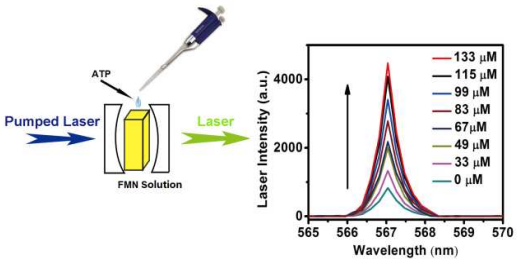
RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances



Flavin mononucleotide and lumiflavin, the fluorescent biomolecules, were firstly demonstrated as the ATP Sensors.

Journal Name

RSCPublishing

COMMUNICATION

Fluorescent/laser dual-channel ATP sensors based on flavins

Cite this:

Yu Liu, Peng Li, Hongwei Ma, Ming Zhang* and Feng Li*

DOI: 10.1039/x0xx00000x

www.rsc.org/

Flavin mononucleotide (FMN) and lumiflavin, the fluorescent biomolecules, were firstly demonstrated as the ATP Sensors. FMN and lumiflavin are totally compatible with the human body and can be easily obtained with low cost. The laser and fluorescence of FMN and lumiflavin in aqueous solution can efficiently discriminate the ATP from other common anion such as Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^- and PPi.

Adenosine-5'-triphosphate (ATP) serves as a phosphate donor in kinase-catalyzed protein phosphorylation and the extracellular ATP released from the cell membrane mediates many cell-to-cell signals in a wide range of physiological and pathological conditions [1].Therefore, the determination of ATP is essential to biochemical studies as well as clinical diagnosis. There have been considerable efforts to develop fluorescent or colorimetric sensors for ATP [2-21]. For examples, Hamachi et al. developed a series of binuclear zinc complexes as fluorescent chemosensors to ATP [2-5]. Yoon et al. reported a pyrene derivative that can display a unique ratiometric fluorescence change only with ATP binding [6,7]. Mohr et al. synthesized silica nanoparticles for ATP detection [9]. Hong et al. presented a off-on switching fluorescent sensor shows a moderate selectivity for ATP [11]. Bencini et al. developed a phenanthroline-containing polyammonium receptor which can discriminate ATP from other nucleosides through a quantitative quenching of its fluorescence emission [12,13]. Morii et al. reported a covalently linked fluorescent ribonucleopeptide (RNP) sensors that are capable of detecting ATP and GTP simultaneously [14]. However above achievements still need chemical or biological synthesis process which two or more procedures should be gone through. Water solubility and biocompatibility are in urgent need of practical application.

Flavin mononucleotide (FMN), a kind of fluorescent biomolecules produced from vitamin B2, serves as coenzyme in a series of oxidation–reduction catalysts and is found in many types of human tissue including heart, liver, and kidney tissue, thus is totally compatible with the human body and can be easily obtained with low cost. FMN also has a relatively high fluorescent quantum yield of 0.23 [15; 16], which is superior to many other fluorescent biomolecules in the human body whose fluorescent quantum yields are very low. Nizamoglu and Yun [17] demonstrated FMN to be an efficient laser gain material, and the laser based on it has the lasing thresholds as low as tens of nanojoules. The merits of FMN mentioned above indicate it should be good fluorescent biosensors, but there is no report about this until now.

In this work, we firstly used the laser and fluorescence of FMN to detect ATP, which has the following advantages: I) FMN is totally compatible with the human body and can be easily obtained with low cost; II) the laser of FMN can efficiently discriminate the ATP from other common anion such as Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^- and PPi; III) It is fluorescence enhancement which is more desirable in sensing than fluorescence quenching, and the limit of detection (LOD) of FMN is 1.0 μM and 7.3 μM for laser and fluorescence, respectively. Moreover, the feasibility for determining ATP in urine samples was also studied, and satisfactory results were obtained. Lumiflavin has the same isoalloxazine core as FMN but lacks the side chain and it has the similar properties with FMN. We performed the parallel experiments of ATP detection using the laser and fluorescence of lumiflavin in aqueous solution, and obtained the similar results to FMN, please see the ESI.

We used two concave lens (interspacing: L=12.7mm; curvatures: 50 mm) and a 400 uL quartz container to constructed a simple low-loss optical resonator. Both the mirrors had a dichroic coating with high reflectivity (R>99%) in a wavelength range of 500-560nm and high transmission at λ<450nm. The quartz container was completely filled with 100 μM aqueous solution of FMN or lumiflavin and is fixed between the two mirrors. The concave surfaces of the mirrors was used as internal reflective cavity. A Nd:YAG laser (λ =355 nm, pulse width: 5 ns) was used to optically pump the resonator. The pump energy was adjusted by a set of neutral density filters. The output-light of the cavity was collected through a fibre-optics probe connecting a computer. The details of the materials and the measure methods can be found in the ESI.

