



A new green approach to synthesizing MIP-202@porous silica microspheres for positional isomer/enantiomer/hydrophilic separation

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ABSTRACT

Metal-organic frameworks (MOFs) with superior physicochemical properties have great potential for applications in chromatographic separation. However, currently popular methods for the synthesis of MOF-based silica composite materials usually require the use of harmful organic solvents and long-term high-temperature sealing reactions. In order to respond to the needs of green chromatography, it is urgent to develop a new green organic-solvent-free strategy for the synthesis of MOF@SiO₂ composites. MIP-202 is a zirconium-MOF constructed from zirconium ion and L-aspartic acid, which features green synthesis as well as good hydrolytic stability and chemical stability. In this paper, SiO₂-NH₂ was first prepared in a hydrophilic deep eutectic solvent, and then an amino acid-based MOF material (MIP-202) was modified on the surface of the SiO₂-NH₂ in an aqueous solution to obtain a MIP-202@SiO₂ composite material. The multi-mode separation performance of MIP-202@SiO₂ as a promising liquid chromatographic stationary phase was particularly evaluated and the separation mechanisms were discussed. The MIP-202@SiO₂ column exhibited excellent separation ability for aromatic positional isomers. In addition, chiral enantiomers and hydrophilic analytes were also satisfactorily detected and separated. This work provides a new approach for the facile synthesis of MOF-based liquid chromatographic separation material by using green deep eutectic solvent and water as the reaction media.

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Aromatic positional isomers have important applications in the chemical industry. However, it is a challenge to separate aromatic positional isomers in modern analytical chemistry due to their similar physicochemical properties [1-3]. Therefore, it is crucial to develop a facile and low-cost method to achieve the efficient separation of aromatic positional isomers. Chromatographic technology is one of the most important methods for separating aromatic positional isomers, including gas chromatography [4,5], high-performance liquid chromatography (HPLC) [6,7] and capillary electrochromatography [8,9]. As the key component of chromatography, stationary phases play a decisive role in the development of chromatographic technology [10-16]. In recent years, multifarious porous materials such as metal-organic frameworks (MOFs) [17], covalent organic frameworks [18,19] and metal-organic cages [20,21] have been developed as novel stationary phases for separating positional isomers. In particular, the application of new

porous composite materials based on SiO₂ in HPLC has received an extensive attention [22-24].

MOFs are a class of crystalline porous materials with periodic network structures, which are formed by inorganic metal nodes and bridging organic ligands through coordination self-assembly [25]. Due to the advantages of large specific surface area, superior thermostability and diverse topological structure, MOFs have been widely applied in catalysis [26], gas storage and separation [27], drug delivery [28], electrochemistry [29], as well as separation science [30]. Yan and co-authors have attempted to directly pack MOF materials such as MIL-101(Cr) [31] and MIL-100(Fe) [32] into the stainless steel columns by the slurry method to separate aromatic positional isomers. However, due to the uneven morphology and poor monodispersity of MOF particles, these MOF-packed columns have some disadvantages such as high column pressure and low column efficiency. To address these issues, MOF@SiO₂ composite materials that combine the advantages of MOF materials and porous silica microspheres have been developed as promising HPLC stationary phases for the separation of positional isomers

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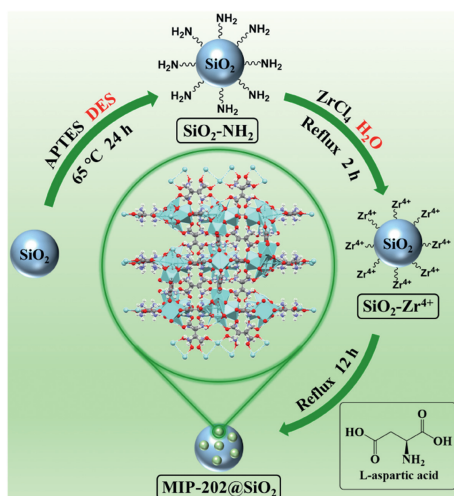


Fig. 1. Scheme for the green synthesis of MIP-202@SiO₂ composite material.

