Fluorescent/laser dual- channel ATP sensors based on flavins

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Materials

Riboflavin-5'-phosphate sodium salt dihydrate (FMN) and lumiflavin were purchased from J&K Scientific(~ 63 $，10g). Adenosines 5′-triphosphate disodium salt hydrate (ATP), adenosine 5′-diphosphate sodium salt (ADP) and adenosine 5′-monophosphate sodium salt (AMP) were purchased from Sigma and kept in the freezer under −20 ° C. Tetra-sodium pyrophosphate (PPi) was purchased from J&K. Acetate acid gracial and hydrochloric acid 37% were purchased from J&K.

Laser sensor

Experimental setup: 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 two mirrors were used as internal reflective cavity. A Nd:YAG laser (λ=355 nm, pulse width: 5 ns) was used to optically pump the resonator at left side (**Figure S1**) which was focused in the middle of the mirror. The pump energy was adjusted by a set of neutral density filters. On the right side the output-light of the cavity was collected through a fibre-optics probe connecting a computer (**Figure S1**).

100 μM FMN/lumiflavin solution was obtained after dissolving them in the acetate sodium buffer solution at pH 5.0. Each time we injected 0.5 μL ATP (10 mM) into the 300μL FMN solution. The mixture solution needs to be waited for 5 min at the room temperature. The laser spectrogram was measured using 355nm excitation light source.

Fluorescent sensor

Fluorescence spectra were taken with a fluorescence spectrophotometer (Shimadzu RF-5301PC) equipped with a xenon lamp excitation source. 100 μM FMN/lumiflavin solution was obtained after dissolving them in the acetate sodium buffer solution at pH 5.0. Each time we injected 0.5μL ATP (10 mM) into the 300 μL FMN solution. The mixture solution needs to be waited for 5 min at the room temperature.

Figure S1 Schematic of laser detection measurement

Laser properties of FMN and lumiflavin aqueous solutions

Figure S2 The spectra of FMN (a) and lumiflavin (b) as a function of the pumping power. The emission spectra are narrowed and the FWHMs become smaller as the pumping power gradually increases from10 to 20 $kWcm^{-2}$.

Figure S3. The laser spectra of FMN (a) and lumiflavin (b) with different length of optical resonator. When we adjust the position of one mirrors to increase the length of optical resonator, the wavelengths of the laser change. The wavelengths firstly show redshift (moving from 567 to 579 nm) when the length of optical resonator changes from 12.7 to 21.7 mm and then show blue shift (changed to 560 nm) when the length of optical resonator reaches 30.2 mm.

Figure S4. The peak intensity of spectra of FMN (a) and lumiflavin (b) as a function of the pumping power. The threshold values of the laser are 15 and 13 $kWcm^{-2}$ for FMN and lumiflavin, respectively.

Properties of the fluorescence and laser of lumiflavin aqueous solutions as the ATP sensors

Figure. S5 The fluorescence (a) and laser (b) spectra of lumiflavin (100 μM) upon incremental addition of ATP sodium salt in aqueous solution. With the incremental addition of ATP, the intensity of the fluorescent and laser spectra linearly increased and are enhanced by 4.5 and 5.6 fold until the amounts of ATP reach 163 μM and 133 μM.

Figure. S6 Change ratios of laser and fluorescence of lumiflavin (100 μM) upon the addition of various anions with the concentration of 99 μM in aqueous solution. Inset: Change ratios of laser and fluorescence of lumiflavin (100 μM) upon the addition of ATP (99 μM) in different buffer solution with the PH ranging from 4.0 to 8.0. The change ratios caused by inorganic anions are less than 19 % for laser sensor and 14 % 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 (301 % for laser, 62 % for fluorescence), following by ADP (127 % for laser, 30% for fluorescence) and AMP (48 % for laser, 17 % for fluorescence).

Figure S7 The fluorescence (a) and laser (b) spectra of lumiflavin(100 μM) in aqueous solution upon incremental addition of one urine sample. 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 350% and 560% after adding 175 μL urine, respectively.