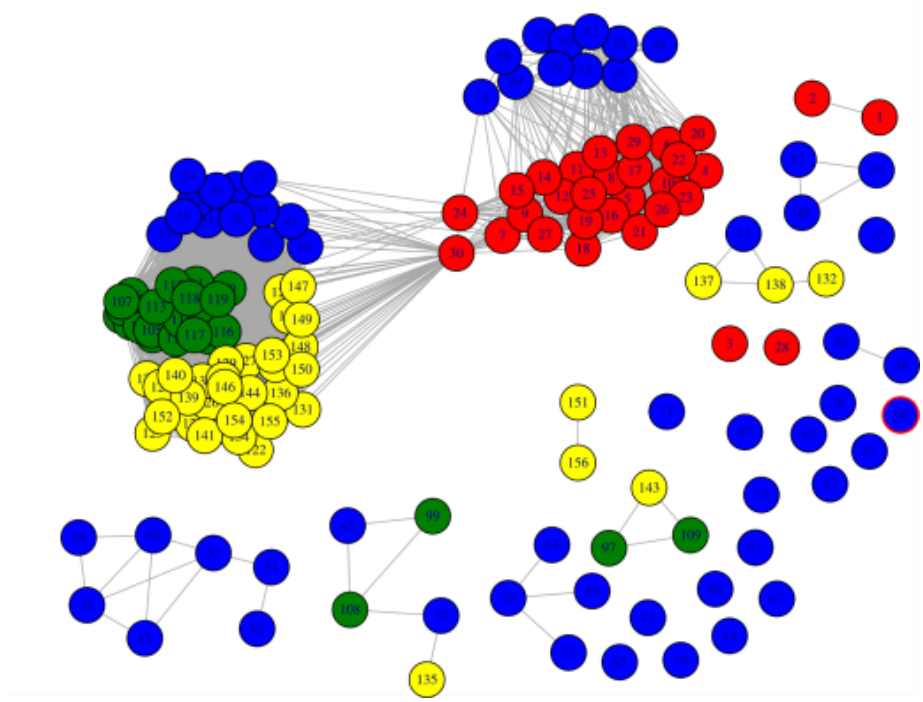

2015 Annual Report

Institute for Biophysics

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

2015 Annual Report
Institute for Biophysics

DEPARTMENT OF NANOBIOTECHNOLOGY
University of Natural Resources and Life Sciences, Vienna
March 5, 2016

Third annual report and we still are going on. This is good news. We are growing scientifically little by little. Unfortunately, the bureaucracy has also been present during 2015 as main opponent of the "working time" needed to the creative work. I have always thought that bureaucrats should work for professors and not the opposite...but...

As last year, we have continued to manage laboratories that are constantly used by DNBT coworkers and external scientists (mainly from BOKU, ACIB and the Austrian Institute of Technology). We have participated in several commissions related to teaching, research and ethical matters: DokStuko, Forschungsprecher, Departmenleiterkonferenz, ethics platform, and FachStuko.

Teaching is going well. This year we have achieved about 50 students in master lectures (more than last year, especially in Dietmar's courses). I hope this will bring us some MSc students that we need to start and develop basic research topics that will constituted the foundations of future PhD topics. In addition, the evaluations of the teachers (Dietmar Pum and myself) has been very positive. Let us keep that for 2016. A major teaching improvement has been the organization of the *Microscopy lab in Biophysics (803311)*, a practical lecture that will define and will show to the BOKU students our experimental expertise. I am sure of that because the most experience researcher-teachers of the institute will participate directly or indirectly in the practical lecture. Finally related to teaching, I was honoured to be offered a visiting professorship, from the University of Science and Technology (AGH) in Krakow in the frame of the "Marian Smoluchowski Kraków Research Consortium Matter-Energy-Future". Teaching Biophysics and Scientific writing at Faculty of Physics and Applied Computer Science was a real challenge and if I can I will repeat. The students were great, and Violetta Golebiewska came in summer to do her MSc thesis. But even more important, BOKU awarded Rafael Benítez with a visiting professorship and he ended up teaching us mathematical and computing methods in biophysics and an introduction to programming in R. Now our young and senior researchers are using analysis programs that he developed.

Our research activities kept the level of 2015. Briefly, our numbers are: 13 (SCI) publications, 1 book chapter and 14 communications to conferences, workshops or seminars (including invited lectures). As usual I do not want to get anxious about numbers. Important is to work in a continuous way, learning as much as we can and enjoying our work. Our numbers are

good an institute of about 13 people on average. This year we have had a highlight: we have got funding form the high competitive agency AFOSR. The project with Dietmar as PI (and Uwe and myself as Co-PI) will deal with S-layer and fluid mechanics at the nanoscale. For 2016 I wish to get deeper in the question of how cell mechanics is influence by cell/substrate interactions, and to explore different ways to study molecular interactions (as a whole). I do not want to forget the main S-layer questions, which are part of the institute's life.

During 2015, Dr. Daniel Wastl came from the University of Regensburg to try expand the knowledge of the SPM laboratory in the field of soft matter. Another very useful incorporation has been Amsatou Andorfer-Sarr, who definitely has put order in the chaotic cell culture lab (where visitors seems to work without gloves). We should also welcome Julia Miholich and Laia GIli Solé as new MSc students.

This year we had again the luck of receiving visitors. We love visitors. They bring new ideas, technical and scientific, knowledge and also good mood. Their stay has been very productive and I am really thankful. We had the luck and the pleasure of receiving five visiting professors (Rafael Benitez, Luis M. Gonzalez, Vicente Bolos, Xavier Garcia and Spela Zemlijc-Jokhadar), and four guest students (Agnes Weiss, Ana Viana, Violetta Golebiewska, Michaela Zrelski), who did the main part of their diploma works with us. We will continue hosting (foreign) students and scientist.

We had our second summer school. As long as we have global money we will do it. I am convince that such event helps us to get better scientifically and to improve the group dynamics of the institute. Actually, the atmosphere was very nice and constructive.

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

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

José L. Toca-Herrera

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

Contents

Institute members and visitors

- Univ. Prof. Dr. José L. Toca-Herrera (Director)
- Ao. Univ. Prof. Dr. Dietmar Pum (deputy Director)
- O. Univ. Prof. em. Dr. Uwe B. Sleytr (emeritus, former Director)
- Dr. Andreas Breitwieser (post-doctoral research assistant)
- Dr. Jagoba J. Iturri (univ. assistant)
- Dr. Daniel Wastl (univ. assistant)
- Dr. Med. Michael Handler (PhD student, collaboration with Sports Univ. Innsbrück)
- Mag. Jacqueline Friedmann (techn. assistant)
- Mag. Amsatou Andorfer-Sarr (techn. assistant)
- Mag. Eva Ladenhauf (PhD student)
- MSc. Maria Sumarokova (PhD student, Erasmus Mundus Iamonet Program)
- MSc. Elham Ghorbani Gorji (PhD student, collaboration with Inst. Food Sci. - BOKU)
- MSc. Alberto Moreno Cencerrado (PhD student)
- Claudia König (apprentice)
- Laia Gili Solé (MSc Student, FH-Technikum, Vienna)
- Julia Miholich (MSc student)
- Assoc. Prof. Dr. Rafael Benítez (UNEX, Spain)
- Assoc. Prof. Dr. Luis M. González (University of Valencia)
- Ass. Prof. Dr. Vicente Bolós (University of Valencia)
- Ass. Prof. Dr. Xavier Garcia-Mass (University of Valencia)
- Dr. Spela Zemlijc-Jokhadar (University of Ljubljana, Slovenia)
- MSc. Agnes Weiss (Apothekerin, Universität des Saarlands, Germany)
- Violetta Golebiewska (MSc student, AGH-Krakow, Poland)
- Eline de Meyer (Erasmus student, Ghent University, Belgien)
- Margareta Mittendorfer (BSc student)
- Michaela Zrelski (BSc Student, University of Vienna)
- Ana Carol Vianna (IAESTE student, Brasil)

1 Research Topics

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

Dietmar Pum, Eva M. Ladenhauf, Daniel S. Wastl, Uwe B. Sleytr

Aim/Objective

Molecular imprinting based on S-layer proteins as templates was one of the scientific topics over the last three years and was concluded in May 2015. A brief summary is given below.

Results

The main objective of the proposed work was 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. The unique feature of these imprints is the precisely controlled repetition of surface functional groups and topographical features - induced by the crystalline character of the reassembled S-layer protein lattice. 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 supports that were used as stamps. First of all, the rigidity (expressed by the Young (elastic) modulus) 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. Quartz crystal microbalance (QCM) and Surface Plasmon Resonance (SPR) studies demonstrated outstanding sensitivity and selectivity of the S-layer imprinted sensing layers. The response to rebinding SbpA - the S-layer protein which was used for printing - was linear up to 200 ng/ml protein, and no cross-selectivity to Bovine serum albumin (BSA) or a different S-layer protein (SbsB; G. stearothermophilus PV72/p2) was observed. Based on the successful imprinting of planar surfaces, the molecular imprinting of spherical architectures, such as S-layer coated liposomes and silica particles, was investigated too. Unfortunately, those experiments did not yield the expected results since liposomes were not stable in DMSO (dimetylsulfoxide) that is used in the polymer synthesis and silica particles were completely enclosed by the polymer and subsequently could not be removed from the imprint. Nevertheless, in the course of theses experiments, S-layer coated bacterial cells (*L. sphaericus CCM2177*) were successfully used as templates. Atomic Force Microscopy (AFM) demonstrated the successful imprinting of the bacterial cells and the attached S-layer lattice. However, despite the initial assumption, it was really challenging to resolve the imprinted S-layer lattice by AFM. We assume that several factors are responsible for this such as plastic deformation at molecular level upon removing the template, water cushions between the S-layer template and the polymer preventing a successful transfer of the S-layer topography into the polymer, or too shallow

surface corrugations in relation to the roughness of the polymer. However, in addition, Peak Force Quanto Nanomechanical property Mapping (PF-QNM) was able to show that the adhesion of an S-layer coated AFM-tip at the (blank) S-layer imprint was significantly higher than that at the blank reference area. The use of S-layers as carriers for other molecules and imprinting of the whole assemblies were investigated too. For this purpose, Polycationic ferritin (PCF) (mean diameter ca. 12 nm) was bound in dense packing on the S-layer on the stamp first and subsequently used for generating a square pattern of 12 nm cup-shaped indentations. Successful rebinding of PCF proved that the S-layer lattice can be used as template for making imprints of densely packed and, probably, perfectly oriented biologically functional molecules, a concept that can in principle be extended to a wide range of other biomolecules (e.g. antibodies, or affinity surfaces) as well as in the material sciences.

Conclusions

In summary, we would like to anticipate that our approach provides a key enabling technology for the fabrication of nano patterned molecular imprints by using self-assembled S-layer lattices. Applications will be found in the life and non-life sciences wherever well defined repetitive topographic and (bio)chemical features in the nanometer range are required.

Acknowledgments

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

S-layer directed nanoscale fluid mechanics

Dietmar Pum, Jose Luis Toca-Herrera, Daniel S. Wastl, Uwe B. Sleytr

Aim/Objective

The main objectives of this research proposal are the detailed investigation of the anti-fouling and the hydrodynamic surface properties of S-layers. We assume that the S-layer modifies the structure of the first "locked-in" water layer(s) and, in this way, makes it to the liquid-repellent and slippery surface. This project started end of 2015 and thus an Executive Summary is given as an introduction to subsequent annual reports.

Executive Summary

Two-dimensional crystalline S(urface)-layers are the most commonly observed cell surface structures in prokaryotic organisms (Bacteria and Archaea). S-layers are isoporous protein mesh works with unit cell sizes in the range of 3 to 30 nm, thicknesses of 5 to 10 nm (up to 70 nm in archaea), and pore sizes of 2 to 8 nm. S-layer lattices are formed in solution or at various interfaces including solid supports by self-assembly of the constituent native or recombinant proteins. Although no general biological function has been found so far, many of the specific functions assigned to S-layers depend on the completeness of the covering and the structural and physicochemical repetitive uniformity down to the nanometer scale.

A striking feature of S-layers is their excellent anti-fouling property that can be deduced from the always perfectly clean surfaces of bacterial cells seen in TEM-micrographs of freeze-etched preparations. In this context, it may be speculated that this phenomenon may also lead to a reduced flow resistance in water and consequently to a higher motility of the flagella driven cell - an advantage when taking their extremely low Reynolds numbers into account! The investigation of both phenomena, anti-fouling and low flow resistance, as benefits for the bacterial cell are the main objectives of this research proposal and will be studied on the basis of the assumption that the water layer "locked-in" by the S-layer acts as the liquid-repellent and slippery surface. But this hypothesis seems to be only plausible when the nano metric topography and charge distribution of the S-layer protein matrix modifies the structure of the water layer on molecular scale and in this way might prevent the miscibility with the bulk water.

Preliminary work has already been started concerning the reassembly and patterning of S-layer proteins on solid surfaces. Investigations regarding the advancing and receding contact angles of different fluids on (tilted) S-layer coated substrates will provide first results on the anti-fouling properties of S-layers.

Conclusions

The results of this work are primarily relevant for basic research concerning fluid mechanics on molecular scale as determined by the natural S-layer self-assembly system. Nevertheless, we would like to anticipate that the achievements might provide the basis for novel developments in biocompatible non-wetting surfaces as required in micro- or nano total analysis systems (μTAS or nTAS), mass sensitive (bio)chemical sensors, High Throughput Screening (HTS) devices for DNA analysis, or ink jet printing of complex fluids.

Acknowledgments

This work is funded by the Air Force Office of Scientific Research (AFOSR), Agreement award FA9550-15-1-0459.

Optimizing and utilizing of functionalized fluorescent tips as AFM probes

Daniel S. Wastl, José L. Toca-Herrera

Aim/Objective

Optimizing and utilizing of functionalized fluorescent tips as AFM probes e. g. for pH sensing at the micro and nanoscale have been one of the scientific topics over the last six month and is an ongoing project in 2016. Fist important step, characterize pH sensitivity; find an ideal candidate for the further experiments.

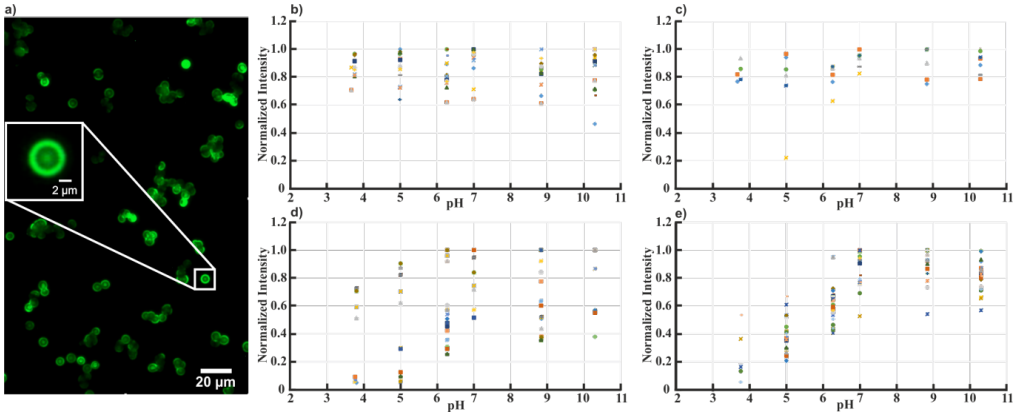

Figure 1: Fluorescence microscopy a) image of BSA-FITS-MC, b)-e) Maximal normalized intensity versus variation of the pH value of the environment: b) for BSA-FITS-MC, c) for BSA-FITS-MP, d) EGFP-SbpA -PEMMC and Fig e) EGFP -SbpA -PEMMP

Results

Fluorescence microscopy was used to characterize the pH sensitivity of functionalized fluorescent BSA/BSA-FITS- micro particle (MP) and -microcapsules (MC) as well as polyelectrolyte multilayer (PEM)-MP and -MC coated with a SbpA-EGFP surface layer. The MC and MP (center particle: CaCO3-core) differ in the way they are produced. To create MC the CaCO3-core of MP is dissolved by EDTA. Starting from the full particles the CaCO3 core is dissolved to achieve hollow capsules.

The MP and MC have been adsorbed directly on a sample surface consisting of PEI coated glass coverslips. The particle position is fixed, while the pH level of the environment was changed to different pH values while the maximal fluorescence intensity was measured. Data were collected on different substrate (equally prepared) and sample regions for a general view. The buffer solution used for the pH levels 10.3, 8.84 and 7.0 a glycerin buffer and for lower pH values 3.8, 4.99 and 6.27 citric buffer was used. The intensity was measured for 6 different pH values 10.3, 8.84, 7.0, 6.27, 4.99 and 3.8 averaged and normalized

to the maximal intensity of each particle (up to 30 single particles). To avoid interferences and disturbance form other particles in close proximity, only single particles without a close neighbour have been taken into account for the intensity measurement (see Figure 1a and inset).

