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Visible Enantiomer Discrimination via Diphenylalanine-based Chiral Supramolecular Self-assembly on Multiple Platforms

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ABSTRACT: The development of enantioselective recognition is of great significance in medical science and pharmaceutical industry, which associates with molecular recognition phenomenon widely observed in biological systems. In particular, the facile and straight achievement of visual enantioselective recognition has been drawing increasing consideration but still challengeable. Herein, a heterochiral diphenylalanine-based gelator (LFDF) is synthesized, presenting left-handed nanofibers during self-assembled in ethanol, which accomplish the phenylalaninol enantiomer recognition on multiple platforms. When adding *L*- or *D*-phenylalaninol into LFDF supramolecular solution followed by ultrasonic treatment, precipitate and gel are formed, respectively. Meanwhile, LFDF supramolecular gel completely collapses in a minutes after dropping *L*-phenylalaninol, while the gel almost remains when *D*-type is employed. Moreover, a fluorescent supramolecular xerogel (ThT-LFDF) is fabricated by introducing Thioflavine T (ThT), which could detect *L*-phenylalaninol accompanying with fluorescence quenching and *D*-type with barely decreasing. And the ThT-LFDF xerogel system shows a good sensitivity for the detection of *L*-phenylalaninol reaches to ppm. It is found that the chirality of the assembled nanofibers, as well as amino and carboxyl of phenylalaninol play critical role on the discrimination process. The multiple and visible enantioselective recognition of phenylalaninol through chiral supramolecular self-assemblies show potential applications in the fields of medical science and pharmaceutical industry.

KEYWORDS: Supramolecular chemistry, chiral assembly, visible recognition, diphenylalanine, multiple platforms

INTRODUCTION

Enantioselective recognition is of pivotal importance not only in biomedical sciences but also in pharmaceutical industry, and has been attracting tremendous research interests in the past decades.[1-4] Numerous tools and strategies such as colorimetric or chromogenic methods, nuclear magnetic resonance (NMR), gas chromatography (GC), crystal morphology, circular dichroic (CD), and wettability switching, etc. are available to achieve this goal, yet accompanied by specialized setups, strict test conditions and tedious procedures.[5,6] These shortcomings and invisible results severely restrict their application scopes, therefore simple, real-time, and convenient techniques for this purpose are badly in demand.[7] With the advantages of stimuli-responsiveness and reversibility, the chiral supramolecular architecture formed through self-assembly provides an ideal model for mimicking natural biopolymers to recognize the chiral guest.[8] Inspired by this, we introduce a new chiral recognition system using the fabricated chiral supramolecular nanostructures as the host, which shows visible enantiomer discrimination of phenylalaninol enantiomers on multiple platforms. This naked-eye detectable platform based on artificial chiral assemblies offers a facile, straightforward approach for chiral molecular recognition, which is of great significance in scientific research and biomedical applications.[9-11]

To date, chiral sensors have mainly been established based on noncovalent interactions between a host and an enantiomeric guest at molecular level.[12-17] However, it is difficult for molecular enantiomer to express their chirality in a range of available analytical tool.[18] In recent years, the enantioselective recognition system has been

focused on the chiral polymer-based fluorescence sensors owing to amplification effects among multiple chiral units.[3] Nonetheless, covalent systems are often too rigid and unable to adapt their shapes to the guest molecules, which results in less than optimal binding affinities and selectivity. Another proverbial drawback is their labor-intensive synthesis, which leaves little potential for structural variations in the polymer chain.[19] Inspired by the supramolecular architectures found in biological living organisms (e.g. DNA, RNA, proteins), chiral supramolecular self-assemblies, such as double helices, twists, and rolled-up nanotubes have been attracting great interests owing to their fascinating structural features, attractive functions, and possible applications in chiral sensing and separation.[20,21] Recently, significant efforts have been made to achieve visible chiral recognition and discrimination through supramolecular systems.[22-25] Typically, Liu[26] reported a chiral supramolecular assembly formed by an organogelating procedure, which can be functionalized as an enantioselective system. The noticeable fluorescence color change of chiral nanofibers demonstrated that the chiral supramolecular assemblies are potentially valuable for visual chiral recognition. In spite of this, the visual discrimination of enantiomers via chiral supramolecular on multiple platforms is still urgently needed but rarely being reported.

Herein, we developed a new heterochiral diphenylalanine-based gelator (LFDF), which was able to form supramolecular assemblies in ethanol (supramolecular solution) after heating-cooling process and gelated ethanol via a solution-to-gel transformation under sonication. The supramolecular solution and organogel systems could visually recognize *L*/*D*-phenylalaninol enantiomers through the observation of precipitate or gel

reservation, respectively. In addition, the supramolecular xerogel doped with fluorescent dye ThT can sensitively examine *L*/*D*-phenylalaninol enantiomers through fluorescence quenching or not. It was found that the multiple chiral discrimination capacities were achieved by the different interactions between chiral supramolecular nanofibers and phenylalaninol enantiomers, which resulted in different phenomena. On the other hand, the chirality of the assembled nanostructures, as well as amino and carboxyl of phenylalaninol played critical roles on the discrimination process. These outcomes not only clearly present the multiple and visible enantioselective recognition of phenylalaninol enantiomers based on the same chiral supramolecular substrate, but also enhance our understanding of molecular recognition and greatly expand the scope of potential applications in the fields of medical science and pharmaceutical industry.

EXPERIMENTAL SECTION

Materials

Boc-L-phenylalanine (Boc-L-Phe-OH), D-phenylalanine methyl ester hydrochloride (H-D-Phe-OMe·HCl), 1-hydroxybenzotriazole (HOBt), N-(3-dimethylaminopropyl)-n'-ethylcarbodiimide hydrochloride (EDC·HCl), N;N-diisopropylethylamine (DIEA), *L*/*D*-phenylalaninol and 1,4-benzenedicarbonyl chloride (BDC) were purchased from Adamas-beta reagent Co. Ltd. Trifluoroacetic acid (TFA), dichloromethane (DCM), triethylamine (Et_3N), methanol, sodium hydroxide (NaOH), sodium bicarbonate ($NaHCO_3$), citric acid (CA) and sodium chloride (NaCl) were purchased from Sinopharm Chemical Reagent Beijing Co. Ltd. All those chemicals were used without further purification.

