Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

A dual-responsive pH-sensor and its potential as a universal probe for assays of pH-changing enzymes

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Stern-Volmer plot

The Stern–Volmer equation ($F_o/F = 1 + K_{sv}[Q]$) was employed to calculate bimolecular quenching. F_o and F are fluorescence signals observed in the absence and presence of the quencher, poly-L-histidine (Q), respectively. [Q] is the quencher concentration and K_{sv} is the Stern-Volmer constant. Fluorescence intensity of Co-NTA-Atto488 (100 nM) was measured with varying concentration of the quencher (0, 25, 40, 50, and 80 nM) at three different temperatures (25, 30 and 37ºC).

Quenching by hexahistidine (His_6)

His_6-Co-NTA-Atto488 was prepared by incubation of Co-NTA-Atto488 (100 nM) with varying concentrations of His_6 (0 – 6000 nM) purchased from Abbiotec (USA). Fluorescence intensity of the complex was measured at room temperature.

Dynamic light scattering

The hydrodynamic size of PLH (1 μM) was measured on the ZS90 Zetasizer Nano series spectrometer (Malvern, UK) at pH 4, 7, and 10 (in 10 mM Tris-HCl buffer). Before measurement, solutions were pipetted several times for even distribution of the aggregated peptide. Measurements were conducted 5 times for each solution at room temperature.

Table S1. Stern-Volmer quenching constant K_{sv} at different temperatures.

T (°C)	K_{sv} x 10^{-7} (L/mol)
25	9.76
30	7.55
37	5.97

Table S1. Stern-Volmer quenching constant K_{sv} at different temperatures.

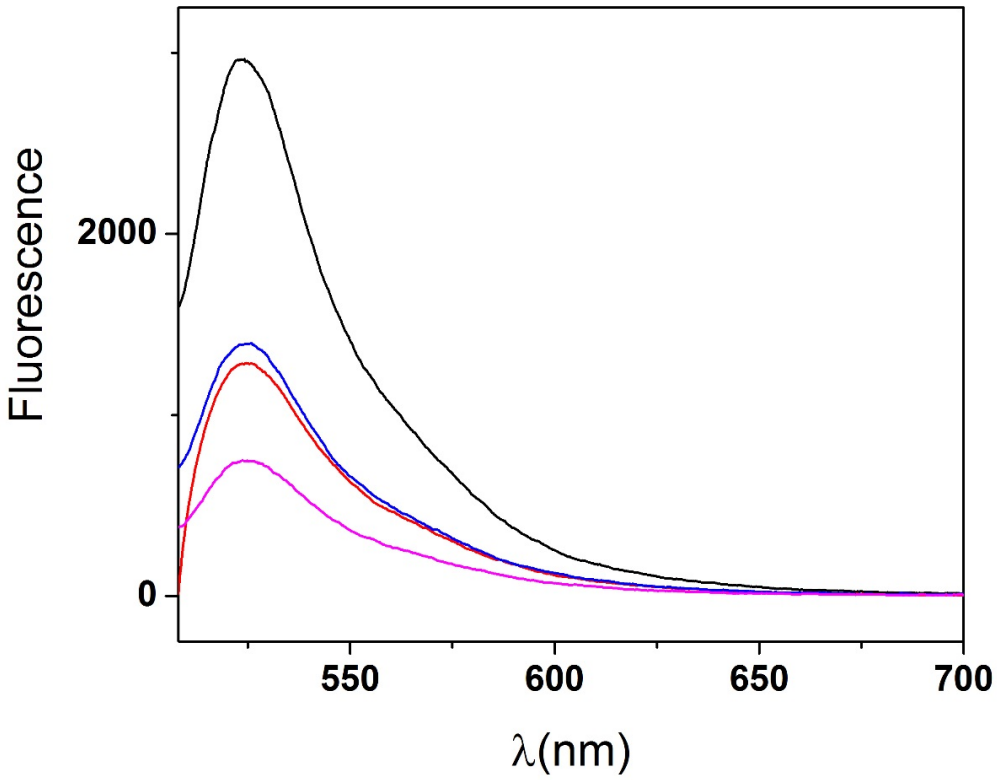

Fig. S1. The fluorescence emission spectra were measured in PBS for Atto488 (black trace), NTA-Atto488 (red trace), Co-NTA-Atto488 (blue trace), and PLH-Co-NTA-Atto488 (magenta trace) at the concentrations providing the same absorption value at 488 nm. The excitation wavelength was 488 nm. The determined relative quantum yields were 34% for NTA-Atto488, 37% for Co-NTA-Atto488, and 20% for PLH-Co-NTA-Atto488, respectively, based on the previously reported quantum yield of Atto488 in PBS (80%)[1]

