



MINISTRY OF EDUCATION AND RESEARCH
National University of Science and Technology
POLITEHNICA Bucharest

DOCTORAL THESIS

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GRAPHENE-BASED OPTICAL SENSITIVE
PLATFORM FOR OLIGONUCLEOTIDE
EXTRACTION AND DETECTION

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Abstract

This PhD thesis investigates the role of graphene-based derivatives and carbon nanotubes (CNTs) in modifying nitrocellulose (NC) membranes in order to develop a novel approach for the extraction and fluorescence-based detection of short oligonucleotides from complex ionic media. The overall goal is to design a novel, simple, low-cost, and reliable optical platform capable of overcoming current limitations in biosensing associated with the isolation and detection of low-abundance nucleic acids, as well as platform stability in biological samples. To achieve this goal, three research directions were pursued, focusing on the progressive design and optimization of NC membranes modified with graphene derivatives and CNTs. To begin with, the primary research direction explored the development of a graphene oxide (GO)-modified NC membrane as a baseline platform, enabling the investigation of extraction and fluorescence detection of short oligonucleotides in complex media. This stage provided fundamental insight into the role of incubation time and medium composition in governing extraction efficiency, signal generation, and platform stability in aqueous environments.

Building on these findings, the second research direction focused on the modification of NC membranes with amino-polyethylene glycol-functionalized reduced graphene oxide (NH₂-PEG-rGO), resulting in enhanced extraction and fluorescence-based detection of oligonucleotides. Particular attention was given to the role of complex ionic media, demonstrating that divalent ions, especially Mg²⁺, significantly influence adsorption-desorption processes and fluorescence response, leading to improved extraction efficiency under optimized conditions.

In the final research, the platform design was further advanced through the combined use of NH₂-PEG-rGO and single-walled carbon nanotubes (SWCNTs), exploiting their complementary adsorption capacity and fluorescence quenching properties. These results highlighting the synergistic effect between SWCNTs and NH₂-PEG-rGO, where increased surface area, improved structural stability, and enhanced electronic interactions, together with the complexity of the ion-containing media, contribute to stronger oligonucleotide adsorption and more efficient fluorescence signal modulation.

Overall, this thesis contributes to the field of biosensing by providing a systematic understanding of how graphene-based nanomaterials, carbon nanotubes, and ionic environments interact at the solid-liquid interface to control oligonucleotide extraction and fluorescence-based detection. The findings support the development of efficient, scalable, and application-oriented optical platforms, with potential to advance optical biosensing technologies and enable their integration into practical diagnostic and point-of-care applications.

Summary of the doctoral thesis

General objectives

Recent developments in biosensing technology have shown the necessity for analytical platforms that are not only highly sensitive and selective but also easy to use, reliable, and adaptable to challenging biological conditions. These factors are particularly important in regenerative medicine, especially in the study of bone-related diseases such as osteosarcoma, multiple myeloma, bone metastasis, and osteoporosis where the detection of low-abundance biomolecules, particularly short oligonucleotides, is essential for monitoring early molecular changes, cell behavior, osteogenic differentiation, extracellular matrix remodeling, and therapeutic response. Yet, the small size of oligonucleotides presents challenges, as even conventional techniques struggle to extract, and to detect them efficiently due to their low abundance and increased susceptibility to degradation in biological samples. Driven by these challenges and the growing need for rapid, sensitive, and reliable analytical methods, this thesis addresses the gap in biosensing by investigating the use of graphene derivatives and carbon nanotubes to develop optical sensitive platforms for short oligonucleotide extraction and detection.

The main goal of this thesis is to investigate how graphene-based derivatives and carbon nanotubes can be used to modify nitrocellulose (NC) membranes in order to develop a novel approach for the extraction and fluorescence-based detection of short oligonucleotides, while examining the role of material composition, surface modification, and the complexity of the ionic adsorption medium in modulating platform analytical performance. Through this approach, the thesis provides a deeper understanding of how graphene derivatives, carbon nanotubes, and the ionic conditions influence short oligonucleotide extraction and fluorescence response at the NC membrane interface, where close-range adsorption and interfacial electron transfer govern signal quenching, leading to improved analytical contrast, enhanced stability, and reduced membrane degradation under complex biological conditions.

The thesis is organized into two main parts: the first part provides the current state of the art in biosensing, outlines the general objectives of the thesis, and offers an overview focused on analytical methods, transduction strategies, and advanced materials used in oligonucleotide detection and related biomedical applications, while the second part presents the original research contributions of this thesis, with focus on the design and optimization of graphene derivatives and carbon nanotubes based optical sensitive platforms for short oligonucleotide extraction and

detection. All the data supporting this thesis are presented throughout the following chapters that are briefly described below:

Chapter 1 introduces the state of the art in biosensing and outlines the general objectives that underpin the structure of this thesis. Building on this foundation, **Chapter 2** establishes the theoretical basis for the selected methods and materials, following the literature review. It concludes that fluorescence-based optical approaches, using fluorophores such as fluorescein in combination with graphene derivatives and carbon nanotubes as quenchers, integrated on NC membranes as solid supports, can effectively overcome the analytical challenges in biosensing associated with the extraction and detection of short oligonucleotides in biological samples.

Following these considerations, **Chapter 3** presents the first original part of this thesis, focusing on the development of an optical platform based on the modification of NC membranes with graphene oxide (GO) for oligonucleotide extraction. The successful fabrication of the platform was confirmed through physicochemical characterization using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and wettability measurements. The adsorption performance of the developed platform was evaluated using fluorescently labeled ssDNA sequence in complex media at pH 7.0, based on water, Alpha Minimum Essential Medium (α MEM), and α MEM supplemented with fetal bovine serum (FBS), over different incubation periods. The fluorescently labeled single-stranded deoxyribonucleic acid ssDNA was then detected by incubating the membranes in a desorption solution at pH 8.0, allowing the release of oligonucleotides from the obtained platform into a solution suitable for fluorescence analysis. The results demonstrated that extraction efficiency and fluorescence responses are strongly influenced by both the surface modification of the NC membrane with GO and the experimental conditions, particularly incubation time and medium composition. These findings enabled the identification of the most suitable working conditions for subsequent studies, forming the basis of this thesis for further optimization of the platform in **Chapter 4**.

Representing the second original part of this thesis, this chapter focuses on the modification of NC membranes with amino-polyethylene glycol functionalized reduced graphene oxide (NH_2 -PEG-rGO) to enhance oligonucleotide extraction and fluorescence-based detection. The surface modification of the developed platform was investigated in detail, supported by physicochemical characterization using FTIR, SEM, and wettability analysis, which revealed significant morphological and interfacial changes, including increased hydrophobicity of the modified membrane. In parallel, particular attention is given to the role of the optimized complex media identified in the previous chapter, demonstrating that both the supplementation and the type of

divalent ions, such as Mg^{2+} and Mn^{2+} , strongly influence oligonucleotide immobilization and fluorescence response. The results indicated that MgCl_2 -containing media provide the most favorable conditions, significantly enhancing extraction efficiency compared with the unmodified platform, thereby highlighting the critical role of both ionic composition and surface modification in modulating platform performance at the membrane interface.

Extending these findings, **Chapter 5**, which represents the third original part of this thesis, further advances the platform design through the combined use of NH_2 -PEG-rGO and single-walled carbon nanotubes (SWCNTs), aiming to improve extraction efficiency and fluorescence signal modulation by exploiting the complementary adsorption and quenching properties of these carbon-based nanomaterials. This chapter supports the platform modification through comprehensive physicochemical and structural analyses, including FTIR, Raman Spectroscopy, X-ray Photoelectron Spectroscopy (XPS), SEM, and wettability measurements, which confirm successful integration and interfacial changes. In this context, the influence of ionic conditions is further elucidated, demonstrating that divalent ion-containing media, particularly MgCl_2 , promote stronger interactions between the modified membrane and oligonucleotides, leading to enhanced fluorescence response and higher extraction yields. The optimized platform exhibits an improved analytical performance compared with previously developed systems, confirming the synergistic effect of material composition and complexity of the ionic conditions.

