MaSC
Users' Group for **Mass Spectrometry and Chromatography**

10th International MaSC Meeting

Mass Spectrometry and Chromatography in Cultural Heritage

29-30th September 2022

Musée d'Aquitaine
Grand Théâtre de Bordeaux
Bordeaux, France

Preface

The Committee of the Users' Group for Mass Spectrometry and Chromatography (MaSC) welcomes you to the 10th MaSC Meeting, which will be held at two of Bordeaux's most iconic locations: the Musée d'Aquitaine and Grand Théâtre de Bordeaux.

We are extremely pleased to be able to meet again, after an unusually long time since our last meeting, in Ottawa in 2019. In the nearly 20 years that have passed since MaSC was founded, our Users' Group has developed as a critical international forum for scientific interaction and discussion on chromatographic and mass spectrometric techniques for the study of objects of cultural heritage. MaSC currently has more than 100 members, representing 85 institutions – primarily cultural and academic organisations – in 26 countries.

As in previous years, the present meeting was preceded by a three-day workshop, led by Prof. Caroline Tokarski and colleagues. The workshop involved instruction on both the theoretical and practical aspects of protein characterization in artistic, archaeological, and paleontological cultural heritage objects. Participants were also trained in the bioinformatics approaches needed to handle the large data sets obtained from such analyses. We would like to cordially thank the workshop instructors for sharing their expertise with our members.

The tenth MaSC meeting has a very diverse programme, reflecting the specialities and research interests of our members. Research is presented through oral presentations and poster sessions, encompassing new chromatographic and mass spectrometric techniques, as well as instrumentation and applications in general. The selected presentations highlight challenges and strategies for sample derivatization, data interpretation, and cover a wide range of materials across a variety of cultural heritage objects. The studies add to the understanding of historic materials, manufacturing techniques, and artists' methods, and in turn, inform conservation treatments and preservation strategies.

We are immensely grateful to the local organisers, particularly Dr. Francesca Galluzzi, Dr. Aleksandra Popowich, and Prof. Caroline Tokarski for their huge efforts in organising and hosting the workshop and meeting. The wonderful venues in this beautiful city add much cachet to the 10th MaSC meeting. We hope you enjoy the meeting, and your visit to Bordeaux!

The MaSC Committee:

Klaas Jan van den Berg,
Christopher Maines,
Ester Ferreira,
Catherine Higgitt,
David Peggie,
Ken Sutherland

Message from the organisers

First, we would like to start by welcoming you all to Bordeaux! To the MaSC committee, thank you for the opportunity to host this meeting. We hope that over the next two days you will not only have fulfilling scientific discussions, but will also have gotten a taste of what Bordeaux and the surrounding region have to offer.

We have the opportunity to host the two days of the MaSC meeting in two historic buildings in Bordeaux, the Musée d'Aquitaine and the Grand Théâtre.

We have the honour to welcome Dean Lewis, the President of the University of Bordeaux and Jacques Maddaluno, the Director of the CNRS Chemistry Institute. We would like to thank them for participating in this conference and for supporting scientific research in cultural heritage in Bordeaux. We must also thank their teams, in particular Etienne Duguet, Vice-President of Innovation of the University of Bordeaux and Erick Dufourc, CNRS Director, previously Deputy Director of the Chemistry Institute, who will participate in this conference. We are extremely thankful to Pierre Hurmic, the Mayor of Bordeaux for his presence and support as well as Laurent Védrine, the Director of the Musée d'Aquitaine, who offered the space and a guided tour of the museum for the first day of the meeting. We also offer our sincere thanks to Emmanuel Hondré, Director of the Bordeaux Grand Théâtre and National Opera, for their help in organising the event in such a historic venue.

We must also thank our excellent panel of invited speakers, for presenting their ground-breaking research in this conference: Julie Arslanoglu, Ilaria Bonaduce, Matthew Collins, Juergen Cox, Victor Etgens and Remy Chapoulie.

This event would not have been possible without the generous support of the sponsors of the event, including the Université de Bordeaux, the Region Nouvelle Aquitaine, the CNRS, the French Society of Mass Spectrometry, the French Proteomics Society, the Bordeaux Summer School, the Excellence Initiative of the University of Bordeaux and MaSC.

Finally, we offer our thanks to the workshop organising committee for putting together a truly exceptional 3 days of practical and theoretical courses, we are proud to be able to share our expertise in the field of mass spectrometry applied to cultural heritage. We have an excellent scientific and social programme for the meeting, enjoy!

MaSC local organisers,
Francesca Galluzzi - Univ. of Bordeaux, Proteome Platform, CBMN, and Archéosciences Bordeaux - CNRS, France
Aleksandra Popowich - The Metropolitan Museum of Art, New York, USA
Caroline Tokarski – Univ. of Bordeaux, Proteome Platform, CBMN - CNRS, France

Workshop organizing committee,

Katell Bathany - Univ. of Bordeaux, Proteome Platform, CBMN - CNRS, France
Landry Blanc - Univ. of Bordeaux, Proteome Platform, CBMN - CNRS, France
Stéphane Chaignepain - Univ. of Bordeaux, Proteome Platform, CBMN - CNRS, France
Stéphane Claverol - Proteome Service Platform, France
Nicolas Desbenoit - Univ. of Bordeaux, Proteome Platform, CBMN - CNRS, France
Jean-William Dupuy - Proteome Service Platform, France
Francesca Galluzzi - Univ. of Bordeaux, Proteome Platform, CBMN, and Archéosciences Bordeaux - CNRS, France
Florent Grélard - Univ. of Bordeaux, Proteome Platform, CBMN - CNRS, France
Catherine Gilbert - Univ. of Bordeaux, Proteome Platform, CBMN - CNRS, France
Vaclav Krupicka - Univ. of Bordeaux, Proteome Platform, CBMN - CNRS, France
Anne-Marie Lomenech - Proteome Service Platform, France
Aleksandra Popowich -The Metropolitan Museum of Art, New York, USA
Michael Tuck - Univ. of Bordeaux, Proteome Platform, CBMN - CNRS, France
Caroline Tokarski - Univ. of Bordeaux, Proteome Platform, CBMN - CNRS, France
Daniel D. Vallejo, Georgia Institute of Technology

Meeting schedule

Thursday 29th September, 2022

Musée d'Aquitaine

20 Cr Pasteur, 33000 Bordeaux

The Museum of Aquitaine in its current form was officially created in 1963 by museologist Georges-Henri Rivière, who was tasked with reorganising France's museums of history, archaeology and ethnology after the war. The museum initially shared the city's Museum of Fine Arts building, occupying its north wing. In 1970, the decision was taken to move the museum to the former University of Bordeaux Faculty of Arts and Sciences on Cours Pasteur, constructed at the end of the nineteenth century by local architect Pierre-Charles Durand, on the site of the Feuillant convent where philosopher Michel de Montaigne was buried in 1592. The museum is the result of the merger of several public collections built up by the city since the sixteenth century. In 1963, collections from other museums in Bordeaux were brought together at the Musée Lapidaire, which was then renamed the Museum of Aquitaine.

Today, the museum boasts over 1.3 million pieces, illustrating the history of Bordeaux and the local region from prehistory to the twentieth century. Prestigious collections of regional and extra-European archaeology, history and ethnography retrace the lives of the Aquitaine people and their relationship with the rest of the world.

08:00 - 08:30	**Welcome & Registration**
08:30 - 09:00	**Opening Remarks** *Pierre Hurmic*, Mayor of Bordeaux *Etienne Duguet, Vice-President of Innovation, University of Bordeaux* *Klaas Jan van den Berg, European Coordinator of MaSC* *Caroline Tokarski, Director of Bordeaux Proteome, CBMN CNRS, University of Bordeaux, MaSC 2022 host*
09:00 - 09:20	**Welcome remarks and Introduction to the Collection of Aquitaine Museum & Associated Research** *Laurent Védrine* Aquitaine Museum, Bordeaux, France Chair: Caroline Tokarski, Bordeaux Proteome, University of Bordeaux, CBMN CNRS
09:20 - 09:40	**Study and Conservation of Works of Art at the C2RMF and investment in European Infrastructures** *Victor Etgens* Centre of Research and Restoration of French Museums (C2RMF), Paris, France Chair: Caroline Tokarski, Bordeaux Proteome, University of Bordeaux, CBMN CNRS
Session 1: Paint binders Chair: Klaas Jan van den Berg, Cultural Heritage Agency of the Netherlands and University of Amsterdam, European Coordinator of MaSC	
09:40 - 10:00	**Analysis of binders and additives in Talens ETA and Rembrandt tempera paints** *Inez Dorothé van der Werf,* Cultural Heritage Agency of the Netherlands
10:00 - 10:20	**A mass spectrometric study of the oil paint curing using methyl linoleate as a model binder** *Silvia Pizzimenti,* University of Pisa
10:20 - 10:40	**A retrospective and critical review of binding media analysis in wall paintings** *Joy Mazurek,* Getty Conservation Institute

10:40 - 11:00	**14 Flash Poster Presentations** Chair: Catherine Gilbert, Univ. of Bordeaux, Proteome Platform, CBMN CNRS
11:00 - 11:30	**Coffee break & posters***
11:30 - 12:00	**Ancient Proteins - Future Directions in the Past** *Matthew Collins* University of Cambridge, England and Natural History Museum of Denmark, University of Copenhagen, Denmark Chair: Caroline Tokarski, Bordeaux Proteome, University of Bordeaux, CBMN CNRS
12:00 - 13:30	**Lunch** Atrium, Campus Victoire, historic campus of the University of Bordeaux
13:30 - 14:15	**Aquitaine Museum tour guided by the Director - Mr. Laurent Vedrine**
14:15 - 14:45	**Computational paleoproteomics** *Juergen Cox* Max Planck Institute, Germany Chair: Francesca Galluzzi, Univ. of Bordeaux, CBMN CNRS and Archéosciences Bordeaux
Session 2: Protein analysis Chair: Francesca Galluzzi, Univ. of Bordeaux, CBMN CNRS and Archéosciences Bordeaux	
14:45 - 15:05	**Identifying emulsion-cured leather in museum collections using proteomics** *Aleksandra Popowich*, The Metropolitan Museum of Art
15:05 - 15:25	**Discrimination of plant gum polysaccharides using MALDI-MS: developments, limitations, and exploration of a complementary proteomics approach** *Clara Granzotto*, Art Institute of Chicago

15:25 - 15:45	**An ongoing quest to identify 2000-year-old proteinaceous residues on ceramics from the Late Iron Age and Roman Period** *Tania F.M. Oudemans*, Kenaz Consult & Laboratory Bloody Proteins
15:45 - 16:00	**Coffee break & Posters***
16:00 - 16:20	**Poster Session****
16:20 - 16:50	**Chemistry of Paint Film Formation** *Ilaria Bonaduce* University of Pisa, Italy Chair: Klaas Jan van den Berg, Cultural Heritage Agency of the Netherlands and University of Amsterdam, European Coordinator of MaSC
16:50 - 17:00	**Departure by bus to Chateau Château Malartic-Lagravière** Tour of the vineyards, wine tasting (Grand Cru Classé de Graves), and Gala Dinner

* The presence of poster authors not necessarily required

** The presence of poster authors required

Lunch, Thursday Midday

Atrium, Campus Victoire

3ter Pl. de la Victoire - 33000 Bordeaux

In the heart of Bordeaux, this historic site is one of the most beautiful monuments in the city, with its 19th century library in which 40 generations of students have worked. A historic site in the heart of Bordeaux, this campus is the place of study and research in Human Sciences.

