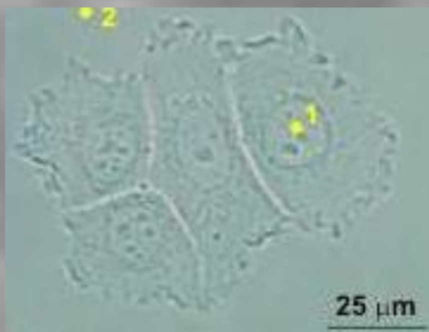
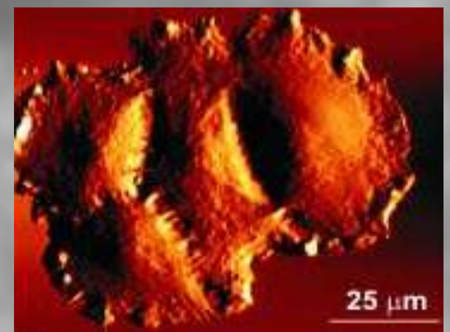
2013 Annual report

Institute for Biophysics

DNBT, BOKU-Vienna





2013 Annual Report
Institute for Biophysics

DEPARTMENT OF NANOBIOTECHNOLOGY
University of Natural Resources and Life Sciences, Vienna
March 30, 2016

I am glad to present finally the first annual report of the Institute for Biophysics. The motivation is multiple. On one hand it is a good way to present ourselves to the "outsiders". On the other hand, it could be considered a guide for future work while it represents already our "history".

2013 has been a good year. The final balance is positive. From the laboratory point of view, we have got the necessary space to carry out our activities maintaining a dynamical equilibrium with the other two institutes of the department. This fact also represents responsibility from our part since we have to manage such labs (including the training of users), something that we will learn by doing it.

An important activity that we have consolidated during 2013 is teaching. This is important because teaching and research will define our identity for the years to come. By now we have established our main teaching core that I hope will provide us with motivated students willing to do a master or a PhD thesis with us. Between Dietmar Pum and me we cover a wide range of topics: nanobiotechnology, experimental techniques applied to nanosystems, modern microscopy techniques including high resolution electron microscopy, physical chemistry, biophysics, mathematical methods in biological physics, modern physics and scientific writing.

Our research activities have improved in comparison to 2012. Our numbers are: 16 (SCI) publications, 1 book, 3 book chapters and 15 communications to conferences, workshops or seminars (including invited lectures). These numbers for a young institute of about 14 people (plus visitors) are a good start.

In particular, Dietmar Pum has published very successfully and has opened new questions about the S-layer proteins that could lead to new interesting results and future projects. He has also shown his ability to interact with other BOKU and external groups. Uwe Sleytr has shown how an emeritus professor can still be active and creative and contribute to high impact factor papers (carried out in cooperation with members of the Institute of Synthetic Bioarchitectures and the Austrian Institute of Technology). My own group has also contributed to the development of the institute's research topics being the most important item the book about scanning probe microscopy (and its combination with other experimental techniques) that is currently used in our teaching activities.

During 2013 we have started new research lines concerning physical activity, that without being a priority, we will try to develop during 2014. Such research activities will allow us to develop physical and mathematical models that are suitable for other topics related to life sciences.

In 2014 we need to establish two main research lines: i) mechanical properties of biomaterials (mainly cells) and ii) dynamic molecular interactions. We hope to be able to do that with the incorporation of two new university assistants. Finally, we have had the luck and the joy of receiving guest scientists and Erasmus students, with what that means in terms of knowledge and human exchange. We will continue doing it in future.

Last but not least, I would like to thank all the members of the institute that made this possible and especially those who left to have a professional career somewhere else during 2013. All the best!

José L. Toca-Herrera

PS: Many thanks to Alberto, who edited the final version.

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Institute members and visitors

- Univ. Prof. Dr. José L. Toca-Herrera
  (Director)
- Ao. Univ. Prof. Dr. Dietmar Pum
  (deputy Director)
- O. Univ. Prof. em. Dr. Uwe B. Sleytr
  (emeritus)
- Assoc. Prof. Dr. Luis M. Gonzalez
  (visiting professor, Univ. of Valencia, Spain)
- Dr. Susana Moreno-Flores
  (univ. assistant)
- Dr. Kathryn Melzak
  (univ. assistant)
- Dr. David Schuster
  (researcher)
- Dr. Xavier Garcia-Masso
  (visiting researcher, Univ. of Valencia, Spain)
- MMSc. Ewa Oprezska-Zingrebe
  (PhD student)
- MSc. Jone Munoz
  (PhD student, collaboration with Univ. of Basque Country, Spain)
- Mag. Eva Ladenhauf
  (PhD student)
- MSc. Elham Ghorbani Gorji
  (PhD student, collaboration with Inst. Food Sci. - BOKU)
- Mag. Sudarat Tharad
  (PhD student, collaboration with Mahidol University, Thailand)
- Mag. Jacqueline Friedmann
  (techn. assistant)
- MSc Batirtze Prats Mateu
  (FFG grant student)
- Andreas Mark
  (MSc student, University of Bayreuth, Germany)
- Remedios Gomez Infante
  (Erasmus student)
- Antonio Miranda del Alamo
  (Erasmus student)
- Beatriz Lucas Delgado
  (Erasmus student)
- Manfred Tesarz
  (techn. Assistant)
- Claudia König
  (apprentice)

1 Research Topics

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

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

Aim

The main objective of this project is the development of a key enabling technology for the fabrication of nano patterned thin film imprints by using functional S-layer protein arrays as templates. Molecular imprinting is a generic technology where compounds with functional groups reciprocal to those of a template are selected for making a scaffold around it. In this way an imprint is formed that is chemically and sterically complementary to the chosen template. Due to the crystalline character of the S-layer template the unique feature of such novel imprints will be the precisely controlled repetition of surface functional groups and domains, and topographical features.

Results

The start of the project focused on the selection, composition and chemical modification of suitable polymers (Methacrylic acid (MAA), Vinylpyrrolidone (VP)) as well as on the preparation and reassembly of the S-layer protein SbpA from *Lysinibacillus sphaericus* CCM2177 on silicon surfaces. The compressibility of the S-layer with respect to that of the polymer was investigated in order to make sure that the S-layer is mechanically robust enough to leave an imprint behind. It was found that the Young modulus of the S-layer is ca. 7 times higher compared to that of the polymer at the time of starting the imprinting. First stimulating results were obtained by successfully imprinting supported S-layers and rebinding the same S-layer protein on the cleaned imprints -as demonstrated by QCM (Quartz crystal microbalance) studies (see Figure 1). In addition to the imprinting of planar S-layer templates the imprinting of spherical S-layer architectures was investigated too since it offers the advantage of increased surface area and consequently higher number of functional groups or domains per unit area. For this purpose, mono disperse silica particles (100 nm and 500 nm in diameter) were fabricated based on the sc. Stöber process and coated with S-layer protein SbpA from *L. sphaericus* CCM2177. Zeta-potential measurements confirmed a complete coverage of the particles.

The visualization of the S-layer topography by atomic force microscopy (AFM) is more difficult than anticipated. Due to high roughness of the polymer (up to 100 nm) compared to the depth of the imprints (5-8 nm) it is often difficult and time consuming to find suitable areas on the imprint. Thus, a silicon wafer with a unique micro lithographically fabricated pattern was developed and, now, successfully serves as a new support for the S-layer reassembly.

Moreover, as a first step in using S-layer imprints as patterning elements in material sciences, polycationic ferritin (PCF) was bound in dense packing on

reassembled S-layer protein monolayers and imprinted. After removal of the template and cleaning of the imprint it was possible to bind PCF in the cavities of the imprinted areas again. As expected, the PCF molecules resembled the original S-layer pattern, and in this way demonstrated the advantage of using S-layer imprints as molecularly precise patterning elements in material sciences.

Summary

Although it is still challenging to image the S-layer topography on the imprints, QCM measurements - comparing the signals from the molecularly imprinted and the non-imprinted electrode - unambiguously demonstrated the proof-of-concept of the S-layer approach. Its main characteristics are the anti-fouling properties of the S-layer and the possibility to generate perfectly ordered functional arrays. It is anticipated that the application potential of S-layer imprints will be great with a particular focus on material science aspects where geometrically and surface chemically well-defined synthetic arrays are required.

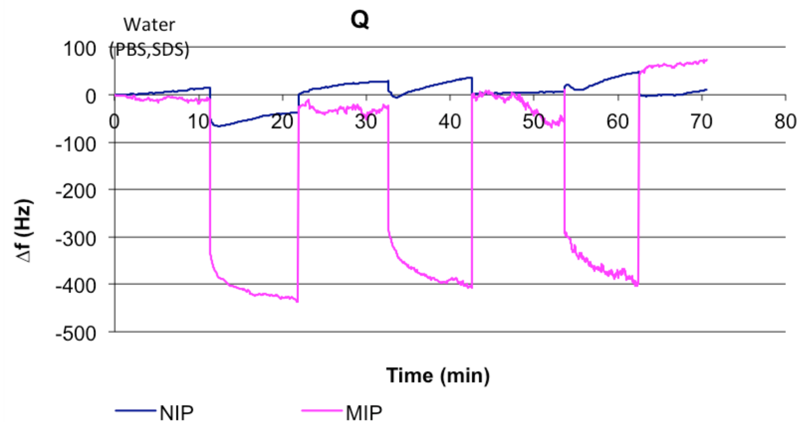


Figure 1: Diagram showing three repeats of rebinding S-layer protein (1 mg / ml) on the molecularly imprinted electrode. Measurement was paused during cleaning (injection of PBS- SDS).

