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Array-Based Biosensors for Bacteria Detection: From the Perspective of Recognition

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Array-based biosensors have shown as effective and powerful tools to distinguish intricate mixtures with infinitesimal differences among analytes such as nucleic acids, proteins, microorganisms, and other biomolecules. In array-based bacterial sensing, the recognition of bacteria is the initial step that can crucially influence the analytical performance of a biosensor array. Bacteria recognition as well as the signal readout and mathematical analysis are indispensable to ensure the discrimination ability of array-based biosensors. Strategies for bacteria recognition mainly include the specific interaction between biomolecules and the corresponding receptors on bacteria, the noncovalent interaction between materials and bacteria, and the specific targeting of bacterial metabolites. In this review, recent advances in array-based bacteria sensors are discussed from the perspective of bacteria recognition relying on the characteristics of different bacteria. Principles of bacteria recognition and signal readout for bacteria detection are highlighted as well as the discussion on future trends in array-based biosensors.

1. Introduction

Bacterial infection has caused multifaceted issues closely related to human life, such as food safety, water pollution, biodefense, diagnosis, and treatment of bacterial infectious diseases.[1,2] Bacterial identification, especially distinguishing different bacterial species in a mixed sample of various bacteria, is crucially important that can guide the treatment of bacterial infection.[3] Current techniques for bacterial identification rely on culture-based method or molecular biotechnology such as polymerase chain reaction, which requires either long-running process or complicated instruments, unable to meet the requirements of rapidness and high throughput in clinical settings.[4–6] Optical and electrochemical methods can provide facile readouts for bacteria analysis.[7–9] By implementing optical or electrochemical approaches, highly selective biosensors have been developed based on the specific "lock-and-key" molecular recognitions.[10,11] In spite of the high specificity, these "lock-and-key" biosensors lack in sensitivity because of the one-component target recognition and signal transduction that may not sensitively work for other multiple analytes in the mixture. In addition, it is unpractical to synthesize highly selective receptors for each analyte in a sample of complex bacterial mixture. Traditional "lock-and-key" recognition strategies are inefficient for the analysis and differentiation of subtle differences in the composition of complex bacterial mixtures.[12]

To address these issues, array-based biosensors have been developed that aim to detect subtle changes in multiple samples by transforming relevant chemical or physical properties of bacterial species into an analytically useful output.[13–15] Array-based biosensors are composed of a set of statistics that derives from olfactory system of animals, also known figuratively as "optoelectronic nose."[16] In a typical array-based biosensor, the multiple components of the sensor array interact with the target by specific binding or recognition, further transducing the molecular event to result in a detectable signal (**Scheme 1**).[17] The optical or electrochemical signal can then be detected and processed with statistical methods, thus generating patterns of different regions with clear boundaries by mathematical analysis.[18–20] Compared with a traditional single-component biosensor, an array-based biosensor is capable of discriminating a variety of targets spontaneously. More importantly, a subtle difference in the chemical structure or the physical property of the objects under test can be identified by an array-based biosensor.[21]

In recent years, array-based biosensors have been developed for bacterial identification based on versatile recognition mechanisms. Bacteria differ in surface physicochemical properties and metabolites that can be used for targeting. Besides, specific or noncovalent interactions between materials and bacteria in terms of electrostatic interaction and hydrophobic/hydrophilic interaction also contribute to distinguishing bacteria. By implementing versatile recognition principles of sensor arrays, it can generate different pattern signals for bacterial identification.[22] In this review, recognition strategies are outlined from the view of bacterial characteristics: 1) specific molecular receptors on bacterial

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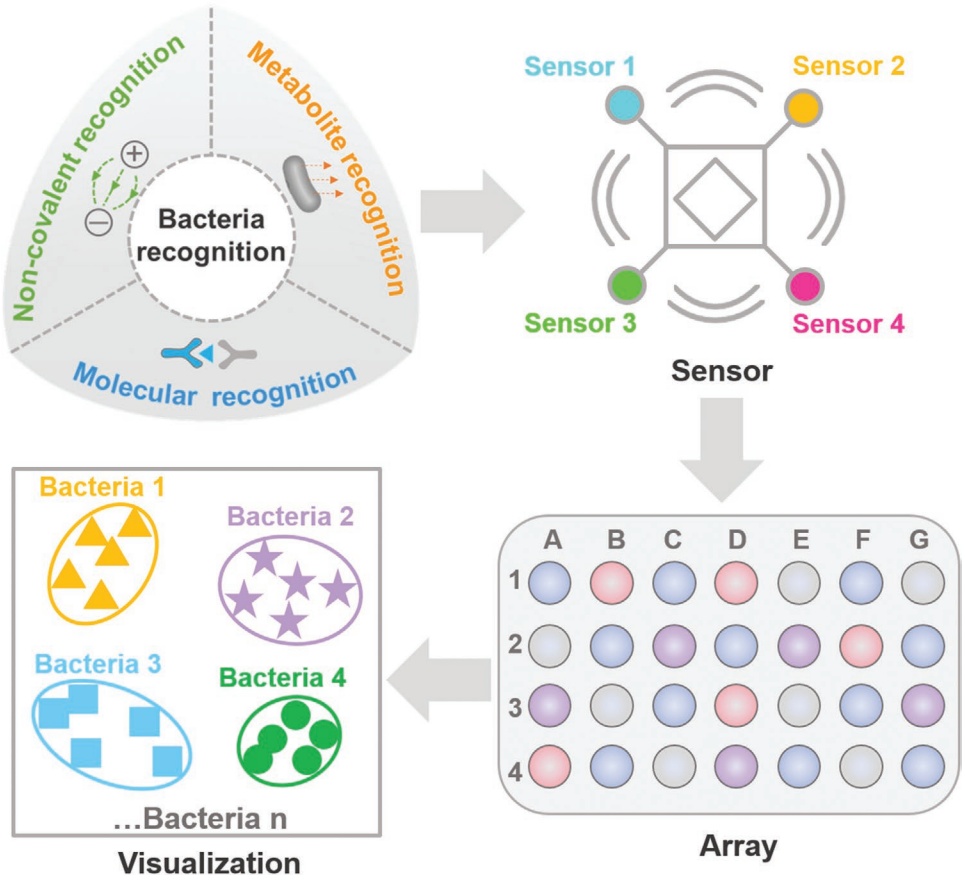
DOI: 10.1002/smll.202006230



Scheme 1. The workflow of array-based biosensors including bacteria recognition, multiple components of the sensor, generation of the signal array, and visualization of the biosensor.

cell membranes, 2) electrostatic and hydrophobic interactions between negatively charged bacteria and positively charged materials, and 3) specific metabolites of bacteria (**Scheme 2**). Recent advances in array-based biosensors for bacteria detection are discussed from the perspective of recognition, highlighted together with the signal readout in those biosensor arrays.

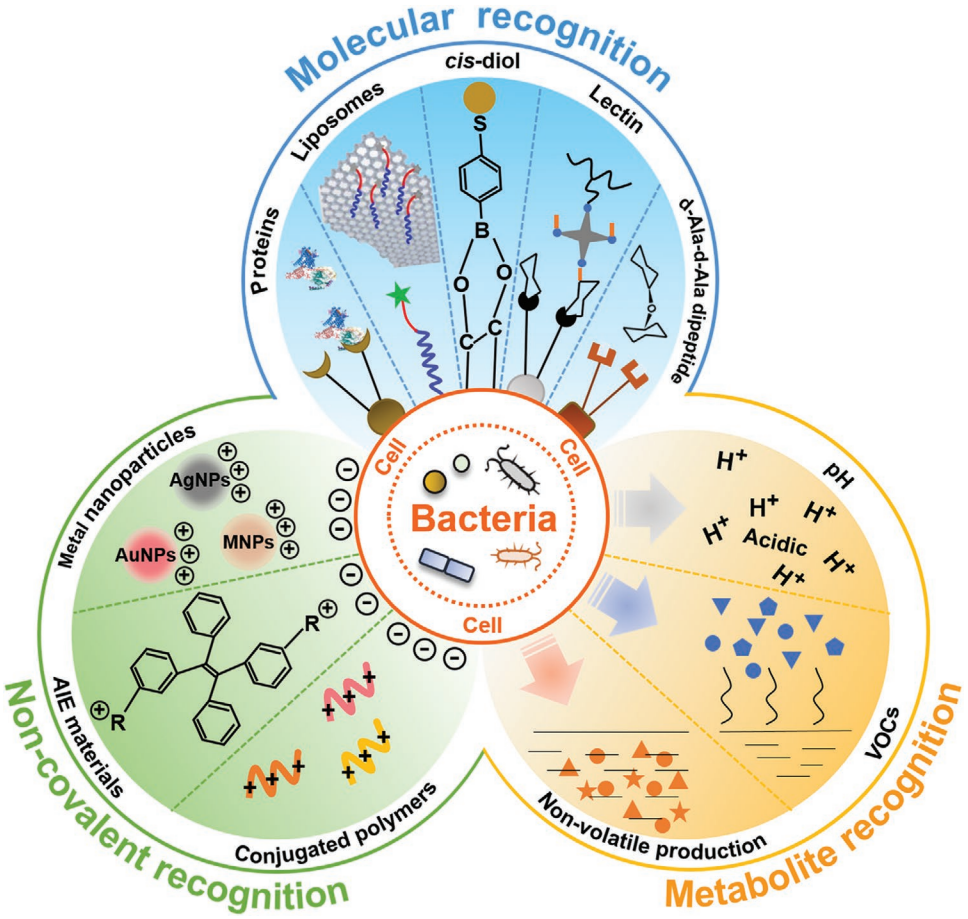
2. Molecular Recognition with Bacterial Surface

Specific biomarkers on bacterial surface such as saccharide, protein, peptide, and other membrane structures can be identified as recognition molecules for rapid and direct bacterial detection. These biomarkers can specifically interact with the multiple components of the sensor array though selective binding including antibiotic, peptide, protein, and carbohydrate, thus triggering a signal generation and an optical signal readout. In this section, bacterial detection is discussed according to different molecular recognition mechanisms.

2.1. Boronic Acid with *Cis*-Diol Group of Saccharide

Boronic acid can specifically interact with *cis*-diol group to form cyclic ester,[23] which has been widely used for detection and quantification of carbohydrates, glycoproteins, bacteria, and other biomolecules containing *cis*-diol groups.[23–25] Boronic acid and its derivatives have demonstrated as recognition molecules for bacteria detection due to the different saccharides and glycosylated biomolecules on their surface.[26] Zhang et al.[27] reported a colorimetric detection method for Gram-negative bacteria based on antiaggregation of 4-mercaptophenylboronic acid (MPBA)-functionalized silver nanoparticles (AgNPs). The principle was that MPBA as a boronic acid derivative could react with *cis*-diol group-containing saccharides on bacteria cells. The MPBA-functionalized AgNPs could bind to the surface of bacteria to keep them separated and dispersed, resulting in the hindering of a color change from yellow to brownish red (**Figure 1**A). The concentration of bacteria dictated the color change that could be conveniently determined by UV–vis or the naked eye.

The affinity of boronic acid and *cis*-diol can be regulated by the pH value. The covalent complexes form at a high pH value (basic) and are dissociated at a low pH value (acidic).[28,29] Wu et al.[30] reported a sensor array for bacterial detection at various pH values using MPBA-mercaptoethylamine (MA)-cofunctionalized AgNPs (Figure 1B). In this research, Wulff-type boronate that has amines adjacent to boronic acid was selected as the recognition molecule. As a consequence, the affinity of boronic acid and *cis*-diol at pH 7 was improved due to the formation



Scheme 2. A schematic overview of versatile recognition methods of array-based biosensors for bacterial detection.

