Supporting Information for

Fabrication of dual-stimulating responsive films assembled by flavin mononucleotide and layered double hydroxides

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Experimental section:

Reagents and materials: Flavin mononucleotide (FMN) and poly (styrene sulfonic acid) (PSS, M_w=70,000) was purchased from J&K Chemical Co. Ltd. $Mg(NO_3)_2 \cdot 6H_2O$, $Al(NO_3)_3 \cdot 9H_2O$, urea, $NaNO_3$, HNO_3, melamine and formamide were purchased from Aladdin Chemical Co. Ltd. Ethyl alcohol and HCl were purchased from Sinopharm Chemical Reagent Co. Ltd. All other chemicals were analytical grade and without further purification.

Synthesis of MgAl-LDH nanasheets: $Mg(NO_3)_2 \cdot 6H_2O$ (0.002 mol), $Al(NO_3)_3 \cdot 9H_2O$ (0.001 mol) and urea (0.012 mol) were dissolved in aqueous solution (70 mL). The mixture was sealed in a Teflon-lined stainless steel autoclave and heated at 100 °C for 24 h. The obtained MgAl-CO_3-LDH was washed with water and dried at 60 °C in the air. Conversion of MgAl-CO_3-LDH to MgAl-NO_3-LDH was achieved by treating a sample of following method: MgAl-CO_3-LDH (0.3 g) with 300 mL of an aqueous solution containing $NaNO_3$ (0.50 mol) and HNO_3 (0.0015 mol) whilsts purged with nitrogen and shaken at ambient temperature (25 °C) for 1 day. The obtained MgAl-NO_3-LDH was washed with hot distilled water and then dried under vacuum at 60 °C. Subsequently, 0.1 g of MgAl-NO_3-LDH was shaken in a 100 mL formamide solution for 24 hours to produce a colloidal suspension of exfoliated MgAl-LDH nanosheets.

Preparation of (FMN-PSS/LDH)$_n$ UTFs: The quartz glass substrate was cleaned in concentrated NH_3/30% H_2O_2 (7:3) and concentrated H_2SO_4 for 30 min each, and then was washed thoroughly with deionized water. FMN was modified with PSS to create more negatively charged atmosphere. Then the ultrathin films consisting of LDH nanosheets and FMN-PSS adducts were prepared on the quartz glass substrate

through the LBL technique: the substrate was alternately immersed in a colloidal suspension (0.1 g/mL) of LDH nanosheets and the FMN-PSS solution (0.1 mM) for 10 min. Subsequently, multilayer films of $(FMN\text{-}PSS/LDH)_n$ were fabricated for n cycles (n = 4-20).

Characterization techniques: The UV-vis absorption spectra were recorded on Shimadzu U-3000 spectrophotometer in the range from 200 to 800 nm. The fluorescence spectra was monitored on Hitachi F-7000 fluorescence spectrophotometer with excitation wavelength of 468 nm and the slits width were set to 3 nm. X-ray diffraction (XRD) patterns of the as-prepared materials were obtained on a Rigaku 2500VB2+PC diffractometer. The morphology of the UTFs was investigated by a scanning electron microscope (SEM Hitachi S-3500). Surface roughness was investigated by using atomic force microscope (AFM) software (Digital Instruments, Version 6.12). Fluorescence measurements of the UTFs and powder were recorded with a CRAIC UV-Visible-NIR Microspectrophotometer at different temperature (in the range from -150 °C to 210 °C, 30 °C a step). Fluorescence images of UTFs were obtained by using a confocal fluorescence microscope (Leica TCS-SP5). The decay curves were obtained on an Edinburgh Instruments FLS980 fluorimeter, and the excitation light source was 440 nm laser with slit width of 5 nm, and emission was 530 nm with slit width of 5 nm. The background test condition was the same as the UTFs measurements and the weighted average fluorescence lifetime was calculated by using multiple-exponential fitting with Edinburgh Instruments F980 software.

