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Dipartimento di
Biotecnologie Molecolari
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Dipartimento di
Chimica

Giornate di Bioanalitica 2026

DALLE SCIENZE OMICHE AI LIGAND BINDING ASSAY

13-15 Aprile 2026, Torino

GIORNATE DEDICATE AL CONTRIBUTO DELLA CHIMICA BIOANALITICA E WORKSHOP “AI E MACHINE LEARNING PER LA BIOANALITICA”

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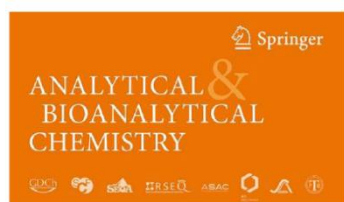

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Programma Scientifico

Lunedì, 13 Aprile 2026

AULA MAGNA DEL RETTORATO, UNIVERSITÀ DI TORINO

10:00 REGISTRAZIONE

11:00 SALUTI ISTITUZIONALI
Prof.ssa Stefania Beolé Delegata della Rettrice per la Ricerca Scientifica
Prof. Lorenzo Maschio, Vicedirettore alla Ricerca del Dipartimento di Chimica

11:15 - 13:00 SESSIONE SCIENTIFICA
Chair: **Prof.ssa Maria Careri,** Università degli Studi di Parma e **Prof. Alessandro Porchetta,** Università degli Studi di Roma Tor Vergata

Keynote

11:20 **KN1 - S. Fortunati,** *Advances and challenges in IoT-integrated biosensors for Point-of-Care diagnostics*, Università degli Studi di Parma

Presentazioni orali

11:55 **O1 - C. Orecchio,** *Image-based modeling of three-way data for butter authentication*, Università di Torino

12:10 **O2 - A.C. Di Pede**, *Triplex DNA clamp regulates Cas12a activation for ssDNA and RNA sensing*, Università degli Studi di Roma Tor Vergata

12:30 **O3 - G. Gambardella,** *Synthetic Gene Networks for Tunable Regulation of Enzyme-Inhibitor Complexes*, Università degli Studi di Roma Tor Vergata

12:45 **O4 - I. Peralta,** *Untargeted UHPLC-HRMS combined with multivariate statistics to reveal the effect of agricultural sustainable treatments on metabolomics of basil*, Università degli Studi di Parma

13:00 -14:00 *Lunch*

14:00 -15:45 *TAVOLA ROTONDA "**OPEN SCIENCE, FAIR DATA E QUALITÀ DEI DATI**"*

Moderatori: **Prof. Marco Aldinucci**, Università di Torino e **Prof.ssa Sandra Furlanetto**, Università degli Studi di Firenze

Intervengono:

Prof. Marco Aldinucci, Università di Torino
Prof. Alessandro Enrico Cogo, Università di Torino
Dr.ssa Michaela Kuepferling, Istituto Nazionale di Ricerca Metrologica (INRIM) Torino
Dr.ssa Francesca Rita Novara, Wiley-VCH.

15:45 -16:15 SESSIONE POSTER

16:15 -16:45 *Coffee break*

16:45 -18:30 SESSIONE SCIENTIFICA

Chair: **Prof. Dario Compagnone**, Università degli Studi di Teramo e **Prof.ssa Monica Mattarozzi**, Università degli Studi di Parma

Keynote

16:45 **KN2 - S. Giordani**, *From nano sentinels to nature-based drug carriers: exploring the potential of EVs via AF4-based analytical platforms*, "Alma Mater Studiorum" Università di Bologna

Flash Presentations

17:10 **FP1 – E. Belforte,** *Magnetically separable HaloTag–NanoLuc reporter for bioluminescent CRISPR/Cas12 nucleic acid detection*, Università degli Studi di Roma Tor Vergata

17:15 **FP2 - T. Lomonaco,** *Exhaled breath acetone: a non-invasive marker of disease severity across the spectrum of heart failure*, Università di Pisa

17:20 **FP3 - V. Rondinini,** *AF4-MD Characterization of Bio-Conjugation on SiNPs for highly sensitive TCL-POCT Applications*, "Alma Mater Studiorum" Università di Bologna

17:25 **FP4 – S. Ducoli**, *The broad-spectrum absorption capability of true-to-life micro- and nanoplastics,* Università degli Studi di Brescia

17:30 **FP5 – A. Ghignone,** *An Optimized Workflow for Untargeted Serum Lipidomics: Impact of Sample Handling and Acquisition Strategies,* Università del Piemonte Orientale

Presentazioni orali

17:40 **O5 - A. Cerrato**, *Regioisomer-resolved annotation of cholesteryl esters by photochemical derivatization and negative-ion-mode LC-MS/MS*, Sapienza Università di Roma

17:55 **O6 - D. Biagini**, *From Plastics to Cellular Pathways: Activation of the Arachidonic Acid Cascade in Cardiomyocytes Exposed to Virgin and Aged Microplastics*, Università di Pisa

18:10 **O7 - P. Sfragano,** *Electrochemical characterization of sustainable soot-based nanocomposites*, Università degli Studi di Firenze

18:30 CONCLUSIONE

20:00 CENA SOCIALE, Ristorante *Villa Glicini*

Martedì, 14 Aprile 2026

AULA MAGNA DEL RETTORATO, UNIVERSITÀ DI TORINO

09:00 -10:30 SESSIONE SCIENTIFICA

Chair: **Prof. Luigi Reschiglian**, "Alma Mater Studiorum" Università di Bologna e **Prof.ssa Laura Anfossi**, Università di Torino

Keynote

09:00 **KN3 - F. Eugelio**, *Quinolizidine alkaloids in lupins: from contaminants to markers of traceability*, Università degli Studi di Teramo

Presentazioni orali

09:25 **O8 - A. Silvestri**, *High-Sensitivity Electrochemical Detection of IL-18 in 3D Patient-Derived ALS Models*, Università Ca' Foscari Venezia

09:40 **O9- C. Reale**, *Direct mass spectrometry analysis of mollusks lipidome for deciphering bio-signatures of habitat and climate change*, Università degli Studi di Messina

09:55 **O10 - E. Taglioni**, *A Flow-Splitting Orthogonal 2D-LC Platform for Reliable Impurity Quantification of Therapeutic Oligonucleotides*, Sapienza Università di Roma

10:10 **O11 - S. Fornasaro**, *How Methodological Flaws Distorted a Decade of Serum SERS Studies*, Università degli Studi di Trieste

10:25 **O12 - E. Paialunga**, *3D-Printed Microneedle-Based Electrochemical Aptasensor for Vancomycin Monitoring in Interstitial Fluid*, Università degli Studi di Roma Tor Vergata

10:40 -11:10 *Coffee break*

11:10 -13:00 SESSIONE SCIENTIFICA

Chair: **Prof.ssa Anna Laura Capriotti**, Sapienza Università di Roma e **Prof.ssa Paola Agata Eustochia Donato**, Università degli Studi di Messina

Keynote

11:10 **KN4 - C.M. Montone** *Green and Automated Platforms for Biomarker Analysis*, Sapienza Università di Roma

Presentazioni orali

11:35 **O13 - L. Floris,** *Systematic development and optimization of a cIEF method for the charge variants analysis of basic monoclonal antibodies using the Analytical Quality by Design strategy*, Università degli Studi di Firenze

11:50 **O14 - T. Lomonaco,** *Towards non-invasive kidney monitoring: sweat-based measurement of creatinine and Cystatin-C proteoforms in CKD*, Università di Pisa

12:05 **O15 - D. Paolini,** *Lab-on-3D-printed devices for integrated bioelectroanalytical sensing*, Università degli Studi di Teramo

12:20 **O16 - G. Iula**, *Enhanced Photoluminescence and Electrical Conductivity of Carbon Quantum Dot–UiO-66-$(OH)_2$ Composites for Sulphur Dioxide and Sulfamethoxazole Sensing*, Università degli Studi di Napoli Federico II

12:35 **O17 - J. Brandi,** *Integration of NanoMIPs on D-Shaped POF-SPR Platform for High-Affinity SARS-CoV-2 RBD Sensing*, Università degli Studi di Verona

12:50 -14:00 *Lunch*

14:00 -15:30 SESSIONE SCIENTIFICA

Chair: **Prof. Claudio Baggiani**, Università di Torino e **Prof.ssa Barbara Roda**, “Alma Mater Studiorum” Università di Bologna

Keynote

14:00 **KN5 - A. Bonini,** *Single-molecule proteomics with nanopores*, Università degli Studi di Firenze

Presentazioni orali

14:25 **O18 - F. Di Francesco**, *Identification and Real-Time Monitoring of Emotional Status Chemosignals in Human Body Odours*, Università di Pisa

14:40 **O19 - R. Di Lecce,** *Volumetric urine microsampling and fully automated cannabinoids analysis*, “Alma Mater Studiorum” Università di Bologna

14:55 **O20 - D. Marra**, *A sensitive and simple smartphone-based lateral flow assay for PSA detection using core-satellite magnetic nanoparticles*, Università degli Studi di Napoli Federico II

15:10 **O21 – E. Primiceri**, *Development of Innovative Electrochemical MIP-based Sensors for Biomedical and Environmental Applications*, CNR Nanotec, Lecce

15:25 Assegnazione dei riconoscimenti “Analytical and Bioanalytical Chemistry Best Oral”

SALUTI CONCLUSIVI

WORKSHOP “AI E MACHINE LEARNING PER LA BIOANALITICA”

Aula Magna del Rettorato, Via Verdi, 8 - Torino

15:30 -16:00 REGISTRAZIONE e *Coffee break*

16:00 -18:00: *Methods Validation App (MVA)*
Giovanni Solarino, Prof. Eugenio Alladio

Mercoledì, 15 aprile 2026

AULA MAGNA CAVALLERIZZA REALE, UNIVERSITÀ DI TORINO

9:00 -10:30 *CACTUS: Chemometric and Analytical Chemistry Tools, Parte I*
Alessandra Olarini, Ciro Orecchio, Prof. Eugenio Alladio

10:30 -11:00 *Coffee break*

11:00 -12:30 *CACTUS: Chemometric and Analytical Chemistry Tools, Parte II*
Alessandra Olarini, Ciro Orecchio, Prof. Eugenio Alladio

SESSIONE POSTER 13 Aprile 15:45 -16:15

P1 - A. Bonini, *An electrochemical aptamer-based sensor for the therapeutic drug monitoring of anti-TNF-α monoclonal antibodies: A preliminary study*, Università degli Studi di Firenze

P2 – A. Magnani, *HPLC–MS/MS for advanced uremic toxin profiling in nephrology*, Università di Torino

P3 – A. L. Capriotti, *High-Resolution LC–MS/MS Peptidomics and Metabolomics for Non-Invasive Biomarker Discovery in Cardiac Damage*, Sapienza Università di Roma

P4 - C. De Lucia, *Multi-Analytical approaches to assess the physicochemical stability of biotherapeutic products: application to bevacizumab*, Università degli Studi di Firenze

P5 – F. Della Pelle, *Paper-based Sensors and Direct Electron Transfer Type Biosensors based on Graphenic Films Integrated on Ecoinnovative Substrates*, Università degli Studi di Teramo

P6 – T. Serra, *Biocompatible aldehyde–gum arabic/gelatin scaffolds for 3D cell culture: physicochemical, morphological and stability characterization*, Università di Torino

P7 – V. Testa, *Integration of Molecularly Imprinted Nanoparticles into Competitive and Sandwich pseudo-Immunoassays*, Università di Torino

P8 – Z. Yousefniayejahromi, *Innovative aptasensor for the rapid detection of Escherichia coli in real matrices*, Università di Pisa

P9 – T. Pacini, *Is a Standard-Free HRMS-QTOF Workflow a reliable tool for Semi-Quantitative Pesticide Screening in Biological Samples?,* Istituto Zooprofilatico Sperimentale dell’Umbria e delle Marche “Togo Rosati”

P10 – S. Stanzani, *Development of a Lateral Flow Immunoassay for the differential diagnosis of European Brown Hare Syndrome Virus and Rabbit Haemorrhagic Disease Virus-2*, Università di Torino

P11 – A. Di Bernardo, Untargeted lipidomics of thymic tissue: insights into aging-associated lipid remodeling, Università di Torino

Keynotes

KN1 - Advances and challenges in IoT-integrated biosensors for Point-of-Care diagnostics

Simone Fortunati
Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università di Parma, Parma

In recent years, there has been a growing trend towards the implementation of on-board wireless connections in electrochemical biosensors, including genosensors and immunosensors, enabling the integration into smart environments, such as the Internet of Things (IoT) [1]. Electrochemical transduction is particularly well-suited for IoT-integrated systems because it facilitates the creation of compact, portable, and easily miniaturized sensor devices compared to other mechanisms such as optical transduction. This integration can be exploited for data encryption and transfer to a cloud service for storage and/or further processing through advanced data deconvolution aimed at developing classification and prevision models enabled by Machine Learning algorithms. These features are particularly promising for developing IoT-integrated point-of-care (PoC) biosensors for clinical diagnostics, as they can provide cost-effective, rapid, robust and portable devices for diagnosis at the point of care near the site of patient care [2]. However, while integration with IoT protocols provides several benefits, including advanced data processing and rapid remote monitoring, significant challenges remain. These include the computational complexity required to process large datasets, sensitivity of wireless signal to environmental changes, and stringent requirements for data security, patient privacy, and system interoperability [3,4].
Over the course of several research projects, our group has devised biosensors for PoC applications integrating micro- and nano-material-based immobilisation substrates such as carbon nanotubes [5], gold nanoparticles [6] and micromagnetic particles [2,7] with novel receptors, including DNA mimics [7] for liquid biopsy as a theranostic tool, aptamers [8], antibodies [9], engineered proteins [10] for the detection of viral infections and peptides for the detection of tumour biomarkers [11]. These genosensors and immunosensors have been interfaced with prototypes of a smart and portable device capable of performing differential pulse voltammetry analysis [1]. The device has undergone continuous improvement of its capabilities, starting from a WiFi-enabled electrochemical acquisition device [12] to finally become a multichannel platform implementing Machine Learning data processing [13]. The integration of the device with electrochemical biosensors allowed to successfully detect several biomarkers of interest for PoC applications, including the diagnosis of celiac disease [6], colorectal cancer [7], and Sars-CoV-2 infection [10] and immunity [9].
The synergy between electrochemical biosensors and portable smart acquisition devices offers a significant advantage for clinical applications, enabling rapid diagnosis in decentralized settings with real-time sharing of results, as well as the opportunity to create a patient data repository for long-term monitoring of relevant biomarkers. As a result, the application of IoT in PoC analysis plays a key role in reducing healthcare costs and improving treatment outcomes.

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13. A. Boni, V. Bianchi, S. Fortunati, M. Giannetto, M. Careri, I. De Munari, IEEE Trans. Instrum. Meas. 2022, 72, 1–12. DOI:10.1109/TIM.2022.3228004.

