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Highly sensitive salicylic fluorophore for visual detection of picomole amounts of Cu^{2+}†

Nakorn Niamnont,[a] Nattaporn Kimpitak,[b] Gamolwan Tumcharern,[c] Paitoon Rashatasakhon[d] and Mongkol Sukwattanasinitt*[d]

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Four new fluorophores containing multiple fluorogenic branches of phenylene-ethynylene and salicylic acid Cu^{2+} binding sites are synthesized and evaluated as Cu^{2+} sensors in aqueous media. The fluorophore, **F4**, having a triphenylamine core and three fluorogenic branches, possesses superior sensitivity, selectivity and emission properties for fluorescence quenching detection of Cu^{2+}. This fluorophore exhibits the highest Cu^{2+} quenching sensitivity in aqueous solution; $K_{sv} = 5.79 \times 10^6\ M^{-1}$. The relatively high fluorescence emission of this fluorophore provides excellent visual contrast in a solid state paper-based sensor that enables naked eye detection of Cu^{2+} at the picomole level.

Introduction

Cu^{2+} is an important ion which plays a critical role as a catalytic cofactor for a variety of metalloenzymes, including superoxide dismutase, cytochrome c oxidase, tyrosinase and nuclease.[1] Several research works to establish the detection of Cu^{2+} ions in living cells at the micromolar level have been reported.[2–6] However, under overexposure conditions, Cu^{2+}can accumulate within tissues and organs and exhibit toxicity causing oxidative stress and disorders associated with neurodegenerative diseases *e.g.* Alzheimer's, Wilson's and Parkinson's diseases.[7–10] A Cu^{2+} level above 0.6 μmol in urine collected over 24 hours is strongly indicative of Wilson's disease and other abnormal conditions.[11–13] The World Health Organization (WHO) recommends that the concentration of Cu^{2+} in drinking water should not exceed 2 mg L^{-1} (31 μM).[14] Thus, there is the need for sensing methods which can detect low doses of Cu^{2+} in biologically relevant aqueous samples. Among various modes of sensing, the observation and measurement of fluorescence signal change is one of the most sensitive, rapid, simple and non-destructive.[15–18] In certain systems, a naked eye detection of Cu^{2+} is possible even at nanomole levels.[19–26] Conjugated polymers (CPs) have been selected for detection of $Cu^{2+}$for their high fluorescence quenching sensitivity caused by the amplification effect originating from their efficient intramolecular energy and electron transfer-processes.[27–31]

In comparison with a polymer, a small fluorescent molecule has a better defined structure but lacks signal amplification as it usually contains only one fluorogenic unit. To attain the amplification effect, a well-defined structure and sufficient water solubility, we have recently focused on the molecular design of sensing compounds based on star-shaped and dendritic structures with multiple fluorogenic units and ionisable peripheral groups.[32–35] We have found that the 1,3,5-triphenylbenzene fluorophore **F1** containing salicylic end groups was an excellent Cu^{2+} fluorescence quenching sensor with a quenching efficiency of $1.6 \times 10^6\ M^{-1}$.[34] Despite high selectivity and sensitivity, **F1** possesses a low quantum efficiency (0.007) which required the addition of a non-ionic surfactant to enhance its initial fluorescence signal. For naked eye detection of fluorescence quenching, a fluorophore with a higher quantum efficiency is more desirable. We noted that the poor quantum yield of **F1** was partially caused by self-quenching, originating from its hydrophobic planar structure which tends to form some sort of H-aggregate in water and the solid state. In this work, we therefore decided to synthesize four new analogues (**F2–F5**) of **F1** (Fig. 1) with the aim that these fluorophores would have a higher quantum efficiency suitable for applications for naked eye detection.

Experimental section

Apparatus, reagents and chemicals

Methyl 4-iodosalicylate, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), copper(I) iodide, iodine monochloride and sodium chloride were reagent grade and purchased from Sigma-Aldrich.

[a]*Organic Synthesis & Natural Products Research unit, Department of Chemistry, Faculty of Science, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand. Fax: +66 24708843*

[b]*Program of Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*

[c]*National Nanotechnology Center, National Science and Technology Development Agency, Pathumthani 12120, Thailand*

[d]*Organic Synthesis Unit, Department of Chemistry, Faculty of Science and Nanotec-CU Center of Excellence on Food and Agriculture, Chulalongkorn University, Bangkok 10330, Thailand. E-mail: smongkol@chula.ac.th; Tel: +66 2218 7620*

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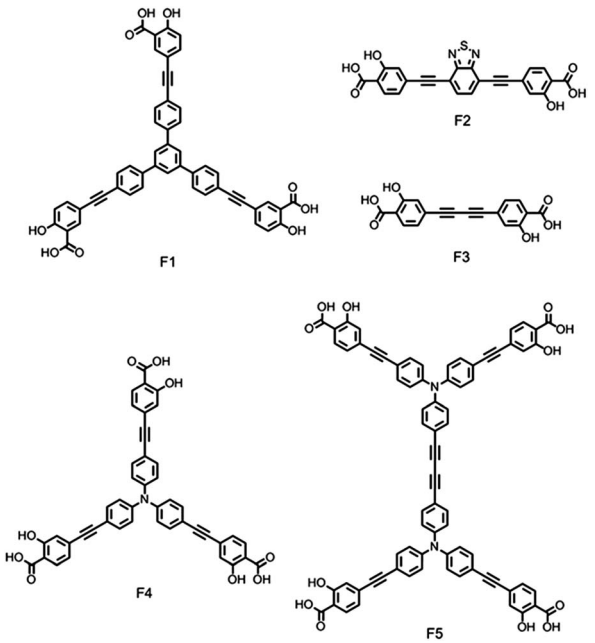

Fig. 1 Structures of molecules **F1–F5**.

