Received: 7 February 2018 | Revised: 4 April 2018 | Accepted: 17 May 2018
DOI: 10.1002/bio.3518

RESEARCH ARTICLE

WILEY LUMINESCENCE
The Journal of Biological and Chemical Luminescence

A highly selective colorimetric and long-wavelength fluorescent probe for the detection of Hg^{2+}

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Funding information
The National Nature Science Foundation of China, Grant/Award Numbers: 21607053 and 21777053; Major Livelihood Projects of Jinan, Grant/Award Number: SHMS2015-3-01; Municipal Development and Reform Commission Project of Jinan, Grant/Award Number: 2016JSFW02Z0307; National Science and Technology Support Planning Subjects, Grant/Award Number: 2015BAD20B02-05; Shandong Province Key Research and Development Project, Grant/Award Number: 2016GSF117010; Outstanding Young Scientists Award Fund of Shandong Province, Grant/Award Number: BS2013HZ007

Abstract

Currently, the fluorescent probe is an important method for detecting heavy metal ions, especially mercury ion (Hg^{2+}), which is harmful to the health of humans and the environment due to its toxicity and extensive use. In this paper, we designed and synthesized a colorimetric and long-wavelength fluorescent probe Hg-P with high sensitivity and excellent selectivity, which could detect Hg^{2+} by the changes of visual color, fluorescence and absorption spectroscopy. With the addition of Hg^{2+} to probe Hg-P solution, its color changed from yellow to pink, and showed a 171 nm red-shifted absorption spectrum. Probe Hg-P was used in real water and soil solution samples to detect Hg^{2+}, and the result is satisfactory. Therefore, this new probe shows great value and application in detecting Hg^{2+} in the environment.

KEYWORDS

colorimetric fluorescent probe, internal charge transfer (ICT), long-wavelength, mercury ion (Hg^{2+})

1 | INTRODUCTION

Mercury is a metal element that exists widely in nature. It usually has three forms in living organisms and the environment, including elemental mercury, inorganic mercury and organic mercury.[1–4] As one of the most serious heavy metal pollutants, more and more attention has been attracted to mercury pollution, due to its bioaccumulation, durability, long residence, and easy transference.[5–7] Furthermore, very small amounts of mercury causes serious harm to humans. Once mercury is absorbed through the skin or respiratory tract, it will pose a threat to the body's central nervous system, digestive system and kidney, etc.[8–12] Therefore, it is significant to develop a highly selective and excellently sensitive method for the detection of mercury ions (Hg^{2+}).

In recent years, many methods have been reported to detect Hg^{2+} in the world, including atomic absorption spectroscopy, optical probes, electrochemical analysis, and so on.[13–18] Specifically, the colorimetric fluorescence probes stand out from numerous detection methods because they allow the target analytes to be detected using the naked-eye, therefore needing no help from other sophisticated instruments.[19,20] Also, they have the advantages of simple synthesis, high selectivity and sensitivity.[21–24] Due to the long-wavelength the fluorescent probe possesses several merits, such as more obvious color change, better

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Abbreviations used: Hg^{2+}, mercury ion; PBS, phosphate-buffered saline.

tissue penetration, and minimum interference from background autofluorescence by biomolecules,[25–30] therefore it has attracted intense interest of researchers. So far, numerous colorimetric or fluorescent probes for detecting Hg^{2+} have been synthesized and reported;[31–33] however, some shortcomings still existed in most of them, such as bad water solubility, color/fluorescence quenching, and short-wavelength.[34–37] Hence, simple long-wavelength fluorophores (> 600 nm) with excellent fluorescence-on property are still in high demand.

In this paper, a long-wavelength and highly selective colorimetric probe Hg-P with 171 nm absorption shift was designed and synthesized for monitoring Hg^{2+}. The probe Hg-P was constructed using the 2-dicyanomethylene-3-cyano-4,5,5-trimethyl-2,5-dihydrofuran (TCF) as the fluorophore which possesses a strong internal charge transfer (ICT) structure. This fluorophore was designed and synthesized by our team for the first time and was used to detect the various target analytes.[38,39] Furthermore, the carbonothioate moiety was selected as the receptor.[40] This new probe has the advantages of (1) excellent water solubility (ethanol/ultrapure water = 1:9, *v*/*v*); (2) colorimetric fluorescence probe (with a large red-shift of 171 nm); (3) a low detection limit of 17 nM (on the basis of 3σ/slope); (4) high selectivity; (5) practical applicability in real water and soil solution samples.

