Biosensors Hot Paper

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Magnetic-Field-Driven Extraction of Bioreceptors into Polymeric Membranes for Label-Free Potentiometric Biosensing

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Abstract: *We report here the concept of a magnetically controlled extraction of hydrophilic bioreceptors into polymeric membranes for bioassays. The potentiometric assay relies on the intrinsic charges of an antimicrobial peptide and its unique recognition abilities, which can eliminate the probe labeling and indicator addition. The target binding event could effectively prevent the extraction of the peptide into the polymeric membrane doped with an ion exchanger, thus resulting in a potential change. The potentiometric response properties of the peptide assembled on magnetic beads can be dynamically controlled and modulated by applying a magnetic field. Staphylococcus aureus, as a model of food-borne pathogens, was measured at levels down to 10 CFU mL^{-1}. Based on this sensing strategy, a potentiometric array was developed for the pattern recognition of bacteria. The proposed general platform can be used for potentiometric biosensing using other hydrophilic bioreceptors.*

There is currently an urgent need for simple, rapid, low-cost, and accurate monitoring systems in a variety of fields including healthcare, industrial process control, and environmental monitoring. As a well-established routine analytical technique, polymeric membrane ion-selective electrodes (ISEs) have experienced solid growth with new breakthroughs in sensitivity, selectivity, and stability.[1] Currently, ISEs have become important analytical tools in environmental trace analysis and potentiometric biosensing.[2] The key components of ISEs are ion-binding chemical receptors (that is, ionophores) which are usually lipophilic and well-dissolved in the hydrophobic polymeric membranes. In recent years, bioreceptors such as antibodies and aptamers have been used for potentiometric detection of a broad range of different target molecules.[3] However, the target-binding events are commonly indirectly measured by using labels and/or indicators such as enzyme tags, nanoparticles and indicator ions, which could cause problems of decrease of enzyme and indicator ion activities.[2c] Notably, many bioreceptors have net electrical charge polarities, and a potentiometric biosensor can therefore be designed directly according to the charge density change of the bioreceptor induced by the recognition and interaction with its target. Unfortunately, ISE membranes doped with bioreceptors are unsuitable for direct potentiometric sensing due to the incompatibilities of these hydrophilic receptors with the hydrophobic polymer membrane matrixes, which can cause the rapid leaching of the receptor from the membrane into the sample solution.[4] As an alternative, the hydrophilic bioreceptor can be immobilized on the surface of the solid-state electrode so that the target-binding process induces a surface charge change.[5] However, the low efficiency of the immobilization procedures and high interference from the sample matrixes could restrict the wide applications of these solid-state sensors. Therefore, it is still a big challenge to achieve the direct potentiometric sensing of analytes using the hydrophilic bioreceptors.

Herein, we propose a magneto-controlled potentiometric sensing system based on hydrophilic bioreceptor-assembled magnetic beads (MBs). Peptides, amino acid sequences linked by the peptide bonds, have been used as promising bioreceptor for the fabrication of novel biosensors.[6] Peptide-based potentiometric biosensors show promise for sensing but are still rare.[7] Previously, we reported a short antimicrobial peptide (AMP) pair-based potentiometric sandwich assay, in which enzyme labels and/or additional indicators were used to report the receptor–bioanalyte interaction.[7a] Clearly, a general approach for direct potentiometric sensing of bioanalytes without probe labeling and indicator addition is desired. As the building blocks of peptides, amino acids with different side chains give peptides zwitterionic and amphipathic properties. In principle, peptides can be positively or negatively charged, which could induce a potentiometric response on the polymeric membrane ISEs. Inspired by the potentiometric responses of anionic polyamino acids and protamine,[7b,8] we envision that a general and direct potentiometric sensing platform can be designed by using peptides for both molecular recognition and signal transduction. To achieve high sensitive and direct potentiometric measurement, a simple, general strategy based on magnetic-field-driven accumulation and reconfiguration of a network of peptide-assembled MBs was designed.

