Supporting information

Full-color peptide-based fluorescent nanomaterials assembled under the control of amino acid doping

Yuhe Shen, [a,1] YuLin Sun, [a,1] Yaoyu Liang, [a] Xiaojian Xu, [a] Rongxin Su, [a, b, c] Yuefei Wang, [a, c] ,* Wei Qi [a, b, c],*

a. *State Key Laboratory of Chemical Engineering, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, P. R. China.*
b. *Collaborative Innovation Centre of Chemical Science and Engineering (Tianjin), Tianjin 300072, P. R. China.*
c. *Tianjin Key Laboratory of Membrane Science and Desalination Technology, Tianjin University, Tianjin 300072, P. R. China.*
1. *These authors contributed equally to this work.*

Supplementary Materials and Methods

Chemicals and Materials

The Boc-Tyr, and amino acids (≥98%, CP) were purchased from Sigma-Aldrich (Beijing, China). Mushroom tyrosinase (≥500 U/mg) were obtained from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). All other reagents used were analytical grade commercially available. The water used in all experiments was prepared by a three-stage Millipore Milli-Q Plus 185 purification system (Millipore Corp., Bedford, MA) and had a resistivity of 18 MΩ·cm. Phosphate buffered saline (PBS, pH 6.5; 10 mM phosphate, 138 mM sodium chloride, and 2.7 mM potassium chloride) was prepared in the laboratory and used as the reaction buffer.

Preparation of fluorescent pigments

30 mmol·L^{-1} Boc-$_L$-Tyr solutions were prepared in phosphate buffer (pH 6.5), and 10 U·mL^{-1} mushroom tyrosinase was added to catalyze the reaction solution with stirring at 37 °C. After 2 h, mix each amino acid (45 mmol·L^{-1}, dissolved in water) with the oxidized mixture of tyrosine derivatives in a 1:1 volume ratio and placed at room temperature for further reaction. Pigments supernatants were filtered through an ultracentrifugal filter (MWCO 3 kDa and 10 kDa) for analysis, and further lyophilized at −80°C, 0.08 mbar.

Cell Culture and Cytotoxicity Assay

The application of new dyes requires safety assessment. Cell viability was measured in HEK293T cells using CCK8 assays as a function of the prepared biomimetic pigments at doses ranging from 5 to 1000 μg/ ml for 24 hours. HEK293T cells were seeded in 96-well plates with the density of 3×104 cells/ well and incubated in DMEM medium containing 10% FBS and 1% antibiotics (104 units/ ml penicillin, 104 ug/ml streptomycin) at 37 ℃ in 5% CO_2 atmosphere for 24h. Then the culture mediums were replaced by 100uL fresh mediums containing different concentrations of pigments. After 18 hours, 100 uL of fresh medium and 10 uL CCK8 solution were added to each well and the cells were further incubated for 1-4 h. The absorbance was measured at a wavelength of 450 nm to evaluate the cell viability by EnSpire Multilabel Reader. The cell viability was calculated as follows:

Cell viability (%) =(As-Ab) / (Ac-Ab) × 100% (3)

Where As, Ac and Ab were showed as absorbance of the sample, control wells and blank wells, respectively. All assays were performed in triplicate.

Confocal fluorescence Imaging

Hela cells were grown on glass-bottomed dish (NEST) for 24 h and then co-incubated with 0.1 mM biomimetic pigment for 4-24 hours. Cells were then washed three times with PBS and fixed in 4% paraformaldehyde (5 min). Nuclei were stained with DAPI (1 min) and observed by confocal microscopy (UltraView Vox, PerkinElmer).

Simulation Calculation of UV-Vis Absorption Spectrum

We use Gaussian 09 to calculate the UV absorption spectra of the presumed representative pigment structures by the following steps: firstly, we optimized the ground state structure of representative pigment moleculars on

SMD/B3LYP/6-31+G(d,p) level. Then, we calculated the excitation energy of the lowest 20 states of the optimized molecule in the solution ((TD)–DFT SMD/PBE0/6-31+G(d,p)level). And further obtained their ultraviolet visible(UV-vis) absorption spectrum in solution through the spectrum plotting function of Multiwfn.