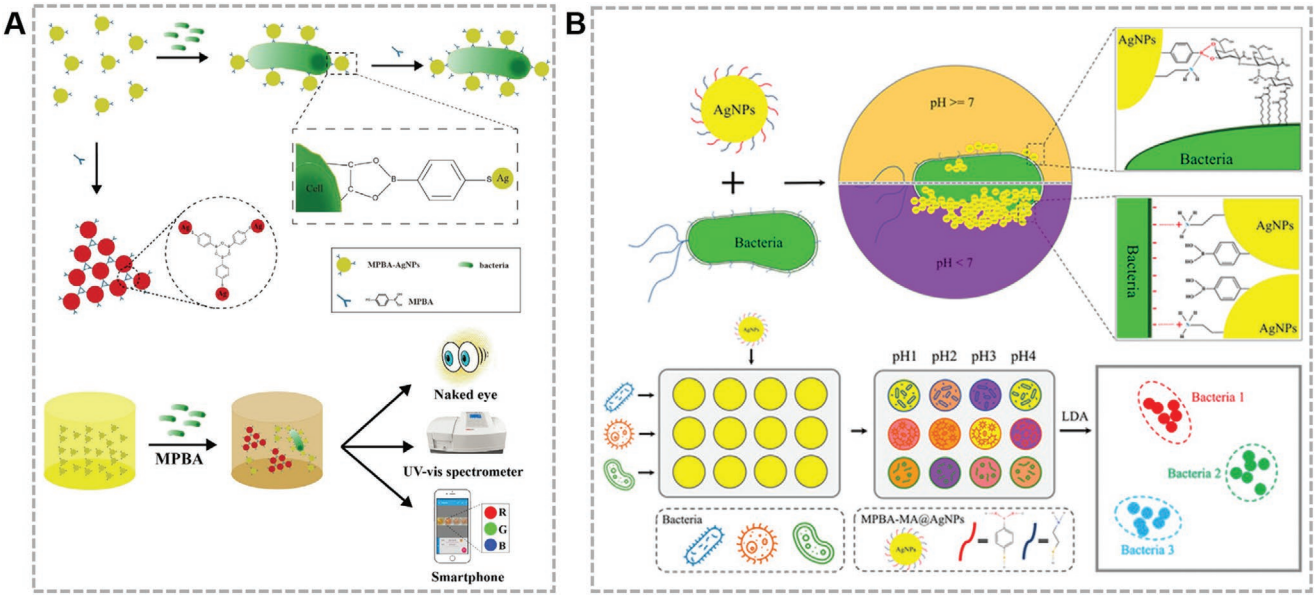


Figure 1. Bacterial recognition through the interaction between boronic acid and *cis-diol* group. A) Gram-negative bacteria detection using MPBA-AgNPs. Reproduced with permission.[27] Copyright 2018, Elsevier. B) Bacteria detection based on pH-modulated binding affinity of MPBA-MA-AgNPs and *cis*-diol on bacterial surface. Reproduced with permission.[30] Copyright 2019, American Chemical Society.

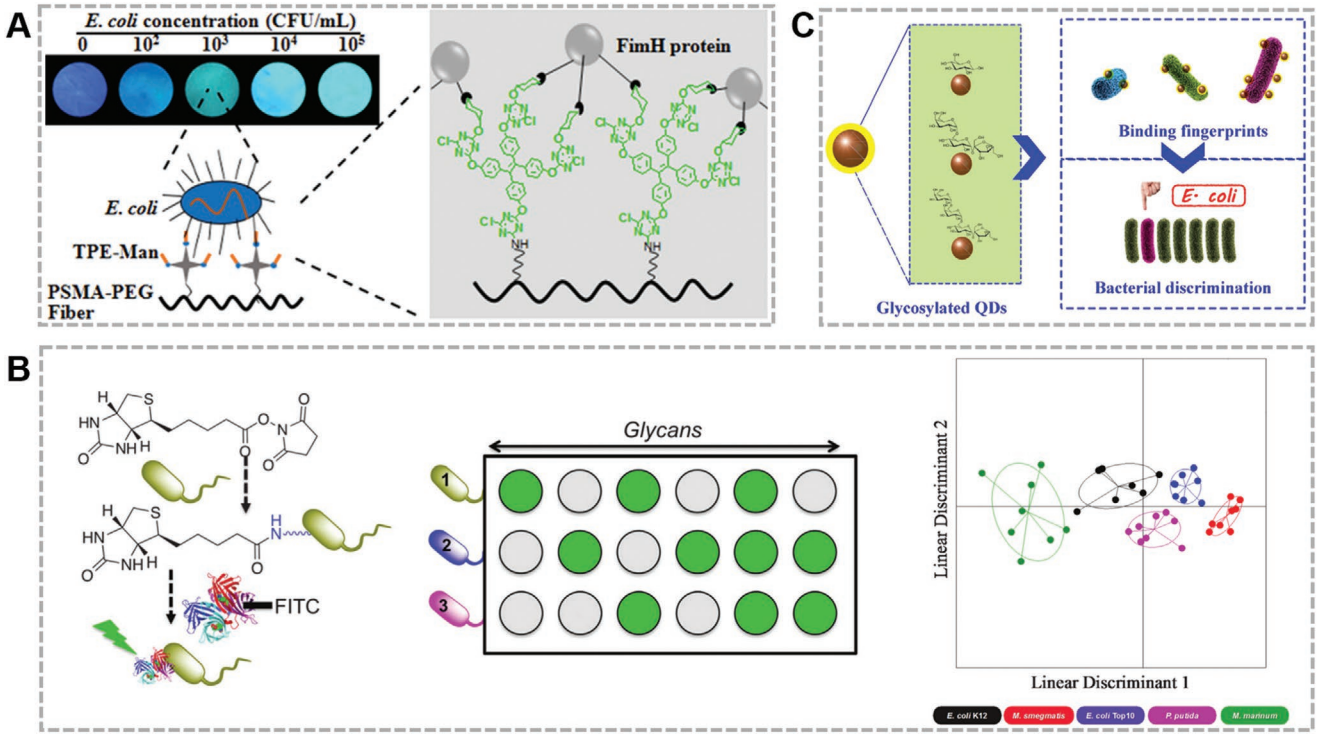


Figure 2. Bacterial recognition through the interaction between glycan and lectin proteins. A) Fluorescent sensing of *E. coli* through the binding of FimH protein and mannose. Reproduced with permission.[37] Copyright 2015, American Chemical Society. B) Glycosylated surfaces used to profile the binding patterns of Gram-negative, Gram-positive, and mycobacteria. Reproduced with permission.[38] Copyright 2016, Royal Society of Chemistry. C) Multivalent glycosylated quantum dots for bacterial discrimination. Reproduced with permission.[39] Copyright 2018, Elsevier.

of an intramolecular B–N coordination. Under neutral and alkaline conditions, the specific interaction between *cis-diol* residues in carbohydrate-rich bacterial cell surface and MPBA resulted in the antiaggregation of MPBA-MA-AgNPs. The color change induced by the specific blinding and electrostatic interaction could be exploited for the classification of seventeen bacterial species with an accuracy of 100%.

2.2. Glycan with Lectin Proteins

Protein–carbohydrate interactions play a crucial role in the initial adhesion step during bacterial infection, where carbohydrate-binding proteins (known as lectins) mediate the adhesion step.[31,32] Lectins are one of the biorecognition proteins binding to the surface of bacteria and can specifically interact with carbohydrates (mono- and oligosaccharide) on bacterial surface. Lectins have demonstrated effective for selectively detecting and directionally killing bacteria.[33–35] For example, *E. coli* is a Gram-negative pathogen and associated with infectious diseases that need an urgent identification. Like most Gram-negative bacteria, *E. coli* adheres to host tissues through the interaction between the bacterial fimbria and the mannose molecules on the surface of host cell. FimH is a two-domain adhesion protein at the end of the fimbria and has sugar-binding properties with *E. coli*. In the research of Li et al.,[35–37] the mannose-binding FimH protein was exploited for the effective detection of *E. coli*, on which a mannose-bearing glycopolymer was present (**Figure 2**A).

A series of glycans with slightly different features could be exploited to generate a sensor array based on their different binding affinities with bacteria. Gibson et al.[38] reported a rapid technique for the identification of bacterial strains based on profiling their differential binding to nine glycans-functionalized surfaces, including arabinose, cellobiose, dextran, galactose, glucose, *N*-acetyl-D-glucosamine, glyceraldehyde, lactose, and mannose (Figure 2B). Multiplexed diagnostics could be developed based on the different binding abilities between different bacterial species and glycans. Five bacteria strains (*E. coli* k12, *M. smegmatis*, *E. coli* Top10, *P. putida*, and *M. marinum*) could be differentiated in the range of Gram-negative, Gram-positive, and mycobacteria.

Based on a similar principle, Zhang et al.[39] reported a multivalent glycosylated Cu:CdS quantum dots (QDs) for rapid bacterial discrimination and detection (Figure 2C). In this research, three natural glycans including glucose, stachyose, and raffinose were coated on the surface of Cu:CdS QDs to construct a glycosylated sensor array. Consequently, six bacteria strains (*V. desulphuricans*, *V. alginolyticus*, *P. aeruginosa*, *M. luteus*, *E. coli*, and *S. shewanella*) could be detected with a 91.6% discriminant accuracy within 30 min.

2.3. Peptides with Liposomes on Membrane

Antimicrobial peptides (AMPs) are small molecules composed of amino acids, exhibiting antimicrobial activities against bacteria as well as other microorganisms.[40,41] AMPs can interact

with bacterial cells to perform their therapeutic functions and can be divided into receptor-binding AMPs,[42–44] membrane-active AMPs,[45–47] and cell wall-inhibiting AMPs [48–50] according to different binding targets. Although the working mechanisms of AMPs are still under investigation,[51,52] their binding capacity with bacterial membrane has been widely applied for bacteria detection.[53–56] Even a single-AMP-based sensor array demonstrated effective for bacterial discrimination. Wu et al.[57] reported a sensor array consisting of an antimicrobial peptide (Ib-AMP4) and a green-fluorescent protein, which allowed for the discrimination of clinical bacterial isolations. Ten most frequent clinical species were classified in 79 trials with an accuracy above 70% by a single measurement, including *A. baunammii*, *E. areogenes*, *E. cloacae*, *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. aureus*, *S. epidermid*, and *S. maltoph*.

Based on the previous work, the same research group reported a turn-off sensor array (**Figure 3**A).[58] In this research, graphene oxide (GO) could absorb aromatic and charged materials due to its high volume-to-surface ratio. GO had binding affinities with two different typical structures of AMPs, including an alpha-helix AMP thanatin and a beta-sheet Ib-AMP4. This fluorescent sensor array enabled the classification of thirteen most frequent bacterial species with accuracies of above 70% in medical field, including *S. aureus*, *S. epidermidis*, *E. faecium*, *E. coli*, *P. aeruginosa*, *E. cloacae*, *S. maltophilia*, *A. baumannii*, *P. mirabilis*, *K. pneumoniae*, *E. aerogenes*, *K. oxytoca*, and *S. marcescens*.

AMPs have different antibacterial activities toward different bacterial strains, which can be employed for bacterial recognition. Bunz et al.[59] reported a polymer/peptide complex-based sensor array, in which four AMPs including protamine, Ib-AMP4, PAF26, and jelleine-I were used (Figure 3B). The different binding abilities of AMPs between bacterial surfaces and poly(para-phenyleneethynylene) (PPE) resulted in the dissociation of AMP-PPE complex composed of negatively charged PPE and positively charged AMP. By the measurement of the fluorescence intensity from free PPE, fourteen different bacteria including six Gram-negative and eight Gram-positive were detected in urine.

Using a similar approach, Zheng et al.[60] reported a four-channel bacterial discrimination assay based on short AMPs (Figure 3C). Four AMPs including FFFLSRIF (SAMP-1), WWWLSRIW (SAMP-2), WWWLKRIW (SAMP-3), and WWWLRKIW (SAMP-4) had different capacities to selectively interact with bacterial surfaces, which were exploited to develop a sensor array for bacterial detection and discrimination. Six bacteria could be effectively and simultaneously discriminated within 30 min, including *E. coli*, *S. sciuri*, *P. aeruginosa*, *L. monocytogenes*, *D. caledoiensis*, and *B. subtilis*.