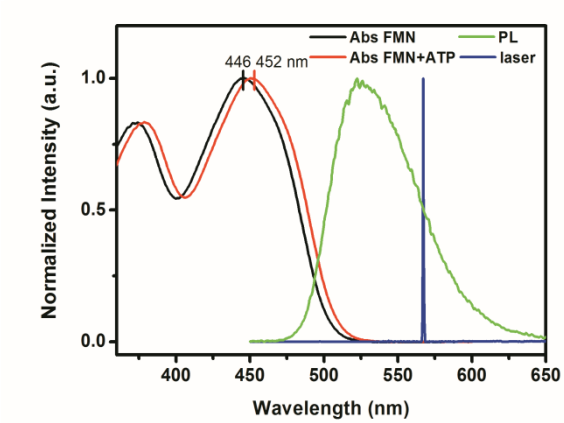


Fig. 1. The Absorption (black line, 100 μM), PL (green line, 100 μM) and laser (blue line, 100 μM) spectra of FMN in aqueous solution and the absorption (red line) spetra of FMN (100 μM) and FMN (100 μM)+ATP (20 μM) in aqueous solution.

Fig.1. shows the absorption, photoluminance (PL) and laser spectra of FMN. As can be seen, the absorption bands peaking at around 446 nm and 375 nm correspond to the transitions of isoalloxazine chromophore from S0 to S1 and from S0 to S2, respectively [15; 18]. The PL spectrum of FMN is characterized by broad structureless band peaking around 522 nm, corresponding to the transition from S1 to S0 [15]. Under the irradiation of a Nd:YAG nanosecond pulse laser (355 nm), the FMN aqueous solution in the optical resonator emits a laser peaking at 567 nm. The details of FMN laser properties can be found in ESI.

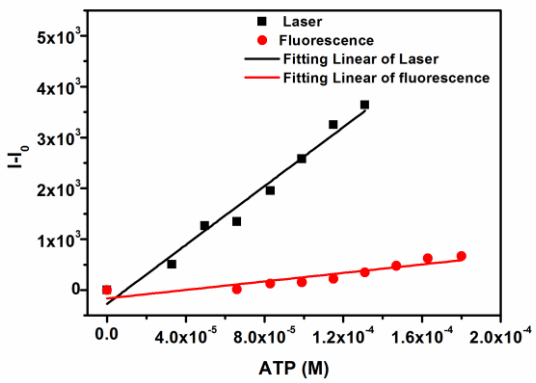


Fig 2. Changes ($I-I_0$) of the fluorescence (red circle) and laser (black square) spectra of FMN (100 μM) as a function of the concentration of ATP sodium salt in aqueous solution.

Upon the incremental addition of ATP, the intensities of the fluorescent and laser spectra increase and are enhanced by 3.4 and 5.5 fold until the amounts of ATP reach 180 μM and 133 μM, respectively. By linearly fitting the changes of the fluorescence and laser (Fig 2) as the function of concentration of ATP, we obtain the slop of 2.9×10^7 for laser and 4.2×10^6 for fluorescence, respectively. According to LOD= $3S_B/m$ [19], where S_B is the standard deviation of blank measurements and m is the slope of intensity versus sample concentration, the LODs of the laser and fluorescence sensor are calculated to be 1.0 μM and 7.3 μM, both of which are much lower than the ATP levels in human body (4 mM in resting muscle; 2 mM in erythrocytes) [20]. The LOD of laser sensor is decreased about one order of magnitude compared to that of fluorescence sensor. This is because laser has the function of amplifying the test signal.

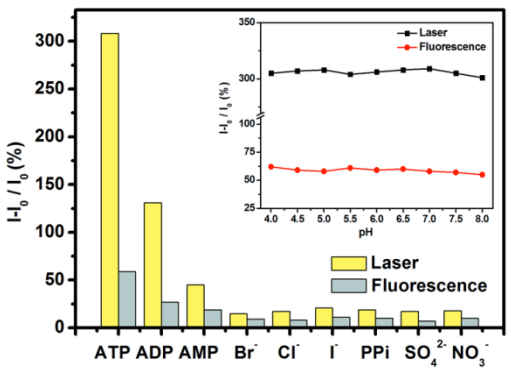


Fig. 3. Change ratios of laser and fluorescence of FNM (100 μM) upon the addition of various anions with the concentrations of 99 μM in aqueous solution. Inset: Change ratios of laser and fluorescence of FNM (100 μM) upon the addition of ATP (99 μM) in different buffer solution with the PH ranging from 4.0 to 8.0.

