

# Emory @ Unisi

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**Chemistry for Life & Environment  
Education, Collaboration, Innovation**

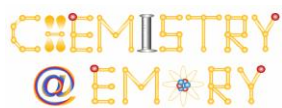
**XIV Edition, 28 May - 04 July 2017**



Università di Siena  
1240



DIPARTIMENTO DI BIOTECNOLOGIE,  
CHIMICA E FARMACIA



celebrating scientific  
discovery and innovation



**EMORY**  
UNIVERSITY



**Department of Biotechnology, Chemistry & Pharmacy,  
University of Siena,**

**warmly welcomes**

**Emory University Teachers and Students**

**The events are organized with the cooperation of**



**Unisi  
International Office**



**Unisi Graduate College  
Santa Chiara**



**Unisi  
Botanical Garden**



**Barone Ricasoli Spa  
Agricola**



**Azienda Agraria  
Sovestro in Poggio**



**ISVEA Srl**

**WebPage**

**<http://www.dbcf.unisi.it/en/research/cooperation/emoryunisi-2017>**



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Gabriella Tamasi  
José Soria  
Vincent Conticello  
Nathan Jui

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**Summer School Emory @ Unisi**  
**Chemistry for Life & Environment**  
**14th Edition, 28 May - 04 July 2017**

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<b>28 May, Sunday</b>	
11.00 am 3.00 pm	Departure from Rome Arrival of Emory Group. Check-in at Refugio residence
4.00	Introductory meeting at Refugio by summer program coordinators. Introduction to the program, teaching aids, internet connection, notes about Siena ...
7.00	Lottery of Contrade that will run the Palio on July 2 <sup>nd</sup>
8.00	Welcome Dinner at Spadaforte, Piazza del Campo
<b>29 May, Monday</b>	
9.00 am	Emory Classes at Refugio
12.00 2.00 pm	Lunch at Mensa Universitaria at Bandini, cards validation Guided visit to the "Museo Civico, Palazzo Comunale, Piazza del Campo"
<b>30 May, Tuesday</b> <b>OPENING OF THE SUMMER SCHOOL (Santa Chiara, Auditorium)</b>	
10.00 am	Introduction and Greetings Agnese Magnani, Gabriella Tamasi and Daniela Valensin Francesco Frati – Rector of Unisi Annalisa Santucci - Director Dept Biotechnology Chemistry & Pharmacy & Director Biochemistry & Molecular Biology PhD School Stefano Mangani - Director Chemical & Pharmaceutical Sciences PhD School
10.30	José Soria & Vincent Conticello - Emory Representative Gabriella Tamasi - Introduction of summer program
11.00	Presentations by Unisi Research Groups Claudio Rossi - USiena-AgriFood Network Coordinator Nutraceuticals
11.15	Ilaria Bonini & Massimo Nepi Botanical Museum of the University of Siena: Conservation, Education and Research
11.30	Gianluca Giorgi Mass spectrometry in organic chemistry: from structural to stereochemical and conformational characterization
11.45	Stefania Butini Development of modulators of the endocannabinoid system as potential therapeutics
12.00	Enrico Tavarnelli The depositional and tectonic history of ridges and basins of Southern Tuscany: geological controls on the quality of wines and food produced in Val d'Orcia and in the Chianti mts
12.30 2.00 pm	Buffet Conclusions

<b>31 May, Wednesday</b>	
9.00 am	Emory Classes at Refugio
<b>01 June, Thursday</b>	
9.00 am	Emory Classes at Refugio
<b>02 June, Friday - Italian Civic Holiday (Festa della Repubblica)</b>	
9.00 am	Emory Classes at Refugio
<b>03 June, Saturday</b>	
	Free Day in Siena
<b>04 June, Sunday</b>	
	Free Day in Siena
<b>05 June, Monday FIELD-TRIP TO BARONE RICASOLI SPA AGRICOLA (BROLIO, GAIOLE IN CHIANTI)</b>	
9.30 am	Departure from Porta Romana
10.00	Arrival at Brolio
10.15	Guided visit and class in the vineyards about Sangiovese and other vines growing, treatments, ... and guided visit and class in the cellars about wine production and aging
12.00	Wine tasting
1.00 pm	Lunch at Restaurant Cantine Barone Ricasoli, typical Chianti foods
2.30	Guided visit to the Ricasoli Castle, old enological laboratory of Barone Bettino Ricasoli, History of Chianti and Italy
4.30	Return to Siena
<b>06 June, Tuesday</b>	
9.00 am	Emory Classes at Refugio
12.00	First program evaluation, forms filling for Emory Students
<b>07 June, Wednesday - Day at San Miniato (Room #12)</b>	
9.30-10.30 am	Unisi Lecture Session Stefano Mangani Ferritin - Chemistry at the protein-mineral interface
10.30-11.30	Evelina Colacino Thinking chemistry differently: Mechanochemistry a disruptive innovation for chemists and biologists
11.30-12.30	Emory students meet Unisi Students Students present their own experiences at Universities. Teachers excluded!
12.30	Lunch time at San Miniato Mensa
2.30-5.30 pm	Parallel Unisi Laboratory Sessions Gianluca Giorgi Mass spectrometry in organic chemistry: from structural to stereochemical and conformational characterization



	<p>Manuela Benvenuti Lysozyme crystallization</p> <p>Evelina Colacino Thinking chemistry differently: Mechanochemistry a disruptive innovation for chemists and biologists</p>
<b>08 June, Thursday</b>	
9.00 am	Emory Classes at Refugio
<b>09 June, Friday</b>	
9.00	Emory Students and Faculties visiting Florence
<b>10 June, Saturday</b>	
	Free Day
<b>11 June, Sunday</b>	
	Free Day
<b>12 June, Monday - Day at San Miniato (Room Aula Magna) 1st Emory-UniSi Research Symposium</b>	
9.30 am	<p>Chairman: Agnese Magnani Introduction and Greetings Francesco Frati – Rector of Unisi Annalisa Santucci - Director Dept Biotechnology Chemistry &amp; Pharmacy at Unisi Stefan Lutz - Director Dept Chemistry at Emory Anna Lisa Poggialini – Office for International Affairs</p>
10.00 - 10.45	<p>Chairman: Gabriella Tamasi Stefan Lutz Engineering biocatalysts: exploring and exploiting flavoenzymes</p>
10.45 - 11.30	<p>Rebecca Pogni Oxidative enzymes: mapping of the catalytic intermediates and immobilization on nanomaterials</p>
11.30 - 12.15	<p>Vincent Conticello Peptide and protein nanomaterials: the design challenge</p>
12.30	Buffet & Posters session
2.30 - 3.15	<p>Chairman: Daniela Valensin Neri Niccolai Structural bioinformatics: a new window to observe the protein universe</p>
3.15 - 4.00	<p>Nathan Jui New reaction development using radical anion intermediates</p>
4.00 - 4.45	<p>Allan Watson Development of Autotaxin inhibitors via structure-based design</p>
4.45 - 5.30	<p>Maurizio Botta The first molecule interacting with a host protein for the inhibition of multiple viruses</p>
8.00	Dinner offered by Emory

