**An Amidochlorin-Based Colorimetric Fluorescent Probe for Selective Cu\(^{2+}\) Detection**

Wenting Li †, Guohua Zhu †, Jinhua Li †, Zhiqiang Wang * and Yingxue Jin *

Received: 5 December 2015; Accepted: 14 January 2016; Published: 18 January 2016

**Abstract:** The design and synthesis of selective and sensitive chemosensors for the quantification of environmentally and biologically important ionic species has attracted widespread attention. Amidochlorin p6 (ACP); an effective colorimetric and fluorescent probe for copper ions (Cu\(^{2+}\)) in aqueous solution derived from methyl pheophorbide-a (MPa) was designed and synthesized. A remarkable color change from pale yellow to blue was easily observed by the naked eye upon addition of Cu\(^{2+}\); and a fluorescence quenching was also determined. The research of fluorescent quenching of ACP-Cu\(^{2+}\) complexation showed the detection limit was 7.5 \(\times\) 10^{-8} mol/L; which suggested that ACP can act as a high sensitive probe for Cu\(^{2+}\) and can be used to quantitatively detect low levels of Cu\(^{2+}\) in aqueous solution. In aqueous solution the probe exhibits excellent selectivity and sensitivity toward Cu\(^{2+}\) ions over other metal ions (M = Zn\(^{2+}\); Ni\(^{2+}\); Ba\(^{2+}\); Ag\(^{+}\); Co\(^{2+}\); Na\(^{+}\); K\(^{+}\); Mg\(^{2+}\); Cd\(^{2+}\); Pb\(^{2+}\); Mn\(^{2+}\); Fe\(^{3+}\); and Ca\(^{2+}\)). The obvious change from pale yellow to blue upon the addition of Cu\(^{2+}\) could make it a suitable “naked eye” indicator for Cu\(^{2+}\).

**Keywords:** fluorescent probe; copper ions; chlorophyll

### 1. Introduction

The design and synthesis of selective and sensitive chemosensors for the quantification of environmentally and biologically important ionic species has attracted widespread attention [1]. Among ionic species, copper is one of the important pollution sources [2]. As a common heavy metal existing widely in Nature and all living organisms, an appropriate amount of copper ion is essential to living organisms because it is a key constituent of the respiratory enzyme complex cytochrome c oxidase [3]. However, excess copper ion may cause physical discomfort and sometimes life-threatening illness [4–7]. Therefore the determination of heavy metal content in living organisms and the environment is particularly important.

Because chromo- or fluoroionophores are highly effective for these determinations, given their easy handling and the simple equipment required, effort has been expended to develop optical chemosensors that selectively respond to the Cu\(^{2+}\) ion. Various methods have been developed in the past decades to determine Cu\(^{2+}\) ion content, such as spectrophotometric [8–10], electrochemical (EM) [11–13], inductively coupled plasma atomic emission spectrometric (ICP-AES) [14–17], atomic absorption spectroscopic (AAS) [18,19] and fluorescence methods [20–22]. Among them, the fluorescence method utilizes a specific chemical reaction between dosimeter molecules and the target species to form a fluorescent or colored product. Thus, high selectivity toward the probe is an advantage of chemodosimeters, making them useful for detecting Cu\(^{2+}\) ions. Meanwhile, as...
paramagnetic Cu(II) ion has a strong ability to quench fluorescence, recent years have seen a growing interest in the development of fluorescent probes for Cu<sup>2+</sup> with different chemical transducers, such as rhodamine and semiconductor quantum dot-based probes [23–27]. Although rhodamine dyes are widely used as fluorescent probes owing to their high photostabilities, high extinction coefficients, and high fluorescent quantum yields, their structural instability in strong acid or base media (pH < 4 or pH > 9) has limited their applications [28]. Semiconductor quantum dots (QDs) have also emerged as an important class of inorganic nanomaterial that affords promising potential in the ion-detection field, yet QDs probes cannot be applied under alkaline conditions, while the morphology, size and surface defects of the nanocrystals could influence the detection sensitivity [29].

