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Short communication

# Non-functionalized carbon nanotube binding with hemoglobin

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ABSTRACT

Carbon nanotube has a high potential to be used as a biosensor and drug carrier. However, its binding behavior with proteins needs to be studied before the full potential of carbon nanotube in biological studies can be realized. Although many studies have been conducted to characterize the affinity of functionalized carbon nanotube to various types of proteins, our present study for the first time reported that hemoglobin can bind with non-functionalized carbon nanotube, and this binding can be identified by Raman spectrum. Further, this binding has not changed Raman luminescence with specific excitation and emission wavelengths. The immediate application of these findings is to use non-functionalized carbon nanotube as a biosensor to measure $H_2S$ in blood in which hemoglobin takes about 37% of the total blood volume. Other potential uses of non-functionalized carbon nanotube to bind selective groups of proteins are also hinted.



## 1. Introduction

Studies on protein binding with carbon nanotube have gained great attention since the discovery of carbon nanotube due to its high potential to be used as a biosensor and drug carrier. It has been reported that small peptides [1], protein streptavidin [2], and nucleic acids [3,4] can bind with and be transported by functionalized acid-oxidized single-walled carbon nanotubes (SWCNTs). Pantorotto et al. [1] employed positively charged peptide-functionalized bundles and aggregates of SWCNTs with a length of several microns and a bundle size of several submicrons to transport peptides. Kam et al. [2] used a combination of the treatment of refluxing and sonication in nitric acids to produce short (50–500 nm) individual or small bundles of SWCNTs with oxygen-containing groups (e.g., –COOH) along the sidewalls and ends of the tubes. They reported that short SWCNTs with various functionalizations are capable of the transportation of proteins (with weight less than 80 kD) and oligonucleotides. Bianco et al. [4] reported that ammonium-functionalized single-walled carbon nanotubes were able to condense DNA and achieve a significant transport behavior *in vitro*. Salvador-Morales et al. [5] studied the binding of proteins to purified carbon nanotubes in human plasma and serum. They found that when multi-walled carbon nanotubes (MWCNTs) were exposed to the human serum and plasma, only a few kinds of proteins, such as fibrinogen and apolipoprotein A1, were able to bind MWCNTs in large quantity. CNTs binding with several other kinds of proteins including apolipoproteins AIV and C-III were visible at lower intensity [5]. In the serum which does not contain fibrinogen, apolipoprotein A1 was the dominant protein that binds with MWCNT, while several other kinds of proteins, including apolipoprotein AIV and C-III, bind with MWCNT in low quantity [5]. It has been well accepted that functionalization of CNTs is necessary in order that they can bind proteins.

Hemoglobin is an iron-containing oxygen-transport metalloprotein in the mammals' red blood cells and has a molecular weight of 68 kD [6]. It makes up about 97% of the red cell's dry content and around 37% of the total content (including water). Normal hemoglobin level in human blood is around 11–17 g/dL [7]. Hemoglobin is a common "sink" for CO to form scarlet carboxyhemoglobin [8], for NO to form nitrosylhemoglobin [9] and for $H_2S$ to form green sulfhemoglobin [10,11]. Interestingly, the redox status of hemoglobin affects the binding of different gasotransmitters. While $H_2S$ only binds with methemoglobin [11], both CO and NO bind with oxyhemoglobin [12].

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In order for carbon nanotubes to be used as biosensors or drug delivery carriers, knowledge regarding whether hemoglobin can bind with carbon nanotube is very important. In this paper, we report that hemoglobin can bind with non-functionalized carbon nanotubes, analyzed with the Raman microscopy frequency change of carbon nanotubes before and after binding with hemoglobin. However, such a binding does not change Raman luminescence of carbon nanotubes under specific excitation and emission wavelengths at which $H_2S$ binding with carbon nanotubes can be observed.

## 2. Materials and methods

### 2.1. Carbon nanotube preparation

In this experiment, the MWCNTS were prepared using microwave plasma enhanced chemical vapor deposition (MPECVD) reactor in a gas mixture of hydrogen and methane [13]. The carbon nanotubes were purified at 450 °C in vacuum. Fig. 1 shows a typical transmission electron microscope (TEM) (Philips CM-10) image of the carbon nanotubes after purification. The average outer diameter of the carbon nanotubes is approximately 25 nm and the average inner diameter is approximately 5 nm.

### 2.2. Hemoglobin sample preparation

Three bovine methemoglobin (Sigma, Cat. # H2500) water solution samples with different concentrations, 3.7, 1.8, and 1.2 mM, respectively, were used. A 100 μL of each sample was used in the experiment. Carbon nanotubes of 0.5 mg were then immersed in each of the three samples for 5 min. Carbon nanotubes of the same amount were also immersed in 100 μL distilled water for 5 min. The distilled water sample was used for control. The carbon nanotubes in the four samples were then taken out and placed on different parts of the same glass slide for Raman microscopy measurement.