The selectivity of the laser and fluorescence sensor of FMN was tested against several common anions. The results are represented in Fig. 3. As can be seen, inorganic anions cannot provide significant interactions with FMN. The change ratios caused by inorganic anions are less than 21 % for laser sensor and 11 % for fluorescence sensor, respectively. On the other hand, ATP, ADP and AMP can interact well with FMN and enhance the laser and fluorescence intensity to a different extent. The highest enhancement is observed in the case of ATP (308 % for laser, 59 % for fluorescence), followed by ADP (131 % for laser, 27% for fluorescence) and AMP (45 % for laser, 19 % for fluorescence). The results indicate that the selectivity of the laser and fluorescence sensor are not satisfied to discriminate ATP, ADP and AMP, but the laser and fluorescence sensor can efficiently discriminate the ATP, ADP or AMP from other common anion, thus can be used as the rough sensors due to the low cost of FMN.

A study was also carried out regarding the influence of pH on the enhancement of laser and fluorescence of FMN towards ATP. For this purpose, the changes of laser and fluorescence intensity were measured in the presence of ATP (99 μM) in acetate sodium buffer solution with the pH ranging from 4.0 to 6.0, potassium dihydrogen phosphate, sodium hydroxide buffer solution with the pH ranging from 6.0 to 7.0 and Tris-HCl buffer solution with the pH ranging from 7.0 to 8.0, as shown in the inset of Fig. 3. The results indicated that the pH values have little effect on the laser and fluorescence sensor of FMN in a large range from 4.0 to 8.0.

According to the fact that the ability of ATP, ADP and AMP to enhance the laser and fluorescence of FMN becomes weaken with the decrease of the number of phosphates, we guess that the hydrogen bonds between the isoalloxazine of FMN and the phosphate groups of ATP, ADP and AMP induce the enhancement of the laser and fluorescence of FMN. The absorption bands of FMN +ATP solution have a little red-shift compared to those of FMN solution suggesting the existence of hydrogen bonds, [21; 15] as shown in Fig. 1. 1H NMR spectra were also employed to clarify the interaction between ATP and FMN. Upfield shifts were observed from Fig. 4 for the aromatic protons (H1, H2 and H9) of isoalloxazine and the purine protons (H10 and H11) of ATP after mixing the ATP and FMN solutions. The upfiled shifts denote the increase of the electron cloudy density of the microenvironment, indicating the strong interaction between ATP and FMN.

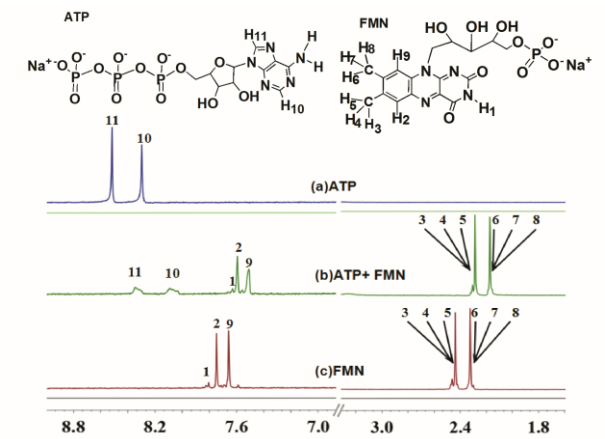


Fig. 4. Partial 500 MHz 1H NMR spectra for (a) ATP (10 mM), (b) ATP (10 mM)+ FMN (100 μM), (c) FMN (100 μM) in D_2O solution.

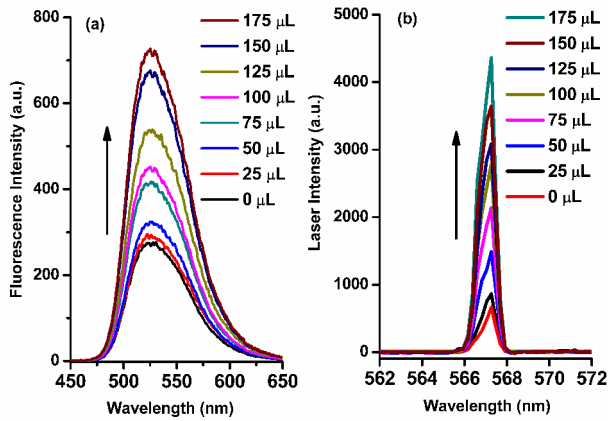


Fig. 5. The fluorescence (a) and laser (b) spectra of FMN (100 μM) in aqueous solution upon incremental addition of one urine sample.