Detection of melamine in the liquid milk sample: The milk sample was pretreated according to the general procedure with some modification. Typically, 5 mL of the samples spiked was mixed with 15 mL of 1% trichloroacetic acid and 3 mL of acetonitrile, and sonicated for 10 min. The mixture was then centrifuged at 10000 rpm for 5 min to remove the impurities. Next, the filtrate was condensed to give a total volume of 5 mL and filtered through a 0.45 μm filter membrane to obtain the samples for detection. Because the milk is free of melamine, the sample was spiked with certain amounts of melamine standard solution directly. Finally, the fluorescence sensor of $(FMN\text{-}PSS/LDH)_{20}$ UTF was immersed in a quartz cell containing the pretreated sample (3 mL) to analysis the fluorescence response, which was recorded by using a Hitachi F-7000 fluorescence spectrophotometer under the optimal conditions. Meanwhile, the same sample was analysis by HPLC technique as control.

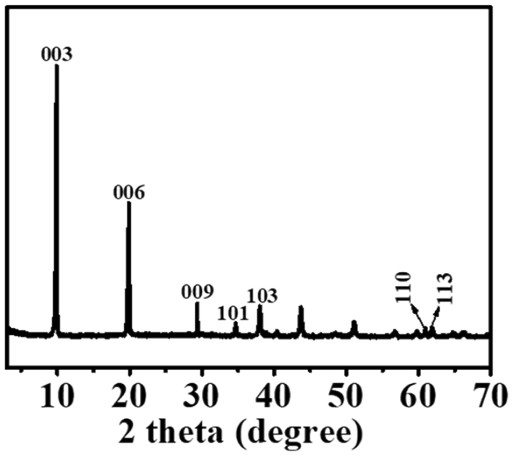

Fig. S1 XRD pattern of MgAl-LDH.

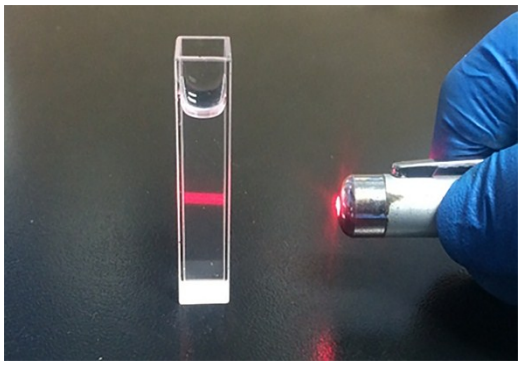

Fig. S2 Digital picture of MgAl-LDH colloidal suspension.

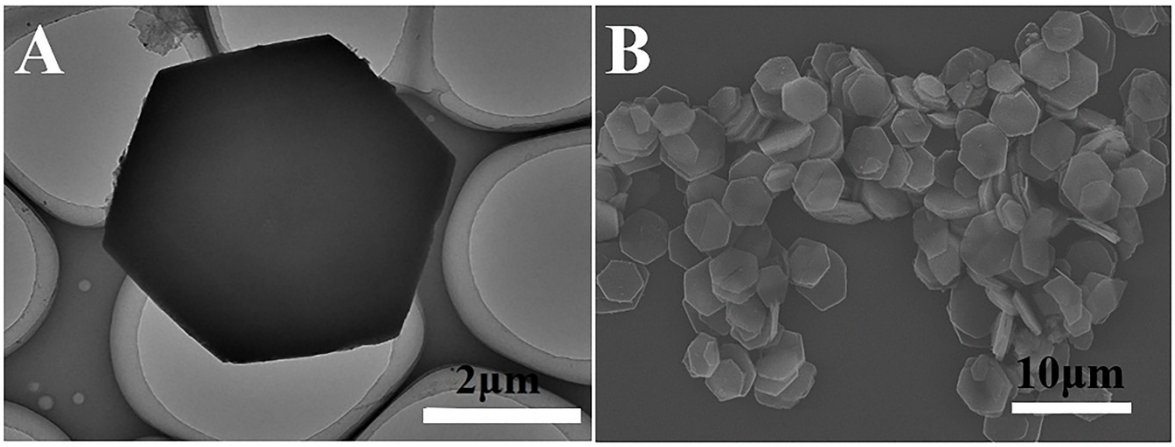

Fig. S3 TEM pattern (A) and SEM pattern (B) of MgAl-LDH.

