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Signal Amplification and Detection via a Supramolecular Allosteric Catalyst

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The development of methods for the construction of supramolecular architectures[1] offers chemists the ability to synthesize many potentially useful species. Ultimately, when one has control over the synthesis of compounds containing large, well-defined cavities, one can design species that can arrange molecules in a specific and predictable fashion for catalysis and detection of guest molecules. Indeed, chemists are now beginning to utilize supramolecular chemistry as a tool toward the realization of catalytic systems with enzymelike properties.[2]

Our research has focused on utilizing the concept of allosteric regulation in nonbiological catalytic systems.[3] Allosteric regulation is a nascent concept in synthetic catalysis despite its widespread presence in biology.[3,4] We have used allosteric regulatory reactions to control both the rate and selectivity of the asymmetric ring-opening of epoxides.[3] In turn, we have demonstrated the reversible in situ modulation of catalysis through the introduction and removal of allosteric effector molecules capable of binding specifically to a structure control element within a supramolecular catalyst.[3b] These studies have prompted us to investigate the plausibility of using allosteric control in a catalytic detection scheme in which the analyte, behaving as an allosteric effector, binds to the sensor and acts as an "on" switch for catalysis. The signal is amplified via the subsequent catalytic cycle,[5] and the products are detected using a convenient fluorophore probe strategy, reminiscent of biological assays such as ELISA.[6] Herein, we present an initial proof-of-concept demonstration of the use of a supramolecular allosteric catalyst in signal amplification and detection (Figure 1).

Complexes **1** and **2**, Figure 1, were synthesized via the Weak−Link approach[1a,1e,7] according to literature procedures.[3a] Compound **1** was previously characterized in the solid-state by a single-crystal X-ray diffraction study.[3a] Each complex contains two structural domains containing Rh(I) metal centers and a catalytic domain containing two Zn(II) metal centers. The macrocyclic cavity of compound **1** can be opened to form **2** by the introduction of CO gas (1 atm) in the presence of Cl^- ions (benzyltriethylammonium chloride) in CH_2Cl_2. Significantly, both CO and Cl^- are required to break the thioether/Rh(I) bonds, leaving the phosphine/Rh(I) bonds intact.[3] The result of the selective breaking of these bonds is a concomitant, significant change in molecular shape. The Weak−Link approach has been demonstrated to be amenable to a host of metal-coordination environments and ligand combinations.[1a,1e,7] A variety of functional groups have been shown to initiate the ring-opening reaction depending on the choice of ligand and metal combination. These functional groups range from cyanide and halide ions to pyridyl-based compounds, nitrile-based compounds, amines, and CO, which provide for a range of potential analytes. Importantly, these small molecules often react selectively with specific metal and ligand combinations.[1a,1e,7] As a preliminary example of the utility of this novel detection strategy, data for the detection of Cl^- ions is presented.

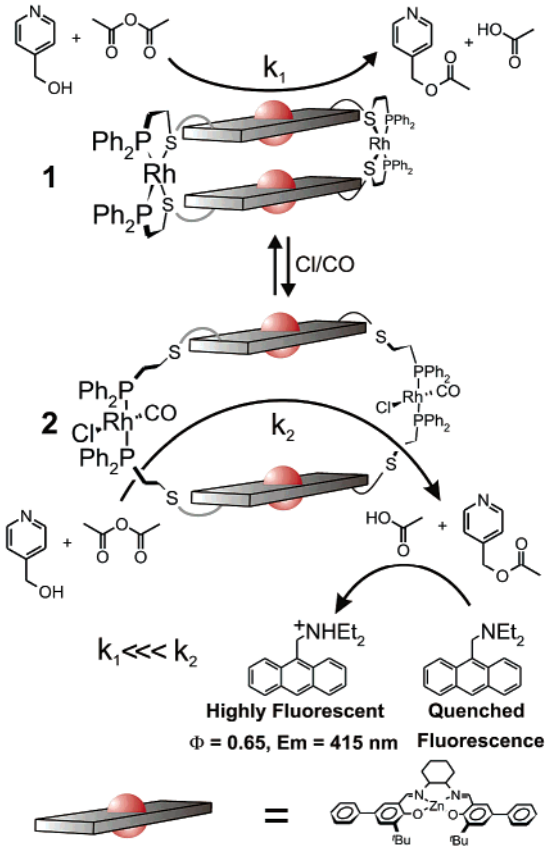

Figure 1. Supramolecular allosteric catalytic signal amplifier. A slow background reaction occurs in the absence of analyte (Cl^- or CO). Analyte binding opens the cavity and allows substrate molecules to enter, where they undergo a fast intramolecular reaction generating acetic acid, which protonates a pH-sensitive fluorescent probe.

