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Supporting Information

In-Situ Detection of Intracellular Tissue Transglutaminase Based on Aggregation-Induced Emission

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Experimental Section

Materials and Reagents. TPE-N_3 was purchased from AIEgen Biotech Co., Limited (Shenzhen, China). PepK: GRKKKR, pepQ: GRQQRR and dialysis membrane were received from Sangon Biotech Co., Ltd (Shanghai, China). Cell culture products were purchased from Nanjing KeyGen Biotech. Inc (Nanjing, China). Tissue transglutaminase (TG2) was purchased from Sigma-Aldrich (Shanghai, China). $CuSO_4$•$5H_2O$, ascorbic acid, DMSO, tri(2-carboxyethyl) phosphine (TCEP), $CaCl_2$, HCl, ammonium sulfate, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) and tris(hydroxymethyl) aminomethane (Tris) were purchased from China National Pharmaceutical Group Corporation (Shanghai, China).

Apparatus. UV-vis spectra were performed on UV-visible spectrometer (Shimadzu UV-2450, Kyoto, Japan). Fluorescence spectra were collected on a fluorescence spectrometer (Fluoromax-4, Horiba Jobin Yvon, Japan). Absorbance of MTT assays was recorded by a microplate reader (MultiskanTM FC, Thermo Scientific). Confocal fluorescence imaging was performed with Olympus FV3000 laser scanning confocal microscopy (Japan). Mass spectra were measured on a HP 1100 LC-MS spectrometer (Brooke Ultraflextreme, USA). High performance liquid chromatography (HPLC) was detected with a HPLC instrument (Shimadzu LC-10A, Japan).

The synthesis of probe TPE-pepK/Q. The TPE-pepK/Q were synthesized by click reaction according to previous methods.[S1,2] 10 μmol TPE-N_3 and 11.94 μmol pepK or pepQ were firstly mixed in DMSO/H_2O (v/v=1:1) solution. To induce the click chemical reaction, 4.78 μmol sodium ascorbate and 9.55 μmol $CuSO_4$ in water were

slowly added into the above solution. The reaction mixture was stirred at room temperature for about 2 days. The final products were purified by dialysis. The TPE-pepK/Q molecules were characterized by liquid chromatography mass spectrometry-ion trap-time-of-flight high resolution-mass spectrometry (LCMS-IT-TOF) and high-performance liquid chromatography (HPLC).

Cell culture. HepG2, A2780/ADR and NIH3T3 cells were obtained from Shanghai Institute of Biochemistry and Cell Biology of Chinese Academy of Science (Shanghai, China). HepG2 cells were seeded in DEME/high glucose medium, A2780/ADR and NIH3T3 cells were seeded in RPMI-1640/high glucose medium containing 10% fetal bovine serum (FBS), 100 μg mL^{-1} streptomycin and 100 U mL^{-1} penicillin at 37 °C in a humidified incubator containing 5% CO_2 and 95% air. The medium was replenished every other day and the cells were subcultured after reaching confluence.

Evaluation of cytotoxicity of the nanoprobe. The cytotoxicity of the nanoprobe was tested with HepG2 cells by MTT assay. After the cells were incubated with 100 μL culture medium containing 25 μL TPE-pepK and 25 μL TPE-pepQ for different times, MTT (50 μL, 1 mg mL^{-1}) was added to the well and incubated at 37 °C for 4 h. Then 100 μL of DMSO was added to each well to dissolve the produced formazan. After shaking, the optical density (OD) at a wavelength of 570 nm was measured with microplate reader (Multiskan GO, Thermo Fisher Scientific, China).

The formation of aggregation-induced emission (AIE) nanoprobe. 10 μM TPE-pepK and 10 μM TPE-pepQ were mixed at the molar ratio of 1:1 in the Tris-HCl buffer solution (pH 7.0) containing 1 mM $CaCl_2$, 0.1 mM Tris(2-

carboxyethyl)phosphine (TCEP) and 150 mM NaCl. Afterward, a certain concentration of TG2 solution was added into the above solution, the reaction mixture was incubated for 60 min at 37 °C. Under excitation at 315 nm, the fluorescence of the nanoprobe was collected from 375-600 nm.

In Situ imaging of TG2 with the nanoprobe. 0.5 mL HepG2 cells (or A2780/ADR and NIH3T3 cells) of 1×10^{6} mL^{-1} were seeded in each confocal dish for 24 h, 25 μL TPE-pepK and TPE-pepQ were then added into each cell-adhered dish. After incubation at 37 ^{o}C for different times, the cell imaging was observed by confocal laser scanning microscopy. Grayscale value of fluorescence intensity was read with ImageJ software.