Therefore, the search for new fluorescence probes with sufficient high sensitivity and a wide application range is still an active field as well as a challenge for the analytical chemistry community. Recently, porphyrins have gained widely attention for their good photophysical properties with large Stokes shifts and relatively long excitation (>400 nm) and emission (>600 nm) wavelengths that minimize the effects of the background fluorescence [30]. A newly reported pyro-pheophorbide-a methyl ester (PPME) could selectively complex with Cu<sup>2+</sup> ions, leading to a distinct change in its absorption spectrum as well as efficient fluorescence quenching [31]. However, the association rate between PPME and Cu<sup>2+</sup> is very slow, and no systematic research on quantitative detection of Cu<sup>2+</sup> has been performed. Moreover, the poor water-solubility of PPME had limited its application in sensing Cu<sup>2+</sup> in aqueous solution, hence improving the water-solubility is necessary and desirable. In this paper, a new chlorophyll-based Cu<sup>2+</sup> fluorescent probe, amidochlorin p6 (ACP), was designed and synthesized. (Scheme 1) Two flexible side chains with hydrophilic hydroxyl groups were introduced to improve the water-solubility of the designed molecule, while the hydroxyl groups may also serve as ligand binding sites to chelate heavy metals. ACP has large absorption, strong fluorescence and a relatively long emission wavelength in visible region, displaying high selectivity for Cu<sup>2+</sup> in aqueous solution among the metal ions examined, with a low detection limit in a wide pH range of 1 to 12. Moreover, ACP exhibited marked fluorescence quenching upon the binding of Cu<sup>2+</sup> ion, thus it has potential applied value for rapid detection of Cu<sup>2+</sup> in aqueous solution.

![Scheme 1. The proposed synthesis of amidochlorin p6.](image)

2. Results and Discussion

2.1. Chemistry

Methyl pyropheophorbide a (Mpa) was synthesized according to the literature procedure [32]. Then propanolamine (1 mL) was introduced to Mpa through a aminolysis reaction of the methyl ester to give the title compound ACP.

2.2. Recognition of Metal Ion

To verify its metal ion sensing abilities, ACP was titrated over a wide range of metal ions, such as Cu<sup>2+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Ba<sup>2+</sup>, Ag<sup>+</sup>, Co<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Cd<sup>2+</sup>, Pd<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>3+</sup>, and Ca<sup>2+</sup>. Stock
1 mM solutions of metallic ions were prepared by dissolving the appropriate salts in doubly distilled water, respectively, and then diluting to a lower concentration of 10 µM. Meanwhile, a stock 1 mM solution of ACP was also prepared in ethanol, and then diluted to a lower concentration of 10 µM. In brief, to a 10 mL volumetric flask, 100 µL of the stock solution (1 mM) of ACP was added, followed by addition of 100 µL of different metal ions stock solutions, the mixtures were diluted to lower concentrations by addition of 50% ethanol (v/v) solution. As a control, the same procedure was performed but in the absence of Cu²⁺.

2.3. Spectral Titration of ACP with Cu²⁺

Copper is a quenching metal ion and the coordination of ACP with Cu²⁺ would quench the fluorescence of ACP. The UV-Vis and fluorescence titration experiments of ACP with Cu²⁺ were performed in 50% ethanol (v/v) solution. Figure 1a shows the UV-visible absorption spectrum of ACP. ACP absorbs throughout the ultraviolet region into the visible region between about 400 and 800 nm with four peaks: a strong Soret absorption peak at 399 nm, two weak absorption peaks at 499 nm and 605.5 nm, and a Qy peak at 660.5 nm. The absorption of ACP is highly affected by the presence of Cu²⁺ ions. Upon addition of Cu²⁺ ions, the absorption intensity of Soret peak at 399 nm decreased with a little red shift, and the peak at 499 nm also decreased, with no peak shift. Meanwhile, the Qy peak gradually reduced in intensity with the formation of a new absorption peak at about 632 nm and with the formation of an isosbestic point at 652 nm. When the concentration of Cu²⁺ increased to the same level as ACP, the Qy peak disappeared yet the absorption intensity at 632 nm reached a maximum. The change of ACP absorption spectra demonstrated the complexation between ACP and Cu²⁺. The value of the shift is indicative of the degree of the interaction between the fluorophore and the bound Cu²⁺. To study the binding stoichiometry of ACP and Cu²⁺, a Job’s plot experiment was carried out by using the UV-Vis absorbance spectrum at 632 nm. Keeping the sum of the initial concentration of Cu²⁺ and ACP at 10 µM, increasing the concentration of Cu²⁺ from 0 to 1. The maximum absorbance occurred when the [Cu²⁺]/[[ACP]+[Cu²⁺]] reached at 0.5.

Figure 1. (a) The absorption spectrum of ACP in water/ethanol (v/v = 50/50) solution (10 µM) with added Cu²⁺; (b) Job’s plot according to the method for continuous variations (the total concentration of ACP and Cu²⁺ is 10 µM. The absorbance was measured at 632 nm; (c) Mole ratio plot for stoichiometric ratio between ACP (10 µM each) and Cu²⁺.
This observation indicates that ACP and Cu\(^{2+}\) formed at 1:1 ratio complex. In order to verify this, the mole ratio plot for stoichiometric ratio between ACP (10 µM each) and Cu\(^{2+}\) was measured. As can be seen from Figure 1c, the molar ratio of ACP to Cu\(^{2+}\) was 1:1.

