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Review Article

Designing Biomimetic Systems Using Nature-Inspired Supramolecular Chemistry Principles**

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The topics covered in this review article are relatively distinct yet based on a common platform of designing biomimetics employing Nature-inspired supramolecular chemistry principles. The first section deals with the design, synthesis and biophysical characterization of carbocyclic and pyrrolidine peptide nucleic acids as nucleic acid mimics. These nucleic acid mimics forms highly stable complexes with complementary RNA compare to DNA with identical sequence. Second section constitutes a preliminary account of our recent effort in developing modular peptide-based materials with nanoscale order. Simplest classes of acyclic or cyclic peptides are cyclic dipeptides which can form (N-H- -O) hydrogen bonded molecular chains and molecular layers. Aromatic cyclic dipeptides have been employed as modular units to build biomimetic materials with structural hierarchy similar to that of natural materials. For instance cyclic dipeptide of (Phe-Phe) forms fiber bundles with high thermal stability and the structural hierarchy resemble that of natural fibrous materials such as spider silk and collagen. In other words, cyclic dipeptide of (Phg-Phg) undergo spontaneous self-assembly to form two-dimensional nano and mesosheets with layered structural hierarchy resembling that of graphene. Similarly, employing Nature-inspired biomimetic approach the morphology of naphthalenediimide (NDI) nanostructures was tuned into nanospheres, nanospheres aggregates, nanobelts, microfibers (nanobelts bundles) and fractals. Third section is focused on the development of chemosensors for metal ions based on supramolecular host-guest chemistry inspired by biopolymer-metal ion interactions. Novel chemosensors based on conformationally constrained fluoroionophore approach were developed for selective sensing of Al^{3+} and Zn^{2+} by means of colorimetric and fluorometric optical response. New class of chemosensors with visible-near infrared (Vis-NIR) optical response for copper detection has been developed recently based on salicylaldehyde-urea/thiourea conjugate platform.

Key Words: Supramolecular Chemistry; Biomimetic Systems; Nucleic Acid; Antisense; Peptide; Nanostructures; Chemosensors

1. Introduction

In recent years, supramolecular chemistry has gained enormous interest in the research community as a valuable tool for designing biomimetic systems close to Nature's perfection with interesting and unexplored properties [1]. Supramolecular Chemistry refers to chemistry beyond molecules and deals with systems obtained through assembly of discrete number of molecules. It also focuses on the self-assembly of modular molecular units driven by noncovalent interactions to produce various forms of molecular systems and materials with nanoscale order [1]. In Nature, the supramolecular interactions plays a crucial role in controlling the intramolecular conformation and the intermolecular ordering of many biopolymers such as peptides, proteins, nucleic acids and host-guest interactions between

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biopolymers and metal ions. Some of the concepts that have been demonstrated utilizing supramolecular chemistry principles include molecular recognition, molecular self-assembly and host-guest chemistry [2]. In particular, nucleic acids are the basic building blocks of all forms of life and perhaps the best known self-assembling system. The double stranded helical structure of DNA is key for its use as genetic material in living organisms [3]. Each strand of DNA is about 2 nm wide and composed of a linear chain of four possible nucleobases (A: adenine, C: cytosine, G: guanine, and T: thymine) on a backbone of alternating sugar molecules and phosphate ions. Two strands of single stranded DNA sequences forms a double helix through complementary A-T (two hydrogen bonds) and G-T (three hydrogen bonds) base paring. The phosphate ion carries a negative charge in the DNA molecule.

Supramolecular chemistry has a strong dependence on the nature of the modular molecular units, the type of noncovalent interactions and the eventual incorporation of functional subunits [4]. The noncovalent interactions may involve complementary hydrogen bonding, π-π interactions, electrostatic forces, electronic donor-acceptor interactions, hydrophobic interactions and metal-ligand interactions [5]. Combinations of two or more of these interactions additively contribute to the overall strength of supramolecular systems and derived materials. Polypeptides and proteins are the building blocks of a range of biological materials. Many of these materials are responsible for variety of essential biological functions in living organisms. Self-assembly based nano and microstructures can play an important role as substitutes for various materials of biological origin which are difficult to obtain in bulk quantity. Metal-ligand interactions that are not only thermodynamically stable, depends on the metal ion and ligand used, can also be kinetically labile, play important role in designing biomimetic host-guest systems. The thermodynamic stability of the metal-ligand interactions will determine strength of the host-guest complex, while their kinetic lability makes them responsive to environmental stimuli. Particularly organic ligands with in-built photoresponsive units make them superior sensors for metal ions and therefore can be used for environmental and biomedical applications. The incorporation of metal ions opens up the possibility of imparting the functional properties such as catalysis, light-emission, ionic conduction, gas absorption and storage to the resulting host-guest systems as well derived materials [6]. In this review article an overview of three different biomimetic systems developed based on supramolecular chemistry principles will be presented. These supramolecular systems were generated by employing the Nature-inspired noncovalent interactions that are prevalent in macromolecular systems such as nucleic acids, proteins and metallo-biopolymer (host-guest system) to construct biomimetic systems as well as derived materials. These biomimetic systems and materials may become indispensable and much anticipated solutions for biomedical, biomaterials, environmental and energy related issues.

2. Biomimetics of Nucleic Acids

Drug design involving small organic molecules against disease related proteins requires structural knowledge of binding site of the target. Understanding specific binding forces between the host (protein) and the guest (drug) is of primary importance for successful drug design. This traditional drug discovery process has serious limitations due to the fact that our knowledge on protein structure-function is very minimal and protein-targeted drugs are far from the concept of magic bullet. In contrast, the nucleotide sequence in DNA and RNA is universal and the detailed information on structure-function is available. Therefore from the drug design point of view nucleic acids are better targets compared to proteins. In principle, a short piece of oligonucleotide itself can act as a drug for DNA/RNA target [7]. The oligonucleotide based drugs are designed to target DNA in the nucleus (antigene)/RNA in the cytoplasm (antisense) sequence specifically and check the production of wrong proteins responsible for particular disease of interest (Fig. 1). Antisense therapy is preferred method as the RNA lies in the cytoplasm and delivering oligonucleotide drug to the target is much easier among other issues compared

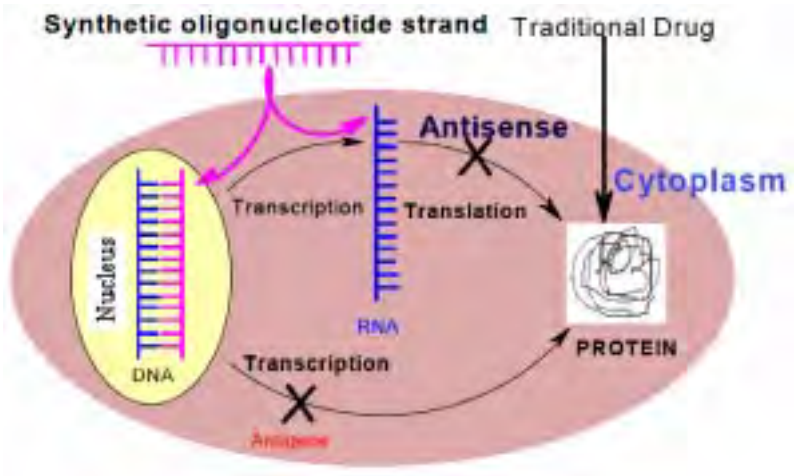

Fig. 1: Principle of action of antigene and antisense oligonucleotides

to targeting DNA. However, the unmodified oligonucleotide drugs are rapidly identified and cleaved by the action of nucleases that primarily hydrolyze the phosphodiester of the internucleoside backbone. Furthermore, the ability of the negatively charged oligonucleotide to cross phospholipid cell membrane is poor. Such drawbacks necessitates the need for structural modifications to enable oligonucleotides more stable to be used as potential antisense therapeutic agents and also to understand the conformational changes that they introduce in the oligonucleotides incorporating them [8]. The structural changes include the modifications in nucleobases, phosphodiester linkage and sugar moiety (Fig. 2) [8]. In the area of nucleic acid analogues, there have been several modifications reported to date. Among all, peptide nucleic acids (PNA) are found to mimic many of the oligonucleotide properties. PNA composed of *N*-(2-aminoethyl) glycine units to which natural nucleobases are attached *via* methylenecarbonyl linker (Fig. 2, *aeg*PNA), an acyclic sugar-phosphate backbone modification [9, 10]. PNA binds complementary DNA and RNA to form duplexes *via* Watson-Crick base pairs and triplexes through Watson-crick base pair and Hoogsteen hydrogen bonding that exhibit thermal stability higher than that of DNA-DNA and DNA-RNA complexes. Because of higher thermal stability and resistant to protease and nuclease cleavage, PNAs are of great interest in medicinal chemistry. PNAs are considered as potential agents for the development of gene-targeted drugs (antigene and antisense) and as reagents in molecular biology and diagnostics. However, PNA suffers from drawbacks such as poor aqueous solubility, cell permeability, ambiguity in binding complementary DNA/RNA in both parallel and antiparallel orientations and equal affinity in binding DNA and RNA attribute to its flexibility (Fig. 3). Some of the PNA deficiencies have been addressed by rational chemical modifications [10]. However a sufficiently important problem from an application perspective that has not been adequately dealt with is nondiscrimination of identical DNA and RNA sequences by PNA antisense molecules [12]. Because of its equal affinity towards DNA and RNA, PNA can access identical sequences but on different targets in the cytoplasm (mRNA) and nucleus (DNA) leading to undesirable consequences.

