An antimicrobial peptide-based colorimetric bioassay for rapid and sensitive detection of *E. coli* O157:H7

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Foodborne diseases

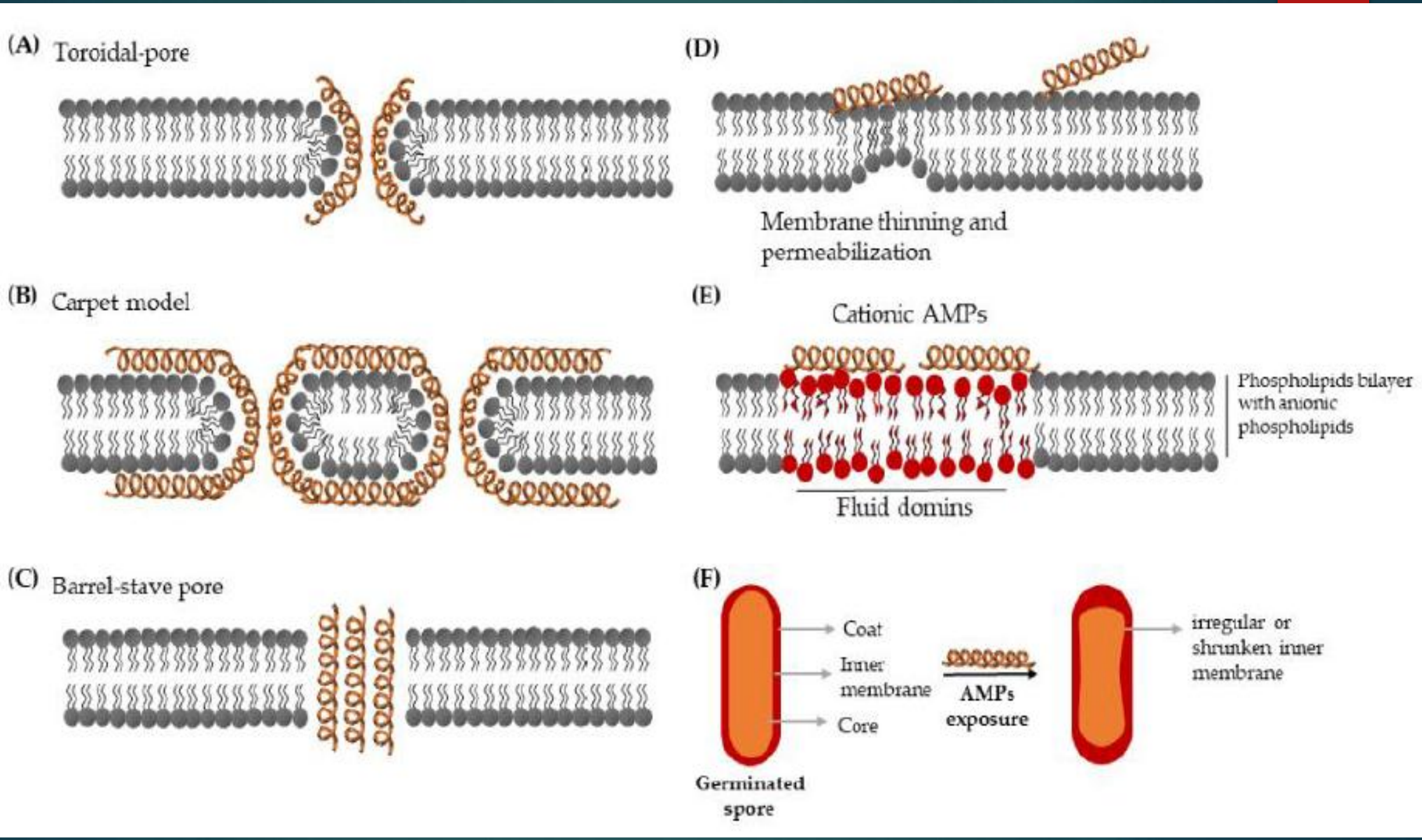
- one of the most threatening public health problems worldwide
- There are 9.4 million cases of foodborne illness every year in the United States caused by foodborne pathogens.
- Escherichia coli (E. coli) O157:H7 is one of the most notorious foodborne pathogens that can cause severe illness such as hemorrhagic diarrhea, vomiting or acute kidney failure
- It was estimated that the infectious dose of E. coli O157:H7 may be as low as 10 to 100 cells and the infections could be lethal without proper medical treatment.5 Therefore, E. coli O157:H7 was classifed as a “zero tolerance” adulterant