The fluorescence titration of Cu\(^{2+}\) was carried out using a solution of 10 µM ACP in ethanol, using 412 nm as excitation wavelength. As illustrated in Figure 2a, the fluorescence intensity of ACP decreases with increasing concentration of Cu\(^{2+}\), which constitutes the basis for the determination of Cu\(^{2+}\) with the fluorescent probe proposed in this paper. Moreover, it can be seen from Figure 2b that the fluorescence intensity at 632 nm showed a linear quenching with the increasing addition of Cu\(^{2+}\). The fluorescent response of ACP toward Cu\(^{2+}\) was calculated to cover a linear range from 1 to 10 µM. The linear equation was \(y = -45.66x + 546.27\) (\(R^2 = 0.999\)), where \(y\) is the fluorescence intensity at 668 nm measured at a given Cu\(^{2+}\) concentration and \(x\) is the concentration of Cu\(^{2+}\) added. The detection limit of Cu\(^{2+}\) is \(7.5 \times 10^{-8}\) mol/L, which is lower than the limit of Cu\(^{2+}\) in drinking water (~20 µM) demanded by U.S. Environmental Protection Agency. This result showed that ACP is sensitive enough to monitor the concentration of Cu\(^{2+}\) in drinking water.

![Figure 2](image-url)  
**Figure 2.** (a) Fluorescence spectra of [ACP-Cu\(^{2+}\)] with emission wavelength 668 nm; (b) The relation between ACP fluorescence intensity and the concentration of Cu\(^{2+}\).

2.4. Selectivity and Interference Studies

Selectivity is a very important parameter to evaluate the performance of a probe. Development of chemosensors with “naked eye” capability has an advantage over traditional fluorescence sensors because they do not need cumbersome labor and a sophisticated instruments [33]. The selectivity of ACP toward Cu\(^{2+}\) and the interference of a number of common ions with the determination of Cu\(^{2+}\) were investigated. The experiments were carried out by fixing the concentration of Cu\(^{2+}\) at 10 µM and then recording the change of the UV-Vis absorbance and fluorescence intensity before and after adding the interferent into the Cu\(^{2+}\) solution (Figure 3). In the presence of other tested metal ions (Zn\(^{2+}\), Ni\(^{2+}\), Ba\(^{2+}\), Ag\(^{+}\), Co\(^{2+}\), Na\(^{+}\), K\(^{+}\), Mg\(^{2+}\), Cd\(^{2+}\), Pd\(^{2+}\), Mn\(^{2+}\), Fe\(^{3+}\), and Ca\(^{2+}\)), the UV-Vis absorbance spectra showed almost no obvious change relative to the free ligand ACP, and the absorbance of ACP was only slightly influenced by the addition of other ions (Figure 3a). When 1 equiv. of Cu\(^{2+}\) and selected metal ions (10 µM) was added into the solution of ACP (10 µM), many of the investigated metal ions do not interfere with detection of Cu\(^{2+}\). The data in Figure 4 clearly reveals that the addition of other common metal ions can hardly affect the fluorescence response of ACP towards Cu\(^{2+}\). There are only slight interfering effects of Mg\(^{2+}\) and Cd\(^{2+}\). These results clearly suggest that the probe ACP shows a high anti-interference ability against other potentially coexisting metal ions.

Furthermore, upon addition of the same amount of the various metal ions, respectively, only Cu\(^{2+}\) induced a striking color change from pale yellow to blue, as observed by the naked eye (Figure 3a). Those observations indicate that ACP has a high selectivity to Cu\(^{2+}\) and can be a good colorimetric sensor for Cu\(^{2+}\) ions. Moreover, upon addition of Cu\(^{2+}\) and selected metal ions (10 µM), only Cu\(^{2+}\) showed distinct quenching (Figure 3a,b), which suggested that ACP can be a selective fluorescent sensor for Cu\(^{2+}\) ions. It’s worth mentioning that upon addition of Cu\(^{2+}\), the color of ACP changed

...
much faster than PPME that previously reported in the literature [31]. In brief, our proposed probe shows extraordinary selectivity to Cu\(^{2+}\) and could meet the selectivity requirements for biomedical and environmental applications.

Figure 3. (a) UV-Vis absorption and (b) fluorescence emission spectra of ACP (10 µM) upon addition of various metal ions (10 µM) in water/ethanol (v/v = 50/50) solution. The color changes of ACP (10 µM) upon addition 1 equiv. of various metal ions under natural light and UV-Vis are also displayed.

Figure 4. The relative fluorescence intensity diagram of ACP (10 µM) to different metal ions (1 equiv.). Excitation was at 412 nm, and emission was at 668 nm.