The results are consolidated in **Chapter 6**, which presents the general conclusions of the thesis and summarizes the main original scientific contributions obtained during the PhD program, emphasizing the potential of the developed graphene-based derivatives and CNTs optical fluorescence platforms for oligonucleotide extraction and detection. In addition, this chapter outlines perspectives for further optimization of the optical platforms, integration into advanced biosensing systems, and potential applications in personalized medicine and real-time monitoring.

In summary, this thesis contributes to the field of biosensing by demonstrating how graphene derivatives and carbon nanotubes can enhance the performance of NC membranes for the extraction and fluorescence-based detection of short oligonucleotides. The findings highlight the importance of surface modification, pH, ionic conditions, and material complementarity in improving extraction efficiency, signal modulation, and analytical reliability in complex ionic media. By elucidating the interaction between optical platform, composition of the complex ionic medium, and fluorescence mechanisms, this thesis provides a pathway for the further development of simple, low-cost, and sensitive optical biosensing platforms, highlighting the need for continued research to address current limitations in biosensors performance.

Research motivation

Bone tissue engineering (BTE) is an area of regenerative medicine focused on restoring or regenerating damaged bone by bringing together biomaterials, cells, and bioactive molecules to support osteogenesis, vascularization, and structural integration. Nevertheless, at the current stage, the intrinsic complexity of bone healing and the limited ability to directly monitor molecular and genetic processes in real time remain key constraints of current approaches, thereby underscoring the need for advanced detection methods [1-3]. In this context, reliable detection methods for bone-related biomolecules are essential for monitoring osteogenic activity, matrix formation, functional integration of regenerative strategies, or even gene expression and suppression pathways associated with bone-related diseases. Detection methods are pivotal analytical approaches in biosensing that transduce molecular interaction into measurable signals for analyte identification and quantification [4-6]. The global biosensors market shows strong growth, valued at approximately 34.71 billion USD in 2026 and projected to reach 63.96 billion USD by 2033, corresponding to a compound annual growth rate of about 9.12%, driven by the accelerated adoption of biosensing mechanism and the increasing integration of artificial intelligence in diagnostic and monitoring applications [7]. So far, biosensing mechanisms encompass a broad range of transduction strategies, such as electrochemical, mechanical, optical, thermal, magnetic, and spectrochemical approaches, each offering distinct analytical advantages in sensitivity, selectivity, response time, and suitability for specific application settings. In this perspective, electrochemical platforms dominate the current commercial applications regardless of their limitations related to sensitivity, stability, and matrix interference [8, 9], while mechanical and thermal methodologies are predominantly constrained to research settings due to fabrication complexity and scalability constraints [10-13]. Likewise, magnetic and spectrometric approaches are advancing towards early commercialization and extensive analytical application, respectively; however, both remain limited in broader usage by specialized instrumentation, labelling techniques, and limited portability [14-17]. Building on this view, optical biosensors are attracting growing interest as complementary platforms, driven by their high intrinsic sensitivity, real-time and label-free detection capacity, and compatibility with miniaturized and point-of-care designs [18, 19].

Optical biosensing is an analytical technique that leverages the interaction between light and biofunctionalized systems to transduce molecular recognition events into detectable optical signals, utilizing fundamental phenomena such as absorption, fluorescence, scattering, plasmonic,

and resonance effects, enabling high-fidelity detection of biological analytes [20, 21]. Absorbance-based detection offers simple instrumentation, high stability, and a direct quantitative output; however, it is fundamentally constrained by low sensitivity and poor analyte selectivity in complex matrices [22, 23]. By comparison, elastic and inelastic scattering processes offer rich, label-free chemical information; yet, they are limited by the weak signal intensities, susceptibility to background interference, and dependence on advanced instrumentation, which restrict rapid analysis and detection at low analyte concentrations [24, 25]. Resonance- and plasmon-based processes offer enhanced sensitivity and label-free detection by leveraging the strong light-matter interactions at the nanoscale; nevertheless, these approaches remain highly dependent on refractive index fluctuations, nanoscale geometry, and precise optical alignment, which can compromise stability and quantitative accuracy [26, 27]. Together, these limitations have driven the shift toward fluorescence-based processes, which remain among the most versatile and widely adopted detection schemes, enabling sensitive and specific monitoring of biomolecular interactions through spontaneous emission from excited fluorophores [28, 29]. Additionally, owing to their inherent high sensitivity, strong molecular specificity, and wide dynamic range, fluorescence-based approaches enable real-time signal transduction using relatively simple and cost-efficient instrumentation, facilitating easy integration at micro- and nanoscale in wearable, point-of-care, and high-throughput diagnostic systems [30-33].

Within fluorescence-based transduction strategies, photoinduced electron transfer (PET), Förster resonance energy transfer (FRET), and intramolecular charge transfer (ICT) are among the most extensively used mechanisms. FRET is a non-radiative energy transfer process in which excitation energy is passed from a donor fluorophore to a nearby acceptor in a manner that strongly depends on the distance between them [31, 34, 35]; PET, on the other hand, involves charge-transfer processes that modulate emission via quenching or enhancement [36, 37], while ICT arises from redistribution of electron density within a single fluorophore upon excitation [38, 39], leading to environment-sensitive spectral shifts. Among these transduction pathways, FRET remains the most effective and widely used mechanism, primarily because it offers a direct, quantitative, and predictable relationship between signal output and nanoscale donor-acceptor separation, providing highly sensitive detection, reduced background interference, and easier reproducibility in complex biosensing environments [40, 41]. As a result, FRET-based systems have been widely implemented in biosensing platforms for detecting pathogenic microorganisms, proteins, particular genes, nucleotide sequences, and small molecules [34, 35, 40-42]. In such platforms, fluorophore selection is decisive, as combinations of metal-ligand complexes, quantum dots, and

organic dyes offer distinct advantages in terms of photostability, emission efficiency, and spectral versatility, arising from differences in excited-state relaxation pathways [43, 44]. Although, metal-ligand fluorophores and quantum dots offer high brightness, the capability to tune their emission as a function of composition and size, and improved photostability [45-48]; however, their practical utilization is limited by complex synthesis routes, potential toxicity associated with metal components, fluorescence blinking phenomena, surface- and environment-dependent quenching, and difficulties in achieving reproducible bioconjugation and long-term colloidal stability [45-50]. In biological media, these limitations may affect signal reliability and prevent quantitative interpretation, regardless of their advantageous optical performance. Therefore, organic fluorophores are the most used and reliable probes, providing well-characterized luminescence, facile chemical functionalization, predictable biocompatibility, and uniform emission characteristics that facilitate robust, quantitative sensing and imaging across a variety of experimental platforms [51, 52]. Within this class, fluorescein holds a pivotal role among widely utilized organic fluorophores, as it is one of the most extensively studied and broadly employed dyes due to its high molar absorptivity, large fluorescence quantum yield in aqueous media, and well-defined photophysical properties [53, 54]. Its precise excitation and emission, strong brightness at physiological pH, and capability for easy structural modification have enabled the careful design of a broad range of probes for metal ions (e.g. Mg^{2+} , Mn^{2+} , small molecules, and biological targets, while predictable environmental response, such as pH sensitivity and controllable PET or FRET pathways, help ensure reliable signal transduction in complex biological media [53-55].

In fluorescence biosensors, both the properties of the fluorophore and its quenching behavior are important, as controlled quenching is the key for signal transduction, background suppression, and improved sensitivity. Fluorescence quenching refers to the reduction of emission intensity through nonradiative energy or electron transfer processes that occur upon interaction with a quencher, thereby modifying the excited-state deactivation pathways [56]. Accordingly, a wide range of quenchers have been broadly integrated in fluorescent biosensing applications, including metal ions, metal oxides (MOFs), covalent organic frameworks (COFs), and carbon-based nanomaterials due to their effectiveness in regulating fluorescence responses [57-60]. Within this group, carbon-based nanomaterials, including graphene, graphene derivatives, and carbon nanotubes (CNTs), have emerged as highly efficient quenchers due to their broad absorption, pronounced distance-dependent quenching, large surface area, and strong electronic coupling with fluorophores [60, 61]. These features facilitate efficient adsorption of

oligonucleotides, and other probes, promote close fluorophore surface proximity required for quenching, and support reliable signal modulation, which has led to the extensive use of graphene-based nanomaterials in fluorescence biosensors, especially for deoxyribonucleic acid (DNA) detection applications [62].