In order to demonstrate the feasibility of the FMN sensor in real samples, the urine samples from 5 healthy volunteers were used. Fig 5 shows the changes of the fluorescence and laser spectra of FMN upon incremental addition of one urine sample. As can be seen, only 25 μL urine can cause the obvious changes of the intensity of fluorescence and laser. The change ratios of the intensity of fluorescence and laser can reach 198% and 557% after adding 175 μL urine, respectively. Thus the fluorescence and laser of FMN aqueous solution can be used to detect the ATP in urine. It should be noted that the change ratios of the intensity of fluorescence and laser caused by the urine samples from different volunteers are different, indicating the various content of ATP in different urine samples.

We also performed the parallel experiments of ATP detection using the laser and fluorescence of lumiflavin in aqueous solution. Similar results to FMN were obtained, please see ESI.

Conclusions

In summary, we demonstrated FMN and lumiflavin can be as the fluorescent/laser dual-channel ATP sensors in 100% aqueous solution. FMN and lumiflavin are totally compatible and non-toxic with the human body and can be easily obtained with low cost. The LOD of FMN laser was found to be much lower than the ATP levels in human body (4 mM in resting muscle; 2 mM in erythrocytes). The feasibility for determining ATP in urine samples was also approved. The ATP sensors from the aqueous solution of flavins offer the possibility to roughly detect ATP with low cost in the future.

This work was supported by NSFC (Grant No. 21374037, 21221063 and 91233113).

Notes and references

State Key Lab of Supramolecular Structure and Materials, Jilin University, 2699 Qianjin Avenue, Changchun 130012, People's Republic of China. E-mail: lifeng01@jlu.edu.cn; zhming@jlu.edu.cn.

1 A. V. Gourine, E. Llaudet, N. Dale and K. M. Spyer, *Nature* , 2005, **7047**, 108.
2 A Ojida, H. Nonaka, Y. Miyahara, S. I. Tamaru, K. Sada and I. Hamachi, *Angew. Chem.-Int. Edit*, 2006, **33**, 5518.
3 A. Ojida, I. Takashima, T. Kohira, H. Nonaka and I. Hamachi, *J AM CHEM SOC*, 2008, **36**, 12095.
4 T. Sakamoto, A. Ojida and I. Hamachi, *Chem. Commun*, 2009, **2**, 141.
5 Y. Kurishita, T. Kohira, A. Ojida and I. Hamachi, *J AM CHEM SOC*, 2010, **38**, 13290.
6 Z. C. Xu, K. S. Kim and J. Y. Yoon, *J AM CHEM SOC*, 2009, **131**, 15528.
7 Z. Xu, D. R. Spring and J. Yoon, *Chemistry-an Asian Journal*, 2011, **8**, 2113.
8 Y. Zhou, Z. C. Xu and J. Y. Yoon, *Chem. Soc. Rev*, 2010, **40**, 2222.
9 A. J. Moro, J. Schmidt and G. J. Mohr, *Chem. Commun*, 2011,**47**, 6066.
10 D. L. Lee, S. Y. Kim and J. I. Hong, *Angew. Chem.-Int. Edit*, 2004, **43**,777.
11 D. L. Lee, S. Y. Kim and J. I. Hong, *Tetrahedron Letters*, 2007, **48**, 4477.
12 C. Bazzicalupi, A. Bencini, *Chem. Commun*, 2006, **39**, 4087.
13 C. Bazzicalupi, A. Bencini, *Chem. Commun*,2005, **20**, 2630.
14 S. Nakano, M. Fukuda, T. Tamura, R. Sakaguchi, E. Nakata and T. Morii, *J AM CHEM SOC*, 2013, **135**, 3456.
15 P.F. Heelis, *Chem. Soc. Rev*,1982, **11**, 15.
16 W. Holzer, J. Shirdel and P. Zirak,, *Chem. Phys*, 2005, **308** , 69.
17 S. Nizamoglu, S.H Yun, *.Adv. Mater*, 2013, **41**, 5943.
18 E. Sikorska, I.V. Khmelinskii and M. Prukala, *J. Phys. Chem. A* , 2004, **108**, 1501.
19 Iternational Union of Pure and Applied Chemistry,*Pure and Applied Chemistry*, 1976, **45**, 99.
20 A. Ghosh, P. Ronner, and E. Cheong, *J. Biol. Chem*, 1991, **266**, 22887.
21 K. Yagi, N. Ohishi, and K. Nishimoto, *Biochemistry*, 1980. **19**, 1553