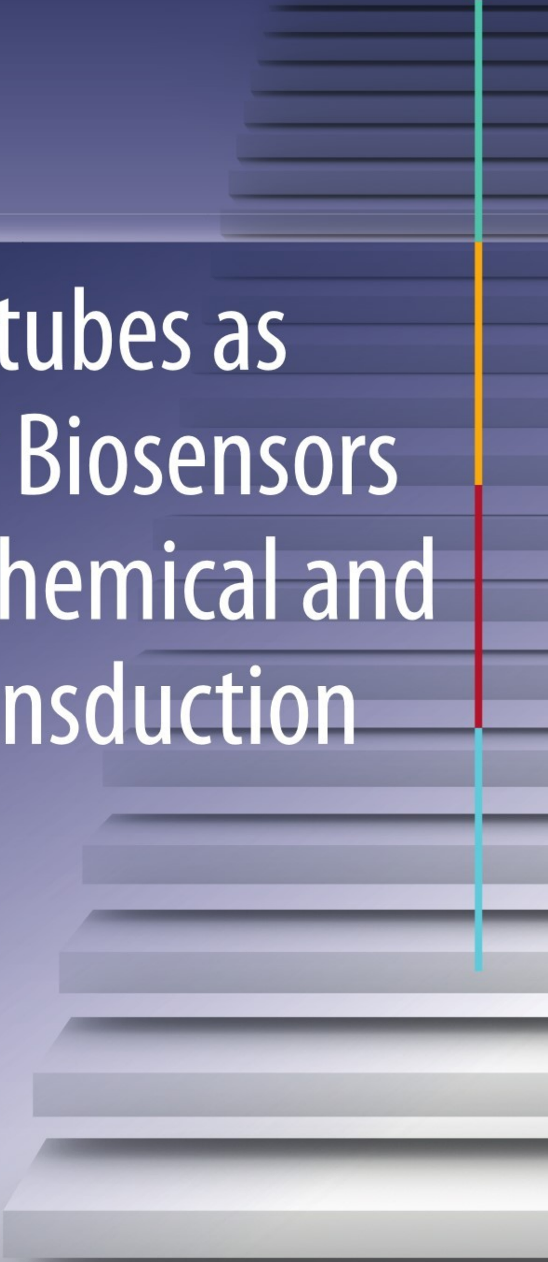
Springer Theses
Recognizing Outstanding Ph.D. Research

Mercè Pacios Pujadó

Carbon Nanotubes as Platforms for Biosensors with Electrochemical and Electronic Transduction



Springer

Springer Theses

Recognizing Outstanding Ph.D. Research

For further volumes:
http://www.springer.com/series/8790

Aims and Scope

The series “Springer Theses” brings together a selection of the very best Ph.D. theses from around the world and across the physical sciences. Nominated and endorsed by two recognized specialists, each published volume has been selected for its scientific excellence and the high impact of its contents for the pertinent field of research. For greater accessibility to non-specialists, the published versions include an extended introduction, as well as a foreword by the student’s supervisor explaining the special relevance of the work for the field. As a whole, the series will provide a valuable resource both for newcomers to the research fields described, and for other scientists seeking detailed background information on special questions. Finally, it provides an accredited documentation of the valuable contributions made by today’s younger generation of scientists.

Theses are accepted into the series by invited nomination only and must fulfill all of the following criteria

- They must be written in good English.
- The topic should fall within the confines of Chemistry, Physics, Earth Sciences, Engineering and related interdisciplinary fields such as Materials, Nanoscience, Chemical Engineering, Complex Systems and Biophysics.
- The work reported in the thesis must represent a significant scientific advance.
- If the thesis includes previously published material, permission to reproduce this must be gained from the respective copyright holder.
- They must have been examined and passed during the 12 months prior to nomination.
- Each thesis should include a foreword by the supervisor outlining the significance of its content.
- The theses should have a clearly defined structure including an introduction accessible to scientists not expert in that particular field.

