### **Diiminic Schiff Bases: An Intriguing Class of Compounds for a Copper-**Nanoparticle-Induced Fluorescence Study

Mainak Ganguly,<sup>[a]</sup> Anjali Pal,<sup>[b]</sup> Yuichi Negishi,<sup>[c]</sup> and Tarasankar Pal<sup>\*[a]</sup>

Abstract: The condensation products of salicylaldehyde and different diamines constitute an important class of diiminic Schiff bases (DSBs). This class of compounds has been rediscovered as reducing as well as capping agents under UV irradiation. UV irradiation of alkaline DSB solutions in the presence of water-soluble copper salts has been employed to produce copper nanoparticles (CuNPs). Intriguing

Keywords: copper · diimines · fluorescence · nanoparticles · Schiff bases

### Introduction

Recently, metal nanoparticles have attracted immense attention in the area of fluorescence spectroscopy in which the particles behave as nanoantennas. The nanoparticles frequently manipulate light and light-matter interactions at the nanoscale. The radiative decay rate of emitters placed in their near field<sup>[1-3]</sup> can easily be influenced owing to the existence of localized plasmon polaritons of metal nanoparticles. Mohammadi et al. studied nanoantennas from spheroidal metal nanoparticles as a function of aspect ratio, volume, background index, and metal.<sup>[4]</sup> Gold and silver nanostructures have also been established as nanoantennas. Owing to the cost of generating gold and silver surfaces, these substrates are unlikely to find widespread metal-enhanced fluorescence (MEF) applications. It is an academic as well as practical challenge to work with stable copper nanoparticles as they are generally susceptible to oxidation.

In biological research, including single-molecule detection, cellular imaging, gene profiling, proteomics, drug discovery, and disease diagnostics, fluorescence techniques have increasingly found applications. Fluorescent markers

[a] M. Ganguly, Prof. T. Pal Department of Chemistry Indian Institute of Technology, Kharagpur Kharagpur-721302, West Bengal (India) Fax: (+91)03222-282252 E-mail: tpal@chem.iitkgp.ernet.in

[b] Dr. A. Pal Department of Civil Engineering Indian Institute of Technology, Kharagpur Kharagpur-721302, West Bengal (India)

[c] Dr. Y. Negishi Department of Applied Chemistry Tokyo University of Science Tokyo-1628601 (Japan)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201201242.

separation distance, molecular dipole orientation with respect to the particle surface, and size of the nanoparticle.[9-11] It was found that comparatively smaller (<30 nm) gold

nanoparticles quench the fluorescence emission owing to the nonradiative transfer from the excited states of luminophore molecules to the gold nanoparticles.<sup>[10]</sup> The nanoparticle scattering efficiency is increased for bigger particles, which results in enhanced fluorescence.<sup>[9-12]</sup> The proposition of Lukomska et al.<sup>[13]</sup> that larger aggregated colloids enhance the fluorescence to a larger extent than smaller ones further

Intense narrow surface plasmon bands (SPBs) have provoked researchers to study silver and gold nanoparticles in

the context of MEF. Gold nanoparticles can enhance or

quench fluorescence depending on the fluorophore-particle

Copper possesses a very large value of the imaginary component of dielectric constant (more than twice) than that of noble metals in the wavelength range of 300 to 600 nm. So, it is expected that in this wavelength range due

Chem. Eur. J. 2012, 18, 15845-15855

© 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

supports this.

🕅 WILEY 师

15845

CuNP-stimulated fluorescence behavior of the solution has been observed. Depending upon the nature of the spacer in between two iminic bonds, fluorescence enhancement or quenching is observed. Such surprising fluorescence contrast has been ascribed to farfield radiation and lossy surface waves.

are very commonly introduced for labeling and cell imaging. Weak fluorescence signals from a low concentration of fluorophores attached to the molecules as well as the photostability of molecular fluorophores are two major drawbacks of this technique.<sup>[5]</sup> Proximal conducting metallic particles, colloids, or surfaces are well known to notably control the emission behavior of luminophores.<sup>[6]</sup> By exploiting metalnanoparticle platforms, the quantum yield and photostability of weakly fluorescing molecules are increased. This is caused by the improved emission efficiency, radiative decay rate, and/or coupling of the emission with far-field through scattering. Thus, for improved surface immunoassay and DNA detection, enhanced wavelength ratiometric sensing, amplified assay detection, and so forth, MEF is very promising.<sup>[7]</sup> On the contrary, quenching by metallic nanoparticles is also fruitfully employed for competitive fluorescence immunoassays, hybridization assays, optical detection of DNA hybridization, and in optoelectronics.<sup>[8]</sup>

to higher ohmic losses, copper nanoparticles will mostly quench the luminescence at their close proximity.<sup>[14]</sup> But for large nanoparticles (>100 nm) with high aspect ratio, the increase of coupling efficiency of the fluorescence emission to the far field through nanoparticle scattering excels MEF. In this case, as effect of ohmic losses on MEF is decreased, less expensive metals such as Al and Cu, which are more lossy than Ag and Au, can be considered as promising candidates for enhancing fluorescence.<sup>[15]</sup>

Salen<sup>[16]</sup> (the condensation product of salicylaldehyde and diamines) and salen-like molecules in which two imine bonds are separated from each other with an aliphatic or aromatic group have been found to act as a potential fluorophore for many applications. Liu et al.<sup>[17]</sup> reported Mn-salen as a promising probe molecule to detect traces of DNA in solution because of the prominent alteration of the fluorescence property of the molecule. A remarkable decrease of fluorescence intensity is noticed for Mn-Schiff bases bound to DNA with a blueshift of excitation and emission peaks. Other reports are available in the literature in which the fluorescence properties of salen, salen derivatives, and their metal complexes were exploited for the determination of trace amounts of hazardous or useful substances, namely, Mg,<sup>[18]</sup> H<sub>2</sub>O<sub>2</sub>, triacetone organic peroxides,<sup>[19]</sup> and cyanide.<sup>[20]</sup> The determination of aliphatic primary amines by flow-injection fluorometry using beryllium-Schiff base complexes has been performed by Akoi et al.<sup>[21]</sup> Very recently, an attempt to make a silver sensor was reported from our laboratory.<sup>[22]</sup> We present herein a very interesting observation for copper-enhanced fluorescence spectroscopy again by using salen and salen derivatives. The spacer (the aromatic/aliphatic group in between two iminic bonds) determines the extent of the enhancement/quenching, which is quantified for the first time with photoproduced copper nanoparticles (CuNPs) in the solution phase.

