Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2024

Supporting Information

A NANO platform harnessing synergistic amino acid browning for biomedical applications

Teh-Min Hu,* Jia-An Liang and Yi-Hua Chiang

Department of Pharmacy, College of Pharmaceutical Sciences, National Yang Ming Chiao Tung University, 112304 Taipei, Taiwan

*Correspondence: tehmin@nycu.edu.tw

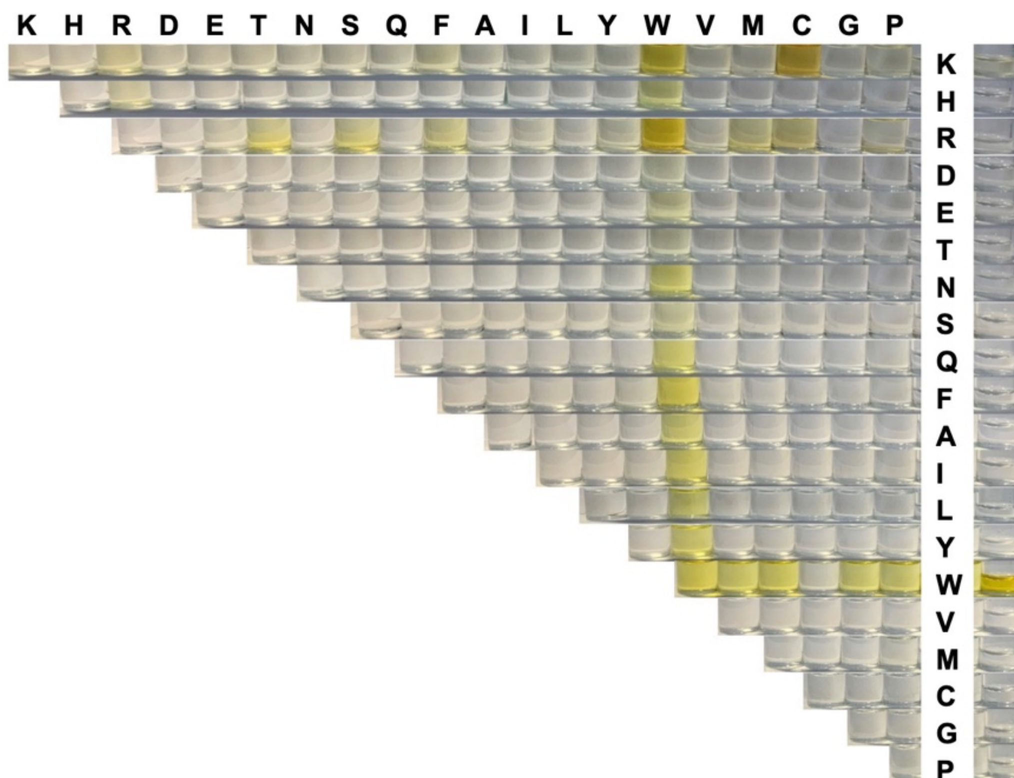


Figure S1. Appearance of solutions containing two amino acids in a solvent mixture of DMSO and acetone at a volume ratio of 9:1.
There are a total of 190 binary combinations for 20 common amino acids. The final concentration for each amino acid is 2.5 mg/mL. The rightmost column displays control solutions containing a single amino acid at a concentration of 5 mg/mL. The reaction was conducted in a glass vial at room temperature for 48 h.

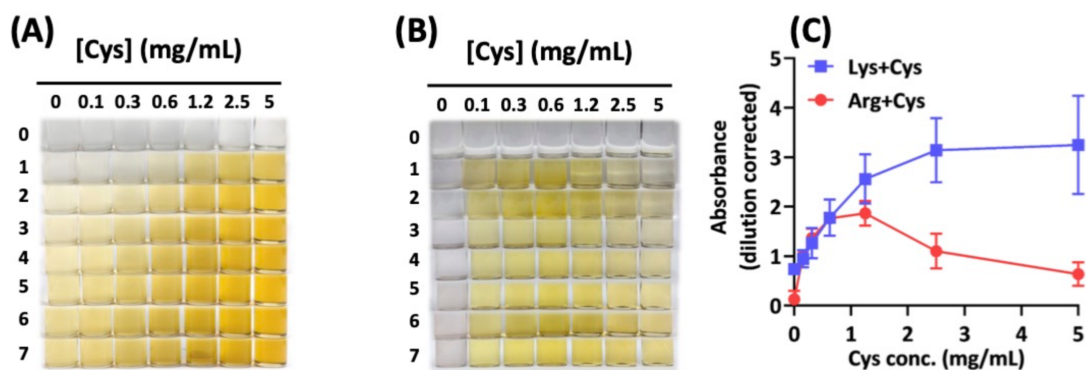


Figure S2. Cysteine concentration-dependent synergistic browning effect in lysine-cysteine and arginine-cysteine systems in a DMSO/Acetone (9:1 v/v) cosolvent mixture at room temperature.

(A) Visual observation of lysine-cysteine browning over a 7-day period with an initial [Lysine] concentration of 1.25 mg/mL.
(B) Visual observation of arginine-cysteine browning over 7 days with an initial [Arginine] concentration of 1.25 mg/mL.
(C) Dilution-adjusted absorbance at 420 nm plotted against varying cysteine concentrations.

