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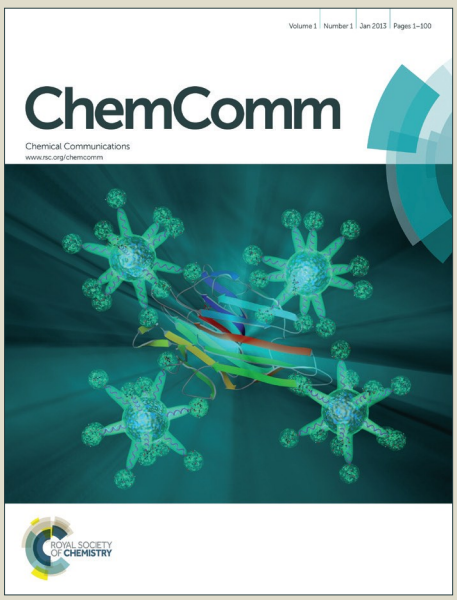





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COMMUNICATION

# Chiral Recognition of Arg Based on Label-Free PET Nanochannel

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**Fabricating chiral arginine (Arg) sensing system with convenient method is significant. Herein, we design a label-free PET nanochannel for enantioselective recognition of Arg by adding bovine serum albumin (BSA) as chiral selector. This method does not require modification on channel surface and has applicability for fabricating other chiral sensor.**

Amino acid which is the building blocks for biological macro-molecular proteins plays vital role in biological process.[1] Among those, arginine (Arg) has attracted much attention because of its significant influence on cell division, human brain chemistry, immune responses, blood vessel dilation and neurotransmission.[2] As we all know, the enantiomer has totally differing effects on biological systems, L-Arg is the precursor of nitric oxide and proteins and it has been used in clinical research.[3] While, D-Arg as one kind of active molecule has significant pharmacological activities in mammalian animals.[4] Nowadays, the major methods for chiral recognition are chromatography[5] and spectrometry.[6] Although these techniques in enantioselective recognition has been achieved during the past decades, but they are generally time-consuming; they require expensive reagents and equipment. Hence, design of structurally simple yet efficient systems for the enantioselective detection of Arg is still a challenge task.

Nanochannels which were inspired by biological channels have drawn enormous research attentions.[7-11] Using modified nanochannels as chiral sensors to directly recognize of amino acids have been exploited.[12] Some of them are based on protein channels but they are not suitable for practical applications.[12b] Comparing with protein nanochannels, synthetic nanochannels have mechanically and chemically robust properties,[13] and they provide a new powerful platform to fabricate enantioselective recognition nanodevice. Recently, Li reported enantioselective recognition of histidine (His) sensing device based on β-cyclodextrin (CD) modified single conical nanochannel in polyethylene terephthalate (PET). [12a] Overwhelming majority of chiral nanochannels sensors need to be modified with functional ligand on the surface. [14] While, after modification the property of nanochannel is not very stable and the ratio of effective modification cannot be controlled precisely.

Here, we design a chiral recognition system based on label-free nanochannels, by adding chiral selector to achieve enantioselective recognition of Arg. Arg has special functional guanidine group, it can interacted with protein the ionized carboxyl groups ($-COO^-$), and forming ion pairs through cooperative interactions. So Arg is commonly used for detection of protein and RNA probe. [15] It is well known that, at pH 7.1, bovine serum albumin (BSA) pI 4.8, was negatively charged ($-COO^-$), it can interact with guanidine group of Arg. At the same time, BSA also can be used to separate enantiomer of drugs, small molecules, amino acids and so on. [16] In this paper, BSA was used as chiral selector to achieve enantioselective recognition of D- and L- Arg. This nanochannel based chiral Arg recognition system, which is simple and requires no external modification on channel surface, provides a strategy to fabricate convenient and stable nanodevice for enantioselective recognition of Arg.

