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Supplementary Information

Visualization detection of mycotoxin patulin in fruit juices by a small-molecule fluorescent probe

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Experimental Section

1. Reagents

Patulin (PAT) was purchased from Shanghai Aladdin Biochemical Technology Co. Ltd. 3',6'-Dihydroxy-5-isothiocyanato-3H-spiro[isobenzofuran-1,9'-xanthen]-3-one[fluorescein 5-isothiocyanate] (fluorescein-5-isothiocyanate) was purchased from Bide Pharmaceutical Technology Co. Ltd. N,N-diisopropylethylamine (DIPEA), piperidine and (((9H-Fluoren-9-yl)methoxy)carbonyl)-L-lysine (Fmoc-Lys-OH) were purchased from Energy Chemical Reagents Ltd. The solvents including tetrahydrofuran (THF), dimethyl sulfoxide (DMSO) and N, N-dimethylformamide (DMF) of analytical grade were dried with molecular sieves. Other solvents used in this study were analytical grade reagents without further purification. The water used in the experiments was the triple-distilled water.

2. Apparatus

Nuclear magnetic resonance (NMR) spectra were measured on Bruker AVANCE III HD 500 MHz NMR spectrometer. ^{1}H NMR was conducted at 400 MHz. ^{13}C NMR was conducted at 126 MHz. High resolution mass spectrometer (HR-MS) was recorded on a Bruker MAXIS IMPACT mass spectrometer. High performance liquid chromatography (HPLC) was conducted on Agilent 1260 Infinity Liquid Chromatograph (with DAD). The absorption spectra were obtained on Hitachi U-3010 absorption spectrophotometer. The fluorescence spectra were obtained on Hitachi F-4700 fluorescence spectrophotometer. All Fluorescence imaging tests were performed on AMI small animal fluorescence imaging system (Spectral Instruments Imaging Co.)

3. Syntheses

Synthesis of Fmoc-Lys-FITC: Fluorescein-5-isothiocyanate (80 mg, 0.2 mmol) and Fmoc-Lys-OH (78 mg, 0.2 mmol) were dissolved in anhydrous DMF/THF (v/v, 1:2) and then treated with DIPEA (20 μL). The reaction mixture was stirred at room temperature for 12 hours under the protection of N_2. After that, the THF was removed under reduced pressure and the residue was added an appropriate amount of ethyl acetate to obtain a yellow solid product (50 mg, 30%). ^{1}H NMR (400 MHz, CD_3OD/DMSO-d_6, Fig. S1) δ 8.19 (s, 1H), 7.80 (t, J = 7.4 Hz,

2H), 7.75 (d, J = 8.2 Hz, 1H), 7.68 (t, J = 6.8 Hz, 2H), 7.45–7.35 (m, 2H), 7.34–7.25 (m, 2H), 7.10 (d, J = 8.2 Hz, 1H), 6.73–6.64 (m, 4H), 6.58–6.53 (m, 2H), 4.37–4.30 (m, 2H), 4.22 (t, J = 7.0 Hz, 1H), 4.14–4.06 (m, 1H), 3.73–3.60 (m, 2H), 1.97–1.61 (m, 4H), 1.55–1.40 (m, 2H).

Synthesis of FITC-Lys: Compound **Fmoc-Lys-FITC** (80 mg, 0.1 mmol) was dissolved in anhydrous DMF (6.4 mL) and then piperidine (1.6 mL) was added slowly dropwise. The reaction mixture was stirred at room temperature for one hour under the protection of N_2. Afterward, the solution was removed and the crude product was purified by forming precipitates in methanol and dichloromethane to obtain a red solid product (20 mg, 37%). ^{1}H NMR (400 MHz, CD_3OD/DMSO-d_6, Fig. S2) δ 8.07 (d, J = 2.2 Hz, 1H), 7.77–7.72 (m, 1H), 7.22 (d, J = 8.2 Hz, 1H), 7.08 (d, J = 9.0 Hz, 2H), 6.58 (d, J = 2.2 Hz, 2H), 6.56 (s, 2H), 3.67 (t, J = 6.8 Hz, 2H), 3.57–3.53 (m, 1H), 2.04–1.80 (m, 4H), 1.60–1.52 (m, 2H). ^{13}C NMR (126 MHz, CD_3OD/DMSO-d_6, Fig. S3) δ 181.89, 174.06, 171.70, 164.48 (2C), 157.39 (2C), 151.27, 141.87, 131.78 (2C), 131.31, 129.10, 126.71, 122.60, 119.89 (2C), 113.19 (2C), 103.95 (2C), 103.69, 55.83, 45.12, 32.01, 29.51, 28.06. HR-MS (ESI) (m/z, Fig. S4) $[M+H]^+$ calculated for $[C_{27}H_{26}N_3O_7S]^+$: 536.1491, found 536.1493.

