Functionalized surfaces for the detection of circulating tumor cells (CTC) and bacteria

3- Surface functionalization strategies for bacteria detection and analysis

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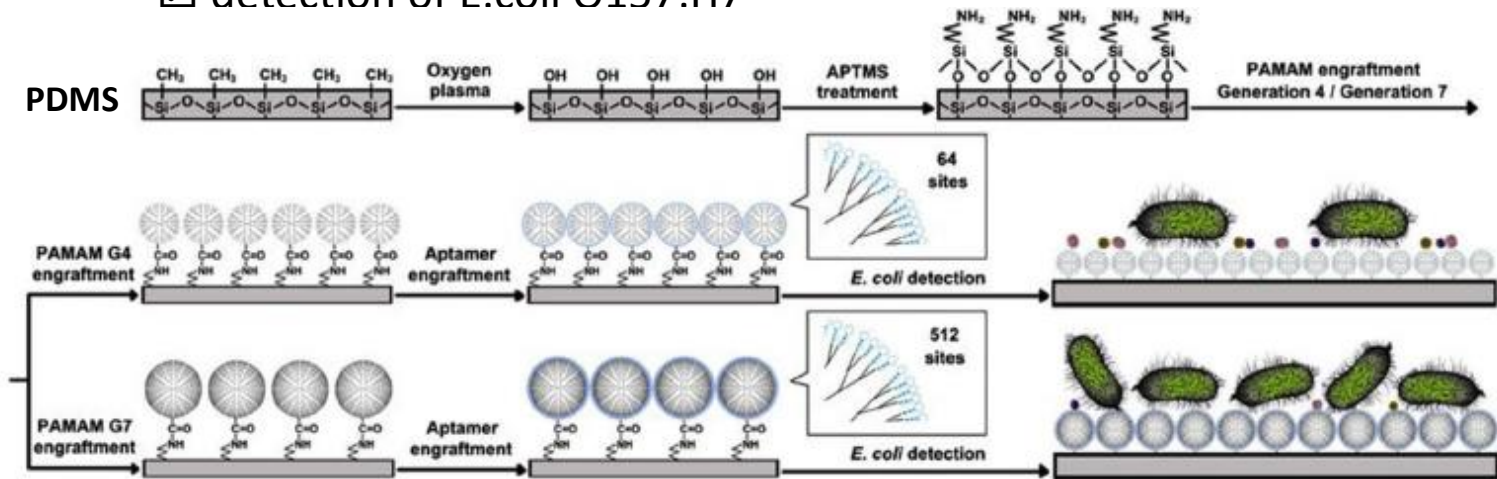# Challenges for bacteria detection

☞ Cell culture is very sensitive and reliable method but time-consuming with multiple steps and numerous amount of skills

☞ Need to develop rapide, sensitive and selective detection for the control of disease progression as it can help decide the course of treatment

☞ Major issue worldwide: foodborne pathogen bacteria
- E.coli O157:H7
- Salmonella enterica
- Listeria monocytogenes
- S. aureus
- Campylobacter jejuni
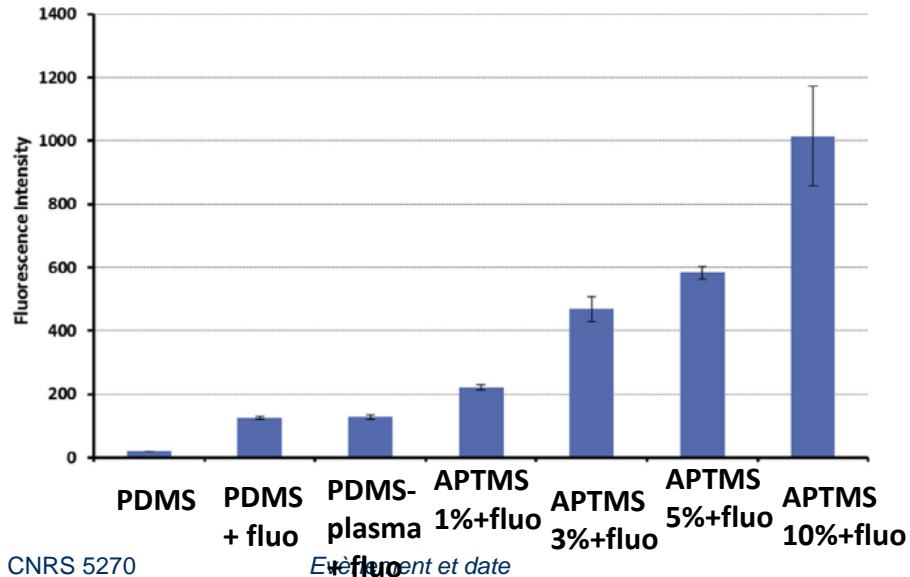
Dendrimers as multi-handled platform

☞ PAMAM dendrimer (G4 and G7) for aptamer immobilization
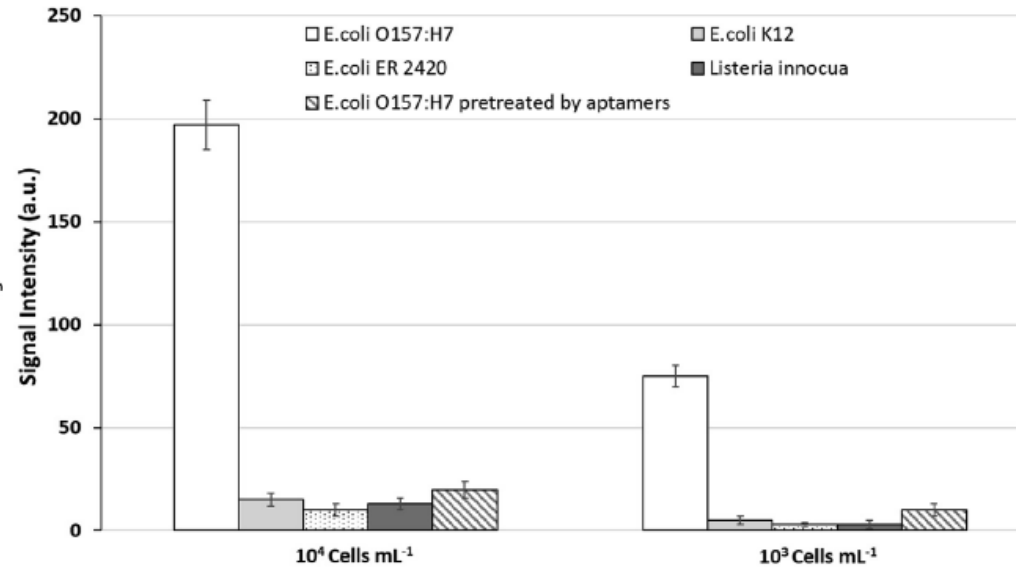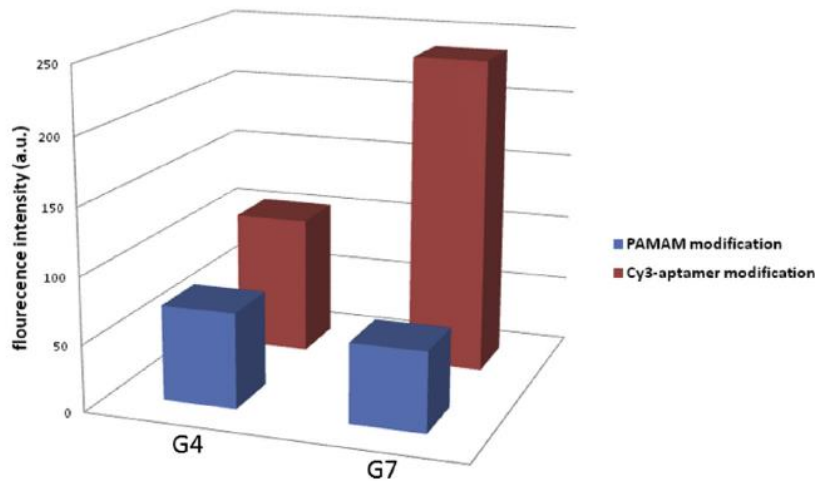
☒ detection of E.coli O157:H7

