A Fluorescent Nanobiosensor for Rapid and Facile Detection of Enrofloxacin in Chicken Products

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Content

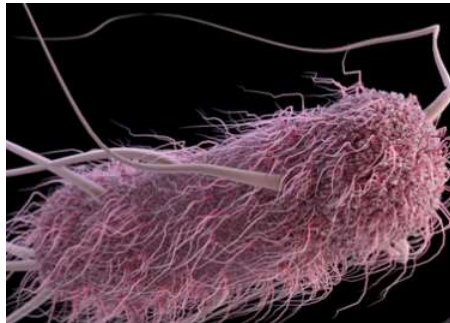# 1. Why antibiotics detection is essential

◆ **Antibiotics**

An antibiotic is a type of **antimicrobial drug** used in the treatment and prevention of bacterial infections.
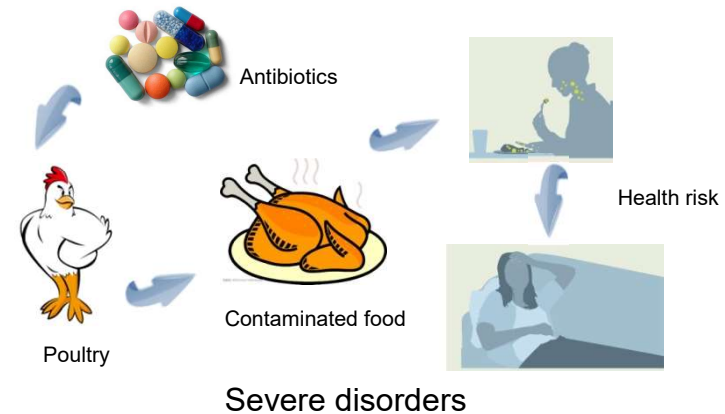
◆ **Antibiotics for poultry in China**

➢ **70%** in the total usage of poultry drugs;

➢ Approximately **6,000 tons** of antibiotics were used as additives in feed every year.

Drug resistance

Antibiotics

Health risk

Poultry

Contaminated food

Severe disorders

https://en.wikipedia.org/wiki/Antibiotic; https://www.cdc.gov/narms/disease.html.

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1. Why antibiotics detection is essential

◆ Enrofloxacin (ENR)

A kind of synthetic antibiotics with broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria by inhibiting their DNA gyrase.
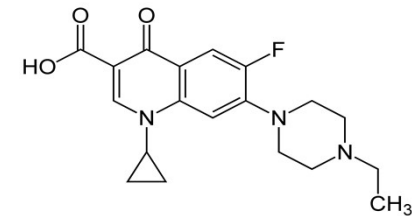
> ➤ Only used in **veterinary medicine**;
>
> ➤ Banned from USA, where growth promoters are still allowed;
>
> ➤ MRL: **100 µg kg⁻¹** (China and the EU).

Food Chem. Toxicol. 2018, 118, 340; Anal. Chim. Acta, 2008, 612, 83.
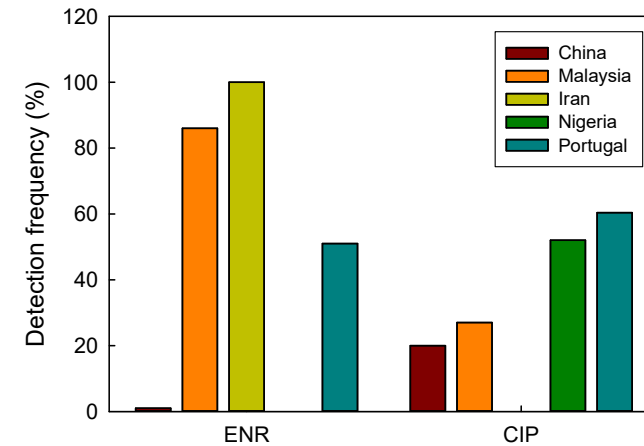
Worldwide detection frequency of ENR and CIP in poultry muscle.

2. Detection methods for antibiotics

➢ **Microbiological methods**
- ✓ Visualization, simplicity
- ✓ Low cost
- ✗ Low sensitivity
- ✗ Low interference resistance

➢ **Instrumental methods**
- ✓ Sensitivity, accuracy
- ✓ Stability
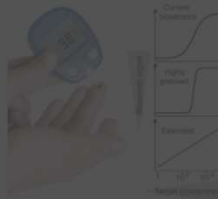- ✗ Tedious procedures
- ✗ Expensive apparatus

How to further improve detection performance?