At the entrance to the campus, the two statues that adorn its forecourt form a symbolic dyad representing Nature unveiling itself in front of Science. If you venture further, in the centre of the Atrium, you will see the motto "Pro Scientia Urbe et Patria" (For science, city and country).

We will walk here together from the Musée d'Aquitaine for lunch. We will walk along Cours Pasteur (following the tracks of the Tram B), where we will arrive at the Place de la Victoire, the Victoire campus is located on the left of the square.

Social Program, Thursday evening

Chateau Château Malartic-Lagravière – Grand Cru Classé de Graves

43 Av. de Mont de Marsan, 33850 Léognan

Since its creation in the 18th century, only four families have owned Malartic. The taste for adventure still flows in the veins of the men and women who steer its destiny. Having enjoyed an excellent reputation from the beginning of the nineteenth century, Château Malartic-Lagravière is one of only six properties to be classified in both red and white wines in the 1953 Graves classification.

At 17h we will travel by bus from the Musée d'Aquitaine to Château Malartic-Lagravière. Here, we will enjoy a tour of the vineyards, a wine tasting and a dinner together.

We will leave the chateau around 10h30-11h, and will make several stops in Bordeaux for your convenience:

1st stop: Gare Saint Jean (train station)

2nd stop: Place de la Bourse

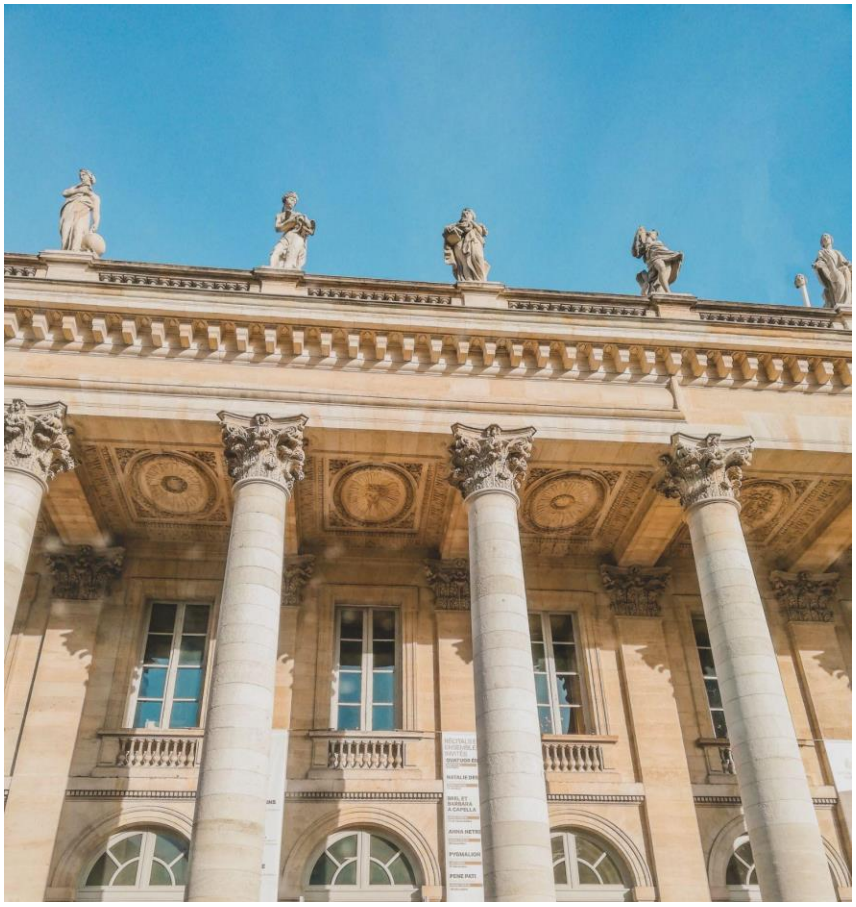
3rd stop: Place de Quinconces

4th stop: Place Gambetta

Final stop: Place de la Republique (closest to Musée d'Aquitaine)

Friday, 30th September, 2022

Grand Théâtre at the Opéra National de Bordeaux



Pl. de la Comédie, 33000 Bordeaux

The history of the Grand Theatre of Bordeaux, inaugurated on 7 April 1780, has been punctuated by various events over the ages. The building is still considered one of the most beautiful theatres in the world and was the work of renowned architect Victor Louis. In addition to its aesthetic value, the building has exceptional acoustics thanks to the wooden structure surrounding it. On its façade, the 12 Corinthian columns are crowned by 9 muses and 3 goddesses from ancient mythology gazing into the distance, designed by Pierre Berruer. Nearly a century after its construction, Charles Garnier drew inspiration from the grand staircase for his design for the one in the Opéra de Paris. The task of decorating the dome in the theatre was entrusted to Jean-Baptiste Robin. The theme chosen by the artist was "Apollo and the muses approving the dedication of a temple built by the city of Bordeaux", a three-fold tribute, both allegorical and realistic, to the arts, the craftsmen who built the theatre and the city of Bordeaux. The current chandelier was installed in 1917. Weighing 1.2 tonnes, it is made of Bohemia crystal and holds an impressive 400 lights.

Some of the most celebrated artists have performed and captivated audiences in this architectural masterpiece in Bordeaux, including Liszt, Cinti-Damoreau, Falcon, Viardot, Talma, Nourrit, Duprez, Rubini, Petipa, Chaliapine, Schipa and, in more recent years, Plácido Domingo, Gustav Leonhardt, Natalie Dessay, Cecilia Bartoli, and Carolyn Carlson.

08:45 - 09:00	**Welcome**
09:00 - 09:40	**Opening Remarks** *Dean Lewis, President of the University of Bordeaux* *Erick Dufourc, CNRS Chemistry Institute, CBMN* *Christopher Maines, National Gallery of Art, North American Coordinator of MaSC* *Caroline Tokarski, Director of Bordeaux Proteome, CBMN CNRS, University of Bordeaux, MaSC 2022 host*
9:40-9:50	**Welcome remarks and Introduction to the History of the Grand Theather** *Emmanuel Hondré* Director-General Opéra National de Bordeaux - Grand-Théâtre, Bordeaux
09:50 – 10:20	**Omics in the Museum World: grit, education, communication, and ethics at the Met** *Julie Arslanoglu* The Metropolitan Museum of Art Chair: Ken Sutherland, Art Institute of Chicago
Session 3: Lacquers Chair: Ester Ferreira, Cologne Institute of Conservation Sciences	
10:20-10:40	**Identification and differentiation of essential oils used as solvents in paints and lacquer works** *Patrick Dietemann*, Doerner Institut
10:40-11:00	**Blended Thitsiol/Urushiol Asian lacquers in cultural heritage: new advances in understanding their curing, ageing, and photo-ageing processes by THM-GC/MS** *Valentina Pintus*, Academy of Fine Arts, Vienna
11:00-11:20	**Differentiation between primary and secondary pyrolysis reactions to improve the identification of Asian lacquer macromolecules** *Jonas Veenhoven*, Royal Institute for Cultural Heritage
11:20-11:40	**Coffee break & posters***
Session 4: Sampling and minimally invasive methods Chair: Christopher Maines, National Gallery of Art, North American Coordinator of MaSC	

11:40-12:00	**Minimally invasive proteomics workflow to determine the species of origin of ivory objects** *Catherine Gilbert*, Univ. of Bordeaux, Proteome Platform, CBMN CNRS
12:000-12:20	**New GC-MS sampling approaches at the Rijksmuseum: current challenges and future perspectives** *Alba Alvarez-Martin,* Rijksmuseum
Session 5: Synthetic polymers Chair: Ken Sutherland, Art Institute of Chicago	
12:20-12:40	**Characterization of historical PMMA sheets used by two Portuguese artists by a comparative study of three analytical methods: EGA-MS, TD-GC/MS and Py-GC/MS** *Anna Micheluz,* Deutsches Museum
12:40-13:00	**PVC "Kunststoffschule" A valuable resource in the research of technical and material development of the PVC industry in Germany 1950-1970. Establishing a link between composition and end use** *Ester S.B. Ferreira,* Cologne Institute for Conservation Science
13:00-14:00	**Lunch & Posters***
Session 6: Technological and methodological advances Chair: Aleksandra Popowich, The Metropolitan Museum of Art	
14:00-14:20	**Rapid characterization of organic material sampled from surfaces of art works and other cultural heritage related objects by atmospheric solids analysis probe – high-resolution mass spectrometry** *Wim Genuit,* Cultural Heritage Agency of the Netherlands
14:20-14:40	**Top-Down MS: the next frontier in MS proteomic analysis of cultural heritage samples?** *Vaclav Krupicka,* Univ. of Bordeaux, Proteome Platform, CBMN CNRS
14:40-15:00	**Native Proteins Relevant to Cultural Heritage at Nanomolar and Picoliter Quantities using Triboelectric Nanogenerator and Ion Mobility-Mass Spectrometry** *Daniel D. Vallejo,* Georgia Institute of Technology

