Supporting Information

Clustering-triggered emission from natural products: Gelatin and its multifunctional application

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Experiment Section

Materials.

Pigskin was purchased from Wal-mart supermarket, Changchun City, Jilin Province. Tetrahydrofuran (THF) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, > 99%) was purchased from Tianjin Guangfu Chemical Reagents Company (Tianjin, China). DMSO, Acetic acid were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) was obtained from Gibco (Grand Island, USA). Fetal bovine serum (FBS) was obtained from Gibco (Grand Island, USA). $AgNO_3$, KI, $NaNO_3$, $Cr(NO_3)_3$, $CuNO_3$, $Mg(NO_3)_2$, KNO_3, $Ca(NO_3)_3$, Na_2S, $Fe(NO_3)_3$ were all obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Amersco (Solon, USA). ^1H NMR spectra was recorded deuterium oxide (D_2O, 99.9% D, abcr GmbH, Germany) was used, the water used in all experiments was distilled water.

Characterizations.

^1H NMR spectra of gelatin dissolved in D_2O was measured by using a Bruker Avance 400 MHz spectrometer (Bruker Corp., Karlsruhe, Germany). The structure of gelatin was characterized by Fourier transform infrared (IRAffinity-1, SHIMADZU). Fluorescence spectra, fluorescent and phosphorescent lifetime, quantum yields of the solutions and solids were all recorded on Steady-state Transient Fluorescence

Spectrometer using an FLS920 fluorescence spectrophotometer (Edinburgh Instrument Ltd., Livingston, UK). The average particle size and size distribution of the gelatin were characterized on a Brookhaven Zeta Plus potential analyzer (Brookhaven Instruments Corporation, USA) at 25 °C. The pH value of gelatin solution was measured via a pH meter (INESA PHS-3C). Cell viability was analysed by GF-M3000 microplate reader (Shandong, China) cell imaging was investigated by confocal laser scanning microscope (LSM 710, Carl Zeiss Microscopy LLC, Jena, Germany).

Purification and Preparation of gelatin

Pigskins are used in 0%, 0.5%, 1% and 2% (v/v) hydrochloric acid solutions at 30°C and immersed in a constant temperature water bath magnetic stirrer for 20 minutes. Take the traditional acid method as a control. The prepared pig skin is swelled with 1% (v/v) hydrochloric acid with a skin-to-liquid ratio of 1:10 (w/v) under constant stirring at room temperature for 8 hours. Then, the skin is pretreated by freezing and thawing and the traditional acid-assisted method is thoroughly washed with distilled water until the washing water is close to neutral. Part of the sample is freeze-dried to make collagen, which is placed in a drying container together with the desiccant and stored until use. The remaining samples were mixed in distilled water at 60°C for 4 hours at a skin/water ratio of 1:3 (w/v) in a constant temperature water bath shaker to extract the gelatin. The mixture was then filtered and dried under vacuum (0.07 MPa) at 60°C to prepare gelatin.[1,2] First, the gelatin powder is dissolved in water at room temperature, and then THF (water/THF, 1/10, v/v) is added as a

non-solvent to precipitate it. The precipitate was collected after centrifuging. After centrifuging, the collected powder was dried in a freeze dryer for 24 hours, and then refrigerated for later use. Next, Preparing gelatin solutions of different concentrations and different pH to measure fluorescence property.

Cellular cytotoxicity study

The cells of A549, HepG2 and LO2 were seeded in 96-well plates with a density of 8000 cells/100 μL DMEM containing 10% full guard then incubated at 37°C for 12 h. When after removing the medium, these cells were washed with PBS and treated with SF at different concentrations from 20 to 100 μg/mL. The cells were cultured for another 24 hours and treated with 15 mL MTT solution (5 mg/mL) for 4 hours. Then the culture medium was taken out, and dissolved the blue-purple formazin crystals in 200 mL DMSO, Shaking for 2 minutes, and measured the absorbance at 492 nm with a GF-M 3000 microplate reader (Shandong, China). The cell survival rate (%) is determined by the ratio of absorbance between the treated group and the untreated group.

