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Session I:
Biochemistry / Biomolecular Engineering

Design strategies for nanoparticles translocating through lipid bi-layers

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Abstract:
Design of nanomaterials able to cross lipid bilayers is a challenging task in nanotechnology. Large variety of shapes, sizes and surface coatings are used for the design of nanomaterials to overcome this barrier. However, the potential barrier is quite high for carbon nanotubes and nanoparticles to cross the lipid bilayer to translocate by thermal motion. It is generally accepted that small hydrophobic nanoparticles are blocked by lipid bilayers and accumulate in the bilayer core, while nanoparticles with sizes larger than 5 nm can only penetrate cells through a slow energy-dependent processes such as endocytosis, lasting minutes.
In one example, we show how variation of hydrophobicity of the nanoparticles can lead to passive translocation of nanoparticles through lipid bilayer. This adsorption transition through reversible destabilization of the structure of the bilayer induces enhanced permeability for water and small solutes.
In another example, we demonstrate that lipid-covered hydrophobic nanoparticles may translocate through lipid membranes by direct penetration within milliseconds. We identified the threshold size for translocation: nanoparticles with diameters smaller than 5 nm stay trapped in the bilayer, while nanoparticles larger than 5 nm insert into bilayer, open transient pore in the bilayer. Using the Single Chain Mean Field (SCMF) theory a mechanism of passive translocation through lipid bilayers is proposed. Observing individual translocation events of gold nanoparticles with 1-dodecanethiol chains through DMPC bilayers we confirm the particle translocation and characterize the kinetic pathway in agreement with our numerical predictions. Mechanism relies on spontaneous pore formation in the lipid bilayer. The observed universal interaction behaviour of neutral and chemically inert nanoparticles with bilayer can be classified according to size and surface properties

Keywords: lipid bilayers, nanoparticles, membranes, translocation

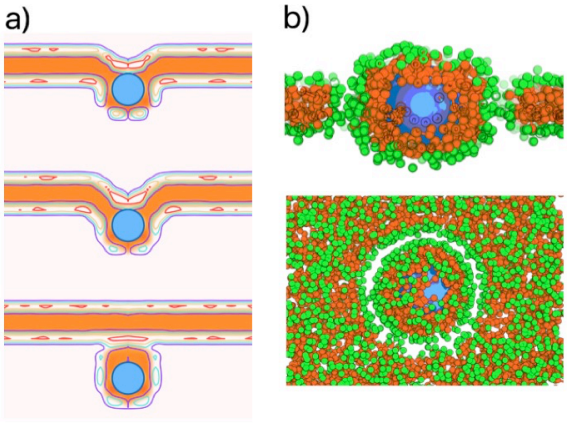

Figure 1: a) spontaneous escape of hydrophobic nanoparticle covered with lipids from lipid bilaer; b) lipid wrapping around nanoparticles and consequent pore formation allowing the nanoparticle to escape the lipid bilayer.

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Establishing Thraustochytrids as a Biotechnology Platform

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Abstract:
Despite a sparse literature describing their biology, thraustochytrid strains are proving useful in microbial bioprocesses. These oleaginous marine protists have been used for over twenty years, by virtue of their prolific ability to produce high-value lipids, in commercial-scale fermentation processes: including the production of omega-3 polyunsaturated fatty acids (PUFAs), carotenoids and squalene[1]. They can be grown heterotrophically to high cell density and, under controlled conditions, produce ~80% of their biomass as oil. This compares favourably with other well-established industrial microorganisms which produce a major portion of their oil profile as saturated or monounsaturated fatty acids, rather than higher-value PUFAs.
A number of desirable biomolecular and cellular properties suggest thraustochytrids can be candidates for engineerable biotechnology: 1) a relatively simple level of cellular/genomic complexity; 2) a biochemical chassis that provides plentiful substrates for organic molecule biosynthesis; 3) a multi-subunit enzyme, the PUFA-synthase, which rapidly assembles PUFAs, 4) post-translational glycosylation profiles which are consistent with applications of recombinant proteins in mammals and 5) the ability to consume a diverse range of carbon sources. Given the number of applications of organic molecules throughout commerce, from fuels to pharmaceuticals, the rapid ability of thraustochytrids to assemble organic building blocks into high-value and commodity products represents a commercial opportunity.
Since its discovery in 2006, Mara's thraustochytrid strain, T18, has been the focus of a broad research program to develop it for commercial-scale fermentation processes[2]. Alongside, Mara also invested in a campaign to exploit and modify the biology of this organism towards its establishment as a microbial biotechnology. Progress has identified many basic molecular biology tools for genomic modification such as: transformation protocols, selectable markers, regulatory elements and targeted genomic modification via homologous recombination. Here, we will provide a progress update in thraustochytrid manipulation and describe examples of engineered versions of our platform organism, *Thraustochytrium* T18.

Keywords: Thraustochytrids, heterotrophs, biotechnology platform, metabolic engineering.

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Thiosemicarbazones as Novel Tyrosinase Inhibitors

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Abstract:
Tyrosinase plays an important role in the biosynthesis of melanins. This enzyme belongs to oxidoreductases and catalyses two reactions which are the initial steps of complicated process leading to skin and hair pigmentation in animals and enzymatic browning in fruits, vegetables and fungi. Excessive melanin production can result in many skin disorders in humans and animals and cause the loss of commercial value of plant and fungi-derived food. Tyrosinase inhibitors have application in a lot of fields including pharmacy, medicine, cosmetology and food industry. There are many tyrosinase inhibitors already described in the literature but cytotoxicity and high price are the barrier in commercial application of those compounds. Thiosemicarbazones seem to be promising tyrosinase inhibitors because of good inhibition parameters and also fast, easy and relatively low-cost synthesis. Tyrosinase was isolated and purificated from mushroom using such methods as extraction with acetone, salting out and column chromatography. Activity, concentration and purity of obtained enzyme were checked using UV-VIS spectroscopy, Bradford method and SDS-PAGE, respectively. Inhibitory kinetics on the diphenolase activity of mushroom tyrosinase was investigated for a group of aryl thiosemicarbazone derivatives. The inhibitory effects of these compounds on mushroom tyrosinase were investigated using UV-VIS spectroscopy. For all investigated compounds K_i (or K_{is}), IC50, mechanism and type of action were determined using computer programs. Relationship between structure of investigated compounds and inhibitory activity on mushroom tyrosinase was also evaluated. Inhibition constants were determined and compared to each other and to kojic acid – literature reference compound.

Keywords: tyrosinase, melanin, hyperpigmentation, enzyme kinetics, enzyme inhibitors, thiosemicarbazone derivatives, UV-VIS spectroscopy.

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Antibodies against difficult to express membrane proteins

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Abstract:
Ion channels such as Kv1.3 have been historically difficult to raise antibodies against due to sequence conservation, paucity of cell surface epitopes, and poor expression levels in heterologous systems. Tetragenetics Inc. is addressing these issues by combining its unique technology for membrane protein expression in Tetrahymena thermophila, and antibody generation in phylogenetically diverse animals, to develop therapeutic antibodies against a range of ion channel targets including Kv1.3, a voltage-dependent channel produced by effector memory T-cells implicated in certain autoimmune disorders.

MonoPEGylated Human Arginase I for the Treatment of Cancers

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Abstract:
Arginine depletion is a new, promising and relatively safe strategy that can be applied as a potential targeted therapy for treating different types of cancers. Use of this therapy is based on the fact that many cancer cells are auxotrophic for L-arginine, whereas normal cells may enter into quiescence and are resistant to L-arginine depletion. L-Arg depletion can be induced via the administration of arginine degrading enzymes, such as recombinant human arginase I that converts arginine into ornithine and urea. We have carried out random PEGylation of human arginase I (rhArg-peg5,000mw) to prolong its circulation half-life and improve its pharmaceutical properties (Cheng et al., 2007), which is undergoing phase II clinical trials in hepatocellular carcinoma (HCC). However, rhArg-peg5,000mw exhibits low catalytic efficiency (k_{cat}/K_M), and contains heterogeneous bioconjugates with each protein molecule being attached to a variable number of PEG molecules as a result of non-specific lysyl conjugation at multiple sites. We have developed a method to produce homogenously monoPEGylated human arginase I molecules which completely retain the activity of the parent arginase, making possible the administration of correct and consistent doses required for clinical uses. This method involves a main step of genetically modifying the gene encoding the human arginase I (HAI) so that the resulting arginase will have only one single free cysteine. Site-specific covalent conjugation of HAI engineered with a Cys^{45} residue to a 20 kDa linear PEG-maleimide is then carried out to produce the monoPEGylated human arginase I (HAI-PEG20).
We establish that following intraperitoneal administration in Balb/c mice at 12.6 and 20.1 mg-HAI-PEG20/kg-weight, the serum L-Arg level could be maintained below detection limit for over 144 h and 192 h, respectively, and the elimination half-life was 30.0 ± 8.1 h and 29.4 ± 2.1 h, respectively. The excellent animal study results can be explained by the fact that HAI-PEG20 had a superior catalytic efficiency (k_{cat}/K_M = 1367.3 ± 79.6 $mM^{-1}s^{-1}$) for serum L-Arg hydrolysis in the mice model. Additionally, the results of enzyme kinetics and circular dichroism spectrometer show that 20 kDa linear PEG attached on the enzyme surface did not affect the catalytic activity and the protein secondary structure. HAI-PEG20 also exhibited anticancer activity against a wide range of cancer cell types *in vitro*. *In vivo* efficacy studies indicate further that HAI-PEG20 gave outstanding results in tumor suppression in HepG2 human HCC tumor xenografts on nude mice. Thus, the favorable pharmaceutical properties of HAI-PEG20, coupled with its homogeneity and human origin, make it a promising candidate for use as an anti-cancer drug.

Keywords: Amino acid deprivation, L-arginase, L-arginine, PEG conjugates, PEGylated drugs, PEGylation, Cancer, Targeted anticancer therapy.

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Acknowledgements:

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Session II: Bioprocess / Biomedical / Food Engineering

Microbial production of high-value chemicals such as medium- to long-chain fatty acid and alcohols from biobased waste streams

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Abstract:
The business unit Biobased Products within the research centre of Wageningen Research, focusses on sustainable production of bulk and fine chemicals, preferably from biobased substrates. For this purpose, a strong conversion platform has been developed for microbial and enzymatic processing of cheap waste streams, and converting these biochemically and/or microbially into high-value chemicals. In our presentation we will explain the various platforms using some practical examples;

1. Conversion/valorization of waste gasses

This platform focusses on the development of cell factories that convert C1-gasses such as CO_2, CO, and combinations with or without H_2, into various (higher) alcohols, such as pentanol, hexanol, octanol and chemical derivatives. For this purpose, synthetic mixed cultures will be constructed combining homacetogenic bacteria such as *Clostridium ljungdahlii* and specific alcohol-producing bacteria such as *C. kluyveri* and *C. beijerinckii*.

2. Microbial production of alcohols

This platform focusses on the anaerobic production of various alcohols (isopropanol, (iso)but-anol, butanediol) using the ABE-fermentation. The innovation in this area is provided by the use of CO_2 as co-substrate, on introducing novel alcohol-production pathways in these organisms, and on conversion of recalcitrant biomass directly into the various alcohols.

3. Microbial production of fatty acids

Various microorganisms such as the yeast *Cryptococcus* and *Yarrowia*, the bacterium *Pseudomonas putida* and several microalgae, are known for their ability to produce specific (unsaturated) fatty acids. Here we discuss these microbial platforms as sustainable alternative sources for palm and coconut trees.

Keywords: C1-to-chemicals, Clostridium, ABE-fermentation, Cryptococcus, waste valorization, fatty acids, alcohols, microbial production, CRISPR/CAS

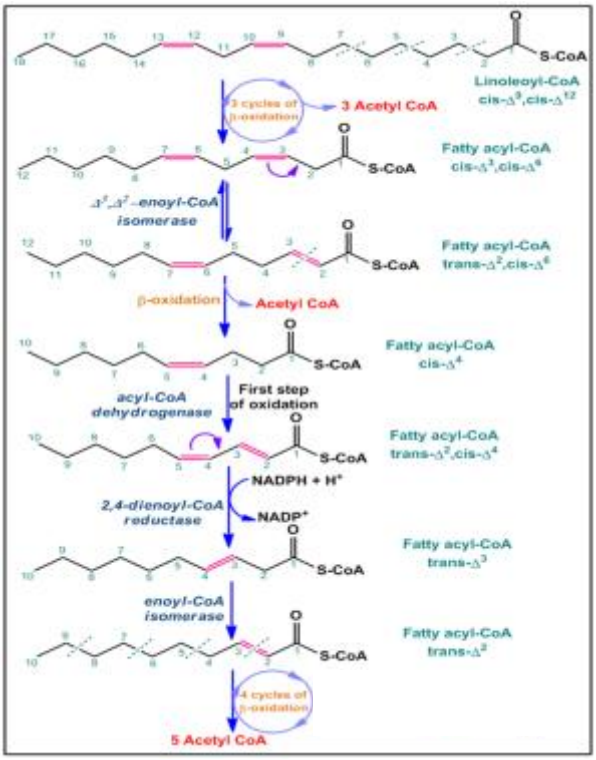

Figure 1*: Cryptococcus* as cell factory for tailor-made fatty acid production.

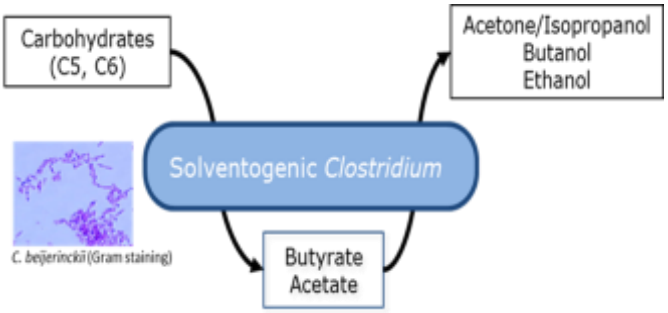

Figure 2. *Clostridium beijerinckii* as cell factory for production of various alcohols.

