Effects of Water Molecules on Photoluminescence from Hierarchical Peptide Nanotubes and Water Probing Capability

Minjie Wang, Shijie Xiong, Xinglong Wu,* and Paul K. Chu*

Photoluminescence (PL) spectra reveal that deficiency of water molecules in the channel cores of bioinspired hierarchical diphenylalanine (l-Phe-l-Phe, FF) peptide nanotubes (PNTs) not only modifies the bandgap of the subnanometer crystalline structure formed by the self-assembly process, but also induces a characteristic ultraviolet PL peak the position of which is linearly proportional to the number of water molecules in the PNTs. Addition or loss of water molecules gives rise to the UV PL redshift or blueshift. Density functional theory calculation also confirms that addition of water molecules to the PNTs causes splitting of the valence-band peak, which corresponds to the shift and splitting of the observed UV PL peak. Water molecules play an important role in the biological properties of FF PNTs and the results demonstrate that the PL spectra can be used to probe the number of water molecules bonded to the FF molecules.

1. Introduction

Diphenylalanine (l-Phe-L-Phe, FF), one of the simplest peptides, is the core recognition motif of the Alzheimer’s disease-associated β-amyloid polypeptide. It can self-assemble into stiff as well as chemically and thermally stable nanotubes (NTs) in aqueous solutions[1] and is also used to create more complex structures, such as vertically aligned nanoforests/nanowires and well-organized films.[2–4] X-ray analysis shows that FF monomers crystallize with hydrogen-bonded head-to-tail chains in the form of helices with four to six peptide molecules per turn and side chains emanating from the channel core filled with water molecules.[5] This implies that water molecules bonded weakly to FF molecules affect the biological activity of the FF peptide NTs (PNTs), which have many biochemical and biomedical applications. In fact, the materials have been used in some nanodevices.[6–8] The interaction between water and PNTs is an important cross-disciplinary research subject encompassing biophysics, nanotechnology, and biomolecular sensing because it provides the platform to study new and not well understood biological, chemical, and physical effects. Hence, systematic investigation and better understanding of these interactions are crucial to device design and applications to biochemistry, biomedical engineering, and medical science.

There have been relatively few papers reporting the photoluminescence (PL) properties of the PNTs. Rosenman et al. investigated the origin of the ultraviolet (UV) PL from FF PNTs, and showed that a crystalline structure with a dimension of 1.0 nm formed by self-assembly was responsible for the observed PL.[9] They also observed UV PL from self-assembled peptide nanospheres and reached a similar conclusion.[10] Further investigation on the self-assembled bioinspired peptide hydrogels indicated that the quantum phenomenon observed previously only from semiconductor...
crystals was also present in self-assembled nanostructures made of biological building blocks. These findings have spurred new research in photonic devices consisting of inexpensive organic substances. Park et al. introduced luminescent complexes composed of photosensitizers and/or lanthanide ions into PNTs and observed enhanced lanthanide PL due to the energy-transfer cascade from the PNTs to photosensitizer molecules. The results also demonstrate the possibility of producing PNTs that exhibit various colors spanning almost the entire visible range. Consequently, the PL properties of FF PNTs constitute a scientifically intriguing and practically significantly research topic.

In the work reported herein, PL spectra are acquired from hexagonal hierarchical FF PNTs fabricated using different FF concentrations and different water vapor pressures. Our results reveal that deficiency of water in the FF PNTs not only changes the bandgap of the subnanometer crystalline structure formed by self-assembly, but also leads to characteristic UV PL spectra with peak positions between 300 and 320 nm. A linear relationship between the UV PL peak position and water content in the NTs is observed, which suggests that the PL spectra can be used to detect the number of water molecules in the FF PNTs. Our density functional theory (DFT) calculation reveals that addition of different numbers of water molecules to the peptide molecules in the channel core leads to significant splitting of the densities of state (DOFs) at the valence-band maximum corresponding to peak splitting into two. This is the reason for the appearance of the UV PL and subsequent shifts observed experimentally.