Single conically nanochannels with tip diameter of about 16 nm and base diameter of about 520 nm (Fig. S1) were prepared by chemical etching of 12 μm thick PET (Hostaphan RN12 Hoechst) membrane containing a single ion track in center.[17] The chiral recognition capability between L- and D-Arg was embodied through the changes in ionic current. Before adding BSA, ionic current matured in D- and L- Arg solution nearly the same, while after adding BSA, ionic current changed by different degree. Label-free nanochannel exhibited excellent chiral recognition property after adding BSA as chiral selector.

The property of chiral amino acids recognition in label-free nanochannel was evaluated by ion current measured in 0.03 M phosphate saline buffer (PBS, pH 7.1) (Fig. 1A). The unmodified conical shaped single PET nanochannel at pH 7.1 was negatively charged that because during the chemical etching process, the ionized carboxyl groups ($-COO^-$) were generated on the nanochannel surface, so it presented a slightly rectified ionic current. When the label-free channel was exposed in 1 mM D- and L-Arg in 0.03 M PBS (pH 7.1), the ionic current change at -2V was very slightly. After adding 1 mg/mL BSA into D- and L-Arg solution, there were different changes in the ionic current. When nanochannel was exposed into L-Arg/BSA, a significant decrease in ionic current at -2 V was observed, while in the presence of 1 mM D-Arg/BSA, the ionic current decreased inconspicuous. Fig. 1B shows the ionic current change ratio (defined here as absolute value of the current change ratio at -2 V i.e., $(I-I_0)/I_0$) of label-free nanochannel before and after addition

of 1 mg/ml BSA into 1 mM Arg solution. It is obvious that nanochannel did not exhibit chiral recognition capability without adding BSA into 1 mM D- and L-Arg solution. While with the addition of BSA, it displayed good chiral selectivity. In this system, BSA was chosen as chiral selector to build the chiral recognition system based on label-free nanochannel. Current changes in the nanochannel upon the addition of 1 mg/mL BSA into different concentrations Arg solutions was described in Fig. 1C and S2. Current at -2 V decreased dramatically after addition of (0 to 1 mM) L-Arg solution/BSA (1 mg/mL), and current decrease with concentration of L-Arg solution increase. Currents at -2 V also show a similar decreasing trend via increasing D-Arg concentration, but reductive degree was much smaller than that measured with L-Arg/BSA at same concentration.

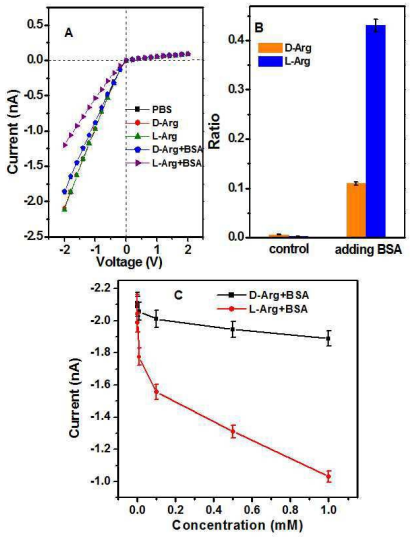



**Fig. 1** (A) I-V curves and (B) current change ratios measured at -2 V of single nanochannel before and after addition of 1 mM Arg and Arg/BSA (1mg/ml) in 0.03 M PBS (pH 7.1) of single nanochannel. (C) Current-concentration (I-C) properties of label-free nanochannel exposed in different concentration of D- and L-Arg (0 to 1 mM) with addition of 1 mg/mL BSA. Before modification, diameter of tip is about 16 nm. This nanochannel can achieve chiral recognition of Arg.

In aqueous solution, BSA is existence in nanoparticles form. Therefore, morphology is an important aspect in reflect the selectivity of nanoparticles with analyte at molecular level. Hence, AFM studies were performed on D-Arg/ BSA, L-Arg/ BSA (Fig. 2A, B). In AFM images on mica surface, D-Arg/ BSA complex covered mica evenly and sizes of them are about 6-10 nm in apparent diameter (Fig. 2A1, B1). While a lot of agglomerates (10-36 nm) appeared in L-Arg/ BSA complex (Fig. 2A2, B2). The difference in nanostructural features confirms that the interaction of L-Arg with BSA is stronger than D-Arg, and BSA is a useful chiral selector to bonding L-Arg selectively.

