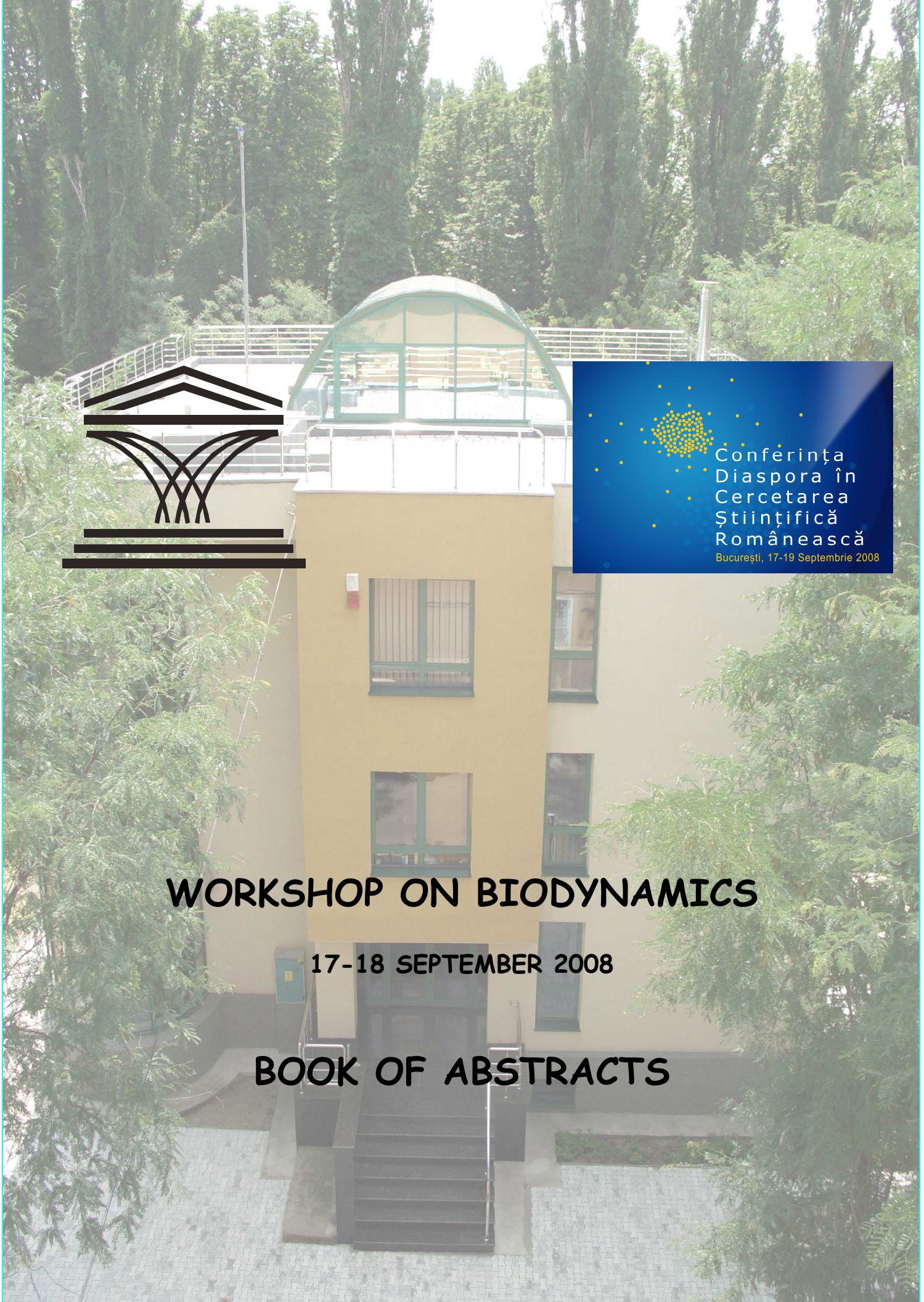

Conferința Diaspora în Cercetarea Științifică Românească
București, 17-19 Septembrie 2008

WORKSHOP ON BIODYNAMICS

17-18 SEPTEMBER 2008

BOOK OF ABSTRACTS

Contents

TOWARDS QUANTITATIVE MODELS OF TRANSCRIPTION ELONGATION AND TERMINATION

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Transcription, the process by which the genetic information encoded in DNA is transferred into RNA, is the first step in protein synthesis and it is the step at which most regulation of gene expression occurs. A detailed understanding of the structural and mechanistic aspects of each step of transcription (initiation, elongation, termination and regulation) is one of the holy grails of biology. Here we characterize the motion of RNA polymerase (RNAP), the multi-subunit molecular motor that carries out the transcription process, as it proceeds along DNA. We argue that during elongation RNAP moves by a complex Brownian ratchet mechanism in which the translocation along DNA and the binding of nucleotides into RNAP's catalytic center are coupled to a fluctuating internal degree of freedom associated with a protein sub-unit (G-loop/F-bridge) of RNAP. This model is used to explain a number of biochemical and kinetic experiments on mutants of RNAP. We also use the same general model to predict pauses in bacterial transcription, places along DNA where RNAP stalls either reversibly (pauses) or irreversibly (arrests). Our pause-prediction algorithm is based on the thermodynamic stability of the elongation complex along the DNA template calculated from the sequence dependent free-energy of DNA-DNA, DNA-RNA and RNA-RNA base pairing associated with (a) the translocation and size fluctuations of the transcription bubble; (b) changes in the associated DNA-RNA hybrid; and (c) changes in the co-transcriptional RNA secondary structure upstream of the RNA exit channel. The same thermodynamic arguments can be used to characterize the stability of the elongation complex at specific termination sequences, places along DNA where, with high probability, RNAP releases the RNA transcript and disengages from the template. In more general terms, some of the modeling to be presented raises fundamental issues related to "model comparison" and "model selection", the problem of identifying and characterizing quantitative models on the basis of limited sets of experimental data.

NONTRIVIAL SYNCHRONIZATION OF MULTIMODE STOCHASTIC OSCILLATORS

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An ensemble of biologically inspired oscillator system is investigated. The oscillators are stochastic elements capable of emitting pulses and detecting the pulse emitted by the others. They have several operational modes, characterized by different oscillating periods. Shifting between these modes is induced by a simple optimization rule: the average output intensity is kept around a fixed G threshold. This simple dynamical rule realizes the coupling of the elements and leads to complex collective behavior. Computer simulations indicate that for a given interval of the G parameter partial synchronization of the elements can occur. The appearance and disappearance of this synchronization as G is changed indicates a phase-transition type behavior. The observed synchronization is highly nontrivial, since no phase-difference minimizing interactions are considered in this system. Synchronization is a co-product of the considered output optimization. Moreover, one can observe that the periodicity of the output for the ensemble is better than the periodicity level of a single element, leading to several possible practical applications as well. A practical realization of the system is also considered. A system of electronic oscillators capable of emitting and detecting light-pulses is build and experimentally investigated. Experimental results confirm the predicted nontrivial collective behavior.

NUCLEOTIDE GENOMIC SIGNALS: A MOLECULAR INVESTIGATION TOOL FOR EARLY DIAGNOSIS AND DETECTION OF PATHOGEN DRUG RESISTANCE

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Nucleotide genomic signal (NuGS) analysis reveals surprising regularities in the distribution of nucleotides and pairs of nucleotides in both prokaryote and eukaryote genomes, regularities that would be difficult to identify by using only standard symbolic sequence analysis. But NuGS analysis is also efficient in the analysis of local structural features, such as genomic inserts or polymorphisms determined by pathogen variability. This is important for the molecular level detection of mutations that induce pathogen drug resistance, providing the clinician with the information needed for fast and accurate decisions

CONFINEMENT EFFECTS ON DIFFUSIOPHORETIC SELF-PROPELLERS

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Abstract — We study theoretically the effects of spatial confinement on the phoretic motion of a particle, which generates density gradients of its solvent by chemical reactions active on certain parts of its surface. In three spatial dimensions, the presence of confining walls to an increase in the composition gradients responsible for propulsion, but the velocity decreases due to a competing increase in the viscous friction exerted by the surrounding medium. In two dimensions, e.g., for the motion through an adsorbed film or a membrane, or at a liquid-gas interface, the composition gradients also increase with confinement while the change in the viscous friction is governed by a length scale that is different from that of the confinement. Depending on the system, the velocity may be increased or decreased by confinement, and an optimal confinement may exist for which the velocity is maximal.

Keywords - diffusion, phoresis, hydrodynamics, confinement

Introduction

Last years have witnessed a growing technological, experimental, and theoretical interest in scaling standard machinery down to micro- and nano-scales, such as producing pumps, motors, or sieves needed for the development of ``lab on a chip'' devices [1]. For applications in, e.g., drug-delivery systems or micromechanics, one of the most challenging problems at this stage is to develop ways to enable small-scale objects to perform autonomous, controlled motion. Inspired by biological nano-engines that are using catalytic reactions to extract energy from the environment (e.g., kinesin moving along microtubules is a molecular motor that uses hydrolization of ATP as the energy source), Whitesides and co-workers proposed a design of self-propelling devices based on an asymmetric decoration of the surface of small objects by catalytic particles promoting a chemical reaction in the surrounding medium [2].

Following the idea of the asymmetric surface distribution of catalysts, Golestanian et al. have recently studied a model system in which the autonomous motion emerges as a result of self-created diffusiophoretic gradients. Based on the model used in [3], here we address the effects of spatial confinement on the diffusiophoretic motion of a self-propelling particle [4].

