The 9th International Electronic Conference on Medicinal Chemistry (ECMC 2023)

01–30 November 2023 | Online

Nucleic acids and analogues: tools for therapeutic and biosensoristic applications

Chaired by Dr. Alfredo Berzal-Herranz and Prof. Dr. Maria Emília Sousa

pharmaceuticals

MDPI

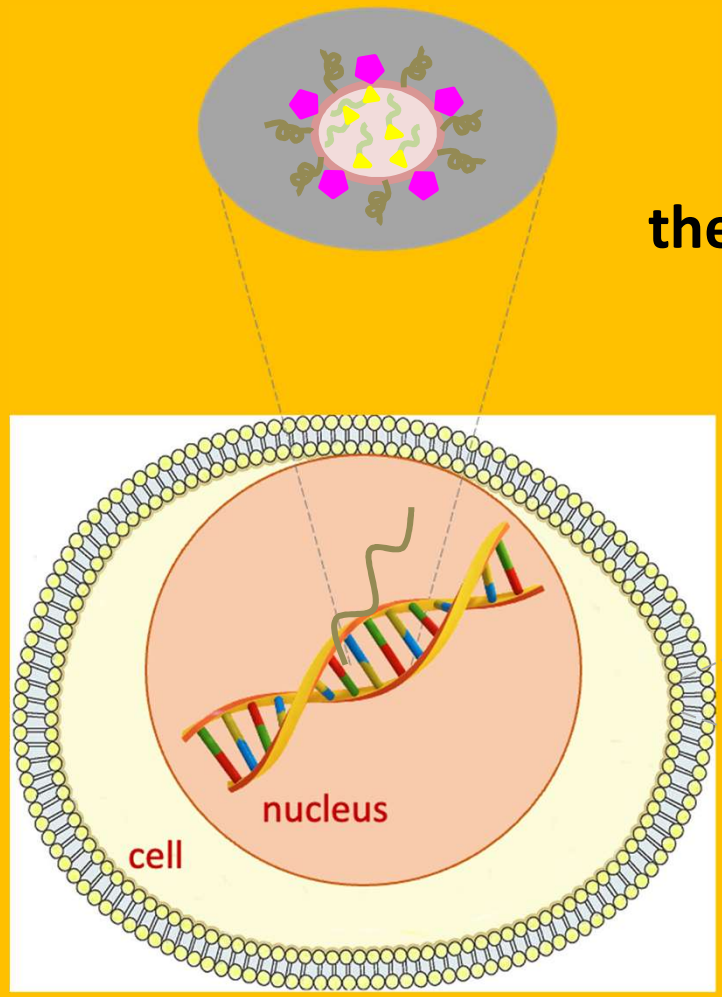
Andrea Patrizia Falanga[1,*], Stefano D'Errico[1], Monica Terracciano[1], Francesca Greco[1], Maria Marzano[1], Maria Grazia Nolli[1], Giorgia Oliviero[2] and Nicola Borbone[1]

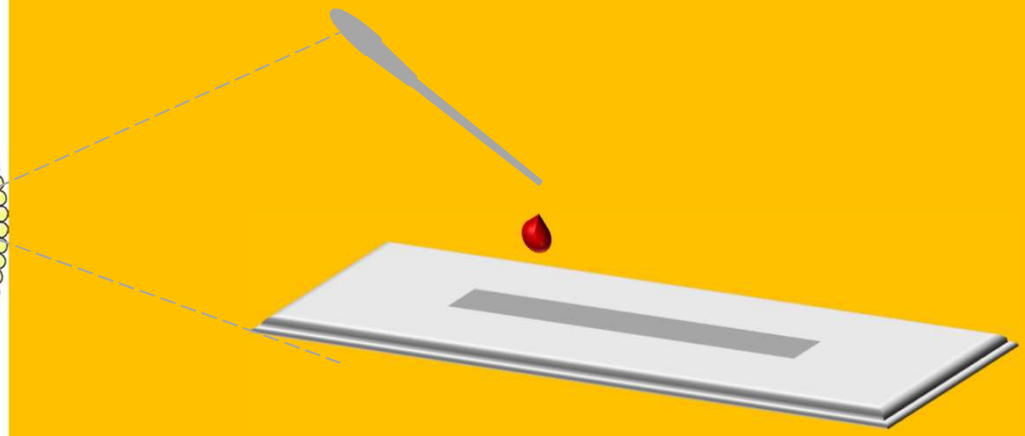
[1] Dipartimento di Farmacia, Università degli Studi di Napoli Federico II, Naples, 80131, Italy ;

[2] Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Naples, 80131, Italy.

* Corresponding author: andreapatrizia.falanga@unina.it

Nucleic acids and analogues: tools for therapeutic and biosensoristic applications





Abstract:

Single-stranded oligonucleotides have been explored in the field of precision medicine both as therapeutics and diagnostic tools. Oligonucleotides that function in a sequence-dependent manner have been used to address diseases that range from neurological to metabolic, as well as to develop vaccines. Until now, their use has been limited by their short half-life in the biological environment. These limiting aspects can now be overcome by resorting to chemical modifications in the drug and using appropriate nanocarriers. Thus, synthetic analogues of oligonucleotides are exploited to increase their application. Peptide nucleic acid (PNA) represents a promising class of synthetic DNA analogues in which the favoured sugar backbone is replaced by N2 aminoethylglycine repeats held together by peptide bonds. In this context, we investigated the relevance of PNAs in therapy and diagnostics. In the first case, we synthesized an antigene anti-Bcl-2 PNA, and we have developed a new delivery system never used before for the transport of PNA based on oncolytic adenoviruses. This promising transport system has already demonstrated its extraordinary effectiveness, as evidenced by the recent development of SARS-CoV vaccines. Furthermore, we have also used PNAs to support the functionalization of the biosensor development. We covalently bound the PNA to the surface of ZnO nanowires for mRNA CD5 detection, a diagnostic marker of Leukemia. The PNA-based biosensor has also been developed to detect mutations responsible for pathologies such as Brugada syndrome. The wide range of applications denotes the versatility of single-stranded oligonucleotides as a robust therapeutic and diagnostic platform

Keywords: Biosensor; drug delivery; nanocarrier; oligonucleotide; PNA

Introduction

Table 1. Some of the most used nucleic acid-based vaccines in Western countries.

Vaccine Name	Carried Nucleic Acid	Developer	Confirmed Efficacy
ChAdOx1-S/AZD1222	DNA	AstraZeneca + University of Oxford	63.1%, based on a median follow-up of 80 days
Ad26.CoV2.S	DNA	Janssen Pharmaceuticals Johnson & Johnson	66.0%, 28 days post-vaccination
BNT162/Comirnaty	RNA	Pfizer/BioNTech + Fosun Pharma	95.0%, measured starting from seven days after the second dose
mRNA-1273	RNA	Moderna + National Institute of Allergy and Infectious Diseases (NIAID)	94.1%, measured starting from two weeks after the second dose

