BioInspired Nanotechnologies:
from concept to application
Trans Domain 1003

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COST Thematic Workshop

„Biomimetic structures and DNA technology in biosensing“

Program and Abstracts

Comenius University in Bratislava
Faculty of Mathematics, Physics and Informatics
Bratislava
8. - 10. 4. 2013

The thematic workshop is organized by Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Slovakia in framework of the COST TD1003 „Bio-inspired nanotechnologies: from concepts to applications“. The workshop is focused on applications of biomimetic structures such as supported lipid and polymer films as well as DNA technologies including DNA origami, DNA/RNA aptamers in development of biosensors and their applications. The workshop will bring together experts and those who are interested in the themes of the workshop. It will include invited plenary lectures, oral and poster presentations.

Local organizing committee

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Program

April 8, 2013

12.30-14.00 **Registration**
12.30-14.00 **Establishment of posters**
14.00-14.15 **Workshop opening**

Biomimetic structures in biosensing

Chairs: **F. Lisdat, C. Bala**

14.15-15.00
Invited Lecture
I1 **D. Pum, U.B. Sleytr**, Reassembly of S-layer proteins at interfaces

15.00-15.40
O1 **D.P. Nikolelis**, G-P. Nikoleli, N. Tzamtzis, T. Hianik, Analytical applications of lipid modified graphene and ZnO electrodes
O2 **A. Fandrich**, J. Buller, E. Wischerhoff, A. Laschewsky, F. Lisdat, Approaching biosensors application of nanoscaled films of responsive polymers on gold electrodes

15.40-16.40 **Coffee break and posters**

16.40-17.25
Invited Lecture
I2 M. Sanmarti**, J. Samitier,** Membrane protein arrays based on natural nanovesicles

17.25-18.05
O3 J. Chlpik, K. Bombarova, **J. Cirak**, Development of ellipsometric methods to the investigation of phenomena at surfaces and interfaces for biosensing
O4 **A. Ebner**, H. Gruber, P. Hinterdorfer, Generating single protein biosensors for atomic force microscopy on protein layers, biomembranes and cells

19.00 **Welcome dinner**

Tuesday, April 9, 2013

DNA technology

Chairs: **K. Gothelf, J-J. Toulme**

09.30-10.15
Invited Lecture
I3 **P. Morales**, C. Cantale, M. Celino, C. Dalmastri, L. Mosiello, L. Pilloni, B. Rapone, M. Caruso, K. Spinella, S. Vespucci, L.Wang, S. Retterer, Nanotechnologies and selfassemblages for the bio-inorganic interface

10.15-10.35
O5 **M. Leitner**, J. Brummeir, T. Hianik, M. Snejdarkova, A. Poturnayova, H. Gruber, P. Hinterdorfer, A. Ebner, Chemically tagged DNA tetrahedra as linker for single molecule force spectroscopy

10.35-11.00 **Coffee break**

11.00-12.00

O6 **A. Tiwari**, Molecularly imprinted polymer for DNA sensor technology

O7 **V. Beni,** Development of an electrochemical genosensors for celiac disease predisposition analysis

O8 **W. Nuansing**, A. Rebollo, A.M. Bittner, Electrospinning of short peptides, proteins and DNA

12.00-14.00 **Lunch**

Affinity interaction at surfaces and DNA aptamers

Chairs: **J. Samitier, B. Strehlitz**

14.00-14.45

Invited Lecture

I4 **J-P. Aime**, Folding of DNA origami, a cooperative self-assembling process

14.45-15.45

O9 **P. Skládal**, Z. Fohlerová, J. Přibyl, S. Angote, Z. Naal, R.M.Z.G. Naal, Piezoelectric biosensors and atomic force microscopy for cellular studies

O10 **V. Viglasky,** General insight on the quadruplex structures and their potential in nanotechnology application

O11 **L. Mosiello**, K. Spinella, F. Vitali, Development of microarrays and biosensors for mycotoxins determination at ENEA

15.45-16.30 **Coffee break**

16.30-17.15

Invited Lecture

I5 **K. Gothelf,** DNA Nano-technology in biosensing

17.15-18.15

O12 **J-J. Toulmé,** E. Dausse, L. Evadé, E. Daguerre, C. Di Primo, S. Da Rocha, HAPIscreen: a fluorescence method for the evaluation of aptamer pools and the detection of analytes

O13 S. Scarano, E. Dausse, F. Crispo, J-J. Toulmé, **M. Minunni,** Development of a piezoelectric biosensor for targeting MMP-9 Protein for molecular diagnostics

O14 **A. Trapaidze,** M. Brut, A. Bancaud, D. Esteve, A. Esteve, A-M. Gue, Thrombin detection with aptadimer technology

18.30 **Dinner**

Wednesday, April 10, 2013

Applications of DNA sensors and nanotechnology

Chairs: **M. Minunni, P. Morales**

09.30-10.10

O15 R. D'Agata, A. Manicardi, R. Corradini, **G. Spoto** , High sensitivity detection of miRNA210 using peptide nucleic acid probes and surface plasmon resonance imaging

O16 **G. Castillo**, A. Miodek, H. Korri-Youssoufi, L. Kvapilova, P. Kois, M. Minunni, T. Hianik, Development of aptamer-based biosensors designed for targeting small molecules and proteins.

10.10-10.40 **Coffee break**

10.40-11.25
Invited Lecture
I6 **W. Knoll**, DNA diagnostics – optical or by electronics?

11.25-12.05
O17 **I. Safarik,** K. Horska, K. Pospiskova, Z. Maderova, M. Safarikova, Magnetically responsive biologically active compounds in bioanalysis
O18 **A. Heuer-Jungemann,** R. Kirkwood, A. El-Sagheer, T. Brown, A.G. Kanaras, Copper-free click chemistry as a novel tool for the programmed ligation of DNA-functionalized gold nanoparticles

12.05 **Workshop closing**

12.15-13.30 **Lunch**

13.30-15.30 **Meeting of the COST Management Committee**

ABSTRACTS

INVITED LECTURES

I1 **D. Pum**, U.B. Sleytr, Reassembly of S-layer proteins at interfaces

I2 M. Sanmarti, **J. Samitier**, Membrane protein arrays based on natural nanovesicles

I3 **P. Morales**, C. Cantale, M. Celino, C. Dalmastri, L. Mosiello, L. Pilloni, B. Rapone, M. Caruso, K. Spinella, S. Vespucci, L.Wang, S. Retterer, Nanotechnologies and selfassemblages for the bio-inorganic interface

I4 **J-P. Aime**, Folding of DNA origami, a cooperative self-assembling process

I5 **K. Gothelf,** DNA Nano-technology in biosensing

I6 **W. Knoll**, DNA diagnostics – optical or by electronics?

ORAL PRESENTATIONS

O1 **D.P. Nikolelis**, G-P. Nikoleli, N. Tzamtzis, T. Hianik, Analytical applications of lipid modified graphene and ZnO electrodes

O2 **A. Fandrich,** J. Buller, E. Wischerhoff, A. Laschewsky, F. Lisdat, Approaching biosensors application of nanoscaled films of responsive polymers on gold electrodes

O3 J. Chlpik, K. Bombarova, **J. Cirak**, Development of ellipsometric methods to the investigation of phenomena at surfaces and interfaces for biosensing

O4 **A. Ebner**, H. Gruber, P. Hinterdorfer, Generating single protein biosensors for atomic force microscopy on protein layers, biomembranes and cells

O5 **M. Leitner**, J. Brummeir, T. Hianik, M. Snejdarkova, A. Poturnayova, H. Gruber, P. Hinterdorfer, A. Ebner, Chemically tagged DNA tetrahedra as linker for single molecule force spectroscopy

O6 **A. Tiwari**, Molecularly imprinted polymer for DNA sensor technology

O7 **V. Beni,** Development of an electrochemical genosensors for celiac disease predisposition analysis

O8 **W. Nuansing**, A. Rebollo, A.M. Bittner, Electrospinning of short peptides, proteins and DNA

O9 **P. Skládal**, Z. Fohlerová, J. Přibyl, S. Angote, Z. Naal, R.M.Z.G. Naal, Piezoelectric biosensors and atomic force microscopy for cellular studies

O10 **V. Viglasky,** General insight on the quadruplex structures and their potential in nanotechnology

O11 **L. Mosiello**, K. Spinella, F. Vitali, Development of microarrays and biosensors for mycotoxins determination at ENEA

O12 **J-J. Toulmé,** E. Dausse, L. Evadé, E. Daguerre, C. Di Primo, S. Da Rocha, HAPIscreen: a fluorescence method for the evaluation of aptamer pools and the detection of analytes

