Chapter 3

Surface Plasmon Coupled Circular Dichroism of Au Nanoparticles on Peptide Nanotubes

Seeding

$\text{hv}$ HAuCl$_4$

L-isomer

D-isomer
3.1 Abstract

Peptide nanotubes (PNTs) of D and L-isomers of diphenylalanine were prepared and characterized by different spectroscopic and microscopic techniques. These nanotubes were found extremely stable to temperature, pH and photoirradiation. Gold nanoparticles were seeded on to the surface of PNT which act as the nucleation sites for the growth of new nanoparticles. Formation of nanoparticle assemblies were observed as bunches on PNTs, which showed bisignated CD signal at their surface plasmon frequency. Interestingly, the Au nanoparticle assembly on the surface of the D- and L-isomers of peptide nanotube showed positive and negative couplets, respectively. Mirror image relationship in the CD spectra clearly indicates that the chiral molecules on the surface of nanotubes drive the asymmetric organization of nanoparticles in two different ways.

3.2 Introduction

Design of hybrid nanostructures with a specific geometry, composition and function is one of the major challenges in the field of nanotechnology. One of the convenient methods to organize molecules into nanomaterials is through self-assembly [Ortega Lorenzo 2000]. The self-assembly of biological systems like peptides and proteins, into regular supramolecular structures has important applications in drug delivery and bioengineering [Yan 2008]. Peptide based nanomaterials have attracted considerable attention due to their biocompatibility, capability of molecular recognition, and well-defined structures. First demonstration of peptide based nanotubes was by Ghadiri et al. [Bong 2001] that a cyclic octa-peptide with alternating D- and L-isomers of amino acid can form tubular structure of nanoscale dimension by self-assembly. However, the peptide nanotubes that formed further assembled into an array of tubes that were aligned as fused crystalline microscopic-
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Figure 3.1. Self-assembly of well-ordered and elongated peptide nanotubes by a molecular recognition motif derived from the β-amyloid polypeptide. (A) TEM images of the negatively stained nanotubes formed by the diphenylalanine peptide. (B) HR-TEM images of negatively stained peptide nanotubes, visualized by field emission gun microscope [Reches 2003].

structures. Gazit et al. [Reches 2003] has reported the self-assembly of a very short peptide, the Alzheimer’s β-amyloid diphenylalanine structural motif, into discrete and stiff peptide nanotubes (PNT). The formed structures were highly organized and appeared to be stiff but without the usual branching and curving, typical of amyloid fibrils (Figure 3.1). Formation of PNTs is an irreversible process, like the denaturation of β-amyloid proteins to fibrils, responsible for (i) Alzheimer’s disease, (ii) type II diabetes and (iii) prion diseases [Gazit 2002] (vide infra). PNT shows remarkable thermal (121°C, 1.2 atm) and chemical stability on treatment with ethanol, methanol, 2-propanol, acetone, and acetonitrile and in acidic (10% TFA) to alkaline (1 M NaOH) conditions. PNTs possess complete stability towards extensive boiling with ionic silver solution and completely retain their ultrastructure in wet or dry conditions [Reches 2003]. Introduction of a thiol group into the PNT allow covalent attachment to gold fabricated electrodes in nanodevices [Kol 2005].

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Figure 3.2. A model for alternative assembly of tubular and spherical peptide nanostructures [Reches 2004].

Chemical modification of diphenylalanine has important consequences on their morphological properties, for e. g., the cysteine modified diphenylalanine does not self-assemble into nanotubes but rather forms nanospheres [Reches 2004]. A stacking interaction between aromatic moieties of the peptides is suggested to provide energetic contribution as well as order and directionality for the initial interaction to form an extended pleated sheet that is stabilized by hydrogen bonds and aromatic stacking interactions. The formation of the tubular structures may occur by a closure of the extended sheet along one axis of the two-dimensional layer. Alternatively, the formation of spherical structures may result from a
closure of the sheet along two axes. The introduction of a thiol group may assist the closure at the second axis (Figure 3.2). The energetic contribution provided by the disulfide bridge formation may allow closure of the two-dimensional layer into more closely packed spherical structures. Thus the differences in the geometrical constraints between the short peptide, direct the mode of assembly. It was reported that linker peptides can bind gold nanoparticles to the surface of peptide nanotubes, through the association of weak hydrogen bonds or by chemical modification [Carny 2006]. The key attractions to use PNT surface as a template for nucleation of nanoparticles are their biological compatibility and remarkable thermal and chemical stability.

