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Molecular Devices

A Fluorophore Capable of Crossword Puzzles and Logic Memory**

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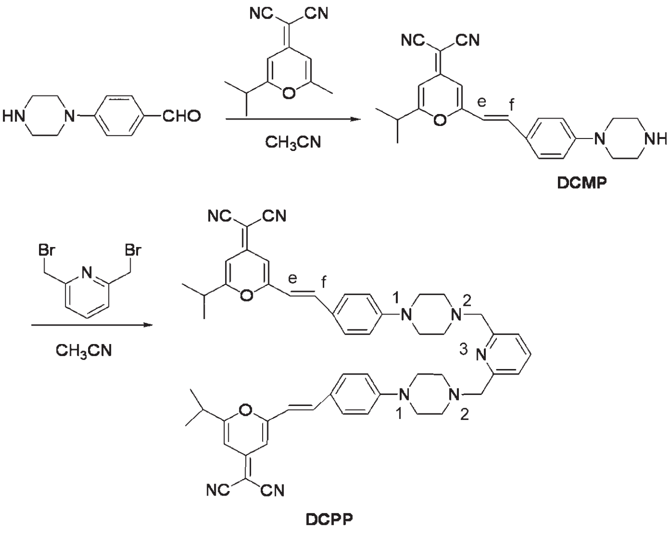
Dedicated to Professor Vincenzo Balzani on the occasion of his 70th birthday

Computer memories have been reaching higher bit densities for decades, although it is becoming ever more difficult to keep up with this long-term trend. Many researchers have therefore begun to think seriously about building memory devices from the bottom up using individual molecules.[1] A very recent proof-of-concept study along these lines showed that molecular memory cells can be made with a bit density of 10^{11} per square centimeter.[2] In addition, by harnessing the principles of molecular Boolean logic, the first example of a molecular system that mimics a keypad and is capable of processing password entries was described. Such molecular devices that are capable of distinguishing between different chemical sequences might have considerable advantages over simple molecular logic gates.[3]

Herein we present the novel fluorescent compound DCPP (see Scheme 1), which possesses both 2-{2-isopropyl-6-[4-(piperazin-1-yl)styryl]-4*H*-pyran-4-ylidene}malononitrile (DCMP) and 2,6-bis(aminomethyl)pyridine moieties. Remarkably, DCCP shows a reversible and controllable fluorescent behavior as a result of competitive coordination of Hg^{2+} and Cu^{2+} ions. The characteristic fluorescence of Hg^{2+}-selective OFF-ON and Cu^{2+}-selective ON-OFF operations can be controlled by varying the sequence of Hg^{2+} and Cu^{2+} inputs, and this has been used to construct sequential logic circuits capable of memory function.[4] The output signals in this molecular logic system are dependent not only upon the appropriate combination of chemical inputs but also upon the exact sequence of these inputs. An easy-to-handle molecular keyboard capable of crossword puzzles can be established based on the above logic operation.

The design and synthesis of chemosensors with high selectivity and sensitivity for heavy and/or transition metal (HTM) ions continues to be an active area of supramolecular chemistry.[5] Fluorescent sensors that change their fluorescence properties upon binding HTM ions (such as Hg^{2+} and Cu^{2+})[6] are one of the most widely used analytical tools, and various molecular switches and logic gates have been proposed that convert a change produced by external stimuli such as chemical species and light into detectable outputs.[7] Chemically triggered logic gates operating on solid supports have stimulated chemists to develop a new class of programmable logic gates that are capable of executing logic functions.[8] However, it is still a great challenge to implement combinatorial logic circuits in a single molecular entity. To date, only a few such examples can selectively and reversibly identify HTM ions and emit distinct signals,[9] thereby limiting the application of these systems as molecular logic gates.