➢ **Immunoassays**
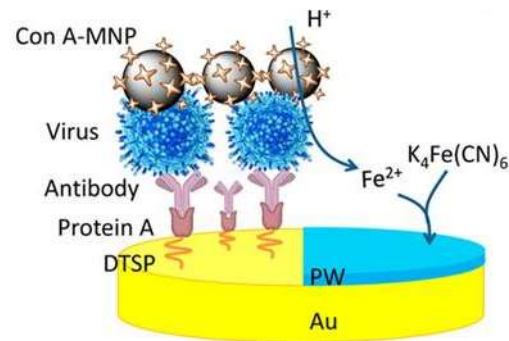- ✓ High specificity
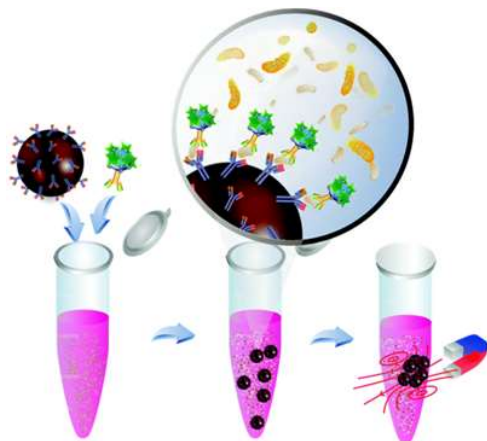- ✗ High cost
- ✗ Low stability

➢ **Biosensors**
- ✓ Sensitivity, rapidness
- ✓ Specificity
- ✓ Simplicity, portability
- ✗ Commercialization

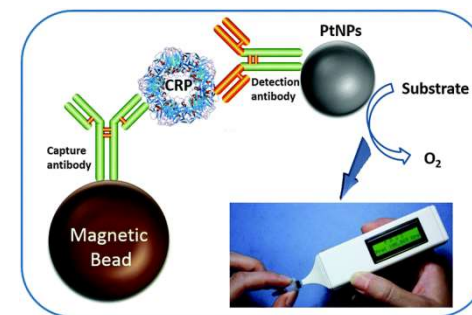Biosens. Bioelectron. 2017, 90, 363; Biosens. Bioelectron. 2017, 91, 504.

3. Fabrication of biosensors

◆ **Immunomagnetic beads (IMBs)**

Magnetic particles composed of magnetic carriers and immune ligands.
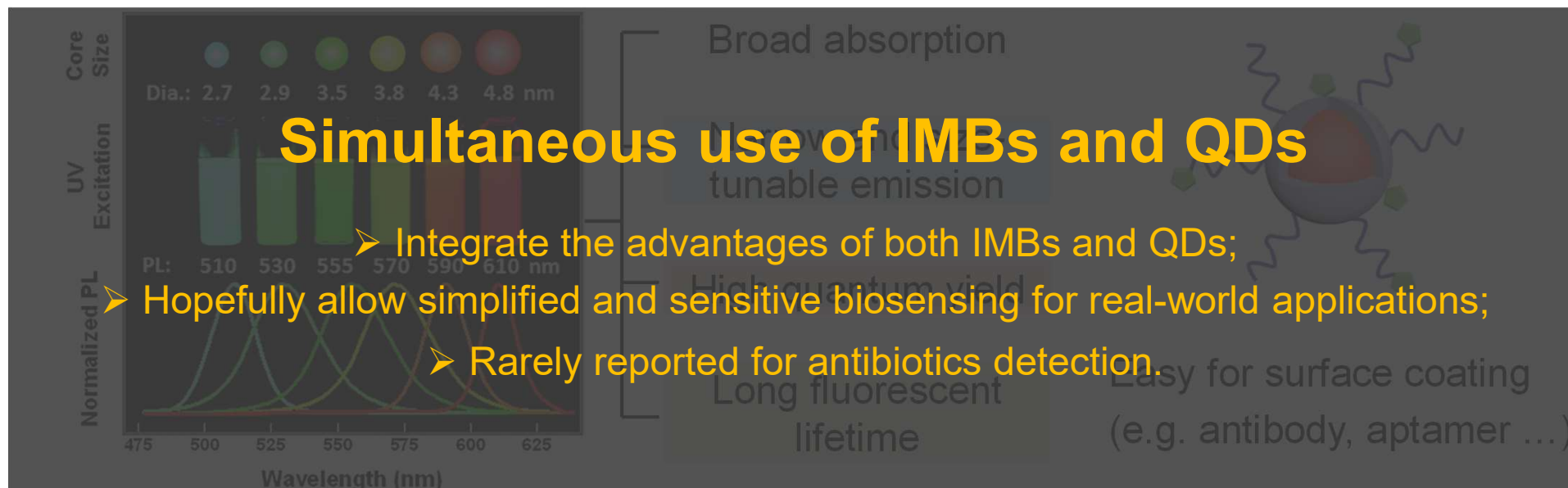

