Nanomaterials based microarray platforms for biodetection

PhD Thesis

Eden Morales Narváez

Doctoral programme in Biomedical Engineering

Departament d'Enginyeria de Sistemes, Automàtica i Informàtica Industrial

Universitat Politècnica de Catalunya

June 2013

Nanomaterials based microarray platforms for biodetection

Dissertation submitted to obtain the degree of Doctor from the Universitat Politècnica de Catalunya by

Eden Morales-Narváez

Universitat Politècnica de Catalunya
Departament d'Enginyeria de Sistemes, Automàtica i Informàtica Industrial
Doctoral programme in Biomedical Engineering

Adviser: **Arben Merkoçi**
Tutor: **Pere Joan Riu Costa**

June 2013

To my wife, my family, my friends, my teachers and my wonderful research group.

Acknowledgments

I will always be grateful for being accepted as a pre-doctoral researcher by Professor Arben Merkoçi at the Catalan Institute of Nanotechnology. I have been honoured to work and learn side by side with extraordinary colleagues. Thanks to all of them for the nice moments we have spend together. I would also like to thank the academic support of my tutor Pere J Riu.

I acknowledge funding from CONACYT (Mexico), through a fellowship grant. MICINN through project MAT2011-25870 and E.U. through FP7 "NADINE" project (contract number 246513) have funded this research.

Preface

This PhD dissertation has been developed from March 2011 to May 2013 at the Catalan Institute of Nanotechnology. This work has been supported by CONACyT (Mexico) through a fellowship grant awarded to the author. Moreover, this research has been supported by the EU through the NADINE project (Nanosystems for early Diagnosis of Neurodegenerative Diseases, Program: FP7 -NMP-2009-4.0-3 –) and the MICINN (Spain) through the project MAT2011-25870.

According to the authorization of the Doctoral School of the UPC, which was received on June 7th 2013, this dissertation is presented as an article thesis in the Doctoral programme in Biomedical Engineering of the UPC. It includes the following peer-reviewed publications, which have until now obtained a total of 42 citations:

- Eden Morales-Narváez, Helena Montón, Anna Fomicheva, Arben Merkoçi, Signal enhancement in antibody microarray using Quantum Dots nanocrystals: Application to potential Alzheimer biomarker screening, *Analytical Chemistry* **2012**, 84:6821. (5 citations)

- Eden Morales-Narváez, Arben Merkoçi, Graphene Oxide as an Optical Biosensing Platform, *Advanced Materials* (front cover article) **2012**, 24:3298. (30 citations)

- Eden Morales-Narváez, Briza Pérez-López, Luis Baptista Pires, Arben Merkoçi, Simple Förster resonance energy transfer evidence for the ultrahigh quantum dot quenching efficiency by graphene oxide compared to other carbon structures, *Carbon* **2012,** 50:2987. (7 citations)

Additionally, the Catalan Institute of Nanotechnology is currently conducting a patentability study on a system for pathogen screening designed by the author. Since the details of this system cannot be revealed at this stage, this system is briefly discussed in the general conclusions.

The author has also written a book chapter that will be published in July/August 2013.

- Medical Nanobiosensors, in Nanomedicine: principles and perspectives, Eds. Yi Ge, Songjun Li, Shenqi Wang, Richard Moore, Springer, New York, 2012. Ch 7, ISBN: 978-1-4614-2139-9.

Part of this PhD thesis has been presented in the following conference proceedings/talks:

- Gaphene based platforms for biosensing applications. Graphene International Conference, Bilbao, Spain. Presentation. 2013

- Graphene and biosensors. IPN-UPIITA, D.F., Mexico. Talk. 2012

- Biosensors based on nanotechnology. IPN-UPIITA, D.F., Mexico. Talk. 2012

- QDs versus fluorescent dyes in microarray based biomarkers screening. Biosensors 2012, Cancun, Mexico. Poster. 2012

- Optical biosensors based on graphene. Graphene International Conference, Brussels, Belgium. Poster. 2012

- Optical biosensors based on graphene. NanoSpain, Santander, Spain. Talk. 2012

- Graphene in biosensors applications, Sectorial Meeting, ICFO, Barcelona, Spain. Talk. 2011

- Alzheimer biomarker screening using microarrays, XVI Transfrontaliar meeting Sensors and Biosensors, Toulouse, France. Talk. 2011

This PhD thesis consists of 4 chapters, which aim at demonstrating how the fusion between nanomaterials and microarray technology exhibits enormous possibilities towards biomarker screening, food safety and environmental monitoring. The first chapter is a brief introduction to nanomaterials, nanobiosensing technology, microarray technology and the endeavour of the integration of nanomaterials into microarray technology. The second chapter describes the advantageous behaviour that microarray technology can display when quantum dots nanocrystals are incorporated in order to screen a potential Alzheimer biomarker (*Anal. Chem.* **2012**, 84:6821). Since graphene oxide is a very recently discovered nanomaterial and microarray technology relies on optical signals, the advantageous photonics properties of graphene oxide in biosensing are widely discussed in the second chapter (*Adv. Mater.* **2012**, 24:3298). In the third chapter, graphene oxide has been studied as a highly efficient quencher of quantum dots by using a microarray scanner and finally such interaction is proposed as a highly sensitive transduction system for biodetection (*Carbon* **2012**, 50:2987). The fourth chapter includes a general discussion and the overall conclusions of this research.

