RATIONAL DESIGN AND MOLECULAR ENGINEERING OF SYNTHETIC RECEPTORS FOR BIOMARKER-BASED PATHOGEN DETECTION

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Rational Design and Molecular Engineering of Synthetic Receptors for Biomarker-Based Pathogen Detection

by

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Dedicated to

My parents, Mrs. Ritu Rajpal and Mr. Vinod Kumar Rajpal

My dear sister, Ms. Shreya Rajpal

CERTIFICATE

This is to certify that the thesis entitled "**Rational Design and Molecular Engineering of Synthetic Receptors for Biomarker-Based Pathogen Detection**" being submitted by **Ms. Soumya Rajpal** to the **Indian Institute of Technology Delhi** for the award of the degree of **Doctor of Philosophy** in Biochemical Engineering and Biotechnology, is a record of the authentic research work carried out by her under my supervision and guidance. She has fulfilled all the requirements for submission of this thesis, which to the best of my knowledge has reached the required standard. The results contained in this thesis have not been submitted in part or full to any other university or institute for the award of any other degree or diploma.

Professor Prashant Mishra

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ABSTRACT

Accurate diagnosis is pivotal in effective healthcare management and containment of antimicrobial resistance. This thesis explores the potential of molecularly imprinted polymers (MIPs), also known as synthetic receptors or artificial antibodies, as a groundbreaking solution for pathogen detection, aiming to address the limitations of traditional diagnostic methods. In an era where rapid and precise diagnosis of infectious diseases is imperative, MIPs emerge as a stable, cost-effective, and versatile alternatives to conventional antibodies. Differentiating between bacterial and viral infections remains a significant challenge due to overlapping symptoms. Diagnostics based on the identification of whole microorganisms can be a biosafety issue, laborious, time-consuming and costly, thus alternative strategies for early clinical diagnosis include biomarker-based detection. By selectively targeting specific biomarkers, MIPs offer a promising avenue to enhance healthcare outcomes through the development of a comprehensive and multiplexed diagnostic platform for a wide array of infectious diseases. This study focusses on designing synthetic receptors for pathogen specific biomarkers like pyocyanin characteristic of *P. aeruginosa* infections, viral coat proteins specific to SARS-CoV-2 and host biomarkers like Myxovirus Resistance proteins expressed in viral infections. To achieve precise structuring of recognition sites within the polymer matrix for biomarkers, this thesis emphasises on rational design and advanced synthesis techniques. For protein-based biomarkers, an epitope imprinting approach complemented by *in silico* screening of polymerization components has been used to fabricate chemically stable and cost-effective synthetic receptors for correct identification of bacterial and viral etiologies. Herein, introducing a novel multi monomer simultaneous docking approach for MIP design highlights the binding mechanics and the intermediates of monomers during the polymerization steps. It serves as a critical bridge between theoretical concepts and practical applications, significantly enhancing our understanding of MIPs' molecular engineering. The adaptability, specificity, and the capacity to imprint a wide spectrum of molecules, regardless of size, paves the way for innovative solutions that can enhance disease diagnosis, treatment, and monitoring, ultimately improving patient care and public health.