For the synthesis of CuNPs, many methods can be adopted: use of supercritical carbon dioxide,<sup>[23,24]</sup> water-in-oil microemulsions,<sup>[25]</sup> high-temperature decomposition of organometallic precursors,<sup>[26]</sup> a polyol reduction method,<sup>[27]</sup> and a photochemical route.<sup>[28]</sup> Herein, we report the synthesis of CuNPs by means of a reproducible photoactivation technique using salen and salen-like Schiff bases (DSBs). The evolution strategy for CuNPs is endowed with high fluorescence enhancement of the exposed reaction mixtures on the one hand and quenching of the fluorescence on the other. The contrast of the luminescence behavior depends upon the spacers (the groups in between the two iminic bonds) of the DSBs.

Two mechanisms have been anticipated for fluorescence enhancement: 1) localized surface plasmon resonance (LSPR) at the surface of the metal NPs causing enhancement of the electromagnetic field and 2) coupling between the surface plasmon field of the metal and the molecular dipole of probe molecules.<sup>[29]</sup> The radiating plasmon (RP) model suggests that the far-field radiation originated from scattering for it to be responsible for the observed fluorescence intensity.<sup>[30]</sup> The extent of the enhancement lies in the geometry of the metallic nanostructures. The size and shape contribute to different surface plasmonic modes with drastic enhancement of the fluorescence signals at "hotspots".<sup>[31]</sup> At very close proximity (<20 nm), the lifetime of the fluorophore drops dramatically and the emission intensity is strongly reduced. This quenching effect in the proximity of metal nanoparticles has been ascribed to lossy surface waves (LSWs), dissipated losses, ohmic losses, and such similar phenomena, all of which are examples of nonradiative dissipation of energy within metals.

#### **Results and Discussion**

Six DSBs containing different spacers in between two imine bonds have been synthesized (Figure 1). It is known that they form coordination complexes with Cu<sup>II</sup>. In the present study, we describe the synthesis of CuNPs using a photo-



Figure 1. Different DSBs synthesized from salicylaldehyde and different diamines.

chemical route for the first time. As a result of the synthetic procedure, we have found interesting fluorescence contrast behavior from CuNPs bearing exposed DSBs with variable spacers.

Nine hours of irradiation of an alkaline reaction mixture of a copper salt in the presence of salen or salen-like Schiff bases, that is, DSBs, causes the formation of CuNPs under UV light. The in situ generated CuNPs exhibit strikingly different fluorescence behavior out of the exposed reaction mixture. An irradiation time of more than nine hours shows a constant fluorescence intensity of the reaction mixture, that is, the fluorescence or absorption property does not change further. Figure 2 represents the formation kinetics of CuNPs (from fluorescence studies).

DSB acts as both a reducing and a stabilizing agent since no other reducing agent and/or stabilizer is introduced into the reaction mixture. We presume semiquinone formation from the phenolic OH group, through the same mechanism of nanoparticle synthesis in the presence of DSB as pro-

15846





Figure 2. Formation kinetics of CuNPs under UV irradiation ( $\approx$ 365 nm) in the presence of C<sub>2</sub> observed from fluorescence measurements. Conditions: C<sub>2</sub> concentration=1.66×10<sup>-4</sup> M; CuSO<sub>4</sub> concentration=83×10<sup>-6</sup> M;  $\lambda_{ex}$ =290 nm.

posed by Selvakannan et al.<sup>[32]</sup> Phenolate ions lose electrons, which in turn reduce Cu<sup>II</sup> to Cu<sup>0</sup>. UV light provides the necessary activation energy for the productive photoactivation process. Without UV irradiation, quenching of fluorescence due to the formation of cupric hydroxide in the alkaline medium occurs. Visible light of variable wavelengths, for example, 450, 550, and 650 nm, shows a similar type of quenching. However, it has been found that only UV irradiation ( $\approx$  365 nm) causes the drastic change in fluorescence (Figure 3).

A new peak in the UV-visible spectrum at approximately 300 nm (see the Supporting Information, Figure S1) and an approximately 52 nm blueshifted fluorescence spectrum ( $\lambda_{cm} = 449$  nm) of the alkaline DSB solution after UV exposure are due to the formation of semiquinone. The blueshift of 20 nm of the emission maxima of the exposed C<sub>2</sub> in the presence of photoproduced CuNPs has been observed as proposed by Geddess and Lakowicz due to polarity considerations.<sup>[33]</sup> From the mass spectra (Figure S2 in the Supporting Information), we observed the molecular-ion peak of C<sub>2</sub> at *m*/*z* 282 before and after UV-light irradiation in the presence and absence of copper salts. The observed molecular-ion peak speaks against the formation of a dimer (molecular weight × 2) or quinone (molecular weight + 32).

The oxidation state of the photoproduced CuNPs has been confirmed by X-ray photoelectron spectroscopy (XPS; Figure 4) studies. The peaks at 932.66 and 952.72 eV are assigned to  $2p_{3/2}$  and  $2p_{1/2}$ , respectively (and no shake-up peak) when copper is in a zero oxidation state. TEM images of the photoproduced CuNPs capped with exposed C<sub>2</sub> reveal that the particles are spherical with diameters of 3– 6 nm. The lattice fringe of 0.208 nm for the [111] plane and the selected-area electron diffraction (SAED) image shown in Figure 5 further support the zero oxidation state of Cu<sup>0</sup>. The TEM images of CuNPs that are capped with other capping agents (C<sub>1</sub>, C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub>, and C<sub>6</sub>) are shown in Figure S3 in the Supporting Information.