Functional derivatized and bio-encapsulated proteins: new strategies for biomaterial preparation and structural characterization

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Abstract:
It is the future strategy in biotechnology to use the processes of nature to create biomaterials. One interesting class of biomaterials is bio-inspired silica, where the polycondensation of silicic acid is directed by the presence of a polycationic templating molecule or by enzymes. Bioinspired silica coprecipitates of enzymes find a wide range of applications for analysis and catalysis in industrial and academic research. Here we used ssNMR as a proxy for the 3D structure of enzymes trapped in bioinspired silica. We show that it is easy to assess whether the enzymes retain their native conformation in atomic detail. We thus propose ssNMR as a rapid and reliable tool to analyze this kind of samples at high resolution. Moreover high-field DNP can be applied to biosilica-entrapped enzymes, and significantly higher sensitivity can be achieved, both with and without cryoprotectant. We reported an integrated strategy for the characterization of both protein and bio-inspired silica scaffold generated.
Another main industrial applications of enzymes is as biological drugs (biologics). However, proteins are characterized by poor pharmacokinetic and safety profiles. PEG-coating of biologics provides several benefits, including an increased half-life related to reduced renal clearance, an increased stability to degradation, and a reduced immunogenic/antigenic response. The confirmation of the preservation of the three-dimensional structure and the activity is a strict requirement. We developed new protocols to prepare samples of PEGylated proteins and we demonstrate that, for PEGylated proteins in the sedimented state, the spectral quality is comparable to - or better than - that of the corresponding crystalline samples. The excellent quality of the ssNMR spectra would make it possible to perform extensive resonance assignment and even a conventional full structure determination. The simplicity of the method should make it attractive for industrial purposes: biologics are expensive drugs, in terms both of design and of manufacturing, and significant savings could be obtained if ssNMR could be used to monitor optimization of the PEGylation procedure.

Antioxidant and cytotoxic properties of non-conventional food plant, *Parkia timoriana* (DC.) Merr.

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Abstract:
The tree bean, *Parkia timoriana* (DC.) Merr. is widely distributed in the north-east India and south-east Asia, and is largely consumed for its rich protein content and characteristic flavor which is due to the presence of thioproline (Figure 1). Thioproline (thiazolidine-4-carboxylic acid) is a cyclic sulfur-containing amino acid, and its endogenous formation is considered as a detoxification pathway of formaldehyde. Some anti-tumor effects of thioproline in cancer patients have been clinically reported[1,2]. Thioproline is an effective nitrite-trapping agent in human body thereby inhibiting endogenous formation of carcinogenic N-nitroso compounds[3]. Supplementation of diet with thioproline has been shown to stimulate lymphocyte functions and increased immune activities in old rats[4]. So far, experiments conducted by various workers have used synthetic compound but studies involving natural thioproline from plant-based food has not been investigated. In this paper, investigations have been made with regard to antioxidant properties of thioproline using DPPH radical assay, hydroxyl radical scavenging and inhibition of superoxide radicals. Cytotoxic properties using MTT [3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay in normal mouse fibroblast cell line (NIH/3T3) which is used as tumor model has been made. Strong free radical scavenging activities could be observed in all the above assays. Methanol extract of *Parkia timoriana* seeds tested for cytotoxicity showed low toxicity up to 400 ug/ml and increased toxicity above this concentration thereby showing that the extract possesses significant anti-tumor potentials for possible extraction and purification of the compound for large scale exploitation in drug and pharmaceutical industries.

Keywords:

Antioxidant, cytotoxicity, non-conventioanl food plant, *Parkia timoriana*, DPPH assay, hydroxyl radical scavenging, superoxide radical, MTT assay, anti-tumor properties, mouse fibroblast cell line NIH/3T3.

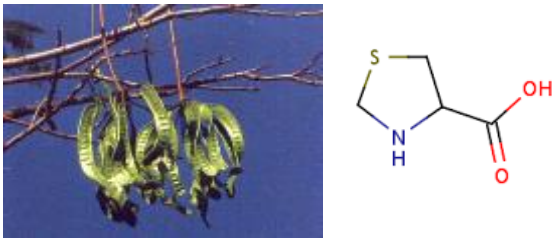

Figure 1: Seed pod of tree bean, *Parkia timoriana* (left) and chemical structure of thioproline (right)

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Changes in lipid and fatty acids composition in wine yeasts by different temperatures

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Abstract:
Winemaking industry employs many fermentation strategies for the production of wine (e.g. different yeast strains, different fermentation temperatures). The waste yeast biomass obtained after the fermentation might be biotechnologically utilized, as opposed to the usual composting or the use as feedstock, as is the common practice. The aim of this work was to determine the changes in lipid and fatty acid composition in 11 wine yeast strains isolated from various sources from the winemaking technology processes, both *Saccharomyces cerevisiae* and non-*saccharomyces* strains. The yeast strains were cultivated in temperatures: *5, 10, 20, 30, 40°C. The growth curve was observed and the biomass content was determined for all 11 strains. GC-MS with* SP-2380 column was used for the determination of the fatty acid content. It was found that the change in cultivation temperature leads to the changes in both total lipid content and fatty acid composition. The content of the palmitoleic acid is a constant in *S. cerevisiae* strains even during great changes in cultivation temperature. This may be exploited in the production of nutritionally important fatty acids from *S. cerevisiae.*

Keywords: wine, yeast, fatty acids, lipids

Leidenfrost like effect exhibited by microbubbles for biological systems such as Anaerobic Fermenters

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Abstract:
Anaerobic fermenters are used ubiquitously in the biochemical processing industry with the primary use being for ethanol production. Conventional production of ethanol depends on the system set up and the microorganism used to produce the ethanol. One of the key processes to make low alcohol drinks or alcohol free drinks is to add unit operations to the brewing process in order to have reverse distillation happen. The alcohol is to be removed without significantly removing or changing the other volatile components of the mixture or denature other products. One of the major ways to achieve this is to use vacuum and boil off the alcohol exploiting its volatility and another is to add a tertiary component to the mixture which doesn't compromise on the taste. In-situ product removal is in vogue with products being removed under operation. This would help remove inhibitors for the process , add nutrients to the system and therefore speed up the fermentation as observed in [1].

Hot microbubble injection in thin liquid layers has now been used for phase separations without significant change in the global liquid temperature has been effectively shown to separate ethanol-water mixtures , break the ethanol-water azeotrope as well as separate ammonia-water mixtures [2,5] These separations tend to have substantially high mass transfer coefficients thanks to the driving force being present in the bubble, the bubble acting as a third phase and the temperature gradient helping the separation coupled with the concentration gradient.

Our study shows that although there is a possibility to rapidly separate out the components in a mixture (high mass transfer rates) with relatviely low heat shock/stress to the bacteria in the culture. Two questions arise from this,

Firstly, what causes high mass transfer whilst keeping global temperatures constant or even lower than the injection temperature. Secondly, does local heating (due to hot bubbles heating locally) cause any cell death in the system ?

These were investigated in our study and it was found that there is an underlying principle where the hot bubble doesn't transfer heat when injected in thin liquid layers due to the Leidenfrost-like effect [6,7] exhibited by the bubbles. A thin layer of vapour surrounds the bubble for a small period of time (Figure1) which dramatically reduces the heat transfer rate until the bubble rise takes place resulting in a negligible heat transfer whereas the concentration gradient ensures sufficient mass transfer to take place. We will be presenting results on this process with effectively 100% EtOH removal with negligible cell death and liquid heating.

Keywords: Anaerobic Fermentation, Phase Separations, Transport Phenomena, ISPR

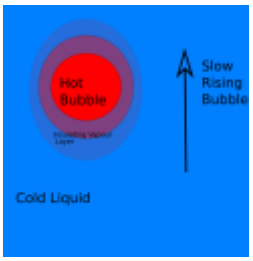

Figure 1: Illustrating the hot bubble interface with the surrounding cold liquid. The Leidenfrost like effect ensures that there is no significant heat transfer across the boundary layer but mass transfer takes place for a brief period of time resulting in effective separations with negligible cell death and global temperatures rising for thin liquid layers.

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Enzymatic synthesis of glycans with Immobilised Leloir-Glycosyltransferases in a microreactor system

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Abstract:
Glycoproteins, glycolipids and proteoglycans are among the important class of glycoconjugates. As such, they serve as multifunctional information carriers in cell-cell and cell-matrix communication. They are involved in the control of infections, cancer invasion and metastasis, viral and bacterial cell attachments as well as inflammation and immunity and developmental processes [1]. Leloir glycosyltransferases (GTs) have already been widely used for the synthesis of glycoconjugates in order to carry out individual glycosylation steps with a high product yield in a relatively short reaction time. GTs, which are in consecutive reactions, have already been described, also for the synthesis of nucleotide sugars, the donor substrates of the GTs [2]. Limitations of the synthesis on a larger scale are the costs of the enzymes and the often different optima of the reaction conditions in each step of the enzymatic cascade. Immobilization on magnetic carriers provides the advantages of high stability, recyclability and easy separation. We developed a novel microreactor system using magnetic carriers for syntheses in a nearly preparative scale (Figure 1). It realizes contactless process control via permanent and electromagnetic fields and syringe pumps. To adapt reaction conditions for ideal syntheses a temperature control is installed and compartmentalized microreaction chambers are generated. Linking reaction cascades with the flexible combination of individual immobilized enzymes allows the syntheses of tailor-made glycoconjugates for innovative biomedical applications. We report the successful synthesis of the glycoconjugate LacNAc-linker-t-Boc from galactose with immobilized β1,4-galactosyl-transferase in the microreactor system.

Keywords: biocatalysis, carbohydrates, glycoconjugates, bioreactor, immobilized microfluidic enzymatic reactor, magnetic particles

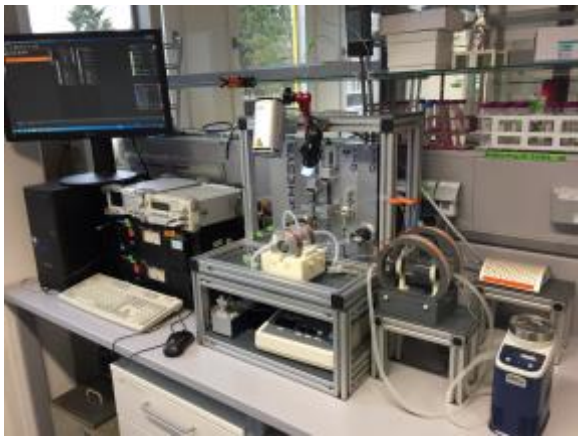

Figure 1: Overview of the microreactor system: On the left you see the computer with a commercial software which controlls the entire reactor system. Commercial pump, valve and spectrometer modules are installed in the framework structure. Connected to this is an FEP capillary, which is guided between two electrocoils mounted on a 3D printed holder. An infrared radiator and a video recording device are installed above and a control desk beneath. To the right a reactor module with temperature control is connected.

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Aerobic fermentation of *Zymomonas mobilis* integrated with *in situ* separation of bio-products using microbubble technology

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*The auther is interested in Biotech Session II: Bioprocess / Biomedical/ Food Engineering

Abstract:
Zymomonas mobilis has long been known as the best microbial producer of ethanol, and it is widely used to produce a large quantity of ethanol in anaerobic conditions, offering many advantages over other ethanol producers such as *Saccharomyces cerevisiae* [(1)]. Under aerobic condition, however, this bacterium produces a reasonable amount of acetaldehyde and carbon dioxide with lower quantity of ethanol[(2)]. Acetaldehyde and carbon dioxide accumulation in the fermentation broth can cause severe inhibition for *Zymomonas* growth. Removing the accumulated acetaldehyde and carbon dioxide, however, reduces the chemical activity of the gaseous products with a negative value change in Gibbs free energy; hence the biological reactions become thermodynamically favourable and provides momentum for the formation of more products. Microbubbles generated by fluidic oscillation were used to remove both acetaldehyde and carbon dioxide from the fermentation broth.
The results show that the *Zymomonas* growth is inhibited by acetaldehyde at concentration as low as 0.1% with several morphological changes seen of the bacterial cells by SEM. This inhibition can be avoided by stripping with microbubbles, which removes acetaldehyde from the fermentation broth with 99% efficiency (Fig. 1 and 2), leading to relatively high microbial growth. Using the microbubble technology periodically, however, gives 45% yield of ethanol and 1% yield of acetaldehyde with 110% yield of microbial biomass in comparison with 70%, 0.5% and 90% yield for ethanol, acetaldehyde and biomass respectively in the initially sparged group. Also, the periodically-sparged group produces 900% more carbon dioxide than the initially sparged group. Additionally, the oxygenation concurrent with the stripping process by microbubbles efficiently maintained the oxygen concentration in the fermentation broth above the critical oxygen concentration, leading to stable aerobic conditions. This approach has potentially high ramifications particularly for fermentation-based industries and it promises to offset many of traditional aerobic fermentation deficiencies.

Keywords: Aerobic fermentation, *In situ* separation, Microbubble technology, *Zymomonas mobilis*.

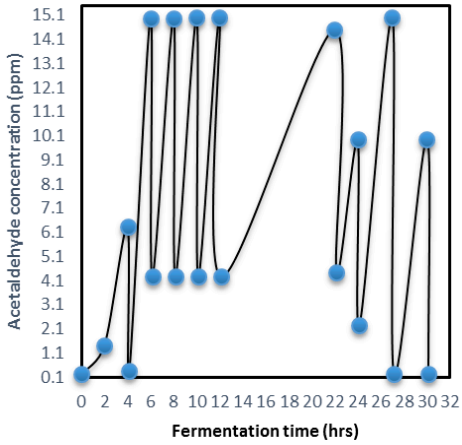

Figure 1: Figure illustrating the fluctuation of the acetaldehyde concentration, which was produced in the fermentation broth by *Z.mobilis* under microbubble-sparged conditions.

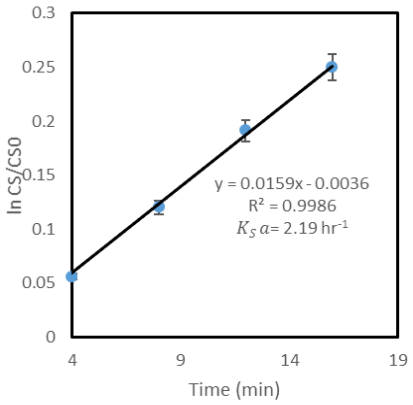

Figure 2: Figure illustrating the Variation of acetaldehyde concentrations with each sparging cycle and calculation of stripping rate constant $K_S a$.

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Fabrication and application of 'all-printed' enzymatic microreactors

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Abstract:
Immobilization of enzymes is a common technique to expand the possibilities for their use in industrial processes. Several approaches to retain the catalyst in the reactor have been proposed depending on the application and the specific enzyme. Scenarios where tagged enzymes are available favour sophisticated immobilization methods depending on covalent or ionic interactions with spacer molecules on surfaces or the crosslinking of enzymes. For process development as well as for screening purposes, physical entrapment of enzymes in biocompatible hydrogels can be applied as it is simple and fast.