2.5. Spike and Recovery Test

Spike and recovery test was conducted in tap water to examine whether there is any positive or negative interference in real drinking water samples. We first examined the effect of tap water on the fluorescence stability and found no quenching effect. The local tap water was filtered first through filter paper to remove any insoluble suspended solids. The recovery study was carried out on a mixture of tap water and the interferent into the Cu\(^{2+}\) solution (Figure 3). In the presence of other tested metal ions (Zn\(^{2+}\), Ni\(^{2+}\), Co\(^{2+}\), Pb\(^{2+}\), Ag\(^{+}\), and Hg\(^{2+}\)), only Cu\(^{2+}\) induced a striking color change from pale yellow to blue, as observed by the naked eye (Figure 3a). The relative fluorescence intensity diagram of ACP (10 µM) toward Cu\(^{2+}\) was calculated to cover a linear range from 1 to 10 µM. The linear equation obtained in Figure 3. The analysis results for the sample water and ethanol (1:1, v/v) which was spiked with 2, 5 and 8 µM Cu\(^{2+}\). Each experiment was done in quintuplicate and the average was presented with relative standard deviation. The contents of Cu\(^{2+}\) were recovered using the linear equation obtained in Figure 3. The analysis results for the sample with spiked Cu\(^{2+}\) were given in Table 1. The result showed that the method had a good recovery at the concentration test, suggesting no serious positive or negative interferences for selectively and sensitively determining copper(II) ion in real water samples.

Table 1. Recovery test of Cu\(^{2+}\) in tap water.

| Tap Water Sample | Cu\(^{2+}\) Added (µM) | Cu\(^{2+}\) Found (µM) | RSD (%\(\times n = 5\)) | Recovery (%) |
|------------------|-----------------------|------------------------|------------------------|--------------|
| Sample 1         | 2                     | 2.242                  | 3.75                   | 112.1        |
| Sample 2         | 5                     | 5.198                  | 1.90                   | 104.0        |
| Sample 3         | 8                     | 8.404                  | 1.43                   | 105.1        |

\(^1\) Values shown were the calculated mean Cu\(^{2+}\) for each sample.
2.6. Effect of pH

The spectroscopic characters of the probe were studied in the pH range 2–13 in sodium acetate-acetic acid buffer solution. Figure 5 shows the fluorescence response of ACP toward Cu\(^{2+}\) in the pH range. The fluorescence intensities of the mixture were very high in the pH range 2–4, yet the fluorescence emission (\(\lambda_{ex}/\lambda_{em} = 561/580\) nm) drastically decreases in pH up to 5 and varies slightly until 11. This may be attributed to the fact that H\(^+\) and Cu\(^{2+}\) competitively bind to ACP in acid solutions, consequently the formation of Cu\(^{2+}\)-ACP complexes are inhibited, thus the mixture displayed high fluorescence intensities in the pH range 2–4. Moreover, in the pH range 11–13 the mixture possess very high fluorescence emission, which is most probably due to the fact that in strongly alkaline solutions OH\(^-\) and ACP competitively bind to Cu\(^{2+}\). The more alkaline of the buffer solution is, the more liable it is to form Cu(OH)\(^4\)\(^-\), and the more difficult it is to form Cu\(^{2+}\)-ACP complexes. Therefore, the Cu\(^{2+}\)-ACP complexes are stable only in the pH range 6–11.

![Figure 5](https://example.com/figure5.png)

**Figure 5.** The effect of different pH values on the spectroscopic characteristics of Cu\(^{2+}\)-ACP.

2.7. Binding Mechanism

As a new kind of porphyrin, ACP is endowed with a cyclic \(\pi\)-aromatic system and exhibits unique coordination chemistry. Owing to the four pyrrole units of ACP, Cu\(^{2+}\) would coordinate with pyrrole N atoms in a square planar shape. We have simulated the ACP-Cu\(^{2+}\) complex through density functional theory (DFT) calculations with the Becke-3-Lee-Yang-Parr (B3LYP) exchange function using the Gaussian 09 package [34]. The 6-31G (d, p) basis sets were used except for Cu\(^{2+}\), where a LANL2DZ effective core potential (ECP) was employed. Figure 6 represents the molecular geometry optimization according to the 1:1 binding stoichiometry of ACP with the Cu\(^{2+}\) ion. The atom distances of N1-N3 and N2-N4 in ACP were 4.272 and 4.115 Å (Figure 6a), respectively, yet in ACP-Cu\(^{2+}\) complex their distances decreased to 3.999 and 3.776 Å, respectively, which can be attributed to the fact that the electron-donating N atom of pyrrole rings have high affinity to bind to Cu\(^{2+}\) with short bond lengths (shown in Figure 6c). Moreover, the four pyrrole rings in ACP are almost planar, yet during the formation of ACP-Cu\(^{2+}\) complex, the Cu\(^{2+}\) ions occupy the coordination center of ACP and the molecular plane was slightly contorted and metamorphosed due to the formation of coordination bonds and steric strain (Figure 6d). According to the experimental results, the ACP-Cu\(^{2+}\) complex exhibits an absorption at 632 nm compared with the absorption at 660.5 for ACP. This can be easily explained by the above mentioned phenomena: the introduction of Cu\(^{2+}\) distorts the conjugate plane of ACP molecule, and to some extent destroys the conjugated \(\pi\)-bond of the four pyrrole rings, thus leading to a blue-shift in the UV-visible absorption spectrum of ACP-Cu\(^{2+}\).