To amplify a detection event using an allosteric catalyst, a suitable reaction is needed in which substrates react at significantly different rates in the presence of the open and closed states of the catalyst. Furthermore, it is critical that the chemistry occurring at the catalytic site be orthogonal to the chemistry occurring at the regulatory site. The acyl transfer reaction between acetic anhydride and pyridyl carbinol was employed here as the signal amplification reaction. This reaction has several features that make it useful for this purpose. First, the reaction can be catalyzed in a bimetallic fashion. Second, acetic acid is a product of the reaction, allowing coupling of the amplification step to a pH-sensitive fluorophore.[8] We hypothesized that this design would provide a switch between a Lewis-acid-catalyzed monometallic (Zn(II)−salen) reaction and a bimetallic reaction in which the acetic anhydride is activated by one Zn(II)−salen moiety while in proximity to a pyridyl carbinol molecule bound to the other Zn(II) center within the supramolecular

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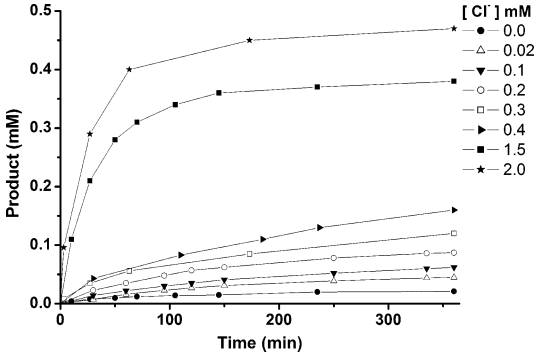

Figure 2. Product (4-acetoxymethylpyridine) concentration vs time for a range of Cl^- ion concentrations. Reactions were monitored by GC. Conditions: CH_2Cl_2, rt, 1 mM pyridyl carbinol, 1 mM acetic anhydride, 1.5 mM biphenyl (standard), 1 mM closed catalyst, CO (1 atm), and appropriate amounts of benzyltriethylammonium chloride.

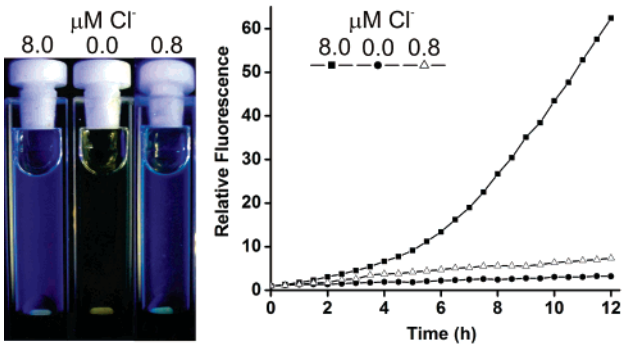

Figure 3. Photo: Taken under a UV lamp (365 nm); reaction time = 6 h. Graph: Fluorescence vs time plot (λ_{ex} = 368 nm, λ_{em} = 415 nm). Conditions: 0.1 mM catalyst, 0.1 mM pyridyl carbinol, 0.1 mM acetic anhydride, 1 mM diethylaminomethylanthracene, CH_2Cl_2, rt, benzyltriethylammonium chloride.

cavity.[2a] Therefore, the bimetallic catalytic pathway takes advantage of proximity effects and the Lewis-acid activation of acetic anhydride. The result of this analyte-induced switch to an intramolecular reaction mechanism was a significant increase in the rate of formation of the acylated product, 4-acetoxymethylpyridine, and acetic acid.

To assess the viability of this approach to analyte detection, solutions containing fixed [mM] quantities of pyridyl carbinol, acetic anhydride and catalyst were treated with CO in the presence of a range of Cl^- ion concentrations (Figure 2). The reactions were initially monitored by gas chromatography (GC) for the formation of 4-acetoxymethylpyridine (see Supporting Information for details). Interestingly, the magnitude of the allosteric effect, which determines the signal-to-noise ratio, is maximized at high loadings of **1** compared with the catalytic substrates. Rate enhancements of approximately 25 times are achievable upon activation of **1** with Cl^- under these conditions. In turn, this novel allosteric catalytic system responds to a range of Cl^- ion concentrations, with the greatest amplification, measurable as 4-acetoxymethylpyridine formed per mole of Cl^-, occurring for the lowest Cl^- to catalyst **1** ratios. By GC, [mM] quantities of Cl^- can be detected; however, it is not an attractive or convenient way of detecting analyte.

Coupling of the catalytic amplification step to a pH-sensitive fluorophore (diethylaminomethylanthracene) provides a straightforward method for visually and spectrophotometrically monitoring the amplification of the signal and corresponding presence of analyte (Figure 3). Concentrations of Cl^- as low as 800 nM could easily be observed using a commercial, handheld UV (365 nm) lamp and differentiated from a system not exposed to Cl^-, but containing the same concentration of pH-sensitive fluorophore, components of the catalytic reaction and CO (1 atm).[9]

This approach brings together three key elements in order to effect efficient and facile recognition, amplification, and detection of analyte. First, the analyte acts to switch "on" an allosteric catalyst. Second, this switch takes the form of a topological change that results in a significant increase in the rate of acyl transfer from acetic anhydride to pyridyl carbinol. Third, the production of acetic acid is easily coupled to a pH-sensitive fluorophore. The essential component is the allosteric effect that gives rise to a significant rate difference and allows one to obtain excellent differentiation between activated and inactive catalyst, by both GC and the easily implemented fluorophore method.

In light of the generality of the Weak−Link approach to the construction of structurally related flexible multimetallic supramolecular entities, we are currently expanding this detection strategy to a range of chemically and biologically relevant analytes.

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Supporting Information Available: Detailed experimental procedures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Commercial chloride ion-selective electrodes typically have a micromolar detection limit and a response time of less than 10 s.

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