Tripeptide-Dopamine Fluorescent Hybrids: A Coassembly-Inspired Antioxidative Strategy

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Materials

Tripeptides (seq: GYK-NH_2, GFK-NH_2, GWK-NH_2, GHK-NH_2) were purchased from Top-Peptide Biotechnology Co. Ltd. (Shanghai, China) with a purity of no less than 97% (verified by HPLC and MS). The lyophilized powder was stored at - 20 ℃. Other chemicals were bought from Sigma-Aldrich Corporation and used without further purification.

Methods

Coassembling of Tripeptides and Dopamine

The peptide solutions (8.0 mM) were prepared by dissolving tripeptide powder in sodium borate buffer ($Na_2B_4O_7$, 10 mM, pH 8) in quartz tubes. The reaction solutions were then stored at 25 °C, followed by the addition of DA (2.6 mM) under vigorous stirring.

Tyrosinase (TYR) catalysis

Tripeptide solutions (seq: GYK-NH_2, 8.0 mM) were prepared by dissolving their powder in sodium borate buffer (10 mM, pH 8) in quartz tubes. Tyrosinase (TYR, 0.1 mg/mL dissolved in Milli Q water) was then mixed with the peptide solutions under vigorous stirring. The TYR was removed by dialysis (3 kDa Mw cut off, Spectrolabs) after reaction.

Electron spray ionization (ESI) - Mass Spectrum (MS)

The samples were analyzed on a Thermofisher Easy-nano LC-Q Exactive Plus Orbitrap mass spectrum (MS) system equipped with a Thermo Acclaim PepMap C18 (diameter 100 μm, length 2 cm) as a pretreat column and a Thermo Acclaim PepMap C18 (diameter 50 μm, length 15 cm) as an analysis column. Electron spray ionization (ESI) was set as spray voltage of 3.6 kV, capillary temperature at 320 ℃, and positive full scan mode was used with a scanning range of 100 - 1200 m/z.

Absorption and fluorescence spectroscopy and kinetics

All fluorescence spectra was recorded with a fluorescence spectrometer (Fluorolog®-MAX 4, Horiba) equipped with a 1.0 cm quartz cell with a fixed excitation wavelength at 350 nm. The excitation and emission slit widths were set at 5.0 nm. The absorption data was obtained on a UV–vis spectrometer (U-2900, Hitachi). And the kinetics data was obtained by using a microplate reader (BioTek Synergy H1).

Dynamic light scattering (DLS)

The hydrodynamic diameter measurements were conducted on a DLS instrument (Malvern Zetasizer Nano ZS90, Malvern Instruments Ltd., Worcestershire, UK) equipped with quartz cuvette at 25.0 ℃ over 100 scans. The buffer was sodium borate (10 mM, pH 8.0, SB8) buffer.