To meet these challenges and increase the number of complex experiments, a scalable reactor setup was developed in our group. Manufactured by rapid prototyping it is suitable for the testing of several conditions for industrial purposes in rapid succession. The system is modular, single modules can be exchanged and the setup is compatible to standard laboratory equipment. For easy manufacturing, a standardized polymer enclosure is produced first, and only the functional insert is exchanged (Figure 1). The reactor is closed by a lid and can be connected to standard tubing and thus to analytical equipment like flow-through spectrometers or pH-electrodes.

For physical entrapment of enzymes in hydrogels, 3D-printed hydrogel lattice structures were developed. Its properties are low degradability, high porosity as well as mechanical stability. The hydrogel cubes containing the enzymes can be stored and inserted into the reactor right before the start of the process. Tests with the model enzyme beta-galactosidase showed stable enzyme activity over a time of ten days – indicating that the amount of leached enzyme is low.– As the number of manual process steps is reduced and components are either standard or produced by rapid prototyping we propose this modular system as a tool for development of enzymatic processes when high flexibility is required.

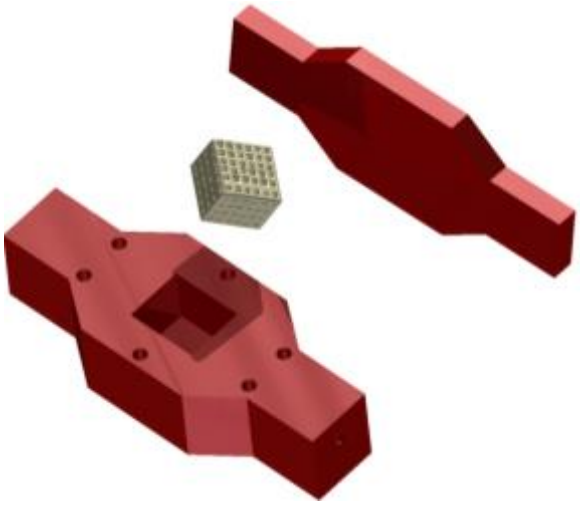

Figure 1: Layout of a modular reactor with hydrogel insert. Reactor housing, lid and hydrogel lattice structure are manufactured by rapid prototyping so that the volume of the reaction chamber and its design offer a great degree of freedom.

Keywords: enzyme immobilization, porous hydrogel structures, rapid prototyping, modular reactor system, scale-up, economic process development.

References: Kazenwadel, F., Biegert, E., Wohlgemuth, J., Wagner, H. and Franzreb, M. (2016), A 3D-printed modular reactor setup including temperature and pH control for the compartmentalized implementation of enzyme cascades. *Eng. Life Sci., 16(6): 560–567.*

EFFECT OF WAVELENGTH TUNING MATERIAL AND CO_2 SUPPLY ON *DUNALIELLA SALINA* GROWTH IN PHOTOBIOREACTOR

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Abstract
The amount of greenhouse gases mainly CO_2 emission from industry has reached to a problematic level during last few decades; hence, scientists are investigating an effective solution for this issue. Thereby, microalgae have been offered for this purpose since microalgae uses high amount of CO_2 in photosynthesis to grow and produces valuable biomass and hydrocarbons. After supplying all other requirements in abundance for the photosynthesis, only the driving factor will be the availability of light since chlorophyll and accessory pigments absorb light at certain wavelengths (450nm and 680 nm) which powers the necessary reactions. More efficient microalgae growth results in more efficient CO_2 capture. Therefore, this project aims to investigate the effect of spectral shifting the wavelengths of light unutilized in photosynthesis such as UV light to blue light as well as green light to red light. For this purpose, *Dunaliella salina* CCAP 19-30, which has high CO_2 uptake ability, was grown in a 3L airlift photobioreactor (ALB) which was coated with specifically produced wavelength tuning materials and under 24 hour UV light/white light illumination and CO_2 supply with different concentration. As a result, there is an obvious enhancement of the *Dunaliella* growth rate and the algal cell density achieved when using the coated ALB compared to the uncoated on well as CO_2 supply one compared to non-CO2 SUPPLY.Moreover, *D. salina* which does not grow under UV light has been shown to grow under these conditions using our wavelength tuning set-up.

Keywords: Fluorescent dye, wavelength shifting, *Dunaliella salina*, airlift photobioreactor.

Structural elucidation of xylan and xylooligosaccharides from selected underutilised African crops

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Abstract:
Lignocellulosic biomass, a principal byproduct of agricultural sector, has great potential to be used as a source for biochemical product synthesis. Xylan, the second predominant biopolymer in plant cell wall offers researchers an avenue to be used as a raw material to produce many value-added products. The structural pattern of xylan mainly depends on the nature of plants and their monosaccharide composition. The xylan structure has an impact on its hydrolysed product, xylooligosaccharides (XOS), which can be used as a dietary fiber in food and pharmaceutical products. In this study, xylan extracted from agricultural residues of bambara, cowpea and sorghum biomass were tested as feedstocks for XOS production. The selected biomasses are from underutilized crops of Africa and have significant amount of carbohydrates in them. The ^{1}H-NMR analysis has confirmed that the xylans present in the selected biomasses were arabinoglucuronoxylan. The xylans were further hydrolysed by GH11 xylanase from *Thermomyces lanuginosus* SSBP to produce XOS. The hydrolysed products were detected by TLC and concentration of the oligomers were quantified by HPLC. The MALDI-ToF MS analysis has proven the presence of oligosaccharides with pentose residues from dimer to oligomers of 13 residues. From these analyses, it was further confirmed that the xylan from all biomasses were arabinoglucuronoxylan with significant variation in monosaccharide content. The xylan could have arabinose, glucuronic acid or both substituted in single or adjacent xylose residues. The assessment of XOS as a prebiotic is in progress which will further be applied in food based applications.

Keywords: Biomass, xylan, dietary fiber, xylooligosaccharides, xylanase, TLC, NMR spectroscopy, MALDI-ToF MS.

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Rheology of Activated Sludge as a means to Indicate Floc Structure Changes and Settling Properties

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Abstract:
Unfavorable influent wastewater quality as well as inappropriate bioreactor arrangement and operational conditions may lead to filamentous or viscous bulking of activated sludge (AS) resulting in poor separability [1] and changes in flow behavior which may affect oxygen mass transfer and energy consumption of pumping as well [2]. Research purpose was to investigate changes in apparent viscosity during the evolution of AS bulking. Continuous-flow lab-scale experiment was carried out by the feed of nutrient (N and P) deficient synthetic wastewater for 25 days. AS floc structure was analyzed by microscopy and Diluted Sludge Volume Index (DSVI) was measured as well as rheological measurements were carried out. From apparent viscosity values measured in the shear rate range of 0.1 – 100 s^{-1} consistency index and flow behavior index were calculated applying Ostwald law [3]. At the applied biomass concentration of ca. 5 g/L, the AS floc structure and settling ability was seriously deteriorated resulted in very high DSVI (>400 cm^3/g on the 11th day, Figure 1). Both production of extracellular polisaccharides (measured by India ink staining) and abundance of filaments reached excessive levels with the remarkable increase of viscosity during the whole experiment. At the same time consistency index went from 0.04 up to 0.38 $Pa.s^n$. Results showed that rheological measurements may be good indicator of AS floc structure changes and powerful tool for follow-up of filamentous and viscous bulking.

Keywords: activated sludge; filamentous bulking; viscous bulking; rheology; viscosity

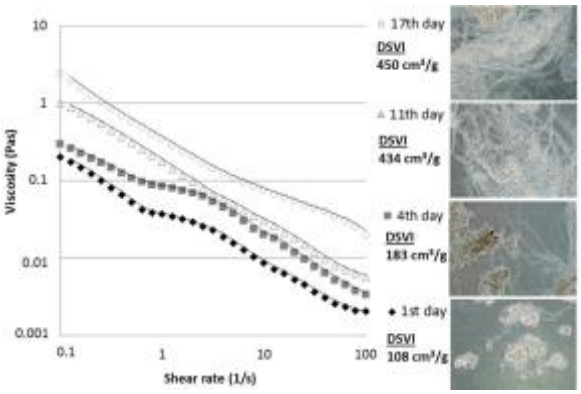

Figure 1: Measured apparent viscosity at different shear rates illustrated in the range of 0.1 – 100 1/s. Native samples were examined with phase contrast, magnification: 200).

Acknowledgements:

Research was supported both by Hungarian National Research, Development and Innovation Office and Campus France.

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Yield and characteristics of keratin hydrolysates obtained from chicken feathers irradiated with accelerated electrons

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Abstract:
We studied the effect of irradiating chicken feathers with accelerated electrons on the extraction yield of hydrolyzed keratin. The feathers were obtained from a poultry farm located in Quito, Ecuador. The irradiation was performed in the linear electron accelerator ELU-6U located at the Nuclear Science Department of *Escuela Politécnica Nacional*. First, the higher yielding process for obtaining hydrolyzed protein extracts was determined through a factorial design 3×2×2, which variables were: Na_2S concentration, temperature and purification method. The selected process allowed to recover 75.55 % of the protein from non-irradiated feathers. Then, a completely randomized design was used to select the irradiation dose that provided the highest recovery of keratin. Doses between 10 and 25 kGy were tested. The best dose corresponded to 25 kGy, and the keratin recovery was 92.78 %. Subsequently, keratin extracts were prepared from irradiated and non-irradiated feathers, under the best obtaining conditions determined experimentally, for their comparison. Physical, chemical, microbiological and sensory characteristics were evaluated. The irradiated feathers constituted the best raw material for the extraction of keratin, since they allowed to obtain hydrolyzates with a higher protein content, which were found to be free of *Salmonella,* one of the most common pathogenic microorganisms in poultry, and it had sensorial characteristics similar to those of the commercial keratin extracts. The functional groups determined through FTIR indicated that there were not noteworthy differences between the hydrolyzates from irradiated and non-irradiated feathers.

Keywords: Keratin; chicken feathers; ionizing radiations; protein hydrolizates; electron accelerator beam, Fourier transform infrared spectroscopy.

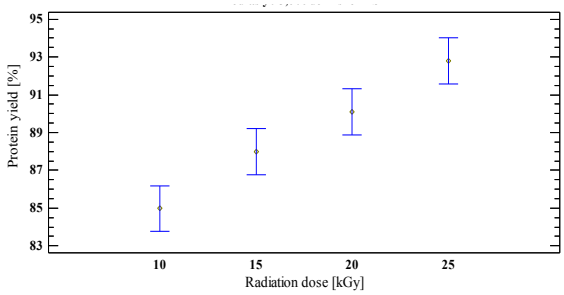

Figure 1: Effects of the irradiation dose on the yield of keratin hydrolizates extracted from chicken feathers (Fisher, 95 % confidence)

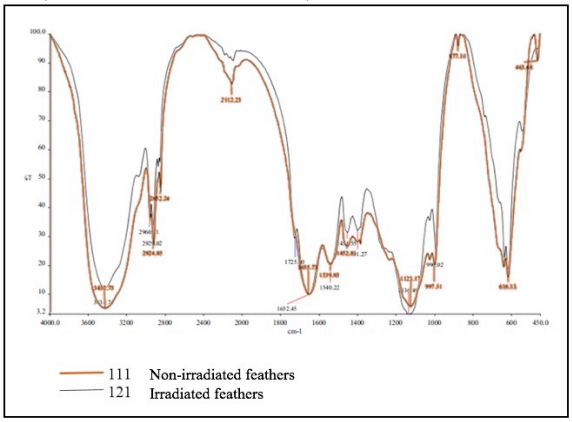

Figure 2: IR spectrum of hydrolyzed keratin samples obtained from irradiated and non-irradiated chicken feathers.

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Nanotech / Biotech Joint Plenary session II

Medical Nanochemistry, the use of reactive inorganic nanoparticles in medicine.

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[2] Vall d Hebron Institute of Research, Barcelona, Spain
[3] Institut Català de Recerca i Estudis Avançats, Barcelona, Spain

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Abstract:
Chemical processes performed or promoted by inorganic nanoparticles in physiological conditions allows to monitore and manipulate biological states, from its catalytic properties, as ROS quenchers CeO2 NPs, to coordination chemistry, for the pH controlled release of antitumoral agents, first developed with polynmers and then gold NPs, or the use the aggregate state of a compound in the form of a NP which progressively dissolves or corrodes, as a carrier of itself, as curcumin or iron oxide nanoparticles, and providing cations and electrons when dissolving affecting cellular REDOX state (metabolsim). A case that is already in the clinic is the use of feromuxitol to treat ferropenic aenemia. Other more subtless alterations of the reactivity of the biomolecules is observed in the case of therapeutic antibodies linked to NPs. In this chemical approach is also important to unveil the special features that rise in the pharmacokinetic models when working with nanoparticles, very different from the small drug standard pharmacokinetical models.
Due to their higher percentage of surface atoms and their colloidal nature, NPs experience processes that transform them towards more stable thermodynamic states -driven by the minimization of their reactivity- which is translated into high rates of oxidation, dissolution, aggregation and interaction with proteins. Aggregation, that is colloidal stability, has a significant influence on the reactivity, bioavailability and pharmacokinetic of NPs, having long been recognized that the effective size can mediate their toxicity, as in the case of asbestosis, industrial aerosols and ambient particulate matter. Similarly, oxidative dissolution favors the chemical dissolution of the NPs, affecting their persistence and promoting the release of ionic species. The physicochemical state of the NPs also play a role in their interaction with media proteins, and the subsequent nature of the protein corona around them (the so-called soft and hard corona), inevitably providing them with new biological identity, which determines their physiological response including cellular uptake, biodistribution and toxicity. Although the extent of each individual process, determined by the intrinsic properties of the NPs (material, size, shape, concentration, crystallinity, surface charge and coating) and the nature of the media in which they are dispersed (ionic strength, protein concentration, pH…), can be studied separately, the fact that the greatest NP transformation occur within the same time scale (from minutes to hours of exposure) it is translated into the overlapping and competition between all the different processes involved. Thus, it is common to observe that NPs are instable and tends to aggregate after their exposure to cell culture media and that they corrode while being coated by proteins. As a result of this complexity, it is critical to fully understand the transformation kinetics of NPs in biological systems. In this regard, the understanding of these transformations not only may help to better interpret the biological effects of NPs, but also assists in designing the desired NPs for specific purposes. Finally, a NP surface must have charges to organize surrounding ions and control interactions with the surrounding media1 (e.g.:Ca2+ ions interferes between surfaces and proteins in implants). Indeed, drug penetration and distribution properties have been related to the electrostatic water envelop that surrounds it. This chemical approach will contribute to the development of tools for medicine and consequently nanomedicine.

