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# Immobilization of Proteins on Boron Nitride Nanotubes

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The discovery of carbon nanotubes[1] (CNTs) followed by their large-scale production[2] have paved the way to wide CNT integration into modern nanotechnology by taking advantage of their excellent mechanical properties and high electrical and thermal conductivities.[3,4] Recently, biological applications of CNTs, such as biosensor devices, have captured the researchers' imagination, and numerous efforts have been made in the field.[5] As a basic idea, the immobilization of biomolecules on CNTs has been pursued, and various approaches have been explored toward the conjugation of proteins with CNTs.[6]

Boron nitride nanotube (BNNT) has a layered structure similar to that of a CNT. In addition, the two systems have close mechanical properties and thermal conductivities.[3,4] However, in many respects, BNNT possesses many advantageous properties compared with CNT; it is chemically inert, structurally stable,[7] and has a wide band gap independent of geometrical parameters,[8] while a CNT may be a metal or semiconductor depending on tube helicity and diameter, which are difficult to control. Recent studies have indicated that through doping BNNT with fluorine or by covalent functionalization[9] the band structure and electrical conductance of BNNT can be tuned. These factors make BNNT particularly suitable for biological applications. In addition, BNNT does not absorb visible and infrared light; this would protect biological molecules from overheating and damage as in the case of CNT usage. However, to date, the usefulness of BNNT for biology-related applications has not been elucidated.

In this paper, we report that proteins may be immobilized on BNNTs. It is found that there is a natural affinity of a protein to BNNTs—it can be immobilized on BNNT directly, without usage of an additional coupling reagent. For the most effective immobilization, noncovalently functionalized BNNTs should be used. The effect of immobilization was studied using high-resolution transmission electron microscopy (TEM) and energy dispersion spectroscopy (EDS).

BNNTs were synthesized via a chemical vapor deposition method with elemental boron and a metal oxide as the precursors (BOCVD).[10] As-grown BNNTs were heated to ~1900 °C in order to remove the catalyst particles and impurities. The synthesized BNNTs were white in color. A BNNT sample purity can reach ~90 vol %.[11] In an attempt to immobilize proteins, the dispersed BNNTs were simply stirred with dilute protein solutions for several hours. Then, the samples were filtered, washed, and placed on copper TEM grids for the detailed TEM characterization.

During TEM, we confirmed that successful immobilization of the ferritin protein onto BNNTs after 120 h stirring indeed took place. Figure 1a demonstrates ferritin molecules (dark contrast particles) immobilized on a BNNT. A ~6 nm iron core of each ferritin molecule is clearly visible in Figure 1. The apoproteins appear amorphous around the cores. All BNNTs are coated by the ferritin molecules.[11] No obvious dependence between the size of BNNTs and density of ferritin was observed. The immobilization process was rather slow. For the sample stirred over 48 h, only very sporadic ferritin molecules were found on BNNTs. Longer time stirring resulted in numerous ferritin molecules anchored on BNNTs. EDS analysis verified the ferritin immobilization on BNNTs; the Fe peaks appeared after immobilization (Figure 1b). Although FeO was used to synthesize BNNTs,[10c] the regarded Fe signals were totally absent in the starting BNNTs.[11] In addition, some ferritin molecules were found inside BNNTs (Figure 1c), due to numerous open tip-ends. Independent of a sample tilting angle during TEM, these ferritin molecules are visible inside the hollow channels but not on the external tube walls. This implies that they are encapsulated inside BNNTs, but are not stuck to the tube periphery. Provided that BNNTs are chemically inert and structurally stable, their internal channels would be perfect nanoscale chambers to perform further delicate chemical and/or biological experiments with the protein in a confined space. To further demonstrate a wide range of biomolecules that can be analogously anchored to BNNTs, the same experimental procedure was utilized to immobilize cytochrome *c*, streptavidin, and glucose oxide molecules. The similar positive results were obtained. Figure 2 displays TEM images of protein-functionalized BNNTs taken after 120 h stirring of the protein solutions. Although the contrast is not that sharp as in the previous case because of the absence of heavy-atom originated marks, the amorphous layers consisting of proteins on the BNNT surfaces are fairly well visible. Importantly, these contrasts are quite different from those seen for starting pure BNNTs

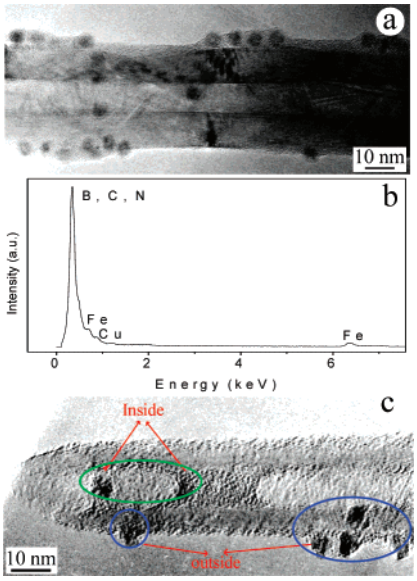


***Figure 1.*** (a) TEM image of ferritin molecules on a BNNT. (b) EDS spectrum of a ferritin-covered BNNT. Note the characteristic Fe peak peculiar to the ferritin; the Cu signal originates from a TEM grid. (c) Ferritin filled in a BNNT.