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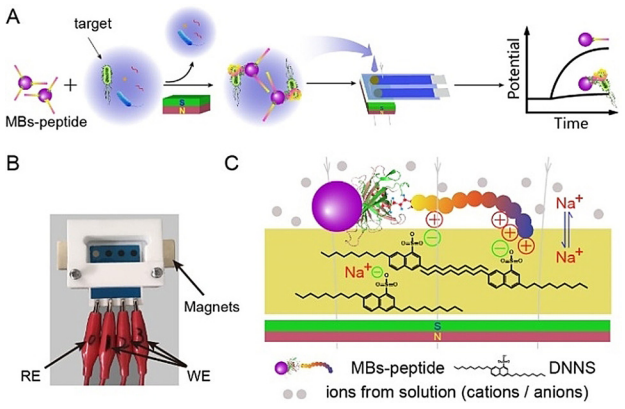
As a proof of concept experiment, AMP-based potentiometric biosensing platforms for bacterial strains were designed. The AMP-RVRSAPSSS for *Staphylococcus aureus* (*S. aureus*, a major cause of food-borne diseases) was selected as a model. The AMP can bind a 78-KDa cell-division protein, which is specific to *S. aureus*, on the bacterial cell surface with high affinity.[9] Scheme 1 illustrates the magneto-controlled potentiometric sensing mechanism. Since the voltage changes induced by the peptide in the solution on an ISE are rather small, especially in the presence of a high electrolyte background (as indicated below), the peptide serving as both the bioreceptor and signal reporter was linked to the MBs through the biotin–streptavidin reaction. MBs functionalized with the bioreceptor can act as highly sensitive, easily manipulated concentration carriers and used for the development of biosensors.[10] More importantly, the bioreceptor can be attached to the surface of polymeric membrane with ion exchanger in the presence of a magnetic field and lead to a rapid and obvious potential change. In the presence of bacteria, the linear cationic AMP can electrostatically and specifically bind to the bacterial cell surface, which could prevent the extraction of the peptide into the polymeric membrane, thus induces a potential decrease (Scheme 1 A).

Screen-printed electrodes (SPEs) were designed and used in this work owing to the advantages of disposability, simplicity, and rapidity.[11] By drop-casting the membrane cocktail (see the Supporting Information), the SPEs with polymeric membrane were fabricated and placed on the bottom of the cell for the potentiometric detection of antimicrobial peptides (Scheme 1 B). By applying a magnetic field, MBs-peptide can be assembled and reconfigured on the membrane surface efficiently. Meanwhile, in situ cooperative ion-pairing interactions between the ion exchanger in the membrane and the bioreceptor on the MBs can be dramatically enhanced by increasing the mass transfer at micrometer levels (Scheme 1 C). The enhanced mass transfer of the peptide molecules to the polymeric membrane can lead to an improved sensitivity and potential stability. Moreover, cross talk among the sensing units in sensor arrays as noted below can be minimized by the magnetic-field-driven accumulation of the MBs-peptide.

In this study, the peptide (for *S. aureus*) was rationally designed by incorporating specific amino acids into the sequence (Supporting Information, Table S1). To immobilize the peptides on the MBs, biotin was added to the N-terminal of the sequences. Three glycine residues act as a spacer to impart chain flexibility. Arginine residues carrying positive charges were linked to the C-terminal of the sequence to increase the charges and subsequently the potentiometric responses.[12] No obvious circular dichroism (CD) spectra and folded structures (antiparallel) change were observed for peptides with the incorporation of even 3 arginine residues (Supporting Information, Figure S1). While the charges of the peptides increase with the incorporation of arginine residues, the potential responses of the peptides on the conventional ISE are rather small. As shown in Figure 1 A, a small potential change (ca. 1–2 mV) was observed even for the high concentration of peptide S5 (1.0 μM) during the measurements. Compared to the potential response of the peptides on the conventional ISE, MBs-peptide can be attached to the polymeric membrane surface by applying a magnetic field, which can lead to a rapid, stable and reproducible potential change (Figure 1 A). The potentiometric responses can be further enhanced with a low background electrolyte (for example, 1.0 mM PBS, Figure 1 B). Control experiments reveal that the presence of the peptides on the MBs and their interactions with the ion exchanger (dinonylnaphthalenesulfonate, DNNS) in the membrane lead to the large potential responses. Moreover, the potentiometric responses of peptides can be enhanced with increase in the number of the positively charged arginine (Supporting Information, Figure S2).[7b] It should be noted that the distribution of the hydrophilic and hydrophobic residues and the amount of the hydrophobic residues such as Val, Ala, could also make