Mercè Pacios Pujadó

Carbon Nanotubes as Platforms for Biosensors with Electrochemical and Electronic Transduction

Doctoral Thesis accepted by
the Universitat Autònoma de Barcelona, Spain

Springer

Author
Dr. Mercè Pacios Pujadó
Universitat Autònoma de Barcelona
Spain

Supervisors
Prof. Dr. Maria José Esplandiu Egido
Centre d'Investigació en Nanociència
i Nanotecnologia (CIN2)
Barcelona
Spain

Prof. Dr. Manel del Valle Zafra
Universitat Autònoma de Barcelona
Spain

ISSN 2190-5053 ISSN 2190-5061 (electronic)
ISBN 978-3-642-31420-9 ISBN 978-3-642-31421-6 (eBook)
DOI 10.1007/978-3-642-31421-6
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2012941424

© Springer-Verlag Berlin Heidelberg 2012
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed. Exempted from this legal reservation are brief excerpts in connection with reviews or scholarly analysis or material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work. Duplication of this publication or parts thereof is permitted only under the provisions of the Copyright Law of the Publisher's location, in its current version, and permission for use must always be obtained from Springer. Permissions for use may be obtained through RightsLink at the Copyright Clearance Center. Violations are liable to prosecution under the respective Copyright Law.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
While the advice and information in this book are believed to be true and accurate at the date of publication, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Parts of this thesis have been published in the following journal articles:

M. J. Esplandiu, M. Pacios, E. Bellido, M. del Valle. Carbon nanotubes and electrochemistry. Zeitschrift fuer Physikalische Chemie (Muenchen, Germany). **2007**, 221 (9–10), 1161–1173.

M. Pacios, M. del Valle, J. Bartrolí, M. J. Esplandiu. Electrochemical behaviour of rigid carbon nanotube composite electrodes. Journal of Electroanalytical Chemistry. **2008**, 619–620, 117–124.

M. J. Esplandiu, M. Pacios, L. Cyganek, J. Bartrolí, M. del Valle. Enhancing the electrochemical response of Myoglobin with carbon nanotube electrodes. Nanotechnology. **2009**, 20 (35), 5502.

M. Pacios, M. del Valle, J. Bartrolí, M. J. Esplandiu. Electrocatalyzed O_2 response of myoglobin immobilized on multi-walled carbon nanotube forest electrodes. Journal of Nanoscience and Nanotechnology. **2009**, 9 (10), 6132–6138 (7).

M. Pacios, I. Martín-Fernández, R. Villa, P. Godignon, M. Del Valle, J. Bartrolí and M. J. Esplandiu (**2011**). Carbon Nanotubes as Suitable Electrochemical Platforms for Metalloprotein Sensors and Genosensors, Carbon Nanotubes - Growth and Applications, Dr. Mohammad Naraghi (Ed.), ISBN: 978-953-307-566-2, InTech.

M. Pacios, Nihan Yilmaz, I. Martín-Fernández, R. Villa, P. Godignon, M. Del Valle, J. Bartrolí, M. J. Esplandiu. A simple Approach for DNA detection on Carbon Nanotube Microelectrode Arrays. Sensors & Actuators: B. Chemical. **2012**, 162 (1).

To my parents

A very neat job in a small space

Supervisors' Foreword

The convergence of nano and biotechnology, also known as nanobiotechnology, is a promising up-the-front science topic for improving materials and processes. Within this new field, the development of (bio)sensors from nanomaterials is an exciting area which is already providing real-life applications in the biotechnological, clinical, and environmental fields. In this context, electrochemical nanobiosensors are a preferred choice thanks to their advantages in terms of simplicity, robustness, low cost, miniaturization capability, and integration in microfabricated devices.

Historically, carbon has been widely used as electrode material due to its desirable properties for electrochemical applications. Available in a variety of forms, carbon electrodes are recognized as versatile and easy handling devices, also praised by their rich surface chemistry which has been exploited to influence surface reactivity. More recently, the discovery and popularization of carbon nanotubes (CNTs) has fostered their use as electrode materials, improving reference properties, and propelling in an unprecedented way their electrochemical and electroanalytical applications. CNTs nanometric size and high aspect ratio are the distinct features which have contributed in a larger degree to innovate in electrochemical applications.

The doctoral thesis of Mercè Pacios aims at exploiting the properties of CNTs to design novel electrochemical (bio)sensing devices. Thanks to the prominent electrochemical properties of carbon nanotubes, the design of diverse electrode configurations was possible. This fact, combined with their chemical properties and (bio)functionalization versatility, have made these materials ideal candidates for the development of electrochemical biosensors.

