

Supplementary Materials

For

**Colorimetric Detection and Killing of Bacteria by
Enzyme-Instructed Self-Aggregation of
Peptide-Modified Gold Nanoparticles**

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Table S1. High performance liquid chromatography (HPLC) peaks of CF₄KY^P.

Peak	Time	Height	Area	Conc.
1	7.433	1498.536	15566.402	0.2921
2	8.840	1201.502	22311.703	0.4187
3	9.638	8790.235	89428.344	1.6783
4	9.832	305713.250	4919143.000	92.3199
5	10.188	25168.014	171152.031	3.2121
6	10.479	6480.615	45130.223	0.8470
7	10.872	3519.667	53393.016	1.0021
8	11.752	1166.092	12243.196	0.2298

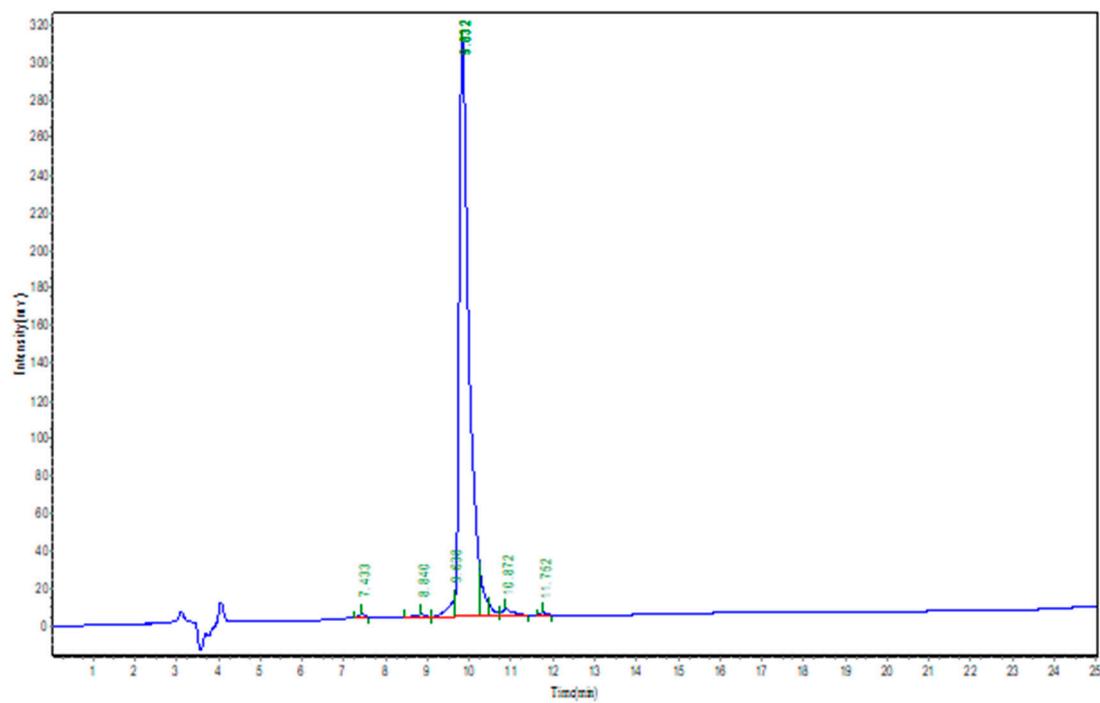


Figure S1. HPLC spectra of CF₄KY^P.

