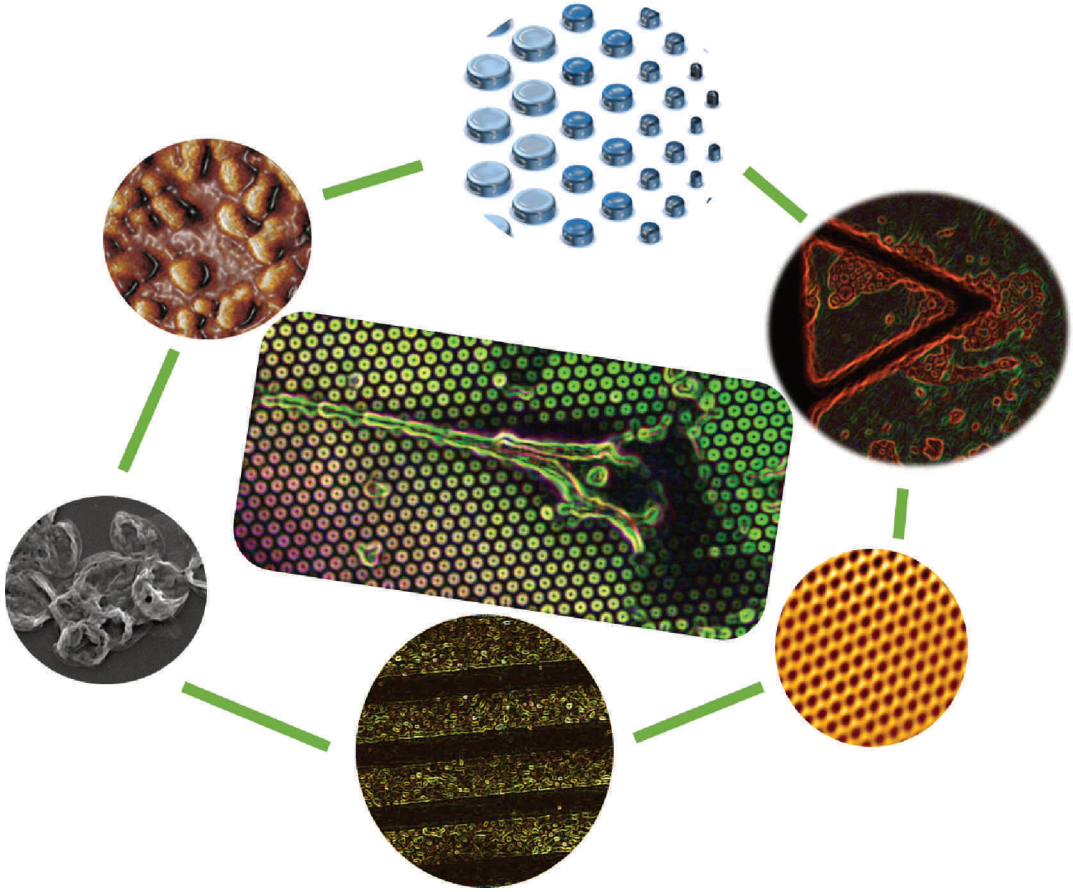

2014 Annual Report

Institute for Biophysics

Department of Nanobiotechnology
University of Natural Resources and Life Sciences,
Vienna

2014 Annual Report
Institute for Biophysics

DEPARTMENT OF NANOBIOTECHNOLOGY
University of Natural Resources and Life Sciences, Vienna
March 7, 2015

This is already our second annual report. This is good news. We go further. 2014 has been a year of changes. Actually, a good one. I think that it has been better than 2013 in terms of institute development and group dynamics.

In general terms, we have contributed for the Department of Nanobiotechnology in several ways. We have managed laboratories that are used by the two other DNBT institutes and guest scientist (mainly from BOKU and the Austrian Institute of Technology). We have also improved our teaching, reaching registrations of about 35 students in master lectures. I hope that this positive development will enhance in the near future the interest of the students to do bachelor and master diplomas with us. Concerning the teaching, we will also reorganise the laboratory of "Methods in Ultrastructure Research" focusing on new biological systems that can be investigated with electron and scanning probe microscopy. The lecture will be coordinated by Dietmar Pum. In addition to the necessary burocratical work, we have participated in BOKU commissions and meetings such as DokStuko, Forschungsprecher, Department Head Conference, Ethics, and FachStuko among others.

Our research activities have maintained the level of 2013. Briefly, our numbers are: 13 (SCI) publications, 1 book chapters and 18 communications to conferences, workshops or seminars (including invited lectures). This performance is not bad for an institute of about 13 people on average (the total number of people working during 2014 was 24; this includes research visitors and guest students). Again this year, we have got good contributions about S-layer research from Dietmar Pum, who has also proved that he has very good collaborations. Uwe Sleytr's ideas about the interaction of S-layer with cells (inside and outside microfluidic devices) have been successfully published (through the collaboration with the Austrian Institute of Technology and former coworkers of his that are now part of the Institute of Synthetic Bioarchitectures). Finally, my own group contributed to basic research about cell/substrate interactions, the influence of the substrate on cell uptake and the use of mathematical techniques on biosciences.

I do not want to forget to mention that our emeritus Uwe continues getting international recognition. If in 2013, he was awarded with an honorary professorship at the Jiao Tong University in Shanghai; during 2014 he has been appointed as Honorary Member of the Austrian Business Association and Member of the Genetic Commission of the Austrian Academy of Sciences (what a long name, impressive!). In another context, from my part,

I accepted to be part of the editorial board of Microscopy Research and Technique (Wiley).

I am especially pleased to say that new people joined our time. Dr. Jagoba Iturri (from the Max-Planck Institute for Polymer Research, Mainz) brings to us a large expertise is surface science modification and analytical techniques. He will stay for six years to do his habilitation on the cell/substrate interaction and its influence on cell mechanics, a topic that I believed will grow in the community in the near future. New students (that I hope will "polished" the labs) started their PhD thesis with us: Alberto Moreno, Michael Handler and Maria Sumarokova. Let us wish them a lot of success.

During 2014, we also had the luck and the pleasure of receiving guest students (PhD, Erasmus), who did the main part of their diploma works with us. They have contributed to increase our scientific and technical knowledge. Also, they have improved the group dynamics (with their sense of humor) and boosted cultural and human exchange in general (which was of chaotic nature, I must say). We will continue inviting (foreign) students and scientist.

In 2015 we need to go further with two main research lines: i) mechanical properties of biomaterials (mainly cells) and ii) dynamic molecular interactions. I hope that a new university assistant (with AFM expertise) will help us to consolidate such difficult but promising task.

Finally, as last year, I would like to thank all the members of the institute and visitors that made this possible, and especially to those who left during 2014 to continue a professional career somewhere else.

José L. Toca-Herrera

PS: As last year, many thanks to Alberto for the final editing!

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Institute members and visitors

- Univ. Prof. Dr. José L. Toca-Herrera (Director)
- Ao. Univ. Prof. Dr. Dietmar Pum (deputy Director)
- O. Univ. Prof. em. Dr. Uwe B. Sleytr (emeritus, former Director)
- Dr. Jagoba J. Iturri (univ. assistant)
- Dr. Kathryn Melzak (univ. assistant)
- Dr. Med. Michael Handler (PhD student, collaboration with Sports Univ. Innsbrück)
- MMSc. Ewa Oprezska-Zingrebe (PhD student)
- Mag. Jacqueline Friedmann (techn. assistant)
- Mag. Eva Ladenhauf (PhD student)
- MSc. Maria Sumarokova (PhD student, Erasmus Mundus Iamonet Program)
- MSc. Elham Ghorbani Gorji (PhD student, collaboration with Inst. Food Sci. - BOKU)
- MSc. Sudarat Tharad (PhD student, collaboration with Mahidol University, Thailand)
- MSc. Alberto Moreno cencerrado (PhD student)
- MSc. Batirtze Prats Mateu (FFG grant student, collaboration with AIT)
- MSc. Elisa Rocchi (Erasmus student, Univ. of Milano, Italy)
- MSc. Denisse Bender Bojalil (visiting researcher, CONACYT, Mexico)
- Piero Sabella (Erasmus student, Univ. of Parma, Italy)
- Remedios Gomez Infante (Erasmus student, Univ. of Extremadura, Spain))
- Antonio Miranda del Alamo (Erasmus student, Univ. of Extremadura, Spain))
- Beatriz Lucas Delgado (Erasmus student, Univ. of Extremadura, Spain))
- Teresa Leon Sala (Erasmus student, Univ. Politécnica de Valencia, Spain)
- Ana Carol Vianna (IAESTE student, Brasil)
- Margareta Mittendorfer (BSc student, BOKU)
- Claudia König (apprentice)

1 Research Topics

S-layer based bio-imprinting - Synthetic S-layer polymers

Dietmar Pum, Eva Ladenhauf, David Schuster, Uwe B. Sleytr

Topics

Molecular imprinting based on S-layer proteins as templates was one of the scientific topics in the year 2014.

Results

Based on the experience in making S-layer coated liposomes, work continued with the imprinting of such liposomes. As an alternative to planar S-layer templates, spherical structures offer the advantage of higher surface area and consequently higher number of functional groups per projected unit area. Although considerable knowledge is available in the group concerning the preparation of S-layer coated liposomes, it was not possible to make successful S-layer-liposome imprints. Liposomes are not stable in DMSO which is mandatory in the course of the polymer preparation. TEM and AFM studies showed that S-layer coated (water filled) liposomes are destroyed before a cavity is formed. It is assumed that DMSO penetrates the pores and dissolves the lipid bilayer of the liposome. Nevertheless, AFM demonstrated that S-layer coated bacterial cells (L. sphaericus CCM2177) can be successfully used as templates in the printing process. This is not surprising since the bacterial cell wall is much more rigid than the bilayer of the liposomes.

Further on, Surface Plasmon Spectroscopy (SPR) confirmed the results from the QCM measurements and the fact that the S-layer based imprinting is successful despite the difficulties in obtaining high-resolution AFM images. As shown in the figure below, the rebinding of SbpA S-layer protein at imprinted areas led to a clear response (left) while no signal was obtained at the non-imprinted regions (right). The high noise level is due to the relatively large thickness of the polymer.

However, Peak Force Quanto Nanomechanical Mapping (Peak Force QNM), whose application is completely new in the field of molecular imprinting, was used to demonstrate the selective binding of S-layer protein on its respective imprints. Peak Force QNM is an AFM technique and a suitable mean to demonstrate, for example, biological layers on corrugated surfaces by probing the local adhesion with a tip. As a result, an adhesion map and a corresponding height image are obtained. By comparing the adhesion maps of imprinted and non-imprinted areas the specific rebinding of SbpA S-layer protein could be shown.