In summary, CNTs have been assayed as electrochemical transducers, finding the factors that most influence the electrode activity. The possibility to arrange CNTs in different geometrical layouts also permitted the design of successively improved transducer platforms and biosensing devices, specially those incorporating enzymes, proteins, and DNA. Exploiting the semiconductor character of CNTs, a last variant has been assayed, which is the field effect transistor configuration (FET). The CNT-FET device, optimized for operating in liquid environment, was used to probe in real-time protein/CNT adsorption and protein/aptamer interactions. Results of this thesis work have shown that these electrochemical and electronic CNT devices can indeed become highly promising for biomolecule sensing and for the sensitive monitoring of biological processes. Throughout this work, new and unexpected gateways have been opened to keep and continue exploring the fascinating world of nanoscience.

Barcelona, May 2012

Dr. Maria José Esplandiu Egido
Dr. Manel del Valle Zafra

Contents

Abbreviations and Symbols

AC	Alternating current
AFM	Atomic force microscopy
BSA	Bovine serum albumin
Cat	Catalase
CC	Chronocoulometry
C-C	Carbon–carbon bond
C_{dl}	Double-layer capacitance
CNT	Carbon nanotube
CNT-FET	Carbon nanotube field-effect transistor
CPE	Constant phase element
CV	Cyclic voltammetry
CVD	Chemical vapour deposition
(D)	Drain electrode
D	Dimensional (1D, 2D, 3D)
DC	Direct current
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
DOS	Density of electronic states
DPV	Differential pulse voltammetry
ΔE_p	Peak potential difference
EDC	1-ethyl-3(3-dymethyl amino propyl carbodiimide hydrochloride
EDS	Energy-dispersive X-ray spectroscopy
E_f	Fermi energy
EG	Ethylene glycol
EIS	Electrochemical impedance spectroscopy
ESCA	Electron spectroscopy for chemical analysis
ET	Electron transfer
eV	Electron volt
F	Faraday’s constant
FET	Field-effect transistor
G	Conductance
(G)	Gate electrode
GC	Glassy carbon

GEC	Graphite-epoxy composite
g_m	Transconductance
GOx	Glucose oxidase
HOPG	Highly ordered pyrolytic graphite
i	Electrical current
K^o	Electron transfer rate constant
LEDs	Light-emitting diodes
LOD	Limit of detection
Mb	Myoglobin
MOSFET	Metal-oxide-semiconductor field-effect transistor
MWNT	Multi-walled carbon nanotube
NHS	N-hydroxysuccinimide
NSB	Non-specific binding
NSL	Nanosphere lithography
1-PBASE	1-(pyrene)butyric acid N-hydroxysuccinimide ester
PBS	Phosphate buffer solution
PECVD	Plasma Enhanced Chemical Vapour Deposition
PEG	Polyetylenglycol
PL	Photoluminescence
PMMA	Polymethylmethacrylate
Q	Charge
R	Ideal gas constant
RBM	Ring breathing modes
R_{ct}	Charge transfer resistance
RIE	Reactive Ion etching
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RTCVD	Rapid-thermal CVD
(S)	Source electrode
SB	Schottky barrier
SB_W	Schottky barrier thickness
SB_H	Schottky barrier height
SCE	Saturated calomel electrode
SELEX	Selection evolution of ligands with exponential enrichment
SEM	Scanning electron microscopy
STM	Scanning tunnelling microscopy
SW	Stone-wales
SWNT	Single-walled carbon nanotube
T	Temperature
TEM	Transmission electron microscopy
TRIS	Tris(hydroxymethyl)aminomethane
UMA	Ultramicroelectrode array
VACNT	Vertically aligned carbon nanotube
V_G	Gate-to-source voltage
V_{SD}	Source-to-drain voltage

V_{thr}	Threshold gate voltage
W	Warburg impedance
XPS	Spectroscopy X-ray photoelectron
Z	Electrochemical impedance

