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Chromene "Lock", Thiol "Key", and Mercury(II) Ion "Hand": A Single Molecular Machine Recognition System

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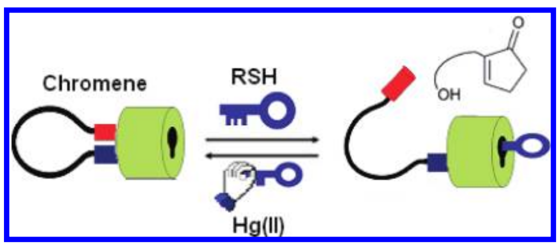
ABSTRACT



A regenerative, molecular machine-like "ON−OFF−ON" chemosensor based on a chromene molecule with the pyran ring "OFF−ON−OFF" cycle is reported for the first time. It behaves as a molecular lock that requires a thiol "key" to open the lock and a mercury(II) ion "hand" that unlatches the key for unsheathing the key to close the lock.

There is considerable interest and intense activity in constructing molecular-level devices with molecular recognition functionality and signal transduction ability in chemistry,[1] biology,[2] and sensors.[3] Especially important and significant in this regard are sensors that detect sulfhydryl-containing amino acids and peptides, cysteine (Cys), homocysteine (Hcy), and glutathione (GSH), as they play many crucial roles in many physiological processes.[4] In recent years, great effort has been put into the development of thiol detection by means such as high-performance liquid chromatography,[5] capillary electrophoresis,[6] Ellman's reagent,[7] cleavage reactions by thiol,[8] cyclization reactions with aldehyde,[9] and others.[10]

One of the most attractive approaches involves the construction of receptors of a thiol-based reaction through the powerful

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"click" chemisty.[11,12] A few excellent receptors have been exploited such as OND,[12a] squaraine,[12b,c] coumarin dyes,[12d,e] maleimide,[12f,g] propiolate,[12h] and quinone.[12i,j]

Very recently, we demonstrated a colorimetric probe for thiol based on the ring-opening mechanism of the chromene molecule, 7-nitro-2,3-dihydro-1*H*-cyclopenta[*b*]chromen-1-one, in aqueous solution.[13] Herein, we report a new, regenerative, fluorescence "ON−OFF−ON" probe for thiol and the mercury(II) ion, 7-chloro-2,3-dihydro-1*H*-cyclopent-a[*b*]chromene-1-one (**1**) (Figure 1, prepared by Baylis−Hillman

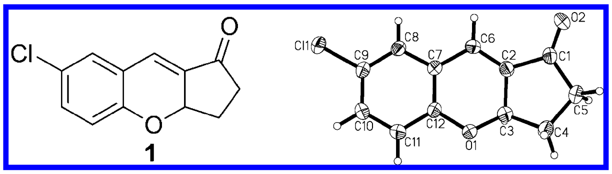
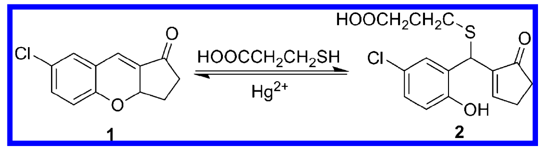


Figure 1. Structure and thermal ellipsoids of probe **1** are drawn at the 50% probability level.

and intramolecular Michael addition reactions (Figure S1, Supporting Information)).[14] We also report a single molecular lock based on the following phenomenon: a thiol-chromene "click" nucleophilic pyran ring-opening reaction with a thiol key to open the lock and Hg^{2+}-promoted desulfurization and subsequent intramolecular Michael addition to the pyran ring with the mercury(II) ion as a "hand" unsheathing the key to close the lock.