The alkaline DSB solutions exhibit fluorescence spectra with an emission maximum at 501 nm. After 365 nm UV-



Figure 3. Fluorescence spectra of a C<sub>2</sub> solution recorded under different experimental conditions. A) An unexposed C<sub>2</sub> solution (1), a C<sub>2</sub> solution after UV exposure (2), a mixture of C<sub>2</sub> and CuSO<sub>4</sub> solution after UV exposure (3), a mixture of C<sub>2</sub> and CuSO<sub>4</sub> solution without UV exposure (4). Inset: fluorescence microscopic images of solutions (2) and (3) drop-casted on glass slides. B) Comparative fluorescence spectra of the mixture of C<sub>2</sub> and CuSO<sub>4</sub> solution after UV (Y) and visible light (X) exposure. Conditions: C<sub>2</sub> concentration =  $1.66 \times 10^{-4}$  m; CuSO<sub>4</sub> concentration =  $83 \times 10^{-6}$  M;  $\lambda_{ex} = 290$  nm.

light exposure, this peak is greatly decreased and formation of a new peak at 449 nm is observed. Formation of semiquinone as a result of UV irradiation causes a loss of resonance in the fluorophore system, which results in a higher energy emission (blueshift of the emission maxima) but with lower intensity.<sup>[34]</sup> If UV irradiation of the DSB is performed in the presence of the copper salt, the in situ generated Cu<sup>0</sup> produces a dramatic enhancement of the fluorescence ( $\lambda_{em}$  $\approx$ 429 nm) for the individual exposed reaction mixtures for the cases of C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, and C<sub>4</sub> (Scheme 1).

Interestingly, for  $C_5$  and  $C_6$  the quenching phenomenon is observed. When the two iminic bonds are separated by aliphatic groups ( $C_1$ – $C_3$ ), a huge enhancement of the emission intensity is observed. On the contrary, an aromatic group between the two iminic bonds exhibits a low enhancement

www.chemeurj.org



Figure 4. XPS spectra of  $C_2$ -capped CuNPs: A) wide angle and B) high resolution.



Scheme 1. Formation of CuNPs under UV-light exposure from an alkaline DSB solution in the presence of a copper salt (DSB/Cu<sup>II</sup>=2:1).



Figure 5. A) TEM image, B) SAED pattern, and C) HRTEM image of the photoproduced CuNPs prepared from  $C_2$ .

of the fluorescence ( $C_4$ ) and sometimes quenching ( $C_5$  and  $C_6$ ) in the presence of photoproduced  $Cu^0$ . So, the spacer in between the iminic bonds dramatically tunes the fluorescence behavior of the exposed DSBs in the presence of CuNPs produced in situ in the reaction mixture as a consequence of UV-light irradiation. The bar diagram (Figure 6) shows the extent of different fluorescence intensities in the presence of different spacers when  $CuSO_4$  is employed. The  $C_2$ -capped CuNP shows the maximum enhancement of fluorescence and the  $C_6$ -capped CuNP exhibits the most efficient quenching capability of the DSBs.

It was observed that the fluorophore emission is strongly quenched by metallic surfaces when the metal to fluorophore proximity falls by approximately 50 Å.<sup>[33]</sup> The optimum distance for the probe molecules from the metallic surface to exhibit maximum fluorescence enhancement is approximately 100 Å, whereas Sokolov et al.<sup>[35]</sup> claimed the distance to be approximately 600 Å. The DSBs containing an aromatic group in between two iminic bonds (C<sub>4</sub>, C<sub>5</sub>, and C<sub>6</sub>) are more rigid than those with aliphatic spacers (C<sub>1</sub>, C<sub>2</sub>, and C<sub>3</sub>). Aliphatic spacers can acquire different rotameric conformations that hinder a closer approach of the DSBs towards the metal-nanoparticle surface, thereby showing a higher degree of enhancement; whereas the rigid capping agents entangle the metal nanoparticles, that is, enable a closer approach, which thereby produces quenching (or less

15848



Figure 6. Fluorescence spectra (A–F) showing enhancement/quenching of exposed DSBs in the presence of photoproduced CuNPs. The bar diagram represents different degrees of enhancement from the exposed DSBs in the presence of photoproduced CuNPs (I=fluorescence intensity of exposed C<sub>2</sub> in the presence of photogenerated CuNPs;  $I_0$ =fluorescence intensity of exposed C<sub>2</sub> solution without copper). Conditions: C<sub>2</sub> concentration=1.66×10<sup>-4</sup> m; CuSO<sub>4</sub> concentration=83×10<sup>-6</sup> m;  $\lambda_{ex}$ =290 nm.

enhancement). The oscillations created in the CuNPs at the close proximity of  $C_5$  and  $C_6$  are not capable of radiating owing to optical constraints at the metal–sample interface. Induced plasma at short metal–fluorophore distances are trapped and decay as heat.<sup>[30]</sup> The phenomena of fluorescence enhancement and quenching are dependent on the scattering cross section and absorption cross section. When the scattering cross section dominates, the induced plasmons radiate, which results in enhancement of the fluorescence as in the cases of  $C_1$ ,  $C_2$ ,  $C_3$ , and  $C_4$ .