☒ Characterisation:
- contact angle
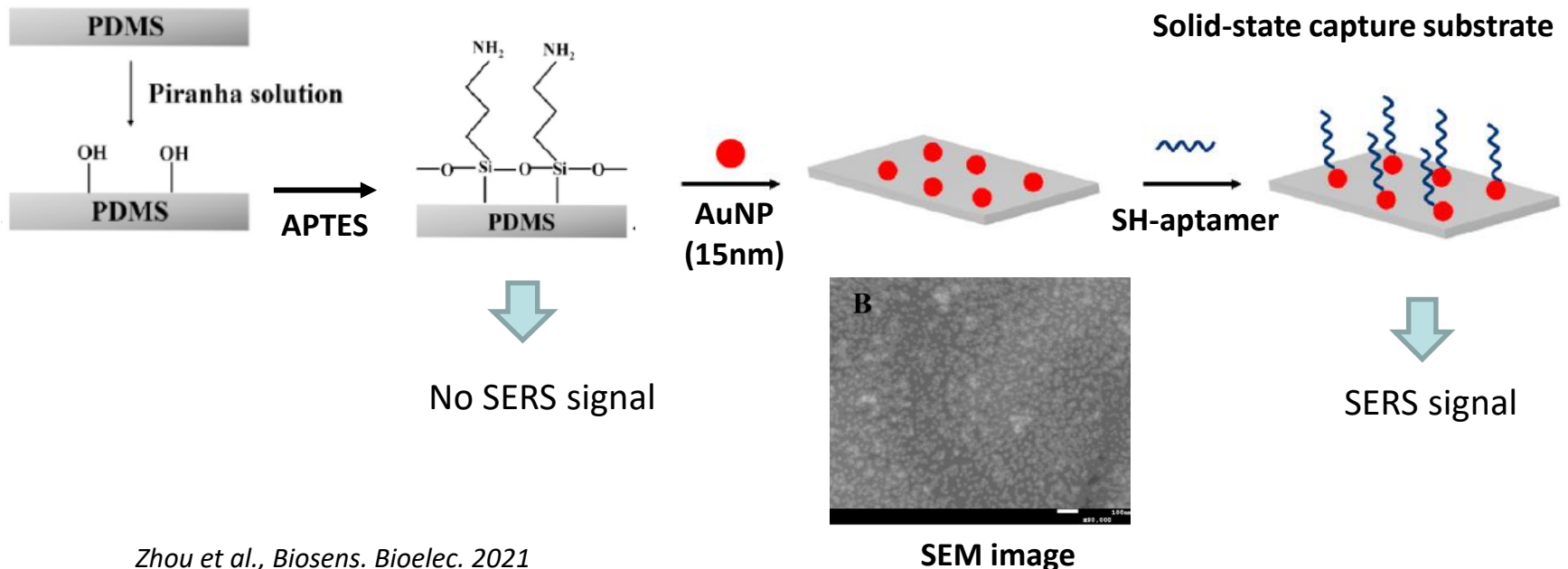- fluorescence
- XPS
- IR

Hao et al., Anal.Chem. Acta 2019

☒ Higher capturing rate on PAMA-G7-aptamer
☒ Good specificity of the dendrimer-aptamer system
☒ LOD=10^2 bact/mL
☒ Possibility to regenerate the capturing surface by heating
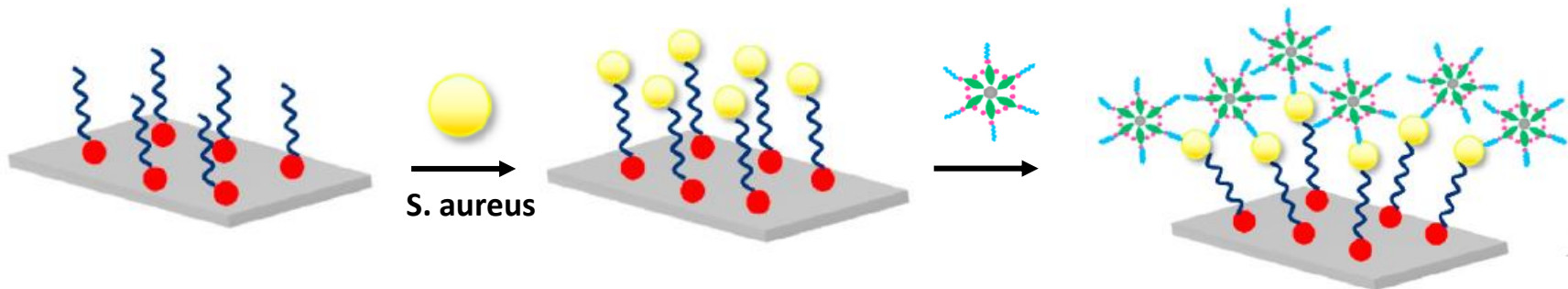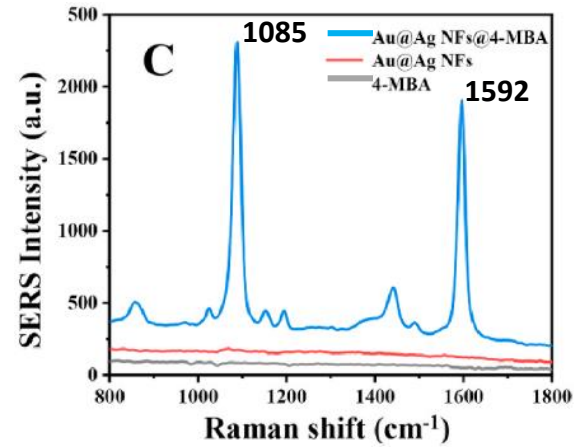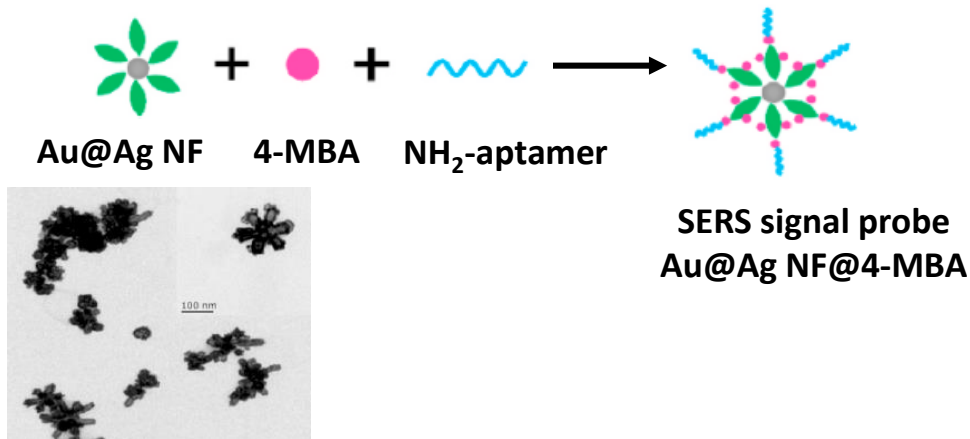
SERS for sensitive bacteria detection

☞ SERS= Surface-Enhanced Raman Spectroscopy

    ☒ fast, sensitive, separation-free and label-free technology for analyte detection