Single-stranded DNA is a single-chain oligonucleotide that enables highly selective molecular recognition and sensitive genetic analysis, playing a central role in monitoring biological and regenerative processes, while its reliability depends on extraction and detection methods that preserve purity and sequence integrity. The effective isolation and detection of ssDNA is essential as sequence-specific identification, hybridization-driven assembly, and signal transduction processes are fundamentally dependent on nucleic acid integrity. Therefore, variations in yield, purity, length distribution, and conformational state directly influence detection sensitivity, quantitative reliability, and functional performance in diagnostic, biosensing, and regenerative systems. Despite its importance, ssDNA extraction remains challenging, as current extraction methods (e.g., silica column, magnetic beads, enzymatic extraction) often rely on multistep chemical or physical processes that suffer from low recovery, susceptibility to degradation, and co-extraction of inhibitors or contaminants, while method-dependent trade-offs between purity, efficiency, cost, and reproducibility continue to hinder standardization across applications [63, 64].

This thesis addresses these limitations by following an optical transduction strategy, identified through a comparative evaluation of electrochemical and optical biosensing approaches as the most appropriate framework for oligonucleotide extraction and detection from complex media. On this basis, this thesis combines fluorescence-based detection of short oligonucleotides with graphene derivatives, CNTs and nitrocellulose (NC) membranes as solid supports to enable efficient ssDNA isolation and optical measurements. The use of these materials in combination comes from the synergy between NC membranes and carbon-based materials, which arises from their complementary physicochemical properties: NC provides a high binding capacity and strong electrostatic interactions with single-stranded DNA, facilitating efficient adsorption and isolation, while graphene derivatives enhance surface functionality and capture efficiency and simultaneously act as efficient fluorescence quenchers. This process is governed by close-range surface adsorption and interfacial electron transfer, resulting in effective signal quenching and improved analytical contrast, while also contributing to reduced membrane degradation and increased stability under complex biological conditions.

State-of-the-art

One of the main contributions of this thesis to the current state-of-the-art lies in the integration of graphene derivatives and CNTs with NC membranes for the development of optical sensitive platforms dedicated to short oligonucleotide extraction and fluorescence-based detection. While carbon nanomaterials are extensively studied in biosensing, and NC membranes are commonly used as solid supports in analytical assays, their integration into a single platform for both extraction and optical detection of short oligonucleotides remains limited, as most studies have addressed either the fluorescence quenching properties of the carbon nanomaterials or NC membrane-based separation techniques as independent approaches. Moreover, although fluorescence-based optical approaches using fluorophores such as fluorescein in combination with graphene derivatives or CNTs as quenchers have been previously reported, their integration with NC membranes, together with the influence of complex ionic media and pH variation on the modulation of adsorption-desorption efficiency of short oligonucleotides and fluorescence response, has not been comprehensively investigated. In particular, the influence of divalent ions such as Mg^{2+} and Mn^{2+} on oligonucleotide surface interactions and signal modulation remains insufficiently studied in the context of solid-phase extraction and detection platforms. To the best of our knowledge, as the studies associated with the main objective of this thesis span the period 2023-2025 (based on publication years), no prior studies in biosensing field had investigated this combination of methods and materials for the extraction and detection of short oligonucleotides.

The present thesis builds upon these gaps in biosensing for extraction and detection of short oligonucleotides by proposing a stepwise development of optical-based fluorescence sensing platforms, progressing from GO-modified NC membrane platform to a more complex platform based on amino-polyethylene glycol functionalized reduced graphene oxide ($\text{NH}_2\text{-PEG-rGO}$) and single-walled carbon nanotubes (SWCNTs). This approach allows not only the evaluation of individual material contributions but also the investigation of their complementary effects, together with the influence of the complex ionic medium components, on extraction performance and fluorescence-based detection of short oligonucleotides. In this context, the thesis provides a more integrated perspective on how complementarity of the material composition, surface chemistry, pH, and ions, collectively influence biosensing performance, contributing to the advancement of simple, low-cost, and reliable optical platforms for short oligonucleotide extraction and detection in complex biological systems.

General and Specific Objectives of the Thesis.

Building on the considerations and gaps identified in the previous subchapter, the general objective of this thesis is to investigate how graphene-based derivatives and carbon nanotubes can be used to modify nitrocellulose membranes, as novel approach for the extraction and fluorescence-based detection of short oligonucleotides from complex ionic media, while exploring the role of material composition, surface modification, and complexity of the media in modulating platform analytical performance. To achieve this objective, the present thesis was structured around three secondary objectives:

First objective: Development and evaluation of GO-modified NC platform for short oligonucleotide extraction and detection. To accomplish this, a baseline optical platform was first fabricated through the modification of NC membranes with GO, followed by the evaluation of its extraction performance and fluorescence response under relevant experimental conditions, as described in the activities (A) presented below:

- A1.** Fabrication of GO-modified NC platform.
- A2.** Physicochemical characterization of the obtained platform using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and wettability analysis.
- A3.** Evaluation of the extraction performance using fluorescently labeled ssDNA (≤ 50 nt) as target oligonucleotide in aqueous and complex media, including Alpha Minimum Essential Medium (α MEM), and α MEM supplemented with fetal bovine serum (FBS), at physiological pH (≈ 7.0), over different incubation times (30, 45, and 60 minutes).
- A4.** Investigation of the desorption process using alkaline conditions (Tris-HCl buffer, pH ≈ 8.0) to enable oligonucleotide release and fluorescence-based detection.
- A5.** Development and validation of an equation for converting fluorescence intensity (r.f.u.) into oligonucleotide mass (pg), based on calibration with known concentrations of fluorescently labeled ssDNA.
- A6.** Identification of optimal working conditions by analyzing the influence of incubation time, medium composition, and pH on extraction efficiency and fluorescence response.

Second objective: Building on the previous objective, this stage focuses on enhancing the stability and efficiency of the optical platform for oligonucleotide extraction and detection through the modification of NC membranes with NH_2 -PEG-rGO and the optimization of complex solution

conditions through the control of the ionic medium. To accomplish this, the following activities were performed:

- A1.** Fabrication of the NH₂-PEG-rGO modified NC platform.
- A2.** Investigation of the NH₂-PEG-rGO influence in the modification of NC membrane through structural, morphological, and wettability analysis.
- A3.** Supplementation of the optimized complex media identified in the first objective with various divalent ions (Mg²⁺, Mn²⁺), followed by the evaluation on the oligonucleotide extraction performance of the obtained platform after 60 minutes of incubation.
- A4.** Analysis of the effect of ionic complex media composition and strength on fluorescence response of the optical platform their correlation with extraction performance.

Third objective: Following the optimization achieved in the previous objective, the third secondary objective introduces SWCNTs with NH₂-PEG-rGO to modify NC membranes as an additional modification component, aiming to further improve stability in aqueous environment, extraction performance, and fluorescence response through complementary adsorption and quenching effects. To achieve this, the following described activities were performed:

- A1.** Fabrication of the optical platform based on NC membranes modified with a combination of NH₂-PEG-rGO and SWCNTs.
- A2.** Structural and physicochemical investigation of the obtained optical platform using FTIR, Raman spectroscopy, X-ray Photoelectron Spectroscopy (XPS), SEM, and wettability analysis.
- A3.** Investigation of the optical platform performance after incubation in ionic complex media, obtained in the second objective, focusing on oligonucleotide-surface interactions and extraction efficiency.
- A4.** Evaluation of fluorescence-based signal generation and modulation after incubation of the obtained optical platform in the ionic complex media, with emphasis on signal intensity, quenching efficiency, and their correlation with extraction performance.

General conclusions

The field of biosensing, particularly in the context of nucleic acid analysis, has evolved significantly in recent years; however, the efficient extraction and detection of short oligonucleotides from complex biological environments remain limited due to their low abundance and increased susceptibility to degradation in biological samples. Despite these limitations, the results obtained in this thesis demonstrate that further research in this direction is warranted, given the potential to improve analytical performance and to develop more rapid, sensitive, and reliable biosensing platforms.

