ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

DIPARTIMENTO
DI CHIMICA
"GIACOMO CIAMICIAN"

ACCADEMIA DELLE SCIENZE
DELL'ISTITUTO DI BOLOGNA

Società Chimica Italiana
Divisione di Chimica Analitica
Gruppo di lavoro di Bioanalitica

"Attività organizzata nell'ambito dell'iniziativa
Dipartimenti di Eccellenza MUR 2023-2027
(L. 232 del 01/12/2016)"

Giornate di Bioanalitica 2024

ONE HEALTH nuove frontiere per la chimica bioanalitica

15-17 Aprile 2024, Bologna

GIORNATE DEDICATE AL CONTRIBUTO DELLA CHIMICA BIOANALITICA E WORKSHOP SUL CONTROLLO DI QUALITÀ PER LA SFIDA «ONE HEALTH», CONSEGNA DEI PREMI «ALESSANDRO MANGIA» E «CRISTINA GIOVANNOLI»

Giornate di Bioanalitica
One Health: nuove frontiere per la chimica bioanalitica

BOLOGNA
15-17 APRILE 2024

Con il patrocinio del Dipartimento di Chimica G. Ciamician dell'Università di Bologna

ATTI
E
Programma Finale

Società Chimica Italiana
Divisione di Chimica Analitica
Gruppo di lavoro di Bioanalitica

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Società Chimica Italiana
Divisione di Chimica Analitica
Gruppo di lavoro di Bioanalitica

Giornate di Bioanalitica
Bologna 15-17 Aprile 2024
ONE HEALTH: NUOVE FRONTIERE PER LA CHIMICA BIOANALITICA

Programma

- **Lunedì, 15 Aprile 2024**

SALA ULISSE, ACCADEMIA DELLE SCIENZE DELL'ISTITUTO DI BOLOGNA

10.00 - **REGISTRAZIONE**

11.00 - **SALUTI ISTITUZIONALI**

11.10 - **PREMIAZIONE MEDAGLIA "ALESSANDRO MANGIA" E PREMIO "CRISTINA GIOVANNOLI"**
MODERANO: ALESSANDRO MANGIA, LAURA ANFOSSI, SANDRA FURLANETTO, ALESSANDRO PORCHETTA, BARBARA RODA
11.30-12.00 Lecture Premio "Alessandro Mangia"
DARIO COMPAGNONE, UNIVERSITÀ DI TERAMO
12.00-12.20 Keynote Premio "Cristina Giovannoli"
VALENTINA MARASSI, UNIVERSITÀ DI BOLOGNA

SESSIONE I
12.20 - Stepbio S.r.l., 35 anni di supporto all'innovazione.
STEFANO GIOVANNINETTI, STEPBIO S.R.L. BOLOGNA
12.35 - **OC1** Exploiting sequestration mechanism to tune dose response of synthetic receptors for improved sensitivity of biosensing platforms.
ALEJANDRO CHAMORRO-GARCIA, UNIVERSITÀ DI ROMA "TOR VERGATA"
12.50 - **OC2** Detection of 25 PFAS in human blood by quantitative dried blood spots microsampling for large population monitoring.
MARTINA GALLETTO, UNIVERSITÀ DI TORINO

CORTILE DEL POZZO

13.15-14.15 LIGHT LUNCH E SESSIONE POSTER

SALA ULISSE, ACCADEMIA DELLE SCIENZE DELL'ISTITUTO DI BOLOGNA

SESSIONE II

MODERANO: ALDO LAGANÀ, GIOVANNA MARRAZZA

14.15 - **OC3** Streamlined Monolith Preparation for Online Extraction and LC-MS Analysis of β-Estradiol in Serum Using a Simple Multicomponent Reaction.

CARMELA MARIA MONTONE, UNIVERSITÀ DI ROMA LA SAPIENZA

14.30 - **OC4** Dopamine-related parameter involved in neurological disorders monitored using wearable sensing platform.

ILARIA ANTONIA VITALE, UNIVERSITÀ DI FIRENZE

14.45 - **OC5** p- jet biosensor for testing picogram level of different protein biomarkers.

CONCETTA DI NATALE, UNIVERSITÀ DI NAPOLI

15.00 - **OC6** Are aptamers really promising as bioreceptors? A multitechnique approach to bridge the gap between aptamer selection and analytical applications.

MONICA MATTAROZZI, UNIVERSITÀ DI PARMA

15.15 - **OC7** Electrochemical magneto-assay for BRD4 detection with theranostic applications in cancer diseases.

SIMONE FORTUNATI, UNIVERSITÀ DI PARMA

15.30 - **OC8** On-chip device for flow-driven release of extra-cellular vesicles and their detection as diagnostic biomarkers.

ALESSIA FOSCARINI, CNR NANOTEC, LECCE

PRESENTAZIONI FLASH

MODERANO: ANDREA ZATTONI, ILARIA PALCHETTI

15.45 - **PF1** Smartphone-based bioluminescent paper sensor for water toxicity monitoring.

DENISE GREGUCCI, UNIVERSITÀ DI BOLOGNA

15.50 - **PF2** A fast and native approach for the characterization of functionalized bacteriophages for photodynamic therapy.

STEFANO GIORDANI, UNIVERSITÀ DI BOLOGNA

15.55 - **PF3** Development of a ScreenPrinted Electrochemical biosensor for organophosphates detection directly on fruit peels

ANTONELLA MIGLIONE, UNIVERSITÀ DI NAPOLI

16.00 - **PF4** Landfill waste fire: oxidative stress and elements accumulation in bees.

MARCELLO MESSI, UNIVERSITÀ DI ROMA LA SAPIENZA

16.05 - **PF5** Hempseed-derived peptide mixtures with multifunctional properties for metabolic syndrome prevention.

SARA ELSA AITA, UNIVERSITÀ DI ROMA LA SAPIENZA

16.10 - **PF6** Lab-made fructose amperometric third-generation biosensors based on laser-patterned reduced graphene oxide films.

D. PAOLINI, UNIVERSITÀ DI TERAMO

CORTILE DEL POZZO

16.15-16.45 COFFEE BREAK E SESSIONE POSTER

SALA ULISSE, ACCADEMIA DELLE SCIENZE DELL'ISTITUTO DI BOLOGNA

SESSIONE III

MODERANO: PIERLUIGI RESCHIGLIAN, ANNA LAURA CAPRIOTTI

16.45 - **OC9** Extraction of phytosterols by fast synthesized molecularly imprinted polymers (MIPs) from food matrices.
FEDERICO FANTI, UNIVERSITÀ DI TERAMO

17.00 - **OC10** Unveiling biochemical profiles of peri-implant crevicular fluid using SERS spectroscopy.
STEFANO FORNASARO, UNIVERSITÀ DI TRIESTE

17.15 - **OC11** One-phase extraction coupled with photochemical reaction allows the in-depth lipid characterization of hempseed by untargeted lipidomics.
ENRICO TAGLIONI, UNIVERSITÀ DI ROMA LA SAPIENZA

17.30 - **OC12** Analytical characterization of microlgae for their use in health products.
GABRIELA BERMUDEZ, UNIVERSITÀ DI BOLOGNA

PRESENTAZIONI FLASH

17.45 - **PF7** A comparison between HF5 and SEC in the isolation of extracellular vesicles from human plasma.
ANNA PLACCI, UNIVERSITÀ DI BOLOGNA

17.50 - **PF8** Rapid and green discrimination of bovine milk according to fat content, thermal treatment, brand and manufacturer via colloidal fingerprinting.
NICHOLAS KASSOUF, UNIVERSITÀ DI BOLOGNA

17.55 - **PF9** Fast synthesis of molecularly imprinted polymers for selective extraction of phomopsins in lupin samples by UPLC-MS/MS analysis.
SARA PALMIERI, UNIVERSITÀ DI TERAMO

18.00 - **PF10** Exploring innovative solutions in Point-of-Care diagnostics: unveiling the potential of gold silica nanoparticles and molecularly imprinted nano polymers.
THEA SERRA, UNIVERSITÀ DI TORINO

18.05 - **PF11** CRISPR/Cas-based cell-free biosensor for antibody detection.
FRANCESCA C. MICELI, UNIVERSITÀ DI ROMA TOR VERGATA

18.10 - **PF12** Development of an optimised icIEF method for harmonising Quality Control of Monoclonal Antibodies.
VIRGINIA GHIZZANI, ISTITUTO SUPERIORE DI SANITÀ, ROMA

18.15 APERITIVO

- **Martedì, 16 aprile 2024**

SALA ULISSE, ACCADEMIA DELLE SCIENZE DELL'ISTITUTO DI BOLOGNA

SESSIONE IV
MODERANO: DARIO COMPAGNONE, CLAUDIO BAGGIANI
09.00 - **OC13** NanoMIP as synthetic receptor for rabbit IgG: effect of different crosslinker amount.
VALENTINA TESTA, UNIVERSITÀ DI TORINO
09.15 - **OC14** Electrochemical sensing of wound healing biomarkers using a smart dressing.
FEDERICA MARIANI, UNIVERSITÀ DI BOLOGNA
09.30 - **OC15** Biocatalytic electrochemical method for online monitoring of wastewater.
LORENZO QUADRINI, UNIVERSITÀ DI FIRENZE
09.45 - **OC16** Wearable and Edible Enzyme based Bioelectronics as Point of Care Devices.
PAOLO BOLLELLA, UNIVERSITÀ DI BARI
10.00 - **OC17** Reagent-free paper-based electrochemical sensor modified with carbon black for the detection of essential oils.
LUCA FIORE, UNIVERSITÀ DI ROMA TOR VERGATA
10.15 - **OC18** Quality Control of recombinant proteins: the case of infliximab.
BENEDETTA PASQUINI, UNIVERSITÀ DI FIRENZE

10.30-11.00 COFFEE BREAK E SESSIONE POSTER

11.00 - **OC19** CO2-laser approach for nano-metal equipped paper-based opto-colorimetric analytical devices manufacturing.
ANNALISA SCROCCARELLO, UNIVERSITÀ DI TERAMO
11.15 - **OC20** An untargeted analytical workflow based on Kendrick mass defect filtering reveals dysregulations of acylcarnitines in prostate cancer tissue.
ANDREA CERRATO, UNIVERSITÀ DI ROMA LA SAPIENZA

11.30-13.00 **TAVOLA ROTONDA "LA SFIDA ONE HEALTH"**
MODERANO: ALDO RODA, MARIA CARERI
INTERVENGONO:
ALESSANDRA SCAGLIARINI - DIPARTIMENTO DI SCIENZE MEDICHE E CHIRURGICHE, UNIVERSITÀ DI BOLOGNA
ALESSANDRA BONOLI - DIPARTIMENTO DI INGEGNERIA CIVILE, CHIMICA, AMBIENTALE E DEI MATERIALI, UNIVERSITÀ DI BOLOGNA
VITTORIO SAMBRI - DIPARTIMENTO DI SCIENZE MEDICHE E CHIRURGICHE, UNIVERSITÀ DI BOLOGNA
FRANCESCO CUBADDA - ISTITUTO SUPERIORE DI SANITÀ
CECILIA BERGAMINI – ARPA EMILIA ROMAGNA

CORTILE DEL POZZO

13.00-14.30 LIGHT LUNCH E SESSIONE POSTER

14.30 CONCLUSIONI GIORNATE DI BIOANALITICA 2024

SALA ULISSE, ACCADEMIA DELLE SCIENZE DELL'ISTITUTO DI BOLOGNA

WORKSHOP CONTROLLO DI QUALITÀ PER LA SFIDA ONE HEALTH: LA QUALITÀ DEL DATO
MODERANO: SANDRA FURLANETTO, BARBARA RODA
14.30-16.00
Importanza della tracciabilità del dato analitico per campioni clinici
e tossicologici. SIMONE DONZELLI, WATERS CORPORATION
Produzione farmaceutica: cosa cambia con l'introduzione della ICH Q14/Q2(R2).
ANDREA GHEDUZZI, WATERS CORPORATION

16.00-16.45 COFFEE BREAK

16.45-18.00
Challenges of Data Management in One Health Model.
GIOVANNA CANTONI, PQE – PHARMA QUALITY EUROPE GROUP

- **Mercoledì, 17 aprile 2024**

SALA ULISSE, ACCADEMIA DELLE SCIENZE DELL'ISTITUTO DI BOLOGNA

WORKSHOP CONTROLLO DI QUALITÀ PER LA SFIDA ONE HEALTH: SVILUPPO E VALIDAZIONE DI DISPOSITIVI DIAGNOSTICI PER LA SFIDA ONE HEALTH
MODERANO: LAURA ANFOSSI
09.15-09.45
Introduzione
LAURA ANFOSSI, UNIVERSITÀ DI TORINO
09.45-10.30
Il campionamento degli odori e il ruolo del cittadino, Prj EU H2020 ODORPREP.
IVANO BATTAGLIA, LABSERVICE ANALYTICA
10.30-11.00
Frontiers in MIP-based artificial receptors: new opportunities in advanced sensing technologies.
ELISABETTA PRIMICERI, CNR NANOTEC - INSTITUTE OF NANOTECHNOLOGY

11.00-11.30 COFFEE BREAK

11.30-13.00
Validazione di dispostivi diagnostici: criteri, normative e rete degli enti organizzazioni nazionali ed internazionali in ambito veterinario.
SANTINA GRAZIOLI, ISTITUTO ZOOPROFILATTICO SPERIMENTALE DI LOMBARDIA ED EMILIA ROMAGNA

COMUNICAZIONI POSTER

P01 - IMPROVING THE SENSITIVITY AND THE COST-EFFECTIVENESS OF A COMPETITIVE VISUAL LATERAL FLOW IMMUNOASSAY THROUGH SERIAL DESIGNS OF EXPERIMENTS
SIMONE CAVALERA, Fabio Di Nardo, Thea Serra, Valentina Testa, Stefano Bertinetti, Alessandro Gelli, Laura Ozella, Claudio Forte, Claudio Baggiani, and Laura Anfossi

P02 - ELEMENTAL AND FATTY ACID PROFILES IN VOLCANIC MARINE ENVIRONMENTS: THE CASE STUDY OF PANAREA ISLAND (ITALY)
Federico Girolametti, Silvia Illuminati, Cristina Truzzi, Behixhe Ajdini, Matteo Fanelli, Lorenzo Massi, Teresa Sani, Arianna Mancuso, Stefano Goffredo, Mauro Marini, ANNA ANNIBALDI

P03 - FAST SONOCHEMICAL SYNTHESIS OF MOLECULARLY IMPRINTED POLYMERS FOR SELECTIVE CONTAMINANT DETECTION IN FOOD
DOUNIA ELFADIL, Sara Palmier, Flavio Della Pelle, Filippo Silveri, Aziz Amine, Dario Compagnone

P04 - COTTON THREADS BIO-CHEMILUMINESCENT DEVICES FOR SUSTAINABLE AND ACCESSIBLE POINT-OF-CARE
EMANUELA MAIORANO, Maria Maddalena Calabretta, Riccardo Desiderio, Elisa Michelini

P05 - "ONE HEALTH" STRATEGY, EMERGING POLLUTANTS AND GREEN PHARMACY. A NEW UHPLC-QTOF QUANTITATIVE METHOD TO STUDY READY BIODEGRADATION OF THERAPEUTIC PRODUCTS EXCIPIENTS.
ENRICO FLAMINI, Antonio Di Ruberto, Giada Fodaroni, Luca Massa, Mattia Gianni, Emiliano Giovagnoni, Luisa Mattoli

P06 - TRIPLEX-BASED CRISPR REACTION NETWORK FOR HIGHLY SPECIFIC DETECTION OF NUCLEIC ACIDS
Andrea Celeste Di Pede, ERICA BELFORTE, Alessio Palone, Neda Bagheri, and Alessandro Porchetta

P07 - COLLOIDAL CARBON NANOPARTICLES AS LABEL IN LATERAL FLOW IMMUNOASSAY
FABIO DI NARDO, Francesco Barbero, Simone Cavalera, Laura Anfossi, Ivana Fenoglio, Claudio Baggiani

P08 - ELECTROANALYTICAL PLATFORMS EQUIPPED WITH NANOSTRUCTURED SENSING SURFACES FOR (BIO)ANALYTICAL PURPOSES PRODUCED VIA SUSTAINABLE APPROACHES
FLAVIO DELLA PELLE, Annalisa Scroccarello, Filippo Silveri, Davide Paolini, Selene Fiori, Ida Valeria Di Cristoforo, Paolo Bollella, Luisa Torsi, Keisei Sowa, Dario Compagnone

P09 - NOVEL CANDIDATES FOR METAL RECOGNITION IN BIOLOGICAL FIELD: PHENANTHROLINE-COUMARIN LIGANDS
F. MELONI, M.G. Cabiddu, E. Cadoni, S. Masuri and T. Pivetta

P10 - VALORIZATION OF MEDITERRANEAN BIODIVERSITY FOR MEDICINAL PURPOSES: A BIOANALYTICAL APPROACH
F. PETTINAU, A. Orrù, B. Pittau, A. Cao, E. Cadoni, A.C. Rinaldi, T. Pivetta

P11 - POLY-L-AMINOACIDS-BASED NANOCOMPOSITES: CHARACTERIZATION AND APPLICATION IN PHTHALATES BIOSENSING

GIULIA SELVOLINI, Costanza Scopetani, Agnese Bellabarba, Tania Martellinia, Alessandra Cincinellia, Carlo Viti, Alessandra Adessi, Giovanna Marrazza

P12 - SYNTHESIS OF MULTIVALENT ACTIVATABLE APTAMERS FOR ULTRASENSITIVE DETECTION OF SALMONELLA TYPHIMURIUM
MENGYUE LIU, Shouyi Dou, Giovanna Marrazza, Yemin Guo, Xia Sun

P13 - EXPANDING CRISPR-BASED MOLECULAR DIAGNOSTICS BEYOND DETECTION OF NUCLEIC ACIDS
NEDA BAGHERI, Luca Capelli, Federica Pedrini, Andrea C. Di Pede, Alejandro Chamorro, Andrea Idili, Roberto Corradini, Alessandro Bertucci, Alessandro Porchetta

P14 - LIPIDOMIC INVESTIGATION IN PLASMA OF PARKINSON'S DISEASE, MULTIPLE SYSTEM ATROPHY AND PROGRESSIVE SUPRANUCLEAR PALSY DIAGNOSED PATIENTS
NICOLÒ INTERINO, David Chamoso-Sanchez, Alessandro Perrone, Manuela Contin, Giovanna Calandra Buonaura, Giovanna Lopane, Francisco Javier Rupérez, Jessica Fiori

P15 - A HYBRID NANOCOMPOSITE OF GOLD NANOPARTICLES AND NANO-GRAPHENE OXIDE FOR THE ELECTROCHEMICAL DETECTION OF ESTRONE
PATRICK SEVERIN SFRAGANO, Serena Laschi, Chiara Ingrosso, M. Lucia Curri, Ilaria Palchetti

P16 – VACUUM-ASSISTED HEADSPACE-SOLID PHASE MICROEXTRACTION FOR SHORT-CHAIN FATTY ACIDS IN FAECAL SAMPLES
ROSSANA COMITO, Emanuele Porru, Nicolò Interino, Francesco Saverio Violante, Jessica Fiori

P17 - MICROFLUIDIC AND SENSING TOOLS FOR CANCER IMMUNOTHERAPY
MARIA SERENA CHIRIACÒ, Elisabetta Primiceri, Antonio Turco, Valeria Garzarellia, Giulia Siciliano, Alessia Foscarini, Giuseppe Gigli, and Francesco Ferrara

P18 - A NEW BIOLUMINESCENT BIOASSAY BASED ON A "CAGED" LUCIFERIN FOR THE SCREENING OF BILE SALT HYDROLASE ACTIVITY IN HUMAN COMPLEX MATRICES
ANGELA PUNZO, Alessia Silla, Patrizia Simoni, Antimo Gioiello, Giada Moroni, Vanessa Rezende Bevilaqua, Vadim Viviani, Barbara Roda, Aldo Roda, Cristiana Caliceti

P19 - PORTABLE AND AFFORDABLE 3D PRINTED BIOSENSOR FOR NERVE AGENT DETECTION IN DRINKING WATER USING CARBON BLACK/THERMOPLASTIC POLYURETHANE COMPOSITE ELECTRODES
LUDOVICA GULLO, Vincenzo Mazzaracchio, Noemi Colozza, Leonardo Duranti, Luca Fiore, Fabiana Arduini

P20 - A RAPID LIQUID MICROEXTRACTION METHOD FOR THE DETERMINATION OF F2-ISOPROSTANES IN ORAL FLUID BY MEANS OF PALME COUPLED WITH UHPLC-MS/MS ANALYSIS
FRANCESCO BARTOLINI, Paola De chirico, Ilenia Bracaglia, Martina Croce, Gaia Di Francesco, Gianmarco Pezzuti, Federico Fanti, Dario Compagnone, Camilla Montesano, Manuel Sergi.

