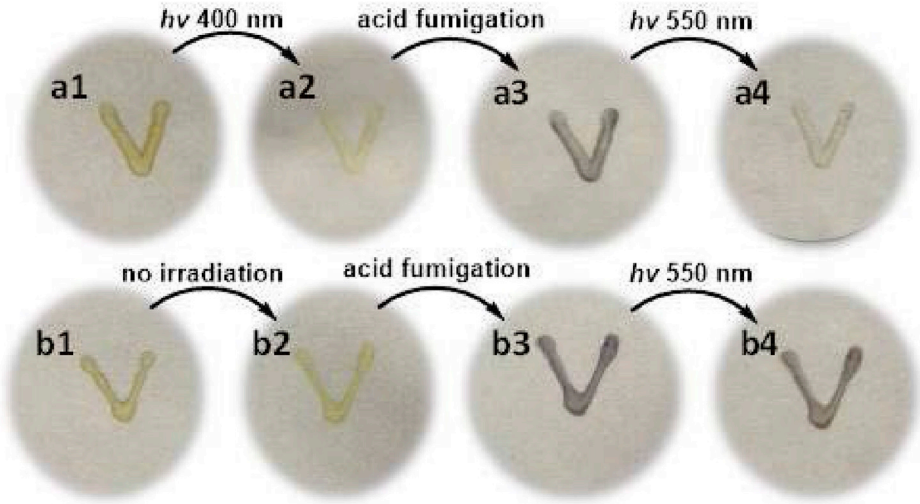


Fig. 5. Letters written with **V3** solution (**a1**, **b1**), **a2** was irradiated with 400 nm light for 2 min, **b2** without irradiation, **a3** and **b3** were acidified with fumigation, **a4** and **b4** were irradiated with 550 nm within 2 min.

environment, retinal protonated Schiff bases (Fig. 1) extend the absorption spectrum to 700 nm [24,25]. In the absence of a protein environment, the absorption of protonated **V3** and **V3-cis** completely covered the visible range 400–700 nm (Fig. 4a, 4c). Protonated/deprotonated imine of retinylidene chromophore trigging a large change in the absorption has been reported and proved [13]. The absorption change of protonated **V3** and **V3-cis** is just an ordinary example. Surprisingly, the protonated **V3-cis** is able to respond sensitively to 550 nm light, which show that the absorption spectra have a tendency to return to deprotonated state (Fig. 4d). Protonated **V3-cis** efficiently undergo a light induced deprotonation reaction to theirs neutral form, with the concomitant release of a proton to the environment. Compared with Fig. 4b and d, protonated **V3** has no response to 550 nm light, showing minimal degradation caused by photobleaching. It may be attributed to the steric hindrance effect of **V3** is indistinctively, which is advantageous to the stable existence of proton. The Photo-controlled deprotonization process was further proven by ^{1}H NMR spectroscopy. The ^{1}HNMR of protonated **V3-cis** changed dramatically as a whole before and after 550 nm irradiation. Especially, the proton chemical shift of H–C=N shifted to downfield after irradiation (Fig. S4b inset). The ^{1}H NMR of protonated **V3** kept stable before and after 550 nm irradiation. The detailed protonation, deprotonation process of **V3** and **V3-cis** is shown in Scheme 3. Therefore, protonated **V3-cis** are excellent candidates for photo-acid generator [26].

Chromotropic molecular materials are widely used in information storage and encryption [27,28]. Responsible measures can be taken quickly when information disclosure is discovered in time. Here, the chromism of **V3** is applied to verification of information security. Photoisomerization occurred once **V3** was irradiated with 400 nm light for 2 min. The photoisomerized product was acidified to be black and less stable, which would fade within 2 min of exposure at 550 nm (Fig. 5 a1-a4). This means that the information has been read under natural light containing 400 nm. The letter without 400 nm irradiation would remain stable and not fade (Fig. 5 b1-b4). This means that the letters have been properly preserved and been not exposed for long time.

4. Conclusions

In summary, this work mimicked the response mechanism of visual pigments in the protein environment, and constructed three light-sensing chromophores with different electronic distribution states based on retinal. In the absence of a protein environment, the sensitive response to visible light was achieved by cis-trans isomerization and protonation, deprotonation. The response mechanism similar to that in the protein environment has been confirmed by copper ion coordination. A preliminary application based on **V3**, information security verification is also implemented. Furthermore, this work provided an important reference for the application of retinal derivatives in the absence of a protein environment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Yang Li: Validation, Formal analysis, Investigation, Data curation. **Haichuang Lan:** Writing - original draft, Writing - review & editing, Supervision, Funding acquisition, Conceptualization. **Xia Yan:** Formal analysis. **Xiaotao Shi:** Funding acquisition. **Xiao Liu:** Funding acquisition. **Shuzhang Xiao:** Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dyepig.2019.108177.

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