NEW TRENDS IN BIOSCIENCE 3

České Budějovice, 6.9.-8.9. 2025

September 6, Monday

L1

130 YEARS OF SEEING THE INVISIBLE: THE STORY OF X-RAY DISCOVERY

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In November 1895, Wilhelm Conrad Röntgen discovered X-rays, a new type of invisible radiation that can pass through the body and create images of bones and organs. His meticulous experiments not only revealed a new branch of physics but also transformed medicine by introducing diagnostic imaging. Röntgen's wife, Anna Bertha, was part of this story—her hand was the subject of the very first X-ray picture, which showed her wedding ring and became a powerful symbol of the new invention.

News about X-rays spread very fast. Within weeks, newspapers in Europe and the United States were reporting the discovery. This quick publicity helped bring X-rays from the laboratory into medical practice almost immediately.

Today, X-rays are used every day in hospitals and clinics to diagnose broken bones, lung diseases, and many other conditions. They are also important in cancer treatment, dentistry, airport security, and scientific research. From Röntgen's first experiment to modern technology, X-rays have become one of the most valuable tools in both medicine and science.

L2

X-RAY IN STRUCTURAL BIOLOGY

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How do we know what proteins look like? How can we study the invisible machinery of life at the atomic level? The answer lies in the powerful use of X-rays in structural biology. By analyzing how X-rays diffract on crystals of biomolecules, we can reconstruct detailed 3D structures that reveal how these molecules work – and how we can target them in medicine, biotechnology, and research. This talk explores how X-ray crystallography has become one of the most essential tools in structural biology, changing our understanding of life, health, and disease, and how it is still driving major discoveries today. We will walk through the principles behind the technique, its historical milestones, and explain why seeing is believing when it comes to biology.

L3

TRACING THE ORIGIN OF THE TIMBERS OF NOTRE-DAME DE PARIS: SR–ND ISOTOPIC AND MULTI-ELEMENTAL FINGERPRINTS

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The wooden framework of Notre-Dame de Paris Cathedral was almost entirely destroyed by fire on 15 April 2019. Although the charred timbers could not be reused for the reconstruction, they were of great interest to historians and archaeologists. Their study provided an unique opportunity to explore over 800 years of the monument's history and to gain new insight into cathedral construction in the Central Middle Ages (11^{th}–13^{th} centuries). A key question for understanding medieval forestry management and the timber trade was the provenance of the beams.

Provenance determination was based on a multidisciplinary approach combining the study of historical archives, the observation of archaeological features, and the analysis of the geochemical (multi-elemental and Sr–Nd isotopic) composition of the wood. Tracing with geochemical signatures consists of discriminating sites based on their geological and pedological contexts. Trees take up nutrients from the bioavailable soil pool during their growth. Concentrations of mineral nutrients and isotopic ratios in wood are therefore linked to the geochemical composition of soils and underlying rocks. Absorption of certain elements (e.g., Mn, Ca) is controlled by soil pH, while isotope ratios reflect the type and age of the bedrock. These tracers provide complementary information on the environment where trees grow. In the case of archaeological wood, geochemical signatures may be further modified by post-depositional conditions [1].

The case study of the Notre-Dame timbers required (1) verification of the stability of geochemical tracers in carbonized wood, and (2) the establishment of a reference database of multi-elemental and isotopic signatures in present-day woods around Paris.

High-temperature exposure did not alter the Sr and Nd isotopic composition, but caused volatilization and the loss of some elements. Elemental tracers were therefore selected based on their thermostability (i.e., elements showing < 20% decrease at 800 °C) [2]. The reference database comprised 12 forest sites, each representing a distinct type of substrate, covering the geological and soil diversity of the Seine River catchment area. Provenance of present-day wood could be resolved with 93% accuracy, with site discrimination primarily controlled by the $^{87}Sr/^{86}Sr$ isotopic ratio and the Mn/Ca and Sr/Ca ratios [3]. The carbonized Notre-Dame timbers were analysed for felling dates and geochemical signatures, which were compared both internally and with source forests from reference database. The signatures of most medieval beams were characteristic of stands growing on deep silty soils, consistent with archival evidence placing their origin southeast of Paris.