P21 - DEVELOPMENT OF ANALYTICAL PROCEDURES FOR THE SIMULTANEOUS DETERMINATION OF INDIGOTIN AND INDIRUBIN IN INDUSTRIALLY PRODUCED NATURAL INDIGO
ELIA FRIGNANI, Laura Pigani, Fabrizio Roncaglia

P22 - FAST CLASSIFICATION OF CANNABIS SATIVA L. SAMPLES ACCORDING TO THE TOTAL THC CONTENT
ALESSANDRO MONARI, Giorgia Foca, Alessandro Ulrici, Chiara Zanardi, Laura Pigani

P23 - LABEL-FREE ELETTROCHEMICAL IMMUNOSENSOR FOR THE DETECTION OF PSEUDOMONAS AERUGINOSA IN CONFINED ENVIRONMENT

ANTONIO CECCARELLI, Rocco Cancelliere, Elisa Paialunga, Giulia Sarpi, Giuseppina Rea, Laura Micheli

P24 - SIZE SEPARATION AND CHARACTERIZATION OF HUMAN POLYSOMES BY FLOW FIELD-FLOW FRACTIONATION

JUNJIE WANG, Stefano Giordani, Barbara Roda, Valentina Marassi, Pierluigi Reschiglian, Lorenzo Montanaro, Marianna Penzo, Andrea Zattoni

Lecture Premio “Alessandro Mangia”

From enzyme electrodes to lab on strip devices; challenges and opportunities for food and biomedical analysis

Dario Compagnone[a]

[a]Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Via R. Balzarini 1, 64100 Teramo

In the electroanalytical scenario, the use of commercial electrodes is highly widespread, nevertheless, they present limitations regarding their default design, rigid substrate, and analytical performance. To overcome these limitations, recently, different low-cost strategies to fabricate (bio)devices have emerged as smart alternatives to the more expensive and cumbersome clean-room and lithography-based methods. In particular, the manufacturing of flexible and paper-based devices integrating nanomaterials (NMs) conductive films is a hot topic. The talk will review electrochemical sensing strategies from the development of enzyme electrodes based on the classical Pt elecrodes and membrane based devices to the recent development of stencil printing, laser printing and film production on flexible and paper substrates. The use of electrochemical mediators as Prussian Blue as successful electrochemical mediator coupled to different graphite substrates for different food quality and biomedical markers will be given. The potential of a controlled nanostructuration of the electrochemical surface to overcome fouling and improve sensitivity will be reported with examples related to the use of carbon black, transition metal dichalchogenides, gold and silver nanoparticles. The use of molecules such ad cathechins both for the functionalisation of the electrodes and/or the production of exfoliated nanomaterials will be critical addressed for their exploitation in the development of electrochemical sensors. Xurography and stencil printing for the realisation of ultra-low cost electrochemical sensors and lab on strip devices composed only of nanomaterial will also described. Examples of electrochemical devices for monitoring of oxidative stress in cell cultures, detection of polyphenols, antioxidant activity, pesticides, glutathione, fructose, in different biological and food samples will demonstrate the feasibility of the approach and the maturity of the approaches for real applications.

Keynote Premio “Cristina Giovannoli”

KN – FFF in bioanalytical chemistry: the gentle touch

Valentina Marassi[a,b], Stefano Giordani[a], Anna Placci[a], Junjie Wang[a], Barbara Roda[a,b], Andrea Zattoni [a,b], Pierluigi Reschiglian [a,b]

[a] Department of Chemistry G Ciamician, University of Bologna, Italy
[b] byFlow srl, Bologna, Italy

Many efforts of the scientific community have been directed to investigate innovative methods for sorting subpopulations from dispersed biological media (cell culture, lymph, extracellular matrix) and devising advanced detection strategies to identify and characterize peculiar structures (aggregates, vesicles, nucleic strands) as biomarkers of diseases, as well as working in the development and characterization of new diagnostic devices in the most representative conditions, preserving both sample and information.
In the field of bioanalytical chemistry, Flow Field Flow Fractionation (FFF) is a challenging yet incredibly promising opportunity.
FFF coupled on-line with multiple detectors can separate and characterize populations (proteins, colloids, polymers and particulates) with uncorrelated parameters: together with the intrinsic features of being a native, stationary phase-free size-based separation method, FFF offers highly resolved size distribution, separation of the unbound reagents from labelled species and information on the interactions between a target analyte and recognition elements such as antibodies or aptamers. FFF-based analytical platforms may significantly improve the knowledge and development of nanobased systems for bioanalytical applications, such as the analysis of different proteins and biologically relevant species, from protein drugs to bio-vesicles, and nanoparticles of biopharmaceutical interest. It is fundamental in the optimization of sample-labelling methods (drug development purposes and diagnostic selection). Last, the multidimensional data obtained can be elaborated into predictive models and assist grouping for data-driven diagnosis of clinical conditions. I will illustrate the contribution of FFF to the advancement of bioanalytical chemistry and detail the important aspects in native, low-impact analytical/preanalytical platforms to achieve improved sample processing, better data quality and reduced environmental impact of analyses. Key applications will be presented, including the monitoring of protein/drug aggregation, aptasensor development, the size characterization, quantification and enrichment of biologically relevant nanoparticles and of extracellular vesicles, and the perspectives on the future of FFF as a tool for liquid biopsy.

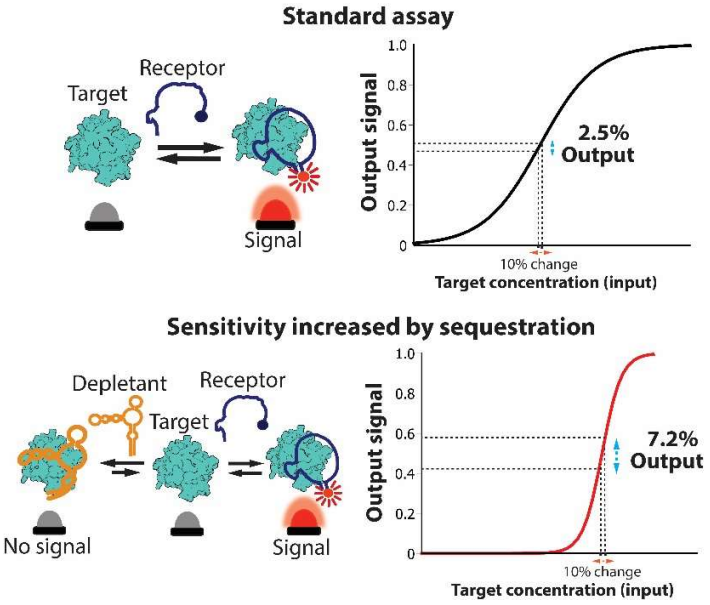
Comunicazioni Orali

OC01-Exploiting sequestration mechanism to tune dose response of synthetic receptors for improved sensitivity of biosensing platforms

Alejandro Chamorro-Garcia[a], Claudio Parolo[b], Gabriel Ortega[c], Andrea Idili[a], Kevin W. Plaxco[d], Francesco Ricci[a]

[a] University of Rome Tor Vergata. Via della Ricerca Scientifica 1, 00133 Rome, Italy.
[b] ISGlobal–Barcelona Institute for Global Health, Carrer del Rosselló 132, 08036 Barcelona, Spain
[c] Precision Medicine and Metabolism Laboratory, CIC BioGUNE, Basque Research and Technology Alliance, Parque Tecnológico de Bizkaia, 48160 Derio, Spain
[d]Department of Chemistry and Biochemistry University of California Santa Barbara (UCSB), Santa Barbara, CA 93106, USA

Biosensors and bioassays have seen significant success largely due to the extraordinary versatility, affinity, and specificity of biomolecular recognition elements. Nevertheless, these receptors suffer from two inherent limitations: the single saturable binding site hyperbolic relationship (Langmuir isotherm) between target concentration and receptor occupancy, and the position of the linear response region with respect of the detection range of interest. Therefore, tuning the dynamic range and the dose response output of bioreceptors is crucial to be able to perform accurate (high precision) measurements at a specific range of analyte concentrations. Nature, to overcome this limitation, has developed a number of mechanisms ( ), such as sequestration, allostery or cooperativity, to easily tune (shift, narrow or extend) the dynamic range of response to target and optimize sensitivity and specificity. In this talk I will give a short overview and examples of successfully engineered bioreceptors( ), to later focus on the case of the sequestration( ), a mechanism that relies on the use of two biorecognitions elements: a higher-affinity, non-signaling "depletant", and a lower affinity, signal-generating "receptor", this mechanism increases the steepness of the input/output curves and improves our ability to measure small relative changes in target concentration. Finally, I will explain how we successfully applied sequestration onto 3 different sensing approaches( ): an Aptamer Based electrochemical sensor (EAB-sensor), lateral flow immunoassay (LFIA) and enzyme linked immunoassay (ELISA).



1. *Ricci F., Vallée-Bélisle A., Simon A.J., Porchetta A., Plaxco K.W., Acc. Chem. Res. 49 (2016), 1884.*
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OC02 - Detection of 25 PFAS in human blood by quantitative dried blood spots microsampling for large population monitoring

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Background: PFASs represent a class of synthetical chemicals, widely used in industrial, domestic, and consumer applications since the 1940s. They are ubiquitous in the environment, and they tend to bioaccumulate in tissues and fluids of human body. Following repeated exposure to PFASs, a broad range of adverse effects has been described: immunosuppression, hormones disruption, carcinogenicity, and lipids profile alteration [1]. Therefore, monitoring PFAS levels in human blood is of paramount importance for public health policies. Compared to traditional venous blood, Dried Blood Spots (DBS) is an attractive and reliable sampling technique to assess the individual *exposome* [2].
Purpose: This study aimed to develop and validate an innovative analytical method based on quantitative DBS microsampling to identify and quantify a selected panel of 25 PFAS. The proposed method is highly promising for a simple, rapid, and cheap monitoring activity in exposed or at-risk populations, such as pregnant women, newborns, or certain categories of workers.
Methods: A quantitative volume of 10 μL of fortified blood was deposited on *Capitainer®B* card. After 3 hours of drying, 500 μL of methanol and internal standards were added. After 30 minutes of sonication and 10 minutes of centrifugation, the extraction solvent was evaporated under nitrogen flow and then reconstituted with 20 μL of 75:25 aqueous:organic mobile phases solutions. Finally, 3 μL of sample were injected into the UHPLC-MS/MS for targeted analysis. The calibration curve was built at six different concentration levels (2-5-10-20-50-100 ng/mL). The validation for sensitivity, specificity, linearity, accuracy, and precision was performed during three non-consecutive days, with three calibration curves for each session. Additional experiments were performed for matrix effect and recovery assessment. Stability was evaluated under three different storage temperature (-20°C, 4°C, 25°C) and time conditions (1 day, 2 weeks, 1 month).
Results: The developed method enabled to achieve LOD and LOQ values in the range from 0.4 (PFODA, PFOS) up to 1.0 ng/mL (PFOA, 3,6-OPFHpA) and from 0.8 up to 2.0 ng/mL, respectively. Accuracy and precision fulfilled the acceptability criteria within ± 20% for each analyte at all concentrations. Extraction process showed high recovery above 80% for all analytes, whereas matrix effect experiment demonstrated ion enhancement for 13 molecules (+50%) and a moderate result for others (<50%). Consequently, the extraction protocol revealed a process efficiency higher than 100%. Real samples collected from *non-exposed* volunteers showed negligible levels of PFAS.
Conclusions: The validation results demonstrate that the proposed workflow, which combines the DBS microsampling with UHPLC-MS/MS instrumentation, is reliable, fit-for-purpose, and easily adaptable in the laboratory routine. The proposed approach provides a straightforward and effective solution to monitor PFAS levels in selected population.

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OC03 - Streamlined Monolith Preparation for Online Extraction and LC-MS Analysis of β-Estradiol in Serum Using a Simple Multicomponent Reaction

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Multicomponent reactions present effective and eco-friendly approaches for crafting monoliths suitable for analytical chemistry applications [1;2]. In this study, we employed a multicomponent reaction to achieve one-pot, scaled-down synthesis of a poly(propargyl amine) polymer within silica-lined PEEK tubing readily available in the market. The reaction utilized minimal reagents and demonstrated high atom economy. The resulting monolithic column was integrated into an autosampler system for online extraction and purification of β-estradiol from human serum. Sample pretreatment involved straightforward dilution with methanol and subsequent protein removal via centrifugation. This platform enabled LC-MS analysis in multiple reaction monitoring mode for β-estradiol quantification. Validation in serum showcased practical utility for monitoring fertile women, with recoveries exceeding 94% and LOD and LOQ values of 0.008 and 0.18 ng mL^{-1}, respectively. Our developed platform proved competitive with prior solid-phase microextraction methods for β-estradiol in serum, offering comparable recovery and sensitivity while significantly enhancing automation. Environmental impact assessment deemed acceptable owing to monolith synthesis miniaturization and extraction automation. Potential limitations associated with LC-MS analysis can be mitigated by incorporating additional analytes within a single investigation. This work underscores the versatility, cost-effectiveness, and environmentally friendly nature of multicomponent reactions for generating reversed-phase and mixed-mode sorbents, facilitating the downsizing of the entire analytical procedure from extraction preparation onwards.

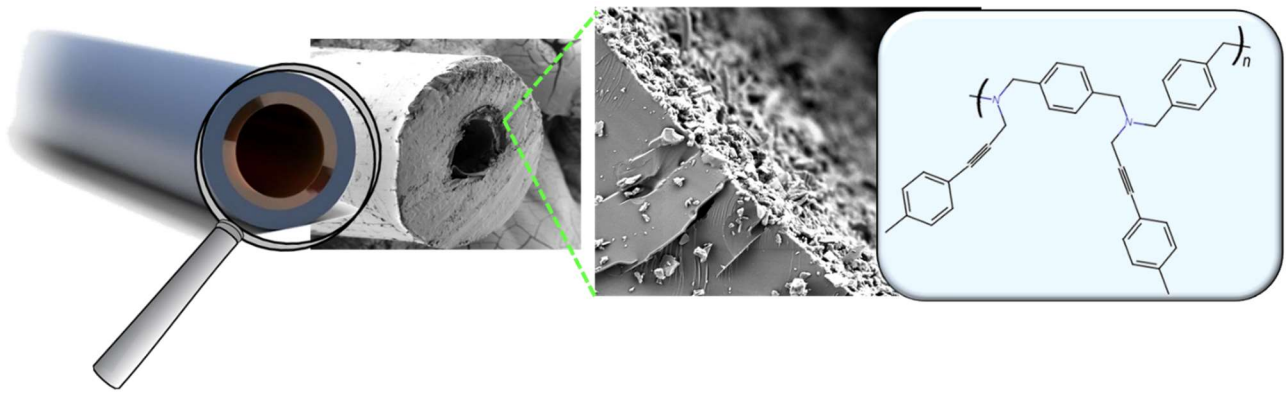

Figure. High-resolution SEM image of the inside of functionalized PEEK-sil capillary showing the porous structure of the polymer layer; structure of the poly(propargyl amine) polymer showing aromatic and amine moieties.

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OC04 - Dopamine-related parameter involved in neurological disorders monitored using wearable sensing platform

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Neurological disorders (NDs) are different and heterogeneous diseases, such as Alzheimer's, Parkinson and other, that affect the body's autonomic, peripheral and central nervous system. The onset of ND leads to a progressive loss of cognitive and motorial abilities, making difficult to perform daily activities and resulting in a lack of autonomy. The main protein biomarkers involved in the onset of NDs resided in poorly accessible fluids, such as cerebrospinal fluid and blood, and their continuous monitoring result painful and discomfortable. On the other hands, several candidate biomarkers or metabolites reside in peripheral biofluids such as saliva, urine and sweat, facilitating the development of wearable devices capable of detect them in continuous and sensitive manner.
Electrochemical-based sensors offer amazing potential for the development of point-of-care-testing devices. Indeed, thanks to the miniaturization it is possible to integrate the sensors into comfortable wearable devices (bracelets, smartwatches and others).
This study introduces a new analytical wearable platform for screening and monitoring dopamine, a molecule that plays a key role in neurodegenerative disorders. Specifically, carbon screen-printed electrodes were enhanced with a biocompatible polymeric conductive layer. To this end, different amino acids were studied as biocompatible and biodegradable functional monomers. Then, the polymeric layer was further modified by electrodepositing, gold nanoparticles for the direct detection of electroactive moleculesor for the enzyme covalent immobilization. The modified platforms were characterized using cyclic voltammetry and electrochemical impedance spectroscopy. The analytical performances were evaluated in both standard solution and in synthetic biological fluids.

Acknowledgements
This work was supported by the European Union by the NextGenerationEu project ECS00000017 'Ecosistema dell'Innovatione' Tuscany Health Ecosystem (THE, PNRR, Spoke 3: Nanotechnologies for diagnosis and therapy).

OC05 - P-jet biosensor for testing picogram level of different protein biomarkers

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Our continued exposure to pollutants causes an excessive production of reactive oxygen species (ROS), that allows to neurodegeneration onset. In this context, the structural aggregation of some biomolecules as β -amyloid or Tau protein, in different areas of the brain, seem to play a key role in the pollution related neurodegeneration becoming putative targets for developing innovative biosensors for an early diagnosis, it is indeed largely documented that neurodegenerative diseases can be effectively treated only if quickly diagnosed. Hereby, we propose an innovative biosensor for detecting the aggregation of different neurodegeneration biomarkers, at low picogram level by using a pyro-electrohydrodynamic jet (p-jet) system. The protein aggregation phase was evaluated by spectroscopic techniques such as circular dichroism (CD) and InfraRed Attenuated Total Reflection (IR-ATR). The detection limit of the system was obtained by an immunofluorescence- based test in complex media as artificial urine. Our approach could represent an innovative breakthrough in monitoring the early-stage neurodegeneration syndromes giving the possibility to therapeutically intervene before the onset of symptoms.

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OC06 - Are aptamers really promising as bioreceptors? A multitechnique approach to bridge the gap between aptamer selection and analytical applications

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In the last years, increasing attention has been devoted to aptamers as biorecognition elements in the analytical field, creating new opportunities for the design and development of biosensing and sample treatment strategies [1]. Aptamers are short single-stranded DNA or RNA oligonucleotides selected from randomized libraries through Systematic Evolution of Ligands by EXponential enrichment (SELEX) exhibiting high-affinity and selectivity towards target molecules. After initial enthusiasm for aptamers as highly promising alternative to antibodies, their reliability as bioreceptors in analytical applications has sometimes been questioned, with recent articles pointing out how the field of aptamer research is particularly susceptible to poorly reproducible results [2,3]. In this context, the scientific community is now faced with the need for rigorous strategies for the multifaceted characterization of the aptamer-target interaction to assess the real analytical potential of aptamers and prevent misinterpretation of the results [3,4].

The present work represents the first study aimed at developing a multitechnique approach to get further insights into the binding performance of the DNA aptamers commonly used in the literature for the biorecognition of lysozyme, which is an important clinical biomarker and a major allergenic protein in egg white [4]. For this purpose, a strategy based on a magneto-electrochemical apta-assay, circular dichroism spectroscopy, fluorescence spectroscopy and asymmetrical flow field-flow fractionation with UV detection was devised and applied to investigate the aptamer–lysozyme interaction [4,5]. The rigour and reliability of the proposed approach rely on the use of negative controls, i.e. randomly scrambled sequences and a 40-mer thymine sequence, to assess the sequence specificity of the interaction, and on the inclusion of the SELEX-selected RNA aptamer acting as positive control. It was observed that in the investigated buffers an interaction occurs in the low micromolar range and binding is not associated with a conformational change of either the protein or the DNA aptamer. The similar behaviour of the anti-lysozyme DNA aptamers compared to that of the negative controls showed sequence-aspecific interactions, thus demonstrating that oligonucleotides can interact with lysozyme regardless of their sequence.

This study moves in the direction of calling for adequate and rigorous testing of candidate aptamers with appropriate controls and exploring different binding conditions, recognized as a unique way to advance the aptamer field within the analytical scenario.

