A highly efficient, colorimetric and fluorescent probe for aliphatic primary amines based on a unique cascade chromophore reaction

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Scheme S1. Synthetic routes of PPAB-Py.



Scheme S2 Schiff base hydrolysis mechanism in the alkaline media.



Scheme S3. The proposed reaction mechanism of PPAB-Py with n-hexylamine in dioxane/H₂O (1:1, v/v).

1. Experimental section

1.1. Instrumentation and materials

All chemicals used in this study were analytical reagent grade. The UV-vis absorption spectra were recorded using a Helios Alpha UV-Vis scanning spectrophotometer with a 1 cm quartz cell. Fluorescence spectra were quantitatively measured by FluoroMax-4 spectrofluorometer with a xenon lamp and 0.5 cm quartz cells. High-resolution mass spectra were carried on LCQ Fleet LC-MS System (Thermo Fisher Scientific). Mass spectra were recorded on Waters ACQUITY TQD liquid chromatograph-mass spectrometer using APCI ionization. ¹H NMR, ¹¹B NMR, ¹⁹F NMR and ¹³C NMR spectra were carried on a Bruker spectrometer.

1.2 Preparation of the samples for the UV-vis and fluorescence measurement of PPAB-Py nanoaggregates in the presence of amines.

PPAB-Py nanoaggregates (10 μ M) were freshly prepared for each measurement. Stock solutions of 10.0 mM amines in dioxane were freshly prepared for each measurement. The solution of **PPAB-Py** in dioxane /water (1/1, v/v) were mixed with various solutions of amines. The resulting solutions were left at room temperature for desired time and used to record UV-vis and fluorescence spectra.

1.3 Measurement of detection limit.

The detection limit was gained from the fluorescence titration data. On the basis of the results of the fluorescence spectra titrating experiment, a good linear relationship between the fluorescence intensity of **PPAB-Py** nanoaggregates (10 μ M) at 550 nm and the n-hexylamine concentration ranging from 0 to 10 μ M was obtained. A detection

limit (LOD) was calculated by means of equation (1):

Detection limit =
$$(3 \times \sigma) / k$$
 (1)

Where σ is the standard deviation of blank measurements, *k* is the slope between fluorescence intensity at 550 nm versus n-hexylamine concentration.

1.4 Preparation and determination of n-hexylamine vapor. A volume of 5 mL of nhexylamine solution in dioxane with various concentrations was placed at the bottoms of sealed 100 mL bottles. After n-hexylamine vapor reached equilibrium by standing overnight at room temperature.

1.5 Preparation of Test Strips. Preparation of Test Strips. **PPAB-Py**-based test strip was fabricated by immersing filter paper into the dioxane solution of **PPAB-Py** (1.0 mM) and drying in air, then cut into test strips for testing.

1.6 Detection of shrimp freshness with PPAB-Py-loaded test strips

Fresh samples of shrimps were purchased from a local supermarket. To first examine if the test strip would show any response, three sealed Petri dishes with test strips containing 1) no sample, and samples of shrimps kept at 2) 28 °C and 3) -4 °C were prepared. After 2d, 3d, 4d, the test strips sealed with Petri dishes was used to check colorimetric and fluorescence change by photos.

2 Synthesis

Synthetic procedures. The synthetic route for compound **PPAB-Py** was shown in Scheme S1. 6-(tert-Butyl)benzo[d]thiazol-2-amine (1), 6-(1,2,2-triphenylvinyl)-benzo[d]thiazol-2-amine (2), 3-bromo-4-(octyloxy)benzonitrile (3') were prepared as previously described^{1, 2}.

Synthesis of compound 4. Synthesis of compound **4** was prepared as previously described ³. ¹H NMR (CDCl₃, 400 MHz, ppm): 11.04 (s, 1H), 9.59 (s, 1H), 8.57 (s, 1H), 8.34 (d, *J* = 8, 1H), 7.84 (m, 2H), 7.64 (s, 1H), 7.47 (d, *J* = 8, 1H), 6.64 (m, 2H), 3.80 (m, 4H), 1.75 (m, 2H), 1.50-1.40 (m, 2H), 1.38 (s, 9H), 1.37-0.94 (m, 19H), 0.91 (m, 3H). ¹³C NMR (CDCl₃, 100 MHz, ppm):169.12, 164.59, 159.57, 157.29, 151.66, 149.14, 148.36, 147.01, 138.74, 127.54, 125.00, 122.40, 120.32, 118.24, 116.93, 114.35, 113.78, 113.06, 111.50, 68.94, 34.98, 31.90, 31.48, 29.48, 29.29, 26.07, 25.70, 22.74, 14.17. HRMS (ESI): m/z [M + Na] ⁺ calcd for C₄₅H₅₃BBr₂F₂N₄NaO₃S: 959.2158, found: 959.2188.