DCPP, which can competitively coordinate Hg^{2+} and Cu^{2+} ions, is derived from the well known laser dye 4-dicyanomethylene-6-[4-(dimethylamino)styryl]-2-methyl-4*H*-pyran (DCM).[10] The synthesis of DCPP is straightforward and is depicted in Scheme 1. The intermediate DCMP was synthesized in 68 % yield by treating 4-(piperazin-1-yl)benzaldehyde with 2-isopropyl-6-methyl-4-dicyanomethylene-4*H*-pyran. DCPP was finally obtained from the reaction between DCMP and 2,6-bis(bromomethyl)pyridine[11] in acetonitrile with a yield of about 26%. The characteristic coupling between protons e and f in the ^1H NMR spectrum of DCMP and DCPP ($J = 16.0$ Hz) is indicative of the predominant *trans* isomer.



Scheme 1. Synthetic route to sensor DCPP.

DCPP is an A-π-D type laser dye with a broad absorption band resulting from an ultra-fast internal charge-transfer (ICT) process. As shown in Figure 1, free DCPP has a characteristic emission at around 621 nm with moderate

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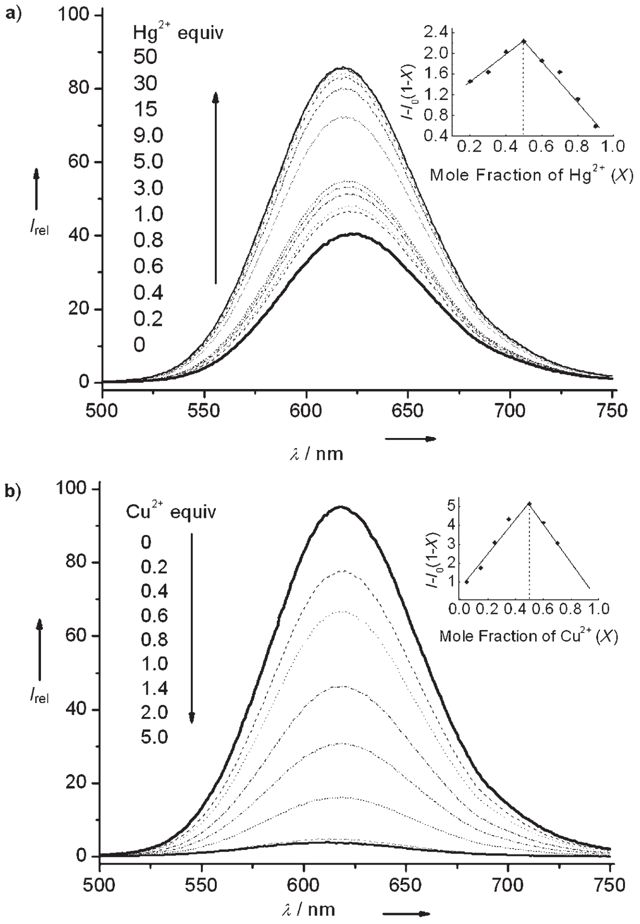


Figure 1. Emission spectra of DCPP (5.0 μM) in a mixture of H_2O and CH_3CN (4/6, v/v) with a buffer solution of Na_2HPO_4/KH_2PO_4 (0.01 M, pH 7.0) upon titrating different molar ratios of Hg^{2+} (a) and Cu^{2+} ions (b) with excitation at 440 nm. $I_{rel} = 100$ corresponds to $I = 1000$ in the case of (a) and to $I = 390$ in the case of (b). Insets: Job's plots; the total concentration of DCPP and Hg^{2+} or Cu^{2+} ion is 10.0 μM.

efficiency ($\Phi_{DCPP} = 0.09$)[12] resulting from this ICT process. There are three kinds of nitrogen atoms in DCPP that can coordinate to metal ions (Scheme 1), namely the aniline nitrogen (N^1), the piperazine nitrogen (N^2), and the pyridine nitrogen (N^3) atom, which play different roles in the fluorescence. Thus, metal ions will increase the fluorescence intensity (fluorescence ON) when coordinated to N^2 and N^3 as this coordination will stop the photo-induced electron transfer (PET) from N^2 to N^1.[5f] On the other hand, metal ions bonded to N^1 will tend to reduce the fluorescence (fluorescence OFF) due to a decrease in the electron-donating ability of this nitrogen atom.[13] Such a design is likely to yield distinct fluorescence signals depending on the target species coordinated to specific nitrogen atoms.

