# SUPPORTING INFORMATION

## An "off-on" Electrochemiluminescence Biosensor Based on CRISPR-Cas12a for Ultrasensitive Detection of Aflatoxin B1

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## Table of contents

## 1. Preparation of Pdots.

Pdots were prepared via a nano-coprecipitation method.[1,2] First, a THF solution of 100 μg/mL polymer was as the stock solution. Then, 2 mL polymer solution was quickly injected into the ultrapure water, which was degassed by sonication before use. After continued sonication for 3 min, the solution was concentrated to 2 mL by vacuum distillation through a rotary evaporator. Finally, the reaction solution was filtered to obtain a Pdots suspension with carboxyl groups.

## 2. Treatment of real samples

For the spiked sample assays, Anoectochilus roxburghii was first pulverized using a grinder to yield a homogeneous powder. Spiked samples were then prepared by incorporating specific amounts of AFB1 standards into the homogenized powder. Subsequently, 30 mL of a mixed organic solvent (methanol/water, 70:30, v/v) was added, and the mixture was stirred thoroughly for 30 min. Following vacuum filtration, the resulting filtrate was collected, appropriately diluted, and subjected to ECL scanning analysis.

## 3. $^1$H NMR spectrum of polymer

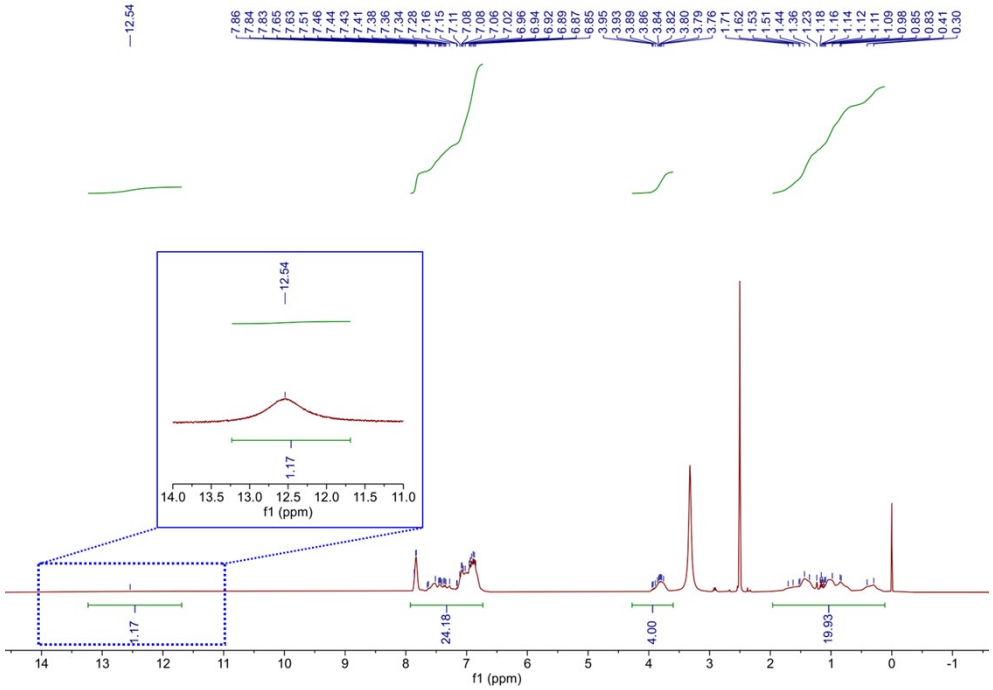


**Figure S1.** Nuclear magnetic resonance spectroscopy ($^1$H NMR) of polymer. Inset: enlarged region of dashed box.

$^1$H NMR (400 MHz, DMSO-$d_6$) δ (ppm): 12.54 (s), 7.86-6.85 (m, 24H), 3.95-3.76 (m, 4H), 1.71-0.30 (m, 20 H).

