Biosensors and Bioelectronics 179 (2021) 113075

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: http://www.elsevier.com/locate/bios

Adenine deficient yeast: A fluorescent biosensor for the detection of Labile Zn(II) in aqueous solution

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ARTICLE INFO

Keywords:
Biosensors
Yeast
Autofluorescence
Labile Zn(II)

ABSTRACT

Labile Zn(II) species play key roles in inducing bioresponse. Thus the development of a biosensor for labile Zn(II) quantification is important. In this study, we demonstrate that the autofluorescence intensity (FITC channel) of an adenine deficient yeast (Ade(−) yeast) was enhanced in the presence of Zn^{2+}. Yeast cells were firstly cultured for 24 h to obtain the Ade(−) yeast, and the biomass (OD value) was optimized to be 0.03. After pre-culturing in D-glucose at 2.5 g/L for 1 h, the cells were transferred to 2.5 g/L D-glucose containing Zn^{2+} and the autofluorescence intensity was determined by flow cytometry after 1 h. The biosensor could detect Zn^{2+} at ultralow concentration (0.01 μM) in the optimized medium and accurately quantify the extracellular concentrations of Zn^{2+} ranging from 0.01 to 0.5 μM. High tolerance of Ade(−) yeast to salinity, pH variation and other metals enabled its application as a biosensor for labile Zn detection in complex media. Determining dissolved Zn^{2+} from a viscous sample (zinc cream), Ade(−) yeast accurately quantified the labile Zn^{2+} with a lower quantification limit than the chemosensor and higher simplicity than the conventional method (ICP-MS coupled with ultrafiltration). The study provides a novel biosensor based on an Ade(−) yeast and could be potentially used to detect labile Zn(II) species at trace levels in complex media.

1. Introduction

Excess Zn from either natural or anthropogenic activities can lead to threats to biota and to human health (Councell et al., 2004; Long et al., 2011), especially in the case of labile Zn^{2+} which tends to bind with biomolecules. Therefore, determining concentrations of labile Zn^{2+} in aqueous environments is important due to its high bioavailability. Concentrations of Zn in several aqueous environments have been determined to be within the range of 0.001–0.3 μM (Ma et al., 2020; Sun et al., 2019) and thus the concentrations of labile Zn^{2+} must be much lower. It is important to develop methods to measure the labile Zn^{2+} concentration, and thus the potential toxic effects of Zn^{2+} on organisms can be estimated. To quantify metals in environmental samples, traditional methods including inductively coupled plasma/atomic emission spectrometry, atomic absorption spectrometry, microparticle-induced X-ray emission, synchrotron radiation X-ray spectrometry, cold vapor atomic fluorescence spectrometry and electron paramagnetic resonance are generally employed because of their high specificity and accuracy (Xu et al., 2011; Zhou et al., 2016). However, applications of these methods are limited due to their high cost and needs for complicated pretreatment, sophisticated instrumentation with skilled personnel, and especially the use of complicated extraction procedures in which the speciation of metals is altered.

The design of organic fluorophores is based on the reaction of fluorescein, quinolone, coumarins and naphthalene with Zn^{2+}, and these fluorophores can potentially be used due to their high sensitivity and cost effectiveness (Gan et al., 2016). Though the quantification range of the probe is mostly 0.06–1 μM (Leung et al., 2019; Liu et al., 2016; Wang et al., 2010, 2011), limitations of organic fluorophores are obvious, such as the tedious synthesis process, complicated purification, hydrophobicity, and poor photostability (Xu et al., 2011). Nanoprobes such as silica-based nanoparticles, metal-based nanoparticles and semiconductor quantum dots have been proposed to lower the quantification limit to 0.1 μM (Rastogi et al., 2011; Xu et al., 2011). Nevertheless, the

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https://doi.org/10.1016/j.bios.2021.113075
Received 1 January 2021; Received in revised form 1 February 2021; Accepted 2 February 2021
Available online 6 February 2021

highest sensitivity of fluorescence probes was only effective within a narrow range of pH values. Using iron oxide/graphene composites to detect Zn^{2+} at 0.015 μM, the pH value should be optimized to be 4.5 (Lee et al., 2016). Nanoprobes such as CdSe/ZnS core/shell nanoparticles released components which were more toxic than Zn^{2+} (Ruedas-Rama and Hall, 2008). Compared with chemosensors for detecting other metals, designs of the Zn^{2+} specific chemosensors are limited, resulting from the lack of intrinsic spectroscopic signals (Li et al., 2010). With similar chemical properties as Zn^{2+}, Cd^{2+} interferes with the specificity of chemosensors (Choi et al., 2014; Hu et al., 2015). Any presence of Cu^{2+} also interferes with the detection of Zn^{2+} due to the formation of Cu–Zn intermetallic species (Hwang et al., 2019).

Biosensors with their advantages of high specificity, fast response, low cost, high portability, and ability to obtain real time signals, display high potential in quantifying Zn^{2+} and also provide toxicological relevance (Geun et al., 2000). Specific enzymes, metalloproteins and antibodies are involved in protein-based biosensors, while individual-based biosensors are usually genetically engineered microorganisms (Chouteau et al., 2005; Lu and Liu, 2003; Ren et al., 2020). Enzyme-based biosensors generally have a higher Zn^{2+}quantification limit of 5 μM as compared to other metals: Hg^{2+}, Ag^{+}, Pb^{2+}, Cu^{2+} with quantification limits of 0.002, 0.02, 0.2, 0.5 μM, respectively. Regeneration of biosensors requires the introduction of ethylenediaminetetraacetic acid (EDTA), KCN and dithiothreitol, making the procedure complicated (Verma and Singh, 2005). Controlling the gene expression of *E. coli*, the quantification limit of Zn^{2+} was over 16 μM (Gireesh-Babu and Chaudhari, 2012; Ravikumar et al., 2012). The high quantification limit is mainly due to the highly expressed metal efflux proteins in microorganisms and the application of *E. coli* may lead to potential biosafety issues. Yeast cells with high bioengineering potential and few biosafety concerns have been used as a whole cell sensor system for fatty acids (Baumann et al., 2018). Furthermore, yeast cells are sensitive to metals especially the transition metals, making them potential candidates to have bioresponses to a variety of metals in the environment (Van Ho et al., 2002).

