

Aggregation-induced emission enhancement effect of gold nanoclusters upon interaction with bacterial lipopolysaccharide

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Bacterial lipopolysaccharides cause numerous undesirable immune responses in humans, underscoring the need for selective and sensitive methods for their detection. In the present work, the interaction between glutathione-stabilized gold nanoclusters and bacterial lipopolysaccharide was studied by fluorescence spectroscopy. The lipopolysaccharide was isolated from *Pseudomonas putida* biofilm cells. The interaction of the nanoclusters with supramolecular particles in the lipopolysaccharide preparation gave rise to aggregation-induced emission enhancement (AIEE effect). This effect manifested as an increase in fluorescence intensity and a slight blue shift of the nanoclusters' fluorescence emission maximum. The enhancement of the nanoclusters' fluorescence intensity was found to depend linearly on the lipopolysaccharide concentration over a wide range. The emission enhancement upon lipopolysaccharide addition was retained in the presence of various interfering chemical agents. These results can be used to develop a sensor system for the detection of lipopolysaccharides in pharmaceutical products.

Keywords: fluorescence spectroscopy, gold nanoclusters, aggregation-induced emission enhancement, lipopolysaccharide.

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Introduction

Endotoxins or lipopolysaccharides (LPS) are amphiphilic biomolecules included in cell walls of gram-negative bacteria [1]. Even a small dose of LPS molecules entering a human body can induce various undesired immune system reactions, including fever, complement activation and blood coagulation enhancement [2]. The fact that LPS is a key factor in the abdominal sepsis pathogenesis additionally emphasizes a diagnostic significance of this biomolecule. It is especially important to detect the content of endotoxins in pharmaceutical products because modern biotechnological production uses microbe cells as producers. LAL test where *Limulus* amebocytes react with LPS molecules is a standard endotoxin detection method. This method is highly sensitive, however, it takes much time (about 60 min) and is expensive. The quality of reagents in diagnostic LAL tests can vary considerably due to a complicated amebocyte production procedure [3]. Thus, an important task involves the development of alternative LPS detection methods.

Fluorescent methods for LPS detection have good prospects because they combine high analyte detection sensitivity with well-established instrumentation available for most analytical laboratories. When creating fluorescent sensors for LPS detection, some researchers use a technique where fluorophore fluorescence is „enabled“ in the presence of analyte molecules in an analyte mixture [4]. To increase the interaction specificity, some works used recognition

biomolecules such as aptamers [5–7] and peptides [8], which selectively interact with LPS.

An alternative approach allows LPS detection sensors to be created on the basis of an aggregation-induced emission enhancement effect (AIEE effect). These analytical systems include fluorescent agents (AIEE-phores) whose emission is enhanced when intramolecular structure rigidity changes [9]. Optical signal enhancement during direct interaction between AIEE-phores and endotoxins is caused by the fact that LPS in solutions form supramolecular particles, which considerably limits intramolecular rotations and oscillations in AIEE-phores upon binding to them [10]. Tetraphenyl ethylene and its derivatives are most commonly used as fluorophores in such sensor systems [11]. A certain restriction of organic AIEE-phores is in that the AIEE effect shows also when water is added, thus necessitating differentiation between a background signal and a signal directly induced by adding the analyte.

Fluorescent gold nanoclusters (AuNCs) are also classified as AIEE-phores [12]. These nanomaterials consist of gold atoms and stabilizing organic ligands. Their benefits include simple synthesis, large Stokes shift, high biocompatibility and spectral adjustment of fluorescence emission [13]. Using AuNCs as fluorescent agents for quantitative detection of LPS may be a promising method. Compared with organic AIEE-phores, many AuNCs feature hydrophilicity, which simplifies analysis in aqueous media. Our previous work uses a molecular simulation method to show that

gold nanoclusters stabilized with glutathione residues (GSH-AuNCs) have affinity for a polysaccharide component of the LPS molecule [14].

This work characterized the AIEE effect that occurs when GSH-AuNCs interact with simulated LPS isolated from *Pseudomonas putida* biofilms. Effect of some physical and chemical factors on AuNCs fluorescence intensity enhancement was studied with AuNCs exposed to LPS. Potential for using AuNCs for quantitative detection of endotoxin without using additional recognition molecules was first reported.

