

**DESIGN, SYNTHESIS AND VESICULAR
SELF-ASSEMBLY OF CYCLIC, ACYCLIC AND LIPID
BASED MOLECULES**

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**DEPARTMENT OF CHEMISTRY
INDIAN INSTITUTE OF TECHNOLOGY DELHI**

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SELF-ASSEMBLY OF CYCLIC, ACYCLIC AND LIPID
BASED MOLECULES**

by

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Submitted

In fulfillment of requirement of degree of **Doctor of Philosophy**

to the



Indian Institute of Technology Delhi

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Dedicated to my beloved

'PARENTS' AND 'RAO BROTHERS'

CERTIFICATE

This is to certify that the thesis entitled, "**Design, synthesis and vesicular self-assembly of cyclic, acyclic and lipid based molecules**", being submitted by **Mr. Appa Rao Sapala**, 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. Appa Rao Sapala** 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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ABSTRACT

The thesis entitled "**Design, synthesis and vesicular self-assembly of cyclic, acyclic and lipid based molecules**" deals with the design, synthesis and mechanistic insight from the self-assembling study of triazole-based cyclic, acyclic and lipidated molecules. We have demonstrated a new hypothesis regarding the mechanism of vesicle formation from non-amphiphilic molecules, which provides much needed insight into the long standing debate on the vesicular mechanism. The research work presented in this thesis is divided into five chapters.

Chapter I

Chapter I comprises of the recent advancements in supramolecular chemistry of morphogenesis of vesicles. The focus has been majorly on the design principles and vesicular self-assembly of molecules belonging to a variety of classes ranging from polymers, amphiphilic molecules, peptide molecules, to recently emerged non-peptidic molecules. A special attention was devoted to explaining their self-assembling properties emphasizing molecular structure.

Chapter II

Chapter II embodies the design, synthesis and self-assembly of a new class of triazolophanes with a hierarchical mechanism of self-assembly. The concentration dependent self-assembly from hemi-toroid to vesicle through the intermediacy of a toroid was demonstrated using ultramicroscopic and crystallographic investigations.

Chapter III

Chapter III comprises of a rational-design strategy for the synthesis of triazole based large ring cyclic and acyclic molecules with amide bonds to unravel the vesicular self-assembly mechanism. The effect of architecture of molecules and chemical moieties, which are responsible for the formation of vesicles, has been studied.

Chapter IV

Chapter IV deals with the investigation of the self-assembly of hybrid peptide molecules with varying curvatures. The concentration dependent formation of toroids and vesicles was demonstrated with simple acyclic molecules. The morphological transformations in solution, has been studied using fluorescence spectroscopy and a variety of microscopic methods.

Chapter V

Chapter V describes the design principles and synthesis of a series of triazole-based acyclic molecules and amino acid-based lipidated molecules to understand the structure-assembly relationship. The formation of self-assembled structures such as fibers, dendritic structures, flower like morphology and vesicles were investigated in this chapter.

सारांश

थीसिस "साइकल, एसाइकल और लिपिड आधारित अणुओं के डिजाइन, संश्लेषण और वेश्युलर स्वयं-असेंबली" शीर्षक, त्रिज्या-आधारित चक्रीय, एनासिक और लिपिडेटेड अणुओं के स्व-संयोजन अध्ययन से डिजाइन, संश्लेषण और यंत्रवत अंतर्दृष्टि से संबंधित है। हमने गैर-एम्फ्रीफिलिक अणुओं से पुटिका गठन के तंत्र के बारे में एक नई परिकल्पना का प्रदर्शन किया है, जो वेश्युलर तंत्र पर लंबे समय तक बहस में बहुत आवश्यक जानकारी प्रदान करता है।

इस थीसिस में प्रस्तुत अनुसंधान कार्य को पांच अध्यायों में विभाजित किया गया है।

अध्याय I

अध्याय I में वेसिकल्स के मॉर्फोजेनेसिस के सुपरमोलेक्युलर रसायन विज्ञान में हाल ही में प्रगति शामिल हैं। पॉलिमर, एम्फ्रीफिलिक अणुओं, पेप्टाइड अणुओं से हाल ही में गैर-पेप्टाइडिक अणुओं के उभरने वाले विभिन्न वर्गों से जुड़े अणुओं के डिजाइन सिद्धांतों और वेश्युलर स्वयं-असेंबली पर ध्यान केंद्रित किया गया है। आणविक संरचना पर बल देते हुए अपने स्वयं-संयोजन गुणों को समझाते हुए एक विशेष ध्यान केंद्रित किया गया था।