Figure 1(b-e) show the results for the change of the normalized maximal intensity. Figure 1b represents the intensity change of BSA-FITS-MC, figure 1c BSA-FITS-MP, figure 1d SbpA-EGFP-PEMMC and figure 1e SbpA-EGFP-PEMMP with chancing pH value of the environment. The intensity is normalized to the maximal intensity of the single MP or MC. To reduce environmental disturbance an average over three to five images per particle was used before normalizing to the maximum.

For BSA-FITS-MC (Figure 1b) and BSA-FITS-MP (Figure 1c) there is no clear tendency to a higher or lower normalized maximal intensity while varying the environmental pH value. The maximal change of intensity by a pH step is for single particles in the range of 5-10%. To built sensors out of particles, a clear predictable (continuous) change of the intensity by changing the pH value is needed. A clear trend, is hard to define for BSA-FITS-MC and MP. The pH sensitivity and intensity variation of the BSA-FITS-systems used here is not high and constant enough for usage as sensors.

Figure 1d and 1e represent the maximal normalized intensity of the SbpA-EGFP covered PEMMC and PEMMP. The EGFP-SbpA-PEMMC (Figure 2d) and the EGFP-SbpA-PEMMP (Figure 2e) the pH sensitivity related to the change in normalized maximal intensity is 10-20%, much higher in contrast to the BSA-FITS systems discussed before. Furthermore the changes by pH are more defined for single EGFP-SbpA-PEMMC but scatter strongly in the case of several capsules. For EGFP-SbpA-PEMMP there is a clear pH dependence for all particles. Due to slight variations the maximum (100%) for the normalized intensity is not fixed for every particle to the same pH value, therefore the average situation will be discussed, 100% at a pH of 7.03. From low pH values the particle intensity is increasing form the off state to ≈20% of the normalized maximal intensity (100%) at a pH of 3.76 by increasing the pH to 4.99 the normalized maximal intensity increases to ≈40% (see Fig1e). A further increase of the pH to 6.27 and 7.03 leads to rise of the normalized maximal intensity to the level of ≈80% and ≈100% respectively. For higher pH values the intensity decreases again to ≈85% at a pH of 8.84 and ≈80% at a pH of 10.3.

Conclusions

The use of EGFP-SbpA-PEMMP as a pH sensor would be possible for pH ranges between ≈3.5 to 7 and if a sample system is calcified for positive pH changes in the high pH ranges the sensor is usable for the pH regime of ≈7 to ≈10. The application of particles coated with fluorophores labeled S-layer proteins or fusion proteins will be the further task.

Acknowledgments

The help of Batirtze Prats Mateu Institute for Physiks and Materials Science, Department of Physics and Process Engineering, University of Natural Resources and Life Sciences Vienna (BOKU) with the EGFP-SbpA coated polyelectrolyte multilayers is grateful acknowledged. Dr. Andreas Breitwieser is acknowledge for technical assistance.

Determining and Tuning the Viscoelastic Characteristics of Gelatin Nanoparticles for Drug Delivery

Agnes-Valencia Weiss[1], *Marc Schneider*[1], *José L. Toca-Herrera*
[1] *Institut of Biopharmaceutics and Pharmaceutical Technology, Saarland University, Germany.*

Aim/Objective

In the research of drug delivery systems nanoparticles are focused due to their advantages in drug targeting. To use their beneficial characteristics like size, uptake behavior or modifiable surface characteristics in therapy nanoparticles have to be composed by biodegradable and biocompatible polymers, such as gelatin or Polylactic-co-glycolid acid (PLGA). A large and growing field of drug molecules is based on macromolecular hydrophilic molecules. To efficiently incorporate and transport such molecules a new gelatin-based system was introduced. In recent years the elasticity was shown to have a high impact on the retention time in the blood and the cellular uptake, both important parameters for delivery of drugs. Even though conventional crosslinked gelatin nanoparticles are well studied nothing is known about their stiffness and its tunability as a key parameter for modern drug delivery systems.

The aim of this study was to design a setup to measure and evaluate the viscoelastic parameters of the gelatin nanoparticles developed in our lab. Therefore the commonly prepared, crosslinked gelatin nanoparticles with an average hydrodynamic diameter of around 200 nm are used as a model system. First of all we investigated different crosslinking reaction times which should lead to differently crosslinked and hence differently elastic particles.

Results

The viscoelasticity can be determined by force-distance-measurement with the atomic force microscope (AFM). In the contact time which was set to 10 seconds, either the force or the height is kept constant. The resulting creep and force relaxation curves can be fitted to the Zener model by a single exponential decay and the parameters: c_0, c_1 and x_1 or a_0, a_1 and tau_1 are extracted (Fig. 1). To check the linear relation of stress and strain we applied different forces between 0.5 and 2.5 nN. The read out were the time dependent parameters x_1 and tau_1 their consistency is shown and proves the linearity of the relation. The linear dependence between stress and strain was examined for particles with crosslinking times 3, 15 and 18 hours. Unfortunately we did not get any fitting curves for x_1 at the 3h crosslinked particles as the influence from outside overlaid the forces of this experiment.

For all the other values we could extract fitting parameters of at least 30 curves. For the following experiments a load of 1 nN was chosen, as this force is in the linear range, in addition the particle deformation is not more than 10% of the particle size. This allows to evaluate the obtained results by Zener's model.

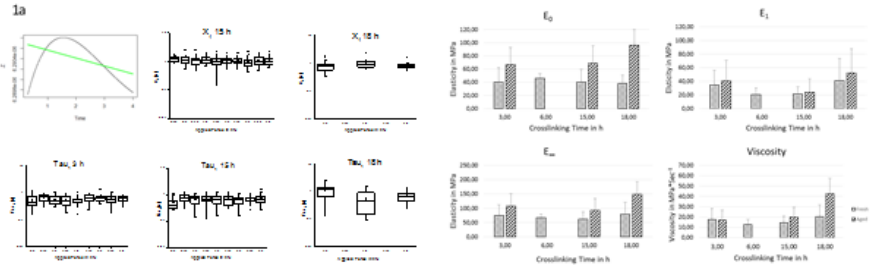

Figure 1: (a) Relaxation and creep characteristic times for the applied load which show linearity. (b) Elastic moduli E_0, E_1 and E_∞ ($\sum E_i$) and viscosity calculated from the extracted fitting parameters.

From the extracted parameters the elasticity is calculated. For the 6 hours incubated particles we did not get results for aged particles as we prepared them in the last week of the experiment run. For E_1 and E_∞ there is a slight increase visible in the elasticity from 6 to 18 hours crosslinking time (Fig 1b). The particles which are crosslinked for 3h only do not show this trend but the standard deviation is very high for these particles. Even there is a little increase in the elasticity it is clear that the aging of crosslinked gelatin nanoparticles after a storage time of approximately 4 weeks at 4° C has an influence on the stiffness which is much bigger. For E_0 there is no influence observed of the crosslinking time for the freshly produced particles. With the influence of the storage time this changes whereas the elasticity increases from 40 to 69 MPa for the 15h crosslinked particles the values for the particles with 18 h crosslinking are more than double as high after storage (38 to 96 MPa). The viscous characteristics show the same tendency as E_1 and E_∞. With a very high increase for the 18h crosslinked particles.

Conclusion and Outlook

From the performed experiments, we can conclude that the crosslinked gelatin nanoparticles show linear elasticity in the examined range. Furthermore, the crosslinking time of the particles seem only to have a small or no influence on the stiffness of the particles. In contrast, the aging of the particles showed a high influence to our particles and changed the stiffness clearly.

The obtained results have to be repeated as all the particles we examined in the measurement series are from one batch per crosslinking time. Afterwards we plan on a publication. It will be the first data on viscoelastic properties of hydrogel nanoparticles as before only die Young's modulus was. Furthermore a closer look into the hardening is of interest to understand what is happening during this process and if we reach a plateau after a certain time or not. In a next experiment series the examination of our newly developed formulation.

Acknowledgments

I want to thank Prof. Marc Schneider and the Institute of Biopharmacy and Pharmaceutical Technology for the financial support as well as the scientific input for this work. I want to thank Dr. Daniel Wastl, Dr. Jagoba Iturri and Alberto Moreno-Cencerrado for scientific and technical support. Acknowledgements go as well to Prof. Dr. Rafael Benitez for the introduction to R and the coding of the evaluation script. Last but not least I would like to thank the biophysics group for the warm welcoming atmosphere and my Saarbrücken colleagues for covering the daily work during my stay at BOKU.

Tuning of cell mechanics via substrate stiffness variation

Jagoba J. Iturri, Spela Zemlijc-Jokhadar[1], Amsatou Andorfer-Sarr, José L. Toca-Herrera

[1] *Department of Biophysics, Medicine Faculty, University of Ljubljana, Vrazov trg 2, 1000 Ljubljana, Slovenia*

Aim/Objective

Atomic Force Microscopy technique was employed to study cell mechanics on Human Umbilical Vessel Endothelial cells (HUVEC). Cells were seeded on elastomeric PDMS of different stiffness, by tuning of the pre-polymer/cross-linker ratio during their preparation. Variation on the mechanical properties of such films induce a different degree of cell spreading, affecting the formation of focal adhesions, which reflects on the final cell membrane mechanical response: (stress relaxation, creep, Young's modulus). In turn, as a result of such variation, activity of the cell upon different processes (i.e. particle absorption) might become highly influenced.

Results

Force Spectroscopy was applied on HUVEC cells cultured on PDMS-coated glass substrates. The AFM tip can be approached and placed in contact with a chosen cell following well-defined coordinates, which allows for obtaining the characteristic F-d curves of the indented location. Subsequent fitting of the plot by Hertz model equations permits to calculate the corresponding Young's modulus. Furthermore, application of different modes at the Dwell time offer the possibility to determine either the stress relaxation capability of the sample or its progressive deformation at a constant temperature, respectively.

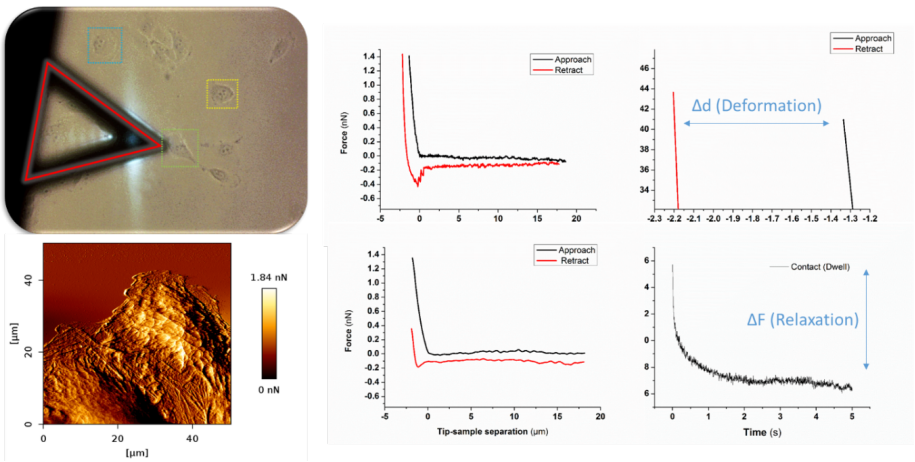

Figure 1: (Left) Optical microscopy image of HUVEC cells deposited on PDMS and below, cell topography by Deflection Error image obtained in contact mode. (Right) Examples of the different Force Spectroscopy modes employed: F-d plots and recording at the Dwell time in both Constant Force (above) and Constant Height (below) approaches.

Studying the antifouling properties of bacterial S-layer

Ana C. Vianna Cintra[1], *Jagoba J. Iturri, José L. Toca-Herrera*

[1] *IAESTE student, Dept of Chemistry, Universidade São Paulo, Ribeirão Preto, São Paulo, Brasil.*

Aim/Objective

The mimicking of (the physicochemical) properties bacterial surface layers (S-layers), the outermost cell envelope component of prokaryotic organisms, enables infinite possibilities for technological processes and scientific studies. S-layers are composed of single (glyco)protein units acting as building blocks, which reassemble into crystalline arrays when exposed to different types of supports (i.e. lipid films, polymers, silica). This assembly in a regular arrangement in the case of Lysinibacillus sphaericus (SbpA) is driven by the presence of divalent cations (Ca2+) in the crystallization buffer, which also contributes to its high stability. Among other features, such biomimetic films are characterized by their antifouling activity while forming the crystalline structure, which is subjected to analysis in this work.

Results

Studies focused on the recrystallization of S-layer from Lysinibacillus sphaericus (SbpA) on both hydrophobic and hydrophilic silicon surfaces. Subsequently, the formed crystalline films were exposed to different chemical treatments (metal chelator EDTA and/or pH variations) in order to disrupt the SbpA crystalline structure, without causing full protein removal. In a second step, the ability of the protein layer to bind different molecules (BSA, polyelectrolytes) was investigated. Quartz crystal microbalance with dissipation (QCMD) technique was used to monitor the real time variations of mass deposited per unit area along the crystallization, as well as the kinetics of the process. Complementary atomic force microscopy (AFM) measurements allowed for a detailed following of the topographical changes and mechanical properties of the structures formed.

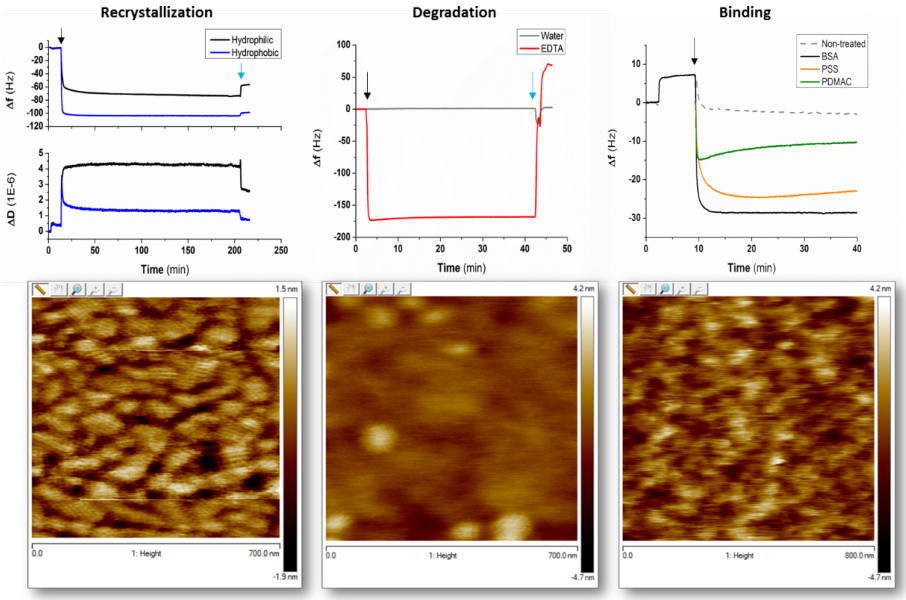

Figure 1: QCM real time monitoring (above) and AFM topography images (below) at the end of each step of the process labeled on top.

Kinetics and Structure of SbsB Bacterial Protein Crystal Formation

Michaela Zrelski[1], *Jagoba J. Iturri, José L. Toca-Herrera*

[1] *Dept. Microbiology, Immunobiology and Genetics, University of Vienna, Dr. Bohr-Gasse 9, 1030 Wien, Austria.*

Aim/Objective

In the study of different S-layer bacterial proteins, besides the widely extended SbpA from Lysinibacillus sphaericus bacterium, some other systems, such as the SbsB obtained from the bacterium Geobacillus stearothermophilus, offer an interesting alternative to study the recrystallization capability of these type of biomolecules.

Among other features, one could envisage a mixture of the proteins afore mentioned to form mixed layers and, by extension, to address the formation of new forms of crystal. This may lead to a full bunch of completely novel applications. In order to achieve this, the adsorption behaviour and kinetics of the individual systems has to be fully characterized.

Results

Recrystallization process of SbsB S-layer protein has been followed via Quartz Crystal Microbalance with Dissipation (QCM-D), which permit to calcu-

late the amount of adsorbed mass and the viscoelastic properties of the absorbed film upon variation of factors such as the protein concentration employed, the pH of the bulk solution or the substrate wettability. Additionally, the crystal structure formation has been followed in parallel by Atomic Force Microscopy (AFM).

According to the observations done, surface wettability and the choice of pH show to exert a critical influence on the protein adsorption. Overall, SbsB protein activity and crystal formation capability stays far below that of SbpA, which would definitely jeopardize the formation of a mixed crystalline layer.