Finally, it was found in the course of the printing experiments that the polymer is partly detached from the gold electrodes when removing the stamp.

Coating of the gold electrodes with L-cysteine improved the adhesion of the polymer considerably and, thus, is part of the standard preparation protocol now.

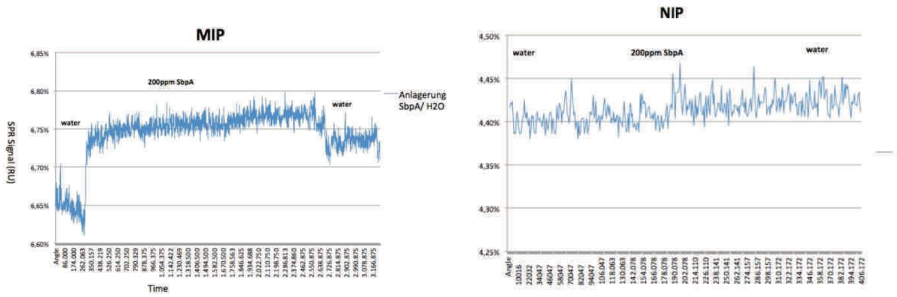

Figure 1: SPR measurements demonstrating proof-of-principle for S-layer based molecular imprinting. Binding of SbpA on imprinted (left) yields a clear response while non-imprinted areas (right) no signal is obtained.

Outlook

The next steps will focus on the synthesis of metallic nanoparticles on the imprints and the double imprinting where an imprint of the imprint is formed.

Acknowledgements

The support of Prof. P. Lieberzeit, Institute of Analytical Chemistry, University of Vienna and his team is gratefully acknowledged. This work is funded by the Air Force Office of Scientific Research (AFOSR), Agreement award FA9550-12-1-0274.

On the interaction between resveratrol and carrier proteins

Elisa Rocchi[1], *Denisse Bender-Bojalil*, *Elham Ghorbani-Gorji*[2], *Laura Piazza*[1], *Gerhard Schleining*[2], *José L. Toca-Herrera*

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[2]*Institute of Food Science, Department of Food Science & Technology, BOKU-Vienna, Austria*

Aim

Study the interaction of resveratrol (RES) with three different carrier proteins (β-lactoglobulin, β-casein and bovine serum albumin (BSA)) in order to elucidate the strongest bonding for future encapsulation strategies.

Results

In order to evaluate the interaction between the carrier proteins and RES, the samples were homogenized at various concentrations, using four different molar ratios RES-protein: 1:4, 1:2, 1:1, 2:1.

The stability of the system was evaluated measuring zeta potential. At 20mM, all pure protein solutions show negative values relatively close to the isoelectric point ($< |30|$ mV [1]), suggesting that hydrophobic aggregation easily takes place. The response of β-casein solution after the addition of increasing concentrations of resveratrol is almost constant, thus it can be assumed that there is no significant interaction between the components (fig. 1). On the contrary, both β-lactoglobulin and BSA have a maximum peak in presence of an equimolar concentration of resveratrol (fig. 1): this leads to the supposition that in these cases the ligand interacts with polymers forming 1:1 complexes. The influence of resveratrol on the microstructure of proteins was investigated by means of TEM. The sample was absorbed on the surface of a carbon coated copper grid and fixed with a negative staining method. The images of the isolated carrier proteins (20 μM) reveal the presence of aggregated structures while it can be observed a reduction of the aggregates size consequently the addiction of resveratrol (20 μM) in a molar ratio 1:1. In particular, the complexes with BSA and β-casein form structures that are significantly smaller than the ones formed by β-lactoglobulin. On the contrary the addition of an excess of the bioactive compound (80 μM) to the protein solutions generates larger aggregates compared to lower concentrations of resveratrol. The investigation was completed in collaboration with the Department of Food Technology, where analyses of fluorescence spectroscopy and circular dichroism were carried out.

[1]Salgın, S., Salgın, U., & Bahadır, S. (2012). Zeta Potentials and Isoelectric Points of Biomolecules: The Effects of Ion Types and Ionic Strengths. *Int. J. Electrochem. Sci*, *7*, 12404-12414.

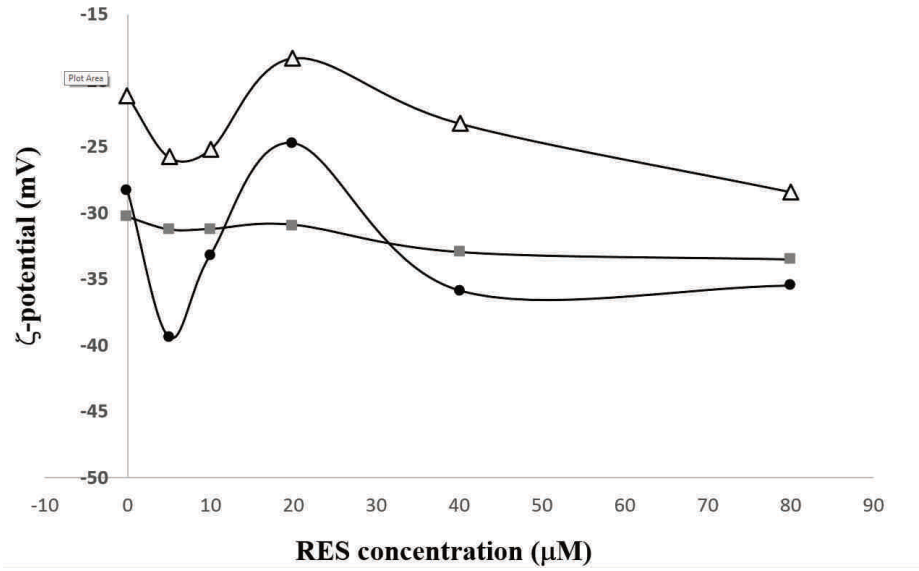

Figure 1: Variation of zeta potential of protein-resveratrol solutions as a function of the bioactive concentration; (•) β-lactblobulin, (□) β-casein, (△) bovine serum albumin.

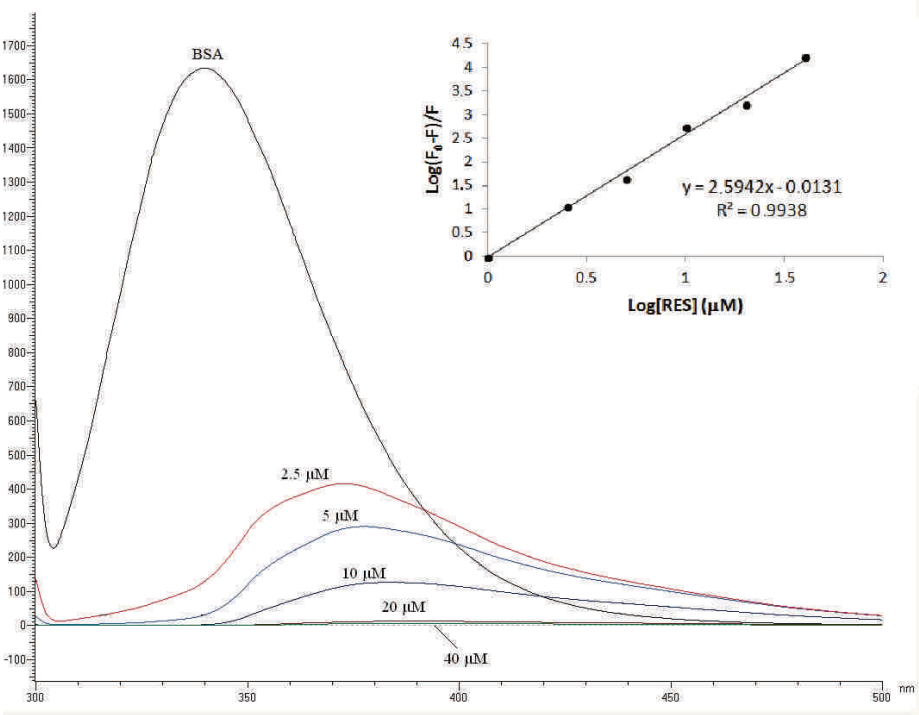

Figure 2: Fluorescence emission spectra of 20 μM BSA in the presence of 2.5, 5, 10, 20 and 40 μM of resveratrol in 10mM phosphate buffer at pH 7.4

The evaluation of the emitted fluorescence allowed us to calculate the binding constants:

- BSA-RES $K_a = 3.84x10^6 M^{-1}$
- BSA-RES $K_a = 3.84x10^6 M^{-1}$
- β-casein-RES $K_a = 2.68x10^6 M^{-1}$
- β-lactoglobulin-RES $K_a = 1.8510^6 M^{-1}$

Circular dichroism gave some information about the proteins structure, in particular the addiction of resveratrol does not affect significantly the far-UV spectra of the proteins so it can be assumed that the interaction RES- protein has no effect on their secondary structure.

Conclusions

The study shows that resveratrol can interact with β-lactoglobulin (BLG), β-casein (BCN), bovine serum albumin (BSA) to form strong complexes, which can be considered as potential carriers of resveratrol. More experiments concerning binding strength and complex structure are currently carried out.

Laser-Assisted Generation of Nickel Nanoparticles in Liquid

Niusha Lasemi[1], *Ulrich Pacher*[1], *Jacqueline Friedmann*, *Dietmar Pum*, *Christian Rentenberger*[2], *Herwig Peterlik*[2], *Wolfgang Kautek*[1].

[1]*University of Vienna, Department of Physical Chemistry, Vienna, Austria;*

[2]*University of Vienna, Faculty of Physics, Vienna, Austria*

Summary

Nanoparticle generation by pulsed laser ablation at a solid-liquid interface was first reported with iron in water.[1] The advantages and potential of laser-assisted nanoparticle productions are high purity, simple starting materials, high efficiency, and laser parameter control of the NP-size.[2][3]

In the present work, nickel nanoparticles were generated from a nickel target by laser irradiation at 532nm (Nd:YAG laser, pulse length 5ns, repetirion rate 20Hz) in ethanol and distilled water without stabilizer. The influence of polyvinyl pyrrolidone (PVP) on size distribution of nanoparticles was investigated as a steric hindrance factor. By increasing the PVP concentration the average size of nickel nanoparticles could be reduced down to the critical radius. The size distribution, the amount of aggregation, the crystallinity and chemical nature of the nanoparticles were investigated by transmission electron microscopy (TEM), selected area (electron) diffraction (SAD), small angle x-ray scattering (SAXS) and energy dispersed x-ray analysis (EDX).