Real-time monitoring of intracellular TG2 activity. The monitoring of intracellular TG2 activity was obtained by treating HepG2 cells with different concentrations of TG2 inhibitor cysteamine for 24 h, then detecting the TG2-triggered fluorescence intensity with the nanoprobe through confocal imaging and reading the grayscale value with ImageJ software.

Scheme S1. The mechanism of pepK and pepQ crosslinking by active TG2.

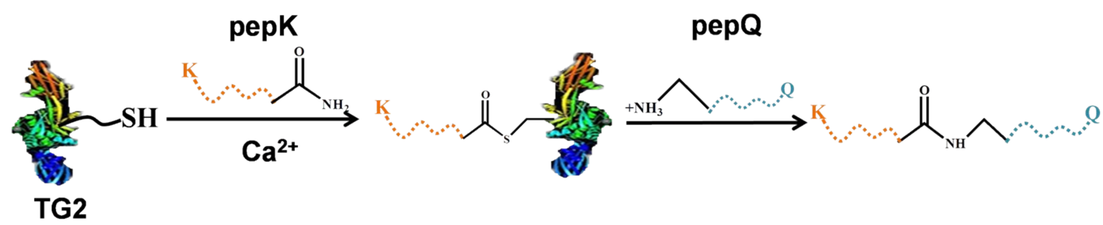
Figure S1. LCMS-IT-TOF characterization of (A) TPE-pepK, m/z: $[M+H]^+$, calcd for 1197.5, found 1197.5. (B) TPE-pepQ, m/z: $[M+H]^+$, calcd for 1224.6, found 1224.6.

Figure S2. HPLC characterization of (A) TPE-pepK, peak time 5.8 min (B) TPE-pepQ, peak time 5.6 min.

Figure S3. (A) The FL intensity of TPE-N3 in DMSO/H_2O mixture with different water ratios. (B) UV-vis absorbance and FL intensity of 10 μM TPE-N3 (red line), TPE-pepK (green line) and TPE-pepQ (blue line) in DMSO/H_2O (v/v=1/99). λex=315 nm. The inset image showed the corresponding photographs of TPE-N3 (a), TPE-pepK (b) and TPE-pepQ (c) taken under UV lamp illumination (λex=365 nm).

Figure S4. FL intensity at 459 nm vs the pH value of Tris-HCl buffer (A), the concentration of Ca^{2+} (B), the incubation time of TPE-pepK, TPE-pepQ and TG2 (C).

Table S1. Comparison of the performance for TG2 detection by using the proposed approach with other reports.



Scheme S1. The mechanism of pepK and pepQ crosslinking by active TG2.

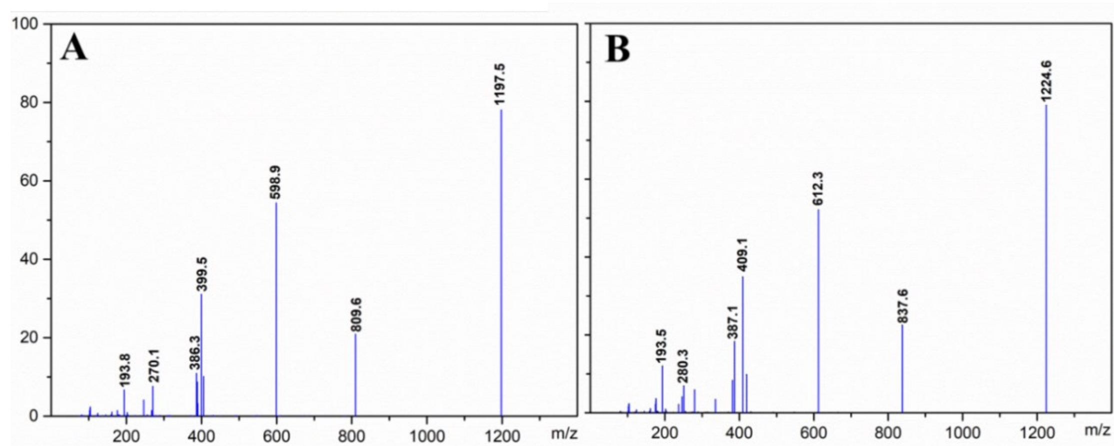


Figure S1. LCMS-IT-TOF characterization of (A) TPE-pepK, m/z: $[M+H]^+$, calcd for 1197.5, found 1197.5. (B) TPE-pepQ, m/z: $[M+H]^+$, calcd for 1224.6, found 1224.6.