Materials and methods

A method described by Luo et al. [15] was used to synthesize glutathione-based fluorescent AuNCs (GSH-AuNCs). All glassware used for synthesis was preliminary washed in aqua regia ($\text{HCl}/\text{HNO}_3 = 3:1$) and then rinsed with ethanol and water. 1.5 mL of 100 mM reduced glutathione solution and 43.5 mL of miliQ H_2O were mixed in a glass flask and stirred. Then, 5 mL of 20 mM-HAuCl₄ was added to the resulting solution. The resulting mixture was stirred for 2 min. Then, the reaction mixture was incubated in a thermostat for 24 h at 70 °C without stirring.

GSH-AuNCs characterization was performed using various physical and chemical methods. Extinction spectra were recorded using the Specord S600 spectrophotometer (Analytic Jena, Germany) in a 300–800 nm spectral range. Fluorescent properties of nanoclusters were evaluated using the Cary Eclipse spectrofluorometer (Agilent Technologies, USA). A slit width of 10 nm was used for measurement. Electron-microscopic measurements were performed using the Libra 120 transmission electron microscope (TEM) (Carl Zeiss, Germany). The sample was applied to Formvar-coated nickel grids and dried. Electron-microscopic examination was performed at an accelerating voltage of 120 kV.

Gram-negative *Pseudomonas putida* TSh-18 (IBPPM 358) were provided by the Collection of Rhizosphere Microorganisms of the Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences (IBPPM RAS) (<http://collection.ibppm.ru>). To produce plankton cultures, bacteria were grown in LB medium [16] with constant stirring on a vibration table (100–120 rpm, 30 °C) for 24 h until the end of the exponential growth phase. To form biofilms at the air–liquid interface, cells were cultivated in LB medium in steady-state conditions at 30 °C during 72 h.

Formed *P. putida* TSh-18 biofilms were separated from the plankton cells using a nylon coarse-mesh filter, suspended in phosphate buffer (pH 7.2) and underwent two sonications (37 kHz, 40 °C) for 30 min each. From the resulting suspension, cells were pelleted by centrifugation at $3000 \times g$ for 40 min. LPS extraction was performed from finely powdered and acetone dried biofilm cell biomass using a hot phenol–water mixture in accordance with the

modified Westphal method without separating the water and phenol layers [17]. Phenol was removed from the resulting extract by dialysis through membranes with an exclusion limit of 12–14 kDa against running water during 120 h and concentrated on a rotary evaporator (Laborota 4000; Heidolph, Germany). Protein impurities were precipitated from the LPS solution by adding 40% trichloroacetic acid to final values of pH 2.7 followed by centrifugation at $16000 \times g$ (20 min). After removal of acid residues by dialysis against distilled water (48 h), the LPS extract was lyophilized using the Benchtop 2K dryer (Virtis, USA).

Supramolecular particle sizes in the LPS preparation were measured by dynamic light scattering using the Malvern Nano-ZS system (Malvern, UK). Light scattering intensity and correlation function were measured at 173° with a helium–neon laser wavelength $\lambda = 633$ nm. Measurements were performed in plastic four-sided cuvettes (SARSTEDT, Germany) with fixed laser focusing into the center of the cuvette and constant diaphragm diameter. Measurements were performed at 37 °C. For measurements, lyophilized LPS preparations were dissolved in miliQ H_2O in a concentration of 1 mg/mL. The LPS solution was held for 2 min at the specified temperature before each measurement.

During experiments, nanocluster and LPS suspensions were mixed in various proportions in plastic four-sided cuvettes. Resulting suspensions were dissolved to the final volume of 3 mL miliQ H_2O . Experiments investigating the effect of pH on the AIEE effect severity used the acetate buffer (pH 4.5, 20 mM), phosphate buffer (pH 7.4, 20 mM) and borate buffer (pH 8.8, 20 mM) as solvents. After mixing, the preparations were held for 3 min at room temperature. Fluorescence spectra of the suspensions were recorded using the Cary Eclipse spectrofluorometer (Agilent Technologies, USA).