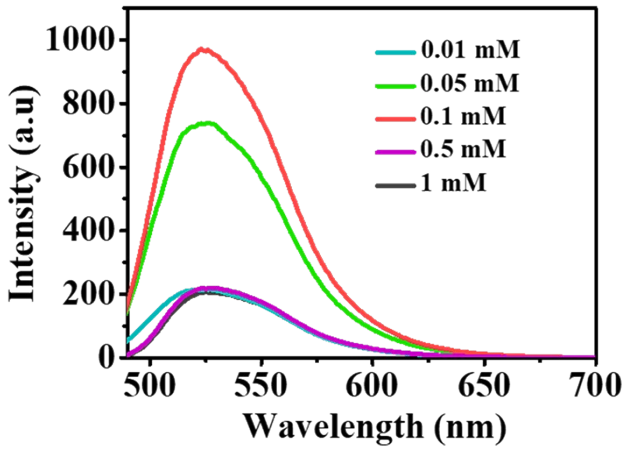

Fig. S4 Fluorescence spectra of FMN solution with the different concentration.

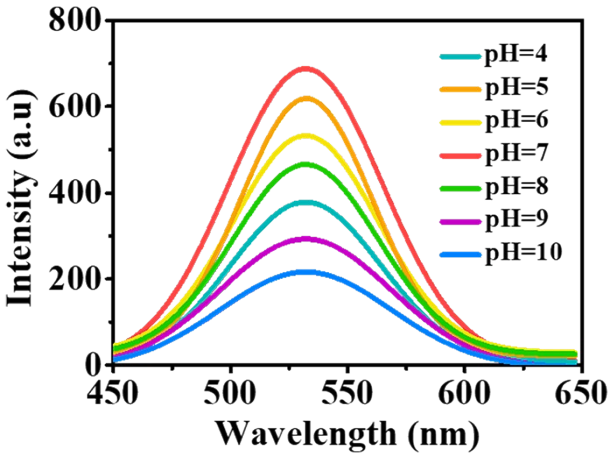

Fig. S5 Fluorescence spectra of FMN solution under different pH.

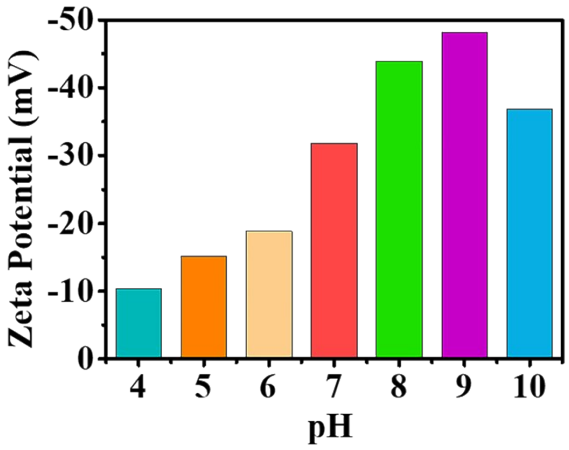

Fig. S6 Zeta potential of FMN solution with different pH

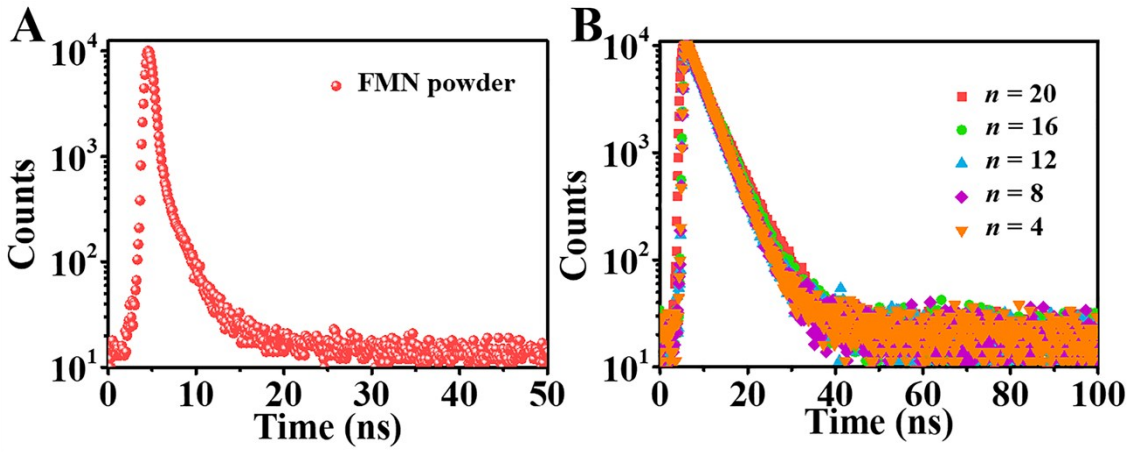

Fig. S7 Time-resolved fluorescence decays of (A) FMN powder and (B) $(FMN\text{-}PSS/LDH)_n$ UTFs (n = 4, 8, 12, 16 and 20).