<b>13 June, Tuesday</b>	
9.00 am	Emory Classes at Refugio
<b>14 June, Wednesday – A cultural field trip organized by After’s Cool</b>	
8.30 am	Departure from Porta Romana
9.50	Arrival to Chiusdino
10.00	Visit to the Civic Museum of Chiusdino
11.00	Guided trekking to San Galgano Abbey (6 Km)
1.00 pm	Lunch at Casale San Galgano with traditional Tuscan Food
2.45	Guided visit to the San Galgano Abbey and the Chapel (Eremo di Montassiepi)... <i>The sword in the Stone...</i>
4.00	Departure to Sovicille
4.30	Conference at the After’s Cool The Origin of Chemistry: History and Alchemy
6.00	Departure to Siena
<b>15 June, Thursday</b>	
9.00 am	Emory Classes at Refugio
<b>16 June, Friday</b>	
9.00 am	Emory Classes at Refugio
<b>17 June, Saturday</b>	
	Free Day in Siena
<b>18 June, Sunday</b>	
	Free Day in Siena
<b>19 June, Monday</b>	
9.00 am	Emory Classes at Refugio
<b>20 June, Tuesday – Day at San Miniato (Room #8)</b>	
9.30-10.30 am	Unisi Lecture Session Andrea Atrei Magnetic hydrogels: from biomedical to environmental applications
10.30-11.30	Enrico Tavarnelli The depositional and tectonic history of ridges and basins of Southern Tuscany: geological controls on the quality of wines and food produced in Val d’Orcia and in the Chianti mts
11.30-12.30	Lia Millucci The story of a rare genetic disease: Alkaptonuria
12.30	Lunch time at San Miniato Mensa
2.30-5.30 pm	Parallel Unisi Laboratory Sessions Gabriella Tamasi and Alessio Pardini. Visit to Toscana Life Sciences Foundation (Laura Salvini) Chemical characterization of nutraceuticals in vegetable products and by-products: green tomatoes and tomato leaves. HPLC-MS application

	<p>Ottavia Spiga and Andrea Bernini A structural account on the amyloidogenic process of the atrial natriuretic peptide: a multi technique approach using nuclear magnetic resonance, electron microscopy and bioinformatics</p> <p>Marco Consumi ToF-SIMS analysis for food chemistry</p>
<b>21 June, Wednesday</b>	
9.00 am	Emory Classes at Refugio
<b>22 June, Thursday</b> <b>FIELD-TRIP TO ISVEA AND TO SAN GIMIGNANO</b>	
9.00 am	Departure from Porta Romana
9.40 10.00	Arrival at ISVEA Srl (laboratory for enological and food analyses) Guided visit to chemical and microbiological laboratories
11.30	Departure to San Gimignano
12.00	Arrival at Fattoria Sovestro in Poggio, and visit to the Vineyards and Cellars
13.00	Lunch at Fattoria Sovestro in Poggio
2.30 pm	Visit to San Gimignano, the town and main historical places
5.00 pm	Departure to Siena
<b>23 June, Friday</b>	
9.00 am	Emory Classes at Refugio
3.00 pm	Massimo Nepi Botanical Museum of the University of Siena: Conservation, Education and Research
<b>24 June, Saturday</b>	
	Free day
<b>25 June, Sunday</b>	
	Free day
<b>26 June, Monday</b>	
9.0 am	Emory Classes at Refugio
3.00 pm	Visit to a Contrada Museum, Church, ... Contrada....
<b>27 June, Tuesday- Day at San Miniato (Room #8)</b>	
9.30-10.30 am	Unisi Lecture Session Sonia Carmignani Consumer protection
10.30-11.30	Luisa Galgani The sea-surface microlayer: biogenic composition in a changing ocean
11.30-12.30	Rita Vignani Molecular authentication of food and wine

12.30	Lunch time at San Miniato Mensa
2.30-5.30 pm	Parallel Unisi Laboratory Sessions Daniela Valensin NMR Applications for Food Chemistry  Vanessa Volpi & Alessandro Donati HH-XRF of silver coins and archaeological finds from the "Colline Metallifere district" (South Tuscany)  Giulia Bernardini Biochemical alkaline phosphatase assay
<b>28 June, Wednesday</b>	
9.00 am	Emory Classes at Refugio
<b>29 June, Thursday</b>	
9.00 am	Emory Classes at Refugio
12.00	Horse lottery in Piazza del Campo
<b>30 June, Friday</b>	
9.00 am	Emory Classes at Refugio
12.00	Second and final Evaluation, forms filling for Emory Students
<b>01 July, Saturday</b>	
7.00 pm	Horse trials in Piazza del Campo
8.30	Eve Palio Dinner in a Contrada
<b>02 July, Sunday - Palio Day</b>	
07.00 am	Blessing Mass celebrated by Archbishop in Piazza del Campo for Fantino (Jockey)
02.00 pm	Attending to the horse blessing in a Contrada
03.00	Attending to Contradas blessing from the Archbishop at Duomo
04.30	Piazza del Campo ...waiting for Palio Race
... ..	Palio Race
<b>03 July, Monday</b>	
	Free day
12.00	Meeting of Evaluation Committee of Unisi and Emory Teachers Conclusions, Remarks and Perspectives.
<b>04 July, Tuesday</b>	
	Check-out

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**ABSTRACTS  
of  
LECTURES  
&  
LABORATORY SESSIONS**

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## 30 May 2017 - OPENING OF THE SUMMER SCHOOL

### Nutraceuticals

Claudio Rossi

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Nutraceuticals is a new discipline aimed to study bioactive substances from vegetable products. Unlike pharmaceutical, studying synthesis of new drugs for therapeutical uses, nutraceuticals, analyse natural molecules, found in vegetables, to evaluate their ability preventing pathology development. In addition, in chronic pathological processes, nutraceuticals quite often work better than traditional drugs, without causing undesired effects even for long-term treatments. Nutraceuticals are belong to a number of chemical families: polyphenols (flavonoids, stilbenoids, lignans, tannins, secoiridoids), carotenoids (terpenoids), amino acid derivatives, sugar derivatives, lipids, antioxidant alkaloids. These molecules are typically present at very low concentration in plants, but show high bioactivity. From a biochemical point of view, their mechanisms are very specific for each different metabolic pathway. The main mechanism is reducing inflammation caused by pathogens and other exogenous and/or endogenous factors. As a consequence, they prevent degenerative processes of cardiovascular system, nervous system, osteo-articular system. Nutraceuticals also act on the cellular metabolism promoting pro-apoptotic processes, and removing cells showing altered metabolic profiles. The study of the nutraceutical properties of vegetables will allow to formulate new natural supplements, able to prevent and/or delay serious degenerative diseases development.

**30 May 2017 - OPENING OF THE SUMMER SCHOOL**  
**23 June 2017 - LAB SESSION**

**Botanical Museum of the University of Siena: Conservation, Education and Research**

Claudia Perini, Ilaria Bonini, Paolo Castagnini, Massimo Nepi

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The Botanical Museum includes the Garden and the Herbarium. These two museums make education activities for all types of schools and cooperate with the Department's research activities, according to the indications of Botanic Garden Conservation International (BCGI), that so defines: "Botanic gardens are institutions holding documented collections of living plants for the purposes of scientific research, conservation, display and education" (1).

Moreover it also works in the surrounding of Siena, in collaboration with other Museums (2). Botanical paths have been realized in the Provincial Natural Reserve and in Ethnographic Museum "Museo del Bosco" (3).

The Garden plays an extremely important role in studying, naming, cataloguing and displaying plant diversity and their conservation. All these roles are developed with the presentation of thematic collections on Tuscan native and threatened species. Tourists and students can enjoy observing directly the plants, reading the explaining poster or participating at the guided tours (4). In 2005 the Siena Botanical Garden, with those of Pisa and Florence, was acknowledged by the Tuscany Region, as Centrum for *ex situ* conservation Flora (CESFL) and it collaborates with Italian Seeds Bank Network for native species conservation (RIBES) (5).

In 2003 the Herbarium of Siena made a collaborative project with University of Camerino and Perugia: a open source software to support researchers and students to archive and retrieve botanical data "An Archive for Botanical data" (<http://erbaritaliani.unipg.it>) (6).

