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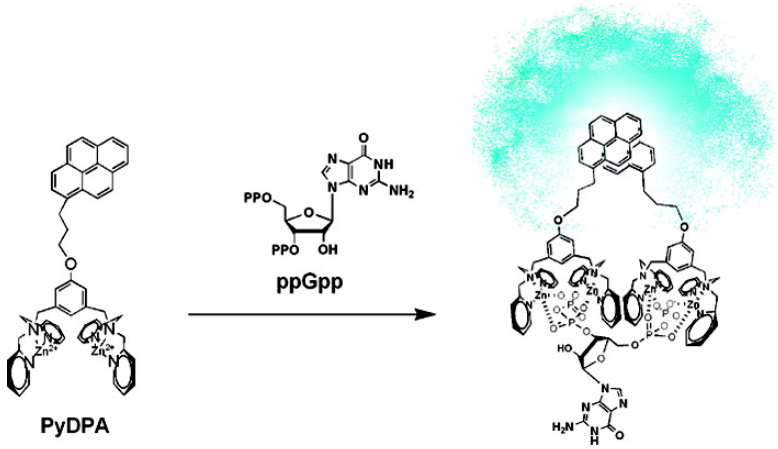

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Selective Fluorescent Chemosensor for the Bacterial Alarmone (p)ppGpp

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Guanosine 3′-diphosphate-5′-di(tri)phosphate, (p)ppGpp, is a bacterial and plant alarmone that generates a stringent response under conditions of nutritional deprivation.[1] When bacteria are placed in stress circumstances such as amino acid starvation, ppGpp is swiftly synthesized from ATP and GDP by RelA on the bacterial ribosomal complex. (p)ppGpp is synthesized by the transfer of a pyrophosphate from ATP to the 3′-OH of GDP (or GTP) (Figure 1). (p)ppGpp influences many bacterial survival mechanisms,[1d] and this facilitates bacteria persistence under stress conditions. Further, (p)ppGpp acts as a global regulator of the gene expression in microorganisms.[2] Therefore, precise and real-time detection of (p)ppGpp would enable deeper understanding of bacterial physiology.

Since Cashel and Gallant discovered (p)ppGpp in *Escherichia coli*,[1a] (p)ppGpp is usually detected by the isotope (^{32}P) in thin layer chromatography or by UV/vis absorption of (p)ppGpp in HPLC.[3] However, these methods lack sensitivity and are laborious, and hence, they cannot be applied for the real-time detection of (p)ppGpp.

Therefore, there is an urgent need to develop selective fluorescent chemosensors for (p)ppGpp because such chemosensors would enable the sensitive and real-time detection of (p)ppGpp. To the best of our knowledge, there has been no report on the development of fluorescent chemosensors for (p)ppGpp. Here, we report the first fluorescent chemosensor, **PyDPA**, which can detect (p)ppGpp extremely selectively from among other nucleotides in water.

As indicated in Scheme 1, **PyDPA** consists of two components: pyrene and bis(Zn^{2+}-dipicolylamine) (dipicolylamine = DPA). Bis-(Zn^{2+}-DPA) is well-known for its strong binding to pyrophosphate groups in water.[4] Pyrene was used for its unique excimer emission (Em = 470 nm).[5]

Since the Zn^{2+}-DPA group of **PyDPA** can bind each pyrophosphate moiety at the 3′- and 5′-hydroxyl positions of (p)ppGpp, two **PyDPA**s come close to each other upon binding to (p)ppGpp to generate pyrene-excimer fluorescence due to the stacking of their pyrene groups (Scheme 1). In fact, our observations were as expected. Upon the addition of ppGpp (or pppGpp) to **PyDPA** (20 μM) in water (pH 7.4, 1 mM HEPES buffer, 20 °C), the pyrene-excimer fluorescence of **PyDPA** was swiftly enhanced. Job plot between **PyDPA** and (p)ppGpp shows 2:1 stoichiometry in water. When ppGpp (or pppGpp, each 7.0 μM) was added to **PyDPA** (20 μM) in water, the fluorescence intensity at 470 nm increased approximately 17- and 14-fold for ppGpp and pppGpp, respectively (Figure 2A and B).[6]

When other nucleotides (ATP, GTP, CTP, TTP, UTP, cAMP, or cGMP) and inorganic pyrophosphate (PPi) were added to **PyDPA**