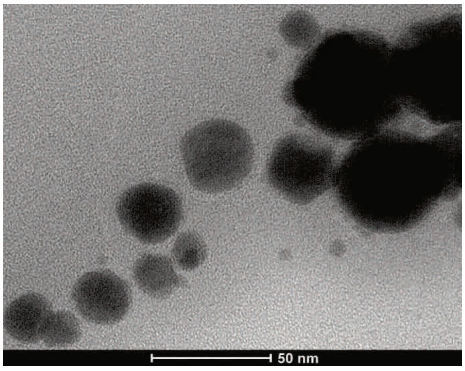

Figure 1: TEM micrograph of Ni nanoparticles generated in ethanol (0.1mM PVP); pulse energy 43 mJ.

[1]P. P. Patil, D. M. Phase, S. A. Kulkarni, S. V. Ghaisas, S. K. Kulkarni, S. M. Kanetkar, and S. B. Ogale, Phys. Rev. Lett., 1987, **58**, 238.

[2]S.C. Singh, H.B. Zeng, C. Guo, W. Cai (eds.), *Nanomaterials: Processing and Characterization with Lasers,* Wiley, 2012.

[3]G. Yang (ed.), *Laser Ablation in Liquids: Principles and Applications in the Preparation of Nanomaterials,* Pan Stanford Publishing, Singapore, 2012.

Characterization of S-layer proteins mixtures at different concentrations: looking for crystalline phase coexistence

Ana Carolina Vianna Cintra[1]*,Alberto Moreno Cencerrado, José L. Toca-Herrera*
[1]*Department of Chemistry, University of São Paulo, Brazil*

Introduction

The main objective of this project is to achieve a complete overview of the physicochemical phenomena between biomolecules and surfaces. In particular we will focus on the called S-proteins and their biophysical interactions: we will study the recrystallization pathways of the bacterial proteins SbpA and SbsB recrystallized on silica, analysing the biophysical interaction between protein/surface and protein/protein, as well as the phase coexistence between crystalline domains.

Surface layer proteins (*S-layer proteins*, or also called *S-proteins*) are one of the commonest envelopes of prokaryotic cells and archaea with outstanding properties that derive from their (sub) molecular morphology and extreme supramolecular symmetry. They have the ability to self-assemble in solution and at interfaces on various surfaces, with functional groups located in an identical position and orientation.

Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) will be used to carry out real time recrystallization processes from SbpA, SbsB and SbpA/SbsB mixtures. We will follow the kinetics of the process, the protein mass deposited per unit area and the steps that lead to the formation of the protein layer, these being adsorption, self-assembly and recrystallization, and crystal reorganization.

Atomic Force Microscope (AFM) will be carried out to characterize the final structure of every mixture. Among the parameter to be considered are: lattice parameters and protein domains size.

Imaging and spectroscopy of S-layers using Scanning Tunnelling Microscopy

Alberto Moreno Cencerrado, José L. Toca-Herrera

Aims

Testing the Scanning Tunnelling Microscopy as a feasible technique to characterize surface layer proteins from bacteria. Estimation of the electric (tunnel) current through s-layer crystals deposited on different chemically modified gold substrates.

Summary

In the last decade, bacterial surface layer proteins (s-layers proteins) have become a major subject due to their ability to build protein crystal layers up with nanometer regularity on many different substrates. This key feature turn them into a feasible template for nanoelectronics. To elucidate this question, the scanning tunnelling microscopy (STM) appears as the best technique: on one hand, due to its ultrahigh-resolution, we can characterize the s-layers at submolecular resolution; and on other hand we can investigate at the same time the electrical properties of bacterial membranes.

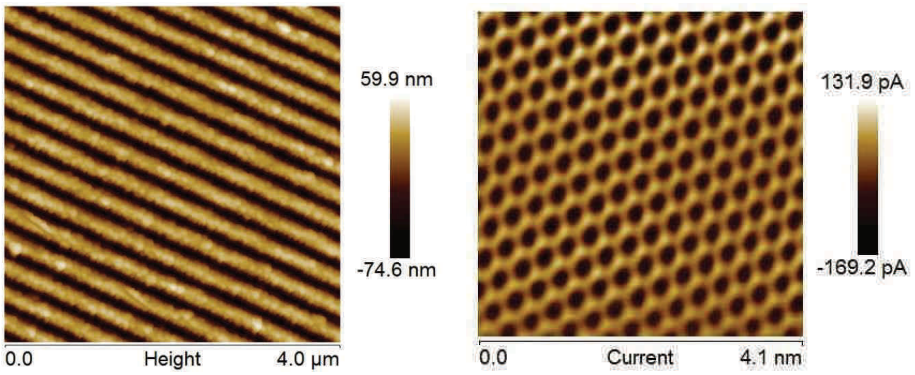

Figure 1: Left image: Calibration's standard for scanning probe microscopes in the X or Y direction. Right image: Highly Ordered Pyrolytic Graphite (HOPG) at atomic resolution. Both STM images show the wide resolution range of the STM techniques.

The only prerequisite to work with the scanning tunnelling microscope is to have sufficient electrical conductivity of the sample. Therefore metals, ultra-high vacuum and low temperatures are the best conditions to research with this technique. However, if the deposited monolayer is thin enough compared to the conducting substrate, we will be able (at room temperature and in air or liquid conditions) to achieve images of the molecular structure and to measure the difference of the electrical tunnelling current from different systems.

In the present project, we are using SbpA as s-layer protein (formed by

Lysinibacillussphaericus CCM2177), which has a monolayer thickness of about 9 nm; and as substrate we prepared modified gold substrates, what include either self-assembled monolayers of thiols with different end-groups, or layer-by-layer deposited oppositely charged polyelectrolytes. We plan to recrystallize the SbpA proteins onto these surfaces and to analyse their electrical and structural properties.

The first modified gold surface consists of different pathways of thiol polymers: ATRP thiol Initiator, and Ethylene glycol. These pathways were printed on an ultra-flat gold surface (Au (111)) using the relief patterns on a polydimethylsiloxane (PDMS) stamp.

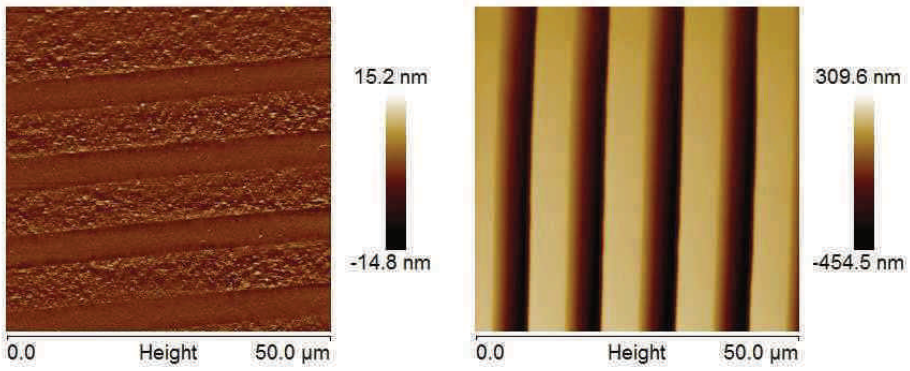

Figure 2: AFM images of micro-contact printing: Left image is the PDMS stamp before immersion in ethylene glycol; right image corresponds to the gold modified surface after immersion overnight in ATRP 1mM dissolution and printed with the ethylene glycol PDMS stamp.

After immersing the PDMS stamp into a 1mM ethylene glycol dissolution, the hydrophobicity of the PDMS keeps the thiol inside the micro-pathways of the stamp. Then we dried the stamp with N2 obtaining a micro-contact printing (μCP) PDMS of ethylene glycol. If we apply this μCP onto a surface (e.g. gold surface), we obtain a well-defined pathways of ethylene glycol. The last step will be to immerse overnight this surface into an ATRP thiol 1mM dissolution, to obtain the two different pathways of thiols.

The second modified gold surface consists of layer-by-layer deposited oppositely charged polyelectrolytes (PEMs). These systems allow us to control the thickness of the surface (with nanometric precision) where we will recrystallize the s-layer proteins on. Starting with a cationic polyethylenimine (PEI), we deposited a combination of anionic polystyrene sulfonates (PSS) and cationic Polyallylamine hydrochlorides (PAH) building different charged surfaces up.

Once we have prepared the gold surfaces, we start the s-layer recrystallization process, following the standard protocols.

Outlook

The main problem that we found in this experiment corresponds to the denaturation of the protein crystals when they come into contact with air. Our STM works in air conditions at room temperature, therefore we have to find a method to keep the crystalline structure.
It was tried to keep the structure using Glutaraldehyde and Uranyl acetate (following the standard protocols of Transmission Electron Microscopy), but for now these attempts were unsuccessful. Another possibilities (supported by scientific literature) to be tested are:

- By controlling the protein hydration: the STM can work in humidity conditions. Under controlled humidity percentage (which the protein needs to form crystals) we will be able to measure with STM in air conditions.
- Conductive metal coatings: probably the easiest way. The problem from this method is that we need to resolve the electrical contribution of the thin metal layer and the S-layer contribution.
- Immersion of the samples into an alkane liquid solution: Alkanes do not conduct electricity. Therefore we could measure our samples with STM in liquid conditions. The question should be if the S-layer protein crystals keep their structure into this medium.

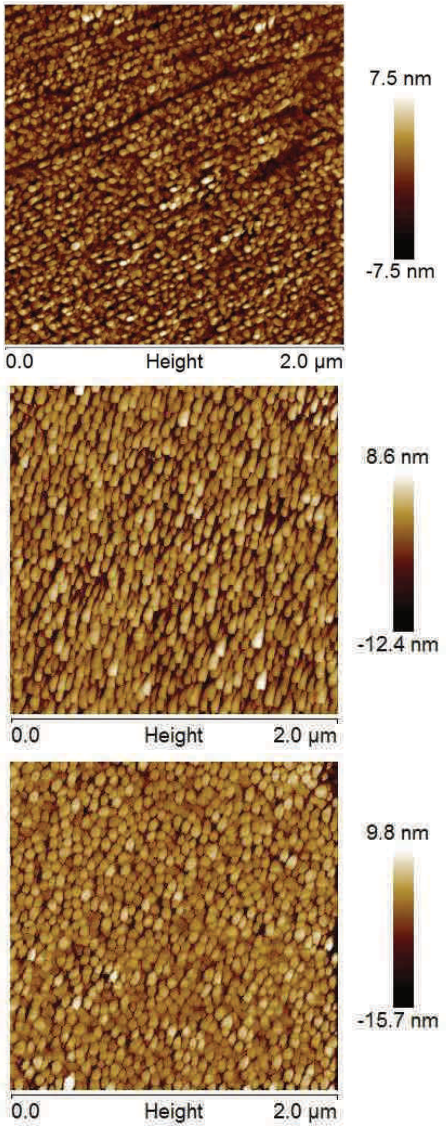

Figure 3. Different STM images of polyelectrolyte systems deposited on Au (111): first PEI, second PEI+PSS, third PEI+PSS+PAH.