15:00-15:30	**Archaeometry, mass spectrometry and 3D technologies: how can they match?** *Remy Chapoulie* Archéosciences Bordeaux / Archéovision, Université Bordeaux Montaigne Chair: Florent Grélard, Univ. of Bordeaux, Proteome Platform, CBMN CNRS
15:30 – 15:50	**Coffee break & posters***
15:50 - 16:30	**Round Table** **Contribution of modern mass spectrometry to major questions of cultural heritage** *Klaas Jan van den Berg,* Cultural Heritage Agency of the Netherlands and U. Amsterdam *Christopher Maines,* National Gallery of Art *Ken Sutherland,* Art Institute of Chicago *Ester Ferreira,* Cologne Institute of Conservation Sciences *Julie Arslanoglu*, The Metropolitan Museum of Art Chair: Caroline Tokarski, Bordeaux Proteome, University of Bordeaux, CBMN CNRS
16:30 - 17:00	**MaSC Business Meeting chaired by MaSC committee and Closing Remarks**

* The presence of poster authors not necessarily required

MaSC 2022 Poster Presentations

Exploring possibilities of a combined analysis of binding medium and yellow lake pigments by Py-GC/MS
Charlotte Hoffmann, Ester S. B. Ferreira

The high- and the low-molecular weight components of ambers revealed by evolved gas analysis-mass spectrometry (EGA-MS) and double-shot pyrolysis-gas chromatography/mass spectrometry (DSPy-GC/MS)
Marco Mattonai, Lucia Andrei, Federica Nardella, Erika Ribechini

Application of laser-induced breakdown spectroscopy (LIBS) and pyrolysis gas chromatography mass spectrometry (Py-GC/MS) for identification of mahogany in 18th- and 19th-century furniture
Richard R. Hark, Randy Wilkinson, Chandra S. Throckmorton, Monica Grasty, Ivy Vuong, Patrick Chu, Anikó Bezur

Comparison of thermal hydrolysis and methylation versus in situ trimethylsilylation pyrolysis-gas chromatography-mass spectrometry applied to the analysis of Asian lacquer of a Burmese Buddha sculpture
Jonas Veenhoven, Delphine Mesmaeker, Steven Saverwyns, Nathalie Vandeperre, Henk van Keulen, Maarten van Bommel, Frederic Lynen

The derivatization of amino acids
Giulia Caroti, Alessandro Arrigo, Ilaria Bonaduce

Modifying analytical protocols of organic analyses with GC/MS and HPLC-DAD at the Rathgen-Forschungslabor to improve efficiency and sustainability
Elke Cwiertnia

Non-destructive identification of prehistoric adhesives by HS-GCxGC-TOFMS: preliminary study
Anika Lokker, Pierre-Hugues Stefanuto, Roné Oberholtzer, Dries Cnuts, Veerle Rots, Jean-François Focant

The use of Kendrick diagrams in high-resolution mass spectrometry
Wim Genuit

Blurred lines: issues distinguishing between alkyds and oils
Corina E. Rogge, Michael Schilling, Joy Mazurek

Wild Kingdom: an Excel-based tool for species identification using MALDI-ToF data
Rosie Grayburn, Catherine Matsen, Mike Szelewski

Preliminary results on the development of a workflow for simultaneous extraction of dyes and keratins in dyed wool textiles
Ilaria Serafini, Gwenaelle M. Kavich, Gabriele Favero, Roberta Curini, Caroline Solazzo

Revisiting the imperial past - comprehensive conservation and scientific investigation of historic lacquer coatings of Prince carriages from the collection of the Wagenburg in Vienna
Václav Pitthard, Sabine Stanek, Mathias Manzini, Judith Gagl and Martina Griesser

Cold paint on the stained glass windows from the Park Abbey, Leuven, Belgium.
Louise Decq, Marina Van Bos, Leen Wouters, Steven Saverwyns

SuPerStAr - Sustainable Preservation Strategies for Street Art: a new Italian project on the safeguard and preservation of street art
Silvia Pizzimenti, Dominique Scalarone, Laura Cartechini, Silvia Prati, Lucia Toniolo, Francesca Izzo, Cosima Damiana Calvano, Beatrice Campanella, Maria Sileo, Jacopo La Nasa, Ilaria Degano, Ilaria Bonaduce, Celia Duce, Valentina Brunella, Giulia Pellis, Bernadette Doherty, Giorgia Sciutto, Francesca Ramacciotti, Lucrezia Gatti, Rocco Mazzeo, Sara Goidanich, Laura Pagnin, Stefano Legnaioli, Manuela Scavone, Nicola Masini, Francesca Modugno

Invited Speakers

Julie Arslanoglu

Julie Arslanoglu joined the Department of Scientific Research at The Metropolitan Museum of Art in in 2006. She investigates the organic materials of paints, coatings, and adhesives, using mass-spectrometric and immunological techniques, with emphasis on natural and synthetic polymer identification and degradation. She introduced routine identification and localization antibody-based methods at the Metropolitan Museum to study artworks and in 2010 was awarded an NSF grant to study the impact of age, pigments and environment on protein-based paints and their identification by immunological methods and in 2021 was awarded an NEH grant applying a novel tripartite omics approach to the identification of chia oil use in art from New Spain. She is co-founder of Art Bio Matters (ABM; https://www.artbiomatters.org/), a one-of-a-kind conclave of scientists, conservators, art historians and curators who are interested in the analysis of biological materials found in cultural heritage. She is also co-founder of ARt and Cultural HEritage: Natural Organic Polymers by Mass Spectrometry (ARCHE; https://arche.cnrs.fr/) with Dr. Caroline Tokarski (University of Bordeaux), an International Laboratory (IRP) through the National Center for Scientific Research (CNRS) of France to study the material dimension of museum collections on a molecular level with mass spectrometry, especially for conservation and preservation of artworks, and to reconnect the relationship between history and natural products. She has a graduate degree in organic chemistry from the Pennsylvania State University and a postgraduate degree in paintings conservation from the Courtauld Institute of Art. She has held positions at the Getty Conservation Institute, the Victoria & Albert Museum, the University of Texas at San Antonio, and the National Institutes of Health.

Ilaria Bonaduce

Ilaria Bonaduce received her Ph.D. in Chemical Science in 2006 and is currently Associate Professor in Analytical Chemistry at the Department of Chemistry and Industrial Chemistry, University of Pisa. She teaches undergraduate and Masters level lecture courses, and associated practical courses, in Analytical Chemistry. Her research centres on understanding modifications undergone by organic materials in paint and archaeological polychrome artifacts as an effect of pre-treatments, ageing and interaction with other organic and inorganic materials, with particular focus on lipids and proteins. On these grounds she works on three main research lines: i) the development of analytical methodologies and procedures, based on mass spectrometry (GC-MS, Py-GC-MS, HPLC-MS and proteomics), as well as analytical models for data interpretation, aimed at the reliable identification of organic materials in art and archaeological samples; ii) understanding the physicochemical behaviour of paint layers to aid the further development of appropriate conservation and preservation strategies; iii) the characterisation of organic materials in paintings and archaeological polychrome artifacts, to reconstruct artistic techniques and technologies of the past. This research has resulted in over 80 peer reviewed scientific publications.

Matthew Collins

Matthew Collins is an international leading authority on palaeoproteomics and the 'Godfather' of bioarchaeology. He is a Fellow of the British Academy, the United Kingdom's national academy for the humanities and social sciences. He is a Niels Bohr Professor at the University of Copenhagen and the McDonald Chair of Palaeoproteomics, based at the McDonald Institute for Archaeological Research within the Department of Archaeology and Anthropology. He has also a Professor position at the University of Cambridge, U.K. He previously founded BioArCh, a biomolecular archaeology laboratory at the University of York collaboration between the departments of biology, chemistry and archaeology (BioArCh: Biology Archaeology, Chemistry). He helped develope ZooMS (Zooarchaeology by Mass Spectrometry) a way to rapidly identify bone and other collagen based materials using peptide mass fingerprinting. Matthew Collins recently received a prestigious ERC grant for research on old animal skins. He has coordinated Marie Curie Training Sites (Biogeochemistry and GeneTime), and acted as coordinator of a third (Palaeo) as well as a member of the management team of both LeCHE (reported in a Nature Extended News Article, August 2013) and EUROTAST, and directs the MSC European Joint Doctoral Site, ArchSci2020. Recently he is involved in ChemArch and PlaCE ITNs. He was a panel speaker at the MSCA organised Future of the Doctorate meeting in Riga. MC has supervised more than 30 PhD students, seven of whom have won tenured positions and six Marie Curie Fellows all of whom have enjoyed successful careers. His research focuses on the decay pathways of proteins, enabling him to both recover sequences in deep time, and to use patterns of protein decay as a geochronological tool. He pioneered the analysis of amino acid decay in closed systems, thermal age modelling of protein decay, proteins from ancient dental calculus and rapid species identification of bone, antler and parchment. Collins's research into ancient proteins has made significant contribution to the discipline of Archaeology as evidenced by his election to the Fellowship of the British Academy.

Jurgen Cox

Juergen Cox is full Professor and research group leader at Max Planck Institute of Biochemistry (Martinsried, Germany). He is a world leading authority on computational proteomics. He is best known as the creator of the MaxQuant suite of computational proteomics algorithms and software programs, which have revolutionized the field of quantitative, high resolution proteomics. Prof. Cox was originally trained as a theoretical physicist. He worked on computational modeling in statistical and particle field theory, for his PhD degree, which he obtained from the Massachusetts Institute of Technology (MIT) in Cambridge, US, in 2001. His work was awarded by the Gilbert S. Omenn Computational Proteomics Award from US HUPO in 2019 and Mass Spectrometry in the Life Sciences Award from the German Society for Mass Spectrometry in 2013. He is the coordinator of the highly attended MaxQuant summer school. Dr. Cox is the author of numerous peer reviewed publications in the field of data analysis in mass spectrometry and quantitative proteomics.

Victor Etgens

Victor Etgens was appointed head of Research of the Centre de Recherche et de Restauration des Musées de France (C2RMF) in 2022. Before joining the C2RMF, he was director of IPANEMA (European Photonic Institute for Non-Destructive Analysis of Ancient Materials), a research platform dedicated to ancient materials. Professor of physics at the University of Versailles Saint-Quentin-en-Yvelines (University of Paris-Saclay), he has acquired extensive experience in the development and study of materials. During his scientific career, he has carried out various integrated and varied experiments as a researcher (as CNRS research director at the INSP-Institut des NanoSciences de Paris, with more than 150 international publications), as an experimenter (with large instruments, in particular synchrotron SOLEIL and ESRF), team leader (INSP team dedicated to the development and study of semiconductor materials, metals and oxides).

