Nanozyme-Based AuNCs for Enzymatic Detection

Sadegh Zomorodimanesh[1-3], Seyed Hadi Razavi[1-3*] and Parivash zomorodimanesh[4]

[1]Bioprocess Engineering Laboratory (BPEL), Department of Food Science, Engineering and Technology, Faculty of Agricultural Engineering and Technology, University of Tehran, Karaj, Iran

[2]Department of Food Science, Engineering and Biotechnology, Faculty of Agricultural Engineering and Technology, Aras international campus of university of Tehran, Jolfa, Iran

[3]Agricultural and Natural Resources Engineering Organization of Iran, Tehran, Iran

[4]Department of Chemistry, Central Tehran Branch, Islamic Azad University, Tehran, Iran

* Corresponding Authors: Professor Seyed Hadi Razavi

E-mail: srazavi@ut.ac.ir

ABSTRACT

Gold nanoclusters (AuNCs) have emerged as multifunctional nanozymes with exceptional potential in biosensing and food safety applications. Their ultra-small size, quantum confinement effects, and strong photoluminescence enable them to serve as both catalytic agents and optical reporters. This review focuses on the synthesis of AuNCs within enzymatic frameworks, particularly the TetX2@AuNCs system, which demonstrates dual-mode detection of tetracycline via enzymatic degradation and fluorescence signaling. The integration of AuNCs into protein scaffolds enhances biocompatibility, stability, and substrate specificity. Multi-modal detection strategiesincluding luminometric, colorimetric, and spectrofluorimetric methods offer rapid, sensitive, and equipment-free monitoring. Compared to conventional enzymes, AuNC-based nanozymes exhibit superior thermal stability, broader pH tolerance, and longer shelf life. Despite challenges in scalability, toxicity assessment, and regulatory compliance, advancements in green synthesis and ligand engineering continue to drive their development. AuNCs are poised to become key components in next-generation biosensors for food safety and medical diagnostics.

Keywords: Gold nanoclusters (AuNCs), Nanozymes, Tetracycline detection, Protein-templated synthesis, Multi-modal biosensing, Food safety, Enzymatic degradation, Fluorescence signaling, Ligand engineering, Green synthesis.

1. Introduction

Nanozymes are a class of nanomaterials that exhibit enzyme-like catalytic properties, offering a synthetic alternative to natural enzymes. Unlike their biological counterparts, nanozymes are more stable under harsh environmental conditions, such as extreme pH and temperature, and can be engineered to possess specific catalytic functions (Zomorodimanesh et al., 2024). This tunability makes them highly attractive for applications in biosensing, environmental monitoring, and medical diagnostics. Among various nanozyme platforms, gold nanoclusters (AuNCs) have gained significant attention due to their unique physicochemical properties. AuNCs typically consist of several gold atoms (less than 2 nm in diameter), which confer quantum confinement effects and strong photoluminescence. These features allow AuNCs to serve not only as catalytic centers but also as optical reporters in sensing systems (Khan et al., 2023). The integration of AuNCs into protein frameworks has opened new avenues for constructing hybrid nanozymes with dual functionality. Proteins such as enzymes or structural scaffolds can stabilize AuNCs and enhance their biocompatibility, while also contributing to substrate specificity and catalytic efficiency. For instance, the incorporation of AuNCs into the TetX2 monooxygenase enzyme results in a nanozyme capable of detecting tetracycline through both enzymatic degradation and fluorescence signaling (Zomorodimanesh et al., 2024). Furthermore, the protein-templated synthesis of AuNCs allows for precise control over cluster size, surface chemistry, and spatial orientation, which are critical parameters for optimizing catalytic performance. This approach also facilitates the development of biosensors that are responsive to specific analytes, such as antibiotics, toxins, or biomarkers, with high sensitivity and selectivity (Li et al., 2018). In summary, nanozyme-based AuNCs represent a promising frontier in the design of multifunctional biosensing platforms. Their ability to mimic enzymatic activity while offering optical readouts makes them ideal candidates for next-generation diagnostic tools, particularly in food safety and environmental applications. The following sections will explore their synthesis, functionalization, and practical deployment in antibiotic detection systems.