4. HPLC analysis

The probe FITC-Lys and patulin were dissolved in methanol, respectively. The time course test of the reaction of patulin with FITC-Lys was performed immediately after the solution of patulin was added into the solution of probe FITC-Lys. The mobile phase was methanol/water (v/v, 3/1), and the flow rate was 1.0 mL/min.

5. The probe's fluorescent response toward patulin with different mycotoxin, reaction time and temperature

The anti-interference test of FITC-Lys toward patulin against other mycotoxins was performed, the mycotoxins were obtained from several contaminated foods (barley, wheat, peanuts and maize).

The reaction temperature and reaction time were evaluated from 25°C to 45°C and 0 to 4

h, respectively. The excitation wavelength of fluorescence spectra was 490 nm, and the scanning range was 510−650 nm, and both the excitation and emission slits were 5 nm.

6. Fluorescence spectra and fluorescence imaging measurements

The stock solutions of the probe **FITC-Lys** (1 mg/mL) and PAT (1 mg/mL) were prepared in DMSO. Briefly, various concentrations of patulin (0, 0.05, 0.015, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 10 μg/mL) were added into the probe **FITC-Lys** (5 μg/mL) in water at 37°C. After 2 h of reaction, the fluorescence spectra and fluorescence imaging were recorded at room temperature. All fluorescence imaging tests were carried out on AMI small animal fluorescence imaging system (Excitation filter: 500 nm, emission filter: 630 nm).

8. Detection of patulin in real juice samples.

All original juice samples were extracted from the normal apples and pears without any rotting as well as the apples and pears with different degrees of rotting with a juicer. Then, all juice samples were filtered through 0.22 μm nylon filter to obtain final juice samples.

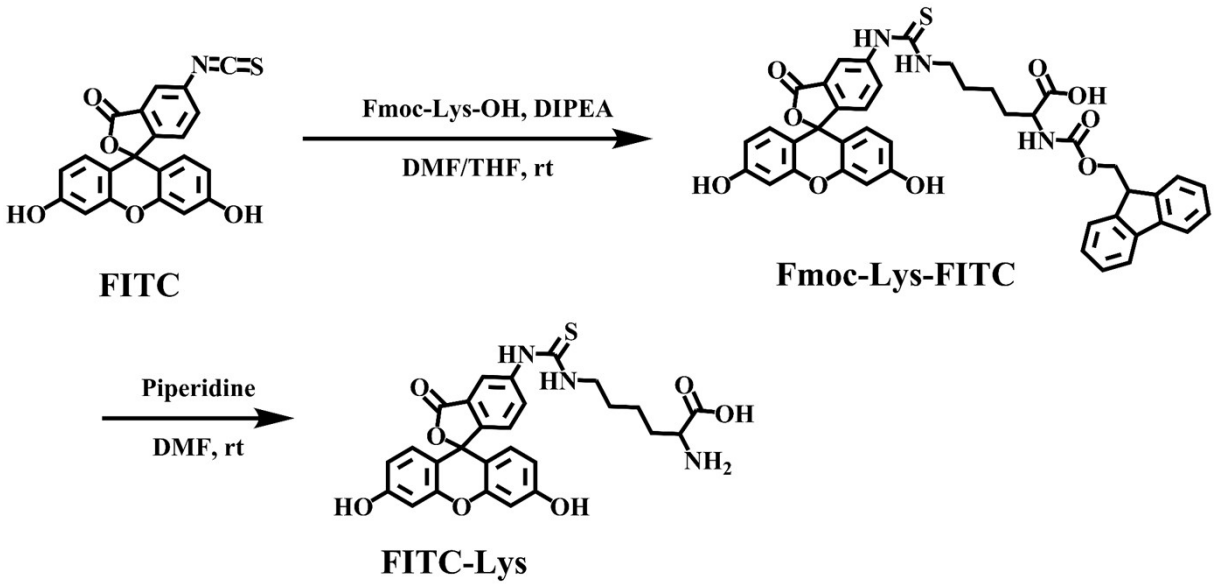
As for spiking experiments, patulin was added into the patulin-free apple or pear juice samples made from normal apples or pears without any rotting (final patulin concentration: 0.5 μg/mL and 1.5 μg/mL, respectively), and the probe **FITC-Lys** was added into the juice samples (final probe concentration: 5 μg/mL). Subsequently, fluorescence spectra and fluorescence imaging tests were carried out after the reaction at 37°C for 2 h. The measured patulin concentration in each sample was calculated based on the regression equation.