Figures

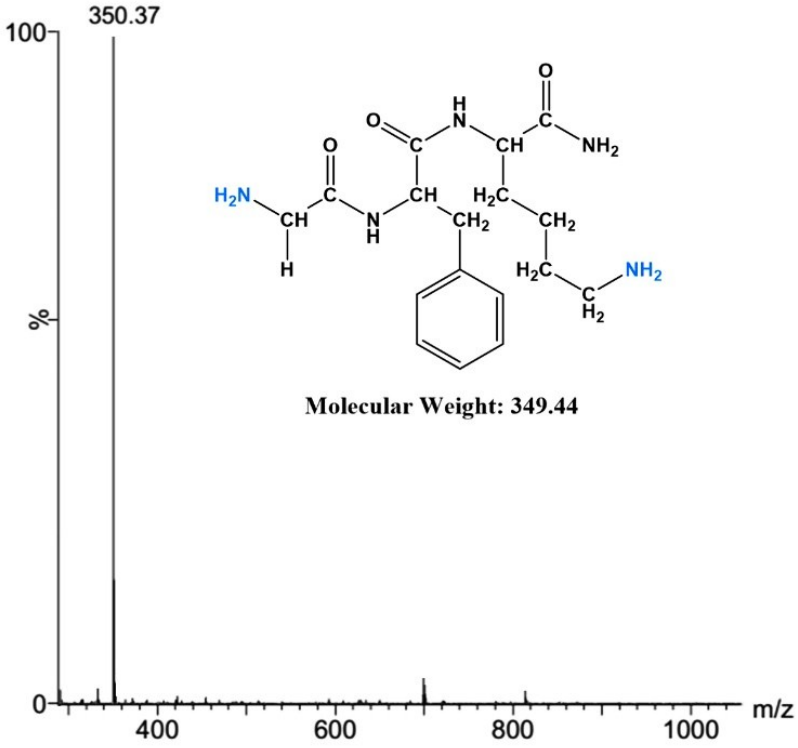

Fig. S1 Mass spectrum of GFK.

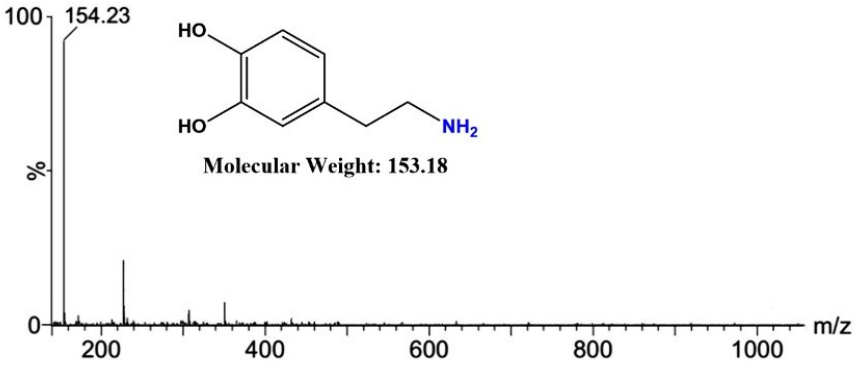

Fig. S2 Mass spectrum of DA.

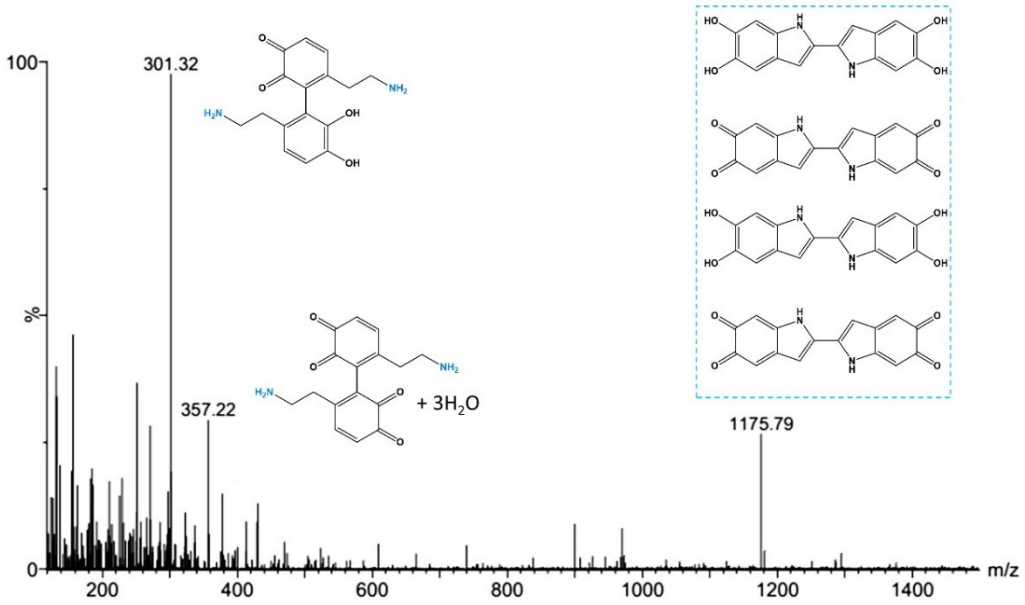

Fig. S3 Mass spectrum of diDA**.** The proposed molecular structures were drawn corresponding to the m/z values.