Scheme 1. A) Illustration of the potentiometric assay for bacterial cells based on the MBs-peptide. B) Photograph of the setup for potentiometric detection. The screen-printed electrodes include working electrodes (WE, acting as indicator electrodes for the potentiometric assay) and a reference electrode (RE). C) Illustration of the response mechanism of MB-peptide on the polymeric membrane ion-sensitive electrode.

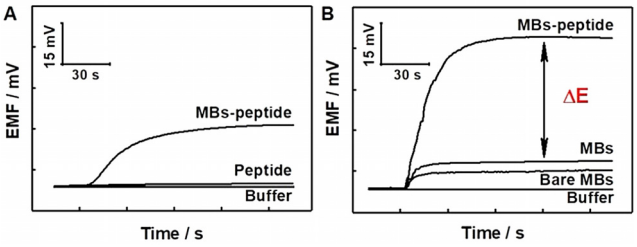


Figure 1. Potential responses of the ISEs to MBs-peptide S5, and different kinds of magnetic beads recorded in 50 mM (A) and 1.0 mM (B) PBS (pH 7.4) containing 1.0 mM NaCl, respectively. Bare MBs (1 μm), magnetic beads with a magnetite core (Fe_3O_4), and a SiO_2-coated surface; MBs, streptavidin modified magnetic beads; MBs-peptide, peptide modified MBs. Bare MBs, MBs and MBs-peptide (10 mg mL^{-1}) of 5 μL were used. Unless stated otherwise, the membrane components of the electrode contained 49 wt% poly(vinyl chloride) (PVC), 49 wt% *o*-nitrophenyloctyl ether (*o*-NPOE), 1 wt% dinonylnaphthalenesulfonate (DNNS) and 1 wt% tetradodecylammoniumtetrakis(4-chlorophenyl)borate (ETH 500). Potential responses of the peptide S5 on the ISE were measured in the galvanic cell with stirring.

contributions to the potentiometric responses. The small potential change induced by the bare MBs or streptavidin modified MBs on the membrane with DNNS or that by MBs or MBs-peptide on the membrane without DNNS (Supporting Information, Figure S3) could be due to the ion redistribution near the membrane surface induced by the non-specific adsorption on the electrode surface.

As shown in Scheme 1 C, the positively charged peptide immobilized on the MBs can be accumulated/concentrated on the surface of the polymeric membrane by using the magnetic force, which could facilitate the extraction of the peptide molecules into the polymeric membrane via the formation of ion pairs with the lipophilic ion exchanger (that is, DNNS) in the membrane phase. Such extraction results in the ion exchange between the positively charged peptide in the sample solution and sodium ions in the membrane, thus inducing a significant change in the phase boundary potential at the membrane/sample interface. The ion exchanger-peptide binding equilibrium could be achieved rapidly in the presence of magnetic fields. Indeed, the field-emission scanning electron microscopy (FE-SEM) shows that the MBs-peptide can be adsorbed on the membrane and peptide groups even extracted into the membrane (Figure 2 A,B). Furthermore, the appearances of silicon (SiO_2) and iron elements (Fe_3O_4) in the energy dispersive spectrograms (EDS) indicate that the MBs-peptide can be indeed adsorbed on the membrane after strong washing with freshly deionized water (Figure 2 C,D). The molecular docking analysis also reveals that DNNS can interact with the peptides (Figure 2 E; Supporting Information, Figure S4).

