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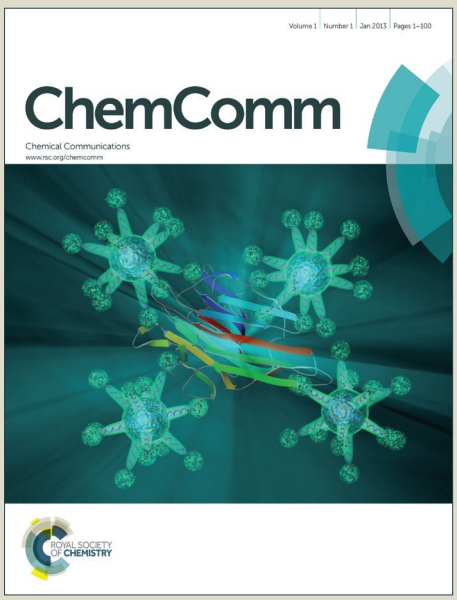

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COMMUNICATION

Chiral Recognition of L-Tryptophan with Beta-Cyclodextrin-Modified Biomimetic Single Nanochannel

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A stable system of enantioselectively recognising L-tryptophan based on ß-cyclodextrin-modified single nanochannel fabricated in a polyimide membrane was demonstrated, which is the first time to realize chiral recognition of essential amino acid with this systems.

In nature, chirality is a widespread phenomenon and plays a crucial role in the metabolism of organisms. The "picky" organisms may exhibit different physiological responses to different enantiomers. That is to say, one isomer is effective, while the other one may be ineffective or even produce some side-effects.[1] For example, the R-enantiomer of thalidomide is responsible for the sedative ability while the S-enantiomer is associated with teratogenic and antitumor properties, which is related to birth defects.[2] The painful thalidomide events always remind scientists to pay more attention to chiral discrimination, which has been an area of considerate research interests because of its importance in almost fields of biological, chemical, and pharmaceutical science in recent decades.[3] In order to realize chiral discrimination, different types of chiral receptors, including cyclodextrins (CDs) and their derivatives,[4] crown ethers[5] and maltodextrins[6] etc, have been developed. Among them, ß-CD has drawn our attention in stereoselective recognition because of its unique properties such as excellent chiral recognition and relatively low cost.[7] Amino acids are biologically important substances, especially essential amino acids, which cannot be synthesized by the organism being considered. L-tryptophan (L-Trp), as an essential amino acid, is so important for human and animals that the unbalance or deficiency of it may cause several chronic diseases.[8] Although various discrimination techniques about enantiomers have been developed in the past decades, such as electrochemical detection,[9] fluorescence detection[10] and so on,[11] high cost, time consumption and complexity limit their practical application.[7, 10b] Therefore, it is valuable and challenging to develop an efficient and cost-effective system to enantioselectively recognize Trp enantiomers.

Recently, biomimetic nanochannels have attracted increasing attention in biotechnology because of their well tunable geometry and chemistry.[12] With proper modification, artificial nanochannels have proven to be a promising platform for the detection of DNA,[13] proteins,[14] glucose,[15] some organic molecules,[16] and even some enantiomers.[17] Although artificial α-hemolysin nanochannels have been employed in enantioselective detection,[17b, 17c] their fragility out of the living environment prompts scientists to develop chemically stable and biocompatible solid-state nanochannels to realize chiral recognization.

Herein, we demonstrate a stable system of enantioselectively recognizing L-Trp based on a single conical nanochannel fabricated in a polyimide (PI) membrane. This system possesses properties of PI membrane with stability[18] and ß-cyclodextrin with excellent chiral recognization to L-Trp, which is the first essential amino acid to be chirally recognised by this biomimetic nanochannel. Such a device can lead to a better understanding of chiral recognition in biological systems and may help develop new techniques in chiral discrimination.[9b]

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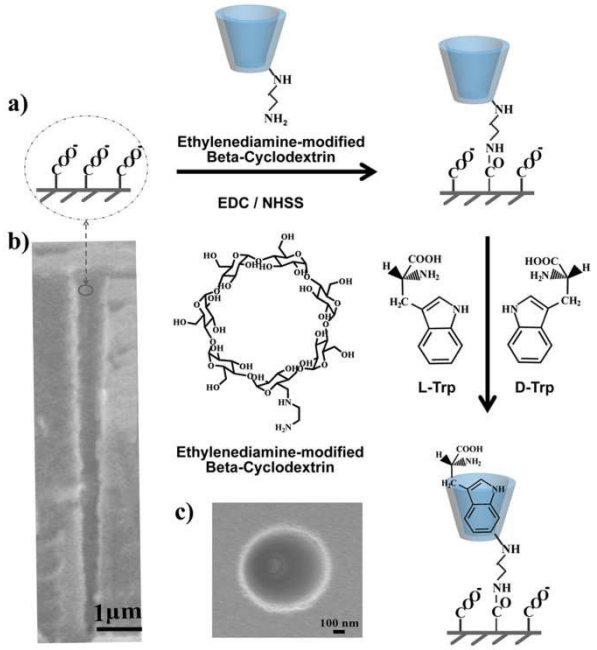