अध्याय II

अध्याय 2 स्वयं-असेंबली के एक पदानुक्रमित तंत्र के साथ त्रिजोलोफेन के एक नए वर्ग के डिजाइन, संश्लेषण और स्वयं-असेंबली का प्रतीक है। हेरोमी-टॉयरोइड से पुतली से टोरोइड की मध्यस्थता के माध्यम से कंसंट्रेशन पर निर्भर स्वयं-असेंबली का प्रदर्शन अल्ट्रा-मैक्रोस्कोपिक और क्रिस्टलोग्राफिक जांच के माध्यम से किया गया।

अध्याय III

अध्याय III में वेश्युलर स्व-असेंबली तंत्र को सुलझाने के लिए एमेड बॉन्ड के साथ ट्रयाज़ोल आधारित बड़े अंगूठी चक्रीय और एसाइड अणुओं के संश्लेषण के लिए एक तर्कसंगत-डिजाइन रणनीति शामिल है। अणुओं और रासायनिक मोईएटीएस, जो वेसिकल्स के गठन के लिए जिम्मेदार हैं की वास्तुकला का असर का अध्ययन किया गया है।

अध्याय IV

अध्याय IV भिन्न वक्र के साथ संकर पेप्टाइड अणुओं की स्वयं-स्वयं-असेंबली की जांच से संबंधित है। टोराइड्स और वेसिकल्स के कंसंट्रेशन पर निर्भर निर्माण सरल एसाइकल अणुओं के साथ प्रदर्शित किया गया था। समाधान में रूपांतरिकीय परिवर्तनों का अध्ययन, प्रतिदीप्ति स्पेक्ट्रोस्कोपी और सूक्ष्म तरीकों की विविधता का अध्ययन किया गया है।

अध्याय V

अध्याय V संरचना-असेंबली संबंधों को समझने के लिए त्रिज्या-आधारित एसाइकल अणुओं और एमिनो एसिड-आधारित लिपिडित अणुओं की एक श्रृंखला के डिजाइन सिद्धांतों और संश्लेषण का वर्णन करता है। इस अध्याय में स्वयं-इकट्टे संरचनाओं जैसे फाइबर, डेनड्रिटिक के समान संरचनाएं, आकारिकी और वेसिकल्स जैसी फूलों की जांच की गई।

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LIST OF ABBREVIATIONS

%	Percent
δ	Chemical shift
$^{\circ}\text{C}$	Degree centigrade
AFM	Atomic force microscopy
aq.	Aqueous
Ar	Aryl
ArH	Aromatic proton
Ac	Acyl
Boc	t-butyloxycarbonyl
br	Broad
CD	Circular dichroism
Conc.	Concentrated
CuAAC	Copper catalyzed azide alkyne cycloaddition
d	Doublet
DCM	Dichloromethane
dd	Double doublet
DCC	N,N'-dicyclohexylcarbodiimide
DIPEA	N,N'-Diisopropylethylamine
DMF	N,N-dimethylformamide
DMSO	Dimethylsulfoxide
ESI	Electrospray ionization
FIB	Focused ion beam
Fmoc	Fluorenylmethyloxycarbonyl
g	Gram
h	Hour
Hz	Hertz
HRMS	High resolution mass spectrum
HRTEM	High resolution transmission electron microscope
IR	Infrared
J	Coupling constant
m	Multiplet
MD	Molecular dynamics
MeOH	Methanol
μM	Micro molar
μm	Micro meter
mg	Milli gram
mL	Milli liter

min	Minutes
mmol	Milli moles
mol	Mole
Mp	Melting point
m/z	Mass/charge
NR	Nile red
NMR	Nuclear magnetic resonance
ppm	Parts per million
q	Quartet
RT	Room temperature
s	Singlet
t	triplet
TFA	Trifluoroacetic acid
TLC	Thin layer chromatography

