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An "*Off-On*" Type UTP/UDP Selective Fluorescent Probe and Its Application to Monitor Glycosylation Process

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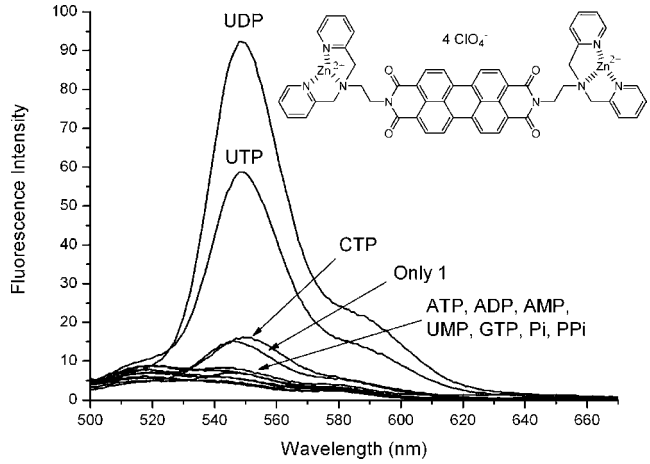
ABSTRACT



A New fluorescent sensor based on a perylene−dpa-Zn platform was synthesized. Selective "Off-On" type fluorescence changes were observed upon the addition of UTP and UDP, which was also applied to monitor glycosylation processes.

The recognition and sensing of anionic analytes has recently emerged as a significant goal of research programs.[1] A variety of anions, phosphates, pyrophosphates, and nucleotides are important species whose recognition and sensing can play a major role in understanding and evaluating key biological processes. Sensors of these anions, which are based on anion-induced changes in fluorescence intensities, are attractive in this regard owing to their potentially high levels of simplicity and sensitivities.[1] In the past decades, considerable effort has been devoted to the development of rapid and convenient detection systems for phosphate (Pi),[1c,2] pyrophosphate,[1c,3] ATP,[4] GTP,[5] phosphorylated peptide/protein,[6] phosphatidylserine[7] and lysophosphatidic acid.[8] Uridine triphosphate (UTP) and uridine diphosphate (UDP), key building blocks for the synthesis of RNA and in glycotransfer pathways, are widespread in living cells. As a result, they play pivotal roles in various biological events. For

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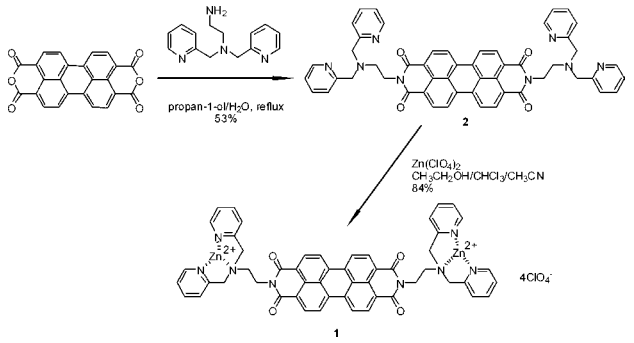
example, UTP serves as a donor in energy transduction in organisms and as a control element in metabolic processes by its participation in enzymatic reactions. Besides, UTP and UDP are involved in many glycosylation processes that are catalyzed by glycosyltransferases. A pioneering work regarding glycosyltransferase assay was recently reported by Hamachi. et al. utilizing anthacene-Zn derivative.[9] However, this probe displayed high binding affinities toward Pi, *p*-Tyrosin, ATP, and ADP as well.[10]

Despite having many biological roles, to our knowledge, no fluorescent chemosensors that selectively respond to UTP/UDP in aqueous solution have been reported. Below, we describe the results of studies that have led to the development of a new fluorescent sensor **1** for this important target that is based on a perylene−dpa-Zn platform [dpa; bis(2-pyridylmethyl)amine]. This sensor operates under physiological conditions and exhibits high selectivity for UTP and UDP relative to other phosphate derivatives.