The accuracy of the analysis was evaluated by the recoveries of patulin spiked into the apple juices and pear juices, which were compared with known spiked concentrations of patulin (0.5 and 1.5 μg/mL), and the recoveries were calculated by the following equation.

$$Recovery\ (\%) = \frac{Patulin\ concentration\ measured\ from\ the\ spiked\ samples}{Patulin\ concentration\ used\ for\ spiking\ the\ samples} \times 100$$

For juice samples made from apples and pears with different degrees of rotting, the probe **FITC-Lys** was added into the juice samples (final probe concentration: 5 μg/mL). Subsequently, fluorescence spectra and fluorescence imaging tests were carried out after the

reaction at 37°C for 2 h. The measured patulin concentration in each sample was calculated based on the regression equation.



Scheme S1. The synthetic route of **FITC-Lys**.

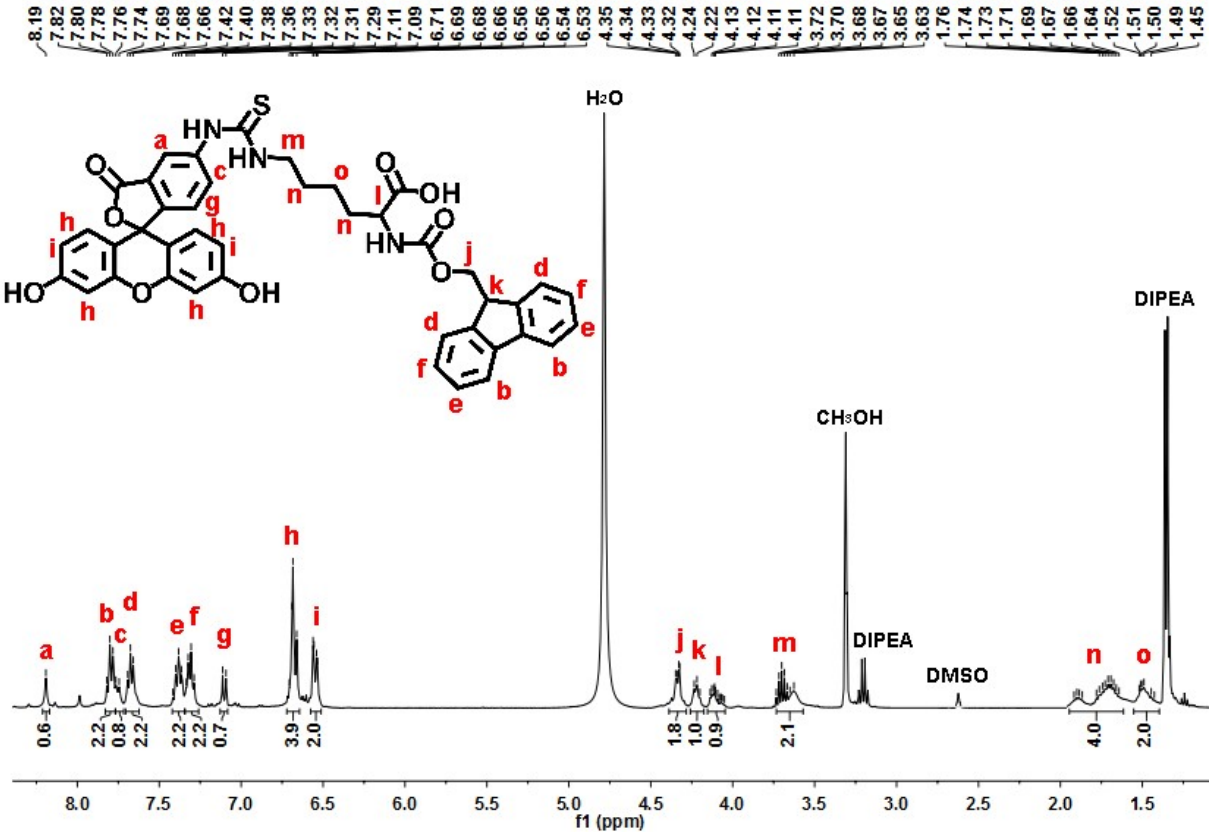


Fig. S1. ^{1}H NMR spectrum of **Fmoc-Lys-FITC** in CD_3OD/DMSO-d_6.