In addition, we analysed the frontier molecular orbitals (FMO's) of bare ACP and the ACP-Cu\(^{2+}\) complex. This will help us to understand the quenching phenomenon upon addition of Cu\(^{2+}\) ions. The calculated FMO's are shown in Figure 7.
were destroyed, resulting in a colour change and fluorescence quenching; the other because of the paramagnetic nature of Cu$^{2+}$. These results support our expectation that ACP could serve as a sensitive fluorescent probe as well as a naked-eye probe for Cu$^{2+}$.

In the absence of Cu$^{2+}$, the solution of ACP is pale yellow and fluorescent, and from the figure it is seen that the electron density in HOMO and LUMO are both localized on the pyrrole rings, mainly involving the $\pi-\pi^*$ electronic transitions of conjugated $\pi$-bonds. Upon the addition of Cu$^{2+}$ into the ACP solution, the HOMO of ACP-Cu$^{2+}$ is distributed over the pyrrole rings, Cu$^{2+}$ and amide in the side chain, while in the LUMO the electron density is mainly localized on the pyrrole rings. This is mainly involved in the charge-transfer (CT) from Cu$^{2+}$ and amide in the side chain to the pyrrole rings. Therefore, the quenching phenomenon may be explained by two factors: one is that the coordination of Cu$^{2+}$ to ACP decreases the electron-donating ability of the nitrogen atoms of ACP, and to some extent the conjugated-$\pi$ bond, whereby the most important ultraviolet absorption and fluorescence were destroyed, resulting in a colour change and fluorescence quenching; the other because of the paramagnetic nature of Cu$^{2+}$. These results support our expectation that ACP could serve as a sensitive fluorescent probe as well as a naked-eye probe for Cu$^{2+}$.

Figure 6. Energy-minimized structures by DFT calculations: (a) viewed from the front for ACP; (b) viewed from the side for ACP; (c) viewed from the front for ACP-Cu$^{2+}$ complex; (d) viewed from the side for ACP-Cu$^{2+}$ complex.

Figure 7. Frontier molecular orbitals of ACP and ACP-Cu$^{2+}$ complex (1:1) obtained at B3LYP/6-31G(d, p) and B3LYP/LANL2DZ level, respectively.
3. Experimental Section

3.1. General Information

All chemicals used in this paper were obtained from commercial suppliers and used without further purification. Ultrapure water was used for aqueous solution preparation. All samples were prepared at room temperature and promptly used for UV-Vis and fluorescence determination. Zinc chloride (98%), copper(II) chloride dihydrate (99%), nickel(II) chloride hexahydrate (98%), barium chloride (99.5%), silver nitrate (99.8%), cobalt(II) chloride hexahydrate (90%), sodium chloride (99.5%), potassium chloride (99.5%), magnesium chloride hexahydrate (98%), cadmium chloride (99%), lead(II) nitrate (99%), ferric chloride hexahydrate (99%) and calcium chloride anhydrous (96%) were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All the chemical reactions were performed under argon protection and away from sunshine.

$^1$H-NMR and $^{13}$C-NMR spectra were recorded at 400 and 100 MHz, respectively, on an AMX400 spectrometer (Bruker, Bremen, Germany) with tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded with a VG-7070 spectrometer (Hitachi, Manchester, UK). UV-Vis absorption and emission spectra were recorded using a UV-160A spectrophotometer (Shimadzu, Kyoto, Japan) and spectrofluorophotometer with a 150 W xenon lamp as a visible excitation light source (RF-5301PC, Shimadzu), respectively. All measurements were made at room temperature (about 25 °C). All spectra were obtained in a quartz cuvette (path length = 1 cm). The excitation and emission slit widths were both 10 nm, and PMT voltage of 700 V. The fluorescence intensities/spectra were measured at $\lambda_{ex}/em = 412/668$ nm.