2 | EXPERIMENTAL

2.1 | Materials and general methods

All the chemicals were obtained from commercial suppliers and none of them needed to be further purified. The ultrapure water which was used in all of the experiments was produced by the Sartorious Arium 611DI system. Column chromatography using Silica gel 200–300 mesh from the Qingdao Haiyang Chemical Co. (China) was used to purify the reaction product. The chemical shifts of the ^{1}H-NMR and ^{13}C-NMR which were reported as parts per million (ppm) [in deuterated dimethyl sulfoxide (DMSO-d_6), tetramethylsilane (TMS) as internal standard] were recorded on a Bruker AV-400 spectrometer (Bruker, Karlsruhe, Germany). Fluorescence spectra were gained from a Horiba FluoroMax-4 (HORIBA Jobin Yvon, Paris, France), and the UV-3101PC spectrophotometer (Shimadzu, Kyoto, Japan) was used to obtain the absorption spectra.

2.2 | Synthesis of 1

4-Hydroxybenzaldehyde (200.5 mg, 1.64 mol) and phenyl thioxochloroformate (425.3 mg, 2.46 mol) were dissolved in 10 ml dichloromethane, and a few drops of *N*,*N*-diisopropylethylamine (DIPEA) were added. Then the solution was stirred for about 10 h at room temperature. After the reaction was completed, the pure product **1** was obtained through purifying by silica column chromatography.

2.3 | Synthesis of 2

3-Hydroxy-3-methyl-2-butanone (3.2006 g, 31.37 mol) was dissolved in 20 ml of pyridine. Then malononitrile (4.1005 g, 62.12 mol) and 2–3 drops of acetic acid was added to the mixed solution. The reaction was stirred in an oil bath at 25°C for 24 h. When the reaction was completed, the product was combined with 200 ml of ice ultrapure

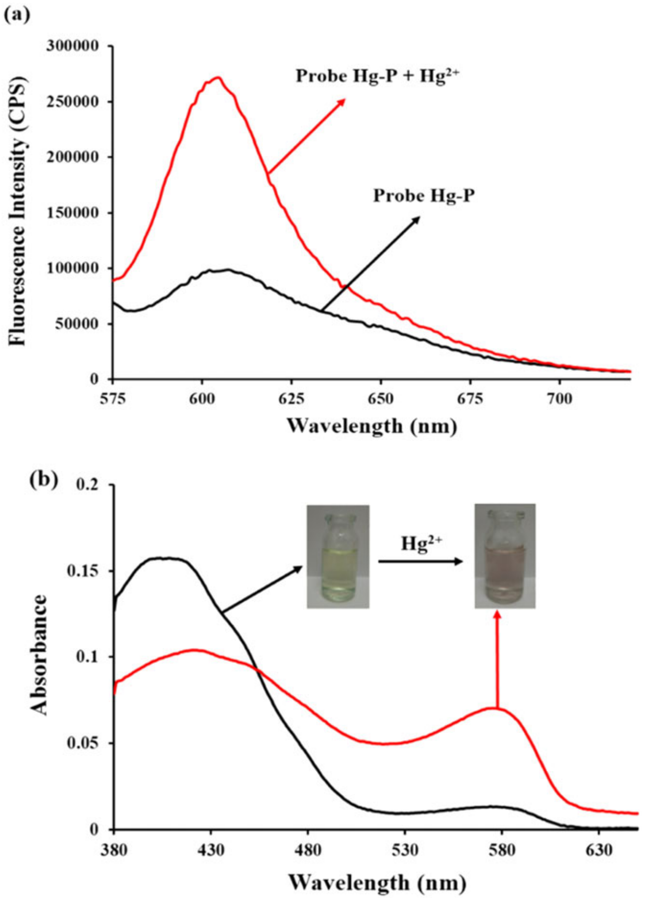
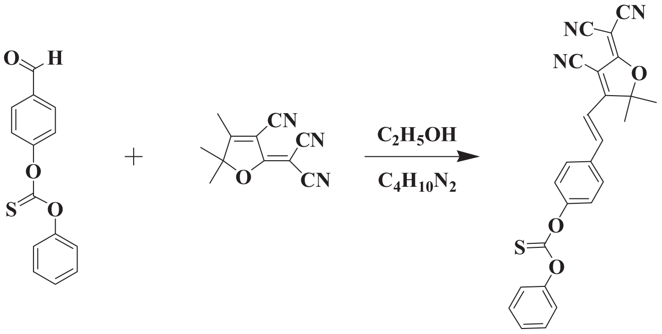