Scheme 1 The operation principle of ß-CD-modified single nanochannel system. a) Enantioselective recognition system of L-tryptophan based on biomimetic single nanochannel. The etched PI nanochannel was modified with ß-CDs, which worked as a chiral receptor for L-tryptophan. When the functional nanochannel was exposed to L-Trp solution, the L-Trp molecules would strongly binds with modifed ß-CDs which resulted in a significant change in the transmembrane ionic current. Meanwhile, D-Trp would not produce a similar change. b) The cross section of typical conical PI nanochannel. c) Enlarged SEM image of single nanochannel from the base side, which was about 770 nm.

Scheme 1a describes the operation principle of this designed system. To achieve the goal of chiral recognition, ß-CDs were modified into the carboxyl-exposed nanochannel by a well-studied EDC/NHSS coupling reaction (see the ESI† for details). The modified ß-CD[19] would adsorbed the Trp by including the hydrophobic aromatic ring into its hydrophobic cavity and leaving the polar part out to interact with the hydroxyl groups and form hydrogen bond of the mouth. Difference in favourability of hydrogen bond formation results in selective adsorption of the L-enantiomer and endows the nanochannel with an excellent capability of chiral recognition.[20] This recognition can be demonstrated by measuring ion current flowing through the nanochannel in 0.2 M phosphate buffer (PB, pH 5.9). Once chemical modification or host-guest complexing occurs, the ion current will accordingly change. Upon exposing this functional nanochannel to the solution, the modified ß-CD would selectively bind with L-Trp, thereby changing the surface wettability and producing some changes in transmembrane ion current. Meanwhile unlike the L-Trp-activated phenomenon, ionic current of nanochannel remained unchanged with the addition of D-Trp or the other aromatic amino acids.[20] Herein, the used single nanochannel was prepared by the well-developed ion track-etching technique with a track-etched PI membrane (Hostaphan RN12 Hoechst, 12 μm thick, with a single ion track in the center).[21] The diameter of large opening (base) of this conical nanochannel was ~770 nm (Scheme 1b and 1c), and the corresponding narrow opening (tip) was ~15 nm (see the ESI† for details).

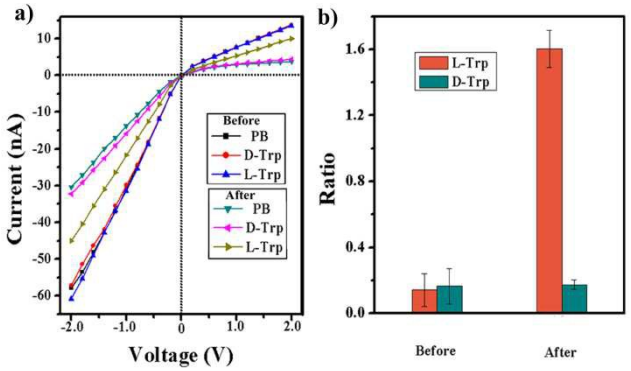

Fig. 1 a) I-V changes of the single nanochannel before and after modifying with ß-CDs in the presence of D-Trp or L-Trp in 0.2 M PB (pH 5.9). b) current change ratios $[(I-I_0)/I_0]$ at +2 V of this designed system before and after modifying with ß-CDs in 0.2 M PB (pH 5.9) upon addition of 1 mM D-Trp or L-Trp. The change in transmembrane ionic current indicates that this functional nanochannel can realize chiral recognition of tryptophan.

