

SELF-ASSEMBLED PEPTIDE NANOSTRUCTURES AS A
VERSATILE TOOL FOR FABRICATION OF NANOSTRUCTURED
MATERIALS

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By

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in scope and in quality, as a dissertation for the degree of Doctor of Philosophy.

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ABSTRACT

SELF-ASSEMBLED PEPTIDE NANOSTRUCTURES AS A VERSATILE TOOL FOR FABRICATION OF NANOSTRUCTURED MATERIALS

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Ph.D. in Materials Science and Nanotechnology

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Several peptides such as amyloid like peptides (ALPs) and peptide amphiphiles (PAs) were synthesized by solid phase peptide synthesis technique. These peptides were utilized in fabrication of nanostructured materials. ALPs with amino (Ac-KFFAAK-Am) and carboxylate (Ac-EFFAAE-Am) functional groups were used in growth of titanium dioxide (titania) in solution. On the other hand, PA with amino group (lauryl-VVAGK-Am) was used in growth of titania by atomic layer deposition method. Obtained nanostructured titania in each case was used in construction of dye-sensitized solar cells.

Peptide amphiphile with phytochelatin mimetic sequence and adamantyl functionality (ADAc-6-Ahx-GECECECG-Am) was used in noncovalent functionalization of electrospun hydroxypropyl- β -cyclodextrin (HP β CD) mesh; HP β CD mesh functionalized with peptide was further used in metal ion scavenging.

PA with sequence lauryl-VVAGH-Am was used in noncovalent encapsulation of zinc phthalocyanine derivative. New photophysical properties of encapsulated chromophore were studied and ultrafast energy transfer was observed. L and D peptide amphiphiles (L/D-pyrenebutyryl-6-Ahx-VVAGH-Am and L/D-lauryl-VVAGH-Am) were used to induce chiral organization of achiral chromophore (pyrene) in self-assembled nanofibers of PAs. Two strategies were involved to induce chiral organization: first strategy envisioned conjugation of pyrene to peptide sequence, whereas second strategy involved direct encapsulation of the

chromophore. Achiral pyrene molecules organized in supramolecular chiral manner were observed to demonstrate circular dichroism.

Keywords: peptide, bio-inspired mineralization, nanostructured materials, programmed self-assembly, one-dimensional nanostructures, template directed synthesis, nanotechnology.

ÖZET

KENDİLİĞİNDEN DÜZENLENEN PEPTİT NANOYAPILARIN NANOMALZEME ÜRETİMİNDE ÇOK YÖNLÜ KULLANILMASI

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Amiloid benzeri peptit (ABP) ve peptit amfifil (PA) gibi çeşitli moleküller katı faz peptit sentezi tekniğiyle sentezlenmiştir. Bu peptitler, nanoyapılı malzeme üretiminde kullanılmıştır. Amino (Ac-KFFFAAK-Am) ve karboksilat (Ac-EFFFAAE-Am) fonksiyonel grupları taşıyan ALP'ler solusyonda titanya büyütmek için kullanılmıştır. Ayrıca, amino grup taşıyan PA (loril-VVAGK-Am) atomik katman kaplama yöntemiyle titanya büyütmek için kullanılmıştır. İki yöntemle de elde edilmiş olan nanoyapılı titanya, boya duyarlı güneş hücreleri yapımında kullanılmıştır.

Sekansıya fitokelatini taklit eden ve adamantil fonksiyonel grubu taşıyan peptit amfifil (ADAc-6-Ahx-GECECECG-Am), elektro lif çekimi yöntemiyle üretilmiş hidroksipropil- β -siklodekstrin (HP β CD) ağını kovalent olmayan bir yolla fonksiyonelleştirmek için kullanılmıştır. Peptide fonksiyonelleştirilmiş HP β CD ağı sonrasında sudan metal iyonlarının uzaklaştırılmasında kullanılmıştır.

loril-VVAGH-Am sekanslı peptit, çinko ftalosiyanın türevini kovalent olmayan yolla enkapsüle etmek için kullanılmıştır. Enkapsüle olmuş kromoforun yeni fotofiziksel özellikleri incelenerek, ultrahızlı enerji transferi gözlemlenmiştir. L ve D PA'lar (L/D-pirenbutiril-6-Ahx-VVAGH-Am ve L/D-loril-VVAGH-Am), akiral kromoforun (piren), kendiliğinden oluşan PA fiberlerinin içinde kiral düzenlenmesini tetiklemek için kullanılmıştır. Bunun için iki yol izlenmiştir: Birinci yol, pirenin peptit sekansına konjuge edilmesini esas alırken, ikinci yolda ise, kromoforun doğrudan enkapsüle edilmesi sözkonusuydu. Supramoleküler bir şekilde, kiral olarak

düzenlenmiş akiral piren moleküllerinin dairesel dikroizm sinyali sergilediği gözlemlenmiştir.

Anahtar Kelimeler: peptit, biyo-esinlenme mineralizasyon, nanoyapılı malzemeler, kendiliğinden programlı düzenlenme, tek-boyutlu nanoyapılar, kalıp güdümlü sentez, nanoteknoloji.

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Chapter 1.

1. Introduction: Peptide synthesis and programmed assembly codes

Solid phase peptide synthesis method paved the way to simple and robust peptide synthesis. This technological leap made peptide chemistry and related fields grow enormously fast. Nowadays, researchers actively exploit designer peptides to achieve programmed assembly, and look for diverse applications of the peptide nanostructures.¹

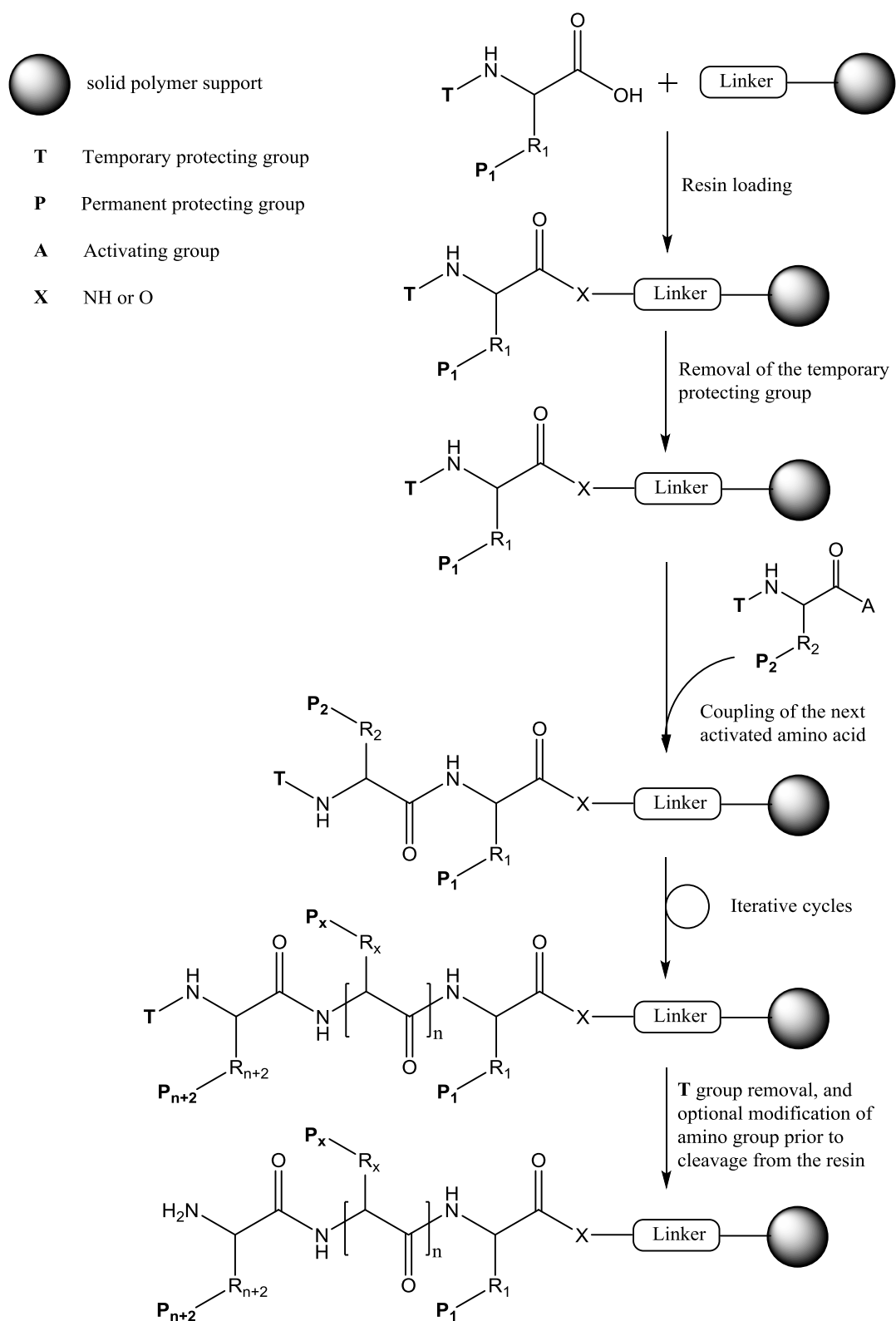
Solid phase peptide synthesis is a widely used method, which allows synthesizing long amino acid sequences with high yield. One of the variations of SPPS includes protection of N-terminus of an amino acid with 9-fluorenylmethoxycarbonyl (Fmoc) protecting group. Nowadays, Fmoc SPPS is the most widely used variation of peptide synthesis on a solid support.² Peptide synthesis based on this method will be discussed in details in this chapter.

Programmed peptide self-assembly represents a field of study with enormous potential. Programmed assembly is based on non-covalent weak interactions which can be used as an input on a design level. Designed peptides can assemble into various supramolecular architectures with specified complexity. Several examples of designed peptides will be presented throughout this chapter.

1.1 Solid Phase Peptide Synthesis

It is important to utilize a peptide synthesis method that results in a high product yield. In 1963, Robert Bruce Merrifield publishes a paradigm shifting manuscript³ on a solid phase peptide synthesis (SPPS). He achieves a synthesis of a tetrapeptide on a polymer support. His method circumvents drawbacks of solution phase peptide synthesis approaches and paves the way to high yield peptide synthesis, even for long amino acid sequences. A 100 amino acid sequences are in the reach of SPPS method. Development of a SPPS approach was a significant leap in synthetic peptide chemistry. Importance of this achievement was fully appreciated and in 1984 the Nobel Prize in chemistry was awarded to Robert Bruce Merrifield "for his development of methodology for chemical synthesis on a solid matrix". Moreover, Merrifield's SPPS also permitted the development of automation⁴⁻⁶ and the extensive range of robotic instrumentation is now available. After defining a synthesis strategy and programming the amino acid sequence of peptides, machines can automatically perform all the synthesis steps required to prepare peptide samples. SPPS has now become the method of choice to produce long peptides and short proteins.⁷

The main idea of SPPS is a stepwise elongation of an amino acid sequence on a solid polymer support.⁸ The principles of SPPS are illustrated in Scheme 1. The N-protected C-terminal amino acid residue is tethered via its carboxyl group to a hydroxyl (or chloro) or amino resin to yield respectively an ester or amide linked peptide that will ultimately produce a C-terminal acid or a C-terminal amide peptide. After loading the first amino acid, the desired peptide sequence is assembled in a linear fashion from the C-terminus to the N-terminus (the C→N strategy) by repetitive cycles of N^α deprotection and amino acid coupling reactions.



Scheme 1.1. SPPS principles.

Side-chain functional groups of amino acids must be protected with permanent protecting groups (Pn) that are stable in the reaction conditions used in the process of peptide growth. The α -amino group is protected by a temporary protecting group (T) that is usually an urethane derivative. The temporary protecting group (T) can be easily removed under mild conditions that preserve peptide integrity and reduce the rate of epimerization, which can occur via 5(4H)-oxazolone formation of the activated amino acid. Ability of urethanes to prevent epimerization also explains the superiority of the C $\rightarrow$ N strategy. After coupling, the reactant excess is removed by series of filtration and washings. Next, the removal of temporary N-terminal protecting group takes place, thus allowing the addition of the next N-urethane protected amino acid by activation of its α -carboxylic group. This deprotection/coupling cycle is repeated until the target sequence is obtained. Finally, the peptide is cleaved from the resin and the side-chain protecting groups are simultaneously removed.

In SPPS, two main strategies are utilized: Boc/Bzl and the Fmoc/tBu approaches for T/Pn protecting groups. The Boc/Bzl strategy makes use of the unequal acid lability of the side-chain protecting groups. In this approach, the Boc group is removed by pure TFA or TFA in dichloromethane, and side-chain protecting groups and peptide-resin bonds are cleaved at the end of the synthesis by treating the resin with a strong acid such as anhydrous hydrofluoric acid (HF). While this method results in efficient synthesis of large peptides and small proteins, the use of highly toxic HF and the need for special teflon-lined apparatus seriously limit the usability of this approach. Moreover, the strongly acidic conditions can cause deleterious and irreversible changes in the structure of peptides with sensitive sequences.

The Fmoc/tBu method utilizes an orthogonal protecting group strategy. This strategy uses the base-labile 9-fluorenylmethoxycarbonyl (Fmoc) group for protection of the α -amino functional group, acid-labile side-chain protecting groups and acid-labile peptide-resin linkers. The advantage of this strategy is that temporary and permanent orthogonal protecting groups are removed by different mechanisms, thus allowing the use of milder acidic conditions for final deprotection and peptide cleavage from the resin. For all these reasons, Fmoc-based SPPS technique² is the method of choice for the routine peptide synthesis, and it is actively utilized in laboratories worldwide.

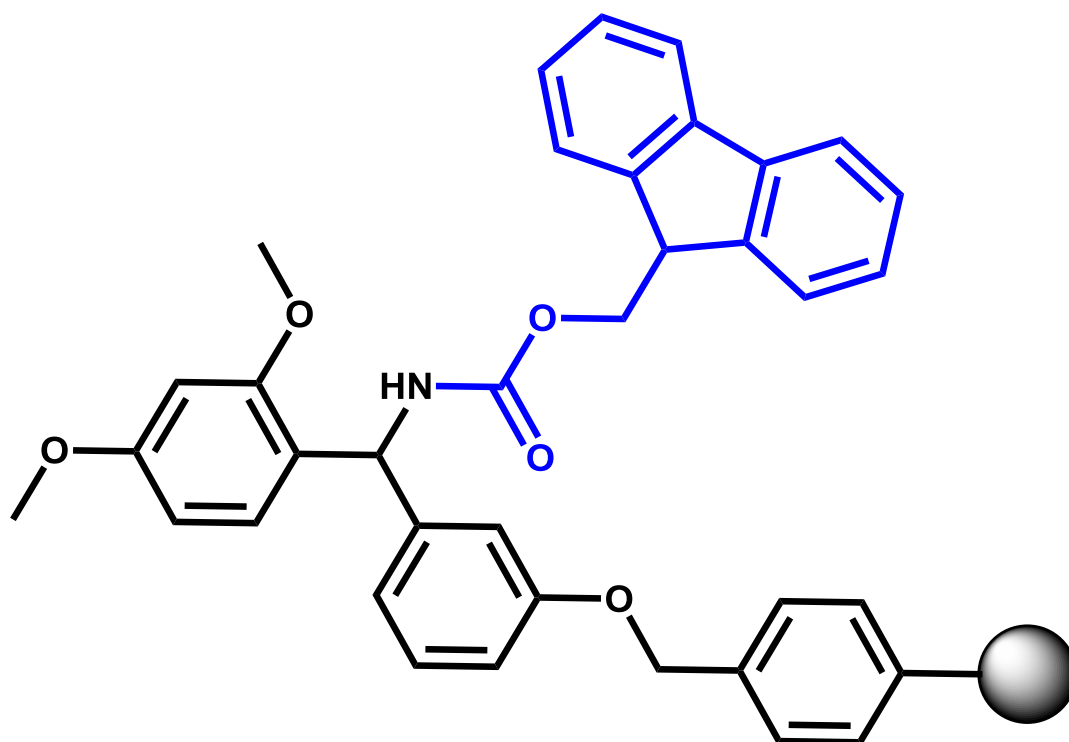
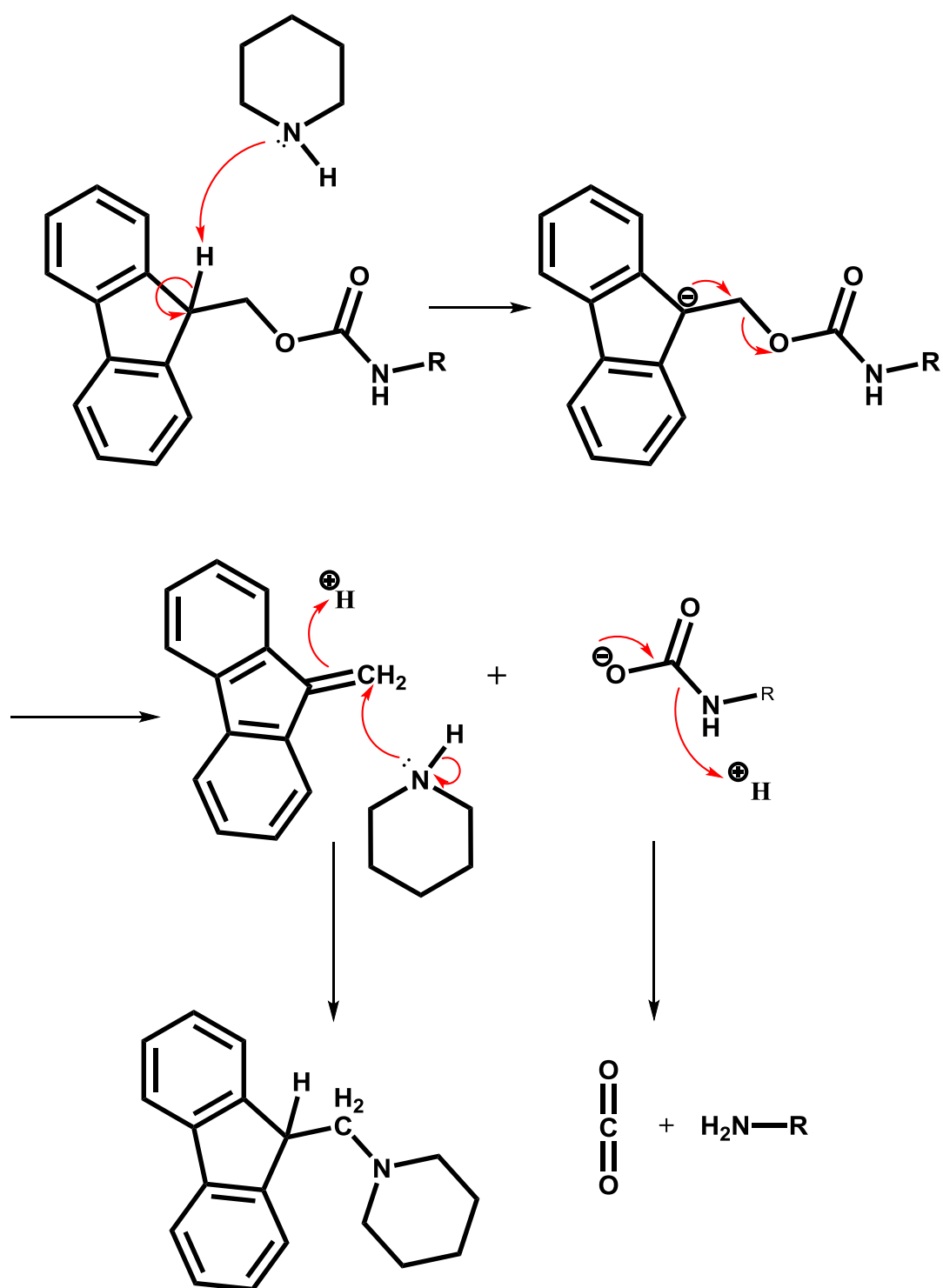


Figure 1.1. Rink amide resin, Fmoc protecting group is shown in blue color.

In the Fmoc/tBu method, general route to target peptide sequence comprises three main parts. First part is loading of a resin with the first Fmoc-protected amino acid. If

we consider a widely used Rink Amide resin (Figure 1.1),⁹ it is first required to remove Fmoc protecting groups of amino functionalities present on polymer support. Fmoc removal mechanism is depicted in Scheme 1.2; Fmoc removal is generally achieved by treatment of the resin with 20% (v/v) piperidine solution in DMF.²



Scheme 1.2. Fmoc cleavage mechanism.

After deprotection of the resin, the first amino acid can be loaded onto resin. For this purpose, Fmoc-protected amino acid needs to be activated. Several activators such as HBTU, HATU, HCTU, TBTU, PyBOP are utilized in the peptide synthesis (Figure 1.2).² These coupling reagents resolve racemization issue and therefore, have effectively replaced carbodiimides. Activation of amino acid by HBTU and its consequent reaction with amino group are given in Scheme 1.3. Deprotonation of amino acid is generally achieved by organic base such as diisopropylethylamine (DIPEA). Loaded with the first amino acid resin becomes available for peptide construction.

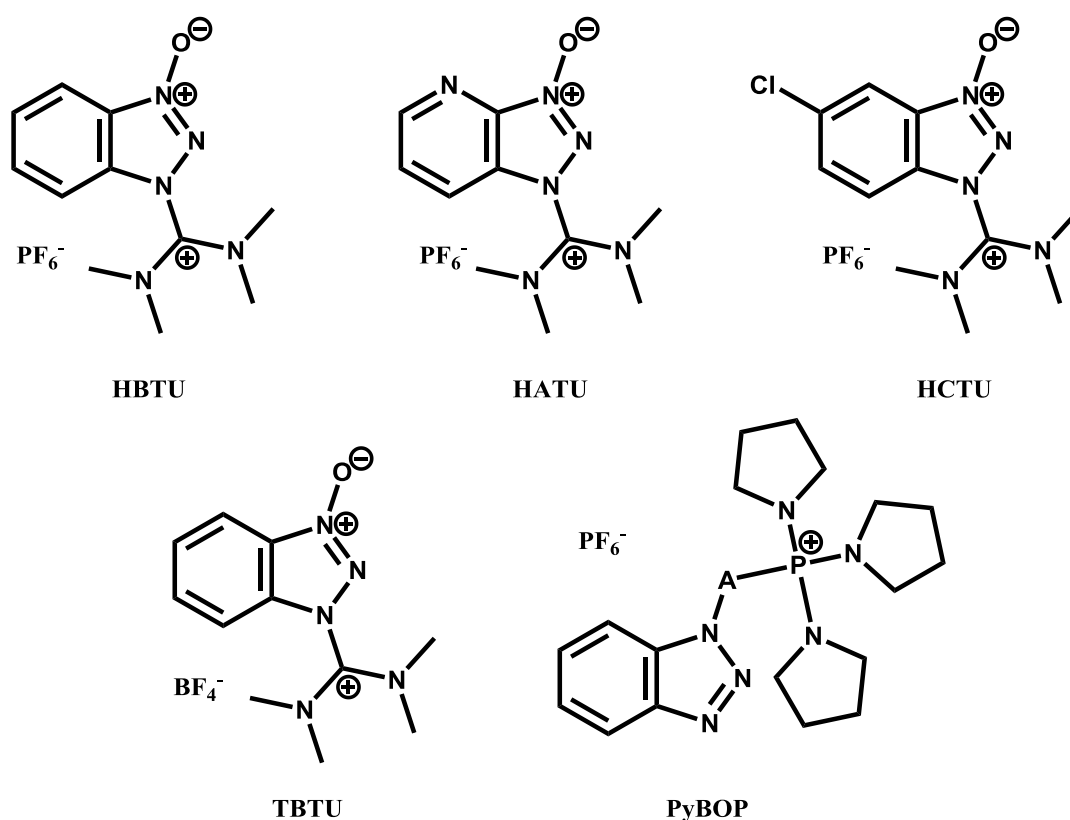
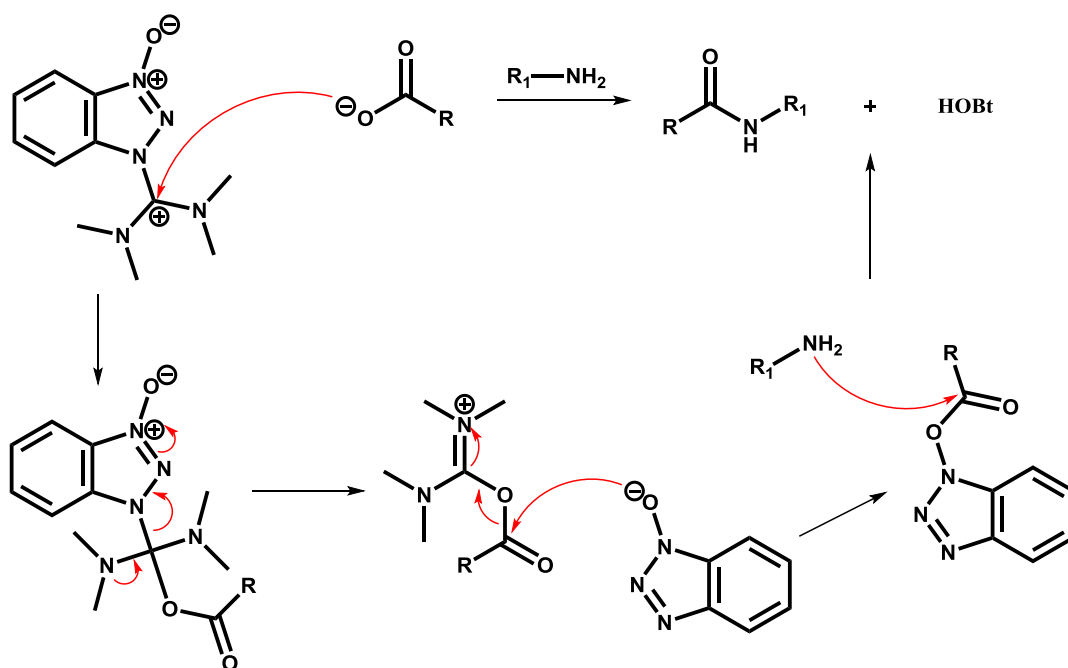
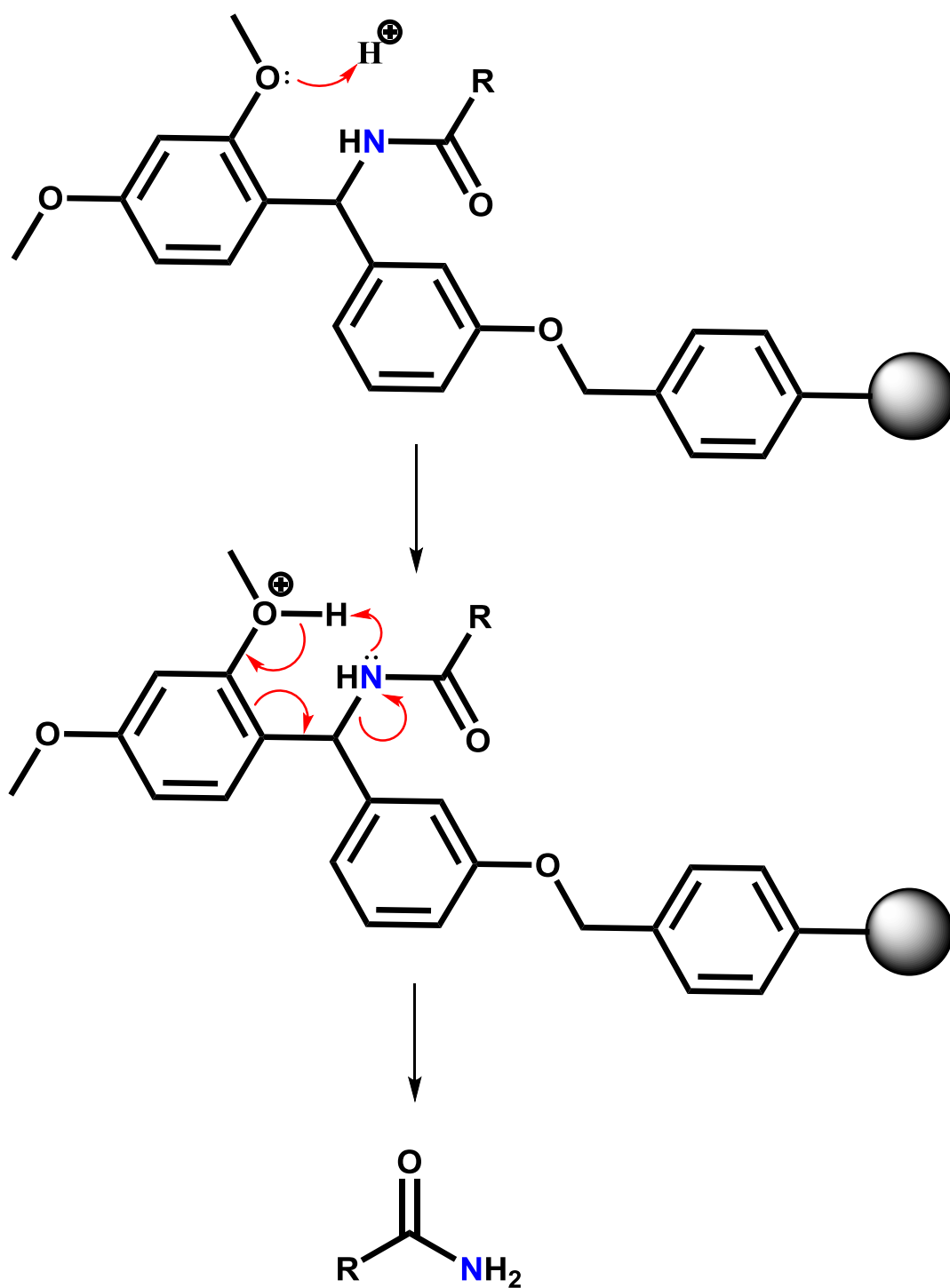


Figure 1.2. Benzotriazole based coupling reagents (activators).



Scheme 1.3. Activation of α -carboxylic acid of N-urethane protected amino acid residue and its consequent reaction with amino group.

Peptide construction is carried on with deprotection/coupling cycles until desired sequence is obtained (Schemes 1.1-1.3). When desired sequence is synthesized, it is cleaved from the resin by a concentrated TFA (~95%). Peptide cleavage mechanism is given in Scheme 1.4. Side-chain protecting groups are also cleaved under these highly acidic conditions. Side-chain group deprotection may yield highly active carbocations, which are generally suppressed by introduction of so-called “scavengers” into cleavage mixture. Standard scavengers are triisopropylsilane (TIS) and water (H₂O) molecules.²



Scheme 1.4. Rink Amide resin cleavage mechanism, R represents amino acid sequence.

1.2 Programmed assembly codes

Peptides are actively used as building blocks for supramolecular nanostructures such as nanofibers, nanotubes, nanovesicles, nanoribbons and etc.¹⁰ By controlling sequence it is possible to establish a relative control over morphology of a target nanostructure and thus achieve programmed assembly.¹¹ Moreover, it is possible to obtain nanostructures with appropriate functionalities on the surface of the structures.

There are several inputs on a design level that allow controlling an assembly process. These inputs are based on weak noncovalent interactions such as electrostatic interactions, coordination binding, hydrogen bonding, π - π interactions, solvophobic effects and van der Waals interactions (Table 1.1).¹²

Table 1.1. List of non-covalent interactions.¹²

Type of Interaction	Strength (kJ/mol)	Range	Properties
Electrostatic	50-300	long	Non-selective
Coordination binding	50-200	short	Directional
Hydrogen bonding	5-120	short	Selective, directional
π - π interaction	0-50	short	Directional
Solvophobic	Depends on solvent type	short	Directional constraint
van der Waals	< 5	short	Non-directional, non-selective
Covalent*	350	short	Irreversible

* Covalent bonding is a primary (strong) interaction type and given as a reference.

1.2.1 Electrostatic Interactions

Electrostatic interaction is a Coulomb-type interaction between electrically charged species. Electrostatic interactions play crucial role in formation of quaternary structure of proteins and have profound impact on protein functionality. Two protein domains or subunits can be brought and hold together by electrostatic interactions.¹³ This kind of interaction can be used to bring together short peptides and thus trigger assembly processes. There are many studies that utilize this kind of input to direct assembly process and fold peptide into certain morphology. Synthetic α -helical coiled coils are an example of nanostructures stabilized by electrostatic interactions (Figure 1.3). The amphipathic α -helical peptides are designed to dimerize by interchain hydrophobic interactions at positions *a* and *d* and interchain salt bridges between lysine and glutamic acid residues at positions *e* and *g* of the repeating heptad sequence of Glu-Ile-Glu-Ala-Leu-Lys-Ala (g-a-b-c-d-e-f).¹⁴ Salt bridges formed between chains are an explicit example of designed electrostatic interactions.

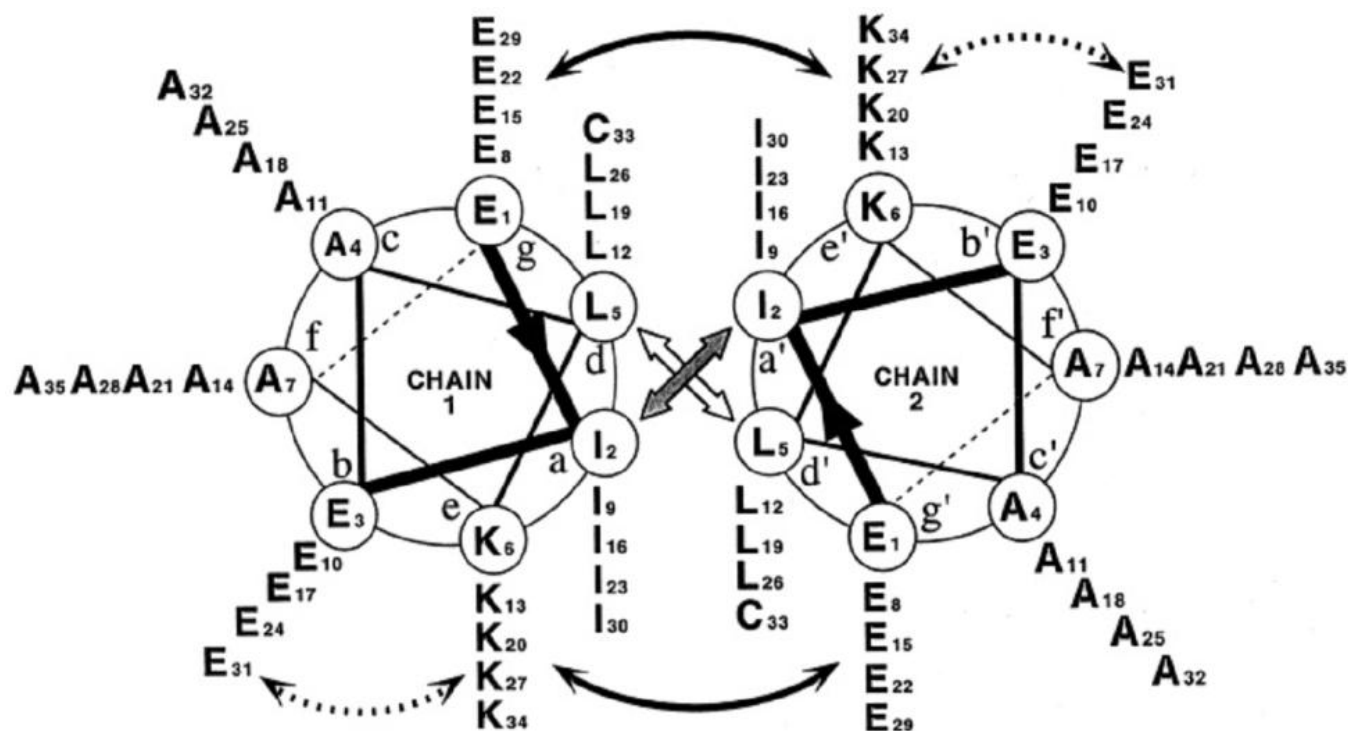


Figure 1.3 Schematic representation of two-stranded, α -helical coiled coils formed by the dimerization of 35 residue sequences. Positions in the heptad repeat are denoted by letters a to g and a' to g' . The number following the capital letter indicates the position of the amino acid residue in the peptide sequence starting from the N-terminus. The hydrophobic residues at a and a' and d and d' interact and stabilize the coiled coil. Intrachain electrostatic attractions could occur between b and e (b' and e'), that is $i, i+3$ interactions, or e and b (e' and b'), that is, $i, i+4$ interactions (dashed arrows). Interchain electrostatic attractions $i, i'+5$ ($g-e'$ or $g'-e$) are shown by the solid arrows (Reproduced from Ref. 14 with permission from American Chemical Society).

Another interesting example is an ionic self-complementary peptide RADARADARADARADA (RADA16-I) that forms a well defined nanofiber scaffold.¹⁵ This 16-residue peptide exhibits stable β -sheet organization and undergoes molecular assembly into nanofibers and eventually forms a hydrogel (Figure 1.4).

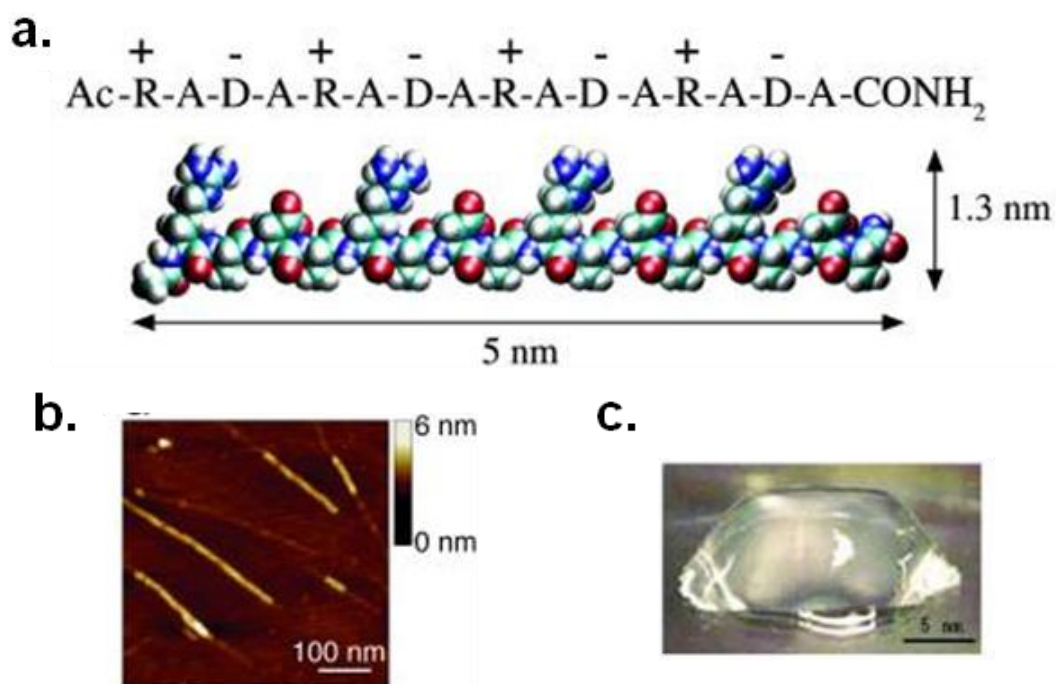


Figure 1.4. Peptide RADA16-I. **a.** Amino acid sequence and molecular model of peptide, **b.** AFM image of peptide nanofiber, and **c.** 0.1 wt% hydrogel in TRIS buffer at pH 7.5 (Reproduced from Ref. 15 with permission from National Academy of Sciences of the U.S.A.).

Next study suggests hydrogel formation by interaction of oppositely charged complimentary modules, Ac-KWKAKAKAKWK-Am and Ac-EWEAEAEAEWE-Am.¹⁶ Further, role of electrostatic interactions in gel formation is investigated by gradually converting glutamic acid residues (E) into glutamine residues (Q) and tracking changes in mechanical properties of a gel by dynamic rheology.

Aliphatic peptide amphiphiles¹⁷ is an interesting variation of peptide molecules where fatty acid is conjugated to N-terminal end. Peptide amphiphiles involve a number of non-covalent interactions in assembly process.¹⁸ Two oppositely charged peptide amphiphiles have been shown to assemble into nanofibers by electrostatic attractions in aqueous medium at neutral pH (Figure 1.5).¹⁹ Peptide assembly and nanofiber formation took place upon mutual charge screening of interacting peptides.

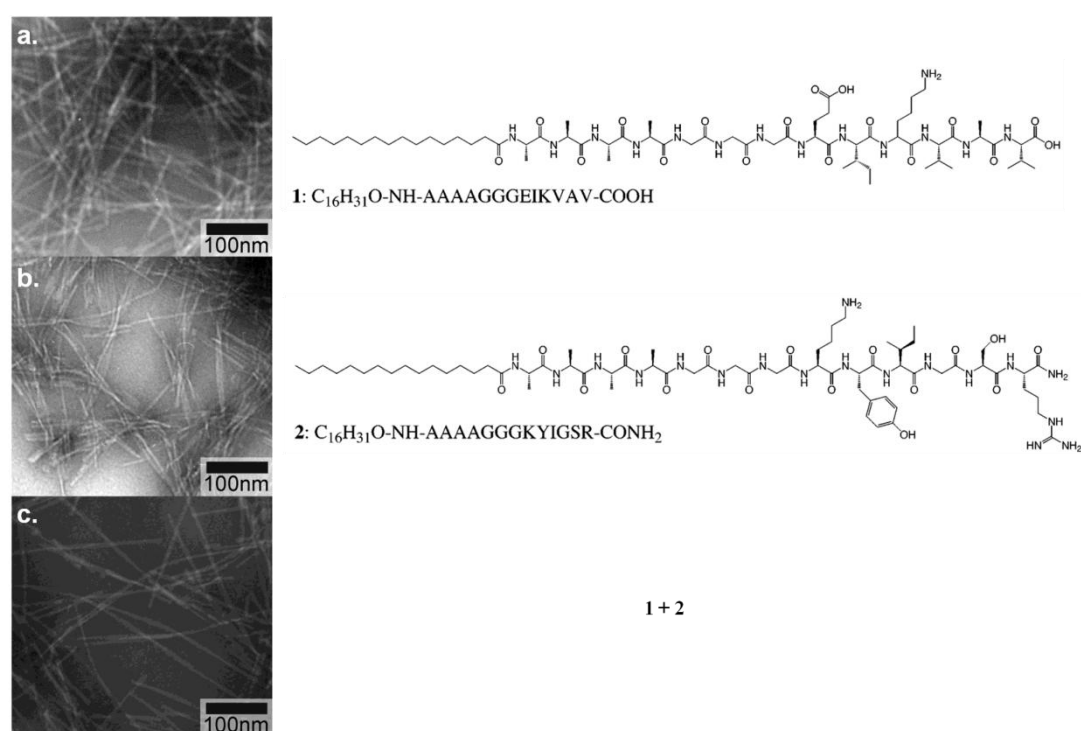


Figure 1.5. TEM images of nanofibers of **a.** **1** at acidic pH, **b.** **2** at basic pH, and **c.** co-assembled **1** and **2** at neutral pH (Reproduced from Ref. 19 with permission from American Chemical Society).

