UNIVERSITÀ DI PARMA

EDUCATIONAL PLAN

ACADEMIC YEAR 2020/21

PhD Course, cycle:	**CHEMICAL SCIENCES, 34**
PhD Student:	FEDERICA CURTI
Supervisor:	WOLFGANG KNOLL, ROBERTO CORRADINI, MARIA CARERI,

EDUCATIONAL ACTIVITIES

SPECIFIC COURSES (TABLE A) AND MULTI-DISCIPLINARY ACTIVITIES

	ACTIVITY	DATE	Scientific Discipline *	N. of hours	ECTS	Vote – Judgment – Attendance (V/J/A)	Teacher	Level (PhD, Master, etc.)
***COURSES from* TABLE A:** https://scvsa.unipr.it/it/node/2294 (at least 10 ECTS over the 3-year period) 1 ECTS = 8 h								
	Semiconductor Journey (AIT)	20-24/05/2019		24	3	Id	Various	PhD
	Dispositivi sensoristici portatili e metodi di screening rapido per point-of-care testing	21-24/09/2020	SSD CHIM/01	8	1	Id	Prof. Marco Giannetto	PhD
	Processi promossi dalla luce visibile	Gen/Feb 2021	SSD CHIM/06	8	1	Id	Prof. Giovanni Maestri	PhD
	Interface Design and Characterization For Optical and Electronic Bio-Sensing	June 2021		24	3	Id	Prof. Wolfgang Knoll	PhD
WORKSHOPS 1 ECTS = 12 h								
	Symposium “Bioelectrochemistry, surfaces, and more…2019” (AIT)	6/02/2019	Multi	8	0.666	A	Various	PhD
	AMYC-BIOMED 2020 Virtual Conference	13-14/10/2020	Multi	8	0.666	A	Various	PhD
	Giornata bioanalitica 2021. Chimica bioanalitica per il benessere: ambiente, salute ed alimentazione	13/07/2021	CHIM/01	8	0.666	A	Various	PhD
	SCI2021	14-23/09/2021	Multi	48	4	A	Various	PhD
SEMINARS 1 ECTS = 12 h								
	Reduced-graphene oxide transistors:towards wearable e-	13/03/2021		1	0.083	A	Dr. Stefano Casalini	PhD

	platform featuring biochemical sensitivity (AIT)							
	Engineering functional and responsive surfaces by chemical vapour deposition for biotechnology (AIT)	13/03/2019		1	0.083	A	Prof. Anna Maria Coclite	PhD
	Seminar on Biosensing (AIT): - Electrolyte gated transistors with floating gate - Top-down fabricated nanoscale interfaces of metal-graphene based materials for electrical and optical biosensing - Neuromorphic organic electronic biosensors - New psychoactive substances: current challenges in health care and forensic toxicology	15/03/2019		3	0.250	A	Various	PhD
	8 seminars, various titles, organized by SCI (venerdì della SCI)	Nov/Dec 2020 Jan/Feb 2021	Multi	8	0.666	A	Multiple	PhD
	La chimica ai tempi del covid	2/12/2020	Multi	4	0.333	A	Multiple	PhD
	Recent advancements in organometallic chemistry and homogeneous catalysis	25/02/2021	SSD CHIM/06	2	0.166	A	Prof. Gianpiero Cera Prof. Michal Szostak	PhD
	Hybrid materials for medicine	9/03/2021		1	0.083	A	Prof. Luisa De Cola	PhD
COURSES within UNIPR from other degrees 1 ECTS = 8 h								
SCHOOLS 1 ECTS = 8 h								
	Hirshegg AIT Winter School – 18th VBST 2019	10-15/02/2019	Multi	24	3	A	Various	PhD
	8th AIT PhD Winter School	9-14/02/2020	Multi	24	3	A	Various	PhD
TOTAL: 21.662 **TOTAL FROM TABLE A:** 8								

- **(as a reference see 'Aree disciplinari' at https://it.wikipedia.org/wiki/Settore_scientifico-disciplinare)**

INTER-DISCIPLINARY ACTIVITIES (SOFT SKILLS)

Interdisciplinary courses dedicated to general skills or links among disciplines, e,g, communication, computer skills, research management or networking and intellectual property

UNIVERSITÀ DI PARMA

ACTIVITY		DATE	Scientific Discipline	N. of hours	ECTS	Vote – Judgment/ Attendance	Teacher	Level (PhD, Master, etc.)
***COURSES from* TABLE B:** https://scvsa.unipr.it/it/node/2294 (at least 3 ECTS over the 3-year period) 1 ECTS = 8 h								
	Scientific English	June 2021		20	2.5	A	Adrian Wallwork	PhD
WORKSHOPS 1 ECTS = 12 h								
	From lab to market- workshop on entrepreneurship and technology commercialization in Parma and Vienna	2-7/05/2019		20	1.666	A	Prof. Buddy Ratner and other experts	PhD
SEMINARS 1 ECTS = 12 h								
	Webinar con FEDERCHIMICA e ALFASIGMA	21/05/2021		4	0.333		Dott. Vittorio MAGLIA Dott. Mariano LEONE Dott. Francesco BONVICINI	PhD
SCHOOLS 1 ECTS = 8 h								
TOTAL: 4.5 **TOTAL FROM TABLE B: 2.5**								

