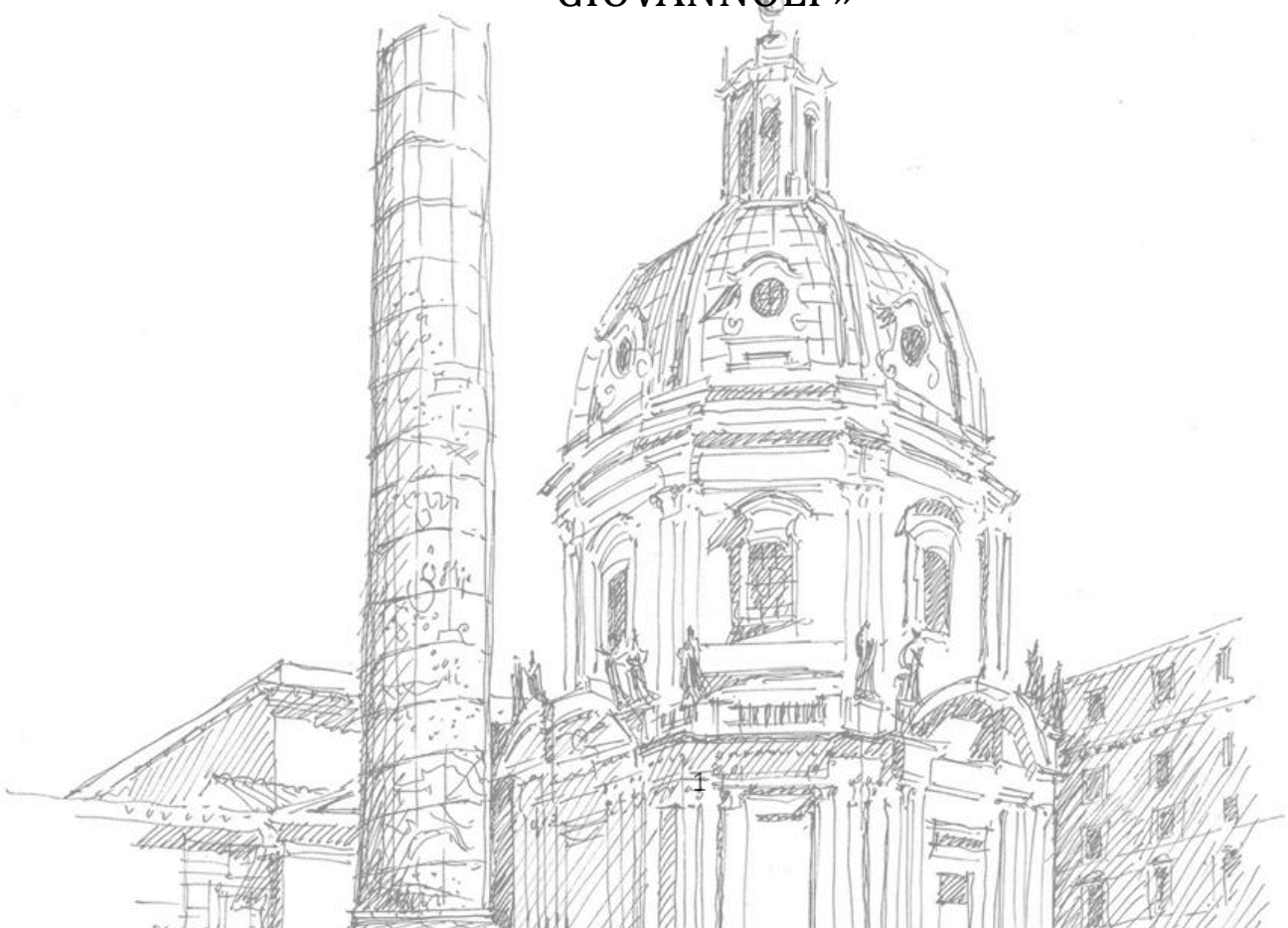
Giornate di Bioanalitica 2025

NUOVI ORIZZONTI PER LA BIOANALITICA: SALUTE, NUTRIZIONE, SPORT

7-9 APRILE 2025, ROMA

GIORNATE DEDICATE AL CONTRIBUTO DELLA CHIMICA BIOANALITICA E WORKSHOP "Bioanalitica e Citizen Science: innovazione e collaborazione"

CONSEGNA DEI PREMI «ALESSANDRO MANGIA» E «CRISTINA GIOVANNOLI »



Giornate di Bioanalitica 2025

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7-9 Aprile 2025, Roma

Con il patrocinio del Dipartimento di Chimica dell'Università degli Studi di Roma "Tor Vergata"

ATTI

E

PROGRAMMA FINALE

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

ISBN 978-88-94952-53-7

Editor: Società Chimica Italiana in coedizione con Divisione di Chimica Analitica e Gruppo di Bioanalitica

7 Aprile 2025, Palazzetto Mattei, sede della Società Geografica Italiana, Roma

Pubblicazione elettronica a cura di Alessandro Porchetta e Valentina Marassi

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Si ringraziano i seguenti Sponsor per il contributo economico dato all'organizzazione:

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Giornate di Bioanalitica

Roma 7-9 Aprile 2025

NUOVI ORIZZONTI PER LA BIOANALITICA: SALUTE, NUTRIZIONE, SPORT

Programma Scientifico

- **Lunedì, 7 Aprile 2025**

SOCIETÀ GEOGRAFICA ITALIANA, ROMA

10.00 – 11.00 **REGISTRAZIONE**

11.00 – 11.30 **SALUTI ISTITUZIONALI**

11.30 – 13.00 **TAVOLA ROTONDA "*NUOVI ORIZZONTI PER LA BIOANALITICA: SALUTE, NUTRIZIONE, SPORT*"**
MODERANO: DARIO COMPAGNONE, MARIELLA CARERI

INTERVENGONO:
FRANCESCO BOTRÉ - UNIVERSITÀ DEGLI STUDI DI FIRENZE
MARIA ALESSANDRA CALEGARI - FONDAZIONE POLICLINICO UNIVERSITARIO "A. GEMELLI"
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PAOLO STACCHINI - ISTITUTO SUPERIORE DI SANITÀ
CLAUDIA ZOANI - AGENZIA NAZIONALE PER LE NUOVE TECNOLOGIE, L'ENERGIA E LO SVILUPPO ECONOMICO SOSTENIBILE (ENEA)

13.00 – 14.00 **PAUSA PRANZO E SESSIONE POSTER**

PRIMA SESSIONE - PRESENTAZIONI ORALI

MODERANO: SANDRA FURLANETTO, CLAUDIO BAGGIANI

14.00 - **OC1** Zeolite-based biomaterial for drugs' remediation: evaluating removal efficiency towards ibuprofen with an electrochemical device
ANTONELLA MIGLIONE, UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II

14.15 - **OC2** Non-invasive diagnosis of colorectal cancer: from fecal biomarker discovery to electrochemical magneto-immunoassay
LORENZO TOMA, UNIVERSITÀ DEGLI STUDI DI PARMA
14.30 - **OC3** Activity based (bio)sensor for the monitoring of MutyH DNA glycosylase
ERICA BELFORTE, UNIVERSITÀ DEGLI STUDI DI ROMA "TOR VERGATA"
14.45 - **OC4** Seaweed (G.gracilis) protein hydrolysates: a valuable source of short- and medium-chain peptides with multifunctional properties
ENRICO TAGLIONI, UNIVERSITÀ DEGLI STUDI DI ROMA "LA SAPIENZA"
15.00 - **OC5** Selective extraction of Phytoprostanes from food samples using molecularly imprinted polymers and confirmation by UPLC-QqQ-LIT-MS/MS and UHPLC-Q-OrbitrapMS
FEDERICO FANTI, UNIVERSITÀ DEGLI STUDI DI TERAMO
15.15 - **OC6** Determination of Aflatoxin B1 (AFB1) level in whole blood by means of quantitative Dried Blood Spots (DBS) microsampling coupled to Enzyme Linked ImmunoSorbent Assay (ELISA)
MARTINA GALLETTO, UNIVERSITÀ DEGLI STUDI DI TORINO
15.30 - **OC7** SERS spectroscopy for bioaerosol analysis and characterization: challenges and future perspectives
STEFANO FORNASARO, UNIVERSITÀ DEGLI STUDI DI TRIESTE
15.45 - **OC8** Empowering precision medicine and well-being: A fully 3D printed miRNA detection platform for liquid biopsy applications
PANAGIOTA KALLIGOSFYRI, UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II

16.00 – 16.30 **COFFE BREAK E SESSIONE POSTER**

SECONDA SESSIONE - PRESENTAZIONI ORALI
MODERANO: FABIANA ARDUINI, BARBARA RODA

16.30 - **OC9** Smartphone-based colorimetric paper sensors for free chlorine and pesticides detection in clinical and environmental matrices
CATERINA CAMBREA, ALMA MATER STUDIORUM – UNIVERSITÀ DI BOLOGNA
16.45 - **OC10** Advances in electrochemical magneto-genoassays for multi-allergen detection in food
SIMONE FORTUNATI, UNIVERSITÀ DEGLI STUDI DI PARMA
17.00 - **OC11** Human fecal short chain fatty acids determination by SPME-GC-MS: disease monitoring by a chemometric approach in pediatric Crohn's Disease
BENEDETTA PASQUINI, UNIVERSITÀ DEGLI STUDI DI FIRENZE
17.15 - **OC12** Multichannel laser-induced graphene electrochemical device for paper-based flow-analysis of phenolic antioxidants
IDA VALERIA DI CRISTOFARO, UNIVERSITÀ DEGLI STUDI DI TERAMO

SESSIONE PRESENTAZIONI FLASH
MODERANO: FABIANA ARDUINI, BARBARA RODA

17.30 - **PF1** Advancing cancer management: a paper-based electrochemical sensor for detecting H_2S in murine tissue lysates

ALESSANDRA GLOVI, UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II
17.35 - **PF2** Highly sensitive printed pH sensor using CB/PANI nanocomposite for POC diagnosis of orthopedic infections
CHRISTIAN GOSTI, UNIVERSITÀ DEGLI STUDI DI ROMA "TOR VERGATA"
17.40 - **PF3** Overcoming the challenges of adeninosine template in nanoMIP design
VALENTINA TESTA, UNIVERSITÀ DEGLI STUDI DI ROMA TORINO
17.45 - **PF4** Microfluidic 3D-printed electrochemical device for ischemic stroke point-of-care testing
DAVIDE PAOLINI, UNIVERSITÀ DEGLI STUDI DI TERAMO
17.50 - **PF5** Flexible micro thread/paper-based wearable device (μTPAD) for pH monitoring in chronic wounds
LAURA BELCASTRO, UNIVERSITÀ DEGLI STUDI DI ROMA "TOR VERGATA"
17.55 - **PF6** Waste from oil industry: a possible beneficial resource
MYRIAM PERRUCCI, UNIVERSITÀ DEGLI STUDI "GABRIELE D'ANNUNZIO" DI CHIETI-PESCARA
18.00 -**PF7** On-Body Real-Time Lactate Detection in Sweat Using a 3D-Printed Flexible Wearable Sensor
GABRIELLA IULA, UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II
18.05 - **PF8** Synergistic Integration of Molecularly Imprinted Polymers and Nanozymes for Enhanced Selectivity and Catalytic Efficiency
ABDELHAFID KARRAT, UNIVERSITÀ DEGLI STUDI DI ROMA "TOR VERGATA"

20.00 **CENA SOCIALE AL RISTORANTE "OSTERIA CIRCO"**

• Martedì, 8 aprile 2025

9.00 – 10.00 **PREMIAZIONE MEDAGLIA "ALESSANDRO MANGIA" E PREMIO "CRISTINA GIOVANNOLI"**
MODERANO: ALESSANDRO MANGIA, LAURA ANFOSSI, SANDRA FURLANETTO, ALESSANDRO PORCHETTA, BARBARA RODA

9.00 - Premio "Alessandro Mangia"
GIUSEPPE PALLESCHI, UNIVERSITÀ DEGLI STUDI DI ROMA "TOR VERGATA"

9.30 - Premio "Cristina Giovannoli"
Keynote - The bioanalytical chemistry behind the development of lateral flow immunoassays: new approaches and paradigms
SIMONE CAVALERA, UNIVERSITÀ DEGLI STUDI DI TORINO

TERZA SESSIONE - PRESENTAZIONI ORALI
MODERANO: ALDO RODA, GIOVANNA MARRAZZA

10.00 - **OC13** An AF4 approach for the characterization of innovative diagnostic tools in biological fluids
STEFANO GIORDANI, ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA
10.15 - **OC14** Point-of-care device for hemoglobin detection in capillary blood
LUCA FIORE, SENSE4MED

10.30 - **OC15** FFF-driven biomarker profiling via improved isolation of Extracellular Vesicles
ANNA PLACCI, ALMA MATER STUDIORUM - UNIVERSITÀ DI BOLOGNA
10.45 - **OC16** Extracellular vesicles detection by exploiting electrochemical magneto-assays
PATRICK SEVERIN SFRAGANO, UNIVERSITÀ DEGLI STUDI DI FIRENZE

11.00 – 11.30 **COFFE BREAK**

QUARTA SESSIONE - PRESENTAZIONI ORALI

MODERANO: ANNALAURA CAPRIOTTI, PIERLUIGI RESCHIGLIAN

11.30 - **OC17** Oxidized cholesterol compounds in the development stages of zebrafish analyzed by LC-MS/MS and machine learning approaches
FABIOLA EUGELIO, UNIVERSITÀ DEGLI STUDI DI TERAMO
11.45 - **OC18** Unraveling the data treatment issues of untargeted metabolomics: cholangiocarcinoma as a case study
ANDREA CERRATO, UNIVERSITÀ DEGLI STUDI DI ROMA "LA SAPIENZA"
12.00 - **OC19** An all-paper-based analytical platform for electrochemical H_2O_2 detection in breath
VINCENZO MAZZARACCHIO, UNIVERSITÀ DEGLI STUDI DI ROMA "TOR VERGATA"
12.15 - **OC20** A sensor-based strategy for the rapid quantification of nucleic acids in lipid nanoparticle formulations
WANDA CIMMINO, UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II
12.30 - **OC21** Integrated four-leaf clover-like MIP/PAD for maleic hydrazide colorimetric determination
DOUNIA EL FADIL, UNIVERSITÀ DEGLI STUDI DI TERAMO
12.45 - **OC22** Biochar-based plastics electrodes for sustainable electrochemical detection of marine pollutants
MONICA MOSQUERA, UNIVERSITÀ DEGLI STUDI DI UDINE
13.00 - **OC23** Portable Multifunctional Potentiostat for Static and Flow-Based Electrochemical Biosensing: Application to Pseudomonas aeruginosa
ELISA PAIALUNGA, UNIVERSITÀ DEGLI STUDI DI ROMA "TOR VERGATA"

13.15 – 14.30 **PAUSA PRANZO**

QUINTA SESSIONE - PRESENTAZIONI ORALI
MODERANO: STEFANO CINTI, LAURA ANFOSSI

14.30 - **OC24** Cut out the middlemen: centrifugation-free isolation of EVs from conditioned medium
VALENTINA MARASSI, ALMA MATER STUDIORUM – UNIVERSITÀ DI BOLOGNA
14.45 - **OC25** MAIGRET: a diagnostic assay that combines antibody-triggered cell-free transcription and CRISPR/Cas systems
FRANCESCA MICELI, UNIVERSITÀ DEGLI STUDI DI ROMA "TOR VERGATA"

15.00 - **OC26** Chemical profile assessment of food supplements by HPLC-DAD
ERIKA MARIA RICCI, UNIVERSITÀ DEGLI STUDI "GABRIELE D'ANNUNZIO" DI CHIETI-PESCARA
15.15 - **OC27** Electrochemical Platform Development by Multivariate Approach for Uric Acid Detection in Disease Diagnosis
GIULIA SELVOLINI, UNIVERSITÀ DEGLI STUDI DI FIRENZE
15.30 - **OC28** CRISPR-Powered Monitoring of DNA Repair Activities Using Synthetic DNA Transducer
NEDA BAGHERI, UNIVERSITÀ DEGLI STUDI DI ROMA "TOR VERGATA"
15.45 - **OC29** Development of a Lateral Flow-Based biosensor for the early detection of Ovarian Cancer-specific microRNAs
THEA SERRA, UNIVERSITÀ DEGLI STUDI DI TORINO

16.00 – 16.30 **COFFE BREAK**

Workshop "Bioanalitica e Citizen Science: innovazione e collaborazione"

PRIMA SESSIONE - PRESENTAZIONI ORALI
MODERA: SANDRA FURLANETTO

16.30 – 17.00 Citizen Science: Principi ed Applicazioni Pratiche
AMEDEO BOLDRINI, UNIVERSITÀ DEGLI STUDI DI SIENA

17.00 – 17.15 Domande e chiarimenti

17.15 – 17.45 Sviluppo di Sensori per la Citizen Science
RICCARDO CIRRONE, UNIVERSITÀ DEGLI STUDI DI SIENA

17.45 – 18.00 Domande e chiarimenti

18.00 – 18.30 Pianificazione delle attività del giorno successivo

● MERCOLEDÌ, 9 APRILE 2025

SECONDA SESSIONE – ATTIVITÀ DI GRUPPO
COORDINANO: AMEDEO BOLDRINI, RICCARDO CIRRONE

9.00 – 10.00 Ideare un biosensore con applicazioni in progetti di CS

11.00 – 11.30 **COFFE BREAK**

11.30 – 12.15 Ideare un biosensore con applicazioni in progetti di CS

12.15 – 13.00 Presentazione delle idee e conclusione dell'attività

COMUNICAZIONI POSTER

P01 - USING A CLAMP-LIKE TRIPLEX SWITCH TO CONTROL CRISPR-CAS12A CLEAVAGE ACTIVITY
Andrea Celeste Di Pede, Erica Belforte, Alessio Palone, Marianna Rossetti, Neda Bagheri, Alessandro Porchetta

P02 - MUTYH CATALYSED 8-OXOG REPAIR MONITORING THROUGH AN ELECTROCHEMICAL SCAFFOLD
Ankita Sinha, Alejandro Chamorro, Alessandro Porchetta

P03 - LASER-INDUCED NANOZYMATIC METAL NANOPARTICLES FOR PAPER-BASED ANALYTICAL DEVICES
Annalisa Scroccarello, Flavio Della Pelle, Paolo Di Battista, Dounia El Fadil, Dario Compagnone

P04 - ADVANCED WEARABLE SENSORS FOR NON-INVASIVE EMOTIONAL STATE ASSESSMENT
Antonio Licheri, Beatrice Ercolani, Chiara Balestrieri, Ciro D'Elia, Antonio Maffucci, Silvia Orlanducci, Laura Micheli

P05 - HYBRID NANOZYME SYSTEMS: GOLD-DECORATED NANODIAMONDS FOR ADVANCED ELECTROCHEMICAL SENSING
Beatrice Ercolani, Antonio Licheri, Sara Tosi, Leonardo Sablone, , Valeria Guglielmotti, Roberto Matassa, Juan G. Lozano, Ana Maria Beltrán, Riccardo Salvio, Lucia Sansone, Laura Micheli, Silvia Orlanducci

P06 - ELECTROCHEMILUMINESCENCE ENHANCEMENT VIA REDOX-MEDIATOR FOR BEAD-BASED IMMUNOASSAYS
Claudia Martínez-Asenjo, Alessandro Fracassa, Francesco Paoluccia, Giovanni Valentia

P07 - A SIMPLE COLORIMETRIC PAPER-BASED DEVICE FOR ZINC IONS DETECTION IN WATER
Fabio Di Nardo, Thea Serra, Simone Cavalera, Valentina Testa, Laura Anfossi, Claudio Baggiani

P08 - ENZYME-MEDIATED DISSIPATIVE HYBRIDIZATION CHAIN REACTION FOR DYNAMIC DNA NANOMATERIALS
Federica Pedrini, Luca Capelli, Alessandro Bertucci, Erica Del Grosso

P09 - ECO-INNOVATIVE PAPERS INTEGRATING NANOSTRUCTURED GRAPHENIC FILMS WITH MULTIFUNCTIONAL SENSING CAPABILITIES
Flavio Della Pelle, Davide Paolini, Annalisa Scroccarello, Dario Compagnone

P10 - EMPLOYING DNA CONDENSATES AS A CRISPR-CAS μBIOREACTOR FOR BIOSENSING APPLICATIONS

Laura Beltrán, Corinne Giancaspro, Raj Paul, Alessandro Porchetta

P11 - ORIGAMI PAPER-BASED BIOSENSOR TO SARS-COV-2 ON THE SURFACE
Laura Fabiani, Rachele Petrocchini, Giorgia Grilli, Riccardo De Santis, Florigio Lista, Fabiana Arduini

P12 - SUSTAINABLE PAPER-BASED (BIO)SENSORS FOR AN INNOVATIVE ORIGAMI ORGAN-oN-CHIP DEVICE: PHOENIX-OoC
Luca Fiore, Laura Belcastro, Fabiana Arduini

P13 - 3D PRINTED PLATFORM FOR PHYTIC ACID DETECTION IN SPINACH LEAVES: INTEGRATED SAMPLE TREATMENT AND PAPER-BASED ELECTROCHEMICAL BIOSENSING
Ludovica Gullo, Igor Gabriel Silva Oliveira, Achref Chebil, Luca Fiore, Vincenzo Mazzaracchio, Willyam Róger Padilha Barros, Fabiana Arduini

P14 - DNA-BASED DIMERIZATION NETWORKS TO CONTROL IN-VITRO TRANSCRIPTION
Miriam Quattrociocchi, Simone Brannetti, Erica Del Grosso, Francesco Ricci

P15 - PHOTOLUMINESCENT PAPER-BASED PLATFORM INTEGRATING LASER-INDUCED ALUMINUM NANOSTRUCTURES FOR SMARTPHONE-BASED SELECTIVE DETERMINATION OF ORTHO-DIPHENOLS
Paolo Di Battista, Annalisa Scroccarello, Flavio Della Pelle, Dario Compagnone

P16 - PAPER-BASED ELECTROCHEMICAL BIOSENSOR FOR NT-PROBNP DETECTION IN CAPILLARY BLOOD
Rachele Petrocchini, Laura Fabiani, Viviana Scognamiglio, Luca Fiore, Giulia Volpe, Fabiana Arduini

P17 - DNA CONDENSATES AS BIOSENSING PLATFORM FOR THERAPEUTIC
Raj Paul, Laura Beltran, Alessandro Porchetta

P18 - BACTERIAL CHEMICAL SIGNALING: A NOVEL HPLC-MS/MS APPROACH FOR QUORUM SENSING MOLECULES ANALYSIS
Serena Arpaia, Andrea Magnani, Vincenzo Cantaluppi, Federica Dal Bello

P19 - IN VIVO QUANTIFICATION OF THERAPEUTIC BIOLOGICS USING ELECTROCHEMICAL DNA-SENSORS
Myriam Alfonsini, Alejandro Chamorro, Lisa Fetter, Tod Kippin, Giovanni Valenti, Kevin Plaxco, Alessandro Porchetta, Andrea Idili

P20 - SHADING NEW LIGHT ON THE SYSTEMIC RESPONSE FOLLOWING NEW SYNTHETIC OPIOID INTAKE THROUGH METABOLOMICS
Ludovica Chiodo, Ilenia Bracaglia, Francesco Bartolini, David Serafini, Matteo Marti, Manuel Sergi, Camilla Montesano

P21 - DEVELOPMENT OF A SANDWICH ELISA-BASED METHOD FOR FAST AND EFFECTIVE DETECTION OF PATHOGENIC Y. ENTEROCOLITICA IN FOOD SAMPLES: PRELIMINARY RESULTS

Giulia Volpe, Silvia Piermarini, Samra Mannan, Elisabetta Delibato

P22 - DYNAMIC NUCLEIC ACID SYSTEMS FOR BIOANALYTICAL APPLICATIONS

Daniela Sorrentino, Simona Ranallo, Mahdi Dizani, Eiichi Nakamura, Francesco Ricci, Elisa Franco

Premio “Alessandro Mangia”
Assegnato al Prof. Giuseppe Palleschi

per il suo contributo nell'ambito dello sviluppo di biosensori e metodi analitici innovativi.

