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Enantiomorphic Microvortex-Enabled Supramolecular Sensing of Racemic Amino Acids using Achiral Building Blocks

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Abstract: Chiral analysis of bioactive molecules is of increasing significance in chemical and life sciences. However, the quantitative detection of a racemic mixture of enantiomers is a challenging task, which currently relies on complicated and time-consuming multiple steps of chiral derivatization, chiral separation, and spectroscopic measurement. Herein, we show that, without the use of any chiral molecules and pretreatment steps, the co-assembly of amino acids with achiral TPPS₄ monomers controlled by enantiomorphic microvortices allows for quantitative detection of racemic or enantiomeric amino acids, through analysis of sign and magnitude of supramolecular chirality in different outlets of a microfluidic platform. A simulation model demonstrates that chiral microvortices can induce an initial chiral bias by bending the sheet structure, resulting in supramolecular self-assembly of TPPS₄ and amino acids of compatible chirality by the self-sorting. This enantiomorphically controlled supramolecular sensing system may find versatile applications in chiral sensing.

Chirality is a fundamental aspect of nature, playing important role in chemical and biological processes such as asymmetric catalysis, protein folding, and DNA duplication.[1] Certain bioactive chiral molecules, i.e. amino acids, occur in both L- and D-forms, but exhibiting significant difference in the physiological activities.[2] Chiral analysis of amino acids is of increasing significance in chemical science, pharmaceutical industry, and other related fields.[3] Circular dichroism (CD) spectroscopy is one of the most widely applicable techniques for determining the absolute configuration of chiral analytes.[4] Despite its growing use, CD spectroscopy is difficult to detect amino acids lacking strong chromophoric groups as well as the racemic mixture of amino acid enantiomers, both of which show weak chiro-optical response.[5]

The assembly of chiral amino acids with achiral building blocks can transfer the chiral information to the supramolecular aggregates through the weak intermolecular interactions. For example, D- and L-amino acids promote the formation of J-aggregates of tetra-(4-sulfonatophenyl) porphyrin (TPPS₄) and induce opposite CD signals of the supramolecular assembly.[6] The self-assembly of racemic alanine derivatives leads to formation of supramolecular twisted ribbons, allowing for enantioselective detection of various amino acids based on the chiral interactions.[7] The investigation of self-induced diastereomeric diastereomeric anisochronism effect enables characterization of racemic and enantiopure samples based on their different chemical shifts in nuclear magnetic resonance spectroscopy.[8] In addition, the induction of large chiral hydrodynamic gradients by a stirring vortex can also select the chiral sign of J-aggregates of TPPS₄ in the absence of chiral molecules.[9] The coupling of chiral mechanical effect to the supramolecular system may provide a tremendous opportunity to detect the racemic mixture of amino acids.[10]

Figure 1. Schematic of enantiomorph microvortex-enabled detection of racemic Lys by co-assembly with achiral TPPS₄ monomers. (a) Chemical structures of l-Lys (blue), d-Lys (red), and TPPS₄ (gray). (b) P and M helical microvortices in the opposite microchambers controlling the co-assembly of TPPS₄ and racemic Lys at the initial state, leading to the formation of supramolecular assemblies of M and P chirality, the sign and intensity of which indicate the presence and concentration of racemic Lys.

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Supporting information for this article is given via a link at the end of the document.
Here, we show that the enantiomorph microvortex-controlled co-assembly of amino acids with achiral TPPS₄ building blocks enables quantitative detection of amino acids in racemic or enantiomeric form. Using a microfluidic platform to generate right-handed (P) and left-handed (M) helical microvortices in the opposite microchambers, the chirality of initially formed TPPS₄-Lys co-assemblies is determined by that of microvortices (Figure 1). Through analysis of sign and magnitude of supramolecular chirality in different outlets, the presence of racemic or enantiopure Lys is detected.