In this study, an adenine deficient yeast (Ade(−) yeast) was cultured to quantify labile Zn^{2+} in aqueous solution. Based on the results of flow cytometry, a good linear relationship between extracellular [Zn^{2+}] and autofluorescence increase has been developed, indicating the potential of using Ade(−) yeast in quantifying Zn^{2+}. We have used Ade(−) yeast to quantify the dissolution of zinc cream and compared the results with a fluorescence probe and with inductively coupled plasma mass spectrometry (ICP-MS) coupled with ultrafiltration. This work, for the first time, proposes a yeast-based biosensor to determine labile Zn^{2+} in aqueous solution, with ultrahigh sensitivity, a low quantification limit and great potential for biological assessments.

2. Materials and methods

2.1. Yeast culture and observation by microscope

Wild type *Saccharomyces cerevisiae* (Yeast W303) was used in this study. Cells were inoculated in YPD broth (Sigma) at around 185,000 cells/mL and cultured (30 °C, 200 rpm) for 24 h to obtain the Ade(−) yeast. The obtained Ade(−) yeast could be preserved at 4 °C for at least one week for further use. Optical Density (OD) values (600 nm) at different time points were measured by a microplate reader (FlexStation 3, Molecular Devices, USA) to develop the growth curve. Cells were obtained after 24 h and washed by ultrapure water for 3 times prior to the test. Ade(−) yeast cells were cultured in the medium with 10 μM Zn^{2+} for 10 min. A 0.5 mg/mL stock solution of concanavalin A (C2010, Sigma) was prepared and spread out on the dish to facilitate the immobilization of Ade(−) yeast on the culture dish. Fluorescence intensities of cells at channels including AF405, AF488 and AF633 were observed using a Celldiscoverer 7 Automated Microscope (Zeiss, USA). The location of mitochondria and nucleus was indicated by MitoTracker™ Deep Red FM (459 nM, Ex/Em-644/665 nm, M22426, Thermo Fisher Scientific, USA) and NucBlue™ Live ReadyProbes™ (2 drops per milliliter, Ex/Em-360/460 nm, R37605, Thermo Fisher Scientific, USA), respectively.

2.2. Determination of fluorescence intensity by flow cytometry

Ade(−) yeast cells were cultured in YPD broth and different metals including Ag, Al, As, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Se, Ti, and Zn (instrument calibration standard in 2% HNO_3, PerkinElmer, USA) were added at 10 μM. After co-culturing for 10 min, the fluorescence intensity of 10,000 cells was recorded by flow cytometry (BD FACSAria™ III sorter, USA). Fluorescence intensity was recorded at channels including FSC, SSC, DAPI (Ex/Em 358/461 nm), Alex Fluor 430 (Ex/Em 434/540 nm), FITC (Ex/Em 494/519 nm), PE (Ex/Em 496/578 nm), PE-Texas Red (Ex/Em 496/615 nm), PerCP-Cy5-5 (Ex/Em 482/695 nm) and PE-Cy7 (Ex/Em 496/785 nm). The fluorescence increase (%) was calculated as the (fluorescence intensity in the test group-fluorescence intensity in the control group)/fluorescence intensity in the control group × 100%. To determine the influence of growth stage on fluorescence increase, yeast cells at different time points (14 h, 19 h and 24 h) were collected. A medium of mixed YPD broth and ultrapure water (4:0/3:1/2:2/1:3/0:4) was used to culture Ade(−) yeast cells. Thus the influence of ratio of YPD broth to water on Zn^{2+} directed fluorescence increase was determined. D-glucose was added in ultrapure water as the carbon source, with the final concentrations of glucose at 0, 2.5, 5, 10, 20 g/L to determine the influence of glucose on fluorescence. Cells were diluted for different times to obtain the cells at different OD values (i.e. around 1.2, 0.6, 0.3, 0.24, 0.16, 0.12). Flow cytometry was used to determine the fluorescence increase of cells after adding 10 μM Zn^{2+} in the medium. To explore the time dependent change of Zn^{2+} directed fluorescence increase, the fluorescence intensity at different time points was recorded.

2.3. Quantification of Zn^{2+} in the medium

To obtain the highest sensitivity to Zn^{2+} and facilitate the direct determination by flow cytometry, the biomass of the Ade(−) yeast was diluted to be 0.03 (Fig. S1). Cells (OD value = 0.03) were put in glucose-based medium (2.5 g/L) and pre-cultured for 1 h. After that, the medium was replaced by a Zn^{2+} containing medium with the final concentrations of Zn^{2+} at 0, 0.01, 0.04, 0.06, 0.1, 0.2, 0.5, 0.6, 0.8, 1 μM, followed by the detection of fluorescence intensity by flow cytometry after 1 h. The relationship between [Zn^{2+}] and fluorescence increase was thus obtained. Zn^{2+} at 0.1 μM was added to the medium to determine the reproducibility of different batches of yeast. Fig. S2 shows that the increase of Zn^{2+}-induced autofluorescence of different batches of yeast was unchanged. To quantify the total Zn contents, Ade(−) yeast cells (OD value = 0.03) were cultured as described above, washed with ultrapure water for 4 times, digested with 1 mL 69% nitric acid (trace metal grade) and analyzed using ICP-MS (NexION 300X, PerkinElmer, USA). The detection limit of the concentration of Zn^{2+} (c_L) was obtained according to the International Union of Pure and Applied Chemistry:

$$c_L = \frac{k s_{b1}}{S}$$

where s_{b1} and S represent the standard deviation of the blank sample and the sensitivity at low concentration (slope value of the standard curve with concentrations of Zn ranged from 0 to 0.1 μM), respectively, with $k = 3$.