KN2 - From nano sentinels to nature-based drug carriers: exploring the potential of EVs via AF4-based analytical platforms

Stefano Giordani *[a,b,c] , Virginia Rondinini [a], Mario Kurtjak [d], Hrvoje Križan [e], Anna Placci [a,b,c], Andrea Zattoni [a,b,c], Barbara Roda [a,b,c], Pierluigi Reschiglian [a,b,c], Mladenka Malenica [e], Valentina Marassi [a,b,c]

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[e] Department of Medical Chemistry, Biochemistry and Clinical Chemistry, University of Rijeka, 51000 Rijeka, Croatia;

One of the most promising trends in nanomedicine is the study of extracellular vesicles (EVs), a heterogeneous group of nanoparticles involved in intercellular communication. These vesicles can act both as “nano sentinels,” reflecting physiological and pathological states, and as nature-inspired drug carriers with enhanced biocompatibility and targeting capabilities [1]. However, their structural complexity, heterogeneity, and low abundance in complex biological environments pose significant analytical challenges, requiring robust, high-resolution characterization strategies. In this context, the analytical chemist plays a central role, not only in developing and optimizing isolation methodologies, but also in critically interpreting multidimensional data, ensuring measurement reliability, and bridging the gap between physicochemical characterization and biological function. Among current methodologies, Asymmetrical Flow Field-Flow Fractionation (AF4) coupled with multiple detectors (DAD, MALS, dRI) stands out as a versatile platform for gentle, label-free purification and in-depth analysis of vesicular systems across a broad size range [2]. These outstanding features have led to the widespread use of AF4 in supporting innovative diagnostic and prognostic approaches, for instance, in colorectal cancer [3], as well as in enabling the selection and isolation of vesicle-based nanocarriers. In this work, the dual potential of AF4 in this field is explored. On the diagnostic side, a method is presented to isolate and characterize EVs directly from untreated human cerebrospinal fluid (CSF). CSF samples from traumatic brain injury (TBI) patients were collected at multiple time points post-injury, including both individual and pooled samples. The pooled sample was used to optimize the AF4 method, identify the EV elution region, and perform initial molecular and morphological characterization via Western blot and Atomic Force Microscopy (AFM). The optimized method was then applied to individual samples, where AF4 allowed collection of EV-enriched fractions, revealing a temporal trend: EV/protein signal intensity increased up to day 4 post-injury and then decreased. This pattern is consistent with a transient neuroinflammatory response followed by stabilization, highlighting the potential of CSF-derived EVs as biomarkers for monitoring TBI progression and providing time-resolved information to support clinical applications. Vesicle-like nanoparticles naturally present in bee products, such as royal jelly (RJ), are instead promising candidates for drug delivery, although their exploration is limited by challenges in identification and isolation. To address this, a semi-preparative AF4 setup was employed to separate and characterize nanoscale components directly from RJ samples under native conditions. The method identified multiple nanoparticle populations with distinct sizes and compositions. AF4 fractions were further analysed by AFM, confirming particle morphology, size, and concentration, and highlighting the most promising fractions to be exploited as nanocarriers. The semi-preparative approach also allows scale-up for isolation of larger quantities, supporting the development of RJ-derived nanocarriers for nanomedicine. Overall, this work demonstrates the versatility of AF4 both in the diagnostic and preparative field, offering a comprehensive platform for the characterization and exploitation of vesicular nanoparticles in medical applications.

Acknowledgments: This work has been supported by the University of Rijeka, uniri-mzi-25-34 grant, Nano-delivery particles from bee products for better bioavailability of bioactive compounds with antitumor activity, BEE-nano.

1. *Y. Tsai, L. Chang, C. Lan, Y. Chen, J. Yang. Int J Nanomedicine. 2025; 20:12767–12781. doi: 10.2147/IJN.S541042*

2. *S. Giordani, V. Marassi, A. Placci, A. Zattoni, B. Roda, P. Reschiglian. Molecules. 2023; 28:6201. doi: 10.3390/molecules28176201*

3. *V. Marassi, S. Giordani, A. Placci, A. Punzo, C. Caliceti, A. Zattoni, P. Reschiglian, B. Roda, A. Roda. Sensors. 2023; 23: 9432. doi: 10.3390/s23239432*

KN3 - Quinolizidine alkaloids in lupins: from contaminants to markers of traceability

Fabiola Eugelio*[a]

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Lupins (*Fabaceae* family) have gained increasing attention as a sustainable and nutritionally valuable crop, due to their high protein and fiber content and favorable agronomic properties. However, lupin seeds also contain quinolizidine alkaloids (QAs), secondary metabolites derived from L-lysine and involved in plant defense mechanisms. These compounds are responsible for the characteristic bitter taste of lupins and have traditionally been considered antinutritional factors, being subject to regulatory control due to their known neurotoxicity in humans and animals. The research presented here aims to go beyond this traditional view by exploring whether the chemical complexity of QAs can serve as a source of useful information in food science, while addressing their analytical challenges. From an analytical standpoint, QAs show high chemical diversity, including compounds such as lupanine, sparteine, and multiflorine, requiring analytical methods capable of both high selectivity and broad coverage. Matrix effects further complicate accurate quantification, especially at low concentration levels required for regulatory purposes.

To address these aspects, a multi-step analytical strategy was applied, progressively expanding both the analytical approaches and data interpretation. Each stage aimed not only to improve analytical performance, but also to evaluate whether QA variability could provide information on the identity and origin of lupin seeds.

A first validated ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) method, based on selective solid phase extraction (SPE) clean-up, was applied to *Lupinus albus* L. samples under different agronomic conditions, establishing a robust basis for alkaloid quantification and revealing significant differences in QA profiles [1].

Building on this, a hybrid triple quadrupole–linear ion trap (QqQ-LIT) platform, combining Multiple Reaction Monitoring (MRM) with information-dependent acquisition (IDA) and enhanced product ion (EPI) experiments, enabled the putative identification of additional alkaloids and improved structural understanding. The application of multivariate analysis demonstrated clear geographical discrimination of samples from four Italian regions, supporting the potential of QA fingerprinting for provenance classification [2].

The final step of the investigation broadened the scope further, moving from targeted and semi-untargeted alkaloid profiling to untargeted metabolomics by means of high-resolution mass spectrometry (UHPLC-Q-Orbitrap-HRMS). This allowed a comprehensive investigation of the lupin metabolome, capturing the influence of both geographical origin and agricultural practices. Even in this unbiased framework, alkaloids emerged again among the most discriminant markers, alongside amino acids, lipids, and fatty acids, providing strong evidence of their intrinsic informative value, independently of targeted strategies [3].

Overall, these results show how mass spectrometry, applied through complementary approaches and combined with multivariate chemometric analysis, can change the way quinolizidine alkaloids are interpreted. Compounds traditionally considered mainly as safety concerns can, when studied in sufficient detail, also act as informative molecular markers. In this context, their chemical complexity should be seen not as a limitation, but as a useful feature that can be exploited for food authentication, quality control, and traceability of lupin-based products.

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2. F. Eugelio, S. Palmieri, M. Mascini, F. Della Valle, F. Fanti, E. Oliva, M. Del Carlo, D. Compagnone, M. Sergi. Food Chemistry X, 2024, 23, 101769. doi:10.1016/j.fochx.2024.101769

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KN4 - Green and Automated Platforms for Biomarker Analysis

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Despite the need for reliable and rapid workflows, accurate quantification of biomolecules in biological matrices remains a major analytical challenge due to their low abundance and significant matrix effects. In this context, online extraction and enrichment strategies using *in situ* synthesized monoliths in SPME tubing offer significant advantages, such as miniaturization, near-complete automation, and reduced environmental impact.

In particular, the use of multicomponent reactions and click chemistry (thiol-ene) enables the one-pot preparation of functionalized monoliths with minimal reagent consumption and precise control of surface chemistry [1]. These materials feature tunable porosity and multiple interaction mechanisms (hydrogen bonding, π–π, dipole–dipole, interactions with sulfur-containing sites), allowing efficient enrichment of diverse analytes such as β-estradiol [2] and neurotransmitters directly from serum or plasma, without derivatization.

Integration of these monoliths with LC–MS/MS systems for online extraction and quantitative analysis has demonstrated performance comparable or superior to traditional methods, with recoveries >94% and detection limits in the pg–ng mL^{-1} range. This versatile, green approach not only simplifies sample preparation but also opens new perspectives for multiplexed analysis of systemic biomarkers, with potential applications in clinical diagnostics, pharmacology, and neurochemical research.

Figure 1: Analytical workflow enabling rapid implementation at the clinical and diagnostic level.

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KN5 - Single-molecule proteomics with nanopores

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Biological nanopore-based technologies have proven highly successful in DNA analysis, forming the basis of third-generation DNA sequencing methods that enable long-read, high-throughput, and cost-effective sequencing, greatly expanding the use of sequencing technologies in genomics [1]. For a long time, extending nanopore technology to protein analysis was considered a major challenge due to the intrinsic differences between DNA and proteins, including their structural complexity, heterogeneity, and the presence of post-translational modifications (PTMs) [1,2].

More recently, thanks to advances in nanopore engineering and improved control over biomolecular translocation, nanopores are emerging as promising tools for real-time peptide and protein sensing and potentially for the sequencing of full-length proteins [3-7]. These approaches make it possible to shift protein observation from bulk measurements to the single-molecule level and may help address key challenges in proteomics, such as the detection of PTMs, the quantification of low-abundance proteins, and the characterization of protein variants. Although nanopore-based peptide and protein analysis and sequencing technologies are still under development, these methods could complement established analytical techniques, such as mass spectrometry-based approaches, by expanding the toolbox available for studying proteome complexity [2].

In this talk, an overview of recent advances in nanopore-based peptide and protein analysis will be presented, with particular focus on the emerging field of single-molecule protein sequencing [2,7].

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Comunicazioni orali

O1 - Image-based modeling of three-way data for butter authentication

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In the last years, the issue of food authentication has received increasing attention due to the growing consumer demand for high quality and regionally distinctive food products [1]. As a result, food safety and authenticity detection play a crucial role in safeguarding consumers' interest, especially in cases involving high value-added ingredients [2]. Among these, butter, is practically susceptible to intentional adulteration with low-quality additives for economic profit.

Currently, some of the most effective analytical techniques for detecting food adulteration rely on three-way methods, such as fluorescence spectroscopy and GC-MS. Classical approaches for analysing such multiway data include well-established methods like PARAFAC and Tucker decomposition. However, these techniques are not always suitable for building robust and reliable discrimination or authentication models.

This study explores an alternative approach: treating three-way datasets as images, so two modes (e.g. excitation and emission wavelength) serve as spatial dimensions and the spectral intensities represent pixels values. This transformation allows the application of powerful image analysis models, particularly Convolutional Neural Networks (CNN) – that are broadly used for classification and anomaly detection. These models can be pre-trained on large image datasets and subsequently fine-tuned for specific cases, thereby reducing the need for extensive domain-specific training data.

To validate this strategy, a butter authentication case study was conducted. Samples from six different commercial brands of butter were collected from various retail sources. A subset of these samples was adulterated with 5% and 10% w/w of palm oil, resulting in a total of 91 samples. Excitation emission matrices (EEMs) were acquired for each sample.

Since the focus is on identifying a single target class (pure butter), a one-class modelling approach was adopted [3]. Specifically, a Variational Autoencoder (VAE), a class of deep learning models designed for image representation - was combined with the SIMCA algorithm to construct a one-class classifier [4]. Furthermore, canonical Data-Driven SIMCA (DD-SIMCA) was trained on entirely unfolded EEM profiles as benchmark method. All experimental results and methodological specifics – especially related to some innovative data augmentation strategies – will be disclosed during the presentation.

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O2 - Triplex DNA clamp regulates Cas12a activation for ssDNA and RNA sensing

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The extensive implementation of CRISPR-based technologies in molecular diagnostics has been driven by the discovery of collateral, non-specific nuclease activities exhibited by type V (Cas12) and type VI (Cas13) CRISPR systems following the recognition of specific DNA or RNA targets. [1,2] In particular, Cas12 is an RNA-guided endonuclease that integrates single- and double-stranded DNA recognition with collateral cleavage, which can be harnessed for signal generation through the Cas12-mediated digestion of fluorophore–quencher-labeled hairpin DNA reporters.[3]

Here we present a modular molecular platform for the conditional control of CRISPR–Cas12a cleavage activity based on a rationally engineered DNA hybridization network driven by Triplex Clamp formation. [4] The strategy exploits clamp-like triplex structures to enhance the affinity and specificity of homopurine ssDNA or RNA target recognition, while allosterically regulating a toehold-mediated strand displacement reaction embedded within a designed DNA hairpin architecture, termed the PAM-Switch. Upon target-induced triplex folding, the hybridization network undergoes structural reconfiguration that restores protospacer adjacent motif (PAM) accessibility, enabling activation of the Cas12a ribonucleoprotein complex. This activation event initiates collateral trans-cleavage activity, which is converted into a quantitative fluorescent readout. By uncoupling target recognition from direct interaction with the Cas12a–crRNA complex, the proposed strategy removes the requirement for sequence-specific crRNA redesign and enables parallel detection of multiple nucleic acid targets within a single CRISPR reaction mixture. The use of triplex-based clamps further improves discrimination of single-nucleotide variants and supports the detection of short ssDNA and RNA sequences (10–20 nt). Collectively, this approach overcomes key constraints of conventional Cas12a-based diagnostics and establishes a versatile framework for highly specific and modular nucleic acid sensing.

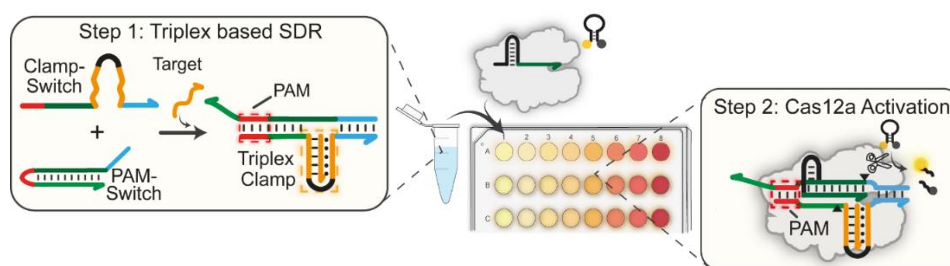

Figure 1. Schematic representation of the two-step sensing strategy. In step 1 (left), target recognition triggers a triplex-based strand displacement reaction, leading to the formation of the Triplex Clamp that enables reconfiguration of the PAM-Switch and exposure of the PAM motif. In step 2 (right), PAM availability allows Cas12a activation, resulting in collateral cleavage and generation of a measurable fluorescent signal.

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O3 - Synthetic Gene Networks for Tunable Regulation of Enzyme-Inhibitor Complexes

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Precise control of biomolecular interactions is central to synthetic biology, therapeutics, and biosensing [1]. Enzyme-inhibitor complexes, in particular, represent a powerful but underutilized axis for programmable regulation of enzymatic activity due to their inherent reversibility and specificity. However, conventional approaches to modulate these enzyme-inhibitor interactions often lack dynamic tunability and contextual responsiveness. Here, we present a modular platform that harnesses the programmability of synthetic nucleic acids and the versatility of cell-free gene networks to dynamically regulate enzyme-inhibitor complexes. Specifically, synthetic gene circuits are engineered to respond to specific targets and trigger the transcription of functional RNA strands in a highly programmable and orthogonal manner. The so transcribed RNA strands act as regulatory elements for the modulation of enzyme-inhibitor interactions. By encoding these logic-driven responses in transcriptional modules, we achieved repression or activation of the enzymatic activity, thus demonstrating how transcriptionally encoded RNA regulators can be used to achieve input-specific and dose-responsive control in a tunable and reversible way (Figure 1). We demonstrate this using different enzyme-inhibitor systems. Specifically, as proof-of-principle of our system, we employed the alpha amylase as enzyme [2] and its inhibitor derived from *Triticum aestivum*. We characterized the enzymatic activity and the cell-free gene network and we demonstrated the possible application of our system as a biosensing platform for the detection of nucleic acids and target antibodies. For all the above reasons, we believe our platform may represent a new route to achieve a dynamic biochemical control with broad implications for therapeutic enzyme regulation, biosensing and synthetic biology.