Nature often adopts biomolecular templates for creating inorganic structures. More interesting is the transfer of asymmetry of chiral templates at the molecular level into asymmetry at the nanometres, micrometres and microscopic level which is still poorly understood. There are many examples of chiral shapes in biologically formed minerals, and a lot can be learned about how they are created, by studying the information about chirality transfer from molecules to crystalline surfaces [Addadi 2001]. Chiral molecules can induce optical activity in achiral chromophores and electronic circular dichroism (CD) has been used to investigate chirality transfer in biomolecular systems [Berova 1997]. Biomineralization of silver nanoparticles on a chiral poly(dG)-poly(dC) double stranded DNA scaffold induces chirality at the silver particle surface plasmon resonance (SPR) frequency (Figure 3.3). In-situ nucleation of Ag nanoparticles with the pre-complexed Ag\(^{+}\) cations on DNA surface induces chirality. Ag nanoparticle formed on DNA scaffold shows the CD spectrum intact for hours, indicating relatively high kinetic stability. Bisignated CD
Figure 3.3. (A) CD spectra measured on Ag nanoparticles grown directly on the DNA; (B) TEM image of Ag nanocrystals grown on the DNA template and the corresponding schematic representation showing the organization of Ag nanoparticles on DNA template [Shemer 2006].

signal with a positive followed by negative couplet with a zero cross-over at the wavelength maximum in the SPR region of the Ag nanoparticles indicate an induction of the chirality from the DNA scaffold and/or the chiral plasmon coupling between the nanoparticles [Shemer 2006]. A reversal in the CD signal is not possible with the present method since all the naturally and synthetically prepared stable DNA templates are of M-form and can only provide a chiral induction as shown in Figure 3.3.

Post-synthetic modification of gold nanoparticles with calix[4]arene enantiomers 2a and 2b exhibits a circular dichroism (CD)-active SPR band [Ha 2009]. Calix[4]arene 2a and 2b capped gold nanoparticles exhibit two characteristic bands in the CD spectrum corresponding to (i) the calixarene ligand’s asymmetric π-π* transition in the 270-300 nm window, and (ii) the SPR band in the 460-600 nm window (Figure 3.4). The ellipticities of CD bands in these two regions systematically increase with addition of chiral calixarene
Figure 3.4. (A) Structure of aminocalixarene enantiomers (2a and 2b); (B) selected CD results of TOAB-stabilized gold nanoparticles (black), gold nanoparticles capped with 160 μM (red) of 2a and 2b, respectively. The concentrations of Au atoms and TOAB were 450 μM and 8 mM, respectively [Ha 2009].

ligand, and are zero for gold nanoparticles free of calixarene. A mechanism responsible for the CD-active SPR bands is proposed, which is based upon the influence of the asymmetric center of the chiral adsorbate on the electronic states of the metal nanoparticle core. One of the limitations of this chiral modification is the very low intensity of the CD signal, due to weak electronic interaction between post-synthetically modified chiral adsorbate and the metal core.

Recent studies have shown that the inorganic nanoparticles having chiral molecules in their vicinity show optical activity. In all the systems, except the report on Ag nanoparticles grown on double stranded DNA, nanoparticles are capped with chiral ligands. Significant progress has been made in the design and study of such chiral nanoclusters, but chirality transfer from surfaces with reduced elements of symmetry to nanomaterials is not
well understood. Herein, we report the *in-situ* growth of Au nanoparticles on D- and L-diphenylalanine peptide nanotubes and their chiroptical properties.

### 3.3 Result and discussion

The objective of the present investigation is to grow metal nanoparticles on the surface of peptide nanotubes (PNTs) prepared from their D- and L-isomers and investigate the chiral optical behavior of the assembly. PNT selected for the current study is obtained by the self-assembly of a very short peptide, the diphenylalanine structural motif (*vide supra*). It is reported that diphenylalanine based molecular systems self-organize to form homogeneous nanotubular assemblies. Molecular structure of D- and L-isomers of diphenylalanine used for the preparation of PNT is presented in the **Chart 3.1**.

![Chart 3.1. Structure of D- and L-diphenylalanine enantiomers](image_url)

L-isomers of diphenylalanine were prepared through the sequence of reactions shown in **Scheme 3.1**. The carboxylic and amino groups of L-phenylalanine (1) were protected using acetyl chloride and 9-fluorenylmethyloxycarbonyl-N-hydroxysuccinimide (Fmoc-OSu), in two successive steps, to yield compound 3 which was further hydrolyzed to yield Fmoc-N-phenylalanine acid (4). The diphenylalanine derivative 5 was synthesized by reacting Fmoc-N-phenylalanine acid, isobutylchloroformate and compound 2 by following a reported procedure [Sladojevich 2007]. L-isomer of diphenylalanine was prepared by
Scheme 3.2. (i) MeOH, CH₃COCl, 70°C, 3 h; (ii) fmo-OSu, THF, NaHCO₃, r. t., 12 h; (iii) 5 N HCl, THF, r. t., 18 h; (iv) isobutylchloroformate, Et₃N, THF, r. t., 6 h; (v) 5 N HCl, THF, r. t., 18 h; (vi) piperidine, DMF, r. t., 6 h.

the hydrolysis of compound 5 with 5 N HCl and followed by deprotection of f-moc group by piperidine. All the derivatives were purified by passing through silica gel column. Lyophilized form of (S,S)-L-FF is used for the preparation of self-assembled PNTs. Details about the synthesis and characterization of intermediates and final compounds are presented in Section 3.5.2.