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OC07 - Electrochemical magneto-assay for BRD4 detection with theranostic applications in cancer diseases

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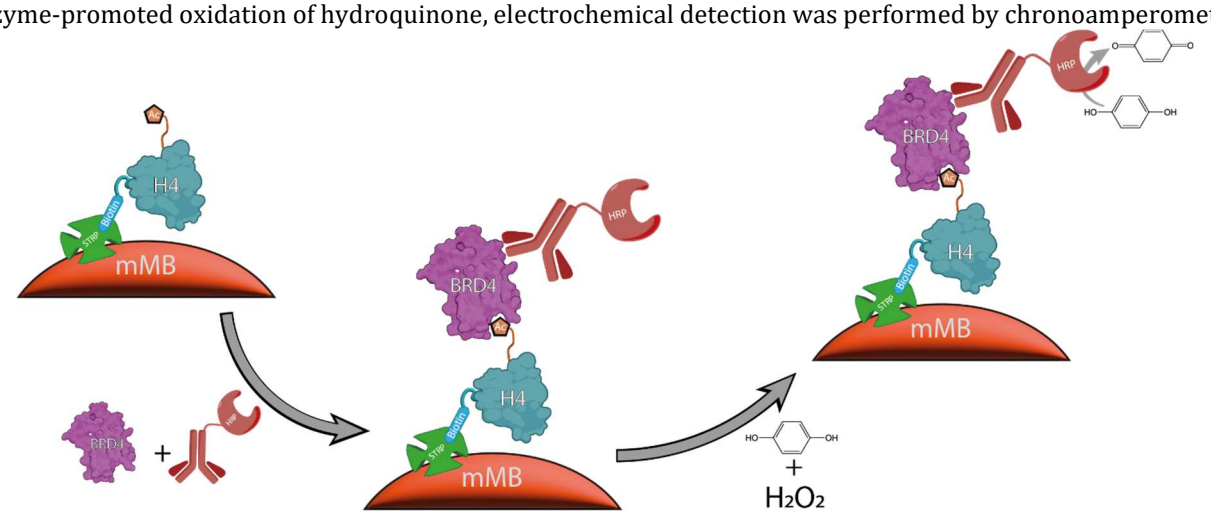
The Bromodomain and Extra-Terminal (BET) proteins are a class of well-known epigenetic regulators involved in gene expression mechanisms. Within the BET family, bromodomain-containing protein 4 (BRD4) has demonstrated to play a prominent role in various cancer diseases, including breast and prostate cancer, glioblastoma and melanoma.[1] Consequently, the downregulation of these genes through BRD4 inhibition or degradation has shown significant therapeutic potential for the treatment of several tumour diseases.[2] In this context, the detection of BRD4 is useful both to assess its overexpression as well as the efficiency of treatments aimed at its inhibition. However, to our knowledge, no biosensor for the determination of BRD4 protein has been developed to date.
In the context of the development of magneto-electrochemical platforms for the detection of protein biomarkers,[3] we devised a novel electrochemical magneto-assay for the detection of the BRD4 protein. The method is based on the immobilization of a biotinylated BET-specific ligand on magnetic micro-beads (mMBs) functionalized with streptavidin, followed by BRD4 target capture and binding of an HRP-conjugated anti-BRD4 antibody. After enzyme-promoted oxidation of hydroquinone, electrochemical detection was performed by chronoamperometry.



This protocol proved to be extremely fast, allowing the entire complex to be assembled in a single incubation step in as little as 10 minutes. The challenges of matrix effects on measurements were addressed, showing no significant differences ($p>0.05$) in the results obtained in undiluted human plasma compared to those obtained in PBS buffer. Analytical validation was performed in human plasma at different concentrations of BRD4 obtaining good sensitivity with a LOD of 1.99 nM, although with a limited response range. Interestingly, by changing the receptor concentration on mMBs, it has been observed that it is possible to increase the dynamic response range and, to some extent, tune the analytical performance of the assay. Finally, the magnetoassay was applied to detect BRD4 in untreated cell lysates from HEK cells, with results consistent with those obtained using a commercial ELISA kit.
The proposed method paves the way for the development of rapid tests to evaluate the effectiveness of therapeutic treatments and monitor the progress of various cancer diseases.

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OC08 - On-chip device for flow-driven release of extra-cellular vesicles and their detection as diagnostic biomarkers

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Precision and personalized medicine advancements, allow to tailor the clinical approach, depending on the makeup of patients' DNA and expression issues. Liquid biopsy and the chance to use selected biomarkers from biological fluids, could strongly contribute to the improvement of an increasingly patient-oriented method, avoiding invasive assays and tissue biopsies[1]. Extracellular Vesicles (EVs) act both as a snapshot of the cells from which they originate and as depository of important information, facilitating direct extracellular transfer of proteins, lipids, and miRNAs/mRNAs/DNAs. For all these reasons, EVs are on the rise for the possibility to be considered as powerful biomarkers[2]. In this study, we realized a Lab-On-Chip (LoC) device on fluid dynamic simulations and microfabrication techniques (micro-milling and 3D printing) to study the release of EVs from different cell lines in response to dynamic conditions and mechanical stimuli.
Molded plastic substrates assembled on a glass slide create a microfluidic chamber where Oral Squamous Carcinoma (OECM-1), neuroblastoma (SH-SY5Y) and microglial (CHME-5) cell lines were seeded reaching 50% of confluence and exposed to a controlled culture medium flow for different time-points. A complete medium replacement in the dynamic condition was obtained setting the flow medium rate, avoiding shear stress. The large- and small-EVs released from cells in dynamic and static conditions were isolated by differential ultracentrifugation and the different EV populations were characterized using high-resolution flow cytometry and western blot assays.
Our purpose is to build a benchtop-ready device able to identify arrays of biomarkers associated to the size and to different functions of EVs subclasses, customizable according to clinical needs. The aim of this platform is to integrate the function of microfluidic sorting and electrochemical characterization of EVs in order to identify a panel of EVs related biomarkers on a single lab-on-chip. A key point will be the effort in removing all the highly impacting processing procedures to operate in the most native conditions possible, reducing time and cost, and preserving EV morphology and properties.

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OC09 - Extraction of phytosterols by fast synthesized molecularly imprinted polymers (MIPs) from food matrices

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Phytosterols (PSs) are bioactive compounds structurally and functionally similar to cholesterol. They contain an extra methyl, ethyl group, or double bond, and most of their side chains contain 9-10 carbon atoms. PSs have been classified as 4-desmethyl sterols of the cholestane series, which all have double bonds at the C5 position of the B-ring[1]. These molecules are particularly known for a wide range of properties including reduced intestinal cholesterol absorption and potential contributions to preventing cardiovascular diseases[2]. PSs are generally classified into three groups based on the number of methyl groups on carbon-4, two (4-dimethyl), one (4-monomethyl), or none (4-desmethyl). Moreover, 4-dimethyl esters and 4-monomethyl sterols are metabolic intermediates in the biosynthetic pathway leading to the final product, 4-desmethyl phytosterols, but are usually present at low levels in most plant tissues. These compounds are present in a wide range of food matrices, such as seeds, grains, and legumes, both in free and conjugated form and they can be found in the form of fatty acyl esters, glycosides, and fatty acyl glycosides. Molecularly imprinted polymers (MIPs) represent a promising approach for selective extraction of target molecules due to their tailor-made binding sites[3]. In this scenario, a low-cost molecularly imprinted polymers (MIPs) approach for selecting these compounds was not fully explored. In this work, a fast chemical MIPs synthesis approach for selective extraction of PSs was performed, using cholesterol as a dummy template. The synthesis of MIPs involved the use of cholesterol as a dummy template molecule, methacrylic acid as the functional monomer, ethylene glycol dimethacrylate as the cross-linker, and azobisisobutyronitrile as the initiator. The resulting MIPs exhibited specific recognition sites complementary to the molecular structure of phytosterols, ensuring high selectivity during extraction. The MIPs were used as an adsorbent phase for SPE and combined with a targeted approach using ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) with atmospheric pressure chemical ionization (APCI). The results showed high selectivity with significant extraction performances and low matrix effect, a principal goal in complex plant matrices. The chromatographic separation allowed for the simultaneous detection of multiple phytosterols with high resolution and accuracy. The developed method was validated providing sensitive and reliable quantification of phytosterols on 12 different food matrices. The chromatographic separation allowed for the simultaneous detection of multiple phytosterols with high resolution and accuracy. Furthermore, the method demonstrated excellent reproducibility and recovery rates, indicating its suitability for routine analysis. The integration of MIPs with UPLC-MS/MS represents a robust and versatile approach to the extraction and analysis of phytosterols. The superior selectivity and efficiency of MIPs over traditional SPE techniques make this method highly promising for applications requiring precise quantification of phytosterols in a wide range of complex matrices, proving the high versatility of the MIPs approach. In the end, the proposed strategy can be considered as a fast and effective method to produce MIPs as an extraction tool for the determination of these target compounds.

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This research/publication/volume was funded the European Union – Next Generation EU. Project Code: ECS00000041; Project CUP: C43C22000380007; Project Title: Innovation, digitalization and sustainability for the diffused economy in Central Italy - VITALITY;

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OC10 - Unveiling biochemical profiles of peri-implant crevicular fluid using SERS spectroscopy

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The exact identification and categorization of peri-implant diseases without the need for invasive procedures is critical for the successful clinical treatment and long-term durability of dental implants. Analysing peri-implant crevicular fluid (PICF) samples using a combination of surface-enhanced Raman scattering (SERS) spectroscopy and advanced chemometrics is a new emerging approach aimed to provide an unbiased assessment of implant health. SERS is a spectroscopic technique that offers several advantages over traditional bioanalysis for analysing biological samples ranging from in vitro cell culture models to ex vivo tissues and biofluids. SERS datasets obtained from biofluids provide a wealth of metabolic fingerprint information, however access to this information is not always straightforward. Bioanalytical SERS is a complex field requiring a complete understanding of the chemical and physical interactions between photons, nanomaterials, and biological systems. This communication will present results from a recent clinical study. A thorough investigation was conducted on PICF samples collected from a cohort of patients displaying varying degrees of peri-implant health, including implants without infection, implants affected by peri-implantitis, and implants with peri-implant mucositis. The canonical-powered partial least squares (CPPLS) method was used to analyse the acquired SERS spectra and determine the distinct biochemical features linked to each inflammatory state. Importantly, peri-implant mucositis and peri-implantitis both exhibit a comparable inflammatory SERS spectral pattern. Further, a linear discriminant analysis (LDA) classifier was used to combine the SERS-based values acquired from CPPLS with established clinical scores. The method's ability to distinguish between various implant conditions was validated using repeated double cross-validation. The integrated method showed great promise as a non-invasive diagnostic tool for early diagnosis of inflammatory diseases and real-time implant monitoring, thanks to its high sensitivity and specificity and overall balanced accuracy of 92%.

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OC11 - One-phase extraction coupled with photochemical reaction allows the in-depth lipid characterization of hempseed by untargeted lipidomics

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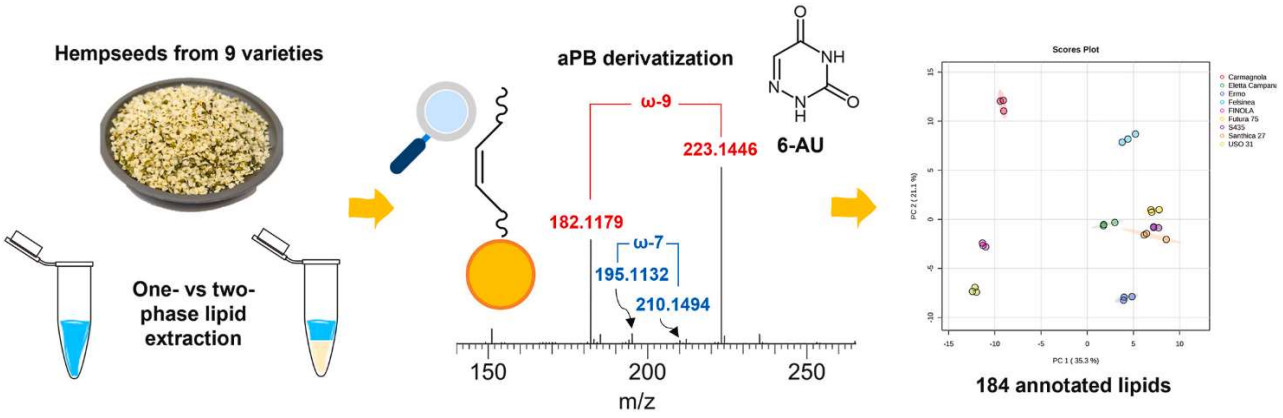
Due to their valuable nutritional content, several hemp-derived products from hempseeds have recently been placed in the market as food and food ingredients [1]. In particular, the lipid composition of hempseeds has raised interest for their rich content in biologically active polyunsaturated fatty acids with an optimum ratio of omega-3 and omega-6 compounds [2]. At present, however, the overall polar lipidome composition of hempseeds remains largely unknown. In the present work, an analytical platform was developed for the extraction, untargeted HRMS-based analysis, and detailed annotation of the lipid species. First, five one- and two-phase solid-liquid extraction protocols were tested and compared on a hempseed pool sample to select the method that allowed the overall highest efficiency as well as easy coupling with lipid derivatization by photochemical [2 + 2] cycloaddition with 6-azauracil. Underivatized lipids were annotated employing a data processing workflow on Compound Discoverer software that was specifically designed for polar lipidomics, whereas inspection of the MS/MS spectra of the derivatized lipids following the aza-Paternò-Büchi reaction allowed pinpointing the regiochemistry of carbon-carbon double bonds [3]. A total of 184 lipids were annotated, i.e., 26 fatty acids and 158 phospholipids, including minor subclasses such as N-acylphosphatidylethanolamines. Once the platform was set up, the lipid extracts from nine hempseed samples from different hemp strains were characterized, with information on the regiochemistry of free and conjugated fatty acids. The overall analytical approach helped to fill a gap in the knowledge of the nutritional composition of hempseeds.



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OC12 - Analytical characterization of microalgae for their use in health products

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We report here the results of the analytical characterization of microalgal biomasses to highlight their micro and macro nutrients content1. The research was oriented towards the development and validation of accurate, fast and reproducible methods for the nutritional assessment of algal biomasses, aiming to provide a guiding methodology.
We focused on the disclosure of bioactive molecules such as pigments, proteins, fatty acids, polysaccharides, vitamins, and antioxidants, all of which are of great interest in the preparation of a wide range of food, cosmetics and nutraceuticals, all contributing to wellbeing. The lipid profiles of algal matrixes were analysed for the content of saturated, unsaturated and polyunsaturated fatty acids. A GC-MS method was applied to get a fingerprinting of MUFA and PUFA omega fatty acids, for qualifying microalgae species.
Raw protein content of the algal samples and amino acids composition were estimated by optimizing the extraction of the protein fraction from the microalgae matrix, and their qualification by bottom-up LC-MS approach.
The determination of pigments (total carotenoids, chlorophylls and tocopherols) in the lipid extracts was obtained through the development of an HPLC-DAD analysis.
A toxicological study2 was also performed to determine by GC-MS the bis-phenol (BPA) content as contaminant according to the European legislation limits.
Once characterized in terms of their micro and macro nutrients and safety profile, microalgae were clustered in view of their potential use in cosmetics, food supplements, nutraceuticals and as food for maintaining a healthy status of individuals.

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OC13 - NanoMIP as synthetic receptor for rabbit IgG: effect of different crosslinker amount

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The binding selectivity typical of molecularly imprinted polymers (MIPs) is of fundamental importance in many analytical applications, such as solid phase extraction, immunochemical assays, and sensoristics. The Solid-Phase Polymerization Synthesis (SPPS) represents an innovative approach to preparing Molecularly Imprinted nanoParticles or "nanoMIPs". SPPS technique showed its validity for different types of polar templates such as small organic molecules, peptides and proteins, nucleic acid, and whole cells.
Despite this, SPPS[1] uses a pre-polymerization formulation with a nearly fixed composition between the different components of the mixtures, typically composed of acrylic acid (AA) as a functional monomer, t-butylacrylamide (tBAM), and isopropyl acrylamide (NIPAM) respectively as moderately hydrophobic and thermoresponsive co-monomers. Regarding the cross-linker, the most used is methylene-bis-acrylamide (BIS) at 2% mol concentration[2]. In literature, it is possible to find some polymerization in which BIS is used at 16% molar concentration, or in 0% applicate in linear molecularly imprinted polymer approach[3].
Until now the effect of different amounts of crosslinker has never been reported in detail because,usually, the binding properties have been related to the interactions with the functional monomers rather than the crosslinker. Here we report the effect of different amounts of BIS ranging between 0 (non-crosslinked) - 50% mol concentrations towards binding properties of nanoMIPs imprinted with rabbit IgG.
Binding properties have been tested with equilibrium partition towards rabbit IgG and, to get the selectivity, against bovine IgG. The results show that the degree of cross-linking defines three distinct types of nanoMIPs: (i) those with a low degree of cross-linking, including nanoMIPs without cross-linker (0–05 mol%), showing a low binding affinity about $1.0 \pm 0.2 \times 10^6$ mol L^{-1}, high density of binding sites, and low selectivity; (ii) nanoMIPs with a medium degree of cross-linking (1–18 mol%), showing higher binding affinity about o $14.4 \pm 0.7 \times 10^6$ mol L^{-1}, low density of binding sites, and high selectivity; (iii) nanoMIPs with a high degree of cross-linking (32–50 mol%), characterized by non-specific nanopolymer–ligand interactions, with low binding affinity about $1.6 \pm 0.3 \times 10^6$ mol L^{-1}, high density of binding sites, and no selectivity.
In conclusion, the results are particularly relevant in the synthesis of high-affinity, high-selectivity nanoMIPs as they demonstrate that a significant gain in affinity and selectivity could be achieved with pre-polymerization mixtures containing quantities of cross-linker up to 10–20 mol%, well higher than those normally used in this technique.

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OC14 - Electrochemical sensing of wound healing biomarkers using a smart dressing

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The increasing demand for wearable technologies is giving rise to a strong push for the design of innovative chemical sensors targeting the real-time acquisition of vital parameters. Among the most challenging applications, nonhealing wounds monitoring is a scarcely explored medical field that still lacks quantitative and minimally invasive tools for the management of the healing process. This contribution deals with the development of smart bandages for the real-time quantification of wound exudate pH and uric acid (UA) concentration, which correlate with the healing stages and can potentially give access to the wound status without unnecessary dressing changes that perturb the wound bed.

Fully textile sensors were obtained by screen-printing an optimised ink formulation containing a biocompatible organic semiconductor, i.e., poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS), in the desired sensing pattern. In particular, an organic electrochemical transistor (OECT) configuration was exploited for the design of the uric acid sensor, which proved to selectively and reversibly detect UA concentration within the biologically relevant range of 220−750 μM[1]. Moreover, a two-terminal pH sensor was realised by functionalising the screen-printed polymeric ink with IrOx particles, which spontaneously exert an electrochemical gating on the polymer and reversibly modulate its conductivity in a pH-dependent fashion[2] within the medically relevant range for wound monitoring (pH 6−9).

The textile sensors were combined with other medical gauzes with different absorption properties, thus leading to a final smart dressing ensuring the delivery of a continuous wound exudate flow across the sensor area. This setup allowed us to assess the analytical performances in flow conditions for better mimicking the final use of these wearable devices. Thanks to the careful selection of the textile materials and to the compactness of the final assembly, as well as the robustness of the sensing elements and transduction mechanisms, the smart dressings showed excellent resiliency to mechanical deformations and temperature variations.

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OC15 - Biocatalytic electrochemical method for online monitoring of wastewater

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Enzymatic catalytic processes have the potential to reduce pollutants in wastewater treatment facilities and promote sustainable bioremediation strategies. Monitoring urea levels in wastewater is crucial due to its widespread use as a fertilizer and cattle feed supplement[1], which contributes to environmental contamination through soil runoff, leading to algal blooms and eutrophication[2]. In this study, we propose a real-time urea measurement method that combines enzyme reactors with flow injection analysis (FIA) potentiometric analysis. FIA-based bioreactors are ideal for continuous monitoring and automated sample processing. Various bioreactors were constructed by immobilizing enzymes onto solid-phase materials (e.g., glass beads, plastic tube inner walls)[3] and connected to solid-state ammonium sensors for electrochemical readings. Analytical performance was assessed based on factors such as cross-linker concentration, immobilized enzyme quantity, and flow rate. The FIA system was optimized to achieve maximum signal in minimal time, and key analytical parameters were evaluated. Ultimately, the FIA system should be fully automated, enabling its installation at strategic locations within wastewater treatment plants for real-time monitoring.