### 2.3. Hemoglobin in serum sample preparation

Fetal bovine serum (F1050, Sigma) was used in our experiment. Serum of 200 μL was equally divided into two samples, sample 1, and sample 2, respectively. Methemoglobin was then added to each of the two samples; in particular, sample 2 has twice as much hemoglobin as in sample 1. The 0.5 mg carbon nanotubes were immersed in each of the two samples for 5 min. Thereafter, the carbon nanotubes were taken out of the samples and placed on different parts of the same glass slide for measurement.

### 2.4. Raman microscopy measurement

Raman microscope (Renishaw system 2000) was used in the experiment for data or information acquisition in two ways. One is to measure the frequency (Raman spectrum) change between original carbon nanotubes and carbon nanotubes immersed in hemoglobin or serum where hemoglobin is added, and the other is to measure the luminescence change between these two situations with carbon nanotubes. In both ways, the excitation wavelength for Raman microscopy was 514 nm, the collection wave number range for Raman spectrum was from 1000 to 4000 $cm^{-1}$, and the collection wavelength range for Raman luminescence was from 520 to 600 nm.

## 3. Results

The results of the Raman luminescence for the hemoglobin samples are shown in Fig. 2. The Raman luminescences of carbon nanotubes immersed in different concentrations of methe-

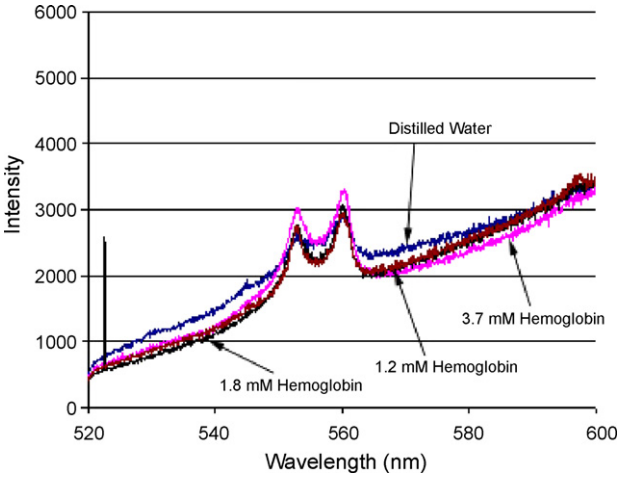


**Fig. 2.** Raman luminescence of carbon nanotubes in distilled water, in methemoglobin with concentrations of 3.7, 1.8, and 1.2 mM, respectively.

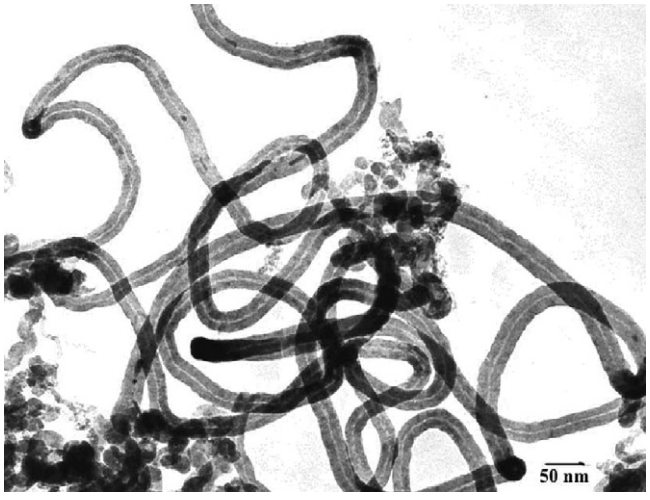


**Fig. 1.** TEM image of carbon nanotubes.

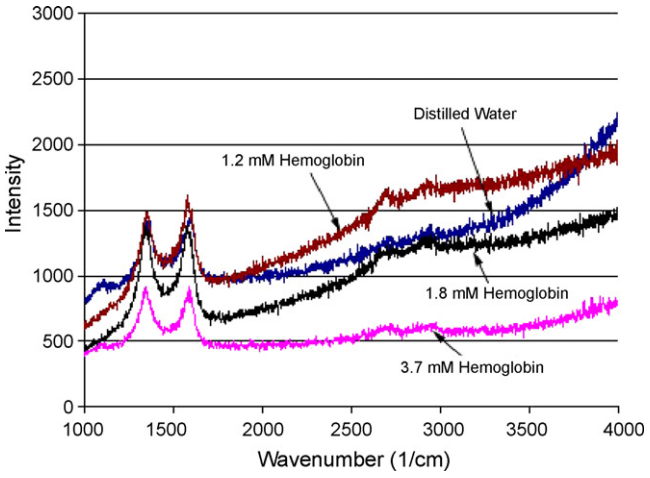


**Fig. 3.** Raman spectrum of carbon nanotubes in distilled water, in methemoglobin with concentrations of 3.7, 1.8, and 1.2 mM, respectively.