3.3.1 Preparation of diphenylalanine peptide nanotubes (PNTs)

Peptide nanotubes of D- and L-isomers of diphenylalanine were synthesized by following a reported procedure [Reches 2003]. Fresh stock solution was prepared by dissolving the lyophilized form of the peptide in 1,1,1,3,3,3-hexafluoro-2-propanol (100 mg/mL, 320 mM) which was further diluted to 3.2 mM (1 mg/mL) in water. The peptide
Figure 3.5. FTIR spectrum of L-diphenylalanine peptide nanotubes in aqueous medium recorded using the ATR facility (the broad nature of amide I band at 1630 cm$^{-1}$ corresponds to β-sheet structure during peptide nanotube formation).

appeared to be highly soluble in 1,1,1,3,3,3-hexafluoro-2-propanol, a rapid assembly of diphenylalanine into a faint white turbid solution was observed visually within seconds after the dilution into aqueous solution at a final concentration of 3.2 mM. Fourier-transformed infrared spectroscopy (FTIR) was used to understand the molecular configuration of the assembled structures. Spectral analysis of the assemblies showed a sharp peak at 1630 cm$^{-1}$ representing the amide I band (Figure 3.5). This peak is due to the β-sheet like conformation of the single amide bond, as was suggested for peptide nanotubes built of larger building blocks [Bong 2001, Reches 2003]. The dipeptide contains only a single amide bond and the vibrational assignment of this peak gives conclusive evidence for β-sheet conformation (it does not represent an averaged contribution of several bonds, as occurs when longer peptide assemblies are analysed). Moreover, no other vibrational peaks are observed (such as random coil peak around 1654 cm$^{-1}$), which further suggest a uniform conformation of all the building blocks.
UV-Visible spectroscopy suggests that both the isomers of PNT possess absorption at 258 nm and a high extinction band at 220 nm (Figure 3.6A). The characteristic band at 258 nm is due the $\pi-\pi^*$ transition of benzene rings in the diphenylalanine after aggregation. The peak around 220 nm in the CD spectra was used to compare the secondary structure of the nanotubes (Figure 3.6B). The mirror image relationship in the CD signal for D- and L-isomer of PNTs indicates opposite conformation of the building units in the peptide nanostructures. Freshly prepared peptide stock solution (320 mM) was diluted to final concentration of 3.2 mM (1 mg/mL) in double distilled water. Then a 10 µL aliquot of 1 day aged solution of PNT was placed on 400 mesh copper grid, dried overnight. Transmission electron microscopy (TEM) analysis of the above sample indicates that the peptide forms tubular structures which are well-ordered and elongated (Figure 3.7). Almost all the assemblies as observed by TEM had tubular structures with negligible amorphous
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Figure 3.7. (A) Low and (B) high resolution thermionic emission gun TEM images of PNT formed after one day of aging. Inset of (A) shows a large scale image of the PNT aggregates (<1%). High-resolution thermionic emission gun TEM (HR-TEM) provided further indication of the regular structures of the tubular walls (Figure 3.7B).

PNT shows remarkable chemical stability to alkaline pH (10) and photoirradiation for 3 h (vide supra). The tubes retain their strong absorption at 258 nm upon increasing the pH from 7 to 10, followed by photoirradiation at 300 nm in a Rayonet photochemical reactor for 3 h (Figure 3.8A). Functional groups on the surface of peptide nanotube exist in its deprotonated form at pH 10. A decrease in the intensity of the CD signal was observed for both D- and L-isomers of peptide nanotubes on varying the pH from 7 to 10 (Figure 3.8B) which may be due to the formation of carboxylate ion on the surface of PNT. No changes in the CD intensity were observed on irradiating the solution of peptide nanotube at pH 10 for 3 h and the possibility of any deformation is ruled out. These aspects were further confirmed through TEM studies (Figure 3.9).
Figure 3.8. (A) Absorption and (B) circular dichroism (CD) spectra of PNT at (a) pH 7 (black trace), (b) pH 10 (red trace) and (c) pH 10 on photoirradiation for 3 h (blue trace). Subscripts 1 and 2 denote L- and D-isomers of PNT, respectively (note that the traces b and c of L- and D-isomers are merged together in the CD spectra).