Table S1 The fitting of fluorescence decay data for FMN powder and $(FMN\text{-}PSS/LDH)_n$ UTFs (n = 4, 8, 12, 16 and 20).

Samples	τ_i (ns)	A_i (%)	$<\tau_i>$ (ns)	χ^2
FMN powder	0.3628	87.61	0.58	1.410
	2.126	12.39		
$(FMN\text{-}PSS/LDH)_4$ UTF	3.016	27.16	4.19	1.356
	4.630	72.84		
$(FMN\text{-}PSS/LDH)_8$ UTF	3.626	21.03	4.46	1.302
	4.690	78.97		
$(FMN\text{-}PSS/LDH)_{12}$ UTF	2.246	19.40	4.37	1.271
	4.882	80.60		
$(FMN\text{-}PSS/LDH)_{16}$ UTF	2.905	23.17	4.57	1.129
	5.073	76.83		
$(FMN\text{-}PSS/LDH)_{20}$ UTF	3.415	24.13	4.63	1.127
	5.100	75.87		

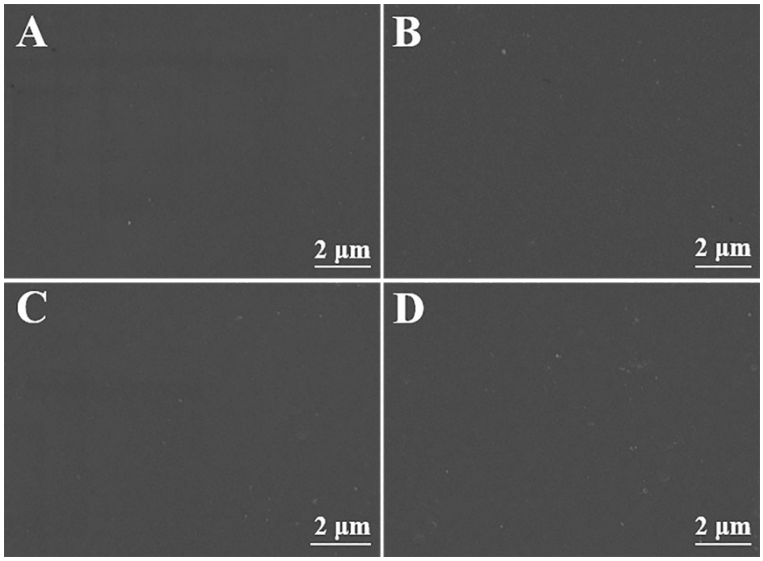

Fig. S8 Top-view SEM images of $(FMN\text{-}PSS/LDHs)_n$ UTFs**,** from (A) to (D): n = 4, 8, 12 and 16, respectively.