➤ Signal readout   ➤ Labeled with reporter molecules

➤ Separate and concentrate target analytes;
➤ Improve sensitivity and specificity;
➤ Accelerate the binding kinetics;

➤ Facilitate automation;
➤ Integrate with analytical devices (optical, electrochemical, etc.).

Nanoscale, 2016, 8, 1944; Anal. Chem. 2017, 89, 12145;
Biotechnol. Adv. 2017, 35, 51; Chem. Commun. 2016, 52, 8452.

3. Fabrication of biosensors

◆ **Quantum Dots (QDs)**
Inorganic nanocrystals of around 1-6 nm with atoms from groups II-VI or III-V of the periodic table.

Simultaneous use of IMBs and QDs

➢ Integrate the advantages of both IMBs and QDs;
➢ Hopefully allow simplified and sensitive biosensing for real-world applications;
➢ Rarely reported for antibiotics detection.

Anal. Chem. 2011, 83, 8826; Anal. Chem. 2012, 84, 224;
Adv. Mater. 2018, 30, 1706356.

4. Objectives

➢ Extract ENR from chicken samples using a facile pre-treatment method;

➢ Detect ENR in chicken using an IMBs-QDs based biosensor;

➢ Coordinate this biosensing method with a portable and automated instrument.

In-field detection of ENR in poultry supply chain.

5. Materials and methods

◆ **Detection principle**

IMBs ENR QDs-BSA-ENR

λ_{ex}=450 nm

λ_{em}=620 nm

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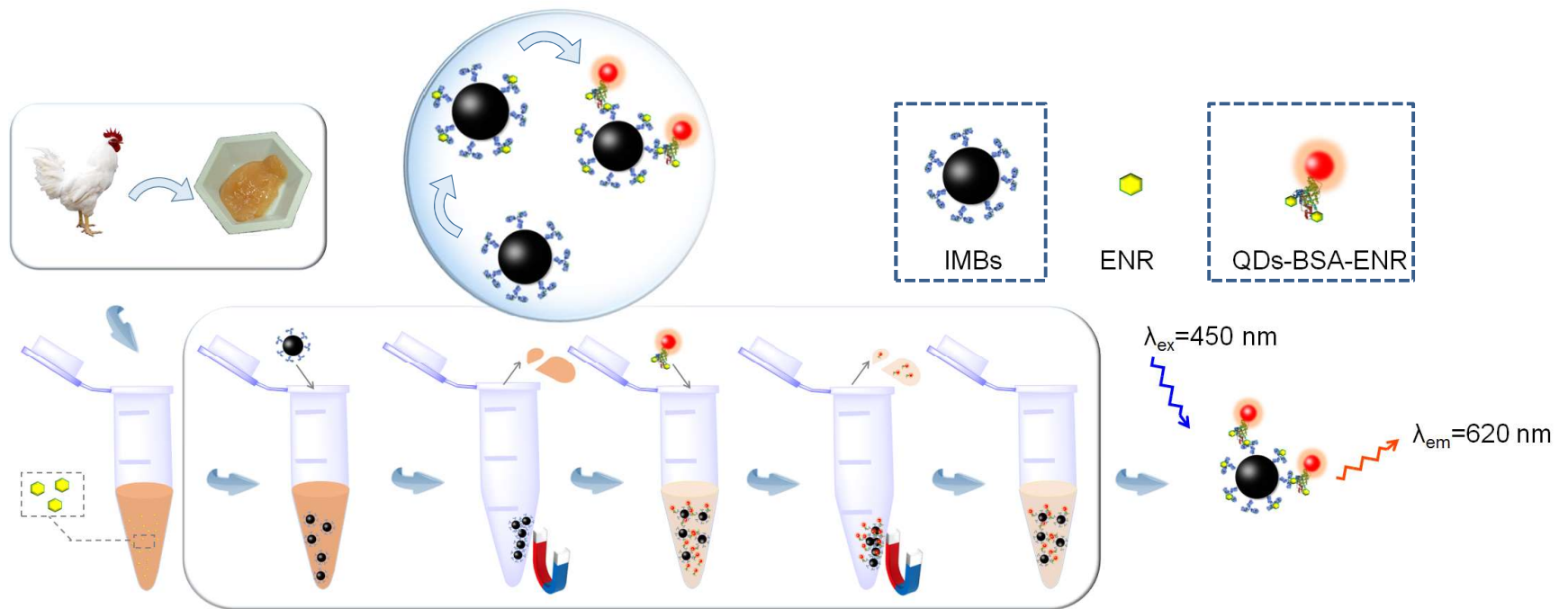
5. Materials and methods