The synthesis of sensor **1**, begins with reaction of 3,4,9,10-perylenetetracarboxylic dianhydride and (2-aminoethyl)bis(2-pyridylmethyl)amine,[11] carried out in refluxing propan-1-ol/H_2O for 24 h (Scheme 1). This reaction produces the

Scheme 1. Synthesis of Compound **1**



perylene diimide **2** in 53% yield. Treatment of **2** with $Zn(ClO_4)_2 \cdot 6H_2O$ in a $CHCl_3-CH_3CH_2OH$ mixture then affords sensor **1** in 84% yield. The experimental details for this preparative route and characterization data for **1** and **2** are given in the Supporting Information.

Figure 1 showed the fluorescence changes of **1** (1 μM) upon the addition of $H_2PO_4^-$ (Pi), pyrophosphate (PPi), ATP, CTP, GTP, ADP, AMP, UMP, UDP and UTP at pH 7.4

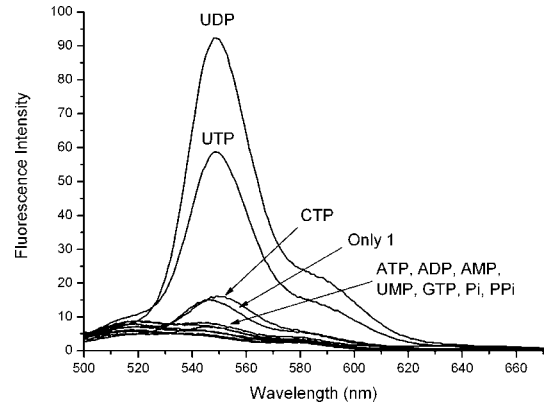


Figure 1. Fluorescence changes of **1** (1 μM) with Pi, PPi, ATP, GTP, CTP, ADP, AMP, UMP, UDP, and UTP (100 equiv) in CH_3CN-HEPES buffer (0.01 M, pH 7.4) (0.05:99.95, v/v) (excitation at 485 nm, slit: 10 nm/5 nm).

[CH_3CN-HEPES buffer (0.01 M, pH 7.4) (0.05:99.95, v/v)]. The fluorescence spectra were obtained by excitation of the perylene fluorophore at 485 nm. Compound **1** displays selective fluorescence enhancements ($\lambda_{max} = 550$ nm) in the presence of 100 eq. of UTP and UDP while a negligible fluorescence enhancement is promoted by CTP comparing with UTP and UDP. On the other hand, Pi, PPi, ATP, GTP, ADP, AMP and UMP induced a little decrease in fluorescence intensity. The emission band is centered at 550 nm, which is an ideal wavelength for biological applications. From the fluorescence titration experiments using 1 μM sensor (Figure S-5, see Supporting Information), the association constants for formation of UTP-**1** and UDP-**1** were calculated as 6.0×10^3 M^{-1} and 1.1×10^4 M^{-1} respectively (error $<11\%$).[12]

The fluorescence enhancement of **1** is associated with UTP/UDP promoted strengthening of the Zn^{2+} coordination

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with nitrogens in the tertiary amine moieties of dpa. As **1** showed a weak fluorescence in aqueous buffer, we speculate it exists predominantly as a mononuclear Zn^{2+} complex. The addition of UDP or UTP induces a second Zn^{2+} complexation, probably due to its electrostatic and coordination assistance, to form the UDP-**1** or UTP-**1** complex. This binding mode can induce inhibition of the electron transfer from tertiary amine donors to perylene singlet excited state,[1c] then results in fluorescence enhancement. Other analytes with diphosphate group (ATP, GTP, ADP, PPi) did not lead to the intensification of fluorescence, shown in Figure 2, indicate