To address this deficiency of flexible PNA, we have applied a different rationale for structural design to induce binding selectivity towards RNA that involves preorganization of the PNA backbone to conformational features attained in PNA:RNA complexes [11]. An analysis of X-ray and NMR structural data [13] suggests that the preferred values of dihedral angle β in PNA:RNA duplex is in the range of 65-70° while that for the PNA:DNA duplex is about ~140°, suggesting that it may be possible to

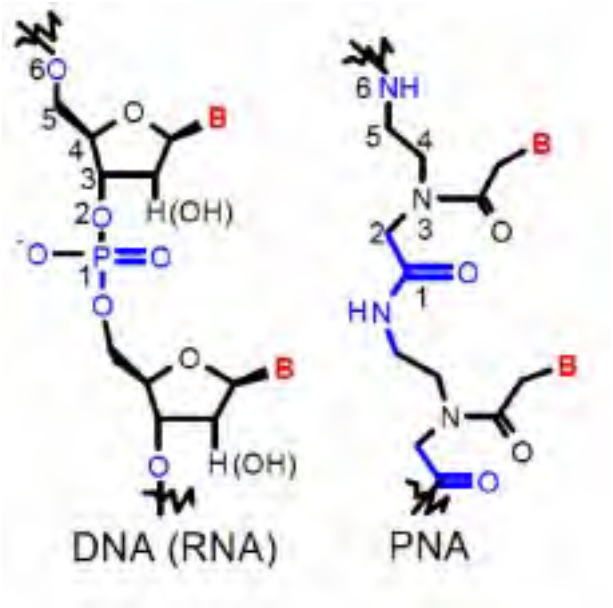

Fig. 2: Chemical structures of DNA (RNA) and PNA. B = Nucleobases: Adenine (A)/Thymine (T)/Guanine (G)/ Cytocine (C).

impart DNA/RNA binding selectivity by restricting β to 65-70° range through suitable modification. We designed PNAs with *cis*-cyclohexyl constraint in the backbone, where the dihedral angle β would be around 65° (Fig. 4) [12, 14-16]. The enantiomeric *ch*PNAs derived through such a strategy will provide entropic advantage and the chirality introduces the orientational selectivity in binding to complementary RNA sequences. In an attempt to tune the dihedral angle β and to strike a balance between flexibility and rigidity, rather to keep a situation where the PNA:RNA/DNA complex formation should facilitated by "balanced enthalpy and entropic contribution", *cis*-cyclopentyl PNAs (*cp*PNA) were designed (Fig. 4, *cp*PNA) [17, 18]. The flexible *cis*-cyclopentane ring [19] in the pre-organized PNA structure makes it to bind complementary DNA/RNA sequence with greater affinity, represents the study of reduced dihedral angle β in *cp*PNA compare to *ch*PNA.

Cyclohexyl and cyclopentyl building blocks were accessed in their enantiomerically pure form through a synthetic routes involving enzymatic resolution of key intermediates [12, 14-18]. The synthetic routes also provide an easy access to chiral *cis*-cyclohexyl and cyclopentyl diamines, which are otherwise difficult to obtain in their optical pure form. The torsion angles were determined from the single crystal X-ray structures of monomers (Table 1) [12, 17].

Various PNA sequences were constructed incorporating *ch*PNA and *cp*PNA monomers including a biologically relevant *ch*PNA sequence corresponding to a part of the gene code for mutated version of p53 protein [15, 20]. The binding selectivity, specificity and discrimination of *ch*PNAs

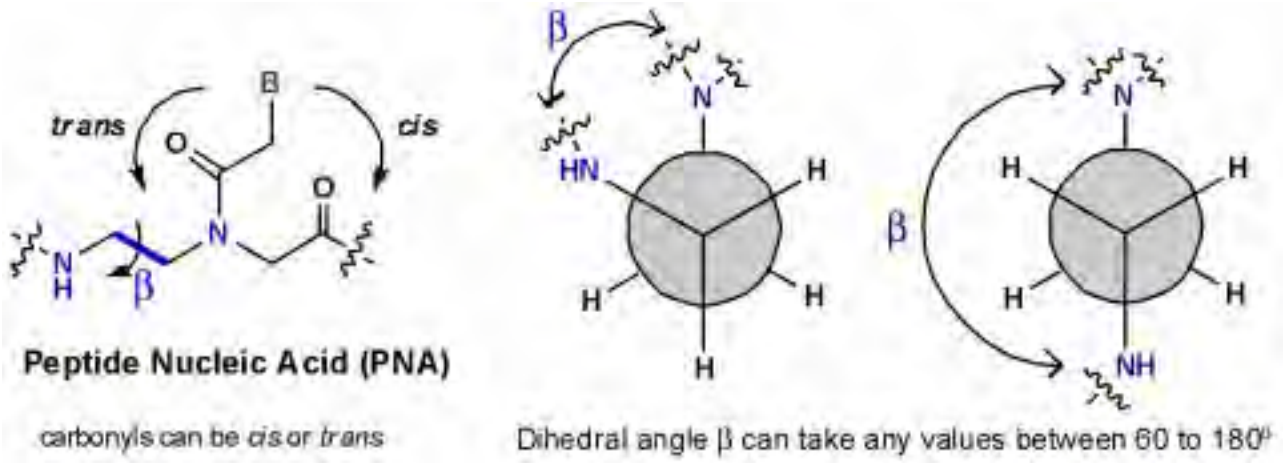

Fig. 3: The flexibility of *aeg*PNA is attributed to i) ethylenediamine portion of the backbone (Dihedral angle β) and ii) side chain linker connecting nucleobase to main chain (cis or trans). B = Nucleobases: Adenine (A)/Thymine (T)/Guanine (G)/Cytocine (C).

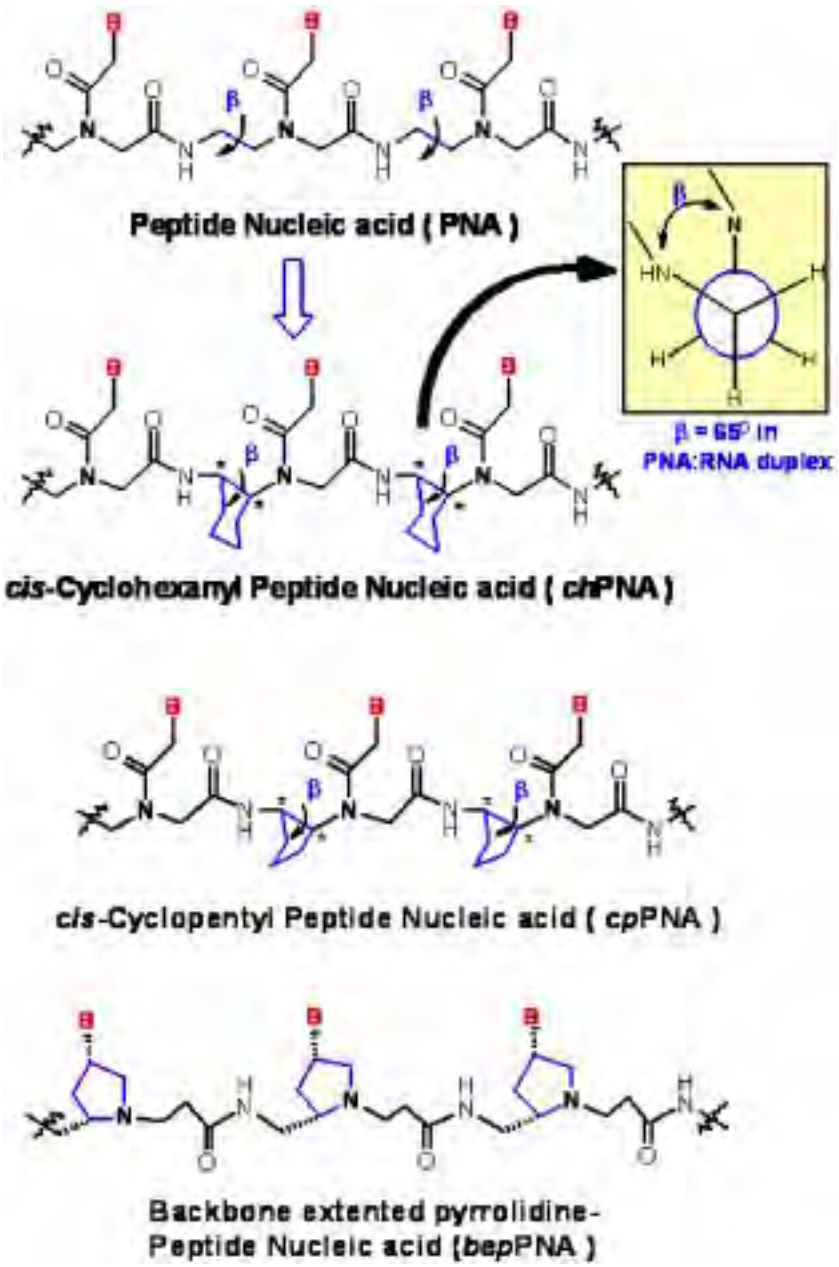

Fig. 4: Tuning the dihedral angle over ethylene diamine portion (blue) of PNA to that observed in PNA:RNA duplex (65°) using cis-cyclohexyl ring constraint (*ch*PNA). Also shown are the chemical structure of cis-cyclopentyl peptide nucleic acid (*cp*PNA) and backbone extended pyrrolidine peptide nucleic acid (*bep*PNA). Where B = Nucleobases: Adenine (A)/Thymine (T)/Guanine (G)/Cytocine (C).

and *cp*PNAs towards complementary DNA and RNA were investigated using various biophysical techniques such as UV-thermal denaturation studies, circular dichroism, competitive binding-gel electrophoresis shift assay and Isothermal titration calorimetry in comparison with corresponding *aeg*PNA. The UV-T_m thermal stability data of the *ch*PNA and *cp*PNA duplexes with complementary DNA/RNA were shown in Table 2 (*ch*PNA) and Table 3 (*cp*PNA) [15, 17, 18]. The most important feature of the data in Table 2 is that *SR*/*RS*-*ch*PNAs destabilize the DNA duplex but enormously stabilize the RNA duplex, while *SR/RS cp*PNAs remarkably stabilize both RNA and DNA complexes (Table 3).

Table 1: Dihedral angles (º) in PNA and PNA:DNA/RNA complexes [12], [17]

Compound	α	β	γ	δ
PNA_2:DNA	–103	73	70	93
PNA:DNA	105	141	78	139
PNA:RNA	170	**67**	79	84
*ch*PNA(1*S*,2*R*)*	128	**–63**	76	119
*ch*PNA(1*R*,2*S*)*	–129	**66**	–78	–119
*cp*PNA(1*S*,2*R*)*	84	–24	86	90
*cp*PNA(1*R*,2*S*)*	–84	25	–86	–90

*dihedral angles from *ch*PNA and *cp*PNA monomer crystal structures.