Table 1 Photophysical properties of **F2–F5** in PBS (10 mM, pH 7.4)

	Absorption		Emission	
Compound	λ_{abs} (nm)	log ε	λ_{em} (nm)	Φ^a (%)
F1[b]	316	4.28	465	0.7
F2	395	3.61	532	0.2
F3	322	4.44	434	0.3
F4	373	4.84	506	3.2
F5	379	4.80	480	0.4

[a] Quinine sulfate in 0.1 M H_2SO_4 ($\Phi = 54\%$) was used as the standard. [b] The data are taken from ref. 34.

Bis(triphenyl-phosphine)palladium(II) dichloride ($PdCl_2(PPh_3)_2$), potassium hydroxide, potassium carbonate, potassium chloride, potassium hydrogen carbonate and dipotassium hydrogen phosphate were purchased from Fluka (Switzerland). Trimethylsilylacetylene was purchased from GFS Chemical. Metal ions (Ag^+, Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Sr^{2+} and Zn^{2+}) for fluorescent measurements were purchased from Sigma-Aldrich and Fluka (Switzerland). All column chromatography was operated using Merck silica gel 60 (70-230 mesh). Thin layer chromatography (TLC) was performed on silica gel plates (Merck F245). Solvents used for extraction and chromatography were commercial grade and distilled before use. Milli-Q water was used in all experiments unless specified otherwise.

Dendritic fluorophore was characterized with ^{1}H-NMR and ^{13}C-NMR spectra from sample solutions in CD_3OD by a Varian Mercury NMR spectrometer (Varian, USA) at 400 MHz and Bruker 400 MHz, respectively. Absorption spectra were measured by a Varian Carry 50 UV-Vis spectrophotometer. Fluorescence spectra were acquired on a Varian Carry Eclipse spectrofluorometer (Varian, USA) from solution samples in a 1×1 cm^2 quartz cuvette cell. The fluorophore solutions were prepared in 10 mM phosphate buffer saline (pH 7.4). The stock solutions of metal ions were prepared in Milli-Q water.

Synthesis and characterization

Compound 1 in Scheme S1†. A mixture of methyl 4-ethynyl-2-hydroxybenzoate (0.50 g, 2.84 mmol), $PdCl_2(PPh_3)_2$ (0.11 g, 0.16 mmol), CuI (0.03 g, 0.16 mmol) in toluene (15 mL), was added to DBU (1.0 mL) and the mixture was stirred at RT for 24 h. After the combined filtrate was evaporated, the residue was eluted through a silica gel column by gradient solvents from pure hexane to methylene chloride–hexane (3/1 v/v) as an eluent. **1** was obtained as a light-yellow solid (0.44 g, 88% yield), ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.81 (s, 2H), 7.86 (d, $J = 8.0$ Hz, 2H), 7.83 (s, 2H), 7.28 (s, 2H), 7.18 (d, $J = 12.0$ Hz, 2H), 3.98 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2, 161.4, 130.1, 129.9, 122.8, 122.7, 120.8, 112.7, 91.3, 52.6; MALDI-TOF m/z calcd for $C_{20}H_{14}O_6$, 350.079; found, 350.367.

Compound 2 in Scheme S1†. A mixture of 4,7-dibromobenzo-[c]-1,2,5-thiadiazle (2BrDZ) (133.5 mg, 0.45 mmol), $PdCl_2(PPh_3)_2$ (28 mg, 0.04 mmol), CuI (8.0 mg, 0.04 mmol) and methyl 4-ethynyl-2-hydroxybenzoate (179.6 mg, 1.02 mmol) in toluene (15 mL) was added to DBU (0.5 mL) and the mixture was stirred at 70 °C for 12 h. After the combined filtrate was evaporated, the residue was eluted through a silica gel column by gradient solvents from pure hexane to methylene chloride–hexane (1/1 v/v) as an eluent to afford **2** which was obtained as a yellow green solid (139.5 mg, 64% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.81 (s, 2H), 7.86 (d, $J = 8.0$ Hz, 2H), 7.83 (s, 2H), 7.28 (s, 2H), 7.18 (d, $J = 12.0$ Hz, 2H), 3.98 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2, 161.4, 154.4, 133.0, 130.1, 130.0, 122.8, 121.0, 117.3, 113.0, 96.6, 88.0, 52.6.

