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Applications of polymer single nanochannels in biosensors

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There are many elaborate masterpieces exist in natural world. Learning from nature, people developed serial intelligent biomimetic devices. Biomimetic smart nanochannels received widespread attention for mimicking biological processes in bodies. Excellent stability, tailorable surface characteristics and nano-size effects rend polymer single nanochannel an ideal candidate for constructing sensitive and reproducible biosensors. Nanochannels are responsive for special analytes while appropriate recognition elements are modified in channels wall. In this review, we summarized recent works in contructing biosensors that are using polymer single nanochannels for detecting various analytes.

polymer, single, nanochannels, biosensors, ion track-etching

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Biological ion channels located at the cell walls and composed of protein complexes could open and close in response to external stimuli for dominating ion translocation and play crucial role in the creature metabolism process [1]. Inspired by ion channels, researchers have developed various kinds of artificial nanopores or nanochannels to construct biosensors or other devices [2–13]. Strictly speaking, under the premise of insuring at least one dimension in the range of one to several dozens of nanometers, pore diameter larger than its depth is simply defined as nanopore while oppositely defined as nanochannel. The nanochannels are more suitable for biosensing than nanopores due to its larger contact area with bio-molecules [14]. Biological materials such as lipid bilayer confront limitation for further applications in biosensors due to their unsatisfied physical stability, therefore solid-state nanochannels are expected to conquer the drawbacks. Tailorable synthetic nanochannels possessing great physical stability and mechanical robustness meet the increasing demands on imitating biological ion channel research. It is easy to obtain and the size of nanochannels can be accurately controlled, and dimensions comparable to biological molecules also makes it a preferential choice for extremely sensitive biosensors [15]. Throughout the artificial nanochannels field, polymer single nanochannels behave unique advantages in nanoscale analytical sensing applications. Synthetic polymer single nanochannels functionalized with special molecules can be designed as specific biomimetic nanosensors [16–19]. Both the physical properties and the chemical properties especially channel inner surface characteristics make them suitable for sensing. Modification of channel surface properties plays an essential role in biosensing performance because incorporated groups serve as recognition sites for numerous passing analytes [20]. The inherent virtues of polymer materials simplified the modification process. For example, chemically etched PET single nanochannel generates carboxyl groups on the inner wall. During the chemical modification procedure, the carboxyl groups provide combination sites for introducing chemical functionalities which are vital for sensing [21]. Solvents or reagents that would be harmful or incompatible with the polymer surface or biomaterials were unnecessary for incorporation of external functionalities in the nanosacle confinements. Compared with multiple nanochannels, single nanochannel performing

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as a sensitive sensor provides an ideal platform for probing transport properties of trace analytes. The behavior of an individual analyte crossing a specific nanochannel can be studied clearly without averaging the effects of multiple nanochannels [22].

At present, the most widely used polymer materials for single nanochannels preparation are polyethylene terephthalate (PET), polyimide (PI) and polycarbonate (PC). Single nanochannel based sensors are usually fabricated in a polymer membrane by latent ion track-etching technique [23,24]. Polymer membranes were penetrated by barely one high energy heavy ion forming single connected damage latent track [25], followed by proper chemical etching treatment. Etching procedure is monitored by applying voltage at both sides of conductivity cells. Advisable etching parameters like concentration of the etchant, temperature and etching duration are beneficial for trimming channel structures at nanoscale precision. There are two major approaches for chemical modification of ion-track etched polymer nanochannels. Firstly, amide bonds between inherent carboxyl groups on channel surface and amino-terminated biorecognition molecules are accomplished in present of the coupling reagent 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) [26]. Secondly, electroless deposition of gold followed by chemisorptions of thiol derivatives onto the gold surface. Successful chemical modification can be confirmed by recording current-voltage curves [27–31].

This review is focused on recent applications of single track-etched polymer nanochannels for constructing biosensors. In this review, we summarized various polymer single nanochannel based biosensors on the basis of different kinds of analytes. Adoption of emerging nanochannel technology is beneficial to develop novel detection methods aiming at typical biomolecules, metallic ions and other analytes.

1 DNA sensors

DNA sequencing is one of the most prominent technologies in genetics. Single nanochannels have attracted enormous attention and imposed profound effects on DNA sequencing since 1990s [32,33,36]. DNA segments spurred by applied voltage pass through a proper nanochannel leading to transient obstruction of the nanochannel, hence the transmembrane ionic current decreased partially compared with its initial magnitude [34,35]. Efforts by John J. Kasianowicz and co-worker [37] were regarded as the first attempt to detect single DNA segments with a single nanochannel which made by α-hemolysin decorated lipid bilayer. Although it made distinction between homo- and block polynucleotides with different base compositions, the major limitation of nanochannel's backbone embodying in electrical leakage currents, high capacitance per unit area and the innate physical instability of the lipid bilayer switched people's eye on synthetic nanochannels [38].