Not only  $CuSO_4$ , but also other common water-soluble copper salts have been tested to check the efficacy of anions for the fluorescence enhancement of exposed  $C_2$  (Figure 7; Figure S4 in the Supporting Information). The extent of the fluorescence enhancements was comparable for all the copper salts except for the chloride salts. This indicates that in situ produced Cu<sup>0</sup> is mainly responsible for the drastic enhancement of fluorescence, not the counter anions. However, if CuCl<sub>2</sub> is employed, the degree of enhancement is comparatively lower. This is not a very unusual occurrence. The fluorescence quenching capability of Cl<sup>-</sup> reported by Garcia et al.<sup>[36]</sup> and several other groups is observed in our case also (Cl<sup>-</sup> causes spin–orbit coupling effects).<sup>[37]</sup>

We have also performed a fluorescence study of exposed  $C_2$ , unexposed  $C_2$ , and exposed  $C_2$  in the presence of  $CuSO_4$  with temperature variation (Figure 8). In all three cases, the fluorescence intensity decreases with the increase of temperature.<sup>[38]</sup> The increase of temperature assists important Brownian motions that facilitate energy loss through dynamic quenching by the solvent molecule. Consequently, a decrease of fluorescence intensity as well as fluorescence quantum yield is observed with the rise of temperature. The

www.chemeurj.org



Figure 7. Effect of different counter anions of Cu<sup>II</sup> on the fluorescence enhancement of the exposed C<sub>2</sub> solutions. Conditions: C<sub>2</sub> concentration =  $1.66 \times 10^{-4}$  M; Cu<sup>II</sup>X concentration =  $83 \times 10^{-6}$  M (X=counter anion);  $\lambda_{ex}$  = 290 nm.

emission peak at 501 nm for the unexposed  $C_2$  rapidly decreases with the increase of temperature. On the contrary, the fluorescence intensity of the emission peak is slowly reduced for the exposed  $C_2$  and exposed  $C_2$  containing in situ generated CuNPs. But, the extent of the lowering of the fluorescence for the exposed  $C_2$  and for the CuNP-induced enhanced fluorescence of exposed  $C_2$  is exactly same; this implies that the enhancement by CuNPs is not very sensitive to temperature variation. Figure 8C shows that the  $I/I_0$  value (I=fluorescence intensity of exposed  $C_2$  in the presence of  $Cu^0$ ;  $I_0$ =fluorescence intensity of exposed  $C_2$  in the absence of  $Cu^0$ ) remains unaltered with an increase of temperature.

If we add ethylenediaminetetraacetic acid disodium salt (Na<sub>2</sub>-EDTA) along with the copper salts in the aqueous alkaline solution of the DSB and then expose it to UV light, instead of enhancement of fluorescence, damping of the fluorescence takes place. Again, very insignificant enhancement is noticed when we irradiate a cuproammonium complex in the presence of a DSB. Both Na2-EDTA and ammonia form highly stable complexes with Cu<sup>II</sup> (see Figure S5 in the Supporting Information). At room temperature, the stability constant of the former complex is  $5 \times 10^{18}$  and for the latter it is  $1.1 \times 10^{13}$ . DSBs could not at all reduce Cu<sup>II</sup> to Cu<sup>0</sup> from such stable complexes. The phenomenon of quenching is due to the effect (spin-orbit coupling effect) of Cu<sup>II</sup>, which is very commonly reported in the literature.<sup>[39]</sup> Interestingly, the introduction of an individual complexing agent, Na<sub>2</sub>-EDTA or ammonia, to the already exposed reaction mixtures, that is, the reaction mixtures with preformed CuNPs, had no effect on the fluorescence behavior.

The fluorescence-lifetime measurement (Figure 9) has been performed with exposed  $C_2$  in the presence of photoproduced CuNPs. It is observed that 31% of molecules have a lifetime of 1.5 ns and 69% of molecules possess a lifetime of 3.6 ns. The average lifetime of exposed  $C_2$  in presence of CuNPs is 2.9 ns. Owing to insignificant fluorescence intensity, the time-correlated single-photon counting (TCSPC) in-



Figure 8. A) Temperature-dependent fluorescence intensity study. B) Plot of  $I_T/I_{\rm RT}$  versus the temperature of exposed C<sub>2</sub> in the presence of in situ generated CuNPs and unexposed and exposed C<sub>2</sub> solutions in the absence of copper ( $I_T$ =fluorescence intensity at temperature *T*;  $I_{\rm RT}$ =fluorescence intensity at room temperature (30 °C)). C) Plot of  $I/I_0$  versus temperature (I=fluorescence intensity of exposed C<sub>2</sub> in the presence of photogenerated CuNPs;  $I_0$ =fluorescence intensity of exposed C<sub>2</sub> solution without copper). Conditions: C<sub>2</sub> concentration =  $2.5 \times 10^{-4}$  M; CuSO<sub>4</sub> concentration =  $83 \times 10^{-6}$  M;  $\lambda_{ex} = 290$  nm.



Figure 9. Fluorescence-lifetime measurement of the exposed C<sub>2</sub> solution (gray color) in the presence of in situ generated CuNPs. Conditions: C<sub>2</sub> concentration =  $1.66 \times 10^{-4}$  M; CuSO<sub>4</sub> concentration =  $83 \times 10^{-6}$  M;  $\lambda_{ex} = 290$  nm.

strument cannot measure the fluorescence lifetime of the exposed  $C_2$  in the absence of copper salts.

Excitation energy is an important parameter for observing fluorescence enhancement (Figure 10). We chose 290 nm as the excitation wavelength for all the cases mentioned above and observed the highest  $I/I_0$  value. Above and below this particular excitation wavelength, the  $I/I_0$  ratio becomes less. It is worth mentioning that just by changing the excitation wavelength,  $I/I_0$  can be tuned many times larger than 1 to a fraction (<1). Figure 10 shows that in between 260 and 350 nm,  $I/I_0 > 1$ , that is, there is enhancement. Above and below the range, quenching occurs, that is,  $I/I_0$  becomes <1.