3.2. General Procedure for Synthesis of the title Compound

Methyl pyropheophorbide a (Mpa) was synthesized according to the literature procedure [32]. Then propanolamine (1 mL) was added to a solution of Mpa (66.73 mg, 0.11 mmol) in chloroform and the reaction stirred under a nitrogen atmosphere for 24 h at rt. The reaction mixture was then concentrated, and the residue was dispersed in dichloromethane (30 mL), and then washed by water (30 mL) for three times. After drying and evaporation of the solvent, the residue was purified by silica gel chromatography with methanol: dichloromethane (1:15) as the eluent to give pure ACP (76%).

$^1$H-NMR (CDCl$_3$) $\delta$ (ppm): 1.00~1.10 (m, 2H, 13$^4$-CH$_2$), 1.26~1.28 (m, 2H, 15$^5$-CH$_2$), 1.67 (t, $J = 7.6$ Hz, 3H, 18$^3$-CH$_3$), 1.68 (d, $J = 7.2$ Hz, 3H, 18-CH$_3$), 1.73~1.81 (m, 2H, 15$^4$-CH$_2$), 1.86~1.91 (b, 2H, 13$^3$-CH$_2$), 2.22~2.28 (m, 2H, 172-CH$_2$), 2.41~2.45 (m, 2H, 17$^1$-CH$_2$), 3.27 (s, 3H, 7-CH$_3$), 3.48 (s, 3H, 2-CH$_3$), 3.51 (s, 3H, 12-CH$_3$), 3.68 (s, 3H, 17$^3$-OCH$_3$), 3.72 (q, $J = 3.76$ Hz, 2H, 8$^1$-CH$_2$), 3.75~3.88 (m, 4H, 13$^5$-CH$_2$), 4.31 (b, 1H, 17-H), 4.35 (q, $J = 7.2$ Hz, 1H, 18-H), 4.46 (d, $J = 7.2$ Hz, 3H, 18-CH$_3$), 5.29 (d, $J = 18.5$ Hz, 1H, 15-H), 5.39 (d, $J = 18.5$ Hz, 1H, 15-H), 6.13 (d, $J = 2.8$ Hz, 1H, 3$^2$-H (Z)), 6.33 (d, $J = 2.8$ Hz, 1H, 3$^2$-H (E)), 7.32 (bs, 1H, 15$^3$-NH), 8.09 (dd, $J_1 = 6.0$ Hz, $J_2 = 11.6$ Hz, 1H 3$^1$-H)), 8.81 (s, 1H, 20-H), 9.62 (s, 1H, 10-H), 9.64(s, 1H, 5-H); $^{13}$C-NMR (MeOD) $\delta$ (ppm): 10.0, 11.5, 11.8, 17.8, 19.8, 23.5, 29.5, 31.8, 32.0, 33.2, 33.3, 33.9, 37.4, 38.6, 38.8, 50.4, 52.8, 54.4, 56.0, 60.4, 60.9, 70.5, 94.7, 98.9, 101.8, 103.5, 121.2, 129.5, 130.5, 130.9, 135.1, 135.6, 136.1, 136.8, 139.6, 145.3, 149.8, 154.8, 171.8, 175.1, 175.2. Anal calc'd for C$_{41}$H$_{52}$N$_6$O$_6$: C 67.93, H 7.23, N 11.59; found C 67.78, H 7.46, N 11.28.

4. Conclusions

In summary, we have prepared ACP, a simple but effective colorimetric and fluorescent probe for Cu$^{2+}$ detection, from methyl pheophorbide-a. It shows excellent sensitivity and selectivity for Cu$^{2+}$ over other common metal ions in aqueous media. More importantly, the color change upon the addition of Cu$^{2+}$ to ACP solutions could make it a suitable “naked eye” indicator for Cu$^{2+}$. Meanwhile, our study of the fluorescence quenching of ACP-Cu$^{2+}$ complex showed the detection limit was $7.5 \times 10^{-8}$ mol/L, which suggested that ACP can act as a highly sensitive probe for Cu$^{2+}$ and can be used to quantitatively detect low levels of Cu$^{2+}$ in aqueous solution.
Acknowledgments: This work was supported by the National Nature Science Foundation of China (No. 20972036, 21272048) and the Program for Scientific Technological Innovation Team Construction in Universities of Heilongjiang Province (No. 211TD010). The theoretical calculations were conducted on the ScGrid and Deepcomp7000 the Supercomputing Center, Computer Network Information Center of Chinese Academy of Sciences.