Indeed, a distinct enhancement of the fluorescence intensity for DCPP was observed upon interaction with Hg^{2+} (Figure 1a). An enhancement factor (EF, I/I_0) of 2.2 at 621 nm was found in the presence of about five equivalents of Hg^{2+}, thus indicating that the PET quenching pathway is efficiently blocked by the likely coordination of the Hg^{2+} ion to N^2 and N^3 in DCPP. In contrast, a quenching was observed upon titration with Cu^{2+}, with the fluorescence being almost completely quenched to the baseline upon addition of two equivalents of Cu^{2+} (Figure 1b). This quenching is not unexpected because this ion can, and in fact does, quench most excited states by energy and/or electron transfer since it is easily reduced. Notably, the fluorescence peak of DCPP is not so strongly affected by the titration with other metal ions. None of the above phenomena were observed for DCMP, which suggests that the configuration of DCPP is critical for the selectivity and sensitivity to metal ions.

^{1}H NMR studies of the interaction of Hg^{2+} with DCPP give useful information consistent with the above analysis. The peaks at around $\delta = 7.75$ ppm (Figure 2), which were assigned to pyridine proton d (Scheme 2) in free DCPP, shift significantly downfield to around $\delta = 8.06$ ppm upon metal coordination. The signals of the protons b of the piperazine moiety also undergo a downfield shift from $\delta = 2.62$ to

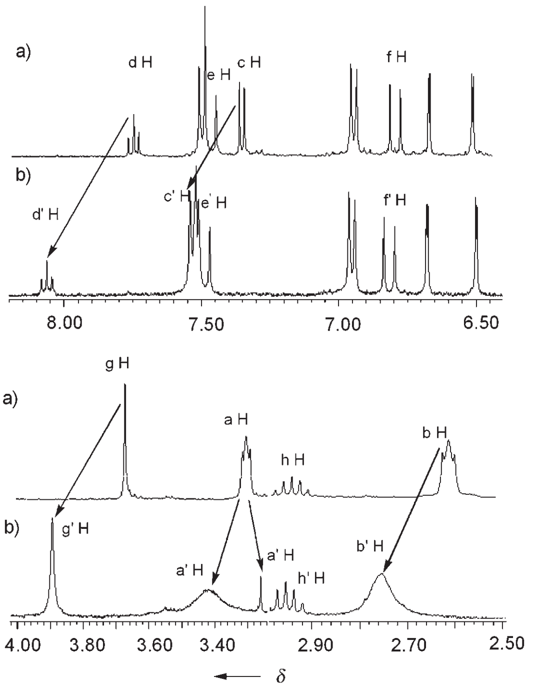
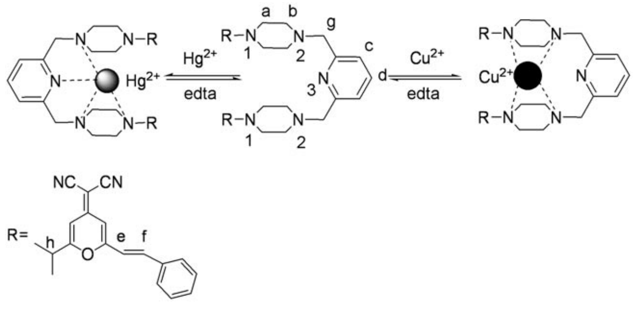


Figure 2. Parts of the ^{1}H NMR spectra of: a) free DCPP and b) DCPP-Hg^{2+} in CD_3CN.



Scheme 2. Proposed sensing process of DCPP by characteristic square-planar coordination with Cu^{2+} and tetrahedral coordination with Hg^{2+}, both of which can be reset by addition of edta.