[33,34]. Previously reported MOF@SiO₂ composite materials are usually synthesized by using hazardous organic solvents such as *N,N*-dimethylformamide (DMF) under high-temperature and high-pressure conditions. In addition to the relatively high cost of experimentation, the use of toxic solvents will be harmful to the environment and researchers. Therefore, it is necessary to develop a new strategy for the green and facile synthesis of MOF@SiO₂ materials under mild conditions.

Deep eutectic solvents (DESs) are a new class of ionic liquid analogs consisting of hydrogen bond donors and hydrogen bond acceptors. In comparison with the conventional organic solvents, DESs are often considered as renewable, low-cost and green solvents, which have the prominent advantages of low toxicity, affordable raw materials and good dispersibility [35]. Qiu *et al.* demonstrated the feasibility of applying DESs as the reaction media for the surface modification of silica microspheres with different silane reagents [36]. Meanwhile, the same research group successfully synthesized silica stationary phases in DESs, further validating the application of DESs in the preparation of chromatographic stationary phases [37].

Herein, the silica microsphere was first reacted with 3-aminopropyltriethoxysilane (APTES) in a green hydrophilic DES composed of choline chloride and ethylene glycol to obtain SiO₂-NH₂, and then an amino acid-based MOF material (MIP-202) was functionalized on the surface of SiO₂-NH₂ to synthesize a MOF@SiO₂ composite material by an organic-solvent-free cyclic reaction method (Fig. 1). Currently, there are few reports on the green synthesis of MOF@SiO₂ materials by using DES and water as the reaction media for multi-mode liquid chromatographic separation. MIP-202 is a zirconium-MOF constructed from zirconium ion and L-aspartic acid, which features green synthesis as well as good hydrolytic stability and chemical stability [38]. The synthesis of MIP-202 does not require the use of hazardous organic solvents and high-temperature reactions, which is more ecologically friendly compared to that of the other MOFs [39]. In addition, due to the presence of chiral amino-acid containing functional amino and carboxyl groups, the multi-mode separation performance of MIP-202@SiO₂ as a promising HPLC stationary phase was particularly evaluated. A series of analytes including positional isomers, chiral enantiomers and hydrophilic compounds were selected to investigate the separation ability of the MIP-202@SiO₂ slurry-packed column. Furthermore, separation mechanisms and influencing factors were also studied.

The morphology of the MIP-202@SiO₂ was characterized by SEM and TEM (Figs. S1a and b in Supporting information), confirm-

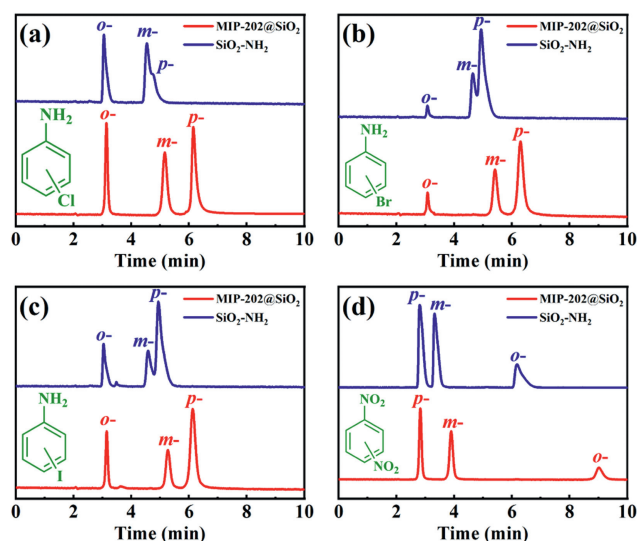


Fig. 2. Chromatograms of positional isomers of chloroanilines (a), bromoanilines (b), iodoanilines (c) and dinitrobenzenes (d) on the MIP-202@SiO₂ column and the SiO₂-NH₂ column. Chromatographic conditions: mobile phase: *n*-hexane/isopropanol = 85/15 (v/v); column temperature: 25 °C; flow rate: 1.0 mL/min; detection wavelength: 254 nm.