2.4. Interference test

Ade(−) yeast cells were firstly cultured in 2.5 g/L glucose for 1 h, followed by transfer to the medium with different salinity/pH/metals

and 0.5 μM Zn^{2+}. NaCl was added to obtain media with different salinities (i.e. 5, 10, 15, 20, 25 and 35). The pH values of the 2.5 g/L glucose solution were adjusted to 3.28, 5.20, 8.78 and 10.56 with 1 M NaOH and 1 M HNO_3. Acidic solution (pH = 3.28) was neutralized to pH = 7.50 using NaOH, followed by the additions of Zn^{2+} at 0.1 or 1 μM, yeast culture and detection by flow cytometry. Interference effects of other metals (i.e., Ag, Al, As, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Se, Ti) at 0.5 μM on the 0.5 μM Zn^{2+} directed fluorescence increase were explored. The time dependent influence of Ag and Cu on Zn^{2+} directed fluorescence increase was further determined. After culturing in these media for 1 h, fluorescence intensity was recorded by flow cytometry.

Mountain water was collected from the campus of Hong Kong University of Science and Technology, using a precleaned low-density polyethylene bottle. The sample was transferred to a cooler at 4 °C immediately without any filtration and preconcentration treatments. After culturing in 2.5 g/L glucose solution for 1 h-pretreatment, Ade(−) yeast cells were transferred to the sampled mountain waters added with 2.5 g/L glucose. As the internal standard, 0.01 μM Zn^{2+} was added to determine the possible influence of components in the mountain water on the sensitivity to Zn^{2+}. The culturing time was limited to 15 min in case of any side effects induced by other metals in the mountain water. Following culturing for 15 min, flow cytometry was used to detect the fluorescence intensity of cells. The mountain water at 1 mL was mixed with 1 mL of 10% HNO_3 and heated at 80 °C for 24 h, followed by quantification by ICP-MS (NexION 300X, PerkinElmer, USA).

2.5. Zn dissolution from Zn cream detected by a chemosensor, Ade(−) yeast cells and ICP-MS coupled with ultrafiltration

Zinc cream (OVELLE, zinc oxide 15% w/w) was dissolved in ultrapure water to prepare the homogenized emulsions at 182 mg zinc cream/L. The stock solution was diluted to 0.1 mg zinc cream/L for the test. A chemosensor ((9-anthrylmethyl)bis(2-pyridylmethyl)-amine, APA) at 10 μM was used in this study. After mixing the probe and Zn^{2+} (0, 0.25, 0.5, 0.8 and 1 μM) for 10 min, the fluorescence intensity at 420 nm emission wavelength was recorded by a microplate reader (Excitation wavelength at 375 nm, FlexStation 3, Molecular Devices, USA). As an internal standard, 0.5 μM Zn^{2+} was used to determine the influence of zinc cream on the fluorescence increase. Ade(−) yeast cells were precultured in glucose solution (2.5 g/L) for 1 h and Zn^{2+} was added in medium with final concentrations at 0, 0.02, 0.04, 0.06, 0.2, 0.4 μM. After 1 h, yeast cells were loaded in the flow cytometry system to determine the fluorescence intensity. In this case, 0.1 μM Zn^{2+} was added as the internal standard. EDTA-2Na (1 μM) was added to chelate the extracellularly dissolved Zn^{2+} and thus the influence of intracellular dissolved Zn^{2+} could be determined (Fig. S3). The fluorescence increase induced by extracellularly dissolved Zn^{2+} from zinc cream was recorded as: fluorescence increase (%)-fluorescence increase in the EDTA group (%). As a conventional method, ICP-MS coupled with ultrafiltration (10,000 kDa, 8000 g, 10 min, VIVASCIENCE, USA) was applied to determine the dissolution of zinc cream. To reduce the nonspecific adsorption of $Zn^{2+}$by the filtration membrane, two methods were used in this study. The sample firstly experienced 7 times ultrafiltration and the permeates were then collected at the 8th time. In the other method, Cu^{2+} at 1000 mg/L was used to occupy as many adsorption sites as possible, followed by ultrafiltration of the sample. In both methods, 0.2 μM Zn^{2+} was added as the internal standard to explore the efficiency of these two methods.

2.6. Statistical analysis

Data were expressed as the mean ± standard deviation and performed in triplicate. Statistical significance was determined using one-way analysis of variance and compared using LSD's test in SPSS 22.0.

3. Results and discussion

3.1. Increased autofluorescence of adenine deficient yeast by Zn^{2+} addition

Growing in YPD broth for over 20 h, yeast W303 was in the stationary phase with nearly unchanged OD values (Fig. 1a). One significant character of yeast W303 in this phase was the red color appearance which resulted from the accumulation of the red pigment (P-ribosylamino imidazole, AIR) in the adenine biosynthetic pathway (Weisman and Wickner, 1988). Continuous consumption of nutrients from the medium led to the deficiency of adenine after 20 h, further resulting in the necessity of synthesizing adenine intracellularly and the over-accumulation of AIR mediates (Fig. 1b). A recession of red pigment was induced in Ade(−) yeast within 10 min after the addition of 10 μM Zn^{2+} (Fig. 1a). The recession of red pigment could be due to decreased synthesized AIR and the simultaneous transformation from AIR to adenine, suggesting that a side reaction was accelerated by Zn^{2+} and resulted in a reduction of products (i.e. AIR). The pentose phosphate pathway (PP pathway) was the former pathway of adenine synthesis (Humbelin et al., 1999). In the PP pathway, many fluorophores are synthesized because their carbon skeletons come from glucose-based molecules (Maslanka et al., 2018). Using fluorescence microscopy, we documented an increased autofluorescence in the yeast W303 after addition of Zn^{2+}, especially when the cells were excited by a 488 nm-laser (Fig. 1c). The autofluorescence part was not overlaid with mitochondria or nuclei on the fringe but was concentrated in the vacuole part of the yeast cells. Therefore, it can be speculated that the introduction of Zn^{2+} has accelerated fluorophore synthesis in the PP pathway and fluorophores were finally transported to vacuole parts.