    ☒ 2 types of enhancement due to nanostructures

        • physical due to surface plasmon resonance

        • chemical due to charge transfer between metal surface and analyte
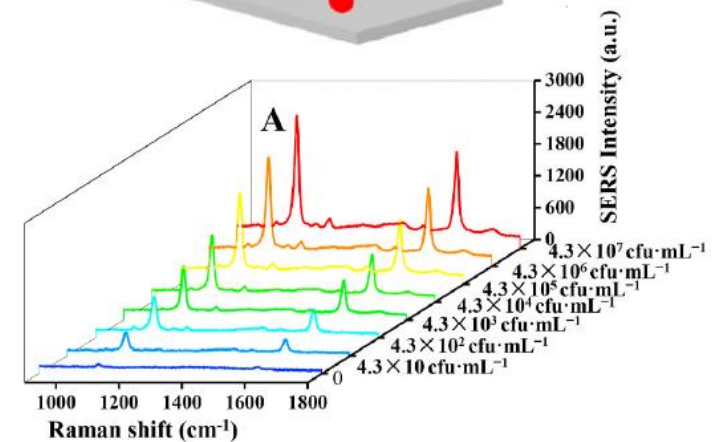
  ☞ Detection of Staph. aureus with AuNP-apatmer

Solid-state capture substrate

PDMS → Piranha solution → APTES → AuNP (15nm) → SH-aptamer

No SERS signal

SEM image

SERS signal

Zhou et al., Biosens. Bioelec. 2021

Au@Ag NF   4-MBA   NH₂-aptamer

SERS signal probe
Au@Ag NF@4-MBA

S. aureus

☒ LOD = 13 cfu/mL
☒ Specificity to S. aureus
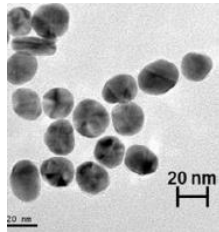
☞ Detection of S. typhimurium with MNP

☒ foodborne pathogen found in raw, uncooked, unwashed food products

☒ cause human gastroenteristis and bloodstream infection

- SERS signal probes: AuNP@MBA-Ab and AuNP@DSNB-Ab
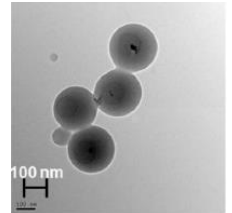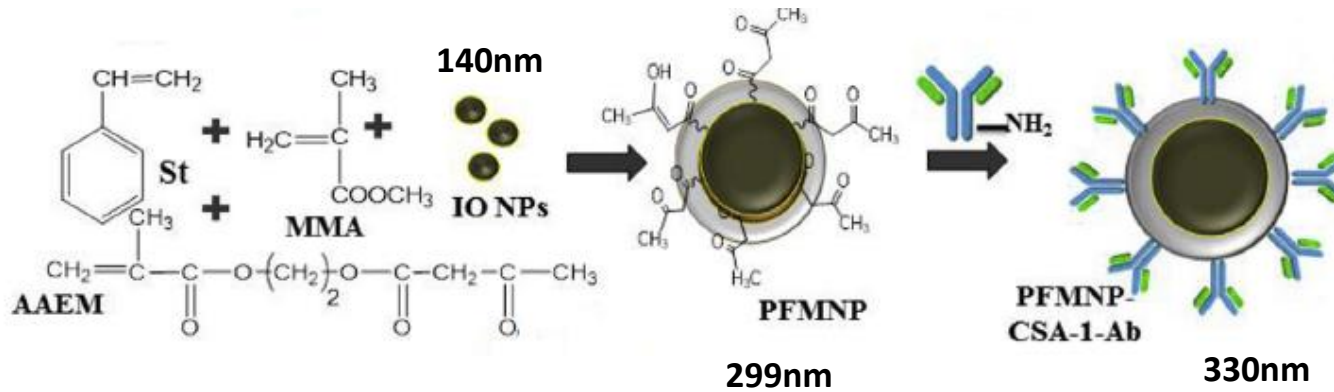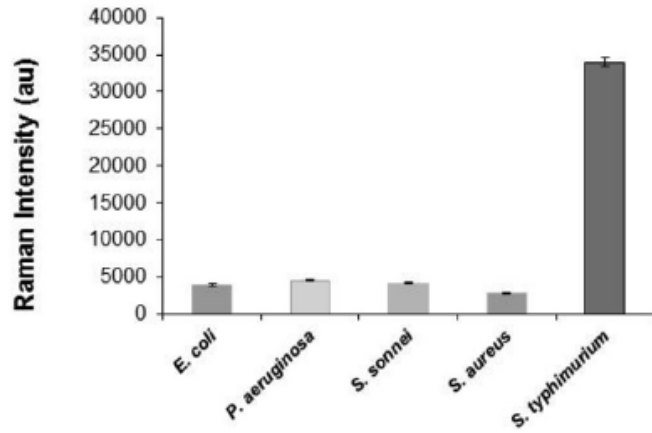
AuNP=31nm
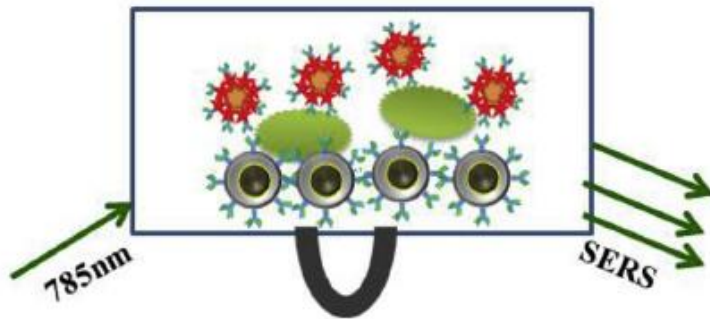

41nm

48nm

- Capture probe: MNP@PS-PMMA-Ab

140nm

299nm

330nm

Chattopadhyay et al., Anal. Chim. Acta 2019

LOD= 100 cells/mL

LOD= 10 cells/mL

$y = 4057.x + 3435.$
$R^2 = 0.976$

☒ Good specificity of AuNP@DSNB-Ab
☒ 80% detection in real samples (food)