The Botanical Museum contributes to research activities of Department of Life Sciences: ecophysiology of plant reproduction, mycology, biology of sexual plant reproduction, agronomy, phytoremediation technology and community ecology. Just some examples: a research program on reproductive biology of *Cucurbita pepo* L. (7); study on the cytological, and biochemical characteristics of *Nicotiana tabacum* L. pollen tubes (8); study on the relation between productivity and species richness (9); studies on wild Italian orchids and their relation with fungi (10).

## References

- [1] A.A.V.V. (2001) *Informatore Bot. Ital.*, 33,1-66.
- [2] C. Perini, V. De Dominicis (1993) *Giunta Regionale Toscana & Quattroemme srl Perugia*, pp. 83-92.
- [3] I. Bonini (1993) *Toscana musei, Protagon Editori Toscani*, pp. 19-73.
- [4] P. Castagnini, F. Casini, C. Perini, V. De Dominicis (2006), *Informatore Bot. Ital.*, 38 (1): 248-253.
- [5] M. Clauser, G. Bedini, C. Perini, A. Casadio (2005) *Informatore Bot. Ital.*, 37(1):128-129.
- [6] I. Bonini (2006) *Annali di Storia delle Università*, 10:255-275.
- [7] M. Nepi, E. Pacini (2001) *Acta Soc. Botanicorum Poloniae* 70(3):165-172.
- [8] M. Scali, A. Moscatelli, M. Cresti (2000) *Carpologia*, 53(1):45-54.
- [9] A. Chiarucci, C. Alongi, W. J Bastow (2004) *I. of Vegetation Science*, 15:789-796.
- [10] C. Perini, E. Salerni, L. Pecoraro, P. Leopardi, V. De Dominicis (2006) *Abstr. B. 1, PS9-184-0590*: 85.



**30 May 2017 - OPENING OF THE SUMMER SCHOOL**  
**07 June 2017 - LAB SESSION**

**Mass spectrometry in organic chemistry: from structural to stereochemical and conformational characterization**

Gianluca Giorgi

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Mass spectrometry (MS) is a powerful methodology for identifying, structurally characterizing, investigating the reactivity and quantitating wide classes of naturally occurring organic molecules or obtained by chemical synthesis.

A number ionization techniques, depending on the chemico-physical properties of the molecules, and a wide range of analyzers are available.

Given an organic molecule, a lot of information can be obtained by mass spectrometry: molecular weight, elemental formula (high resolution MS), structural characterization (tandem mass spectrometry), quantitation, but also it is possible to determine stereochemical properties, to study conformations (ion mobility mass spectrometry), to make a molecular mapping of a surface, such as a tissue or a leaf (mass spectrometry imaging), to make ion spectroscopy in the gas phase [1-2]. The use of soft ionizations, such as electrospray, MALDI, and a wide variety of ambient mass spectrometry techniques allows to study wide classes of analytes and to characterize non covalent complexes and supramolecular aggregates.

The mass spectrometer can be used as a complete chemical laboratory for investigating gas phase ion chemistry and the reactivity of radical ions, cations and anions, to carry out collision-induced dissociation reactions, ion activation by photons and electrons, ion-molecule and ion-ion reactions.

The coupling of mass spectrometry with different separative techniques, such as gas chromatography and xPLC allows the study of complex mixtures.

Applications of mass spectrometry in organic chemistry developed by this research group [3] will be presented.

#### Laboratory session

Mass Spectrometry in Practice: What is Your Weight and What is Your Structure?

ESI MS and MS/MS experiments on natural organic compounds will be carried out.

#### References

- [1] Chapman J. R., Practical Organic Mass Spectrometry: A Guide for Chemical and Biochemical Analysis, 2<sup>nd</sup> ed., Chichester, John Wiley & Sons, 1995.
- [2] De Hoffmann E., Stroobant V., Mass Spectrometry: Principles and Applications, 3rd Ed., Wiley, 2007.
- [3] Giorgi G., Overview on Mass Spectrometric Based Techniques Applied in the Cultural Heritage Field, In Organic Mass Spectrometry in Art and Archaeology, M.P. Colombini & F. Modugno Eds, Wiley Chichester, 2009, pp. 37-74.

## 30 May 2017 - OPENING OF THE SUMMER SCHOOL

### Development of modulators of the endocannabinoid system as potential therapeutics

Stefania Butini

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The main endocannabinoids (ECs) anandamide (AEA) and 2-arachidonoylglycerol (2-AG), by stimulating cannabinoid CB1 and CB2 receptors (CB1R and CB2R), regulate relevant signaling pathways. The key enzymes involved in ECs catabolism are fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Their unique role in terminating ECs signaling and regulating the intracellular levels of AEA, 2-AG and other ECs supports their potential as therapeutic targets. Selective inactivation of ECs degrading enzymes represents an attractive approach for eliciting the desirable effects of CBR activation, while avoiding the negative (psychotropic, among others) effects of CB1R stimulation.

Multiple sclerosis (MS) is a chronic, inflammatory autoimmune disease characterized by nerve demyelination that affects up to 2.5 million people worldwide. At present there is no drug available for controlling the disease progression of patients with progressive forms of MS, and no means to repair injured axons or protect neurons from further damage. In the past few years, an increasing body of evidence has suggested that ECs may have beneficial effects on the symptoms of MS [1] and ECs have been suggested to be neuroprotective in this context. Therefore MAGL appears as a promising and reliable target for the treatment of MS.

We have recently characterized a new class of beta-lactam-based MAGL inhibitors typified by NF1819 [2]. NF1819 is a highly potent and selective MAGL inhibitor, it increases the levels of 2-AG by blocking its degradation, thus acting as CBRs' indirect agonist. NF1819 was effective in reducing the clinical scores in mice suffering from experimental autoimmune encephalomyelitis and in limiting the associated demyelination and inflammation in the spinal cord in mice at the acute stage of the disease.

#### References

- [1] Pryce, G.; *CNS Neurol. Disord. Drug Targets* 2012, 11, 624-41.
- [2] Brindisi, M.; *J. Med. Chem.* 2016, 59, 2612-32.

**30 May 2017 - OPENING OF THE SUMMER SCHOOL**

**20 June 2017 – LECTURE**

**The depositional and tectonic history of ridges and basins of Southern Tuscany:  
geological controls on the quality of wines and food produced in Val d’Orcia and in  
the Chianti mts**

Enrico Tavarnelli

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Tuscany is traditionally regarded as a not-to-be-missed stop when visiting Italy, not only for its early human history dating back to the Etruscan age, and for the consequent richness in arts and architecture that have flourished ever since, but also for the quality of its renowned wines and for the taste of its food products, whose combination makes the traditional tuscan cuisine worldwide known and appreciated. Tuscan food and wine owe their specific character and taste to the variety of the landscape and climate, combined with a great diversity of bedrock and soils, that collectively make the unicity of this terroir. But which are the relationships between geology and wine? And between geology and food? How do the composition of the substratum, weathering, soil development, climate and landscape evolution influence the character of wine and the quality of food? These questions, once considered solely as matters to be treated in nice convivial events, are now receiving increasing attention amongst the geological community, to the point that thematic sessions and disciplinal conferences are organized on these topics, with a consequent impact in the scientific literature at the highest international level.