[1] More than a century ago, Franz Hofmeister recognized that ions with identical charge precipitate proteins to differing extents. It was later discovered that equally charged ions exhibit opposite tendencies. Indeed, the Hofmeister series can be observed for many interfacial phenomena, usually when the Debye length is small, as in the NPs object of this study.

Engineering porous silicon nanoparticles for smart drug delivery

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Melbourne Centre for Nanofabrication, Australian National Fabrication Facility, Clayton, VIC, Australia

Abstract:
Targeted approaches to deliver anti-cancer drugs have the potential to achieve improved efficacy and at the same time reduced side effects. In fact, this is one of the cornerstones of nanomedicine.
We are exploring the use of high porosity biodegradable porous silicon and genetically engineered biosilica nanoparticles that are loaded with chemotherapy drugs or siRNA and also display on the particle's periphery targeting moieties such as cell-surface antibodies recognising cognate ligands highly expressed on the surface of tumour cells.
One approach centers around porous silicon nanodiscs. The process relies on a combination of colloidal lithography and metal-assisted chemical etching. Height and diameter of the pSi nanodiscs can be easily adjusted. The nanodiscs are degradable in physiological milieu and are non-toxic to mammalian cells. In order to highlight the potential of the pSi nanodiscs in drug delivery, we carried out an *in vitro* investigation which involved loading of nanodiscs with the anti-cancer agent camptothecin and functionalization of the nanodisc periphery with an antibody that targets receptors on the surface of neuroblastoma cells. The thus prepared nanocarriers were found to selectively attach to and kill cancer cells [1,2]. In a second approach, we used natural nanoporous biosilica from the diatom *Thalassiosira pseudonana*. The biosilica was genetically engineered to display GB1, an IgG binding domain of protein G, on the biosilica surface, which allowed for the attachment of cancer cell targeting antibodies and the adsorption of nanoparticles loaded with anti-cancer drugs. Adherent neuroblastoma cells and B lymphoma cells in suspension were selectively targeted and killed by drug-loaded biosilica displaying specific antibodies (Figure 1). In a subcutaneous mouse xenograft model of neuroblastomamice, regression of SH-SY5Y tumour growth was evident in immunodeficient Balb/c nude mice that were when treated with drug-loaded anti-p75NTR-labelled biosilica. Histological analysis confirmed accumulation of antip75NTR-labelled biosilica in the tumours. This result established the efficacy of targeted drug-loaded-biosilica in a relevant clinical model [3]. In a final approach, we engineered porous silicon nanoparticles to deliver siRNA to successfully downregulate drug transporter proteins in brain tumour cells [4,5]. Coating of nanoparticles with cationic polymers improved sustained DNA and siRNA release and suppressed burst release effects. In addition, polymer coating significantly enhanced the uptake of nanoparticles across the cell membrane. Histopathological analysis of liver, kidney, spleen and skin tissue collected from mice receiving nanoparticles further demonstrates their biocompatible and non-inflammatory properties.

Keywords: drug delivery, nanoporous silicon, diatom biosilica.

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Nanotech / Biotech joint Session III: NanoBioMedecine / Nanosafety

Gold Nanoparticles can shuttle Ions across Phospholipid Bilayer Membranes

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Abstract:
Gold Nanoparticles capped with mercaptocarborane ligands have a number of unusual properties that make them of interest as membrane transporters in biological and bio-mimetic systems.[1] Most pertinent for this application is the ability of the particles to adjust their hydrophilicity. The particles, as prepared, are negatively charged by excess electrons in the metal core. This charge is partially balanced by Na ions that reside within the voids of the ligand shell. The resulting net charge of the particles makes them dispersible in water. Under condition of less or no net charge, the particles become less hydrophilic and, if possible, preferentially associate with the phospholipid membrane of vesicles as shown in Figure 1. In the presence of a membrane potential and a remaining small net charge of the particles, the particles can be forced by the electric field to flip across the membrane. This process can lead to the transport of ions by the particle from one side of the membrane to the other. Here we will present the results of monitoring this process using two different experimental systems. The first is based on measuring the depolarization of a vesicle membrane as Na ions are shuttled across by the particles. The second system measures electrochemically the particle-mediated charge transfer across an artificial membrane supported by a small hole in a plastic cell. Both experimental setups give consistent and complementary information, and it is evident that the particles do not act as ion channels but as membrane potential dependent electrostatic shuttles. Examples of nanoparticles carrying charge across membranes are to date very scarce.[2]

Keywords: Gold Nanoparticles, Phospholipid Membranes, Charge Transfer, Vesicles, Electrochemistry, Mercaptocarborane, Ion Shuttles, Ion Channels, Transporters, Fluorescence Spectroscopy, Membrane Potential.

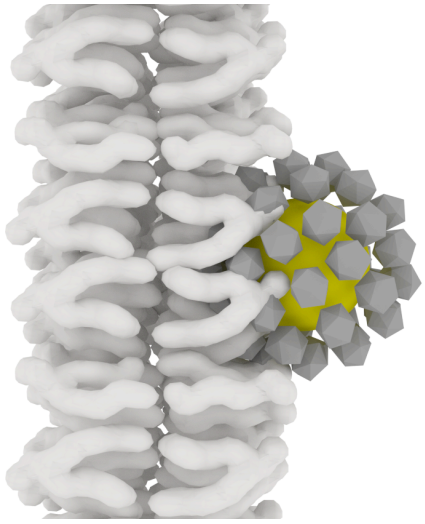

Figure 1: To-scale cartoon of a phospholipid bilayer membrane and a *ca*. 3 nm mercaptocarborane-capped gold nanoparticle. Note the open structure of the ligand shell, which allows for the inclusion of Li, Na or K ions counterbalanced by electronic charge in the metal core. Electrostatic charging of the particles makes them dispersable in water, while neutral particles are very hydrophobic and tend to bury into the nonpolar part of the membrane. This adaptable behaviour and the ability to carry cations in the ligand shell enables the particles to shuttle charge across the membrane.

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Nanomedicine for Molecular Imaging and Targeted Therapy of Cardiovascular Diseases

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Abstract:
One hundred years ago, Paul Ehrlich, the founder of chemotherapy, received the Nobel Prize in Physiology or Medicine. His postulate of creating 'magic bullets' for use in the fight against human diseases inspired generations of scientists to devise powerful molecular imaging agents and therapeutics. After the use of various synthetic polymers, natural polysaccharides have received a lot of attention in the biomedical field thanks to their biological properties and to recent progresses in polysaccharide chemistry and nanotechnologies. These new dedicated nanosystems may be designed to fight atherothrombotic pathology, which remains the main cause of morbidity and mortality in the industrialized world.

The presentation will intend to provide an overview on polysaccharide-based nanosystems as drug delivery systems and targeted contrast agents for molecular imaging with an emphasis on the treatment and imaging of cardiovascular pathologies

Multilayer Nano-Films for Controlled Release of Therapeutics

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Chung Ang University, School of Chemical Engineering and Material Science, Republic of Korea

Abstract

Recent developments in biomedical engineering, novel cell culture techniques, and newly discovered chemicals as a replacement of the growth factors have opened new area toward the engineered technique of biomedical applications, for use in both research and clinical applications. Ideal bio-platform must be biocompatible that is to say, they should show both proper surface stability for the promotion of cell attachment and functions with drug delivery. However, plenty of challenges are still in progress, as the reason for the high compatibility of polymer film in cell culture environment and precisely controlled functional release of their drugs..

Layer-by-layer (LbL) self-assembly technic has been developed and used to prepared biocompatible multilayer films and polyelectrolyte capsules for drug delivery. Certain drug molecules, such as active proteins, cytokine, growth factors, enzymes, nucleic acids, and DNA, have been immobilized into nano-sized multilayer films. The advantages of LbL multilayer films as drug delivery systems include the sustained drug release through controlling the film physical & chemical properties, in addition multilayer films have the potential to protect drug molecules from losing their biological functions, and the film preparation process is simple and can be automated.

In this presentation, we prepared the cell friendly platform by take full advantages of LbL assembly with evenly distributed drug loading by nano-sized layer assembly. The nano-films are prepared by various materials including not only synthetic-, natural-polymers but also functional materials such as growth factors, cytokines which are resulting different film degradation profiles. These results lay a cornerstone for future studies to achieve the multi-functional nanofilms including programmed loading/release of drugs for therapeutic purposes

Keywords: multilayers, layer-by-layer, self-assembly, protein, growth factor, controlled release, nano-film

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A targeted vaccine delivery system using functionalized gold nanocages and an intestinal Trojan horse

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Abstract:
The intestinal epithelium forms an essential element of the mucosal barrier and plays a critical role in the pathophysiological response to different enteric disorders and diseases. It is also a barrier for oral vaccine delivery. Here, we demonstrated the feasibility of purposefully designing immunogen-carrying gold nanocages functionalized with σ-1 proteins from T1L reovirus to deliver immunogens across the mucosal barrier to enhance immunological responses. The immunogen delivery was further enhanced by a Trojan horse system. The Trojan horse system with the synergy of nanotechnology and host cells can achieve better therapeutic efficacy in specific diseases. Primary isolated intestinal stem cells were used to create these Trojan horses. M-cells, which are the targets for σ-1 functionalized nanocages, were induced in these Trojan horses to allow easy pass of the nano-vaccine complexes into the Trojan horses.

Here, we demonstrated this proof-of-concept intestinal Trojan horse nano-vaccine system that will have a wide variety of applications in the development of oral vaccines for infectious diseases.

Keywords: σ-1 protein, gold nanocages, nano-vaccines, intestinal stem cells.

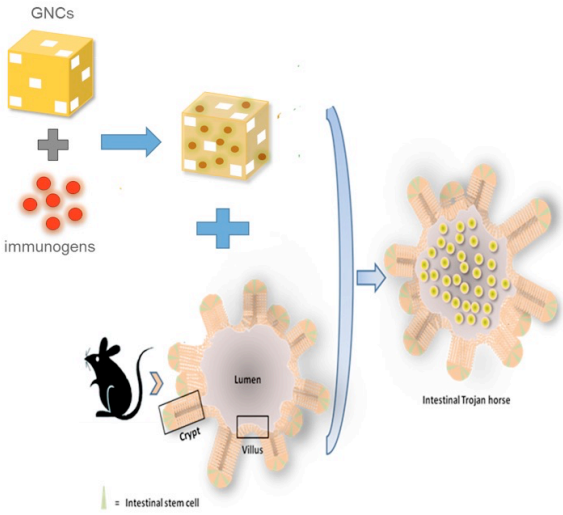

Figure 1: Figure illustrating the mechanism of targeted vaccine delivery using gold nanocages functionalized by σ-1 proteins from reovirus to deliver immunogens into Trojan horse made from small intestinal stem cell to facilitate vaccine delivery across mucosal barrier to stimulate strong immune-responses.

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Asymmetric flow field-flow fractionation: A new approach for analysis of the protein corona

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Abstract:
Targeted drug delivery by nanosized particles is a potent and promising medical concept, especially in cancer therapy. The synthesized drug carriers are injected in the blood stream and as soon as they enter any biological fluid proteins adsorb to its surface and form the so called "protein corona". As a consequence the chemical identity of the particle only plays a minor role; instead the new biological identity is responsible for the fate of the nanoparticle in the body. (1)
To operate safely in nanomedicine, it is essential to understand and predict the *in vivo* behavior of the nanomaterial and therefore analyze the "protein corona". However, this analysis is challenging, since there are not many methods to separate free proteins from those, which are forming the protein corona, without influencing the complex system. Right now, methods requiring a separation step provide access to the identification of only the strongly bound proteins, so that those methods have to be combined with other techniques that allow characterization directly in the biological medium. (2) Those techniques can give information about the loosely bound proteins, but not directly about the type of bound proteins.
A new approach to address this separation problem is the asymmetric flow field-flow fractionation (AF4). Hereby, very small particular species like single proteins can be separated from larger nanoparticle-protein complexes. The shear stress affecting the sample is very low in comparison to centrifugation, what makes it a rather mild method.
In our study, polystyrene-nanoparticles served as model systems which were incubated with human blood plasma.
After separation of the protein corona from free proteins by AF4, the obtained fractions are characterized with regards to size and protein composition by using dynamic light scattering, SDS-polyacrylamide gel electrophoresis and liquid-chromatography mass-spectrometry. We could show that the separation is possible using the above mentioned methods. Moreover, the composition of the protein corona separated by AF4 differed significantly from repetitive centrifugation as the alternative separation method. Specifically, more serum albumin with a generally lower binding affinity was present in the extracted corona. This suggests that we were able to extract the nanoparticles with strongly as well as loosely bound proteins.

Keywords: nanoparticles, protein corona, drug delivery, asymmetric flow field-flow fractionation.

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Simultaneous Live Cell Monitoring and Gene Therapy with Multi-functional Carbon Nanoparticles

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Abstract:
Light-emitting nanoparticles are emerging as promising imaging agents and therapeutic carriers for biological and medical applications.1, 2 The ability to track light-emitting nanoparticles inside the living tissues and organisms opens a range of opportunities for in vivo or in vitro biosensing or targeted drug delivery. 3, 4 Functionalization of nanoscale light-emitting sources for selective and sensitive signalling allows observation and eventually engineering of biological processes at the subcellular level.5, 6

Detection of nucleic acids (NA) at the subcellular level is especially interesting due to their vital role in gene expression and disease diagnostics. Nanoparticle platforms with combined light-emitting properties and surface functionality are particularly suitable for intracellular NA sensing. The molecular interactions between the nanoparticles surfaces and fluorescently-tagged NA can provide a signalling system for sensing.

We report on a novel method for fabrication of carbon coated alumina nanoparticles (CANPs), using the plasma-induced carbonization method that effectively modifies the surface of the alumina nanoparticles, producing a conformal ultrathin carbon layer over the entire surfaces of the particles. The hybrid nanoparticles exhibit multiple functionalities, such as fluorescence, tuneable surface chemistry, quenching ability and water solubility. A proof of concept study is demonstrated to show that CANPs can be used as an effective fluorescent sensing platform for nucleic acid detection. The fluorescently-tagged probe nucleic acids quench upon adsorption on the surface of the CANPs and the fluorescence recovers after selective hybridization with target nucleic acids.