Owing to the magnetic-field induced accumulation and the rapid ion-exchange process, the effect of magnetic field intensity on the potentiometric response was investigated. The magnetic field was controlled with the number of the applied magnets. For each single magnet, the magnetic field strength is a fixed value (surface magnetic field strength, 750 gausses; magnetic force, 3 kg). Since the degree of adsorption and extraction of the peptide could be modulated by the magnetic field intensity, with increase in the amount of the magnet up to 3, the potential change can be increased. With further increase of the magnetic force, the potential response decreases probably due to the high background signal induced by the extraction of more MBs (Figure 3 A). It should be noted that the separation of the conjugates of MBs and bacteria after the incubation can eliminate the interferences of inner substrates released from the bacteria in the incubation solution.

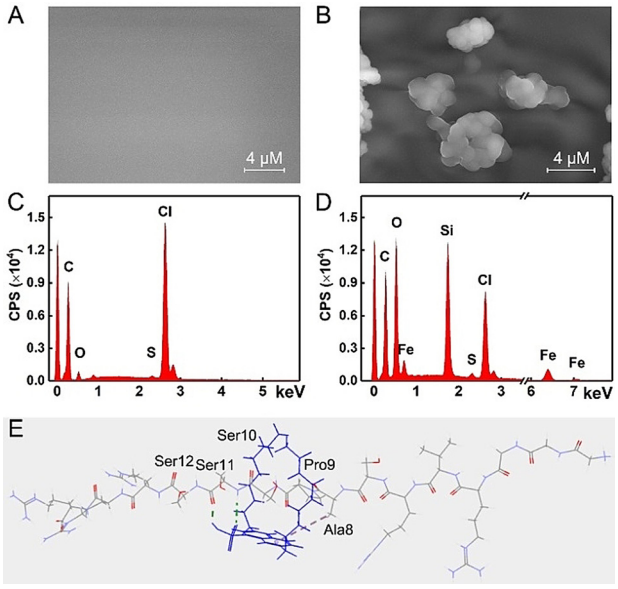


Figure 2. A),B) SEM images of the membrane of the electrode without (A) and with (B) addition of MBs-peptide under magnetic force. C),D) EDS element analyses of the membrane of the electrode without (C) and with (D) addition of MBs-peptide under magnetic force. E) Molecular docking of DNNS (blue) to the receptor Peptide S4. The CDOCKER interaction energy is −25.51 kcal mol^{-1}.

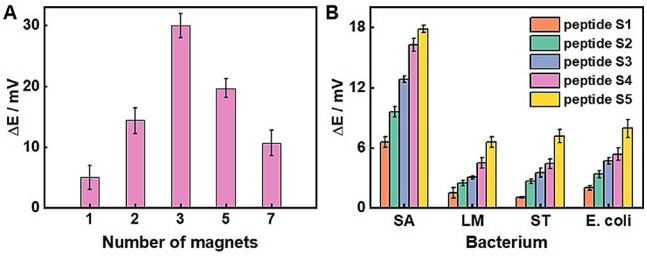


Figure 3. A) Potential variation of MBs-peptide S4 with different amounts of magnets. The potential difference between the MBs-peptide S4 and MBs was used for optimization of magnetic field. B) Potential responses of peptides-modified MBs to different targets at the concentrations of 1.0×10^4 CFU mL^{-1}: *Staphylococcus aureus* (SA), *Listeria monocytogenes* (LM), *Salmonella typhimurium* (ST), and *Escherichia coli O157:H7* (*E. coli*). The potential difference between the MBs-peptides in the absence and presence of bacteria cells was used for selection of the peptides. Error bars represent one standard deviation for three measurements.