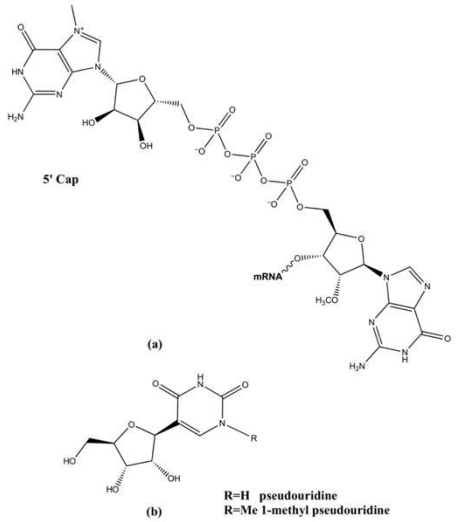
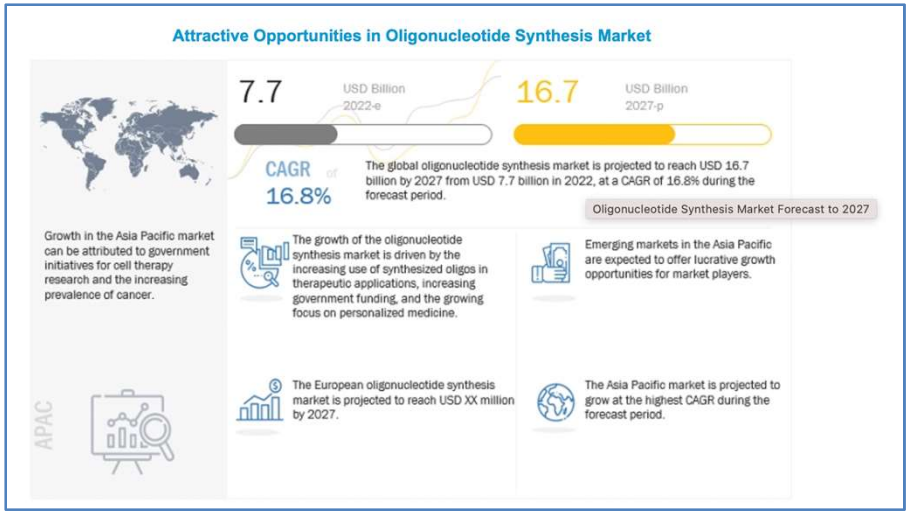
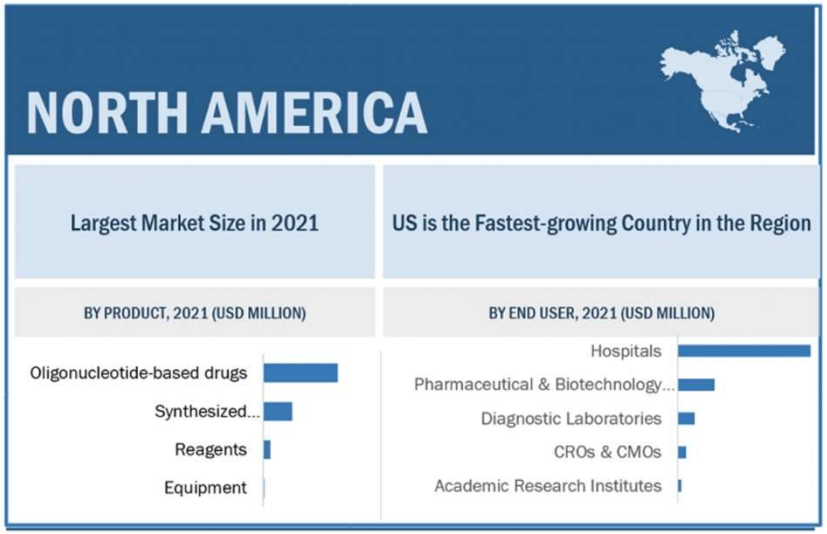
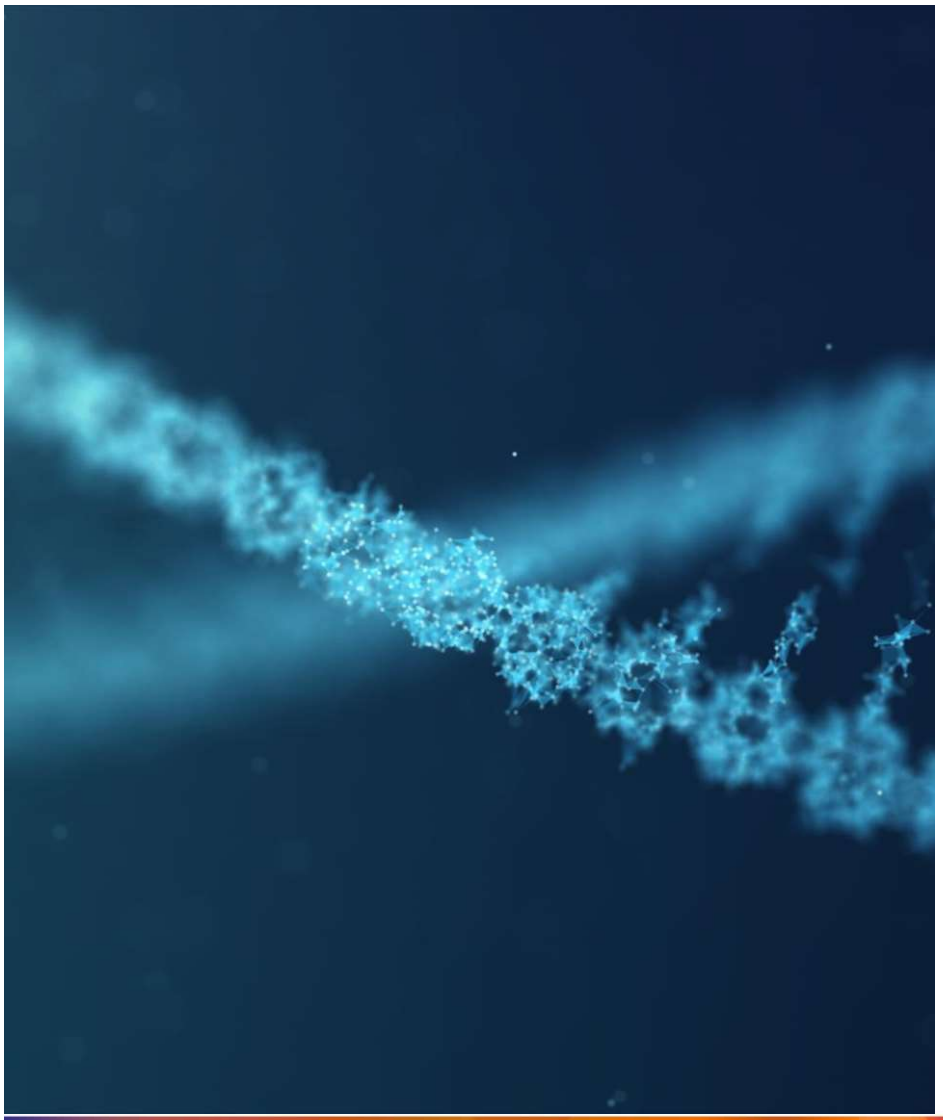


Figure 3. Schematic representation of some typical modifications of synthetic mRNAs contained in the COVID-19 vaccines: (**a**) 5′ capping via cap1 structure (m7GpppNm); (**b**) uridines are replaced with pseudouridine or 1-methyl pseudouridine units.


Attractive Opportunities in Oligonucleotide Synthesis Market
7.7
USD Billion
2022-e
16.7
USD Billion
2027-p
CAGR of 16.8%
The global oligonucleotide synthesis market is projected to reach USD 16.7 billion by 2027 from USD 7.7 billion in 2022, at a CAGR of 16.8% during the forecast period.
Oligonucleotide Synthesis Market Forecast to 2027
Growth in the Asia Pacific market can be attributed to government initiatives for cell therapy research and the increasing prevalence of cancer.
The growth of the oligonucleotide synthesis market is driven by the increasing use of synthesized oligos in therapeutic applications, increasing government funding, and the growing focus on personalized medicine.
Emerging markets in the Asia Pacific are expected to offer lucrative growth opportunities for market players.
APAC
The European oligonucleotide synthesis market is projected to reach USD XX million by 2027.
The Asia Pacific market is projected to grow at the highest CAGR during the forecast period.


NORTH AMERICA
Largest Market Size in 2021
US is the Fastest-growing Country in the Region
BY PRODUCT, 2021 (USD MILLION)
Oligonucleotide-based drugs
Synthesized...
Reagents
Equipment
BY END USER, 2021 (USD MILLION)
Hospitals
Pharmaceutical & Biotechnology...
Diagnostic Laboratories
CROs & CMOs
Academic Research Institutes



- Gene Therapy
- Biosensors

ODNs as therapeutics

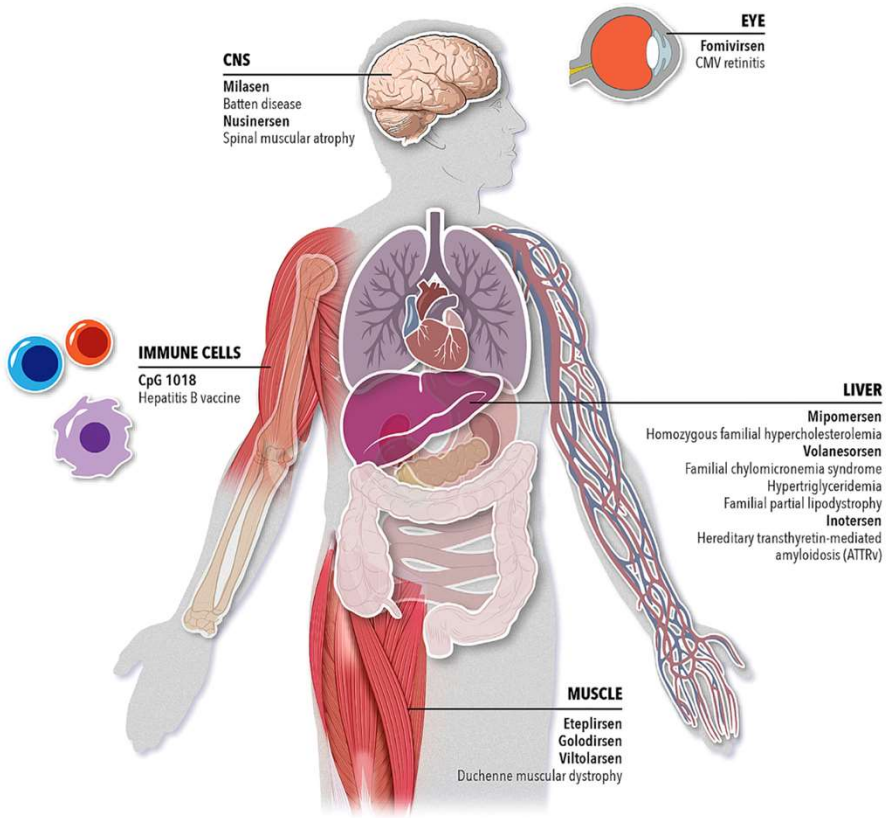
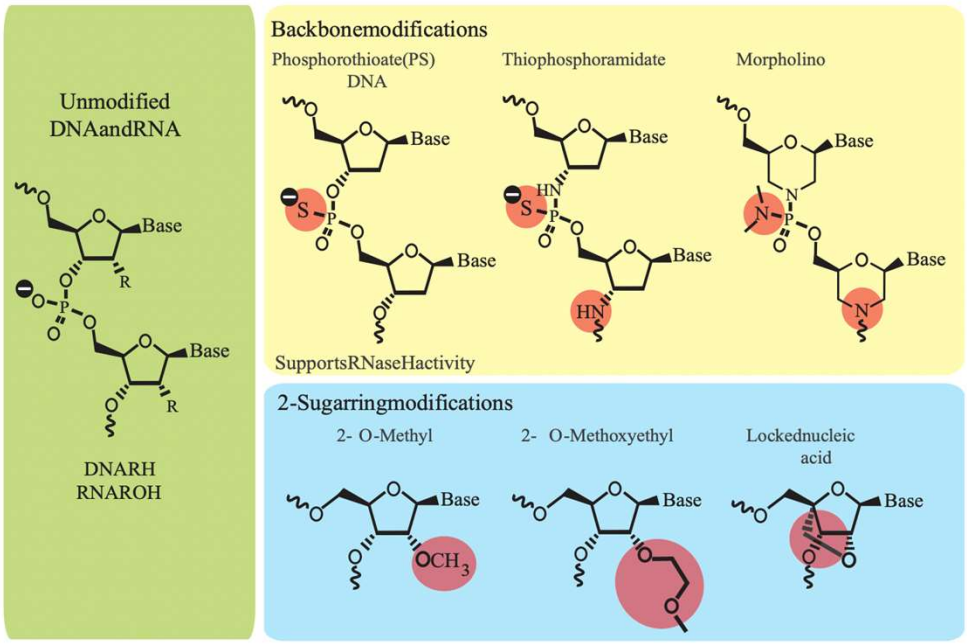