FIGURE 1 (a) Fluorescence spectra of probe Hg-P (5 μM) in the presence and absence of Hg^{2+}. (b) Absorption spectra of probe Hg-P (5 μM) in the presence and absence of Hg^{2+}, inset: the color changes of probe Hg-P (5 μM) from yellow to pink with the addition of Hg^{2+}. Conditions: the ultrapure water and ethanol system (9:1, *v*/*v*) containing phosphate-buffered saline (PBS) solution (5 mM, pH 7.4)



SCHEME 1 Synthesis of probe Hg-P

water drop-by-drop, and then the solids were precipitated. After mixing evenly, the mixture was filtered to obtain the filter cake. The filter cake was dissolved in 50 ml dichloromethane. Some anhydrous sodium sulfate was added to the solution, and mixed well. Then the solution was poured in to a bottle, and pure product **2** was acquired through steaming.

2.4 | Synthesis of probe Hg-P

Products **1** (200.2 mg, 0.77 mol) and **2** (260.5 mg, 1.31 mol) were dissolved in 15 ml absolute ethanol, and a piece of piperazine was added to the mixture. Then the reaction bottle was wrapped in aluminum foil, and stirred for 12 h at room temperature. Finally, the reaction mixture was filtered to obtain the pure probe Hg-P (Scheme 1).

^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.82 (s, 6H), 7.27 (d, J = 16.0 Hz, 1H), 7.35–7.40 (m, 3H), 7.50–7.55 (m, 3H), 7.60 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 16.0 Hz, 1H), 8.06–8.09 (m, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 25.53, 55.23, 99.99, 100.53, 101.81, 104.12, 111.21, 112.25, 116.59, 122.17, 123.39, 127.57, 130.41, 131.54, 133.65, 146.24, 153.61, 155.80, 157.53, 175.41, 177.52, 186.22, 192.49, 194.12.

3 | RESULTS AND DISCUSSION

3.1 | Characteristic spectra

At first, there was a weak peak at 604 nm (λ_{ex} = 560 nm) in the fluorescence spectra without Hg^{2+}. Subsequently, a considerable fluorescent enhancement was observed at 604 nm (Figure 1a) when the solution was added Hg^{2+} (Scheme 2). The absorption peak of the probe Hg-P without Hg^{2+} was at 404 nm. With the addition of Hg^{2+} to the probe solution, it showed a large red-shift (171 nm) and resulted in a new absorption band at 575 nm (Figure 1b). The color of the solution changed from yellow to pink (Figure 1b, inset). Thus, this is a great superiority of probe Hg-P relative to other analytical techniques because it can detect Hg^{2+} using the naked-eye. In other words, our probe can detect Hg^{2+} by fluorescence spectra and absorption spectra.

3.2 | Reaction time on detecting Hg^{2+}

As an important factor of the probe, the reaction time of probe Hg-P with Hg^{2+} was studied at room temperature, and other conditions were all as mentioned earlier. The results are shown in Figure 2. The fluorescence intensity was continuously increased at first and maintained when the reaction time was more than 40 min. So, the whole time of the probe Hg-P to detection of Hg^{2+} was about 40 min.