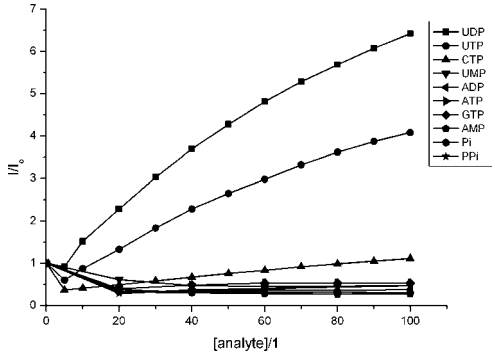
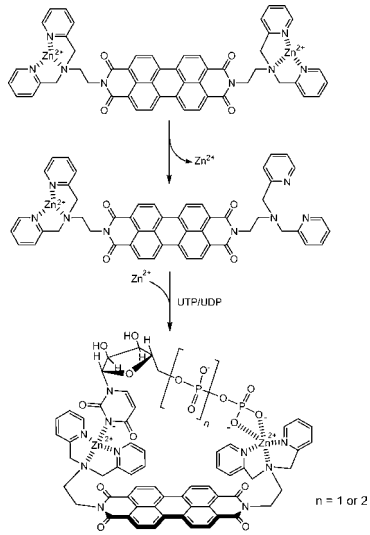


Figure 2. Dependence of the relative fluorescence intensity of **1** (1 μM) at 550 nm on the various anions at different concentrations in CH_3CN-HEPES buffer (0.01 M, pH 7.4) (0.05:99.95, v/v).

uridine group plays a key role. The selectivity for UTP and UDP may be the result of two important interactions illustrated in Scheme 2. In the complex formed between **1**

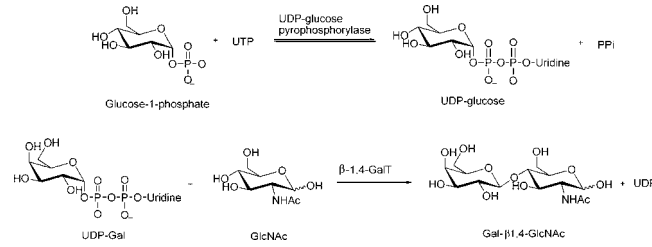
Scheme 2. Proposed Binding Mechanism of Chemosensor **1** with UTP/UDP



and UTP/UDP, one of the Zn^{2+} moieties of **1** can bind specifically to the uridine group in UTP/UDP while the other Zn^{2+} ion can bind to the phosphate group in UTP/UDP. Importantly, there is ample precedence for specific binding of Zn^{2+} to uridine.[13] Thus, the cooperative binding of the Zn-centers in **1** provided by perylene platform may lead to the high level of selectivity observed with this sensor. An optimum spacer is necessary for the cooperation between phosphate group and uridine group because the mixture of PPi (1 mM) and UMP (1 mM) did not lead to effective enhancement in the fluorescence of **1** (Figure S-6, see Supporting Information).

To determine if our sensor was applicable to the biology, we attempted to monitor UTP consumption and UDP production during glycosylation processes. Scheme 3 dis-

Scheme 3. Schematic Illustration of Glycosylation Procedure Involved with UTP and UDP



played the two glycosylation reactions involved in UTP and UDP. In the first reaction, uridine 5′-diphosphate (UDP)-glycoside is produced from UTP and glucose-1-phosphate in the presence of UDP-glucose pyrophosphorylase. The second reaction was initiated by β-1,4-galactocosyltransferase (β-1,4-GalT) in the presence of UDP-Gal and GlcNAc as glycosyl donor and acceptor, respectively. The fluorescence response to UTP and UDP inspired us to perform the chemosensor-based glycosyltransferase assays using sensor **1**. In the first reaction, with the addition of UDP-glucose pyrophosphorylase in the HEPES buffer containing UTP (1 mM) and glucose-1-phosphate (1 mM), the fluorescence intensity at 550 nm gradually decreased, which indicates that UTP was gradually consumed (Figure 3). In contrast, no fluorescence change was observed without pyrophosphorylase (Figure S-7, see Supporting Information). Similarly, in the second reaction inducing by β-1,4-galactocosyltransferase, the fluorescence intensity at 550 nm gradually increased. The change is identical to that of UDP, which indicates that this fluorescence effect arises from the production of UDP in the presence of UDP-Gal and GlcNAc as glycosyl donor and acceptor respectively (Figure 4). In previous report, Hamachi. et al. has carried out the real-time assay only for the second reaction using anthacene-Zn derivative.[6] These results strongly suggest that sensor **1** can monitor both two steps of glycosylation processes in the convenient and quantitative manner.

