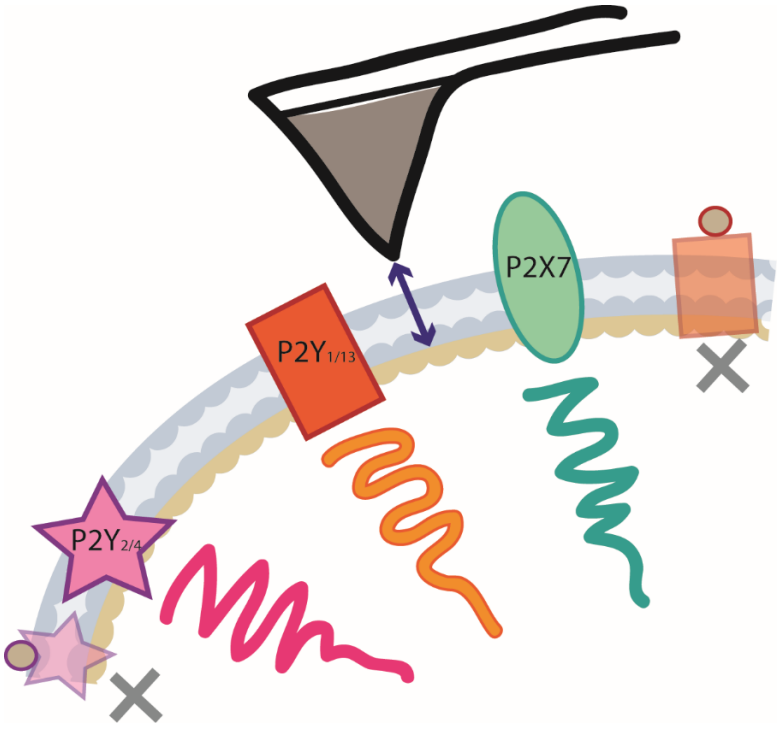
2019 Annual Report

Institute for Biophysics

Department of Nanobiotechnology

University of Natural Resources and Life Sciences

Vienna, Austria



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Foreword

2019. First Bi-annual report although it will be just called “annual report”. The reason is that the even years we will prepare a year summary with the most important achievements.

With respect to BOKU and DNBT duties, we still support and manage laboratories that are heavily used by DNBT coworkers and “external” researches. We also participate in rewarding commissions concerning leadership, teaching, research and ethical matters (DLK, DokStuko, FachStuko, Forschungsprecher, ethics platform, habilitation group). No more comments about that.

Concerning the institute, 2019 has been a good year. We are still growing. I am not only talking about people but about topics and experience. Still our budget and third party money is enough to do the research we are interested in. Looking at the numbers this year we have published about 25 articles as main or supportive authors. Notburga Gielinger managed a cover picture in Advanced Science, which shows the importance of the topic she is investigating. In addition, we all have presented our results in about 17 conferences, workshops or invited talks.

Following the “international” tradition of our institute, we have received this year 10 visiting scientist from professor to student level. A highlight of the year was to have Reinhard Miller as visiting professor at BOKU. Reinhard was a leading scientist on interface science at MPI for Colloids and Interfaces (Golm, Germany) when I just was a PhD student. His lecture “Adsorption of surfactants/proteins al liquid interfaces, kinetics, thermodynamics, interfacial visco-elasticity” received the best possible reviews. We will keep inviting as many exciting lecturers as we can. Other highlights were the habilitation of Ioana Giouroudi (in Nanotechnology) and the doctoral degree of Elham Ghorbani. The case of Ioana is relevant since her research work was mainly done at the TU-Vienna, and she decided to do the habilitation with us. We also have a couple of nice improvised seminars by Stephan Schmidt (Heinrich-Heine-Universität, Germany), Antonio Lucas-Alba (University of Zaragoza, Spain), and Francisco Blanco (BioGUNE, Spain).

Looking back at our research, I think that we should improve the use (and the knowledge) of our experimental techniques. There is still room for new developments in optics, scanning probe and spectroscopy that post-docs and students should work out. We should also not forget that part of our research needs from theoretical and computing support that we might need to look for.

Teaching is still our weak point since the institute does not have real mandatory teaching. My wish would be to teach our expertise in basic courses in the bachelor program (biophysics, optics, spectroscopy or basic mathematical methods)... it sounds redundant to say that physics knowledge is important for any experimental device anyone uses in the lab… Anyway, we will keep going teaching our master courses. The students attending such courses always give us very good reviews.

In summary, during 2019, we have been 40 people between members and visitors. Among them: 11 PhD students, 6 MSc students, and 2 BSc students. We have to care about our students, the future relay more in their hands than in ours.

I would like to thank all the people who made this report possible. I would also wish all the best to the coworkers who left the institute to continue a professional career somewhere else.

Thank you.

José L. Toca-Herrera

Contents

1. Institute members and visitors

Univ. Prof. Dr. José L. Toca-Herrera (director)
Ao. Univ. Prof. Dr. Dietmar Pum (deputy director)
O. Univ. Prof. em. Uwe B. Sleytr (emeritus, former director)
Assoc. Prof. Dr. Rafael Benitez Suarez (University of Valencia)
Assoc. Prof. Dr. Notburga Gierlinger (group leader)
Assoc. Prof. Dr. Luis Millan Gonzalez (University of Valencia)
Assoc. Prof. Dr. Tonya Andreeva (Reutlingen University, WTZ exchange program)
Dr. Habil. Reinhard Miller (BOKU visiting professor)
Dr. Andreas Breitwieser (post-doctoral research assistant)
Dr. Avgustina Danailova (Bulgarian Academy of Sciences, WTZ program)
Dr. Xavier García Massó (University of Valencia)
Dr. Jessica Huss (post-doctoral research assistant)
Dr. Jagoba Iturri (univ. assistant)
Dr. Itziar Otazo (tech. assistant)
Dr. Alberto Pardo (University of Valencia)
Dr. Svetozar Stoychev (Bulgarian Academy of Sciences, WTZ program)
Dr. Sudarat Tharad (univ. assistant)
Mag. Amsatou Andorfer-Sarr (techn. assistant)
Mag. Jacqueline Friedmann (tech. assistant)
MSc Sebastian Antreich (PhD student)
MSc. Peter Bock (PhD student)
MSc. Martin Feldhofer (PhD student)
MSc Juan Carlos Gil (PhD student, collaboration with Complutense University of Madrid)
MSc. Elham Ghorbani Gorji (PhD tudent, collaboration with Inst. Food Sci. - BOKU)
Dr. Med. Michael Handler (PhD student, collaboration with Sports University of Innsbruck)
MSc Martin Niedermeier (PhD student)
MSc. Naroa Sadaba (PhD student, collaboration with University of the Basque Country)
MSc. Nadia Sasani (PhD student)
MSc. Nannan Xiao (PhD student)
MSc. Andreas Weber (PhD student)
BSc Jakob Bachmayr (MSc Student)
BSc Alice Buytaert (Erasmus student, Ghent University)
BSc Ulrich Fuchs (MSc Student)
BSc Lukas Krismer (MSc student)
BSc Christoph Pötschner (MSc student)
BSc Charlotte Verlinde (Erasmus student, Ghent University)
Flavio Hoeck (BSc student)
Luis Ponce González (BSc student, collaboration with the University of Valencia)
Hannah Blaschka (apprentice)
Walter Klug (IT technician)

2. Research projects

Micro-contact printing - a useful feature to structure surfaces

Jacqueline Friedmann[1], Jagoba Iturri[1] and J. L. Toca-Herrera[1]

[1]Institute for Biophysics, Department of Nanobiotechnology, University of Natural Resources and Life Science, Vienna, Austria

Objective

Complementary to previous work µ-contact printing experiments regarding the growth of patterned polymer brushes are continued in the following directions:

(1) DOPA (3,4-dihydroxyphenylalanin) is used as a patterning agent which increases possible applications such as patterning on different substrates especially silicon prior to brush polymerisation or S-Layer recrystallization (experiments using ATRP as a polymerisation initiator need activated gold surfaces)

(2) Structuring S-Layer substrates by printing blocking agents (stabil blot or stabil guard) followed by protein recrystallization.

Results & Conclusion

DOPA as well as the blocking agents (stabil blot and stabil guard) show good results in attracting or repelling further growth of molecules such as polymer brushes or S-Layer proteins and can therefore be used to build complex sandwich structures in various pattern.

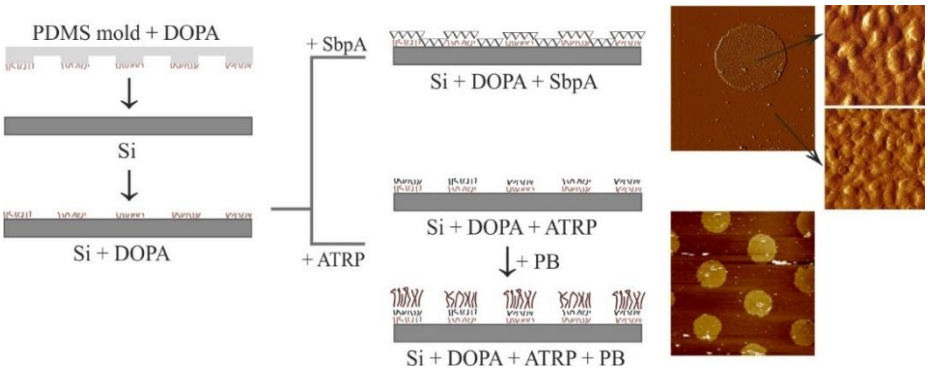

Figure 1. Patterning on silicon followed by SbpA recrystallization above and polymer brush growth below AFM images showing patterning: size 50µm / images up right showing SbpA lattice: size 700

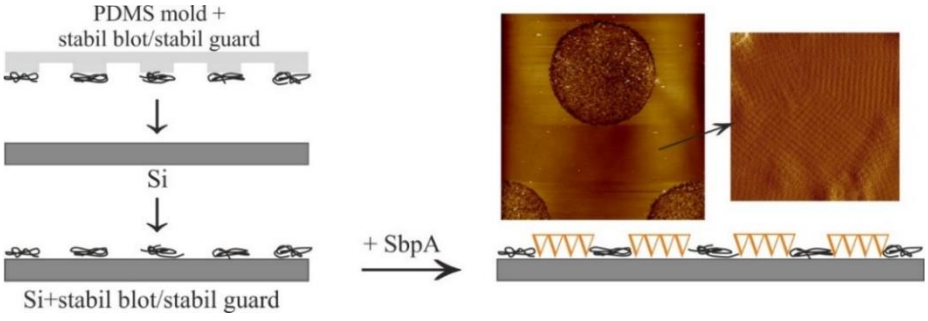

Figure 2. S-Layer blocking with stabil blot / stabil guard
AFM images show blocking and S-layer lattice on patterned stabil blot surface - left: size 100µm, right: size 400nm

Insitu recrystallization of Annexin V on lipid bilayers

Jacqueline Friedmann[1], Sudarat Tharad[1], Rafael Benítez Suárez[2], José L. Toca-Herrera[1]

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[2]Departamento de Matemáticas para la Economía y la Empresa, University of Valenica, Spain

Objective

Annexin V is a protein that binds to membranes containing negatively charged phospholipids in a calcium-dependent manner. The protein was recrystallized insitu to observe the growing of the crystalline patches over time. Several substrates, buffer concentrations, lipid mixtures and recrystallization techniques were tested to achieve optimal images.

These results will be used to test the probabilistic crystal growth model that was used for SbpA recrystallization [1].

Results & Conclusion

SUVs (small unilamellar vesicles) in the composition of POPC/DOPS fused on freshly cleaved mica sheets were the most suitable substrates for performing these insitu experiments. Annexin was added in the concentration of 10µg/ml in recrystallization buffer containing 2mM $CaCl_2$.

To overcome the problem of slow dissolution of the mica sheet from the AFM stub before finishing the recrystallization measurement, the mica sheet was first glued to a scotch tape prior fixation to the AFM stub and furthermore a liquid blocker pen was used the keep the droplet in shape and avoid covering the whole mica sheet with solution.

AFM measurements were performed in contact mode in liquid with a standard tip (spring constant 0.06N/m) treated with UV ozone prior use.

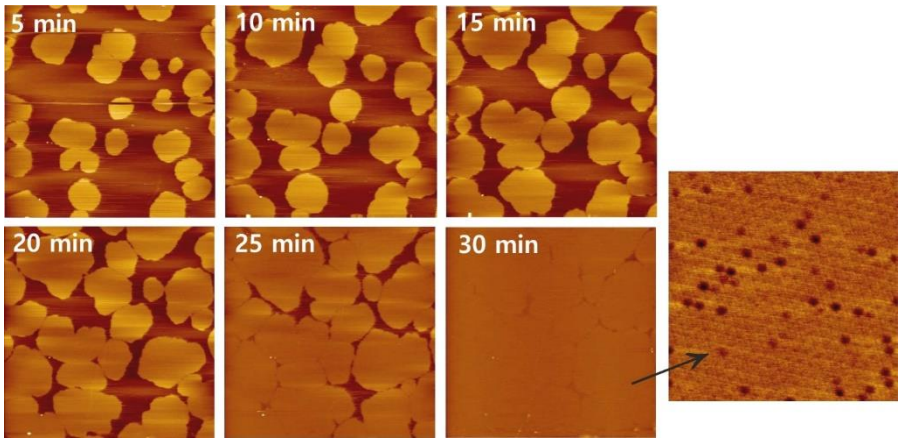

Figure 1. Insitu AFM measurement: growth of Annexin V layer – height images, size 10µm (z-limit 5nm)
far right: Annexin V pattern - protein recrystallization in p6 symmetry is clearly visible – height image, size 400nm (z-limit 1.2nm)

[1] V.J. Bolos, R. Benitez, A. Eleta-Lopez and J.L. Toca-Herrera, *Materials,* 2019, 12, 479.

Recrystallization of SbpA on Latex particles visualized with AFM

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[1]Institute for Biophysics, Department of Nanobiotechnology, University of Natural Resources and Life Science, Vienna, Austria
[2]Istituto Italiano di Tecnologia, Central Research Labs, Genova, Italy

Objective

The quality of recrystallized bacterial S-layer protein on polystyrene particles was proved. As main issue a proper fixation/stabilisation of the particles on a solid support is necessary to perform imaging measurements in AFM.

Results & Conclusion

Several glues and surface precoatings e.g. polyelectrolytes, secondary cell wall polymer or filter surfaces were tried out prior to finding a proper way to stabilize the latex particles on a solid surface.

The technique which finally led to good results was following: degassed PDMS was spin coated on silicon and precured at 65°C. Diluted 3µm latex particles were added before curing PDMS completely over night at 65°C. Subsequently S-Layer protein (SbpA) was recrystallized overnight - the pattern was resolved in AFM with a standard cantilever (0.12 N/m) in contact mode in liquid.

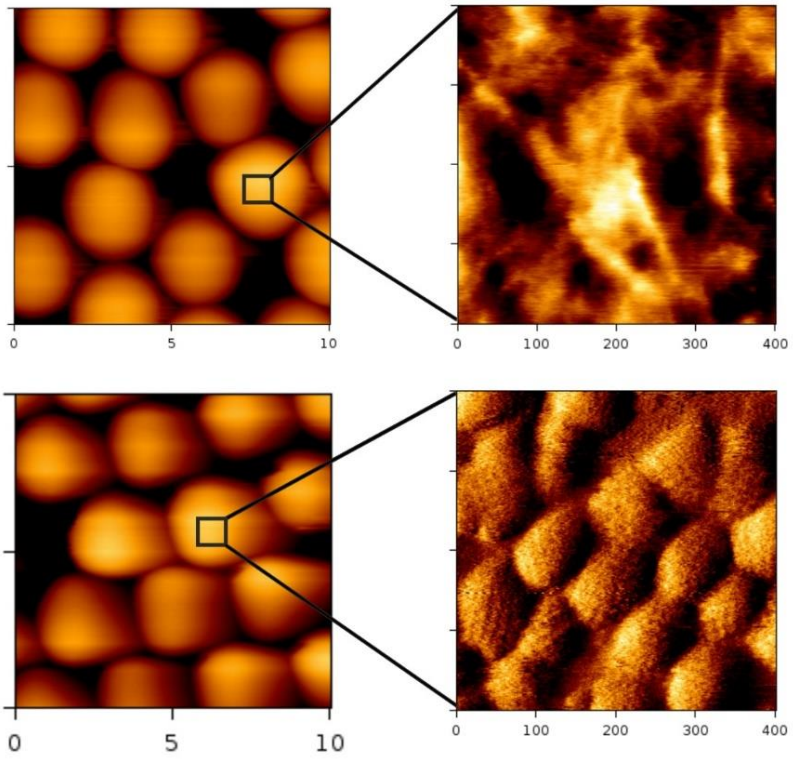

Figure 1. above: AFM images of pure latex particles fixed in PDMS (size 10µm / 400nm, z-limit: 2µm / 7nm)
below: subsequent recrystallization of SbpA on latex particles (size 10µm / 400nm, z-limit: 2µm / 25nm) – the square lattice structure featuring an optimal assembly and recrystallization is clearly visualized

Attaching microspheres to AFM cantilevers

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Objective

Microsphere cantilever tips can be used for performing force measurements in atomic force microscopy (AFM) e.g. mechanical testing of biological samples. These cantilevers can be purchased or prepared by gluing a hard sphere (e.g. silicon particle) to a tipless cantilever. Homemade spherical cantilevers offer a wide range of applications by choosing spheres of different materials and sizes. This allows measuring with defined and known tip geometry and surface parameters.

Results & Conclusion

For the attaching procedure a tipless cantilever (NP-O silicon nitride probe, Bruker) was approached to a surface coated with a freshly prepared thin glue layer and subsequently moved to a surface covered with particles (e.g. 10 or 20µm silicon particles). Particles were picked up by approaching slowly onto the particle and retracting the cantilever in small steps allowing the particle to be captured and lifted. Dependent on the glue used for the procedure a subsequent UV curing was necessary. These preparations were performed with both JPK I and JPK III devices. Quality control of the gluing procedure was performed by scanning electron microscopy (SEM).

