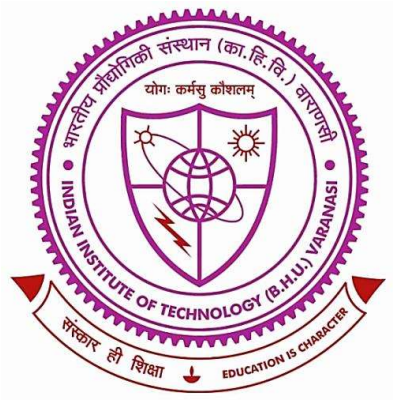
Bioinspired Bionanozymes: Design, Synthesis, and Innovations in Biocatalysis



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Doctor of Philosophy

By

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CHAPTER-6

Conclusion and Outlook

The intricate biological catalysts known as enzymes are fundamental to countless physiological processes and offer immense potential for technological innovation. However, their widespread adoption in industrial and in vitro applications remains constrained by significant hurdles, including high production costs, inherent operational instability, and practical challenges in recovery and reuse. This thesis, "Bioinspired Bionanozymes: Design, Synthesis, and Innovations in Biocatalysis," endeavours to address these limitations through the bioinspired design and synthesis of minimalistic biomolecular nanomaterials, termed bionanozymes. These engineered materials are developed to emulate the catalytic functions of natural enzymes, presenting robust and promising alternatives with broad prospective applications in critical areas such as environmental remediation, advanced biosensing, and biomedicine.

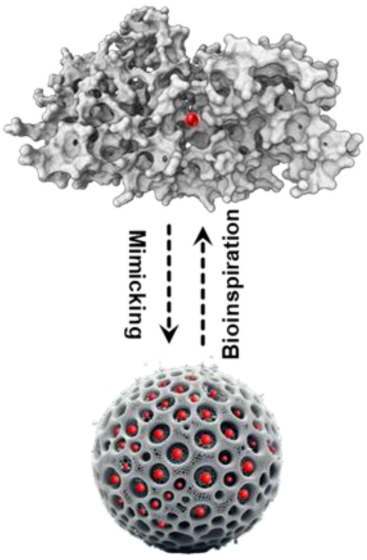
This concluding chapter summarizes the pivotal findings derived from the systematic investigation into the design, synthesis, and characterization of various amino acid- and nucleobase-based nanoassemblies. It discusses the significant implications of these discoveries for the field of biocatalysis, outlining their contributions to existing knowledge and highlighting the versatility, tunability, and functional potential of single component bionanozymes. Furthermore, this chapter provides an outlook on future research directions, identifying avenues for continued exploration and advancement, and concludes with a definitive statement on the overall impact and transformative potential of this research.

6.1 Summary of Key Findings

This thesis systematically explored the development of bioinspired bionanozymes, demonstrating their effectiveness as scalable and sustainable alternatives to natural enzymes across diverse applications.

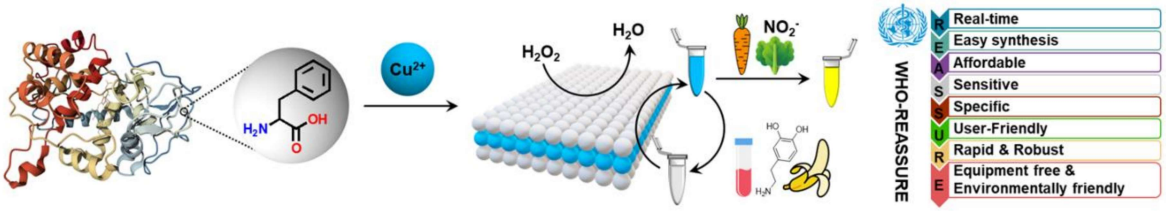
6.1.1 Chapter 1: Introduction

Chapter 1 provided a comprehensive introduction to oxidoreductase enzymes, detailing their biological significance, functional roles, and established industrial applications, while critically examining their inherent limitations, such as high production costs, poor stability, and reusability challenges. It reviewed contemporary strategies for enzyme mimicry, particularly



those centred on peptides and nucleic acids, discussing their respective advantages and constraints. In response to these persistent challenges, this chapter introduced the concept of bionanozymes—nanoscale biomolecular assemblies possessing enzyme-like catalytic activity—as a novel and practical approach to enhance the applicability of biocatalysis across various domains. This foundational chapter set the stage for the experimental investigations that followed, proposing bionanozymes as a viable and promising solution to overcome the long-standing hurdles associated with natural enzymes.