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O16 **G. Castillo**, A. Miodek, H. Korri-Youssoufi, L. Kvapilova, P. Kois, M. Minunni, T. Hianik, Development of aptamer-based biosensors designed for targeting small molecules and proteins

O17 **I. Safarik,** K. Horska, K. Pospiskova, Z. Maderova, M. Safarikova, Magnetically responsive biologically active compounds in bioanalysis

O18 **A. Heuer-Jungemann,** R. Kirkwood, A. El-Sagheer, T. Brown, A.G. Kanaras, Copper-free click chemistry as a novel tool for the programmed ligation of DNA-functionalized gold nanoparticles

POSTERS

P1: **C. Ocaña**, M. del Valle, Signal amplification for thrombin impedimetric aptasensor: use of gold-streptavidin nanoparticles

P2: **M. Šnejdárková**, A. Poturnayová, I. Neundlinger , A. Ebner , T. Hianik, DNA aptamer-based biosensor for sensitive thrombin detection

P3: **A. Poturnayová**, M. Šnejdárková, I. Neundlinger , A. Ebner , T. Hianik, The topography of lipid layers containing calixarene-cytochrome c complexes by atomic force microscopy

P4:**L. Kvapilova**, G. Castillo, T. Hianik, P. Kois, Comparison of different fabrication techniques in aptamer biosensing for VEGF detection.

P5: **K. Spinella**, G. Palleschi, L. Mosiello, F. Vitali, Development of a label free immunoassay quartz crystal microbalance (QCM) based for Aflatoxin B1 determination

P6: **P. Tóthová**, K. Tlučková, P. Krafčíková, V. Viglaský, Use of G-quadruplexes as biosensors

P7: **K. Tlučková**, P. Tóthová, Ľ. Bauer, V. Viglasky, Novel G-quadruplex structures found in Human Papillomaviruses

P8: **L. Traxler** , D. Zauner, A. Scherfler, M. Leitner, V. Hytönen, M. Kuloma, P. Hinterdorfer, Andreas Ebner, Reversible functionalization of biosensing gold surfaces with a pH-sensitive avidin mutant

P9: **S. Weich**, A. Paananen, M. Linder, P. Hinterdorfer, A. Ebner, Exploring the energy landscape of hydrophobin-hydrophobin interactions using AFM single molecule force spectroscopy

P10: P. Vitovic **V. Subjakova**, T. Hianik, The physical properties of lipid monolayers and bilayers modified by calixarenes with the affinity towards cytochrome c

P11: **I. Safir**, K. X. Ngo, D. Kedracki, N. Gour, C. Vebert-Nardin, Crystal morphological characterization of novel self-assembling chitosan-ssDNA hybrids

P12: **S. Melikishvili**, M. Poliakova, Z. Garaiova, T. Hianik, PAMAM dendrimers modified with DNA and dextran sulfate in drug delivery

P13: **E. Catalano**, S. Ferraris, E. Vernè, M. Miola, A. Follenzi, L. Rimondini, Labelling of living cells with magnetic nanoparticles for applications of cell patterning

I1
Reassembly of S-layer proteins at interfaces

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S-layers are the most commonly observed cell surface structures in prokaryotic organisms (bacteria and archaea). They are composed of a single protein or glycoprotein species (Mw = 40 to 200 kDa) and exhibit oblique, square or hexagonal lattice symmetry with unit cell dimensions in the range of 3 to 30 nm, and a thickness of ~10 nm. They are highly porous protein meshwork with pores of uniform size and morphology in the range of 2 to 8 nm. One of the key features of isolated S-layer proteins is their intrinsic tendency to self-assemble into monomolecular arrays in suspension, at solid supports (e.g. silicon wafers), at the air-water interface, at planar lipid monolayers, and at liposomes and nanocapsules [1].

Basic research on S-layer proteins laid foundation to make use of the unique self-assembly properties of native and, in particular, genetically functionalized S-layer protein lattices in a broad range of applications in the life and non-life sciences. Thus, the overall goal of our research is dedicated towards the development of an S-layer-based biomolecular construction kit.

This contribution briefly summarizes the knowledge about structure, genetics, chemistry, morphogenesis, and function of S-layer proteins and pays particular attention to self-assembly in solution, at differently functionalized solid supports, and at lipid layers.

Acknowledgements
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I2
Membrane protein arrays based on natural nanovesicles

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Natural vesicles produced from genetically engineered cells with tailored membrane receptor composition are promising building blocks for sensing biodevices [1]. This is particularly true for the case of G-protein coupled receptors (GPCRs) present in many sensing processes in cells, whose functionality crucially depends on their lipid environment. However, the controlled production of natural vesicles containing GPCRs and their reproducible deposition on biosensor surfaces are among the outstanding challenges in the road map to realize practical biomolecular devices based on GPCRs.

Dealing with membrane receptors is challenging due to the fact that they are difficult to produce, in comparison with other biomolecules, such as, for instance, soluble proteins or oligonucleotides. Current strategies developed for biosensing applications with membrane receptors include immobilization into supported lipid bilayers or into lipid vesicles (liposomes), made from artificial [2] or native membranes [3] as well as their inclusion into free-standing lipid bilayers lying on nanoporous substrates [4]. Isolation of native membrane fractions from a cell source, which integrate membrane receptors artificially expressed in the cell line, constitutes one of the preferred approaches as it provides the same lipidic environment found in the native cell, thus preventing the protein denaturation during the insertion into an artificial membrane.

We present the production and characterization of membrane nanovesicles containing heterologously expressed olfactory receptors - a member of the family of GPCRs - and study their deposition onto substrates used as biosensor supports [5,6].

References

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I3
Nanotechnologies and selfassemblages for the bio-inorganic interface

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The perspectives of the exciting branch of bioelectronics and biosensing are bound to the ability to control the interface between biomolecules and solid state electronic components at the micro and possibly nanometric scale. To this aim different micro and nanofabrication technologies should be developed in an integrated view, devoted to fabrication of bioelectronic and sensing components, and more generally to the problem of the bio-inorganic interface. This talk will describe some of the instruments and technologies developed, and will show the results obtained for micro- and nanopatterned ultrathin layers of metals and active biomolecules.

Laser-SPM assisted carvings and depositions will also be described, involving radiation enhancement effects at the tip. Micro and nanometric ablations and spectroscopically selected metal depositions will be described.

One outstanding example of hybrid fabrication/characterization technique is the use of scanning probe instruments under electron microscope observation to precisely address the probe to the required locations while minimizing sample and probe damage due to high scanning speeds of the tips.

A further subject of interest is the use of micro-and nano biological structures as sensors, or moulds and stencils to be used in nanotechnology and nanoscience experiments. Extremely simple and inexpensive microlithography achievements and micro-nano wires based on biological structures will be shown.

Control of the adhesion of biomolecules on inorganic surfaces is a central issue. Short peptides selected by phage or cell display methods are particularly interesting systems where computational simulation can supply important insight into the mechanisms of specific adhesion. The case of adhesion on flat graphite vs carbon nanotubes will be shown.

Finally the talk will describe some of the ongoing projects, that are focussed on the fabrication of hybrid bio-inorganic device-oriented structures exploiting DNA based architectures and specific peptide-inorganic adhesion together with advanced lithography.

References

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I4
Folding of DNA origami, a cooperative self-assembling process

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DNA based nanostructures built on a long single stranded DNA scaffold, known as DNA origamis, offer the possibility to organize various molecules at the nanometer scale in one pot experiments. The folding of the scaffold is guaranteed by the presence of short, single stranded DNA sequences (staples), that hold together separate regions of the scaffold. In this paper, we first consider simple structures made of three single-stranded oligonucleotides. Based on experimental (UV absorption) and numerical (replica exchange molecular dynamics simulations) data, we show that cooperativity is key to understand the thermodynamics of these constructions. In a second part, we derive a model of the annealing-melting properties of DNA origamis. The model captures important features such as the hysteresis between melting and annealing, as well as the dependence upon the topology of the scaffold. We also obtain temperature dependent average conformations that compare well to AFM images of quenched states of the partially folded origamis.