Rémy Chapoulie

Rémy Chapoulie graduated in applied physics at the university of Bordeaux (Sciences and Technology). He took his PhD in 1988 at the university of Bordeaux Montaigne (Human and Social Sciences) and his Habilitation at the university of Bordeaux in 2004 in sciences and technology. Full Professor of Physics in archaeometry at the University of Bordeaux Montaigne, he was a member of the scientific commission at the CNRS in Social and Human Sciences, from 2000 to 2004 and was a member of one of the commissions of research for the Aquitaine region from 2005 to 2010. His main research activities currently concern the multiphysical study and archaeometry of ceramics and pigments from pre-Columbian Peru, the taphonomy of walls in decorated prehistorical caves in Dordogne (France), and also the archaeometric study of different ancient materials: antique marble, Roman amphorae, lithic materials, Japanese prints, French faïence. One of his major and present interests lies in the development of mobile analytical systems for in situ measurements.

His scientific and administrative responsibilities are very closely linked to the sciences dedicated to the study of the cultural heritage. Between 2016-2021 he was head of the laboratory IRAMAT-CRP2A (Institut de recherche sur les archéomatériaux-Centre de recherche en physique appliquée à l'archéologie) UMR 5060 CNRS at Bordeaux, and a member of the scientific advisory board of the LabEx "Laboratoire des Sciences Archéologiques de Bordeaux". This cluster gathered three research teams from Bordeaux with competences in archaeology, archaeometry, history, physical anthropology and prehistory. Rémy Chapoulie became, between 2018-2021, the head of the laboratory Archeovision (UMS 3657 CNRS), involved in 3D technologies: digitizing and surveying, modeling and rendering, promoting and data preservation. Since 2022, he is the head of the archeovision platform. He is member of UMR 6034 Archéosciences Bordeaux and board member of the Department of Archaeological Sciences of the University of Bordeaux.

Laurent Védrine

Laurent Vedrine was appointed head of the Musée d'Aquitaine in Bordeaux in 2017. For the past nine years, he has been director of the Marseille History Museum, of which he led the complete renovation in 2013. After studying history and archeology in Bordeaux, Laurent Vedrine completed his military service as an archaeologist in the Army Historical Service in the mid-1990s. From 1996 to 2004, he directed the ecomuseum de Margeride and the Haute-Auvergne museum. He was then project manager for the heritage development of the territory of the Basque Country and Béarn until 2008, when he won the heritage curator competition. It is precisely at the Aquitaine Museum, which he is preparing to take over as director, that Laurent Vedrine is doing his specialty internship, as part of his training at the National Heritage Institute (INP). For nine years, he has been at the head of the Marseille City History Museum for which he undertook a vast renovation operation which led to its reopening in 2013.

Oral Presentation Abstracts

Analysis of binders and additives in Talens ETA and Rembrandt tempera paints

Inez Dorothé van der Werf , Saskia Smulders , Klaas Jan van den Berg

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ETA is a casein/oil emulsion paint that was produced by Royal Talens in Apeldoorn (The Netherlands) from the 1920ies till the 1970ies. It was developed as a rain-water resistant decoration paint, but it was also employed by artists because of its versatility and matt finish, for instance in mural paintings. Alternatively, tempera paints of the Rembrandt series were put on the market by Talens, specifically meant for artists. New information on the formulations of these paints has recently become available from the examination of the Talens archives. [1]

In this study various historic ETA and tempera paints from Royal Talens were analysed. Thermally assisted hydrolysis and methylation (THM) gas chromatography-mass spectrometry (GC/MS) in combination with ultrafast thermal desorption (UTD) was used to get an overall picture of their formulation, including organic additives and pigments, while identification of casein could be better achieved with pyrolysis (Py) – GC/MS at lower temperatures (T_{Py} 350°C). [2] Proteomics with nanoLC-Orbitrap was applied for confirmation.

The results were used for comparison with the information from the Royal Talens archives and represented a useful database for the investigation of some case studies.

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A mass spectrometric study of the oil paint curing using methyl linoleate as a model binder

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The structure of the polymeric fraction in an oil painting is strongly connected to the stability of the paint layers over time[1-3], but its molecular characterisation is extremely difficult given the complex composition of oil-based polymers. We implemented a methodological approach for the systematic mass spectrometric investigation of the molecular features of the products of oxidative degradation and cross-linking of oil paint layers upon curing. The approach was based on the use of methyl linoleate as a simplified model of an oil paint binder. Gas-chromatography coupled with mass spectrometry, solid-phase microextraction gas chromatography-mass spectrometry, flow injection electrospray mass spectrometry and evolved gas analysis mass spectrometry, were used to analyse the evolution of compounds produced over seven months of natural ageing, from the volatile products to the cross-linked fractions. The aim was to improve our molecular understanding of the curing process of oil paints and to investigate the balance between oxidative degradation and cross-linking when specific binder-pigment combinations are in place. Model paintings were prepared using lead white and ultramarine blue as pigments, as these pigments are known to produce paint layers with different stability over time. The use of methyl linoleate as a model oil binder simplified the mass spectral features of the organic fraction, enabling the detection of products of oxidation and cross-linking with a new high level of molecular detail. Data clearly showed that crucial differences between paints containing the two pigments established with time, which are mostly related to the cross-linked fraction.

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A retrospective and critical review of binding media analysis in wall paintings

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The conservation of wall paintings presents many difficult analytical challenges. Gas Chromatography Mass Spectrometry (GC/MS) often plays a critical role in studying the artists' materials and painting technique. In the past 20 years, various paint samples were tested for organic binding media at the GCI including, King Tutankhamun's Tomb, Herculaneum, Bamiyan murals in Afghanistan, and the Mogao Caves in China, as well as many others. The aim of this presentation is to present a critical overview of the challenges and lessons learned from analyzing a variety of mural paintings.

The instrument primarily used to identify the organic components was GC/MS and it can identify a wide variety of organic materials such as waxes, oils, plant resins, sugars, organic acids, and proteins and is commonly used in the field of conservation science. Plant gum and protein analysis can be especially difficult because samples are matched with known materials in a database. For example, an animal glue can be degraded from biological, chemical, and environmental causes. It is well known that certain pigments cause degradation of proteins in artificial aging experiments, but the chemical reactions that alter the amino acid profiles in situ are not well understood.

To add to these challenges, wall paintings are often consolidated with organic materials and samples sizes are often less than 1 mg. If an original binder was present in the samples, it may be masked by the treatment materials or the sample size was too small, thus the organic material may have been below the detection limit. The wall painting samples presented in this retrospective highlight the causes of interpretation error, including biodeterioration, changes in the chemical profile of the organic binders due to burial for thousands of years, the artist's preparation technique, and exposure to heat and humidity, as well as contamination due to consolidation or biocide applications.

Identifying emulsion-cured leather in museum collections using proteomics

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Multiple cultures around the globe – from the Tungus people in Asia to the Zulus in Africa, to Native Americans, use the ancient technique of emulsion (organ) curing for leather. Touching every habited continent, emulsion curing demonstrates a cross-cultural desire for a durable, flexible, water-resistant material. This efficient technique exploits the natural emulsifying agents (lipids), generally from brain, to preserve animal skin against putrefaction. Despite the abundance of emulsion-cured leather worldwide, throughout history, and in museums, there is no scientific method to accurately identify or characterize this complex material. Understanding the mechanisms of deterioration and effects of curing on leather proteins at the molecular level will improve the conservation, storage, and display of these objects.

An extraction protocol and liquid chromatography Orbitrap tandem mass spectrometry (LC-MS/MS) method was developed to distinguish trace amounts of brain tissue-specific proteins from the overwhelming collagen background of the leather. This was done using minimal sample sizes compatible with irreplaceable cultural heritage collections. This work will describe the challenges of trace protein analysis and the steps for identifying tissue-specific protein markers. The method was used to characterize a 19th century Taiwanese painted leather wall hanging from The Metropolitan Museum of Art (accession #09.3), making it the first 'unknown' object to be definitively identified as brain-cured leather.

Discrimination of plant gum polysaccharides using MALDI-MS: developments, limitations, and exploration of a complementary proteomics approach.

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Polysaccharide-based plant exudates have been used as paint binders and adhesives since Antiquity. A method for their discrimination using partial enzymatic digestion and analysis of the released oligosaccharides by matrix assisted laser desorption ionization-mass spectrometry (MALDI-MS) has shown promise in particular for differentiation of Acacia species, which cannot readily be achieved with other techniques. In this paper we report on new developments using this analytical approach, including expansion of the MALDI-MS database of reference gums and the use of statistical analysis for their discrimination. While the Acacia gums show groupings that can be related to their taxonomy, examples from Astragalus and Prunus species have proven more perplexing, with spectral differences apparently determined by additional (as yet unidentified) factors. Challenges are compounded when studying polysaccharide binders in painted works of art from less studied locations and cultures, for which research becomes a "treasure hunt" for relevant reference materials. Examples presented include a 200-30 BCE painted cartonnage from ancient Egypt, a 300-750 A.D. wooden box fragment from Teotihuacan (Mexico), and a 10th century painting from Thanjavur (India). To overcome the limitations encountered with MALDI-MS, a complementary approach based on mass spectrometry-based proteomics is being explored to discriminate plant gums at the species level by their proteinaceous fraction, with reference to constantly expanding publicly available databases for plant proteins.

Bloody Proteins – An ongoing quest to identify 2000-year-old proteinaceous residues on ceramics from the Late Iron Age and Roman Period.

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Archaeologist studying ceramics from the Netherlands and Belgium frequently discover a specific kind of red-brown residue on vessels from the Late Iron Age and Roman period. Many archaeologists have proposed these residues consist of blood.
Combined analysis using Fourier Transform IR-spectroscopy (ATR-FTIR) and Direct Temperature-resolved Mass Spectrometry (DTMS) has regularly shown the presence of a preserved protein fraction. However the identification of the exact protein origin is another matter. In this paper we present the results of our ongoing quest for the bloody proteins. A study using pyrolyse-gas chromatography-mass spectrometry (THM-Py-GCMS) suggests the presence of an albumine, possibly originating from blood. However, a small proteomics pilot-study using nano LC-MS/MS analysis determined that the proteins were seriously fragmented and only one significant identification of a goat/sheep milk residue could be established. Can newer proteomic techniques or better post-analysis data processing help?

Identification and differentiation of essential oils used as solvents in paints and lacquer works

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Analyses of 20th century tube paints of the paint manufacturer Fritz Behrendt led to the identification of so far rather uncommon solvents, copaiba oils. However, other seemingly specific markers for additional essential oils were found as well, sometimes certainly added as solvents, but sometimes probably only present as mono- or sesquiterpenoid components of resins added to the products. Since some of the essential oils were also used for centuries, analytical findings of Baroque lacquers are included in the study as well.