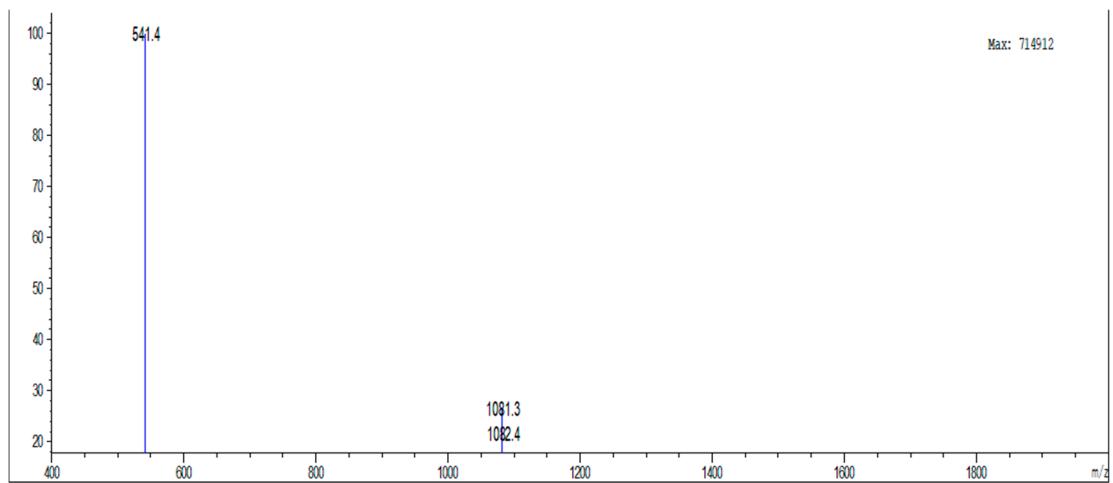


Figure S2. Mass spectra of $\text{CF}_4\text{KY}^{\text{P}}$ (MW= 1081.17)

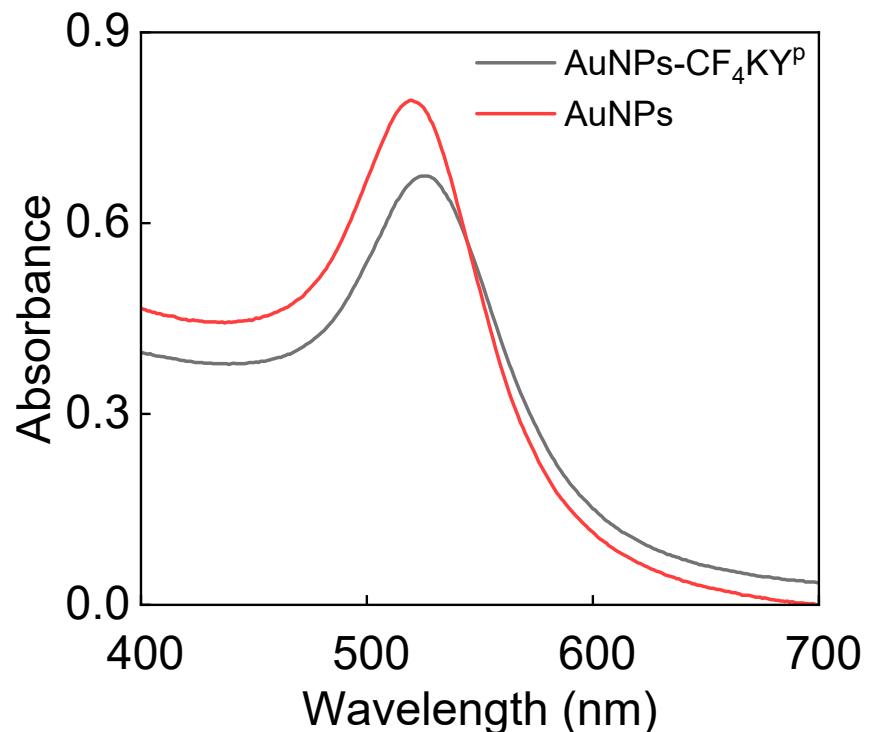


Figure S3. UV-vis spectra of AuNPs and $\text{AuNPs-}\text{CF}_4\text{KY}^{\text{P}}$.