ing that MOF particles were attached on the surface of silica microsphere. Moreover, the selected area electron diffraction (SAED) result of MIP-202@SiO₂ is shown in Fig. S1c (Supporting information). The FTIR spectra of MIP-202, SiO₂-NH₂ and MIP-202@SiO₂ are shown in Fig. S2a (Supporting information). A strong peak at 1100 cm⁻¹ is attributed to the Si-O-Si stretching vibration. The characteristic peaks around 1510 and 3400 cm⁻¹ are caused by the stretching vibration of -COOH and -NH₂ in the aspartic acid ligand of MIP-202. The thermogravimetric curve of MIP-202@SiO₂ is shown in Fig. S2b (Supporting information), and a weight loss of 8.76% was observed in the range of 35–800 °C. The XRD results of the synthesized MIP-202, the simulated MIP-202 and the MIP-202@SiO₂ are displayed in Fig. S2c (Supporting information). The characteristic diffraction peaks of the synthesized MIP-202 are consistent with the simulated MIP-202, indicating the successful synthesis of MIP-202. In addition, it may be due to the small shell thickness of MIP-202 on the surface of SiO₂, which results in the indistinct characteristic diffraction peaks of MIP-202 on the MIP-202@SiO₂. Moreover, Figs. S2d and S3 (Supporting information) show the nitrogen adsorption-desorption isotherms and pore size distribution curves of the MIP-202@SiO₂ and the MIP-202, respectively. The specific surface area/average pore size of the MIP-202@SiO₂ and the synthesized MIP-202 are 264.60 m²/g, 11.29 nm and 3.01 m²/g, 7.10 nm, respectively (Table S1 in Supporting information). Furthermore, the C, H and N contents of MIP-202@SiO₂ are determined to be 4.10%, 1.77% and 1.21%, respectively (Table S2 in Supporting information). All the above characterization results verify the successful fabrication of MIP-202@SiO₂ stationary phase.

Disubstituted benzenes may be utilized in a variety of fields such as insecticides, dyes and medicines depending on their different structures. However, it is difficult to effectively separate positional isomers due to their extremely similar polarity and molecular structure. Therefore, developing a highly selective stationary phase for the effective separation of such positional isomers is necessary. Herein, positional isomers of chloroanilines, bromoanilines, iodoanilines and dinitrobenzenes were selected to investigate the separation performance of MIP-202@SiO₂ column, and the separation effect of these positional isomers on the SiO₂-NH₂ column was used for comparison. As shown in Fig. 2, baseline separations

of positional isomers of chloroanilines, bromoanilines and iodoanilines were achieved on the MIP-202@SiO₂ column, and the elution sequence was *o*-, *m*-, *p*-, while the elution sequence for the positional isomers of dinitrobenzenes was *p*-, *m*-, *o*-. The separation results including column efficiency, selectivity (α) and resolution (R_s) are summarized in Table S3 (Supporting information). The *p*-/*m*-dinitrobenzenes, *o*-/*m*-chloroanilines and *m*-/*o*-dinitrobenzenes were separated with high-resolutions, reaching up to 5.71, 8.06 and 15.34, respectively. Compared with the SiO₂-NH₂ and the other reported MOF materials (Table S4 in Supporting information), the highest column efficiency of 64,440 N/m for *m*-dinitrobenzene was achieved on the MIP-202@SiO₂ column, suggesting a good separation performance of MIP-202@SiO₂ for positional isomers. The successful separation of positional isomers probably results from a combination of multiple analyte-stationary phase interactions, such as hydrogen bonding, hydrophilic/hydrophobic and dipole-dipole interactions [18]. For chloroanilines, bromoanilines and iodoanilines, the elution order of positional isomers is the same as the order of their molecular length/width (L/W) ratio. As shown in Fig. S4 and Table S5 (Supporting information), the molecular L/W ratio of chloroaniline, bromoaniline and iodoaniline isomers is *o*- < *m*- < *p*-, which is exactly the same as their elution order, indicating that the larger the L/W ratio, the stronger the retention. However, the elution order of dinitrobenzene isomers is *p*- < *m*- < *o*-, which is opposite to the order of the L/W ratio. The Log*P* value of *p*-dinitrobenzene, *m*-dinitrobenzene and *o*-dinitrobenzene is 1.37, 1.62, 1.84, respectively, which is the same as the elution order, implying that the hydrophobic interaction may play a more significant role in the separation of dinitrobenzene isomers [18].