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

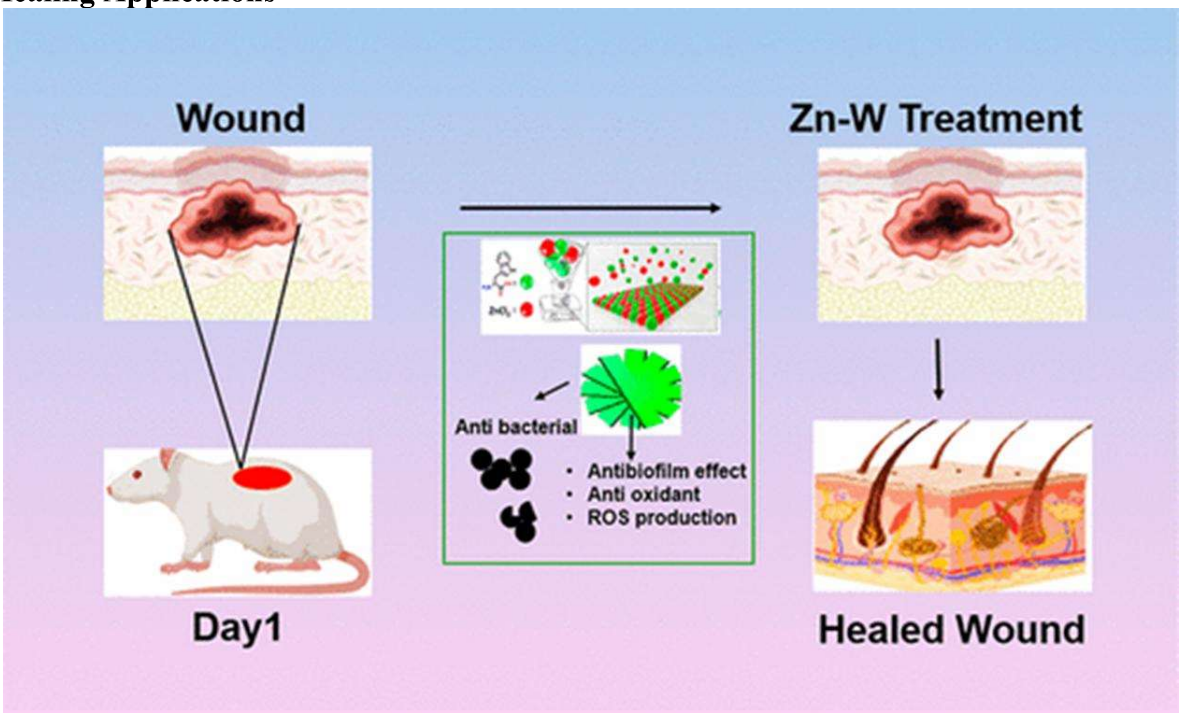


Chapter 2 focused on engineering an affordable, easily synthesized, and environmentally friendly single amino acid bionanozyme (F-Cu) designed for ultrasensitive biomarker detection, aligning with WHO-REASSURE standards crucial for resource-constrained environments. Conventional peroxidase (POD) enzymes, essential for sensitive colorimetric assays, face challenges including high production costs, operational instability, and limited reusability. To overcome these, the F-Cu bionanozyme was synthesized by integrating phenylalanine (F) with redox-active Cu^{2+} ions, forming robust 2D hierarchical van der Waals crystals.

The F-Cu nanosheets exhibited rapid and real-time POD-mimicking catalytic activity, efficiently reducing hydrogen peroxide (H_2O_2). A key finding was their remarkable robustness, maintaining stability under extreme pH conditions, prolonged storage, and elevated temperatures. Furthermore, this bionanozyme facilitated user-friendly, sensitive, and specific equipment-free visual detection of crucial biomarkers, including hydrogen peroxide (H_2O_2), dopamine (DA), and nitrite ions (NO_2^-). The achieved detection limits were impressively low: 5 µM for H_2O_2, 1.5 µM for DA, and 4 µM for NO_2^-. These results emphatically underscore the

F-Cu bionanozyme's alignment with WHO-REASSURE criteria, highlighting its significant potential in point-of-care diagnostics, biotechnology, food safety, and analytical chemistry applications, particularly for addressing health risks associated with H_2O_2, neurological functions linked to DA, and food safety concerns with NO_2^-.