RESEARCH ACTIVITY

PARTECIPATION IN RESEARCH PROJECT

Title of the International or National projects of which the research was part and brief description of the activity carried out (max 100 characters)

TITLE OF THE PROJECT	WHERE Institution/Venue	WHEN		ECTS[4]
		FROM	TO	
Synthesis of PNAs as possible aptamer mimics	Chimica – SCVSA – Università di Parma	05/2020	01/2021	40
antimiR-PNAs probes as PET tracers for lung cancer detection	Chimica – SCVSA – Università di Parma	02/2021	04/2021	15
Electrochemical aptasensor for protein-related virus detection	Chimica – SCVSA – Università di Parma	05/2021	10/2021	25

RESEARCH PROJECTS ABROAD

TITLE OF THE PROJECT	WHERE	WHEN	ECTS[4]

	Institution/Venue	FROM	TO	
Biological sensing with optical and optoelectronic platforms	Austrian institute of technology GmbH, Vienna (Austria)	11/2018	04/2020	85

ACHIEVEMENT

Publications (also submitted), **Posters**, **Oral communications**, **Patent applications**, **Scientific Awards**

1.Fortunati, S, Rozza, A, **Curti, F**, Giannetto , M, Corradini, R, Careri, M. novel amperometric genosensor based on peptide nucleic acids (PNA) probes immobilized on carbon nanotubes-screen printed electrodes for the determination of trace levels of non-amplified DNA in genetically modified (GM) soy. *Biosensors Bioelectron*. **2019**, 129, 7-14.
2. Fortunati, S, Rozza, A, **Curti, F**, Giannetto , M, Corradini, R, Careri, M. Single-walled carbon nanotubes as enhancing substrates for PNA-based amperometric genosensors. *Sensors* **2019**, 19, 588
3.D. Kotlarek, F. Curti, M. Vorobuu, R. Corradini, M. Careri, W. Knoll, C. Rodriguez-Emmenegger, j. Dostalek. Surface plasmon resonance based aptasensor for direct monitoring of thrombin in a minimally processed human blood. Sensors Actuators B. Chem. 2020, Vol. 320, pp. 128-380
4. Poster – DNA and PNA aptamers for the detection of thrombin – AMYC BIOMED 2020 Virtual Conference
5. Poster - Fluorescence anisotropy analysis for studying DNA-based- and PNA-based aptamer - thrombin interactions. - Bioanalitica 2021. Chimica bioanalitica per il benessere: ambiente, salute e alimentazione
6. Poster – DNA vs PNA as thrombin aptamers: the role of electrostatic interactions. – SCI2021

OTHER ACTIVITIES:

Didactic activity at University of Parma (40 hours max), tutoring, exercises, lab traning, other…

1.Tutor Tesi Magistrale: Maddalena Montanini, LM Chimica, “Development of an opto-electronic sensing setup using a combined OFET/SPR approach” AA 2018/2019 (24h)
2. Tutor Tesi Triennale: Clelia Ferrari, Chimica, “Valutazione delle interazioni tra biorecettori molecolari e proteine: la trombina come caso di studio” AA 2019/2020 (12h)
3. PLS_SCVSA-02/2021_biotec – 70 h
4.

NOTES: Rows can be added or deleted.

[1] Indicate the discipline (or MIUR-SSD code) for specific courses or "Multi" for Schools/Workshops where modules of different disciplines are offered;

[2] Indicate as approved by the Academic Board (8 hours = 1 ECTS for courses and schools, 12 hours= 1 ECTS for seminars and workshops);

[3] Indicate "Vote" if at the end of the activity there was an exam with espression of a vote; "Id" if only an idoneity has been awarded by the teacher;

[4] Approximately 5 ECTS per month of full-time work (excluding holidays and educational and other activities); PhD students must Accumulate 180 ECTS in three years.

