Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Rapid paper-based optical sensing of *Spilosoma Obliqua Nucleopolyhedrovirus* via ester hydrolysis

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EXPERIMENTAL SECTION

Materials and Methods

All reagents, solvents, and chemicals, including silica gel for thin liquid and column chromatography, were obtained from the best-known commercial sources and used without further purification. Fourier transform infrared (FT-IR) spectra were recorded on a PerkinElmer FT-IR Spectrometer BX system and were reported in wave numbers (cm^{-1}). ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker Advance DRX 400 spectrometer operating at 400 and 100 MHz for ^{1}H and ^{13}C NMR spectroscopy, respectively. Chemical shifts were reported in ppm downfield from the internal standard, tetramethylsilane. Mass spectra were recorded on a Micromass Q-tof Micro mass spectrometer. Fodder was cultivated on the farm at the ICAR-National Institute of Animal Nutrition and Physiology (ICAR-NIANP) without involving any pesticides. The vegetables, fruits, and cereal samples were collected from organically grown farms.

UV-Visible and Fluorescence Experiment

The UV-Vis and fluorescence spectra were recorded on a Shimadzu model 2100 UV-Vis spectrometer and Cary Eclipse spectrofluorimeter. In the fluorescence emission spectral studies, the slit widths for both the excitation and emission channels were kept at 5 mm, and the excitation wavelength was kept at 370 nm. To monitor the effect of pH, a sensing experiment was performed in buffered media of different pH (HCO_2Na/HCl buffer for pH 2, Tris/HCl for pH 7, and $Na_2B_4O_7 \cdot 10H_2O$/NaOH for pH 12). Again, during the temperature-dependent experiments, 5 min of incubation was given in each case for thermal equilibrium. Every measurement was done in replicates to check the reproducibility of the system.

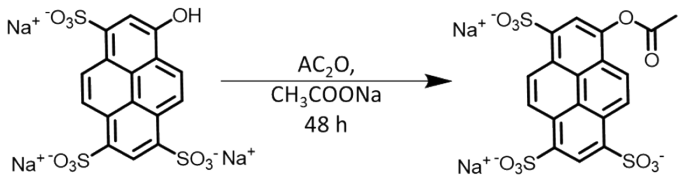
Real-Life Sample Analysis

The crops were thoroughly washed using deionised water to remove dirt and chopped into small pieces (especially the vegetables, fruits, and fodders). Almost every case, about 1 g of the processed samples (cereals were weighed as they were) were added to a pH 7.4 buffer (10 mL) solution, and the mixture was sonicated at 50 °C for 1 h. Finally, the mixtures were centrifugated at 1000 rpm for 10 min, and the clear supernatants were used for fluorometric spectral analysis. During studies, the diluted suspension of each supernatant (20% in pH 7.4 buffer) was mixed with **1**. The emission spectra were recorded in the presence and absence of the added SpobNPV. The detection limit in each case was calculated using the blank variation method.

Preparation of Paper Discs for Sensing

To prepare the compound-coated paper strips, 40 μL of methanolic solution of **1** (0.02 mM) was drop-coated on the filter paper strips using a micropipette to form a square luminescent spot. The concentration of **1** in the solution (pH 7.4) and immersion time were crucially maintained to avoid aggregation (cyan emission) to begin with. The solution was completely absorbed in the filter paper strips within 15 minutes, and then, the strips were kept overnight to dry in open ambient air. Finally, the air-dried paper strips were ready for sensing studies.

Synthetic Scheme



Synthesis of compound 1: Compound **1** was synthesised using the reported procedure in the literature.[S1] ^{1}H NMR (400 MHz, D_2O) δ (ppm): 9.25 (s, 1H), 9.20 (d, 1H, *J* = 10.0 Hz), 9.16 (d, 1H, *J* = 10.0 Hz), 9.10 (d, 1H, *J* = 10.0 Hz), 8.48 (s, 1H), 8.39 (d, 1H, *J* = 10.0 Hz), 2.63 (s, 3H).