The paper discusses the questions, which essential oil solvents have been used in the past, and whether they can be found and distinguished in tube paints or lacquer works today, or not. It turns out that many mono- or sesquiterpenoid components are contained in several or many products, but examples from analyzed tube paints and lacquers on Baroque objects indicate, that a distinction by the relative ratios are possible nevertheless, which is supported by the evaluation of written sources.

Blended Thitsiol/Urushiol Asian laquers in cultural heritage: new advances in understanding their curing, ageing, and photo-aging processes by THM-GC/MS

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A blend of Thitsiol with Urushiol Asian lacquers was a mixture often used for lacquerware exported to Europe from Japan in the XVII century [1]; it is also used to produce new lacquer by the coating industry [2]. Despite the importance of East Asian lacquer's use in the west, there is a lack of scientific studies for understanding their curing, aging, and photo-ageing processes.
In this work, blend-models of Thitsiol with Urushiol in different percentage concentrations were prepared and for the first time their curing process, as well as ageing, and photo-ageing processes, were studied by analytical pyrolysis in thermally assisted hydrolysis and methylation mode, coupled with gas chromatography and mass spectrometry (THM-GC/MS). For the curing, the specimens were dried at 20 °C and 80 % rH% and then aged in dark-environmental conditions for two years. The light-aging was then carried out for a maximum of one-month in a daylight chamber, which uses radiation with wavelengths from 320 nm.
The data obtained within this research highlights unknown oxidation markers for the Thitsiol-Urushiol blends, and their mechanism of reactions are proposed according to the THM-GC/MS results obtained. These results are of high interest for professionals dealing with Asian lacquers.

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Differentiation between primary and secondary pyrolysis reactions to improve the identification of Asian lacquer macromolecules

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The analysis of polymerised Asian lacquers is challenging due to the complex cross-linked matrices. The carbon to carbon (C-C) and carbon to oxygen to carbon (C-O-C) nature of the cross-links prohibits wet chemical pre-treatments to break down the lacquer polymer to its monomers, making analysis using state of the art NMR, or chromatographic techniques coupled to mass spectrometry difficult.
Pyrolysis hyphenated with gas chromatography-mass spectrometry (Py-GC-MS) is suitable for the analysis of Asian lacquers and relies on the introduction of solid samples and online thermal decomposition of the lacquer polymer into small molecules amenable to gas chromatography, followed by mass spectrometry of individually separated compounds. Differentiation between primary and secondary pyrolysis mechanisms, is required to be able to identify the intermolecular covalent bonds, and to characterise the macromolecular architecture.
In this contribution we show that the stepwise application of progressive flash pyrolysis temperatures, ranging between 300-700 °C, is useful to differentiate between primary pyrolysis and secondary dehydration products. This optimisation has shown to improve the characterisation of the polymeric compositions of *thitsiol*, *laccol* and *urushiol* based polymers and to distinguish between the different, intermolecular covalent bonds.
The research was conducted within the framework of PHySICAL: the Profound study of Hydrous and Solvent Interactions in Cleaning Asian Lacquer, dedicated to investigating the molecular effects of cleaning solvents or aqueous solutions on light degraded and thus extremely sensitive Asian lacquer surfaces. Identification of the, aged, lacquer polymers prior treatment, is a foundation on which to base cleaning methods and to study the molecular modifications resulting from the cleaning interventions, found in Asian lacquers.

Minimally invasive proteomics workflow to determine the species of origin of ivory objects

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Ivory is a highly sought-after material in many cultures, both for its medicinal properties and its use as a structural medium. Ivory is essentially an elongated tooth, and like other mineralised tissues, the structural protein collagen composes 90% of the organic material in ivory. Structural proteins such as collagens are well preserved in mineralised tissues such as bones, teeth, and ivory, and can remain a source of species specific information long after the degradation of DNA. For this reason, the analysis of ancient proteins is often used to enable species identification of morphologically unidentifiable objects. In this study, we perform species identification on ivory objects from the collections of the Metropolitan Museum of Art, using optimised proteomics methods adapted to trace level detection.

In order to perform successful minimally invasive analysis by rubbing with microabrasive films, it is necessary to miniaturise and simplify the sample preparation procedure. This can be done, for example, by integrating different chemical treatments into a single or few steps, or using miniaturized analytical workflows, both of which have already proven to improve recovery in the most challenging ancient samples. In addition to this, we have used data acquisition methods adapted to low sample amounts (Orbitrap Fusion Lumos, Thermo Fisher Scientific) and adapted our data processing steps to target for the high heterogeneity of the collagen proteins.

In this presentation we will demonstrate the optimisation we have performed in order to robustly achieve proteomic analysis using minimal amounts of starting material, as well as introducing new methods that we are developing to further increase the sequence coverage of collagens. These new methods are essential in order to establish minimally invasive sampling with microabrasive films in the cultural heritage analysis workflow and to offer a method of species identification where visual assessment is not possible.

In many cases we were able to identify several hundred peptides attributable to both collagen alpha1-(I) and alpha2-(I), from objects as old as 3200 years old; e.g. 957 AAs COL1A1 fragment identified by 92% sequence coverage and 343 peptides. This means that we were also able to perform confident species identification based on several unique peptides that were confirmed with y and b fragment ions covering the sequences.
In addition to this, since many of the objects that we are analysing in this study originate from Egypt, the objects are likely to originate from either elephant or hippopotamus ivory. A challenge here is that the proteomes of these species are not available on public databases, and so we must rely on protein sequences that are either incomplete, sequences that have been calculated from data sequences, and on de novo sequencing. While de novo sequencing is commonly required in the field of palaeoproteomics, it has, as of yet, rarely been applied to cultural heritage objects to identify the species of origin.

New GC-MS sampling approaches at the Rijksmuseum: current challenges and future perspectives

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Gas chromatography-mass spectrometry (GC-MS) analysis of art samples has been traditionally associated with a destructive analytical technique. Thanks to improvements to the instrumentation and sampling methods, more sensitive, less-invasive and non-destructive analyses are now possible.

This presentation will focus on the implementation of a very sensitive and semi-automated sampling approach: high-capacity sorptive extraction technique (HiSorb), coupled with gas chromatography-mass spectrometry (GC-MS). This new methodology integrates sampling, extraction, concentration and sample introduction to an analytical instrument into one solvent-free step. In this context, HiSorb probes are great candidates as screening tools when it is not possible to take a sample from a historical artifact.

For the first time, two recently commercially available HiSorb probes were tested, and their performance was compared with the complementary solid phase microextraction fibers. Additionally, different GC-MS methods have been optimized in order to increase the sensitivity of detection of target compounds. HiSorb has shown great performance for the identification of volatile compounds accumulated in enclosed areas at the Rijksmuseum. Another application of this method includes the analyses of the molecular profile emitted from the back of a canvas for monitoring purposes like the condition of paintings.

The final addition is to automated part of the workflow to replace long-time consuming passive sampling strategies while working more effectively and reducing instrumental variables.

Characterization of historical PMMA sheets used by two Portuguese artists by a comparative study of three analytical methods: EGA-MS, TD-GC/MS and Py-GC/MS

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Remnants of acrylic sheets from the 1960s used by the two well-known Portuguese artists Ângelo de Sousa and Lourdes Castro made by Plásticos do Sado (P), Röhm & Haas (DE, Plexiglas®) and Altulor (FR, Altuglas™), respectively, were analysed. The aim was to understand their chemical composition, especially regarding possible additives and associated ageing effects, but also to evaluate and improve our analytical methodology. Therefore, three different on-line sample preparation techniques were applied: evolved gas/ mass spectrometry (EGA-MS), thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS) and single-shot pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS).

EGA-MS provided thermal degradation profiles of the samples and differences regarding the chemical composition. With the analysis of the volatile fractions by TD-GC/MS, a comprehensive list of additives including initiator, polymerisation reagents, plasticizers, UV-stabilizers and lubricants was built. Pyrolysates obtained with Py-GC/MS showed further variations between the samples, probably due to the different production processes of the companies. Archival research aid interpretation of the analytical results.

The results from the analysed PMMA samples confirmed that the two Portuguese artists worked with acrylic sheets of various compositions. Their intrinsic properties and past use help to interpret their ageing behaviour and plan a preservation strategy for these artworks.

PVC "Kunststoffschule" A valuable resource in the research of technical and material development of the PVC industry in Germany 1950-1970. Establishing a link between composition and end use.

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In the 1950s, the German plastics industry working group in Frankfurt a.M. initiated the development of the *Kunststoffschule* (plastics school), a publication in several volumes, each dedicated to one type of plastic. As far we know today five editions were produced between 1950s and 1980s, the first of which published in 1955. An exemplar of the 1957 edition belonging to a private collection was made available for study. In the current presentation particular focus is given to volume VI dedicated to PVC (polyvinylchloride). The volume opens to reveal on the left hand side a schematic of the synthesis of PVC from the synthesis of the monomer, the polymerisation of PVC, the introduction of additives and the different moulding processes to produce a wide range of products. On the right hand side material samples for each of the steps are provided: from PVC powder to pigmented granulate to final products of hard and plasticized PVC such as different tubing, flooring, and decorative foil types. These were characterised with Py-GCMS, SEM-EDX, Raman and FTIR for the identification of polymer and the additives, showing significant composition variation depending on product type, including different plasticizers types, stabilizers, fillers etc. Since the provenience and date of the volumes are known, the material characterisation of polymer and additive profile provides a unique insight into the material development of the plastics industry in Germany and can assist in developing targeted preservation strategies.

Rapid characterization of organic material sampled from surfaces of art works and other cultural heritage related objects by atmospheric solids analysis probe – high-resolution mass spectrometry (ASAP-HRMS)

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An atmospheric solids analysis probe (ASAP) is a novel analytical application that has been applied in combination with high resolution mass spectrometry (HRMS) to characterize a large number of reference materials which are of interest in studies of cultural heritage objects and their conservation materials.

The reference materials cover a broad range of different organic compound classes: organic colourants, natural resins, glycerolipids, waxes, polysaccharides and synthetic polymers.
The potential of Kendrick diagrams has been explored for a rapid visual distinction and identification of various classes of materials.

ASAP sample technique will be introduced and compared with other direct mass spectrometric sampling techniques. Representative mass spectra and the Kendrick diagrams will be presented from reference materials and original and conservation materials in Old Master paintings.