Cellular uptake and imaging

The cells of A549, HepG2, and LO2 were respectively plated onto 6-well culture plates (8,000–10,000 cells per well), incubated with sterilized coverslips at 37°C for 12 hours, and then incubated with gelatin at a concentration of 20 μg/mL for 4 hours. Washing the cells three times with phosphate buffer. Last, placing the coverslip on the glass microscope slide and observing the sample with a laser confocal microscope.

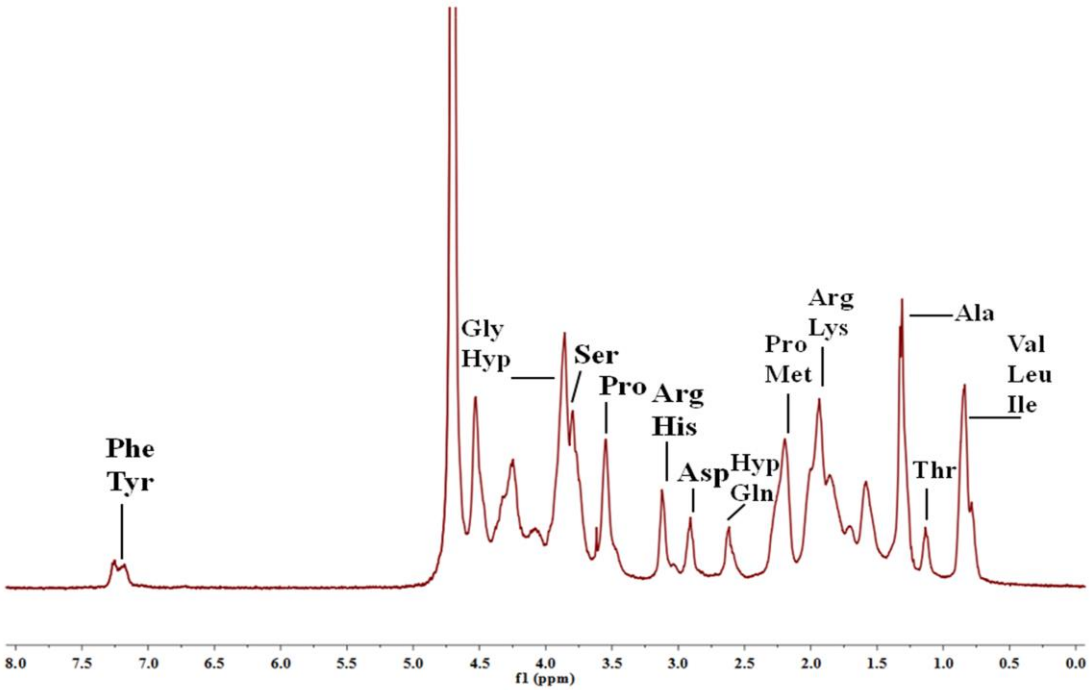

Figure S1. ^{1}H NMR of gelatin.