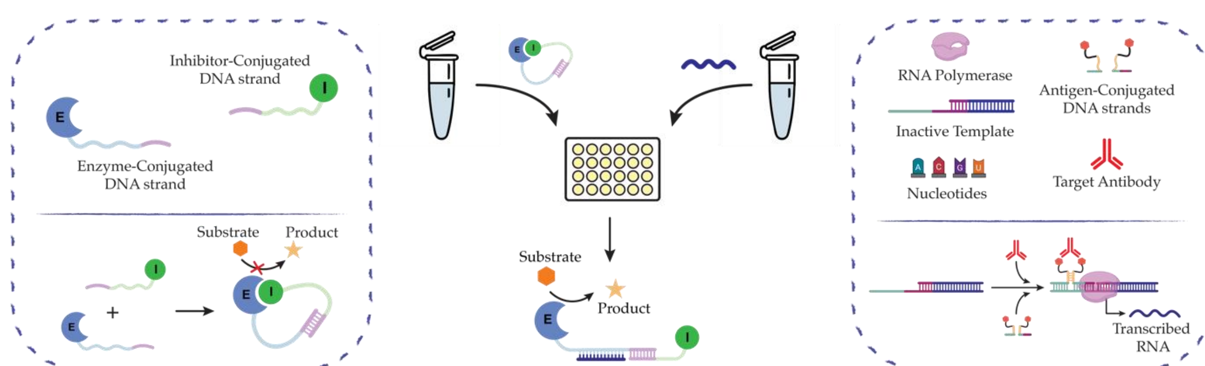

Figure 1: Representative scheme of the platform. (Left) Two proteins conjugated to two different DNA strands with a complementary region (in purple). (Right) The gene network involves a pair of antigen-conjugated DNA strands designed to hybridize to the single-stranded portion of a synthetic DNA template of the gene network and reconstitute the incomplete T7 RNA Polymerase promoter domain only upon recognition of a specific target antibody. The transcribed RNA will be able to remove the inhibitor from the enzyme through base pair interactions thus letting the conversion from substrate to product occur.

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O4 - Untargeted UHPLC-HRMS combined with multivariate statistics to reveal the effect of agricultural sustainable treatments on metabolomics of basil

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Advanced mass spectrometry-based techniques are providing new insights into the complexity of plant metabolism by identifying primary and secondary metabolites that play a crucial role in processes such as plant growth, stress responses, and nutritional value [1]. Plant metabolomics relies on a multidisciplinary approach aimed at agricultural improvement, drug development and sustainable ecosystem management [2]. As untargeted metabolomics evolves toward big data science, proper multivariate statistical tools are required to handle high-dimensional omics data and extract reliable information on metabolic processes [1].
In this study, untargeted metabolomics by ultra-high performance liquid chromatography-electrospray ionization Orbitrap high-resolution mass spectrometry (UHPLC-ESI Orbitrap HRMS) was applied to investigate the metabolome of basil (*Ocimum Basilicum* L.) in response to sustainable treatments, i.e., the addition of biochar alone and in combination with two different consortia of commercial plant growth promoting microbes. Field experiments were carried out to test three fertilization treatments as well as the control (untreated), with two harvests to account for variability related to different plant growth phases. The MS acquisition mode was Data Dependent Analysis (DDA, Top 4), implementing a scheduled exclusion list created from procedural blanks.
UHPLC-HRMS data analysis was performed by processing the raw data by Compound Discoverer 3.3 software (Thermo Fisher Scientific); after alignment and Quality Control (QC) area correction, features were initially filtered based on signal-to-noise ratio, analytical quality parameters, availability of the MS/MS spectrum in DDA acquisition and univariate *p*-value (ANOVA), shortlisting hundreds of features. ANOVA-simultaneous component analysis (ASCA) was applied as a multivariate extension of ANOVA particularly suitable when dealing with a high number of correlated variables as in metabolomic studies, often exceeding the number of samples [3,4]. This was feasible because the multivariate data were acquired according to a balanced experimental design at two factors (fertilization and harvest time). The significance of the fertilization factor was assessed by separating the variance attributable to its effect from the total variance, obtaining a *p*-value lower than 0.001. The score plot of the ASCA model for the fertilization effect allowed us to distinguish and separate the four groups using three Simultaneous Components (SCs). Further feature selection was carried out by considering the most significant loadings associated with the three SCs; these compounds were then submitted to annotation. The differentiation of the metabolic profile was mainly driven by sugars, amino acids, vitamins and essential nutrients, reflecting the impact of soil amendment and its combination with microbial consortia on the bioactive profile and overall quality of basil.
The multivariate data analysis approach applied in study to identify biomarkers able to differentiate among treatments highlights the triggered metabolic pathways, underpinning the decision-making process for a more sustainable food system.

This work was supported by the project by Emilia Romagna Region under the ERDT projects “SAFER” and “STREAM2B” (PR FESR 2021-2027 program -Action 1.1.2) with the support of the European Union.

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O5 - Regioisomer-resolved annotation of cholesteryl esters by photochemical derivatization and negative-ion-mode LC-MS/MS

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Cholesteryl esters (CE) play central roles in lipid transport and storage, yet their structural characterization remains challenging due to their extreme hydrophobicity and poor ionization efficiency [1]. Conventional CE lipidomics workflows typically rely on positive-ion-mode analysis and report CE at the sum-composition level, without resolving double-bond (C=C) positional isomerism. Chemical derivatization strategies enabling isomer-resolved analysis of CE are therefore highly desirable but remain largely unexplored [2]. In the present study, the use of the aza-Paternò-Büchi (aPB) reaction with 6-azauracil [3] was extended to CE, enabling negative-ion-mode LC–HRMS/MS analysis with annotation of C=C bond regiochemistry. Optimization of the derivatization conditions allowed efficient reaction of highly hydrophobic CE while maintaining compatibility with electrospray ionization. Tandem mass spectrometry revealed a previously unreported set of diagnostic fragment ions that proved particularly suitable for quantitative applications. The workflow enabled both relative and absolute quantitation of CE regioisomers with good linearity, repeatability, and trueness over a wide dynamic range, using a single dominant diagnostic ion to simplify data processing. Application to complex biological matrices of clinical interest demonstrated the feasibility of the approach, providing direct access to CE regioisomer distributions in human plasma and bovine liver.

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O6 - From Plastics to Cellular Pathways: Activation of the Arachidonic Acid Cascade in Cardiomyocytes Exposed to Virgin and Aged Microplastics

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Microplastics (MPs) are now recognized as a global environmental threat, widely distributed across ecosystems and increasingly entering the human body through ingestion and inhalation. Emerging evidence suggests that these pollutants may trigger vascular inflammation and negatively affect cardiovascular health. Among the key regulators of inflammation are oxylipins i.e., highly potent lipid mediators derived from polyunsaturated fatty acid oxidation, which play a crucial role in shaping and resolving inflammatory responses.

In this study, advanced oxylipin profiling based on micro-extraction by packed sorbent (MEPS) coupled with ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-ESI-MS/MS) was integrated with biological assays to evaluate the effects of polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and high- and low-density polyethylene (HDPE and LDPE) microplastics on the inflammatory status of human induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs). Both pristine and artificially aged MPs (four weeks at 40 °C under simulated solar irradiation (750 W/m^2)) were tested at a concentration of 1 mg/mL to reproduce realistic exposure conditions.

Using this comprehensive analytical platform, we demonstrated activation of the arachidonic acid cascade in cardiomyocytes exposed to MPs, including progression from the pro-inflammatory phase toward inflammation resolution, particularly evident in LDPE-exposed samples through the detection of lipoxin B4. Moreover, a polymer-specific inflammatory response emerged, with LDPE, HDPE, and PS acting as the strongest inducers of inflammation and oxidative stress. Finally, potential mechanisms underlying these effects were proposed, highlighting the link between microplastic exposure and the fine regulation of inflammatory pathways.

O7 - Electrochemical characterisation of sustainable soot-based nanocomposites

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The sustainable upcycling of carbonaceous waste into high-value functional materials represents an interesting opportunity in contemporary materials chemistry and electrochemical sensing. Carbon soot NPs, a pervasive by-product of incomplete hydrocarbon combustion, represents both a significant environmental challenge and a largely unexploited graphitic resource. In this contribution, we report the valorisation of ethylene-derived carbon soot nanoparticles (NPs) as a low-cost electroactive scaffold for advanced and sustainable electrochemical sensing, aligning with the principles of circular economy. Pristine soot NPs, collected from a quenched ethylene diffusion flame, present a promising graphitic core, but show limited intrinsic electrochemical activity due to poor surface functionality and sluggish electron transfer. To overcome these limitations, the soot NPs were subjected to a nitric acid oxidation treatment, introducing oxygen-containing functional groups, while preserving the underlying graphitic network. This oxidation step significantly improved surface wettability and provided anchoring sites for subsequent metal deposition. Thus, the oxidised soot NPs were decorated with Au NPs *via* two distinct *in situ* routes: spontaneous galvanic reduction and citrate-assisted reduction.

The electrochemical properties of the resulting hybrid nanocomposites were rigorously evaluated and compared by modifying both carbon paste electrodes (CPEs) and screen-printed carbon electrodes (SPCEs) by drop casting. Cyclic voltammetry and electrochemical impedance spectroscopy studies demonstrated that the platforms modified with the hybrid nanocomposite formed of soot NPs decorated using Au NPs obtained through citrate-mediated reduction consistently showed significantly enhanced charge transfer kinetics, with lower charge transfer resistances and higher electrochemically active surface areas when compared to the bare graphite surface or pristine soot NPs.

The analytical utility of these platforms was assessed by studying the detection of two analytes: hydrogen peroxide, utilised as a standard electrocatalytic benchmark, and diclofenac (DCF), a high-impact pharmaceutical pollutant. While bare carbon surfaces suffered from slow kinetics and high overpotentials, the Au NPs-decorated soot NPs exhibited a marked electrocatalytic response, benefiting from the synergistic effect of Au NPs intrinsic activity. These findings were further confirmed by diclofenac detection. Here, Au NPs-modified sensors significantly outperformed bare and undecorated carbon surfaces in terms of sensitivity. Moreover, selectivity tests showed minimal interference, and real-sample analysis of pharmaceutical tablets yielded recoveries near 100%. Preliminary experiments on the use of these hybrid nanocomposite platforms for the coupling with selected enzymes were also evaluated.

Acknowledgements

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O8 - High-Sensitivity Electrochemical Detection of IL-18 in 3D Patient-Derived ALS Models

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Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder that currently lacks validated molecular biomarkers for early diagnosis and prognosis, a gap that severely delays the implementation of personalized care.[1] While the proinflammatory cytokine Interleukin 18 (IL-18) has emerged as a promising marker linked to NLRP-3 inflammasome activation, traditional colorimetric ELISAs lack the analytical sensitivity required to distinguish subtle variations between Fast- and Slow-progressing ALS phenotypes. [2,3] To address this limitation, we developed a highly sensitive electrochemical ELISA (e-ELISA) by systematically optimizing key parameters, including the capture antibody immobilization strategy, the electrochemical mediator, and reagent concentrations. We validated this platform using sophisticated 3D innervated skin models composed of 3D-printed methacrylated hyaluronic acid (MeHA) and electrospun polylactic acid (PLLA) fibers, colonized with patient-derived fibroblasts and neuronal cells. Reaching a limit of detection of 1.77 pg mL^{-1}, the e-ELISA not only differentiated ALS models from healthy controls but, most critically, distinguished between Fast- and Slow-progressing ALS models based on significantly different IL-18 concentrations. By identifying IL-18 as a quantifiable prognostic biomarker within a biomimetic human context, this work validates a high-performance diagnostic framework ready for personalized clinical use and provides a robust foundation for the development of portable, point-of-care diagnostic devices.

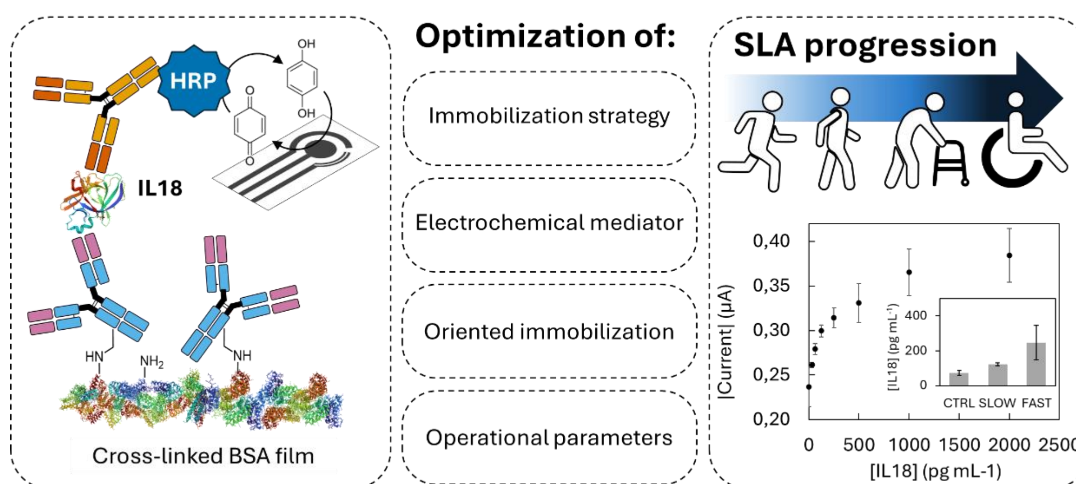

Figure 1. Development of an e-ELISA assay for the highly sensitive detection of IL18 in patient-derived ALS 3D skin models

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O9 - Direct mass spectrometry analysis of mollusks lipidome for deciphering bio-signatures of habitat and climate change

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Cephalopods represent a class of marine mollusks occupying intermediate trophic levels, thereby fulfilling a pivotal role in marine food chain. Owing to their high physiological plasticity and sensitivity to environmental fluctuations, these organisms are increasingly regarded as potent bioindicators for assessing the impact of anthropogenic disturbances on marine ecosystems. This study aimed to investigate the biochemical variations in cephalopods associated with seasonal dynamics and varying habitat conditions. The lipidomic profiles of three species, namely *Octopus vulgaris*, *Loligo vulgaris*, and *Sepia officinalis*, were investigated. Samples were collected during the summer and winter seasons from two ecologically distinct areas in Sicily (Mediterranean Sea): a Marine Protected Area (MPA), characterized by minimal human disturbance, and an adjacent unprotected area subject to significant anthropogenic influence.
Analytical profiling was performed using Rapid Evaporative Ionization Mass Spectrometry (REIMS) coupled with an electrosurgical knife, a technique that allows for real-time, high-throughput lipidomic fingerprinting directly from biological tissues without prior sample preparation. The fatty acid composition was further validated through gas chromatography coupled with mass spectrometry and flame ionization detection (GC-MS/FID). The integration of chemometric tools enabled the rapid differentiation of samples and the identification of key discriminant lipid biomarkers. The findings revealed significant shifts in the lipidomic profiles of the cephalopods, driven by both seasonal transitions and habitat quality, confirming that environmental stressors and anthropogenic factors substantially modulate the metabolic profiles of these invertebrates.
These outcomes provide novel insights into the physiological responses of marine mollusks to human-induced environmental changes. Furthermore, this study highlights the potential of lipid-based biomarkers as robust tools for ecological risk assessment and for monitoring the health and integrity of marine ecosystems.