Abstract

Analytical disciplines are an important field for the progress of healthcare and medicine. In fact the technologies related to analytical disciplines may reveal important information for early diagnosis, treatment of diseases, food safety and environmental monitoring. In this regard, novel advances in analytical disciplines are highly desired. As a promising tool, biosensors are useful systems that enable the detection of agents with diagnostic interest. Since nanotechnology enables the manipulation and control at the nanoscale, biosensors based on nanotechnology offer powerful capabilities to diagnostic technology. In this dissertation, the advantages of the integration of nanomaterials into microarray technology are widely studied, generally in terms of sensitivity. Particularly, the performance of cadmium-selenide/zinc-sulfide (CdSe@ZnS) quantum dots (QDs) and the fluorescent dye Alexa 647 as reporter in an assay designed to detect apolipoprotein E (ApoE) has been compared. The assay is a sandwich immunocomplex microarray that functions via excitation by visible light. ApoE was chosen for its potential as a biomarker for Alzheimer's disease. The two versions of the microarray (QD or Alexa 647) were assessed under the same experimental conditions. The QDs proved to be highly effective reporters in the microarrays, although their performance strongly varied in function of the excitation wavelength. At 633 nm, the QD microarray, at an excitation wavelength of 532 nm, provided a limit of detection (LOD) of ~62 pg mL^{-1}, five times more sensitive than that of the Alexa microarray (~307 pg mL^{-1}). Finally, serial dilutions from a human serum sample were assayed with high sensitivity and acceptable precision and accuracy (*Anal. Chem.* **2012**, 84:6821).

Since graphene oxide (GO) is a recently discovered nanomaterial and microarray technology relies on optical signals, the photonic properties of GO are discussed and the state-of-the-art of GO in optical biosensing has been widely documented (*Adv. Mater.* **2012**, 24:3298). Furthermore, GO has been studied as a highly efficient quencher of QDs, reporting a quenching efficiency of nearly 100%. Finally, such interaction between GO and QDs has been proposed as a highly sensitive transduction system for microarray-based biodetection (*Carbon* **2012**, 50:2987). This research aims at demonstrating how the endeavour of the fusion between nanomaterials and microarray technology exhibits enormous possibilities towards biomarker screening, food safety and environmental monitoring.

Keywords: Biosensors, Quantum dots, Fluorescence resonance energy transfer, Graphene oxide, microarray technology.

Table of Contents

List of figures and tables

Simple Förster resonance energy transfer evidence for the ultrahigh quantum dot quenching efficiency by graphene oxide compared to other carbon structures

Nanomaterials Based Microarray Platforms for Biodetection

Introduction

The concept of diagnosis based on biological samples dated back several thousand years ago documented from the ancient China, Egypt to the Middle Ages of Europe.[1] Nevertheless, it was not until the 60's when Professor Leland C Clark Jnr., as the father of the biosensor concept, described how to perform reliable and robust measurements of analytes (molecules of interest) presents in the body.[2] Presently, cancer can be diagnosed by screening the levels of the appropriate analytes existing in blood and likewise diabetes is inspected by measuring glucose concentrations. Moreover, the most conventional techniques of diagnostic technologies are the enzyme-linked immunosorbent Assay (ELISA) and the polymerase chain reaction (PCR). Nevertheless, these techniques report different handicaps such as high cost and time required, significant sample preparation, intensive sample handling, and can become troublesome to patients. Accordingly, novel advances in diagnostic technology are highly desired.

Diagnostic technology is an important field for the progress of healthcare and medicine. In fact this technology may reveal important information for early diagnosis, treatment of diseases, food safety and environmental monitoring.

A biosensor is defined by the International Union of Pure and Applied Chemistry (IUPAC) as a "device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles, or whole cells to detect chemical compounds usually by electrical, thermal, or optical signals".[3] Generally, biosensors include biorecognition probes (responsible for the specific detection of the analytes) and a transducer element (which converts a biorecognition event into a suitable signal). In the 21st century, nanotechnology has been revolutionizing many fields including medicine, biology, chemistry, physics, and electronics. In this way, biosensors have also been benefited by nanotechnology, which is an emerging multidisciplinary field that entails the synthesis and use of materials or systems at the nanoscale (normally 1 to 100 nm). The rationale behind this technology is that nanomaterials possess optical, electronic, magnetic or structural properties that are unavailable for bulk materials. Since nanomaterials range in the same scale of the diagnostic molecules, when linked to biorecognition probes (such as antibodies, DNA and enzymes), nanostructures allow the control, manipulation and detection of molecules with diagnostic interest, even at the single molecule level. Normally, nanobiosensors are based on nanomaterials or nanostructures as transducer elements or reporters of biorecognition events. Figure 1 displays the schematic representation of a nanobiosensor.

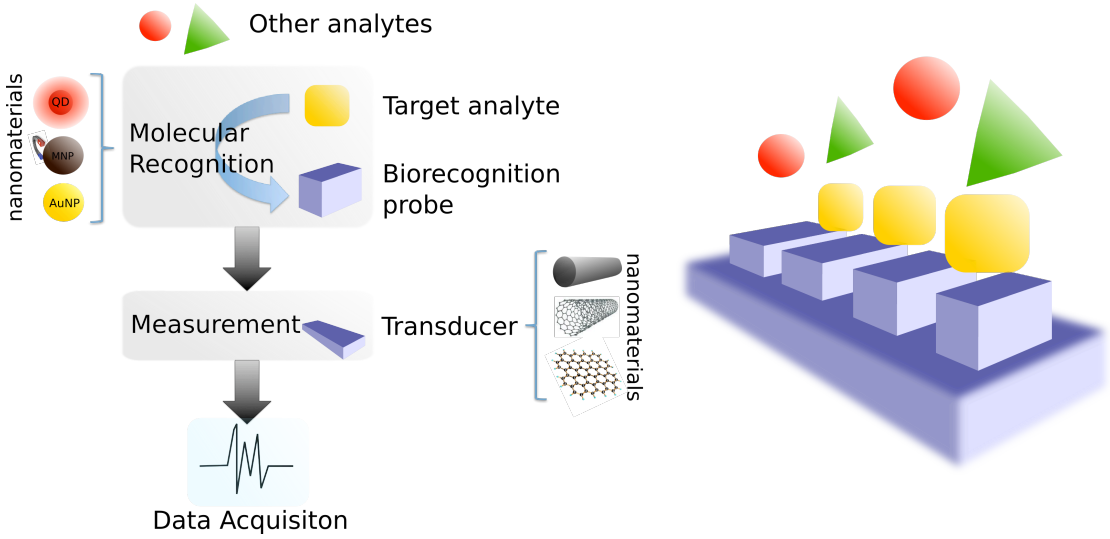

Figure 1. Schematic representation of a nanobiosensor. Normally, a nanobiosensor relies on nanomaterials as transducer elements or reporters of biorecognition events.