2. Results and Discussion

The samples produced using different FF concentrations and water vapor pressures have tubelike hierarchical structures. Figure 1a depicts the field-emission scanning electron microscopy (FESEM) image of a typical hierarchical PNT fabricated using an FF concentration of 90 mg mL$^{-1}$ at the relative humidity (RH) of 33%. The tail is composed of many overlapping hexagonal NTs and the head has a clear hexagonal shape with a larger size (see Figure 1a, inset). Although similar PNT morphologies have been reported together with theoretical prediction, the formation mechanism of the self-assembled hexagonal structures is still unclear. Based on the UV–visible–near infrared (UV–vis–NIR) diffuse reflectance spectra (DRS) obtained from three FF PNTs (FF concentrations of 30, 90, and 160 mg mL$^{-1}$ at the RH of 1), the bandgap is derived to be $\approx 4.4$ eV ($\approx 280$ nm) according to the Kubelka–Munk absorption function versus wavelength plot (Figure 1b). The bandgap increases clearly with FF concentration (see inset). Obviously, the bandgap is closely related to the highly ordered subnanometer crystalline structure with weak water bonding formed during self-assembly of the FF molecules into NTs (a hexagonal crystalline unit cell is plotted in Figure 1c). Here, the two absorption peaks at 256 and 226 nm over the band edge arise from the absorption of FF molecules.

Figure 2a shows the PL spectra of the FF PNTs produced using an FF concentration of 30 mg mL$^{-1}$ and different RH

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**Figure 1.** a) Typical FESEM image of the hierarchical FF PNTs fabricated using an FF concentration of 90 mg mL$^{-1}$ at RH = 0.33 and 22 °C. The inset shows the enlarged head of the NT. b) Calculated Kubelka–Munk absorption function versus wavelength according to the UV–vis–NIR DRS of three FF PNT samples fabricated using FF concentrations of 30, 90, and 160 mg mL$^{-1}$ at the RH value of 1. The absorption band related to a defect state (the UV PL peak) has a smaller intensity (black arrow). c) Schematic of the highly ordered subnanometer crystalline structure (hexagonal unit cell) with weak water molecule bonding (red balls) onto the FF molecule of the channel core (inset).
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It can be observed that only one peak at about 282 nm is from the band edge recombination (Figure 1b). When the FF concentration is raised to 105 mg mL$^{-1}$, the band edge PL peak appears at 279 nm and another UV PL peak emerges at ≈304 nm. Although the 304 nm peak does not change in position, the intensity diminishes gradually with increasing RH, and the peak eventually vanishes when the RH reaches 1.00 (Figure 2b). If the FF concentration is increased further to 160 mg mL$^{-1}$, the band edge PL peak blueshifts and goes beyond our measurement range. At the same time, the UV PL peak appears at 310 nm and redshifts with increasing RH (Figure 2c). The same is observed from the sample produced with an FF concentration of 300 mg mL$^{-1}$. That is, the bandgap of the FF PNTs increases with FF concentration (Figure 1b), whereas the UV PL peak redshifts with increasing FF concentration or RH. It should be emphasized that the RH value and FF concentration together dictate the UV PL peak position. Different RH values and FF concentrations can give rise to different NT structures and morphologies, as shown in Figure 3a–c. These NTs with different structures and morphologies can contain different numbers of water molecules. The NTs fabricated using high FF concentrations may have a slightly larger content of water for the same RH value due to NT divarication. Consequently, the UV PL peak exhibits a larger redshift.

The experimental results shown in Figure 2c appear to indicate that the UV PL peak is closely related to the number of water molecules bonded to the FF PNTs. The increase and decrease in the number of molecules may be responsible for the redshift and blueshift of the UV peak, respectively. This may also imply that the bandgap increases with the number of water molecules. The band edge peak blueshifts as shown in Figure 2a–c and is accompanied by a redshift in the UV peak. Since the bandgap of the materials cannot be easily modified by free water molecules, these water molecules must be bonded, albeit weakly, to the FF molecules (inset in Figure 1c) and are thus not totally free in the channel cores. This also implies that the crystalline unit cell structure of the PNTs is distorted slightly due to the water molecules.

To investigate this issue further, we examined the X-ray diffraction (XRD) patterns of the PNT samples fabricated using an FF concentration of 160 mg mL$^{-1}$ at the RH values of 0.33, 0.52, and 0.83. b) Complete XRD pattern from the PNT sample fabricated using an FF concentration of 160 mg mL$^{-1}$ at the RH of 0.83.