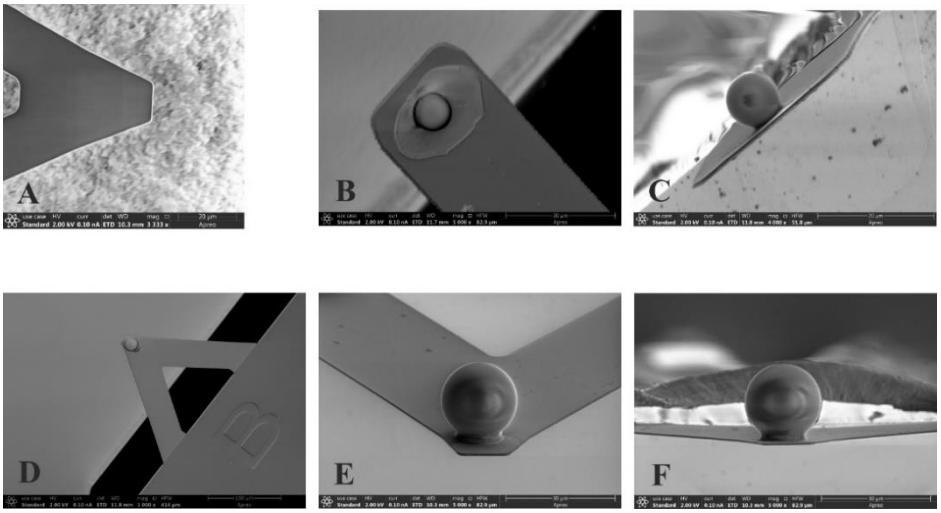

Figure 1. **A:** tipless silicon nitride cantilever, triangular, prior particle gluing, SEM image
B: tipless silicon nitride cantilever, rectangular, 10µm silicon particle attached, SEM image (tilt 45°)
C: tipless silicon nitride cantilever, rectangular, 10µm silicon particle attached, SEM image (tilt ~90°)
D: tipless silicon nitride cantilever, triangular, 20µm silicon particle attached, SEM image (tilt 45°)
E: tipless silicon nitride cantilever, triangular, 20µm silicon particle attached, SEM image (tilt 75°)
F: tipless silicon nitride cantilever, triangular, 20µm silicon particle attached, SEM image (tilt 90°)

Structure and mechanics of hard nutshells

J.C. Huss[1,2], S.J. Antreich[1], J. Bachmayr[1], N. Xiao[1] and N. Gierlinger[1]

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[2]Department of Biomaterials, MPI of Colloids and Interfaces, Potsdam, 14476, Germany

The aim of this project is to understand how plants build hard and strong shell structures. In order to clarify this issue, we study both the structural development and the mechanical properties of commonly encountered nutshells.

As part of the project, samples of pine nuts (*P. koraiensis*), walnuts (*J. regia*), pistachios (*P. vera*), hazelnuts (*C. maxima*), pecans (*C. illinoiensis*) and macadamia (*M. integrifolia*) nutshells were tested under tensile loading. In parallel, we investigated the shell structure in 2D and 3D by means of histochemical staining, x-ray micro-tomography and serial block-face scanning electron microscopy. The results indicate that the cellular arrangement plays a key role for the mechanical properties of the nutshells. Recently, we have already identified a new cell type in walnut shells – the polylobate sclereid cells (3D puzzle cells, shown in Fig. 1) [1] – which are able to withstand higher tensile loads than shells without lobed cells. The high tensile strength arises from the interlocking of the 3D puzzle cells, which is so effective that individual cells can only be separated by fracture formation through the thick cell walls [1].

Future experiments will focus on the biochemical changes in the cell walls during development of the 3D puzzle cells to understand which cell wall compounds are involved in the development of these intricate cell shapes. Studies on 2D puzzle cells have already revealed that pectin and cellulose are initiating morphogenetic events in pavement cells of Arabidopsis [2,3]. Moreover, we will also focus on the relevant structural properties of mature nutshells, which allow germination of the seed and emergence of the radicle.

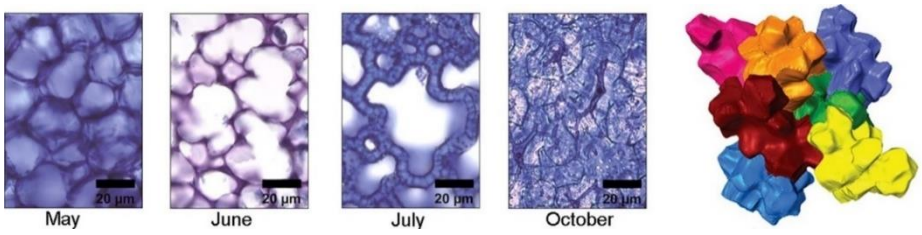

Figure 1. Toluidine blue stained sections of walnut shells, showing the development of the poly-lobate sclereid cells (May-October) and the intricate 3D interlocking of the cells at maturity in October (cells in colour obtained from segmentation of micro CT scans). Original figure adapted with permission [1].

Acknowledgements:
This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No. 681885). Furthermore, we would like to thank our collaborators M. Eder, N. Horbelt, R. Weinkamer, D. Werner and P. Leibner from the MPI of Colloids and Interfaces (Potsdam, Germany) for their support.

[1] S. J. Antreich, N. Xiao, J. C. Huss, N. Horbelt, M. Eder, R. Weinkamer and N. Gierlinger, *Advanced Science,* 2019, 1900644.
[2] B. Altatouri, A. J. Bidhendi, T. Tani, J. Suzuki, C. Conrad, Y. Chebli, N. Li, C. Karunakaran, G. Scarcelli and A. Geitmann, *Plant Physiology*, 2019, **181**, 127-141.
[3] A. J. Bidhendhi, B. Altatouri, F. P. Gosselin and A. Geitmann, *Cell Reports*, 2019, **28**, 1237-1250.

Shed new light on heartwood formation: A progress report

Martin Felhofer[1] and Notburga Gierlinger[1]

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Trees use nutrients derived from photosynthesizing cells and translocate or store these materials in special cells, called parenchyma cells (rays). After the use of the required substances, trees, unlike animals, have to use space within their tissue to get rid of secondary metabolites. At this point, heartwood formation takes place by impregnation with these secondary metabolites (e.g. extractives as pinosylvin) and the death of these cells in the innermost sapwood (transition zone). Coordinated transportation by vesicles and falling dry of the innermost xylem are supposed to be key steps in heartwood formation.[1] The aim of this work is to reveal the changes that extractives undergo from their synthesis during their transport and the final impregnation of wood cells making up the heartwood.

During the first half of the ongoing project, several questions have been addressed: Which mechanism is behind the transport of extractives? Are there any chemical/structural differences along sapwood and heartwood and how the cell wall gets impregnated with extractives. In the first project publication[2] in July 2018 several questions have been answered in order to improve our understanding of heartwood formation, but some keep us busy. The most important finding is that the transport of extractives for heartwood formation is in the form of lipid vesicles generated in the parenchyma rays. These vesicles undergo a chemical transition from sapwood (lipids) to heartwood (phenolics). In addition, they are found attached to the lumen sided S3 cell wall layer and to the pit membranes. The highest concentration of extractives is found in the middle lamella, indicating as well a transport way for the extractives. The most surprising fact was the possibility to mimic these vesicles in-vitro by creating emulsomes with isolated ethanol extract, rich in pinosylvins. However, the question around the cell wall impregnation concerns us still. Furthermore, we have developed and successfully screened several experiments in our lab in order to improve the framework around the study of heartwood formation. We also worked on the analysis of the thousands of Raman spectra acquired during Raman imaging. Herein, we showed that multivariate data analysing approaches have a high potential as the whole wavenumber region of all thousands of spectra is analysed at once.[3]

Despite everything, there are still some gaps to fulfil to understand how wood achieves its long-lasting and waterproofing properties: can heartwood formation be done artificially? Are the other components present in wood rearranging during heartwood formation? Which is the main driving force for heartwood formation? These questions will be assessed during the final period of the project, which will be finalised by August 2020.

Acknowledgements:
This work is supported by a fellowship of the Austrian Academy of Science (ÖAW) [24763] and the START Project [Y-728-B16] from the Austrian Science Fund (FWF) and from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme grant agreement No. 681885.

[1] Charles M. Stewart (1966) Excretion and Heartwood Formation in Living Trees. Science, 1966 Sep; Vol. 153, Issue 3740, pp. 1068-1074; doi.org/10.1126/science.153.3740.1068
[2] Felhofer M. et.al. (2018) Antifungal stilbene impregnation: transport and distribution on the micron-level. Tree Physiology, Volume 38, Issue 10, 1 October 2018, Pages 1526–1537; doi.org/10.1093/treephys/tpy073
[3] Prats Mateu B. et.al. (2018). Multivariate unmixing approaches on Raman images of plant cell walls: new insights or over interpretation of results? Plant Methods 14:52. https://doi.org/10.1186/s13007-018-0320-9

The puzzle of the walnut shell: a novel cell type with interlocked packing [1]

S. J. Antreich[1], N. Xiao[1], J. C. Huss[1,2], M. Eder[2], R. Weinkamer[2], N. Gierlinger[1]

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[2]Department of Biomaterials, MPI of Colloids and Interfaces, Potsdam, Germany

The outer protective shells of nuts can have remarkable toughness and strength, which are typically achieved by a layered arrangement of sclerenchyma cells and fibers with a polygonal form. Here, the tissue structure of walnut shells is analyzed in depth, revealing that the shells consist of a single, never reported cell type: the polylobate sclereid cells. These irregularly lobed cells with concave and convex parts are on average interlocked with 14 neighboring cells. The result is an intricate arrangement that cannot be disassembled when conceived as a 3D puzzle. Mechanical testing reveals a significantly higher ultimate tensile strength of the interlocked walnut cell tissue compared to the sclerenchyma tissue of a pine seed coat lacking the lobed cell structure. The higher strength value of the walnut shell is explained by the observation that the crack cannot simply detach intact cells but has to cut through the lobes due to the interlocking. Understanding the identified nutshell structure and its development will inspire biomimetic material design and packaging concepts. Furthermore, these unique unit cells might be of special interest for utilizing nutshells in terms of food waste valorization, considering that walnuts are the most widespread tree nuts in the world.

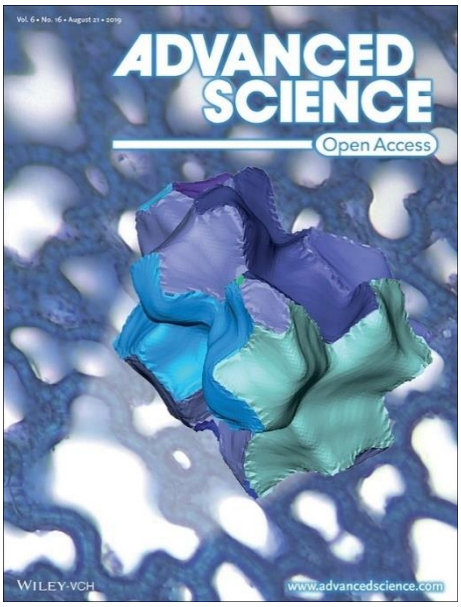

Figure 1. Cover picture of the Advanced Science Journal, showing a single cell in 3D and a section of a walnut shell tissue stained with Toluidin blue in the background

Acknowledgements:
We thank Karl Refenner (BOKU, Versuchszentrum Jedlersdorf) for access to the walnut trees in the BOKU Versuchszentrum Jedlersdorf and Peter Bock and Martin Felhofer for help in sampling. SJA, NX, NG acknowledge funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme grant agreement No 681885 and NG from the Austrian Science Fund (FWF) START Project [Y-728-B16].

[1] S. J. Antreich, N. Xiao, J. C. Huss, N. Horbelt, M. Eder, R. Weinkamer and N. Gierlinger, *Advanced Science,* 2019, 1900644

3D-Printing for scientific applications

S.J. Antreich[1] and P. Bock[1]

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The institute of biophysics has recently put a 3D-printer into use, which helps us in experimental design as well as getting an understanding of structures otherwise difficult to visualize.

The machine uses a filament of polylactic acid (PLA), a biodegradable polymer that is heated up to its melting point and extruded of a nozzle during printing. The nozzle diameter currently used is 0.4 mm – this is also the lateral resolution. The object is formed by subsequent addition of layers which can be up to 0.05 mm thin. Objects can be sized up to 135 x 120 x 100 mm and several objects can be printed within this volume simultaneously.

The 3D-printer is actually used to help understanding the interlocking of cells of different nutshells. We use 3D-stacks from different methods like µ-CT or SBF-SEM to reconstruct 3D-surface models from cells of different nut species in the software Amira-Aviso (FEI, SAS) [1]. These models are loaded into the currently employed software Inventor (Autodesk) to create the needed file format for the controlling software Cura (Ultimaker B. V.) that the printer uses (Fig. 1, left). In this way, we are able to print interlocked cells in a magnification of 1:500 (Fig.1, middle). With the printed cells, we better understand, how the interlocking between the cells contribute to the tested mechanical properties of the different nutshells (Fig.1, right).

In future, we will print 3D-models from other nutshells with different cell types, so we can better understand the different strategies in building a tough nutshell.

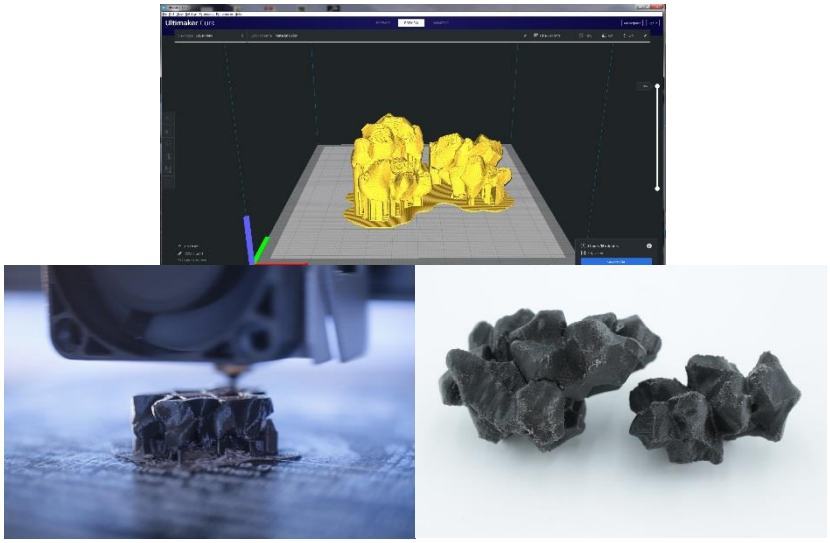

Figure 1. 3D-model of three walnut cells loaded into the Cura software (left), which is printed in the 3D-printer (middle), finished model (right).

Acknowledgements:
This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No. 681885).

[1] S. J. Antreich, N. Xiao, J. C. Huss, N. Horbelt, M. Eder, R. Weinkamer and N. Gierlinger, *Advanced Science,* 2019, 1900644.

From the soft to the hard: Changes in microchemistry during cell wall maturation of walnut shells

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[1]Institute for Biophysics, Department of Nanobiotechnology, University of Natural Resources and Life Science, Vienna, Austria
[2] Department of Botany and Biodiversity Research, Division of Structural and Functional Botany, University of Vienna, Austria

Aim. The aim of this study is to understand the structure and composition of the mature walnut shell and its development. Analyzing chemical changes of nutshells in the context of microstructure during maturation (lignification) by FT-IR and Confocal Raman Microscopy will provide valuable insights into composition of the interlocked polylobate walnut cells and their "fabrication"[1].

Results. In the soft (developing) walnut shell the cells towards the green husk have the thickest and most lignified cell walls. With secondary cell wall thickening, the amount of all cell wall components (cellulose, hemicellulose and especially lignin) increase as revealed by the FTIR microscopy. On the cell wall level, Raman imaging showed that lignin is deposited first into the pectin network between the cells and cell corners when the secondary cell wall is added onto the primary cell wall as found in the inner July shell (shown in Fig. 1a). Furthermore, Raman imaging of fluorescence visualized numerous pit channels within every cell, connecting all the interlocked polylobate walnut shells with each other and enabling transport during development (shown in Fig. 1b). In the final mature stage, fluorescence was increasing throughout the cell wall, but also a remarkable fluorescent layer towards the lumen was detected in the inner part (shown in Fig. 1c).

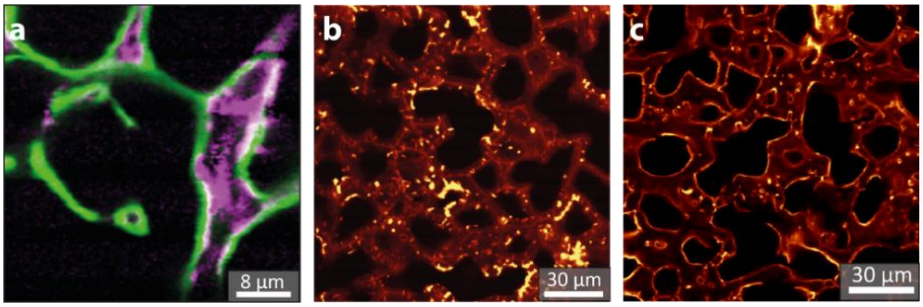

Figure 1. a. NMF analysis of the inner July shell showing the two representative endmember (EM) abundance maps of cell wall (green, cellulose), intercellular space and cell corner (magenta, lignin). b. Raman images of the outer July shell were calculated by integrating over the fluorescence background. c. shows Raman images of the inner of October shell by integrating the fluorescence background.

Outlook. As the next step we will focus on lignin degradation caused by 532 nm laser bleaching, which may be helpful to show differences in composition of the walnut shell cell wall or in lignin substructures. We also continue to focus on the pistachio (*P. vera*) shell and explore the reason for its higher tensile strength compared to the walnut shell. As both employ a similar puzzle cell shape, we aim our research on the microchemistry as well as the nanostructure and nanomechanics by atomic force microscopy. Correlated measurements will allow us to draw conclusions on structure-function relationships.

Acknowledgements:
This work has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 681885) and the Austrian Science Fund (FWF) START Project [Y-728-B16].

[1] S. J. Antreich, N. Xiao, J. C. Huss, N. Horbelt, M. Eder, R. Weinkamer and N. Gierlinger, *Advanced Science,* 2019, 1900644.