Figure 3.9. TEM images of PNT after photoirradiation at pH 10.
3.3.2 Characterization of seed Au nanoparticles

Triethylene glycol thiol protected Au nanoparticles (EG₃-S-Au) with ~4.5 nm diameter were synthesized and characterized and the details are presented in Section 2.5.3. Gold nanoparticles protected with triethylene glycol thiol, possess a characteristic surface plasmon absorption band centered at 510 nm (Figure 3.10A). The plasmon absorption in Au nanoparticles originates from the interaction of external electromagnetic radiation with the highly polarizable Au $5d^{10}$ electrons of gold atoms [Hostetler 1998]. X-ray diffraction studies can provide information about the average size and structure of Au colloidal crystals.

![Figure 3.10](image)

*Figure 3.10. (A) Absorption and (B) Powder XRD spectra of ethylene glycol thiol protected Au seed nanoparticles.*

*Figure 3.10B* shows the powder X-ray diffraction pattern in a wide-angle range recorded from the colloidal crystals. Debye-Scherrer equation was used for calculating the average size of the Au nanoparticles and details are presented below.

Debye-Scherrer equation ($t = \frac{K\lambda}{\beta \cos \theta}$)

$$K = 0.9, \lambda = 1.54056, 2\theta = 38.24^\circ \text{ and } \theta = 19.12^\circ$$
\[ \beta \text{ (FWHM)} = 3.5294^\circ \times 3.14 / 360^\circ = 0.0307 \text{ rad} \]

Thickness or diameter of Au NPs = 0.9 x 1.54056 / 0.0203 x cos (19.12°) = \textbf{4.8 nm}

HRTEM images of gold nanoparticles were obtained by drop casting dilute solution on to a formvar coated copper grid (Figure 3.11). It has been observed that nanoparticles are randomly distributed all over the grid and the average diameters of Au nanoparticles were estimated to be 4.5 nm.

\textbf{Figure 3.11.} (A, B) TEM images of ethylene glycol thiol protected Au seed nanoparticles (average diameter of nanoparticles is 4.5 nm).

3.3.3 Growth of Au nanoparticle assemblies on PNT

With the objective of investigating the chirality transfer to metal nanoparticles, \textbf{EG}_3-S-Au nanoparticles (0.032 mM) were added to both D- and L-isomers of PNT (3.2 mM). The adsorption of nanoparticles was further confirmed through the TEM studies and the seed nanoparticles exist as small clusters containing two or three nanoparticles in several locations on the surface of peptide nanotubes (Figure 3.12). It was reported that gold nanoparticles bound on the surface of PNT can act as nucleation sites for the reduction of gold ions [Carny 2006]. PNT seeded with \textbf{EG}_3-S-Au nanoparticles was further mixed with
Figure 3.12. (A, B) TEM images of ethylene glycol protected Au seed nanoparticles adsorbed on the surface of PNT.

An aqueous solution of HAuCl₄ (1.06 mM) and the solution was irradiated at 300 nm in a Rayonet photochemical reactor for 3 h. A substantial increase in the intensity of the surface plasmon band was observed, indicating the reduction of gold ions to gold nanoparticles (trace c; Figure 3.13). Isolated seed nanoparticles in solution possess absorption at 510 nm and strong binding of nanoparticles on to the surface of peptide nanotube shift the plasmon band to red region (527 nm in trace b; Figure 3.14). The shift in the surface plasmon resonance band can be either due to particle-particle coupling or by the ligand modification on the surface of the Au nanoparticles. Binding of Au nanoparticles on the surface of PNT is not strong at neutral pH, as compared to that of basic pH. Due to the strong binding of the nanoparticles on PNT at pH 10, SPR band further red shift to 548 nm (trace b; Figure 3.14, Table 3.1). It is earlier reported that the carboxylate ions of amino acids strongly bind to Au nanoparticles which results in a red shift in the plasmon absorption band [Joshi 2004]. HAuCl₄ forms complexes with the Au atoms of the seed nanoparticles, which resulted in a
Figure 3.13. Absorption spectra of (a,) L-diphenylalanine peptide nanotubes (L-PNT, 3.2 mM) in water/HFP (99:1) on subsequent addition of (b,) EG-Au nanoparticles (0.032 mM) and (c,) HAuCl₄ (1.06 mM, pH 10), followed by photochemical irradiation (3 h). Absorption spectrum of L-PNT up to 200 nm is shown as dotted trace (a shoulder is observed at 220 apart from the peak at 258 nm).

