

MOLECULAR RECOGNITION AND SELF- ASSEMBLING PROPERTIES OF PEPTIDE- BASED SYSTEMS

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Dedicated to my dear parents

(P. K. Prabhakaran and K. V. Sunandha)

CERTIFICATE

This is to certify that the thesis entitled, “**Molecular Recognition and Self-assembling Properties of Peptide-Based Systems**”, being submitted by **Mr. Praveen Kumar P. P**, 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. Praveen Kumar P. P** 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

This thesis, “**Molecular recognition and self-assembling properties of peptide-based systems**” deals with the design and synthesis of various peptide-based molecules. We have demonstrated the utility of these molecules for binding various ions and as self-assembled materials for various applications. Molecular recognition of cations, anions and neutral molecules play a significant role in biology and chemistry. Cation transport is an important biological event that keeps the ionic balance in the body. Anions regulate the flux of key metabolites into and out of cells while maintaining osmotic balance. By keeping this idea in mind, we have designed and synthesized various peptide-based molecules and their sensing abilities has been studied in detail by using various spectroscopic techniques and by DFT studies. The self-assembling properties of these peptide-based molecules were studied in detail by various microscopic techniques.

Chapter 1 deals with a brief history and scope of the application of peptide-based fluorescent systems in molecular recognition, and as self-assembling materials for various applications.

Chapter 2 describes the design and synthesis of cysteine-based “turn-on” sensors for metal ions. In this chapter, we have designed three cysteine based molecules **B3-B5**, and their metal binding ability was studied in detail by UV-visible, fluorescence spectroscopy, ITC, NMR and by DFT calculations. Experimental results showed that **B3** can sense Cu^{2+} , **B4** can sense Ag^+ and **B5** can sense Hg^{2+} . The self-assembling properties of these molecules and their stimuli response with metal ions were also studied by using various microscopic techniques.

Chapter 3 addresses the design and synthesis of cystine based fluorescent sensors (**C2-C4**) for metal ions. Detailed spectroscopic studied revealed that **C2** can sense Cu^{2+} , **C3** can sense Hg^{2+} , and **C4** can sense Cd^{2+} colorimetrically. We have demonstrated the formation of a

supramolecular heterodimer (**C2:C3**) from **C2** and **C3**, and which detects Pb^{2+} by a fluorescent resonance energy transfer mechanism (FRET). The heterodimer formation was supported by UV-visible, fluorescent spectroscopy, ^1H NMR, ITC, DSC and by DFT calculations.

Chapter 4 discusses about design, synthesis, self-assembly and anion binding properties of tryptophan based molecules (**D2**, **D4**, **D6**, **D8**, and **D10**) which were arranged at different positions on a benzene aromatic core. Self-assembling properties of these molecules were studied in detail by various microscopic techniques. The binding property of these peptide molecules towards various anions were studied in detail.

Chapter 5 deals with the design, synthesis, self-assembly and mechanical properties of lipidated Asp/Glu- based dendrons and dendrimers were studied. The results showed that most of these dendrons and dendrimers can form excellent organogels and the guanidinylated dendrimer **E16** can form hydrogel. The rheological studies showed that the higher lipidated Glutamic acid based dendrimer **E13'** possess high mechanical strength of the order 10^7 Pa.

LIST OF FIGURES

Figure No.	Description	Page No.
Figure 1.1	Cartoon diagram showing the specificity for host-guest complex formation.	2
Figure 1.2	Cartoon representation showing the various self-assembly structures from a molecular entity	3
Figure 1.3	Cartoon representation of Guest binding by a fluorescent molecule	5
Figure 1.4	Cyclodecapeptide A1 for Hg ²⁺ and A2-5	6
Figure 1.5	Asparagine based sensors A6-16 for selective detection of Cu ²⁺	7
Figure 1.6	Cysteine and Cystine based molecules A17 and A18 respectively for Hg ²⁺	7
Figure 1.7	Lysine-based metal receptor systems A19-22	8
Figure 1.8	Peptide-based sensor A23 for Hg ²⁺ and A24 for Cd ²⁺	9
Figure 1.9	Peptide-based sensors A25 and A26	9
Figure 1.10	Tryptophan based receptor A27 for Pb ²⁺ and Hg ²⁺	10
Figure 1.11	Peptide-based chemosensors A28-A29 for Cd ²⁺ and Hg ²⁺ respectively	11
Figure 1.12	Aminoacid containing calix[4]arene based receptors A30-A33 for anions and carboxylates.	12
Figure 1.13	Aminoacid containing calix[4]arene based receptors A34-A36 for carboxylates	13
Figure 1.14	Aminoacid containing receptors A37-39 for enantioselective recognition of aminoacids	14
Figure 1.15	Cartoon diagram for self-assembled peptide amphiphile showing different self-assembled structures	15
Figure 1.16	Cartoon representation of gel formation process	16
Figure 1.17	Lysine-based dendritic organogelators A40-A42	17
Figure 1.18	Structures of organo gelator units A43-A46	18