Synthesis of LFDF gelator

The synthesis of the gelator mainly involves four steps described in Scheme S1 in supporting information. Firstly, compound 1 was obtained by conventional solution-phase approach for dipeptide synthesis: Boc-*L*-Phe-OH (5.31 g, 20 mmol) and HOBt (3.51 g, 26 mmol) in dry DCM (100 mL) was added to a DCM (100 mL) solution containing DIEA (11.12 g, 80 mmol) and H-*D*-Phe-OMe·HCl (4.75g, 22 mmol) in ice-water bath. After stirring 30 min, EDC (7.67 g, 40 mmol) was then added and the mixture was stirred for another 12 h. The solvent was evaporated under vacuum, and the residue was subsequently dissolved in DCM (100 mL). Washed successively with saturated citric acid solution, $NaHCO_3$ solution and brine, the obtained organic phase was dried over anhydrous $MgSO_4$. Purification by recrystallization (ethyl acetate/light petroleum) afforded Compound 1 (6.39 g, 75%). Secondly, TFA (12.4 mL, 168 mmol) was added to the solution of Compound 1 (4.80 g, 11.2 mmol) in dry DCM (10 mL) at 0 ℃ and stirred for 90 min to remove the Boc-group. The reaction was quenched by adding an excess amount of methanol. Evaporation of all solvents under vacuum afforded Compound 2 (3.54 g, 97%). Thirdly, BDC (0.609 g, 3.0 mmol) in dry DCM (30 mL) was slowly added dropwise to a cooled (0 °C) solution of Compound 2 (2.14 g, 6.2 mmol) in dry DCM (100 mL) and Et_3N (5 mL, 36.45 mmol). After stirring overnight at room temperature precipitate was formed, filtered and washed in ethanol and dried to give white product Compound 3 (1.67 g, 71%). Finally, for the hydrolysis, aqueous NaOH (30 mL, 2M) was added to suspension of Compound 3 (1.56 g, 2.0 mmol) in methanol (30 mL). The mixture was stirred at room temperature for 24 hours

and then acidified with HCl (3M) solution until pH value less than 3.0. The precipitate was collected and dried under vacuum to give the target Compound 4 LFDF (1.25 g, 83%). ^{1}H- and ^{13}C-NMR and HR-MS were checked, shown in Figure S1-2.

Preparation of supramolecular self-assemblies

Transparent LFDF supramolecular solution is obtained by dissolving 4 mg gelator in 1 mL ethanol under heating-cooling process. Translucent organogel could be formed when 4 mg LFDF added in 1 mL ethanol under sonication for 2 min. Fluorescent supramolecular xerogel is fabricated by incorporating 0.1 mg fluorescent dye thioflavin T (ThT) into the gel (4 mg/mL) and further drying at ambient temperature overnight. The prepared supramolecular solution, organogel and xerogel are directly utilized for visible discrimination of enantiomeric phenylalaninol.

Characterizations

NMR spectra were performed on a Bruker Advance DRX 400 spectrometer operating at 500/400 and 125/100 MHz for ^{1}H and ^{13}C NMR spectroscopy, respectively. High-resolution mass spectrometry was measured on a Bruker Micro TOF II 10257 instrument. Field emission scanning electron microscopy (FE-SEM) measurements were carried out using an FEI QUANTA 250 microscope. Samples were prepared by drying the gels on silicon slice and coating with gold. Transmission electron microscopy (TEM) observation was conducted on a Tecnai G2 spirit Biotwin at an accelerating voltage of 120 kV. The circular dichroism (CD) spectra of self-assemblies were measured using a JASCO J-815 CD spectrometer with bandwidth of 1.0 nm. UV-Vis absorptions were recorded on an Evolution 201 Thermo Fisher Company, USA.

Attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR) FTIR spectra were collected in transmission mode on a Thermofisher Nicolet iS5 FTIR spectrometer with smart orbit diamond ATR (iD7) and OMNIC software. The rheological properties of gels are measured with a Rotary Rheometer (KINEXUS LAB+, Malvern Company, UK). Fluorescent spectra were recorded on Perkin Elmer LS 55. Laser scanning Confocal Microscopy (LSCM) measurements were performed on Leica TCS SP8 STED 3X. Sonication treatment was performed in a KQ-3200DA ultrasonic cleaner of Kunshan ultrasonic instruments Co. Ltd.

RESULTS AND DISCUSSION

A C_2 symmetrical structure with two heterochiral diphenylalanine arms connected to para-disubstituted phenyl group (named LFDF) was synthesized. The choice of phenylalanine is due to its favorable steric characteristics, which is expected to play a major role in the stereospecific orientation of supramolecular building units and to least steric hinder conformation.[27-29] On the other side, visible enantioselective recognition of chiral amino alcohols has attracted considerable research interests,[30-33] since chiral amino alcohols are usually indispensable as intermediates for synthesizing a variety of biologically active compounds.[34,35] However, the enantioselective recognition of chiral phenylalaninol through chiral supramolecular architectures has not been investigated previously. On account of the prepared chiral supramolecular self-assemblies with different states, we have successfully accomplished the discrimination of *L*/*D*-phenylalaninol enantiomers through heterochiral diphenylalanine-based supramolecular solution, organogel and xerogel. The related results are shown in detail

in Figure 1.