Italy has a long tradition in promoting its cuisine, which reflects a high vocation in quality assessment for its agricultural production. Yet there are to date relatively few studies on the geological factors that control the quality of Italian food and wine [1]. The Apennines are a mountain range that represents the backbone of the italian peninsula. These mountains are made of a stack of tectonic slices, consisting of Mesozoic and Tertiary mainly marine sedimentary rocks; these were detached from a Palaeozoic basement and piled northeastwards since Upper Cretaceous time largely during the Alpine orogeny. The structurally highest units are unconformably covered by younger sediments, that were deposited in fluvial-lacustrine and marine basins whose formation began in Late Tertiary time, following the orogenic climax [2].

The Mesozoic-Tertiary sequence differs greatly from younger sediments, thus reflecting a significantly different depositional environment. This lecture, conceived as an introduction to the reader who is little familiar with the geological history of Italy, outlines the main sedimentary and tectonic events that led to the development of the Apennine belt. The lecture aims at providing a framework for the study of the relationships between geology and food/wine in Southern Tuscany. Emphasis is given to the history of the Mesozoic-tertiary sedimentary sequence, whose deformation gave rise to the Chianti Mountain Range famous for its wine production. The development of younger sedimentary basins, whose fill extensively crops out in the Val d’Orcia, will also be illustrated, with the aim at providing a clue for the understanding why the cereal production of this province is greatly appreciated by food experts and gourmets alike worldwide.

**References**

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[2] Alvarez, W. The Mountains of Saint Francis. Norton, New York-London, 2009. ISBN 978-0-393-06185-7.

**07 June 2017 – LECTURE**

**Chemistry at the protein-mineral interface**

Stefano Mangani

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Ferritins are ubiquitous multimeric protein systems showing a nanocage structure able to include thousands of iron atoms as oxoferric biomineral. In mammals, these twentyfour-mer protein shells are generally heteropolymers composed by two different types of subunits classified as heavy (H) and light (L). The relative ratios between the two types in heteropolymers is tissue-dependent. The H-subunit possess enzymatic activity being able to rapidly oxidize  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$ , while L-subunits only assist spontaneous iron oxidation. Both subunits deposit the iron oxides within the internal protein cavity.

We have developed a soaking/flash freezing method to allow aerobic and anaerobic addition of iron(II) to human ferritin crystals. The properties of multi-wavelength anomalous x-ray diffraction have been exploited to reveal the iron pathway and the chemistry occurring in both H and L ferritins.

**07 June 2017 – LECTURE**  
**07 June 2017 – LAB SESSION**

**Thinking chemistry differently: Mechanochemistry a disruptive innovation for chemists and biologists**

Evelina Colacino

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A major concern for the development of a sustainable chemical synthesis is to find alternatives to the use of organic solvents, very often toxic and volatile, the halogenated ones creating major damages to the environment.

Mechanochemistry is a challenging approach - applied also on an industrial scale - to develop solvent-free reactions for the synthesis of important organic molecules, including pharmaceutically active ingredients, or palladium-catalyzed reactions.

## Lysozyme crystallization

Manuela Benvenuti

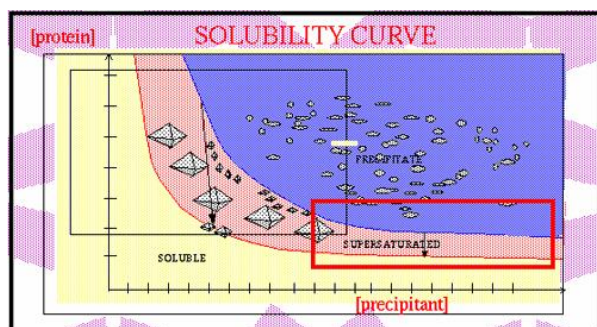
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Crystallization is the process, governed by both thermodynamic and kinetic factors, by which molecules arrange themselves in a natural manner to form a repetitive three-dimensional reticulum we call crystal. Thermodynamically, protein crystallization is not very different from the crystallization of NaCl. In both cases, we need to bring the solution into a supersaturated state after which the salt or the protein will hopefully start to crystallize. However, protein crystallization methods are very different. In the case of NaCl supersaturation may be achieved by first preparing a saturated solution of the salt at some high temperature (for example, 40°C) and then leaving it at room temperature for some time. At room temperature the solution will be in a thermodynamically metastable state. The result is that after a short while salt crystals will be found at the bottom of the glass. In the case of proteins, heating is not a method to use, proteins may quickly denature at high temperatures (unless it is a protein from a thermophilic organism). We are helped by the fact that protein solubility depends on many factors and not only on temperature. Among these factors is the concentration and type of salt present in the buffer, the pH of the buffer, the presence of possible co-factors, etc. Depending on the protein, different crystallization methods may be used to bring the solution into supersaturation, normally through a gradual decrease of the solubility of the protein. The most common way to reduce protein solubility for crystallization is by the addition of so-called precipitants (see Diagram). A precipitant binds water molecules, essentially competing with the protein for water, thus reducing water availability, which mimics higher protein concentration. Popular precipitants include polyethylene glycol and ammonium sulfate, probably the most widely used, but there are many other precipitants. When precipitant concentration is gradually increased, for example by using the method of vapor diffusion, the amount of solvent available for the protein is decreased, which in turn may lead to protein precipitation, or if the conditions are correct, to crystallization of the protein.

The objective of the Laboratory session is to provide "hands on" experience on the crystallization of Enzyme Lysozyme, which has been well characterized with respect to crystallization properties.

We will use the sitting drop methods that rely on vapor diffusion, in which a drop containing lysozyme/precipitant solution is allowed to equilibrate in a closed system containing a reservoir of precipitant.

With vapor diffusion, the sample is at 50% of the concentration of the precipitant compared to the reservoir solution and is less than that required for protein crystallization. Thus because the precipitant is the major solute present, vapor diffusion in the closed system results in the net transfer of water from the protein solution to the reservoir, until the precipitant concentration is the same in both solutions. Upon equilibration this transfer of water ceases and the resultant protein solution stays at the optimal precipitant concentration for crystallization.



Generic diagram showing the different areas of a protein-precipitant equilibrium in terms of the concentrations of both components.

**20 June2017 -LECTURE**

**Magnetic hydrogels: from biomedical to environmental applications**

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Hydrogels are 3D networks of cross-linked polymer chains capable to absorb a large amount of water with respect to their weight. Hydrogels made of suitable polymers can swell or shrink in response to external stimuli like temperature, pH, ionic strength. The introduction of magnetic nanoparticles (NPs) into hydrogels allows one to obtain materials which are able to respond to static and alternating magnetic fields. Matter at nanometric scale has properties different from those in the bulk form. For instance, NPs of magnetic materials exhibit a superparamagnetic behavior. Beads of magnetic hydrogels can be used for the targeting of drugs by means of a static magnetic field. The release of molecules loaded in thermoresponsive hydrogels can be triggered by the temperature increase of the magnetic NPs in the hydrogels induced by alternating magnetic fields. In this lecture the magnetic properties of NPs will be reviewed. An overview of the methods to prepare magnetic NPs and magnetic hydrogels and of the experimental techniques to study the physical and chemical properties of these systems will be presented. Some examples of applications of the magnetic hydrogels to controlled drug release and to remediation of contaminated water will be presented.

## 20 June 2017 – LECTURE

### The story of a rare genetic disease: Alkaptonuria

Lia Millucci

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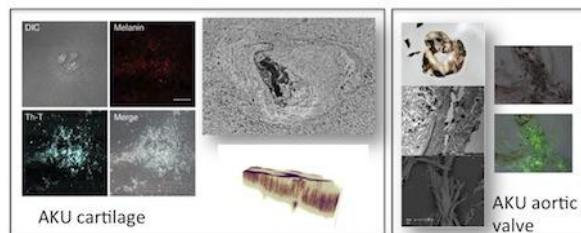
AKU was first described as an inherited disease by Sir Archibald Garrod in 1902. Sir Archibald was a true pioneer, relying upon simple observations to understand fundamental aspects of human biology.