Combined with rapid microsampling techniques employing small , the novel technique provides rapid characterization of minute samples of organic material. ASAP-HRMS will be demonstrated to be a powerful addition to the palette of techniques for rapid analysis of materials from cultural objects, such as DTMS (or DEMS) and ESI-MS.

Top-Down MS: the next frontier in MS proteomic analysis of cultural heritage samples?

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The study of proteins in Cultural Heritage is key to deciphering ancient materials in order to reveal new historical insights or to aid in the preservation of valuable objects. Bottom up proteomics is becoming a mainstream method to identify the primary sequence of ancient protein, as well as their biological origins and chemical modifications. While bottom-up proteomics is shown to be a highly informative and sensitive method, it has some limitations, in particular in the identification of protein breakdown or truncations and the loss of combinatorial posttranslational modifications (PTMs) patterns. Here we describe a top down proteomic method to analyze ancient proteins from limited paint samples of a few micrograms, well below levels considered for robust top down experiments. This presentation will describe our analytical workflow showing how top down proteomics represents a valuable support to the study of ancient proteins revealing sample heterogeneity through the "bird's eye" view offered.

Tempera paint models were prepared using egg white and yolk mixed with the pigment lead white ($(PbCO_3)_2 \cdot Pb(OH)_2$) and spread on a glass slide. Proteins were extracted using an adapted filter aided protocol. The resulting protein extracts were analyzed using nanoLC (C4) coupled to an Orbitrap Fusion Eclipse using a combination of fragmentation modes (CAD, EThcD, UVPD). Raw data was analyzed using several available search engines including TopPic, MSPathFinderT, and ProSight.

A particular focus was given in this work to egg proteins. Utilizing lysozyme (14 kDa) and ovalbumin (45 kDa without PTMs) protein standards first, and then from egg and paint models, the intact spectra at femtomolar concentrations were acquired. Both nanoLC separation and MS settings for MS and MS/MS were optimized for an improved separation and identification of proteins and their breakdown products. The combination of CAD, EThcD and UVPD fragmentation modes allowed to achieve a protein coverage over 50% for lysozyme starting from trace amounts of model paint sample. The successful deconvolution and assignment of the spectra allowed the identification of various chemical modifications and isoforms. For example, highly heterogeneous patterns such as those observed for ovalbumin due to its extensive N-linked glycosylation (N292) and phosphorylation were acquired allowing a confident protein identification despite several overlapping patterns.

The presentation will describe the full method from sample preparation to protein analysis and data processing. It will discuss the challenges of working on ancient proteins considering the additional protein heterogeneity provided by deamination and protein *in situ* cleavages. The first applications to historic samples will be used to illustrate how a top down approach can increase our knowledge of the sample and allow access to proteins in their original state.

Native Proteins Relevant to Cultural Heritage at Nanomolar and Picoliter Quantities using Triboelectric Nanogenerator and Ion Mobility-Mass Spectrometry

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Native mass spectrometry (MS) has found widespread success in measuring native-like protein structures in the gas-phase and, when combined with ion mobility (IM), capable of measuring their collision cross sections (CCS) and stabilities. These methods are well validated, but often rely on samples that are abundantly available through repeated recombinant expression. For ultra-precious and irreplaceable samples from paintings, protein content can be far below the micromolar and microliter levels required for robust protein experiments. Triboelectric nanogenerators (TENG) and IM-MS are capable of measuring protein size and stability rapidly from ultra-small sample quantities. Here, TENG-IM-MS is used to characterize standard proteins and proteins relevant to paintings, showing native protein structures can be obtained even at nanomolar and picoliter quantities.

Eight proteins ranging between 5-800 kilodaltons were analysed under native conditions. Human serum albumin (HSA) was used as a positive control, and three proteins relevant to cultural heritage were measured: ovalbumin (OVA), ovotransferrin (OVT), and lysozyme from egg white. Direct infusion electrospray ionization was performed using a TENG or DC nanospray ion sources and CCS measurements were calculated for both methods and compared. We measured CCS values of three standard proteins using either TENG or a DC power supply to power the nanoelectrospray ionization process. We found that Bovine Serum Albumin (BSA), Cytochrome C (CytC), and Alcohol Dehydrogenase (ADH) generated native-like charge states and presented native CCS measurements under both conditions. For BSA, ConA, and ADH under DC conditions CCS measurements were 4499±50, 5980±29, and 7533±35 $Å^2$ and under TENG conditions were 4523±55, 5957±26, and 7526±28 $Å^2$, respectively. These measurements are within 0.25% of each other and all within 1.5% of the Bush protein CCS database.

Confirming TENG could sufficiently generate native-like structures, we obtained CCS measurements of 4575±6.5 and 3366±1.6 $Å^2$ for HSA and OVA, respectively. We evaluate that these experimental protein CCS are within 3.34% and 0.27% of their theoretical CCS values as derived from the CCS database, respectively. Additionally, native charge states for OVA were measured at several concentrations ranging from 10 µM to 100 nM. At low concentrations, 500 nm and 100nm, for OVA we measured the RMSD of the CCS to be 3.4% and 5.9% vs. the 10 µM samples, respectively. The pulsed nature of TENG required less sample than the average nESI experiment with each pulse using approximately 20 pL of sample. For low concentrations, two-minute acquisitions (~24 pulses or ~10 nL) were required for adequate signal intensity, but for highly concentrated samples as little as two pulses were needed requiring only 800pL of sample. Our presentation will detail our most recent data and analysis for proteins at nanomolar and picoliter quantities.

Poster Abstracts

Exploring possibilities of a combined analysis of binding medium and yellow lake pigments by Py-GC/MS

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Although the use of yellow lake pigments, based on flavonoid-containing plant extracts, is often reported in historical written sources, they are seldom clearly identified in paint samples. This can be related to their known poor stability and high sensitivity to light induced degradation for example. Analyses targeting the identification of yellow dyes and their degradation products are possible using well-established ultra-high performance liquid chromatography (UHPLC) with photo diode array (PDA) or mass spectrometric (MS) detection. However, in order to obtain information on the binding medium in addition to data on lake pigments, further sample material is required. Identification of textile dyestuff with GC/MS was first proposed by Poulin (2018) using TMTFTH as derivatising agent. The work presented here further explores the possibilities of a combined analysis of binding medium and lake pigments in paintings in a single microsample, using pyrolysis gas chromatography (Py-TMAH-GC/MS). Optimal pyrolysis temperature was determined by evolved gas (EGA) MS analysis of pure dyestuffs and artificially aged paint samples containing lake pigments in linseed oil. Subsequent Py-TMAH-GC/MS measurements on reference samples were evaluated with the AMDIS deconvolution software. Results obtained show that it is possible to simultaneously detect fragments of dyestuff and binding medium. Possibilities and limitations of analysing oil paint samples containing lake pigments with Py-TMAH- GC/MS will be discussed, thus contributing to the further development of such analytical applications.

Reference

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The high- and the low-molecular weight components of ambers revealed by evolved gas analysis-mass spectrometry (EGA-MS) and double-shot pyrolysis-gas chromatography/mass spectrometry (DSPy-GC/MS)

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EGA-MS and DSPy-GC/MS were used to study the thermal behaviour and the low and high-molecular weight fractions of a set of ambers with different geographical origins. EGA-MS was carried out to obtain information on the thermochemistry of ambers and to establish the optimal temperatures for Py-GC/MS experiments. Double-shot experiments were then performed by first heating the sample at 280 °C to desorb low-molecular weight compounds, which were cryo-focused using a liquid nitrogen trap before GC-MS analysis. The high-molecular weight fraction of the sample was pyrolyzed in a second run at 480 °C. Hexamethyldisilazane (HMDS) was used as silylating agent in both the steps.
Both EGA-MS and DSPy-GC/MS data were processed with principal component analysis, using the average mass spectra and the percentage peak areas of identified desorption/pyrolysis products, respectively, to highlight similarities or differences among the various ambers. Both the techniques showed promising potential to discriminate ambers with different botanical and geological origins. The use of EGA-MS is highly recommended, as it can provide fundamental insights into the thermal behaviour of amber and guide the choice of optimal pyrolysis temperatures. DSPy-GC/MS also offers a significant advantage, minimizing co-elution of peaks and separating the desorption and pyrolysis steps.

Application of laser-induced breakdown spectroscopy (LIBS) and pyrolysis gas chromatography mass spectrometry (Py-GC/MS) for identification of mahogany in 18th- and 19th-century furniture

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Laser-induced breakdown spectroscopy (LIBS), pyrolysis gas chromatography-mass spectrometry (Py-GC/MS), and machine learning techniques were used to evaluate the level of discrimination between mahogany and wood species with a very similar appearance and amongst the three species of "true" mahogany (*Swietenia*) for the purpose of classification of materials found in the Yale University Art Gallery (YUAG) furniture collection. Mahogany was a valuable commodity sourced from the Caribbean in the 18th and early 19th centuries that was used in high-end furniture made in Great Britain and North America. Wood identification is important to appreciate the connections between the raw material sources and furniture manufacturing centers, to understand the choices individual craftsmen made in constructing these objects, and to aid object conservators when treating pieces. Identification of wood in cultural heritage objects is typically done by a wood anatomist using a variety of physical characteristics coupled with a visual examination of anatomical features found in microscopic images of thin sections. This poster will describe the very promising results of a chemotaxonomic approach that were obtained after analysis of a wood library contained hundreds of pieces of mahogany and look-alike woods as well as samples removed from furniture.

Comparison of thermal hydrolysis and methylation versus in situ trimethylsilylation pyrolysis-gas chromatography-mass spectrometry applied to the analysis of Asian lacquer of a Burmese Buddha sculpture

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Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) is a powerful technique for the analysis of solid samples of Asian lacquer polymers. Pyrolysis involves a thermal fragmentation step leading to many polar pyrolysates bearing hydroxylic and carboxylic groups, which are less suitable for gas chromatographic analysis, resulting in peak broadening, bad peak shapes, and memory effects.
Derivatisation is required to substitute the hydroxylic and carboxylic groups with less polar moieties, which increases the volatility of the pyrolysates. The most common derivatisation procedures in use are alkylation and trimethylsilylation (TMS).
Alkylation can be performed using thermally assisted hydrolysis and methylation (THM) using tetramethylammonium hydroxide (TMAH). Hexamethyldisilazane (HMDS) is commonly used as a TMS reagent, where derivatisation is performed offline or in situ.
In this contribution we show that, even though using TMAH proved more robust, it showed to be detrimental for compounds comprising multiple unsaturations. The selectivity and sensitivity for those molecules was enhanced when HMDS was used. The comparison between TMAH and HMDS has already been described previously [1], on aged Asian lacquer mock-up samples, but is now for the first time applied on a Burmese Buddha sculpture decorated with Asian lacquer. The layer structure on the wooden substrate of the sculpture comprised foundation layers and lacquer layers, which were sampled separately. Each layer sampled was analysed in separate series using TMAH and HMDS to allow comparison of both derivatisation methods.