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OC16 - Wearable and Edible Enzyme based Bioelectronics as Point of Care Devices

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Over the last six decades, enzyme-based biosensors have evolved for in situ analysis and Point-of-Care (PoC) devices, especially in glucose and lactate monitoring (fig.1). [1] Wearable technologies, like lab-on-skin devices, are replacing traditional testing platforms. Despite early-stage development of electronic textiles (e-textiles), they hold promise for continuous health monitoring. Researchers are exploring methods such as microfluidic integration and direct electrode application on clothing. [2]
Commercial wearable sensing platforms, apart from Continuous Glucose Monitoring (CGM), face challenges like limited sensor stability and understanding the correlation between sweat and blood analytes. Establishing this connection requires extensive clinical trials. Challenges for microneedles include ensuring reproducible skin penetration, considering skin elasticity and thickness variations based on age, gender, and ethnicity. [3]
The latest evolution of enzyme-based amperometric biosensors is represented by edible biosensors that can now detect a variety of parameters, ranging from basic physiological measurements such as temperature and pH to complex analyzes of organic and biological gases, and provide the data in real time. They can monitor a wide range of biomarkers, including those related to gastrointestinal health, enzymes, hormones, glucose levels, and even drug concentrations. [4]

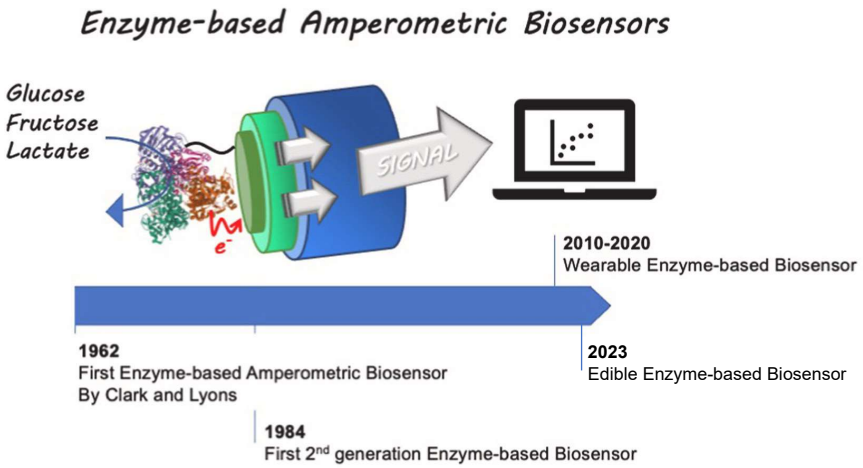

Fig.1 Evolution of Enzyme-based Amperometric Biosensors

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OC17 - Reagent-free paper-based electrochemical sensor modified with carbon black for the detection of essential oils

Luca Fiore,[a,b] Arianna Antinucci,[a] Giorgia Leotta,[a] Laura Fabiani,[a] Pierluca Galloni,[a] Riccardo De Santis,[c] Andrea Ciammaruconi,[c] Giorgia Grilli,[c] Elisa Recchia,[c] Florigio Lista,[c] Fabiana Arduini*[a,b]

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Paper-based electrochemical (bio)sensors have established a new route in the electrochemical sensing field[1,2], because paper-based electrochemical biosensors have been not only environmentally friendly devices, but recently our group demonstrated further advantages, including the simple combination with vertical microfluidics and their use as a reservoir to deliver smart electrochemical (bio)sensors able to i) contain the reagents, ii) preconcentrate the target analyte, and iii) synthesize the nanomaterials inside the paper network. Furthermore, these devices have demonstrated their ability to overcome the limitations of the other printed electrochemical sensors in the measurement of entirely liquid samples by detecting the target analyte in the aerosol phase or solid sample, without the additional sampling system[3]

In the era of sustainability, the use of natural compounds as antimicrobial compounds is the rational selection to avoid the release of pollutants into the environment. Among natural compounds, essential oils are characterized by reliable antimicrobial activity and their use is estimated to grow in the future, thus their detection is an asked point. Herein, we report an electrochemical paper-based device for the detection of essential oils, namely thymol, eugenol, and carvacrol by adding a few µL of solution onto the electrode, as well as by sampling the target analyte on the surface and in the aerosol phase, demonstrating its capability to work as both sampling system and sensor. We functionalized the working electrode by drop casting with carbon black, demonstrating improved sensitivity using this affordable nanomaterial. To deliver a reagent-free device, the paper-based electrode was loaded with the working buffer for asking the end-user only the contact with the sample. This sensor detects the selected essential oils in a dynamic linear range of up to 16 ppm, with a detection limit equal to 0.1, 0.1, and 0.2 ppm for thymol, eugenol, and carvacrol, respectively. The reliable results demonstrated the versatility of the paper-based electrochemical sensor, enlarging its use in essential oil detection.

Acknowledgements
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OC18 - Quality Control of recombinant proteins: the case of infliximab

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Recombinant proteins are complex molecules expressed by living organisms that present numerous possible conformations, post-translational modifications and microheterogeneity that should be pointed out in order to assure the quality, efficacy and safety of the final product. The Quality Target Product Profile (QTPP) is a concept described in the International Council for Harmonisation (ICH) Guideline Q8(R2) [1] and includes all the Critical Quality Attributes of the product. One or more Analytical Procedures can be established to address a particular (or various) product's Quality Attribute(s), being the analytical performance requirements based on the QTTP. In order to set up a horizontal analytical platform for monoclonal antibodies by Analytical Quality by Design (AQbD), infliximab was used as case study. Systematic method development based on AQbD is performed in different steps: the first step is the development of the Analytical Target Profile, which defines the purpose of the analytical method and the application scope; the second step is Quality Risk Assessment, in which all potential influencing factors are grouped by category in order to facilitate the examination of their influence on the method performances. In this presentation, a preliminary overview of the characterization of the monoclonal antibody infliximab by AQbD approach will be shown.

Acknowledgements
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OC19 - CO_2-laser approach for nano-metal equipped paper-based opto-colorimetric analytical devices manufacturing

Annalisa Scroccarello [a], Flavio Della Pelle [a], P. Di Battista, Dario Compagnone [a]

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The implementation of affordable technologies to fabricate optical and colorimetric paper-based analytical devices (PAD) integrating nanomaterials is a compelling challenge for the (bio)analytical community. In particular, the controlled decoration of flexible substrates with optical active metal nanoparticles (MNP), can offer infinite opportunities for the development of (bio)sensors and integrated analytical devices. However, implementing strategies to fabricate optical and colorimetric PAD integrating MNPs with a controlled pattern is still a difficult task.

Recently, our group proposed an innovative and versatile approach to in-situ synthesize on-paper plasmonic active gold, silver, platinum (Pt), copper and nickel nanostructures employing a CO_2-laser plotter [1]. This approach allows to 'write' MNPs onto paper according to the required design. The obtained MNPs demonstrated to possess two useful features, i.e., (i) the localized surface plasmon resonance that results in distinguishable visible colors, and (ii) catalytic features, these two phenomena are related to the MNPs' intrinsic chemistry and nanostructure. Laser-induced MNPs (LIMs) were integrated into different fully lab-made PAD coupled to smartphone-based readouts.

In this presentation will be presented a Flip-PAD for the selective determination of ascorbic acid, where Pt-LIM oxidase-like activity allows a dye-based colorimetric readout without the need for external reagents (**Figure A**). The Pt-LIM was fully characterized, and the oxidase-like activity was deeply investigated. The proposed lab-on-paper device was designed in an analytical configuration conceived to facilitate analysis steps and was manufactured via low-cost benchtop technologies (i.e., laser/cutter-plotter, thermal-laminator, etc.) using office-grade substrates (i.e., polymeric-sheets, cellulosic substrates, etc.).

Further, a paper-based device integrating LIMs based on aluminum (Al), for the selective detection of o-diphenols will be also presented; in this case, the analytical quantification of the analyte relise on the selective Al-nanostructures fluorescence enhancement induced by the interaction with the phenolic structures (**Figure B**). The Al-LIM based PAD sensing ability was carefully studied and optimized, eventually the PAD was employed for the selective detection of o-diphenols in foods.

The proposed laser writing strategy can be considered a new nanopatterning technique, particularly prone to generate optical sensing zones in tailored colorimetric PADs.

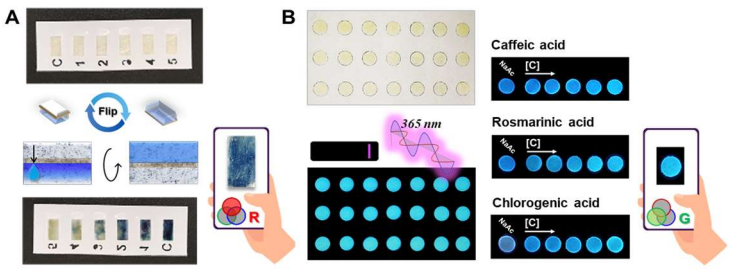

Figure. Scheck of the (**A**) Flip-PAD for ascorbic acid determination and (**B**) Al-LIM based PAD for the selective detection of o-diphenols

Acknowledgments

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OC20 - An untargeted analytical workflow based on Kendrick mass defect filtering reveals dysregulations of acylcarnitines in prostate cancer tissue

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Acylcarnitines play important roles in fatty acid oxidation and branched-chain amino acid metabolism and their disturbance has been associated with the occurrence and development of many diseases[1]. Previous studies have correlated altered levels of acylcarnitine with the progression of prostate cancer (PCa)[2], one of the leading causes of cancer-related deaths in males. Currently, the early detection of PCa is based on the measure in blood of the prostate-specific antigen (PSA), followed by magnetic multiparametric resonance and targeted prostate biopsy for final diagnosis. At present, the measurement of the PSA levels in blood is affected by limited sensitivity and specificity and cannot discriminate PCa from benign prostatic pathologies. In this regard, untargeted metabolomics represents a powerful strategy for the identification of novel and more specific biomarkers[3]. For structurally related classes of compounds, strategies could be put in place to simultaneously make data analysis more straightforward and achieve more confident identification results. In the present study, the acylcarnitine profile of prostate tissue was investigated. A cohort of 25 PCa patients undergoing radical prostatectomy was collected at the time of the surgical removal. For each patient, samples from both cancer and non-malignant adjacent tissue were collected and analyzed by high-resolution mass spectrometry to obtain a patient-matching control sample for each cancer tissue. Given the homogenous nature of acylcarnitines, an innovative data processing workflow based on Kendrick mass defect filtering was implemented on Compound Discoverer software. Later, an extra layer of validation on the putatively identified compounds was obtained by a retention time prediction model. The obtained data matrix comprising the 71 annotated acylcarnitine was finally analyzed by partial least square–discriminant analysis (PLS-DA) in double cross-validation. The classification results were 93.36 ± 0.05 for the cancer tissue and 92.96 ± 0.05 for the sane tissue, demonstrating the key role of acylcarnitine in the progression of PCa.

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Comunicazioni Flash

PF01 - Smartphone-based bioluminescent paper sensor for water toxicity monitoring

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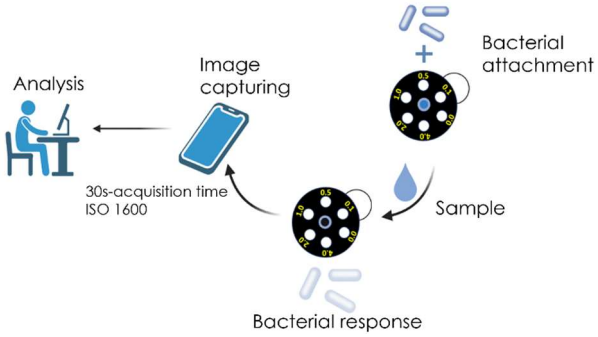
Water is vital for human survival and ecosystems. Organic carbon, nutrients (such as nitrogen and phosphorus), and metals (such as potassium, copper, and silver) are commonly found in waste streams and can be recovered through circular economy principles. However, to enable water reuse the presence of potential contaminants, such as heavy metals, personal care products and pharmaceutical residues, plastics, pesticides, and pathogens, should be monitored. Again, microbiological contaminants, such as bacteria, viruses, and protozoa, pose serious threats to water ecosystems and human health[1]. The United Nations Agenda 2030 Sustainable Development Goal 6 (SDG6)[2] aims at ensuring the availability and sustainable management of water and sanitation for all. Several chemical analytical techniques have been developed to monitor the aquatic environment, but they are time and labour-consuming, costly, and specialized personnel and sophisticated equipment are needed. Innovative, eco-friendly methods are sought for sustainable water management. Ideal solutions are cost-effective, sensitive, and enable on-site detection. Chemical and biosensors emerge as promising tools for real-time toxicological assessment, aligning with environmental sustainability goals and enhancing human well-being and economic progress.



To achieve this goal, we immobilized bioluminescent marine bacteria, on paper and used them for rapid toxicity testing of different water samples. *Aliivibrio fischeri* bacteria were chosen thanks to their natural emission of photons of visible light as a by-product of their metabolic respiration metabolism. Their bioluminescence is affected by toxic agents, resulting in a decrease in light emission. Freshly prepared or freeze-dried *A. fisheri* are already widely used to monitor water quality according to the ISO 11348 method[3]. We implemented this method into a user-friendly analytical sensing platform by immobilizing bacteria, employing a smartphone as a light sensor, and developing a tailored application to translate the reduction in light intensity into quantitative data on sample's toxicity level. NaClO was first chosen as model toxic analyte to test the performance of the sensor. Bioluminescent signals were acquired with a smartphone camera and evaluated by brightness analysis both with ImageJ software and the dedicated mobile-based application, achieving a limit of detection of 0.04 ppm for NaClO with both approaches. Other analytes were also measured with the sensor, including lead, 3,5-dichlorophenol, and microcystin-LR, selected since they are relevant pollutants in fresh or marine water. Spiked drinking water and wastewaters samples were tested for the analysis of the presence of NaClO, lead, 3,5-dichlorophenol, and microcystin-LR providing promising results for the on-site application of the sensor.

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PF02 - A fast and native approach for the characterization of functionalized bacteriophages for photodynamic therapy

Stefano Giordani[a], Roberto Saporetti [a], Tommaso Rossi[b], Paolo Emidio Costantini[b], Matteo di Giosia [a], Barbara Roda [a,c], Andrea Zattoni [a,c], Pierluigi Reschiglian [a,c], Alberto Danielli[b], Matteo Calvaresi[a], Valentina Marassi[a,c]

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Bacteriophages are viruses that infect bacteria but not eukaryotic cells. They are biocompatible, uniform in size and morphology, and stable across various conditions. They are gaining attention as a protein-based platform for nanostructured materials. Phages are innovative, safe delivery vectors, with flexible genetic engineering for targeting. Compared to common drug delivery systems, they offer high target avidity and numerous functionalization sites, leading to increased loading capacity and multivalency [1]. In particular, the possibility to conjugate phages with suitable sensitizers conferring photokilling activity makes them interesting vectors for photodynamic therapy applications. From production to clinical testing, fast and powerful screening tools to assess the quality of the synthesis as well as of the chemical functionalization and purification are required. However, most of the common approaches such as spectrophotometry, fluorimetry and SDS page are time consuming, not automatable, and even complex (SDS-PAGE). Techniques such as HPLC-MS and NMR are not suitable due to the complexity of the systems, providing results that are challenging to interpret. With the aim of fill this technological gap we exploited a Hollow Fiber Flow Field Flow Fractionation (HF5) multidetection (MD) platform to analyse different bacteriophages as well as the products resulting from their conjugation with a fluorophore and a photosensitizer for photodynamic therapy (PDT). Our semiautomatic method allowed in less than 40minutes a spectroscopical size and shape native characterization of the bacteriophages and provided quick information concerning the success of the conjugation and purification processes. In particular we observed a successful conjugation between bacteriophages and the fluorophore while no clear conjugation to the PDT photosensitizer has been detected, moreover we were also able identify impure samples still characterized by the presence of polyethylene glicole used as precipitating agent during the production of those systems. These results were coherent with the ones stemming from a traditional technique (SDS-Page). Overall, these findings indicate the key role of HF5-MD as the missing powerful tool for a fast quality control screening over the long production pipeline of those nanosystems.

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PF03 - Development of a Screen-Printed Electrochemical biosensor for organophosphates detection directly on fruit peels

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One of the greatest challenges for the future is represented by the conservation of global agricultural production, severely affected by the climate change and the exponential growth of the population. For this reason, it is necessary to implement early monitoring of the health conditions of crops through new technologies that allow sustainable development, as reported in the 2030 agenda established by the United Nations; in addition, as demonstrated by the Covid-19 pandemic, the necessity of portable and wearable solutions for analysing samples, from the medical to the environmental field, is growing day by day: an example is precision agriculture, where sensing devices are required to be in close contact with fruit, leaf or soil, lowering the risk of contamination, waste disposal, especially in remote areas. In this scenario, the use of (bio)sensors capable of detecting the state of health of crops has proved to be a useful strategy for optimizing agricultural production.[1] In this work, a miniaturized, electrochemical strip is developed in order to detect organophosphorus pesticides directly on fruits peels, only by scrubbing their surface. The system has been suitably modified by loading bio-hybrid nanosized probes (Prussian blue, carbon black, and butyrylcholinesterase enzyme) able to detect the amount of organophosphorus pesticide.[2] The portable system has been characterized by a low detection limit of 0.2 ppb in standard solution and it was successfully applied for in situ quantification of Dichlorvos deposed at different concentration on orange and apple peels; the quantification was performed only by scrubbing the surface of the peels with the modified sensor and proceeding with the electrochemical measurement, obtaining a limit of detection of 10 ppb for both the fruit peels tested. Results demonstrated the ability of these modified biosensors to be used as valuable tools for precision agriculture in decentralized monitoring settings.

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PF04 - Landfill waste fire: oxidative stress and elements accumulation in bees

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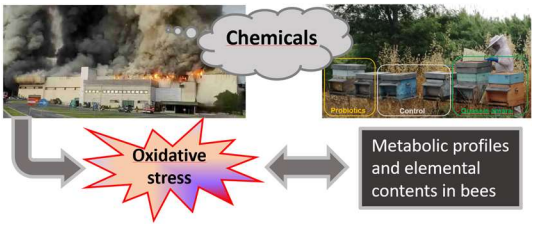
Landfill fires are relatively frequent incidents that can contribute significantly to air pollution and have environmental and health impacts (1). Various pollutants can be emitted during waste combustion, such as carbon monoxide and dioxide, nitrogen oxides, volatile organic compounds, persistent organic pollutants, aldehydes, and toxic or potentially toxic elements (1,2). Bees are important pollinating insects sensitive to environmental contaminants (3). Toxic elements, such as As, Cd, Hg, and Pb, can weaken their immune system and induce oxidative stress in these valuable insects (4,5). The present study aimed to evaluate the impact of air pollution from waste fires on bee health, in terms of oxidative stress and metal accumulation, as well as the possible beneficial effect of feeding probiotics and *Quassia amara* to bees. Six beehives were considered near the Malagrotta landfill, central Italy (41°51'49.9 N 12°19'46.5 E) before and after a landfill fire event on 15 June 2022. Bees, fed with different nutrients (placebo, probiotics, and *Q. amara*), were analyzed for elemental content by a quadrupole inductively coupled plasma mass spectrometry and cold vapor atomic fluorescence spectrometry (3,4), oxidative stress by hydrogen peroxide and protein carbonyl group contents (5), and metabolic profiles by ^{1}H-NMR (4). Compared with control bees, lower concentrations of As, B, Ba, Cd, Co, Fe, Li, Mn, Ni, Pb, Sn, Ti, and U were found in probiotic-fed bees, and Ba, Be, Cd, Co, Fe, Li, Mn, Sn, Ti, and U in *Q. amara*-fed bees, indicating a possible protective action of probiotics and medicinal plants against the accumulation of toxic or potentially toxic elements (3,4). The administration of probiotics and *Q. amara* to bees has also shown a protective effect against the oxidative stress caused by the fire of landfill waste (4). The comparison of the metabolic profiles through pre- and post-event PCA analyses showed that bees treated with different feeds react differently to the environmental event. The greatest differences in metabolic profiles are observed between the placebo-fed bees compared to the others. This study can help to understand how some stress factors can affect the health of bees and to take measures to protect these precious insects.



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PF05 - Hempseed-derived peptide mixtures with multifunctional properties for metabolic syndrome prevention

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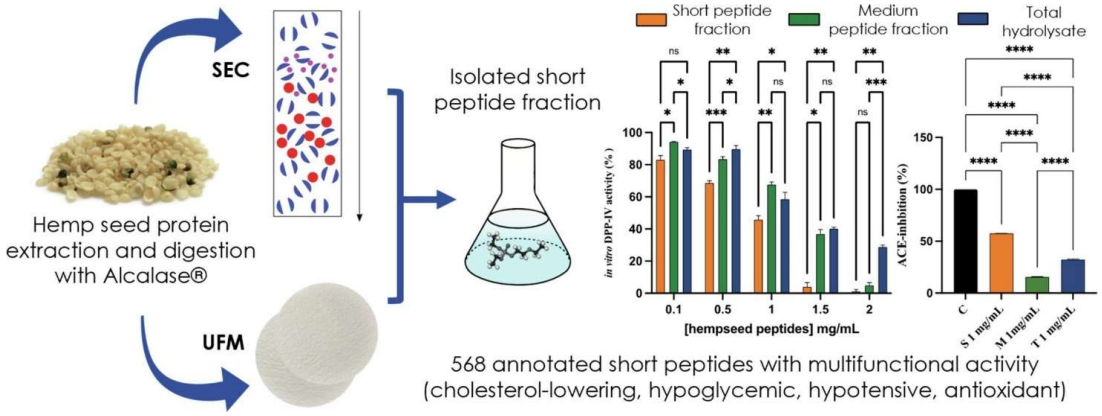
Metabolic syndrome (MetS) constitutes a grouping of conditions that collectively heighten the likelihood of cardiovascular disease, type 2 diabetes, and stroke, which are the primary contributors to mortality [1]. Addressing MetS typically requires a combined approach involving lifestyle adjustments and ongoing medication, often involving expensive pharmaceuticals [2]. Given this, exploring bioactive compounds presents a promising avenue for crafting innovative functional foods aimed at MetS prevention.
In this work [3], a dedicated analytical platform was devised to isolate and characterize short peptide sequences derived from hemp seed proteins. The Alcalase® hydrolysate underwent peptide separation using two distinct methods: size exclusion chromatography (SEC) and membrane ultrafiltration (UFM). SEC yielded two fractions containing medium and short-chain peptides, while UFM, employing a molecular cut-off <1kDa, produced a single fraction comprising solely a mixture of short-chain peptides. The fraction containing short-chain peptides underwent analysis through a suspect screening untargeted approach using ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS), whereas a conventional proteomics experiment utilizing nanoLC-HRMS was employed to analyze the fraction containing medium-chain peptides. Following meticulous manual validation, 559 short peptides and 557 peptides were tentatively identified in hemp seed by SEC and UFM, respectively.
The biological activity of the hemp seed hydrolysates was evaluated for bioactivities directly associated with metabolic syndrome. The results indicated that the short-chain peptide mixture exhibited approximately three-fold greater activity compared to the medium-chain peptide mixture and total hydrolysate in inhibiting angiotensin-converting enzyme. Additionally, the short-chain peptide mixture demonstrated twice the activity as a dipeptidyl peptidase IV inhibitor and showed a two-fold enhancement in modulating the cholesterol metabolism pathway, particularly through its impact on the low-density lipoprotein receptor.