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Results and discussion

Figure 1 shows the characteristic of synthesized GSH-AuNCs. There is no pronounced peak on the GSH-AuNCs absorption spectrum (Figure 1, c). This suggests that a size of all synthesized nanoclusters was less than 5 nm, and no plasmon-resonance nanoparticles were formed. Electron-microscopic characteristic proves this. According to the TEM data, the GSH-AuNCs size was 1.5 ± 0.5 nm (Figure 1, a, b). Maximum fluorescence excitation of GSH-AuNCs was $\lambda = 410$ nm, and maximum emission was $\lambda = 613$ nm (Figure 1, d).

Figure 2 shows the GSH-AuNCs fluorescence spectra recorded with various LPS concentrations. It can be seen that as the LPS concentration increased, nanocluster fluorescent signal intensity in the 550–650 nm range increased significantly (Figure 2, a). Fluorescence intensity changes were easily visible to the naked eye in UV light

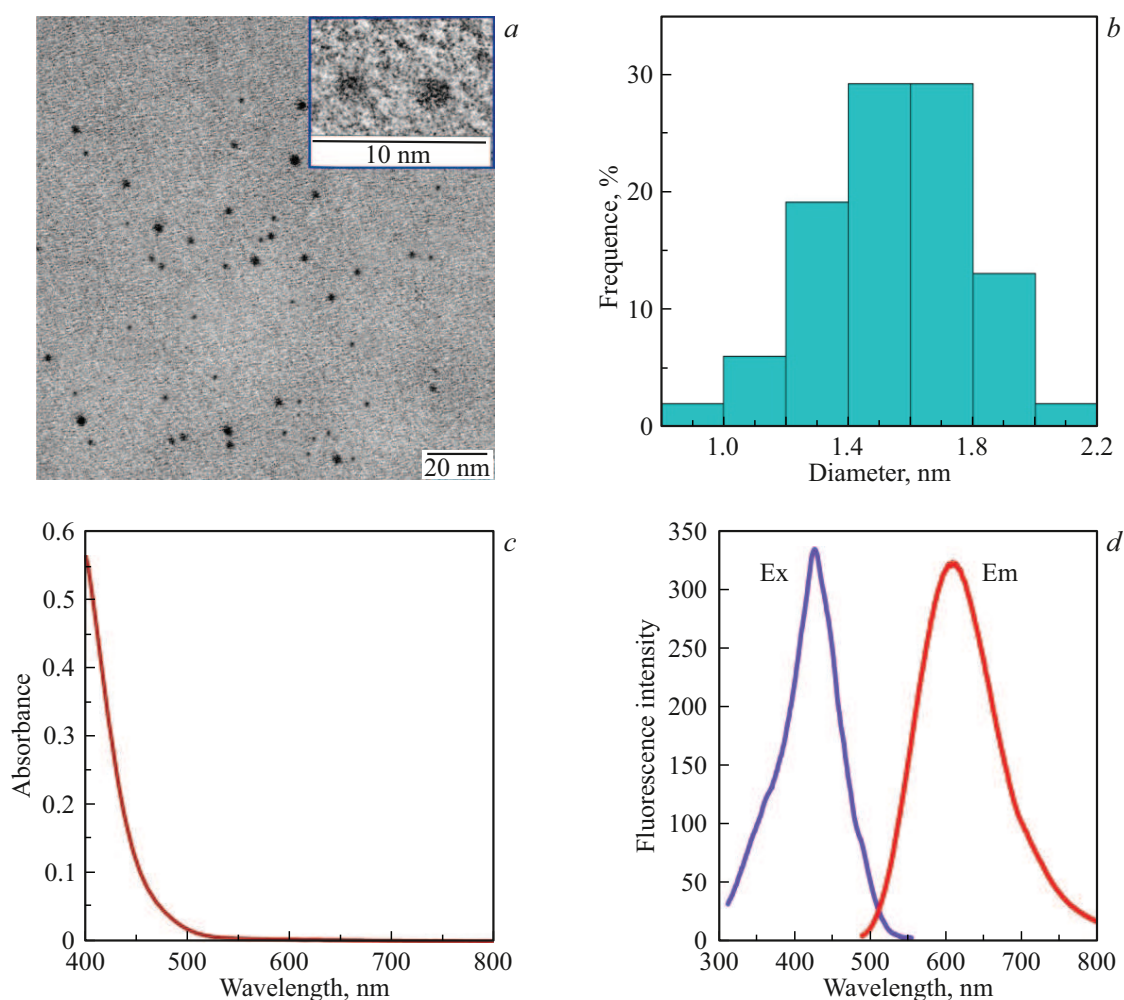


Figure 1. Characteristic of the synthesized GSH-AuNCs: electron-microscopic image of GSH-AuNCs (a); diagram of numerical size distribution of nanoclusters (b); GSH-AuNCs absorption spectrum (c); GSH-AuNCs fluorescence spectra, blue line. excitation spectrum (Ex), red line — emission spectrum (Em) (d).

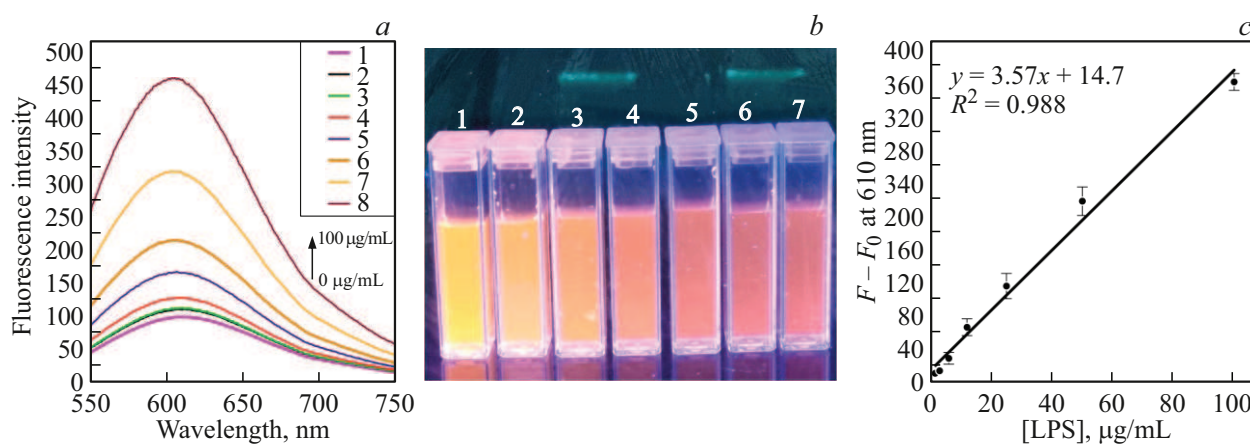


Figure 2. Dependence of GSH-AuNCs fluorescence intensity on *P. putida* TSh-18 LPS concentration: 1 — 0 µg/mL, 2 — 1.5 µg/mL, 3 — 3 µg/mL, 4 — 6 µg/mL, 5 — 12 µg/mL, 6 — 25 µg/mL, 7 — 50 µg/mL, 8 — 100 µg/mL (a); View of the GSH-AuNCs suspension exposed to various concentrations of LPS: 1 — 100 µg/mL, 2 — 50 µg/mL, 3 — 25 µg/mL, 4 — 12 µg/mL, 5 — 6 µg/mL, 6 — 3 µg/mL, 7 — 0 µg/mL. Photographs were made in UV light ($\lambda = 365$ nm) (b); Dependence of the GSH-AuNCs fluorescence increment at $\lambda_{em} = 610$ nm on the LPS concentration (c).