Figure 1.19	Phenylalanine containing discotic amphiphiles A47	19
Figure 1.20	Structures of linear and branched gelators (A48-A51) derived from lysine	20
Figure 1.21	Multi-stimuli response organogels from lipidated alanine derivatives (A48-A50)	21
Figure 2.1	Schematic representation of choice of Cysteine for the sensors	31
Figure 2.2	(a) UV-visible spectra of B3 alone and with the addition of $\text{Cu}(\text{ClO}_4)_2$ in CH_3CN . (b) UV-visible spectra for B3 with and without metal ions in CH_3CN . Cu^{2+} ions 10 equiv. and other metal ions 50 equiv.	33
Figure 2.3	(a) Fluorescence spectra of B3 (1.2×10^{-5} M) with and without addition of $\text{Cu}(\text{ClO}_4)_2$ (1.2×10^{-4} M) in acetonitrile, $\lambda_{\text{ex}} = 340$ nm. The addition of metal ion results in the enhancement of excimer band at 470 nm. (b) Fluorescence spectral studies of B3 with various metal ions in CH_3CN , the results obtained are for 5 equiv. of Cu^{2+} and for 25 equiv. of other metal ions, $\lambda_{\text{ex}} = 340$ nm.	33
Figure 2.4	(a) ESI-HRMS of B3 -Cu(II) complex (b) Job's plot of B3 with Cu^{2+} (c) Excitation spectra for B3 with 5 equiv. of Cu^{2+} in CH_3CN . (d) Competitive binding studies of B3 towards various metal ions. The binding was studied by steady-state fluorescence spectroscopic analysis	34
Figure 2.5	(a) UV-visible spectra of B4 alone and with the addition of $\text{Ag}(\text{ClO}_4)$ in CH_3CN . (b) UV-visible spectra for B4 with and without metal ions in CH_3CN . Ag^+ ions 10 equiv. and other metal ions 50 equiv.	36
Figure 2.6	(a) Fluorescence spectra of B4 (1.8×10^{-5} M) alone and upon the addition of AgClO_4 (2×10^{-4} M) in acetonitrile, $\lambda_{\text{ex}} = 290$ nm. The fluorescence intensity increases upon addition of AgClO_4 , indicating the "turn-on" behavior. (b)	37

Fluorescence spectral studies of **B4** with various metal ions in CH₃CN, the results obtained are for 5 equiv. of Ag⁺ and for 25 equiv. of other metal ions, λ_{ex} = 290 nm. (c) Job's plot of **B4** with Ag⁺. (d) ESI-HRMS of **B4**-Ag(I) complex. (d) Benesi-Hildebrand plot for **B4** with Ag⁺. (e) Competitive binding studies of **B4** towards various metal ions. The binding was studied by steady-state fluorescence spectroscopic analysis

- Figure 2.7 (a) UV-visible spectra of **B5** (1.2 x 10⁻⁵ M) alone and upon the addition of Hg(ClO₄)₂ (2 x 10⁻⁴ M) in acetonitrile (b) Job's plot of **B5** with Hg²⁺. (c) ESI-HRMS of **B5**-Hg(II) complex. (d) Benesi-Hildebrand plot for **B5** with Hg²⁺ 39
- Figure 2.8 ITC titration data of (a) **B3** [200 μM] with Cu²⁺ (n = 2.09, K_a = 1.95 x 10⁵ M⁻¹) (b) **B4** [150 μM] with Ag⁺ (n = 0.48, K_{a1} = 1.26 x 10⁵ M⁻¹ and K_{a2} = 1.97 x 10⁸ M⁻¹) (c) **B5** [200 μM] with Hg²⁺ (n = 1.02, K_a = 1.57 x 10³ M⁻¹). 40
- Figure 2.9 SEM images of (a) **B3** (b) **B4** (c) **B5** (d) **B3** + Cu²⁺ (e) **B4** + Ag⁺ (f) **B5** + Hg²⁺ in CH₃CN 42
- Figure 2.10 AFM images of (a) **B3** (b) **B4** (c) **B5** (d) **B3** + Cu²⁺ (e) **B4** + Ag⁺ (f) **B5** + Hg²⁺ in CH₃CN 42
- Figure 2.11 Possible binding modes of Cu²⁺ with **B3**. Initial structures resembling these configurations are subjected to DFT level optimization 44
- Figure 2.12 Final Optimized structures of Cu²⁺ complex of **B3** obtained by DFT calculation. 44
- Figure 2.13 The optimized structures of eight different possible binding modes (**1-8**) in the decreasing order of stability for **B4**-Ag⁺ complex 45
- Figure 2.14 B3LYP/6-31G(d) optimized structure of (a) **B3**-Cu²⁺-**B3** and (b) Ag⁺-**B4**-Ag⁺. (c) HOMO of **B3**-Cu²⁺-**B3**, (d) LUMO of **B3**-Cu²⁺-**B3**. (e) HOMO of Ag⁺-**B4**-Ag⁺ and (f) LUMO 46