Compound 3 in Scheme S1†. A mixture of 4,4′,4″-triiodotriphenylamine (**T3I**) (2.00 g, 3.21 mmol), $PdCl_2(PPh_3)_2$ (0.11 g, 0.16 mmol), CuI (0.03 g, 0.16 mmol), methyl 4-ethynyl-2-hydroxybenzoate (1.24 g, 7.06 mmol) in toluene (30 mL) was added to DBU (2.0 mL) and the mixture was stirred at RT for 24 h. After the combined filtrate was evaporated and the residue was eluted through a silica gel column by gradient solvents from pure hexane to methylene chloride–hexane (3/1 v/v) as an eluent. The fractions were combined and the solvents were removed to afford the desired product as a yellow-orange solid (0.32 g, 13% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.78 (s, 3H), 7.80 (d, $J = 8.0$ Hz, 3H), 7.45 (d, $J = 8.0$ Hz, 6H), 7.08–7.12 (m, 9H), 7.00–7.03 (m, 3H), 3.96 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3, 161.4, 147.2, 133.3, 130.8, 130.0, 124.2, 122.5, 120.3, 117.6, 112.1, 92.6, 88.7, 52.5. MALDI-TOF m/z calcd for $C_{48}H_{33}NO_9$, 767.216; found, 766.807.

Compound 4 in Scheme S1†. A mixture of **T3I** (2.00 g, 3.21 mmol), $PdCl_2(PPh_3)_2$ (0.11 g, 0.16 mmol), CuI (0.03 g, 0.16 mmol), methyl 4-ethynyl-2-hydroxybenzoate (1.24 g, 7.06 mmol) in toluene (30 mL) was added to DBU (2.0 mL) and the mixture was stirred at RT for 24 h. After the combined filtrate was evaporated and the residue was eluted through a silica gel

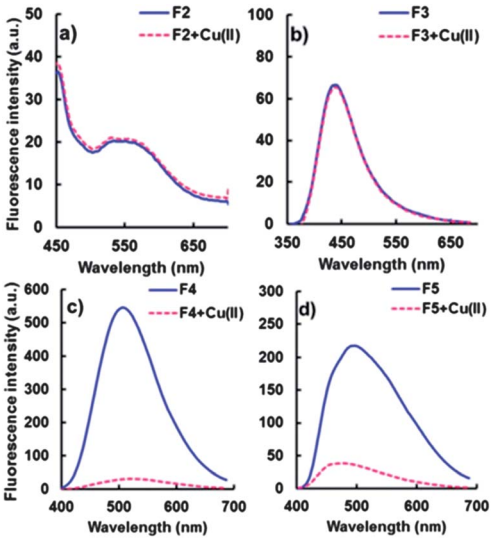

Fig. 2 Emission spectra of (a) **F2**, (b) **F3**, (c) **F4** and (d) **F5** (1 μM) in the absence and presence of Cu^{2+} (10 μM) in PBS pH 7.4.

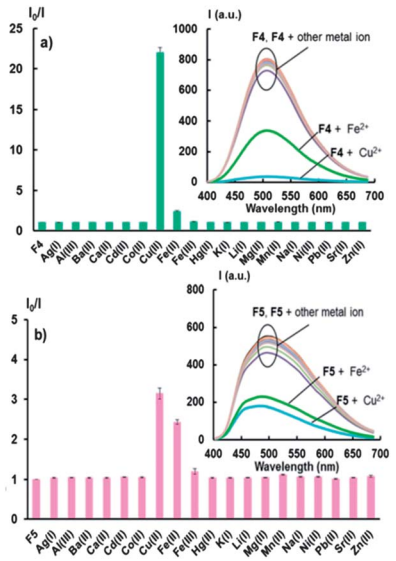

Fig. 3 Fluorescence quenching of (a) **F4** and (b) **F5** (5 μM) by various metal ions (5 μM) in PBS (10 mM, pH 7.4). The corresponding fluorescence spectra are shown in the insets.

column by gradient solvents from pure hexane to methylene chloride–hexane (2/1 v/v) as an eluent. The fractions were combined and the solvents were removed to afford the desired product as a dark yellow solid (0.76 g, 33% yield), ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.78 (s, 2H), 7.80 (d, $J = 8.0$ Hz, 2H), 7.59 (d, $J = 8.0$ Hz, 2H), 7.43 (d, $J = 8.0$ Hz, 4H), 7.11 (d, $J = 8.0$ Hz, 2H), 7.00–7.06 (m, 6H), 6.89 (d, $J = 8.0$ Hz, 2H), 3.96 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2, 161.3, 147.1, 146.4, 138.6, 133.1, 130.7, 129.8, 127.0, 123.6, 122.3, 120.2, 117.2, 111.9, 92.5, 88.5, 87.5, 52.4.