Alkaptonuria, also known as AKU or Black Bone Disease, is an extremely rare genetic condition, which can cause significant damage to the bones, cartilage and tissues of those affected. AKU normally only affects one in every 250,000 people worldwide. AKU is a recessive condition that is caused by a mutation of one chromosome, this means that if two people carry the faulty gene, their child still only has a 25% chance of developing AKU.

AKU stops patients' bodies from breaking down a chemical called homogentisic acid (HGA) which the body naturally produces during the digestion of food. Due to this, HGA builds up in the body and, over time, leads to black and brittle bones and cartilage, and early onset osteoarthritis. The build up of HGA in the body can also lead to other, sometimes more serious health complications.

There is still no approved, effective treatment for alkaptonuria and although there have been many publications on AKU, there is little understanding of the mechanism of ochronosis. To study AKU, we are part of an international research network. The university will lead on analysis of biochemical samples, indicating inflammation, amyloidosis and oxidative stress in samples taken from AKU patients. We develop cell and tissue models reproducing the disease in laboratory conditions, allowing the study of molecular mechanisms. Our focus is the comprehension of physio-pathological mechanisms.

Overall, the results obtained, helping the clarification of AKU pathogenesis and related amyloidogenesis, could lay the basis to set up appropriate pharmacological interventions for the disease.



Ochronotic pigment from different sources and analyzed by different techniques. AKU cartilage: Left) Thioflavin T stained cartilage from AKU patient. Co-localization of melanin and amyloid was revealed by merge of Th-T and melanin fluorescence. Right upper) TEM image of an ochronotic chondrocyte. Right lower) 2 photon image of pigment and amyloid through AKU tissue. AKU aortic valve: Left) Upper: Light microscopy; Middle: TEM; Lower: SEM. Right) Ochronotic pigment (upper) co-localized with SAA amyloid (lower, immunofluorescence).

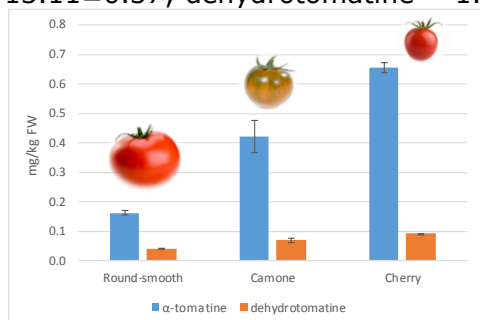


**Chemical characterization of nutraceuticals in vegetable products and by-products: green tomatoes and tomato leaves. HPLC-MS application**

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In the present work, the nutraceutical properties of tomato fruits (*Solanum lycopersicum L.*) were investigated. Particular attention was focused on the study of tomatine, a glycoalkaloid synthesized by tomato plants and green tomatoes as a defense against the adverse effects of pathogens as fungi and bacteria. Indeed, the tomato glycoalkaloid referred to as tomatine is composed by a 10:1 mixture of  $\alpha$ -tomatine and dehydrotomatine. Tomatoes represent a source of other bioactive compounds, as polyphenols, important for their antioxidant properties and their ability to reduce the risk of triggering of chronic diseases, like some types of cancers and cardiovascular diseases.

Firstly, the whole fruits of three tomato varieties were analyzed: round-smooth, cherry, and camone tomatoes. Camone tomato is an Italian variety, cultivated in Sicily and Sardinia, which remains green in the upper part of the fruit also at the mature stage of ripeness. Quantification of  $\alpha$ -tomatine and dehydrotomatine in all the samples was performed by reverse phase liquid chromatography coupled with electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) on a triple quadrupole analyzer. The two glycoalkaloid concentrations found in camone tomatoes ( $\alpha$ -tomatine =  $5.27 \pm 0.69$ ; dehydrotomatine =  $0.86 \pm 0.09$  mg/kg DW, dry weight) were intermediate with respect to the amount found in round-smooth tomatoes ( $\alpha$ -tomatine =  $3.24 \pm 0.13$ ; dehydrotomatine =  $0.78 \pm 0.03$  mg/kg DW) and cherry tomatoes ( $\alpha$ -tomatine =  $13.11 \pm 0.37$ ; dehydrotomatine =  $1.83 \pm 0.01$  mg/kg DW).



$\alpha$ -Tomatine and dehydrotomatine in three tomato varieties.

Subsequently, different parts of camone tomato fruit were studied, and analyses were carried out on peel, pulp and locular gel. The concentrations of  $\alpha$ -tomatine and dehydrotomatine in the locular gel were  $38.73 \pm 3.323$  mg/kg DW and  $4.90 \pm 0.01$  mg/kg DW, respectively, and they resulted about ten times lower in the peel, and were not detected in the pulp.

Also tomato leaves were analyzed, as by-product (not directly edible) very rich in tomatine. The contents of  $\alpha$ -tomatine and dehydrotomatine were  $12.24 \pm 0.31$  and  $0.16 \pm 0.01$  g/kg DW, for baby plants; ranged  $5.57 \pm 0.11$  -  $8.79 \pm 0.17$  and  $0.07 \pm 0.01$  -  $0.11 \pm 0.01$  g/kg DW for productive plants; and  $27.89 \pm 0.65$  -  $47.22 \pm 0.68$  and  $0.23 \pm 0.02$  -  $0.45 \pm 0.01$  g/kg DW for productive plants showing yellowish-leaves or fruits affected by parasites.

All samples were also analyzed for total polyphenols content (via Folin-Ciocalteu assay), antioxidant activity as Trolox Equivalent Antioxidant Capacity (TEAC; via ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) and DPPH (di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium) assays). Selected targeted polyphenols were also quantified via HPLC-ESI-MS/MS. The results showed that chlorogenic acid and caffeic acid were the main hydroxycinnamic acids in all the varieties, while rutin was the most abundant flavonoid. Rutin occurred mainly in the peel of the camone variety, while chlorogenic acid was primarily in the locular gel.

On the basis of these preliminary results, spontaneously hypertensive rats (SHR) were fed daily with locular gel obtained from camone tomatoes, to evaluate its efficiency as a potential nutraceutical product endowed with antihypertensive activity.

20 June 2017

## VISIT TO TOSCANA LIFE SCIENCES FOUNDATION

Laura Salvini

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Toscana Life Sciences Foundation (TLS) was established in Siena in 2005 by different public and private subjects, to support research activities in the field of life sciences and, in particular, fostering the development of projects from basic research to industrial application in the field of Life Sciences.

In particular, TLS is a bio-incubator that supports the start-up of companies in the Life Sciences sector. The bio-incubator is located in Siena at the "Torre Fiorentina" Science Park, where Achille Sclavo founded the homonymous Tuscan Serotherapeutic and Vaccinogenic Institute in 1904 and where GSK has located its Research and Development Center, a flagship of Italian and international research with over 2000 employees.

TLS currently hosts biomedical companies, non-profit research groups and service companies that work in the R&D of new drugs, diagnostics and medical devices, and provides access to a wide range of specialized services, technological platforms and advanced facilities to both incubated companies and external subjects.

The incubation service package includes access to offices and laboratories and the use of instrumentation at competitive costs with respect to market standards. The bio-incubator offers over 3000 m<sup>2</sup> of spaces equipped with laboratory modules of up to 150 m<sup>2</sup> that are equipped with modern technologies. A BSL3 laboratory, an animal facility and common spaces dedicated to analytical equipment are also available to incubated companies. Moreover, a team of professionals with scientific and industrial backgrounds who are specialized in technology transfer processes is responsible for supporting incubated companies at every phase of their development, also by making a consolidated network of experts and consultants available.

