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Supporting Information

Acid-Assisted Self-Assembly of Pyrene-Capped Tyrosine Ruptures Lysosomes to Induce Cancer Cells Apoptosis

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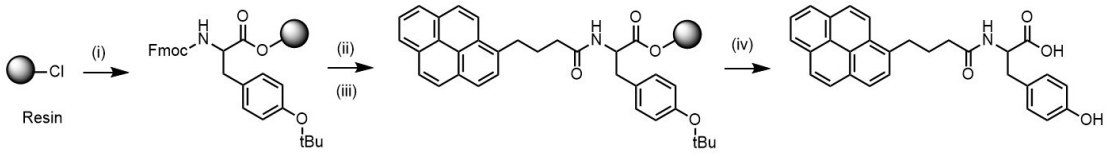
Content

Synthesis



Scheme S1. Solid-phase synthesis of Py-Tyr. Reagents and conditions: (i) Fmoc-Tyr(tBu)-OH, DIEA; (ii) 5%Piperazine, 2%DBU, DMF; (iii) 1-Pyrenebutyric acid, HBTU, DIEA, DMF; (iv) 95%TFA, 5%DCM.

Target compound **Py-Tyr** was synthesized by solid phase synthesis on 2-chlorotritylchloride resin (Scheme S1). Resin (0.5g, approximate 0.5 mmol) was swollen in 5mL of DCM for 20 min and washed with DMF for 3 times. Fmoc-Tyr(tBu)-OH (0.57 g, 1.25 mmol) dissolved in 5mL anhydrous DMF in the presence of DIEA (0.43 mL, 2.5 mmol) was linked to resin for 30min. The resin was then washed with anhydrous DMF 3 times and unreacted sites on the resin were blocked with DIEA: MeOH: DCM (5: 15: 80) solution for 20min. The Fmoc capping group was removed with a 5% piperazine and 2% DBU in DMF for 10min. 1-Pyrenebutyric acid (0.43g, 1.5mmol) dissolved in 5mL anhydrous DMF in the presence of DIEA (0.52 mL, 3.0 mmol) and HBTU (0.57 g, 1.5 mmol) was added and reacted for 1 h at room temperature. The compound was cleaved off the resin by 95% TFA in DCM for 2h. The crude product was purified on an EClassical P3140 HPLC (Elite, China) equipped with a SinoChrom C18 column (ODS-BP, 10μm, 20.0mm × 250mm) to obtain white powders. Yield 0.18g, 79.8%.

Supplementary Figures

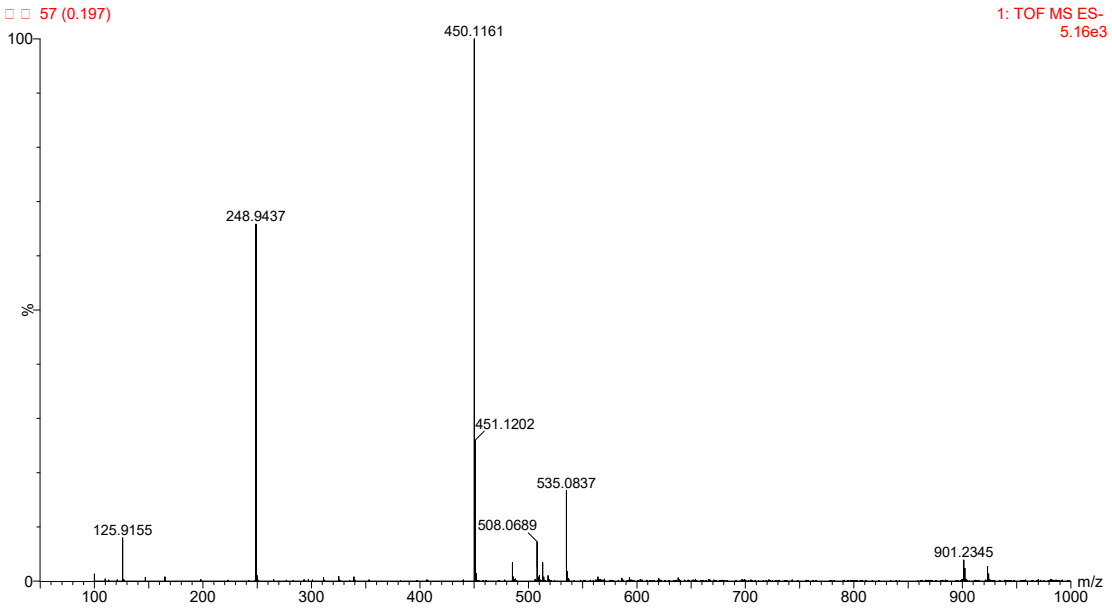


Figure S1. Mass spectrum of **Py-Tyr** in MeOH, negative mode.