The concentration of the DSB used in the experiment is  $1.66 \times 10^{-4}$  M and with this concentration, a  $83 \times 10^{-6}$  M copper(II) concentration shows maximum fluorescence enhancement. The phenomenon of enhancement starts to appear when the concentration of  $Cu^{II}$  becomes  $11 \times 10^{-6} M$ . A gradual increase of the Cu<sup>II</sup> concentration causes the increase of the fluorescence emission peak. Above  $83 \times 10^{-6}$  M, a further increase of the copper concentration causes a gradual decrease of fluorescence. Consequently, when the DSB/  $Cu^{II}$  ratio is 2:1, the highest fluorescence intensity is noticed. Salen-like compounds form copper complexes in which the ligand/copper ratio is 2:1.<sup>[40]</sup> So, it is thought that the copper-salen complex is formed first. Then, owing to UV irradiation, it is converted to Cu<sup>0</sup>. The CuNPs maintain an exact distance from the exposed DSBs as a result of the synthetic strategy, which is responsible for the huge fluorescence intensity enhancement from the DSB in the reaction mixture. If we do not use UV exposure, such Cu<sup>II</sup> to Cu<sup>0</sup> reduction does not take place at all. The Cu<sup>II</sup> ion is prone to coordination and is weakly bonded to DSB and therefore easily forms CuNPs in solution upon UV irradiation. As a matter of fact, without UV exposure, no enhancement of the fluorescence is observed from the unexposed solution. When a higher copper concentration is employed, reduction



Figure 10. A) A selection of optimum excitation wavelengths ( $\lambda_{ex}$ ) to indicate maximum fluorescence enhancement of exposed C<sub>2</sub> in the presence of in situ produced CuNPs with respect to exposed C<sub>2</sub> (without copper). B) A pie diagram of  $I/I_0$  at different excitation wavelengths ( $\lambda_{ex}$ ). I=fluorescence intensity of exposed C<sub>2</sub> in presence of in situ generated CuNPs,  $I_0$ =fluorescence intensity of exposed C<sub>2</sub> solution. Conditions: C<sub>2</sub> concentration =  $1.66 \times 10^{-4}$  M; CuSO<sub>4</sub> concentration =  $83 \times 10^{-6}$  M.

of Cu<sup>II</sup> is not quantitative and quenching is observed due to the spin–orbit coupling effect.<sup>[37]</sup> It may also be possible that Cu<sup>0</sup> comes closer to the DSBs, that is, some DSBs may be present at the quenching zone of Cu<sup>0</sup>. A copper concentration of less than  $83 \times 10^{-6}$  M supports the idea that some DSBs are deprived of the effect of Cu<sup>0</sup>, which is responsible for the fluorescence enhancement (Figure 11).

www.chemeurj.org



Figure 11. Fluorescence intensity of the exposed C<sub>2</sub> solutions with different concentrations of CuNPs. The CuNP concentration varies because of the use of different concentrations of aqueous copper salt solution. Conditions: C<sub>2</sub> concentration =  $1.66 \times 10^{-4}$  M;  $\lambda_{ex}$  = 290 nm.

Copper, a base metal, is prone to corrosion. Metallic copper is easily oxidized in air and the oxidation reaction is facilitated in alkaline solution. Copper hydroxide is formed under ambient conditions owing to the interaction of alkali and copper metal. With this idea in mind, we have designed an experiment to monitor the corrosion of a piece of copper wire 51 cm long and 126 µm wide (measured optically) in 0.1 M NaOH. The submerged commercial copper wire corroded in alkaline solution. The copper ions from the alkaline solution were estimated fluorometrically regularly for seven days after a time interval of one day by using our own protocol with DSB as described above. Alkaline C<sub>2</sub> solution was added to an aliquot of the corrosion-produced copper(II) solutions and exposed under 365 nm UV light for nine hours. Then, from the fluorescence studies, copper dissolution and quantification were performed (Figure 12).

Copper is generally susceptible to oxidation and it is even more so at the nanoscale. The CuNPs prepared by the proposed UV irradiation routes are exceptionally stable and robust as revealed from fluorescence measurements. No perceptible change of the fluorescence intensity was observed even after keeping DSB-bound CuNPs in solution for more than one month at ambient temperature. An unaltered fluorescence intensity proves that no aggregation of the CuNPs or decomposition of the fluorophore takes place (Figure 13). Thus, CuNPs enjoy exceptional stability even in alkaline solution. This may be due to the presence of a large excess amount of exposed DSBs that act as capping agents and protect the particles for a longer time. Diamines,<sup>[41]</sup> alkanethiols, oleic acid,<sup>[42]</sup> and so forth are well-known stabilizers for CuNPs in solution. In the present case, exposed DSBcapped CuNPs do not undergo oxidation, presumably as a result of a strong Cu-N bond affinity. Again, zeta-potential measurements for representative DSB-capped CuNPs (C2capped CuNPs) indicate a high surface-charge density (-30.3 mV), which is also responsible for making the copper



Figure 12. A) Experimental setup showing Cu<sup>II</sup> leaching from the copper wire in NaOH (0.1 m) solution. B) Time-dependent Cu<sup>II</sup> leaching, quantified from a fluorescence study after UV irradiation. Conditions: C<sub>2</sub> concentration =  $1.66 \times 10^{-4}$  m;  $\lambda_{ex} = 290$  nm. C) A plot of Cu<sup>II</sup> concentration leached in NaOH (0.1 m) solution at different time intervals.

hydrosol stable for a longer time The particle size and morphology are not changed even when the solution becomes aged (>30 days). It speaks in favor of the excellent capping capability of exposed DSBs for CuNPs.

15852



Figure 13. Fluorescence intensity of exposed C2 CuNPs after ageing.

The uniqueness of  $Cu^0$  is very important. A drastic enhancement of fluorescence is observed when any copper salt solution is added to the alkaline  $C_2$  solution and is irradiated

for nine hours. We have repeated the experiment for other metal ions, such as, Co, Ni, Zn, Hg, Mn, Ag, Au, Mg, K, and Na. The fluorescence enhancement was not observed from these metal ions under the proposed experimental conditions. As a consequence, copper is an exceptional candidate in the family of coinage metals for displaying such metal-enhanced fluorescence unlike gold and silver. This observation clearly indicates that DSBs become a simple potential fluorometric sensor for copper. We have also studied the copper-enhanced fluorescence intensity of the exposed DSBs in presence of other metal ions. The enhanced fluorescence is slightly dampened<sup>[37]</sup> in the presence of other metal ions. Nevertheless, the copper-enhanced fluorescence (in the presence of other metal ions) is much higher than the othermetal-induced fluorescence. So this strategy is reliable and convenient for generating a copper sensor (Figure 14).