of Ag⁺-**B4**- Ag⁺. Many hydrogen atoms are omitted for clarity

Figure 2.15	Left panel ¹ H NMR spectra for B4 (a) with and (b) without Ag ⁺ ion in CD ₃ CN (0-10 equiv. metal ion was added). Right panel ¹³ C NMR spectra (75 MHz) of (a) B4 (b) B4 + Ag ⁺ ion in CD ₃ CN. The blue colored carbon atoms are involved in Ag ⁺ ion binding	49
Figure 2.16	¹ H NMR (D ₂ O, 300 MHz) spectrum of <i>S-tert</i> -butyl-cysteine	57
Figure 2.17	¹³ C NMR (D ₂ O, 75 MHz) spectrum of <i>S-tert</i> -butyl-cysteine	57
Figure 2.18	ESI-Mass spectrum of <i>S-tert</i> -butyl-cysteine	58
Figure 2.19	¹ H NMR (D ₂ O, 300 MHz) spectrum of B2	58
Figure 2.20	¹³ C NMR (D ₂ O, 75 MHz) spectrum of B2	59
Figure 2.21	ESI-Mass spectrum of B2	59
Figure 2.22	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of B3	60
Figure 2.23	¹³ C NMR (CDCl ₃ , 75 MHz) spectrum of B3	60
Figure 2.24	ESI-Mass spectrum of B3	61
Figure 2.25	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of B4	61
Figure 2.26	¹³ C NMR (CD ₃ CN, 75 MHz) spectrum of B4	62
Figure 2.27	DEPT-135 NMR (CD ₃ CN, 75 MHz) spectrum of B4	62
Figure 2.28	ESI-Mass spectrum of B4	63
Figure 2.29	¹ H NMR (CDCl ₃ +D ₂ O, 300 MHz) spectrum of B5	63
Figure 2.30	¹³ C NMR (CDCl ₃ , 75 MHz) spectrum of B5	64
Figure 2.31	ESI-Mass spectrum of B5	64
Figure 3.1	Conformational equilibrium of C2	71
Figure 3.2	UV-visible absorption spectra for C2 and C3 in CH ₃ CN. (a) UV-visible spectra for C2 with and without Cu(ClO ₄) ₂ . (b) UV-visible spectra for C2 with Cu(ClO ₄) ₂ (15 equiv.) and the other metal ions tested (50 equiv.). (c) UV-visible spectra for C3 with and without Hg(ClO ₄) ₂ . (d) UV-visible spectra for C3 with Hg(ClO ₄) ₂ (21 equiv.) and the other metal ions tested (50 equiv.)	72

Figure 3.3	UV-visible absorption spectra for C4 in CH ₃ CN. (a) UV-visible spectra for C4 with and without Cd(ClO ₄) ₂ . (b) UV-visible spectra for C4 with Cu(ClO ₄) ₂ (25 equiv.) and the other metal ions tested (50 equiv.)	73
Figure 3.4	(a) Concentration vs I ₄₈₀ /I ₃₈₀ of C2 (b) Excitation spectra of C2 in CH ₃ CN	74
Figure 3.5	(a) Fluorescence spectral studies of C2 with various metal ions in CH ₃ CN, the results obtained are for 4 equiv. of Cu ²⁺ and Hg ²⁺ and for 20 equiv. of other metal ions, λ _{ex} = 340 nm. (b) Fluorescence spectra of C2 [10 μM] in acetonitrile with and without the addition of Cu(ClO ₄) ₂ , λ _{ex} = 340 nm. (c) Stern-Volmer plot for C2 with Cu ²⁺ (d) Selectivity study of C2 towards various metal ions. The binding was studied by steady-state fluorescence spectroscopic analysis	75
Figure 3.6	(a) CD Spectra of C2 alone and with 3 equiv. of Cu ²⁺ (b) Job's plot for C2 with Cu ²⁺	76
Figure 3.7	(a) Fluorescence spectral studies of C3 in CH ₃ CN, 14 equiv. other metal ions and 3 equiv. of Cu ²⁺ and Hg ²⁺ , λ _{ex} = 290 nm. (b) Fluorescence spectra of C3 [15 μM] in acetonitrile with and without the addition of Hg(ClO ₄) ₂ , λ _{ex} = 290 nm. (c) Job's plot C3 with Hg ²⁺ . (d) Colorimetric response of C3 with various metal ions	77
Figure 3.8	¹³ C NMR (75 MHz) in CD ₃ CN (i) C3 alone (ii) C3 with 5 equiv. of Hg ²⁺ . The red dotted lines represent the carbons that show maximum shift upon Hg ²⁺ binding	78
Figure 3.9	(a) Selectivity study of C3 towards various metal ions. The binding was studied by steady-state fluorescence spectroscopic analysis. (b) Stern-Volmer plot for C3 with Hg ²⁺	79
Figure 3.10	CD Spectra of C3 alone and with 3 equiv. of Hg ²⁺ in CH ₃ CN	80