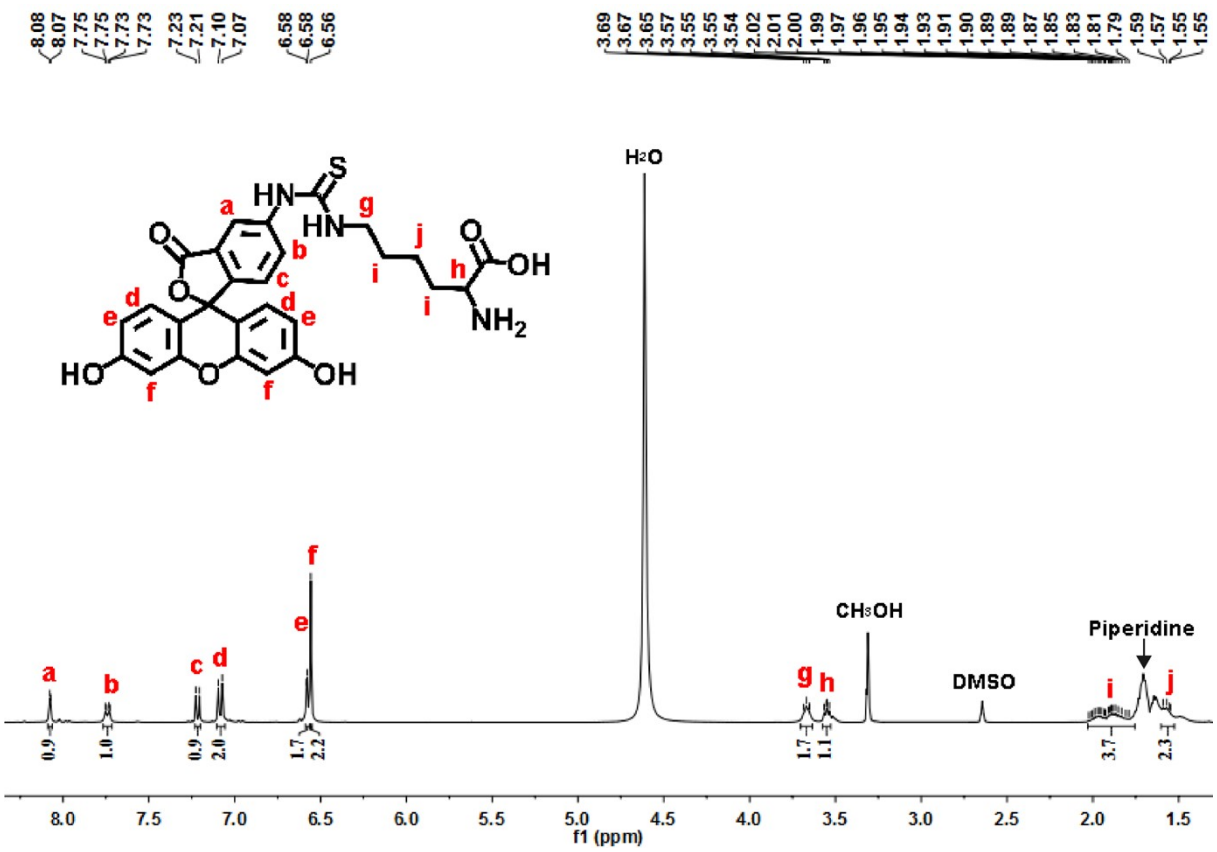


Fig. S2. ^{1}H NMR spectrum of **FITC-Lys** in CD_3OD/DMSO-d_6.