The high fluorescence enhancement of the fluorophore in the presence of metal nanoparticles is due to an increased rate of excitation and intrinsic radiative decay rate. Metal nanoparticles concentrate the local field on the fluorophore, which increases the rate of excitation (a phenomenon



Figure 14. A) Bar diagram indicating different  $I/I_0$  values in the presence of different metal ions. A1) Fluorescence intensity of exposed C<sub>2</sub> in the presence of different metal ions. B) Bar diagram indicating different  $I/I_0$  values in the presence of copper and other metal ions. B1) Fluorescence intensity of exposed C<sub>2</sub> in the presence of copper and other metal ions. C<sub>2</sub> concentration= $1.66 \times 10^{-4}$  M; CuSO<sub>4</sub> concentration= $83 \times 10^{-6}$  M;  $\lambda_{ex} = 290$  nm, Other metal-ion concentration= $83 \times 10^{-6}$  M.

Chem. Eur. J. 2012, 18, 15845-15855

© 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

www.chemeurj.org

- 15853

known as the "lightening rod effect").<sup>[34,43-45]</sup> Metal nanoparticles can also modify the rate at which a fluorophore emits a photon.<sup>[46]</sup> According to the radiating plasmon (RP) model, instead of the fluorophore it is the metal that radiates after energy transfer from fluorophore. Far-field radiation from fluorophore-induced plasmons and trapped plasmons are accountable for enhancement and quenching of fluorescence.

In our earlier paper, a silver- and gold-nanoparticle-induced fluorescence contrast phenomenon was reported.<sup>[22]</sup> AgNPs or AuNPs were produced upon UV irradiation from alkaline DSB solutions as has been reported herein for the copper hydrosol evolution. The irradiation time was 3 h for silver and 11 h for gold systems. AgNPs always induce fluorescence enhancement (except for  $C_5$ ), whereas the present CuNPs exhibit enhancement for  $C_1$  to  $C_4$ . On the contrary, a stable gold hydrosol is obtained after prolonged photoactivation only from C2 and C5. The gold particles quench the fluorescence of these DSB solutions. But all the DSBs (except C<sub>5</sub>) show an enhancement of fluorescence in the presence of photoproduced AgNPs. In this context it is important to mention that with the increase of irradiation time (>3 h), the stability of the silver hydrosol decreased, and as a result, so did the extent of the fluorescence enhancement. It is imperative to use silver in a twofold excess over DSBs, that is, the metal/DSB ratio remains at 2:1 for photoactivation, to observe maximum fluorescence enhancement ( $\approx$ 9fold for C<sub>2</sub>). Using this ratio and 3 h of irradiation time did not produce any significant fluorescence enhancement for the copper system. More precisely, for the copper system, the copper/DSB ratio needs to be 1:2 and the required irradiation time is 9 h for maximum enhancement ( $\approx$ 10-fold for  $C_2$ ). So under the present experimental conditions silver does not show any significant fluorescence enhancement whereas copper does (i.e., no interference from silver). Thus by tuning the experimental conditions, a silver(I) and copper(II) sensor can be designed with a proper DSB.

Copper-enhanced fluorescence in the solution phase is rarely reported. Although gold and copper possess similar optical constants, copper exhibits normally larger losses than gold, as evidenced from its broader plasmon resonance.<sup>[4]</sup> Surprisingly, we have observed efficient quenching phenomena for in situ generated AuNPs (Figure 14), whereas both enhancement and quenching are obtained for in situ produced CuNPs depending on the spacer in between two iminic bonds of the DSB. Usually, the scattering efficiency is smaller for copper, and CuNPs having an especially smaller size are known for quenching of fluorescence. For larger nanoparticles, copper-enhanced fluorescence is found in the literature.<sup>[15]</sup> The synthetic protocol disclosed herein generates CuNPs of a smaller size (3-6 nm) showing fluorescence enhancement. The concept of the RP model can adequately explain the enhancement phenomena. Our synthetic strategy can eliminate the interband absorption for C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, and C<sub>4</sub> responsible for quenching. Low-quantum-yield fluorophores (exposed DSBs) readily transfer energy to CuNPs, which radiate more efficiently than the fluorophore in free space. Smaller CuNPs are able to have a stronger interaction with the metal, which facilitates energy transfer from the fluorophore. So, we have found a new type of fluorophore showing an intriguing fluorescence behavior. The virtue of our synthetic procedure keeps the fluorophore, that is, capping agent (the exposed DSBs), at an interesting position from the CuNPs thereby sometimes causing quenching (due to lossy surface waves) or enhancement (far-field radiation by means of the nanoantenna) of fluorescence.

### Conclusion

The dramatic fluorescence contrast (enhancement and quenching) of exposed DSBs in the solution phase has been reported in the presence of in situ generated CuNPs. The spacers in between the two iminic bonds have been found to be responsible for such dramatic contrast phenomena. The nature of the synthetic route and that of the spacer increase the fluorescence enhancement in the solution phase, which may prove useful in metal-enhanced fluorescence studies in the solution phase. The unique fluorescence enhancement under these experimental conditions may provide promising information for the design of copper sensors.

#### **Experimental Section**

Materials and instruments: All the reagents were of AR grade. Throughout the experiments, triple-distilled water was used. Copper salts, salicylaldehyde, all the diamines, and ethylenediaminetetraacetic acid disodium salt were obtained from Sigma-Aldrich. NaOH was purchased from HiMedia Laboratories Pvt. Ltd. Ammonia was purchased from Merck. All glassware was cleaned with freshly prepared aqua regia, subsequently rinsed with copious amounts of distilled water and dried well before use. The sample solution was irradiated with a TUV 15W/G 15 T8 ultraviolet light (Philips India) source. All UV/Vis absorption spectra were recorded in a SPECTRASCAN UV 2600 digital spectrophotometer (Chemito, India). FTIR spectra were recorded in a FTIR Nexus spectrophotometer (Thermo Nicolet). <sup>1</sup>H NMR spectrum was obtained with a 400 MHz Bruker NMR instrument. XPS analysis was carried out with a VG Scientific ESCALAB MK II spectrometer (UK) equipped with a Mg<sub>Ka</sub> excitation source (1253.6 eV) and a five-channeltron detection system. The fluorescence measurements were carried out at room temperature using a LS55 fluorescence spectrometer (Perkin-Elmer, USA). TEM analysis was performed with a H-9000 NAR instrument (Hitachi) using an accelerating voltage of 300 kV.