O10 - A Flow-Splitting Orthogonal 2D-LC Platform for Reliable Impurity Quantification of Therapeutic Oligonucleotides

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Oligonucleotide (ON) therapeutics are an innovative and rapidly emerging class of pharmaceuticals, which aid in the treatment of human diseases. These therapeutics function by preventing translation of messenger RNA (mRNA) into protein, with more than ten approved therapies and numerous others advancing in clinical trials[1]. Conventional impurity profiling of therapeutic oligonucleotides (ONs) by one-dimensional liquid chromatography (1DLC) commonly employs a combination of UV and mass spectrometric (MS) detection. While impurities chromatographically resolved from the full-length product (FLP) are quantified by UV, species that co-elute with the FLP require MS-based quantification. This approach can potentially lead to a systematic bias and inaccurate purity assessment unless relatively complex countermeasures are implemented in HPLC-UV-MS analytical procedure and supporting validation. Although two-dimensional liquid chromatography (2DLC) has the potential to address these shortcomings, traditional heart-cutting 2DLC is limited by small transferable fraction volumes and dependence on non-selective UV detection in the first dimension, thereby hindering reliable quantification and identification of low-abundance, critical impurities[2,3]. In this work, the development and thorough optimization of a compact flow-splitting single heart-cut 2DLC method for the impurity profiling of two model oligonucleotide therapeutics is presented. This platform integrates ion-pairing reversed-phase LC (IP-RPLC) with an orthogonal ion-pairing hydrophilic interaction LC (HILIC)[4] separation using flow-splitting stationary-phase-assisted modulation (SPAM), which supports the transfer of substantially larger fractions and enables simultaneous UV and high-resolution MS data acquisition in both dimensions. Optimization of the dilution flow and implementation of in-line mixing effectively mitigated solvent incompatibility between dimensions. Application of the method to a model 16-mer antisense single-stranded oligonucleotide (ASO) mixture and a 23-mer single stranded small interfering RNA (siRNA) antisense strand revealed multiple impurities in the second dimension that were unresolved and co-eluted with the FLP in 1DLC. Compared to UV-based 2DLC-UV quantification, conventional 1D-LC-UV-MS, has the potential to overestimate sample purity by 2 to 4% dependant on the calibration approach applied in each case. Collectively, this approach could provide a promising, simple alternative with potentially to be a more reliable and less biased alternative strategy for impurity quantification and purity determination of therapeutic ON with less reliance on complex calibration approaches.

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O11 - How Methodological Flaws Distorted a Decade of Serum SERS Studies

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Surface-enhanced Raman scattering (SERS) spectroscopy has been widely adopted for serum and plasma analysis in disease diagnosis [1], yet recent work has revealed that methodological flaws have distorted the interpretation of SERS spectra over the past decade [2]. Misinterpretation has proliferated due to the uncritical propagation of flawed band assignments, confusion between Raman and SERS spectra, and underappreciation of binding affinities in complex biofluids. In this contribution we demonstrate that, contrary to widespread claims in the literature, the vast majority of untargeted serum SERS signals arise from just two purine metabolites—uric acid and hypoxanthine—owing to their strong affinity for silver surfaces. Through rigorous comparison, spiking, depletion experiments, and analysis of samples from 81 donors, we show that these two metabolites account for nearly all observed spectral features and variability, highlighting the critical importance of methodological rigor, robust spectral interpretation, and an awareness of biochemical context for reliable SERS-based diagnostics. Guidelines are proposed to align SERS applications with diseases where uric acid and hypoxanthine are established biomarkers and to prioritize scientific quality over publication quantity to ensure the integrity of bioanalytical science.

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O12 - 3D-printed microneedle-based electrochemical aptasensor for vancomycin monitoring in interstitial fluid

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Microneedle-based electrochemical sensors (MES) provide a minimally invasive interface to interstitial fluid (ISF), enabling real-time monitoring of therapeutic agents[1]. Here, we report a microneedle patch platform integrating an electrochemical aptamer-based (EAB) sensor for vancomycin detection. The microneedle arrays were fabricated via stereolithographic 3D printing and coated by sputtering with nanostructured gold and silver layers, providing high conductivity, mechanical resilience, and antibiofouling properties[2]. An engineered vancomycin-specific aptamer delivered a reproducible electrochemical response across the clinically relevant therapeutic range (6-35 μM)[3]. The fully integrated three-electrode configuration enabled voltammetric measurements in buffer, artificial ISF, and porcine skin models, maintaining high sensitivity and stability under physiologically relevant conditions. Repeated insertion tests confirmed structural integrity and consistent analytical performance, while extended monitoring experiments ensured robust, drift-minimized operation. Moreover, preliminary in vivo studies demonstrated reliable skin penetration, stable sensor response, and operational durability. These findings establish a versatile and robust microneedle-based platform for minimally invasive vancomycin monitoring, paving the way towards the future development of wearable EAB sensors for precision pharmacotherapy.

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O13 - Systematic development and optimization of a cIEF method for the charge variants analysis of basic monoclonal antibodies using the Analytical Quality by Design strategy

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Monoclonal Antibodies (mAbs) are the most important biopharmaceuticals, chemically defined as glycoproteins and derived from a monoclonal cell line. As complex proteins, mAbs exhibit inherent structural heterogeneity as primarily due to post-translational modifications, such as deamidation, glycation, sialylation, methionine oxidation, etc. Therefore, different charged isoforms, often referred to as charge variants, are inextricably present in mAbs; their characterization and monitoring is a critical requirement to the quality control, and it has been considered as a Critical Quality Attribute (CQA).

Capillary Isoelectric Focusing (cIEF) is a gold-standard technique for the high-resolution separation of mAb charge variants mainly addressed at the pI assessment. The separation by cIEF is influenced by several parameters and in the present work, Analytical Quality by Design (AQbD) approach was used to develop a Platform Analytical Procedure (PAP) able to analyze molecules that are sufficiently similar with respect to the attributes is intended to measure [1]. Infliximab, a murine mAb mainly used in the treatment of inflammatory diseases, was selected as a model compounds.

The Analytical Target Profile required a PAP able to ensure accurate measurement of mAbs isoforms pI with a Bias lower than 4%. The risk analysis step allowed the identification of several potential Critical Procedure Parameters (pCPPs) *e.g.*, urea and methyl cellulose concentrations, cathodic and anodic stabilizer concentrations, and mainly the composition of the carrier ampholytes (CAs), the most important components establishing the pH gradient throughout the capillary.

The ratio and type of CAs, namely ampholyte pH 3-10, pH 5-8 and pH 8-10.5 were recognized as Critical Procedure Parameters (CPP). The optimization of the composition was carried out by the mixture design, an approach in which the factors under study are considered as the components of a mixture, and the response depends on their relative proportions rather than on their absolute amounts. By means of Monte Carlo simulation, the Method Operable Design Region, accounting for model uncertainty and experimental variability, was defined with a probability level of 5%.

Under the found AQbD conditions, the optimized cIEF method was validated for accuracy of the pI determination, linearity, accuracy and precision of the infliximab quantitation. Interestingly, selectivity was demonstrated by the ability of the cIEF system in differentiating between therapeutic proteins based on their specific charge heterogeneity. Despite being analyzed under identical experimental conditions, bevacizumab and daratumumab exhibited discrete and well-resolved isoform profiles confirming the ability of the optimized cIEF method to be a robust horizontal platform for quality control of mAbs.

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O14 - Towards non-invasive kidney monitoring: sweat-based measurement of creatinine and Cystatin-C proteoforms in CKD

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Chronic kidney disease (CKD) represents a major global health burden and requires accurate, timely assessment of kidney function for diagnosis and management. The estimated glomerular filtration rate is commonly derived from serum creatinine or Cystatin-C (CysC). Although blood-based measurements are standard in nephrology, venipuncture is invasive and not ideal for frequent or long-term monitoring. Within the EIC-funded KERMIT project (Kidney Disease Sweat Sensor Patch for Early Diagnosis and Remote Monitoring), we conducted a pilot study (Ethics Committee approval n. UOI1.110) to evaluate a fully non-invasive protocol for the simultaneous quantification of creatinine, native CysC, and its proteoforms in sweat, and to assess their relationship with corresponding blood concentrations. Twenty CKD patients were enrolled during routine follow-up visits. Paired sweat and blood samples were collected in the morning under fasting conditions in a controlled environment (25 ± 2 °C; 50–60% relative humidity). Sweat was induced on both forearms by pilocarpine iontophoresis (0.5%) using the clinically established Macroduct® Advanced system (1.5 mA for 5 min; 25 cm^2 electrodes). After stimulation, the skin was rinsed and dried, and BSA-pretreated cellulose filter papers were applied for 30 minutes for passive sweat collection. Patients avoided skincare products for 24 hours prior to sampling. Blood creatinine and CysC were measured according to standard clinical procedures, while sweat analyses were performed using validated analytical method [1]. For the first time, native CysC, 3prOH-CysC, and two oxidized proteoforms (Ox(M14)-CysC and Ox(M41)-CysC) were identified and quantified in human sweat. Median (IQR; min–max) sweat concentrations were 2.9 μg/L (1.2; 1.0–5.0 μg/L) for native CysC, 3.7 μg/L (2.5; 1.7–7.9 μg/L) for 3prOH-CysC, 0.5 μg/L (0.3; 0.1–1.6 μg/L) for Ox(M14)-CysC, 0.6 μg/L (0.4; 0.3–1.4 μg/L) for Ox(M41)-CysC, and 0.2 mg/dL (0.1; 0.1–0.6 mg/dL) for creatinine. The 3prOH-CysC/native CysC ratio in sweat (approximately 1.3) mirrored that observed in blood, supporting analytical consistency. Significant correlations were found between sweat and blood levels for native CysC ($\rho = 0.56$, $p = 0.017$), 3prOH-CysC ($\rho = 0.75$, $p < 0.001$), and creatinine ($\rho = 0.62$, $p = 0.003$) (Figure 1). Multivariate linear regression adjusted for age and BMI confirmed these associations (adjusted r = 0.52–0.61), with no significant covariate effects ($p > 0.2$). Bland–Altman analysis demonstrated no systematic bias for CysC and 3prOH-CysC, while creatinine showed a moderate proportional trend, with smaller sweat–blood differences at higher concentrations (slope = 0.70, $p = 0.002$). This pilot study demonstrates the feasibility of simultaneously measuring creatinine, native CysC, and its proteoforms in sweat using a completely non-invasive approach. The strong agreement with blood measurements supports sweat as a reliable surrogate matrix for kidney biomarkers and highlights its potential for non-invasive, longitudinal CKD monitoring in remote and point-of-care settings.

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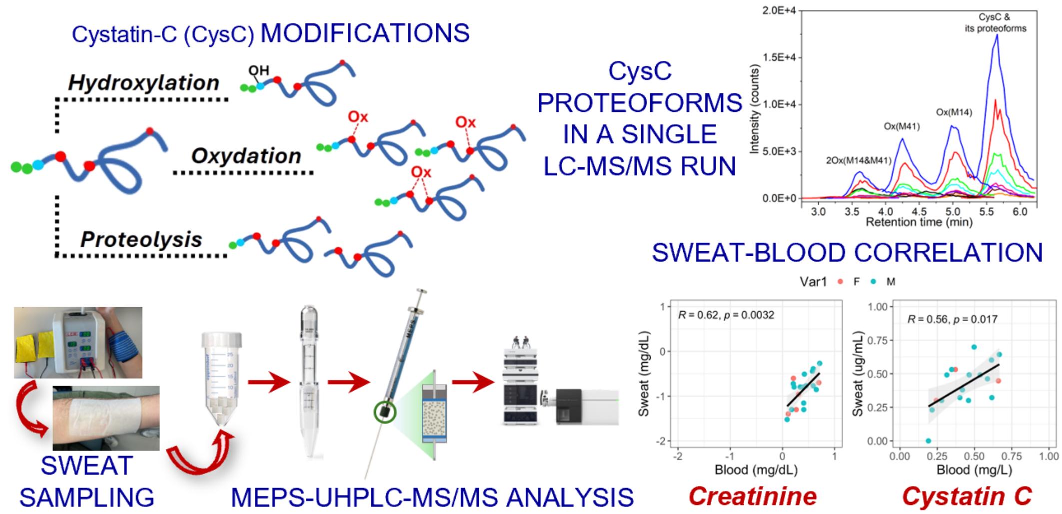

Figure 1. Analytical protocols for sweat creatinine and Cystatin-C proteoforms analysis.

O15 - Lab-on-3D-printed devices for integrated bioelectroanalytical sensing

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Additive Manufacturing (AM), commonly known as 3D printing, has emerged as a powerful technology in electrochemistry, offering rapid prototyping, design freedom, and the ability to produce integrated devices, providing a unique route to on-demand, low-cost (bio)analytical platforms. AM enables the integration of hybrid architectures within a single device, in which 3D printing provides the structure, fluidic system, mechanical frame, and functional components, manufactured using complementary technologies (e.g., paper-based sensors, stencil-printed electrodes, immunochemical elements), can be readily integrated as needed. In this way, compact, portable, reproducible systems can be produced in series, minimizing human involvement. From an analytical perspective, this approach enables the automation of analytical procedures, yielding fully integrated analysis systems on board 3D devices.

In this presentation, two representative 3D-printed electrochemical platforms are presented.

(i) A 3D-printed analytical device integrating functional paper components for the direct determination of amitraz in apicultural matrices. The platform combines a 3D-printed microfluidic architecture with interchangeable paper-based electrochemical sensors and a ‘hydrolysis-paper’ component that enables in situ conversion of amitraz into its electroactive metabolite, 2,4-dimethylaniline (2,4-DMA). The integration of functional paper substrates into the 3D-printed structure enables sample processing and sensing on a single device, achieving sensitive, reproducible, and low-cost detection of amitraz in honey and beeswax within a few minutes, without the need for sample pretreatment. Studies are underway to analyze 2,4-DMA directly in minimally treated bees to assess its ecotoxicity and bioaccumulation, with the aim of using it as an ecotoxicological marker.