Keywords: carbon materials, surface modification, DNA sensing, cell imaging, Gene delivery.

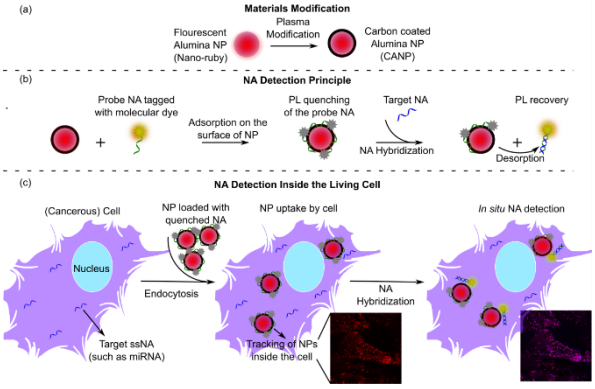

Figure 1: Schematic (not to scale) of the platform for simultaneous cellular monitoring and targeted gene release. NA loaded nanoparticles are internalized by the cell, and the NA releases from the surface of the particle upon hybridization with its complementary pair.

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Elaboration of nanohybrid based on titanate nanotubes for bio-medical applications: new nanovectors of resveratrol derivatives

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Abstract:
Titanate nanotubes (TiONts) synthesized *via* hydrothermal processes have attracted extensive interest since their first synthesis owing to their physicochemical properties such a high aspect ratio[1-2]. In spite of their interesting physico-chemical properties and their low cytotoxicity [3-4], bare titanate nanotubes (TiONts) present some shortcomings such as strong aggregation and a poor suspension stability which limit their use as biomaterials. One needs to circumvent these drawbacks to turn TiONts into valuable nanohybrids[5].
The aim of this work is to elaborate nanotubes-based nanohybrid materials suitable for biomedical uses. For this purpose, two essential features have been enhanced: colloidal stability and biocompatibility. The grafting of 3,4-dihydroxyhydrocinnamic acid (DHCA), amino acid 3,4-dihydroxy-L-phenylalanine (L-DOPA) and citric acid have been studied to enhance the long-term stability of TiONts under physiological conditions. A comparative study between the three types of grafting has been made.
The biocompatibility of TiONts in biological systems has also been improved through their surfaces modification with 3-aminopropyl triethoxysilane (APTES) and chitosan grafting, with two different methods.
Experimental conditions (pH, temperature, reaction time, molar ratio) have been tested and optimized in order to obtain the highest grafting efficiency for all the studied compounds. The obtained compounds were characterized by a large range of techniques (TEM, XPS, IR, Raman spectroscopy, zeta potential measurements, UV-vis, TGA, *etc.*).
Finally, an original study of TiONts functionalization with trans-resveratrol as a cancer therapeutic molecule will be also exposed with the results of the first bioassays.

Keywords: titanate nanotubes, surface functionalization, drug delivery, resveratrol derivative.

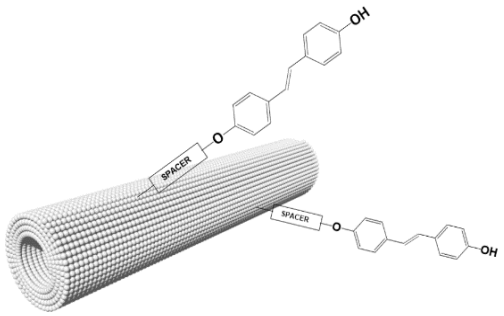

Figure 1: TiONts-trans-resveratrol nanohybrids

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Enhancing transdermal delivery of hydrophilic compound via synergistic action of d-limonene loaded PEGylated lipid nanocarriers

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Abstract:
The aim of this study was to investigate the effect of d-limonene (DL) loaded three types of PEGylated lipid nanocarriers [liposomes (PL), niosomes (PN) and solid lipid nanoparticles (PSLN)] on the skin penetration of hydrophilic compound, sodium fluorescein (NaF). The physicochemical characteristics of nanocarriers and their effects on *in vitro* skin penetration were evaluated. Confocal laser scanning microscopy (CLSM) was used to visualize the penetration pathways. Fourier transform infrared spectroscopy (FTIR) was used to confirm the enhancing effect on stratum corneum lipids. The results showed that all formulations had small particle sizes in the range from 31 nm to 197 nm, which was in the order: PL-DL < PL < PN-DL < PN < PSLN-DL < PSLN. TEM image also showed the spherical in shape. This indicated that DL reduced the particle sizes of all nanocarriers. The percent entrapment efficiency of NaF was in the range from 21% to 51%, which was in the order: PL < PL-DL < PN < PSLN-DL < PN-DL < PSLN. For the skin penetration, the penetrated flux of NaF-loaded PL-DL was significantly higher (93-fold enhancement from NaF solution) than PN-DL, PL, PSLN, PN, PSLN-DL, and NaF solution, respectively. DL loaded PL and PN vesicles increased the NaF flux due to enhanced permeability of the stratum corneum. While DL loaded PSLN decreased the NaF flux, suggesting that DL might not be released from lipid particles to skin surface. CLSM images of NaF loaded PL-DL treated skin exhibited brighter fluorescence intensity of entrapped NaF and PL-DL vesicles both in the deeper skin layer and hair follicles than other formulations. The niosome and SLN formulations had little or no vesicles in the skin, suggesting that NaF may released from these nanocarriers before permeation. For FTIR results, PL-DL provided a significant change in stratum corneum lipid, which DL-containing liposomes play an important role to deliver NaF by increasing the fluidity of stratum corneum intercellular lipid. In conclusion, PL-DL serves as a potentially effective strategy to transdermally deliver of hydrophilic compound. The combination of d-limonene (as a skin-penetration enhancer) and the small size of liposome vesicles might provide a synergistic effect to enhance the skin permeability.

Keywords: skin penetration, d-limonene, lipid nanocarriers, liposomes, niosomes, solid lipid nanoparticles, hydrophilic compound

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Plasma Engineered Nanorough Substrates For Stem Cells In Vitro Culture

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[2] University of Liverpool, Liverpool, UK

Abstract:
Stem cells based therapies are one of the greatest promise of new-age medicine due to their potential to help curing most dreaded conditions such as cancer, diabetes and even auto-immune disease. However, establishing suitable in vitro culture materials allowing to control the fate of stem cells remain a challenge.[1] Amongst the factor influencing stem cell behavior, substrate chemistry and nanotopogaphy are particularly critical.[2] In this work we used plasma assisted surface modification methods to produce model substrates with tailored nanotopography and controlled chemistry.[3] Three different sizes of gold nanoparticles were bound to amine rich plasma polymer layers to produce homogeneous and gradient surface nanotopographies. The outer chemistry of the substrate was kept constant for all substrates by depositing a thin layer of our patented biocompatiple polyoxazoline plasma polymer on top of the nanofeatures. For the first time, protein adsorption and stem cell behaviour (mouse kidney stem cells and mesenchymal stem cells) were evaluated on nanorough plasma deposited polyoxazoline thin films. Compared to other nitrogen rich coatings, polyoxazoline plasma polymer support the covalent binding of proteins.[4, 5] Moderate surface nanoroughness, in both size and density, triggers cell proliferation. In association with polyoxazoline coating, cell proliferation is further enhanced on nanorough substrates.[6] (Figure 1).
Results are discussed in term of substrates wetting properties. These findings provide valuable insights on the mechanisms governing the interactions between stem cells and their growth support.

Keywords: Nanotopography, stem cells, differentiation, plasma polymer, oxazoline

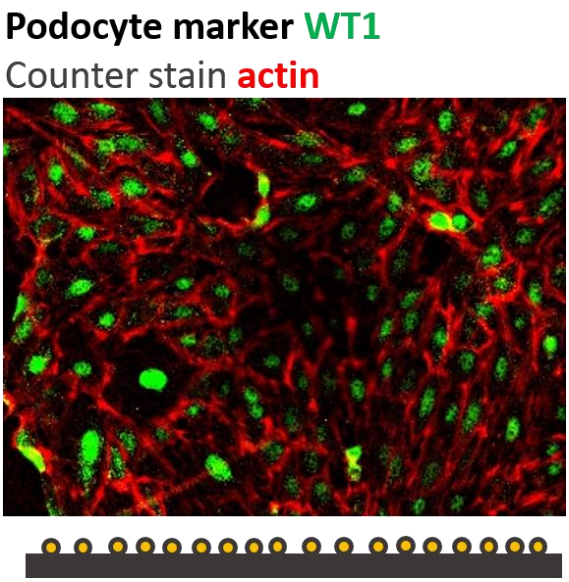

Figure 1: Kidney derived kidney stem cells grown on nanorough (decorated with 68 nm gold nanoparticles) plasma deposited polyoxazoline film.

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Providing an alternative to covalent stealth modification of nanomaterials by using tunable surfactants

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Abstract:
In the field of nanomedicine, nanocarriers are currently being investigated as potential drug delivery devices. Such nanocarriers will present a foreign interface to the body when applied into biological fluids such as blood. This results in the formation of a protein coating, called the 'protein corona'. In order to achieve a suffienct blood circulation time, unspecific cell uptake of the nanocarriers has to be avoided. Recently, it was shown that the covalent modification of the nanocarriers surface with poly(ethylene glycol) or biodegradable polyphosphoesters leads to an enrichment of specific 'stealth' proteins in the protein corona, which effectively reduced uptake in phagocytotic cells. (1) Furthermore, our group analyzed the influence of surfactants present on the nanocarriers surface after synthesis on the interaction with serum albumin. A significant correlation between surfactant concentration and protein binding affinity was found, which means that not only covalently bound functional groups will affect the protein corona. (2) Thus, we decided to take advantage of the properties of surfactants for providing an easy alternative to covalent 'stealth' modification. Therefore, different biodegradable poly(phosphoester) based surfactants were synthesized and characterized with regard to their thermodynamic binding properties on model nanocarriers. Depending on the length and structure of their hydrophilic and hydrophobic building blocks, their binding affinity could be maximized. It was found that with higher surfactant binding affinity also the tendency towards unspecific protein adsorption could be reduced. At the same time, all surfactants enabled an enrichment of the 'stealth' proteins clusterin and apolipoprotein A-I in the protein corona, which successfully reduced phagocytotic cell uptake. This non-covalent approach facilitates the tuning of protein adsorption and with it the biological fate of nanocarriers. Because of the surfactant adsorption principle, it can potentially applied to any kind of nanomaterial with sufficient binding optimization. (3)

Keywords: nanoparticles, protein corona, surfactants, stealth effect, poly(ethylene glycol), poly(phosphoester)s, isothermal titration calorimetry.

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Investigating the Mechanical Properties of Soft Biomaterials Using the Atomic Force Microscopy (AFM)

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Abstract:
Biomaterials are used widely in medical applications. They are used in both diagnostic and therapeutic applications. Microbubbles (MBs) are a type of biomaterials which are used in diagnosis as ultrasound contrast agents (UCAs) and in therapy for drug/gene deliveries. They encapsulate an inert gas in very thin monolayer phospholipid shell. The aim of this study was to investigate MBs from the engineering perspective, to assess their mechanical properties and their response under applied forces using the atomic force microscope (AFM) to perform a real direct mechanical test. The AFM was used with a conical-tipped cantilever at constant speed and constant force to investigate the impact of the MBs' size on their mechanical properties. Many mechanical properties were calculated, including plasticity, instability, hysteresis, adhesion forces, Young's modulus and the stiffness. Young's modulus was estimated by three different models, where two of them calculate the elasticity of the shell of the MB while the third model evaluates the elasticity of the whole MB body. A contact mode and a force spectroscopy regime were used with the AFM to acquire the force-deformation curves, as shown in Figure 1. The gradient of the linear part of the force-deformation curves expresses the stiffness of the MB.

Keywords: AFM, Microbubbles, Young's modulus, stiffness, Biomaterials, plasticity, instability, hysteresis, adhesion forces.

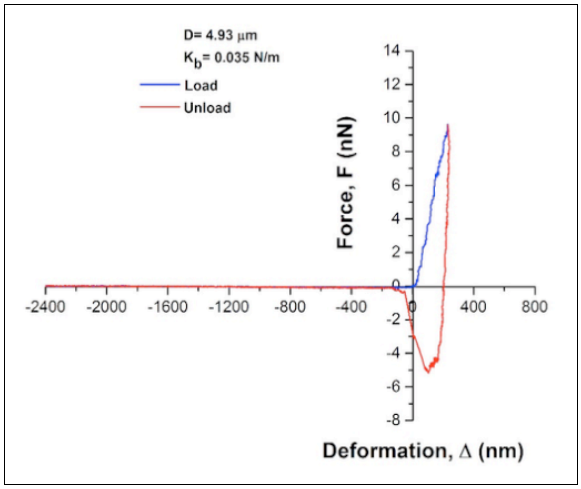

Figure 1: Load and unload curves with large hysteresis, where the MB behaves as a pure plastic material (η=0.95).

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Human Pancreatic Islet Secretory Fingerprint Analysis using a Microfluidic Impedance Spectroscopy Platform

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Abstract:
Diabetes mellitus affects 37M adults in the North America and Caribbean region [1]. This disease arises from secretory defects in the pancreatic islets of Langerhans, an endocrine cluster of cells mainly composed of α, β, δ, PP, and ε cells, which cooperate to secrete hormones in response to metabolic changes [2]. Although many research groups are interested in studying the paracrine interactions between islet cells [3] or insulin and glucagon secretion [4], not much works report on monitoring a secretory fingerprint (SF) involving more than two hormones. Advances in microfluidic perfusion systems (MPS) have enabled reproduction of the islet *in vivo* environment and allow different aspects of islet physiology to be studied [5]. In parallel, non-faradaic impedance biosensors have been developed for the detection of insulin in the femto-molar concentration range using a chemically adsorbed zwittorionic polymer with attached monoclonal insulin antibodies [6]. The aim of this worl is To develop a microfluidic multiplexed impedance biosensor platform for monitoring different hormones of interest secreted by islets Interdigitated microelectrodes (IDE) with various geometries were fabricated (Figure 1). To validate the impedance measurement, a surface plasmon resonance imaging biosensor was also employed with the same immobilization strategy as the impedance sensor. To achieve maximum sensitivity, insulin was immobilized on top of a self-assembled monolayer (SAM) of a carboxyl terminated alkyl thiol and a competitive immunoassay was performed. The IDE with a 10x10 micron inter-digit spacing and electrode width led to the largest impedance change and therefore was selected for insulin sensing. Significant changes in impedance spectra between 10 and 50 KHz were observed after flowing hormones and antibodies in the microfluidic device, demonstrating the feasibility of the proposed objective. The ongoing work consists of designing a 3-layer MPS to immobilize pancreatic islets and collect their secretions. This setup allows continuous delivery of secretagogues to the islet while avoiding the formation of concentration gradients within the chamber. Our results confirmed that it is possible to sense insulin using impedance spectroscopy in a continuous flow regime. The proposed microfluidic platform with integrated impedance biosensors is promising for real time monitoring of a pancreatic islet SF and could be used to discriminate between healthy and diseased islets.