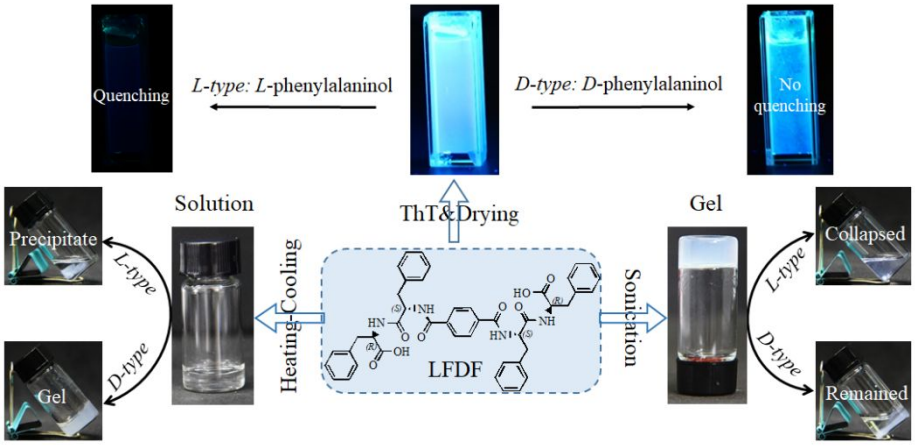


Figure 1. Middle: The molecular structure of prepared gelator LFDF; Left: The supramolecular solution through heating-cooling transformed into precipitate/gel upon L/D-phenylalaninol, respectively; Right: The supramolecular organogel formed under ultrasound experienced collapse with addition of L-phenylalaninol; Up: Fluoresce quenching was observed when dropping *L*-phenylalaninol to the ThT doped fluorescent supramolecular xerogel. *L*/*D*-type represent the disriminate target *L*/*D*-phenylalaninol.

L/*D*-Phenylalaninol Discrimination via LFDF supramolecular solution

Transparent LFDF solution was prepared by dissolving LFDF in ethanol through heating-cooling process. The nanostructure and handedness of supramolecular self-assemblies were confirmed by CD, FE-SEM and TEM characterizations. CD experiments presented a splitting Cotton effect at the position of the π–π* band, with a zero crossing close to the absorption maximum at 250 nm (Figure 2a), indicating the exciton coupling among aromatic chromophores. The existence of chiral nanofibers were further confirmed by FE-SEM and TEM observations (Figure 2b and inset). In

addition, the molecularly dispersed state CD was checked under diluted ethanol solution shown in Figure S3, in which no obvious cotton effect was observed.

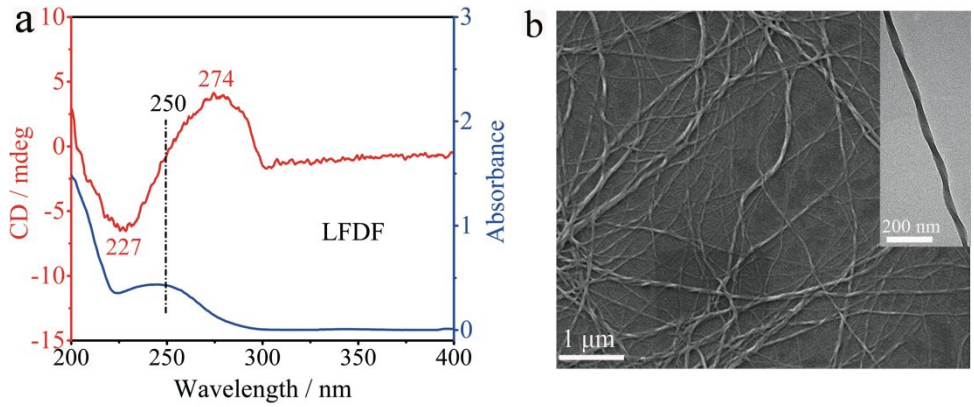


Figure 2. (a) CD spectrum, (b) SEM and TEM images (inset) of supramolecular LFDF self-assembled nanofibers in ethanol.

As presented in Figure 3a, when *D*-phenylalaninol was added to the above prepared LFDF supramolecular solution, a translucent and stable gel was formed under sonication. On the contrary, a totally different situation was observed upon addition of *L*-phenylalaninol, which strongly inhibited gel formation under the same condition. As a blank control, the LFDF solution without *L* or *D*-phenylalaninol was directly sonicated, gernerating a homogeneous and stable gel. To further clarify such different changes response to *L*/*D*-phenylalaninol, the SEM images and CD spectra of the systems were checked. As shown in Figure 3b, II, the nanostructure of the gel formed from pure LFDF solution showed uniform nanofibers, and the same apperance observed for LFDF with *D*-phenylalaninol (Figure 3b, III). While, only a few micron-sized fractions were occurred when *L*-phenylalaninol was appended in (Figure 3b, I). Besides, the significantly different SEM results were also verified by their corresponding CD spectra. The addition of an equal amount of *D*-phenylalaninol induced a distinct

enhancement of the CD signals at 280 nm, with an increase in the intensity from 8 to 17 mdeg, whereas the chiral signal was completely suppressed by the addition of *L*-phenylalaninol (Figure 3b, IV), indicating the destruction of chiral assembly. As shown in Figure S3, compared with the pure LFDF, the shoulder peak around 290 nm in UV-Vis almost disappeared after adding L-phenylalaninol, indicating the breakage of π-π interactions. And only slight derease was occurred when changing L phenylalaninol into D-phenylalaninol. The above results clearly demonstrate that such chiral recognition could be attributed to the difference in the interaction between the LFDF chiral nanaofibers and the enantiomeric phenylalaninol. LFDF supramolecular solution can visually discriminate L/D-phenylalaninol through the observation of precipitate or gel.