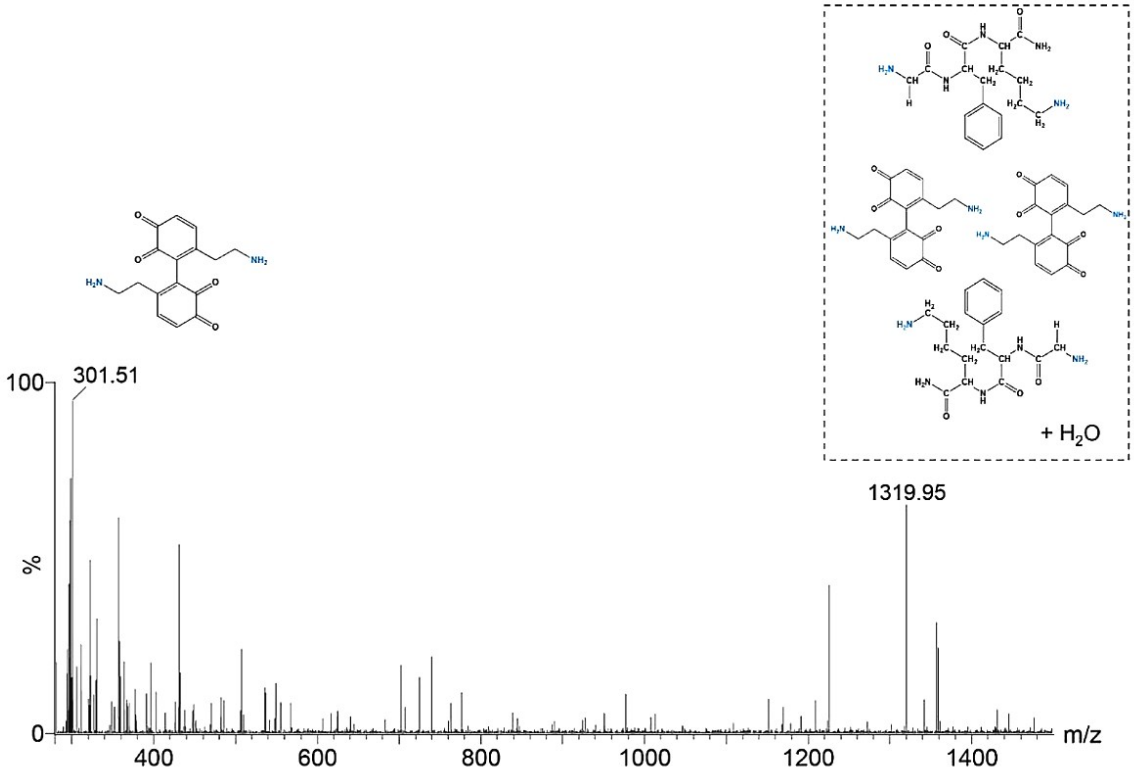

Fig. S4 Mass spectra of GFK-diDA coassembled nanostructures. The proposed molecular structures were drawn corresponding to the m/z values.

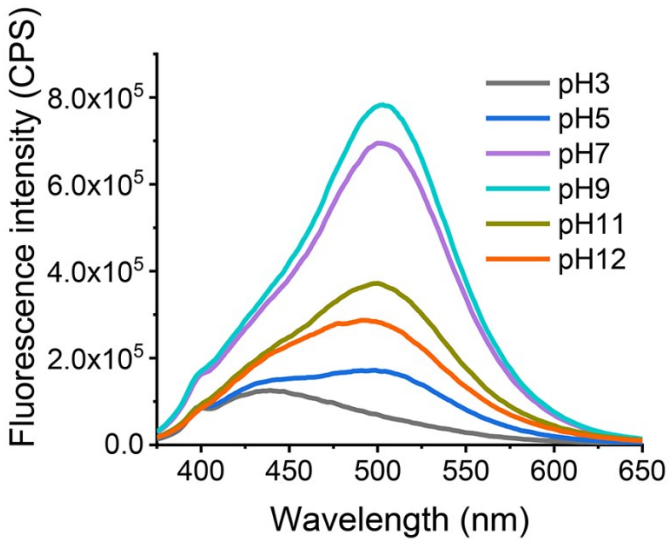

Fig. S5 Fluorescence spectra of GFK-diDA coassembled nanostructures at different pH conditions.