RESUME

EDUCATIONAL ACTIVITIES	RESEARCH ACTIVITIES	OTHER ACTIVITIES

UNIVERSITÀ
DI PARMA

SPECIFIC COURSE AND MULTIDISCIPLINARY ACTIVITIES	INTER-DISCIPLINARY ACTVITIES (SOFT SKILLS)		
ECTS 21.7	ECTS 4.5	ECTS 165	ECTS 4.24

(N.B. The sum of the annual credits should be 60)

Date, ___16/12/2021_____

Federica Gusti
_____
(Signature of PhD student)

_____
(Signature of the Supervisor)

Signature of the Coordinator of the PhD course

_____

This form must be fill in and signed by the PhD student, approved and signed by the tutor. A .pdf copy **must be submitted to the Coordinator by E-mail**

Signiert von: Wolfgang Knoll
Datum: 16.12.2021 19:20:46

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UNIVERSITÀ DI PARMA

Ph.D. course in Chemical Sciences, XXXIV cycle, III-year report,
Dr. Federica Curti

Title: DNA aptamer- and PNA-based sensory systems
Supervisors: Prof. Wolfgang Knoll, Prof. Roberto Corradini, Prof. Maria Careri

INTRODUCTION

Aptamer-based sensors have recently emerged as a novel biosensor class to cover the growing need to detect specific analytes in fields like food safety, clinical diagnostics and forensic analysis. Aptamers are single-stranded oligonucleotides tailored to recognize a non-nucleotide target via hydrogen bonding, van der Waals forces or electrostatic and stacking interactions. They exhibit many advantages as recognition probes in biosensing compared to natural receptors (e.g. enzymes and antibodies), offering high affinity binding and selectivity as they can be engineered in vial and produced by chemical synthesis[1].

During my staying at the Austrian Institute of Technology in Vienna in the research group of prof. Wolfgang Knoll, the development of a surface plasmon resonance (SPR) sensor based on DNA aptamers for the detection of human thrombin was carried out[2]. The affinity interaction of three different thrombin binding aptamers (HD1-sort, HD1 and HD22) with thrombin was investigated, as well as changes in their performance with other abundant molecules present in human blood, like prothrombin, human Immunoglobulin-G (h-IgG) and human serum albumin (HSA).
Driven by these results, it was evaluated the possibility of employing Peptide Nucleic Acids (PNAs) as aptamer probes. This is a new strategy since in the literature PNAs have been poorly investigated as potential aptamer ligands for biosensing[3]. In the research group of Prof. Roberto Corradini at the University of Parma, two main biosystems were interrogated: the complex between thrombin and HD1 aptamer, and the complex between troponin and cTnI aptamer. The former was analysed via fluorescence anisotropy and Fiber-Optic SPR (FO-SPR), and the latter through Graphene Field Effect Transistor (GFET).
Moreover, an electrochemical aptasensors for fast and direct detection of spike protein (S1) of SARS-CoV-2 was developed in the laboratory of Prof. Maria Careri at the University of Parma. The affinity interaction of a methylene blue labelled Cov2-6C3 aptamer with S1 was investigated, as well as with other the spike proteins from MERS and influenza A H1N1.
Finally, the outstanding hybridization properties of PNAs were explored towards the identification of genes overexpression related to microRNAs, whose dysregulation is involved in several pathologies like tumors or neurodegenerative/autoimmune diseases. Indeed, PNAs were confirmed to function as modulator of the biological activity of microRNAs.[4] Three PNAs sequences targeting microRNA-21, -146a and -155 bearing two different chelators were synthetized in order to perform a labelling process with radio-metals. Their cellular uptake was validated in vitro.

Hereinafter a more detailed description of the work is reported.

SURFACE PLASMON RESONANCE (SPR) APTASENSOR DEVELOPMENT

The assembly chosen is based on a *sandwich* architecture as presented in the scheme below (Fig. 1). The immobilization of the biological species is performed in situ on the SPR sensor chips: the surface architecture was obtained by modifying a gold substrate with mixed thiol oligo-ethylene glycol (OEG) self-assemble monolayer (SAM) bearing biotin residues, which were used to bind a layer of neutravidin. A solution of biotinylated aptamer in PBS was used to link this recognition element to the layered surface. The sensor was then used in affinity interaction experiments, where concentrations of thrombin ranging from 5-500 nM were flowed sequentially over the sensor surface with a NaCl 2M regeneration step in between. The same protocol was applied to check the cross-reactivity of interferent species present in human blood with these aptamers, as prothrombin, HAS and h-IgG.

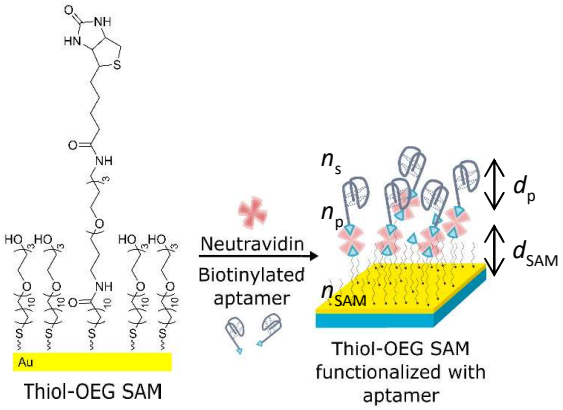

Figure 1. Schematic functionalization protocol for thrombin detection.