La premiazione è stata introdotta da un contributo orale del Prof. Francesco Ricci (UniTOV), allievo del Prof. Palleschi, che ha ricordato gli studi e gli interessi scientifici del prof. Palleschi

Premio Giovane Ricercatore ”Cristina Giovannoli”
Assegnato al Dr. Simone Cavalera

KN01

The bioanalytical chemistry behind the development of lateral flow immunoassays: new approaches and paradigms

Simone Cavalera*[a]

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More than sixty years after its introduction to the world of scientific research, and to the market for in-vitro diagnostic (IVD) devices, lateral flow immunoassay (LFIA) is still the predominant technology for many Point-of-Care (POC) applications, particularly those related to infectious diseases. Typical LFIA consists of the use of antibodies for specific detection and a colorimetric probe (usually colloidal gold nanoparticles) for visual qualitative detection. This configuration has enabled the success of LFIA, whose characteristics overlap with the REASSURED criteria, universally recognised as the reference for POC testing [1]. As with any established technique, innovation and evolution is sought in the literature in order to improve the technology while complying with the generally associated limitations, namely sensitivity. Innovation often, involves the type of detection or signal reporter. This, however, results in the loss of one or more of these figures of merit in the face of the possible increase in sensitivity.

However, it is possible to apply a different approach, that does not involve modifying the technology or the configuration of the test, but rather is based on a more critical study of the phenomena underlying the functioning of the test and the attempt to develop intelligent strategies aimed at optimising the specific interaction or yield of traditional materials and reagents. This approach sees as its reference the fundamental principles of pure bioanalytics, which also acts as a bridgehead in the encroachment from theory to application. Indeed, the key role of the bioanalytical chemist makes it possible to understand and develop the best performing system on the basis of the specific need, thereby overcoming specific analytical challenges. This research can lead to the breaking of long-standing dogmatisms, allowing us to come across discoveries whose impact goes beyond the single application, taking on a broader, if not sometimes universal, significance. The dual-antigen strategy, for instance, considered unfeasible by theory, exploits a recombinant antigen to both capture and detect specific antibodies in a biological sample more effectively than other formats. This approach has been exploited for the detection of antibodies to SARS-CoV-2 on >100 sera samples from hospitalised patients [2] and then applied at other sites with the same finding. Another aspect discovered and later named ‘antigen saturation hook effect’, is a particular hook effect that occurs in a LFIA using the same antibody in capture and detection. This phenomenon was demonstrated and modelled during the development of a multiplex LFIA for the diagnosis of infection with 5 serotypes of bovine foot-and-mouth disease virus [3]. Its validity was confirmed each time a similar system was encountered. The use of DoE was the key to remedying this problem and was the opportunity to include in the development of IVDs a discipline that was generally considered alien to it. In addition to allowing efficient optimisations on sandwich systems [4], this allowed us to gain experience in achieving sub-nanomolar sensitivities in a LFIA for cortisol detection by proposing a universal method for optimising competitive LFIAs [5], which are much more complicated to optimise.

This critical approach, which has resulted in discoveries and new paradigms in the development of LFIA, has enabled us to make a significant contribution to the field of bioanalytics without, in fact, having included more expensive or complicated, new materials, technologies or methods, but focussing on the REASSURED-ness of the developed LFIA.

References

[1] K.J. Land, D.I. Boeras, X.S. Chen, A.R. Ramsay, R.W. Peeling, Nat. Microbiol. 4 (2019) 46–54. https://doi.org/10.1038/S41564-018-0295-3.

[2] S. Cavalera, B. Colitti, S. Rosati, G. Ferrara, L. Bertolotti, C. Nogarol, C. Guiotto, C. Cagnazzo, M. Denina, F. Fagioli, F. Di Nardo, M. Chiarello, C. Baggiani, L. Anfossi, Talanta. 223 (2020) 121737. https://doi.org/10.1016/j.talanta.2020.121737.

[3] S. Cavalera, A. Russo, E.A. Foglia, S. Grazioli, B. Colitti, S. Rosati, C. Nogarol, F. Di Nardo, T. Serra, M. Chiarello, C. Baggiani, G. Pezzoni, E. Brocchi, L. Anfossi, Talanta. 240 (2022) 123–155. https://doi.org/10.1016/J.TALANTA.2021.123155.

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[5] S. Cavalera, A. Gelli, F. Di Nardo, T. Serra, V. Testa, S. Bertinetti, L. Ozella, C. Forte, C. Baggiani, L. Anfossi, Microchem. J. 208 (2025) 112450. https://doi.org/10.1016/J.MICROC.2024.112450.

Comunicazioni orali

OC1

Zeolite-based biomaterial for drugs' remediation: evaluating removal efficiency towards ibuprofen with an electrochemical device

Antonella Miglione[a], Dalila Capocotta[a], Panagiota M. Kalligosfyri[a], Gabriella Iula[a], Sossio F. Graziano[a], Stefano Cinti[a,b,c]

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[b] *Bioelectronics Task Force at University of Naples Federico II, Via Cinthia 21, Naples 80126, Italy*
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The increasing presence of emerging contaminants, such as pharmaceuticals, pesticides, and industrial chemicals, presents significant threats to water quality, ecosystems, and public health [1]. Among these, non-steroidal anti-inflammatory drugs (NSAIDs), like ibuprofen, are of particular concern due to their persistence in wastewater and their harmful effects on aquatic biodiversity. Long-term exposure to these contaminants in water sources can result in adverse human health outcomes, including hormonal disruptions and the development of antibiotic resistance [2]. As a result, effective remediation practices and in-situ monitoring of these contaminants are becoming increasingly critical.

Zeolites, microporous aluminosilicate minerals known for their exceptional adsorption capacity and ion-exchange properties, have emerged as promising biomaterials for sustainable wastewater remediation. Their ability to efficiently remove contaminants, combined with their regenerability, makes them an eco-friendly alternative for water treatment [3]. Additionally, electrochemical sensors have proven to be innovative tools for real-time monitoring of these contaminants, enabling the detection and quantification of trace levels and supporting more effective wastewater management strategies [4].

In this study, we developed and analytically characterized a polyester-based electrochemical sensor for detecting ibuprofen in wastewater, reaching a limit of detection of 1.6 µg/mL and a repeatability of 8% in wastewater. Zeolite-based remediation strategies were evaluated for their effectiveness in removing ibuprofen. Both surfactant-modified and unmodified zeolites were tested under various conditions of quantity and exposure time to optimize remediation performance. The best-performing system, using modified zeolites, achieved a 73% removal efficiency for ibuprofen. When integrated with a portable potentiostat, the sensor enabled real-time, on-site monitoring of ibuprofen levels, allowing for the assessment of zeolite remediation performance.

This study demonstrates the potential of zeolites as an effective and sustainable biomaterial for wastewater treatment and highlights the value of electrochemical sensing in evaluating the efficiency of remediation strategies.

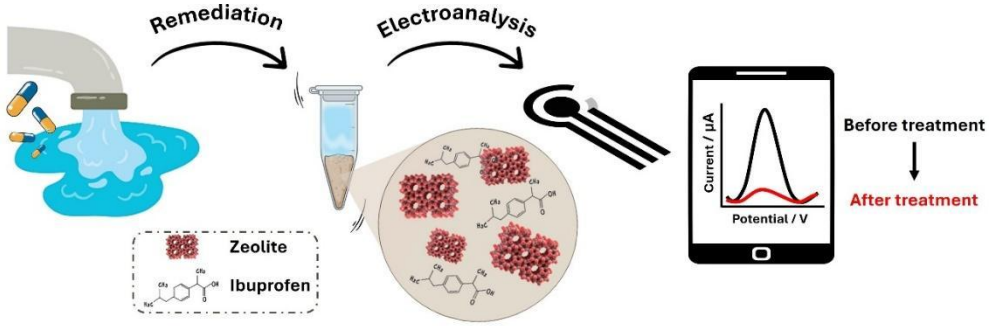

Figure: Workflow for electrochemical monitoring of zeolite-based remediation effectiveness against ibuprofen.

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OC2

Non-invasive diagnosis of colorectal cancer: from fecal biomarker discovery to electrochemical magneto-immunoassay

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Colorectal cancer (CRC) is the second-leading cause of cancer death worldwide.[1] Current screening programs are characterized by high false-positive rate, and, in case of positivity, they are followed by colonoscopy that involves an invasive procedure. Consequently, there is a strong need for innovative and non-invasive diagnostic tests to guide screening programs and early patient stratification.[2] The discovery and investigation of new CRC biomarkers in non-invasive stool samples is crucial to boost population engagement in preventive care screening.

The present work aims to combine the potential of mass spectrometry (MS)-based proteomics for the discovery of fecal proteins as CRC biomarkers with the implementation of electrochemical bioassays for candidate biomarker detection to be used for point-of-care testing.[3,4] Starting from a large cohort of patients who underwent colonoscopy, stratification was performed into 4 different groups, from healthy control to adenocarcinoma tumor. As for biomarker discovery, stool samples were subjected to a procedure which involves cell lysis, sonication and protein precipitation, enabling the extraction of 100 proteins per sample (on average). Protein extracts were analyzed by an untargeted MS-based shotgun protocol, using a nano-LC coupled to an Orbitrap Exploris 480 with a High-Field Asymmetric Waveform Ion Mobility System. Statistical analysis identified two promising biomarker candidates, here coded as PROT1 and PROT2, which show upregulation during cancer progression, also in accordance with previous literature findings.

Subsequently, the study focused on the development of electrochemical immunoassays for rapid, point-of-care detection of the two CRC biomarker candidates. The devised strategy involves a sandwich-type configuration which exploits carboxylated magnetic micro-beads as sensing platforms for monoclonal capture antibody immobilization. Monoclonal reading antibody was previously conjugated to HRP labelling enzyme in order to generate a chronoamperometric current signal associated to the reduction of quinone generated by enzymatic oxidation of hydroquinone, in presence of hydrogen peroxide as substrate. Initially, the focus was on PROT1 and an experimental design was applied to optimize the concentration of the capture and reading antibodies, that could ensure the highest signal with respect to the blank. A calibration curve was constructed in extraction buffer (Calprest® NG extraction solution, Eurospital Diagnostics), permitting to assess a LOD at ng/mL level of antigen; RSD% values lower than 15% were obtained in terms of inter-assay precision (*n*=3). Current efforts are aimed at refining non-denaturant protein extraction from stool, assessing the presence of matrix effect, and performing selectivity study against potential interfering proteins. After validation of the assay in matrix, it will be applied to sample of patients selected for external validation to assess clinical utility and usefulness in determining the selected biomarker by the developed biosensing analytical platform. A similar bioanalytical strategy will be applied also to PROT2 candidate biomarker.

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OC3

Activity based (bio)sensor for the monitoring of MutyH DNA glycosylase

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DNA is continually subject to interactions with mutagens which can lead to structural damage of the molecule, affecting human health. The most frequent oxidation-induced lesions in DNA are 8-oxo-7,8-dihydroguanine, often resulting in G:C to T:A transversion mutations. When this modified base pairs with cytosine on the double helix, it is recognized by the DNA glycosylase OGG1 and removed, initiating the base excision repair (BER). However, when OGG1 fails or the damage occurs during replication, it leads to a mismatch of 8-oxo-guanine:A, recognized by a specific glycosylase, MUTYH, which removes the adenine.
The primary disease associated with mutations in the MUTYH gene is MUTYH-associated polyposis (MAP). MAP is a hereditary cancer syndrome characterized by an increased risk of developing colorectal cancer and other types of cancer.
The literature presents various molecular methods for assessing DNA repair activity, primarily centred on the use of indirect techniques.[1] However, these methods have a variety of issues, including long analysis times and limited applicability in clinical settings. To enable direct monitoring of MUTYH activity, only few examples using chemically modified DNA probes with fluorescence readout have been reported.[2] they exhibit limited sensitivity and involve a high degree of chemical synthesis complexity. Here we propose developing a synthetic biology toolkit for real-time analysis of MUTYH activity. We design programmable nucleic acid capable of transducing glycosylase activity into downstream CRISPR-powered ultrasensitive detection. This is achieved by using a rationally designed DNA-based hybridization network based on the switching activity of a DNA probe called “DNA activator module” whose structural switch is controlled by glycosylase activity. Only when the DNA activator module populates an ON state the CRISPR/Cas12a is activated, thus generating a fluorescence signal output. (Figure 1)

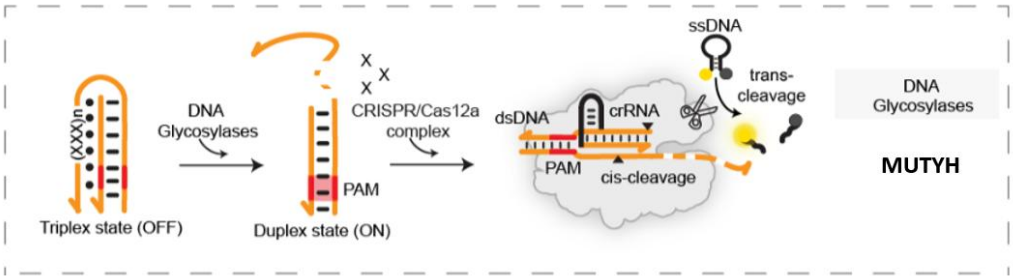

Figure: Description of Glycosylase-triggered CRISPR-Cas12 activity assay where the DNA activator module is identified in a DNA Triplex module.

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OC4

Seaweed (*G.gracilis*) protein hydrolysates: a valuable source of short- and medium-chain peptides with multifunctional properties

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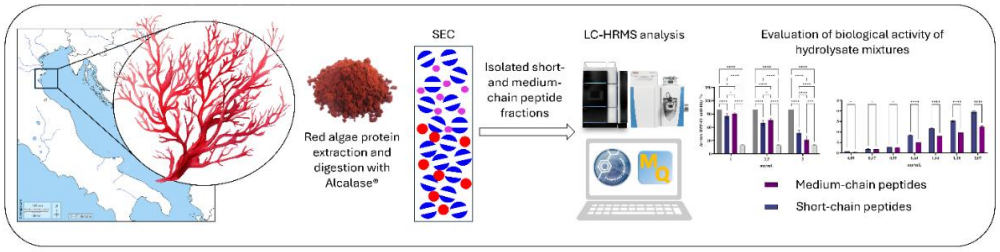
Seaweed-derived bioactive compounds are gaining attention due to their potential applications in the nutraceutical and pharmaceutical industries [1]. This study investigates the protein hydrolysates from *Gracilaria gracilis*, a red macroalga, as a valuable source of short- and medium-chain peptides with multifunctional properties. Enzymatic hydrolysis was employed to generate peptide fractions, characterized using liquid chromatography coupled with high-resolution mass spectrometry and peptidomic analysis, as previously described [2-3]. In total, 97 medium-chain peptides were identified by database confrontation with most of the identified medium-chain peptide sequences deriving from α and β subunits of phycoerythrin. Using a dedicated data processing workflow for short peptidome identification [3], 362 short-chain peptides were putatively identified after manually interpreting the MS/MS spectra. Biological activity evaluations demonstrated that short-chain peptides exhibited higher antioxidant potential and superior ACE inhibitory activity, whereas medium-chain peptides showed enhanced DPP-IV inhibition, highlighting their potential antidiabetic effects. The antioxidant properties were assessed using DPPH, ABTS, and FRAP assays, confirming the ability of these peptides to scavenge free radicals and reduce oxidative stress. The ACE and DPP-IV inhibitory activities were tested both *in vitro* and *in situ* using Caco-2 cells, revealing significant functional differences between short- and medium-chain peptides in their enzyme-inhibitory effects. The findings highlight the potential of *G. gracilis* hydrolysates as functional food ingredients with antihypertensive and antidiabetic properties, supporting their sustainable valorization in the blue economy and bio-based circular economy [4].



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OC5

Selective extraction of Phytoprostanes from food samples using molecularly imprinted polymers and confirmation by UPLC-QqQ-LIT-MS/MS and UHPLC-Q-Orbitrap-MS.

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Phytoprostanes (PhytoPs) are non-enzymatic products of lipid peroxidation derived from α-linolenic acid. While PhytoPs are not essential for the metabolic activity of living cells, they are considered key components in oxidative damage detection systems and serve as excellent biomarkers for oxidative degradation in plant-based foods. These compounds have gained increasing attention due to their potential role in assessing the quality and stability of food products, as well as their implications in plant stress responses. Despite their importance, there is a limited number of analytical methods for PhytoPs reported in the literature. Most methodologies rely on homemade chemical synthesis, and only a few PhytoPs standards are commercially available. Various techniques have been developed for the qualitative and quantitative determination of PhytoPs in food matrices. Among these, ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) has emerged as a powerful tool, providing high sensitivity, selectivity, and rapid analysis [2]. Furthermore, recent advancements in analytical chemistry have introduced high-resolution mass spectrometry (HRMS), including Orbitrap-based technologies, which offer enhanced mass accuracy and structural elucidation capabilities. Additionally, the integration of biosensors and other novel detection approaches is being explored to refine the quantification of PhytoPs in complex biological and food matrices. The extraction of PhytoPs from food samples presents several challenges, including matrix complexity, compound stability, and extraction efficiency. Conventional extraction methods, such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE), may not always provide optimal recovery due to the susceptibility of PhytoPs to oxidation and degradation. In recent years, molecularly imprinted polymers (MIPs) have been introduced as a promising approach for selective extraction of target compounds, including PhytoPs [3]. MIPs offer high specificity, stability, and reusability, making them suitable for complex sample preparation processes. In this study, we propose an alternative strategy for the rapid synthesis of MIPs and their application in the selective extraction of PhytoPs from commercial food samples. The synthesized MIPs were evaluated in combination with UHPLC-MS/MS as the targeted analytical method. Additionally, an Orbitrap IQ-X Tribrid mass spectrometer was employed for confirmatory analysis, leveraging high-resolution mass spectrometry (HRMS) for precise identification and characterization of PhytoPs. The MIPs were synthesized using a cost-effective and rapid sonochemical free-radical polymerization method, which required only 5 minutes for completion. 4-Cyclopentene-1,3-dione was selected as a dummy template to mimic the structural characteristics of PhytoPs. Methacrylic acid (MAA) and methacrylamide (MMA) were tested as functional monomers, with ethylene glycol dimethacrylate (EGDMA) serving as the cross-linker and 2,2'-azobisisobutyronitrile (AIBN) as the radical initiator. The performance of MIP-based dispersion solid-phase extraction (dSPE) was evaluated using five isoprostane standards: 8R-Isoprostane (8-ISOR), 8S-Isoprostane (8-ISOS), 8-Keto-Isoprostane (8-KETO), 11β-Isoprostane (11β), and 5-IPF2α-VI. Among the synthesized MIPs, MAA-based MIP demonstrated the best performance in terms of recovery, selectivity, and reproducibility. The developed MIP-dSPE method was successfully applied to food samples, proving its capability to selectively extract and enrich PhytoPs. Putative identification of extracted PhytoPs was confirmed using HRMS, further supporting the applicability of MIPs in food analysis. In conclusion, this study introduces a novel and efficient MIP-based extraction method for PhytoPs, combining rapid synthesis, high selectivity, and compatibility with advanced mass spectrometric techniques. The results highlight the potential of MIPs as valuable tools in food metabolomics, offering an innovative approach for the selective enrichment and analysis of oxidative stress biomarkers in plant-based products. Future research may focus on optimizing MIP formulations for broader applications, including environmental and biomedical sample analysis.