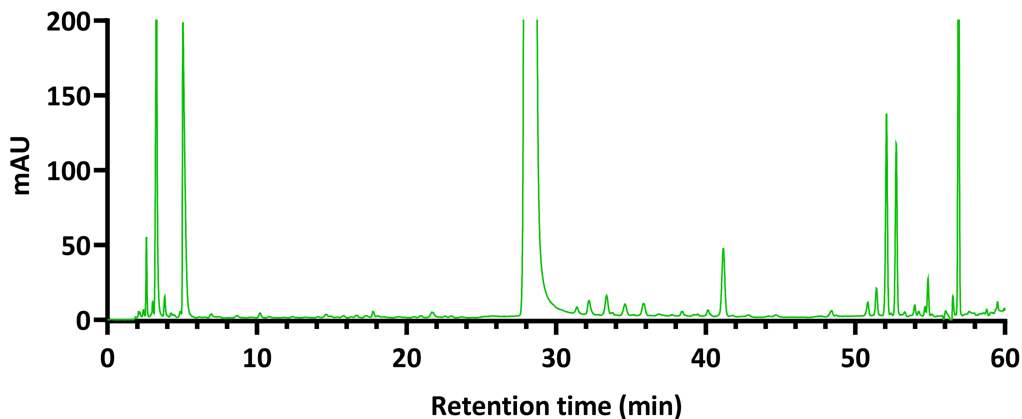


Figure S3. Reverse-phase HPLC chromatograms for amino acid solutions containing cysteine and lysine, each at 1.25 mg/mL, reacting in DMSO/acetone (9/1 v/v) for 7 days at room temperature. The chromatogram detected at 242 nm is presented.

HPLC conditions:
Instrument: Agilent 1260 Infinity II HPLC system with diode array detection; column: Mightysil octadecyl silica (ODS; 4.6 mm × 250 mm, 5 μm); column temp: 25 °C; flow rate: 1 mL/min; injection volume: 20 μL (5-fold diluted sample); mobile phase: gradient elution with varying ratios of 0.05% formic acid and methanol.

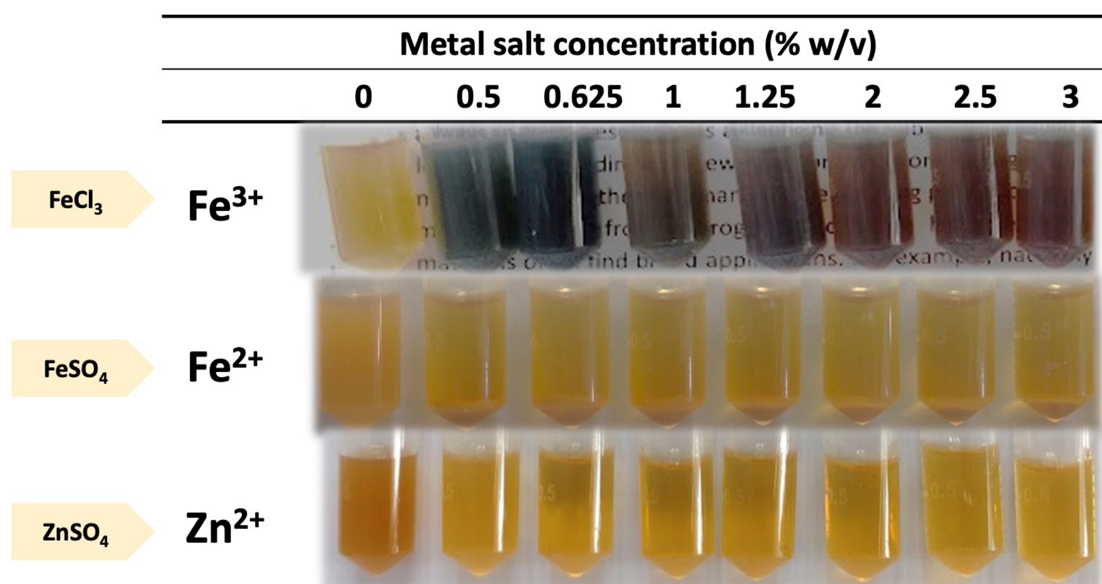


Figure S4. Comparison of metal salts in interaction with browned lysine-cysteine.

The two amino acids ([lysine] = 5 mg/mL; [cysteine] = 10 mg/mL) underwent browning in DMSO/Acetone (9:1 v/v) over 7 days. The final solution mixture was prepared by combining 0.2 mL of browned lysine-cysteine with 0.8 mL of aqueous solutions containing metal salts at various concentrations, as indicated.

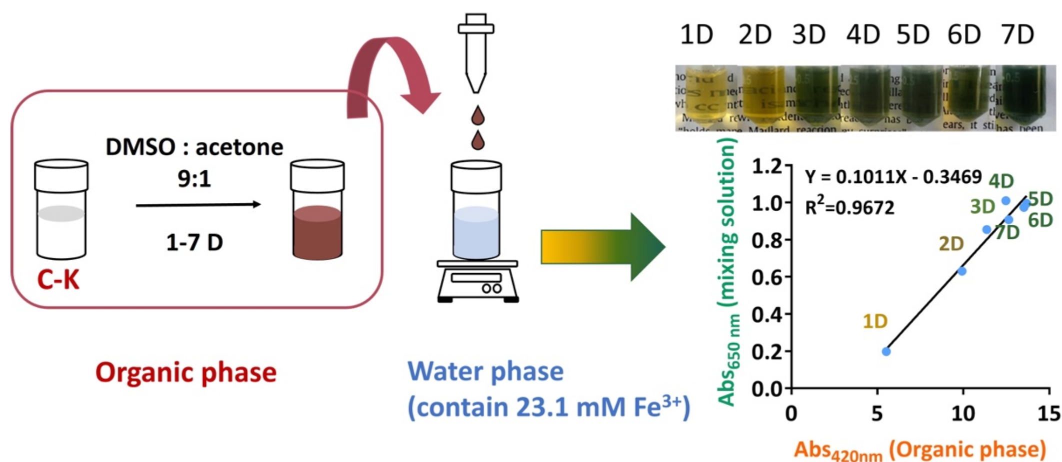
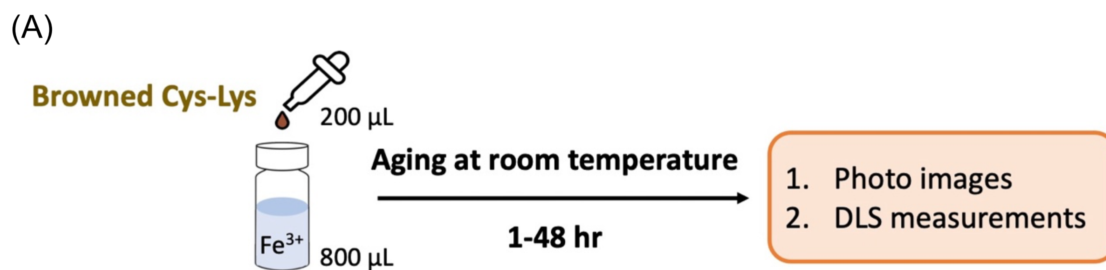