1.2.2 Coordination Interactions

Engineered peptides and proteins can assemble in response to metal coordination (Figure 1.6).²⁰ Peptides are attractive building blocks for systems assembling in

programmed manner because they are chemically stable, can be synthesized in large amounts and can be modified with unnatural functionalities at specific sites. The interplay between peptide assembly and metal coordination has been used to induce secondary/tertiary structure formation in short peptides,²¹⁻²³ to construct artificial metalloenzymes^{24,25} and to direct the peptide assembly for binding biological targets.²⁶

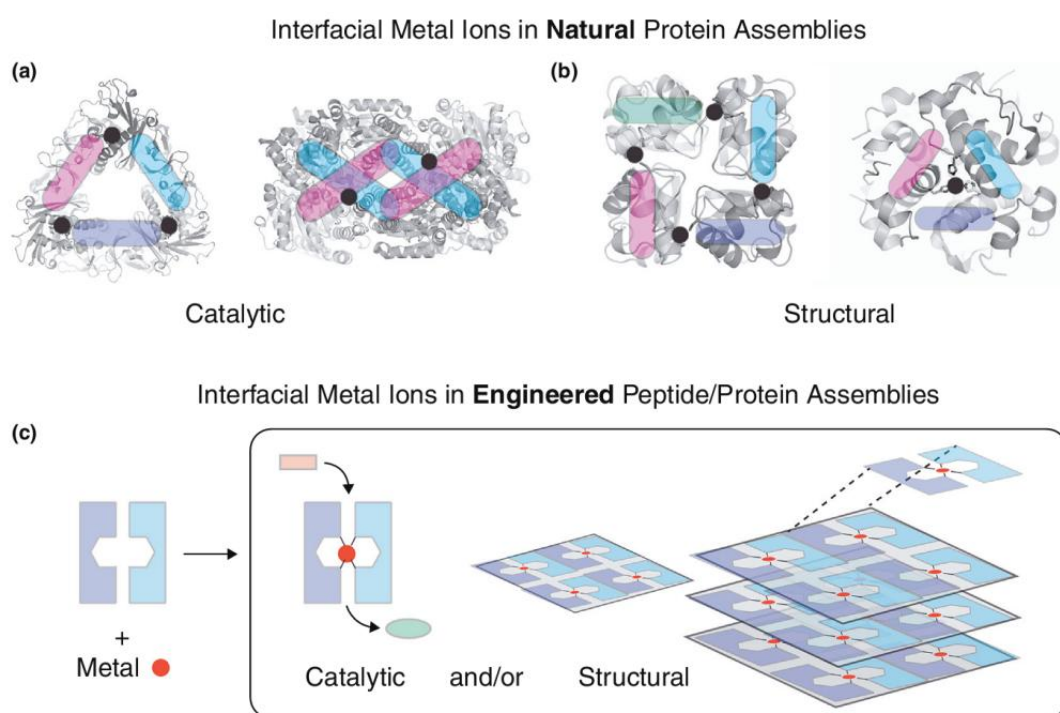


Figure 1.6. Interfacial metal ions in natural and engineered protein assemblies.

a. Representative examples of multimeric proteins that contain interfacial metals as catalytic sites: dicamba monooxygenase and MoFe-protein from nitrogenase. Symmetric protein subunits are shown as colored ovals, and black spheres represent interfacial metal sites. **b.** Protein complexes containing structural metal sites in their interfaces: voltage-gated potassium channel and insulin. **c.** A schematic representation of metal-mediated peptide/protein assembly (Reproduced from Ref. 20 with permission from Elsevier).

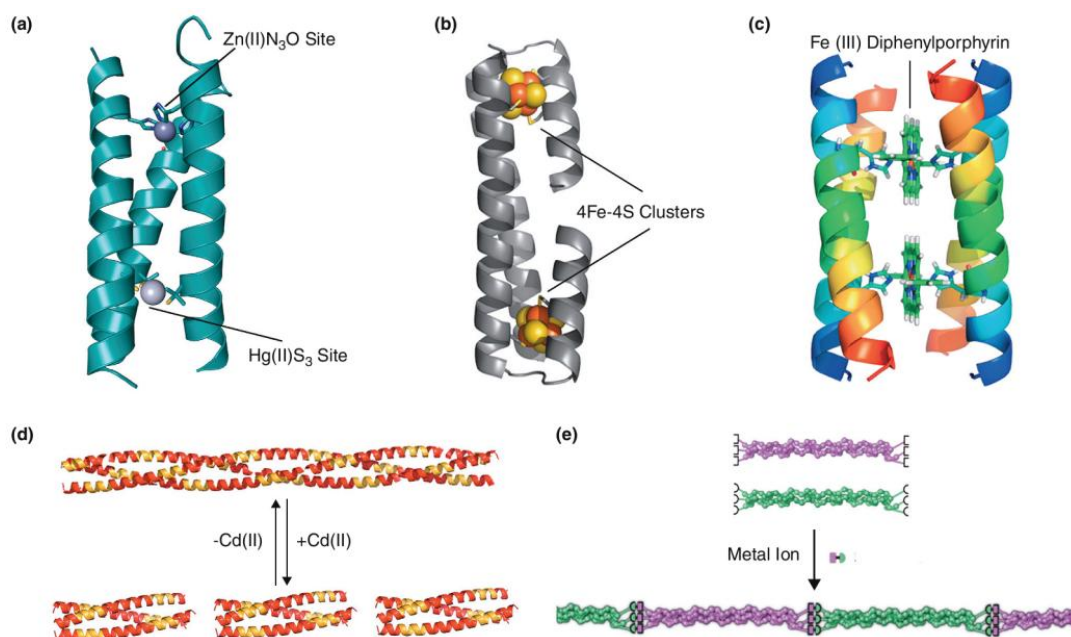


Figure 1.7. Examples of metal-mediated peptide assemblies. **a.** A trimeric coiled-coil peptide housing a structural Hg(II), and a catalytic Zn(II) site.²⁷ **b.** A homodimeric triple helix protein scaffold with two redox-active 4Fe-4S clusters bound at interfacial positions.²⁸ **c.** A membrane-soluble four-helix peptide assembly bearing two Fe-diphenylporphyrin cofactors in its interior.²⁹ **d.** Cd(II)-mediated conformational switching of a peptide with heptad-repeat motifs between nanofibrils and discrete three-helix bundles (yellow: Cys-containing heptads and red: Cys-free heptads).³⁰ **e.** Metal-dependent assembly of collagen bundles functionalized with complementary coordination motifs into banded architectures³¹ (Reproduced from Ref. 20 with permission from Elsevier).

Previously mentioned α -helical coiled-coil peptides with heptad-repeats are the most widely used scaffolds in protein engineering, because their structures and assembly route can be programmed up to certain extent at the amino acid sequence design level.^{32,33} For instance, introverted hydrophobic residues (positions *a* and *d*) can be replaced with metal-coordinating residues to obtain internal coordination sites, at the

same time, extroverted residues can be modified for metal-directed supramolecular assembly. Several studies have shown that even multiple metal ions can be selectively introduced into assembling trimeric or tetrameric helical scaffolds. Some of the examples are assemblies with interfacial Cu (I/II),³⁴ Ni(II)/Cu(II)³⁵ and Hg(II)/Zn(II) (Figure 1.7a)²⁷ mixed metal ion pairs, Cu(I)-thiolate³⁶ or Cd(II)-thiolate clusters,³⁷ and 4Fe-4S clusters (Figure 1.7b).²⁸ All of the mentioned assemblies are constructed via appropriate spatial organization of histidine and cysteine residues. Regarding designer transmembrane protein complexes, the peptide sequence Ac-AIYGILAHSLASILALLTGFLTIW-Am assembling upon binding a pair of Fe(II/III) diphenylporphyrins into four-helix bundles has been reported (Figure 1.7c).²⁹

1.2.3 Hydrogen Bonding

Hydrogen bonding (H-bonding) is inherent interaction mode of peptides and proteins. β -sheet and α -helix structural motifs³⁸ (Figure 1.8) are the most abundant organizational elements in folding and supramolecular assembly of proteins. In polypeptides, mainly amide bonds which connect adjacent amino acids are responsible for emergence of structural H-bonding. H-bonding in tandem with engineered sequence can be used as an input on design level that will direct and determine the fate of assembling structure. H-bonding being weak on its own gains considerable weight when reaches cumulative effect, i.e. hydrogen bonds have additive nature.

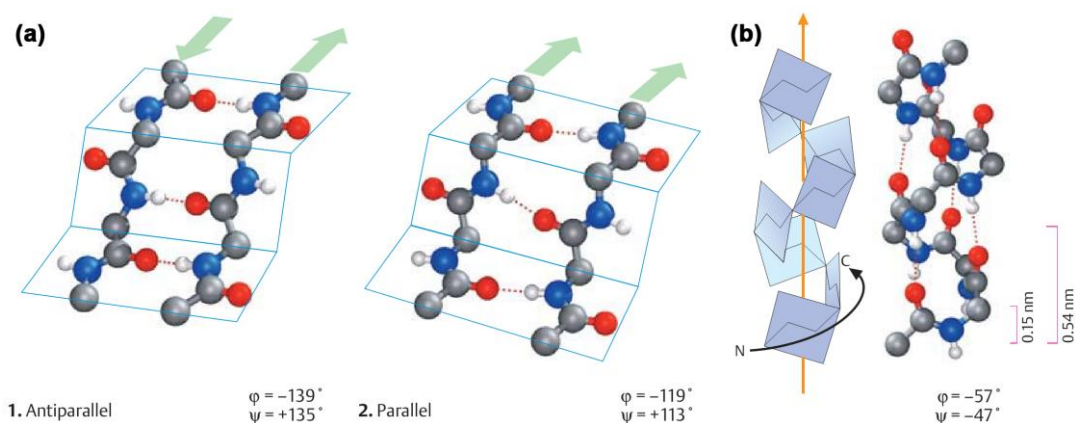


Figure 1.8. H-bonding based structural motifs. a. Antiparallel and parallel pleated β -sheets and **b.** α -helix (Reproduced from Ref. 38 with permission from Thieme).

All peptide-based assembling materials in one way or the other involve hydrogen bond mediated interaction mode. In many of our aliphatic peptide amphiphile designs³⁹⁻⁴¹ we have utilized VVAG sequence, which is strong β -sheet forming segment (Figure 1.8a). Active intermolecular hydrogen bond formation takes place between these four residues which actually “glue” assembling molecules together.

Mentioned earlier coiled coil peptides involve intramolecular H-bonding, which is known as α -helix motif (Figure 1.8b). Next example includes assembly of ring-shaped peptide molecules into nanotube structures (Figure 1.9).⁴² A cyclic octapeptide with the sequence of cyclo[-(aEaQ)₂-] forms antiparallel stacks via intermolecular H-bonding along peptide backbone. Formation of contiguous β -sheet structure provides nanotube growth. Alternating L and D amino acids ensure that all side chains are situated on the exterior of the nanotubes.

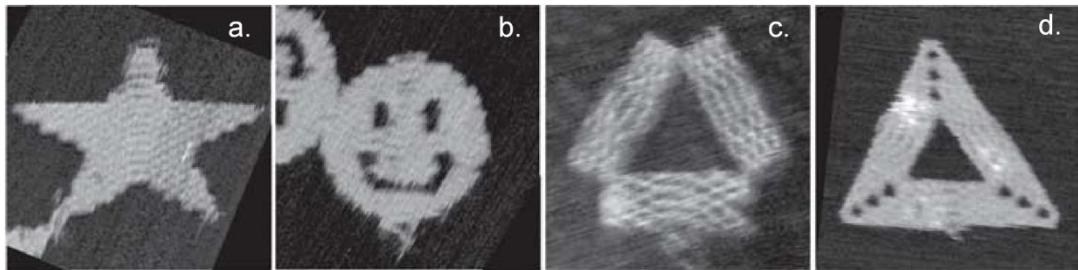


Figure 1.10. a. DNA origami shapes. a. star, **b.** disk with three holes; **c.** triangle with rectangular domains, **d.** sharp triangle with trapezoidal domains and bridges between them (Reproduced from Ref. 43 with permission from Nature Publishing Group).

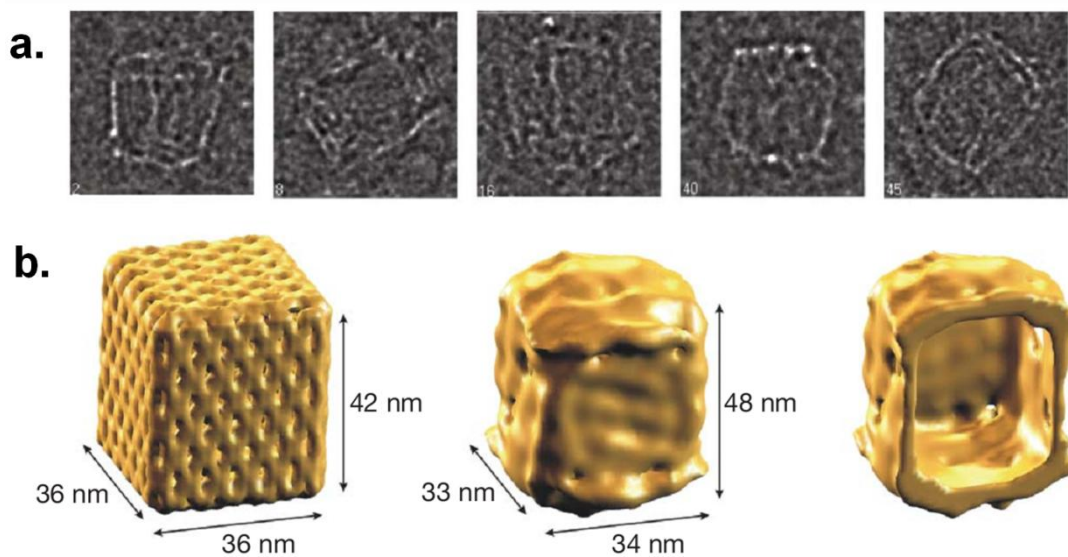


Figure 1.11. a. Single-particle cryo-TEM images of box-shaped ensembles. **b.** Single-particle reconstruction of the DNA box applying D2 symmetry. Left, theoretical model. Middle, surface representation of the cryo-TEM map. Right, cut-open view showing the interior cavity of the cryo-TEM map (Reproduced from Ref. 44 with permission from Nature Publishing Group).

1.2.4 π - π interactions

π - π interactions are a widely used type of input in programmed assembly. π -rich systems can provide an extra structural motif and stabilize overall architecture. The strongest π - π interaction is stacking of aromatic moieties. Effective overlap between aromatic π -systems can significantly stabilize assembling structure, and thus, actively participate in assembly process.⁴⁵ Several studies including our own have used this kind of input; for instance, diphenylalanine (FF) unit has taken place in several peptide designs. The simplest peptide of this kind which is dipeptide FF assembles into highly-ordered nanotubular structures.⁴⁶ Peptide designs such as Ac-KFFAAK-Am and Ac-EFFAAE-Am have been realized in our own studies;^{47,48} these peptides effectively assemble into fibrillar structures (Figure 2.5). Moreover, even a single amino acid – phenylalanine was reported to assemble into fibrils.⁴⁹

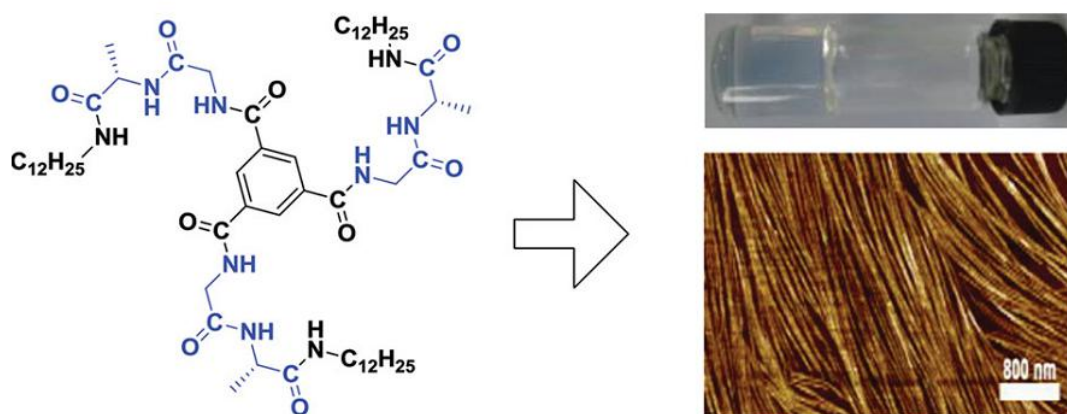


Figure 1.12. A peptidic molecule assembles into fibers and forms a gel in THF (Reproduced from ref. 50 with permission from John Wiley & Sons Ltd).

A peptidic molecule (Figure 1.12) consisting of trimesic acid core (π - π stacking), Gly-Ala dipeptidic pendants (H-bonding), and long alkyl chains terminals (solvophobic interaction) is another example for π - π interaction stabilized

ensemble.⁵⁰ These molecules with C₃-symmetry undergo supramolecular polymerization in THF and form a gel.

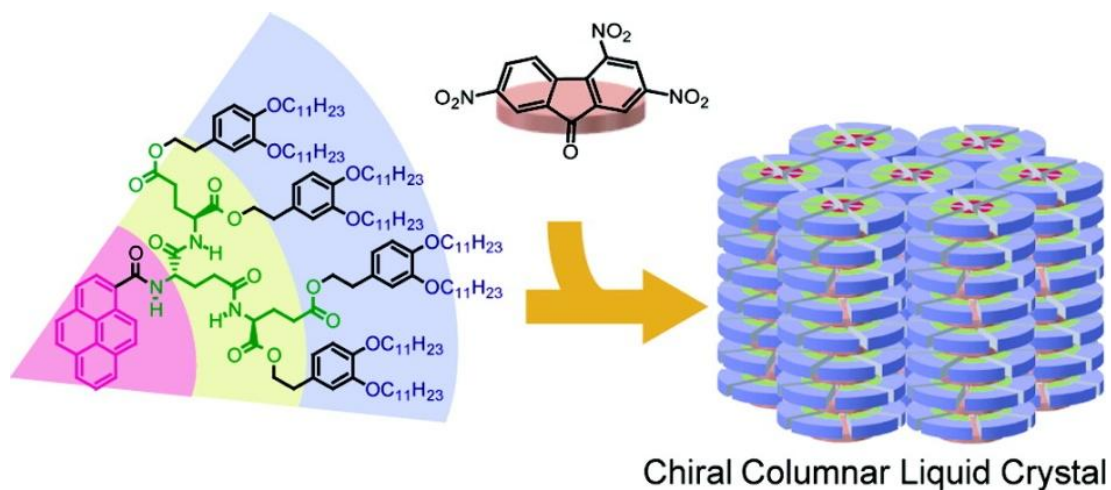


Figure 1.13. A schematic representation of assembly of chiral nanocolumns (Reproduced from Ref. 51 with permission from American Chemical Society).

A supramolecular chirality tuning of a π -conjugated molecule through cooperative π - π interaction and H-bonding was reported.⁵¹ A compound having molecular triblock structure, pyrene moiety at focal point, branched glutamic acids as chiral hydrogen-bonded parts, and lipophilic alkyl parts, was expected to form cylindrical pillars by nanosegregation between the molecular block structures (Figure 1.13).

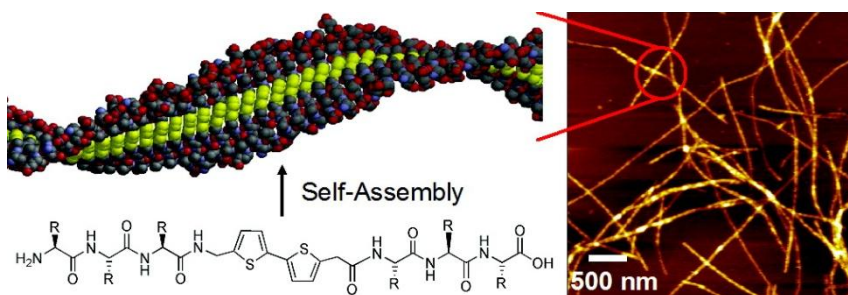


Figure 1.14. An assembly of oligopeptide-flanked bithiophene into nanofibers (Reproduced from Ref. 52 with permission from American Chemical Society).

Next intriguing example represents a π -conjugated system flanked by oligopeptide sequence at each side (Figure 1.14).⁵² This molecule with embedded bithiophene unit assembles into discrete one-dimensional nanostructures in aqueous medium. π - π stacking of bithiophenes and H-bonding between oligopeptides give rise to programmed assembly. The peptide-based molecular design provides intimate π - π communication within the supramolecular ensemble upon charge screening. These nanostructures can be utilized in optical or electronic applications in biological environments.

1.2.5 Solvophobic interactions

Solvophobic interaction is an important mode of interaction which can be encountered in animal cells. Phospholipids such as phosphatidylcholines (Figure 1.15a) can assemble in aqueous medium due to hydrophobic effect. Phospholipid bilayer, liposome and micelle are explicit examples of supramolecular architectures formed as a result of solvophobic interactions (Figure 1.15b). Solvophobic aggregation is common for amphiphilic molecules. The solvophobic parts of the molecules tend to aggregate to minimize their surface area contacting the solvent, whereas the solvophilic parts try to remain solvated. Solvophobic aggregation takes

place mainly due to favorable entropy rather than enthalpy and is thought to be achieved in two stages. The stages are solvation and solvophobic escape from the solvent; in the later stage solvent molecules are liberated, and consequently total entropy is increased.

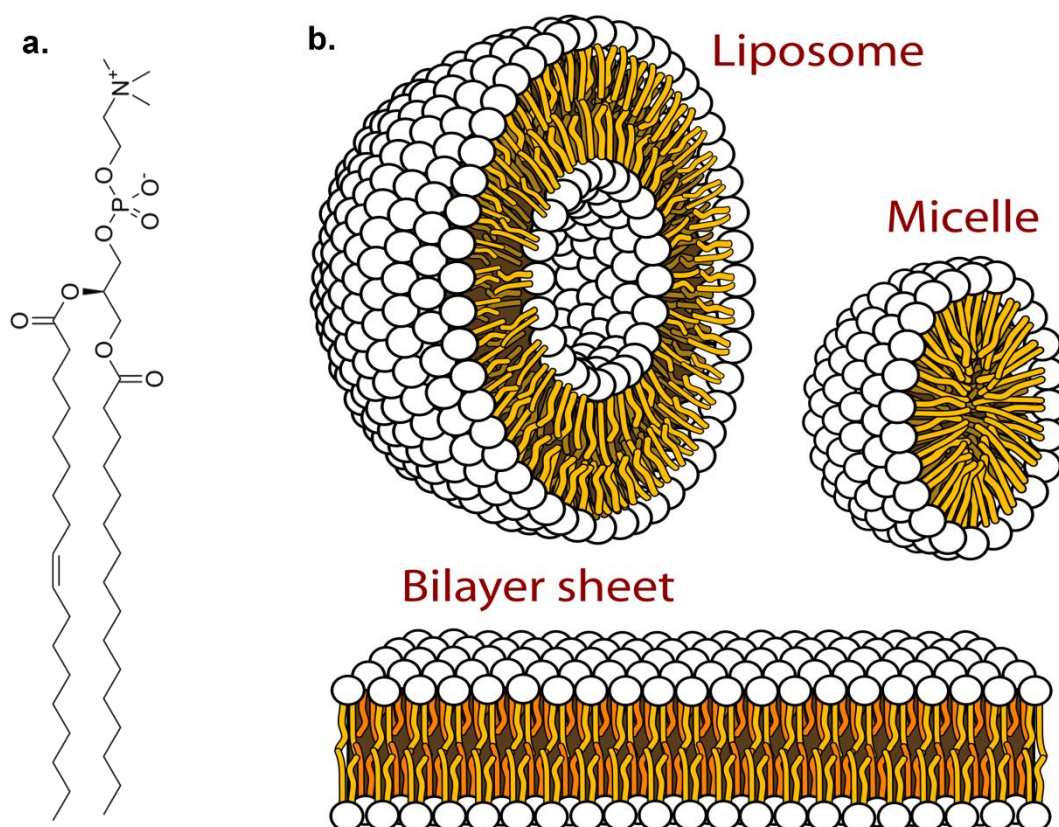


Figure 1.15. **a.** Phosphatidylcholine molecule. **b.** Supramolecular architectures formed by phosphatidylcholine.

Folding of globular proteins is also accompanied with solvophobic interactions.⁵³ Hydrophobic residues in globular proteins tend to burry themselves in forming hydrophobic core, whereas hydrophilic residues tend to stay in contact with the solvent on the surface of a globe. For instance, hemoglobins from higher organisms

are multi-subunit globular proteins. Each subunit of the hemoglobin complex is a globular protein with an embedded heme group. These subunits are organized in characteristic three-dimensional globin fold typically consisting of eight α -helices; the folding process is primarily governed by sterics and hydrophobic interactions of the amino acid side chains near the helix interfaces. On the higher design level, the noncovalent interactions stabilizing the quaternary structure of the complex comprise solvophobic interactions, van der Waals forces, H-bonding, and electrostatic interactions (salt bridges).

Many low molecular weight compounds can assemble and gel a solvent. Gel formation can be defined as an extensive solute precipitation into three-dimensional network which entraps a solvent. An assembly of these compounds into fibrous networks is driven by noncovalent interactions such as electrostatic interactions, π - π interactions, dipolar interactions, H-bonding, and van der Waals interactions. Solvophobic effects also play a crucial role. These solvophobic effects stem from moieties or functional groups in the gelator molecule that are poorly soluble in the solvent to be gelled, and contribute to the gel forming ability by reducing the overall solubility of the gelator in that solvent.

A large group of gelators is related to amphiphilic molecules (Figure 1.16). Amongst the oldest gelators known to humankind are metallic soaps.⁵⁴ An example of a metallic soap gelator is the lithium salt of 12-hydroxystearic acid **1**.⁵⁵ This gelator assembles by means of several intermolecular interactions: ionic interactions between the metal ions, van der Waals interactions between the alkyl tails and H-bonding interactions between hydroxyl groups. Cationic surfactants are structurally

related to the metallic soaps. Quaternary ammonium salts with long alkyl chains, like compound **2** exhibit gel forming behavior due to association driven by electrostatic interactions, as well as the poor solubility of the quaternary ammonium head in many organic solvents.⁵⁶ Less obvious amphiphilic gelators are compounds **3** and **4**. Gelator **3** is a perfluorocarbon-hydrocarbon block-compound, of which in hydrocarbon solvents the perfluorocarbon block is insoluble and hence solvophobic, whereas the aliphatic part is soluble, and hence solvophilic.⁵⁷ At room temperature compound **3** is immiscible with aliphatic hydrocarbons, leading to extensive precipitation and gel formation at weight concentrations as low as 2 %. Compound **4** can be also considered as an amphiphile for hydrocarbon solvents, with the aliphatic hydrocarbon and anthracene moieties as solvophilic and solvophobic parts, respectively.⁵⁸ Apart from solvophobic effects aggregation of **4** is also governed by π - π stacking and van der Waals interactions. In addition to solvophobic effects and specific intermolecular interactions, structural rigidity may have its own contribution to the gelling potency. For example, many steroids like cholesterol molecule **5** efficiently gel various organic solvents.^{59,60} The strong aggregation behavior of these compounds is thought to stem from their molecular rigidity, which reduces entropic losses upon assembly. 1,3:2,4-di-*O*-benzylidene-D-sorbitol **6** is also a fairly rigid molecule with reduced conformational freedom, and it is known as an efficient gelator for both water and organic solvents.^{61,62}

In the examples given above H-bonding is mostly absent or its contribution to assembly mechanism is minimal. However, for many gelator molecules H-bonding is essential for their ability to gel a solvent. For instance, the peptides like **7** have been reported to gel a range of different organic solvents, due to the strong H-bonding

between the peptide amide groups.⁶³ Another well-known example of an amino acid based gelator is Fmoc-diphenylalanine **8**, this aromatic peptide amphiphile is a very potent hydrogelator capable of gelling water at weight concentrations as low as 0.22%.⁶⁴ With this compound again solvophobic effects, namely hydrophobic effects, and π - π interactions between the pendant benzyl groups make a major contribution to the stability of its supramolecular aggregates.

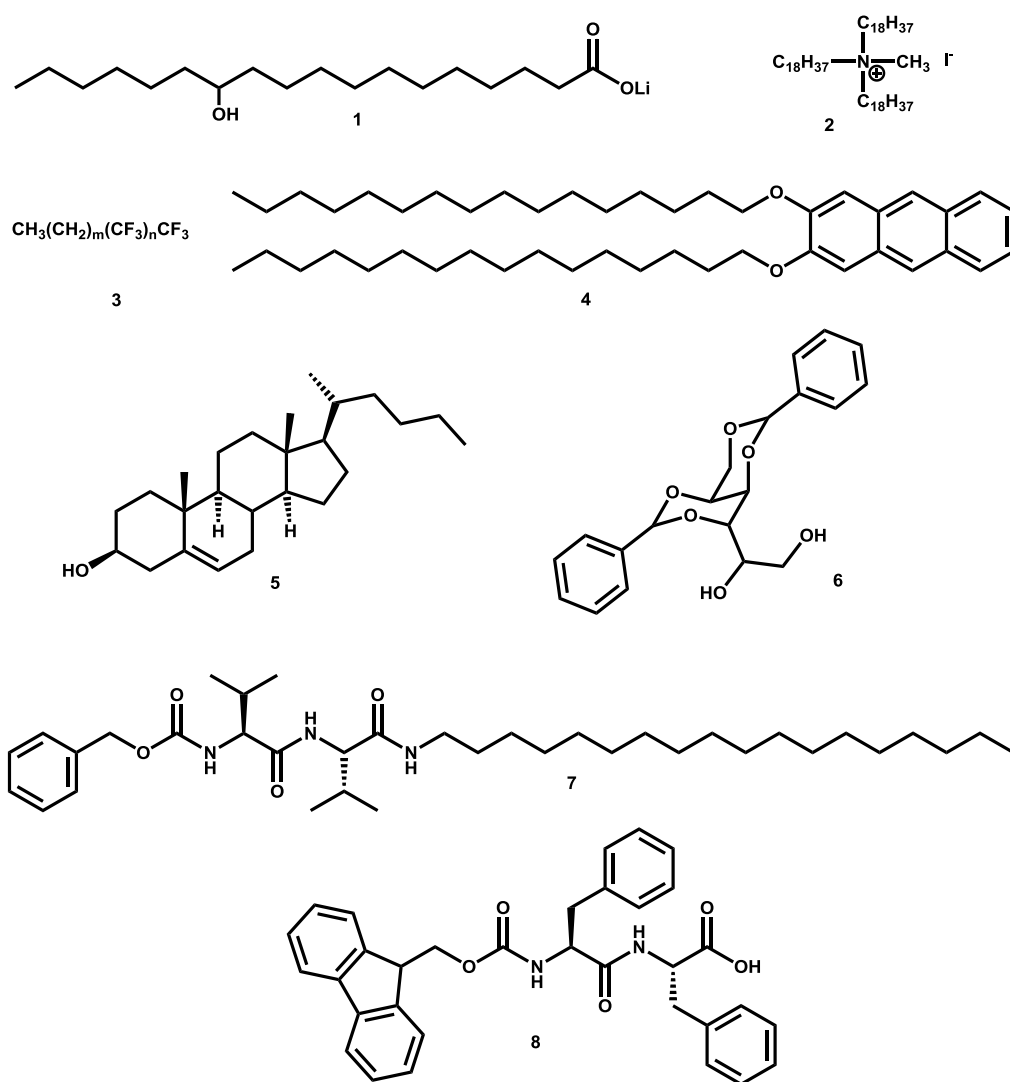


Figure 1.16. Amphiphilic low molecular weight gelators.

1.2.6 Van der Waals interactions

The van der Waals force is the attractive or repulsive force between molecules other than those due to covalent bonds or to the electrostatic interactions of ions. The van der Waals force originates from dipole or induced-dipole interactions at the atomic and molecular levels. There are three different types of van der Waals forces:

1. Keesom interactions: permanent dipole-permanent dipole interactions.
2. Debye interactions: permanent dipole-induced dipole interactions.
3. London dispersion forces: induced dipole-induced dipole interactions.

The van der Waals forces play crucial role in protein folding. Since atoms are in constant oscillation, the dipole induction is a continuous process. In comparison with other forces determining protein conformation, single van der Waals interactions are extremely weak. However, the large number of closely arranged van der Waals forces in proteins makes these interactions significant to the preservation of tertiary structure. Striking example of cumulative effect of van der Waals interactions is adhesion mechanism of gecko setae to various surfaces.⁶⁵

Aliphatic and aromatic peptide amphiphiles mentioned earlier, once assembled, are also stabilized by van der Waals forces. Alkyl tails or aromatic moieties closely packed in hydrophobic core of nanostructures are effectively kept together by cumulative effect of van der Waals interactions.

Chapter 2.

2. Peptide nanonetworks templated titania for dye sensitized solar cell

2.1 Amyloid-like peptide nanofiber templated titania nanostructures as dye sensitized solar cell anodic materials

This work is partially described in the following publication:

Acar, H., Garifullin, R., Aygun, L.E., Okay, A.K., Guler, M. O., “Amyloid-Like Peptide Nanofiber Templated Titania Nanostructures as Dye Sensitized Solar Cell Anodic Materials”, *Journal of Materials Chemistry A*, 2013,1, 10979

2.1.1 Introduction

Solar energy is an important source for sustainable energy efforts. Dye sensitized solar cells (DSSCs) are promising and inexpensive alternatives to silicon based solar cells.⁶⁶ Although there are many semiconductor materials available for fabrication of DSSCs, titania (TiO₂) is the most widely used one owing to its several advantages such as abundance, biocompatibility, eco-friendliness and inexpensiveness.⁶⁷ DSSCs have several components for light harvesting, electron transport, and hole transport. Optimization of each component affects the overall performance of the cell.⁶⁸ Since electrons and holes are transported in different media, separate optimization in each interface can be studied to enhance the yield of DSSCs. There are several parameters to enhance the efficiency of DSSCs including TiO₂ component such as obtaining pure anatase phase, greater surface area for better dye adsorption, hole conduction, higher pore volume and diameter,

and well-connected network of individual nanostructures.^{69,70} On the other hand, application of TiO₂ nanoparticles in DSSCs limits the power conversion efficiency of DSSCs by electron trapping in the nanostructured film. The time scale for injection and transport of the electron by TiO₂ is comparable with the time scale of the recombination by the electrolyte.⁷¹ The competition between these time scales determines the photon-to-current conversion efficiency of the DSSC. One of the major problems of DSSCs is this loss of electron at the TiO₂/electrolyte interface.⁷²

The transport of charge carries through a one-dimensional morphology of TiO₂ electrode is more facile because of its inherent nature to produce lower diffusion resistance.⁷³ One-dimensional nanostructures including nanowires⁷⁴ and nanorods⁷⁵ are able to transport electrons before recombination process takes place.⁷⁶ Highly ordered architectures offer longer electron diffusion paths and shorter electron transport time constants than randomly oriented titania nanoparticle films.⁷⁷ In fact, cylindrical (nanowire) and tubular (nanotube) architectures act as a “box” that delimits medium through which electron travels. If the diameter of the “box” is smaller than the mean free path of the electron, enhancement in electron mobility could be expected.⁷⁸

Proteins and peptides can assist synthesis of nanostructured inorganic materials in an eco-friendly strategy via biomineralization process. Nature inspired synthetic peptide nanofiber networks have wide applications including bioactive tissue scaffolds,^{79,80} carrier agents,^{81,82} and template-directed synthesis of inorganic materials.⁸³ Self-assembled amyloid-like peptides (ALPs) can be successfully used to obtain one-dimensional inorganic nanostructures,^{47,84} which may find

applications in electronics⁸⁵ and sensors.⁸⁶ Synthesis of TiO₂ hybrid nanowires using amyloid protein fibrils as templates, and their application in hetero-junction hybrid solar cells were previously reported.⁸⁷ The peptide assemblies can be effectively used as soft templates for synthesis of inorganic and organic-inorganic hybrid nanostructures.⁸⁸ Previously, we demonstrated titania and silica mineralization on the self-assembled ALP templates.⁴⁷

In this chapter, we demonstrate peptide nanofiber templated synthesis of TiO₂ nanostructures. Bottom-up approach, realized through mineralization process of self-assembled organic templates, leads to high-surface area hybrid titania nanofiber network. Calcination of hybrid material network on the surface of fluorine doped tin oxide (FTO) coated glass yields in functional electrode with nanostructured anatase titania layer. Staining of obtained titania layer with N719 photosensitizer dye provides it with photoactivity. Photoactivity and overall performance of functional devices based on our engineered materials were assessed in dye sensitized solar cell application. One-dimensional TiO₂ nanostructures synthesized with self-assembled peptide templates exhibit high surface area with abundant mesopores, which is convenient for high dye loading, and also exhibit improved open circuit voltages (V_{oc}); as a result, enhanced photovoltaic performance is observed compared to peptide nanofiber template-free TiO₂ particles.

2.1.2 Results and Discussion

Designed peptides were constructed on MBHA Rink Amide resin. Amino acid coupling reactions were performed with 2 equivalents of Fmoc-protected amino acid,

1.95 equivalents of HBTU and 3 equivalents of DIEA for 2 h. The Fmoc protecting group removal was performed with 20 % piperidine in DMF solution for 20 min. Cleavage of the peptides from the resin was carried out with a mixture of TFA:TIS:H₂O in ratio of 95:2.5:2.5 for 3 h. Excess TFA was removed by rotary evaporation. The remaining peptide was triturated with ice-cold diethyl ether; the resulting white precipitate was dissolved in water, freeze-dried at -80 °C and lyophilized. The peptides were characterized by a quadrupole-time of flight mass spectrometry (Q-TOF MS). The mass spectra (Figures 2.1 and 2.3) show the corresponding mass of the peptides; the purity of the peptides were assessed by RP-HPLC and found to be more than 95 % (Figures 2.2 and 2.4).

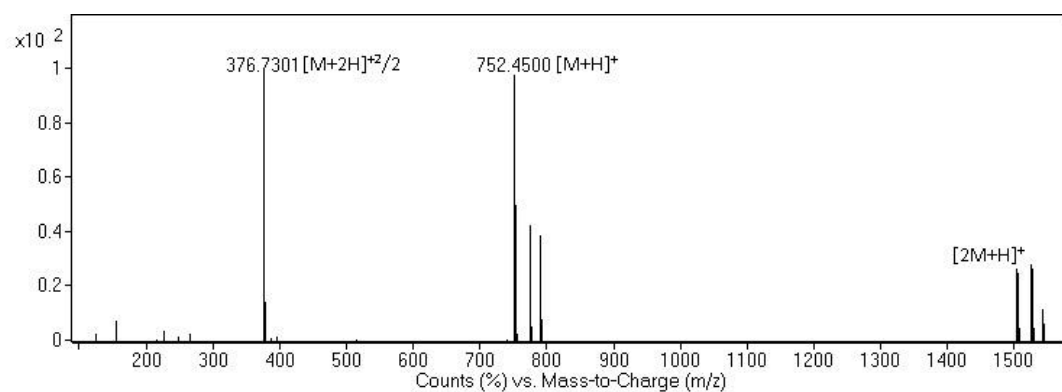


Figure 2.1. Mass spectrum of Ac-KFFAAK-Am (Peptide-1) molecule. MS: (m/z) calculated 751.4381, $[M+H]^+$ found 752.4500, $[M+2H]^{2+}/2$ found 376.7301.

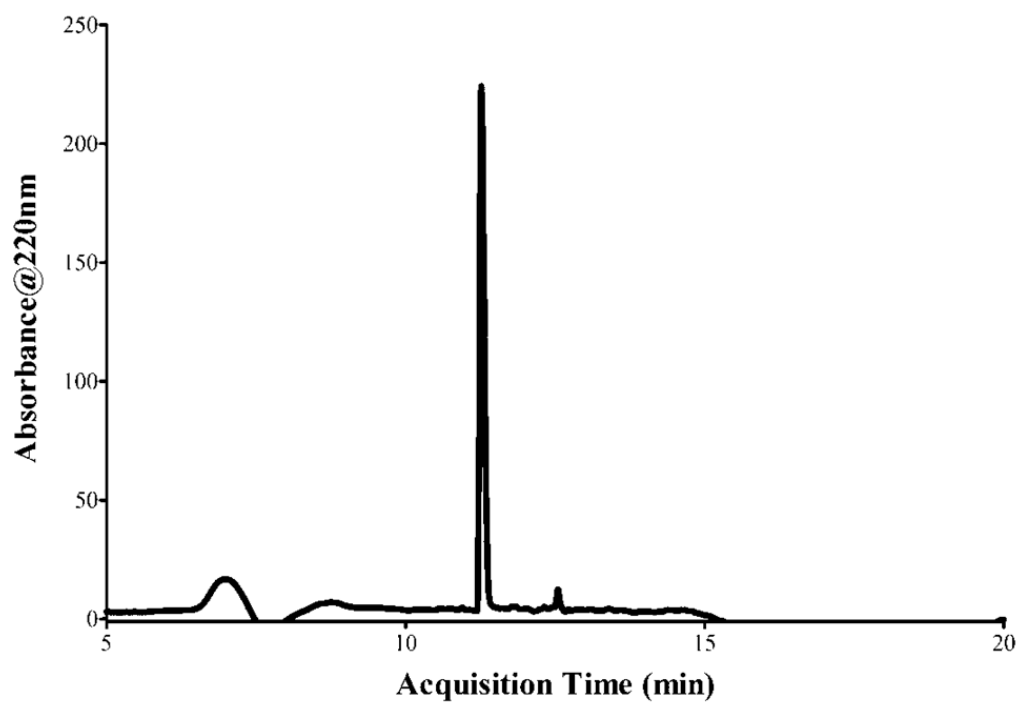


Figure 2.2. Liquid chromatogram of Ac-KFFAAK-Am peptide.