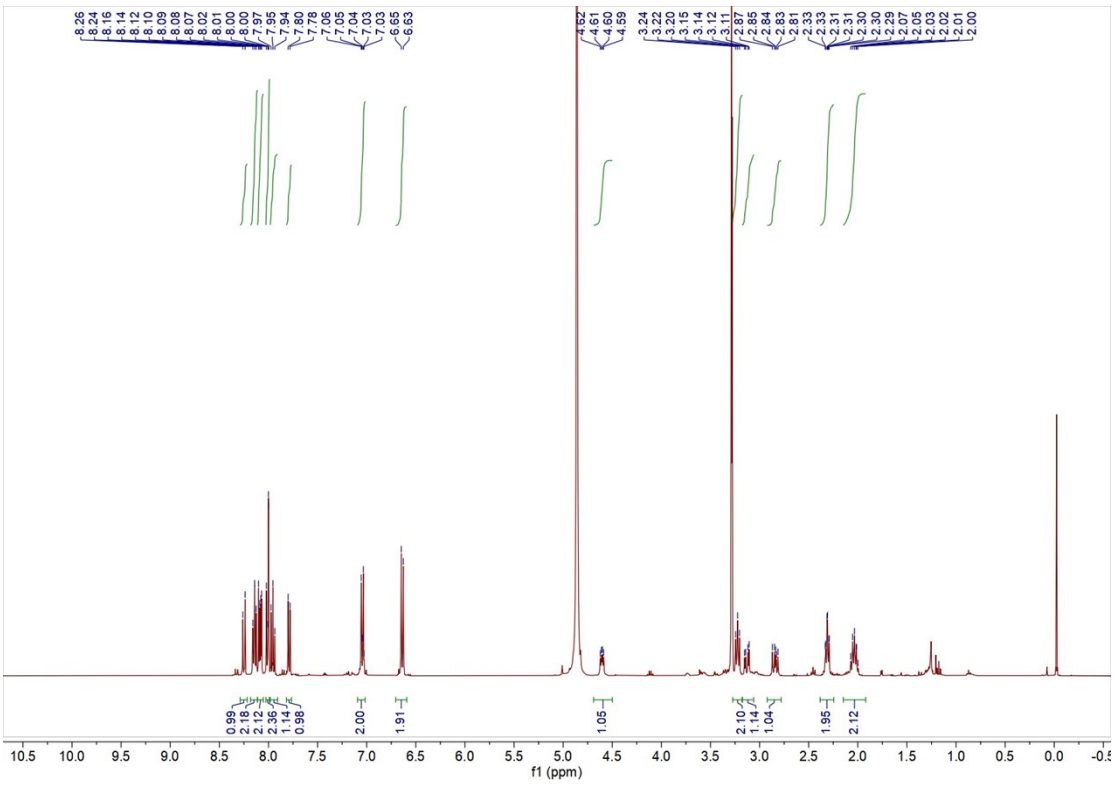


Figure S2. ^{1}H NMR spectrum of **Py-Tyr** in methanol-d4, 400MHz.