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L4

REVEALING OF LEAD AND MERCURY SOAPS IN MINIATURE PORTRAITS

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Saponification of paint layers often induces unwanted changes of the original appearance of paintings caused by the increased transparency and/or opacity of the affected paint layers. The as formed soap aggregates have a strong tendency to growing in time. They could, therefore, threaten the stability of works of art when the protruding and efflorescing soaps result in the loose of paint layers' adhesion. [1] In the studies of saponification processes, the attention is paid in the first place to zinc and lead-based pigments which are apparently the most sensitive ones to interact with fatty binding media under formation of metal soaps. Nevertheless, our research of portrait miniatures has revealed presence of different types of crystalline metal carboxylates frequently in a conjoined occurrence of lead white ($2PbCO_3 \cdot Pb(OH)_2$) and cinnabar (HgS) in paint layers, exceptionally even without presence of any lead-based pigment, indicating that HgS assisted to the formation of Pb and/or Hg carboxylates. [2] However, the lack of reliable reference structural data for mercury carboxylates limited both their proper identification in artworks and the experimental research of HgS interactions with binders on molecular level.

Therefore, we synthesized long chain simple and mixed mercury (II) carboxylates of the general formula $Hg(C16)_x(C18)_{2-x}$ (where C16 and C18 stand for palmitate and stearate, resp., and (0 ? x ? 2) in the form of pure polycrystalline powders and characterized them primarily by XRPD and ssNMR. The crystal structure description of the synthesized mercury carboxylates [3] enabled us the successful identification of mercury and lead carboxylates in miniature portraits [4]. Moreover, application of the Rietveld refinement on the collected XRPD patterns provided a detailed insight into the chemical composition of detected crystalline mercury and lead carboxylates, showing their mixed character (i.e., both palmitate and stearate anions are incorporated in one compound). In addition, specification of chemical composition of detected mercury and lead soaps allowed us to estimate consumption of cinnabar and lead white by saponification reaction based on mass balance relations, indicating the original composition of degraded paint layers.

This contribution demonstrates results of the first ever analyses of portrait miniatures by various non-invasive methods with the special focus to XRPD. It also summarizes the fundamental structural characteristics of synthesized reference mercury carboxylates and their invaluable role in the identification of mercury soaps found in painted artworks.

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The authors thank to all colleagues who participated in the research and analyses, namely E. Kočí, S. Garrappa, M. Pech, D. Hradil, J. Hradilová, J. Plocek, L. Kobera, J. Rohlíček, and J. Hermans, as well as to restorers, curators and artworks' owners collaborating in the investigation of paintings.

L5

COUNTERFEIT ANALYSIS IN REAL FORENSIC PRACTICE

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The current world market is flooded with counterfeits in every sector, and the art market and market for art objects are no exception. Most estimates agree that approximately 30% of items in the art market are counterfeit. Very often, it is modern or contemporary art where it is easier to introduce counterfeit pieces. Unfortunately, identifying these counterfeits is also more difficult when they are of high quality.

In the forensic field, the tendency is to apply a complex analytical approach that enables the analysis of all components of a piece of art. The area of painting analysis is probably the most extensive. Complex analyses of the paint layers and other materials can generally be divided into completely non-destructive methods and those requiring the collection of microsamples.

Methods classified as non-destructive analyses include initial imaging in the visible spectrum, infrared and UV spectrum, multispectral photography, and X-ray imaging. X-ray imaging is used for non-destructive detection of the composition, structure, and condition of artworks, for identifying pre-paintings and underpaintings, and for mapping stylistic differences between originals and counterfeits. Other non-destructive methods include X-ray fluorescence (XRF) and Raman spectroscopy. These methods are typically used as screening tools to obtain initial information about the examined item and to specify locations from which microsamples should be collected for further analysis.