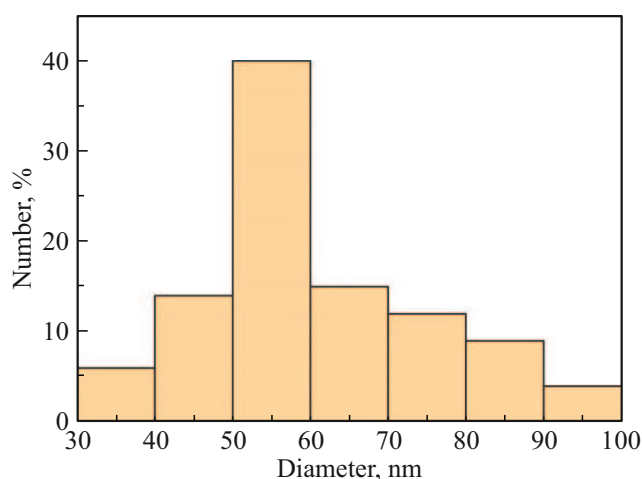


Figure 3. Diagram of numerical size distribution of particles in the *P. putida* TSh-18 LPS aqueous solution.

($\lambda = 365$ nm) (Figure 2, *b*). Dependence of the nanocluster fluorescence emission enhancement indicator ($F - F_0$ at $\lambda = 610$ nm) on the LPS concentration was linear in the 1–100 $\mu\text{g/mL}$ range ($R^2 = 0.988$) (Figure 2, *c*). The established detection limit was 0.7 $\mu\text{g/mL}$. Attention should be also paid to the fact that, when LPS was added to the medium, there was a minor, but detectable blue shift of the GSH-AuNCs emission maximum position by 3 nm: from $\lambda = 613$ nm to $\lambda = 610$ nm. The fluorescence intensity growth as well as the emission maximum shift towards short wavelengths are typical signs of the AIEE effect in metal nanoclusters [18].

Formation of supramolecular complexes in LPS water preparations separated from bacterial cells is based on the amphiphilic nature of these biomacromolecules. It is commonly believed that single-layer micelles are primarily formed from bacterial LPS. They include a hydrophobic core from fatty acid residues and a hydrophilic carbohydrate shell. Dynamic light scattering is a very convenient tool for characterizing a supramolecular arrangement of such dispersion systems [19]. The study used dynamic light scattering to determine a mean hydrodynamic radius of supramolecular structures in the *P. putida* TSh-18 LPS preparation and particle size distribution (Figure 3). Mean hydrodynamic radius of supramolecular micellar complexes in the LPS preparation was 62.3 ± 17.4 nm.

Our previous work [14] used a molecular docking method (Lamarckian genetic algorithm) to show that GSH-AuNCs are able to bind to the simulated endotoxin due to formation of hydrogen bonds between carboxyl, carbonyl and amine group of GSH-AuNCs and hydroxyl groups of the LPS carbohydrate shell. In this case, the AIEE effect manifestation was caused by interaction between GSH-AuNCs and supramolecular complexes (micelles) in the LPS preparation, leading to limitation of intramolecular nanocluster mobility and predominance of radiative transition processes over energy dissipation processes in the form of heat.

Influence of other bioorganic compounds on the GSH-AuNCs emission intensity enhancement was also evaluated (Figure 4, *a*). Pectin preparation, bovine serum albumin (BSA), glucose and glycine were used as interfering compounds. Only the *P. putida* TSh-18 LPS preparation had a significant effect on the fluorescence signal enhancement. Other compounds in the given concentration had no effect on the signal. Thus, it can be suggested that the AIEE effect induced by interaction between nanoclusters and LPS is selective in nature.

It was shown that acidity of the medium, where interaction between GSH-AuNCs and LPS preparation took place, affects the AIEE effect severity (Figure 4, *b*). With neutral (pH 7.3) and alkaline (pH 8.9) pH values, nanocluster fluorescence signal intensity enhancement is virtually identical. On the contrary, with acidic reaction of the medium (pH 4.5), 80%-decrease in $F - F_0$ takes place. This is probably due to the fact that, at low pH values, the pattern of LPS micelle formation changes, and smaller supramolecular complexes are formed.