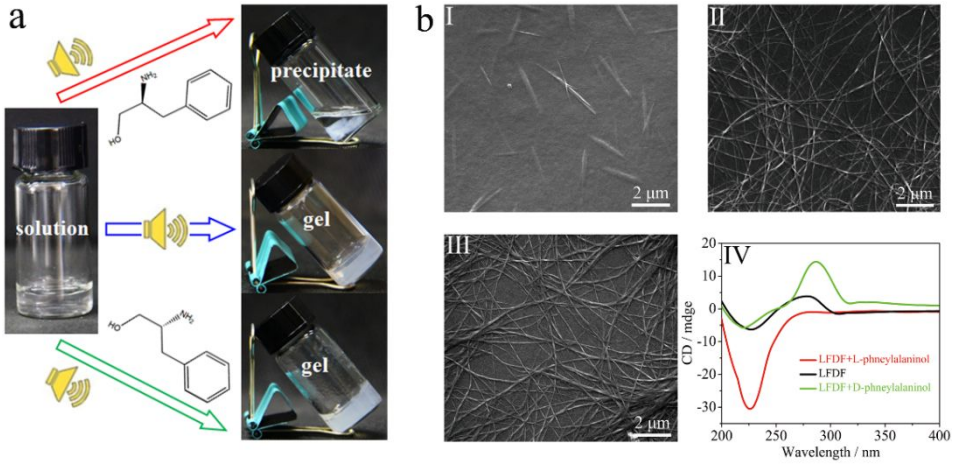


Figure 3. (a) Visual chiral recognition of L/D-phenylalaninol through LFDF supramolecular solution (0.4 wt %) under sonication for 2 min, enantioselective precipitate for L and gel for D-type; (b) SEM images of LFDF+ L-phenylalaninol (I), LFDF (II), and LFDF+ D-phenylalaninol (III) in ethanol after sonication for 2 min, and their corresponding CD spectra (IV).

L/*D*-Phenylalaninol Discrimination via LFDF supramolecular organogels

After the first example of enantioselective gel collapsing reported by Pu,[22] as a new means of visual chiral sensing, chiral supramolecular gel formation and collapsing have been shown to be valuable in visual sensing because supramolecular gels are found to be functional materials with sensitive responses.[36] In this report, unlike the formation of supramolecular solution through heating-cooling, LFDF gelator can directly transform into stable organogel by trapping ethanol under ultrasound at room temperature (gelator concentration: 5 mg/mL), as shown in Figure 4a. The critical gelation concentration of LFDF was measured to be 3 mg/mL. Rheology experiment was performed to investigate the viscoelastic properties of the supramolecular gel. At the condition of strain sweep = 0.1%, both of storage modulus (G′, representing elasticity) and loss modulus (G″, representing viscosity) exhibited an independence to the applied frequency (0.1-100 rad s^{-1}). Meanwhile, G′ values were almost one order higher than that of G″ (Figure S5a), suggesting the formation of stable gel.[37] It was also confirmed by the result of variable-temperature CD, in which the CD intensity under 60 ℃ still kept half of that under 20 ℃ (Figure S5b). Figuring out the sonication-induced self-assembly mechanism could be valuable for understanding the molecular recognition happened to the chiral self-assembly. Thus, we particularly utilized ATR-FTIR spectra and temperature-dependent ^{1}H-NMR to investigate the driving forces that promote LFDF organization. Compared with the molecular state in DMSO, red-shift of carboxyl, amide I (C=O stretching vibration) and amide II (N-H bending vibration) from 1730, 1676/1651, 1543 cm^{-1} to 1718, 1661/1631, 1541 cm^{-1} were obviously occurred in supramolecular gel state, suggesting the existence of strong hydrogen bonds

among amide groups and carboxylic acid units (Figure S5c).[38,39] Despite the molecules that constitute a gel are not observable by solution-state ^{1}H NMR spectroscopy, it is still possible to derive valuable information about the interactions associated with gelation by investigating the chemical-shift variations of the gelator molecule.[40] As shown in Figure S5d, the signals were strongly broadened in the gel state and underwent significant sharpening when the gel was heated from 20 to 60 ℃. Meanwhile, the chemical shift located at 7.726 (attributed to aromatic protons) presented up-field shift to 7.707 ppm. These results strongly suggested that aromatic rings of LFDF were involved into the π-π interaction during the self-assembly.[41,42] In brief, we can conclude that non-covalent hydrogen bonding and π-π stacking afforded by amide, carboxyl groups and benzene rings are the main driving forces for molecular aggregation to produce chiral nanofibers.

As a kind of smart soft material with fascinating properties, the sonication-activated chiral gel is versatile with reversible functionality and has drawn increasing attention as an effective platform for chiral enantiomers recognition.[10,23,43] However, very few examples of naked-eye detectable recognition of phenylalaninol enantiomers has been realized, particularly, based on the supramolecular gel state.

In this part, phenylalaninol enantiomers 1 equivalent weight were introduced into the LFDF supramolecular gel, and the different responses of the gel toward *L* or *D*-phenylalaninol were observed. When *D*-phenylalaninol was appended on the surface of the gel, the system almost maintained stable with only small partial gel-sol transition. Remarkably, gel system totally collapsed into translucent solution in 30 min when *L*-

phenylalaninol was introduced, as shown in Figure 4b, d and S6. We further examined the structural changes during such transitions using the SEM at different time points. It was found that no noticeable changes of LFDF nanostructures were detected with adding *D*-phenylalaninol, that is to say, the system still presented uniform nanofibers without production of any new architectures (Figure 4e and Figure S8). However, along with the time development, obvious phase separation occurred in the LFDF gel with adding *L*-phenylalaninol, in which the non-chiral nano-rods, nanofibers and other aggregates coexisted (Figure 4c and Figure S7).