**Preparation of DSBs**: Methanolic solutions of ethylenediamine  $(10^{-2} \text{ M})$ and salicylaldehyde  $(2 \times 10^{-2} \text{ M})$  were mixed with constant stirring. Then the mixture was heated at reflux for approximately 4 h and a yellow product (C<sub>1</sub>) was obtained after cooling. The product was recrystallized from methanol. In a similar procedure, C<sub>2</sub> (salprn), C<sub>3</sub> (salben), C<sub>4</sub>, C<sub>5</sub>, and C<sub>6</sub> were synthesized by using 1,3-propylenediamine, 1,4-butanediamine, *o*-phenylenediamine, *m*-phenylenediamine, and *p*-phenylenediamine, respectively. The only difference is that in place of ethylenediamine different amines were used. Figure 1 indicates the structures of all the six DSBs.<sup>[16]</sup>

The melting points (see the Supporting Information), <sup>1</sup>H NMR spectra (see Figure S6 in the Supporting Information), and IR spectra (see Figure S7 in the Supporting Information) confirm the synthesis of the DSBs. **Synthesis of the CuNPs**: DSBs are insoluble in water. So, we dissolved them in an alkaline medium. A stock solution of  $2.5 \times 10^{-3}$  M DSB was

prepared by dissolving an appropriate amount of DSB in 0.1 M aqueous NaOH solution. In a fluorescence cuvette, 0.1 M NaOH (3.3 mL), DSB solution (0.2 mL), and  $10^{-2}$  M CuSO<sub>4</sub> solution (0.025 mL) were mixed together. The final concentration ratio of DSB to CuSO<sub>4</sub> was maintained at 2:1. Then the well-stoppered cuvette was irradiated under a UV lamp ( $\approx$ 365 nm) for 9 h. The plasmon band for the CuNPs remained masked within the absorption band of the Schiff bases (see Figure S1 in the Supporting Information).

### Acknowledgements

The authors are thankful to the UGC, DST, NST, BRNS, and CSIR, New Delhi, India, and the IIT Kharagpur for financial assistance. They are also thankful to Miss. Isozaki of Tokyo University of Science, Japan, for XPS measurements.

- [1] R. Ruppin, J. Chem. Phys. 1982, 76, 1681-1684.
- [2] P. Anger, P. Bharadway, L. Novotny, Phys. Rev. Lett. 2006, 96, 113002.
- [3] S. Kühn, U. Håkanson, L. Rogobete, V. Sandoghdar, *Phys. Rev. Lett.* 2006, 97, 017402.
- [4] A. Mohammadi, F. Kaminski, V. Sandoghdar, M. Agio, Int. J. Nanotechnol. 2009, 6, 902–914.
- [5] E. Le Moal, E. Fort, S. Leveque-Fort, F. P. Cordelieres, M. P. Fontaine-Aupart, C. Ricolleau, *Biophys. J.* 2007, 92, 2150–2161.
- [6] C. D. Geddes, J. R. Lakowicz, J. Fluoresc. 2002, 12, 121-129.
- [7] a) K. Aslan, I. Gryczynski, J. Malicka, E. Matveeva, J. R. Lakowicz, C. D. Geddes, *Curr. Opin. Biotechnol.* 2005, *16*, 55–62; b) K. Aslan, C. D. Geddes, *Analyst* 2008, *133*, 1469–1480; c) K. Aslan, J. R. Lakowicz, H. Szmacinski, C. D. Geddes, *J. Fluoresc.* 2005, *15*, 37–40; d) K. Aslan, C. D. Geddes, *Plasmonics* 2008, *3*, 89–101.
- [8] a) N. Kato, F. Caruso, J. Phys. Chem. B 2005, 109, 19604–19612;
  b) L. Ao, F. Gao, B. Pan, R. He, D. Cui, Anal. Chem. 2006, 78, 1104–1106;
  c) W. Zai-Sheng, J. Jian-Hui, L. FU, S. Guo-Li, Y. Ru-Qin, Anal. Biochem. 2006, 353, 22–29;
  d) B. Dubertret, M. Calame, A. J. Libchaber, Nat. Biotechnol. 2001, 19, 365–370;
  e) H. Imahori, S. Fukuzumi, Adv. Mater. 2001, 13, 1197–1199.
- [9] P. P. Pompa, L. Martiradonna, A. Della Torre, F. Della Sala, L. Manna, M. De Vittorio, F. Calabi, R. Cingolani, R. Rinaldi, *Nat. Nanotechnol.* 2006, 1, 126–130.
- [10] a) E. Dulkeith, M. Ringler, T. A. Klar, J. Feldmann, J. A. Munoz, W. J. Parak, *Nano Lett.* **2005**, *5*, 585–589; b) M. Ganguly, A. Pal, T. Pal, *J. Phys. Chem. C* **2012**, *116*, 9265–9273.
- [11] K. Aslan, S. N. Malyn, C. D. Geddes, J. Fluoresc. 2006, 17, 7-13.
- [12] K. Aslan, S. N. Malyn, C. D. Geddes, Chem. Phys. Lett. 2008, 453, 222–228.
- [13] J. Lukomska, J. Malicka, Z. Gryczynski, Z. Leonenko, J. R. Lakowicz, *Biopolymers* 2005, 77, 31–37.
- [14] Y. Zhang, K. Aslan, M. J. R. Previte, C. D. Geddes, *Appl. Phys. Lett.* 2007, 90, 173116–173113.
- [15] H. Mertens, A. Polman, in ArXiv e-prints. 2007, 711.
- [16] a) P. Pfeiffer, E. Breith, E. Lübbe, T. Tsumaki, Justus Liebig's Annalen der Chemie 1933, 503, 84–130; b) R. Gheorghe, P. Cucos, M. Andruh, J. P. Costes, B. Donnadieu, S. Shova, Chem. Eur. J. 2005, 11, 187–203; c) A. Rezaeifard, M. Jafarpour, M. A. Nasseri, R. Haddad, Helv. Chim. Acta 2010, 93, 711–717; d) S. R. Lane, N. Sisay, B. Carney, S. Dannoon, S. Williams, H. P. Engelbrecht, C. L. Barnes, S. S. Jurisson, Dalton Trans. 2011, 40, 269–276; e) N. M. Geller, A. G. Ivanov, L. B. Nadezhdina, V. V. Shamanin, L. A. Shibaev, A. V. Gribanov, Russ. J. Appl. Chem. 2008, 81, 1398–1402; f) N. Kumari, R. Prajapati, L. Mishra, Polyhedron 2008, 27, 241–248.