Synthesis of compound PPAB. Compound **PPAB** was synthesized under similar reaction conditions to **4**. ¹H NMR (CDCl₃, 400 MHz, ppm): δ = 8.80-8.84 (m, 2H), 8.48-8.39 (m, 2H), 7.92 (d, *J* = 8, 1H), 7.72-7.66(m, 2H), 7.57-7.52 (m, 1H), 7.32 (s, 1H), 7.19-6.99 (m, 18H), 4.18 (t, *J* = 12, 4H), 1.97-1.86 (m, 4H), 1.45-1.41 (m, 4H), 1.38 (s, 9H), 1.35-1.24 (m, 16H), 0.91 (t, *J* = 12, 6H). ¹³C NMR (CDCl₃, 100 MHz, ppm):169.15, 157.67, 153.51, 153.32, 152.24, 151.75, 148.32, 142.27, 142.14, 142.03, 141.43, 140.60, 138.41, 138.29, 137.79, 136.28, 130.38, 130.21, 127.03, 126.89, 126.69, 125.96, 125.81, 121.74, 117.76, 117.28, 116.55, 116.08, 110.79, 110.51, 68.36, 34.08, 30.79, 30.37, 28.25, 28.21, 27.95, 24.97, 21.66, 13.12. HRMS (ESI): m/z [M + H] + calcd for C₇₂H₇₁B₂Br₂F₄N₆O₂S₂: 1371.3563, found: 1371.3587.

Synthesis of compound PPAB-Py. Compound PPAB (140 mg, 0.1 mmol), pyridin-4ylboronic acid (49 mg, 0.4 mmol) potassium carbonate solution (2 M, 0.2 mL) and $Pd(PPh_3)_4$ (30 mg) was added to a 100 mL two necked, round-bottom flask, then 15 ml toluene and 5 ml ethanol were injected into the flask. The flask was evacuated under vacuum and flushed with dry nitrogen three times. After the reaction was further refluxed for 3 h, the solution was poured into water and extracted with CH₂Cl₂. The organic layer was washed with brine and dried over magnesium sulfate. After filtration and solvent evaporation, the residue was purified by silica gel column chromatography, using petroleum ether/CH₂Cl₂/ethyl acetate (10/8/1, v/v) as eluent. PPAB-Py (a dark green solid) was obtained in 62% yield. ¹H NMR (CDCl₃, 400 MHz, ppm): δ = 8.66-8.74 (m, 4H), 8.64-8.51 (m, 4H), 7.93 (d, J = 12, 1H), 7.79-7.66 (m, 6H), 7.57 (d, J = 8, 1)1H), 7.36 (s, 1H), 7.22-7.01 (m, 18H), 4.18 (t, J = 16, 4H), 1.50-1.43 (m, 4H), 1.39 (s, 9H), 1.37-1.24 (m, 16H), 0.91 (t, J = 12,6H). ¹³C NMR (CDCl₃, 100 MHz, ppm): 170.23, 159.68, 154.85, 154.67, 154.46, 153.97, 149.64, 145.66, 143.35, 143.24, 143.15, 142.65, 141.87, 139.35, 138.87, 135.31, 133.04, 131.70, 131.50, 131.32, 129.12, 128.31, 128.17, 128.06, 127.83, 127.21, 126.97, 126.07, 125.38, 124.44, 121.71, 118.90, 118.57, 117.65, 117.14, 111.94, 68.91, 35.25, 31.87, 31.50, 29.31, 29.29, 29.09, 26.21, 22.76, 14.23. HRMS (ESI): m/z [M + H] + calcd for C₈₂H₇₉B₂F₄N₈O₂S₂: 1369.5884, found: 1369.5908.



Fig. S1. ¹H NMR spectrum of PPAB-Py in CDCl₃.





Fig. S2. ¹³C NMR spectrum of PPAB-Py in CDCl₃.



