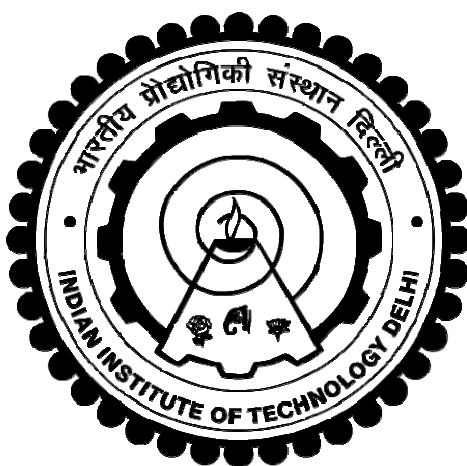


RATIONAL DESIGN AND MOLECULAR ENGINEERING OF SYNTHETIC RECEPTORS FOR BIOMARKER-BASED PATHOGEN DETECTION

SOUMYA RAJPAL



**DEPARTMENT OF BIOCHEMICAL ENGINEERING AND
BIOTECHNOLOGY**

INDIAN INSTITUTE OF TECHNOLOGY DELHI

MARCH 2024

© Indian Institute of Technology Delhi (IITD), New Delhi, 2024

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

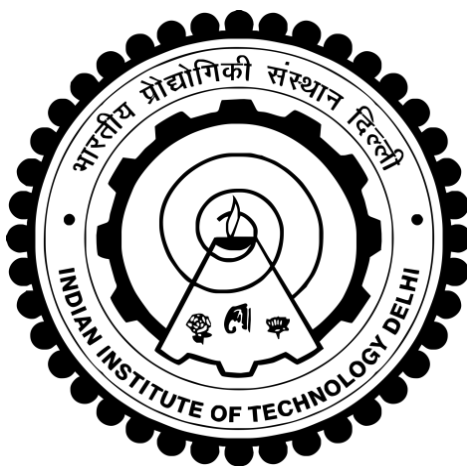
by

SOUMYA RAJPAL

Department of Biochemical Engineering and Biotechnology

Submitted

**in fulfilment of the requirements of the degree of Doctor of Philosophy
to the**



INDIAN INSTITUTE OF TECHNOLOGY DELHI

March 2024

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

Department of Biochemical Engineering and Biotechnology
Indian Institute of Technology Delhi

ACKNOWLEDGEMENTS

Completing a thesis marks a significant milestone, which symbolises the conclusion of an arduous academic journey. It serves as a platform to showcase one's research acumen and intellect in a specific domain. I am profoundly grateful for the opportunity and wish to convey my heartfelt appreciation to all those who have contributed to its fruitful culmination.

First and foremost, I express my sincerest gratitude to **Prof. Prashant Mishra** for his unwavering support, insightful guidance, and encouragement throughout the intricate research process. His invaluable inputs and wise critique have been pivotal in shaping the outline of this composition. I am deeply appreciative of his patience and willingness to go the extra mile in ensuring my understanding of the fundamental conceptual framework and theoretical support that underscore my research. His commitment to academic excellence has served as a motivation and inspiration and I consider myself immensely fortunate to have had him as my erudite mentor.

I extend my heartfelt thanks to **Prof. Boris Mizaikoff**, my supervisor during the DAAD exchange program at Ulm University. His persistent support, invaluable guidance and constant encouragement have been pivotal throughout my research expedition. His expertise, scholarly erudition and discerning feedback have been instrumental in realizing my academic objectives. I am grateful for his patience, enthusiasm and dedication in fostering the development of innovative research. His mentorship has been an abiding source of inspiration and the invaluable lessons imbibed shall always resonate within me.

I am thankful to my research committee members, **Prof. Ritu Kulshreshtha**, **Prof. Preeti Srivastava** and **Prof. Samresh Das** for their invaluable feedback and sustained support. Their wisdom in the field have been instrumental in moulding the trajectory of my research and

ensuring its adherence to exacting standards. Their constructive criticism has served to refine my arguments and fortify my research methodology. I am sincerely appreciative of the time and effort they dedicated to meticulously scrutinizing my work and providing me with the necessary guidance and support.