2.76 ppm upon metal coordination and they clearly broaden. These deshielding effects confirm the coordination of Hg^{2+} to N^2 and N^3 of DCPP (Scheme 2). The protons a behave differently upon addition of Hg^{2+}, with one of the two peaks shifting downfield from $\delta = 3.30$ to 3.41 ppm and the other shifting slightly upfield from $\delta = 3.30$ to 3.26 ppm. This behavior is attributed to a shielding effect induced by the coordination of Hg^{2+} to N^2, which might indicate that the Hg^{2+} ion coordinates to only one of the two N^1 atoms in DCPP (Scheme 2). This observation is consistent with a previous report concerning the regular tetrahedral coordination with Hg^{2+}.[6d]

Unfortunately, the ^1H NMR spectrum of DCPP-Cu^{2+} does not provide useful information due to the paramagnetic nature of the metal ion. An intense peak for DCPP-Cu^{2+} at *m/z* 910.4 (100 %), with the correct isotope distribution, in the electrospray ionization (ESI) HPLC mass spectrum, however, provides strong evidence for the formation of this complex (see Figure S14 in the Supporting Information). It is likely that DCPP is coordinated to the Cu^{2+} ion in a characteristic square-planar coordination, as shown in Scheme 2. The energy-minimized conformations of DCPP-Hg^{2+} and DCPP-Cu^{2+} were also simulated with the Hyperchem software to further support the above-mentioned square-planar coordination with Cu^{2+} and the characteristic tetrahedral coordination with Hg^{2+} (see Figures S15–S17 in the Supporting Information).

Fluorescence titrations of DCPP with various metal ions (see Figures S4 and S5 in the Supporting Information) clearly showed that the fluorescence change due to the addition of metal ions other than Cu^{2+} and Hg^{2+} is negligible. Both the linear decrease of fluorescence intensity within the equivalent range of Cu^{2+} ion and the Job's plot (Figure 1 b) indicate that DCPP forms a 1:1 complex with Cu^{2+}. The association constant (K_{ass}) of the DCPP-Cu^{2+} complex, as calculated from the titration curve, is $4.36 \times 10^5\,\text{M}^{-1}$.[14a] Similarly, the appropriate Job's plot also suggests that DCPP forms a 1:1 complex with Hg^{2+}, with a K_{ass} of $1.85 \times 10^5\,\text{M}^{-1}$, as calculated from a non-linear least-squares equation (see the Supporting Information). The detection limits for Cu^{2+} and Hg^{2+} can be estimated from the titration results to be 1.2×10^{-7} and $3.0 \times 10^{-7}\,\text{M}$, respectively.[14b]

Since the K_{ass} values of DCPP-Cu^{2+} and DCPP-Hg^{2+} are approximately equal Cu^{2+} and Hg^{2+} can compete against each other to coordinate with DCPP. As shown in Figure 3, a significant quenching of the fluorescence of DCPP (fluorescence OFF) is obtained upon adding Cu^{2+} due to the formation of DCPP-Cu^{2+}. Sequential titration with 50 equivalents of Hg^{2+} and 30 equivalents of Cu^{2+} caused the fluorescence to vary from its initial maximum intensity (ON) to quenched fluorescence (OFF). The competing coordination of Cu^{2+} and Hg^{2+} with DCPP therefore results in a transformation between DCPP-Cu^{2+} and DCPP-Hg^{2+}. Moreover, a specific signal pattern was also observed upon changing the addition sequence of Hg^{2+} (5 equiv) and Cu^{2+} (3 equiv) (see Figures S6 and S7 in the Supporting Information).

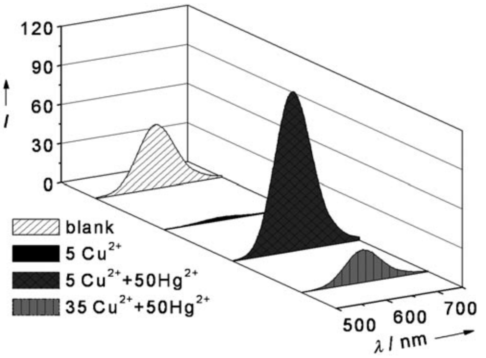


Figure 3. Emission spectra of DCPP (5.0 μM) in a mixture of H_2O and CH_3CN (4/6, v/v) with a buffer solution of Na_2HPO_4/KH_2PO_4 (0.01 M, pH 7.0) upon titrating different molar ratios of Cu^{2+} and Hg^{2+} when excited at a wavelength of 440 nm: Cu^{2+}-Hg^{2+}-Cu^{2+} OFF-ON-OFF.