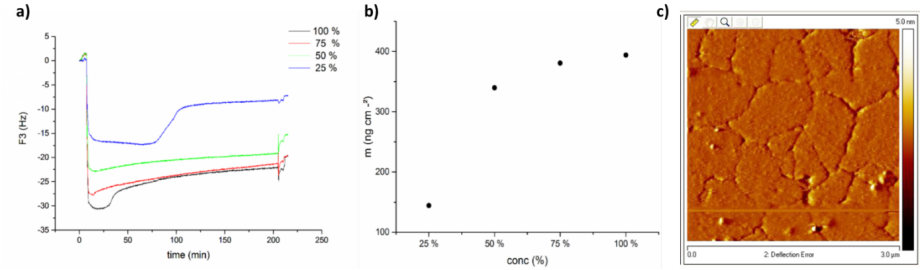

Figure 1: (a) Frequency vs time variation upon injection of SbsB bacterial protein in different concentrations. The frequency values at the end of the incubation process were converted to adsorbed mass by means of the Sauerbrey equation, as shown in (b). (c) AFM deflection error image of a formed SbsB film on SiO2.

Mechanical properties of fibrin hydrogels

Jagoba J. Iturri, Eline de Meyer[1], Michaela Purtscher[2], Carina Huber-Gries[2], Timothy Douglas[1], Andre Skirtach[1], José L. Toca-Herrera

[1] *Department of Molecular Biotechnology, University of Ghent, Ghent, Belgium.*

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

Aim/Objective

Fibrin is a fibrous protein with the capacity of forming 3D structures. Fibrin is naturally formed by the polymerization of fibrinogen plasma glycoprotein caused by platelet-attached thrombin, forming a protective mesh over the platelet which plays a key role in the formation of blood cloths. Mechanical properties of fibrin are undoubtedly essential for a proper performance of its functions (haemostasis, thrombus formation). Its use in the field of biomaterials in topics related to tissue regeneration has also focused the efforts of scientific community. In this work, Atomic Force Microscopy technique was employed to characterize the mechanical properties of fibrin hydrogels in varying contents of thrombin in a liquid environment. Thus, from the characteristic Force vs distance plots, calculation of factors such as the elastic modulus (E) or the adhesive force can be determined.

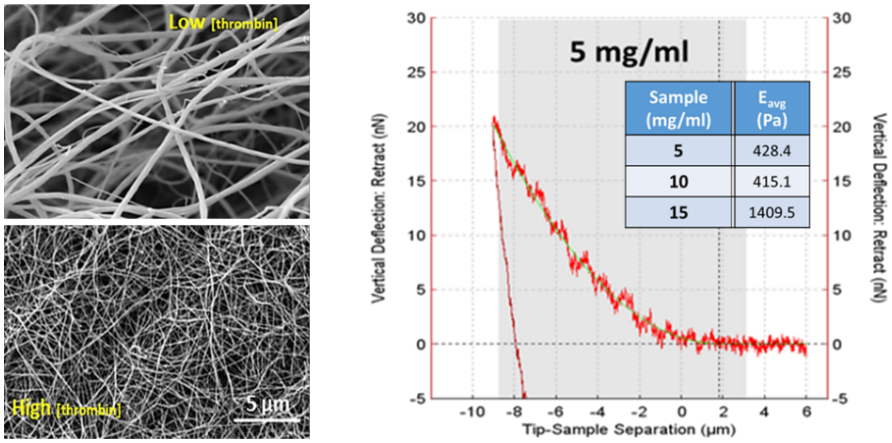

Figure 1: (Left) Electron microscopy images comparing the thrombin content-induced changes in the Fibrin structure. (Right) Characteristic force-distance plot and Hertz model fitting. The inlet table shows the average Young's modulus values for the Thrombin concentrations employed.

Results

Three different concentrations of thrombin were employed to synthesize the hydrogels under analysis. Variation of the Thrombin content does not only affect to the structural appearance of the fibers being formed (see picture below) but also impacts their mechanical response. First results speak for themselves

about a noticeable difference in terms of surface strengthening as there is more thrombin being employed in the mixture with fibrinogen during the hydrogel preparation process.

Atomic Force Microscopy characterization of biodegradable polymer grafts

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Aim/Objective

Polymer-based biodegradable materials have found an increasing number of medical applications in the recent yeas. In addition to their unique properties, these materials contribute with an on-demand adaptability which turns them into a very useful tool for the development of new devices.

In this work, Atomic Force Microscopy technique was employed to characterize the influence of degradation on both topographical and mechanical features of electro-spun biodegradable TPU polymer fiber-like structures, commonly used as catheters (around 60% degraded in 9 months). Samples were exposed to cultures of macrophages on top, for different periods of time, after which a cleaning of the surface takes place prior to their measurement.

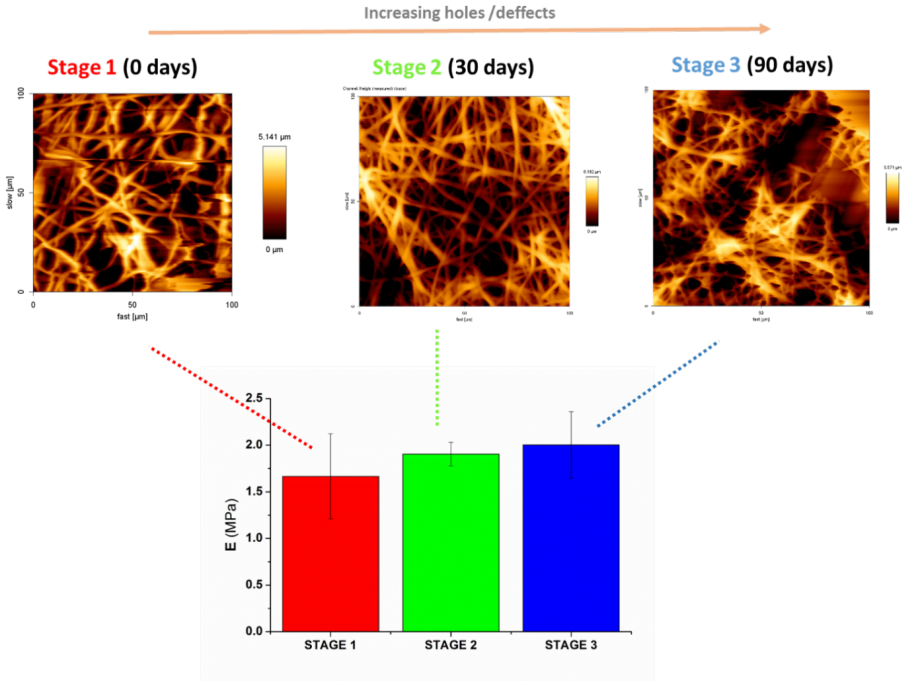

Figure 1: Degradation time dependent AFM height micrographs in the 3 stages under analysis: 0, 30 and 90 days. Below, the corresponding average Young's modulus calculated for each of the systems.

Results

A topographical visualization of TPU films could be first obtained in contact mode in both dry and wet states. Films were featured by an increasing number of defects as the exposure time to macrophages was prolonged in time (see figure). Furthermore, it could be easily observed the appearance of residues from the macrophage extra-cellular matrix. However, analysis of the mechanical properties by means of colloidal probes, brought almost no differences between the samples compared and therefore, the creation of defects in the structure might only affect to the material performance in a scales above the nano- or micro-meter range.

Polyelectrolyte capsules: about core dissolution influence on topography and mechanical properties.

Jagoba J. Iturri, Jacqueline Friedmann, Claudia König, , José L. Toca-Herrera.

Aim/Objective

Polyelectrolyte (PE) capsules prepared by layer-by-layer coating of micrometer sized CaCO3 or Melamine Formaldehyde (MF) particles, followed by the subsequent core dissolution, represent a very well-known model for fabrication of drug delivery devices. The success on the last step mainly depends on the capability of the core-degrading agents (EDTA or HCl, respectively) to diffuse through the multilayered structure. An incomplete removal of the core induces the formation of a broad variety of structures which differ not only in their appearance but also in their response upon indentation, which focuses the study of this work.

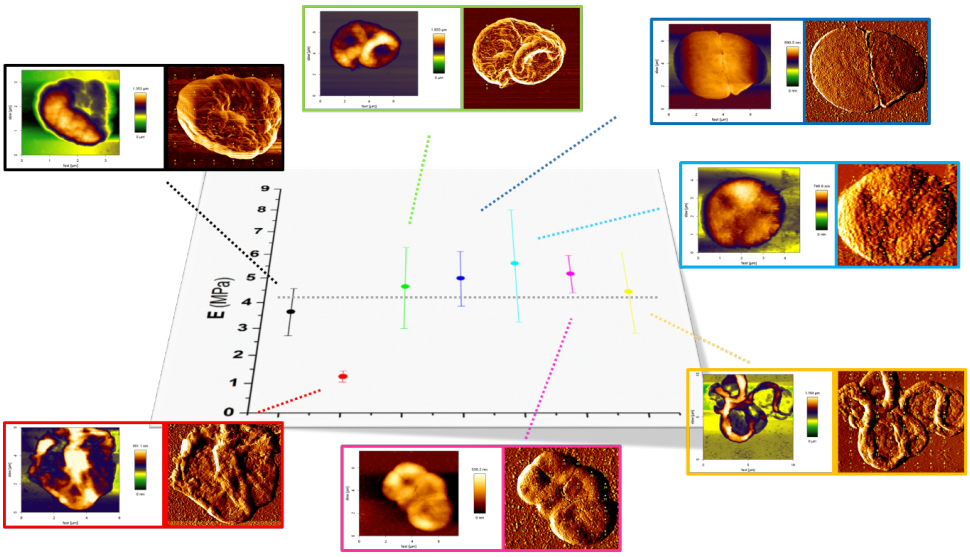

Figure 1: Full range of 3D structures obtained for PAH/PSS multilayered capsules and the corresponding elastic modulus calculated.

Results

Capsules composed of alternate layers of Poly(Allylamine Hydrochloride) (PAH) and Poly (Sodium StyreneSulfonate) (PSS), were attached to PE-coated glass substrates, to induce an electrostatically driven deposition in the presence of NaCl. Samples were then taken to the Atomic Force Microscope, on which topographical imaging and force spectroscopy measurements could be performed.

Effect of the Concentration of Cytolytic Protein Cyt2Aa2 on the Binding Mechanism on Lipid Bilayers Studied by QCM-D and AFM

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Aim/Objective

Bacillus thuringiensis is known by its insecticidal property. The insecticidal proteins are produced at different growth stage including the cytolytic protein (Cyt2Aa2), which is a bioinsecticide and an antimicrobial protein. The aim of this work was to investigate the interaction between Cyt2Aa2 proteins and cholesterol-containing surface-supported lipid bilayers (SLBs). Figure 1 shows formed results from the project.

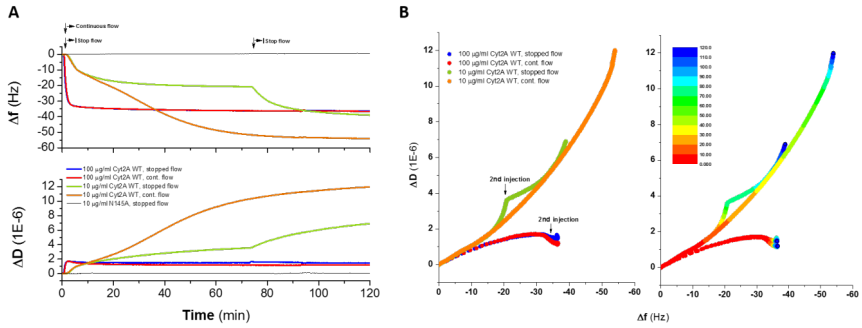

Figure 1: Concentration-dependent Cyt2Aa2 protein binding. (A) The protein solutions were exposed to the lipid bilayer in either stop-flow or continuous flow conditions, as shown on top of the image. Vertical arrows point out the injection and vertical lines indicate flow interruption events. The frequency curves of 100 μg/ml protein concentration overlap to each other (in both flow and stop-flow conditions). (B) $\Delta D/\Delta f$ plot (left) of the binding processes shown in (A). Axis (0,0) position corresponds to the first injection while the second protein injection is indicated. (Right) The time evolution of the experiment is indicated by the color bar (0-120 minutes).

Results

We found that the binding mechanism is concentration dependent: whereas at 10 μg/ml, Cyt2Aa2 binds slowly on the lipid bilayer forming a compliance protein/lipid layer with aggregates, at higher protein concentrations (100 μg/ml), the binding is fast, and the protein/lipid layer is more rigid including holes (of about a lipid bilayer thickness) in its structure. Our study suggests that the protein/lipid bilayer binding mechanism seems to be carpet-like at low protein concentrations and pore forming-like at high protein concentrations.

QCM-D measurements show the high dependence of Cyt2Aa2/lipid bilayer binding mechanism on the Cyt2Aa2 protein concentration employed. Furthermore, the protein concentration selected will also impact the viscoelastic properties of the adsorbed protein film. At high protein concentration, Cyt2Aa2 binds quickly (in approx. 8 min) onto the SLB and forms a rigid protein-lipid complex. On the contrary, when the bilayer is exposed to the lowest protein concentration, the full binding process is up to 10-fold slower and the protein-lipid interaction results into a more viscoelastic structure, as indicated by the higher dissipation values observed.

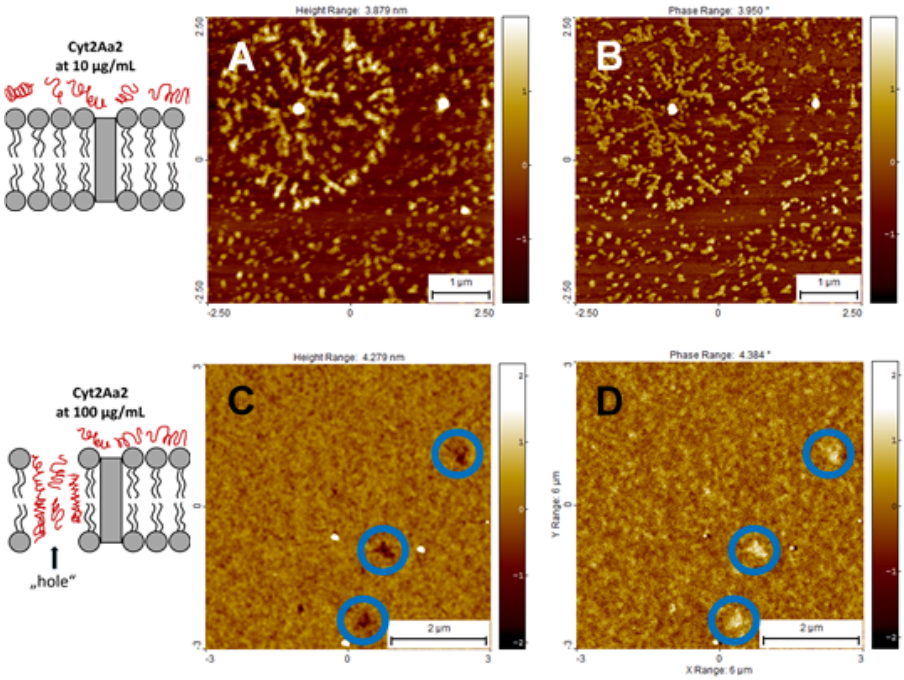

Figure 2: (A, C) Topography and (B, D) Phase micrographs of a 10 μg/ml and 100 μg/ml Cyt2Aa2 protein films, respectively, measured in tapping mode in the presence of PBS buffer (pH 7.4). Emphasized by blue circles it is shown the formed pores, at both modus: height and phase imaging.

The influence of the toxin concentration on the final structure of the protein-lipid layer is shown in Figure 2. The figure depicts height (A and C) and phase images (B and D) after 1 hour exposing the lipid bilayer to either 10 μg/ml (A and B) or 100 μg/ml (C and D) of Cyt2Aa2 protein. Height images provide information about the different structures whereas phase images detect variations in viscoelasticity, composition, adhesion or friction. Both type of images show two different behaviours between the two different lipid/protein layers (at 10 and 100 μg/mL): softer aggregates of similar size were found at low concentrations; a crystal lattice-like layer formed in the first minutes at high concentration of Cyt2Aa2 protein (with a random distribution of inserted holes).

Conclusions

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

Acknowledgments

The authors thank Jacqueline Friedmann for artistic and technical support. This work was supported by the Thailand Research Fund (TRF) through the Royal Golden Jubilee Ph.D. Program (RGJ) (Grant No. PHD/0116/2551), and the National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Thailand (NSTDA). Financial support from RGJ to S.T. and B.P. is gratefully acknowledged. This work was partially supported by the International Graduate School BioNanoTech (IGS) Program of the Federal Ministry for Science and Research, Austria.