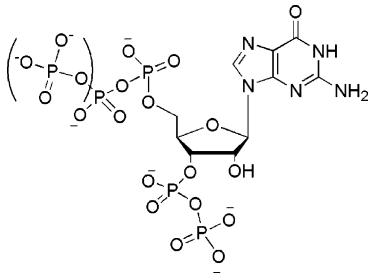
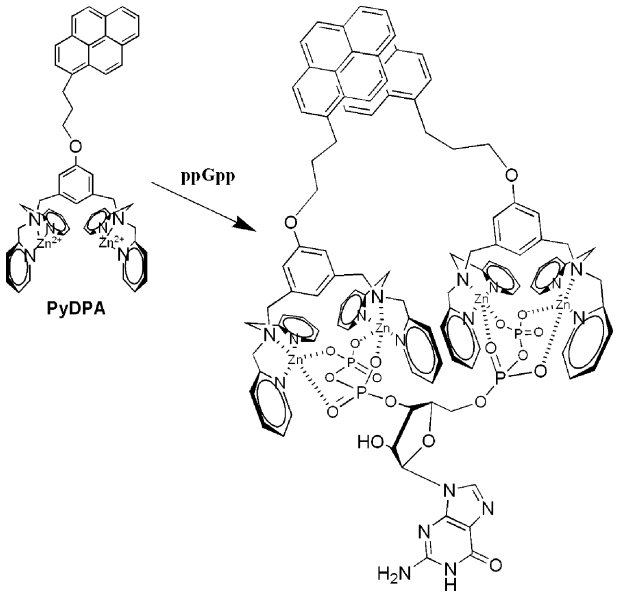


Figure 1. Chemical structure of (p)ppGpp.

Scheme 1. Binding Conformation between **PyDPA** and ppGpp



(20 μM) in water, there were no pyrene-excimer fluorescence emissions at 470 nm, only the pyrene monomer fluorescence at 380 nm was enhanced due to the increased hydrophobicity of the bound NTP or PPi.[4d] The fluorescence emission ratios (I_{470nm}/I_{380nm}) of **PyDPA** upon the addition of (p)ppGpp, other nucleotides, and PPi are listed in Figure 2D. Under a UV lamp (excitation at 365 nm), **PyDPA** emitted strong pyrene-excimer fluorescence only with (p)ppGpp addition (Figure 2E). These results indicate that **PyDPA** can be an extremely selective and sensitive chemosensor that targets (p)ppGpp from among other nucleotides.

We achieved the real-time fluorescent detection of in vitro ppGpp synthesis by using extracted *E. coli* ribosomal complexes (Figure 3). By using ATP (4 mM) and GDP (2 mM), ppGpp synthesis by the ribosomal complex was detected in real time by the increasing pyrene-excimer fluorescence of **PyDPA** (see the Supporting

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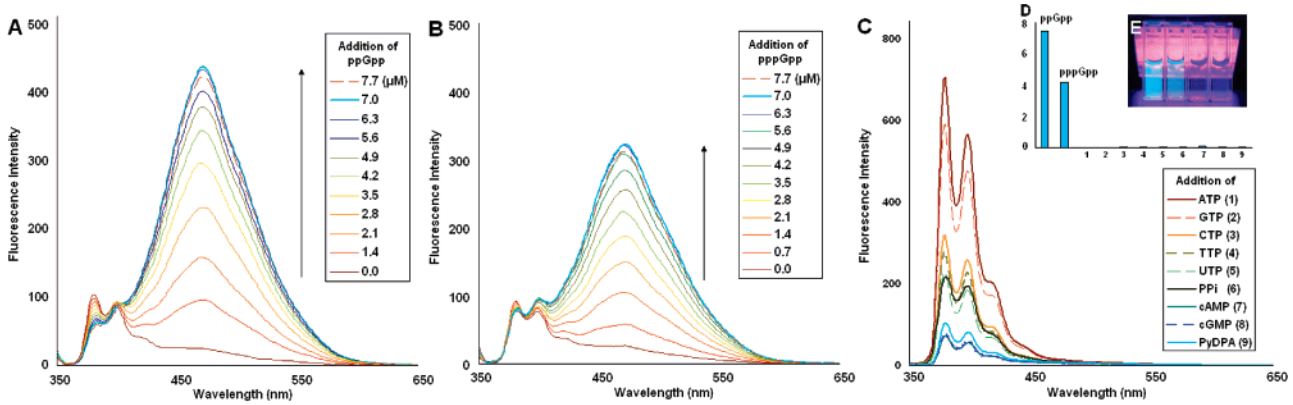