Figure 6. Single-Stranded Oligonucleotide Therapies
Summary of approved drugs to date and their target tissue. Milasen is a clinical investigational treatment under an Expanded Access-Investigational New Drug application.

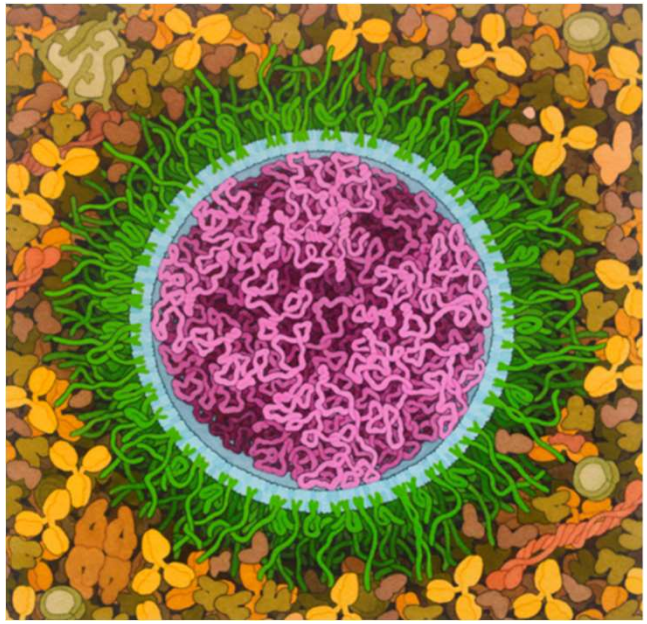
Scharner et al . Molecular Therapy Vol. 29 No 2 February 2021

LIMITATIONS:

- Degradation by endo/eso nucleases
- Affinity/selectivity for the target
- Poor pharmacokinetics
- Poor cellular uptake



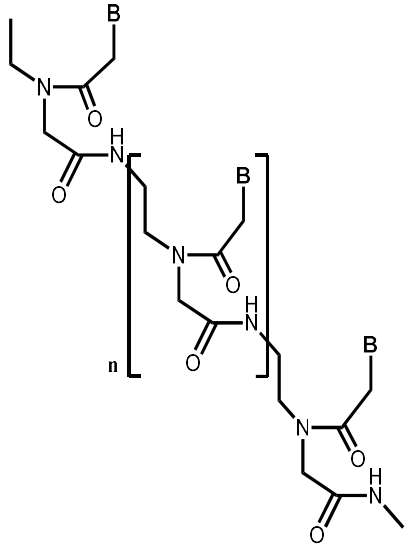
Neurotherapeutics **10**, 486–497 (2013)

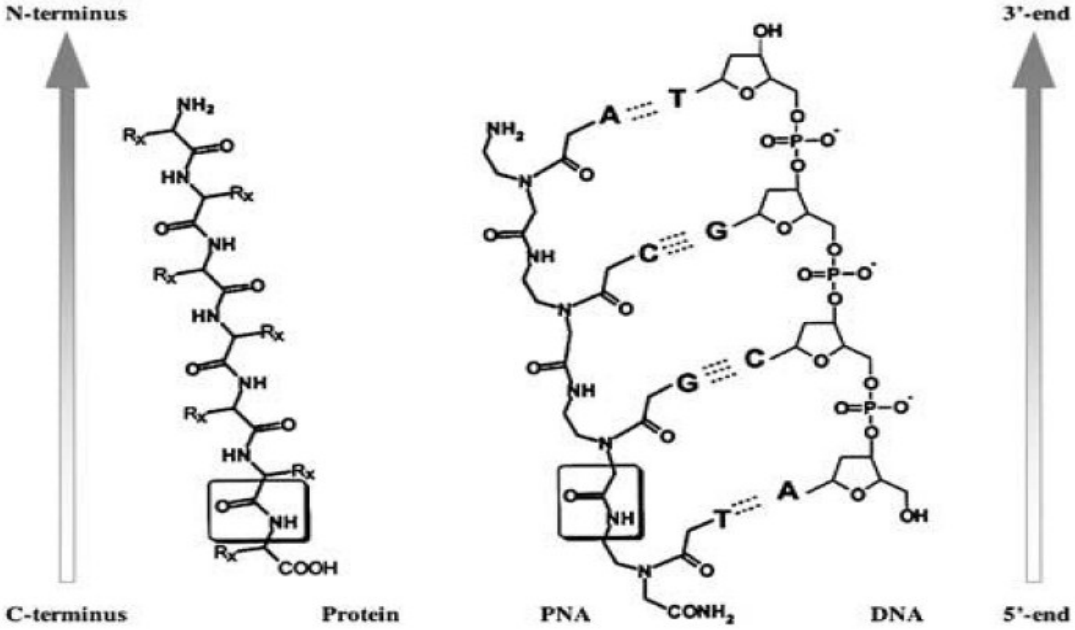


https://www.rcsb. org/structure/7B3Y,

Peptide Nucleic Acid (PNA)

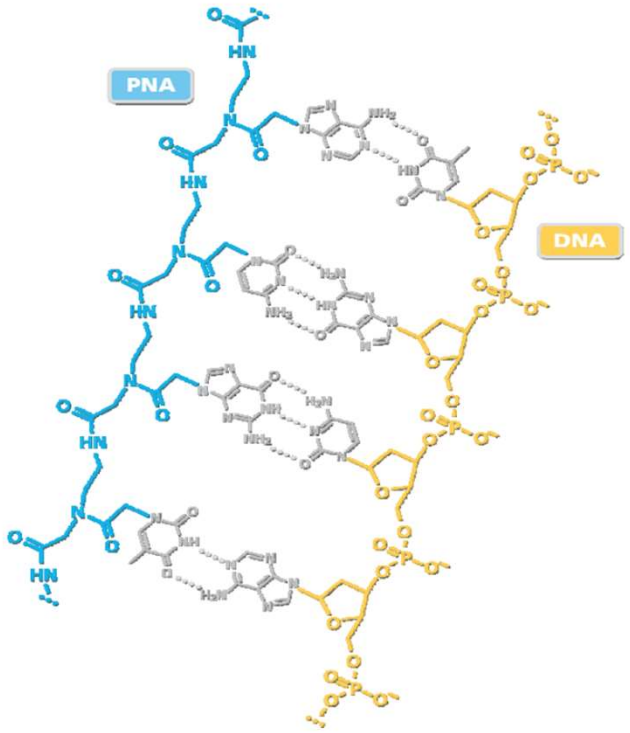
«What DNA could do, PNA can do it better»





- Increased affinity of hybridization
- Increased biological stability
- Increased chemical stability

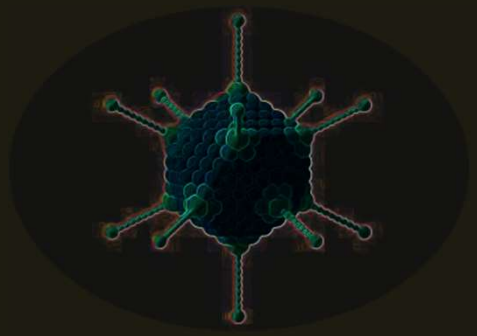
Peptide Nucleic Acid (PNA)



Characteristics of PNA

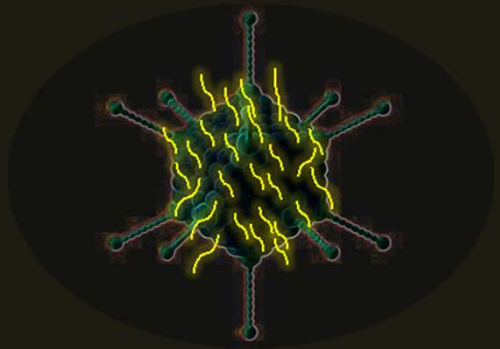
- Radically different from DNA
- Neutral backbone
- Higher affinity to complementary nucleic acid
- Hybridization independent of salt concentration
- Greater specificity and sensitivity of interaction
- Thermal and chemical stability
- Resistance to nucleases and proteases
- Triplex formation