◆ Materials

➢ Carboxyl CdSe/ZnS core/shell QDs (emission wavelength: 620 nm)

➢ Carboxyl magnetic beads (150 nm), Ocean NanoTech, San Diego, CA;

➢ Monoclonal antibody, Cusabio Biotech, Wuhan, China;

➢ BSA, Sangon, Shanghai, China;

➢ ENR, Aladdin, Chemistry, Shanghai, China.

◆ Apparatus

➢DynaMag^{TM}-2 Magnetic (0.35 – 0.37 T), Thermo-Fisher Scientific, Waltham, MA;

➢ Synergy H1 Hybrid Multi-Mode Microplate Reader, BioTek, Winooski, VT.
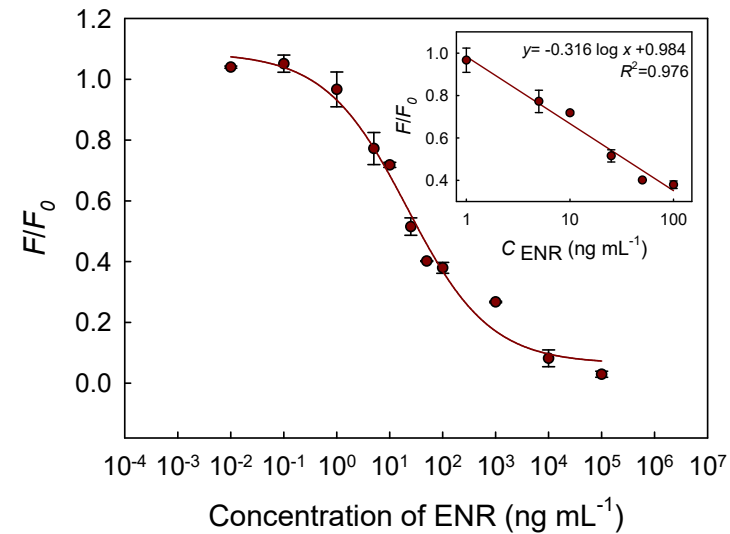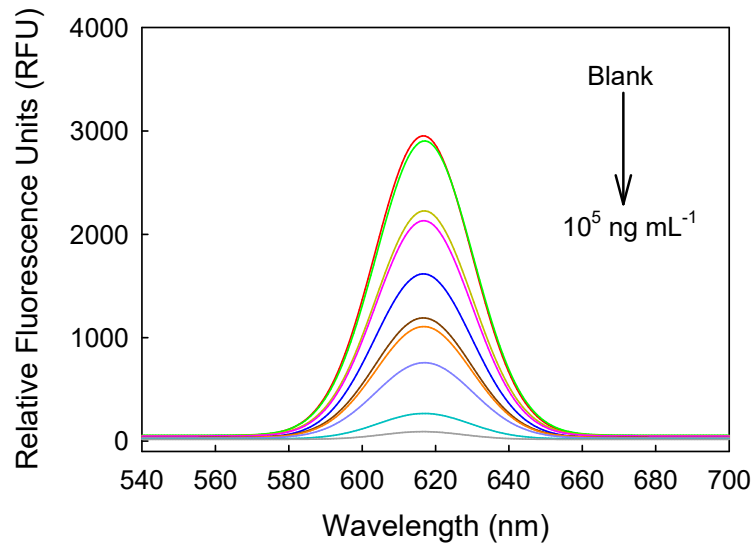
6. Experimental results