A wide range of devices and techniques are used for further instrumental analysis. The most important are:

a) Optical and electron microscopy and microanalysis (SEM/FIB and EDS/WDS, mXRF) – these are basic methods applied to most inorganic samples. Their advantage is non-destructiveness, allowing the sample to be reused. If cross-section examination of paint layers is not necessary, the item can be placed directly into the microscope chamber without adjustment (for sizes up to 20 by 20 cm). For many items, such as gemstones and jewelry, this is the only option. To determine quantitative characteristics (for example, morphological parameters of mineral grains), imaging analysis methods are used. The data from individual samples are compared using multivariate statistical analyses. To compare textile fibers and their fragments, methods of quantitative color measurement at the microscale are used. For studying microelements and admixtures, an XRF device built directly into the SEM chamber (mXRF) with an analyzed surface size of about 30 μm is beneficial. This device also enables simultaneous EDS/XRF analysis and is successfully used to analyze microelement admixtures, for example, in synthetic gem materials that imitate natural gemstones. Based on microelement content, it is possible, for instance, to determine the origin of cubic zirconia (ZrO2), etc. FIB methods are applied to study thin layers and metal material structures (e.g., imitations of gold items coated with titanium nitride). Items are evaluated comprehensively, and canvases are examined—the type of weave is identified, fibers are analyzed, and fiber pigments and dyes are studied. The materials used for frames are also examined, etc.

b) X-ray powder diffraction and microdiffraction (XRD, mXRD) – these methods enable direct phase analysis of substances even within mixtures. The mXRD allows obtaining complete structural information from an area of approximately 100 μm, making it possible to analyze individual pigment grains, thin layers, etc., directly.

c) Infrared spectroscopy (IR, FTIR) and Raman spectroscopy – these are important complementary methods. Problems can arise when analyzing mixtures due to significant overlapping of absorption bands. In IR spectroscopy, so-called mirror reflections often appear in spectra from polished sections. In Raman spectroscopy, fluorescence can completely prevent measurement at the laser wavelength used.

d) Gas chromatography coupled with mass spectrometry (GC-MS, MS/MS) – these methods are especially useful for identifying organic substances in mixtures. In the case of counterfeit paintings, they are used to detect impregnants that create the appearance of "aged" canvases.

Other methods include botanical techniques (identification of wood species), biological methods, and genetic analysis (for example, analysis of binders of biological origin). Signature analysis is also performed, though it is very challenging since signatures usually do not represent continuous script and the texts are very short.

The multidisciplinary approach has enabled the collection of a large dataset for both the art materials database and the database of painting techniques of important Czech painters. Research using mobile spectroscopic methods has yielded very satisfactory results. Thanks to these methods, detailed characterizations of individual artists' palettes have been possible. The data obtained are fundamental for assessing the authenticity of artworks. All data, including primary documentation, are stored in specially programmed databases, which will later be used for authenticity assessment of paintings. Although these databases are still in their initial stages, they already contain more than eleven thousand data entries.

In practice, a few thousand cases are processed annually. Large cases may contain several hundred items. Besides pigment and color analysis and comparison in the broader sense, a significantly wider spectrum of materials is examined. These include complex analyses of samples with petrological and mineralogical character (e.g., remnants of statues, sculptures, gemological items, etc.), analyses of metal materials in a broader sense, fiber identification and textile material analysis, analysis of tool marks (e.g., on painting frames, comparison with paintings, detection of tool marks), and more.

L6

ANALYSIS OF FINE ART FORGERIES IN FORENSIC PRACTICE

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Investing in fine art is widely perceived as a safe and prestigious form of long-term capital appreciation. However, when the authenticity of an artwork is questioned, such an investment may become a significant financial risk. The exponential rise in auction prices over the past decades has made the production and sale of art forgeries an increasingly lucrative and thus dangerous field of criminal activity. In many cases, counterfeits are so sophisticated that they cannot be distinguished from originals by visual inspection alone, which makes the application of advanced forensic and analytical methods indispensable.

The Institute of Criminalistics of the Police of the Czech Republic, in close cooperation with the National Gallery in Prague and, more recently, with the University of Pardubice, has been developing robust and court-defensible procedures for the authentication of fine art. These procedures are based on systematic research of original works by significant Czech and European painters, especially from the late 19th and 20th centuries, and on building comprehensive databases of pigments and other artistic materials. The goal is to provide forensic experts and law enforcement authorities with reliable, reproducible, and scientifically verifiable tools for distinguishing genuine works from forgeries.