3.2. Specificity of the Zn^{2+} directed autofluorescence increase

Fluorescence intensity in 488 nm-laser excited channels was significantly increased by Zn^{2+} addition while other metals including Ag, Al, As, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Se, and Ti did not induce significant autofluorescence change in Ade(−) yeast (Fig. 2). By comparing the Zn^{2+} directed fluorescence increase (%) in 488 nm-laser excited channels, the fluorescence increase became lower as emission wavelength increased (Fig. 2). Therefore, the FITC channel would be the highly efficient one to get high sensitivity to Zn^{2+}. The fluorescence intensity was slightly changed by Zn^{2+} in the channels of DAPI, Alexa Fluor 430 and APC, and the trend was consistent with the change of FSC values (Fig. S4). A high value of FSC represented a large volume of cells, leading to high fluorescence recorded by flow cytometry (Trumpp et al., 2001). The positive relationship between FSC and fluorescence intensity also supported this finding (Table S1).

3.3. Optimization of factors influencing the sensitivity of Ade(−) to Zn^{2+}

Given that the autofluorescence intensity could be enhanced by Zn^{2+} addition, we explored whether there existed a certain relationship between [Zn^{2+}] and fluorescence increase. In Fig. S5, a standard curve of [Zn^{2+}] and fluorescence increase was developed and [Zn^{2+}] (less than 20 μM) was positively correlated with the fluorescence increase. However, the yeast used was in a certain growth phase (stationary phase) with certain biomass (OD value around 1.2), and the experiment was carried out in YPD broth for 10 min. Factors such as growth phase, biomass, medium and time were then optimized for higher sensitivity to Zn^{2+}.

In Fig. 3a, yeast cells in different growth phases (14, 19 and 24 h) were collected, and the color of yeast cells changed from white to pink and further to red. Given that the color depth of yeast cells implied the quantity of accumulated red pigment, more pigment was accumulated in yeast cells in the stationary phase. As a result, more fluorescent substances were synthesized in the former reaction in red cells, as shown by

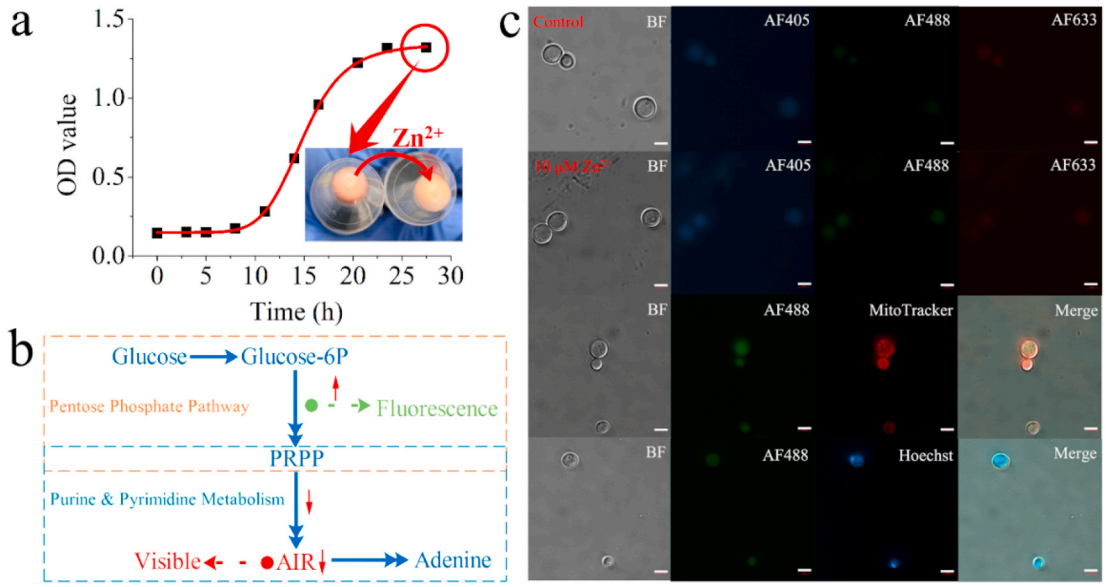

Fig. 1. Zn^{2+} directed recession of red pigment in Ade(−) yeast. a, Growth curve of yeast W303 (in YPD broth, 30 °C, 200 rpm). b, Pathways involved in the production of adenine. c, Intracellular distribution of the overproduced fluorescence substance. Scale bar: 5 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

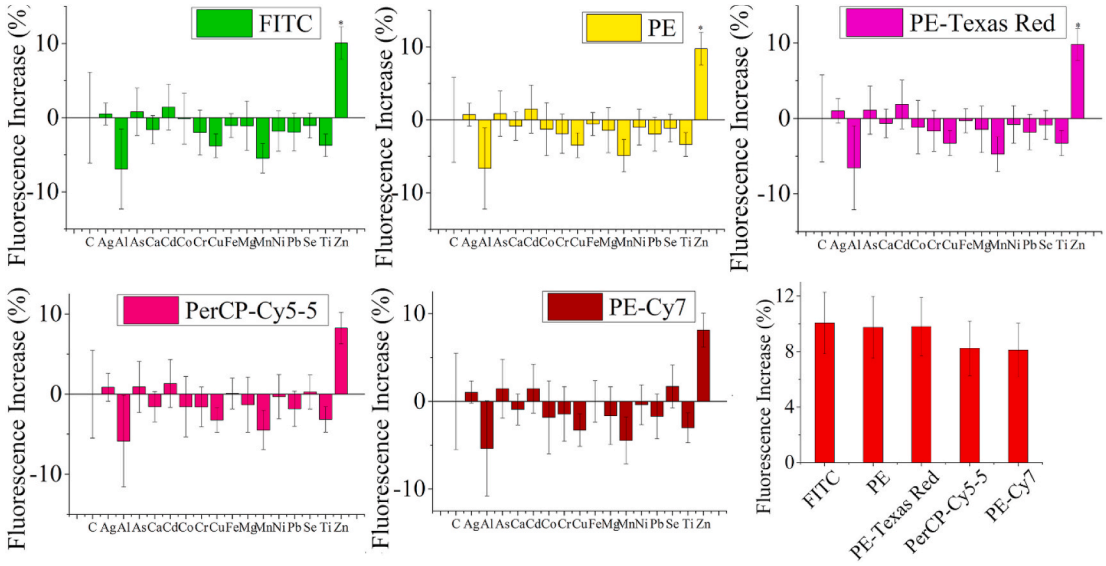

Fig. 2. Influence of metal ions on the fluorescence intensity of Ade(−) yeast in five channels (OD = 1.2 in YPD broth, 10 μM metal ions, Excitation 488 nm).

the gradual increase of fluorescence intensity from 14 h to 24 h-cultured yeast cells (Fig. 3a). Providing Zn^{2+} in the medium, the Zn^{2+}-directed fluorescence increase could only be observed in red yeast cells after culture for 24 h (Fig. 3a), while white and pink cells were not particularly sensitive to Zn^{2+}.