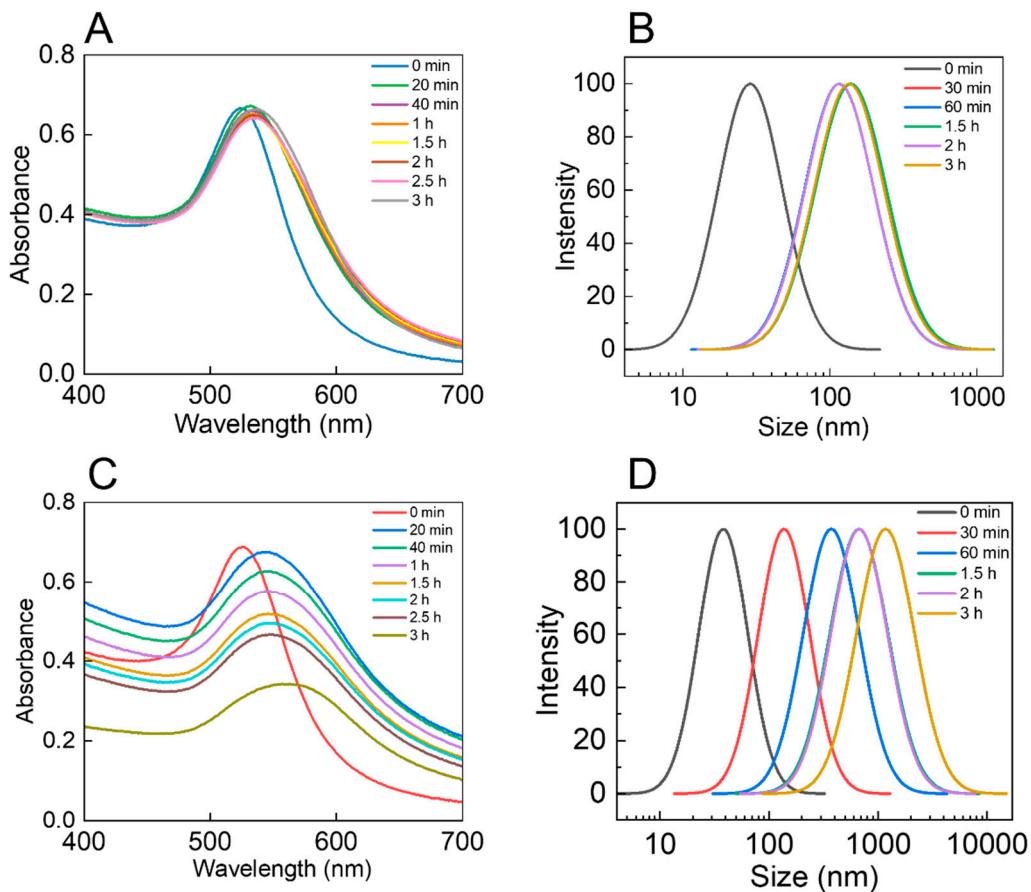


Figure S4. (A) UV–vis spectra and (B) hydrodynamic size profiles of AuNPs-CF4KY^p-1 before and after the addition of ALP. (C) UV–vis spectra and (D) hydrodynamic size profiles of AuNPs-CF4KY^p-2 before and after the addition of ALP. Experiments were repeated three times.