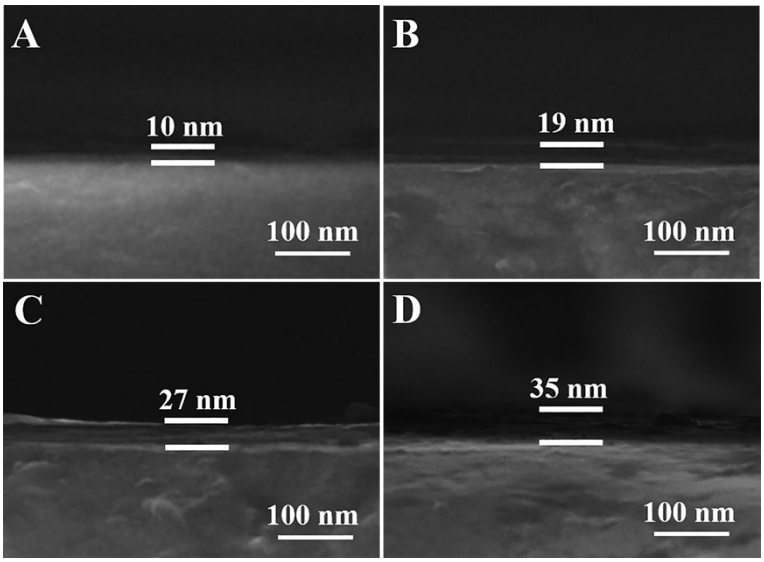

Fig. S9 Side-view SEM images of **(**FMN-PSS/LDHs)$_n$ UTFs, from (A) to (D): n = 4, 8, 12 and 16, respectively.

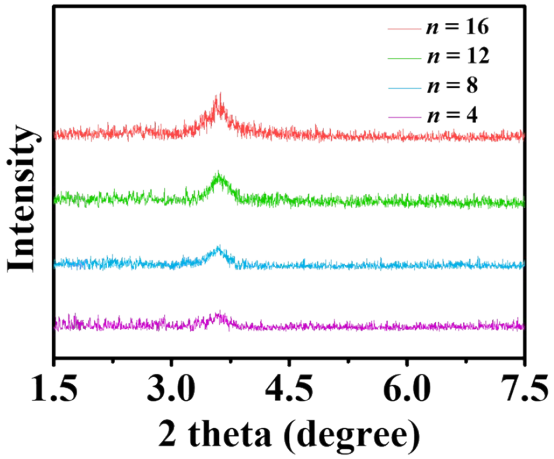

Fig. S10 XRD of (FMN-PSS/LDHs)$_n$ UTFs, from bottom to top: n = 4, 8, 12 and 16, respectively.

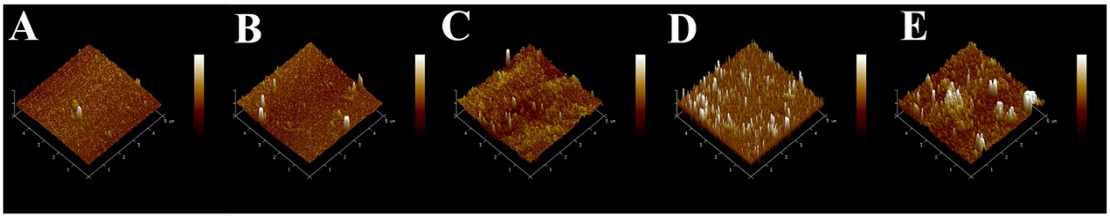

Fig. S11 Tapping-mode AFM image of (FMN-PSS/LDH)$_n$ UTFs (n = 4, 8, 12, 16 and 20).

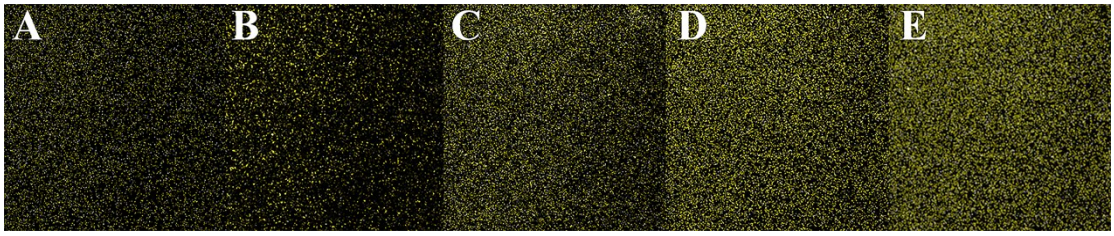

Fig. S12 Confocal fluorescent microscopy images of $(FMN\text{-}PSS/LDH)_n$ UTFs (n = 4, 8, 12, 16 and 20).

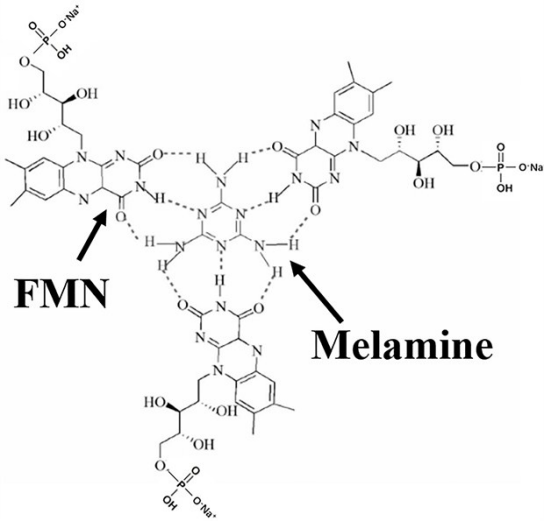

Fig. S13 Schematic model of the hydrogen bonding between FMN and melamine.