2. Synthesis of AuNCs within Enzymatic Structures (Expanded Version)

The synthesis of gold nanoclusters (AuNCs) within enzymatic frameworks represents a cutting-edge strategy for developing multifunctional nanozymes. One of the most illustrative examples is the TetX2@AuNCs system, in which AuNCs are embedded within the TetX2 monooxygenase enzyme. This hybrid structure enables simultaneous enzymatic degradation and optical detection of tetracycline, making it a powerful tool for biosensing applications (Zomorodimanesh et al., 2024). The synthesis process typically involves the in situ reduction of gold precursors (e.g., $HAuCl_4$) in the presence of the enzyme scaffold. The amino acid residues within the protein particularly cysteine, tyrosine, and histidine act as reducing and stabilizing agents, facilitating the formation of ultra-small AuNCs with strong fluorescence properties (Li et al., 2018). This method ensures that the nanoclusters are well-dispersed and embedded within the protein matrix, preserving both enzymatic activity and optical responsiveness. Protein-templated synthesis offers several advantages over conventional nanoparticle fabrication. It allows for precise control over the size and distribution of AuNCs, which directly influences their catalytic and photophysical properties. Moreover, the protein environment provides a biocompatible and protective shell that prevents aggregation and enhances stability under physiological conditions (Guo et al., 2021). Ligand engineering is another key strategy used to improve the performance of AuNC-based nanozymes. By modifying the surface chemistry of the clusters with specific ligands—such as glutathione or polyethylene glycol—researchers can tailor the solubility, charge, and targeting capabilities of the nanozyme (Khan et al., 2023). These modifications also play a crucial role in enhancing the selectivity of the system toward specific analytes, such as antibiotics or toxins. The TetX2@AuNCs nanozyme exhibits dual-mode detection capabilities. Upon interaction with tetracycline, the enzymatic activity of TetX2 leads to substrate degradation, while the AuNCs generate a measurable fluorescence or luminometric signal. This dual functionality enables rapid, sensitive, and quantitative detection without the need for external labeling or complex instrumentation (Zomorodimanesh et al., 2024).

To better understand the synthesis and stabilization strategies, the following table 1 summarizes key parameters and their roles:

Parameter	***Role in Synthesis***	***Impact on Nanozyme Performance***	***Reference***
Enzyme scaffold (TetX2)	Template for AuNC formation	Maintains enzymatic activity and structure	Zomorodimanesh et al., 2024
Amino acid residues	Reduction and stabilization of AuNCs	Controls cluster size and dispersion	Li et al., 2018
Ligand engineering	Surface modification of AuNCs	Enhances solubility and targeting	Guo et al., 2021
In situ synthesis method	Gold precursor reduction within protein matrix	Enables hybrid functionality	Khan et al., 2023

So, the synthesis of AuNCs within enzymatic structures such as TetX2 represents a promising approach for developing smart biosensors. The combination of enzymatic specificity and optical signaling offers a versatile platform for detecting a wide range of analytes with high sensitivity and selectivity. Future research should focus on optimizing synthesis protocols, exploring alternative enzyme templates, and scaling up production for industrial applications.

3. Application in Antibiotic Detection

The application of gold nanocluster-based nanozymes in antibiotic detection has garnered significant attention due to their high sensitivity, rapid response, and biocompatibility. Among these, the TetX2@AuNCs system stands out as a pioneering example. This nanozyme is synthesized by embedding gold nanoclusters within the TetX2 monooxygenase enzyme, enabling a multifunctional platform for detecting tetracycline residues in food products (Zomorodimanesh, Razavi et al., 2024). The TetX2@AuNCs nanozyme operates through a dual-mode mechanism. Upon exposure to tetracycline, the enzymatic activity of TetX2 facilitates the degradation of the antibiotic, while the embedded AuNCs produce distinct optical signals. These signals can be measured via luminometric, colorimetric, and spectrofluorimetric methods, offering flexible and accurate detection across various time intervals 1, 15, and 30 minutes respectively (Zomorodimanesh, Razavi et al., 2024). This multi-modal detection strategy eliminates the need for complex instrumentation and allows for real-time monitoring of antibiotic contamination in food matrices. The fluorescence response is particularly advantageous due to its high sensitivity and visual clarity, while the colorimetric change provides a rapid and user-friendly readout. Luminometry, on the other hand, enables quantitative analysis with minimal sample preparation (Duan, Yao et al., 2022).