Scheme 1



Scheme 1 depicts the reversible character of the thiol-chromene "click" nucleophilic pyran ring-opening reaction of **1** and Hg^{2+}-induced cyclization of **2** to chromene as a sensing mechanism. The nucleophilic addition of mercaptopropionic acid (MPA) to the probe **1** leads rapidly to the formation of the ring-opened, low fluorescent product **2** and demonstrates a fluorescence quenching effect. 1D ^{1}H NMR, ^{13}C NMR, 2D ^{1}H−^{1}H COSY, and ESI mass spectrometry confirmed a thiol-quantitative reaction (Figure S2, Supporting Information). A kinetic study of the response of MPA to probe **1** under pseudofirst-order conditions (30 μM probe **1** and 300 μM MPA) is shown in Figure S3 (Supporting Information). The nucleophilic ring-opening reaction was completed in less than 3 min at room temperature in aqueous media.

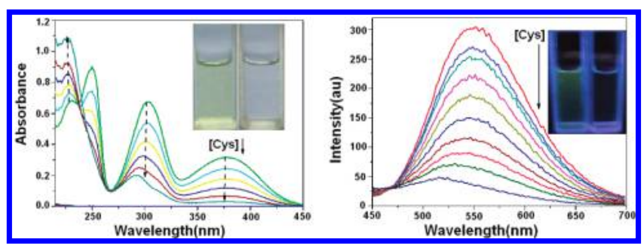


Figure 2. (Left) Absorption spectral changes of **1** (30 μM) in Tris-HCl 10 mM containing 0.15% EtOH, pH 8.0 aqueous buffer upon addition of Cys. Cys was added gradually at [Cys] = 0−90 μM. Each spectrum was recorded 3 min after Cys addition. (Right) Fluorescence spectral changes of **1** (3 μM) upon addition of Cys (0−9 μM) (λ_{ex} = 380 nm, λ_{em} = 550 nm; slit, 10 nm/10 nm) in 10 mM Tris-HCl containing 0.15% EtOH, pH 8.0 aqueous buffer upon addition of Cys. Each spectrum was recorded 3 min after Cys addition. Inset: color (left) and visual fluorescence (right) change photographs of **1** (100 μM) upon addition of Cys in Tris-HCl/ethanol (200:1, v/v, 10 mM, pH 8.0) buffer solution under illumination with a UV 365 nm lamp.

Figure 2 (left) shows the change in the UV/visible spectrum when the Cys solution was added to Tris-HCl buffer (10 mM, pH 8.0) containing probe **1** (30 μM). With increasing Cys concentration, the probe **1** absorption peaks at 250, 302, and 377 nm gradually decreased, and a new peak appeared at 227 nm (blue-shifted 23, 75, and 150 nm, respectively) with an isosbestic point at 236 nm, indicating the formation of the product **2**. Figure 2 (right) displays the

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(14) See Supporting Information. The probe **1** molecule: ^{1}H NMR (600 MHz, 25°C, DMSO-d_6): δ 7.54 (s, 1H), 7.34 (d, 1H), 7.25 (s, 1H), 6.96 (d, 1H), 5.32 (t, 1H), 2.58−2.63 (m, 1H), 2.45−2.49 (m, 1H), 2.36−2.42 (m, 1H), 2.00−2.08 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 27.99, 37.05, 75.91, 117.87, 123.21, 126.30, 127.07, 129.55, 131.91, 132.73, 157.46, 201.08. Elemental analysis (calcd %) for $C_{12}H_9ClO_2$: C, 65.32; H, 4.11. Found: C, 65.64; H, 4.21. ESI-MS *m/z* 220.9 (30%, $[M]^+$): calculated 220.7 $[M]^+$. Crystal data for **1**: $C_{12}H_9ClO_2$, FW = 220.64, crystal size: 0.1 × 0.1 × 0.1 mm, orthorhombic, space group *Pbca* (No.61), a = 15.103(3) Å, b = 6.0078(12) Å, c = 21.747(4) Å, β = 90.000°, V = 1973.2(7) Å^3, Z = 8, T = 183 K, θ_{max} = 25.35°, 7615 reflections measured, 1766 unique (R_{int} = 0.041). Final residual for 137 parameters and 1575 reflections with $I > 2\sigma(I)$: R_1 = 0.0442, wR_2 = 0.1037, and GOF = 1.09.

emission peak of **1** at 550 nm (λ_{ex} =380 nm) that decreased rapidly upon addition of Cys and eventually stopped with a saturation point around 3 equiv of Cys. When 3 equiv of Cys was added to the solution of **1**, a more than 10-fold decrease in fluorescent intensity at 550 nm was observed.