Keywords: Human Pancreatic Islets; Secretory Fingerprint Analysis; Microfluidics, Dielectric Spectroscopy, Diabetes

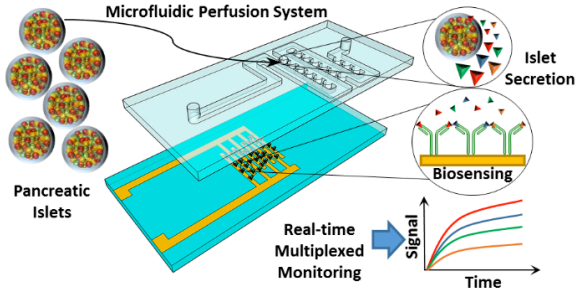

Figure 1: Microfluidic perfusion system for pancreatic islet secretion analysis.

Acknowledgements: Canadian Institutes for Health Research (CHRP, POP), National Science and Engineering Council of Canada (CHRP, Discovery, Strategic).

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Ni ions containing DNA nanowire devices

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Abstract:
DNA is a multifunctional biopolymer which can be used in the field of biomedical clinic or/and researches. Moreover, the usage of DNA has been focus in the field of nanomachines and nanoelectronics, recently, due to nanofabrication and self-assembly technology improvment. DNA exhibits high stability, adjustable conductance, self-organizing capability, programmability and vast information storage. It is an ideal linear material in the applications of nanodevices, nanoelectronics, and molecular computing. By chelating nickel ions within the base-pairs of DNA (Ni-DNA), the conductance of this complex improved dramatically. Further studies showed that nickel ions containing DNA (Ni-DNA) nanowires exhibited characteristics of memristor and memcapacitor making them a potential mass information storage and computing system. In summary, Ni-DNA has promising applications in a variety of fields, including nanoelectronics, biosensors and memcomputing.

Keywords: Metal ions, nucleic acid, self-assembled layers, Ni-DNA, nanodevice, nanoelectronics memristor, memcapacitor, redox induced negative resistant, memcomputing.

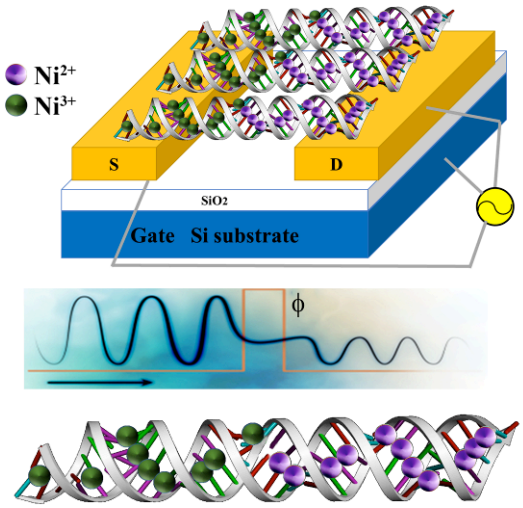

Figure 1: Figure illustrating the Ni-DNA devices (upper panel) and the charge transport properties of single based mismatched containing Ni-DNA. In this device the purple spheres denoted Ni^{2+} ions and the green spheres denoted N^{3+} ions. In the single based mismatched containing Ni-DNA device the resistance increase expernatially.

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Managing industrial accident risks in the Nanomaterials value Chain

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Abstract:
Nanomaterials industry is developing, with significant amounts of materials already being produced and transported and many new applications under development. As for any industrial activity, the whole nanomaterials value chain present a series of risks, that include accident risks. Due to specific properties of nanomaterials the management of these risks raises a number of issues that need to be solved in order to ensure a sustainable development of this promising industry and waive concerns that may arise among populations and workers.
The present study was focused on nano powders, which exhibit the highest hazards. For each step of the value chain, a generic risk analysis allows to identify the main accidental scenarios that need to be taken into account : fire, explosion, massive releases and dispersion. For each scenario, it identifies the key aspects that need to be considered in the risk assessment process and the limits of currently available tools. This includes issues relative to the characterization of hazardous properties such as flammability, explosivity, dustiness, toxicity and eco-toxicity. But it also considers the limitations of existing models for air dispersion, fire and explosion modelling. The issues raised by engineering controls that can be implemented to limit risks are also outlined.
A last part of the paper is dedicated to presenting some of the solutions that are presently being developed to overcome these limitations. The first aspect refers to the characterization of physico-chemical hazards for which recent works have lead to a better understanding of the influence of agglomeration proceses of the explosion violence[1]. They also resulted into recommendations for testing protocols which are now proposed in dedicated standardization committees.
Recent advances in modelling dispersion of nanoparticles resulting from a breach in a pneumatic transport pipe[2] are also briefly described as an illustration of a more general approach to better estimate the potential consequences of accidental events involving massive release of nanoparticles.

Keywords: nanopowders, accident risk, air dispersion modelling, explosion, fire.

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Probing the size- and charge dependent cytotoxicity of silica nanoparticles and their application in biosensors

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Abstract:
Despite the growing enthusiasm for nanotechnologies, nanoparticles (NPs) might put environmental safety and human health at risk, as they can interact with biological systems and affect their behavior. It is therefore essential to know their mechanisms of interactions in order not only to prevent their potential risks but also to benefit from their unique properties, such as in biosensors design. In this context, we study the cytotoxicity of silica NPs, with diverse sizes and charges, on the morphological and nanomechanical properties of *Escherichia coli* ($Gram^-$) and *Bacillus subtilis* ($Gram^+$) bacteria, by means of atomic force microscopy (AFM) and viability tests. These tests show that negatively charged NPs (NPs^-) with a diameter φ lower than 50-80 nm (critical diameter φ_c) lead to the isolation of bacteria, initially in aggregates, their interactions with cells being stronger than inter-cells ones. Conversely, positively charged NPs (NPs^+), whatever their diameter, lead to a strong aggregation of the cells, due to the electrostatic interactions with the two bacteria, which are negatively charged. AFM observations specify these phenomena by also showing the antibacterial activity of such NPs. In *E. coli*, NPs^- with $\varphi < \varphi_c$ induce a "spherification" of the cell initially rod shaped, the formation of pore-like lesions in the outer membrane and a reorganization of its structure. All of these damages potentially lead to the cell lysis, a particularly strong one for φ = 4nm (Figure 1A). In *B. subtilis*, although the morphology is unchanged during treatment with such NPs^-, similar damage to the membrane (peptidoglycan layer) is also observed. For both strains, NPs^- with a diameter larger than φ_c have no effect on population, morphology or bacterial structure. Moreover, independently of this critical diameter, NPs^+ tend to favor the formation of membrane invaginations, not necessarily involving cell lysis. To conclude, we have thus shown that the decrease in diameter and a positive surface charge favor the antibacterial activity of silica NPs. This fundamental study is currently being used to develop an electrochemical biosensor for bacteria (Figure 1B). NPs involved in such tools offer a fast, high-sensitive and low-cost way of detection. The size and charge of the NPs, previously optimized, will allow not only to detect (harmless NPs) but also to kill (toxic NPs) targeted bacteria.

Keywords: cytotoxicity, silica nanoparticles, bacteria, AFM, electrochemistry.

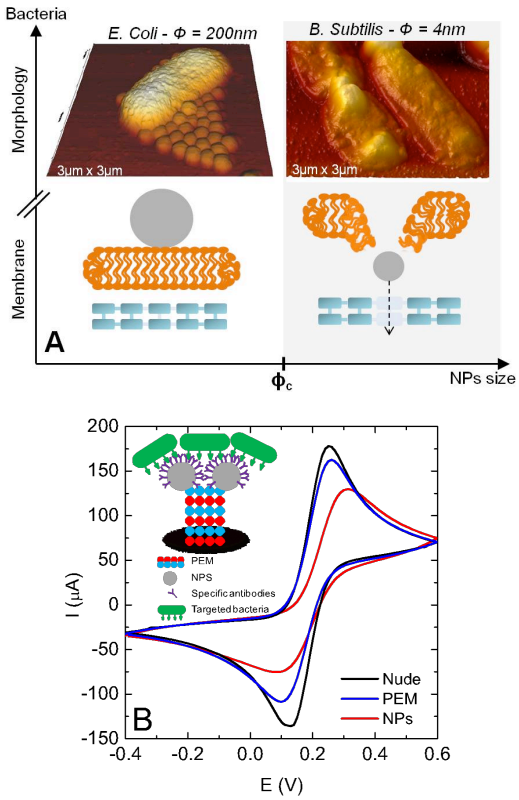

Figure 1: Illustration of (A) the potential anti-bacterial activity of silica NPs depending on their diameter and (B) their potential application in electrochemical biosensors.

Nanogap Capacitive Biosensor For Lable-Free Aptamer-Based Protein Detection

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Abstract:
Recent advances in nanotechnology offer a new platform for the label free detection of biomolecules at ultra low concentrations. Nano biosensors are emerging as a powerful method of improving device performance whilst minimizing device size, cost and fabrication times. Nanogap capacitive biosensors are an excellent approach for detecting biomolecular interactions due to the ease of measurement, low cost equipment needed and compatibility with multiplex formats.[1] Initial work in the field was limited to high frequency measurement only, since at low frequency there is large electronic thermal noise ($<V^2>=4k_BTR$) from the electrical double layer (EDL). This was a significant drawback since this masked most of the important information from biomolecular interactions. A novel approach to remove this parasitic noise is to minimize the EDL impedance by reducing the capacitor electrode separation to less than the EDL thickness.[2] In the case of aptamer functionalized electrodes, this is particularly advantageous since device sensitivity is increased as the dielectric volume is better matched to the size of the biomolecules and their binding to the electrode surface.
In this work we have fabricated a large area vertically oriented capacitive nanogap biosensor with a 40 nm electrode separation between two gold electrodes. A silicon dioxide support layer separates the two electrodes and this is partially etched, leaving an area of the gold electrodes available for thiol-aptamer functionaliztion. We present results on the electrical characterization of the device as well as its capability for detecting label-free proteins.

Keywords: Nanogap capacitive biosensor, label-free detection, Aptamer functionalized surfaces, protein detection.

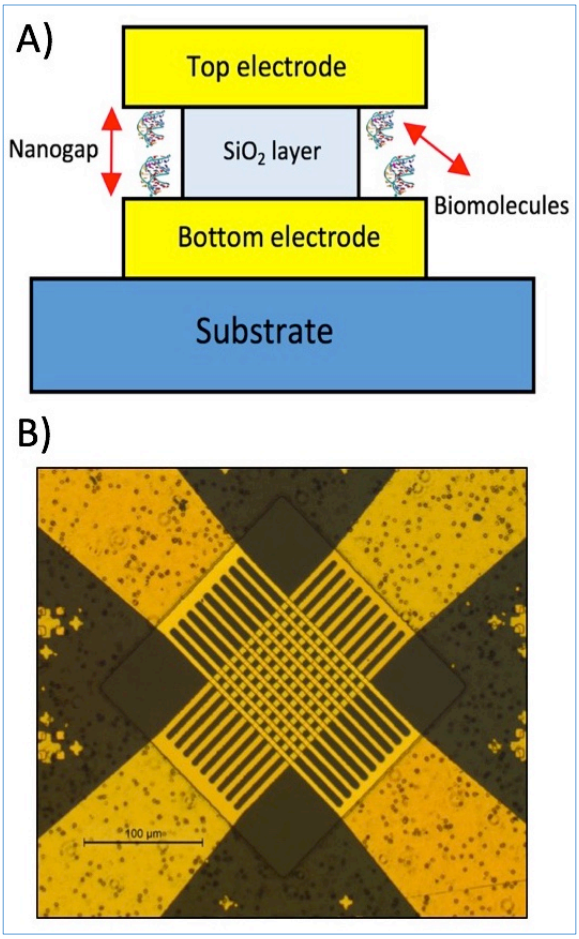

Figure 1: Schematic of the nanogap capacitor (A) and image of the final device (B).

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Focused session on Nanomedicine for cancer diagnosis and therapy

Magnetic Nanoparticles for magnetic hyperthermia and cytotoxic action: from the synthesis to their *in vitro* and *in vivo* characterization

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Abstract:
Chemotherapy together with surgery are the main modalities to treat tumors in clinics. Although there are several FDA approved chemotherapeutic agents, including the most common doxorubicin, cisplatin, paclitaxel etc., they all show together with beneficial actions against tumor cells also various side effects due to their non-specific action against healthy cells.

On the other hand, the use of heat to reduce tumor mass is very ancient. Nowadays, many techniques allow to precisely focalizing the heat in very specific body regions resulting in treatments that are more efficient and minimize side effects. Magnetic nanoparticles can act as heat mediators under oscillating magnetic fields in the so-called "magnetic hyperthermia". The field of magnetic hyperthermia has received a renewed interest since the development of colloidal preparation by non-hydrolytic methods. These approaches have shown several merits over conventional wet chemical hydrolytic processes as the magnetic nanoparticles obtained by non-hydrolytic methods can achieve a better control in terms of size, size distribution and crystallinity. All these parameters affect structural and magnetic properties of nanomaterials and thus their heat performance. Here, I will first focus on our recent progress on the combination of cubic shaped iron oxide magnetic nanoparticles with thermo-responsive coatings to combine both magnetic hyperthermia and heat-mediated drug delivery. I will cover all topics from the synthesis, to the functionalization and characterization, to the drug loading and in vitro characterization, to the *in vivo* long-term study (up to 5 months after hyperthermia treatment) on xenograft tumor murine model. In addition, our bio-distribution studies at the iron dose needed for hyperthermia have indicated the absence of toxicity of such thermo-responsive iron oxide nanocubes and their *in vivo* degradation over three months.

I will also provide a comparative study of magnetic hyperthermia based on polyethylene glycole stabilized iron oxide nanocubes to another type of nanocubes made of spinel cobalt ferrites ($Co_{0.6}Fe_{2.3}O_4$ NCs) providing also in this case the *in vitro* and in vivo study. In a murine model, the slow release of cytotoxic cobalt ions together with the heating effects of the $Co_{0.6}Fe_{2.3}O_4$ NCs under the oscillating magnetic field of clinical use, has brought to a complete tumor regression in case of locoregional treatment of small tumors (less than 1 cm in diameter).