The main objective of this thesis proposed a novel approach by investigate how graphene-based derivatives and carbon nanotubes can be used to modify NC membranes, for the extraction and fluorescence-based detection of short oligonucleotides from ionic complex media, while examining the influence of material composition, surface modification, and complexity of the ionic conditions on platform analytical performance. This objective was structured into three secondary objectives, each contributing to the progressive optimization of the platform and to a deeper understanding of the relationship between material properties, interfacial interactions, and analytical performance.

The first objective was to develop a GO-modified NC platform for the extraction and fluorescence-based detection of short oligonucleotides. The successful fabrication of the GO-modified NC membrane was confirmed through FTIR, SEM, and wettability analyses evidenced by surface chemistry and morphology. These changes translated into an improved stability and interaction between the membrane and oligonucleotides, enhancing adsorption and subsequent detection. The extraction performance of the developed platform was evaluated using fluorescently labeled ssDNA samples immersed in three complex solutions at pH 7 based on H₂O, α MEM, and α MEM supplemented with FBS, over different incubation periods (30, 45, and 60 minutes). The fluorescently labeled ssDNA was then detected by incubating the membranes in a desorption solution at pH 8.0, allowing the release of oligonucleotides from the obtained platform into a solution suitable for fluorescence analysis. After converting the obtained fluorescence results into quantitative units, the results showed that, among the tested media, the α MEM-containing medium provided the most favorable conditions, highlighting the importance of medium composition in modulating nucleic acid-surface interactions. In addition, the influence of incubation time was clearly demonstrated, with optimal performance achieved after 60 minutes, indicating that adsorption kinetics play a critical role in determining extraction efficiency. Compared to the

unmodified nitrocellulose membrane, which exhibited a limited immobilization capacity reflected by low fluorescence signals (106.5 r.f.u., corresponding to 124.3 pg after 30 minutes in α MEM + FBS), the the GO-modified NC membrane showed a substantial improvement, reaching a maximum fluorescence signal of 294.4 r.f.u. after 60 minutes of FAM-ssDNA adsorption in α MEM, corresponding to 343.6 pg of recovered oligonucleotide. The results demonstrated that extraction efficiency and fluorescence responses are strongly influenced by both the surface modification of the NC membrane with GO and the experimental conditions, particularly incubation time and medium composition.

The second objective was to investigate the influence of surface chemistry on oligonucleotide extraction and detection by modifying NC membranes with NH₂-PEG-rGO. The successful fabrication of the NH₂-PEG-rGO modified NC membrane was confirmed through comprehensive physicochemical characterization, including FTIR, SEM, and wettability analysis, which revealed the formation of a uniform coating, and a transition toward a hydrophobic surface behavior. The developed platform was evaluated under the optimized conditions established in the previous objective, with particular emphasis on the role of the ionic condition from the complex media. The results demonstrated that both the composition of the medium and the nature of the divalent ions significantly influence oligonucleotide immobilization and fluorescence response. Among the tested conditions, MgCl₂-containing media provided the most favorable environment, leading to a substantial increase in extraction efficiency compared with the unmodified NC membrane. Quantitatively, while the pristine NC membrane exhibited limited adsorption capacity, with fluorescence values around 250 r.f.u. corresponding to approximately 286 pg of ssDNA, the NH₂-PEG-rGO-modified NC membrane showed markedly enhanced performance, reaching fluorescence intensities above 520 r.f.u., corresponding to approximately 611 pg of extracted ssDNA under identical conditions. This improvement highlighted the synergistic effect of surface functionalization and ionic environment in promoting stronger ssDNA surface interactions. Overall, this objective demonstrated that NC membrane functionalization with NH₂-PEG-rGO, together with the modulation of ionic composition, provided an efficient approach for enhancing short oligonucleotide extraction and fluorescence response, enabling the development of an improved optical platform.

The third objective was to further enhance the analytical performance of the developed platform by modifying the NC membranes with SWCNTs and NH₂-PEG-rGO aiming to investigate the complementary adsorption and fluorescence quenching properties of these carbon-based nanomaterials. The successful fabrication of the SWCNTs/NH₂-PEG-rGO modified NC

membrane was confirmed through comprehensive physicochemical and structural characterization, including FTIR, Raman spectroscopy, XPS, SEM, and wettability analysis, which revealed the formation of a more uniform surface, increased hydrophobicity, and significant interfacial modifications. The developed platform was evaluated under the same optimized adsorption and desorption conditions established in the previous objectives, with particular emphasis on the effect of the complex ionic media. The results demonstrated that divalent ions play a critical role in modulating ssDNA surface interactions, with MgCl_2 -containing medium providing the most favorable conditions for oligonucleotide immobilization and fluorescence response. Compared with the unmodified NC membrane, which exhibited fluorescence values in the range of 245-265 r.f.u. (290-320 pg), the SWCNTs/ NH_2 -PEG-rGO modified NC membrane showed substantially enhanced performance, reaching 420 r.f.u. (490 pg) in MnCl_2 , 510 r.f.u. (590 pg) in $\text{MnCl}_2/\text{MgCl}_2$, and up to 670 r.f.u. (780 pg) in MgCl_2 -containing medium. These results highlighted the synergistic effect between SWCNTs and NH_2 -PEG-rGO, where the increased surface area, improved structural stability, and enhanced electronic interactions contribute to stronger ssDNA adsorption and a more efficient fluorescence signal modulation. Thus, this objective demonstrated that the complementarity of SWCNTs and NH_2 -PEG-rGO in modification of NC membranes, together with complexity of the ionic medium, represents a highly effective strategy for improving short oligonucleotide extraction and detection, enabling the development of advanced optical biosensing platforms with superior analytical performance.

Overall, this thesis demonstrates the substantial impact of graphene-based nanomaterials and CNTs on the development of advanced optical platforms for short oligonucleotide extraction and fluorescence-based detection. Through the systematic design of the optical platforms based on GO, NH_2 -PEG-rGO, and SWCNTs, this thesis contributes to the field of biosensing by providing consistent experimental evidence that NC membrane surface modification, in direct correlation with material complementarity and complexity of the ionic medium, governs the platform stability, the efficiency of short nucleic acid immobilization, and the fluorescent-signal modulation. Moreover, the results obtained throughout this thesis support the development of simple, low-cost, and scalable biosensing platforms with improved analytical performance, offering a viable alternative to the state-of-the-art nucleic acid extraction methods, which are often limited by multi-step procedures, the need for specialized equipment, higher operational costs, and the risk of sample loss or contamination, particularly when handling short oligonucleotides. At the same time, the thesis provides a novel approach that goes beyond the current state-of-the-art by integrating graphene derivatives and CNTs with NC membranes into a single platform capable of both

extraction and fluorescence-based detection of short oligonucleotides, while systematically examining how surface chemistry, and complexity of the ionic medium, particularly divalent ions such as Mg^{2+} and Mn^{2+} , influence adsorption-desorption processes and fluorescence response. The favorable outcomes achieved across all objectives of this thesis underline the relevance of continued research in this area, with clear potential to advance optical biosensing technologies and facilitate their integration into practical diagnostic and point-of-care applications.

Original Contributions

This chapter summarizes the main original contributions derived from the research conducted within this doctoral thesis, correlated with each paper, while also reflecting the overall research of the PhD candidate undertaken during the doctoral program.