The methodological framework combines a wide range of complementary analytical techniques. Optical microscopy provides initial information on the stratigraphy of paint layers, the morphology of pigment particles, and evidence of retouching or later interventions. Scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM/EDS) enables high-resolution imaging and elemental analysis of pigments, fillers, and fibers. Fourier-transform infrared spectroscopy (FTIR) is applied to identify binding media and organic components within paint layers, while Raman spectroscopy supplies highly specific molecular information, particularly valuable for identifying pigments and dyes in microsamples. These techniques are complemented by X-ray diffraction (XRD), which is crucial not only for the precise identification of crystalline phases but also for detecting historical changes in pigment production, such as those associated with titanium white or Naples yellow.

The applicability of these procedures has been confirmed in a number of forensic cases involving disputed artworks. Frequently investigated materials include not only pigments and binders but also textile fibers and other components of cultural heritage objects, which are often essential for dating or verifying both artworks and historical artifacts. For example, the Institute has collaborated with restorers during the examination of the historic theatre curtain of the Jewish Museum in Prague, as well as with the Náprstek Museum in Prague, where indigenous South American ritual artifacts, including shrunken heads (tsantsas), were subjected to detailed material analysis. These case studies highlight not only the analytical challenges but also the necessity of interdisciplinary collaboration between forensic scientists, conservation specialists, and art historians.

The development of standardized methodologies, supported by this interdisciplinary approach, ensures that the results are both scientifically valid and legally defensible in court. This research has been financially supported by the project of the Ministry of the Interior of the Czech Republic: The Development of a Strategic Cluster for Effective Instrumental Technological Methods of Forensic Authentication of Modern Artworks (VJ01010004).

September 7, Tuesday

L8

PROTEIN PURIFICATION IN AND BEYOND THE CRYSTALLOGRAPHY WORLD

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The X-Ray Structural Biology field relies on the production of abundant, pure and homogeneous protein samples for protein crystallization [1]. Besides its importance in Structural Studies, proteins are used at different purity levels in economic sectors such as feed industry, pharmaceuticals or human health. Purification methodologies have improved dramatically in the past decades, but they represent multifactorial-processes highly dependent (but not limited to) protein production hosts, recombinant construction design, lysis methods, protein stability (degradation, aggregation propensity, thermostability,…) and/or sample composition (pH, ionic strength, temperature, additives,…) [2,3].

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L9

THE ROLE OF PROTEIN CRYSTALS IN BIOTECHNOLOGY AND PHARMA

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The crystallization of biomolecules has played a pivotal role in pharmaceutical innovation, with insulin being the first hormone successfully crystallized to obtain a pure and stable form that transformed diabetes treatment and improved the lives of millions of patients, its crystallization not only enabled large-scale purification and stabilization, but also established the foundation for modern protein-based therapeutics (Figure 1). Building on this legacy, protein crystals have become essential tools for diverse applications ranging from structural biology to advanced drug delivery. In the field of biocatalysis, enzymes exploit their versatility, selectivity, and specificity to catalyze industrially relevant reactions under mild conditions. To extend their lifetime under extreme conditions and enhance efficiency, enzymes can be immobilized in different supports, with Cross-Linked Enzyme Crystals (CLECs) offering one of the most robust and recyclable solutions [1]. CLECs are currently experiencing a renaissance, boosted by advances in protein crystallization over the last two decades and by the development of novel scaffold materials. In this lecture, I will present how knowledge gained from crystallization in convection-free environments has been applied in our lab to produce protein crystals for biotechnological applications, including: i) CLECs for the development of enhanced, robust biosensors [2], ii) reinforced cross-linked enzyme crystals (rCLECs) for large-scale enzymatic reactions with durable auto-supported catalysts [3], and iii) reinforced protein crystals for controlled drug delivery systems [4].

Figure 1. In May 1921, Fredrick Banting met in John Macleod's lab to begin their experiments with insulin. In January 1922, the first person received an insulin injection. By 1923, insulin was commercially available. Banting and Macleod received the 1923 Nobel Prize in medicine.