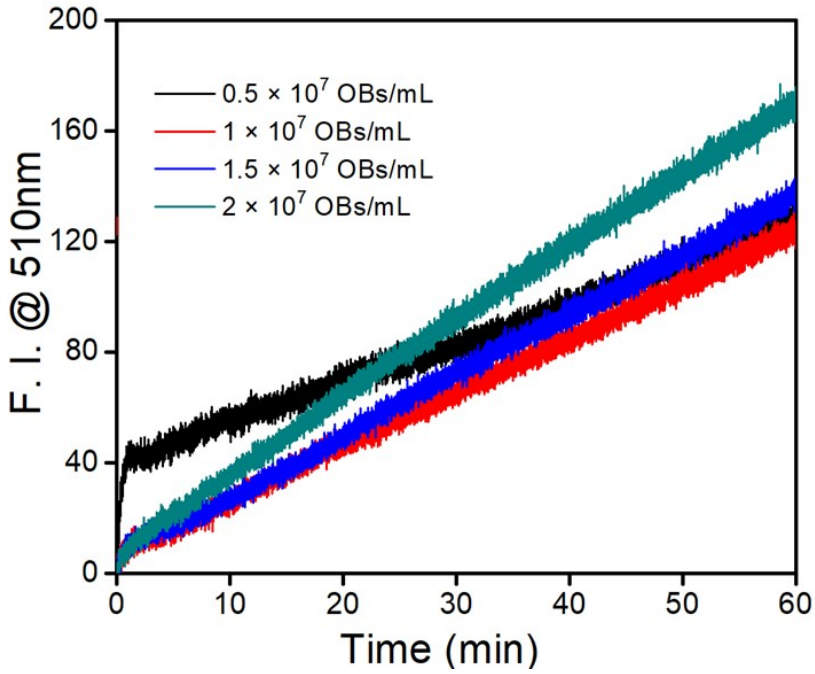


Figure S1: Change in the emission spectra of compound **1** (10 μM) at 510 nm upon the addition of different concentrations of SpobNPV.

(a)

[Comp] in μM	Rate (sec^{-1}) × 10^{-5}
5	3.24
10	5.92
15	10.98
20	19.27
25	34.29
30	53.89

(b)

Conc. of Virus (OBs/mL)	**Rate (sec^{-1}) × 10^{-5}**
0.5 × 10^7	4.83
1.0 × 10^7	5.92
1.5 × 10^7	7.98
2.0 × 10^7	10.50

Table S1. Table showing the rate constant calculation for the ester hydrolysis of **1** by SpobNPV at different (a) substrate concentrations. (b) viruses' concentration.

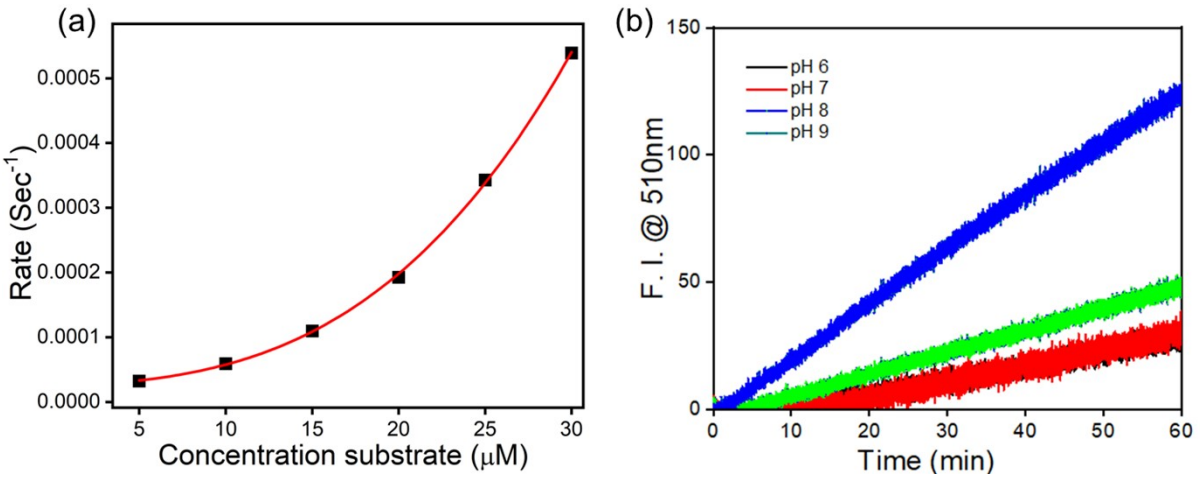


Figure S2. (a) The plot shows the dependence of the substrate on the rate constant. (b) Effect of pH on the extent of SpobNPV mediated emission change of **1** (10 μM, λ_{ex} = 370 nm) at 510 nm. (b) Calculation of rate for ester-mediated hydrolysis of SpobNPV by Compound **1** (10 μM).

(a)

Temperature (K)	Rate (sec^{-1}) × 10^{-5}
293	2.81
303	5.76
313	8.98
323	20.35

(b)

pH	Rate (sec^{-1}) × 10^{-5}
6	1.42
7	1.80
8	1.85
9	4.86

Table S2. Tabulation of rate constant calculation for the hydrolysis of **1** by SpobNPV at (a) different temperatures, keeping pH fixed, and (b) different pH, keeping temperature fixed.