In order to assess the performance of the sensor chip to enable direct detection of thrombin, a regular mixed thiol-OEG SAM was incubated on the surface, which exposed OEG-OH and OEG-Biotin headgroups. This biointerface is highly suitable to minimize the blocking of the sensor surface when model biological matrices are used[5]. The immobilisation of the three biotinylated aptamers - HD1-short, HD1 and HD22 - was monitored by SPR and both reflectivity curves $R(\theta)$ and the signal kinetics $R(t)$ upon the surface reaction were acquired. These results lead to a surface density of Γ/MW= 0.013 pmol mm^{-2}, 0.020 pmol mm^{-2} and 0.022 pmol mm^{-2} for HD1 short, HD1 and HD22 respectively. Interestingly, the density ratio of the aptamer to the tetrafunctional neutravidin, which served as a linker between the biotin groups in the SAM and aptamer biotin terminal group, was in all cases close to one. This confirms that the density of these bioreceptors was controlled by the density of immobilized neutravidin linker.

The affinity interactions between the immobilised aptamers and thrombin were evaluated via SPR: the kinetic data $R(t)$ are reported in Fig. 3b), c), d). Different thrombin concentrations in PBS ranging from 5 to 500 nM were flowed over the sensor surface and the measured curves were fitted assuming 1:1 interaction model. The longer HD1 and HD22 aptamer sequences exhibited higher affinity, leading to an equilibrium dissociation constants K_d <5 nM (Tab. 3a). Indeed, upon interaction with thrombin they fold into a rigid G-quadruplex configuration, resulting into a stable complex thanks to their longer binding site with respect to that of HD1-short aptamer.

Furthermore, specificity of HD1 and HD22 was evaluated against some biomolecules that are present in human blood at high concentration. These include prothrombin (the direct precursor of thrombin), HSA and h-IgG. The measured curves revealed a high affinity of HD1 aptamer for prothrombin at concentration below 100 nM with K_d = 39 ± 9 nM nM, while HD22 did not manifest interactions

a)

K_d [nM]	Thrombin	Prothrombin
HD1 short	12	-
HD1	<5	39.9
HD22	<5	>1000

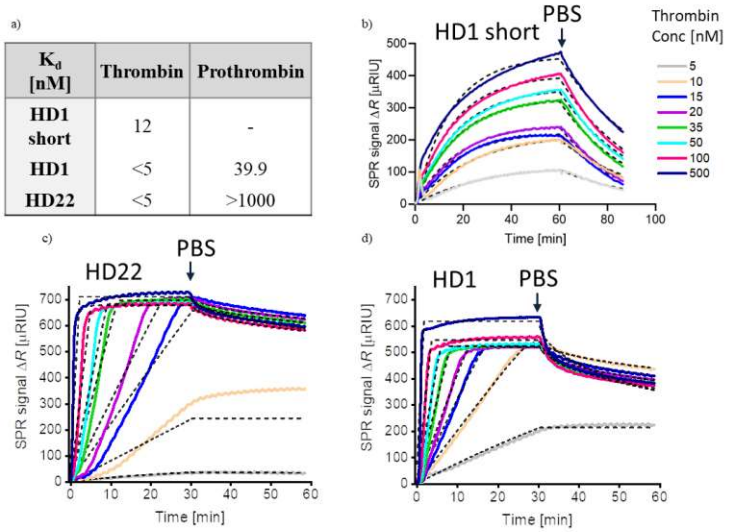

Figure 2. Global affinity binding measurements of thrombin with three aptamer ligands: (b) HD1 short, (c) HD1 and (d) HD22.

up to 1 μM (Fig.4a). Indeed, the HD1 aptamer is known to predominantly interact with thrombin exosite I, which is located on the surface of prothrombin as well, while HD22 recognizes the binding exosite II that is hidden inside this prothrombin[6]. Concerning HSA and h-IgG interaction, both aptamers revealed a negligible response for concentrations over the micromolar range (Fig. 4b). Moreover, performing the same experiments on the surface with a scrambled sequence of HD1 aptamer, the response suggests that the measured negligible change in SPR signal is attributed to unspecific sorption to the thiol SAM-based biointerface.

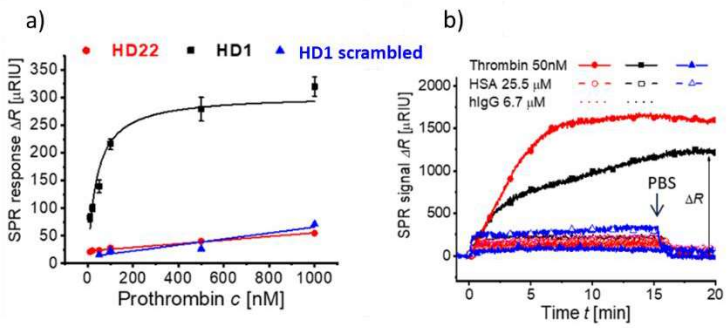

Figure 3. Specificity binding measurements of prothrombin, HSA and h-IgG.