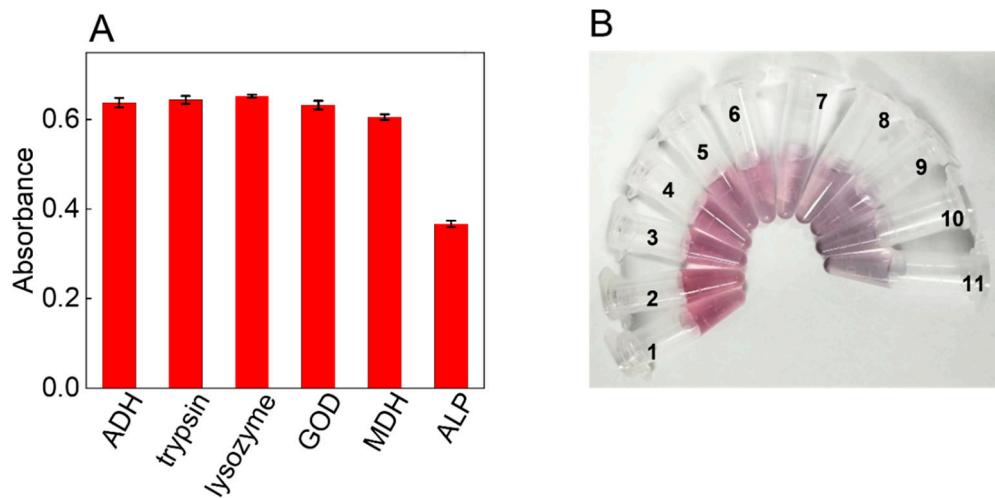


Figure S5. (A) The absorbance changes (530 nm) of the AuNPs-CF4KY^p after incubating with different enzymes. Data are presented as mean \pm S.D. Error bars were obtained from three replicate experiments. (B) The picture of AuNPs-CF4KY^p solutions at different concentrations of ALP. 1, 2 U·mL⁻¹; 2, 2.2 U·mL⁻¹; 3, 2.4 U·mL⁻¹; 4, 2.6 U·mL⁻¹; 5, 2.8 U·mL⁻¹; 6, 3.0 U·mL⁻¹; 7, 3.2 U·mL⁻¹; 8, 3.4 U·mL⁻¹; 9, 3.6 U·mL⁻¹; 10, 3.8 U·mL⁻¹; 11, 4 U·mL⁻¹). Experiments were repeated three times.

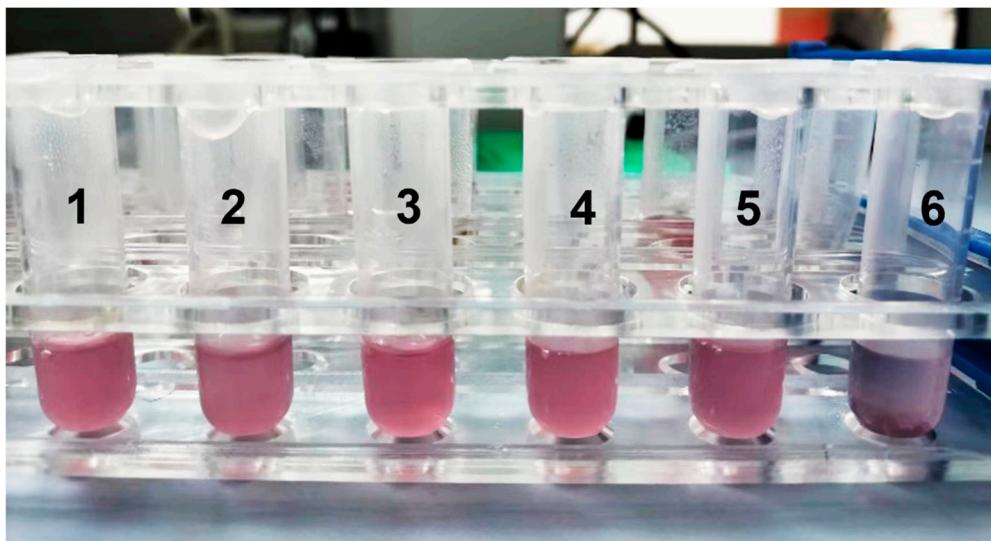


Figure S6. The picture of AuNPs- CF₄KY^P solutions after incubating with different bacteria. 1, *E. coli*; 2, *K. pneumoniae*; 3, *A. baumannii*; 4, *S. aureus*; 5, DH-5 α ; 6, *S. typhimurium*. Experiments were repeated three times.