Physiological and anatomical response of two larch species and their hybrid to drought

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Using a plant robot experimental setup (Ref.) the effect of drought on physiological and wood anatomical aspects was investigated on three larch species: European larch, Japanese larch and their Hybrid. Biomass, transpiration, relative water content, water use efficiency and root/shoot ratio (R/S) were assessed in control (C-trees) and stressed (S-trees) trees as well as cell wall thickness and lumen diameter of tracheidsby light microscopy. The response to drought resulted in lower biomass, transpiration and relative water content and higher water use efficiency in S-tress compared to C-trees for all species. The highest water use efficiency under the impact of drought had Japanese larch, the lowest European larch. The initial R/S was highest in European larch and lowest in Japanese larch. S-trees showed a significant increase in final R/S, with a maximum increase in European larch. In non-stressed early wood, conduit wall reinforcement in the tangential direction was significantly higher in European larch than in Hybrid or Japanese larch. The radial lumen diameter and tangential wall thickness from the entire last annual ring was used to show the effect of drought in different relative distances. At the end of the experiment, lumen diameter of stressed European larch was significantly higher than in stressed Japanese and Hybrid larch, indicating that the latter two started already to produce latewood. It is concluded that larch species responded to drought by minimizing loss of water through transpiration and more efficiently utilizing the available water (water use efficiency).

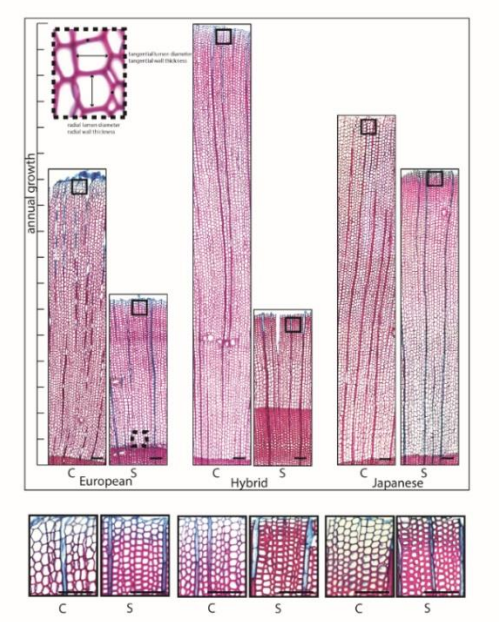

Figure 1. Transverse section of xylem growth increment showing species difference between control(C) and stressed (S)trees .Boxed regions are shown at high magnification (scale bar=100 µm)corresponding to low magnification (scale bar=100 µm). Sections were stained with safranin-astrablue.

Acknowledgements:
This work is supported by the START Project [Y-728-B16] from the Austrian Science Fund (FWF).

Infrared and Raman spectra of lignin substructures: Dibenzodioxocin[1]

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Vibrational spectroscopy is a very suitable tool for investigating the plant cell wall *in-situ* with almost no sample preparation. The structural information of all different constituents is contained in a single spectrum. Interpretation therefore heavily relies on reference spectra and understanding of the vibrational behavior of the components under study.

For the first time we show infrared (IR) and Raman spectra of dibenzodioxocin (DBDO), an important lignin substructure. A detailed vibrational assignment of the molecule, based on quantum chemical computations, is given in the supplementary material, the main results are found in the paper. Furthermore, we show IR and Raman spectra of synthetic guaiacyl lignin (dehydrogenation polymer - G-DHP). Raman spectra of DBDO and G-DHP both differ with respect to the excitation wavelength and therefore reveal different features of the substructure/polymer.

This study confirms the idea previously put forward that Raman at 532 nm selectively probes end groups of lignin, whereas Raman at 785 nm and IR seem to represent the majority of lignin substructures.

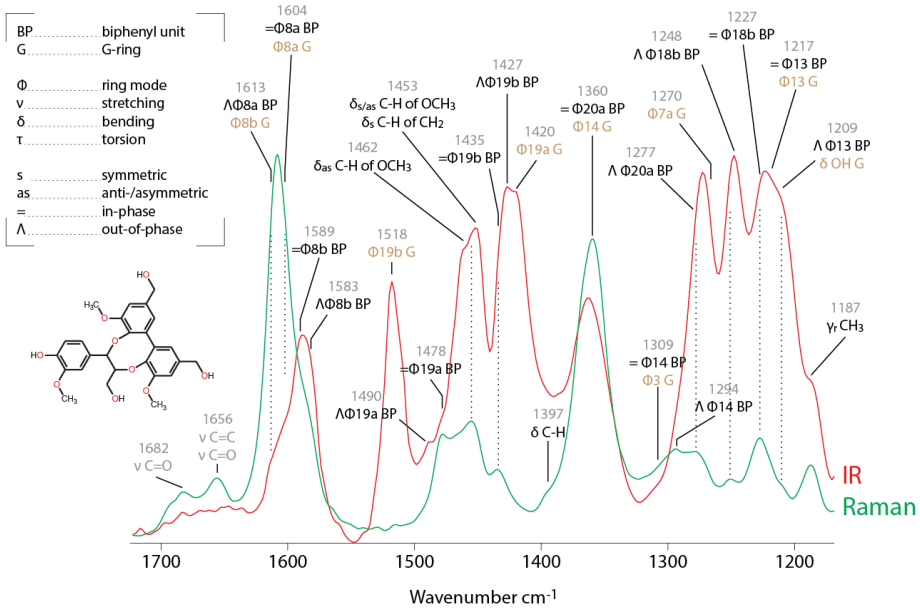

Figure 1. Assigned IR and Raman spectra of DBDO in the region 1700 – 1200 cm^{-1}.

Acknowledgements:
This work received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program grant agreement No. 681885 and from the Austrian Science Fund (FWF) START Project [Y-728-B16].

Grigory Zinovyev is thanked for the kind provision of the G-DHP sample. Adya Singh read through the manuscript with keen eyes – we really appreciate his dedicated work! This work used the Extreme Science and Engineering Discovery Environment (XSEDE), which is supported by National Science Foundation grant number ACI-1548562. Specifically, it used the Stampede 2 at the Texas Advanced Computing Center (TACC), both via MCB-090159, Gregg T. Beckham, PI.

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Infrared and Raman spectra of lignin substructures: Coniferyl alcohol, abietin and coniferyl aldehyde[1]

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Anatomical and chemical information can be linked by Raman imaging. Behind every pixel of the image is a Raman spectrum, which contains all the information as a molecular fingerprint. Yet to understand the spectra, the bands have to be assigned to components and their molecular structures. Although the lignin distribution is easily tracked in plant tissues, the assignment of the spectra is not good enough to allow in-depth analysis of the composition.

Assignments of three lignin model compounds were derived from polarization measurements and quantum-chemical computations. Raman spectra of coniferyl alcohol crystals showed orientation dependence, which helped in band assignment. Abietin showed a Raman spectrum that was very similar to the spectrum of coniferyl alcohol, whereas its IR spectrum was very different due to bands of the sugar moiety. The Raman spectrum of coniferyl aldehyde is affected by the crystal order of molecules.

All three compounds show much stronger band intensities than unconjugated single aromatic rings, indicating that the bulk of the lignin structure has significantly reduced contribution to Raman band intensities. Therefore, it is possible to highlight certain structures of lignin with Raman spectroscopy, because low amounts of a compound do not necessarily mean weak features in the spectrum.

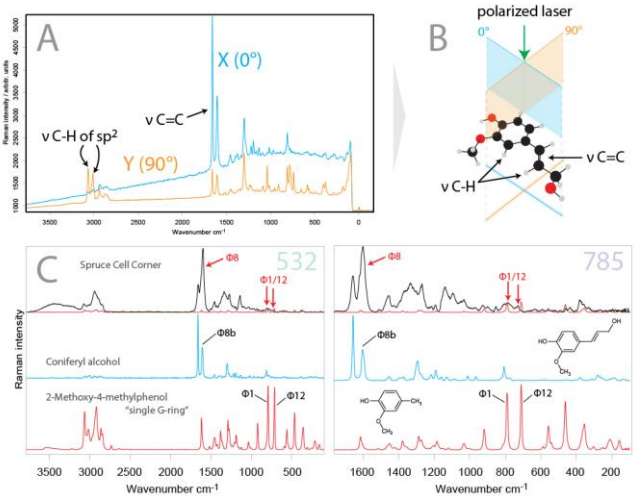

Figure 1. A) Different spectra of the molecule are recorded, depending on its molecular orientation shown in B). C) Conjugated molecules are stronger Raman scatterers than unconjugated ones and therefore overrepresented in the lignin spectrum.

Acknowledgements:
P. B. and N. G. acknowledge funding from the Austrian Science Fund (FWF) START Project (Y-728-B16) and from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme grant agreement No 681885. We especially want to express our gratitude towards Martina Schroffenegger for helping in the reduction experiments. Christian Knoll from the Institute of Chemical, Environmental & Biological Engineering of TU Wien is acknowledged for helping with the computational chemistry. Martin Felhofer is acknowledged for his precise microtome cutting. Adya Singh is cordially thanked for English correction of the manuscript.

[1] P. Bock and B. Gierlinger, *Journal of Raman spectroscopy,* 2019, 50, 778.

Calcium oxalate crystals in different nut species confirmed by Raman microscopy

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It is well known that calcium oxalate crystals are present in numerous plant species. In contrast to animals, the presence of these crystals are not considered to be pathological in plants. Contrary, crystal formation can be seen as intracellular detoxification process to remove excess oxalic acid and a surplus of calcium [1]. Literature about the occurrence of oxalic acid or calcium oxalate in nuts mainly concentrate on the kernel, to obtain information about nutritional values. Nevertheless, it is known that calcium oxalate monohydrate (COM) crystals are present in the pecan nutshell (*Carya illinoiensis*) [2]. With this background the question arose, if COM crystals can be found in several tissues of nuts from different species. Additionally the chemical composition, distribution, crystal development during nut growth and the classification of crystal holding cells are main objectives for my ongoing work. Results are going to be obtained with various microscopic and analytical methods.

So far it could be proven that several nut species inherit COM crystals. Crystals could be found in different tissues. Crystal distribution seems to be species dependent. For instance in pecan, crystals can be found easily. They appear to be randomly distributed in the husk and mainly the outer part of the shell. While the husk contains druse shaped COM crystals [Fig. 1], the shell contains crystals with different morphology (e.g. rhombic, druses, cubic; results not shown). All found crystals seem to have the same chemical composition (CaC_2O_4 * H_2O).

Upcoming work will concentrate on the classification of crystal containing cells and the crystal development during the nut growth.

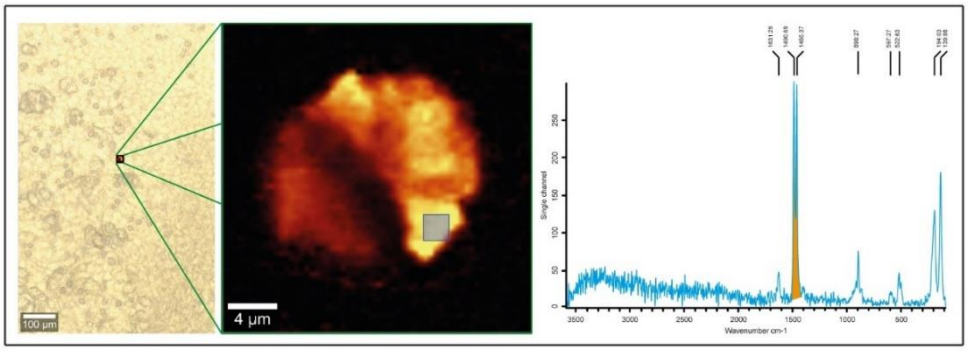

Figure 1. Light microscopic image and Raman measurement of a druse shaped COM crystal, found in the husk of a pecan. Raman image shows the integration at 1472 cm^{-1} (orange area in the spectrum); Spectrum shows measurement from an area indicated by the blue area on the crystal

Acknowledgements:
This work has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 681885) and the Austrian Science Fund (FWF) START Project [Y-728-B16].

[1] V.R. Franceschi, H.T. Horner. Calcium oxalate crystals in plants. Bot. Rev. 1980, 46(4), 361-427.
[2] J. Nicolás-Bermudez et al.. Morphological and micromechanical characterization of calcium oxalate (CaOx) crystals embedded in the pecan nutshell (*Carya illinoinensis*). Plant Physiol. Biochem. 2018, 132, 566-570.

S-layer protein and carbon nanotube construction kit

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Aim/Objective. The main objective of the project is to conduct fundamental studies on the reassembly of S-layer proteins on carbon nanotubes (CNTs) and to learn how these hybrid architectures can be used to produce new materials. The reassembly and binding properties of S-layer proteins, which enable a highly specific and sensitive functionalization of the CNT surface, are crucial. Moreover, novel organic-inorganic nanostructures (e.g. nanocontainers for drug delivery) might be made possible by using the S-layer coating as a template for the biomineralization of silicon dioxide, metals or other technologically important materials.

Results. Carbon nanotubes (CNTs) are cylindrical nanostructures made of carbon atoms. Due to their excellent mechanical and electrical properties as well as their thermal conductivity, they are already used as additives in various novel materials. However, since CNTs are chemically inert and insoluble in water, they must be chemically functionalized or coated with biomolecules to carry payloads or interact with the environment. Proteins bound to the surface of CNTs are preferred because they offer better biocompatibility in medical applications. In general, they are only bound irregularly, so that arrangement and density of functional groups for binding further biomolecules on the surface varies greatly. Bacterial surface layer proteins (S-layer proteins), which have already attracted much attention for the functionalization of surfaces and support structures for biomembranes, offer an alternative approach to the functionalization of CNTs with the advantage of an additionally closed and precisely ordered arrangement.

We have shown already that S-layer proteins (SbpA of *Lysinibacillus sphaericus* CCM 2177 and the recombinant fusion protein r$SbpA_{31\text{-}1068}$GG consisting of the S-layer protein and two copies of the IgG binding region of protein G) can be used for the dispersion and functionalization of oxidized multi-walled CNTs [1,2]. We decided to work with multi-walled nanotubes (MWNTs) with diameters of 30 - 200 nm because the diameters of single and double-walled nanotubes (typically below 10 nm) could be too small for the S-layer unit cell size of 13.1 x 13.1 nm. The addition of S-layer protein to aggregated CNTs led to their immediate dispersion. Moreover, the CNTs were completely covered by the S-layer over dozens of micrometers.

Conclusions. We would like to anticipate that the S-layer and carbon nanotube construction kit introduced in this new project will offer the advantage of producing completely new supports and containers when used as a catalyst, template, framework or affinity matrix. In particular, the large variety of functional S-layer fusion proteins has the potential for a wide range of functionalized CNTs.

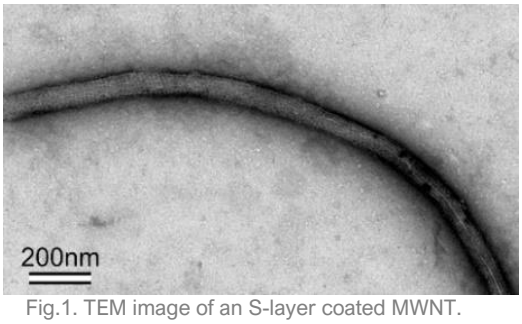

Fig.1. TEM image of an S-layer coated MWNT.

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Lipid phase influences the binding of *Bacillus thuringiensis* Cyt2Aa2 toxin on model lipid membranes

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Objective

Cytolytic toxin Cyt2Aa2 binds directly to the lipid membrane without a specific protein receptor requirement. In this work, the effect of lipid phase state on Cyt2Aa2-lipid membrane binding was investigated.

Results

By means of atomic force microscopy (AFM), Cyt2Aa2 toxin selectively bound to L_d lipid bilayers of POPC. In turn, it did not bind to the L_o and S_o phases of DPPC. Lipid mixtures of DPPC/POPC revealed lipid phase separation with the ratio of 1:1 and 4:1 (by mole). Interestingly, the exposure of Cyt2Aa2 toxin to these lipid bilayers revealed distinct binding location for Cyt2Aa2 toxin. The POPC-enriched domain were a target membrane in the 1:1 DPPC/POPC bilayer. On the contrary, the binding of Cyt2Aa2 was localized at the lipid phase boundary for the 4:1 DPPC/POPC bilayer. In addition, quartz crystal microbalance with dissipation (QCM-D) implied the POPC-enriched domain binding with mass quantification. In particular, the amount of protein bound to 1:1 DPPC/POPC (with phase separation) was half of the binding quantified for the L_d phase lipid bilayer (pure POPC and 1:4 DPPC/POPC mixture).

Conclusions

These results indicate that the lipid phase (lipid acyl chain) of lipid membrane influences the Cyt2Aa2-lipid interaction.

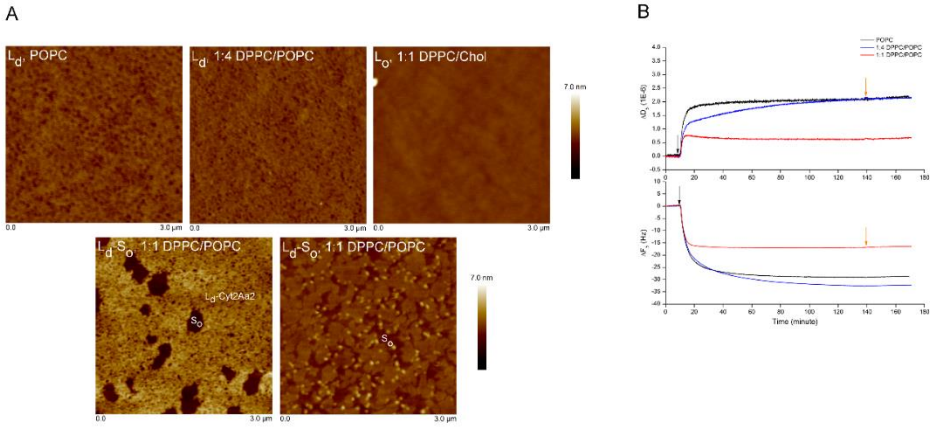

Figure 1. The interaction of Cyt2Aa2 toxin with different lipid phase bilayers. (A) AFM-height topographic image and (B) QCM-D result

Acknowledgements: This work was supported by The Austrian Science Fund (FWF) grant number: P29562-N28.

T144A point mutation of cytolytic toxin Cyt2Aa2 interacts specifically with lipid bilayers

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Objective

Cyt toxin exerts its activity by direct binding to lipid membrane and disturbing the membrane integrity of target cells including erythrocyte cells. In this report, the interaction of Cyt2Aa2 toxin and lipid membrane models of erythrocyte was investigated for the wild type (WT) and the T144A mutant.