To take advantage of the reversibility, selectivity, and fast response (see Figures S10 and S11 in the Supporting Information) of this system, a sequential logic circuit capable of memory function was built. The appropriate choice of addition or subtraction chemical inputs induced by Cu^{2+} or Hg^{2+} ions was used to control the algebraic operation. The metal ions Cu^{2+} (3 equiv) and Hg^{2+} (5 equiv) are defined as In 1 and In 2, respectively, in Figure 4. The value of I/I_0 at 621 nm is designated as either output "0" ($I/I_0 < 1.20$) or "1" ($I/I_0 > 1.20$). The sequence of adding Cu^{2+} and Hg^{2+} ions, which leads to the input string 11, determines the values of the output. Thus, when the first input signal is Cu^{2+} (3 equiv) as "1" and the second input signal is Hg^{2+} (5 equiv) as "1", the output digit is "0" (Figure 4 a). However, changing the sequence of adding Cu^{2+} and Hg^{2+} ions gives the output digit "1" (Figure 4 b). Accordingly, the output value in the previous string is memorized and maintained when both inputs become "1".

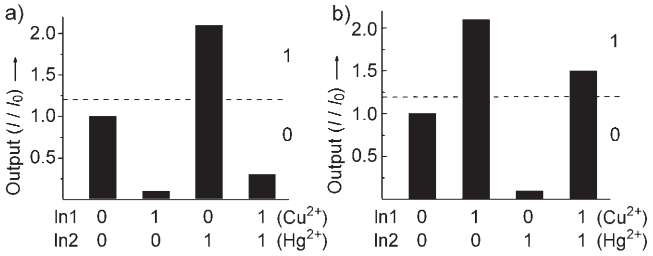


Figure 4. Output (I/I_0) resulting from different input sequences: a) In 1 (Cu^{2+} (3 equiv) first) and In 2 (Hg^{2+} (5 equiv) second); b) In 1 (Hg^{2+} (5 equiv) first) and In 2 (Cu^{2+} (3 equiv) second); the dashed line shows the threshold (1.20) determined after 5 min.

To visualize these sequence-dependent phenomena directly, this sequential logic operation with memory function can be constructed as a crossword puzzle. As illustrated in Figure 5, the input of Hg^{2+} (5 equiv) is designated as the character "U" and the input of Cu^{2+} (3 equiv) as the character "S". When the input signal "U" is added first, emission as the character "E" (strong fluorescence signal, ON) is observed as a result of memorizing the input sequence. This combination of inputs gives the word "USE" in the crossword. Inverting

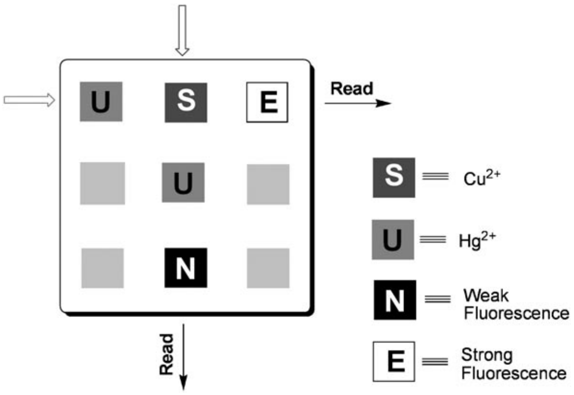


Figure 5. Crossword puzzles that allow direct visualization of the sequence dependence. For details see text.

the addition sequence of Cu^{2+} and Hg^{2+} gives an obvious fluorescence quenching (OFF, designated as the character "N"). This input sequence gives the word "SUN" in the crossword, therefore different character strings are produced by changing the input sequences (Figure 5). Furthermore, the cycle can be repeated by adding a strong chelate such as ethylenediamine tetraacetic acid (edta; see Scheme 2 and Figure S8 in the Supporting Information) due to the large difference in association constants (K_{ass}) between edta and DCPP with these metals ($\log K_{ass}$ of edta with Cu^{2+} and Hg^{2+} is 18.70 and 21.80,[15] respectively, which indicates that edta binds much more strongly with Cu^{2+} and Hg^{2+} ions than DCPP). This means that the logic operation reported here can be reset.