To begin with, the first paper, addressed in chapter 3,

1. *“Graphene Oxide/Nitrocellulose Non-Covalent Hybrid as Solid Phase for Oligo-DNA Extraction from Complex Medium”*,

corresponding to the first research objective of this thesis, focuses on the development and investigation of a GO modified NC membrane used for the extraction and fluorescence-based detection of short oligonucleotides. The thesis introduce a novel approach that represents a departure from conventional strategies by integrating the surface modification of the NC membrane with the intrinsic adsorption and fluorescence quenching properties of graphene derivatives, an approach that, to the best of my knowledge, has not been previously explored in biosensing for the extraction and detection of short oligonucleotides. A first key contribution of this study is the comprehensive physicochemical characterization of the GO-modified NC membrane, through which the authors provide a deeper understanding of the structural, morphological, and interfacial changes, as well as of how graphene derivatives influence platform stability and performance. A second contribution of this study is the development of an experimental protocol for the adsorption, extraction, and detection of short oligonucleotides from complex solutions. These solutions were prepared using three binding media based on H_2O , α MEM, and α MEM supplemented with FBS. Each medium was prepared under controlled conditions and contained FAM-ssDNA as target oligonucleotide, SDS, BSA, NaCl, and Tris-HCl at pH 7.0, ensuring a biologically relevant ionic and molecular environment for oligonucleotide adsorption. Following the desorption of the GO-modified NC membrane in Tris-HCl (pH 8.0) and subsequent fluorescent analysis of the solution, the study established that a 60-minute incubation

period in the complex medium with α MEM is optimal for achieving efficient extraction and sensitive detection. Another important contribution is the development of a method for converting fluorescence intensity, expressed as r.f.u., into oligonucleotide mass, expressed as pg, based on calibration with known concentrations of fluorescently labeled ssDNA.

Overall, this study contributes to the biosensing field by introducing a new approach based on a GO-modified NC membrane platform for short oligonucleotide extraction and fluorescence detection, while also providing an experimental protocol for adsorption, desorption, and detection, as well as a method for converting fluorescence intensity into oligonucleotide mass. This contribution is significant as it enables the identification of optimal working parameters and establishes a solid basis for the subsequent development and optimization of advanced optical platforms in biosensing for short oligonucleotide extraction and detection.

Building on these findings, the second study from chapter 4, published as:

2. *“Solid Phase Oligo-DNA Extraction from Complex Medium Using an Aminated Graphene/Nitrocellulose Membrane Hybrid”*

advances the platform design through the modification of NC membranes with NH_2 -PEG-rGO, introducing a more controlled surface chemistry, enabling improved interaction with short oligonucleotides under complex ionic conditions. The contribution of this study lies in showing that the performance of the oligonucleotide extraction and fluorescence detection platform depends on both NC membrane modification and the ionic composition of the binding medium. The results of this study showed that NH_2 -PEG-rGO modification produced clear morphological and interfacial changes, confirmed by FTIR, SEM, and wettability analysis, including improved membrane stability and a marked increase in surface hydrophobicity, which can influence oligonucleotide-membrane interactions. Using the optimized conditions from the previous study, the modified membranes were further evaluated in media supplemented with Mg^{2+} and Mn^{2+} ions. The results showed that divalent ions play an important role in oligonucleotide immobilization and fluorescence response, with Mg^{2+} -containing media providing the most favorable conditions for enhanced extraction and fluorescence-based detection of short oligonucleotides. To the best of the authors' knowledge, the modification of NC membranes with NH_2 -PEG-rGO, together with the implementation of tailored ionic binding conditions for modulating oligonucleotide adsorption-desorption mechanisms and fluorescence response, has not been previously explored within a single solid-phase extraction platform. Thus, the combined analysis of surface modification and

ionic effects provides a clearer mechanistic perspective on interfacial interactions, representing an important step toward rational optimization of optical biosensing platforms.

Extending these findings, the third study from chapter 5, published as:

3. *“Efficient Solid-Phase Extraction of Oligo-DNA from Complex Media Using a Nitrocellulose Membrane Modified with Carbon Nanotubes and Aminated Reduced Graphene Oxide”*

further advances the platform design through the combined use of NH₂-PEG-rGO and SWCNTs, demonstrating that the complementary adsorption and fluorescence quenching properties of these carbon-based nanomaterials enhance both extraction efficiency and fluorescence-based detection. The results showed in this study that the obtained platform exhibited improved stability in complex media, together with a uniform surface, increased hydrophobicity, and significant interfacial modifications. These features were further correlated with the enhanced functional behavior of the platform compared with the previously developed systems, supporting stronger extraction capacity and higher fluorescence response, while Mg²⁺-containing media provided the most favorable ionic environment for promoting oligonucleotide-surface interactions and improving overall analytical efficiency. To the best of the authors’ knowledge, the combined use of SWCNTs and NH₂-PEG-rGO for NC membrane modification, together with controlled incubation in ionic complex medium, has not been previously investigated in the field of biosensing for the extraction of short oligonucleotides. This novel approach provides new insight into how the complementary between material composition, interfacial structure, and the complexity of the ionic medium governs the platform performance and establishes a solid basis for the further optimization of high-performance optical detection platforms.

Perspectives

The results obtained in this thesis demonstrate that graphene-based derivatives and CNTs optical sensitive platforms, developed through surface modification of NC membranes and careful tuning of the complex ionic medium, represent a viable approach for the extraction and fluorescence-based detection of short oligonucleotides. However, several directions remain open for further investigation and optimization, particularly in the context of improving analytical robustness, selectivity, and practical applicability. A first important perspective concerns the refinement of interfacial design at the nanoscale, with emphasis on achieving a more controlled and reproducible surface chemistry. Although the present work highlights the role of graphene

derivatives and CNTs in enhancing adsorption and fluorescence modulation, further studies are required to better control surface heterogeneity and binding-site distribution. This aspect is essential for improving reproducibility and reducing variability in complex biological media, where non-specific interactions and matrix effects remain significant limitations.

Another relevant direction involves the deeper integration of photophysical mechanisms into platform design. The findings of this thesis, together with recent advances in fluorescence biosensing, indicate that analytical performance is governed by the combined action of multiple processes, including FRET with PET, and charge-transfer interactions. Future work should aim to design platforms in which these mechanisms are not only exploited but deliberately engineered, enabling more predictable and tunable signal responses. Such an approach would allow the transition from empirical optimization to mechanism-driven design strategies.

The optimization of ionic media and binding conditions also represents a critical area for future research. The presented results from this thesis clearly demonstrate the influence of divalent ions, particularly Mg^{2+} , on oligonucleotide adsorption and fluorescence response. Extending this investigation toward more complex and physiologically relevant systems, as biological fluids, would provide a more accurate assessment of platform performance. In this context, the use of simulated biological media should be complemented by validation in clinical samples.

From a technological perspective, an important direction is the integration of the developed platforms into miniaturized and portable systems. As highlighted in recent developments in optical biosensing, there is a growing interest in point-of-care devices, microfluidic architectures, and smartphone-based detection systems. The efficiency in biomolecule immobilization, together with the simplicity and low cost of the NC-based platforms developed in this thesis, makes them suitable candidates for such integration. Future work may therefore focus on coupling these materials with microfluidic channels, optical readout modules, or digital imaging systems, enabling real-time and on-site analysis.

In parallel, the incorporation of data-driven approaches and artificial intelligence (AI) represents a promising direction for enhancing analytical performance. Machine learning algorithms can be used for signal processing, spectral deconvolution, and pattern recognition, particularly in complex datasets affected by noise, background fluorescence, or overlapping signals. Moreover, AI-assisted design strategies could accelerate the selection of optimal material combinations and operating conditions, reducing experimental trial-and-error.

Another key perspective relates to improving long-term stability and robustness of the sensing platforms. Issues such as photobleaching, signal drift, and material degradation remain

relevant, especially for repeated measurements or prolonged use. Future efforts should therefore address the stabilization of fluorescent signals and the development of more durable surface modifications, potentially through covalent functionalization strategies or protective coatings.

Finally, an important direction for future research is the extension of the platform toward broader analytical applications. While this thesis focuses on short oligonucleotide extraction and detection, the underlying principles can be adapted for the detection of other biomolecules, including microRNAs, proteins, or small metabolites. This versatility opens the possibility of developing multiplexed sensing platforms capable of addressing complex diagnostic needs in biomedical and environmental contexts.

Publications List and Participation to Scientific Conferences

This PhD thesis is based on the following scientific research articles published during the course of the doctoral program, with a cumulative impact factor of 13.2.

