FROM SEQUENCE TO STRUCTURE: HIERARCHICAL SELF-ASSEMBLY INTO STRUCTURALLY DISTINCT ARCHITECTURES

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JANUARY 2026

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by

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(Entry No. 2021CYZ8386)

Submitted

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

to the

Indian Institute of Technology Delhi

Hauz Khas, New Delhi, India-110016

January 2026

DEDICATED TO

MY PARENTS AND MY SISTER

CERTIFICATE

This is to certify that the thesis, entitled "**From Sequence to Structure: Hierarchical Self-Assembly into Structurally Distinct Architectures**", being submitted by **Mr. Souvik Dutta**, to the Indian Institute of Technology Delhi, for the award of degree of **Doctor of Philosophy** in Chemistry, is a record of bonafide research work carried out by him. **Mr. Souvik Dutta** has worked under my guidance and supervision and has fulfilled all the requirements for the submission of this thesis, which to my knowledge has reached the requisite standard. The results embodied in this thesis have not been submitted in part or in full, to any other University or Institute for award of any degree or diploma.

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ACKNOWLEDGEMENTS

It is with great pleasure and deep respect that I express my profound gratitude to my supervisor, **Prof. V. Haridas**, for his invaluable guidance, encouragement, and unwavering support throughout the course of my doctoral research. His scientific ideas, insightful discussions, and constant motivation have been instrumental in shaping my research ideas and nurturing my overall growth as a researcher. His patience, generosity with time, and high standards of academic rigor have greatly inspired me and will continue to influence my future endeavours. I feel extremely fortunate for having the opportunity to work under his supervision.

I am also sincerely thankful to the members of my Student Research Committee (SRC), **Prof. Nalin Pant, Prof. Janakiram Vaitla** and **Prof. Bishwajit Kundu**, for their valuable feedback, constructive criticism, and kind encouragement during the course of my research. Their suggestions not only helped me refine my work but also gave me new perspectives to pursue challenging problems with confidence.

I extend my heartfelt thanks to the **Indian Institute of Technology Delhi, Hauz Khas, New Delhi**, for providing me with the opportunity to pursue my doctoral studies in such a vibrant academic environment and for my fellowship. I am grateful to the **past** and **present Heads of the Department of Chemistry**, **Prof. A. J. Elias, Prof. S. Pandey** and **Prof. S. Nagendran**, for their constant support in building and maintaining a stimulating and resourceful research atmosphere within the department.

I also take this opportunity to thank all the **faculty members of the Department of Chemistry, IIT Delhi**, for their inspiring lectures, critical discussions, and encouragement during my academic journey. I am equally indebted to all the **staff members of the department**, whose help and cooperation have been indispensable in the smooth functioning of both academic and research activities.

I gratefully acknowledge the use of **CRF and SATHI facilities at IIT Delhi**, which provided access to instrumentation and resources essential for the successful completion of my research work.

I owe a special note of thanks to all my **past and present lab members**, whose scientific discussions, and collaborative spirit made the lab a lively and motivating place to work in. Their companionship has been a source of both professional learning and personal joy.

Finally, I express my deepest gratitude to my **parents, sister** and **friends**, whose unconditional love, encouragement, and sacrifices have been my greatest strength throughout my life. Their constant support during difficult times and belief in my abilities gave me the confidence to overcome challenges and persevere in my work. This thesis is as much their achievement as it is mine, and I dedicate this accomplishment to them with immense affection and respect.

Souvik Dutta.

January 2026 **Souvik Dutta**

ABSTRACT

This thesis, entitled "**From Sequence to Structure: Hierarchical Self-Assembly into Structurally Distinct Architectures**", explores the design, synthesis, and self-assembly behaviour of pseudopeptides functionalized with phenylene urea motifs, demonstrating how subtle changes in molecular architecture and amino acid sequence can lead to diverse supramolecular structures. Through a combination of spectroscopic, crystallographic, and microscopic techniques, we investigate the role of non-covalent interactions, such as π–π stacking, hydrogen bonding, and hydrophobic clustering, in directing the formation of nanotubes, fibers, vesicles, toroids, and zipper-like assemblies. The autofluorescent properties of these assemblies further enhance their potential for bioimaging applications. Overall, this work highlights the versatility of urea-based scaffolds in mimicking biological folding and in forming complex architectures with the help of self-assembly.