All the services, including access to instrumentation and technological platforms, are available not only to incubated and affiliated companies but also to companies and public and private research groups external to the structure.

TLS is open to evaluating new applications from private individuals, companies, and organizations that wish to enter the bio-incubator in order to begin or develop research activities and services in the biotech, diagnostics, medical devices, nutraceuticals and cosmeceuticals, and new technologies applied to life sciences fields.

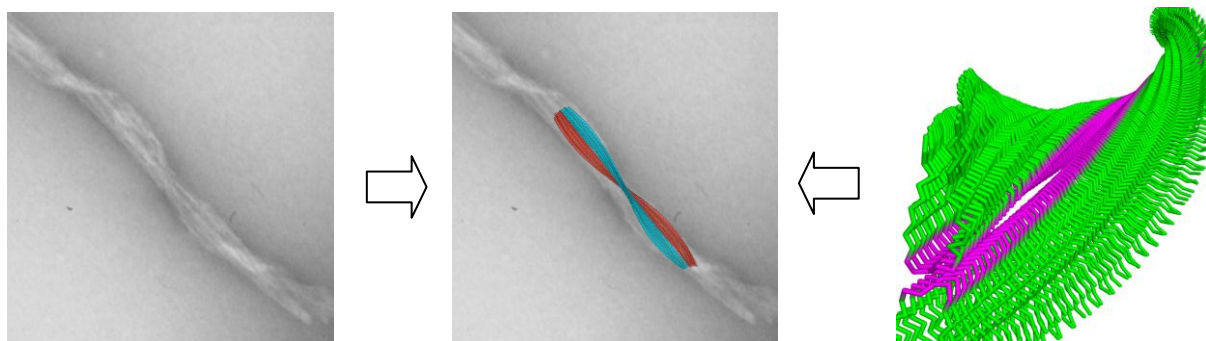
Furthermore the TLS Foundation has explicitly identified acting in the orphan disease field among its institutional objectives by trying, in particular, to act as a link between basic research and industrial application in the biomedical and pharmaceutical field. More explicitly, TLS is trying to implement some actions that do not regard basic research, which is the first phase of study, with the objective of making the consequent scientific results industrially developable.

In 2013, the project "A feasibility study for the in vitro development of an enzyme replacement therapy targeted to the treatment of cblC type methylmalonic acidemia with homocystinuria", led by researcher Laura Tinti, was financed by Telethon as part of the "2013 exploratory projects call" dedicated to the support of hitherto neglected diseases of a genetic origin.

**A structural account on the amyloidogenic process of the atrial natriuretic peptide: a multi technique approach using nuclear magnetic resonance, electron microscopy and bioinformatics**

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Natriuretic peptides are peptide hormones that are synthesized by the heart, brain and other organs. The release of these peptides by the heart is stimulated by atrial and ventricular distension, as well as by neurohumoral stimuli, usually in response to heart failure. The main physiological actions of natriuretic peptides is to reduce arterial pressure by decreasing blood volume and systemic vascular resistance. Atrial natriuretic peptide (ANP) is a 28 amino-acid peptide that is synthesized, stored, and released by atrial myocytes in response to atrial distension, angiotensin II stimulation, endothelin, and sympathetic stimulation (beta-adrenoceptor mediated). Therefore, elevated levels of ANP are found during hypervolemic states (elevated blood volume), which occurs in congestive heart failure (CHF). ANP is first synthesized and stored in cardiac myocytes as prepro-ANP, which is then cleaved to pro-ANP and finally to ANP. ANP is the biologically active peptide, characterised by a cysteine pair forming either an intra-chain disulphide bridge leading to a cyclic, monomeric peptide ( $\alpha$ -ANP), and a cross-chain, double disulphide bridge leading to an antiparallel dimer ( $\beta$ -ANP).  $\alpha$ -ANP amyloid is responsible of isolated atrial amyloidosis (IAA), a fibril deposit known to play an important role in the pathophysiology of CHF [1,2]. In the present study, the structural behaviour of  $\alpha$ -ANP amyloidogenic process has been investigated by a multi-technique approach, combining Nuclear Magnetic Resonance (NMR), Transmission Electron Microscopy (TEM) and bioinformatics tools. NMR studies in aqueous media has demonstrated the process to rely mostly on addition of monomers to the elongating fibril rather than on formation of large, soluble intermediates. Such behaviour, together with the evidence of the monomers being completely unstructured, make the elongating fibril the structural target of choice for rational design of modulator molecules. Matching of simulated  $\alpha$ -ANP protofilaments with TEM micrographs of amyloids, followed by refinement with molecular docking/dynamics simulations, allowed for a the modelling of the fibril structure at atomic level, resulted to be of the 2-ring type, similar to that found already in amylin and A $\beta$  amyloids [3]. Such model will be the starting point for structure-based, de-novo design of small molecule disruptors of elongation interfaces. Furthermore, the peculiarity of  $\alpha$ -ANP cyclic structure and of its monomer-based elongation model make it a suitable system for atomistic studies of amyloidogenic processes.



TEM image of an  $\alpha$ -ANP fibril obtained at pH 4: the twist and flatness of the structure are apparent.

Matching of the simulated structure of the 2-ring type fibril onto the TEM image. Coiled protofilaments are highlighted in cyan and red.

2-ring type model of the fibril: the opposing peptide loops, constituting the protofilament interface, are highlighted in magenta.

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## 20 June 2017 – LAB SESSION

### ToF-SIMS analysis for food chemistry

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Nowadays there is a growing interest of researchers for the application of sophisticated analytical techniques in conjunction with statistical data analysis methods to the characterization of natural products to assure their authenticity and quality, and for the possibility of direct analysis of food to obtain maximum information. In this work, time-of-flight secondary ion mass spectrometry (ToF-SIMS) in conjunction with principal components analysis (PCA) are applied to study the chemical composition and variability of different food matrices:

- Sardinian myrtle (*Myrtus communis* L.): analysis of both berries alcoholic extracts and berries epicarp. through the. ToF-SIMS spectra of berries epicarp show that the epicuticular waxes consist mainly of carboxylic acids with chain length ranging from C20 to C30, or identical species formed from fragmentation of long-chain esters. PCA of ToF-SIMS data from myrtle berries epicarp distinguishes two groups characterized by a different surface concentration of triacontanoic acid. Variability in anthocyanins, flavonols,  $\alpha$ -tocopherol, and myrtucommulone contents is showed by ToF-SIMS PCA analysis of myrtle berries alcoholic extracts.

- Seggianese olives and olive oil: three different groups of Seggianese olives: (i) treated with an insecticide (dimethoate) and a fungicide (copper oxychloride) (TU); (ii) untreated (UT); and (iii) treated-washed (TW) have been analyzed by TOF-SIMS. Intact olive slices and olive oil were analyzed. Principal component analysis (PCA) of the ToF-SIMS spectra was used to investigate similarities among samples. Results showed that TW olives were more similar to UT samples than to TU samples. However, the washing process was not totally effective, since the treatment was able to induce alterations in the composition of the olives.

- Peaches and nectarine: samples of yellow-fleshed peaches (*Prunus persica* L. Batsch) and nectarines (*Prunus persica* L. Batsch, var. Nectarina) of four different cultivars (RomeStar, Zeelady, peaches; Venus, Nectaross, nectarines) from two geographic areas of Southern Italy, "Piana di Sibari" and "Piana di Metaponto", located on the Ionian coast of Calabria and Basilicata regions. ToF-SIMS analysis confirmed the presence of Cyanidin and Phosphatidylcholine in the skin of peaches and nectarines, and Cyanidin, Phosphatidylcholine, Oleic Acid and Coniferyl Alcohol in the skin of seed. The Cluster analysis obtained from the skins of the seeds, constitutes a viable option for the geographical characterization of peaches and nectarines. ToF-SIMS with statistical data analysis is a promising method for thoroughly investigating the chemical composition and variability of food and natural products, allowing to extrapolate information on geographical origin and possible adulteration.