Table 2 : T_m (ºC) of PNA:DNA/RNA hybrids [15]

PNA sequences		DNA	RNA
*aeg*PNA	H -G T AGATCACT-$LysNH_2$	55.0	55.4
*ch*PNA	H-G T_{SR} $AGAT_{SR}CACT_{SR}$-$LysNH_2$	25.0	58.0
*ch*PNA	H-G T_{RS} $AGAT_{RS}CACT_{RS}$-$LysNH_2$	35.0	>85
p53 sequence			
*aeg*PNA	H-GGCAGTGCCT-$LysNH_2$	59.5	64.0
*ch*PNA	H-GGCAGT_{SR}GCCT-$LysNH_2$	58.5	74.5
*ch*PNA	H-GGCAGT_{SR}GCCT_{SR}-$LysNH_2$	55.5	>85.0
*ch*PNA	H-GGCAGT_{RS}GCCT-$LysNH_2$	57.0	83.0
*ch*PNA	H-GGCAGT_{RS}GCCT_{RS}-$LysNH_2$	56.0	>85.0

Table 3: T_m (ºC) of PNA:DNA/RNA hybrids [17], [18]

PNA sequences	DNA	RNA
*cp*PNA , H-$(T_{SR})_8$-$LysNH_2$	66.6	>85.0
*cp*PNA, H-$(T_{RS})_8$-$LysNH_2$	72.0	>85.0
*aeg*PNA. H-G T AGATCACT-$LysNH_2$	55.0	55.4
*cp*PNA. H-G T_{SR} $AGAT_{SR}CACT_{SR}$-$LysNH_2$	77.1	84.0
*cp*PNA. H-G T_{RS} $AGAT_{RS}CACT_{RS}$-$LysNH_2$	78. 8	>85.0

In the process, the *ch*PNAs induce remarkable differences in duplex stabilities among their DNA and RNA complexes, with ΔT_m (RNA/DNA) = +33°C for *SR-ch*PNA 2 and +50°C for *RS-ch*PNA 3. This is a highly significant, exceptional binding selectivity of conformationally modified PNA to RNA over the DNA with identical sequence [14, 15].

These results show the strong preference of (*SR*/*RS*)-*ch*PNA to bind RNA is in consonance with our strategy of adjusting dihedral angle β through chemical modifications to achieve structure-based selectivity in PNAs. This is perhaps the first report of any PNA analogue that overwhelmingly discriminates identical DNA and RNA sequences with a strong preference for RNA. The observed differences in DNA/RNA affinity among *cis*-cyclohexanyl and *cis*-cyclopentanyl PNAs arise from differences in their relative backbone flexibility. The rigidity of the *cis*-cyclohexane ring results in a fixed dihedral angle, β ~ 65°, appropriate for RNA selectivity, whereas the flexible *cis*-cyclopentane ring with a reduced angle, β ~ 25°, is prone to adapt for complementation with both DNA and RNA due to inherent ring puckering [17, 18]. Delineating the relative contributions of these properties may help to achieve optimal fine tuning of PNA chemical modifications to attain balanced affinity/selectivity towards target sequences. Here we assume that the RNA binding selectivity of *ch*PNA over *aeg*PNA is a case of *steric fit* model [21], where preorganized *ch*PNA accommodate sterically fit RNA sequence as shown in Fig. 5. Where as flexible *aeg*PNA follows *induced fit* mechanism and attain conformations suitable for both DNA and RNA binding as guided by the target DNA/RNA sequence. The chiral pyrrolidine PNA with an extended seven-atom backbone (Fig. 4, *bep*PNA) was designed [22]. The positive charge and chirality in the backbone have additional advantage such as increased water solubility and orientational selectivity in binding to complementary RNA/DNA strand. UV-T_m data along with gel shift assay clearly suggested that *bep*PNA monomer introduced the DNA/RNA binding selectivity and affinity depending on the position and the number of units present in PNA sequence [22, 23].

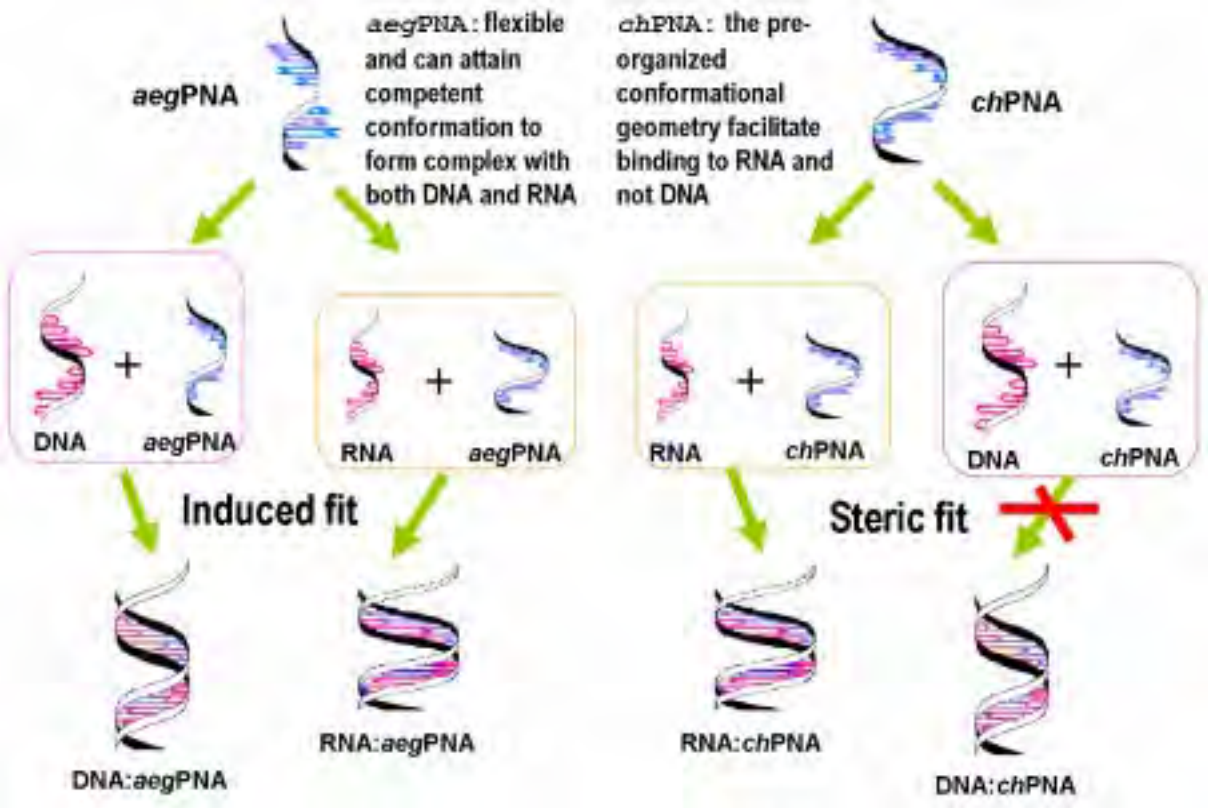

Fig. 5: Cartoon showing proposed binding models of *aeg*PNA and *ch*PNA

3. Biomimetic Materials

Molecular self-assembly based bottom up approach is a fascinating area of research for fabricating nano-meso- and microscopic structures with unexplored properties. Biopolymers are the basic building blocks of a range of natural materials with essential biological functions [24]. An active area of research in our laboratory is to pursue the design and synthesis of self-assembly driven small peptide-based materials possessing well defined nano-, meso- and micro-structures with properties similar to natural materials such as spider silk, collagen and graphene through molecular self-assembly approach. The self-assembly based materials with nanoscale order play an important role as substitutes for various materials of biological origin which are difficult to obtain in bulk quantity [25]. In particular, peptide-based materials are of great interest due to their modularity and variety of chemical modifications are possible to tune the structure and physico-chemical properties to suit a desired application [26]. Polypeptides and proteins are the building blocks of a range of biological materials such as spider silk, collagen, actin and keratin (Fig. 6) [24, 27]. These materials have essential biological functions such as structural stability, mechanical (cytoskeletal) strength, self-defense, and many other physiological functions. Spider silk has been at the center stage as a topic of special interest to scientists of various disciplines [28]. Spider silk is five times stronger by weight than steel and comparable to that of synthetic polymer kevlar. Thus, spider silk is one of the most sought after materials, gaining a reputation as the "Holy Grail" of biomaterials. A balanced combination of properties such as high tensile strength, stiffness, and elasticity makes the spider silk fibers as one of the

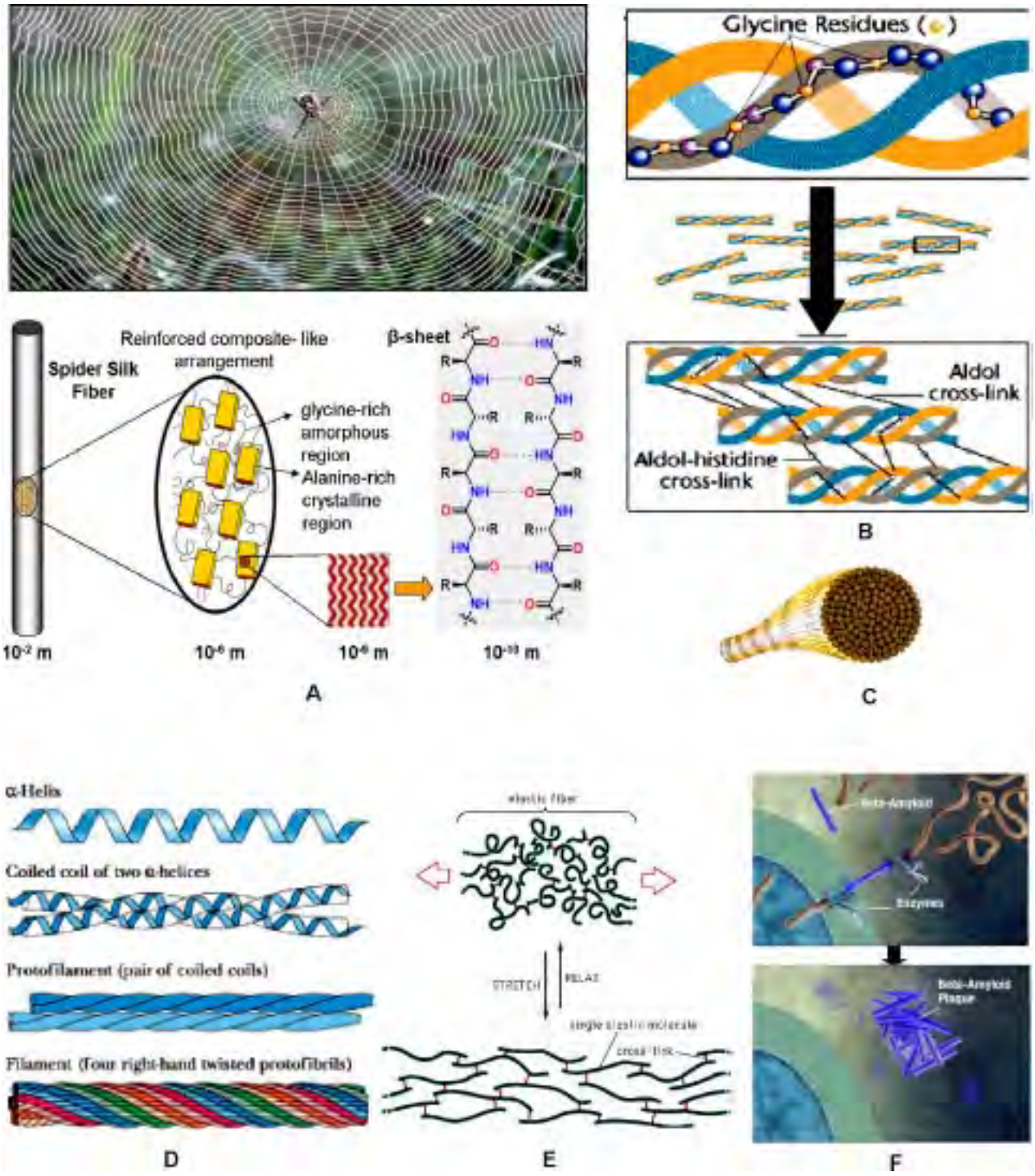

Fig. 6: Representative natural protein based materials and their basic structural characteristics. (A) spider silk, (B) collagen, (C) hierachical structure of natural fibers (Fiber bundle), (D) keratin, (E) elastin and (F) β-Amyloid fibrillar aggregates. Source: (B) and (C) www.mun.ca; (D) and (E) www.imb-jena.de; (F) www.researcher.nsc.gov.tw.