Figure 3.14. (A) Surface plasmon resonance (SPR) band of Au nanoparticles in a mixture (99:1) of water and hexafluoro-2-propanol (pH 10), at various stages of growth: (a, black trace) EG-Au nanoparticles (0.032 mM); (b, red trace) EG-Au nanoparticles (0.032 mM) adsorbed on PNT (3.2 mM); (c, green trace) on subsequent addition of HAuCl₄ (1.06 mM) and (d, blue trace) surface plasmon resonance (SPR) band after photochemical irradiation (300 nm) of the resultant solution for 3 h.
**Table 3.1:** Change in the surface plasmon resonance (SPR) band of Au nanoparticles during each step of nanoassembly formation.

<table>
<thead>
<tr>
<th>Name</th>
<th>pH 7 ($\lambda_{\text{max}}$)</th>
<th>pH 10 ($\lambda_{\text{max}}$)</th>
<th>HAuCl₄ ($\lambda_{\text{max}}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG₃-S-Au</td>
<td>510</td>
<td>510 (a)</td>
<td>528</td>
</tr>
<tr>
<td>EG₃-S-Au + PNT</td>
<td>527</td>
<td>548 (b)*</td>
<td>560 (c)*</td>
</tr>
<tr>
<td>EG₃-S-Au + PNT</td>
<td>--</td>
<td>--</td>
<td>533 (d)*</td>
</tr>
</tbody>
</table>

(*note that the unbound nanoparticles observed in the TEM grid, which may also contribute to the overall absorption*)

Strong shift in the plasmon band to 560 nm. Nucleation occurs immediately on the surface of the seed nanoparticles, due to lower potential of ~1.0 eV for the reduction of Au³⁺ to Au¹⁺ ions ($\text{Au}^{3+} \rightarrow \text{Au}^{1+} = 1.5 \text{ eV in water}$) [Perrault 2009]. Within 3 h of photoirradiation, the SPR band got blue shifted to 533 nm indicating the reduction of Au³⁺ ions to Au nanoparticles (trace d; Figure 3.14, Table 3.1).

### 3.3.4 Surface plasmon coupled circular dichroism (SP-CD)

The assembly of gold nanoparticles on peptide nanotubes was further probed using CD spectroscopy, which provided valuable information about the geometrical properties of these hybrid systems. Small decrease in the signal intensity of PNT seeded with EG₃-S-Au nanoparticles may be due to the screening of diphenylalanine molecules by Au nanoparticles. However, no CD signal was observed at the gold surface plasmon frequency, ruling out the possibility of chirality transfer. The asymmetry in the electromagnetic coupling between the nanoparticles is deficient or negligible on the surface of PNT.
Addition of HAuCl₄ at basic pH (10) causes a slow nucleation and growth of new Au nanoparticles, adjacent to the adsorbed EG₃-S-Au nanoparticle seed on PNT and bunches of Au nano-assemblies are thus formed. Interestingly, gold nanoparticle bunches formed on D- and L-isomers of PNT showed bisignated CD signal with symmetrical mirror images at the gold surface plasmon frequency (traces c₁ and c₂; Figure 3.15). The D-isomer of PNT showed positive followed by negative cotton effects (positive couplet). In contrast, an inversion of CD signal was observed with negative followed by positive cotton effects for the L-isomer (negative couplet). The surface plasmon coupled CD signals originate from the asymmetry in the electromagnetic coupling between the nanoparticles in the Au nanoassemblies. Anisotropic factor (g-factor) of gold nanoparticle bunches on D- and L-isomers of PNT are in the range of ± 5 x 10⁻⁶, comparable with earlier reports [Schaaff 1998, Schaaff 2000, Yao 2005, Gautier 2006, Gautier 2008].

The formation of bunches of nanoassemblies were further confirmed through different microscopic analysis. SEM images of Figure 3.16 indicate the presences of large number of PNTs. Au nanoassemblies are not visible on the surface of PNTs due to low resolution of the SEM images. High-resolution thermionic emission gun TEM (HR-TEM) images were obtained, well separated gold nanoparticle bunches were observed on the surface of PNT (Figure 3.17). EG₃-S-Au nanoparticles seeded on the PNT can act as the nucleation site and the chiral molecules on their surface provide a restricted environment, which drives the selective growth of nanoparticles as bunches. The close proximity of Au nanoparticles in each of these bunches (Figure 3.17B) on PNT induce coupling of their plasmon oscillations, resulting in a shift in the absorption band to 533 nm. The position of
Figure 3.15. CD spectra of (a) diphenylalanine peptide nanotubes in a mixture (99:1) of water and HFP at pH 10 on subsequent addition of (b) EG-Au nanoparticles and (c) HAuCl₄ (1.06 mM), followed by photochemical irradiation (3 h). Subscripts 1 and 2 denote L- and D-isomers of PNT, respectively.