Both the accumulation of red pigment and fluorescence increase were nutrient-dependent (Nevzglyadova et al., 2011); the former could be promoted by deficiency of adenine while the latter could be enhanced by providing more source of carbon. As the ratio of YPD broth to ultrapure water increased, the Zn^{2+} directed fluorescence increase remained nearly unchanged except for an obvious fluorescence increase when the ratio was 1/3 (Fig. 3b). Because of the gradually limited carbon source as the ratio of YPD broth to ultrapure water was reduced, fluorescence intensity in the control group decreased when the ratio was reduced to less than 1/3 (Fig. S6). However, in the Zn^{2+}-added groups, a significant reduction of fluorescence intensity only emerged when the medium was completely replaced by ultrapure water. Thus, with low fluorescence intensity in the control group but high fluorescence intensity in groups added with Zn^{2+}, we obtained a high fluorescence increase (%) in the medium containing 1/3 YPD broth and 2/3 ultrapure water.

Given that a carbon source was necessary to increase the sensitivity of Ade(−) yeast to Zn^{2+}, extra carbon was needed for the quantification of Zn^{2+} in samples. In natural (mountain) water, the concentration of glucose was ultralow, i.e., 1–10 mg/L, which was far less than the concentration of glucose in YPD broth (20 g/L) (Wright and Hobbie, 1966). Thus, it was necessary to add glucose as an extra carbon source to increase the sensitivity of Ade(−) yeast for the quantification of Zn^{2+} in water samples. At 2.5 g/L, glucose significantly enhanced the Zn^{2+} directed fluorescence increase in ultrapure water (Fig. 3c). At this concentration of glucose, the fluorescence intensity of the control group was the lowest while that of the group with added Zn^{2+} was the second

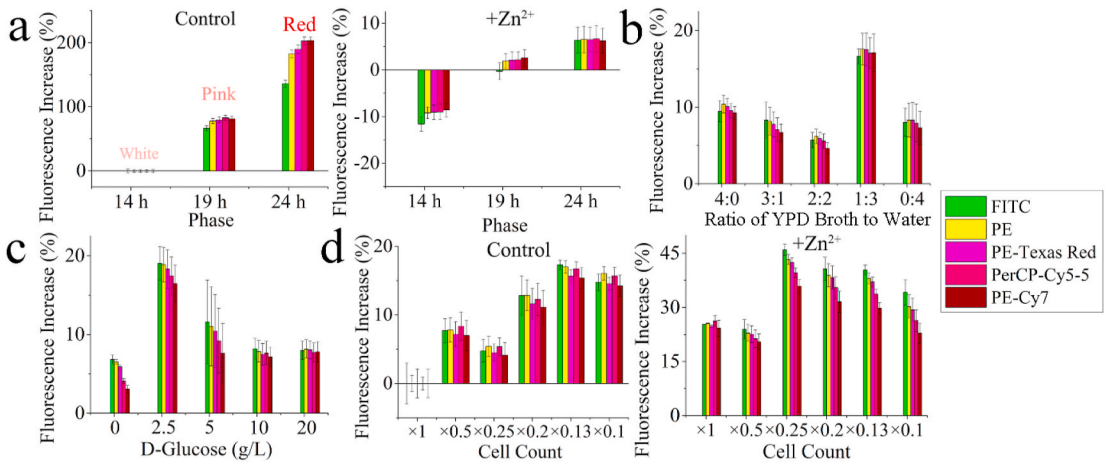

Fig. 3. Optimization of factors influencing the performance in Zn^{2+} detection. a, Performance of Ade(−) yeast in different growth phases. b, Performance of Ade(−) yeast in YPD broth with different amounts of water addition. c, Performance of Ade(−) yeast in water with different concentrations of D-glucose. d, Performance of Ade(−) yeast with different biomass. (10 μM Zn^{2+}, Excitation 488 nm).

lowest (Fig. S7), leading to the highest fluorescence increase in this case.

Diluting the original biomass (OD value around 1.2) by different factors, we found the higher fluorescence increase as dilution factors increased, even in the control group (Fig. 3d). Fewer cells meant more nutrients per cell, causing a higher fluorescence intensity in groups with lower biomass. Adding Zn^{2+} into the medium, the highest fluorescence increase was found when the biomass was diluted by 4 times, suggesting that there exists an optimum cell number for quantification. Putting Ade (−) yeast cells (OD value = 0.3) into medium containing 2.5 g/L glucose and 10 μM Zn^{2+}, a time dependent change of fluorescence increase was observed and the fluorescence stabilized after around 10 min of exposure (Fig. S8).