toughest known fiber to man [28, 29]. Therefore mimicking its structure and properties has been an ongoing challenge in material science. Spider dragline silk consists of two reinforced protein segments, spidroins I and II [30]. These protein segments can be described as block copolymers with alternating

polyalanine and glycine-rich blocks. The polyalanine domains within the fully formed silk form anti-parallel β-sheets which constitute mainly crystalline region providing high strength and stiffness [31]. The glycine-rich regions constitute amorphous region which bestow extensibility [31]. The crystalline and amorphous regions reinforced to form a strong spider silk fibers. Overall, a generalized structure of spider silk is considered to be crystalline regions in an amorphous matrix. However it is not entirely clear how the protein molecules align and undergo self-assembly to form silk but it involve mechanical and frictional forces that arise during passage through the spider's spinning organs. Hence the extraordinary strength of spider silk is the right combination of chemistry and engineering. Utility of spider silk is limited as the bulk production of spider silk is still a challenge. Harvesting spider silk from spiders is difficult as they are just too territorial and predate on each other. Many research groups and companies worldwide have strong interest and programmes on producing spider silk in bulk quantity [32]. Nexia Biotechnologies has grown spider silk in genetically engineered goats. The spider's gene was inserted into goats such that the silk protein was expressed in milk [32c, 33]. The silk protein was precipitated from the milk as fibrous material which is named BioSteel. The major limitation being the scale of production, three liters of milk would give 2-3 mg of silk protein which is far from realizing the practical applicability. Another drawback was spinning the protein into fibers with properties similar to that of natural spider silk. Similarly production of spider silk proteins in tobacco and potato have been reported [34]. In a different approach peptide blocks with (Gly-Ala) repeating units have been used as conjugates of π-conjugated systems or as amphiphilic systems to study their self-assembly behaviour [35]. Some of these peptide conjugates have been shown to form nanofibers [35]. However these conjugates were studied for specific purpose and not as mimics of spider silk. Fibrillar aggregates of polypeptides and proteins have also been implicated in various human diseases such as Alzheimer's, Parkinson's, type II diabetes, and prion diseases (Fig. 6F) [36]. The reasons for studying peptide self-assembly are of twofold; i) to understand and provide insight into the molecular mechanisms underlying the formation of fiberillization, ii) to discover new synthetic peptide-based biomimetic materials with potential for applications. Molecular self-assembly driven by noncovalent interactions is the main driving force that Nature finally evolved to produce various forms of materials [37]. Modular peptide-based biomimetic materials with nanoscale order may find applications in material science and biomedical research [26].

The main goal in our laboratory is to design natural or unnatural modular peptide-based building blocks and scaffolds to produce synthetic biomimetic fibers with nanoscale order. The strategy was to combine the available knowledge of natural fibers such as spider silk and collagen (hydrogen bonding) in combination with toxic fibrillar aggregate formation (aromatic interaction) that is responsible for various neurodegenerative diseases to design and produce biomimetic fibers. The π-π interactions between aromatic side chains of amino acids in polypeptides are known to induce peptide fibrillation [38]. Thus aromatic π-π interactions will serve as one of the important noncovalent forces together with hydrogen bonding in the design of biomimetic fibers.

Based on this design concept we have demonstrated the potential of simplest aromatic cyclic dipeptides (CDP) as modular building blocks for producing biomimetics of natural materials (Fig. 7). CDPs are the simplest members of the cyclic peptides exhibiting exceptional rigidity and stability. Aromatic cyclic dipeptides can undergo spontaneous molecular self-assembly to form 1D and 2D materials with high thermal stability. CDPs are known to form (N-H- -O) hydrogen bonded molecular chains and in few cases molecular layers in crystalline solids [39]. These (N-H- -O) hydrogen bonded motifs along with aromatic π-π interactions through α-substituents have been successfully employed to produce fiber bundles and 2D sheets [40, 41, 42]. Aromatic CDP of (Phe-Phe) undergo molecular self-assembly by means of (N-H- -O) hydrogen bonding supported by aromatic π-π interactions to form fibers. A detailed study on the spontaneous self-assembly of aromatic cyclic dipeptide (Phe-Phe) revealed that the fibers are indeed

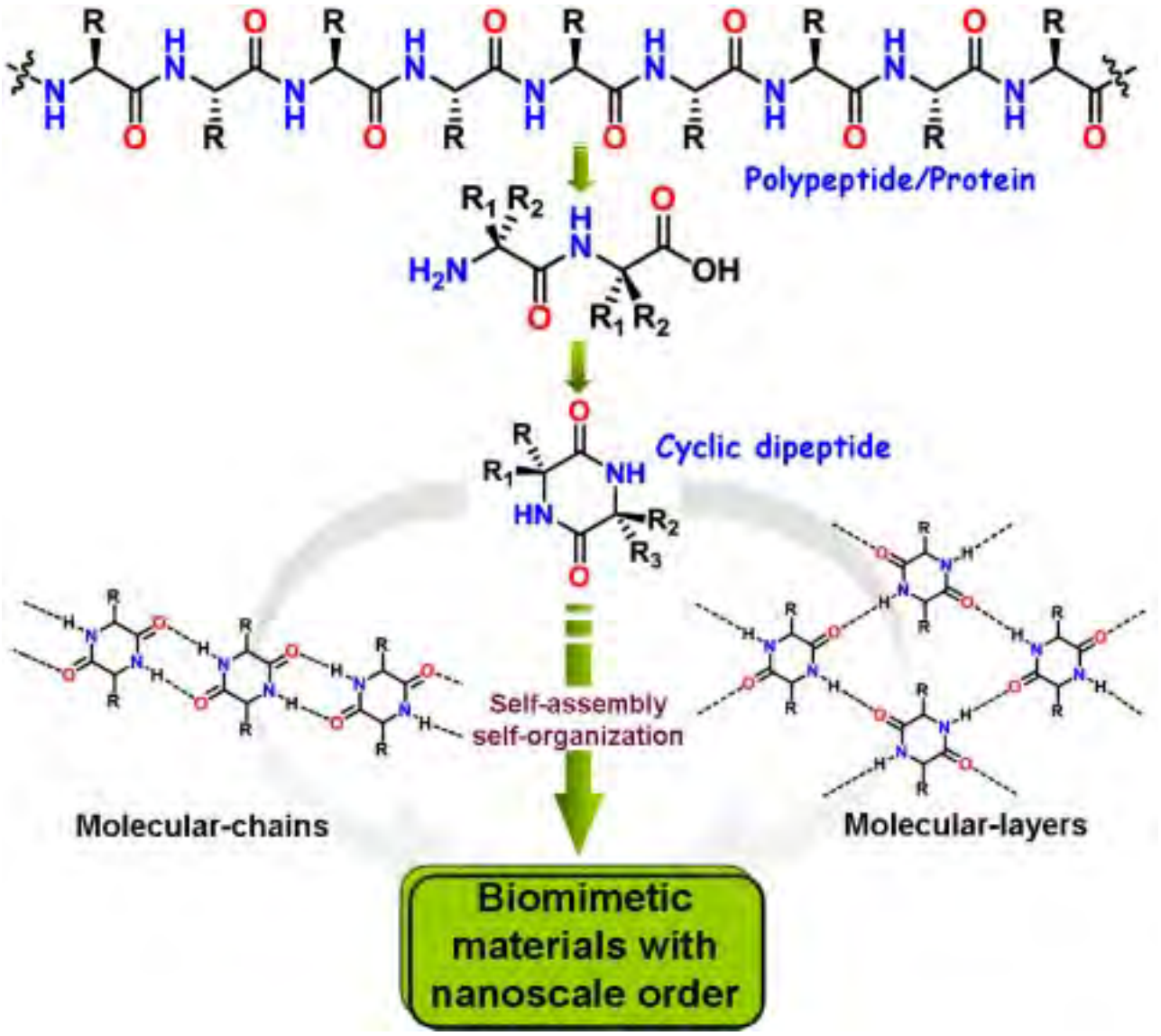

Fig. 7: Designing modular cyclic dipeptides (a retro-approach to Nature's route) based biomimetics materials with well defined morphology and nanoscale order

fiber bundles which are 1-2 μm thick and several millimeters long as shown in Fig. 8a-c [41]. These fiber bundles exhibit the structural hierarchy that is found in natural fibers such as spider silk and collagen (Fig. 8d). The structural hierarchy involves the nano- and sub-micrometer fibers which self-organize to form micrometer thick and several millimeter long fiber bundles. The solubility studies in various solvents provided more insights on the existence and nature of fiber bundles. The fiber bundles form gel in chloroform at critical concentration of added trifluoroacetic acid (Fig. 8e). Thermogravimetric analysis data indicated high thermal stability of the fiber bundles comparable to that of natural protein-based fibrous materials (Fig. 8f). NMR studies further revealed that aromatic π-π interactions along with intermolecular (N–H--O) hydrogen bonding are mainly responsible for the formation of fiber bundles with high thermal stability. The strength of this protocol is the ability to achieve large scale production of CDP fibers compared to relatively inefficient recombinant technology-based production