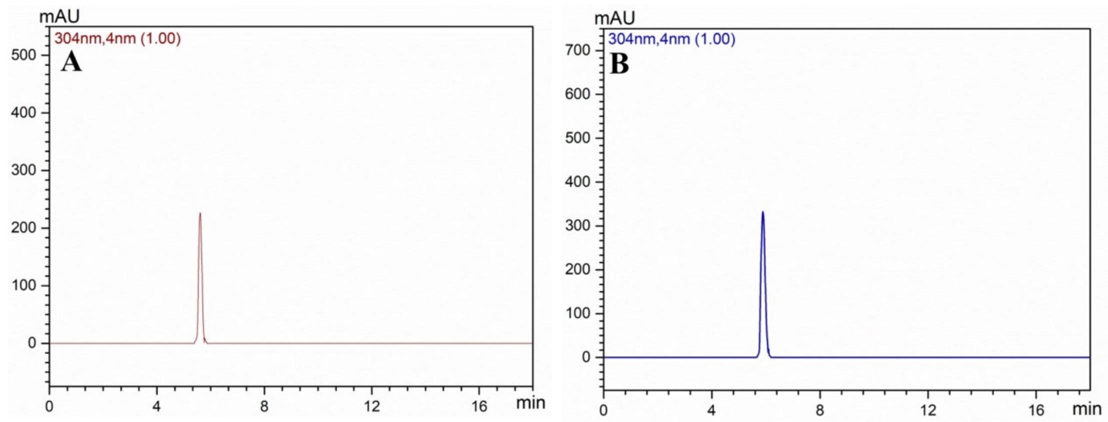


Figure S2. HPLC characterization of (A) TPE-pepK, peak time was 5.8 min (B) TPE-pepQ, peak time was 5.6 min.

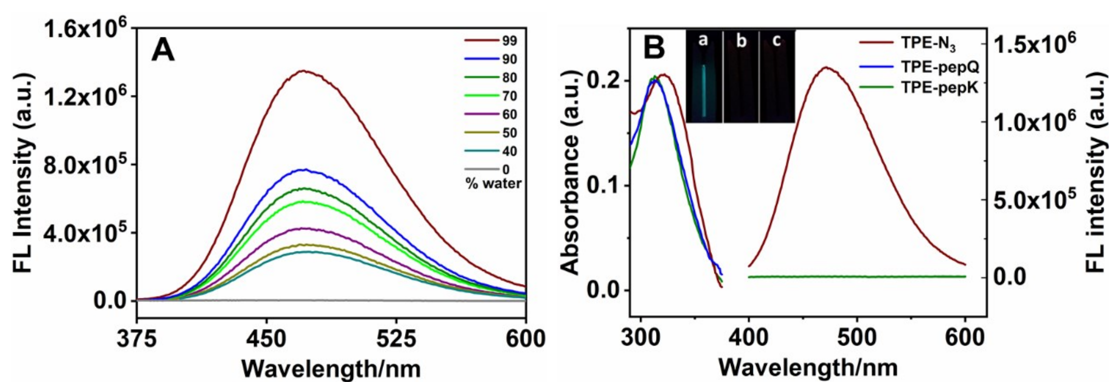


Figure S3. (A) The FL intensity of TPE-N_3 in DMSO/H_2O mixture with different water ratios. (B) UV-vis absorbance and FL intensity of 10 μM TPE-N_3 (red line), TPE-pepK (green line) and TPE-pepQ (blue line) in DMSO/H_2O (v/v=1/99). λex=315 nm. The inset image showed the corresponding photographs of TPE-N_3 (a), TPE-pepK (b) and TPE-pepQ (c) taken under UV lamp illumination (λex=365 nm).