**27 June 2017 – LECTURE**

**Consumer protection**

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Consumer protection, from the original mention in the Article 2 of the Treaty of Rome, has experienced a constant evolution, moving from the protection of contractual reliance toward the protection of market reliance. The new course of the consumer protection, starting from the European Charter of the Rights of the Consumers and continued by the Treaty of Maastricht, Treaty of Amsterdam and lastly with the Treaty of Lisbon. This path has influenced the internal law of European member states, including Italian law.

A specific protection has been established for specific markets, in view of the special protection needs of consumers. This is what happened in the case of the consumer of foods, also due to food scandals of the 90's that revealed the inadequacy of the European food safety system. A whole new system of food safety is born with the Regulation No 178/2002, that establishes in the hands of the consumer non-negotiable rights such as life, information and safety.

**27 June 2017 – LECTURE**

**The sea-surface microlayer: biogenic composition in a changing ocean**

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The sea-surface microlayer (SML) is the oceanic uppermost boundary in contact to the atmosphere, a sort of biofilm matrix where microorganisms and organic material largely contribute to its physical and chemical structure. By accumulating organic material, this surface film damps capillary waves and influences air-sea gas exchange across the ocean's surface. Furthermore, organic compounds in the SML contribute to organic enrichment of bursting bubbles at the sea-surface, creating droplets that later dry out in the air as organic marine aerosols. This presentation addresses the organic composition of the SML in response to anthropogenic-driven climate and ocean changes. Sea-surface warming, ocean acidification, and reduced oxygen concentrations by influencing ocean's biological dynamics, may affect the turnover of organic material in the SML with potential effects on air-sea exchange processes and climate.

**27 June 2017 – LECTURE**

**Molecular authentication of food and wine**

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Gourmet food and wine markets move annually, millions of Euros/dollars and especially in Mediterranean Countries, genuine food production is of strategic importance for the internal food production. At the same time, food fraud and counterfeiting are emerging issues within the food and wine sectors. It is a major concern not only for consumers, but also for producers, distributors and authorities. These illegal activities result in considerable monetary losses worldwide and diminishing consumer confidence. Ensuring the authenticity of food and wine requires powerful and reliable tools for food analysis, traceability, and control. Therefore, there is a great need to identify foods and wines with regard to declared composition. The research on food authentication is of strategic importance for the EU and that's why many Universities and Research center in the world, have posed attention to the innovation in the biotechnology potential in solving the current issues that are faced in this matter. The aim of the seminar consists mainly in a general survey of the molecular approaches available to the consumer and scientific community for authenticating food composition. The teaching will focus on the need of authentication as an essential anti-counterfeiting tool for supporting policy oriented in defending local markets in relation to general international trading exchange of food. Even if focusing on molecular approaches, the lesson will consider also the possibility of integrating different analytical procedures, including metabolomic and chemical approaches. Special attention will be given to molecular techniques applied to plant-derived food and wine varietal characterization, discussing several cases of wine complexity and responding to specific experimental questions.

**27 June 2017 – LAB SESSION**

**NMR Applications for Food Chemistry**

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NMR techniques clearly provide powerful tools for the study of the physical and chemical properties of foods [1,2]. Foods are very complex and highly heterogeneous systems comprising a large number of chemical compounds, the composition of which is dependent on agronomical or slaughter practices, industrial processes, storage, maturation, etc. 1D liquid or solid-state high-resolution NMR spectroscopy can provide in a single experiment a huge number of structural and quantitative information. These experiments need no separation of the various food components and no serious sample pre-treatment. Moreover, NMR spectroscopy is sensitive to dynamics, which allows differentiation between molecules or groups of molecules with different mobility through spin-lattice and/or spin-spin relaxation measurements

Practical information about NMR instrumentation, experiment setup, acquisition and spectral analysis for the study of different food categories will be explored and discussed.

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**HH-XRF of silver coins and archaeological finds from the "Colline Metallifere district"  
(South Tuscany)**

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The archaeological study of the excavated remains and the chemical analysis of archaeological finds and materials represent multidisciplinary efforts to reconstruct the human past. The application of scientific techniques to the study of archaeological artifacts is an increasingly important dimension of archaeological research. For this reason there is a strong moral and practical requirement to extract the maximum information from material remains recovered during archaeological investigations. All the finds are materials that require chemical analysis to be understood fully and these provide a sharper lens for us to use when explaining material culture. Using suitable analytical methods it is possible to reveal data about the selection of raw materials and manufacturing processes (temperature, pressure, time etc...), the provenance of materials and the early trade of archaeological finds.

Research into metallurgy has long focused on the scientific analysis of production debris with the aim of elucidating the technical processes that created these artifacts. Metalworking activities and more the specific areas in these activities played out tend to imprint its signs in soil contexts that can be detected by geochemical techniques. Chemical investigation are requested to know the purity of the metal produced, the composition of the waste slag and to give an understanding of the general nature of the technology involved as well as a more detailed knowledge of the operating conditions of the process.

The non-destructive character of the handheld X-ray fluorescence analysis makes it ideal to determine the major, minor and trace elements composition of precious metal artefacts, in particular coins and archaeological finds.

This study is part of a wider project named "Origins of a new economic union (7<sup>th</sup>-12<sup>th</sup> centuries): resources, landscapes and political strategies in a Mediterranean region" (nEU-Med), that received a five year ERC grant in 2015. Its main objective is the study of the Colline Metallifere landscapes features in order to reconstruct and understand the deep changes occurred in settlements patterns, trade routes and, more in general, in the economical background of the area between Late Antiquity and the 12<sup>th</sup> century. The core of the project is the multidisciplinary approach to the study of the territory to identify the major factors of the economic growth in relation both to the exploitation of agriculture and natural resources to the production and circulation of money.

Among the factors able to strongly promote the economic changes, we are focussed on the well known and long-standing tradition of ore mining and processing in the Colline Metallifere district. Metal deposits are mostly characterized by mixed sulphides ores Cu-Pb-Zn(Ag) and were exploited for the production of copper, silver, lead and iron since Etruscan times. The aims of the research is mainly to establish metal sources for coinage in the period of interest, and, in particular, the role played by the Colline Metallifere copper/silver ores.

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## 27 June 2017 – LAB SESSION

### Biochemical alkaline phosphatase assay

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The process of bone matrix mineralization is relatively poorly understood; crucial steps are the initial extrusion of calcium-rich vesicles, the enzymatic cleavage of pyrophosphate and the following formation of the first mineralized nuclei.

As osteoblasts differentiate from their precursors, they express alkaline phosphatase and begin to secrete bone matrix proteins, mainly collagen type 1, osteocalcin, matrix gla protein, osteopontin, bone sialoprotein, and, growth factors such as BMPs and TGF- $\beta$ .

Alkaline phosphatase enzymatic activity is essential for efficient osteoblastic mineralization. It hydrolyzes the ester bond of organic phosphate compounds under alkaline conditions, playing an important role in bone calcification: on one hand, it hydrolyzes substances that inhibit calcification, such as pyrophosphate and ATP, on the other it is indispensable for increasing the levels of phosphate required for hydroxyapatite crystallization.