Model

The system we consider is shown in Fig.1 It consists of an impermeable, spherical particle (disk in *2d*) of radius R with a point-like catalytic site (black dot in Fig.1) on its surface (perimeter in *2d*), which promotes a chemical conversion of a surrounding solvent into product particles (small hatched circles in Fig.1). The particle and the surrounding solvent of viscosity μ_3 are enclosed in a concentric, impermeable, spherical shell of radius $R_1 = \eta R$ ($\eta > 1$). For the *2d* system, the disk and the surrounding solvent (adsorbed monolayer, thin film, or membrane) of viscosity μ_2 are enclosed by a concentric circular ring of radius R_1, while the subphase of viscosity μ'_3 and depth h is assumed to be practically unbounded laterally [5]. The thicknesses of the disk, of the solvent phase, and of the ring are assumed to be negligible.

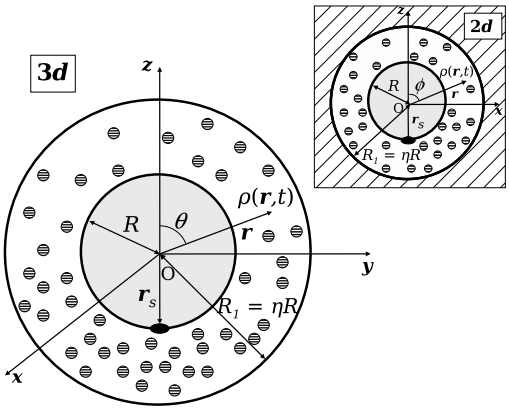

Figure 1. Schematic drawing of the model system. In *2d* the diagonally hatched area indicates the subphase.

We assume that the reaction at the catalytic site acts effectively only as a point-like source of product molecules, which are diffusing in the solvent with diffusion coefficient D; we will consider explicitly only the particular case in which the activity of the catalytic site is time independent for t > 0. We also assume the typical velocities to correspond to small Reynolds numbers Re (which is a reasonable assumption for phoresis [6]) and the diffusion of the product molecules to be fast, corresponding to the limit of small Peclet number. (Thus the diffusion field is unperturbed by the flow.) The time evolution of the number density $\rho(\mathbf{r},t)$ of product molecules around the moving particle is then governed by the diffusion equation:

$$\partial_t \rho = D\nabla^2 \rho + A(t)\delta^3(r - r_s) \qquad (1)$$

subject to the initial condition of zero density of product molecules and to the boundary conditions (BC) of zero normal current on the surface of the particle and on the confining wall.

Such an asymmetric, non-uniform distribution of products $\rho(\mathbf{r},t)$ induces solvent flow around the particle and leads to phoretic motion along the z direction [6]. Specifically, the density distribution $\rho(R,\theta;t)$ [$\rho(R,\phi;t)$ in *2d*] on the surface of the particle (characterized by the tangent unit vector $\mathbf{t}$), which is obtained by solving Eq. (1) subject to the corresponding BC, is mapped onto a distribution of slip velocities which serves as BC for the solvent [3,6]. After solving the Stokes hydrodynamics for the solvent, subject to the slip BC on the surface of the particle and stick BC on the wall at R_1 [7], the velocity of the particle is obtained by requiring that its motion is 'force-free' [6]. Therefore, knowledge of the distribution of products $\rho(\mathbf{r},t)$ as a function of η for confined (i.e., $\eta > 1$ and finite) *3d* and *2d* systems will allow us to quantify the effect of confinement on the phoretic motion of the particle.

Discussion and Conclusions

The solution of Eq. (1) reduces to a calculation of the Green's function for the corresponding boundary condition, and it is computed in a standard manner via Laplace transform and decomposition into a singular part picking-up the point source and a regular part that is a solution of the homogeneous Helmholtz equation. The general result is given by rather involved expressions and thus we will discuss here only the asymptotic $u = D\ t/R^2 >> 1$ behavior of the velocity, $V^{(\infty)}$, which in both *3d* and *2d* is given by the product between the unbounded space ($\eta \to \infty$) value V_0 and a confinement factor:

$$V^{(\infty)}(\eta)/V_0^{(3d)} = \frac{\eta^3+2}{\eta^3-1}\left(\frac{\eta(\eta^5-1)}{\eta^6-(9/4)\eta^5+(5/2)\eta^3-(9/4)\eta+1}\right)^{-1}, \quad \text{in } 3d, \quad 4)$$

$$V^{(\infty)}(\eta)/V_0^{(2d)} = \frac{\eta^2+1}{\eta^2-1}\begin{cases} 1, & \text{for } \eta > \eta_b = \xi/R, \\ \dfrac{\ln(2\eta)-\gamma}{\ln(2\eta_b)-\gamma}, & \text{for } \eta < \eta_b, \end{cases} \quad \text{in } 2d, \quad 5)$$

where $\gamma \cong 0.577$ is Euler's constant and the length scale ξ is given by. $\xi = (h\ \mu_2/\ \mu_3)^{1/2}$ [4]. These results are shown in Fig. 2.

In *3d*, with increasing confinement the viscous friction increases and is dominant, thus the velocity $V^{(\infty)}$ decreases and approaches zero as $\eta \to 1$. Thus, the efficiency of the self-propelling device decreases, and (extrapolating to more general geometries of the confining wall) this may become critical once one of the typical length scales of the systems is comparable with the size of the particle.

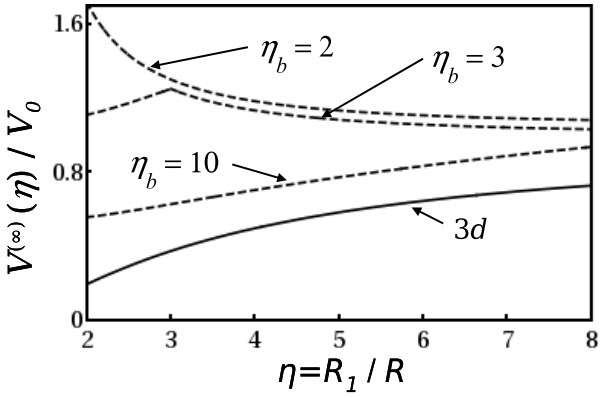

Figure 2. Asymptotic velocity as a function of the confinement.

In contrast to the *3d* case, in *2d* the behaviour of $V^{(\infty)}$ of depends on the additional length scale ξ as discussed below and shown in Fig.2. If $\xi > \approx R$, one typically has $\eta > \eta_b$, in Eq. (5) only the first line applies, and $V^{(\infty)}(\eta)$ increases with increasing confinement (thus the propulsive mechanism is enhanced in the presence of confining walls); this is the case $\eta_b = 2$ shown in Fig.2. If $\xi >> R$, typically only the second line in Eq. (5) applies and the velocity is a decreasing function of confinement); this is the case $\eta_b = 10$ shown in Fig.2. Finally, if ξ is just somewhat greater than R, e.g., $\eta_b \sim 3$, for weak confinement the wall effects on the propulsive gradients will dominate and the velocity will increase with increasing confinement, while for strong confinement the wall effects on the viscous friction will dominate and the velocity decreases with increasing confinement. Therefore, in this case there will be an optimal confinement corresponding to $\eta \cong \eta_b$, for which the velocity of the self-propeller is maximal.

A direct experimental realization of the co-moving geometry discussed above seems to be difficult. But the theoretical results are expected to apply (at least qualitatively) to more

general situations, such as the motion of spherical particles along cylindrical tubes or disk-like particles along a long rectangular stripe of an adsorbed layer. Therefore, an experimental testing may be possible.

References

[1] W.E. Paxton, A. Sen, and T.E. Mallouk, Chem. Eur. J. 11, 6462 (2005); E. Paxton, S. Sundararajan, T.E. Mallouk, and A. Sen, Angew. Chem. Int. Ed. 45, 5420 (2006).

[2] R.F. Ismagilov, A. Schwartz, N. Bowden, and G.M. Whitesides, Angew. Chem. 114, 674 (2002); Angew. Chem. Int. Ed. 41, 652 (2002).

[3] R. Golestanian, T.B. Liverpool, and A. Ajdari, Phys. Rev. Lett. 94, 220801 (2005).

[4] M.N. Popescu, G. Oshanin, S. Dietrich, *in preparation*.

[5] See, e.g., P.G. Saffman., J. Fluid. Mech. 73, 593 (1976).

[6] J.L. Anderson , Ann. Rev. Fluid. 21, 61 (1989).