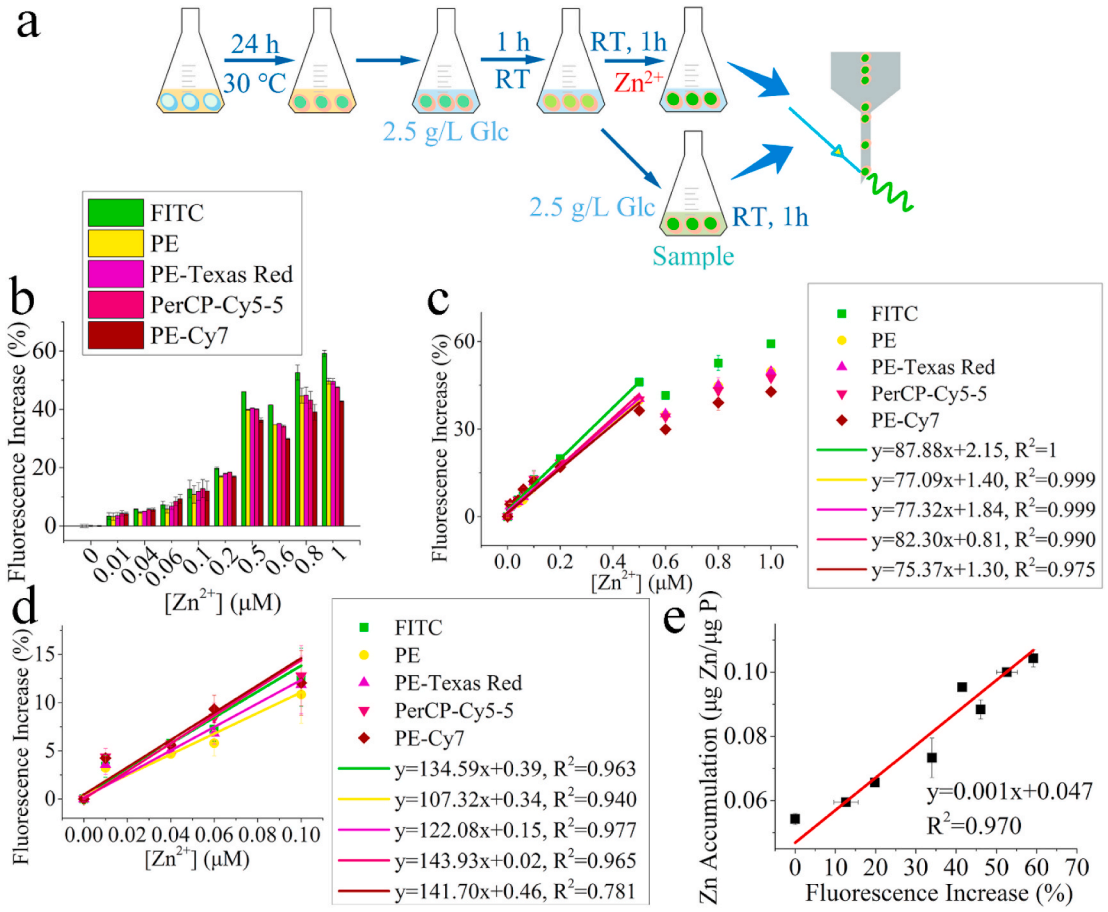

Fig. 4. Quantification of Zn^{2+} using Ade(−) yeast. a, Experimental protocol based on the optimized factors. b, Fluorescence increase by the addition of Zn^{2+}. c, Relationship between [Zn^{2+}] and fluorescence increase. d, Relationship between [Zn^{2+}] (low concentration range: 0.01–0.1 μM) and fluorescence increase. e, Relationship between Zn accumulation and fluorescence increase. (OD = 0.03, in 2.5 g/L glucose medium, Excitation 488 nm).

3.4. Quantification of labile Zn^{2+} using Ade(−) yeast

After the optimization of the key factors determining the sensitivity of Ade(−) yeast to Zn^{2+}, the method was developed as follows: 1. Yeast W303 cells were firstly cultured for 24 h to obtain Ade(−) yeast; 2. The medium of the cells was replaced by 2.5 g/L glucose and the Ade(−) yeast was put into this medium for 1 h to make it fully adapted to this environment; 3. For developing a standard curve, different amounts of Zn^{2+} were added for 1 h and the fluorescence intensity per cell was recorded by flow cytometry; 4. For determining the concentration of labile Zn^{2+} in a water sample, the original medium was replaced by the water sample with addition of glucose at 2.5 g/L, followed by determination of fluorescence by flow cytometry (Fig. 4a).

The fluorescence intensity of Ade(−) yeast cell was significantly enhanced even when the concentration of Zn^{2+} was as low as 0.01 μM (Fig. 4b). In previous studies, the quantification limit of most Zn^{2+} specific fluorescence probe was around 0.1–1 μM (Leung et al., 2019; Wang et al., 2010, 2011). However, the use of Ade(−) yeast cells could determine the concentration of Zn^{2+} at least 10 times lower than the limit of chemosensors. A positive correlation was found between [Zn^{2+}] and fluorescence increase (Fig. 4c), suggesting that it was possible to use the Ade(−) yeast cell as a sensitive biosensor to measure concentrations of labile Zn^{2+} less than 0.5 μM. The relationship was the strictest in the FITC channel ($R^2 > 0.999$ as the highest), and gradually decreased as the emission wavelength increased (from $R^2 > 0.999$ to < 0.980). Thus, the FITC channel would be the best channel for quantification. Using the correlation between [Zn^{2+}] at ultralow concentration (0–0.1 μM) and fluorescence increase (Fig. 4d), the detection limit of [Zn^{2+}] was calculated to be 0.01 μM, which was just equal to the lowest value we used in the standard curve. At concentrations higher than 0.5 μM, the assimilated Zn^{2+} was saturated, leading to slightly enhanced fluorescence increase. The strict correlation between bioaccumulated Zn^{2+} and extracellular [Zn^{2+}] was found when [Zn^{2+}] was 0–0.5 μM ($R^2 = 0.981$, Fig. S9), which was consistent with the abovementioned correlation. In contrast, a strong correlation between bioaccumulated Zn^{2+} and fluorescence increase was found even when the corresponding extracellular [Zn^{2+}] was as high as 1 μM ($R^2 = 0.970$, Fig. 4e), suggesting that the fluorescence increase caused by Zn^{2+} was largely caused by the bioavailable Zn concentration. The unavailable Zn^{2+} could not be assimilated by Ade(−) yeast cells, leading to the ineffectiveness in enhancing fluorescence increase and further resulting in the limited detection range of extracellular Zn^{2+}.