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The derivatization of amino acids

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The analysis of proteins is fundamental in several research fields, such as food, health, environment and cultural heritage. In paintings, animal proteins such as egg, casein and glue were frequently used as binders for pigments.[1]
Gas chromatography coupled with mass spectrometry is particularly useful for the detection of proteinaceous materials in paint media thanks to its high sensitivity and specificity. GC-MS requires low molecular weight molecules and so the macromolecular nature of proteinaceous materials means that pretreatments of the sample are required. In particular a hydrolysis step is necessary in order to free amino acids.[2]
Amino acids can't be directly analyzed by GC-MS, as they are not volatile, they have low thermal stability and they may interact with the column stationary phase. A derivatization step is thus required.
This work presents the study of two different types of derivatization: acylation with ethyl chloroformate (ECF) and silylation with N-tert-butyldimethylsilyl-N-methyl-trifluoroacetamide (MTBSTFA) with 1% of tert-butyldimethylchlorosilane (TMCS).
Acylation is an instantaneous reaction, which proceeds without heating in aqueous media. Amino acids are treated with ethyl chloroformates in the presence of ethanol and pyridine, and the ethyl chloroformate derivatives are subsequently extracted from the reaction mixtures with organic solvents.[3] The major advantage of this protocol is its speed and the great stability of the derivatized amino acids, enabling the automatization of the analysis step.
Silylation requires more time than the acylation (30-40 min) and it must be conducted in anhydrous solvent. Amino acids are mixed with a silyl agent and heating of the reaction mixture is required (60°C).[4] The main drawbacks of the silylation are the low stability of the derivatives in the presence of environmental humidity, and the sensitivity towards inorganic salts, making purification steps prior derivatization necessary. On the other hand, the sensitivity of MS analysis is greater when silyl derivatives are concerned in comparison to ethyl chloroformate derivatives. This work discusses the optimisation of the conditions of reaction, and presents a systematic comparison and critical evaluation of the advantages and disadvantages the two analytical approaches.

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Modifying analytical protocols of organic analyses with GC/MS and HPLC-DAD at the Rathgen-Forschungslabor to improve efficiency and sustainability

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Standard organic analyses using GC/MS and HPLC-DAD at the Rathgen-Forschungslabor have been evaluated in regard to aid sustainability goals of the Stiftung Preußischer Kulturbesitz and improve efficiency to answer analysis requests by conservators and curators.

After presenting some general sustainability ideas for laboratories (energy, solvents, disposables), changes to the standard operation procedure of the dye analysis with HPLC-DAD as well as gum analysis with GC/MS will be discussed. Changes include for example kind and amount of solvents and reagents, analysis time, reaction temperatures and vials.

Identifying and translating LODs of components to the conservator into measurable sample sizes of real samples to decrease these but also obtain enough material where the scientists are not able to take samples themselves (to reduce traveling) was another part in that study, improving transparency of the laboratory work and collaboration with conservators.

The present study aims to foster discussions around efficiency and sustainability in the analysis of cultural heritage samples and provides ideas and practical solutions to start implementing sustainable strategies.

Non-destructive identification of prehistoric adhesives by HS-GCxGC-TOFMS: preliminary study

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Identification of prehistoric adhesives on stone tools is valuable as it might reveal something about tool use. Currently, prehistoric glues are chemically analysed by gas chromatography coupled to mass spectrometer (GC-MS) which requires extraction and derivatization of the residues[1]. This is a major drawback as it is destructive for the glue and often, the required amount is not present. Moreover, the adhesives cover a wide range of materials (e.g., resin, animal glue, gum). Therefore, sensitive, universal, and non-destructive identification methods are needed. Headspace solid phase micro-extraction (HS-SPME) in combination with GC-MS and with comprehensive GC-time-of-flight mass spectrometer (HS-SPME-GCxGC-TOFMS) has been proposed[2,3]. But the sensitivity remains a problem.
In this study dynamic headspace (DHS)-GCxGC-TOFMS is tested on several adhesives and compared with HS-SPME-GCxGC-TOFMS. The DHS method is optimized and validated via design of experiment on pine resin and hide glue.

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The use of Kendrick diagrams in high-resolution mass spectrometry

Wim Genuit

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In this tutorial lecture, the concept of the Kendrick diagram will be presented as a useful tool for the visualization of high-resolution mass spectra of complex materials. The concept was originally proposed in the 1960's by Kendrick [1] to facilitate the interpretation of high-resolution mass spectra of petroleum samples. However, Kendrick plots nowadays find increasingly wider application in the visualization of mass spectrometric data on various materials such as natural organic matter (NOM), synthetic polymers, bio-oils, etc [2]. Kendrick diagrams have been applied as well in the characterization of paint and varnish samples from old master paintings by direct temperature-resolved mass spectrometry (DTMS) [3]. Their potential in this latter field of cultural heritage studies will be highlighted.

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Blurred lines: issues distinguishing between alkyds and oils

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Alkyd paints are oil-modified polyesters manufactured from poly-ols, aromatic polybasic acids (PBAs), and fatty acids or oils. Fourier transform infrared spectroscopy (FTIR) is often used to identify alkyds although fillers and extenders can obscure the characteristic peaks ester and aromatic peaks. Solvent micro-extraction of the media can remove these interferences and FTIR is often considered a reasonably reliable means to identify alkyds. Recent work on a collection of studio materials from Hélio Oiticica samples from Edward Kienholz sculptures has called this reliability into question. Comparison of FTIR data with GC/MS data processed using Synthetic ESCAPE revealed that the former method failed to identify some paints as alkyds that were subsequently shown to be alkyds by GC/MS. Synthetic ESCAPE, which creates an overview of oils and alkyds by breaking their main constituents down into four main material categories (oils, polybasic acids, polyols, and pine resin), provides an assessment of relative amounts through the summation of peak areas. While FTIR was able to reliable identify paints as alkyds that contained high amounts of PBAs (<20% as assessed by Synthetic Escape), paints with lower amounts of BPAs were often mis-identified by FTIR. Therefore, FTIR analysis alone cannot be considered reliable when distinguishing between these media.

Wild Kingdom: an Excel-based tool for species identification using MALDI-ToF Data

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In 2018 Winterthur Museum, Garden & Library was awarded an NEH grant to catalog "overlooked organic" objects in the collection through physical identification and analytical research. This project focused on cataloging a group of objects with a high standard of accuracy, acquiring information through visual and scientific analysis, research, and expert consultation. Where visual identification was inconclusive, peptide mass fingerprinting (PMF) provided a species identification, to varying degrees of certainty, based upon existing reference tables. The grant allowed the museum to invest in the equipment required to prepare samples for PMF, while MALDI-ToF analysis is conducted at the University of Delaware's Mass Spectrometry Facility.

While working on PMF analysis with infrequent or one-off users of the technique (art conservation students, material culture students, museum cataloguers), Winterthur scientists started looking into more user-friendly ways to cross-reference MALDI-ToF data of collagen markers to known, open-source collagen marker reference lists. The tool known as the "Wild Kingdom Helper" was developed to suggest possible species identifications with a certainty factor, drawing from known collagen markers for a range of species.

Users input calibrated MALDI-ToF data (m/z and intensity) into the Excel-based tool. Wild Kingdom Helper then finds collagen and keratin markers from the list and determines whether the sample is deamidated or not. The input data is compared to all m/z values in a table of species. The closest match, high or low, for each m/z is reported. Acceptance of these is based on a user defined tolerance. A list of possible species (listed from best to worst match) is then outputted, alongside the certainty factor: percentage of total markers found. The table of species is open ended for additional entries.

The tool is currently still in development but has been used successfully to corroborate results from manual cross-referencing of data tables in several instances where data quality is high. Compatibility is Excel 2013 or newer. It is hoped that the Wild Kingdom Helper will be a usefulteaching tool for students in the future.

Preliminary results on the development of a workflow for simultaneous extraction of dyes and keratins in dyed wool textiles

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The analysis of dyed archaeological textiles is a challenging undertaking [1-3]. The preservation of archaeological textiles varies greatly depending on the site from which they have been excavated. In the best-case scenarios, traces of color are still visible; in the worst, such as carbonized textiles, little is left besides the physical outline of the yarns. To characterize the composition of these precious remains, highly specialized, sensitive analytical tools are requested: Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) can be applied to the analysis of both dyes and the proteins of animal-based fibers. This poster will present the first phase of the PARCA project that aims at developing an innovative streamlined protocol to recover both dyes and proteins in a single extraction (thus minimizing sampling size), and the preliminary results of the LC-MS/MS analysis on Thermo's Elite Orbitrap. Building on the ammonia-based protocol used for dyes extraction [4] and proteomics methodologies specifically developed for wool, a series of steps were tested to optimize the extraction and purification of proteins and dyes in modern madder-dyed wool. The ultimate goal of the project is a protocol of high information content for archaeological dyed wool, in particular applicable to highly degraded samples such as carbonized textiles.

Acknowledgements

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Revisiting the imperial past - comprehensive scientific investigation and conservation treatment of historic lacquer coatings of Prince carriages from the collection of the Wagenburg in Vienna

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The Imperial Carriage Museum Vienna (Kaiserliche Wagenburg Wien) holds an exceptional and precious collection of various carriages of the former transport pool of the Viennese court and vehicles from numerous aristocratic families. The aim of this research project was to establish a suitable conservation strategy for four prominent 18th century Prince´s state carriages supported by extensive chemical research. This included study of exact construction and composition of the lacquered surfaces combining optical microscopy (LM, SEM/EDS) and gas chromatography - mass spectrometry (GC-MS) technique. The microscopical results revealed a multiple stratigraphy with up to 20 coloured lacquer layers: Several packages of alternating pigmented layers (chalk, lead white, ochre, red lead or vermilion), metal interlayers (gold, silver or brass) and organic coatings give an impression of the eventful prehistory of the object. GC/MS analyses proved the use of complex oil-resinous mixtures containing drying oil, namely linseed oil with addition of essential oils and various diterpenous and triterpenous resins: spike oil, amber, sandarac, pine resin and mastic were often detected.