Self-assembly processes which lead to the formation of helical superstructures can be investigated by CD,[21] herein, the CD was used to dynamically monitor the gel disassembly process. Not surprisingly, only a negligible decrease towards the CD signal intensity in 30 min when adding *D*-phenylalaninol (Figure 5b). However, the Cotton peak at 280 nm decreased rapidly and disappeared completely in 10 min when *L*-phenylalaninol was utilized (Figure 5a). Meanwhile, the corresponding UV-Vis absorbance showed that an obvious blue shift was happened along with the time after adding L-phenylalaninol, indicating the weakening of aromatic interactions (Figure 5c). On the contrary, the UV-Vis absorbance almost kept the same with the addition of Dphenylalaninol (Figure 5d). All these results indicated that the left-handed nanofibers interacted more efficiently with *L*-phenylalaninol than with *D*-phenylalaninol, leading to the disruption of the self-assembly, which were in accordance with the SEM results. Besides, the FTIR and XRD were also checked to determine the variations of molecular interactions and rearrangements. Since the negligible transformation with the addition

of D-phenylalaninol into the gel, herein, we just focused on the changes resulted from the addition of L-phenylalaninol. As shown in Figure S9, the peaks at 1661, 1631 and 1540 cm^{-1} attributed to amide I and II presented obvious blue-shift, suggesting the weakening of intermolecular hydrogen bounding. Meanwhile, as shown in Figure S10, the XRD presented different peaks and intensities that only the main peak at 18 degree remained and the other peaks reduced or disappeared, indicating the changes of molecular arrangements after adding L-phenylalaninol. These results clearly confirmed the visual discrimination ability of the LFDF supramolecular organogel toward chiral phenylalaninol enantiomers. It enables us a better understanding of the assembly behavior of chiral gelators, and can also be used to add new "smart" features to organogels.[36]

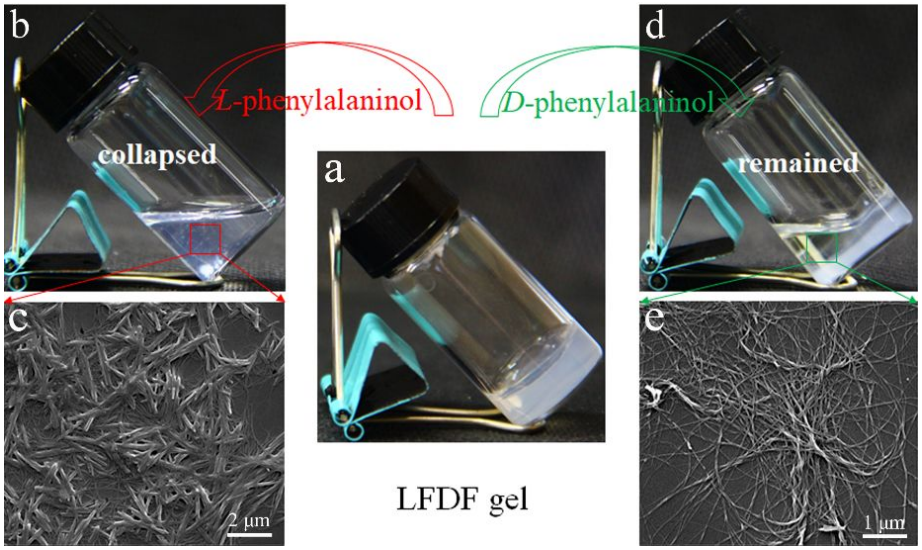


Figure 4. (a) Stable LFDF supramolecular organogel (0.4 wt %) formed under ultrasound; Visual gel collapse for *L*-phenylalaninol (b) and remained for *D*-phenylalaninol (d); and their corresponding SEM images shown in (c) and (e) after 30 min.

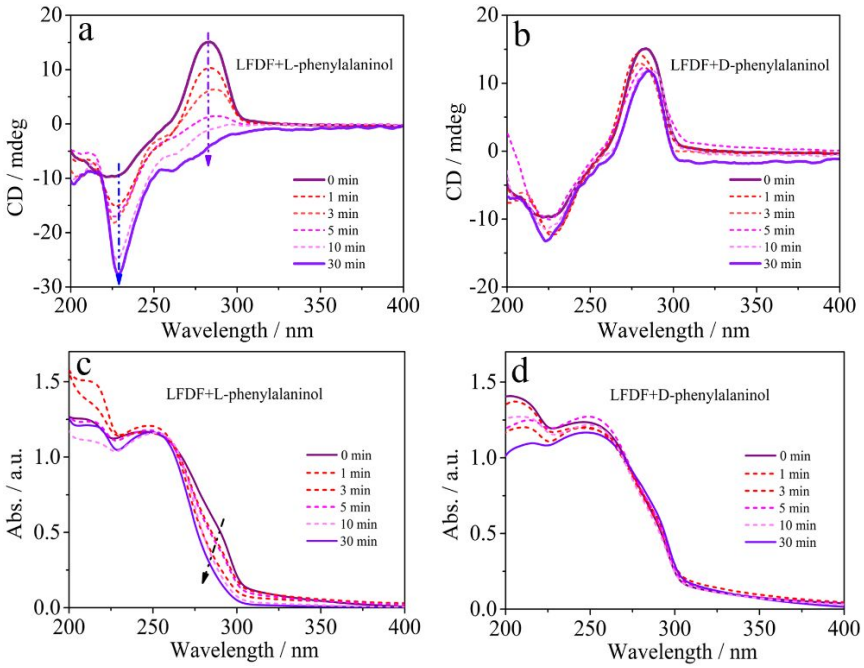


Figure 5. Time-dependent CD spectra of LFDF suopramolecular organogel adding *L*-phenylalaninol (a) and *D*-phenylalaninol (b), and their corresponding UV-Vis absorption (c) and (d).

L/*D*-Phenylalaninol Discrimination via LFDF supramolecular xerogel

Compared with low molecular weight chiral organic molecules, chiral supramolecules based fluorescence sensors can afford a number of benefits for the chiral enantioselective recognition, such as enhancement of fluorescence efficiency and possible collaborative effects between multiple chiral units.[44] In particular, fluorescence variation induced by physical or chemical stimuli is especially attractive because the response process is often instant, switchable, and visible. Moreover, the change in the fluorescence intensity was commonly used to track the assembled or disassembled process in the macro state.[11] Taken together, attribute to the abundant chiral units and the noncovalent versatile interactions in the supramolecular self-assembly, it is thus anticipated that xerogel exhibits high sensitivity towards external

additives.