Molecular Dynamics Simulations (MDs)

GROMACS 2022.1 [1, 2] was used for 200 ns molecular dynamics simulations of representative monomers. The simulation was carried out with a step size of 2 fs under Amber ff99SB-ILDN force field[3]. The initial model was constructed using Discovery Studio Visualizer. The topology file of pigment residue was generated by *acpype.py*[4-6], and the RESP charge was calculated by Gaussian 09[7] combined with Multiwfn[8]. Gaussian 09[6, 7] was used to calculate the single point energies of pigment monomers using B3LYP theoretical method[9] and 6-311G** basis[10]. Then 150 of the structures were placed in a rectangle box with a margin of 15 nm, and the box was consequently filled with tip3p water[11]. Energy minimization was performed using the conjugate gradient method. Subsequently, the system was pre-equilibrated under isothermal-isobaric (NPT, 0.5 ns, 298.15 K) ensemble. Then, 200 ns production simulation at 298.15 K was carried out. The particle mesh Ewald (PME) method was used to deal with the system charges. Then the molecular structures and interactions were illustrated by visual molecular dynamics (VMD) [12] and PyMOL.

Cryogenic Transmission electron microscopy (Cryo-TEM)

The morphology of the pigment assemblies was further assessed using Talos F200C G2 cryo-transmission electron microscope. A droplet of solutions (~10 μL) was deposited on the surface of copper grid, and blotted with a filter paper for 1−2 s and plunged into a liquid ethane cooled by liquid nitrogen to obtain a vitrified thin film. Mount the grid in a cryogenic sample holder and perform cryogenic transfer to Cryo-TEM to investigate the morphologies of pigments.

Size Distribution

Size distribution of the prepared pigments were determined by dynamic light scattering (DLS) on a Malvern Panalytical Instruments Zetasizer nano ZS90. All samples were studied in quartz cuvettes at 25 °C with triplicate measurements.

Fluorescence Spectroscopy (FL)

The fluorescence signals of the pigment solutions (45 mM, 3 mL) were measured in quartz cuvette on an Agilent Cary Eclipse (Agilent Technologies) fluorescence spectrometer. The excitation slit and emission slit were set to 5, and the PMT detector voltage was set to 700 V. For time-resolved fluorescence, the fluorescence intensity of pigment solutions was measured on an EnSpire Multilabel Reader (PerkinElmer) and the intensity was recorded at 10 min intervals. The effect of pigment fluorescence luminescence was observed by 365 nm hand-held ultraviolet lamp irradiation.

Supplementary Figures

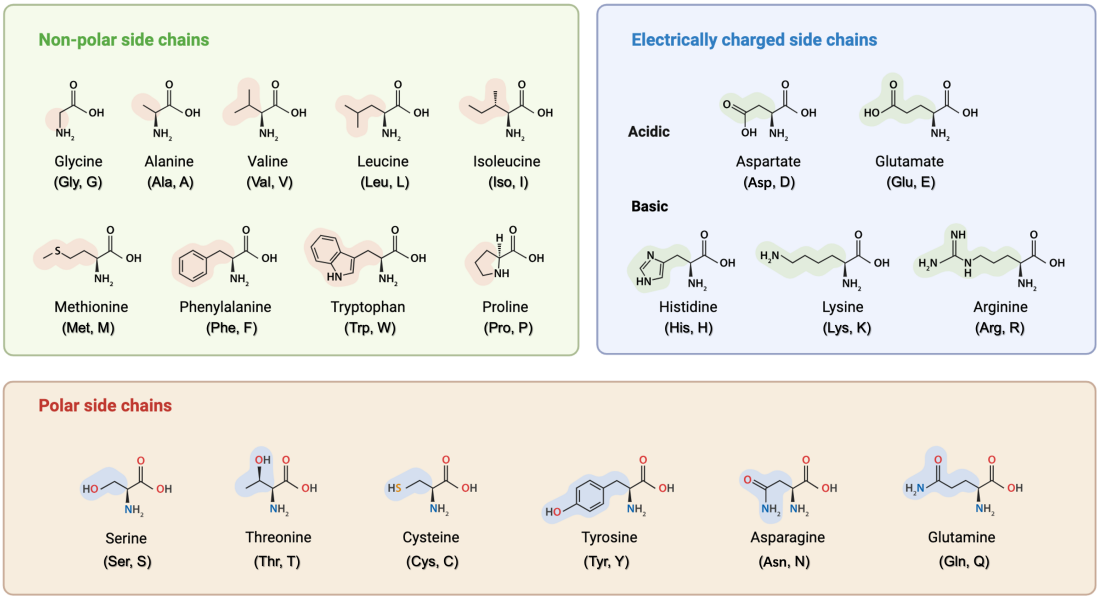

Fig. S1 Names, structural formulae, corresponding three-letter and single-letter abbreviations for 20 common amino acids.