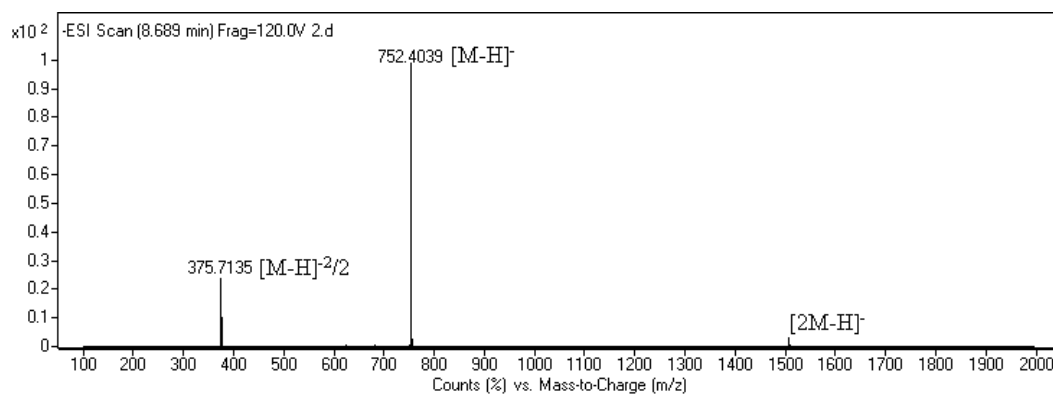


Figure 2.3. Mass spectrum of Ac-EFFAAE-Am (Peptide-2) molecule. MS: (m/z) calculated 753.3334, $[M-H]^{-}$ found 752.4039, $[M-2H]^{-2/2}$ found 375.7135.

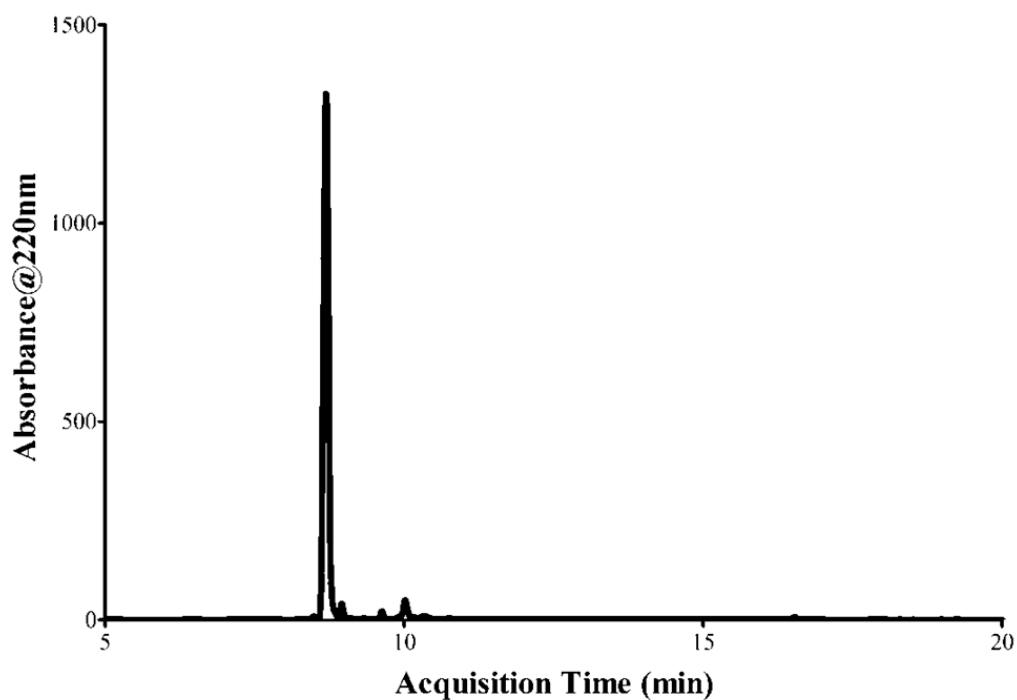


Figure 2.4. Liquid chromatogram of Ac-EFFAAE-Am peptide.

Amyloid-like peptides (ALP) are able to self-assemble into one-dimensional nanofibrillar structures through supramolecular interactions between individual peptide molecules. Here, two de novo designed peptides (Figures 2.5 a and b) with high binding affinity to metal ions were used in synthesis of nanostructured TiO₂. The nanostructured TiO₂ was obtained through bottom-up approach, where self-assembled peptide nanofibers (Figures 2.5 c and d) were used as a template. Amine group in the lysine residues in the peptide 1 (Figure 1a) and carboxylate group in glutamate residues in the peptide 2 (Figure 1b) act as nucleation and successive growth centers for TiO₂. Owing to the side chains of lysine residues, the peptide 1 is several atoms longer than peptide 2 (Figures 2.5 a and b) and the self-assembled peptide nanofibers formed by peptide 1 are slightly thicker than the peptide 2 nanofibers (Figures 2.5 c and d). The average diameters of peptide 1 and peptide 2 nanofibers are found to be 11.4 ± 0.42 and 9.1 ± 0.61 nm, respectively. The difference in nanofiber thicknesses is dictated by the self-assembly mechanism; while hydrophobic amino acids in the structure of the peptides escape from polar solvent and bury themselves in the core of the nanofibers, hydrophilic residues, on the contrary, expose them on the nanofiber surface. Solvophobic escape and consequent nanofiber formation is enhanced by π - π stacking of diphenylalanine motif.

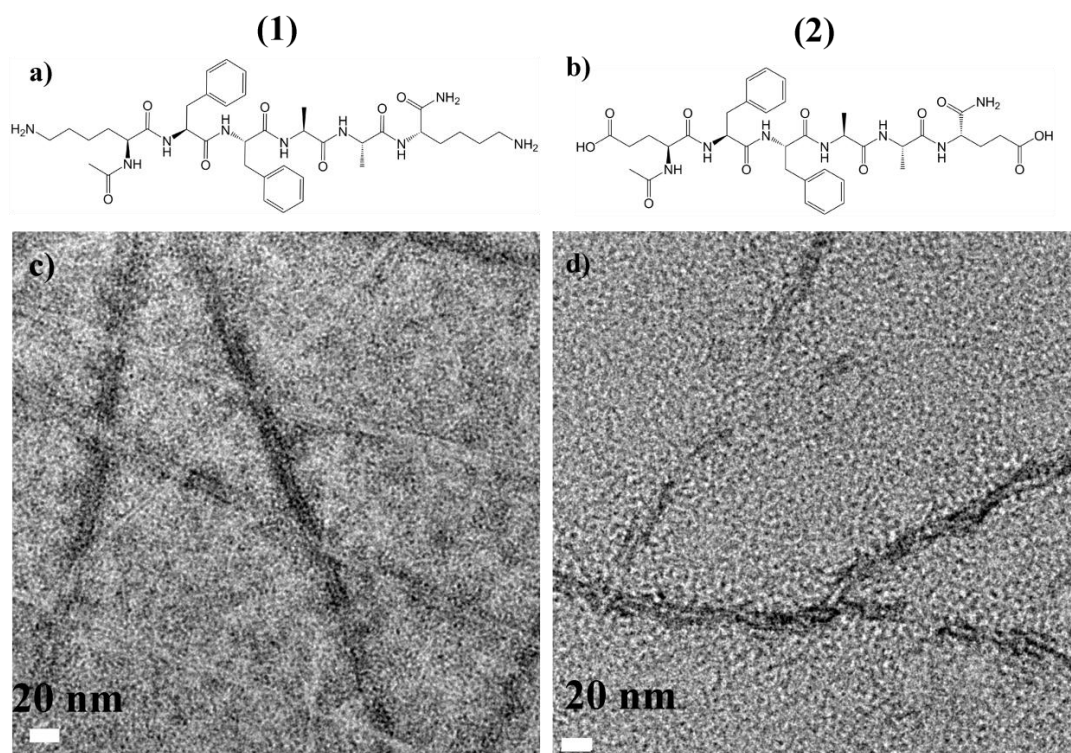


Figure 2.5. Amyloid-like peptides. **a.** Ac-KFFAAK-Am (peptide 1), **b.** Ac-EFFAAE-Am (peptide 2), **c.** TEM image of the peptide **1** nanofibers, **d.** TEM image of the peptide **2** nanofibers.

Peptide self-assembly and nanofiber surface mineralization both take place in solution, thus making this approach appealing for bulk production procedures. To compare the template-effect of peptide nanofibers and their effect on peptide templated TiO_2 morphology, template-free TiO_2 particles were synthesized under the identical conditions.

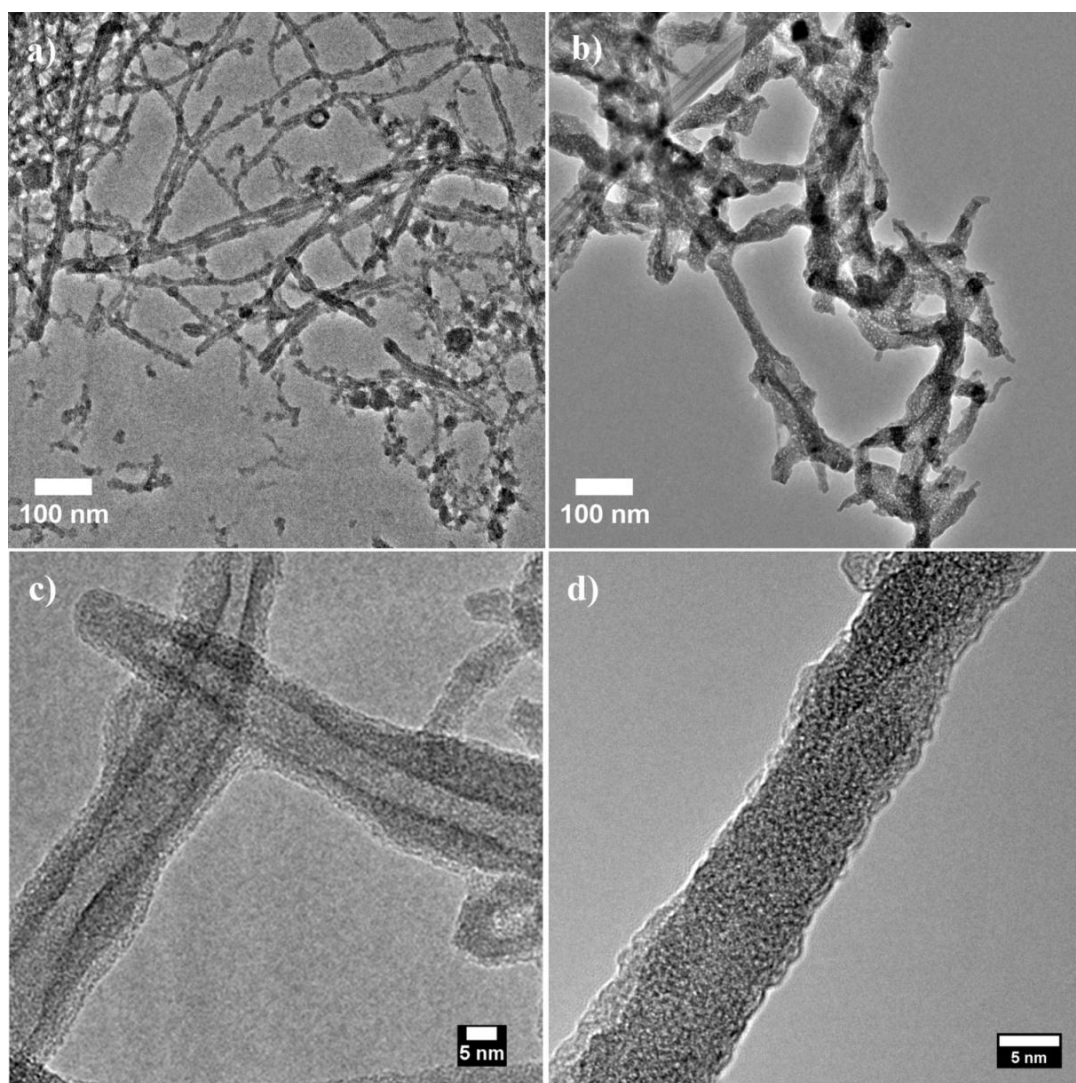


Figure 2.6. One-dimensional titania nanostructures after calcination. TEM images of peptide **1**-templated titania **a.** nanotube network and **c.** nanotubes; TEM images of peptide **2**-templated titania **b.** nanowire network and **d.** nanowire.

Exploiting soft nanofibrillar templates in nanofabrication processes enables synthesis of high-aspect-ratio materials with high surface area.⁸³ Here, we obtained highly porous network of one-dimensional TiO_2 nanostructures by using peptide nanofiber templates (Figure 2.6). Due to the shape of the template-directed TiO_2 growth, fast and directional charge transfer to the conductive

transparent oxide layer (anode) should be possible. This charge transfer enhancement should substantially decrease conduction losses, due to recombination process in the electrode. Moreover, nanostructured titania with high surface area provides increased interaction between TiO_2 and the dye in DSSC devices. To understand the effect of nanostructured TiO_2 on DSSC photovoltaic performance, three sets of solar cells were built from three different TiO_2 materials (peptide 1-templated TiO_2 , peptide 2-templated TiO_2 and template-free TiO_2). Peptide 1 leads to nanotubular TiO_2 structures, while peptide 2 favors TiO_2 nanowire architecture (Figures 2.6 a and b). As mentioned above, the lysine residues have longer side chains compared to glutamate residues. This small difference affects final diameter of the nanofibers. Thicker peptide 1 nanofibers prevent complete sintering of the material into nanowire during the calcination process, therefore nanotubes are observed. The thickness of the self-assembled peptide nanofibers affects the resultant architecture of one-dimensional TiO_2 nanostructures (Figure 2.6). It is crucial to obtain one-dimensional TiO_2 in its anatase phase – a semiconductor phase used for DSSC construction. The mineralized titania nanostructures were annealed at 450 °C to obtain anatase phase. The XRD pattern of the anatase phase obtained during sintering and annealing process is shown in Figures 2.7. The organic peptide template was removed during the calcination stage. It was previously demonstrated that the thermal decomposition of peptide completes at 350 °C.⁴⁷ Thus, 450 °C is sufficient for both thermal decomposition of the organic peptide template and phase transformation of titania.

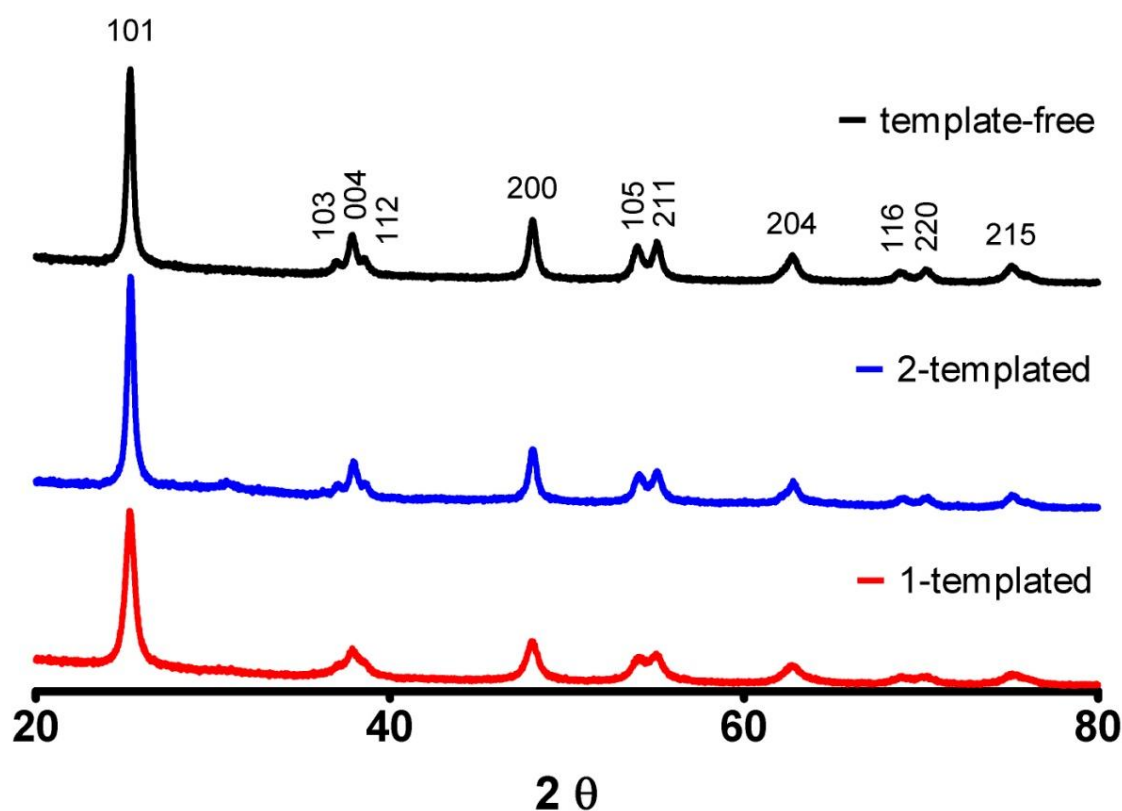


Figure 2.7. XRD spectra of template-free TiO_2 (black), peptide 1-templated TiO_2 (blue), peptide 2-templated TiO_2 (red) powders.

Calcination process takes place directly on FTO glass, which minimizes the solar cell assembly steps. Stained with sensitizer (N719), peptide-templated materials were probed in dye sensitized solar cell experiments. In fully functional solar cell devices, nanostructured titania was sandwiched between two electrodes with addition of liquid I_2/I^- electrolyte. The amount of TiO_2 on the FTO surface is an important parameter, which affects the overall efficiency of the DSSC. Accurate measurement of the TiO_2 amount was achieved by inductively coupled plasma – mass spectrometry (ICP-MS). The amount of template-free synthesized TiO_2 was found to be about two times greater than TiO_2 amounts of nanowires and

nanotubes synthesized by peptide nanofiber templates. It could be due to the three-dimensional structure of bulk TiO_2 nanowires and nanotubes, which inhibit the sintering and aggregation of titania during the calcination process. On the other hand, since template-free titania particles have no particular shape and size, they re-assemble on the surface during the calcination to form denser aggregations. Thus, after the calcination, the amount of adhered TiO_2 on the surface was higher for template-free synthesized nanoparticles.

The specific surface area of the TiO_2 nanostructures were analyzed by using nitrogen gas adsorption method, which relies on Brunauer-Emmett-Teller (BET) theory.⁸⁹ The measurements showed that the surface area of peptide 1-templated nanotube network was more than five times and area of peptide 2-templated nanowire network was more than three times greater than that of template-free synthesized TiO_2 particles (Table 2.1) The pore size of the TiO_2 in DSSC should be large enough to allow easy diffusion of electrolyte, while avoiding the recombination of redox species in the electrolyte.⁶⁶ Therefore, one-dimensional nanostructures offer the best morphology. Since N719 dye molecules are not likely to aggregate, it could be assumed that dye should be adsorbed as monolayer;^{90,91} therefore, increasing the surface area causes increase in the amount of adsorbed dye and as a result, enhance the efficiency of the solar cell.⁹² The amount of adsorbed dye is expected to be greater for nanotubes and nanowires compared to template-free synthesized TiO_2 . Indeed, the average amount of adsorbed dye was found to be 3 times greater for nanotubes and 1.5 times greater for nanowires compared to template-free particles, which does not contradict the surface area measurements (Table 2.1). Nevertheless, increase in

the dye loading observed for template synthesized materials is smaller than increase in the surface area. This incomplete dye loading can be rationalized by imperfect diffusion of the dye into porous structures.

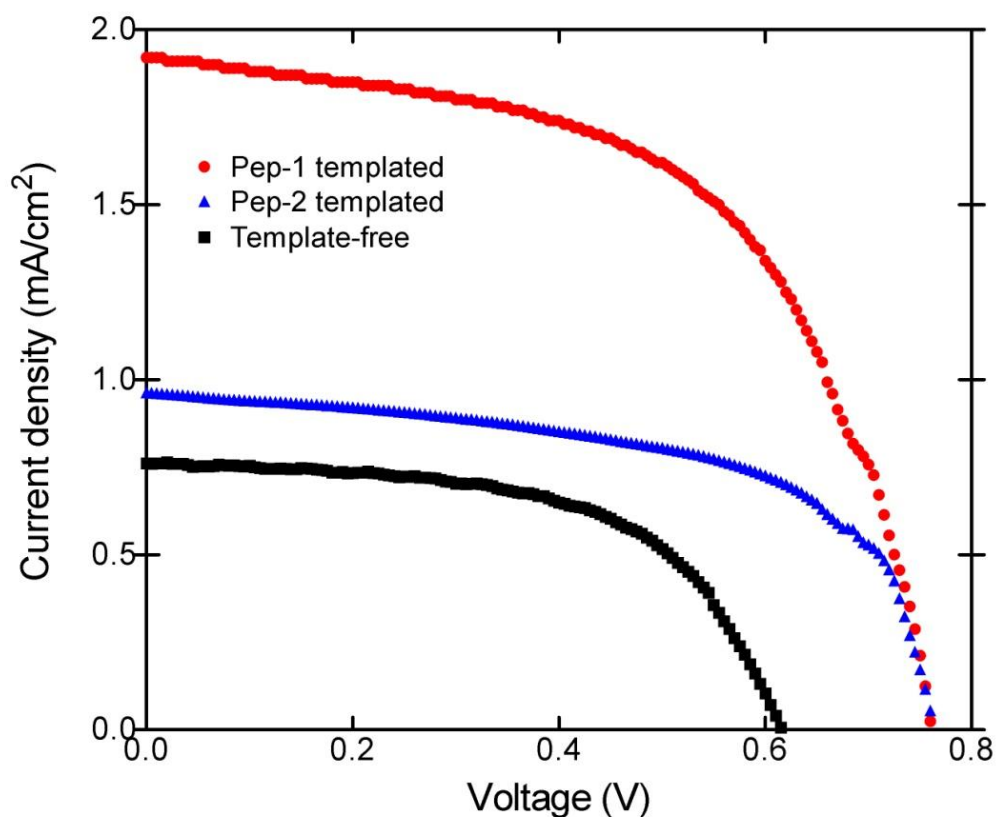


Figure 2.8. Representative J–V spectra of devices based on peptide 1 (Pep-1), peptide 2 (Pep-2) templated and template-free TiO₂ materials.

Photoconversion efficiencies of DSSCs were analyzed by a solar simulator set-up. The J-V characteristics of the photovoltaic devices are shown in Figure 2.8. The devices with nanostructured materials exhibited significantly greater photovoltaic performance. The cells produced on peptide 1 templated TiO₂ nanotube electrodes revealed significant enhancement in short circuit current compared to

peptide 2 templated TiO_2 nanowire electrodes and template-free TiO_2 electrodes. This is attributed to dramatic increase in the surface area of the electrode. Although fill factor values were comparable, open circuit voltage values for templated materials were around 760 mV, while template-free synthesized material did not exceed 620 mV (Table 2.1). Observed V_{oc} enhancement is attributed to bandgap widening caused by physical confinement of electrons in nanostructured materials. Diffuse reflectance spectra indicate blue-shifted bandgap edges for templated materials (Figure 2.9). This clearly demonstrates important role of the peptide-templated materials in enhancing overall efficiency of the device performance.

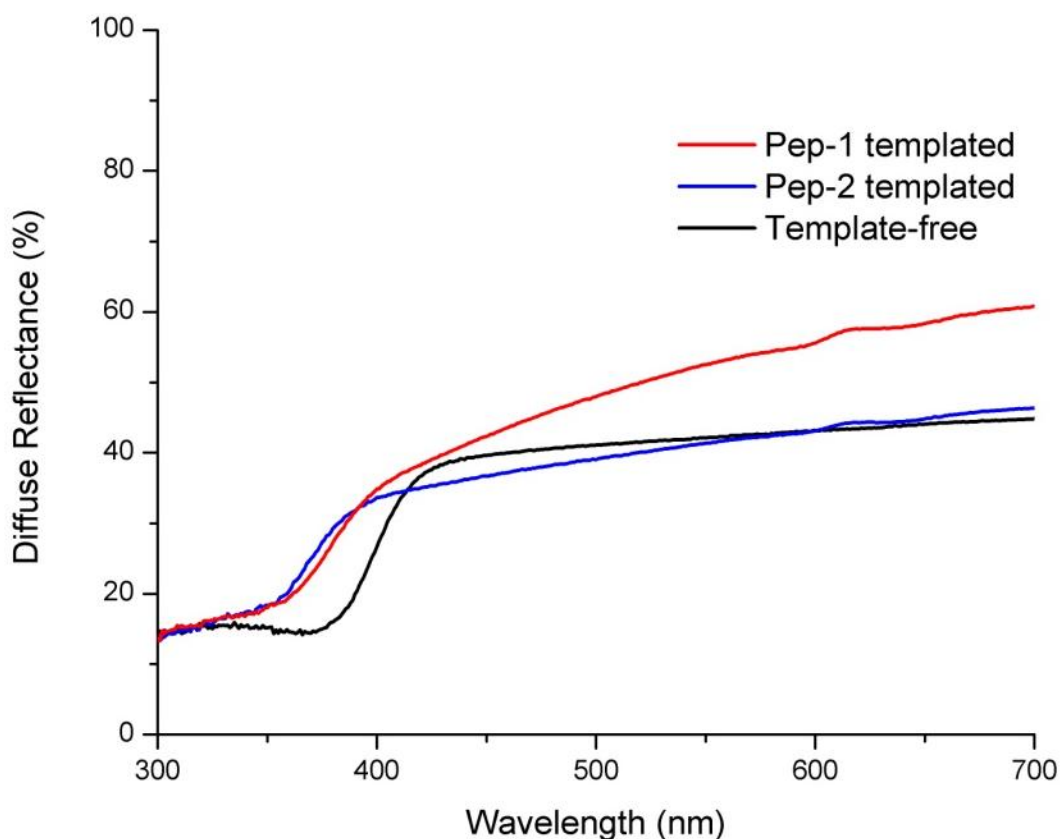


Figure 2.9. Diffuse reflectance spectra of TiO_2 materials.

As discussed above, the efficiency of the DSSC directly depends on the amount of electrocyclic TiO_2 and the amount of dye adsorbed on electrode. Thus, the measurement of all of the parameters is crucial for comparing efficiencies of DSSCs constructed from different TiO_2 structures. Due to the nanostructured properties, TiO_2 nanotubes are capable of adsorbing more dye on their surface. Therefore, the efficiency results were normalized by TiO_2 and adsorbed dye amounts (Table 2.2). By taking all of the parameters into account, the efficiency of TiO_2 nanotube network prepared by peptide 1 template was significantly higher than that of peptide 2 templated TiO_2 nanowire network (Figure 2.8 and Table 2.1). Also, the efficiency of DSSCs prepared from peptide-2 templated TiO_2 was higher than that of template-free TiO_2 particles. It should be also noted that obtained absolute device efficiencies are low and serve primarily for comparative purposes. Low efficiencies are the result of poor adherence of titania materials to FTO surface; only thin layers of anodic titania could be achieved.

Table 2.1. Parameters of representative DSSCs.

TiO_2	J_{sc} (mA/cm ²)	V_{oc} (mV)	Fill Factor	Efficiency (%)	Surface Area (m ² /g)	Adsorbed Dye (mmol/g)
Peptide 1- templated	1.92	760	0.57	0.83	150.63	1.99
Peptide 2- templated	0.96	765	0.59	0.44	102.94	0.97
Template- free	0.76	620	0.57	0.27	27.01	0.65

Table 2.2. Relative efficiencies of different devices normalized to dye and titania amounts.

Sample	Peptide-1 templated TiO ₂	Peptide-2 templated TiO ₂	Template-free TiO ₂
% Efficiency / (Conc _{Dye} × Conc _{TiO₂})	3.59	2.18	1.00

2.1.3 Conclusion

Green synthesis method of one-dimensional TiO₂ nanostructures for dye sensitized solar cells by using peptide nanofiber templates can offer an attractive and promising way for the growing field of sustainable energy. The differences between efficiencies of DSSCs prepared by nanotubes, nanowires and template-free nanoparticles were compared and discussed. Using peptide self-assembly and mineralization under ambient conditions enabled convenient synthesis of titania nanostructures. Self-assembled peptide nanofibers offer unique templating possibilities, which allow synthesis of nanostructured materials with high surface area. The mineralization of titania around the peptide nanofibers also occurs by deposition of inorganic ions. Network of these one-dimensional titania nanostructures possesses intriguing features, such as greater surface area and improved open circuit voltage, which result in enhanced photoactivity. Dye sensitized solar cell experiments have demonstrated superiority of nanostructured materials and emphasized importance of bottom-up approach realized via self-assembled soft templates.

2.2 Atomic layer deposition of titania on peptide amphiphile nanonetworks for dye sensitized solar cells

2.2.1 Introduction

Peptide amphiphiles with their intrinsic property to self-assembly into nanostructures such as fibers and micelles represent a versatile tool in an inorganic material templating. PAs are mentioned as templates in many research articles. Most of these articles are focused on in-solution material deposition on the surface of a template. Even though this approach allows successful material deposition, precise control over material thickness and high conformality are not always achievable. In order to circumvent this handicap, atomic layer deposition technique was deployed in material growth over PA nanonetwork template. ALD approach allowed obtaining TiO₂ nanonetworks with tunable wall thickness and high template conformality. Organic template was removed upon calcination at 450 °C, a temperature at which titania transforms to its anatase form. In principle, anatase titania can be used as a photoanode material. The aim of this study is to analyze and describe the effect of conformal coating on dye sensitized solar cell device, because near to perfect one-dimensional nanostructures can have an impact on cell parameters such as open circuit voltage (V_{oc}) and short circuit current (I_{sc}).

The nanostructured photoanodes with 150 deposition cycles were fabricated. TiO₂ nanonetworks in photoanodes are a system of interconnected nanotubes, which can facilitate electron transfer. Moreover, these networks are porous high surface area materials. High surface area of the material drastically increases number of sensitizer

molecules attached to titania. In addition, all devices have sub-milligram TiO_2 content; average titania content is 133.38 μg .

2.2.2 Results and discussion

The peptide with a sequence lauryl-VVAGK-Am (Figure 2.10) was synthesized by solid phase peptide synthesis method. Peptide sequence was constructed on Rink Amide MBHA resin with a standard loading of 0.59 mmole/g. 0.5 mmole peptide synthesis was carried out; 2 equivalents (with respect to resin) of amino acids and lauric acid were used in each coupling. Amino acids or lauric acid (2 eq.) were activated with DIPEA (3 eq.) and HBTU (1.98 eq) prior to coupling. All couplings were carried in dimethylformamide (DMF). At the end of the coupling cycles resin was cleaved from the resin by concentrated trifluoroacetic acid (TFA). For this purpose, a 10 mL cleavage cocktail ($\text{TFA}:\text{TIS}:\text{H}_2\text{O} = 9.5:0.25:0.25$) was prepared; distilled water (H_2O) and triisopropylsilane (TIS) were used as scavengers. Cleaved peptide was collected by dichloromethane (DCM), which was later removed alongside with TFA by rotary evaporation. Residual material was triturated by cold diethyl ether. White peptide precipitate was completely separated from ether by centrifugation; the centrifugate was dissolved in water, frozen at $-80\text{ }^\circ\text{C}$ and finally lyophilized. Lyophilized peptide was characterized by high resolution time-of-flight mass spectrometer coupled to liquid chromatography system (Agilent 1200/6210). A peak of 654.4798 a.m.u corresponding to peptide ($\text{M}+\text{H}$) was registered in mass spectrum (Figure 2.11). Purity of the peptide was c.a. 95% (Figure 2.12).

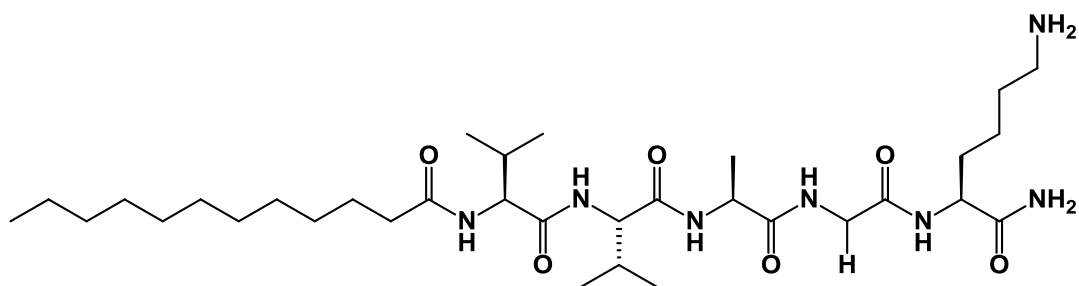


Figure 2.10. Structural formula of lauryl-VVAGK-Am peptide amphiphile.

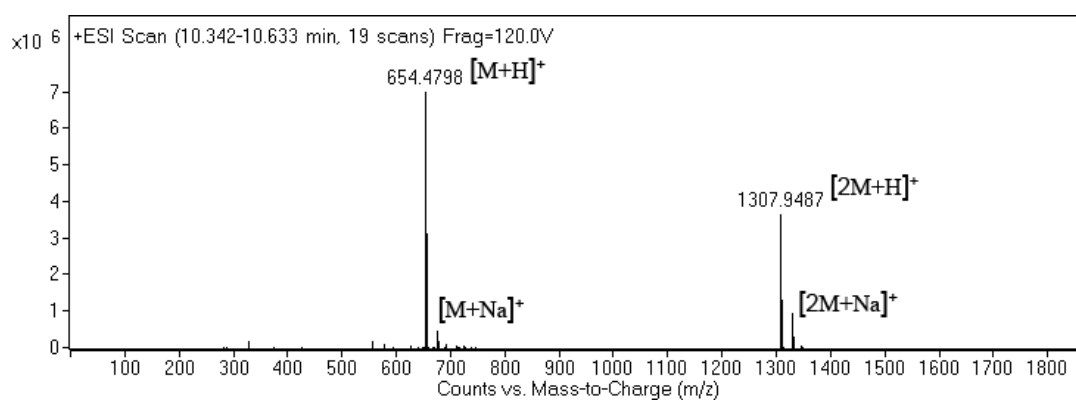


Figure 2.11. Mass spectrum of the lauryl-VVAGK-Am peptide amphiphile. Calculated m/z is 653.4840 (M), found m/z is 654.4798 (M+H) and 1307.9487 (2M+H).

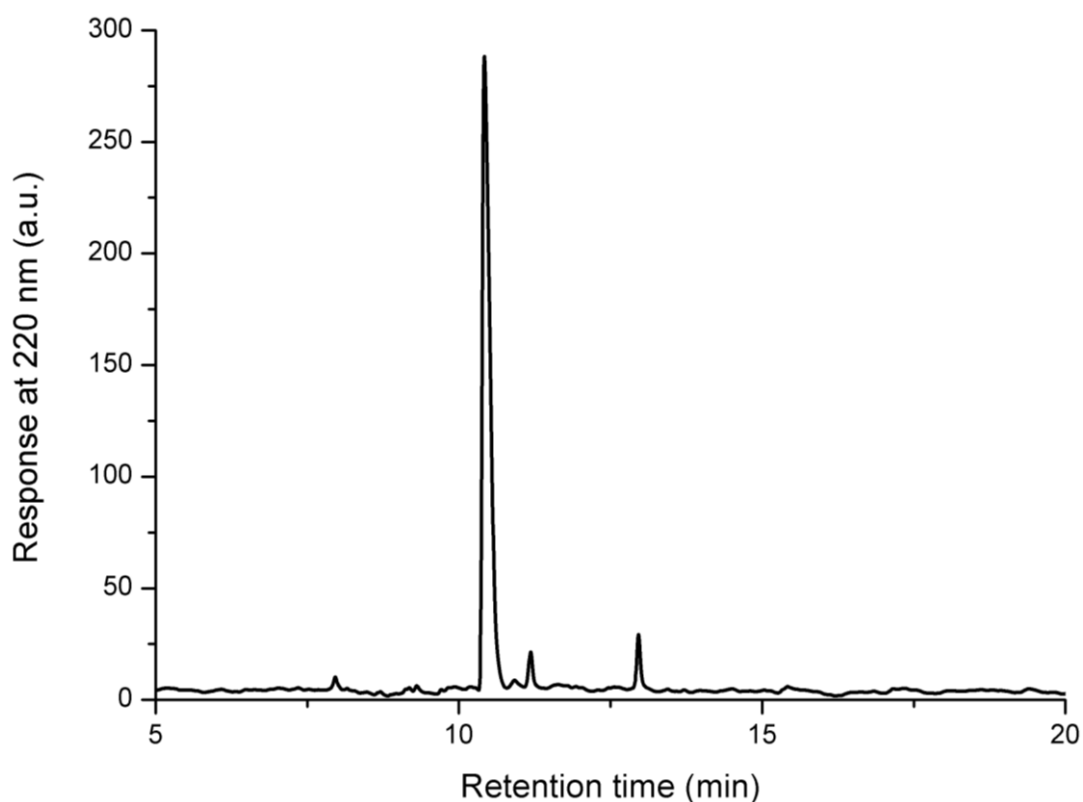


Figure 2.12. Liquid chromatogram of lauryl-VVAGK-Am peptide amphiphile.

The PAs are molecular entities designed to self-assemble into nanofibers providing a highly porous and homogenous 3-D nanonetwork with a small diameter.⁹³ In this study, PA molecule with a sequence lauryl-VVAGK-Am (Figure 2.10) was synthesized to serve as a programmed unit assembling into a template with amino functionalities responsible for nucleation and growth of TiO₂. Designed peptide is able to form a hydrogel which can be further converted to aerogel (Figure 2.13) by critical point drying technique.

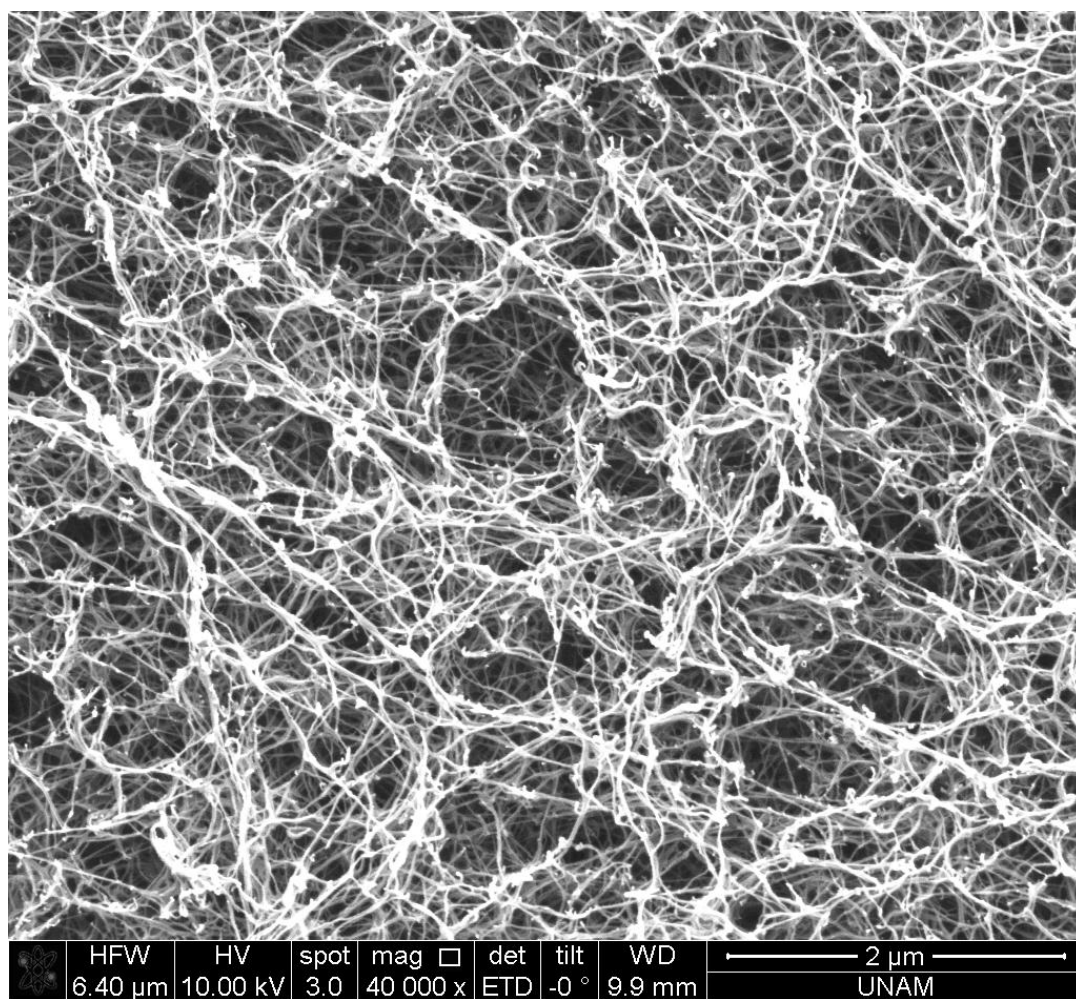


Figure 2.13. SEM image of critical point dried gel on FTO (6 nm Pd/Au).

Because ALD can deposit coatings with ultimate conformality and uniformity, it provides thin film coatings with a sub-nanometer thickness resolution on high surface area nano-template materials such as the peptide nanofiber networks.⁹⁴ Here, we utilized ALD technique to produce TiO₂ nanonetworks. First, aerogel was subject to 150 cycles of ALD coating. Tetrakis(dimethylamido)titanium (Ti[N(CH₃)₂]₄) and water (H₂O) were used as a titanium (Ti) and oxygen (O) precursors, respectively. Obtained hybrid organic-inorganic material (Figure 2.14) was analyzed by energy dispersive X-ray (EDX) scattering and X-ray diffraction (XRD) techniques. EDX analysis revealed intense titanium and oxygen peaks representing TiO₂, and also intense carbon (C) peak pertaining to organic peptide content (Figure 2.15). XRD analysis has shown that inorganic titania part exists in its amorphous phase (Figure 2.18). In order to remove organic part and convert titania to its anatase form, hybrid nanonetwork was calcined at 450 °C (Figure 2.16). This thermal processing substantially decreased content of carbonaceous material (Figure 2.17) and transformed amorphous titania to anatase titania (Figure 2.18). Raman spectrum of calcined sample supports phase transformation as well (Figure 2.19).⁹⁵ TEM imaging revealed formation of nanotubes in the form of a nanotube network (Figure 2.20).