Figure 3.11	(a) Fluorescence spectra of C4 [12 μ M] in acetonitrile with and without the addition of $\text{Cd}(\text{ClO}_4)_2$, $\lambda_{\text{ex}} = 290$ nm (b) Job's plot of C4 with Cd^{2+}	81
Figure 3.12	(a) ESI-HRMS of C4 - Cd^{2+} complex (b) Colorimetric response of C4 with various metal ions (c) CD Spectra of C4 alone and with 3 equiv. of Cd^{2+} in CH_3CN	83
Figure 3.13	Optimized structures of (a) C4 (d) C4 - Cd^{2+} complex	84
Figure 3.14	(a) UV-visible spectra of 1:1 mixture of C2 and C3 in CH_3CN (b) Partial ^1H NMR spectra of C2 , C3 , and C2:C3 in CD_3CN (c) ESI-HRMS of C2 and C3 interaction (d) Job's plot of C2 and C3 .	85
Figure 3.15	CD spectrum of C2:C3 in CH_3CN	86
Figure 3.16	Optimized structures of (a) C2 (b) C3 (c) the heterodimer C2:C3 (d) HOMO-LUMO orbital diagram for C2:C3 heterodimer using B3LYP/6-31G* DFT method	88
Figure 3.17	(a) ITC titration data of C2 [200 μ M] upon titrating against C3 [50 μ M]; ($n = 1.01$, $K_a = 3.2 \times 10^3 \text{ M}^{-1}$). (b) ITC titration plots C2 with C3 as a titrant (c) control experiment in which CH_3CN titrated against C3	89
Figure 3.18	SEM images of (a) C2 (b) C3 (c) C4 (d) C2:C3 in acetonitrile	90
Figure 3.19	DSC thermograms of (a) C2 (b) C3 (c) C2:C3	91
Figure 3.20	UV-visible titration spectra for C2:C3 with Pb^{2+} in CH_3CN	91
Figure 3.21	(a) Observed color change under UV light upon binding of C2:C3 [10 μ M] with Pb^{2+} (b) fluorescence spectra of C2:C3 ($1.1 \times 10^{-5} \text{ M}$) in acetonitrile with and without addition of $\text{Pb}(\text{ClO}_4)_2$, $\lambda_{\text{ex}} = 290$ nm (c) ESI-HRMS of C2:C3 - Pb^{2+} complex (d) Job's plot of C2:C3 with Pb^{2+}	92
Figure 3.22	(a) Benesi-Hildebrand plot for C2:C3 with Pb^{2+} (b) ^1H NMR titration plot for C2:C3 interaction with Pb^{2+} in	94

	CD ₃ CN (c) Optimized structure of the heterodimer with Pb ²⁺ (d) CD spectra of C2 : C3 alone and with 3 equiv. of Pb ²⁺	
Figure 3.23	The plot of τ_0/τ and F_0/F against Cu(II) concentration	100
Figure 3.24	¹ H NMR (DMSO- <i>d</i> ₆ , 300 MHz) spectrum of C2	104
Figure 3.25	¹³ C NMR (CDCl ₃ , 75 MHz) spectrum of C2	104
Figure 3.26	ESI-Mass spectrum of C2	105
Figure 3.27	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of C3	105
Figure 3.28	¹³ C NMR (CDCl ₃ , 75 MHz) spectrum of C3	106
Figure 3.29	ESI-Mass spectrum of C3	106
Figure 3.30	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of C4	107
Figure 3.31	¹³ C NMR (CDCl ₃ , 75 MHz) spectrum of C4	107
Figure 3.32	ESI-Mass spectrum of C4	108
Figure 4.1	SEM images (a) for D2 (b) for D4 (c) for D6 (d) for D8 (e) for D10 (g) gel of D8 in CHCl ₃ /Hexane	116
Figure 4.2	HR-TEM images (a) for D2 (b) for D4 (c) for D6 (d) for D8 (e) for D10 (g) gel of D8 in CHCl ₃ /Hexane	116
Figure 4.3	AFM images (a) for D2 (b) for D4 (c) for D6 (d) for D8 (e) for D10 (g) gel of D8 in CHCl ₃ /Hexane	117
Figure 4.4	Concentration dependent ¹ H NMR (300 MHz) studies for (a) for D2 (b) for D4 (c) for D6 (d) for D8 (e) for D10 in 1:1 Me-OH- <i>d</i> ₃ +CDCl ₃	120
Figure 4.5	Frequency sweep experiment for the organogel from D8 gel.	121
Figure 4.6	(a) Crystal structure of D10 . DMSO molecules are omitted for clarity, (b) represents the hydrogen bonding between DMSO to the amide –NHs of D10 . (c) Crystal structure showing the intermolecular association in D10 . Some of the hydrogen atoms and methyl groups are removed for clarity	122
Figure 4.7	(a) Structure of Carboxyfluorescein (CF). (b) Confocal microscopic images of (i) D2 (ii) D4 (iii) D6 (iv) D8 (v)	125