(ii) An integrated 3D-printed electrochemical microfluidic device for immunocapture and electrochemical assessment of transferrin saturation (TSAT) in human serum. This device is entirely produced via AM and comprises a platform that incorporates a rotary-valve-controlled microfluidic network, an on-device immunoassay module, and an electrochemical cell fabricated by combining conductive filaments (CB-PLA) and non-conductive filaments (PLA). The system enables selective isolation of transferrin (Tf) and simultaneous electrochemical detection of Tf and Tf-bound iron directly from untreated human serum, providing clinically relevant information with significantly reduced analysis time compared with conventional methods. (iii) Eventually, if worthy of note, additional findings and future perspectives on 3D manufacturing of bioelectroanalytical devices will be presented.

The proposed devices offer clear advantages in versatility, portability, and cost-effectiveness, positioning 3D printing as a pivotal technology for developing next-generation electrochemical systems for on-field and Point-of-Care testing. In summary, this presentation demonstrates that AM offers possibilities beyond the fabrication of PLC-CB-based electrochemical sensors, enabling the seamless integration of heterogeneous functional components into all-in-one analytical devices that encompass complete analytical processes.

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O16 - Enhanced Photoluminescence and Electrical Conductivity of Carbon Quantum Dot–UiO-66-$(OH)_2$ Composites for Sulphur Dioxide and Sulfamethoxazole Sensing

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This work investigates the effect of incorporating carbon quantum dot (CQD) nanomaterials into the UiO-66-$(OH)_2$ metal–organic framework (MOF), with a focus on fluorescence and electrical conductivity. The composite material, entitled CQD-UiO-66-$(OH)_2$, exhibits remarkable improved fluorescence and electrical conductivity as result of band alignment and charge transfer between CQD-UiO-66-$(OH)_2$ and carbon quantum dots. Therefore, as result of enhanced electronic properties, we evaluated CQD-UiO-66-$(OH)_2$ as probe for sensing/detection of sulphur dioxide and sulfamethoxazole. Specifically, for SO_2 sensing via fluorescence spectroscopy, CQD–UiO-66-$(OH)_2$ exhibits significantly enhanced sensitivity and a lower detection limit compared to the pristine MOF, also improving gaseous SO_2 detection. For Sulfamethoxazole (SMX), the engineering of a screen-printed electrode (SPE) with CQD-UiO-66-$(OH)_2$ allows to reach a detection limit down to 600 nM with a linearity up to 50 µM, highlighting the efficacy of CQDs nanomaterials incorporation with respect both the bare SPE and the SPE engineered with MOF only. In order to evaluate the real-world application, the CQD-UiO-66-$(OH)_2$ engineered SPE was interrogated in real wastewater samples, yielding satisfactory recoveries in the range of 87–118%, which further confirms the reliability of the proposed sensing platform. Thus, CQDs nanomaterials incorporation into MOFs shows to be a promising strategy to boost fluorescence and electrical conductivity in MOFs, and, subsequently, employing MOFs in sensing/detection.

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O17 - Integration of NanoMIPs on D-Shaped POF-SPR Platform for High-Affinity SARS-CoV-2 RBD Sensing

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) enters host cells via interactions mediated by the spike protein receptor-binding domain (RBD), which represents a critical target for diagnostic applications. Although RT-PCR remains the gold standard for COVID-19 diagnosis, its limited suitability for point-of-care testing has prompted the development of alternative rapid, portable, and highly sensitive biosensing approaches [1]. Among these, surface plasmon resonance (SPR) allows label-free, real-time monitoring of biomolecular interactions and provides access to binding kinetics [2].

In this work, RBD-specific molecularly imprinted polymer nanoparticles (nanoMIPs) were synthesized as synthetic receptors and employed in a portable SPR sensor. NanoMIPs were prepared using acrylamide-based monomers templated by RBD and characterized by dynamic light scattering (DLS) and scanning electron microscopy (SEM). Affinity and selectivity were evaluated by isothermal titration calorimetry (ITC), using non-target proteins (HSA and Cyt C) as controls. The nanoMIPs were immobilized onto a gold-coated D-shaped plastic optical fiber (POF) SPR platform via self-assembled monolayer formation and EDC/NHS-mediated coupling.

DLS and SEM characterization demonstrated that the synthesized nanoMIPs form a uniform, spherical nanoparticle population suitable for surface functionalization. Thermodynamic analysis confirmed that RBD binding by the nanoMIPs is favourable and selective relative to non-target proteins, supporting their use as synthetic receptors. Functionalization of the POF-SPR sensor with nanoMIPs resulted in measurable plasmonic wavelength shifts consistent with successful surface modification. Upon exposure to RBD solutions across a wide concentration range, the SPR sensor exhibited a clear, concentration-dependent signal response and differentiation from non-specific protein interactions, enabling the detection of RBD in the attomolar range [3].

The portable nanoMIP-SPR sensor platform demonstrates robust performance for ultralow-concentration detection of the SARS-CoV-2 RBD, with high specificity and potential suitability for point-of-care diagnostics. These findings underscore the promise of combining nanoMIPs with plasmonic sensing for rapid, sensitive bioanalytical detection.

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O18 - Identification and Real-Time Monitoring of Emotional Status Chemosignals in Human Body Odours

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This study presents a novel methodology for real-time monitoring of chemosignals in human body odours, combining direct mass spectrometry and comprehensive two-dimensional gas chromatography (GC×GC) for highly selective compound identification. The setup involved custom 3D-printed samplers and in-house-manufactured PTFE connectors, ensuring low contamination and reproducibility. Volatile organic compounds (VOCs) were collected in real-time from the armpit of participants in fear induction experiments using a high-sensitivity proton transfer reaction time-of-flight mass spectrometer (PTR-TOFMS) and complemented by HiSorb sampling for GC×GC analysis. A rigorous cleaning protocol and quality control measures eliminated interfering signals, ensuring high data integrity for trace analysis.

Data analysis identified 260 unique features, integrating real-time PTR-TOFMS data with complementary GC×GC results for isomer differentiation. Key findings included the identification of seven VOCs potentially associated with fear responses, including notable aldehydes, acids, and furans. These compounds were corroborated using a novel HiSorb-GCxGC-TOFMS library and compared to public VOC libraries, enhancing confidence in their identification. Time-course clustering revealed significant temporal patterns correlating with self-reported fear levels and physiological arousal, confirming the involvement of specific sweat-derived volatiles during high-stress scenarios.

This approach highlights the feasibility of combining real-time mass spectrometry with complimentary GC×GC analysis to elucidate chemosignal dynamics. The identified VOCs hold promise for further behavioural and physiological testing to validate their role as fear chemosignals, with potential applications in human olfactory studies and stress-related diagnostics.

O19 - Volumetric urine microsampling and fully automated cannabinoid analysis

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Monitoring cannabinoids in biological samples is becoming increasingly challenging, owing not only to the established relevance of classical phytocannabinoids and their metabolites, but also to the rapid emergence of semi-synthetic cannabinoids. First detected in late 2022, with hexahydrocannabinol (HHC) among the earliest identified compounds, semi-synthetic cannabinoids accounted for over 40% of substances notified to the European Early Warning System in 2024, highlighting the urgent need for updated, broad-coverage bioanalytical strategies[1,2].

Here, we present an advanced analytical workflow based on urine volumetric absorptive microsampling (VAMS), fully automated sample pretreatment and UHPLC-MS for the determination of an extended cannabinoid panel. Target analytes include Δ9-tetrahydrocannabinol (Δ9-THC), 11-hydroxy-THC (THC-OH), 11-nor-9-carboxy-THC (THC-COOH), cannabidiol (CBD), 7-hydroxy-cannabidiol (7-OH-CBD), 7-carboxy-cannabidiol (7-COOH-CBD), as well as Δ8-THC, Δ9-THC acetate, HHC, HHC acetate, Δ9-THCP, Δ8-THCP, HHCP and HHCP acetate. Urine VAMS enables the accurate volumetric collection of minute sample amounts, reducing pre-analytical variability and simplifying sample handling, storage and transport, while the automated platform manages the entire pretreatment sequence, including microsample elution, analyte extraction and transfer of the final extract into injection vials, with minimal operator intervention[3].

The developed method showed good linearity ($R^2 \geq 0.9990$), precision in the 2–13% RSD range, satisfactory extraction yield and minimal matrix effect, while ensuring high sensitivity and reproducibility. By combining volumetric urine microsampling, end-to-end automation and mass spectrometry, this workflow provides a reliable, scalable and high-throughput solution for modern cannabinoid bioanalysis, with interesting applications to clinical and forensic fields[4].

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O20 - A Sensitive and Simple Smartphone-Based Lateral Flow Assay for PSA Detection Using Core–Satellite Magnetic Nanoparticles

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Prostate cancer is one of the most prevalent malignancies among men worldwide, highlighting the need for rapid and accessible diagnostic tools. Prostate-specific antigen (PSA) is the most widely used biomarker for screening and monitoring prostate cancer; however, conventional laboratory methods rely on complex instrumentation, trained personnel, and centralized facilities, which limits their applicability in point-of-care settings. In this work, we report the development of a lateral flow immunoassay (LFA) for PSA detection based on core–satellite magnetic nanoparticles (CSMPs) [1-2]. These nanostructures consist of superparamagnetic Fe_3O_4 nanoparticles aggregates decorated with gold nanoparticles (AuNPs), with an overall diameter of ≈ 450 nm. Compared to conventional spherical AuNPs typically employed in LFAs, CSMPs provide an enhanced optical response due to their larger effective optical cross-section and the high local density of plasmonic nanostructures. This configuration makes them more visually detectable than individual AuNPs and results in an amplified colorimetric signal on the LFA in the presence of PSA. Although similar signal enhancement could theoretically be achieved using larger gold nanoparticles, CSMPs offer a significant additional advantage by retaining magnetic functionality. The particles were functionalized with anti-PSA antibodies using photochemical immobilization (PIT), which promotes stable and oriented antibody attachment onto the gold surface [3]. The resulting functionalized CSMPs were used as reporters in a sandwich-format LFA as showed in Figure 1 (a). During the assay, undiluted human serum samples containing PSA, migrate along the strip by capillary action and interact with the CSMPs, forming PSA–particle immunocomplexes. These complexes are captured at the test zone by immobilized anti-PSA antibodies, generating a visible signal, while excess particles are retained at the control zone to confirm correct assay operation. Quantitative analysis was performed through simple smartphone image acquisition followed by grayscale analysis of the test and control lines. The proposed assay achieved a limit of detection of 50 pg mL^{-1} (Figure 1b), demonstrating that CSMPs are effective plasmonic reporters for LFA platforms and enable sensitive PSA detection while preserving the simplicity, portability, and rapid response of lateral flow assays, without the need for additional instrumentation or external techniques to enable quantitative analysis.

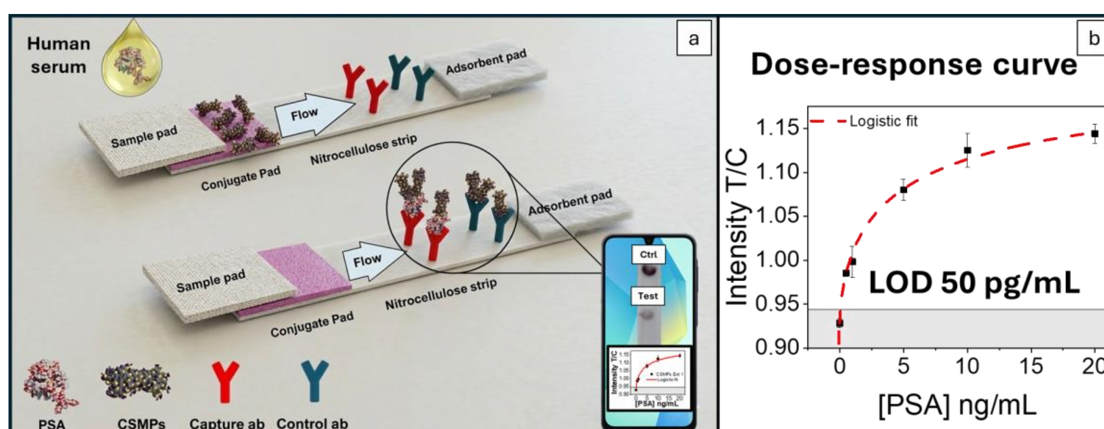

Figure 1. (a) Schematic representation of the CSMPs-based lateral flow immunoassay. The sample selectively interacts with the CSMPs, forming immunocomplexes that migrate along the strip and are captured at the test and control zones, producing a visible signal. (b) Image analysis of the acquired strips enabled the construction of the dose–response calibration curve, yielding a limit of detection of 50 pg mL^{-1}.

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O21 - Development of Innovative Electrochemical MIP-Based Sensors for Biomedical and Environmental Applications

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The increasing demand for rapid, sensitive, and cost-effective analytical tools for both biomedical diagnostics and environmental monitoring is driving the development of advanced electrochemical sensing platforms. In this context, molecularly imprinted polymers (MIPs) have emerged as powerful biomimetic receptors capable of overcoming the intrinsic limitations of antibody-based systems, offering enhanced stability, reproducibility, and scalability.
Here, we report the development of electrochemical MIP-based sensors for the selective detection of both clinically relevant protein biomarkers and environmental contaminants. MIPs were synthesized via electropolymerization of o-phenylenediamine on platinum microelectrodes in the presence of target templates, followed by template removal to generate highly selective recognition sites.
In the biomedical field, we developed a dual, label-free electrochemical sensor for the simultaneous detection of nerve growth factor (NGF) and its precursor proNGF, whose imbalance is associated with Alzheimer's disease. The platform exhibited high selectivity, excellent isoform discrimination, and picomolar sensitivity, enabling antibody-free quantification of both neurotrophins in real samples of cerebrospinal fluid under native conditions.
In parallel, a carbon nanotubes (CNTs)-modified electrochemical MIP sensor was developed for the detection of cyromazine, a triazine pesticide of environmental concern. CNTs were employed to enhance electrode surface area and conductivity, significantly improving electron-transfer kinetics and analytical performance. The sensor demonstrated high sensitivity and selectivity, with negligible interference from structurally related compounds such as atrazine, and was successfully validated in real food samples.
Overall, these results highlight the versatility of electrochemical MIP-based sensors as a unified platform for applications ranging from liquid biopsy to environmental monitoring, paving the way for the development of portable, cost-effective, and high-performance devices for point-of-care and on-site analysis.

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Comunicazioni Flash

FP1 - Magnetically separable HaloTag–NanoLuc reporter for bioluminescent CRISPR/Cas12 nucleic acid detection

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CRISPR-based diagnostics have emerged as powerful tools for rapid, specific, and portable nucleic acid detection, exploiting the programmable recognition of CRISPR-associated nucleases and their target-activated collateral cleavage activity [1]. Most current CRISPR assays rely on fluorescence-based readouts, which require excitation light and dedicated optical components, increasing instrumental complexity and background signal.

Here, we present a bead-based bioluminescent extension of CRISPR/Cas12 diagnostics that integrates HaloTag–NanoLuc as a covalently defined luminescent reporter within a magnetically separable architecture. In this system, the HaloTag–NanoLuc fusion protein is associated with magnetic beads through a programmable nucleic acid scaffold. Upon target recognition, Cas12 activation induces collateral cleavage of the linker region, triggering the release of HaloTag–NanoLuc into solution. Magnetic separation provides a clear physical partition between intact and cleaved states, and the analytical signal originates exclusively from the released bioluminescent reporter (Figure1).