Results

The point mutation at position of threonine 144 to alanine (T144A) resulted in a decreasing of hemolytic activity. Consequently, atomic force microscopic (AFM) image revealed the less efficiency of lipid binding for the T144A compared to the WT. The T144A mutant was unable to bind to the liquid disordered phase (L_d) bilayer of POPC. Addition of cholesterol (Chol) or sphingomyelin (SM) into lipid membrane enhanced the T144A binding on the liquid ordered phase (L_o) bilayers despite it was not necessary for the WT. Cyt2Aa2 WT entirely bound the lipid membrane surface. Unlike WT, AFM images unveiled the small aggregates for the T144A binding on the 1:1 SM/POPC and 1:1 SM/DOPC bilayers. Moreover, the S_o domain of sphingomyelin was not the binding place for both the WT and T144A mutant.

Conclusions

The single mutation T144A can contribute a specific-L_o bilayer binding to Cyt2Aa2 toxin and consequently lead to the less activity.

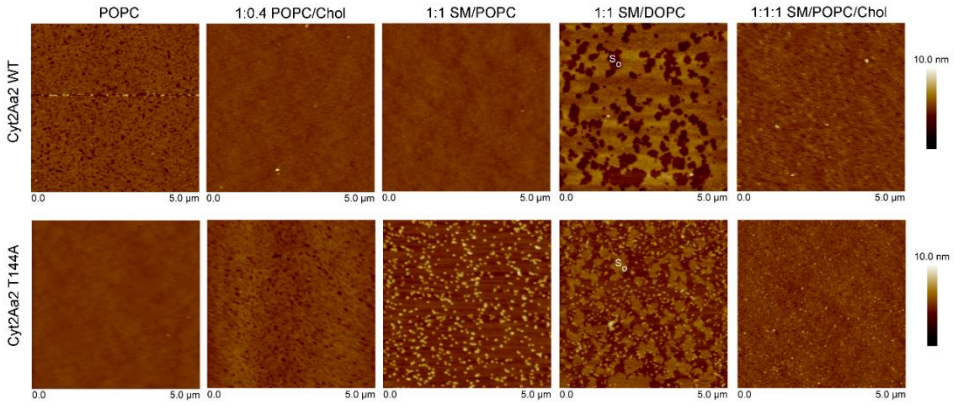

Figure 2. The interaction of Cyt2Aa2 toxin with different lipid bilayers of the wild type and T144A mutant toxins.

Acknowledgements:

This work was supported by The Austrian Science Fund (FWF) grant number: P29562-N28.

Formation of lipid bilayers of different composition and their subsequent interaction with Cyt2Aa2 toxin

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Supported lipid bilayers (SLBs) are popular models of cell membranes. They are really useful for the in vitro study of interactions with biomolecules and other elements with potential bio-technological application. In this regard it is important to control both their formation and properties. Besides, Bacillus thuringiensis is a cytolytic toxin showing activity against a variety of cells, including bacteria. In this work the influence of the membrane composition regarding the Cyt2Aa2 interaction will be studied in two steps. QCM-D experiments were carried out to study the influence of the lipid composition on both the bilayer formation and the subsequent Cyt2Aa2-lipid interaction.

From our measurements, the use of SiO_2 sensors was observed to be more adequate for the formation of the SLB. While alternative TiO_2 substrates improved the vesicle absorption, formation of SLB was not fully achieved. The use of Ca^{2+} ions has also shown to be critical in the adsorption and fusion of the formed vesicles. Indeed, depending on the liposome composition, different amounts of Ca^{2+} will be necessary for the vesicles to fuse Furthermore, the presence of Ca^{2+} can convert the lipid bilayer into a more elastic structure.

The influence of the different structures achieved on the subsequent interaction with the cytotoxin Cyt2Aa2 was also studied. In general, the protein seemed to interact much better in the presence of Ca^{2+} containing buffer, while in its absence interaction drops (basic running buffer: 10 mM Tris + 100 mM NaCl). This behaviour was measured for different bilayer compositions: POPG 100%, POPG:POPC (1:1),POPG:POPC (1:2), POPG:POPE (1:1) and POPG:POPE (1:5),

For a better understanding of the interaction taking place, mutant versions of the Cyt2Aa2 were also used. As observed, replacement of one original amino acid by a positively charged amino acid (T148K and I150K), did not show any interaction with the bilayer. In the particular case of T144A mutant (original amino acid replaced by Alanine), the interaction seemed to occur at longer (> 2h) incubations.

Electrochemical studies on the interaction between Cyt2Aa2 cytotoxin and substrate supported lipid bilayers

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Cyclic Voltammetry (CV), and Impedance (EIS) measurements have been performed simultaneously to regular QCM-D experiments in order to have a better understanding of the interaction between Cyt2Aa2 and substrate-supported POPC/Cholesterol (13:1) lipid bilayers. Cyclic Voltammetry is useful to determinate the packing of a layer, as well as to discard the presence of defects, while Impedance measurements display the resistance that the layer shows.

According to the results, high concentrations of the cytolytic protein induced the formation of densely packed films with low ion permeability, high electrical resistance and low capacitances, almost independently from the time kept in incubation. Cyclic Voltammetry experiments (Fig. 1a), for instance, confirmed a better ion passage insulation as the number of layers increased. This is, the electrical current (in µA) dropped when going from bare surfaces to SLB and SLB/Cyt2Aa2 containing systems. In this last case, the performance of the films created from both low (10 µg/ml) and high (100 µg/ml) toxin concentrations was almost identical, with the exception of a current peak in the -0.4 to -0.6 V range observed for the high concentration film. The intensity of this peak resembles those values from uncoated SiO_2 substrates. Also a detailed look over Bode plots (Fig. 1b, c) brings again a subtle variation in the behavior of 100 µg/ml Cyt2Aa2 systems: in the 1-100 Hz range this sample stays closer to the trend followed by bare substrates rather than to SLB and 10 µg/ml toxin systems.

On the other hand, hybrid toxin-lipid layers from low Cyt2Aa2 concentrations evolved over time to yield electrical properties which oppose the previous ones (high permeability, low resistance, and high capacitance) and, at the same time, resemble an intermediate-like state between the bare substrate and an intact bilayer. This could be indicative of major membrane defects/disruption taking place upon sufficiently long incubations under low toxin concentrations. However, still, a deeper analysis would be required to address the exact ongoing mechanism.

a) Cyclic voltametry b) Bode plot c) Nyquist plot d) Faraday Impedance plot

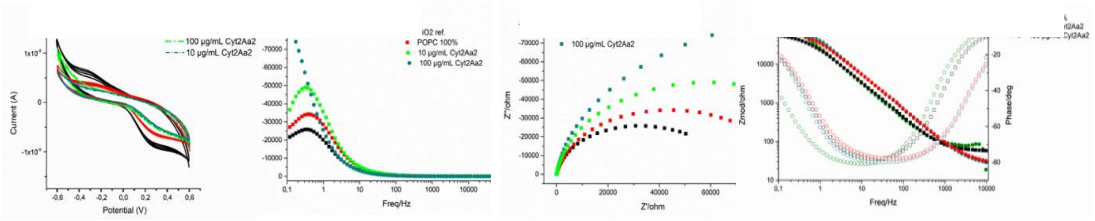

Figure 1: a) CV curves,
b) Bode Plots and
c) Nyquist plot of SiO2 ref. (black), POPC 100% bilayer on SiO2 (red), 10 µg/mL Cyt2Aa2 interaction with the POPC 100% bilayer (green), 100 µg/mL Cyt2Aa2 interaction with POPC 100% bilayer (blue).
d) . Faradic Impedance Spectra (Zmod & Phase vs Frequency Plot). Closed and open symbols represent Zmod and Phase values, respectively, for SiO2 (black), POPC bilayer (red), 10 µg/ml Cyt2Aa2 (dark cyan) and 100µg/ml Cyt2Aa2 (green). Experimets had been performed in presence of 0.1 M LiClO4 + 1 mM K4Fe(CN)6 in water

Discolouration of synthetic dyes by Laccase

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The use of oxidative enzymes as a tool for removing toxic compounds from the environment has increased in recent years. In this regard, the use of Laccase (from *T. Pubescens*) can be an interesting approach, since this enzyme is able to oxidize a great variety of phenolic and non-phenolic compounds. Then, measurements aimed testing real efficiency of Laccase in the discoloration of synthetic dyes such as RBBR (Remayol Brilliant Blue R) and MG Methyl Green. For this purpose, QCM-D and UV-Vis Spectrophotometry techniques were employed. While QCM-D allows following in real time Laccase's immobilization process, UV-Vis Spectrophotometer will allow to quantify the discoloration process.

First, in order to determine the maximum dye concentration that the enzyme is able to process, pre-purified Laccase was immobilized on Silicon Dioxide sensors (either directly or on top of polyelectrolytes) and subsequently exposed to increasing concentrations of the colored solution at a 10µl/min rate. These solutions were collected at the end of their incubation, and the color shift was compared with the reference UV spectrum. According to the results, the discoloration percentage varies with the starting concentration but, in general terms, it presents a linear relationship (Figure). As observed, the ability of Laccase for dye discoloration is out of any doubt.

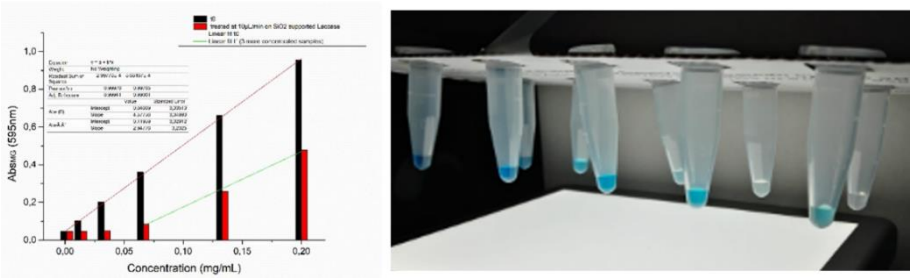

Figure 1: (Left) MG dye Absorbance at 595 nm before and after the treatment with Laccase. (Right) Image of the MG discoloration before and after the treatment.

Acknowledgements:

I would like to thank MSc. Andreas Weber and Dr. Jagoba Iturri for the technical support. We also thank Prof. Susana Rodriguez Couto for providing the organism.

Life under Continuous Streaming: Recrystallization of Low Concentrations of Bacterial SbpA in Dynamic Flow Conditions

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The well-known bacterial S-layer protein SbpA from Lysinibacillus sphaericus CCM2177 induces spontaneous crystal formation via cooperative self-assembly of the protein subunits into an ordered supramolecular structure. Recrystallization occurs in the presence of divalent cations (i.e., Ca^{2+}) and finally leads to producing smooth 2-D crystalline coatings composed of squared (p4) lattice structures. Among the factors interfering in such a process, the rate of protein supply certainly plays an important role since a limited number of accessible proteins might turn detrimental for film completion. Studies so far have mostly focused on high SbpA concentrations provided under stopped-flow or dynamic-flow conditions, thus omitting the possibility of investigating intermediate states, in which dynamic flow is applied for more critical concentrations of SbpA (i.e., 25, 10, and 5 µg/mL). In this work, we have characterized both physico-chemical and topographical aspects of the assembly and recrystallization of SbpA protein in such low concentration conditions by means of in situ Quartz Crystal Microbalance with Dissipation (QCMD) and atomic force microscopy (AFM) measurements, respectively. On the basis of these experiments, we can confirm how the application of a dynamic flow influences the formation of a closed and crystalline protein film from low protein concentrations (i.e., 10 µg/mL), which otherwise would not be formed.

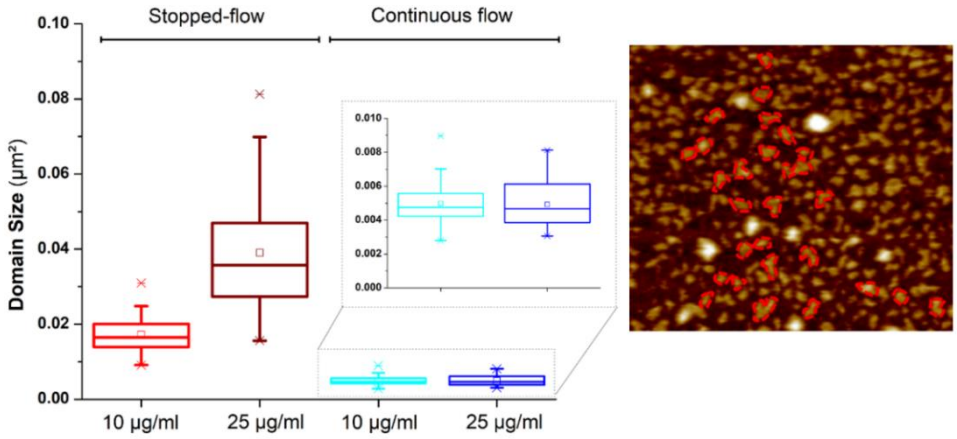

Figure 1. (Left) Boxplots showing the SbpA S-layer domain dimension values after an incubation of 30 minutes. The number of domains considered for the statistical analysis was N = 50.
(Right) Height micrographs of the crystalline layer formation, with the protein domains highlighted in red.

Acknowledgements:

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Miscibility, interactions and antimicrobial activity of PCL/chloramphenicol blends

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Poly(ε-caprolactone) (PCL) has been blended with Chloramphenicol (CAM), a well-known bacteriostatic antibiotic, in order to obtain new biomaterials with antibacterial properties. The resulting samples have been thoroughly characterized regarding both their physicochemical behavior and antimicrobial efficacy by means of very diverse techniques. Hence, PCL/CAM blend miscibility has been analyzed by Differential Scanning Calorimetry (DSC) using the single glass transition temperature (Tg) criterion, intermediate between those corresponding to the two components in the blend. In turn, the interaction parameter has been obtained from the analysis of the melting point depression in both PCL-rich and CAM-rich blends. Fourier-Transform Infra-Red (FTIR) spectroscopy and X-Ray Diffraction (XRD) analysis have been used - in the pure components and in the blends- to analyze both the specific interactions and the crystallization behavior, respectively. The morphology of PCL/CAM blends obtained by spin-coating has been also studied by means of Atomic Force Microscopy (AFM). Finally, drug release kinetics of different PCL/CAM systems as well as their antibacterial efficacy against Escherichia Coli have been investigated, indicating that CAM can be released from the PCL/CAM blends in a controlled way while keeping intact the antibacterial efficiency.

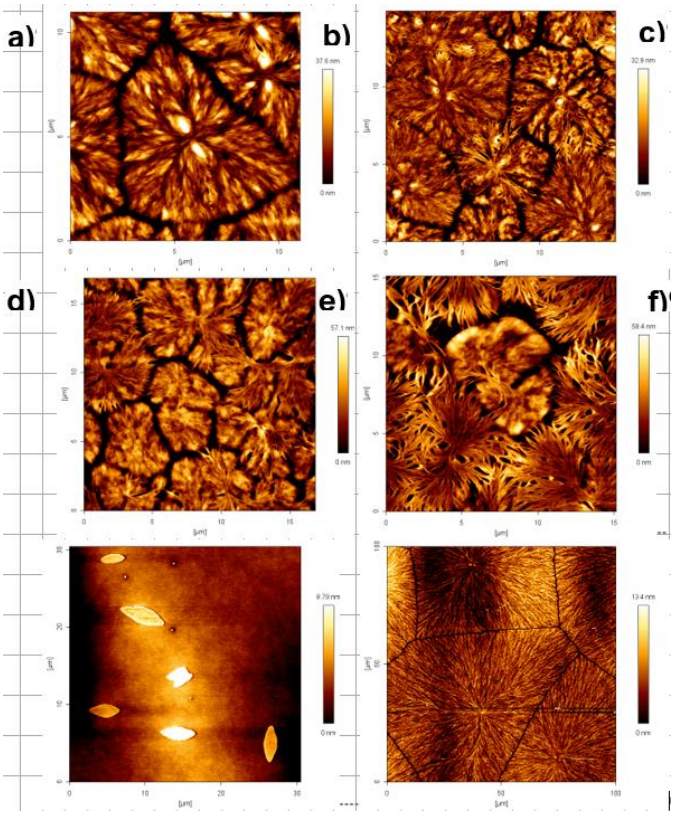

Figure. AFM topographic images: (a) pure PCL; (b) PCL/CAM 80/20; (c) PCL/CAM 60/40; (d) PCL/CAM 40/60; (e) PCL/CAM 20/80; and (f) PCL/Erythromycin 50/50 (immiscible). Films thicknesses were about 800 nm.

In-situ 2D bacterial crystal growth as a function of protein concentration: An atomic force microscopy study

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The interplay between protein concentration and (observation) time has been investigated for the adsorption and crystal growth of the bacterial SbpA proteins on hydrophobic fluoridefunctionalized SiO2 surfaces. For this purpose, atomic force microscopy (AFM) has been performed in real-time for monitoring protein crystal growth at different protein concentrations. Results reveal that (1) crystal formation occurs at concentrations above 0.08 μM and (2) the compliance of the formed crystal decreases by increasing protein concentration. All the crystal domains observed presented similar lattice parameters (being the mean value for the unit cell: a = 14.8 ± 0.5 nm, b = 14.7 ± 0.5 nm, γ = 90° ± 2). Protein film formation is shown to take place from initial nucleation points which originate a gradual and fast extension of the crystalline domains. The Avrami equation describes well the experimental results. Overall, the results suggest that protein-substrate interactions prevail over protein–protein interactions.

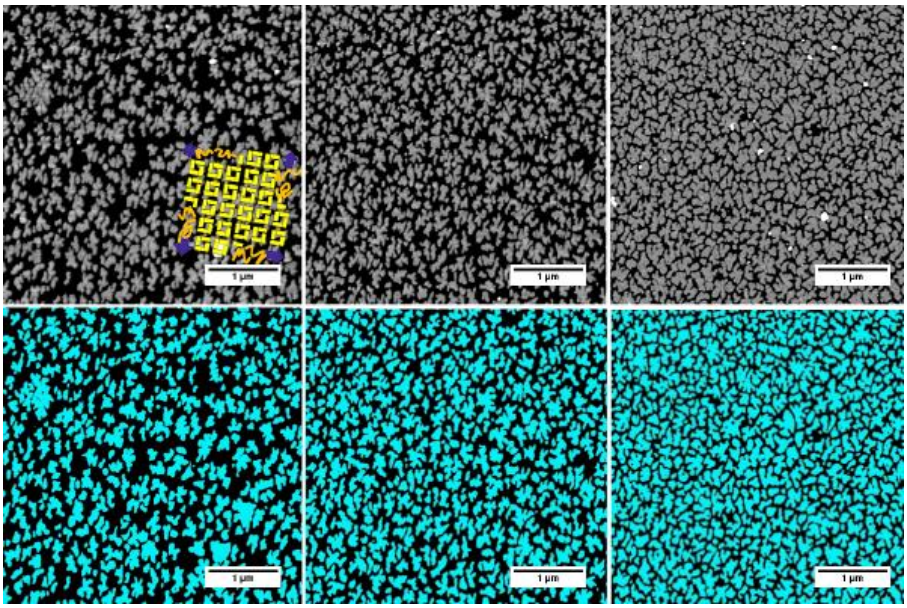

Figure. Comparison of AFM height images (top) with the surface profiles calculated by ImageJ (bottom) for the in situ crystalline S-layer growth at a fixed protein concentration (0.2 μM) at three time points: 30 min (left), 60 min (center), and 120 min (right).

