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Sensitive sulfide ion detection by optofluidic catalytic laser using horseradish peroxidase (HRP) enzyme

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ABSTRACT

We report an optofluidic catalytic laser for sensitive sulfide ion detection. In the catalytic reaction, horseradish peroxidase (HRP) enzyme is used for catalyzing the non-fluorescent substrate, 10-Acetyl-3,7-dihydroxyphenoxazine (ADHP), to produce highly fluorescent resorufin, which was used as gain medium for lasing. Using sulfide ions as inhibitors, the catalytic reaction slows down, resulting in a delay in the lasing onset time, which is used as the sensing signal. The sensing mechanism of the catalytic laser is theoretically analyzed and the performance is experimentally characterized. Sulfide anion is chosen as a model ion because of its broad adverse impacts on both environment and human health. Due to the optical feedback provided by the laser, the small difference in the sulfide ion concentration can be amplified. Consequently, a detection limit of 10 nM is achieved with a dynamic range as large as three orders of magnitude, representing significant improvement over the traditional fluorescence and colorimetric methods. This work will open a door to a new catalytic-laser-based chemical sensing platform for detecting a wide range of species that could inhibit the catalytic reaction.

1. Introduction

Ions play unique and vital roles at different scales, ranging from cell biochemistry (Brown et al., 1993) to ocean acidification (Orr et al., 2005). Optical methods are one of the mainstream technologies for ion concentration detection. Traditionally, the fluorescent and colorimetric mechanisms are used. The former relies on the quenching (Barati et al., 2016; Tang et al., 2013; Wang et al., 2013) or recovery (Gore et al., 2013; Sun et al., 2016; Wang et al., 2015; Z.X. Wang et al., 2014) of fluorescence, whereas the latter uses the absorption of light (Chen et al., 2015; Hatamie et al., 2014; Zhang et al., 2011). Since these technologies inherently do not have high sensitivity, novel nanomaterials such as nanoparticles (Hatamie et al., 2014; Zhang et al., 2011), nanowires (H. Wang et al., 2014), nanosheets (Wang et al., 2016), nanoclusters (Gao et al., 2016; Wang et al., 2015; Z.X. Wang et al., 2014; Xu et al., 2016), and quantum dots (Gore et al., 2013), are being explored to improve the sensitivity. The drawbacks of complex synthesis process, fluctuations in uniformity, tendency to aggregate, limit the potential of nanomaterials-based ion sensors. Here, as an alternate option, we develop an optofluidic catalytic laser for sensitive ion detection.

Optofluidic lasers has been extensively investigated for sensitive intra-cavity biochemical analysis (Fan and Yun, 2014; Humar and Yun, 2015; Sun and Fan, 2012; Wu et al., 2014). In comparison with the traditional fluorescence and colorimetric based detection, the optical feedback mechanism of laser is able to amplify small changes in the gain medium, thus achieving high sensitivity (Fan and Yun, 2014; Wu et al., 2014). Here, we explore the possibility to combine the optofluidic laser technology and enzyme catalytic reaction inside a laser cavity for the detection of ion concentration. The fluorescent product generated by the enzyme-substrate reaction is used as the gain medium. The concentration of the product increases over the reaction time. When the gain reaches a concentration threshold, the laser emission starts to emerge. However, in the presence of ions, which act as an inhibitor, the catalysis process slows down. Consequently, it takes longer time for laser emission to occur. The delay in the laser onset time can thus be used as the sensing signal. In this work, we theoretically analyze the sensing mechanism of the catalytic laser and experimentally character-

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Table 1
Comparison of different methods for sulfide ion detection.

Technology platform	Sensing principle	Detection limit (μM)	Dynamic range (μM)	Dynamic range, 10Log (C_{max}/C_{min})[a]	References
Fluorescence	Quenching and recovery	0.18	0.5–5	10.0	Wang et al. (2013)
		1.35	1.35–10	8.7	Tang et al. (2013)
		0.28	0.5–8	12	
		0.88	0.88–25	14.5	H. Wang et al., 2014
		0.32	2–10	7	Barati et al. (2016)
	Quenching	50	50–1000	13	Xu et al. (2016)
		0.42	0.5–12	13.8	Wang et al. (2016)
		0.2	0.2–12	17.8	Gao et al. (2016)
		1.1	5–100	13	Sun et al. (2016)
		6.5	3.1–56.2	12.6	Gore et al. (2013)
		0.31	0.31–700	33.5	Z.X. Wang et al., 2014
		0.38	0.5–80	22	Wang et al. (2015)
	Enzyme inhibition	1.0	1.0–10	10	This work
Colorimetric	Absorbance by UV–vis	0.28	0.5–15	14.8	Chen et al. (2015)
	spectroscopy	8.1	12.5–50	6	Hatamie et al. (2014)
		0.3	0.3–10	15.2	Zhang et al. (2011)
	Plasmonic wavelength shift	0.5	0.5–5	10	Huang et al. (2014)
Phosphorescence	Quenching	0.138	2.67–596	23.5	Zhou et al. (2010)
Resonance Rayleigh scattering	Conformation changes	0.24	0.81–308.4	25.8	Yan et al. (2016)
Amperometric	Enzyme inhibition	0.3	1.1–16.3	11.7	Savizi et al. (2012)
Optofluidic laser	Enzyme inhibition	0.01	0.01–10	30	This work