A similar sequence-dependent phenomenon has been reported in the literature,[1c] although the short timescale limited its application. Our system overcomes this problem as it remains stable for a reasonably long time (> 12 h; see Figures S12 and S13 in the Supporting Information) over the threshold of 1.2. It should be pointed out, however, that the Hg^{2+}:Cu^{2+} ratio is critical to the sequence dependence.[16] Thus, changing the ratio of Hg^{2+} and Cu^{2+} to (50 and 5 equiv, respectively (Figure 3 and Figure S9 in the Supporting Information), gave only one fluorescence signal (ON), even when changing the addition sequence. Thus, the coordination of DCPP with Hg^{2+} and Cu^{2+} is a competitive process that occurs under kinetic control.

In summary, a novel fluorescent probe (DCPP) that possesses both DCMP and 2,6-bis(aminomethyl)pyridine moieties has been designed for the detection of Hg^{2+} and Cu^{2+} ions. The characteristic fluorescence of Hg^{2+}-selective OFF-ON and Cu^{2+}-selective ON-OFF operations can be monitored and controlled reversibly by the addition sequence and ratio of Hg^{2+} and Cu^{2+} inputs, and this has been used to construct a crossword puzzle and a logic memory at the molecular level. This molecular logic system has the following advantages over other reported combinatorial logic circuits: 1) a characteristic signal pattern that performs distinct algebraic operations solely in the fluorescence mode; 2) a single fluorescence intensity measurement setup for direct "reading" of the arithmetic results at the same excitation wavelength; 3) a reset function; and 4) is easy to handle by controlling the input sequence. This concept can be expected to greatly enhance combinatorial logic operations in sequential logic circuits with a memory function.

Experimental Section

The synthesis of DCPP was performed as follows. A mixture of DCMP (135 mg, 0.363 mmol), 2,6-di(bromomethyl)pyridine (44 mg, 0.165 mmol), potassium carbonate (5 mg) as catalyst, and acetonitrile (30 mL) was stirred and refluxed for 15 h under nitrogen. After cooling to room temperature the mixture was concentrated under vacuum to afford a dark red solid. The product was purified by column chromatography with dichloromethane/methanol (15/1, v/v) as eluent to give DCPP (85 mg, yield: 28%); m.p. 188–190°C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.33$ (d, $J = 6.8$ Hz, 12H; $CH(CH_3)_2$), 2.69 (t, $J = 4.8$ Hz; 8H; CH_2), 2.88 (m, 2H; $CH(CH_3)_2$), 3.37 (t, $J = 4.8$ Hz; 8H; CH_2), 3.75 (s, 4H; CH_2-pyridine), 6.46 (s, 2H; pyran-H), 6.51 (d, $J = 16.0$ Hz, 2H; CH=), 6.58 (s, 2H; pyran-H), 6.89 (d, $J = 8.4$ Hz, 4H; phenyl-H), 7.33 (d, $J = 16.0$ Hz, 2H; CH=), 7.38 (d, $J = 7.6$ Hz, 2H; pyridine-H), 7.43 (d, $J = 8.4$ Hz, 4H; phenyl-H), 7.69 ppm (2d, $J = 7.6$ Hz, 1H; pyridine-H); ^{13}C NMR (400 MHz, $CDCl_3$): $\delta = 20.29$, 32.96, 47.60, 52.93, 58.00, 64.33, 103.51, 106.06, 114.20, 114.79, 115.57, 121.65, 124.74, 129.44, 136.98, 138.02, 152.51, 156.66, 157.68, 159.94, 169.89 ppm; MS(ESI positive ion mode for M+H): calcd. 848.4400; found 848.4416.

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