2.4. Glycopeptide Antibiotics with D-Ala-D-Ala Dipeptide

Glycopeptide antibiotics, such as vancomycin and polymyxin, have high affinity to D-Ala-D-Ala dipeptide on bacterial cell walls, thus they have strong binding abilities to the surface of both Gram-positive and Gram-negative bacteria.[61–63] Antibiotics can act as recognition molecules for bacteria detection. For example, Zhang et al.[26] reported a fluorescence sensor array based on carbon dots functionalized with three kinds of receptors. Vancomycin as a glycopeptide antibiotic possesses a strong affinity to D-Ala-D-Ala dipeptide on bacteria. In this research, vancomycin, boronic acid, and polymyxin-functionalized carbon dots had the ability to bind to different bacteria, leading to a pattern of the fluorescence response for the classification of *D. desulfuricans*, *P. aeruginosa*, *E. coli*, *S. sciuri*, *S. aureus*, and *L. monocytogenes* (**Figure 4**A).

A competitive binding strategy can be employed to develop multidimensional sensing arrays based on antibiotic-targeted bacterial identification. Wang et al.[64] reported a sensor array using gold-silver alloy nanoclusters and gold nanoparticles (AuNPs). The sensor array was composed of negatively charged vancomycin-template AuNPs and positively charged cetyltrimethylammonium bromide (CTAB)-templated gold nanoclusters (AuNCs), which could help promote the discrimination of sulfur-containing species and the identification of sulfur bacteria (Figure 4B). For the performance of the sensor array, ten types of Gram-negative bacteria could be selectively discriminated at a low concentration level of OD_{600} = 0.015, including two strains of nonresistant *E. coli* and three strains of antibiotic-resistant *E. coli*, two species of *P. aeruginosa* and three common pathogenic bacteria, *Y. mollaretii*, *P. putida*, and *P. vulgaris*.

2.5. Proteins with Specific Receptors

Due to the multiple protein receptors on bacterial surface, protein could act as recognition probes for bacterial identification. For example, the peptide motifs on human serum albumin (HSA) can interact with the cell wall of bacteria. Lactoferrin has a high binding affinity with lactoferrin receptor on bacteria. Lysozyme can catalyze the hydrolysis of peptidoglycan on the cell wall of bacteria. These three proteins could be used as recognition probes in an array-based biosensor that discriminated analytes for bacterial identification (**Figure 5**A).[62] Protein-encapsulated AuNCs and vancomycin were used in the sensor array. This sensor array responded to the subtle changes in the physicochemical properties of the surfaces of different bacteria. Discrimination and classification of six bacteria could be achieved with a 100% accuracy through the analysis of UV–vis data, including four common bacteria (*A. faecalis*, *B. subtilis*, *E. coli*, and *S. aureus*) and two drug-resistant bacteria (kanamycin-resistant *E. coli*, KREC and methicillin-resistant *S. aureus*, MRSA).

Bovine serum albumin (BSA) can also serve as one of the components of a sensor array. Yan et al.[65] reported a fluorescence sensor array based on different AuNCs functionalized with proteins including BSA, HSA and Lysozyme (Figure 5B). Three protein-stabilized AuNCs were conjugated with two metal ions (Zn^{2+} and Cd^{2+}) to form six types of metal ion-protein-AuNCs, which were employed as the sensing elements to develop the fluorescent sensor array. In the presence of bacterial protein, the intensity of fluorescence changed due to the interaction between bacterial protein and AuNCs. According to the data analysis of fluorescence response and linear discriminant analysis, five different bacteria were identified including *Bacillus*, *B. aceticus*, *B. aeruginosa*, *E. coli*, *B. natto*, and *P. aeruginosa* with an accuracy of 100%.

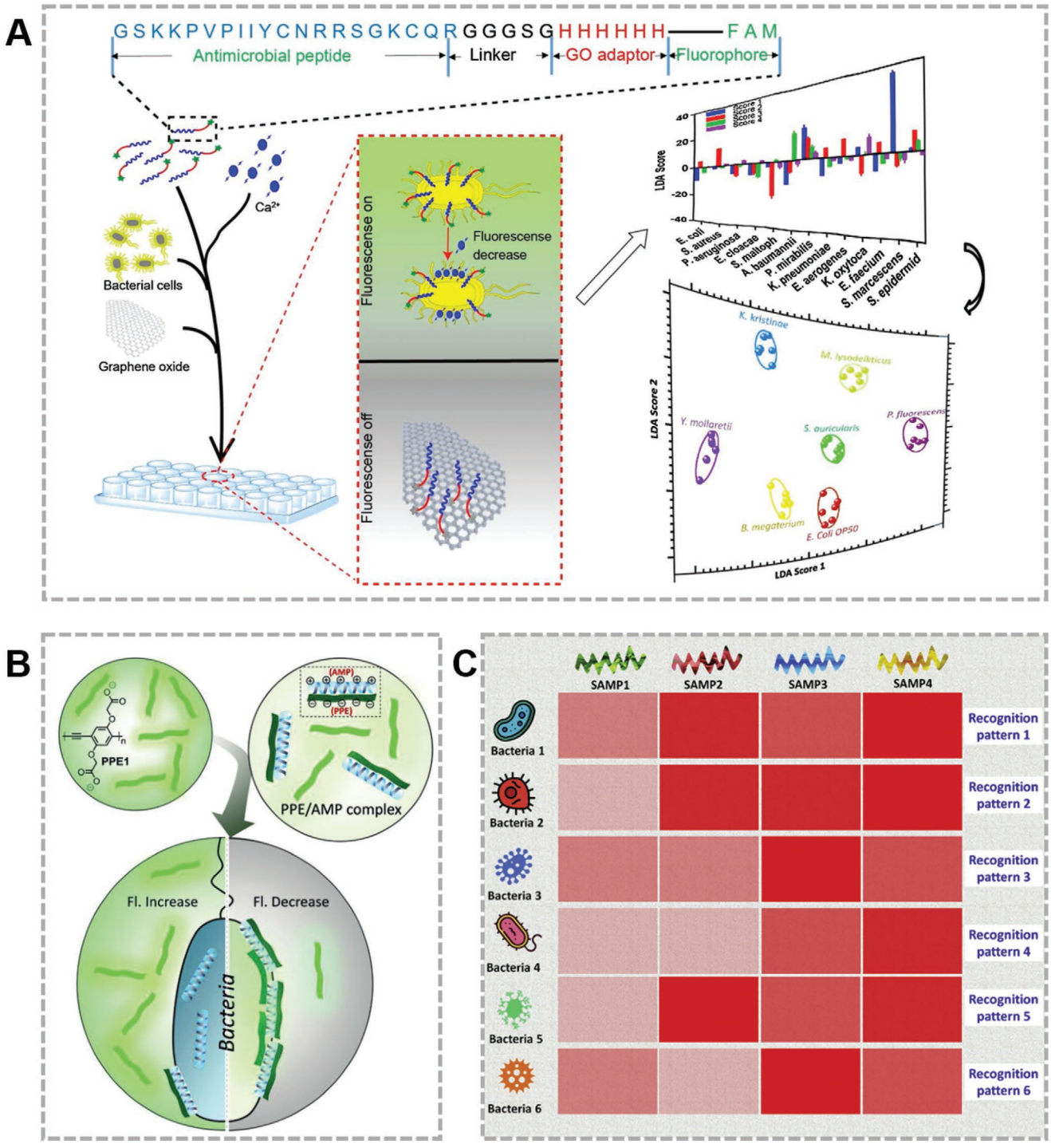


Figure 3. Bacterial recognition through the interaction between peptides and liposomes. A) Classification of clinical bacterial isolates with a sensor array based on graphene oxide and AMPs. Reproduced with permission.[58] Copyright 2019, Elsevier. B) Discrimination of bacteria in urine through a polymer/peptide complex array. Reproduced with permission.[59] Copyright 2017, Wiley-VCH. C) Multichannel bacterial discrimination based on four short AMPs. Reproduced with permission.[60] Copyright 2020, Elsevier.

3. Noncovalent Recognition at Bacteria–Material Interfaces

The surface of bacteria contains saccharide, proteins, and liposomes. Given that these biomolecules are composed of complex chemicals with versatile properties including different surface charges and hydrophobic/hydrophilic groups, materials that have different features can be used to interact with bacteria to develop biosensor arrays for bacteria detection. The noncovalent interactions including the electrostatic interaction and/or

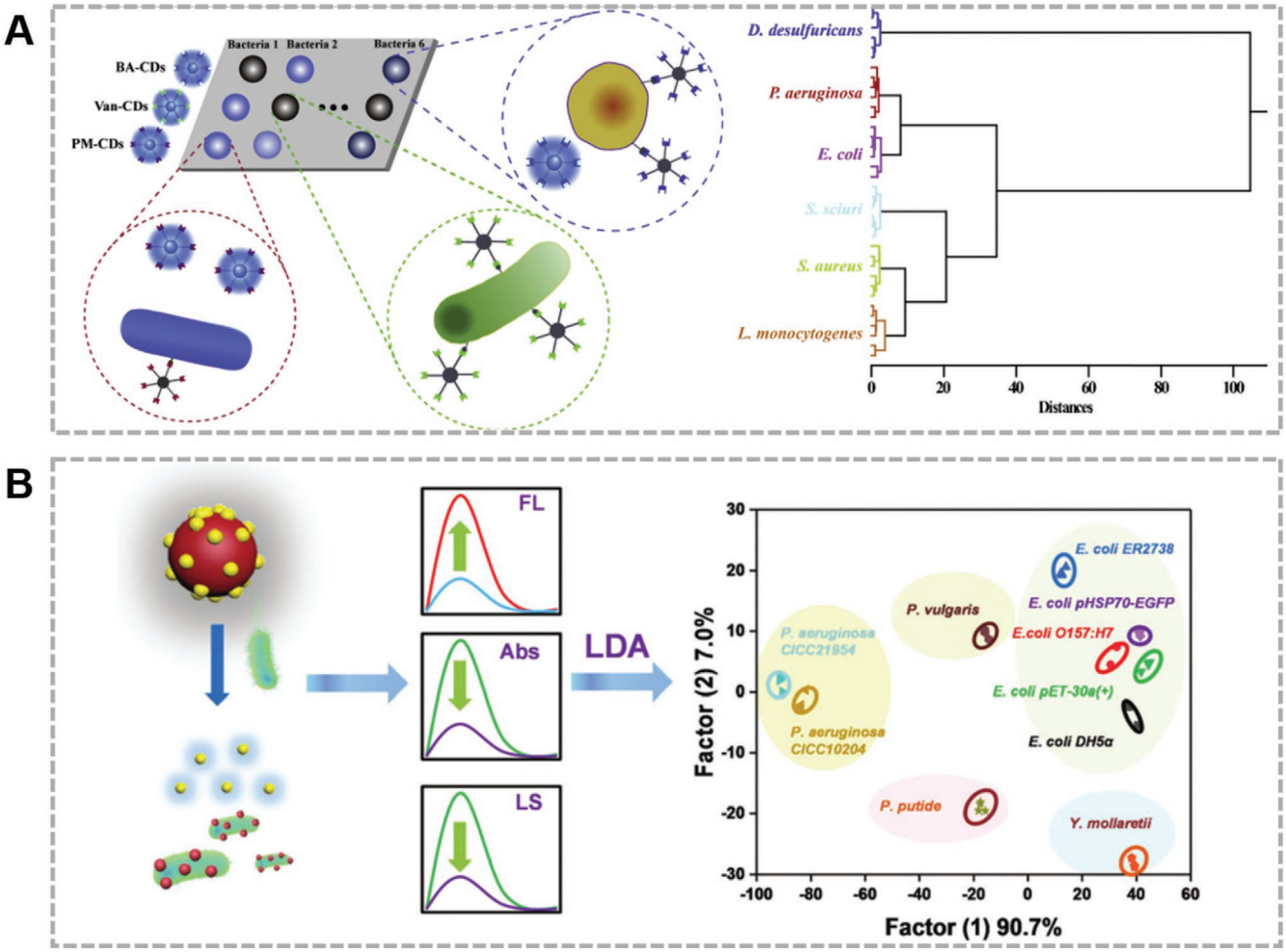


Figure 4. Bacterial recognition through the interaction between glycopeptide antibiotics and D-Ala-D-Ala dipeptide. A) Identification of bacteria by a fluorescence sensor array with vancomycin-functionalized carbon dots. Reproduced with permission.[26] Copyright 2019, Elsevier. B) Gram-negative bacteria identification using a sensor array with antibiotic-modified nanoparticles. Reproduced with permission.[64] Copyright 2020, Royal Society of Chemistry.

hydrophobic interaction can be used for bacterial recognition at the bacteria–material interface for designing the sensor array. In this section, materials that can be functionalized with different surface charges and hydrophobic/hydrophilic groups were highlighted and discussed.