Foodborne diseases

- The conventional culture-based methods are the golden standard methods for the detection of E. coli O157:H7.
- these methods are labor-intensive and time-consuming, which cannot satisfy the requirement of rapid detection
- PCR based techniques are highly sensitive and can even achieve single-cell detection
- require the extraction of nucleic acids and are limited in portability
- The immunoassays based on antigen–antibody reaction are simple and sensitive methods for the high-throughput detection of bacteria with high specificity
- However, the specific antibody–antigen interactions limit the recognition sites leading to the insensitivity for measurement of bacteria at low concentrations.

AMPs

- found in multiple niches in nature
- providing the first line of defense against infection by acting as natural antibiotics.
- Currently, more than 3200 natural AMPs are recorded in The Antimicrobial Peptide Database (http://aps.unmc.edu/AP/main.php).
- Most of antimicrobial peptides contain 12–50 amino acid residues, including two or more positively charged residues and a large proportion of hydrophobic residues.
- They can attach on the surface of bacteria mainly through electrostatic and hydrophobic interactions
- Compared with antibodies, AMPs have advantages of stableness in harsh environment, low-cost synthesis, and easy modification


(A) Toroidal-pore
(B) Carpet model
(C) Barrel-stave pore
(D)
Membrane thinning and permeabilization
(E)
Cationic AMPs
Phospholipids bilayer with anionic phospholipids
Fluid domins
(F)
Coat
Inner membrane
Core
Germinated spore
AMPs exposure
irregular or shrunken inner membrane

AMPs

- These methods are simple and practicable, but with low sensitivity due to the lack of the processes of signal amplification.
- Hydrophobic residues of AMPs can attach on to the amphiphilic negatively charged lipopolysaccharide (LPS) in the bacterial membrane
- Moreover, each E. coli cell contains approximately 3.5 106 LPS molecules occupying three-quarters of the bacterial surface
- Therefore, in conjunction with proper signal reporters, AMPs can be adopted as effective signal amplifiers.

AMPs

- an immunomagnetic assay for detection of E. coli O157:H7 based on Cy5-labeled AMPs, revealing a 10-fold higher sensitivity compared with Cy5-antibody.
- non-specific binding to the immunomagnetic beads resulted in high noise in background and relatively low sensitivity.
- Filtration has been proven to be effective for the rapid isolation and concentration of foodborne pathogens.
- Herein, combined with filtration, we developed an AMP-based colorimetric bioassay utilizing AMP–HRP as the whole cell surface binding probes for rapid and sensitive detection of E.coli O157:H7.

AMPs

- Magainin I
- an AMP with 23 amino acid residues, exhibiting high affinity toward E. coli O157:H7,13 was selected as the recognition element.

Magainin I

≥97% (HPLC)