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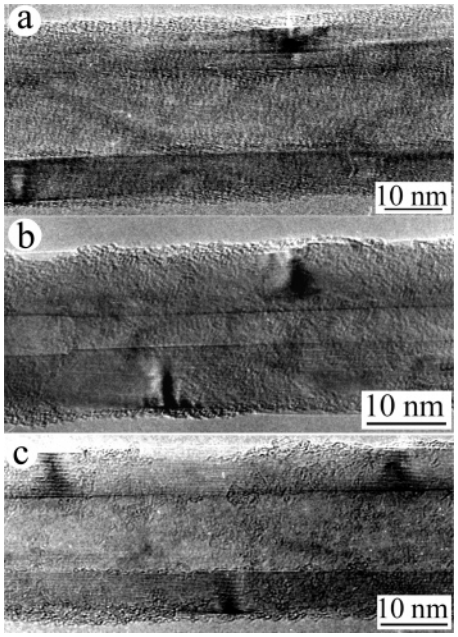


***Figure 2.*** (a) Cytochrome *c*, (b) glucose oxide, and (c) streptavidin immobilized on BNNTs and leading to the amorphous-like appearance of the outermost tube surface (see Supporting Information).

on which only marginal traces of amorphous residues were detected since BNNTs are typically nonwetted by most of materials.[11]

The contradictory data on the protein affinity to CNTs have been presented. Strong natural affinity to single-walled CNTs was observed,[6b,d] by contrast, Dai et al.[6a] have not detected the meaningful natural adsorption of ferritin to CNTs. Such inconsistency presumably indicates that the protein−NT conjugation interactions are a complex function of the experimental conditions, the synthesis method, and NT sample history.[12] We realized that amine functional groups may exist at the surface and/or open tip-ends of BNNTs due to the specific growth conditions in a high reduction atmosphere.[9b] Thus it is suggested here that, contrary to the amidation of a CNT-bound carboxylic acid, the interactions between BNNTs and a protein may originate from the carboxylation of BNNT-bound amine groups. Therefore, it takes a long time to obtain the evident immobilization because the carboxylation process is much slower and less efficient. Another possible mechanism of the effective BNNT−protein bonding may be the electrostatic interactions. After a long time stirring, the BNNT surface may become charged because of its poor conductivity. In fact, we have frequently observed that the BNNTs stick to the wall of a plastic bottle due to an electrostatic charge.[11] Experiments are underway to exploit the exact nature of interactions between a protein and BNNTs.

Finally, to make the immobilization process more efficient, 1-pyrenebutyric acid *N*-hydroxysuccinimide ester (PAHE)[11] functionalized BNNTs were utilized to anchor the ferritin protein. A highly aromatic pyrenyl group in PAHE, which is known to have strong $\pi-\pi$ interactions with a basal plane of graphite and sidewalls of CNTs,[6a] has also been found to strongly interact with the sidewalls of BNNTs. Typically, BNNTs may have profound interactions with some chemicals via $\pi-\pi$ stacking due to the electrical polarization phenomena induced by a BNNT broken symmetry.[9b] Thus, the efficient immobilization may be based upon the formation of an amide bond via the nucleophilic substitution of *N*-hydroxysuccinimide by an amine group on the ferritin. The BNNTs and PAHE were mixed and stirred in an organic solvent, dimethylformamide (DMF), for 2 h. Then the solution was filtered and repeatedly washed with DMF to remove the reagent excess.

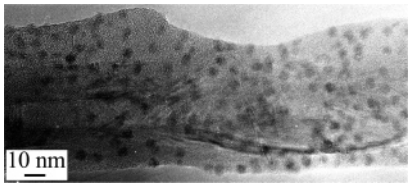


***Figure 3.*** Ferritin immobilized on a PAHE-functionalized BNNT.

As we expected, the functionalized BNNTs immobilized ferritin in the same way as pure BNNT did. However, the stirring time was shortened to 24 h. Figure 3 depicts a BNNT with numerous ferritin molecules on its surface. The BNNTs are covered with a thick amorphous PAHE layer[11] on which many ferritin molecules are effectively immobilized.

In summary, we have demonstrated that proteins may be immobilized on BNNTs. The inner nanoscale channels of BNNTs may also be useful for further intratube chemical and/or biological operations with encapsulated proteins. The immobilization is thought to be due to carboxylation of BNNT-bound amino groups or prominent tube/protein electrostatic interactions. Noncovalently functionalized BNNTs have been finally used to further improve the efficiency of protein immobilization. Given the fact that electrical properties of BNNTs are much more consistent and reproducible than those of CNTs, and they can be reliably adjusted through doping or functionalization, the present study is thought to stimulate the applications of BNNTs in novel biosensor materials.

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**Supporting Information Available:** SEM image of pure BNNTs (S1); EDS of pure BNNTs (S2); image of BNNTs sticking to plastic bottle (S3); TEM image of pure BNNT (S4); structure formula of PAHE (S5); TEM image of PAHE functionalized BNNT (S6); enlarged profile of Figure 2 (S7); low-magnification TEM image of ferritin−BNNTs (S8); FTIR spectrum of BNNTs. This material is available free of charge via the Internet at http://pubs.acs.org.

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