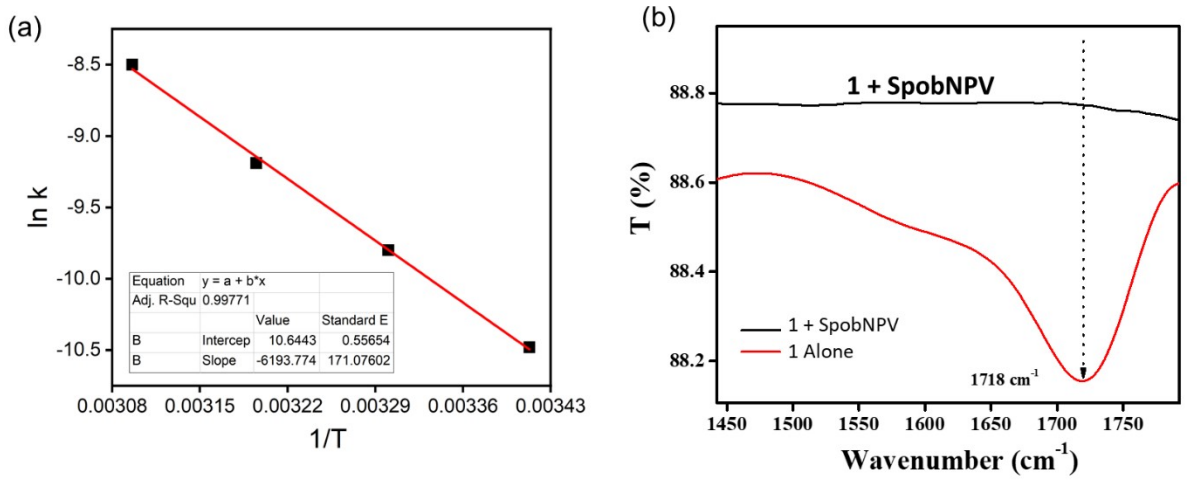


Figure S3. (a) Arrhenius plot to determine kinetic parameters of SpobNPV mediated ester hydrolysis of **1**. (b) FT-IR spectra of **1** before and after treatment with SpobNPV.

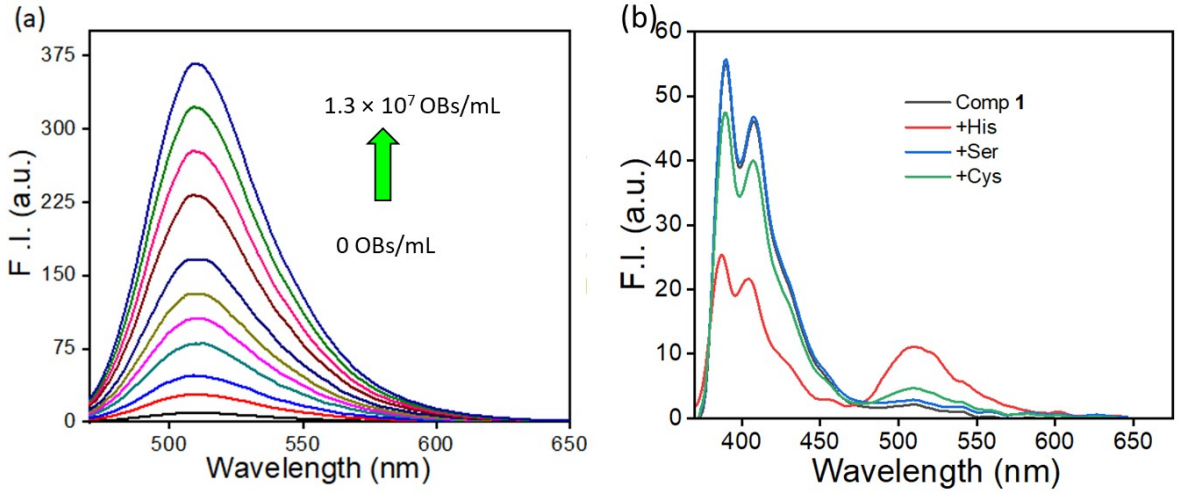


Figure S4. (a) Fluorescence titration of **1** (10 μM, λ_{ex} = 454 nm) in the presence of SpobNPV (0-1.3 × 10^7 OBs/mL) in water. (b) Emission spectra of **1 (**10 μM, λ_{ex} = 370 nm) in the presence of different amino acids (50 μM) in water.

Reference:

S1. P. S. P. Wang, J. B. Nguyen and A. Schepartz, Design and High-Resolution Structure of a β3-Peptide Bundle Catalyst. *J. Am. Chem. Soc.* 2014, **136**, 6810-6813.