To better explore the chromatographic properties of MIP-202@SiO₂ column, the influencing factors including isopropanol (IPA) content in the mobile phase and column temperature were studied. As shown in Fig. S5 (Supporting information), the retention factors (*k*) of the positional isomers decrease with the increase of IPA content, revealing a classical retention mechanism of normal-phase liquid chromatography. When the amount of IPA increases, the polarity of the mobile phase will be enhanced, leading to a stronger elution ability of the mobile phase, thereby reducing the retention of the analytes.

Column temperature is also very important for the analyte retention. The relationship between column temperature (*T*) and *k* can be explained by the van't Hoff equation:

$$\ln k = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln \Phi \quad (1)$$

where ΔH and ΔS represent enthalpy change and entropy change associated with the analyte transfer between the two phases; *R* is molar gas constant; Φ is phase ratio. The van't Hoff plots for positional isomers of chloroanilines and dinitrobenzenes are shown in Fig. S6 (Supporting information), and the fitting results are summarized in Table S6 (Supporting information). The good linearity indicates no changes in the retention mechanism in relation to column temperature in the studied temperature range. The retention of positional isomers of chloroanilines and dinitrobenzenes gradually decreases as the temperature increases (Fig. S7 in Supporting information), and the ΔH is negative, indicating that the separation process is exothermic [24]. In principle, more negative ΔH should be thermodynamically favorable for the analyte transfer between the two phases, thus resulting in a stronger analyte retention [31]. The *p*-chloroaniline and *o*-dinitrobenzene with the most negative ΔH are eluted last, indicating that the selective separation of chloroanilines and dinitrobenzenes isomers on the MIP-202@SiO₂ column is controlled by ΔH .

In this study, an enantioselective L-aspartic acid was used as the organic ligand to synthesize MIP-202, therefore, it can be reasonably inferred that MIP-202@SiO₂ also has chiral recognition abil-

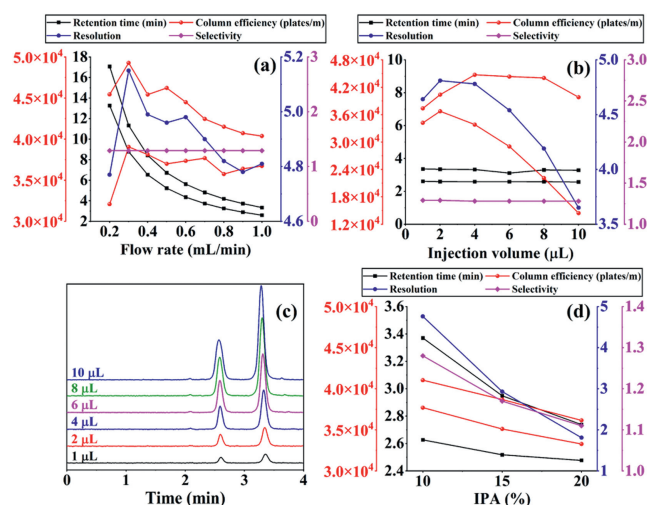


Fig. 3. Influence of flow rate (a), injection volume (b) and IPA content (d) on the retention time, column efficiency, resolution and selectivity. (c) Chromatograms of 1-phenylethanol enantiomers on the MIP-202@SiO₂ column with different injection volumes. Chromatographic conditions: (a) mobile phase: *n*-hexane/isopropanol=90/10 (v/v), injection volume: 2 μ L; (b, c) mobile phase: *n*-hexane/isopropanol=90/10 (v/v), flow rate: 1.0 mL/min; (d) flow rate: 1.0 mL/min, injection volume: 2 μ L. Column temperature: 25 $^{\circ}$ C; detection wavelength: 254 nm.