Figure S5. Correlation between ferric ion complexation and the degree of amino acid browning in the DMSO/acetone mixture.

Amino acid concentrations: [lysine] = 2.5 mg/mL; [cysteine] = 10 mg/mL.

Various levels of browning were regulated by the duration of the reaction, ranging from the initial day (0D) to day 7 (7D). In each instance, the organic phase (0.2 mL) was combined with a 0.8 mL water phase containing 23.1 mM ferric ions to yield the ultimate greenish solutions. These solutions exhibited a prominent peak in visible light absorption at 650 nm. The absorbance at 650 nm (Y-axis) for the final solution mixture was graphed against the absorbance at 420 nm (X-axis) of the browned organic phase.

(A)



(B)

	Cys/Lys = 10/2.5 (mg/mL) [Fe^{3+}] (mM)			Cys/Lys = 10/5 (mg/mL) [Fe^{3+}] (mM)			Cys/Lys = 15/2.5 (mg/mL) [Fe^{3+}] (mM)		
Aging time (hours)	23.1	37.0	46.2	23.1	37.0	46.2	23.1	37.0	46.2
1									
2									
4									
6									
24									
48									

Figure S6. Optimization of nanoprecipitation conditions involved varying concentrations of amino acids (in the organic browning reaction) and ferric ions (in the water phase), with color transformation observed within 48 hours of aging at room temperature. The amino acid browning process was conducted over a 7-day period at room temperature.

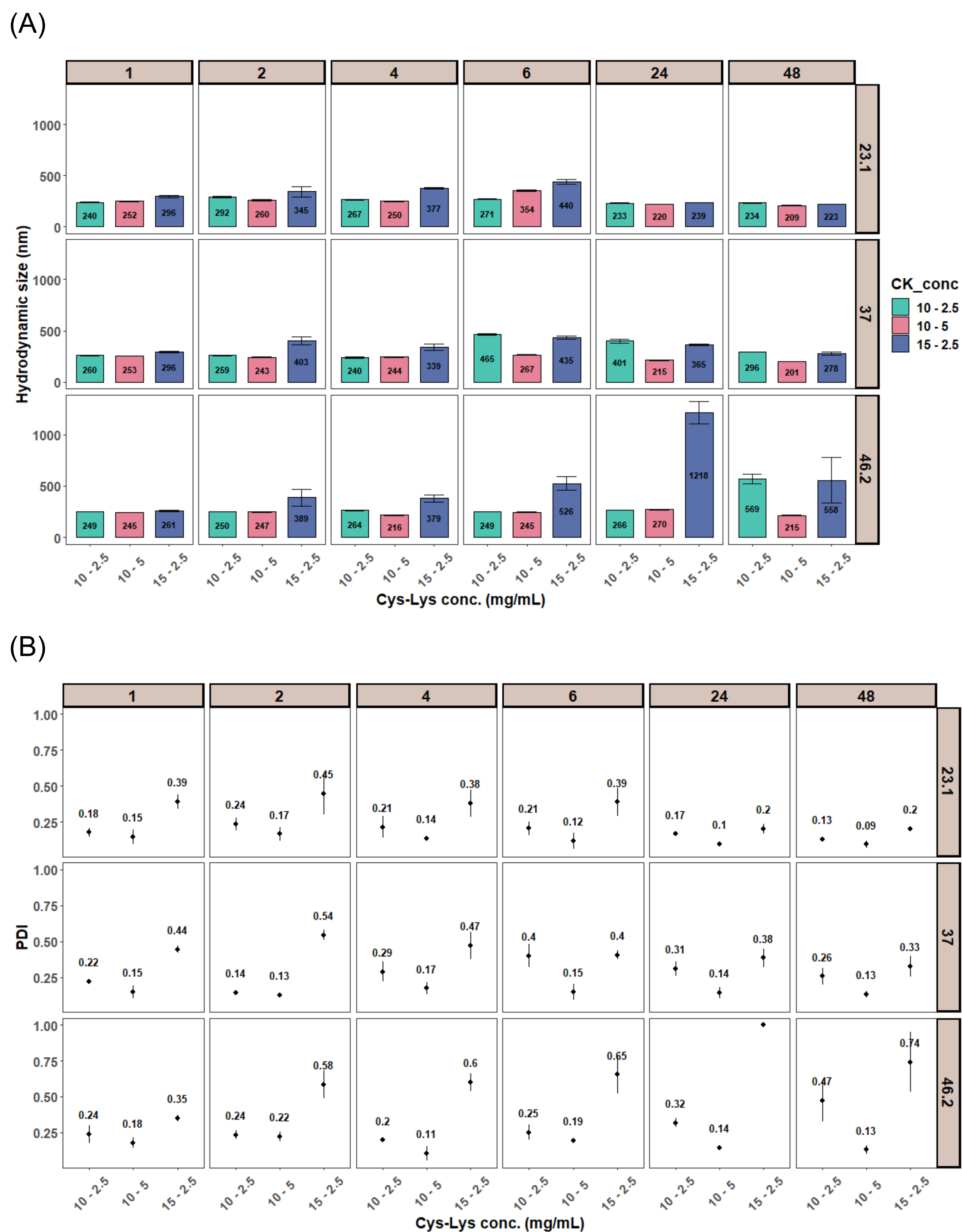


Figure S7. Hydrodynamic sizes (A) and PDI (B) of nanoprecipitation solutions during aging (as illustrated in figure S5) from 1 to 48 hours.