Fig. 1a shows the I-V changes of this designed system before and after modifying with ß-CDs in the presence of L-Trp or D-Trp. Firstly, when the unmodified nanochannel was immersed in L-Trp or D-Trp solution, no difference of ion current was observed. After ß-CDs modification, a remarkable increase of ion current from 3.73 nA to 9.94 nA was observed in the presence of 1 mM L-Trp solution at +2 V, while the current almost kept unchanged in the presence of D-Trp. Additionally, the decrease of ~47% in ion current after modification confirmed the success of binding ß-CDs onto the nanochannel surface. Actually, the results of contact angle measurements and X-ray photoelectron spectroscopy (XPS) analysis (see Table S1, Table S2 and Fig. S2 in ESI†) also indirectly revealed that ß-CDs have been modified onto the etched surface of PI membrane successfully. Such a change could be shown by current change ratio (R) (Fig. 1b), which was defined as $(I-I_0)/I_0$, where I_0 and I stand for current at +2 V before and after adding D- or L-Trp solution into system, respectively. Fig. 1b depicts R changes before and after ß-CDs modification in the presence of 1 mM L-Trp or D-Trp. Obviously, the modified nanochannel displayed good chiral selectivity to L-Trp.

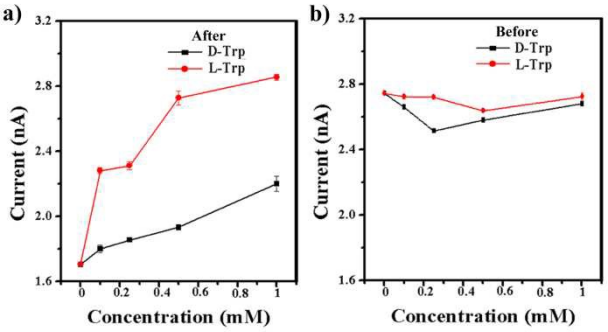

Fig. 2 Current-concentration property of the single nanochannel a) after and b) before ß-CD was immobilized into nanochannel. Both systems were tested with two enantiomers in concentration range 0-1 mM. The modified nanochannel responded differently to two enantiomers, especially to L-Trp, while the unmodified had the similar responses to

them. The result demonstrates that such designed device is a sensitive and useful tool to enantioselectively recognize L-Trp.

It is well known that sensitivity is important for a sensing platform. Fig. 2 shows the concentration-dependent ionic current of this designed system before and after ß-CD modification at +2 V upon the addition of different concentrations D-Trp or L-Trp solution under the same conditions. When the ß-CD-modified nanochannel was tested in a series of different concentrations of D-Trp and L-Trp (0 mM, 0.1 mM, 0.25 mM, 0.5 mM and 1 mM), we found that the nanochannel responded differently to two enantiomers in concentration range 0-1 mM (Fig. 2a). Firstly, the ion current rose rapidly from 1.70 nA to 2.28 nA with increasing L-Trp concentrations from 0 to 0.1 mM, whereas the current changed slowly from 1.70 nA to 1.80 nA for D-Trp. Secondly, at higher concentrations (0.1 mM to 0.25 mM), both of their current growth rates tended to be the same. When the concentration increased from 0.25 mM to 0.5 mM, the system with L-Trp experienced a rapid augment in current from 2.31 nA to 2.73 nA. While the growth rate of D-Trp system kept almost the same as before. Lastly, with the concentration increasing from 0.5 mM to 1 mM, these two systems recovered to the same rate again. Meanwhile when the unmodified nanochannel was tested, no obvious change in ion current was observed in both kinds of solutions at different concentrations (Fig. 2b). Therefore, although the D-Trp also experience current increase in this ß-CD-modified nanochannel system, the difference was large enough to discriminate the L-Trp and made it a sensitive and practically useful tool to enantioselectively recognize L-Trp.

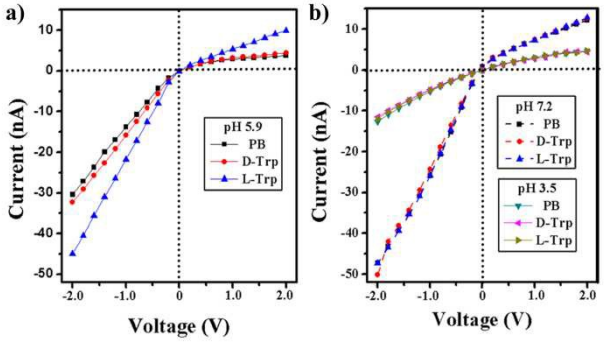

Fig. 3 I-V changes of 1 mM D-Trp or L-Trp at (a) pH 5.9, (b) pH 7.2 and pH 3.5. At pH 5.9, the ion current increased ~50% with addition of L-Trp; while there were no changes at pH 7.2 and 3.5. The outcome implies that the ß-CD-modified nanochannel was capable to recognize L-Trp at pH 5.9.