ity. Herein, 1-phenylethanol enantiomers were selected to investigate the enantioseparation performance of MIP-202@SiO₂ column, and the influence of chromatographic conditions on the chiral resolution were investigated. As the flow rate increases, the retention time of 1-phenylethanol enantiomers decreases, while the column efficiency and resolution show a certain degree of variation, and the selectivity remains unchanged (Fig. 3a). The baseline separation of two enantiomers of 1-phenylethanol can be achieved over a wide flow rate range. Therefore, a high flow rate (1.0 mL/min) was selected to shorten the analysis time and reduce the use of organic phase. The injection volume will also affect the separation efficiency, so it is necessary to select the appropriate injection volume [24]. As shown in Fig. 3b, with the increase of injection volume, the retention time and selectivity remain basically unchanged, while column efficiency and resolution first increase and then gradually decrease. The resolution for the two enantiomers of 1-phenylethanol is greatest and column efficiency is reasonably high when the injection volume was 2 μ L. The chromatograms of 1-phenylethanol enantiomers with different injection volumes are shown in Fig. 3c. Moreover, the effect of IPA content in the mobile phase on the chiral separation was also investigated (Fig. 3d). The retention time, column efficiency, resolution and selectivity all decrease as the amount of IPA increase. The *n*-hexane/isopropanol (90/10, v/v) was chosen as the optimum mobile phase since the column efficiency and the resolution are the highest. Under the optimal separation conditions, the ideal chiral resolution of 1-phenylethanol enantiomers was achieved (Fig. S8 in Supporting information). The obtained resolution, selectivity and the highest column efficiency are 4.76, 1.28 and 41,040 N/m, respectively. Fig. S9a (Supporting information) shows the chromatograms of 1-phenylethanol enantiomers continuously injected six times on the same day, and Fig. S9b (Supporting information) shows the chromatograms of 1-phenylethanol enantiomers injected twice daily for three consecutive days. The relative standard deviations (RSDs) of retention factors for the intra-day and inter-day repeatability are in the range of 0.18%–0.22% and 0.05%–0.21%, respectively. In addition, the MIP-202@SiO₂ column shows minimal changes in the retention of 1-phenylethanol enantiomers after 6 months of use. These results indicate that the MIP-202@SiO₂ column has excellent repeatability and stability. Furthermore, a chiral resolution of 1-(*p*-

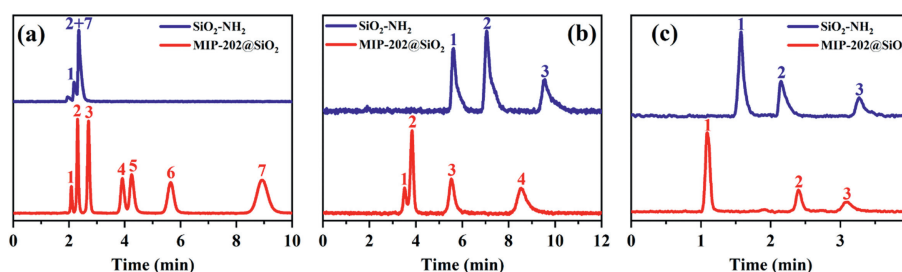


Fig. 4. Chromatograms of (a) sulfonamides (1. *o*-toluenesulfonamide, 2. sulfanilamide, 3. sulfamethazine, 4. sulfalene, 5. sulfadiazine, 6. sulfathiazole, 7. sulfacetamide), (b) nucleosides/nucleobases (1. β -thymidine, 2. uracil, 3. adenine, 4. hypoxanthine) and (c) B vitamins (1. thiamine, 2. nicotinamide, 3. riboflavin) on the MIP-202@SiO₂ column and the SiO₂-NH₂ column. Mobile phase: (a) ACN/H₂O = 95/5 (v/v); (b) ACN/H₂O = 90/10 (v/v); (c) ACN/H₂O = 70/30 (v/v). Column temperature: 25 °C; flow rate: 1.0 mL/min; detection wavelength: 254 nm.

tolyl)ethanol enantiomers was also obtained on the MIP-202@SiO₂ column, as shown in Fig. S10 (Supporting information), demonstrating satisfactory enantioseparation ability of the MIP-202@SiO₂ column.

The polar carboxyl and amino groups of aspartic acid may make the MIP-202@SiO₂ stationary phase potentially suitable for the separation of hydrophilic analytes. Herein, sulfonamides, nucleosides/nucleobases and B vitamins were selected to evaluate the hydrophilic separation performance of MIP-202@SiO₂ column.