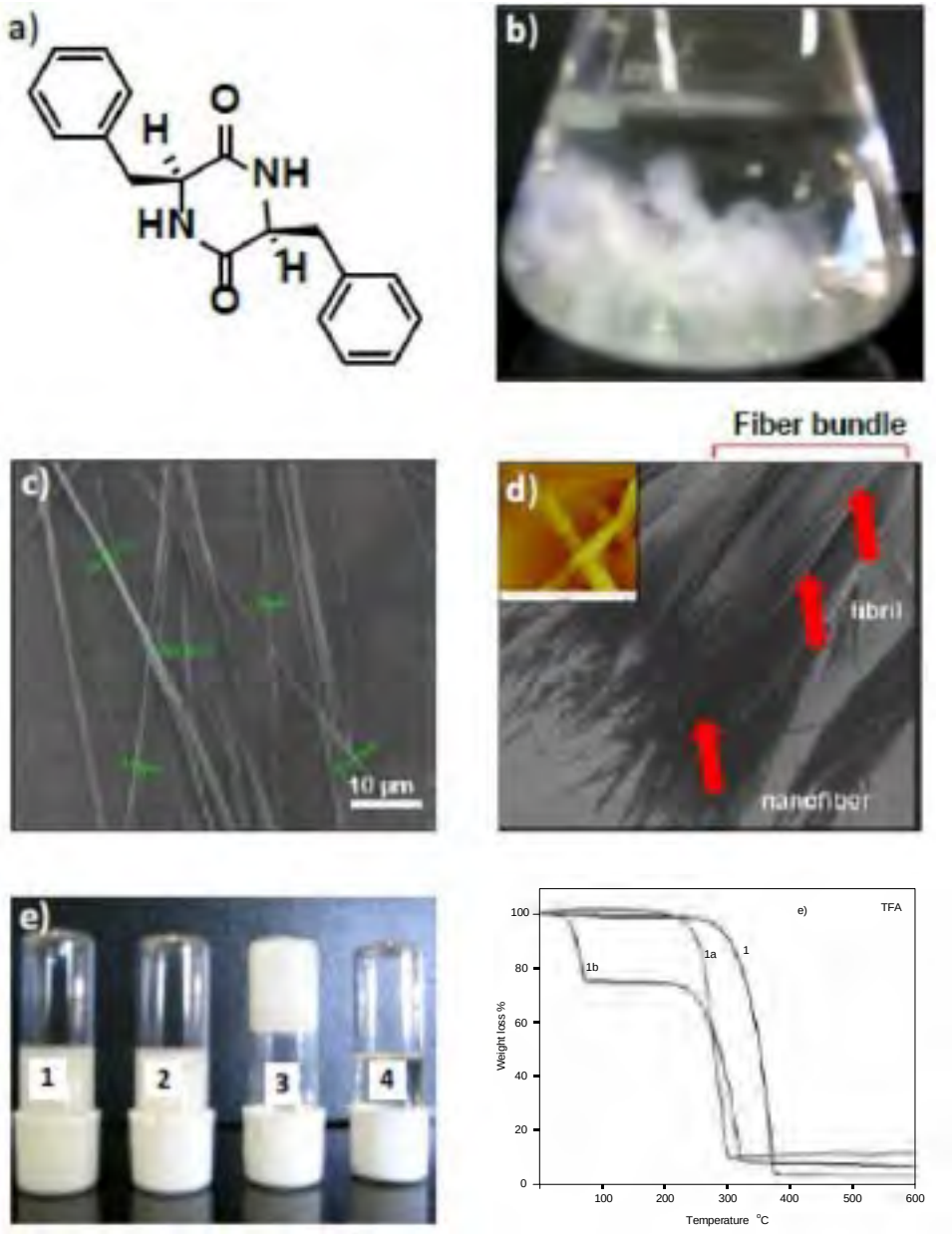

Fig. 8: a) Fiber bundles of aromatic cyclic dipeptide (CDP) of (Phe-Phe). b) Photograph of fiber bundles suspended in dichloromethane. c) FESEM micrograph of CDP fibers. d) micrograph of fiber bundles showing the structural hierarchy similar to that of natural fibers (inset: AFM micrograph of fiber bundle). e) Gelation study. Insoluble CDP of (Phe-Phe) in Chloroform (vials 1 and 2) gives clear solution with addition of trifluoracetic acid (vial 4), if the added TFA is controlled to a critical concentration forms a gel. (vial 3). f) Thermogravimetric analysis. 1: Fiber bundles (solid), 1b: fiber bundles with minimum amount of TFA and 1b: gel formation at critical concentration of TFA. Adapted from Ref. [41]

of biological fibers. The study of structural hierarchy, solubility, gelation, and thermal stability demonstrate the robustness of aromatic cyclic dipeptide (Phe-Phe) to form fiber bundles and may constructively help the future design and production of biomimetic fibrous materials from modular building blocks in accordance with supramolecular chemistry principles. The materials based on fiber bundles of aromatic cyclic dipeptide with natural and unnatural amino acids can be viewed as potential candidates for the biomaterials applications such as neurological regeneration, organ replacement,

production of suture and fabrics, optoelectronic and composite materials.

Recently, we have also demonstrated formation of 2D nano and mesosheets by employing simplest aromatic CDP of (Phg-Phg) spanning several micrometers in lateral dimensions (Fig. 9) [42]. The self-assembly of cyclo (*L*-Phg-*L*-Phg) begins by formation of 2D nanosheets, followed by self-organization of these nanosheets to form 2D mesosheets (thickness = 300 nm). Cyclo (*L*-Phg-*L*-Phg) 2D mesosheets are insoluble and possess rhomboid shape and large lateral dimensions (> 10 μm) (Fig. 9a). Interestingly, the 2D mesosheets revealed the existence of a layered hierarchy (Fig. 9a, inset) similar to that observed in grapheme [43]. Attempts to solubilize the self-assembled mesosheets in organic solvents provided valuable insights. The solution of cyclo (*L*-Phg-*L*-Phg) in $CHCl_3/CH_2Cl_2$–TFA upon solvent evaporation formed well separated 2D nanosheets (Fig. 9b) and was used as an indirect method for the exfoliation of nanosheets from 2D mesosheets. Structural calculations and energy minimized structure indicated the cis-conformation for cyclo (*L*-Phg-*L*-Phg) as shown in Fig. 9c. Model constructed based on the solution and solid state NMR spectroscopic studies revealed that cyclo (*L*-Phg-*L*-Phg) form (N-H- -O) hydrogen bonded molecular chains which are further supported by aromatic (through phenyl ring on phenylglycine) π-π interactions to form 2D nano and mesosheets (Fig. 9d).

Cyclo (*D*-Phg-*L*-Phg) was also found to form 2D nano and mesosheets similar to Cyclo (*L*-Phg-*L*-Phg) with slightly reduced thickness (200 nm). More interestingly, we were also able to grow single crystalline 2D sheets as shown in Fig. 9e. The shape of 2D single-crystalline sheets resembled that of 2D mesosheets obtained by self-assembly based aggregation (Fig. 9e, inset). These 2D single-crystalline sheets were much larger in dimension compare to self-assembly based 2D mesosheets (> 600 μm). Single crystal X-ray data revealed that Cyclo (*D*-Phg-*L*-Phg) adopt trans-conformation (two phenyl groups in reverse direction) and forms (N-H- -O) hydrogen bonded molecular layers (Fig. 9f). The molecular layers supported by aromatic π-π interactions (stacks along the *c*-axis) to form 2D single-crystalline sheet. The single-crystalline 2D sheets do not possess the layered structural hierarchy that was observed in 2D mesosheets obtained from self-assembly based aggregation.

The 2D mesosheets exhibited high thermal stability as revealed by their thermogravimetric analysis data. The cyclo (*L*-Phg-*L*-Phg) and cyclo (*D*-Phg-*L*-Phg) mesosheets (solid sample) showed two transitions which are attributed to a hierarchical arrangement present in the 2D sheets. Major thermal transitions were observed at 220 and 310 ºC for *LL*-isomer (Fig. 9g) and at 306 and 497 °C for *DL*-isomer (Fig. 9h). High thermal decomposition temperatures are indicative of high stability associated with 2D mesosheets. This provides supporting evidence for the existence of morphological hierarchy involving the self-organized nanosheets to form stable mesosheets. The observed difference in the thermal stability of *LL* and *DL*-isomers of cyclo (Phg-Phg) are due to (N-H- -O) and π-π interactions driven two-dimensionally extended molecular chains and molecular layers (network-like arrangement) respectively. Taken together, the formation of such 2D sheets with large lateral surface area, their topographical hierarchy, high thermal stability, and in particular strong hydrogen bonds along with aromatic π-π interactions opens up new avenues for the design of novel biomaterials. For instance, 2D nano and mesosheets of cyclo (Phg-Phg) can be viewed as potential candidates for applications such as biomineralization, template for cell culture and tissue culture, 2D sheets derived composites and optoelectronic material scaffolds.

Noncovalent interactions and their contribution to molecular organizations are the basis for various biological and non biological systems. The molecular recognition is complicated process as it involves the interplay of several factors. Nature provides an exhaustive illustration of the elegance with which the noncovalent interactions have been employed in the design strategy catering to various requirements. Supramolecular chemistry concepts can be guiding principles to mimic Nature's versatility in controlling