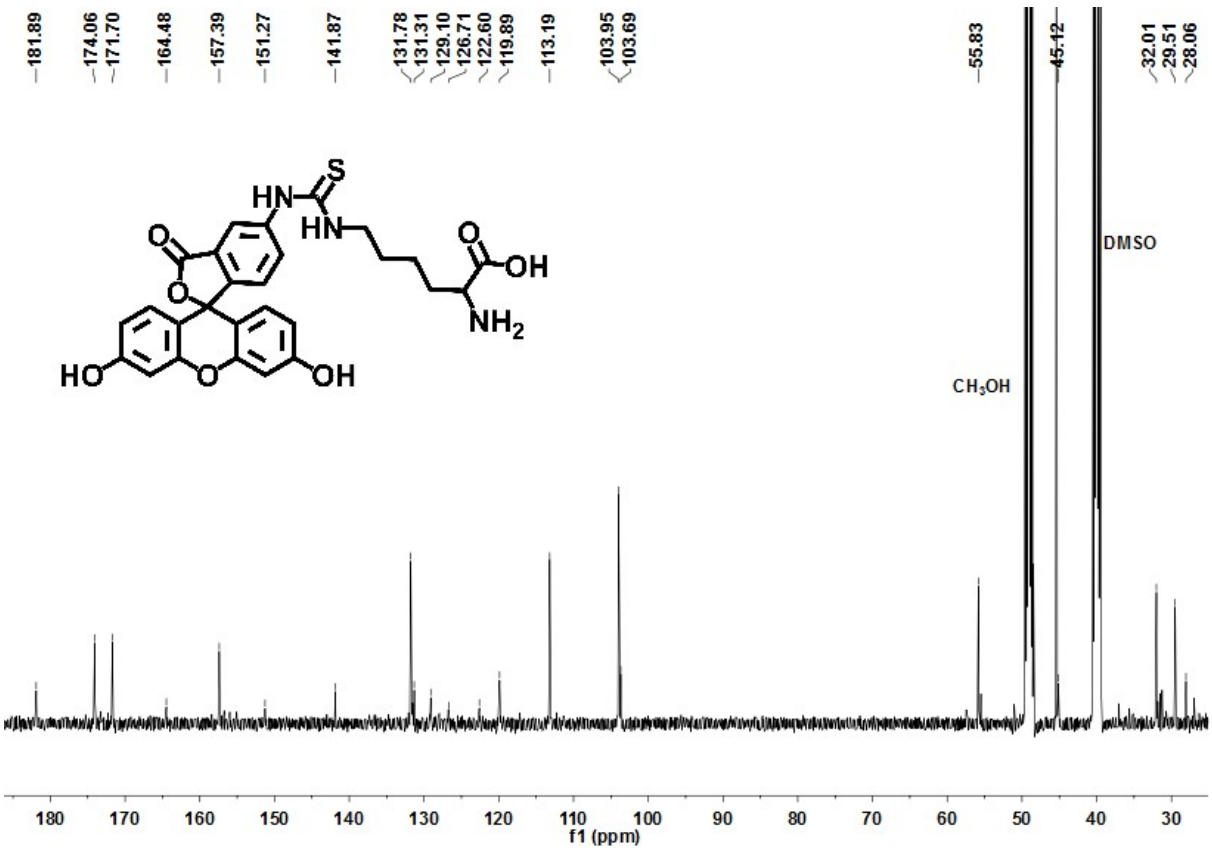


Fig. S3. ^{13}C NMR spectrum of **FITC-Lys** in CD_3OD/DMSO-d_6.

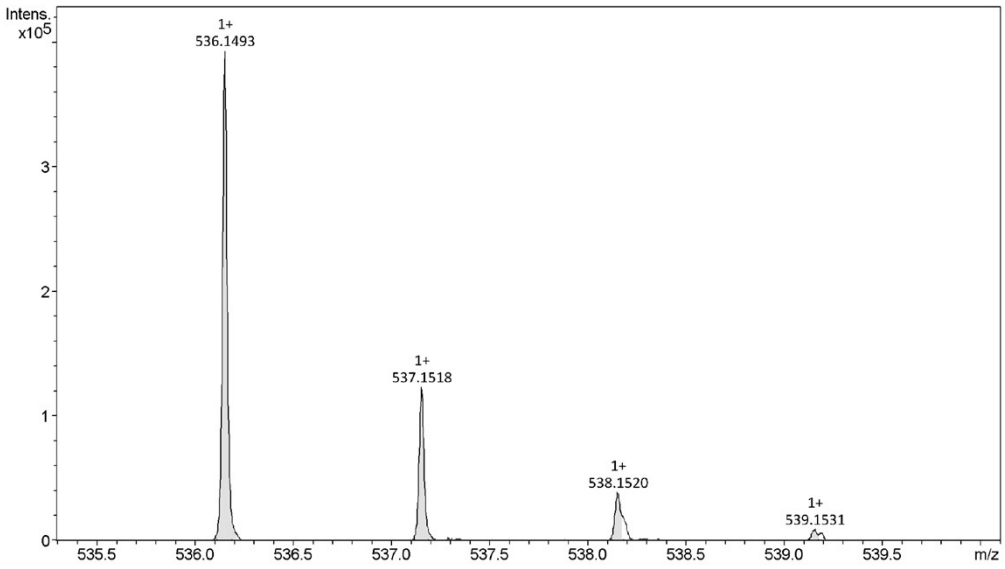
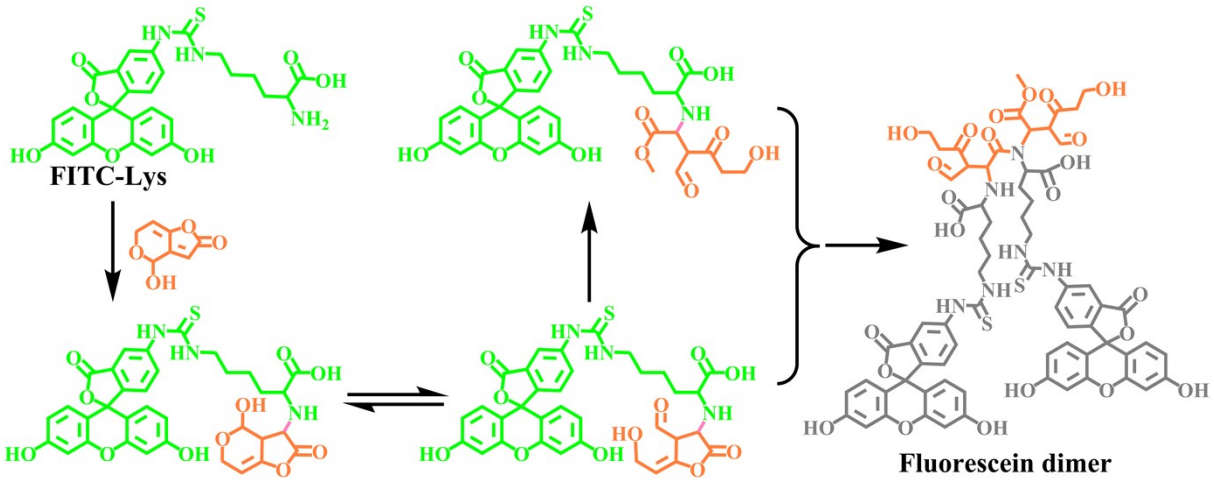