3.1. Metal Nanoparticles

AuNPs can present a visible color change based on their dispersion-to-aggregation transformation in liquid solution, due to the localized surface plasmon resonance phenomenon.[66,67] More importantly, AuNPs can be easily functionalized with biomolecules with different features such as charged groups or hydrophobic/hydrophilic molecules.[68,69] Therefore, AuNPs functionalized with recognition receptors can act as promising probes to construct colorimetric sensors for bacterial identification.

AuNPs can be used for both qualitative and quantitative colorimetric detection.[70,71] For example, Rotello et al.[72] reported an enzyme-nanoparticle conjugate system capable of quantifying bacteria at a concentration of 1×10^2 bacteria mL^{-1} in solution. In this research, functionalized AuNPs acted as enzyme inhibitors that regulated the reaction between β-galactosidase and the substrate. As the size of AuNPs is tens to hundreds of nanometers, much smaller than microorganisms, one microorganism can aggregate multiple AuNPs. Aggregation-based quantitative detection allows the colorimetric recognition of a single microorganism with high sensitivity.[70] Compared with quantitative detection, the qualitative detection method can simultaneously identify multiple microorganisms.

A multichannel sensor array was developed based on functionalized AuNPs and fluorescent protein by Rotello et al.[73] Two types of AuNPs were fabricated with a cationic hydrophobic and a hydrophilic functional group, respectively (**Figure 6**A). Fluorescent protein was functionalized on AuNPs forming the protein-AuNPs conjugate that could give fluorescent readouts in the presence of bacterial biofilms, generating an essentially instantaneous readout. Through this AuNPs-based sensor array, six bacteria species including *E. coli* CD-3,

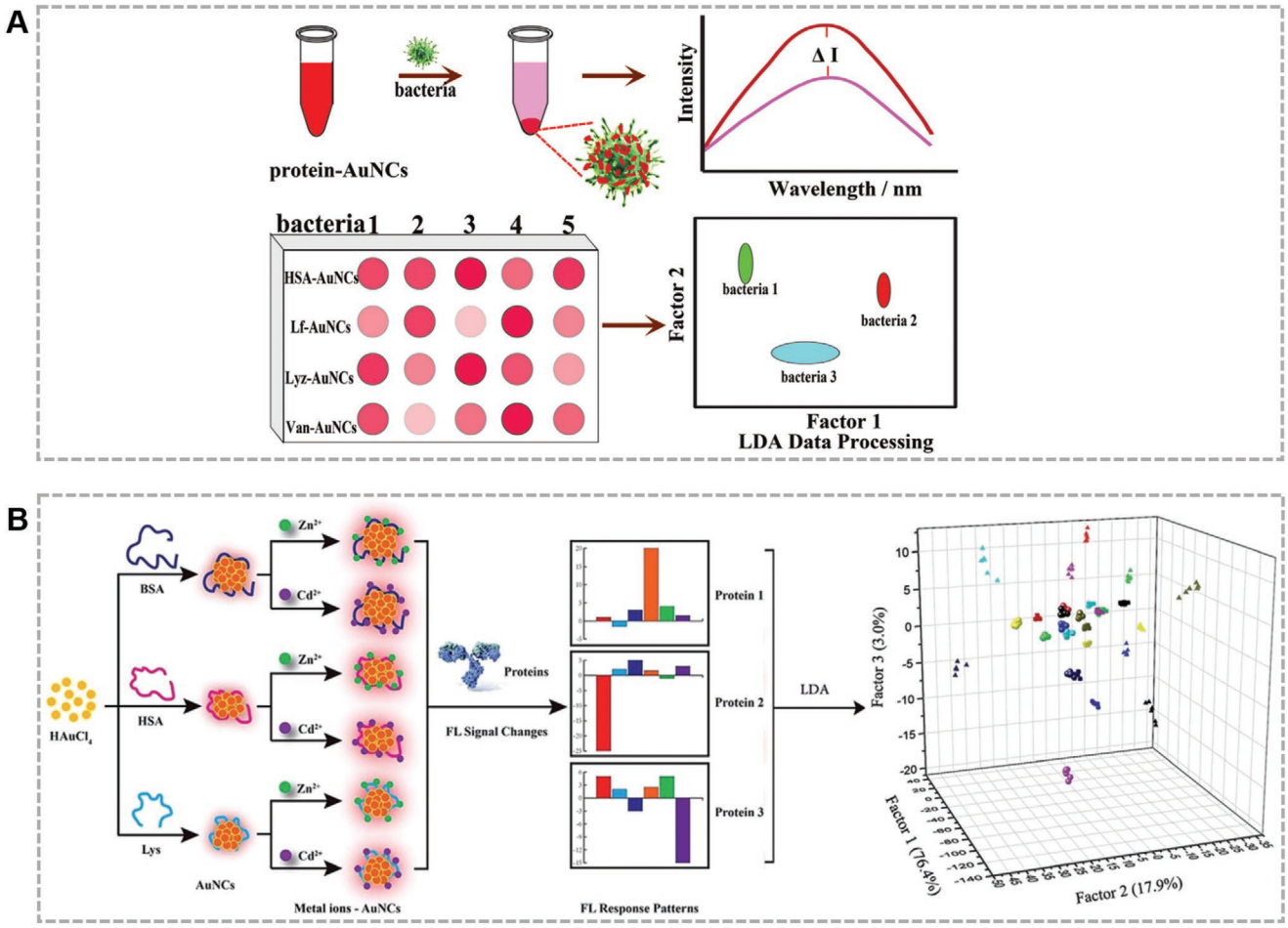


Figure 5. Bacterial recognition through the interaction between proteins and specific receptors on bacterial surface. A) Protein-encapsulated AuNCs for bacterial identification. Reproduced with permission.[62] Copyright 2018, Wiley-VCH. B) Fluorescent sensor array based on metal ion-AuNCs for bacteria detection. Reproduced with permission.[65] Copyright 2018, Royal Society of Chemistry.

P. aeruginosa CD-1006, *E. coli* DH5a, *A. azurea*, *B. licheniformis*, and *B. megaterium* could be discriminated, two of which were uro-pathogenic bacteria.

Meanwhile, the plasmonic property of AuNPs could be directly used in a sensor array for the signal readout. Wu et al.[74] reported a colorimetric sensor array in which AuNPs with diverse surface charges were used as sensing elements (Figure 6B). Four different AuNPs functionalized with mercaptopropionic acid, mercaptosuccinic acid, cysteamine, and CTAB were used to sense the microorganism in water. These AuNPs acted as colorimetric sensing elements with bacteria due to the different surface charges of AuNPs. By analysis of UV–vis data, fifteen microorganisms including twelve bacteria and three fungi were differentiated with a classification accuracy of 100%.

Similar to AuNPs, AgNPs have been also widely applied for colorimetric sensing of biomolecules due to their high extinction coefficient. AgNPs-based colorimetric detection of bacteria relies on target-induced aggregation of AgNPs. For example, 4-MPBA functionalized AgNPs demonstrated by Zhang et al.[27] had different binding abilities toward different bacteria species in solution. When incubating with Gram-negative bacteria, it resulted in the aggregation of MPBA-AgNPs that could distinguish *E. coli* from the other four Gram-positive bacteria (*S. sciuri*, *B. safensis*, *B. velezensis*, and *S. aureus*) in 20 min.

Magnetic nanoparticles (MNPs) can be also used to develop biosensor arrays for bacterial detection.[75,76] MNPs feature in magnetism that enables a simple and convenient method for separation. Zhang et al.[77] reported an MNPs-fluorescent polymer sensor array in which MNPs functionalized with different hyamines had the ability to bind with fluorescent chiral polymer functionalized with side-chain carboxylic acid group (PFBT) (Figure 6C). Negatively charged bacteria could competitively bind to MNPs leading to the dissociation of PFBT, thus it could be used for detection and identification of bacteria in liquid. Through the measurement of the fluorescence intensity of supernatant and the calculation of the data matrix, eight bacteria were differentiated including *S. oneidensis*, *V. fischeri*, *M. luteus*, *E. tarda*, *E. coli*, *V. alginolyticus*, *P. aeruginosa*, and *P. pastoris*.

In the research of Guo et al.,[78] a urease nanosensor was reported that has three types of quaternized MNPs with excellent sensitivity for both Gram-positive and Gram-negative bacteria (Figure 6D). This sensor array took advantage of quaternized MNPs that had a stronger ability to bind with bacteria than urease that was conjugated noncovalently. The

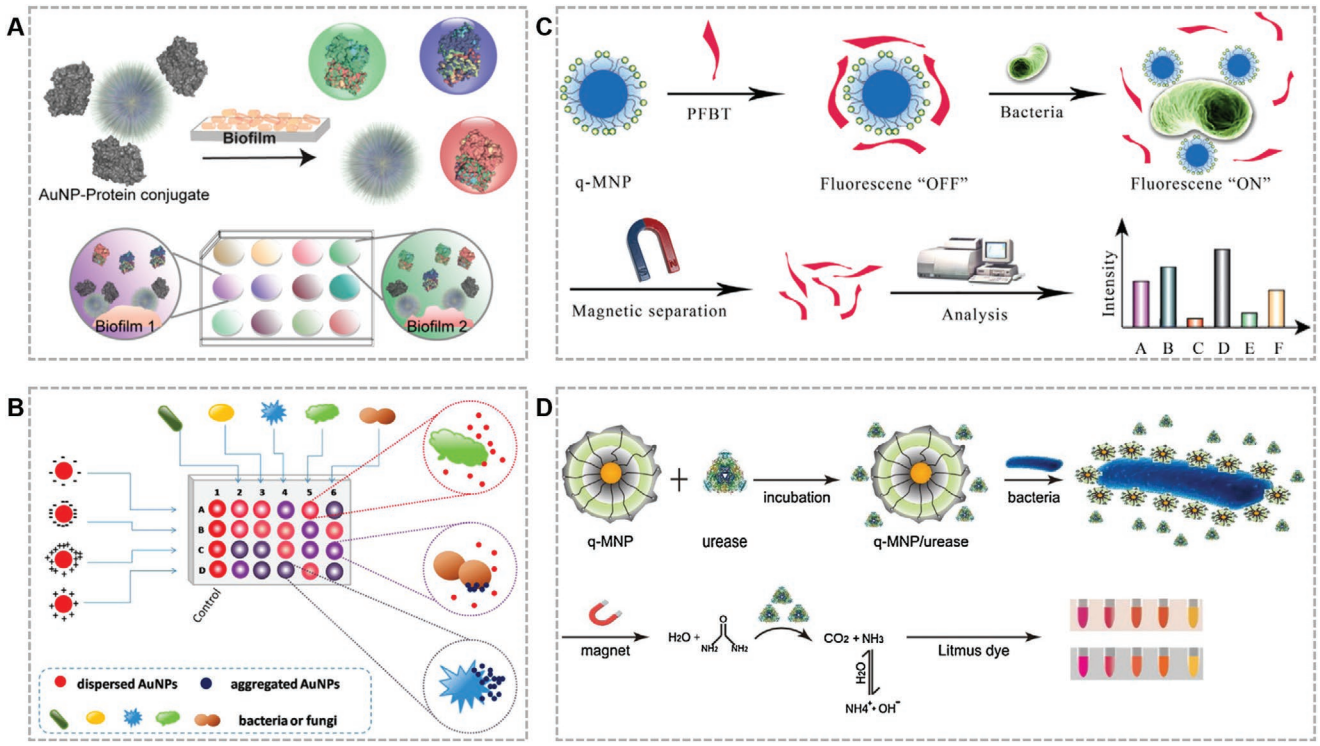


Figure 6. Bacterial recognition through noncovalent interaction between bacteria and metal nanoparticles. A) A AuNPs-based multichannel sensor to detect bacteria based on the physicochemical properties on biofilms. Reproduced with permission.[73] Copyright 2014, American Chemical Society. B) A colorimetric sensor array based on AuNPs with diverse surface charges for microorganism identification. Reproduced with permission.[74] Copyright 2017, American Chemical Society. C) A quaternized MNPs-fluorescent polymer system for bacteria detection. Reproduced with permission.[77] Copyright 2013, Elsevier. D) An MNPs-urease nanosensor for bacterial detection with a colorimetric readout by litmus dye. Reproduced with permission.[78] Copyright 2017, Elsevier.

hydrolysis of urea by free ureases produced ammonia that could be detected and quantified by the color change of litmus dye. Seven pathogens could be detected with an accuracy of 90.7% within 30 min, including *S. aureus*, *A. junii*, *V. harveyi*, *M. luteus*, *E. tarda*, *V. parahemolyticus*, and *E. coli*.