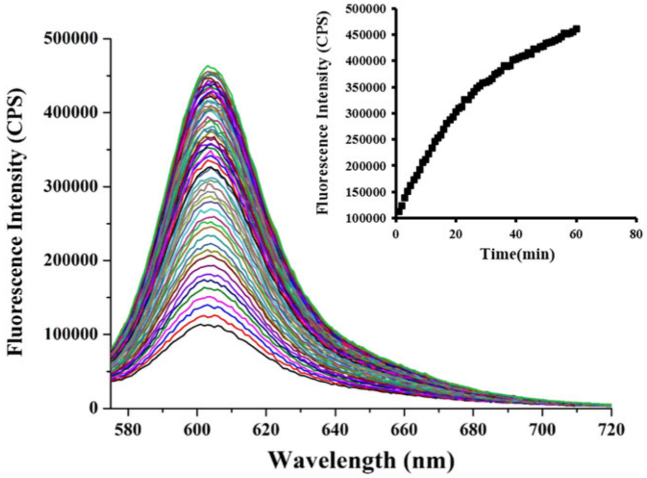


FIGURE 2 Reaction time for probe Hg-P (5 μM) with the addition of Hg^{2+} (20 μM)

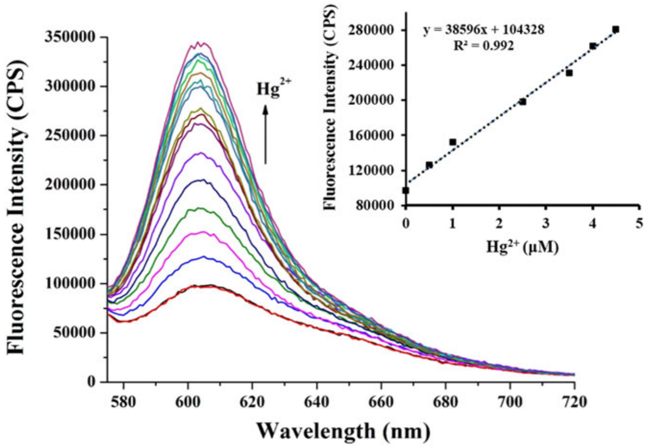
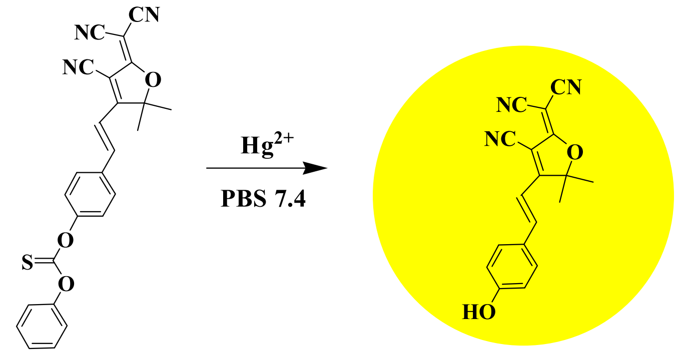


FIGURE 3 Fluorescence spectra of probe Hg-P (5 μM) toward the various concentrations of Hg^{2+} (final concentration: 0, 0.1, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 9, 10, 12, 14, 16 μM). The fluorescence intensity at 604 nm of probe Hg-P increased with the increasing concentrations of Hg^{2+}. Excitation wavelength = 560 nm, emission wavelength = 604 nm. Conditions: in PBS (5 mM, pH 7.4). Solution: ethanol/ultrapure water = 1:9, *v/v*



Probe Hg-P
Fluorescence OFF

Fluorophore Hg-P-Hg
Fluorescence ON

SCHEME 2 Reaction mechanism of probe Hg-P for Hg^{2+}

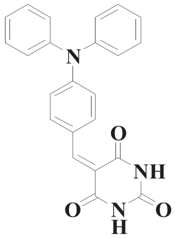
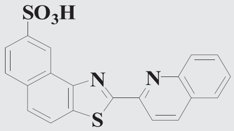
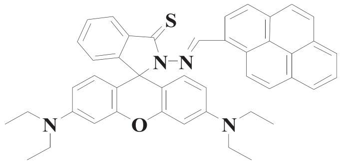
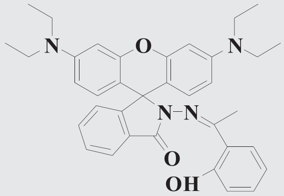
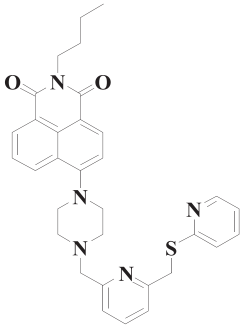
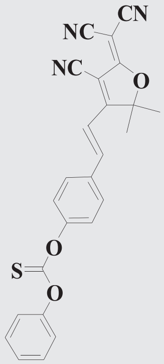
3.3 | Quantification of Hg^{2+}