J. Happel and H. Brenner, *Low Reynolds Number Hydrodynamics* (Noordhoff International, Leyden, 1973), p. 130

ON THE VORTICAL STRUCTURES OF COMPLEX FLUIDS IN MICRO-CHANNELS

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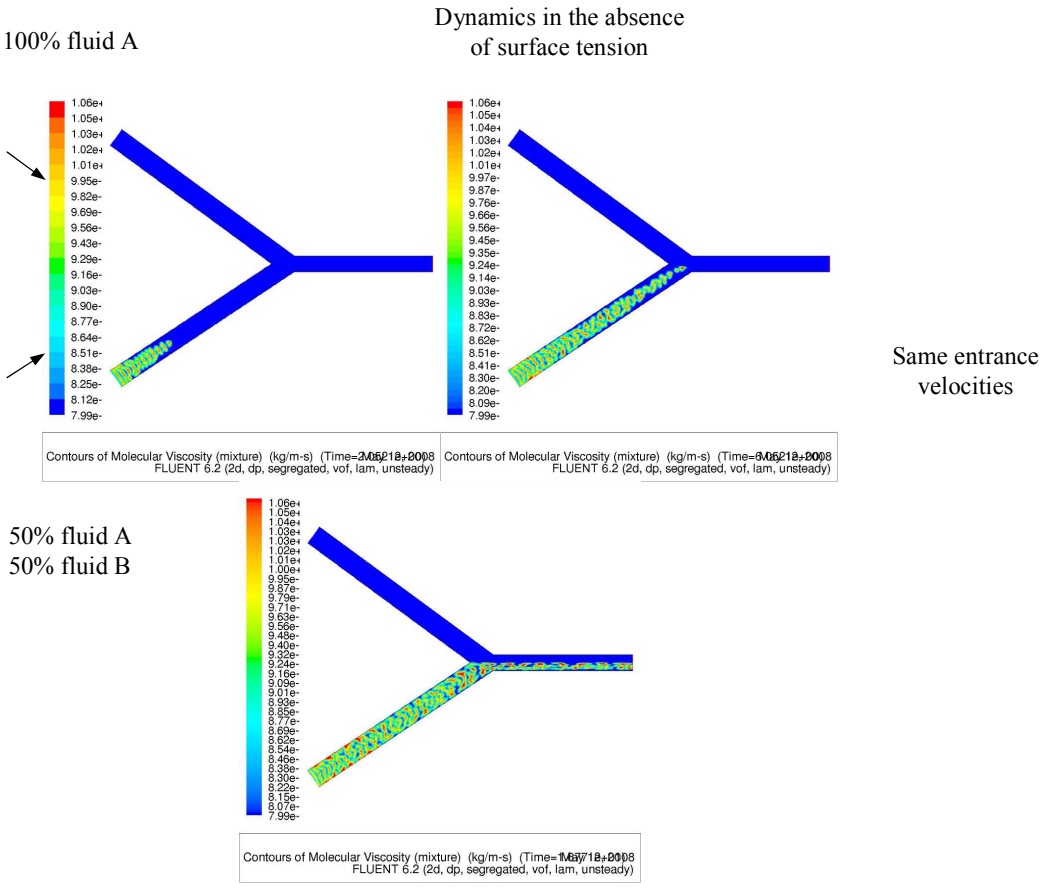
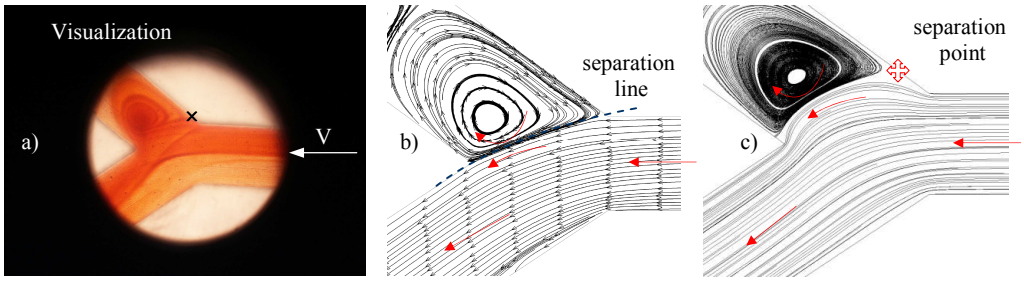
The lecture is dedicated to the experimental studies and modelling of complex fluids flow in a micro-channel bifurcation. The work is focused to the investigation of vortices generation in the vicinity of the separation boundary between two immiscible fluids: (i) a weakly elastic polymer solutions based on the mixing between polyacrylamide and water and (ii) a Newtonian oil of low viscosity and low density. One main goal of the study is to establish the influences of surface tension and viscosity ratio on the developing and structure of the vortex in one branch of the bifurcation. The experiments are performed with special designed setups based on optical and confocal microscopic devices, numerical simulations being performed with FLUENT and POLYFLOW codes.

The application of the work is directed to micro-fluidics of immiscible fluids (micro-bifurcations located in a Lab on a chip devices), in particular in mixing liquids with medium and high interfacial tension.



Numerical simulation of a flowing mixture (0.75 viscosity ratio) in a branched geometry.



Visualizations and numerical simulations of flow pattern in a bifurcation for the Newtonian fluid (the upper branch is blocked); separation point is marked (channel width: 0.7 mm, V = 0.255 m/s, *Re* = 180).

Analytical approach for strain and piezoelectric potential in conical self-assembled quantum dots

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The problems of strain and piezoelectric effect are solved for a buried semiconductor quantum dot (QD) of conical shape with wetting layer, within continuum elastic model. An extension of Eshleby's continuum elasticity theory of inclusions [J. D. Eshelby, Proc. R. Soc. London, Ser. A, 376 (1957)] to QDs with wetting layers is adopted. The results for self-assembled InAs/GaAs QDs are compared with numerical results obtained with an atomistic model based on the valence force field method. We find good agreement between the results obtained with the two methods. Analytical results for the strain obtained for the isotropic case of the elasticity, are presented in Figure 1. For the isotropic dielectric constant case, we provide an analytical expression of the piezoelectric potential. Results are presented in Figure 2.

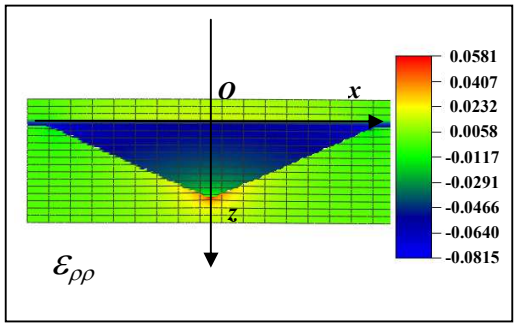


Figure 1a

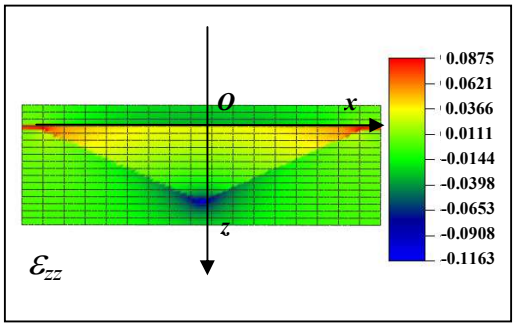


Figure 1b

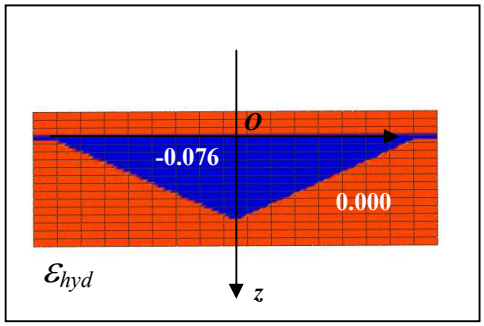
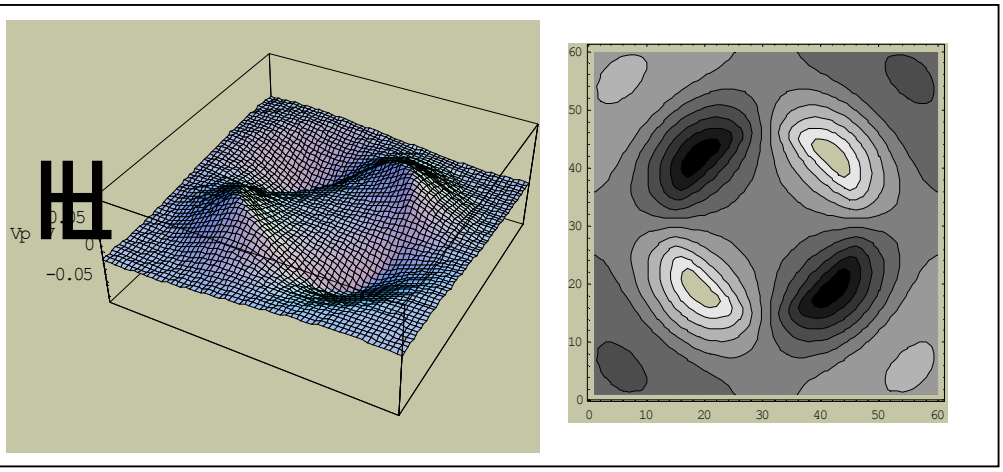
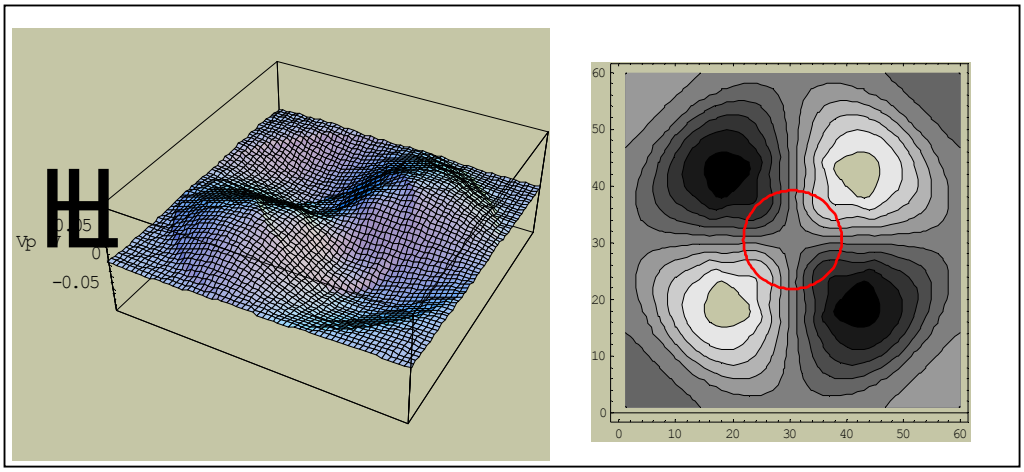


Figure 1c

Figure 1. The strains in the plan perpendicular on the base of the conical QD and containing the *Oz* axes: **a)** the diagonal strains ε_{zz}; **b)** the strains $\varepsilon_{\rho\rho}$. Simulations for QD with cone angle $\alpha = \pi/3$, and radius $R = 100\text{Å}$, and wetting layer $d = 5\text{Å}$.