For the purpose of improving performance of nanochannel based DNA sensors, Kamme's group [39] fabricated a conically shaped single nanochannel in a 12 μm thick kapton membrane by ion track-etching method. The diameter of the conical channel's small opening is 4 nm, providing an optimized size for DNA translocation studies. Various DNA fragments with different lengths can be separated by the nanochannel sensor. Primitive transmembrane ionic current is 710 pA under 120 mV applied voltage before DNA added, and the smooth line showing that there was no blockage in the nonochannel (Figure 1(a)). After 284 bp and 4.1 kb DNA fragments were added at the small opening of the kapton membrane, some decrease of ionic currents were close to 70 percent of the primitive values (Figure 1(b)). In view of the extreme narrow size of the nanochannel, only one double-stranded DNA molecule was expected to translocate at a time. Different decreases of currents recorded at 20000 ms time duration reflected different extent of blockages and conformational changes when the DNA fragment passing through the nanochannel. "Train" effect implying two or more blockage events occurred within 2 ms was observed in the data collecting process. This fancy phenomenon may be explained as the hybridization of double-stranded DNA fragments' sticky ends.

Similarly, outstanding works were carried out by using resistive pulse current method. Martin's group [40] proposed single PC conical nanochannel for separating single-stranded DNA and double-stranded DNA. The single-stranded DNA pass through the pore inducing transient blockage of the ionic current while double-stranded DNA

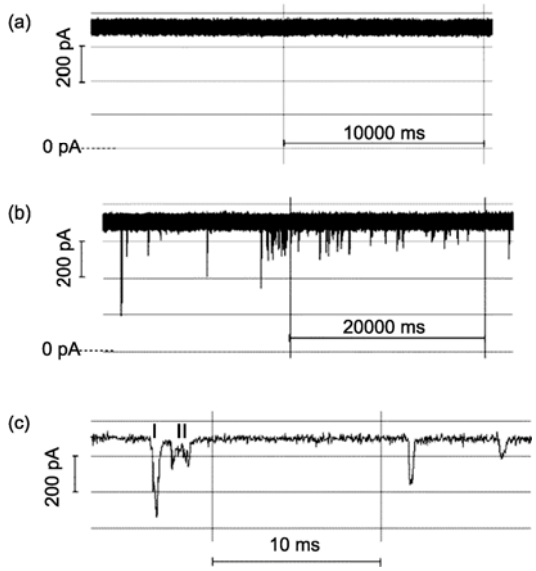

Figure 1 Resistive pulse current recording in 1 mol/L KCl. (a) With 284 bp and 4.1 kb dsDNA fragments. (b) Without 284 bp and 4.1 kb dsDNA fragments. (c) 284 bp and 4.1 kb dsDNA fragments added to the small side result in double event, or so called "train" event with various shapes of blockage at high resolution. Reprinted with permission from [39].

can hardly cross the pore. Siwy and co-worker [41] presented conical PI nanochannel for individual DNA sensing measurement. Various pulse current signals reflect varying extent blockages induced by different DNA configurations while passing through the pore. Conical PET channel sensor prepared by Martin and co-worker [42] that bound with neutral and hydrophilic ethanolamine could be applied for short double-stranded DNA sense. DNA molecules containing 50 and 100 bp can be separated by distinguishing resistive pulse signals.

In contrast to the resistive pulse current based DNA sensor mentioned above, Ensinger's group [43] developed a novel PNA/DNA hybridization sensor based on the alteration of *I-V* curves and current rectification. A single conical nanochannel was formed by chemical ion track-etching technique in a 12 μm thick PI membrane. As shown in Figure 2, uncharged peptide nucleic acid (PNA) probes can be covalently linked to the channel's inner wall profit from the exposed carboxyl groups after etching [44]. PNA probes serving as shielding layer dilutes effective surface charge density and considerable depression of transmembrane ionic current was observed consequently. PNA probes act as binding sites for negatively charged single-stranded DNA [45–47]. PNA/DNA hybridization was confirmed by increased ionic current and current rectifications. Moreover, Martin and co-worker [48] proposed gold plating PC nanochannel for selective DNA transport. Fixed DNA-hairpin molecule could selectively recognize and transport complementary DNA strand.

2 Ion sensors

Certain ion channels exist in muscle cells and nerve cells regulate metallic ions concentrations in a proper level. Normal metabolism strongly depends on such kinds of ion channels [49–53]. Biomimetic polymer single nanochannels provide a great platform for imitating biological ion channels, which reveal these processes occurred in organisms.