Acknowledgements

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

Cell shaped induced changes of cell functions: controlled uptake using ionic interfaces

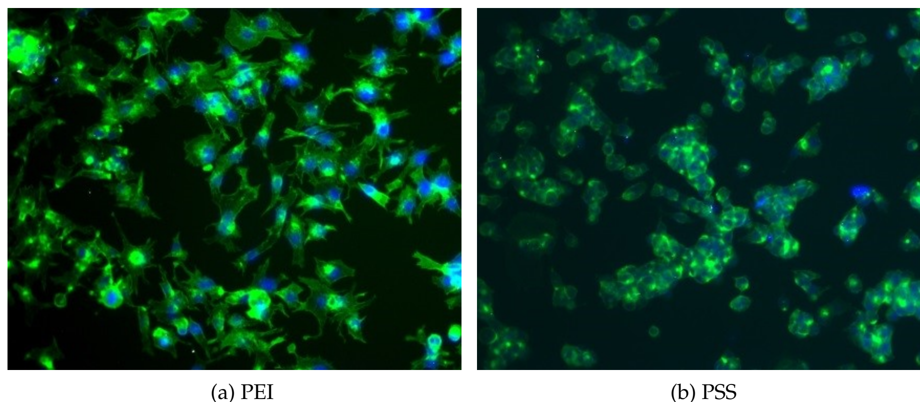
Batirtze Prats Mateu, Peter Ertl[1], José L. Toca-Herrera
[1]*Austrian Institute of Technology, Vienna, Austria*

Aim

- Fabrication of polyelectrolyte interfaces though "Layer by Layer" coatings to induce cell shape changes.
- Give insight of cellular uptake mechanisms in the context of cell shape and cell mechanics.
- Relation between cell uptake, underlying polyelectrolyte layer and cell shape.

Results and Summary

In the current project, we have investigated the effect of cationic and anionic interfaces on cell shape and activity. We demonstrated with this study that cellular uptake can be influenced by controlling the biomechanical properties of adherent cells through advanced biointerfaces. Cell biomechanics, an emerging field in cell biology, provides insights in cell responses to mechanical stimuli, which is an important parameter known to influence a variety of cell functions. For instance, understanding the interdependence between mechanical stimulus, cell shape and function is key in controlling the cell culture microenvironment for pharmacological/medical applications such as the enhancement of transfection of mammalian cells or targeted drug delivery in cancer treatments.



(a) PEI (b) PSS

Figure 1: Fluorescence microscopy images of HepG2 cells on two different polyelectrolyte substrates: anionic polystyrene sulfonate (PSS) and cationic polyethylenimine (PEI). Adherent cells on PEI show a tendency to spread and cover higher surface area than cells on PSS or PS. HepG2 cells morphology was visualized with DAPI (blue) and Phalloidin 488A (green) for staining the nucleus and the cytoskeleton, respectively.

Previous results of the laboratory indicated that surface modifications made by the Layer-by-Layer technique using different cationic and anionic polyelectrolytes delivered differences in the Young modulus of HepG2 cells. We have started to investigate how a shift in biomechanical properties effect cellular activity of various human cell lines including HepG2, NHDF and HUVEC cells. Changes in cell shape were induced using functionalized glass slides exhibiting either polyethylenimine (PEI) or polystyrene sulfonate (PSS) nanolayers. Our results showed that the engineered coatings induce an alteration in cell shapes and activities. The effects of cell shape on activity and function are demonstrated by monitoring cell uptake and internalization of fluorescent polystyrene particles using fluorescent microscopy, flow cytometry, fluorimetry and confocal microscopy. Cell spreading and cell shape interaction were monitored by fluorescence microscopy (see Figure 2).

The following figure shows the nanoparticle uptake of HepG2 cells cultured on PEI and PSS functionalized coverslides. Our results indicate that cell shape can be changed using ionic interfaces. It seems that active cellular uptake is influenced by the cell shape and therefore by the physicochemical properties of the substrate. In this context, plasmatic membrane mechanical properties might be crucial for nanoparticle cellular uptake, in which the Young modulus could be considered as a parameter for cell functionality.

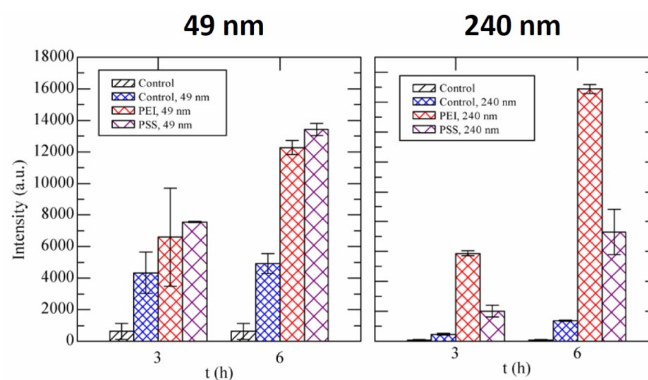


Figure 2: Nanoparticle uptake vs polyelectrolyte substrate. Left: 49-nm particles, right: 240-nm particles. Cells on PEI were able to uptake 240 nm particles about 2-fold more than cells seeded on PSS, whilst 49 nm particles internalization differences between the distinct PEM coating was not significant.

Investigation of S-layers mechanical stability by Atomic Force Microscopy

Batirtze Prats Mateu, Jone Muñoz[1]*, José R. Sarasua*[1]*, José L. Toca-Herrera*
[1]*Biopolymers and Thermoplastics Materials Group, Faculty of Engineering, University of the Basque Country, Bilbao, Spain*

Aim

The present project aims to resolve protein crystal mechanical properties of the so-called S-layers. S-layers are the outermost surface proteins present on most archea and bacteria. They have the ability to recrystallize in vitro with different symmetries and form ordered 2D assemblies on different surfaces such as polymers and glass.

In order to assess the mechanical stability of such crystal, we will use a thermo-dependent copolymer, Poly-lactide-caprolactone (PLCL) that changes its structure depending on the temperature and the surrounding media. The S-layer will be reassembled on PLCL polymer and thermal variations will be applied. Atomic Force Microscopy will be used to analyze the recrystallization process as well as variations of the original protein crystal due to latter temperature dependent polymer movements. In deep, forces between morphological units will be elucidated by force spectroscopy curves.

Elastic energies and morphologies of the first stages of the discoechinocyte transition

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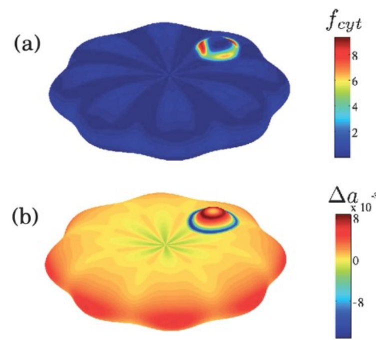
[2]*Departament de Fisica Fonamental, Universitat de Barcelona, Spain*

Aim

Role of the structural components of the RBC membrane in cell deformation.

Results

Expansion of the outer RBC lipid leaflet forces the membrane to bend, leading to the deformation of the biconcave discocyte into increasingly spiculated shapes, in a well-defined series of cell shapes known as the discoechinocyte transition. We explored the first stages of this transition by means of an elastic membrane energy model that accounts for the bilayer and cytoskeleton contributions. The morphological evolution is explained in terms of the elastic response of these membrane components. Our results highlight the importance of the cytoskeleton as a stabilizing component and how it determines the strong sequential character of the development of different morphologies. In general, cells develop undulations around the cell contour prior to the growth of out-of-plane bumps; this was found to be due to the high energetic penalty relative to a limited area-difference benefit.



(a) Shows the stress-energy density of the cytoskeleton in the discoechinocyte II stage. (b) Local area-difference between layers for a discoechinocyte II. The out-of-plane bumps are penalized for a limited area difference benefit. Therefore, they appear when the bilayer has stored more energy than the in-plane undulations.

Summary

The mechanisms driving cell deformations and the role of each structural component of the RBC membrane have been identifed. The results highlight the relevance of understanding the process of ripping, and how it interplays with the cytoskeleton remodelling, in order to establish the time-dependent behaviour of the shape transformation. Active processes seem fundamental to clarify the kinetic behaviour of the cell morphology and elasticity; whereas recent theories have incorporated the effect of ATP into several mechanisms affecting membrane elasticity, the approach to study cell morphology still as-

sumes a static picture, with active processes being considered negligible or of little importance. In that regard, more studies are required to elucidate the resting shape of the cytoskeleton, ideally including local relaxation, as it seems realistic that the network is able to conveniently change its connectivity at certain regions depending on the environmental conditions. All these questions are crucial to throw additional light on the issue of how environment and time affect the membrane elastic conditions and their consequences to cell ageing and preservation during blood storage.

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

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

Aim

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

Results and Summary

End-tethered brushes of poly(N,N-dimethyl acrylamide) (PDMA) were prepared at high initial graft densities. Polymer chains were then cleaved off by alkaline hydrolysis of the single ester bond linking the chains to the substrate. The rate of hydrolysis of the brushes was found to be dependent on the graft density: a high hydrolysis rate was observed at higher graft densities, with a sharp transition to a lower rate as the graft density decreased. This transition takes place at a point where the average separation distance between the chains is much lower than the radius of gyration, so that the polymer chains are expected to be moderately stretched. We suggest that the hydrolysis is enhanced due to mechanical activation of the ester bond in the high density brush. A corollary of these results is that for polydisperse samples, hydrolysis becomes selective for longer chains at one stage of the reaction.

Partial molar area for cholesterol in lipid bilayers

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[1]*Foundation for Research and Technology Hellas, Institute of Electronic Structure and Laser, Crete, Greece*

Aim

We are interested in finding a way to analyse cholesterol-phospholipid interactions using a system that is closer to the native environment. For this purpose the size of liposomes before and after removal of cholesterol will be measured.

Results

The partial molar area for cholesterol in lipid mixtures is a measure of the non-ideality of the system: it reflects the fact that the total area occupied by the mixture will not be equal to the sum of the areas of the individual components. This has previously been studied on monolayers at the air-water interface. However in cell membranes, lipids should not be expected to behave in the same way as lipids in Langmuir trough studies. One example of the difference between monolayers and bilayers is the solubility limit for cholesterol: monolayers of pure cholesterol can be formed on Langmuir troughs, but the solubility limit is 66 mol% or less in lipid bilayers.

If the liposome surface area can be determined accurately (in this case, from the light scattering Rg or Rh values), and if all the cholesterol can be removed, then this would provide a way to measure the partial molar area of the cholesterol in the mixture.