Compound 5 in Scheme S1†. A mixture of methyl 2-hydroxy-5-iodobenzoate (**I2SA^0**) (**4**) (2.00 g, 2.78 mmol), $PdCl_2(PPh_3)_2$ (0.06 g, 0.08 mmol), CuI (0.02 g, 0.08 mmol) and trimethylsilylacetylene (0.33 g, 3.3 mmol) in toluene (10 mL) was added to DBU (1 mL) and the mixture was stirred at room temperature for 3 h. The reaction mixture was then filtered and the solid was washed with toluene (3 × 15 mL). The filtrate was evaporated and the residue was eluted through a silica gel column by gradient solvents starting from pure hexane to dichloromethane–hexane (1/4 v/v) as an eluent to afford **5** as a yellow solid (0.81 g, 42% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.78 (s, 2H), 7.80 (d, $J = 8.0$ Hz, 2H), 7.41 (d, $J = 8.0$, 8.0 Hz, 6H), 7.11 (s, 2H), 7.03 (dd, 8H), 3.96 (s, 6H), 0.25 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2, 161.3, 147.1, 133.1, 130.7, 129.8, 123.9, 122.3, 120.2, 117.2, 111.9, 104.8, 94.2, 92.5, 88.5, 52.4, 0.0.

Compound 6 in Scheme S1†. A mixture of **5** (1.00 g, 1.45 mmol) and K_2CO_3 (0.059 g, 0.15 mmol) in dichloromethane (15 mL) and methanol (15 mL) was stirred at room temperature for 24 h. The organic layer was separated and the aqueous phase was extracted with dichloromethane (2 × 50 mL) and was then dried over anhydrous $MgSO_4$. The solvent was evaporated and the residue was eluted through a silica gel column by gradient solvents starting from pure hexane to dichloromethane–hexane (1/4 v/v) as an eluent to afford H2SA^0 (**6**) as a brown solid (0.74 g, 83% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.79 (s, 2H), 7.80 (d, $J = 8.0$ Hz, 2H), 7.59 (d, $J = 8.0$ Hz, 1.5H), 7.43 (d, $J = 8.0$ Hz, 4.5H), 7.00–7.12 (m, 8.5H), 6.89 (d, $J = 8.0$ Hz, 1.5H), 3.96 (s, 6H), 3.08 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3, 161.4, 147.2, 133.6, 133.3, 130.0, 124.4, 124.1, 122.5, 120.3, 117.5, 112.1, 92.6, 88.7, 83.5, 52.5.

Compound 7 in Scheme S1†. A mixture of **6** (0.50 g, 0.41 mmol), $PdCl_2(PPh_3)_2$ (0.02 g, 0.02 mmol), CuI (0.004 g, 0.02 mmol) in toluene (15 mL) was added DBU (1.0 mL) and the mixture was stirred at RT for 24 h. After the combined filtrate was evaporated, the residue was eluted through a silica gel column by gradient solvents from pure hexane to methylene chloride–hexane (3/1 v/v) as an eluent. **7** was obtained as a dark yellow solid (0.33 g, 65% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 10.79 (s, 4H), 7.80 (d, $J = 8.0$ Hz, 4H), 7.45 (d, $J = 8.0$ Hz, 12H), 7.01–7.12 (m, 20H), 3.96 (s, 12H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3, 161.4, 147.0, 133.3, 130.8, 130.0, 124.4, 122.5, 120.4, 117.8, 116.6, 112.1, 92.5, 88.8, 81.9, 74.3, 52.5. MALDI-TOF m/z calcd for $C_{80}H_{52}N_2O_{12}$, 1232.352; found, 1232.357.

F2. A mixture of **2** (0.50 g, 1.03 mmol) in THF (15 mL) and methanol (15 mL) was added to saturated KOH aqueous solution (0.5 mL) and the mixture was heated to 70 °C. After 24 h, the solution was evaporated and the residue was dissolved in water (20 mL). Approximately 50 g of ice was then added to the aqueous solution. The mixture was acidified and stored in the refrigerator for 1 h. The product was filtered to afford **F2** as a green solid (0.34 g, 72% yield). ^{1}H NMR (DMSO-d6, 400 MHz): δ (ppm) 8.00 (s, 2H), 7.85 (d, $J = 8.0$ Hz, 2H), 7.16–7.18 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 171.1, 160.7, 153.5, 133.3, 130.9, 128.1, 122.3, 119.6, 116.0, 114.1, 95.7, 87.8.

F3. A mixture of dimethyl **1** (0.50 g, 1.43 mmol) in THF (15 mL) and methanol (15 mL) was added to saturated KOH aqueous solution (0.5 mL) and the mixture was heated to 70 °C. After 24 h, the solution was evaporated and the residue was

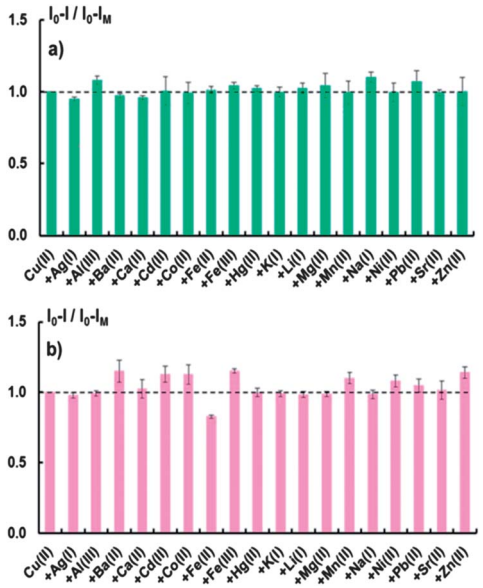

Fig. 4 Bar chart displays fluorescence intensity ratio (($I_0 - I$)/($I_0 - I_M$)) of (a) **F4** and (b) **F5** in the presence of Cu^{2+} (5 μM) and another interfering ion (50 μM).