Take those into account, in this part, a kind of fluorescent xerogel (ThT-LFDF) was facilely obtained by incorporating fluorescent dye thioflavin T (ThT) into the gel, followed by drying at ambient temperature. Therein, ThT can specifically recognize β-sheet second structures, accompanying a strong enhancement of the fluorescence with emission maximum at around 488 nm,[45] whereas no fluorescence for individual LFDF xerogel and ThT (Figure 6a). The fluorescent property of ThT-LFDF xerogel was also verified under ultraviolet radiation (365 nm), yielding bright blue-green fluorescence (Figure 6a inset), which supplies an advantage for highly efficient sensing system based on fluorescence changing. LSCM imaging (Ex. 457 nm) of ThT-LFDF xerogel revealed fibers of several mms in length under bright field, (Figure 6d), and the corresponding green fluorescence (Figure 6c) confirmed the specific binding between ThT and β-sheets.[46]

The rich existence of β-sheet was further confirmed from the CD spectra with a negative peak at about 230 nm. The relative red-shift compared with typical β-sheet second structure occurred at 215 nm might due to the strong π-π stacking of the aromatic phenylalanine residues.[47,48] Different to LFDF xerogel, the CD signal at 270 nm shifted to longer wavelength 290 nm for ThT-LFDF xerogel, suggesting that the aromatic interaction became more compact due to the implantation of ThT into the assembly.[49] Notably, a strong negative Cotton effect emerges at 445 nm corresponding to the maximum absorption of ThT (Figure 6b), indicating that chirality of ThT is induced in the matrix of the helix formed by LFDF.[50]

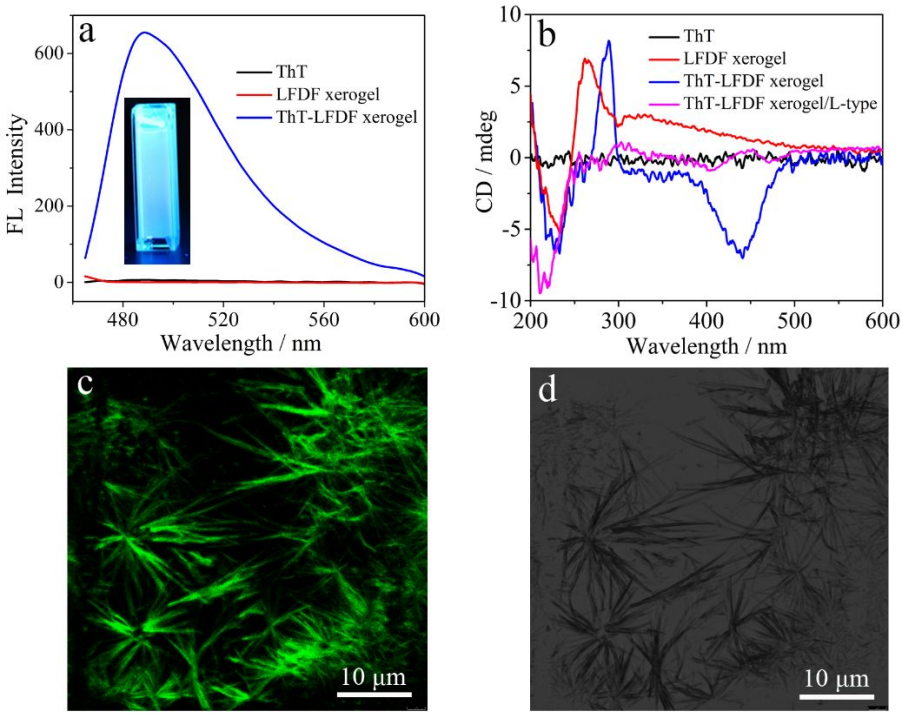


Figure 6. (a) Fluorescence spectra and (b) CD spectra of ThT, LFDF xerogel, ThT-LFDF xerogel and ThT-LFDF xerogel after adding *L*-phenylalaninol, inset in (a) showed ThT-LFDF xerogel fluorescence emission under ultraviolet radiation (365 nm); LSCM images of ThT-LFDF xerogel under ultraviolet irradiation (c) and corresponding bright field image (d).

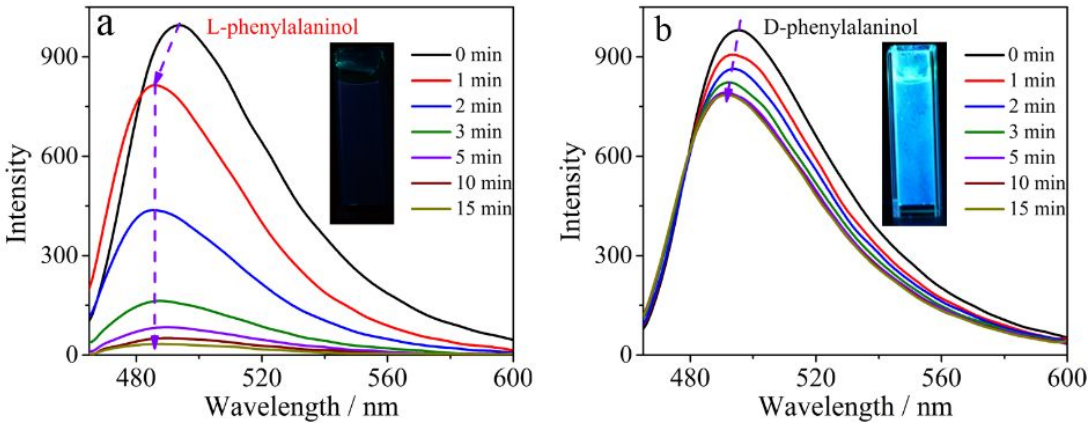


Figure 7. Fluorescence spectra of ThT-LFDF xerogel upon adding *L*-phenylalaninol (a) and *D*-phenylalaninol (b) determined at different time points. Insets: corresponding digital photographs after 15 min at 365 nm irradiation.