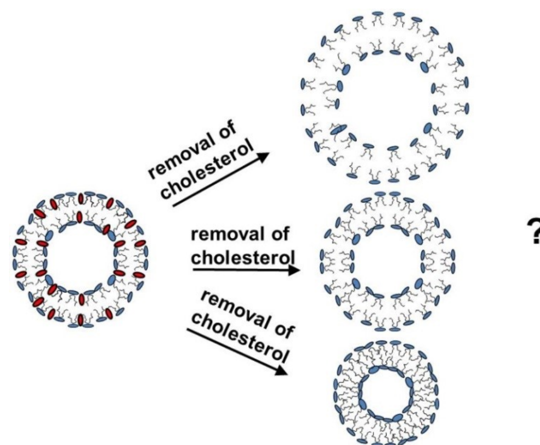


Figure 1: Summary of the experiments in the diagram. Liposomes were prepared with different mole fractions of cholesterol, and added methyl-β-cyclodextrin (which will remove some of the cholesterol). Light scattering measurements were done to see if the liposome diameter changed. (According to the Langmuir trough measurements, it is possible for the total area to increase, decrease, or stay the same when cholesterol is removed, depending on the cholesterol mole fraction, and on the pressure applied during the measurements.)

Preliminary results for the 30% cholesterol liposomes showed that the size increased on removal of the cholesterol, this is the reverse of what would be predicted based on Langmuir trough data (experiments with giant vesicles at 30% cholesterol- matched the Langmuir trough predictions).

Conclusions

More investigations are required to clarify the underlying contradictory results. Possible additional experiments include: new light scattering measurements and comparison with results obtained with giant unilamellar vesicles.

Mechanical force experiments on elastin like polymers

Jacqueline Friedmann, Xavier Garcia[1], *Stefan Schiller*[2], *José L. Toca-Herrera*
[1]*Facultad de Magisterio, University of Valencia, Valencia, Spain*
[2]*Freiburg Institute for Advanced Studies, University of Freiburg*

Aim

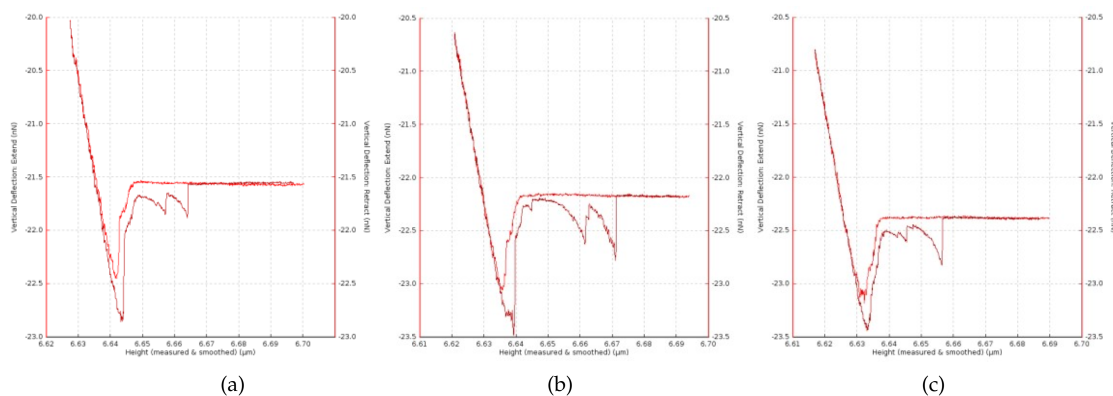
Elucidation of the molecular nature of the elasticity of elastin-like polymers.

Preparation

Flat gold surfaces were cleaned with EtOH and Milli-Q water and finally treated in the plasma cleaner. A drop of a 1:5 dilution from Elastin protein Nr. 9 with Milli-Q water was adsorbed to the gold substrate and washed with Milli-Q or buffer solution after an incubation time of 10 minutes (dilution is important to avoid picking more than one polymer per pulling trial). Force distance curves were measured in aqueous solutions: Milli-Q water, 150 mM NaCl and 150 mM $CaCl_2$. Force-distance curves were carried out at different pulling speeds with the JPK NanoWizard instrument using the standard NP-S tip with a nominal spring constant of around 0.15N/m. Prior measurements the cantilever was calibrated. From each experimental set-up about one hundred force curves were captured to obtain enough data for the corresponding statistical analysis (which is not shown in this report, it is still under work).

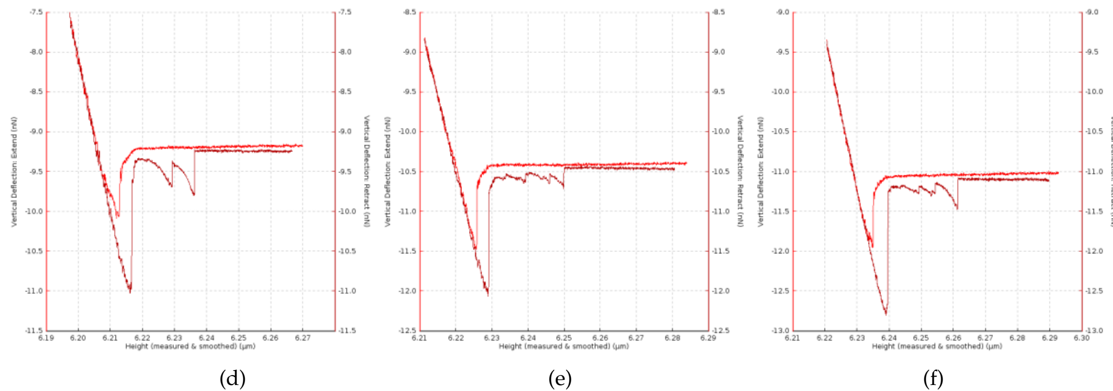
Results

(1) The force-distance curves, raw data, for elastin-like polymer measured in Milli-Q water shows a strong attractive force (lower than 1 nN) when the tip approaches the sample (light red curve). In the retracting curve (dark red) an adhesion peak (stronger than 1 nN) followed by peaks related to the elasticity of the elastin polymer (unfolding at forces in a range from 50 pN to 300 pN) can be observed. The pulling speed was 0.5μm/s.



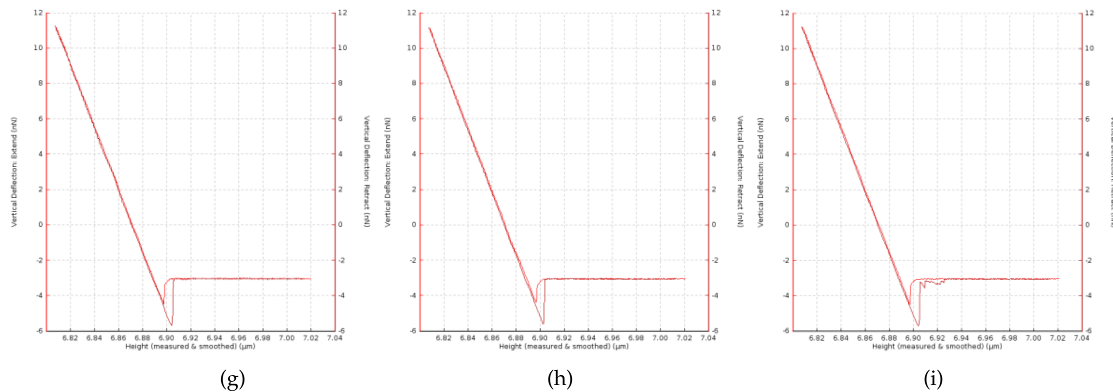
(a) (b) (c)

(2) Force curves of elastin measured in 150 mM $CaCl_2$. Force curves measured with an extent speed of 0.5μm/s. The curves show a similar trend to those reported in (1).



(d) (e) (f)

(3) Finally force-distance curves of elastin like polymer measured in 150 mM NaCl (at pulling speed of 0.8μm/s) depicted a different behavior. Approaching and retracting curves show an attractive and an adhesion force, respectively. More importantly, no elastic peaks like in (1) and (2) could me observed.



(g) (h) (i)

Conclusions

The main result of this preliminary study is that no significant stretching peaks were measured in 150 mM NaCl, which would imply a critical conformational change of the elastin polymer that has to be investigated in more detail.

AFM/STM Calibration standard

Jacqueline Friedmann, Susana Moreno-Flores, Harald Mayer[1], Erik Reimhult[1], José L. Toca-Herrera
[1]*Institut für Biologisch-inspirierte Materialien, DNBT, BOKU Vienna, Austria*

Aim

To fabricate calibration standards for AFM in order to fill the (market) gap of a two dimensional calibration standard for the A-scanner in the nanometer range. The idea is to produce a dried S-Layer calibration standard (S-layers are surface layer proteins which can be crystallised on various supports forming regular arrays in nanometer scale).

Preparation and results

* Step 1: Preparation of ultraflat surfaces to minimize height defects caused by the substrate: An 80nm thick gold layer was evaporated on freshly cleaved mica. A substrate (p.ex. grid, silicon chip...) was glued to the gold surface with a 2-K glue. After complete drying of the glue the gold layer on the edges of the substrate was scratched and the substrate covered with gold was stripped from the mica surface. Finally, the ultraflat gold layer first heading to the flat mica surface was then exposed on the new substrate. The conductivity was checked with a voltmeter.

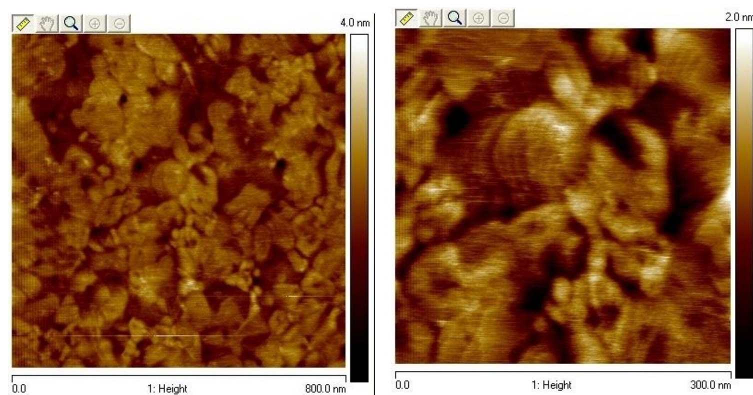


Figure 1: AFM height images of the stripped Au surface: Au terraces can be observed (Z-range: left: 4nm, right 2nm).

* Step 2: S-Layer sample preparation: The stripped Au surface was plasma cleaned and coated with thiolated secondary cell wall polymer (SCWP). SbpA from *Lysinibacillus sphaericus* CCM 2177, was recrystallised onto the SCWP layer and finally stabilised with fixing agents.