Author Contributions: Yingxue Jin and Zhiqiang Wang conceived and designed the experiments; Guohua Zhu and Wenting Li performed the experiments; Guohua Zhu and Jinghua Li analyzed the data; Yingxue Jin contributed reagents/materials/analysis tools; Zhiqiang Wang wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Quang, D.T.; Kim, J.S. Fluoro- and chromogenic chemodosimeters for heavy metal ion detection in solution and biospecimens. Chem. Rev. 2010, 110, 6280–6301. [CrossRef] [PubMed]
2. Duffus, J.H. “Heavy metals” a meaningless term? (IUPAC Technical Report). Pure Appl. Chem. 2002, 74, 793–807. [CrossRef]
3. Malvankar, P.L.; Shinde, V.M. N,N-dibromodiethylbarbituric acid as an analytical reagent. Part 1. Determination of some pharmaceutically important hydrazine derivatives. Analyst 1991, 116, 1081–1085. [CrossRef] [PubMed]
4. Malik, A. Metal bioremediation through growing cells. Environ. Int. 2004, 30, 261–278. [CrossRef] [PubMed]
5. Fatemi, N.; Sarkar, B. Molecular mechanism of copper transport in Wilson disease. Environ. Health Perspect. 2002, 110, 695–698. [CrossRef] [PubMed]
6. Gaggelli, E.; Kozlowski, H.; Valensin, D.; Valensin, G. Copper homeostasis and neurodegenerative disorders (Alzheimer’s, prion, and Parkinson’s diseases and amyotrophic lateral sclerosis. Chem. Rev. 2006, 106, 1995–2044. [CrossRef] [PubMed]
7. Lovell, M.A.; Robertson, J.D.; Teesdale, W.J.; Campbell, J.L.; Markesbery, W.R. Copper, iron and zinc in Alzheimer’s disease senile plaques. J. Neurol. Sci. 1998, 158, 47–52. [CrossRef]
8. Sabel, C.E.; Neureuther, J.M.; Siemann, S. A spectrophotometric method for the determination of zinc, copper, and cobalt ions in metalloproteins using Zincon. Anal. Biochem. 2010, 397, 218–226. [CrossRef] [PubMed]
9. Yamini, Y.; Tamaddon, A. Solid-phase extraction and spectrophotometric determination of trace amounts of copper in water samples. Talanta 1999, 49, 119–124. [CrossRef] [PubMed]
10. DeWitt, R.; Watters, J.I. Spectrophotometric investigation of a mixed complex of copper(II) ion with oxalate ion and ethylenediamine. J. Am. Chem. Soc. 1954, 76, 3810–3814. [CrossRef]
11. Grujicic, D.; Pesci, B. Electrodeposition of copper: the nucleation mechanisms. Electrochim. Acta 2002, 47, 2901–2912. [CrossRef]
12. Bond, A.M.; Wallace, G.G. Simultaneous determination of copper, nickel, cobalt, chromium(VI), and chromium(III) by liquid chromatography with electrochemical detection. Anal. Chem. 1982, 54, 1706–1712. [CrossRef]
13. Etienne, A. Electrochemical method to measure the copper ionic diffusivity in a copper sulfide scale electrochemical science—Technical papers. J. Electrochem. Soc. 1970, 117, 870–874. [CrossRef]
14. Otero, R.J.; Moreda, P.A.; Bermejo, B.A.; Bermejo, B.P. Evaluation of commercial C18 cartridges for trace elements solid phase extraction from seawater followed by inductively coupled plasma-optical emission spectrometry determination. Anal. Chim. Acta 2005, 536, 213–218. [CrossRef]
15. Rao, K.S.; Balaji, T.; Rao, T.P.; Babu, Y.; Naidu, G.R.K. Determination of iron, cobalt, nickel, manganese, zinc, copper, cadmium and lead in human hair by inductively coupled plasma-atomic emission spectrometry. Spectrochim. Acta B 2002, 57, 1333–1338. [CrossRef]
16. Murillo, M.; Benzo, Z.; Marcano, E.; Gomez, C.; Garaboto, A.; Marin, C. Determination of copper, iron and nickel in edible oils using emulsified solutions by ICP-AES. J. Anal. At. Spectrom. 1999, 14, 815–820. [CrossRef]
17. Rahil-Khazen, R.; Bolann, B.J.; Myking, A.; Ulvik, R.J. Multi-element analysis of trace element levels in human autopsy tissues by using inductively coupled plasma atomic emission spectrometry technique (ICP-AES). J. Trace Elem. Med. Biol. 2002, 16, 15–25. [CrossRef]
18. Pourreza, N.; Hoveizavi, R. Simultaneous preconcentration of Cu, Fe and Pb as methylthymol blue complexes on naphthalene adsorbent and flame atomic absorption determination. *Anal. Chim. Acta* 2005, 549, 124–128. [CrossRef] 