In conclusion, we have uncovered a water-soluble, "Off-On" type fluorescence sensor that effectively and selectively recognizes UTP and UDP in aqueous solution at physiological pH. The perylene sensor **1** that displays a fluorescence

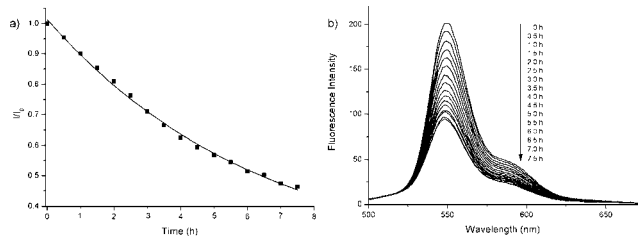


Figure 3. Fluorescence real-time detection of UDP-glucose pyrophosphorylase-catalyzed disappearance of UTP with **1**. (a) Time-trace plot of UTP disappearance monitored by the emission ratio I/I_0 at 550 nm. (b) Difference spectra obtained with UDP-glucose pyrophosphorylase (2 U) after different reaction time (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 h). Conditions: [**1**] = 10 μM, $[UTP]_0 = [Glucose\text{-}1\text{-}phosphate]_0 = 1$ mM, CH_3CN-HEPES buffer (0.01 M, pH 7.4) (0.5:99.5, v/v) (excitation at 485 nm, slit: 5 nm/5 nm).

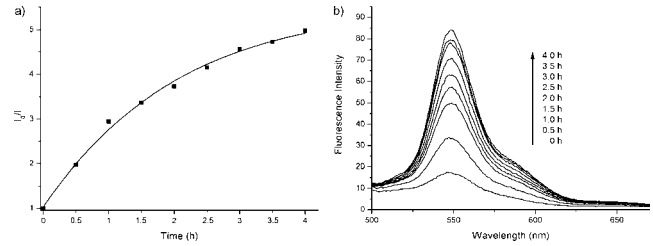


Figure 4. Fluorescence real-time monitoring of the glycosyl-transfer reaction catalyzed by β-1,4-GalT with **1**. (a) Time-trace plot of UDP produce monitored by the emission ratio I/I_0 at 550 nm. (b) Difference spectra obtained with β-1,4-GalT (50 mU) after different reaction time (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 h). Conditions: [**1**] = 10 μM, $[UDP\text{-}Gal]_0 = [GlcNAc]_0 = 1$ mM, CH_3CN-HEPES buffer (0.01 M, pH 7.4) (0.5:99.5, v/v) (excitation at 485 nm, slit: 5 nm/5 nm).

enhancement associated with formation of a complex with UTP/UDP. The selective UTP/UDP sensing may be a consequence of cooperative binding of the Zn^{2+} ions in **1** with the uridine and phosphate moieties of UTP/UDP. As a result, the perylene moiety in **1** might play two important roles, the first being the key fluorophore and the second being an optimum spacer template binding. Furthermore, we successfully demonstrated that sensor **1** can be applied to fluorometric monitoring of the enzyme-catalyzed glycosylation processes.

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Supporting Information Available: Synthesis and characterization of **1** and **2**. Fluorescence spectra of sensor **1** with UTP, UDP, and the mixture of PPi/UMP. Fluorescence real-time detection of glycosylation processes with sensor **1** in the absence of UDP-glucose pyrophosphorylase and β-1,4-GalT. This material is available free of charge via the Internet at http://pubs.acs.org.

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