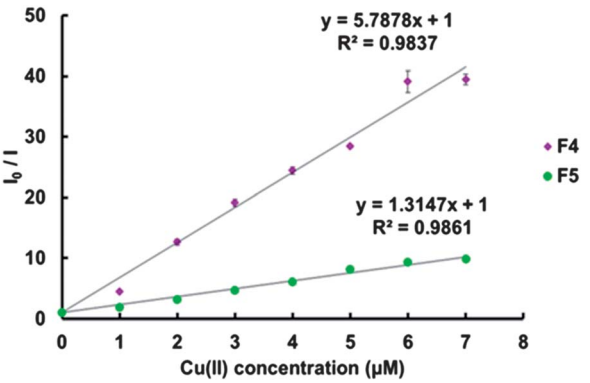

Fig. 5 Stern–Volmer plots for fluorescence quenching of **F4** and **F5** (1 μM) by Cu^{2+} in PBS (10 mM, pH 7.4).

dissolved in water (20 mL). Approximately 50 g of ice was then added to the aqueous solution. The mixture was acidified and stored in the refrigerator for 1 h. The product was filtered to afford $2SA^-$ as a light-yellow solid (0.42 g, 91% yield). ^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 7.82 (d, J = 8.0 Hz, 2H), 7.00–7.03 (m, 8H), 7.64; ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 131.9, 131.8, 130.4, 123.7, 123.2, 120.9, 91.5.

F4. A mixture of **3** (0.50 g, 0.41 mmol) in THF (15 mL) and methanol (15 mL) was added to saturated KOH aqueous solution (0.5 mL) and the mixture was heated to 70 °C. After 24 h, the solution was evaporated and the residue was dissolved in water (20 mL). Approximately 50 g of ice was then added and the aqueous solution was acidified and stored in the refrigerator for 1 h. The product was filtered to afford **F4** as a yellow solid (0.43 g, 92% yield). Mp: >200 °C to decompose. ^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 7.72 (d, J = 8.0 Hz, 3H), 7.35 (d, J = 8.0 Hz, 6H), 6.84–6.96 (m, 12H). ^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 173.02, 162.82, 148.38, 134.23, 131.62, 131.55, 125.28, 123.14, 120.58, 118.87, 113.77, 92.94, 89.45. MALDI-TOF m/z calcd for $C_{45}H_{27}NO_9$, 725.169; found, 725.210.

F5. A mixture of **7** (0.50 g, 0.41 mmol) in THF (15 mL) and methanol (15 mL) was added to saturated KOH aqueous solution (0.5 mL) and the mixture was heated to 70 °C. After 24 h, the solution was evaporated and the residue was dissolved in water (20 mL). Approximately 50 g of ice was then added to the aqueous solution, acidified and kept in a refrigerator for 1 h. The product was filtered to afford **F5** as a yellow solid (0.39 g, 82% yield). Mp: >200 °C to decompose. ^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 7.91 (d, 4H), 7.57 (m, 12H), 7.14 (m, 20H); ^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 173.0, 162.7, 148.0, 143.5, 135.2, 134.4, 131.6, 125.4, 123.4, 120.7, 119.0, 113.5, 93.3, 89.9; MS-ES$^-$ m/z calcd for $C_{76}H_{44}N_2O_{12}$, 1176.2894, 293.07 $[M]^{4-}$ found: 292.83 $[M]^{4-}$.

Paper-based sensor

F4 solution (1.0 mM) in ethanol was pipetted at 1.0 μL onto the surface of marked filter paper and allowed to air dry to generate fluorescent spots under an ordinary 20 W black light lamp. Filtered water samples, *i.e.* drinking, mineral, Milli-Q and river (Chaophraya), were pipetted on top of the fluorescent spots. After air drying, the images of the filter paper under the black light were photographically recorded.

Results and discussion

The synthesis of fluorophores **F2–F5** was accomplished by Sonogashira coupling[36,37] and acetylenic homocoupling[38,39] followed by base-catalyzed hydrolysis of the salicylate esters (Scheme S1†). The photophysical properties of **F2–F5** (spectra provided in Fig. S1†) in phosphate buffer saline (PBS), pH 7.4 are compiled in Table 1 along with those of **F1**. The fluorophores showed the maximum absorption wavelength (λ_{ab}) in the range of 316–395 nm. The order of λ_{ab} (**F2** > **F5** > **F4** > **F3** > **F1**) denotes

Table 2 Comparison of Cu^{2+} detection limit of **F4** with other optical sensing materials