Fig. S4. HR mass spectrum of **FITC-Lys**.
m/z $[M+H]^+$ $[C_{27}H_{26}N_3O_7S]^+$ 536.1493
(The isotopic peaks are due to the contribution from isotopes of the elements)



Scheme S2. Proposed mechanism of the reaction of **FITC-Lys** with PAT.

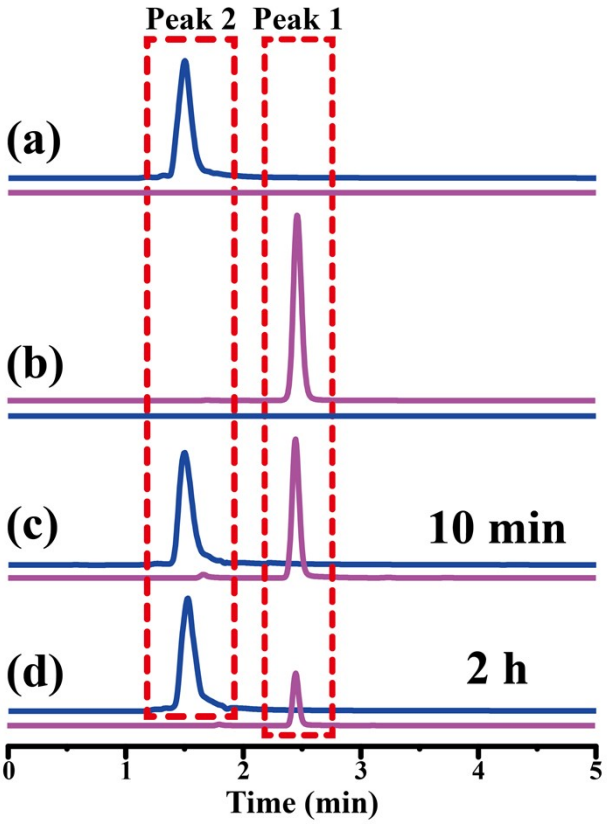


Fig. S5. HPLC chromatograms for (a) FITC-**L**ys, (b) Patulin, (c) FTIC-Lys treated with patulin for 10 min, and (d) FITC-Lys treated with patulin for 2 h.
Purple lines represent signal acquired at 276 nm, blue lines represent signal acquired at 500 nm.