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OC6

Determination of Aflatoxin B1 (AFB1) level in whole blood by means of quantitative Dried Blood Spots (DBS) microsampling coupled to Enzyme Linked ImmunoSorbent Assay (ELISA)

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Aflatoxins represent a class of carcinogenic mycotoxins produced as secondary metabolites by *Aspergillus flavus* and *Aspergillus parasiticus* fungi in several crops (corn, wheat, rice, peanut). Among the over 20 existing aflatoxins, Aflatoxin B1 (AFB1) is the most prevalent and toxic and it was classified in the Group I of carcinogen compounds by the International Agency for Research on Cancer [1]. The main route of exposure to AFB1 for humans and animals is the ingestion of contaminated food, whilst acute intoxications are more often observed in occupational settings through the inhalation of airborne grain dusts [2].

This study aimed to develop an innovative, straightforward, rapid, and cost-effective approach to measure the AFB1 level in whole blood. The whole procedure is based on a quantitative Dried Blood Spot (DBS) microsampling, by the *Capitainer®50* card, ensuring the collection of 50 µL of capillary blood. The samples are then processed by a competitive direct ELISA immunoassay , which was previously optimized in terms of dilution factor of the polyclonal antibody AFB1-selective coated, the analyte labeled AFB1-HRP concentration and the incubation times.

During the analytical method development, fortified blood at 1.2, 3.0, 24 ng/mL was deposited on the card. After 3 hours of drying, the spots were peeled off and placed into 96-well plates to perform the extraction by adding 150 µL of methanol:water 70:30 and shaking at room temperature for 30 minutes. After a further dilution with 150 µL of water, the extracts were analyzed by means of the ELISA method.

The preliminary results showed an IC90 value of 0.1 ng/mL and adequate recovery (85-100%) in fortified extracts of whole blood, demonstrating no significant matrix interference. The inter- and intra- day and inter-operator precision fulfilled the bioanalytical guidelines requirements (below 20%).

These data are promising for the potential application of easy DBS microsampling to a cohort of potentially exposed subjects, offering the possibility of monitoring programs in remote geographical area without the need of trained healthcare professional. The coupling of DBS and the detection by the ELISA test allows the application even in rural locations and in low-income countries, where the risk of exposure to AFB1 appears to be higher than other regions.

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OC7

SERS spectroscopy for bioaerosol analysis and characterisation: challenges and future perspectives

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Bioaerosols are microscopic airborne biological particles such as bacteria, fungal spores, pollen, viruses, and their derivatives. The global spread of the COVID-19 pandemic as well as the rise of antibiotic resistance in healthcare settings demonstrate how bioaerosols have become serious public health concerns in both outdoor and indoor settings. Identifying and quantifying bioaerosol components is critical for assessing risks and setting appropriate exposure limits.

Specifically, new research opportunities have arisen thanks to the proliferation of real-time (RT) methods for autonomous, online detection and characterization of bioaerosols features [1]. However, effective online bioaerosol monitoring is hindered by the complexity, diversity, and great spatiotemporal variability of bioaerosols, as well as their mixing with abiotic components, both internally and externally. Though they are effective, traditional methods for bioaerosol analysis—such as culture-based and molecular approaches—are not always portable, have long processing periods, and can't always handle real-time analysis.

Because of its high sensitivity and specificity, and ability to be performed in complex biological mixtures, using portable and relatively inexpensive devices, surface enhanced Raman scattering (SERS) spectroscopy has attracted interest as a feasible method for detecting airborne pathogens at environmentally relevant concentrations and sensing trace environmental contaminants, among many other potential applications [2]. Unlike spontaneous Raman scattering, SERS spectroscopy has a short assay time and requires far smaller concentrations of analytes, making it particularly suited for bioaerosol studies. The possibility of a direct (also known as “label free”) detection can reduce the need for complex sample preparation, while the availability of portable instrumentation can facilitate on-site and RT monitoring.

However, the difficulty of standardising procedures to ensure consistent and comparable results, however, grows with the development of new technologies. Because of the lack of standardised methodologies, SERS sensing of bioaerosols remains challenging. Here we present state of the art in the use of SERS spectroscopy to examine bioaerosols. A critical evaluation of the experimental aspects involved in the collection of SERS spectra is presented, and the potential applicability and weaknesses of various experimental setups are highlighted, helping to provide a solid foundation for further research and the practical implementation of SERS spectroscopy for bioaerosol analysis and characterisation in various environmental and clinical settings.

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OC8

Empowering precision medicine and well-being: A fully 3D printed miRNA detection platform for liquid biopsy applications

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The demand for robust and precise point-of-care devices is particularly high in personalized medicine, where rapid and accurate detection of biomarkers is crucial for tailored healthcare and well-being[1]. In this context, electrochemical sensors offer significant advantages due to their sensitivity, specificity, and adaptability[2]. The emergence of 3D printing technology has further expanded their potential by enabling the creation of innovative electrochemical well designs, making them suitable for a wide range of applications in electrochemical sensing[3]. This study aimed to develop a fully 3D printed electrochemical sensing device featuring a three-electrode system fabricated from conductive printing materials and incorporating a microwell as the sensing platform. The assay principle of a robust electrochemical screen-printed sensor[4] was adapted to this platform, integrating a well structure to enhance fluid control and enable the detection of a microRNA biomarker associated with lung cancer. The detection mechanism was based on the hybridization of the target miRNA with an immobilized DNA probe labeled with methylene blue as a redox mediator. The sensor was thoroughly characterized and optimized, achieving a dynamic detection range of 0.001 to 400 nM and a lower limit of detection in the picomolar range, surpassing conventional screen-printed sensors. Furthermore, the sensor demonstrated high selectivity for the target miRNA over other sequences, confirming its specificity. These findings highlight the potential of 3D printing technology in advancing personalized diagnostics and prognostic healthcare solutions, ultimately contributing to improved patient outcomes and well-being. The successful integration of this sensor into clinical settings could significantly enhance diagnostic capabilities and medical decision-making by providing a reliable, cost-effective, and customizable sensing platform for detecting critical biomolecules such as miRNAs.

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OC9

Smartphone-based colorimetric paper sensors for free chlorine and pesticides detection in clinical and environmental matrices

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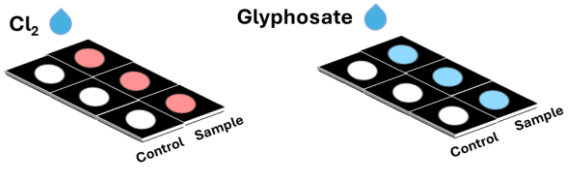
Chlorination is a widely used method for disinfecting drinking water, involving the addition of chlorine or its compounds to generate disinfecting agents like hypochlorous acid (HOCl) and hypochlorite ions (OCl^-). Nevertheless, it is crucial to monitor the concentration of free residual chlorine, defined as the amount of disinfectant remaining in water after treatment because the disinfection by-products (DBPs) such as trihalomethanes (THMs) and haloacetic acids (HAAs), are potentially harmful to human health[1]. In addition to water analysis, the detection of chlorine could be important in diagnostics of respiratory diseases. It has been reported that significant increased levels of chlorine (Cl_2) are found in breath condensate of patients with allergy during pollen season compared to post-season[2]. The ISO 7393-2 standard[3] detects free residual chlorine by its reaction with N, N-diethyl-p-phenylenediamine (DPD), producing a pink-colored complex whose intensity is proportional to the residual chlorine concentration. However, the method requires sophisticated instrumentation and trained personnel, making routine monitoring expensive and time-consuming. To address these limitations, we developed a rapid, portable and user-friendly colorimetric paper-based sensor with an estimated cost per sample of less than 0.1 euro. The sensor leverages the color change resulting from the DPD reaction enabling both visual detection of free residual chlorine quantitative analysis via smartphone image capture. To assess potential applications both for water monitoring and for detection of chlorine in clinical samples the paper sensor was used to detect chlorine in bottled water samples and exhaled breath condensate, showing adequate analytical performance and good recoveries with spiked real samples.
Pesticides are another category of water contaminants that urgently requires a rapid screening method to safeguard the environment and human health. Glyphosate (Glyp), which is still worldwide used, is suspected to have severe health effects causing respiratory, myocardial, and neuromuscular dysfunctions. Hence, designing a straightforward, precise, and affordable method for glyphosate detection with high sensitivity and selectivity is strongly valuable. To this end, a colorimetric reaction based on the competition between Cu^{2+}, TMB and Glyp was also implemented in a portable paper-based sensor. Indeed, free Cu (II) ions catalyze the oxidation of colorless TMB into his blu oxidated form, but the presence of glyphosate inhibits the reaction by forming the Glyp-Cu^{2+} chelate[4].



Reflectance signal detection by smartphone

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OC10

Advances in electrochemical magneto-genoassays for multi-allergen detection in food

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The presence in foods of undeclared allergenic ingredients, emerging allergens or hidden allergens resulting from accidental contamination can cause serious reactions in sensitized individuals and clearly represents a significant worldwide public health issue [1]. Given the risk they pose to consumers, their detection is of paramount importance for food safety. Mass spectrometry (MS) detection methods, particularly liquid chromatography-mass spectrometry (LC-MS), have been emerging as confirmatory strategies for unambiguous identification and accurate quantification of allergens over approximately the last 10-20 years [1]. Antibody-based assays such as enzyme-linked immunosorbent assay (ELISA) and DNA-based assays have also expanded significantly over the past two decades, achieving challenging sensitivities. Although a number of ELISA kits are available for specific allergens, a challenge in ELISA detection is the variability of data generated across different kits targeting the same allergen, thus providing considerably different results for the same analyte as well as the risk of antibody-cross-reactivity with non-target antigens from similar species [2]. On the other hand, DNA based assays such as PCR offer molecular specificity but have complex sample preparation process and laboratory equipment, hindering their wide applications in the portable on-site detection of food allergens [2]. In this context, electrochemical genoassays provide a valuable tool to maintain the specificity afforded by DNA sequence detection while enabling rapid testing using inexpensive and portable instrumentation.
Taking into account the challenges of managing multiple food allergies, the aim of the present study is the development of an electrochemical magneto-genoassay-based portable platform for multi-allergen detection. First, a sandwich magneto-genoassay, which has been shown to be highly specific and sensitive in our previous studies [3,4], was devised to detect a specific soybean (*Glycine max*) sequence at trace levels in wheat flour samples. Promising analytical performance of the assay was achieved, reaching picomolar detection limit on synthetic target soy DNA. The effect of the size of magnetic beads was tested by assembling the sandwich assay on 2.8 and 1 µm beads. Our findings prove that enhancing the surface properties of the beads is crucial for improving detection, as smaller bead sizes result in higher signal-to-noise outputs. Preliminary tests on genomic DNA extracted from wheat flour containing 0.1 to 5% soy highlighted the effectiveness of enzymatic digestion with respect to conventional ultrasonic treatment, thus improving the efficiency and reproducibility of the procedure. Furthermore, the assay proved highly selective, as no signal was detected by incubation of genomic DNA extracted from wheat flour. Future developments of the assay will involve the implementation of PNA-based probes able to covalently link the reporter probe to the microbeads thanks to a proximity-induced bio-orthogonal ligation [5]. The reaction will be triggered by the recognition of the target DNA and covalent link will avoid signal loss during the required washing steps, thus significantly improving the assay sensitivity. Finally, the developed electrochemical magneto-genoassay will be extended to detect multiple allergens using magnetic beads functionalised with probes targeting specific DNA sequences of different allergenic species. The present study is expected to provide a rapid and portable platform capable of detecting multiple allergen species at trace levels in foods, providing a valuable analytical tool in addressing the issue of hidden allergen determination.

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Acknowledgement

Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No. 341 of 15/03/2022 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU Award Number: Project code PE0000003, Project title “Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods” (ONFOODS). Wheat flour samples were kindly provided by Barilla G. & R. Fratelli S.p.A.

OC11

Human fecal short chain fatty acids determination by SPME-GC-MS: disease monitoring by a chemometric approach in pediatric Crohn's Disease

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The incidence of pediatric Crohn's Disease (CD) is increasing worldwide, particularly in countries with previously reported low rates of inflammatory bowel diseases (IBD). The dramatic rise in incidence of IBD in newly industrialized countries, shifting to western dietary habits, represents one of the foremost clues of the relevant influence of environmental factors, among which diet, in the pathogenesis of CD. Despite numerous available medications, achieving prolonged remission remains challenging for many patients, often requiring long-term immune suppression. Guidelines recommend dietary therapy for children with mild-to-moderate CD, especially exclusive enteral nutrition (EEN). EEN involves liquid medical formula as the sole energy source for 6–8 weeks with excellent remission together with many limitations that include poor adherence, social and mental-health impacts, and need for multidisciplinary support. In response to these challenges, the Crohn's Disease Exclusion Diet (CDED) has emerged as an alternative approach. CDED is a whole-food diet specifically designed to minimize exposure to potentially inflammatory dietary components and avoid exposition to noxious substances hypothesized to have harmful effect on microbiome, intestinal permeability, and immunity[1].
From disease monitoring point of view, the quantitative determination of the short chain fatty acids (SCFAs) in biological fluids has gained much attention in the last years because of their relevant implication as fermentation end products of gut microbiota. The fecal sample has gained a major role being the most accessible biological matrix which can directly probe the connection between intestinal bacteria and the physiology of the holobiont. SCFAs abundance depends on the amount of microbiota present in the colon, the substrate source, and the gut transit time. Alterations of SCFAs circulating concentrations reflect shifts in gut microbiota diversity and richness, classified as a dysbiosis status that has been strongly associated with IBD[2].
The present study was aimed to evaluate a possible correlation among SCFAs, therapy and disease remission status. The content of SCFAs, in fecal samples of 17 pediatric patients affected by CD, was measured by gas chromatography coupled to mass spectrometry, using solid phase microextraction as the sample preparation technique according to a previous developed method[3]. In total, 71 biological samples of patients who received CDED were collected longitudinally and population characteristics were recorded. All the patients included in our cohort had active disease, documented by an increase of fecal calprotectin and others biochemical parameters. Quantitative data of fecal fatty acid content (C2-C5) were studied using the multivariate principal component analysis (PCA) technique together with clinical, demographic, anthropometric and laboratory data. The impact of biological therapy on gut SCFA-producing bacteria was also analysed. PCA highlighted that the content of SCFAs was correlated to the severity of the disease.

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OC12

Multichannel laser-induced graphene electrochemical device for paper-based flow-analysis of phenolic antioxidants

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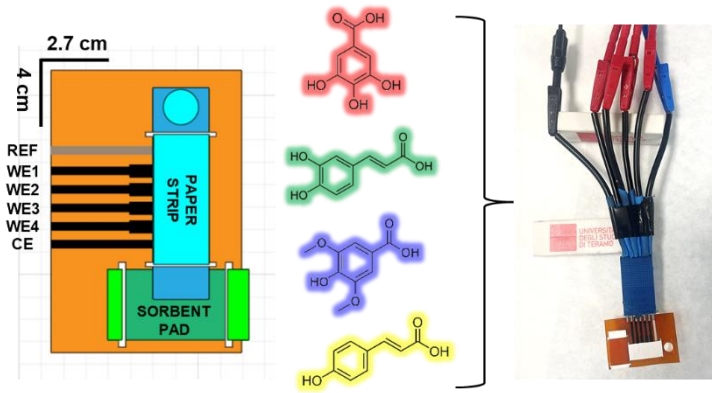
In the last decades, the use of graphene in the sensoristic scenario has emerged predominantly, thanks to its favorable electrical, mechanical, and chemical features, resulting particularly appealing in the form of self-supporting conductive films. These features represent a significant opportunity for manufacturing highly performing electrochemical (bio)analytical devices and microfluidic systems. Unfortunately, the 'synthesis' of graphenic films and their integration into lab-made devices, using basic laboratory equipment, remains a critical issue, which makes this material prerogative of a few.

Herein, an integrated paper-fluidic graphenic device with a multichannel configuration has been developed for the simultaneous quali-quantitative analysis of polyphenols in food samples. For the device construction, a polyimide foil has been used as the device base and 'source of carbon' for laser-induced graphene (LIG) generation. The integrated device consists of 4 working and 1 counter electrodes of LIG and a stencil-printed reference electrode; LIG and device geometries were obtained and defined using a CO_2-laser plotter. Eventually, the device has been equipped with a sampling paper-strip, that moves the sample to the sensing multielectrode systems without the need for external pumps.

At first, the CO_2-laser parameters were optimized to produce conductive LIG with useful features. Then, the optimal LIG sensing elements' electrochemical and morphochemical features were carefully studied, and their sensing ability toward the different classes of analytes was studied. Then, the complete device was challenged for the capillary-fluidic analysis of about twenty food samples characterized by the simultaneous presence of different phenolic compounds. The analysis was performed via chronoamperometry charging the sample onto the paper strip and applying at each of the 4 LIG working electrodes a different overpotential. The proposed analytical platform, thanks to the LIG electrocataliticiy, allows the discrimination and simultaneous determination (at micromolar levels) of the sample phenolic compounds, characterized by different structures and antioxidant capacities.

Summing up, this work demonstrates how CO_2-laser-based technologies can allow the easy production of graphenic-films and analytical device components useful to develop tailor-made point-of-need/care testing.



OC13

An AF4 approach for the characterization of innovative diagnostic tools in biological fluids

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Precision and personalized medicine aim to customize treatments by considering each patient's unique biological profile. Liquid biopsy, which analyzes biomarkers from biological fluids, enhances these patient-centered approaches by providing a non-invasive alternative to traditional tissue biopsies. Among the most promising biomarkers are extracellular vehicles (EVs), which are key mediators of intercellular communication and play a crucial role in disease development[1]. Common strategies for EV biomarker analysis rely on immunological methods and biosensors, utilizing antibodies, aptamers, and, more recently, bacteriophages as recognition elements. Bacteriophages, already employed in various diagnostic and therapeutic strategies, are versatile tools that offer several advantages over traditional recognition systems[2]. Due to their high stability and ease of genetic engineering, they can be modified to selectively bind multiple units of a biological target, thereby improving sensitivity[3]. Furthermore, as self-replicating biological entities, bacteriophages can be produced on a large scale in an economical and sustainable manner, overcoming the cost limitations of antibodies. Their filamentous structure also facilitates immobilization on sensor surfaces, further enhancing signal sensitivity and reproducibility. From production to application, fast and reliable screening tools are needed to assess the quality of bacteriophage synthesis, chemical functionalization, and purification. However, conventional techniques such as spectrophotometry, fluorimetry, and SDS-PAGE are time-consuming, not easily automated, and, in some cases (e.g., SDS-PAGE), technically complex. Advanced methods like HPLC-MS and NMR are unsuitable due to the complexity of bacteriophage systems, often yielding results that are difficult to interpret. To address this technological gap, we utilized a Hollow-Fiber Flow-Field-Flow Fractionation (HF5) multi-detection (MD) platform to analyze bacteriophages differing according to purification methodology, engineering process, and conjugation. Our semi-automated method enabled a native spectroscopic characterization of bacteriophage size and shape in under 40 minutes, while also providing rapid insights into their purification degree. Furthermore, by exploiting the intrinsic filtration properties of AF4, we developed an additional approach to evaluate the phage's conjugation degree in less than 25 minutes. While mainly focused on characterization, this work also suggested AF4's potential as a preparative tool due to its non-destructive nature and scalability. Overall, these findings highlight the dual potential of HF5-MD as a powerful purification and quality control tool for bacteriophage-based nanosystems, streamlining the long and complex production pipeline.

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Acknowledgment

Financed by the European Union – NextGeneration EU through the Italian Ministry of University and Research under PRIN 2022 RESOLVE, 202233FTW8 CUP B53D23013360006.