Chapter 1
Introduction

Carbon nanotubes (CNTs) have become one of the most exciting and extensively studied materials of the last two decades. They have captured the interest as nanoscale materials due to their nanometric structure and their impressive list of superlative and outstanding properties. All these ingredients have encouraged their exploitation for promising applications. One of the most interesting ones is related with the use of CNTs as electrochemical platforms for biosensing purposes, the topic in which the present thesis is framed. Accordingly, the main aim of this introductory chapter is to explain the fundamental concepts of the building blocks that constitute this thesis. Therefore, Sect. 1.1 introduces the transducer element: carbon nanotubes (CNT). In this section, the properties of CNTs, their synthesis and purification are explained. Section 1.2 describes the different carbon nanotube platforms developed for biosensor purposes and their fundamentals. Section 1.3 describes the biological recognition elements used for sensing events on the different CNT platforms. Finally, Sect. 1.4 provides an overview of the fundamentals of the main techniques that have allowed characterizing the biosensor devices and following the sensing events.

1.1 Novel Sensing Materials

Nanoscience and Nanotechnology address the study, control, manipulation, and assembly of nanometre(nm) scale components into materials, systems and devices for human interest and needs [1]. The rapid progress of nanotechnology and advanced nanomaterial production offer significant opportunities for designing powerful sensing devices with enhanced performances. Such nanomaterials can exhibit properties and functions different from the ones corresponding to bulk or macroscopic version of them. Additionally, such nanostructures can become suitable materials that favour the integration with biomaterials or biological systems. Under this context, carbon nanotubes have been exploited as a novel material with huge potential in bioanalytical and biosensing applications.

M. Pacios Pujadó, *Carbon Nanotubes as Platforms for Biosensors with Electrochemical and Electronic Transduction*, Springer Theses, DOI: 10.1007/978-3-642-31421-6_1,

1.1.1 Carbon Nanotubes: Structure and Properties of Carbon Nanotubes

This section will be addressed to the discovery, structure and properties of carbon nanotubes. Specifically, special attention will be given to the electronic, mechanical, chemical, electrochemical and optical properties which lead to immediate applications of the CNTs.

1.1.1.1 Discovery, Structure and Electronic Properties

The ability to form very long chains interconnecting C–C covalent bonds allows carbon to form an almost infinite number of compounds. Being one of the most versatile elements, carbon is the chemical basis of all known living systems on Earth; it is the fundamental element of many important biological compounds including sugars, DNA and proteins.

Until few decades ago, it was thought that there were only three structurally different forms or allotropes of carbon since they were abundant in nature: the hardest substance, diamond; one of the softest known substances, the layered graphite and the non-crystalline form, amorphous carbon. However, in the last time we have been witnesses of the discovery of two other carbon allotropes, the fullerene and the carbon nanotubes.

The true identity of the discoverers of carbon nanotubes is a subject of some controversy. For years, scientists assumed that Sumio Iijima, a Japanese physicist, had discovered CNTs in 1991. He published a paper describing his discovery which initiated a flurry of excitement and could be credited by inspiring the many scientists now studying applications of carbon nanotubes. Though Iijima has been given much of the credit for discovering carbon nanotubes, it turns out that the timeline of CNTs goes back much further than 1991.

In 1952, two Russian scientists gave the world its first clear look at carbon nanotubes. LV Radushkevich and VM Lukyanovich published clear images showing multi-walled carbon nanotubes (MWNTs) with a 50 nm diameter (the first known, transmission electron microscope images of carbon nanotubes). Unfortunately, their findings were not given much publicity: their paper was in Russian, published in a Russian journal (Journal of Physical Chemistry of Russia), and was the period of the Cold War.

Before they came to be known as carbon nanotubes, in 1976, Endo, Koyama and Oberlin, observed hollow tubes of rolled up graphite sheets synthesised by a chemical vapour-growth technique [2]. The first specimens observed would later come to be known as single-walled carbon nanotubes (SWNTs). The three scientists were also the first ones to show images of a nanotube with a solitary graphene wall.

In 1981, Russian scientists published more findings. The carbon multi-layer tubular crystals (as they were known then) were made by rolling graphene layers into cylindrical shapes. In 1987, Howard Tennet was given a patent for his cylindrical discrete carbon fibrils.

In 1985 the three Nobel Price Winners, Robert F. Curl, Sir Harold W. Kroto and Richard E. Smalley performed experiments that aimed at understanding the mechanisms by which long chained carbon molecules are formed in interstellar space and circumstellar shells. Graphite was vaporized by laser irradiation, producing a remarkably stable cluster consisting of 60 carbon atoms: the first buckminsterfullerene C_{60} [3].