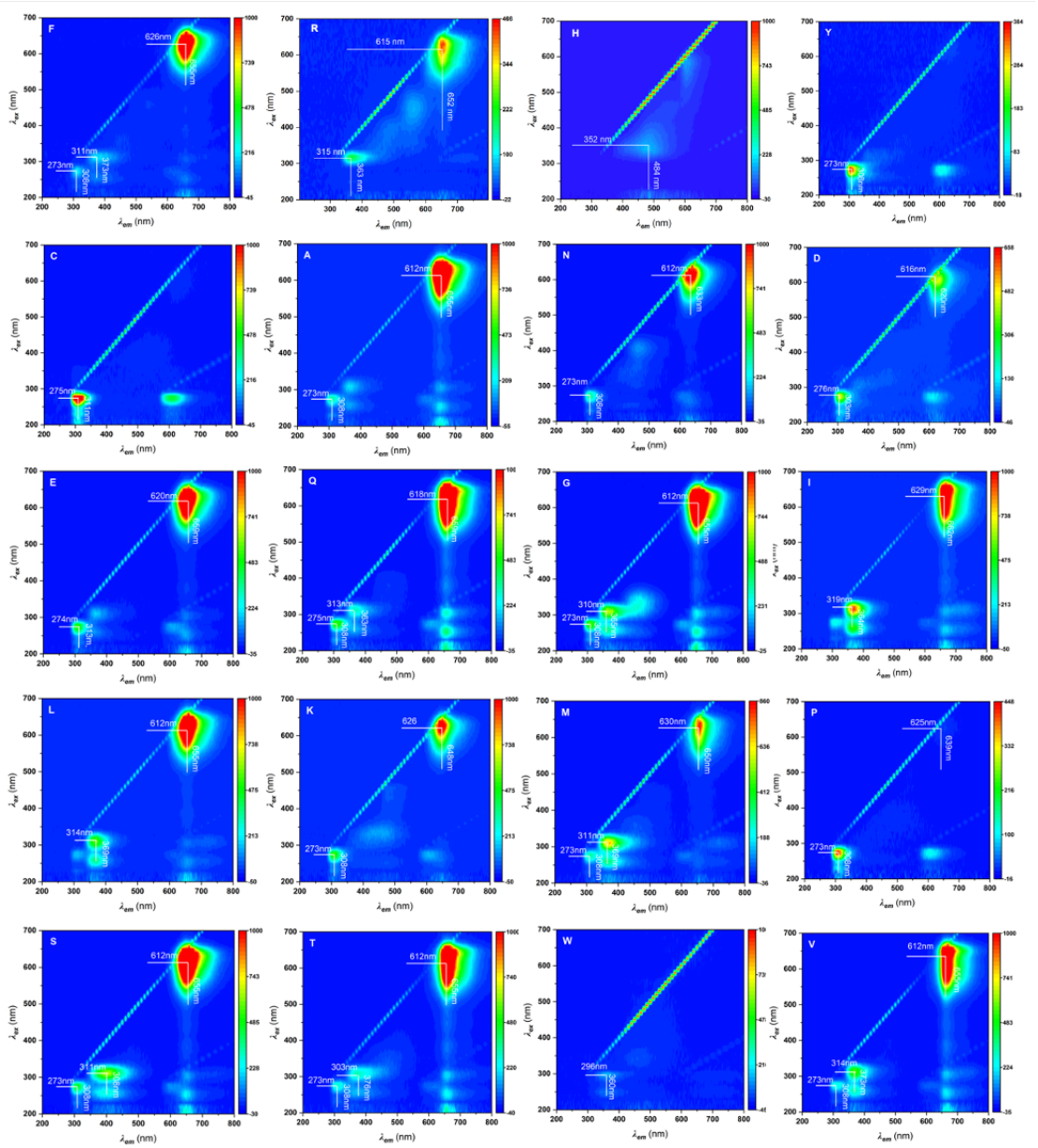

Fig. S2 Excitation–emission matrix of the reaction mixture of the Tyr-peptide system doped with different amino acids for 48 h. The white letters in the upper left corner of the picture represent the types of amino acids doped into the system.