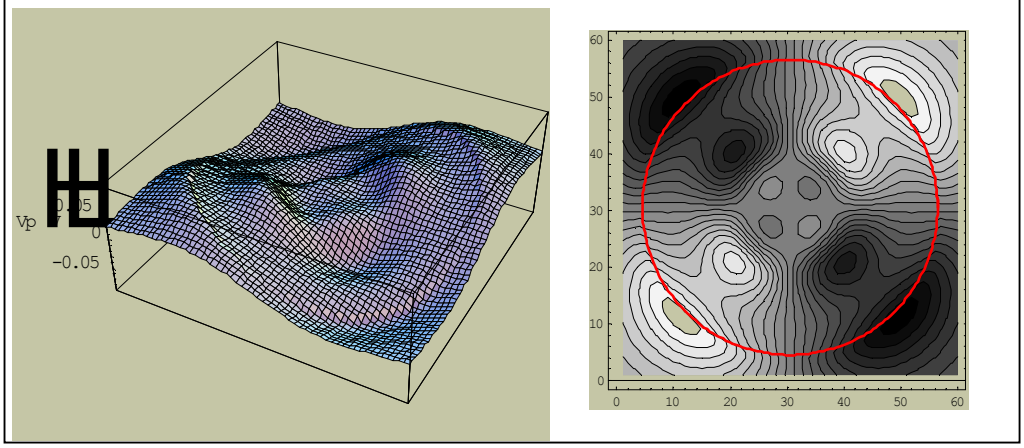


Figure 2. The piezopotential V_p: **a)** in the horizontal plan $z = 0$ containing the top of the cone; **b)** in the horizontal plan $z = H/3$; **c)** in the horizontal plan $z = H$. The left side figures are the three dimensional plots, and the right side figures are the contour plots. The red circles represent the cross-section of the QD with the horizontal plans. In the left side figures the positive equipotential surfaces are plotted. The right side figures are plots with a top view. Conical QD with the $H = 150$ Å and $\alpha = \pi/3$.

ASPECTS OF PATTERN FORMATION OUTSIDE OF EQUILIBRIUM

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Abstract **–** We present here some examples on some research of complex systems which focuses on pattern formation outside of equilibrium. The concepts and methods are largely inspired from the physics of disordered systems, but the basic ideas are related to structural stability and the physics of phase transitions as classes of universality.
In one example, a film of dimyristoylphosphatidylethanolamine (DMPE), made by Langmuir-Blodgett (LB) technique, was used to study the conditions and topology of coexistence of both liquid-expanded (LE) and liquid-condensed (LC) phases. Other examples described here are detachment pattern formation experiments using viscous liquids, the swarm pattern formation, and cellular automata modelling of pattern formation. From these and other experiments we reinforce the idea that emergence of order and stability of pattern are a typical results of the nonlinear complex systems even they do not evolve under long range forces that could be sometimes responsible for stability of the system.

MICROSISTEME ADAPTIVE IN MEDICINA - DE LA PROTEZE AUDITIVE LA CHIRURGIE NON-INVAZIVA

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Tehnologia microsistemelor se gaseste la confluenta dintre doua tendinte de evolutie moderne: microelectronica si tehnologia informatiei.
Prezenta lucrare dezbate necesitatea unei priviri unitare in proiectarea si simularea microsistemelor, unde reducerea de scara nu intotdeauna favorizeaza performantele ca senzori, si noi arhitecturi de sistem trebuie abordate. Posibilitatea unor bucle de reactie stranse intre domenii multiple energetice, specifica microsistemelor, avantajeaza o gandire de tip functional. In arhitecturile clasice functionalitatea e concentrata preponderent in anumite subsisteme, urmand ca celelalte sa efectueze corectii ce tin cont de imperfectiunile intalnite. Proiectarea e modulara, cu interfete ce reduc interactiunile intre diversele blocuri. Alternativa in gandire la microscara este de a genera o functionalitate care rezulta din interactiunea dintre module, de regula neliniara datorita legilor fizice. Aceasta alternativa poate conduce la aplicatii complet noi, sau imbunatati performantele unor aplicatii clasice.
Doua asemenea exemple sant prezentate. In primul dintre ele, cuplajul neliniar dintre o microstructura mecanica si un circuit electric este folosit pentru a genera un analizator de spectru mecanic in timp real.
Arhitectura poate fi folosita pentru definirea unui nou gen de proteza auditiva, unde separarea in frecventa a semnalului audio nu mai necesita operatii intensive, implementate de regula cu un procesor digital de semnale, si care reduc substantial timpul de viata al bateriilor.
Un alt doilea exemplu are ca scop dezvoltarea unui accelerometru foarte sensibil si de dimensiuni reduse, care sa fie folosit in chirurgia cu invazivitate redusa (« minimally invasive surgery »). Senzorul va face parte dintr-un grup de senzori care se ataseaza pe microscalpel si ii monitorizeaza pozitia in interiorul corpului pacientului.
Constrangerile unor dimensiuni reduse limiteaza sensibilitatea senzorilor, si din nou cai alternative, la nivel de sistem, trebuie cautate. In acest caz sistemul opereaza la granita lui de stabilitate prin aplicarea controlata a unei tensiuni putin peste valoarea critica de « pull-in » static. Dinamica microstructurii in regimul metastabil, care e traversat in evolutia catre instabilitate (« pull-in »), este foarte sensibila la acceleratii externe. Sensitivitatea ridicata e demonstrata prin posibilitatea de a masura zgomotul 1/f la nivel de microstructuri mecanice.

AUTOREGRESSIVE DESCRIPTION OF NATURAL PHENOMENABY

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Many natural phenomena can be described by power-laws of the temporal or spatial correlations. The equivalent in frequency domain is the 1/f spectrum. A closer look at various experimental data reveals more or less significant deviations from a 1/f characteristic. Such deviations are especially evident at low frequencies and less evident at high frequencies where spectra are very noisy. We ex-emplify such cases with four different types of phenomena offered by molecular biology (series con-sisting of the atomic mobility of the protein main chain), cell biophysics (flickering of red blood cells), cognitive psychology (series of mentally generated series of apparent random numbers) and astrophysics (the X-ray flux variability of a galaxy). Some of these cases can be better approximated by AR(1) -a first order autoregressive model, which is a short-range memory model - than by a 1/f model (long-range memory). The same spectra can be more or less easily confused and/or approximated by power-laws. On the other hand, an AR(1) model is only a zero approximation, which can be improved if more complex short-range correlation models, such as high order AR(p), ARMA, FARIMA models, are used. A key step to detect non-power laws in the spectra, already suggested by Mandelbrot, is to average out the spectra.

ASTRO-BIO-GEODYNAMICS - A TRANSDISCIPLINARY APPROACH TO THE LOVELOCK'S GAIA HYPOTHESIS.

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The Planet Earth System, is, as any complex system (or system supporting life), extremely difficult to define and characterize properly and completely. In the same time, presently mankind can be described as being now in an intense process of globalization in which the Informational Society is currently unfolding while, at the same time, the Knowledge-based Society may be regarded as emerging. Taking into account this general context, we attempt a definition of the Planet Earth System that extends beyond the natural structures (interactions between inorganic, organic / living systems as in the Gaia model) that are typically the object of study for *Geosciences* and/or *Biology* and *Biochemistry*. However, besides the Natural structures, man-made Artifacts have also been produced throughout the entire human history and, after adding onto/combining with the Natural ones, resulted in the present days in a complex symbiot. Natural and Artificial systems are now intermingled, interacting, inter-related and interdependent, thus permanently defining and influencing each other, giving rise to an entirely new and qualitatively different dynamics. This novel symbiosis and its dynamics are only partly controllable or predictable, and in order to be understood (even if only partially) they have to be examined using an interdisciplinary approach, a re-evaluation of the classical sciences, a fundamental change in the points of views and techniques used to study, model and predict such complex systems.

The paper presents some ideas, concepts, models and methodologies in order to reveal which are the best directions of study to be followed for the examination and understanding of this Artificial-Natural hybrid in order to extend the Lovelock's Gaia hypothesis and to define useful recommendations for a sustainable development.

BIODYNAMICS: WAYS AND MEANS TO APPRAISE THE INTERACTION BETWEEN SELECTED ENVIRONMENTAL STIMULI AND BIOSYSTEMS OF VARIOUS HIERARCHIES

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The concept of sensing and detection has to be readdressed in view of the huge number of analytes & stimuli including the environmental and biological risks of nanobiotechnology, to be assayed. Tremendous progress has been made in the ability to measure particular compounds at very low concentrations. However, evaluation of rare or previously unknown compounds, metabolites and mixtures is still presenting considerable analytical challenges, while being particularly relevant in terms of possible health effects.

Aiming to appraise gentle, non-lethal, effects triggered by different (environmental) stimuli, we have developed specific analytical tools (measuring devices as well as system modeling & advanced data analysis) suitable to assess:

1. A population of fish in aquarium;

The behavior of fish populations (carps and sturgeons) has been monitored using a multi channel echo-acoustic system. Analysis of related time series reveals "patterns" depending on day/light cycle and availability Oxygen. The effect of feeding has been investigated, as well.

2. Initially synchronized cell suspensions;

Cell cycle progression has been monitored by impedance / dielectric assay on cell suspensions with various degrees of synchrony. Advancing our previous studies, we have: a) prolonged the length of the time series (duration of cell growth) by preserving the "quality" of the culture medium; b) increased the signal-to-noise ratio by "local" enhancement of cell concentration and c) find an effective way to "freeze" the evolution of a cell suspension on a particular phase of the cell cycle.

3. Ensembles of cells adhered on a chip;

With the advent of concerns regarding hazardous effects of engineered (nano)particles and the documented "cocktail effect", novel analytical and predictive tools based on the interaction with bio-macromolecules or living cells are highly required.