Alkaline phosphatase activity will be evaluated on monolayer cultures of human osteoblastic-like cells, namely SaOS-2, grown in complete medium or in osteogenic medium (3.5 mM  $\beta$ -glycerophosphate, 50  $\mu$ g/mL ascorbic acid, and  $10^{-8}$  M dexamethasone). Briefly, cell lysates will be incubated with p-nitrophenyl phosphate and the production of p-nitrophenol (yellow) will be monitored spectrophotometrically at 405 nm. The amount of p-nitrophenol corresponds to alkaline phosphatase activity. The results of the activity assay will be normalized by the total protein content of the cells.



DIPARTIMENTO DI BIOTECNOLOGIE,  
CHIMICA E FARMACIA



EMORY  
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OF ARTS AND  
SCIENCES

Department of Chemistry

# 1st Emory-UniSi Research Symposium & DBCF Research Day with Poster Session

**12 June 2017 (9.00 – 18.00)**

**Aula Magna – Polo Scientifico S. Miniato  
Via A. Moro, 2 – 53100 Siena**

All students of Chemistry, Chemical Sciences, Phar

maceutical Chemistry and Technology, Pharmacy are warmly welcome to participate in the Symposium where they will have the opportunity to familiarize with the current research activities of DBCF

Gli studenti dei Corsi di Studio in Chemistry, Scienze Chimiche, Chimica e Tecnologia Farmaceutica, Farmacia sono gentilmente invitati a partecipare al Simposio dove avranno l'opportunità di conoscere le attività di ricerca del DBCF



PEGASO TUSCANY REGION PhD SCHOOL IN  
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PhD SCHOOL IN  
CHEMICAL AND PHARMACEUTICAL SCIENCES



# FIELD TRIPS

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## **05 JUNE 2017**

VISIT TO BARONE RICASOLI VINEYARD AND CELLAR

## **22 JUNE 2016**

VISIT TO ISVEA

VISIT TO SOVESTRO IN POGGIO

Note - The abstracts reported in the following pages related to the companies/factories are downloaded from the respective websites or from previous editions.



05 JUNE 2017

## VISIT TO BARONE RICASOLI VINEYARD AND CELLAR

<http://www.ricasoli.it/>

Ricasoli is the oldest winery in Italy, the second oldest in the world according to the leading American magazine Family Business. Today it is the largest winery in the Chianti Classico area: Brolio Castle, where Baron Bettino Ricasoli invented the Chianti formula in 1872, is surrounded by 1,200 hectares in the communes of Gaiole and Castelnuovo Berardino. Valleys, hills, woods of oak and chestnut trees, 240 hectares of vineyards and 26 hectares of olive groves, all enjoying the beauty and the wide variety of soils and climate in this central Chianti area.

In the middle of the 1990s, Barone Ricasoli started a huge project to renew the vineyards in its Chianti Classico land. They were old vineyards (all planted at the end of the 1960s and the beginning of the 1970s), ripped apart by Esca, with low densities per hectare and containing all the varieties belonging to Chianti Classico, but distributed randomly. So an excellent opportunity arose to renew and improve the vineyards, while introducing international varieties, such as merlot and cabernet, at the same time.

Considering that most of the land was made up of rock and that the breaking up of the land had to be done using ploughs and explosives, the problem of reclamation proved to be complicated and hard right from the start.

So far 204 hectares have been replaced in Brolio, using modern preparation techniques and genetically selected material, all aimed at obtaining long-lived vineyards capable of producing high-quality grapes.

Plant spacing was based on high density and the number of vines varies from 5500 to 6600 per hectare. The training system is spurred cordon, 50 cm from the ground. There are 8 buds per plant and crop thinning enables a yield of about 1 kg of grapes per vine (65-70 quintals/ha). The white grape varieties, on the other hand, are guyot trained so as to exploit bud fertility to the full.

The attention paid to the *terroir*, which influences Barone Ricasoli's decisions, is at the base of the zoning study.

The harvest is done separately for every vineyard plot. The grapes are taken to the vat room in containers with a maximum capacity of 200 kg; vinification takes place in small steel vats, enabling us to carefully control the fermentation process and to keep all the characteristics of every single vineyard plot separate. Experiments and a thorough knowledge of the land have led us to vinify separately also within the same plot and according to the morphological similarities of the subsoil. The structure of the vinification vat room was devised so that the vats can be filled by means of gravity, which allows a gentle punching down so as to extract the noblest substances from the skins. At the end of the fermentation process the wines are transferred to barrels and oak barriques. The frequent organoleptic and laboratory controls accompany Ricasoli wines throughout their development right up to the long (sometimes very long) bottle maturation, in appropriate heat-controlled rooms, before being sent off to the four corners of the earth.

Brolio has always been a prime place for innovation and experimentation. It was here that Bettino Ricasoli, in search of a "sublime wine", invented the Chianti formula in 1872, after three decades of patient research and meticulous experiments, a compromise between art, passion and science: "...meanwhile, we beat our path to you, science and a little bit of art; to me more art than science" (Bettino Ricasoli, 1873).

**22 JUNE 2017**

**VISIT TO ISVEA LABORATORIES**

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Recently the chemical and biochemical analysis on enology science are a “fundamentals” for vinification processes. The fast improvements of the knowledge about chemical and biological transformation processes from grape to wine, start new needs for enologists to improve the quality of the products new analytical methods, by higher precision, high accuracy, and efficiency. National and European Community Regulations were introduced to preserve population health and discovering illegal procedures and for identify noxious compounds. From the size and complexity of this mechanism origin specialized analytical laboratories, where the expertise of enologists and chemists have been combined. The choice of proper analytical methods has fundamental importance for a laboratory: it is therefore mandatory a dip knowledge on several analytical techniques and on the principal characteristics of the methods (like precision, accuracy and sensitivity) with the goal to choice the optimum compromise for the client needs.

In enological laboratory different types of analytical determinations have to be carried out:

- Standard enological techniques (determination of alcoholic degree, volatile and total acidity, sugar, sulfur dioxide, ...)
- Analysis for product certification (check for the selected parameters to be under the maximum allowed values)
- Analysis of toxic species in food beverages (heavy metals, micotoxins, pesticides, ...)
- Analysis and characterization of the products (poly-phenol profile, aromatic profile, acidic profile, flavor, and tasting defects, ...).

As regards the first type of analysis a fast and highly reproducible analytical response is requested with the aim to compare the analytical data for the same amount of wine as function of time, and to be able to assure a fast response in the case the analysis shows unacceptable parameters. This fact requires the usage of sound, reliable and highly efficient procedures. The accuracy is less important than precision.

On the contrary the analyses for certifications require accurate and officially accepted analytical methods. In this country the analytical laboratories are request to reach accreditation by the health authorities on the basis of ISO 17025 regulations to be allowed to certify wine quality.

The research of contaminants demands highly sensitive and accurate methods because the determination of trace species, for which the maximum allowed amount are very low, is needed. The analysis aimed to characterize the species are the most interesting ones: in fact the applied research allow the validation of new methods through very innovative techniques.



**22 JUNE 2017**

**VISIT TO SOVESTRO IN POGGIO VINEYARD AND CELLAR**

<http://www.sovestroinpoggio.it/>

Sovestro in Poggio is a farm located on the hills of Chianti near San Gimignano. We are a small farm and we produce wine, olive oil, grappa and sweet wine. We delight our self in receiving people from all over the world and we are pleased to involve everybody in our activities.

The visit to our farm consists in explanation of viticulture, visit to the cellars, vinification, technical wine tasting with typical Tuscan foods.

We love what we are doing, we love nature and what nature gives us: our wines and the simplicity of our life.

Mostly we love people, particularly when people appreciate the products from this land.

We are waiting you.