Inspired by such ion channels, Jiang's group [54] proposed a potassium-responsive nanochannel. The effective pore size of the single channel depends on conformational changes of the G4 DNA chains. As shown in Figure 3(a), loosely packed single-stranded G4 DNA transformed into rigid i-motif structure while K^+ was added [55,56]. Therefore, reduced conductance observed due to partially blocked small opening. Hybridization of the complementary DNA strands with G4 DNA generating the rigid double-strands shaped DNA structure and showing smaller currents. On the basis of above research, another outstanding work reported by the same group [57] proposed biomimetic zinc activated ion channels which based on the modification of zinc fingers on the channel wall. The Zn responsive mechanism was clarified clearly in Figure 3(b). Zinc finger protein molecules cause volumetric obstruction leading to weak conductance. After Zn^{2+} was added, the channel was activated showing an obvious increase in ion conductance. Zinc finger proteins shrank to finger-like conformations, thus enlarged the effective pore size [58]. Appropriate opening size of nanochannel is necessary for a successful zinc biosensor. Zinc finger proteins cannot transform to folding state when the channel opening size is too small. Moreover, the folding protein cannot reduce the effective pore size if the opening is too large. Control experiments carried out with other ions and control peptides showed that zinc fingers specifically react with Zn^{2+} rather than Ca^{2+} or Mg^{2+} (Figure 3(c)). This kind of biosensor not only imitated biological conformational alteration from natural ion channels but also sophisticated component.

For the purpose of investigating polyvalent ions on

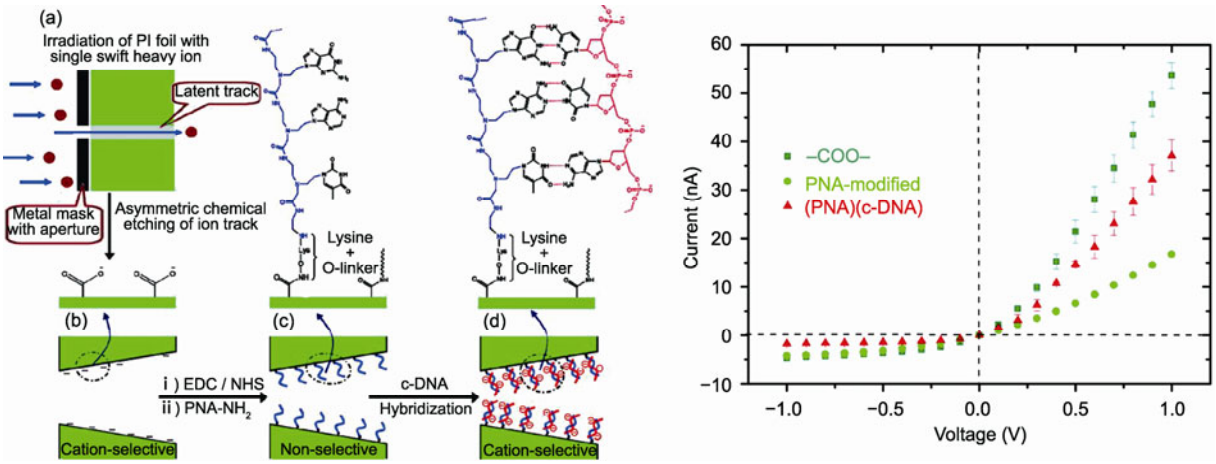

Figure 2 (Color online) (a) Schematic of fabrication of ion track-etched polymer nanochannel after irradiation with single swift heavy ion. (b) Fabrication of a single conical nanochannel. (c) Amide linkage between inherent carboxyl groups and amino-terminated PNA probes. (d) Hybridization of ssDNA with the fixed PNA probe. (e) Current-Voltage curves measured in 0.1 mol/L KCl (pH 7.6) solution of as-prepared conical channel bearing $-COO^-$ groups (■), modified PNA probe (●), and PNA/DNA hybridization (▲) on the channel surface, respectively. Reprinted with permission from [43].

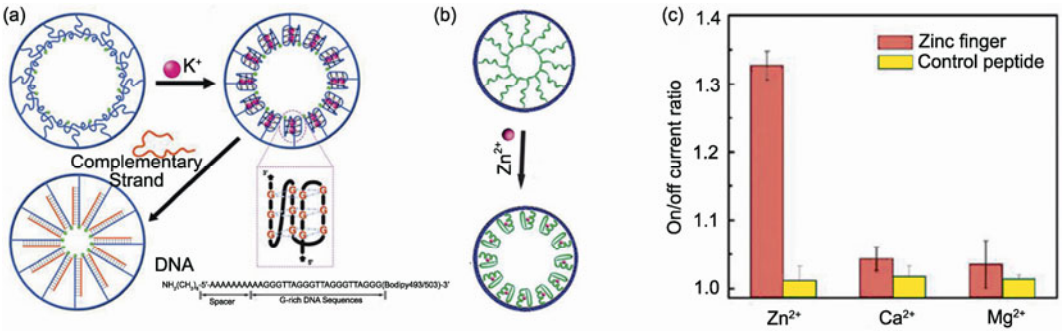

Figure 3 (Color online) (a) Conformational transition of G4 DNA immobilized in a single nanochannel from a loosely packed single-stranded structure to a rigid quadruplex structure in response to K^+ and the close arrangement of the double strand after the addition of complementary DNA strands. (b) Scheme of effective pore size change caused by conformational transition of modified zinc fingers in single nanochannel. (c) On/off current ratios of the zinc fingers and the control peptides modified in nanochannels interacting with different ions (measured at −1 V); Reprinted with permission from [54,57].