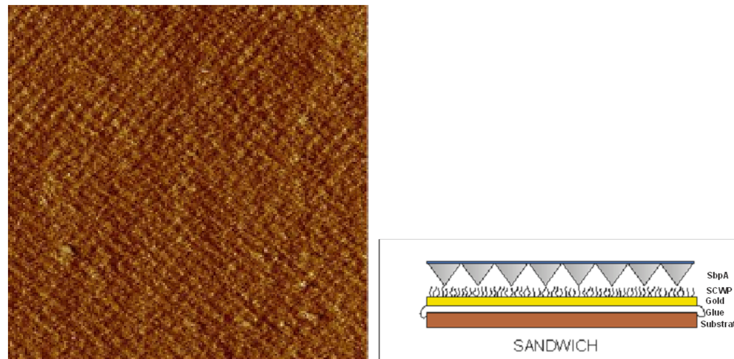


Figure 2: Left: Recrystallised S-Layer: Pattern with 13.1nm size (image 400x400nm). Right: Scheme of the sample preparation

* Step 3a: Freeze drying of the sample sandwich: As the recrystallised S-layer is just stable when kept in liquid, it was decided to perform freeze-drying experiments on BAF 400 to obtain a dried sample for easier handling. After freezing and drying the sample, the surface was coated with a platinum layer of 2 nm at an angle of 90°.

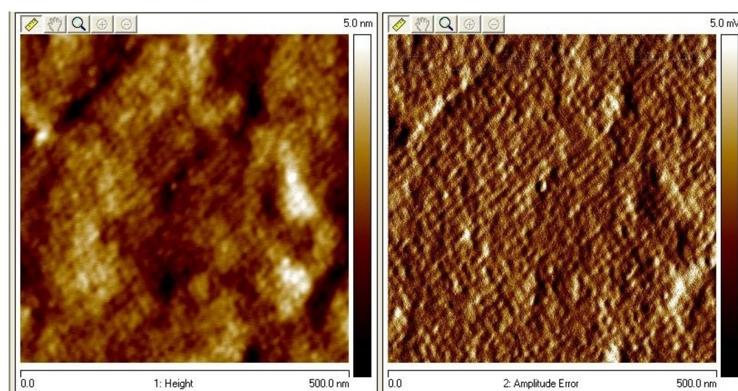
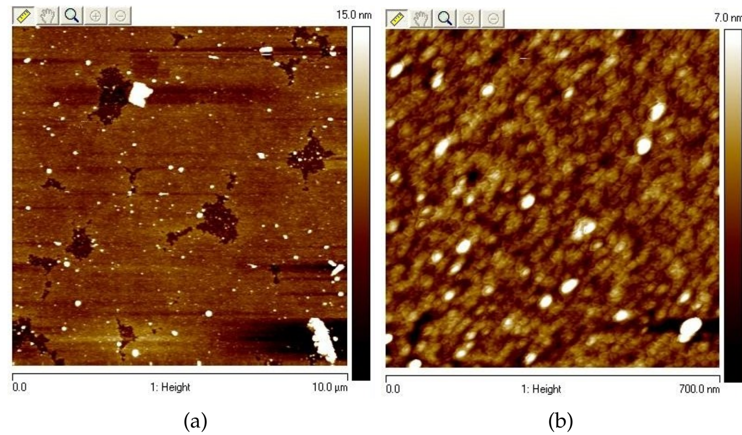


Figure 3: Recrystallised S-Layer, freeze dried: pattern imaged with contact and tapping mode in air. The freeze dried sample showed weak stability. Optimisation of storage and fixation did not improve the results.

* Step 3b: Critical point drying (CPD) of the sample sandwich: Another possibility to dry the sample offers the critical point drying instrument Leica EM CPD 030. Samples were dried in different solvents following the basic instruction of the instrument.



(a) (b)

Figure 4: Recrystallised S-Layer after critical point drying in Ethanol: surface overview with patches (left), after zoom in (right) no structure was found anymore; imaged with contact and tapping mode in air

Weak reproducibility, most of the experiments failed, just a few show regular array. CPD performed in Acetone or Ethanol with various kinds of pre-fixation of the S-layer trying to improve the stability of the pattern.

Conclusions

Although some progress has been achieved, new experimental conditions should be found to assure the stability of the calibration standard.

AFM Cantilever used as nanobalance

Jacqueline Friedmann, Claudia Koenig, José L. Toca-Herrera

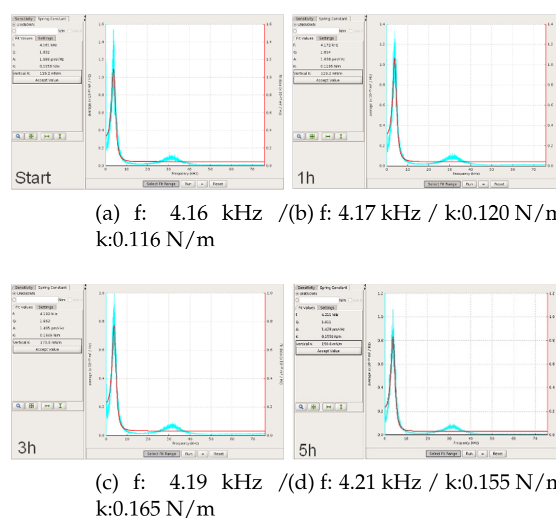
Aim

The AFM tip is a flexible and very sensitive spring. Beside imaging the topography of a surface or measuring forces between the tip and the surface it can as well be used to measure small weights. The aim of this investigation, using this idea, is to follow the formation of S-layers on AFM cantilevers. Such crystallization process (and protein denaturation) would be monitored and expressed by changes in the total spring constant, which is determined by thermal noise. SbpA protein from *Lysinibacillus sphaericus* CCM 2177 was used for these experiments.

Preparation

NP-S tips with a nominal spring constant of around 0.1N/m (the most sensitive one) were used for the nanobalance experiments. Each cantilever was calibrated prior use. For protein adsorption the cantilever was dipped into the protein solution (pH 9 and 10 mM $CaCl_2$) and kept in there for several hours. The change in frequency (and spring constant) was measured hourly. After 5 hours of adsorption/recrystallisation the cantilever was dipped into a buffer solution with a pH value of 3 to monitor potential desorption or denaturation of the protein.

Results



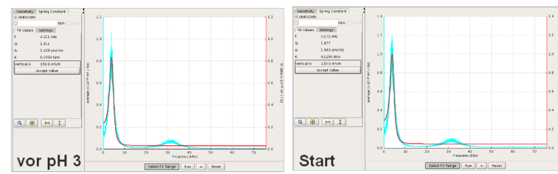
(a) f: 4.16 kHz / k:0.116 N/m

(b) f: 4.17 kHz / k:0.120 N/m

(c) f: 4.19 kHz / k:0.165 N/m

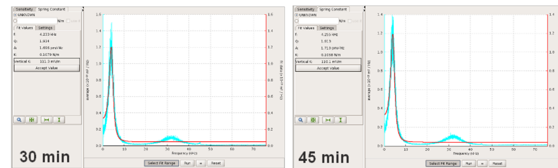
(d) f: 4.21 kHz / k:0.155 N/m

Figure 1: Protein adsorption/recrystallisation on the cantilever: the self-assembly protein on the cantilever should shift the frequency. In our case, it varies in about 0.5 kHz after 5 hours.



(e) f: 4.21 kHz / k:0.155 N/m

(f) f: 4.17 kHz / k:0.130 N/m



(g) f: 4.23 kHz / k:0.108 N/m

(h) f: 4.26 kHz / k:0.107 N/m

Figure 2: Desorption/denaturation at pH 3: After 45 minutes of incubation at pH 3 the values shift to 4.26 kHz.

Conclusions

The method seems to be sensitive to follow self-assembly (protein crystallization) and surface coating. New experiments to investigate the denature process and the topography of the coated cantilevers are needed. The quantification and the explanation of the coating influence on the final spring constant is also required.

Molecular interaction between lipid membrane and a cytolytic protein from *Bacillus thuringiensis*

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

Aim

To verify a kinetic interaction between lipid bilayers and cytolytic protein, and lipid membrane associated protein complex by quartz crystal microbalance with dissipation (QCM-D).

Results

The interaction of lipid membrane and cytolytic protein was determined on supported lipid bilayer (SLB). The lipid mixture of 1-palmitoyl,2-oleoyl-sn-glycero-3-phosphocholine (POPC) and cholesterol (13:1 mole ratio) in PBS pH 7.4 formed a bilayer on SiO_2 coated quartz crystal. The frequency and dissipation of the bilayer were -25.03 ± 2.74 Hz and $(1.06 \pm 0.47)x10^{-6}$, respectively (see figure 1). Different protein concentrations of cytolytic protein were flow into QCM-D chamber for 3 hours.

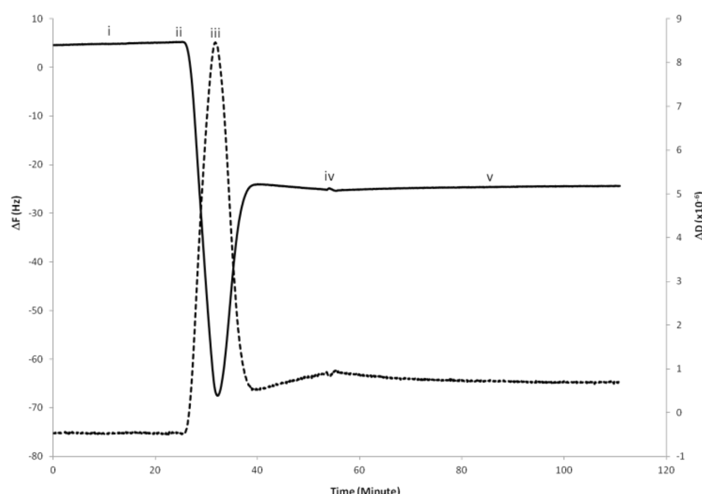

Figure 1: Lipid bilayer formation on SiO_2 coated quartz crystal. POPC/Cholesterol liposome of 0.1 mg/ml in PBS pH7.4 was injected in QCM-D chamber at flow rate 50 μl/min. Frequency shift (dark line) and dissipation shift (Dash line) were plotted from overtone 5th. Position (i) Buffer, (ii) Liposome injection, (iii-iv) Stop flow and (v) Buffer.