I5
DNA Nanotechnology in Biosensing

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Several inventive designs for sensors based on electrochemical readout have appeared in recent years. The combination of electrochemistry and nanotechnology with molecular biology provides very powerful tools for the development of highly sensitive and highly specific biosensors. In this field we have developed new methods to electrochemically graft DNA on surfaces and to detect complementary sequences [1]. We have also explored new approaches for electrochemical detection of DNA, by using e.g. quantum dots [2] and lipase enzymes [3] to amplify the signal from the binding event. The quantum dot based amplification system was also applied to a sensor based on the human estrogen receptor [4]. Oligonucleotides, in particular DNA and RNA aptamers, have also been used to develop a small molecule electrochemical sensors for theophylline [5] and sensors for neurotransmitters are currently being developed [6]. Recent results on development of optical sensors based on DNA strand displacement reactions will also be presented as well as recent progress on immobilization of conducting polymers on DNA origami scaffolds.

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I6
DNA diagnostics – optical or by electronics?

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The race in DNA diagnostics between optical detection principles (fluorescence, surface plasmons) and electrical/electrochemical/electronic concepts is not decided yet. Both scientific communities continue to offer solutions for fast, multiplexed, simple and cheap detection of oligonucleotides, PCR amplicons, genomic DNA (fragments), etc. Most likely, the competition will never see a single winner that meets all needs because the different practical formats and applicational environments, as well as, market requirements may ask for specific and unique solutions that could be better achieved in one case by optics and in another situation by electronics.
Along these lines, we will briefly review the state of the art in both categories of DNA diagnostics and will then present a number of examples of what has been demonstrated for the sensitive detection of DNA in (from) solution by monitoring surface hybridization reactions of target strands binding from the analyte solution to surface-attached capture oligonucleotides. A particular emphasis will be put on the physico-chemical principles of these surface recognition and binding (or dissociation) reactions in order to be able to develop criteria of how to optimize sensitivity, selectivity, e.g., for the detection of single nucleotide polymorphisms (SNPs), etc., in DNA diagnostics.
The examples given include

- SNP detection in singly and doubly mismatched oligonucleotides and amplicons;
- demonstration of the limit of detection in these surface hybridization reactions;
- multiplexed recording of surface hybridization reactions via quantum dot- labelled targets;
- surface polymerase chain reactions;
- the use of uncharged peptide nucleic acids (PNAs) as capture probes instead of the highly charged DNA;
- label-free detection of DNA by electrochemical techniques, and
- organic field-effect transistor- (OFET-) based monitoring of hybridization reactions.

O1
Analytical applications of lipid modified graphene and ZnO electrodes

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Lipid membrane based biosensors have been exploited in biosensor technology during the last 30 years. Lipid membrane serves as a biocompatible matrix into which ion channels or bioreceptors are incorporated. However, a major obstacle that limited their applications and commercialization was their fragility. Recent advances in stabilization of supported lipid membranes have been achieved by polymerization on glass fiber filters and provides a novel route for commercialization of these devices. This presentation summarizes achievements in the field of biosensors utilizing polymerized lipid films on ZnO and graphene electrodes. A number of toxicants such as urea, uric acid, cholesterol, etc. were potentiometrically determined by simple means of inserting the biosensor into the analyte solution and measuring the potential difference with a simple MVmeter. The detection limits are down to nmolar levels and the life time of the device is more than a month.

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O2
Approaching biosensors application of nanoscaled films of responsive polymers on gold electrodes

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Electrode interfaces switchable by external stimuli such as temperature or pH changes may allow the construction of sophisticated systems for applications in biosensing, biotechnology and biomedicine [1]. Such interfaces can show variations of surface properties “on demand” and control of the access to the modified surface for chemical species [2].

Here thermoresponsive polymers covalently bound to modified gold are investigated regarding the application as matrix for biorecognition processes. Oligo(ethylene glycol)methacrylate based thermoresponsive polymers were synthesized and immobilized as films (10 – 20 nm) on gold. Cyclic voltammetric and impedimetric measurements with modified electrodes were realized. The results show successful immobilization of the polymer on gold by significantly increased interface impedance. Significant changes in the voltammetric peak current and peak separation values for the electrochemical conversion of ferro-/ferrocyanide by varying the temperature in the range 25 – 50 °C demonstrate the thermally induced phase transition. The experiments indicate modified access for the redox couple after the change in polymer conformation. These results are also verified by surface plasmon resonance [2].

Further experiments concern the idea of using this interface as carrier for biorecognition elements and its utility for the bioanalysis. The switchable system is combined with biological components (peptide, antibody) and the effect on the responsive behavior is tested.

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O3
Development of ellipsometric methods to the investigation of phenomena at surfaces and interfaces for biosensing

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The method of ellipsometry, particularly spectroscopic ellipsometry, is a very sensitive, non-destructive experimental technique of thin film characterization. The method is based upon the registration of changes in the polarisation of light on reflection from the investigated surface. In case of known materials it is possible to detect the thickness of layers with an accuracy under 0.1 nm. It is evident that the prospects of spectroscopic ellipsometer are not fully exploited and their further research may bring a number of new results. In this presentation we bring the results of investigation of molecular ordering in ultrathin organic layers deposited using various technologies: Langmuir – Blodgett, spin – coating, vacuum evaporation. The recently proposed method of total internal reflection ellipsometry [1] (also called plasmon enhanced ellipsometry) combines the advantages of the spectroscopic ellipsometry and the Kretchmann type SPR geometry of total internal reflection (TIRE) [2]. This method was employed and proved to be more accurate and sensitive if compared to SPR [3]. TIRE method is found to be extremely suitable for detecting changes in adsorbed layers (at the metal / dielectric interface) caused by specific binding of an analyte from the surrounding solution.

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O4
Generating single protein biosensors for atomic force microscopy on protein layers, biomembranes and cells

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Atomic Force Microscopy (AFM) has developed to a key tool in nano-medicine offering methodologies to understand molecular and cellular biology at the single molecule level. Some important advanced bio-AFM techniques like Molecular Recognition Force Spectroscopy (MRFS) and Topography and RECognition Imaging (TREC) are based on the use of monomolecular biological sensors tips

Here we present recently developed and new strategies to upgrade commercial AFM silicon or silicon nitride tips to real single molecule biosensors. As a first step the inert AFM tip surface has to be activated, e. g. by aminofunctionalization techniques. In the next step a heterobifunctional crosslinker, typically containing a poly(ethylenglycol) (PEG) chain, is covalently bound to the amine groups. Finally the specific bio-ligand is tethered on the outer PEG end. Depending on the chemical nature of the ligand, different coupling protocols have been developed allowing to bind target molecules via their lysine residues, thiols, his-tags and many more.

In MRFS such single molecule biosensors have successfully been used to explore the energy landscape of receptor-ligand interaction on the molecular level. In addition, the performance of TREC, a recently developed AFM imaging technique allowing simultaneously mapping of the sample topography and localization of molecular recognition sites at near physiological conditions and with a lateral resolution on the nanometer range is directly related to the performance of these bio-sensor tips. TREC was successfully used on artificial and natural membranes as well as on different cells using bio-sensing tips functionalized with single proteins, specific antibodies, hormones or other single bio-ligands.

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O5
Chemically tagged DNA tetrahedra as linker for single molecule force spectroscopy

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Atomic force microscope (AFM) has developed to one of the key techniques in nanoscience. The enhancement of the cantilever to a molecular biosensor yielded to techniques that allow for the detection of forces between single receptor ligand complexes in the pico newton range down to a single chemical bond (MRFS). Furthermore, the combination of recognition and topographical measurements, simultaneous topography and recognition imaging (TREC), is capable of determining receptor distributions on surfaces at the nano scale. Nevertheless there are still limitations for a wide use of these techniques as standard tool in applied life science and in the clinical field. The main limitation is the complex chemical tip functionalization of the AFM cantilever, which restricts these techniques to experts in basic science.

In this work we present an alternative way of functionalizing the AFM tip for MRFS and TREC by the use of DNA building blocks. For this, tetrahedra shaped DNA building blocks were bonded to gold coated cantilevers via three disulfide vertices. For a test system the forth vertex carried a biotin tag. Alternatively, it was equipped with a short single strand DNA and used in combination with DNA aptamers containing the corresponding counter sequence. Compared to commonly used complex standard tip functionalization, the here described procedure can be done with basic chemical knowledge via a simple incubation or pick up procedure. It could thus be one important step to enable the use of functionalized cantilevers to many research labs.

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This work was supported by the Slovak Research and Development Agency (project No. SK-AT-0009-12), FFG MNTera.net (project 823980) and by the Austrian Science Foundation project (N00104-NAN).