Furthermore, I wish to express my profound gratitude to the German Academic Exchange Service (**DAAD**) for bestowing upon me a scholarship that facilitated my participation in an enlightening exchange program at Ulm University. This prestigious scholarship has afforded me an extraordinary opportunity to broaden the horizons of my academic pursuits, gain novel perspectives, and refine my research acumen. I am highly obliged to DAAD for their benevolent financial support which has enabled me to pursue my academic aspirations and manifest my scholarly ambitions. The exchange program has been a transformative experience, and I shall forever cherish the privilege of being an active participant therein.

I wish to express my profound gratitude to IIT Delhi and Ulm University for endowing me with the necessary resources, state-of-the-art facilities, dedicated personnel and enriching opportunities to embark upon this scholarly endeavour. The university's unyielding commitment to academic excellence has served as a beacon of inspiration, and I am deeply appreciative of the unfailing support and astute guidance offered by the profound academic staff. The vast repository of library resources, meticulously curated research facilities, and comprehensive academic programs have been indispensable in configuring my research and ensuring its excellence.

I wish to express special gratitude to the senior postdocs Dr. Snehasis Bhakta, Dr. Senthil Guru Kulanthaivel, Dr. Alex Domingues Batista and colleagues, Dr. Sanjay Singh, Dr. Neeti Kalyani, Dr. Shereena Joseph, Benedikt Keitel for their scientific insights and valuable suggestions throughout the course of my research journey.

Finally, my heartfelt appreciation goes out to my beloved family and cherished friends for their steady support and encouragement throughout this transformative expedition. Their love and support have served as a pillar of strength and motivation. I am sincerely grateful for their patience and understanding during the challenging times encountered in the pursuit of this research.

With utmost gratitude, I extend my profound appreciation to all who have traversed this academic voyage alongside me.

Thank you all for being invaluable companions on this momentous journey of intellectual growth.

Soumya Rajpal

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 रोगजनक 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 रोगजनक 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 पोलिमराइजेशन तत्वों की कम्प्यूटेशनल स्क्रीनिंग के साथ एक epitope इंप्रिंटिंग प्रक्रिया का उपयोग किया गया है। इस थीसिस ने पोलिमराइजेशन 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।

Table of Contents

Certificate	i
Acknowledgements	ii
Abstract.....	v
Table of Contents	vii
List of Figures.....	x
List of Tables.....	xiii
List of Abbreviations	xiv
Chapter 1	
1.1 Introduction	1
1.2 Objectives.....	6
Chapter 2	
2.1 Background	7
2.2 Molecular imprinted polymers based on biomimetics	14
2.3 Strategies for molecular imprinting and evolution of MIPs.....	16
2.4. Design and molecular engineering of synthetic receptors	20
2.4.1 Rational selection of polymerization components	22
2.4.2 Rational template selection	26
2.5 Synthetic receptors for biomarkers	29
2.6 MIPs based biosensors for IVD and POC applications	32
2.7 Standalone MIP assays based on nanomaterials	37
2.8 Commercial prospects	39
Chapter 3	
3.1 Introduction	42
3.2 Materials and Methods	47
3.2.1 Synthesis of magnetic nanoparticles and surface functionalization	47
3.2.2 Surface modification of magnetic nanoparticles.....	47

3.2.3 Synthesis of imprinted polymers using different monomers	48
3.2.4 Binding studies.....	49
3.2.5 Specificity studies	50
3.2.6 Growth and culture conditions of <i>P. aeruginosa</i>	50
3.2.7 Pyocyanin extraction from culture.....	51
3.2.8 Measurements of pyocyanin in bacterial cultures.....	52
3.2.9 Binding assay with <i>Pseudomonas</i> culture.....	53
3.2.10 Binding assay of MIP with different biological media	53
3.2.11 Control experiment for large scale pyocyanin extraction	54
3.3 Results and Discussion.....	54
3.3.1 Synthesis of core-shell type plastibodies for pyocyanin	54
3.3.2 Binding performance and target selectivity towards pyocyanin.....	55
3.3.3 Characterization of pyocyanin specific MIPs	58
3.3.4 Biocompatibility and binding analysis in complex biological medium.....	63
3.3.5 <i>In vitro</i> pyocyanin extraction for commercial applications.....	66
3.4 Summary and Conclusion	68
Chapter 4	
4.1 Introduction	69
4.2 Materials and Methods	73
4.2.1 Computational simulations	73
4.2.2 Silica particle synthesis and functionalization	75
4.2.3 Synthesis of MIP and NIP	75
4.2.4 Binding studies.....	76
4.3 Results and Discussion.....	77
4.3.1 Rational design of MxA epitope specific-imprinted polymers	77
4.3.2 MMSD guided combinatorial screening of monomers for MxA epitope imprinting	83