The three highest protein concentrations (100, 50 and 25 μg/ml) rapidly bound to lipid membrane and the membrane was saturated with protein at

frequency Δf of -36.67±1.24, -37.76± 0.58 and -39.70±2.21 Hz, respectively. On the other hand, the lowest protein concentration (10 μg/ml) protein gradually bound to lipid membrane and the membrane was saturated with protein at $\Delta f = -48.93 \pm 5.80$Hz (see figure 2a).

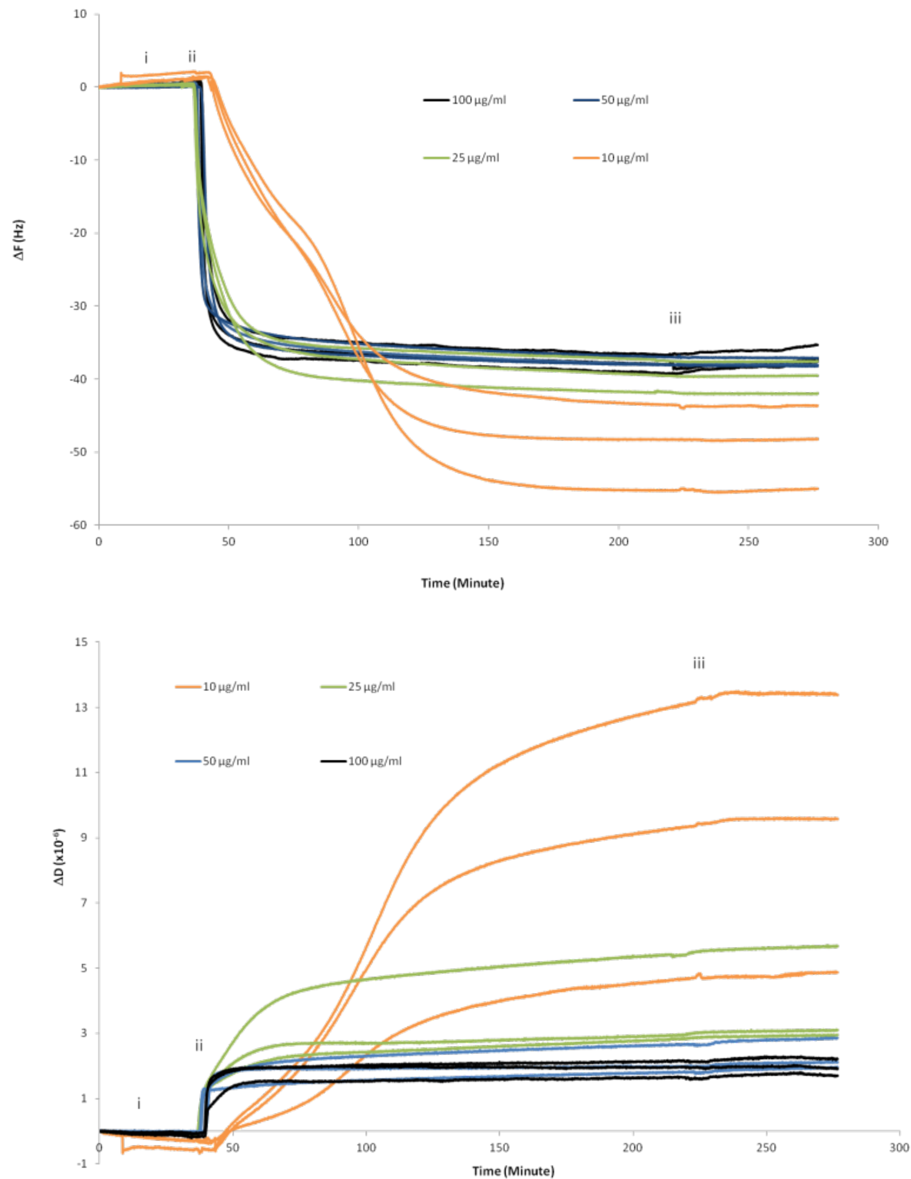

Figure 2: Cytolytic protein interaction with a POPC/Cholesterol lipid bilayer. Different protein concentrations of cytolytic protein were exposed to lipid bilayer at flow rate 4.0 μl/min. A; Frequency (Δf) and B; dissipation (ΔD) were monitored at overtone 5th. In the graph: (i) introducing buffer in the chamber, (ii) protein injection and (iii) buffer again in the chamber to remove remaining protein.

The viscoelasticity of the protein-lipid membrane complex was determined by the dissipation values ΔD, which were consistent with the frequency. The dissipation of protein-lipid membrane of each protein concentration were $(1.94 \pm 0.25)x10^{-6}$, $(2.32 \pm 0.48)x10^{-6}$, $(3.90 \pm 1.53)x10^{-6}$ and $(9.27 \pm 4.26)x10^{-6}$, respectively from high to low protein concentration (see figure 2b).

The largest difference of Δf and ΔD could be observed for protein concentrations of 25 μg/ml and 10μg/ml. Moreover, Δf and ΔD of 10 μg/ml was not accurate determined by standard deviation value (SD). The dissipation values indicated that protein/lipid complex was more rigid (low dissipation value) for large protein concentration in solution. Unlike, low protein concentration showed high viscoelasticity behavior for the protein-lipid membrane complex.

Conclusions

The POPC/cholesterol mixture (13:1 mole ratio) was formed a bilayer on SiO_2 surface with Δf and ΔD were -25.03$\pm$2.74 and $(1.06 \pm 0.47)x10^{-6}$, respectively. QCM-D reveal the different kinetic lipid membrane binding, mass absorption and protein complex formation at protein concentration $\leq 10\mu$g/ml.

Acknowledgement

This work was supported by Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (PHD/0116/2551).

Wetting properties of leaves as a function of pH and aging

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Aim

The goal of the project was to study the wetting properties (Lotus effect) of 4 different species of leaves, two of them representing Mediterranean forest (*Quercus robur* and *Fagus sylvatica*), the other two, representing riverside woodland (*Populus nigra* subsp. *italica* and *Betula pendula*) as a function of its nanostructure and aging.

Methods

Contact angle measurements were carried out using the sessile drop method (Easy Drop Equipment, Krüss, Germany). The aqueous solutions were prepared with Millipore water. The following aqueous solutions were used: 1M NaCl, pH 4, pH 2 and pH 10 (citric acid, hydrochloric acid and sodium hydroxide were used to adjust the pH).

The nanostructure of the leaves was investigated by scanning electron microscopy (SEM) (FEI Inspect S50, Japan). The wax of the leaves was eliminated with chloroform and deposited on microscopy glass slides for observation. The contact angle and the nanostructure of the leaves without wax were investigated by sessile drop and SEM.

Results

The results obtained with sessile drop method are summarized below:

1. The difference in contact angle for different species at fixed pH as a function of storage time (aging) ranges from 10° to 20° (significant variations in the data).
2. Contact angle for the same specie at different pHs and at different storage time (aging): The difference in contact angle is about 10°.
3. The contact angle values for leaves without waxes (fixed pH and different pH) are smaller than the values obtained for leaves with waxes. The difference in value is more than 20°

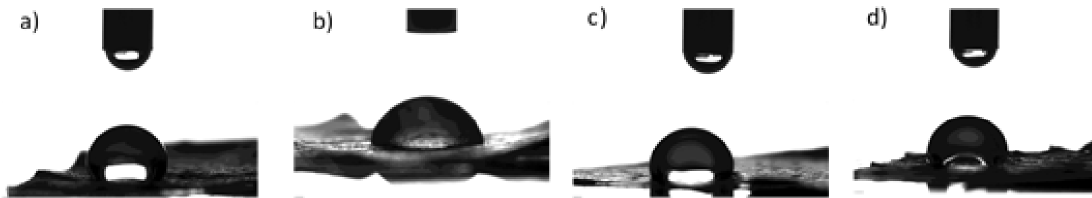

Figure 1: Photographs obtained while doing experiments using drops of 10 μl and a syringe of 3 mm diameter. The images were taken immediately after drop deposition. (a) shows a water drop on the surface of *Betula pendula* while (b) depicts a drop of pH 10 solution on *Quercus robur*. (c) shows a drop of pH 2 solution over *Fagus sylvatica*, (d) depicts a drop of water over *Populus nigra subsp. italica.*

The following figure is a representative micrograph obtained with SEM:

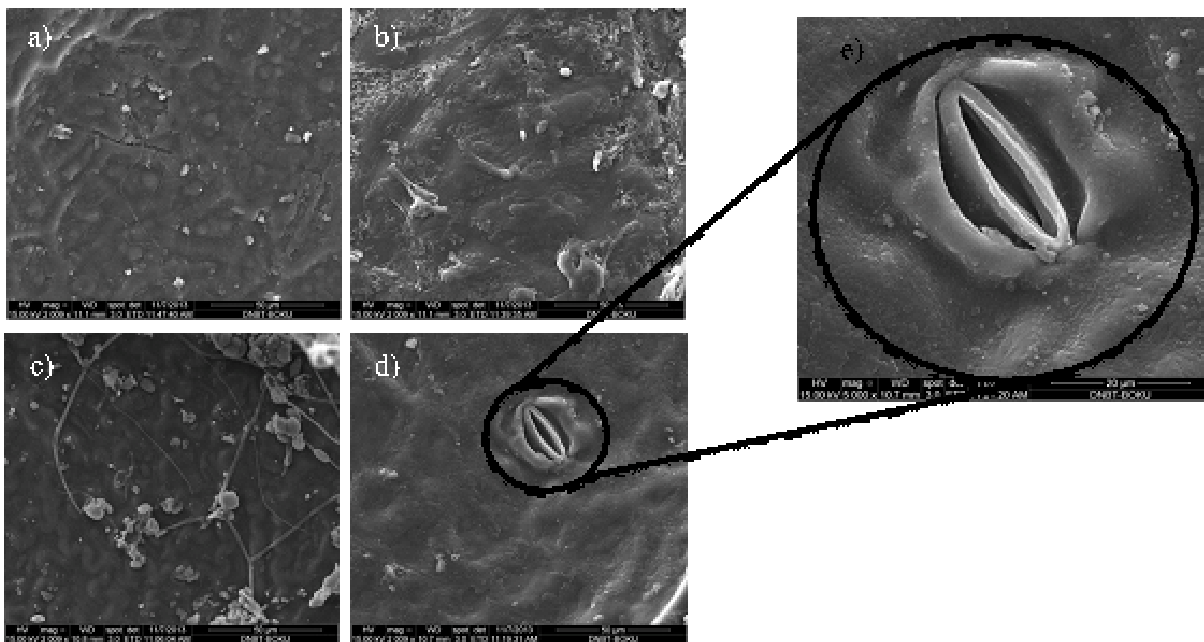

Figure 2: Micrographs obtained with SEM at a pressure of $2x10^{-4}$ mba. The micrograph (a) corresponds to a surface of *Betula pendula*. The micrograph (b) is the surface of *Quercus robur*. (c) Represents *Fagus sylvatica*. In micrograph (d), it can be seen the surface of *Populus nigra subsp. italica*. (e) is a zoom of (d) where a stoma and wax can be observed. Each sample was covered with 3 nm of Au (scale bar: 50 μm).