O6
Molecularly imprinted polymer for DNA sensor technology

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The molecularly imprinted polymers (MIPs) have attracted much attention due to their multifarious affinity such as in sensors, separations, catalysis, drug delivery and waste water management [1]. MIPs have the ability to mimic biological functions through their three-dimensional cavities with specific size, shape, and functionality for mimetic recognition of target molecules and have already been applied for interesting applications including separation and recognition of deoxyribonucleic acid (DNA) [2]. The high selectivity and affinity of MIPs for the DNA templates offers a promising approach to develop a new generation of DNA biosensors [3]. A typical scheme for fabrication of MIP-electrode for DNA biosensing is shown in Fig. 1. Compared to conventional DNA biosensors, which use single-stranded or sequence specific oligodeoxyribonucleotide onto the electrode surface as sensing elements, DNA biosensors based on MIPs offer three advantages: 1) high affinity and selectivity to the imprinted template (i.e., the target molecules); 2) superior stability compared with those using DNA in the biosensor structure; and 3) ease of fabrication and adaptation for range of DNA biosensors. And, the talk will be devoted on novel methodologies and strategies of MIPs for DNA biosensing.

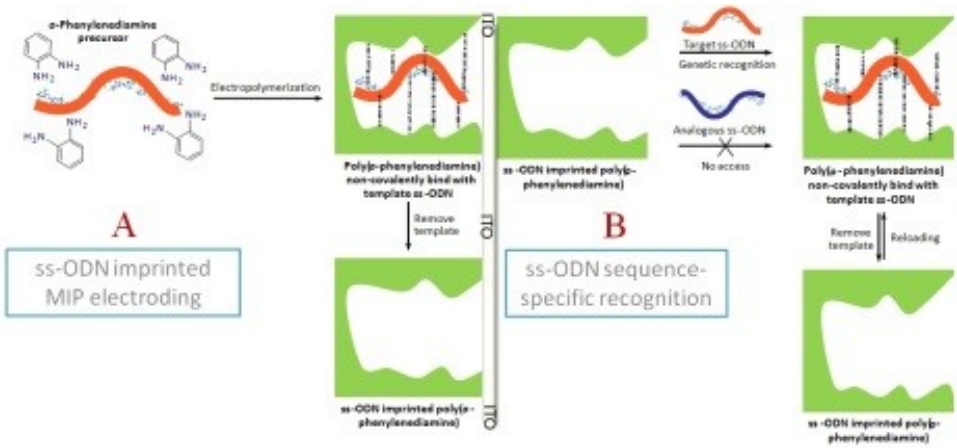

Fig. 1. (A) Electrochemical preparation of ss-ODN imprinted MIP electrode and (B) Re-usable biosensor to recognise sequence-specific ss-ODN.

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O7
Development of an electrochemical genosensors for celiac disease predisposition analysis

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Low density DNA arrays are of growing interest in the field of fast clinical/environmental analysis; for example in clinical analysis these can find application in areas as early diagnosis of genetic diseases or as support in the diagnosis of genetically associated diseases.

Coeliac disease, a small intestinal inflammation triggered by the intake of gluten, has been shown to affect only genetically predisposed individuals. Relation between Coeliac disease and two Human Leukocyte Antigens (HLA) genes, DQ2 and DQ8, has been reported, with almost 100% of the affected patients carrying at least one of them.

In this seminar the development of a low density electrochemical genosensor array for medium resolution typing of Coeliac disease associated HLA genes, is presented.
The proposed array was based on an enzymatic sandwich assay format performed at a photolitographically fabricated electrode array.
The optimisation of different aspects as surface chemistry, assay conditions, probe's design and single stranded DNA generation, together with real sample analysis will be present.

O8
Electrospinning of short peptides, proteins and DNA

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Biomolecules such as peptides and proteins can assemble into supramolecular structures, e.g. nanofibers. This self-assembly is entirely based on noncovalent interactions, especially on electrostatics. An example is amyloid assembly, which takes years *in vivo*, and can result in neurodegenerative diseases (e.g. Alzheimer‘s or Parkinson‘s) [1, 2]. The smallest model system for amyloids is the aromatic dipeptide diphenylalanine (Phe-Phe). Its assembly to fibers is based on ion pairs, but additional π-stacking interactions come into play [3].

We explored new routes for assembly, such as the electrospinning technique. This simple and fast procedure fabricates biomimetic fibers from a jet that is drawn from a single droplet of solution. We show that it speeds up the amyloid-like assembly of model peptides, and of proteins. The substances were dissolved in highly polar solvents, and droplets of the solution were polarized to up to 15 kV, resulting in fiber formation. In order to investigate the role of π-stacking, we chose various short aromatic peptides, e.g. Phe-Phe, Gly-Phe, and Fmoc-Phe-Gly. We also investigated several globular proteins that normally do not form fibers, e.g. insulin, and we tested DNA.

The spun peptide fibers were probed with optical microscopy, SEM, confocal Raman and infrared spectroscopy [4, 5]. The results show that natural molecular assembly can be tuned by electrospinning for many peptides and proteins. We can produce albumin protein fibers with diameters from about 10 nm to a few hundred nm. In analogy to synthetic polymers, DNA molecules appear to bundle into nanoscale fibers of practiaclly infinite length; we found centimeter-long fibers with diameters down to 10 nm.

We demonstrated that small molecules and pure proteins can be spun without the normally required addition of synthetic polymers. This should translate into enhanced biocompatibility of our fibers, which is crucial for applications such as wound dressing or tissue engineering. Moreover, electrospinning of DNA would be a useful technique for preparing thin and ultralong DNA fibers onto a substrate for further characterization, or for applications in solid-state devices.

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O9
Piezoelectric biosensors and atomic force microscopy for cellular studies

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Mammalian cell-based biosensors exploit the sensitivity of cells as a recognition element responding to the wide range of chemical agents including toxins, pharmaceutical and environmental compounds. The adherent cells and stable attachment on the planar sensor surface are crucial requirements for the sensor construction. The right choice of the adhesion promoting coating must be pre-tested. The cellular response to tested compounds and other external effects might be detected using different transducers depending on the processes and parameters of interest. The most common transducers connected with adherent cells include piezoelectric quartz crystal microbalance (QCM) combined with the energy dissipation or the resistance measurements and impedance devices. Both provide sensitive, real-time and label free information relating to cellular adhesion, proliferation, differentiation, exocytosis and apoptosis since the adhesiveness, spreading, morphology and viscoelasticity of the cells are changed. Recently, scanning probe techniques as atomic force microscopy (AFM) were successively applied for detailed visualization of surface-bound cells. QCM and AFM were used for complementary investigation of IgE-mediated degranulation of mast cells; these are involved in many allergic diseases due to the proallergic and proinflammatory substances released from granules when they become activated. The initial step of sensitization requires IgE antibodies to bind to high affinity FcεIR receptors. The following activation of cells becomes triggered by multivalent antigen-induced oligomerization of the IgE/FcεIR complexes. The sensitized RBL-2H3 mast cells were cultured on poly-L-lysine coated gold surface of quartz crystal integrated in the flow cell and connected with the detector providing resonance frequency and resistance of QCM. The activation of mast cells by antigen – dinitrophenyl conjugated to bovine serum albumin (DNP-BSA), was continuously followed for several hours. Furthermore, quercetin as the efficient inhibitor of the degranulation process was tested in micromolar concentrations. The observed response was also confirmed using atomic force microscopy for imaging topography and morphological changes of the mast cells fixed at different stages of the degranulation. The piezoelectric biosensor proved to be sensitive, fast and label free tool for screening of anti-allergic drugs and development of cellular biosensors.

O10
General insight on the quadruplex structures and their potential in nanotechnology application

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Short nucleic acids tend to form non-canonical structural motifs, e.g. Z-DNA, cruciform, slipped DNA, triplex, tetraplex, quadruplex, etc. Many of these structural motifs occur naturally in living organisms, but some of them were artificially designed, e.g. the aptameric molecules. DNA and RNA aptamers are molecules specifically recognizing other type of molecules based on surface complementarity. In some aspects they have some better properties than the antibodies. Currently, the biologically important molecules derived from nucleic acids became a base of different micro/nano devices such as fluorescent beams DNA, biochips (DNA microarray) or biosensors with functionalized surfaces with different electrochemical and optical characteristics [1,2]. DNA chips let analyze wide spectra of mutations and polymophisms in different genes that cause hereditary diseases. They represent one of many possibilities of fastening DNA diagnoses and lowering their costs. Most of such devices are based on complementarity of the nucleic acids, so-called Watson-Crick pairing between bases of the nucleic acids. However, there are also some other hydrogen interactions which can take their place in the DNA alternative structures formation, Hoogsteen pairing [1].. Within the last 15 years in the field of DNA self-assembling structures there has been more attention paid to G-quadruplexes (≈2500 current publications in journals) thanks to their specific structural characteristics, high level of polymorphism and biological regulating potential on gene expression. G-quadruplex motifs are able to form intra- and intermolecular/ supramolecular structures which are considered to be very promising candidates for construction of various nanodevices.