Keywords: magnetic nanoparticles, thermo-responsive polymers, drug delivery, magnetic hyperthermia, photoablation

Acknowledgements: The author acknowledges the Italian AIRC project (Contract No. 14527), the Cariplo foundation (Contract No. 2013 0865), the EU- Horizon 2020 MSCA RISE call, (COMPASS project – 691185) and the European Research Council (ERC) (starting grant ICARO project, Contract N. 678109) for supporting this research.

Raspberry-Like Magneto-Fluorescent Nanoassemblies as Versatile Platforms for *In Vitro* and *In Vivo* Diagnostics

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Abstract:
Multifunctional nanoassemblies comprising units with complementary properties have emerged as must-have systems in the field of bioimaging for the last decade. In this respect, magneto-fluorescent nanostructures represent attractive multimodal imaging agents that can bridge the gap between *in cellulo* and *in vivo* investigations since they enable high-resolution diagnosis under simple handling and addressing conditions. We have thus designed original high-payload structures comprising a shell of superparamagnetic iron oxide nanoparticles arranged around an organic core of self-assembled fluorophores. The resulting raspberry-like nanostructures *fluo@mag* (~100 nm large) could easily be functionalized with various stabilizing polyelectrolytes and proteins to target specific membrane receptors of tumor cells or bacteria. Proton NMR experiments revealed significantly distinct behaviors as a function of the polyelectrolyte coating, showing that accurate control of the polymer chain using RAFT polymerization brings high benefits. Cell endocytosis studies using ten different cell lines have evidenced strong correlations between their transcriptomic signatures and uptake capabilities as a function of the polyelectrolyte coating. All these results point out the need of a multifold parameter screening before concluding on the efficiency of functional nanomaterials as bioimaging probes.

Keywords: multimodal nanomaterials, fluorescent organic nanoparticles, iron oxide nanoparticles, superparamagnetism, bioconjugation, MRI.

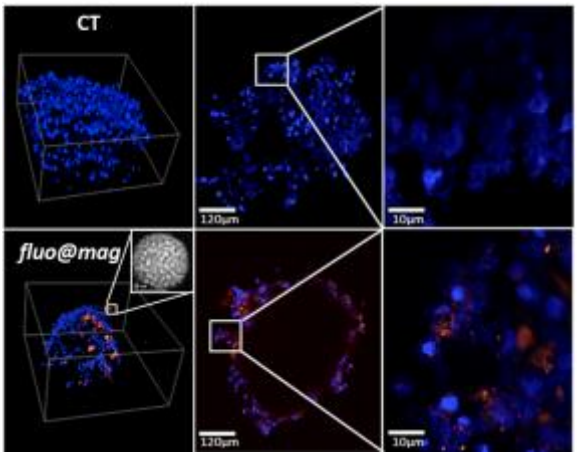

Figure 1: Fluorescence confocal imaging of *fluo@mag* nanoassemblies (red spots) internalized in multicellular tumor cell spheroids (DAPI-stained nuclei). Top : control cell (CT). Bottom: *fluo@mag* uptake.

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Nano-modified natural therapeutic agents to target copper homeostasis in Neuroblastoma

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Abstract

Neuroblastoma is frequently diagnosed at the advanced disease stage and treatment includes high dose chemotherapy and surgery. Despite the use of aggressive therapy, survival rates are poor and children that survive the disease experience long term side effects from their treatment, highlighting the need for effective and less toxic therapies. Catechin is a natural polyphenol with anti-cancer properties and limited side effects, however its mechanism of action is unknown. Here we report that Dextran-Catechin, a conjugated form of catechin that increases serum stability, is preferentially and markedly active against neuroblastoma cells that have high levels of intracellular copper, without affecting non-malignant cells. Copper transporter 1 (CTR1) is the main transporter of copper in mammalian cells and it is upregulated in neuroblastoma. Functional studies showed that depletion of CTR1 expression reduced intracellular copper levels and led to a decrease in neuroblastoma cell sensitivity to Dextran-Catechin, implicating copper in the activity of this compound. Mechanistically, Dextran-Catechin was found to react with copper, inducing oxidative stress and decreasing glutathione levels, an intracellular antioxidant and regulator of copper homeostasis. In vivo, Dextran-Catechin significantly attenuated tumour growth in human xenograft and syngeneic models of neuroblastoma. Thus, Dextran-Catechin targets copper, inhibits tumour growth, and may be valuable in the treatment of aggressive neuroblastoma and other cancers dependent on copper for their growth.

Keywords: catechin; copper transporter; copper metabolism; childhood cancer

Polymer radionuclide conjugates - the selected stories from the Institute of Macromolecular Chemistry AS CR

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Abstract:
For many important research topics in polymer science the use of radionuclides brings significant benefits concerning nanotechnology, polymer drug delivery systems, tissue engineering etc. This contribution describes important achievements of the radionuclide laboratory at the Institute of Macromolecular Chemistry AS CR in the area of polymers for biomedical applications.[1] Particular emphasis will be given to water-soluble polymer carriers of radionuclides such as multistage-targeted delivery system for Auger electron emitters (see Figure 1), thermoresponsive *N*-substituted acrylamide-based and poly(2-alkyl-2-oxazoline)-based polymer radionuclide carriers, thermoresponsive polymers for local brachytherapy including multimodal systems fro combined immuno- and radiotherapy and polymer copper chelators for the therapy of Wilson´s disease (hereditary disease characterized by copper hyperacumulation in organism).
The authors acknowledge financial support from the Ministry of Education, Youth and Sports of the Czech Republic (grant # POLYMAT # LO1507), from the Ministry of Health of the Czech Republic (grant # 16-30544A) and from the Czech Science Foundation (grant # 16-03156S).

Keywords: water-soluble biocompatible polymers, thermoresponsive polymers, micelles, polymer nanoparticles, drug delivery systems, multimodal probes, noninvasive imaging, radiotherapy, positron emission tomography, single photon emission computed tomography, polymer chelators.

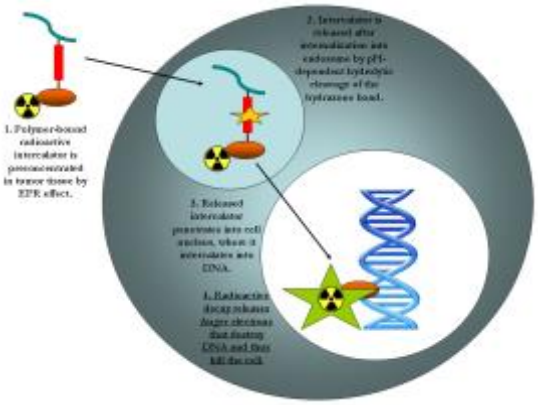

Figure 1: Scheme of the multistage-targeted Auger electron emitter delivery.

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Synthesis and In Vitro Evaluation of a Cancer-Specific Dual-Targeting ^{177}Lu-Nanoradiopharmaceutical

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Abstract:
For tumor growth and metastasis, angiogenesis process must be present during the cancer development[1]. The receptors overexpressed on the surface of cancer cells during angiogenesis process represent promising targets for cancer diagnosis or therapy[2].
The aim of this research was to synthesize and characterize the physicochemical properties of a nanoconjugate labelled with ^{177}Lu and formed by gold nanoparticles (AuNP), the nuclear localization peptide (NLS), the RGD tripeptide sequence and an aptamer analogous to Pegaptanib. The in vitro potential of this compound (^{177}Lu-AuNP-NLS-RGD-Aptamer) as cancer-specific dual targeting probe was also evaluated. The receptors overexpressed on the surface of cancer cells such as α(*v*)β(3) integrins and the Vascular Endothelial Growth Factor (VEGF), both involved in the angiogenesis process, are the main targets of this theranostic nanoconjugate. RGD sequence specific recognize integrins and the same occurs between the apatamer and the VEGF[3]. These two bonds have also high affinity and stability, being one of the reasons for designing this nanoconjugate. Gold nanoparticles were functionalized with DOTA-GGC, NLS-RGD and the apatamer. In all cases by instant reaction of thiol groups with AuNP surface. ^{177}Lu was later incorporated into the molecule through DOTA.
The chemical characterization of the DOTA-GGC-AuNP-NLS-RGD-Aptamer nanoconjugate was carried out by UV-Vis, Infrared spectroscopies. The size and size distribution were determined by Dynamic Light Scattering and TEM images. After ^{177}Lu labelling, the stability in human serum, and in vitro affinity assays (integrins and VEGF molecular targets) were evaluated. In vitro binding studies were carried out in C6 glioblastomas cancer cells. Epifluorescence studies demonstrated the internalization of the radioconjugated into the cell. ^{177}Lu-AuNP-NLS-RGD-Aptamer may be useful as an imaging agent for cancer tumors overexpressing integrins and also VEFG as well as targeting radiotehrapy.

Keywords: Cancer detection and theraphy, nuclear medicine, angiogenesis, molecular targeting, Nanoradiopharmaceutical.

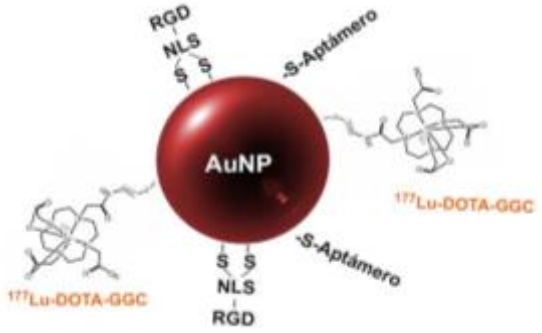

Figure 1: Overall scheme of the multifunctional Cancer-Specific Dual-Targeting system of ^{177}Lu-AuNP-NlS-RGD-Aptamer.

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Upconverting nanophosphors for bioimaging: Preparation Strategies for Hydrophilic Colloidal Stable Particles

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Abstract:

Lanthanide doped nanophoshors are capable of converting near-infrared (NIR) radiation into visble light. Biological tissues have an "optical transparency window" in the near-infrared (NIR) range of 650−950 nm which allows a deeper light penetration and reduced photodamage effects, but also offers lower autofluorescence, reduced light scattering, and phototoxicity.(1) In this study we have focused on the controllable synthesis of sub-10 nm upconverting nanoparticles that can be excited by 800 nm lasers.

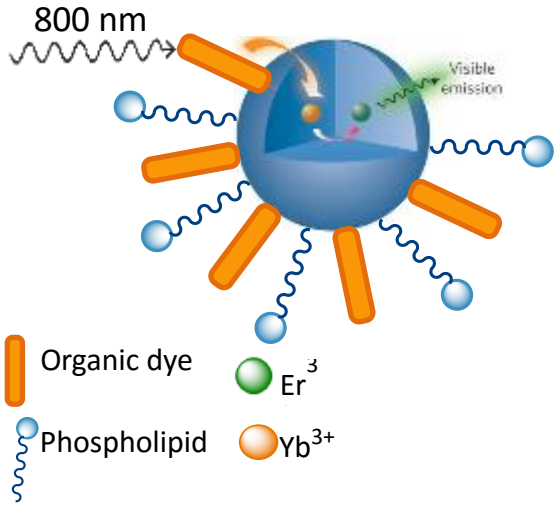
The preparation of ultrasmall, monodisperse and hydrophilic UCNPs that display intense luminescence remains a challenging issue , as smaller UCNPs generally have lower luminescence efficiency. The decrease of the luminescence is due to quenching effects caused by surface defects, ligands and solvents that possess high phonon energy. Only a few examples of ultrasmall and hydrophilic UCNPs have been reported. [2-4] In this work we report a convenient and versatile strategy to dramatically enhance the luminescence efficiency of sub- 10 nm particles by using organic dyes and functional phospholipids. With this approach, the dye molecules function as antennas, absorbing incident light and transferring their excitation energy to lanthanide ions in the UCNP core (figure 1). The assemblies are all water-soluble and fluorescent in the visible region of the spectrum when excited with 800 nm near-infrared laser.

Futhermore the luminescence properties can be tuned by varying the dopants and relative proportions.

The size and shape of the particles will be influenced by controlling the reaction time and temperature. This study will also allow establishing information about the formation of protein corona for ultrasmall UCNPs.

Keywords: **:** Upconversion, lanthanide, Sub 10nm particles, surface functionalization, organic dye

Figure 1: Schematic design illustrating energy transfer pathway from the dye to the lanthanide ions[+] in the core. The Er^{3+} ion accepts the energy from the excited Yb^{3+} ion, giving rise to upconverted emission



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Active targeting and *in vivo* multimodal imaging of renally excretable polymer nanoparticles

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Abstract:

Multimodal imaging represents a strategy to integrate multiple modalities on a single carrier molecule so as to increase the detection sensitivity and to obviate the need to administer compounds with different pharmacokinetics. In this regard, dendritic polyglycerols are highly biocompatible nanoscale scaffolds with multiple attachment sites, anti-fouling properties and small size (2-20 nm).[1] The great versatility of the dendritic polyglycerols allows to fine tune physicochemical parameters such as the size, water solubility, surface charge that are relevant for the successful preparation of theranostic systems. Previous experiments showed that the dendritic polyglycerols (>10kDa) show a fast renal clearance with negligible uptake in the mononuclear phagocytic system (MPS) organs such as the liver and spleen.[2-3]

The purpose of this work to design a PET/OI dual modal construct based on dendritic polyglycerols for epidermal growth factor receptor (EGFR) targeting. In this regard, a one-pot strategy was employed for simultaneous attachment of fluorescent labels for optical imaging (cy3/cy7) and macrocyclic chelators based on a 1,4,7-triazacyclononane system for ^{64}Cu (PET tracer) to thiol anchoring groups of the dPGs. A small camelid single-domain antibody (sdAb) representing a potential recognition agent for EGFR as targeting vector was attached (**1**). In parallel, a probe with similar surface characteristics but an EGFR unspecific sdAb (control) was synthesized (**2**). The conjugates were purified using affinity chromatography, which selectively separates the antibody-conjugated multimodal conjugates. *In vitro* and *in vivo* studies were conducted to assess its diagnostic potential. The *in vitro* results revealed a highly specific receptor mediate uptake of **1** in EGFR expressing A431 and FaDu cell lines using confocal microscopy and radio detection.