Sensing materials	Solvent	Signal detected	Detection limit	Ref.
Iridium(III) complex (ZIr2)	PIPES buffer, pH 7.0	Turn-on emission	35 ppb	15
Imidazole-CP microspheres	Acetate–acetic buffer, pH 6.5	Turn-off emission	1 ppb	55
AuNPs	Tris–HCl, pH 7.4	Absorption	0.6 ppb	56
F4	PBS buffer, pH 7.4	Turn-off emission	0.5 ppb	This work
	Paper-based	Turn-off emission (visual observation)	10 pmol	

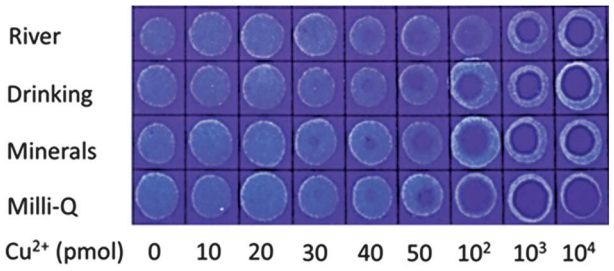

Fig. 6 Photographic images of **F4** (0.1 nmol) paper-based detection of Cu^{2+} under a 20 W black light.

the HOMO–LUMO gap associated with the delocalization of the π-conjugated system. The observed molar extinction coefficients of all fluorophores were well above 1×10^4 except for that of **F2** which was probably underestimated due to its poor solubility in water at this physiological pH .

All fluorophores showed maximum emission wavelengths (λ_{em}) in the range of 434–532 nm with the fluorescence quantum yield of 0.2–3.2%. Among the four new fluorophores, only **F4** showed higher quantum yield than **F1** probably due to the decrease of the self-quenching process, originating from its propeller-like conformation which precludes the intermolecular H-type π–π interaction.[40–44]

To evaluate the Cu^{2+} sensing ability of these new salicylic fluorophores, the fluorescence signals of **F2–F5** solution in PBS pH 7.4 were recorded in the absence and presence of Cu^{2+} (10 μM). As illustrated in Fig. 2, only the fluorescence signals of **F4** and **F5** are quenched by Cu^{2+}, while those of **F2** and **F3** do not show any response. The results indicated that not only the salicylic acid binding sites but also the π-conjugated systems are important for Cu^{2+} sensing ability. Since the fluorescence quenching mechanism of Cu^{2+} is mostly accepted as a photo-induced electron transfer (PET) process,[45–48] the quenching ability should thus depend on the energy of the LUMO level of the electron donating fluorophore matching that of the Cu^{2+} complex, the electron accepting unit. In other words, the LUMO level of **F2** is probably too low while that of **F3** is too high to allow the transfer of an excited electron from the fluorophores to the Cu^{2+}–salicylate complex.

The fluorescence quenching selectivity of **F4** and **F5** by metal ions (5 μM) such as Ag^+, Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Sr^{2+} and Zn^{2+} was evaluated. We found that the fluorescence signal of **F4** was most effectively quenched by Cu^{2+} (over 20 times) and somewhat quenched by Fe^{2+} (~2 times) (Fig. 3a). On the other hand, **F5** showed lower quenching sensitivity and selectivity for Cu^{2+} over Fe^{2+} (Fig. 3b). The interference test with the interfering metal ions at 10 times concentration showed negligible interference of all ions tested on **F4** (Fig. 4a) but discernible interference of Fe^{2+} tested on **F5** (Fig. 4b).

The Stern–Volmer plots of the fluorescence quenching against the Cu^{2+} concentration gave linear lines with the slopes corresponding to the quenching efficiency (K_{sv}) of 5.79×10^6 M^{-1} and 1.31×10^6 M^{-1} for **F4** and **F5**, respectively (Fig. 5). Clearly, **F4** is a better Cu^{2+} sensor than **F5** in terms of sensitivity, selectivity and its initial fluorescence quantum yield. In PBS pH 7.4, **F4** and **F5** gave the detection limits (at 3× noise) of 0.5 ppb and 6.9 ppb, respectively, well below the WHO limit of 2 mg L^{-1} (2 ppm) recommended for drinking water.[14]

Paper-based solid state sensors are very economical, environmentally friendly and convenient to use.[49–53] It is important to emphasize here that, compared with our previous fluorophore **F1**, **F4** not only possesses a 5 times higher quantum yield but also has a K_{sv} value 3 times higher.[34] This new fluorophore is thus tested for feasibility as a Cu^{2+} sensor detectable by the naked eye. A series of 1.0 μL **F4** solutions in ethanol was dropped onto a piece of filter paper and allowed to air dry to generate rows of bright blue fluorescent spots containing 1.0 nmol of **F4**. In the sensing test, 1.0 μL of each water sample, *i.e.* drinking, mineral, Milli-Q and river water containing various concentrations of Cu^{2+} (0–500 μM) was dropped on top of the fluorescent dots. Under an ordinary black light, a dark quenching area within the blue emission spots of **F4** was readily observed for the water samples containing at least 40 pmol of Cu^{2+}, representing the naked eye detection limit of this method.