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L10

PROTEIN CRYSTALLIZATION IN LIVING CELLS - PUSHING THE LIMITS

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Although it has been known for more than a century, protein crystallization in living cells was considered an artificial, pathogenic phenomenon for a long time. However, during the past decades, it has been observed surprisingly often in all domains of life and is now recognized as a physiological assembly process that is also accessible for recombinant proteins in host cells [1]. The advent of high-brilliance synchrotron sources, X-ray free-electron lasers, and improved serial data collection strategies has allowed the use of these micrometer-sized crystals for structural biology [2-7]. Thus, *in cellulo* crystallization offers exciting new possibilities for the structural investigation of proteins in a quasi-native environment, complementing conventional X-ray crystallography approaches.

This lecture will present an overview of the current knowledge about *in cellulo* crystallization of native and recombinant proteins, complemented with a discussion of the current method developments to successfully collect X-ray diffraction data from intracellular crystals. Efforts to systematically exploit living insect cells as protein crystallization chambers and to streamline this process for structural biology resulted in the establishment of a pipeline to elucidate the structural information of *in cellulo* crystallized target proteins in short time, denoted as '*InCellCryst*' [8]. After cloning of the target gene into baculovirus transfer vectors, the associated recombinant baculoviruses are generated to infect insect cells, and crystal formation is detected at day 4 to 6 after infection. If intracellular crystal -lization is successful, X-ray diffraction data of tens of thousands of crystals are collected directly within the living cells using recently developed serial crystallography approaches at XFELs [2,3,6,7] or synchrotron sources [4,8]. However, a recent proof-of-principle experiment demonstrated that a full electron diffraction (mED) dataset can be collected only using a single intracellular crystal in the low µm size-range [10]. Since low numbers of crystal containing cells are frequently obtained within a cell culture, *in cellulo* mED holds the promise to overcome this major bottleneck of *in cellulo* protein crystallization, which currently restricts a wider application in structural biology.

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L11

ANAEMIA, CULTURAL/RELIGIOUS HERITAGE AND DIETARY PREFERENCES

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Anaemia globally affects roughly one-quarter of the world population. It affects vulnerable populations, particularly children, pregnant women, and women of reproductive age, leading to severe health consequences like increased maternal and child mortality, impaired cognitive and physical development, and reduced productivity.

Religious heritage, cultural and dietary preferences significantly influence anaemia prevalence by restricting access to essential nutrients like iron, vitamin B12 and folate as seen in persons with food taboos, gender-based food restrictions, or cultural/religious dietary rules, with the hazard of an increasing anaemia risk.

Addressing anaemia requires – besides culturally sensitive interventions and community involvement – tailored nutrition education and precision medicine to ensure effective anaemia prevention and management.

Nutrition education – respecting religious heritage, cultural and dietary preferences – starts with a deeper understanding of the physiological and biochemical background of iron uptake.

Selected readings

https://www.who.int/news-room/fact-sheets/detail/anaemia

Biology of Anemia: A Public Health Perspective. Britterham et al., 2023, J. Nutr. https://doi.org/10.1016/j.tjnut.2023.07.018

Iron insight: exploring dietary patterns and iron deficiency among teenage girls in Sweden. Stubbendorff et al., 2025, Eur. J. Nutr. https://doi.org/10.1007/s00394-025-03630-z

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Health effects associated with consumption of unprocessed red meat: a Burden of Proof study. Lescinsky et al. 2022, Nature Med. https://www.nature.com/articles/s41591-022-01968-z

Associations of unprocessed and processed meat intake with mortality and cardiovas-cular disease in 21 countries [Prospective Urban Rural Epidemiology (PURE) Study]: a prospective cohort study. Iqbal et al., 2021, Am. J. Clin. Nutr. https://doi.org/10.1093/ajcn/nqaa448