Figure 2. (A) Fluorescence emission titration of **PyDPA** (20 μM) upon ppGpp addition; Ex = 344 nm, Em = 470 nm. (B) Fluorescence emission titration of **PyDPA** (20 μM) upon pppGpp addition; Ex = 344 nm, Em = 470 nm. (C) Fluorescence emission spectra of **PyDPA** upon the addition of 7 μM of each nucleotide and PPi; Ex = 344 nm. (D) Fluorescence emission intensity ratio (I_{470nm}/I_{380nm}) of **PyDPA** (20 μM) upon the addition of ppGpp, pppGpp, and other nucleotides (each 7 μM). ppGpp and pppGpp are depicted on the graph, and the others are numbered in panel C. (E) Fluorescence emission of **PyDPA** (20 μM) under a UV lamp (excitation wavelength = 365 nm) upon the addition of ppGpp, pppGpp, ATP, and GTP (left to right, each 7 μM).

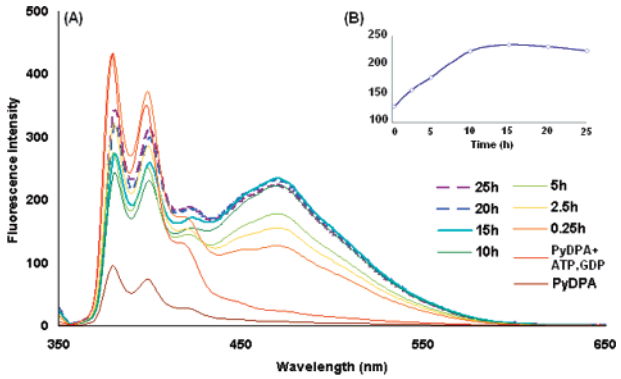

Figure 3. (A) Real-time fluorescent detection of in vitro ppGpp synthesis from ATP (4 mM) and GDP (2 mM) by extracted *E. coli* ribosomal complexes. At regular intervals, the fluorescence of the reaction mixture was measured after dilution (×2000) in **PyDPA** (20 μM). Excitation wavelength = 344 nm. (B) Changes in the fluorescence intensity at 470 nm with time. *X*-axis: elapsed time (h), *Y*-axis: fluorescence intensity at 470 nm.

Information). As shown in Figure 3B, excimer fluorescence at 470 nm gradually increased as 15 h elapsed; this implies that ppGpp was synthesized in the reaction mixture. After 15 h, the fluorescence at 470 nm decreased slowly as the synthesized ppGpp was degraded by other factors of the bacterial ribosomal complex. These results support the theories of in vitro ppGpp synthesis[7] and degradation[8] by the bacterial ribosomal complex.

PyDPA can also be used to detect ppGpp produced in the starved bacterial cells.[9] As shown in Figure S6, bacterial ppGpp can be detected either by HPLC (UV absorption) or by **PyDPA**. However, the fluorescent detection by **PyDPA** was much more selective and sensitive than the HPLC method.

In summary, we have developed the first fluorescent chemosensor (**PyDPA**) for the detection of (p)ppGpp, a bacterial and plant alarmone. **PyDPA** can detect (p)ppGpp extremely selectively from among other nucleotides or pyrophosphate in water by using pyrene-excimer fluorescence. **PyDPA** was used for the real-time detection of in vitro ppGpp synthesis by the *E. coli* ribosomal complex.

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Supporting Information Available: Synthesis of **PyDPA**, Job's plot between **PyDPA** and (p)ppGpp, PPi competition experiment results with (p)ppGpp, fluorescence emission titration of **PyDPA** with more than 7.7 μM (p)ppGpp, experimental data for the detection of in vitro ppGpp synthesis by extracted bacterial ribosomal complexes, experimental data for the detection of internal ppGpp in the starved bacterial cells. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Cashel, M.; Gallant, J. *Nature* **1969**, *221*, 838. (b) Cashel, M.; Gentry, D. R.; Hernandez, V. J.; Vinella, D. *Escherichia coli and Salmonella: Cellular and Molecular Biology*, 2nd ed.; ASM Press: Washington, DC, 1996; Vol. 1, pp 1458−1496. (c) Braeken, K.; Moris, M.; Daniels, R.; Vanderleyden, J.; Michiels, J. *Trends Microbiol.* **2005**, *14*, 45. (d) Jain, V.; Kumar, M.; Chatterji, D. *J. Microbiol.* **2006**, *44*, 1.