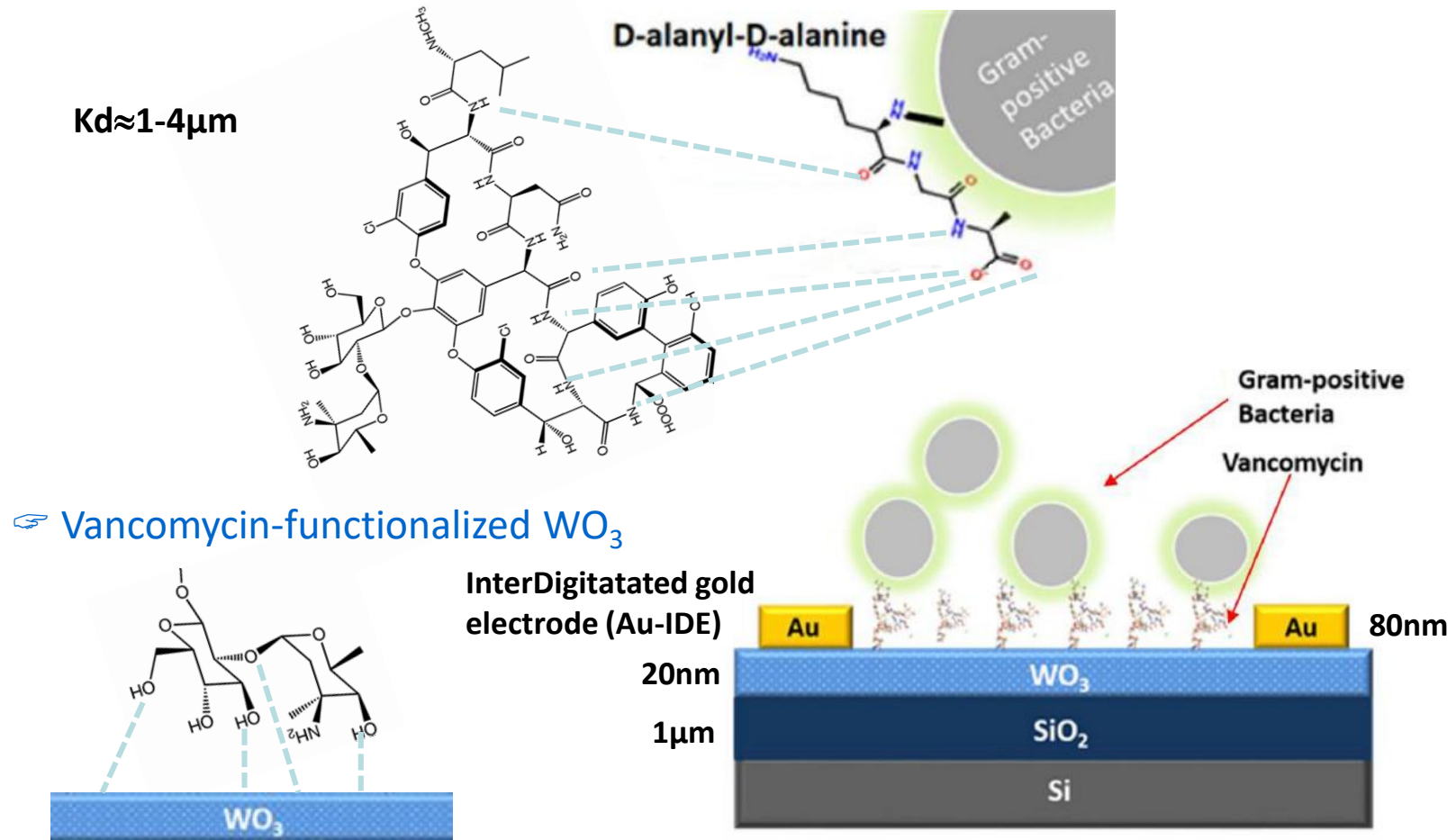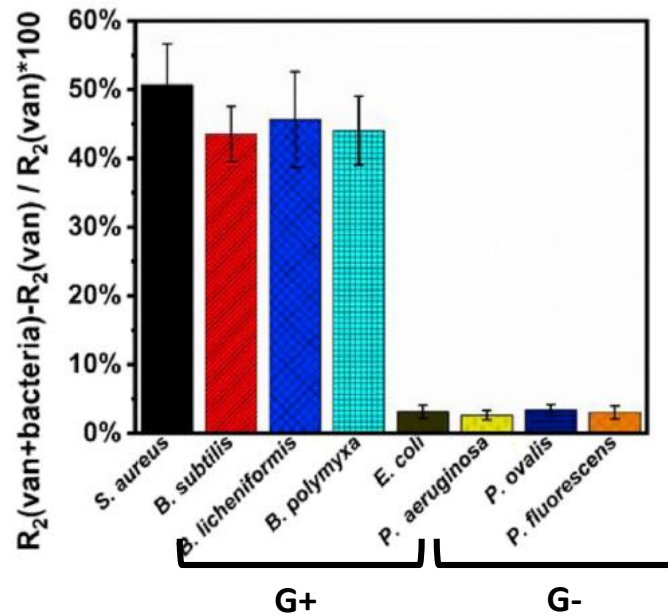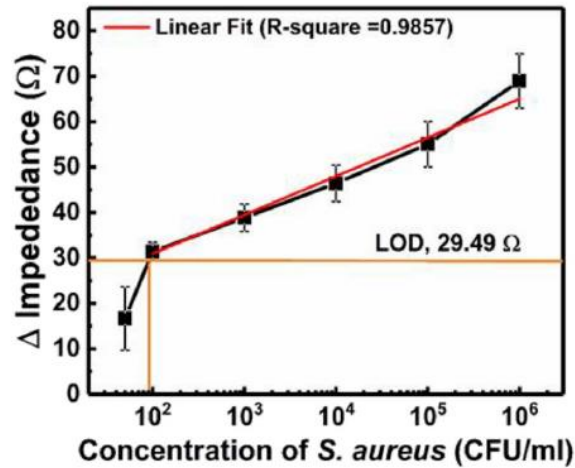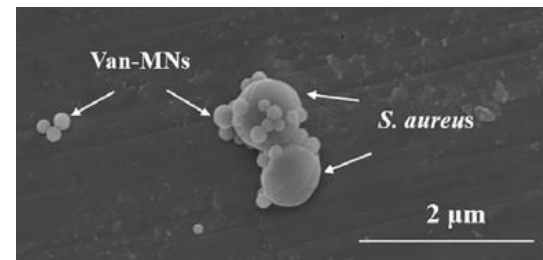# Antibiotic probe for selective bacteria detection

☞ Vancomycin (van) = glycopeptide antibiotic interacting with D-alanyl-D-alanine terminus of peptidoglycan extending the cell wall of G+ bacteria but not G- bacteria

Kd≈1-4μm

☞ Vancomycin-functionalized WO₃

InterDigitatated gold electrode (Au-IDE)

20nm

1μm

80nm

Singh et al., Biosens. Bioelec. 2019

☒ Selective detection of G+ bacteria
☒ Same results in physiological condition
☒ Impedence allows to discriminate dead and alive cells

☞ Vancomycin-functionalized MNP

Wang et al., Anal Chim Acta 2022

S. aureus

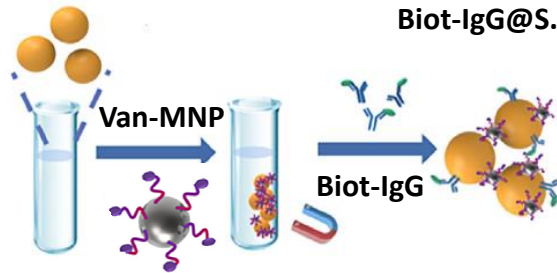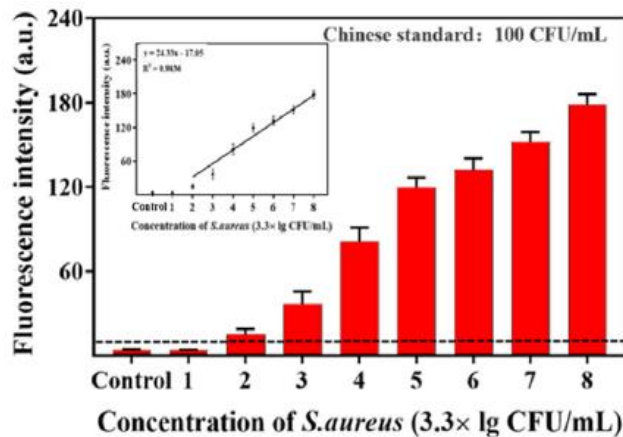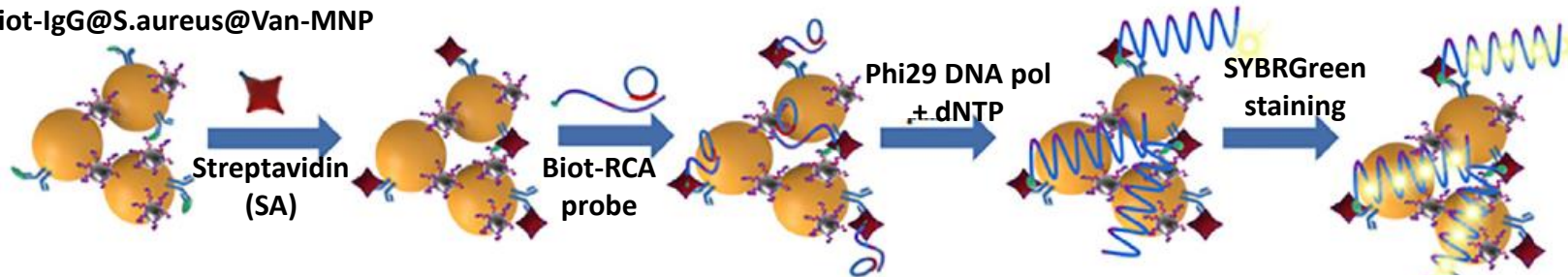**Biot-IgG@S.aureus@Van-MNP**