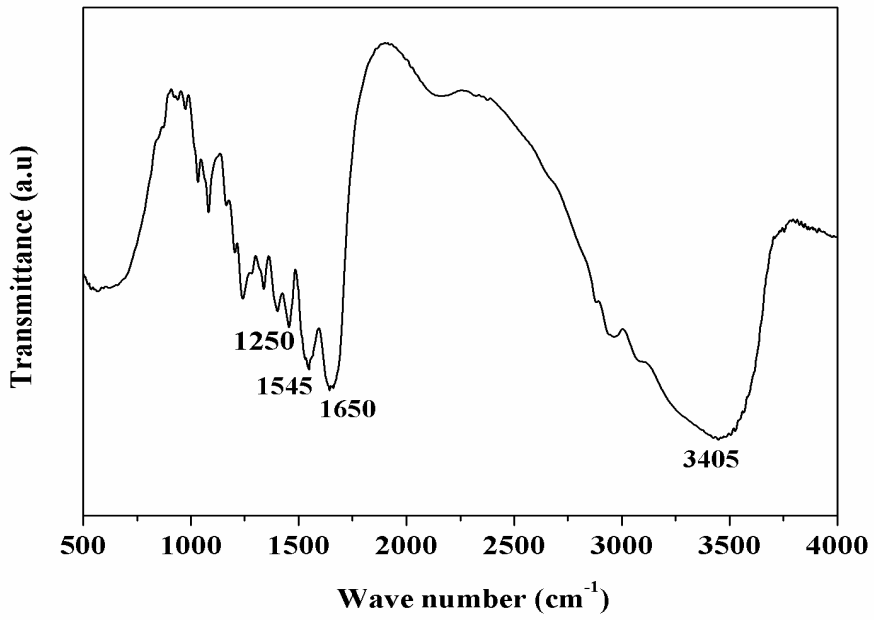

Figure S2. FT-IR spectra of gelatin.

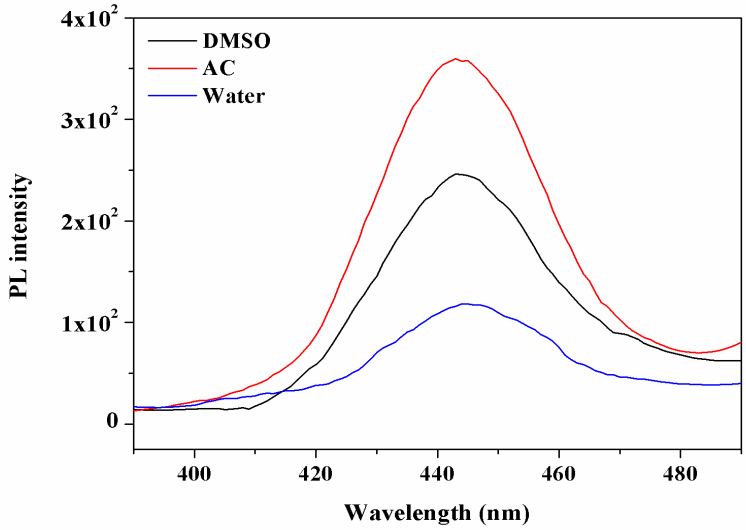

Figure S3. PL spectra of gelatin in Water, DMSO, and Acetic acid. Excitation wavelength: 365 nm. Slit width: 5 nm.

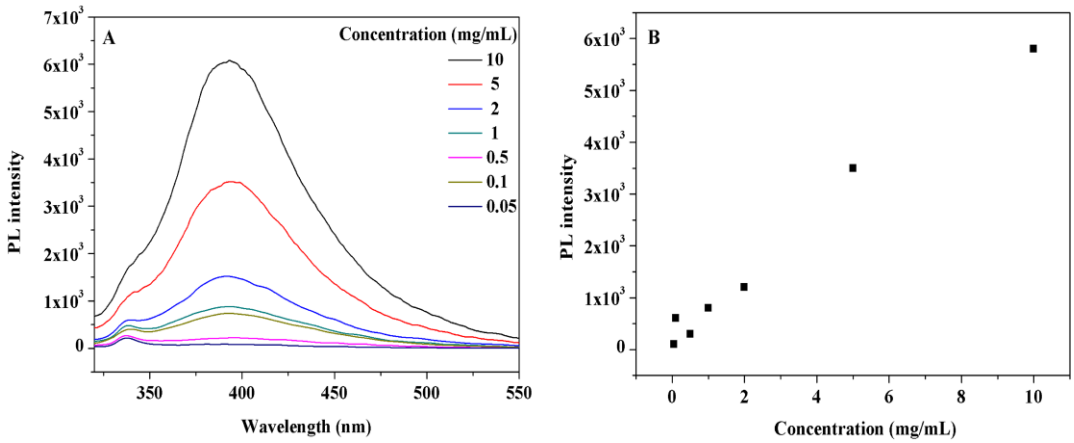

Figure S4. (A) Emission spectra of gelatin (λ_{ex} = 300 nm), and (B) Increase of the peak intensity of gelatin aqueous solutions at different concentrations.