Fig. S3. HRMS spectrum of PPAB-Py.



Fig. S4. Dynamic light scattering (DLS) of PPAB-Py (10 μ M) in dioxane/H₂O (1:1,

v/v).



Figure S5 (a) SEM images of **PPAB-Py** nanoaggregates, (b) Enlargement of image a.



Figure S6 (a) TEM images of **PPAB-Py** nanoaggregates, (b) Enlargement of one particle.





Fig. S7. MS spectra of PPAB-Py reaction with n-hexylamine in 50% water in dioxane.



Fig. S8. Partial ¹H NMR spectra of **PPAB-Py** and after adding 10 eq. n-hexylamine in CDCl₃.



Fig. S9. Partial ¹H NMR spectra of **PPAB-Py** and after adding 10 eq. hexylamine in CDCl₃.



Fig. S10¹¹B NMR spectra of PPAB-Py and after adding 10 eq. n-hexylamine in CDCl₃.



Fig. S11¹⁹F NMR spectra of PPAB-Py and after adding 10 eq. n-hexylamine in CDCl₃.



Fig. S12 Absorption spectra of PPAB-Py (10 μ M) in dioxane/H₂O (1:1, v/v) in presence

of varying concentration of n-hexylamine.



Fig. S13 Dynamic light scattering (DLS) of PPAB-Py (10 µM) in dioxane/H₂O (1:1,

v/v) solution with 40 eq. n-Hexylamine.



Fig. S14 The emission spectra of PPAB-Py (10 $\mu M)$ in dioxane/H2O (1:1, v/v) in

presence of varying concentration of n-hexylamine.



Fig. S15 a linear calibration curve between the fluorescent intensity ($\lambda_{em} = 550 \text{ nm}$) of **PPAB-Py** (10 μ M) in dioxane/H₂O (1:1, v/v) and the concentration of n-Hexylamine in the range of 0 to 10 μ M.



Fig. S16 Reaction kinetics of PPAB-Py (10 μ M) in dioxane/H₂O (1:1, v/v) with 400 μ M n-hexylamine.



Fig. S17 (a) UV-vis and (b) emission spectra of **PPAB-Py** (10 μ M) in dioxane/water (1:1, v/v) after the addition of 40 equiv. Cys, Hcy and GSH.



Fig. S18 Reaction kinetics of PPAB-Py (10 μ M) in dioxane/H₂O (1:1, v/v) with different amounts of amine.



Fig. S19 Photographs of **PPAB-Py** loaded on filter paper exposure to increasing nhexylamine vapor concentration from left to right 0 M, 2 mM, 4 mM, 8 mM, 10 mM, 20 mM, 30 M, 40 M and 50 M under daylight and 365 nm UV light.



Fig. S20 Reaction kinetics of PPAB-Py (10 μ M) in dioxane/H₂O (1:1, v/v) with 40 eq.

putrescine, cadaverine and trimethylamine.



Fig. S21 Photos of PPAB-Py-loaded filter paper sealed with shrimp at -4 °C after 4

days storing time.

Probes	LOD	Remark	Reference
NC	No data	"turn-on"; primary amines: 5 min	J. Am. Chem. Soc. 2014, 136, 15493- 15496
	dimethylamine: 0.4 ppm Ammonia: 0.5 ppm	Colorimetric; 5 min	Chem. Eur. J. 2017, 23, 3562-3566
NH NH O O	Ammonia vapor: 8.4 ppm	"turn-on"; 5 min	ACS Sens. 2016, 1, 179-184
	volatile amines: low ppm range	fluorescence quenching;	Angew. Chem. Int. Ed. 2014, 53, 9792-9796
S NO ₂	BAs: ppm level	absorption response; putrescine: 20 min cadaverine: 60 min	Sensors & Actuators: B. Chemical, 2018, 271, 183-188
of the second se	BAs: 0.2 μM	Ratiometric fluorescence; 5 min	Analyst, 2016, 141, 827-831
	Put: 0.78 μM; Cad:1.20 μM	"turn-off"	Sensors & Actuators: B. Chemical, 2018, 254, 842-854
	Ammonia vapor: 690 ppb	colorimetric and fluorescence; 5 min	Chem. Eur. J. 2017, 23, 14911-14917
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Table S1 The comparison of **PPAB-Py**-based amine probe with others.

References

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