- [17] G. D. Liu, J. P. Liao, S. S. Huang, G. L. Shen, R. Q. Yu, Anal. Sci. 2001, 17, 1031–1036.
- [18] C. E. White, F. Cuttitta, Anal. Chem. 1959, 31, 2083-2087.
- [19] M. E. Germain, M. J. Knapp, Inorg. Chem. 2008, 47, 9748-9750.
- [20] J. H. Lee, A. R. Jeong, I. S. Shin, H. J. Kim, J. I. Hong, Org. Lett. 2010, 12, 764–767.
- [21] I. Aoki, A. Takahashi, K. Watanabe, Anal. Sci. 1992, 8, 323-327.
- [22] M. Ganguly, A. Pal, T. Pal, J. Phys. Chem. C 2011, 115, 22138– 22147.
- [23] G. L. Williams, J. K. Vohs, J. J. Brege, B. D. Fahlman, J. Chem. Educ. 2005, 82, 771–774.
- [24] H. Ohde, F. Hunt, C. M. Wai, Chem. Mater. 2001, 13, 4130-4135.
- [25] L. Qi, J. Ma, J. Shen, J. Colloid Interface Sci. 1997, 186, 498-500.
   [26] a) C. Crouse, A. R. Barron, J. Mater. Chem. 2008, 18, 4146-4153;
- b) S. Shen, J. Zhuang, X. Xu, A. Nisar, S. Hu, X. Wang, *Inorg. Chem.* 2009, 48, 5117–5128.
- [27] B. K. Park, S. Jeong, D. Kim, J. Moon, S. Lim, J. S. Kim, J. Colloid Interface Sci. 2007, 311, 417–424.
- [28] S. Giuffrida, L. L. Costanzo, G. Ventimiglia, C. Bongiorno, J. Nanopart. Res. 2008, 10, 1183–1192.
- [29] a) J. R. Lakowicz, Anal. Biochem. 2001, 298, 1–24; b) M. H. Chowdhury, K. Aslam, S. N. Malyn, J. R. Lakowicz, C. D. Geddes, Appl. Phys. Lett. 2006, 88, 173104.
- [30] J. R. Lakowicz, Anal. Biochem. 2005, 337, 171-194.
- [31] A. Bek, R. Jansen, M. Ringler, S. Mayilo, T. A. Klar, J. Feldmann, *Nano Lett.* 2008, 8, 485–490.
- [32] P. R. Selvakannan, A. Swami, D. Srisathiyanarayanan, P. S. Shirude, R. Pasricha, A. B. Mandale, M. Sastry, *Langmuir* 2004, 20, 7825– 7836.
- [33] C. D. Geddes, J. R. Lakowicz, J. Fluoresc. 2002, 12, 121-129.
- [34] V. G. Malshet, A. L. Tappel, *Lipids* 1973, *8*, 194–198.
- [35] K. Sokolov, G. Chumanov, T. M. Cotton, Anal. Chem. 1998, 70, 3898–3905.
- [36] N. H. García, C. F. Plato, J. L. Garvin, *Kidney Int.* 1999, 55, 321– 325.
- [37] a) L. R. Naik, H. M. S. Kumar, S. R. Inamdar, N. N. Math, Spectrosc. Lett. 2005, 38, 645–659; b) R. T. Gephart, N. J. Williams, J. H. Reibenspies, A. S. De Sousa, R. D. Hancock, Inorg. Chem. 2009, 48, 8201–8209; c) A. L. Koner, P. P. Mishra, S. Jha, A. Dutta, J. Photochem. Photobiol. A 2005, 170, 21–26.
- [38] J. R. Albani, Principles and Applications of Fluorescence Spectroscopy, Blackwell, Oxford, 2007, p. 154.
- [39] Y. Rahimi, A. Goulding, S. Shrestha, S. Mirpuri, S. K. Deo, Biochem. Biophys. Res. Commun. 2008, 370, 57-61.
- [40] a) L. Rigamonti, F. Demartin, A. Forni, S. Righetto, A. Pasini, *Inorg. Chem.* **2006**, *45*, 10976–10989; b) R. Paschke, D. Balkow, E. Sinn, *Inorg. Chem.* **2002**, *41*, 1949–1953.
- [41] C. Barrière, K. Piettre, V. Latour, O. Margeat, C.-O. Turrin, B. Chaudret, P. Fau, J. Mater. Chem. 2012, 22, 2279–2285.
- [42] P. Kanninen, C. Johans, J. Merta, K. Kontturi, J. Colloid Interface Sci. 2008, 318, 88–95.
- [43] T. Hayakawa, S. T. Selvan, M. Nogami, Appl. Phys. Lett. 1999, 74, 1513–1515.
- [44] S. T. Selvan, T. Hayakawa, M. Nogami, J. Phys. Chem. B. 1999, 103, 7064–7067.
- [45] J. F. Le, D. W. Brandl, Y. A. Urzhumov, H. Wang, J. Kundu, N. J. Halas, J. Aizpurua, P. Nordlander, ACS Nano 2008, 2, 707–718.
- [46] A. Kinkhabwala, Z. Yu, S. Fan, Y. Avlasevich, K. Muellen, W. E. Moerner, *Nat. Photonics* 2009, *3*, 654–657.

Received: April 12, 2012 Revised: August 15, 2012 Published online: October 12, 2012