3.5. Possible interference using Ade(−) yeast to quantify Zn^{2+}

Components in the water sample were complex and thus it was necessary to determine the interference effects of these physical-chemical factors on the sensitivity of Ade(−) yeast to Zn^{2+}. The yeast's tolerance to salinity was high, even when the salinity was as high as that of seawater (Fig. 5a), suggesting that Ade(−) yeast has the potential to quantify Zn^{2+} in seawater samples. Unlike chemosensors, biosensors especially an individual-based biosensor have the ability to adjust the local pH. Due to the strong tolerance of yeast cells to pH variation, the sensitivity to Zn^{2+} remained unchanged when the pH value changed from 5.20 to 8.78 (Fig. 5b). However, the fluorescence increase induced by 0.5 μM Zn^{2+} was obviously decreased in water samples with ultralow pH (3.28) or ultrahigh pH (10.56). In acidic environment, the Ade(−) yeast was insensitive to Zn^{2+} due to the death of the yeast cells, as shown by the enhanced amount of debris (Fig. S10). No extra debris was found when the pH value was as high as 10.56, indicating that the decreased fluorescence was not due to the loss of biofunction of yeast cells. The pH value of the medium could influence the dissociation rate of Zn, affecting the form of Zn both extracellularly and intracellularly. Thus, the insensitivity of the Ade(−) yeast at this pH was mainly due to the formation of $[Zn(OH)_4]^{2-}$ which was less bioavailable than labile Zn^{2+} (Lee et al., 2016). Given the pH dependent change of the sensitivity, the acidic and alkalic samples should be neutralized to the neutral value before the detection. Fig. S11 shows that the extra influence of pH change can be alleviated by adjusting the pH of medium to the neutral value, indicating the potential use of this biosensor in samples with ultrahigh or ultralow pH values.

While other metal ions could not induce the equivalent fluorescence increase in the Ade(−) yeast as Zn^{2+}, Ag^{+} and Cu^{2+} caused a significant decrease of the 0.5 μM Zn^{2+}-induced fluorescence increase over 1 h exposure (Fig. 5c). Toxic effects of Ag^{+} and Cu^{2+} to yeast *Saccharomyces cerevisiae* were obvious (Yang and Pon, 2003). However, the exposure time of Ag^{+} and Cu^{2+} that could influence the sensitivity to Zn^{2+} was more than 15 min and 25 min, respectively (Fig. 5d and e). Therefore,

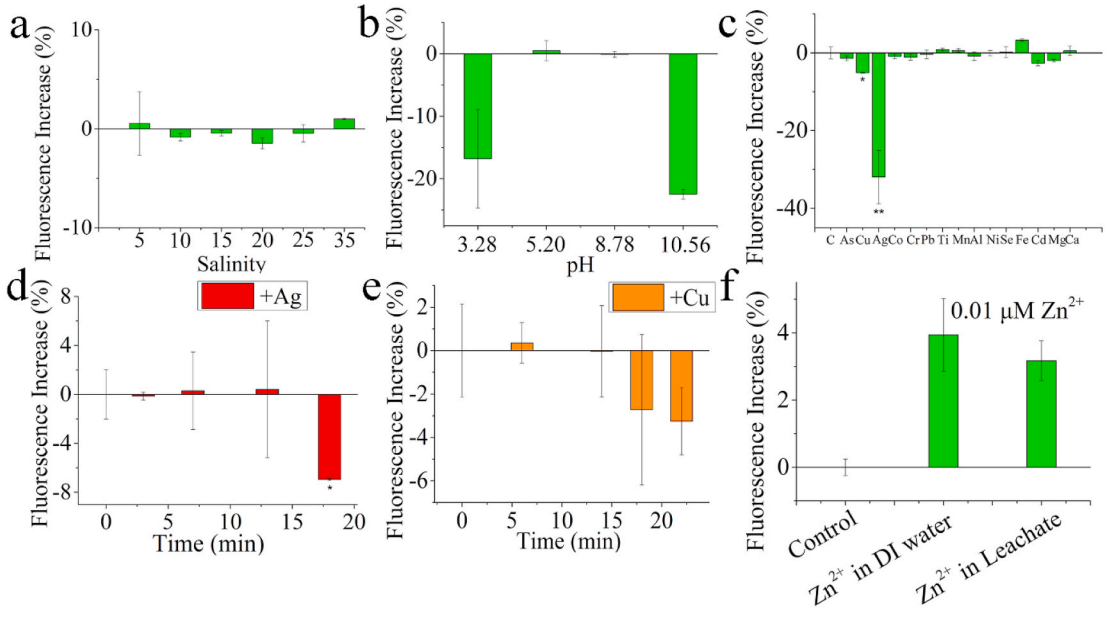

Fig. 5. Interference tests. a, Influence of salinity on fluorescence increase. b, Influence of pH values on fluorescence increase. c, Influence of other metal ions on fluorescence increase. d, Influence of Ag^{+} at different time points. e, Influence of Cu^{2+} at different time points. f, Performance of Ade(−) yeast in detecting labile Zn^{2+} in the mountain water sample. (OD = 0.03, Excitation 488 nm, FITC channel).

shortening the exposure time was necessary when quantifying Zn^{2+} in metal-enriched water samples. Mountain water was collected to explore the possible practical use of Ade(−) yeast in quantifying labile Zn^{2+}. Results show that although components in the mountain water did not influence the detection accuracy of Zn^{2+} at 0.01 μM, no labile Zn^{2+} from the mountain water sample could be determined (Fig. 5f). The total Zn content in the sample was only 0.12 μM. Metal ions in the mountain water were largely complexed with dissolved organic carbon (Christensen and Christensen, 2000), making them less easily internalized by yeast cells and causing fewer ecological concerns. Nevertheless, organic substances in the mountain water did not influence the uptake of added Zn^{2+}, indicating the high ability of yeast cells to compete for labile Zn^{2+} against nonspecific adsorption onto such organic matter present.