channel current-voltage characteristics, Siwy's group [59] discovered that addition of small amount of Ca^{2+} to the monovalent ionic solution leading to an oscillating transmembrane current through the conical nanochannel. Instantaneous formation and dissolution of nanoprecipitates temporarily block the ions passing through the channel. The obtained *I-V* curves exhibit nonlinear fashion and negative incremental resistance observed at negative voltages (Figure 4(a) and (b)). Next year, Siwy and co-worker [60] reported conical PET nanochannel placing at asymmetric electrolyte conditions. Polyvalent cations like Ca^{2+} and trivalent cobalt sepulchrate with different species and concentrations were filled in each sides of conical channel, inducing neutralization and inversion of the surface charge polarity. Only when the polyvalent concentrations are high enough, inversion of surface charge polarity can be accomplished. Selectivity and transport functions can be adjusted by reversible charge inversion effects (Figure 4(c) and (d)).

3 Protein sensors

Polymer nanochannel based protein sensors confront crucial challenges for chemical modification of bio-recognizable elements and biomolecular conjugations treatments. Generally speaking, there are two widely adopted routines for attaching biomolecules on the channel walls. Firstly, amino-containing biomolecules can be covalently attached on the carboxylic channel surfaces by forming amide bonds. Secondly, electrostatic self-assembly of charged protein

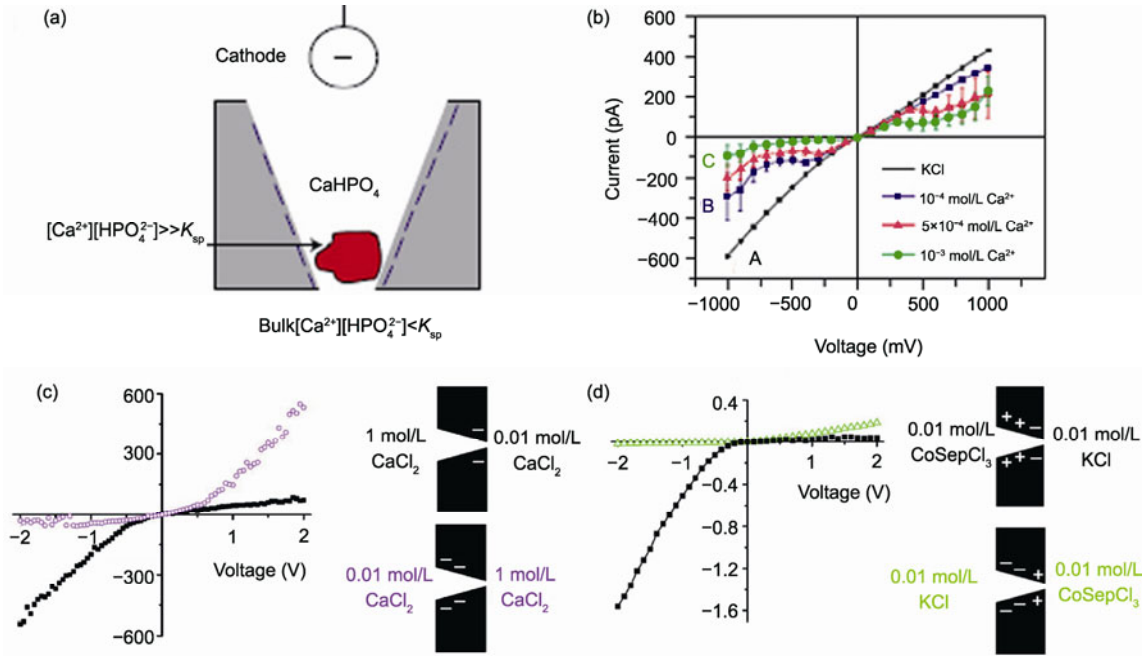

Figure 4 (Color online) (a) Current-voltage curves recorded at asymmetric Ca^{2+} concentrations. (b) $[CoSep]^{3+}$, K^+ placed at each side of the single conical channel with equal concentrations. (c) Current-voltage curves recorded in asymmetric Ca^{2+} concentrations. (d) Current-voltage curves recorded with 0.01 mol/L $CoSepCl_3$ placed on one side of the membrane and 0.01 mol/L KCl on the other side of the membrane. Reprinted with permission from [59,60].

also considered as an effective method for conjugation between introduced analytes. Martin's group [61] reported protein sensor which behave covalent linkage between thiol-terminated biomolecules (such as ligand-receptor and protein-antibody) and gold deposited polymer channel surface. Ensinger's group [62] explored noval interactions for conjugation of biomolecules on the channel wall. In his work, mannose-riched Horse Radish Peroxidase (HRP) enzymes were attached to the channel wall by carbodiimide coupling chemistry. After that, lectin-riched Concanavalin A molecules react with HRP by sugar-lectin affinity. As shown in Figure 5(a), ionic conductance of the channel decreased with addition of HRP and Con A due to the blocking effects in the nanoscale confinement that described quantitatively in accordance with pore radii changes despite the channel shape. The cylindrical nanochannel exhibit linear *I-V* characteristics in contrary to obvious ionic rectification observed from conical nanochannel at pH 7.6. Both HRP and Con A were negatively charged at neutral pH circumstances, so *I-V* curves in Figure 5(b) perform the equivalent rectify direction. The specificity and reusability of the protein sensor were confirmed according to Figure 5(c) and (d). The evident increases of ionic conductance were obtained after free glucose was added in Con A–HRP conjugation nanochannel system. However, galactose can hardly lead to significant alteration in ionic conductance. All the above results verified the truth that Con A shows high affinity towards free glucose instead of galactose.