Van-MNP

Biot-IgG

☒ IgG combines with Protein A properties of the surface of S. aureus

☒ RCA amplification of detected S. aureus in juice fruit samples

Biot-IgG@S.aureus@Van-MNP

Streptavidin (SA)

Biot-RCA probe

Phi29 DNA pol + dNTP

SYBRGreen staining

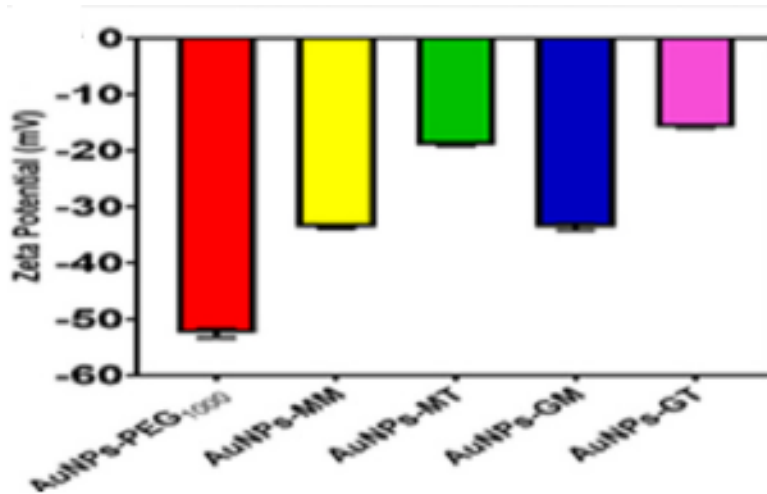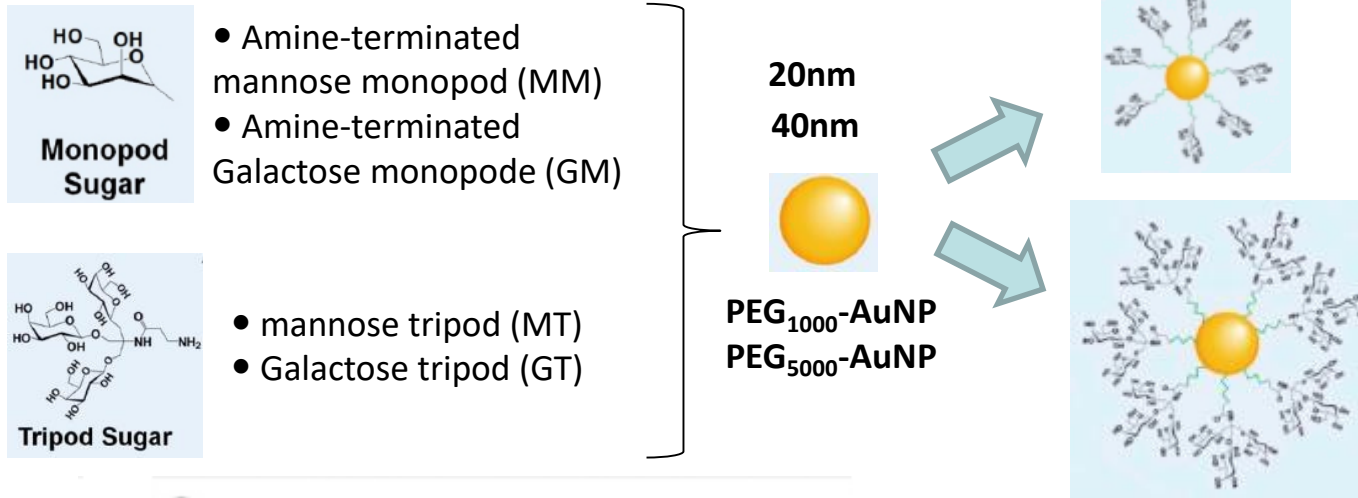
- LOD=$3,3 \times 10^2$ CFU/mL

  - Mixture 1 = 10^5 CFU/mL of same volume of non-target bacteria
  - Mixture 2 = $3,3 \times 10^5$ CFU/mL S. aureus + 10^5 CFU/mL non target bacteria

Nanoglycocluster based diagnostic platform

☞ AuNP functionalized with multivalent glycans
  ☒ carbohydrate-lectins interactions (ConA-mannose; PNA-galactose)
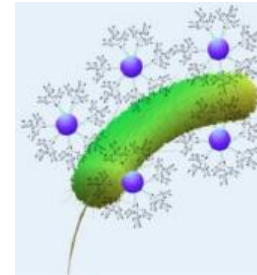 ☒ multivalency enhances binding affinity with higher specificty and kinetic stability

- Amine-terminated mannose monopod (MM)
- Amine-terminated Galactose monopode (GM)

Monopod Sugar

20nm
40nm

- mannose tripod (MT)
- Galactose tripod (GT)

Tripod Sugar

PEG_{1000}-AuNP
PEG_{5000}-AuNP

☒ Less immobilization on PEG1000 compared to PEG 5000 due to steric hindrance
☒ Better AuNP aggregation with 20nm AuNP and smaller PEG
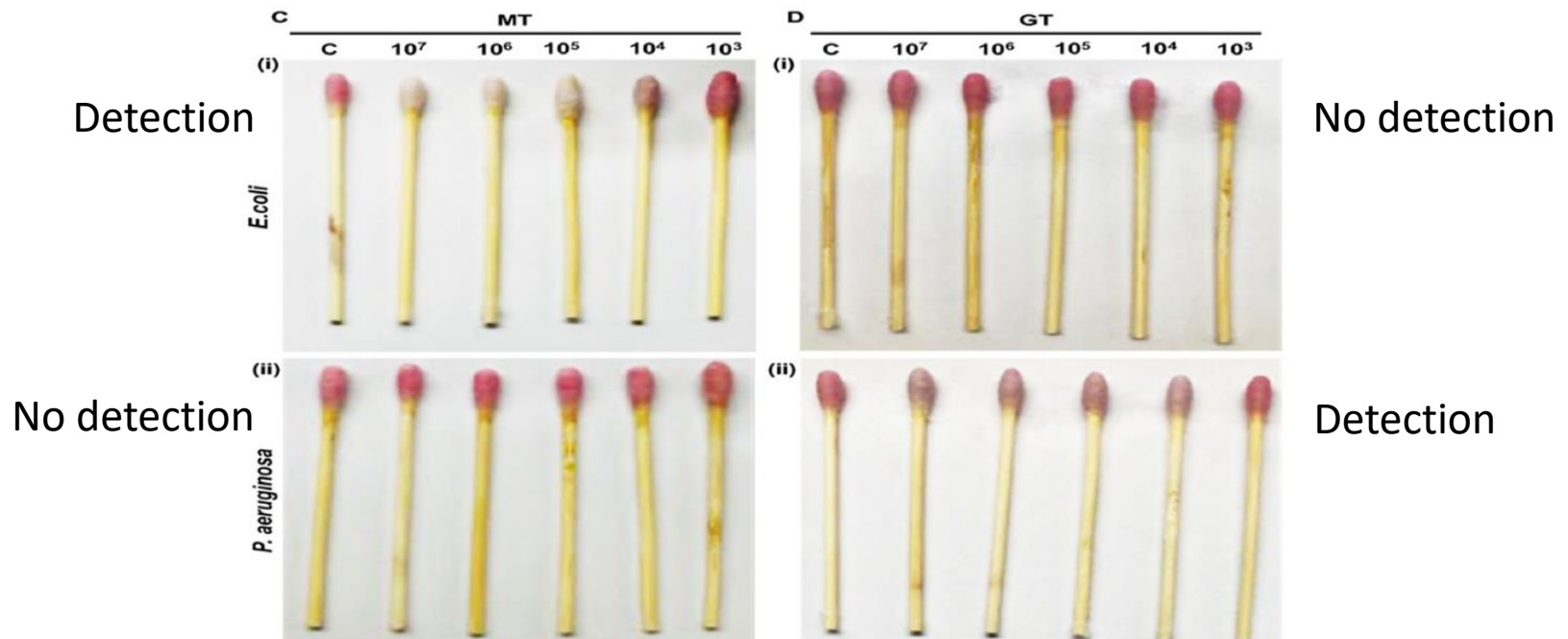
Priyadarshi et al., Biosens. Bioelec. 2022