The XRD patterns show that the lattice spacing expands gradually with increasing RH, and all the diffraction peaks that can be indexed to the self-assembled crystalline structure of FF molecules gradually shift to the low 2θ side. That is, the lattice spacing expands gradually and so the unit cell becomes bigger. Simple calculation yields the expansion of the hydrophilic channel in the plane perpendicular to the channel to be 4.6% from the 0.83 to 0.33 RH samples, whereas the

Figure 2. PL spectra of three FF PNT samples formed using low, intermediate, and high FF concentrations: a) 30, b) 105, and c) 160 mg mL$^{-1}$ at RH values of 0.33, 0.52, 0.67, 0.83, and 1.00.

Figure 3. SEM images of the FF PNTs fabricated using the same RH value (0.67%) but different FF concentrations: a) 30, b) 105, and c) 160 mg mL$^{-1}$. Scale bar: 30 μm.

Figure 4. a) Three local XRD patterns of the PNT samples fabricated using an FF concentration of 160 mg mL$^{-1}$ at the RH values of 0.33, 0.52, and 0.83. b) Complete XRD pattern from the PNT sample fabricated using an FF concentration of 160 mg mL$^{-1}$ at the RH of 0.83.
aromatic stacking distance along the channel is only contracted by less than 0.06%,\textsuperscript{[12,13]} If we consider a subnanometer scale unit cell with two sidewalls \( a = b = 2.407 \text{ nm} \) and \( c = 0.545 \text{ nm} \) as reported by Gorbitz,\textsuperscript{[16]} the 4.6% expansion only increases the distance in the \( a \) or \( b \) direction by about 0.094 nm, which is far less than the diameter (0.4 nm) of one water molecule. This implies that the arrangement of the water molecules is mainly along the channel. The expansion of the nanochannel decreases the bandgap due to the quantum size effect.\textsuperscript{[9–11]} In addition, the XRD results indicate that the UV PL peak shifts are not due to random aggregation of FF molecules because no obvious broadening in the diffraction peaks can be observed. This is also consistent with the absorption result (Figure 1b).\textsuperscript{[11]} Therefore, the UV PL peak shift is most likely associated with addition and loss of weakly bonded water molecules, but this conclusion needs to be verified further by both experiments and theory.

Since the UV PL spectra in Figure 2c show the same shape but different positions, we can rule out that the redshift with RH arises from some luminescent metallic impurities. Generally, the PL associated with metal impurities has a narrow but different positions, we can rule out that the redshift with RH arises from some luminescent metallic impurities. Generally, the PL associated with metal impurities has a narrow linewidth.\textsuperscript{[12]} Our energy-diffuse X-ray spectroscopy (EDXS) results also indicate that no new impurities are introduced to the PNTs formed at different RH values (Figure SI-1 in the Supporting Information). To further identify the origin of the PL shift, the FF PNT sample was divided into five pieces. One sample was dried at 90 °C for 16 h and the initial 310 nm PL peak (curve 1) is observed to blueshift to 301 nm (Figure 5, curve 2). After this sample is exposed to saturated water vapor at 90 °C for 16 h, the 301 nm peak returns to 306 nm (Figure 5, curve 3). If the sample is first exposed to saturated water vapor at 90 °C for 16 h, the 310 nm peak redshifts to 318 nm (curve 4), and after further drying at 90 °C for 16 h, the 318 nm peak returns to 314 nm (Figure 5, curve 5). Furthermore, with the exception of the different peak positions, curves 1, 4, and 5 show the same spectral shape as that in Figure 2c because of the separation from the band edge peak. With regard to curves 2 and 3, owing to overlapping between the UV and band edge peaks, the shape on the high-energy side shows some deviations from curves 1, 4, and 5. This implies that the PL spectra acquired from the PNTs after drying and water treatment have an origin similar to those shown in Figure 2c.