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



Chapter 3 addressed the complex process of wound healing, which is frequently compromised by infection, oxidative stress, and inflammation, especially in chronic wounds. Leveraging the known therapeutic potential of zinc (Zn) for its antibacterial, antioxidant, and anti-inflammatory properties, and the vital role of tryptophan (W) in protein synthesis and tissue repair, this study successfully developed zinc-tryptophan (Zn-W) nanosheet assemblies.

The synthesized Zn-W nanoassemblies exhibited enhanced multifunctional properties, including potent antioxidant, antibacterial, and antibiofilm activities, along with significant wound-healing potential. In vitro assays confirmed their efficacy against both Gram-negative (Escherichia coli) and Gram-positive (Bacillus subtilis) bacteria, alongside robust free radical scavenging abilities. Critically, in vivo wound models demonstrated that Zn-W treatment markedly accelerated tissue regeneration, showcasing their promise as therapeutics for managing infected and chronic wounds. This work successfully combined the benefits of Zn and W while overcoming their individual limitations such as poor bioavailability and

instability. The biocompatibility, cost-effectiveness, and scalability of these Zn-W nanoassemblies further underscore their potential for broad accessibility in healthcare applications. The study also highlighted the critical issue of antimicrobial resistance (AMR) in wound infections, positioning Zn-W as a promising strategy to mitigate this growing threat.

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

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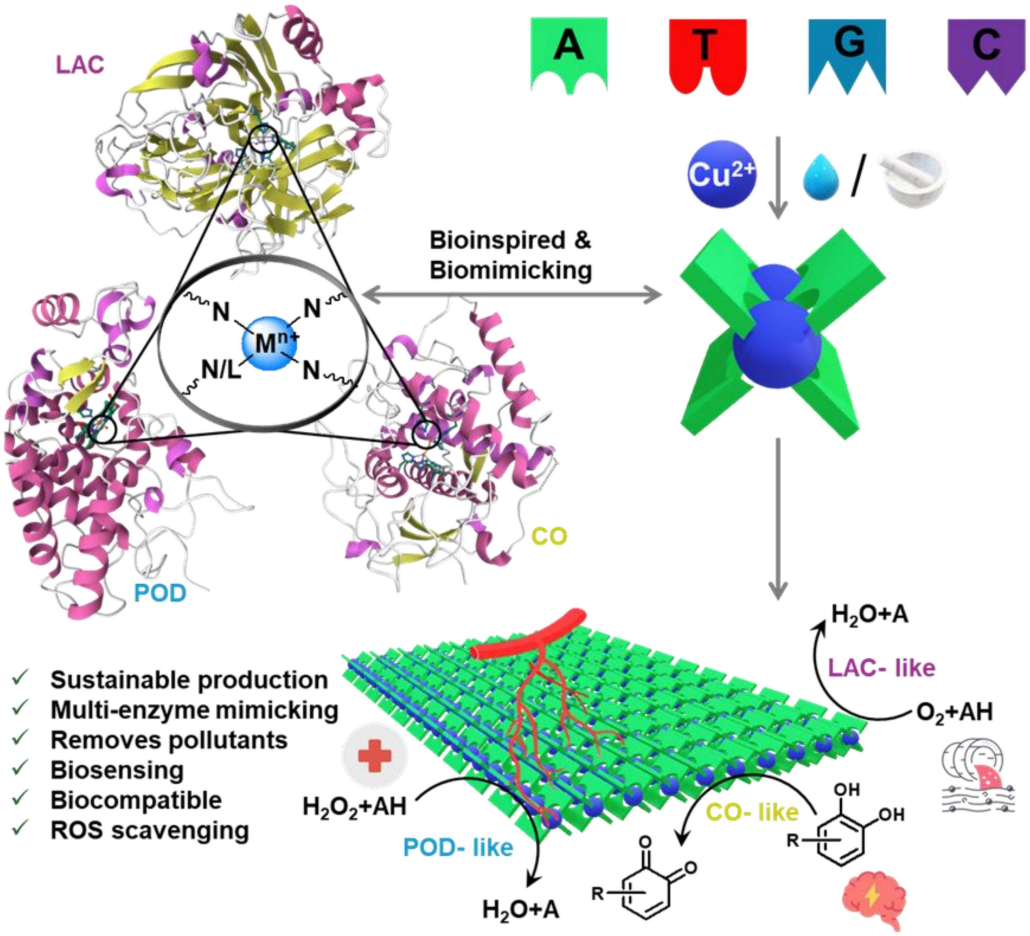
6.2.5 Chapter 5: A Peroxidase-Mimicking Single Nucleobase Bionanozyme for Biomedical Applications