Institut des Nanotechnologies de Lyon UMR CNRS 5270   *Evènement et date*   http://inl.cnrs.fr

☞ ConA lectin on E. coli
  ☒ interaction with mannose
  ☒ kd=34nM with MM; kd=18nM with MT

☞ PNA lectin on P. aeruginosa
  ☒ interaction with galactose
  ☒ kd=37nM with GM; kd=16nM with GT

From red color (non aggregated AuNP) to purple color when bacteria is detected (aggregation of AuNP)

Detection — E.coli — No detection (top row)
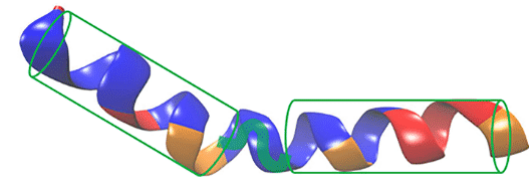
No detection — P. aeruginosa — Detection (bottom row)

Antimicrobial peptide (AMP) based detection
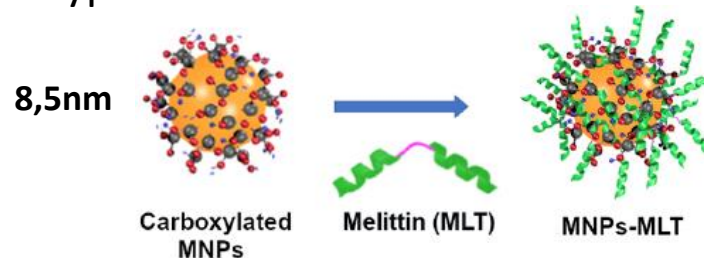
☞ AMP have low selectivity: active against viruses, bacteria and fungi
  ☒ Importance of the choice of the active molecule

☞ Low sensitivity can be impove by the combination with nanomaterials

☞ Melittin (MLT) = cationic amphipathic molecule of 26aa

Peptide	Amino Acid Sequence *												
Melittin	1	2	3	4	5	6	7	8	9	10	11	12	13
	G	I	G	A	V	L	K	V	L	T	T	G	L
	14	15	16	17	18	19	20	21	22	23	24	25	26
	P	A	L	I	S	W	I	K	R	K	R	Q	Q

* Blue: hydrophobic; Orange: hydrophilic; Red: charged

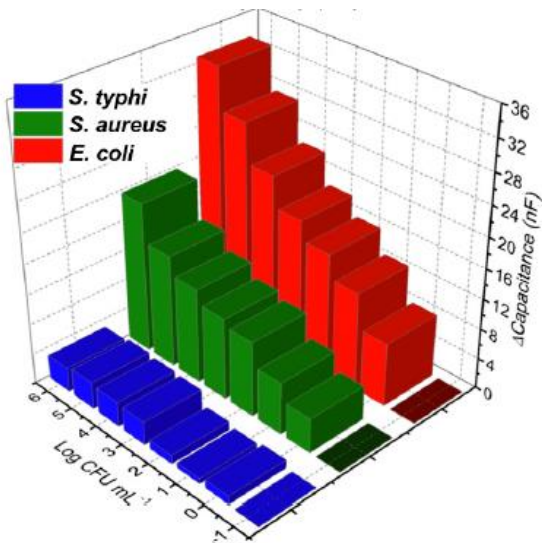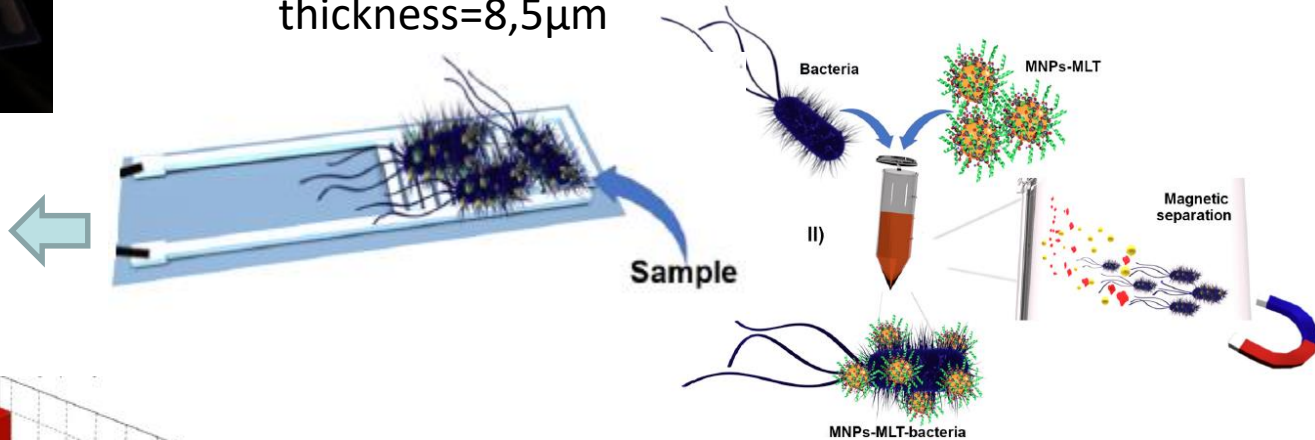☒ Detection of contaminated food with G+ and G- bacteria: E. coli, S. aureus, S. typhimurium

8,5nm

Carboxylated MNPs   Melittin (MLT)   MNPs-MLT

Wilson et al., Talenta 2019

☞ Screen-printed silver interdigitated electrode (SPIDE) on PET
  ☒ 10 pairs of electrode of 180µm width and separation, thickness=8,5µm
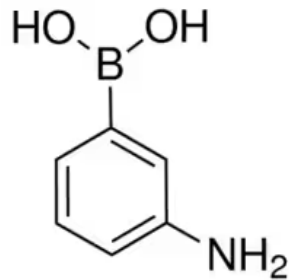
Impedence measurement

☒ E. Coli detection

Sample	Added (CFU mL^{-1})	Found	Recovery
Apple juice	5.5	5.25	95.4
	15	14.21	94.7
Potable water	2.0	1.79	89.5
	15	14.62	97.5

☒ S. Typhi not reproducible detection
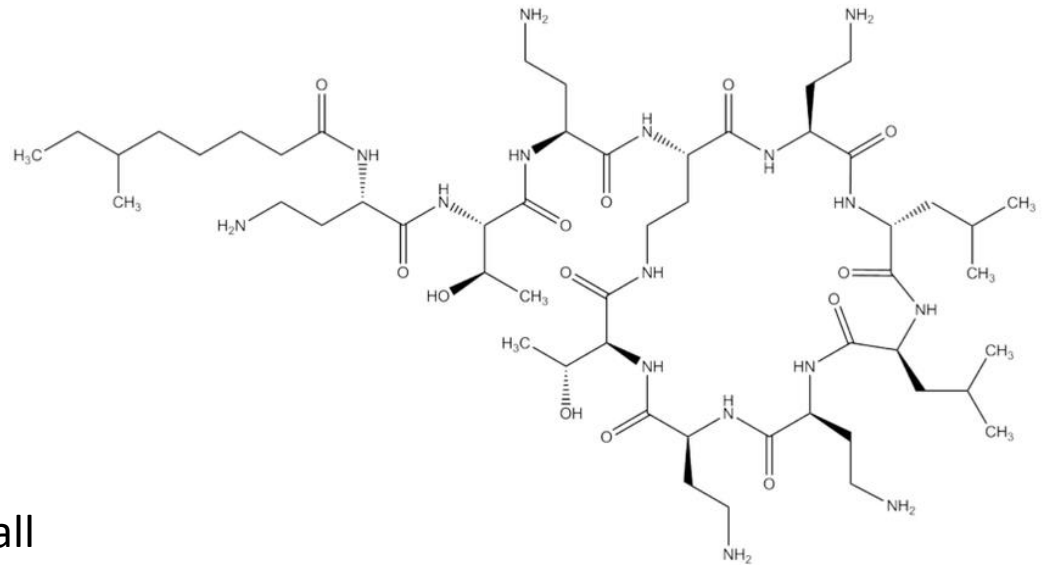☒ E. Coli LOD= 1 CFU/mL
☒ S. Aureus LOD= 10 CFU/mL