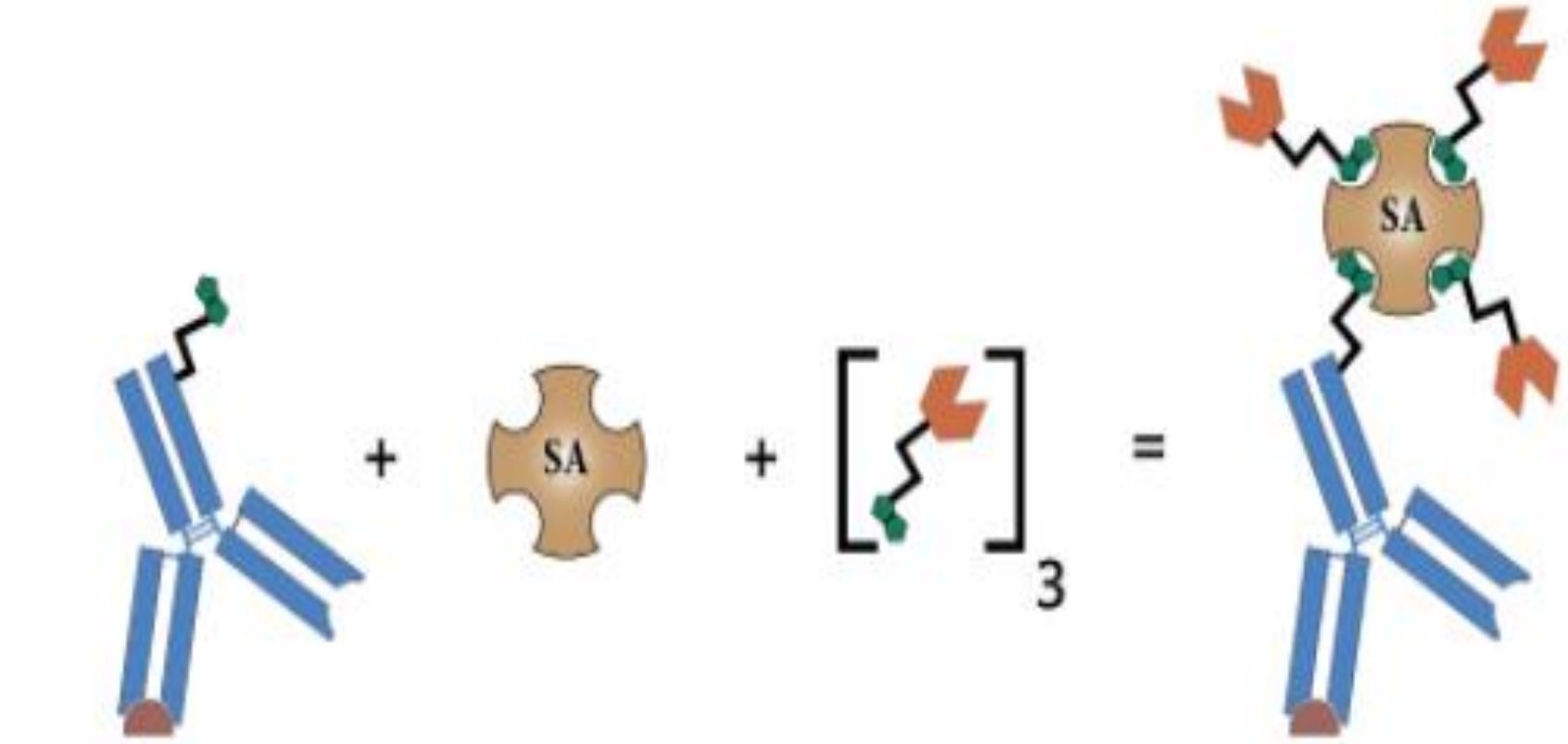
CAS Number 108433-99-4 | Empirical Formula (Hill Notation) $C_{112}H_{177}N_{29}O_{28}S$ | Molecular Weight 2409.85 | MDL number MFCD00133521 | PubChem Substance ID 329818042 |

NACRES NA.32

AMP–HRP

- AMP–HRP conjugates were fabricated via streptavidin and biotin interaction.
- AMP–HRP probes can bind on the surface of bacteria rapidly with high density through the interaction between AMP and amphiphilic negatively charged LPS.
- small size of the biotin and streptavidin molecules themselves, which allows for extensive binding to biologically active macromolecules, such as antibodies, without impedance to their functions
- Streptavidin tetramers have an extraordinarily high binding affinity for biotin
- This tight and specific binding is rapid and able to withstand extremes in pH, temperature, organic solvents, and denaturing reagents.