L12

ECO-CULTURAL HERITAGE: CONCEPTUAL INNOVATIONS AND APPLIED METHODOLOGIES FOR THE 21ST CENTURY

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This paper proposes a reconceptualization of heritage epistemology through the integration of eco-cultural perspectives with contemporary theoretical frameworks in heritage studies. In response to the increasing urgency of environmental degradation, climate change, and cultural fragmentation, heritage scholarship must move beyond traditional dichotomies of natural versus cultural heritage. Eco-cultural heritage, understood as the entanglement of ecological systems and cultural practices, offers a dynamic lens for interpreting, preserving, and managing heritage in the Anthropocene. Drawing on interdisciplinary theories from environmental humanities, critical heritage studies, and Indigenous epistemologies, this paper develops a conceptual model that bridges ecological interdependence with cultural continuity. The model is then tested through selected case studies in East-Central Europe, demonstrating how this integrated approach reorients heritage practice toward sustainability, community engagement, and resilience. The paper argues that embracing eco-cultural heritage as a core analytical category not only enriches our understanding of place and identities, but also fosters innovative strategies for heritage governance and policy. Ultimately, this study contributes to the formulation of a new heritage epistemology, one that is reflexive, holistic, and attuned to the complexities of contemporary socio-environmental realities. The proposed approach - central to KreativEU Alliance as well - is intended to serve as both a conceptual advancement and a practical tool for scholars, practitioners, and policymakers alike.

L13

PLANT-BASED BIOCIDES FOR THE SUSTAINABLE PRESERVATION OF BUILT CULTURAL HERITAGE

Dina Mateus

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Biological colonization is among the most pressing threats to the integrity of built cultural heritage, accelerating material deterioration and compromising long-term preservation. Microorganisms—including fungi and bacteria—produce acids, pigments, and enzymes that deteriorate both organic and inorganic substrates, resulting in discoloration, cracking, surface detachment, and structural weakening. Traditional mitigation strategies rely heavily on synthetic biocides such as quaternary ammonium compounds, phenols, or commercial formulations, which, although effective, present significant drawbacks: their toxicity to humans, persistence in the environment, and potential to cause irreversible alterations to heritage materials. In line with global sustainability goals and ecological engineering principles, there is increasing interest in developing bio-based, less harmful alternatives for preventive and curative conservation practices.

This research explores the use of plant-derived essential oils (EOs) as natural biocides for the sustainable preservation of cultural heritage materials, with a focus on mural paintings, stone, and ceramics materials. EOs obtained from fennel (*Foeniculum vulgare* Mill.), pennyroyal (*Mentha pulegium* L.), lavender (*Lavandula viridis* L'Hér.), and thyme (*Thymus mastichina* L.) were tested against microorganisms isolated from emblematic Portuguese heritage sites, including the Convent of Christ in Tomar [1], the Roman city of Conímbriga [2], and the 18th-century murals of the House of Moscadim [3]. The tested strains comprised both bacteria and fungi, representative of biodeteriorative communities affecting built heritage.

Biocidal efficacy was assessed through direct-contact (disk diffusion) and micro-atmosphere methods. The latter proved particularly promising for fragile surfaces, as it capitalizes on the volatility of EOs, enabling antimicrobial activity without physical contact or risk of chemical interaction with original substrates. Field trials were performed to validate laboratory results under real environmental conditions.

EOs from fennel, pennyroyal, lavender, and thyme showed significant antimicrobial activity against the microorganisms isolated from stone and mural paintings, although generally less effective than the commercial biocide Biotin T®. Pennyroyal and fennel EOs proved the most potent antifungal overall, even surpassing Biotin T® against fungi. Mixtures of EOs revealed synergistic effects, enhancing biocidal efficacy. The results confirm that the effectiveness of plant-based biocides depends on both the microorganism species and the chemical composition of the EOs, highlighting the need for context-specific evaluation in cultural heritage preservation. Field trials confirmed the practical potential of EO-based treatments for cultural heritage preservation.

Overall, this research provides compelling evidence that plant-derived essential oils represent a viable and eco-friendly strategy for the sustainable preservation of built cultural heritage. Their demonstrated antimicrobial efficacy, renewable origin, reduced environmental impact, and potential for non-invasive application support their integration into conservation practice. Further optimization of EO-based formulations and application methods can strengthen their role as effective and sustainable substitutes for synthetic biocides, aligning heritage conservation with environmental responsibility.

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L14

NO LABELS NEEDED – ANALYZE YOUR ORGANELLES USING HOLOGRAPHY

Kateřina Lónová

Altium International s.r.o., Na Jetelce 69/2, 190 00 Praha 9

Cell metabolism plays a pivotal role in both health and disease, yet conventional fluorescence-based techniques are often constrained by phototoxicity, photobleaching, and demanding sample preparation. Nanolive's holotomographic technology enables label-free, high-resolution, and continuous monitoring of metabolic activity without compromising cell viability.