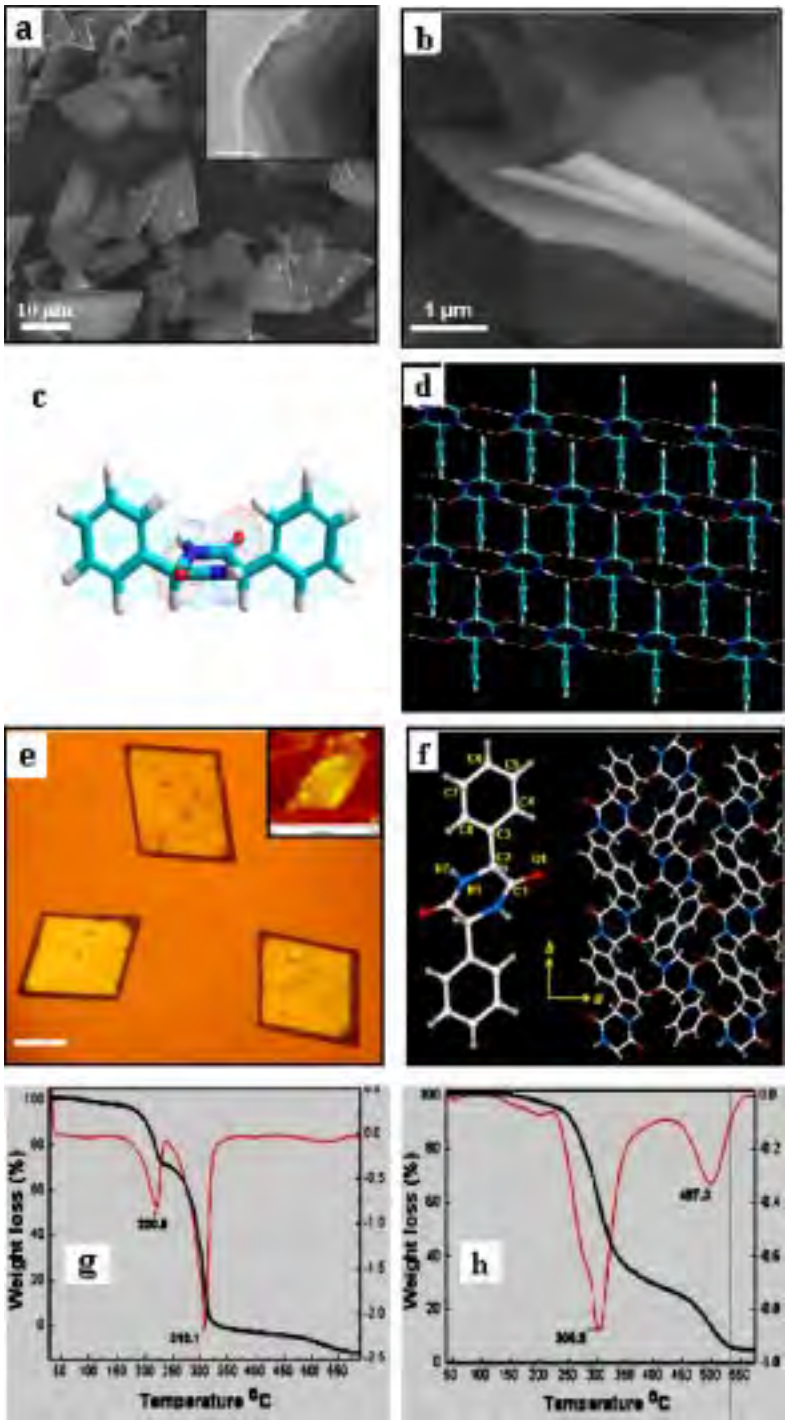

Fig. 9: (a) FESEM micrograph of 2D mesosheets (inset: HRTEM image show layered hierarchy). (b) FESEM micrograph of exfoliated nanosheets. (c) cis-conformation of cyclo (*L*-Phg-*L*-Phg). (d) A model of sheet formed by self-assembly of cyclo (L-Phg-L-Phg) through (N-H- -O) hydrogen bonding and aromatic π-π interactions. (e) Single-crystalline rhomboid 2D sheets of cyclo (*D*-Phg-*L*-Phg) (scale bar: 350 µm). [inset: Self assembled mesosheet of cyclo (D-Phg-L-Phg)]. (f) Ortep-diagram and molecular interactions of cyclo (*D*-Phg-*L*-Phg) to form molecular-layers. (g) thermogravimetric analysis (TGA) of (a) cyclo (*L*-Phg-*L*-Phg) and (h) cyclo (*D*-Phg-*L*-Phg) 2D mesosheets. Adapted from Ref. [42]

molecular interactions and organizations to design novel biomimetic materials. Despite significant advances in supramolecular chemistry, direct control of molecular organization remains to be conquered by elegant design concepts. Noncovalent assembly of aromatic moieties with variable functionality into well defined architectures remains a challenging task. Control and optimizing the relatively strong π-π interactions in cooperation with those of the substituents is a main hurdle in organizing aromatic moieties such as arylene dimides. The most commonly employed strategy to achieve arylene diimide organization trough long chain alkyl, alkoxy or the phenyl imide-substitution [44, 45]. All these substituent's are rather restricted to a simple, specific interaction such as hydrophobic, hydrophilic or aromatic and lack a combination of noncovalent forces that can act in a cooperative manner. A novel bioinspired design strategy involving amino acids is appealing as they are the important structural and signaling biomolecules due to their molecular recognition and distinctive sequence-specific self-assembly properties [26, 35, 46-48]. To demonstrate the utility of this approach, naphthalenediimide (NDI) was used as representative arylene diimides to tune the molecular organization into well defined architectures [46]. NDIs are among the most promising n-type semiconductors and may find potential applications in organic field effect transistors, supramolecular switches, fluorescent chemosensors, electron and energy transfer

systems [49-56]. Equally important characteristics of NDI are planarity and high π-acidity which is ideal for face to face π-stacking [57]. The enhanced solubility of NDI system compare to other arylene imides offers better solution processability into variety of possible nanostructured scaffolds.

In spite of several advantages associated, the morphology tuning of the NDI nanostructures is largely unexplored [58-60]. We have recently reported an exclusive investigation of the molecular organization of NDI appended with two tryptophan moieties (Fig. 10). Tryptophan was selected as preferred imide substituent due to its polar carboxylic acid group and an indole aromatic heterocycle offering both the hydrophilic and hydrophobic properties [46]. In addition, tryptophan provides sites for metal interaction and biocompatibility. A small structural modification such as methylester of tryptophan in NDI system renders better solubility in nonpolar solvents and has huge influence on molecular organization into interesting nanostructures. Overall J-type aggregation was induced by hydrophobic forces in the tryptophan appended NDIs and H-type aggregates by the addition of sodium hydroxide which involves sodium cation-π interactions. All these features were responsible for drastic changes in the morphology of NDIs with well defined architectures. The molecular organization of NDIs was transformed into nanospheres, particles, nanobelts, microfibers (nanobelts bundles) and fractals (Fig. 10). Such drastic change in the morphology of NDIs architectures is a clear evidence of the importance of the weak, complicated noncovalent forces. Thus bioinspired design strategy provides enough opportunities to modulate the molecular organization into nanomaterials with novel properties. Future work would involve the different amino acid, peptide modifications of NDI and core-modified NDIs which will for sure leads to interesting nanostructures with unexplored properties. These bioinspired nanostructures find potentials applications as biomaterials and in organic electronics.

4. Chemosensors

Metal ions are beneficial as well as harmful to human health [61]. The beneficial metal ions are toxic above critical concentrations and responsible for variety of diseases [62]. For example Zn^{2+}, Cu^{2+}, Fe^{2+}, Ni^{2+}, Co^{2+}, Mg^{2+}, Ca^{2+}, Na^{+}, K^{+} and many others are all found in living organisms including humans and act as cofactors to enzymes in many biochemical reactions or involved in some kind of physiological functions [61, 63, 64]. Metal ions such as Hg^{2+}, Cd^{2+}, Al^{3+} and Pb^{2+} are highly toxic to humans even at very low concentrations [65]. In fact these metal ions have been already implicated in many diseases [66]. The ability to selectively detect and remove specific metal ions from waste streams, the environment and human body will become essential analytical tools in years to come [66, 67]. Aluminium which is generally acquired by the use of antiperspirants, deodorants, antacids, drinking water supplies can be deadly for humans if it exceeds the average daily human intake, approximately 3-10 mg/day reported by WHO. Aluminium is toxic even in small amounts if deposited in brain. Alzheimer's disease, osteoporosis, colic, rickets, decreased liver and kidney function, softening of bones are the symptoms associated with aluminium toxicity [68]. Surprisingly, until now Al-toxicity has not drawn enough interest of scientists and needs immediate attention to tackle the adverse effects of aluminium to agriculture and human health. The environmental acidification (soil acidity) due to increased solubility of Al-minerals at lower pH increases the amount of available Al^{3+} which is harmful for growing plants (~50% of the world's soil and in particular 25 million hectares of Indian farming land is acidic and hence infertile). Copper (II) is generally soluble in water and acts as bacteriostatic substances and fungicides at low concentration level [69]. It is an essential trace nutrient for plants and used as cofactors for various enzymes in humans. It is the third most abundant soft transition metal and human body normally contains copper at a level of about 1.4 to 2.1 mg/kg of body weight. But accumulation of Cu in body tissues is believed as the cause of liver cirrhosis, Wilson's disease and other neurodegenerative diseases [69]. Toxicity due to copper can occur from eating acidic food that has

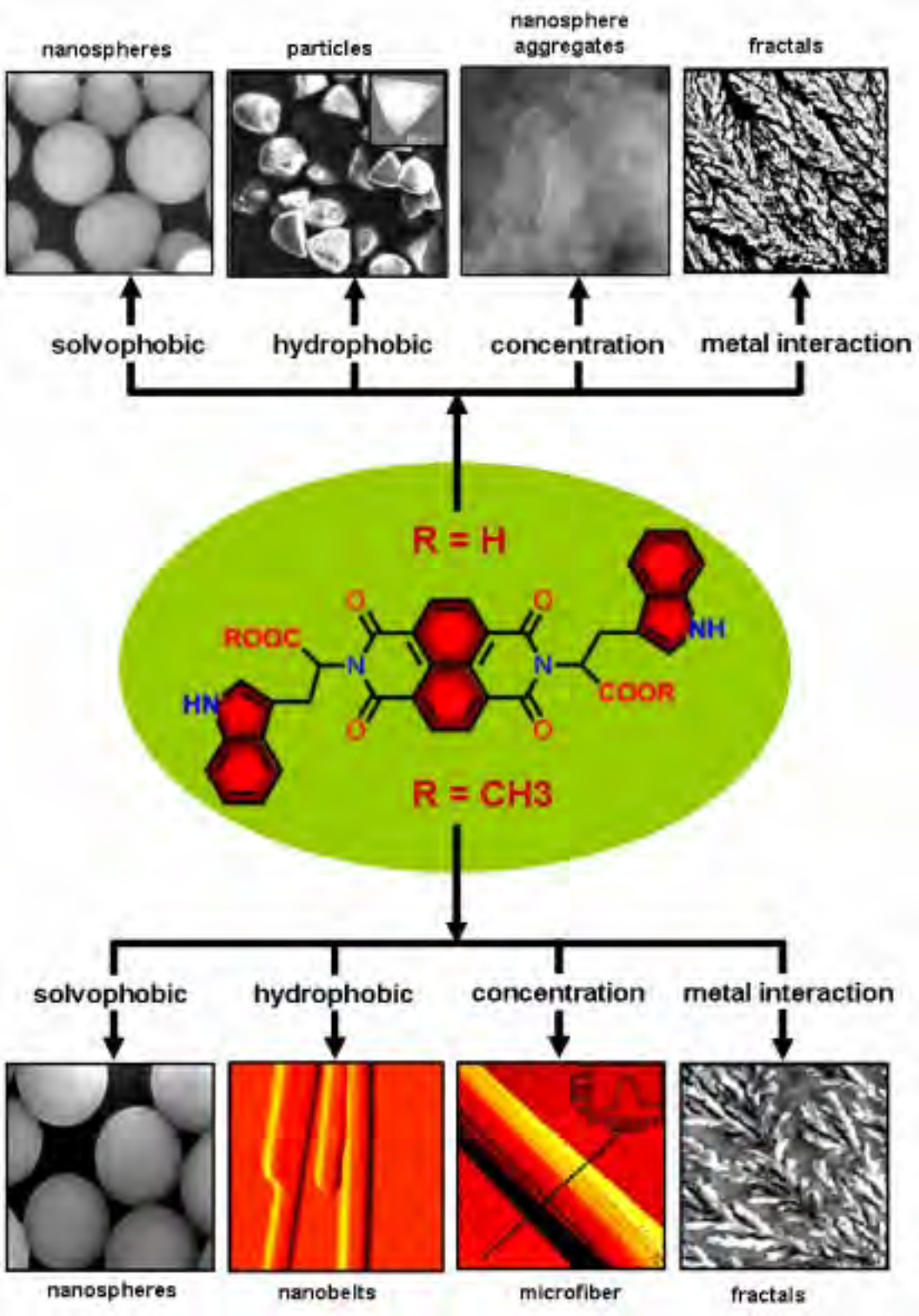

Fig. 10: Biomimetic approach for tuning the morphology of naphthalenediimides (NDI). Morphology of tryptophan appended NDI was tuned into nanospheres (solvophobic effect), particles (hydrophobic effect), nanospheres aggregates (concentration effect) and fractals (metal interaction). Similarly Morphology of tryptophan methylester appended NDI was tuned into nanospheres (solvophobic effect), nanobelts (hydrophobic effect), microfiber (bundle of nanobelts, concentration effect) and fractals (metal interaction). Adapted from Ref. [46]

been cooked in Cu cookware among other sources. Zn has been implicated in neurodegenerative diseases such as Alzheimer's and Parkinson's diseases [69, 70].