AMP–HRP


SA
+
SA
+
3
=
SA
Biotinylated Antibody
Streptavidin
Biotin Conjugate
Biotin Streptavidin Complex

Preparation and culture of bacteria

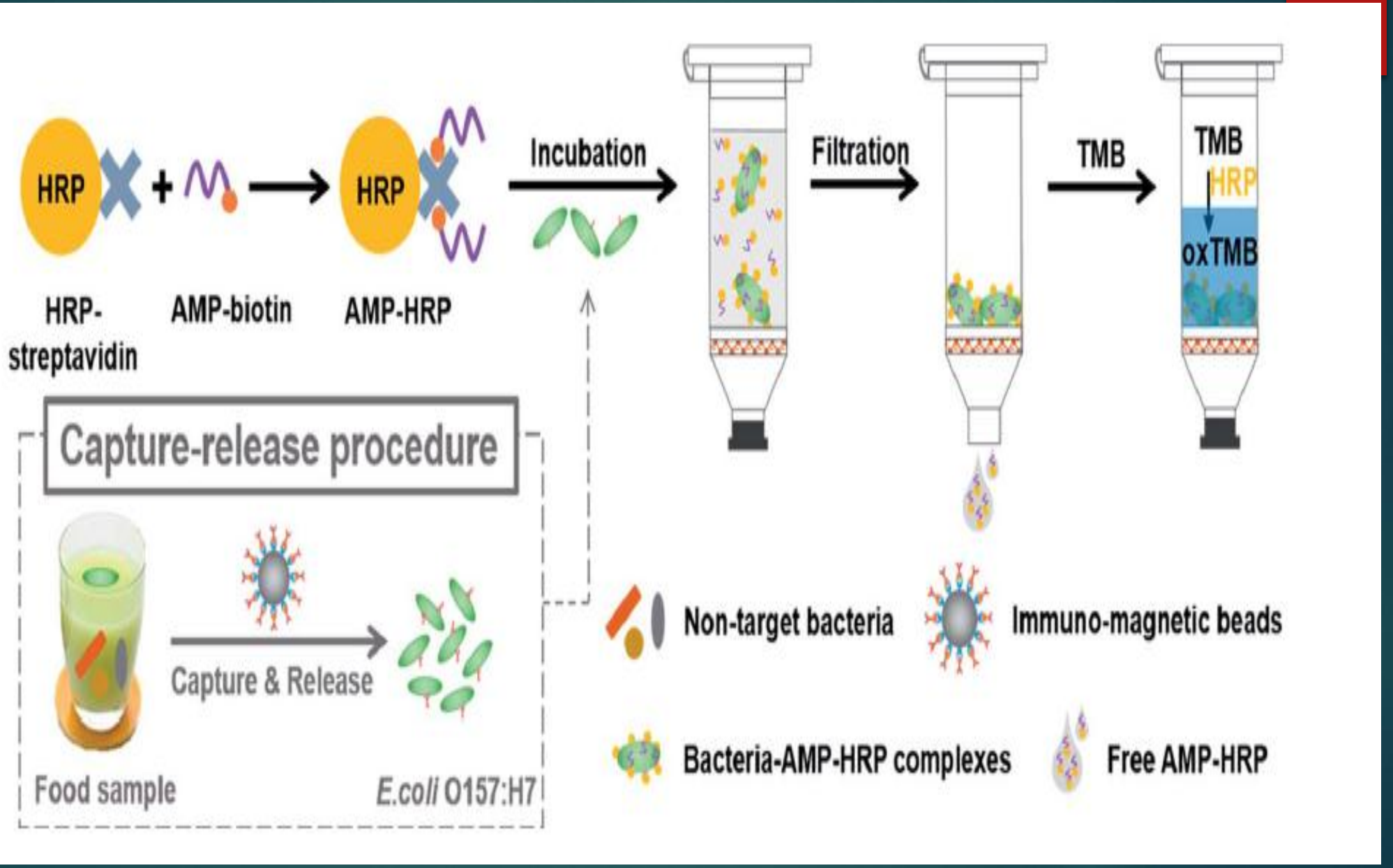
- E. coli O157:H7, as well as other bacteria used as non-target cells, was prepared by growing the stock cultures in brain heart infusion (BHI) broth at 37 C for 16–20 h. After washed three times by centrifuging at 8000 rpm for 5 min
- the bacteria solutions were 10-fold diluted with sterile PBS to obtain the samples with different concentrations ranging from 10 to 107 cfu mL1
- To determine the cell numbers, 100 mL of proper dilutions were plated onto the surface of corresponding agars.
- After incubation at 37 C for 24 h (for bacteria except L. monocytogenes) or 48 h (for L. monocytogenes),
- colonies on the plates were counted to determine the number of viable cells in terms of colony forming units per milliliter (cfu mL)