Novel PNA Delivery Platform



Oncolytic Adenovirus (OAd)

PNA PNA

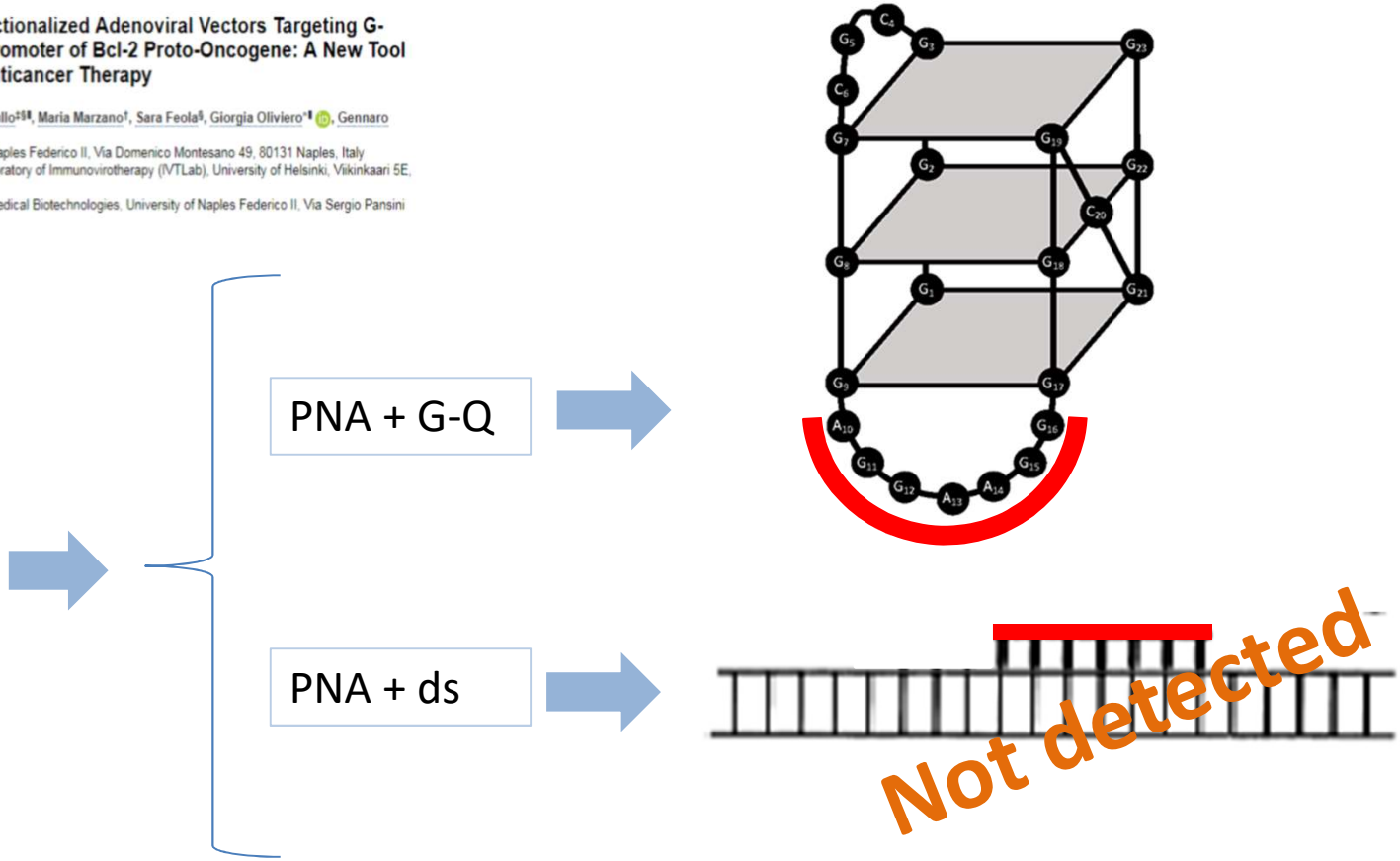


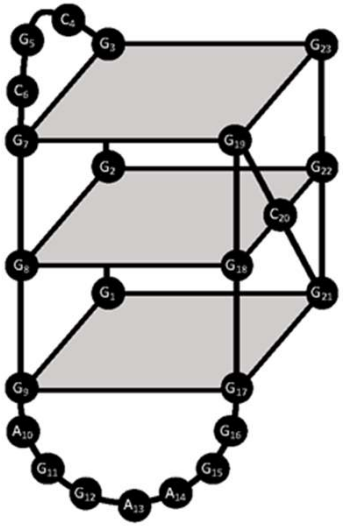
PNA-functionalized OAd

Bioconjugate Chemistry

Peptide Nucleic Acid-Functionalized Adenoviral Vectors Targeting G-Quadruplexes in the P1 Promoter of Bcl-2 Proto-Oncogene: A New Tool for Gene Modulation in Anticancer Therapy

Andrea Patrizia Falanga†, Vincenzo Cerullo‡§‖, Maria Marzano†, Sara Feola§, Giorgia Oliviero*†, Gennaro Piccialli†, and Nicola Borbone†
† Department of Pharmacy, University of Naples Federico II, Via Domenico Montesano 49, 80131 Naples, Italy
‡Drug Research Program (DRP) and §Laboratory of Immunovirotherapy (IVTLab), University of Helsinki, Viikinkaari 5E, 00790 Helsinki, Finland
‖ Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Via Sergio Pansini 5, 80131 Naples, Italy





GQ-Bcl-2

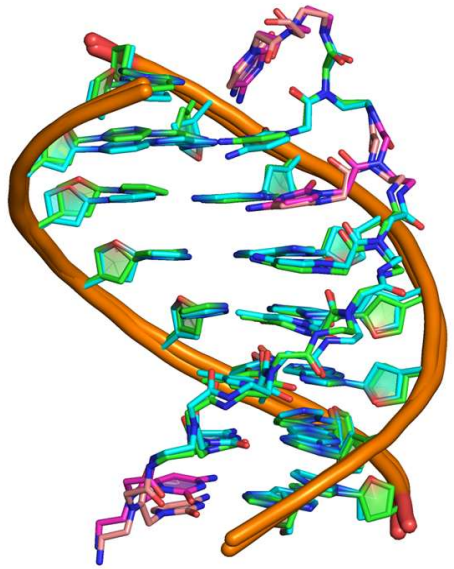
C-rich

5' 3'
G C
G C
G C
C G
G C
C G
G C
G C
G C
A T
G C
G C
A T
A T
G C
G C
G C
G C
G C
C G
G C
G C
G C
3' 5'

PNA

ds-Bcl-2

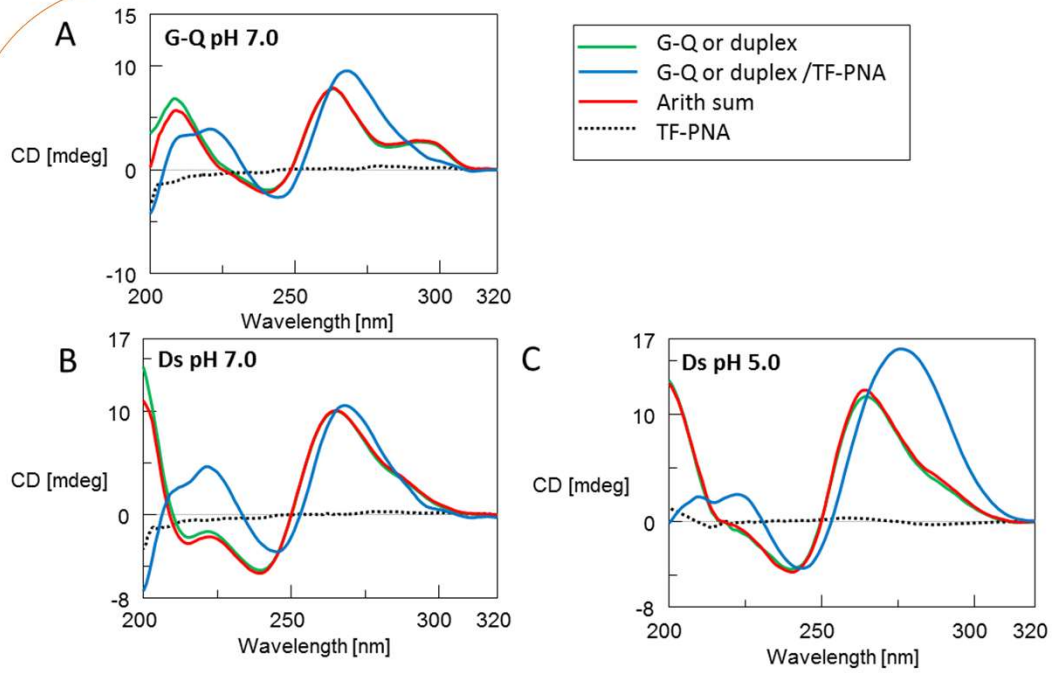
5' 3'
G C
G C
G C
C G
G C
C G
PNA
G C
G C
G C
T A T
C G C
C G C
T A T
T A T
C G C
C G C
C G C
C G C
C G C
G C
G C
G C
3' 5'