[a] C_{max} and C_{min} are the maximum and minimum detectable concentrations of S^{2-}.

ize the laser performance. Sulfide anion (S^{2-}) is chosen as a model ion due to its wide negative impacts in both environment and healthcare (Bagarinao, 1992; Yang et al., 2009). A detection limit of 10 nM is achieved with a dynamic range of three orders of magnitude, which represents a significant improvement over the state-of-the-art (see Table 1 for comparison). The results agree well with the theoretical analysis. Potential improvement in the sensing performance is also discussed.

2. Theoretical analysis

As illustrated in Fig. 1, the enzyme catalytic reaction occurs when the enzyme, the substrate and hydrogen peroxide (H_2O_2) are mixed, which converts the transparent substrate into the highly fluorescent product that is used as the gain medium. The product concentration increases with the reaction time. Laser emission can be observed under a fixed optical pump when the product concentration reaches a threshold for lasing. In the presence of inhibitors such as S^{2-}, the enzyme activity is suppressed at first (Fig. 1b) due to the binding of inhibitors to the enzyme-substrate complex. Then S^{2-} is consumed and oxidized by H_2O_2, leading to the recovery of the enzyme activity and the catalytic reaction rate. A higher S^{2-} concentration results in a longer recovery time and hence a longer laser onset time, which can be used as the sensing mechanism for inhibitor detection.

We develop a theoretical model to analyze ion detection mechanism based on the optofluidic catalytic laser described above. The laser emission can be expressed as (Wu et al., 2014)

$$I_{Laser}(t) = I_0 \cdot \left[W_{pump} \left(\frac{[P]}{\gamma [C]_0} - 1 \right) - 1 \right], \quad (1)$$

where I_0 is a constant, W_{pump} is the pump intensity, $[P]$ is the concentration of the fluorescent product. $[C]_0$ is the initial concentration of the substrate. γ is the fraction of the product molecules in the excited state at the lasing threshold. Note that γ remains constant for a given optofluidic laser.

According to the laser theory, once the pump intensity exceeds the threshold intensity, i.e., $W_{pump} \geq W_{th}$, laser emission emerges. In our experiment, by using a fixed W_{pump}, the laser threshold can be represented by the threshold concentration of the product (Wu et al., 2014)

$$[P]_{th} = [C]_0(\gamma / W_{pump} + \gamma). \quad (2)$$

As the concentration of product increases with reaction time, the threshold concentration can be further converted to the threshold time, named laser onset time in this work. The temporal changes of $[P]$ can be calculated by

$$[P](t) = \int_0^t v(t)dt, \quad (3)$$

where $v(t)$ is the reaction rate of the enzyme catalytic reaction, which, according to the Michaelis–Menten theory, is given by Cornish-Bowden (1974)

$$v(t) = \frac{V_{max}[C]}{K_m + (1 + [S]/k_i)[C]}, \quad (4)$$

where K_m is the Michaelis-Menten constant. $[C] = [C]_0 - [P]$ is the substrate concentration after certain reaction time. $V_{max} = k_{cat} \cdot [E]_0$ denotes the maximum reaction rate. k_{cat} is the turnover number, i.e., the maximum number of substrate molecules converted to product per enzyme molecule per second. $[E]_0$ is the enzyme concentration. $[S]/k_i$ is an inhibition term, with $[S]$ the concentration of inhibitor S^{2-} and k_i the inhibition constant.

In the mixture, S^{2-} is oxidized by H_2O_2. The decreasing of $[S]$ can be described by the rate equation (Millero et al., 1989)

$$\frac{d[S]}{dt} = -k \cdot [S] \cdot [H_2O_2], \quad (5)$$

where k is the rate constant. $[H_2O_2]$ is the concentration of H_2O_2, given by

$$[H_2O_2] = [H_2O_2]_0 - ([S]_0 - [S]) = \Delta + [S], \quad (6)$$

where $\Delta = [H_2O_2]_0 - [S]_0$ is the initial concentration difference between H_2O_2 and S^{2-}. By solving Eqs. (5) and (6), we arrive at

$$[S] = \frac{\Delta}{\beta e^{\Delta kt} - 1}, \quad (7)$$

where $\beta = [H_2O_2]_0/[S]_0$.

In the Supplementary material, simulation results based on the theory are given. Fig. S1 shows temporal changes of the S^{2-} concentration. It decreases exponentially with time. The calculated reaction rate as a function of time is shown in Fig. S2 under different initial S^{2-}