The performances of this binding system were further interrogated for analysis in 10% blood. Daria Kotlarek in the research group of Prof. Jakub Dostalek, developed a sensing architecture where the mixed thiol-OEG-SAM was replaced by poly(HPMA-co-CBMAA) brushes, in order to increase the antifouling properties to assess thrombin detection at clinically relevant level in real matrix. The LOD of 0.7 nM achieved with HD1 aptamer was sufficient to predict a thrombotic in time.

SYNTHESIS OF PNAs AS POTENTIAL APTAMERS

In a further development of this research, a similar binding system was designed by replacing the DNA thrombin aptamer backbone with a DNA-mimic probe based on PNAs. PNAs are well known ligands that offer enhanced base pairing binding properties compared to DNA/RNA system, can be easily synthetized in laboratory, and are very resistant to both chemical and enzymatic degradation. A biotin-labelled PNA with HD1 aptamer-like sequences was synthesized following the standard Fmoc-based solid phase synthesis (SPS) strategy, purified with HPLC and fully characterised using UPLC-MS. The affinity interactions were first analysed through FO-SPR in the research group of Prof. Wolfgang Knoll. In figure 4 is reported the home-made setup developed by Roger Hasler. A sputtered-gold fiber tip was functionalized with the biotinylated HD1-PNA aptamer (1 μM in PBS) after the deposition of a mixed thiol-OEG SAM architecture, as previously described in figure 1. Then, the modified fiber tip was immersed in different concentration of thrombin in PBS (50-500 nM), each of them followed by a rinsing step with PBS, and changes of resonance wavelength were observed as a function of time. Unfortunately, an extremely week interaction was observed at high concentrations (Fig. 5a), which is probably related to bulk changes/unspecific binding. It is worth mentioning that the DNA-HD1 aptamer interaction with thrombin exosite I is also driven by electrostatic forces, as it is an anion binding site, and the G-quadruplex structure is highly stabilized by Na^+ and K^+ ions. Conversely, PNA single strands are known and employed as biosensing probes for their reduced dependence on changes of ionic strength in solution. As a result, the secondary G-quadruplex structure may not occur with HD1-PNA aptamer. A comparative analysis employing HD1-DNA aptamer was performed on the FO-SPR system as well. As shown in figure 5b, increasing concentration of thrombin led to a shift of the resonance wavelength as expected, with K_D comparable to that found in the literature.

Figure 4.FO-SPR setup

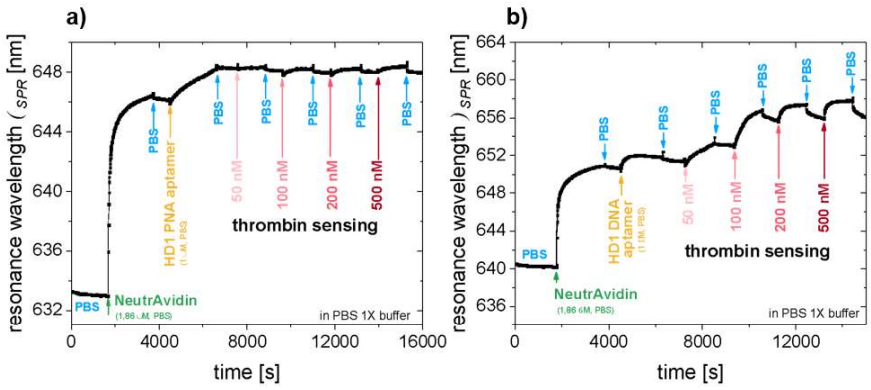

Figure 5. Affinity binding experiments with PNA (a) and DNA (b) aptamers in a FO-SPR setup.

Secondly, TAMRA-labelled-PNA sequences were also synthesised in order to perform interaction studies with thrombin via fluorescence anisotropy (FA). Specifically, a completely neutral PNA-HD1 sequence and a PNA-HD1 bearing glutamic acid residues, synthesized in order to introduce negatively charges into the probe, were compared. The aim was to further investigate the effect of the electrostatic vs protein-nucleobase interactions on protein binding. Before FA analysis, both PNAs and DNA aptamers were mixed with increasing concentrations of human thrombin, using ammonium formate buffer at pH=6.8 with different ionic strength. The DNA aptamer-thrombin complex revealed a significant change in its behavior when exposed to different ion strength solutions, leading to a destabilization of the complex by increasing the ionic strength from 2 to 200 mM. Conversely, a weak signal was observed when the PNA aptamer was used, highlighting the predominant electrostatic nature of this aptamer-protein system. Moreover, neither the PNA HD1 modified with glutamic acid units showed improved interactions with thrombin, suggesting that the key factor is not only the presence of charges in the aptamer sequence, but also that they require to be placed in the near proximity of the binding site.

