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Catalytic Signal Amplification Using a Heck Reaction. An Example in the Fluorescence Sensing of Cu(II)

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Signal amplification for ultrasensitive molecular sensing has attracted considerable attention in the past decades.[1] Among the current literature methods, the "molecular wire" approach is attractive because highly sensitive analyte detection results from energy migration in conjugated semiconducting polymeric assemblies.[2] This technique has found applications in the detection of toxic ions[3] and explosive compounds.[4] Another amplification routine exploits conformational changes in helical polymers that result from chiral molecular interactions, giving discrimination of the sense of chirality.[5]

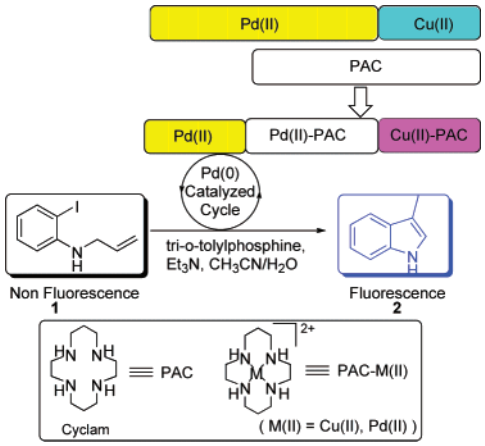
The most common signal amplification routines involve biochemical catalytic processes. For example, catalytic RNA cleavage has been used to give both direct detection and quantification of RNAs in situ.[6] Similarly, nucleic acid-templated metal-catalyzed ester hydrolysis has been used to amplify signals.[7] In addition, exponential amplification of RNA cleavage was achieved via substrate deactivation, followed by seeding the cleavage reaction with an initiator.[8] Of course, enzyme-linked immunosorbent assay (ELISA) is a renowned tool, proven to link antibody detection to enzymatic signal amplification.[9]

In contrast to the aforementioned methods using polymers and bio-inspired systems, we have started exploring the use of common organometallic reactions to amplify signals in molecular sensing routines to detect and quantify various analytes in aqueous solution.[10] Our general signal amplification protocol relies upon deliberate deactivation, by an exogenous ligand, of an organometallic reaction that catalytically creates a fluorophore or chromophore. The ligand also binds an analyte, and a competition is set up between the analyte and the catalyst. To whatever extent the deactivating ligand is sequestered by the analyte, the catalytic reaction will proceed.

To test this strategy, we choose Cu(II) as the analyte and a polyaza cyclam (PAC) as the deactivating ligand. Scheme 1 shows the strategy in the context of a Heck cross-coupling reaction. In our design strategy, an equivalent amount of ligand should result in complete sacrifice of the Pd(II) due to creation of Pd(II)−PAC. However, upon pretreatment of PAC with Cu(II), which has a larger affinity than Pd(II), the deactivating ligand is only able to fractionally capture the Pd(II), thereby leaving an equal amount of Pd(II) to be reduced to the Pd(0) catalyst in a Heck coupling cycle. Each equivalent of Cu(II) should free up a set amount of catalyst, and as a result, the fluorescent product will be formed at a rate linearly proportional to analyte concentration. Further, in such an approach, the fluorescence is catalytically "turned-on".

Although several Cu(II) fluorescent chemosensors have been reported, very few have sensitivity in the nanomolar range. In addition, they involve fluorescence quenching processes rather than fluorescence enhancement.[11] To date, the most sensitive Cu(II) detection method involves an optical fiber with an immobilized fluorophore-tagged Cu(II) binding protein. The system reached an astounding 0.1 pM Cu(II) detection limit, even though it was based on a fluorescence quenching and lifetime reduction approach.[10d] Further, 0.2 pM copper ion measurement was achieved using an electrochemical sensor, in which the tripeptide Gly-Gly-His was covalently attached to a modified gold electrode.[12]

Scheme 1. Cu (II) Sensing Protocol[a]



[a] Fluorophore **2** is generated from nonfluorescent aniline **1** through Heck reaction. When 1 equiv of PAC is introduced into the system, PAC−Pd(II) is formed, which causes the absence of fluorescence. However, upon addition of Cu(II) prior to Pd(II), the preferred PAC−Cu(II) complex is formed, and fluorescence is catalytically "turned on".

The Heck reaction of **1** was chosen for our proof-of-principle study because of its ease of operation, as well as the completely different fluorescent activities between the starting material and product. Through a Pd(0)-catalyzed Heck reaction,[13] the nonfluorescent starting material 2-iodo-*N*-allylaniline **1** can be converted into the fluorescent indole product **2** ($\Phi = 0.343$).[14] Excitation of **2** was performed at 275 nm (absorption maximum), and emission was recorded at 351 nm (emission maximum). The reaction was monitored by measuring fluorescence intensity as a function of time. As expected, introduction of 1.0 equiv of ligand (PAC) deactivates the Pd(II) by the formation of a Pd(II)−PAC complex. This deactivating ligand nearly completely silences the emission over time. However, when 1.0 equiv of Cu(II) is incubated with PAC prior to addition of Pd(II), the remaining Pd(II) indeed yields a successful Heck reaction with a rate equal to that of no ligand. These control experiments are shown in Supporting Information.