OC14

Point-of-care device for hemoglobin detection in capillary blood

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In recent years, one of the great challenges in current medicine is the diagnosis of diseases at an early stage, allowing both an increase in the chances of treatment of patients, a reduction in the cost of treatment, and optimizing the use of health care resources. Point-of-care (POC) devices are a promising alternative to fulfill this goal, as they enable rapid, cost-effective, and close-to-patient diagnosis without the need to transport and store samples to the laboratory. The future implementation of these devices in the healthcare field will allow for reliable and rapid analysis without sample pretreatment, avoiding the use of expensive instrumentation and highly skilled personnel [1]. Among the various applications of POC, the assessment of hemoglobin levels is of particular clinical relevance, as it provides essential information for diagnosing and managing numerous medical conditions [2]. Hemoglobin (Hb) is a tetrameric heme protein found in red blood cells (RBCs). It contains four iron atoms essential for oxygen transport from the lungs to various tissues in the body. Additionally, the same molecule is responsible for carrying a portion of carbon dioxide from the tissues back to the lungs. Conventionally, the Hb test is a specific and sensitive indicator for diagnosing anemia and other disorders related to abnormal Hb levels in the blood. Normal hemoglobin levels are strictly dependent on age and sex. According to the World Health Organization (WHO), the normal Hb range for adult males is 13–18 g/dL, while for adult females, it is 12–16 g/dL. The conventional method used for hemoglobin measurement is the Drabkin test [3]
Herein, an innovative smart POC device for hemoglobin detection was developed. The POC device consists of a miniaturized electrochemical sensor combined with a portable potentiostat, exploiting a Bluetooth module for data transmission. The electrochemical cell consists of one silver-reference electrode, one graphite-counter electrode, and two graphite-working electrodes able to detect hemoglobin by an easy sampling of few µL of capillary blood, providing a revolutionary clinical analytical tool capable of transforming the medical approach and the hospital system that we know today, promising a healthcare landscape characterized by speed, accuracy, and accessibility. Our device utilizes an innovative electrochemical approach based on chronoamperometry, complemented by impedance spectroscopy, to measure hemoglobin concentration with high sensitivity and specificity. A disposable screen-printed electrode modified with a selective redox mediator, namely Metilen Blue, enables the direct detection of hemoglobin through its electroactive properties. The integration of impedance spectroscopy further enhances accuracy by accounting for variations in blood matrix resistance. The developed system is characterized by its user-friendly operation, requiring only a small volume of capillary blood (≤10 µL), no sample treatment, and a short analysis time (<2 minutes). Calibration experiments were conducted using blood samples (>40 patients) with known hemoglobin concentrations at the Molinette hospital in Turin, demonstrating a strong correlation with standard laboratory methods (R^2= 0.827), with a linear regression described by the following equation: y= 0.1022x -0.757. The obtained results by using the POC device ensure its applicability in routine screening and point-of-care diagnostics.

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OC15

FFF-driven biomarker profiling via improved isolation of Extracellular Vesicles

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Extracellular vehicles (EVs) are spherical nanoparticles secreted by cells, playing crucial roles in biological processes such as intracellular communication and in the development of various diseases. Consequently, EVs are increasingly studied as diagnostic biomarkers and therapeutic tools. However, effective isolation of EVs remains challenging due to their low concentration and high heterogeneity. Traditional isolation techniques, including ultracentrifugation, size-exclusion chromatography (SEC), and ultrafiltration, often require large sample volumes and exhibit low efficiency in terms of time and purity. Additionally, these methods may compromise EV integrity, potentially affecting their biological activity and hindering their characterization under native conditions.

In this context, Hollow Fiber Flow Field-Flow Fractionation (HF5) emerges as a promising solution for EV isolation [1]. Operating under native, gentle conditions, HF5 preserves vesicle structure and biological activity. It can be coupled with various detectors, enabling detailed characterization of EVs' size, composition, and shape. Furthermore, due to its non-destructive nature, HF5 allows for the collection of fractions for downstream analysis.

In this study, we utilized an HF5 system to collect EV-enriched fractions from low amount of plasma and confirmed the presence of vesicles through Western Blot analysis of common vesicular markers. Based on these results, the fractions were further characterized using a multi-detection HF5 platform and a multiplex bead-based system to profile surface markers, assess size and shape, and evaluate HF5 performance in EV isolation.

Our method effectively isolated biologically active EVs directly from human plasma, highlighting their roles in biological processes and their potential as disease biomarkers. HF5 achieved high EV purity, reduced aggregation, and reproducibility. Moreover, the FFF isolated EVs were enriched in EV and platelet markers, overlapping with markers of SEC-derived vesicles. Thus, this technique enables sequential isolation of EVs across a wide size range while maintaining sample integrity, making it suitable for cancer translational and basic research, surpassing benchmark approaches.

In conclusion, the HF5 platform not only ensures efficient and native separation of EVs from plasma but also facilitates their downstream molecular characterization, overcoming limitations of traditional approaches. These findings underscore HF5 as a powerful tool for studying EVs in both physiological and pathological conditions, paving the way for improved biomarker discovery and functional studies.

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Acknowledgment

Financed by the European Union – NextGeneration EU through the Italian Ministry of University and Research under PRIN 2022 RESOLVE, 202233FTW8 CUP B53D23013360006.

OC16

Extracellular vesicles detection by exploiting electrochemical magneto-assays

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Initially considered simple cellular debris, extracellular vehicles (EVs) are now gaining significant attention due to their potential correlation with cancer diseases. These are particular membrane-enclosed nanometric particles found in biological fluids. However, the classification of EVs and most of their roles inside the human body remain unclear.
Gaining information on the type and amount of these vesicles in biological samples might be of great help as a further advanced step in the fight against cancer. To this end, several approaches have been used in recent years to detect and characterise extracellular vesicles but have often been hampered by the high costs related to the complex equipment required, the demand for high sample volumes, and the incompatibility with point-of-care testing approaches. Lately, a simpler and cost-effective solution is increasingly being found in the field of biosensors.
This study examined a simple yet flexible bioanalytical magneto-assay to detect extracellular vesicles by relying on an electrochemical readout and superparamagnetic microparticles. These particles were coated with biorecognition elements (*e.g.*, antibodies or aptamers) able to recognise and bind to specific structures embedded over the surface of the extracellular vesicle (*e.g.*, tetraspanins). After the capture, the vesicles could be simply isolated through the application of a magnetic field. Then, the resulting conjugate was recognised by another biorecognition element marked with an enzyme and magnetically immobilised on the surface of a screen-printed sensor. The addition of the appropriate enzymatic substrate started an enzymatic reaction that yielded a vast number of electroactive molecules that could be detected *via* electrochemical strategies, thus achieving a signal amplification mechanism. After studying the reliability of this biosensing scheme in the detection of nucleic acids and cancer-related EVs through manually operated steps, the introduction of microfluidic approaches was considered to (semi)automate the overall procedure. Indeed, careful optimisations were necessarily introduced, given the particular susceptibility to stresses of EVs, especially during handling procedures, and the general lack of similar standardised biosensing workflows in the field of extracellular vesicles. The assay was then applied to the detection of extracellular vesicles extracted from breast cancer, colon cancer, and glioblastoma cell lines.

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Acknowledgement

Regione Toscana Bando Salute 2018 (Research project CUP n. D78D20000870002), the European Climate, Infrastructure and Environment Executive Agency, HORIZON-MISS-2022-OCEAN-01, project iMERMAID, grant number 101112824, and Fondazione Umberto Veronesi (Post-Doctoral Fellowship 2025) are acknowledged for financial support.

OC17

Oxidized cholesterol compounds in the development stages of zebrafish analysed by LC-MS/MS and machine learning approaches

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The investigation on oxysterols profile in a suitable in vivo system could provide useful information on the amount, roles and biological functions of these molecules that are enzymatically generated by cytochrome P450 (CYP450) family, or via autooxidation, or even via both pathways in synergy. Oxysterols, which are oxidized derivatives of cholesterol, are reliable indicators of oxidative stress and lipid oxidation. Zebrafish (Danio rerio), due to their genetic similarity to humans and transparent embryos, serve as an excellent model for such studies. Zebrafish is used in toxicology to estimate the effects of xenobiotics and their teratogenic consequences; this animal model presents several advantageous features as high fecundity, rapid embryonic development (24 h) and external fertilization [1]. The knowledge of the oxysterols profile in zebrafish, during early embryonic stages, provides important information on the role and biological function of these molecules [2]. This research focuses on the determination of oxidative stress in zebrafish embryos by assessing oxysterols as biomarkers at different developmental stages: 3, 24-, 48-, 72-, and 96-hours post fertilization (hpf). The study employs liquid chromatography-tandem mass spectrometry (LC MS/MS) for the precise quantification and analysis of oxysterols in zebrafish embryos. The methodology involves the rapid extraction of lipids using a modified solid-phase extraction (SPE) technique based on the method described by Fanti et al. [3] in their study on oxysterol profiling in zebrafish embryos exploiting the significant profile mutation during development stages using multivariate analysis. Following extraction, separation and detection are conducted using LC-MS/MS, which offers high sensitivity and specificity. The method was validated according to European Medicines Agency (EMA) guidelines, ensuring robustness and reliability. Results demonstrate that specific oxysterols are significantly elevated in embryos, proposing their use as biomarkers.

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OC18

Unraveling the data treatment issues of untargeted metabolomics: cholangiocarcinoma as a case study

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Cholangiocarcinoma (CCA) is a deadly cancer of the biliary epithelium whose etiopathogenesis remains largely unknown. With incidence and mortality rates currently increasing, it is now the second most common type of primary liver cancer and represents up to 3% of all gastrointestinal malignancies [1]. Metabolomics is a valuable tool for discovering disease-associated markers since changes in the concentration of metabolites in biofluids reflect alterations in the physiological status of an individual [2]. This approach can be used to understand altered metabolic pathways and to identify biomarkers suitable for early detection and diagnosis, prognostic stratification, and therapy response monitoring. Untargeted approaches, in which ideally the whole metabolome is analyzed, provide the most appropriate route for observational studies to detect unexpected changes in metabolites. However, metabolomics studies in untargeted fashion are inherently more complex, given the enormous acquired datasets, which introduce the need for careful data pre-processing, statistical treatment, and metabolite annotation. Variations in ionization efficiency, signal overlap, and incomplete databases can hinder accurate metabolite identification. Moreover, high-dimensional data require advanced computational methods to identify meaningful patterns while avoiding overfitting and batch effects. In this study, three cohorts of patients were recruited, i.e., localized CCA patients (n = 33), metastatic CCA patients (n = 18), and healthy controls (n = 17), and metabolomics datasets were obtained by untargeted MS. The experiments were carried out in agreement with the guidelines of the Metabolomics Quality Assurance and Quality Control Consortium (mQACC) [3]. Later, the acquired datasets were pre-processed by Compound Discoverer software for alignment, adduct annotation and grouping, prediction of the molecular formulas, gap filling, and peak area normalization. Once the data matrices were obtained, partial least square discriminant analysis (PLS-DA) in repeated double cross validation (rDCV) was employed to build the classification models, and variables of importance in projection (VIP) analysis was used to highlight the most relevant features to the models. After careful MS and MS/MS spectral annotation, all compound of exogenous origin (e.g., drugs and food-related compounds) were discarded, and the models were re-run. This process was iterated until the selected variables from VIP scoring were endogenous, and the contribution of the exogenous compounds to the models was canceled. The final models had classification rates of over 99% for CCA vs controls, and over 93% for localized CCA vs metastatic CCA vs controls, with several dysregulated sulfated bile acids among the annotated compounds. These results are one of the first attempts to define possible CCA biomarkers as well as discriminate localized and metastatic CCA forms, thus providing insights into the pathogenesis and progression of the disease.

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OC19

An all-paper-based analytical platform for electrochemical H_2O_2 detection in breath

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Point-of-care sensing devices play a crucial role in assisting clinicians with quick analyses, easy management, and painless monitoring of patient diseases. These sensing tools closely align with the ASSURED criteria set by the World Health Organization, where ASSURED stands for affordable, sensitive, specific, user-friendly, rapid, equipment-free, and deliverable to end-users. This concept was further updated to REASSURED, emphasizing real-time connectivity and ease of specimen collection.

In this context, in the past ten years, there has been significant progress in creating new point-of-care (POC) sensors that exploit the various properties of paper, such as porosity, capillary forces, and ease of modification. Indeed, paper has become a focus for developing electrochemical POC sensors due to its many advantages, including i) not requiring external equipment for liquid flow, ii) the ability to create reagent-free devices, and iii) the ease of performing multistep analyses using origami techniques. Additionally, an important aspect of using paper is its capacity to hold reagents needed for analysis. This enables the paper-based sensor to be exposed to aerosols, such as breath, allowing the paper to become wet and dissolve the stored reagents. As a result, the exhaled breath aerosol can be easily sampled by incorporating collecting paper into a face mask and further analysed.

In this overall context, we are developing a whole-paper-based analytical tool for hydrogen peroxide detection in exhaled breath, taking into account the level of H_2O_2 in lung-associated diseases, i.e., cystic fibrosis. In this work, we leverage the use of an office paper electrochemical screen-printed electrode and a paper collector integrated into a face mask. Specifically, the paper-based collector enables the non-invasive sampling and the further treatment-free analysis of the biological sample. The office paper-based screen-printed electrode is modified with i) an electrodeposited gold nanoparticle layer, and ii) a Carbon black-Prussian Blue nanocomposite, enabling reliable detection of hydrogen peroxide in the condensed aerosol breath sample.

After a first optimization of the parameters to obtain an optimal gold nanoparticles electrodeposition (namely, potential and time deposition), the development of the sensor focused on studies related to hydrogen peroxide detection, optimizing the applied reduction potential. A linear correlation between the H_2O_2 concentration and the current was obtained in the range 3 µM-0.01 M, described by the calibration curve equation $y = (7.4 \pm 0.1) x – (0.5 \pm 0.4)$, $R^2 = 0.998$, with a limit of detection equal to 0.9 µM, calculated as $(3 \times \sigma_b / S)$, where σ_b and S refers to the standard deviations of 10 blanks and the slope of the calibration curve, respectively. Interference studies were performed using compounds typically found in breath samples (including glucose, urea, NaCl, $NaNO_3$, Valine, Arginine, Tryptophane, Glycine, Histidine, and Lactic acid), showing a negligible variation in the recorded current.

The final application on breath samples is currently under investigation, to assess the reliability of the developed analytical platform for real sample analysis.

In conclusion, the proposed analytical platform has the potential to open up new possibilities for the design of face masks as active analytical platforms for breath analysis, aiming to facilitate health management and improve the quality of patient life.

OC20

A sensor-based strategy for the rapid quantification of nucleic acids in lipid nanoparticle formulations

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Lipid nanoparticles (LNPs) represent a versatile delivery platform proposed for a wide range of nucleic acid-based therapies, including microRNAs (miRNAs) and precursor microRNAs (pri-miRNAs). Their ability to encapsulate and protect RNA from degradation, as well as to promote targeted cellular uptake, has led to the clinical approval of RNA-based medicinal products, such as COVID-19 vaccines [1]. In this context, a growing number of LNP formulations with enhanced transfection efficiency and biocompatibility are under development, requiring rapid, sensitive, and reliable quality control methods, particularly for the quantification of encapsulated RNA [2]. Currently, classical analytical techniques such as fluorescence, UV-vis spectrophotometry, and chromatography are widely used; however, cost-effective and user-friendly alternatives are needed to facilitate real-time monitoring of RNA encapsulation and provide immediate quality control. A novel sensor-based approach was recently demonstrated for the quantification of a model drug encapsulated in liposomes [3]. Building on this methodology, the present work explores the development of an electrochemical biosensor for the quantification of encapsulated nucleic acids in LNPs. As a model target in this study, miR-218 was selected, given its widely reported antitumor effects. The system employs commercial screen-printed gold electrodes modified with a DNA probe fully complementary to miR-218, integrated with a 3D-printed chamber that enables the simultaneous analysis of multiple samples. The use of Triton X-100 surfactant allows the controlled disruption of LNPs and the release of the encapsulated miR-218, achieving detection limits as low as 1 nM (Figure1). Furthermore, preliminary results are presented on a dual working electrode configuration, enabling the simultaneous detection of two different miRNAs within the same formulation. This was achieved by immobilizing distinct DNA probes on separate working electrodes, allowing multiplexed analysis within a single measurement. The results show a strong correlation with traditional fluorimetric methods, highlighting the potential of this electrochemical platform as a rapid, sensitive, and cost-effective tool for nucleic acid quantification in lipid-based formulations.

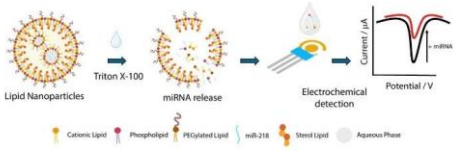

Figure: Workflow of the whole experimental setup.

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OC21

Integrated four-leaf clover-like MIP/PAD for maleic hydrazide colorimetric determination

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Sensitive and rapid detection of pesticide residues in foods are crucial for ensuring human safety. This study presents a laser-manufactured colorimetric Paper Analytical Device (PAD) integrating Molecularly Imprinted Polymer (MIP) for the detection of maleic hydrazide (MH), an anthropogenic plant growth regulator commonly found in vegetables. The PAD features a four-leaf clover-like design (4L-MIP/PAD) that facilitates a complete MH analysis process.

Fabricated using a CO_2 laser plotter, the PAD incorporates a CO_2 laser-activated fiberglass layer functionalized with UV-synthesized MIP. The central region houses the MIP, while each foldable leaf performs a specific analysis step. The MH analysis involves depositing a 40 µL sample extract on the MIP. Unbound components are removed using capillarity by folding the 'absorption-leaf,' followed by washing with water and folding the 'washing-leaf.' Finally, an alkaline solution elutes the analyte, triggering a colorimetric reaction on the 'colorimetric-leaf' pre-loaded with Folin-Ciocalteu (FC) reagent. The 4L-MIP/PAD graphical sketch is reported in Figure. The analysis is completed within 30 minutes, and results are captured via smartphone camera. The 4L-MIP/PAD achieves MH quantification in the range of 5–60 mg/kg (RSD ≤ 17%, n=3) with a detection limit (LOD) of 1.4 mg/kg, meeting regulatory limits for vegetables like potatoes (50 mg/kg), carrots (30 mg/kg), and garlic/onions (15 mg/kg). Despite the non-selectivity of FC reagent, the 4L-MIP/PAD demonstrates high selectivity for MH over other pesticides and food matrix components. Recovery rates for garlic, onion, and carrot samples ranged from 82.0% to 123% (RSD ≤ 21%, n=3). This innovative laser-fabricated cloverleaf PAD offers an efficient and reliable approach for MH detection in food matrices.

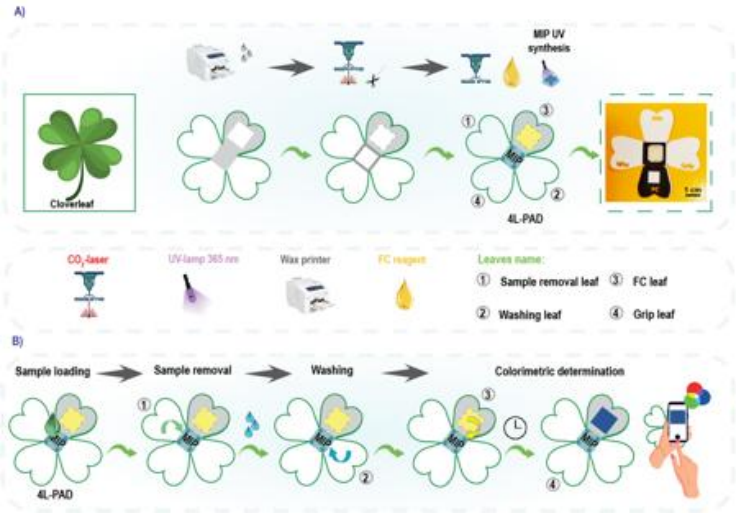

Figure: Graphical sketch of the Four-Leaf Clover-like MIP/PAD

Acknowledgment

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.

OC22

Biochar-based plastics electrodes for sustainable electrochemical detection of marine pollutants

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Anthropogenic activities are the primary source of marine pollution, with heavy metals such as cadmium (Cd) and lead (Pb) being of particular concern due to their toxicity, environmental persistence, and bioaccumulative potential [1]. Previous studies on Adriatic Sea sediments have reported concentrations of 3, 70, and 150 µg/g (dry weight) for Cd, Pb, and Zn, respectively [2]. Inductively coupled plasma mass spectrometry (ICP-MS) is the gold-standard analytical technique for the determination of these contaminants. However, its high operational cost and lack of portability limit its application in rapid analyses and point-of-need scenarios. Electrochemical techniques and electroanalytical sensors offer cost-effective and efficient alternatives for environmental contaminant detection, standing out for their selectivity, robustness, and portability. In this context, biochar, derived from biomass pyrolysis, emerges as a sustainable and promising material for the detection of Cd, Pb, and Zn [3].In this study, plastic electrodes [4] were fabricated using biochar obtained from the pyrolysis of sunflower at 750 °C, followed by chemical activation with chitosan (CS) and NaOH. New electrodes, composed of 75% graphite (GP) and 25% activated biochar (BCS), designated as GP_{75}_BCS_{25}, were characterised by cyclic voltammetry in $Fe(CN)_6^{3-/4-}$ (10 mM in 0.1 M KCl) (Fig. 1a). The results indicated that chemical activation significantly enhanced the electrochemical performance of the electrodes compared to those composed solely of GP, yielding electroactive surface areas of 0.0404 cm² for GP_{75}_BCS_{25} and 0.011 cm² for GP, calculated from peak current (Ip) measurements at different scan rates (10–400 mV/s) (Fig. 1b).