The research gained new impetus when it was shown in 1990 that C_{60} could be produced in a simple arc-evaporation apparatus readily available in all laboratories. It was just by analysing samples from such evaporator that the Japanese scientist Sumio Iijima of the NEC Corporation discovered fullerene-related carbon nanotubes in 1991 [4]. These were elongated fullerenes with diameters as small as 0.7 nm and lengths of up to several microns which were termed carbon nanotubes.

The graphene layers have become the starting point to explain the structure of carbon nanotubes. A single-walled carbon nanotube is a rolled-up tubular shell of graphene sheet which is made up of benzene-type hexagonal rings of carbon atoms. The structure is conveniently expressed in terms of a one-dimensional unit cell. Indeed, the appearance of a closed cage in CNTs can be easily rationalized by considering the presence of high energy dangling bonds at the boundaries of a finite graphene layer. The total energy of carbon atoms in a layer can be reduced by promoting the formation of a closed structure which eliminates the dangling bonds, even at the expense of increasing the strain energy.

The way the graphene sheet is wrapped up can be described by a pair of indices (n, m) that define the chiral vector, $\vec{C} = n\vec{a}_1 + m\vec{a}_2$, *in* which $\vec{a}_1$ and $\vec{a}_2$ are the basis vectors of the hexagonal graphene lattice [5–10] as shown in Fig. 1.1. Three different types of nanotube structures can be generated by rolling up the graphene layer: zigzag $(m = 0)$, armchair $(n = m)$ and chiral nanotubes (the rest of vectors).

Although CNTs are closely related to 2D graphene, the tube curvature and the quantum confinement[1] in the circumferential direction of the nanotube bring about the unique properties that make CNTs different from graphene. One of these unusual properties is the electronic conductivity which strongly depends on the chirality and CNT diameter. CNTs can exhibit singular electronic band structures and can show metallic and semiconducting behaviour. As a general rule, (n, m) tubes with $n - m$ being an integer multiple of 3 are metallic while the remaining tubes are semiconducting. The band gap of semiconducting tubes can be approximated by the relation $E_g = 0.8\,\mathrm{eV}/d$, with d being the diameter of the nanotube [11, 12]. Therefore, the bigger the diameter, the more metallic behaviour is found.

The verification of the electronic properties of carbon nanotubes was of great interest in light of the theoretical predictions. It turned out to be very challenging to measure them due to the small diameter of the tubes. At the beginning, many of

---

[1] The electron wavelength around the circumference of a nanotube is quantized due to periodic boundary conditions. Along the tube the electrons are not confined.

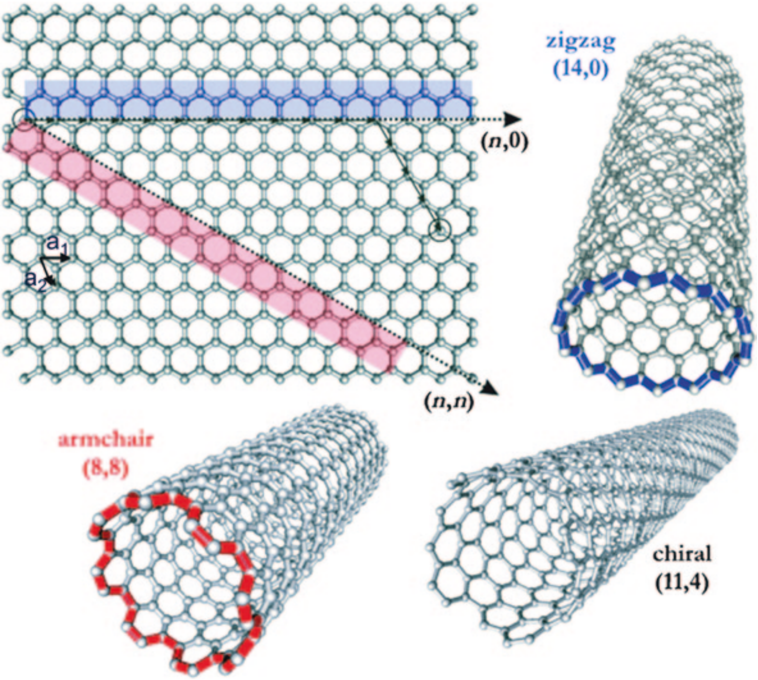


Fig. 1.1 Roll-up of a graphene sheet leading to three different types of SWNTs. Reprinted with permission from Ref. [10]