As shown in Fig. 3, further pH-dependent studies were conducted with this system. When the pH of solution was close to the isoelectric point of Trp (5.89) (Fig. 3a), the Trp molecule was neutral and the surface stayed negative because of the unmodified carboxyl groups, therefore ß-CD could successfully complex with L-Trp whose hydrophilic groups (amino and carboxyl) were exposed from the hydrophobic CD cavity, which resulted in a decrease of contact angle [20, 22] (see Fig. S2 in ESI†) and lead to an increase of ~50% in ion current. At pH 7.2 (Fig. 3b), the addition of Trp did not produce any difference in current, because the negative Trp and negative channel surface existed electrostatic repulsion. At pH 3.5, the L-Trp could decrease surface charge density and decrease the contact angle as pH 5.9, while the ultimate result of ionic current was almost unchanged.[17a] This is to say, the behavior of chiral recognization is the result of changes of surface charge and wettabillity. Therefore, the designed system was capable to recognize L-Trp enantioselectively at pH 5.9.

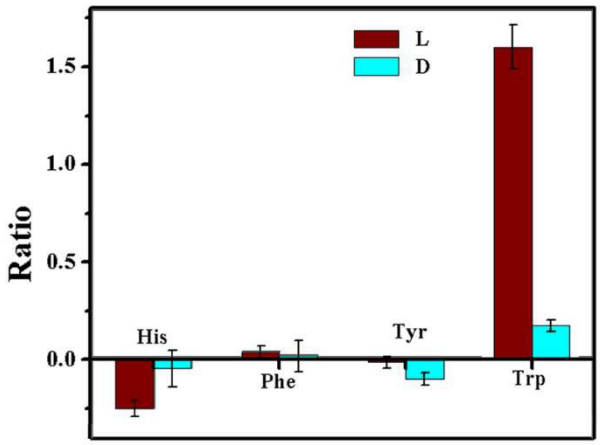

Fig. 4 Current change ratio for the ß-CD-modified single nanochannel with 1 mM L- or D-His, L- or D-Phe, L- or D-Tyr and L- or D-Trp under the condition of 0.2 M PB and pH 5.9, respectively. Among these amino acids, Trp had the most obvious difference of current change ratio. The result indicates that the ß-CD-modified single nanochannel can selectively recognize L-Trp.

The selectivity of this functional system was detected by repeating the same experiments with the other amino acids, including histidine (His), phenylalanine (Phe) and tyrosine (Tyr), under the same conditions, respectively. As Fig. 4 depicts, the designed system was capable to realize chiral recognition to L-Trp and the other parallel experiments did not show similar difference to Trp at pH 5.9. This can be explained by deduction in pH-dependent part. As the isoelectric points of Phe and Tyr are respectively 5.48 and 5.66, at pH 5.9, the amino acids and channel surface are negative to produce electrostatic repulsion, which prevent them from complexing each other. Therefore the addition of both amino acids hardly affected the system's electric property. Moreover, because the isoelectric point of His is 7.59, to some extent, it can be discriminated by this system at pH 5.9.[17a] However, the difference from Trp enantiomers is much larger than His. It is reasonable to conclude that L-Trp can be selectively recognised by this ß-CD-modified single nanochannel at pH 5.9.

In conclusion, we have designed a biomimetic single nanochannel system based on ß-CD-modified PI membrane to enantioselectively recognize L-tryptophan. The biomimetic nanochannel system combined the properties of ß-CD, such as selectivity to L-Trp, with the mechanical and thermal stability of PI membrane. This functional nanodevice provided an excellent sensing platform for discriminating tryptophan, which was the first essential amino acid to be chirally recognized in such a single nanochannel. Furthermore, if appropriate chiral receptor was modified, this PI single nanochannel system may have potential applications in fields such as drug detection and the analysis.[23]

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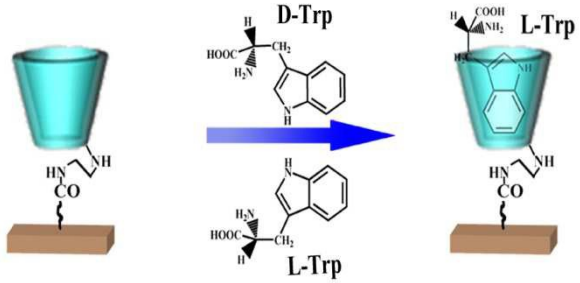

It is the first time to realize chiral recognition of essential amino acid with such a biomimetic nanochannel system.