The matrix columns correspond to aging hours, while the matrix rows represent ferric ion concentrations. Within each matrix cell, three amino acid concentration pairs were compared. Prior to DLS measurements, the nanoprecipitated solutions underwent centrifugation at 10,000 rcf for 15 minutes, followed by redispersing the particles with de-ionized water.

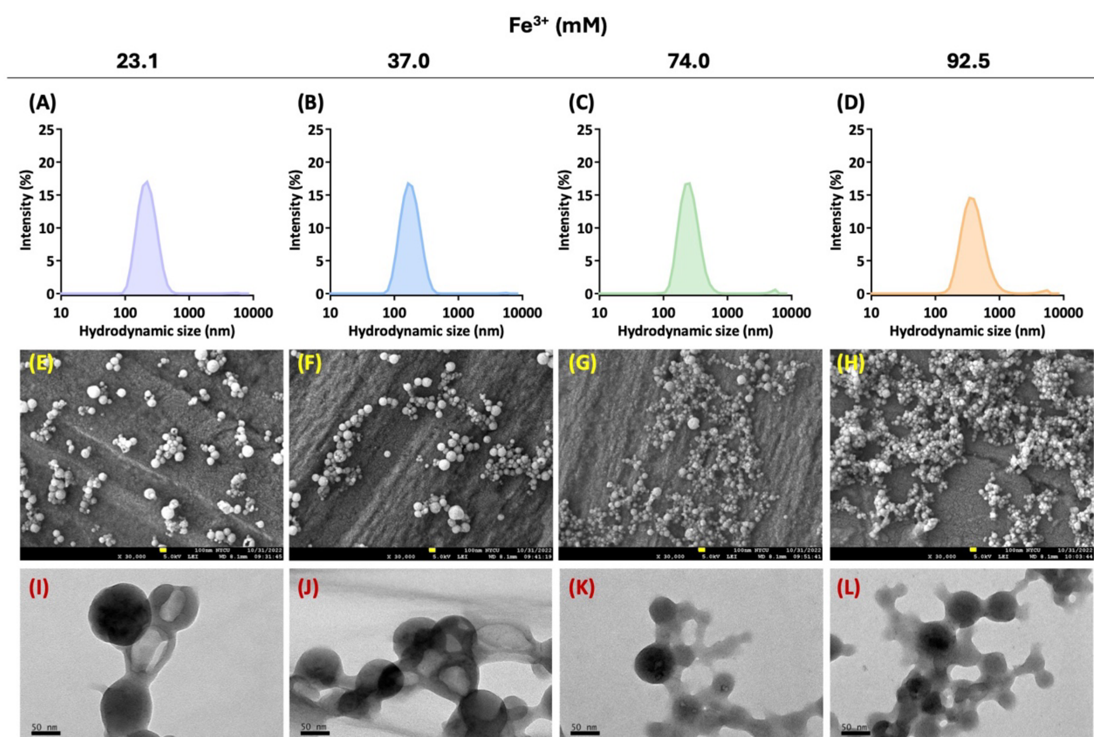


Figure S8. Particle size distribution of particle dispersions prepared from nonprecipitating browned organic phase in water with varying concentrations of ferric ions. (A-D) Dynamic Light Scattering (DLS); (E-H) Scanning Electron Microscopy (SEM); (I-L) Transmission Electron Microscopy (TEM).

Experimental details:
Browned organic phase: DMSO/acetone = 9/1 v/v, containing 10 mg/mL cysteine and 5 mg/mL lysine, reacting at room temperature for 7 days.
Nanoprecipitation conditions: 8 mL of the organic phase added to 2 mL of water containing various concentrations of ferric chloride equivalent to 23.1, 37.0, 74.0, and 92.5 mM of ferric ions.
All particle dispersions were collected after aging at room temperature for 24 hours, followed by repeat centrifugation/redispersion (twice) at 10,000 rcf for 15 minutes.