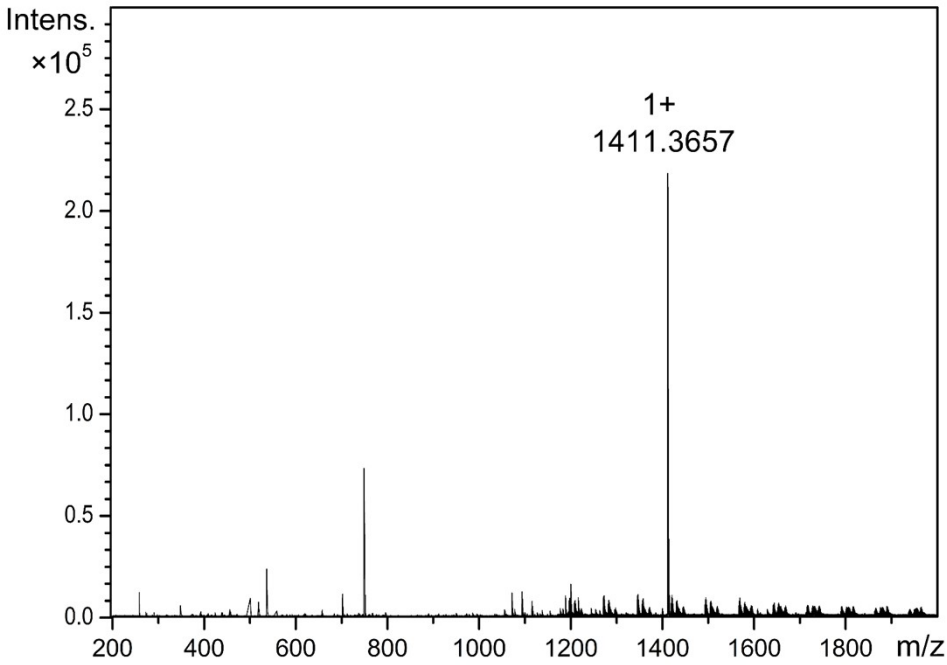


Fig. S6. HR mass spectrum of the product (fluorescein dimer) from the reaction between **FITC-Lys** and PAT.
m/z $[M+H]^+$ $[C_{69}H_{67}N_6O_{23}S_2]^+$ 1411.3657

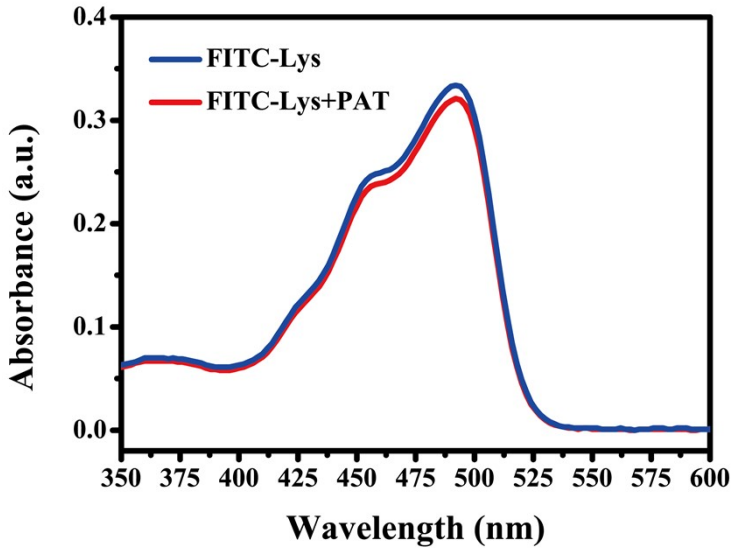


Fig. S7. The absorption spectra before and after the probe **FITC-Lys** (5 μg/mL) responds to patulin (PAT, 10 μg/mL).

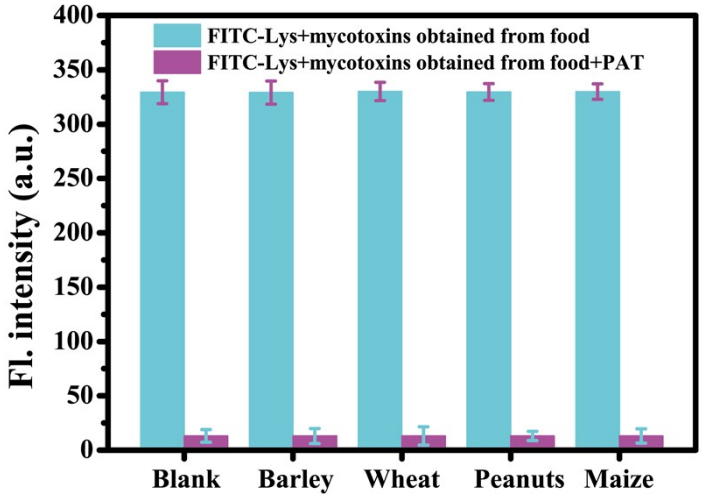


Fig. S8. The fluorescence intensity at 544 nm (excited at 490 nm) of **FITC-Lys** (5 μg/mL) with patulin (PAT, 10 μg/mL) in presence of other mycotoxins as well as FITC-Lys with other mycotoxins (obtained from several foods contaminated with mycotoxins), respectively.