Siwy's group [63] invented antibody-protein complex system in a single conical PET channel for the purpose of detecting poly-γ-glutamic acid (γDPGA) from the capsule of Bacillus anthracis. Monoclonal antibody (mAb F26G3) was covalently attached to the conical channel's small side. The as-prepared sensor exhibits severe dependence on ambient pH circumstances. As shown in Figure 6(a), rectification direction of the sensor reversed when pH changed from high to low. Linear *I-V* curve at pH 6.0 implies that the isoelectric point of the monoclonal antibody is about the same value, and free charge of the channel's small side. Fully protonated nanochannel with acidic solution leaded to enormous augment on rectification degrees comparing to alkali environment. After γDPGA was introduced into the biosensing system, distinctive rectifying behavior emerged at the corresponding pH situations mentioned before. We are informed from Figure 6(b) that analyte conjucted sensor showed a cation-selective characteristic at pH values exceeds 4.2. This can be explained as deprotonated polypeptide resulting in profoundly negatively charged nanochannel surface when pH >4.

PI membrane provides an excellent alternative platform

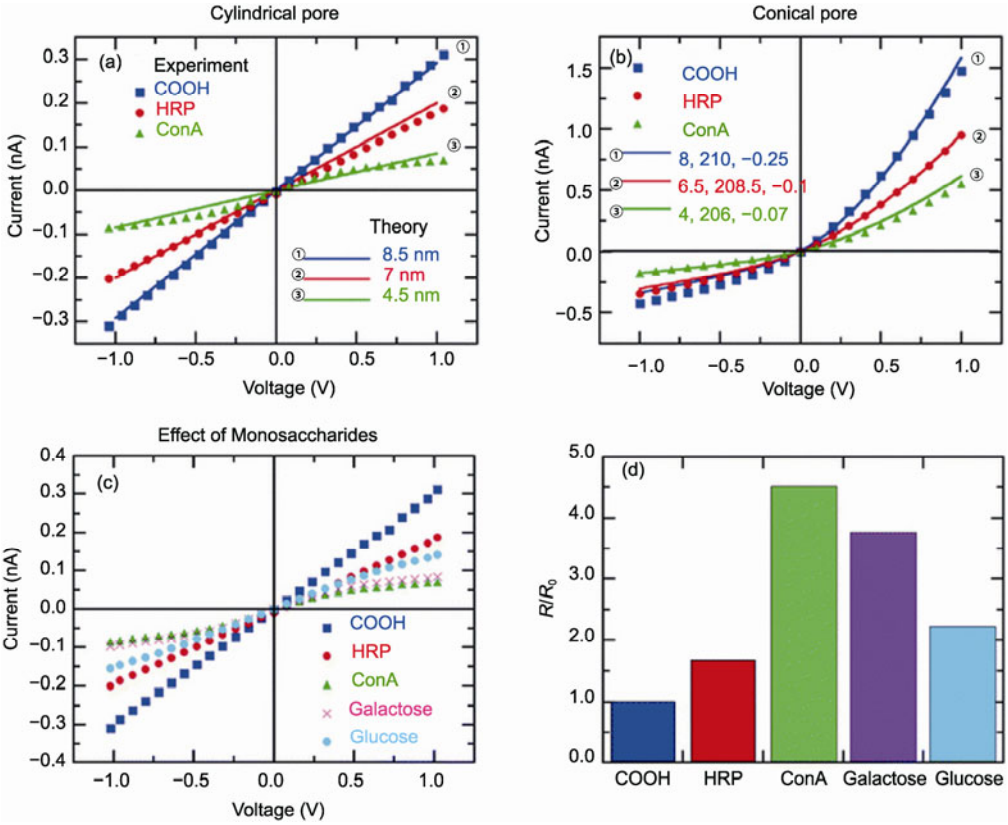

Figure 5 (Color online) (a) Comparison between theoretical (lines) and experimental (symbols) *I-V* curves for a cylindrical pore with COOH, HRP and Con A groups. (b) Conical pore immersed in 0.1 mol/L KCl (pH 7.6). (c) *I-V* curves and (d) normalised resistance response for a cylindrical pore upon treatment with HRP enzyme, Con A, free galactose, and free glucose analytes. Reprinted with permission from [62].