The formation of agglomerates appeared in L-Arg/BSA complex may because BSA selectively interacted with L-Arg, the charge of BSA changed, so stability and electrostatic repulsion among BSA decreased and, thus, caused aggregation. So it is necessary to investigate charge change of Arg/BSA complex. We measured Zeta potential to investigate charge change of D- and L-Arg/BSA solution respectively (Fig. 2C). At neutral pH values, BSA (pI 4.7) is negatively charged. Zeta potential of 0.1 mg/mL BSA at pH 7.1 was about -12.6 mV. When adding 0.4614 mM D- and L-Arg solutions, zeta potential changed into -8.57 and -6.27 mV respectively. Comparing with adding D-Arg, charge of BSA sharply reduced with addition of different concentration of L-Arg solution. That may because BSA selectively interacts with L-Arg. So the negative charge of L-Arg/BSA complex reduced significantly than D-Arg/BSA complex.

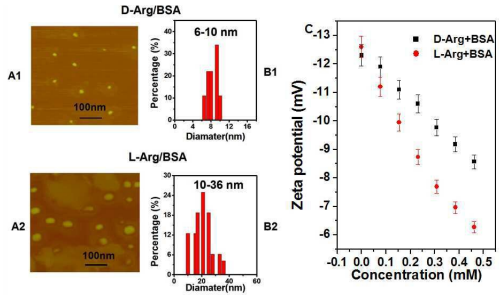


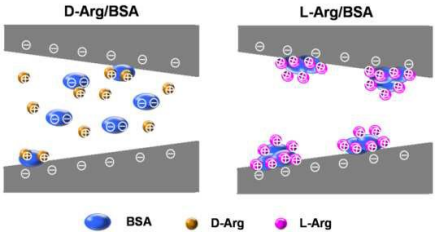

**Fig. 2** AFM images of (A1) D-Arg/BSA, (A2) L-Arg/BSA complexes absorbed on mica surface (tapping mode AFM) and corresponding (B1, B2) size distributions. (C) Zeta potential measurement of different concentrations of D- and L-Arg solution (0-0.4614 mM) with 1 mg/ml BSA in 0.03 M PBS (pH 7.1). Addition of BSA can realize enantioselective recognition of L-Arg.

The pH influence on enantioselective recognition of Arg was also investigated. We investigated I-V curves and current change ratios of nanochannel before and after addition of BSA at different pH values (using 0.1 M KCl as the electrolyte and NaOH and HCl to adjust pH) in Fig. S3. At pH 10, with addition of Arg/BSA complexs, the I-V curves changed only a little, but it still showed enantioselectivity to chiral Arg. That because at pH 10, close to the isoelectric point of Arg (10.76), Arg molecules tack very small amount of positive charge, so the negative charge of BSA decreased very small. At the same time, BSA preferential interacted with L-Arg and, thus BSA bonding much more L-Arg than D-Arg. So the negative charge of L-Arg/BSA complex reduced larger than D-Arg/BSA complex. At pH 7.3, when film was exposed into L-Arg/BSA, a significant decrease in current at -2V was observed, while in the presence of D-Arg/BSA complexs, ionic current decreased slightly. This phenomenon shows a good agreement with experimental results in Fig. 1. The previous study have shown that BSA can readily adsorb on channel surface by hydrophobic interaction at pH 3,[18] so pH influence on enantioselective recognition of Arg was not investigate at pH 3.