Conclusions

First of all, it was necessary to create a protocol for the preservation and further study of the samples; as a result of this, the samples were stored at 4°C against N_2.

The preliminary contact angle study as a function of pH and aging time was the starting point to get insight about the role of plant wax. The SEM study provided information about the structure of the leaves with and without wax and its influence on the leaves wetting properties.

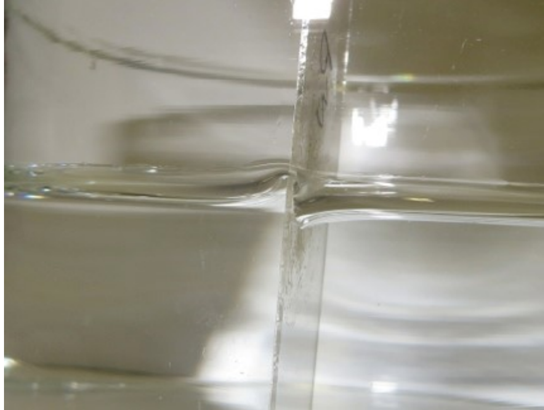

Figure 3: The picture shows the hydrophobicity of wax of *Betula pendula*. The right side of the glass slide is covered with waxes while the left side is bare glass. Thus, it can be observed the repellent effect that wax produces on water.

At the moment, surface analysis of the obtained images after removing these protective layers is carried out with specialized software.

Acknowledgements

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

Pilot study about the behavior of visitors in Viennese urban and natural environments

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Aim

This study had the objective of understanding the flow of visitors in natural recreational areas and in the city center of Vienna. We have studied the influence of the environment, either urban or natural, on the behavior of visitors and the final spatial distribution of their activity.

More specifically, the study focused on the movement of people along the nature trails in natural environments (Lobau and Viennese forest) and most important shopping streets in Vienna. Furthermore, the characteristics of the existing roads and their environment are considered as potential possible determinants of the spatial behavior of visitors.

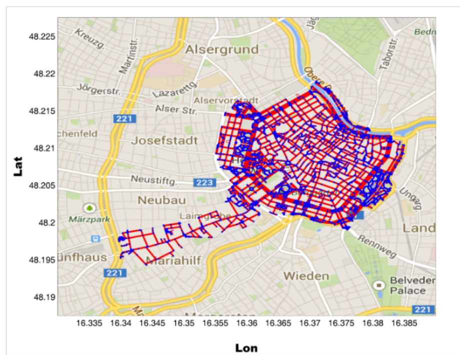
Results

	Mean	**Standard-deviation**	**Minimun**	**Maximun**
Vienna (City)				
Distance walked (km)	3.63	2.04	1.38	6,65
Mean velocity (km/h)	3.16	0.95	1.73	4.45
RMS velocity (km/h)	3.78	0.97	2.19	4.9
Total time walking (h)	1.85	0.77	1.03	3
Energy expenditure (kcal)	155.06	85.41	105.11	327.73
Lobau (park)				
Distance walked (km)	3.21	0.81	2.65	4.81
Mean velocity (km/h)	3.65	0.72	2.23	4.30
RMS velocity (km/h)	4.18	0.80	2.64	4.85
Total time walking (h)	1.51	0.48	1.00	2.12
Energy expenditure (kcal)	111.65	9.81	104.69	190.75

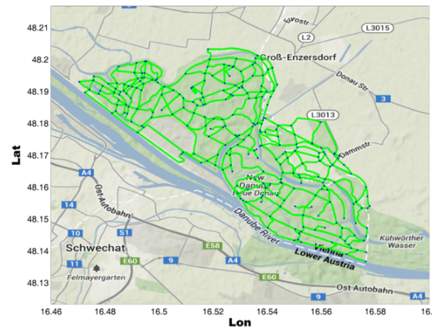
Table 1: Parameters calculated for Vienna visitors (wanderer): natural parks vs urban context

Summary

The spatial behavior of seven people in the natural area of Lobau (2400 hectares) and in the so-called "Ring" of Vienna has been determined. The experiments, carried out with GPS, were performed under similar weather conditions. During the months of November and December two excursions were done in the city center and the Lobau area. The wanderers walked for an hour. The latitude, altitude and height of the wanderers were determined, with the GPS, every two seconds. The data were analyzed with Matlab and Pajek software in order to quantify the speed, distance of each subject. Graph theory was used to create the functional network form the visitor's paths and to evaluate the importance of the nodes (also the places were the wanderers stopped). Future work will be devoted to develop mathematical (programming) tools based on graph theory and to refine calculation of the energetic loss.



(a) Ring nodes and vertices



(b) Lobau Park nodes and vertices

(c) Track of a subject within the Ring (downtown)

(d) Figure 4 : Track of a subject in the Lobau Park

Acknowledgments

BLD thanks the Erasmus program of the EU commission.

Mechanical properties of giant lipid vesicles

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Aims

Understand the physical behaviour of living cells and its relation with cell structure in a scaled way.

Elucidation of the mechanical properties of cells and models systems (e.g. giant vesicles).

Confinement of vesicles in defined geometries.

Methods

First, Egg-PC (multivesicular) vesicles were created by electroformation and water-in-oil (w/o) emulsion transfer. Results and conclusions Giant (multilamellar) vesicles were formed by electroformation in aqueous solution. The lipid composition was eggPC:DOPS (80:20 mol/mol) and fluorescent NBD-PC (0.8% mol). The voltage range varied between 0.2 and 3.24V for an application time of 15 min and 3.24 V for 3 hours.

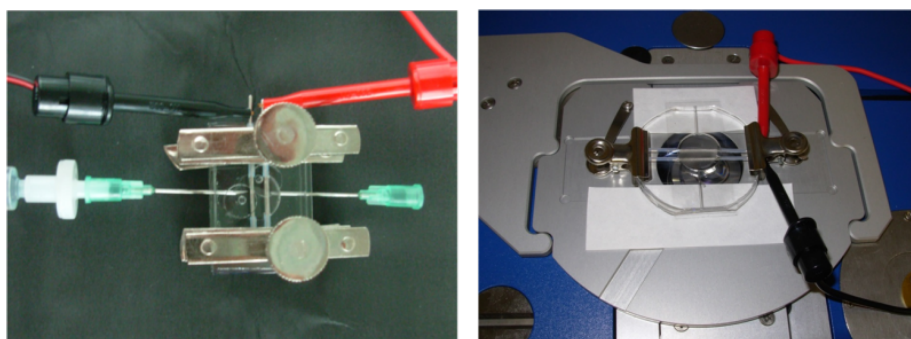

Figure 1: The figure shows the experimental device consisting in Pt- electrodes (1 mm diameter) arranged in parallel (note that Inlet-outlet flow is oriented at 90 degrees with respect to the electrodes). The left image shows the device on a bench while the right image depicts how to mount the electrodes on an AFM stage.

In order to confine the vesicles laser ablation was used to fabricate wells glass. This was achieved by using a picosecond pulse Nd:YVO4 laser (RAPID, Coherent, Germany) integrated in a micromachining workstation by 3D-Micromac. The laser source delivers 10 ps pulses at 1064 nm wavelength with energy of 12 μJ operating at a maximum repetition rate of 1 MHz. In addition to the fundamental mode, the laser emits at second and third harmonic wavelengths of 532 and 355 nm, with maximum energies of approximately 2.5 and 1.5 μJ, respectively, at the same repetition rate (1 MHz). The laser beam was focused over the sample by a focusing lens placed in air that has a focal length of 100mm

for light of wavelengths 532 and 1064 nm, and of 103 mm for light of 355 nm wavelength. Spot sizes of 30 μm and 20.5 μm were obtained at energy of 0.2 μJ for wavelengths of 532 nm and 355 nm, respectively, by selecting optional fixed beam expanders. Sample position can be selected by a XY stage with lateral resolution in the μm-range and a Z positioning system with a vertical resolution of roughly 10 nm. By means of pulse overlapping we can obtain different trenches, whose width and depth can be controlled by appropriately selecting the laser power, frequency and mark speed. By using a galvanometric scanner and appropriate control strategies, any desired topography and geometry can be generated on the workpiece. For obtaining an array of wells on a glass surface, we applied the 355nm wavelength at a frequency of 10 kHz and an energy of 53 μJ. The distance between laser pulses is of 60 micrometers. The application of these laser parameters allows us to obtain single-craters of approximately 30 μm of diameter on the sample. The well depth was varied from 4 μm to 10 μm.

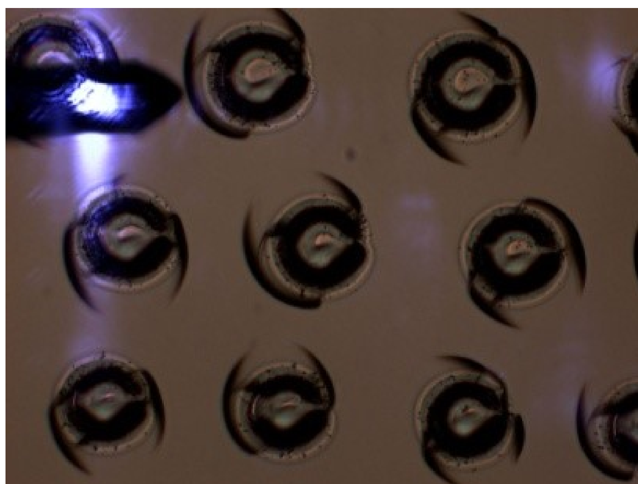

Figure 2: Fabricated well on glass (the AFM tip can be seen on the upper left corner).

Finally, Atomic force microscopy (Nanowizard III, JPK) and optical fluorescence microscopy (Zeiss) were used to study non-contact cell interactions as well as cell contact mechanics.