Simultaneously, an elaborate assay was carried out by fluorescence titration (Figure S4, Supporting Information) and showed a linear response of probe **1** to increasing amounts of even low Cys concentration from 3×10^{-7} to 3.9×10^{-6} M. GSH and HCy can also play the same roles. These indicate that probe **1** can respond to thiol at low micromolar levels. Other amino acids did not affect the detection of Cys (Figure S5, Supporting Information).

It is very exciting and noteworthy that probe **1** could be regenerated only by adding Hg^{2+} ions to the solution containing compound **2** (a regenerative process of **1**, Figure S6, Supporting Information). Common metal ions such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Al^{3+}, Ga^{3+}, Sn^{2+}, Zr^{4+}, Ce^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Er^{3+}, Tb^{3+}, Ho^{3+}, Yb^{3+}, Ag^+, and Pb^{2+} did not generate the same result, which indicates these ions cannot cause the regeneration of **1** (Figure 3 (above)). These novel facts show that compound **2** can act as a fluorogenic chemosensor for the Hg^{2+} ions with high selectivity and sensitivity (Figure 3 (below)). The detection limit for Hg^{2+} in aqueous buffer is low micromolar level, e.g., 0.15−9.0 μM. The regeneration of probe **1** should involve a Hg^{2+}-promoted desulfurization such as the one reported by Ros-Lis:[12c,15] intramolecular Michael addition of **2** to the pyran ring. In other words, Hg^{2+} ions trigger C−S bond cleavage of compound **2**, and subsequent intramolecular nucleophilic attack of the phenolic oxygen on the cyclopentenone group results in the recovery of the cyclized probe **1** (Scheme 1). Furthermore, the regeneration of probe **1** after exposure to thiols and Hg^{2+} was examined by spectra. The change of spectra is regenerative over several cycles of "ON−OFF−ON" (Figure S7, Supporting Information). The results strongly proved that probe **1** can be not only used to detect thiols and Hg^{2+} but also readily regenerated.

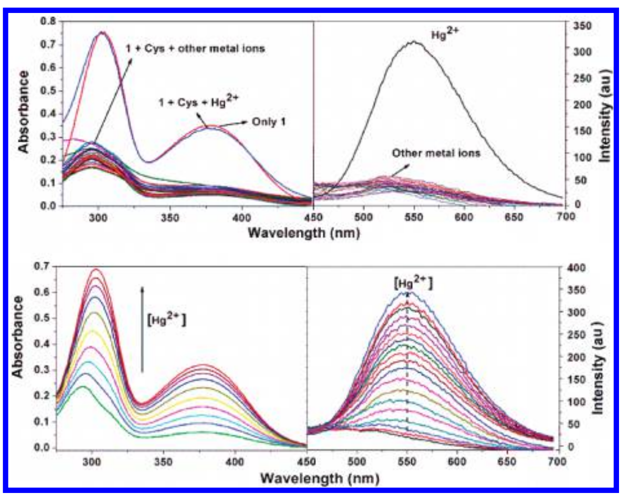
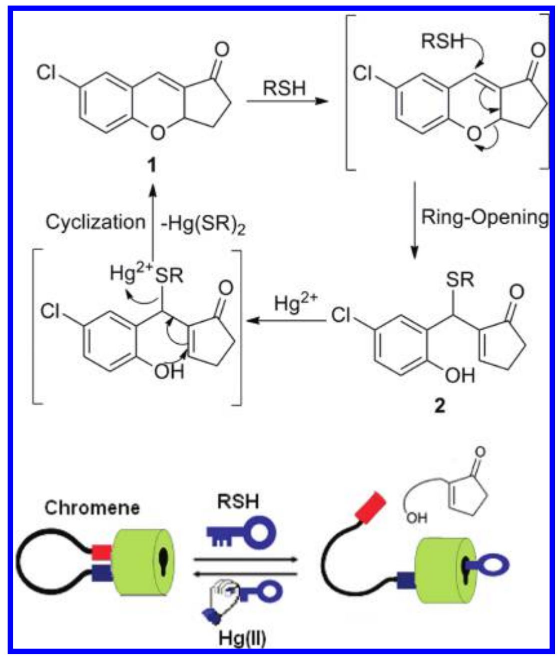