Chapter 1

Chapter 1 provides a comprehensive introduction to self-assembly in biological systems, emphasizing the fundamental principles and significance of this phenomenon in nature. It discusses how weak, non-covalent interactions, including hydrogen bonds, hydrophobic interactions, and electrostatic forces, drive the ordered organization of molecules into functional structures such as proteins, membranes, and DNA. The chapter highlights the central role of peptides as minimal, versatile building blocks for creating a wide array of nanostructures like fibers, nanotubes, and vesicles, outlining the advantages of synthetic peptides over natural proteins. Additionally, it states the diverse applications of self-assembled peptides and pseudopeptides in areas such as drug delivery, tissue engineering, biosensing, catalysis, nanoelectronics, and antimicrobial therapies, while addressing current challenges and future research directions in the field.

Chapter 2

Chapter 2 introduces a novel peptide design strategy where a phenylene urea aglet at the N-terminus induces a reverse turn conformation via edge-to-face π–π interactions. This leads to the formation of polygonal peptide tubes through a Phe-zipper arrangement, as confirmed by X-ray crystallography and electron microscopy. Single-crystal X-ray diffraction analysis

reveals that one of the derivatives adopts a left-handed triple-helical conformation, stabilized through a combination of intermolecular hydrogen bonding and hydrophobic interactions.

Chapter 3

Chapter 3 extends the design to include aliphatic residues (Leu/Ile) alongside Phe, resulting in tubular assemblies via Leu/Ile-Phe zipper motifs. Single-crystal X-ray analysis reveals a double-helical arrangement stabilized by intermolecular hydrogen bonds and hydrophobic interactions. The resulting nanostructures exhibit concentration-dependent autofluorescence and red-edge excitation shift (REES), enabling confocal imaging of the self-assembled architectures.

Chapter 4

Chapter 4 investigates the concentration-dependent morphological evolution of urea-cored pseudopeptides, which transition from vesicles to toroids and eventually to multi-torus and honeycomb-like structures. This study provides insights into the fusion mechanisms of synthetic peptide-based vesicles and highlights the influence of sequence and concentration on supramolecular topology. The autofluorescence of these structures is morphology-dependent, allowing optical visualization of assembly pathways.

Chapter 5

Chapter 5 demonstrates the use of phenyl urea as an aglet to stabilize the trans conformation of prolyl peptide bonds via n→π* interactions. X-ray crystallography confirms the exclusive trans geometry and reveals an Ile-Phe zipper-like clustering of hydrophobic side chains in non-aqueous media. This chapter underscores the ability of urea tags to control peptide conformation and promote biomimetic hydrophobic assembly even in organic solvents.

सारांश

"**अनुक्रम से संरचना तक: संरचनात्मक रूप से विशिष्ट संरचनाओं में पदानुक्रमित स्व-संयोजन**" शीर्षक वाला यह शोध प्रबंध, फ़िनिलीन यूरिया रूपांकनों से क्रियाशील स्यूडोपेप्टाइड्स के डिज़ाइन, संश्लेषण और स्व-संयोजन व्यवहार का अन्वेषण करता है, यह दर्शाता है कि आणविक संरचना और अमीनो अम्ल अनुक्रम में सूक्ष्म परिवर्तन कैसे विविध अतिआणविक संरचनाओं को जन्म दे सकते हैं। स्पेक्ट्रोस्कोपिक, क्रिस्टलोग्राफिक और सूक्ष्म तकनीकों के संयोजन के माध्यम से, हम नैनोट्यूब, रेशों, पुटिकाओं, टोरॉइड और ज़िपर जैसी संरचनाओं के निर्माण को निर्देशित करने में गैर-सहसंयोजक अंतःक्रियाओं—जैसे π–π स्टैकिंग, हाइड्रोजन बॉन्डिंग और हाइड्रोफोबिक क्लस्टरिंग—की भूमिका की जाँच करते हैं। इन संरचनाओं के स्व-प्रतिदीप्ति गुण जैव-चित्रण अनुप्रयोगों के लिए उनकी क्षमता को और बढ़ाते हैं। कुल मिलाकर, यह कार्य जटिल जैविक वलन और संयोजन परिघटनाओं की नकल करने में यूरिया-आधारित मचानों की बहुमुखी प्रतिभा को उजागर करता है।