In comparison with other sensing materials used for Cu^{2+} detection in aqueous solution, the Cu^{2+} detection limit of **F4** is one of the lowest as shown in Table 2.[15,19,54] To the best of our knowledge, the paper-based sensor constructed from micro-liter-drops presented in this work is also one of the most sensitive and convenient techniques for the naked eye detection of Cu^{2+} (Fig. 6).

Conclusion

Our comparison study on fluorophores **F1–F5** containing phenylene-ethynylene fluorescent units with salicylic acid termini identified **F4** as the most sensitive and selective sensor for Cu^{2+} detection in aqueous media. Not only its multiple salicylic binding units but also its optimal LUMO level are important for efficient fluorescence quenching by Cu^{2+}. **F4** also possesses high absorptivity and emission efficiency for Cu^{2+} which provides excellent visual contrast for naked eye detection of Cu^{2+} at the picomole level on a solid state paper-based sensor.

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Notes and references

1 H. L. Lelie, A. Liba, M. W. Bourassa, M. Chattopadhyay, P. K. Chan, E. B. Gralla, L. M. Miller, D. R. Borchelt, J. S. Valentine and J. P. Whitelegge, *J. Biol. Chem.*, 2010, **286**, 2795.
2 M. Kumar, N. Kumar, V. Bhalla, P. R. Sharma and T. Kaur, *Org. Lett.*, 2012, **14**, 406.
3 D. C. Kennedy, R. K. Lyn and J. P. Pezacki, *J. Am. Chem. Soc.*, 2009, **131**, 2444.
4 Y. Zhao, X.-B. Zhang, Z.-X. Han, L. Qiao, C.-Y. Li, L.-X. Jian, G.-L. Shen and R.-Q. Yu, *Anal. Chem.*, 2009, **81**, 7022.
5 D. Huster, T. D. Purnat, J. L. Burkhead, M. Ralle, O. Fiehn, F. Stuckert, N. E. Olson, D. Teupser and S. Lutsenko, *J. Biol. Chem.*, 2007, **282**, 8343.
6 S. C. Dodani, S. C. Leary, A. C. Paul, D. R. Winge and C. J. Chang, *J. Am. Chem. Soc.*, 2011, **133**, 8606.
7 D. J. Waggoner, T. B. Bartnikas and J. D. Gitlin, *Neurobiol. Dis.*, 1999, **6**, 221.
8 D. Strausak, J. F. B. Mercer, H. H. Dieter, W. Stremmel and G. Multhaup, *Brain Res. Bull.*, 2001, **55**, 175.
9 N. Kumar and P. A. Low, *J. Neurol.*, 2004, **251**, 747.
10 T. R. Halfdanarson, N. Kumar, C.-Y. Li, R. L. Phyliky and W. J. Hogan, *Eur. J. Haematol.*, 2008, **80**, 523.
11 A. Ala, A. P. Walker, K. Ashkan, J. S. Dooley and M. L. Schilsky, *Lancet*, 2007, **369**, 397.
12 I. G. Macreadie, *Eur. Biophys. J.*, 2008, **37**, 295.
13 E. Gaggelli, H. Kozlowski, D. Valensin and G. Valensin, *Chem. Rev.*, 2006, **106**, 1995.
14 Copper in Drinking-water, World Health Organization (WHO), 2004.
15 A. Alvarez-Diaz, A. Salinas-Castillo, M. Camprubi-Robles, J. M. Costa-Fernandez, R. Pereiro, R. Mallavia and A. Sanz-Medel, *Anal. Chem.*, 2011, **83**, 2712.
16 X. Zhou, F. Su, Y. Tian, C. Youngbull, R. H. Johnson and D. R. Meldrum, *J. Am. Chem. Soc.*, 2011, **133**, 18530.
17 X. J. Zhao and C. Z. Huang, *Biosens. Bioelectron.*, 2011, **30**, 282.
18 K. Sasakura, K. Hanaoka, N. Shibuya, Y. Mikami, Y. Kimura, T. Komatsu, T. Ueno, T. Terai, H. Kimura and T. Nagano, *J. Am. Chem. Soc.*, 2011, **133**, 18003.
19 X. Yang and E. Wang, *Anal. Chem.*, 2011, **83**, 5005.
20 N. R. Chereddy and S. Thennarasu, *Dyes Pigm.*, 2011, **91**, 378.
21 S. Dalapati, S. Jana, M. A. Alam and N. Guchhait, *Sens. Actuators, B*, 2011, **160**, 1106.
22 R. Sheng, P. Wang, Y. Gao, Y. Wu, W. Liu, J. Ma, H. Li and S. Wu, *Org. Lett.*, 2008, **10**, 5015.
23 Y. Yang, W. Gao, R. Sheng, W. Wang, H. Liu, W. Yang, T. Zhang and X. Zhang, *Spectrochim. Acta, Part A*, 2011, **8**, 14.