Acknowledgements
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O11
Development of microarrays and biosensors for mycotoxins determination at ENEA

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ENEA is an Italian national public research institution dealing basically with energy, new technologies and environment, organized in 11 research centres spread over the entire national territory operating mainly in support of Scientific Research Ministry's programmes. The aim of this presentation is to report some activities carried out at Casaccia Research Center, Rome in biosensors field. Casaccia is the our main research Center, multidiciplinary campus, hosting scientists with expertise in many different fields encompassing physics, chemistry, information science, engineering and our research team is working in Laboratory "Agroindustrial Innovation" mainly on microsystems and biosensors development and application for food contaminants determination. In ENEA before to devote our attention on biosensors for MTXs (Mycotoxins) determination we developed potentiometric device for s-triazines and E.coli determination [1,2]. We also developed a microarray antibodies based for suitable for two MTXs (Aflatoxin B1 and Fumonisine) determination. Actually we focused our attention on mass sensitive biosensor, based on Quartz Crystal Microbalance (QCM). QCM based biosensors are popular for their sensitive mass detection capabilities and their ability to monitor in real-time. The general operating principle of a QCM biosensor is adecrease in the crystal's resonance frequency following binding of the target organism. When a rigid mass binds to the oscillating crystal surface, the shift in the resonance frequency (Δf) is proportional to the mass bound following the Sauerbrey equation. Hence, the QCM can potentially be used to develop affinity biosensor in order to identify and quantity specific target molecules in a sample. During a collaboration with Prof.Tibor Hianik we explored this method for label-free detection of low molecular toxicant and developed a QCM [3] and impedimetric biosensor [4] for detection ochratoxin A using DNA aptamers as specific receptors with a limit of detection (LOD) of 0.12 nM . We decided to continued in the field of mass sensitive QCM biosensor development also in ENEA [5].

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O12
HAPIscreen: a fluorescence method for the evaluation of aptamer pools and the detection of analytes.

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Aptamers are oligonucleotides obtained through a combinatorial method (SELEX) followed by cloning and sequencing of the selected pool. The most represented candidates are then synthesized and their binding properties are evaluated, thus leading to the identification of aptamers. These post-selection steps introduce a bias in particular to the expense of poorly amplified strong binders. We designed a method than weakens this problem. We described the High-throughput APtamer Identification screening (HAPIscreen) based on fluorescence measurements [1]. It takes advantage of the Alphascreen methodology and allows the blind evaluation of SELEX pools and individual candidates. This approach was used for evaluating the properties of aptamers raised to RNA hairpins and to the M1 protein of the Influenza virus.

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O13
Development of a piezoelectric biosensor for targeting MMP-9 Protein for molecular diagnostics

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Piezoelectric based aptasensing was developed for metalloprotein MMP-9 detection in human fluids. MMP-9, Matrix MetalloProtease-9 or 'gelatinase', 92 kDa, is a pProtein involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis, intracerebral hemorrhage, and metastasis. For all these reasons MMP-9 is of interest in molecular diagnostics.

In this work we developed an Aptasensor based on piezoelectric transduction for MMP-9. Aptamers previously characterized (1) specific for the analyte were immobilized on the sensor surface via streptavidin-biotin chemistry.

The assay design was optimized and finally resulted in a sandwich-like assay using a secondary aptamer, specific for a different region of the MMP-9. The analytical parameters of the sensor were evaluated. Matrix effects were also studied exploring possible sample pre-treatments using magnetic particles covered with Protein A and Protein G to minimize immunoglobuline interfering effects.

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O14
Thrombin detection with aptadimer technology

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Targeting two sides of the target protein simultaneously gives us opportunity to improve and facilitate the detection of proteins, by increasing specificity, selectivity and affinity, and by decreasing non-specific interactions. As an aptadimer model we use the construct bearing the well-studied aptamers for thrombin HD1 and HD22. These anticoagulant aptamers provide a starting point for merging the principles of DNA nanotechnology with aptameric functions.

The aptadimer is essentially composed of two aptamers separated by a spacer, which is composed of complementary strands (stem) of 5-10 bases interconnected with the loop to form a hairpin. The stem also contains a fluorophore and a quencher. This hairpin is connected to the aptamers by flexible and inert poly-dT sequence. Folding of the stem into a DNA double-helix leads to the quenching of the fluorophore. However, binding of the target to the aptadimer disrupts the stem, and directly triggers fluorescence emission.

In order to construct the aptadimer, we used software created for this purpose, which generates spacer sequence obeying predefined specific design rules, so that the inserted spacer doesn't alter the structure and stability of the aptamers.

Experimental validation of the action principle of the aptadimer comprises endorsement of spacer folding, and confirmation of dual aptamer interaction with target. Spacer folding was confirmed with fluorospectormeter, measuring increase of fluorescence during thermal actuation and in presence of a detergent, since these factors promote dehybridization of the stem.

In the context of aptamer functionality ordinary fluorescence and fluorescence anisotropy measurements were performed. We mapped specific changes in the fluorescence in the presence of thrombin, indicating existence of the specific interaction between analyte and dimer. Whereas, detected change in rotational relaxation properties of dimer enabled exact evaluation of the binding stoichiometry.

We believe aptadimer technology will be step forward in development of aptamer-based sensors.

O15
High sensitivity detection of miRNA210 using peptide nucleic acid probes and surface plasmon resonance imaging

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MicroRNAs (miRNAs) are short RNAs that regulate gene expression, playing an important role in various regulatory processes such as differentiation and development. miRNAs expression level have also a role in a number of different diseases and provide a valuable molecular diagnostic and prognostic indicator [1]. To fulfill the potential of miRNAs as diagnostic tool, several challenges must be overcome. The small size of miRNA sequences, the low abundance and close sequence similarity make difficult to design probes capable to bind such small miRNA templates thus making ineffective most conventional amplification tools. The low sensitivity of the time-consuming Northern blot procedures, the golden standard for miRNA analysis, togheter with disadvantages associated to the fluorescence detection of microarray-based methods, require considerable efforts to be put to develop new methods for high-throughput and multiplexed analysis of miRNA [2]. In this context, Surface Plasmon Resonance Imaging (SPRI) can offer rapid, sensitive, and label-free analysis and substantial advances toward high-throughput screening [3,4]. Recently, we have shown [5,6] that an ultrasensitive detection of DNA is obtained by using nanoparticle-enhanced SPRI and Peptide Nucleic Acids (PNA) probes. Herein, we describe a SPRI-based miRNA sensing methods where specific PNA probes targeting miRNA-210 are immobilized on gold surfaces. A PNA tethered to the surface through lysine-modified backbone was used, so that the probe had both termini accessible, thus allowing to modify miRNAs directly at either 3' hydroxyl or 5'-monophosphate terminus after the hybridization. Further amplification is achieved by the subsequent hybridization of gold nanoparticles functionalized with poly(T) or streptavidin, which respectively bind to the appended poly(A) or biotin tails on modified miRNA. The presence of the nanoparticle greatly enhances the SPRI signal, demonstrating its utility in recognition of miRNA-bound PNA probes by allowing the detection of miRNAs in the low nanomolar range.

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O16
Development of aptamer-based biosensors designed for targeting small molecules and proteins

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Development and implementation of high sensitive and effective biosensors heading to detection of a wide range of proteins and small molecules belong to one important, challenging and still emerging field of research [1]. The purpose of this work is to present different biosensing arrays, competent enough to recognize affinity interactions occurring between single stranded DNA oligonucleotides -aptamers- and specific biomolecules involved in physiological functions and food analysis.

Among investigated analytes are: Human thrombin, a serine protease responsible for coagulant and anticoagulant process in blood, Ochratoxin A (OTA) a contaminant mycotoxin present in foodstuff [2], Cellular prions (PrP^c) referred to pathogenic agents able to induce abnormal folding of specific normal cellular proteins causing neurodegenerative brain disorders, and Vascular Endothelial Growth Factor (VEGF) a signal protein that stimulates vasculogenesis and angiogenesis, crucial processes related to cancer tumor formation [3].

Different chemistry assessments for immobilization of probes on solid supports, as well as diverse transducers, have been employed in order to assemble the referred aptamer-based biosensors.