SAAR

प्रभावी स्वास्थ्य देखभाल प्रबंधन और रोगाणुरोधी प्रतिरोध की रोकथाम में सटीक निदान महत्वपूर्ण है। यह थीसिस रोगज़नक़ का पता लगाने के लिए एक अभूतपूर्व समाधान के रूप में, सिंथेटिक रिसेप्टर्स या कृत्रिम एंटीबॉडी के रूप में जाने जाने वाले आणविक रूप से मुद्रित पॉलिमर (MIP) की क्षमता का पता लगाती है, जिसका लक्ष्य पारंपरिक निदान विधियों की सीमाओं को संबोधित करना है। ऐसे युग में जहां संक्रामक रोगों का त्वरित और सटीक निदान जरूरी है, MIP पारंपरिक एंटीबॉडी के एक स्थिर, लागत प्रभावी और बहुमुखी विकल्प के रूप में उभरे हैं। ओवरलैपिंग लक्षणों के कारण बैक्टीरिया और वायरल संक्रमण के बीच अंतर करना एक महत्वपूर्ण चुनौती बनी हुई है। संपूर्ण सूक्ष्मजीवों की पहचान पर आधारित निदान एक जैव सुरक्षा मुद्दा, श्रमसाध्य, समय लेने वाला और महंगा हो सकता है, इस प्रकार प्रारंभिक निदान के लिए वैकल्पिक रणनीतियों में बायोमार्कर-आधारित पहचान शामिल है। विशिष्ट बायोमार्करों को चुनिंदा रूप से लक्षित करके, MIP संक्रामक रोगों की एक विस्तृत श्रृंखला के लिए एक व्यापक और बहुसंकेतन निदान मंच के विकास के माध्यम से स्वास्थ्य देखभाल परिणामों को बढ़ाने के लिए एक आशाजनक अवसर प्रदान करता है। यह थीसिस रोगज़नक़ विशिष्ट बायोमार्कर जैसे *P. aeruginosa* संक्रमण की विशेषता pyocyanin, SARS-CoV-2 के लिए विशिष्ट वायरल कोट प्रोटीन और वायरल संक्रमण में व्यक्त Myxovirus resistance प्रोटीन (MxA) जैसे मेजबान बायोमार्कर के लिए सिंथेटिक रिसेप्टर्स को डिजाइन करने पर केंद्रित है। बायोमार्कर के लिए पॉलिमर मैट्रिक्स के भीतर पहचान की सटीक संरचना प्राप्त करने के लिए, यह थीसिस तर्कसंगत डिजाइन और उन्नत संश्लेषण तकनीकों पर जोर देती है। प्रोटीन-आधारित बायोमार्कर के लिए, बैक्टीरिया और वायरल संक्रमण की सही पहचान के लिए रासायनिक रूप से स्थिर और लागत प्रभावी सिंथेटिक रिसेप्टर्स बनाने के लिए पोलीमराइजेशन तत्वों की कम्प्यूटेशनल स्क्रीनिंग के साथ एक epitope इंप्रिंटिंग प्रक्रिया का उपयोग किया गया है। इस थीसिस ने पोलीमराइजेशन प्रक्रिया के दौरान बाइंडिंग मैकेनिक्स और मोनोमर्स के मध्यवर्ती को उजागर करने के लिए MIP डिजाइन के लिए एक नई मल्टी मोनोमर समवर्ती डॉकिंग प्रक्रिया की शुरुआत की। यह सिद्धांत और प्रयोग के बीच एक संबंध के रूप में कार्य करता है, जो MIP की आणविक इंजीनियरिंग के बारे में हमारी समझ को महत्वपूर्ण रूप से बढ़ाता है। अनुकूलनशीलता, विशिष्टता और किसी भी आकार के अणुओं को छापने की क्षमता, नवीन समाधानों का मार्ग प्रशस्त करती है जो रोग निदान, उपचार को बढ़ा सकते हैं, अंततः रोगी देखभाल और सार्वजनिक स्वास्थ्य में सुधार कर सकते हैं।

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List of Abbreviations

ACN	Acetonitrile
ACR	Acrylamide
AGM	Alternating gradient field magnetometry
AgNP	Silver nanoparticles
AHL	*N*-acyl homoserine lactones
AMR	Antimicrobial resistance
APTES	3-Aminopropyltriethoxysilane
APTMS	3-Aminopropyltrimethoxysilane
ATCC	American type culture collection
AuNP	Gold nanoparticles
BC	Binding capacity
BE	Binding energy
BSA	Bovine serum albumin
CDC	Center for Disease Control and Prevention
CIPs	Cell imprinted polymers
CRP	C-reactive protein
DIDMS	Diisobutyldimethoxysilane
DLS	Dynamic light scattering
DVB	*p*-divinyl benzene
EDX	Energy dispersive X-ray spectroscopy
EGDMA	Ethylene glycol dimethacrylate

ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
FTIR	Fourier transform infrared spectroscopy
HR-TEM	High-resolution transmission electron microscopy
HSA	Human serum albumin
IBTES	Isobutyltriethoxysilane
IF	Imprinting factor
IVD	*in vitro* diagnostics
LB	Luria broth
LGA	Lamarckian genetic algorithm
Lys	Lysozyme
MAA	Methacrylic acid
MagNP	Magnetic nanoparticles
MC	Monomer combinations
mg	milli gram
MHB	Mueller Hinton broth
MIP	Molecularly imprinted polymers
mL	milli litre
mM, M	milli molar, molar
MPS	3-methacryloxypropyltrimethoxysilane
MPTMS	3-Mercaptopropyltrimethoxysilane
MTMS	Methyltrimethoxysilane
MxA	Myxovirus resistance protein

NIP	Non-imprinted polymer
nm	Nanometre
PC	Polymer composition
PCR	Polymerase chain reaction
PDB	Protein data bank
POC	Point-of-care
PTES	Phenyltriethoxysilane
SASA	Solvent accessible surface area
SEM	Scanning electron microscope
SERS	Surface enhanced Raman spectroscopy
SiNPs	Silica nanoparticles
SPR	Surface plasmon resonance
TEOS	Tetraethoxysilane
UPTMS	Ureidopropyltrimethoxysilane
VIPs	Virus imprinted polymers
VLP	Virus like particles
WHO	World Health Organization