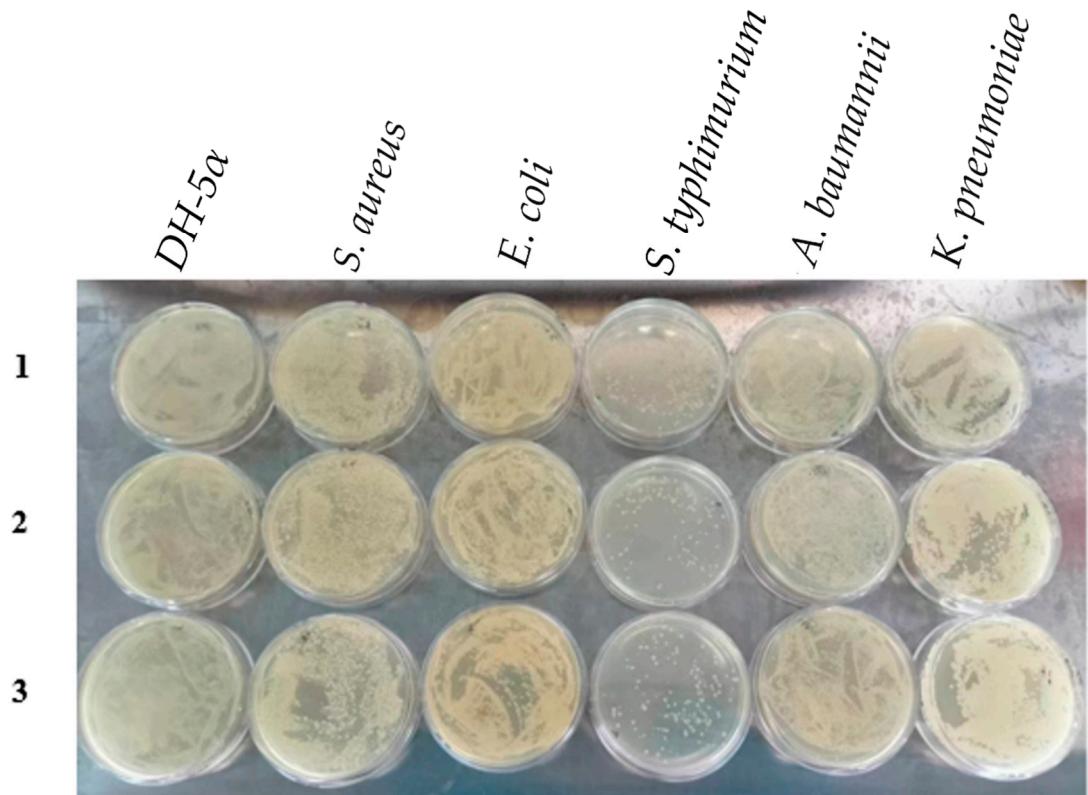


Figure S7. Colony growth image of different bacteria after incubating with AuNPs- CF₄KY^P.

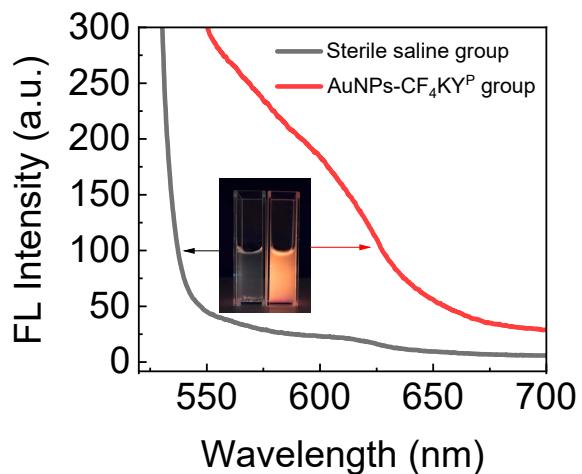


Figure S8. ROS fluorescence spectra of *S. typhimurium* after incubating with AuNPs-CF₄KY^P or sterile saline. The inset fluorescence image is the *S. typhimurium* solutions after incubating with a commercial bacteria ROS kit (BBoxiProbeO13).