This strategy offers several conceptual advantages. First, NanoLuc-based bioluminescence eliminates the need for external excitation, thereby reducing optical complexity and minimizing background compared with fluorescence-based systems [2]. Second, HaloTag chemistry enables covalent, site-specific conjugation between the luciferase and the nucleic acid scaffold, ensuring structural definition and reproducibility of the reporter assembly [3]. Third, bead immobilization introduces spatial control over the reaction and allows straightforward magnetic separation, enhancing signal discrimination and analytical contrast. Finally, recent bead-based CRISPR diagnostic platforms have demonstrated the value of surface-engineered reaction formats in improving sensitivity and multiplexing potential [4]. Overall, this work extends CRISPR/Cas12 detection into a magnetically controlled, bioluminescent framework that combines enzymatic collateral cleavage, covalent protein–DNA conjugation, and physical separation in a unified diagnostic concept.

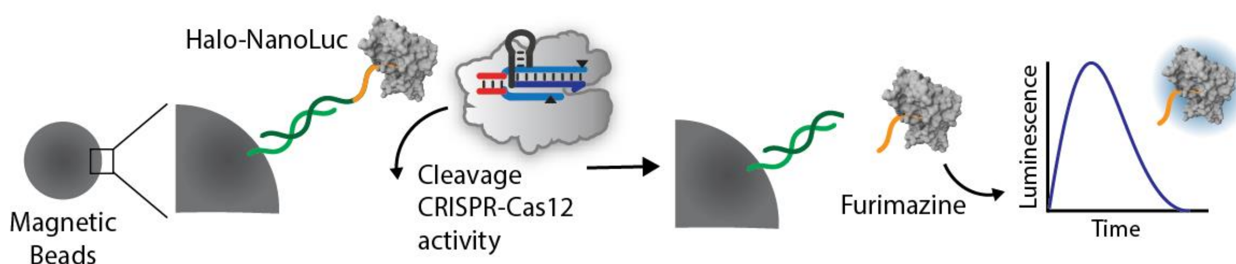

Figure 1 Bead-based HaloTag–NanoLuc reporter is released upon Cas12 activation, producing a bioluminescent signal

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FP2 - Exhaled breath acetone: a non-invasive marker of disease severity across the spectrum of heart failure

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Exhaled breath acetone (EBA) is a marker of impaired myocardial energetics in heart failure (HF), and is associated with disease severity in HF with reduced ejection fraction (LVEF, HFrEF) (1). Much less is known about the role of EBA in HF with preserved ejection fraction (HFpEF), or about EBA kinetics during exercise in this population. We explored the relation between EBA, cardiovascular structure and function, and exercise capacity in patients with HF irrespective of LVEF. We enrolled 153 patients in a tertiary hospital: 50 patients at risk of developing HF, 62 with HFpEF, and 41 with HFrEF. These subjects underwent a clinical and laboratory evaluation, resting transthoracic echocardiography, and a combined cardiopulmonary-echocardiographic stress test with EBA monitoring at rest (EBArest) and during exercise (EBAex). EBArest and EBAex, as well as exercise-induced EBA increases, were significantly higher in patients with overt HF than in patients at risk of HF. When patients were divided according to EBArest tertiles, patients in the higher tertile displayed higher serum levels of the N-terminal prohormone of brain natriuretic peptide (NT-proBNP), more marked abnormalities in cardiovascular structure and function, more prevalent congestion by ultrasound, and more impaired exercise capacity compared to the other tertiles. At multivariable regression analysis, both EBArest and EBAex resulted as independent predictors of NT-proBNP (Figure 1).

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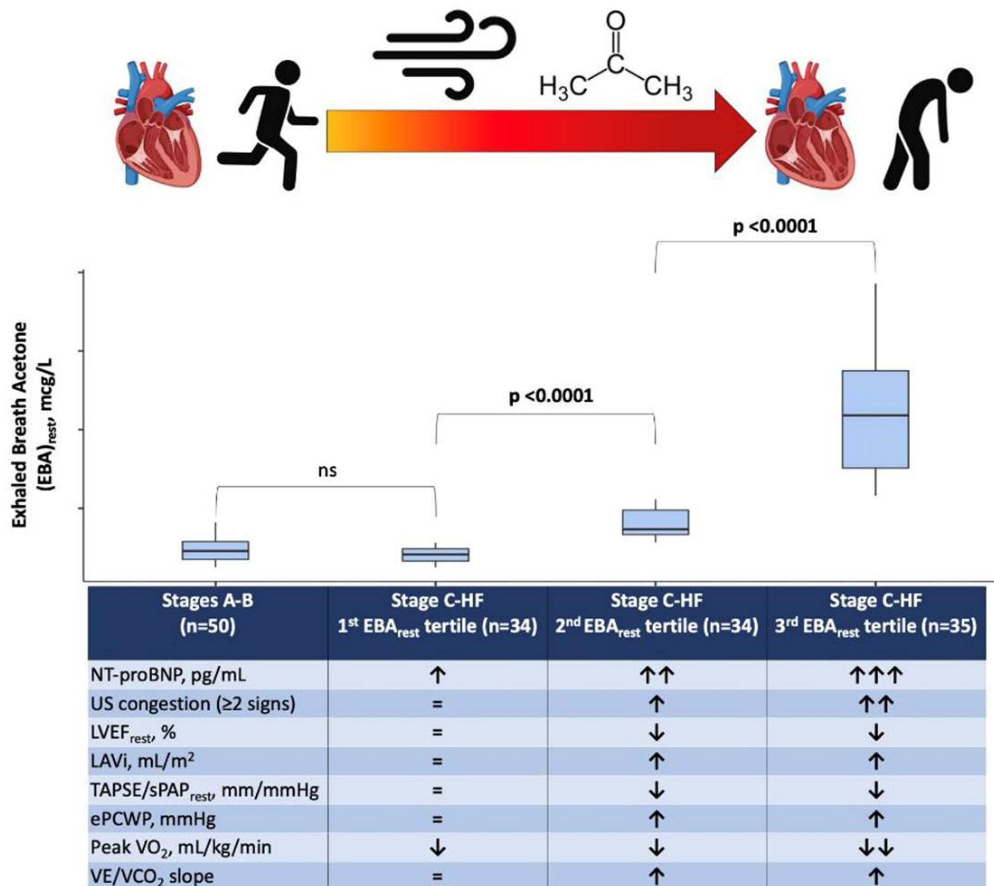

	Stages A-B (n=50)	Stage C-HF 1st EBA$_{rest}$ tertile (n=34)	Stage C-HF 2nd EBA$_{rest}$ tertile (n=34)	Stage C-HF 3rd EBA$_{rest}$ tertile (n=35)
NT-proBNP, pg/mL		↑	↑↑	↑↑↑
US congestion (≥2 signs)		=	↑	↑↑
LVEF$_{rest}$, %		=	↓	↓
LAVi, mL/m^2		=	↑	↑
TAPSE/sPAP$_{rest}$, mm/mmHg		=	↓	↓
ePCWP, mmHg		=	↑	↑
Peak VO_2, mL/kg/min		↓	↓	↓↓
VE/VCO_2 slope		=	↑	↑

Figure 1. Increased EBArest concentrations are associated with higher NT-proBNP, more marked ultrasound signs of congestion, advanced cardiovascular abnormalities, and impaired exercise tolerance in patients with HF, irrespective of LVEF. EBArest: exhaled breath acetone at rest; ePCWP: estimated pulmonary capillary wedge pressure; HF: heart failure; LAVi: left atrial volume index; LVEF: left ventricular ejection fraction; NT-proBNP: N-terminal prohormone of brain natriuretic peptide; sPAP: systolic pulmonary arterial pressure; TAPSE: tricuspid annular plane systolic excursion; VCO2 : carbon dioxide production; VE: minute ventilation; VO2 : oxygen consumption.

FP3 - AF4-MD Characterization of Bio-Conjugation on SiNPs for highly sensitive TCL-POCT Applications

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Chemiluminescence (CL)-based bioassays offer significant advantages in terms of sensitivity, instrumental simplicity, and the absence of external light sources compared to photoluminescence, as demonstrated by the widespread adoption of CL principles in automated clinical chemistry analyzers, and in point-of-care testing (POCT) devices. Among CL approaches, thermo-chemiluminescence (TCL) represents an elegant and effective alternative, as it relies on a "reagent-less" mechanism in which photon emission originates from a thermally induced molecular fragmentation that generates an excited-state species. TCL allows for simpler and more efficient microfluid system that improve the CL applicability on POCT devices [1].

Here we present the preliminary phase of a broader project aimed at developing a POCT device based on silica nanoparticles (NPs) of different sizes doped with TCL-active molecules for the simultaneous detection of the main prostate cancer (PCa) biomarkers, prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA), in both serum and urine. The initial phase of the work focused on optimizing the covalent conjugation of antibodies (Abs) onto carboxylated silica NPs. At this stage, non-TCL-doped NPs and model fluorescent Abs were employed to rigorously validate the bioconjugation protocol. The conjugated NPs were characterized by DLS and, most importantly, by Asymmetric Flow Field-Flow Fractionation (AF4) coupled with multiple (Multi-Angle Light Scattering; MALS, UV–Vis, and fluorescence) detectors.

Multidetection AF4 (AF4-MD) proved to be a key analytical tool for investigating the system. Compared to conventional techniques such as TEM and SEM, AF4 enables simultaneous dimensional and spectroscopic characterization under fully "soft" and non-destructive conditions. Hyphenation with MD provides structural and optical information within a single analysis while preserving NP integrity, allowing the conjugated NPs to be collected after detection and reused [2].

Another central aspect of this study was the investigation of the Ab layers covalently bound to the NP surface. Since the NPs are intended for biosensing applications, the presence of a controlled monolayer is crucial, as the thickness and organization of the Ab coating directly influence antigen binding and biorecognition efficiency. Quantification of surface-bound Abs by BCA assay, combined with data fitting according to Langmuir and Freundlich models adapted to the covalent Ab–NP system [3], enabled the determination of the average number of Abs per particle and discrimination between monolayer and multilayer configurations. The obtained fits suggest the formation of a stable Ab monolayer around the NPs.

Based on the multiparametric characterization provided by AF4 and the demonstration of a controlled Ab monolayer, the NPs are structurally optimized and effectively functionalized for the next phase of the project: validation of antigen recognition for PSA and PSMA and integration into a TCL-based POCT device.

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FP4 - The broad-spectrum adsorption capability of true-to-life micro- and nanoplastics

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Micro- and nanoplastics have become significant environmental pollutants, posing potential threats to aquatic ecosystems and human health. Despite growing concerns, there is a lack of harmonized methods for accurately identifying and assessing the impact of these particles. A major challenge in developing more relevant test micro- and nanoplastics is finding processes that closely mimic their environmental counterparts[1]. Current studies still predominantly employ pristine spherical micro- and nanoplastic particles (micro/nanobeads). To address this issue, we have developed protocols for producing true-to-life micro- and nanoplastics, along with comprehensive analytical approaches for their identification, characterization, and impact assessment.

We developed and optimized protocols of mechanical fragmentation in cryogenic conditions to produce true-to-life micro- and nanoplastics from daily life plastic[2]. Through centrifugation protocols, we successfully isolated nanosized fractions from micrometric plastic powders. In addition, we investigated the broad-spectrum adsorption capabilities of true-to-life micro- and nanoplastics, including eco- and protein-corona formation on nanoplastics, and biofilm formation on microplastics[2,3]. The results showed that the morphological and structural characteristics of microplastics varied based on the fragmentation approach and this in turn affect the biofilm formation onto microplastic surface, in terms of amount and types of bacterial strains. The importance of using realistic test micro- and nanoplastics for eco-toxicological studies were highlighted by comparing the protein corona adsorbed on true-to-life nanoplastics and pristine nanobeads upon incubation in human plasma. The protein corona profile adsorbed on true-to-life nanoplastics differed from that of nanobeads, with further differences observed among various types of nanoplastics. Eco-corona formation on nanoplastics was also investigated and demonstrated using microFTIR analysis, which revealed characteristic peaks of humic acids in the polystyrene spectrum. DLS measurements revealed an increase in the hydrodynamic diameters of nanoplastics due to the formation of this coating, suggesting a partial destabilization of nanoplastic suspensions. This study highlights the importance of employing true-to-life materials with greater environmental relevance as a crucial step toward bridging the gap between experimental laboratory investigations and real-world observations.

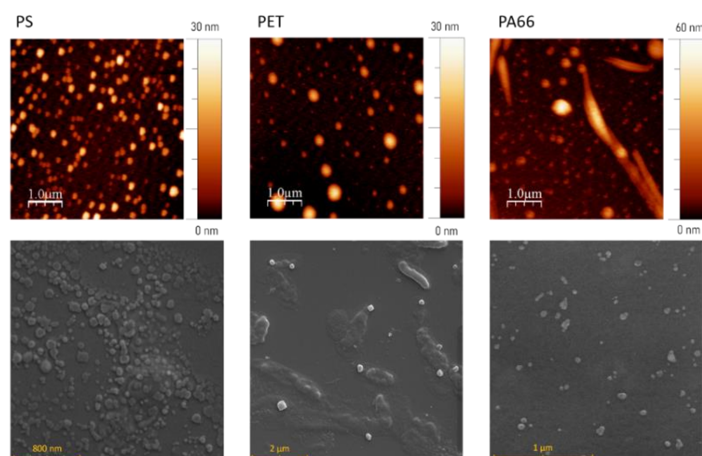

Fig. 1. True-to-life nanoplastics of polystyrene (PS), polyethylene terephthalate (PET), and polyamide (PA) 66 analyzed with AFM and SEM.

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FP5 - An Optimized Workflow for Untargeted Serum Lipidomics: Impact of Sample Handling and Acquisition Strategies

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Lipidomics is emerging as a powerful tool for understanding complex biochemical processes in health and disease. Untargeted lipidomics, in particular, enables comprehensive profiling of lipid species in biological matrices, providing valuable insights into metabolic pathways and biomarker discovery [1]. In this context, the development of robust and reproducible analytical workflows is essential to ensure accurate lipid characterization.

This study presents an analytical workflow integrating optimized sample preparation, instrumental method development, and advanced data processing for untargeted lipidomics of biological samples, with the aim of investigating real serum samples in the biomedical field. Particular attention was dedicated to pre-analytical variables that may affect lipid stability and extraction efficiency.

Serum samples underwent pretreatment following standard procedures reported in the literature [2, 3]. These protocols were further refined to enhance lipid extraction efficiency and reproducibility. In addition, specific experiments were conducted to evaluate the impact of different storage and thawing conditions on lipidomic profiles. In particular, three different sample handling strategies were compared: frozen serum, serum precipitated in methanol prior to storage, and dried extracts. Furthermore, different thawing procedures were assessed to determine their influence on lipid recovery and stability. Samples were subjected either to vortex mixing or sonication after thawing, in order to identify the most suitable approach for ensuring efficient reconstitution and minimal lipid degradation.

In parallel, different acquisition methods were evaluated during these experiments to assess their impact on lipid detection, coverage, and reproducibility under varying sample conditions. These combined investigations allowed the identification of optimal conditions for sample preservation, handling, and data acquisition prior to analysis.