From above results we suggested the possible mechanism (Scheme 1) of chiral recognition. At pH 7.1, Arg and BSA (pI 10.76 and 4.8) was positively and negatively charged respectively. Arg has special functional guanidine group, it can interacted with protein the ionized carboxyl groups ($-COO^-$), and forming the ion pairs to neutralize the negative charge of BSA. BSA preferential interacted with L-Arg and, thus BSA bonding much more L-Arg than D-Arg. So the negative charge of L-Arg/BSA complex reduced larger than D-Arg/BSA complex, which was confirmed by Zeta potential measurement, therefore the electrostatic

repulsion between L-Arg/BSA complexes and the negatively charged channel surface decreased obviously. At the same time, larger aggregates (L-Arg/BSA complex) were formed. Thus L-Arg/BSA complex was easily absorbed on channel surface and partial blockage of nanochannel. Hence, ionic current decreased significantly when exposed in L-Arg/BSA solution, while in presence of D-Arg/BSA solution, ionic flux decreased a little.

The enantioselective recognition and L-Arg/BSA complex absorbed on the channel surface was also verified by laser scanning con-focal microscopy (LSCM, Fig. 3B) focused on the inner membrane surface. We used Arg fluorescent derivative (Arg-F), which was synthesized by derivatization of the α-amine group with 5-(dimethylamino) naphthalene-1-sulfonyl chloride (DNS-Cl)[19] in Fig.3A to interact with BSA. Porous PET membranes was filled with D-Arg-F/BSA and L-Arg/BSA complexes solution for 1 h. On nanochannel surface, the significant fluorescence signal was too weak to observe, when PET membrane immersed in D-Arg-F/BSA complex solution. While strong fluorescence signal appeared, after film was immersed in L-Arg/BSA complexes solution (Fig. 3B). The data indicated that BSA enantioselective interacted with L-Arg-F and L-Arg-F/BSA complex absorbed on channel surface. So the the strong fluorescence signal appeared.




**Scheme 1**. Enantioselective recognition of D-, L-Arg by adding BSA as chiral selector.

Moreover, the selectivity of chiral detection among D- and L-Asp, Glu, Trp, Tyr, Leu, Thr and Arg (Fig. S4) was also investigated. It clearly shows that this system showed a high selective on chiral recognition for D-, and L-Arg. That may because other chiral amine acids don't have guanidine group they may not forming ion pairs with BSA. And at pH 7.1, other chiral amine acids and BSA were all negatively charged, if they may interact with BSA, the negative charge of BSA/amine acids complex will increase, so the electrostatic repulsion between BSA and channel surface enhance, so they cannot absorbed on channel surface and influence the current.

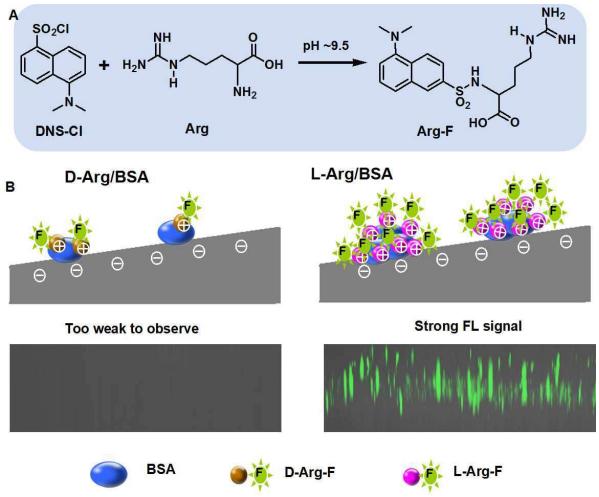



**Fig. 3** A) reaction equation of Arg derivatization with DNS-Cl; B) The enantioselective recognition process and LSCM observation of the Arg fluorescent derivative D-Arg-F/BSA and L-Arg-F/BSA complex in nanochannel. The strong fluorescence signal that appeared indicated that L-Arg-F/BSA complex absorbed on the channel surface selectively.

## Conclusions

In summary, we have designed a label-free nanochannel for enantioselective recognition of amino acids by addition of chiral selector. This method expands potentialities of chiral nanochannels sensors, adding chiral selector into enantiomer solution with label- free nanochannel would also provide a new strategy and universal applicability platform for fabrication of enantioselective recognition nanodevice. Furthermore, this label-free nanochannel system may has potential applications in fields such as chiral separation of drugs. And it also has general applicability for fabrication of other chiral sensing device.

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## Notes and references

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