Heterotriplex: ds + PNA

Falanga et al.,Heliyon, 2023, under review

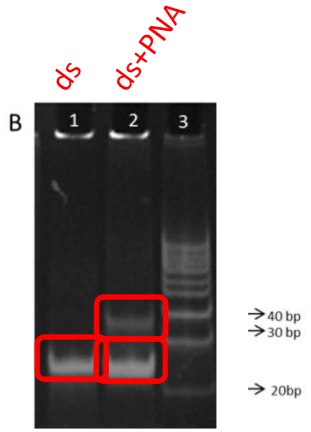
Circular Dichroism analysis



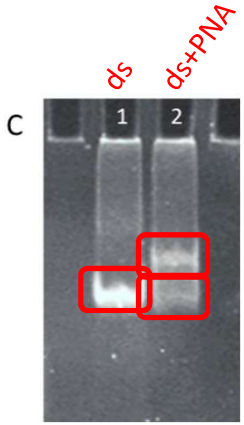
	Sample	λ min (nm)	λ max (nm)
pH 7.0	bcl2midG4-G4	240	208-263-293
	bcl2midG4-G4/TF-PNA	244	220-268
pH 7.0	bcl2midG4-ds	240	265-290
	triplex	245	222-268
pH 5.0	bcl2midG4-ds	240	265-290
	triplex	243	223-276

	Species	$T_{1/2}$ (°C)
pH 7.0	bclmidG4-G4	72
	bcl2midG4-G4/TF-PNA1K	30-72
pH 7.0	bcl2midG4-ds	72
	bcl2midG4-ds/TF-PNA1K	28-73
pH 5.0	bcl2midG4-ds	75
	bcl2midG4-ds/TF-PNA1K	40-78

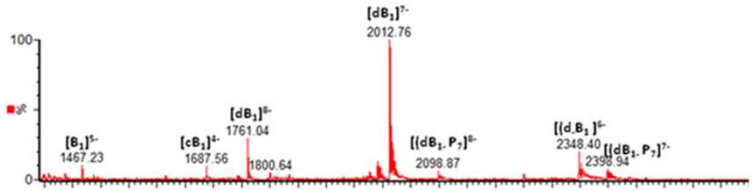
Falanga et al.,Heliyon, 2023, under review



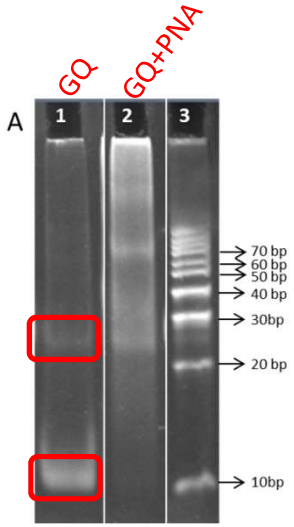
pH 7.0



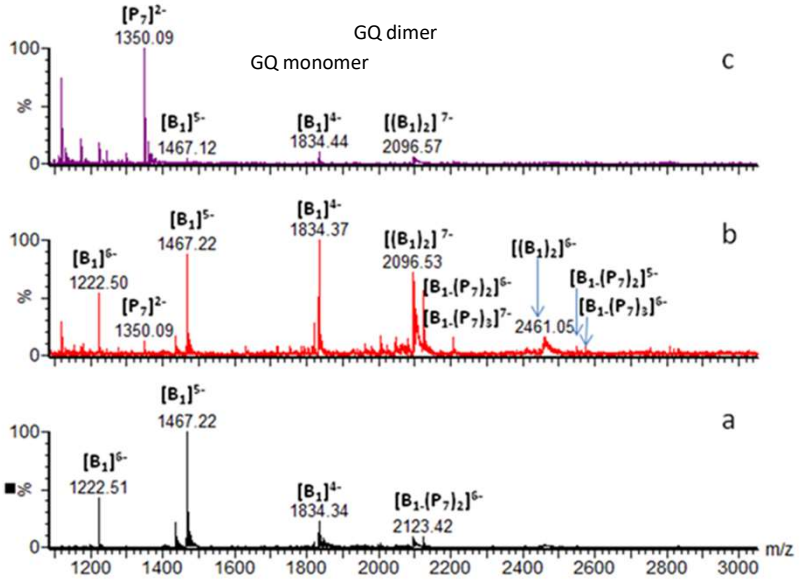
pH 5.0



1:4



pH 7.0



1:4_DNA:PNA

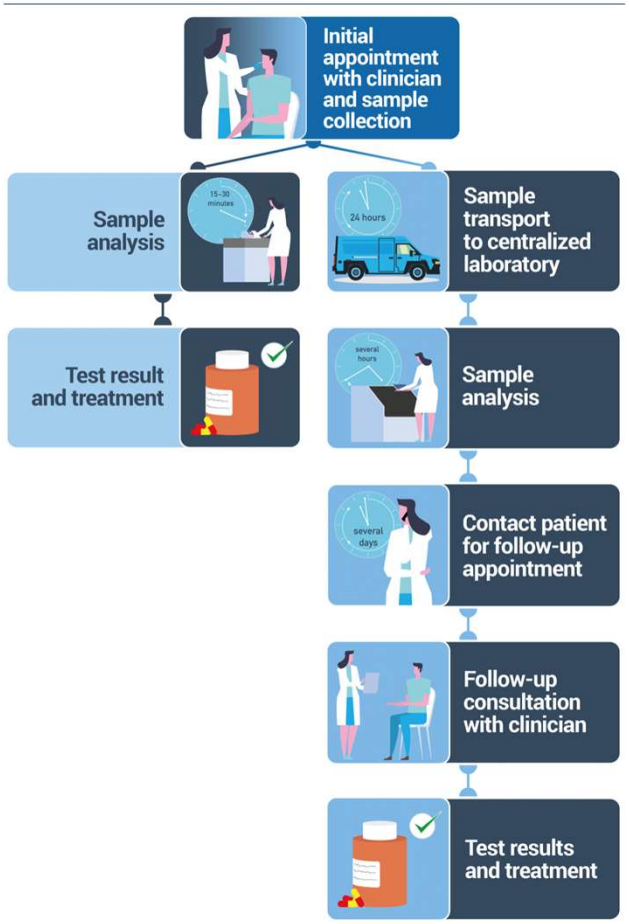
1:2

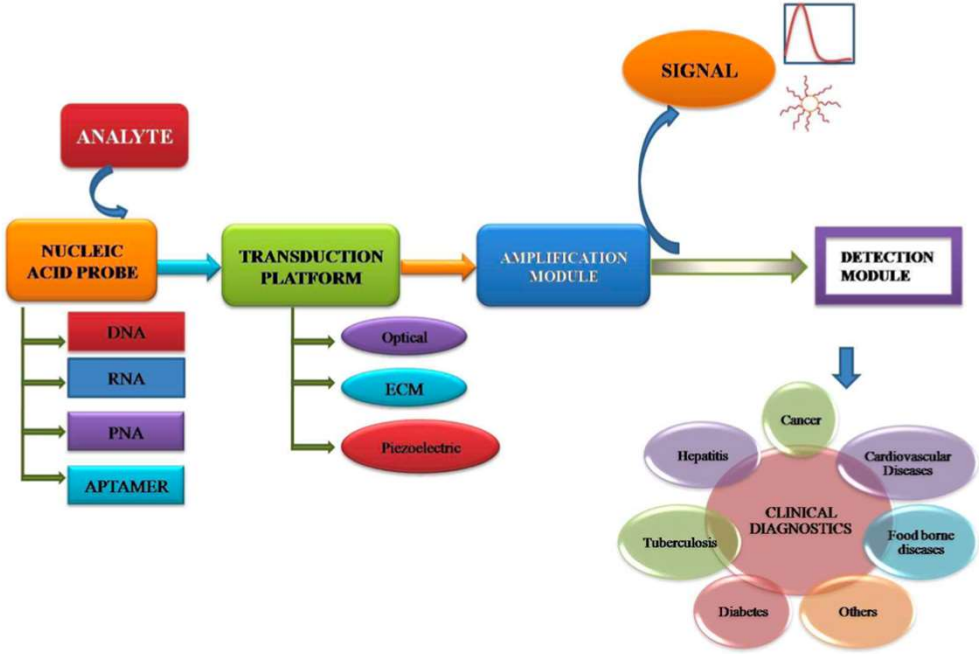
1:1

ODNs for biosensors

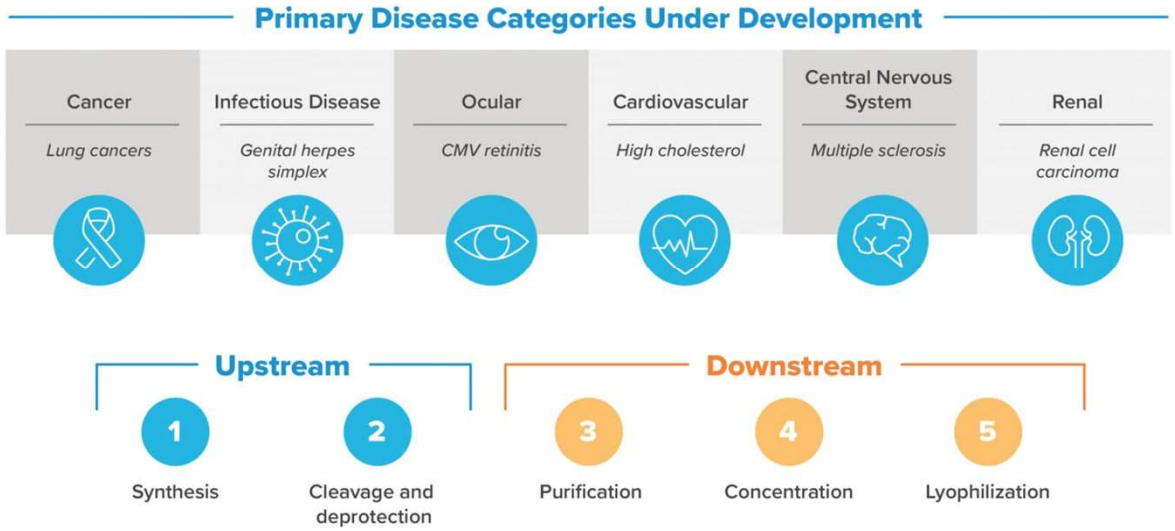
- Advances in biosensor technology
- Growing demand