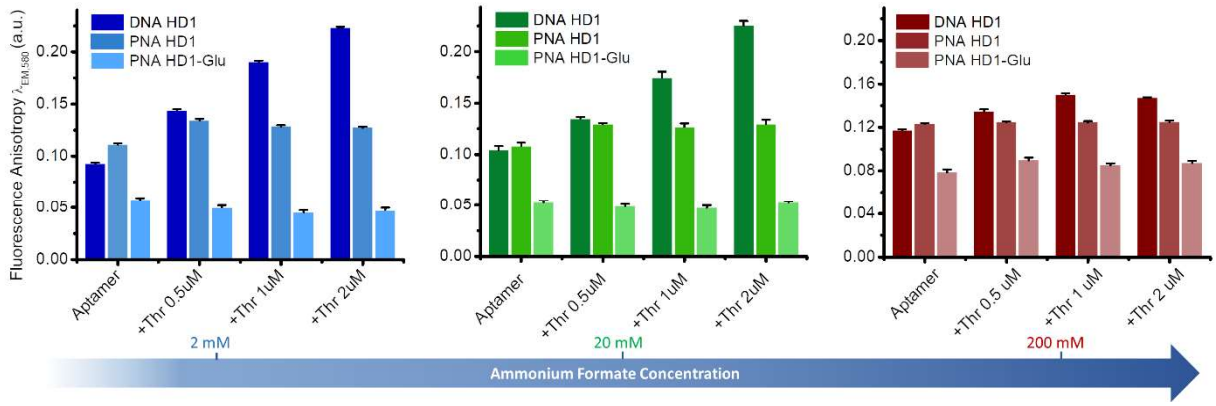

Figure 6. Binding interaction of thrombin (c= 0.5, 1 an 2 uM) with DNA HD1, PNA HD1 and PNA HD1 modified with glutamic acids residues analysed with fluorescence anisotropy at different ionic strength values: 2 mM (blue), 20 mM (green) and 200 mM (red).

The specificity of these systems was monitored with human serum albumin and no significant interactions were observed in both complexes. The intramolecular G-quartet structure of both DNA and PNA systems was monitored with UV-melting profiles, which can reveal the presence of quadruplex structures. The DNA HD1 showed the typical sigmoidal curve, as a result of a transition phase phenomenon after the G-quadruplex formation. The PNA HD1 revealed a more complex behavior, as the significant decrease of the absorbance value at λ= 290 nm denotes the predominant presence of intermolecular interactions occurring among the single strands in solution.

In collaboration with the research group of Prof. Sabine Szunerits at the University of Lille, the troponin I (cTnI)-aptamer binding complex was interrogated as well. The research group had previously developed a GFET biosensor where the azido-modified cTnI-DNA aptamer was tethered at the sensing surface via "click-chemistry".[7] More interestingly, they recently observed an almost negligible dependence of the complex formation from charges in buffer solution. Therefore, a 41-mer PNA probe with cTnI-DNA aptamer-like sequence was synthetized via Boc-SPS. A preliminary amino-modification was left at the C-term, as the strong acidic conditions necessary to cleave the Boc-protected PNA from the resin are not compatible with azide groups. Then, the cTnI-PNA aptamer was immobilized on the carboxy-modified GFET via EDC/NHS (Fig. 7a). The exposed carboxylic terminations were previously inserted by adsorption of 1-pyrene butyric N-hydrosuccinimide ester (PBSE) on the graphene electrode. Different concentrations of troponin were deposited on the modified electrode (5-800 pg/mL) and I_DV_G curves were collected. A cathodic shift in the Dirac point was observed by increasing the protein concentration, without any changes in the ambipolar shape. A K_D of 155 pg/mL was estimated with LOD of 6 pg/mL, revealing an incredible sensitivity (relevant concentration of troponin is 26 pg/mL for human without heart damage tissue), together with a good selectivity against another cardiac-related biomarker BNP-32. This is an amazing step forward in the design of new synthetic probe aimed to assess biologically relevant detection. Hence, an azido-modified cTnI-PNA aptamer was synthetized to fully compare the two receptor binding systems. The synthesis was carried out as follows: after the solid-phase synthesis the Boc-protected PNA aptamer was firstly cleaved from the resin and then a NHS-azidoacetic acid was linked to the PNA C-term in solution at 37°C overnight. The performance related to this binding probe are currently under investigation.