Preparation of AMP–HRP probes

- AMP–HRP conjugates were fabricated via streptavidin and biotin interaction.
- In a typical procedure, biotinylated AMPs (5mmol L1) were mixed with HRP-conjugated streptavidin (50 mg mL1) and then the mixture was placed in a programmable rotating-mixer (Grant-bio, Grant Instruments (Cambridge) Ltd., UK) at 15 rpm for 30 min at room temperature (RT)
- The molar ratio of AMPs and HRP is 10 : 1 in order to guarantee every HRP molecule was conjugated with AMPs
- After that, the mixture was centrifuged at 10 000 rpm for 10 min. To make sure unbound AMPs removed completely, the mixture was washed thrice by 0.5 mL of PBS and added PBS to make HRP with a final concentration of 50 mg mL

Preparation of AMP–HRP probes

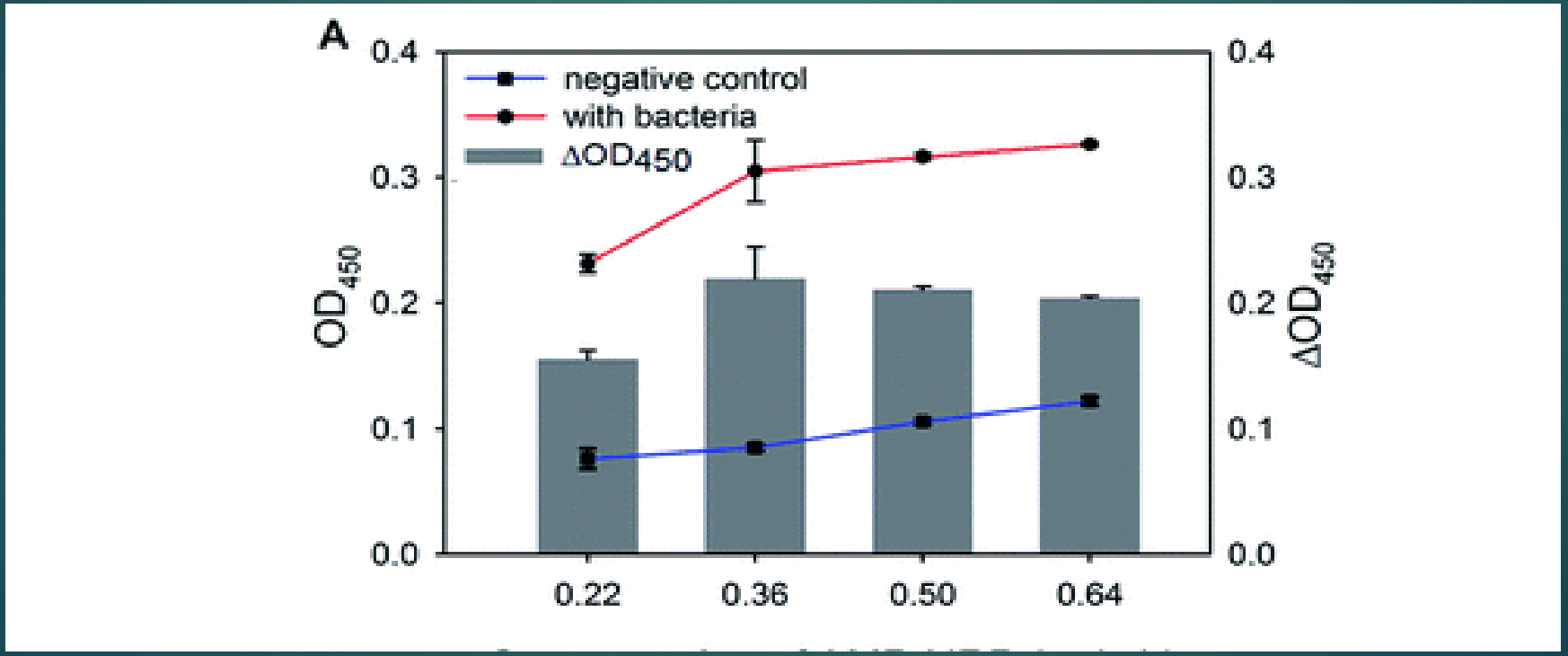
- An aliquot of 800 mL of serially diluted E. coli O157:H7 were mixed with 0.36 mg mL1 of AMP–HRP and incubated for 10 min in filter tubes at RT when the tubes outlets were sealed.
- The same amount of AMP–HRP probes was added to 800 mL of PBS as negative control
- After the incubation, the outlets were open and the mixtures were filtered through the membrane under centrifugation at 4000 rpm for 1 min
- To reduce the non-specific attachment, the membrane was washed thrice by flowing 500 mL PBS through the membrane
- 100 mL of TMB was added into the tube for color reaction, followed by adding 100 mL of 2 mol L1 H2SO4 to stop the reaction 5 min later
- Finally, the UV-vis absorption spectrum of each sample was measured by the microplate reader.