अध्याय 1

अध्याय 1 जैविक प्रणालियों में स्व-संयोजन का एक व्यापक परिचय प्रदान करता है, और प्रकृति में इस परिघटना के मूलभूत सिद्धांतों और महत्व पर बल देता है। यह चर्चा करता है कि कैसे हाइड्रोजन बंध, जलभीतिक अंतःक्रियाएँ और स्थिरवैद्युत बल सहित दुर्बल, असहसंयोजक अंतःक्रियाएँ अणुओं को प्रोटीन, झिल्लियों और डीएनए जैसी कार्यात्मक संरचनाओं में व्यवस्थित रूप से व्यवस्थित करती हैं। यह अध्याय रेशों, नैनोट्यूब और पुटिकाओं जैसी नैनो संरचनाओं की एक विस्तृत श्रृंखला के निर्माण के लिए न्यूनतम, बहुमुखी निर्माण खंडों के रूप में पेप्टाइड्स की केंद्रीय भूमिका पर प्रकाश डालता है, और प्राकृतिक प्रोटीन की तुलना में सिंथेटिक पेप्टाइड्स के लाभों को रेखांकित करता है। इसके अतिरिक्त, यह औषधि वितरण, ऊतक अभियांत्रिकी, जैवसंवेदन, उत्प्रेरण, नैनोइलेक्ट्रॉनिक्स और रोगाणुरोधी चिकित्सा जैसे क्षेत्रों में स्व-संयोजन पेप्टाइड्स और स्यूडोपेप्टाइड्स के विविध अनुप्रयोगों को बताता है, साथ ही इस क्षेत्र में वर्तमान चुनौतियों और भविष्य की अनुसंधान दिशाओं पर भी प्रकाश डालता है।

अध्याय 2

अध्याय 2 एक नवीन पेप्टाइड डिज़ाइन रणनीति प्रस्तुत करता है जहाँ N-टर्मिनस पर एक फेनिलीन यूरिया एग्लेट, किनारे-से-सामने π–π अंतःक्रियाओं के माध्यम से एक विपरीत मोड़ संरचना उत्पन्न करता है। इससे Phe-ज़िपर व्यवस्था के माध्यम से बहुभुज पेप्टाइड नलिकाओं का निर्माण होता है, जैसा कि एक्स-

रे क्रिस्टलोग्राफी और इलेक्ट्रॉन माइक्रोस्कोपी द्वारा पुष्टि की गई है। एकल-क्रिस्टल एक्स-रे विवर्तन विश्लेषण से पता चलता है कि व्युत्पन्नों में से एक, अंतर-आणविक हाइड्रोजन बंधन और हाइड्रोफोबिक अंतःक्रियाओं के संयोजन द्वारा स्थिर, एक बाएँ-हस्त त्रि-हेलिकल संरचना को अपनाता है।

अध्याय 3

अध्याय 3 डिज़ाइन का विस्तार करते हुए Phe के साथ-साथ एलिफैटिक अवशेषों (Leu/Ile) को शामिल करता है, जिसके परिणामस्वरूप Leu/Ile-Phe ज़िपर रूपांकनों के माध्यम से नलिकाकार संयोजन बनते हैं। एकल-क्रिस्टल एक्स-रे विश्लेषण से अंतर-आणविक हाइड्रोजन बंधों और हाइड्रोफोबिक अंतःक्रियाओं द्वारा स्थिर एक द्वि-हेलिकल व्यवस्था का पता चलता है। परिणामी नैनो संरचनाएँ सांद्रता-निर्भर स्वप्रतिदीप्ति और लाल-किनारा उत्तेजन विस्थापन (REES) प्रदर्शित करती हैं, जिससे स्व-संयोजित संरचनाओं की कॉन्फोकल इमेजिंग संभव होती है।

अध्याय 4

अध्याय 4 यूरिया-कोरयुक्त स्यूडोपेप्टाइड्स के सांद्रता-निर्भर रूपात्मक विकास की जाँच करता है, जो पुटिकाओं से टोरॉइड और अंततः बहु-टोरस और छत्ते जैसी संरचनाओं में परिवर्तित होते हैं। यह अध्ययन सिंथेटिक पेप्टाइड-आधारित पुटिकाओं के संलयन तंत्रों में अंतर्दृष्टि प्रदान करता है और सुपरमॉलेक्यूलर टोपोलॉजी पर अनुक्रम और सांद्रता के प्रभाव पर प्रकाश डालता है। इन संरचनाओं का स्वप्रतिदीप्ति आकारिकी-निर्भर है, जिससे संयोजन पथों का प्रकाशीय दृश्यीकरण संभव होता है।