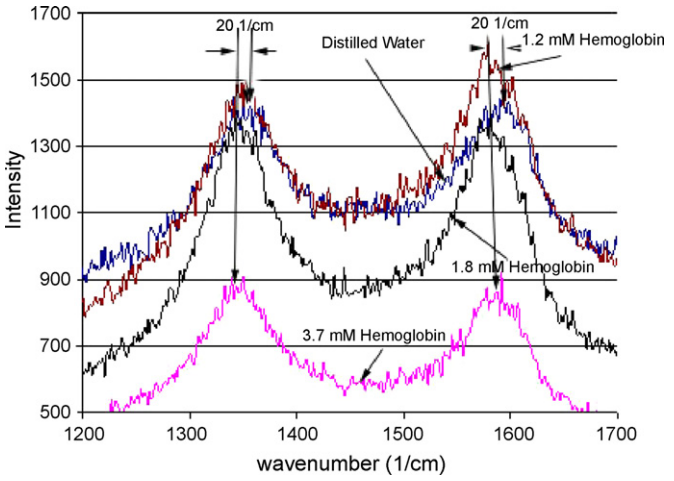


**Fig. 4.** Enlarged Raman spectrum of carbon nanotubes in distilled water, methemoglobin with concentration of 3.7, 1.8, and 1.2 mM in the wave number range from 1200 to 1700 $cm^{-1}$.

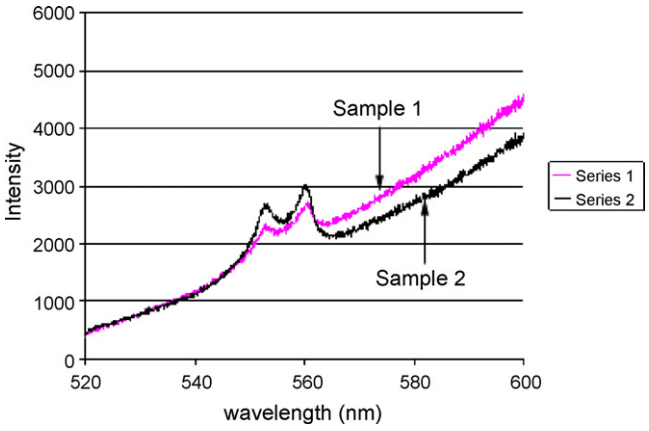


**Fig. 5.** Raman luminescence of carbon nanotubes that are immersed in sample 1 and sample 2 (sample 2 has twice as much methemoglobin as sample 1).

moglobin are the same, and these luminescences are also the same as that of the carbon nanotubes immersed in distilled water. However, in Raman spectrum, with reference to the peak of carbon nanotubes immersed in distilled water, the peaks of carbon nanotubes immersed in methemoglobin have 20 $cm^{-1}$ wave number shift at about 1500 $cm^{-1}$ towards the direction of lower wave number as shown in Figs. 3 and 4. However, the peak position is the same for all the samples immersed in methemoglobin with different concentrations.

The Raman luminescence of carbon nanotubes that are immersed in serum where methemoglobin was added is shown in Fig. 5. It can be seen from this figure that the carbon nanotubes immersed in serum with higher methemoglobin concentration (sample 2) have less intensity in Raman luminescence. Fig. 6 shows the Raman spectrum of samples 1, 2, and carbon nanotube samples immersed in distilled water, respectively. It is clear that there is no wave number shift for all these samples.

## 4. Discussion

In Raman spectrum, the incident wavelength can be observed via elastic and inelastic scattering, a relatively small amount of radiation with different wavelengths, referred to as Stokes and anti-Stokes Raman scattering. The energy increase or decrease due to