	D10 after mixing with CF in 1:1 MeOH/CHCl ₃ H ₂ PO ₄ ⁻	
Figure 4.8	Fluorescence spectral analysis for loading of CF in vesicles of D2 , D4 , D6 , D8 , and D10 ; $\lambda_{\text{ex}} = 490 \text{ nm}$	125
Figure 4.9	SEM images of compounds D2 , D4 , D6 , D8 , and D10 after interaction with CF . (a) D2 with CF (b) D4 with CF (c) D6 with CF (d) D8 with CF (e) D10 with CF	126
Figure 4.10	(a) UV-visible spectra of D2 with and without TBAH ₂ PO ₄ in DMSO. (b) Job's plot for D2 with TBAH ₂ PO ₄ . (c) Benesi-Hildebrand plots for D2 with TBAH ₂ PO ₄	127
Figure 4.11	UV-visible spectra of D4 (a) with and without the addition of TBAF in DMSO (b) with and without TBACl in DMSO (c) with and without TBAI in DMSO (d) with and without the addition of TBAH ₂ PO ₄ in DMSO (e) with and without the addition of TBAHSO ₄ in DMSO	128
Figure 4.12	Jobs plot for D4 (a) with TBAF (b) with TBACl (c) with TBAI (d) with TBAH ₂ PO ₄ (e) with TBAHSO ₄	129
Figure 4.13	Benesi-Hildebrand plots for D4 (a) with TBAF (b) with TBACl (c) with TBAI (d) with TBAH ₂ PO ₄ (e) with TBAHSO ₄	130
Figure 4.14	UV-visible spectra of D6 (a) with and without the addition of TBAH ₂ PO ₄ in DMSO (b) with and without the addition of TBAHSO ₄ in DMSO	131
Figure 4.15	Jobs plot for D6 (a) with TBAH ₂ PO ₄ (b) with TBAHSO ₄	131
Figure 4.16	Benesi-Hildebrand plots for D6 (a) with TBAH ₂ PO ₄ (b) with TBAHSO ₄	132
Figure 4.17	(a) UV-visible spectra of D8 with and without TBAH ₂ PO ₄ in DMSO (b) Job's plot for D8 with TBAH ₂ PO ₄ (c) Benesi-Hildebrand plot for D8 with TBAH ₂ PO ₄	133
Figure 4.18	UV-visible spectra of D10 (a) with and without TBAH ₂ PO ₄ (b) with and without TBAHSO ₄ in DMSO	133
Figure 4.19	Jobs plot for D10 (a) with TBAH ₂ PO ₄ (b) with TBAHSO ₄	134

	and Benesi-Hildebrand plot for D10 (c) with TBAH ₂ PO ₄ (d) with TBAHSO ₄	
Figure 4.20	ESI-MS spectra for the complex formation (a) D2 with TBAH ₂ PO ₄ (b) D4 with TBAF (c) D4 with TBAI (d) D4 with TBAH ₂ PO ₄ (e) D6 with TBAH ₂ PO ₄ (f) D8 with TBAH ₂ PO ₄ (g) D10 with TBAH ₂ PO ₄	136
Figure 4.21	(a) Emission spectra of D2 with and without H ₂ PO ₄ ⁻ in DMSO. (b) Emission spectra of D2 with various anion salts in DMSO; λ _{ex} = 290 nm	137
Figure 4.22	(a) Emission spectra of D4 with and without F ⁻ in DMSO. (b) Emission spectra of D4 with and without Cl ⁻ in DMSO. (c) Emission spectra of D4 with and without I ⁻ in DMSO. (d) Emission spectra of D4 with and without H ₂ PO ₄ ⁻ in DMSO. (e) Emission spectra of D4 with and without HSO ₄ ⁻ in DMSO; λ _{ex} = 290 nm	138
Figure 4.23	a) Emission spectra of D6 with and without H ₂ PO ₄ ⁻ in DMSO. (b) Emission spectra of D6 with and without HSO ₄ ⁻ in DMSO; λ _{ex} = 310 nm	139
Figure 4.24	(a) Emission spectra of D8 with and without H ₂ PO ₄ ⁻ in DMSO; λ _{ex} = 290 nm	140
Figure 4.25	(a) Emission spectra of D10 with and without H ₂ PO ₄ ⁻ in DMSO. (b) Emission spectra of D10 with and without HSO ₄ ⁻ in DMSO; λ _{ex} = 310 nm	140
Figure 4.26	¹ H NMR titration plot of D2 with TBAH ₂ PO ₄ in DMSO- <i>d</i> ₆	141
Figure 4.27	¹ H NMR titration plots of D4 (a) with TBAF (b) with TBAI (c) with TBAH ₂ PO ₄ in DMSO- <i>d</i> ₆	143
Figure 4.28	¹ H NMR titration plot for D6 with TBAH ₂ PO ₄ in DMSO- <i>d</i> ₆	144
Figure 4.29	¹ H NMR titration plot for D8 with TBAH ₂ PO ₄ in DMSO- <i>d</i> ₆	145
Figure 4.30	¹ H NMR titration plot for D10 with TBAH ₂ PO ₄ in DMSO- <i>d</i> ₆	146