Thus, possibility to detect LPS using the AIEE effect of AuNCs was first reported. This analytical procedure features simplicity, high speed of execution and high selectivity. Low sensitivity is the main limitation of the procedure. Detection limit of the developed method is 0.7 $\mu\text{g/mL}$, while the reported AIEE-sensors for LPS detection make it possible to detect these biomolecules with a detection limit of 1–2 ng/mL [10]. Further investigations are required to improve the sensitivity of the developed technical approach. Selection and introduction into the analytical system of heavy metal ions that quench nanocluster fluorescence followed by restoration of the fluorescent signal when LPS is added [20]. Such approach can be used in future to create a turn off /turn on sensor with a higher endotoxin detection sensitivity.

Conclusion

This work first detected and characterized the fluorescence emission enhancement effect (AIEE effect) that occurs when GSH-AuNCs type hydrophilic nanoclusters interact with *P. putida* TSh-18 LPS. It was demonstrated that, among a wide range of bioorganic compounds, only LPS selectively enhanced the GSH-AuNCs emission. This effect is probably induced by a decrease in the intramolecular GSH-AuNCs mobility upon binding to supramolecular particles in the LPS preparation. It is shown that the GSH-AuNCs fluorescence intensity increased as the LPS concentration increased. Dependence of the increase in the nanocluster fluorescence intensity on the LPS concentration was linear in concentrations ranging from 1 to 100 $\mu\text{g/mL}$. Established LPS detection limit was 0.7 $\mu\text{g/mL}$. Acidity of the medium affected the AIEE effect severity during interaction between GSH-AuNCs and LPS.

The findings can be used for development of a sensor system for LPS detection in pharmaceutical products.

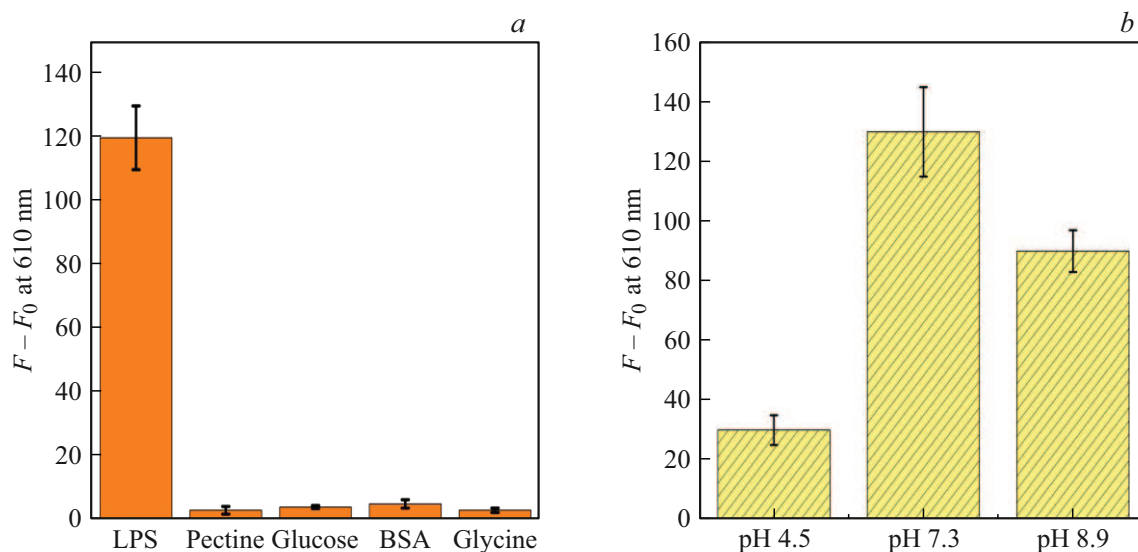


Figure 4. Influence of various substances on the GSH-AuNCs fluorescence emission enhancement. LPS and interfering agent concentration — 50 $\mu\text{g/mL}$ (a). Dependence of the GSH-AuNCs fluorescence increment on pH of the medium exposed to 50 $\mu\text{g/mL}$ of *P. putida* TSh-18 LPS (b).

Further research shall be focused on increasing the technical approach sensitivity.

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Conflict of interest

The authors declare no conflict of interest.