The detection capability of the plastic electrodes was assessed in acetate buffer solution and in real marine samples (sea sand), without aggressive acid digestion, a procedure commonly used in heavy metal determination. The metal extraction procedure included the treatment of 1 g of sea sand with 5 mL of acetate buffer (0.1 M, pH 4.5), the solution was sonicated for 1 h in an ultrasonic bath and shaking overnight at room temperature. Finally, the solid was separated by centrifugation and subsequent filtration through a 0.22 µm filter. Electrochemical detection of Cd, Pb, and Zn was carried out using square wave anodic stripping voltammetry (SWSAV) in a range of 25-600 ng/g (Fig. 2A), with an optimal Bi^{3+} concentration of 500 ng/mL (Fig. 2B). SWASV was performer with accumulation potential of -1.2 V for 300 s, a relaxation time of 60 s, a scanning potential range from -1.1 to 0.6 V, with a pulse amplitude of 40 mV, and a frequency of 10 Hz. Final concentrations were quantified by linear regression of current response using the standard addition method, and the limits of detection (LOD) calculated by signal to noise ratio=3 were 15.88 ng/g, 9.86 ng/g, and 6.66 ng/g for Zn, Cd, and Pb, respectively (n=3), which are less than the legal limit for tap and marine water. These results suggest the success of the potential applications of biochar as an electrode material when chemical activation treatment is carried out after pyrolysis. In this case, CS and NaOH induce structural and morphological changes that enhance ion exchange processes with heavy metals through interactions with functional groups such as OH-, NH_2- and CO-. In line with the principles of green chemistry, these results highlight the potential of activated biochar as a sustainable electrochemical material for the detection of environmental pollutants.

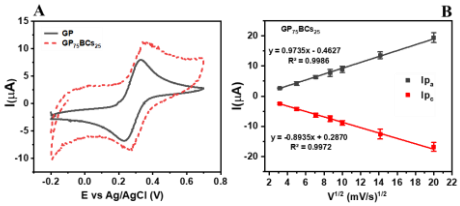

Figure 1. Cyclic voltammetry of $Fe(CN)_6^{3-/4-}$ redox system (1 mM in 0.1M KCl at 50 mV/s) at: plastic electrodes manufactured using of GP and $GP_{75}BCS_{25}$ (A). Dependence of the current (I µA) vs concentration of $Fe(CN)_6^{3-/4-}$ redox system using a potential window between -0.2 to 0.7 V for the plastic electrodes GP and $GP_{75}BCS_{25}$ (B).

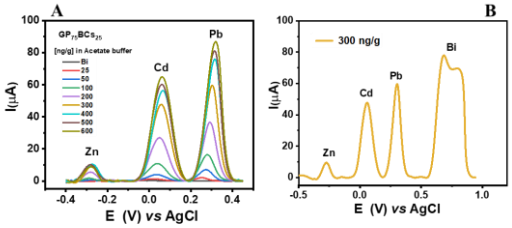

Figure 2. SWSAV curves of Zn, Cd and Pb solutions (25-600ng/g) with plastic electrodes $GP_{75}BCS_{25}$ in presence of Bi (500 ng/mL)(A-B) in acetate buffer (0.1M, pH 4.5).

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Acknowledgement

The authors acknowledge the Interreg Italy-Croatia Programme 2021-2027 for funding this research, conducted as part of the Standard project BRIGANTINE (ID: ITHR0200237).

OC23

Portable multifunctional potentiostat for static and flow-based electrochemical biosensing: application to *Pseudomonas aeruginosa*

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Multifunctional potentiostats are advanced instruments that improve the efficiency of electrochemical measurements, offering adaptable solutions for diverse applications. The integration with microfluidic systems enhances sample handling, reducing contamination risks and ensuring high analytical reproducibility.[1] This study presents the development and analytical characterization of FACILE 2.0, a portable multifunctional potentiostat designed to support electrochemical biosensors in both static and dynamic conditions, ensuring reliability under controlled flow. To validate its integration capabilities, the system was tested with a label-free electrochemical immunosensor for detecting Pseudomonas aeruginosa, a clinically and environmentally relevant Gram-negative bacterium known for antibiotic resistance.[2] Initial validation was conducted under static conditions using tap water samples. In this configuration, the Square Wave Voltammetry (SWV)-based sensor employed biochar, a carbonaceous nanomaterial derived from waste, to facilitate covalent binding of the immunological chain and enhance electrochemical performance.[3] The system achieved a Limit of Detection (LOD) of 30 CFU/mL. To enable real-time target monitoring, a custom-designed system for flow-based electrochemical measurements was developed and integrated. This internally designed platform combined 3D printing for structural components and laser cutting for the flow chamber. Strategically positioned magnets facilitated target recognition in flow, improving detection efficiency. Electrochemical measurements were conducted using antibody-functionalized magnetic microparticles, ensuring high sensitivity and specificity. Electrochemical analysis confirmed the device's reliability, showing comparable performance in static and dynamic configurations. These results highlight the potential of the potentiostat-immunosensor system for environmental monitoring, food safety, and diagnostics, providing effective solutions for on-site analysis and advanced biosensing.

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OC24

Cut out the middlemen: centrifugation-free isolation of EVs from conditioned medium

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Extracellular vehicles (EVs) play a pivotal role in intercellular communication and have emerged as promising biomarkers in liquid biopsy and regenerative medicine. However, their routine analysis remains hindered by time-consuming, multi-step isolation procedures, often requiring ultracentrifugation or precipitation-based pre-processing. These conventional approaches not only demand specialized equipment and large sample volumes but can also compromise EV integrity, leading to potential loss of biological information.
In contrast, native flow-based fractionation techniques, such as Flow Field-Flow Fractionation (FFF), offer a powerful alternative by enabling high-resolution separation of EVs in physiological conditions, minimizing sample perturbation and preserving their functional state [1].
Here, we present an optimized FFF workflow that enables direct isolation of EVs from conditioned cell culture medium, entirely bypassing centrifugation pre-processing. By integrating a high-volume injection step followed by in-channel reconcentration, we successfully separated EV subpopulations from free proteins and cellular debris in a single step, while preserving vesicle morphology and bioactivity, reducing dilution greatly. Real-time characterization via multi-angle light scattering (MALS) and dynamic light scattering (DLS) confirms the structural integrity of the isolated EVs, while Western blot analysis of the EV fraction validated the enrichment of exosome markers (CD9, CD81, Alix, Flotillin).
This streamlined, centrifugation-free approach significantly reduces processing time, minimizes sample handling bias, and enhances compatibility with on-chip biosensing platforms, paving the way for direct medium-to-sensor EV analysis. By eliminating intermediate steps, we propose a more efficient and scalable strategy for EV isolation, accelerating their integration into both diagnostic applications and therapeutic research.

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Acknowledgment

Financed by the European Union – NextGeneration EU through the Italian Ministry of University and Research under PRIN 2022 RESOLVE, 202233FTW8 CUP B53D23013360006.

OC25

MAIGRET: a diagnostic assay that combines antibody-triggered cell-free transcription and CRISPR/Cas systems

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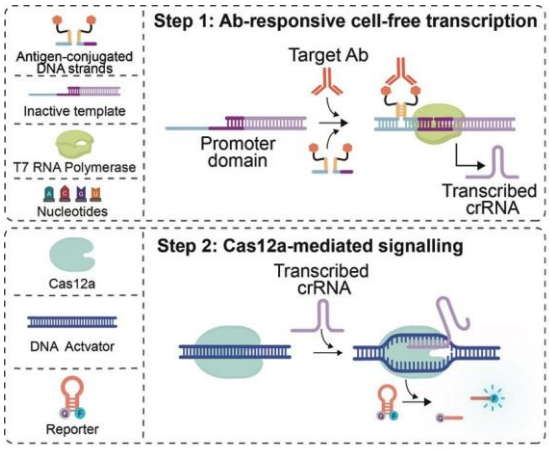
CRISPR-associated (Cas) nucleases coupled to a guide RNA are sequence-dependent nucleic acid-targeting systems that are able to recognize and cleave nucleic acid sequences. The programmability of CRISPR-Cas systems and the ability to combine target recognition, signal transduction and amplification have enabled the development of revolutionary approaches in the field of diagnostics[1]. Here we present a two-step CRISPR-based immunoassay, that, we have named MAIGRET[2] (Molecular Assay based on antibody-Induced Guide-RNA Enzymatic Transcription). MAIGRET enables the versatile detection of specific antibodies and other proteins with high sensitivity by combining the advantageous properties of CRISPR-based sensors with those of cell-free transcription systems[3]. In the first step of the assay, the binding of a target antibody to a pair of antigen-conjugated DNA strands induces the formation of a bimolecular complex that can hybridize to the single-stranded part of an incomplete inactive synthetic template encoding the CRISPR RNA (crRNA) guide strand specific of Cas12a enzyme. Only when such a bimolecular complex is formed the synthetic template is activated and cell-free transcription of the crRNA strand can begin. In the second step, an aliquot of the reaction from the first step is transferred to a solution containing Cas12a, its double-stranded DNA activator and a DNA hairpin reporter labelled with a fluorophore/quencher pair. The presence of the crRNA transcribed in step 1 induces the DNA collateral activity of Cas12a, leading to cleavage of the reporter and the resulting increase in the measured fluorescence signal (Figure 1). MAIGRET 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 six different antibodies. Due to the programmable nature of the sensing platform, we have also adapted MAIGRET to a competitive approach for the detection of specific antigens and for orthogonal detection of two antibodies in the same solution using a different Cas enzyme (i.e., Cas13).



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OC26

Chemical profile assessment of food supplements by HPLC-DAD

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In order to ensure micro-and macro- nutrient requirements and reduce disease risk factors, various types of products have been introduced to the market including food supplements [1]. Herbal-based food supplements (PFS) are plant-based preparations, but their use raises several concerns, such as misuse, poor product quality, presence of multiple herbal ingredients that may interact with each other and with other drugs [2]. Several factors can affect the quality and thus the safety of a herbal-based supplements. Some plants are toxic and their use is obviously prohibited in supplements, while others may become toxic depending on factors like the part of the plant used (e.g. roots, leaves, fruit), environmental conditions (e.g climate), cultivation practices (e.g. pesticide use, adherence to good agricultural practices). Furthermore, the presence of pharmacologically active substances such as anticoagulants, anticonvulsants, non-steroidal anti-inflammatory drugs, beta-blockers and anorectics, not listed on the label, has been revealed. This adulteration can have serious consequences for consumer health. The extracts obtained from fruit skin and seed (grape seeds) of *Vitis Vinifera* contain active ingredients with biological and pharmacological activity such as proanthocyanidins, resveratrol, fatty acids, flavonoids, minerals and vitamins. Consequently, the multicomponent pattern and the biological characterization of plant material are essential for the pharmaceutical industry in quality control procedures for food supplements and other plant-derived products. The aim of this study is to compare the multicomponent pattern of phenolics in grape seed extratc supplements using validated liquid chromatography coupled with a diode array (HPLC-PDA) methods for the quantitative analisys of twenty-two phenolic compounds among those most commonly found in supplements [3].

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OC27

Electrochemical platform development by multivariate approach for uric acid detection in disease diagnosis

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A rapid, accurate, and user-friendly approach recognizing predictive biomarkers in the human body is essential for health monitoring and pharmaceutical guidance. Specifically, a real-time measurement of uric acid (UA) is important, given its elevated level in physiological samples, known as hyperuricemia, which has been suggested as an indicator for multiple disorders associated with purine metabolism. The typical range for uric acid concentration is reported to be between 0.13 and 0.46 mM in serum and from 1.49 to 4.46 mM in urinary excretion. Excessive production of uric acid can be linked to several health conditions such as obesity, high blood pressure, pneumonia, leukemia, and cardiovascular illnesses, whereas an abnormally low UA level in serum fluids may trigger multiple sclerosis and oxidative stress[1].

In the last years, a consistent part of the research in sensing and biosensing field was also devoted to synthesize new nanocomposites to improve the performance of a sensor 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 enhanced 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 performance of electrochemical sensors, 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 selectivity, stability, and reproducibility, thus promoting the development of sensors for assays and bioassays. In this scenario, the Design of Experiments (DoE) is a powerful statistical approach for optimizing different parameters of the assay and biosensor performance, reducing the number of experiments while achieving high-quality information. It estimates interactions between variables and builds predictive models linking responses to variables[4].

A nanocomposite-based biosensing platform is developed in order to monitor in a fast and user-friendly way the levels of UA in biological fluids. Several types of poly(l-amino acids) (*e.g.*, poly-(l-lysine), 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. 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. A screening phase was followed by Response Surface Methodology (RSM). The obtained nanocomposite platforms were electrochemically characterized by electrochemical techniques and applied in the quantification of UA in biological fluids.

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OC28

CRISPR-powered monitoring of DNA repair activities using synthetic DNA transducer

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DNA glycosylases are a class of DNA repair enzymes crucial for maintaining genomic stability by identifying and excising damaged nucleotide bases, thereby initiating the base excision repair (BER) pathway. BER plays a vital role in protecting the genome from mutations and associated diseases like cancer. Monitoring DNA glycosylase activity is pivotal for developing targeted therapies and facilitating clinical diagnosis.[1] However, traditional assays for measuring DNA repair activity in biological samples often face challenges such as multi-step procedures, dependency on auxiliary enzymes, or sensitivity issues.

Here, we introduce a CRISPR-Cas12a-powered detection platform tailored for rapid and sensitive measurement of both mono- and bi-functional DNA glycosylases. Our activity-based assay exploits CRISPR-Cas12a-mediated signal amplification[2] triggered by the molecular reconfiguration of a lesion-containing hairpin DNA probe (i.e., DNA Transducer, Figure 1). This DNA Transducer serves as both the substrate for the DNA repair enzyme and the activator for Cas12a. By utilizing a uracil-containing DNA Transducer, we enable single-step monitoring of uracil-DNA glycosylases (e.g., UDG, HSMUG1). UDG excises uracil bases incorporated into the DNA Transducer, leading to the generation of apurinic/apyrimidinic (AP) sites and subsequent destabilization of the hairpin structure. Repair-induced hairpin reconfiguration triggers Cas12a nuclease activity and fluorescence signal transduction. We demonstrate that the kinetics of hairpin reconfiguration are specifically induced by UDG activity and can also be temporally regulated by the number of uracil incorporated into the DNA transducer. Moreover, by incorporating 8-oxo guanine (8-oxoG) into the hairpin DNA probe, we assess the activity of 8-oxo guanine DNA glycosylases, including Fpg and hOGG1, via a release-upon-repair mechanism induced by the enzyme secondary lyase activity.

Our assay demonstrates precise quantification of cellular DNA glycosylases in single-step, with recovery values ranging between 98-122%. Furthermore, our platform is adaptable for inhibitor screening, making it a versatile tool for clinical diagnosis and drug discovery.

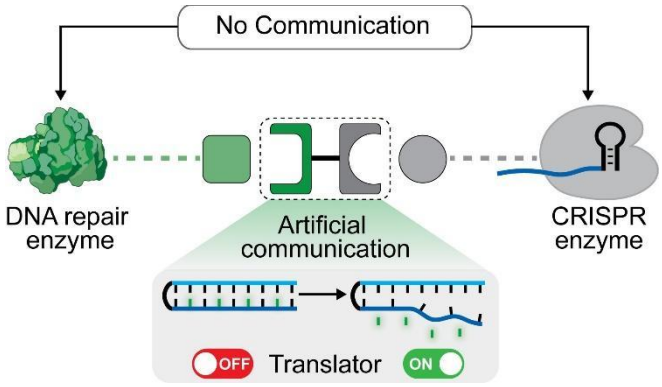

Figure: Schematic of the artificial communication pathway between DNA repair and CRISPR-Cas12 enzymes controlled by a DNA-based molecular transducer.

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OC29

Development of a Lateral Flow-Based biosensor for the early detection of Ovarian Cancer-specific microRNAs

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Ovarian cancer (OC) is the fifth most common gynecological malignancy in women[1]. Despite its low incidence, it has a high mortality rate due to the absence of symptoms in the early stages (I/II) and the lack of reliable early diagnostic tools. This often results in late-stage diagnosis (III/IV) when metastasis occurred, dropping the five-year survival rate below 30% while early detection can improve survival rates to up to 90% [2,3]. The current diagnostic approaches involve invasive and expensive exams such as pelvic examinations and transvaginal ultrasonography. In addition, only the CA-125 biomarker is analysed in bodyfluids, but it lacks diagnostic sensitivity and specificity, making it unsuitable for routine screening[4]. Therefore, many efforts are focused on identifying new specific biomarkers in biological fluids and developing advanced screening technologies to enhance early detection and expedite treatment decisions. In this context, specific microRNA profiles (miRNAs), which are small regulatory RNA molecules generally spanning 18-25 nucleotides, have been suggested to permit the distinction of ovarian cancer (OC) patients from healthy individuals, highlighting their potential as diagnostic biomarkers due to their dysregulated expression in cancerous tissues and their release into the bloodstream during the early stages of disease[5]. Nevertheless, traditional miRNA detection methods like Northern blot, microarrays, qRT-PCR, in situ hybridization, and high-throughput sequencing are labor-intensive and impractical for large-scale screening. To address these issues, this work aims to develop a rapid, cost-effective, and portable optical biosensor for point-of-care detection of miRNAs using the Lateral Flow Assays (LFAs) platform. The developed LFA could offer a non-invasive and decentralized approach to cancer diagnostics applications detecting and quantifying multiple miRNAs associated with OC in capillary blood samples. The design of this optical biosensor integrates nucleic acid lateral flow assay (NALFA) technology using gold nanoparticles (AuNPs) for the visual readout and horseradish peroxidase (HRP) for the chemiluminescent (CL) quantitative detection. A dual functionalization of the surface of AuNPs was performed by covalently immobilizing thiolated DNA probes and by physically absorbing the HRP (AuNPs@HRP@DNA_probe). This enables signal amplification, allowing NALFA to provide quantitative results related to the miRNA target concentration in blood samples. The principle of the biosensor involves a sandwich hybridization format, wherein target miRNAs are captured by specific DNA probes immobilized on a nitrocellulose membrane (test line) and the detection is achieved through hybridization with the AuNPs@HRP@DNA_probe, enabling plasmonic and CL detection. By leveraging LFA's simplicity and accessibility, this platform may help with early OC diagnosis, facilitating large-scale screening at reduced costs enhancing early cancer detection, expedite treatment decisions, and ultimately improve patient outcomes. Moreover, its integration with smartphone-based signal quantification ensures user-friendly, decentralized diagnostics, potentially enhancing early cancer detection and patient outcomes, especially in remote and resource-limited settings.

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Presentazioni Flash

PF1

Advancing cancer management: a paper-based electrochemical sensor for detecting H_2S in murine tissue lysates

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Hydrogen sulfide (H_2S) is the most recently identified endogenous gasotransmitter, produced in mammalian tissues. It has been identified as a crucial mediator in various physiological processes, including cellular homeostasis, and also plays a role in several pathological conditions, such as cancer. Depending on its concentration and exposure time, H_2S has diametrically opposite effects on cancer cells: at low levels, it can promote tumor cell growth and proliferation, while at higher concentrations, it can inhibit growth or induce apoptosis[1,2]. This dual role makes H_2S a promising biomarker for cancer diagnosis and therapy, with potential applications in liquid biopsy for non-invasive early detection of tumor progression treatment response. Given the complexity of accurately detecting H_2S in biological samples, there is an increasing demand for sensitive and precise analytical techniques, such as electrochemical methods. To address these challenges, a paper-based novel electrochemical sensor was developed. The sensor was fabricated by screen-printing onto filter paper and enhanced with a dispersion of Prussian blue (PB), synthesized directly on the paper substrate (Fig. 1). After the optimization of several experimental parameters, the analytical characterization was performed in standard solutions, achieving a detection limit of 3 µM with an adequate repeatability, lower than 10 %. The electrochemical platform was tested on several murine tissue lysates, including a murine skin lysate, two murine cutaneous melanoma tissue lysates and a murine cutaneous melanoma tissue lysate from PAG-treated mice. The results aligned closely with those obtained using the methylene blue assay, with the benefit of analyzing very small sample quantities, which cannot be measured using the standard reference method. This novel sensor represents a significant step forward in point-of-care (PoC)[3] cancer diagnostics, offering fast, on-site analysis with minimal sample requirements. By simplifying traditional workflows, this technology enables timely, non-invasive cancer detection and monitoring, demonstrating the applicability of the developed electroanalytical method for liquid biopsy.

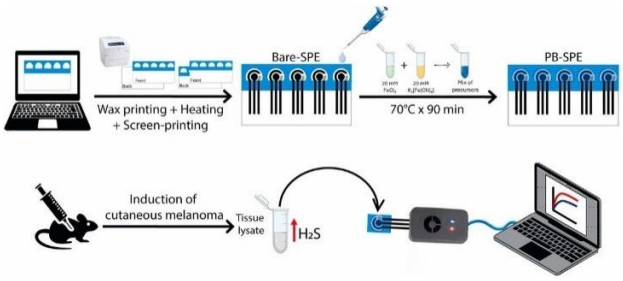

Figure: Schematic representation of the paper-based screen-printing process, modification, and electrochemical analysis of the murine tissue lysates[4].