List of publications included in the thesis

1. **Toader G.A.**, Nitu F.R., Ionita M., *Graphene Oxide/Nitrocellulose Non-Covalent Hybrid as Solid Phase for Oligo-DNA Extraction from Complex Medium*; *Molecules*, Volum 28; Pages 4599-4612; [10.3390/molecules28124599](https://doi.org/10.3390/molecules28124599); 2023; Impact factor 4.2.

2. Chiticaru E.A., **Toader G.A.**, Ionita M., *Towards Point-Of-Care Medical Applications Using Electrochemical Biosensor*; University Politehnica of Bucharest, Scientific Bulletin, Series B-Chemistry and Materials Science; Volum 86; Pages 59-70; 2024; Impact factor 0.3.

3. **Toader G.A.**, Titus G.V., Ionita M., *Solid Phase Oligo-DNA Extraction from Complex Medium Using an Aminated Graphene/Nitrocellulose Membrane Hybrid*, *Biomolecules* (14), no. 3: 366, <https://doi.org/10.3390/biom14030366>; 2024; Impact factor 4.8.

4. **Toader, G.A.**, Mihalache, D.I., Grigorean, V.T., Chiticaru E.A., Pandele M.A., M. Ionita., *Efficient Solid-Phase Extraction of Oligo-DNA from Complex Media Using a Nitrocellulose Membrane Modified with Carbon Nanotubes and Aminated reduced Graphene Oxide*; *Scientific reports* 15, 5325, <https://doi.org/10.1038/s41598-025-89705-7>; 2025; Impact factor 3.9.

Other publications that are not included in the thesis:

1. **Toader G.A.**, Vlasceanu G.M., Serafim A., Banciu A., Ionita M.; *Double-Reinforced Fish Gelatin Composite Scaffolds for Osteochondral Substitutes*; Materials; Volum 16; Pagini 1815-1833; <https://doi.org/10.3390/ma16051815>; 2023; Impact factor 3.1.

2. Ionita M., Vlăsceanu G.M., **Toader G.A.**, Manole M., *Advances in Therapeutic Contact Lenses for the Management of Different Ocular Conditions*, Journal of Personalized Medicine (13), no. 11: 1571. <https://doi.org/10.3390/jpm13111571>, 2023, Impact factor 3.

3. Cojocaru E., Oprea M., Vlasceanu G.M., Nicolae M.C., Popescu R.C., Mereuta P.E., **Toader G.A.**, Ioniță M., *Dual Nanofiber and Graphene Reinforcement of 3D Printed Biomimetic Supports for Bone Tissue Repair*, RSC advances, 14(44), 32517-32532., DOI: <https://doi.org/10.1039/D4RA06167E>; 2024, Impact factor 3.9.

4. Dobrisan M.R., **Toader G.A.**, Chiticaru A.E, Vlasceanu G.M., Popescu R.C., Nicoara A.I., Podaru A.I., Toader G., Rotariu T., Ionita M., *Nanostructured Gelatin-Gellan Inks Double Reinforced with Cellulose Nanofibrils and Graphene Derivatives for 3D Printing in Bone Regeneration Applications*; Materials Today Advances; DOI: <https://doi.org/10.1016/j.mtadv.2025.1006211>, 2025, Impact factor 8.0.

5. Cojocaru E., Chiticaru E.A., Vlasceanu G.M., Nicolae M.C., Popescu R.C., Dobrisan M.R., **Toader G.A.**, Ionita M., *3D Printing of Multicomponent Polymeric Inks Enables Hierarchical Hybrid Architectures for Bone Tissue Regeneration*; Materials Today Advances. DOI: <https://doi.org/10.1016/j.mtadv.2025.100683>, 2025, Impact factor 8.0.

The results obtained during this program were disseminated at the following national and international conferences:

- 13th International Advances in Applied Physics & Materials Science Congress & Exhibition (APMAS 2022); Fethiye–Muğla, Turkey; 13-19 October 2022; poster presentation: “Graphene oxide/Nitrocellulose non-covalent hybrid as solid phase for oligoDNA extraction from complex media”, authors: **Toader G.A.**, Nitu F.R., Ionita M.
- 3rd International Conference on Bioengineering and Polymer Science; Bucharest, Romania; 7-10 June 2023; poster presentation: “Graphene oxide/Nitrocellulose non-covalent hybrid as solid

phase for oligoDNA extraction from complex medium”, authors: **Toader G.A.**, Nitu F.R., Ionita M.

- 13th World Biomaterials Congress (WBC 2024); Daegu, South Korea; 26-31 May 2024; poster presentation: “Process for Fabricating 3D Printed Scaffolds Based on Sodium Alginate and Graphene Oxide for Personalized Tissue Engineering”, authors: **Toader G.A.**, Cojocaru E., Ionita M.
- 23rd Romanian International Conference on Chemistry and Chemical Engineering (RICCCE 2024); Constanța, Romania; 4-7 September 2024; oral presentation: “Oligo-DNA Extraction from Complex Media Using Nitrocellulose Membranes Modified with Carbon Nanotubes and PEG-Aminated Graphene”, authors: **Toader G.A.**, Ionita M.
- Napoca Biodent; Cluj-Napoca, Romania; 27-28 September 2024; oral presentation: “Graphene-Based Materials for Bone Tissue Engineering: Advancements in Bioinspired 3D Printable Scaffold Design and Nucleic Acid Extraction Techniques”, authors: **Toader G.A.**, Ionita M.
- 4th International Conference on Bioengineering and Polymer Science (BPC 4); Brașov, Romania; 2-5 June 2025; oral presentation: “Efficient antisense oligonucleotide (ASO) extraction using graphene-based platforms for designing bioinspired 3D printable scaffolds employed in bone tissue engineering”, authors: **Toader G.A.**, Dobrișan M.R., Chiticaru E.A., Cojocaru E., Ionita M.

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Selective bibliography:

- [1] Z. Rezaei, N. Wang, A. D. J. A. Rodriguez, S. Higashi, and S. R. Shin, "Biosensors in biomedical research: Bridging cell and tissue engineering and real-time monitoring," *Current Opinion in Biomedical Engineering*, vol. 34, 2025, doi: 10.1016/j.cobme.2025.100582.
- [2] A. H. Hoveidaei *et al.*, "Clinical challenges in bone tissue engineering - A narrative review," *Bone*, vol. 192, p. 117363, Mar 2025, doi: 10.1016/j.bone.2024.117363.
- [3] L. V. Bauso, V. La Fauci, C. Longo, and G. Calabrese, "Bone Tissue Engineering and Nanotechnology: A Promising Combination for Bone Regeneration," *Biology (Basel)*, vol. 13, no. 4, Apr 2 2024, doi: 10.3390/biology13040237.
- [4] Y. Sun, Z. Sun, and W. Chen, "The evolution of object detection methods," *Engineering Applications of Artificial Intelligence*, vol. 133, 2024, doi: 10.1016/j.engappai.2024.108458.
- [5] Y. Luo *et al.*, "Detection methods for antibiotics in wastewater: a review," *Bioprocess Biosyst Eng*, vol. 47, no. 9, pp. 1433-1451, Sep 2024, doi: 10.1007/s00449-024-03033-0.
- [6] S. Cheng, B. Huang, K. Lu, J. Li, H. Wen, and J. Zhang, "Recent advances in upconversion nanoparticles for biomolecule detection: a review," *Anal Methods*, vol. 17, no. 27, pp. 5563-5578, Jul 10 2025, doi: 10.1039/d5ay00631g.
- [7] G. V. Research. "'Biosensors Market Size, Share & Trends Report'." (accessed 29.12.2025).
- [8] S. Ben-Aissa, S. Tripathy, and A. E. G. Cass, "Implantable electrochemical biosensors: Challenges, strategies, and applications," *Current Opinion in Electrochemistry*, vol. 54, 2025, doi: 10.1016/j.coelec.2025.101745.
- [9] E. A. Chiticaru, L. Pilan, and M. Ionita, "Electrochemical Detection Platform Based on RGO Functionalized with Diazonium Salt for DNA Hybridization," *Biosensors (Basel)*, vol. 12, no. 1, Jan 13 2022, doi: 10.3390/bios12010039.
- [10] B. McCann, B. Tipper, S. Shahbeigi, M. Soleimani, M. Jabbari, and M. Nasr Esfahani, "A Review on Perception of Binding Kinetics in Affinity Biosensors: Challenges and Opportunities," *ACS Omega*, vol. 10, no. 5, pp. 4197-4216, Feb 11 2025, doi: 10.1021/acsomega.4c10040.
- [11] A. Gautam, "Towards Modern-Age Advanced Sensors for the Management of Neurodegenerative Disorders: Current Status, Challenges and Prospects," *ECS Sensors Plus*, vol. 1, no. 4, 2022, doi: 10.1149/2754-2726/ac973e.
- [12] T. Chalklen, Q. Jing, and S. Kar-Narayan, "Biosensors Based on Mechanical and Electrical Detection Techniques," *Sensors (Basel)*, vol. 20, no. 19, Sep 30 2020, doi: 10.3390/s20195605.
- [13] N. Alanazi, M. Almutairi, and A. N. Alodhayb, "A Review of Quartz Crystal Microbalance for Chemical and Biological Sensing Applications," *Sens Imaging*, vol. 24, no. 1, p. 10, 2023, doi: 10.1007/s11220-023-00413-w.