Additionally, the incorporation of nanomaterials including carbon nanotubes, synthetic polymers and gold nanoparticles as signal enhancers or assay supporters, have made possible the construction of more compact and stable biosensors.

Validation of the sensors has been carried out both in buffering and in analyte-spiked samples by means of electrochemical and optical techniques obtaining satisfactory responses. Achieved results might potentially be considered for practical applications and certainly contribute to broadening aptamers using in biosensing.

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O17
Magnetically responsive biologically active compounds in bioanalysis

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Magnetic techniques based on the application of magnetic nanoparticles and microparticles as carriers for the immobilization of target biologically active compounds have been successfully used for bioanalysis of different types of analytes. Examples of beneficial applications of immobilized antibodies, enzymes, DNA and aptamers for the determination and detection of different types of xenobiotics (e.g. herbicides, insecticides, fungicides, aromatic and polyaromatic hydrocarbons, pentachlorophenol and heavy metal ions), biologically active compounds (e.g., clinically important molecules or toxins), viruses, microbial pathogens (e.g., *Salmonella, Listeria* or verocytotoxin producing *Escherichia coli*), protozoan parasites (*Cryptosporidium* and *Giardia*) and tumour cells will be presented [1,2].

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O18
Copper-free click chemistry as a novel tool for the programmed ligation of DNA-functionalized gold nanoparticles

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Controlling nanoparticle self-assembly in an effective and easy way can have many applications in several scientific fields such as nanophotonics and metamaterials, nanoelectronics and nanodiagnostics [1]. The employment of DNA as a scaffold for the organization of nanoparticles is exceptionally attractive and has been utilized to arrange nanoparticles into dimers, trimers or more complex structures. Among various strategies to covalently link nanoparticles, the use of 'biomolecular tools' has been shown as a unique approach to prepare gold nanostructures with a high level of programmability and complexity [2]. In those studies, restriction and ligation enzymes have been employed for the selective manipulation of DNA sites on gold nanoparticles (AuNPs). The utilization of these biomolecules has inherent advantages deriving from their cleavage or ligation specificity at desired sequences. This enables multistep nanostructure syntheses. However, these enzymes are only functional at specific temperatures, certain pH and ionic strength conditions. Additionally, they can be easily denatured or interact with the surface of particles. These limitations make their general applicability difficult.

Here, we demonstrate a new method to program the ligation of single stranded DNA-modified gold nanoparticles using copper–free click chemistry. AuNPs functionalized with a discrete number of 3'-azide or 5'-alkyne modified oligonucleotides can be brought together via a splint strand and covalently 'click', in a simple one-pot reaction. This new approach is inherently advantageous in comparison to traditional enzymatic ligation. The chemical ligation takes place at room temperature by simply mixing the particles without the need for special enzymatic conditions. Additionally, using a strained cyclooctyne as the reactive alkyne allows for clicking via a ring-strain promoted alkyne-azide [3+2] cycloaddition and thus eliminates the need for a Cu^{I} catalyst [3]. The yield of 'clicked' nanoparticle dimers was measured as high as 92%. The ease of the copper-free, 'click-ligation' method allows for its universal applicability and opens up new avenues in programmed nanoparticle organization.

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P1
Signal amplification for thrombin impedimetric aptasensor: use of gold-streptavidin nanoparticles

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Electrochemical impedance aptasensor are a powerful and sensitive alternative for the development of biosensors detecting proteins. Some of their advantages are they are easy-to-use, simplicity of concept, cost-efficiency and high sensitivity. In this work, we report a highly specific aptamer biosensing method with specific amplification strategy, used for ultrasensitive detection of thrombin using gold-streptavidin nanoparticles (Strep-AuNPs) and silver deposition. Biotinylated aptamer of thrombin (Apt1) was immobilized onto avidin-graphite epoxy composite (AvGEC) electrode surface by affinity interaction between avidin and streptavidin, and the impedance measurement was performed in a solution containing the redox marker ferrocyanide/ferricyanide. The change of interfacial charge transfer resistance (R_{ct}) experimented by the redox marker, was recorded to confirm aptamer complex formation with target protein, thrombin (Thr), in a labelless first stage. A biotinylated second thrombin aptamer (Apt2), with complementary recognition properties, was next used in a sandwich approach. The addition of strep-AuNPs and silver enhancement treatment led to a further increment of R_{ct} thus obtaining significant signal amplification. The Apt1-Thr-Apt2 sandwich formation was inspected by confocal microcopy after incubation with streptavidin quantum dots. In order to visualize the presence of gold nanoparticles, the same silver enhancement treatment was applied to electrodes already modified with the nanoparticles-sandwich conjugate, allowing direct observation by scanning electron miscroscopy (SEM). Results showed high sensitivity and selectivity for thrombin detection, in an improvement from ca.4.7 pM in a simple assay, to 0.3 pM in the amplified reported scheme.

P2
DNA aptamer-based biosensor for sensitive thrombin detection

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Thrombin, a kind of serine protease, plays important role in the coagulation cascade, thrombosis and hemostasis. The high-picomolar range of thrombin in blood was known to be associated with diseases so that it was important to assess this protein at trace level with high sensitivity. Development of the sensitive method of the thrombin detection is important for clinical practice. The traditional methods of thrombin detection are based on the antigen-antibody immunoassay, clotting – based assay etc. Alternative method of thrombin detection is based on biosensors using DNA aptamers as receptors.

The binding of thrombin to the aptamers has been detected by thickness shear mode method in flow measuring cell. We used biotinylated DNA aptamer (BF, 5´- GGT TGG TGT GGT TGG TTT TTT TTT TTT TTT -3´- biotin). We compared two types of the aptamer`s surfaces formed on the gold electrode. First one, was formed from neutravidin (NA) on which BF was attached. Second surface was formed from tetrahedron DS3BT1 for oriented attachment of NA and than for BF binding. We studied the changes of resonant frequency, f_s, and the motional resistance, R_m, of the quartz crystal transducer after addition of thrombin. We observed decrease of f_s and increase of R_m. The changes of both parameters started already at 50 nM of thrombin. The increase of R_m suggests on increase of surface viscosity. The kinetics analysis of aptamer-thrombin system was performed. Binding experiments carried out using BF immobilized on neutravidin layer at gold surface showed that constant of dissociation is K_d = 114 nM. This constant is measure of the affinity of the interactions. The lower K_d, the higher affinity. However, lower affinity of thrombin to the aptamer was observed when DS3BT1 structures were used for aptamer immobilization (K_d =151 nM).

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P3
The topography of lipid layers containing calixarene-cytochrome c complexes by atomic force microscopy

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Cytochrome c (cyt c) participates in electron transport and it is responsible for activation of the apoptotic pathway through releasing from mitochondria into the cytosol. In particularly it has been shown that cyt c induced transition of lamellar phase composed of phosphatidylcholine, which is favorable for transport of cyt c through hydrophobic part of the membrane. The detection of endogenous concentration of cyt c is of high importance for diagnosis of possible pathological processes in the organism. Calixarenes (CX) are being used for detection of a wide range of compounds such as metal ions, amino acids or proteins. The synthetic receptor CX incorporated in supported lipid films was used for the detection of the cyt c.

Using atomic force microscopy we studied the topography of the self assembled lipid films composed of dimyristoylphosphatidylcholine (DMPC) contained CX and formed on freshly cleaved mica surface. The height difference between mica surface and upper part of DMPC layer was between 3 and 4 nm, which correspond to the lipid bilayer and agrees well with results published earlier. Interesting result were obtained for the layers incubated with 30 nM cyt c. The roughness of CX layer was about 5 times higher in comparison with that of DMPC, probably due to formation of monolayers and even multilayers of CX. Novel and surprising result has been obtained for mixed DMPC-CX layers at presence of cyt c. Incubation of these layers with 30 nM of cyt c resulted in transformation of rather rough multilayers into the relatively flat layers contained sharp fibers of cyt c.

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P4
Comparison of different fabrication techniques in aptamer biosensing for VEGF detection

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The vascular endothelial growth factor (VEGF) is important protein with multiple functions involving angiogenesis, vasculogenesis and blood vessel leakage. VEGF is also now known as highly specific mitogen for endothelial cells, which plays important role in tumor vascularisation and several other diseases [1]. Therefore, the development of method allowing detection of VEGF with high sensitivity is desirable for medical diagnostics. The biosensor technology is rather suitable for this purpose. Recently, the DNA aptamers, sensitive to VEGF were reported. Aptamers are short synthetic single-stranded RNA or DNA oligonucleotides that can selectively bind wide range of target molecules with high specificity and selectivity. It is possible to modify aptamers with various functional groups, which allowing their immobilisation on various supports [2]. Thus, the aptamers can be used as recognition elements in biosensors.