Detecting metal ions qualitative and quantitatively is very crucial to balance their levels in the environment as well as to sustain good human health [61-67]. The sensing of metal ions with high selectivity and sensitivity remains a challenge in environmental and biological applications [71]. With the help of supramolecular chemistry principles, the design and synthesis of optically responsive chemical sensors for the selective detection of metal ions has become a growing field of research [72]. A chemical sensor generally includes two components, a reporter unit for example a fluorophore and a recognition unit (ionophore), which can be either independent species or covalently linked as a conjugate, and additionally a mechanism for communication between them [73]. When the analyte bind to the recognition center, changes occur in the optical properties (e.g., changes in the absorption or emission properties) of the chemosensor. Fluorophores are particularly attractive optical molecules and have recently found applications in self-assembled chemosensors [74] for signal amplification by allosteric catalysis [75], in supramolecular analytical chemistry [76], and as fluorescent and photochromic chemosensors [77]. The fluorescent chemosensors composed of a cation recognition unit (ionophore) together with a fluorogenic reporting unit (fluorophore) and thus the conjugate is described as fluoroionophore [78, 79].

Designing conformationally constrained fluoroionophores based on supramolecular host-guest interactions is particularly interesting as it increases the selectivity and sensitivity of chemosensor ligand towards metal ion of interest (Fig. 11). A straight forward and easily accessible synthetic routes for accessing conformationally constrained fluoroionophores was established employing high yielding azide-alkyne cycloaddition (click reaction) and amide-bond formation as key intermediate reactions [79, 80]. Based on conformationally constrained fluoroionophore approach bipyridyl-dansyl (BD) and coumarin-bipyridyl (CB) ligands were designed with pyrrolidinyl-triazole rigid linker (Fig. 12). It should be noted that azide-alkyne click reaction between linker molecule and ionophore results in the formation *triazole ring*, which will become a part of the ionophore making it a new recognition unit [79]. The BD-ligand selectively sense Al^{3+} at micromolar concentration by means of fluorometric response and at millimolar concentration by colorimetric change (Fig. 12a). Additionally, dual responsive BD ligand sense Al^{3+} ratiometrically which minimize the error arise from the physical or chemical fluctuations in the sample. Interestingly, the coumarin-bipyridyl (CB) ligand was found to be 'turn-on' fluorescence sensor with sub-micromolar detection limit (Fig. 12b) [80]. In BD and CB ligands the dansyl and coumarin fluorophore moieties plays a dual role as a reporting unit and cation binding site. The BD and CB ligands will find application as aluminium detection in soil, environment and biological samples and scavenger/drug for the selective removal of toxic-aluminium from the human body. A similar design strategy was used to develop novel reversible fluorescent chemosensor for Zn^{2+} (Fig. 13) [81]. Hydroxyquinoline moieties were linked to pyridyl-unit through triazole linker. The ligand turns out be selective for Zn^{2+} and free ligand can be reversibly regenerated by using aqueous ammonia.

A rationally different approach was undertaken to develop colorimetric sensors with near-infrared (NIR) optical response. Although chemosensors with visible signal has important roles in various research endeavors, molecular probes with near infrared (NIR, 700–1000 nm) optical response are useful for sensing and imaging of metal ions [82]. Unlike UV/Vis, NIR radiation can penetrate much deeper into the sample as there is no or little absorption and scattering. In the NIR region there is no interference of autofluorescence generated from the endogenous chromophores [83]. Very few studies have been reported for cation and anion sensing based on UV/Vis-NIR absorption spectroscopy [84, 85]. Recently we have reported Schiff base ligands based on 4-(diethylamino) salicylaldehyde conjugates of carbohydrazide/thiocarbohydrazone as NIR

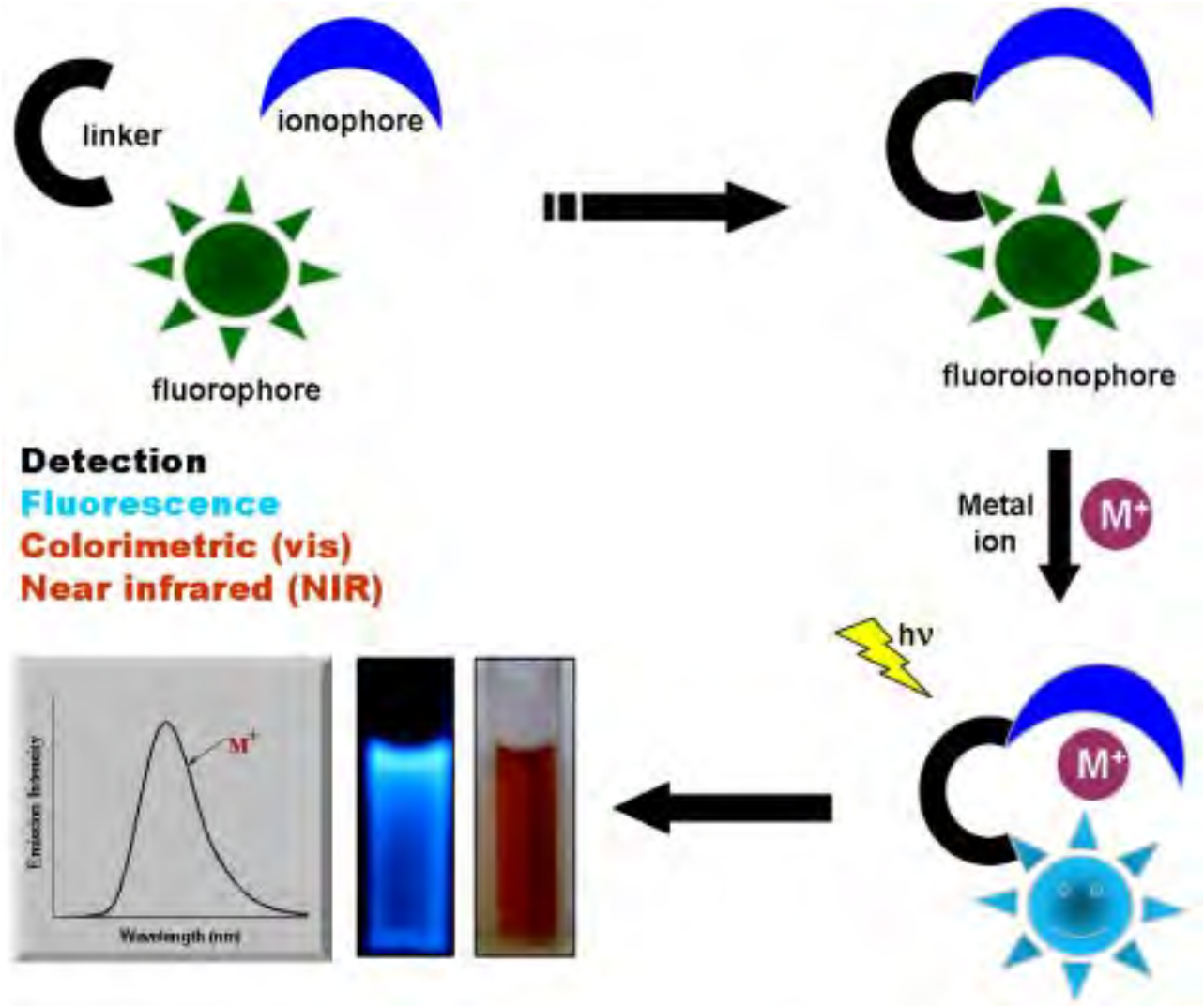

Fig. 11: Design concept for developing novel chemosensors for metal cations. Fluoroionophore obtained by conjugating ionophore (metal binding unit) and fluorophore (reporting unit) through a suitable linker

colorimetric chemosensors for selective detection of Cu^{2+} (Fig. 14) [86]. Two Cu^{2+} binds to 4-(diethylamino) salicylaldehyde-thiocarbono-hydrazide (TC) ligand through double deprotonation of phenolic hydroxyl groups. TC has showed distinct colour change from light green to purple (535 nm) on addition of Cu^{2+} in presence of various other metal ions tested (Fig. 14). In TC, all the atoms are almost in one plane and planarity of the molecule further supported by deprotonation as it supports maximum delocalization of the negative charge over entire molecule (Fig. 14). In addition both carbon and sulphur atoms have near similar electronegativity values and thioamide undergoes conjugation with nitrogen to stabilize weak C=S bond. Additional stability gained through conjugation and coordination with Cu^{2+} which leads to the appearance of characteristic NIR signature (936 nm). This design principle can be used to develop colorimetric and fluorometric chemosensors with NIR optical response for various other metal ions. In general, fluoroionophores will be used as i) chemosensors for the detection of toxic metals in waste streams, drinking water, industrial effluents, ii) biosensors for the detection of metal ions in plants and humans, iii) scavengers/drugs for the removal of toxic metals from