the studies on the electrons properties were on bulk CNT material by performing, for instance, electron spin resonance. Then, a more sophisticated methodology was employed based on scanning tunnelling microscopy (STM) which allowed addressing individual carbon nanotubes. The tip of the STM was used as a spectroscopic probe to collect the tunnelling conductance of each tube, providing a direct measure of the local electronic density of states (DOS)[2] of the nanotube. Since the STM has the additional power to obtain atomically resolved images of the tube's hexagon lattice, the electronic structure could be correlated with the chiral structure of the tube and with the carbon nanotube semiconducting or metallic properties.

Figure 1.2 shows the density of electronic states for a metallic and a semiconducting carbon nanotube. The DOS is not a continuous function of energy as in the case of bulk 3D materials (e.g. graphite) but they present discontinuous spikes which are typically present in one-dimensional materials (Van Hove singularities).

---

[2] Density of states (DOS) of a system describes the number of electron states per unit volume and per unit energy that are available to be occupied.

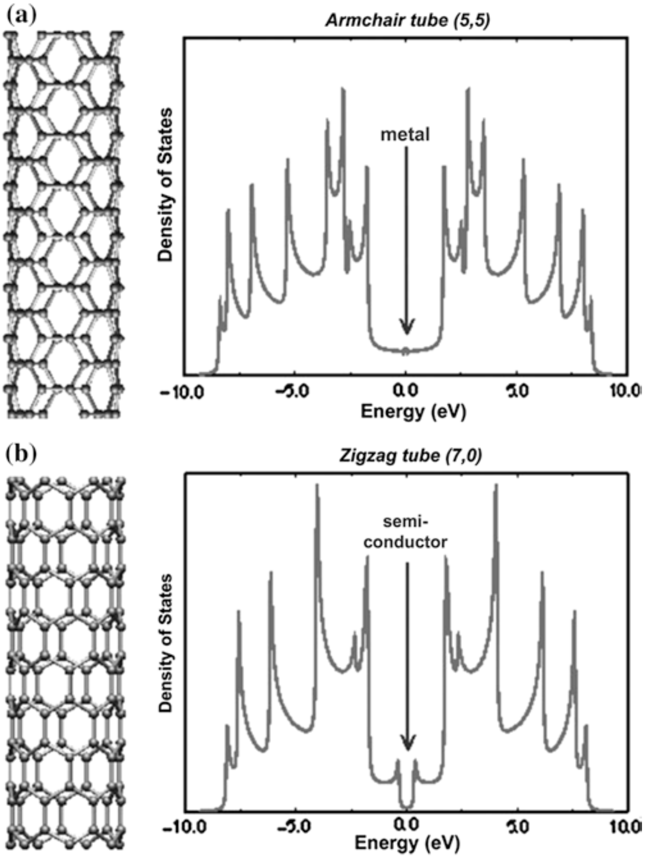


Fig. 1.2 Density of states (DOS) for a metallic (**a**) and semiconducting (**b**) carbon nanotube with the sharp Van Hove singularities. The density of states at the Fermi energy (E = 0) (Fermi energy is the energy of the highest occupied state at the absolute zero temperature.) for the metallic tube is finite but zero for the semiconducting one, providing a band-gap in the latter case. The DOS at the right of the Fermi energy are part of the conduction band (unoccupied states) whereas the one at the left corresponds to the valence band (occupied states)

Another way to verify the electronic properties of carbon nanotubes is by performing electron transport studies by contacting them in a field-effect transistor (FET) configuration. In this configuration, three electrodes are employed. Two of them (source and drain electrodes) are used to contact the nanotube and allow the flow of current through the tube when a voltage is applied between them. The third electrode (gate) is separated from the nanotube by a dielectric, and when a voltage is applied through such electrode, a modulation of the tube current is produced. The charge carriers of the carbon nanotube are modulated in a big extent if the contacted nanotube is a semiconducting but the nanotube conductance remains almost constant if the nanotube is metallic. Therefore, by sweeping the