Real-time monitoring of biomolecular recognition processes in living cells is a significant challenge for the next phase of genomics and proteomic technologies leading to improved understanding of cell – environment interactions and to powerful tools for fundamental research and applications.

Whereas common label free analytic assays cope with single techniques e.g., Surface Plasmon Resonance (SPR), Quartz Crystal Microbalance (QCM), Surface Acoustic Wave (SAW), or Impedance Spectroscopy (IS), we are addressing this challenge by developing **hybrid sensing platforms** able to integrate recognition elements (affine compounds and/or cells immobilized on top of chips) and combined electro-optical analytic systems with Flow Injection Analysis (FIA), **suitable for time based assays**.

Aiming to assess cell – environment interaction (in particular with noxious agents), we have developed hybrid (bio)affinity and cellular platforms allowing for time based dual electro-optical assays i.e., IS and SPR & Total Internal Reflection Fluorescence Microscopy (TIRF).

Such platforms comprising FIA have been advanced to assess the interaction between selected analytes, as well as normal/malignant cells and nano-patterned and/or chemically modified surfaces revealed by the related changes exhibited by cell membrane, morphology, adhesion and monolayer integrity.

Whereas impedance/dielectric analysis of cellular platforms (e.g., epithelial cells) usually ignore the behavior of interconnected cells we aim to relate impedance data both to cell-substrate and to cell monolayer properties using microscopic models of confluent cells (according to cell morphology).

I will present ICB results on dielectric modeling of interconnected cells as well as the virtues of our electro-optical assays to appraise the effect of minute amounts of detergents, peptides and pathogen cells on confluent epithelial cells and lipid supported membranes.

In a "nutshell", ICB skills & collaboration offer:
- Analytic, platforms integrating several techniques (e.g., IS & SPR, IS & TIRF) and microfluidics able to simultaneously address the same chip.
- Cellular platforms (suitable for epithelial cells adhered on engineered substrates) analyzed by a complementary set of combined assays i.e. AFM, IS, (L)SPR, TIRF, Electro-Physiology, Electrochemistry- detection of Reactive Oxidative Species.

ENVIRONMENTAL AND CLINICAL APPLICATIONS OF ADVANCED NANOMATERIALS

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Unique electrical, optical, catalytic and magnetic properties of nano-architectural materials (nanoparticles, nanofibers, nanotubes, nanoporous silica nanocrystalline films, etc) have attracted considerable interest in many areas of science and technology. These facilitated (i) development of new classes of powerful analytical devices, e.g. chemical and biological sensors, (ii) numerous applications in the biomedical field including uses as therapeutic agents, nanoprobes for monitoring neurological activity and as carriers for drug delivery, as well as in (iii) environmental monitoring for waste remediation and detection. However, while many types of nanostructures have been reported in literature, their implementation into routine functional devices still remains a great challenge. This presentation will discuss recent advances in the study and use of various nanomaterials for designing biologically active systems, and provides an overview of our research activity in this area with selected examples from our lab in the development of biosensors,[1-8] nano-based systems for bio-remediation and bioethanol production[9] and preliminary data on toxicological implications. We will focus on clinical and environmental applications.

In the biosensors field, we have used different nanomaterials such as carbon nanotubes, Au, Ni and metal oxide nanoparticles and composites of these materials to enhance the performance of these devices by decreasing the overpotential, ensuring direct electron transfer between electrodes and redox proteins, and providing high sensitivity and selectivity for the detection of H_2O_2,[2] dopamine,[3] glutamate, NO, glucose,[4] pesticides and phenolic compounds.[5-7] Different strategies used to functionalize nanostructures with biomolecules,[6,8] development and characterization of the nanostructure assembly, evaluation of biological activity and generation of response and sensing function will be described. For example, we have studied the surface chemistry and the interactions of Au and Ni nanoparticles with native and genetically engineered proteins and use this system to site-specifically immobilize proteins onto electrode surfaces and develop a simple and highly selective biosensor for glucose detection.[4] Another interesting example is an electrochemical sensor based on mixed metal oxide nanocomposites, used as "oxygen-rich" materials, for *in-vivo* monitoring of clinically important analytes. The sensor is able to operate in environments with a limited oxygen content due to the redox, catalytic and oxygen storage properties of the metal oxides.[3] This approach provided significantly better characteristics than conventional macroscopic materials including a wide linear range of three orders of magnitude, fast response time of ~3-4 sec and increased sensitivity.

On the other hand, the use of advanced nanomaterials in conjunction with natural biopolymers and sol-gels has allowed our laboratory to develop multifunctional core-shell structures with immobilized enzymes and cells for applications in environmental bioremediation and in fermentation processes, in the production of bioethanol from cellulosic biomass.[9] In these systems, both enzymatic and chemical reactions are performed in the

interior of a core/shell structure with multifunctional properties including biocatalytic, and magnetic capabilities for easy removal and handling. Examples of core-shell structures with encapsulated biomaterials (enzyme, yeast and cells) developed in our lab are presented in Figure 1. These structures are stable to ambient conditions, provide opportunities to conduct continuous-flow processes, are reusable and environmentally benign. We will discuss the fabrication, optimization, and performances of these systems.

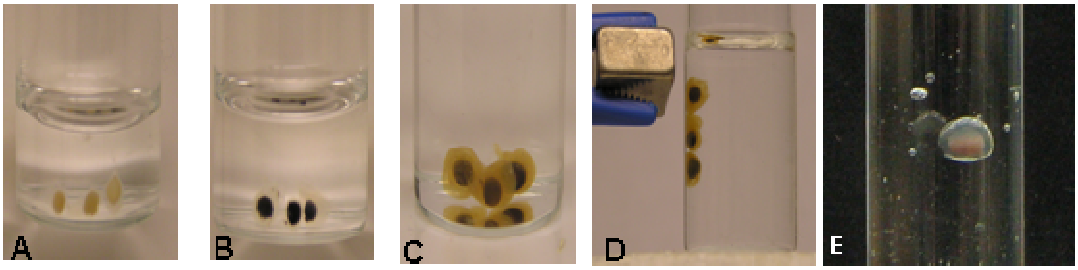

Figure 1. A and B: nanoparticle-based microcapsules containing biocatalytic material for environmental bioremediation before (A) and after (B) removal of the contaminant. C: biomagnetic capsules. D: magnetic properties under an external magnetic field. E: biocapsule for ethanol production with encapsulated yeast/enzymes in a liquid core.[9]

Finally, the growing interest and the extensive utilization of nanometer scale materials have created the need to establish procedures for evaluating toxicological, environmental and health impact and determine safety risks associated with the widespread use of these materials. In a collaborative project, we have used zebrafish embryos as a model target for probing toxicological effects of engineered nanomaterials, mainly of metal and metal oxide nanoparticles (e.g. Fe_3O_4, $Au@Fe_3O_4$, TiO_2, CeO_2, Ni) of different sizes, dose, surface coverage and composition. We will discuss preliminary results that will allow us to correlate the toxic effect with the type and nature of the nanomaterial and establish if, in addition to toxicity, these assays could also be used for detection and identification purposes.

References

1. C. Ispas, I. Sokolov, S. Andreescu, *Anal. Bioanal. Chem.*, In press, 2008.
2. C. Ispas, J. Njagi, M. Cates, S. Andreescu, *J. Electrochem. Soc.*, 155(8), 2008, 1-8.
3. J. Njagi, C. Ispas, S. Andreescu, *Anal. Chem.*, In revision, 2008.
4. S. Andreescu, L.A. Luck, *Anal. Biochem.*, 375, 2008, 282-290.
5. J. Njagi, S. Andreescu, *Biosens. Bioelectron.*, 23, 2, 2007, 768-775.
6. S. Andreescu, J. Njagi, C. Ispas, in "The New Frontiers of organic and composite nanotechnology" Ed. V. Erokhin, M.K. Ram, O. Yavuz, *Elsevier* (ISBN 978-0-0804-5052-0), 2008, 355-394.
7. G. Istamboulie, S. Andreescu, J-L Marty, T. Noguer, *Biosens. Bioelectron.,* 23, 4, 2007, 506-512.
8. S. Andreescu, J.-L. Marty, *Biomolecular Engineering*, 23 (1), 2006, 1-15.
9. B.J. Frederick, P.A. Christiansen, S. Andreescu, *US Patent* application, US 60 / 924,967, 2008.

ANTENELE IN FOTOSINTEZA - QUO VADIS?

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Suntem obisnuiti sa anlizam membranele celulare, printre care si membranele fotosintetice prin prisma modelului "fluid mozaic" al lui Singer si Nicholson. Pe scurt, imaginea membranelor celulare este aceea a unui lac alcatuit din fosfolipde (mai ales fosfatidyl colina si fosfatidyl serina) pe care plutesc ca intr-un roman de Jules Verne insule proteice care se misca alene si se ciocnesc cateodata. Daca doua sau mai multe sisteme proteice se intalnesc, se pot stabili intre ele interactii mai mult sau mai putin puternice, mai mult sau mai putin specifice. Daca nu, nu.

Membrana fotosintetica

Acest model idilic, calm si pastoral se regaseste rareori in structurile reale. In tilacoid, membrana fotosintetica, el pare a fi in directa contradictie cu realitatea. Mai mult de 50% din membrana este alcatuita din proteine care ocupa practic intreaga suprafata a membranei lipidice, in care fosfatidyl colina, fosfatidyl serina si fosfatidyl etanolamina, lipide amfipatice care formeaza membrana plsmatica sunt inlocuite cu lipide polare, de origine procariotica, cum sunt monogalactosyl diacylglicerol-ul si digalactosil dicacylglicerol-ul. Asa cum apar in imaginile de microscopie electronica, structurile membranei fotosintetice sunt departe de pasnicul model al lui Singer si Nicholson, aparand ca structuri complexe, incluzand supercomplexe fotosintetice care sunt apropiate cu forta unul in altul din cauza lipsei de spatiu lateral, ajungand cateodata chiar la formarea de structuri pseudocristaline. Toata aceasta aglomeratie este complicata in plante si in majoritatea algelor verzi de organizarea morfologica a dublului strat membranal in structuri proteo-lipidice lamelare, departate una de alta si aproximativ paralele, care sunt bogate in sistemul fotosintetic I (PSI). Din loc in loc, aceste lamelele numite lamele stromatice,se bifurca dand nastere unor structuri cilindrice (grane), alcatuite din straturi lipidice compresate unul in altul si care contin cu predilectie sistemul fotosintetic II (PSII).