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PF06 - Lab-made fructose amperometric third-generation biosensors based on laser-patterned reduced graphene oxide films

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In this presentation, a strategy to transfer laser-produced reduced graphene oxide (rGO) onto flexible polymers is proposed, proving for the first time its effectiveness for direct bioelectrocatalysis.
Laser-patterned rGO films were transferred onto three flexible polymeric substrates (PET, PVC, and EVA) by a here-proposed strategy named "roll-to-roll thermal stamping", taking advantage of a simple office-grade thermal laminator. The obtained films were compared with the native rGO (untransferred) via depth morpho-chemical and electrochemical characterizations. The rGO transferred onto plastic substrates presents morphology and chemical characteristics similar to native laser-produced rGO. Given these properties, the rGO-films were coupled to the enzyme fructose dehydrogenase (FDH) and their ability to give direct electron transfer (DET) has been carefully investigated. Particularly, the DET reaction between FDH and rGO-based transducers was investigated evaluating the influence of the enzyme unit amount on the catalytic process.
The most performing DET-type biosensor was obtained by transferring rGO on PET, further modified with 15 mU of FDH. This biosensor demonstrated superior performance, thanks to its preserved rGO features and reduced capacitive current, proving reproducible (RSD = 3%, n=3) and competitive electro-analytical features (LOD = 0.2 μM) for the determination of D-fructose. Noteworthy, the enzyme units required to reach the highest catalytic currents resulted significantly lower (20-times less) compared to biosensors based on commercial electrodes, while the obtained performances resulted superior to the majority of FDH-biosensors. Eventually, the proposed biosensor was successfully used to monitor fructose evolution in bananas during post-harvest ripening (recoveries 109-90 %; RSD ≤ 7%, n= 3).
This work demonstrates how laser-obtained rGO films can be transferred onto different flexible substrates using simple equipment, allowing the manufacturing of complete biosensors with fascinating features.

Acknowledgments
The authors acknowledge financial support of MUR PRIN 2022 Project No. 2022T2E7NT_01, CUP C53D23003850006, under the National Recovery and Resilience Plan (NRRP), Mission 4 Component C2 Investment 1.1—MUR call No. 104 on 2 February 2022, funded by the European Union—NextGenerationEU

PF07 - A comparison between HF5 and SEC in the isolation of extracellular vesicles from human plasma

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§ Contributed equally

Extracellular vesicles (EVs) are spherical nanoparticles secreted by cells in the extracellular environment, therefore they can be found in many body fluids. These vesicles are involved in several processes, like intracellular communication and immune responses. In particular, they play a role in tumor growth and resistance to therapy. For these reasons they are raising a growing attention both as diagnostic analytes and therapeutic tools. Isolating and characterizing EVs from body fluids is challenging due to their low concentration and high heterogeneity. The isolation techniques usually exploited (ultracentrifugation, size-exclusion chromatography (SEC), ultrafiltration) present many drawbacks like the requirement of large amount of sample, and low efficiency in time and purity. They also risk compromising the integrity of the vesicles with consequent loss of biological activity hence not allowing a characterization in native conditions.

We present an approach to isolate EVs from low amount of plasma (60 µl/subject) in physiological and native conditions exploiting a Hollow Fiber Flow Field Flow Fractionation Multidetection (HF5-MD) platform. The described platform was employed to separate and analyse plasma samples from healthy donors. The developed method allowed for a fast (<25min) separation, based on hydrodynamic size, and a simultaneous spectroscopic, dimensional and shape characterization of the analytes, then enabling the downstream collection of EV-enriched fractions, with minimal dilution. In addition, EVs from the same plasma samples were isolated through SEC with collection of EV-enriched fractions.

To evaluate and compare the performance of the innovative HF5-MD system with the EV isolation protocol employing SEC, the fractions resulting from both separations were ultrafiltered as a pre-concentration step and then again analysed with HF5-MD platform, to estimate the isolation efficacy and their differences in content/size distribution. This allowed to point out the superiority of HF5-MD approach in terms of analysis time and low amount of plasma and to demonstrate the efficient isolation of EVs with minimal pressure and damage to vesicles. The obtained results suggest that the HF5-MD platform could be a great approach to isolate biologically active EVs from plasma and therefore to understand the role of circulating EVs in tumour and healthy microenvironment.

PF08 - Rapid and green discrimination of bovine milk according to fat content, thermal treatment, brand and manufacturer via colloidal fingerprinting

Nicholas Kassouf[a], Alessandro Zappi[a], Andrea Zattoni[a,b], Stefano Giordani[a], Barbara Roda[a,b], Dora Meluccia[a], Valentina Marassi[a,b]

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Addressing food safety and detecting food fraud while fulfilling greenness requisites for analysis is a challenging but necessary task. The use of sustainable techniques, with limited pretreatment, non-toxic chemicals, high throughput results, is recommended. A combination of Field Flow Fractionation (FFF), working in saline carrier and with minimal preprocessing, and chemometrics was for the first time applied to bovine milk grouping.

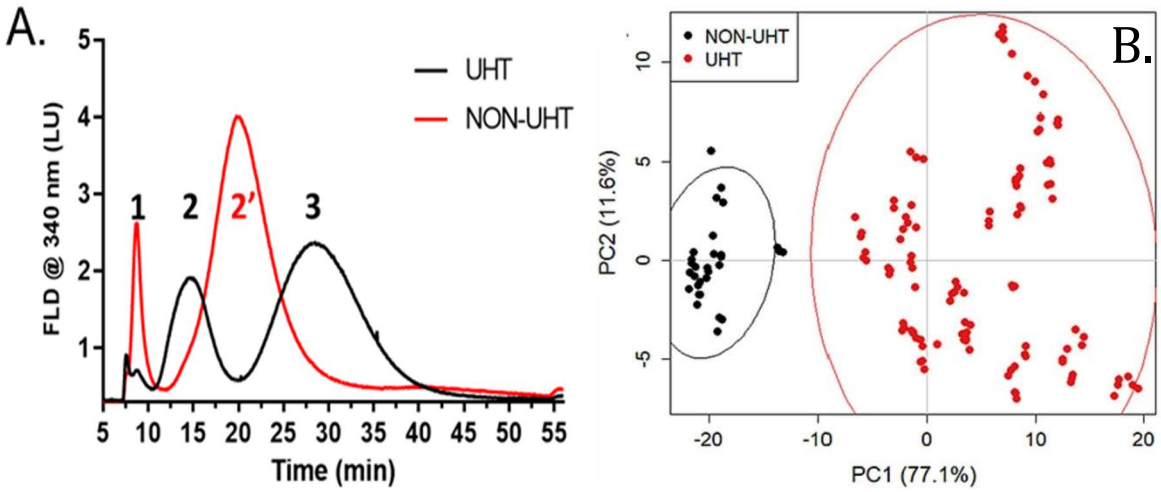

Figure A shows the difference between the colloidal fraction of UHT and not UHT milk. Figure B shows the grouping of these two classes. A set of 50 bovine milk samples was analyzed: a single analysis yielded a characteristic multidimensional colloidal dataset, that once processed with multivariate tools allowed simultaneously for different discriminations: fat content, thermal treatment, brand and manufacturing plant. The analytical methodology is fast, green, simple, and inexpensive and could offer great help in the field of quality control and fraud identification[1]. This work represents also the first attempt to identify milk sub-typologies based on colloidal profiles, and the most complete study concerning multivariate analysis of FFF fingerprint.

PF09 - Fast synthesis of molecularly imprinted polymers for selective extraction of phomopsins in lupin samples by UPLC-MS/MS analysis

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Phomopsins (PHOs) are a group of toxins generated by the fungus *Phomosis Leptostromiformis*, commonly found in legumes, particularly in grains like lupine. Their presence in legumes can result in a condition known as lupinosis in both humans and animals, characterized by symptoms such as dizziness and significant liver toxicity [1]. With the increasing use of sweet lupins in food and feed production, concerns have emerged regarding fungal contamination and the consequent existence of PHOs. Among these toxins, in the literature is reported as only Phomopsin A (PHO-A) is the most abundant in foods than other forms; PHO-A has been regulated in Australia, the maximum regulatory level was set at 5 µg/kg in lupine seeds and related products. Various methodologies are employed to qualitatively and quantitatively detect PHO-A in food and feed. Among different approach, high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) stands out as the most effective analytical technique for the sensitive and selective identification of PHO-A. For this reason, given the complexities of the matrices involved, a specialized cleanup approach was necessary to eliminate interference as conventional cleanup methods such as Solid Phase Extraction (SPE), µSPE, and immunoaffinity columns. In recent years, researchers introduced molecularly imprinted polymers (MIPs) extraction for target contaminants. Anyway the producing of MIPs usually required large time consuming protocol, so it is difficult to apply on routine analysis. In this work, an alternative strategy for fast MIPs synthesis was proposed and apply to the PHOs toxin, such as PHO-A, PHO-B, PHO-E and PHO-P in lupin samples detected by multiple reaction monitoring (MRM) coupled with enhanced product ion (EPI) scan in an information-dependent acquisition (IDA) experiment. MIPs were synthesized via a low-cost and rapid (5 min) sonochemical free-radical polymerization, using hexapeptide-11 as a dummy template. To this aim, we tested acrylamide (AA), methacrylic acid (MAA), methacrylamide (MMA), and methacrylic acid +2-vinylpyridine (MAA-VP) as monomers, using ethylene glycol dimethacrylate (EGDMA) as the cross-linker and 2,2 azobisisobutyronitrile (AIBN) as the initiator. MAA-VP-MIP based in solid phase extraction (SPE) yielded the best result than the others. For this reason, the performance of MAA-VP-MIP-SPE obtaining satisfactory recovery rates (80–85%) and high reproducibility (RSD < 6%) for PHO-A, validated following SANTE guidelines. Proposed MIP was tested on lupin samples contaminated by the fungus *Phomosis Leptostromiformis* proving the selective extraction of PHO-A and other toxin forms by their putative identification.

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PF10 - Exploring innovative solutions in Point-of-Care diagnostics: unveiling the potential of gold silica nanoparticles and molecularly imprinted nano polymers

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In the realm of immunodiagnostics, antibodies have long been regarded as the gold standard for molecular recognition, playing a pivotal role in various applications [1]. However, the challenges associated with antibody production, such as high cost, batch-to-batch variability, and ethical concerns, have spurred exploration into alternative molecular recognition systems. Among these alternatives, molecularly imprinted nano polymers (nMIPs) have emerged as promising biomimetic materials with the potential to overcome these limitations. Unlike antibodies, nMIPs are synthetic, highly cross-linked materials designed with cavities that exhibit selective molecular recognition [2]. Although the current state-of-the-art applications predominantly employ nMIPs for tasks such as molecular separation, sensing, and controlled release, their adoption as primary recognition elements in diagnostics remains limited. Notably, the use of nMIPs in lateral flow assay (LFA) platforms for diagnostics is still in its nascent stage. While LFA has become a widespread and user-friendly tool for rapid diagnostics, traditional antibodies continue to dominate as the recognition element. The potential of nMIPs in LFA, with their advantages of stability, reproducibility, and cost-effectiveness, is an area ripe for exploration. [4]
This study presents a novel approach to lateral flow immunoassay (LFIA) diagnostics, aiming to reshape point-of-care testing (POCT) by minimizing reliance on biomolecules. The innovation centers around integrating nMIPs with core-shell gold silica nanoparticles (Au@SiO2), a strategically chosen marker widely utilized in colorimetric LFIA [3]. nMIPs, emerging as promising biomimetic materials, were synthesized to selectively target IgG bovine, establishing a molecularly imprinted framework. Functionality and binding properties of the composite nanomaterial were investigated through batch rebinding to estimate affinity and binding capacity. Characterization studies, including transmission electron microscopy (TEM) and dynamic light scattering (DLS), validated the structural integrity of the material. To enhance their functionality, the nMIPs were conjugated with core-shell gold silica nanoparticles, ensuring a synergistic integration of their selective recognition properties with the optical advantages of gold nanoparticles. Experiments were meticulously designed to evaluate the applicability of the new hybrid material in the LFIA diagnostic platform. Key objectives included establishing the visual limit of detection, unraveling the dynamic interaction between nMIP and IgG, and conducting selectivity studies were crucial to defining the potential of this innovative detection system.
This proof-of-concept challenges conventional LFIA paradigms, showcasing the power of nMIP for the advancement of synthetic receptors in rapid bioanalytical tests. This approach paves the way for a future where LFIA becomes more accessible, robust, biomolecule-free, and user-friendly, signaling a significant advancement in rapid screening tests.

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PF11 - CRISPR/Cas-based cell-free biosensor for antibody detection

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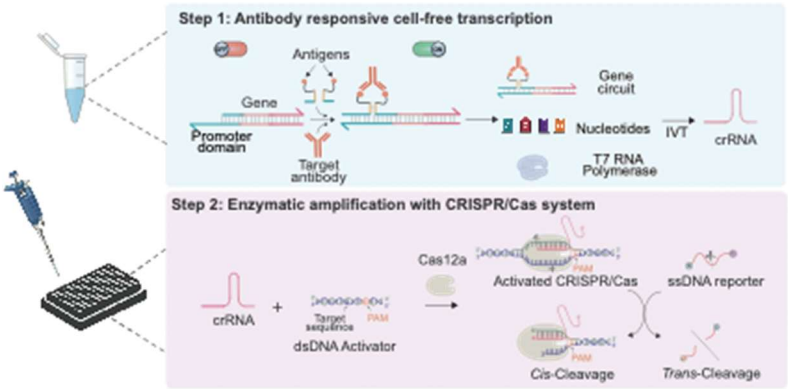
Detection of specific antibodies and other protein biomarkers plays a crucial role in disease diagnosis, infections, and other pathologies such as cancer and autoimmune diseases. Among the variety of nucleic acid-based sensors developed so far, cell-free biosensors, by exploiting the enzyme machinery of RNA Polymerase, provide advantages in terms of sensitivity and specificity over the existing diagnostics methods for the detection of a wide range of targets.[2] In recent years, several cell-free biosensors for the detection of different target, including antibodies, proteins and small molecules have been reported to date.[3] Despite this, most of the cell-free biosensors for the detection of antibodies do not reach the level of sensitivity typical of the early stages of infections. Thus motivated, we report here a cell-free biosensor for antibody detection that takes advantage of CRISPR/Cas system. We have designed a synthetic gene to contain the transcriptional sequence of crRNA for Cas enzyme and an incomplete T7 RNA polymerase promoter domain that prevents efficient binding of the enzyme. The binding of the specific antibody to a pair of antigen-conjugated DNA strands triggers the reconstitution of the T7 RNA polymerase promoter domain and thus the *in-vitro* transcription of the crRNA. The transcribed crRNA, by binding its activator strand, induces the activation of the cleavage activity of Cas enzyme. A fluorophore/quencher-labelled reporter strand that acts as substrate for Cas enzyme provides information about the presence and concentration of the target antibody. The developed cell-free biosensor, by coupling the advantageous features of DNA-based sensors (i.e., high programmability) with those of cell-free and Cas-based diagnostic methods (i.e., high sensitivity and specificity) allows the sensitive (low picomolar detection limit), specific (no signal is observed in the presence of non-specific antibodies) and selective (the system can be employed in complex media, including 50% blood serum) detection of antibodies. Thanks to the programmable nature of the sensing platform, the method can be adapted to different target molecules: we demonstrate the detection of different antibodies, also clinically relevant (i.e., Anti-MUC1 antibody; Cetuximab) and protein (i.e., Mucin1 and EGFR). Given all these advantages, the CRISPR/Cas-based cell-free biosensors may be of utility for different applications including point-of-care diagnostics.



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PF12 - Development of an optimised icIEF method for harmonising Quality Control of Monoclonal Antibodies

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Today, more than 75 monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs) have been approved as biotherapeutic products by the European Medicines Agency (EMA) and the Food and Drug Administration (FDA). mAbs are becoming a commonly used pharmacological therapy for several oncological, inflammatory, and autoimmune diseases principally due to their high specificity in target antigen binding, reducing the need for frequent dosing[1]. During their production in cell culture and storage, mAbs are prone to Post-Translational Modifications (PTMs) such as deamidation, glycosylation or oxidation which produce charge heterogeneities. Since some charged-based variants can have an impact on pharmacokinetics, biological activity, and long-term storage, charge heterogeneities are considered a Critical Quality Attribute (CQA) by regulatory authorities which must be monitored and evaluated during biotherapeutics life cycle. This quality control (QC) approach is commonly achieved using ion exchange chromatography (IEC) or isoelectric focusing (IEF) techniques.[1]
The imaged-capillary IEF (icIEF) technique has gained wide applications in biopharmaceutical QC due to its improved sensitivity and robustness. Compared to conventional cIEF, icIEF allows faster separation, higher resolution, and a simpler method development procedure. These advantages offer in the regulatory context a potential analytical platform for an effective detection of several PTMs-related charge-isoforms. To accomplish this, icIEF utilizes whole capillary imaging at 280nm - without a mobilisation step - to separate analytes and allowing the determination of the isoelectric point (pI).[2,3.]
To strengthen their independence in QC activities, Official Medicines Control Laboratories (OMCLs) are currently involved in the effort to harmonize analytical procedures, trying to develop less product-specific method which still ensure their performance and reliability.[4] In Ph. Eur. is published a general chapter which presents a horizontal method - involving an overlapping of broad and narrow range carrier ampholytes - to analyse mAbs with icIEF technique.
The pI value is not typically presented in the biotherapeutics release specifications as an identity parameter because of its variability depending on the instrument employed to conduct the analytical test.[4] Instead, the comparison of the Drug Product (DP) isoform pattern is lead with the one of the Reference Standard (RS). However, in certain situations – for example, the fight against counterfeit drugs - when a RS for comparison is unavailable, the measure of an accurate, precise, and universal pI value can be crucial in distinguishing one biotherapeutic from another.
Firstly, we tested this Ph. Eur. method on a panel of therapeutic mAbs observing a good resolution but a poor accuracy of the measured pI values, according to those available on the literature.[5] Therefore starting from that model we decided to develop a new independent, transversal, and effective icIEF analytical method tuning its fundamental parameters and exploiting two types of contiguous narrow range ampholytes.
As evidenced in some preliminary results, we are confident of the achievement of a method with an enhanced accuracy in the measure of the pI value of mAbs, thanks to a better system calibration linearity.

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Comunicazioni Poster

P01 - Improving the sensitivity and the cost-effectiveness of a competitive visual lateral flow immunoassay through serial designs of experiments

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The colorimetric Lateral Flow Immunoassay (LFIA) based on the visual read-out interpretation is the most diffused point-of-care test. Despite its strong field establishment in the biosensing panorama, a drawback is the low sensitivity. Nevertheless, a systematic unbiased optimisation of the experimental conditions is rarely performed to maximise the sensitivity of the available material for an LFIA device development. The need of many experiments and bioreagents (antibodies and antigens) consumption discourages the exhaustive exploration of the influent variables. On the other hand, by means of an appropriate strategy, the number of required experiments can be restricted. In fact, for some non-competitive LFIA this approach led to a significant increase in the sensitivity [1]. In this work we treasured the experience gained over the experimental-design-based optimisation of non-competitive LFIAs for optimizing a competitive LFIA. We aimed at improving the sensitivity of a competitive LFIA for the detection of cortisol. The sensitive detection of cortisol is clinically relevant (3.6, 2.5 ng/mL morning and afternoon, respectively, basal in saliva, higher with Cushing syndrome or stress related diseases[2,3]). High sensitivity is required especially for challenging real samples (such as saliva) requiring dilution to solve matrix effects. The LFIA includes the antigen (cortisol-BSA) spotted onto the test line, a labelled monoclonal antibody as the signal reporter and ruby red gold nanoparticles as the label (mAb_AuNP). Our optimization strategy is composed by four actions: Start, Shift, Sharpen, Stop (4S). The idea underlying the 4S process was the execution and the elaboration of one or more progressive designs of experiments (DoE), up to achieving the maximal sensitivity with the available materials. The 4S can be resumed as follows: the first DoE should be designed for the exploration of the influent variables into a reasonable interval (Start); after the elaboration of the data, the operator must decide whether: i) not furtherly proceeding (Stop) in case of satisfying sensitivity; ii) proceeding with another exploration with different intervals of the variables (Shift) in case of insufficient sensitivity; iii) zooming in a narrower interval, even tightening the criteria (Sharpen) in case of satisfying sensitivity, to see if the sensitivity can be furtherly improved beyond the initial goal. Taking inspiration from the ELISA theory [4], we considered, as the variables, the amount of mAb conjugated with gold nanoparticles (antibody, Ab), the amount of gold conjugate in the assay (optical density, OD), the concentration of the antigen on the test line (T). The number of experiments was defined by using D-optimal algorithm. A key role for making proper decisions along the 4S, had the definition of a convenient figure-of-merit for the evaluation of each DoE. Therefore, each combination was tested in the absence (NEG, true if > 80 a.u., false otherwise) and in the presence of cortisol (POS, true if < 15 a.u., false otherwise). The colour intensity on the test line was acquired with a scanner and the image digitally processed. The presence of a neighbourhood of combination of variables providing TP+TN (true positive + true negative) response on the experiment was used as an indicator of achieved goal and the decision about being satisfied (stop) or pursuing a higher sensitivity tightening the criteria (Sharpen) can be freely rated. In the absence of these sectors, information could be acquired by observing the trend of the response surface, in order to design a different experimental design in another experimental space (shift). In our 4S path, we performed the Start DoE, obtaining a very thin neighbourhood of achieved goal, then moved outside for the second DoE (shift) obtaining TP+TN neighbourhoods, into which we tightened the criteria for POS (Sharpen), obtaining the maximal sensitivity with two conditions (Stop). At the end of the optimization process standard curves were performed and the starting and optimized LFIAs were compared in terms of analytical features and reagent consumption. The differently optimized conditions were named LFIA 0 (unoptimized), LFIA 1 (optimized after 1 DoE, 18 experiments), LFIA 2a, and LFIA 2b (optimized after 3 DoEs, 34 experiments). The analytical performances were compared in terms of sensitivity and reagents consumption. In the LFIA 0, 10µg/mL of cortisol limitedly inhibited the test line intensity (-28.6%) and a reliable visual limit of detection was not determined, with a consumption of reagents of 480ng/test of mAb and 400ng/test of antigen. After 1 DoE, the obtained LFIA 1 showed a remarkably higher half maximal inhibition capacity (IC_{50}: 1.9 ± 0.4 ng/mL), a dynamic range of 6.6 - 0.6 ng/mL and a calculated vLOD (concentration for test line intensity = 15 a.u.) of 20.5 ± 1.0 ng/mL. The reagent consumption was 20.6 ng/test of antibody and 80 ng/test of antigen. The LFIA 2a represents another significative improvement compared with the LFIA 1 (IC_{50}: 0.5 ± 0.1 ng/mL), the dynamic interval of 1.9 - 0.1 ng/mL and vLOD of 2.2 ± 0.1 ng/mL. The reagent consumption was 3.6 ng/test of antibody and 20 ng/test of competitor/antigen. The LFIA 2b showed similar results compared with the ones obtained with the LFIA 2a. In conclusion, through the 4S progression, including 3 experimental designs, 34 experiments, allowed for a sensitivity improvement by a factor >5000 compared to the unoptimized condition, with a 37.3-fold lower overall bioreagents consumption, using the same materials and immunoreagents. In addition, the progression allowed us for the identification of the "directions", renouncing to futile experiments in redundant experimental areas. This resulted, also, in time and cost effectiveness of the optimizing process itself. This demonstrates the power of a systematic unbiased approach.

