



Decoration of carbon nanotubes with biological entities for electronic device applications

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1. Introduction & Motivation

The chemical modification of nanoscale materials has created increasing interest in novel hybrids for application in molecular devices. Both single- and multi-wall carbon nanotubes are an excellent platform for both covalent and non-covalent functionalisation. We have made recent progress towards understanding hydrophilic and hydrophobic based interactions with entities of a biological nature (enzymes and proteins) and polymers (water soluble and biodegradable). Immobilisation of biological moieties on carbon nanotubes has been motivated by the prospects of using nanotubes as a new type of sensor material. The enhanced electronic properties of carbon nanotubes prelude their use as exceptional electrical transducers. Further, the covalent attachment of biological entities, such as antibodies, results in stable and specific reaction sites for sensing applications. In a first step to understanding covalent attachment to multi-wall carbon nanotubes, the reaction of amide functionalised multi-wall carbon nanotubes with inorganic metal complexes containing acyl chloride linkers resulted in the formation of both T- and Y- heterojunctions.

3. Interactions with Enzymes

Preparation of CNTs: 2mg MWCNT (COOH/NH₂ functionalized) in 10mL of distilled water (1% Triton X100)

Preparation of immobilisation: SBP was reacted with CNT. The CNTs were centrifuged and washed any remaining unreacted sites on the CNTs were blocked with BSA. The CNTs were then characterized by enzyme and protein assays.

Preparation of Lateral flow assay: nitrocellulose membrane were sprayed with different proteins (BSA, ovalbumin, and SBP), and the strips were prepared as shown in Fig 1. CNT (with and without SBP immobilised) were allowed to run up the nitrocellulose strip. Two other functionalized CNTs were investigated (SWNT-chitosan and SWNT-hyaluronic acid). These preparations were investigated as before.

Detection of protein and SBP activity: Detection of protein immobilised onto CNT was performed by the BCA (Smith *et al.*, 1985) and micro-BCA assay (Pierce) and SBP activity was determined by the TMB assay (Ryan *et al.*, 1994)

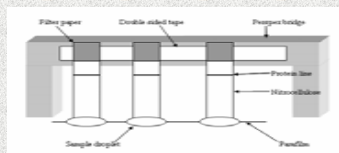
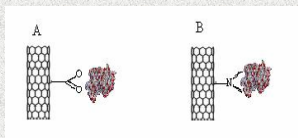


Figure 1: Schematic representation of a lateral flow setup.

Soybean peroxidase immobilization on CNT's, remained active and was used to visualize migration on a nitrocellulose membrane in a lateral flow device (Fig 1). Enzyme immobilised CNT's (blocked with BSA) did not bind to protein lines sprayed on the membrane in contrast to the binding exhibited by untreated CNT's (Fig 2). Untreated CNT's concentrated at both protein lines (Fig 3) indicating that the protein line does not inhibit CNT migration per se. When 3 different proteins were sprayed onto the membrane the untreated CNT's concentrate at all 3 lines (Fig 4). It is suggested that this approach may provide a novel methodology for studying the immobilisation of CNT's with proteins.



Schematic of SBP attached to the MWCNT through the carboxyl group (A) and the amine group (B).

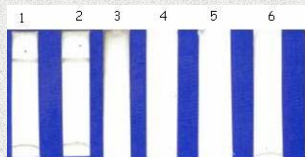
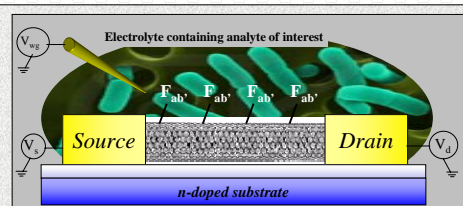


Figure 2: Demonstration of CNT binding to protein using lateral flow. 1; CNT-COOH, 2; CNT-NH₂, 3; CNT-COOH-SBP, 4; CNT-NH₂-SBP, 5; SBP only and 6; CNT only.

5. Nanoscale Sensors

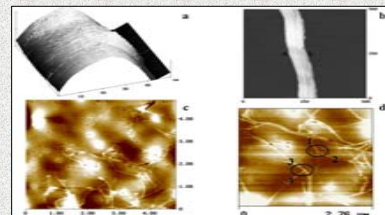
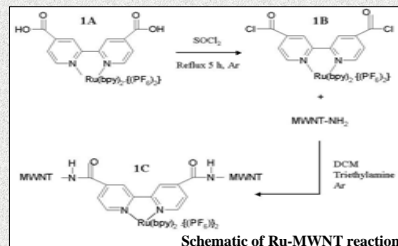


Schematic of a nanoscale electrochemical device using functionalised carbon nanotubes



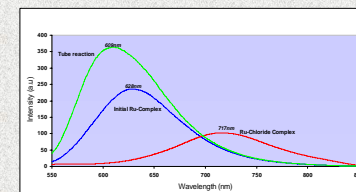
Scanning electron microscope image of carbon nanotube FET type device

2. Inorganic Metal Complex Attachment



a + b) Topographic STM images of individual MWNT-NH₂ and c + d) tapping mode AFM images of MWNT junctions.

The attachment of Ru(bpy)₃(PF₆)₃ to amino-functionalised multi-wall nanotubes was carried out as follows: 10mg of **1A** was dissolved in 15mL of thionyl chloride. The reaction mixture **1B** was refluxed under argon for 5h. The excess SOCl₂ was removed by vacuum distillation and the remaining solid was partially dissolved in dichloromethane. 2mg of NH₂-MWNT was sonicated in 5ml of DCM and then added to the refluxed mixture. The solution was stirred under argon for 72h. After reaction the product was filtered and washed with DCM and sonicated for 20min, to create a stable suspension. Unreacted MWNT settled at the bottom of the flask and the 'functionalised ruthenium MWNT' **1C** product was held in suspension. A colour change from dark red-orange (**1B**) to dark brown-green (**1C**) was observed after the reaction was completed.

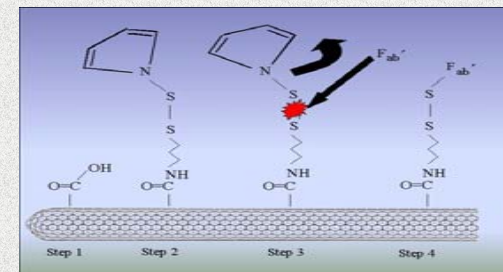


Emission spectra for the Ru-complex, Ru-Cl complex and after reaction with MWNT

After the reaction was completed a characterisation of the resultant material showed the formation of mono-substituted and interconnected nanotubes. These interconnected nanotubes appeared to be present in both T- and Y-junctions.

4. Antibody Attachment

Antibody fragments were attached to the nanotube lattice through an amide terminated linker group - adipylhydrazidyl (pyridyldithio) propionate - using a condensation reaction between the amide and carboxylic groups along the nanotube surface. Addition of the cleaved F_{ab}' substitutes at the disulfide bond (as shown). The success of this reaction can be monitored through the disappearance of the fluorescence signal during the substitution reaction shown in Step 3. Initial characterisation is being carried out.



Schematic showing covalent attachment of antibody fragments to the nanotube.

In building nanoscale based devices for sensing platforms, it is important to remember the differences in scale of the two objects that are being combined. That is, biological entities are of the order of up to several microns, which is 10³ - 10⁴ times larger than the nanotubes that you are attempting to functionalise.

Minett, A.I. *et al.*, *React Funct Polym* 53 (2002) 217; *Anal Chim Acta* 475 (2003) 37.