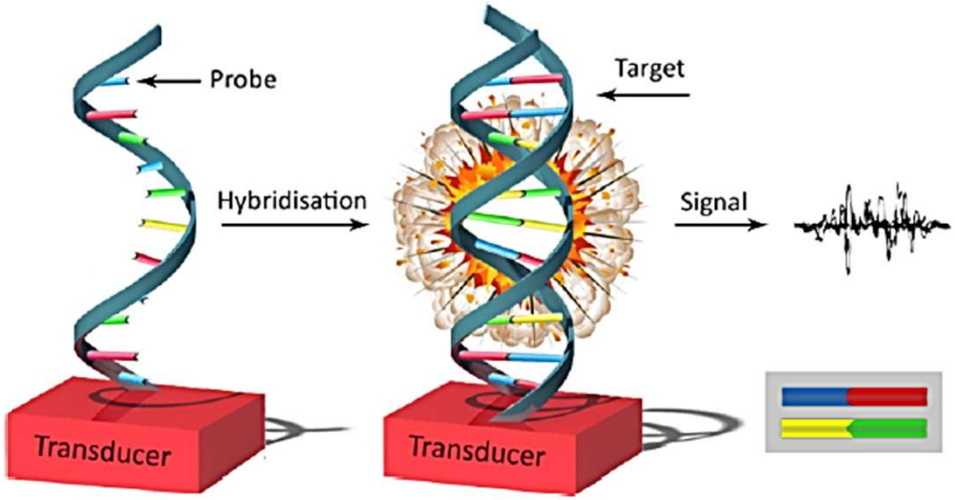
ODNs for biosensors

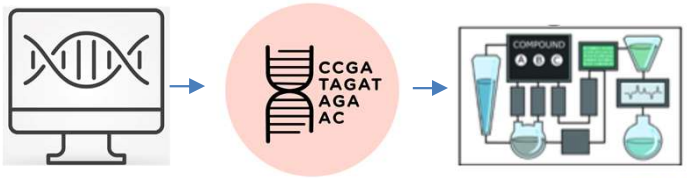




Bora et al., Biosens J 2013, 1:1


Primary Disease Categories Under Development
Cancer
Lung cancers
Infectious Disease
Genital herpes simplex
Ocular
CMV retinitis
Cardiovascular
High cholesterol
Central Nervous System
Multiple sclerosis
Renal
Renal cell carcinoma
Upstream
Downstream
1
Synthesis
2
Cleavage and deprotection
3
Purification
4
Concentration
5
Lyophilization

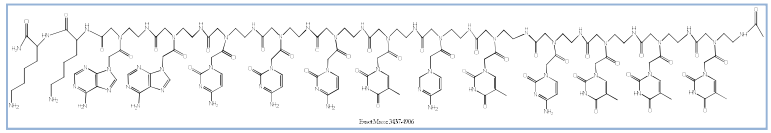

Probe
Target
Hybridisation
Signal
Transducer
Transducer

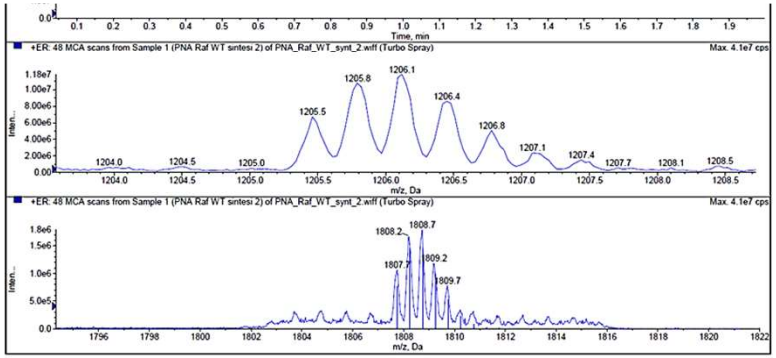


PNA SYNTHESIS

nanomaterials
Article

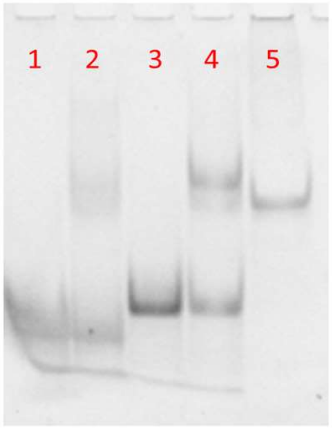
PNA-Based Graphene Oxide/Porous Silicon Hybrid Biosensor: Towards a Label-Free Optical Assay for Brugada Syndrome



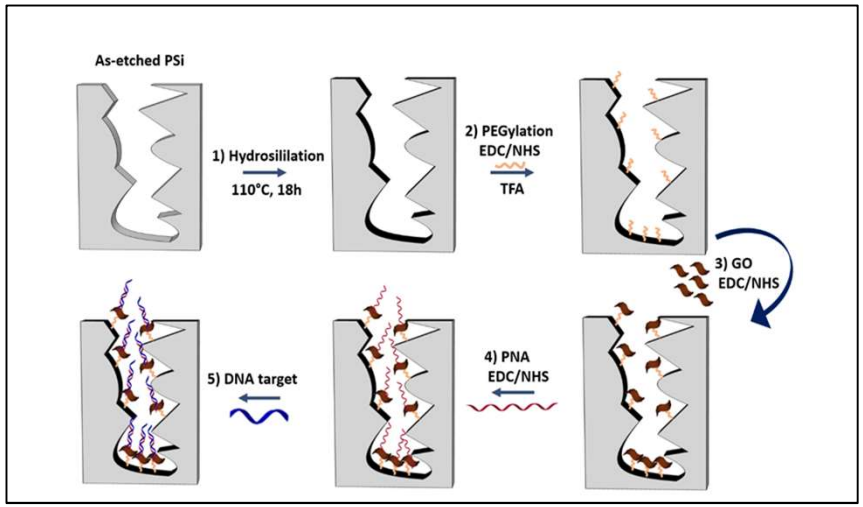


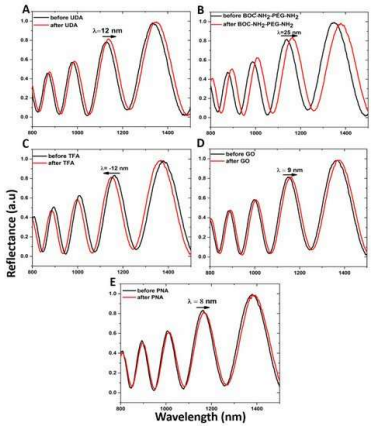
DNA 5’- TTGGGAGAGAAA- 3’

PNA KK AACCCTCTCTTT-Ac

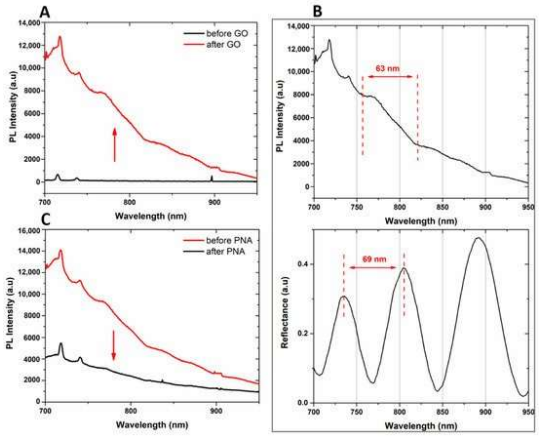


PAGE of DNA-FAM or DNA annealed with PNA in 100 mM PBS solution. Lane 1: DNA alone; lane 2: DNA/PNA mixture; lane 3: DNA FAM alone; lane 4: DNA FAM/PNA mixture, lane 5: 23-bp duplex DNA marker.