Acknowledgements:
Jacqueline Friedmann for technical support.
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Mechanical properties of gelatin nanoparticles in dependency of crosslinking time and storage

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Mechanical properties of nanoparticles are an important characteristic for drug delivery and therefore, they have gained interest in pharmaceutical research during the last years. Among others, cellular uptake, blood circulation time and accumulation in organs are influenced by the elastic modulus of nanoparticles. Thus, by varying the stiffness of nanoparticles a more specific drug targeting might be achieved. Gelatin nanoparticles (GNPs) show advantageous characteristics in respect to encapsulation and delivery of hydrophilic drugs such as antibodies or other biologicals. Furthermore, the GNPs as hydrogel-nanoparticles offer adjustable elastic behavior. In this study, a method for GNP sample preparation and the determination of the mechanical properties by nanoindentation experiments using atomic force microscopy (AFM) was developed. The obtained force-distance curves were evaluated and fitted with the Hertzian model, in order to calculate the Young's modulus. GNPs were crosslinked with glutaraldehyde (GTA) for different incubation times to investigate a possible modification of the Young's modulus. In addition, this study addresses the influence of storage on the mechanical characteristics of GNPs. The results provide first insights about the elastic properties of GNPs and their development over time. In the tested range of crosslinking times no notable differences in the mechanical properties occurred. In turn, the influence of the storage on the mechanical particle properties was observed: particle stiffness raised over time. Furthermore, it could be observed that the cellular uptake in a model cell line (A549) was increased for harder particles.

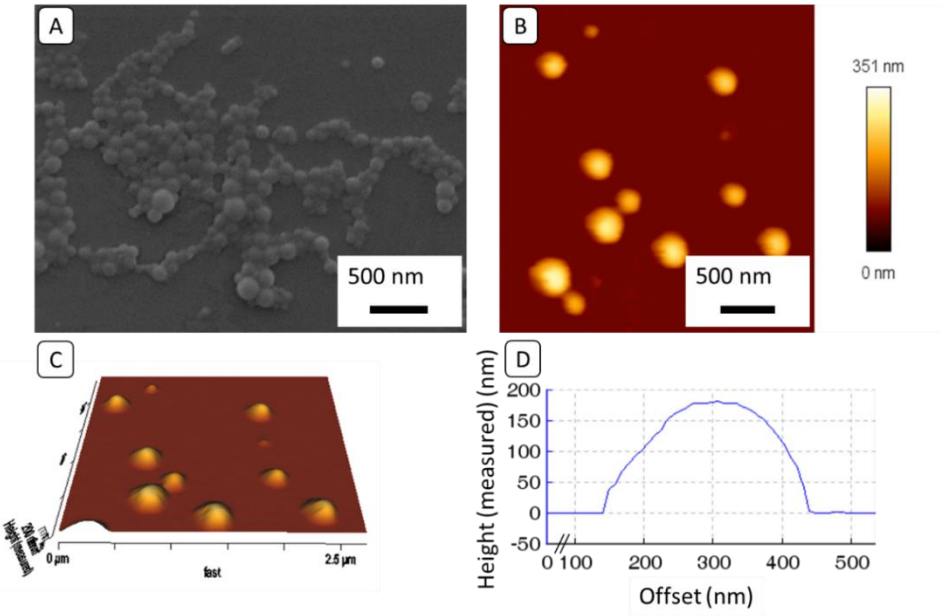

Figure 1. Morphological overview of gelatin nanoparticles. A) SEM image of dried GNPs on a SiO2 wafer sputtered with a 10 nm gold layer. B) AFM height image taken in tapping mode in deionized water in 2D view C) 3D view of the same image as shown in B) and D) cross section of a GNP imaged in tapping mode in water showing a nearly round particle. All particles shown here are from 6 h crosslinked GNPs after 4 weeks of storage.

Electrochemical-QCMD Control over S-Layer (SbpA) Recrystallization with Fe^{2+} as Specific Ion for Self-Assembly Induction

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The critical role of divalent ions (M2+) in the self-assembly of SbpA S-layer proteins (from Lysinibacillus sphaericus CCM 2177) into crystalline structures has been reported in several studies. Hence, ions such as magnesium, barium, nickel and, most commonly, calcium (Ca2+) have proven to trigger both protein-protein and protein-substrate interactions involved in the two-stage non-classical pathway recrystallization followed by SbpA units.

As a result, two dimensional, crystalline nanometric sheets in a highly ordered tetrameric state (p4) can be formed on top of different surfaces. The use of iron in its ferrous state (Fe2+) as self-assembly inducing candidate has been omitted so far due to its instability under aerobic conditions, tending to natural oxidation to the ferric (Fe3+) state. In this work, the potentiality of assembling fully functional S-layers from iron (II) salts (FeCl2 and FeSO4) is described for the first time. A combination of chemical (oxidation retardants) and electrical (−1 V potential) factors has been applied to effectively act against such an oxidizing trend. Formation of the respective crystalline films has been followed by means of Electrochemical Quartz Crystal Microbalance with Dissipation (EQCM-D) measurements and complementary Atomic Force Microscopy (AFM) topography studies, which prove the presence of squared lattice symmetry at the end of the recrystallization process.

Both techniques, together with additional electrochemical tests performed over the ion permeability of both types of S-layer coatings formed, show the influence of the counterion chosen (chloride vs. sulphate) in the final packing and performance of the S-layer. The presence of an underlying Secondary CellWall Polymer (SCWP) as in the natural case contributes to pair both systems, due to the high lateral motility freedom provided by this biopolymer to SbpA units in comparison to uncoated substrates.

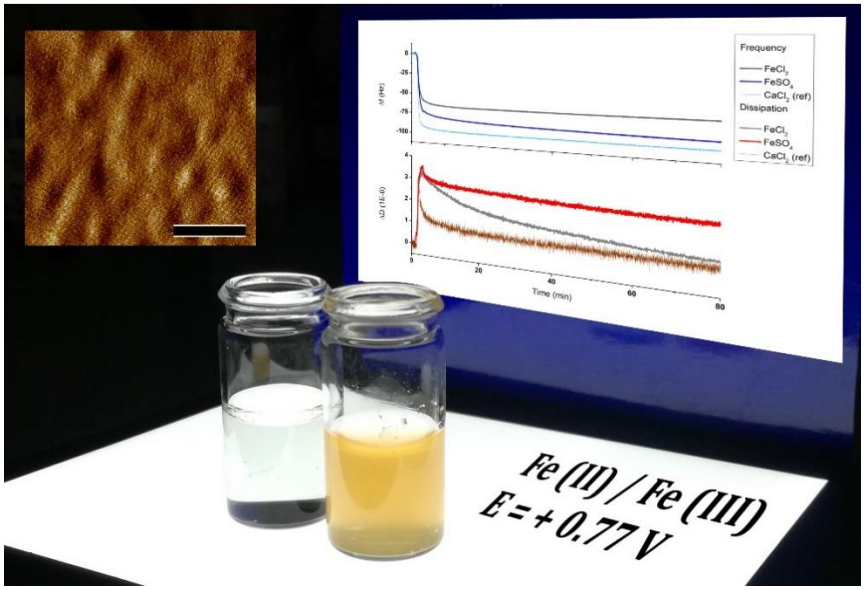

Figure. SbpA solutions in crystallization buffer upon application of an-external potential (E = -1 V) or left exposed to natural oxidation, with the featuring Fe (III) yellow color. Inset on the left shows the AFM micrograph of the square lattice film obtained in the presence of Fe (II). Inset on the right hand side shows the QCMD monitoring of S-layer formation in the presence of Fe (II) salts (chloride vs sulfate) when compared to crystallization in Ca^{2+}.

Influencing the adhesion properties and wettability of mucin protein films by variation of the environmental pH

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Mucins, the main component of the mucus secretions of goblet and epithelial cells, are known for exhibiting a different behaviour in accordance with their surrounding environment (i.e. among others the environmental pH), which induces a drastic change in their measured mechanical properties. In this work, we have first employed Atomic Force Microscopy (AFM) in Force Spectroscopy mode to evaluate the adhesion of porcine mucin films at the nanoscale, and the changes caused in this particular factor by a pH variation between 7.0 and 4.0, both quite common values in biological conditions. Measurements also involved additional varying factors such as the indenting tip chemistry (hydrophobic vs hydrophilic), its residence time on the measured film (0, 1 and/or 2 seconds), and increasing pulling rates (ranging from 0.1 up to 10 µm/s).

A second approach regarded the macroscale behaviour of the films, due to their potential applicability in the development of a new set of stimuli-responsive biomaterials. This was possible by means of complementary Wilhelmy plate method (to test the wetting properties) and cell proliferation studies on films previously exposed to the corresponding pH solution. According to our results, treatment with lowest pH (4.0) provides porcine mucin with a more hydrophilic character, showing a much stronger adhesion for analogous chemistries, as well as enhanced capability for cell attachment and proliferation, which opens new pathways for their future use and consideration as scaffold-forming material.

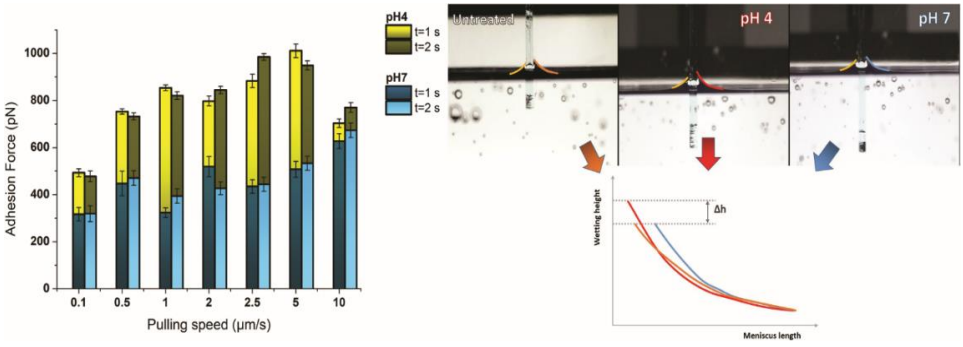

Figure. (Left) Adhesion force of mucin films as obtained from its contact with a hydrophilic tip for varying dwell times, and after exposure of the film to two different pH values: 4.0 – yellow- and 7.0 –blue-.Wettability test performed following the Wilhelmy plate method. Coloured lines highlight the meniscus profiles resulting from immersion of the untreated (orange), pH 4 (red) and pH 7 (blue) mucin films in water. Left side of the glass slide remained uncoated.

Acknowledgements:
Dr. Sudarat Tharad, Jacqueline Friedmann and Amsatou Andorfer-Sarr for technical support (AFM, SEM, cell culture). Special thanks to Dr.Spela Zemljic-Jokhadar (Univ. Ljubljana, Slovenia) for provision of the HUVEC cells employed in this work.

Resveratrol-Induced Temporal Variation in the Mechanical Properties of MCF-7 Breast Cancer Cells Investigated by Atomic Force Microscopy

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Atomic force microscopy (AFM) combined with fluorescence microscopy has been used to quantify cytomechanical modifications induced by resveratrol (at a fixed concentration of 50 μM) in a breast cancer cell line (MCF-7) upon temporal variation. Cell indentation methodology has been utilized to determine simultaneous variations of Young's modulus, the maximum adhesion force, and tether formation, thereby determining cell motility and adhesiveness. Effects of treatment were measured at several time-points (0–6 h, 24 h, and 48 h); longer exposures resulted in cell death. Our results demonstrated that AFM can be efficiently used as a diagnostic tool to monitor irreversible morpho/nano-mechanical changes in cancer cells during the early steps of drug treatment.

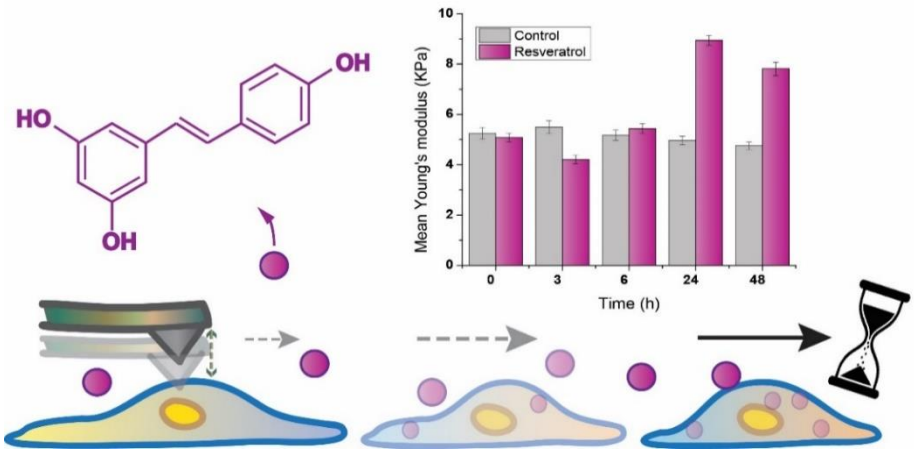

Figure. Schematic picture showing the time evolution of MCF7 cells in the presence of Resveratrol (purple).
The chart on top shows the variation of the elastic modulus in comparison with control experiments.

Acknowledgements:

Amsatou Andorfer-Sarr for the technical support with the cell culture.

Novel biodegradable and non-fouling systems for controlled-release based on PCL/Quercetin blends and biomimetic bacterial S-layer coatings

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Quercetin is a strong antioxidant with low bioavailability due to its high crystallinity. A further drawback is that Quercetin has potentially toxic effects at high concentrations. To improve this low water solubility as well as control the concentration of the flavonoid in the body, Quercetin is incorporated into a polymeric matrix to form an amorphous solid dispersion (ASD) stable enough to resist the recrystallization of the drug. For this purpose, miscible poly(ε-caprolactone) (PCL) and Quercetin (Q) blends are prepared, provided that they have complementary interacting groups.

For compositions in which the flavonoid remains in an amorphous state thanks to the interactions with polymer chains, various PCL/Q drug release platforms are fabricated: micrometric films by solvent casting, nanometric films by spin coating, and nanofibers by electrospinning. Then, the potential use of bacterial S-layerproteins as release-preventive membranes is tested on PCL–Quercetin blends, due to their ability to construct a biomimetic coating including nanometric pores. For all the platforms, the SbpA coating can maintain a stable release under the toxicity level of Quercetin. Accordingly, a PCL/Q system with an S-layer coating allows the design of versatile bioavailable Quercetin eluting devices that prevent toxicity and biofouling issues.

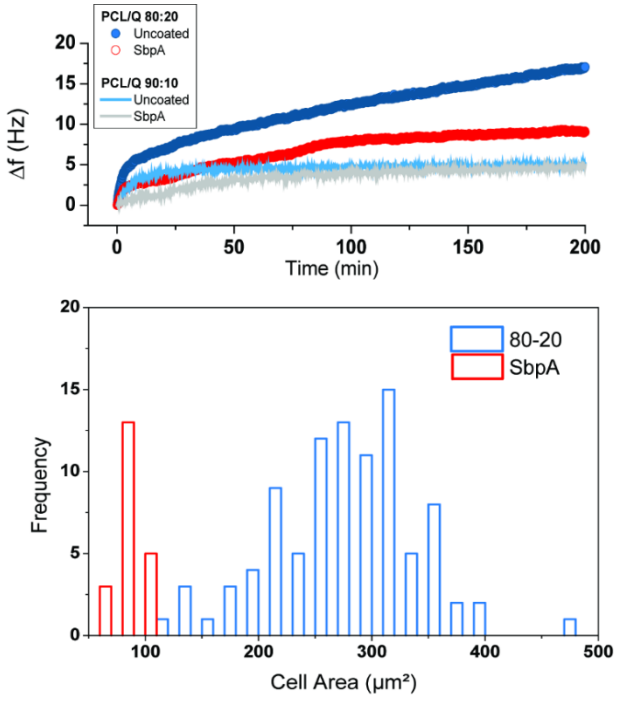

Figure. (Top) Real time monitoring of the influence of an S-layer coating on the Quercetin release from PCL/Q blends. (Bottom) cell area distribution on PCL/Q 80/20 systems with and without S-layer coating.

Acknowledgements:
Jacqueline Friedmann and Amsatou Andorfer-Sarr for technical support with the SbpA purification and cell culture, respectively.

Controlled release of Levofloxacin from PLLA-based polymer blends

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By means of Quartz Crystal Microbalance with Dissipation and Atomic Force Microscopy the uptake and release of Levofloxacin antibiotic from PLLA-based 2D films was followed. PLLA was first doped with Dopamine (DA), either as a blend with a self-synthesized PLLA (low MW)-DA catechol end-functionalized polylactide or with a thin DA coating on top, as control experiment.

The catechol groups of DA interact with Levofloxacin in a non-specific and pH dependent manner, depending on the protonation degree of the OH groups from catechol. By this approach, the pH range could be tuned in order to obtain a drug adsorbing or drug-release system. UV-Spectrophotometry was employed to complementarily quantify the amount being released over time. Results showed that under certain mixing ratios, uptake and release of the drug from hybrid PLLA/PLLA-DA films could be successfully observed and measured.