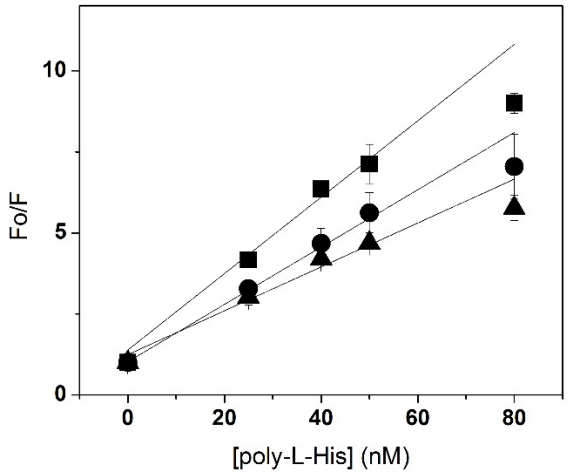

Fig. S2 Stern-Volmer plots for quenching of Co-NTA-Atto488 by poly-L-His at different temperatures: (■)25°C, (●) 30°C and (▲) 37°C. F_o and F denote the fluorescence intensity of Co-NTA-Atto488 in the absence and presence of poly-L-His, respectively.

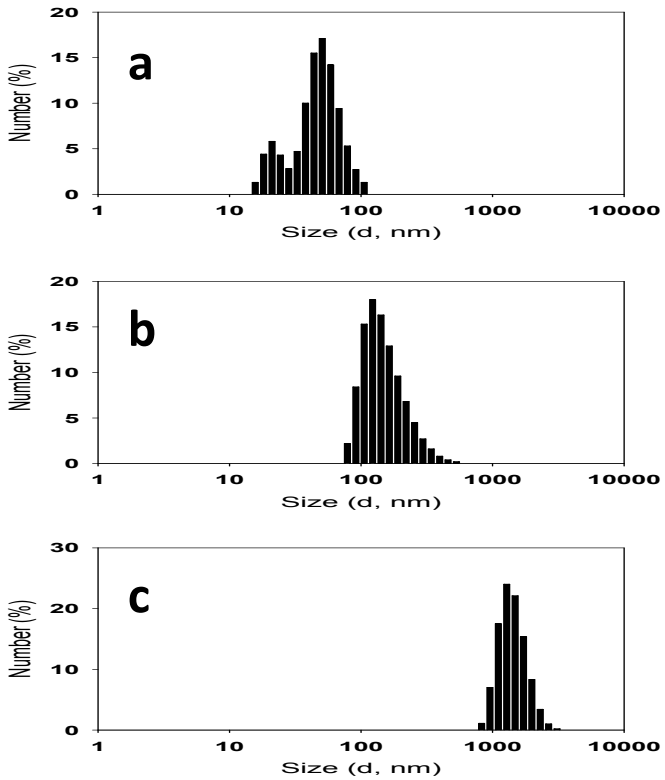

Fig. S3 The hydrodynamic size of PLH at pH (a) 4, (b) 7, and (c) 10 by using dynamic light scattering.

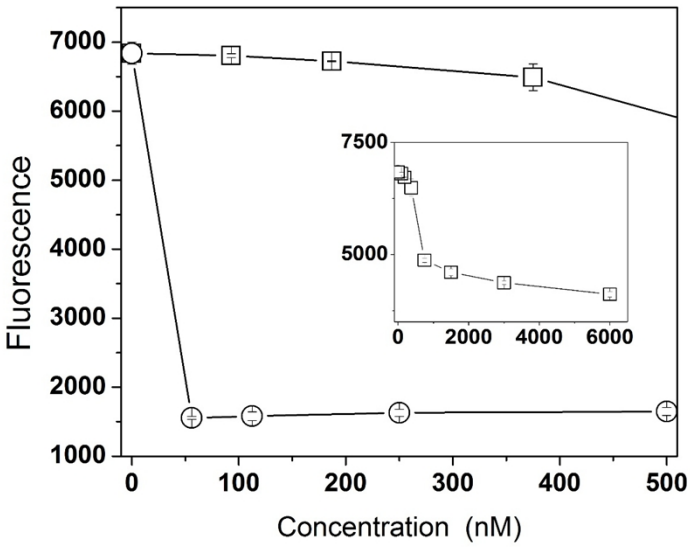

Fig. S4 Quenching of the fluorophore in the presence of PLH (circles) or His_6 (squares). The inset graph shows the fluorescence profile in extended concentration range (0 – 6000 nM) of His_6