In this work we compared immobilisation techniques involving aptamers modified with different functional groups. The simplest method investigated was direct chemisorption of the thiolated aptamer to a gold surface of the electrode. Uncovered areas on the electrode were blocked with 2-mercaptoetanol to prevent non-specific interactions between the protein and gold surface. This method is simple, efficient and results in high sensitivity biosensor.

Another approach involved additional molecules tethered to the surface of the electrodes. When immobilizing aptamer modified with carboxylic functional group, we chose to implement multi-walled carbon nanotubes (MWCNTs) and poly(amidoamine) dendrimers (PAMAM) of fourth generation (G4), to promote electron transfer and to enlarge the area of the sensor. In this protocol, individual components were linked with peptide bond between carboxylic and amine groups of the nanostructures. The peptide bond formation was promoted using coupling reagents EDC and NHS.

Different fabrication method was used with aptamer modified with amine functional group. In this case we used cysteamine self-assembled monolayer at gold electrode surface. Glutaraldehyde was used to crosslink cysteamine molecules with dendrimers, which provided enhancement of the area available for aptamer immobilisation. The crosslinking between aptamers and dendrimers, both containing amine functional groups, was again carried out with glutaraldehyde. The Schiff bases formed on glutaraldehyde during crossliking were reduced with $NaBH_4$.

Obtained data were analyzed and plotted to determine the nature of interactions in the fabricated systems. The results of cyclic voltammetry and electrochemical impedance spectroscopy measurements show potential of these electrochemical aptasensors in future research and applications. The proposed approach offering cheaper, faster and more sensitive option in comparison with antibody-based assays.

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P5
Development of a label free immunoassay quartz crystal microbalance (QCM) based for Aflatoxin B1 determination

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Quartz crystal microbalance (QCM) has gained exceptional importance in the fields of (bio)sensors, environmental monitoring, and electrochemistry due to the phenomenal development in QCM-based sensing during the last two decades [1,2]. The QCM immunosensor is usually comprised of a quartz crystal with an antigen or antibody immobilized on its surface, which allows the label-free detection with a direct quantification of the immunocomplex (Ab–Ag). In this study, we have used a direct immunoassay where the simple binding between antigen and an antibody is detected [3]. Immunoassays were performed in a drop system, monitoring the frequency decrease of the quartz–crystal microbalance device because of mass increasing during immunoreaction. The QCM sensor was coated on both sides by gold electrodes, only one side of the crystal (liquid side) was in contact with the solution; the other side (contact side) was always dry. We tested a piezoelectric immunosensor for aflatoxin B1 (AFLA-B1) mycotoxin detection through the immobilization of DSP–anti-AFLAB1 antibody (AFLA-B1–Ab anti AFLAB1) on gold-coated quartz crystals (AT-cut/5 MHz). The DSP (3,3'-Dithiodipropionic-acid-di-N-hydroxysuccinimide ester) was used for the covalent attachment of the proteins. The DSP-coated crystals were installed in a sample holder and exposed to the anti-AFLAB1 antibody and to the AFLA-BI. Frequency and resistance shifts (Δf and ΔR) were measured simultaneously. Δf versus AFLA-BI concentrations in the range of 0.5–10 ppb exhibited a perfect linear correlation with a coefficient of above 0.995. The QCM based sensing label free procedure for Aflatoxin B1detection, developed in our laboratory, can be considered a simple, cost effective, real time and no time and labor consuming technique in comparison with conventional assay procedures, as GC chromatography.

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P6
Use of G-quadruplexes as biosensors

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DNA and RNA play key roles in controlling the function and behavior of cells through the central processes of transcription and translation. The formation of DNA secondary structures called G-quadruplexes can interfere the biological processes that are crucial to sustain cellular homeostases and metabolism via mechanisms that include transcription, translation, splicing, telomere maintenance and DNA recombination [1]. The G-quadruplex structure consists of planar stacks of guanine tetrads stabilized by eight Hoogsteen and Watson–Crick hydrogen bonds. The overall G-quadruplex structure may be formed from either intramolecular or intermolecular arrangement of guanine rich oligonucleotides [2].

The extensive structural polymorphism of G-quadruplexes has rendered them as attractive signal-transducing elements for the development of DNA-based probes. In the recent years, immense efforts have been invested into the development of specific probes for detecting and distinguishing the G-quadruplexes from other DNA conformations likely to be present in the cellular environment, including the predominant double helix [3]. Guanine quadruplex structures are one kind out of numerous structures which are capable of adopting aptamers. DNA and RNA aptamers are single stranded oligonucleotides which, under certain conditions, fold into 3D structures containing specific binding sites for low or macro molecular compounds of various types, including cells, cell surface proteins, bacteria and viruses. DNA aptamers composed of G-quadruplexes usually contain supporting sections which allow them to be immobilized on solid substrates and serve as recognition elements in biosensors [4].

In this work, we analyze quadruplex-forming oligomers, which can participate in the creation of aptamers. The results were obtained using spectral (UV-Vis spectroscopy, circular dichroism) and electrophoretic methods (PAGE, TGGE).

The versatility of the G-quadruplex motif has vastly expanded the number of potential applications of G-quadruplexes in fields such as diverse therapeutic and diagnostic tools. The study and characterization of the properties of G-quadruplex structures can bring new insights to development of the binding of cancer therapeutics to G-quadruplexes.

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P7
Novel G-quadruplex structures found in Human Papillomaviruses

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Human papillomaviruses (HPV) are nonenveloped viruses composed of a double-stranded, circular DNA genome, approximately 8 kilobases in size. The genome is divided into long control region (regulating transcription and replication), 'early' region (composed of 6 open reading frames) and 'late' region (coding viral structural proteins) [1]. There are approximately 118 known types of HPV, which are characterized as high-risk (causing malignant transformation) or low-risk types (causing benign warts and lesions) [2]. HPV genomes were screening for purpose of finding new potential G-quadruplex sequences.

G-quadruplexes are non-B DNA structures consisting of π–π stacking planar G-tetrads, cyclically bound to each other through eight hydrogen bonds according to the Hoogsteen base pairs. G-quadruplexes show a high degree of structural polymorphism depending on the nucleotide sequences, the orientation of the strands, the syn/anti glycosidic conformation of guanines, the loop connectivities, and environmental factors such as cations, molecular crowding and dehydration [3,4].

Some potential G-quadruplex sequences were found in long control region (HPV 52, HPV 58) and inside the coding regions of L2 (HPV 57), E1 (HPV 32, HPV 42) and E4 (HPV 3, HPV 9, HPV 25).

The found sequences were studied by spectroscopic (circular dichroism- CD and UV spectroscopy) and electrophoretic methods (polyacrylamide – PAGE and temperature gradient gel electrophoreses-TGGE). Using these methods we are able to distinguish among the forms parallel/antiparallel conformation and where there are the multimeric conformers present.

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P8
Reversible functionalization of biosensing gold surfaces with a pH-sensitive avidin mutant

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Quartz crystal microbalance (QCM) is an extremely sensitive technique allowing to detect mass changes in the nanogram/cm^2 range with a wide dynamic range extending into the 100 microgram/cm^2. An AT-cut quartz crystal which has one gold electrode deposited on each side gets excited to oscillate at its resonance frequency by a voltage controlled oscillator (VCO). According to Sauerbrey's equation, the observed change in oscillation frequency of the crystal is in relation to the mass change per unit area at the gold-electrode surface of the quartz crystal. With the used QCM-device, a resolution up to 0.03 Hz or 0.37 ng/cm2 can be achieved. QCM can either be used in gas phase, under vacuum or in liquid environment, which allows a broad field of application like determining the affinity of molecules to surfaces functionalized with specific bait molecules.
For studying interactions between biomolecules such as the avidin-biotin-interaction, the quartz crystal has to get cleaned under harsh conditions (mixture of hydrogen peroxide and ammonia) after each functionalization. It would be desirable to reuse a sensor chip after a measurement series for cost and time reasons, a regeneration method was investigated. To allow subsequent functionalization with new biotinylated bait molecules, a pH-sensitive avidin mutant (M96H) was used, which is very robust and remains fully functional in weak acids (pH > 3). However, this mutant can easily be partly denatured by a mixture of citric acid (pH 2.0) and 0.25% SDS. As QCM is able to detect the binding of avidin M96H to a biotinylated surface due to a drop in resonance frequency, the regeneration process can be observed as an increase to the initial value, too. For demonstrating the reversible biofunctionalization of the quartz crystal, QCM measurements as well as surface plasmon resonance (SPR) measurements were deployed.