Influencing bacterial S-layer protein recrystallization on polymer brushes through surface charge and accessible volume: a combined AFM and QCMD analysis

Alberto Moreno-Cencerrado, Jagoba Iturri, Dietmar Pum, Uwe Sleytr and José L. Toca-Herrera

Aim/Objective

S-layer recrystallization is part of, at least, two major scientific and technological challenges: a model to study 2-D polymer crystal formation and a bottom-up approach to build biomimetic and functional interfaces. In this work, we are taking advantage of the versatility of polymer brushes to study the self assembly mechanism of the S-protein SbpA. In particular, the recrystallization pathway of the protein has been modified by the underlying polymer charge and accessible volume offered by the positively charged poly{[2-(methacryloyloxy)ethyl] trimethylammonium chloride} (PMETAC), the negative poly(sulfo propyl methacrylate) (PSPM) and the non-charged poly(N -isopropyl acrylamide) (PNIPAAm).

Conclusions

The analysis by complementary AFM and QCMD measurements on the SbpA interaction with different types of polymer films has proofed the critical influence of the substrate charge and volume features in both the protein film formation and crystallization capability.

Acknowledgments

The authors thank Jacqueline Friedmann for technical support. Part of this work was supported by the International Graduate School BioNanoTech (IGS) Program of the Federal Ministry for Science and Research, Austria, and the Air Force Office of Scientific Research (AFOSR) (Grant FA9550-12-1-0274).

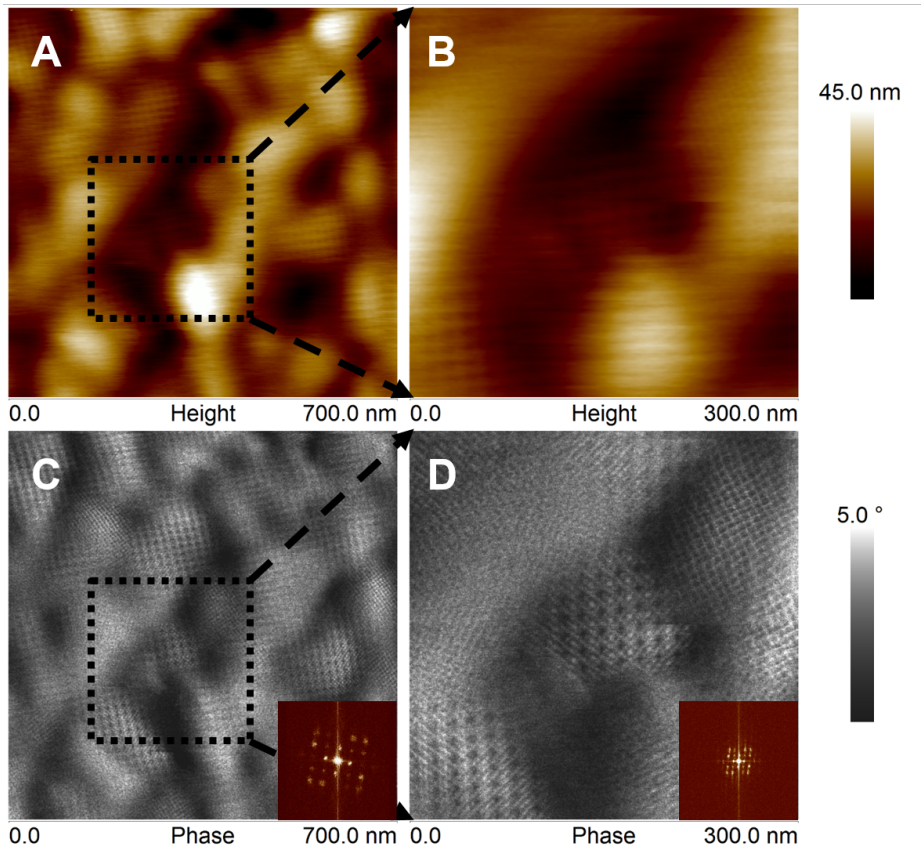

Figure 1: AFM height (A, B) and phase (C, D) micrographs with Fourier spectra images for SbpA crystal film. Images B and D were obtained from the selected regions highlighted by a dashed square.

Influence of surface wettability in the S-layer recrystallization at different protein concentrations

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Aim/Objective

The aim of this work was to clarify the role of the surface wettability on the protein adsorption from the bulk solution to the surface, as well as the subsequently diffusion and recrystallization onto the substrate. Different protein concentrations were employed in order to control the kinetics of the process.

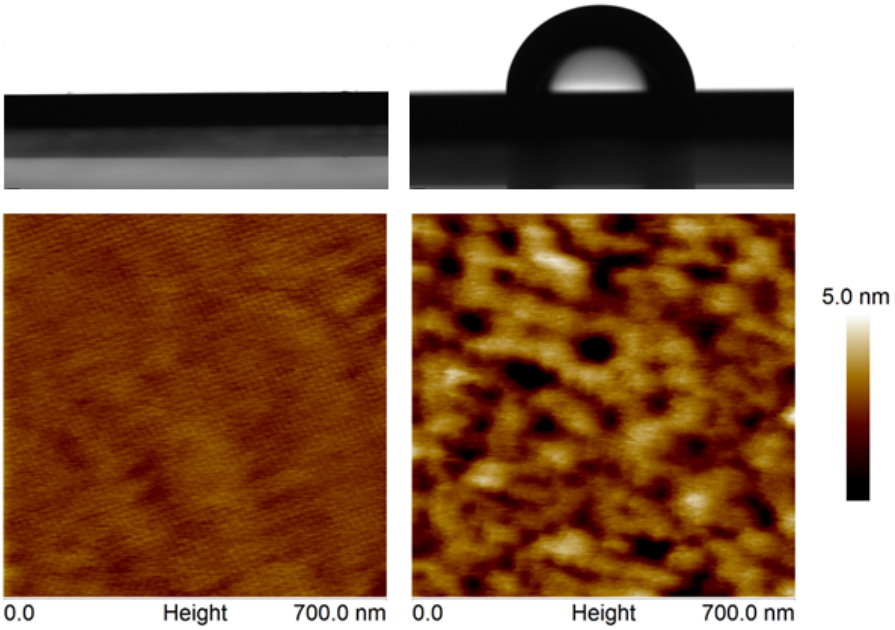

Figure 1: Contact-angle measurements of hydrophilic (A) and hydrophobic (B) SiO2 surfaces. AFM micrographs after protein recrystallization on both cases (C and D, respectively) illustrate the differences of each behaviour.

Results

The measurements were carried out by means of Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) and Atomic Force Microscopy (AFM). It could be observed that in the case of hydrophobic substrates the adsorption rate after protein injection is very high in comparison to hydrophilic

one as almost all of the mass is being bound to the surface in 5 minutes. In general, dissipation-frequency (DF) curves from QCM-D show the dependence between the particular stages of protein crystal formation and mechanical properties of the formed S-layer.

Figure 2 illustrates the basis of the behaviour on both cases, hydrophobic and hydrophilic, for the adsorption/diffusion/recrystallization phenomena. Whereas DF plots related to hydrophobic surfaces show a maximum peak at the beginning of the crystal formation process, hydrophilic silicon dioxide substrates follow a different trend: there is a gradual increase of mass adsorbed on the surface of the substrate. The layer recrystallized on hydrophilic support dissipates the most energy which indicates that it is becoming more and more viscoelastic within the time. It can also be assumed that in this case a formation of much softer layer takes place in comparison to the hydrophobic substrate.

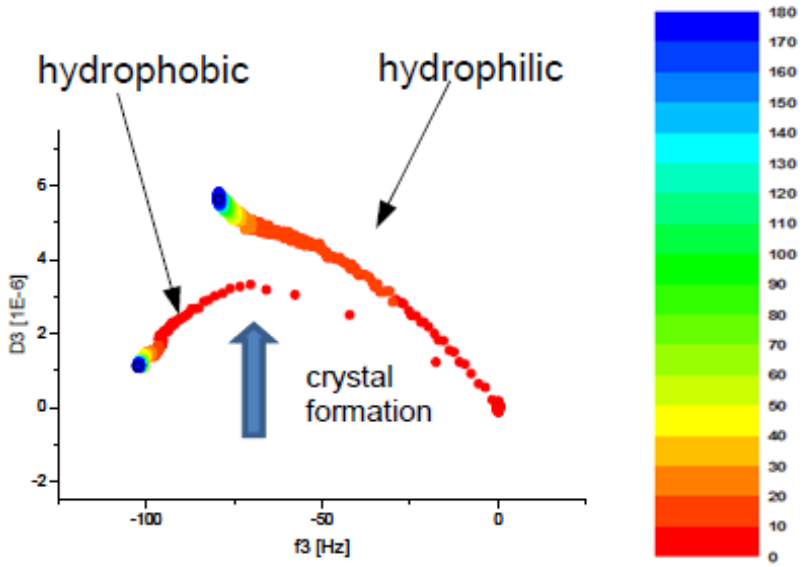

Figure 2: Dissipation of energy as a function of frequency (DF plots) corresponding to hydrophobic and hydrophilic silicon dioxide substrates.

Above all, the surface wettability influences in an obvious way the protein crystal formation. The adsorption process occurs quicker on hydrophobic than on hydrophilic surface. AFM images show that there is also a visible difference in percentage of the surface coverage and in size of crystalline domains obtained in both cases. In addition, experiments with relatively high and low protein concentrations were performed on surfaces with different hydrophobicity and the results will help in the understanding of the protein-protein and protein-surface interactions.

Acknowledgments

Part of this work was supported by the International Graduate School Bio-NanoTech (IGS) Program of the Federal Ministry for Science and Research, Austria, and the Air Force Office of Scientific Research (AFOSR) (Grant FA9550-12-1-0274).

Waveguide-Enhanced Diffraction for Observation of Responsive Hydrogel Nanostructures

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[1] *AIT-Austrian Institute of Technology, BioSensor Technologies, Vienna, Austria.*

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Aim/Objective

UV-nanoimprint lithography was employed for the preparation of arrays of nanopillars from photo-cross-linkable N-isopropylacrylamide - based hydrogel (pNIPAAm). The objective of this work was to study in situ and at real-time the thermo-response of these hydrogels in liquid environment through different temperatures. The measurements were carried out by atomic force microscopy (AFM) and subsequently the results were used to clarify the capability of these systems as candidates for optical nano-waveguides of diffraction.

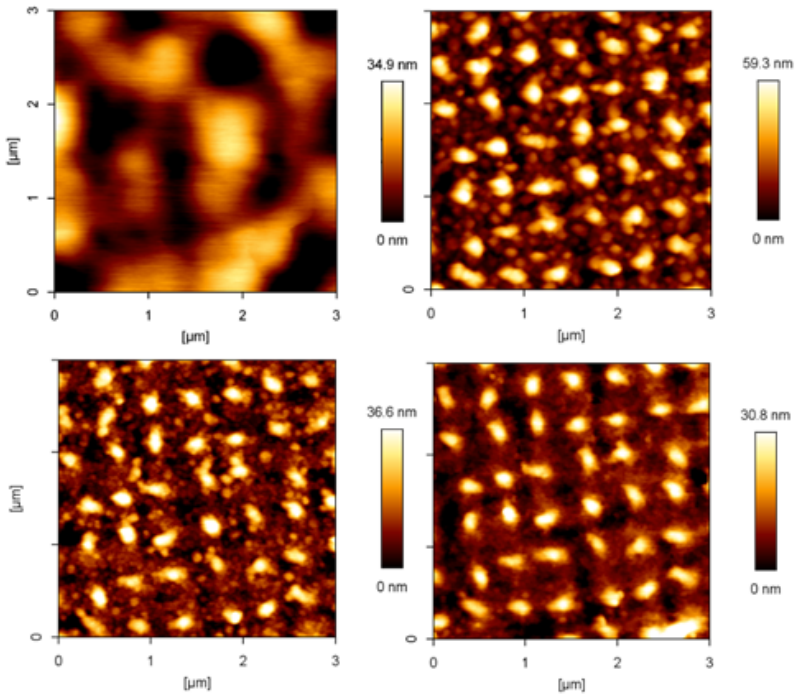

Figure 1: In situ AFM observation of swelling of pNIPAAm hydrogel nanopillars in water at different temperatures.

Acknowledgments

The authors would like to acknowledge the partial support for this work provided by Austrian Science Fund (FWF) through the project ACTIPLAS (P 244920-N20). AMC acknowledges the International Graduate School BioNanoTech (IGS) for financial support (PhD Grant). Program of the Federal Ministry for Science and Research, Austria, and by the Italian FIRB 2011 NEWTON (RBAP11BYNP).

Patterning of silicon wafers with S-layer proteins

Andreas Breitwieser, Uwe B. Sleytr, Jose Luis Toca-Herrera, Dietmar Pum.

Aim/Objective

The patterning of biomolecules at micro and nano scale on solid substrates is relevant to many areas of science and technology, such as bio-sensing devices, diagnostic immunoassays, cell culturing, DNA microarrays and the development of high-throughput screening. S(urface)-layer proteins reveal the ability to self assemble into two dimensional crystalline arrays in suspension, at the air water interface, on lipid films and also on solid supports. In combination with the repetitive surface properties down to the nanometer range presenting function groups in a highly ordered way or genetically introduced biologically active protein moieties in the case of fusion proteins, S-layer proteins are an interesting tool for patterning and functionalization of surfaces. Some routinely used patterning methods cannot be exploited for S-layer proteins as e.g. direct patterning of proteins using stamps interfere with the desired formation of a crystalline protein monolayer. Here, in this study an alternative method was investigated where first patterning was achieved with blocking agents followed by a recrystallization step with the S-layer protein onto the non blocked regions resulting in an inverse patterning of the S-layer protein in a crystalline state.

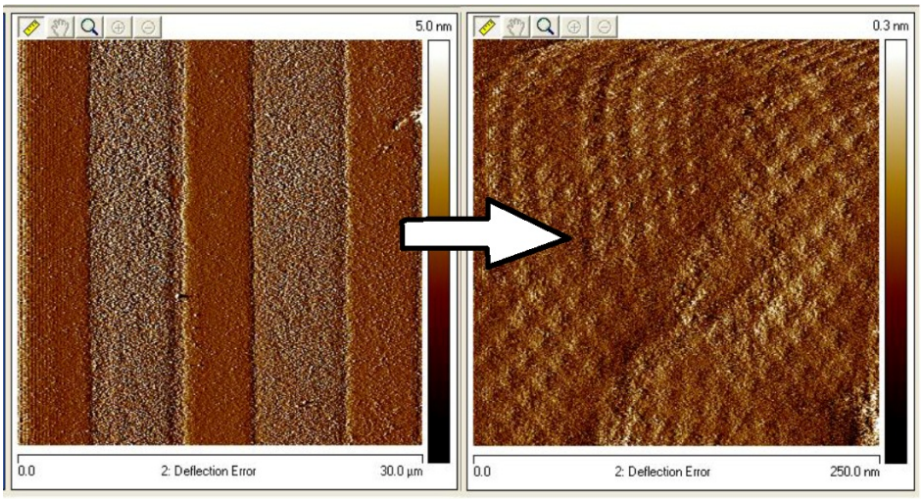

Figure 1: AFM confirms the patterning success and the formation of the S-layer crystalline structure within distinct regions following the pattern of the PDMS stamp.

Results

StabilGuard and StabilBlot (Surmodics) were incubated on the surface of micro patterned PDMS stamps and after drying the blocking agent was transferred onto silicon wafers. Subsequently, S-layer (wtSbpA) recrystallization was

performed (30 min) and after washing away the surplus of S-layer lattice formation was allowed to take place by an incubation in buffer containing CaCl2 over night. The formation of the crystalline monolayer and the overall patterning success could be visualized with atomic force microscopy = AFM (Fig. 1).

To investigate more diverse geometries, again blotting reagents were transferred onto glass slides using the stamping technology. Subsequently rhodamine labeled S-layer protein was allowed to recrystallize over night as described above. The success of patterning could be confirmed with fluorescence microscopy (Fig. 2).