4.3.3 Core-shell type MIP nanoparticle synthesis for templating MxA epitope	88
4.3.3.1 MMSD simulation guided MIPs and validation of imprinting parameters	89
4.3.3.2 Core-shell type MIP nanoparticle characterization	93
4.4 Summary and Conclusion	97
Chapter 5	
5.1 Introduction	99
5.2 Materials and Methods	104
5.2.1 Computational simulations	105
5.2.2 Silica particle synthesis and functionalization	106
5.2.3 Synthesis of MIP and NIP	106
5.2.4 Binding studies.....	107
5.3 Results and Discussion.....	108
5.3.1 Rational design of epitope imprinted MIPs for SARS-CoV-2.....	108
5.3.2 Design of multi-monomer computational screening.....	111
5.3.3 Multi-monomer combinatorial screening for predictive MIP design	113
5.3.4 Evaluation of monomer effects	118
5.3.5 Experimental screening based on predictive MIP design	123
5.3.6 Core-shell type artificial antibodies	129
5.3.7 Artificial antibody binding with virus-like particles	133
5.4 Summary and Conclusion	136
Chapter 6	
6.1 Summary and Conclusion.....	138
6.2 Future Perspectives.....	141
References	144
Appendices	176
Author's Biography	183

List of Figures

Figure. 2.1: Biomimetic strategies employing MIPs for analysis.....	15
Figure 2.2: Schematic representation of different types of direct micro-contact imprinting...	19
Figure 2.3: Graphical summary of the computational approach to rational selection of a monomer for protein-imprinted film synthesis.	25
Figure. 2.4: Schematics of biosensor development for various bio-analytes employing molecularly imprinted polymers (MIPs) as bio-recognition elements.	33
Figure 2.5: COVID-19 diagnostics principle by ncovNP based MIP sensor analysing the samples prepared from nasopharyngeal swab specimens of patients..	34
Figure 2.6: Timeline showing different imprinting processes and strategies that have enabled the evolution of the currently used advanced MIPs.....	40
Figure 3.1: Chemical modifications on the surface of iron oxide magnetic nanoparticles after each step of functionalization..	45
Figure 3.2: Growth curve of <i>Pseudomonas aeruginosa</i>	51
Figure 3.3: Pyocyanin estimation in different bacterial cultures	52
Figure 3.4: Binding studies performed using different combinations of MIP and NIP nanoparticles formed and calculation of the imprinting factor (IF) and binding capacity (BC)	57
Figure 3.5: Structures of different molecules employed for selectivity studies.	58
Figure 3.6: FTIR spectra of iron-oxide magnetic nanoparticles (MAGNP), silica coated magnetic nanoparticles (SiMAGP), MPS-functionalized magnetic nanoparticles (SiMAGP-MPS) and (iv) surface imprinted nanoparticles formed using MAA–DVB as polymerization agents (DVB–MAA–MIP).....	59
Figure 3.7. FTIR analysis of MIP-NP made with different combinations of monomers and cross linkers.	60
Figure 3.8: HRTEM images of MAGNP, SiMAGP, SiMAGP-MPS and surface imprinted nanoparticles using MAA–DVB as polymerization agents (DVB–MAA–MIP).	61
Figure 3.9: Size distribution analysis of different particles using DLS	61