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P9
Exploring the energy landscape of hydrophobin-hydrophobin interactions using AFM single molecule force spectroscopy

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Hydrophobins are fascinating proteins which were until now only isolated in filamentous fungi. They are surface-active proteins consisting of a hydrophobic and a hydrophilic side. In nature they fulfill a lot of different functions in growth and development of fungi (e.g. they enable a growth through the air-water interface, they are responsible for the adhesion to the surface and they can form a protective coating on various fungal structures). Hydrophobins can be distinguished in two classes (class I and class I). The difference between these two classes is the solubility. Class I forms very insoluble aggregates compared to class II.

A feature that all hydrophobins have in common is the eight conserved cysteine residues. The average size of them is about 100 amino acids. Furthermore they are very stable, they can withstand temperatures near 100 °C. A further characteristic of hydrophobins is the ability to form 2D crystal (especially at the air-water interface). Due to their special properties, hydrophobins are expected to have a high potential in biochemical and biomedical applications (e.g. adhesion domains for immobilization of proteins and as foam forming agents).

Within this study we investigated the interaction forces of hydrophobins at the molecular level using AFM single molecule force spectroscopy. For this one single hydrophobin (containing an addressable thiol) was attached to the outer tip apex whereas the support surface, a gold coated chip, was functionalized with hydrophobins directly using the thiophil properties of sulfur. Performing multiple force distance cycles using this configuration allowed us to investigate the interactions and to determine the off-rate of this system.

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P10
The physical properties of lipid monolayers and bilayers modified by calixarenes with the afinity towards cytochrome c

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We studied physical properties of the monolayers and bilayer lipid membranes (BLM) formed by calix[6]arene carboxylic acid derivative (CX) and its mixtures with diphytanoylphosphatidylcholine (DPhPC) by means of measurement surface pressure, surface dipole potential and electrostriction. CX forms stable monolayers at an air-water interface. It forms complexes in mixed monolayers containing DPhPC. Calixarenes increase the elastic modulus of both monolayers and BLM. Cytochrome c (cyt c) affected the physical properties of CX monolayers depending on their initial surface pressure. Addition of cyt c into the water subphase induced increase of surface pressure of CX monolayers at relatively low initial pressure (15 mN/m) when monolayer was in liquid-expanded state (LE). This may be due to the interaction of positively charged cyt c with negatively charged carboxylic groups of CX, and also by its penetration into the air-water interface. However, much subtle changes were observed for higher initial surface pressure (20 and 35 mN/m) when monolayer is in liquid-condensed (LC) and solid (S) state, respectively [1]. Then, we compared effect of cyt c with lysine on the surface properties of the monolayers composed of CX. We found that lysine induces increase of surface pressure of CX monolayer at relatively low initial surface pressure value (5 mN/m). However, at least 10 fold higher concentration of lysine was necessary to induce comparable effect with that caused by cyt c.

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P11
Crystal morphological characterization of novel self-assembling chitosan-ssDNA hybrids

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Nowadays, much research efforts are devoted to the self-assembly mechanism especially for potential applications in nanotechnology. By using the self-assembling nature of artificially designed molecules, chemists have succeeded in constructing various types of nanometer-sized molecular assemblies, e.g. polymer assemblies [1,2], self-assembled monolayers [3] and so on. A crystal is a typical example of the ordered integrated states spontaneously formed by "self-assembly". The phenomenon of crystallization affects the self-assembled nanostructures and creates unique and interesting morphologies [4,5]. We are particularly focusing on the coupling between a DNA fragment (ssDNA) and a suitable hydrophobic segment to achieve self-assembling biocompatible copolymers. Our recent results on morphological characterization of newly synthesized self-assembling chitosan-ssDNA hybrids through surface characterization techniques such as Atomic Force Microscopy (AFM) will be presented (Fig. 1). Crystallization was carried out on several substrates such as silica, gold and mica aiming at elucidating the mechanism of crystallization and the role of intra- and intermolecular interactions between the self-assembled biopolymer-oligonucleotide hybrids to achieve a comprehensive understanding of the process of organization of these novel copolymers.

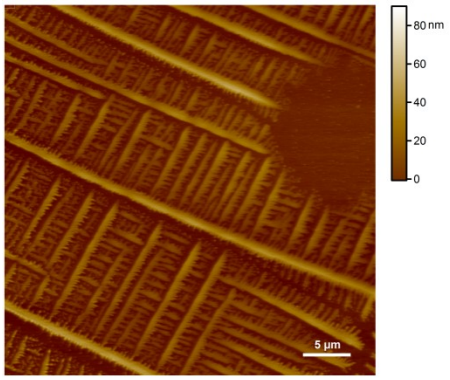

Fig. 1. Crystallization self-assembly of chitosan-g-ssDNA on silica surface

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P12
PAMAM dendrimers modified with DNA and dextran sulfate in drug delivery

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The transport of deoxyribonucleic acids (DNA) into the cells is crucial for gene therapy interest and promises to treat a variety of serious human diseases. To achieve this goal, the external DNA has to reach the cell cytoplasm and nucleus, where the transcription and exertion of its intended action will take place. However, there are several obstacles, that DNA has to face with on this road. Firstly, DNA has to cross the cell membrane. In order to avoid a degradation of DNA, nucleic acids have to be complexed together with gene carriers.

In this work we have studied interaction of potential non-viral vectors - cationic poly(amidoamine) dendrimers (PAMAM) of fourth generation (G4) and their complexes with anionic polyelectrolyte dextran sulphate (DS) and DNA with 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) lipid monolayers and large unilamellar vesicles (LUV). The aim of this work was to explore the mechanisms of interaction of pure G4 dendrimers and G4 dendrimers modified with DS and DNA with lipid membranes via measuring the surface pressure of lipid monolayers by means of Wilhelmy method. We used also the ultrasonic spectroscopy for investigation thermodynamic properties of LUV at presence of PAMAM and those modified by DS and DNA. The complexes were characterized by measuring the size and Zeta potential using dynamic light scattering method. We have shown that dendrimers can be incorporated into the monolayer at subphysiological surface pressures (< 30 mN/m), so they are expected to be useful in the design of dendrimer-based drug carriers. Dendrimer-DS and Dendrimer-DS -DNA complexes were able to induce increase of the lipid monolayer pressure, which is evidence on their incorporation into the monolayer. The effects of polymer nanoparticles on the thermodynamic properties of LUV are discussed as well.

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P13
Labelling of living cells with magnetic nanoparticles for applications of cell patterning

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Aim of the work: The use of magnetic nanoparticles (MNPs) for labelling living cells is useful for numerous biomedical applications such as biosensing applications, cell manipulation, cell patterning for tissue engineering and magnetically-assisted cell delivery. The greater advantage of magnetic-based methods is to activate or monitor cell behaviour by a magnetic field. Cell labelling methods using iron oxide nanoparticles have been previously developed [1], showing no adverse effect on cell proliferation and functions while conferring magnetic properties to various cell types. The perspective is to develop a soft lithography magnetically facilitated to control the spatial organization of mammalian cells assembled with MNPs. This technique will be based on *MNPfunctionalized cells* and the use of poly(dimethylsiloxane) (PDMS) [2].

Materials and methods: This work shows a method to functionalize several cell lines with magnetic nanoparticles (Magnetite) coated with silicon and calcium via a single-step biocompatible process by mean of electrostatic interactions. The nanoparticles were characterized with XRD, TEM, FESEM and DSC-TGA. The content of the superficial layer was characterized by spectroscopic analysis. The interaction between cells and nanoparticles were characterized concerning cytocompatibility. In addition, cells were functionalized by immersion of sterile MNPs suspension (0.6 mg mL^{-1}) in 0.15 M NaCl and after separating *MNPfunctionalized cells using a permanent magnet.*

Essential results: Magnetic nanoparticles were localized on the cellular membranes and do not penetrate cross the plasma membrane. The magnetically responsive cells were viable and able to colonize and grow on culture plates. In summary magnetically facilitated positioning and migration of functionalized cells into viable living clusters was demonstrated.

Conclusion: Magnet-facilitated spatial distribution on the culture plates could be potentially extended to controlled deposition and magnetic-field-oriented growth of functionalized cells into more complex systems. This technique could be used to study intercellular communication in patterned co-cultures, to realize cell-based sensors and to develop a wound healing assay based on migration of murine endothelial cells (MS1) magnetically oriented or a migration in the liver of a mouse after a tail vein injection.

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