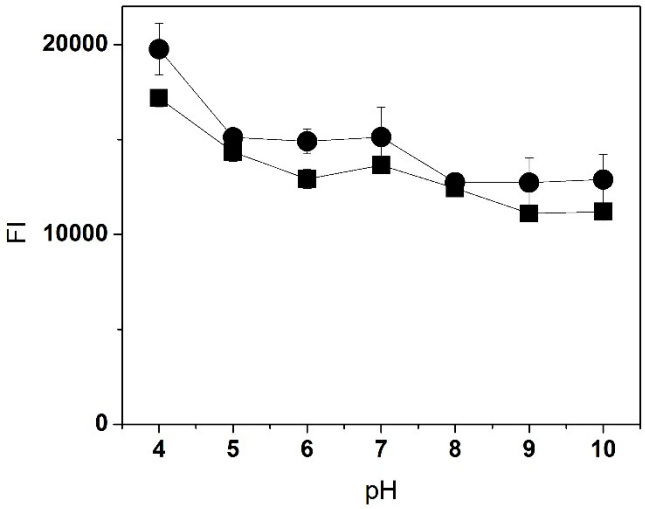

Fig. S5 The fluorescence intensity (FI) of 100 nM of NTA-Atto488 (●) and Co-NTA-Atto488 (■) at different pH values.

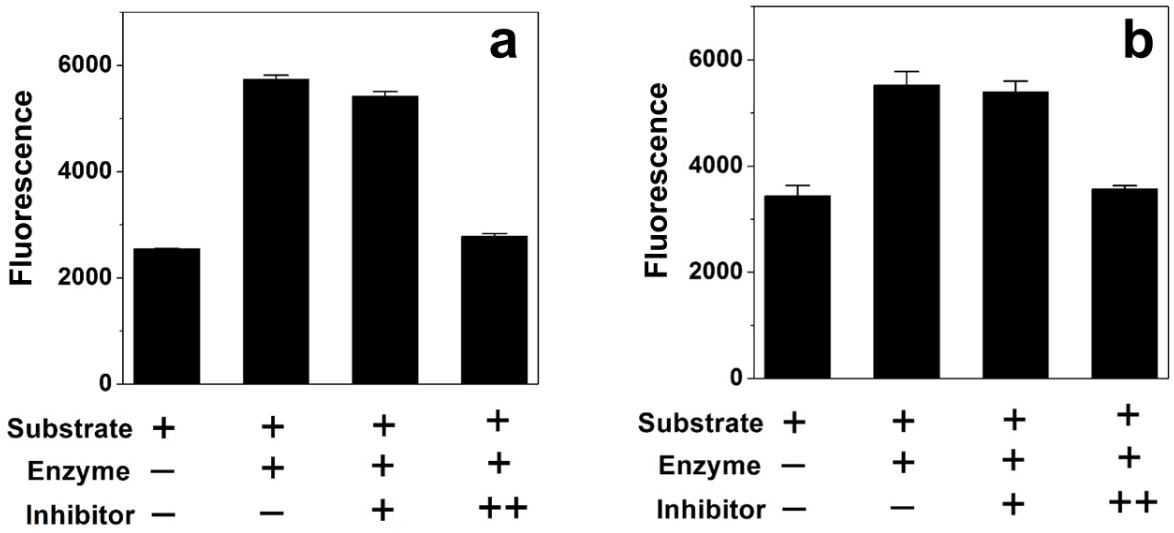

Fig. S6 Inhibition assays for (a) penicillinase and (b) ADA monitored by the dual-responsive probe. The activity of enzymes were measured in the absence of inhibitor (-) and presence of inhibitor at low concentration (+, 0.01 μM postassium clavulanate and 0.01 mM EHNA) versus high concentration (++, 500 μM postassium clavulanate and 2 mM EHNA);

Reference

1. M. Gabba, PhD Thesis, Heinrich Heine University Düsseldorf, 2013, p. 69.

Table S2. Characteristics of β-lactamase-positive bacterial strains and their % difference of fluorescence at 30, 60, and 90 minutes.