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PF2

Highly sensitive printed pH sensor using CB/PANI nanocomposite for POC diagnosis of orthopedic infections

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Modern surgical procedures in the orthopedic field focus on addressing bone-related issues, such as fractures, by implanting medical devices. Nevertheless, this widespread approach leads to a significant risk of post-surgery periprosthetic infections, which may trigger implant failure and prolonged hospitalization, requiring costly antibiotic treatments.[1] The rise of infections is strongly promoted by the bacterial adhesion to the orthopedic implant surface, inducing a pH decrease within the local synovial fluid, from its normal physiological level (pH = 7.4 - 7.7) to more acidic values (pH < 7).[2-3] Relevant limitations, namely storage conditions and inadequate sensitivity for biomedical purposes, hamper the breakthrough of earlier developed electrochemical pH sensors as point-of-care diagnostic devices.[4-5] Given this scenario, we introduced a potentiometric pH screen-printed electrode harnessing the combination of Carbon Black and Polyaniline to provide a point-of-care detection of tiny pH variations with a high sensitivity, aiming to fast-up orthopedic infection diagnosis and treatment. Drop-casting technique was used to easily deposit a dispersion of Carbon Black/Polyaniline nanocomposite on the working electrode to deliver an attractive manufacture of the printed sensor. A deep investigation of the optimal functionalization was carried out by assessing the size and the stability of the nanocomposite within several Carbon Black/Polyaniline mixtures, through DLS and ELS respectively. Moreover, a morphological characterization of the different compositions was provided using TEM. Following the evaluation of analytical performances, we selected CB 90% - 10% PANI nanocomposite to functionalize the printed working electrode, as exhibited an efficient dispersibility resulting in a reliable trade-off between linearity in the pH range 3-8 (R2 = 0.994), lack of memory effect and valid reproducibility (RSD% = 0.9% , n = 3) along with an outstanding sensitivity (- 74 ± 3 mV/pH unit). Furthermore, the optimized sensor exhibited negligible pH deviations in the presence of several interfering candidates of synovial fluid. Then, the storage stability was weekly monitored over 28 days by recording the potentiometric response of the developed sensor, kept dry at room temperature. The potential stabilization has been achieved since the second week, ensuring consistent performances up to one month. Healthy and acidified synovial fluid was used to evaluate sensor's response towards pH variations in real samples. The designed sensor demonstrated remarkable effectiveness for both physiological and infected range, as it revealed a healthy synovial fluid pH equal to 7.7 ± 0.1 and an acidified sample pH of 6.4 ± 0.2, closely matching the pH-meter readings (8.0 ± 0.1, 6.7 ± 0.2, respectively).

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Acknowledgments

This work was supported by SENSEIF project.

PF3

Overcoming the challenges of adenosine template in nanomip design

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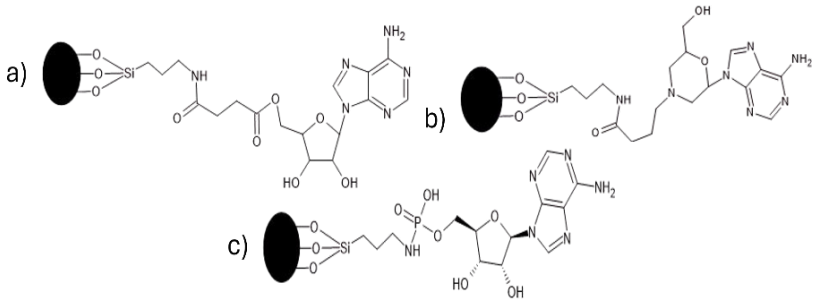
Bioanalytical methods generally require selectivity and sensitivity to ensure reliable target analyte recognition. Traditionally, antibodies, receptors, or natural enzymes have been used to fulfill these requirements. One notable biological target is adenosine, an interesting biomarker involved in physiological and pathological pathways. This nucleoside plays an important role in conditions such as cardiovascular risk, brain disease, stress response, and metabolic dysfunctions, making its monitoring essential. Currently, several methods are available to detect adenosine such as ELISA assays or optical/electronic sensors *[1]*. In the specific context of the molecular imprinting technique, nanoMIPs offer high stability, cost-effectiveness, and adaptability to various environmental conditions, making them suitable for applications in both healthcare and diagnostics. However, not all biological targets are easily imprinted. Among them, adenosine has proven to be a particularly challenging template due to its chemical properties. This difficulty primarily arises from solid phase polymerization synthesis (SPPS)*[2]*, used to produce nanoMIPs which requires the immobilization of the template on the nanobead's surface during the imprinting step.

The present work explores different template functionalization strategies to obtain and optimize an imprinted polymer. The key objective was to maintain the purine base (adenine) exposed, thereby maximizing the imprinting effect. To achieve this, adenosine was functionalized through its sugar moiety, as the ribose unit is chemically unreactive. Several modifications were introduced to functionalize the diol and hydroxyl function of ribose while leaving the adenine structure intact. Specifically, ribose has been modified alternatively with amine, carboxyl, aldehyde, succinate functional group or directly linked via periodate oxidation to the aminated solid phase. Along with these modifications, adenosine monophosphate (AMP) was also tested as a template to reduce steric hindrance. The polymerization mixture initially consisted of acrylic acid, N-isopropylacrylamide, tert-butylacrylamide, and methylen-bis-acrylamide. The binding behavior of nanoMIPs was tested through equilibrium partition experiments. Once the optimal polymer composition was identified, the formulation was further improved by introducing a modified thymine, the complementary base of adenosine. Although this approach presents several challenges, it allows to obtaining a nano MIP with a binding affinity of about 7.6 x 10^5 M^{-1} . While further optimization is required to enhance binding performance and selectivity, these nanoMIPs show promising potential for integration into microplate-based assays and possible application in ELISA-like diagnostic platforms.



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PF4

Microfluidic 3D-printed electrochemical device for ischemic stroke *point-of-care* testing

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Ischemic stroke represents a global health issue, being one of the main causes of death and disability, affecting approximately 15 million people every year [1]. Numerous clinical studies have revealed a strong correlation between brain damage caused by ischemia and elevated iron levels in the affected tissues [2]. In this context, transferrin (Tf), a key protein involved in iron transport and metabolism, has emerged as an important biomarker. In particular, the transferrin saturation (TSAT) ratio, defined as the proportion of transferrin-bound iron (Tf-bound iron) to the total iron-binding capacity (TIBC), has emerged as a critical parameter in the diagnosis and monitoring of conditions such as anemia and ischemia [3].
Recent advancements in micro- and nanotechnologies have facilitated the development of smart biosensors and advanced analytical tools. Among these, additive manufacturing, particularly 3D printing, has gained significant traction due to its affordability, versatility, and customizability. Indeed, electrochemical microfluidic devices produced using 3D printing (3D-EMDs) are emerging as promising tools for the development of the next generation of *point-of-care* testing (POCT) devices [4].
In the present study, we report on the development of a 3D-printed electrochemical microfluidic device for the assessment of transferrin saturation (TSAT). The device has been designed and manufactured to integrate the immunoassay step, a crucial component in isolating transferrin from serum samples. This innovation addresses a key challenge in this field, which has limited the development of smart analytical devices. The 3D device thanks to its tailor-made design incorporates the immunoassay procedure, enabling the simultaneous electrochemical detection of Tf-bound iron and TIBC. This approach allowed for accurate TSAT assessment in serum samples obtained from patients diagnosed with ischemic stroke.
The results obtained with the 3D-EMDs are extremely encouraging, combining cost-effectiveness, integration of complex analytical steps, and ease of use, contributing to the advancement in the field of decentralized medicine and personalized healthcare, in the context of ischemic stroke diagnosis and management.

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Acknowledgment

This work has been funded by the European Union - NextGenerationEU, Mission 4, Component 1, under the Italian Ministry of University and Research (MUR) National Innovation Ecosystem grant ECS00000041 - VITALITY - CUP: C43C22000380007. Grant PID2023-152298NB-I00 funded by MCIN/AEI/10.13039/501100011033 and FEDER, UE and the Community of Madrid grant number Y2020/NMT6312 (NEURO-CHIP-CM). The authors also thank Dr. Teresa Gasullc of the Germans Trias i Pujol Research Institute, Barcelona (Spain) for the donation of the samples.

PF5

Flexible micro thread/paper-based wearable device (µTPAD) for pH monitoring in chronic wounds

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Skin wound healing is a condition affecting people worldwide, from simple, small to more important, and even chronic wounds. The healing pathway is often corrupted by systemic or local factors, e.g. infections, that compromise the curative microenvironment, interfere with the repair progression and potentially lead to chronic or non-heling wounds. Within this intricate and delicate multi-phase process, wearable devices could be of the outmost importance for less/non-invasive and user-friendly monitoring and management of the healing progresses. Textile-based electrochemical sensors are gathering widespread interest, enabling the development of smart wearable clothes and accessories. Common threads used for everyday apparels, namely cotton or polyester, can be functionalized by coating with conductive materials and integrated in electronic circuit, maintaining their stretchability and adaptability. Microfluidics plays a lead role in wearable devices, providing reagent and/or samples to the reaction and detection zone. Threads can passively transport liquids along their length, enabling microfluidics without the need of micro-scale channels and hydrophobic barriers. Aiming to provide a self-sampling sensing device, paper was used as a support for threads embroidery. When in contact with the wound area, paper adsorbs the biological fluids containing the biomarkers of interest for healing monitoring, namely pH. Paper's versatile properties combined with flexible textile threads have been exploited for conceiving a wearable microfluidic thread/paper-based device (µTPAD). The device included silver threads coated with graphite and silver/silver chloride (Ag/AgCl) inks for working (WE) and reference (RE) electrodes, respectively. Iridium oxide (IrOx) was chosen as the pH-sensitive material, since it allows to measure a wide range of pH with high reproducibility and robustness, stability over time and fast response, ability to operate in complex matrices such as blood, and biocompatibility.[1,2] Iridium oxide was electrodeposited via cyclic voltammetry onto the graphite-coated thread-based WE surface. A paper-based sampling pad was designed to have circular hydrophilic sampling zones, delimitated by hydrophobic wax. Recycled filter paper was used as support for thread-based Ag/AgCl RE and IrOx functionalized WE embroidery. Moreover, a 3D printed flexible bracelet was designed as scaffold for the wearable device. The µTPAD was tested using Open Circuit Potentiometry, by loading 15 µL of standard pH solution (pH from 4 to 8) prepared in Britton-Robinson buffer solutions onto the hydrophilic area of the device, containing the thread-based electrodes. A slope of 0.054±0.004 V/pH decade was obtained, with good inter-µTPAD repeatability, showing an RSD% of 6%, calculated with three different µTPADs at pH 6. µTPAD was then tested with a self-developed Arduino module connected to a laptop, which was then also connected via Bluetooth to a smartphone enabling a readout throughout a downloadable application. Calibration curve obtained with the Arduino module showed a sensitivity of 0.053±0.003 V/pH decade, comparable with the one obtained with the potentiostat, and an inter-µTPAD repeatability with an RSD% of 7% evaluated with three different µTPADs at pH 6.

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Acknowledgment

This work was conducted in collaboration with European Horizon 2020 STRENTEX Project.

PF6

Waste from oil industry: a possible beneficial resource.

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It's common to buy olive oil from Italy, being one of the largest producers. On the other hand, these industries generate a large number of residues, which require a significant amount of energy to be wasted. The benefits of olive oil are widely known, which is why it was thought that even waste could contain bioactive compounds. Based on type of industry, olive oil could be obtained through two-, two and half and three phases extraction procedure, obtaining olive oil, solid waste and olive pomace and, sometimes, wastewater and solid suspension. Starting from two and half waste products (the middle of three considered producers), a green extraction method was validated with Design of Experiment protocol. The analysis was conducted with a common instrumentation such as HPLC-PDA, through a method which permits to separate more than twenty analytes, specifically polyphenols [1, 2]. Firstly, type of extraction method was evaluated, between solid- liquid extraction and ultrasound assisted extraction (UAE). Once UAE was chosen, various parameters were optimized such as solid: liquid ratio, type of solvents, time, and temperature. The application of the optimized protocol to the others waste products has shown excellent results in terms of yield. The extracted products were additionally subjected to biological tests to evaluate antioxidant activity. Based on this approach, Green Chemistry and bioeconomy are matching, showing that many scientific fields could be "greener". This study opens the possibility to re-use waste products from olive industry, useful in many fields, for example in cosmetic products, being natural compounds very attractive for people and industry both.

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PF7

On-body real-time lactate detection in sweat using a 3D-printed flexible wearable sensor

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Real-time monitoring of sweat lactate offers valuable insights into exercise performance and physiological responses. Traditional lactate detection techniques often lack the sensitivity, portability, and ease of use needed for in-body applications. To overcome these limitations, electrochemical biosensing has emerged as a promising method for non-invasive, real-time analysis. This study presents a wearable lactate electrochemical biosensor based on custom screen-printed electrodes modified with a bio-hybrid probe containing Prussian blue, carbon black, and lactate oxidase. After optimizing key parameters, the biosensor achieved a detection limit of 0.06 mM and a linear range up to 20 mM. A filter paper strip was incorporated to enhance sweat collection and act as the sample chamber, resulting in 6% repeatability and effective sweat handling. The system's accuracy was validated with three sweat samples, showing excellent correlation (96–101%) with LC-MS/MS, a standard laboratory method. The biosensor was integrated into a 3D-printed thermoplastic polyurethane (TPU) armband, which facilitates efficient sweat collection and transport, combining lightweight durability with a customizable, ergonomic design for active use. This affordable, wearable system represents a significant advancement in non-invasive, continuous health monitoring, offering a practical solution for personalized, real-time tracking of physiological parameters.

PF8

Synergistic integration of molecularly imprinted polymers and nanozymes for enhanced selectivity and catalytic efficiency

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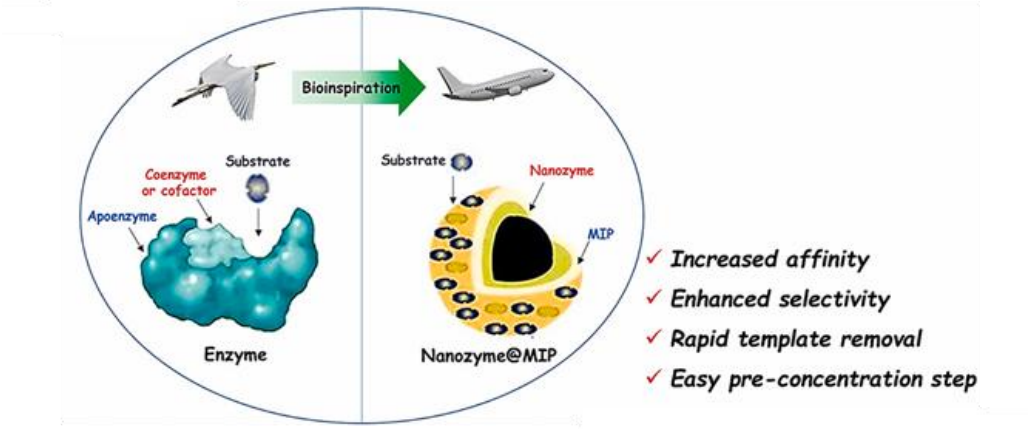
Nanozymes offer several advantages, including high stability and catalytic activity, but their selectivity is lower than that of natural enzymes. This is because enzymes typically consist of a protein component (apoenzyme) that enhances substrate affinity and a non-protein coenzyme that facilitates catalytic activity. To replicate this system, a hybrid approach combining MIPs and nanozymes is proposed, where MIPs function as the apoenzyme to provide substrate selectivity, while nanozymes act as the coenzyme to enable catalysis. In this study, Fe_3O_4-Lys-Cu nanozymes with peroxidase-like activity were integrated into a MIP specifically designed for L-DOPA. The MIP's pre-concentration capability enhanced catalytic efficiency by a factor of twenty. Additionally, the synergy between MIPs and nanozymes significantly accelerated template removal during MIP synthesis, reducing the extraction time from several hours to just one minute. Furthermore, the study addresses the issue of non-specific adsorption in MIPs caused by functional groups located outside the MIP cavities. To solve this, oleic acid was used to eliminate non-specific adsorption in MIPs. By modifying the MIP surface with oleic acid, the selectivity was improved, leading to more reliable and accurate sensing applications.



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Presentazioni Poster

P01

Using a clamp-like triplex switch to control CRISPR-Cas12a cleavage activity

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The discovery of the collateral a-specific cleavage activities of CRISPR type V (Cas12) and type VI (Cas13) systems, which are triggered by DNA/RNA binding, has significantly advanced the application of CRISPR technology in diagnostics.[1] Specifically, CRISPR-Cas12 systems are capable of recognizing both single- and double-stranded DNA targets, triggering enzyme reconfiguration that activates collateral cleavage activity.[2] When paired with FRET-based DNA reporters, this collateral activity amplifies fluorescence signals upon target binding, thereby enhancing the sensitivity of diagnostic assays.[3] However, Cas12a-based detection methods still face challenges in achieving high specificity for detecting single-base mutations within DNA targets. This study presents a novel strategy to regulate Cas12a cleavage activity using a DNA-based hybridization network involving Clamp Triplex DNA formation. Clamp Triplex probes, which exhibit higher specificity and affinity for homopurine DNA/RNA targets compared to traditional linear probes, undergo a conformational change upon binding to single-stranded DNA or RNA. This conformational shift activates a reaction network that triggers Cas12a activation and results in fluorescence output. Our molecular design addresses two critical challenges in CRISPR-Cas diagnostics: it enables the highly specific detection of single nucleotide mutations within target sequences of 12 to 20 nucleotides, while maintaining the same limit of detection (LOD) as conventional CRISPR-Cas12-based systems.

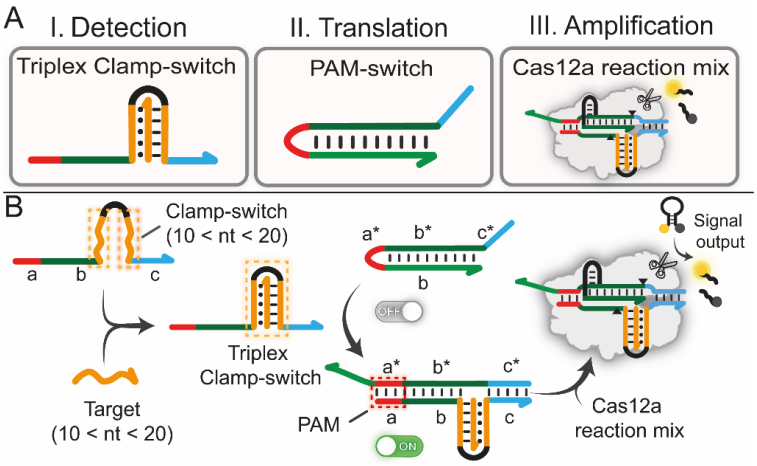

Figure: Design of a one-pot triplex-based CRISPR reaction network for sensing applications. (A) Schematic representation of the three functional modules required to activate Cas12a cleavage activity. (B) Description of the Triplex Clamp-switch DNA reaction network Triplex for triggering Cas12a double-cutting activity.

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P02

MUTYH catalysed 8-oxoG repair monitoring through an electrochemical DNA scaffold sensor

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MUTYH (MutY bacterial homolog) is a crucial DNA glycosylase enzyme involved in the base excision repair (BER) pathway, responsible for preventing mutagenesis caused by oxidative DNA damage. The enzyme primarily recognizes and removes adenine, recruited against 8-oxoguanine (8-oxoG), a common lesion induced by reactive oxygen species (ROS). By excising the mismatched adenine, MUTYH accelerates the subsequent downstream repair processes in BER pathway that restores genomic integrity, thereby diminishing the risk of G:C to T:A transversion mutations and oncogenic transformations [1].

Employing programmable nucleic acid nanoswitches as diagnostic tool, an electrochemical bioanalytical platform is envisioned to be developed in the present study demonstrating molecular activities underlying the MUTYH repair function in BER pathway. MUTYH mediated recognition of altered guanine 8-oxoG lesion, installed within single strand DNA labelled with a redox indicator, results in sensitive signal changes and can be conveniently detected by an electrochemical readout in-vitro.

The study will help to understand MUTYH's function in DNA repair for developing targeted therapies to prevent cancer, specifically MUTYH-associated polyposis, a genetic colorectal cancer syndrome and thus contributing to better management of the disease.

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P03

Laser-induced nanozymatic metal nanoparticles for paper-based analytical devices

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Nowadays, manufacturing easy-to-use and sustainable point-of-need devices is a hot topic. In this framework, the colorimetric approaches, which employ optical variation easily monitorable by naked eye or smartphone, can offer captivating opportunities. Due to its high availability, 3D structure, capillarity, mechanical resistance, flexibility, and biocompatibility, the paper represents an excellent substrate for fabricating analytical devices (PAD). However, there is still a need for affordable technologies that enable precise patterning/cutting of paper substrates, as well as strategies for integrating functional nanomaterials on them.

Herein, a CO_2-laser plotter-based versatile strategy to in-situ synthesize on paper plasmonic active gold (Au), silver (Ag), platinum (Pt), copper (Cu), cerium (Ce), nickel (Ni), and aluminium (Al) nanostructures will be presented [1]. This approach allows the formation of metal nanostructures, named Laser-Induced Metal nanoparticles (LIMs), on cellulosic substrates, including Whatman papers, conventional office paper, and recycled/byproducts-based papers. LIM formation mechanisms will be discussed together with the main variables to modulate their properties. In brief, the laser allows LIMs' design, shaping, and anchoring onto paper in a single stroke in a few seconds with the desired configuration. LIMs possess useful features for sensing purposes, resulting in plasmonically active, catalytic, and photoluminescent according to their chemistry and morphology.