24 S. Basurto, O. Riant, D. Moreno, J. Rojo and T. J. Torroba, *J. Org. Chem.*, 2007, **72**, 4673.
25 C.-H. Lu, Y.-W. Wang, S.-L. Ye, G.-N. Chen and H.-H. Yang, *NPG Asia Mater.*, 2012, **4**, e10.
26 Y.-R. Ma, H.-Y. Niu, X.-L. Zhang and Y.-Q. Cai, *Chem. Commun.*, 2011, **47**, 12643.
27 T. M. Swager, *Acc. Chem. Res.*, 1998, **31**, 201–207.
28 B. Liu and G. C. Bazan, *J. Am. Chem. Soc.*, 2006, **128**, 1188.
29 S. W. Thomas III, G. D. Joly and T. M. Swager, *Chem. Rev.*, 2007, **107**, 1339.
30 D. Xie, A. Parthasarathy and K. S. Schanze, *Langmuir*, 2011, **27**, 11732.
31 F. Vögtle, S. Gestermann, C. Kauffmann, P. Ceroni, V. Vicinelli and V. Balzani, *J. Am. Chem. Soc.*, 2000, **122**, 10398.
32 N. Niamnont, W. Siripornnoppakhun, P. Rashatasakhon and M. Sukwattanasinitt, *Org. Lett.*, 2009, **11**, 2768.
33 P. Rashatasakhon, K. Vongnam, W. Siripornnoppakhun, T. Vilaivan and M. Sukwattanasinitt, *Talanta*, 2012, **88**, 593.
34 S. Sirilaksanapong, M. Sukwattanasinitt and P. Rashatasakhon, *Chem. Commun.*, 2012, **48**, 293.
35 W. Yuanboonlim, W. Siripornnoppakhun, N. Niamnont, P. Rashatasakhon, T. Vilaivan and M. Sukwattanasinitt, *Biosens. Bioelectron.*, 2012, **33**, 17–22.
36 P. Hrobárik, V. Hrobáriková, I. Sigmundová, P. Zahradník, M. Fakis, I. Polyzos and P. Persephonis, *J. Org. Chem.*, 2011, **76**, 8726.
37 D. Jana and B. K. Ghorai, *Tetrahedron Lett.*, 2012, **53**, 196.
38 J.-H. Li, Y. Liang and Y.-X. Xie, *J. Org. Chem.*, 2005, **70**, 4393.
39 J. Yan, J. Wu and H. Jin, *J. Organomet. Chem.*, 2007, **692**, 3636.
40 H. D. Burrows, V. M. M. Lobo, J. Pina, M. L. Ramos, J. Seixas de Melo, A. J. M. Valente, M. J. Tapia, S. Pradhan and U. Scherf, *Macromolecules*, 2004, **37**, 7425.
41 P. Kaur, H. Yue, M. Wu, M. Liu, J. Treece and D. H. Waldeck, *J. Phys. Chem. B*, 2007, **111**, 8589.
42 Q. Chu and Y. Pang, *Macromolecules*, 2003, **36**, 4614.
43 S. Masuo, H. Yoshikawa, T. Asahi and H. Masuhara, *J. Phys. Chem. B*, 2003, **107**, 2471.
44 Q. Chu and Y. Pang, *Macromolecules*, 2005, **38**, 517.
45 S. Kumar, P. Singh and S. Kaur, *Tetrahedron*, 2007, **63**, 11724.
46 Y. Zhou, K. Liu, J.-Y. Li, Y. Fang, T.-C. Zhao and C. Yao, *Org. Lett.*, 2011, **13**, 1290.
47 X. Xie and Y. Qin, *Sens. Actuators, B*, 2011, **156**, 213.
48 Y. Liu, X. Lv, Y. Zhao, J. Liu, Y.-Q. Sun, P. Wang and W. Guo, *J. Mater. Chem.*, 2012, **22**, 1747.
49 A. W. Martinez, S. T. Phillips, E. Carrilho, S. W. Thomas III, H. Sindi and G. M. Whitesides, *Anal. Chem.*, 2008, **80**, 3699.
50 A. W. Martinez, S. T. Phillips, G. M. Whitesides and E. Carrilho, *Anal. Chem.*, 2010, **82**, 3.
51 W. Thongmalai, T. Eaidkong, S. Ampornpun, R. Mungkarndee, G. Tumcharern, M. Sukwattanasinitt and S. Wacharasindhu, *J. Mater. Chem.*, 2011, **21**, 16391.
52 S. Pumtang, W. Siripornnoppakhun, M. Sukwattanasinitt and A. Ajavakom, *J. Colloid Interface Sci.*, 2011, **364**, 366.
53 T. Eaidkong, R. Mungkarndee, C. Phollookin, G. Tumcharern, M. Sukwattanasinitt and S. Wacharasindhu, *J. Mater. Chem.*, 2012, **22**, 5970.
54 Y. You, Y. Han, Y.-M. Lee, S. Y. Park, W. Nam and S. J. Lippard, *J. Am. Chem. Soc.*, 2011, **133**, 11488.
55 A. Alvarez-Diaz, A. Salinas-Castillo, M. Camprubi-Robles, J. M. Costa-Fernandez, R. Pereiro, R. Mallavia and A. Sanz-Medel, *Anal. Chem.*, 2011, **83**, 2712.
56 C.-H. Lu, Y.-W. Wang, S.-L. Ye, G.-N. Chen and H.-H. Yang, *NPG Asia Mater.*, 2012, **4**, e10.