Effect of the concentration of cytolytic protein Cyt2Aa2 on the binding mechanism on lipid bilayers studied by QCM-D and AFM

Sudarat Tharad, Jagoba Iturri, Margareta Mittendorfer, Chartchai Krittanai[1]*, Boonhiang Promdonkoy*[2]*, José L. Toca-Herrera*

[1]*Institute of Molecular Biosciences, Mahidol University, Salaya Campus, Nakhonpathom, 73130, Thailand;*

[2]*National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathumthani 12120, Thailand*

Aims

We would like to elucidate: (1) the binding mechanism of the toxine Cyt2Aa2 on lipid bilayers, and (2) the structure of the final protein-lipid layer.

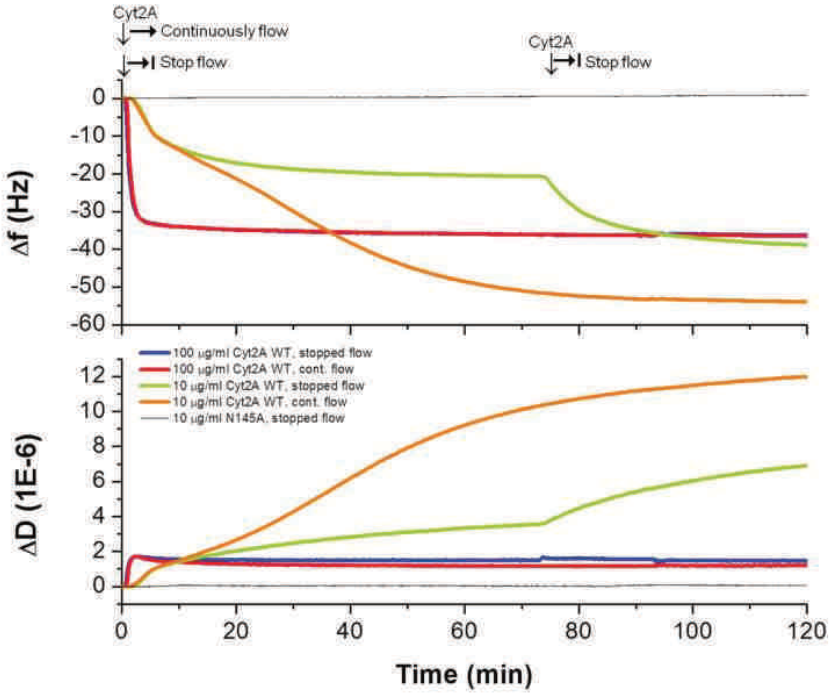

Figure 1: Cyt2Aa2 binding on lipid bilayer determined by QCM-D. The lipid bilayer was represented with 0 value. The protein solutions were exposed to the lipid bilayer by stopped flow or continuous flow. The schematic flow was showed on the upper image. The data was represented at the 5th overtone.

Results and conclusions

This work represents a step forward in understanding the Cyt2Aa2/lipid bilayer binding mechanism. The results show that such mechanism depends

on the Cyt2Aa2 protein concentration. Furthermore, the protein concentration also changes the structure and the mechanical properties of the initial protein/bilayer. At high protein concentration, Cyt2Aa2 binds quickly on the lipid bilayer and forming a rigid protein-lipid layer with inserted holes. This structure might support the putative pore-forming model. On the contrary, when the bilayer is exposed to the lowest protein concentration, the binding process is slower and aggregates are formed in the lipid bilayer. The final layer is more compliance than the layer formed at high protein concentration possibly because aggregation induces the entrapment of water molecules. Cyt2Aa2 aggregation could correlate to a proposed carpet mechanism model. The thickness of both the crystal-like and the aggregation-derived structures correlate with the size of the core β-sheet of Cyt2Aa2.

Outlook

This investigation has led to two new main questions that are currently being investigated in our laboratory. The first one refers to the possible existence of a protein threshold concentration above which the hole mechanism dominates. The second one concerns the (negative) repulsive force between protein/lipid layer and the AFM-tip; we aim to elucidate the nature of such interaction, especially if it is driven by anions surrounding the protein/lipid layer.

Micro-structured bioactive surfaces based on chemical gradients

Jagoba J. Iturri, Carina Huber-Gries[1], Dominik Rünzler[1], Jose L. Toca-Herrera

[1]*Department of Biochemical Engineering, University of Applied Sciences Technikum Wien, Höchstädtplatz 5, 1200 Wien, Austria*

Aim

Combination of (bio)chemical surface modification methods together with micro-structuring techniques (i.e. *μ-contact printing*), allows the formation of a new range of interfaces on which different physico-chemical parameters can be tuned on demand. The presence of topographically restrictive boundaries, in addition to the inherent surface properties variation set by the gradient, provides a full bunch of possibilities in terms of fabrication. Such novel interfaces will be used, in the first run, to study cell adhesion and cell proliferation, as well as to analyze detailed aspects of the cell mechanics by means of Atomic Force Microscopy (AFM). As previously studied in our group, substrate influences cell mechanics and function. Hence, Force vs distance plots are employed to measure stiffness variations due to cell-surface interactions.

Results

First approaches in this project focused on the analysis and characterization of the mechanical properties of different materials (polymer films, proteins) and cell types (Osteogenic and Adipogenic differentiated Human Adipose-derived Stem Cells, ASCs). Results obtained so far not only certificate the usefulness of the measuring technique employed, but they also provide a batch of reference data for next experiments. In the closer future, the choice and characterization of new active materials as well as the development of gradient preparation methodologies will be regarded.

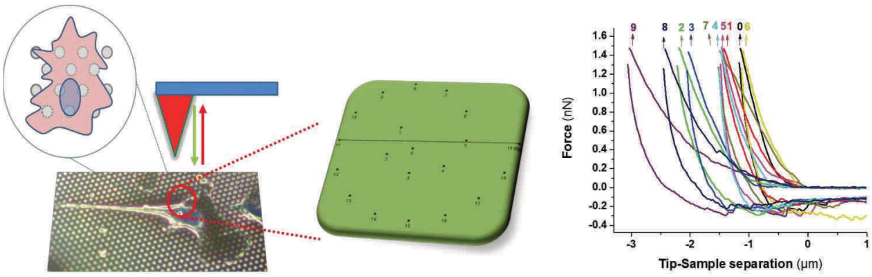

Figure 1: Schematic diagram of the cell mechanics experiments performed by means of AFM. (Left) Optical microscopy image of HUVEC cells deposited into Fibronectin-imprinted microstructures. (Center) Spiral-shaped grid applied for controlled sample indentation. (Right) Force vs distance curves obtained, highlighting the position-dependent mechanical behavior.

Assembly and characterization of layer-by-layer films based on charged polyelectrolyte capsules

Jacqueline Friedmann, Jagoba J. Iturri, Jose L. Toca-Herrera

Aim

Calcium Carbonate ($CaCO_3$) particles were used as template for electrostatically driven layer-by-layer assembly (LBL) of oppositely charged polyelectrolytes. These micro-sized particle are featured by their high porosity, which allows for adsorption and loading of different molecules (i.e. proteins) prior to their coating. Subsequently, the $CaCO_3$ core in the LBL colloids could be dissolved by action of EDTA to obtain protein-filled polyelectrolyte capsules, which are described for their potential applications as drug delivery devices. The surface charge of these capsules, defined by the polyelectrolyte in the outer layer, allows for the subsequent formation of 3D structures of higher complexity. Thus, by the same LBL protocol as that applied for deposition of polyelectrolyte layers, oppositely charged capsules can be adsorbed on flat SiO_2 surfaces to form soft, multilayered reservoirs of these drug-delivery devices.

Results

In this work poly (Allylamine Hydrochloride) (PAH) and/or poly (Diallyldimethylammonium chloride) (PDADMAC) polycations were combined with negatively charged poly (Sodium styrenesulfonate) (PSS) to form multilayered colloidal particles of odd or even number of layers. The formation of $CaCO_3$ microparticles included varying preparation conditions (salt concentration, mixing speed, molar ratio) in order to exert a control over particle size, dispersity or rugosity. Electrostatical-driven assembly of the complex LBL films, with either EDTA-treated or non-treated colloids, was followed and characterized by SEM, AFM and Confocal Microscopy techniques.

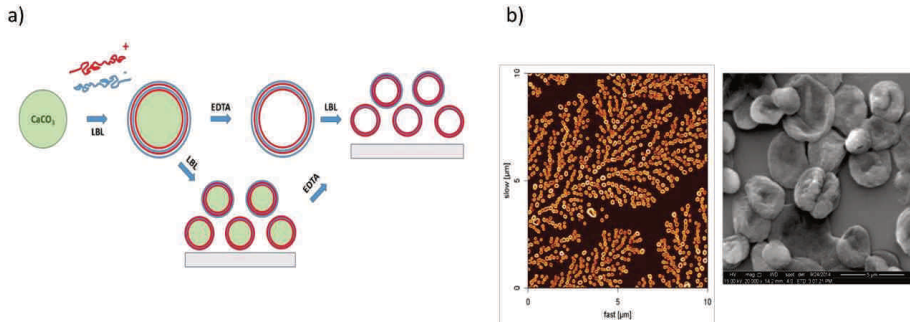

Figure 1: (a) Schematic diagram of the multilayer film preparation protocol. (b) Atomic Force (left) and Scanning Electron (right) micrographs showing the resulting 3D assemblies.

Resveratrol-protein interaction analysis by QCMD

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[1]*Department of Food, Environmental and Nutritional Sciences, University of Milan, 20133 Milan, Italy*

[2]*Dept. of Food Science and Technology, University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna Austria*

Aim

The interaction between resveratrol, a well-known anti-oxidant reservoir of natural source, and surface-anchored BSA protein was followed by means of Quartz Crystal with Microbalance with Dissipation (QCM-D) technique. The QCM-D responses, i.e., the resonance frequency (f) and the energy dissipation (D) of the shear oscillatory motion of a piezoelectric quartz crystal sensor, are highly sensitive to the variation in mass (in the order of a few ng/cm$_2$) and elastic properties of the surface-bound layer, respectively. This allows for a real time characterization and quantification of the ongoing processes at the sensor interface.