Biorecognition probes

Biorecognition probes, or molecular bioreceptors, are the key in the specificity of biosensors (a non-specific biorecognition event can yield a false result). Biomolecular recognition generally entails different interactions such as hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, pi-pi interactions and electrostatic interactions. In this section the most common biorecognition probes in nanaobiosensors are briefly discussed (see figure 2).

Antibodies

Antibodies are soluble forms of immunoglobulin containing hundreds of individual amino acids arranged in a highly ordered sequence. These polypeptides are produced by immune system cells (B lymphocytes) when exposed to antigenic substances or molecules. Proteins with molecular weights greater than 5000 Da are generally immunogenic. Antibodies contain in their structure recognition/binding sites for specific molecular structures of the antigen. Since an antibody interacts in a highly specific way with its unique antigen, antibodies are the key point of the so-called immunoassays and are widely employed in biosensing.

Aptamers

Aptamers are novel artificial oligonucleic acid molecules that are selected (in vitro) for high affinity binding to several targets such as proteins, peptides, amino acids, drugs, metal ions and even whole cells.[4–6]

Enzymes

Enzymes are protein catalysts of remarkable efficiency involved in chemical reactions fundamental to the life and proliferation of cells. Enzymes also possess specific binding capabilities and were the pioneer molecular recognition elements used in biosensors[7] and continue still used in biosensing applications.[8,9]

Nucleic Acids

Since the interaction between adenosine and thymine and cytosine and guanosine in DNA is complementary, specific probes of nucleic acids offer sensitive and selective detection of target genes in biosensors.[10]

Transduction modes

In order to detect biorecognition events, biosensors require a transduction mode. Transduction modes are generally classified according to the nature of their signal into the following types: 1) optical detection, 2) electrochemical detection, 3) electrical detection, 4) mass sensitive detection and 5) thermal detection.

Optical detection

Optical biosensing is based on several types of spectroscopic measurements (such as absorption, dispersion spectrometry, fluorescence, phosphorescence, Raman, refraction, surface enhanced Raman spectroscopy, and surface plasmon resonance) with different spectrochemical parameters acquired (amplitude, energy, polarization, decay time and/or phase). Among these spectrochemical parameters, amplitude is the most commonly measured, as it can generally be correlated with the concentration of the target analyte.[11]

Electrochemical detection

Electrochemical detection entails the measurement of electrochemical parameters (such as current, potential difference or impedance) of either oxidation or reduction reactions. These electrochemical parameters can be correlated to either the concentration of the electroactive probe assayed or its rate of production/consumption.[11]

Electrical detection

Electrical detection is often based on semiconductor technology by replacing the gate of a metal oxide semiconductor field effect transistor with a nanostructure (usually nanowires or graphitic nanomaterials). This nanostructure is capped with biorecognition probes and a electrical signal is triggered by biorecognition events.[12,13]

Mass sensitive detection

Mass sensitive detection can be performed by either piezoelectric crystals or microcantilevers. The former relies on small alterations in mass of piezoelectric crystals due to biorecognition events. These events are correlated with the crystals oscillation frequency allowing the indirect measurement of the analyte binding.[14] Microcantilever biosensing principle is based on mechanical stresses produced in a sensor upon molecular binding. Such stress bends the sensor mechanically and can be easily detected.[15]

Thermal detection

Thermal biosensors are often based on exothermic reactions between an enzyme and the proper analyte. The heat released from the reaction can be correlated to the amount of reactants consumed or products formed.[16]

Nanomaterials: The nanobiosensors toolbox

Recent advances of the nanotechnology focused on the synthesis of materials with innovative properties have led to the fabrication of several nanomaterials such as nanowires, quantum dots, magnetic nanoparticles, gold nanoparticles, carbon nanotubes and graphene. These nanomaterials linked to biorecognition probes are generally the basic components of nanobiosensors. In order to attach nanomaterials with biorecognition probes, nanomaterials are either electrostatically charged or functionalized with the suitable chemically active group.[17–21] In the following section the most widely used nanomaterials in biosensing are briefly described and they are sketched in figure 2.

Zero-dimensional nanomaterials

Quantum Dots (QDs)

QDs are semiconductors nanocrystals composed of periodic groups of II–VI (e.g., CdSe) or III–V (InP) materials. QDs range from 2 to 10 nm in diameter (10 to 50 atoms). They are robust fluorescence emitters with size-dependent emission wavelengths. For example, small nanocrystals (2 nm) made of CdSe emit in

the range between 495 to 515 nm, whereas larger CdSe nanocrystals (5nm) emit between 605 and 630 nm. [22] QDs are extremely bright (1 QD ≈ 10 to 20 organic fluorophores).[23] They have high resistance to photobleaching, narrow spectral linewidths, large stokes shift and even different QDs emitters can be excited using a single wavelength, i.e. they have a wide excitation spectra.[24,25] Because of their properties QDs are used in biosensing as either fluorescent probes[26,27] or labels for electrochemical detection[28].