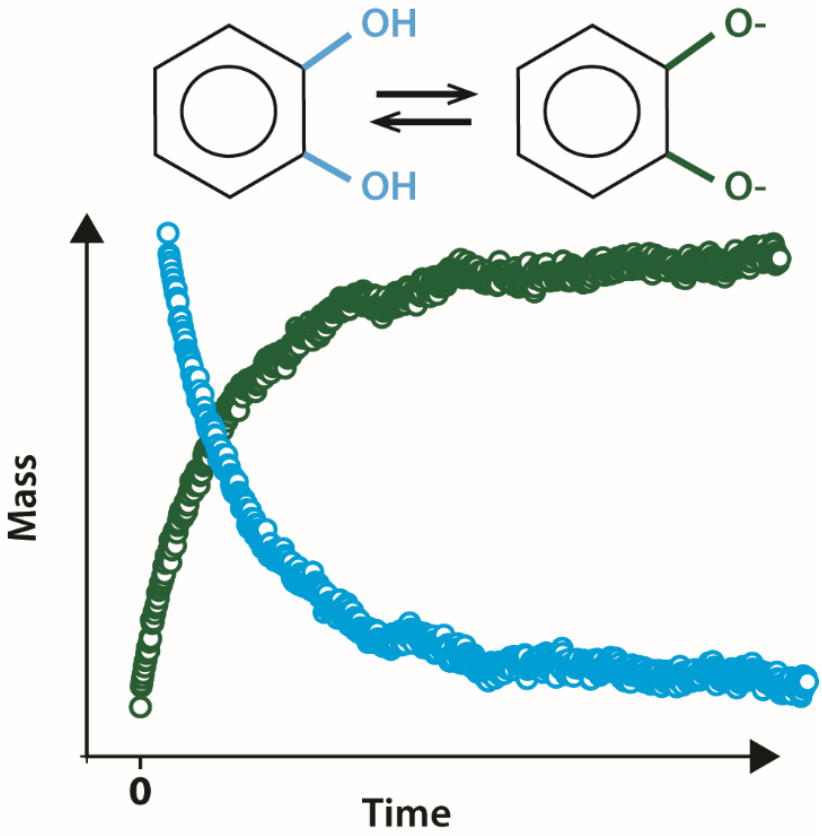

Figure. Schematic picture of the drug uptake/release process, as monitored by QCMD, attending to the pH-dependent oxidation state of the catechol groups.

Mechanical effects of purinergic signaling in Astrocytes

J.-C. Gil-Redondo[1], J. Iturri[2], A. Weber[2], J.-L. Toca-Herrera[2], E. Garcia-Delicado[1], M.-T. Miras-Portugal[1], F. Ortega[1]

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The mechanical response of Astrocytes (using HUVEC as reference line) was tested upon exposure to different purinergic agonists such as UTP, 2MeSADP and BzATP. These compounds interact with P2Y and P2X receptors, which modulate different active processes in the cell (production of actin stress fibers, migration, etc). Therefore, variations in factors such as the cell elastic modulus, the adhesion force and the formation of membrane tethers, might correlate to changes in the cell stiffness, stickiness and membrane-cortex interactions, respectively. Our results confirmed the validity of Atomic Force Microscopy as a mechanical tool to elucidate the effects of signaling pathway activation in the cell lines under study.

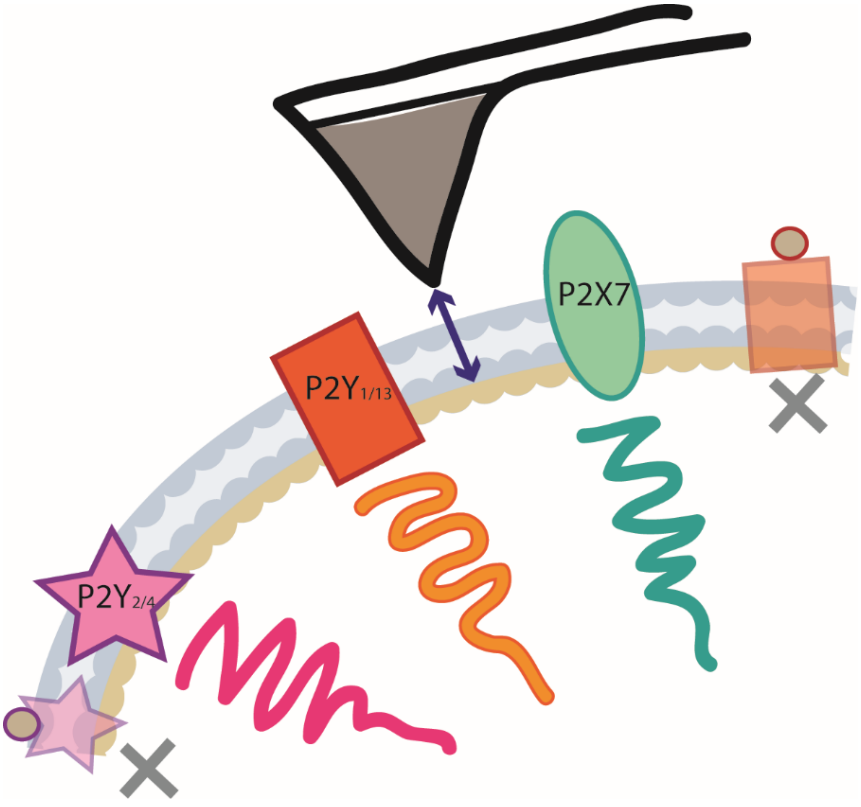

Figure 1. Schematic view of the indentation experiments performed on Astrocytes. The cell membrane shows the three types of membrane receptors (P2Y2/4, P2Y1/13 and P2X7) targeted in our studies. The crossed figures indicate receptor passivation by attachment of the corresponding agonist molecule.

Measurement of Hydrophobic forces by means of AFM Force Spectroscopy

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Atomic Force Microscopy in Force Spectroscopy mode allowed for the characterization of the existing attractive-repulsive interactions between colloidal probes and 2D surfaces. Both probes and the underlying surfaces were chemically modified, with either silanes of increasing hydrophobicity or by plasma treatment, which induced optimal wetting (hydrophilic) conditions. Experiments involved liquid environments of varying polarity and pH, and aimed exploring the kind of interactions derived from confronting symmetric and asymmetric systems, under pre-defined approach/retract rates.

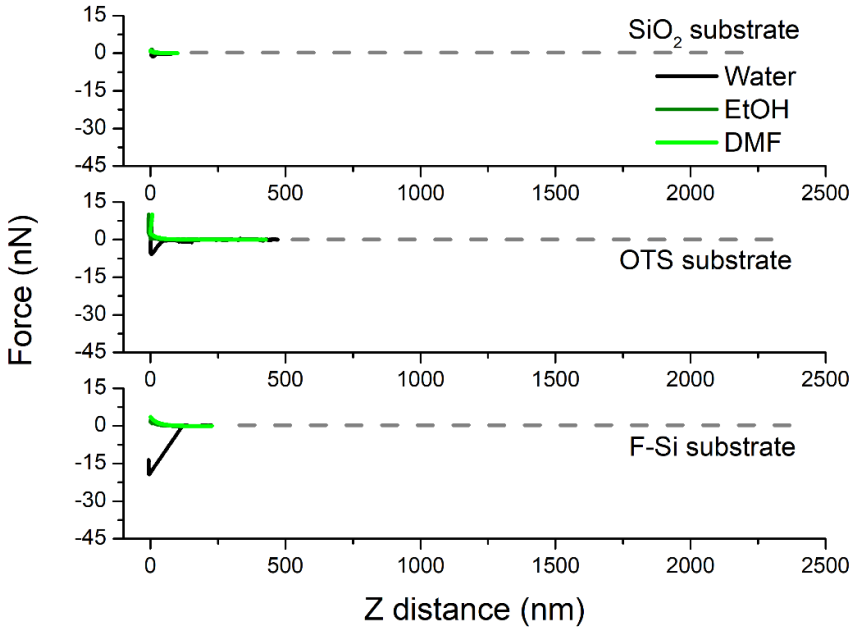

Figure. Force vs distance plots for the retraction segment, showing the adhesion peak obtained for uncoated colloidal SiO_2 probes on uncoated SiO2 wafers, in comparison with OTS- and FSi-coated substrates.

Influence of Cadmium on mechanical properties of *Dunaliella tertiolecta* microalgae

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Aim. Microalgae play a fundamental role in aquatic ecosystems and have developed strategies to cope with higher concentrations of environmental heavy metals. They are frequently used as model organism to evaluate toxicity of certain materials. Cadmium is a non-essential trace metal that is known to be a major pollution factor in marine environment. The response of microalgae under stress of heavy metal leads to an allocation of carbon flux, variation of chlorophyll account and is hypothesized to alter the ultrastructure and many other properties of marine microalgae. The aim of this study was to offer a multi-method approach to study the influence of cadmium on the properties of microalgae. Our laboratory was tasked with determination of mechanical properties of *Dunaliella tertiolecta* and study the effect of Cadmium thereon, using Atomic Force Microscopy (AFM).

Methods. Cells (either untreated or treated with 1 mg/L Cd) were fixed on a PEI coated glass substrate by drying and measured in 200 µL of filtered seawater. We have employed AFM in force spectroscopy at room temperature, using soft, triangular cantilevers (0.12 N/m), a loading/unloading rate of 5 µm/s and a maximum force set-point of 1 nN. A Hertz elastic model with Sneddon extension was used for determination of the sample Young's Modulus, using the first 200 nm of the retract segments (assumptions of model met for this part of the curve).

Results. The used elastic model was suitable to fit the Force-Distance-Curves. For untreated cells, an average Young's Modulus of 26.4 ± 0.4 kPa was determined, while for cadmium-treated cells, the value was 47.9 ± 0.8 kPa. For the treated cells, the distribution of the values slightly deviated from a normal one. Therefore, a stiffness increase of around 80% was determined.

Conclusions. Together with the techniques employed by all the other groups involved in the project (fluorescence imaging, electrochemical adhesion study, cell viability studies and more) we could show that indeed significant changes occur due to incubation of the studied microalgae cells with cadmium [1]. The obtained values are in agreement with another study obtained at the same time. Thus one can conclude that the studied algae due indeed possess adaptation mechanism to toxic environmental concentration of cadmium.

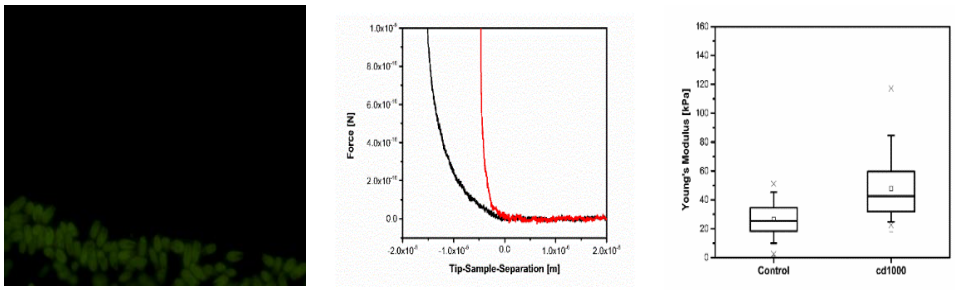

Figure 1. (Left) Fluorescence microscopy micrograph of fixed Dunaliella t. cells, showing the chlorophyll autofluorescence.
(Middle) Representative Force-Distance-Curve for control (black) and Cadmium-treated cells (red).
(Right) Boxplots of Young's Modulus for control and treated cells.

Acknowledgements: The authors acknowledge networking effort within COST Action CA15126 ARBRE MOBIEU.

[1] DeNardis et al., *European Biophysics Journal,* 2019, **48**, 231-248.
[2] Pillet et al., *Bioelectrochemistry*, 2019, **127**, 154-162.

Measuring biomaterials mechanics with atomic force microscopy. 1. Influence of loading rate and applied force (pyramidal tips)

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Aim. Atomic force microscopy (AFM) is today an established tool in imaging and determination of mechanical properties of biomaterials. Due to their complex organization, those materials show properties such as viscoelasticity. One has to consider that the loading rate at which the sample is probed will lead to different mechanical response. The aim of this study was to determine experimental parameter ranges suitable for measuring cellular mechanics.

Methods. In this work, we studied the dependence of the mechanical properties of endothelial cells on the loading rate using AFM in force spectroscopy mode. We employed a sharp, four-sided pyramidal indenter and loading rates ranging from 0.5 to 20 µm/s. In addition, by variation of the load (applied forces from 100 to 10,000 pN), the dependence of the cell properties on indentation depth could be determined.

Results. We showed that the mechanical response of endothelial cells depends nonlinearly on the loading rate and follows a weak power-law. In addition, regions of different viscous response at varying indentation depth could be determined. Based on the results we obtained, a general route map for AFM users for design of cell mechanics experiments was described.

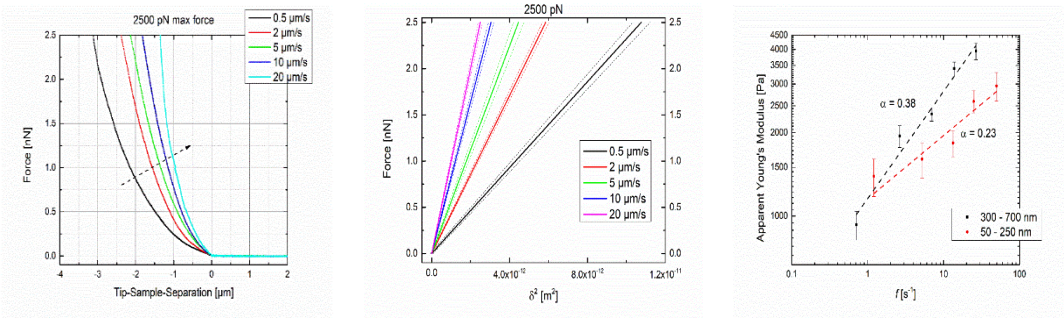

Figure 2

Conditions	Results
• **Fixed forces, changing loading rates**	• Properties change with loading rate (viscoelasticity) • Higher loading rates, higher noise levels • Low loading rates, long experimental time
• **Fixed rates, changing forces**	• Different indentations depths, different cell sub-materials are felt • Too high forces led to nucleus and substrate indentation • Too low forces lead to bad signal to noise ratio • No material history effects (high force also no effects)
• **Fixed force, fixed speed**	• For all experiments comparable curves where achieved
• **Independent of speed and force**	• Two slopes in F-δ^2 curves (below 50 pN first, above second) (contact geometry change, actin cortex)
• **Speed below 2 µm/s, force above 3.5 nN**	• Substrate (+ nucleus) visible in curves
• **Indentation rate between different points**	• Viscosity of material • Dependence of viscosity on indentation depth (anisotropy and heterogeneity of cells)

Acknowledgements: The authors want to thank Amsatou Andorfer-Sarr for expert advice in cell culture.

[1] Weber et al., *Microscopy Research and Technique*, 2019, **9**, 1392-1400.

Microtubule disruption changes endothelial cell mechanics and adhesion

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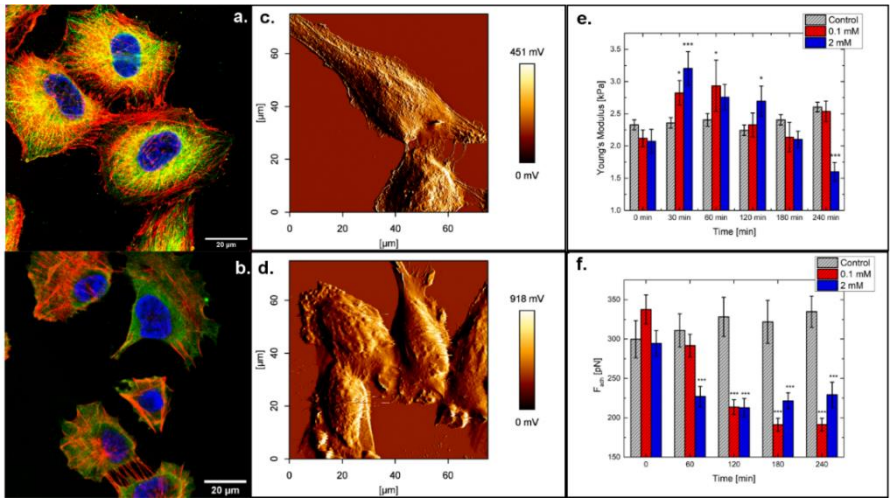
[2]Department of Mathematics for Economics and Business, Universitat de Valencia, Valencia, Spain
[3]Department of Biophysics, Medicine Faculty, University of Ljubljana, Slovenia

Aim. The interest in studying the mechanical and adhesive properties of cells has increased in recent years. The cytoskeleton is known to play a key role in cell mechanics. However, the role of the microtubules in shaping cell mechanics is not yet well understood. We have employed Atomic Force Microscopy (AFM) together with confocal fluorescence microscopy to determine the role of microtubules in cytomechanics of Human Umbilical Vein Endothelial Cells (HUVECs). Additionally, the time variation of the adhesion between tip and cell surface was studied. The disruption of microtubules by exposing the cells to two colchicine concentrations was monitored as a function of time.

Results. Microtubule disruption lead to time- and concentration-dependent changes, as seen in Fig. 1 a-d. Cell height increased, while overall cell area was reduced. CLSM studies showed that already after 30 min of incubation, microtubules were completely disrupted. We were not able to neither prove nor disprove that notion that microtubule disruption leads to more profound formation of actin stress fibers. With respect to cell mechanics, an initial increase of the cell Young's modulus (Fig 1e), accompanied by an increase in relaxation times was reported. A double exponential decay model was best suited to fit the datasets, thus enabling differentiation of two distinct materials. For the high concentration after incubation for 240 min, both stiffness and relaxation times decreased significantly in comparison to the control. Tip-cell-adhesion was drastically reduced after already 60 minutes (see Fig. 1f), after that the value remained approx. constant. Similarly, the number of rupture events decreased while in parallel they appeared nearer to the sample surface. The rupture forces didn't show any time or concentration dependent changes.

Conclusions. This work extensively studied the impact of microtubule depolymerisation on cell mechanics, adhesion and the cytoskeleton. The reported changes followed a similar time-trend and were therefore thought to be intertwined.



Acknowledgements:
The authors want to thank Amsatou Andorfer-Sarr for expert advice in cell culture. In addition, the authors want to thank Monika Debrezcny from the BOKU Imaging Center for advice and teaching.

[1] Weber et al. *Scientific Reports*, 2019, 9, 14903.