- [14] K. Wu, D. Tonini, S. Liang, R. Saha, V. K. Chugh, and J. P. Wang, "Giant Magnetoresistance Biosensors in Biomedical Applications," *ACS Appl Mater Interfaces*, vol. 14, no. 8, pp. 9945-9969, Mar 2 2022, doi: 10.1021/acsami.1c20141.
- [15] V. O. Jimenez *et al.*, "Magnetoimpedance Biosensors and Real-Time Healthcare Monitors: Progress, Opportunities, and Challenges," *Biosensors (Basel)*, vol. 12, no. 7, Jul 12 2022, doi: 10.3390/bios12070517.
- [16] J. Hu, F. Liu, Y. Chen, G. Shangguan, and H. Ju, "Mass Spectrometric Biosensing: A Powerful Approach for Multiplexed Analysis of Clinical Biomolecules," *ACS Sens*, vol. 6, no. 10, pp. 3517-3535, Oct 22 2021, doi: 10.1021/acssensors.1c01394.
- [17] F. Chu, W. Wei, N. S. Shuaibu, H. Feng, X. Wang, and Y. Pan, "Mass Spectrometry-Based Biosensing and Biopsy Technology," *Chemosensors*, vol. 11, no. 8, 2023, doi: 10.3390/chemosensors11080419.
- [18] M. Xu *et al.*, "Recent advances in nanomaterial-based optical biosensors and their biomedical and biopharmaceutical applications," *J Pharm Anal*, vol. 16, no. 1, p. 101349, Jan 2026, doi: 10.1016/j.jpha.2025.101349.
- [19] S. Oliveira *et al.*, "Advances in optical devices for the detection of contaminants in food and water," *TrAC Trends in Analytical Chemistry*, vol. 184, 2025, doi: 10.1016/j.trac.2025.118139.
- [20] B. A. Taha *et al.*, "Unlocking new frontiers in healthcare: The impact of nano-optical biosensors on personalized medical diagnostics," *J Biotechnol*, vol. 400, pp. 29-47, Apr 2025, doi: 10.1016/j.jbiotec.2025.02.005.
- [21] N. Sagar Shrikrishna, R. Sharma, J. Sahoo, A. Kaushik, and S. Gandhi, "Navigating the landscape of optical biosensors," *Chemical Engineering Journal*, vol. 490, 2024, doi: 10.1016/j.cej.2024.151661.
- [22] T. P. Patil, A. K. Parthasarathy, D. Malavekar, J. Kim, and A. P. Tiwari, "Optical Biosensing of SARS-CoV-2 RNA Based on Positively Charged Poly-l-Lysine Functionalized Gold Nanoparticles," *Journal of Cluster Science*, vol. 35, no. 7, pp. 2525-2538, 2024, doi: 10.1007/s10876-024-02678-x.
- [23] S. Mohamed *et al.*, "UV-VIs Spectrophotometry Study of Escherichia Coli Bacteria and Microfluidic Channel Chip Fabrication For Optical Biosensing Application," presented at the 2024 41st National Radio Science Conference (NRSC), 2024.
- [24] Y. S. Tekin, S. M. Kul, O. Sagdic, N. Rodthongkum, B. Geiss, and T. Ozer, "Optical biosensors for diagnosis of COVID-19: nanomaterial-enabled particle strategies for post pandemic era," *Mikrochim Acta*, vol. 191, no. 6, p. 320, May 10 2024, doi: 10.1007/s00604-024-06373-6.
- [25] K. Brzozowski *et al.*, "Tandem Raman microscopy: integration of spontaneous and coherent Raman scattering offering data fusion analysis to improve optical biosensing," *Optica*, vol. 12, no. 1, 2025, doi: 10.1364/optica.537479.
- [26] P. I. Diriwari, "Resonance Energy Transfer within and to optical Nanoparticles for Bioimaging and Biosensing Applications," Doctoral, CHIMIE, l'Université de Rouen Normandie, HAL open science, 2024.
- [27] S. Dasgupta and K. Ray, "Plasmon-enhanced fluorescence for biophotonics and bio-analytical applications," *Front Chem*, vol. 12, p. 1407561, 2024, doi: 10.3389/fchem.2024.1407561.
- [28] Y. Wang, K. Jia, and J. Lin, "Optical biosensors for the detection of foodborne pathogens: recent development and future prospects," *TrAC Trends in Analytical Chemistry*, vol. 177, 2024, doi: 10.1016/j.trac.2024.117785.

- [29] S. Kumar, A. Iadicicco, S. Kim, D. Tosi, and C. Marques, "Introduction to the feature issue: Advances in Optical Biosensors for Biomedical Applications," *Biomed Opt Express*, vol. 15, no. 5, pp. 3183-3190, May 1 2024, doi: 10.1364/BOE.527613.
- [30] M. Wang, Y. Da, and Y. Tian, "Fluorescent proteins and genetically encoded biosensors," *Chem Soc Rev*, vol. 52, no. 4, pp. 1189-1214, Feb 20 2023, doi: 10.1039/d2cs00419d.
- [31] A. K. Verma, A. Noumani, A. K. Yadav, and P. R. Solanki, "FRET Based Biosensor: Principle Applications Recent Advances and Challenges," *Diagnostics (Basel)*, vol. 13, no. 8, Apr 8 2023, doi: 10.3390/diagnostics13081375.
- [32] F. Tian, G. Xu, S. Zhou, S. Chen, and D. He, "Principles and applications of green fluorescent protein-based biosensors: a mini-review," *Analyst*, vol. 148, no. 13, pp. 2882-2891, Jun 26 2023, doi: 10.1039/d3an00320e.
- [33] D. Semeniak, D. F. Cruz, A. Chilkoti, and M. H. Mikkelsen, "Plasmonic Fluorescence Enhancement in Diagnostics for Clinical Tests at Point-of-Care: A Review of Recent Technologies," *Adv Mater*, vol. 35, no. 34, p. e2107986, Aug 2023, doi: 10.1002/adma.202107986.
- [34] S. Valdez, M. Robertson, and Z. Qiang, "Fluorescence Resonance Energy Transfer Measurements in Polymer Science: A Review," *Macromol Rapid Commun*, vol. 43, no. 24, p. e2200421, Dec 2022, doi: 10.1002/marc.202200421.
- [35] A. S. Sharma and N. Y. Lee, "Nanosurface Energy Transfer: Principles, Biosensing Applications, and Future Prospects," *Chemosensors*, vol. 14, no. 1, 2026, doi: 10.3390/chemosensors14010011.
- [36] D. Udhayakumari, D. Duraisamy, and A. Nanthakumar, "PET Coordination Mechanism for the Detecting of Environmental Toxic Analytes: Current Approaches and Future Directions," *J Fluoresc*, vol. 35, no. 10, pp. 9299-9315, Oct 2025, doi: 10.1007/s10895-025-04237-7.
- [37] R. Pramanik, "Nanomaterial-enhanced fluorescence sensors for dopamine neurotransmitters: a photophysical perspective," *Anal Methods*, vol. 17, no. 21, pp. 4251-4292, May 29 2025, doi: 10.1039/d5ay00300h.
- [38] M. Essid and E. U. Mughal, "Push-pull heterocycles and beyond: recent developments in absorption, emission, and ICT properties," *RSC Adv*, vol. 15, no. 45, pp. 37609-37644, Oct 8 2025, doi: 10.1039/d5ra06623a.
- [39] C. Chen and C. Fang, "Fluorescence Modulation by Amines: Mechanistic Insights into Twisted Intramolecular Charge Transfer (TICT) and Beyond," *Chemosensors*, vol. 11, no. 2, 2023, doi: 10.3390/chemosensors11020087.
- [40] W. C. Yang, S. Y. Li, S. Ni, and G. Liu, "Advances in FRET-based biosensors from donor-acceptor design to applications," *Aggregate*, vol. 5, no. 2, 2023, doi: 10.1002/agt2.460.
- [41] A. Kaur and S. Dhakal, "Recent applications of FRET-based multiplexed techniques," *TrAC Trends in Analytical Chemistry*, vol. 123, 2020, doi: 10.1016/j.trac.2019.115777.
- [42] R. Govindaraju and J. Kim, "MXene-based fluorescent aptasensors: advances and prospects in diagnostics and environmental monitoring," *Analyst*, Jan 28 2026, doi: 10.1039/d5an00945f.
- [43] R. Huang *et al.*, "Molecular design and architectonics towards film-based fluorescent sensing," *Chem Soc Rev*, vol. 53, no. 13, pp. 6960-6991, Jul 1 2024, doi: 10.1039/d4cs00347k.