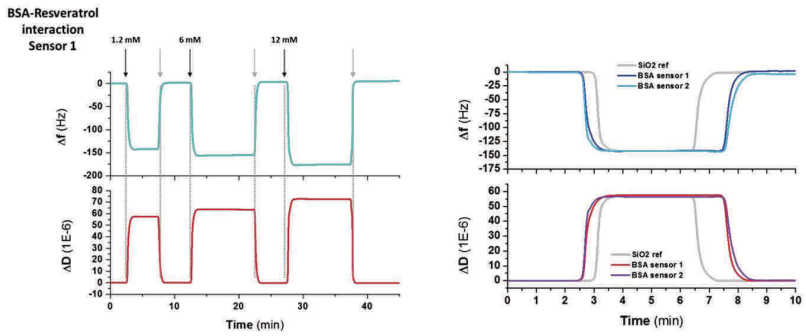

Figure 1: Left: f and D real time variations upon injection of increasing concentrations of Resveratrol (black arrows) and their respective rinses in EtOH (grey arrows). Right: Enlargement of the 1.2 mM Resveratrol injection step. The two BSA-modified sensors employed are compared to a reference $SiO_2$2 surface (grey line). Recovery of the pre-injection state after a final EtOH rinse seems to be complete for sensor n1, while for sample n2 frequency shows a value ca. -5 Hz.

Results

Graphics below show the results obtained for 2 BSA-modified sensors running in parallel. Both systems started in an EtOH baseline. Then, increasing concentrations of the resveratrol reservoir (1.2, 6 and 12 mM) were injected and their interaction with the protein was tested in-situ. Injections led to sudden frequency decreases and simultaneous dissipation increases, originated from the higher density of the incoming solution in comparison to molecule-free EtOH used for the baseline. As can be observed, once the resveratrol concentration increased, the recorded f and D jumps were higher. Between each new solution, thorough rinses in ethanol were performed, causing an almost full recovery of the frequency and dissipation signals, indicative of a poor/unsteady interaction.

Rheological studies on Umami-flavored LBL polyelectrolyte capsules

Piero Sabella, Jagoba J. Iturri, Jose L. Toca-Herrera

Aim

The hetero-aggregation of oppositely charged fat droplets has been used in food industry to create highly viscous or paste like materials with reduced fat contents. This approach has been widely used in non-food science applications for a variety of reasons, such as controlling the rheological properties of ceramics, and/or encapsulating and targeting of biomolecules. In this regard, the layer by layer buildup of polyelectrolyte multilayer (PEM) capsules from oppositely charged polyelectrolytes is a well-known technique for the preparation of supramolecular nano-architectures with drug trapping/delivery capability.

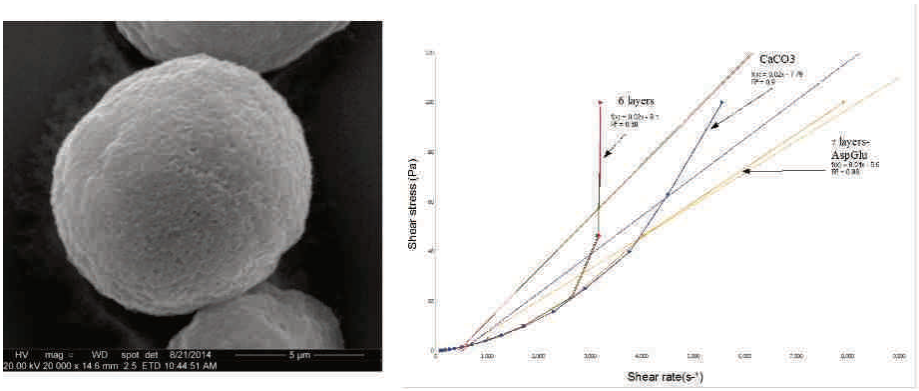

Figure 1: SEM image of Asp-Glu peptide-modified PAH/PSS colloids. (Right) Shear stress vs shear rate curves. Blue line represents the reference $CaCO_3$ micro-particle response.

Furthermore, binding of peptides and proteins to polyelectrolytes, either adsorbed or embedded in the PEM films, have been shown to retain their biological activities. Salt, sour, sweet and bitter are generally seen as the four basic taste sensations, but umami, or pleasantly savory, may be considered as the fifth taste sensation. Umami is the taste sensation brought about by monosodium L-glutamate (MSG) and the ribonucleotides inosine-5@-monophosphate (IMP) and guanosine-5@-monophosphate. These compounds are present in natural foods, and are also widely added in food industry as flavor enhancers. However, the occurrence of alternative compounds to the ones mentioned that can elicit an umami taste sensation has been reported over the years. In particular the so-called umami peptides are often mentioned in this respect. By combination of polyelectrolyte capsules with the so-called *umami* peptides could lead to the design of novel fat trapping systems which could simultaneously act as flavor enhancers.

Results

In this project, the binding of an umami-tasting peptide (Asp-Glu) to LBL-assembled polyelectrolyte capsules composed of poly (Allylamine Hydrochloride) (PAH) and poly(Sodium Styrene Sulfonate) (PSS) was performed. The preparation of the systems was followed by complementary SEM and Z-sizer measurements and final rheology of the bulk was determined.

Conformation-specific hydrolysis of poly(N,N-dimethyl acrylamide) brushes

Kathryn Melzak, Kai Yu[1], Jayachandran Kizhakkedathu[1], Jose L. Toca-Herrera
[1]*Centre for Blood Research, University of British Columbia, Canada*

Aims

Is the rate of hydrolysis of the brushes depending on the graft density?
Is the hydrolysis enhanced due to mechanical activation of the ester bond in the high density brush?

Results

End-tethered brushes of poly(*N,N*-dimethylacrylamide) (PDMA) were prepared at high initial graft densities. Polymer chains were then cleaved off by alkaline hydrolysis of the single ester bond linking the chains to the substrate. The rate of hydrolysis of the brushes was found to be dependent on the grafting density: a high hydrolysis rate was observed at higher graft densities, with a sharp transition to a lower rate as the grafting density decreased.

This transition takes place at a point where the average separation distance between the chains is much lower than the radius of gyration, so that the polymer chains are expected to be moderately stretched. We suggest that the rate of hydrolysis may be enhanced due to mechanical activation of the ester bond in the high density brush. A corollary of these results is that for polydisperse samples, hydrolysis becomes selective for longer chains at one stage of the reaction, which in turn means that analysis of the partial hydrolysate may not give an accurate picture of the tethered brush.

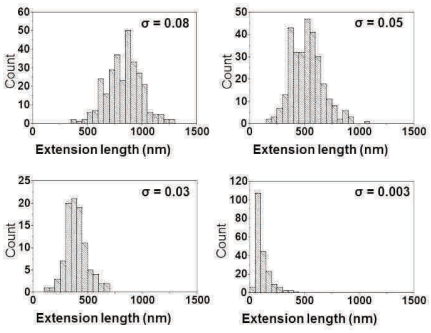

Figure 1: AFM extension lengths for a polymer series with $Mn = 3.8 * 10^5$ and $PDI = 1.8$, measured in an aqueous environment with an unmodified AFM tip. The graft density σ in chains/nm^2 is shown on each graph.

Summary

Accelerated hydrolysis of esters at high graft densities seems to take place. This effect should be associated with shear forces in the plane of the substrate. For polydisperse samples, the accelerated reaction rates have the effect of making the reaction selective for longer chains. This could potentially be exploited when preparing degradable polymer brushes based on hydrolysates.

Wetting properties and structure of Quercus robur and Fagus sylvatica

Antonio Miranda del Álamo[1], *Claudia König, Jacqueline Friedmann, Rafael Benítez Suárez*[1], *Jose L. Toca-Herrera*

[1]*Department of Mathematics, University of Extremadura, Avda. Virgen del Puerto, 10600 Plasencia, Spain*

Aim

The goal of the project was to study the wetting properties (Lotus effect) of 2 different species of leaves representing Mediterranean forest (*Quercus robur* and *Fagus sylvatica*) as a function of it nanostructure and aging.

Methods

Contact angle measurements were carried out using the sessile drop method (Easy Drop Equipment, Krüss, Germany). The nanostructure of the leaves was investigated by sessile drop and SEM (FEI, USA).

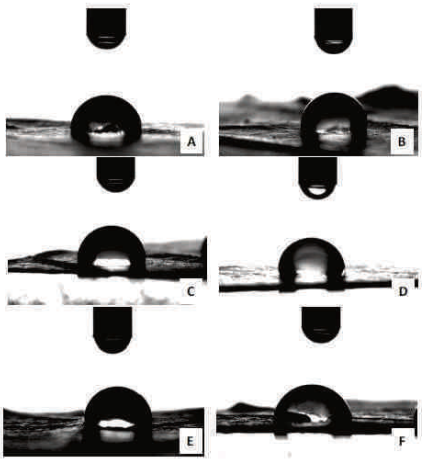

Figure 1: Comparison between *Quercus robur* and *Fagus sylvatica*. Contact angle images obtained with the pendant drop method. The foliar surface was conserved at 4°. (A) *Fagus sylvatica* against aqueous solution of pH 5. The contact angle was 93.3°. (B) For pH10, the contact angle rises to 101°. (C) For pH2 the contact angle is 96.2°. (D) For pH5, *Quercus robur* shown an angle of 93.7°. (E). For pH10, *Quercus robur*, has an angle of 91 °. (F) For pH2, *Quercus robur*, the contact angle is 82.1°.

Results and conclusions

Fagus sylvatica and *Quercus robur* have similar contact angles. The contact angle for fresh samples is about 100°. The contact angle seems to change with time since after 10-15 days due to a variation in roughness and a loss of the hydrophobic properties (note: the roughness is higher in the first conservation weeks). The contact angles measured at pH 10 are lower and lead to the degradation of the wax and the epicuticular epidermis. These sample lose their hydrophobic behavior.

Fagus sylvatica present wax in a plaque fashion like while *Quercus robur* shows a crystalline tubular structure. The last conservation days delivered data with high statistical spread. This might be due to dehydration and structural damage (epicutilar wax protect the leave and confers them hydrophobicity).

Acknowledgements

The following work was performed during an Erasmus Training stay; therefore AMA thank the Erasmus program of the EU.

Wetting properties and structure of Betula pendula y Populus nigra var. italica

Remedios Gómez Infante[1], *Claudia König*, *Jacqueline Friedmann*, *Rafael Benítez Suárez*[1], *Jose L. Toca-Herrera*

[1]*Department of Mathematics, University of Extremadura, Avda. Virgen del Puerto, 10600 Plasencia, Spain*

Aim

The goal of the project was to study the wetting properties (Lotus effect) of 2 different species of leaves representing riverside woodland (*Betula pendula* y *Populus nigra var. italica*) as a function of it nanostructure and aging.