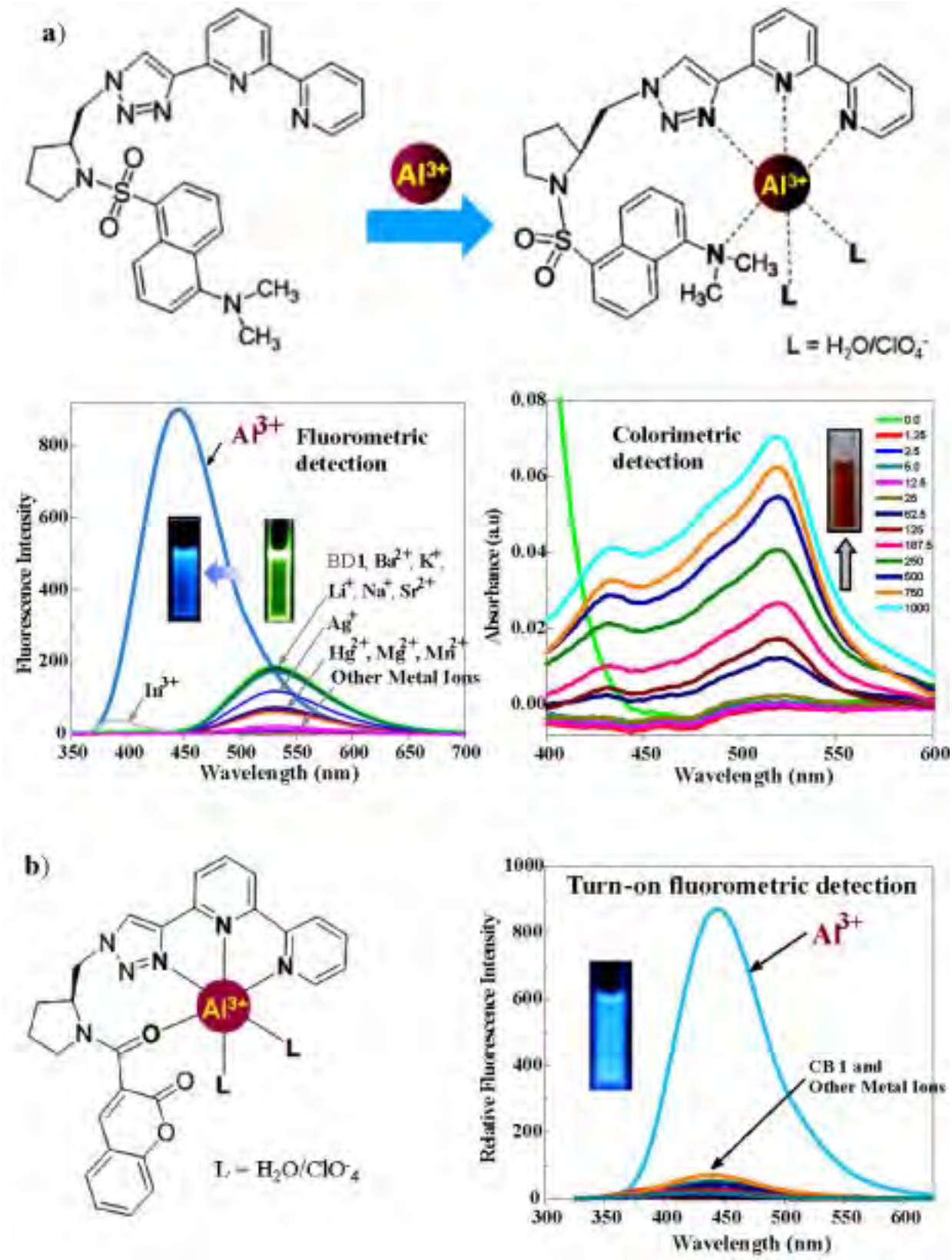

Fig. 12: Conformationally constrained bipyridyl-dansyl (BD**) ligand as dual responsive (fluorometric and colorimetric) for Al^{3+} sensing (a). Improved coumarin-bipyridyl (**CB**) ligand for sensitive detection of Al^{3+} (b). Adapted from Ref. [79] and [80]**

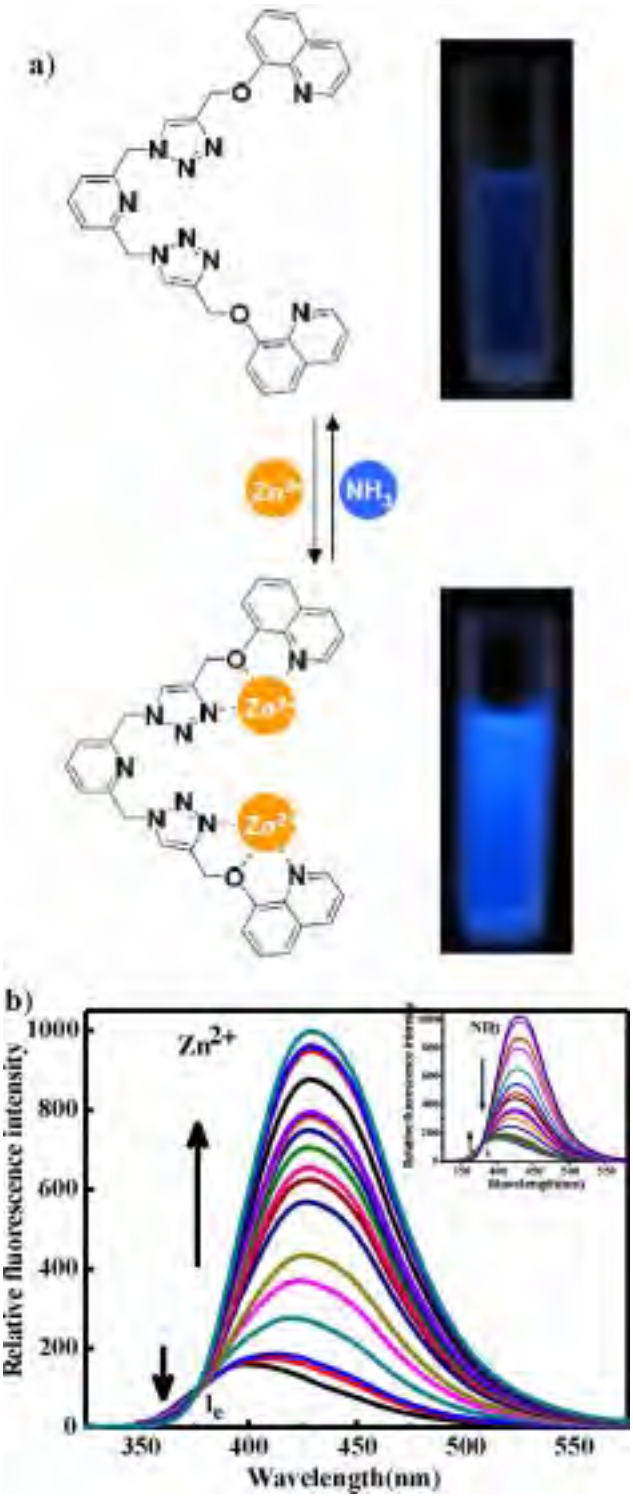

Fig. 13: a) Reversible fluorescence sensing of Zn^{2+} using pyridine-constrained Bis(triazole-linked hydroxyquinoline) ligand. b) Fluorescence spectra of ligand upon addition of increasing concentration of Zn^{2+}. Inset: fluorescence spectra of ligand -Zn^{2+} complex upon adding increasing amount of aq. Ammonia. Adapted from Ref. [81]

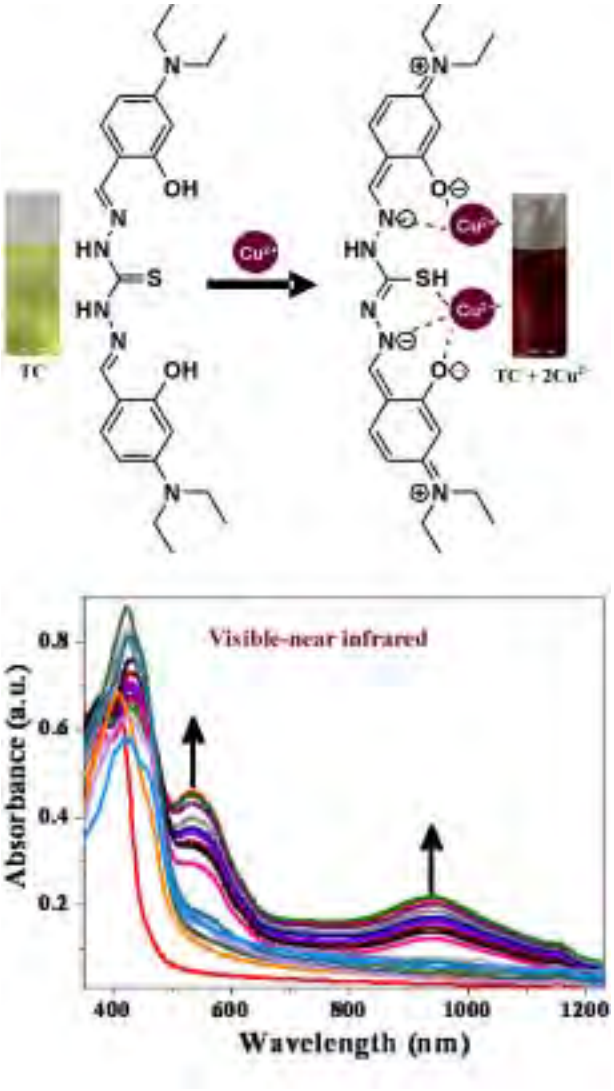

Fig. 14: Thiocarbonohydrazone (TC) designed as colorimetric sensor for Cu^{2+} with characteristic NIR response. Adapted from Ref. [86]

the body and iv) DNA binding/cleaving agents and in designing hybrid-(bio) materials.

4. Conclusion

In summary, great potential in employing Nature-inspired supramolecular chemistry principles to develop wide variety of biomimetic systems and materials has been exemplified with three different topics such as nucleic acid mimics, modular peptide-based biomimetic materials and chemosensors. The nucleic acid mimics can be used as drugs, reagents in molecular biology and diagnostics. In addition, the

nucleic acid mimics may play an important role in nucleic acid nanotechnology to produce hybrid nanomaterials with novel properties. Biomimetic materials with nanoscale order was produced based on modular cyclic dipeptides utilizing orthogonal noncovalent interactions such as hydrogen bonding and aromatic π-π interactions. This will have huge implications on future development of biomimetic materials with structure and function matching the perfection of natural materials. An equally important application of biomimetic approach is the tuning the morphology of naphthalenediimide an arylene dimide and well known n-type organic semiconductor molecular system. Biomimetic approach will play important role in tuning the morphology of various organic materials with improved electronic and bioviable properties. Application of supramolecular host-guest chemistry in developing biomimetics of biopolymer-metal ion interactions was demonstrated with conformationally constrained fluoroionophores and Schiffs base conjugates of urea/thiourea for the colorimetric, fluorometric and NIR detection of metal ions such as Al^{3+}, Zn^{2+}, and Cu^{2+}. These chemosensors can be used for environmental and biological monitoring of metal ions and the very concept will guide the development of improved sensors for various other metal ions. Overall the biomimetic systems discussed in this review article will find applications as drugs, biomaterials, organoelectronics, composites, scavengers and sensor applications.

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