A new peak at 604 nm can be seen with the continuous addition of Hg^{2+}. As shown in Figure 3, there was a good linearity ($y = 38596\times + 104328$, $R^2 = 0.992$) between the Hg^{2+} concentrations of 0 to 5 μM and the fluorescence intensity at 604 nm with a detection limit of 17 nM (3σ/slope).[41] Therefore, the probe Hg-P can detect low concentrations of Hg^{2+}. The results showed that the probe Hg-P could detect Hg^{2+} quantitatively by the fluorescence spectrometry method with excellent sensitivity (Table 1).

3.4 | Selectivity to Hg^{2+}

The selectivity is also an important factor of a probe. Other common metal ions, including Co^{2+}, K^+, Sn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Cr^{3+}, Ca^{2+}, Mg^{2+}, Cd^{2+}, Fe^{2+}, Zn^{2+}, Cu^{2+}, Fe^{3+}, Al^{3+} cannot be detected using

TABLE 1 Comparison of fluorescent probes for Hg^{2+}

Probe	λ_{em} (nm)	Naked-eye	Solution (v/v)	Limit of detection	References
	594	Yes	THF/water =3:7	3.4 μM	[16]
	504	Yes	CH_3CN/water =1:1	34 nM	[17]
	582	Yes	Ethanol/water =1:1	434 nM	[23]
	586	Yes	Ethanol/water =2:1	150 nM	[35]
	525	Yes	THF/water =1:1	18 μM	[37]
	604	Yes	Ethanol/water =1:9	17 nM	This work

Note: THF, tetrahydrofuran; CH_3CN, acetonitrile.