Fotoreactii in fotosinteza

Aceasta organizare moleculara si supra-moleculara determina functionarea optima a procesului fotosintetic. De-a lungul unei bune jumatati de veac, sute de publicatii au stabilit ca fotoreactiile in procesul fotosintetic includ cu predilectie tranferul unui electron de la fotosistemul II din grane la fotosistemul I din lamelele stromatice. Acest electron este transferat de la perechea de clorofile din centrul de reactie fotosintetic prin itermediul reducerii unei molecule de plastoquinona la o molecula de citocrom b6f care la randul sau il transfera catre fotosistemul I care il foloseste intr-o reactie alta reactie redox la formarea unei molecule de NADPH care este generatoare de energie in ciclul Calvin.

Tranzitia de stare

Reactiile fotosintetice descrise pe scurt mai sus, sunt insotite de schimbari structurale menite sa adapteze membrana tilacoida la lumina incidenta. Principala adaptare pe termen scurt este asa-numita trazitie de stare, care este menita sa reduca numarul de fotoni captati de fotosistemul II si sa creasca corespunzator numarul de fotoni captati de fotosistemul I, reducand eficienta globala a sistemului fotosintetic atunci cand intensitatea luminoasa este prea mare. Se presupune ca tranzitia de stare are ca substrat molecular fosforilarea unei fractiuni din antenele potosistemului II urmata de separarea acestora si migrarea catre fotosistemul I.

E pur si muove?

Lucrarea de fata isi propune sa aduca argumente structurale in sprijinul ipotezei ca membrana fotosintetica in care complexele proteice au o concentratie ridicata restrange posibilitatea de deplasare laterala a acestora.

1. Imagini de microscopie confocala prezinta cianobacterii ale caror antene sunt intr-adevar mobile ca urmare a iradierii cu un flux luminos puternic, dar ale caror membrane fotosintetice par a nu mai fi intacte.
2. Imagini de microscopie electronica prezinta schimbari strucurale masive ale structurii membranei fotosintetice, induse de iradierea cu 30 Ei/m2 sec timp de 20 min, interval in care se produce trazitia de stare.
3. Imagini de microscopie electronica coroborate cu masuratori de fluorescenta arata ca, desi structura membranei bacteriilor fotosintetice crescute sub diferite intensitati luminoase este diferita, eficienta fotosintetica este similara.

Concluzia imediata este ca este mai probabil ca reorganizarea purtatorilor de electroni datorata iluminarii tilacoidului, atat in tranzitia de stare cat si in alte fotoreactii din fotosinteza plantelor si a bacteriilor sa se produca prin dezorganizarea si reorganizarea membranei tilacoide decat prin simpla migrare a complexelor fotosintetice in planul membranei.

ROLE OF THE V-ATPASE B SUBUNIT ISOFORMS IN RENAL PROTON SECRETION

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The vacuolar proton-pumping ATPase (V-ATPase) plays an essential role in numerous membrane trafficking processes by mediating the acidification of intracellular organelles. When expressed on the plasma membrane, as in kidney collecting duct (CD) A-type intercalated cells (A-ICs), the V-ATPase mediates transmembrane proton (H^+) secretion. The V-ATPase is a hetero-oligomeric enzyme, composed of a transmembrane (V_1) and a cytosolic sector (V_0), with each sector containing several distinct subunits. The B subunit is a component of the catalytic cytosolic domain and is expressed in animal tissues in two highly homologous 56 kDa isoforms, B1 and B2. B1 is highly expressed in epithelia specialized for regulated H^+ transport while the B2 subunit is quasi-ubiquitous.

Defects in H^+ secretion underlined by mutations in the gene encoding for B1 V-ATPase cause distal renal tubular acidosis (dRTA), also associated in certain patients with progressive sensorineural deafness. Interestingly, mice lacking B1 do not develop dRTA. Therefore, we set out to investigate the role of B1 and B2 in renal H^+ secretion, hypothesizing that B2 might serve as back-up for the active role played by B1.

We report that the V-ATPase B2 subunit isoform has a widespread distribution in the kidney and is expressed at significant levels in ICs of the renal CD. Furthermore, we show that although V-ATPases containing B2 mostly localize to the IC cytosolic domain under baseline conditions, these enzymes are also detected under certain circumstances (e.g., upon chronic carbonic anhydrase inhibition by acetazolamide) on the apical plasma membrane and can consequently contribute to transmembrane H^+ secretion.

B1-deficient mice were found to maintain body acid-base homeostasis under normal conditions, but not when exposed to an acid load. Compensatory mechanisms involving B2 were examined to explain the persistence of baseline pH regulation in these animals. By immunocytochemistry, the mean pixel intensity of apical B2 immunostaining in medullary A-ICs was twofold greater in B1-/- mice than in wild type controls. B2 mRNA and protein expression were not significantly upregulated in B1-/- compared to B1+/+ mice. Increased apical B2 staining is thus due to relocalization of B2-containing V-ATPases from the cytosol to the plasma membrane. This recycling of B2-containing holoenzymes was confirmed by the intracellular accumulation of B1-deficient V-ATPases in response to colchicine. V-ATPase membrane expression is further supported by the presence of rod-shaped intramembraneous particles seen by freeze fracture microscopy in apical membranes of normal and B1-deficient A-ICs. pH_i recovery assays show that significant (28-40% of normal) V-ATPase function is preserved in medullary ICs from B1-/- mice. We conclude that the activity of apical B2-containing V-ATPases in A-ICs is sufficient to maintain baseline acid-base homeostasis in B1-/- mice.

We investigated this compensatory mechanism in order to assess the possibility of novel treatment strategies by isoform upregulation or replacement therapy for dRTA in humans. In general, understanding how epithelial cells respond to different stimuli by modulating the trafficking of transport proteins (like V-ATPase or various ion channels) between cytosol and plasma membrane is essential in approaching many diseases underlined by trafficking defects, such as nephrogenic diabetes insipidus, polycystic kidney disease, or cystic fibrosis.

THE THEORY OF THE PULSATORY LIPOSOME

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ABSTRACT. I consider a greater lipid vesicle filled with an aqueous solution of a solute, for which the vesicle membrane is impermeable. This vesicle is introduced into an hypotonic aqueous medium. Because of the mechanical tension induced by osmotic flow, the vesicle swells up to critical size, triggering a transient lipidic pore. Due to osmotic gradient and transbilayer pore appearance, the vesicle dynamics is a periodic processe. In this paper we analyse the differential equations of the vesicle dynamics and characteristic parameters of periodic processe (swelling time, pore lifetime, number of cycles, the time length of vesicle activity, material quantity leaked out during a cycle). Also, we present the condition to design a n-cycles working vesicle.

PLASMONICS-BASED NOVEL NANO-PROBES AND NANO-TOOLS FOR BIOLOGICAL AND MEDICAL INVESTIGATION

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Noble-metal nanostructures exhibit an enhanced optical interaction with visible light due to the resonant excitation of localized electronic waves known as plasmons [1-2]. As result, plasmonic nanostructures promise to create entirely new prospects for guiding light on the nanoscale and provide probes and tools for investigation at bio-nano interface. Dramatic changes are observed in the optical properties of biomolecules when adsorbed on metallic nanoparticles. The best-known example is the detection and identification of single-molecule by optical spectroscopy, namely by surface-enhanced Raman scattering (SERS). Metal nanoparticles have been also reported to decisively influence the molecular fluorescence rate.

Here, we report the fabrication of plasmonic nanoparticle arrays *via* nanosphere lithography, chemical synthesis and self-assembling routes. In the first procedure self-assembled polystyrene nanospheres are used as lithographic masks to deposit metal and generate periodic arrays of nanoparticles or nanoholes. The second procedure of fabrication involves chemical synthesis of nanoparticles and subsequent attachment of as synthesized nanoparticles onto solid surfaces via amine-terminated molecular layer. Scanning and transmission electron microscopy, atomic force microscopy, and optical transmission and reflectivity measurements have been employed to correlate the nanometer-scale morphology and topography of fabricated nanostructures with their optical properties.

The fabricated plasmonic nanostructures have been investigated as unique multifunctional platforms for spectroscopic detection of low-concentration analytes [3-5]. The SERS efficiency was evaluated with p-aminothiophenol as probe molecule at different excitation laser lines. Both FT-SERS and SEIRA spectra of p-aminothiophenol absorbed onto nanoparticle gold films were successfully recorded from the same metallic substrate. Moreover, morphological changes induced in the initial assemblies of gold nanoparticles by annealing allowed us to convert the SERS substrate into a sensitive sensor based on local surface plasmon resonance (LSPR). We also examined the fluorescence of Eosin Y – protein (BSA) in the presence on gold colloidal nanoparticles immobilized on glass substrate in comparison with the emission of Eosin Y – BSA deposited on bare glass.

The potential use of fabricated plasmonic nanostructures as highly SERS and SEIRA-active substrates as well as LSPR-based and metal-enhanced florescence sensors for biomolecules detection can foster many exciting applications, from biology, biochemistry and DNA sequencing to detection and identification of single molecules.