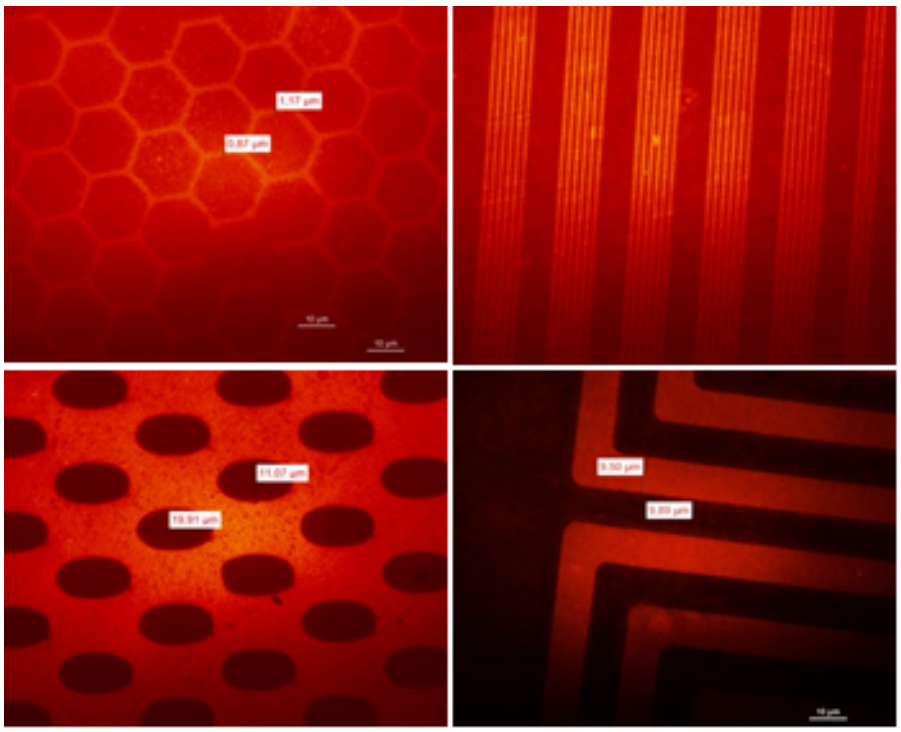

Figure 2: Fluorescence microscopy could confirm the patterning of glass surfaces. Patterning was achieved by transferring blocking agents with the stamping technology followed by a recrystallization of the rhodamine labeled S-layer protein on the non blocked regions of glass slides.

Conclusions

So far approaches to obtain patterned surfaces in combination with S-layer proteins were achieved by etching or exploiting the soft lithography technique, micro-molding in capillaries (MIMIC), as a non-lithographic patterning tool. These techniques have some limitations especially to obtain a patterning over larger areas. The here described method overcomes these limitations by transferring the blocking agents using micro patterned PDMS stamps onto surfaces

followed by incubation of S-Layer protein over the entire surface. As the S-layer proteins do not bind to the blocked regions the crystalline monolayer is only achieved on the non-blocked areas.

With AFM the square lattice of the wtSbpA S-layer protein could be easily seen confirming the success of patterning and the crystalline state of the S-layer protein on silicon wafers. Using the identical technique on glass slides in combination with the usage of rhodamine labelled S-layer allowed the visualization of more complicated patterned geometries using fluorescence microscopy.

First results demonstrated that the new approach to structure surfaces resulted in a patterned crystalline monolayer of S-layer proteins. As blocking agents can be easily handled the indirect pattering method opens new approaches e.g. micro contact printing; ink jet printing, stamping or MIMIC to achieve functionalized, patterned surfaces.

Acknowledgments

Jagoba Itturi did the production of the PDMS stams.

New insights of the crystallization properties of wtSbpA

Andreas Breitwieser, Uwe B. Sleytr, Jose Luis Toca-Herrera, Dietmar Pum.

Aim/Objective

Assembly of S-layer protein wt SbpA from Lysinibacillus sphaericus on mica does not follow the classical pathway of crystal growth. In the non-classical pathway two routes lead to the final ordered low-energy state after adsorption of the S-layer protein resulting in a closed crystalline monolayer with p4 lattice symmetry. While in one pathway early crystal clusters are formed leading directly to the final state, the other possesses a kinetic trap associated with conformational differences between a long-lived transient state and the final stable state (Seong-Ho Shina, et al., PNAS, 2012).

Standard recrystallization of wt SbpA is routinely performed by applying a 100μg /ml protein solution in recrystallization buffer (5mM Tris, 10 mM CaCl2, pH = 9.0) onto various solid supports over night. The basic idea of this study was that after adsorption of the S-layer protein onto the solid support enough S-layer protein should be available on the surface to form a closed crystalline monolayer. As adsorption of proteins is known to be extremely fast the surplus of S-layer can be removed after the short incubation step and crystal formation can be allowed to take place by further incubation in the presence of CaCl2 without any additional S-layer protein.

Results

Even small volumes (1, 2 μl) and / or short incubation time of the S-layer protein solution (5min) are sufficient for the generation of a closed crystalline monolayer if the adsorption/crystallization step is followed by an incubation step with crystallization buffer. In all of these approaches the S-layer protein lattice with p4 symmetry could be easily visualized with AFM confirming the crystalline state of the monolayer. If only Tris buffer without CaCl2 was used for incubation of the S-layer or in the buffer incubation step after the adsorption no lattice or no closed crystalline monolayer could be detected.

The S-layer protein solution could even be sprayed onto solid supports and if drying was avoided the S-layer protein lattice could be visualized after further incubation in the presence of Ca++ ions. First results of ongoing experiments with QCMD and contact angle measurements confirm these findings. With QCMD the characteristic graphs for crystal formation could only be seen in the presence of CaCl2 in the buffers and the contact angle and therefore the hydrophobicity increase if a crystalline layer is formed compared to only adsorbed wtSbpA protein.

Conclusions

The crystallization of S-layer protein wtSbpA could be separated into 2

steps, adsorption and lattice formation confirming that S-layer recrystallization follows the non-classical pathway. After 5 minutes enough S-layer wt SbpA protein is adsorbed to Si wafers to allow the formation of a closed monolayer if in a second step lattice formation is allowed to take place in the presence of CaCl2. This 2 step incubation set up opens new possibilities for functionalization of solid supports with S-layer proteins as small volumes and / or decreased incubation times are sufficient for the coating process. S-layer protein solutions can be sprayed or might even be used in contact printing if after the adsorption process the surfaces are transferred to buffer solution containing CaCl2.

Automated detection of unfolding events in atomic force microscopy force curves

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[1] *Department of teaching music, arts and body expression. University of Valencia. Spain.*

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[3] *Univ. of Valencia, Dept. of Physical Education and Sport, Spain*

Aim/Objective

The first objective is to obtain an automated detection system of unfolding events in atomic force microscopy force curves. The second objective is to analyze the effect of the electrolyte charge on elastin structure.

Results

The automated detection system obtained a sensibility of 79 % and a positive prognostic value of 92.4 %. The distribution of the variables obtained from the force curves (i.e., contour length and force of each event) did not show a change between a manual detection and automated detection (figure 1). Moreover, electrolyte charge had an effect in the contour length ($U2 = 215.32$; $p < 0.001$). Concretely, in NaCl the contour length was higher than using CaCl2 and Milli-Q ($p < 0.05$). Nevertheless, it was not found and effect of electrolyte charge in the unfolding force (figure 2).

Conclusions

The automated detection system of unfolding showed a good performance and the analysis of force curves using this method showed similar results than that obtained using a manual detection system. Moreover, the electrolyte charge has an influence in the elastin structure.

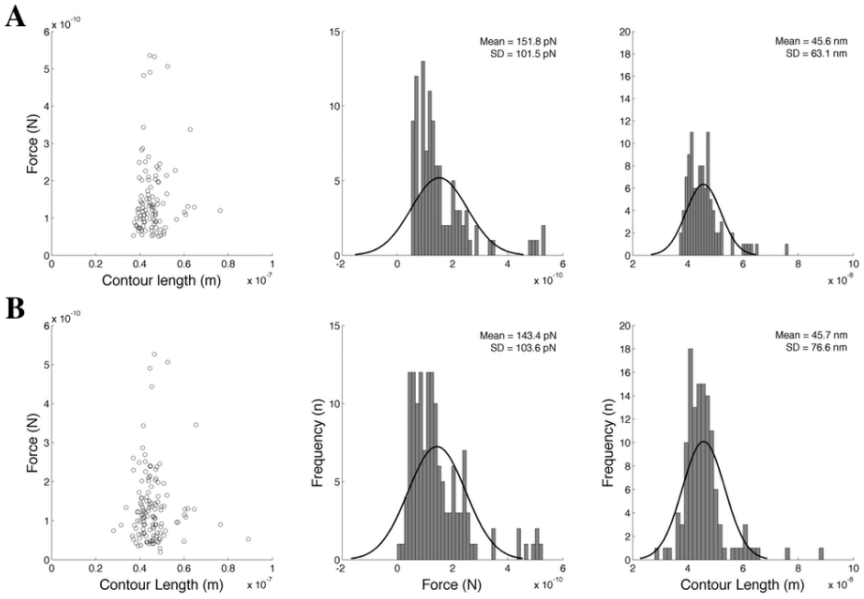

Figure 1: Force and contour length distribution using manual and automatic unfolding detection. A. From left to right appear the Force-Contour length plot, force histogram and contour length histogram of automatic unfolding detection. B. From left to right appear the Force-Contour length plot, force histogram and contour length histogram of manual unfolding detection.

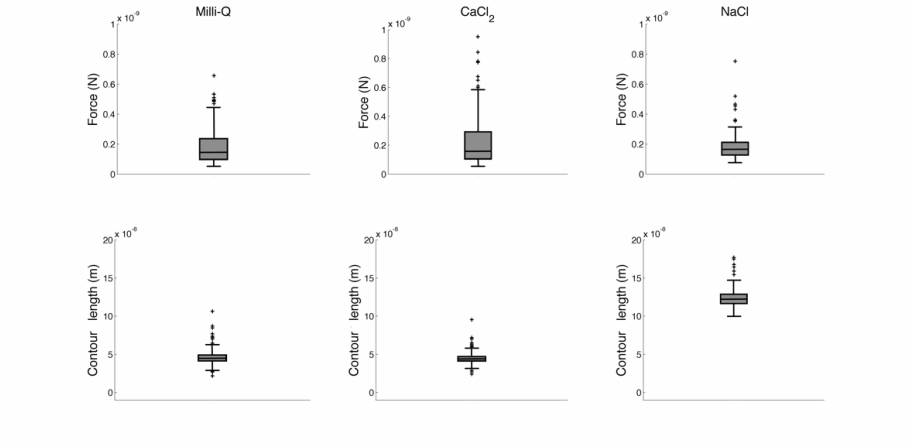

Figure 2: Boxplot of force and contour length variables of elastin force curves using Milli-Q, CaCl2 and NaCl as electrolyte.

On the interaction between resveratrol and carrier proteins

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

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

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Aim/Objective

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

Results and Conclusions

Based on our results, resveratrol interacts with three milk proteins (β-casein, BSA and β-lactoglobulin) mainly via hydrophobic interactions with a high affinity of the bioactive for the fluorescent residue. However, BLG has the highest accessible fluorophore of 82%. Moreover, the occurrence of quenching phenomena reveals that the binding site is located nearby the tryptophan residue. Although the interaction between resveratrol and β-lactoglobulin has no major effect on the secondary structure of the protein, it causes partial disruption of tertiary structure by the slight opening of hydrophobic cavity. Although BCN and BSA have lower accessible fluorophore, they both have a high value of binding constant. Accordingly, BLG could be the best carrier candidate for resveratrol among these proteins.

Acknowlegdments

The authors thank Claudia König for assistance with the TEM measurements. Elisa Rocchi acknowledges the Erasmus program of the EU. Denise Bender acknowledges CONACYT from the Government of Mexico. This project was partially financed by the IGS BioNanoTech graduate school (Bundesministerium für Bildung und Forschung - BMBF, Austria). The authors thank the COST Action "Food Structure Design" (COST FA1001) for financial support.

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

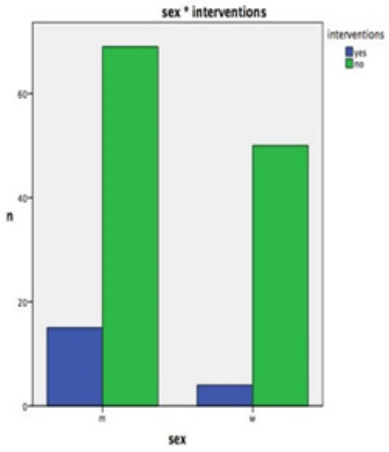
M. Handler[1], *M. Moser*[2], *J. L. Toca-Herrera*, *M. Burtscher*[1]

[1] *Institute of Sport Science - Medical Section, University of Innsbruck, Innsbruck, Austria.*

[2] *Austrian Judo Association, Vienna - Austria.*

Introduction

The first Austrian national championships were held in 1947. After some changes in the tournament organization the fighters were since 1967 divided in different weight categories for women and men. Since then, there has not been systematic scientific literature concerning technical, tactical, physical preparation and health aspects of the participants, as well as trauma statistics of the tournament.



sex * interventions cross table 2x2

Anzahl

		interventions		
		yes	no	TOTAL
sex	m	15	69	84
	w	4	50	54
TOTAL		19	119	138

Chi-square by Pearson for interventions

	value	df	asymptotic significance (2-sided)
Chi-square by Pearson	3,023	1	,082

Material and Methods

The analysis of an audio-visual record of all medal fights of the weight categories organized by cup system during the Austrian national championships in 2014 and 2015 will be the center point of the research process. The judo fight caused injuries will be documented by a specially designed trauma protocol.

Results

All injuries were not serious enough for a hospital treatment. The medical doctor was treating all injured fighters less than 10 minutes pro intervention, which means that competition level no severe injuries happen due to the good physical preparation of the competitors (see figure). The rate of medical intervention was 15/84 by men and 4/54 by women. From this data we can say that in medal fights the percentage of injuries of men was about 18% and much

lower for women (7%), although one fighter was disqualified. This result is significant by p value <0,01.

Quantification of S-layer/secondary cell wall polymer interactions

Rafael Benítez[1], *Vicente J. Bolós*[2], *Uwe B. Sleytr, Dietmar Pum, José L. Toca-Herrera*

[1] *University Center of Plasencia, Dept. of Mathematics, University of Extremadura, Plasencia (Spain).*

[2] *Faculty of Economics, Dept. of Business Mathematics, University of Valencia, Valencia (Spain).*

Aim/Objective

In this work, we have recrystallized the bacterial protein SbpA on silicon dioxide and modified Atomic Force Microscopy-tips with SCWP. Furthermore, we have measured the forces between the functionalized AFM-tips and the S-layer proteins as a function of the loading rate in order to determine the different interaction regimes. Due to the complexity of the experimental results, we present a mathematical procedure that is able to extract the information contained in the AFM force-distance curves.

Methods

Methodology: All experiments were carried out in MilliQ water using the Force Probe from Asylum Research. Silicon nitride (Si_3N_4) cantilevers of nominal 0.06 N/m were utilized. Adhesion, unbinding and unfolding events were measure as a function of the loading rate (250, 500, 1000 and 1500 nm/s).*(see Fig. 1)*

Automatic data analysis: The automatic counting of force peaks, adhesion energies and unfolding events was carried out with R. From the Force-distance curve, another signal Delta which describes the variation in the slope of the original signal, was obtained. Peaks on Delta are related to jumps in the Force-Distance curve. (Fig. 2)

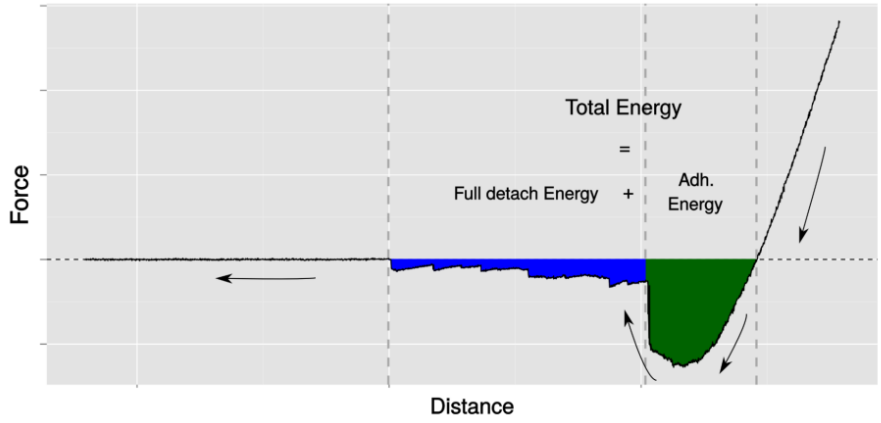


Figure 1: Representative F-D curve and the corresponding evaluated quantities.

Results

Two different regimes were found: for high speed rates (1000 and 1500 nm/s), adhesion energies are very similar and much larger than for 250 nm/s. At 500 nm/s the adhesion energy has the form of a bimodal distribution which indicates a transitional speed: the system react either showing small energy values (low pulling speeds) or larger ones (faster pulling speeds) (See Fig. 3).