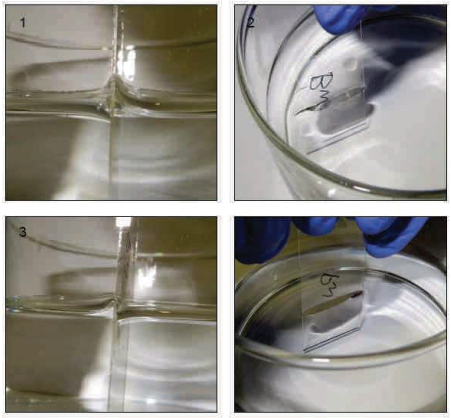

Figure. (1 - 2) microsccpy slide with *Betula pendula* wax. In the first image, it can be observed how water wets the right side (without wax) while it get repelled in the left side. (3 - 4) microscopy slide with *Populus nigra var. italica* wax. The behavior is similar to the shown by *Betula pendula*.

Methods

Contact angle measurements were carried out using the sessile drop method (Easy Drop Equipment, Krüss, Germany). The nanostructure of the leaves was investigated by sessile drop and SEM (FEI, USA).

Results and conclusions

1. None of the studied species presented super-hydrophobic behaviour. However, *Betula pendula* is more hydrophobic than *Populus nigra var. italica*, showing a contact angle value of about 100 °.

2. Sample conservation influences the final contact angle (the wetting properties of the leave). The samples conserved in an atmosphere of N2 were more hydrophobic than the ones conserved in air at (4 °C).

3. The type of solution influences the contact angle. The values were larger for solutions of (millipore) H2O, NaCl y pH 4 than for solutions of pH 10 y pH 2.

4. The higher the roughness the larger the contact angle. The roughness depends on the type of leave. Wax contain is the most determining factor in influencing hydrophobicity. This can be seen in *Betula pendula* which presents a larger contact angle and a wax hierarchical distribution.

Acknowledgements

The following work was performed during an Erasmus Training stay, therefore RMI thanks the Erasmus program of the EU.

Evaluation of hiking trails in recreational areas using data analysis and graph theory

Beatriz Lucas Delgado[1], *Luis M. González*[2], *Karolina Taczanowska*[3], *Rafael Benitez*[1], *José L. Toca-Herrera*

[1]*Department of Mathematics, University of Extremadura, Plasencia, Spain.*

[2]*Department of Physical Education and Sport, University of Valencia, Valencia, Spain*

[3]*Institute of Landscape Development, Recreation and Conservation Planning, University of Natural Resources and Life Sciences, Vienna, Austria*

Aim

This study had the objective of understanding the flow of visitors in natural recreational areas (and in the city center) of Vienna and the Monfragüe National Park (Extremadura, Spain). Interesting is the influence of the environment on the behavior of visitors and the final spatial distribution of their activity.

Methodology

The study focused on the movement of people along the nature trails in natural environments Lobau and Viennese forest. R was used for data analysis and graph theory was utilized for path representation and interpretation,

Results and conclusions

In recreational areas numerous outdoor leisure activities are usually performed. Understanding the trails function is crucial for the managers of these areas to balance the needs of the visitors. This study presents an approach to evaluate the structure of the trails using the data collected (with GPS) with Graph Theory. The three areas of study were: i) the center of Vienna (Austria, 10 tracks), ii) the Lobau National Park (Austria, 10 tracks) and iii) the Monfragüe National Park (Spain, 191 tracks). The following parameters were extracted from the routes: structural and functional network, speed, node centrality (degree, closeness and betweenness) as well as the movement direction. The results for the functional graph were heterogeneous. This methodology is suitable for the evaluation of any network of trails either in cities or in natural environments.

In particular, we can stress the following: i) Hikers walked faster in the center of Vienna than in the Lobau park, ii) Hikers used more non-structural that structural routes in the Monfragüe park, being the most important route the one connected between node 12 and 3 (bid bridge), iii) the nodes with more flow of visitors of the Monfragüe Park were (11) and (3).

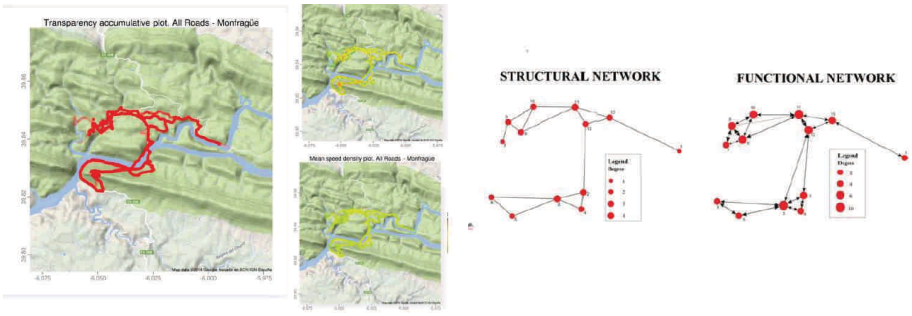

Figure 1: Figure. Left: all routes of Monfragüe. Middle: "all degree" calculation of the nodes centrality of the undirected graph. Right: "all degree" calculation of the nodes centrality of the directed graph.

Acknowledgments

BLD thanks the Erasmus program of the EU commission.

Laser surface functionalization of poly-L-lactide to anchor stem cells, control adipocyte morphology, and improve differentiated cell adherence

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[2]*Micro and Nanoengineering Unit, CIC microGUNE, Goiru Kalea 9, 20500, Arrasate-Mondragón, Gipuzkoa, Spain;*

[3]*Cell Biology & Stem Cells Unit, CIC bioGUNE, Technology Park of Bizkaia, Ed. 801A, 48160 Derio, Spain;*

[4]*University of the Basque Country (UPV/EHU), School of Engineering, Department of Mining and Metallurgy Engineering & Materials Science, Alameda de Urquijo s/n, 48013 Bilbao, Spain*

Aim

Evaluation of the suitability of the Picosecond Laser Micromachining technology (PLM) for surface modification of biomaterials to control stem cell behavior. Assessing the role of predesigned surface microstructures on mesenchymal stem cells (MSCs) adhesion, orientation, shape and co-differentiation.

Results and conclusions

Defined surface microstructures were produced on biodegradable (PLLA: poly (L-lactide)). On laser-structured PLLA, undifferentiated MSCs adapted their shape to the groove size and direction, showing the well-known contact guidance effect.

Additionally, under certain surface topographical conditions, these cells modified their shape by a long-term anchorage to specific locations of the grooves. Considering MSCs after treatment with differentiation protocols, adipocytes responded to changes on substrate height and depth by adapting the intracellular distribution of their fat lipid vacuoles to physical constraints imposed by the laser surface patterning method. Additionally, enhancement of adhesion of differentiated cells on laser-treated PLLA was observed. These findings show that PLM technology can be used to directly manufacture 3D microstructures on biomaterials,

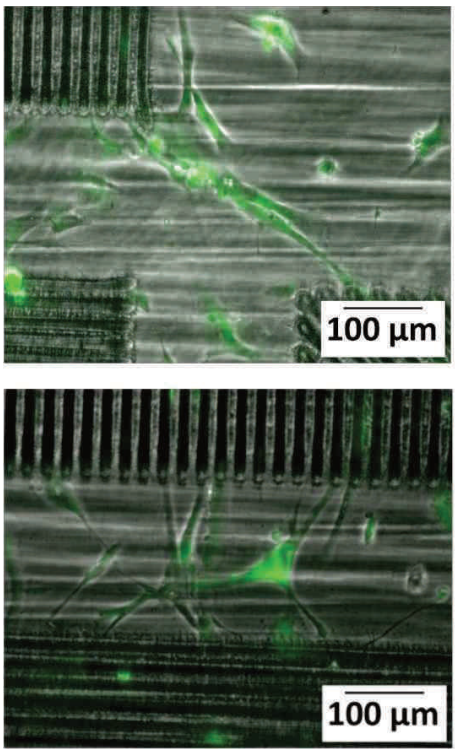


Figure 1. Undifferentiated MSCs between three (above) and two (below) grooved-patterned areas were visualized by inmunofluorescence after 1 day in culture.

promoting cell proliferation guidance, control adipocyte morphology and improving the surface adhesion of bone and fat tissue without the need of a specific biochemical functionalization on the material surface. Further investigation are required to elucidate underlying molecular mechanisms, the present study shows that substrate topography at the micrometer scale affects the morphology and orientation of human mesenchymal stem cells and adipocytes without the interplay of biochemical factors (biochemical functionalization).

Acknowledgments

UE09+ programme of the Basque Government. Spanish Science and Innovation Department under the PID-600300-2009-16 programme. Department of Industry of the Basque Government (Etortek 07/27-IE07/201). Institute of Salud Carlos III and the Health Department of the Basque Government.

Physical activity, physical fitness and academic achievement in adolescents: a self-organizing maps approach

M. Pellicer-Chenoll[1], X. Garcia-Massó[2], J. Morales-Aznar[3], P. Serra-Añó[4], M. Solana-Tramunt[3], L. M. González[1], José L. Toca-Herrera

[1]*Department of Physical Education and Sport, University of Valencia. Valencia, Spain.*

[2]*Departament de Didàctica de l'Expressió Musical, Plàstica i Corporal, Universitat de Valencia, Spain,*

[3]*Facultat de Ciències de l'Esport Balanquerna. Ramon Llull University. Barcelona. Spain.*

[4]*Department of Physiotherapy, University of Valencia, Spain*

Aim

The working hypothesis has been that students with a higher level of physical activity and better physical fitness should be clustered in the area with better academic performance.

Experimental

In the present study we have applied a Self-Organizing Map (SOM). This analytical method employs competitive non-supervised neural networks. The principal goal of the SOM is to transform an incoming signal pattern of arbitrary dimension into a two-dimensional discrete map, performing this transformation in a topologically ordered fashion.

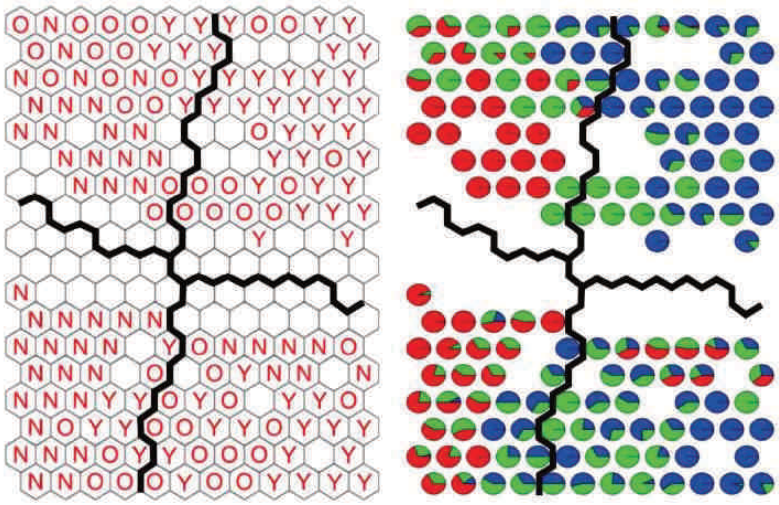


Figure 1: The label map is placed in the left side showing the winning value of each neuron (Y= regular formal physical activity; O=occasional formal physical activity; N=no formal physical activity). The right side shows the pie plane representing the percentage of subjects practicing physical activity on each neuron (blue= regular formal physical activity; green=occasional formal physical activity; red=no formal physical activity). The four areas of interest are overlap with a thicker line.