The extracted lipid fractions were analyzed using ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS) (Orbitrap Exploris 120, Thermo Scientific, Waltham, Massachusetts, USA). The instrumental method was carefully optimized to achieve broad lipid class coverage and high sensitivity.

Raw data were processed using Compound Discoverer 3.4 software, with integrated Lipid Search functionality, enabling peak alignment, feature detection, and lipid species annotation. The data processing workflow was further optimized to improve robustness and reproducibility of the analysis. A comprehensive chemometric approach was applied to extract biologically relevant information and identify potential biomarkers. Multivariate statistical analyses, including principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), allowed clear differentiation between experimental groups and highlighted lipidomic alterations associated with different conditions.

The developed workflow provides a reliable and reproducible platform for untargeted lipidomics, significantly enhancing the ability to identify novel lipid biomarkers and to investigate lipid metabolism dysregulation. Moreover, the evaluation of sample storage, thawing conditions, and acquisition strategies represents an important step toward the standardization of pre-analytical and analytical procedures in lipidomics studies. This approach holds significant potential for applications in biomedical research and disease biomarker discovery.

This study is part of the Pharma-HUB project, a research initiative funded by the Piano di Sviluppo e Coesione Del Ministero della Salute 2014–2020, aimed at establishing a pharmaceutical HUB for drug repositioning.

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Comunicazioni Poster

P1 - An electrochemical aptamer-based sensor for the therapeutic drug monitoring of anti-TNF-α monoclonal antibodies: A preliminary study

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Therapeutic drug monitoring (TDM) is becoming an essential practice in clinical settings to improve patient management and support the implementation of personalized medicine. TDM involves the quantitative measurement of drug concentrations in blood or in other biological fluids whose drug levels correlate with systemic blood concentrations, enabling clinicians to adjust dosing regimens according to individual patient needs. This approach is particularly relevant for biologic therapies, where pharmacokinetic variability and immunogenicity may compromise therapeutic response. Biologic agents are designed to selectively modulate molecular targets involved in inflammatory pathways; among the most widely used, TNF-α inhibitors such as infliximab and adalimumab neutralize this key pro-inflammatory cytokine, thereby reducing inflammation in immune-mediated disorders [1].
Currently, analytical methods used for TDM are mainly based on chromatographic techniques and optical immunoassays, which are expensive, time-consuming, and require specialized instrumentation and trained personnel. In this context, the development of low-cost and easy-to-use biosensing platforms could significantly improve routine clinical practice. Among these, aptamer-based electrochemical sensors represent a promising analytical tool [2].
Here, we present preliminary studies on the development of aptamer-based electrochemical assays aimed at the detection of anti–TNF-α monoclonal antibodies. Two different detection strategies are currently being explored, both based on competitive binding interactions involving TNF-α, a TNF-α-specific aptamer, and the target therapeutic monoclonal antibody, enabling the quantitative evaluation of drug concentration. The sensing platform consists of a screen-printed graphite electrode modified by the electrodeposition of poly-L-amino acid and gold nanoparticles, tailored according to the specific assay design [3,4].
Initial development and optimization steps, including surface characterization and preliminary analytical performance evaluation, have been carried out, and the comparative preliminary results of the two approaches will be presented.

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P2 - HPLC–MS/MS for advanced uremic toxin profiling in nephrology

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Uremic toxins represent a heterogeneous group of endogenous metabolites that accumulate in the blood as a result of impaired renal function, contributing to the development of chronic kidney disease (CKD). This condition is characterized by systemic alterations involving multiple organ systems, including cardiovascular and neurological systems [1]. Uremic toxins are commonly classified into three categories: small water-soluble molecules (such as creatinine), middle molecules, and protein-bound molecules. An increase in the plasma levels of these molecular categories and small molecules may serve as a warning sign of impaired renal function [2].

In this context, the analysis of uremic toxins plays a fundamental role in diagnosis, monitoring, and in assessing the effectiveness of therapeutic procedures. Among these, the use of membranes functionalized with polymyxin B represents a promising therapeutic strategy [3].

The aim of this study was the development and validation of an HPLC-MS/MS method for the simultaneous determination and quantification of six clinically relevant uremic toxins: potassium phenyl sulfate, potassium p-tolyl sulfate, potassium 3-indoxyl sulfate, sodium 4-ethylphenol sulfate, creatinine, and trimethylamine N-oxide. The validated method was subsequently applied to the analysis of plasma samples from patients undergoing treatment with polymyxin B–based membranes in order to evaluate the efficiency of protein-bound uremic toxin removal.

Sample preparation consisted of a liquid–liquid extraction using methanol, which simultaneously enabled protein precipitation. The supernatant was collected, diluted, and directly analyzed without any additional processing steps.

Chromatographic separation was optimized by evaluating different LC columns. Among those tested, an HILIC column provided the best performance, ensuring satisfactory peak resolution and separation efficiency.

The tandem mass spectrometry method was optimized using analytical standards of the selected uremic toxins. Instrumental parameters, including gas settings, ion source potentials, collision energy, and product ions, were carefully optimized and recorded to achieve optimal sensitivity and selectivity.

Method validation was performed in accordance with FDA guidelines [4]. The evaluated validation parameters included linearity, assessment of heteroscedasticity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). Validation was successfully completed for potassium phenyl sulfate, potassium p-tolyl sulfate, potassium 3-indoxyl sulfate, and sodium 4-ethylphenol sulfate, all of which met the acceptance criteria.

The application of the method to real plasma samples enabled the identification and quantification of five target analytes within a concentration ranging from 0.07 μg/L (TMAO) to 261.28 μg/L (*p*-tolyl sulfate). Moreover, in some of the analyzed samples, a decrease in analyte concentration was observed in relation to the therapeutic treatment. This trend suggests the reliability of polymyxin B-based treatment for the removal of uremic toxins in nephrological patients, supporting its potential effectiveness in clinical practice.

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P3 - High-Resolution LC–MS/MS Peptidomics and Metabolomics for Non-Invasive Biomarker Discovery in Cardiac Damage

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The CUOREGRAVI project aims to identify and validate salivary and plasma biomarkers associated with cardiac damage in individuals exposed to physical stress, with potential applications in both aerospace and terrestrial settings. Within this framework, the present contribution describes the peptidomic and metabolomic characterization performed using liquid chromatography–mass spectrometry (LC–MS) platforms.

The study population included: (1) patients with ischemic myocardial injury; (2) patients with systolic heart failure (reduced ejection fraction); (3) patients with diastolic heart failure (preserved ejection fraction); and (4) age-matched healthy controls of both sexes (30–60 years). For each participant, saliva and blood samples were collected following comprehensive clinical and instrumental cardiovascular assessment to define their pathophysiological profile.

Molecular analyses were carried out using: (i) low-resolution HPLC–MS/MS for targeted quantification of low molecular weight compounds (<1000 Da), including established cardiac damage biomarkers reported in the literature; and (ii) high-resolution LC–MS/MS using a hybrid quadrupole-Orbitrap mass spectrometer for untargeted profiling of endogenous peptides and metabolites. Particular attention was devoted to the characterization of small peptides (2–4 amino acid residues), and low molecular weight metabolites[1;2].

Peptide identification was achieved through MS/MS fragmentation pattern analysis and database interrogation. Raw data were subjected to preprocessing steps, including peak alignment, normalization, and noise filtering, to generate structured data matrices for multivariate chemometric analysis (PCA, PLS-DA, and supervised models), enabling the identification of discriminant molecular features among clinical groups. Similarly, untargeted metabolomic data underwent feature extraction, normalization, and annotation prior to multivariate statistical analysis to identify candidate biomarkers.

Integration of omics-derived molecular signatures with conventional clinical and diagnostic parameters enabled the identification of patterns associated with different forms of cardiac injury and supported the development of preliminary diagnostic and risk stratification models.

This work was supported by the Project “GRAVI-CUORE: Biosensore multiparametrico per il monitoraggio di marcatori salivari di danno cardiaco in corso di volo umano spaziale” (CUP F83C23000490005).

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P4 - Multi-Analytical approaches to assess the physicochemical stability of biotherapeutic products: application to bevacizumab

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Recombinant monoclonal antibodies (rmAbs) represent the fastest-growing class of biopharmaceuticals due to their pivotal role in the treatment of chronic and life-threatening diseases, including inflammatory disorders and cancer. The expiration of patents and the increasing demand for biological therapies have further accelerated the development of biosimilars and intensified the need for robust analytical strategies to ensure their quality and stability [1]. In hospital settings, rmAbs are frequently compounded into galenic preparations to provide personalized dosing. These preparations must comply with stringent quality standards to guarantee patient safety and efficacy. In this context, we investigated the chemical and physical stability of bevacizumab (BVZ), a clinically relevant anti-angiogenic monoclonal antibody (mAb) widely used in ophthamology, formulated as solution for intravitreal injections. A comprehensive one-month stability study was performed using a multi-analytical platform including Matrix-Assisted Laser Desorption Ionization–Time of Flight mass spectrometry (MALDI-ToF), Capillary Isoelectric Focusing (cIEF) and nano Liquid Chromatography–High Resolution Mass Spectrometry (nanoLC-HRMS, LTQ-Orbitrap).

MALDI-ToF qualitative analysis of the antibody enabled rapid assessment of molecular integrity, detecting potential fragmentation or aggregation events induced by thermal, chemical, or mechanical stress. To further investigate structural features, analyses were also performed on reduced, deglycosylated, and reduced/deglycosylated forms to characterize heavy and light chains and evaluate N-glycosidic chains. Charge heterogeneity was assessed by cIEF, a gold-standard technique for qualitative and quantitative characterization of rmAbs. The cIEF protocol used for the BVZ isoelectric point determination and isoforms profile was derived from a previous study confirming its robustness as a horizontal quality control platform for rmAbs. Finally, nanoLC-HRMS peptide mapping following tryptic digestion enabled in-depth structural characterization, confirming sequence integrity and identifying potential post-translational modifications such as oxidation, deamidation, and glycosylation variants.

Overall, this multi-level analytical workflow provides a comprehensive and orthogonal strategy for monitoring the stability of rmAbs, supporting hospital pharmacy practices and contributing to the safe and cost-effective management of high-value biopharmaceuticals.

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P5 - Paper-based Sensors and Direct Electron Transfer Type Biosensors based on Graphenic Films Integrated on Ecoinnovative Substrates

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The synthesis and integration of nanostructured films into paper-based analytical devices remain significant challenges. In this framework, benchtop-scale CO_2 laser plotter-based technologies offer an exciting opportunity to produce graphenic and graphitized films.

This presentation focuses on the production of various functional nanostructured films using a CO_2 Laser plotter and their integration into fully lab-made paper sensors and biosensors. This study examines the integration of laser-induced graphene oxide (rGO) and laser-induced graphene (LIG) into cellulosic substrates, enabling the fabrication of complete nanostructured paper sensors and biosensors able to address diverse analytical requirements. To achieve this, eco-friendly cellulosic substrates were explored, including recycled papers and those derived from textile and agro-industrial wastes, and manufactured from fiber sources other than trees. Paper sensors were produced in batches via stencil printing, and rGO/LIG films were incorporated using pressure.

The paper/graphene morphological, structural/chemical, and electrical/electrochemical features were thoroughly examined. In brief, each paper interacts with the rGO/LIG differently, resulting in a unique graphene film formation and chemical rearrangements that impact their electrochemistry and electroanalytical properties. The presentation will cover: (i) electrochemical sensors based on laser-induced rGO films integrated into eco-friendly papers capable of detecting various analytes in different samples, including supplements/medications, and biological fluids. For each application, a dedicated paper sensor proved to be more effective (tree-free/rGO, recycled fibers/rGO, and kiwi by-products/rGO), highlighting the role of the cellulosic substrate even in its final use. Reproducible data (RSD ≤ 7%; n = 3), with nano- to micromolar detection limits and satisfactory recoveries (91-108%), were achieved across all applications. (ii) Additionally, bamboo-derived paper was used to host LIG obtained from polyimide and to accommodate Fructose Dehydrogenase (FDH), with the ultimate goal of fabricating a third-generation enzymatic biosensor for detecting inulin, a natural fructose polymer used as an exogenous marker for glomerular filtration rate (GFR) estimation to evaluate kidney function. LIG integration into office, recycled, and industrial by-product-containing papers was attempted, and the resulting bioelectrocatalytic features were investigated. LIG-biosensors assembled on bamboo fiber 'tree-free' paper proved to be more effective, thus bamboo paper biosensors were employed to measure inulin in real urine and serum samples at clinically relevant levels, yielding satisfactory recoveries (90–111%; RSD ≤ 7.9%, n = 3).

This presentation aims to demonstrate how CO_2-laser plotter-based technologies can create effective nanostructured sensing surfaces and transducers that are accessible and easy to interface through everyone-reach technologies on paper-based substrates, opening new opportunities for developing on-demand, sustainable analytical devices that meet specific needs.

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P6 - Biocompatible aldehyde–gum arabic/gelatin scaffolds for 3D cell culture: physicochemical, morphological and stability characterization

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Three-dimensional (3D) cell culture systems require biomimetic scaffolds with controlled porosity, stability in culture media, and tunable swelling behavior. In this work, we report the development of biocompatible hydrogel scaffolds based on aldehyde-functionalized gum arabic (GA-CHO) and gelatin, exploiting Schiff-base crosslinking between aldehyde and amine groups to form stable 3D networks under mild conditions. [1][2] Formulations were rationally selected to achieve a two-step gelation profile compatible with 3D cell culture workflows, enabling early manipulation with cells while preserving their integrity, followed by rapid stabilization prior to immersion in culture medium.

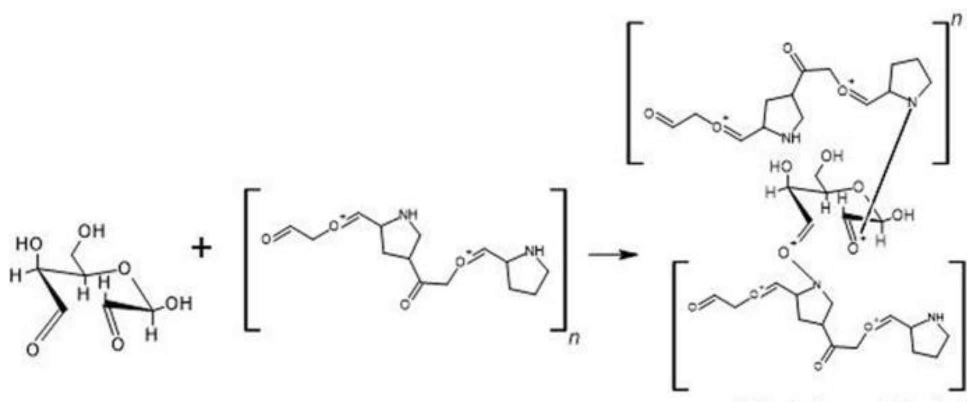

Figure 1. Scheme of the structure oxidated gum arabic and linkage of CHO- group with collagen molecules [1]

The physicochemical and morphological properties of the materials were systematically characterized. Selected formulations exhibited an initial compaction within approximately 10 minutes, suitable for gentle handling and homogeneous cell incorporation, and complete gel formation within 1 hour, providing adequate mechanical integrity for subsequent immersion in culture medium. Scaffold porosity and internal architecture were investigated by scanning electron microscopy (SEM), revealing a porous and interconnected network. Swelling behavior was quantified as equilibrium swelling ratio in aqueous media, while resistance to dissolution was assessed by incubating the hydrogels in standard cell culture medium over time and monitoring their structural stability and shape retention. Optical stability was evaluated by assessing time-dependent yellowing together with light scattering/opalescence, since maintenance of transparency is crucial for subsequent optical microscopy analyses aimed at monitoring cell growth within the 3D matrix.

