Screening Your Sushi: A Selective Fluorescent Chemosensor for Mercury

By Andrew L. Chang

A recent investigation by the New York Times discovered high levels of mercury in tuna sushi sold in Manhattan restaurants and groceries. Mercury levels were so high, in fact, that eating as few as six pieces of sushi a week could surpass levels recommended by the Environmental Protection Agency (1). Consumption of tuna and other fish, including pike, bass, and swordfish, may increase the risk of mercury exposure, as mercury accumulates in the aquatic food chain and reaches its highest concentration in predatory fish such as these (2).

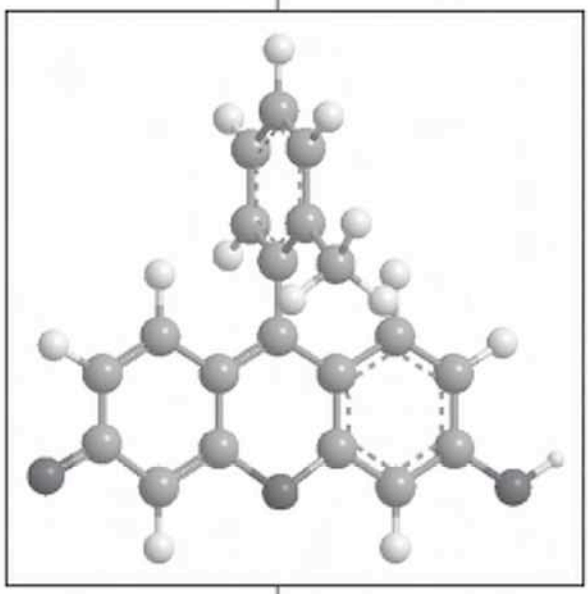

Figure 1. Mercury Green 1 (MG1). The chemosensor consists of (a) a water soluble and fluorescent reporter, (b) an ortho-methyl phenylene linker, and (c) an Hg^{2+}-selective thioether-rich crown receptor. The addition of an ortho-methyl group to the phenylene linker of MF1 to form MG1 restricts rotation about the carbon-carbon bond between the linker and the reporter. When excited, the fluorophore is more likely to return to its ground state through fluorescence rather than through heat loss due to the vibration or rotation of its chemical bonds, resulting in a higher quantum yield.

Emitted by volcanoes and released from the burning of coal, mercury vapor (Hg_0) also evaporates naturally from the earth's surface. Oxidized in the atmosphere to water-soluble Hg^{2+} ions, the mercury is then carried down by rainwater and metabolized by aquatic microbes to methyl mercury (CH_3Hg^+). A potent neurotoxin, methyl mercury subsequently enters the aquatic food chain (2).

Consumption of mercury is connected to significant cognitive and motor disorders (2). The ability to monitor Hg^{2+} in both environmental and biological samples is thus an attractive target. Yet, current methods for screening mercury are often costly or require complex sample preparation or instrumentation. Fluorescent small-molecule sensors offer an appealing approach, but synthetic challenges remain. For instance, the probe must be water-soluble. Additionally, it must be highly selective for Hg^{2+} over other competing metal ions such as Cu^{2+} and Pb^{2+}. Reporting the synthesis of a selective fluorescent sensor for mercury, Mercury Green 1 (MG1), Professor Christopher Chang of the University of California, Berkeley has been able to track changes in mercury levels within aqueous solutions, living cells, and fish tissue (3).

In the design of a fluorescent chemosensor, three key properties were desired: high fluorescence quantum yield, water-solubility, and Hg^{2+} selectivity. Quantum yield, the ratio of the number of photons emitted to the number of photons absorbed, gives the probability that the excited state of the molecule formed after absorption of a photon will return to its ground state through fluorescence rather than through the generation of heat from the vibration or rotation of chemical bonds (Figure 1). Thus, the most active fluorophores have a quantum yield close to one.

The synthesis of MG1 attaches a thioether-rich crown receptor, a phenylene-derived linker, and a fluorescent xanthene reporter unit. The water-soluble fluorescein-based probe has a quantum yield of $\Phi = 0.72$, the highest reported value for an Hg^{2+}-specific sensor in water (3). In comparison, natural green fluorescent protein from the jellyfish *Aequorea victoria* has a quantum yield between 0.72 and 0.78 (4). Furthermore, MG1 only displays high fluorescence when bound to Hg^{2+}. In its unbound state, MG1 is nearly nonfluorescent due to photoinduced electron transfer quenching of the fluorophore by the electron-donating receptor. Once bound to the mercury ion, the receptor is no longer able to quench the fluorophore, allowing for a significant 44-fold turn-on response accompanying the addition of Hg^{2+}.