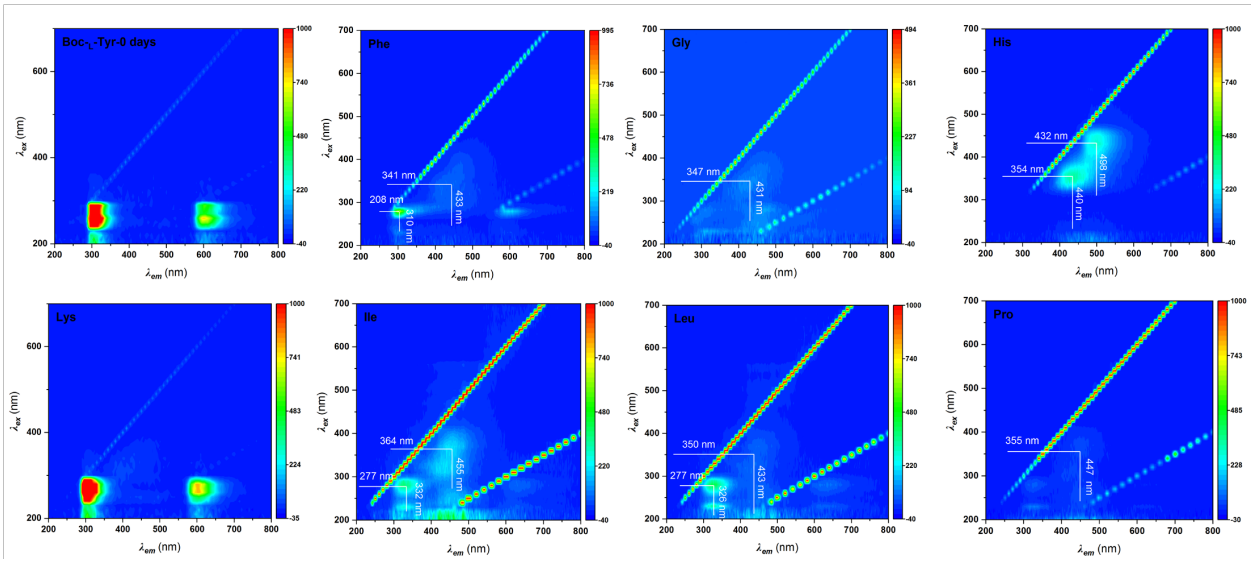

Fig. S3 Excitation–emission matrix images of the Boc-L-Tyr reaction solution and unreacted representative original amino acid solutions (45 mM). The contour graphs were measured after incubation for 48 h under the same environmental conditions as the prepared pigments.

Supplementary reference

1. M. J. Abraham, T. Murtola, R. Schulz, S. Páll, J. C. Smith, B. Hess and E. Lindahl, *SoftwareX*, 2015, **1-2**, 19-25.
2. P. B. B. H. E. Lindahl, *Journal*, 2022, DOI: 10.5281/zenodo.6451567.
3. K. Lindorff-Larsen, S. Piana, K. Palmo, P. Maragakis, J. L. Klepeis, R. O. Dror and D. E. Shaw, *Proteins*, 2010, **78**, 1950-1958.
4. J. M. Wang, W. Wang, P. A. Kollman and D. A. Case, *J Mol Graph Model*, 2006, **25**, 247-260.
5. J. M. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman and D. A. Case, *Journal of Computational Chemistry*, 2004, **25**, 1157-1174.
6. A. W. Sousa da Silva and W. F. Vranken, *BMC Research Notes*, 2012, **5**, 367.
7. G. W. T. M. J. Frisch, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, *Journal*, 2016.
8. T. Lu and F. W. Chen, *Journal of Computational Chemistry*, 2012, **33**, 580-592.
9. P. J. Stephens, F. J. Devlin, C. F. Chabalowski and M. J. Frisch, *The Journal of Physical Chemistry*, 1994, **98**, 11623-11627.
10. R. Krishnan, J. S. Binkley, R. Seeger and J. A. Pople, *The Journal of Chemical Physics*, 1980, **72**, 650-654.
11. W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey and M. L. Klein, *The Journal of Chemical Physics*, 1983, **79**, 926-935.
12. W. Humphrey, A. Dalke and K. Schulten, *Journal of Molecular Graphics*, 1996, **14**, 33-38.