Sulfonamides synthesized based on *p*-aminobenzenesulfonamide are a class of broad-spectrum antibacterial drugs, which are widely used in the growth, prevention and treatment of livestock and poultry meat [40]. As shown in Fig. 4a, seven sulfonamides including *o*-toluenesulfonamide, sulfanilamide, sulfamethazine, sulfalene, sulfadiazine, sulfathiazole and sulfacetamide were effectively separated on the MIP-202@SiO₂ column with the highest column efficiency for sulfanilamide reaching up to 52,980 N/m. However, only *o*-toluenesulfonamide, sulfanilamide and sulfacetamide were eluted on the SiO₂-NH₂ column, which may be due to that the SiO₂-NH₂ column has a stronger hydrophilic effect with sulfonamides. The effect of acetonitrile (ACN) content in the mobile phase on the retention time of sulfonamides was investigated. As shown in Fig. S11 (Supporting information), as the content of ACN changes in the range of 20%–90%, the retention time of sulfonamides first decreases and then increases, exhibiting an obvious U-shaped curve. This indicates that the retention of sulfonamides on the MIP-202@SiO₂ column is affected by multiple mechanisms [41].

In addition, a selective separation of four nucleosides/nucleobases including β -thymidine ($\log P = -1.11$), uracil ($\log P = -0.71$), adenine ($\log P = -2.12$) and hypoxanthine ($\log P = -0.91$) was also realized on the MIP-202@SiO₂ column (Fig. 4b). The elution order of the four nucleosides/nucleobases is inconsistent with the related $\log P$ value. The molecular size of β -thymidine (11.23 nm) is equivalent to the pore size of MIP-202@SiO₂ (11.29 nm), hence, β -thymine is excluded from the pore, resulting in the shortest retention time [33,42]. Although the molecular size of hypoxanthine (7.14 nm) is the same as that of adenine (7.14 nm), hypoxanthine was eluted later, which may be that other interaction forces such as hydrogen bonding and electrostatic interactions are also involved in the separation of nucleosides/nucleobases in addition to hydrophilic effect. Compared with the MIP-202@SiO₂ column, a stronger retention of nucleosides/nucleobases on the SiO₂-NH₂ column was found, and an effective resolution of the four nucleosides/nucleobases was not achieved on the SiO₂-NH₂ column under the tested conditions.

In the end, a fast separation of three B vitamins including thiamine ($\log P = -4.16$), nicotinamide ($\log P = -0.68$) and riboflavin ($\log P = -1.28$) was achieved within 4 min on the MIP-202@SiO₂

column (Fig. 4c). It was also found that the elution order of the three B vitamins was not directly related to the $\log P$ value, suggesting that the retention mechanism of B vitamins in HILIC mode may be the result of multiple interactions. The separation effect of B vitamins on the SiO₂-NH₂ column was not very satisfactory with more severe peak tailing.

In this study, a MIP-202@SiO₂ composite material was successfully synthesized through a green approach by using DES and water as the reaction media. The MIP-202@SiO₂ exhibited favorable separation ability for positional isomers, chiral enantiomers as well as hydrophilic sulfonamides, nucleosides/nucleobases and B vitamins. It was found that multiple interaction mechanisms such as hydrophilic/hydrophobic, hydrogen bonding and electrostatic interactions were conducive to achieving the effective separation of various analytes by the MIP-202@SiO₂ in different chromatographic modes. The chromatographic evaluation revealed that developed MIP-202@SiO₂ was an excellent chromatographic separation material. Moreover, the MIP-202@SiO₂ slurry-packed column exhibited satisfactory repeatability and stability. To sum up, this article provides a new idea for the green preparation of promising MOF@SiO₂ composite materials for multiple applications in liquid chromatographic analysis. What remains to be improved is the homogeneous surface modification of MOF material on the SiO₂ in green reaction media. Therefore, the improvement of green synthesis methods for MOF@SiO₂ composites will be continuously explored in the future studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Zhefei Hu: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. **Jingwen Liao:** Data curation, Investigation, Methodology. **Jiawen Zhou:** Data curation, Investigation, Validation. **Lulu Zhao:** Data curation, Validation. **Yanjuan Liu:** Methodology, Resources. **Yuefei Zhang:** Resources, Writing – review & editing. **Wei Chen:** Resources, Supervision. **Sheng Tang:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ccl.2024.109985.

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