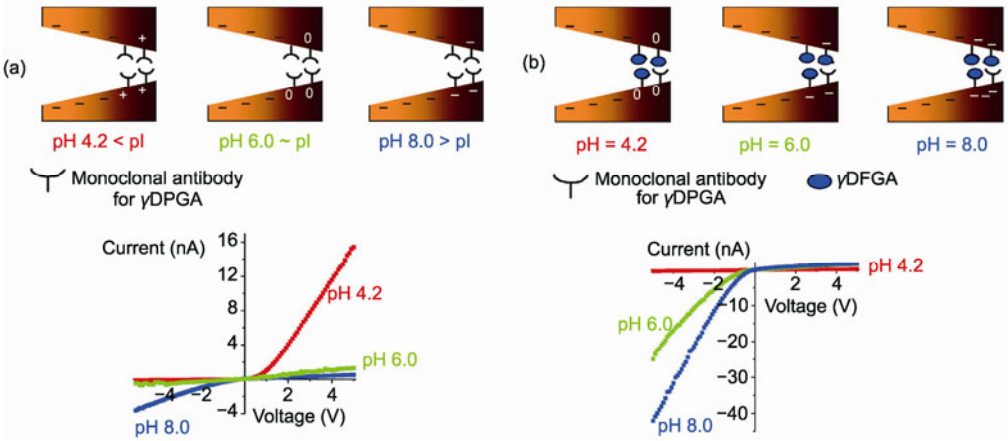

Figure 6 (Color online) (a) Identification of the isoelectric point of the monoclonal antibody for the bacterial polyglutamic acid (γDPGA). (b) Sensing γDPGA with a nanochannel that contained mAb F26G3 at the small opening. Reprinted with permission from [63].

for biosensing due to its inherent chemically robust and extremely stable ionic current signals, especially the stable distribution and polarity of the surface charges in compare with other polymer materials. Ensinger's group [64] described a polyimide channel based protein sensors that biotin recognition elements (biotin-PEO_3-amine) can be covalently bound on the single PI nanochannel inner surface by EDC + PFP activation process. As shown in Figure 7(a) and (b), both of ionic conductance and rectification degrees scaled down after biotin bond in channel at neutral pH solutions. This should be ascribed as partial blockage of the channel and decrease on surface charge density when inherent carboxyl groups were replaced by biotin molecules. For the sensing measurements, various receptors were introduced in biotinylated channel. Addition of lysozyme and Bovine serum albumin (BSA) barely induced current signal changes indicating both lysozyme and BSA are nonspecific to biotin-PEO_3-amine. Nevertheless, incorporation of streptavidin caused significant reduction of ionic conductance due to partial blockage of tight noncovalent binding of

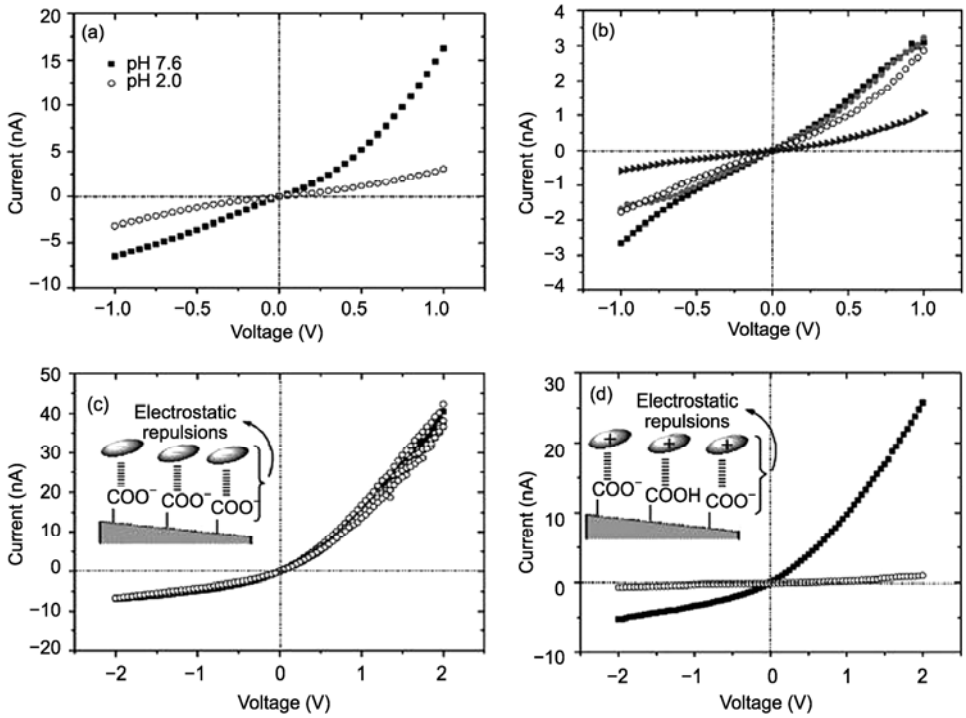

Figure 7 (Color online) (a) *I-V* curves of single asymmetric PI nanochannel with 9 nm small opening in 1 mol/L KCl. (b) *I-V* curves of biotin immobilized asymmetric nanochannel in 1 mol/L KCl at pH = 7.6, (▪) without any proteins, and with adding 10^{-7} mol/L of (•) lysozyme, (○) BSA, and (▸) streptavidin, respectively. (c) *I-V* curves of a single asymmetric carboxylated channel (d = 10 nm) recorded in 1 mol/L KCl solution at neutral pH. (d) *I-V* curves of a single asymmetric carboxylated channel (d = 10 nm) recorded in 1 mol/L KCl solution at acidic pH = 3.0, (▪) prior and (○) after the addition of BSA (10^{-7} mol/L) in the electrolyte, respectively. Reprinted with permission from [64].