Table 2. Multi-Modal Detection Strategies of TetX2@AuNCs Nanozyme for Tetracycline Monitoring.

Detection Method	***Mechanism***	***Response Time***	***Advantages***	***Reference***
Luminometric	Enzyme-catalyzed light emission	~1 min	Quantitative, rapid	Zomorodimanesh, Razavi et al., 2024
Colorimetric	Visual color change due to nanozyme interaction	~15 min	Simple, no equipment needed	Zomorodimanesh, Razavi et al., 2024
Spectrofluorimetric	Fluorescence quenching/enhancement upon binding	~30 min	High sensitivity, visual confirmation	Zomorodimanesh, Razavi et al., 2024

The biosensor's performance is further enhanced by the intrinsic properties of AuNCs, such as long fluorescence lifetime, large Stokes shift, and excellent photostability. These features ensure consistent signal output even under varying environmental conditions, making the system suitable for deployment in smart packaging and on-site testing (Li, Chen et al., 2018). Moreover, the protein-templated synthesis of TetX2@AuNCs contributes to its biocompatibility and environmental safety. The use of natural enzymes and food-grade proteins as scaffolds aligns with sustainable manufacturing practices and regulatory standards for food-contact materials (Guo, Amunyela et al., 2021). In summary, the TetX2@AuNCs nanozyme exemplifies the potential of gold nanocluster-based biosensors in antibiotic detection. Its multi-modal sensing capabilities, rapid response, and compatibility with food systems position it as a promising tool for enhancing food safety and public health monitoring.

Advantages Over Conventional Methods

Nanozyme-based gold nanoclusters (AuNCs) offer a transformative alternative to conventional biosensors and natural enzymes, particularly in food safety and biomedical applications. Their advantages stem from their unique physicochemical properties, synthesis flexibility, and operational robustness:

1. Thermal and Chemical Stability

Unlike natural enzymes that denature under heat or chemical stress, AuNCs maintain their structural integrity and catalytic activity at elevated temperatures and across a wide pH range. This makes them ideal for processed food environments and high-temperature packaging systems (Li et al., 2018; Lin et al., 2020).

2. Biocompatibility and Low Cytotoxicity

AuNCs stabilized by natural proteins (e.g., BSA, gelatin) demonstrate excellent biocompatibility, allowing safe interaction with biological systems. Their low cytotoxicity enables direct application in food-contact materials and biosensors (Guo et al., 2021; Bai et al., 2020).

3. No Need for Refrigeration or Strict pH Control

Traditional enzyme-based sensors often require cold-chain storage and tightly regulated pH conditions. In contrast, nanozymes based on AuNCs are shelf-stable and functional across diverse environmental conditions, reducing logistical complexity (Khan et al., 2023; Kawasaki et al., 2010)

4. Multi-Modal Detection Capabilities

AuNCs can be engineered for luminometric, colorimetric, and spectrofluorimetric detection, enabling rapid and sensitive monitoring of contaminants like tetracycline at different time intervals (Zomorodimanesh et al., 2024).

5. Eco-Friendly and Scalable Synthesis

Green synthesis using plant extracts or protein templates allows for sustainable production of AuNCs, minimizing environmental impact while maintaining high functionality (Sayadi et al., 2021; Mohamad et al., 2014).

Table 3. Comparative Analysis of Nanozyme-Based AuNCs and Conventional Enzyme Systems in Food Safety Applications.