Previous research has shown that the AMP can recognize and capture the *S. aureus* cells by interacting with the 78-KDa cell-division protein on the membrane to form a helical conformation.[9] Such target binding effectively prevents the adsorption or extraction of the peptide assembled on MBs into the polymeric membrane, thus resulting in a potential decrease. Indeed, the zeta potential of MBs-peptide could decrease in the presence of *S. aureus* (Supporting Information, Figure S5). To validate the recognition efficiency of the designed peptides, the peptides with different charges were explored for potentiometric detection of different bacteria. As shown in Figure 3 B, as the increase of number of arginine residues linked on the peptides, the potentiometric change increased. However, the sensing method based on peptide S5 showed poor selectivity (Figure 3 B). Thus, the peptide S4 was selected for *S. aureus* as a counterbalance of sensitivity and selectivity. Meanwhile, in order to achieve sensitive detection of *S. aureus*, the experimental conditions including the amount of MBs, membrane components, background solution were optimized (Supporting Information, Figure S6A–D). The membrane containing PVC and *o*-NPOE in a weight ratio of 1:1 and 1 wt% DNNS was used for further experiments. 5 μL MBs and 1.0 mM PBS (pH 7.4) containing

1.0 mM NaCl were used for potentiometric measurements. It should be noted that a relatively small potential difference of less than 30 mV was observed for the present system when measuring the same amount of MBs-peptide in 1 mM PBS buffers containing 1 and 50 mM NaCl, respectively (Supporting Information, Figure S6D). This implies that the magnetic field-driven extraction of the peptide into the polymeric membrane might be not as strong as that of protamine,[12] which is probably due to the high hydrophilicity of the peptide and the relatively weak interaction between the peptide and DNNS in the membrane phase (see the discussion in the Supporting Information).

Under optimized experiments, the potential change of the electrode increases with the increase of the amount of *S. aureus* (Figure 4A). The target *S. aureus* can be detected with a concentration linear range of 1.0×10^2 to 1.0×10^6 CFU mL^{-1} (Figure 4B). The potentiometric sensing assay is able to detect the target bacterium at concentrations down to 10 CFU mL^{-1}. It should be noted that no obvious aggregations of MBs-peptide can be observed at a high concentration of *S. aureus* (Supporting Information, Figure S7). Moreover, aggregation preventing agents such as Tween-20 could be used to reduce the aggregations of the magnetic beads. Meanwhile, the developed sensor shows good selectivities (Figure 4C). The control experiment using a scrambled peptide as the capture fragment validates the specificity of this method (Figure 4D). Such sensing systems have the flexibility of choosing different peptides for potentiometric sensing of a broad range of analytes.

To expand the applications of this methodology, one disposable potentiometric sensor array for use as an electronic tongue can be designed to achieve multiple bacteria classification and identification in a rapid way (Figure 5A). From the sensor array perspective, antimicrobial peptides with broad-spectrum antimicrobial activity have a high potential for use in the design of potentiometric-based sensor array. Four AMPs were then selected to form a preliminary group of receptors having different affinities. The peptides used for the array are listed in the Supporting Information, Table S2. CD spectra of the peptides show that the structures of peptide A1, peptide A3, and peptide A4 are α-helix, and the structure of peptide A2 is partly β-sheet and partly random coil (Supporting Information, Figure S8). A potentiometric sensor array was developed for the pattern recognition of bacteria by analyzing the potential responses to bacterial strains using linear discriminant analysis (LDA). To confirm the discrimination ability of the proposed sensor array, eight bacterial strains were selected as models for the array. The potentiometric responses (4 peptides × 8 bacteria × 5 replicates) of the sensor array for the bacterial strains are shown in Figure 5B. By converting the potentiometric data into canonical factors using LDA, a two-dimensional (2D) plot can be obtained (Figure 5C; Supporting Information, Table S3). It can be seen that eight bacterial stains were clustered into 8 different clusters by LDA, indicating that the bacterial stains can be effectively discriminated. Meanwhile, the bacteria with different properties (Gram-positive or Gram-negative) can be discriminated by analyzing the canonical scores. It should be noted that, for the unknown sample, the discrimination of bacteria can be carried out with the sensor array and thereafter the concentration of specific bacterium strain can be determined by using a peptide sequence with high selectivity.