Table S2. Typical research work on colorimetric detection of bacteria in the past five years.

Detection of bacteria	Detection limit (CFU·mL ⁻¹)	Antimicrobial performance	References
<i>Salmonella typhimurium</i>	7	80%	This work
<i>Salmonella typhimurium</i>	61	—	[1]
<i>Escherichia coli</i>	24	—	[2]
<i>Bacillus subtilis</i>	2	—	[2]
<i>E. coli</i> ATCC 25922	10	80%-90%	[3]
<i>S. aureus</i> ATCC 25323	10	80%-90%	[3]
<i>Escherichia coli</i>	3.38×10^6	90%	[4]
<i>Salmonella typhimurium</i>	2.65	—	[5]
MRSA	1	—	[6]
<i>E. coli</i> WT3110	10^2	—	[7]
<i>S. aureus</i> ATCC 25923	10^2	—	[7]
<i>S. aureus</i> and <i>E. coli</i>	—	>90%	[8]
<i>Escherichia coli</i> O157:H7	4.1	—	[9]
<i>Escherichia coli</i> O157:H7	10	—	[10]
<i>Escherichia coli</i> ATCC25922	1.02×10^3	—	[11]
gram-positive bacterium	10^2	—	[12]
gram-negative bacterium	10^2	—	[12]
<i>Escherichia coli</i> ATCC 8739	100/44	—	[13]
<i>Salmonella typhimurium</i>	1	—	[14]
<i>Streptococcus pneumoniae</i>	65	—	[15]
<i>S. aureus</i> (ATCC 29213)	80	—	[16]

<i>Escherichia coli</i> ATCC25922	7.48×10^3	—	[17]
<i>S.aureus</i> ATCC29213	3.3×10^3	—	[17]
<i>Salmonella typhimurium</i>	56	—	[18]
<i>Staphylococcus aureus</i>	120	—	[19]

References

- [1] Ahmed A., Rushworth J.V., Hirst N.A., Millner P.A. Biosensors for Whole-Cell Bacterial Detection. *Clin. Microbiol. Rev.* **2014**, *27*, 631–646.
- [2] Chen F., Chen D.A., Deng T., Li J.S. Combination of Alkaline Phosphatase/Graphene Oxide Nanoconjugates and D-Glucose-6-Phosphate-Functionalized Gold Nanoparticles for the Rapid Colorimetric Assay of Pathogenic Bacteria. *Biosens. Bioelectron.* **2022**, *216*, 114611.
- [3] Roh S.G., Robby A.I., Phuong P.T.M., In I., Park S.Y. Photoluminescence-Tunable Fluorescent Carbon Dots-Deposited Silver Nanoparticle for Detection and Killing of Bacteria. *Mater. Sci. Eng. C.* **2019**, *97*, 613-623.
- [4] Zhang X., Ren C.H., Hu F., Gao Y., Wang Z.Y., Li H.Q., Liu J.F., Liu B., Yang C.H. Detection of Bacterial Alkaline Phosphatase Activity by Enzymatic in Situ Self-Assembly of the Aiegen-Peptide Conjugate. *Anal. Chem.* **2020**, *92*, 5185-5190.
- [5] Wang L., Wu X., Hu H., Huang Y., Yang X., Wang Q., Chen X. Improving the Detection Limit of Salmonella Colorimetry Using Long Ssdna of Asymmetric-Pcr and Non-Functionalized Aunps. *Anal. Biochem.* **2021**, *626*, 114229.
- [6] Yang H., Xiao M., Lai W., Wan Y., Li L., Pei H. Stochastic DNA Dual-Walkers for Ultrafast Colorimetric Bacteria Detection. *Anal. Chem.* **2020**, *92*, 4990-4995.
- [7] Le T.N., Tran T.D., Kim M.I. A Convenient Colorimetric Bacteria Detection Method Utilizing Chitosan-Coated Magnetic Nanoparticles. *Nanomaterials.* **2020**, *10*(1), 92.
- [8] Kang E.B., Mazrad Z.a.I., Robby A.I., In I., Park S.Y. Alkaline Phosphatase-Responsive Fluorescent Polymer Probe Coated Surface for Colorimetric Bacteria Detection. *Eur. Polym. J.* **2018**, *105*, 217-225.
- [9] Wang K.-Y., Bu S.-J., Ju C.-J., Li C.-T., Li Z.-Y., Han Y., Ma C.-Y., Wang C.-Y., Hao Z., Liu W.-S., Wan J.-Y. Hemin-Incorporated Nanoflowers as Enzyme Mimics for Colorimetric Detection of Foodborne Pathogenic Bacteria. *Bioorganic Med. Chem. Lett.* **2018**, *28*, 3802-3807.
- [10] Wang J., Cao Y., Li Z., Dong M., Dou W., Xu X., He S. Bridge-DNA Synthesis Triggered by an Allosteric Aptamer for the Colorimetric Detection of Pathogenic Bacteria. *Anal. Methods.* **2023**, *15*, 275-283.
- [11] Huang J., Sun J., Warden A.R., Ding X. Colorimetric and Photographic Detection of Bacteria in Drinking Water by Using 4-Mercaptophenylboronic Acid Functionalized Aunps. *Food Control.* **2020**, *108*, 106885.
- [12] Liu Y., Zhao C., Zhao W., Zhang H., Yao S., Shi Y., Li J., Wang J. Multi-Functional MnO₂-Doped Fe₃O₄ Nanoparticles as an Artificial Enzyme for the Colorimetric Detection of Bacteria. *Anal. Bioanal. Chem.* **2020**, *412*, 3135-3140.