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P02 - Elemental and fatty acid profiles in volcanic marine environments: the case study of Panarea Island (Italy)

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Volcanic islands are ever-changing landscapes, shaped by ongoing geological processes. Understanding the chemical composition of marine environments in volcanic settings is essential for comprehending their ecological complexities and safeguarding their conservation amidst environmental changes. As a part of the Aeolian archipelago near Sicily (Italy), Panarea Island is characterized by active volcanic vents. In this study, the elemental composition of marine sediments and organisms (*Posidonia oceanica* and *Caryophyllia inornata*) from two different sites (Site A and Site B) was studied. At Site A, two sites were examined: site 1, located outside a crateric zone, where marine sediment and specimens of *P. oceanica* were sampled, and site 4, situated on the crater's border 35 meters away from site 1, where only marine sediment was sampled. At Site B, site 1 was positioned outside a cave, where samples of coral *C. inornata* and marine sediment were collected, while site 4 was inside the cave, 5 meters away from site 1, with similar sample types. Utilizing techniques such as TDA-AAS and ICP-OES, we analyzed samples to quantify the presence of 21 elements. Fatty acids (FAs) in biota were also investigated as proxies for environmental changes. Gravimetric analysis coupled with GC-MS allowed to discern the array of FAs present in biotic samples, both as percentage on total FA and percentage on sample dry weight. Our findings indicate that samples naturally exhibited distinct elemental fingerprints based on their type, whether sediment, *P. oceanica* or *C. inornata* specimens (**Figure 1a**). Considering sediments, patterns indicative of unique geochemical signature shaped by proximity to volcanic vents were revealed. Sediments from Site A site 1 exhibited an enrichment of Mn, Ag, Pb, Zn and Co. In contrast, Site A site 4 showed elevated levels of Ca, Al, Hg and Si. Meanwhile, samples from Site B revealed distinctive compositions characterized by heightened concentrations of As, Mg, Na, Fe and Cd. Coral samples from Site A site 1 exhibited higher lipid content, with significant representation of 18:1n-9, while corals from site 4 displayed elevated levels of saturated fatty acids 16:0 and 18:0. *P. oceanica* samples showed higher lipid content and greater proportions of 18:2n-6 and 18:3n-3 compared to corals (**Figure 1b**).

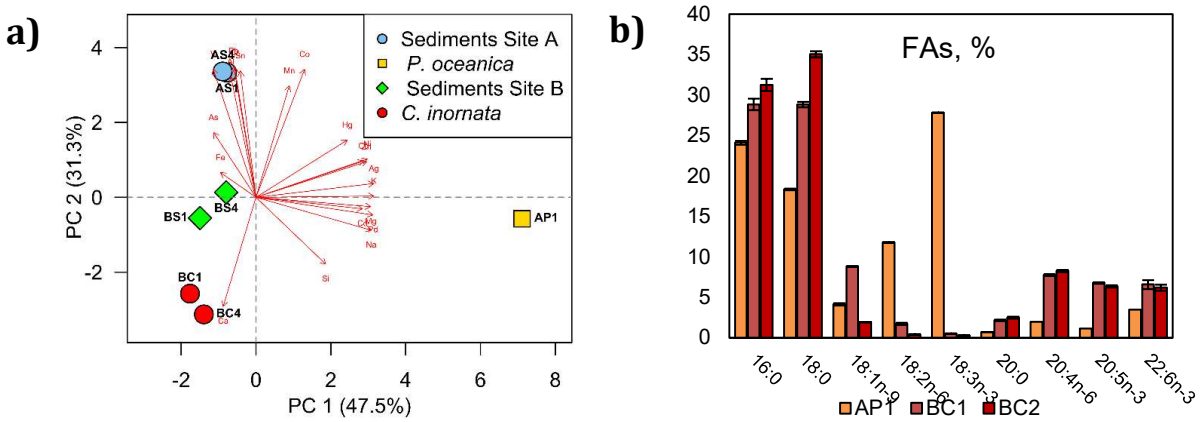

Figure 1. PC1 vs PC2 biplot of elemental composition (a) and major FAs profile in biotic samples (b). In sample names, the first letter stands for the location (A: Site A and B: Site B), the second for the type (S: sediments, C: *C. inornata* and P: *P. oceanica*) and the final number for the site (1: site 1 and 4: site 4).

P03 - Fast Sonochemical Synthesis of Molecularly Imprinted Polymers for Selective Contaminant Detection in Food

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High-power ultrasound has recently emerged as a captivating strategy to synthesize nanomaterials and polymers, allowing a significant reduction in synthesis time compared to conventional techniques. In the framework of the increasing need for food safety, this research focuses on the rapid preparation of Molecularly Imprinted Polymers (MIP) exploiting high-power ultrasounds.
MIP synthesized in 5 min for maleic hydrazide (an herbicide) coupled with an electrochemical determination will be presented. The MIP-extraction/clean-up and electrochemical determination using carbon black functionalized screen-printed electrodes allowed maleic hydrazide selective and sensitive determination (LOD = 40 ppb) in food samples (potatoes, onions, and garlic) revealing reproducible data (RSD ≤ 6%, n = 3) [1].
An electrochemical sensor for Citrinin (CIT) incorporating graphene nanoflakes (GF) produced through fast solvent-free water-phase exfoliation will be also presented. The GF-SC sensor, combined with a MIP-based selective extraction/clean-up allowed the successful detection of CIT in complex food samples; also in this case MIP was produced in a few minutes using a rapid sonochemical approach. The GF-SC sensor exhibits superior sensing ability for CIT compared to other carbon nanomaterials, enabling reproducible (RSD = 4%, n = 8) determination below the maximum residual limit in food (LOD = 5 ppb). The GF-SC sensor, combined with MIP, was able to successfully analyze CIT in complex food samples, demonstrating a strong correlation with LC-MS/MS used as a reference method [2].
Eventually, MIP for aflatoxins (AFB1, AFB2, AFG1, and AFG2) was proposed, the MIP was synthesized in 5 min using a sonochemical polymerization approach which did not require radical initiators; the MIP was applied for the extraction of aflatoxin in 17 dietary supplements. MIP-based solid-phase extraction results in an alternative and selective approach more affordable compared to classical immunoaffinity extraction columns [3].

Acknowledgments
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P04 - Cotton Threads Bio-Chemiluminescent Devices For Sustainable And Accessible Point-Of-Care

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Microfluidic thread-based analytical devices (μTADs) are emerging as a new attractive platform for analytical assays due to their advantages in point-of-care (POC) settings such as their large availability, their light weight as well as their low cost. Generally, thin strands of cotton, nylon, wool or other fibers are used for μTADs whose wicking property facilitates the fluidic flow without using external pumping system (Weng et al., 2019). We report a proof-of-principle application of bio-chemiluminescence biosensing on cotton threads (figure 1a). Bioluminescence (BL), naturally occurring in some living organisms (e.g. fireflies), takes place thanks to the oxidation of luciferin catalysed by an enzyme, called luciferase, in presence of molecular oxygen to form an excited-state species that emits light. We first designed a BL thread biosensor in which luciferin and luciferase are immobilized onto two separate threads after twisted together to trigger the reaction. This spatial separation of the light-emitting molecules and of the enzymes increases the shelf-life of the device and the possibility of quantifying different analytes in a small-volume sample (2 μL) in short time (5-10 min). The preliminary tests were performed for detecting ATP, used as an indicator for microbial contaminants in food or hygiene monitoring (Calabretta et al., 2020). In parallel, a thread-based chemiluminescent (CL) device which, to the best of our knowledge, has not been reported in literature yet, was tested. In this case, preliminary tests were performed exploiting the enhanced luminol/H_2O_2/horseradish peroxidase (HRP) CL system (figure 1b), which can be adapted for different diagnostic applications such as the monitoring of the lactate levels for sport medicine (Roda et al., 2014). To prompt future applications in point-of-care and point-of need settings we implemented smartphone detection for easy monitoring of the thread-based devices. The camera embedded in smartphones, in fact, gave the possibility to acquire different pictures with sufficient level of sensitivity and to store the analyte values for further data elaboration, providing the end user with a simple and quantitative readout.

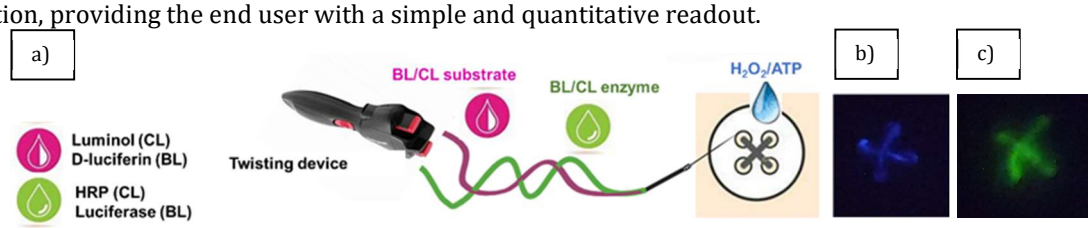

Figure 1. a) Schematic representation of the optimized thread-based bio-chemiluminescent biosensor. b) and c) Respectively, pictures of luminol/H_2O_2/horseradish peroxidase (HRP) CL system and of luciferin/ATP/luciferase BL system obtained with OnePlus 5 smartphone camera at 3200 ISO settings (30-s acquisition time).

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P05 - “One Health” strategy, emerging pollutants and green pharmacy. A new UHPLC-qToF quantitative method to study ready biodegradation of therapeutic products excipients

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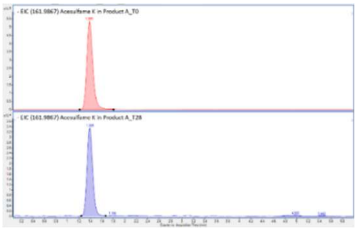
Environmental pollution is increasing day by day and imposing severe and irreversible damage to the world. Pharmaceuticals and personal care products (PPCPs) contribute to increase the level of environmental pollution due to the presence of active pharmaceutical ingredients and additives, such as artificial sweeteners and dyes, which are currently recognized as emerging contaminants of the environment. Biodegradation is the process defined as a conversion of organic compounds generally into a nontoxic and environmentally acceptable products that are able to return to the life cycle. One of the main problems is that many of these artificial and synthetic compounds are not easily biodegradable and persistent. Among therapeutical products, those based on complex natural matrices, used all over the world to realize dietary supplements, traditional medicines and medical devices, due to their nature are unlikely to result in a significant risk to the environment. In view of the new European "one health" strategy a new culture at the level of physicians, pharmacists and patients must be promoted for a more conscious and environmentally sustainable use of therapeutic products. In a green pharmacy perspective, it is possible to undertake a wide spectrum of possible actions that could minimize the dispersion of PPCPs into the environment. An example is choosing to sell therapeutic products which, for the same effect, have a lower impact on the environment. Indeed, the risk-benefit assessment of pharmaceutical should be extended to the environment effects, because what we put into the environment can backfire on our health. One of the tests more used to evaluate biodegradation of pure chemical and pharmaceutical compounds is the ready biodegradability test (RBT). Currently, the RBTs protocols most used are published by OECD, comprising OECD 301 test series. RBTs are known to have a number of well-documented limitations. To overcome some of them, we proposed to implement these tests by testing complex formulation - as the formula can affect the biodegradation - and by adding accurate measurements based on UHPLC-qToF technique. Here, in this work, we evaluated the ready biodegradability of a commercial Vitamin C-based dietary supplement by the OECD 301 F protocol, which contained a series of synthetic ingredients. A specific quantitative UHPLC-qToF method was developed to quantify the residuals of Acesulfame K and Sucralose - two artificial sweeteners, ingredients of the tested product – at the end of the RBT. Each analytical standard was acquired in data dependent mode at three different collision energy. While the acquisition of the sample under investigation was performed in All-Ions mode using a fixed collision energy value. As possible to observe in **Figure 1** and **Figure 2**, it was evident at the end of the RBT that Acesulfame K and Sucralose, respectively, were significantly still present, confirming their resistance (recalcitrance) to biodegradation.



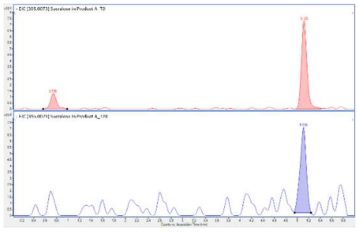


Figure 1. Acesulfame K. Extract ion chromatogram at the beginning (T0) and at the end (T28) of the RBT. **Figure 2.** Sucralose. Extract ion chromatogram at the beginning (T0) and at the end (T28) of the RBT.

In conclusion, the improvement of technology will progressively increase our ability to detect the potential effects of artificial substances in the environment. But to decrease pharmaceuticals form the environment a significant role is played by patients, physicians and pharmacists, the latter being central in the green pharmacy practice.

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P06 - Triplex-based CRISPR reaction network for highly specific detection of nucleic acids

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The extensive use of CRISPR technology in diagnostic applications has been fueled by the revelation of the collateral a-specific cleavage activities exhibited by CRISPR type V (Cas12) and type VI (Cas13) systems upon DNA/RNA binding. Specifically, Cas12, a RNA-guided CRISPR enzyme, integrates single- and double-stranded DNA target recognition and signal amplification within a singular system [1]. The activation of collateral cleavage occurs upon target binding and is accountable for signal generation. Generally, this is achieved by leveraging Cas12-mediated digestion of single-stranded DNA probes that are functionalized with a fluorophore-quencher pair, serving as fluorescence reporters [2]. Here, we describe a strategy for controlling Cas12a cleavage activity by using a rationally designed DNA-based hybridization network based on the formation of Clamp Triplex DNA and the presence of a bio-transducer element. Clamp Triplex are DNA probes that can recognize homopurine DNA/RNA target with superior specificity and affinity compared to standard linear or hairpin DNA probe. When a ssDNA target is present, the Clamp Triplex probe alters its conformation that is associated to a reaction network leading to Cas12a activation and fluorescence output (Figure 1). To do so, our detection strategy takes advantage of the development of a rationally-designed toehold switch DNA probe that we recently reported as a biotransducer element operating as an activator of Cas12a enzyme in response to external inputs. [2]
Our triplex-based reaction network allows us to overcome two hurdles that limit the application of CRISPR-Cas systems in molecular diagnostics: 1) it enables highly specific discrimination of a single base mutation within the target sequence while maintaining the same Limit of Detection (LOD) as standard CRISPR-Cas12-based detection systems; 2) it allows to detect different ssDNA/RNA targets without the need of changing the guide RNA, as it takes advantage of the biotransducer element.

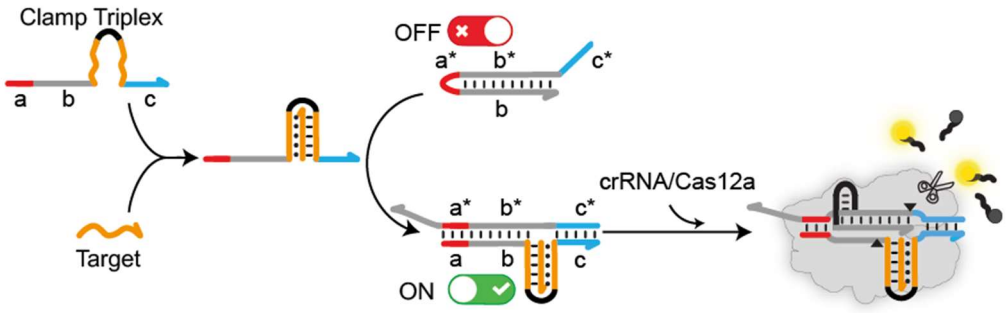

Figure 1: Schematic of the Triplex-based CRISPR reaction network.

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P07 - Colloidal carbon nanoparticles as label in lateral flow immunoassay

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In 2022, the global immunoassays market in term of revenue was estimated to be worth $40.2 billion, and almost the half ($20.5 billion) was accounted by the lateral flow immunoassay (LFIA)[1]. Considering the future of LFIA we can identify two clear trends, one is much more "ultra-sensitivity, more complex, integrated" and the other is "simple and convenient". Strategies to achieve the ultra-sensitivity in LFIA usually exploit fluorophores and chemiluminescent molecules but have disadvantages like requiring additional steps, additional chemicals and different detection methods that rely on the use of detectors or readers making the analysis more complicated, expensive, and time-consuming. To maintain the simplicity of the colorimetric-LFIA, in the last years huge efforts have been devoted to evaluating the use of alternative colorimetric label instead of the most used gold nanoparticles[2].

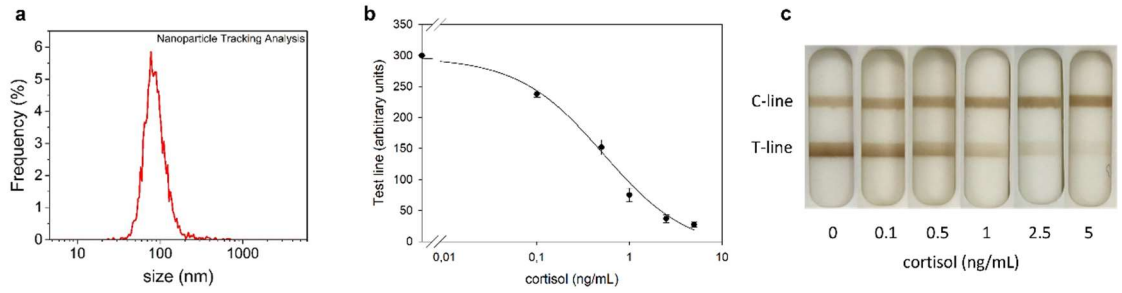

Figure 1 (a) carbon nanoparticles size distribution; (b) inhibition curve for cortisol; (c) strips image.

In this work, carbon nanoparticles (mean hydrodynamic diameter 91.6 ± 1.2 nm, figure 1a) obtained through a hydrothermal carbonization method of glucose[3], have been successfully applied as colorimetric label in LFIA. Preliminary results (figure 1b,c) showed interesting analytical performance for the cortisol detection, outlining the potential benefits of using non-metal NPs as label in LFIA.

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P08 - Electroanalytical platforms equipped with nanostructured sensing surfaces for (bio)analytical purposes produced via sustainable approaches

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Commercial electrochemical electrodes and sensors present limitations regarding their default design, rigid substrate, and analytical performance; recently, different low-cost strategies to fabricate (bio)devices have emerged as smart alternatives to conventional procedures. In particular, the manufacturing of tailored analytical devices integrating nanomaterials (NMs) based conductive films is still a hot topic; to overcome tedious, expensive, and not sustainable conventional fabrication techniques, several efforts are devoted to implementing effective and affordable technologies to produce nanostructured analytical devices. In this framework, low-cost and sustainable substrates/nanomaterials rappresent a captivating opportunity, and their assembling in complete (bio)analytical devices via emerging manufacturing technologies represents a a fervent research field.