NOTES

1. All amino acids used in the reactions were of L-configuration. Standard single/triple letter codes are used to represent the amino acids.
2. All solvents employed in the reaction were distilled or dried from appropriate drying agent prior to use. Unless otherwise stated, all reagents were used without further purification.
3. Melting points were recorded in a Fisher-Johns melting point apparatus and were uncorrected.
4. IR spectra were recorded on a Nicolet, Protégé 460 spectrometer as KBr pellets.
5. ^1H NMR spectra were recorded on Bruker-DPX-300 (^1H , 300 MHz; ^{13}C , 75 MHz) spectrometer using tetramethylsilane (1H) as an internal standard. Coupling constants are in Hz and the ^1H NMR data are reported as s (singlet), d (doublet), br (broad), br d (broad doublet), t (triplet), q (quartet), m (multiplet).
6. Reactions were monitored wherever possible by thin layer chromatography (TLC). Silica gel G (Merck) was used for TLC and column chromatography was done on silica gel (100-200 mesh) columns, which were generally made from slurry in hexane, hexane/ethyl acetate or chloroform.
7. CD measurements were made using AVIV-420/ Jasco spectropolarimeter. Quartz cell of 0.1 cm was used for the measurements.
8. UV-Vis spectroscopy - The absorption spectra were recorded on a Shimadzu UV-2450 spectrometer.
9. SEM images were recorded using ZEISS EVO Series Scanning Electron Microscope EVO 50 operating at an accelerating voltage of 0.2 – 30 kV. For SEM, a 10 μL aliquot

of the sample solution was drop-casted on a glass cover slip, dried and coated with ~10 nm of gold.

10. FIB-SEM A 10 μ l aliquot of the sample solution was put on a fresh piece of glass, which is attached to a stub via carbon tape. The sample was dried at room temperature and coated with ~10nm of gold. Samples were analyzed using FEI Quanta 3D FEG High resolution scanning electron microscope (FE-SEM) combined with High-current ion column with Ga liquid-metal ion source.
11. HR-TEM images were recorded on a TECHNAI G2 (20S-TWIN) electron microscope operated at an accelerating voltage of 200 kV. Samples were prepared by drop-casting the sample on 200 square mesh carbon-coated copper grids.
12. AFM images were recorded using Bruker Dimension Icon atomic force microscope. Tapping mode is used for the analysis. About 10 μ l aliquot of the sample solution was transferred onto freshly cleaved mica and allowed to dry and imaged using AFM.
13. Optical microscopy: Samples for optical microscope were prepared by dissolving compound in methanol. A 5 mL aliquot of the sample solution was placed on a glass slide and allowed to dry in air at room temperature. The glass slide was then covered using a cover slip and analysed using a Nikon Ti Eclipse inverted optical microscope.
14. X-ray diffraction study was carried out on a BRUKER AXS SMART-APEX diffractometer with a CCD area detector (Mo Ka = 0.71073Å, monochromator: graphite). Frames were collected at T = 298 by ω , ϕ and 2θ -rotation at 10 s per frame with SMART. The measured intensities were reduced to F2 and corrected for absorption with SADABS. Structure solution, refinement, and data output were carried out with the SHELXTL program. Non-hydrogen atoms were refined anisotropically. C-H hydrogen

atoms were placed in geometrically calculated positions by using a riding model. Image was created with the diamond program.

15. Confocal microscope (NANONICS, Israel) was made with CW argon-ion laser (Modu-Laser, Model: Stellar Pro. Select) and the laser. The fluorescence from the samples was collected with the same objective and focused onto an avalanche photo diode (Model: SPCM-AQRH-14, CANADA), fitted with a 50 μm confocal pinhole, using a non-polarizing beamsplitter. The samples were scanned using a separate *x-y* closed loop piezo scanner (Nanonics Imaging). For all experiments, piezo scanning and data acquisition was controlled with a HV Piezo Driver (Nanonics Imaging) and software (NWS11). For image acquisition the size of the field of view, resolution points and time exposure per points were 80 x 80 μM , 300 and 3 ms/point respectively.
16. Nile red (9-Diethylamino-benzo[α]phenoxazin-5-one), PRODAN (1-(6-Dimethylaminonaphthalen-2-yl)-propan-1-one) and fluorescein sodium salt (2-(3-Oxo-6-oxido-3H-xanthén-9-yl)benzoate di sodium) were purchased from Sigma-Aldrich Chemical Co. (USA) and were used as received.
17. For Fluorescence measurements, All the steady-state fluorescence experiments of sample solutions were carried out on the FLS900 spectrofluorometer (Edinburgh, UK). The fluorescence measurements were carried out by using 1 cm quartz cuvettes and the temperature was maintained at 25 °C using a Peltier based cooler (Quantum Northwest).