3.2. Aggregation Induced Emission (AIE) Materials

AIE materials have been used for bacterial identification based on their strong fluorescence which is triggered by the restriction of intramolecular motion.[79,80] With the predominant capability of versatile functional groups, positive/negatively charged AIE molecules have demonstrated as excellent probes for bacteria detection.[80,81] For instance, Jiang et al.[82] reported a fluorescent array composed of five different AIE molecules with various electrostatic properties (**Figure 7**A). These designed AIE molecules were synthesized with one or two positively/negatively charged groups. After incubating with a variety of bacteria, the AIE sensor array demonstrated to be effective for the identification of eight bacteria including *E. coli*, *K. pneumonia*, *S. choleraesuis*, *S. aureus*, *S. epidermilis*, *B. subtilis*, and two antibiotic-resistant bacteria multi-drug resistance (MDR) *E. coli* and MRSA with an accuracy of 93.75%.

A series of AIE materials could be designed and synthesized with versatile electrostatic and hydrophobic properties, bearing both different positive, negative, or zero charges, and different hydrophobic groups. Zhou et al.[83] reported an artificial tongue that contained a series of AIE molecules functionalized with one or two aldehyde, carboxylic acid, and quaternary ammonium groups (Figure 7B). Since different AIE molecules had different binding abilities with bacteria, they could be differentiated by the quantitative analysis of relative fluorescence intensity. Using this artificial tongue, eight bacteria species were distinguished including *E. coli*, *S. aureus*, *B. subtilis*, *P. aeruginosa*, *V. cholera*, *K. pneumoniae*, *L. monocytogenes*, and potential bioterrorism agent *Y. pestis*.

Different from the above research, Xu et al.[84] reported an imidazolium-derived pyrene aggregation that had similar aggregation-induced fluorescence properties with AIE. A bacterial identification sensor could be developed by using imidazolium based on its binding ability to anionic bacterial surface through electrostatic interaction (Figure 7C). Pyrene was a classic fluorophore that can emit fluorescence. Pyrene could be aggregated by imidazolium and the aggregation resulted in a fluorescence quenching. After the introduction of bacteria, synergistic effects of electrostatic interaction and hydrophobic force led to a competing binding between bacteria surfaces and the molecules, leading to the disassembly of the molecules to give a fluorescence turn-on signal. Based on the output signals of two fluorescent channels, ten species of bacteria and fourteen clinical isolated multidrug-resistant bacteria were identified through the sensor including both Gram-positive and Gram-negative bacteria.

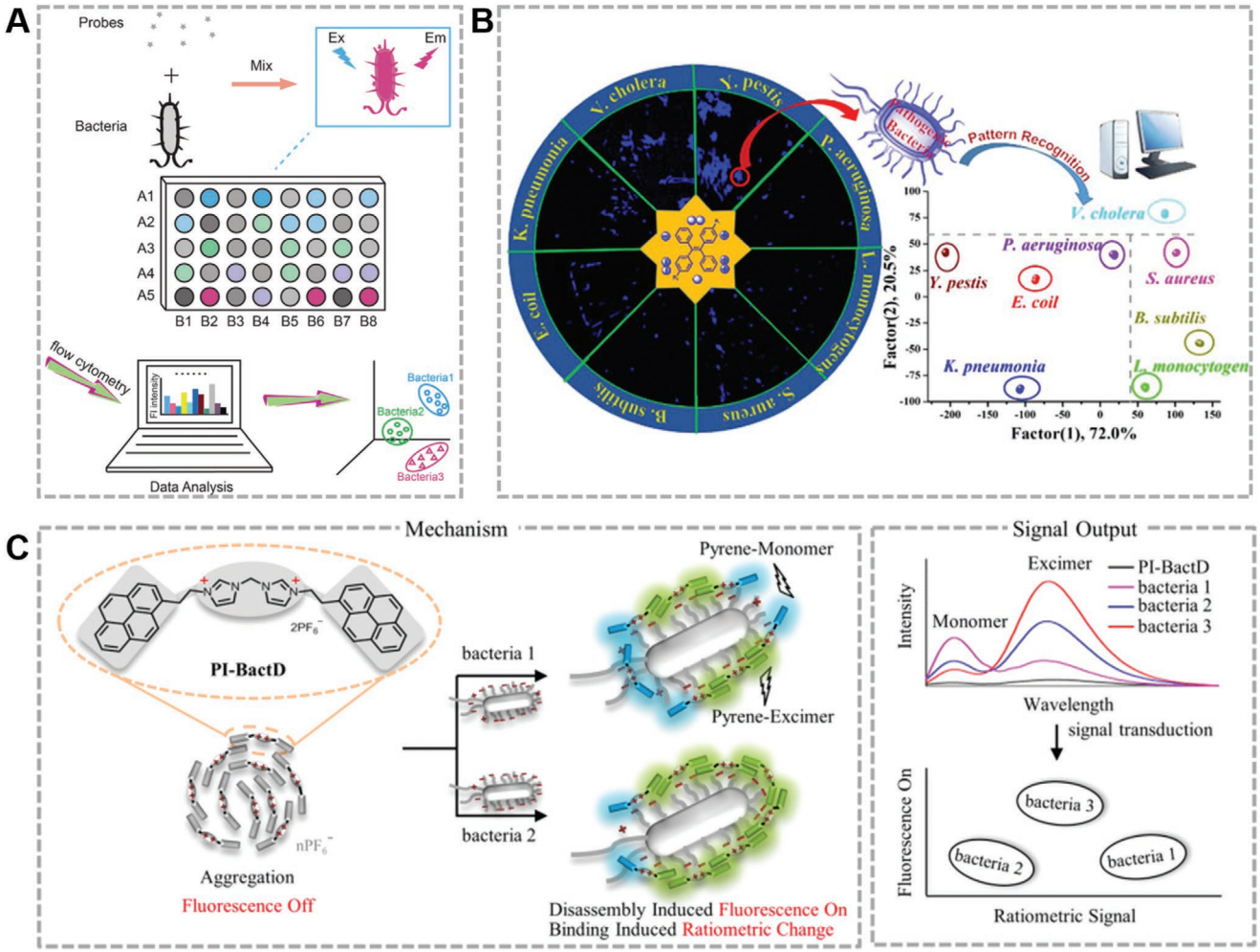


Figure 7. Bacterial recognition through noncovalent interaction between bacteria and AIE materials. A) A fluorescent array with five AIE probes for bacterial identification. Reproduced with permission.[82] Copyright 2014, Wiley-VCH. B) An artificial tongue with six AIE molecules for pathogenic bacterial discrimination. Reproduced with permission.[83] Copyright 2017, American Chemical Society. C) A pyrene derivative membrane-responsive aggregation fluorescence sensor for bacterial identification. Reproduced with permission.[84] Copyright 2019, American Chemical Society.

3.3. Conjugated Polymers

Conjugated polymers with different structural designs can have light harvesting properties due to their conjugated backbones that can be directly used for signal readout.[85,86] Functionalized conjugated polymers can undergo a microbial-mediated aggregation or a conformational transformation due to the multivalent specific interaction with bacteria.[87–89] The noncovalent interaction can be used for the identification of microorganisms, because bacteria with different surface properties have different binding affinities with polymers.[90] A conjugated polymer-based supramolecular sensor was designed for discrimination of virus and microbes by Wang et al. (**Figure 8**A).[86] In this approach, a cationic polythiophene derivative and barrel-shaped macrocyclic molecular cucurbit uril acted as the supramolecular components in the sensor. Through the detection and analysis of fluorescent signals, bacteria and virus could be distinguished including tobacco mosaic virus (TMV), *E. coli*, *S. aureus*, and *C. albicans*.

Conjugated polymers can combine with other functional molecules to enhance the response of multivalent interactions for bacteria detection. For example, Tang et al.[91] developed a sensor array using both AIE luminogens and tetraphenylethylene (TPE) derivatives for detection and discrimination of pathogens (Figure 8B). In this sensor array, a series of AIE-active TPE derivatives (TPE-ARs) were designed and synthesized with positively charged ammonium group and different hydrophobic groups, leading to the different electrostatic and hydrophobic interactions with pathogens. By recognizing the fluorescence pattern, seven bacteria including normal and drug-resistant bacteria, Gram-positive and Gram-negative were identified with a nearly 100% accuracy.

With remarkable light harvesting ability and amplifying effects of optical signals, conjugated polymers have been used as the energy donor of the fluorescence resonance energy transfer (FRET) process for biosensing and bioimaging. Wang et al.[92,93] reported a sensor array based on conjugated polymer-quantum dot hybrid material through FRET process, in which cationic poly (fluorene-alt-phenylene) derivative (PFP)/QDs

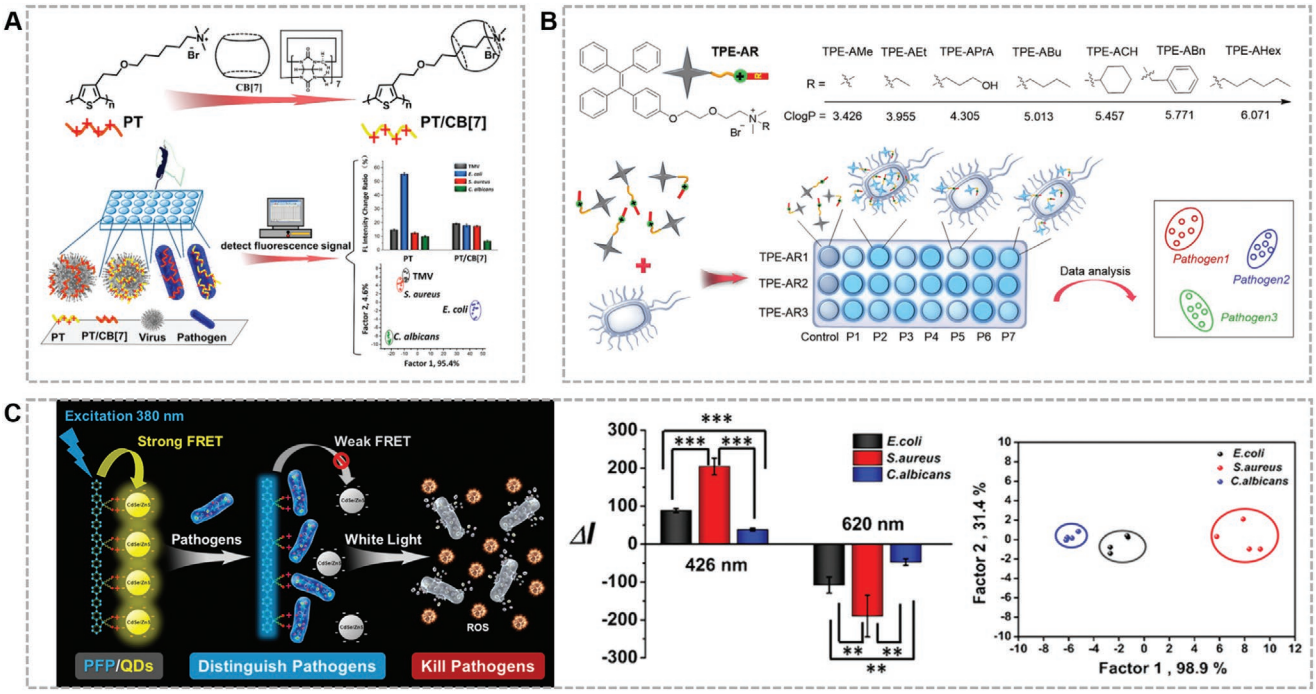


Figure 8. Bacterial recognition through noncovalent interaction between bacteria and conjugated polymers. A) A conjugated polymer-based supramolecular system for discrimination of virus and microbes. Reproduced with permission.[86] Copyright 2018, American Chemical Society. B) An AIE-TPE derivatives sensor for pathogen identification. Reproduced with permission.[91] Copyright 2018, Wiley-VCH. C) A competitive fluorescence sensor composed of conjugated polymer-quantum dot hybrid materials for discrimination of three types of bacteria. Reproduced with permission.[92] Copyright 2019, American Chemical Society.

hybrid material was used to interact with various pathogens to result in a fluorescence recovery of PFP (Figure 8C). By adding different types of bacteria, the FRET process from PFP to QDs was interrupted because of the competitive binding between PFP and the pathogens. *E. coli*, *S. aureus*, and *C. albicans* could be detected due to their different binding abilities with PFP that could result in distinct fluorescence responses.