Estrogen receptor drug binding and breast cancer cell viscoelasticity

Andreas Weber[1], Jagoba Iturri[1], Rafael Benitez[2], Maria del Mar Vivanco[3] and José Luis-Toca-Herrera[1]

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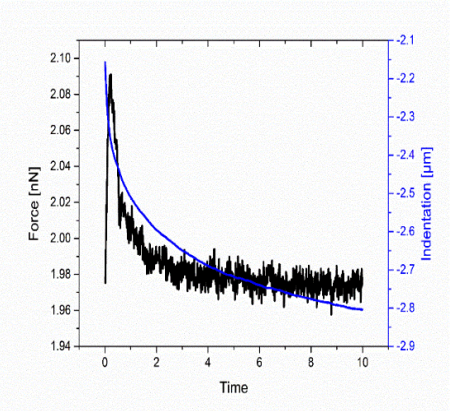
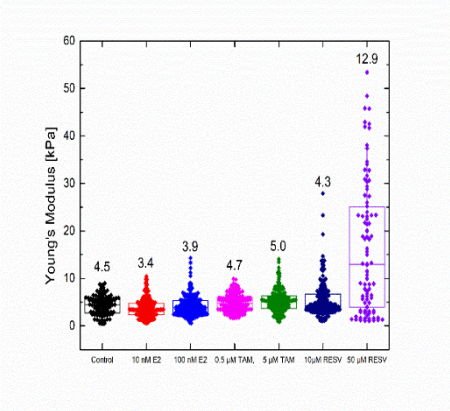
Aim. Breast cancer is the most frequently diagnosed form in females, with around 70% of the cases being estrogen receptor α positive. Receptor signalling is known to also involve non-classical intracellular pathways, such as activation of the wnt-pathway, which acts on adhesion, migration and cytoskeletal remodelling. The aim of this work was to study changes in cellular mechanics related to estrogen receptor signalling, with focus on the role of cell constituents and viscoelastic models.

Methods. AFM time-dependent measurements using stress relaxation (constant deformation) and creep (constant force) measurements with sharp tip (12 nm) and high indentation depth (around 1.5 µm). Cells were disturbed using estradiol, tamoxifen and resveratrol, using different concentrations as well as different incubation times (24 and 48 hours). A Zener model [1] was used to fit the data and calculate viscoelastic parameters.

Results. Fitting measurements with the proposed model produced well-fitting curves; nevertheless, calculation of compressibility moduli and viscosities led to inconsistent and not possible (being negative) values. Careful error search showed that in the creep curves, there was a stress relaxation segment present (see Fig. 1, left), with a magnitude of around 250 pN and a relaxation time of 2 s. Since the model assumptions were therefore not fulfilled, we decided on using only the indentation and stress relaxation curves. From these, we calculated Young's Modulus and relaxation parameters. Of the used drugs, only resveratrol led to significant changes. For resveratrol, cellular stiffness increased while also the relaxation times were increased. In addition, for the high concentration (50 µm) for both 24 and 48 hour of incubation, at least 3 distributions were present.

Outlook. Another mechanical model will be used to describe the stress relaxation data to get a more profound estimation of the values (since for double exponential decay fittings, we do not have enough variable to calculate all the parameters for the present mechanical model). Also, fluorescence microscopy studies will help in understanding the changes present.





Acknowledgements:
In case necessary, must be located at the end of the abstract, just before the references.

[1] Moreno-Flores et al. *Nanotechnology*, 2010, **21**, 445101.

Mechanical unfolding of polyproteins using AFM – cloning, Protein expression and more

Ulrich Fuchs[1], Andreas Weber[1], José Luis Toca-Herrera[1]

[1] Institute of Biophysics, Department for Nanobiotechnology, University of Natural Recourses and Life Sciences, Vienna, Austria

Aim. Proteins are among other things one of the most important components in nature. They are of central importance in enzymatic reactions, membrane transport, inter- and intracellular signaling, protein expression, cellular movement, and many other processes. The function of a protein is linked to its 3D-Structure. Therefore, understanding protein folding is very important in many areas of science and industry. A protein is built up by many amino acids and these are defining the structure of the protein. The interaction forces between amino acid side chains govern the structure. To name some of those interactions, van der Waals interactions, hydrophobic interactions, hydrogen bonding, electrostatic interactions, and others are important for protein stability. The aim of this work is to investigate the protein folding problem from the perspective of mechanical unfolding, using the atomic force microscope (AFM). This enables single molecule studies with a definite reaction coordinate.

Methods. To perform those measurements, we will produce polyproteins consisting of multiple identical well-studied protein domains. We have designed a polyprotein consisting of several consecutive titin 27 domains with a 5'His tag and a 3'Cys tag. This domain was chosen because it is already very well studied and therefore existing data can be used as reference. The first part of the project was the usage of a novel cloning approach, using golden gate cloning. We came up with seven parts with specific four base pair overhangs to clone together in a vector with the Golden Gate Cloning Method.

Results. The golden gate cloning approach did not produce properly defined plasmids as wanted. Currently we hypothesize that the DNA fragments interact with each other during the cloning, therefore disabling amplification of the single parts. We therefore switched to an already published plasmid, provided by the group of Piotr Marszalek [1]. This plasmid contains 8 titin I27 (I93) domains, together with a C-terminal strep-tag and a N-terminal His-tag. Currently we are transfecting different E. coli strains to produce the vector and later on the polyprotein.

Outlook. After correctly produced the homopolyprotein, we will perform AFM force spectroscopy mechanical unfolding studies of the protein to investigate protein unfolding. Later we will change environmental factors to determine the influence of them on protein folding. Finally, we will introduce another protein in our construct to study it in this well-defined system.

Because the cloning method showed no results, we ordered another plasmid. We got this from addgene from Piotr Marszalek. Then we transformed the plasmid into different expression systems to get our polyprotein of interest. The system is IPTG induced and we did different approaches. The purification takes place via a nickel column due to the His tag. Different Analysis methods are used like SDS-Page or an His-Plot.

[1] Scholl et al. *Biomacromolecules*, 2016, **8**, 2502-2505.

Modelling- Atomic Force Microscopy indentation experiments of biomaterials (gels and cells)

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Aim/Objective. For many decades, cells have been explored biochemically, while the physical research has been accounted for at seldom. More and more studies indicate that physical stresses on cells have a major impact on the health of organisms. The insight into the behaviour of cells under mechanical stress is therefore of utmost importance. The major objective of this work is to converge Atomic Force Measurements of single endothelial cells to a Finite Element Model in order to predict the deformation of HUVECs under mechanical stress as well as to get a better understanding of the stress-strain relationship on soft, biological matter.

Methods. Atomic Force Microscopy (AFM) is a method for gaining physical knowledge of micro- and nanostructures. Using Force-distance curves and applying mechanical models enables to study the mechanical properties of the underlying material. Often, the Youngs Modulus is obtained by applying the Hertz Model on the force-distance curves. Adhesion is not taken into account.

Finite element modelling is a numerical method that enables an operator to calculate mechanical stresses and strains, by discretizing a complex body into a finite number of smaller, simpler elements. It is commonly used for civil engineering, but the spectrum of applicability is large. Measuring force-distance-time curves for single Human umbilical vein endothelial cells (HUVECs) with AFM allows the computation of the Youngs-Modulus, Shear-Modulus, Bulk-Modulus, force-relaxation time and creep. The provided values are the main inputs for the FEM Model. The Model will be set-up in different layers to account for the different behaviour of the cell's nucleus, cytoplasm, cytoskeleton and membrane. Each layer differs in the material behaviour but shows no special distribution dependence within itself. The nucleus is modelled as Hooke elements, the cytoplasm as a gel-like-porous elastic material, the cytoskeleton as Maxwell elements and the membrane as a shell under tension. The composition of the different layers will show a non-linear behaviour.

The different properties are gained by three different AFM measurements: Cantilever-Glass, Cantilever-Gel, and Cantilever-HUVEC. The obtained results will be applied in three different FEM models. The first and second model will be intermediate steps to compute the last model what represents a HUVEC's stress-strain behaviour.

Results. There are no results at the current state, since the modelling is not finished yet.

A Probabilistic Model for Crystal Growth Applied to Protein Deposition at the Microscale

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[2]Self-Assembly Group, CIC nanoGUNE, Tolosa Hiribidea 76, 20018 Donostia/San Sebastián, Spain
[3]Institute for Biophysics, Department of Nanobiotechnology, University of Natural Resources and Life Science, Vienna, Austria

Objective

To obtain a probabilistic model to explain and predict 2D crystal growth at the microscale.

Results & Conclusion

We built a model that simulates the spatial spread of the bulk crystal without considering the individual constituents of the crystal (e.g. orientation, nucleation process, etc.). The model takes into account the available space to be filled. It can describe growing processes due to the versatility of its parameters. The accuracy of the simulation has been tested against a real recrystallization experiment carried out with the bacterial protein SbpA (from Lysinibacillus sphaericus CCM2177). The model showed high agreement between the simulation and the experimental results. Although the core of the model algorithm is probabilistic, once the available space for growing is small enough, the general evolution of the crystal ceases to be random and, from a macroscopic point of view (microscale), becomes a deterministic process. In our case, when the model was initialized at t = 20 min (occupied fraction was slightly higher than 50%), it yielded practically the same results at each repetition of the simulation. An open question is if such model would describe other adsorption/recrystallization processes leading to 2D regular structures (e.g., self-assembly monolayers and particle deposition). Finally, it was also addressed how the regularity of the interface (i.e., the curve that separates the crystal from the substrate) affected the evolution of the simulation.

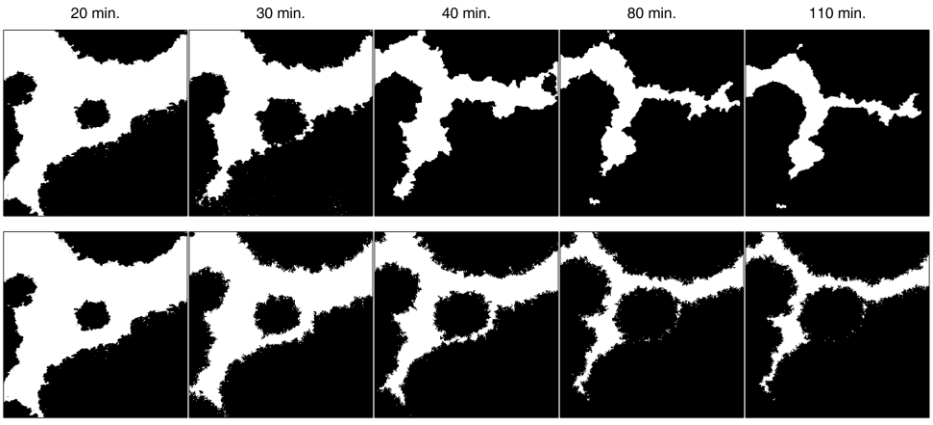

Figure 1. Comparison between real images (above) and simulation (below) at different times, with initial data corresponding to the real data at 20 min. The values of the structural parameters taken in the simulation are b = 1, a = 0.5, and rinf = n/8 (with n = 256). The kinetic parameters have been set to fit the real data, according to the Avrami–Gompertz model. For details, please go to Materials 12 (2019) 479, doi: 10.3390/ma12030479.

Actively Tunable Collective Localized Surface Plasmons by Responsive Hydrogel

Nestor Gisbert Quilis[1], Marcel van Dongen[2], Priyamvada Venugopalan[3], Daria Kotlarek[1], Christian Petri[4], Alberto Moreno Cencerrado[5], Sorin Stanescu[1], Jose Luis Toca Herrera[5], Ulrich Jonas[4], Martin Möller[2], Ahmed Mourran[2], and Jakub Dostalek[1]

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[2]DWI – Leibniz Institute for Interactive Materials, 52056 Aachen, Germany
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[4]Macromolecular Chemistry, Department Chemistry-Biology, University of Siegen, Germany
[5]Institute for Biophysics, Department of Nanobiotechnology, BOKU, Vienna, Austria
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Objective. Implementation of collective localized surface plasmons by using responsive hydrogel materials. Development of a new approach to prepare periodic arrays of gold nanoparticles supported by thermo-responsive hydrogel membranes by using laser interference lithography (LIL) and template stripping.

Results & Conclusion

Collective (lattice) localized surface plasmons (cLSP) with actively tunable and extremely narrow spectral characteristics are reported. They are supported by periodic arrays of gold nanoparticles attached to a stimuli-responsive hydrogel membrane, which can on demand swell and collapse to reversibly modulate arrays period and surrounding refractive index. In addition, it features a refractive index-symmetrical geometry that promotes the generation of cLSPs and leads to strong suppression of radiative losses, narrowing the spectral width of the resonance, and increasing of the electromagnetic field intensity. Narrowing of the cLSP spectral band down to 13 nm and its reversible shifting by up to 151 nm is observed in the near infrared part of the spectrum by varying temperature and by solvent exchange for systems with a poly(N-isopropylacrylamide)-based hydrogel membrane that is allowed to reversibly swell and collapse in either one or in three dimensions. The reported structures with embedded periodic gold nanoparticle arrays are particularly attractive for biosensing applications as the open hydrogel structure can be efficiently post-modified with functional moieties, such as specific ligands, and since biomolecules can rapidly diffuse through swollen polymer networks.

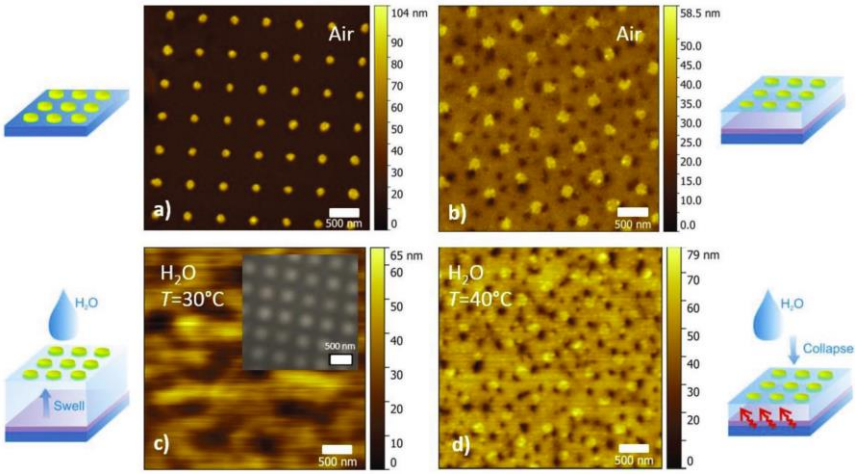

Figure 1. AFM observation of gold particle arrays on a) glass substrate after the UV-LIL fabrication, b) after their embedding to pNIPAAm-based polymer in a structure A followed by the rinsing with water and drying at a temperature above the pNIPAAm LCST (both images were taken in air). Observation of the tethered structure A in contact with water c) at T = 30 °C in swollen state and d) at T = 40 °C in the collapsed state. The inset in figure (c) represents an optical microscopy image of the swollen free-standing hydrogel film in water at room temperature. For details, see Adv. Optical Mater. 7 (2019) 1900342, doi:10.1002/adom.201900342.

Survival Analysis of Author Keywords: An Application to the Library and Information Sciences Area

F. Peset[1], F. Garzon-Farinos[2], L. M. Gonzalez[3], X. Garcia-Masso[4], A. Ferrer-Sapena[1], Jose L. Toca-Herrera[5], Enrique A. Sanchez-Perez[1]

[1]Instituto Universitario de Matemática Pura y Aplicada, Universitat Politècnica de València, 46022 Valencia, Spain
[2]Universidad Católica de Valencia San Vicente Mártir, Quevedo 2, 46001, Valencia, Spain
[3]Departamento de Educación Física y Deporte, Universitat de València, 46010, Valencia, Spain
[4]Departament de Didàctica de l'Expressió Musical, Plàstica i Corporal, University of Valencia, 46021 Valencia, Spain
[5]Institute for Biophysics, Department of Nanobiotechnology, University of Natural Resources and Life Science, Vienna, Austria

Objective

Our purpose is to adapt a statistical method for the analysis of discrete numerical series to the keywords appearing in scientific articles of a given area.

Results & Conclusion

We have applied our methodological approach to the study of the keywords in the Library and Information Sciences (LIS) area. Our objective is to detect the new author keywords that appear in a fixed knowledge area in the period of 1 year in order to quantify the probabilities of survival for 10 years as a function of the impact of the journals where they appeared.

Many of the new keywords appearing in the LIS field are ephemeral. Actually, more than half were never used again. In general, the terms most commonly used in the LIS area come from other areas. The average survival time of these keywords is approximately 3 years, being slightly higher in the case of words that were published in journals classified in the second quartile of the area. We believe that measuring the appearance and disappearance of terms will allow understanding some relevant aspects of the evolution ofa discipline, providing in this way a new bibliometric approach.

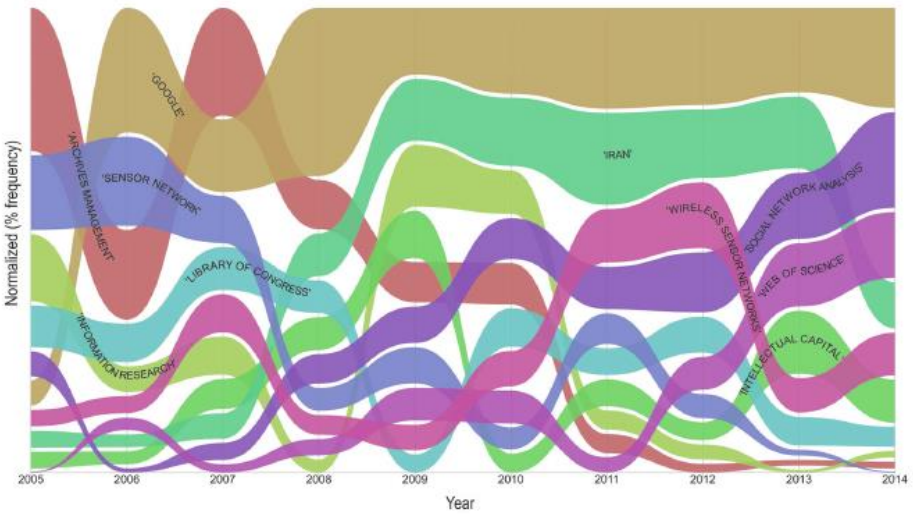

Figure 1. Bump chart of keyword ranking throughout the survival period. The figure has been created considering only the 10 most frequent words that appeared as new keywords in 2004 and indicates the changes in their ranking throughout the period. For details, please see J. Assoc. Inf. Sci. Technol., published on-line, doi: 10.1002/asi.24248.