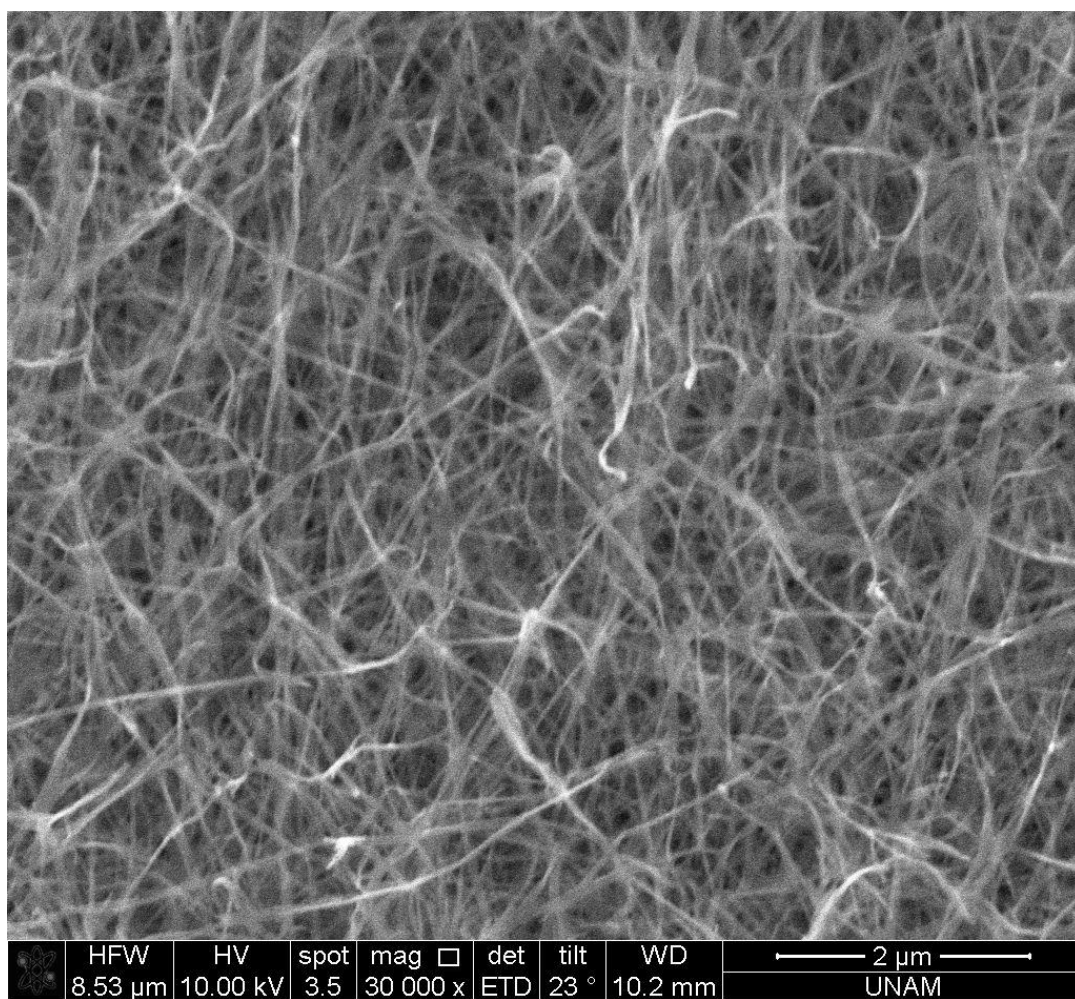


Figure 2.14. SEM image of ALD-coated TiO₂.

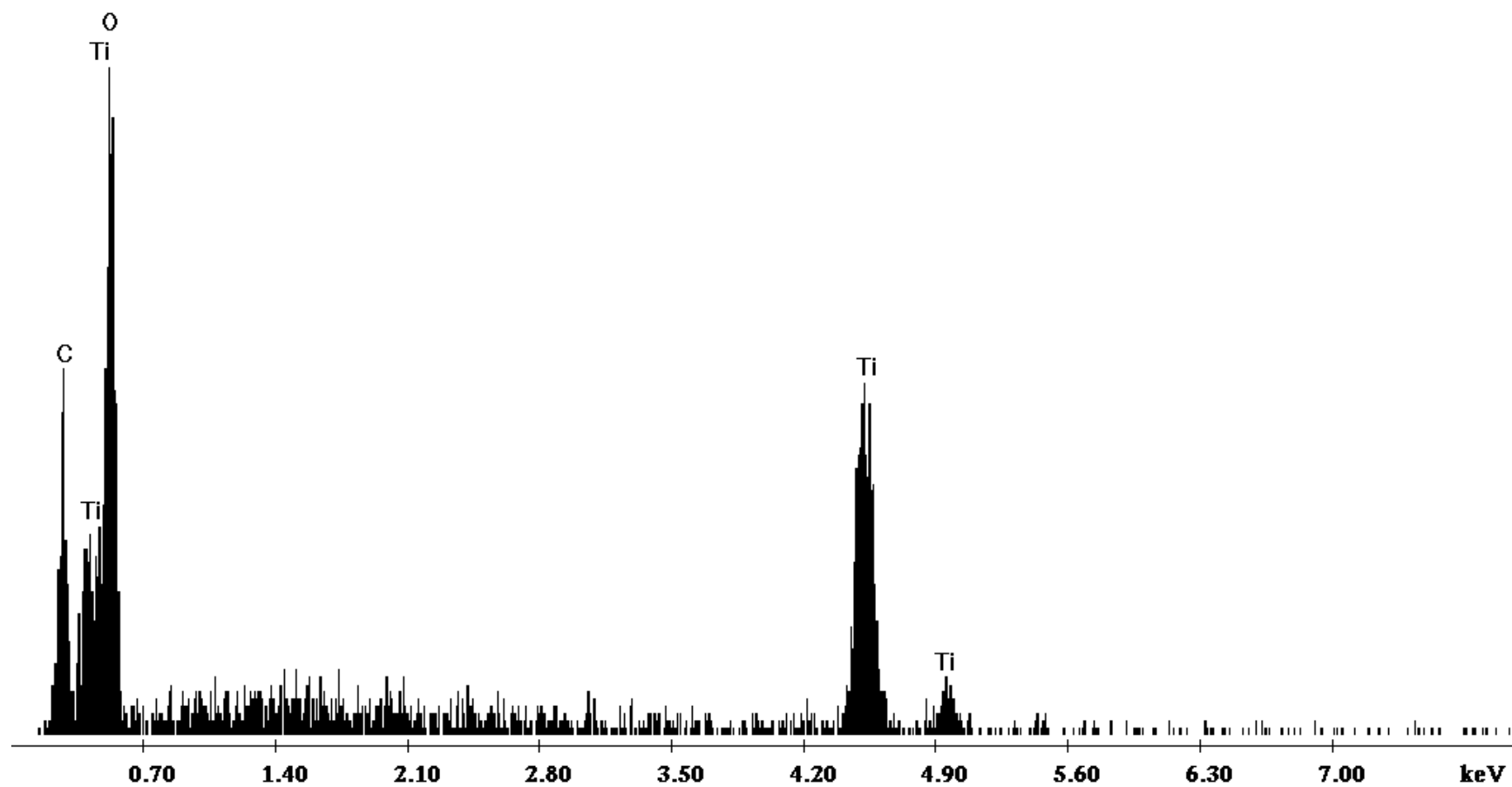


Figure 2.15. EDX spectrum of templated TiO_2 surface.

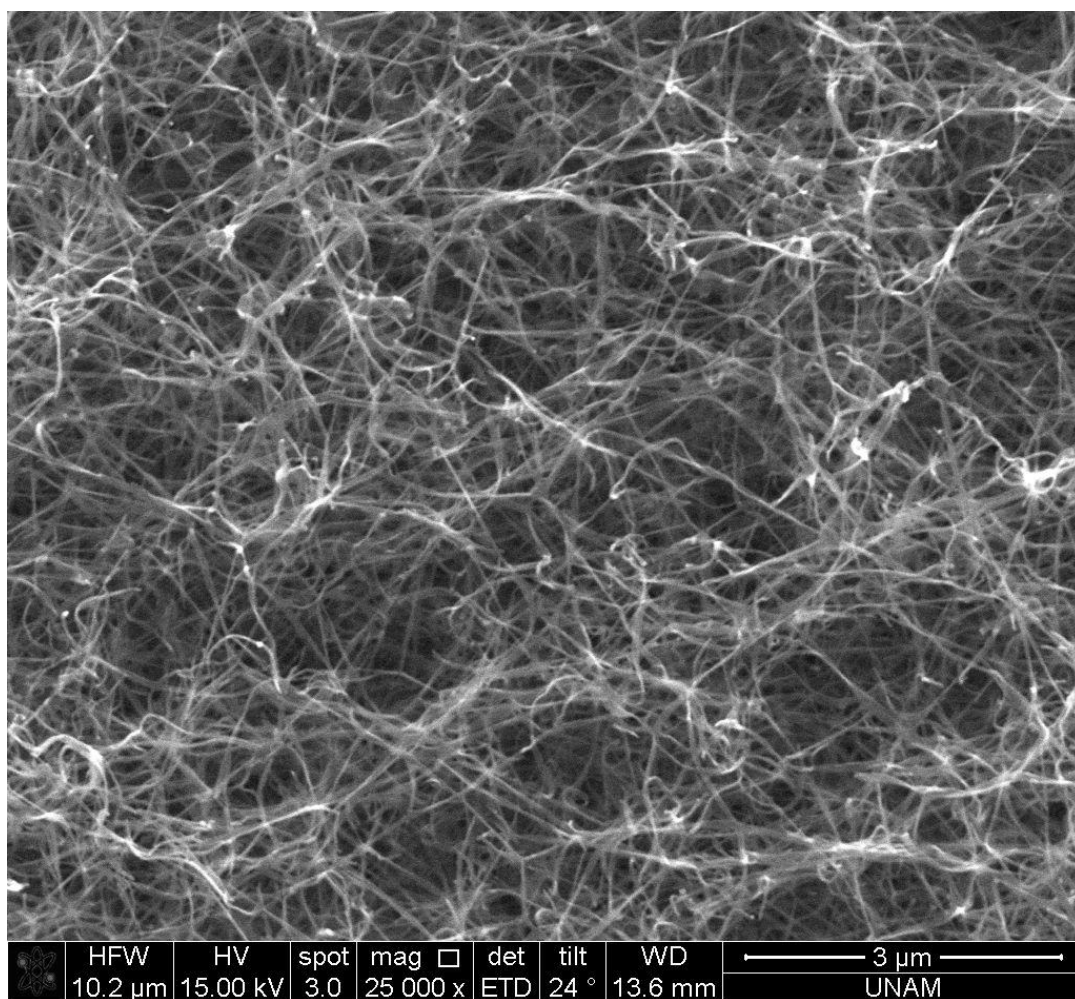


Figure 2.16. SEM image of calcined ALD-coated TiO₂ nanonetwork.

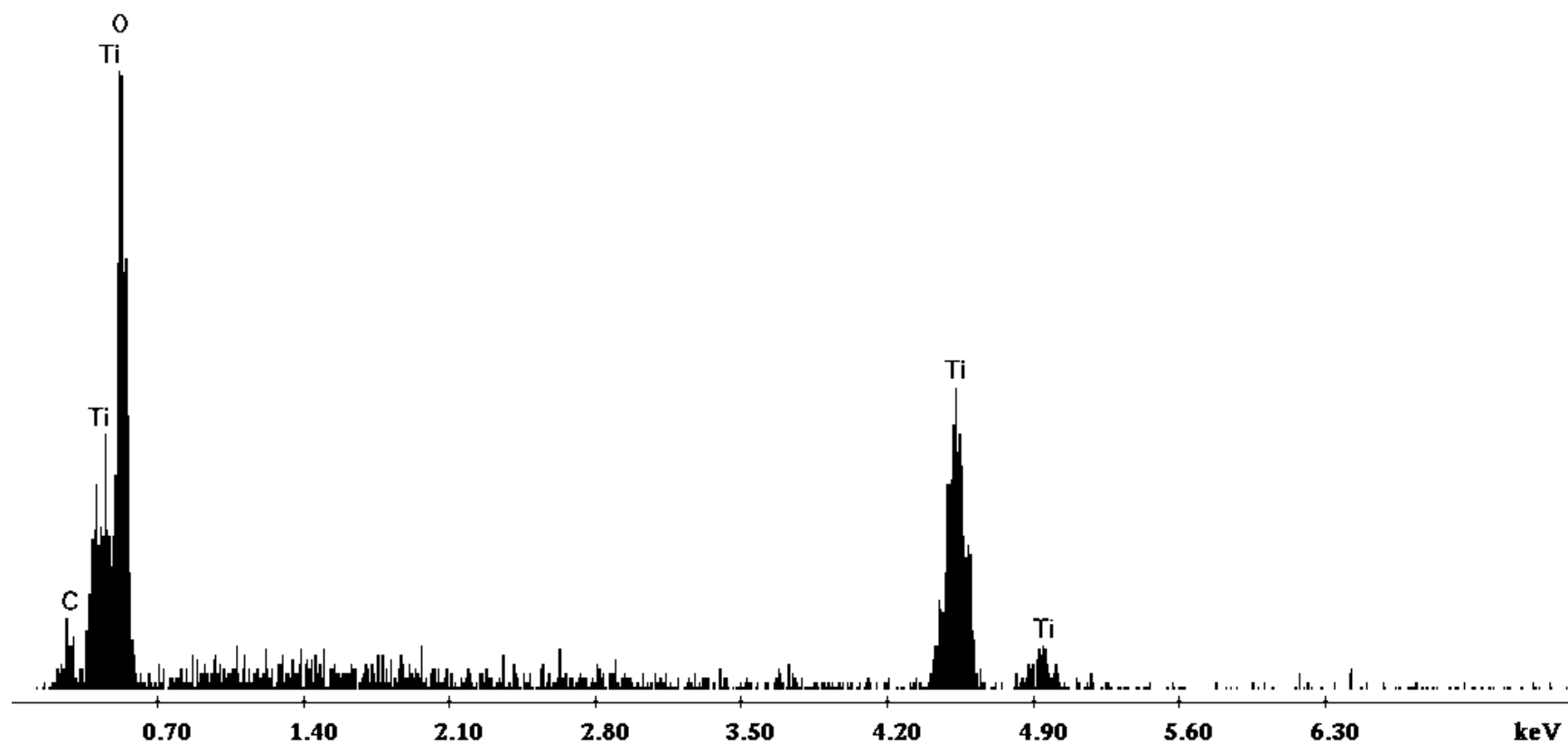


Figure 2.17. EDX spectrum of templated TiO_2 surface after calcination.

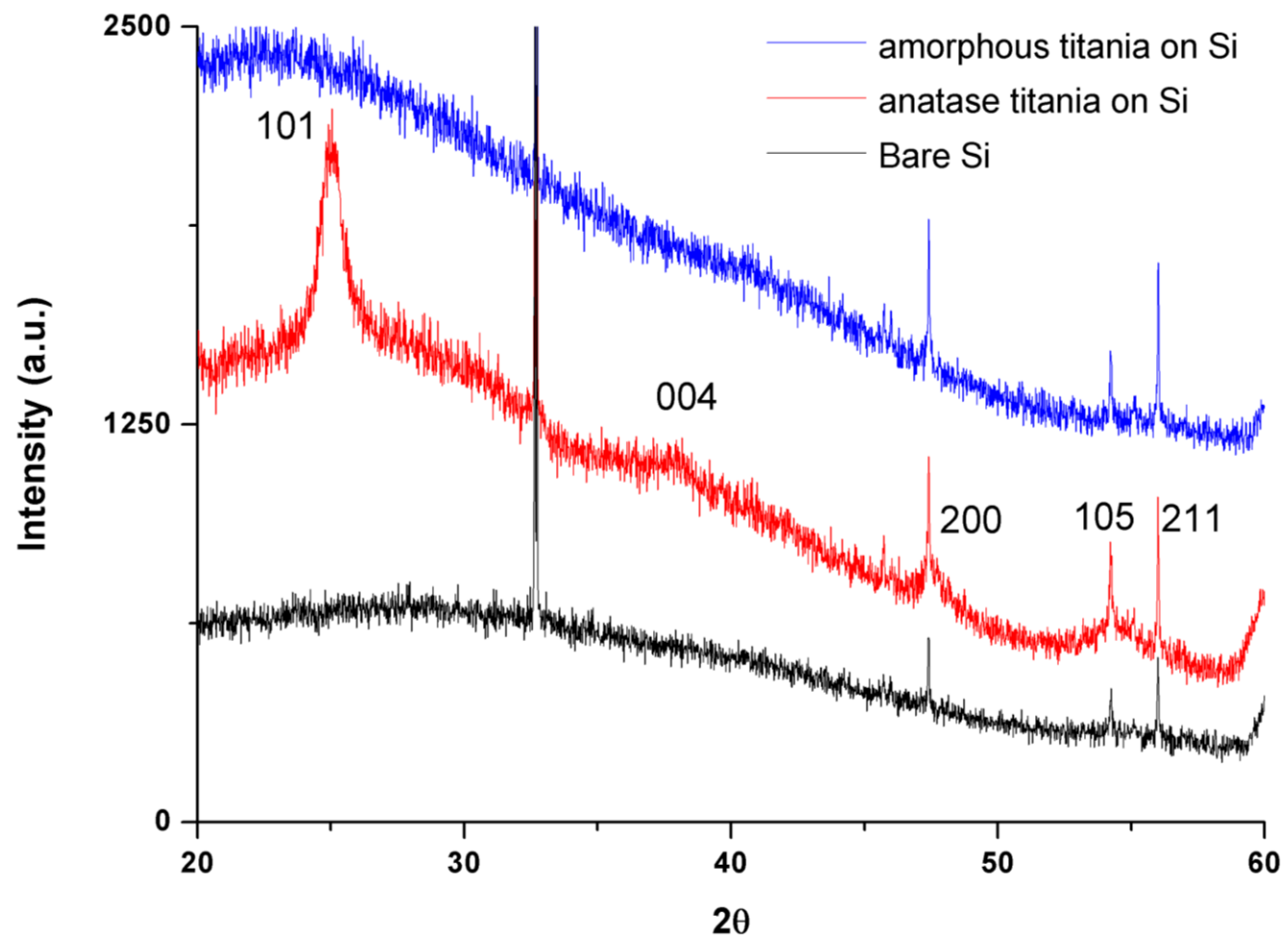


Figure 2.18. XRD spectra of amorphous TiO_2 , anatase TiO_2 and Si.

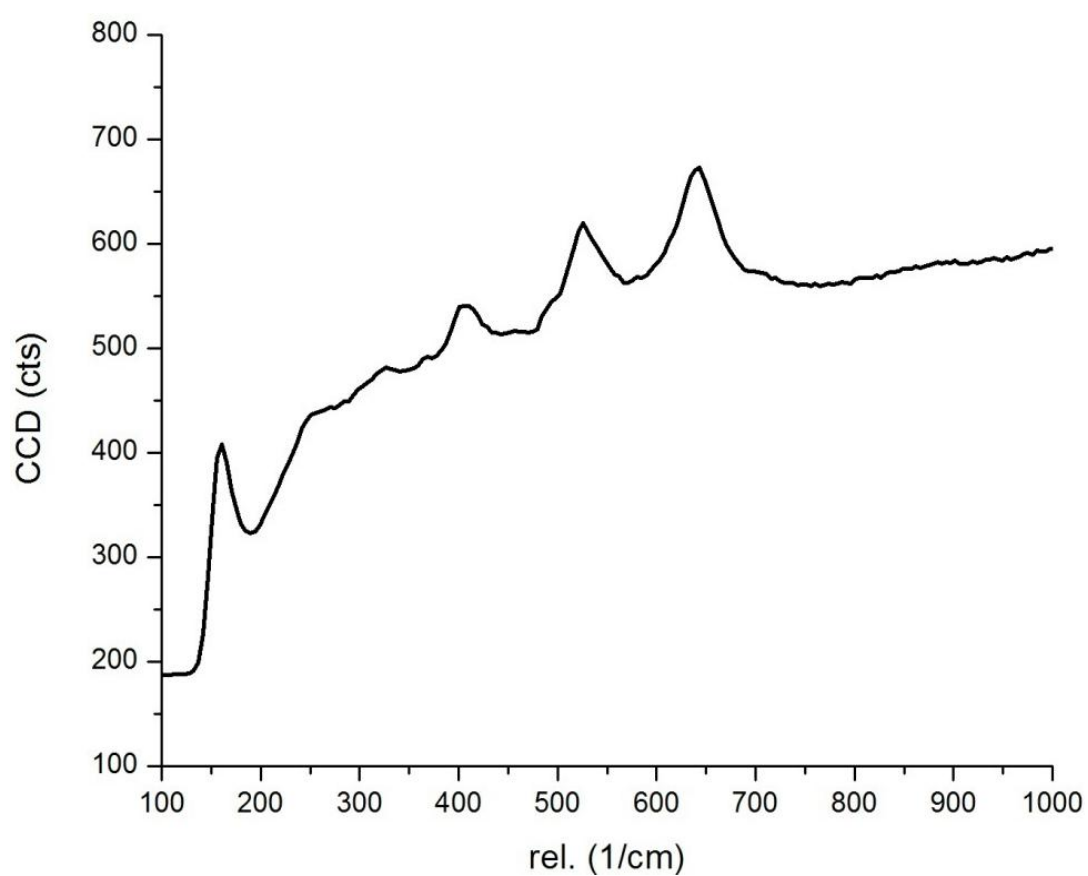


Figure 2.19. Raman spectrum of anatase TiO₂.

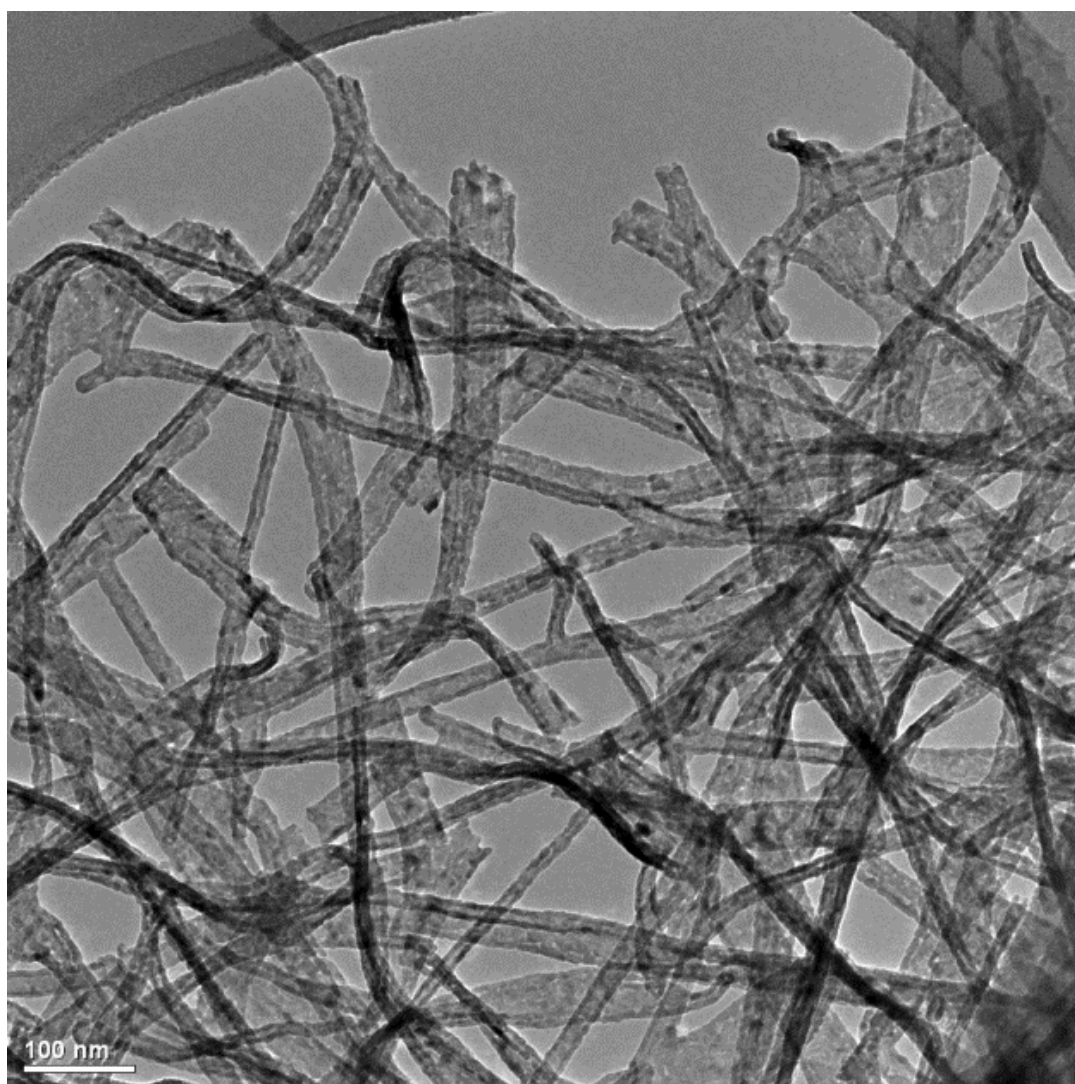


Figure 2.20. TEM image of TiO₂ nanotubes.

Obtained anatase titania nanotube network was employed in assembly of a functional DSSC device. Titania nanonetworks were grown directly on FTO surface, thus minimizing solar cell assembly steps. Representative photoanode is given in Figure 2.21 and its cross-sectional view is given in Figure 2.22. A TiO_2 nanonetwork layer with an average thickness of 28 μm was obtained (Figure 2.22). For a photoanode to be fully functional it needs to be sensitized by a molecular dye. A dye of a choice was ruthenium-based N719 (Figure 2.23). Stained, the photoanode was used in assembly of a complete device, where Pt counter-electrode with a thickness of 25 nm was utilized. The iodine/iodide redox couple was used as an electrolyte ensuring electron circulation. To understand effect of the template on the device, the photoanode with thin layer titania was fabricated as a control. The fully functional devices were irradiated with a solar light simulator (1.5 AM) and simultaneous I-V data in voltage sweep mode were recorded (Figure 2.24). It was observed that devices with nanostructured TiO_2 demonstrate cell parameters superior to those of devices with thin layer TiO_2 . As it can be seen from Figure 2.24 and Table 2.3, devices with nanostructured material have open circuit voltage (V_{oc}) of c.a. 0.7 V, while V_{oc} of devices with thin layer titania are c.a. 0.2 V. Template effect also manifests itself in considerably higher short circuit current. Templated nanonetworks offer greater surface area, and thus, provide dye chemisorption in greater amounts. An efficiency of 0.8% was achieved for nanostructured material, while thin layer material had an efficiency of only 0.0024%. Fill factor of nanostructured materials was also considerably higher.

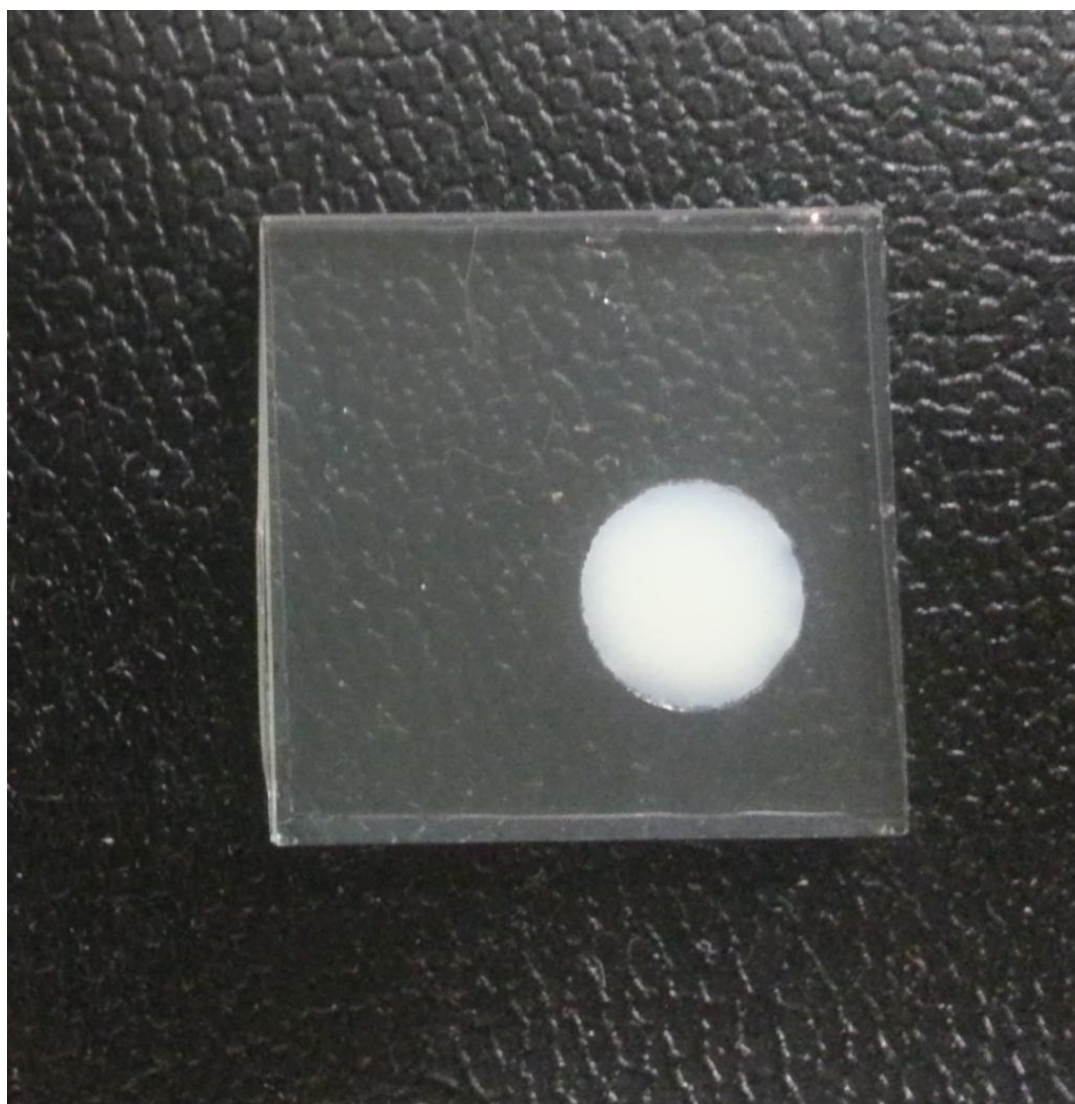


Figure 2.21. Representative photoanode. Nanostructured TiO₂ on FTO after calcination.

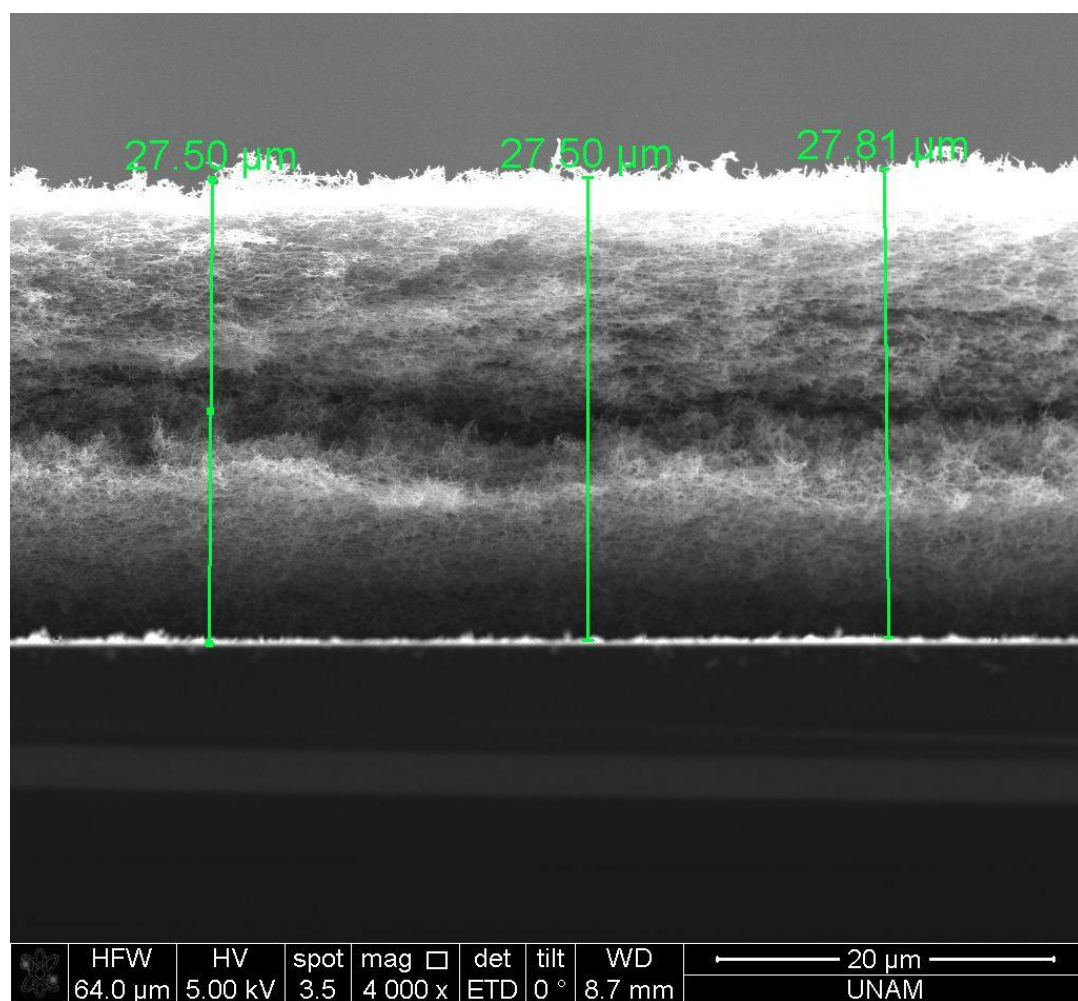


Figure 2.22. Cross-sectional view of anatase TiO_2 nanonetwork layer.

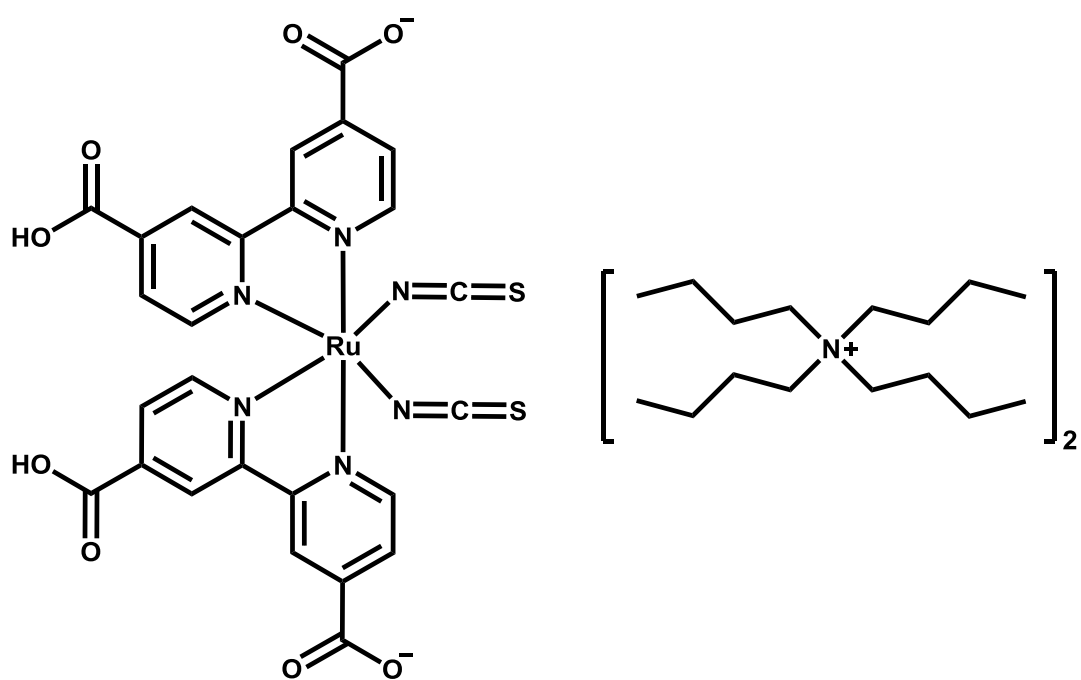


Figure 2.23. Structural formula of N719 dye – a Ru(II)-based sensitizer.

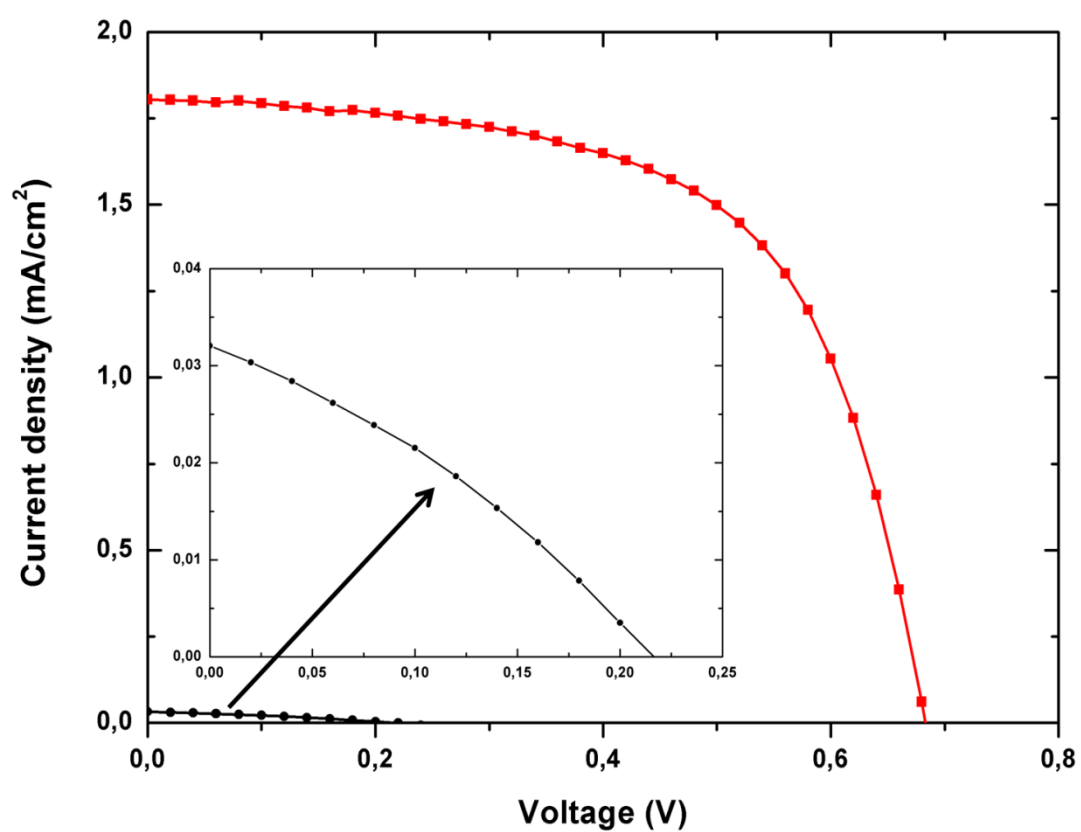


Figure 2.24. J-V curve of the DSSC devices with templated TiO₂ (red) and with template-free thin layer TiO₂ (black).

Table 2.3. Cell parameters of the devices in Figure 2.24.

TiO ₂	V _{oc} (V)	J _{sc} (mA/cm ²)	FF	η
Peptide-templated	0.69	1.805	0.63	0.79
Template-free	0.21	0.032	0.35	0.0024

2.2.3 Conclusion

Peptide-templated material obtained by atomic layer deposition technique in this study has demonstrated superior cell parameter compared to template-free thin layer material. Open circuit voltage and short circuit current values were considerably higher for devices with nanostructured photoanodes. Nevertheless, overall efficiency for these devices is still low. The possible reason could be an increase in number of electron-hole recombinations in obtained high surface material. An extra blocking layer of zinc oxide or hafnium (IV) oxide could reduce recombinations and increase overall efficiency.

Chapter 3.

3. Noncovalent functionalization of a nanofibrous network with a bio-inspired heavy metal binding peptide

This work is partially described in the following publication:

Garifullin, R., Ustahuseyin, O., Celebioglu, A., Cinar, G., Uyar, T., Guler, M.O.,
“Noncovalent Functionalization of Nanofibrous Network with Bio-inspired Heavy
Metal Binding Peptide” RSC Advances, 2013, **3**, 24215

3.1 Introduction

Nature is a constant inspiration source for researchers solving a wide range of critical problems of modern age. Metal ions are essential in many processes in nature such as photosynthesis⁹⁶, water oxidation⁹⁶, respiration⁹⁷ and nitrogen fixation⁹⁸. Although metal ions are essential part of metalloproteins and beneficial at optimum concentration levels, heavy metal ions pose a serious threat for human health even at low concentrations.^{99,100,101} These metal ions can contaminate and spread through natural water sources. Several plants, fungi, nematodes and algae have mechanisms to cope with heavy metal contaminations.¹⁰² Their defense mechanism utilizes phytochelatins, which are known for their heavy metal detoxification capability.¹⁰³ The phytochelatins are short peptides consisting of γ -Glu-Cys repeats and capable of binding heavy metal ions via thiolate coordination.^{104,105} The α -carboxylic group of glutamic acid and sulfhydryl group of cysteine in phytochelatins coordinate to positively charged metal ions and act as chelating agent. Similar to γ -Glu-Cys units of phytochelatins, it was previously reported that Glu-Cys repeating units in

phytochelatin-related peptides showed binding abilities toward various heavy metals.¹⁰⁶ Also, synthetic peptides inspired by phytochelatins were used for different applications such as size determination of the nanocrystals produced in biomimetic synthesis processes,^{107,108} and binding of heavy metals for biosensor applications.¹⁰⁹

Functionalized fibrous polymeric materials and natural polymers have been used for different purposes such as increase of fiber mechanical stability¹¹⁰ or drug delivery.¹¹¹ In addition, straightforward immobilization of peptides on the polymeric materials can provide vast potential for new materials design. Noncovalent interactions can offer a suitable tool in achieving easy functionalization of polymers. For example, cyclodextrins are toroid shaped cyclic oligosaccharides with a hydrophobic inner cavity and a hydrophilic exterior; and they can form stable noncovalent host-guest inclusion complexes.^{112,113} An adamantyl moiety is known to be included and held strongly in cyclodextrin.¹¹⁴⁻¹¹⁶ Nearly perfect match and strong binding between the cavity of β -cyclodextrin and guest adamantyl group were shown previously.¹¹⁷

In this chapter, a bioinspired metal binding peptide was noncovalently immobilized on the surface of polymer network through host-guest inclusion mechanism (Scheme 1). We synthesized a phytochelatin mimetic peptide (PMP) which includes Glu-Cys repeating unit conjugated to an adamantyl moiety. The PMP molecule was immobilized on a fibrous solid support consisting of water-insoluble, crosslinked, electrospun hydroxypropyl-beta-cyclodextrin nanofibers (CDNF). Noncovalent functionalization of CDFN nanofibers with PMP molecule was achieved by host-guest inclusion complex formation between the cyclodextrin and the adamantyl units.