To an acetonitrile−water (10:1 v/v) solution of **1** (0.5 mM), tri-*o*-tolylphosphine (0.05 mM), PAC (0.025 mM), and TEA (1 mM) under argon were added varied amounts of Cu(II), followed by addition of Pd(II) (0.025 mM). A series of reactions at 60 °C were performed in quartz cuvettes. To reduce the slight extent of photocyclization of **1**,[15,16] the emission spectra were taken every 10 minutes, rather than using continual excitation. Figure 1 shows the relationship between fluorescence intensity and time, with a series of different Cu(II) concentrations (from 500 to 30 nM). The slopes of the lines represent initial rate kinetics. As expected, when

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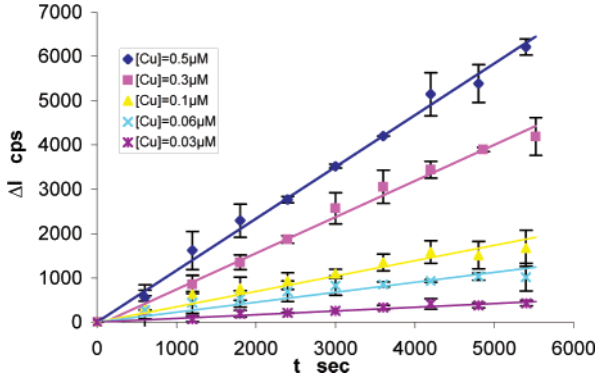

Figure 1. Initial rate analysis. Reaction conditions: [**1**] = 0.5 mM, [PAC] = 0.025 mM, [Pd(II)] = 0.025 mM in CH_3CN/H_2O (10:1 v/v), under argon, temperature = 60 °C.

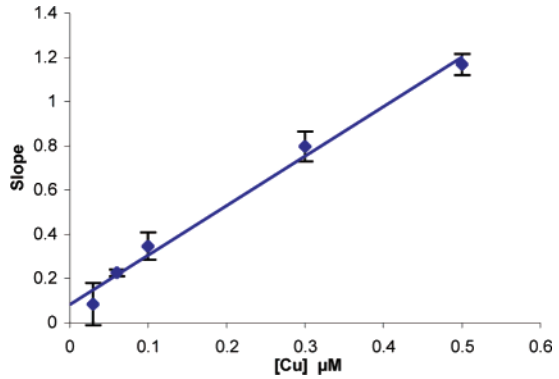

Figure 2. Slope (initial rates) vs [Cu(II)]. [Cu(II)] was decreased from 500 to 30 nM. Reaction conditions are the same as in Figure 1.

decreasing the Cu(II) concentration, the initial rates dropped correspondingly. A plot of slope values as a function of Cu(II) concentration is linear, as shown in Figure 2. This linear relationship implies that 1 equiv of Cu(II) frees a consistent amount of Pd(II).

Many transition metals have high affinities to poly-aza macrocyclic ligands.[17] Therefore, we sought to test the selectivity of our sensing protocol to various metals. Cu(II) has the highest affinity to cyclam **1**. Hence, we used the same protocol as with Cu(II) described above, but for the metal ions Ni(II), Co(II), and Cd(II), all of which have lower affinities. Figure 3 shows that the catalytic production of fluorophore **2** tracks the affinities of the cyclam for Cu(II), Ni(II), Co(II), and Cd(II). The higher the binding affinity, the greater the initial rate observed. As indicated in Figure 3, when Cu(II) was present in the system the initial rate was almost 3-fold that of Co(II) and 6-fold those of Ni(II) and Cd(II). These relative selectivities support the mechanism given herein for the sensing method. While a particular metal ion in a mixture would be difficult to distinguish with these small selectivity ratios, other ligands may improve the selectivity.

In conclusion, signal amplification using an intramolecular organometallic catalytic reaction has been shown to yield a very sensitive sensing technique. Our specific example using a Heck reaction has high sensitivity and reasonable selectivity for Cu(II). Admittedly, to achieve a response for 30 nM Cu(II) required a long

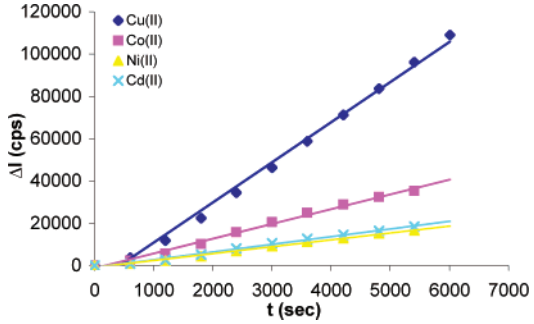

Figure 3. Selectivity analysis toward Cu(II), Co(II), Cd(II), Ni(II). Reaction conditions are same as in Figure 1 except that Cu(II), Co(II), Ni(II), and Cd(II) concentrations are 0.015 mM.

time, nearly 1.5 h. However, the study described here demonstrates a new principle for signal enhancement in molecular sensing. Clearly the use of faster organometallic reactions will significantly improve the practicality of the general method.