◆ Characterizations and optimizations
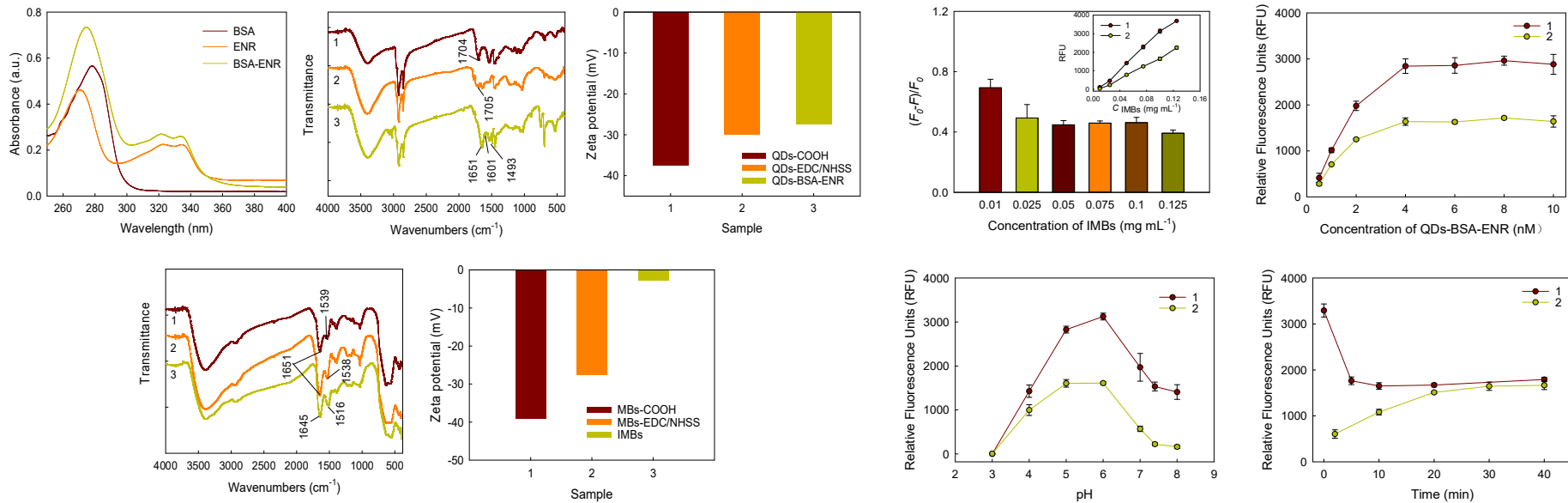
➢ UV-Vis spectra demonstrated the successful synthesis of BSA-ENR composites;

➢ FTIR spectra and Zeta potential measurments confirmed the successful preparation of QDs-BSA-ENR conjugates and IMBs.
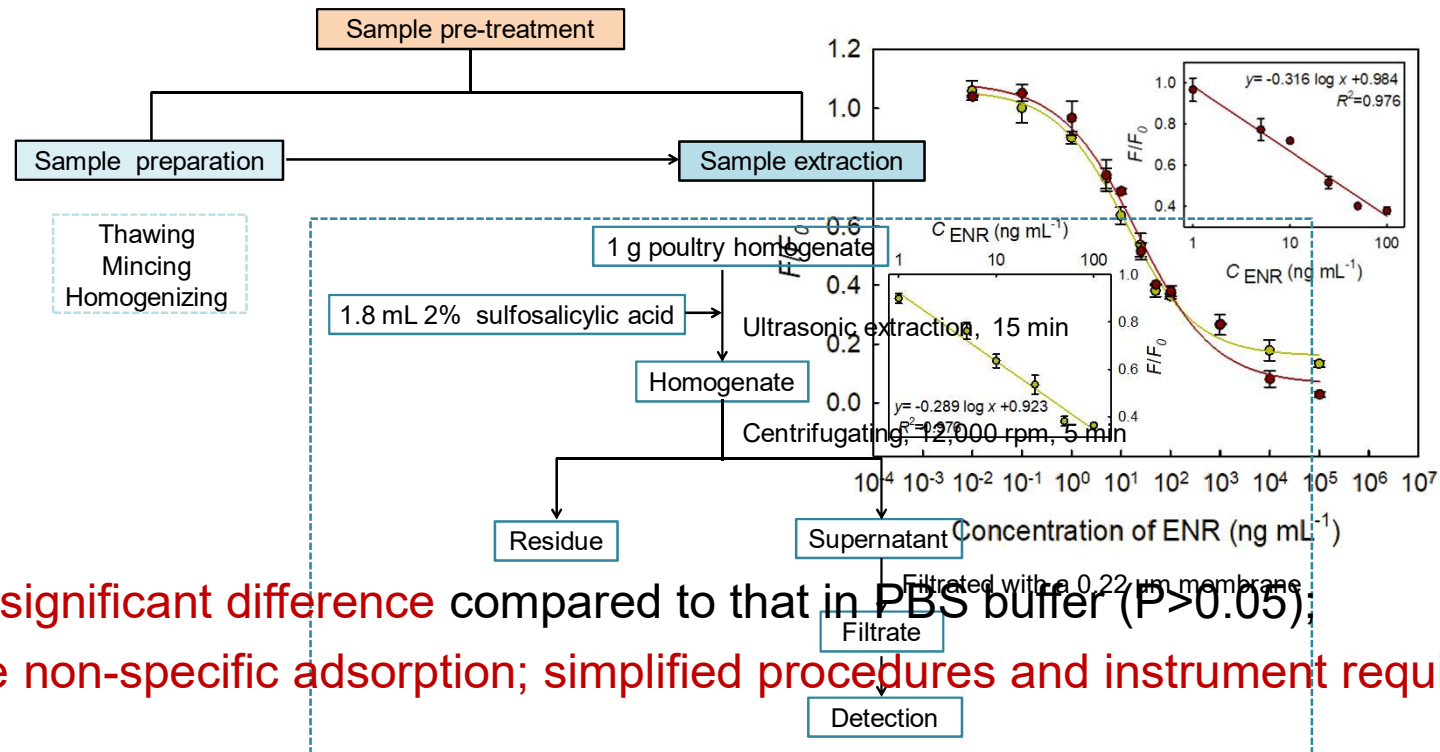
6. Experimental results