HRP
HRP
HRP-
streptavidin
AMP-biotin
AMP-HRP
Incubation
Filtration
TMB
TMB
HRP
oxTMB
Capture-release procedure
Capture & Release
Food sample
E.coli O157:H7
Non-target bacteria
Immuno-magnetic beads
Bacteria-AMP-HRP complexes
Free AMP-HRP

Results and discussion

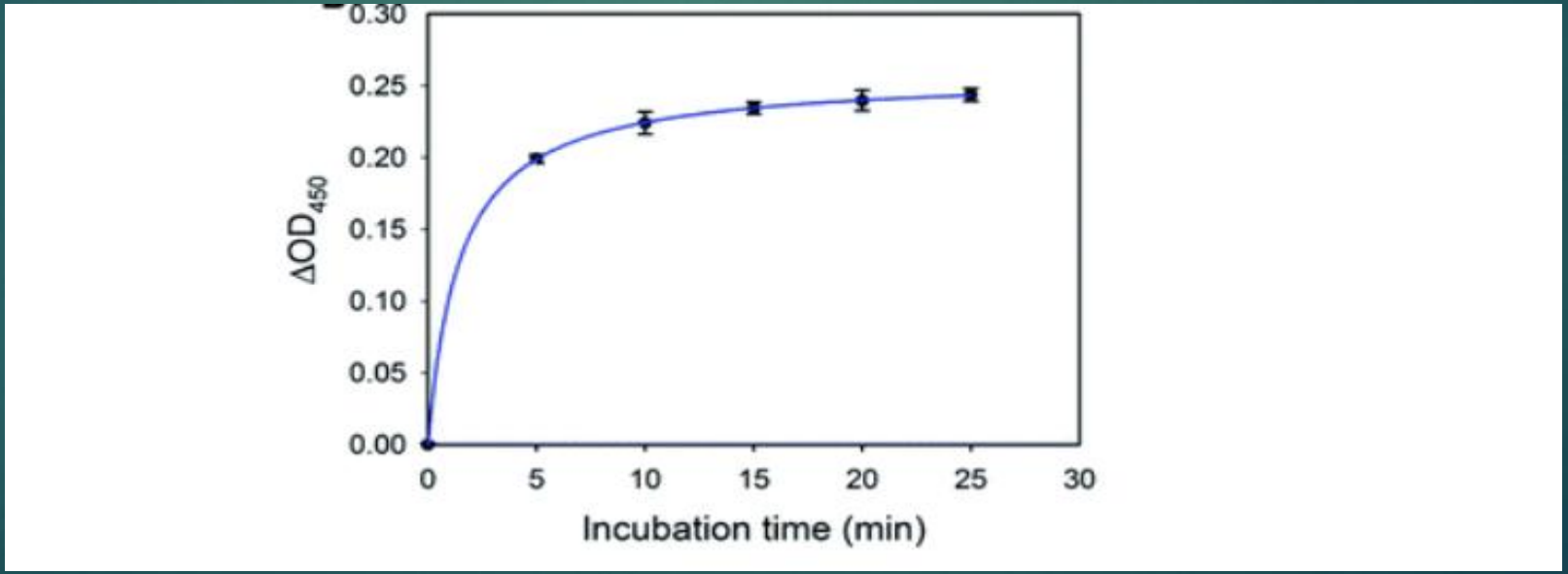
- two parameters including the concentration of AMP–HRP probes and incubation time between AMP–HRP and bacteria were optimized