This presentation will be focused on the use/production of functional NMs and NMs-films and their integration in completely lab-made analytical devices. An overview of NMs and nanostructured surface production, nano-/micro-architectures assembling, and their integration into freestanding conductive films, flexible sensors and biosensors, and all-in-one devices will be given. Particular attention will be paid to (i) sensing surfaces' nano-structuration via smart approaches and (ii) fabrication of disposable devices using low-cost/sustainable substrates (i.e., polymeric sheets, paper, etc.) and benchtop microfabrication technologies as stencil-printing, CO_2 laser scribing/cutting, CO_2 laser nanostructuration and inks activation/decoration, cutter-plotting, thermal-lamination, etc. [2-3]. The exploitability of the developed devices will be demonstrated for the analysis of agri-food quality and safety markers, and biological interest compounds in model solutions and real samples.

This presentation aims to prove how NMs and nanostructured surfaces produced/assembled using benchtop technologies can be easily integrated into designed on-demand sustainable (bio)analytical devices, manufactured with technologies within everyone's reach.

Acknowledgments

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P09 - Novel candidates for metal recognition in biological field: phenanthroline-coumarin ligands

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The recognition and quantitation of metal ions are of great importance in various fields, such as biomedical[1] and environmental ones.[2] The most common instrumental analytical techniques exhibit good detection limits, but they require expensive instrumentation, difficult and time-consuming sample preparation and, furthermore, their application it is not easy in diagnosis. Conversely, analytical methods based on fluorescence emission spectroscopy are relatively inexpensive, while providing high sensitivity, selectivity and very low detection limits (up to nanomolar concentration[3]). When designing fluorescent chemo-sensors, the analyte recognition mechanism needs to be considered. In fact, three behaviors could be expected: an increase of the emission intensity (turn-on mode), a reduction (quenching) of the emission intensity (turn-off mode) and, finally, a variation of the emission wavelength (ratiometric response). The turn-on and/or the ratiometric modes are generally preferred for analytical applications due to their enhanced sensitivity and lower detection limits.
Coumarins constitute an important class of candidate fluorophores, thanks to their ability of coordinating different metal ions. In addition, many coumarin-based molecules show low toxicity due to their natural origin.[4] Organic ligands that can form chelate rings with the metal ion of interest (polydentate ligands) , are promising candidates for the design of fluorescence chemo-sensors. For these reasons, we aimed to use the 1,10-phenanthroline (phen) as a building block to create new conjugated sensors with coumarins. The relative inertness of phen towards chemical reactions other than salt formation or chelation, is a significant asset in its analytical applications. Phen absorbs in the UV spectral region, showing emission at 360 nm with low fluorescence quantum yield ($F_F \leq 0.01$). This behavior makes phen an ideal component of a turn-on fluorescence sensor, being the resulting photophysical properties easily tunable according to the substituents in the sensor backbone.[5] Based on that, a fluorescence sensor based on joined coumarin and phenanthroline units is expected to be optimal candidate for the recognition of metal ions in different fields. For these reasons we prepared and characterized four new coumarin-phenanthroline molecules as potential fluorescence chemo-sensors for various metal ions (Figure 1).

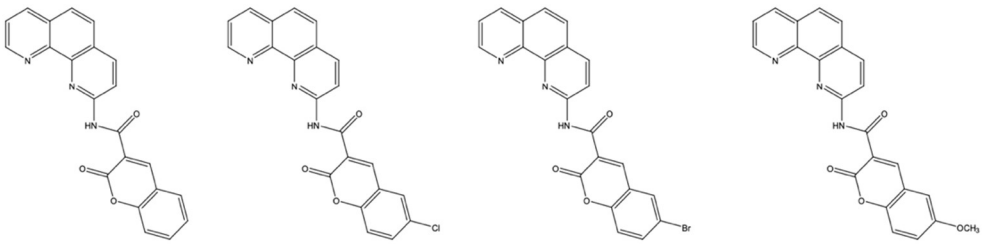

Figure 1. Synthesized ligands

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P10 - Valorization of Mediterranean biodiversity for medicinal purposes: a bioanalytical approach

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This work focuses on the study of two natural matrices: *Withania somnifera* (L.) dunal and fungi. *Withania somnifera* (WS, family *Solanaceae*), also known as Ashwagandha, is a perennial herb representing one of the most important plants used in Ayurvedic medicine (India's traditional medical system). It has, among the others, adaptogenic, anticancer and anti-inflammatory properties [1,2]. Its pharmacological effects are attributed to two main classes of compounds, i.e. withanolides (steroidal lactones) and alkaloids, found in roots and leaves. Although native in India, this species is widespread in many parts of the world and it has recently been identified also in Sardinia, where it represents an allochthonous species. Interestingly, WS specimens growing in Sardinia present a characteristic chemical profile that might imply a particular medicinal value. The main aim of this project is to compare the chemical profile of WS specimens growing in Sardinia with the different WS chemotypes. Moreover, we want to evaluate the potential benefits of the WS hydroponic cultivation (fig. 1), since this method offers more controlled environments, improving and speeding up the plant growth, reducing, at the same time, the use of soil and water and potentially influencing the chemical profile of the plant. As regards fungi, they are an exceptionally species-rich group with more than 150000 described species and a much higher estimated diversity. Although macrofungi have been the source of novel compounds suitable for application as pharmaceuticals, functional food supplements and cosmetics, they are still largely understudied, and thus, underused (3,4). The study of macrofungi could represent an attractive strategy to identify new valuable natural compounds and therapeutic agents. We selected two mushroom-forming fungi occurring in two different ecosystems of the Mediterranean area, to focus on the characterization of their chemical composition and biological activities. In particular, we aim to correlate their chemical composition with their antioxidant, antimicrobial, anti-inflammatory activity, enzymatic inhibition and antitumor properties. Different analytical techniques will be exploited, in particular GC-MS, HPLC-MS, UV-Vis, FT-IR and NMR spectroscopy, in order to identify the potential bioactive molecules. All the experimental data are expected to be analyzed by chemometric tools to find quantitative structure-activity relationships.

Fig 1. Hydroponic cultivation

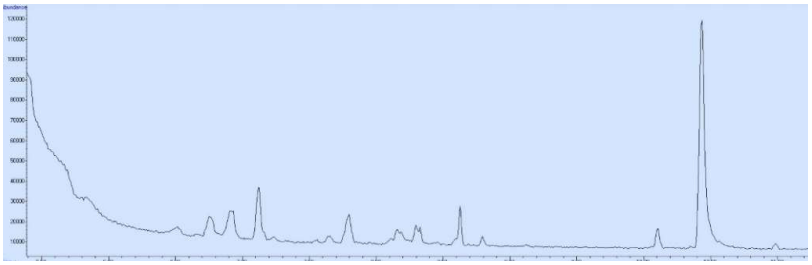

Fig 2. GC-MS chromatography of a fungal extract

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P11 - Poly-L-aminoacids-based Nanocomposites: Characterization and Application in Phthalates Biosensing

Giulia Selvolini[a], Costanza Scopetani[a], Agnese Bellabarba[b,c], Tania Martellini[a,d], Alessandra Cincinelli[a,d], Carlo Viti[b,c], Alessandra Adessi[b], Giovanna Marrazza[a]

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Mulching is an agricultural technique, aiming at improving crop quality, which often involves the use of polyethylene or other plastic-based films: these materials contain and release additives (e.g., phthalates, known as endocrine disruptors) that can enter the food webs *via* soil biota and crop plants, representing a threat for both human health and the terrestrial environment[1]. A smart and rapid sensing of these substances in soil and crops is thus urgently needed. In the last years, a consistent part of the research in sensing and biosensing field was also devoted to synthesize new nanocomposites to improve a sensor performance in terms of sensitivity, selectivity, and biocompatibility. Of those synthesized nanomaterials, conducting polymers composites have been widely used in the construction of sensor surfaces. Conducting polymers display advantages due to their charge transport properties: among them, a great attention was also attracted by polyaminoacids, which own many significant advantages such as non-toxicity, biocompatibility, biodegradability, electrochemical stability, electro-optical properties and a wide number of side functional groups. Moreover, the great potential of these biomaterials for self-assembling into ordered and stable conformations makes them suitable materials in biomimetic structures[2]. For a greater enhancement of the electrochemical sensor performance, different electrode systems have been developed based on surface modification with nanomaterials[3]. The synergy of multifunctional materials, recognition elements, and electrochemical methods is improving the selectivity, stability, and reproducibility, thus promoting the development of sensors for assays and bioassays. A small-scale experiment has been set up to reproduce the use of mulches under field conditions. A nanocomposite-based biosensing platform is developed in order to monitor in a fast and user-friendly way the release of contaminants from the mulches to soil and crop plants. Several types of conductive poly-L-aminoacids (poly-L-glycine, poly-L-cysteine, poly-L-aspartic acid, etc.) were used to obtain a nanopatterned surface at graphite screen printed electrodes (GSPEs) with applications in the biosensors field. The obtained polymeric films were electrochemically characterized by cyclic voltammetry and electrochemical impedance spectroscopy in different redox probes. Different architectures were obtained by depositing noble metal nanoparticles (*e.g.*, AuNPs) at the modified electrodes and the performance of the hybrid composites was assessed. Applications of the newly developed polymer-based electrodes will also be presented, with emphasis on quantification of phthalates contaminants.

Acknowledgements

This research is supported by the European Union-Next GenerationEU, UNIFI Young Independent Researchers Call – MuSC (Analysis and sensing of contaminants in agriculture: from Mulches to Soil and Crops).

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P12 - Synthesis of multivalent activatable aptamers for ultrasensitive detection of *Salmonella typhimurium*

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Aptamers have superior structural properties and have been widely used in bacterial detection methods. However, the problem of low affinity still exists in practical complex sample detection. In contrast, hybridization chain reaction (HCR)-based model I and rolling circle amplification (RCA)-based model II multivalent activatable aptamers (multi-Apts) can fulfill the need for low-cost, rapid, highly sensitive and high affinity detection of *Salmonella typhimurium* (*S. typhimurium*). In our report, two models of multi-Apts were designed. First, a monovalent activatable aptamer (mono-Apt) was constructed by fluorescence resonance energy transfer (FRET) with S. typhimurium aptamer and its complementary chain of BHQ1. Next, the DNA scaffold was obtained by HCR and RCA, and the multi-Apt was obtained by self-assembly of the mono-Apt with a DNA scaffold. In model I, when target was presented, the complementary chain BHQ1 was released due to the binding of multi-Apt to the target and was subsequently adsorbed by UIO66. Finally, a FRET-based fluorescence detection signal was obtained. In mode II, the multi-Apt bound to the target, and the complementary chain BHQ1 was released to become the trigger chain for the next round of amplification of HCR with a fluorescence detection signal. Based on affinity experiments, 25- and 9-fold higher binding affinity of multi-Apt compared with mono-Apt. HCR and RCA based multi-Apts were able to detect *S. typhimurium* as low as 2 CFU mL^{-1} and 1 CFU mL^{-1} respectively. The multi-Apt amplification strategy could be readily expanded for the detection of various pathogenic bacteria, providing a new approach for early diagnosis of pathogenic microorganisms in food samples.

P13 - Expanding CRISPR-Based Molecular Diagnostics Beyond Detection of Nucleic Acids

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CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology has revolutionized molecular diagnostics, renowned for its precision, efficiency, and adaptability. While traditionally recognized for its role in nucleic acid detection, the potential of CRISPR extends far beyond this domain, opening avenues for non-nucleic acid detection applications.[1] This abstract delineates our strategies that combine CRISPR's programmability with the advantages of nucleic acid-based nanotechnology. Our approaches revolve around establishing artificial communication between nucleic acid biotransducers and non-nucleic acid targets through engineered nucleic acid-based circuits, facilitating probing, processing, and conversion of target binding into an amplified CRISPR-based signal. Specifically, we introduce a novel class of Cas12a regulators termed PAM-engineered Toehold Switch DNA, featuring re-engineered locked protospacer adjacent motif (PAM) within the loop.[2] This design enables precise control of Cas12a activities in response to specific molecular targets via structure switching and PAM complementation. PAM-engineered Toehold Switch DNA reconfiguration is precisely controlled by a proximity-based reaction network, facilitating single-step detection of diverse targets like IgG antibodies, small molecules, and microRNAs with high sensitivity and specificity, even within complex matrices (Figure 1A). Additionally, we developed a second strategy for monitoring protease activity, exemplified by matrix metalloproteinase 2 (MMP2), integrating an activity-based method with Cas12a-assisted signal amplification.[3] This approach employs a chimeric peptide-PNA conjugate as a molecular translator, converting protease inputs into nucleic acid activators for Cas12a, enabling highly sensitive detection of diverse proteins and enzymes (Figure 1B). The outcomes of our research highlight the imminent expansion and practical implementation of CRISPR technology in diagnostics, offering a promising horizon for enhanced diagnostic capabilities beyond nucleic acid targets.

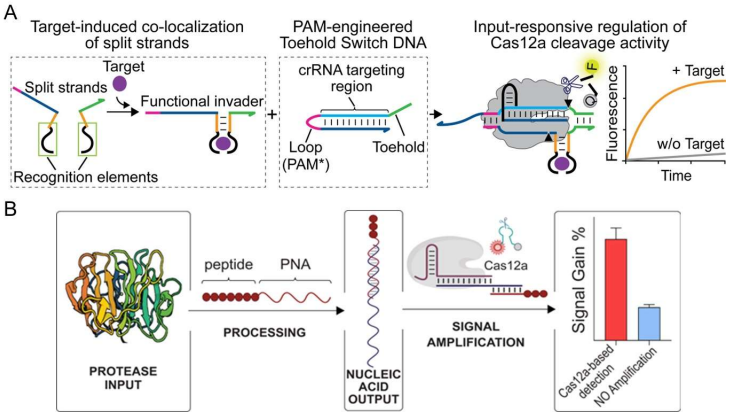

Figure1. (A) PAM-engineered Toehold Switch DNA reconfiguration enables precise control of Cas12a activities in response to specific molecular targets via structure switching and PAM complementation within a proximity-based reaction network. (B) Utilization of a peptide-PNA chemical translator to convert protease activity into a nucleic acid activator for CRISPR-Cas12a-based signal amplification.

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P14 - Lipidomic Investigation in Plasma of Parkinson's Disease, Multiple System Atrophy and Progressive Supranuclear Palsy Diagnosed Patients

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Parkinson's disease (PD), ranking as the second most prevalent global neurodegenerative disorder, presents a multifaceted clinical profile. [1]. It is primarily distinguished by motor symptoms, including characteristic tremors, bradykinesia, and akinesia, which significantly impact patients' daily lives. The diagnostic process for PD remains a clinical endeavour, reliant on the expertise of healthcare professionals to recognize specific motor symptoms. This lack of definitive diagnostic tests poses significant hurdles, especially during the early stages of the disease, where subtle presentations may lead to misdiagnoses. Furthermore, distinguishing PD from related neurodegenerative disorders, such as Multiple System Atrophy (MSA) and Progressive Supranuclear Palsy (PSP), poses its own set of complexities. These conditions often exhibit overlapping clinical symptoms, making accurate differentiation challenging. This investigation aimed to characterize possible existing lipid biomarkers for PD, MSA and PSP by untargeted and semitargeted lipidomic analysis using LC-HRMS,. The lipidomics investigation was carried out on a HPLC (Agilent® 1290) coupled with a QTOF (Agilent® 6545) in an iterative DDA (Dat Dependent Acquisition) mode, after data acquisition two different processing pipeline were essayed and compared, specifically a fully untargeted approach and a pseudo-targeted one (illustrated in Figure 1). More the 400 compounds per polarity were measured and putatively identified. Data analysis provided various important insights both on the differences in results on the different investigation approaches followed, as well as, for clinically relevant information. Regarding the former, while comparing the results of untargeted and semitarget approaches, it became clear that analytical signal drift had occurred. This issue was observable only in the semitarget approach, potentially causing the issue to go undetected in the untargeted approach due to the intricate nature of data interpretation. Regarding clinical data, while initially we aimed to identify significant differences among the three disease lipid profiles, we encountered only limited evidence of such distinctions. Consequently, we refined our analysis to compare just two different groups: Parkinson's and non-Parkinson's. Employing this approach [3], we found 40 lipids that exhibited significant differences between these two groups and achieved an acceptable separation through PLS-DA [4,5]. Further analyses and affected pathway investigation could provide more useful and discriminant information rendering a biochemical differential diagnosis finally feasible.

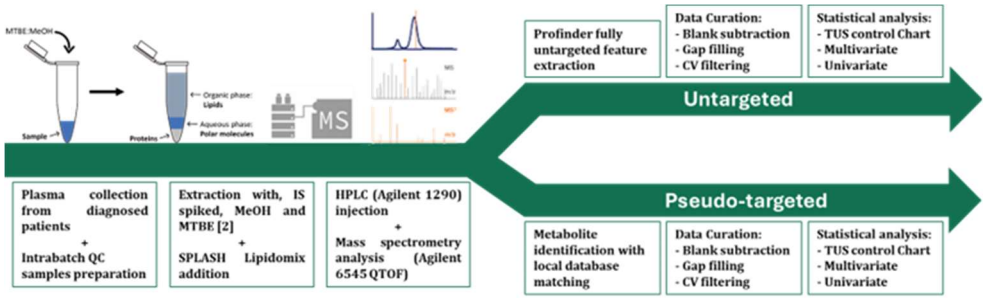

Figure 2 Analytical pipeline followed during lipidomics investigation

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P15 - A hybrid nanocomposite of gold nanoparticles and nano-graphene oxide for the electrochemical detection of estrone

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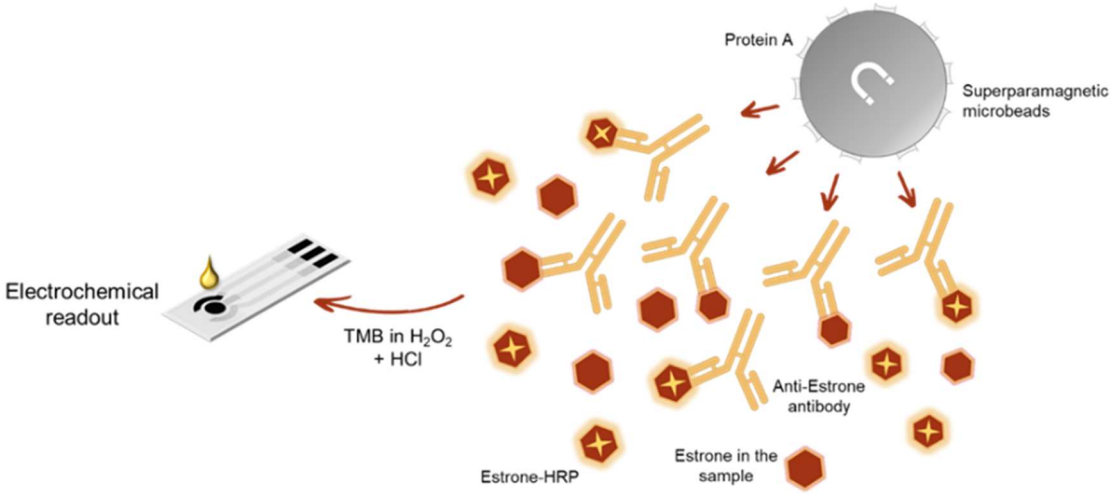
A hybrid nanocomposite composed of nano-graphene oxide (nGO) flakes and gold nanoparticles (AuNPs) was exploited to modify disposable and low-cost screen-printed electrodes (SPE). Compared to bare carbon-based sensors, this surface nanostructuration was able to effectively enhance the electrocatalytic effect, the electron transfer rate, and the sensitivity of the platform. First, a general electrochemical characterisation was conducted. Then, the sensors were challenged for the detection of estrone by designing an immunomagnetic electrochemical assay. Estrone is one of the most important estrogens and it is associated with a variety of environmental and health issues. Indeed, it is considered a contaminant of emerging concern, especially in aquatic environments where its detection gains relevance due to the potential adverse impacts. Moreover, recent studies linked it to proinflammatory and pro-oncogenic activities. Therefore, robust biosensing tools able to monitor the presence of this contaminant in environmental and clinical samples are highly desirable.



Acknowledgements
Funding by the European Climate, Infrastructure and Environment Executive Agency, HORIZON-MISS-2022-OCEAN-01, project iMERMAID, grant number 101112824, is acknowledged.