Taking advantage of self-assembly as an amplification system for molecular recognition, we assumed that the prepared ThT modified LFDF system could be applicable to a visual, real-time and highly sensitive enantiomer recognition purpose.

As shown in Figure 7a, upon the immersion of *L*-phenylalaninol, rapid and dramatic fluorescence quenching was taken place for ThT-LFDF xerogel in 3 min, and completely disappeared in 15 min, which was clearly visible under UV light. By contrary, in the presence of *D*-phenylalaninol, only a slight fluorescence decrease was detected, consistent with the bright fluorescence emission under UV light (Figure 7b and S11). Compared with an only 19.9% decrease upon the addition of *D*-phenylalaninol, the fluorescence intensity of ThT-LFDF xerogel showed an obvious rapid decrease as much as 96.8 % upon the addition of *L*-phenylalaninol (Figure 7a). The selective recognition of chiral guests is related to the enantiomeric fluorescence difference ratio, *ef*, according to $ef=(I_D-I_0)/(I_L-I_0)$, in which I_0 represents the fluorescence emission intensity in the absence of *L* or *D*-phenylalaninol and I_D, I_L are the fluorescence intensities in the presence of *D*- and *L*-phenylalaninol, respectively.[44] The *ef* value for supramolecular ThT-LFDF xerogel is as high as 4.86, which indicates its excellent enantioselective fluorescence ability towards *L*-phenylalaninol. Furthermore, compared with a gradually slight blue-shift in the presence of *D*-phenylalaninol, a remarkable blue-shift phenomenon was happened when adding *L*-phenylalaninol, which might be attributed to the stronger interaction between LFDF and *L*-phenylalaninol, and eventually causing the disruption of β-sheet second structure and decay of fluorescence. The corresponding CD and UV-Vis spectra of ThT-LFDF

xerogel after adding L-phenylalaninol is checked (Figure 6b and S4), in which two new Cotton peaks at 210 and 220 nm are occurred. The result further suggests the disappearance of beta-sheet second structure.

The chirality difference of enantiomeric *L*- and *D*-phenylalaninol was manifested successfully through the ThT loaded LFDF xerogel, which could be visualized as the distinct FL intensities and accordingly results in the enantioselective recognition. As for its high sensitivity, the detection limit towards *L*-phenylalaninol was carried out through fluorescence quenching titrations by subjecting different concentration of *L*-phenylalaninol to ThT-LFDF xerogel. Each of the spectra was recorded after 15 min of incubation at room temperature. No obvious fluorescence decreases when the concentration lower than $5*10^{-4}$ mg/mL, while, distinct fluorescence quenching was observed when the concentration reached to 10^{-3} mg/mL (Figure 8). Namely, the detection limit of the ThT-LFDF xerogel system for *L*-phenylalaninol is measured to be about 10^{-3} mg/mL (1 ppm), suggesting a good sensitivity for the detection of *L*-phenylalaninol.

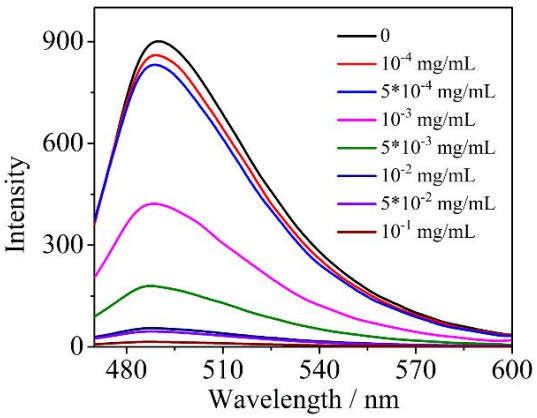
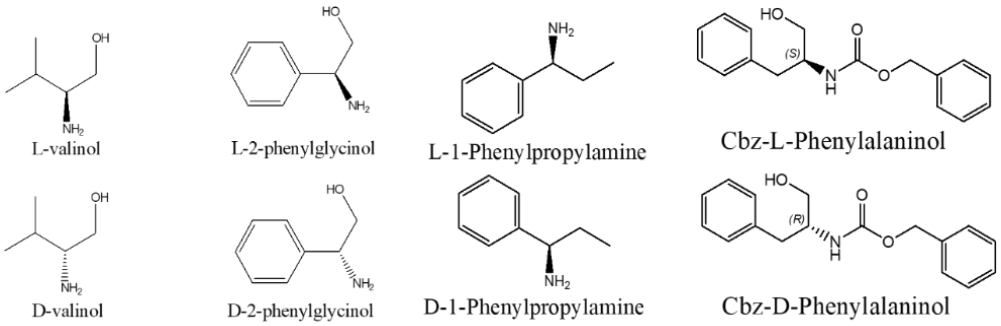


Figure 8. Fluorescence spectra of ThT-LFDF xerogel in the presence of different *L*-phenylalaninol concentrations recorded after 15 min at room temperature.

The recognition mechanism of LFDF towards *L*-phenylalaninol

Molecular recognition is a process in which a molecule selectively recognizes and binds to another substrate molecule through various non-covalent interactions.[51] In a set of comparable experiments, we also studied the behaviors of supramolecular LFDF systems on some other guest enantiomers, like *L*/*D*-valinol and *L*/*D*-phenylglycinol, as shown in Scheme 1. However, no apparent differences were discovered between these two enantiomers, respectively. For *L*-phenylglycinol, the addition into supramolecular LFDF solution, organogel, or ThT-modified xerogel resulted to precipitates, translucent solution, and no fluorescence intensity reduction. And the same phenomenon occurred when using *D*-phenylglycinol. On the contrary, both *L* and *D*-valinol could disassemble the LFDF supramolecular gel and further facilitate fluorescence quenching, without any enantioselective recognition behavior (Figure S12, 13). These outcomes demonstrated that the designed supramolecular chiral LFDF supramolecular system exhibit highly selective response towards phenylalaninol enantiomers.