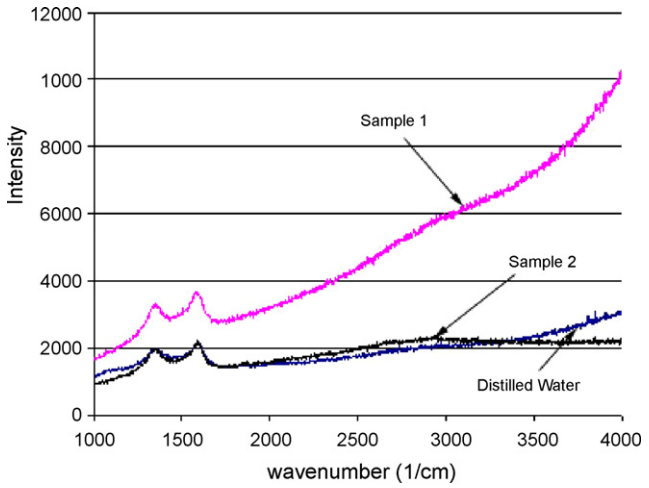


**Fig. 6.** Raman spectrum of carbon nanotubes in distilled water, sample 1, and sample 2.

the elastic scattering is related to the so-called irrational energy spacing in the ground electronic state of the molecule, and therefore the wave number of the Stokes and anti-Stokes scattering is a direct measurement of the irrational energy of the molecule. Thus, the Raman wave number shift is an indication of irrational energy changes in the sample, which provides the chemical and structural information of materials [14–17].

The Raman wave number shift of the carbon nanotubes immersed to the distilled water which contains only methemoglobin, as opposed to the free carbon nanotubes (i.e., the one immersed to the distilled water without methemoglobin), must be due to the binding of methemoglobin to carbon nanotubes. The shift towards lower wave number means that the sample has lower frequency, compared to the free carbon nanotubes, which is consistent with the notion that the natural frequency of a system is inversely and non-linearly proportional to the mass of the system. It is interesting, however, that the binding of methemoglobin to carbon nanotubes is not observable with the Raman luminescence.

In the second experiment—i.e., the experiment with the serum samples, no Raman wave number shift can be found. This result may imply that the free carbon nanotubes are not bound with methemoglobin; otherwise according to the result of the first experiment, there must be wave number shift in the Raman spectrum (because in both serum samples, proper amounts of methemoglobin were added). The plausible cause for the result of the second experiment is that the added methemoglobin might be completely occupied by $H_2S$ to form sulfhemoglobin. Furthermore, serum may contain other proteins, such as albumin, alpha1-globulin, alpha2-globulin, beta globulin, and gamma globulin. The lack of shift of wave number in the Raman spectrum can infer that these proteins do not bind to the carbon nanotubes or their interaction with carbon nanotubes do not cause the shift of wave number in Raman spectrum. This result is consistent with our previous study [18] which showed that non-functionalized carbon nanotubes can bind with $H_2S$ in the serum, observed with the Raman luminescence. The results of the experiments reported in this paper appear to provide further evidence to support our previous finding [18].

The result shown in Fig. 2 further means that the binding with methemoglobin does not change the Raman luminescence of carbon nanotubes in the range of 520–600 nm with the excitation of 514 nm; however, from our previous study [19], these wavelengths will definitely excite Raman luminescence from carbon nanotubes binding with $H_2S$. This result is possible because Raman

luminescence and Raman spectrum can be viewed as two different modalities.

The change in Raman luminescence of carbon nanotubes in the serum samples is observed (Fig. 5). The cause of this phenomenon is still unclear.

## 5. Conclusions with further discussion

Methemoglobin can bind to multi-wall carbon nanotubes which are not functionalized. The binding will not affect the Raman luminescence of the carbon nanotubes under the specific excitation and emission wavelengths (excitation: 514 nm; emission: 520–600 nm). The binging can be observed from the change in frequency by Raman spectrum.

Knowledge of methemoglobin binding to non-functionalized carbon nanotubes is important for the use of carbon nanotubes as a biosensor to measure $H_2S$ in blood and as a carrier of hemoglobin. Because carbon nanotubes can bind with both $H_2S$ and hemoglobin, the interaction of carbon nanotubes with hemoglobin may complicate the interpretation of the role of carbon nanotubes as a sensor to measure $H_2S$ in a biological environment where hemoglobin presents. However, good news is that the binding of carbon nanotubes with hemoglobin does not change the Raman luminescence, while the binding of carbon nanotubes with $H_2S$ does cause the change in Raman luminescence under certain excitation and emission wavelengths [18,19]. As such, the dual affinities of non-functionalized carbon nanotubes to $H_2S$ and hemoglobin can be differentiated such that the role of carbon nanotube as a biosensor for $H_2S$ measurement will not be jeopardized.

Finally, the result in this study is contrary to a common dogma—only functionalized carbon nanotubes can bind with proteins, our finding demonstrates the possibility of bindings between non-functionalized carbon nanotubes and proteins, hemoglobin in this case, but perhaps more other proteins as well. The mechanism behind our finding is open for further discussion.

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