Gold Nanoparticles (AuNPs)

Synthesis of AuNPs often entails the chemical reduction of gold salt in citrate solution. Their scale is less than about 100nm. AuNPs have interesting electronic, optical, thermal and catalytic properties. [29,30] AuNPs enable direct electron transfer between redox proteins and bulk electrode materials and are widely used in electrochemical biosensors, as well as biomolecular labels.[31,32]

Magnetic nanoparticles (MNPs)

MNP are often composed by iron oxide and due to their size (20 – 200 nm) they can possess superparamagnetic properties. MNP are used as contrast agents for magnetic resonance imaging and for molecular separation in biosensors devices.[33–35]

One-dimensional nanomaterials

Carbon Nanotubes (CNTs)

CNTs consist of sheets (multi-walled carbon nanotubes, MWCNTs) or a single sheet (single-walled carbon nanotubes SWCNTs) of graphite rolled-up into a tube. Their diameters range about from 5 to 90 nm. The lengths of the graphitic tubes are normally in the micrometer scale. CNTs seem a remarkable scheme of excellent mechanical, electrical and electrochemical properties[36,37] and even can display metallic, semiconducting and superconducting electron transport.[38] The properties of carbon nanotubes are highly attractive for electrochemical biosensors and also has been used as transducer in bio-field-effect transistors.[39,40]

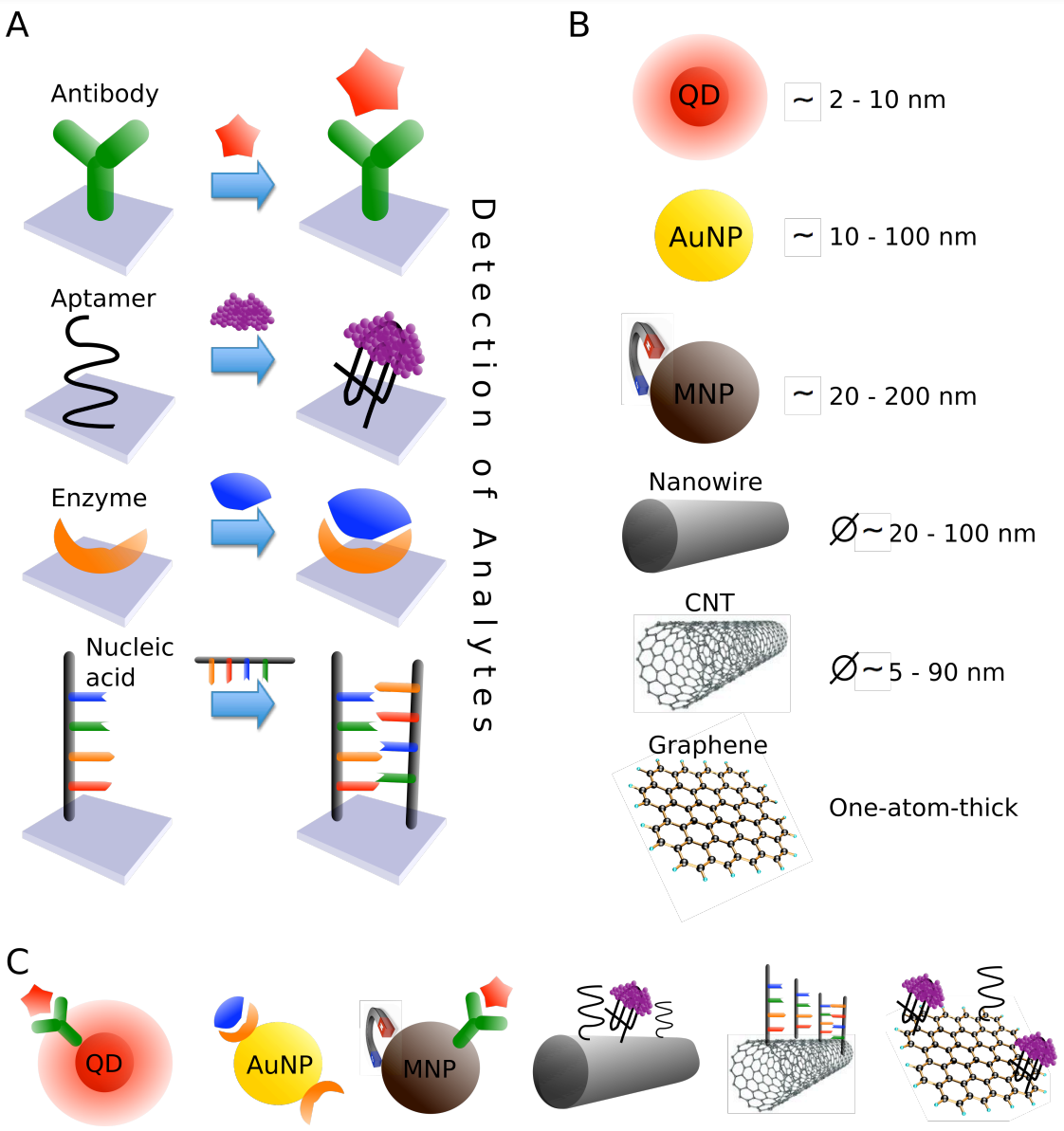

Figure 2. Biorecognition probes and nanomaterials. **A**. Biorecognition probes. **B**. Nanomaterials. **C**. Nanomaterials decorated with biorecognition probes. QD, Quantum Dot; AuNP, gold nanoparticle; MNP, magnetic nanoparticle. Sketches are not at scale.

Nanowires

Nanowires are planar semiconductors with a diameter ranging from 20 to 100 nm and length from submicrometer to few micrometer dimensions. They are fabricated with materials including but not limited to silicon, gold, silver, lead, conducting polymer and oxide.[41,42] They have tunable conducting properties and can be used as transducers of chemical and biological binding events in electrically based sensors such as bio-field-effect transistors.[43–45]

The innovative two-dimensional material: graphene

Graphene is a recently discovered one-atom-thick planar sheet of sp^2 bonded carbon atoms ordered in a two-dimensional honeycomb lattice and is the basic building block for carbon allotropes (eg. fullerens, CNTs and graphite). Graphene has displayed fascinating properties such as electronic flexibility, high planar surface, superlative mechanical strength, ultrahigh thermal conductivity and novel electronic properties.[46] Owing to its properties, graphene has been employed as transducer in bio-field-effect transistors, electrochemical biosensors, impedance biosensors, electrochemiluminescence, and fluorescence biosensors, as well as biomolecular labels.[47,48]

Antibody Microarray Technology

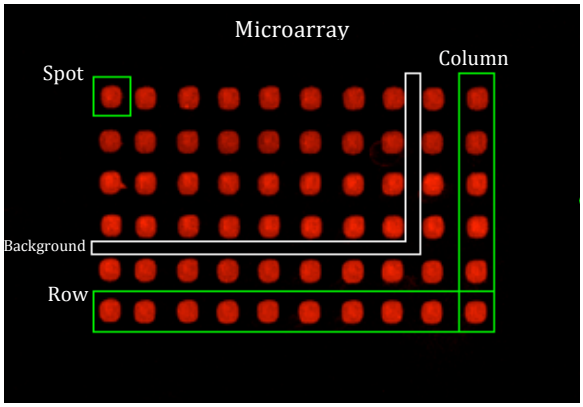

Figure 3. Image of an antibody microarray. Microarrays foreground is integrated by microscopic target elements (spots) and is ordered by rows and columns. Background contains unspecific signals.