(2) (a) Magnusson, L. U.; Farewell, A.; Nystrom, T. *Trends Microbiol.* **2005**, *13*, 236. (b) Artsimovitch, I.; Patlan, V.; Sekine, S.; Vassylyeva, M. N.; Hosaka, T.; Ochi, K.; Yokoyama, S.; Vassylyev, D. G. *Cell* **2004**, *117*, 299. (c) Wang, J. D.; Sanders, G. M.; Grossman, A. D. *Cell* **2007**, *128*, 865. (d) Paul, B. J.; Berkmen, M. B.; Gourse, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 7823. (e) Choy, H. E. *J. Biol. Chem.* **2000**, *275*, 6783.

(3) (a) Fischer, M.; Zimmerman, T. P.; Short, S. A. *Anal. Biochem.* **1982**, *121*, 135. (b) Takahashi, K.; Kasai, K.; Ochi, K. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 4320. (c) Saito, N.; Xu, J.; Hosaka, T.; Okamoto, S.; Aoki, H.; Bibb, M. J.; Ochi, K. *J. Bacteriol.* **2006**, *188*, 4952.

(4) (a) Lee, D. H.; Kim, S. Y.; Hong, J.-I. *Angew. Chem., Int. Ed.* **2004**, *43*, 4777. (b) Lee, D. H.; Im, J. H.; Son, S. U.; Chung, Y. K.; Hong, J.-I. *J. Am. Chem. Soc.* **2003**, *125*, 7752. (c) Kruppa, M.; Konig, B. *Chem. Rev.* **2006**, *106*, 3520. (d) Jang, Y. J.; Jun, E. J.; Lee, Y. J.; Kim, Y. S.; Kim, J. S.; Yoon, J. *J. Org. Chem.* **2005**, *70*, 9603. (e) Ojida, A.; Mitooka, Y.; Inoue, M.; Hamachi, I. *J. Am. Chem. Soc.* **2002**, *124*, 6256. (f) Leevy, W. M.; Gammon, S. T.; Jiang, H.; Johnson, J. R.; Maxwell, D. J.; Jackson, E. N.; Marquez, M.; Piwnica-Worms, D.; Smith, B. D. *J. Am. Chem. Soc.* **2006**, *128*, 16476.

(5) (a) Cho, H. K.; Lee, D. H.; Hong, J.-I. *Chem. Commun.* **2005**, 1690. (b) Kim, S. K.; Lee, S. H.; Lee, J. Y.; Lee, J. Y.; Bartsch, R. A; Kim. J. S. *J. Am. Chem. Soc.* **2004**, *126*, 16499. (c) Nishizawa, S.; Kato, Y.; Teramae, N. *J. Am. Chem. Soc.* **1999**, *121*, 9463. (d) Kim, J. S.; Choi, M. G.; Song, K. C.; No, K. T.; Ahn, S.; Chang, S.-K. *Org. Lett.* **2007**, *9*, 1129.

(6) Because of the 2:1 binding mode, the excimer fluorescence of **PyDPA** decreased over 7.7 μM of (p)ppGpp. See the Supporting Information.

(7) (a) Cashel, M. *Methods in Molecular Genetics*; Academic Press: New York, 1994; Vol. 3, p 341. (b) Haseltine, W. A.; Block, R. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 1564.

(8) (a) Sy, J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5529. (b) Heinemeyer, E. A.; Richter, D. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4180. (c) Heinemeyer, E. A.; Richter, D. *FEBS Lett.* **1977**, *84*, 357.

(9) (a) Pizer, L. I.; Merlie, J. P. *J. Bacteriol.* **1973**, *114*, 980. (b) Kuroda, A.; Murphy, H.; Cashel, M.; Kornberg, A. *J. Biol. Chem.* **1997**, *272*, 21240.

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