The PMP-CDNF hybrid fibrous solid network was used to scavenge highly toxic metal ions including Cd^{II} , Ni^{II} and Cr^{VI} , from aqueous solutions.

3.2 Results and Discussion

Peptide molecule (Figure 3.1a) was constructed on MBHA Rink Amide resin. Amino acid couplings were performed with 2 equivalents of Fmoc-protected amino acid, 1.95 equivalents of HBTU and 2 equivalents of DIEA (N,N-diisopropylethylamine) for 3 hours. Fmoc removals were performed with 20% piperidine / DMF solution for 20 minutes. Cleavage of the peptide from the resin was carried out with a mixture of TFA:TIS:H₂O:EDT in ratio of 92.5:2.5:2.5:2.5 for 2 hours. Excess TFA was removed by rotary evaporation. The remaining viscous peptide solution was triturated with cold ether and the resulting white product was dried under vacuum. The peptide molecule was purified with dialysis. For this purpose, Spectra/Por[®] Biotech Cellulose Ester dialysis membrane with 100-500D molecular weight cut-off was used. The peptide solution was dialyzed for 4 days. After the dialysis, the peptide solution was freeze-dried at -80°C and lyophilized. After lyophilization peptide was characterized by Agilent 1200-6530 quadrupole time of flight (Q-TOF) mass spectrometer with electrospray ionization (ESI) source equipped with Zorbax SB-C8 4.6 x 100 mm column operating on principles of reverse-phase analytical high performance liquid chromatography (HPLC). Mass spectrum and liquid chromatogram are given in Figure 3.1b and Figure 3.2c, respectively. Peptide purity after dialysis was c.a. 95%.

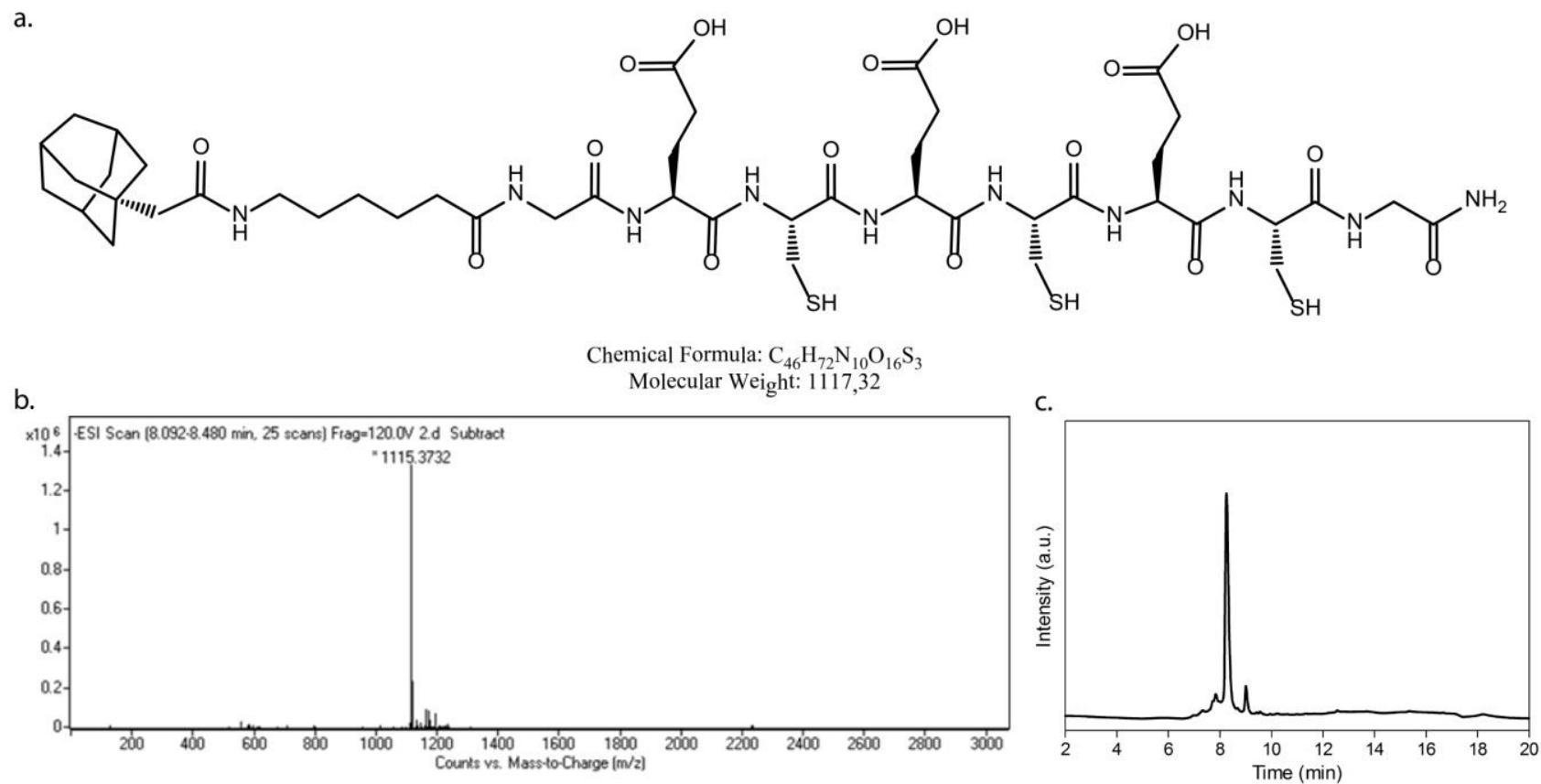
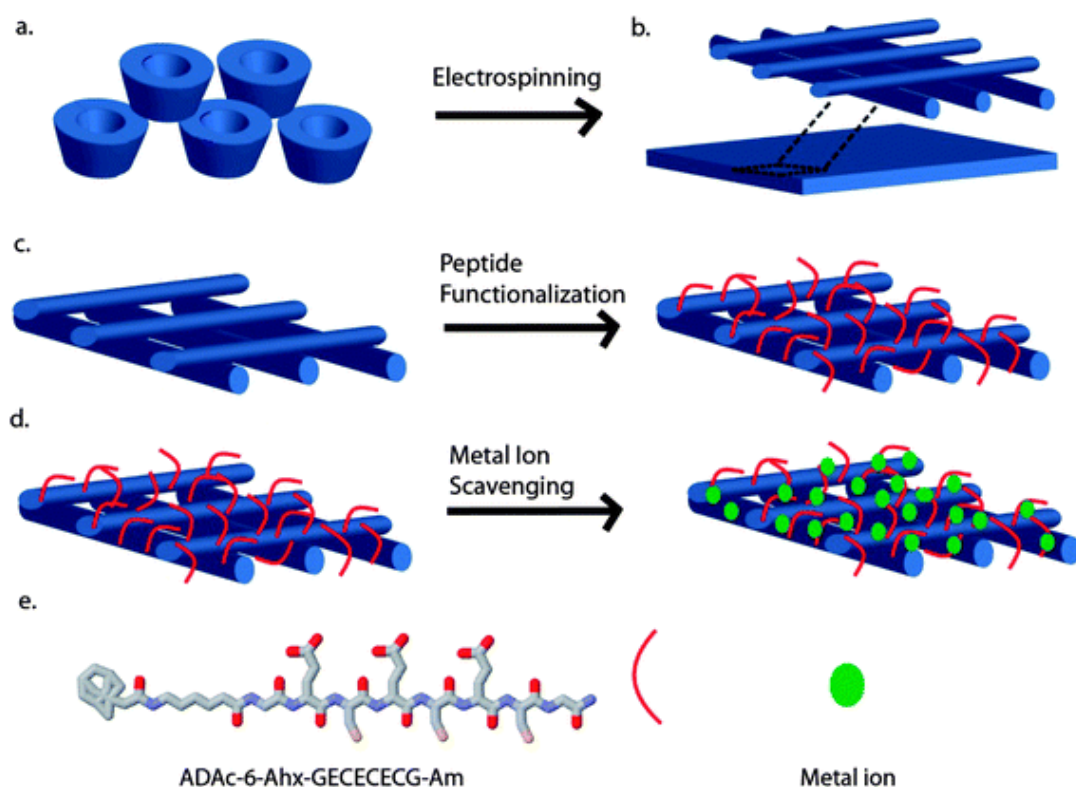


Figure 3.1. **a.** Chemical structure of PMP. **b.** Mass spectrum of PMP after subtracting mass spectra of water sample at that time interval. Mass data [M] (calculated) =1116.43; [M-H]⁻ (observed) = 1115.37. **c.** Liquid chromatogram of PMP after dialysis.



Scheme 3.1. **a.** Schematic drawing of HPβCD molecules. **b.** Electrospun CDNF as a water-insoluble support. **c.** CDNF support functionalization with the metal ion binding peptide via its adamantyl moiety. **d.** Metal ion scavenging by PMP molecules. **e.** Chemical structure and representative illustration of the peptide molecule and metal ion.

Peptide-polymer hybrid systems form a new class of soft materials combining advantages of chemical structure and functionality of biomolecules and benefits of synthetic polymers.¹¹⁸ In addition to exploiting peptides and proteins as novel structural components, a great deal of interest in harnessing their unique functions in a materials context exists.¹¹⁹ Peptide-polymer hybrid materials can find application in water remediation applications.¹²⁰ However, aforementioned macromolecules usually possess poor handling and processing characteristics and are difficult to immobilize

for the purposes of removal and recycling. Therefore, easy immobilization of a peptide on a polymer surface by noncovalent interactions would be useful for developing new functional polymers.

In this work, phytochelatin inspired peptide functionalization of a polymer network was achieved via host-guest interactions. Electrospinning is a widely used technique for development of functional sub-micron fibres using polymers for different applications.¹²¹ Water-insoluble CDNF network (Figure 3.2a-c) was obtained by electrospinning technique. The CDNF network includes cyclodextrin (CD) moieties and an adamantyl group is known to form host-guest inclusion complex with β -CD.¹¹⁷ To immobilize PMP molecule on CDNF, an adamantyl unit was conjugated covalently to N-terminus of the peptide sequence by solid phase peptide synthesis method. A 6-amino-hexanoic acid molecule was used as a spacer before conjugation of 1-adamantaneacetic acid to the peptide backbone. A glycine residue was added to both C and N termini of $-(\text{Glu-Cys})_3-$ peptide sequence (Scheme 3.1e and Figure 3.1a). Host-guest interaction between adamantyl moiety of PMP and β -CD was measured by ITC (Figure 3.3). Titration of β -CD with PMP revealed a moderate binding affinity ($N=1.17$, $K_d=3.85 \times 10^4 \text{ M}^{-1}$). Binding constant of the PMP and β -CD is similar to binding constant of adamantane acetate and β -CD provided in the literature.¹¹⁷

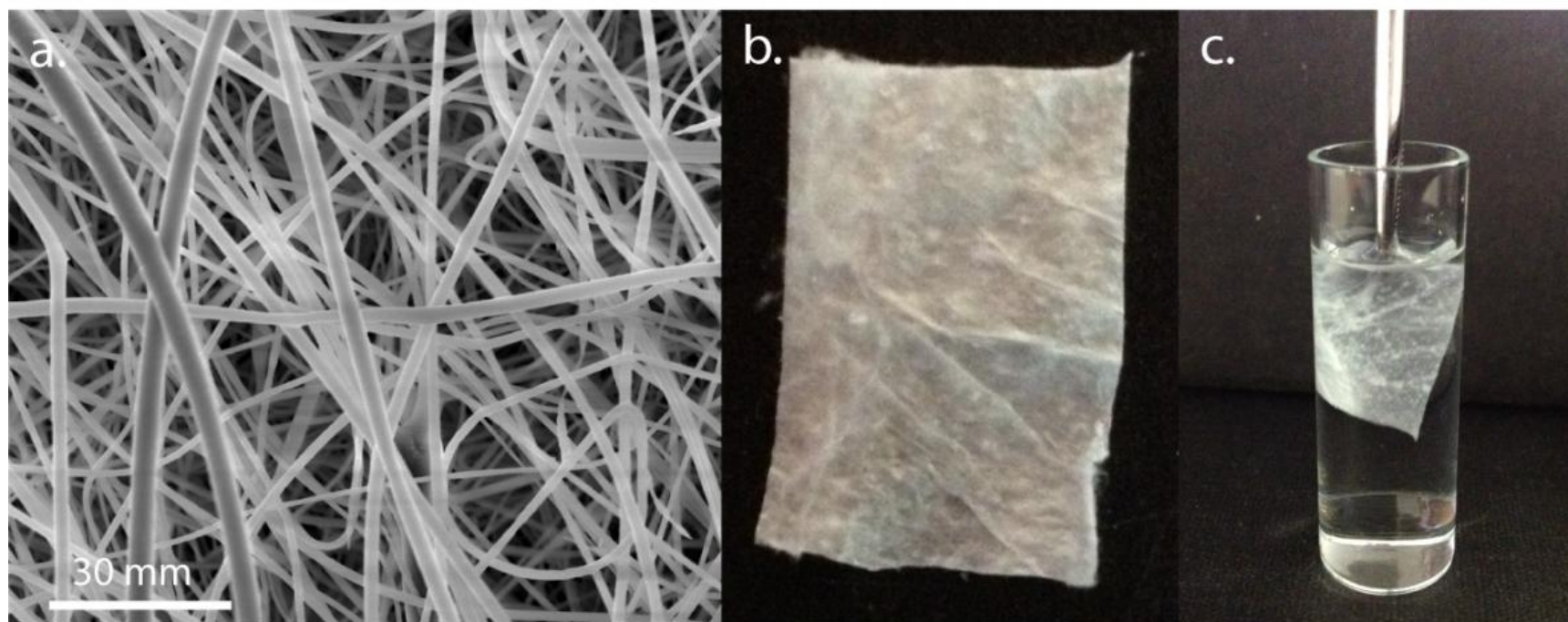


Figure 3.2. **a.** SEM image of electrospun CDNF. **b.** A macroscale photographic image of CDNF. **c.** Water-insoluble nature of CDNF.

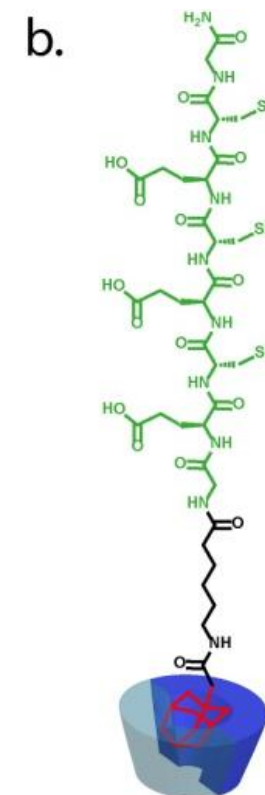
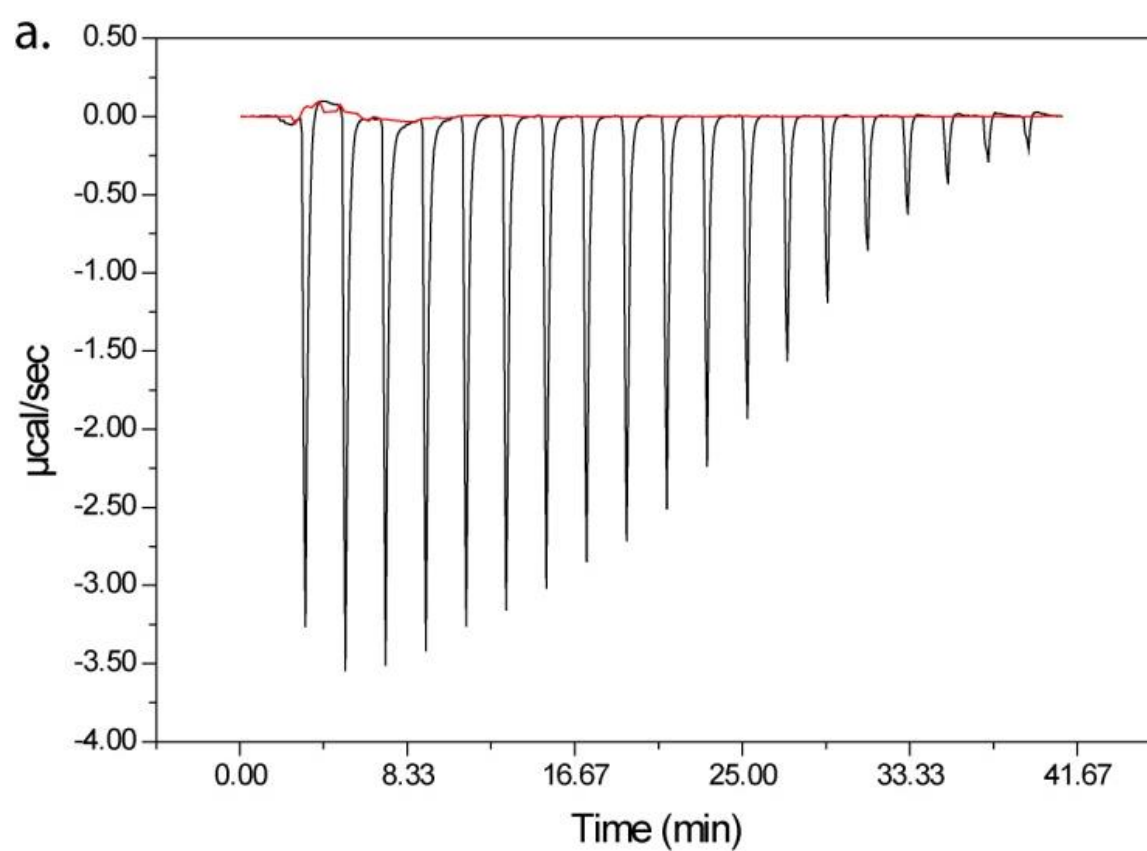


Figure 3.3. **a.** ITC curve obtained from titration of β -CD with adamantane conjugated peptide molecule. **b.** Schematic representation of interaction between β -CD and the peptide molecule. Adamantyl moiety of peptide molecule formed an inclusion complex with β -CD. The PMP molecule can bind to β -CD present in CDNF network.

The CDFN is a fibrous solid support consisting of water-insoluble crosslinked HP β CD nanofibers with submicron diameter. The CDFN can withstand water (pH ~5-8) without losing its integrity (Figure 3.2c). Non-covalent functionalization of CDFN with PMP molecule was achieved by incubation of CDFN network in the PMP solution. The PMP-CDFN network was characterized by scanning electron microscopy and no significant morphology change was observed in CDFN after addition of the PMP molecules (Figures 3.4). Physical appearance and insoluble character of CDFN were preserved after functionalization with the PMP molecules (Figure 3.4).

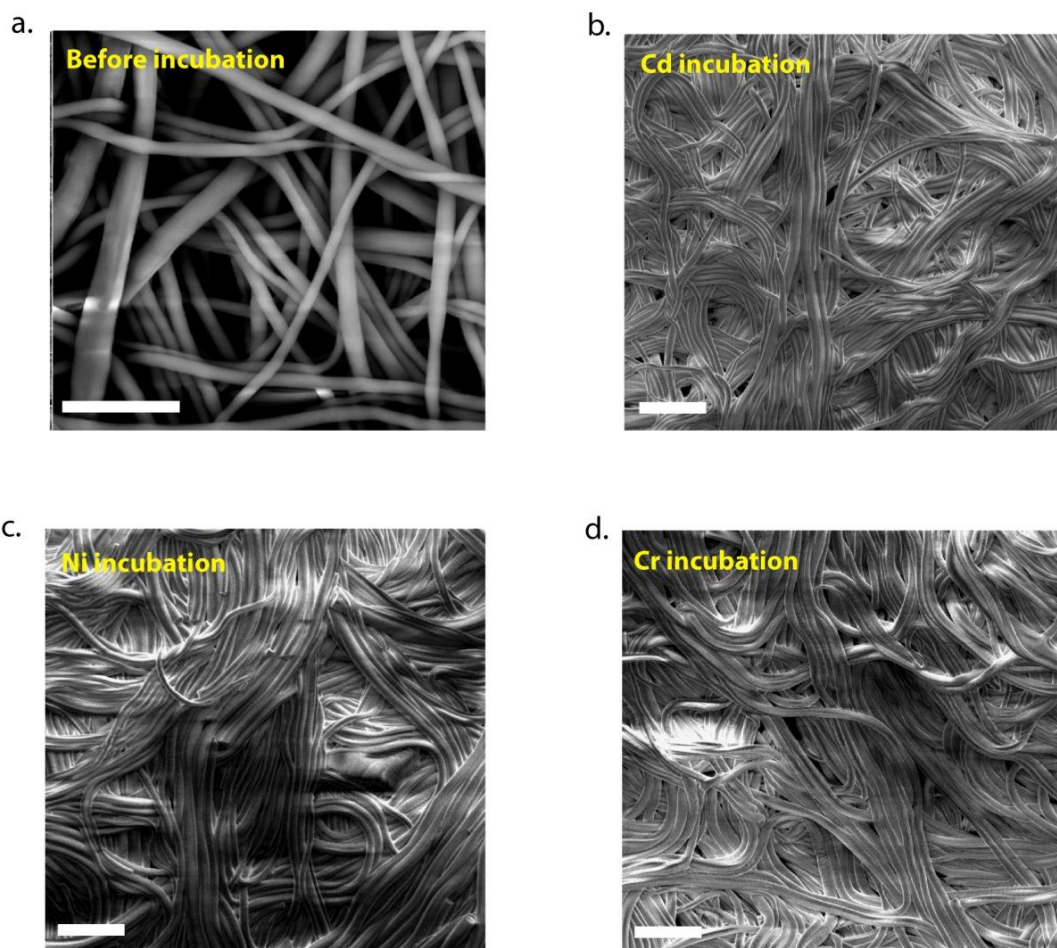


Figure 3.4. SEM images of PMP-CDNF. **a.** before and after incubation in **b.** Cd(II), **c.** Ni(II) and **d.** Cr(VI) solutions (scale bars: 10 μm).

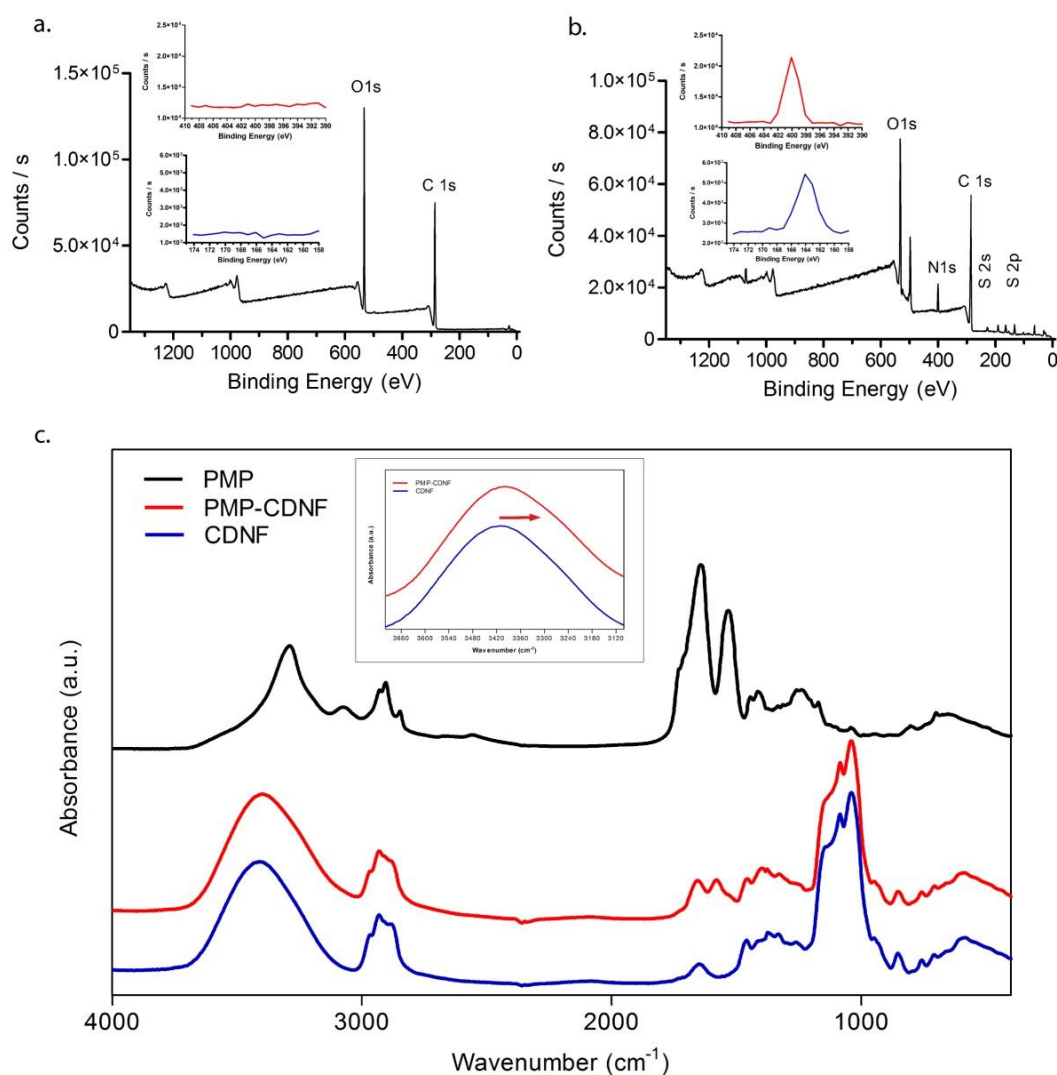


Figure 3.5. XPS spectra of **a.** CDNF and **b.** PMP-CDNF. Insets show N1s (red line) and S2p (blue line) XPS spectra. After PMP-CDNF complex is formed, N1s and S2p peaks are present in XPS spectrum of PMP-CDNF **c.** FTIR spectrum of PMP, CDNF and PMP functionalized CDNF. Inset indicates red shift in PMP-CDNF spectra compared to CDNF spectra from approximately 3440 cm⁻¹ to 3408 cm⁻¹.

The nature of interaction between PMP and CDNF was investigated by using X-ray photoelectron spectroscopy (XPS), FT-IR spectroscopy and Raman spectroscopy. Surface characterization of CDNF by XPS revealed presence of only C and O atoms (Figure 3.5a). On the other hand, PMP-CDNF network contained additional N and S element peaks indicating the presence of PMP (Figure 3.5b). Amount of N and S elements were found to be 5.90% and 2.04%, respectively on the surface of PMP-CDNF, (Table 3.1). The FT-IR spectra of CDNF and PMP-CDNF complex were obtained to investigate adamantane-cyclodextrin complex (Figure 3.5c). Signal caused by multiple -OH groups on HP β CD red-shifted from 3410 cm^{-1} to 3398 cm^{-1} due to interactions of hydroxyl groups with N-terminal carbonyl of the guest molecule.^{122,123} Moreover, amide II band of lyophilized PMP¹²⁴ blue-shifted from 1533 cm^{-1} to 1581 cm^{-1} upon complexation with CDNF. Observed shift is indicative of increased in-plane N-H bending, which is a result of significantly reduced peptide-peptide interactions. Complexation of PMP with CDNF disrupts interactions of individual PMP molecules (mainly H-bonds). Raman spectrum of CDNF (Figure 3.6a) showed significant β -CD Raman bands¹²⁵ such as symmetric stretching of -C-O-C at 938 cm^{-1} , stretching vibration of -CH₂ at 2934 cm^{-1} , scissoring vibration of -CH₂ and -CH at 1344 cm^{-1} , and scissoring vibration of -OH at 1409 cm^{-1} . Raman spectrum of PMP-CDNF revealed additional bands at 2450 cm^{-1} and 1650 cm^{-1} corresponding to stretching vibration of S-H and amide I vibration¹²⁶, respectively. Confocal Raman image of CDNF at 2934 cm^{-1} demonstrated HP β CD presence in the network (Figure 3.6b).

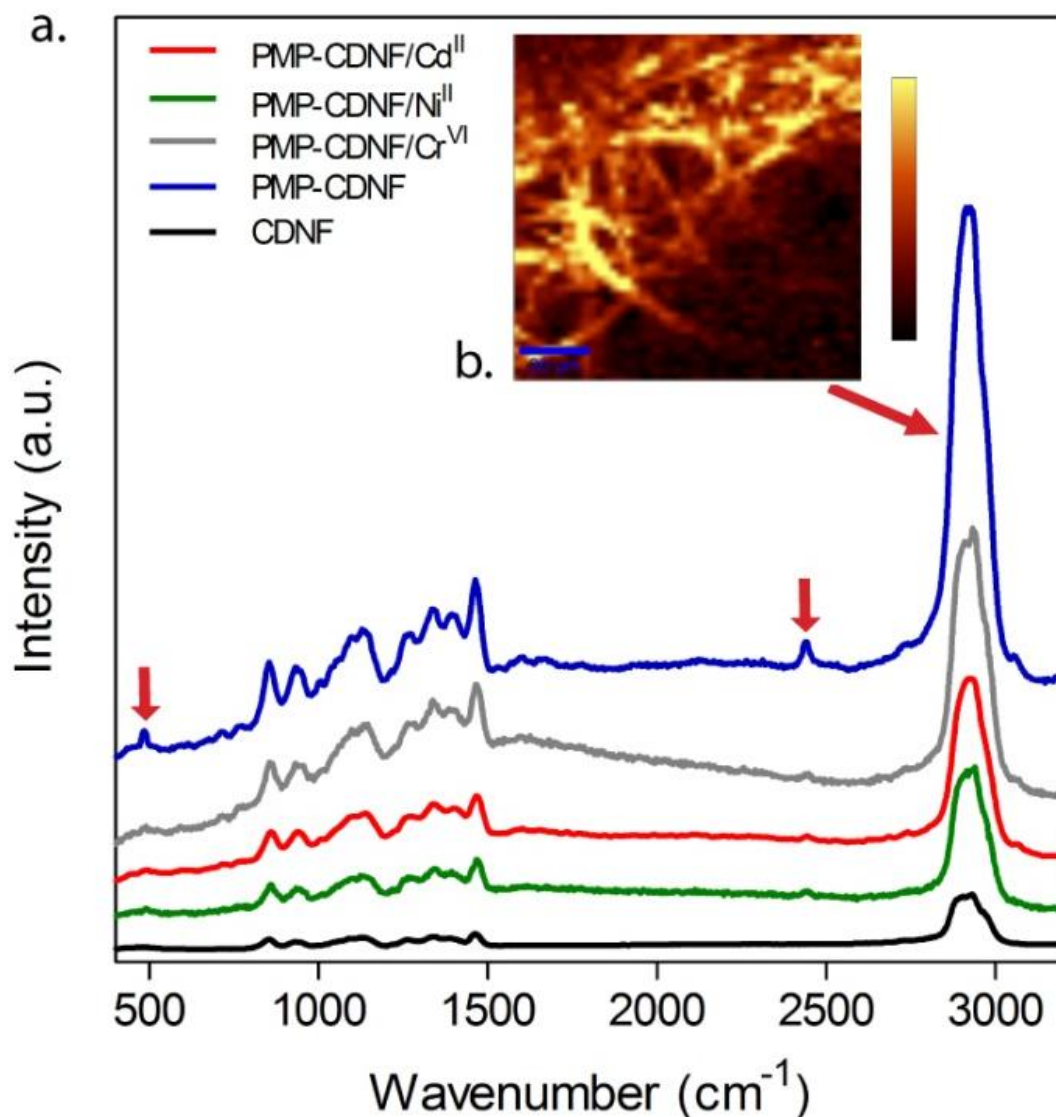


Figure 3.6. a. Raman spectra of CDNF, PMP-CDNF and PMP-CDNF after incubation with different metal solutions and mixture of the metal solutions. As amide I band at 1650 cm⁻¹ and as -SH vibration at 510 and 2450 cm⁻¹ was seen in PMP-CDNF. **b.** Raman spectral image at 2934 cm⁻¹ of 100 μm x100 μm scan area of CDNF.

PMP content in CDNF was determined quantitatively by elemental analysis. PMP, CDNF, CDNF treated with buffer and tris(2-carboxyethyl) phosphine hydrochloride (TCEP-HCl), and PMP-CDNF samples were analyzed with CHNS-O analyzer after drying under ambient conditions (Table 3.2). Mass difference in C, H, N, and S of PMP between experimental and theoretical values was only 1% (Table 3.3). Negligible amount of N was detected in CDNF treated with buffer, although there is no N in the CDNF. The N content could be due to nonspecific adsorption of TRIS buffer molecules.

Metal ions including Cd^{II} , Ni^{II} , and Cr^{VI} were used to evaluate metal binding affinity of the PMP molecule. Interaction between metal ions and the PMP molecule was first studied by isothermal titration calorimetry (ITC). Solutions of Cd^{II} , Ni^{II} , and Cr^{VI} were titrated with PMP solution at pH 8 (Figures 3.7a, 3.8a and 3.9a). ITC measurements showed greater affinity of PMP towards Cd^{II} ions. The interaction between HP β CD molecule and metal ions was negligible (Figures 3.7d, 3.8b, and 3.9b). Interaction between metal ions, Cd^{II} , Ni^{II} , and Cr^{VI} , and the PMP molecule was also monitored through changes in UV absorption of metal ion solutions. Increase in absorption peak at ca. 240 nm was observed for Cd^{II} (Figure 3.7b) and Cr^{VI} metal ions (Figure 3.9c)¹²⁷⁻¹³⁰ due to ligand-to-metal charge transfer ($\text{S} \rightarrow \text{M}^{\text{n}}$) of Cd-S and Cr-S bonds; the increase leveled off after 1.5 molar eq. of PMP was titrated into the solution. Titration of Cr^{VI} with the PMP molecule also revealed decrease in absorption bands at ca. 280 and 375 nm. Titration of Ni^{II} revealed peak increase around 270 nm (Figure 3.8c) due to $\text{S} \rightarrow \text{Ni}^{\text{II}}$ charge transfer¹³¹, which also leveled off at 1.5 molar eq. of PMP. Interaction between metal ions and PMP-CDNF system was verified by using PMP-functionalized and pristine CDNF, which were immersed into

metal ion solutions (Cd^{II} , Ni^{II} and Cr^{VI}). Metal ions can be extracted from water by immersion of the PMP-functionalized CDNF network into solution. Metal ion concentration in solution was monitored by inductively coupled plasma-mass spectrometer (ICP-MS) at different time intervals for 24 h incubation. Increase in metal binding also indirectly showed us the stability and functionality of PMP-CDNF scavenging system during incubation. The PMP-CDNF network captured $0.041 \pm 0.004 \text{ } \mu\text{mol/mg Cd}^{\text{II}}$, $0.010 \pm 0.001 \text{ } \mu\text{mol/mg Ni}^{\text{II}}$ and $0.008 \pm 0.005 \text{ } \mu\text{mol/mg Cr}^{\text{VI}}$ after metal incubation. However, pristine CDNF captured less than $0.005 \text{ } \mu\text{mol/mg Cd}^{\text{II}}$, Ni^{II} and Cr^{VI} (Figure 3.10).

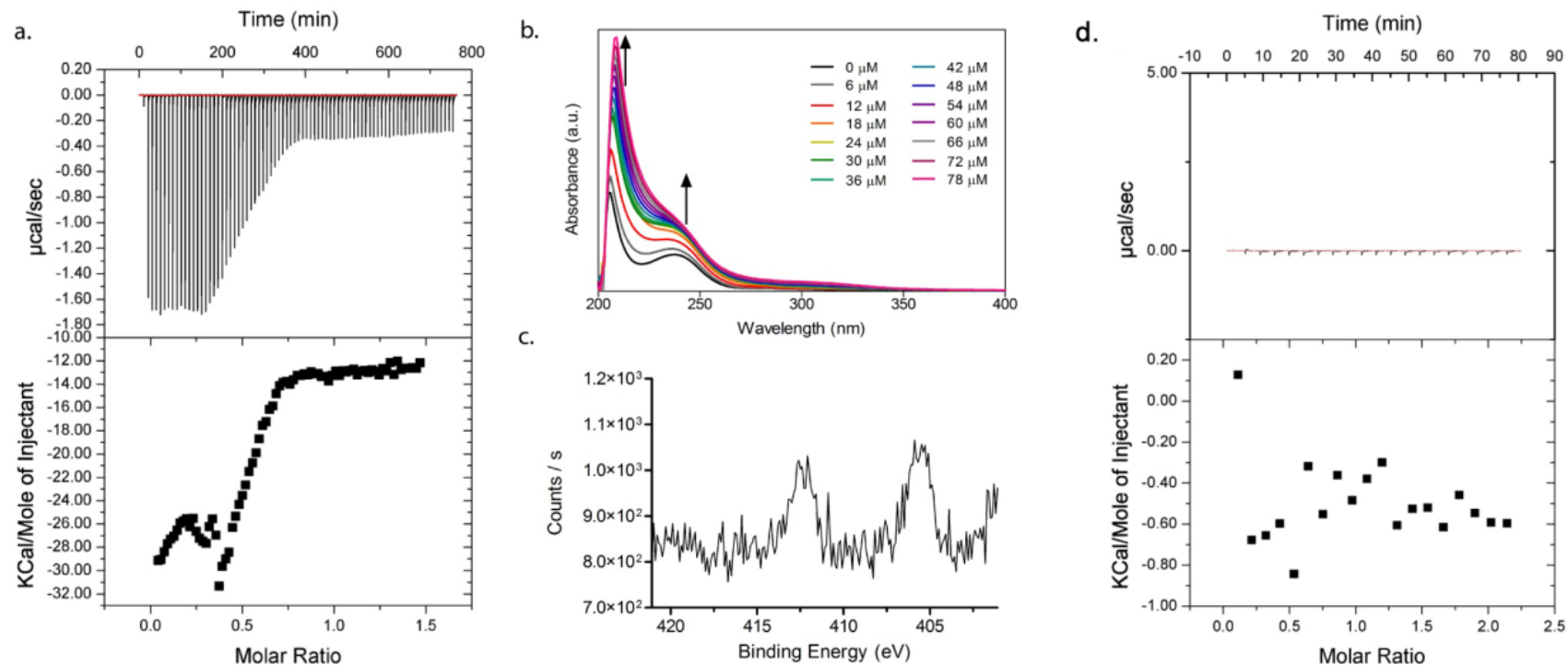


Figure 3.7. a. Isothermal titration curve of PMP with CdCl₂ solution b. Cd 3d XPS spectrum of PMP-CDNF after incubation in Cd^{II} solution. c. Absorption change within 20 μM CdCl₂ titration with PMP solution in 50 mM TRIS buffer at pH 8.0. d. Isothermal titration curve of HPβCD molecule with CdCl₂ solution.

In addition, no significant change was observed in morphology of the PMP-CDNF network upon binding to metal ions and the fibrous structure of the sample was preserved (Figures 3.4b, c and d). After immersion of the sample into metal ion solution, a decrease in S-H vibration at PMP-CDNF sample was observed (Figure 3.7a), which indicates interaction between sulfhydryl groups and metal ions. The XPS analysis of PMP-CDNF samples incubated in metal ion solutions demonstrated greater affinity of the system to Cd^{II} (Figure 3.7c). On the other hand, XPS spectra of Cr^{VI} and Ni^{II} did not show significant binding of these metal ions (Figures 3.7d and 3.8d).

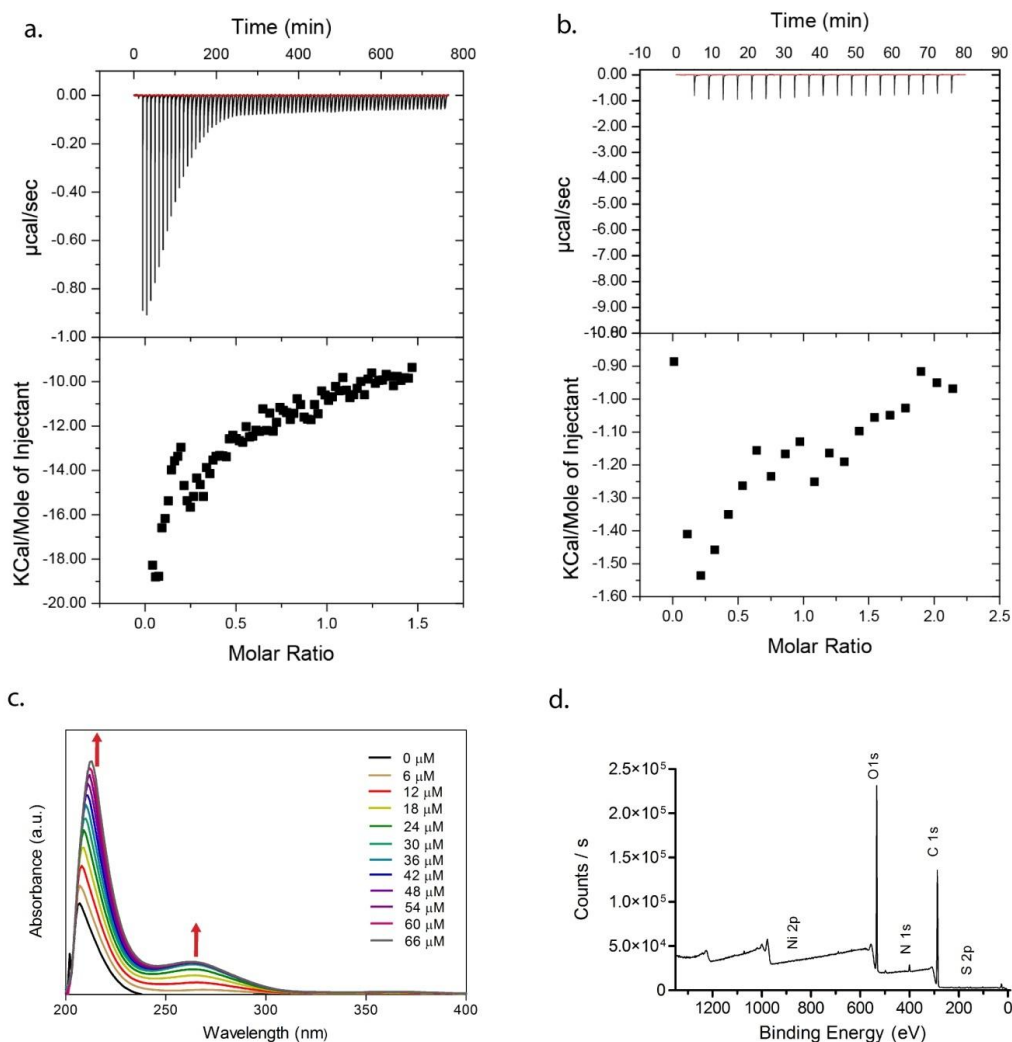


Figure 3.8. Isothermal titration curve of **a.** PMP and **b.** HPβCD molecule with Ni(NO₃)₂ solution. **c.** Absorption change in 20 μM Ni(NO₃)₂ titration with PMP solution in 50 mM TRIS buffer at pH 8.0. **d.** XPS spectrum of PMP-CDNF after incubation in Ni^{II} solution.