We also examined more samples and some typical results are displayed in Figure 6. For the sample showing a peak at 317 nm, the UV PL peak blueshifts and redshifts after drying and water vapor treatment for different time durations (within 12 h), respectively. When the sample undergoes repeated water treatment and drying for different times within 12 h, the PL peak redshifts and blueshifts, respectively, and reproducibly. It should be noted that EDXS verifies that no new impurities have been introduced to the samples during the above treatment. The absorption spectra obtained from the PNT samples (curves 1–3 in Figure 5) in Figure SI-2 (Supporting Information) demonstrate a clear band edge redshift (not the UV PL peak) and there is no indication of random aggregation of FF molecules.\textsuperscript{[11]} To rule out new bonding defects which may cause the observed PL, we examined the Fourier transform infrared (FTIR) spectra in the wave number range of 400–4000 cm\(^{-1}\) (curves 1–3) and the corresponding results are exhibited in Figures SI-3a and SI-3b. With the exception of the intensity changes in the vibration bands associated with water molecules at \( \approx 3400 \text{ cm}^{-1} \) (arrows),\textsuperscript{[20]} no other chemical bonding changes can be observed. Therefore, the 300–320 nm UV PL is closely related to the water content and the PL shift is a result of addition or loss of water molecules weakly bonded to and from the FF PNTs.

Since water molecules are hydrogen-bonded to FF molecules in the channel, post-processing of even the same NT samples (drying and exposure to saturated water vapor) cannot ensure the same and reproducible water addition and loss in all cases. This is the reason for the different spectral shifts in Figure 5. Here, it should be emphasized that these FF PNTs are not intended to be water sensors based on the conventional sense that they are able to detect water in air or other media. Instead, our results demonstrate that the PL spectra obtained from the PNTs can be used to probe the number of water molecules bonded to the FF molecules. This is important because water molecules play an important role in the biological properties of FF PNTs. To determine the dependence of addition or loss of water on the UV PL peak position, we conducted

\begin{figure}
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\caption{PL spectra of the initial FF PNT samples fabricated using an FF concentration of 120 mg mL\(^{-1}\) at the RH value of 1 and 22 °C after different post-processing procedures. Curve 1: as-fabricated; curve 2: dried at 90 °C for 16 h and then exposed to saturated water vapor at 90 °C for 16 h (curve 3); curve 4: exposed to saturated water vapor at 90 °C for 16 h and then dried at 90 °C for 16 h (curve 5). The inset displays the PL spectra obtained from the FF PNT sample fabricated using an FF concentration of 160 mg mL\(^{-1}\) at the RH value of 1 and 22 °C for 0, 1, 5, 15, and 60 min by 256 nm UV irradiation.}
\end{figure}
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thermogravimetric analysis (TGA) on nine PNT samples (five samples from Figure 2c and four samples from Figure 6) and determined the ratio of water to FF molecules from each sample. Subsequently, we calculated the average number of water molecules per FF molecule[21,23] and plotted the UV PL peak position versus the average water molecule number. As shown in Figure 7, a good linear relationship is obtained, clearly indicating that the UV PL peak position and shift can be used to identify the amount of water in the PNT samples.

As shown in the unit cell structure of the FF PNTs (Figure 1c), water molecules are connected to the FF molecules in the channel cores by weak hydrogen bonds (see inset) and these water molecules can be depleted easily. If the FF concentration is low, only small numbers of water molecules are needed. These FF PNTs are water rich and do not exhibit low-energy UV PL. When the FF concentration is increased, more water molecules are required and the channel cores in the FF PNTs become water deficient. Consequently, the low-energy PL peak appears and its position varies with the water content.