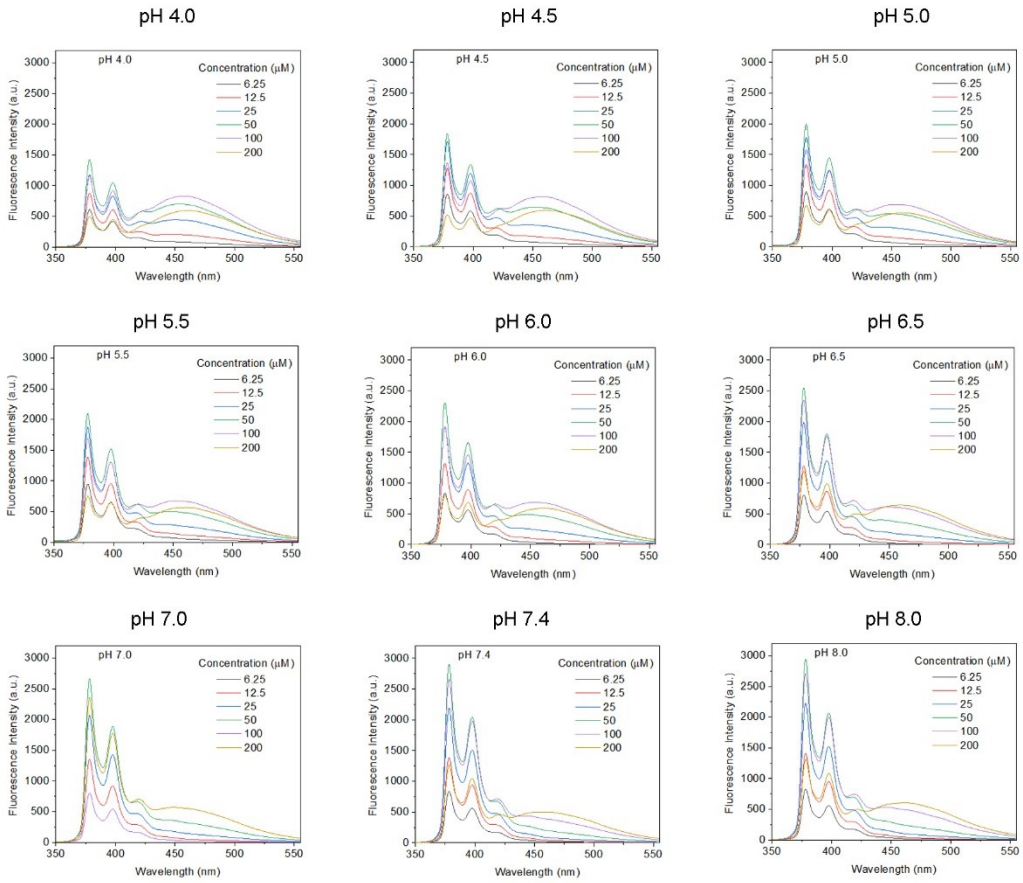


Figure S3. Emission spectra of **Py-Tyr** in citrate-phosphate buffer under different pH values. The concentration of **Py-Tyr** varied from 200, 100, 50, 25, 12.5 and 6.25 μM. λex = 342 nm.