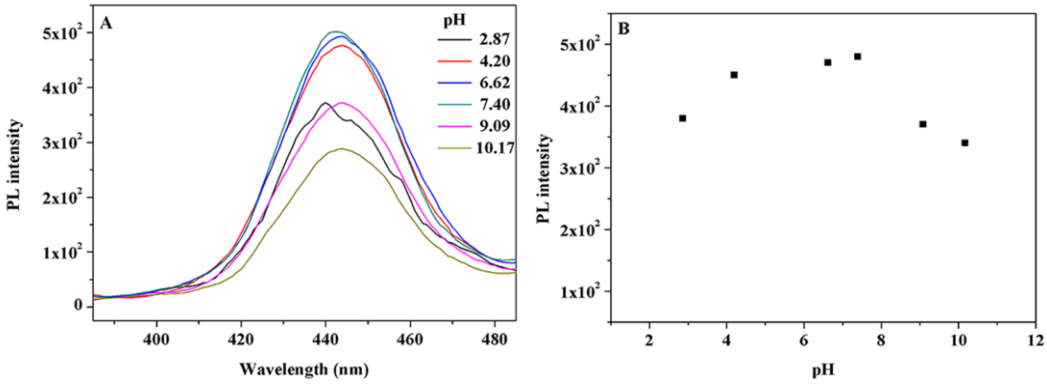

Figure S5. (A) Emission spectra (λ_{ex} = 365 nm) and (B) peak intensities of gelatin aqueous solutions (1 mg/mL) at varying pH values.

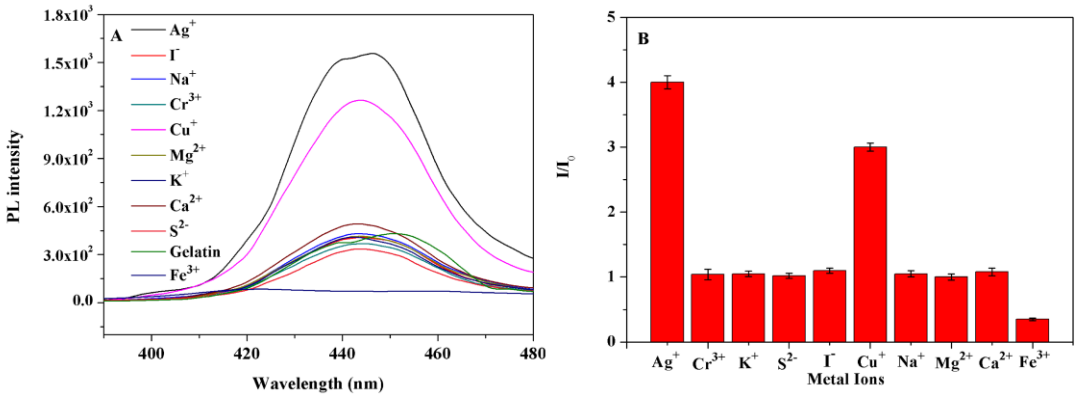

Figure S6. (A) Fluorescence emission spectra of gelatin of different metal ions ($AgNO_3$, KI, $NaNO_3$, $Cr(NO_3)_3$, $CuNO_3$, $Mg(NO_3)_2$, KNO_3, $Ca(NO_3)_2$, Na_2S, $Fe(NO_3)_3$). (B) The change in fluorescence intensity with (I) metal ions concentration and (I_0) pure gelatin. The λ_{ex}= 365 nm.

References

(1) Sompie, M., Surtijono, S.E.; Pontoh, J.H.W., Lontaan N.N. The Effects of Acetic Acid Concentration and Extraction Temperature on Physical and Chemical Properties of Pigskin Gelatin. *Procedia Food Science*., **2015**,3, 383-388.

(2) Chen, X. K., Ma, L., Guo, T., Yu, Y., Li, X. Y., Xia, W. Y., Zhang, Y. H. Effects of Freezing-Thawing Pretreatment Combined with Liquid Nitrogen and Dilute Acid on the Gelatinization of Collagen. *Int J Biol Macromol.*, **2018**, 118, 435-441.