अध्याय 5

अध्याय 5 $n\rightarrow\pi^*$ अंतःक्रियाओं के माध्यम से प्रोलिल पेप्टाइड बंधों के ट्रांस संरूपण को स्थिर करने के लिए एग्लेट के रूप में फेनिल यूरिया के उपयोग को प्रदर्शित करता है। एक्स-रे क्रिस्टलोग्राफी विशिष्ट ट्रांस ज्यामिति की पुष्टि करती है और गैर-जलीय माध्यम में हाइड्रोफोबिक पार्श्व श्रृंखलाओं के इले-फे ज़िपर जैसे समूहन को प्रकट करती है। यह अध्याय यूरिया टैग्स की पेप्टाइड संरचना को नियंत्रित करने और कार्बनिक विलायकों में भी बायोमिमेटिक हाइड्रोफोबिक संयोजन को बढ़ावा देने की क्षमता को रेखांकित करता है।

ENCLOSURES

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LIST OF ABBREVIATIONS

%	Percent
δ	Chemical shift
°C	Degree celsius
ACN	Acetonitrile
ACQ	Aggregation-Caused Quenching
AFM	Atomic Force Microscopy
AIE	Aggregation-Induced Emission
Amoc	9-anthracenemethoxycarbonyl
Boc	tert-Butyloxycarbonyl
CAC	Critical Aggregation Concentration
$CDCl_3$	Deuterated chloroform
CD_3CN	Deuterated acetonitrile
CSI	Chemical Shift Index
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane

DMF	N, N-dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
DOPA	Dihydroxy-L-phenylalanine
ECM	Extracellular Matrix
ee	Enantiomeric Excess
EtOAc	Ethyl Acetate
FET	Field-Effect Transistor
FIB	Focused-Ion Beam
FF	Phe-Phe (Diphenylalanine)
Fmoc	9-fluorenylmethoxycarbonyl
HRMS	High-Resolution Mass Spectrometry
JAK/STAT	Janus Kinase/Signal Transducer and Activator of Transcription
LLPS	Liquid-Liquid Phase Separation
MeOH	Methanol
MP	Melting Point
$NaHCO_3$	Sodium Bicarbonate

Na_2SO_4	Sodium Sulphate
NMR	Nuclear Magnetic Resonance
NEt_3	Triethylamine
NHS	N-Hydroxysuccinimide
PBS	Phosphate Buffered Saline
PDA	Polydiacetylene
Phg	Phenylglycine
REES	Red-Edge Excitation Shift
SEM	Scanning Electron Microscopy
SH2	Src Homology 2
SPPS	Solid Phase Peptide Synthesis
TEM	Transmission Electron Microscopy
TLC	Thin Layer Chromatography
TFA	Trifluoroacetic acid
Trpzip	Tryptophan zipper
UV	Ultraviolet

NOTES

1. All amino acids used in syntheses were of L-configuration, unless stated otherwise, and were purchased from SRL India. Unless otherwise stated, the standard single/triple letter codes are used to represent amino acids.
2. All commercial chemicals and reagents used in chemical syntheses were purchased from Sigma-Aldrich or SRL India unless otherwise stated, and used as received without any further purification.
3. All the solvents employed in the reactions were distilled/dried by standard protocols prior to use.
4. All air sensitive reactions were carried out in oven dried glassware under an inert atmosphere of argon.
5. All the reactions were monitored by silica gel thin layer chromatography (TLC), wherever possible.
6. All the synthesized compounds were purified by silica gel (100-200 mesh) column chromatography. The slurry was generally made in chloroform, DCM and/or MeOH.
7. The characterization of synthesized compounds was done by ^{1}H NMR, ^{13}C NMR, IR and High-Resolution Mass Spectrometry (HRMS).
8. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker-DPX-400/500 MHz spectrometer and the chemical shifts are reported downfield relative to tetramethylsilane (TMS). ^{1}H NMR data are reported as br (broad), s (singlet), d (doublet), q (quartet), t (triplet), dd (doublet of doublet) and m (multiplet). ^{1}H NMR coupling constants are reported in Hz.
9. High resolution mass spectra (HRMS) were recorded in Bruker MicrO-TOF-QII model using Electrospray Ionization (ESI) technique.
10. IR spectra were recorded on Agilent-Cary 660 Series FTIR spectrometer/Nicolet, Protégé 460 spectrometer as KBr Pellets.
11. Melting points were recorded on a Fisher-Scientific melting point apparatus.