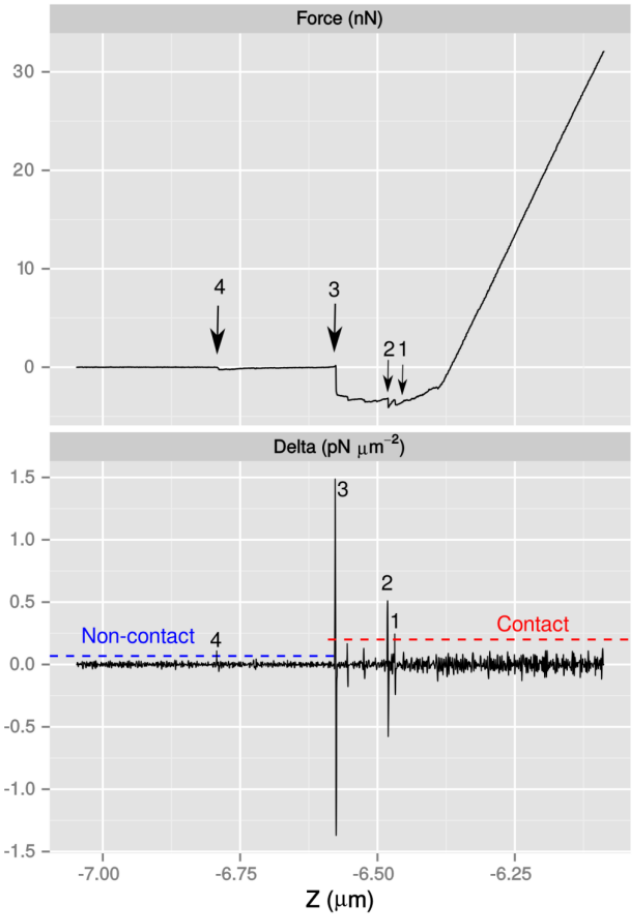


Figure 2: Typical AFM Force-distance curve (top) with the characteristic events to be determined. Delta signal (bottom) obtained from the signal, reflecting the events. Two thresholds were set, since two different noises were found: one for the part of the curve in which the tip was in contact with the sample (red) and another for the non-contact regime (blue).

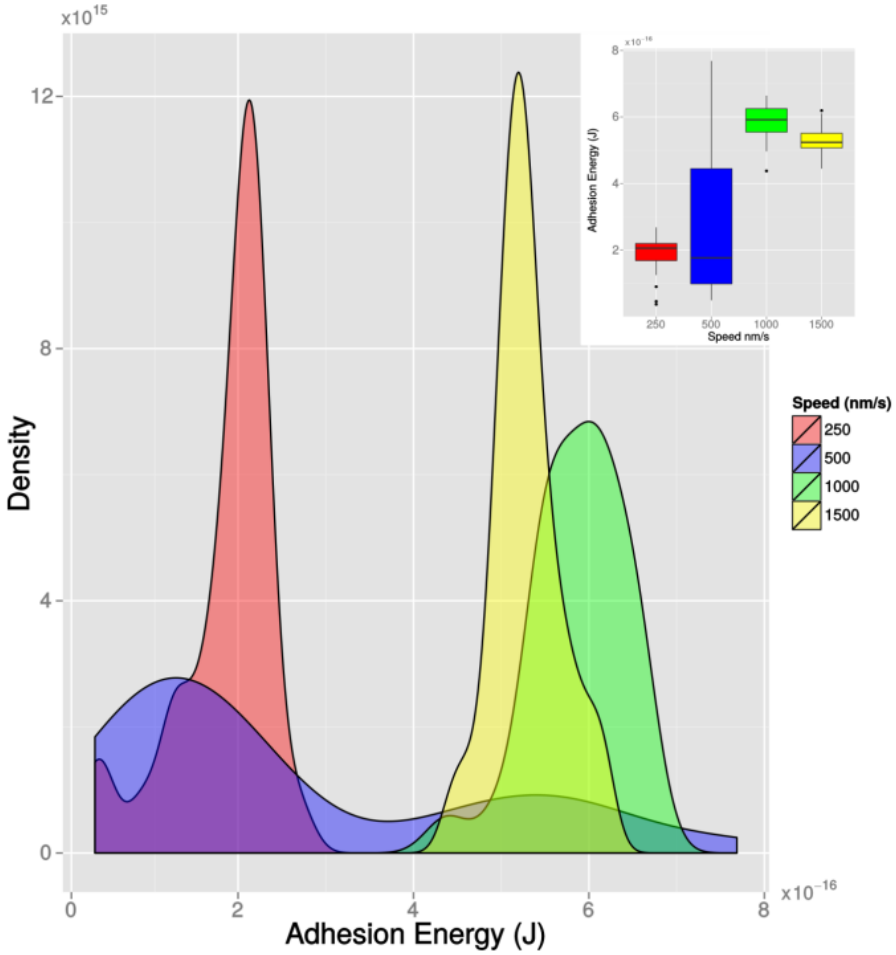


Figure 3: Kernel density functions for the histograms of the adhesion energies for the different approaching rates. The inset shows the box-plot for the same data. Note that high speed rates have similar energy distributions, while (250 nm/s) delivers the lowest adhesion energies and the transition speed (500 nm/s) shows a wider range of energies.

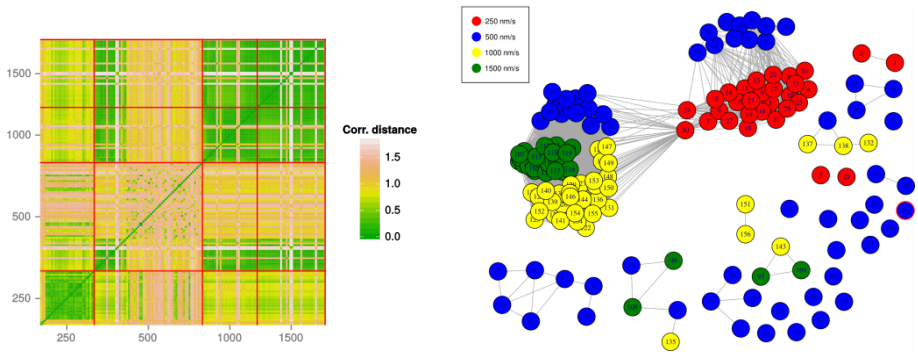


Figure 4: Left: correlation distance matrix: for the whole set of f-d curves, grouped by approaching rates (250, 500, 1000 and 1500 nm/s), the Pearson correlation coefficient is computed for each pair of curves (from the zero force point to the final detach of the tip). From the correlation coefficients rij, the correlation distance, defined by dij=(2(1-rij))1/2 is computed. The resultant matrix is plotted as an image. Low values of the correlation distance (green) can be interpreted as f-d curves with similar shapes. Thus, two groups are clearly distinguished: low speed rates (250 nm/s) and high speed rates (1000 and 1500 nm/s). For the middle speed (500 nm/s), a subgroup with similar patterns than the high speed rate curves is found. Right: graph defined from the correlation matrix. Each node represents an F-d curve and an edge is placed between each pair of curves whose correlation distance is smaller than 0.5. Two distinct groups are found (high and low speed rates) and it is clear how the 500 nm/s speed group is present in both clusters.

Acknowlegdments

The authors acknowledge the IGS BioNanotech program of the Austrian Government (BMBF).

Microscale probabilistic model for protein crystal growth

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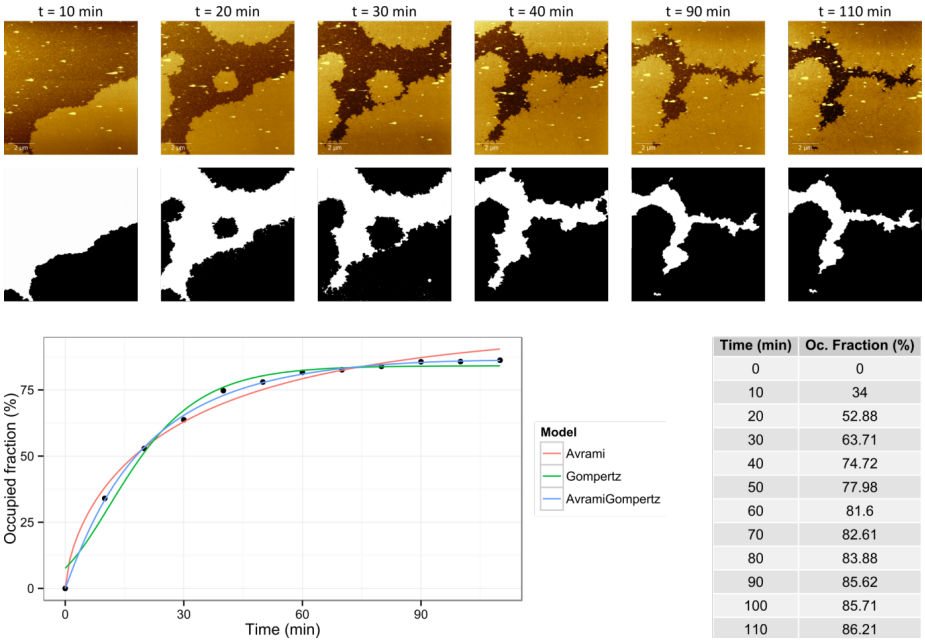
[2] *Faculty of Economics, Dept. of Business Mathematics, University of Valencia, Valencia (Spain).*

[3] *Biosurfaces Unit, CIC biomaGUNE, Donostia/San Sebastián (Spain).*

Aim/Objective

This work is devoted to the definition of an empiric mathematical model which simulates the large scale growth of a protein crystal. The crystal is formed by the deposition of PSba proteins onto a substrate in such manner that only free substrate space can be occupied by the protein, and thus no vertical growth of the crystal takes place and a pure 2D model suffices to describe the general behaviour of the crystallization process.



Time (min)	Oc. Fraction (%)
0	0
10	34
20	52.88
30	63.71
40	74.72
50	77.98
60	81.6
70	82.61
80	83.88
90	85.62
100	85.71
110	86.21

Figure 1: Top row: sequence of images of the protein crystal growth at different time stamps. Middle row: black and white 8-bits images obtained from the original ones. Bottom right: Occupied surface fraction (in %) estimated from the b&w images. Bottom left: comparison of three growth models (Avrami, Gompertz and the Avrami-Gompertz model).

Material and Methods

From images of the crystal growth at different time stamps (Fig. 1, top row), the total surface area occupied by the crystal was estimated by transforming the images into bw 8-bits images and using a self-developed programming code in MATLAB®(Fig. 1, middle row). A modified Avrami growth model

(Avrami-Gompertz model) was fitted to the growth data in order to obtain the kinetic properties of the crystal growth (Fig.1, bottom plot).

Probabilistic Model

We assume a discrete-time / discrete-space evolution model focused on the substrate. The substrate is considered a square grid of fixed cell width. At each time k, the state of the crystal is defined by a binary state-matrix, $M_{ij}^{(k)}$, which will take the value 1 if the cell i-j is occupied and will take the value 0 otherwise.

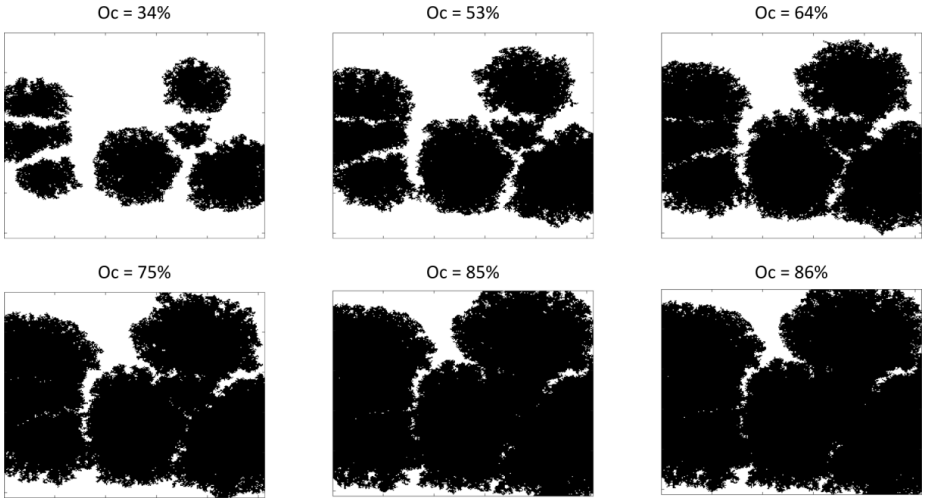


Figure 2: Results of a Monte Carlo simulation of the crystal growth. The images are taken for values of the Occupied Fraction corresponding to the Time Stamps depicted in Fig. 1 (table).

The evolution of the system will be characterized by a probability matrix, $P^{(k)}$, whose i-j element will be the probability that the cell i-j will be occupied at time k+1. This probability matrix, that will be written in terms of a relative probability matrix $C^{(k)}$, needs to be recalculated at each time step following three simple rules:

1. If a cell is occupied, it cannot be occupied again (we have a full 2D model).
2. If a cell is occupied, the probability of occupation of the adjacent free cells is increased.
3. The probability of occupation of a free cell depends also on the available area in a neighborhood of that cell.

Results

From these 3 rules, we parametrized the model, using several structural and kinetic parameters. Once the parameters are tuned up from the data gathered from the original images, a Monte Carlo simulation is used to simulate de growth behavior. Simulations were performed with MATLAB®, obtaining

different types of crystal growth, depending on the values of the parameters (Fig. 2).

Gap-filling and data imputation in the temporal distribution of visitors of recreational areas

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[1]*University Center of Plasencia, Dept. of Mathematics, University of Extremadura, Plasencia (Spain).*

[2]*Faculty of Economics, Dept. of Business Mathematics, University of Valencia, Valencia (Spain).*

[3]*Institute for Landscape Development, Recreation and Conservation Planning; Dept. of Landscape, Spatial and Infrastructure Sciences; University of Natural Resources and Life Sciences, Vienna.*

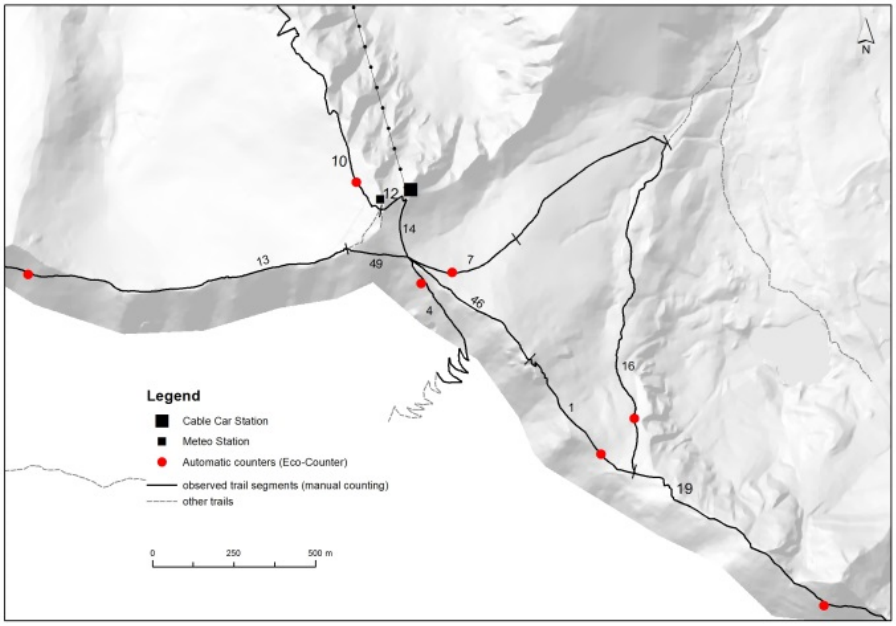

Figure 1: Map of the routes and the positions of the automatic and manual counters in the Kasprowy Wierch area.

Aim/Objective

The aim of this work is to find sensible estimates for the missing values in the data logging of the number of hitchhikers crossing through some specific points of the routes in the Kasprowy Wierch area (Poland).

Material and Methods

Both, automatic and human counters were placed in several specific points in the route network of the Kasprowy Wierch Park (Fig. 1). Automatic counters were continuously recording the number of persons passing through their sensors (including the direction of crossing), whereas the human counters only were recording the same data during some specific hours in the day, but also there were moments when, because of unavoidable causes, their counting duties

ceased. Therefore, at each station and almost every day, some of the data were missing.

Using the data from the automatic counting stations and /or the data from days without missing values, templates describing an "average day" were obtained for each path segment in the route map (Fig. 2). Using these templates, the missing data gaps were filled by estimating a value such that the shape of the curve of the number of visitors at this segment path was the same as in the template. Thus, complete data sets were obtained for each day and route network segment (Fig. 3).