To prove the LIM potentialities in PADs, a disposable LIM-PAD for the rapid and selective smartphone-based colorimetric determination of ascorbic acid (AA) will be presented. The PAD is equipped with a laser-induced platinum nanostructured catalytic paper (LIM-Pt) coupled to 3,3',5,5'-tetramethylbenzidine (TMB) loaded fiberglass, assembled in an array format to allow the simultaneous determination of 5 samples in 1 minute. The LIM-Pt enables instantaneous TMB oxidation, given the proven oxidase-mimicking activity, ensuring the instantaneous conversion of uncolored-TMB to blue-colored TMBox, without additional reagents. AA induces a TMB-catalytic conversion inhibition resulting in a colorimetric signal 'switch-off'. The linear AA dose-response ranged from 31 to 250 mg Kg^{-1} (R^2= 0.992), returning a detection limit of 6 mg Kg^{-1}; analytical performance remained constant over 6 weeks (RSD= 4%). The Flip-PAD exploitability was proved through AA determination in food and pharmaceutical samples, returning accurate (Rec. 92−114%; R.E. −11/+4%) and reproducible data (RSD≤ 10%; n= 3).

Summing up, the herein proposed laser writing strategy turns out an innovative and sustainable nanopatterning technique, prone to generate optical sensing zones useful to develop (bio)sensing strategies and manufacturing within everyone's reach PADs.

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Acknowledgment

This research was funded by 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.

P04

Advanced wearable sensors for non-invasive emotional state assessment

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In contexts such as healthcare, safety, and performance optimization, understanding and monitoring emotional and physiological states is crucial [1]. Physiological responses to emotional stimuli such as stress, excitement, or fatigue alter the composition of biological fluids. Some of these, such as sweat and saliva, are suitable for non-invasive analysis. Tracking physic parameters and some biomarker, like conductivity/resistance and cortisol respectively, provides valuable insights into autonomic nervous system activity [3], [4] and hypothalamic-pituitary-adrenal axis function [5].
This work aims to develop a wearable, integrated in textile, multi-analyte sensor able to monitor biomarkers in non-invasive biological fluids. Emotional states like stress, fear, panic, or excitement can lead to a change in perspiration, which in turn results in a variation in sweat ionic concentration. Single-frequency electrochemical impedance spectroscopy (EIS) is used to measure sweat resistance, which is an indicator of ionic concentration, allowing for rapid, low-energy measurements, ideal for continuous monitoring. Experimental data confirm the sensor's ability to detect physiologically relevant variations in sweat resistance, which represent a starting point for emotional assessment. For cortisol detection, a label-free immunosensor is developed utilizing screen printed electrodes modified with innovative carbon nanomaterials. By enhancing electrochemical performance and supporting covalent antibody immobilization, these materials enable precise detection at low concentrations. Through this setup, cortisol, a hormone closely associated with stress, can be monitored, complementing conductivity measurements and providing insights into the user's emotional state.
By combining advanced electrochemical sensing with wearable textile design, this interdisciplinary project bridges the gap between cutting-edge technology and practical, everyday solutions. The multi-analyte approach offers a robust platform for understanding and managing emotional and physiological states in real time. This is a preliminary study of national researcher projects, which aims to develop a unified wearable multi-analyte sensor capable of monitoring a range of physical parameters (pH, temperature, ionic concentration) and biomarkers, including cortisol, and other physiological parameters, for comprehensive health and emotional state tracking.

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Acknowledgment

The research leading to these results has received funding from the Project "SENS-AI, Environmental Sensing with Artificial Intelligence" CUP H53D23000520006 (Italian "Bando Prin 2022 - D.D. 104 del 02-02-2022" by MUR (2023-2025), Project ""Dress the future; novel combined wearable integrated system (Stargate)" CUP E53D22014620001 by MUR (2023-2025)(MUR-PRIN 2022 PNRR Decreto Direttoriale n. 1409 del 14-9-2022) and Grant MUR Dipartimento di Eccellenza 2023-27 X-CHEM project "eXpanding CHEMistry: implementing excellence in research and teaching".

P05

Hybrid nanozyme systems: gold-decorated nanodiamonds for advanced electrochemical sensing

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Nanozymes, nanomaterials with enzyme-like catalytic properties, have gained significant attention in electrochemical sensing due to their superior stability, reusability, efficiency under extreme conditions, and cost-effectiveness compared to natural enzymes[1]. Gold nanoparticles (AuNPs) have demonstrated remarkable peroxidase-like properties, making them highly attractive for sensing applications [2]. However, the conventional synthesis of gold nanoparticles presents several drawbacks, including the use of reducing and stabilizing agents that can impair the catalytic activity of the material. Developing innovative synthesis strategies remains a key challenge to enhance catalytic performance and improve sensor efficiency.
In this work, we explore for the first time the potential of nanodiamonds (NDs) as a novel platform for gold nanoparticle synthesis, leveraging their unique properties for electrochemical sensors and biosensors. Nanodiamonds exhibit exceptional chemical stability, a high surface area, and remarkable functional versatility, making them ideal candidates for surface modification. By decorating NDs with AuNPs [3 4 5], we obtained a hybrid system with enhanced catalytic properties, as demonstrated by UV-vis spectrophotometry. The AuNP-ND hybrid exhibits significant peroxidase-like activity with the added advantage of greater operational stability.
To further expand the applicability of AuNPs, we tested a new carbon platform based on sp^2 nanocarbon particles derived from the pyrolysis of recovered cellulosic waste materials. Using the same synthesis protocols, we successfully produced a carbon-AuNP hybrid, which also demonstrated catalytic activity.To translate this system into an electrochemical platform, the hybrid materials were incorporated onto the surface of graphite working electrodes in screen-printed electrodes on PET substrates. Electrochemical characterization revealed a strong synergistic effect, leading to enhanced electron transfer, improved sensitivity, and robust performance under operational conditions. Furthermore, the application of these hybrid materials for hydrogen peroxide sensing showed promising preliminary results.This study introduces nanodiamonds as a promising new support for nanozyme-based electrochemical sensors, paving the way for innovative, enzyme-free detection strategies in environmental and biomedical applications.

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P06

Electrochemiluminescence enhancement via redox-mediator for bead-based immunoassays

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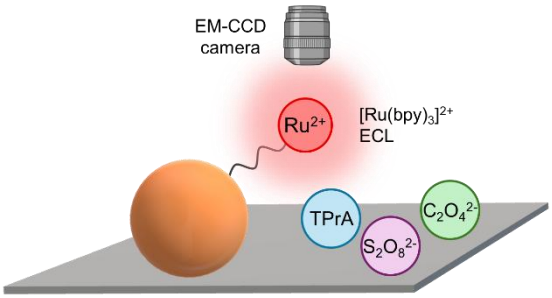
Electrochemiluminescence (ECL) is a powerful bioanalytical technique due to its high signal-to-noise ratio and low detection limits.[1] To make ECL viable for clinical diagnostics, Roche Diagnostic started to produce fully automated analyzers that exploit the bead-based immunoassay technology.[2][3] This system employs biotinylated and dye-functionalized antibodies that specifically recognize a target antigen. When the analyte is present the classical sandwich assay is formed and the generated ECL signal is proportional to the analyte concentration. Commercialized ECL-based immunoassays typically exploit tris(2,2'-bipyridine)ruthenium(II) ($[Ru(bpy)_3]^{2+}$) and tri-*n*-propylamine (TPrA) as ECL luminophore and coreactant, respectively. The beads-based immunoassay is based on the heterogeneous co-reactant ECL mechanism where the Ru(II) complexes are not directly oxidized. Therefore, to produce the emitting $[Ru(bpy)_3]^{2+*}$, the $[Ru(bpy)_3]^{2+}$ labels are first reduced by $TPrA^{\bullet}$ and then excited by the electrogenerated $TPrA^{\bullet+}$. Currently, there are no straightforward methods to enhance the ECL signal of commercial bead-based immunoassays without further modifications (i.e., by employing novel luminophores with higher quantum yield compared to $[Ru(bpy)_3]^{2+}$ or by introducing nanomaterials [3][4]). In this way, we investigate the reaction mechanism underlying the modulation of the ECL signal of $[Ru(bpy)_3]^{2+}$ labels anchored to magnetic beads. Specifically, we analyse the emission of Ru(II) labels on single beads through ECL microscopy, exploring the interaction of the radicals generated through the reported autocatalytic reaction between oxalate and peroxydisulfate.[6]



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Acknowledgement

This work was supported by ECLectic project that has received funding from the European Union's MSCA Doctoral network Horizon Europe programme Grant Agreement Number 101119951.

P07

A simple colorimetric paper-based device for zinc ions detection in water

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Zinc is a prevalent environmental pollutant due to its extensive use in industrial processes such as galvanizing, alloy production, and as a component in pesticides and rubber manufacturing, posing significant risks to both ecosystems and human health. Industrial discharge and urban runoff can elevate zinc concentrations in water, making it crucial to monitor and regulate its levels to prevent environmental damage and ensure water quality. According to DPR n. 236/1988, which aligns with EU Directive 80/778 and WHO guidelines, drinking water with zinc levels exceeding 3 mg/L may be deemed unacceptable to consumers [1].
Zinc detection methods currently include atomic absorption spectroscopy (AAS) with different detectors, such as optical detectors and mass spectrometry [2]. While AAS offers high sensitivity and accuracy, its drawbacks—such as high costs, the requirement for advanced equipment, and extensive sample preparation—restrict its suitability for field applications and real-time monitoring.
To address the limitations of traditional zinc detection methods, there is increasing interest in developing rapid and in situ detection techniques. These analytical methods, commonly known as Point-of-Need (PON) tests, offer significant advantages, including portability, cost-effectiveness, and the ability to deliver immediate results. In this work, a paper-based colorimetric device has been developed for the determination of zinc ions in aqueous samples, outlining its potential use in water quality monitoring.

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P08

Enzyme-mediated dissipative Hybridization Chain Reaction for dynamic DNA nanomaterials

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Supramolecular assemblies in biological systems exhibit dynamic behavior, essential for environmental responsiveness. Mimicking these dynamic aspects in chemically fueled synthetic materials could lead to adaptable materials with unique properties. Dissipative DNA nanotechnology, which leverages the programmability of DNA-DNA interactions and uses nucleic acids as chemical fuels to drive non-equilibrium processes, has recently emerged as a promising strategy for the development of responsive biomaterials. Inspired by this concept, we propose here a novel approach to implement dissipative control over the Hybridization Chain Reaction (HCR), a DNA assembly technique based on the polymerization of two metastable DNA hairpins. By implementing a "tailed HCR" strategy[1], we introduce a dissipative behaviour through enzyme-mediated fuel consumption. Specifically, we have re-designed a fuel that triggers a strand displacement process and induces the disassembly of the DNA polymer into short oligomers. The presence of an enzyme that specifically recognizes the fuel in the oligomer heteroduplex can trigger the degradation of the fuel strand and the consequent reformation of the DNA nanostructure, thus driving a cyclic dissipative reaction.[2] In particular, we have developed two orthogonal strategies that use two different enzyme-fuel pairs: RNA fuel/RNase H and phosphorylated DNA fuel/Lambda Exonuclease. These systems enable programmable assembly and disassembly of the DNA nanostructure, demonstrating the potential of dissipative DNA nanotechnology for time-controlled, responsive biomaterials. By leveraging dissipative strategies, we develop DNA polymers with novel properties, such as concentration-dependent adaptability and reversible multicomponent analysis, which offer exciting possibilities for advanced analytical applications.

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P09

Eco-innovative papers integrating nanostructured graphenic films with multifunctional sensing capabilities

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Laser-induced conductive nanofilm (LIF) electronics have gained increasing attention for their patterning freedom and versatility. Although most systems are based on plastic/polymeric substrates, an open issue regards the manufacturing of performing LIF-based paper devices suitable for real applications.
In this work, light was shed on laser-induced graphene oxide (rGO) integration in cellulosic substrates, to fabricate complete nanostructured paper-sensors able to respond to different analytical needs. Eco-innovative cellulosic substrates were investigated, including recycled papers and papers from textile and agro-industrial wastes, and manufactured with fibers alternatives to those from trees. Paper-sensors have been in series manufactured via an accessible stencil printing approach and rGO-film was easily integrated by pressure. The paper/rGO morphological, structural/chemical, and electrical/electrochemical features were deeply investigated. In brief, each paper accommodates the rGO differently, leading to a peculiar graphene film formation and chemical rearrangements affecting their electrochemistry. The latter appears mainly influenced by the paper's ability to preserve the native rGO-film exfoliated nature dominated by sp^2 carbon domains.
The broad applicability and multifunctionality of the rGO-paper sensors were demonstrated, working at cathodic and anodic potential with different electrochemical techniques, toward different analytes present in agri-food (caffeic acid; mint leaves, apple cider, and pear juice), biological (dopamine Pfizer drug; urine and physiological solution), and pharmaceutical (vitamin C; three different commercial supplements) samples, fulfilling the analytical requires for measuring them. Noteworthy, for each application a dedicated paper sensor turned out more performing (tree-free/rGO, recycled-fibers/rGO, and kiwi by-products/rGO), demonstrating the cellulosic substrate role also toward their final employment. Reproducible data (RSD ≤ %7; n=3), nano/micromolar limits of detections, and satisfactory recoveries (91-108 %) were obtained for all the applications.
Summing up, in this work the use of highly nansostructured rGO conductive films obtained via CO_2 laser-plotter was expanded toward their use on paper, proving the pivotal role of the hosting substrates, and demonstrating how sustainability stuff can lead to scientific advances. This approach offers to the scientific community the basis to obtain on-demand paper-based devices integrating performing nanostructured graphene sensing films using within everyone's reach tools.

Acknowledgment

This work has been funded by the European Union - NextGenerationEU, Mission 4, Component 1, under the Italian Ministry of University and Research (MUR) National Innovation Ecosystem grant ECS00000041 - VITALITY - CUP: C43C22000380007.

P10

Employing DNA condensates as a CRISPR-Cas µbioreactor for biosensing applications

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Liquid-Liquid Phase Separation (LLPS) is a reversible phenomenon by which a homogeneous solution separates into two distinct liquid phases, a solute-dense phase and a diluted phase[1]. In biology, condensates are biomolecule-enriched droplets which arise owing to LLPS, having been recently described to be implicated in many vital mechanisms within cells, where they are referred to as membraneless organelles[1,2]. Although intracellular LLPS is extremely complex, the use of highly programmable sequence-coded biomaterials, such as proteins and nucleic acids, offers a straightforward mechanism to form predictable artificial condensates[2]. These artificial condensates can be used to create biomimetic smart materials capable of selective-partitioning of target molecules of interest, spatiotemporally controlled biochemical reactions and can even be made to be stimuli-responsive[3,4].

Herein, we present our current work on the study and understanding of the thermodynamics and kinetics of condensate formation using DNA nanostars as building motifs, which have been designed to recruit and locally concentrate enzymes *in vitro.* Specifically, we discuss our employed strategy to obtain CRISPR-Cas12a-enriched DNA condensates, which remain catalytically active and accessible to their target DNA in solution, to be used in bioanalytical applications by acting as artificial biocatalytic systems.

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P11

Origami paper-based biosensor to SARS-CoV-2 on the surface

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The European project RELIANCE aims to create modified self-disinfecting surfaces as a way to better control the spread of pathogens. In this context, we present a paper origami electrochemical sensor (Fig. 1) that we are developing, which can measure the effectiveness of the specific surface modification.
Following up on our previously published work in Biosensors and Bioelectronics [1], which was the first publication describing an electrochemical immunosensor for SARS-CoV-2 detection in saliva, we adapted the sensor to allow an easy detection of the virus on surfaces. The method uses the magnetic beads as support for the sandwich-type immunological chain, that thanks to their high surface/volume ratio, permit to load a high number of antibodies improving the assay sensitivity.
Currently, our work is refining the design of the sensor origami model by selecting the type of paper to use and the number of washing steps. We have optimized the working volume (150 µL) as well as the labeled antibody concentration (1 µg/mL), using a Spike protein concentration of 1 µg/mL. The objective is to add the specific recognition elements to wax paper wells and then place the substrate inside a screen-printed paper-based sensor. This approach allows for a very easy workflow: the wax paper well is placed on the surface to be tested, a washing step is performed, and then the well is folded onto the electrode for detection. In this way, our sensor addresses wider goals by providing a simple method to measure disinfecting efficiency on different surfaces.

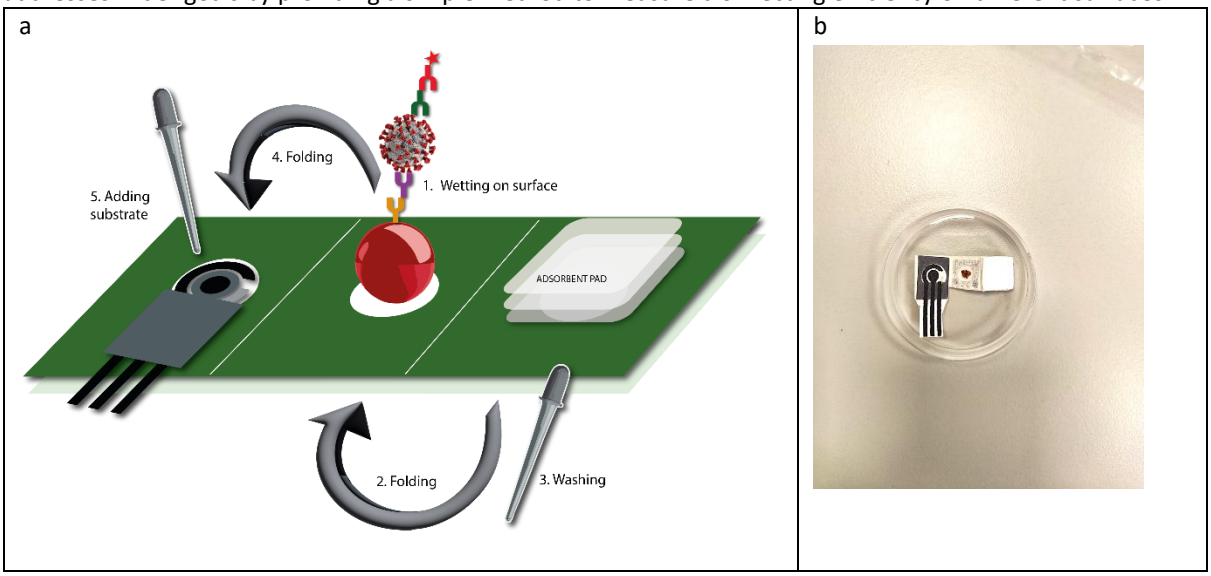

Figure: a) Drawing of the origami-type configuration. b) Photo of the paper experimental setup

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Acknowledgement
This project has received funding from the European Union's Horizon Europe research and innovation program, grant agreement No 101058570 (RELIANCE).

P12

Sustainable paper-based (bio)sensors for an innovative Origami Organ-on-Chip Device: Phoenix-OoC

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In recent years, organ-on-chip (OoC) technology has emerged as a revolutionary approach in the field of biomedical research, offering significant advancements over traditional in vitro and in vivo models. These micro-engineered devices replicate human organs' complex physiological and mechanical properties on a micro-scale, providing a more accurate and reliable platform for studying human biology and disease. Generally, OoC devices consist of microfluidic systems that integrate living cells cultured in a 3D environment, mimicking the architecture and function of specific human organs. This biomimetic approach allows for the recreation of dynamic biological processes, including tissue-tissue interfaces, fluid flow, and mechanical forces, which are critical for organ function.1 Sustainability is one of the most important concepts today, as it can drive activities in several areas, namely environmental, social, and economic. In analytical chemistry, the development of sustainable devices has been boosted by the introduction of paper-based microfluidic analytical devices (µPADs), whose benefits are not limited to the concept of sustainability. Indeed, paper as a functional material gives µPADs unprecedented properties. However, paper-based devices are still used only as analytical tools and have not been adopted by the OoC world. In this context, we present a revolutionary OoC paper-based platform that uses paper in origami configuration for i) cell co-cultures with the aim to better simulate different organ tissues, (ii) (bio)sensors integration with the aim of on-site/continuous monitoring of cells status/response to stimuli, and (iii) with the ultimate goal of performing accurate pharmacological studies. In detail, the OoC device includes a paper-based scaffold consisting of a cage printed using a 3D technique, containing the cell pads in origami configurations preloaded with the different cell lines, the electrochemical sensors modified for the detection of several analytes, and the adsorbent pad as waste. In the first part of the project, electrochemical sensors for pH, nitrate, and glucose were developed and characterized in standard solutions. The sensors were screen printed onto a filter paper support using wax to create a hydrophilic zone delimited by hydrophobic wax barriers. To create layers sensitive to the different ions, the working electrode surface was modified with nanomaterial and ion-selective membranes for nitrate detection, while for H+ detection the surface was modified with a layer sensitive to pH variations. For glucose monitoring, the sensor was modified by drop casting with nanoparticle dispersion and glucose oxidase enzyme.

Calibration curves were obtained by potentiometric measurements in standard solutions with regression equations y= (-0.048±0.002) x + (0.359±0.004); R^2 =0.981, y= (-0.083±0.001) x + (0.763±0. 007); R^2=0.998, for nitrate and pH respectively, and by chronoamperometric technique for glucose detection, a linear regression described by the following equations was obtained: y= (-0.12±0.01) x - (0.7±0.1); R^2 =0.960.

The development and application of OoC systems hold great promise for improving drug discovery, reducing animal testing, and advancing our understanding of human physiology and pathology. As this technology continues to evolve, it is expected to play a critical role in the future of medical research and personalized healthcare.