Figure 3.10: EDX analysis of different functionalized particles to confirm the presence of chemical elements after each step of modification on surface	62
Figure 3.11. Zeta-potential for different particles in aqueous media	63
Figure 3.12: Binding of pyocyanin in medium that mimics physiological conditions relevant to patient samples.....	65
Figure 4.1: Schematic representation of MIPs design specific to MxA-epitope.	72
Figure 4.2: Structures of the silane monomers employed during the screening process. Representation of single monomer docking and multi-monomer simultaneous docking.....	78
Figure 4.3: Representation of peptide-monomer interactions based on single monomer docking results.	82
Figure 4.4: Representation of the MMSD sum and SMD sum for every polymer combination using a radial graph	86
Figure 4.5: Comparison of performance of polymer compositions in terms of BC and IF	90
Figure 4.6: Amount of MxA protein bound to epitope-imprinted MIPs and NIPs synthesized using polymer composition III	93
Figure 4.7: SEM characterization of SiNP, SiNP-Glu and MIP nanoparticles (PC III).	93
Figure 4.8 TEM characterization of MIP and NIP nanoparticles for PC III.	94
Figure 4.9: FTIR of SiNP compared with spectra obtained after APTES functionalization (SiNP-APTES), SiNP-Glu, MIPs	95
Figure 5.1: Schematic representation of design strategy for MIPs specific to epitope (peptide) selected from Spike protein of SARS-CoV-2.	104
Figure 5.2: : Design of MMSD simulations based on single monomer docking.	111
Figure 5.3: Comparison of MMSD and SMD scores.	117
Table 5.4: 2D interaction map representing some common set of interactions with the peptide at multiple residues derived from MMSD calculations..	121
Figure 5.4: Comparison of performance of PC I to VIII in terms of BC and IF.....	127
Figure 5.5 : Specificity studies with human serum albumin (HSA), bovine serum albumin (BSA), lysozyme (Lys), a non-specific epitope peptide relevant to other viruses (P2).....	128

Figure 5.6: SEM and FTIR characterization of SiNP, SiNP-Glu and peptide/epitope imprinted SiNP (MIP).	130
Figure 5.7: EDX of SiNP confirming the presence of Si and O characteristic of silicon dioxide.....	133
Figure 5.8: MIPs bound with virus like particles (VLPs).....	134
Figure 5.9: Analysis of binding of MIPs and NIPs of PC 1 with virus like particles based on fluorescence intensities.	135

List of Tables

Table 2.1 Host biomarkers and pathogen associated biomarkers commonly employed for viral and bacterial infections	10
Table 2.2: Type of monomers commonly used for development of multi-functional and/or stimuli responsive MIPs.....	23
Table 2.3: Different types of biosensors employing MIPs for infectious disease detection in real biological samples that indicates their <i>in vitro</i> diagnostic (IVD) potential.	35
Table 2.4: Different types of nanoparticles used as core-shell format with MIP surfaces to target infectious disease detection	38
Table 3.1: Synthesis of MIP and NIP for pyocyanin with optimized concentrations of different monomer and cross-linker combinations.	49
Table 3.2: Complex biological media spiked with pyocyanin for binding analysis with MIP	54
Table 4.1: Single monomer docking with MxA specific peptide.....	81
Table 4.2: Multi-monomer simultaneous docking (MMSD) scores for 37 monomer combinations used and compared using heat maps.	85
Table 4.3: Four polymer compositions (PC) employed for experimental testing based on rationally selected monomer combinations derived from MMSD based combinatorial screening	88
Table 4.4: Zeta potential of silica nanoparticles after each coating step.....	97
Table 5.1: Single monomer docking with SARS-CoV-2 specific peptide.....	110
Table 5.2: MMSD scores for 36 monomer combinations used and compared with SMD. ...	114
Table 5.3: Single monomer docking scores of monomers qualitatively and largely compared with their scores when applied in multi-monomer simultaneous docking	119
Table 5.4: 2D interaction map representing some common set of interactions with the peptide at multiple residues derived from MMSD calculations.....	121
Table 5.5: Eight polymer compositions (PC) tested experimentally based on rationally selected monomer combinations derived from computational screening.....	124
Table 5.6: Zeta potential of silica nanoparticles after each coating step.....	132

List of Abbreviations

ACN	Acetonitrile
ACR	Acrylamide
AGM	Alternating gradient field magnetometry
AgNP	Silver nanoparticles
AHL	<i>N</i> -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	<i>p</i> -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	<i>in vitro</i> 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