Group	Name	Species	MIC of Cefotaxime (μg/ml)
group 1	CTX-M-1	*S. typhimurium*	> 64
group 1	CTX-M-3	*S. marcescens*	64
group 1	CTX-M-3	*S. marcescens*	64
group 1	CTX-M-3	*S. marcescens*	64
group 1	CTX-M-3	*S. marcescens*	64
group 1	CTX-M-3	*E. coli*	16
group 1	CTX-M-3	*E. cloacae*	64
group 1	CTX-M-3	*E. cloacae*	64
group 1	CTX-M-3	*E. cloacae*	64
group 1	CTX-M-3	*K. pneumoniae*	32
group 1	CTX-M-12	*K. pneumoniae*	32
group 1	CTX-M-15	*E. cloacae*	64
group 1	CTX-M-15	*E. coli*	64
group 1	CTX-M-15	*K. pneumoniae*	64
group 1	CTX-M-15	*C. freundii*	64
group 1	CTX-M-15	*E. coli*	64
group 1	CTX-M-32	*E. coli*	64
group 2	CTX-M-2	*E. coli*	64
group 2	CTX-M-2	*E. coli*	64
group 9	CTX-M-9	*C. freundii*	32
group 9	CTX-M-14	*S. marcescens*	64
group 9	CTX-M-14	*E. coli*	32
group 9	CTX-M-14	*C. freundii*	64
group 9	CTX-M-14	*E. coli*	64

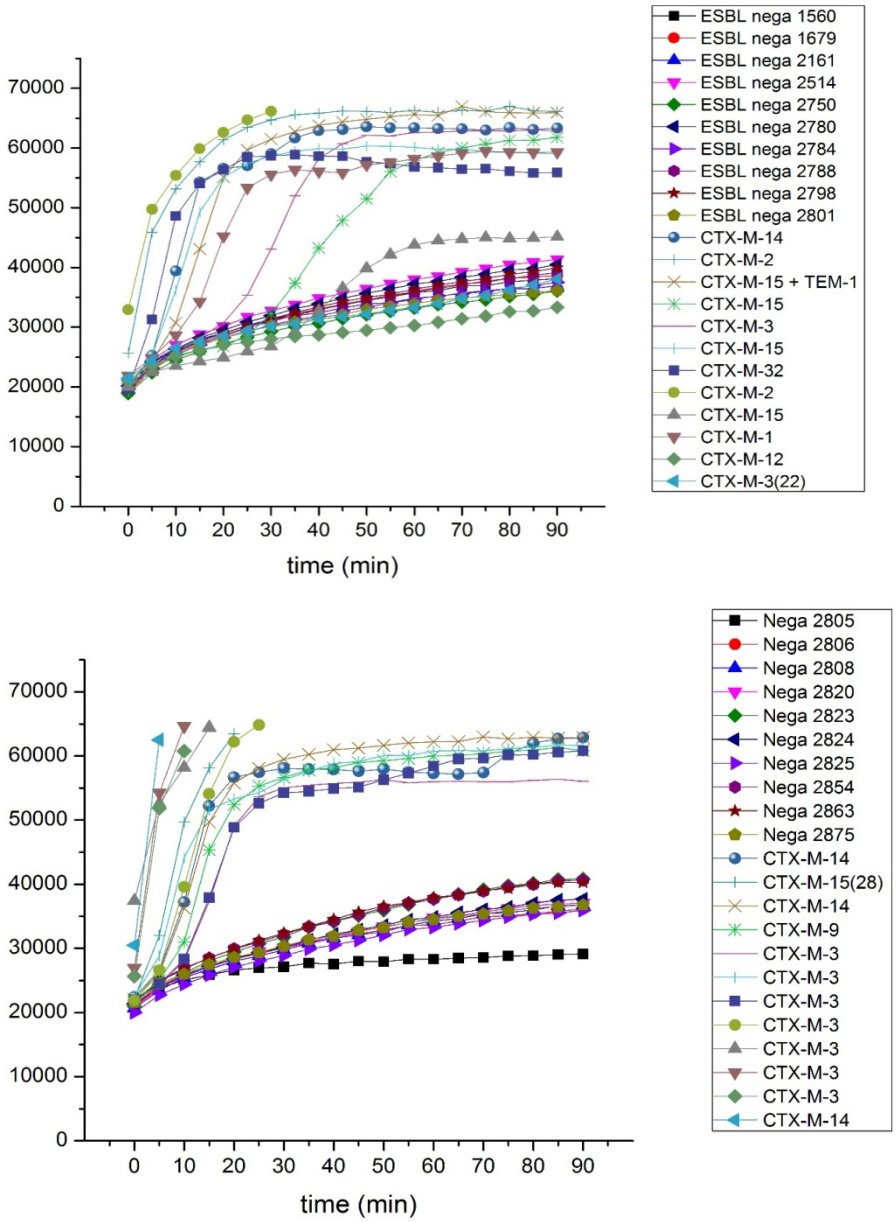

Fig. S7. Detection of penicillin resistant isolates using probe. The fluorescent signal change by time from 0 to 90 min incubation of probe and isolates