The **Smart Lipid Droplet Assay** and **Smart Mitochondrial Assay** provide quantitative analysis of lipid droplet morphology and distribution, as well as detailed assessment of mitochondrial dynamics. By eliminating the need for dyes and enabling long-term real-time observation, Nanolive's technology represents a major innovation for biomedical research, accelerating the discovery of disease mechanisms and drug responses.

September 8, Wednesday

L15

TARGETED PROTEIN DEGRADATION AS A NOVEL ANTIVIRAL STRATEGY

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Enteroviruses (EV), including EV-A71 and EV-D68, represent an increasing public health concern, especially among children [1,2]. These viruses are associated with serious neurological and respiratory illnesses such as hand, foot, and mouth disease (HFMD) [3,4] and acute flaccid myelitis (AFM) [5,6]. Currently, there are no specific antiviral therapies available for these infections.

This project explores a novel approach: **targeted protein degradation**. Instead of inhibiting viral enzymes, we aim to eliminate them by exploiting the ubiquitin-proteasome system. Our target is the **2A protease (2Apro)**, a viral enzyme essential for replication and immune evasion. 2Apro processes the viral polyprotein and interferes with host defense mechanisms by cleaving eIF4G and disrupting interferon signaling through IFNAR1 degradation and cleavage of MAVS and MDA5 [7,8].

To achieve targeted degradation, we propose the development of **proteolysis-targeting chimeras (PROTACs)** that link 2Apro to cereblon (CRBN)-mediated ubiquitination and proteasomal degradation [9,10]. PROTACs provide advantages over conventional inhibitors, including catalytic activity, complete removal of the target protein, and reduced susceptibility to resistance [9,11].

Our strategy combines **structural biology tools** (X-ray crystallography, crosslinking mass spectrometry) with rational design to develop and optimize PROTAC molecules. We established an expression and purification workflow to obtain milligram quantities of active, monodisperse 2Apro using solubility-enhancing fusion tags and chromatography techniques, ensuring suitability for structural and biochemical studies.

This research builds on the vision of the PANVIPREP consortium for broad-spectrum antiviral development and represents a paradigm shift from inhibition to degradation-based antiviral strategies. Ultimately, this work may provide a foundation for new classes of therapeutics against enteroviruses and other RNA viruses of epidemic and pandemic relevance.

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L17

SMALL FLY, BIG IMPACT: *DROSOPHILA* TO UNRAVEL THE PATHOLOGY OF NEURODEVELOPMENTAL DISORDERS

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For over a century, *Drosophila melanogaster* has stood as one of the most powerful and stable model organisms in biology, shaping our understanding of genetics, development, and neuroscience. While far from being a "new trend," the fruit fly continues to adapt to innovative approaches. Today, its genetic tractability and conserved pathways make it invaluable for studying human disease.

Neurodevelopmental disorders (NDDs), such as Intellectual Disability and Autism Spectrum Disorder, affect millions of people worldwide and are often linked to genetic mutations. In our research, we use *Drosophila* to investigate a conserved, yet poorly characterized gene recently implicated in NDDs through clinical studies. Using modern CRISPR/Cas9 genome editing, we generated knockout and patient-specific alleles to model the consequences of these mutations in the nervous system. Cognitive function is assessed through habituation learning, a fundamental and evolutionarily conserved form of non-associative learning, together with additional developmental and morphological analyses.

By combining the long-standing tradition of *Drosophila* research with cutting-edge genetic tools, we advance the understanding of genes implicated in neurodevelopmental disorders while extending the relevance of this classical model organism to human health. This approach demonstrates how a century-old model, continually refined by modern technologies, remains indispensable for uncovering the molecular basis of human disorders and guiding future biomedical research.