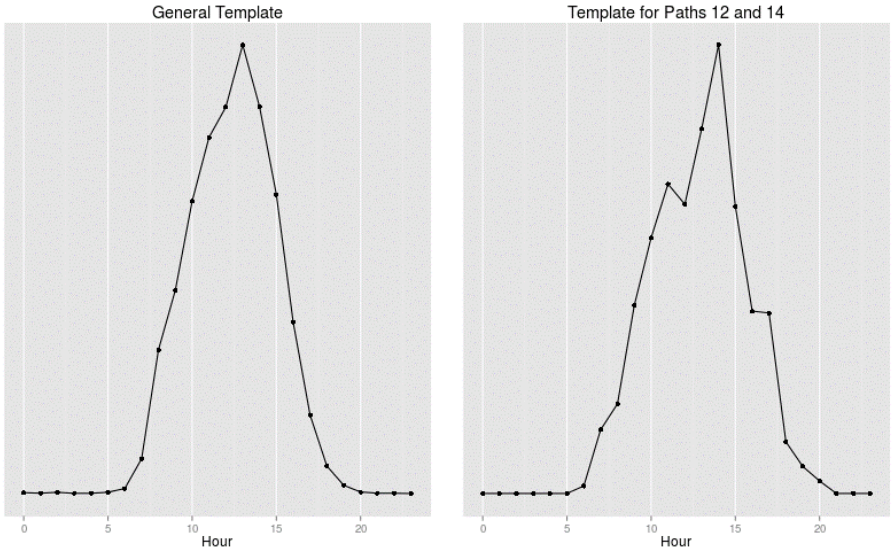

Figure 2: Templates for the average day for the general case (left plot) or for the specific 12 and 14 path routes (right plot).

Results

The method presented work reasonably well for days were not many data was lost, but in the cases where most of the data was not available, the gap-filling procedure gave result which were no better than the template itself (Fig. 4).

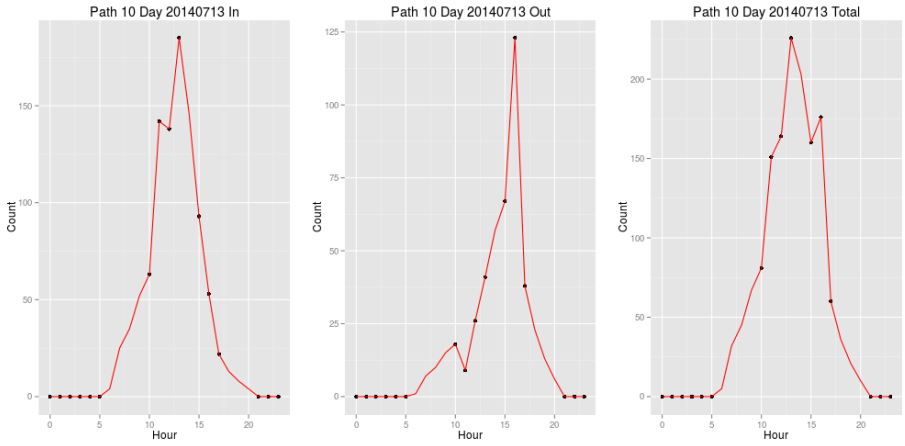

Figure 3: The method presented work reasonably well for days were not many data was lost, but in the cases where most of the data was not available, the gap-filling procedure gave result which were no better than the template itself (Fig. 4).

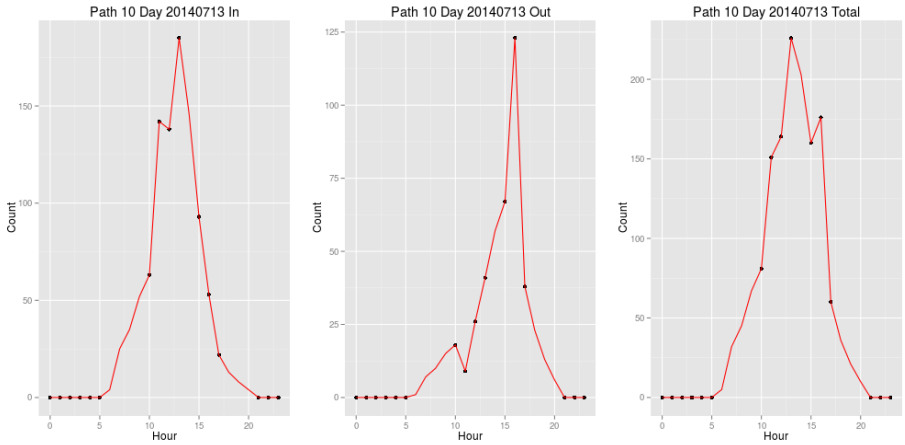

Figure 4: Imputation of data with when only one single datum was available. The gap-filling procedure resulted in a curve very similar to the template.

2 Publications 2015 (SCI articles)

1. **Physical activity, physical fitness and academic achievement in adolescents: a self-organizing maps approach**
M. Pellicer-Chenoll, X. Garcia-Massó, J. Morales, M. Solana-Tramunt, L.-M. González, J. L. Toca-Herrera
Health Education Research 30 (2015) 436 (doi: 10.1093/her/cyv016)

2. **Probing peptide and protein insertion in a biomimetic S-layer supported lipid membrane platform.**
S. Damiati, A. Schrems, E-K. Sinner, U. B. Sleytr, B. Schuster
International Journal of Molecular Science 16 (2015) 2824 (doi:10.3390/ijms16022824)

3. **Atomic force microscopy and cells: indentation profiles around the AFM tip, cell shape changes, and other examples of experimental factors affecting modelling**
K. A. Melzak, J. L. Toca-Herrera
Microscopy Research and Technique 78 (2015) 626 (doi: 10.1002/jemt.22522)

4. **Inspired and stabilized by nature: ribosomal synthesis of the human voltage gated ion channel (VDAC) into 2D-protein-tethered lipid interfaces.**
S. Damiati, S. Zayni, A. Schrems, E. Kiene, U. B. Sleytr, J. Chopineau, B. Schuster, E-K. Sinner
Biomaterials Science 2015 (3) 1406 (doi: 10.1039/C5BM00097A)

5. **Emulsomes Meet S-layer Proteins: An Emerging Targeted Drug Delivery System.**
M. H. Ucisik, U. B. Sleytr, B. Schuster
Current Pharmaceutical Biotechnology 16 (2015) 392 (doi: 10.2174/1389201016041502181126 56)

6. **Characterization of resveratrol-milk protein interaction**
E. Ghorbani Gorji, E. Rocchi, G. Schleining, D. Bender-Bojalil, P. G. Furtmüller, L. Piazza, J. J. Iturri, José L. Toca-Herrera
Journal of Food Engineering 167 (2015) 217 (doi:10.1016/j.jfoodeng.2015.05.032)

7. **Conformation-Dependent Enhancement of Hydrolysis of Poly(N,N-dimethylacrylamide) Brushes via Mechanical Activa-tion of Ester Bonds**
K. A. Melzak, K. Yu, J. N. Kizhakkedathu, J. L. Toca-Herrera
Langmuir 31 (2015) 6463 (doi: 10.1021/acs.langmuir.5b01424)

8. **Relevance of glycosylation of S-layer proteins for cell surface properties**
B. Schuster, U. B. Sleytr
Acta Biomaterialia 19 (2015) 149 (doi: 10.1016/j.actbio.2015.03.020)

9. **Effect of protein concentration to Bacillus thuringiensis cytolytic protein Cyt2Aa2 binding mechanism on lipid bilayer, Studied by QCM-D and AFM.**
   S. Tharad, J. Iturri, A. Moreno-Cencerrado, M. Mittendorfer, B. Promdonkoyb, C. Krittanaia, J. L. Toca-Herrera
   Langmuir 31 (2015) 10477 (doi: 10.1021/acs.langmuir.5b02849)

10. **Anisotropic Crystalline Protein Nanolayers as Multi-Functional Biointerface for Patterned Co-Cultures of Adherent and Non-Adherent Cells in Microfluidic Devices.**
    M. Rothbauer, P. Ertl, B. A. Theiler, M. Schlager, U. B. Sleytr, S.Kupcu
    Advances Materials Interafces 2 (2015) 1400309 (doi:10.1002/admi.201400309)

11. **S-layer based biomolecular imprinting**
    E. M. Ladenhauf, D. Pum, D. S. Wastl, J. L. Toca-Herrera, N. V. H. Phan, P. A. Lieberzeit, U. B. Sleytr
    RSC Advances 5 (2015) 83558 (doi: 10.1039/C5RA14971A)

12. **S-layer fusion protein as a tool functionalizing emulsomes and CurcuEmulsomes for antibody binding and targeting.**
    M. H. Ucisik, S. Küpcü, A. Breitwieser, N. Gelbmann, B. Schuster, U. B. Sleytr
    Colloids Surf B Biointerfaces. 128 (2015) 132 (doi: 10.1016/j.colsurfb.2015.01.055)

13. **On the molecular interaction between albumin and ibuprofen: an AFM and QCM-D study**
    A. Eleta-Lopez, J. Etxebarria, N. Reichardt, R. Georgieva, H. Bäumler, J. L.Toca-Herrera
    Colloids and Surfaces B 134 (2005) 355 (doi: 10.1016/j.colsurfb.2015.06.063)

3 Books and book chapters

- Scanning Probe Microscopy in Biomedical Sciences.
  José L. Toca-Herrera
  In Innovative Technologies in Biomedicine (Ed. H. Figiel, A. Undas, G. Gajos), Technet, 2015, Krakow (ISBN 978-83-7430-400-9)

4 Conferences, workshop and schools

- AUTHOR: J.L. Toca-Herrera
  TITLE: Atomic force microscopy: looking at the macro and nanoscale in life sciences (oral)
  CONFERENCE: Seminars of the Faculty of Health Sciences, University of Ljubljana
  PLACE: Ljubljana, (Slovenia), 2015

- AUTHOR: J. L. Toca-Herrera
  TITLE: Getting to know small and big (soft matter) systems with scanning probe microscopy (oral)
  CONFERENCE: Seminars of the Faculty of Physics and Applied Computer Science, University of Science and Technology (AGH-Krakow)
  PLACE: Krakow, (Poland), 2015

- AUTHOR: J. L. Toca-Herrera
  TITLE:Function and mechanical properties of biomaterials at the nano and macroescale studied with atomic force microscopy (oral)
  CONFERENCE: minars of the Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences
  PLACE: Krakow, (Poland), 2015

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

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

- AUTHOR: J. Iturri, S. Tharad, A. Moreno-Cencerrado, M. Mittendorfer, C. Krittanai, B. Promdonkoi, J.L. Toca-Herrera
  TITLE: Effect of the concentration of cytolytic protein Cyt2Aa2 on the binding mechanism on lipid bilayers studied by QCM-D and AFM (poster)
  CONFERENCE: 6th International BioNanoMed 2015 Congress
  PLACE: Graz (Austria), 2015

- AUTHOR: J. Iturri, J.L. Toca-Herrera
  TITLE:Cell mechanics and stress relaxation variations induced by micro-structured surface gradients of biomolecules (poster)
  CONFERENCE: 5th Conference of the International Association of Colloid and Interface Scientists (together with: Welcome to the 47th Conference of the German Colloid Society)
  PLACE: Mainz (Germany), 2015

- AUTHOR: A. Moreno-Cencerrado, S. Tharad, J. Iturri, M. Mittendorfer, B.Promdonkoy, C. Krittanai, J.L. Toca-Herrera
  TITLE: Interaction cytolytic protein Cyt2Aa2 / lipid bilayer: binding and structural changes (poster)
  CONFERENCE: 15th European Student Colloid Conference (ECIS)
  PLACE: Krakow (Poland), 2015

- AUTHOR: R. Benitez, D. Pum, U. B. Sleytr, J. L. Toca-Herrera
  TITLE: Quantification of S-layer/secondary cell wall interactions
  CONFERENCE: 7th ÖGMBT Annual Meeting
  PLACE: Salzburg (Austria), 2015

- AUTHOR: A. Moreno-Cencerrado, J. Iturri, D. Pum , U. B. Slytr, J L. Toca-Herrera
  TITLE: Influencing bacterial S-layer protein recrystallization on polymer brushes (poster)
  CONFERENCE: 7th ÖGMBT Annual Meeting
  PLACE: Salzburg (Austria), 2015

- AUTHOR: D. S. Wastl
  TITLE: Atomic resolution of calcium and oxygen sub lattices of calcite in ambient conditions using a qPlus sensor with a sapphire tip
  CONFERENCE: 18th International Conference on non-contact Atomic Force Microscopy
  PLACE: France, 2015

- AUTHOR: S. Damiati, A. Schrems, S. Zayni, U. B. Sleytr, E.-K. Sinner, B. Schuster
  TITLE: Reconstitution of peptides/proteins into a native-like lipid environment for retaining their activities (poster)
  CONFERENCE: NANOBIOAPP2015
  PLACE: Barcelona (Spain), 2015

- AUTHOR: R. Ortiz, I. Quintana, S. Moreno-Flores, M. dM Vivanco, J.R. Sarasua, J.L. Toca-Herrera
  TITLE: Ultra-fast laser microprocessing of medical polymers for cell engineering applications (poster)
  CONFERENCE: Joint Annual Meeting of the Austrian Physical Society and the Swiss Physical Society
  PLACE: Vienna (Austria), 2015

- AUTHOR: D. Pum
  TITLE: S-layer based bio-imprinting - Synthetic S-layer polymers
  CONFERENCE: AFOSR Natural Materials and Systems Program Review
  PLACE: Fort Walton Beach, FL (USA), 2015

- AUTHOR: E. Ghorbani Gorji, G. Schleining, J. L. Toca-Herrera
  TITLE: Morphology of encapsulated resveratrol using emulsion-gel method
  CONFERENCE: 29th EFFoST International Conference
  PLACE: Athens (Greece), 2015

- AUTHOR: K. Taczanowska, A. Zieba, C. Brandenburg, A. Muhar, H. Preisel, S. Zieba, J. Krzeptowski, J. Hibner, W. Makaruk, H. Sostmann, B. Latosińska, C. Graf, R. Benítez, V. BolÏÑs, L. M. González, X. García, J. L. Toca-Herrera, S. Ziobrowski
  TITLE: Czasoprzestrzenny rozklad ruchu turystycznego w rejonie kopuly Kasprowego Wierchu w sezonie letnim 2014 (Spatio-temporal distribution of visitors in the Kasprowy Wierch area in the summer season 2014)
  CONFERENCE: Przyroda Tatrzańskiego Parku Narodowego a Czlowiek
  PLACE: Zakopane (Poland), 2015

5 Accepted / Ongoing projects (external funding)

- *S-layer directed nanoscale fluid mechanics*,
  Dietmar Pum (PI), Uwe B. Sleytr (Co-PI), and Jose-Luis Toca-Herrera (Co-PI)
  Air Force Office of Scientific Research (AFOSR), Agreement award FA9550-15-1-0459
- *Testin, an adhesion protein: mechanical, structural and dynamical properties*,
  BMWF (IGS BioNanoTech).
  PI: J. L. Toca-Herrera (Co-PI: A. Meserez).

6 National / International collaborations

- Peter Lieberzeit
  University of Vienna, Inst. of Anal. Chem., Vienna, Austria
- Carole C. Perry
  Nottingham Trent University, Nottingham, UK
- Rafael Benítez
  University of Extremadura, Dept. of Mathematics, Spain
- Luis Millán González
  University of Valencia, Dept. of Physical Education and Sport, Spain
- Stefan Schiller
  University of Freiburg, Inst. of Advances Studies, Germany
- Chartchai Krittanai
  Mahidol University, Institute of Molecular Biosciences, Thailand
- M. Schneider, Institute biopharmacy and pharmaceutical technology, University of Saarland
- A. Göpferich and Miriam Breunig, Institute pharmaceutical technology, University of Regensburg

7 Supervision and Training activities

PhD

- Eva Maria Ladenhauf (AFORS): S-layer based bio-imprinting - Synthetic S-layer polymers
- Alberto Moreno Cencerrado (IGS) : Proteins at (soft) interfaces
- Elham Ghorbani Gorji: Resveratrol milk proteins interactions
- Maria Sumarokova (Erasmus Iamonet): Mechanical properties of hydrogels

MSc/Diploma/Training/Erasmus

- Ana Carol Vianna: S-layer antifouling properties
- Elisa Rocchi: Physico-chemical characterizaiton of the interaction of resveratrol with milk proteins
- Violeta Golebiewska: Absorption kinetics and crystalline structure of S-layer proteins
- Julia Miholich: Mechanical properties of biocompatible polymer systems
- Laia Gili Solé: New designs for drug delivery systems
- Eline de Meyer: Mechanical properties of cell supports

BSc

- Michaela Zrelski: SbsB adsorption kinetics on hydrophobic and hydrophilic interfaces

8 Miscelanea

Workshop on Automatic AFM force-distance curves with R

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[1]*University Center of Plasencia, Dept. of Mathematics, University of Extremadura, Plasencia (Spain).*

[2]*Faculty of Economics, Dept. of Business Mathematics, University of Valencia, Valencia (Spain).*

Aim/Objective

This workshop, held in the Institute of Biophysics of the Dept. of Nanobiotechnology, had as main objective to show the research staff of the Institute the basic capabilities of the free statistical software R, and how can it be used in order to automatize different parts of the processing of AFM force-distance curves.