The new resonance band depends mainly on the interparticle distance and orientation [Jain 2007, Joseph 2006, Padmanabhan 2008, Bakr 2006]. Here, the chiral organization of Au nanoparticles on the surface of the D- and L-isomers of PNT causes an asymmetrically coupled surface plasmon band. Attempts to resolve the arrangement of Au nanoparticles in the assembly using HRTEM at 300 kV were not successful due to the deformation of PNT on prolonged exposure to electron beam (Figure 3.18).
Figure 3.16. SEM images of Au nanoparticle assemblies on peptide nanotubes (Au nanoassemblies are not visible due to low resolution of these images).

Figure 3.17. TEM images (A, B) of Au nanoparticle assemblies formed after photochemical irradiation of diphenylalanine peptide nanotubes (PNT) in the presence of EG-Au nanoparticles for 3 h. Inset of (A) shows low resolution TEM image of the bunches on PNT.
Figure 3.18. TEM images (A-D) of Au nanoparticle assemblies (bunches of nanoparticle assembly imaged after the decomposition of nanotubes on increasing the acceleration voltage).

3.3.5 Mechanism

The obvious question is how the bisignated CD signal, with symmetrical mirror images was observed at the surface plasmon frequency of gold nanoparticles. It is well established in the literature that the induction of chirality can occur when an achiral chromophore and a chiral component are in close contact [Allenmark 2003, Berova 1997]. It occurs mainly through the destruction of the symmetry of the former resulting from the structural perturbation of the latter. In the present case, the hybrid system consists of achiral gold nanoparticles and chiral peptide template as two components. Chiral molecules have
Scheme 3.1. Schematic representation of the formation of Au nanoparticle assemblies on D- and L-isomers of peptide nanotubes in two different ways with opposite chirality (note the direction of arrows).

been shown to reduce the symmetry of growing crystals [Orme 2001, Addadi 2001, Bouropoulos 2001]. Adsorption of \textbf{EG$_3$-S-Au} nanoparticles on to the PNT surface results in the lowering of symmetry due to the fact that the surface does not allow some symmetry elements to occur. The adsorbed nanoparticles act as nucleation sites and the chiral molecules on the surface breaks the symmetry of the growing nanoparticles. It was reported earlier from our group and others that the closely packed linear organization of Au nanoparticles and nanorods can result in the coupling of surface plasmon resonance which depends on the particle size, array spacing, array symmetry, and polarization direction [Jain 2007, Joseph 2006, Padmanabhan 2008, Bakr 2006]. Mirror image relationship in the CD spectra, observed at the surface plasmon frequency, clearly indicates that the chiral molecules on the D- and L-isomers of PNT drive the organization of Au nanoparticles in two different ways, with opposite chirality (note the direction of arrows in Scheme 3.1). CD spectroscopic results clearly indicate that the Au nanoparticles are asymmetrically organized as bunches on the surface of PNTs. Thus the surface plasmon coupled circular dichroism
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Nanoclusters capped with chiral molecules show optical activity at their discrete electronic transition and three different mechanisms have been proposed [Schaaff 2000, Allenmark 2003, Berova 1997]: (i) formation of chiral core, (ii) chiral organization of the chiral adsorbate on an achiral core and (iii) electronic interaction between the chiral molecules and the achiral metal core (a detailed explanation has been given in section 1.5). More recently, CD active surface plasmon resonance bands were observed in gold nanoparticles capped with chiral calix[4]arenes [Ha 2009] and silver nanoparticles adsorbed on DNA template [Shemer 2006, Petty 2004]. In the present study we have demonstrated a different strategy for inducing chirality by growing Au nanoparticles on a chiral template having reduced elements of symmetry. Thus the surface plasmon coupled circular dichroism originates from the asymmetric organization of Au nanoparticles on surface of PNT which result in the formation of bisignated CD signals. It is noteworthy that chiral information and asymmetry at the molecular level on the D- and L-isomers of PNT are transferred to gold nanoparticle assembly by organizing them in an asymmetric fashion.

3.4 Conclusions

Diphenylalanine based peptide nanotubes (PNTs) were found to be extremely stable to temperature, pH and photoirradiation. The D- and L-isomers of PNT showed CD signal with positive and negative cotton effects at their UV absorbing region. Interestingly, Au nanoparticles grown on D- and L-isomers of PNT showed a bisignated CD signal at their
surface plasmon frequency with positive and negative couplets, respectively. The bisignated CD signal originates from the plasmon coupling of the asymmetrically organized Au nanoparticle assemblies on PNT surface. It is further concluded that chiral patterns on the surface of PNTs direct the asymmetric organization of Au nanoparticles in two different ways. In conclusion, we could organize Au nanoparticles on PNT surface in an asymmetric fashion; however, our attempts to separate them were not successful due to the thermal stability of nanotubes.