Feature	*Nanozyme-Based AuNCs*	*Conventional Enzymes*
Thermal Stability	High (up to 150°C) *(Li et al., 2018)*	Low (heat-sensitive) *(Li et al., 2018)*
pH Tolerance	Broad range (pH 4–10) *(Khan et al., 2023)*	Narrow range (typically pH 6–8) *(Khan et al., 2023)*
Biocompatibility	Excellent *(Guo et al., 2021)*	Moderate to high *(Guo et al., 2021)*
Cold Storage Requirement	Not required *(Khan et al., 2023)*	Required *(Khan et al., 2023)*
Detection Modalities	Multi-modal (fluorescence, colorimetry, luminometry) *(Zomorodimanesh et al., 2024)*	Typically single-mode
Shelf Life	Long-term stability *(Li et al., 2018)*	Short-term stability
Production Cost	Lower (especially with green synthesis) *(Sayadi et al., 2021)*	Higher (due to extraction/purification) *(Sayadi et al., 2021)*
Detection Sensitivity	High (down to ng/mL levels) *(Zomorodimanesh et al., 2024)*	Moderate
Surface Modifiability	High (ligand/protein functionalization possible) *(Guo et al., 2021)*	Limited

Key Challenges in the Development and Application of Gold Nanoclusters (AuNCs)

Despite their promising potential in biomedical and food safety applications, gold nanoclusters (AuNCs) face several critical challenges that hinder their widespread adoption and industrial scalability:

1. Synthesis and Production Limitations

- Large-scale production of AuNCs with uniform physicochemical properties remains difficult.
- Variability in cluster size complicates purification and reproducibility.
- Stability and functionality are highly dependent on the choice of ligands and synthesis conditions.

Colloidal and Structural Stability

- AuNCs stabilized by small ligands often exhibit poor colloidal stability in biological environments.
- Encapsulation using polymers, silica, or metal-organic frameworks is often required to enhance durability and prevent aggregation.

3. Biocompatibility and Toxicity Concerns

- Limited understanding of the long-term biological behavior of AuNCs in vivo.
- Comprehensive toxicological assessments are lacking, especially for food-contact applications.
- Absence of standardized regulatory frameworks for human exposure to nanomaterials.

Challenges in Green Synthesis

- Plant-based synthesis methods suffer from variability due to differences in phytochemical composition.
- Lack of standardized protocols affects reproducibility and scalability of eco-friendly approaches.

5. Environmental and Recycling Issues

- Concerns over the environmental impact of nanowaste generated during production and application.
- Need for efficient recycling strategies to recover and reuse gold nanomaterials.

6. Characterization and Property Mapping

- Difficulty in accurately determining local atomic structure and correlating it with functional properties.
- Advanced analytical techniques are required to evaluate quantum yield, photoluminescence, and catalytic behavior.

Table 4. Challenges and Proposed Solutions for Gold Nanoclusters (AuNCs).

Challenge	***Description***	***Proposed Solution***	***Reference***
Industrial-scale production	Difficulty in producing uniform and controlled AuNCs at large scale	Use of encapsulation via MOFs or polymers to enhance stability	Cifuentes-Rius et al., 2021
Colloidal instability	Poor stability in biological environments with small ligands	Encapsulation using silica or biocompatible polymers	Lu & Chen, 2014
Structural characterization	Complexity in determining atomic structure and quantum yield	Development of advanced analytical techniques	Chevrier et al., 2012
Size-controlled synthesis	Variability in cluster size during synthesis	Optimization of purification and separation protocols	Kukreti & Kaushik, 2021
Limitations in green synthesis	Inconsistency due to phytochemical diversity in plant extracts	Establishment of standardized green synthesis protocols	Sayadi et al., 2021
Environmental impact and recycling	Concerns over nanowaste and lack of recycling strategies	Development of efficient recovery and reuse technologies	Pati et al., 2016
Regulatory gaps	Absence of clear legal frameworks for food-related applications	Formulation of strict regulatory guidelines for production and usage	Dhall et al., 2024
Toxicological uncertainty	Lack of long-term safety data for human exposure	Comprehensive in vitro and in vivo toxicity assessments	Yah, 2013

Challenges and Future Perspectives of Nanozyme-Based Gold Nanoclusters (AuNCs)

Nanozyme-based gold nanoclusters (AuNCs) have emerged as promising candidates for applications in food safety, biosensing, and biomedicine. However, several challenges must be addressed to unlock their full potential and enable broader industrial adoption.