For controlled co-assembly of achiral TPPS₄ monomers and Lys, the laminar chiral microvortices were generated within the opposite inclined microchambers of a microfluidic device (Figure S1), while a predominantly P or M chirality of microvortices in the left (L) or right (R) microchambers was verified in previous studies (Figure S2).[11] The mixture of TPPS₄ (20 μM) and racemic Lys (an equimolar mixture of l-Lys and d-Lys, 5 μM) was injected into the two side inlet channels (Inlet 1 and 3) at the flow rate of 30 mL h⁻¹ for each inlet, and the mixture of HCI (1.5 M) and ionic stabilizer C₆H₁₄NH₄⁺ (0.4 M) was injected through the middle inlet (Inlet-2) at the flow rate of 1 mL h⁻¹ (Figure 1b). The acidification of mixing solutions within the microchannel led to symmetry breaking and self-assembly of TPPS₄ with Lys, the process of which was promoted by C₆H₁₄NH₄⁺. Because of the laminar flow characteristics of microfluidics,[12] the majority of co-assemblies experiencing P (or M) helical microvortices in the L (or R) microchambers were inclined to flow out through the L (or R) outlet after 100 ms (Figure S3 and Supporting Information). After keeping the solutions undisturbed for 120 min at room temperature for the growth of TPPS₄-Lys co-assemblies, the appearance of J-aggregates through π–π stacking of TPPS₄ was observed by an absorption peak at 491 nm in ultraviolet-visible (UV-Vis) spectra for solutions collected from both the L and R outlets (Figure 2a). Moreover, the enantiomorph microvortices resulted in almost mirror CD spectra of co-assemblies from the L and R outlets, showing a negative and positive bidisperse Cotton effect centered at 491.5 ± 1.0 nm (Figure 2b). The kinetics of co-assembly process of TPPS₄ and racemic Lys was monitored by atomic force microscopy (AFM) (Figure 2c). A gradual increase in length of supramolecular co-assemblies from 1.0 to 2.5 μm was observed in a time period of 120 min (Figure 2d). In comparison, the length of TPPS₄ aggregates in the absence of racemic Lys increased from 0.8 to 1.8 μm, which was shorter than that of TPPS₄-Lys co-assemblies. Remarkably, the intensity of CD signals (ΔCD = CD$_494$ nm ± 1 nm − CD$_496$ nm ± 1 nm) in TPPS₄-Lys co-assemblies increased with time, suggesting a positive correlation between the size and chirality of co-assemblies (Figure S4).

Next we investigated if it was possible to detect racemic Lys of different concentrations by this enantiomorph microvortex-induced TPPS₄-Lys supramolecular system. The mixture of TPPS₄ (20 μM) and racemic Lys (an equimolar mixture of l-Lys and d-Lys, 0–30 μM) were injected into the side inlets (Inlet 1 and 3) of the microfluidic platform. After microvortex-controlled assembly and hierarchical growth, a negative and positive Cotton effect for TPPS₄-Lys co-assemblies from the L and R outlets were observed in the CD spectra (Figure S5-8). A linear response of the CD signal versus the concentration of racemic Lys from 0 to 30 μM was found for TPPS₄-Lys co-assemblies in both the L and R outlets (Figure 3c). The combination of sign and magnitude of ACD clearly indicated the presence and concentration of racemic Lys.

To illustrate the crucial role of enantiomorph microvortex in detecting racemic Lys, alike experiments using 20 μM of TPPS₄ and 10 μM of racemic Lys were performed in microchambers with rectangular cross-section (Figure S9). In this case, the microvortices displayed no predominant chirality due to the symmetry in chamber geometry (Figure S9a). In the absence of any chiral polarization, the TPPS₄-Lys co-assemblies randomly showed either a positive or negative CD signals from the L and R outlets, which cannot be used to detect the presence of racemic Lys (Figure S9b,c). In contrast, the co-assembly of TPPS₄ with 10 μM l-Lys or d-Lys resulted in the same chiral sign in both outlets, indicated by a negative CD signal for TPPS₄-Lys aggregates, and a positive CD signal for

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Characterization of supramolecular co-assemblies formed by TPPS₄ and racemic-Lys (5 μM). (a) UV–Vis spectra and (b) CD spectra of supramolecular co-assemblies from the L (solid blue line) and the R outlet (dashed red line). (c, d) AFM analysis of growth kinetics of supramolecular co-assemblies (solid circle) and TPPS₄ aggregates (empty circle) up to 120 min. Scale bar, 4 μm. n = 50, mean ± s.e.m.
TPPS-dLys aggregates (Figure S9d,e). Moreover, we performed the co-assembly of TPPS$_4$ and racemic Lys in cuvettes under static and clockwise (CW) stirring conditions (see Supporting Information for experimental details). 15 independent experiments showed 7 positive CD signals and 8 negative CD signals for TPPS$_4$-Lys co-assemblies under static conditions (Figure 3a), while 9 positive CD signals and 6 negative CD signals were observed under CW stirring conditions (Figure 3b). These CD signals with stochastic signs and magnitudes were not indicative of the presence of racemic Lys.