Protein microarrays based biosensing is an area of active research with high potential for the development of novel multiplexed diagnostic assays. This biosensing technology often relies on fluorescent signals, provided from microarrayed labeled molecules over glass slides, so as to estimate the amount of analytes concentration after assay steps. Antibody microarrays is a technology that enables to quantify target proteins into a multiplexed assay (see figure 3). These analytical devices posses four distinct characteristics: (a) microscopic target elements or spots, (b) planar substrates (printing surface), (c) rows and columns of elements and (d) specific binding between microarray biorecognition probes on the substrate (capture antibodies for antibody micorarrays) and the target molecules in solution (analytes). The number of analytes that can be assayed is equal to the number of different spotted biorecognition probes. These analytes are captured through a multiplexed immunoassay, which relies on the reaction between analytes and their specific antibodies.

Generally, microarray based biodetection implies: the printing of the biorecognition probes (i.e. antibodies) onto functionalized glass slides through a spotting robot (figure 4A), carrying out the assay

(including a fluorescent reporter of the performed biodetection, figure 4B), the imaging of the assayed slides with a microarray scanner and the measurement of the obtained images through a specialized software (e.g. GenePix) (figure 4C).

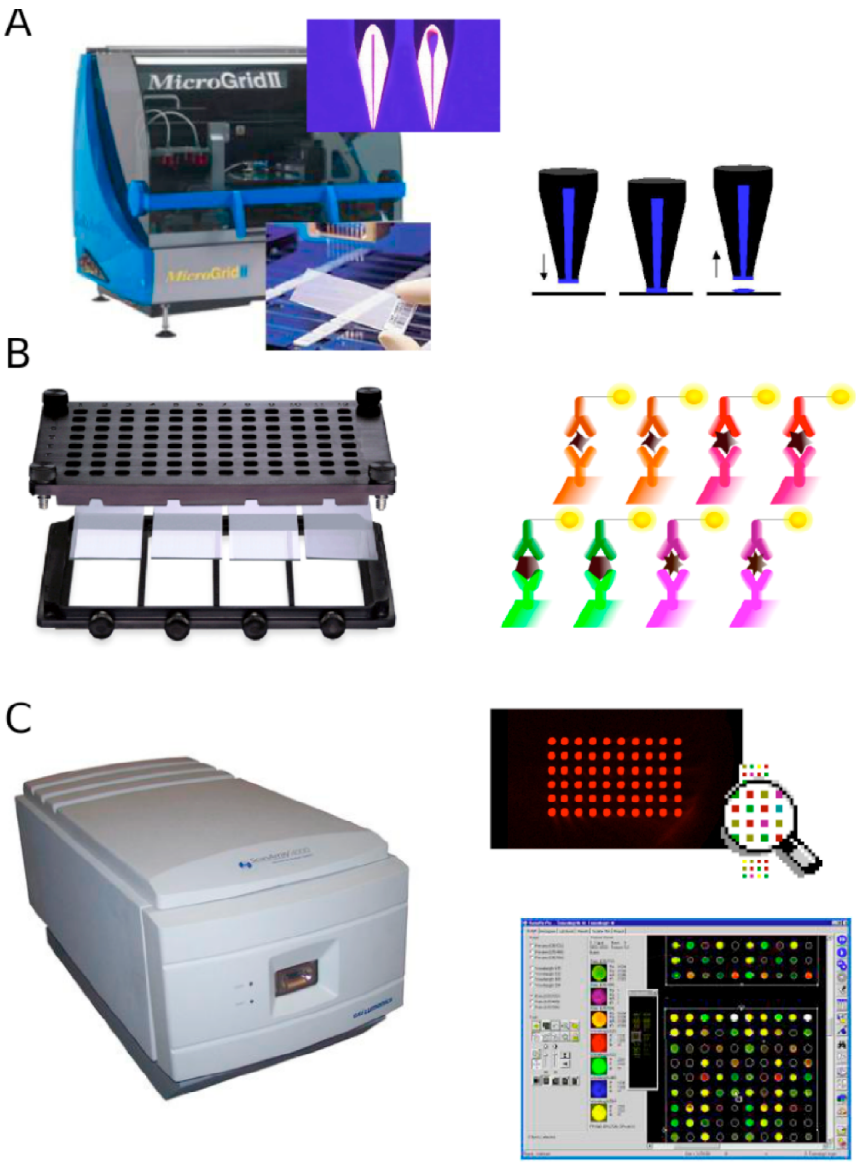

Figure 4. Microarray technology set-up. **A**. Printing process of the microarrays onto functionalized glass slides. **B**. Carrying out the assay and reporting the biodetection through fluorescent probes. **C**. Imaging and measurement of the assayed slides.

The endeavour of the integration of nanomaterials into microarray technology

As a powerful biosensing platform, microarray technology may enable novel biosystems. Moreover, biorecognition probes linked to nanomaterials (such as graphene oxide and quantum dots) provide extraordinary biomolecular receptors/reporters that selectively bind molecules such as: small pesticides, toxins, drugs, biopolymers (e.g. allergens) and complex biological structures like biomarkers, bacteria

and viruses. In spite of possessing enormous capabilities in clinical diagnosis, food safety and environmental monitoring; the endeavour of the integration of nanomaterials into microarray technology is a relatively little-explored field. For example, a search on the Web of Knowledge through the formula Topic=(microarray technology) AND Topic =(nanomaterial*) displays only 22 results (consulted on June 20th 2013). In this dissertation, the advantages of the integration of nanomaterials (such as quantum dots and graphene oxide) into microarray technology are widely studied, generally in terms of sensitivity.