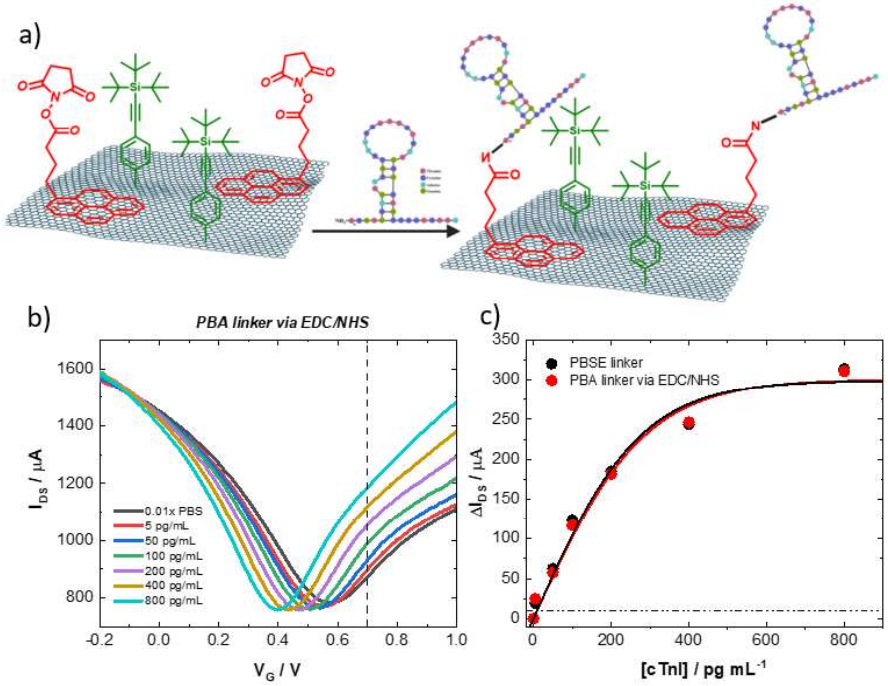

Figure 7. (a) Schematic illustration of surface functionalization with cTnI PNA-aptamer on GFET. (b) I_DV_G curves of the troponin-cTnI aptamer complex and its global fitting (c).

ELECTROCHEMICAL APTASENSOR DEVELOPMENT

Finally, the potentiality offered by aptamer receptors were further evaluated for the detection of S1 Sars-CoV-2 protein virus with an electrochemical readout based on Differential Pulse Voltammetry (DPV). Carbon Screen Printed Electrodes (CSPEs) modified with carboxylate carbon nanotubes (CNTs) were employed as the binding substrates, owing their enhanced electron transfer properties and easy functionalization protocols. The amino-modified Cov2-6C3 aptamer with nucleobase sequence developed by Sun et al.,[8] was tethered on pre-activated CNTs substrate via EDC/NHS coupling. The flexible structure of the 51-mer Cov2-6C3 aptamer allowed the methylene blue (MB) redox mediator, placed at the 5'-term, to easily interact with the sensing substrate giving rise to a certain

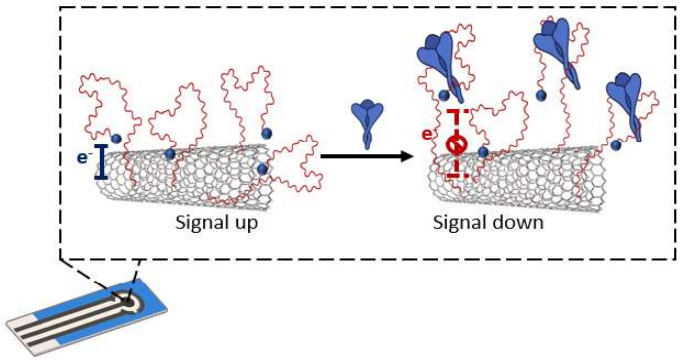

Figure 8. Schematic working principle of the electrochemical biosensor for S1 detection.

electrochemical current (Fig. 1). Then, the aptamer conformational change induced upon binding of S1 protein moved the MB tag far from the electrode surface, resulting into a reduction of the current upon increasing concentration of S1. The biosensor performances were tested in a range between 0.3-500 nM in PBS buffer, and the fitted data showed a LOD = 5.2 ± 1.3 nM and LOQ = 17.5 ± 3.4 nM (Fig. 8b), with a dissociation constant K_D = 57.5 ± 7.3 nM (Fig. 8a). Furthermore, the specificity of the aptamer was evaluated against other coronavirus-related protein: the spike protein of MERS (S1-MERS) and Influenza A H1N1 protein. Both proteins were tested at 100 nM concentration in PBS, owing the saturation state reached at that value with S1-Sars-CoV2. The data showed a signal suppression of 14.8% and 19.2%, respectively, suggesting a promising application in real samples (Fig. 10b). Moreover, the specificity of this binding system was tested with other two aptamer sequences, which are widely known to undergo a conformational modification after binding with their target protein: thrombin aptamer and Platelet-Derived Growth Factor (PDGF) aptamer. Both aptamers were incubated with a concentration of S1 Sars-CoV-2 set at 100 nM in PBS. Also in this case, a good specificity was obtained resulting into a signal suppression of 15% with thrombin aptamer and 21% with PDGF aptamer (Fig. 10a).