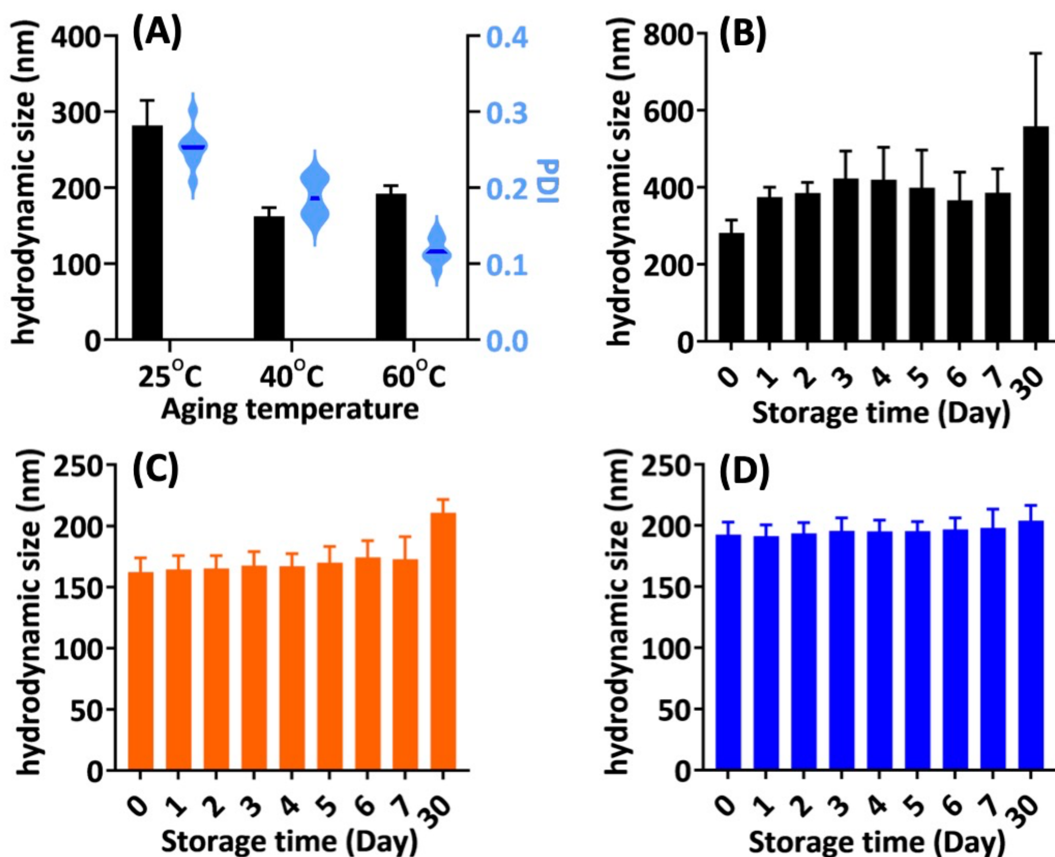


Figure S9. Hydrodynamic size and stability of particles collected at various aging temperatures.

(A) Hydrodynamic diameters (black, left axis) and PDI (blue, right axis) of freshly prepared particles. (B-D) Particle stability monitored over 30 days for preparations obtained from different aging protocols: (B) 25 °C, (C) 40 °C, (D) 60 °C.

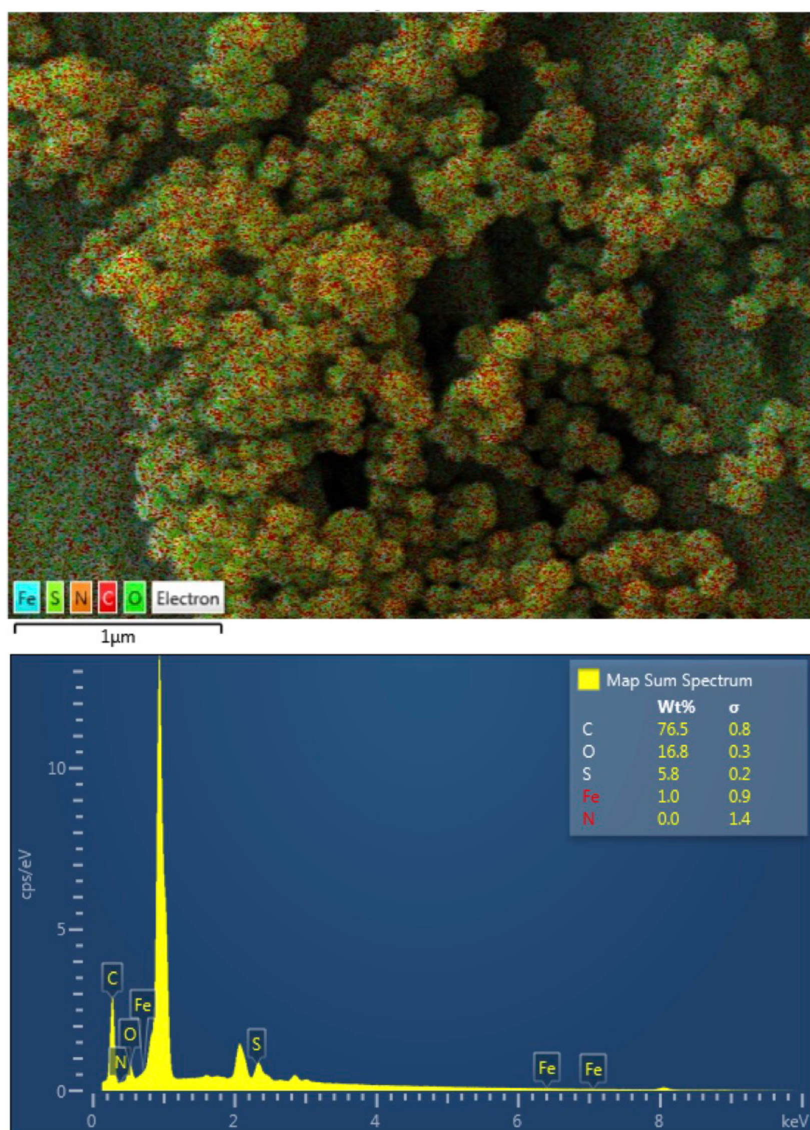


Figure S10. Energy dispersive X-ray spectroscopic (EDS) analysis of the final nanoparticles. Upper panel: elemental map. Lower panel: elemental spectrum.

