Thesis Title: Bioinspired Bionanozymes: Design, Synthesis, and Innovations in Biocatalysis

Enzymes are intricate biological catalysts essential to numerous physiological processes and hold vast potential for technological applications. However, their broader industrial and in vitro implementation is often hindered by challenges such as high production costs, limited operational stability, and difficulties in recovery and reuse. This thesis aims to overcome these limitations by exploring the bioinspired design and synthesis of minimalistic biomolecular nanomaterials—termed *bionanozymes*—that emulate enzymatic catalytic functions. These engineered materials present promising alternatives to natural enzymes, with prospective applications in fields such as environmental remediation, biosensing, and biomedicine.

Chapter 1: Introduction

This chapter introduces enzymes (oxidoreductases), outlining their biological significance, functional roles, and practical applications while addressing their inherent limitations in industrial settings. It reviews emerging strategies for enzyme mimicry, particularly those based on peptides and nucleic acids, discussing both their advantages and constraints. In light of these challenges, the thesis proposes *bionanozymes*—nanoscale biomolecular assemblies with enzyme-like catalytic activity—as a novel and practical approach to enhancing the applicability of biocatalysis across various domains.

Chapter 2: A Peroxidase-Mimicking Single Amino Acid Bionanozyme for Ultrasensitive Biomarker Detection: A WHO-REASSURE-Aligned Approach

Developing reliable, low-cost biosensing technologies aligned with WHO-REASSURE criteria is critical for deployment in resource-limited settings. Conventional peroxidases, central to colorimetric detection assays, often suffer from high production costs, low stability, and limited reusability. To address these issues, we engineered a cost-effective single amino acid-based bionanozyme (F-Cu) by integrating phenylalanine (F) with redox-active Cu^{2+} ions to form two-dimensional hierarchical van der Waals crystals. The resulting F-Cu nanosheets exhibit strong peroxidase-mimicking activity, maintaining stability under extreme pH, high temperatures, and prolonged storage. The F-Cu bionanozyme enables sensitive, equipment-free, and visually interpretable detection of key biomarkers—hydrogen peroxide, dopamine, and nitrite ions—with detection limits of 5 μM, 1.5 μM, and 4 μM, respectively. These properties demonstrate its alignment with WHO-REASSURE standards and highlight its potential in point-of-care diagnostics, biotechnology, food safety, and analytical chemistry.

Chapter 3: Zinc-Tryptophan Nanoassemblies for Antibacterial and Wound Healing Applications

Wound healing is a complex biological process that can be significantly impeded by infection, oxidative stress, and inflammation, particularly in chronic wounds. Zinc (Zn) is well known for its antibacterial, antioxidant, and anti-inflammatory effects, while tryptophan (W) plays a vital role in protein synthesis and tissue repair. In this study, we developed zinc-tryptophan (Zn-W) nanosheet assemblies exhibiting enhanced multifunctional properties, including antioxidant and antibacterial activities, antibiofilm formation, and wound-healing potential. These attributes underscore the Zn-W nanoassemblies' potential in biomedical applications, particularly in promoting effective and accelerated wound healing.

Chapter 4: A Multienzyme-Mimicking Single Nucleobase Bionanozyme for Environmental Remediation

This chapter introduces a novel adenine-copper (A-Cu) bionanozyme, synthesized using a green, solvent-free co-grinding method followed by aqueous evaporation. Featuring a two-dimensional crystalline structure, the A-Cu bionanozyme uniquely mimics laccase (LAC) and catechol oxidase (CO) activities, offering dual catalytic functionality. Its LAC-like properties are applied to the colorimetric detection and degradation of phenolic pollutants, addressing pressing issues in water pollution. Meanwhile, its CO-like activity facilitates the ultrasensitive detection of dopamine. The A-Cu bionanozyme retains over 95% of its activity after 17 catalytic cycles and maintains 99% activity after 30 days of storage, significantly outperforming natural enzymes. These attributes position A-Cu as a durable and effective tool for environmental remediation and biomolecular engineering.

Chapter 5: A Peroxidase-Mimicking Single Nucleobase Bionanozyme for Biomedical Applications

In response to the demand for robust and sustainable solutions in biomedical sensing and therapy, this chapter presents a peroxidase-mimicking A-Cu bionanozyme composed of adenine and copper ions. Synthesized via environmentally friendly techniques such as solvent-free grinding and natural evaporation, this two-dimensional nanomaterial demonstrates strong peroxidase-like catalytic activity. It enables the ultrasensitive colorimetric detection of biomarkers like hydrogen peroxide and glutathione. Furthermore, the A-Cu bionanozyme exhibits high biocompatibility and antioxidant potential through scavenging reactive oxygen species (ROS), indicating its suitability for applications in regenerative medicine and tissue engineering.

Chapter 6: Conclusion and Outlook

This thesis presents a comprehensive exploration of bioinspired bionanozymes—minimalistic, biomolecular nanomaterials designed to mimic the catalytic functions of natural enzymes. Through the systematic development and characterization of various amino acid- and nucleobase-based nanoassemblies, this work addresses longstanding challenges in enzyme stability, cost, and usability, particularly in demanding industrial and biomedical contexts. Beginning with a conceptual framework for enzyme mimicry (Chapter 1), the thesis introduces bionanozymes as a practical and scalable alternative to natural oxidoreductases. This foundational premise is experimentally validated across four distinct applications. In Chapter 2, a phenylalanine-copper (F-Cu) bionanozyme demonstrates robust peroxidase-like activity, meeting WHO-REASSURE criteria for sensitive, low-cost, and equipment-free biomarker detection. Chapter 3 expands this concept with the adenine-copper (A-Cu) bionanozyme, which uniquely mimics dual oxidase activities and offers promising solutions for environmental remediation, particularly in detecting and degrading phenolic pollutants. In Chapter 4, the same A-Cu platform is adapted for biomedical use, showing excellent peroxidase activity, biocompatibility, and ROS scavenging, which are critical for biosensing and tissue regeneration applications. Finally, Chapter 5 introduces zinc-tryptophan (Zn-W) nanoassemblies that combine antimicrobial, antioxidant, and pro-healing properties, advancing the scope of nanomaterials in wound care and regenerative medicine.

These studies highlight the versatility, tunability, and functional potential of single-component bionanozymes. By employing simple, environmentally friendly synthesis techniques—such as solvent-free co-grinding and aqueous evaporation—this research emphasizes not only performance but also sustainability and scalability.

Outlook: The findings in this thesis open new avenues for the rational design of biomolecular nanozymes with targeted functionality. Future work can focus on expanding the molecular palette beyond amino acids and nucleobases to include hybrid assemblies, responsive materials, or multifunctional ligands tailored for specific applications. Integration with flexible electronics, microfluidics, or implantable devices could further enhance the utility of bionanozymes in real-world diagnostics, environmental monitoring, and therapeutic delivery. Moreover, developing machine learning-guided screening and computational modeling tools may accelerate the discovery of next-generation bionanozymes with optimized catalytic performance and substrate specificity. Establishing structure–function relationships at the atomic scale remains a key research priority to unlock the full potential of these biomaterials.

In summary, this thesis not only contributes to the emerging field of bionanozymes but also lays a foundation for their future translation into clinically and industrially relevant platforms. These assemblies' minimalistic yet powerful nature represents a paradigm shift in the design of functional biomimetic materials.