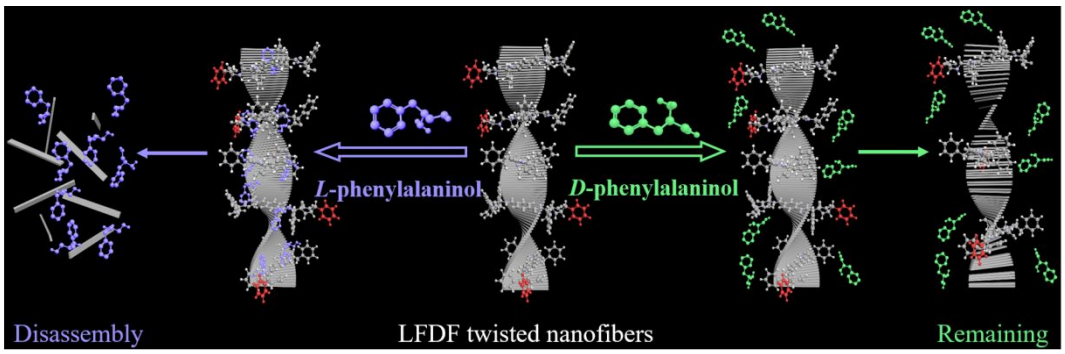
Scheme 1 Molecular structure of the chosen enantiomers.

To verify the decisive role of supramolecular chirality played in the discrimination process, the DFLF gelator, the enantiomer of LFDF was synthesized. Expectedly, in

contrast to left-handed LFDF self-assembly which can discriminate *L*-phenylglycinol, the self-assembled right-handed nanofibers from DFLF is capable of recognizing *D*-phenylglycinol through its supramolecular solution, organogel and xerogel states, accompanied with precipitate formation, gel collapse and fluorescence quenching, respectively (Figure S14). The outcomes above clearly demonstrate that the specific recognition significantly depends on the chirality of the self-assembled nanofibers, which differs to the most reported chiral sensing systems based on noncovalent interactions between an achiral host and an enantiomeric guest at a molecular level.[52] These results exemplify the significant property of chiral self-assembly for the application to enantiomer recognition, which could be identified as the prominent feature realized by the self-assembled supramolecular system.

To further figure out the effects of hydroxyl and amino functional groups during the enantioselective recognition process, we checked the LFDF gel behaviors and CD spectra changes in the presence of phenylalaninol derivatives whose hydroxyl group replaced by hydrogen atom (*L*/*D*-phenylpropylamine) or amino group were protected (*L*/*D*-Cbz-phenylalaninol), as shown in Scheme 1. Upon treatment with *L* or *D*-phenylpropylamine, both the LFDF supramolecular gels collapsed thoroughly without any differences after 30 min. The results were in keeping with the CD spectra of the final reactant mixtures in which the peaks at 280 nm disappeared (Figure S15a). Besides, the gels did not show any damage upon the addition of Cbz-*L* or *D*-phenylalaninol enantiomers. It is worth noting that the corresponding CD signals at 280 nm markedly increased (Figure S15b), which might be ascribed the interactions among Cbz groups

and aromatic residues of Phe. The above results clearly suggested that both the amino and the hydroxyl groups of phenylalaninol played significant functions during the enantioselective recognition process.



Scheme 2 Illustration of LFDF chiral supramolecular nanofibers for discrimination of L-phenylalaninol and D-phenylalaninol.

On account of the expounded recognition results, it's prospective that left-handed nanofibers were more favorable for *L*-phenylalaninol approaching than *D*-phenylalaninol, which might be owing to the minor steric hindrance. The more favorable interactions are markedly amplified into the chiral supramolecular nanostructures, leading to the different responses towards enantioselective phenylalaninol enantiomers, shown in Scheme 2. We therefore propose a very unusual chiral recognition system emerging through chiral self-assembly: that is, a chirality at a molecular level was amplified into chiral self-assembled nanostructures and accordingly leads to a visual output reflecting the initial difference in the enantiomeric information.

CONCLUSIONS

In summary, a C_2 symmetrical diphenylalanine-based gelator (LFDF) was

developed, which could self-assemble into left-handed nanofibers. Based on naked-eye detectable supramolecular gel formation and collapse, *L*/*D*-phenylalaninol enantioselective recognition through LFDF supramolecular solution and organogel was successfully achieved, respectively. Furthermore, by cooperating ThT into the gel, a kind of fluorescent ThT-LFDF xerogel was fabricated, which could instantly discriminate *L*/*D*-phenylalaninol with high sensitivity to 1ppm. Meanwhile, a crucial mechanism toward the recognition was established that left-handed nanofibers were more favorable for *L*-phenylalaninol approaching than for *D*-phenylalaninol owing to the different steric hindrances. In addition, cooperativity in the binding process also played an important role in the observed selectivity. In brief, enantioselective visible recognition of *L*/*D*-phenylalaninol via diphenylalanine-based chiral supramolecular self-assembly on multiple platforms was achieved. We believe that the present work will expand the practical applications of chiral supramolecular assemblies, especially in the fields of visual sensing and screening of chiral drugs.

ASSOCIATED CONTENT

Supporting Information. Synthesis details, additional spectra, characterizations and discrimination results. The Supporting Information is available free of charge on the ACS Publications via the Internet at http://pubs.acs.org.

Notes

The authors declare no competing financial interest.

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