Particularly, the performance of cadmium-selenide/zinc-sulfide (CdSe@ZnS) quantum dots (QDs) and the fluorescent dye Alexa 647 as reporter in an assay designed to detect apolipoprotein E (ApoE) has been compared. The assay is a sandwich immunocomplex microarray that functions via excitation by visible light. ApoE was chosen for its potential as a biomarker for Alzheimer's disease. The two versions of the microarray (QD or Alexa 647) were assessed under the same experimental conditions, and then compared to a conventional enzyme-linked immunosorbent assay (ELISA) targeting ApoE. The QDs proved to be highly effective reporters in the microarrays, although their performance strongly varied in function of the excitation wavelength. At 633 nm, the QD microarray gave an LOD of ~247 pg mL^{-1}; however, at excitation wavelength 532 nm, it provided a LOD of ~62 pg mL^{-1}—five times more sensitive that of the Alexa microarray (~307 pg mL^{-1}) and seven times more than that of the ELISA (~470 pg mL^{-1}). Finally, serial dilutions from a human serum sample were assayed with high sensitivity and acceptable precision and accuracy. (Published in *Analytical Chemistry*, see pages 12-18).

Since graphene oxide is a recently discovered nanomaterial and microarray technology relies on optical signals, the photonic properties of graphene oxide (GO) are discussed and the state-of-the-art of GO in optical biosensing has been widely documented. In fact, as an oxygenated lattice of donor/acceptor molecules exposed in a planar surface, GO enables unprecedented optical biosensing strategies to detect DNA, cancer biomarkers, viruses, and more. It has excellent capabilities for direct wiring with biomolecules, heterogeneous chemical and electronic structures, the ability to be solution-processed, and the ability to be tuned as either an insulator, semiconductor or semi-metal (published in *Advanced Materials*, see pages 19-30).

In this thesis, GO has been studied as a highly efficient quencher of quantum dots; i.e. in Förster resonance energy transfer (FRET). FRET entails the transfer of energy from a photoexcited energy donor to a close energy acceptor. In this regard, quantum dots (QDs), as donors, are quenched when they are next to an acceptor material. Graphite, carbon nanotubes (CNTs), carbon nanofibers (CNFs) and graphene oxide (GO) were explored as energy acceptors of QD FRET donors in the solid phase. In our set-up, using a microarray scanner, the higher estimated values of quenching efficiency for each material are as follows: graphite, 66 ± 17%; CNTs, 71 ± 1%; CNFs, 74 ± 07% and GO, 97 ± 1%. Among these materials, GO is the best acceptor of QD FRET donors in the solid phase. Such an ultrahigh quenching efficiency by

GO and the proposed simple mechanism may open the way to several interesting applications in the field of biosensing (published in *Carbon*, see pages 31-37). For example, it can be exploited in novel nano-enabled systems for food safety and environmental monitoring; particularly, for pathogen screening in microarray platforms. Therefore, as documented in the following content, which includes three peer-reviewed publications, the endeavour of the fusion between nanomaterials (e.g. graphene oxide and quantum dots) and microarray technology exhibits enormous capabilities towards several applications such as clinical diagnosis, food safety and environmental monitoring. These applications might be overall very useful to safeguard/monitor the public health.

References

1. O'Farrel, B. Evolution in Lateral Flow–Based Immunoassay Systems. *Lateral Flow Immunoassay* 1 – 33 (2009).doi:10.1007/978-1-59745-240-3
2. Turner, A. P. F. Biosensors: Past, Present and Future. *Cranfield University 1996* (1996).at <http://www.cranfield.ac.uk/health/researchareas/biosensorsdiagnostics/page18795.html>
3. *IUPAC Compendium of Chemical Terminology 2nd Edition*. (International Union of Pure and Applied Chemistry: Research Triangle Park, NC.: 1997).
4. Song, S., Wang, L., Li, J., Fan, C. & Zhao, J. Aptamer-based biosensors. *TrAC Trends in Analytical Chemistry* **27**, 108–117 (2008).
5. Mairal, T. *et al*. Aptamers: molecular tools for analytical applications. *Analytical and bioanalytical chemistry* **390**, 989–1007 (2008).
6. Ruigrok, V. J. B., Levisson, M., Eppink, M. H. M., Smidt, H. & Van der Oost, J. Alternative affinity tools: more attractive than antibodies? *The Biochemical journal* **436**, 1–13 (2011).
7. Clark, J. & C, L. Electrode systems for continuous monitoring in cardiovascular surgery. *Annals New York Academy of Science* **102**, 29–45 (1962).
8. Li, H., Liu, S., Dai, Z., Bao, J. & Yang, X. Applications of Nanomaterials in Electrochemical Enzyme Biosensors. *Sensors* **9**, 8547–8561 (2009).
9. Leca-Bouvier, B. D. & Blum, L. J. Enzyme for Biosensing Applications. *Recognition Receptors in Biosensors* 177 – 220 (2010).doi:10.1007/978-1-4419-0919-0_4
10. Suman & Ashok, K. Recent Advances in DNA Biosensor. *Sensors & Transducers Journal,* **92**, 122–133 (2008).
11. Kubik, T., Bogunia-Kubik, K. & Sugisaka, M. Nanotechnology on duty in medical applications. *Current pharmaceutical biotechnology* **6**, 17–33 (2005).
12. Gruner, G. Carbon nanotube transistors for biosensing applications. *Analytical and bioanalytical chemistry* **384**, 322–35 (2006).
13. Lee, J.-H., Oh, B.-K., Choi, B., Jeong, S. & Choi, J.-W. Electrical detection-based analytic biodevice technology. *BioChip Journal* **4**, 1–8 (2010).
14. Vo-Dinh, T. & Cullum, B. Biosensors and biochips: advances in biological and medical diagnostics. *Fresenius' journal of analytical chemistry* **366**, 540–51 (2000).
15. Fritz, J. Cantilever biosensors. *The Analyst* **133**, 855–63 (2008).
16. Ramanathan, K. & Danielsson, B. Principles and applications of thermal biosensors. *Biosensors and Bioelectronics* **16**, 417–423 (2001).
17. Hu, X. & Dong, S. Metal nanomaterials and carbon nanotubes - synthesis, functionalization and potential applications towards electrochemistry. *Journal of Materials Chemistry* **18**, 1279–1295 (2008).
18. Martin, A. L., Li, B. & Gillies, E. R. Surface Functionalization of Nanomaterials with Dendritic Groups: Toward Enhanced Binding to Biological Targets. *Journal of the American Chemical Society* **131**, 734–741 (2009).
19. Prencipe, G. *et al*. PEG Branched Polymer for Functionalization of Nanomaterials with Ultralong Blood Circulation. *Journal of the American Chemical Society* **131**, 4783–4787 (2009).
20. Liu, Z., Kiessling, F. & Gaetjens, J. Advanced Nanomaterials in Multimodal Imaging: Design, Functionalization, and Biomedical Applications. *Journal of Nanomaterials* 894303 (2010).