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References:

1. D.A. Stuart, A.J. Haes, C.R. Yonzon, E.M. Hicks and R.P. Van Duyne, Nanobiotech., 152, (2005) 13.
2. B. J. Wiley, S. H. Im, Z-Y. and Y. Xia, J. Phys. Chem. B, 110, (2006) 15666.
3. M. Baia, L.Baia, J.Popp, S. Astilean, Appl.Phys. Lett., 88, (2006) 143121.
5. F. Toderas, M.Baia, L.Baia, S. Astilean, Nanotechnology, 18, (25), (2007) 25570

APLICATII ALE SPECTROSCOPIEI REZONANŢĂ ELECTRONICĂ DE SPIN (RES) ÎN MEDICINĂ ŞI BIOFIZICĂ

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I. Principii generale ale spectroscopiei RES. Rezonanţa electronică de spin (RES) este o tehnică spectroscopică care are la bază tranziţiile induse între nivelele Zeeman ale unui sistem paramagnetic aflat într-un câmp magnetic static. Un electron liber cu spinul S posedă şi un moment magnetic μ_S care este coliniar şi antiparalel cu vectorul asociat spinului: $\mu_S = -g_e \mu_B S$ unde g_e este factorul giromagnetic al electronului liber (g_e=2.0023) iar μ_B -este magnetonul Bohr (μ_B=9.27 $\times 10^{-21}$ erg gauss^{-1}). Energia de interacţiune dintre momentul magnetic şi un câmp magnetic extern este dată de produsul dintre momentul magnetic şi inducţia câmpului magnetic: $E = -\mu_S B = g_e \mu_B B S$. Proiecţia spinului electronului pe direcţia de acţiune al câmpului magnetic are două valori, respectiv $M_S = \pm 1/2$ iar diferenţa dintre energiiile corespunzătoare celor două orientări va fi: $\Delta E = g_e \mu_B B$. Tranziţiile dintre cele două nivele Zeeman pot fi induse prin iradierea sistemului paramagnetic cu radiaţie electromagnetică cu frecvenţa de rezonanţă ν care satisface condiţia: $h\nu = g_e \mu_B B$. Când electronul neîmperechiat face parte dintr-un sistem molecular, valoarea factorului de despicare spectroscopică diferă de valoarea g_e devenind o mărime tensorială întrucât contribuţia la momentul magnetic de spin a nivelelor electronice moleculare poate fi diferită pe direcţii diferite. Deasemeni o serie de nuclee atomice posedă un spin (I) nenul şi ca urmare va exista şi un moment magnetic, coliniar cu vectorul de spin nuclear a cărei valoare este dată de relaţia: $\mu_n = g_n \beta_n I$, g_n fiind factorul de despicare spectroscopică nucleară iar β_n este magnetonul nuclear. Când un centru paramagnetic conţine unul sau mai multe nuclee cu spinul diferit de zero, interacţiunea dintre electronul neînperecheat şi nucleele cu $I \neq 0$ conduce la o despicare a energiilor Zeeman şi ca urmare apar noi tranziţii între aceste nivele (structura hiperfină). Există două tipuri de interacţiuni hiperfine: interacţiunea hiperfină anizotropă şi interacţiunea hiperfină izotropă. Interacţiunea hiperfină anizotropă este interacţiunea de tip dipol magnetic între electronul cu spin neîmperecheat şi nucleul cu moment magnetic diferit de zero. Interacţiunea hiperfină izotropă sau interacţiunea hiperfină de contact (interacţiunea de contact Fermi), are loc între nucleul cu moment magnetic nenul şi electronii cu spini neîmperecheaţi, a căror densitate la locul nucleului este diferită de zero. Interacţiunea hiperfină izotropă este partea principală a interacţiunii hiperfine în radicali liberi, deoarece în multe cazuri interacţiunea hiperfină anizotropă nu se rezolvă şi are ca efect doar o lărgire a liniilor de rezonanţă sau o anizotropie a formei şi lărgimii liniei. Există trei mărimi esenţiale care se obţin din spectrele RES a probelor paramagnetice şi permit analiza structurii speciilor paramagnetice şi anume: (i) factorul de despicare spectroscopică ***g,*** *(ii)* constanta structurii hiperfine, ***A,*** *(iii)* lărgimea liniei derivatei semnalului de rezonantă, ΔH_{pp}.

II. Caracteristici ale spectroscopiei RES în studiul sistemelor biologice. Principalele caracteristici ale spectroscopiei de rezonanţă electronică de spin (RES), date de selectivitate, specificitate, sensibilitate şi caracter non-invaziv, impune această tehnică în domenii medicale şi biofarmaceutice.

●*Selectivitatea.* Pe lângă o mare parte din substanţele constituente ale sistemelor biologice, numai o categorie a acestora manifestă caracteristici paramagnetice, cum ar fi radicalii liberi, incluzând şi speciile reactive ale oxigenului (O_2^-) şi ale oxidului nitric (NO), dar şi un număr de metale tranziţionale, pot fi detectate prin spectroscopie RES. În unele situaţii sunt introduse în mod intentionat specii paramagnetice (numite marcari de spin-*spin labels*) cu

caracteristici specifice, in vederea evaluării cineticii şi evoluţiei sistemului. Esenţial în studiul sistemelor biologice este faptul că prin spectroscopie RES pot fi detectate numai speciile care manifestă caracteristici paramagnetice chiar şi la temperaturi joase.

●*Specificitatea:*Mulţi centri paramagnetici prezintă semnale RES cu forme specifice fiind în unele cazuri amprente ale speciilor paramagnetice naturale sau introduse artificial in sistemele biologice. De exemplu radicalii liberi când nu sunt în vecinătatea nucleelor magnetice (in sistemele biologice principalele nucleee de acest tip sunt ^{1}H, ^{14}N sau izotopi introduşi artificial continând ^{15}N) deşi prezintă forme şi poziţii relativ similare ale semnalelor RES, pot fi puşi în evidenţa prin legăturile chimice care se stabilesc cu capcanele de spin (spin trap). In mod obişnuit, un centru paramagnetic format ca urmare a reacţiei radicalului liber cu capcana de spin (numit *spin-aduct*) prezintă un semnal specific, caracteristic tipului de radical care a iniţiat reacţia. Astfel, metoda capcanei de spin devine o tehnică spectroscopică indispensabilă în măsurarea centrilor paramagnetici de viaţă scurtă (în principal a radicalilor liberi) din sistemele biologice .

●*Sensibilitatea:*Fenomenul de rezonanţă la absorbţie a microundelor, prezintă un caracter stohastic şi în principiu poate fi observat numai într-o populatie de spini. Pentru un sistem spectrometric RES standard, estimările teoretice şi pragul experimental de detecţie este in jur de 10^{11} centri paramagnetici per miligram probă. In practică, particular în detecţia in vivo, această valoare este ce circa 10^{12}.

●*Caracterul non-invaziv*:Datorită selectivităţii, penetrării satisfăcătoare a ţesuturilor biologice de către microunde şi a nivelului relativ slab de absorbţie a energiei de către probe în timpul măsuratorilor, tehnica RES oferă posibilitatea de investigare a întregului sistem şi chiar de a monitoriza *on-line* schimbările proprietăţilor paramagnetice ale acestuia ca urmare a metabolizării marcărilor de spin introduşi în sistem, fără o interferenţă considerabilă cu procesele din sistemul nativ. În ultimii căţiva ani, spectroscopia ESR este intens utilizată in studiile *in vivo*, ca urmare, in special a dezvoltării spectrometrelor RES în banda L (1-2 GHz).

III. Măsurători specifice în sisteme biomedicale.

●*Evaluarea radicalilor liberi***.** Radicalii liberi sunt atomii sau moleculele care conţin unul sau mai mulţi electroni neîmperecheaţi, disponibili pentru formarea unor noi legături chimice. Radicalii liberi, sunt caracterizaţi printr-o reactivitate chimică mare în comparaţie cu atomii şi moleculele corespunzătoare. În consecinţă radicalii liberi sunt structuri instabile, cu timp de viaţă relativ scurt, datorită tendinţei permanente a electronului neîmperecheat de a forma o structură mai stabilă prin asociere într-o nouă legătură chimică. În medicină şi sisteme biologice, radicalii liberi sunt implicaţi într-o mare majoritate a proceselor patologice şi sunt în principal, centraţi pe oxigen, azot, carbon şi sulf, însă apar şi ca intermediari ai medicamentelor şi enzimelor, sub formă de oxid nitric sau radicali de viaţă lungă din ţesuturi tari induşi de radiaţia ionizantă. Valoarea factorului de despicare spectroscopică $\boldsymbol{g}$ apropiată de cea a electronului liber, arată că la radicalii liberi există o interacţiune spin-orbită foarte slabă şi electronii cu spinii neîmperecheaţi au un grad mare de localizare. În general, factorul $\boldsymbol{g}$ nu permite identificarea radicalilor liberi, totuşi unele măsurători RES, foarte precise, au reuşit să pună în evidenţă dependenţa factorului g de structură într-o serie de substanţe organice. Prezenţa structurii hiperfine, $\boldsymbol{A}$, la radicalii liberi se datoreşte interacţiunii magnetice a electronului cu spin neîmperecheat cu momentul magnetic al nucleelor atomilor radicalului. Fiecare radical liber are o structură hiperfină caracteristică, determinată de numărul de nuclee şi de gradul de localizare al electronului la fiecare dintre aceste nuclee. Prin aceasta, structura hiperfină permite, în majoritatea cazurilor, identificarea radicalului liber studiat. În cazul radicalilor liberi, cel de-al treilea parametru spectral, lărgimea liniei $\boldsymbol{\Delta H_{pp}}$, este mică şi permite determinări foarte precise de concentraţie a radicalilor liberi şi separarea efectelor diferiţilor radicali prezenţi în acelaşi timp în proba studiată. Metoda de detecţie a radicalilor liberi prin spectroscopie RES, este de observare directă în cazul când stabilitatea acestora este

mare (radicalii din mediile solide) şi utilizarea capcanelor de spin. Evaluarea cantitativă a concentraţiei acestora se poate face din prin integrarea dublă a spectrelor RES sau din înălţimea intensităţii semnalului, comparat cu etaloane standard (cel mai utilizat fiind semnalul DPPH).