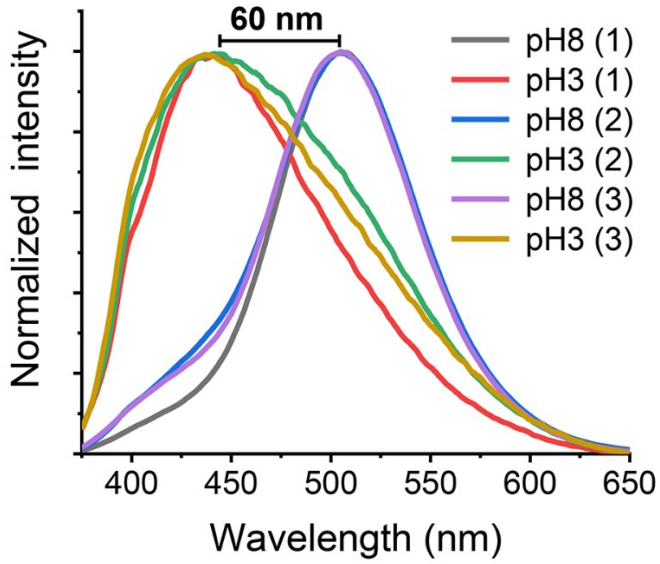

Fig. S6 Fluorescence spectra of GFK-diDA coassembled nanostructures at pH 8.0 and pH 3.0, the numbers in brackets indicate the repeating times.

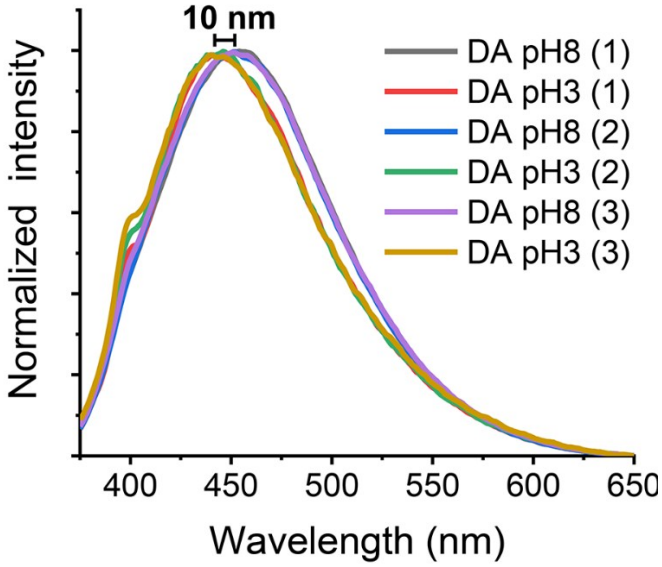

Fig. S7 pH influence on the diDA monomer fluorescence spectra (normalized) between two pH values, the spectrum shift is 10 nm and the numbers in brackets show the repeating times of tuning pH values.

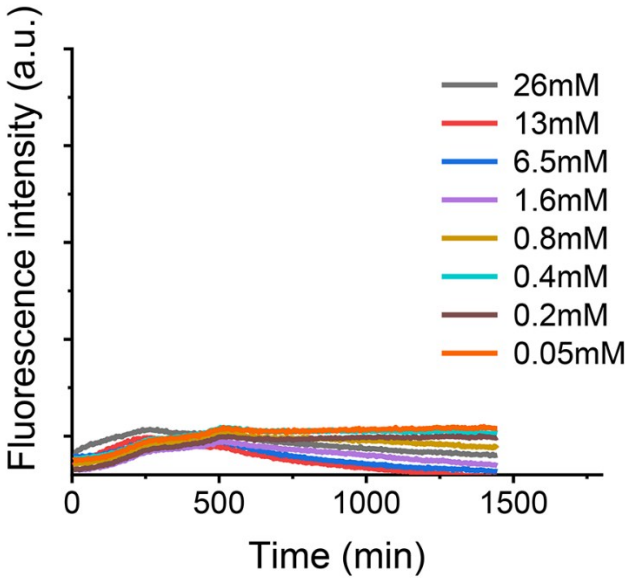

Fig. S8 Fluorescence kinetics of DA alone with different concentrations, which are control experiments for Fig. 1f.