To theoretically explain the dependence of the UV PL peak on the water content in the channel cores of the FF PNTs, a DFT study was conducted on several samples with different water concentrations. The geometry optimization and electron structure were calculated by using the CASTEP package.[22] The norm-conserving pseudopotential method was adopted in the self-consistent calculation[23] in conjunction with the generalized gradient approximation (GGA) of Perdew, Burke, and Ernzerhof for the exchange-correlation function.[24] A kinetic energy cutoff of 500 eV for plane waves is used to represent the single-particle wave functions. In all the systems, a periodic triclinic crystal with lattice parameters $a = b = 28\,\text{Å}$, $c = 5\,\text{Å}$, angles $\alpha = \beta = \frac{\pi}{2}$, $\gamma = \frac{\pi}{3}$, is constructed to perform the band structure calculation. A supercell in this crystal includes six FF molecules forming a circle in the $x$–$y$ plane. The repetition of the circles in the $z$ direction produces a channel core, while the repetition of the supercells in the $xy$ directions forms the FF PNT system. Water molecules of the desired numbers per supercell can be added to the channel core. The lattice structures of the systems are optimized using the BFGS minimizer in the CASTEP package with default convergence tolerances of $2 \times 10^{-6}$ eV for energy, $0.05\,\text{eV}\,\text{Å}^{-1}$ for maximum force, and $0.002\,\text{Å}$ for maximum displacement.[25] The lattice constants of the supercells are fixed to the initial ones and all the ion positions are allowed to relax in the process of optimization in which $1 \times 1 \times 2$ Monkhorst–Pack k-point sampling is adopted. The optimized structures of the supercells in samples with zero, four, and eight water molecules are shown in Figure 8a–c, respectively. Here, we would like to stress that although the water molecules appear to be free in the channel core, they are weakly bonded to the FF molecules as shown in the inset of Figure 1c. The internal reconstruction of the FF molecules in forming the channel core is not substantial and the connection among them is unwound. This is also true for the connections between the water molecules and supercells. Hence, water molecules can be easily inserted into and withdrawn from the FF PNTs depending on the conditions in our experiments.

Based on the optimized structures, the band structures of the electrons in the FF PNTs with different quantities of water molecules in the channel core of a supercell were calculated. According to the DOSs in Figure 9, the transition between the major peak near 4 eV and the valence-band maximum (VBM) at 0 eV corresponds to a 4 eV PL peak. Considering the underestimation of the gap in the DFT calculation with the local density approximation (LDA) or GGA exchange-correlation function[26–28] this is in good agreement with our experimental absorption and PL spectral results. It is interesting to note that incorporation of water molecules into the channel core of the FF PNTs causes splitting of the

Figure 7. UV PL peak position versus average water molecule numbers per FF molecule. The linear dependence indicates that the UV PL peak position and its shift can be used as a fingerprint to identify modification of water content in the PNT samples.

Figure 8. Optimized structures of the supercells consisting of six FF molecules with a) 0, b) 4, and c) 8 water molecules at the channel core of a supercell. Red, gray, blue, and white balls represent oxygen, carbon, nitrogen, and hydrogen atoms, respectively. Lines OA ($x$ axis), OB ($y$ axis), and OC ($z$ axis) are crystal axes of the supercell.
The PL spectra obtained from bioinspired hierarchical FF PNTs reveal a characteristic UV PL peak between 300 and 320 nm, which can be used to determine the number of water molecules in the FF PNTs. The peak position is observed to be tunable depending on addition or loss of the same number of water molecules to and from the channel cores of the FF PNTs. DFT calculation also confirms that addition of water molecules to the PNTs causes splitting of the valence-band peak, which corresponds to the shift and splitting of the observed UV PL peak.

4. Experimental Section

Preparation of PNTs: The humidity-sensitive PNTs were prepared by evaporating a drop of fresh FF solution dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol on a glass substrate. The initial concentrations of the FF solutions were varied between 20 and 300 mg mL⁻¹ and different water vapor pressures were used in the fabrication by adjusting the RH values at a temperature of 22 °C. The FF solution was dried in 3 min and the PNTs produced were characterized after 30 min.

Characterization: The morphology and size of the FF PNT samples were determined by an FESEM instrument equipped with EDXS (Hitachi High-Technologies Corp., Japan) at acceleration voltages from 1 to 15 kV. The samples for UV–vis–NIR spectroscopy were prepared by scraping the samples formed on glass substrates and collecting the powder carefully. The measurements were conducted by placing the powders on impacted barium sulfate (BaSO₄) powders and calibration was performed on pure barium sulfate powders as the white standard. UV–vis–NIR DRS were acquired on a UV-3600 spectrophotometer (Shimadzu Co., Japan) from 200 to 500 nm with a step size of 1 nm. FTIR spectra were acquired from the PNT films on a Nicolet 170SX spectrometer. The PL spectra were obtained using a FLS920 fluorescence spectrophotometer (Edinburgh Instruments Ltd.) equipped with a 450 W Xe lamp as the excitation source. TGA was carried out on a Pyris 1 TGA apparatus (PerkinElmer Corp.) under a nitrogen atmosphere at a scanning rate of 10 °C min⁻¹ with the temperature varied from room temperature to 700 °C.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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