21. Mehdi, A., Reye, C. & Corriu, R. From molecular chemistry to hybrid nanomaterials. Design and functionalization. *Chemical Society Reviews* **40**, 563–574 (2011).
22. Alivisatos, a P., Gu, W. & Larabell, C. Quantum dots as cellular probes. *Annual review of biomedical engineering* **7**, 55–76 (2005).
23. Jiang, W., Singhal, A., Fischer, H., Mardyani, S. & Chan, W. C. W. Engineering Biocompatible Quantum Dots for Ultrasensitive, Real-Time Biological Imaging and Detection. *BioMEMS and Biomedical Nanotechnology* 137 – 156 (2006).
24. Bruchez, M., Morronne, M., Gin, P., Weiss, S. & Alivisatos, A. Semiconductor nanocrystals as fluorescent biological labels. *Science* **281**, 2013–2016 (1998).
25. Chan, W. & Nie, S. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science* **281**, 2016–2018 (1998).
26. Algar, W. R., Tavares, A. J. & Krull, U. J. Beyond labels: a review of the application of quantum dots as integrated components of assays, bioprobes, and biosensors utilizing optical transduction. *Analytica chimica acta* **673**, 1–25 (2010).
27. Rosenthal, S. J., Chang, J. C., Kovtun, O., McBride, J. R. & Tomlinson, I. D. Biocompatible quantum dots for biological applications. *Chemistry & biology* **18**, 10–24 (2011).
28. Wang, C., Knudsen, B. & Zhang, X. Semiconductor Quantum Dots for Electrochemical Biosensors. *Biosensor Nanomaterials* 199–219 (2011).
29. Liu, S., Leech, D. & Ju, H. Application of Colloidal Gold in Protein Immobilization, Electron Transfer, and Biosensing. *Analytical Letters* **36**, 1–19 (2003).
30. Katz, E., Willner, I. & Wang, J. Electroanalytical and Bioelectroanalytical Systems Based on Metal and Semiconductor Nanoparticles. *Electroanalysis* **16**, 19–44 (2004).
31. Yáñez-Sedeño, P. & Pingarrón, J. M. Gold nanoparticle-based electrochemical biosensors. *Analytical and bioanalytical chemistry* **382**, 884–6 (2005).
32. Ambrosi, A., De La Escosura-Muñiz, A., Castañeda, M. T. & Merkoçi, A. Gold Nanoparticles: A Versatile Label For Affinity. *Biosensing using nanomaterialsty* 177 – 197 (2009).
33. Pankhurst, Q. a. Nanomagnetic medical sensors and treatment methodologies. *BT Technology Journal* **24**, 33–38 (2006).
34. Roca, a G. *et al*. Progress in the preparation of magnetic nanoparticles for applications in biomedicine. *Journal of Physics D: Applied Physics* **42**, 224002 (2009).
35. Sandhu, A., Handa, H. & Abe, M. Synthesis and applications of magnetic nanoparticles for biorecognition and point of care medical diagnostics. *Nanotechnology* **21**, 442001 (2010).
36. Wang, J. Carbon-Nanotube Based Electrochemical Biosensors: A Review. *Electroanalysis* **17**, 7–14 (2005).
37. Balasubramanian, K. & Burghard, M. Biosensors based on carbon nanotubes. *Analytical and Bioanalytical Chemistry* **385**, 452 – 468 (2006).
38. Davis, J. J., Coleman, K. S., Azamian, B. R., Bagshaw, C. B. & Green, M. L. H. Chemical and biochemical sensing with modified single walled carbon nanotubes. *Chemistry (Weinheim an der Bergstrasse, Germany)* **9**, 3732–9 (2003).
39. Zhao, Q., Gan, Z. & Zhuang, Q. Electrochemical Sensors Based on Carbon Nanotubes. *Electroanalysis* **14**, 1609–1613 (2002).
40. Compton, R. G., Wildgoose, G. G. & Wong, E. L. S. Carbon Nanotube–Based Sensors and Biosensors. *Biosensing Using Nanomaterials* 1 – 38 (2009).
41. Huo, Z., Tsung, C., Huang, W., Zhang, X. & Yang, P. Sub-Two Nanometer Single Crystal Au 2008. *Nano* 2–5 (2008).
42. Wang, J., Liu, C. & Lin, Y. Nanotubes, Nanowires, and Nanocantilevers in Biosensor Development. *Nanomaterials for Biosensors* 56 – 100 (2007).
43. Cui, Y., Wei, Q., Park, H. & Lieber, C. M. Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species. *Science (New York, N.Y.)* **293**, 1289–92 (2001).
44. Patolsky, F., Zheng, G. & Lieber, C. M. Nanowire sensors for medicine and the life sciences. *Nanomedicine (London, England)* **1**, 51–65 (2006).
45. Hangarter, C. M., Bangar, M., Mulchandani, A. & Myung, N. V Conducting polymer nanowires for chemiresistive and FET-based bio/chemical sensors. *Journal of Materials Chemistry* **20**, 3131–3140 (2010).
46. Wang, Y., Li, Z., Wang, J., Li, J. & Lin, Y. Graphene and graphene oxide: biofunctionalization and applications in biotechnology. *Trends in biotechnology* **29**, 205–12 (2011).
47. Shao, Y. *et al*. Graphene Based Electrochemical Sensors and Biosensors: A Review. *Electroanalysis* NA–NA (2010).doi:10.1002/elan.200900571
48. Pumera, M. Graphene in biosensing. *Materials Today* **14**, 308–315 (2011).