Chapters 4 and 5 collectively introduced a novel adenine-copper (A-Cu) bionanozyme, demonstrating its remarkable versatility in both environmental remediation and biomedical applications. This A-Cu bionanozyme, synthesized using simple, scalable, and green methods, including solvent-free co-grinding and natural aqueous evaporation processes, forms a 2D crystalline structure. A significant breakthrough highlighted in this thesis is the A-Cu bionanozyme's unique ability to exhibit triple-enzyme-mimicking catalytic activity, emulating Laccase (LAC), Catechol oxidase (CO), and Peroxidase (POD).

In the context of environmental remediation (Chapter 4), the A-Cu bionanozyme's LAC-like and CO-like activities were successfully applied. Its LAC-like properties enabled the colorimetric detection and degradation of phenolic pollutants in contaminated water, addressing pressing environmental concerns. The CO-like activity facilitated the ultrasensitive detection of dopamine. Notably, the A-Cu bionanozyme demonstrated exceptional operational stability and reusability, retaining over 95% of its activity after 17 catalytic cycles and maintaining 99% activity after 30 days of storage, significantly outperforming many natural enzymes in durability. These attributes position A-Cu as a durable and effective tool for environmental remediation and biomolecular engineering.

For biomedical applications (Chapter 5), the A-Cu bionanozyme was adapted to leverage its potent peroxidase-like catalytic activity. It enabled the ultrasensitive colorimetric detection of vital biomarkers such as hydrogen peroxide and glutathione. Beyond its sensing capabilities, the A-Cu bionanozyme demonstrated high biocompatibility and significant antioxidant potential through efficient scavenging of reactive oxygen species (ROS). Live cell assays confirmed that A-Cu effectively reduced elevated ROS levels induced by hydrogen peroxide, leading to a notable increase in cell viability and mitigating oxidative damage. These properties underscore its suitability for applications in regenerative medicine and tissue engineering, providing robust and sustainable solutions for biomedical sensing and therapy.

6.2 Implications and Contributions

The findings presented in this thesis hold profound implications for the fields of biocatalysis, materials science, and biomedical engineering, marking significant contributions to existing knowledge. The work fundamentally challenges the reliance on natural enzymes by demonstrating the viability and superior performance of minimalistic, bioinspired bionanozymes.

Firstly, this research directly addresses the longstanding challenges associated with natural enzymes, namely their high production costs, inherent operational instability, and difficulties in recovery and reuse. By designing and synthesizing bionanozymes from abundant and cost-effective biomolecules like amino acids and nucleobases, combined with redox-active metal ions, this thesis offers affordable, stable, and reusable alternatives. The demonstrated robustness of F-Cu under extreme pH and temperature, and the exceptional reusability of A-Cu for numerous cycles, represent significant advancements over their natural counterparts.

Secondly, the thesis showcases the remarkable versatility and tunability of these single component bionanozymes. The A-Cu bionanozyme's ability to exhibit triple-enzyme-mimicking activity (Laccase, Catechol oxidase, and Peroxidase) from a single nucleobase-derived material is a groundbreaking finding. This multifunctionality opens unprecedented avenues for designing highly adaptable catalytic systems capable of performing diverse biochemical transformations. The successful application of the same A-Cu platform for both environmental remediation (phenolic degradation) and biomedical sensing (H_2O_2, glutathione) exemplifies this versatility.

Thirdly, the research emphasizes the importance of sustainable and scalable synthesis techniques. The consistent use of simple, environmentally friendly methods such as solvent-free co-grinding and natural aqueous evaporation across different bionanozyme systems highlights a paradigm shift towards green chemistry in the development of functional biomimetic materials. This approach not only ensures performance but also promotes ecological responsibility and facilitates the large-scale production necessary for industrial translation.