Results and conclusions

Giant (multilamellar) vesicles were formed by electroformation in aqueous solution. The lipid composition was eggPC:DOPS (80:20 mol/mol) and fluorescent NBD-PC (0.8% mol). The voltage range varied between 0.2 and 3.24V for an application time of 15 min and 3.24 V for 3 hours. Note the effect of the electroformation process.

The deposition of vesicles on the wells was not an easy task, actually the confinement of vesicles is a random process, and therefore micromanipulation is needed to introduce the giant vesicles in the well of interest.

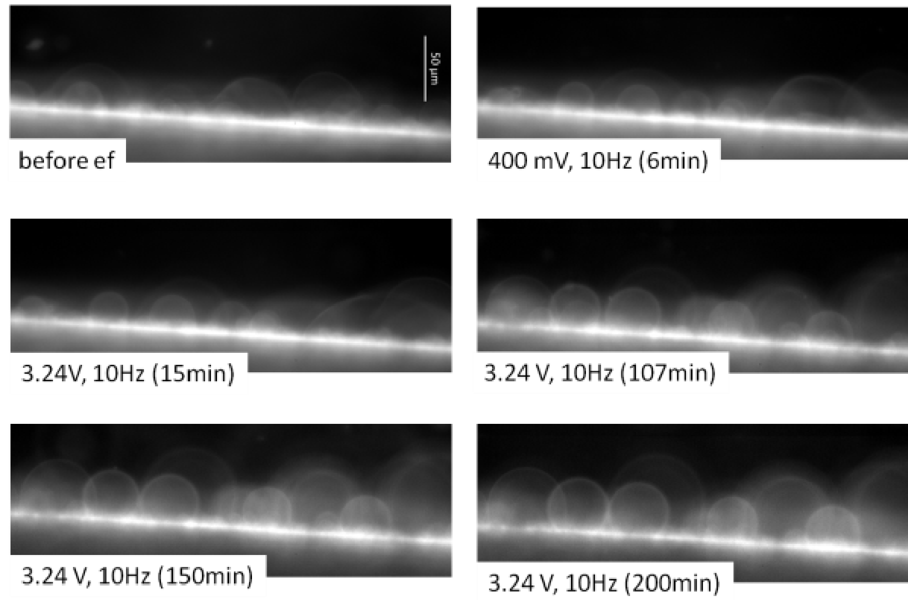

Figure 3: Some "spontaneous" vesicles while after applying a voltage vesicle formation seems to be an organized process, although they were not really homogenous in size.

Figure 4: Random distribution of vesicles around the wells (bright spots).

However, force relaxation experiments (force-distance curves) were performed with atomic force microscopy. These measurements showed two qualitative phenomena. On one hand force relaxation could be observed (vesicle was "immobilized"), and on the other hand the vesicle seemed to move after as a consequence of the applied force. The last figure shows a representative force relaxation curve. New experiments are needed to evaluate the Young modulus of the GUVs. More data are necessary to assess several open questions: i) correlation between the shoulder and force decay with vesicle lateral displacement, ii) size of the vesicle and multilamellarity (i.e. density), and iii) calculation of the elasticity of GV and the effect of vesicle size and multilamellarity.

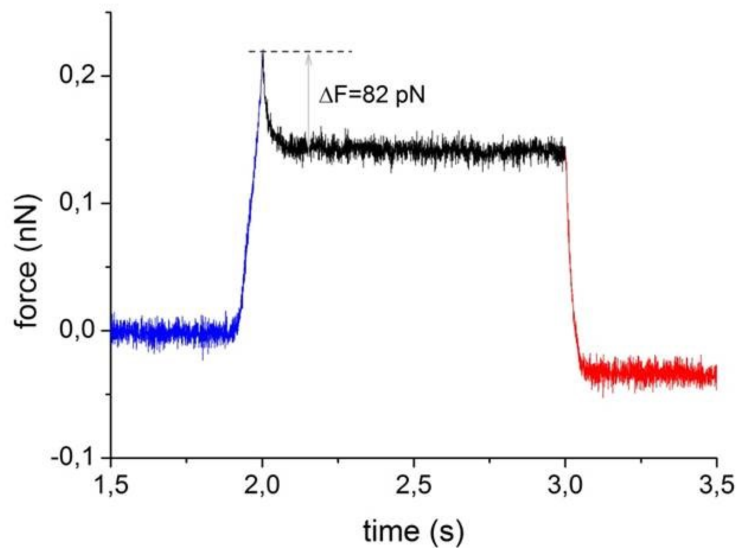

Figure 5: Representative force relaxation curve. The figure shows that the decay of the force is about less than 0.5 seconds. The amplitude of the decay force is of the order of 80 pN. New experiments are needed to evaluate the Young modulus of the GUVs.

Acknowledgements

We happily acknowledge the institute's global budget.

Ultra-fast laser microprocessing of medical polymers for cell engineering applications

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[3] *Cell Biology & Stem Cells Unit, CIC bioGUNE, Derio, Spain*

Aim

The main objective of this project is the fabrication of 3D structured substrates to investigate the influence of substrate topography on cell behavior.

Results

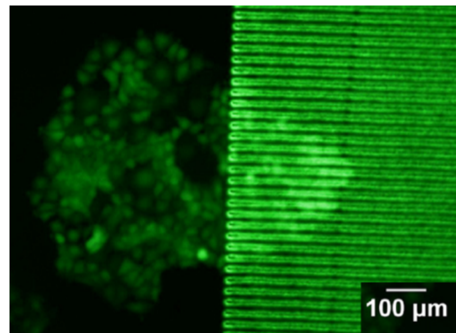

Figure 1: Fluorescence microscopy image shows a cell cluster over a non-patterned area of PS that has reached the patterned area after 5 incubation days. When the cell cluster reaches the grooves, it aligns along the direction of the groove direction. The cells deform inside the pattern exhibiting an elongated shape.

Different micropatterns were PLM-generated on polystyrene (PS) and poly-L-lactide (PLLA) to study cellular proliferation and morphology of breast cancer cells. The laser-induced microstructures included parallel lines of comparable width to that of a single cell (which in this case was roughly 20 μm), and the fabrication of square-like compartments of a much larger area than a single cell (250000 μm^2). The results obtained from this in vitro study showed that though the laser treatment altered substrate roughness, it did not noticeably affect the adhesion and proliferation of the breast cancer cells. However, pattern direction directly affected cell proliferation, leading to a guided growth of cell clusters along the pattern direction. When cultured in square-like compartments, cells remained confined inside these for eleven incubation days. These results imply that laser micromachining with ultra-short laser pulses is a suitable method to modify cell microenvironment in order to induce a predefined cellular behavior, as well as to study the effect of the physical microenvironment on cell proliferation.

Conclusions

Ultra-short laser technology has been used to produce microstructured surfaces on PS and PLLA. Such surfaces influence breast cancer cell elongation and confinement. Contact guidance effect on a supracellular scale is observed when

MCF-7 cells proliferate on microgrooved substrates. These findings indicate that laser micromachining with ultra-short laser pulses can be applied to create a 3D microenvironment that induces predefined behavior of breast cancer cells. Further investigations are in progress in the laboratory to determine the effects of these laser-created features on growth and differentiation of mesenchymal stemcells for regenerative purposes. The control of the microstructural features over cell behavior will have potential applications for expanding stem cells in vitro and guided tissue regeneration.

Acknowledgements

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

2 Publications 2013 (SCI articles)

- **Sensitivity of Aspergillus nidulans to 2,6-Dichlorobenzonitrile: insights from wall-related genes' expression and ultrastructural hyphal morphologies**
  G. Guerriero, L. Silvestrini, M. Obersriebnig, M. Salerno, D. Pum, J. Strauss
  PLoS ONE 8 (2013) e80038

- **SAXS for imaging of S-layers on intact bacteria in the native environment**
  G. Sekot, D. Schuster, P. Messner, D. Pum, H. Peterlik, C. Schäffer
  Journal of Bacteriology 195 (2013) 2408

- **Nanobiotechnology advanced antifouling surfaces for the continuous electrochemical monitoring of glucose in whole blood using a lab-on-a-chip**
  M. M. Picher, S. Küpcü, C. J. Huang, J. Dostalek, D. Pum, U. B. Sleytr, P. Ertl
  Lab on a Chip 13 (2013) 1780

- **S-layer protein self-assembly**
  D. Pum, D., J. L. Toca-Herrera, U. B. Sleytr
  International Journal of Molecular Sciences 14 (2013) 2484

- **Construction of silica enhanced S-layer protein cages**
  D. Schuster, S. Küpcü, D. J. Belton, C. C. Perry, M. Stöger-Pollach, U. B. Sleytr, D. Pum
  Acta Biomaterialia 9 (20139) 5689

- **2D crystalline protein layers as immobilization matrices for the development of DNA microarrays**
  S. R. Scheicher, B. Kainz, S. Köstler, N. Reitinger, N. Steiner, H. Ditlbacher, A. Leitner, D. Pum, U. B. Sleytr, V. Ribitsch
  Biosensensors and Biolelectronics 40 (2013) 32

- **Scientific literature analysis of Judo in Web of Science®**
  F. Peset, A. Ferrer-Sapena, M. Villamón, L.-M. González, J.-L. Toca-Herrera, R. Aleixandre-Benavent
  Archives of Budo 9 (2013) 81-91

- **A new automatic contact point detection algorithm for AFM force curves**
  R. Benítez, S. Moreno-Flores, V. J. Bolós, J. L. Toca-Herrera
  Microscopy Research and Technique, 76 (2013) 870

- **Elastic energies and morphologies of the first stages of the discoechinocyte transition**
  G. R. Lazaro, K. A. Melzak, J. L. Toca-Herrera, I. Pagonabarraga, A.Hernandez-Machado
  Soft Matter 9 (2013) 6430

- **Making novel bio-interfaces though bacterial protein recrystallization on biocompatible polylactide derivative films**
  A. Lejardi, A. Eleta-Lopez, J. R. Sarasua, U. B. Sleytr, J. L. Toca-Herrera
  Journal of Chemical Physics 139 (2013) 121903

- **Surface-Layer Lattices as Patterning Element for Multimeric Extremozymes**
  J. Ferner-Ortner-Bleckmann, N. Gelbmann, M. Tesarz, E. M. Egelseer, U. B. Sleytr
  Small 9 (2013) 3887