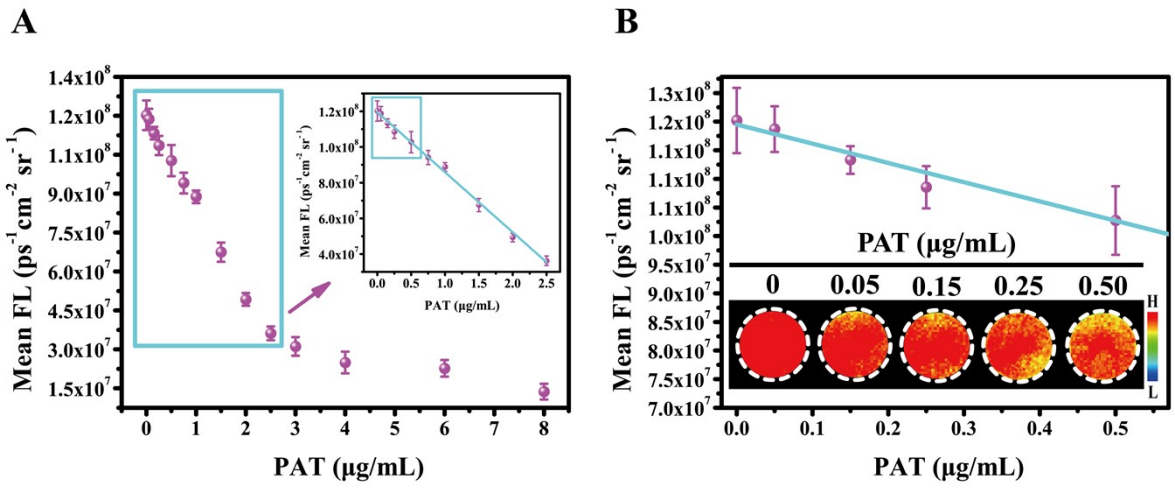


Fig. S9. A) The fluorescence intensities of FITC-Lys (5 μg/mL) in the presence of various patulin (PAT) concentrations (0−8 μg/mL). Inset: the linear relationship between the fluorescence intensity of FITC-Lys and patulin concentrations (0−2.5 μg/mL). B) The linear relationship between the fluorescence intensity of FITC-Lys (5 μg/mL) and patulin concentrations (0−0.50 μg/mL). Inset: the corresponding fluorescence images.

Table S1. The calculated logP of FITC-Lys and Fluorescein dimer.

Compound	Calculated logP
FITC-Lys	-0.8152
Fluorescein dimer	-0.7132

Data obtained from Chemdraw.

Table S2. Several contaminated foods and the mycotoxins in which they may occur.

Susceptible food	Major mycotoxin
Barley	DON
Wheat	OTA, ZEN, DON,
Peanuts	AFB_1
Maize	AFB_1, FB_1, ZEN, DON

Information obtained from reference.[1] Ochratoxin A (OTA), aflatoxin B1 (AFB_1), fumonisin B1 (FB_1), zearalenone (ZEN), and deoxynivalenol (DON).

Table S3. Recoveries of PAT spiked in juice samples detected by **FITC-Lys**.

		Fluorescence spectrum analysis		Fluorescence imaging analysis	
Samples	Spiked (μg/mL)	Detected (Mean ± SD, μg/mL)	Recovery (Mean ± SD, %)	Detected (Mean ± SD, μg/mL)	Recovery (Mean ± SD, %)
Apple juice	0.50	0.503 ± 0.042	100.60 ± 8.40	0.503 ± 0.023	100.60 ± 4.60
	1.50	1.507 ± 0.022	100.46 ± 4.40	1.514 ± 0.020	100.93 ± 1.33
Pear juice	0.50	0.493 ± 0.040	98.60 ± 8.00	0.519 ± 0.022	103.80 ± 4.40
	1.50	1.492 ± 0.042	99.47 ± 2.80	1.513 ± 0.037	100.86 ± 2.47

Data obtained from Figure 3 in the main text.

Table S4. The detection limit comparison between this probe and previous' reports

Recognition element	Linear range	Detection limit	Testing samples	Ref.
Aptamer	20−500 ng/L	4.7 ng/L	Several fruit juices	2
DNA duplex	15 ng/L−35 μg/L	6 ng/L	Apple juice	3
Aptamer	0.01−100 ng/L	8.5 ng/L	Apple juice, grape juice	4
Quantum dots	1 pg/mL−100 ng/mL	0.753 pg/mL	Apple juice	5
Carbon dots	0.1−400 ng/mL	0.053 ng/mL	Apple juice, grape juice	6
Liposomes	0.05−20 ng/mL	0.033 ng/mL	Apple juice, grape juice	7
Aptamer	5.0×10^{-8}–5×10^{-1} μg/mL	30.4 fg/mL	Apple juice	8
DNAzymes	5.0×10^{-6}–50 μg/L	0.92 fg/mL	Apple juice	9
Small-molecular probe	0−2 μg/mL, 0−2.5 μg/mL	8 ng/mL, 12 ng/mL	Juices derived from decayed apples and pears	This work

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