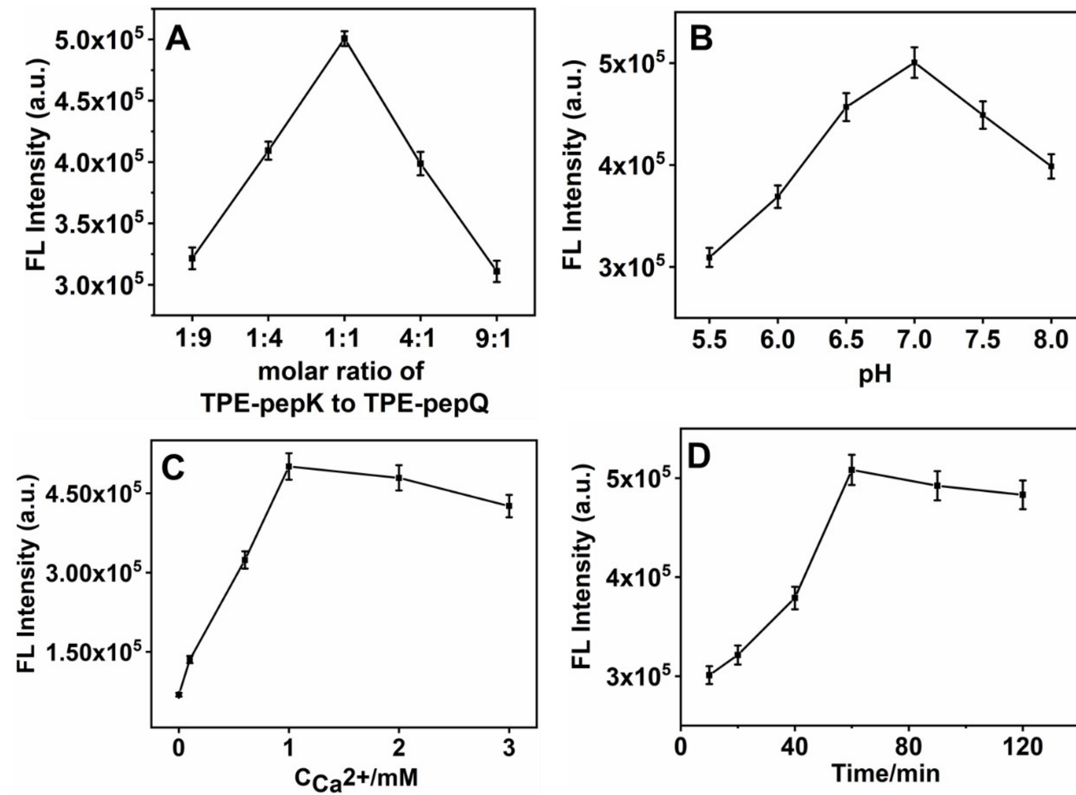


Figure S4. FL intensity at 459 nm vs the molar ratio of TPE-pepK to TPE-pepQ (A), the pH value of Tris-HCl buffer (B), the concentration of Ca^{2+} (C), the incubation time of TPE-pepK, TPE-pepQ and TG2 (D). Data were presented as means ± standard deviation (s.d.) (n=3).

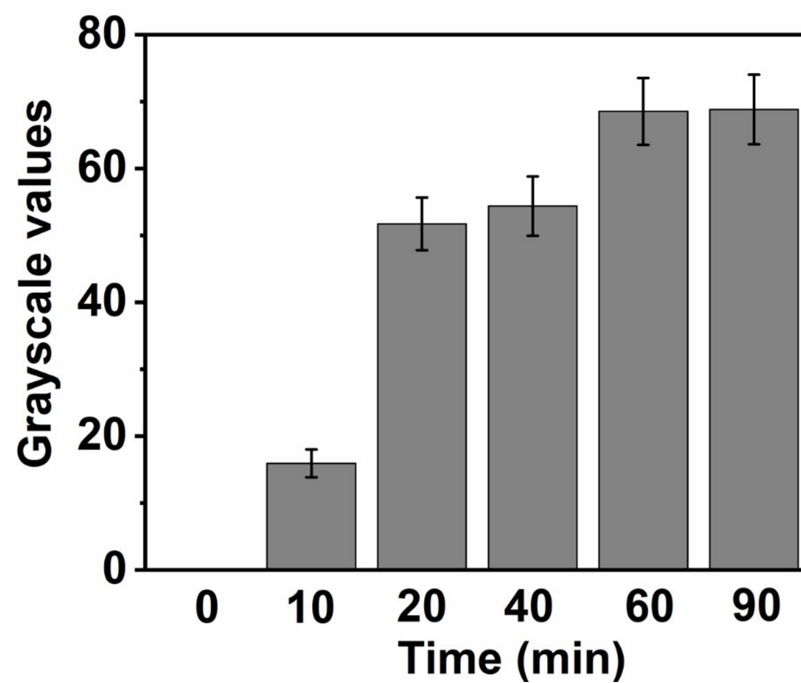


Figure S5. Grayscale values of fluorescence intensity obtained from Figure 3. Data were presented as means ± standard deviation (s.d.) (n=3).

Table S1. Comparison of the performance for TG2 detection by using the proposed approach with other reports.

method	linear range	LOD	Ref
Fluorescence	65.3–653 μM	37.8 μM	[S3]
ELISA	3.3-26.1 nM	1.31 μM	[S4]
ELISA	0.13-6.53 μM	0.02 μM	[S5]
Electrochemical	0.01-0.65 μM	4.30 nM	[S6]
Fluorescence	0.1–2.5 nM	0.02 nM	This work

References:

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