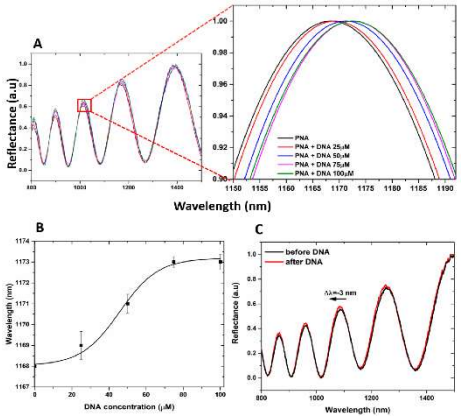




REFLECTIVITY SPECTRA OF PSI (A) before (black line) and after (red line) UDA passivation, (B) before (black line) and after (red line) PEGylation with BOC-NH2-PEG-NH2, (C) before (black line) and after (red line) deprotection of amino group by TFA treatment, (D) before (black line) and after (red line) GO infiltration, (E) before (black line) and after (red line) PNA immobilization.



(A) PHOTOLUMINESCENCE SPECTRA of PSi before (black line) and after (red line) GO infiltration at an excitation wavelength of 442 nm. (B) Comparison between PL spectrum (upper graph) and reflectivity spectrum (lower graph) of GO/PSi hybrid device. (C) PL spectra of PSi before (red line) and after (black line) PNA immobilization, at an excitation wavelength of 442 nm.



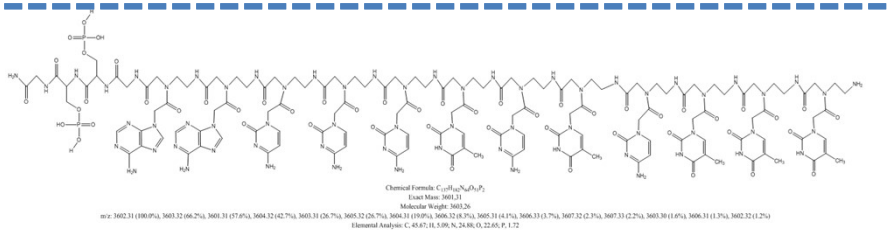
REFLECTIVITY SPECTRA OF PNA-GO/PSi device after the DNA incubation at pH 5. (B) dose–response curve as a function of the DNA concentration (pH 5). Experimental data (black squares) were fitted by using OriginLabTM Dose–response nonlinear curve fit. (C) reflectivity spectra of PNA-GO/PSi device after the DNA incubation at pH 7.5

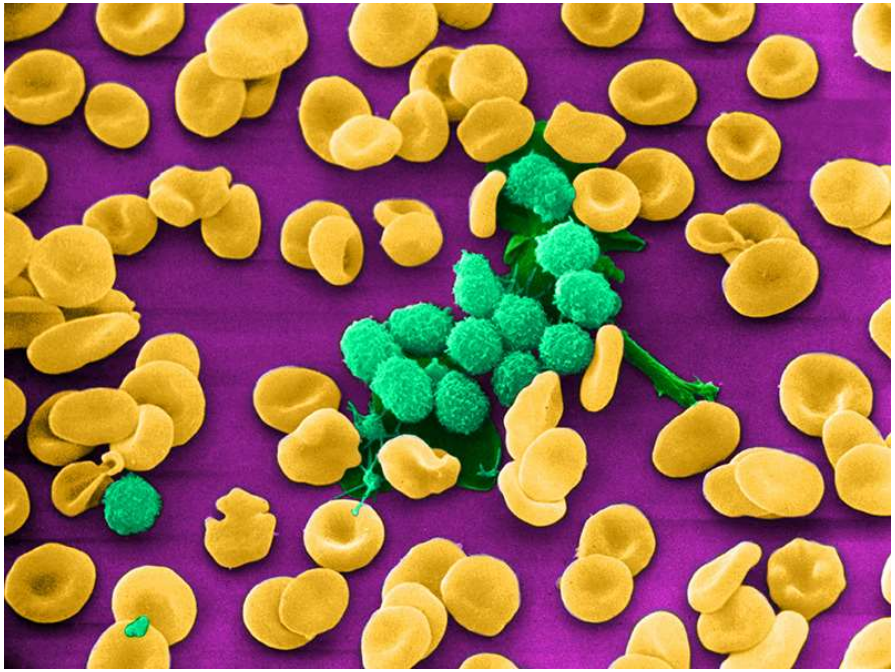
Nanomaterials **2020**, 10(11), 2233; **https://doi.org/10.3390/nano10112233**