In summary, we propose a simple, general potentiometric sensing methodology for the detection of bacteria using peptide-modified MBs as both recognition element and transduction indicator. The potential response properties of

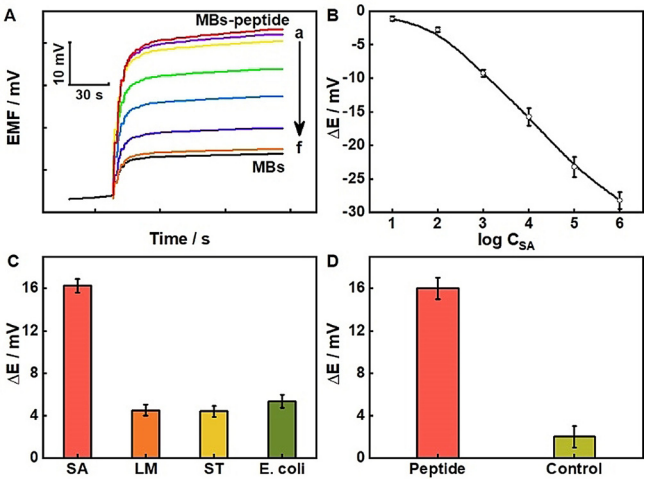


Figure 4. A) Potential responses to *S. aureus* (SA) at increasing concentrations: a) 1.0×10^1; b) 1.0×10^2; c) 1.0×10^3; d) 1.0×10^4; e) 1.0×10^5; f) 1.0×10^6 CFU mL^{-1}. B) Potential changes over the SA concentration range of 1.0×10^1–1.0×10^6 CFU mL^{-1}. The potential difference between the MBs-peptide S4 in the presence and absence of *SA* was used for quantification. C) Potential responses to different bacteria at the concentration of 1.0×10^4 CFU mL^{-1}. D) Potential responses to *S. aureus* at the concentration of 1.0×10^4 CFU mL^{-1} with the peptide S4 and the control. Error bars represent one standard deviation for three measurements.

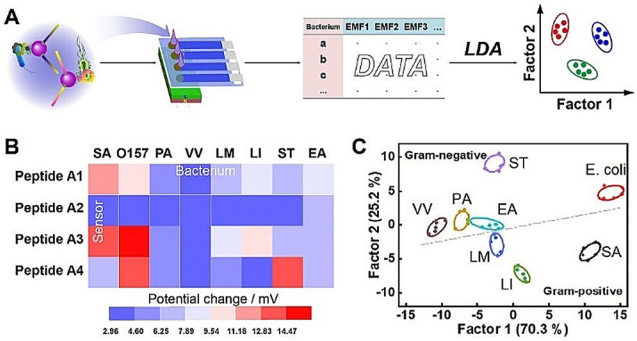


Figure 5. A) Illustration of the potentiometric sensor array for multiple bacteria classification and identification. B) Potential response heat map for each pair, as a function of potential change. Potentiometric responses to different bacterial strains at the concentration of 1.0×10^4 CFU mL^{-1}: *Staphylococcus aureus* (SA), *Escherichia coli O157:H7* (E. coli), *Listeria monocytogenes* (LM), *Listeria iuanuii* (LI), *Salmonella typhimurium* (ST), *Pseudomonas aeruginosa* (PA), *Edwardsiella tarda* (EA), and *Vibrio vulnificus* (VV) were measured. Error bars represent one standard deviation for three measurements. C) 2D canonical score plot obtained from the potentiometric response patterns. Each point represents the response pattern for a single bacterial species to the array.

the peptide on an ion-selective sensor can be dynamically controlled and modulated by a magnetic field, resulting in an improved sensitivity and potential stability. Moreover, the use of MBs can eliminate the interferences from the sample matrix. Meanwhile, a sensor array based on this simple method can be developed to discriminate bacteria. The present methodology can pave a new way to develop label-free potentiometric sensing assay for sensitive and selective detection and identification of various targets. The proposed magneto-controlled sensing principle can be extended to other hydrophilic bioreceptors such as aptamers and antibodies.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: biosensors · magnetic fields · peptides · potentiometry

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