Finally, the alignment of the F-Cu bionanozyme with WHO-REASSURE standards for biomarker detection is a critical contribution, particularly for global health initiatives in resource-constrained settings. The development of equipment-free, sensitive, and user-friendly diagnostic tools has direct societal impact, promising to improve point-of-care diagnostics for diseases and enhance food safety monitoring worldwide. Similarly, the development of Zn-W nanoassemblies with comprehensive antibacterial, antioxidant, and wound-healing properties offers a promising new class of therapeutics for infected and chronic wounds, directly addressing an urgent clinical need exacerbated by rising antibiotic resistance.

In essence, this thesis contributes a foundational framework for the rational design and synthesis of next-generation biomolecular nanomaterials that not only mimic, but often surpass, the performance of natural enzymes in terms of stability, cost-effectiveness, and multi-functionality.

6.3 Future Research Directions (Outlook)

The pioneering findings detailed in this thesis pave significant new avenues for the rational design and application of biomolecular nanozymes with highly targeted functionalities. Future research efforts can substantially expand upon the foundational work presented here by exploring several key directions.

A primary area of investigation involves expanding the molecular diversity beyond the amino acids and nucleobases utilized in this thesis. This could include the incorporation of novel biomolecular building blocks, the creation of hybrid assemblies combining different types of biomolecules or synthetic polymers, or the integration of responsive materials that can modulate their catalytic activity in response to specific environmental stimuli. Furthermore, the design of multifunctional ligands tailored for highly specific applications could lead to unprecedented catalytic efficiencies and selectivity.

Another critical direction lies in the integration of these bionanozymes with advanced technological platforms. This includes their seamless incorporation into flexible electronic devices for wearable biosensors, microfluidic systems for high-throughput diagnostics and environmental monitoring, or implantable devices for continuous therapeutic delivery and *in vivo* biosensing. Such integrations could significantly enhance the real-world utility and practical applicability of bionanozymes in complex biological and environmental matrices.

The acceleration of discovery and optimization of next generation bionanozymes stands as another vital research frontier. This can be achieved through the implementation of machine learning-guided screening and computational modelling tools. These advanced computational approaches could predict optimal molecular designs, reaction pathways, and catalytic mechanisms, thereby drastically reducing the time and resources required for experimental synthesis and characterization, ultimately leading to bionanozymes with optimized catalytic performance and substrate specificity.

Furthermore, a deeper fundamental understanding of these materials is crucial. Establishing precise structure–function relationships at the atomic scale remains a key research priority. Employing advanced characterization techniques such as cryo-electron microscopy, high-resolution atomic force microscopy, and sophisticated spectroscopic methods, coupled with theoretical calculations, will be essential to elucidate how the molecular arrangement and coordination environments within these bionanozymes dictate their catalytic properties. Unlocking this fundamental knowledge will enable more precise control over their design and unlock the full potential of these biomimetic materials.

Finally, a significant focus should be placed on the translation of these bionanozyme platforms into clinically and industrially relevant applications. This involves rigorous testing in more complex biological systems, preclinical validation for therapeutic applications, and scaling up of synthesis methods to meet industrial demands. Collaborations with clinicians, engineers,

and industrial partners will be essential to bridge the gap between fundamental research and practical implementation, bringing these innovative solutions to the forefront of healthcare and environmental sustainability.

6.4 Overall Summary

In summary, this thesis represents a comprehensive and impactful contribution to the burgeoning field of bionanozymes. By meticulously designing, synthesizing, and characterizing minimalistic biomolecular nanomaterials that effectively mimic and often surpass the catalytic functions of natural enzymes, this work successfully addresses critical limitations pertaining to enzyme stability, cost, and usability. The development of multi-enzyme mimicking nucleobase-derived bionanozymes, high-performance amino acid-based biosensors adhering to WHO-REASSURE standards, and multifunctional metal-biomolecule nanoassemblies for wound healing applications collectively underscore the profound versatility and transformative potential of this research. This thesis not only advances our fundamental understanding of bioinspired catalysis but also lays a robust foundation for the future translation of these intrinsically powerful, yet minimalistic, assemblies into clinically and industrially relevant platforms, heralding a significant paradigm shift in the design of functional biomimetic materials for a sustainable future.