- [44] C. Schirripa Spagnolo and S. Luin, "Choosing the Probe for Single-Molecule Fluorescence Microscopy," *Int J Mol Sci*, vol. 23, no. 23, Nov 29 2022, doi: 10.3390/ijms232314949.
- [45] L. Riccardi, V. Genna, and M. De Vivo, "Metal–ligand interactions in drug design," *Nature Reviews Chemistry*, vol. 2, no. 7, pp. 100-112, 2018, doi: 10.1038/s41570-018-0018-6.
- [46] X. Li, Z. Cai, L. P. Jiang, Z. He, and J. J. Zhu, "Metal-Ligand Coordination Nanomaterials for Biomedical Imaging," *Bioconjug Chem*, vol. 31, no. 2, pp. 332-339, Feb 19 2020, doi: 10.1021/acs.bioconjchem.9b00642.
- [47] D. Quesada-Gonzalez and A. Merkoci, "Quantum dots for biosensing: Classification and applications," *Biosens Bioelectron*, vol. 273, p. 117180, Apr 1 2025, doi: 10.1016/j.bios.2025.117180.
- [48] N. Le, M. Zhang, and K. Kim, "Quantum Dots and Their Interaction with Biological Systems," *Int J Mol Sci*, vol. 23, no. 18, Sep 15 2022, doi: 10.3390/ijms231810763.
- [49] I. Sarkar and A. K. Mishra, "Fluorophore tagged bio-molecules and their applications: A brief review," *Applied Spectroscopy Reviews*, vol. 53, no. 7, pp. 552-601, 2017, doi: 10.1080/05704928.2017.1376680.
- [50] A. Acharya *et al.*, "Photoinduced Chemistry in Fluorescent Proteins: Curse or Blessing?," *Chem Rev*, vol. 117, no. 2, pp. 758-795, Jan 25 2017, doi: 10.1021/acs.chemrev.6b00238.
- [51] Z. Jia, A. Kai, and M. Liu, "Fluorescent organic cages and their applications," *Chemical Synthesis*, vol. 6, no. 1, 2026, doi: 10.20517/cs.2024.147.
- [52] L. Zhang, D. Shen, and J. Yang, "Advancing Single-Molecule Biophysics: Next-Generation Organic Fluorophores with Tailored Labeling Strategies," *Chem Biomed Imaging*, vol. 3, no. 9, pp. 572-598, Sep 22 2025, doi: 10.1021/cbmi.5c00007.
- [53] F. Yan *et al.*, "Fluorescein applications as fluorescent probes for the detection of analytes," *TrAC Trends in Analytical Chemistry*, vol. 97, pp. 15-35, 2017, doi: 10.1016/j.trac.2017.08.013.
- [54] K. S. B. Sam, L. George, S. Y. N., and A. Varghese, "Fluorescein Based Fluorescence Sensors for the Selective Sensing of Various Analytes," *J Fluoresc*, vol. 31, no. 5, pp. 1251-1276, Sep 2021, doi: 10.1007/s10895-021-02770-9.
- [55] D. P. Surzhikova, L. A. Sukovaty, E. V. Nemtseva, E. N. Esimbekova, and E. A. Slyusareva, "Functioning of a Fluorescein pH-Probe in Aqueous Media: Impact of Temperature and Viscosity," *Micromachines (Basel)*, vol. 14, no. 7, Jul 18 2023, doi: 10.3390/mi14071442.
- [56] P. R. Callis, "Binding phenomena and fluorescence quenching. I: Descriptive quantum principles of fluorescence quenching using a supermolecule approach," *Journal of Molecular Structure*, vol. 1077, pp. 14-21, 2014, doi: 10.1016/j.molstruc.2014.04.050.
- [57] R. A. Salinas Dominguez, J. Aguila Rosas, S. E. Martinez Tolibia, E. Lima, and A. Dutt, "Opportunities in functionalized metal-organic frameworks (MOFs) with open metal sites for optical biosensor application," *Adv Colloid Interface Sci*, vol. 344, p. 103598, Oct 2025, doi: 10.1016/j.cis.2025.103598.
- [58] Y. Ren *et al.*, "Fluorescence Visualization of Strain by Tetraphenylethylene/Ruthenium Complex Three-Dimensional Covalent Organic Framework Assemblies," *J Am Chem Soc*, vol. 147, no. 23, pp. 20033-20044, Jun 11 2025, doi: 10.1021/jacs.5c05354.
- [59] G. Petresky, M. Faran, V. Wulf, and G. Bisker, "Metal-Ion Optical Fingerprinting Sensor Selection via an Analyte Classification and Feature Selection Algorithm," *Anal*

- Chem*, vol. 97, no. 16, pp. 8821-8832, Apr 29 2025, doi: 10.1021/acs.analchem.4c06762.
- [60] F. R. Nitu, J. S. Burns, and M. Ionitǎ, "Oligonucleotide Detection and Optical Measurement with Graphene Oxide in the Presence of Bovine Serum Albumin Enabled by Use of Surfactants and Salts," *Coatings*, vol. 10, no. 4, 2020, doi: 10.3390/coatings10040420.
 - [61] F. R. Nitu, L. Savu, S. Muraru, I. Stoian, and M. Ionita, "Label-Free Homogeneous microRNA Detection in Cell Culture Medium Based on Graphene Oxide and Specific Fluorescence Quenching," *Nanomaterials (Basel)*, vol. 11, no. 2, Feb 2 2021, doi: 10.3390/nano11020368.
 - [62] S. Muraru, S. Muraru, F. R. Nitu, and M. Ionita, "Recent Efforts and Milestones for Simulating Nucleic Acid FRET Experiments through Computational Methods," *J Chem Inf Model*, vol. 62, no. 2, pp. 232-239, Jan 24 2022, doi: 10.1021/acs.jcim.1c00957.
 - [63] P. Zoruk, M. Morozov, V. Veselovsky, A. Strokach, V. Babenko, and K. Klimina, "Impact of DNA extraction techniques and sequencing approaches on microbial community profiling accuracy," *Frontiers in Microbiomes*, vol. 4, 2025, doi: 10.3389/frmbi.2025.1688681.
 - [64] M. S. Adamowicz, D. M. Stasulli, E. M. Sobestanovich, and T. W. Bille, "Evaluation of methods to improve the extraction and recovery of DNA from cotton swabs for forensic analysis," *PLoS One*, vol. 9, no. 12, p. e116351, 2014, doi: 10.1371/journal.pone.0116351.