streptavidin with the biotin. The biotin/streptavidin complex units were stable enough to keep hindering when electrolyte ions transport through [65]. Bio-specific characteristic was identified when BSA (pI = 4.7) was added in PI channels filled with different pH electrolyte solutions. At neutral pH solution, whether BSA molecules were added or not, *I-V* curves of the channel seem still the same. Both of BSA and PI surface were negatively charged at neutral pH condition [66], BSA failed to link on the surface owing to electrostatic repulsions (Figure 7(c)). At acidic pH solution (pH 3), BSA molecules were positively charged while PI surface was negatively charged. Strong electrostatic interaction made BSA adhering on channel wall tightly, and steric hindrance resulted in minimal conductance (Figure 7(d)). Interestingly, despite low conductance, BSA affined channel still rectified ion transporting to a certain degree. This phenomenon can be attributed to partial ionized $-COO^-$ groups along with protonated carboxyl groups.

4 Other sensors

Chiral selectivity is important in body physiological processes [67]. Jiang and co-worker [68] developed robust polymer single channel based devices for chiral analysis. Mono-6-amino-β-CD molecules serving as enantioselective recognition sites were modified in conical PET single channel. As shown in Figure 8(a), addition of *L*-His trigged considerable drop of transmembrane ionic conductance while nothing has happened after *D*-His was added. Figure 8b suggests that rectification degree of *L*-His incorporated channel is larger than *D*-His containing channel. The results indicate that mono-6-amino-β-CD modified channel exhibits great chiral selectivity to *L*-His [69]. This work paved the way of fabricating various kinds of chiral sensor based on nanochannels and promoted development in this field.

ATP sensor based on conical single PET nanochannel was introduced by Ensinger's group [70] recently. Branched polyethyleneimine (PEI) molecules were firstly bound on channel as recognition sites for ATP sensing. ATP molecules were electrostatically adsorbed on the channel surface, converting the surface charge polarity from negative to positive and resulting in inversion of rectification directions (Figure 8(c)). The shrinking trend of transmembrane conductance may attribute to ATP blockage effect at channel small opening. Great reproducibility of the sensor was confirmed after 5 repetitions. The "on" and "off" states of the sensor can be effectively adjusted by adding ATP molecules.