## 4. PL spectra and PL trends of polymer with different water fractions

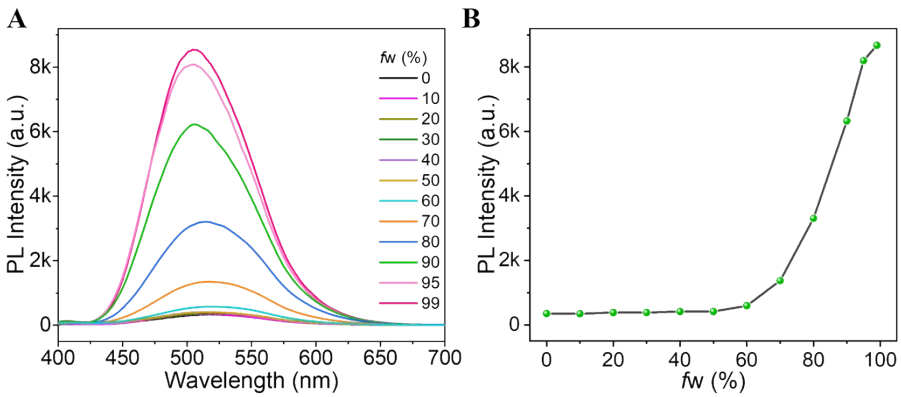


**Figure S2**. PL spectra (A) and PL trends (B) of polymer in THF/$H_2O$ mixtures with different *f*ws ($10^{-5}$ mol $L^{-1}$).