concentration of AMP–HRP increased from 0.22 to 0.36 μg mL



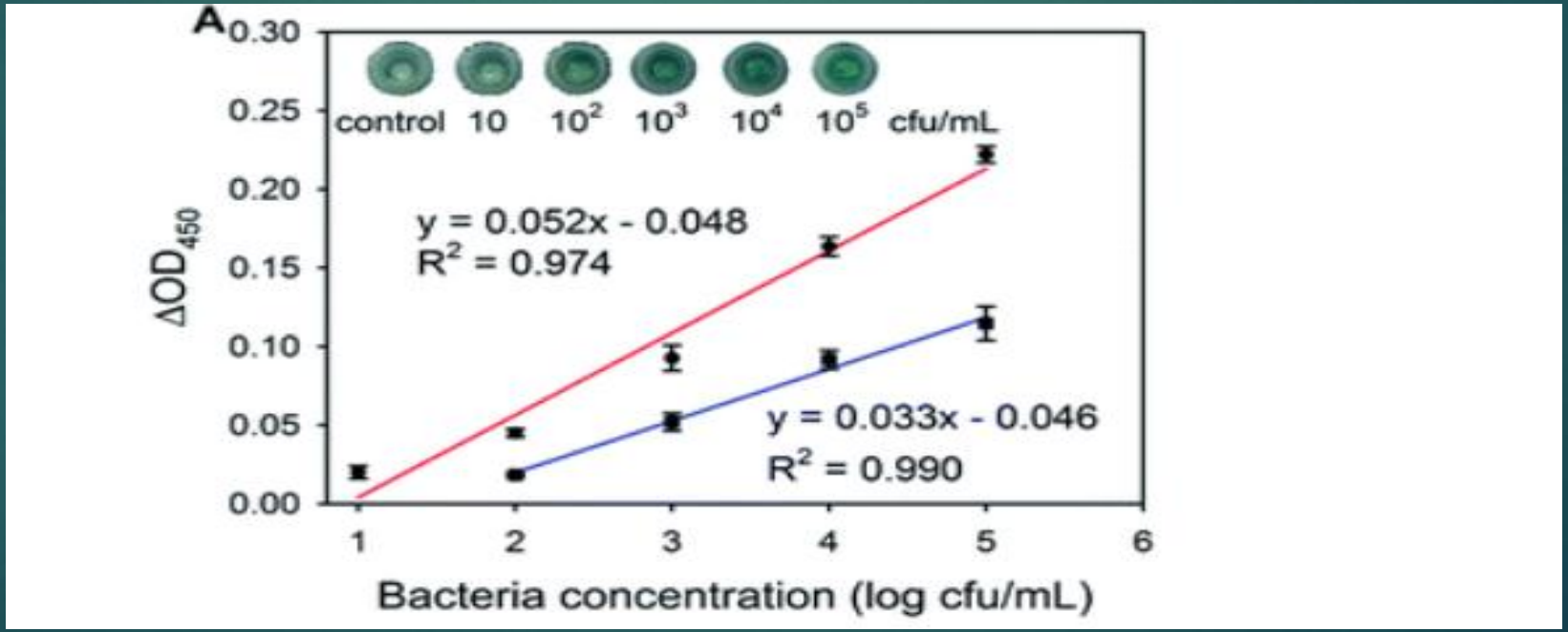
Results and discussion

- a rapid increase of ΔOD450 was observed in the initial 10 min indicating that AMP–HRP quickly bind on the surface of bacteria
- 10 min was used as the optimal reaction time in the subsequent experiment



Results and discussion

- the sensitivity of AMP-based bioassay was much better than that of antibody based assay (blue line)
- These results can be attributed to the fact that there are much more binding sites for AMP on the surface of bacteria than that for antibody



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An antimicrobial peptide-based colorimetric bioassay for rapid and sensitive detection of *E. coli* O157:H7†

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- thanks for your attention