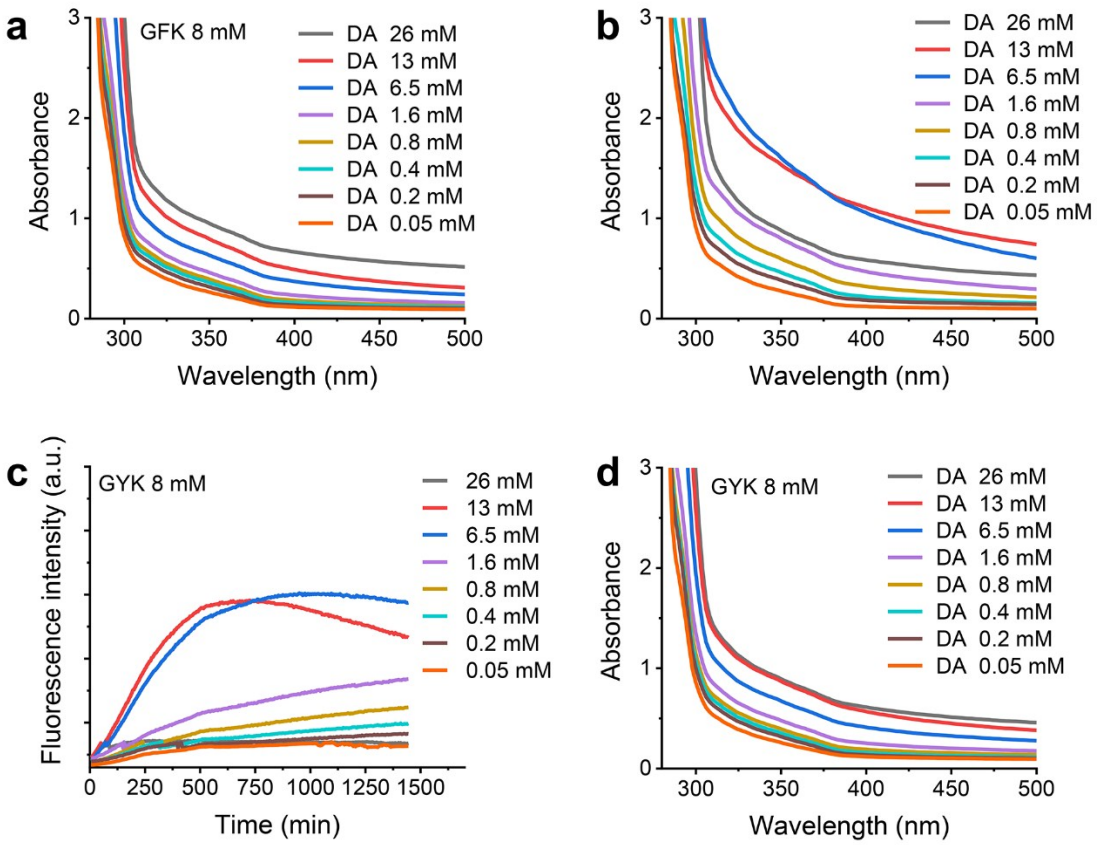

Fig. S9 Optical kinetics of DA oxidation and its coassembling with tripeptides. (a) The absorbance kinetics of GFK (8 mM) mixed with DA of different concentrations. (b) The absorbance kinetics of DA alone with different concentrations, which are the control experimental results for (a). The fluorescence kinetics (c) and absorbance (d) of GYK (8 mM) mixed with DA of different concentrations. All the concentrations are labelled in their own figures.