4. Recognition through Bacterial Metabolites

Bacteria can intake nutrients from external environment such as carbohydrate, proteins, amino acids, and other carbon/nitrogen sources, to ensure normal activities through various biochemical transformations performed internally. Since different bacteria have their own unique biochemical pathways, their metabolites are usually different. By identifying the bacterial metabolites, microorganisms can be distinguished and identified. In this section, bacterial detection will be discussed from the perspective of bacterial metabolites including pH, volatile, and nonvolatile compounds.

4.1. pH

Bacteria produce weak acids such as carbonic acid and lactic acid through metabolic activities such as aerobic respiration and fermentation, thereby reducing the pH of the bacteria-containing solution. pH value can be used as an indicator to evaluate bacterial growth for developing sensors for bacterial identification.[94–98] Dipanjan et al.[99] presented a pH-sensitive fluorescent sensor embedded in agarose to distinguish bacteria growing in a solid medium (**Figure 9**A). Key to this sensor was the pH-responsive yttrium and carbon nanoparticles. A pH change in agarose could cause the aggregation of yttrium and carbon nanoparticles. The fluorescence quenching induced by aggregation could be quantified by the wide-range fluorescent signal. In this way, bacteria including *E. coli*, *B. subtilis*, and *S. mutans* could be differentiated by the fluorescence intensity in both mono- and multiexisting state.

Taking advantage of the metabolism of sugars such as glucose and lactose, bacteria can be accurately distinguished. Wang et al.[100] reported a pH-dependent carbon dots synthesized via a facile one-step hydrothermal method (Figure 9B). When pH-dependent carbon dots were introduced into an aqueous solution containing pathogenic bacteria and sugar molecules, the fluorescent reading would change linearly with the pH caused by the acidic substances produced during the sugar metabolism of bacteria. The pH-dependent carbon dots platform could accurately distinguish bacteria through the readout of fluorescence intensity between *E. coli* and *S. aureus* with a small sample volume (100 µL) and a rapid response (25 min).

4.2. Volatile Organic Compounds (VOCs)

VOCs are the major metabolites in the cycle of bacteria growth and can be classified into six categories, including carboxylic

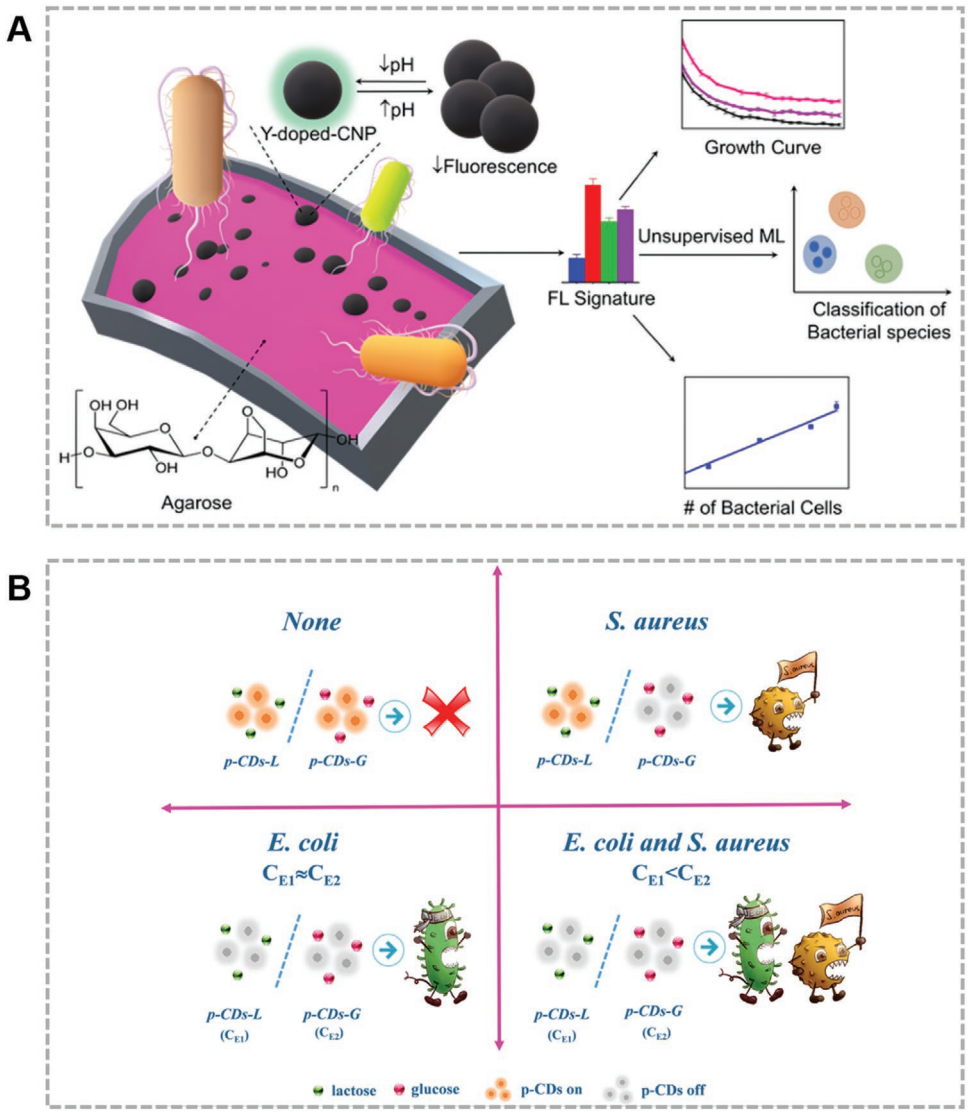


Figure 9. Bacterial recognition through pH changes caused by bacterial metabolism. A) A label-free sensor for bacterial detection based on pH-sensitive fluorescent yttrium and carbon nanoparticles embedded in agarose. Reproduced with permission.[99] Copyright 2019, American Chemical Society. B) Strategies to distinguish between *E. coli* and *S. aureus* based on pH-dependent carbon dots. Reproduced with permission.[100] Copyright 2020, Elsevier.

acids, alcohols, aldehydes, esters, hydrocarbons, and organic sulfur derivatives.[101,102] Some VOCs are common metabolites of different bacterial species while some VOCs only exist in specific bacterial metabolic pathways, which can be utilized to distinguish bacteria of different genera, species, and subspecies.[103–106]

The colorimetric sensor array is usually used to identify bacteria by obtaining the volatile products released during their growth. Banaei et al.[107,108] reported an inexpensive, printed, disposable sensor array that responded to the VOCs emitted by microorganisms into plate headspace. The sensor consisted of 80 different chemical response indicators printed on a polyvinylidene fluoride membrane, including metal ion-containing dyes, pH indicators, dyes with large permanent dipoles, metal salts, and nucleophilic indicators. An automated flat-panel processing system integrated with a digital camera could be used to continuously monitor the VOCs produced by metabolism to detect and identify growing bacteria. Eighteen pathogenic bacterial species in blood culture could be identified at an inoculum concentration as low as eight colony forming units (CFU) per plate with an overall sensitivity of 95.1% through the reading on a smartphone (**Figure 10**A).

Based on previous studies, Hemmateenejad et al.[109] reported a colorimetric assay based on nanoparticle arrays to identify ten infectious bacteria strains belonging to both Gram positive and Gram-negative species. This colorimetric sensor array was composed of sixteen functionalized metal nanoparticles (eight AgNPs and eight AuNPs) synthesized with different reducing and stabilizing agents. The principle of the sensor array was based on the interaction of the volatile metabolites emitted from bacteria with the array of metallic nanoparticles deposited on a paper substrate (Figure 10B). The sensor could be successfully used to detect

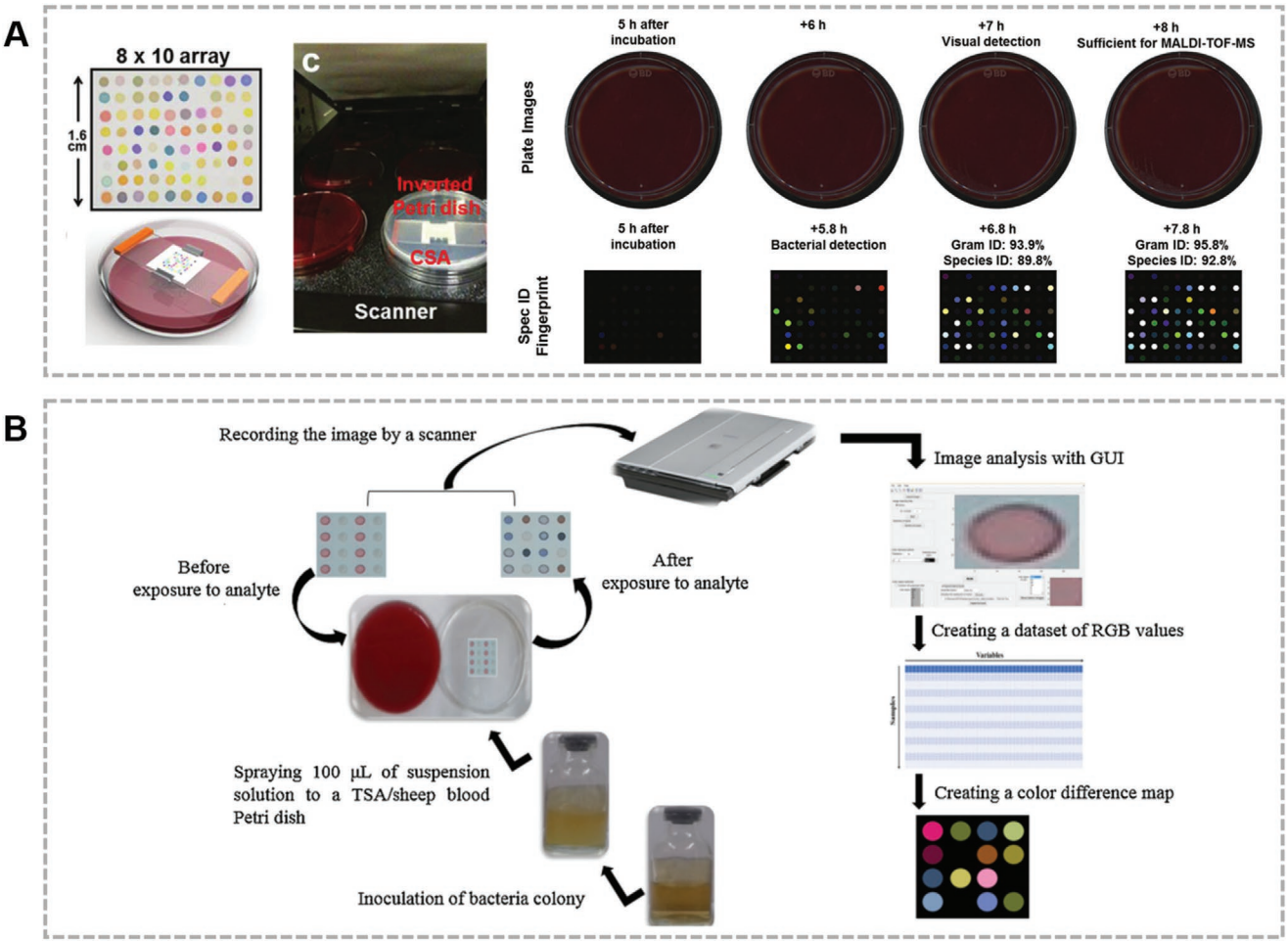


Figure 10. Bacterial recognition through volatile organic compounds produced by bacterial metabolism. A) Fifteen pathogenic bacteria detection and identification in blood agar plates. Reproduced with permission.[107] Copyright 2016, Royal Society of Chemistry. B) Identification of ten infectious bacteria strains based on a colorimetric assay composed of eight AgNPs and eight AuNPs. Reproduced with permission.[109] Copyright 2020, Elsevier.

bacteria in drinking water as well as human urine (containing 177 healthy and 123 patient samples) in less than 50 min, which was dramatically faster than traditional urine culture.