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Supporting Information Available: Spectroscopic and controlling experimental data and general procedure (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Swager, T. M. *Acc. Chem. Res*. **1998**, *31*, 201. (b) Ehret, A.; Spitler, M. T.; Stuhl, L. S. *Comments Inorg. Chem*. **2002**, *23*, 275. (c) Blaedel, W. J.; Boguslaski, R. C. *Anal. Chem*. **1978**, *50*, 1026. (d) Shibata, T.; Yamamoto, J.; Matsumoto, N.; Yonekabo, s.; Osanai, S.; Soai, K. *J. Am. Chem. Soc*. **1998**, *120*, 12157.
(2) Kim, Y.; Zhu, Z.; Swager, T. M. *J. Am. Chem. Soc*. **2004**, *126*, 452.
(3) Kim, T.; Swager, T. M. *Angew. Chem., Int. Ed*. **2003**, *42*, 4803.
(4) Kuroda, K.; Swager, T. M. *Macromol. Symp*. **2003**, *201*, 127.
(5) Yashima, E.; Maeda, k.; Nishimura, T. *Chem. Eur. J*. **2004**, *10*, 42.
(6) Hartig, J. S.; Grüne, I.; Najafi-Shoushtari, S. H.; Famulok, M. *J. Am. Chem. Soc*. **2004**, *126*, 722.
(7) Brunner, J.; Mokhir, A.; Kremer, R. *J. Am. Chem. Soc*. **2003**, *125*, 12410.
(8) Ellington, A. D.; Levy, M. *Proc. Natl. Acad. Sci. U.S.A*. **2003**, *100*, 6416.
(9) (a) Hang, H. C.; Yu, C.; Pratt, M. P.; Bertozzi, C. R. *J. Am. Chem. Soc*. **2004**, *126*, 6. (b) Shibata, H.; Sasaki, N.; et al. *J. Vet. Med. Sci*. **2003**, *65*, 1207. (c) Reymond, J.-L. *J. Immunol. Methods* **2002**, *269*, 125. (d) Jean, J.; Blais, B.; Darveau, A.; Fliss, I. *FEMS Microbiol. Lett*. **2002**, *210*, 143. (e) Steinert, S. A.; Pickwell, G. V. *Mar. Environ. Res*. **1984**, *14*, 229.
(10) Zhu, L.; Anslyn, E. A. *Tetrahedron* **2004**, *60*, 7267.
(11) (a) Marzouk, S. A. M.; AI-Ariqui, W. T.; Hassan, S. S. M. *Anal. Bioanal. Chem*. **2003**, *375*, 1186. (b) Grandini, P.; Mancin, F.; Tecilla, P.; Scrimin, P.; Tonellato, U. *Angew. Chem., Int. Ed*. **1999**, *38*, 3061. (c) Krämer, R. *Angew. Chem., Int. Ed*. **1998**, *37*, 772. (d) Dujols, V.; ford, F.; Czarnik, A. W. *J. Am. Chem. Soc*. **1997**, *119*, 7386. (e) Zeng, H.; Thompson, R. B.; Maliwal, B. P.; Fones, G. R.; Moffett, J. W.; Fierke, C. A. *Anal. Chem*. **2003**, *75*, 6807.
(12) Yang, W.; Jaramillo, D.; Gooding, J.; Hibbert, D. B.; Zhang, R.; Willett, G. D.; Fisher, K. J. *Chem. Commun*. **2001**, 1982.
(13) Genet, J. P.; Blart, E.; Savignal, M. *Synlett* **1992**, 715.
(14) Eftink, M. R.; Jia, Y.; Hu, D. *J. Phys. Chem*. **1995**, *99*, 5713.
(15) (a) Sonawane, H. R.; Nanjundiah, B. S.; Panse, M. D. *Tetrahedron. Lett*. **1985**, *26*, 3507. (b) Ogata, Y.; Takagi, K. *Tetrahedron* **1971**, *27*, 1573.
(16) 2-Iodo-*N*-allylaniline **2** does not display fluorescence because of the internal heavy element effect resulting from the iodine substituting on the aromatic ring, while the iodine is also a potential source to occur photoreaction with terminal alkene. Measurement spectra are shown in Supporting Information.
(17) (a) Izatt, R. M.; Bradshaw, J. S.; Nielsen, S. A.; Lamb, J. D.; Christensen, J. J. *Chem. Rev*. **1985**, *85*, 271. (b) Christensen, J. J.; Eatough, D. J.; Izatt, R. M. *Chem. Rev*. **1974**, *74*, 351.

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