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Acknowledgement

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P13

3D printed platform for phytic acid detection in spinach leaves: integrated sample treatment and paper-based electrochemical biosensing

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Phytic acid, a phosphorylated derivative of myo-inositol and a crucial phosphorus storage form in plants, is also recognized as an anti-nutrient due to its mineral-binding properties (e.g., calcium, iron and zinc), reducing their bioavailability, which makes crucial its monitoring in food[1,2]. The developed integrated system features a biosensor fabricated on office paper screen-printed electrodes, combined with a custom 3D printed grinder for efficient phytic acid extraction using 0.2 M HCl. Spinach leaves are ground and treated in the grinder, then directly applied to the coupled electrode and pre-loaded pad system. The solution passing through the pad redissolves the ammonium molybdate, forming an electroactive phosphomolybdic complex when phosphate ions are released after phytic acid hydrolysis, catalyzed by immobilized phytase on the electrode. The biosensor leverages synergistic effects of carbon black nanocomposite and immobilized phytase, coupled with cyclic voltammetry, to detect phytic acid levels ranging from 1 to 50 µM with a detection limit of 0.8 µM. The device exhibits high selectivity for phytic acid amidst potential interferents as inisitol, ascorbic acid, Cu^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+} and a robust storage stability when maintained at room temperature for a period of up to one week. The reliability of this biosensor was validated through measurements of phytic acid in leaf samples, demonstrating a compact, miniaturized hybrid system that integrates 3D printed components for sample extraction and filtration with a paper-based platform for electrochemical detection. This innovative approach offers a practical tool for monitoring phytic acid in plants. The project received funding from COP-PILOT Horizon Europe project (n° 101189819).

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P14

DNA-based dimerization networks to control *in-vitro* transcription

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Living systems are an inspiring example of how fundamental functions can emerge from networks of interacting molecules [1]. Competitive dimerization networks, for example, are composed by families of proteins that bind to each other to form a combinatorial library of dimers that play a crucial role in the downstream activation of specific signalling pathways [2]. In recent years, synthetic DNA has emerged as an extraordinary nanomaterial to build synthetic molecular networks that exhibit complex input-output behaviour. Inspired by naturally-occurring systems, we demonstrate here a strategy to rationally program in-vitro transcription using synthetic DNA-based dimerization networks. The approach we propose is based on the use of a DNA-based competitive dimerization network consisting of DNA monomers modified with reactive groups that can covalently bond to each other and create a library of DNA dimer outputs [3]. In the presence of specific DNA input strands that sequester DNA monomers, we can trigger the formation of a specific DNA dimer output able to activate an in-vitro transcription system. The strategy is highly versatile and we demonstrate the possibility to finely modulate the in-vitro transcription process using different network sizes and input sets. The programmability of these DNA-based dimerization networks also enables the orthogonal transcription of different fluorogenic aptamers. Finally, the DNA networks proposed here allow to perform complex input-output computations in a highly predictable and programmable manner.

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P15

Photoluminescent paper-based platform integrating laser-induced aluminium nanostructures for smartphone-based selective determination of ortho-diphenols

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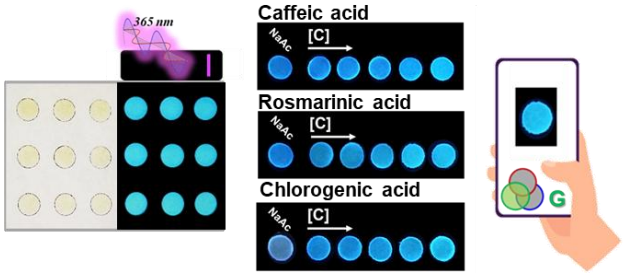
In a balanced diet and nutraceutical foods, antioxidants exert preventive and beneficial roles. European Food Safety Authority defined phenolic compounds (PCs) as functional molecules, recommending their daily intake; moreover, PCs can be considered also 'food' quality markers. Ortho-diphenolic compounds (o-PCs) result in the most antioxidants Among PCs, due to their ability to stabilize free radicals and directly or indirectly limit oxidative stress in living organisms. However, the rapid and selective analysis of o-PCs content in food samples is still an open issue due to the need for time and ad-hoc extraction procedures and complex analysis. In this framework, the manufacturing of easy-to-use paper-based analytical devices (PAD) offers captivating opportunities to overcome analytical limitations, increasing also the overall sustainable.

Herein, a CO_2-laser plotter-based innovative and versatile strategy to synthesize on paper photoluminescent aluminum nanostructures, named 'laser-induced aluminum nanostructures' (L-Al), will be presented [1]. The L-Al demonstrates photoluminescent features under UV lamp irradiation (λex= 365 nm) and can be produced on paper substrates with the required geometries. The L-Al optical properties were deeply investigated and the laser-induced synthesis was carefully optimized to tailor the photoluminescent effect for the sensing purpose. The L-Al was synthesized on paper in an Elisa-Plate format (n= 117 wells) enabling simultaneous analysis, and have been integrated into a lab-made PAD to facilitate photoluminescent results-readout via smartphone.

The L-Al photoluminescent PAD (L-Al_{3xP}) was successfully employed to determine cinnamic o-PCs in food samples. The cinnamic o-PCs induce an L-Al fluorescence turn-on which results in a green color brightness increase, that was exploited as an analytical signal. The L-Al_{3xP} reaction mechanisms and selectivity were carefully studied towards 23 PCs belonging to different phenolic classes. The figures of merit of the device were assessed using caffeic, rosmarinic and chlorogenic acids as representative cinnamic o-PCs. Limit of detections ≤ 3.0 µM were obtained along with linear ranges suitable for food analysis (2.5-15 µM caffeic acid; 2.5-25 µM rosmarinic acid; 10-50 µM chlorogenic acid. R^2≥ 0.990). The exploitability of the L-Al_{3xP} was proved via cinnamic o-PCs determination in 15 food samples including vegetables, spices, juices, purees, and infuses. The data obtained with the L-Al_{3xP} correlate with the ones obtained with HPLC-MS/MS (r= 0.993) and Folin Ciocâlteu assay (r= 0.930), proving the reliability and class selectivity of the proposed platform for food analysis.



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Acknowledgment

This work has been funded by the European Union - NextGenerationEU, Mission 4, Component 1, under the Italian Ministry of University and Research (MUR) National Innovation Ecosystem grant ECS00000041 - VITALITY - CUP: C43C22000380007.

P16

Paper-based electrochemical biosensor for NT-proBNP detection in capillary blood

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The project MHCD (Monitoring Heart and Cardiovascular Disease) aims to create a paper-based electrochemical immunosensor to NT-proBNP in capillary blood samples. In response to cardiac volume overload, cardiomyocytes stretch and release proBNP, which is then cleaved into BNP and NT-proBNP before entering the bloodstream. NT-proBNP is used to monitor progression of heart failure (HF), as its half-life in the body is much longer than BNP. The critical NT-proBNP threshold (1000 pg/mL) helps physicians assess the risk of hospitalization or mortality in HF patients [1]. Due to the variability of the values, it would be helpful for heart failure patients to monitor their condition to reduce the risk of hospitalization. The standard analytical method to detect NT-proBNP is the ELISA that, while highly sensitive, is not suitable for point-of-care diagnostics because it typically requires multistep procedures and laboratory-based equipment. As part of the MHCD project, we are developing a new diagnostic system for monitoring cardiovascular biomarkers. This system is based on a paper-based microfluidic device combined with printed electrochemical sensors. The goal is to create an easy-to-use and eco-friendly device to detect NT-proBNP. This innovative solution aims to significantly improve cardiovascular condition monitoring, enabling more precise and timely diagnoses, enhancing the management and treatment of heart diseases. The development of this device exploits magnetic beads and modified paper electrodes with carbon black, as already demonstrated using carbon-black based polyester printed electrodes [2]. Magnetic beads are selected for the high surface-volume ratio, which enhances sensitivity and specificity. The measurement is performed using an immunological chain (Fig.1) and a modified paper sensor with carbon black to improve performance [3]. The goal is to provide a rapid and sustainable tool for point-of-care diagnostics.

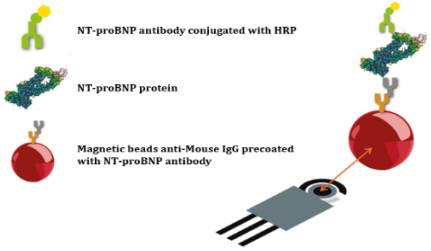

Figure: Representation of the immunological chain using Magnetic Beads anti-Mouse IgG

We report results related to the first step of our study, in which we are fine-tuning the design of the biosensor by choosing the appropriate type of paper. We have optimized the working volume (310 µL) and the labeled antibody concentration (1 µg/mL), using a NT-pro BNP concentration of 1 µg/mL. The goal is to incorporate the specific recognition elements into the paper and then add the substrate into a screen-printed paper-based sensor for a reagent-free measurement. Furthermore, the use of paper as support will manage the microfluidics without the use of any external pump.

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Acknowledgement

The project MHCD has received funding from the Regional Programme of the European Regional Development Fund, prot. N° A0613-2023-077260 - PR FESR LAZIO.

P17

DNA condensates as biosensing platform for therapeutics

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Membraneless organelles, such as DNA/RNA condensates formed via liquid-liquid phase separation (LLPS), provide a flexible and dynamic platform for biosensing applications in therapeutic advancements.[1, 2] The DNA condensates may offer a biomimetic environment for molecular recognition, enabling the detection of disease biomarkers with high sensitivity and specificity. By leveraging multivalent interactions, DNA condensates can be engineered to respond to specific nucleic acid sequences, proteins, or small molecules, making them ideal for diagnostics and targeted drug delivery.[1-3] The condensates also play a crucial role in regulating intracellular machinery and maintaining physiological homeostasis in biological system.[2, 4] In the context of cancer and infectious diseases, DNA condensate-based biosensors may facilitate real-time monitoring of disease progression and therapeutic response. Additionally, their tunable physicochemical properties may allow for controlled drug release, improving therapeutic efficacy while minimizing off-target effects.[2-5]

In this work, we aim to develop nucleic acid condensate as a biosensing platform for cancer and other critical diseases. Formation of DNA droplets via condensation were confirmed using fluorescence microscopy imaging in the presence of divalent Mg^{2+} and monovalent cations like Na^{+}, or K^{+}. The fluorescence microscopy imaging and dynamic lights scattering measurement reveal the avearge size distribution profile of dynamic DNA condensates. In our next approach, therapeutic oligonucleotides e.g., antimicro RNA will be incorporated to investigate the dynamic behaviour and encapsulation efficiency of DNA condensates for therapeutic cargo in physiological microenvironment.

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P18

Bacterial chemical signaling: a novel HPLC-MS/MS approach for quorum sensing molecules analysis

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Quorum sensing (QS) is a sophisticated chemical communication system that allows bacteria to interact through signaling molecules known as autoinducers (AIs). This mechanism enables bacteria to monitor population density and regulate gene expression, triggering specific processes once a "quorum" is reached. In Gram-positive bacteria QS is mediated by oligopeptides, whereas Gram-negative bacteria rely on small organic molecules, primarily N-acyl homoserine lactones (AHLs). These molecules play a crucial role in processes such as biofilm formation, bioluminescence, secondary metabolite production, swarming motility and expression of various virulence factors[1].

This study aimed to develop an advanced analytical method with high selectivity and sensitivity for the detection and quantification of QS molecules, specifically N-acyl homoserine lactones (AHL) and hydroxyquinolones (HQ), in biological samples. An HPLC-MS/MS method was optimized for the identification and quantification of 14 AHLs and one HQ. Several chromatographic columns were tested (Jupiter C4, Luna C18, Kinetex C18, and Gemini C18; Phenomenex, Milan, Italy), along with different mobile phase compositions using acetonitrile in combination with formic and picolinic acids. The best chromatographic separation was achieved using a Luna C18 column (150 × 2 mm, 3 µm) with an elution system consisting of 0.1% formic acid + 0.016% 2-picolinic acid in water and 0.1% formic acid in acetonitrile. The total run time was 10 minutes (figure 1).

Detection was performed using a triple quadrupole mass spectrometer (QTRAP 5500, Sciex, Milan, Italy) equipped with a Turbo V™ source (ESI, positive ionization mode). MRM transitions were optimized via direct injection analysis. Under these conditions, the limits of detection (LOD) and quantification (LOQ) ranged from 0.5 to 4.2 µg/L and from 0.5 to 54 µg/L, respectively. The method was successfully applied to biological samples from patients with septic shock, using a liquid-liquid extraction protocol with ethyl acetate. QS molecules were detected in all analyzed samples, with N-dodecanoyl-DL-homoserine lactone showing the highest plasma concentration (3 µg/L on average).

The developed HPLC-MS/MS method demonstrated excellent performance in terms of sensitivity, selectivity, and speed, making it a powerful tool for studying QS molecules in biological samples. To our knowledge, this is the first study enabling the simultaneous detection of 14 AHLs and one HQ in biological matrices, opening new perspectives for understanding the role of QS in clinical and microbiological settings.

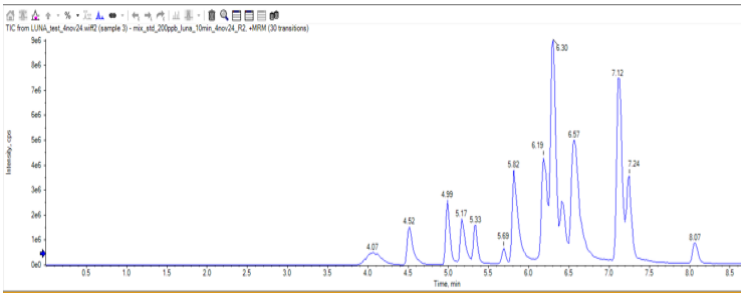

Figure: Chromatographic separation of a standard solution of QS (200 ng/L) using the reverse phase column LUNA C18 (150 × 2 mm, 3 µm).

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P19

In vivo quantification of therapeutic biologics using electrochemical DNA-sensors

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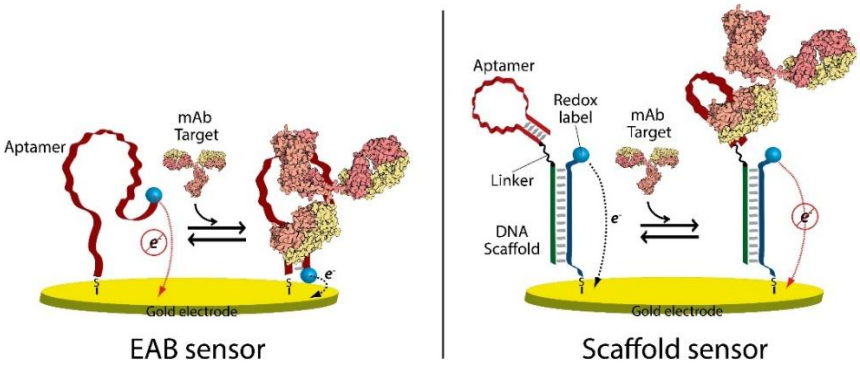
Pharmacokinetic variability between patients is a factor that significantly complicates the optimization of pharmacological therapy, making it difficult to ensure that drug levels remain within the desired therapeutic range. In precision medicine, the therapeutic approach is based on personalizing treatment according to the genetic and physiological characteristics of the individual, aiming to improve the effectiveness and safety of therapy. However, the application of this principle is hindered by pharmacokinetic monitoring methods, which are still slow and dependent on blood draws and complex laboratory analyses.

To address this challenge, we are developing electrochemical DNA-based sensors [1], a technology that enables real-time and minimally invasive monitoring of therapeutic drug levels directly in vivo. These sensors, thanks to their ability to provide rapid readings (even in seconds) and with minimal technical requirements, could represent a significant step towards more effective precision medicine.

In this study, we present the development of DNA sensors specifically designed to monitor the concentration of Trastuzumab, a monoclonal antibody used in cancer treatment, in vitro and in vivo [2]. Specifically, we explored two different DNA-based sensing architectures: the scaffold eDNA sensor and electrochemical aptamer-based (EAB) sensor. We achieved higher analytical performance in terms of signal gain and sensitivity with EAB sensors, and we demonstrate that we can obtain quick and easy measurements of the drug's presence in blood, providing more precise information about the drug's pharmacokinetics. This approach could help to optimise therapeutic dosing, ensuring safer and more effective treatment management



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P20

Shading new light on the systemic response following new synthetic opioid intake through metabolomics

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Novel psychoactive substances (NPS) represent a broad class of new drugs that often allow passing drug-screening tests. They are characterized by a variety of structures, thus creating an ever-changing scenario with evolving analytical targets [1]. This study aimed at developing an indirect screening strategy for NPS monitoring, and specifically for new synthetic opioids, based on assessing changes in endogenous urinary metabolite levels resulting from the systemic response following their intake. The experimental design involved in-vivo CD-1 mice models: 20 animals (10 male, 10 female) received different treatments. At day 0, mice were treated with a vehicle and urine was collected at 0-12h and 12-24h. On day 1, brorphine was administrated to half of the animals of both genders, while the other half received etonitazene; urine samples were collected at the same time intervals. A second drug administration and subsequent urine collection occurred at day 8. Finally, following a one-week washout, urine was collected again at day 15. Urine samples were analyzed by LC-HRMS/MS with an untargeted metabolomics platform. Mass spectra were acquired on an Orbitrap Q-Exactive mass spectrometer in full scan/data dependent acquisition mode; every sample was analysed with both RP and HILIC chromatography in both polarities. Given the large amount of information in the metabolomic matrix, the data underwent a pre-treatment phase to ensure uniformity. By combining multivariate analysis (PLS-DA) and univariate analysis (Volcano plot), it was possible to identify the most altered metabolites following acute drug administration. These included metabolites involved in lipid peroxidation, inflammatory processes, oxidative stress, nucleotide metabolism and others. To assess metabolome changes based on different factors such as collection time, gender, and drug treatment, advanced chemometrics techniques were used. The adopted approach offered a new perspective to address the scarcity of studies on the urinary metabolic profile of opiate-treated mice.

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P21

Development of a sandwich ELISA-based method for fast and effective detection of pathogenic Y. enterocolitica in food samples: preliminary results

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The genus Yersinia has been recently classified to the Yersiniaceae family and comprises 28 species, three of which are pathogenic for humans: Y. enterocolitica; Y. pseudotuberculosis and Y. pestis. Whereas Y. pestis causes plague and is transmitted to humans by rat flea bites or through the inhalation of aerosol from infected patients, Y. enterocolitica and Y. pseudotuberculosis cause gastroenteritis and are mainly transmitted by ingestion of contaminated food and water.

In industrialized countries, most infections are caused by Y. enterocolitica. Among many existing Y. enterocolitica biotypes, only a few strains are pathogenic to humans. These strains have numerous virulence factors encoded by plasmid and chromosomal genes. The chromosomal ail gene, which encodes for an outer membrane protein called ail protein, is present uniquely in the pathogenic strains of Y. enterocolitica. This protein is involved in the initial colonization and penetration phase of the host's intestine.

The standard cultural method (ISO 10273) for detecting and isolating Y. enterocolitica from food samples is time-consuming, taking at least 7-8 days, and requires several phenotypic assays to differentiate pathogenic and non-pathogenic strains. To overcome these limitations, several researchers have successfully detected virulent strains of Y. enterocolitica by amplifying the ail gene via PCR or real-time PCR. Therefore, ISO approved a standard method (ISO/TS 18867:2015) for rapid identification of pathogenic Y. enterocolitica, based on the recognition of this gene by real-time PCR.

The detection of ail protein by rapid, cost-effective, and accurate methods, such as immunological assays, has been poorly explored. Therefore, the present study aims to develop a sandwich ELISA-based method for fast and effective detection of pathogenic Y. enterocolitica in food samples, using ail protein as target. To achieve this goal, rabbit polyclonal antibodies against recombinant ail protein (r-ail protein) were selected and purchased. Recombinant ail protein was obtained by cloning the ail gene of Y. enterocolitica into E. coli, where it is transcribed and translated.

Preliminary ELISA tests were carried out successfully to verify the capability of the polyclonal antibodies (PAb and PAb-HRP) to bind r-ail protein. In particular, two binding curves were constructed by incubating different dilutions of each antibody with a fixed amount of r-ail protein, immobilized in a conventional ELISA plate. Experiments with pathogenic and non-pathogenic strains of Y. enterocolitica and other non-target bacteria are in progress to establish whether the antibodies can selectively recognize the native ail protein exposed on the outer membrane of the pathogenic Y. enterocolitica strains.

P22

Dynamic nucleic acid systems for bioanalytical applications

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Synthetic nucleic acids are emerging as particularly suitable materials for the construction of artificial DNA- and RNA-based systems, paving the way to transform our understanding of cell biology. By building artificial, programmable DNA and RNA systems from the bottom up we have the opportunity to extend the self-assembly capabilities of materials and devices that utilize biological components but operate in a simplified manner. Here, we present the rational design of artificial, multifunctional synthetic gene networks whose design can be adapted to regulate molecular cargo[1] release from biomolecular receptors, as well as to direct the self-assembly of DNA nanostructures[2] for diagnostics and drug delivery as key applications. Further, we introduce the development of DNA motifs whose design parameters can be adapted to regulate the recruitment of many cellular clients at specific times and locations [3], or control the catalytic activity of target proteins[4]. In particular, we focus on how this can be used to build tunable organelles within emulsion droplets that mimic cellular compartmentalization and have potential applications in biosensing and diagnostics. Overall, our approach aims to demonstrate the potential of programmable DNA- and RNA-based systems and their ability to provide a platform for the development of functional biomolecular systems that mimic cellular processes in a controlled environment for bioanalytical applications.

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