4.3. Nonvolatile Compounds

Apart from VOCs produced by bacteria, many bacterial metabolites are nonvolatile compounds that can be directly measured in liquid phase samples.[110] Versatile methods can be used for the identification of bacterial metabolites in solution.

Nuclear magnetic resonance (NMR) is a powerful technique that has the advantages of being nondestructive, intrinsically quantitative and information-rich with respect to the determination of molecular structures, especially in the setting of complex mixture such as bio-fluids. In the research of Herrmann et al.,[111] NMR was applied for the rapid bacterial discrimination relying on untargeted metabolic profiling of supernatants from bacterial culture media (**Figure 11**A). As a consequence, six bacterial species were identified with both Gram-positive and Gram-negative bacteria including *E. coli*, *P. aeruginosa*, *P. mirabilis*, *E. faecalis*, *S. aureus*, and *S. saprophyticus*.

Except for magnetic methods, electrochemical methods can be used for detection of bacterial metabolites as well. In the research of Raguse et al.,[112] an electrochemical sensor was used to detect bacterial metabolites to distinguish bacteria relying on the change of resistance of AuNPs (Figure 11B). The principle was to use the specific resistance of AuNPs in the metabolites of different bacteria to identify bacterial species. Four different bacterial species could be discriminated in liquid based on their metabolic profiles, including *E. coli*, *B. subtilis*, *S. epidermidis*, and *E. aerogenes*.

5. Conclusion

Array-based sensing has demonstrated as effective tools for bacterial identification that can detect mixed microbial components for applications in fields such as biomedical diagnosis and food safety. This review outlines different recognition methods from the views of bacterial characteristics. These recognition strategies can be classified into three categories including 1) specific molecular receptors on bacterial cell membranes, including boronic acids with *cis-diol* groups of saccharides, glycan with

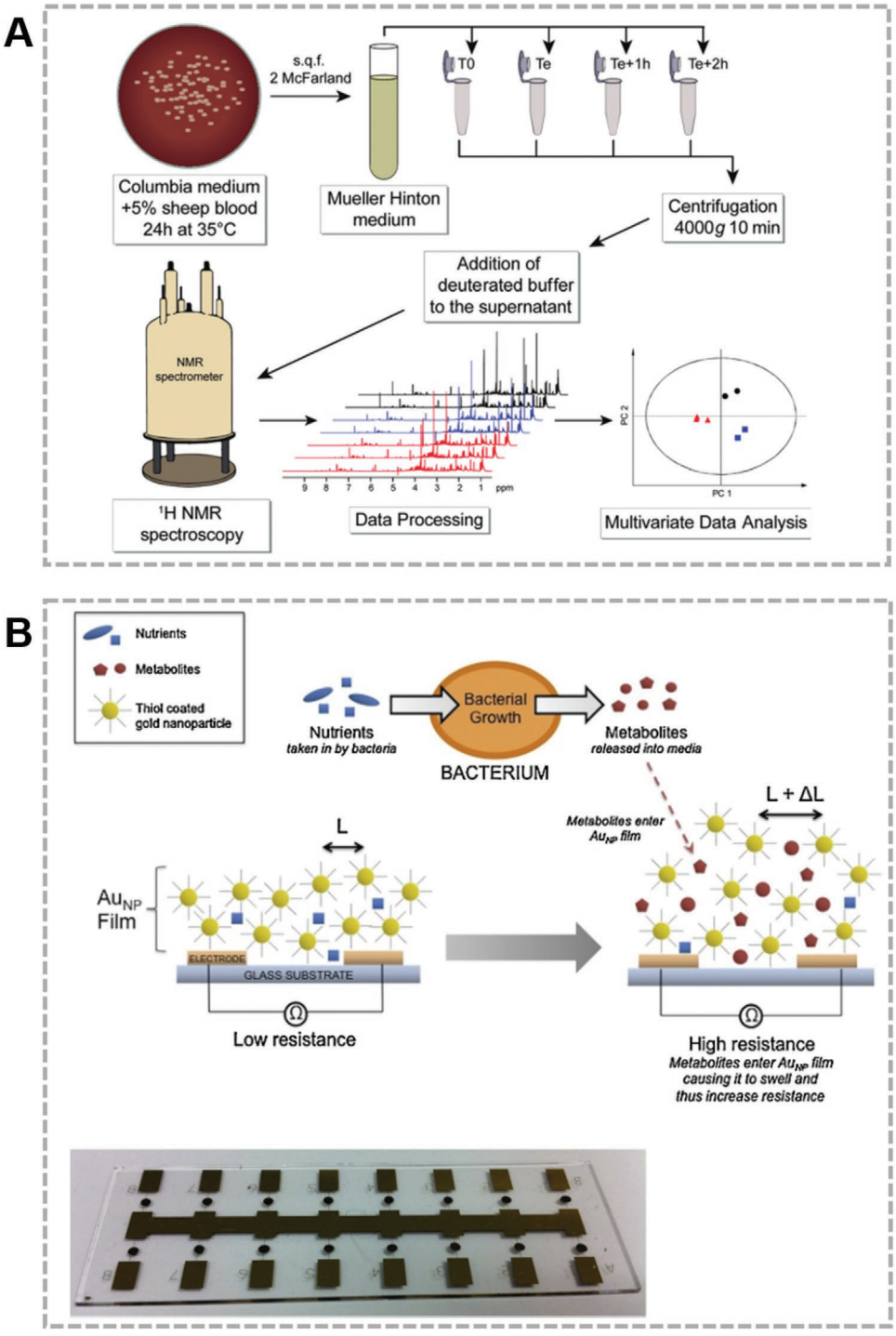


Figure 11. Bacterial recognition through nonvolatile compounds produced by bacterial metabolism. A) Nuclear magnetic resonance method to analyze bacterial metabolites to distinguish microorganisms. Reproduced with permission.[111] Copyright 2016, Royal Society of Chemistry. B) Discrimination of bacterial metabolites utilizing AuNPs chemi-resistor sensors for bacterial detection. Reproduced with permission.[112] Copyright 2015, Elsevier.

lectin proteins, peptides with bacterial membrane, glycopeptide antibiotics with D-Ala-D-Ala dipeptide, and proteins with specific receptors; 2) noncovalent interactions such as electrostatic force and hydrophobic force between negatively charged bacteria and positively charged materials including metal nanoparticles, AIE materials, and conjugated polymers; 3) specific metabolites of bacteria including changes of pH, volatile, and nonvolatile molecules. A summary of versatile recognition methods for array-based biosensors is listed in **Table 1**, including functionalized materials and their target location in different bacteria, the concentration and medium for bacteria preparation, as well as the reaction time and signal readout of the biosensor array.

Table 1. A summary of versatile recognition methods for array-based biosensors.

Functional group	Material	Targeted group	Target location	Bacterial type	Species	Quantity	Reaction time	Sample type	Concentration	Medium	Signal readout	Reference
4-Mercaptophenylboronic acid (MPBA)	AgNPs	*Cis-diol*	Specific receptor on bacterial surface	Gram-negative	*E. coli*	1	20 min	Bacterial suspension	$0.9 \times 10^4 - 1 \times 10^7$ CFU mL^{-1}	PBS	UV–vis	[27]
4-Mercaptophenylboronic acid (MPBA) Mercaptoethylamine (MA)	AgNPs	*Cis-diol*	Specific receptor on bacterial surface	Gram-positive Gram-negative	*E. coli* National Center for Medical Culture Collections, China (CMCC) (B)44102 *E. coli* BL21 *S. aureus* *S. Flexneri* *E. faecalis* *S. epidermidis* *S. thermophilus* *A. aceticus,* *B. subtilis* *L. monocytogenes* *E. sakazakii* *V. parahemolyticus* *P. aeruginosa* *S. paratyphi* *C. albicans* *S. cerevisiae* *S. pneumoniae*	17	15 min	Bacterial suspension	$OD_{600} = 1$	PBS	UV–vis	[30]
Boronic acid Polymyxin Vancomycin	Carbon dots	Lipopolysaccharide molecules D-Ala-D-Ala *cis-diol*	Specific receptor on bacterial surface	Gram-positive Gram-negative	*E. coli* *D. desulfuricans* *S. sciuri* *L. monocytogenes* *S. aureus* *P. aeruginosa*	6	60 min	Bacterial suspension	$OD_{600} = 1$	PBS	Fluorescence	[26]
Glycans: arabinose (Ara) Gellobiose (Cel) Dextran (Dex) Galactose (Gal) Glucose (Glc) *N*-acetyl-D-glucosamine (GlcNAc) Glyceraldehyde (Gly) Lactose (Lac) Mannose (Man)	–	Lectins	Specific receptor on bacterial surface	Gram-positive Gram-negative	*E. coli* Top10 *E. coli* K12 *P. putida* *M. smegmatis* *M. marinum*	5	30 min	Bacterial suspension	$OD_{600} = 1$	PBS	Fluorescence	[38]
Glucose Stachyose Raffinose	Quantum dots	Lectins	Specific receptor on bacterial surface	Gram-positive Gram-negative	*V. desulphuricans* *V. alginolyticus* *P. aeruginosa* *M. luteus* *E. coli* *S. shewanella*	6	30 min	Bacterial suspension	$OD_{600} = 0.3$	PBS	Fluorescence	[39]

Table 1. Continued.