●Studiul procese metabolice, fiziologie şi biochimice in vivo**.** În sistemele biologice o mare majoritate a proceselor metabolice, fiziologice şi biochimice, au loc în urma unor dinamici care implică formarea sau tranzitarea prin intermediul unor structuri paramagnetice (în general radicali liberi tranzienţi) şi care pot fi studiate prin spectroscopie RES prin utilizarea unor specii diamagnetice care pot deveni paramagnetice în urma interacţiunii (capcane de spin). Capcanele de spin (spin traps) sunt capabile să reacţioneze specific asupra diferitelor tipuri de radicali liberi transfomându-i în radicali stabili (spin adducts). Mărimile despicării hiperfine a radicalilor stabili formaţi, sunt dependente de natura chimică a radicaluli captat, astfel că este posibil identificarea radicalului iniţiator. Există mai mulţi compuşi cu caracteristici de capcane de spin cum ar fi PBN (N-tert-Butyl-a-phenylnitrone), POBN(N-tert-Butyl-a-(4-pyridyl)nitrone N'-oxide), DMPO(5,5-Dimethyl-1-pyrrolineN-oxide) care reacţionează caracteristic în prezenţa radicalilor liberi tranzienţi. In principal, procesele şi stările care pot studiate, sunt: stări redox, grupări thiol, activitate redox a ionilor metalici. De asemeni, o serie de structuri, procese şi efecte implicate în procesele clinice pot fi abordate prin stări paramgnetice sau prin utilizarea unor "marcări de spin-spin labels" care să ofere informaţii asupra: interacţiuni intre *situ*-rile membranare, mobilitate macromoleculară, farmacocinetică, metabolismului medicamentelor, parametri biofizici şi de mediu celular şi de ţesut (vâscozitate, pH, temperatură). Metoda de detecţie este utilizarea radicalilor nitroxidici (spin label) şi/sau a unor compuşi paramagnetici solubili. Aceşti compuşi sunt legaţi covalent cu structuri moleculare şi oferă informaţii asupra proceselor în care sunt implicate acestea. Existenţa centrului paramagnetic şi sensibilitatea acestuia la interacţiunile cu mediul în care sunt înseraţi, îi face uşor detectabili prin spectroscopie de razonanţă electronică de spin (RES). Această tehnică de investigare, utilizează proprietăţile de interacţiune din sistemele cuplate şi este o metodă importantă de caracterizare nedistructivă a proprietăţilor dinamice şi structurale la nivel molecular, din sisteme ordonate sau neordonate. Moleculele marcate cu radicali nitroxidici (spin label), fac posibilă cunoaşterea nemijlocită şi în timp real a proprietăţilor dinamice din mediul molecular. În acest fel se pot determina *in situ* şi *in vivo* micropolarităţi, microvâscozităţi, valori ale pH-ului local.

DELAYED LUMINESCENCE OF DRY SEEDS – KNOWN ISSUES AND PERSPECTIVES

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Delayed luminescence (DL), a very weak, but slowly decaying afterglow, following a short light stimulus, is emitted by any kind of dry seeds. The phenomenon is known since more than 20 years and from the beginning Veselova et. al. reported a correlation between the intensity of DL and the germination capacity of the seeds. Further investigations uncovered an additional correlation between seed DL and the biological clock phenomenon, which lead to the speculation that DL emitting structures might be directly involved into the biological clock mechanism. At the moment there is no consistent model, which is unifying the two observations in the sense of explaining possible interference effects in order to avoid misleading interpretations of the signals obtained from DL measurements.

Nevertheless, it has been emphasised by all authors, that DL is a highly sensitive and non-invasive tool, which can be applied in fundamental research on the biology of dry living systems as well as in the development of new and fast seed testing methods. The most recent investigations demonstrated the possibility to develop single grain scanning methods, which might end up in automatic seed sorting for breeding or processing purposes.

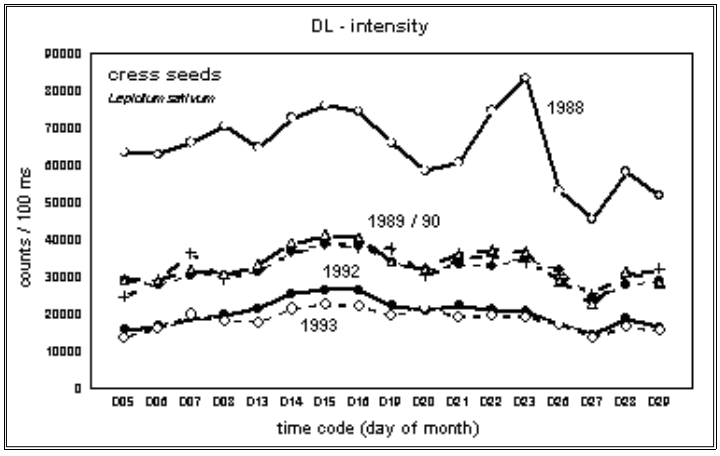
The seminar will cover:

I. The history of research on seed DL, also touching the concept of “Biophotons”, which can contribute to a better understanding of the phenomenon.

II. A summary of the authors experimental work on seed DL, including also some unpublished results.

III. Recent activities, perspectives and funding background in the field of biophotonics in general.

IV. Possibilities to develop an international collaboration for fundamental research and method development.



About the Author

Ralf Neurohr, born in 1959, studied biology in the laboratories of Werner Nachtigall at Saarbruecken University (Germany) and graduated in 1987. During a research period from 1987 to 1994 in biophotonics at the International Institute of Biophysics (Kaiserslautern, Germany), he earned his PhD with a thesis on the delayed luminescence of cress and cress seeds at Saarbruecken University. In 1994 he became an independent consultant, doing research projects for industrial partners in different European countries. Since 1997 Dr. Neurohr is a permanent Senior Consultant of the SGS Institut Fresenius group (Taunusstein, Germany).

Based on some research collaboration with "Politehnica" University of Bucharest (UPB), in 2006 Dr. Neurohr was invited by the Senate of "Politehnica", in order to introduce Bionics at the Faculty of Engineering Taught in Foreign Languages. Since March 2007 he is teaching at UPB and and in February 2008 he started to build up a research team in biophotonics.

EMIL (EUROPEAN MOLECULAR IMAGING LABORATORIES) NETWORK OF EXCELLENCE: EXPERIENCE OF A COLLABORATIVE EUROPEAN PROJECT

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In March 2000, the Lisbon Summit fixed the objective of creating a **European Research Area** (ERA) as an essential instrument that shall allow making Europe a leading knowledge-based economy in the world by the year 2010.
The ERA concept encompasses three inter-related aspects: a European 'internal market' for research, where researchers, technology and knowledge can freely circulate; effective European-level coordination of national and regional research activities, programmes and policies; and initiatives implemented and funded at European level.

Since 1984, the European Union (EU) has implemented several **Research Framework Programmes** (**FP**), each covering 5 years periods, as the main research funding instruments in Europe.
The 6th FP (2002-2006) was explicitly designed to support the creation of ERA with a lasting impact on greater coherence at the European level. In order to achieve durable integration and to build up a necessary 'critical mass' to have real impact; two new instruments have been introduced starting with FP6: the Networks of Excellence (NoE) and the Integrated Projects (IP).
NoEs are aimed at establishing durable, virtual centres of excellence in specific research areas by grouping expertise and research capacities around a joint programme of activities (JPA), while IPs are aimed at large-scale, strategic, objective-driven co-operative research requiring the integration of a critical mass of activities and resources.
EMIL (European Molecular Imaging Laboratories) is one of these Networks of Excellence created under the FP6's **Life sciences, genomics and biotechnology for health** thematic priority of the EU. Started in July 2004 and funded for a 5 years period, EMIL coordinates research efforts in Molecular Imaging of Cancer in Europe of **58 groups** from universities, research centres and industry. The objective of EMIL is to gather leading European Molecular Imaging Laboratories for early stage diagnosis as well as therapeutic and prognosis assessment of cancer. The general objectives of EMIL are:

- to coordinate the current effort in Molecular Imaging of Cancer in Europe by merging together a multidisciplinary task force in physics, chemistry, biology, engineering science, medical science, and assemble the critical mass necessary for international leadership, into ONE virtual excellence center;
- to advance Molecular Imaging of Cancer to the scientific, technical and economical status that should be expected from its value for European citizens;
- to act as a leverage for a strong technological development that can be fuelled through specific research and development projects.

EMIL's **joint programme of activities** is based on:

- Integration activities: sharing facilities and equipments (the Molecular Imaging Training Centres); exchange and mobility of personnel; integration of SMEs in the EMIL network.

- A joint programme of research activities with two dimensions for integration: HORIZONTAL integration = tools for molecular imaging of cancer (imaging technology; imaging probes; animal engineering) and VERTICAL integration = cancer imaging applications (early diagnostic imaging; follow-up of guided therapies; imaging drug development).

- Dissemination activities: training and education; communication; management of common knowledge and intellectual property rights.

EMIL is designed to reach durable integration into the European Research Area through intellectual excellence, strong and durable cooperation with SMEs, education activities, scientific management, and a research programme based on existing tools and ambitious objectives such as assembling the critical mass necessary for international leadership, bridging gaps in the innovation system, raising health expectancies of the European population to the highest levels