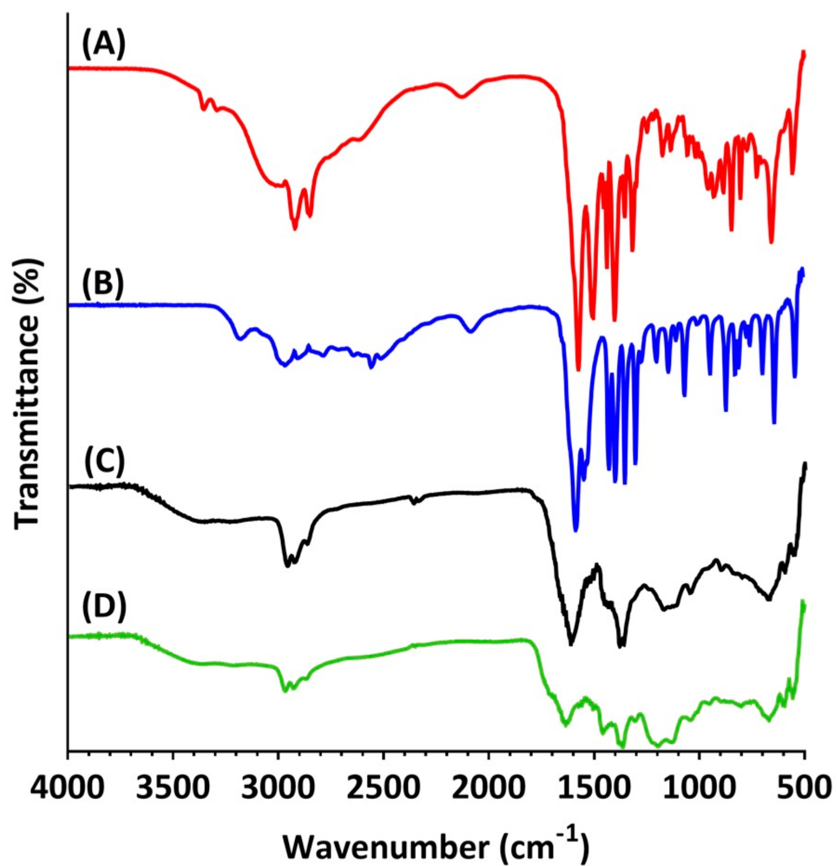


Figure S11. FTIR spectra of lysine (A), cysteine (B), browning-derived particles (without adding ferric ions, C), and the final nanoparticle product (with ferric ion catalysis, D).

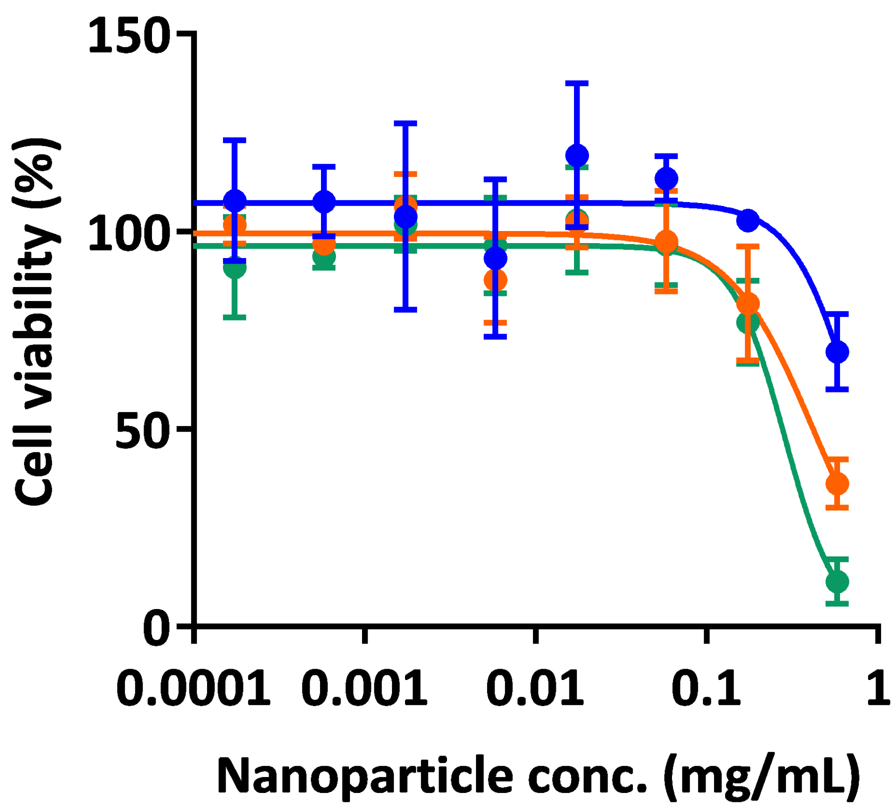


Figure S12. Cytotoxicity of the final nanoparticle product in cancer cells (HCT-116).

Each curve represents the result of the MTT assay after nanoparticle treatment and incubation for 24 hours (blue), 48 hours (orange), and 72 hours (green).