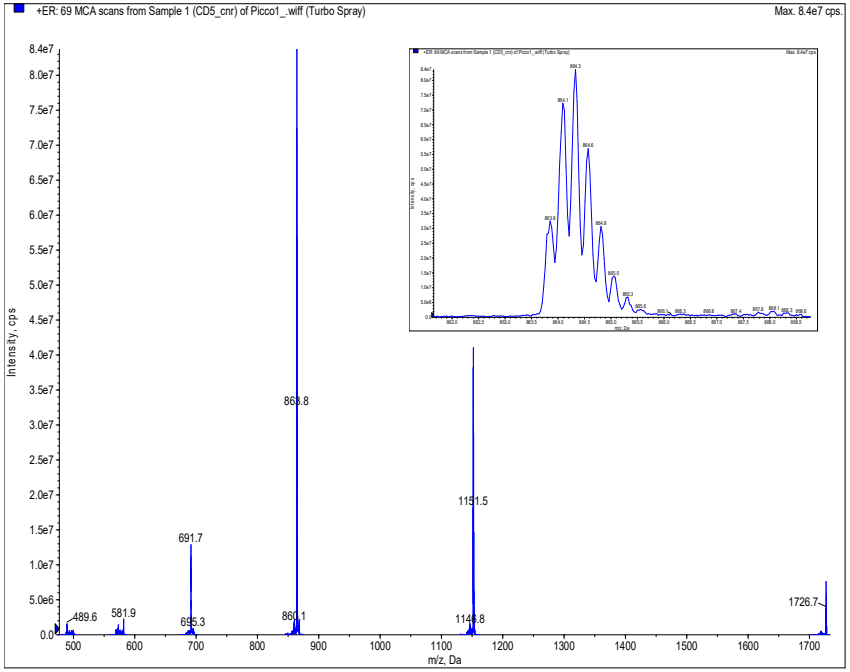
nanomaterials
MDPI

Article

Bioconjugation of a PNA Probe to Zinc Oxide Nanowires for Label-Free Sensing



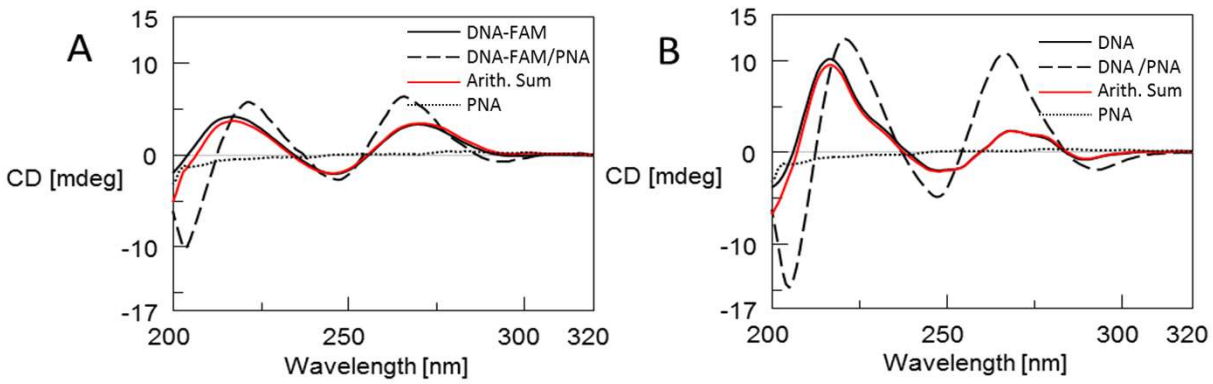


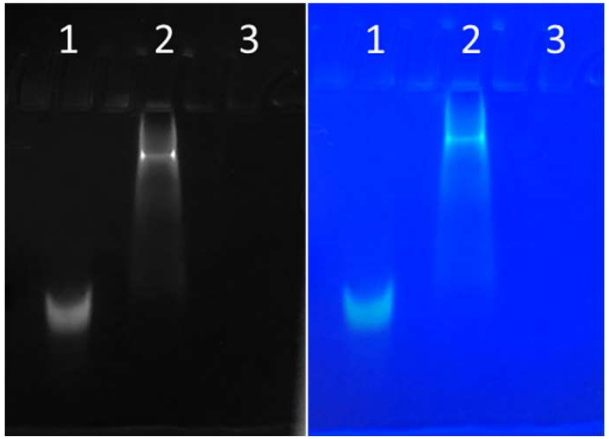


Non denat

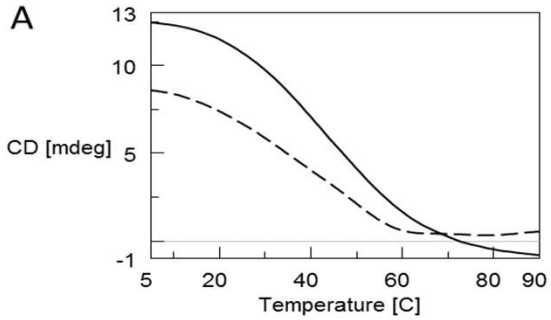
MDPI

Circular Dichroism analysis





CD melting

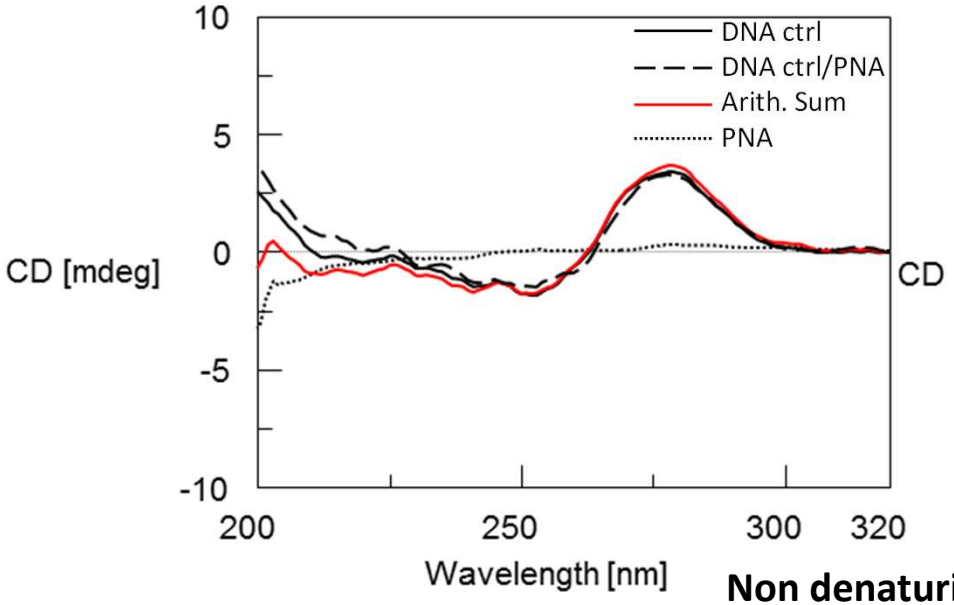


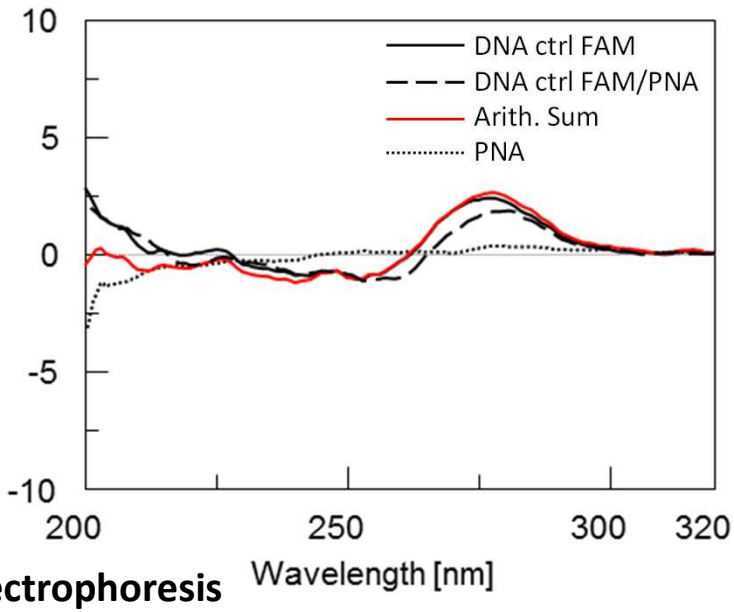
B

Sample	Tm (°C)
DNA-FAM/PNA	40
DNA/PNA	45

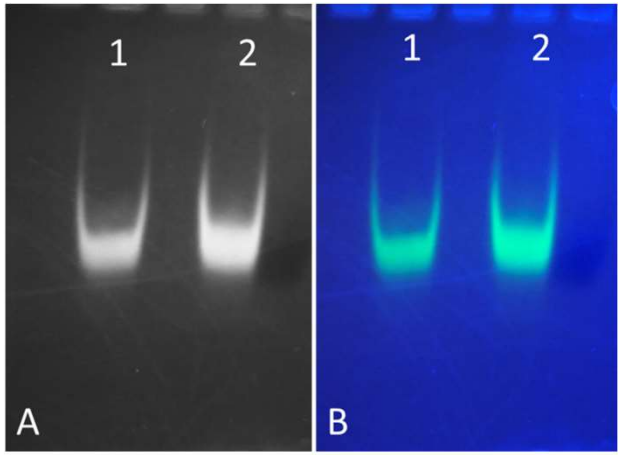
CD melting profiles of 1:1 DNA-FAM/PNA mixture (solid line) and DNA-FAM/PNA (dashed line).

Circular Dichroism of DNA ctrl annealed with PNA

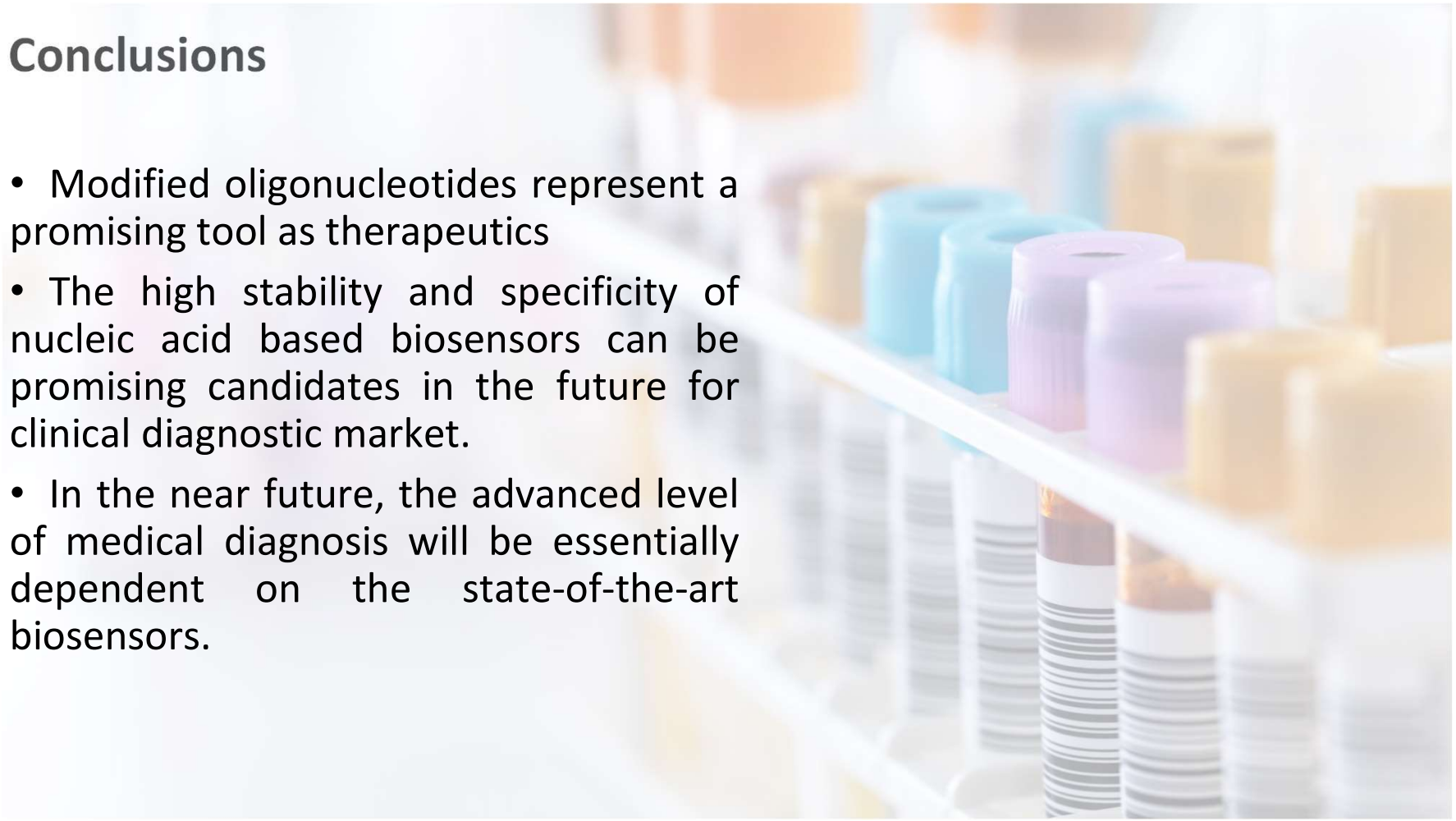




Non denaturing electrophoresis



Conclusions



- Modified oligonucleotides represent a promising tool as therapeutics
- The high stability and specificity of nucleic acid based biosensors can be promising candidates in the future for clinical diagnostic market.
- In the near future, the advanced level of medical diagnosis will be essentially dependent on the state-of-the-art biosensors.

Thank you for your kind attention

Thanks to my research group:

Prof. Giorgia Oliviero
Prof. Nicola Borbone
Prof. Gennaro Piccialli
Dr. Stefano D'Errico
Dr. Monica Terracciano
Dr. Maria Marzano
Dr. Francesca Greco
Dr. Maria Grazia Nolli

Acknowledgment
Vincenzo Cerullo, University of Helsinki
Valerie Gabelica, Inserm, University of Bordeaux