Receptors functionalized carbon dots

☞ 3 recognition molecules = versatile receptors with different ability of binding to bacteria

⊠ Vancomycin (Van): specific binding to D-Ala-D-Ala of peptidoglycan f cell wall

⊠ 3-aminophenyl boronic acid (BA):
Biding to cis-diol molecules od cell wall

⊠ Polymixin B (PM):
High affinity for LPS of cell wall
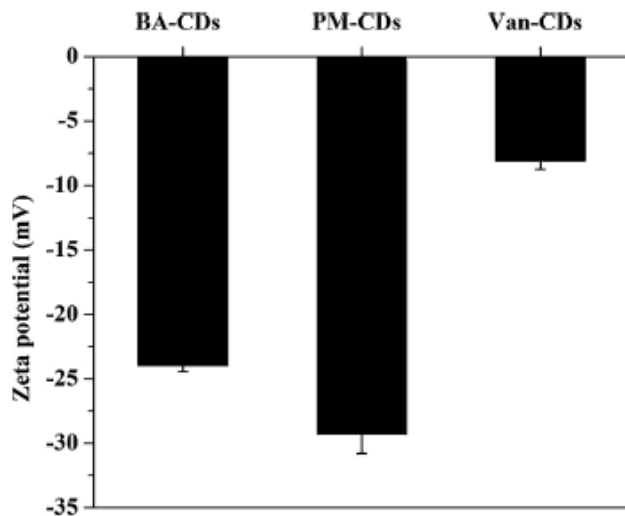
Zheng et al., Sensors and Actuators B 2019

☞ Carbon dots (CD) synthesis using pyrolysis method (1h at 180°C)from di-hydrogen ammonium citrate (DHAC)
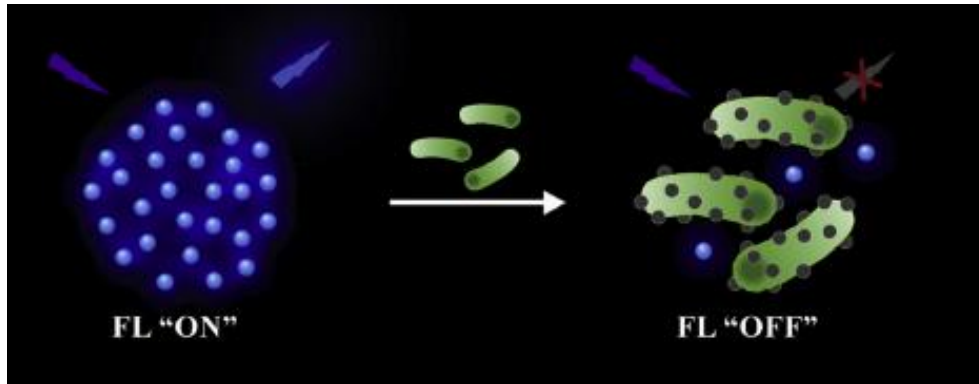
⊠ CD@BA, CD@PM, CD@Van obtained by mixing DHAC with receptor molecule : 4h at 180°C (Dia=3-6nm)
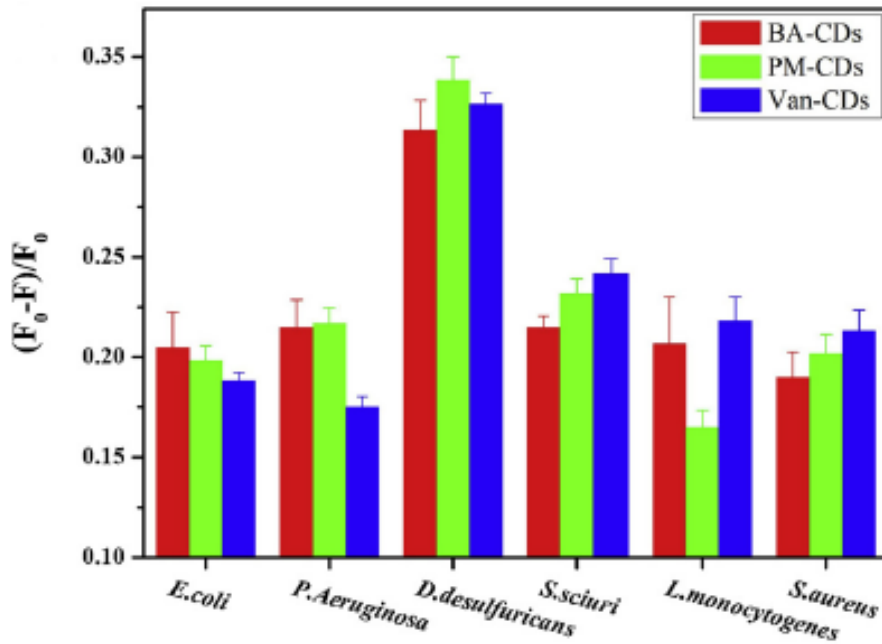
⊠ Characterisation with XPS
⊠ Analysis of fluorescence spectra
- CD@BA: exc = 340nm
- CD@PM: exc=350nm
- CD@Van: exc=350nm
- Em=455nm

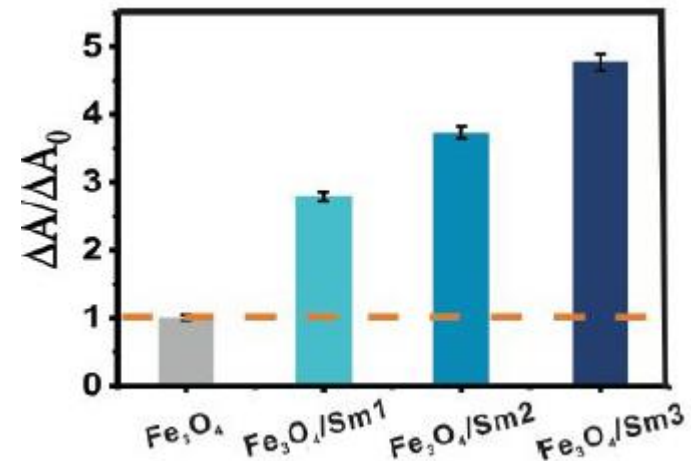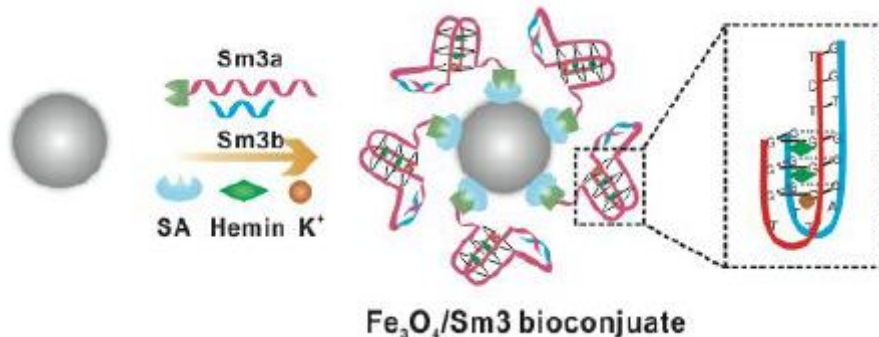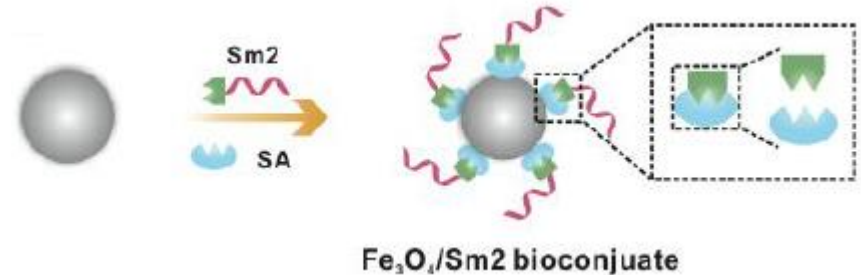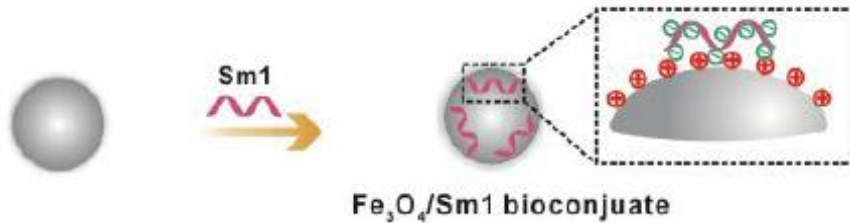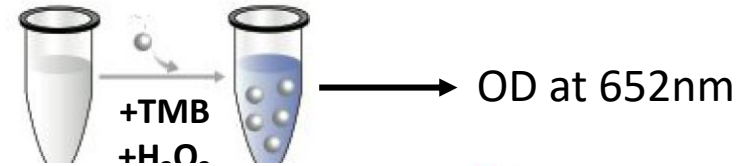☒ Decline of the fluorescence intensity with binding to bacteria