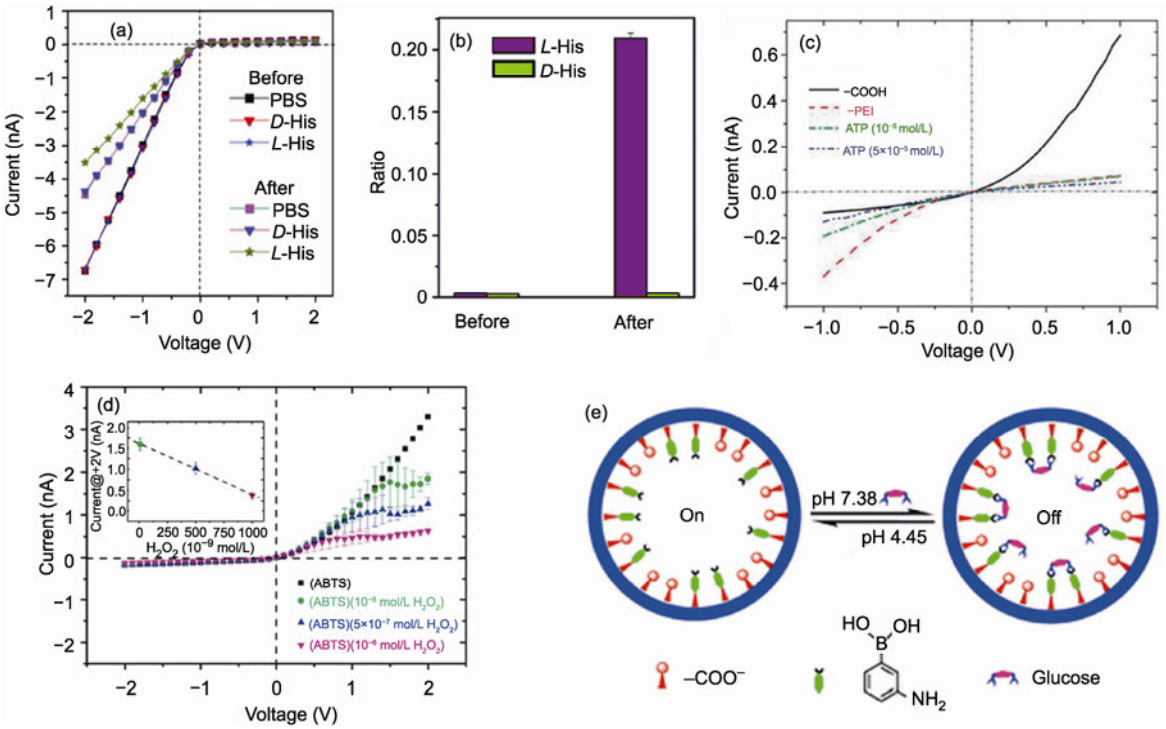

Figure 8 (Color online) (a) Current-voltage curves for the single nanochannel before and after β-CD modification in 0.05 mol/L PBS (pH 7.2) without or with the addition of 0.001 mol/L *L*-His or *D*-His. (b) Comparison of current change ratios for the single nanochannel before and after β-CD modification in 0.05 mol/L PBS (pH 7.2) upon addition of 0.001 mol/L *L*-His or *D*-His. (c) *I-V* curves for a single conical nanochannel (tip $d \approx 9$ nm, base $D \approx 170$ nm in diameter), prior to (black solid line) and after (red dashed line) functionalization with PEI measured in 0.1 mol/L KCl along with different ATP concentrations under symmetric conditions. (d) Current-voltage characteristics of a single asymmetric nanochannel immobilized with the HRP enzymes. The recordings were performed in an electrolyte solution containing 0.0015 mol/L ABTS and various concentrations of hydrogen peroxide. The inset summarizes the changes of ion currents versus H_2O_2 concentration at +2 V. (e) Fabrication of pH gated glucose responsive biomimetic nanochannel. Reprinted with permission from [68,70,71,76].

H_2O_2 is regarded as toxic wastes of cellular metabolism and principal indications of certain redox reactions. Ensinger and co-workers [71] proposed a HRP modified single conical polymer nanochannel based H_2O_2 detection apparatus. Prominent decrease of positive currents accompany with increasing turbulence occurred after addition of various concentrations of H_2O_2 in the HRP-modified system (Figure 8(d)). Such changes can observed only when H_2O_2 and ABTS were presented simultaneously. Serial oxidation and reduction reactions between HRP enzymes and ABTS molecules were triggered by H_2O_2, generating cationic radicals ($ABTS^{\cdot+}$) product [72]. At pH 6.5, positively charged $ABTS^{\cdot+}$ was absorbed electrostatically on the negatively charged polymer channel wall, leading to fluctuations of the surface charge density. Abundant $ABTS^{\cdot+}$ gained by more addition of H_2O_2 finally aggravating neutralization of surface charges. Steric obstruction of the nanochannel induced by radical cation ($ABTS^{\cdot+}$) molecules also result in reduction of ionic conductance [73]. The as-prepared H_2O_2 sensor behaves great sensitivity about 10×10^{-8} mol/L. Merits are confirmed by the experiment such as easy to prepare, free contamination and multiple-use availability.

As abnormal glucose level reflects disease of organisms [74,75], blood glucose detection is imperative for health. Inspired by ion channels, Jiang's group [76] reported pH gated glucose sensor based on boronate ester containing polymer single nanochannel. At pH 7.38, fixed 3-aminobenzeneboronic acid molecules which bear scarcely charges reduced original negative surface charge density, hence pulled the conductance down. Addition of glucose induced a significant decrease of conductance at negative voltage scale. This phenomenon can be explained that each two 3-aminobenzeneboronic acid molecules conjugated with one glucose molecule which contains a pair of *cis*-diol units in the 1,2- and 5,6-positions forming a reversible cyclic ester complex (Figure 8(e)) [77]. Serious steric hindrance and charge screening effects by complexes impeded ions transporting from one side to another side. Saccharides like Man, Xyl and Gal contains only one *cis*-diol moiety failed to form such 2∶1 complexes, consequently no significant alteration of the current was observed [78]. Gate capability of the channel was confirmed by adjusting solution pH. At pH 7.38, the cyclic ester complexes lead to "off" state. The cyclic ester complexes hydrolyzed when pH fall to 4.49 resulting in "on" state. Negligible loss of current indicated excellent reversibility of the glucose sensor.

5 Perspective

Since Coulter [79,80] proposed Coulter counter in 1953, applications of nanochannels in biosensing have undergone tremendous development in fields such as diagnostics, security checks and many industrial uses. Solid-state nanochannels especially polymer single nanochannels provide ideal platform for biosensing due to its mechanical robustness, tailorable surface properties. Extremely small channel opening endow sensors with excellent sensitivity and requiring trace amount of analytes. So far, function mechanisms of nanochannel based sensors are generally summed up in steric hindrance and alteration of surface charge induced by incorporation of bio-recognition groups. For purpose of achieving more efficient sensors, multi-sensing approaches are expected to utilize optical measurements and theoretical analysis as auxiliary means. Emerging nanometerials and fabrication techniques such as nanoimprinting technologies merit great attention so that promising horizon can be opened up in this field.

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