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L18

FROM THE BRAGGS TO BILL ASTBURY AND THE ADVENT OF MODERN STRUCTURAL MOLECULAR BIOLOGY

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The Bragg law underpins interpretation of X-ray diffraction patterns of crystals as well as other partially ordered systems. We will first turn back the clocks to the beginning of the 20th century and look at the early developments of X-ray diffraction and its connection to the University of Leeds where the data underpinning the famous law were collected. Subsequently, Bill Astbury was recruited to the University of Leeds to take over the Bragg legacy and applied X-ray diffraction to textiles. We will discuss how this work fundamentally contributed to the study of biological material and paved way to the discovery of DNA structure. In the end we will return to the present day Astbury Biostructure Laboratory and illustrate the complementarity of X-ray scattering and electron cryo-microscopy in the study of partially ordered biological assemblies.

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L19

EXTREMELY BRILLIANT X-RAY SOURCES AND NEW OPPORTUNITIES IN MACROMOLECULAR CRYSTALLOGRAPHY

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Over the past decade, the advent of X-ray free electron lasers delivering ultra intense X-ray beams has revolutionized biocrystallography. With a brilliance a billion times higher than at synchrotrons, the XFEL beam destroys the sample just after the emission of its diffraction signal in a process called “diffraction before destruction”. While this firepower allows the characterization of smaller crystals than ever (micro or even nanocrystals), the sample needs to be refreshed after each shot and the collection of a full dataset requires series of thousands of crystals. Also, crystal cryocooling is no longer necessary and this type of analysis is mostly performed at room temperature. In these near-to-physiological conditions and thanks to the temporal resolution of XFEL pulses (<100 fs), the dynamics of biological systems (conformational changes, catalytic events) can be probed in crystallo. Similar protocols have been implemented at synchrotron facilities and are widely accessible.

To take advantage of these new approaches, crystal growers need to adapt current protocols mainly devoted to the production of large single crystals, to the preparation of showers of microcrystals with homogeneous size and diffraction quality. Based on crystal growth principles and examples of alternative crystallization approaches including advanced crystallization control or microfluidics technologies [1,2,3].

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L20

PLASMA DEPOSITION OF NANOSTRUCTURED SURFACES: FROM PREPARATION TO (BIO)APPLICATIONS

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Nanostructured surfaces prepared by means of low-temperature plasma represent a rapidly developing field at the interface of discharge physics, solid-state physics, nanotechnology, and materials science. The growth of the field is driven by the expansion of nanomaterials, where structural dimensions on the order of 10–100 nm provide materials/surfaces with unique physicochemical properties. Nanostructured surfaces can be fabricated as homogeneous thin films, 2D nanoislands, or nanoparticles deposited directly on the surface or embedded into the bulk of a host material, with subsequent applications in modern semiconductor electronics, sensing, biomedicine, catalytic processes, and beyond.

The lecture maps the process of plasma-assisted preparation of nanoparticles and nanocomposite films—from the initial phase of growth initiation, through the formation of layers with defined properties, to the utilization of nanostructures for pathogen detection, demonstrated on two sensor design concepts. In the first case, aspects of optimizing the preparation of a nanocomposite of Ag nanoparticles embedded in a plasma polymer C:H:N:O for LSPR (Localized Surface Plasmon Resonance) detection of Lyme disease pathogens will be discussed.

L21

EXPLORING THE MORPHOLOGY AND STRUCTURE OF FE-BASED NANOMATERIALS FOR PHARMACEUTICAL APPLICATIONS USING X-RAY SCATTERING METHODS

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Iron–carboxymaltose (ICM) is an intravenous iron formulation in which ferric hydroxide nanoparticles are stabilized by a carboxymaltose shell. Understanding its internal architecture is essential for elucidating structure–function relationships that govern stability and bioavailability. In this study, the microstructure of ICM was investigated using Small-Angle X-ray Scattering (SAXS) and X-ray Diffraction (XRD). SAXS analysis revealed a core–shell morphology with nanometer-scale ferric oxyhydroxide cores (~2–5 nm radius) and a disordered carbohydrate coating, consistent with a colloidal complex. XRD patterns exhibited broad reflections characteristic of poorly crystalline ferrihydrite, confirming the amorphous-to-nanocrystalline nature of the iron core. The combination of SAXS and XRD provides complementary insights, demonstrating that ICM is composed of ultrasmall ferrihydrite-like domains embedded within an amorphous polysaccharide matrix. These findings contribute to a deeper understanding of ICM's physicochemical stability and its controlled iron release in vivo.