3.6. Comparison of ICP-MS, a chemosensor and Ade(−) yeast in detecting Zn dissolution from zinc cream

Previous studies have shown that Zn^{2+} accelerates wound healing because of its ability to promote cell growth and antibacterial effects (Sharir et al., 2010). Thus detection of the potential dissolution of products containing Zn is important. We used the APA chemosensor which has a low quantification limit of Zn^{2+} as low as 0.1 μM in ultrapure water (Leung et al., 2019). The fluorescence intensity correlated well with [Zn^{2+}] ($R^2 = 0.987$) and non-interference to the detection of an internal standard (0.5 μM) was found after the addition of zinc cream, suggesting that this method had the potential for directly quantifying the dissolved Zn^{2+} from zinc cream (Fig. 6a and b). A strong correlation between [Zn^{2+}] and autofluorescence intensity was also found in Ade (−) yeast cells ($R^2 = 0.990$, Fig. 6c), and the introduction of zinc cream did not reduce the fluorescence increase caused by 0.1 μM Zn^{2+}. The most common methodology for quantifying dissolved Zn^{2+} is to couple ICP-MS with ultrafiltration (Fabricius et al., 2014). However, nonspecific adsorption of Zn^{2+} on the ultrafiltration membrane inhibits ion permeation and underestimates the concentration of dissolved Zn^{2+}. Nonspecific adsorption still existed even though the samples were pre-ultrafiltrated for 7 times (Fig. 6e). A high concentration of Cu^{2+} was used to occupy the adsorption sites on the membrane prior to the adsorption of Zn^{2+} and successfully prevented the adsorption of Zn^{2+} (Fig. 6e). However, the existence of zinc cream blocked the membrane and inhibited the permeation of Zn^{2+} at 0.2 μM. Therefore, ICP-MS coupled with ultrafiltration is not suitable for quantifying the dissolution of zinc cream or other substances with high viscosity. Quantification results by the APA fluorescent probe and Ade(−) yeast cells were similar, and the value was equal to the total Zn determined by ICP-MS (Fig. 6f), suggesting that Zn from zinc cream dissolved completely into the medium. The unchanged value detected by the APA probe within 1 h verified that the co-culturing time of yeast cells and zinc cream would not influence the result.

4. Conclusions

Based on the Zn^{2+} directed autofluorescence increase of the Ade(−) yeast, Zn^{2+} concentrations ranging from 0.01 to 0.5 μM could be accurately quantified using our biosensor (OD value = 0.03) and flow cytometry. The strong correlation between autofluorescence increase and Zn bioaccumulation verified that the Zn^{2+}-directed reaction took place intracellularly. High tolerance of Ade(−) yeast to salinity, pH variation and other metals enabled its application as a biosensor for labile Zn detection in complex media such as seawater, slightly acidic/alkaline water or metal enriched water. Comparing the performance of this biosensor with a conventional method (ICP-MS coupled with ultrafiltration) and a chemosensor in determining the dissolution of zinc cream, Ade(−) yeast could directly determine the labile Zn^{2+} with high accuracy, a low quantification limit (0.01 μM) and low cost. Our work, for the first time, proposes a yeast-based whole cell sensor to quantify extracellular Zn^{2+}. This method utilizes the changed autofluorescence of Ade(−) yeast to expand the use of yeast cells in the field of trace metal detection, and provides an individual based biosensor for detecting Zn^{2+} with the lowest quantification limit. In the future, the underlying biochemical reactions of this Zn^{2+}-directed autofluorescence increase will be further explored. The individual based biosensor may be potentially transferred to an enzyme-based biosensor to further lower

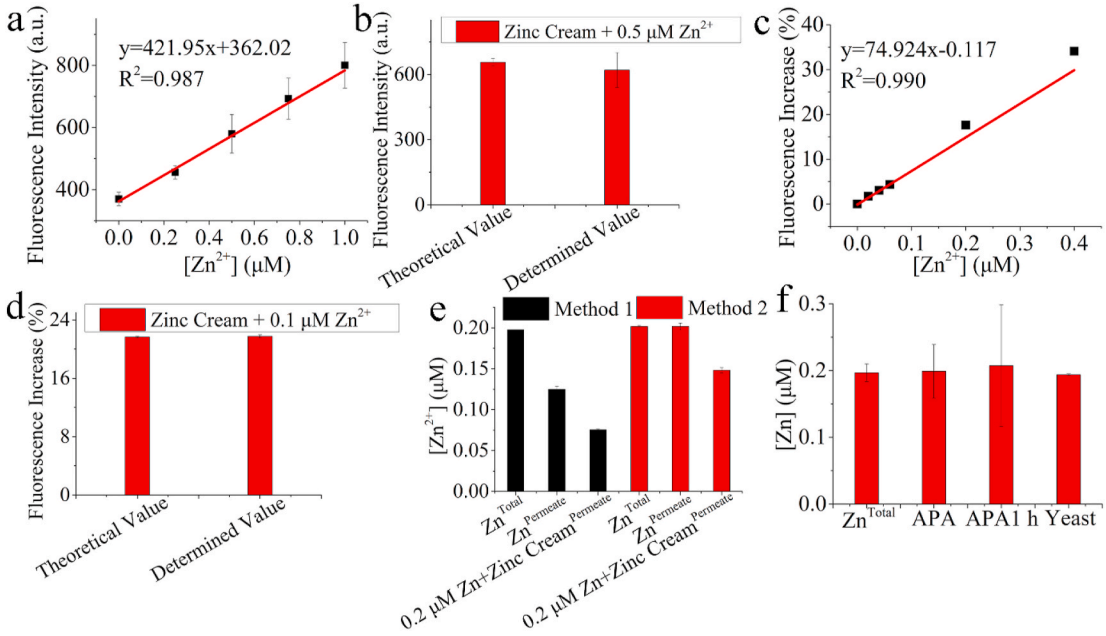

Fig. 6. Detection of dissolved Zn^{2+} from zinc oxide cream: comparison of results by an AIE chemosensor, Ultrafiltration-ICP-MS and yeast-based biosensor. a, Relationship between [Zn^{2+}] and fluorescence increase by the APA probe. b, Influence of components in zinc cream on detection of Zn^{2+} by the APA probe. c, Relationship between [Zn^{2+}] and fluorescence increase of Ade(−) yeast. d, Influence of components in zinc cream on detection of Zn^{2+} by Ade(−) yeast. e, Performance of Ultrafiltration-ICP-MS in detection of Zn^{2+} dissolved from zinc oxide cream. f, Comparison of results by the APA chemosensor and the yeast-based biosensor. (biosensor: OD = 0.03, Excitation 488 nm, FITC channel).

the detection limit and save the preparation time.

CRediT authorship contribution statement

Anqi Sun: Conceptualization, Investigation, Writing. **Wen-Xiong Wang:** Conceptualization, Writing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by a grant from Shenzhen Science and Technology Innovation Committee (SZSTI19SC04).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2021.113075.

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