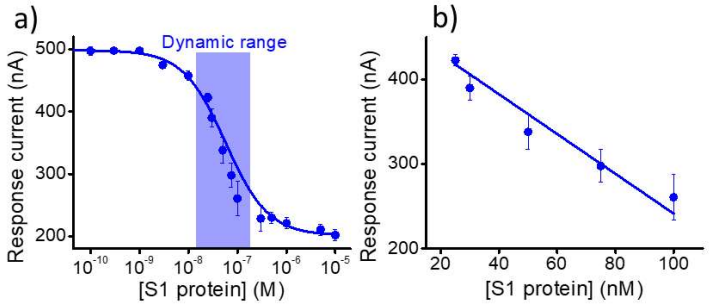

Figure 9. Performances of the biosensor for S1 detection.

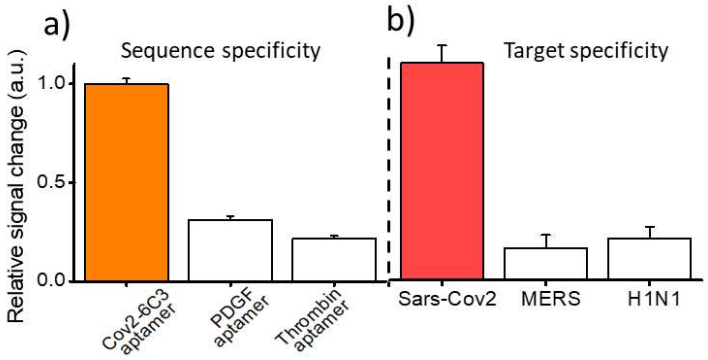

Figure 10. Specificity interaction depending on the aptamer sequence (a) and target analyte (b).

PNA RADIOLABELLED PROBES FOR IN VIVO STUDIES

In the last decade, radiolabelled probes (peptides or nucleic acids) have gained considerable attention for their specific and non-invasive application as Positron Emission Tomography (PET) tracers for the detection of microRNA levels in tumour cells. In this context, three microRNA target sequences were selected, microRNA-155, -146a and -21, which are overexpressed in lung cancers, and PNAs were chosen as targeting probes for their chemical stability and selectivity towards, together with their inhibitory activity of the target microRNA, achieving potential therapeutic effects. The anti-microRNA PNA sequences were synthetised via Boc-based SPS and each of them linked with NODAGA and DOTA chelators. Then, the labelling with the radio-metals ^{64}Cu and ^{68}Ga was performed by Dr. Mattia Asti at the Nuclear Medicine Unit in AUSL-IRCCS Reggio Emilia, being the most common radiometals used for NODAGA and DOTA chelators, respectively. The radiolabelling selectivity of ^{64}Cu with NODAGA- and DOTA-antimiR-PNA-146a was assessed as a function of the PNA amount (Fig. 11a): the radiochemical yield (%) was optimal after 5 min of incubation at 95°C with 1 nmol of PNA and the higher selectivity towards NODAGA chelator was observed as expected. Moreover, the complexes showed a stability > 95% until 18h after radiolabelling in PBS buffer as well as in human serum. These tests were carried out with NODAGA- and DOTA-antimiR-PNA-155 and NODAGA- and DOTA-antimiR-PNA-21, leading to the same selectivity and stability. The cellular uptake of labelled PNAs were measured in A549 cells (adenocarcinoma) expressing normal levels or overexpressing the target miRs (Fig. 11b)

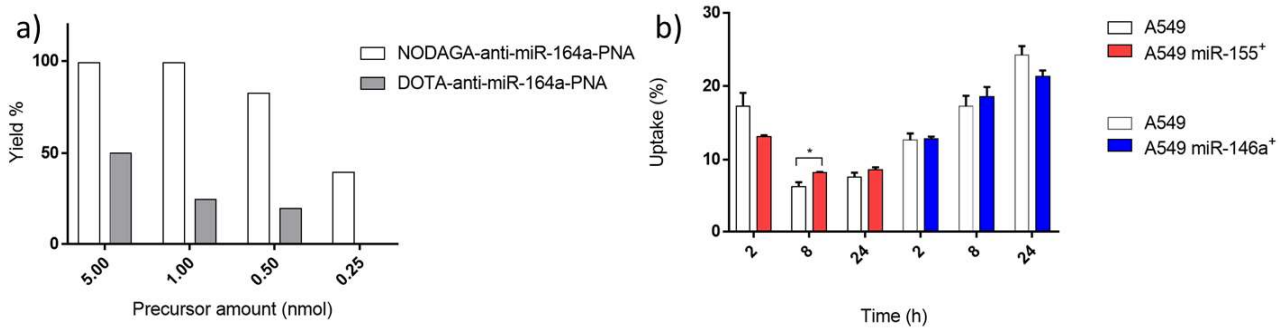

Figure 11. (a) Radiolabelling of NODAGA- and DOTA-antimiR-PNA-146a with ^{64}Cu at different PNA concentrations. (b) Cellular uptake of labelled PNAs in A549 cells.

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