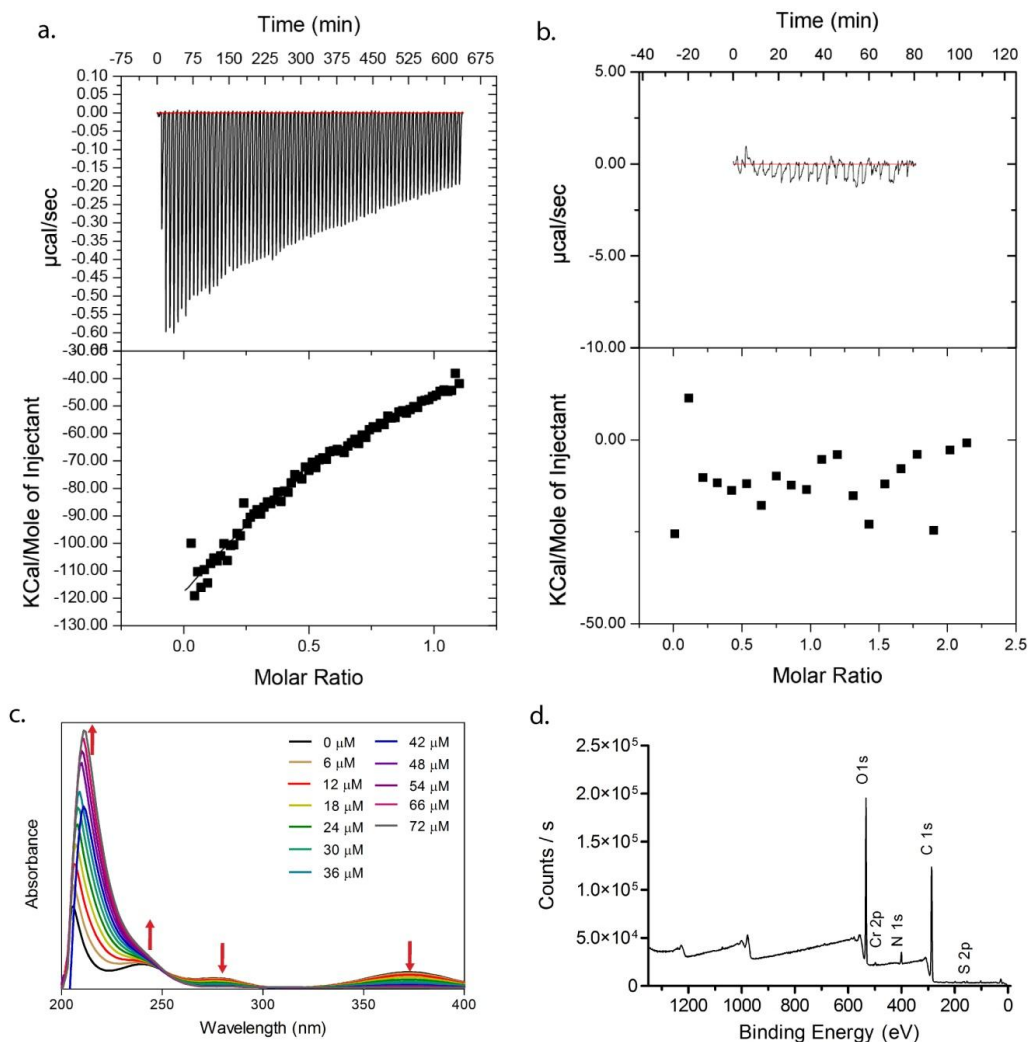


Figure 3.9. Isothermal titration curve of **a.** PMP and **b.** HPβCD molecule with $K_2Cr_2O_7$ solution. **c.** Absorption change in 20 μM $K_2Cr_2O_7$ titration with PMP solution in 50 mM TRIS buffer at pH 8.0. XPS spectra of **d.** PMP-CDNF after incubation in Cr^{VI} solution.

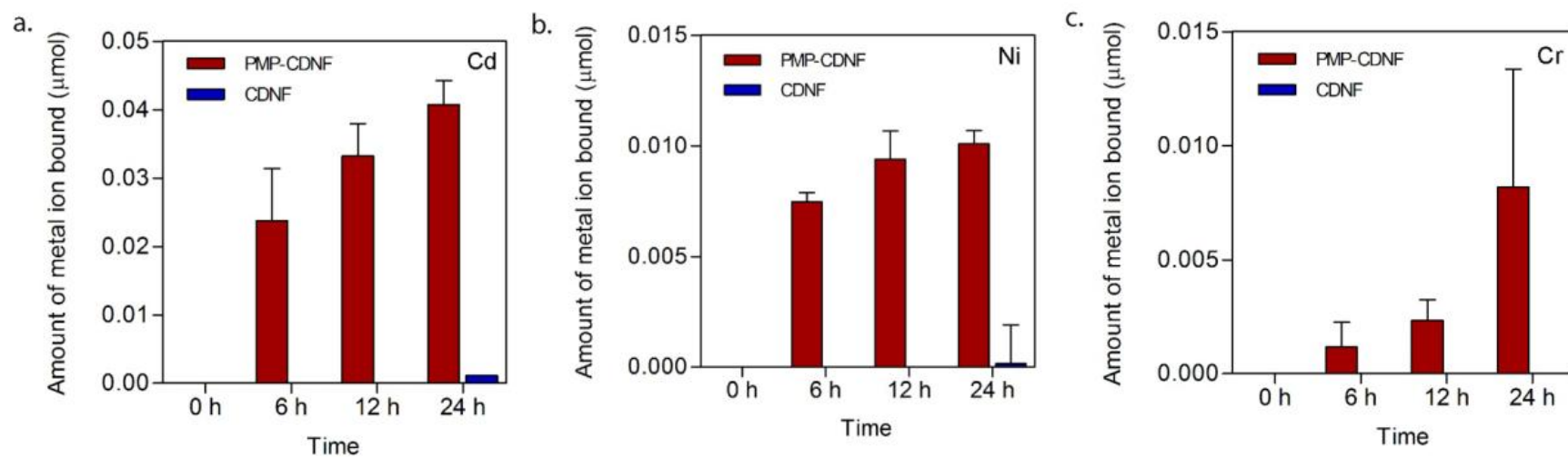


Figure 3.10. The amount of metal ions **a.** Cd, **b.** Ni and **c.** Cr in μmol concentrations bound to per mg of the PMP-CDNF system and bare CDNF from different metal solutions.

Table 3.1. Atom percentages in PMP-CDNF based on XPS spectra.

O1s	C1s	N1s	P2p	S2p
26.97	61.72	5.9	3.38	2.04

Table 3.2. Atom weight percentages of PMP, CDNF, and CDNF treated with TCEP and TRIS and PMP-CDNF in terms of CHNS-O analyzer.

Compound	wt % C	wt % H	wt % N	wt % S
PMP	46.119±0.5075	5.8676±0.2769	11.156±0.1063	7.9138±0.0842
CDNF	46.888±0.6483	6.7914±0.1449	0	0
PMP-CDNF	43.572±0.2839	6.4800±0.012 3	1.2238±0.054 6	0.4751±0.034 9
CDNF treated with TCEP&TRIS	44.670±1.0894	6.8153±0.032 6	0.8670±0.282 4	0

Table 3.3. Comparison of experimental and theoretical [C]/[S], [N]/[S], [H]/[S], and [C]/[N] of PMP.

Compound	[C]/[S]	[N]/[S]	[H]/[S]	[C]/[N]
Experimental-PMP	15.540	3.2222	23.726	4.823
Theoretical -PMP	15.333	3.3333	23.000	4.6000
% Difference	1.0135	0.9667	1.0315	1.0484

3.2 Conclusion

In conclusion, we reported design and synthesis of a heavy metal binding peptide sequence, which can be conjugated to an electrospun CDNF network through adamantane-cyclodextrin host-guest inclusion complex formation. Metal ion binding ability of the PMP molecule was verified with Cd^{II} , Ni^{II} , and Cr^{VI} for PMP functionalized CDNF network in aqueous conditions. ITC measurements by titrations of metal ions with PMP molecule confirmed binding of the PMP molecules to corresponding metal ions. ITC measurement of PMP and β -CD showed that there is moderate binding affinity. Therefore, the PMP-CDNF system was utilized for metal ion scavenging. The amount of metal ions bound to PMP-CDNF fibrous network was quantified by ICP-MS, stability and functionality of the scavenging system was assessed during the metal incubation. Water-insoluble PMP-CDNF fibrous network preserved its fibrous structure without any deformation during metal ion scavenging process. Hence, we successfully demonstrated design, synthesis, characterization, and application of a peptide-polymer hybrid scavenging system by using electrospun CDNF fibrous network as a solid support. On the other hand, metal binding capacity of such a scavenging system can be enhanced using additional modifications on phytochelatin mimetic peptides and also polymer support. The peptide-polymer scavenging system has potential for development of further molecular recognition systems with various peptide sequences or host-guest inclusion complexes.

Chapter 4.

4. Peptide amphiphile-chromophore interactions

4.1 Encapsulation of a zinc phthalocyanine derivative in self-assembled peptide nanofibers

This work is partially described in the following publication:

Garifullin, R., Erkal, T. S., Tekin, S., Ortac, B., Gurek, A. G., Ahsen, V., Yaglioglu, H. G., Elmali, A., Guler, M. O., "Encapsulation of Zinc Phthalocyanine Derivative in Self-Assembled Peptide Nanofibers", *Journal of Materials Chemistry*, 2012, **22**, 2553

4.1.1 Introduction

Construction of metal complex chromophores (e.g. phthalocyanine and porphyrin) containing nanostructures of well-defined size and shape in water is of great interest, because of their attractive photochemical, photophysical and electronic properties.¹³² Zinc phthalocyanine complexes are interesting examples of chromophoric molecules. Various methods such as metal coordination, electrostatic interactions, hydrogen bonding and host-guest interactions have been used to organize phthalocyanine molecules.¹³³⁻¹³⁷

In this study, we presented a straight-forward, one-pot hydrophobic encapsulation method for organizing zinc phthalocyanine derivatives within peptide nanofibers. Our method involves solvophobic effect along with hydrogen bonding in order to achieve encapsulation and nanofiber formation. As a result of encapsulation and nanofiber formation, the zinc phthalocyanine molecules are aligned and ultrafast

energy transfer phenomena is observed upon their organization through the peptide nanofiber core.

Several successful attempts to mimic ultrafast energy transfer mechanism were performed by using polymers,¹³⁸ dendrimers,¹³⁹ porphyrin arrays linked by covalent bonds^{140,141} and by self-assembled systems.¹⁴²⁻¹⁴⁴ It is known that ultrafast energy transfer characteristics of zinc phthalocyanine molecules can be exploited to mimic the light harvesting mechanism in photosynthetic antenna proteins^{145,146} and as it was observed in this work, it can be tuned with the help of self-assembled peptide nanofibers.

4.1.2 Results and Discussion

lauryl-VVAGH-Am peptide (Figure 4.1) was constructed on Fmoc-Rink Amide MBHA resin. Amino acid coupling reactions were performed with 2 equivalents of Fmoc-protected amino acid, 1.95 equivalents of HBTU and 3 equivalents of DIEA for 2 h. The Fmoc protecting group removal was performed with 20 % piperidine/DMF solution for 25 min. Cleavage of the peptides from the resin was carried out with a mixture of TFA:TIS:H₂O in ratio of 95:2.5:2.5 for 2 h. Excess TFA was removed by rotary evaporation. The remaining peptide was triturated with ice-cold diethyl ether and the resulting white precipitate was freeze-dried. Peptide was characterized by Agilent 1200-6530 quadrupole time of flight (Q-TOF) mass spectrometer with electrospray ionization (ESI) source equipped with Zorbax SB-C8 4.6 x 100 mm column operating on principles of reverse-phase analytical high performance liquid chromatography (HPLC). A gradient of 0.1% formic acid/water and 0.1% formic acid/acetonitrile was used as mobile phase for analytical HPLC. A

peak of 663.4996 a.m.u corresponding to peptide $[M+H]^+$ was registered in mass spectrum (Figure 4.2). Peptide actively captures alkali metal ions as peaks corresponding to $[M+Na]^+$ and $[M+K]^+$ were observed. Some other peaks corresponding to dimeric $[2M+H]^+$ and doubly charged $[M+2H]^{+2}$ species were also registered. According to liquid chromatogram (Figure 4.3), the peptide with high purity of c.a. 98% was obtained. After verification of peptide identity and its purity, synthesized molecule was utilized in series of experiments which envisioned encapsulation of octakis (hexylthio) zinc phthalocyanine and further study of its photophysical properties in new environment.

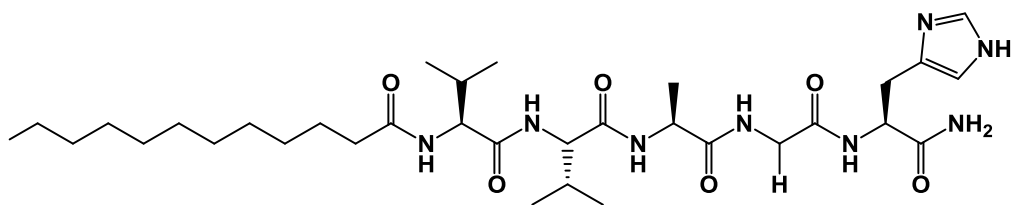


Figure 4.1. Structural formula of lauryl-VVAGH-Am.

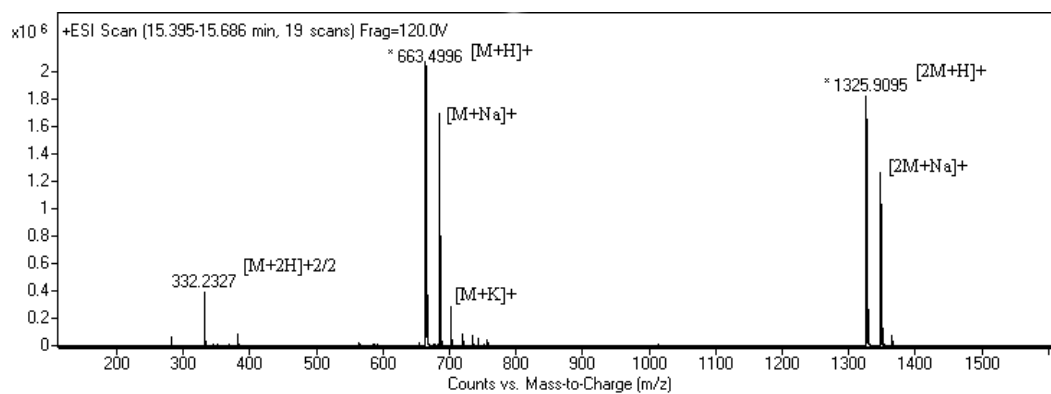


Figure 4.2. Mass spectrum of lauryl-VVAGH-Am.

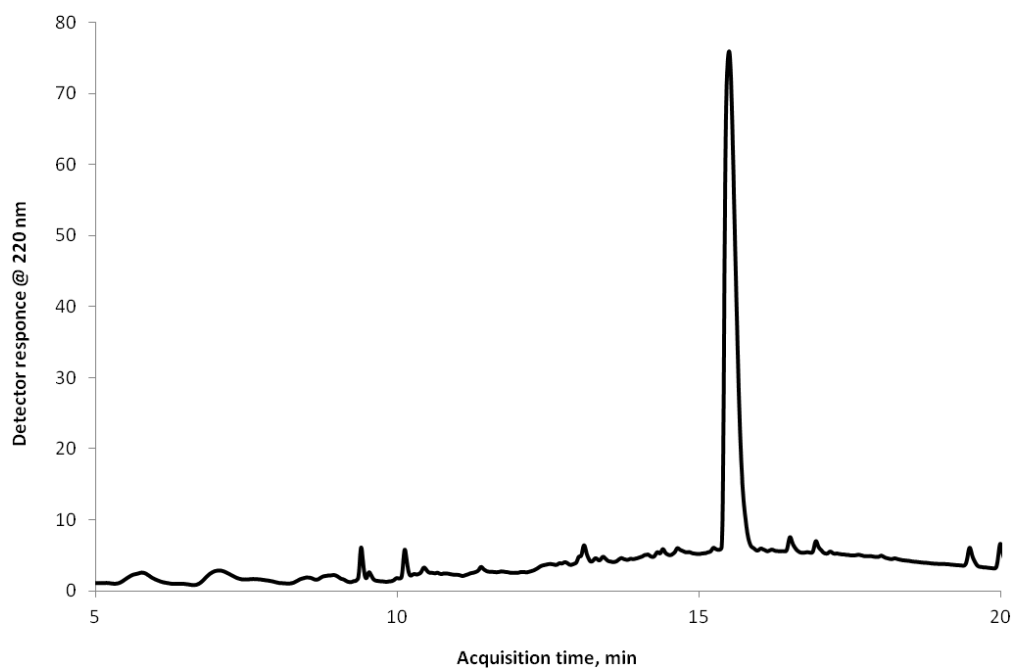


Figure 4.3. Liquid chromatogram of lauryl-VVAGH-Am.

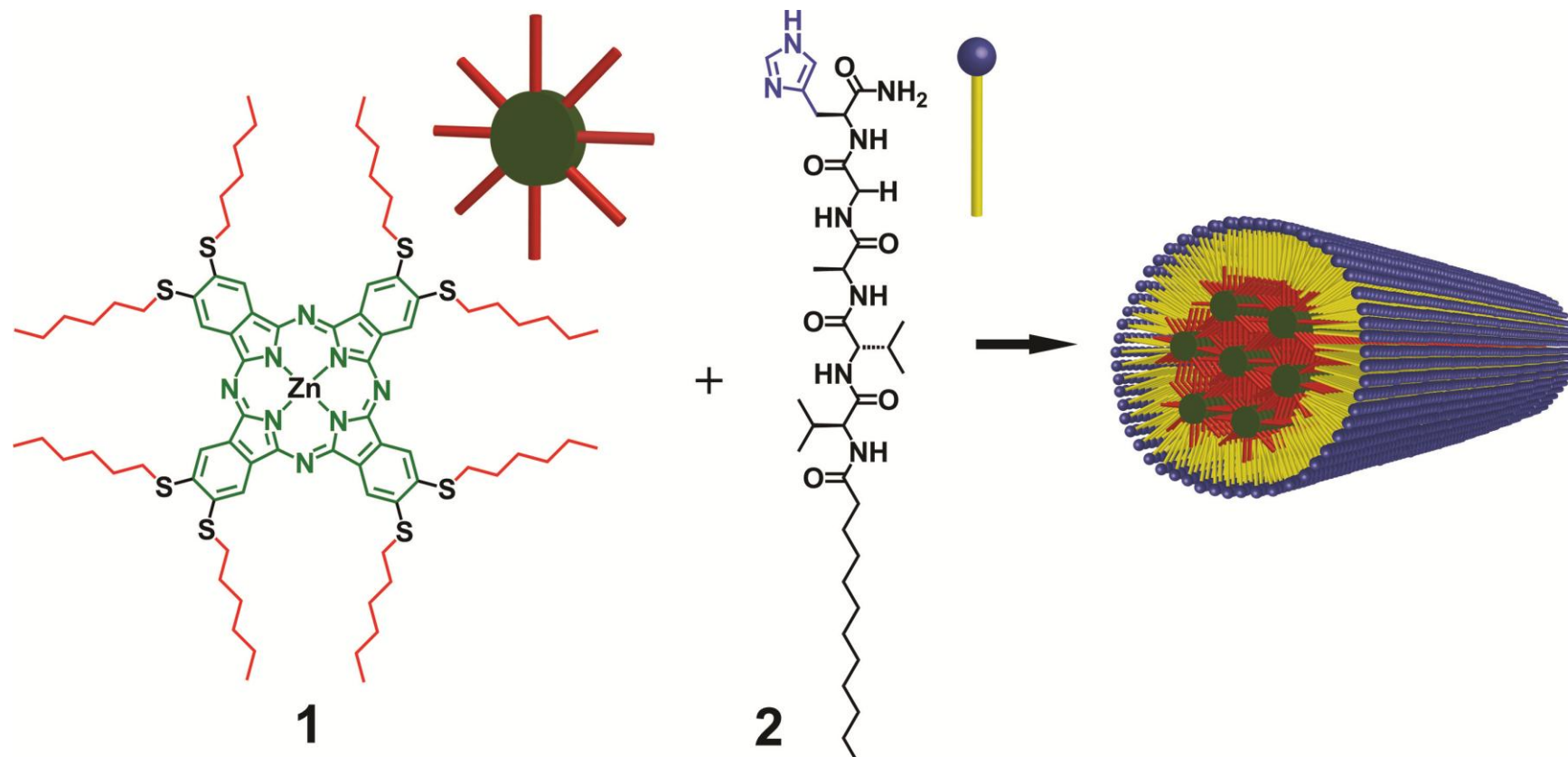
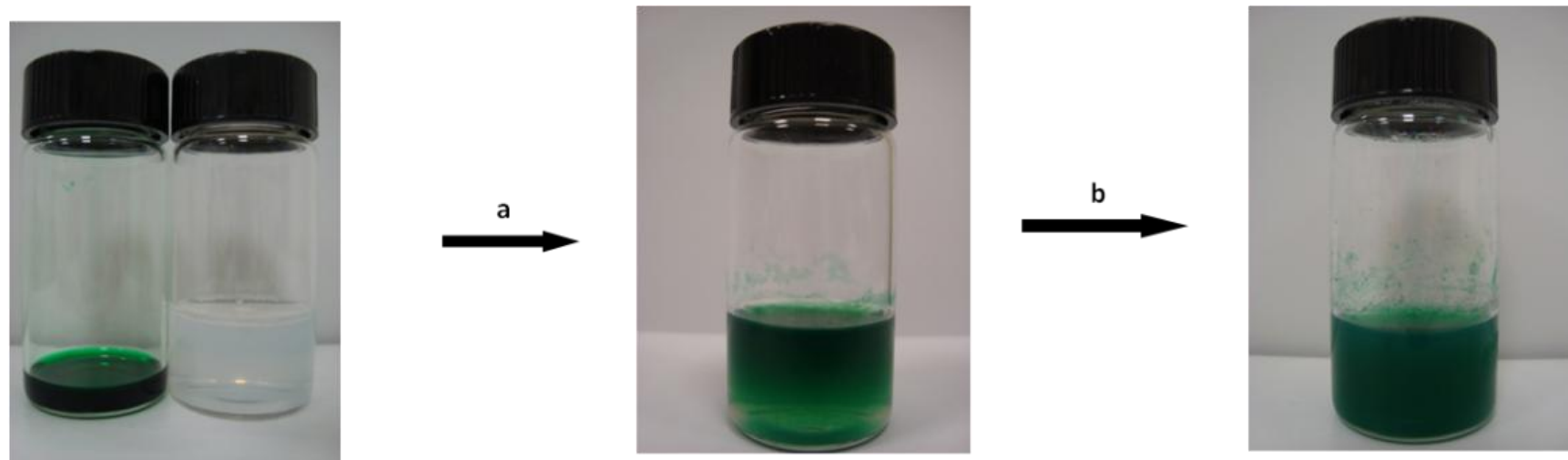


Figure 4.4. Schematic representation of ZnPc (1) encapsulation in PA (2) nanostructures.

In this chapter, encapsulation and non-covalent alignment of octakis (hexylthio) zinc phthalocyanine (ZnPc) molecules (**1**) by using self-assembling peptide amphiphile (PA)^{147,148} molecules (**2**) with the sequence of lauryl-VVAGH-Am is reported (Figure 4.4). The encapsulation of zinc phthalocyanine derivative is performed by hydrophobic interactions between alkylthiol functionalized ZnPc and fatty acid conjugated peptide molecule (Figure 4.4). The hydrophilic parts of peptide amphiphiles render ZnPc molecules soluble in water. The histidine residue provides hydrophilic property to the amphiphilic peptide molecules. The nature of the histidine residue determines the self-assembly properties of the PA molecule depending on the pH of the medium. During the encapsulation process, ZnPc was dissolved in THF and PA was dissolved in water. These two solutions were mixed and the mixture was ultrasonicated for encapsulation of ZnPc molecules within the peptide nanofibers during the evaporation of THF (Scheme 4.1). At the end of the encapsulation process, ZnPc molecules were surrounded by the PA molecules (Figure 4.1) which can self-assemble into nanofibers with a hydrophobic core.¹⁴⁹



Scheme 4.1. Encapsulation process. **a.** Chromophore introduction. **b.** Sonication with heating.

Morphological characterizations

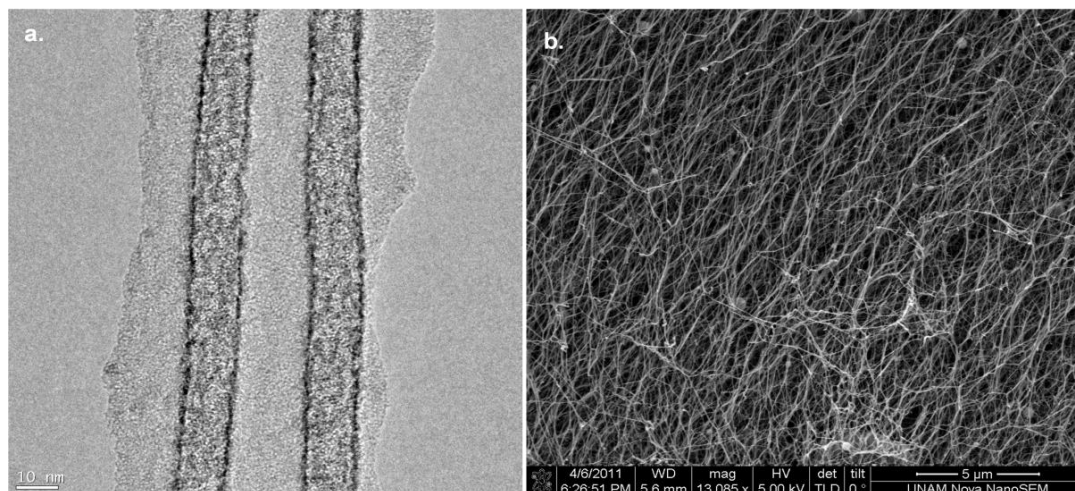


Figure 4.5. **a.** TEM and **b.** SEM images of the ZnPc – containing PA nanostructures.

In this study, hydrophobic ZnPc molecules were located in the hydrophobic core during self-assembly of PA molecules due to hydrophobic interactions. Morphology of the self-assembled structures was studied by means of scanning and transmission electron microscopy techniques. It was observed that mixture of ZnPc and PA indeed yields one-dimensional fibrous nanostructures. SEM and TEM images of ZnPc – containing peptide nanofibers are shown in Figure 4.5. In order to verify the presence of ZnPc molecules within the peptide nanofibers, energy dispersive X-ray spectrum demonstrating presence of zinc was recorded from an individual peptide nanofiber (Figure 4.6).

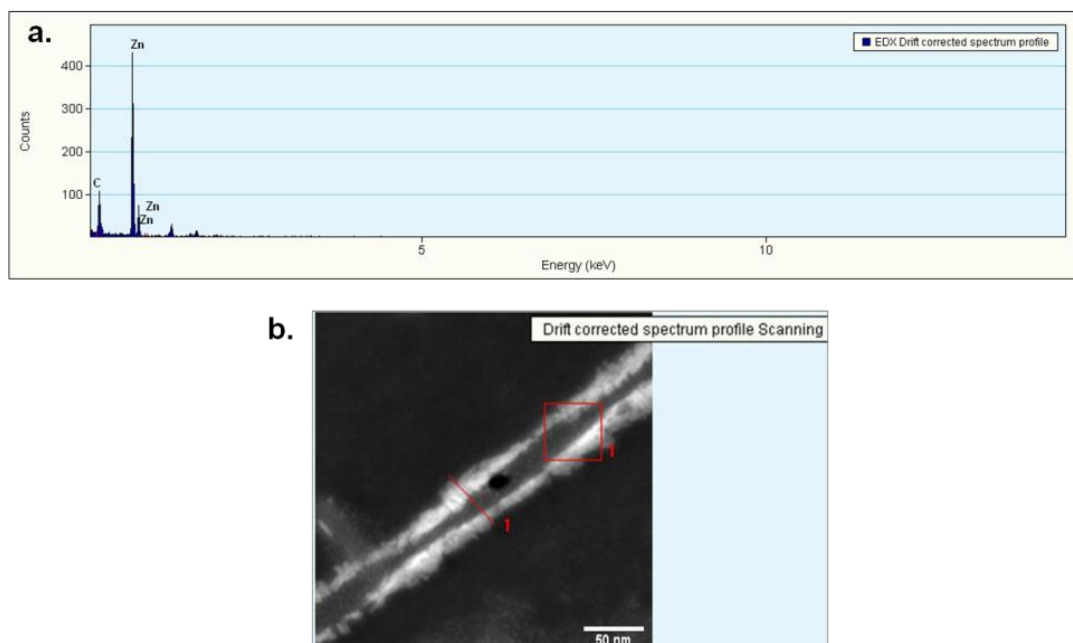


Figure 4.6. a. EDX spectrum of peptide nanofiber surface. **b.** Scanned fiber.

It was important to rule out the possibility of ZnPc presence on the surface of nanofibers. Therefore, we additionally measured the singlet oxygen generation capacity of ZnPc molecules to confirm the encapsulation of ZnPc molecules within the PA nanofibers. First, we demonstrated that ZnPc is capable of singlet oxygen generation. For this purpose, we dissolved ZnPc together with DPBF trap molecule in THF and observed trap degradation upon irradiation with appropriate light source. Next, encapsulated ZnPc sample was mixed with water soluble ADMA trap molecule and again irradiated, as a result no detectable trap molecule degradation was observed. Based on this observation, it was inferred that there are no ZnPc molecules accumulating on the surface of nanowires. But it was still unclear whether ZnPc is still capable of singlet oxygen generation in encapsulated form. To test its activity, ZnPc was co-encapsulated with DPBF, and upon irradiation with light we observed degradation of DPBF. Briefly, singlet oxygen generation and consequent

trap molecule degradation were observed only in cases of direct interaction between the ZnPc and trap molecule (Figure 4.7).

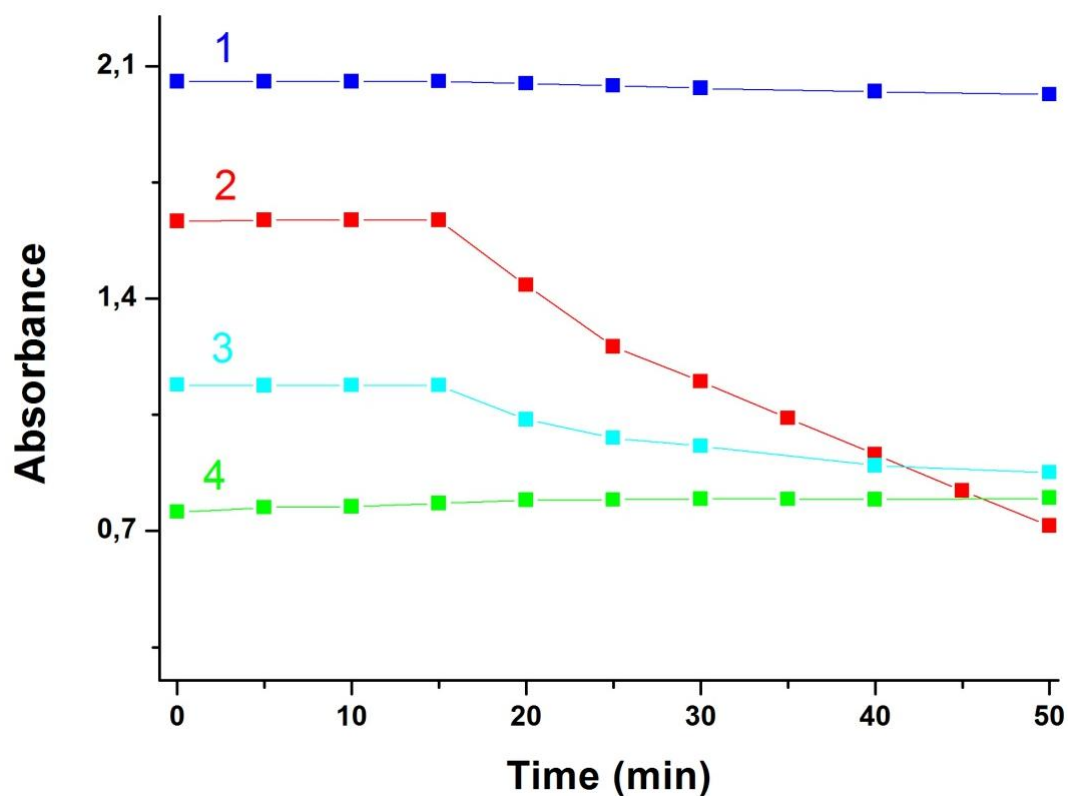


Figure 4.7. Singlet oxygen measurement of ZnPc in THF, DPBF in THF (control), ZnPc within PA in water, ZnPc and DPBF within PA in water. 1) DPBF in THF (at 414 nm), 2) DPBF+ZnPc in THF (at 414 nm), 3) ZnPc+DPBF with PA in water (at 418 nm), 4) ZnPc+PA with ADMA in water (at 381 nm).

Three-dimensional network of the ZnPc – containing peptide nanofibers forms a self-supporting gel at pH 8 upon neutralization of the charges on the peptide sequence. The mechanical properties of the gels were characterized by oscillatory rheology experiments (Figure 4.8).

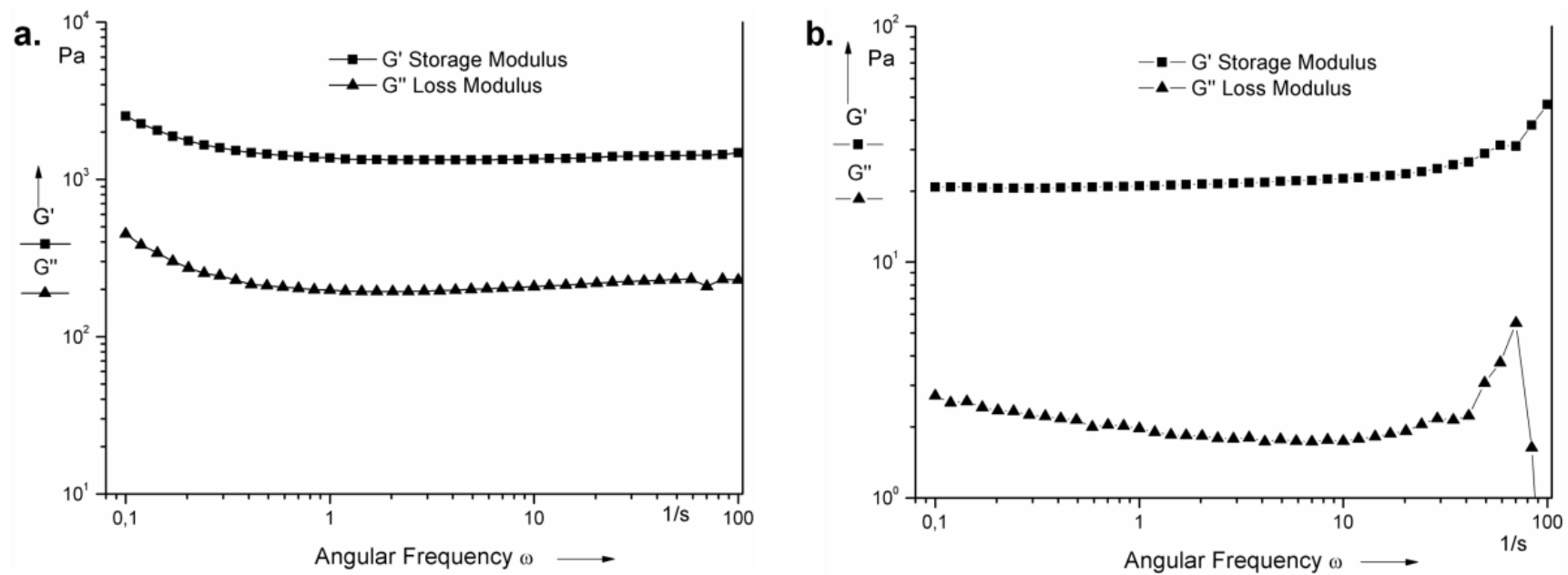


Figure 4.8. Frequency sweep rheology measurement of **a.** ZnPc-containing PA gel and **b.** PA only gel.

Storage modulus of ZnPc – containing peptide gel is greater than that of peptide only gel; it can be indirectly inferred that self-organized π -stacks of phthalocyanines enhance mechanical properties of the gel. Critical point dried gel of ZnPc – containing peptide nanofibers was studied by SEM. Well-defined nanofiber network was observed (Figure 4.5b). The morphology of the nanofibers was also studied by circular dichroism spectroscopy indicating that the self-assembly process involves extended β -sheet structural motif (Figure 4.9).

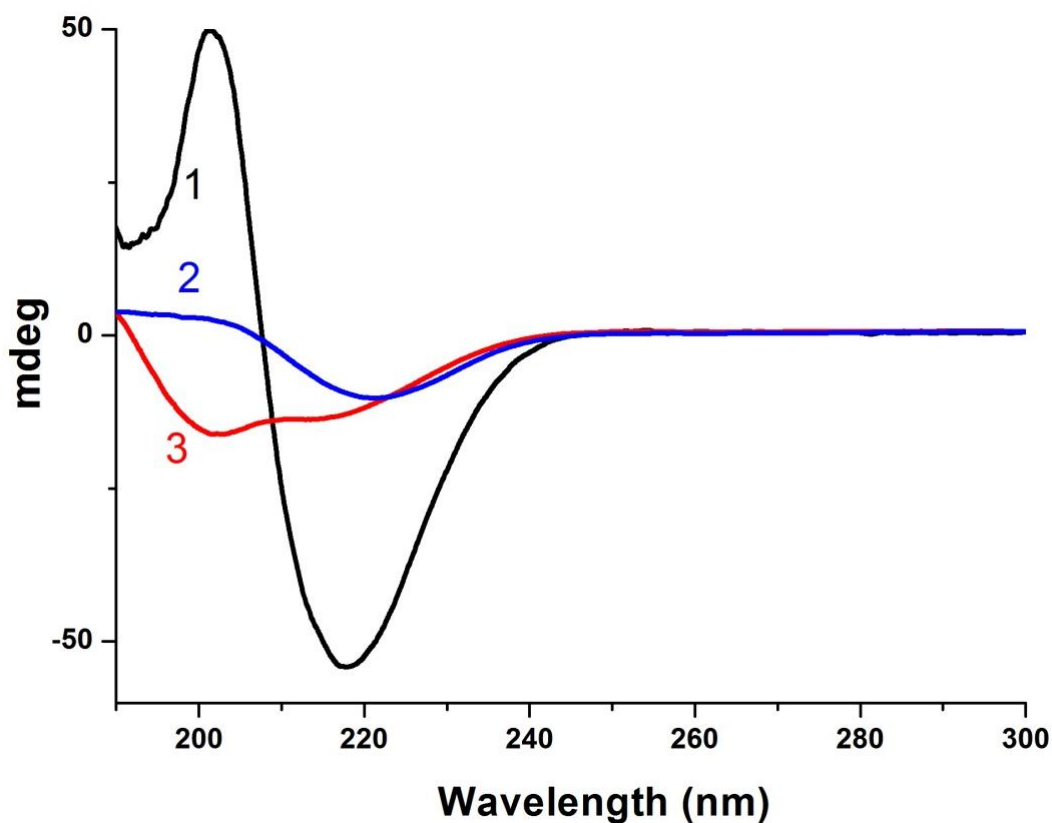


Figure 4.9. Circular dichroism spectra of ZnPc with PA at pH 2, pH 5, and pH 8. 1) ZnPc+PA pH 2, 2) ZnPc+PA pH 5, 3) ZnPc+PA pH 8.

Spectroscopic characterization

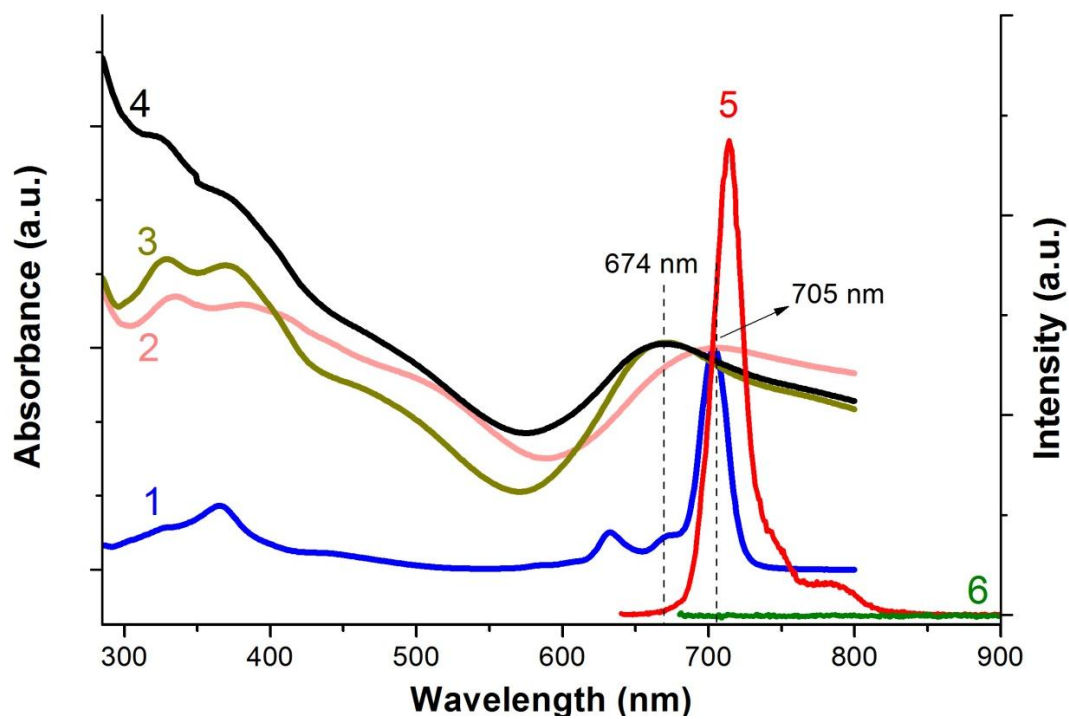


Figure 4.10. Absorbance and fluorescence spectra of ZnPc with and without the PA molecules. 1) ZnPc absorbance in THF, 2) ZnPc with PA absorbance in water at pH 2, 3) ZnPc with PA absorbance in water at pH 5, 4) ZnPc with PA absorbance in water at pH 8, 5) ZnPc fluorescence in THF, 6) ZnPc with PA fluorescence in water.