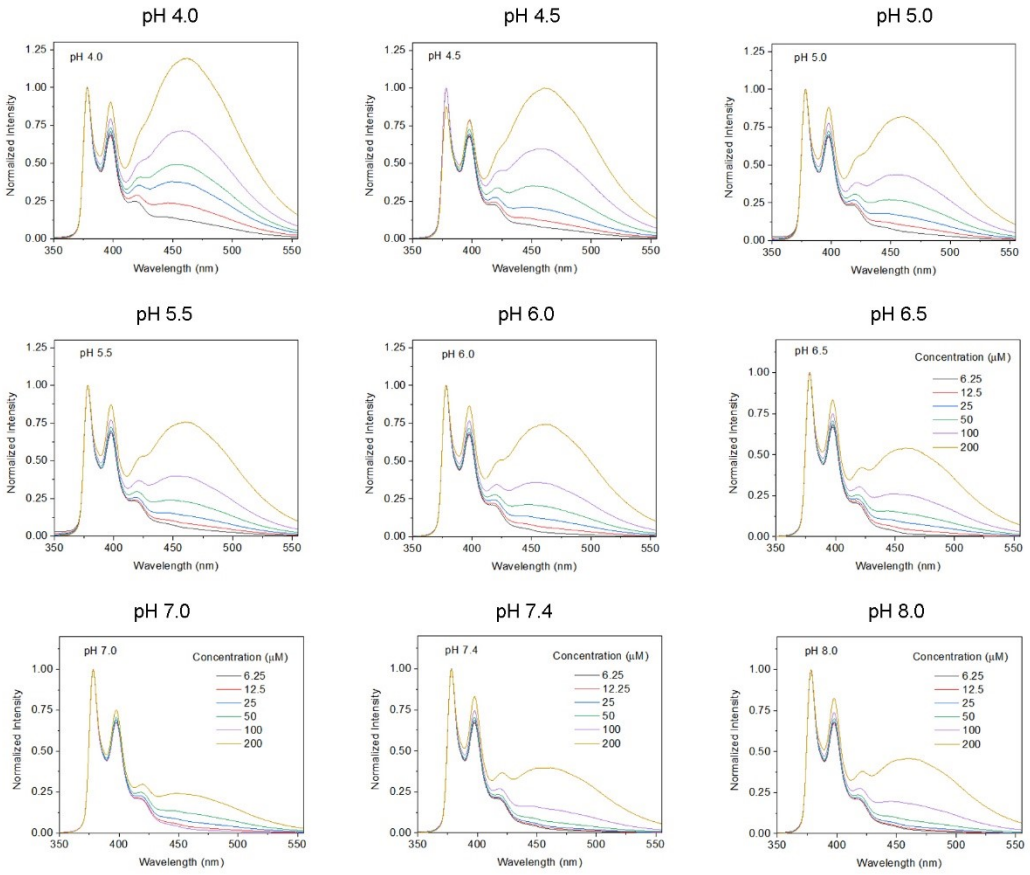


Figure S4. Normalized emission spectra of **Py-Tyr** in citrate-phosphate buffer under different pH values. The fluorescence intensity at 375nm was normalized to 1.0, respectively. The concentration of **Py-Tyr** varied from 200, 100, 50, 25, 12.5 and 6.25 μM. λex = 342 nm. Note that the spectra at pH 4.0 and pH 7.4 were reproduced in **Fig. 1B** for demonstration. The intensity ratio at 458 nm and 375 nm (I_{458}/I_{375}) of **Py-Tyr** in different concentrations and varied pH values was calculated and visualized using a heatmap in **Fig. 1C**.

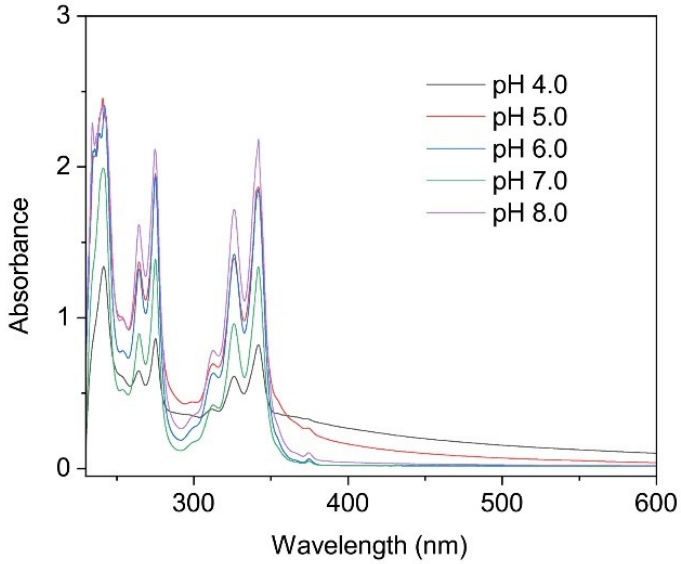


Figure S5. UV-Vis spectra of **Py-Tyr** (100 μM) in citrate-phosphate buffer under different pH values.

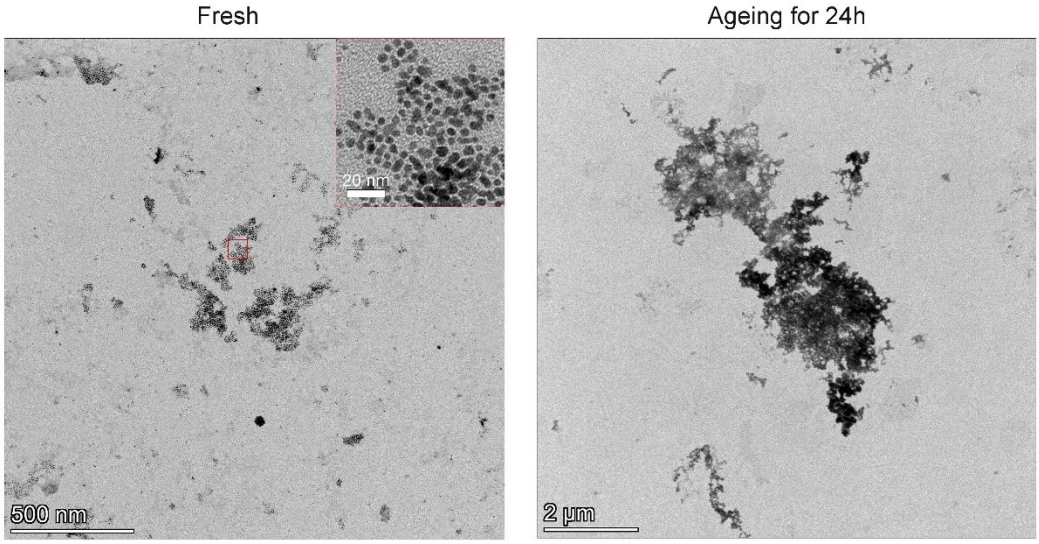


Figure S6 TEM images of freshly prepared 100 μM **Py-Tyr** assemblies at pH 4.5, or after ageing for 24 hrs.