Figure 4.31	^1H NMR (CDCl_3 , 300 MHz) spectrum of D2	153
Figure 4.32	^{13}C NMR (CDCl_3 , 75 MHz) spectrum of D2	153
Figure 4.33	ESI-Mass spectrum of D2	154
Figure 4.34	^1H NMR (CDCl_3 , 300 MHz) spectrum of D4	154
Figure 4.35	^{13}C NMR ($\text{DMSO-}d_6$, 75 MHz) spectrum of D4	155
Figure 4.36	ESI-Mass spectrum of D4	155
Figure 4.37	^1H NMR (CDCl_3 , 300 MHz) spectrum of D6	156
Figure 4.38	^{13}C NMR (CDCl_3 , 75 MHz) spectrum of D6	156
Figure 4.39	ESI-Mass spectrum of D6	157
Figure 4.40	^1H NMR ($\text{DMSO-}d_6$, 300 MHz) spectrum of D8	157
Figure 4.41	^{13}C NMR ($\text{DMSO-}d_6$, 75 MHz) spectrum of D8	158
Figure 4.42	ESI-Mass spectrum of D8	158
Figure 4.43	^1H NMR ($\text{DMSO-}d_6$, 300 MHz) spectrum of D10	159
Figure 4.44	^{13}C NMR ($\text{DMSO-}d_6$, 75 MHz) spectrum of D10	159
Figure 4.45	ESI-Mass spectrum of D10	160
Figure 5.1	Cartoon diagram represents the gel formation	165
Figure 5.2	Inverted vial experiment for the gel formation of dendrons and dendrimers	171
Figure 5.3	SEM images for the xerogels (a) E5 (b) E7 (c) E8 (d) E7' (e) E8' (f) E10' (g) E11' (h) E12' (i) E13' (j) E16	173
Figure 5.4	AFM images for the xerogels (a) E5 (b) E7 (c) E8 (d) E7' (e) E8' (f) E10' (g) E11' (h) E12'	174
Figure 5.5	ATR-FTIR spectra for solid and xerogels from (a) E5 (b) E7 (c) E8 (d) E7' (e) E8' (f) E10' (g) E11' (h) E12' (i) E13' (j) E16	177
Figure 5.6	Panel a, c and e represents the frequency sweep rheometry of the gels formed from E5 , E7 and E8 respectively. Panel b, d and f represents the oscillation stress sweep experiments for E5 , E7 and E8 respectively. The studies were performed at 25 °C	180
Figure 5.7	Panel a and c represents the requery sweep rheometry	181

of the gels formed from **E7'** and **E8'** respectively. Panel b and d represents the oscillation stress sweep experiments for **E7'** and **E8'** respectively. The studies were performed at 25 °C

Figure 5.8	Panel a, c, e, and g represents the frequency sweep rheometry for the gels formed from E10'-E13' respectively. Panel b, d, f and h represents the oscillation stress sweep experiments for the gels from E10'-E13' respectively. The studies were performed at 25 °C	183
Figure 5.9	(a) Frequency sweep rheometry of the hydrogel from E15 and (b) oscillation stress sweep experiments. The studies were performed at 25 °C	184
Figure 5.10	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of E2	199
Figure 5.11	ESI-Mass spectrum of E2	199
Figure 5.12	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of E3	200
Figure 5.13	ESI-Mass spectrum of E3	200
Figure 5.14	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of E5	201
Figure 5.15	ESI-Mass spectrum of E5	201
Figure 5.16	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of E6	202
Figure 5.17	ESI-Mass spectrum of E6	202
Figure 5.18	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of E7	203
Figure 5.19	ESI-Mass spectrum of E7	203
Figure 5.20	¹ H NMR (DMSO- <i>d</i> ₆ , 300 MHz) spectrum of E8	204
Figure 5.21	ESI-Mass spectrum of E8	204
Figure 5.22	¹ H NMR (DMSO- <i>d</i> ₆ +D ₂ O, 300 MHz) spectrum of E9	205
Figure 5.23	ESI-Mass spectrum of E9	205
Figure 5.24	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of compound E5'	206
Figure 5.25	ESI-Mass spectrum of E5'	206
Figure 5.26	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of E6'	207
Figure 5.27	ESI-MS of compound E6'	207

Figure 5.28	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of E7'	208
Figure 5.29	ESI-Mass spectrum of E7'	208
Figure 5.30	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of E8'	209
Figure 5.31	ESI-Mass spectrum of E8'	209
Figure 5.32	¹ H NMR (D ₂ O, 300 MHz) spectrum of E9'	210
Figure 5.33	ESI-Mass spectrum of E9'	210
Figure 5.34	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of E10'	211
Figure 5.35	ESI-Mass spectrum of E10'	211
Figure 5.36	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of E11'	212
Figure 5.37	ESI-Mass spectrum of E11'	212
Figure 5.38	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of E12'	213
Figure 5.39	ESI-Mass spectrum of E12'	213
Figure 5.40	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of E13'	214
Figure 5.41	ESI-Mass spectrum of E13'	214
Figure 5.42	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of E14'	215
Figure 5.43	ESI-Mass spectrum of E14'	215
Figure 5.44	¹ H NMR (CDCl ₃ , 300 MHz) spectrum of Boc-1-H-pyrazole carboxamidamide	216
Figure 5.45	ESI-Mass spectrum of Boc-1-H-pyrazole carboxamidamide	216
Figure 5.46	¹ H NMR (DMSO- <i>d</i> ₆ , 300 MHz) spectrum of E15	217
Figure 5.47	ESI-Mass spectrum of E15	217
Figure 5.48	¹ H NMR (D ₂ O, 300 MHz) spectrum of E16	218
Figure 5.49	ESI-Mass spectrum of E16	218