- **Identification of a novel gene cluster in the upstream region of the S-layer gene sbpA involved in cell wall metabolism of Lysinibacillus sphaericus CCM 2177 and characterization of the recombinantly produced autolysin and pyruvyl transferase**
  M. Pleschberger, F. Hildner, D. Rünzler, N. Gelbmann, H. F. Mayer, U. B. Sleytr, E. M. Egelseer
  Archives of Microbiology 195 (2013) 323

- **S-layer Coated Emulsomes as Potential Nanocarriers**
  M. H. Ucisik, S. Kupcu, M. Debreczeny, B. Schuster, U. B. Sleytr
  Small 9 (2013) 2895

- **Exploitation of S-Layer Anisotropy: pH-Dependent Nanolayer Orientation for Cellular Micropatterning**
  M. Rothbauer, S. Kupcu, D. Sticker, U. B. Sleytr, P. Ertl
  ACS Nano 7 (2013) 8020

- **Insertion of an Anionic Analogue of the Antimicrobial Peptide PGLa in Lipid Architectures Including S-Layer Supported Lipid Bilayers**
  Schrems, A; Larisch, VD; Sleytr, UB; Hohenegger, M; Lohner, K; Schuster,B
  Current Nanoscience 9 (2013) 262

- **Characterization of CurcuEmulsomes: nanoformulation for enhanced solubility and delivery of curcumin**
  M. H. Ucisik, S. Kupcu, B. Schuster, U. B. Sleytr
  Journal of Nanobiotechnology (2013) 11: 37

3 Books and book chapters

- **Mechanical Cues for Cell Culture**
  K.A. Melzak, S. Moreno-Flores, M. dM Vivanco, J.L. Toca-Herrera
  In Handbook of Biofunctional Surfaces (Ed. Wolfgang Knoll), pages: 865-897, Pan Stanford Publishing, Singapore, 2013, ISBN: 9789814316637

- **Hybridizing Surface Probe Microscopies: Towards a Full Description of the Meso- and Nanoworlds (Full book with 8 chapters)**
  S. Moreno-Flores, J. L. Toca-Herrera,
  CRC Taylor and Francis, 2013, ISBN: 978-1-4398-7100-3

- **Nanotechnology with S-layer proteins**
  B. Schuster, U. B. Sleytr
  In: Methods in Molecular Biology, Protein Nanotechnology (Ed. J. A. Gerrard), pages: 153-175, Humana Press, Springer Science+Business Media, 2013, New York, Heidelberg, Dordrecht, London; ISBN 978-1-62703-353-4

- **S-Layer Proteins**
  U. B. Sleytr, D. Pum, E. M. Egelseer, N. Ilk, B. Schuster
  In: Handbook of Biofunctional Surfaces (Ed. W. Knoll), pages 507-568, Pan Stanford Publishing, Singapore, 2013, ISBN 978-981-4316-63-7

4 Conferences, workshop and schools

- AUTHOR: D. Pum
  TITLE: Reassembly of S-layer proteins at interfaces (oral)
  CONFERENCE: COST Thematic Workshop: Biomimetic structures and DNA technology in biosensing
  PLACE: Bratislava (Slovakia), 2013

- AUTHOR: J. L. Toca-Herrera
  TITLE: Implementing scanning probe microscopy with optical techniques (oral)
  CONFERENCE: 2013 Nano and Photonics
  PLACE: Mauterndorf (Austria), 2013

- AUTHOR: R. Ortiz, S. Moreno-Flores, J.L. Toca-Herrera, I. Quintana
  TITLE: Surface modification of poly-L-lactide by picosecond laser irradiation: Effect of substrate topography on proliferation and differentiation of human Mesenchymal Stem Cells (oral)
  CONFERENCE: Euro BioMAT 2013
  PLACE: Weimar (Germany), 2013

- AUTHOR: J.L. Toca-Herrera
  TITLE: High resolution and energy landscapes investigating with AFM (oral)
  CONFERENCE: Mini Symposium Bio Nano Imaging (ACIB)
  PLACE: Vienna (Austria), 2013

- AUTHOR: J.L. Toca-Herrera
  TITLE: Mechanical and surface properties of biomaterials: what atomic force microscopy can tell us (oral)
  CONFERENCE: 5th Erwin Schrödinger Kolloquium 2013
  PLACE: Vienna (Austria), 2013

- AUTHOR: S. Moreno-Flores, K. Melzak, R. Benitez, M. dM. Vivanco, J.L. Toca-Herrera
  TITLE: Cell mechanics through atomic force microscopy (poster)
  CONFERENCE: Joint Annual Meeting of the Austrian Physical Society and the Swiss Physical Society
  PLACE: Linz (Austria), 2013

- AUTHOR: B. Prats Mateu, P. Ertl, J.L. Toca-Herrera
  TITLE: Cell-shaped induced changes of cell functions: controlled cell uptake using ionic biointerfaces (poster)
  CONFERENCE: 5th ÖGMBT Annual Meeting
  PLACE: Innsbruck (Austria), 2013

- AUTHOR: B. Kainz, K. Steiner, D. Pum, U. B. Sleytr, J.L. Toca-Herrera
  TITLE: Novel S-layer fluorescence fusion proteins for bulk and interfacial sensing and protein interaction determination (poster)
  CONFERENCE: MAF 13 - 13th Conference on Methods and Applicaitnos of Fluorescence
  PLACE: Genoa (Italy), 2013

- AUTHOR: B. Prats Mateu, B. Kainz, D. Pum, U. B. Sleytr, J.L. Toca-Herrera
  TITLE: Fluorescent sensors based on bacterial fusion proteins (poster)
  CONFERENCE: MAF 13 - 13th Conference on Methods and Applicaitnos of Fluorescence
  PLACE: Genoa (Italy), 2013

- AUTHOR: J. L. Toca-Herrera
  TITLE: Scanning probe microscopy in biomedical sciences (oral)
  CONFERENCE: 1st International Conference Innovations in nanotechnology and new materials in medicine
  PLACE: Krakow (Poland), 2013

- AUTHOR: R. Ortiz, S. Moreno-Flores, J. L. Toca-Herrera, I. Quintana
  TITLE: Surface modification of poly-l-lactide by picosecond laser irradiation:

effect of substrate topography on proliferation and differentiation of human mesenchymal stem cells (oral)
CONFERENCE: World Conference on Regenerative Medicine
PLACE: Leipzig (Germany), 2013

- AUTHOR: S. Damiati, S. Zayni, S. Küpcü, U.B. Sleytr, J. Chopineau, B. Schuster, E.K. Sinner.
  TITLE: Direct incorporation of cell-free (ex vivo) synthesized human membrane proteins into synthetic bioarchitecture (poster)
  CONFERENCE: 4th International Congress BioNanoMed 2013 - Nanotechnology Enables Personalized Medicine
  PLACE: Krems (Austria), 2013

- AUTHOR: S. Damiati, S. Zayni, U. B. Sleytr, J. Chopineau, B. Schuster, E. K. Sinner
  TITLE: Integrated Membrane Proteins for Synthetic Biosystems (poster)
  CONFERENCE: Nanomedicine 2013 Conference
  PLACE: Barcelona (Spain), 2013

- AUTHOR: D. Pum, E. M. Ladenhauf, D. Schuster, U. B. Sleytr
  TITLE: S-layer based bio-imprinting - Synthetic S-layer polymers. (oral)
  CONFERENCE: AFOSR Biomimetic, Biomaterial and Biointerfacial Sciences Program Review
  PLACE: Washington DC (USA), 2013

- AUTHOR: M. H. Ücisik, S. Küpcü, U. B. Sleytr, B. Schuster
  TITLE: Emulsomes modified with S-layer for lipophilic drug delivery (poster)
  CONFERENCE: European Summit for Clinical Nanomedicine
  PLACE: Basel (Switzerland), 2013

5 Accepted / Ongoing projects (external funding)

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

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

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

6 National / International collaborations

- Peter Lieberzeit
  University of Vienna, Inst. of Anal. Chem., Vienna, Austria
- Carole C. Perry
  Nottingham Trent University, Nottingham, UK
- Murugappan Muthukumar
  University of Massachusetts, Amherst, MA
- Rafael Benítez
  University of Extremadura, Dept. of Mathematics, Spain
- Luis Millán González
  University of Valencia, Dept. of Physical Education and Sport, Spain
- Jose R. Sarasúa
  University of the Basque Country, Faculty of Engineering, Spain
- Aurora Hernandez-Machado
  University of Barcelona, Dept. of Materials Structure, Spain
- Jayychandran Kizhakkedathu
  University of British Columbia, Center for Blood Research, Canada
- Peter Ertl
  Austrian Institute of Technology, Austria
- Stefan Schiller
  University of Freiburg, Inst. of Advances Studies, Germany
- Chartchai Krittanai
  Mahidol University, Institute of Molecular Biosciences, Thailand
- Eva Stöger
  BOKU, Institut für Angewandte Genetik und Zellbiologie, Austria
- Werner Fuchs
  BOKU, Institut für Umweltbiotechnologie, Austria
- Joseph Strauss
  BOKU, Institut für Angewandte Genetik und Zellbiologie, Austria
- Gerhard Schleining
  BOKU, Institut für Lebensmittelwissenschaften, Austria.
- Georg Papastavron
  University of Bayreuth , Dept. Physical Chemistry II, Germany.

7 Training activities

TEM and SEM training activities of BOKU and external students/researchers (Supervisor in parenthesis).

- Andreas Loos, Institut für Angewandte Genetik und Zellbiologie (IAGZ), BOKU (Herta Steinkellner)
- Donatella Tesei, Institut für Angewandte Mikrobiologie (IAM), BOKU (Katja Sterflinger)
- Nicole Pircher, KyuJin Ahn, Anne Neumann, Abteilung für Chemie Nachwachsender Rohstoffe, BOKU (Thomas Rosenau)
- Florian Part, Teresa Gouveia, Institut für Abfallwirtschaft (ABF-BOKU), BOKU
- Pauline Riviere, Institut für Naturstofftechnik, BOKU (Rupert Wimmer),
- Institut für Siedlungswasserbau, Industriewasserwirtschaft und Gewässerschutz (SIG), BOKU (Reinhard Perfler)
- Mario Rothbauer, Austrian Institute of Technology (Peter Ertl)
- Gerhard Sekot, Austrian Center of Industrial Biotechnology (Alois Jungbauer)