ATTENTIÓN ¡¡

Pages 12 to 37 of the thesis are available at the editor's web

- **Eden Morales-Narváez, Helena Montón, Anna Fomicheva, Arben Merkoçi**, Signal enhancement in antibody microarray using quantum dots nanocrystals : application to potential Alzheimer biomarker screening, *Analytical Chemistry* **2012**, 84:6821.
(5 citations)
DOI: 10.1021/ac301369e
http://pubs.acs.org/doi/abs/10.1021/ac301369e

- **Eden Morales-Narváez, Arben Merkoçi**, Graphene Oxide as an Optical Biosensing Platform, *Advanced Materials* (front cover article) **2012**, 24:3298.
(30 citations)
DOI: 10.1002/adma.201200373
http://onlinelibrary.wiley.com/doi/10.1002/adma.201200373/abstract

- **Eden Morales-Narváez, Briza Pérez-López, Luis Baptista Pires, Arben Merkoçi**, Simple Förster resonance energy transfer evidence for the ultrahigh quantum dot quenching efficiency by graphene oxide compared to other carbon structures, *Carbon* **2012,** 50:2987.
(7 citations)
DOI: 10.1016/j.carbon.2012.02.081
http://www.sciencedirect.com/science/article/pii/S0008622312002187

Discussion and Conclusions

The integration of nanomaterials in microarray technology can outperform the conventional microarray technology performance. As en example, the biosensing performance of core−shell quantum dots (QD655) and a fluorescent dye (A647) while being employed in the same experimental conditions as reporters of sandwich immunocomplexes in microarray format has been explored and they are also compared with a conventional ELISA. A potential AD biomarker (ApoE) has been proposed as the model analyte. Regarding the sensitivity, quantum dots (QDs) have been found to become advantageous reporters in microarray technology even though they are not excited with an ideal wavelength (633 nm, LOD of ca. 247 pg mL^{-1}). On the other hand, while QDs are excited at more suitable wavelengths (457, 488, and 532), we obtain up to a 7-fold enhancement in the LOD (ca. 62 pg mL^{-1}) when compared with a conventional ELISA (ca. 470 pg mL^{-1}) and up to a 5-fold enhancement when compared with Alexa 647 as reporter in microarray format (LOD of ca. 307 pg mL^{-1}). Finally, very small volumes (few μL) of human serum were assayed with high sensitivity and acceptable precision and accuracy. This approach could be extended to other kind of biomarkers.

Since the incorporation of QDs into microarrays is a relatively new endeavour, various challenges in this technology remain to be addressed. For instance, many current scanners lack the tools to obtain QD fluorescent signals under suitable excitation conditions. Most of them include excitation sources for red lasers and for green lasers. Furthermore, many of these scanners cannot be configured to simultaneously select the excitation source and the filter emission. Moreover, compared to organic dyes, QDs are very expensive to purchase. However, several synthetic routes to QDs have already been published, and configurable scanners or other setups can be employed for QD signal acquisition in microarray platforms. Fine-tuning of microarrays for use with nanomaterials should enable improved biosensing performance and accelerate incorporation of this technology into real-world bioanalytical scenarios for applications in diagnostics, safety, security, and environmental monitoring.

Graphene oxide (GO) displays advantageous characteristics as a biosensing platform due to its excellent capabilities for direct wiring with biomolecules, a heterogeneous chemical and electronic structure, the possibility to be processed in solution and the ability to be tuned as insulator, semiconductor or semi-metal. Moreover, GO photoluminescences with energy transfer donor/acceptor molecules exposed in a planar surface and is even proposed as a universal highly efficient long-range quencher. The experimental results included here are evidence of the fact that GO is the most powerful acceptor of QDs fluorescence resonance energy transfer (FRET) donors compared with graphite, carbon nanofibers and carbon nanotubes. The demonstrated properties can be of exceptional importance for several kinds of applications in nanobiotechnology. For example, owing to the extraordinary structure and photonic

properties of GO, a pathogen detection system based on the interaction of GO as acceptor of QD FRET donors can be proposed. Since GO bears high-powered antibacterial properties, such proposal might be employed to both detect bacteria and attack bacterial membrane integrity. Furthermore, as a potential diagnosis tool, the proposed system might be extended to different kinds of analyte such as cancer cells, molecular logic operations and other nano-biosystems.

Not only nanomaterials can enhance the performance of microarray technology but also micromaterials. For example, in microarray technology, the transport of the target molecule toward its molecular bioreceptors can play a critical role in its biodetection performance. In this context, the performance of microarray technology might also be enhanced by incorporating microdevices that assist the transport of the analyte (contained in the assayed sample) to the microarray spots (molecular bioreceptors).

The presented approaches are in-vitro applications. In-vivo applications are seldom reported, furthermore the toxic effects of nanomaterials are little known yet. Nevertheless, close consensus with regulatory agencies (e.g. the European Medicines Agency or the US Food and Drugs Administration) to develop comprehensive standards for nanomaterials applications will ensure the operative and realistic transition of nanomaterials based biodetection systems to approved devices. In the future so as to facilitate healthcare and medicine, the studied approaches might be integrated to simple biomolecular detections performed in point of care devices.