LIST OF SCHEMES

Scheme No.	Description	Page No.
Scheme 2.1	Synthetic scheme for cysteine-based sensors (B3-B5)	32
Scheme 3.1	Synthetic scheme for cysteine-based receptors (C2-C4)	71
Scheme 4.1	Synthetic scheme for D2, D4, D6, D8, and D10, with Trp units, placed at different positions on an aromatic ring	115
Scheme 5.1	Synthesis of Lysine alkyne dendron E3	167
Scheme 5.2	Synthesis of Asp/Glu-based lipidated dendrons and dendrimers	168
Scheme 5.3	Synthesis of higher generation dendrons (E10'-E12') and dendrimer (E13'-E14') from Boc-protected glutamic acid	169
Scheme 5.4	Synthesis of guanidine-based dendrimers E15-E16	170
Scheme 5.5	Preparation of Boc-1-H-Pyrazole carboximidamide	195

LIST OF TABLES

Table No.	Description	Page No.
Table 2.1	Parameters calculated from time-resolved fluorescence spectra of B3 alone and B3 with Cu^{2+}	35
Table 2.2	Parameters calculated from time- resolved fluorescence spectra of B4 alone and B4 with Ag^+	38
Table 2.3	ITC studies on the complexation of B3-B5 toward Cu^{2+} , Ag^+ and Hg^{2+}	41
Table 2.4	Theoretically calculated ^{13}C NMR chemical shifts for B4 and B4 + Ag^+ . The red colored carbon atoms showed a maximum shift ($\Delta\delta$) for B4 . 2Ag^+ complex	48
Table 3.1	Parameters calculated from time-resolved fluorescence spectra of C2 alone and with Cu^{2+}	76
Table 3.2	Comparison of ^{13}C NMR Chemical shift values of C3 with and without Hg^{2+} . Carbon atoms in red color showed changes in chemical shift values upon Hg^{2+} complexation	78
Table 3.3	Parameters calculated from time- resolved fluorescence spectra of C3 alone and with Hg^{2+}	80
Table 3.4	Parameters calculated from time- resolved fluorescence spectra of C4 alone and with Cd^{2+}	82
Table 3.5	Parameters calculated from time- resolved fluorescence	86

	spectra of C2:C3 alone and with Pb ²⁺	
Table 3.6	FRET efficiency of C2:C3 ($\lambda_{\text{ex}} = 290 \text{ nm}$) with Pb(II)	94
Table 4.1	Vesicle sizes of D2, D4, D6, D8, and D10 along with the solvent systems	117
Table 4.2	Screening of solvents for gelation of D8 . S (Soluble), NS (not soluble), PS (partially soluble), G (gel), Ppt (Precipitate). Right handed side picture shows the gel of D8 in Chloroform + Hexane. Gel formation was observed for a concentration of 3 mM	118
Table 4.3	Crystal data of D10 (CCDC 1453379)	123
Table 4.4	Crystal data of D10 showing the bond distances for hydrogen bonding and for amide –NH and DMSO interaction	124
Table 4.5	Calculated association constants for the binding of D2, D4, D6, D8, and D10 with various anions. Benesi-Hildebrand method was used for determining the association constants. Nd- Not determined due to very small changes in UV-visible spectrum of compounds with anions	134
Table 5.1	Screening of solvents for gelation of dendrons and dendrimers. NS (not soluble), PS (partially soluble), G (gel formation), S (Soluble), Ppt (Precipitate)	172
Table 5.2	ATR-FTIR spectroscopic data for the dendrons and	178

dendrimers in their solid as well in gel state

Table 5.3 Gelation ability and mechanical strength of dendrons and dendrimers

184

NOTES

1. All amino acids used were of L-configuration. Unless otherwise stated, all reagents were used without further purification.
2. All solvents employed in the reaction were distilled or dried from appropriate drying agent prior to use.
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. HRMS were recorded with Bruker MicrO-TOF- QII model and AB Sciex, 1011273/A model using ESI-technique.
7. 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.
8. UV-Visible spectra were recorded in Shimadzu double beam spectrophotometer, UV-2400.
9. Emission spectra were recorded using HORIBA JOBIN YVON Scientific, fluoromax-4 spectrophotometer, with slit width of 5 nm.
10. Time-resolved fluorescence spectra were recorded using an IBH picosecond single-photon-counting system with an adjustable nano-LED excitation source.

11. Isothermal Calorimetric Titration (ITC) experiments were performed by MicroCal iTC₂₀₀ system at 298 K.
12. SEM measurements were done using ZEISS EVO[®] and JEOL-JSM-5600 LV, instrument with an EHT of 20 kV.
13. AFM measurements were performed using Nanoscope Multimode AFM operating in tapping mode in air.
14. HR-TEM images were recorded using Philips CM 12 electron microscope.
15. ATR-FTIR measurements were performed on Agilent-Cary 660 series FTIR spectrometer.
16. Rheological measurements were done on MODULAR COMPACT RHEOMETER (MCR-150, Anton Par Ltd, India, PAAR PHYSICA, Germany).
17. Differential Scanning Calorimetric (DSC) experiments were carried out using Perkin Elmer Diamond DSC