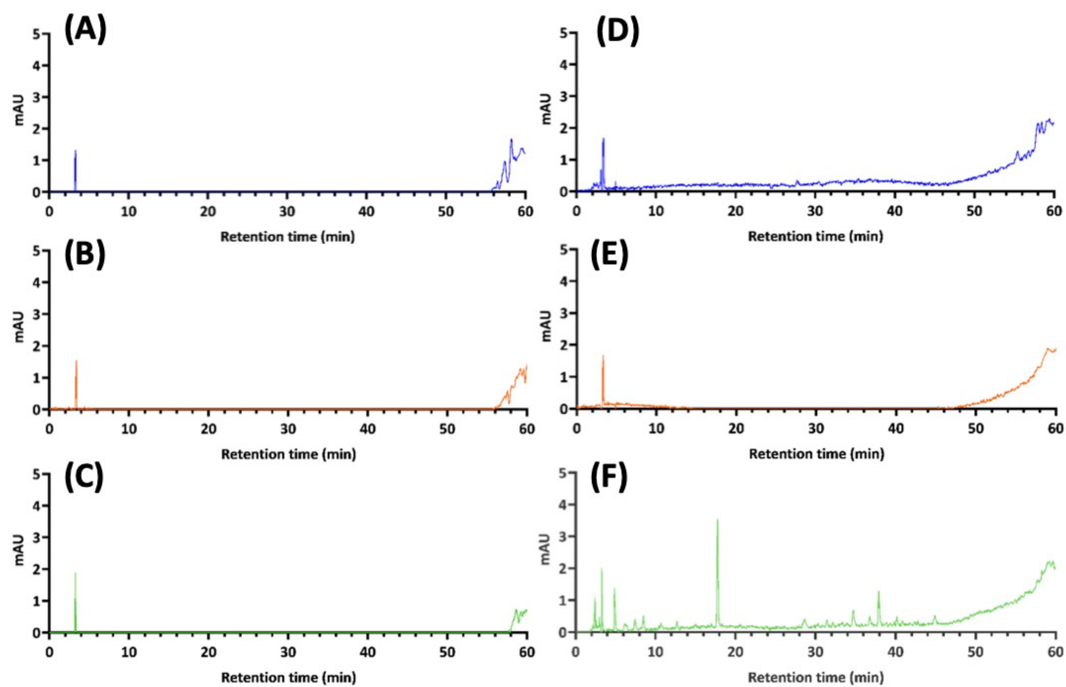


Figure S13. HPLC chromatograms detected at 420 nm for amino acid solutions (1.25 mg/mL) reacting in DMSO/acetone (9/1 v/v) for 5 h (left panel; A-C) and 168 h (right panel; D-F).

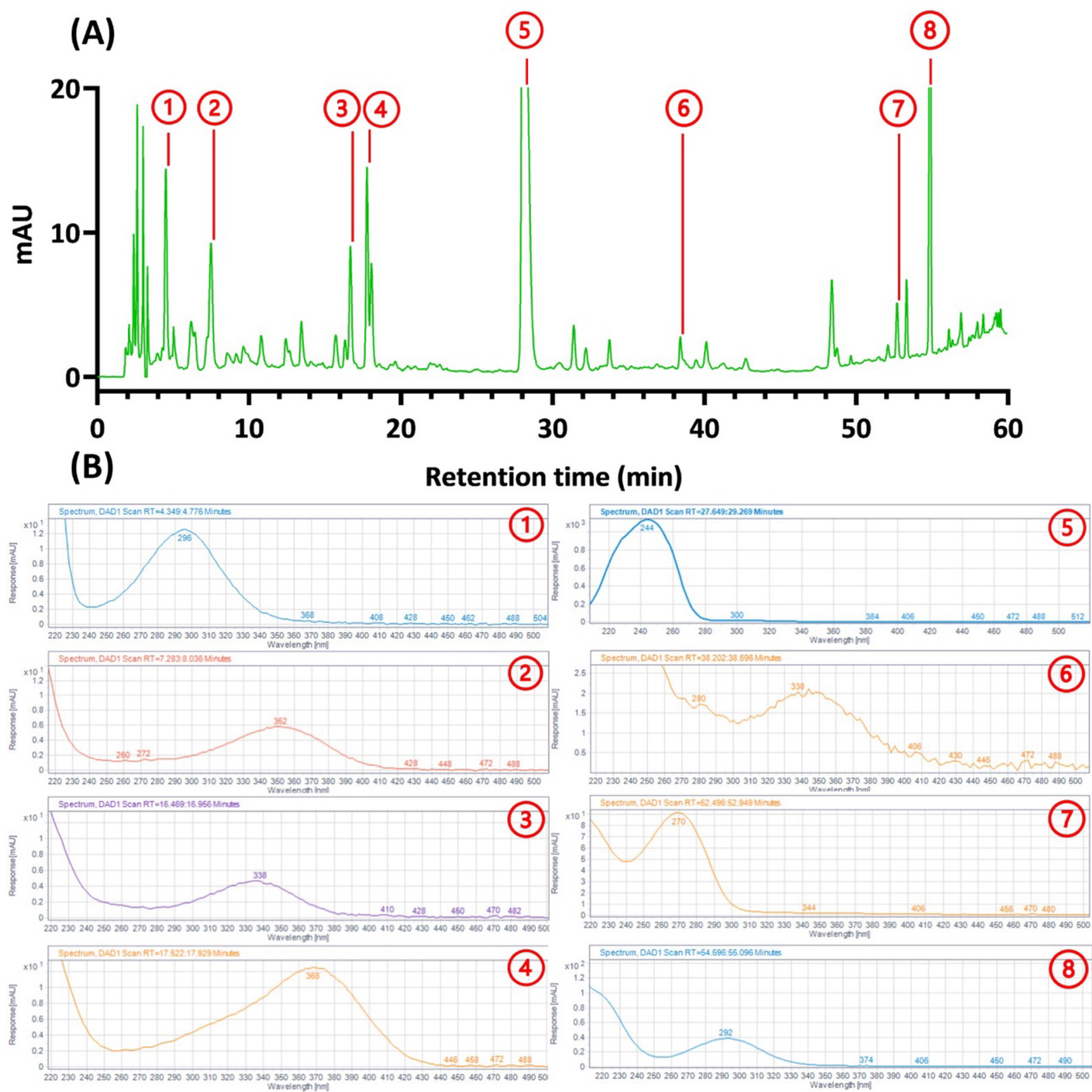
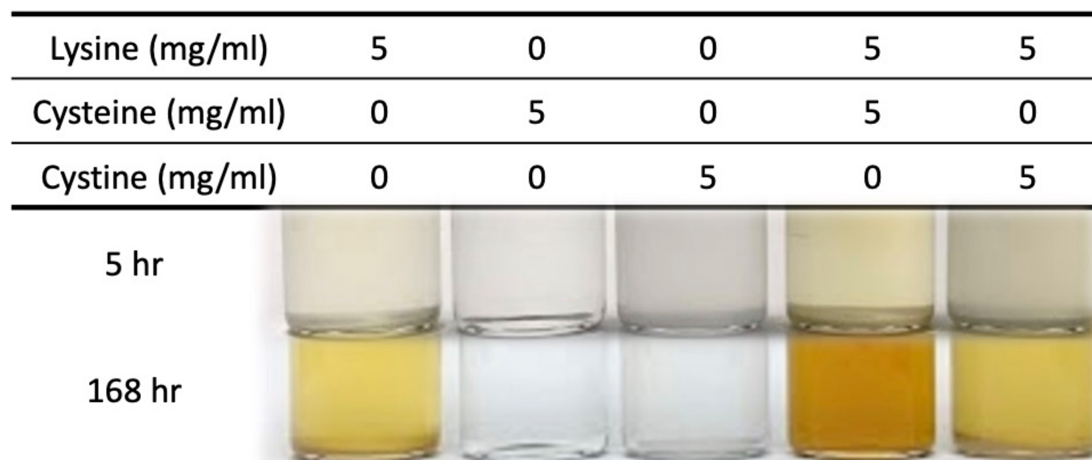