The GA-CHO/gelatin scaffolds exhibited controllable gelation kinetics, significant swelling capacity, and good stability in culture medium compared to non-crosslinked controls. SEM analysis confirmed a homogeneous porous morphology compatible with cell infiltration. The materials showed limited yellowing while maintaining optical transparency, with no significant increase in opalescence over time, thus remaining suitable for optical microscopy-based cell monitoring. Overall, these results demonstrate that aldehyde-modified gum arabic combined with gelatin represents a promising platform for the fabrication of biocompatible and structurally stable scaffolds suitable for advanced 3D cell culture models.

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P7 - Integration of Molecularly Imprinted Nanoparticles into Competitive and Sandwich pseudo-Immunoassays

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Molecularly imprinted polymer nanoparticles (nanoMIPs) are increasingly investigated as synthetic recognition elements for bioanalytical applications. However, their effective integration into conventional immunoassay architectures still requires systematic validation [1]. In this work, we reported the applicability of nanoMIPs in ELISA-like platforms, assessing their performance in both competitive indirect and sandwich-type pseudo-immunoassay formats. To assess the versatility of the approach across distinct analyte classes, two representative targets were selected: a low-molecular-weight, non-immunogenic molecule (adenosine) and a high-molecular-weight protein (bovine IgG). NanoMIPs were synthesized via solid-phase polymerization, enabling the production of homogeneous nanoparticles bearing selective and surface-accessible binding sites [2]. This strategy ensures controlled orientation of the template during synthesis and facilitates direct implementation in surface-based assay formats.

For small-molecule detection, adenosine-imprinted nanoMIPs were immobilized onto microplate wells and integrated into a competitive indirect pseudo-immunoassay. In this configuration, free analyte competes with a labeled analogue for binding to the imprinted sites. The assay generated a characteristic sigmoidal inhibition curve consistent with classical ELISA behavior with an IC_{50} in the 15–18 ng mL^{-1} range and a limit of detection of approximately 4 ng mL^{-1}.

To extend the approach to macromolecular recognition, bovine IgG-imprinted nanoMIPs were used as capture ligands in a sandwich-type pseudo-ELISA. NanoMIPs were grafted onto microplate wells, incubated with the target protein, and subsequently detected using a labeled secondary antibody. The system produced a concentration-dependent response described by a four-parameter logistic model, in agreement with standard immunoassay calibration profiles. Under optimized conditions, the limit of detection was 0.57 μg mL^{-1}. Selectivity was examined through cross-reactivity experiments against IgGs from different animal species, demonstrating preferential recognition of the imprinted protein. Both competitive and sandwich configurations were implemented without substantial modification of established ELISA workflows, highlighting the practical transferability of nanoMIPs into widely used immunoassay procedures. The ability to function in distinct assay formats and across analytes of different molecular weights underlines the methodological flexibility of nanoMIP-based systems. This work demonstrates the applicability of molecularly imprinted polymer nanoparticles in ELISA-like architectures and supports their use as synthetic recognition elements in the development of alternative bioanalytical immunoassay platforms.

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P8 - Innovative aptasensor for the rapid detection of *Escherichia coli* in real matrices

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Pathogenic bacteria provide a significant challenge both for healthcare systems and for the food industry given their rapid detection. Electrochemical biosensors possess some remarkable features, such as low cost, fast response time, and portability, which make them an attractive alternative to conventional methods for pathogen recognition [1,2]. In the fabrication of biosensors, the selection of a new bioreceptor like aptamer is a key factor to enhance biosensor efficiency [3]. In this context, we developed a low-cost and label-free electrochemical biosensor based on a new aptamer for the rapid detection of *Escherichia coli (E. coli)* in real samples. Screen-printed gold electrodes functionalized with aptamer P12-55 [4], and electrochemical impedance spectroscopy was used to evaluate the change of the charge transfer resistance (Rct) as a result of the interaction with *E. coli ATCC 25922*. The aptasensor showed a linear range from 10^0 to 10^4 CFU/mL with a 20-minute response time, and a limit of detection (LOD) of 1.4 CFU/mL under physiological conditions (Figure 1A). In addition, the biosensor's specificity with respect to other bacterial species, including Gram-positive bacteria such as *Staphylococcus aureus* and the Gram-negative bacterium *Pseudomonas aeruginosa* was evaluated, and a non-significant impedimetric signal was observed [5]. Consequently, they represent a promising avenue for achieving swift and precise detection of *E. coli* bacteria in biological specimens, such as tap water, milk and urine (Figure 1B). Future research may further investigate the applicability of this aptasensor for the recognition of *E. coli* bacteria in marine water.

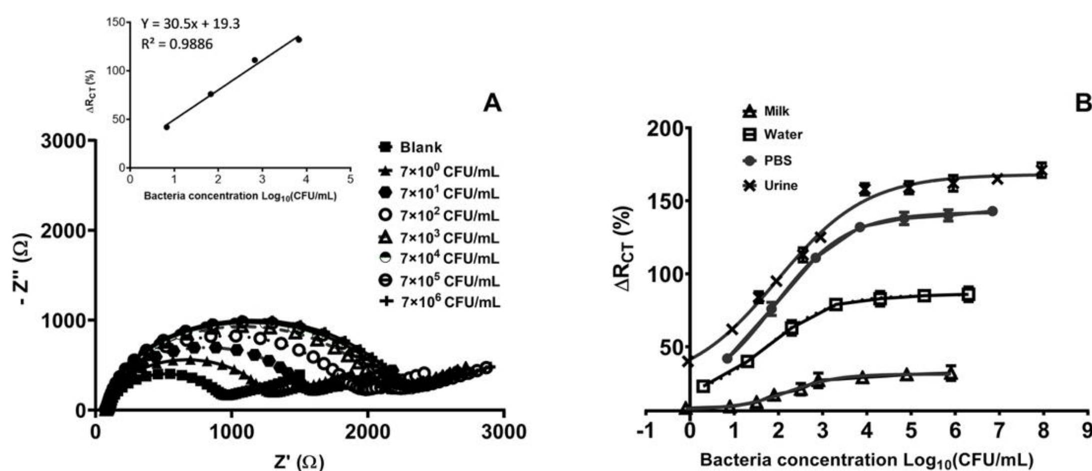

Figure 1. (A) Electrochemical impedance spectroscopy performed in PBS buffer solution (pH 7.4) containing 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ using P12-55 aptamer-based biosensors with increasing concentrations of *E. coli* (0 to 10^6 CFU/mL). (B) Difference in the charge transfer resistance of aptamer-based biosensors towards *E. coli* in different real matrices.

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P9 - Is a Standard-Free HRMS-QTOF Workflow a reliable tool for Semi-Quantitative Pesticide Screening in Biological Samples?

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The growing number of regulated pesticides has increasingly challenged the sustainability and flexibility of conventional targeted approaches, used in official control of biological samples, such as fruit, vegetables and animal origin products, typically relying on large panels of analytical standards for quantification. While HRMS and untargeted screening approaches have substantially improved detection capabilities at the qualitative level, quantitative assessment still largely depends on compound-specific reference standards. In this study, a semi-quantitative, standard-free HRMS-QTOF screening method was internally developed and applied to a European Proficiency Test sample (EUPT-FV-25), consisting in a sample of melon containing incurred and spiked pesticides. Compound identification was achieved through accurate mass measurement, isotopic pattern evaluation, and spectral library matching. Quantitative estimation was performed using Quantem® software, developed by Liigand et al. (2020) [1], which predicts analyte ionization efficiency using a descriptor-calculation software that can calculate more than 1800 2D and 3D descriptors [2]. The developed model relies on a limited number of reference standards per analysis to define response factors (RFs), calculated as the ratio between the Relative Ionization Efficiency (RIE), approximated by the chromatographic peak area, and the known analyte concentration (C). Once RFs are established for compounds with known concentrations and well-characterized molecular structures, the relationship between instrumental response and concentration can be extended to estimate the concentrations of qualitatively identified analytes without other analytical standards [3]. A key aspect of the proposed methodology is therefore the selection of an appropriate and representative set of reference standards, capable of adequately covering the relevant molecular space, i.e. more than 400 pesticides listed in the mass spectral library. The standard selection method was implemented through a new strategy based on three-dimensional clustering using pKa, Rt, and MW, parameters known to strongly influence ESI efficiency. A panel of 150 pesticides from a validated targeted multiresidue method was projected into a three-dimensional chemical space, enabling the identification of clusters of compounds with similar physicochemical behavior. For each cluster, a representative standard was selected by minimizing the cumulative standard deviation across the three variables, leading to the selection of 12 representative standards. Sample extracts were analyzed by LC-HRMS-QTOF, allowing the correct identification of all 18 pesticides present in the fruit sample. Subsequently, a standard-free quantification was carried out. To enable a conventional targeted confirmatory analysis and validate the quantitative results obtained, a strict protocol was developed and applied to determine the appropriate calibration range for the detected analytes only. Two compounds not included in the accredited multiresidue method, Novaluron and Zoxamide, were successfully identified and quantified exclusively through the standard-free approach, yielding z-scores of 3.0 and −2.2, respectively. No false positives were detected. Overall, a z-score < 3.0 was achieved for 55% of the analytes, while eight analytes (45%) showed a z-score > 3.0 with three compounds (Fenitrothion, Mepanipyrim, and Profenofos) typically determined by GC-MS/MS rather than LC-MS/MS, mainly due to their superior ionization efficiency under EI conditions compared with ESI. When these compounds were excluded, the proportion of analytes showing a z-score > 3.0 decreased to 33%, highlighting the robustness of the estimation approach used for analytes suitable for LC-analysis . For 16 out of 18 analytes (88%), the predicted concentration ranges were appropriate for targeted confirmatory analysis, allowing quantitative confirmation through compound-specific calibration in compliance with SANTE/11312/2021 v.2026 criteria [4]. Considering that Fenitrothion is not routinely analysed by LC-MS/MS, its exclusion from the evaluation increased the success rate of correct calibration range prediction to 94%. Overall, the results demonstrate that proposed workflow is reliable, reducing the number of analytical standards required for pesticide multiresidue screening methods, thus improving flexibility, and supporting greener analytical approaches in food safety monitoring.

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P10 - Multiplex lateral flow immunoassay for rapid differential detection of EBHSV and RHDV2

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This study describes the development of a multiplex lateral flow immunoassay (LFIA) for the rapid and simultaneous detection of European Brown Hare Syndrome virus (EBHS) and Rabbit Haemorrhagic Disease Virus 2 (RHDV2), two highly pathogenic lagoviruses affecting both domestic and wild leporids. These viruses represent a major veterinary and epidemiological concern, as they induce very similar clinical signs, including sudden death, haemorrhages, and liver necrosis, making differential diagnosis particularly challenging [1,2]. Conventional diagnostic methods, such as RT-PCR and ELISA [3,4], provide high sensitivity and specificity but require laboratory equipment, specialized personnel, and relatively long processing times, limiting their application in field settings. In this context, LFIA represents a promising point-of-care alternative due to its rapidity, simplicity, and cost-effectiveness [5]. However, most available LFIA systems are limited to single-analyte detection. The aim of this work is therefore to develop a multiplex LFIA capable of simultaneously detecting and distinguishing between EBHS and RHDV2 [6].
A panel of monoclonal antibodies (5F5, 2B2, 4A4, 6G2, 3G9, 4F7, 4H7) targeting the V60 capsid protein was systematically evaluated using a checkerboard screening approach, testing each antibody as both capture (immobilized on the nitrocellulose membrane) and detection (conjugated to gold nanoparticles, AuNPs). A total of 49 combinations were assessed to determine specificity, cross-reactivity, and signal intensity.
Based on the screening results, 2B2 and 4H7 were selected as capture antibodies for EBHS and RHDV2, respectively, while 5F5 and 3G9 were employed to generate a pan-reactive test line. The detection antibody used in the final device was 6G2, conjugated to AuNPs. The resulting multiplex LFIA features three test lines (EBHS-specific, RHDV2-specific, and pan-reactive) along with a control line, allowing rapid and intuitive interpretation of results.
The samples consisted of hare and rabbit livers and were prepared by homogenizing 0.1 g of tissue in 2 mL of running buffer in a 15 mL tube, vortexing for 1 min and allowing 3 min for sedimentation. Subsequently, 100 µL of the supernatant was collected and applied to the sample well. During assay development, critical issues related to matrix interference from liver samples were identified, leading to nonspecific signals. These challenges were successfully addressed by optimizing the composition of the running buffer, particularly through increased ionic strength and the introduction of oxidizing agents. The optimized device demonstrated effective discrimination between EBHS and RHDV2, a significant reduction in false positives, and good stability over time. Overall, this work highlights the feasibility and potential of multiplex LFIA as a rapid and reliable diagnostic tool for field applications, supporting improved surveillance and control of lagovirus infections.

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P11 - Untargeted lipidomics of thymic tissue: insights into aging-associated lipid remodeling

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Population ageing is associated with a progressive decline in immune function, to which thymic involution is a major contributor. The thymus, essential for T-cell development, undergoes early structural and functional deterioration, characterized by epithelial loss and replacement with adipose tissue. While cellular mechanisms of thymic ageing have been extensively studied, less is known about changes in the tissue microenvironment, particularly lipid composition. Given the emerging role of lipid remodeling in ageing across organs, the thymus represents a relevant yet underexplored model. [1-3]

To investigate lipid alterations associated with aging on thymic tissues from young and aged groups, we performed untargeted lipidomic analysis. Thymus samples were obtained from calves (7–9 months old) and adult cows (24–28 months old) to model age-related differences. Lipid extraction was performed using a modified Bligh and Dyer method, followed by separation and detection through liquid chromatography coupled to high resolution mass spectrometry (LC–HRMS) to enable broad and unbiased lipid detection, with data-dependent fragmentation supporting lipid annotation.

Raw data were processed using Xcalibur and MS-DIAL for feature detection, alignment and annotation based on accurate mass and MS/MS fragmentation patterns using reference databases. Statistical analyses were performed to identify lipid species and classes differentially associated with age, allowing a comprehensive assessment of thymic lipid remodeling.

Across all samples, we annotated approximately 300 lipid species, including triacylglycerides (TAGs), diacylglycerides (DAGs), phosphatidylethanolamines (PE), phosphatidylcholines (PC), lysophosphatidylethanolamines (LPE), lysophosphatidylcholines (LPC), ceramides (CER), phosphatidylglycerols (PG), phosphatidylserines (PS), and sphingomyelins (SM). TAGs were the most abundant lipid class detected, followed by PE and PC species.

Overall, our findings contribute to define the lipidomic landscape of the thymus and identify age-related remodeling that may impact tissue function.

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