- [13] Wang C., Gao X., Wang S., Liu Y. A Smartphone-Integrated Paper Sensing System for Fluorescent and Colorimetric Dual-Channel Detection of Foodborne Pathogenic Bacteria. *Anal. Bioanal. Chem.* **2020**, 412, 611-620.
- [14] Chen Q.M., Gao R., Jia L. Enhancement of the Peroxidase-Like Activity of Aptamers Modified Gold Nanoclusters by Bacteria for Colorimetric Detection of *Salmonella Typhimurium*. *Talanta*. **2021**, 221, 121476.
- [15] Bahadoran A., Jabarabadi M.K., Mahmood Z.H., Bokov D., Janani B.J., Fakhri A. Quick and Sensitive Colorimetric Detection of Amino Acid with Functionalized-Silver/Copper Nanoparticles in the Presence of Cross Linker, and Bacteria Detection by Using DNA-Template Nanoparticles as Peroxidase Activity. *Spectrochim Acta A*. **2022**, 268, 120636.
- [16] Bagheri Pebdeni A., Hosseini M. Fast and Selective Whole Cell Detection of *Staphylococcus Aureus* Bacteria in Food Samples by Paper Based Colorimetric Nanobiosensor Using Peroxidase-Like Catalytic Activity of DNA-Au/Pt Bimetallic Nanoclusters. *Microchemical Journal*. **2020**, 159, 105475.
- [17] Sun J., Huang J., Li Y., Lv J., Ding X. A Simple and Rapid Colorimetric Bacteria Detection Method Based on Bacterial Inhibition of Glucose Oxidase-Catalyzed Reaction. *Talanta*. **2019**, 197, 304-309.
- [18] Ma X., Song L., Zhou N., Xia Y., Wang Z. A Novel Aptasensor for the Colorimetric Detection of *S. Typhimurium* Based on Gold Nanoparticles. *Int. J. Food Microbiol.* **2017**, 245, 1-5.
- [19] Verdoort N., Basso C.R., Rossi B.F., Pedrosa V.A. Development of a Rapid and Sensitive Immunosensor for the Detection of Bacteria. *Food Chem.* **2017**, 221, 1792-1796.