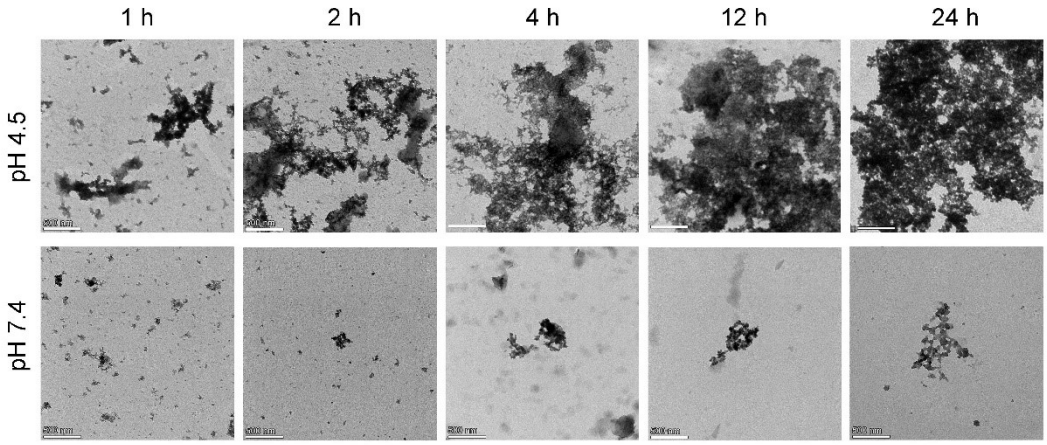


Figure S7 TEM images of 100 μM **Py-Tyr** assemblies at pH 4.5 or pH 7.4 after ageing for 1, 2, 4, 12, and 24 hrs. Scale bars represent 500 nm.

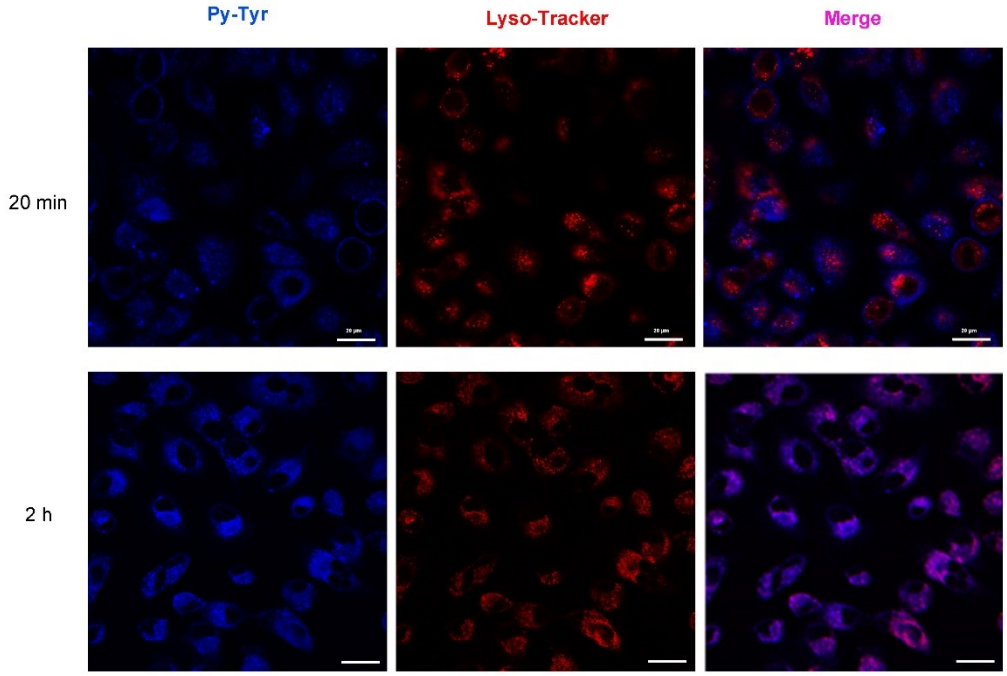


Figure S8 CLSM images of A549 cells treated with 100 μM **Py-Tyr** (blue) for 20min and for 2 hrs. Cells were co-stained with Lyso-Tracker Red DND-99 (Lyso-Tracker, red). Scale bars represent 20 μm.