3. Articles, books and book chapters

Publications (SCI articles)

1. **The Puzzle of the Walnut Shell: A Novel Cell Type with Interlocked Packing**
   S.J. Antreich, N. Xiao, J.C. Huss, N. Horbelt, M. Eder, R. Weinkamer, N. Gierlinger
   Adv. Sci. 6 (2019) 1900644

2. **Infrared and Raman spectra of lignin substructures: Coniferyl alcohol, abietin, and coniferyl aldehyde**
   P. Bock, N. Gierlinger
   J. Raman Spectrosc. 50 (2019) 778

3. **Lignin-based multiwall carbon nanotubes**
   W. Gindl-Altmutter, J. Kohnke, C. Unterweger, N. Gierlinger, J. Keckes, J. Zalesak, O.J. Rojas
   Compos. Part A-Appl. S. 121 (2019) 175

4. **Visualization of the Stimuli-responsive Surface Behavior of Functionalized Wood Material by Chemical Force Microscopy**
   C. Gusenbauer, E. Cabane, N. Gierlinger, J. Colson, J. Konnerth
   Sci. Rep. 9 (2019) 18569

5. **Time-dependent ammonia emissions from fumed oak wood determined by micro-chamber/thermal extractor (mu CTE) and FTIR-ATR spectroscopy**
   E. Hogger, K. Bauer, E. Hollbacher, N. Gierlinger, J. Konnerth, H.W.G. van Herwijnen
   Holzforschung 73 (2019) 165

6. **Comparison of Four Technical Lignins as a Resource for Electrically Conductive Carbon Particles**
   J. Kohnke, N. Gierlinger, B.Prats-Mateu, C. Unterweger, P Solt, A.K. Mahler, E. Schwaiger, F. Liebner, W. Gindl-Altmutter
   Bioresources 14 (2019) 1091

7. **Significant influence of lignin on axial elastic modulus of poplar wood at low microfibril angles under wet conditions**
   M. Özparpucu, N. Gierlinger, I. Cesarino, I. Burgert, W. Boerjan, M. Rüggeberg
   J. Exp. Bot. 70 (2019) 4039

8. **S-Layer Protein Coated Carbon Nanotubes**
   Breitwieser, P. Siedlaczek, H. Lichtenegger, U. B. Sleytr, D. Pum
   Coatings 9 (2019) 492

9. **Russell-Like Bodies in Plant Seeds Share Common Features With Prolamin Bodies and Occur Upon Recombinant Protein Production**
   E. Arcalis, V. Ibl, J. Hilscher, T. Rademacher, L. Avesani, F. Morandini, L. Bortesi, M. Pezzotti, A. Vitale, D. Pum, T. De Meyer, A. Depicker, E. Stoger
   Frontiers in Plant Science 10 (2019) 777

10. **Prevention of allergy by virus-like nanoparticles (VNP) delivering shielded versions of major allergens in a humanized murine allergy model**
B. Kratzer, C. Kohler, S. Hofer, U. Smole, D. Trapin, J. Iturri, D. Pum, P. Kienzl, A. Elbe-Burger, P. Gattinger, I. Mittermann, B. Linhart, G. Gadermaier, B. Jahn-Schmid, A. Neunkirchner,R. Valenta, W.F. Pickl
Allergy 74 (2019) 246

11. **CRISPR-mediated gene silencing reveals involvement of the archaeal S-layer in cell division and virus infection**
I.A. Zink, K. Pfeifer, E. Wimmer, U.B. Sleytr, B. Schuster, C Schleper
Nat. Commun. 10 (2019) 4797

12. **Mechanical properties of gelatin nanoparticles in dependency of crosslinking time and storage**
A.-V. Weiss, T. Fischer, J. Iturri, R. Benitez, J.-L. Toca-Herrera, M. Schneider
Colloids Surf. B: Biointerfaces 175 (2019) 713

13. **Life under Continuous Streaming: Recrystallization of Low Concentrations of Bacterial SbpA in Dynamic Flow Conditions**
J. Iturri, A. Moreno-Cencerrado, J.-L. Toca-Herrera
Coatings 9 (2019), 76

14. **Measuring biomaterials mechanics with atomic force microscopy: 1. Influence of the loading rate and applied force (pyramidal tips)**
A. Weber, J. Iturri, R. Benitez, J. L. Toca-Herrera
Microsc. Res. Techniq. 82 (2019) 1392

15. **Resveratrol-induced temporal variation in the mechanical properties of MCF-7 breast cancer cells investigated by Atomic Force Microscopy**
J. Iturri, A. Weber, A. Moreno-Cencerrado, Maria dM Vivanco, R. Benitez, S. Leporatti, J. L. Toca-Herrera
Int. J. Mol. Sci. 20 (2019) 3275

16. **Novel biodegradable and non-fouling systems for controlled-release based on poly (3-caprolactone)/ Quercetin blends and biomimetic bacterial S-layer coatings**
E.-G. Sánchez-Rexach, J. Iturri, J. Fernandez, E. Meaurio, J.-L. Toca-Herrera, J.-R. Sarasua
RSC Adv. **2019**, 9, 24154

17. **Microtubule disruption changes endothelial cell mechanics and adhesion**
A. Weber, J. Iturri, R. Benitez, S. Zemljic-Jokhadar, J.-L. Toca-Herrera
Sci. Rep. 9 (2019) 14903

18. **Algal cell response to laboratory-induced cadmium stress: a multimethod approach**
N. Ivošević DeNardis, J. Pečar Ilić, I. Ružić, N. Novosel, T. Mišić Radić, A. Weber, D. Kasum, Z. Pavlinska, R.K. Balogh, B. Hajdu, A Marček Chorvatova, B. Gyurcsik
Eur. Biophys. J. 48 (2019) 231

19. **Nature-based Tourism or Mass Tourism in Nature? Segmentation of Mountain Protected Area Visitors Using Self-Organizing Maps (SOM)**
K. Taczanowska, L.M. Gonzalez, X. Garcia-Masso, A. Zieba, C. Brandenburg, A. Muhar, M. Pellicer-Chenoll, J.L. Toca-Herrera
Sustainability 11 (2019) 1314

20. **Study of Interactions between Amlodipine and Quercetin on Human Serum Albumin: Spectroscopic and Modeling Approaches**
Z. Vaneková, L. Hubčík, J.L. Toca-Herrera, P.G. Furtműller, J. Valentová, P. Mučaji, M. Nagy
Molecules. 2019; 24(3) 487

21. **Lipid phase influences the binding of Bacillus thuringiensis Cyt2Aa2 toxin on model lipid membranes**
S. Tharad, B. Promdonkoy, J.L. Toca-Herrera
Biochem Biophys.Res. Co. 511 (2019) 409

22. **A Probabilistic Model for Crystal Growth Applied to Protein Deposition at the Microscale**
V.J. Bolos, R. Benitez, A. Eleta-Lopez, J.L. Toca-Herrera
Materials 12 (2019) 479

23. **Actively Tunable Collective Localized Surface Plasmons by Responsive Hydrogel Membrane**
N.G. Quilis, M. van Dongen, P.Venugopalan, D. Kotlarek, C. Petri, A. Moreno-Cencerrado, S. Stanescu, J.L. Toca-Herrera, U. Jonas, M. Moller, A. Mourran, J. Dostalek
Adv. Opt. Mater. 7 (2019) 1900342

24. **Atomic Force Microscopy Meets Biophysics, Bioengineering, Chemistry, and Materials Science**
J.L. Toca-Herrera
ChemSusChem 12 (2019) 603

25. **Survival analysis of author keywords: An application to the Library and Information Sciences area**
F. Peset, F. Garzón-Farinós, L. M. González, X. García-Massó, A. Ferrer-Sapena, J. L.Toca-Herrera, E. A. Sánchez-Pérez
J. Assoc. Inf. Sci. Technol., published on-line, doi: 10.1002/asi.24248

Books and book chapters

26. **Crystalline Cell Surface Layers (S-Layers)**
U.B. Sleytr, A. Breitwieser, D. Pum
In Encyclopedia of Microbiology (Fourth Edition), Schmidt, T. M., Ed. Academic Press: Oxford, 2019; pp 783-792.

4. Conferences, seminars, workshops and schools

1. **TITLE: Potential and challenges of Confocal Raman and correlative Imaging in Plant Sciences**
   AUTHOR: *N. Gierlinger*
   CONFERENCE: Lunch & Lern (Witec), 2nd International Plant Spectroscopy Conference
   PLACE, YEAR: Berlin (Gemany), 2019

2. **TITLE: Raman imaging of plant cell walls: where we stand and how to move forward**
   AUTHOR: *N. Gierlinger*
   CONFERENCE: 2nd International Plant Spectroscopy Conference
   PLACE, YEAR: Berlin (Gemany), 2019

3. **TITLE: Raman-Mikroskopie an Pflanzenzellen: Chemische Bilder basierend auf Molekülschwingungen**
   AUTHOR: *N. Gierlinger*
   CONFERENCE: Symposium “Moderne bildgebende Verfahren” (ÖAW)
   PLACE, YEAR: Vienna (Austria), 2019

4. **TITLE: The puzzle of the walnut shells: curvature for interlocking**
   AUTHOR: *N. Gierlinger*
   CONFERENCE: Curvature & Biology Workshop
   PLACE, YEAR: Salzburg (Austria), 2019

5. TITLE: **Tip in – light on: Insights into plant cell walls on the micro and nanolevel**
   AUTHOR: *N. Gierlinger*CONFERENCE: Wood Wallenberg Center
   PLACE, YEAR: Stockholm (Sweden), 2019

6. **TITLE: Physics, interfaces and colloids: a bridge between Nanoscience and Medicine. (oral)**
   AUTHOR: J*.L. Toca-Herrera*
   CONFERENCE: “Fortbildungsveranstaltung NephroForum"
   (Univ. Klinik für Nephrologie und Dialyse, AKH-Wien)
   PLACE: Vienna (Austria) 2019

7. **TITLE: Loading rate dependence of mechanical properties of biomaterials studied by AFM (poster)**
   AUTHOR: *A. Weber, J.L. Toca-Herrera*
   CONFERENCE: 25th Congress of the European Society of Biomechanics (ESB 2019)
   PLACE, YEAR: Vienna (Austria), 2019

8. **TITLE: Influence of cytoskeletal features on the mechanics of endothelial cells (poster)**
   AUTHOR: *A. Weber, J. Iturri, R. Benitez, S. Zemljic-Jokhadar, J. L. Toca-Herrera*
   CONFERENCE: 25th Congress of the European Society of Biomechanics (ESB 2019)
   PLACE, YEAR: Vienna (Austria), 2019

9. **TITLE: Resveratrol-Induced Temporal Variation in the Mechanical Properties of MCF-7 Breast Cancer Cells Investigated by Atomic Force Microscopy (Poster)**
   AUTHOR: *J. Iturri, A. Weber, A. Moreno-Cencerrado, MdM Vivanco, R. Benítez, S. Leporatti, J.L. Toca-Herrera*
   CONFERENCE: Cell Physics 2019 conference
   PLACE, YEAR: Saarbrücken (Germany), 2019

10. **TITLE: Does estrogen receptor drug binding influence breast cancer cell viscoelasticity? (Oral)**
    AUTHOR: *A. Weber, J. Iturri, R. Benitez, MdM Vivanco, J.-L. Toca-Herrera*
    CONFERENCE: Cell Physics 2019 conference
    PLACE, YEAR: Saarbrücken (Germany), 2019

11. **TITLE: Microtubule depolymerisation leads to changes in mechanical and adhesive properties of endothelial cells. (Poster)**
    AUTHOR: *A.Weber, J. Iturri, R. Benitez, S. Zemljic-Jokhadar, J.-L. Toca-Herrera*
    CONFERENCE: 12th European Biophysics Congress
    PLACE, YEAR: Madrid (Spain), 2019

12. **TITLE: Microtubule depolymerisation leads to changes in mechanical and adhesive properties of endothelial cells. (Poster)**
    AUTHOR: *A.Weber, J. Iturri, R. Benitez, S. Zemljic-Jokhadar, J.-L. Toca-Herrera*
    CONFERENCE: 12th European Biophysics Congress
    PLACE: Madrid (Spain)

13. **TITLE: Scanning probe microscopy in (bio) soft matter science (oral)**
    AUTHOR: *J.L. Toca-Herrera*
    CONFERENCE: Seminar of the CNR Nanotech (Institute of Nanotechnology of the National Research Council)
    PLACE: Lecce (Italy) 2019

14. **TITLE: Insecticidal Cyt2Aa2 toxin interacts with lipid membranes depending on the lipid phase state. (Poster)**
    AUTHOR: *S. Tharad, B. Promdonkoy, J.L. Toca-Herrera*
    CONFERENCE: 11th ÖGMBT Annual Meeting "INSIDE THE WORLD OF BIOMOLECULES"
    PLACE, YEAR: Salzburg (Austria), 2019

15. **TITLE: Scanning probe and optical microscopy in soft matter (bio)science (oral)**
    AUTHOR: *J.L. Toca-Herrera*
    CONFERENCE: Seminars of the Center of Cooperative Research in Biosciences (CIC bioGUNE)
    PLACE, YEAR: Zamudio (Spain), 2019

16. **TITLE: Specific lipid phase requirement of Bacillus thuringiensis Cyt2Aa2 toxin for protein-lipid interaction (oral)**
    AUTHOR: *S. Tharad, B. Promdonkoy, J.L. Toca-Herrera*
    CONFERENCE: 31st Annual Meeting of the Thai Society for Biotechnology and International Conference (TSB2019)
    PLACE, YEAR: Phuket (Thailand), 2019

17. **TITLE: Importance of lipid phase state in a selective binding of Bacillus thuringiensis Cyt2Aa2 toxin on the lipid membranes (Poster).**
    AUTHOR: *S. Tharad, B. Promdonkoy, J.L. Toca-Herrera*
    CONFERENCE: ASCB/EMBO Meeting 2019 (American Society for Cell Biology and European Molecular Biology Organization)
    PLACE, YEAR: Washington DC (USA), 2019

5. Ongoing projects, national and international collaborations

Accepted / Ongoing projects

- "**Scattering and tapping on soft, hard, open nuts**", ERC-consolidator grant SCATAPNUT, Notburga Gierlinger (PI)
- "**Plant surfaces and Interfaces: Lignin, Suberin and Cutin**", START-Project Y728-B16 SURFINPLANT (Austrian Science Fund, FWF), Notburga Gierlinger (PI)
- "**Meteoriten Chemie und Vergleich mit Kometendaten von Rosetta**", FWF-Projekt (P 26871 - N20), Kurt Varmuza (PI, TU-Vienna), Notburga Gierlinger (Co-author)
- „**S-layer recrystalization though hydrophobic/hydrophilic nanoprotrusions**" FWF-project (P29562-N28), Jose L. Toca-Herrera (PI), Dietmar Pum (co-author)
- "**Shed new light on heartwood formation**" DOC-Programme (24763) from the Austrian Academy of Sciences
- "**S-layer protein and carbon nanotube construction kit**", FWF project (P31927-N28), Dietmar Pum (PI)
- "**Investigation of the biophysical properties of protein nanocarriers for drug delivery**", WTZ project, Jagoba Iturri (PI)

National and international collaborations

- Prof. Peter Lieberzeit (University of Vienna, Spain)
- Dr. Rafael Benítez (University of Valencia, Spain)
- Dr. Luis Millán González (University of Valencia, Spain)
- Dr. Chartchai Krittanai (Mahidol University, Thailand)
- Prof. M. Schneider (University of Saarland, Germany)
- Dr. Maria Vivanco (CICbioGUNE, Spain)
- Dr. Felipe Ortega (Universidad Complutense, Spain)
- Dr. Spela Zemlijc (University of Ljubjana, Slovenia)
- Prof. Hajo Haase and Dr. Claudia Keil (TU-Berlin, Germany)
- Dr. Anders Lundgren (Chalmers University, Sweden)
- Prof. Ingo Burgert (ETH Zurich, Switzerland)
- Dr. Michaela Eder (Max Planck Institute of Colloids and Interfaces, Germany)
- Prof. Anna de Juan (University of Barcelona, Spain)
- Prof. Shawn D Mansfield (University of British Columbia, Canada)
- Prof. Gilbert Neuner (University of Innsbruck, Austria)
- A.o. Univ. Prof. Ursula Lütz-Meindl (University of Salzburg, Austria)
- Prof. Wolfgang Gindl (BOKU, Austria)
- Univ.Prof. Dr. Helga Lichtenegger (BOKU, Austria)
- Dr. Rupert Tscheliessnig (Austrian Center for Industrial Biotechnology)
- Prof. Michael Jantsch and Dr. Mamta Jain (Medical University of Vienna, Austria)
- Dr. Nadica Ivosevic DeNardis (Ruder Boskovic Institute, Croacia)
- Prof. Eva Stöger (BOKU, Austria)
- Dr. Jakub Dostalek (Austrian Institute of Technology)
- Assoc. Prof. Stefano Leporatti (CNR-Nanotec, Italy)

6. Student supervision and institute´s seminars

Student supervision

PhD

- Sebastian Antreich: Shape development of polylobate puzzle cells in walnut
- Peter Bock (START/ERC): Raman and IR spectroscopy on plant aromatics to gain a better understanding of secondary cell walls
- Martin Felhofer: Heartwood formation
- Elham Ghorbani Gorji: Resveratrol milk proteins interactions
- Martin Niedermeier: Calcium oxalate in different nut species
- Naroa Sadaba (Univ. Basque Country): Miscibility, Interactions and Antimicrobial Activity of Poly(ε-caprolactone)/Chloramphenicol Blends (grant of the Regional Basque Government)
- Nadia Sassani (START): Lignin and cutin distribution and composition on the microscale to understand waterproofing and protection of plants
- Andreas Weber: Mechanical properties of biomaterials
- Nannan Xiao (ERC): From soft to hard material: Understanding nut shell development on the micro- and nanoscale

MSc/Diploma/Training/Erasmus

- Jakob Bachmayr:"In a Nutshell: How the cell-shape influences mechanical properties"
- Alice Buytaert (Erasmus student, Ghent University): hydgrogel viscoelasticity
- Ulrich Fuchs: protein elasticity
- Flavio Hoeck: wood technology
- Lukas Krismer: Finite elements on cellsPolymer viscoleasticity
- Christoph Pötcher: Microspectroscopy on Mahagony veneers: a way to understand discolorations? 6. Luis Ponce González (University of Valencia): colloidal forces
- Charlotte Verlinde (Erasmus student, Ghent University): hydrogel viscoelasticity

Institute´s seminars

- **Jun. Prof. Dr. Stephan Schmidt** (Institut für Organische Chemie und Makromolekulare Chemie, Heinrich-Heine-Universität, Duesseldorf, Germany): “Interactive polymer gels for control freaks: Specific adhesion and recognition with carbohydrate scaffolds!” (01.04.2019)

- **Assoc. Prof. Antonio Lucas-Alba** (Facultad de Ciencias Sociales y Humanas, Universidad de Zaragoza, Spain): “WaveDriving: towards uninterrupted traffic flows” (27.08.2019)

- **Ikerbaske Prof. Francisco Blanco** (BioGUNE, Zamudio, Spain): " Molecular recognition of the eukaryotic DNA sliding clamp by NMR” (02.12.2019)