◆ Performance

➤ Limit of detection (LOD): 0.94 ng mL^{-1} , better than or comparable to other analogues;

➤ Linear detection range (LDR): 1 to 100 ng mL^{-1}.
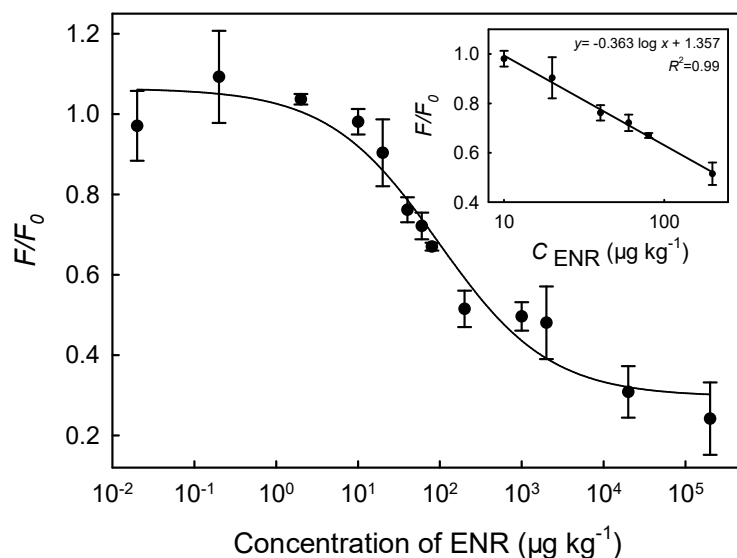
6. Experimental results

◆ Sample pre-treatment

Conventional methods: severe non-specific adsorption, time-consuming , tedious…

> Show no significant difference compared to that in PBS buffer (P>0.05);
> Negligible non-specific adsorption; simplified procedures and instrument requirement.

6. Experimental results

◆Performance

Added (µg kg^{-1})	Detected (µg kg^{-1})	Recovery (%)
50	41.6±9.6	83.3±19.3
100	101.6±17.4	101.6±17.4
200	170.5±7.1	85.2±3.6

➢ LOD: 14.1 µg kg^{-1} in chicken muscle samples;

➢ Good recovery in chicken muscle samples.

7. Conclusions and prospects

➢ We have developed a nanobiosensor based on IMBs and QDs for rapid detection of ENR with a low LOD of 0.94 ng mL^{-1}.

➢ Together with a 5-sulfosalicylic acid-based pre-treatment method, as low as 14.1 µg kg^{-1} of ENR could be detected in chicken muscle samples.

➢ The whole analytical procedure from food sampling to result report could be finished in approximately 1 h.

➢ The on-going research focuses on the implementation of this innovative biosensing method with a portable and automated instrument for in-field detection of ENR in poultry supply chain to enhance food safety.

Acknowledgements

Thank you for your attention!