In our study, the network had a rectangular shape with a size of 18 x 12 neurons height and width respectively. The neurons shape was hexagonal, so that each neuron had 6 neighbour neurons. SOM was carried out with Matlab R2008a program (Mathworks Inc., Natick, USA) and the SOM toolbox (version 2.0 beta) for Matlab.

Results and conclusions

The relation between physical activity, physical fitness and academic achievement in adolescents has been widely studied; however there is still controversy on this topic. The methods used so far for analyzing the relationship between these variables have been mostly traditional lineal analysis according to the available literature. The aim of this study was to perform a visual analysis of this relationship and monitor the subject's evolution during the four years of secondary school. Four main clusters that fit with two main student profiles with slight differences between boys and girls were found. From this clustering it can be understood that students with higher energy expenditure and with better physical fitness show also lower Body Mass Index (BMI) and higher academic performance, while those adolescents that have lower energy expenditure show worst physical fitness, higher BMI and lower academic performance.

Technical-tactical preparation of Austrian judoka at the Austrian national championships and the number of associated injuries

M. Handler[1], *Jose L. Toca-Herrera, M. Burtscher*[1].
[1]*Institut für Sportwissenschaft - Abteilung für Sportmedizin, Universität Innsbruck, Austria*

Aim

In Austria has not been any systematic scientific literature concerning technical, tactical, physical preparation and health aspects of the participants of Austrian Judo Championships, as well as (muscular and skeleton) trauma statistics of the tournament. The analysis of an audio-visual record of all judo fights during the Austrian national championships in 2015 will be the center point of the research process. All effective techniques and documented injuries will be registered and analyzed according to gender, weight category and fighting time. One aim of the study is to make an overview of the technique repertoire of the best Austrian judoka and their effectiveness. Another goal of this research process is the identification of the number of injuries caused by judo at the level of a national championship. The collected data and the results will be used for the optimization of the technical-tactical preparation of Austrian professional judoka who will compete in international tournaments. The expected results will also have a valuable use at human resources and public relations level. With this approach it will be possible to establish a technique-injurie ratio, which can be applied to increase the quality and health of the judo competitors, and for extension, to the rest of judoka.

Uchi komi error rate, muscle function and postural deformity of Austrian judoka - a comparative study between leisure judoka and professional judoka about differences in muscle function and postural deformity caused by false posture during uchi komi

M. Handler[1], *Jose L. Toca-Herrera, M. Burtscher*[1].
[1]*Institut für Sportwissenschaft - Abteilung für Sportmedizin, Universität Innsbruck, Austria*

Aim

Uchi komi are the basic exercise for judoka to learn and automate throwing techniques. So every judoka has to do thousands of uchi komi in his active career to bring his tokui waza (special throwing technique) to perfection. But there is no scientific research about the consequence of false posture during uchi komi on the musculoskeletal system and postural deformity. At all 20 Austrian male and female judoka will be included. They are divided in a group of leisure judoka and a group with professional judoka - participants of Austrian Championships, European Cups, European and World Championships and Youth Olympic Games. Anthropometric data, results of muscle function tests, results of orthopedic tests of the musculoskeletal system and information about

judo caused pain and injuries will be collected, analyzed and compared. Also a computer assisted video analyze of series of uchi komi will be put into practice. The changes of posture, correctness of technique frequency of movement, heart rate and concentration blood lactate acid will be observed and compared. For a correctly executed technique the positions of the centers of gravity of tori (active judoka) and uke (passive judoka) are important and will be investigated. We hypothesize that in both groups the number of uchi komi with false posture will rise with the number of series and the fraction of postural deformity will be higher in the professional group. Furthermore we believe that false posture will cause an ineffective technique.

2 Publications 2014 (SCI articles)

1. **Biomaterial and cellular properties as examined through atomic force microscopy, fluorescence optical microscopies, and spectroscopic techniques**
B. Kainz, E. A. Oprzeska-Zingrebe, and J. L. Toca-Herrera
Biotechnological Journal 9 (2014) 51

2. **Ultra-fast laser microprocessing of medical polymers for cell engineering applications**
R. Ortiz, S. Moreno-Flores, MdM Vivanco, J.R. Sarasua, I. Quintana, and J.L. Toca-Herrera
Materials Science and Engineering: C 37 (2014) 241

3. **Fluorescent sensors based on bacterial fusion proteins**
B. Prats Mateu, B. Kainz, D. Pum, U.B. Sleytr, and J. L. Toca-Herrera
Methods and Applications in Fluorescence 2 (2014) 024002 (8pp)

4. **Interactions in lipid stabilised foam films**
J. L. Toca-Herrera, N. Krasteva, H.-J. Müller and R. Krastev
Advances in Colloids and Interfaces 207 (2014) 93

5. **Influence of HepG2 cell shape on nanoparticle uptake**
B. Prats-Mateu and J. L. Toca-Herrera
Microscopy Research and Technique 77 (2014) 560

6. **Looking at Cell Mechanics with Atomic Force Microscopy: Experiment and Theory**
R. Benitez and J. L. Toca-Herrera
Microscopy Research and Technique 77 (2014) 947

7. **Evaluating the structure and use of hiking trails in recreational areas using a mixed GPS tracking and graph theory approach**
K. Taczanowska, L.-M. González, X. Garcia-Massó, A. Muhar, C. Brandenburg, and J. L. Toca-Herrera
Applied Geography 55 (2014) 184

8. **Reassembly of S-layer proteins.**
D. Pum, U. B. Sleytr
Nanotechnology 25 (2014) 312001

9. **S-layers: Principles and Applications.**
U. B. Sleytr, B. Schuster, E. Egelseer, D. Pum
FEMS Microbiology Review 38 (2014) 823

10. **Chemical foam cleaning as an efficient alternative for flux recovery in ultrafiltration processes.**
B. Gahleitner, C. Loderer, C. Saracino, D. Pum, W. Fuchs
Journal of Membrane Science 450 (2014) 433

11. **The grab-and-drop protocol: a novel strategy for membrane protein isolation and reconstitution from single cells**
A. Schrems, J. Phillips, D. Casey, D. Wylie, M. Novakova, U. B, Sleytr, D. Klug, M. A. A. Neil, B. Schuster, O. Ces
Analyst 139 (2014) 3296

12. **Biomimetic interfaces based on S-layer proteins, lipid membranes and functional biomolecules**
B. Schuster, U. B. Sleytr
Journal of the Royal Society Interface. 11(2014) 20140232

13. **Comment on Mechanical properties of giant liposomes compressed between two parallel plates: Impact of artificial actin shells**
Susana Moreno-Flores, Rafael Benitez
Langmuir 30 (2014) 7928

3 Books and book chapters

- Bacterial membrane formation monitored with atomic force microscopy- and quartz crystal microbalance.
  N. Krska and J. L. Toca-Herrera
  In Biosensors: *Recent Advances and Mathematical Challenges*, (Ed. M. Stoytcheva and J. Faccelo Osma), OmniaScience, 2014, Barcelona , ISBN: 978-84-941872-0-9

4 Conferences, workshop and schools

- AUTHOR: D. Pum, J. L. Toca-Herrera,U. B. Sleytr
  TITLE: S-layer protein reassembly and its application (oral)
  CONFERENCE: E-MRS (Materials Research Society)
  PLACE: Lille (France), 2014

- AUTHOR: J. L. Toca-Herrera
  TITLE: Scanning Probe Microscopy in Biological and Soft matter Sciences (oral)
  CONFERENCE: Seminar - Biological Physics, Faculty of Physics, AGH-University
  PLACE: rakow, Poland, 2014

- AUTHOR: K. Taczanowska., L.-M. Gonzalez., X. Garcia-Masso. A., Muhar., C. Brandenburg, J. L. Toca-Herrera
  TITLE: Combining GPS-tracking and graph theory for evaluating the funcionality of hiking trails in recreational areas (poster)
  CONFERENCE: 7th International Conference on Monitoring and Management of Visitors in Recreational and Protected Areas (MMV)
  PLACE: Tallinn, Estonia, 2014

- AUTHOR: S. Tharad, C. Krittanai, B. Promdonkoy, J. L. Toca-Herrera
  TITLE: Molecular interaction between insect lipid membrane and Bacillus thuringiensis cytolytic toxin (poster)
  CONFERENCE: 18th International Microscopy Congress (IMC)
  PLACE: Prague, Czech Republic, 2014

- AUTHOR: B. Prats-Mateu, J. L. Toca-Herrera
  TITLE: Influence of cell shape on nanoparticle cell uptake (poster)
  CONFERENCE: 18th International Microscopy Congress (IMC)
  PLACE: Prague, Czech Republic, 2014

- AUTHOR: S. Tharad, C. Krittanai, B. Promdonkoy, J. L. Toca-Herrera
  TITLE: Binding kinetics of Bacillus thuringiensis cytolytic protein to lipid bilayers (poster)
  CONFERENCE: 6th ÖGMBT Annual Meeting "Life Sciences meet Entrepreneurship"
  PLACE: Vienna, Austria, 2014

- AUTHOR: E. A. Oprzeska-Zingrebe, J. L. Toca-Herrera
  TITLE: Testin, a focal adhesion protein: the approach to its elastic and structural properties (poster)
  CONFERENCE: 6th ÖGMBT Annual Meeting "Life Sciences meet Entrepreneurship"
  PLACE: Vienna,Austria, 2014

- AUTHOR: J. J. Iturri, J. L. Toca-Herrera
  TITLE: Micro-structured bioactive surfaces based on chemical gradients (poster)
  CONFERENCE: 6th ÖGMBT Annual Meeting "Life Sciences meet Entrepreneurship"
  PLACE: Vienna, Austria, 2014

- AUTHOR: B. Prats Mateu, B. Kainz, D. Pum, U. B. Sleytr, J.L. Toca-Herrera
  TITLE: Fluorescent sensors based on bacterial fusion proteins (poster)
  CONFERENCE: 6th ÖGMBT Annual Meeting "Life Sciences meet Entrepreneurship"
  PLACE: Vienna, Austria, 2014

- AUTHOR: J. L. Toca-Herrera
  TITLE: Atomic force microscopy, life sciences and soft matter (oral)
  CONFERENCE: NanoBio& Med 2014
  PLACE: Barcelona, Spain, 2014