We next assessed the ability of supramolecular system to discriminate Lys in enantiopure form (Figure 4 and Figure S10-13). When the mixture of TPPS$_4$ (20 μM) and l-Lys (10 μM) was added into the microfluidic platform, a negative CD signal was observed in the TPPS$_4$-l-Lys co-assemblies from both the L and R outlets due to the presence of homochiral dopant L-Lys (Figure 4a and Figure S12). In addition, the higher intensity of the co-assemblies from the L outlet with respect to those from the R outlet suggested the formation of more M chiral aggregates in the L outlet. In contrast, the co-assembly of TPPS$_4$ (20 μM) and d-Lys (10 μM) by microfluidics resulted in a positive CD signal correlated with a predominant amount of P chiral aggregates in both the L and R outlets, while the R outlet displayed a higher intensity of the CD signal (Figure 4b and Figure S13). The supramolecular chirality obtained from different outlets in the presence of enantiopure or racemic Lys was summarized in Figure 4c. The two-dimensional chiral signature of TPPS$_4$-Lys co-assemblies was plotted against the L and R outlets in the quadrant, leading to the complete differentiation of l-Lys (L+/R-), d-Lys (L-/R+), and racemic Lys (L+/R+) (Figure 4d).

With the increased concentration of Lys of different forms, the magnitude of CD signals was also increased.

The detection ability of enantiomorphic microvortex-induced supramolecular system was extended to identify a racemic mixture of histidine (His) (Figure S14). The mixture of TPPS$_4$ (20 μM) and racemic His (an equimolar mixture of l-His and d-His, 0–100 μM) was injected into the same microfluidic platform (see Supporting Information for experimental details). The co-assembly of TPPS$_4$ and His resulted in the formation of J-aggregates of opposite chiral signs in different outlets as characterized by CD spectra (Figure S15-18). A negative and positive CD signal corresponding to M and P chirality were observed for TPPS$_4$-His co-assemblies in the L and the R outlet, respectively. The magnitude of CD signals in both the L and R outlets increased linearly with the concentration of racemic His increasing from 0 to 100 μM (Figure S19). This behavior was similar to that of TPPS$_4$-Lys co-assemblies for detecting racemic Lys using enantiomorphous microvortices.

Unlike other supramolecular sensing systems relying on enantiomeric recognition using chiral nanostructures, our detection method applied an enantiomorphic microvortex to control over the emerging chirality of supramolecular co-assemblies of achiral TPPS$_4$ and racemic amino acids. As shown in Figure 5, the rapid mixing of TPPS$_4$, racemic Lys, and HCl in the inlet region of the microchannel resulted in the formation of two-dimensional TPPS$_4$-Lys sheet structure through
The spontaneous mirror symmetry breaking (SMSB) process of amphiphilic porphyrins goes from an achiral monomer substrate to chiral J-aggregates, leading to a stochastic distribution of chiral sign between experiments in the absence of any chiral polarization.\cite{59} This SMSB process is very sensitive to weak chiral polarizations,\cite{17} so that the shear force of hydrodynamic vortexes acting at far from equilibrium conditions can converse a stochastic distribution of chiral signs between experiments in a deterministic one.\cite{58, 9c, 9e} SMSB selection of the chiral sign may also arise by the molecular induction exerted by the induction of a chiral molecule with the monomers and clusters at the bifurcation occurring at the formation of the critical clusters. The effect of a hydrodynamic shear force is an up to down effect and that of a chiral molecule is a down to up effect.\cite{9f} The exploration of both effects in the microfluidic paradigm contributes to discriminative detection of racemic and enantiomeric amino acids.

In conclusion, we present an enantiomorphic microvortex-enabled supramolecular sensing system for detecting the racemic or enantiomeric form of amino acids. The initial chiral bias of TPPS$_2$-amino acid (Lys or His) sheet structure is controlled by chiral microvortices. This chiral bias toward $M$ (or $P$) chirality from the L (or R) outlet leads to supramolecular assembly of TPPS$_2$ and amino acids of compatible chirality. The analysis of the sign and magnitude of supramolecular chirality in different outlets suggests the presence of racemic or enantiopure amino acids. To the best of our knowledge, this enantiomorphic microvortex-enabled sensing system may represent the first example of detecting the racemic mixture of amino acids by one-step supramolecular self-assembly without resorting to any other chiral molecules. We envision that this supramolecular sensing system with unique advantages such as straightforward operation, rapid detection, and no use of chiral additives, can find increasingly versatile applications in chiral sensing.

**Experimental Section**

Experimental details are provided in the Supporting Information.

**Conflict of Interest**

The authors declare no conflict of interest.

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We developed an enantiomorphic microvortex-enabled supramolecular sensing system for quantitative detection of racemic or enantiomeric amino acids, with unique advantages including straightforward operation, rapid detection, and no use of chiral additives.