Figure S14. UV-vis spectra corresponding to selected peaks as indicated in the chromatogram shown above (reproduced from Figure 9F).

A



B

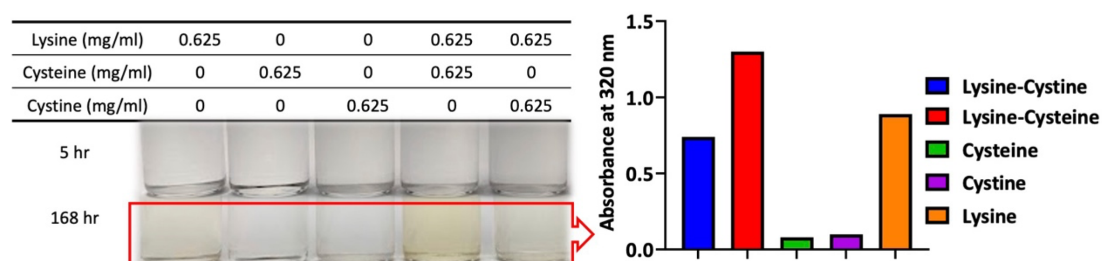


Figure S15. Cystine, the oxidized form of cysteine, does not enhance the browning of lysine. (A) high-concentration amino acid test. (B) Low-concentration amino acid test with accompanying spectrophotometric comparison.

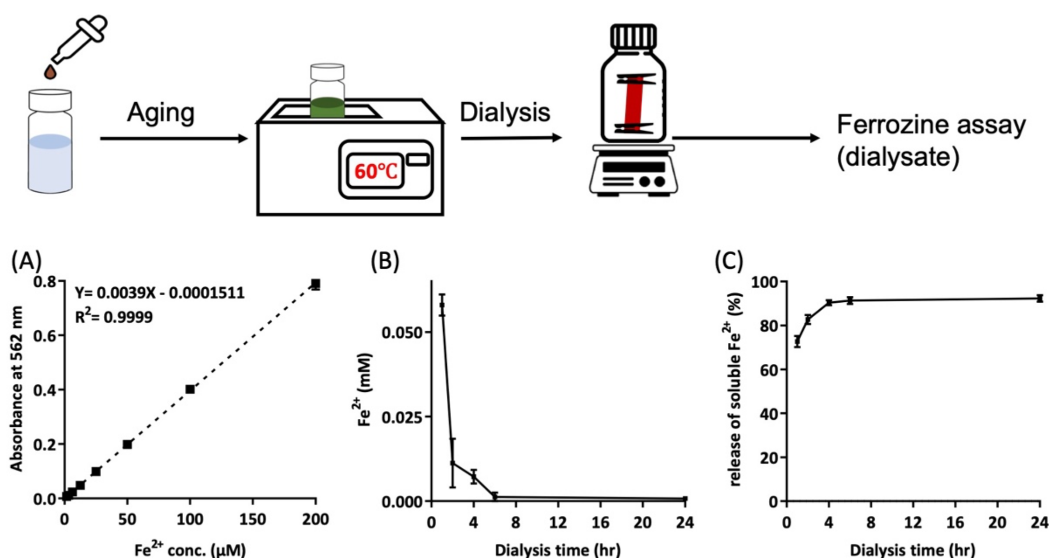


Figure S16. Ferrozine assay for the quantification of soluble ferrous ions in the dialysate.

(A) Standard curve of ferrous ions. (B) Measured concentrations of ferrous ions in the dialysate at different time. (C) Cumulative percentage of soluble ferrous ions measured during the dialysis period.

- Amino acid browning conditions: [lysine] = 5 mg/mL; [cysteine] = 10 mg/mL; DMSO/acetone = 9/1 v/v, room temperature; 7 days.
- Aqueous phase: 23.1 mM ferric ions (0.625% w/v $FeCl_3 \cdot 6H_2O$)
- Aging conditions: temperature 60 °C; 24 hours
- Ferrozine assay for ferrous ions in (1) supernatant of the aged solution (obtained by centrifugation) before dialysis, and in (2) dialysate (outer phase) at different dialysis time.
- Cumulative percentage at time t = (cumulative amount measured in the dialysate/total amount before dialysis) ×100.