Table S2 Comparisons of the linear range and detection limit of this work with the other methods for the determination of melamine.

Method	Modified	Linear range（M）	LOD (M)	*Ref*
GC-MS		$8.0\times10^{-9}-7.9\times10^{-3}$	0.8×10^{-8}	1
HPLC		$1.6\times10^{-7}-7.9\times10^{-5}$	4.8×10^{-8}	2
SERS		$5.0\times10^{-6}-5.0\times10^{-5}$	1.2×10^{-5}	3
Electrochemical	DNA-Based Sensors	−	2.0×10^{-5}	4
Electrochemical	MOFs@XC-72-Nafion	$3.2\times10^{-7}-7.9\times10^{-5}$	4.0×10^{-8}	5
Electrochemical	$d(T)_{20}$/Au	$3.9\times10^{-8}-3.3\times10^{-6}$	9.6×10^{-9}	6
Fluorescence	Ag NCs	$3.6\times10^{-7}-7.4\times10^{-6}$	1.6×10^{-7}	7
Fluorescence	DNA-AgNC−Rh6G	$1.0\times10^{-7}-1.0\times10^{-5}$	2.5×10^{-8}.	8
Fluorescence	UCNPs-AuNPs FRET	$3.2\times10^{-8}-5.0\times10^{-7}$	1.8×10^{-8}	9
Fluorescence	Ag NCs–Hg^{2+}	$0.1\times10^{-3}-3.0\times10^{-2}$	3.0×10^{-8}	[19]
Fluorescence	CTA-AgNCs/LDH	$3.0\times10^{-5}-1.0\times10^{-4}$	4.0×10^{-9}	[20]
Visible and fluorescent	C-dots and AgNPs	$0-1.4\times10^{-5}$	3.0×10^{-8}	[21]
Fluorescence	P1-AgNPs	$1.0\times10^{-9}-1.5\times10^{-6}$	1×10^{-10}	[22]
Fluorescence	$(FMN\text{-}PSS/LDH)_{20}$ UTF	$0-1.6\times10^{-7}$	7.0×10^{-9}	This work

References

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Table S3 Real application of the proposed probe and HPLC for melamine in milk samples

Sample	Added (nM)	Found (nM)		Recovery (%)±SD, n=3	
		This work (nM)	HPLC (nM)	This work	HPLC
milk 1	0.5×10^2	$(0.513 \pm 0.014)\times10^2$	$(0.489 \pm 0.018)\times10^2$	102.6 ± 2.8	97.8 ± 3.6
milk 2	1.0×10^2	$(0.971 \pm 0.033)\times10^2$	$(1.025 \pm 0.029)\times10^2$	97.1 ± 3.3	102.5 ± 2.9
milk 3	1.5×10^2	$(1.536 \pm 0.034)\times10^2$	$(1.542 \pm 0.038)\times10^2$	102.4 ± 2.3	102.8 ± 2.5