Key Challenges:

- **Long-Term Stability:** AuNCs often suffer from structural degradation and reduced catalytic activity under varying environmental conditions, especially in food packaging systems.
- **Reproducibility of Synthesis:** Protein-templated synthesis methods are highly sensitive to pH, temperature, and ionic strength, leading to batch-to-batch inconsistencies (Li et al., 2018).
- **Toxicological Uncertainty:** Comprehensive in vivo and in vitro studies are still lacking, making it difficult to assess the safety of AuNCs in food-contact applications (Suvarna et al., 2022).
- **Regulatory Barriers:** The absence of standardized legal frameworks for nanomaterials in food systems poses a significant hurdle to commercialization.

Future Perspectives:

- **Ligand Engineering:** Rational design of ligands can enhance the stability, biocompatibility, and catalytic efficiency of AuNCs.
- **Scalable Production:** Developing robust and reproducible synthesis protocols is essential for industrial-scale manufacturing.
- **Multi-Modal Sensing Integration:** Incorporating fluorescence, colorimetric, and luminometric detection into a single platform can expand the versatility of AuNC-based sensors (Zomorodimanesh et al., 2024).
- **Regulatory Harmonization:** Establishing clear safety guidelines and approval pathways will facilitate the integration of AuNCs into commercial food safety technologies.

Table ۵. Future Challenges and Strategic Solutions in the Development of AuNC-Based Nanozymes.

Challenge	*Description*	*Strategic Solution*	*Reference*
Long-term stability	Structural degradation under environmental stress	Rational ligand design to enhance durability	Li et al., 2018
Reproducibility of synthesis	Batch-to-batch inconsistency due to environmental sensitivity	Standardization of protein-templated synthesis protocols	Li et al., 2018
Toxicological uncertainty	Lack of comprehensive safety data for food-contact applications	In-depth in vitro and in vivo toxicity studies	Suvarna et al., 2022

Regulatory limitations	Absence of clear legal frameworks for nanozyme use in food systems	Development of international safety and approval guidelines	Suvarna et al., 2022
Limited sensing versatility	Reliance on single-mode detection systems	Integration of multi-modal sensing platforms (fluorescence, colorimetry)	Zomorodimanesh et al., 2024
Scalability of production	Difficulty in industrial-scale manufacturing with consistent quality	Optimization of synthesis for scalable and reproducible output	Zomorodimanesh et al., 2024

Conclusion

Nanozyme-based gold nanoclusters (AuNCs) represent a transformative advancement in biosensing technologies, particularly for antibiotic detection in food safety applications. Their unique combination of quantum confinement effects, photoluminescence, and enzyme-mimicking catalytic activity enables multi-modal detection platforms that are both sensitive and robust. The integration of AuNCs within enzymatic scaffolds—such as TetX2—offers dual functionality, allowing for simultaneous substrate degradation and optical signal generation. This hybrid approach not only enhances detection accuracy but also aligns with sustainable and biocompatible design principles. Despite current challenges in synthesis reproducibility, long-term stability, and regulatory approval, ongoing innovations in ligand engineering and green synthesis methods continue to expand the practical utility of AuNCs. As research progresses, these nanozymes are poised to redefine standards in diagnostic technologies, offering scalable, eco-friendly, and highly adaptable solutions for real-time monitoring in both biomedical and food systems.

References

[1] Zomorodimanesh, S., S. H. Razavi, M. R. Ganjali and S. Hosseinkhani (2024). "Development of an assay for tetracycline detection based on gold nanocluster synthesis on tetracycline monooxygenase: TetX2@ AuNCs." *International Journal of Biological Macromolecules* 283: 137777.

[2] Khan, I. M., S. Niazi, W. Akhtar, L. Yue, I. Pasha, M. K. I. Khan, A. Mohsin, M. W. Iqbal, Y. Zhang and Z. Wang (2023). "Surface functionalized AuNCs optical biosensor as an emerging food safety indicator: Fundamental mechanism to future prospects." *Coordination Chemistry Reviews* 474: 214842.