Functional group	Material	Targeted group	Target location	Bacterial type	Species	Quantity	Reaction time	Sample type	Concentration	Medium	Signal readout	Reference
Poly(paraphenyleneethynylene) (PPE) Antimicrobial peptides (AMPs)	–	Liposomes	Specific receptor on bacterial surface	Gram-positive Gram-negative	*B. megaterium* *S. auriculanis* *M. lysodeikticus* *K. kristinae* *K. marina* *K. rhizophila* *K. salsicia* *K. varians* *P. fluorescens* *Y. mollaretii* *E. coli* K12 *E. coli* HT115 *E. coli* OP50 *E. coli* DH5a	14	30 min	Bacterial suspension	$OD_{600} = 0.1$	PBS	Fluorescence	[59]
6-Carboxyfluorescein-labeled peptides	Graphene oxide	Liposomes	Specific receptor on bacterial surface	Gram-positive Gram-negative	*S. aureus* *S. epidermidis* *E. faecium* *E. coli* *P. aeruginosa* *E. cloacae* *S. maltophilia* *A. baumanni* *P. mirabilis* *K. pneumoniae* *E. aerogenes* *K. oxytoca* *Serratia marcescens*	13	5 min	Bacterial suspension	$OD_{600} = 0.3$	PBS	Fluorescence	[58]
Ib-AMP4	AMPs-fluorescein hybrid	Liposomes	Specific receptor on bacterial surface	Gram-positive Gram-negative	*B. megaterium* *B. subtilis* *M. luteus* *S. agalactiae* *E. faecalis* *S. oralis* *S. epidermidis* *S. aureus* *S. pneumoniae* *S. pyogenes* *E. casseliflavus*	15	24 h	Bacterial suspension	$OD_{600} = 0.3$	PBS	Fluorescence	[57]
Four AMPs, amino acid sequences as follows: FFFLSRIF (SAMP-1) WWWLSRIW (SAMP-2) WWWLKRIW (SAMP-3) WWWLRKIW (SAMP-4)	AMPs-fluorescein hybrid	Liposomes	Specific receptor on bacterial surface	Gram-positive Gram-negative	*E. coli* *S. sciuri* *P. aeruginosa* *L. monocytogenes* *B. subtilis*	6	30 min	Bacterial suspension	$OD_{600} = 1$	PBS	Fluorescence	[60]

Table 1. Continued.

Functional group	Material	Targeted group	Target location	Bacterial type	Species	Quantity	Reaction time	Sample type	Concentration	Medium	Signal readout	Reference
Human serum albumin (HSA) Lysozyme Lactoferrin Vancomycin	AuNCs	Proteins	Specific receptor on bacterial surface	Gram-positive Gram-negative	*S. aureus MRSA* *E. coli KREC* *B. subtilis* *A. faecalis*	6	1 h	Bacterial suspension	$OD_{600} = 1$	PBS	UV–vis	[62]
Bovine serum albumin (BSA) Human serum albumin (HSA) Lysozyme	AuNCs	Proteins	Specific receptor on bacterial surface	Gram-positive Gram-negative	*Bacillus* *B. aceticus* *B. natto* *E. coli* *P. aeruginosa*	5	30 min	Bacterial suspension	$OD_{600} = 0.01$	PBS	Fluorescence	[65]
Boronic acid Polymyxin Vancomycin	Carbon dots	*Cis-diol* D-Ala-D-Ala	Specific receptor on bacterial surface	Gram-positive Gram-negative	*E. coli* *P. aeruginosa* *S. sciuri* *S. aureus* *D. desulfuricans* *L. monocytogens*	6	60 min	Bacterial suspension	$OD_{600} = 1$	PBS	Fluorescence	[26]
Charges	Quaternized MNPs	Electrostatic interaction	Nonspecific binding with bacteria	Gram-positive Gram-negative	*S. oneidensis* *V. fischeri* *M. luteus* *E. tarda* *E. coli* *V. alginolyticus* *P. aeruginosa*	8	20 min	Bacterial suspension	$OD_{600} = 0.2$	PBS	Fluorescence	[77]
Hydrophobic group Hydrophilic group	AuNPs	Electrostatic interaction	Nonspecific binding with bacteria	Gram-positive Gram-negative	*E. coli* CD-3 *E. coli* DH5a *B. megaterium* *B. licheniformis* *A. azurea* *P. aeruginosa* CD-1006	6	45 min	Bacterial suspension	$OD_{600} = 0.1$	PBS	Fluorescence	[73]
Charges	AuNPs	Electrostatic interaction	Nonspecific binding with bacteria	Gram-positive Gram-negative	*S. aureus* *S. epidermidis* *L. monocytogenes* *B. aceticus* *P. aeruginosa* *E. coli* *B. subtilis* *S. paratyphi* *E. sakazakii* *S. flexneri* *V. parahaemolyticus* *C. putrefaciens* *C. albicans* *A. flavus* *Penicillium*	15	5 s	Bacterial suspension	$OD_{600} = 0.05$	PBS	UV–vis	[74]

Table 1. Continued.

Functional group	Material	Targeted group	Target location	Bacterial type	Species	Quantity	Reaction time	Sample type	Concentration	Medium	Signal readout	Reference
Charges	Quaternized MNPs	Electrostatic interaction	Nonspecific binding with bacteria	Gram-positive Gram-negative	*S. aureus* *A. junii* *V. harveyi* *M. luteus* *E. tarda* *V. Parahemolyticus* *E. coli* (EC)	7	30 min	Bacterial suspension	10^2–10^8 CFU mL^{-1}	PBS	UV–vis	[78]
Charges	AuNPs/ AuNCs	Electrostatic interaction	Nonspecific binding with bacteria	Gram-negative	*E. coli* ER2738 *E. coli* DH5a *E. coli* pET-30a *E. coli* pHSP70-EGFP (Enhanced green fluorescent protein)	10	10 min	Bacterial suspension	OD_{600} = 0.015	PBS	UV–vis	[64]
Charges	AIE	Electrostatic interaction	Nonspecific binding with bacteria	Gram-positive Gram-negative	*E. coli* O157:H7 *P. aeruginosa* CICC21954 *P. aeruginosa* CICC10204 *Y. mollaretii* *P. putida* *P. vulgaris*	8	30 min	Bacterial suspension	OD_{600} = 1	PBS	Fluorescence	[82]
Charges	AIE	Electrostatic interaction	Nonspecific binding with bacteria	Gram-positive Gram-negative	*E. coli* *K. pneumonia* *S. choleraesuis* *S. aureus* *S. epidermidis* *B. subtilis* MDR *E. coli* MRSA	8	5 min	Bacterial suspension	10^8 CFU mL^{-1}	PBS	Fluorescence	[83]
Charges	AIE	Electrostatic interaction	Nonspecific binding with bacteria	Gram-positive Gram-negative	*L. monocytogenes* *S. aureus* *B. subtilis* *E. coli* *K. pneumoniae* *V. cholera* *Y. pestis* *P. aeruginosa*	10	At the same time	Bacterial suspension	OD_{600} = 0.5	PBS	Fluorescence	[84]
Charges	AIE	Electrostatic interaction	Nonspecific binding with bacteria	Gram-positive Gram-negative	*A. rhizogenes* *P. aetuginosa* *F. citrobactor* *E. coli* *C. violaceum* *S. epidermidis* *E. faecalis* *B. cereus* *B. subtilis* *S. aureus*							

Table 1. Continued.

Functional group	Material	Targeted group	Target location	Bacterial type	Species	Quantity	Reaction time	Sample type	Concentration	Medium	Signal readout	Reference
Charges	Conjugated Polymers	Electrostatic interaction	Nonspecific binding with bacteria	Gram-positive Gram-negative	*E. coli* *S. aureus* *C. albicans* TMV	4	25 min	Bacterial suspension	$OD_{600} = 1$ for bacteria and $OD_{600} = 2$ for fungi	PBS	Fluorescence	[86]
Charges	Conjugated Polymers AIE	Electrostatic interaction	Nonspecific binding with bacteria	Gram-positive Gram-negative	*E. coli* *E. coliR* *P. aeruginosa* *E. faecalis* *S. aureus* *C. albicans* *S. aureus*	7	15 min	Bacterial suspension	$OD_{600} = 1$ for bacteria and $OD_{600} = 2$ for fungi	PBS	Fluorescence	[91]
Charges	Conjugated Polymers	Electrostatic interaction	Nonspecific binding with bacteria	Gram-positive Gram-negative	*E. coli* *S. aureus* *C. albicans*	3	20 min	Bacterial suspension	$OD_{600} = 1$	PBS	Fluorescence	[92]
Yttrium	Carbon dots	pH-triggered aggregation	Metabolites of bacteria	Gram-positive Gram-negative	*E. coli* *B. subtilis* *S. aureus*	3	25 min	Bacterial suspension	$OD_{600} = 1$	PBS	Fluorescence	[99]
o-phenylenediamine (oPD) Dopamine (DA)	Carbon dots	pH-sensitive	Metabolites of bacteria	Gram-positive Gram-negative	*E. coli* *S. aureus*	2	6–8 h	Bacterial suspension	10, 10^2, 10^3, 10^4, 10^5, 10^6, and 10^7 CFU mL^{-1}	Physiological saline	Fluorescence	[100]
–	–	Nuclear magnetic resonance (NMR)	Metabolites of bacteria	Gram-positive Gram-negative	*E. coli* *E. faefalis* *P. aeruginosa* *P. mirabilis* *S. aureus* *S. saprophyticus*	6	3 h	Bacterial culture supernatant	–	Mueller-Hinton liquid medium	–	[111]
4-(Dimethylamino)pyridine (DMAP)	AuNPs	Electrostatic interaction	Metabolites of bacteria	Gram-positive Gram-negative	*E. coli* *B. subtilis* *S. epidermidis* *E. aerogenes*	4	450 s	Bacterial culture supernatant	–	Tryptone soya broth	UV–vis	[112]
80 Chemoresponsive dyes	AuNPs AgNPs	–	Metabolites of bacteria	Gram-positive Gram-negative	*S. aureus* MRSA *Listeria* *S. agalactiae* *E. faecalis* *E. coli* *Klebsiella* *Proteus* *E. aerogenes* *P. aeruginosa*	10	50 min	Bacterial colony	10^6–10^8 CFU mL^{-1}	Tryptose soya agar/ sheep blood Petri dish	UV–vis	[109]

Table 1. Continued.

Functional group	Material	Targeted group	Target location	Bacterial type	Species	Quantity	Reaction time	Sample type	Concentration	Medium	Signal readout	Reference
80 Chemoresponsive dyes	–	–	Metabolites of bacteria	Gram-positive Gram-negative	*A. baumannii* *S. marcescens* *S. aureus* *E. faecalis* *P. aeruginosa* *P. mirabilis* *S. pneumoniae* *E. coli* *S. maltophilia* *E. faecium* *S. lugdunensis* *S. agalactiae* *K. pneumoniae* *E. cloacae* *S. epidermidis*	15	4 h	Bacterial colony	$\approx 1.5 \times 10^8$ CFU mL^{-1}	Blood agar plates	UV–vis	[107]

The advantage of array-based sensors over traditional single-component sensors is that they are capable of discriminating a variety of targets spontaneously, enabling the multiplexed bacteria detection traditionally limited by the "lock-and-key" sensing strategy. In spite that array-based sensing has offered great opportunities to boost the development of sensors for bacteria detection, current fingerprint-pattern recognition sensing is still in need of further improvements to fully fulfill the requirement for applications in practice.

First, tiny changes or interfering species in the molecular receptors could potentially induce great deviations of the final output. To address this issue, we should focus on the development of more efficient and selective receptors, which could be promising to reduce the interference and improve the specificity.

Second, the contribution of each component in the multiple-component sensor array should be studied in depth. The components in a sensor array can be optimized to achieve a more concise and accurate design for bacteria sensing. The number of components directly relates to the workload, time-consuming process, and accuracy of the identification results.

Third, current biosensor arrays can merely identify bacteria in an existing database, not applicable to unknown and untested microorganisms. Improvement and expansion of the database is in need as well as the development of an intermediate law of bacteria species to forecast.

In addition, it still needs numerous efforts to achieve the real-world application of array-based sensing. Current studies still largely rely on pure bacteria rather than raw samples for bacterial identification, possibly because of the low microbial concentration and high interference of raw samples. Despite numerous bacterial sensors have been demonstrated in laboratory research, it is still far away from applications in practice due to the time-consuming procedures and instrument-based readout. Improvement and refinement should be focused in future research to simplify the procedures and readout, thus allowing timeliness, costless, and convenient visualization of the biosensor arrays.

Acknowledgements

The authors are grateful for the financial support from the National Key Research and Development Program of China (2020YFA0909100) and the "Hundred Talents Program" of Zhejiang University (202001030300).

Conflict of Interest

The authors declare no conflict of interest.

Keywords

array-based biosensor, bacteria detection, bacteria recognition, nanotechnology, signal readout

Received: October 6, 2020
Revised: December 18, 2020
Published online:

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