☞ Binding of CD@molecules depends on the type of molecules and on the kind of bacteria
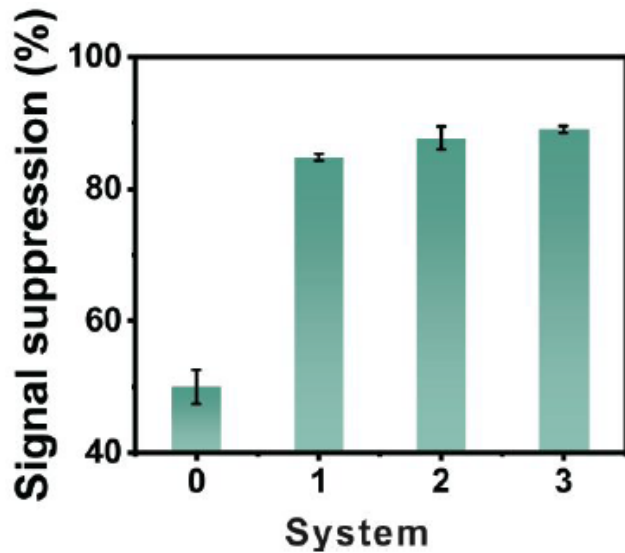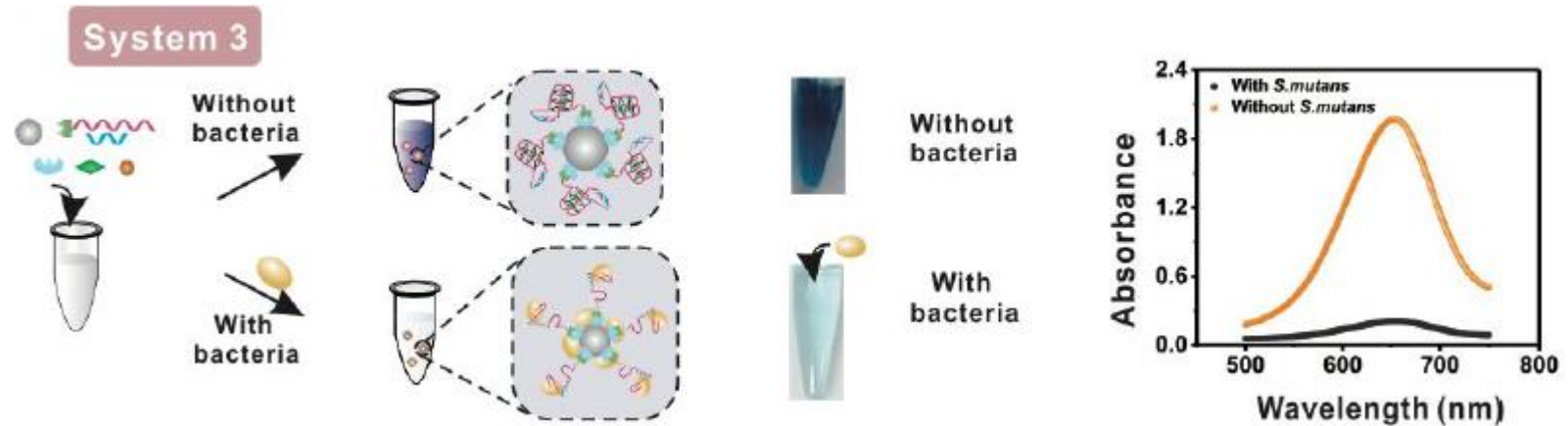
Nanozyme and DNA nanostructure

☞ Nanozyme = nanomaterials with enzyme-like activities

☒ Fe_3O_4 NP (150nm)= peroxidase-like activites
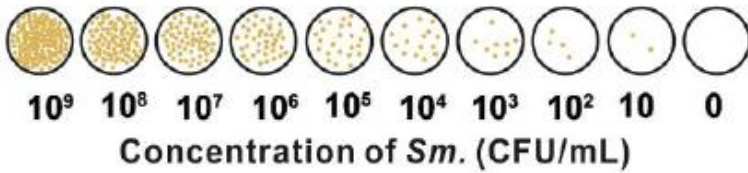
☒ DNA = S. mutans-binding aptamer

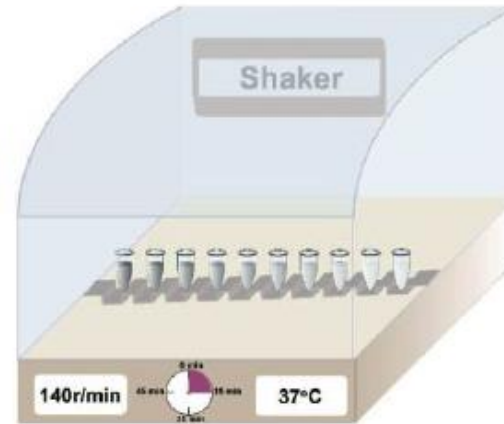OD at 652nm

Zhang et al., ASC Appl. Mater. Interfaces 2019

☒ The binding of bacteria reduces the catalytic activity of the DNAzyme

Samples:

10^9 10^8 10^7 10^6 10^5 10^4 10^3 10^2 10 0
Concentration of Sm. (CFU/mL)

Colorimetric readout:

10^9 10^8 10^7 10^6 10^5 10^4 10^3 10^2 10 0

Shaker

140r/min   37°C

☒ Results in 15min

Detection Limit

96 CFU/mL   41 CFU/mL   12 CFU/mL

Conclusion

☞In recent years: great progress in developing faster, more accurate and more cost-effective detection modalities for monitoring bacterial infections

☞ Use of nanotechnology and chemical modifications to stabilize receptor element and increase transducer sensitivity

☞ Still hurdles translating technologies into commercial devices while maintening low cost

☞ Trends for future developments: wearable devices, microfluidic devices, self-powered technologies

☞ Challenges to adress:
  ☒ simultanous detection of multiple bacteria
  ☒ integration and simplicity of sample treatment