3.5 Experimental section

3.5.1 Materials and methods

HAuCl₄ and tri(ethylene glycol) monomethyl ether were purchased from Sigma-Aldrich and D-diphenylalanine from Bachem Germany. All melting points are uncorrected and were determined on Mel-Temp-II melting point apparatus. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker DPX-300 MHz spectrometer. FAB and High Resolution Mass spectra were recorded on a JEOL JM AX 5505 mass spectrometer. Electronic absorption spectra were recorded on Agilent diode array UV-Visible spectrophotometer (model 8453), infrared spectra on Shimadzu FTIR spectrophotometer (IR Prestige-21), circular dichroism spectra on JASCO spectropolarimeter (J-810), scanning electron microscope images on JEOL (JSM 5600LV) and transmission electron microscope images on HR-TEM (FEI, Tecnai 30G², S-twin; 300 KV) or Hitachi (H-7650; 80KV). Samples for TEM were prepared by drop casting 100 µL of solution on a carbon coated Cu grid and dried by keeping overnight at ambient conditions.
3.5.2 Synthesis and characterization of diphenylalanine

**Synthesis of 2:** Acetyl chloride (2.9 mL, 39.95 mmol) was added dropwise to ice cooled methanol (27.3 mL) over 20 min. Phenylalanine (5 g, 30.27 mmol) was added and the solution was refluxed for 3 h. The reaction mixture was neutralized with NaHCO₃ and extracted with chloroform, the organic layer was washed with water and then brine solution. The organic layer was further dried over anhydrous sodium sulfate and concentrated to get a thick gummy residue. This procedure was followed for both D- and L-isomers in both cases the conversion was nearly quantitative. Yield (5.24 g, 97%): m.p. 149-151 °C. IR (KBr)ν max: 3377 cm⁻¹, 1739 cm⁻¹, ¹H NMR (CD₃OD, 500 MHz): δ 7.37-7.27 (SH, m, Ar), 4.34 (1H, m, CH), 3.82 (3H, s, CH₃), 3.23 (2H, m, CH₂); MS (FAB): 180.10 (M+1)

**Synthesis of 3:** L-phenylalanine methyl ester hydrochloride 2 (2 g, 9.2 mmol) was first neutralized with NaHCO₃. To the above solution, THF (16 mL) and Fmoc-OSu (3.72 g, 11.04 mmol) were added and the reaction mixture was stirred at room temperature for 16 h and diluted with water. The organic layer was extracted with ethyl acetate and washed with brine solution. The crude product was dried over anhydrous sodium sulfate and the solvent was evaporated completely. The crude sample on purification by silica gel column chromatography afforded 3 (3.64 g, 97.8%) as a white solid. m.p. 112°C; IR (KBr)ν max: 3310 cm⁻¹, 1735 cm⁻¹, 1643 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz ): δ 7.7-7.0 (13H, m, Ar), 5.2 (1H, d, NH), 4.6 (2H, d, OCH₂), 4.4 (1H, d, CH), 4.3 (1H, t, fmoc-CH), 3.6 (3H, s, CH₃), 3.00 (2H, m, CH₂); ¹³C NMR (CDCl₃, 125 MHz): δ 29.37, 29.91, 38.34, 47.14, 50.36, 52.38, 54.78, 65.19, 66.95, 120.01, 120.08, 124.72, 125.06, 125.13, 127.07, 127.18, 127.60,
127.73, 128.63, 129.32, 135.72, 141.33, 143.75, 143.85, 155.56, 171.94. By following a similar procedure we have synthesized D-isomer with nearly quantitative yield (3.6 g, 97%).

**Synthesis of 4:** L-isomer of Fmoc-N-phenylalanine methyl ester, 3 (1.2 g, 2.99 mmol) in THF (1 mL) was cooled to 0°C and HCl (5 N, 0.9 mL) was added dropwise with stirring at room temperature for 18 h. The reaction mixture was quenched with saturated sodium bicarbonate solution. THF was removed from the reaction mixture. The organic layer was separated using ethyl acetate and washed with brine solution, dried over anhydrous sodium sulfate and the solvent was evaporated to yield 4 (0.59 g, 52%) as a white powder, m.p. 172°C; IR (KBr)ν<sub>max</sub>: 3316 cm<sup>-1</sup>, 1720 cm<sup>-1</sup>, 1688 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 7.68-7.0 (13H, m, Ar), 5.1 (1 H, d, NH), 4.6 (2H, d, OCH<sub>2</sub>), 4.4 (1H, m, CH), 4.1 (1H, t, fmoc-CH), 3.1 (2H, m, CH<sub>2</sub>).