References

- [1] F. Di Lorenzo, K.A. Duda, R. Lanzetta, A. Silipo, C. De Castro, A. Molinaro. *Chem. Rev.*, **122**, 20 (2021). DOI: 10.1021/acs.chemrev.0c01321
- [2] D.M. Foster, J.A. Kellum. *Int. J. Mol. Sci.*, **24**, 22 (2023). DOI: 10.3390/ijms242216185
- [3] K. Ito, K.Y. Inoue, K. Ino, T. Matsue, H. Shiku. *Analyst*, **144**, 11 (2019). DOI: 10.1039/C9AN00478E
- [4] J. Gao, Z. Li, O. Zhang, C. Wu, Y. Zhao. *Analyst*, **142**, 7 (2017). DOI: 10.1039/c7an00019g
- [5] L.X. Wen, J.J. Lv, L. Chen, S.B. Li, X.J. Mou, Y. Xu. *Mikrochim. Acta*, **186**, 122 (2019). DOI: 10.1007/s00604-018-3218-3
- [6] Z. Zhang, J. Yang, W. Pang, G. Yan. *RSC Adv.*, **7**, 86 (2017). DOI: 10.1039/C7RA10710B
- [7] N.K. Radhika, S.S. Gorthi. *Anal. Methods*, **13**, 2 (2021). DOI: 10.1039/D0AY01906B
- [8] S.K. Lim, P. Chen, F.L. Lee, S. Mochhala, B. Liedberg. *Anal. Chem.*, **87**, 18 (2015). DOI: 10.1021/acs.analchem.5b02270
- [9] X. He, L.H. Xiong, Z. Zhao, Z. Wang, L. Luo, J.W.Y. Lam, R.T.K. Kwok, B.Z. Tang. *Theranostics*, **9**, 11 (2019). DOI: 10.7150/thno.31844
- [10] H. Kimoto, Y. Suzuki, Y. Ebisawa, M. Iiyama, T. Hashimoto, T. Hayashita. *ACS Omega*, **7**, 29 (2022). DOI: 10.1021/acsomega.2c02935
- [11] L. Huang, H. Tao, S. Zhao, K. Yang, Q.Y. Cao, M. Lan. *Ind. Eng. Chem. Res.*, **59**, 17 (2020). DOI: 10.1021/acs.iecr.0c01408
- [12] A. Cirri, H. Morales Hernández, C. Kmíotek, C.J. Johnson. *Angew. Chem. Int. Ed. Engl.*, **58**, 39 (2019). DOI: 10.1002/anie.201907586
- [13] T.S. Sych, A.M. Polyanichko, A.A. Buglak, A.I. Kononov. *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **298** (2023). DOI: 10.1016/j.saa.2023.122796
- [14] S.S. Evstigneeva, D.S. Chumakov, R.S. Tumskiy, B.N. Khlebtsov, N.G. Khlebtsov. *Talanta*, **264** (2023). DOI: 10.1016/j.talanta.2023.124773
- [15] Z. Luo, X. Yuan, Y. Yu, Q. Zhang, D.T. Leong, J.Y. Lee, J. Xie. *J. Am. Chem. Soc.*, **134**, 40 (2012). DOI: 10.1021/ja306199p
- [16] G. Bertani. *J. Bacteriol.*, **62**, 3 (1951). DOI: 10.1128/jb.62.3.293-300.1951
- [17] V.A. Kulshin, A.P. Yakovlev, S.N. Avaeva, B.A. Dmitriev. *Molekulyarnaya genetika, mikrobiologiya i virusologiya*, **5** (1987) (in Russian).
- [18] T. Li, H. Zhu, Z. Wu. *Nanomaterials (Basel)*, **13**, 3 (2023). DOI: 10.3390/nano13030470
- [19] G.L. Burygin, E.N. Sigida, Yu.P. Fedonenko, B.N. Khlebtsov, S.Yu. Shchyogolev. *Biophysics*, **61**, 4 (2016). DOI: 10.1134/S0006350916040059
- [20] H.H. Deng, X.Y. Fang, K.Y. Huang, S.B. He, H.P. Peng, X.H. Xia, W. Chen. *Anal. Chim. Acta*, **1088** (2019). DOI: 10.1016/j.aca.2019.08.047

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