Figure 3. (Above) Absorption spectral changes (30 μM) and fluorescence spectral (3 μM) changes of **2** (λ_{ex} = 380 nm, λ_{em} = 550 nm; slit, 10 nm/10 nm) in 10 mM Tris-HCl, 0.5 mM EDTA, containing 0.15% EtOH, pH 8.0 aqueous buffer upon addition of 10 equiv of various metal ions including: Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Al^{3+}, Ga^{2+}, Sn^{2+}, Zr^{4+}, Ce^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Er^{3+}, Tb^{3+}, Ho^{3+}, Yb^{3+}, Ag^+, Pb^{2+}, and Hg^{2+}. (Below) Hg^{2+} ion was added gradually with [Hg^{2+}] = 0−90 μM for UV/visible spectra with [**2**] = 30 μM and [Hg^{2+}] = 0−9 μM for fluorescence spectra with [**2**] = 3 μM. Each spectrum was recorded 10 min after Hg^{2+} addition.

On the basis of the sensor "ON−OFF−ON" cycle, we propose a cyclic mechanism (Scheme 2 (above), Figure S7, Supporting Information). This reversible system of the thiol-chromene "click" ring-opening process and Hg^{2+}-promoted pyran ring cyclization behaves as a molecular lock that can be unlocked with a thiol key and can be locked with an Hg^{2+} ion "hand", unsheathing the key (Scheme 2 (below)): probe **1** and product **2** represent the locked states and unlocked states, respectively.

Scheme 2



On the basis of structure information for compound **2**, we propose a fluorescence mechanism of **1**, quenched upon the addition of thiols. The probe **1** molecule is planar with a maximum deviation of 0.4914 (2) Å for only the C3 atom and has a strong fluorescence emission at 552 nm. When MPA is added to **1**, the rigid plane of the chromene **1** is destroyed because of the pyran ring opening. To clarify this change, an energy-minimized structure of **2** was produced, with a molecular mechanical calculation which suggests that

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the dihedral angle between the cyclopentene plane and the benzene ring plane is 66.58° (Figure S8, Supporting Information).

In summary, the current study has demonstrated for the first time a regenerative, single molecular machine-like "ON−OFF−ON" chemosensor, based on a chromene molecule with a pyran ring "OFF−ON−OFF" cycle process. The sensor can function as a new, simple fluorescence-off probe for thiol with very high sensitivity and selectivity through the thiol-chromene "click" ring-opening reaction in aqueous media, and it can act as a novel fluorescence-on probe of the Hg^{2+} ion with high selectivity based on a Hg^{2+}-promoted desulfurization, intramolecular Michael addition to the pyran ring. Thus, these results are significant and interesting for a new generation of molecular recognition systems. The above phenomenon behaves as a molecular lock, which requires an inserted key to open and a hand to unlatch the key to close. Further studies on novel molecular machine-like, regenerative probes and their applications are now underway in our laboratory.

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Supporting Information Available: Experimental procedures, spectroscopic data, kinetic study, ^{1}H NMR, ^{13}C NMR, ESI-MS data, and crystal data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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