**Synthesis of 5:** To an ice cooled solution (0°C) of Fmoc-N-L-amino acid (1.5 g, 3.87 mmol) and Et<sub>3</sub>N (0.6 mL, 4.25 mmol) in THF (26 mL), kept under stirring, isobutyl chloroformate (0.51 mL, 3.87 mmol) was added in 1 min. To the above solution, 2 was added (0.72 g, 3.87 mmol) and Et<sub>3</sub>N (0.59 mL, 4.28 mmol) in THF was added successive steps and stirred at room temperature for 4 h. Triethylamine hydrochloride salt was filtered off and the solvent was removed under vacuum, and residue was extracted with ethyl acetate. The organic layer was separated and washed with brine solution and dried over anhydrous sodium sulfate. The solvent was evaporated and the crude sample was purified by using silica gel column chromatography (30% ethyl acetate in hexane), afforded L-isomer of N-fmoc diphenylalanine methyl ester 5 (0.86 g, 58%) as a white solid, m.p. 159°C; IR (KBr)ν<sub>max</sub>: 3300 cm<sup>-1</sup>, 1736 cm<sup>-1</sup>, 1697 cm<sup>-1</sup>, 1647 cm<sup>-1</sup>, 1535 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ
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7.70-6.9 (18H, m, Ar), 6.10 (1H, d, NH), 5.20 (1H, d, NH), 4.7 (1H, m, CH), 4.37 (2H, d, OCH₂), 4.21 (1H, t, CH), 4.10 (1H, t, fmoc-CH), 3.66 (3H, s, CH₃), 3.0 (4H, m, CH₂); ¹³C NMR (CDCl₃, 125 MHz): δ 18.98, 27.96, 29.70, 37.87, 38.31, 47.09, 52.29, 52.34, 53.70, 67.14, 71.33, 120.01, 125.01, 127.10, 127.76, 128.56, 128.73, 129.17, 129.27, 129.39, 135.51, 135.82, 141.30, 143.71, 170.27, 171.29, 172.16; MS (FAB): 550.27 (M+2).

Synthesis of 6: L-isomer of Fmoc-N-L-diphenylalanine methyl ester 5 (0.5 g, 0.91 mmol) dissolved in THF (1 mL). 5 N HCl (0.24 mL) was added slowly to the reaction mixture at 0°C, stirred at room temperature for 18 h. The reaction mixture was quenched with saturated sodium bicarbonate solution. THF was removed from the reaction mixture. The organic layer was separated using ethyl acetate and washed with brine solution, dried over anhydrous sodium sulfate and the solvent was evaporated to yield 6 (0.25 g, 50%) as a white powder, m.p. 146-147°C; IR (KBr) νmax: 3300 cm⁻¹, 1693 cm⁻¹, 1655 cm⁻¹, 1535 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz): δ 7.70-6.8 (18H, m, Ar), 6.3 (1H, d, NH), 5.2 (1H, d, NH), 4.6 (1H, t, CH), 4.35 (2H, d, fmoc-CH₂), 4.2 (1H, t, Fmoc-CH), 3.1 (2H, m, CH₂).

Synthesis of (S,S) L-FF: To an ice cold solution (0°C) of Fmoc-N-L-diphenylalanine 6 (0.2 g, 0.37 mmol) add 5% piperidine in DMF (3.3 mL) and stir the solution for about 6 h at room temperature. The solvent was removed under vacuum, and residue was extracted with ethyl acetate. The organic layer was separated and washed with brine solution and dried over anhydrous sodium sulfate. The solvent was evaporated and the crude sample on purification by silica gel column chromatography; (30% ethyl acetate in hexane), afforded of L-diphenylalanine (S,S) L-FF as a White solid mass. m.p. 148-149°C. ¹H NMR (CDCl₃, 300 MHz): δ 7.28-7.25 (10H, m, Ar), 6.87(1H, d, NH), 5.32(1H, d, NH), 4.70(1H, t, CH), 4.05-
4.00 (1H, t, CH), 3.29-3.24 (2H, m, CH₂), 3.00-2.93 (2H, m, CH₂); $^{13}$C NMR (CDCl₃, 75 MHz): δ 174.8, 171.7, 139.4, 135.9, 129.5, 129.4, 128.7, 128.4, 126.1, 126.0, 55.1, 54.4, 40.2, 36.3; MS (FAB): 313.22 (M+1).