LIST OF ABBREVIATIONS

%	percent
δ	chemical shift
$^{\circ}\text{C}$	degree centigrade
τ	decay-time
eV	electron volt
kV	Killo volt
aq.	aqueous
AFM	Atomic force spectroscopy
Boc	t-butyloxycarbonyl
br	broad
Conc.	Concentrated
d	doublet
dd	double doublet
DCC	N,N'-dicyclohexylcarbodiimide
DIEA	N,N'-Diisopropylethylamine
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
ESI-MS	Electrospray ionization mass spectroscopy
equiv.	Equivalents
F	Fluorescence intensity
g	gram

G'	Storage modulus
G''	Loss modulus
h	hour
Hz	Hertz
HRMS	High resolution mass spectra
HR-TEM	High resolution transmission electron microscope
IR	infrared
J	coupling constant
K	kelvin
Kcal/mol	Kilocalories per mol
KJ/mol	Kilo joules per mol
M	molar
μ M	micromolar
mM	millimolar
m	multiplet
mg	milli gram
mL	milli liter
min	minutes
mmol	milli moles
mol	mole
mp	melting point
m/z	mass/charge
NHS	N-hydroxysuccinimide

NMR	Nuclear magnetic resonance
ns	nano seconds
nm	nanometer
ppm	parts per million
q	quartet
RT	Room temperature
s	singlet
SEM	Scanning electron microscope
TBAF	Tetrabutylammonium fluoride
TBACl	Tetrabutylammonium chloride
TBABr	Tetrabutylammonium bromide
TBAI	Tetrabutylammonium iodide
TBAH ₂ PO ₄	Tetrabutylammonium phosphate
TBAHSO ₄	Tetrabutylammonium sulfate
TEM	Transmission electron microscope
t	triplet
TLC	Thin layer chromatography
UV-vis.	Ultraviolet-visible

TABLE OF CONTENTS

	Page No.
Abstract	i
List of figures schemes and tables	iii
List of schemes	xv
List of tables	xvi
Notes	xix
List of abbreviations	xxi
CHAPTER 1: Introduction	
1.1 An overview of supramolecular chemistry	1
1.2 Fluorescence-based detection techniques	4
1.3 Peptide-based fluorescent sensors for cations and anions	5
1.4 Dendritic peptide-based systems and their self-assembling properties	14
References	22
CHAPTER 2: Cysteine-based fluorescence “turn-on” sensors for Cu²⁺ and Ag⁺	
2.1 Introduction	30
2.2 Results and Discussion	31
2.2.1 Design and synthesis	31
2.2.2 UV-visible and Emission spectroscopic studies for sensing ability of B3 with metal ions	32
2.2.3 Determination of binding constant for B3 with Cu ²⁺	35
2.2.4 UV-visible and Emission spectroscopic studies for sensing ability of B4 with metal ions	35
2.2.5 UV-visible spectroscopic studies for sensing ability of B4 with metal ions	38
2.2.6 Isothermal calorimetric titration experiments (ITC) for metal complexation	39

2.2.7 Electron microscopic studies for the supramolecular interaction	41
2.2.8 Density functional theory (DFT) calculation	43
2.3 Conclusion	49
2.4 Experimental Section	50
References	65
CHAPTER 3: A supramolecular approach to metal ion sensing: Cystine-based designer systems for Cu²⁺, Hg²⁺, Cd²⁺ and Pb²⁺ sensing	
3.1 Introduction	69
3.2 Results and Discussion	70
3.2.1 Design and synthesis	70
3.2.2 UV-visible spectroscopy studies for metal uptake	72
3.2.3 Emission spectroscopy for metal uptake	73
3.2.4 DFT calculations for C4 -Cd ²⁺ complex	83
3.2.5 Supramolecular complex formation	84
3.2.6 DFT calculations for heterodimer supramolecular complex	87
3.2.7 ITC titration experiments for supramolecular interaction	89
3.2.8 SEM and DSC analysis for supramolecular interaction	90
3.2.9 Metal uptake ability of C2:C3 and DFT studies	91
3.3 Conclusion	95
3.4 Experimental Section	95
References	109
CHAPTER 4: Spatially placed tryptophan residues: A strategy for generating molecules with unique self-assembly and molecular recognition properties	
4.1 Introduction	113
4.2 Results and Discussion	114
4.2.1 Design and synthesis	114

4.2.2 Study of self-assembly	115
4.2.3 Rheological measurements for the organogel from D8	120
4.2.4 Crystal structure of D10	121
4.2.5 Encapsulation studies	124
4.2.6. Anion binding studies	126
4.2.7 UV-visible spectroscopic studies for anion binding	126
4.2.8 ESI-MS technique for complex formation	135
4.2.9 Emission spectroscopy for binding studies	137
4.2.10 ¹ H NMR studies for anion binding	141
4.3 Conclusion	146
4.4 Experimental Section	147
References	161
CHAPTER 5: Designer Lipidated Peptide Dendrons and Dendrimers as organogelators-morphological and mechanical studies	
5.1 Introduction	165
5.2 Results and Discussion	166
5.2.1 Design and synthesis	166
5.2.2 Investigation of self-assembly	170
5.2.3 Attenuated total reflectance (ATR-FTIR) spectroscopic studies	174
5.2.4 Rheological measurements for the gels	177
5.3 Conclusion	185
5.4 Experimental Section	185
References	219
Brief Bio-data of the author	223