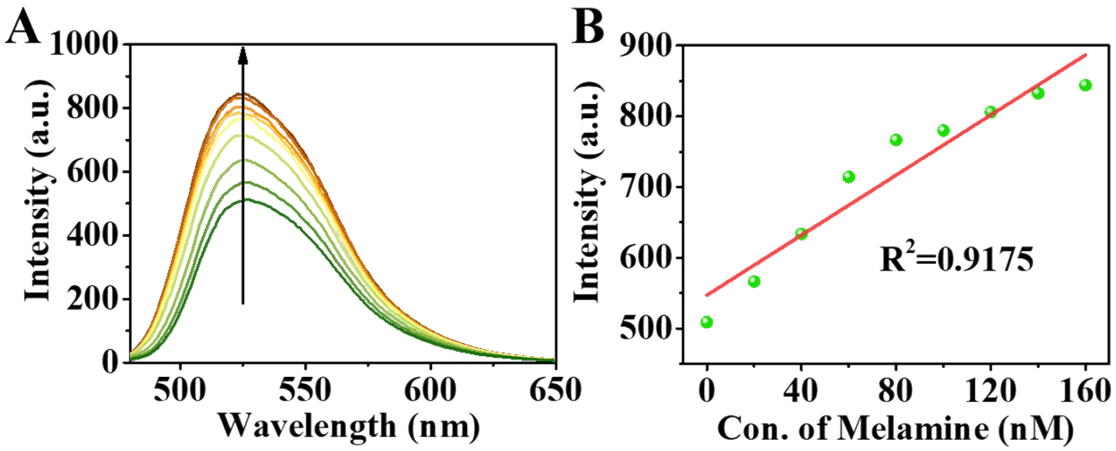

Fig. S14 (A) Fluorescence spectra of FMN solution (0.1 mmol/L) with concentrations of melamine (From bottom: 0, 20, 40, 60, 80, 100, 120, 140, 160 nM). (B) The fitting line of FMN solution with different concentrations of melamine.

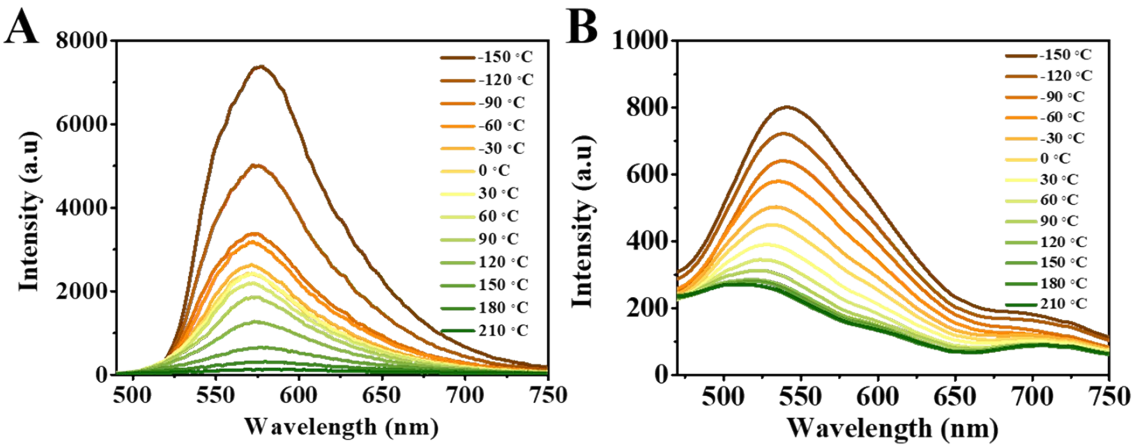

Fig. S15 Fluorescence spectra of FMN powder (A) and $(FMN\text{-}PSS/LDHs)_{20}$ UTF (B) with the different temperature from -150 °C to 210 °C (From Top: -150 °C, -120 °C, -90 °C, -60 °C, -30 °C, 0 °C, 30 °C, 60 °C, 90 °C, 120 °C, 150 °C, 180 °C and 210 °C)

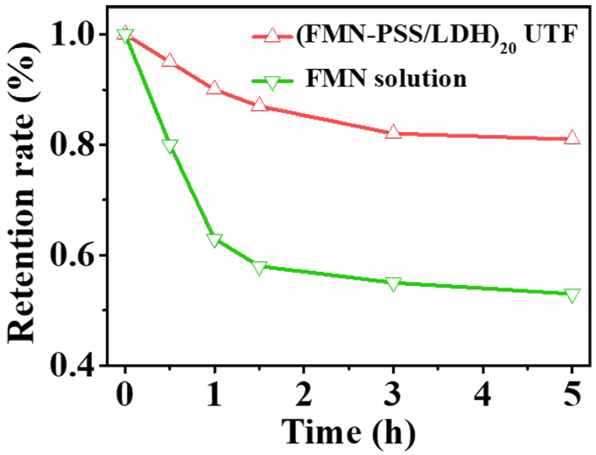

Fig. S16 Photostability of FMN solution and $(FMN\text{-}PSS/LDH)_{20}$ UTF.