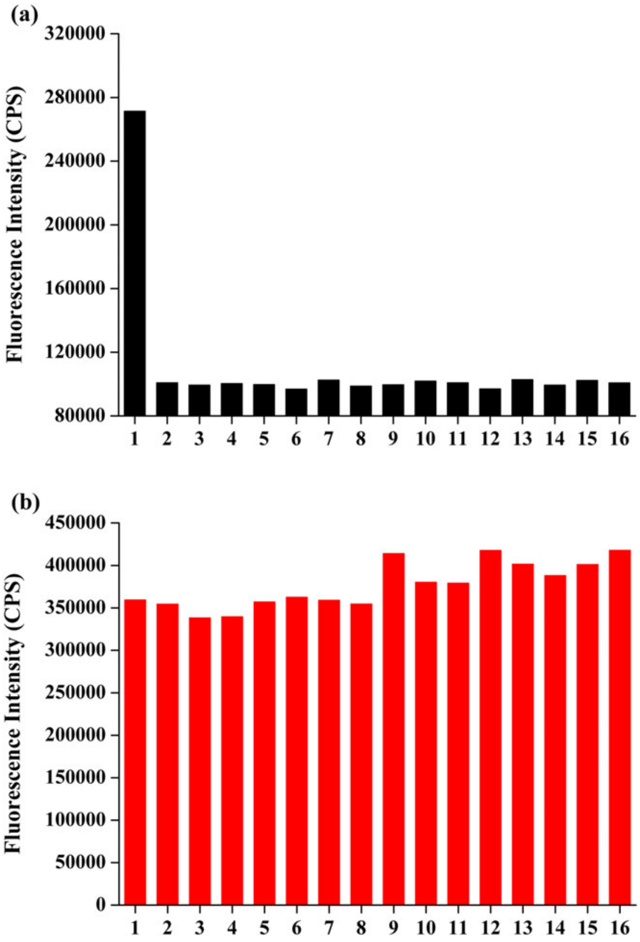


FIGURE 4 (a) The selectivity of probe Hg-P (5 μM) to different analytes containing Hg^{2+} (20 μM). (b) The response of probe Hg-P toward Hg^{2+} in the presence of other metal ions: (1) only Hg^{2+}; (2) Co^{2+}; (3) K^+; (4) Sn^{4+}; (5) Na^+; (6) Ni^{2+}; (7) Pb^{2+}; (8) Cr^{3+}; (9) Ca^{2+}; (10) Mg^{2+}; (11) Cd^{2+}; (12) Fe^{2+}; (13) Zn^{2+}; (14) Cu^{2+};(15) Fe^{3+}; (16) Al^{3+}. Solution: ethanol/ultrapure water = 1:9, *v*/*v* (5 mM, PBS, pH 7.4)

the probe Hg-P, and can only be used to monitor Hg^{2+}. The result of a fluorescence enhancement at 604 nm (Figure 4a) occurred only after adding Hg^{2+}. The interference of the metal ions on the detection of $Hg^{2+}$was also studied. Even though the metal ions were existed in the solution, the probe Hg-P also had a good response to Hg^{2+} (Figure 4b). In a word, it can be seen that the probe Hg-P had excellent selectivity and anti-interference toward Hg^{2+}, and due to the carbonothioate moiety is a good receptor for detecting Hg^{2+}. Thus, the probe Hg-P can provide more accurate detection results in real samples.

3.5 | Analytical application

At the end of the experiment, the practical application of the probe Hg-P was tested. The Hg^{2+} was added to real water and soil solution samples. All the water samples needed to be filtered before analyzing, and every sample was tested three times. At first, Hg^{2+} was not found in the samples, and then 20 μM Hg^{2+} was respectively added to the solution of real water or soil solution/ethanol = 9:1, *v*/*v* (5 mM, PBS, pH 7.4). The results are showed in Table 2. It can be seen that a satisfactory method has been obtained to detect Hg^{2+} in the environment.

4 | CONCLUSION

In this paper, a highly selective colorimetric and long-wavelength fluorescent probe for detection of Hg^{2+} was designed and synthesized. Probe Hg-P could detect Hg^{2+} using just the 'naked-eye', as color changes were observed from yellow to pink when the solution was added into Hg^{2+}. Probe Hg-P could detect Hg^{2+} through the absorption and fluorescence spectrometry, because it showed a very large red-shifted absorption spectrum (171 nm) and fluorescence intensity enhancement. There was a good linear relationship in the range 0–5 μM, and the low detection limit was 17 nM (on the basis of 3σ/slope). More importantly, it showed excellent sensitivity and selectivity in the solution (ethanol/ultrapure water = 1:9, *v*/*v*) and for real water samples and soil solution. All these remarkable advantages of this new probe suggest that this probe will have excellent application prospect in the detection of mercury.

ACKNOWLEDGEMENTS

This work was supported by the National Nature Science Foundation of China (No. 21607053, 21777053), Major Livelihood Projects of Jinan (SHMS2015-3-01), Municipal Development and Reform Commission Project of Jinan (2016JSFW02Z0307), National Science and Technology Support Planning Subjects (2015BAD20B02-05), Shandong Province Key Research and Development Project (2016GSF117010), Outstanding Young Scientists Award Fund of Shandong Province (BS2013HZ007).

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TABLE 2 Analysis results of Hg^{2+} in three water and one soil solution samples [a]

Real water and soil solution samples[a]	Addition Hg^{2+}(μM)	Found Hg^{2+}	Found (μM)	Recovery (%)	Relative standard deviation (*n* = 3) (%)
Water sample A	20	No	21.24 ± 0.67	106.22	3.34
Water sample B	20	No	19.57 ± 0.91	97.84	4.56
Water sample C	20	No	21.69 ± 1.13	108.43	5.67
Soil solution D	20	No	21.46 ± 1.79	108.19	8.94

[a] Sample A, lake water from Jia Zi Lake, University of Jinan; samples B and C from the Xiu Jiang River, at Jinan, China; soil solution D from Jinan, China.

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How to cite this article: Xu J, Wang Z, Liu C, et al. A highly selective colorimetric and long-wavelength fluorescent probe for the detection of Hg^{2+}. *Luminescence*. 2018;1–6. https://doi.org/10.1002/bio.3518