Due to an increase in local concentration of the ZnPc molecules in the core of the nanofibers, the interactions between ZnPc molecules were enhanced and we observed remarkable changes in the spectroscopic characteristics of ZnPc molecule. There is a significant difference between the UV-Vis spectrum of ZnPc dissolved in THF and ZnPc with PA molecules in water (Figure 4.10). The difference in the absorption bands is caused by aggregation of the ZnPc molecules within the PA

nanofibers in aqueous conditions.¹⁵⁰ Encapsulation of ZnPc molecules causes a shift and broadening in the absorption bands.^{136,150} The Q-band absorption of ZnPc in THF blue-shifts from 705 nm to 674 nm compared to ZnPc with the peptide solutions. Smaller blue-shift was observed for sample at pH 2 compared to samples at pH 5 and pH 8 because of the formation of extended peptide nanostructures at higher pH (Figure 4.10). Observed blue-shifts in the Q-band absorption of encapsulated ZnPc upon aggregation in the peptide nanofibers may be caused by formation of H-aggregates (face to face aggregates)¹⁵¹⁻¹⁵³ with effective π - π interactions. However, mechanism of chromophore aggregation should be characterized in more detail in further studies. It was interesting to compare spectroscopic behavior of encapsulated chromophores with aggregated chromophores in solid phase because former ones are completely isolated from the solvent and can resemble up to certain extent latter ones. For this purpose ZnPc thin film was prepared on microscope slide surface. The absorption peak around 670 nm (analogous to blue-shifted peaks around 670 nm for encapsulated ZnPc samples, except pH 2) in spectrum of ZnPc thin provided some information about the aggregation state of the chromophoric units in peptide nanofibers (Figure 4.11). Encapsulated ZnPc molecules in the nanofiber form revealed solid-like behaviour similar to ZnPc film.

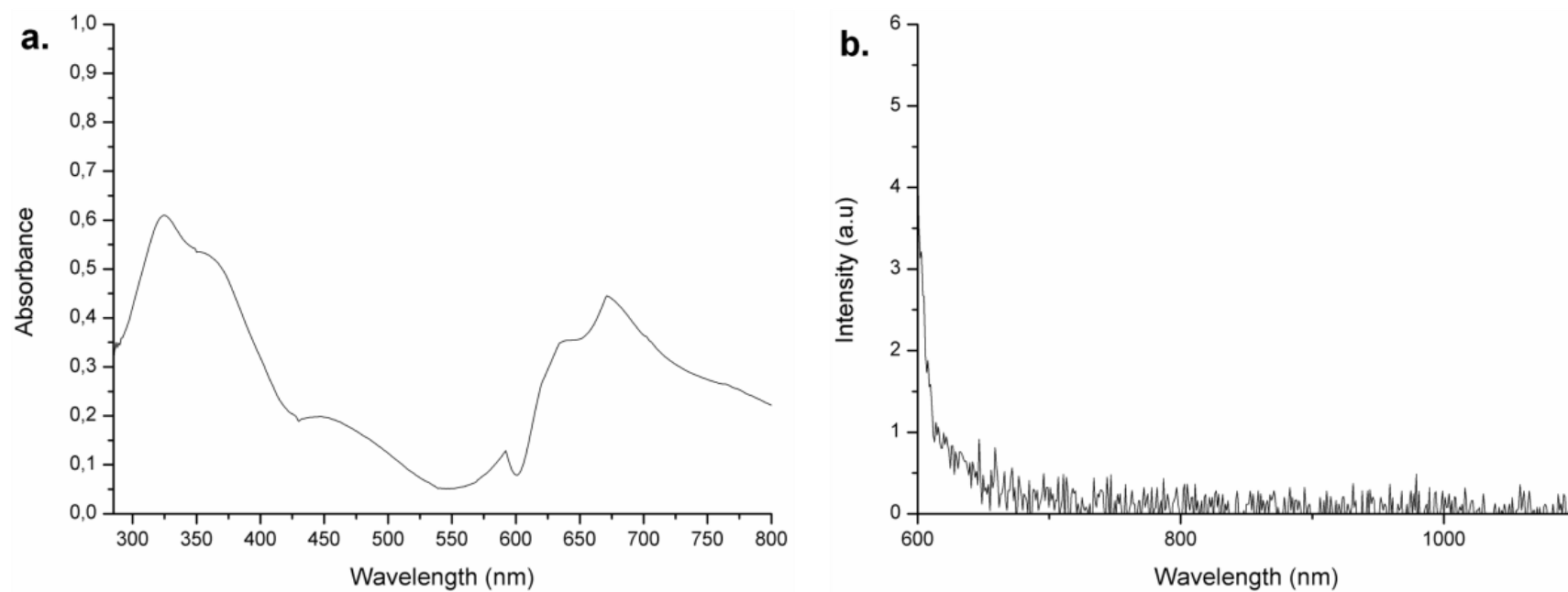


Figure 4.11. a. UV-Vis and **b.** emission spectra of ZnPc film.

Furthermore, fluorescence spectra of ZnPc in THF and in water with PA molecules differ significantly because of the effect of aggregation and change in the environment of the ZnPc molecules. Interestingly, encapsulation of the ZnPc molecules in the peptide nanofibers results in quenching of the fluorescence emission (Figures 4.10). The quenching caused by aggregation was observed for ZnPc film as well (Figure 4.11). The polarity and nature of the environment such as solvent, pH, and temperature is known to have an impact on photophysical properties of fluorescent molecules.^{154,155} The UV-Vis spectra at different pH values indicate indirect effect of pH on the photophysical properties of the ZnPc molecules (Figure 4.10). The change in the pH directly affects the polarity and the self-assembly behavior of the PA molecule because of the presence of the histidine residue. Consequently, the pH change effects aggregation of the chromophores which are surrounded by the self-assembled peptide molecules.

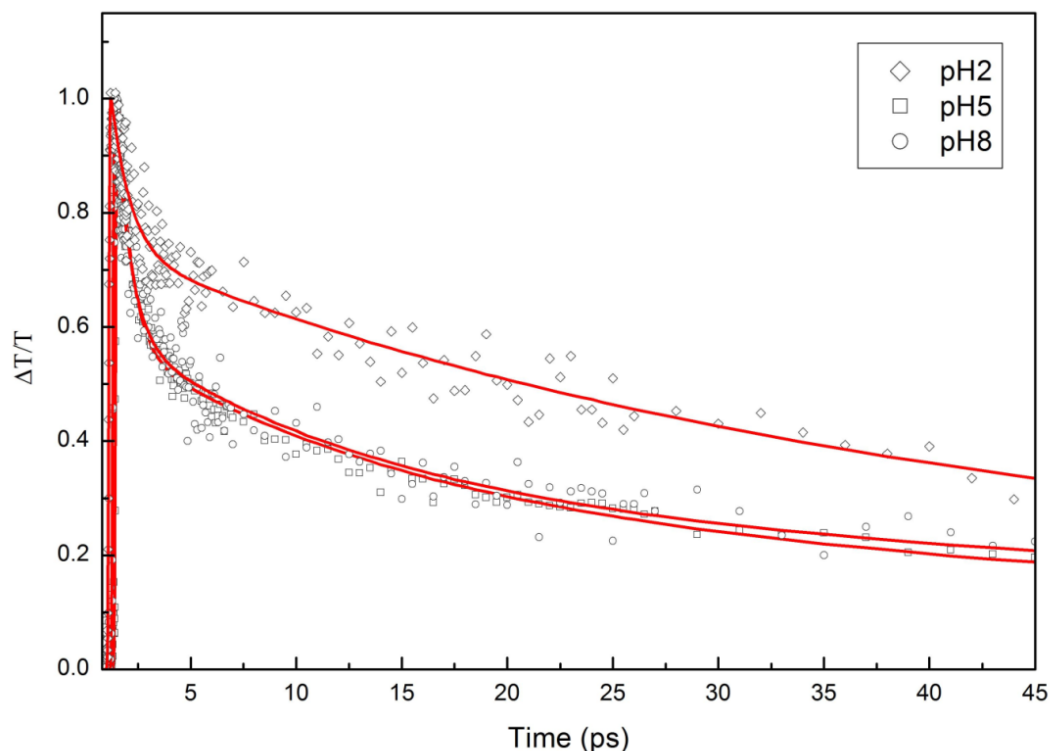


Figure 4.12. Time evolution of the excited state absorption signal at 585 nm for ZnPc in aqueous media with PA at different pH values.

Severe fluorescence quenching observed for encapsulated ZnPc samples has drawn our attention to possible contribution of aggregation to this phenomenon. It is known that aggregation of chromophores may yield fast excited state relaxations due to non-radiative energy transfers between aggregated molecules as observed in the literature.¹⁵⁶ In order to reveal the relationship between aggregation and excited state life times in peptide solutions at different pH values, ultrafast pump-probe experiments were utilized. The time evolution of excited state absorption (ESA) signals for ZnPc in THF reveals long-lived excited state relaxations (on the order of ns). On the other hand the time evolution of ESA signals for encapsulated ZnPc

samples (Figure 4.12) exhibits very short-lived (on the order of ps and less) excited state relaxation pathways depending on the pH values (Table 4.1).

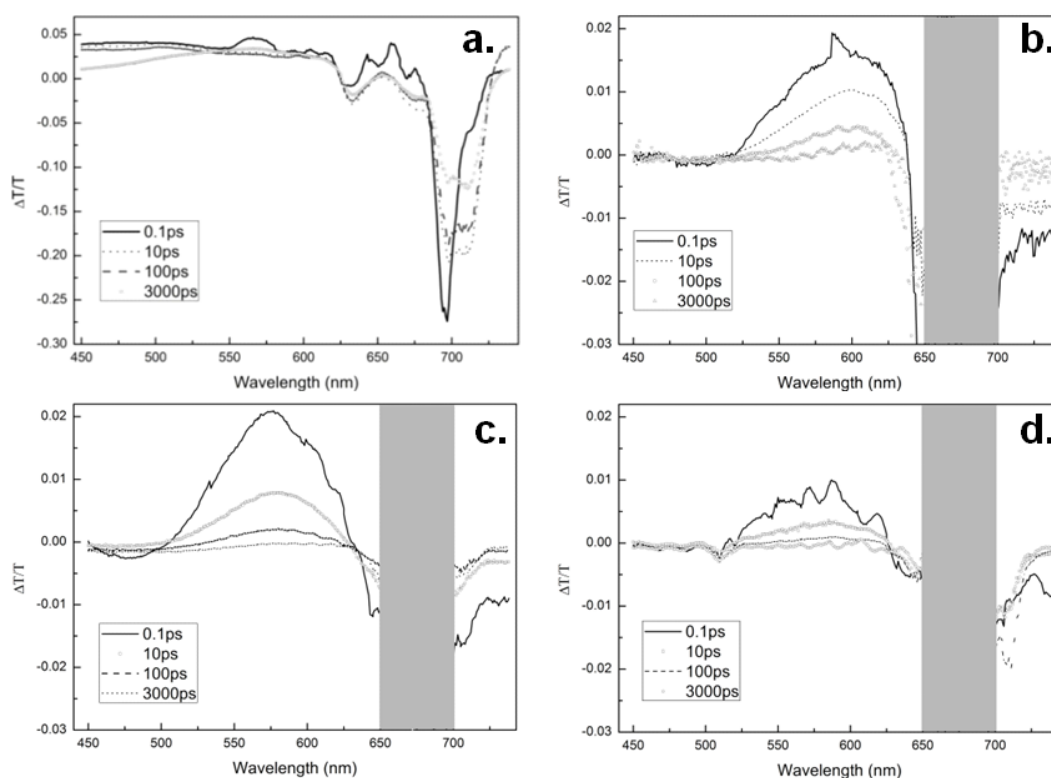


Figure 4.13. Transient spectra of ZnPc. **a.** In THF. In H₂O with PA at **b.** pH 2, **c.** pH 5, and **d.** pH 8.

Transient absorption data versus wavelength for various time delays is shown in Figure 4.13. As shown in Figure 4.13a, ESA signal around 570 nm ($T_1 \rightarrow T_n$ transition) observed for ZnPc in THF decays until 100 ps, then it increases gradually. The ground state bleach and stimulated emission (SE) signal appearing around 695 nm at earlier time delays shifts to 720 nm after couple of ps time delays due to the Stokes shift of ZnPc in THF. The excited state absorption signals versus wavelength

for various time delays for ZnPc in peptide are shown in Figures 4.13b, 4.13c and 4.13d. In these figures a strong scattering signal appears around 690 nm pump wavelength (gray area) due to the peptide nanofibers. Pump-probe data as a function of wavelength for encapsulated ZnPc at different pH values (Figures 4.14 b-d) also differ from ZnPc in THF (Figure 4.14a).

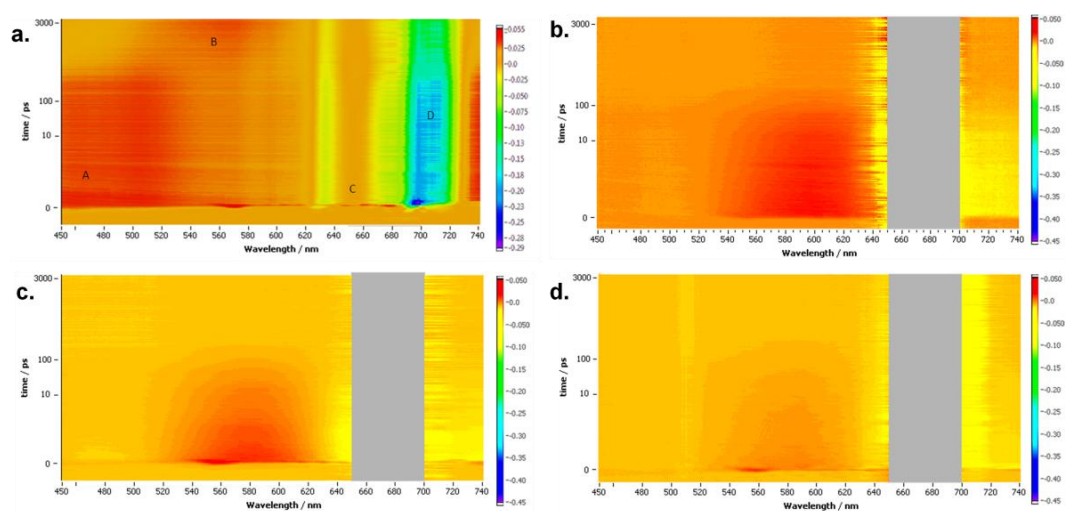


Figure 4.14. Pump-probe data as a function of time (y) and wavelength (x) for **a.** ZnPc in THF [The characteristic features of singlet ESA (A and C), triplet ESA (B) and stimulated emission and bleach (D) are indicated. Pump-probe data for ZnPc in THF revealed broad positive excited state absorption (ESA) bands superimposed with a deep negative signal, due to the ground state bleach and stimulated emission (SE). Similar results were also obtained for zinc phthalocyanine in DMSO and these signals were identified as bleach+SE (D), $S_1 \rightarrow S_{n1}$ ESA (C), $S_1 \rightarrow S_{n2}$ ESA (A), and $T_1 \rightarrow T_n$ ESA (B)], **b.** for ZnPc peptide at pH 2, **c.** for ZnPc peptide at pH 5 and **d.** for ZnPc peptide at pH 8.

It is known that ESA signal with long decay times corresponds to transitions between triplet states ($T_1 \rightarrow T_n$ ESA (B)) of ZnPc in THF (Figure 4.14a).¹⁵⁷ These long decay times do not appear in either of encapsulated ZnPc samples indicating intermolecular fast energy transitions (Figures 4.14 b-d).¹⁵⁶ Similar behavior was also observed in several studies with zinc phthalocyanines at high concentrations or with zinc phthalocyanine thin films.¹⁵⁸⁻¹⁶¹ In addition, strong stimulated emission signal observed for ZnPc in THF around 720 nm does not appear in encapsulated ZnPc samples. This is consistent with the fluorescence quenching observed for the peptide samples.

Pump-probe experiment results also revealed the effect of pH on ZnPc in the peptide nanofibers. The pH 2 sample shows the slowest decay time, while samples at pH 5 and pH 8 showed similar faster decay times (Table 4.1). Based on this observation, it can be inferred that encapsulation of ZnPc in peptide nanofibers not only yields ultrafast energy transfer between chromophores but also provides a route to control the ultrafast decay time by altering the pH of the solution.

Table 4.1. Non-linear absorption decay time constants for ZnPc samples under various conditions.

Medium	t_1 (ps)	t_2 (ps)	t_3 (ps)
Aqueous (pH 2)	142	33	0.92
Aqueous (pH 5)	80	6	0.48
Aqueous (pH 8)	100	6	0.47
ZnPc in THF	2610	482.9	56.7

4.1.3 Conclusion

In summary, successful encapsulation of zinc coordinated phthalocyanines by peptide amphiphile molecules was achieved in water. Most strikingly, encapsulation significantly modifies photophysical properties of chromophores and enables pH controlled ultrafast intermolecular energy transfer. Observed energy transfer mechanism suggests that these systems can be used to mimic light harvesting photosynthetic antenna proteins. Biologically relevant peptide amphiphiles¹⁶² can provide a myriad of useful applications such as bio-imaging and medical applications when combined with properties of various chromophores. This method can also provide a versatile approach for bottom-up fabrication of supramolecular organic electronic devices.

4.2 Induced helical chirality in peptide amphiphile systems

4.2.1 Introduction

Phenomenon of induced chirality is poorly studied and its possible application areas are limited to some laboratory techniques such as monitoring of DNA hybridization in real time,¹⁶³ determination of absolute configuration by zinc phthalocyanine tweezer,¹⁶⁴ and synthesis of chiral plasmonic nanostructures.¹⁶⁵ It was observed that chiral molecules can induce chiral organization of achiral molecules, thus representing interesting tool in control of material properties. Chiral molecules such as peptides are interesting model structures in studying induced chirality phenomenon. Furthermore, a peptide sequence conjugated to hydrophobic tail transforms to a peptide amphiphile (PA) and exhibits self-assembly property. Peptide amphiphiles, depending on a sequence, can assemble into nanofibers and micelles.¹⁶⁶ In our study, we have focused on fiber forming PAs, because nanofiber formation leads to helical arrangement of individual PA molecules. These arrangement forces covalently conjugated or encapsulated chromophore to exhibit induced chirality, namely induced helical chirality. The latter case represented by encapsulated chromophore is of great importance, because it arises solely based on physical restrictions within self-assembled supramolecular cavitand. By switching amino acid sequence from all-L to all-D it is possible to control photophysical properties of a chromophore, i.e. to switch the sign of induced peaks.

In this chapter, two strategies to induce chirality with achiral pyrene chromophore were employed. The first strategy envisioned covalent conjugation of the chromophore to L and D variants of the same peptide sequence (VVAGH) and

formation of self-assembled nanofibers of **1** and **1'** (Figure 4.15). The second strategy included encapsulation of the chromophore in self-assembled nanofibers of **2** and **2'**, which are L and D variants of VVAGH sequence conjugated to lauric acid (Figure 4.15).

4.2.2 Results and Discussion

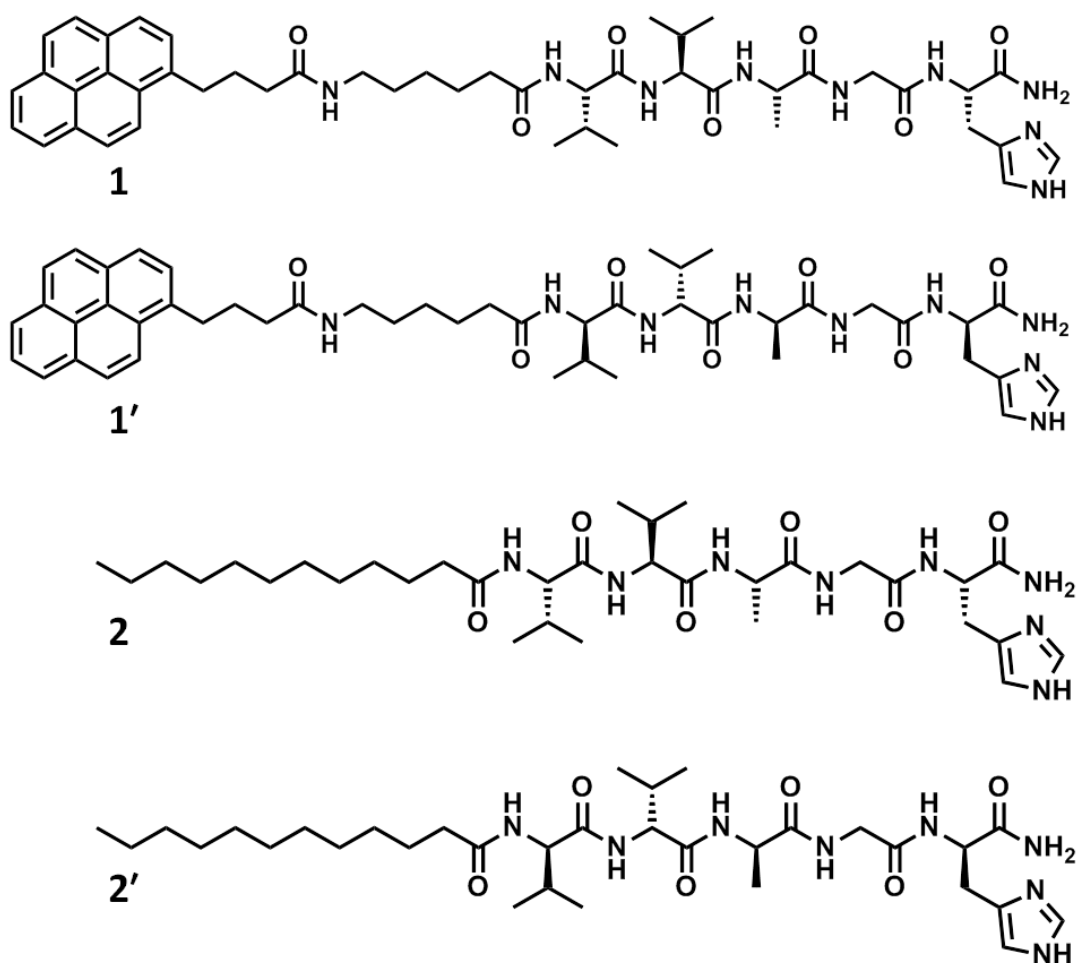


Figure 4.15. Structural formula of pyrenebutyryl- ϵ -Ahx-VVAGH-Am (**1**), pyrenebutyryl- ϵ -Ahx-vvaGh-Am (**1'**), lauryl-VVAGH-Am (**2**), and lauryl-vvaGh-Am (**2'**).

The peptides were synthesized by solid phase peptide synthesis method. Peptide sequences were constructed on Rink Amide MBHA resin (0.59 mmole/g). 0.25 mmole peptide synthesis was carried out in each case; 2 equivalents (with respect to resin) of amino acids, lauric acid or pyrenebutyric acid were used in each coupling. Amino acids, lauric acid or pyrenebutyric acid (2 eq.) were activated with DIPEA (3 eq.) and HBTU (1.98 eq) prior to coupling. All couplings were carried in dimethylformamide (DMF). At the end of the coupling cycles peptides were cleaved from the resin by concentrated trifluoroacetic acid (TFA). For this purpose, a 10 mL cleavage cocktail (TFA:TIS:H₂O = 9.5:0.25:0.25) was prepared; distilled water (H₂O) and triisopropylsilane (TIS) were used as scavengers. Cleaved peptides were collected by dichloromethane (DCM), which was later removed alongside with TFA by rotary evaporation. Residual materials were triturated by cold diethyl ether. Peptide precipitates were completely separated from ether by centrifugation; the centrifugates were dissolved in water, freezed at -80 °C and lyophilized. Lyophilized peptides were characterized by reverse-phase liquid chromatography system coupled to high resolution time-of-flight mass spectrometer (Agilent 1200/6210). For L- and D-variants of pyrenebutyryl- ϵ -Ahx-VVAGH-Am sequence 864.4791 a.m.u. and 864.4759 a.m.u peaks were registered in mass spectra (Figures 4.16 and 4.18), respectively. For L- and D-variants of lauryl-VVAGH-Am sequence 663.4529 a.m.u. and 663.4533 a.m.u peaks were registered in mass spectra (Figures 4.20 and 4.22), respectively. Purity of the peptides was c.a. 98% (Figures 4.17, 4.19, 4.21 and 4.23).

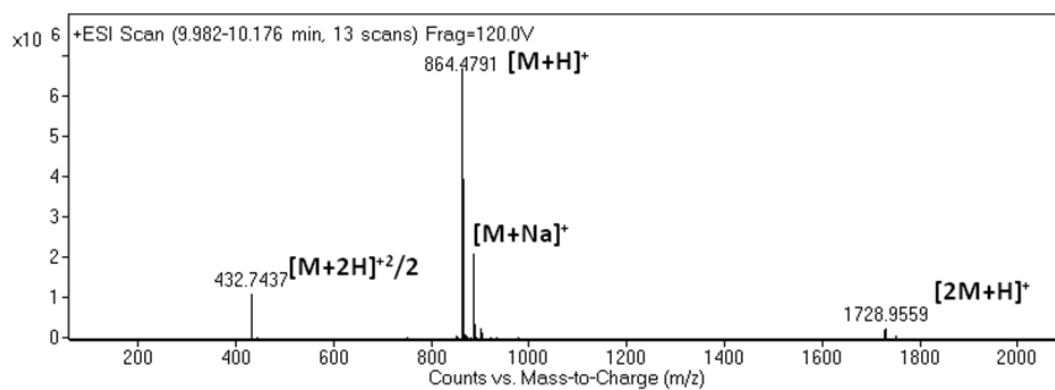


Figure 4.16. Mass spectrum of pyrenebutyryl- ϵ -Ahx-VVAGH-Am.

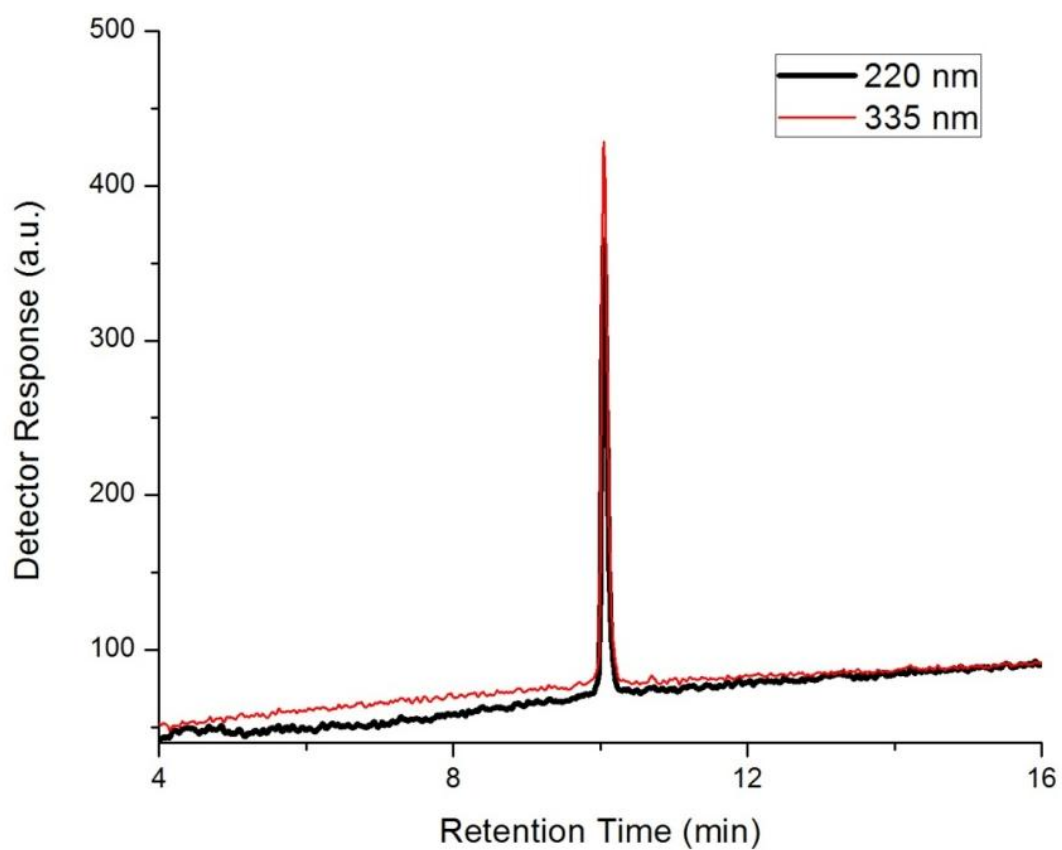


Figure 4.17. Liquid chromatogram of pyrenebutyryl- ϵ -Ahx-VVAGH-Am.

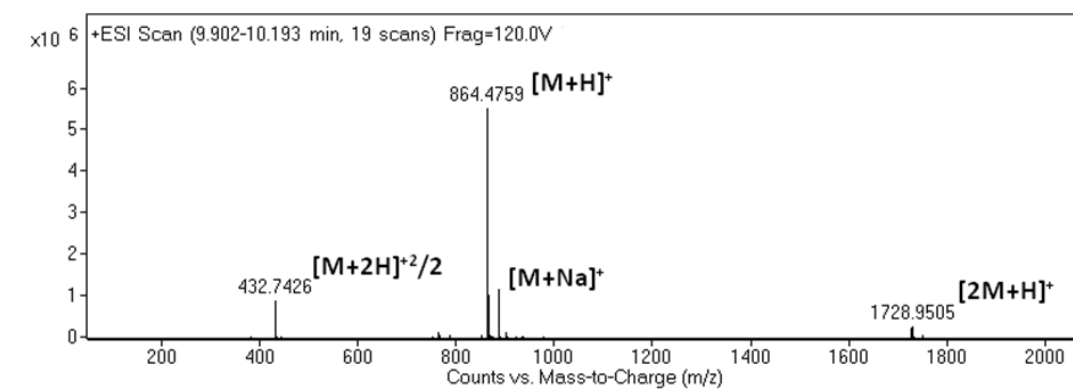


Figure 4.18. Mass spectrum of pyrenebutyryl- ϵ -Ahx-vvaGh-Am.

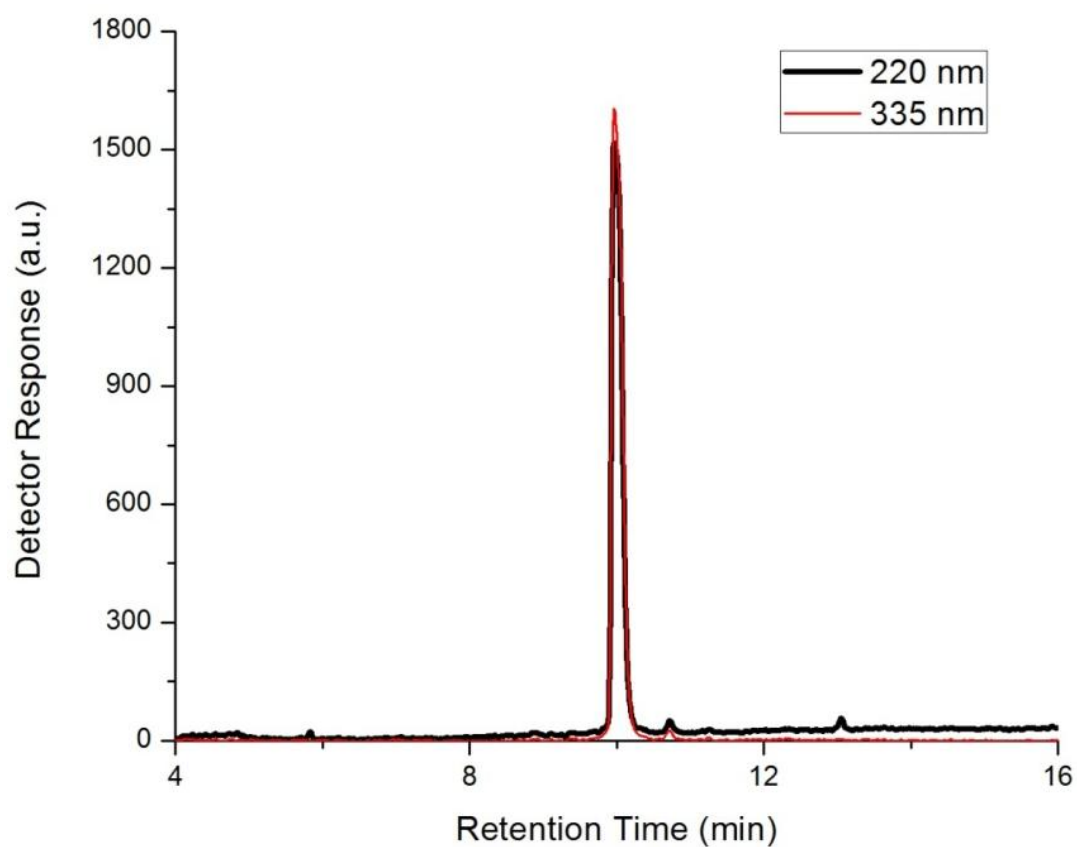


Figure 4.19. Liquid chromatogram of pyrenebutyryl- ϵ -Ahx-vvaGh-Am.

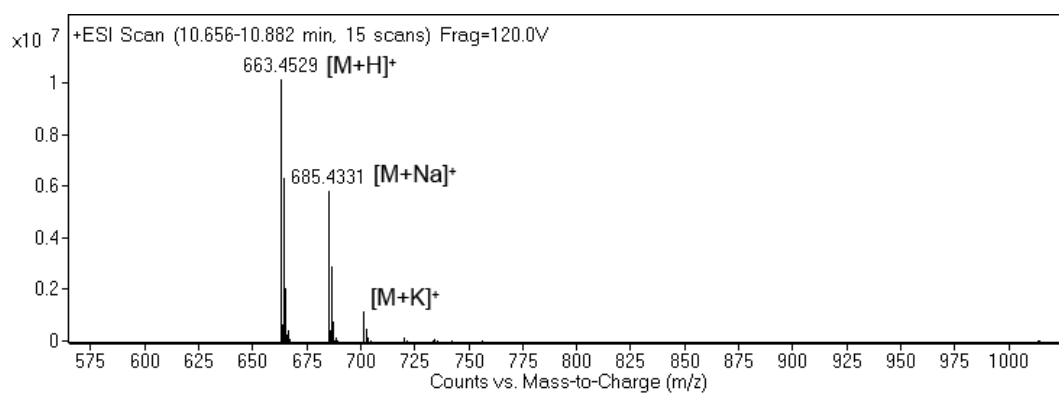


Figure 4.20. Mass spectrum of lauryl-VVAGH-Am.

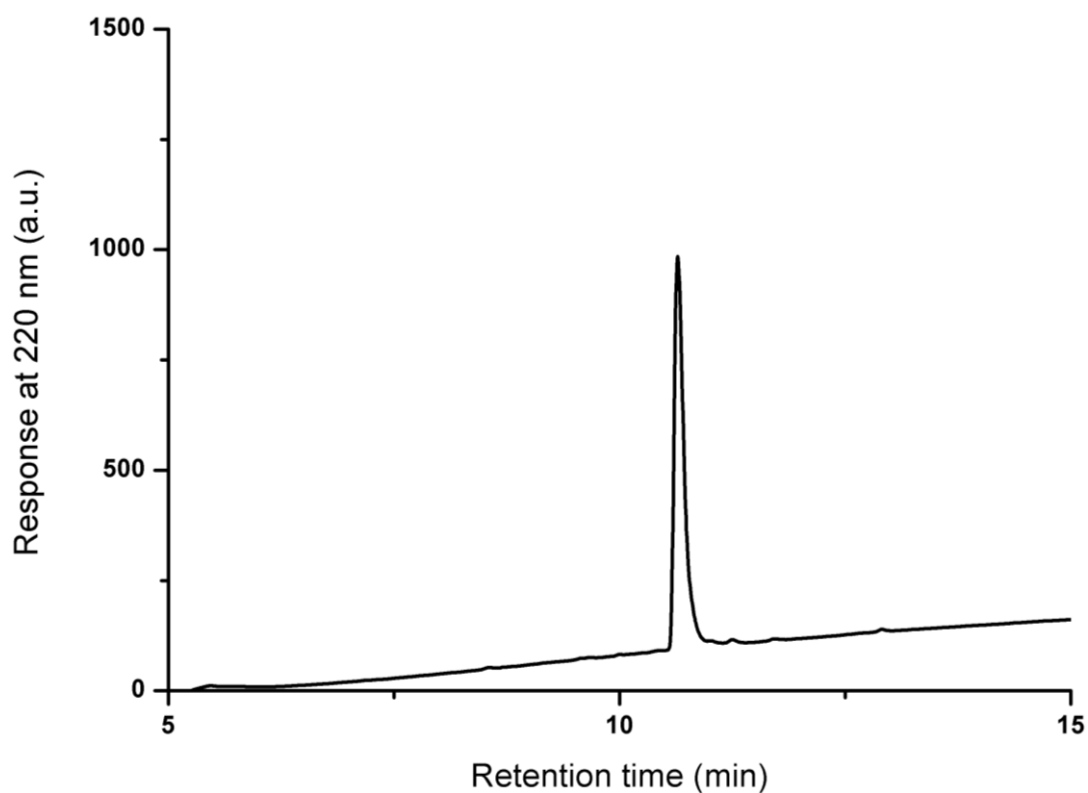


Figure 4.21. Liquid chromatogram of lauryl-VVAGH-Am.

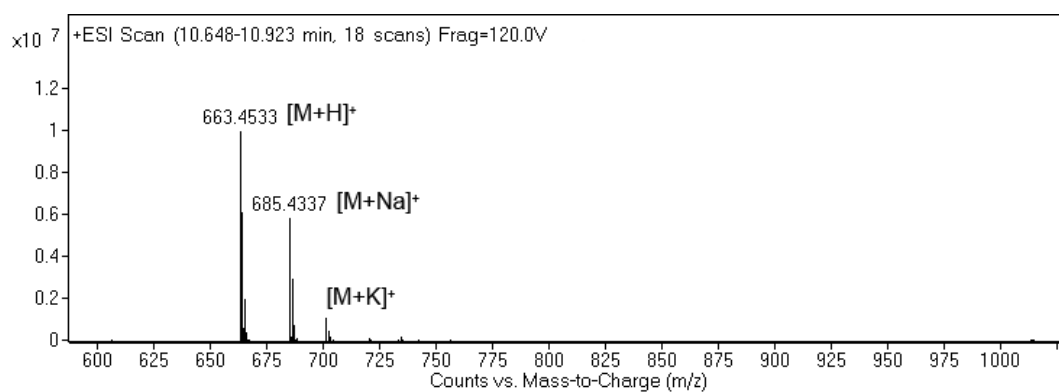


Figure 4.22. Mass spectrum of lauryl-vvaGh-Am.

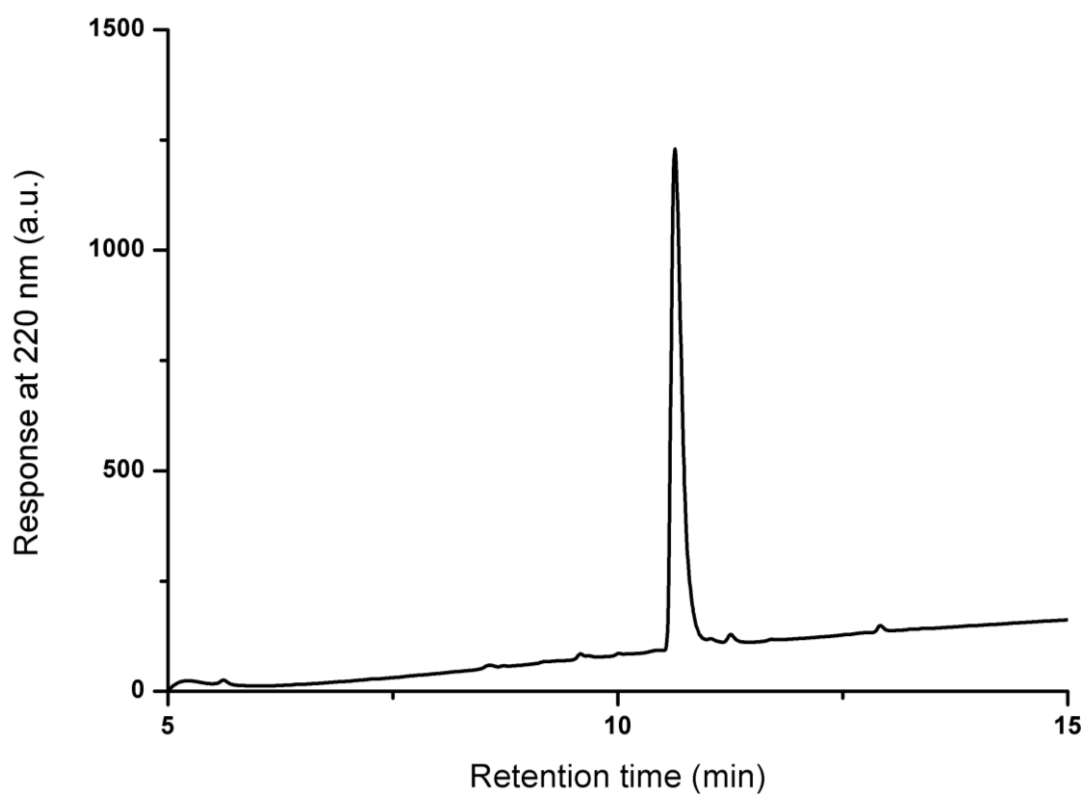


Figure 4.23. Liquid chromatogram of lauryl-vvaGh-Am.