Contents

1. Introduction to R and Rstudio.
2. Data types: vectors, data frames and lists.
3. Importing data into R.
4. Plotting capabilities.
5. JPK^{TM}data import.
6. Contact point determination.
7. Baseline correction and Young's modulus determination.
8. Force relaxation and creep. Exponential decay fits and viscoelastic properties of the samples.
9. Peak and breakout detection in the retract curve. Adhesion energy and protein unfolding events.

Attendants and duration

The workshop lectures were held twice a week in the Meeting Room of the Department of Nanobiotechnology in sessions of two hours of duration. The attendants were members of the Institute of Biophysics, plus some other visiting students.

Results

As a result of this workshop, a new R package, containing most of the function discussed during the lectures, is currently being developed. A preliminary version of the **afmToolkit** package is currently hosted in github. Once it is mature enough it will be uploaded to CRAN (Comprehensive R Archive Network).

15th European Student Colloid Conference

Alberto Moreno-Cencerrado

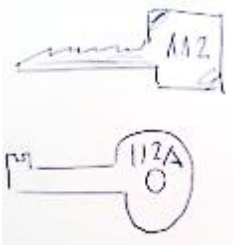
The European meeting for graduate students in colloid and interface science began in 2003 and since then have been celebrated as a biennial scientific congress for PhD students who make their researches in that field. The conferences are based on lectures given by scientists from the field of colloids and interface science.

Since 2007, these meetings have been sponsored by European Colloid and Interface Society (ECIS), a scientific organization founded in 1986 by Prof. Mario Corti (Milano, Italy) and his colleagues Prof. Pierre Bothorel (Bordeaux, France), Prof. Heinz Hoffmann (Bayreuth, Germany), Prof. Bjorn Lindman (Lund, Sweden) and Prof. Vittorio Degiorgio (Milano, Italy). The principal aim of the ECIS is to improve the knowledge in colloid and interface science and to promote cooperation between European scientists. In particular, ECIS has the objective of providing support to young scientists. The last meeting (no. 15) was held at the Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences (ICSC PAS) in Krakow, Poland. The conference was scheduled for four days, from June 8th to June 11th. The students attending the congress mainly came from Europe, in particular from Poland, Spain, Germany, Greece, United Kingdom, France, Switzerland, Italy, Belgium, Czech Republic, Ireland, Sweden, The Netherlands and Ukraine. There were also invited universities from countries outside Europe as Israel, Russia and Australia. In addition to the activities, workshops, posters and oral presentations conducted by PhD students, the conference also provides the opportunity to join master lectures leaded by invited Professors. Following, it is listed the lectures and professors who participated in this year:

- Enrique Lopez-Cabarcos (Complutense University of Madrid).
  Entrapment of Enzymes and Drugs within Colloidal Particles for their Application in Health Sciences.
- Reinhard Miller (Max Planck Institute of Colloids and Interfaces).
  Dynamics of surfactant adsorption layers at water/oil interfaces.
- Jan Hupka (Gdansk University of Technology).
  Filming of bubbles.
- Szczepan Zapotoczny (Jagiellonian University).
  Micellar multilayer films.
- Wuge H.Briscoe (University of Bristol).
  Surfactant layers and polymer brushes under confinement and shear.
- Zbigniew Adamczyk (Jerzy Haber Institute of Catalysis and Surface Chemistry).
  Protein Adsorption-a True Story.
- Brian Vincent (University of Bristol).
  Aggregation in Dispersions Containing Mixtures of Particles.

TEACHING IN POLAND

Univ. Prof. Dr. José L. Toca-Herrera



Depart: BOKU (Vienna) - Arrival: AGH (Krakow). Distance: 332.17 km

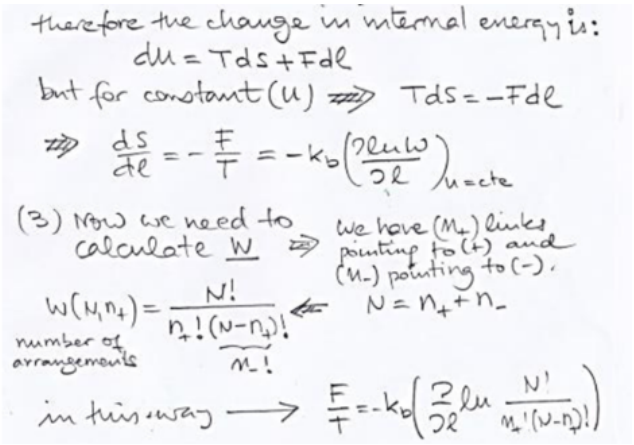
Poland has treated me well since I participated in the International Judo Tournament of Wroclaw in 1987. Years later, in 2010, the University of Science and Technology (AGH) invited me to examine a MSc thesis. Of course, I accepted. The experience was very positive. Therefore, I decided to visit the Faculty of Physics and Applied Computer Science (in 2012 and 2014) to teach some biophysical topics taking advantage of the Erasmus exchange programme. This year I was lucky again and I got the possibility to accept a visiting professorship (from AGH) in the frame of the Marian Smoluchowski Krakow Research Consortium Matter - Energy - Future[1]. Of course, I accepted. The initial (and final) plan for the lectures was to teach "biological physics" and "scientific writing" for master students, a total 6 ECTS confined in 20 working days, which did not coincide (and overlap in time) with my teaching at BOKU[2]. Tiring, but interesting since it was a challenge to prepare lectures for students with a background in physics, mechanical, electrical engineering and mathematics. The first contact with the faculty (immediately after arriving by train) was funny. I approached the porter in order to ask for two keys: my office (which I shared with Prof. Hans-Rainer Trebin, another visiting professor who was teaching "quantum mechanics") and the teaching hall. The porter looked at me and left. One minute later he appeared with another porter. I repeated my request. They looked at me. No reaction. I drew two keys (with the numbers 112 and 112A). I go the keys. I compared them: they were different. (The "bureaucracy" of the second day was easier. The two porters receive me with a big smile. I got the keys without asking.)

The students were responsible of the next positive and best surprise of my first day. They seemed to be young (about 22 years old), interested (they chose

[1]Students from the Jagiellonian University and AGH can attend the lectures delivered in the frame of the Marian Smoluchowski Kraków Research Consortium Matter - Energy - Future , which is composed of: The Faculty of Physics and Applied Informatics (AGH), the Institute of Nuclear Physics and the Institute of Catalysis and Surface Chemistry (both from the Polish Academy of Sciences), and the Faculty of Chemistry and the Faculty of Astronomy and Applied Informatics (both from the Jagiellonian University).

[2]http://www.smoluchowski.fis.agh.edu.pl/wyklady_19.php

to attend my course) and organized (they provided me the whole teaching schedule!). However, they did not ask any question during the lectures (they just took notes). I changed the tactic the second day. I started the second lecture questioning them (*"Sonia, what did you learn yesterday?"*). Slowly they gained in confidence and at the middle of the course all of them were asking questions (later on, some of them confessed some concerns about asking in English).



More problematic was to oblige them to work in couples in the second part of the course ("Scientific writing and presentation"). They wanted to do the task alone. Not in couples. The argument was that by experience only one would do the real job if they work in couples and that in the end both of them would get the same mark. I realized (and was also told by Prof. K. Burda) that the students are very competitive (they can get grants during the studies according to their marks). Even more, the students dress in a formal way to do the exams (suit, tie, dress, good shoes, etc.). Since, I did not care about their opinion, I obliged them to do team work. At the end of the five teaching weeks all students were relatively happy: all of them passed with the highest mark. I had the opportunity to visit the physics labs, and I found that the university was equipped with modern devices, especially the ones related to the Academic Centre for Materials and Nanotechnology, which has no faculty status but works together with other AGH faculties, the Jangiellonian University, and the Cracow University of Technology, and is devoted to carry out interdisciplinary research in modern engineering, physics and chemistry of materials, material diagnostics and nanotechnology. The experience was great and I plan to collaborate "more" with AGH. The first student after my teaching stay, Violetta Golebiewska, is already doing her MSc Thesis with us and I hope she will not be the last one. She helped me to find the information contained in the table. Many thanks.

Ps: Did I forget to say something about the (marvelous) city of Krakow? The last time I saw the porters, I did not get two keys but a bottle of vodka. Not bad.

19 interesting AGH facts (2015):

i. AGH (established in 1919) ranks every year between the best three technical universities in Poland with the Warsaw University of Technology and the Wroclaw University of Technology.

ii. The university campus occupies an area of 38 hectares in Krakow located between the streets of Mickiewicza, Reymonta, Buszka, Tokarskiego, Armii Krajowej, Gramatyka, Nawojki, and Czarnowiejska.

iii. AGH has 18 Faculties plus, an Academic Centre for Materials and Nanotechnology, and a Centre for Transfer of technologies.

iv. AGH offers 59 degree courses and over 200 specialisations.

v. Teaching and research body: 183 full professors, 267 associate professors, 1141 adjunct professors and 373 assistant professors.

vi. About 35531 students (35 % are female students) study in the 18 faculties (full-time studies: 26314, extramural studies: 5931, doctoral studies: 1002, postgraduate studies: 2284, non-national students: 512).

vii. About 500 disabled students study at AGH.

viii. People between 24 and 26 years old graduate from AGH.

ix. Graduation requires: 210 ECTS (engineering studies) and 180 (bachelor studies) for the first cycle of studies, and 90 ECTS (after obtaining engineer degree) or 120 ECTS (master's studies after obtaining bachelor degree) for the second cycle of studies (MSc title).

x. Over 1000 students do a PhD at AGH (14 faculties are authorized to confer the degree of Doctor of Science).

xi. Among the working graduates, a contract of employment was signed by 74.2% of the respondents, 4.8% conducted their own business activity and 19% were offered work on the basis of civil-law agreements.

xii. In Poland, state high education is free for Polish citizens. Non-nationals should pay a fee (e.g. 2000 euros when studying Materials Engineering), although the Rector has the right to exempt the non-national from payment.

xiii. The necessary condition to study at AGH is to have "Matura". The sufficient condition is determined by the Selection Committee of every faculty, which decides who students are accepted based on the result of the following equation: $W = 4G + J$. G is the number of points obtained in the "Matura" exam concerning the main topic, while J is the number of points obtained in the exam of the foreign language. Each faculty accepts a fixed number of students ranging between 120 and 150. For example, this year the candidates applying for a place at Biomedical Engineering needed a threshold of $W = 820$ (a similar score is needed to study medicine at Jagiellonian University).

xiv. The exams have three fixed dates: immediately after the end of the winter or summer semester and in September. If the student fails a topic for the third time, she/he has to register in the course again paying a fee (form 8 to 15 zl per hour of the course; 4 zl = 1 euro).

xv. The notes of the exams are: 2.0 (unsatisfactory, below 50%), 3.0 (satisfactory, 50-60%), 3.5 (satisfactory plus, 61-70%), 4.0 (good, 71-80%), 4.5 (good plus, 81-90%), 5.0 (very good, 91-100%).

xvi. About 10% of MSc students who obtained very good grades in the first part of studies (BSc) can be awarded with scholarships by the Rector (e.g. 670 zl will be given for a mark of the range 4.81-5).

xvii. PhD students earn a minimum of 1470 zl per month (ca. 358 euro). This can be complemented with another grant given by the Rector (about 195 euro). A room in an university residence costs about 100 euro/month.

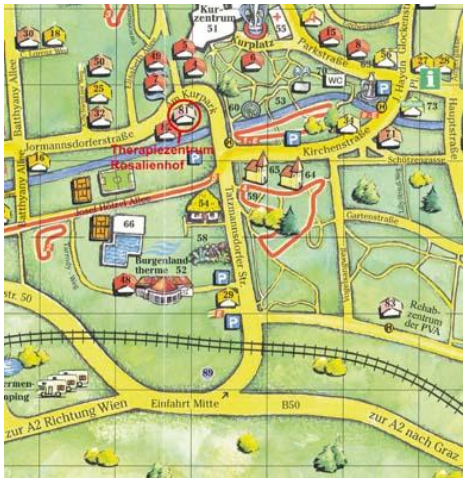
xviii. The AGH Academic Sports Association, divided in 38 units, is one of the largest in Poland.

xix. There is about 100 student scientific organizations where the students can develop their scientific interest (e.g. ”Preservation of the Environment”, ”Biomed”)

References and links:
http://www.agh.edu.pl/en/university/
http://www.agh.edu.pl/biuletyn-informacji-publicznej/agh-w-liczbach

Summer School of the Institute for Biophysics.

University of Natural Resources and Life Sciences Vienna (BOKU)
Bad Tatzmannsdorf, Austria; 15th-17th September 2015.
Book of Abstracts



Participants

Belinda Angjeli (Inst. for Synthetic Bioarchitectures, DNBT, BOKU Vienna), Klemen Bohinc (Faculty of Health Sciences, University of Ljubljana), Andreas Breitwieser (Inst. for Biophysics, DNBT, BOKU Vienna), Jacqueline Friedmann (Inst. for Biophysics, DNBT, BOKU Vienna), Notburga Gierlinger (Inst. of Physics and Material Sciences, MAP, BOKU Vienna), Michael Handler (Institute of Sport Science, University of Innsbruck), Jagoba Iturri (Inst. for Biophysics, DNBT, BOKU Vienna), Dieter Jaeger (DNBT, BOKU Vienna), Claudia König (Inst. for Biophysics, DNBT, BOKU Vienna), Eva Ladenhauf (Inst. for Biophysics, DNBT, BOKU Vienna), Anders Lundgren (Inst. for Bioinspired Materials, DNBT, BOKU Vienna), Alberto Moreno Cencerrado (Inst. for Biophysics, DNBT, BOKU Vienna), Batirtze Prats Mateu (Inst. of Physics and Material Sciences, MAP, BOKU Vienna), Dietmar Pum (Inst. for Biophysics, DNBT, BOKU Vienna), Uwe B. Sleytr (Inst. for Biophysics, DNBT, BOKU Vienna), José L. Toca-Herrera (Inst. for Biophysics, DNBT, BOKU Vienna), Daniel Wastl (Inst. for Biophysics, DNBT, BOKU Vienna), Spela Zemljic Jokhadar (Faculty of Medicine, University of Ljuljana , Faculty of Medicine)

Program

Tuesday - 15/09/2015
14.15 h: Opening of the Biophysics Summer School: José L. Toca-Herrera

First session, chairman: Anders Lundgren
14.20 h - 14.45 h: Jagoga Iturri /Jacqueline Friedmann

14.50 h - 15.15 h: Eva Ladenhauf

Coffee break: 15/20 minutes
15.30 h - 15.55 h: Spela Zemljic Jokhadar
16.00 h - 16.25 h: Belinda Angjeli
16.30 h - 16. 55 h: Andreas Breitwieser

Wednesday - 16 / 09 /2015
Second session, chairman: Klemen Bohinc
9.30 h - 9.55 h: Dietmar Pum
10.00 h - 10.25 h: Anders Lundgren
10.30 h - 10.55 h: Batirtze Prats Mateu

Coffee break: 15/20 minutes
11.15 h - 11.40 h: Notburga Gierlinger
11.45 h - 12.10 h: Michael Handler

Lunch break and relaxing time

Third session, chairman: Dietmar Pum
14.30 h -14.55 h: Klemen Bohinc
15.00 h - 15.25 h: Daniel Wastl

Coffee brake: 15/20 minutes
15.45 h - 16.10 h: Alberto Moreno Cencerrado
16.15 h - 17.00 h: José L. Toca-Herrera - Round table (optional)

Thursday - 17 / 09 /2015
9.30 h - 11.25 h: Brain storming (optional) - Notburga's ERC talk preparation
11.30 h - 13.50 h: Lunch break
14.00 h: Departure to Vienna

Snapshots of the summer school:

A summer school must bring people together and promote scientific collaborations. The really awesome location in Bad Tatzmanndorf was ideal for this purpose: the combination of nature, thermal water and scientific discussion was perfect for strengthening the group(s) bonds. The school consisted mainly of single presentations about the main work performed by the students and postdocs. The pictures below capture some of the moments of the event. one can see JosÃl' and Jagoba (who was in charge of the scientific organization) getting everything ready to start the first presentation given by Eva.

There was also some *"Time to relax"*. Everybody was able to enjoy a delightful dinner in the evening at the hotel's restaurant in an international environment. The outdoors were also vast green extensions perfect for a walk or a jogging session. After a nice stay was *"Time to end"*: Closure ceremony in which the

Biophysics Institute leader made some scientific reflexions that will help to the further scientific development of the group.