19. Ghaedi, M.; Ahmadi, F.; Shokrollahi, A. Simultaneous preconcentration and determination of copper, nickel, cobalt and lead ions content by flame atomic absorption spectrometry. *J. Hazard. Mater.* 2007, 142, 272–278. [CrossRef] [PubMed] 

20. Aksuner, N.; Henden, E.; Yilmaz, I.; Cukurovali, A. A highly sensitive and selective fluorescent sensor for the determination of copper(II) based on a schiff base. *Dyes Pigments* 2009, 83, 211–217. [CrossRef] 

21. Li, Y.; Zhang, X.; Zhu, B.; Xue, J.; Zhu, Z.; Tan, W. A simple but highly sensitive and selective colorimetric and fluorescent probe for Cu^{2+} in aqueous media. *Analyst* 2011, 136, 1124–1128. [CrossRef] [PubMed] 

22. Shao, N.; Zhang, Y.; Cheung, S.M.; Yang, R.H.; Chan, W.H.; Mo, T.; Li, K.A.; Li, F. Copper ion-selective fluorescent sensor based on the inner filter effect using a spiropyran derivative. *Anal. Chem.* 2005, 77, 7294–7303. [CrossRef] [PubMed] 

23. Dujols, V.; Ford, F.; Czarnik, A.W. A long-wavelength fluorescent chemodosimeter selective for Cu^{2+} ion in water. *J. Am. Chem. Soc.* 1997, 119, 7386–7387. [CrossRef] 

24. Kumar, M.; Kumar, N.; Bhalla, V.; Sharma, P.R.; Kaur, T. Highly Selective fluorescence turn-on chemodosimeter based on rhodamine for nanomolar detection of copper ions. *Org. Lett.* 2012, 14, 406–409. [CrossRef] [PubMed] 

25. Nose, K.; Fujita, H.; Omata, T. Chemical role of amines in the colloidal synthesis of CaSe quantum dots and their luminescence properties. *Luminescence* 2007, 126, 21–26. 

26. Chen, Y.F.; Rosenzweig, Z. Luminescent Cds quantum sots as selective ion probes. *Anal. Chem.* 2002, 74, 5132–5138. [CrossRef] [PubMed] 

27. Lee, M.H.; Kim, H.; Yoon, S.; Park, N.; Kim, J.S. Metal ion induced FRET OFF-ON in tren/dansyl-appended rhodamine. *Org. Lett.* 2008, 10, 213–216. [CrossRef] [PubMed] 

28. Emaus, R.K.; Grunwald, R.; Lemasters, J.J. Rhodamine 123 as a probe of transmembrane potential in isolated rat-liver mitochondria: Spectral and metabolic properties. *Biochim. Biophys. Acta Bioenerg.* 1986, 850, 436–448. [CrossRef] 

29. Gaponik, N.; Talapin, D.V.; Rogach, A.L.; Hoppe, K.; Shevehenko, E.V.; Kornowski, A.; Eychmuller, A.; Weller, H. Thiol-capping of CaTe nanocrystals: An alternative to organometallic synthetic routes. *J. Phys. Chem. B* 2002, 106, 7177–7185. 

30. Luo, H.-Y.; Zhang, X.-B.; Jiang, J.-H.; Li, C.-Y.; Peng, J.; Shen, G.-L.; Yu, R.-Q. An optode sensor for Cu^{2+} with high selectivity based on porphyrin derivative appended with bipyridine. *Anal. Sci.* 2007, 23, 551–555. [CrossRef] [PubMed] 

31. Ghosh, I.; Saleh, N.; Nau, W.M. Selective time-resolved binding of copper(II) by pyropheophorbide-amethyl ester. *Photochem. Photobiol. Sci.* 2010, 9, 649–654. [CrossRef] [PubMed] 

32. Wang, J.; Ji, W.-Y.; Han, G.-F.; Wu, X.-R.; Wang, L.M.; Shen, R.J. Synthesis of 3-alkylolyl-3-devinyl-methyl pyropheophorbide-a and the effect of the peripheral carbonyl groups on the 1H-NMR and the visible spectra. *Acta Chim. Sin.* 2004, 62, 302–311. 

33. Caballero, A.; Espinosa, A.; Taftraga, A.; Molina, P. Ferrocene-based small molecules for dual-channel sensing of heavy- and transition-metal cations. *J. Org. Chem.* 2008, 73, 5489–5497. [CrossRef] [PubMed] 

34. Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G.A.; et al. *Gaussian 09, Revision C.01*; Gaussian, Inc.: Wallingford, CT, USA, 2010. 

**Sample Availability:** Samples of the compound ACP are available from the authors.

© 2016 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons by Attribution (CC-BY) license (http://creativecommons.org/licenses/by/4.0/).