The first strategy used to observe induced CD peaks includes assembly of **1** and **1'** into nanofibers. TEM images suggest nanofiber formation (Figure 4.24); spatial organization of PA molecules throughout nanofiber presumably dictates helical organization of chromophores within hydrophobic core. In order to verify this assumption, emission and circular dichroism spectra were recorded for covalently conjugated pyrene samples (**1** and **1'**). First, from emission spectra it is evident that chromophores are situated in the hydrophobic core; ratio of the vibronic peaks (I_3/I_1) in pyrene spectra is greater than unity (Figures 4.25a and b).¹⁶⁷ Second, stacking of the chromophores is evident from excimer formation at 460 nm in emission spectra of pyrene (Figures 4.25a and b).¹⁶⁸ Induced peaks in CD spectra also point toward spatially chiral organization of the chromophores (Figure 4.26). Furthermore, pyrene conjugated peptides were directly dissolved in trifluoroethanol and emission spectra were recorded (Figures 4.25a and b). It was observed that ratio of vibronic peaks (I_3/I_1) in pyrene emission spectra is less than unity, which means that pyrene moiety is in hydrophilic medium, i.e. solvated in TFE. Absence of excimer peaks also points toward solvated peptides. CD spectra (Figure 4.27a) of the same also suggest that peptide molecules are not assembled as there are no induced CD peaks in pyrene absorption region. The only peak present is the wide peak around 220 nm which shows that β -sheet formation is disrupted and disordered interactions are dominant. Similarly, when the aqueous solutions (3.33 mM) containing self-assembled nanofibers of **1** and **1'** were diluted 10 times with trifluoroethanol (TFE) induced CD signals in pyrene absorption region (230-400 nm) disappeared. Moreover, peak centered at about 220 nm developed, thus representing shift of the system from β -sheet dominant state to random state (Figure 4.27b). Another intriguing observation

was made when absorption spectra of **1** and **1'** in TFE were compared to the spectra of the same in H₂O (Figures 4.28 and 4.29). A bathochromic shift with a value of greater than 10 nm was observed; observed shift is indicative of J-aggregate formation. Bulky amino acid sequence seems to interfere with π - π stacking process and results in slipped stacks.

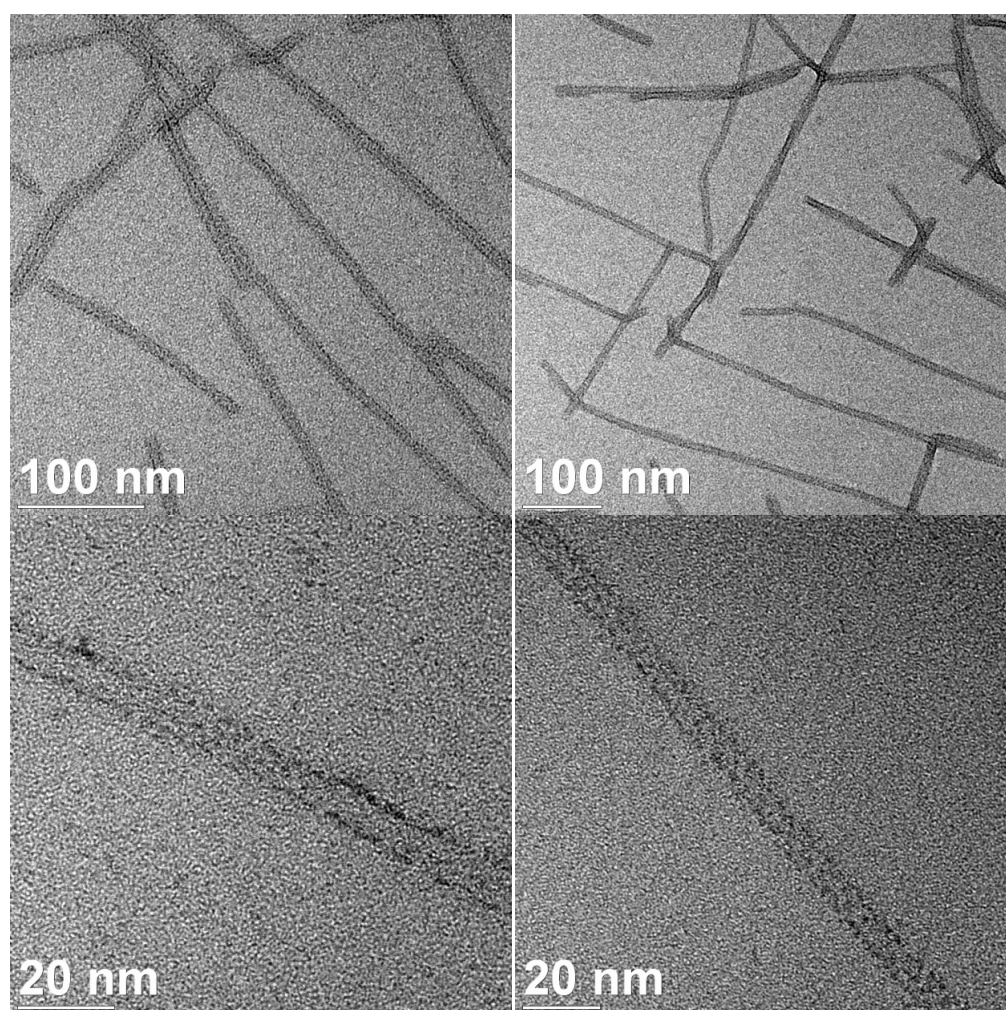


Figure 4.24. TEM images of self-assembled nanofibers of **1**(left) and **1'** (right).

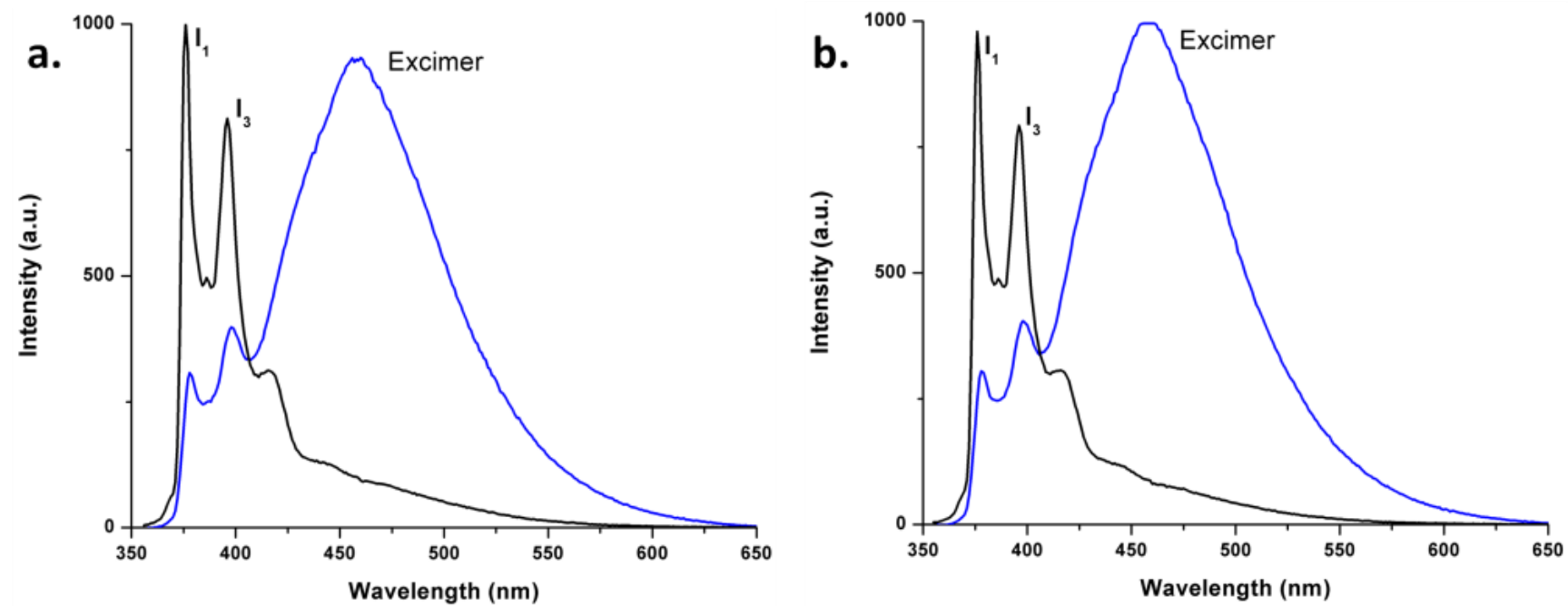


Figure 4.25. Emission spectra of **a. 1** and **b. 1'** in H₂O (blue) and in TFE (black).

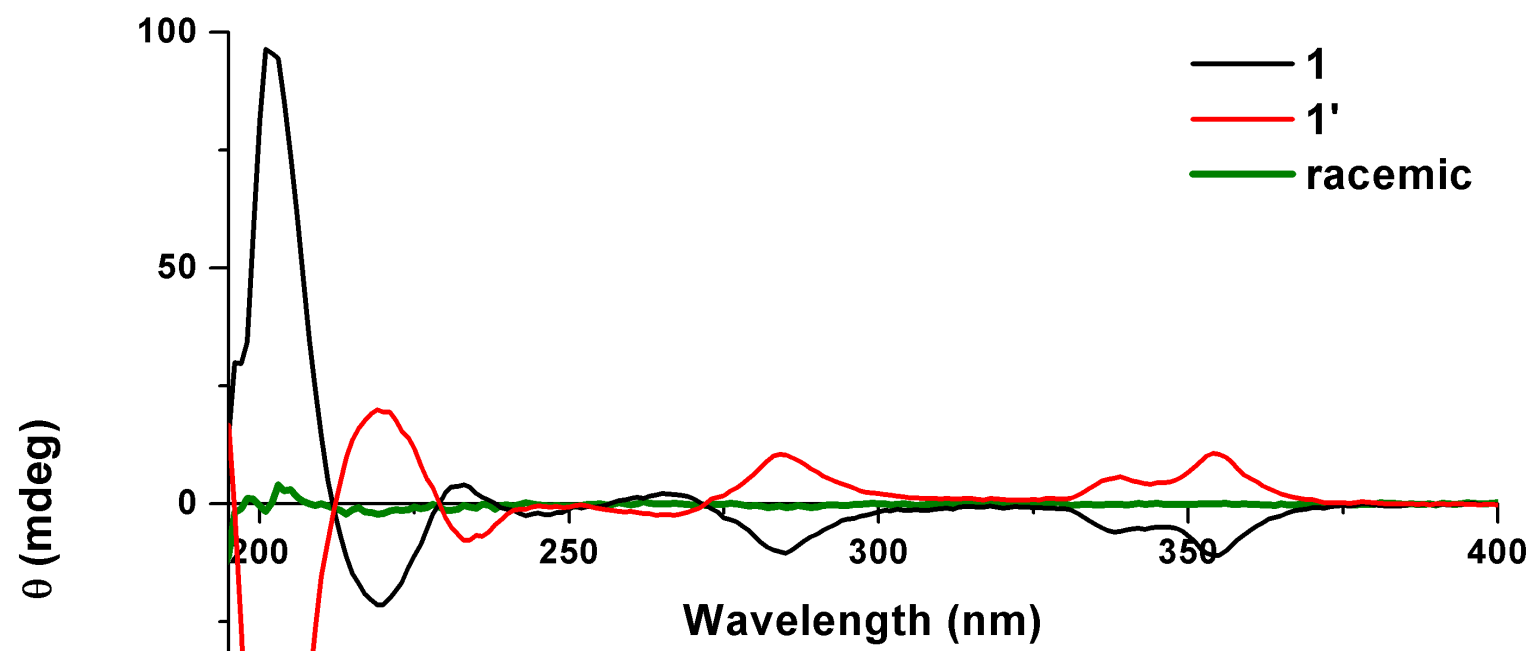


Figure 4.26. CD spectra of self-assembled nanofibers of **1** and **1'** in H₂O (0.333 mM).

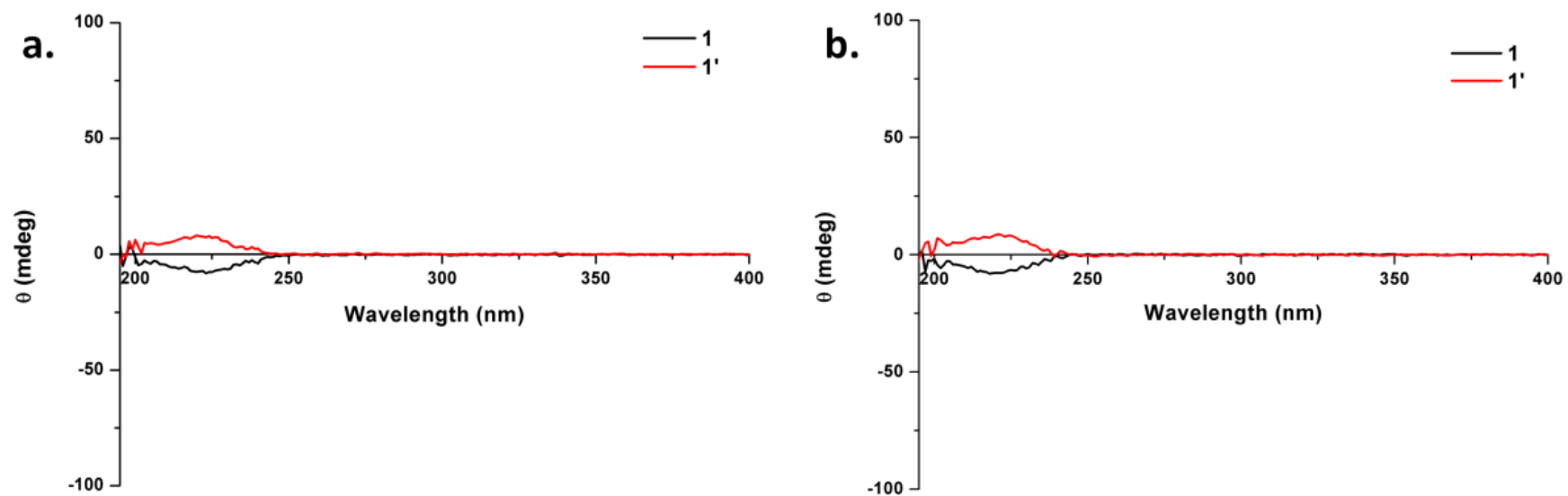


Figure 4.27. CD spectra of **a.** **1** and **1'** directly in TFE (0.333 mM) and **b.** pre-assembled **1** and **1'** in TFE (0.333 mM).

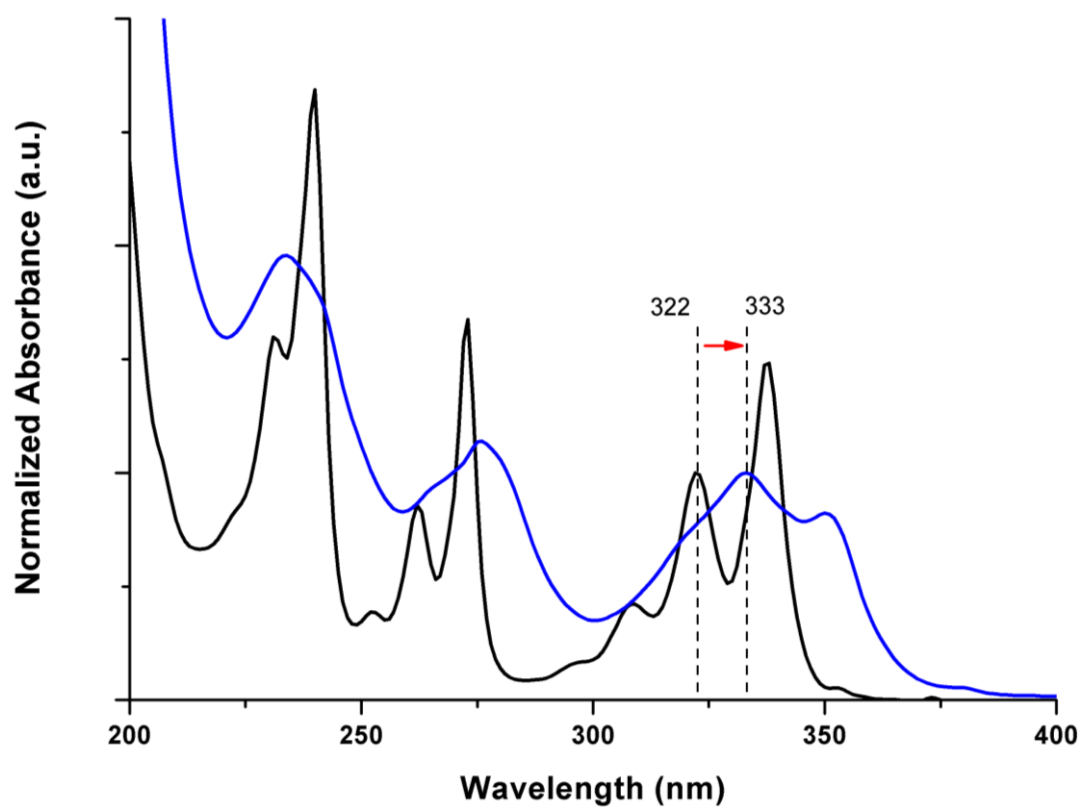


Figure 4.28. Absorption spectra of nanofibers of **1** in H₂O (blue) and **1** dissolved in TFE (black). The same holds for **1'** as well (see Figure 4.29).

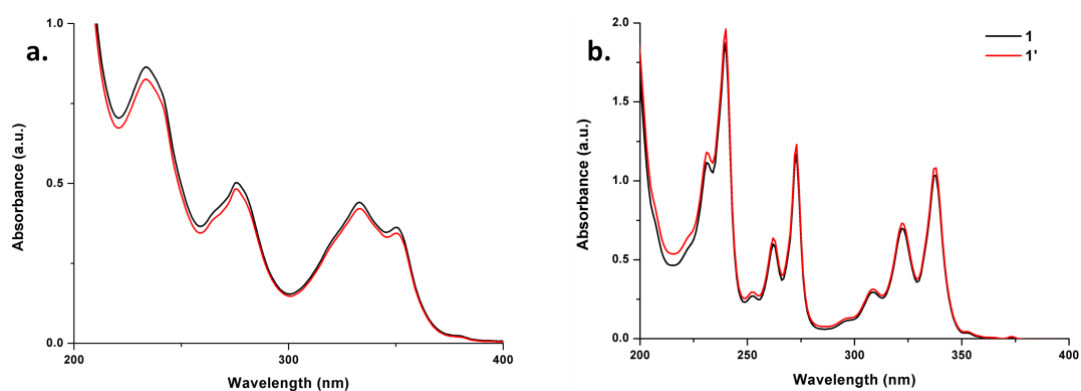


Figure 4.29. Absorption spectra of **a.** nanofibers of **1** and **1'** in H₂O and **b.** of **1** and **1'** dissolved in TFE (pyrene absorption region).

According to the second strategy, encapsulation of pyrene in nanofibers of **2** and **2'** (Figure 4.30) is expected to give induced CD peaks in pyrene absorption region. Indeed, chiral interior of nanofibers impacts organization of the chromophore molecules. Induced CD peaks with opposite signs are observed for pyrene encapsulated in nanofibers of **2** and **2'** (Figure 4.31), β -sheet signals stemming from peptide interaction are also registered (Figure 4.32). CD spectra of pyrene in THF did not exhibit any induced chiral peaks (Figure 4.33). Moreover, emission spectra (Figure 4.34) of encapsulated pyrene systems showed similar results to pyrene conjugated systems. In other words, ratio of vibronic peaks (I_3/I_1) is greater than unity and excimer peak is present at 460 nm. On contrary, pyrene in THF did not show excimer emission (Figure 4.35). Absorption spectra of encapsulated pyrene exhibits slight red-shift (Figure 4.36), which is indicative of J-aggregates. Based on these observations it can be inferred that encapsulated chromophore is forced to acquire chiral 3-D arrangement throughout the nanofiber. Induced helical organization of the system, be it left- or right-handed, is arguably the best 3-D arrangement that accounts for observed induced peaks in CD spectra.

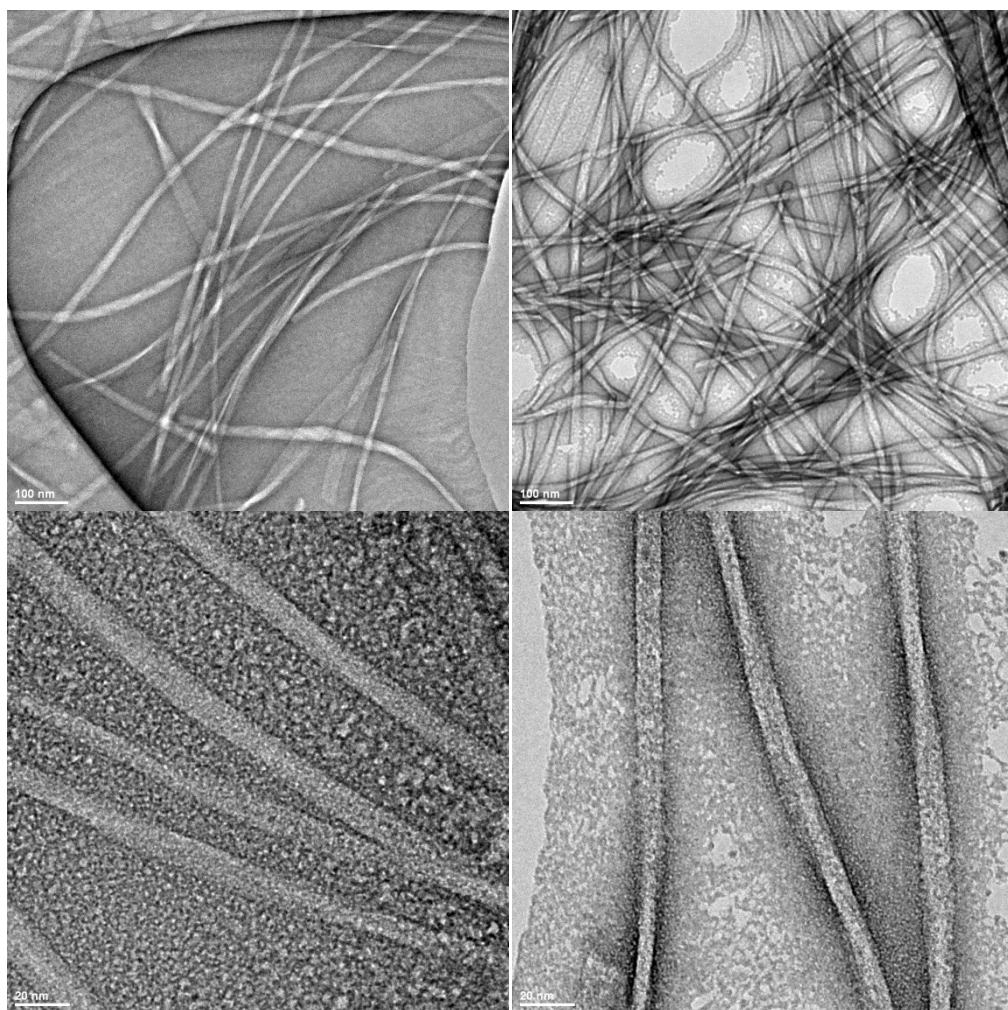


Figure 4.30. TEM images of nanofibers of **2** (left) and **2'** (right) with encapsulated pyrene.

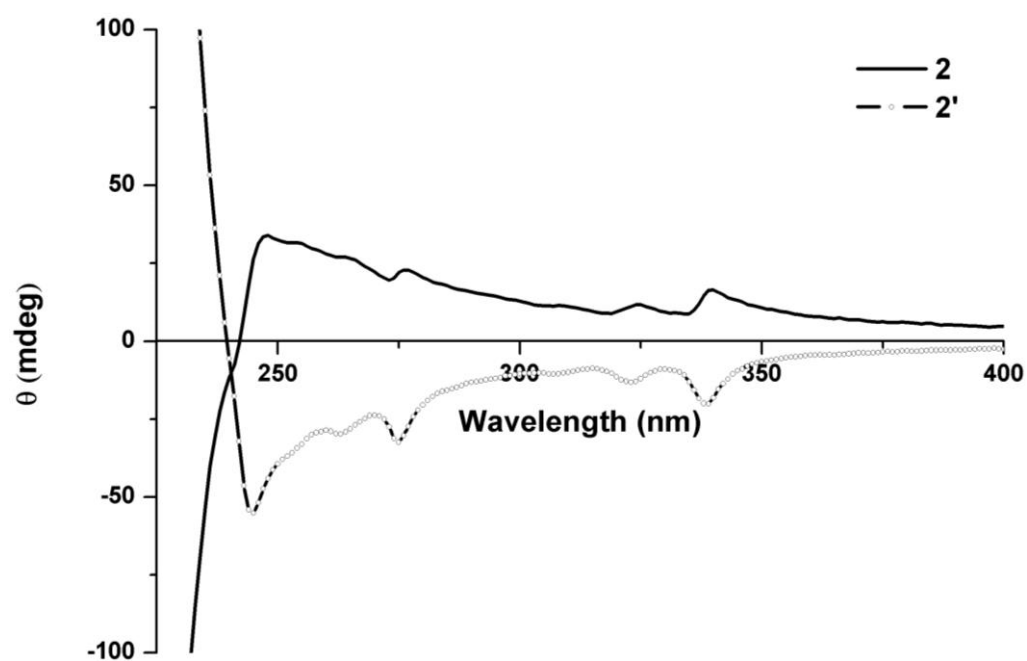


Figure 4.31. CD spectra of pyrene in nanofibers of **2** and **2'** in H₂O (pyrene region).

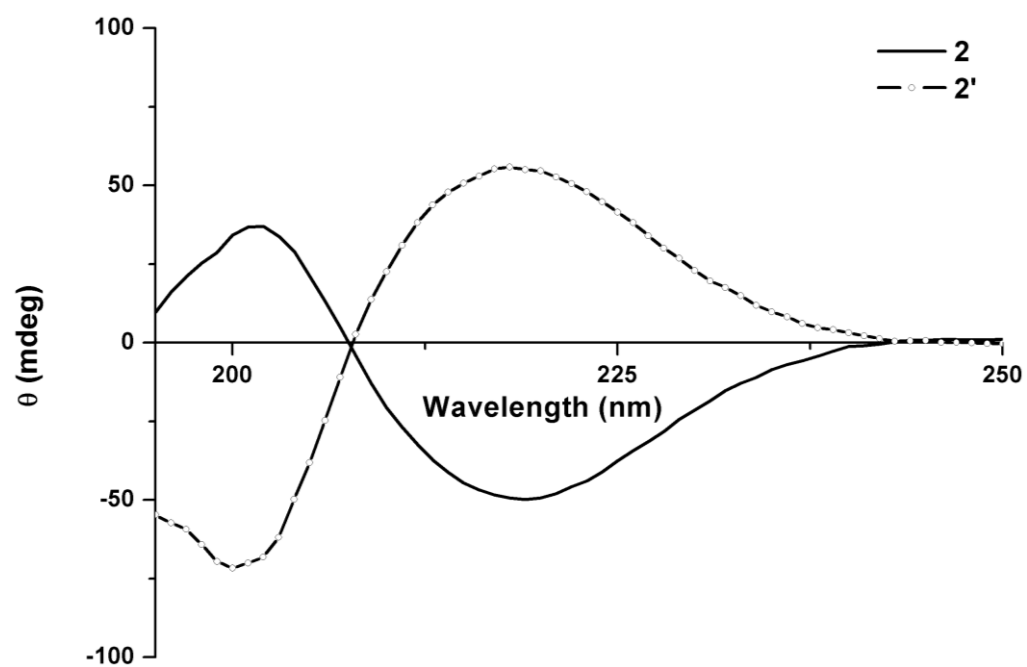


Figure 4.32. CD spectra of **2** and **2'** nanofibers with pyrene (β -sheet region).

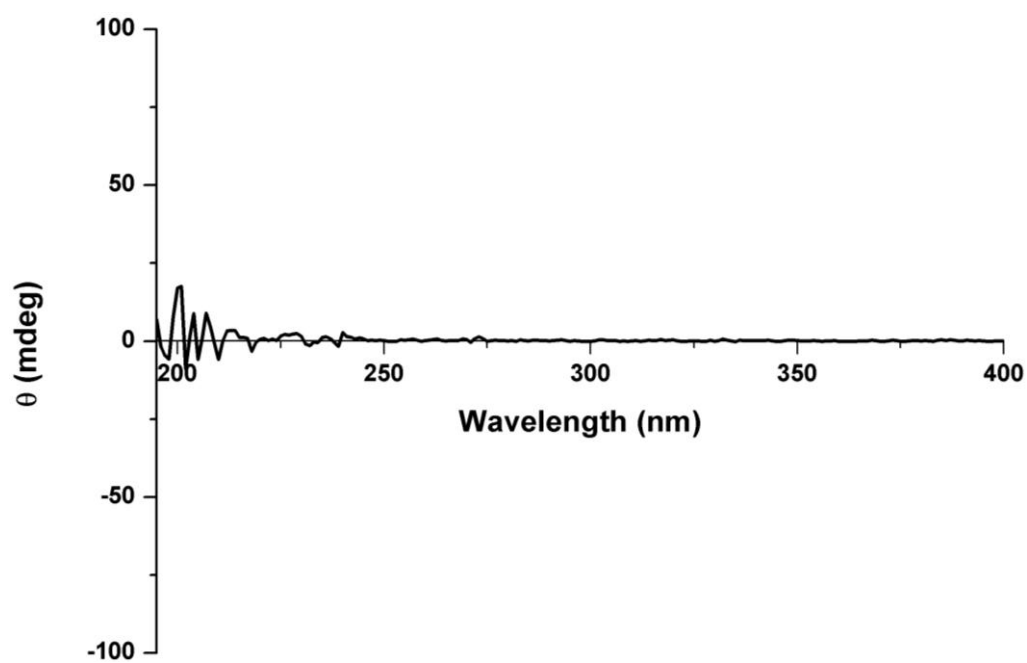


Figure 4.33. CD spectrum of pyrene in THF (0.333 mM).

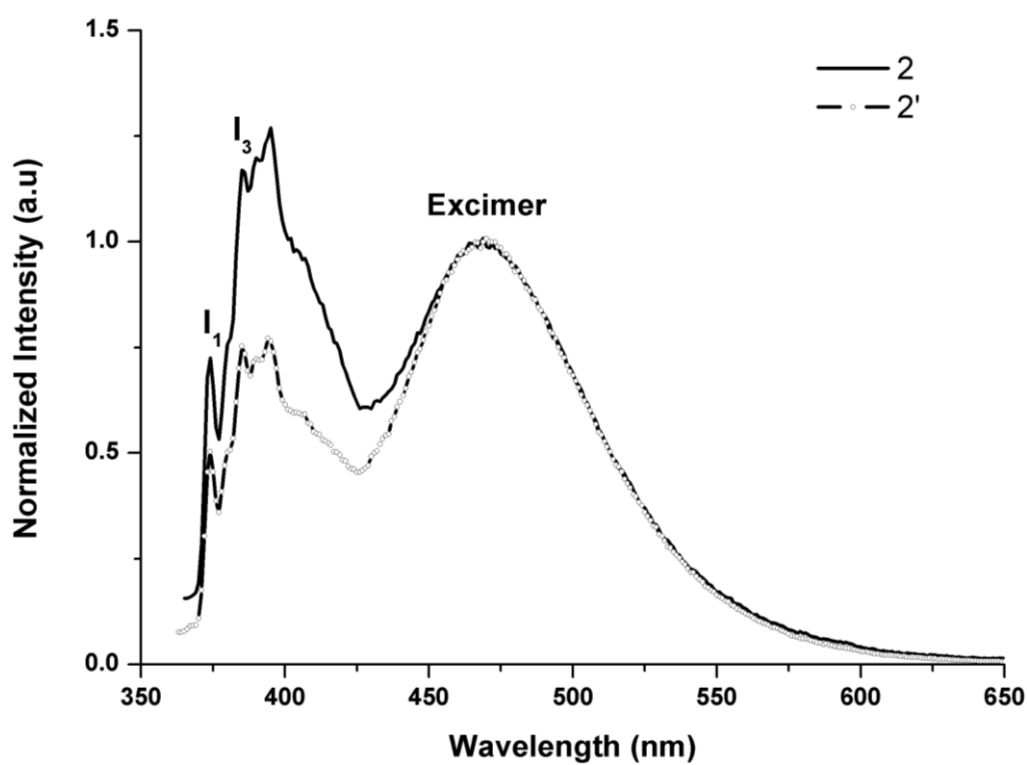


Figure 4.34. Emmission spectra of pyrene in nanofibers of **2** and **2'** in H₂O (0.333 mM pyrene).

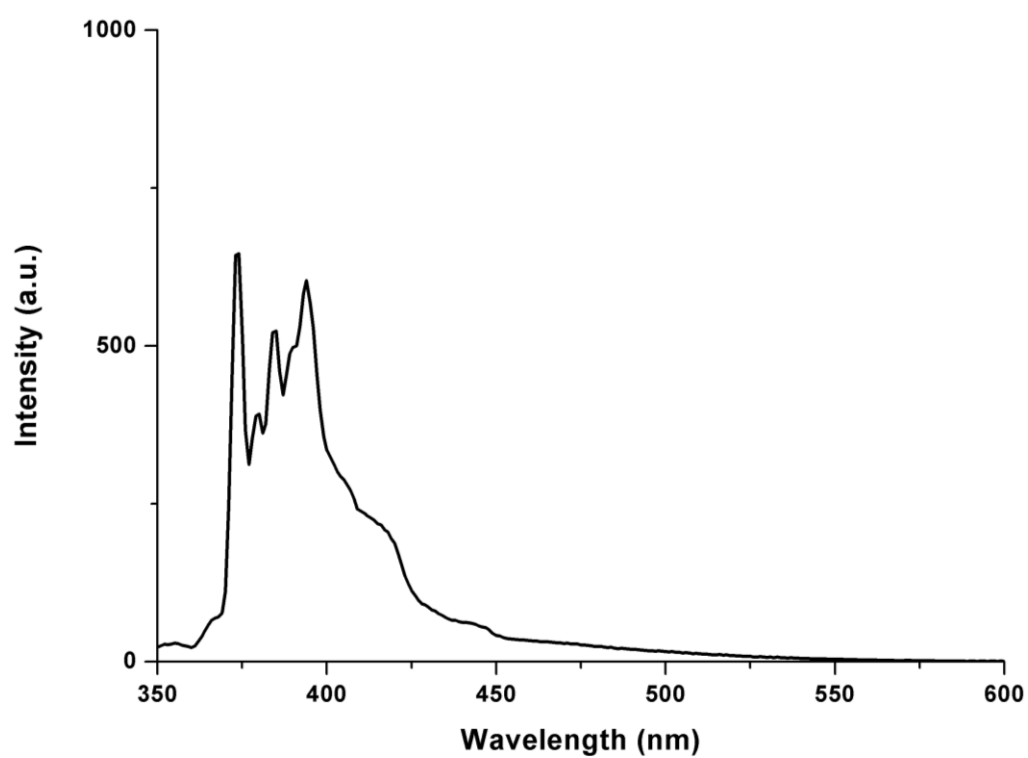


Figure 4.35. Emission spectrum of pyrene in THF (0.333 mM).

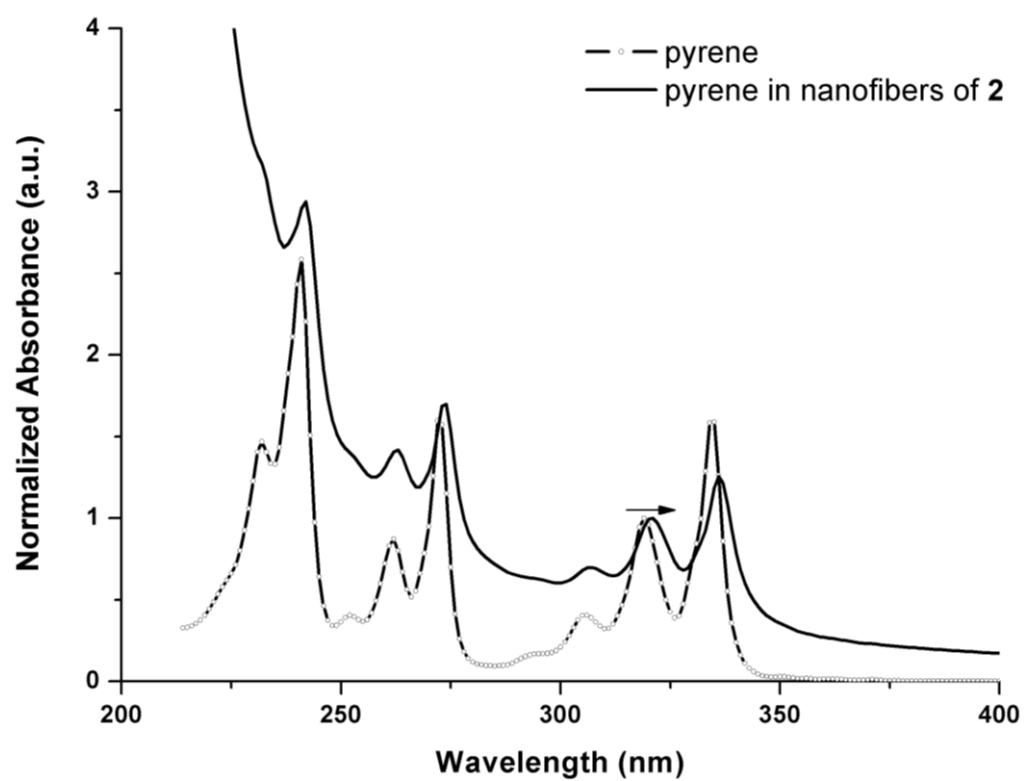


Figure 4.36. UV-Vis spectra of pyrene in THF and pyrene encapsulated in **2** in H₂O.

4.2.3 Conclusion

Both of the strategies lead to induction of chiral signals. CD spectra along with emission spectra suggest that chromophores are organized in helical manner in the core of nanofibers. CD spectra exhibits chiral signals in pyrene absorption range and, thus suggest chiral organization of the chromophores. Emission spectra containing pyrene excimer peak suggest chromophore stacking; moreover, ratio of vibronic peaks in the same spectra suggests that the chromophores are situated in hydrophobic environment, presumably hydrophobic core of peptide nanofiber. Based on the observations discussed above, mutual orientation of the chromophores in helical manner is arguably the best explanation for chirally stacked chromophores throughout nanofiber core.

The most intriguing part of the study is that induced chiral peaks in CD spectra could be switched by changing peptide sequence from all-L to all-D design, thus establishing control over photophysical properties of the chromophore. In addition, systems with encapsulated chromophores represent an approach which utilizes non-covalent interactions and is mainly based on physical restrictions within supramolecular cavitand.

Chapter 5.

5. Conclusion and Perspectives

Peptides are unique and versatile molecules. Peptide function can be tuned and adjusted according to the task to be achieved. Peptides present a design platform with a rich toolbox which includes covalent and non-covalent interactions. Non-covalent interactions are the most interesting part, because it endows peptides with enormous flexibility. Electrostatic interactions, H-bonding, π - π interactions, solvophobic/solvophilic effects and van der Waals forces give an opportunity to design highly sophisticated systems, and thus achieve programmed assembly. Through programmed assembly supramolecular nanostructures with a desired degree of hierarchical complexity can be constructed.

Peptides are real ‘workhorses’, because peptide-based supramolecular architectures are used as templates in inorganic material growth, extracellular matrices in tissue regeneration, interfacing materials in prosthesis integration and artificial enzymes in reaction catalysis.

In my own studies I have worked on fabrication of nanostructured materials such as TiO_2 , ZnO , SiO_2 and Pd. My main focus was on synthesis of peptide-templated TiO_2 nanonetworks and their utilization in photon-to-electron conversion devices, namely dye sensitized solar cells. It was shown that peptide-templated materials provide enhancement in solar cell parameters compared to devices with template-free materials. Further enhancement in photon-to-electron conversion can be achieved by engineering of material surface. Nanostructured titania offers great surface area,

which may increase electron-hole recombination number per unit area. Applying an extra blocking layer of ZnO or HfO₂ may substantially increase overall efficiency.

Development of hybrid peptide-polymer materials is also an interesting field of study. Development of easy polymer functionalization techniques is crucial for this field. In one of our studies we have achieved non-covalent functionalization of HP β CD polymer mesh by phytochelatin mimetic peptide. Functionalization technique is based on host-guest interactions between β -cyclodextrin and adamantyl group. Hybrid peptide-polymer system allowed for heavy metal ion scavenging from water. Fabrication of more sophisticated peptide-polymer systems is possible.

Peptides are intrinsically chiral molecules, and therefore their ensembles demonstrate supramolecular chirality as well. Properly designed peptides can be used to induce chiral organization of achiral chromophores, thus controlling photophysical properties of the later ones. Aliphatic peptide amphiphiles were used to observe induced supramolecular chirality with pyrene chromophore. Aliphatic peptides possess hydrophobic tails, which are fatty acid conjugated to main sequence; this hydrophobic tails upon PA assembly provide hydrophobic medium where hydrophobic chromophores can be encapsulated and organized. In our experiments, we have observed that PAs are capable of inducing supramolecularly chiral order; observed order manifests itself in emergence of phenomenon like circular dichroism. Achiral pyrene molecules start to unequally absorb right and left circularly polarized light, giving rise to elliptically polarized light which is registered as CD peaks. CD induction can be actively used to study supramolecular structures. Moreover, based on whether PA with all-L or all-D sequence was used sign of induced CD peaks can

be switched. Encapsulation of a chromophore in PA nanofibers can have several impacts on photophysical properties. For instance, as we observed in one of our studies highly fluorescent zinc phthalocyanine derivative upon encapsulation ceased to fluoresce and gave rise to ultrafast energy transfer phenomenon.

In conclusion, peptide design and its application areas are enormously extensive. Peptides offer unique possibilities in materials science as a versatile tool allowing fabrication of nanostructured materials.

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