- AUTHOR: J. J. Iturri, A. Moreno-Cencerrado, J. L. Toca-Herrera
  TITLE: "Nanotechnology, we and what it will come" (oral)
  CONFERENCE: Seminar of the cultural program of the Cervantes Institute Vienna
  PLACE: Vienna, Austria, 2014

- AUTHOR: D. Pum, U. B. Sleytr
  TITLE: "S-protein reaasembly" (oral)
  CONFERENCE: 4th ASEM-Workshop: Advanced Electron Microscopy
  PLACE: Vienna, Austria, 2014

- AUTHOR: D. Pum, U. B. Sleytr
  TITLE: S-Layer Morphogenesis (oral)
  CONFERENCE: MRS 2014 Spring Meeting,
  PLACE: San Francisco, CA (USA), 2014

- AUTHOR: E. Ladenhauf, H. F. Sussitz, N. V. H. Phan, U. B. Slyetr, P. A. Lieberzeit D. Pum
  TITLE: S-layer based bio-imprinting (oral)
  CONFERENCE: 8th Int. Congress Mol. Imprinting-MIP2014
  PLACE: Zhenjiang, China, 2014

- AUTHOR: S. Damiati, S. Zayni, A. Schrems,U. B. Sleytr, J. Chopineau, B. Schuster, E. K. Sinner
  TITLE: Construction of An Artificial Membrane Platform for Synthetic Biology: Investigation of a Membrane Proteins in an S-layer Supported Lipid Membrane (oral)
  CONFERENCE: Micro and Bio Technology Conference (NaMiBiTECH)
  PLACE: Akhawayn University of Ifrane, Marocco, 2014

- AUTHOR: V.-D. Larisch, A. Schrems, U. B. Sleytr, M. Hohenegger, B. Schuster
  TITLE: Reconstitution of Ryanodine Receptor/Ca2+ Release Channels in S-Layer supported Lipid Membranes (poster)
  CONFERENCE: Eigth International Conference on Quantum, Nano/Bio, and Micro Technologies
  PLACE: Lisboa, Portugal, 2014

- AUTHOR: A. Schrems, V.-D. Larisch, J. Friedmann, C. Stanetty, A. Kibrom, K. Lohner, U. B. Sleytr, B. Schuster
  TITLE: Fabricating S-layer supported functionalized lipid bilayers (poster)
  CONFERENCE: 1st Erwin Schrödinger Symposium 2014 (Two-dimensional nanostructures)
  PLACE: Vienna, Austria, 2014

- AUTHOR: U. B. Sleytr, B. Schuster, D. Pum
  TITLE: S-layer proteins as basic building blocks for nanobiotechnological application (oral)
  CONFERENCE: 10th Nanoscience and Nanotechnology Conference (NanoTR10)
  PLACE: Istanbul, Turkey, 2014

5 Accepted / Ongoing projects (external funding)

- *S-layer based bio-imprinting - Synthetic S-layer polymers,*
  (funded by the Air Force Office of Scientific Research (AFOSR)).
  Agreement award FA9550-12-1-0274. PI: D. Pum (Co-PI: U. B. Sleytr).

- *Testin, an adhesion protein: mechanical, structural and dynamical properties,*
  BMWF (IGS BioNanoTech).
  PI: J. L. Toca-Herrera (Co-PI: A. Meserez).

- *The relation between physical interactions and structure in living cells,* FWF.
  PI: S. Moreno-Flores (Co-author: J. L. Toca-Herrera).

6 National / International collaborations

- Peter Lieberzeit
  University of Vienna, Inst. of Anal. Chem., Vienna, Austria
- Carole C. Perry
  Nottingham Trent University, Nottingham, UK
- Murugappan Muthukumar
  University of Massachusetts, Amherst, MA
- Rafael Benítez
  University of Extremadura, Dept. of Mathematics, Spain
- Luis Millán González
  University of Valencia, Dept. of Physical Education and Sport, Spain
- Jose R. Sarasúa
  University of the Basque Country, Faculty of Engineering, Spain
- Jayychandran Kizhakkedathu
  University of British Columbia, Center for Blood Research, Canada
- Peter Ertl
  Austrian Institute of Technology, Austria
- Stefan Schiller
  University of Freiburg, Inst. of Advances Studies, Germany
- Chartchai Krittanai
  Mahidol University, Institute of Molecular Biosciences, Thailand
- Elsa Arcalis, Ulrike Hörmann-Dietrich, and Eva Stöger
  Institut für Angewandte Genetik und Zellbiologie (IAGZ), BOKU, Austria

7 Supervision and Training activities

PhD

- Eva Maria Ladenhauf (AFORS): S-layer based bio-imprinting - Synthetic S-layer polymers
- Alberto Moreno Cencerrado (IGS) : Structure and adsorption of proteins at interfaces
- Elham Ghorbani Gorji: Encapsulation of "food" proteins
- Maria Sumarokova (Erasmus Iamonet): Substrate influence on cell mechanics
- Sudarat Tharad (Royal grant, Thailand): Structure and interactions in Lipid-toxine systems

MSc/Diploma/Training/Erasmus

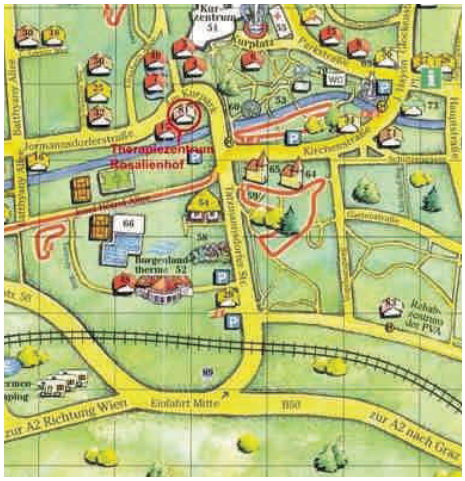
- Denisse Bender (CONACYT, Mexico): Encapsulation of resveratrol
- Remedios Gomez Infante (Erasmus, Spain): Wetting of riverside woodland leaves
- Beatriz Lucas Delgado (Erasmus, Spain): Evaluation of hiking trails using graph theory
- Antonio Miranda del Alamo (Erasmus, Spain): Wetting properties of Mediterranean leaves
- Elisa Rocchi (Erasmus, Italy): Interaction between resveratrol and carrier proteins
- Piero Sabella (Erasmus, Italy): Rheological analysis of Umami capsules

BSc

- Margareta Mittendorfer (BOKU): Lipid-toxin interactions

8 Winter School of the Institute for Biophysics (DNBT)

University of Natural Resources and Life Sciences Vienna (BOKU)
Bad Tatzmannsdorf (Austria), 2-4 December 2014
Book of Abstracts



Participants

Belinda Angjeli (Inst. for Synthetic Bioarchitectures, DNBT, BOKU Vienna), Alberto Moreno Cencerrado (Inst. for Biophysics, DNBT, BOKU Vienna), Gary Dorken (Inst. for Bioinspired Materials, DNBT, BOKU Vienna), Jacqueline Friedmann (Inst. for Biophysics, DNBT, BOKU Vienna), Notburga Gierlinger (Inst. of Physics and Material Sciences, MAP, BOKU Vienna), Jagoba Jon Iturri (Inst. for Biophysics, DNBT, BOKU Vienna), Michael Handler (Institute of Sport Science, University of Innsbrück, Austria, Inst. for Biophysics, DNBT, BOKU Vienna), Dieter Jaeger (DNBT, BOKU Vienna), Claudia König (Inst. for Biophysics, DNBT, BOKU Vienna), Eva Ladenhauf (Inst. for Biophysics, DNBT, BOKU Vienna), Margareta Mittendorfer (Inst. for Biophysics, DNBT, BOKU Vienna), Batirtze Prats Mateu (Inst. of Physics and Material Sciences, MAP, BOKU Vienna), Dietmar Pum (Inst. for Biophysics, DNBT, BOKU Vienna), José L. Toca-Herrera (Inst. for Biophysics, DNBT, BOKU Vienna), Anna Carolina Vianna Cintra (Inst. for Biophysics, DNBT, BOKU Vienna).

Speakers

Belinda Angjeli, Alberto Moreno Cencerrado, Gary Dorken, Jacqueline Friedmann, Notburga Gierlinger, Jagoba Jon Iturri, Michael Handler, Eva Ladenhauf, Margareta Mittendorfer, Batirtze Prats Mateu, José L. Toca-Herrera, Anna Carolina Vianna Cintra.

Program

Tuesday – 02 / 12 /2014

14.15 h - - Opening of the Biophysics winter school: José L. Toca-Herrera

First session, chairman: Gary Dorken
14.30 h - 14.55 h: Eva Ladenhauf: S-layer based bio-imprinting
15.00 h - 15.25 h: Jacqueline Friedmann: Assembly and characterization of layer-by-layer films based on charged polyelectrolyte capsules

Coffee brake: 15/20 minutes

3.45 h - 4.10 h: Margareta Mittendorfer: Molecular interaction between lipid membrane and a cytolytic protein from *Bacillus thuringiensis*
4.15 h - 4.45 h: Anna Carolina Vianna Cintra: Characterization of S-layer proteins mixtures in different concentrations

Wednesday – 03 / 12 /2014

Second session, chairman: Dietmar Pum
9.30 h – 9.55 h: Gary Dorken: Depletion attraction and bacteria
10.00 h - 10.25 h: Belinda Angjeli: Cell fusion between HIV membrane protein-expressing and T-like cells
10.30 h - 10.55 h: Batirtze Prats Mateu: Characterization of surfaces and interfaces of plants by AFM and Raman microscopy

Coffee break: 15/20 minutes

10.15 h – 11.40 h: Notburga Gierlinger: Imaging molecular structure of plant cells by Confocal Raman microscopy
11.45 h - 11.10 h: Jagoba Iturri: Micro-structured bioactive surfaces based on chemical gradients

Lunch break and relaxing time

Third session, chairman: Jagoba Iturri
14.30 h -14.55 h: Michael Handler: Muscle function and postural deformity of austiran judoka - a comparative study between leisure judoka and professional judoka about differences in musle function and postural deformity caused by false posture during uchi komi
15.00 h - 15.25 h: Alberto Moreno Cencerrado: Imaging and spectroscopy of S-layers by STM: Current-Voltage characterization

Coffee brake: 15/20 minutes

15.45 h - 16.10 h: José L. Toca-Herrera: Some of the projects that we are doing and closing remarks
16.15 h – 17.00 h: Round table

Thursday – 04 / 12 /2014

9.30 h - 11.25 h: Brain storming (optional)
11.30 h - 14.00 h: Lunch break
14.30 h: Departure to Vienna