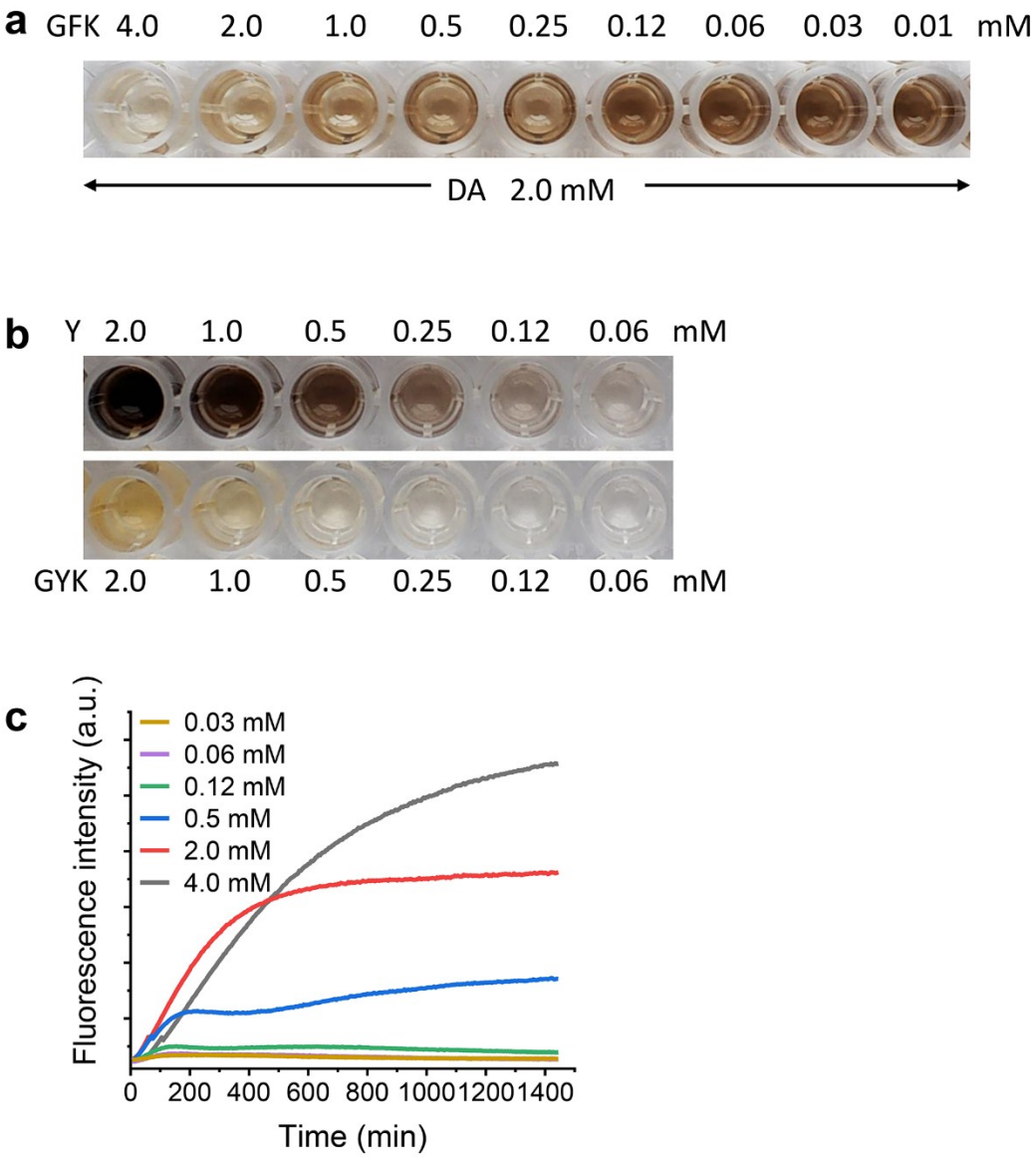

Fig. S10 Colour comparison of solution photos for indicating the oxidation extent of molecules or their coassembled nanostructures. (a) DA (2 mM) mixed with GFK of different concentrations. The more addition of GFK, the stronger protection for DA oxidation. (b) The TYR (of fixed concentration) catalysing results for both Y and GYK with different concentrations. (c) The fluorescence kinetics of DA (2 mM) coassembling with GFK of different concentrations. All the concentrations are indicated in their own figures.