Currently one of the four Prince´s State Carriages is under conservation treatment. One of the main focuses was the removal of extensive overpaint on its panels – the brilliant rococo technique, a gilded panel decorated with painted peacock feathers and flower bouquets adorned with silver filings was covered up with bronzing and multiple layers of varnishes from the 19th century.

The new insights in the accurate composition of the historic coatings certainly endorsed the optimal methodology of their conservation/restoration treatments and gives us important information about the materials used for the original varnishes and the desired surface effect.

Cold paint on the stained glass windows from the Park Abbey, Leuven, Belgium.

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Park Abbey

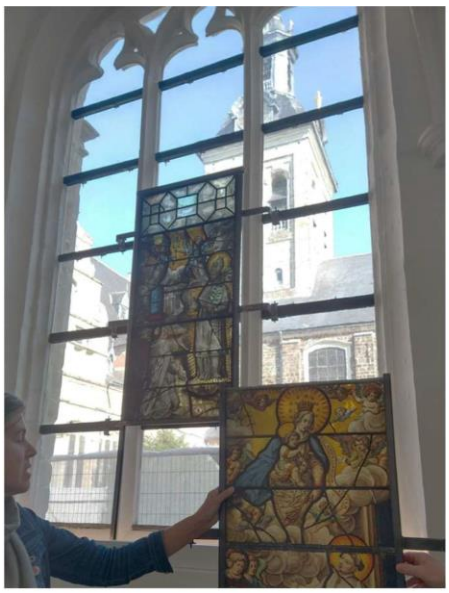
The 12th century Park Abbey, on the outskirts of university town Leuven, grew through a long history into one of the richest and best preserved abbey sites in the Low Countries. The monumental complex, virtually unchanged since 1730, includes an abbey, various outbuildings, entrance gates and four fishponds.

The stained glass windows

The abbey's prosperity translated into numerous lavish possessions, including an impressive series of 41 stained glass windows by Jan de Caumont (1635-1644). Unfortunately, in 1828, they were sold for lack of money. Over the years the panels were altered and distributed internationally. Today, after much effort, 21 of the 41 stained glass windows could already return.

Cold paint

A recent study showed that on some panels so-called cold paint was applied, to adjust the transparency of the panels. Whether any of this cold paint was already applied at the outset is still unclear. Cold paint is regularly removed during cleaning or reuse, but its historical composition has rarely been examined. This poster discusses the different compositions of cold paint on the stained glass windows of the Park Abbey.



SuPerStAr - Sustainable Preservation Strategies for Street Art: a new Italian project on the safeguard and preservation of street art

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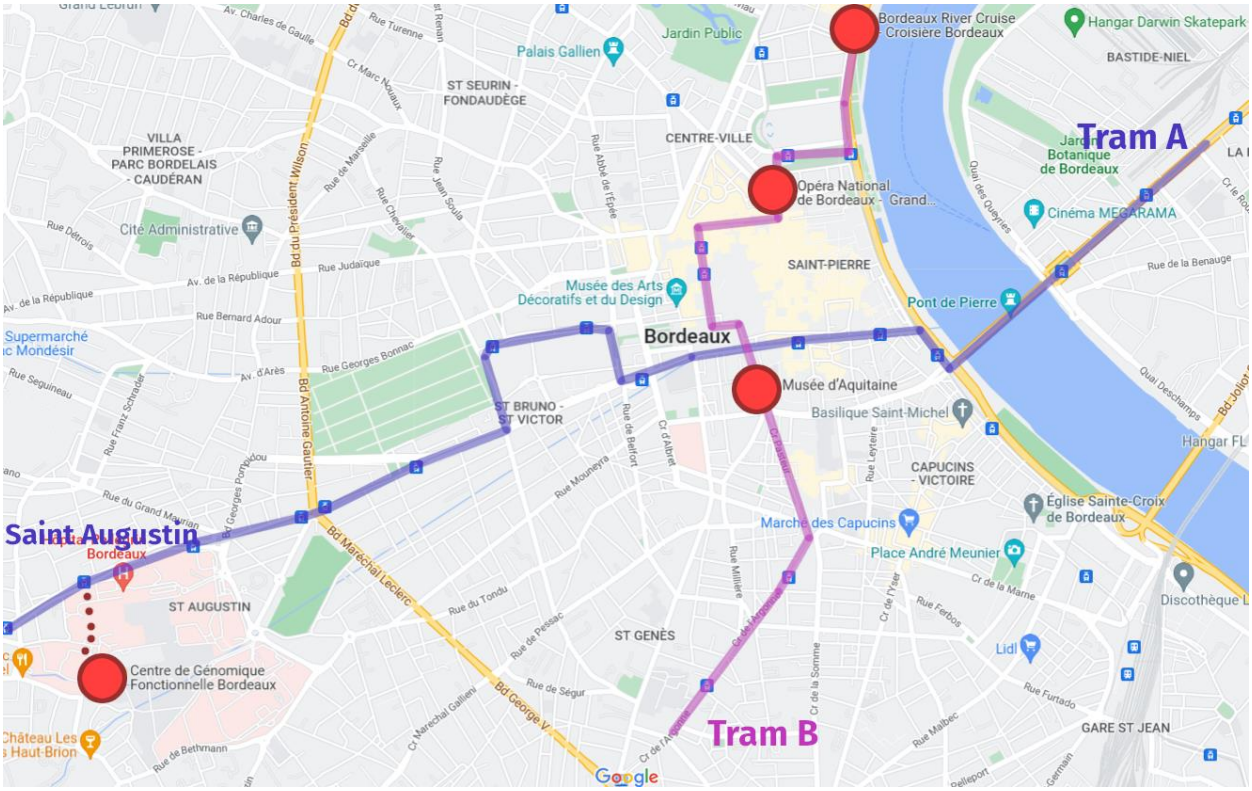
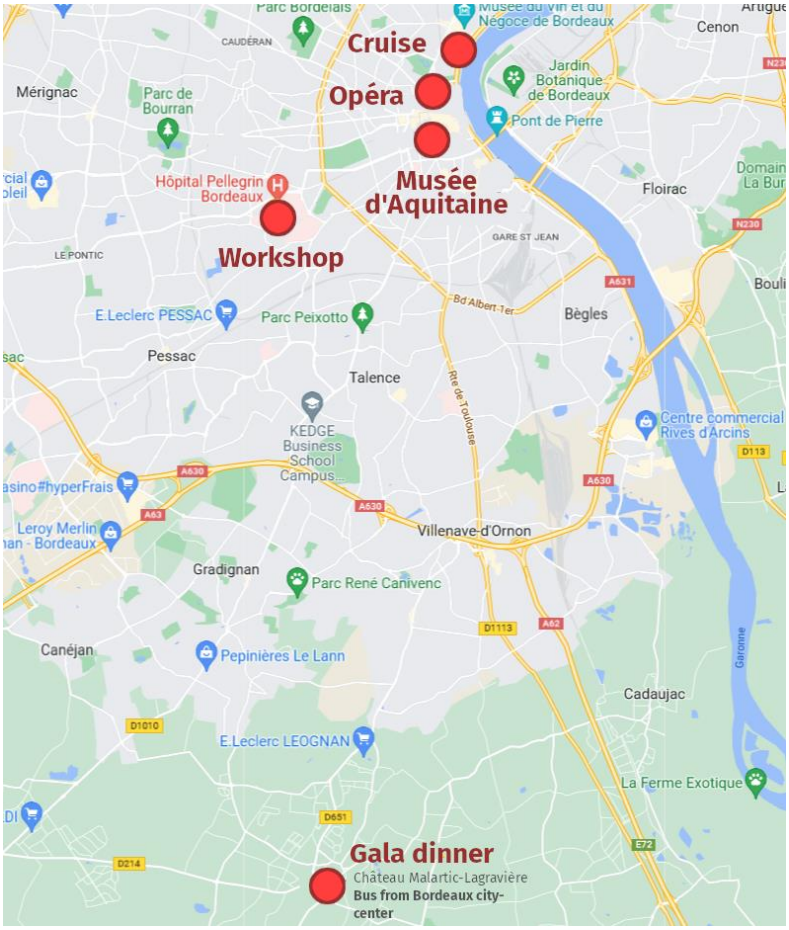
Street art has been recognized part of our cultural heritage only in the latest years. The ephemeral character, free access, and exposure to the environment and anthropic actions, make public paintings vulnerable to neglect, removal, vandalism, and degradation. Beyond that, the strategies aimed at their preservation and fruition are rather unclear or lacking. The project PRIN-2020 SUPERSTAR Sustainable Preservation Strategies for Street Art (2022-2025) sets as a goal the definition of innovative guidelines for the preservation strategy of street art, aimed at safeguarding its powerful social and cultural message in the urban context. The combination of non-invasive and micro-invasive techniques will shed light on the chemical-physical properties and vulnerability aspects of modern paint materials that constitute street artworks. In particular, analytical pyrolysis coupled with gas chromatography and mass spectrometry (Py-GC-MS) and liquid chromatography (HPLC) with spectroscopic and mass spectrometric detection will play a fundamental role in the characterisation of synthetic paint binders and pigments in the formulation of industrial paints used by street artists. The studies performed in the laboratory on reference materials will be supported by research performed on case studies, focused on the materials used by the artists, the environmental risks and anthropic stress. Relevant case studies have been selected, located in different environmental urban contexts in Milan, Torino and Pisa, in collaboration with municipalities and urban art curators. Thanks to the effective collaboration among a wide team of researchers with complementary expertise, involved in the various participating units, and to the interaction with conservation institutions and entities engaged in safeguarding public urban art, the project will contribute to define future preservation strategies. The following outputs are expected: optimized innovative cleaning procedures for the restoration of outdoor murals and for the removal of vandalistic graffiti; selected protective coating materials with particular attention to durability aspects; and an integrated protocol for long-term sustainable monitoring and conservation.

https://prin2020superstar.dcci.unipi.it/

MaSC Locations in Bordeaux





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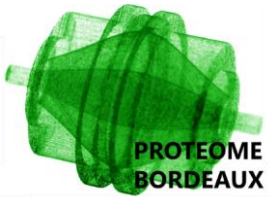
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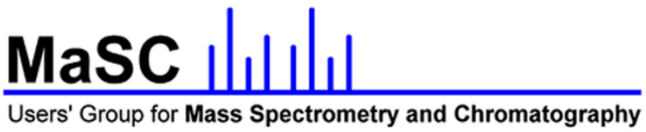
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