## 5. UV-vis absorption spectrum of Pdots

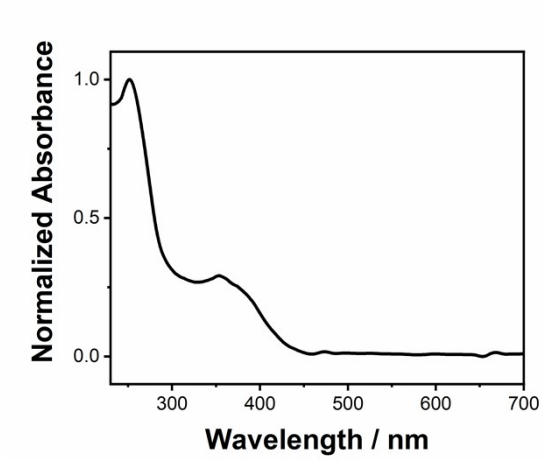


**Figure S3**. UV-vis absorption spectrum of Pdots.

## 6. CV diagram of Pdots in TEA

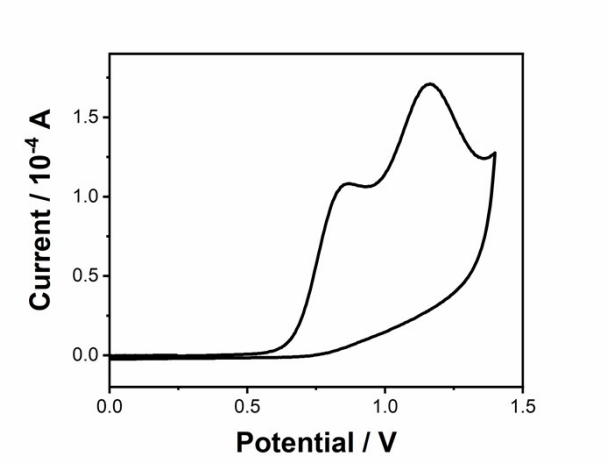


**Figure S4.** CV diagram of Pdots in TEA

## 7. Optimization of oxidative-reductive coreactants

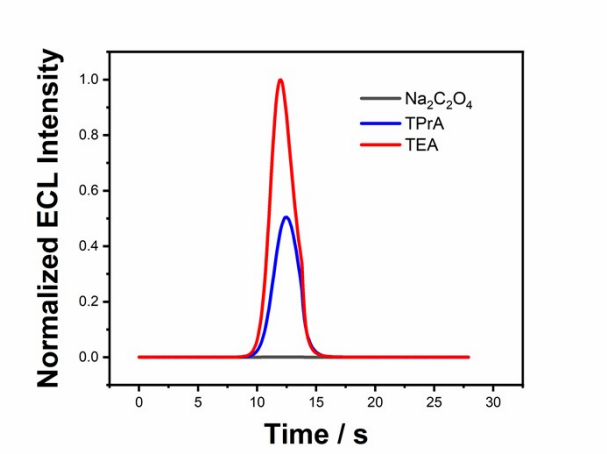


**Figure S5.** ECL intensity-time curves of Pdots/GCE in 0.1 M PBS with different co-reactants (25 mM).

## 8. UV-vis absorption spectrum of BHQ and PL emission spectrum of Pdots

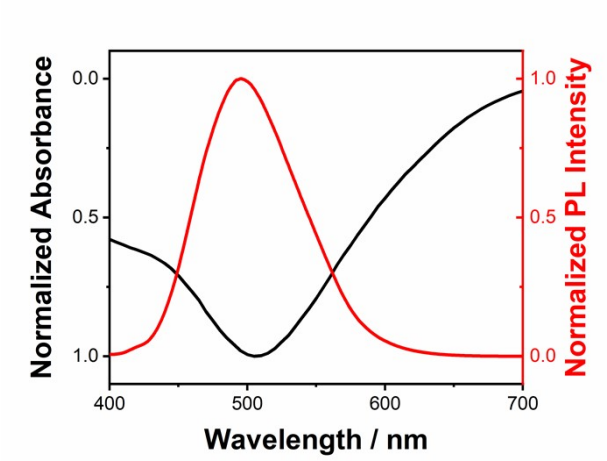


**Figure S6.** UV-vis absorption spectrum of BHQ and PL emission spectrum of Pdots.

**9. PAGE of TDN.**

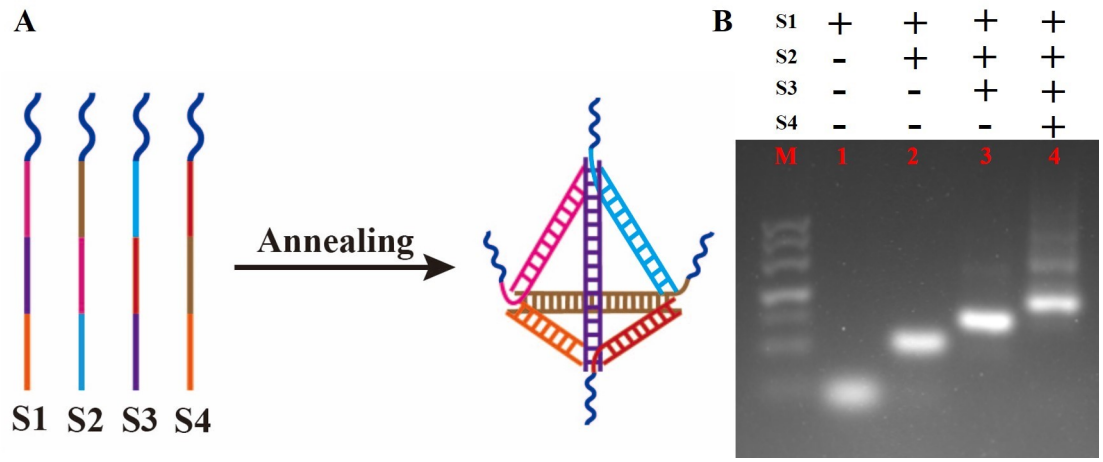


**Figure S7.** (A) Assembling of TDNs. (B) PAGE analysis of TDN.

**10. Optimization of the TEA concentration.**

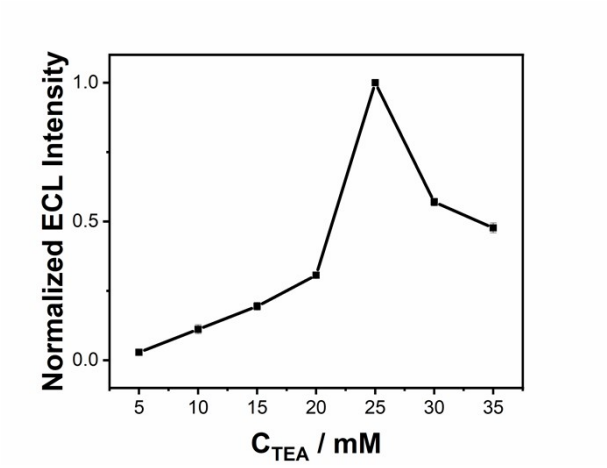


**Figure S8.** Optimization of the TEA concentration.

## 11. Comparison of the proposed ECL biosensor and previous reports on AFB1 detection

**Table S1.** Comparison of the current approach and previous reports on AFB1 detection

| Methods | Dynamic range | Detection limit | Reference |
|---|---|---|---|
| Fluorescence | 0.10-400 ng/mL | 0.09 ng/mL | [3] |
| Fluorescence | 0-3.33 ng/mL | 0.08 ng/mL | [4] |
| Fluorescence | 0.01-100 ng/mL | 5.81 pg/mL | [5] |
| Electrochemistry | 0.05-360 ng/mL | 3.5 pg/mL | [6] |
| Electrochemistry | 0.001-1000 ng/mL | 0.34 pg/mL | [7] |
| Electrochemistry | 0.001-1000 ng/mL | 0.17 pg/mL | [8] |
| ECL | 0.05-100 ng/mL | 0.01 ng/mL | [9] |
| ECL | 0.001-1000 ng/mL | 0.46 pg/mL | [10] |
| ECL | 0.1-1000 pg/mL | 0.06 pg/mL | This work |

## 12. References


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