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P16 - Vacuum-assisted headspace-solid phase microextraction for short-chain fatty acids in faecal samples

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Short-chain fatty acids (SCFAs), the major bacterial metabolites produced after the fermentation of dietary fibre, have been extensively studied in recent years. Indeed, SCFAs or their deficiency may influence the pathogenesis of a wide range of diseases, from allergies and asthma to cancer, autoimmune, metabolic, and neurological diseases1. Gas chromatography-mass spectrometry (GC-MS) is a compelling and reliable method for the analysis of SCFAs. In this context, headspace solid-phase microextraction (HS-SPME) is known to be a fast and effective method for sample preparation. Some research has shown that the use of vacuum can improve the transfer of analytes from the sample to the gas phase and reach equilibrium or at least increase the signal faster and at a lower sorption temperature2. Therefore, vacuum-assisted HS-SPME (VacAC-HS-SPME) can significantly improve the results.
In this study, for the first time, a vacuum-assisted SPME (Vac-HS-SPME) GC-MS method was developed to determine SCFAs in feacal samples. All steps of the analysis such as extraction, VacAC-HS-SPME were investigated to increase the signal and maintain the original concentration and ratio of the fatty acid.
Using a design of experiments (DoE) approach, a highly sensitive GC-MS assay was developed for the quantification of the compounds of interest. The relative influence of the main factors affecting the Vac-HS SPME on the analytical response was investigated using a Plackett-Burman (P-B) design. A D-Optimal design was performed for further optimization and to obtain a more precise optimization of the significant parameters. Principal Component Analysis (PCA) was used to visualize the results.
The Vac-HS-SPME method was validated to achieve high analytical performance in terms of selectivity, linearity (R2 >0.995), accuracy (bias%≤ 4%), precision (CV%≤ 5%), robustness, ruggedness, limit of detection, limit of quantitation, recovery, matrix effect (<90%), and repeatability according to the international guidelines.
As an advantage, our vacuum system was not a custom design2 but we used commercially available tools. The performance was evaluated by comparing the optimized Vac-HS-SPME and regular HS-SPME procedures. For the same extraction time, the Vac-SPME method had faster fiber equilibration time allowing a higher sensitivity to be obtained, with a detection limit (from 0.001 to 0.07 μmol g-1) at least one order of magnitude lower than regular HS-SPME for all target analytes. The Vac-HS-SPME method was successfully applied to quantify FAs in samples of 10 healthy subjects.

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P17 - Microfluidic and sensing tools for cancer immunotherapy

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Immunotherapy with genetically engineered T cells has achieved some spectacular success in clinical trials and has obtained marketing approval. A key need is the widespread availability of small-scale bioreactors providing in-process monitoring. TITAN project aims to the continuous sampling of critical quality attributes, to quickly recognize deviations from the desired range and take appropriated corrective actions[1]. Parameters to be verified and related tools include: bacterial contamination[2]; counting of cells by microfluidic and electrical detection; ratio live/dead cells to be identified by gold nanoparticles on a capacitive sensor; cytokines production identified by electrochemical methods; T-cell function tests through the production of spheroids into droplet microfluidic devices and the electrochemical detection of metal nanoparticles (NPs) through anodic stripping voltammetry.
For each of the developed strategies for sensing and microfluidic, different technologies have been addressed having the common purpose of using a volume of sample as low as possible, since it would originate from patient-derived tissues.

qPCR for contamination check on a POC platform

An innovative protocol for qPCR working with a very low volume of raw sample (0,25 µl) and avoiding the step nucleic acid extraction was optimized for a Point-Of-Care (POC) instrument. The optimized extraction-free protocol in minimal volume analysis combined with the possibility to perform the qPCR assay on a portable device, demonstrates the possibility to avoid the time-consuming bacterial cell culturing. The achieved amplification of the Gram+ S. aureus was performed in a 5 µl total volume of reaction, reaching the limit of detection of 1 CFU/ml.

CAR-T cells efficiency tests

Lymphocytes spilled out from bioreactor will be focused and counted through a simulated and optimized spiral channel which will help in the single cell identification. Detection of Interleukins IL6 and IL10 has been performed down to 2 ng/ml by immobilizing antibodies on the surface of microfabricated gold electrodes, but this limit can be improved by nanostructuring the surface of electrodes. Interleukines and cyitokines detection will be integrated into functional assays to evaluate the efficiency of CAR-T cells in killing tumor cells. To improve reproducibility and standardization of the assays, a microfluidic method to produce spheroids of the same dimensions has been improved, based on computational simulation and fabrication of a droplet generator working with 2-phase emulsion of matrigel containing cells and oil.

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P18 - A new bioluminescent bioassay based on a “caged” luciferin for the screening of bile salt hydrolase activity in human complex matrices

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Bile salt hydrolases (BSH) are a family of microbial enzymes that deconjugate primary bile acids (BA) from the amino acids glycine or taurine. They catalyze the first step of BA metabolism producing unconjugated primary BA and maintaining the BA pool for further modification by the microbiota. Indeed, once deconjugated, free primary BAs can be metabolized (i.e.7-dehydroxylated) to secondary BAs involved in different intestinal pathologies (i.e. Intestinal Bowel Disease and colon cancer). To uncover BSH abundance and their activity in the gut microbiota, different approaches such as biochemical assays and metagenomic analysis in complex biological samples are currently used; however, these approaches have several limitations. In this context, we developed a rapid and cost-effective bioluminescence bioassay for the screening of BSH activity in human feces using an amino luciferin ”caged” with the primary BA chenodeoxycholic acid (aLuc-CDCA), thus mimicking the conjugated CDCA with glycine [1]. Since aLuc- CDCA doesn’t behave as a substrate for the luciferase enzyme being “caged” by the amidation, the reaction occurs after the BSH-catalyzed hydrolysis that allows the release of the “free” amino luciferin that in turn induces the BL reaction in the presence of luciferase and ATP. Firstly, we investigated *in vitro* the deconjugation level of aLuc-CDCA (50 µM) in the presence of pure BSH (range 0-10 µg/mL) using different exposure times (15, 30, and 60 min) at 37°C. The BL reaction was triggered, adding in the solution the firefly luciferase (0.5 mg/mL) in Tris-HCl 0.1 mM, pH 7.5, supplemented with Mg_2SO_4 (80 mM) and ATP (40 mM). Kinetic profiles showed a linear correlation between BL emission intensity and increased amount of BSH, with an R^2 of 0.92 after 60 min of incubation and a LOD and LOQ of 0.8 and 1.6 µg/mL respectively. Next, we moved forward on real human feces, but the light output decreased markedly probably due to interferents in the fecal material that affect the luciferase activity, thereby not allowing an accurate measurement. To address this issue, we explored the possibility of using a whole cell-based BL assay in which human colorectal adenocarcinoma cells (Caco2) genetically encoded with a firefly luciferase (*Amydetes vivianii*), were exposed to solutions containing aLuc-CDCA (50 µM) previously incubated with pure BSH (range 0-10 µg/mL) for 60 min at 37°C. This approach enables the protection of the luciferase enzyme by cell membranes, obtaining a good correlation between BL signal intensity and BSH amount with a LOD comparable to that measured in the cell-free system. As a proof of concept, the whole cell-based assay has been successfully employed for quantifying BSH activity in intact fecal stools (1mg/1ml), demonstrating the potential biomedical application of this sensitive reporter system as a noninvasive diagnostic test.

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P19 - Portable and affordable 3D printed biosensor for nerve agent detection in drinking water using carbon black/thermoplastic polyurethane composite electrodes

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Nerve agents represent a significant threat due to their extreme toxicity and potential use in chemical warfare or terrorism. Detection of these compounds, especially in water sources, is paramount for public safety and environmental protection. The development of biosensors capable of rapid and sensitive detection is crucial for mitigating the harmful effects of nerve agents[1]. Leveraging 3D printing technology for biosensor fabrication offers a cost-effective and versatile approach suitable for various applications, particularly in resource-limited settings or emergency scenarios[2].
In this study, we designed an amperometric biosensor targeting the detection of paraoxon, a widely used nerve agent simulant, in water. The biosensor operates by inhibiting the butyrylcholinesterase enzyme (BChE) upon exposure to paraoxon. To create electrodes suitable for electroanalytical applications, we employed 3D printing to fabricate electrodes using thermoplastic polyurethane (TPU) filled with carbon black. Additionally, carbon black was combined with Prussian blue nanoparticles (CB-PBNPs) to enhance electrocatalytic activity for the oxidation of thiol-containing compounds at lower potentials[3]. The surface morphology and electrochemical properties of the electrodes were characterized using electrochemical impedance spectroscopy and cyclic voltammetry.
Characterization of the electrode surface revealed favorable attributes for electroanalytical applications, including low charge transfer resistance and increased peak current intensity, attributable to the incorporation of CB-PBNPs. The developed biosensor exhibited a linear response up to 20 ppb of paraoxon, with a calculated detection limit of 2 ppb based on 10% enzyme inhibition in drinking water. These results demonstrate the effectiveness of the biosensor for sensitive detection of nerve agent simulants in water offering promise for rapid deployment in emergency situations and proactive monitoring of environmental contamination by nerve agents, thereby contributing to public safety and security.

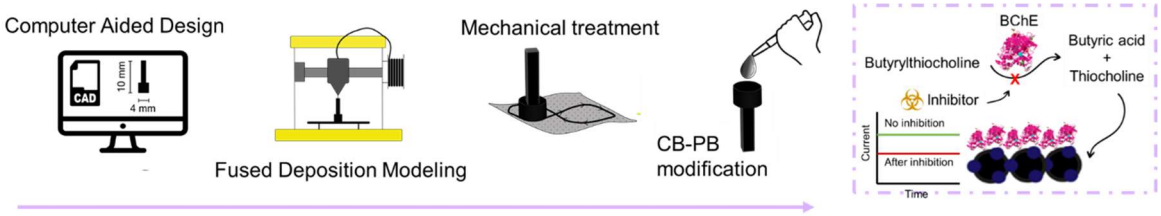

Fig. 1: Schematic representation of the developing process of the 3D-printed device and the biosensor working principle

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P20 - A rapid liquid microextraction method for the determination of F2-Isoprostanes in oral fluid by means of PALME coupled with UHPLC-MS/MS analysis

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A dangerous imbalance between antioxidant defense systems and ROS can occur due to the overproduction of reactive oxygen species (ROS) or their missing degradation; in this scenario ROS exert their toxic action, leading to a condition called oxidative stress (OS)[1]. OS has been implicated in a variety of pathological events such as cardiovascular and neurodegenerative diseases, diabetes, and carcinogenesis. The best-known biomarkers for endogenous oxidative damage to lipids, DNA, and proteins are 8-iso prostaglandin F2α (8-isoPGF2α), 8-hydroxy-2'-deoxyguanosine (8-OHdG), and 3-nitro-l-tyrosine (3-NT)[2], respectively. Since the lipid oxidative stress biomarkers, F2-isoprostanes (F2-IsoPs), are more chemically stable than other analytes, their study is more advantageous for analytical purposes. IsoPs are formed *in situ* from the oxidation of arachidonic acid esterified to phospholipids by the direct action of ROS in the cellular membrane. The determination and analysis of OS markers is usually based on rapid methods such as colorimetric tests, and immunochemical techniques (ELISA and enzyme immunoassays)[3]. Anyway, being IsoPs found at low concentrations in biological fluids, a need to develop highly sensitive methods of analysis, and the use of instrumental analysis such as liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) started to be mandatory in the last years. The elective biological matrices for this proposal are blood, plasma, and urine. However, an alternative matrix, like oral fluid (OF), was used in this work. The sampling of this matrix is favourable because it is a fast patient-friendly approach, moreover, it is characterized by a simpler composition than blood and urine. The aim of this work was the development of a new rapid, reproducible, and sensitive method for the quantification of lipid oxidative stress biomarkers in OF through UHPLC-MS/MS. The procedure involved an extraction step which was developed according with the aim of reducing the volume of organic solvent, using the novel approach based on parallel artificial liquid membrane extraction (PALME)[4]. In the PALME set up, target analytes were extracted through a three phases system, exploiting a pH gradient to facilitate mass transfer of uncharged analytes from the original matrix to an aqueous extracting phase, across an organic solvent immobilised within the pores of a membrane, led to the low consumption of organic solvents for extraction. Moreover, this technique allows an high enrichment factor (40 fold) without an increasing of matrix effect (below 20%). Noteworthy, for the first time, a microextraction technique in the 96-well format was developed for the extraction of IsoPs, to obtain satisfactory extraction levels in the PALME step, it was necessary to pay attention to different parameters: the nature of organic solvent, volumes of biological sample, composition and volume of donator and acceptor solution, pH, ionic strength of donator solution and agitation time.

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P21 - Development of analytical procedures for the simultaneous determination of Indigotin and Indirubin in industrially produced natural indigo

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Historically, indigo has played a crucial role in textile dyeing processes[1]. Traditionally sourced from plants like Indigofera tinctoria, Isatis tinctoria, and Persicaria tinctoria[2], natural indigo consists of two main components: indigotin, responsible for the blue hue, and indirubin, contributing to the red-violet shade[3]. The combination of these compounds imparts a distinct color to the fabric, in contrast to synthetic indigo, which solely contains indigotin. The industrial, sustainable production of indigo necessitates a unique extraction process wherein calcium hydroxide is added to promote hydrolysis of the precursor inside the plant and facilitate the isolation of the pigment. Commercially accepted natural indigo is formed by approximately 80% calcium carbonate and the remaining organic components[4]; the inorganic portion poses a significant challenge for the determination of colored compounds, as it is insoluble in both water and organic solvents. In the industrial sector, the ability to measure both indirubin and indigotin simultaneously represents a very critical point, because on the base of their ration the adjustment of various parameters within the production process is possible. Current methods for analyzing indirubin rely on High-Performance Liquid Chromatography (HPLC). The procedure involves extracting the indirubin from a powdered sample using organic solvents like methanol or acetone. However, indigotin has limited solubility in these solvents, so that a different approach for its detection is necessary. Indigotin analysis typically involves a chemical reduction step. This process transforms indigotin into a water-soluble form called leuco-indigotin, which can then be quantified using spectroscopy.
The main challenge of this study was the definition of analytical procedures for the simultaneous quantification of indigotin and indirubin in the industrially produce natural indigo. To this goal, a chromatographic and a spectroscopic approach were considered. In the first case, dimethyl sulfoxide with the addition of hydrochloric acid was used as a solvent to promote the complete dissolution of indirubin and indigotin. This allowed for the injection of the solution into a UHPLC-PDA system. Separation of indirubin and indigotin was achieved using a reverse-phase C-18 column and a mobile phase consisting of water and acetonitrile. The quantitative results obtained constituted a sort of reference data for the development of the second analytical procedure. In this case, identification of the two analytes was conducted by exploiting their different absorption spectra in the UV-Visible region. Due the high overlapping of the spectra of indigotin and indirubin, as shown, a chemometric analysis exploiting PLS (partially least square) was implemented in the analysis of the UV-Vis signals in order to create a model capable of determining the concentration of the two colored compounds in samples containing different ratios of indigotin and indirubin (Figure 1).

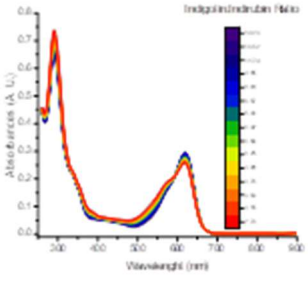

Figure 1. Absorption spectra of DMSO solution with different indigotin:indirubin ratio.

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P22 - Fast classification of *Cannabis sativa* L. samples according to the total THC content

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The widespread diffusion of products deriving from *Cannabis sativa* L. led to the necessity of rapid and reliable methods for the identification of samples containing Δ^9-tetrahydrocannabinol (Δ^9-THC), the psychoactive component of the plant, which imparts mental distortions and hallucinations.
In the *C. sativa* plant, cannabinoids are mostly present in their carboxylated acidic form, mainly including Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA) and cannabidiolic acid (CBDA). By means of a decarboxylation process which occurs spontaneously, under the action of heat and light, cannabinoic acids are converted into their neutral compounds, (Δ^9-THC) and cannabidiol (CBD). These cannabinoids display remarkable similarities as structural isomers but differ in their pharmacological properties. Indeed, only Δ^9-THC shows psychotropic effects while CBD has neuroprotective, anti-inflammatory and anticonvulsant properties. Recreational type *C. sativa*, also known as marijuana, is characterised by the presence of a high content of Δ^9-THC and, for this reason, it is an illicit drug in several countries. On the other hand, fibre-type *C. sativa* (hemp), that was originally used for its stem-fibers, has recently spread in many countries despite its poorer cannabinoid profile, thanks to the noticeable presence of CBD. Usually, the discrimination between legal and illegal *C. sativa* samples is based on the total THC content (THC_{TOT}) which allows for the quantification of all potential Δ^9-THC present in plant material. According to the Common Agricultural Policy of EU, the threshold limit of THC_{TOT} in *C. sativa* has been set equal to 0.3% w/w. If one *C. sativa* crop is detected at harvest to contain a higher quantity of Δ^9-THC, the plants could be confiscated and/or destroyed. According to the Italian legislation, no responsibility is put on the farmer up until THC_{TOT} = 0.6% w/w. Therefore, the development and improvement of methods for the identification of *C. sativa* inflorescences already in the field before harvest and further processing of the plant product is very urgent.
To this purpose, in this work we exploited the combination of a voltametric and a multivariate approach. Screen printed electrodes modified by carbon black (SPEs_CB) [1,2] have been used to collect electrochemical signals in extracts of 50 different *C. sativa* samples. Then, the collected set of signals has been submitted to supervised and unsupervised chemometric analysis methods. The aim was that of using the voltammetric signals recorded in different extracts as a sort of "fingerprint" of the analysed samples, in order to recognize *C. sativa* plants classified as illegal and legal on the base of the THC_{TOT} determined by a HPLC analysis. The responses of the electrode system were first screened via Principal Component Analysis (PCA), which revealed the presence of well-defined clusters of samples with similar THC_{tot} content. Then, classification models were built and validated by Partial Least Squares-Discriminant Analysis (PLS-DA). Discrimination between legal and illegal samples was performed on the base of both the threshold limits corresponding to the maximum value of THC_{TOT} allowed for the cultivation and commercialization of *C. sativa* samples defined by European and by Italian legislations. The excellent classification results obtained confirm the effectiveness of the sensor and of the proposed chemometric procedure, which are suitable for fast routine control.

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P23 - Label-free elettrochemical immunosensor for the detection of Pseudomonas Aeruginosa in a confined environment

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The project concerns the development of an electrochemical immunosensor specifically designed to allow continuous monitoring of the Pseudomonas Aeruginosa bacterium in a confined environment.
All tests were carried out in a chamber built specifically for this study. The chamber was built according to the ISO 16000-36 standard, dimensions, 3m ^{3}, equipped with

- professional nebulizer with particle operating range (1.5 μm ÷3.0 μm) and an atomizer for particle sizes (< 1.0 μm);
- air conditioning system combined with an adjustable internal recirculation system in order to obtain the thermal comfort parameters required by UNI 10339 (speed of 0.25 ± 0.5 m/s, temperature of 20 ± 1 °C and relative humidity of 40 ± 5%, number of air changes per hour equal to 2÷3

The chamber was equipped with a special multi-level test tube holder system in order to verify the distribution of the pathogen in the chamber.
Sampling techniques for environmental air monitoring involve the use of solid and liquid culture media which detect the viable microbial fraction, i.e. metabolically active, which can reproduce and form visible colonies on laboratory plates.
Standards such as UNI EN 17141:2021, UNI 11108:2004, UNI EN 13098:2019, UNI CEN/TS 16115-1:2011, UNI EN 14031:2005 were taken as reference. which establish the requirements, guidelines and methodologies for sampling airborne microorganisms in working environments.
The sampling carried out gave good repeatability and reproducibility over time.
The study was continued by modifying suitable immunosensors already tested in water, to be used to detect the pathogen in Petri dishes placed in an indoor environment.
The plates were modified by adding, in addition to the AGAR-free culture medium for the growth of PA, gellaneous material capable of capturing the pathogen present in the confined environment.
As regards the assembly of the immunosensor, the basic idea will be to exploit the advantages of silk-screen printed electrodes, such as their ease of production, rapid response and low cost, and combine them with the typical characteristics of a immunosensor.
The assembly of the immunological chain traditionally used in ELISA assays on the surface of the electrode will allow us to obtain an immunosensor with electrochemical detection.
All this in order to exploit the immune system and its high specificity in antigen-antibody recognition reactions, in order to avoid the use of chemical markers or fluorescent labels.

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P24 - Size separation and characterization of human polysomes by flow field-flow fractionation

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A polyribosome (or polysome) is a structure of chained ribosomes bound to an mRNA molecule. It consists of an mRNA molecule and two or more ribosomes that act to translate mRNA instructions into polypeptides. Isolated polysomes (also known as translating ribosomes or polyribosomes) are mRNA–ribosome complexes that are frequently used for the in vitro study of the regulation of protein synthesis.
Polysome profile analysis is a popular method for separating polysomes and ribosomal subunits and is typically achieved by sedimentation on a sucrose density gradient (SDG) followed by gradient fractioning coupled to a UV detector. This has remained the gold standard method since ribosomes were first discovered; however, this method is time-consuming and requires multiple steps from making the gradient and long ultracentrifugation to analyzing and collecting the fractions [1].
Asymmetric flow field-flow fractionation (AF4) is a subtechnique of field-flow fractionation that separates molecules according to differences in their diffusion coefficient, which reflects their size and shape. In combination with on-line detectors like UV/vis, fluorescence and multi-angle light scattering, AF4 provides an analytical platform able to separate and characterize biological macromolecules and particles. AF4 has been applied to a wide range of biological analytes [2], including plant and bacterial ribosomes [3].
In this study, we present an AF4-multidetection method for the separation and characterization of human ribosomes and polysomes. The running conditions are set to obtain, in a single run and short analysis time, size resolution of ribosomal subunits and polysomes.
Size-uniform polysome fractions are collected for further, offline characterization. This can consist in low or high throughput analysis of either mRNAs recruited to polysomes or of the proteins constituting or binding ribosomes (ribosomal proteins, translation factors, regulatory proteins, etc.). This kind of analysis allows to define the events which regulate gene expression at the post-transcriptional (translational) level.

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