[3] Li, C., H. Chen, B. Chen and G. Zhao (2018). "Highly fluorescent gold nanoclusters stabilized by food proteins: From preparation to application in detection of food contaminants and bioactive nutrients." *Critical Reviews in Food Science and Nutrition* 58(5): 689–699.

[4] Guo, Y., H. T. Amunyela, Y. Cheng, Y. Xie, H. Yu, W. Yao, H.-W. Li and H. Qian (2021). "Natural protein-templated fluorescent gold nanoclusters: Syntheses and applications." *Food Chemistry* 335: 127657.

[5] Zomorodimanesh, S., S. H. Razavi, S. Hosseinkhani and Z. Emam-Djomeh (2024). "Biosynthesis of gold cluster nanozyme within the structure of TetX2 monooxygenase protein." *Food Bioscience* 62: 105086.

[6] Duan, N., T. Yao, C. Li, Z. Wang and S. Wu (2022). "Surface-enhanced Raman spectroscopy relying on bimetallic Au–Ag nanourchins for the detection of the food allergen β-lactoglobulin." *Talanta* 245: 123445.

[7] Lin, H.-W., J.-Y. Wang, V. K. Hsiao and C.-C. Chu (2020). "“Green” Synthesis and Antioxidant Activity of Thermally Stable Gold Nanoparticles Encapsulated in Carbon Nanosheets." *Applied Sciences* 10(7): 2272.

[8] Bai, Y., T. Shu, L. Su and X. Zhang (2020). "Fluorescent gold nanoclusters for biosensor and bioimaging application." *Crystals* 10(5): 357.

[9] Kawasaki, H., H. Yamamoto, H. Fujimori, R. Arakawa, Y. Iwasaki and M. Inada (2010). "Stability of the DMF-protected Au nanoclusters: photochemical, dispersion, and thermal properties." *Langmuir* 26(8): 5926–5933.

[10] Sayadi, K., F. Akbarzadeh, V. Pourmardan, M. Saravani-Aval, J. Sayadi, N. P. S. Chauhan and G. Sargazi (2021). "Methods of green synthesis of Au NCs with emphasis on their morphology: A mini-review." *Heliyon* 7(6).

[11] Mohamad, N. A. N., N. A. Arham, J. Jai and A. Hadi (2014). "Plant extract as reducing agent in synthesis of metallic nanoparticles: a review." *Advanced Materials Research* 832: 350–355.

[12] Cifuentes-Rius, A., V. G. Deepagan, J. Xie and N. H. Voelcker (2021). "Bright future of gold nanoclusters in theranostics." *ACS Applied Materials & Interfaces* 13(42): 49581–49588.

[13] Lu, Y. and W. Chen (2014). "Progress in the synthesis and characterization of gold nanoclusters." *Gold Clusters, Colloids and Nanoparticles I*: 117–153.

[14] Chevrier, D. M., A. Chatt and P. Zhang (2012). "Properties and applications of protein-stabilized fluorescent gold nanoclusters: short review." *Journal of Nanophotonics* 6(1): 064504–064504.

[15] Kukreti, S. and M. Kaushik (2021). "Gold nanoclusters: An ultrasmall platform for multifaceted applications." *Talanta* 234: 122623.

[16] Pati, P., S. McGinnis and P. J. Vikesland (2016). "Waste not want not: life cycle implications of gold recovery and recycling from nanowaste." *Environmental Science: Nano* 3(5): 1133–1143.

[17] Dhall, S., A. Nigam, M. Harshavardhan, A. Mukherjee and P. Srivastava (2024). "A comprehensive overview of methods involved in nanomaterial production and waste disposal from research labs and industries and existing regulatory guidelines for handling engineered nanomaterials." *Environmental Chemistry and Ecotoxicology*.

[18] Yah, C. S. (2013). "The toxicity of Gold Nanoparticles in relation to their physiochemical properties." *Biomedical Research (0970-938X)* 24(3).

[19] Suvarna, V., A. Nair, R. Mallya, T. Khan and A. Omri (2022). "Antimicrobial nanomaterials for food packaging." *Antibiotics* 11(6): 729.