Having previously reported the similar fluorescent chemosensor Mercuryfluor-1 (MF1), Chang *et al.* sought to improve its optical brightness, as the sensor was limited by its relatively

low quantum yield of $\Phi = 0.16$ (5). The addition of an ortho-methyl group on the phenylene linker to form MG1 restricts the rotation between the previously freely-rotating linker and the xanthene reporter unit (3), keeping the benzene moiety and the fluorophore orthogonal to each other (Figure 2) (6). Thus, the increased structural rigidity of the fluorophore decreases the possibility of vibrational dissipation of energy, resulting in an increase in optical brightness (7).

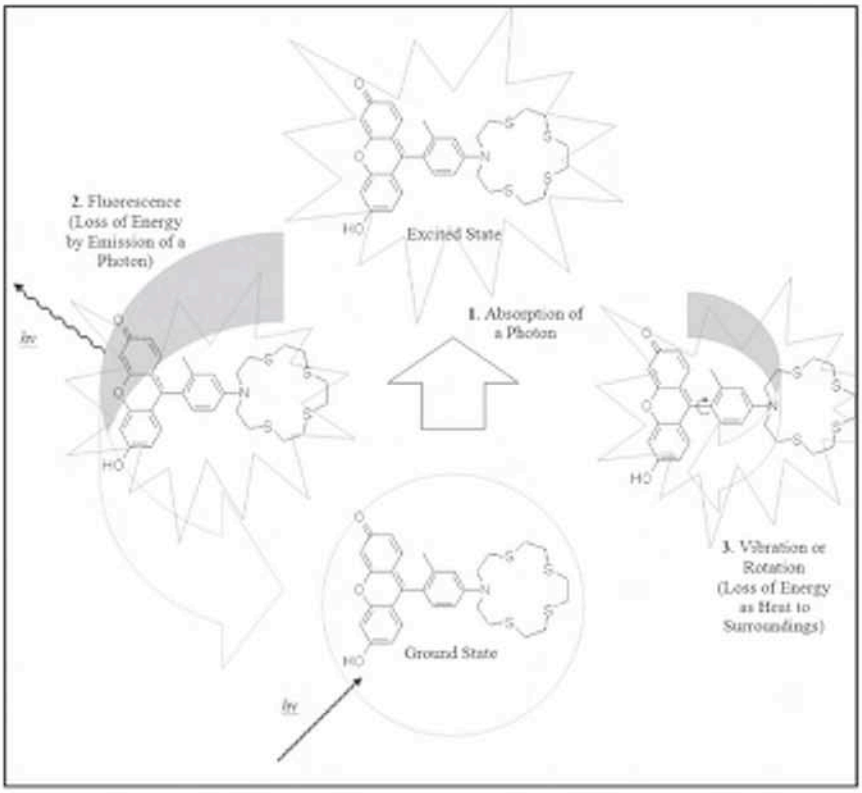

Figure 2. Fluorescence. The fluorophore, initially in its stable ground state, absorbs a photon and reaches a higher energy state, called its excited state (1). Since the molecule is less stable in this higher energy state, it will find a path to release the excess energy. The molecule can return to the ground state either by emitting light, a process called fluorescence (2), or by producing heat through vibration or rotation of its chemical bonds (3). The fluorophore can now absorb another photon and repeat the process.

The thioether-rich receptor of MG1 affords excellent selectivity for soft Hg^{2+} ions in the presence of competing metal ions, including a 5,000-fold excess of alkali and alkaline-earth cations Li^+, Na^+, K^+, Mg^{2+} and Ca^{2+}; 300- to 500-fold excess of first-row transition-metal ions Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^+, and Cu^{2+}; and 500-fold excess of Pb^{2+} and Group 12 metal ions Zn^{2+} and Cd^{2+} (3).

After testing MG1's ability to monitor intracellular mercury levels through live-cell imaging experiments, MG1 was tested on tissue samples from freshwater fish collected in northern California. With mercury concentrations spanning from 0.03 to 13 ppm and measured by atomic absorption spectrometry, the tissue samples were digested with microwave irradiation in nitric acid, basified, brought to pH 7, and analyzed with MG1. Finding a linear correlation between the fluorescence response of MG1 and the mercury content of the fish, MG1 was shown to be able to distinguish between safe and toxic levels of mercury in edible fish (3).

With the highest quantum yield for an Hg^{2+}-bound sensor in water and excellent selectivity for Hg^{2+} ions, MG1 is able to reliably detect environmentally and biologically relevant mercury levels in water, cells, and tissue. With overall processing times of less than fifteen minutes, small sample sizes, and compatibility with high-throughput screening methods (5), MG1 could one day lead to a table-side mercury detector for your sushi. In fact, the small sample sizes (<100 mg) could allow the analysis to be used in conjunction with catch-and-release programs, leading to the development of field-ready kits to rapidly, inexpensively, and accurately monitor mercury levels in fish as they are caught (3, 5). ■

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