Intravenous injection of **1** and **2** on mouse xenografted models studies using PET and optical imaging revealed an overwhelming tumor accumulation of the EGFR-specific **1** in comparison to the EGFR-unspecific **2** and a minimum off-target accumulation of both conjugates. These results unveil the potential of dendritic polyglycerols as efficient multimodal platforms for theranostic applications.

Keywords: dendritic polyglycerols, cancer, biodistribution, radiolabeling, renal clearance, protein corona, biomedical applications.

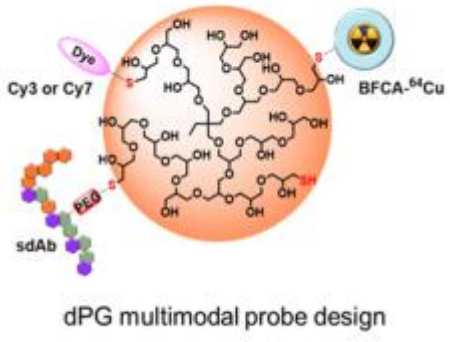

Figure 1: Figure illustrating the design of the multifunctional dPG based imaging probe containing macrocyclic chelators for ^{64}Cu-PET, fluorescence dye for optical imaging and EGFR targeted sdAb or a non-targeted sdAb as a control. Right: Optical imaging of A431 xenograft tumor mice after intravenous injection of the multimodal probes. Ki: Kidneys and Tu: Tumor.

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Mannan conjugates for multimodal imaging

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Abstract:

The aim of this work is development of new hybrid polymer noninvasive multimodal imaging platform based on natural polysaccharide mannan (MN) (Figure 1). Mannan is a biocompatible and biodegradable polysaccharide that could be easily functionalized in order to incorporate imaging moieties with strong self-targeting effect *via* DC-SIGN receptor. Therefore, MN is preferably accumulated in the macrophages and dendritic cells overexpressing the DC-SIGN receptors[1], what is useful in diagnosis of the sentinel lymph nodes and metastases. The polysaccharide was decorated with functional groups (such as fluorophores and gadolinium complexes for magnetic resonance (MR) imaging) in order to be used as a polymer carrier for solid tumor diagnostics or therapy. Grafting with synthetic polymer decreases the biodegradation rate of polysaccharide and allows easy functionalization of the polymer grafts with active payload. Therefore, mannan was reacted with active poly(2-methyl-2-oxazoline) chains, obtained by ring-opening cationic polymerization in anhydrous acetonitrile, followed with a radical-initiated thiol-click chemistry with cysteamine in order to prepare primary amino group containing mannan-based conjugate. The latter precursor was reacted with NHS ester of IR800CW dye as infrared active fluorescence label and NHS ester of MRI T_1 contrast agent – Gd^{3+}-DOTA. The mannan-based conjugates with (MN-Ox) or without oxazoline (MN) were characterized and compared with a commercially available contrast agent (gadoterate meglumine) by ^{1}H-MR and fluorescence imaging. *In vivo* distribution and accumulation of the probes was examined in animals showing accumulation in sentinel lymph nodes.

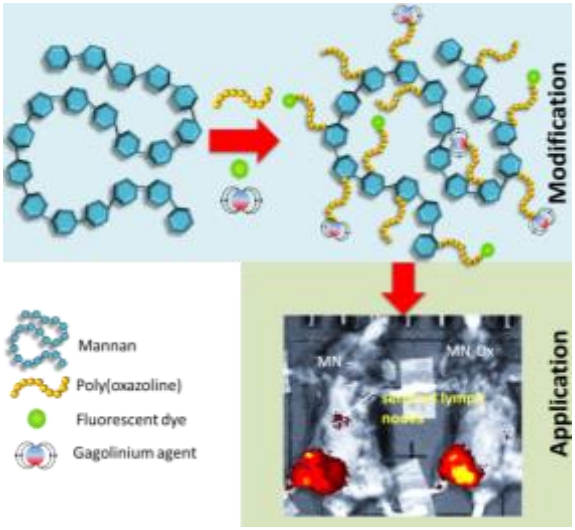

Figure 1: Modification and application of mannan-based conjugates as a multi imaging contrast agents.

Keywords: polysaccharide modification, mannan, multimodal imaging, biomedical applications.

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Acknowledgment

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Biophysical modelisation of gold nanoparticles radiosensitizing effetcts

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Abstract:
The main challenge of radiotherapy is to focus the irradiation dose in cancer cells while preserving the healthy cells surrounding the tumor. Among the different strategies, the use of radiosensitizers aims to amplify the destructive effects of dose in the tumor[1]. Nanoparticles of heavy metals such as gold and gadolinium, are particularly promising radiosensitizers. If their radiosensitizer effect has been studied for about two decades, the origin of this phenomenon is yet quite unknown and barely quantified.
Litterature suggests that irradiation would generate a physical effect called Auger cascades. This effect would lead to a local increase secondary electrons around the nanoparticle, thus implifying the critical cell damages of direct sensible molecules such as DNA, or through a boost of free radicals. These effects are produced at nanometric scales and at very short time (10^{-15} to 10^{-12} seconds) but have consequences on the patient scale.
Because this physical and chemical effects are not directly observable, the simulation tool is therefore mendatory to better understand the initial mecanisms. Our goal is to first develop a simulation that enables us to calculate the spatial dose and free radicals distribution around the nanoparticles, and to quantify the induced boost[2,3]. Secondly, we want to inject the results in the model *Nanox*[4], originally developed in IPNL to calculate the biological dose in hadrontherapy.
These two allow us to assess the the quality of our models, and the relevance of the scenarii offered in literature. The final aim is to guide the development of the nanoparticles and, if possible, to help to planify clinical treatment of nanoparticle-based radiotherapy.

Keywords: modelisation, nanoparticles, radiosensitizer, nanometric scale

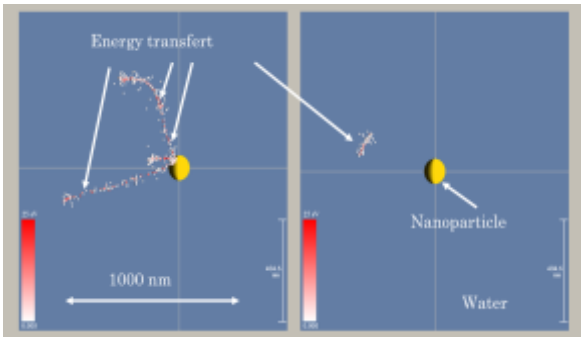

Figure 1: Illusatration of the difference between two tracks (energy transfers) when a 20 keV photon originally interacted in the gold nanoparticle (left) through a photoelectric effect or in water (right) through a Compton effect.

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Thermal and biological study of benignly synthesized silver/reduced graphene oxide nanocomposites

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Abstract:
Graphene oxide (GO) is a pseudo 2D Nobel material useful for constructing wide variety of graphene-based structures known for innumerable captivating properties. Here, we have benignly synthesized silver adorned reduced graphene oxide (GrO) greenish gray (GG-GrO) and navy blue(NB-GrO) colored nanocomposites by tuning silver nitrate proportions with the extract of an indigenous spice plant Murraya Koenigii. The reduction of graphene oxide (GO) and silver nitrate simultaneously accomplished by high contents of extract including polysaccharides as potent reducing agent and other phytoconstutents[4] to produce GG-GrO and NB-GrO nanocomposites. The properties of these nanocomposites were characterized by UV-Vis spectroscopy, SEM-EDS, HRTEM, XRD, FT-IR, TGA, DSC and XPS techniques. These nanocomposites are thermally stable compare to GO and were evaluated for anticancer properties against human lung cancer cell line A549 with adriamycin as the reference drug. A relationship between the amount of silver nanoparticles on the surface of GrO and the anticancer activity of nanoparticles was observed, with an increase in the concentration of silver nanoparticles on the surface of GrO led to enhanced anticancer activity of the nanocomposites. Surprisingly, these nanocomposites were found biocompatible with microbes like Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, Candida albicans. The combined anticancer and microbial compatibility make these nanocomposites a promising candidate for future health cautions.

Keywords: pseudo 2D Nobel material, reduced graphene oxide, spectroscopy, indigenous spice plant extract, anticancer activity of nanoparticles, microbial compatibility.

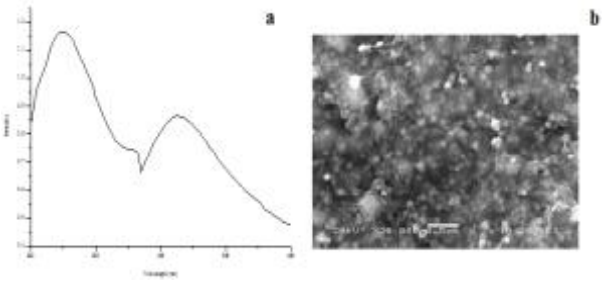

Figure 1: Figure (a) NB-GrO nanocomposite illustrating the UV-vis spectra having characteristic peaks at at 250 nm and 420 nm and (b) SEM-EDS image GG-GrO nanocomposite visualising silver nanoparticles embedded in and on GrO sheets of benignly synthesized nanocomposites.

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Nanotech/Biotech joint Session IV: NanoBioMedecine / Nanosafety

Nanopores: Transistors for Ions in Solution

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Abstract:
Nanopore-based measurements enable to probe the physics of biology at ultrahigh precision. This precision relies on the dimension of the nanopore and our single-layer MoS_2 nanopores could offer sub-nanometer resolution due to its well-defined geometry at the atomic scale(*1*). The key driving force in nanopore research is single molecule DNA sequencing where the sequence of DNA can be extracted based on the modulation of ionic current through the pore caused by individual nucleotides(*2*). In essence, nanopores play a role in ion transport as significant as the role of transistors in electron transport and can also be applied to probe various physical processes beyond sequencing(*3*, *4*). In this talk, I will demonstrate the ability of using nanopores for probing physics and biology at the single molecule level with tunable resolutions: from identification of single nucleotides to fundamental ion transport physics and energy conversion.

Keywords: nanopores, DNA sequencing, ion transport, Coulomb blockade, osmotic power generation

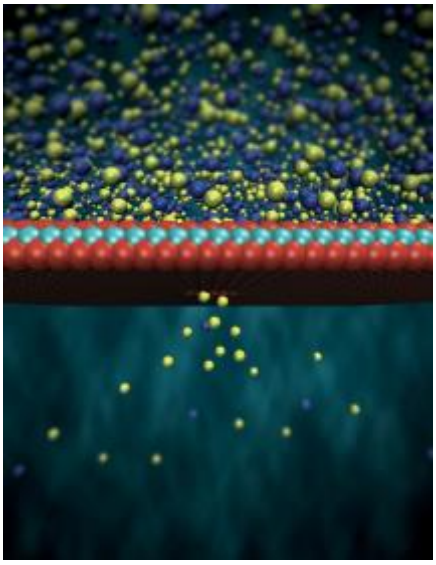

Figure 1: Figure illustrating ion transport through nanopores.
Image credit: Steven Duensing at the National Center for Supercomputing Applications at the University of Illinois at Urbana-Champaign.

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Innovative materials for active food packaging: antimicrobial release from inorganic carrier embedded into biodegradable composites

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Abstract:
Inorganic materials have been long recognized as very promising materials with a wide range of possible applications. Among these, many investigation efforts focused on the exploitation of the porous network provided by such materials as a reservoir for the accommodation of drug molecules. In fact, the well-known opportunity to chemically functionalize the surface of siliceous mesostructures with different organic moieties constitutes a route for controlling the drug release by diffusion under specific conditions. Drug release from mesoporous materials is generally controlled by diffusion. Nevertheless, when the interactions between desorbing molecules and silica pore walls are significantly strong and/or show some kind of specificity, the release also depends by the stability of the complex between the functional groups of the drug and those of the substrate. This phenomenon allows then to fine tune the release of specific molecules from a given mesostructure by simply changing the functional groups that are attached to its pore walls during the synthesis process. In addition to the production of smart drug delivery systems, such approach can be also used in the field of food packaging due to the increasing interest in the concept of "active packaging" materials as compounds which, interacting with the packaged foodstuff, are able to control quality as well as to increase shelf-life.
The aim of the present work is the study and the comparison of the release from active polymeric films of various active compounds embedded or supported into/onto various inorganic carriers i.e. SBA (Santa Barbara Amorphous), Montmorillonite and Halloysite. Migration tests were performed at 25 °C, using 96% v/v ethanol and water as food simulant, from polymer films obtained by embedding active inorganic carriers into biodegradable and polyolefinic matrices. Obtained results show the influence of functionalization of the inorganic carriers on the diffusion of active compounds and thus on their release kinetics into the liquid media.

Keywords: inorganic carrier, chemical functionalization, active compound release.

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Colloidal characterization of surface modified CuO nanosuspensions in media relevant for nano (eco) toxicology

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Abstract:
The relationships between the physicochemical properties of engineered nanoparticles and their adverse health and environmental effects are still unclear. In order to identify key properties that drive nano-bio/eco interactions and to convert this knowledge into "Safety by Design" ($S_{by}D$) strategies, it is essential to study the colloidal properties of ENMs in media relevant to nano (eco) toxicology (1). This work investigates the dispersion stability of copper oxide NPs modified by means of four non-hazardous modifying agents [i.e. polyethylenimine (PEI), sodium ascorbate (ASC), sodium citrate (CIT), and polyvinylpyrrolidone (PVP)]. The modifiers were added to CuO NP suspensions for promoting the in situ coating of particles and compare four design alternatives, achieving positive (PEI), negative (ASC, CIT), and neutral (PVP) surface charging on the NPs. The effects of these four stabilizers on the CuO NPs' physicochemical properties were investigated in different biological and environmental media by combining Dynamic and Electrophoretic Light Scattering (DLS and ELS) with Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Results showed improved dispersion stability for CuO-CIT, CuO-ASC and CuO-PEI in both MilliQ and Phosphate Buffered Saline (PBS) as compared to CuO-pristine and CuO-PVP. Higher ionic strength in artificial fresh (AFW) and marine (AMW) water strongly destabilized all CuO NP suspensions, except for CuO-PEI dispersed in AFW. The presence of proteins and amino acids in the biological test media had a strong influence on the colloidal stability of all dispersions. Characterization of colloidal properties showed the correlation between NP aggregates size and ζ-pot, confirming that coupling DLS with ELS provides an effective tool for colloidal stability evaluation (2). The obtained results in support to (eco) toxicological outputs are highly relevant for hypothesizing early effects of toxicological pathway and deriving criteria and guiding principles for grouping and read-across.

Keywords: CuO nanoparticles; colloidal stability; nano-bio interaction; biological and environmental media.

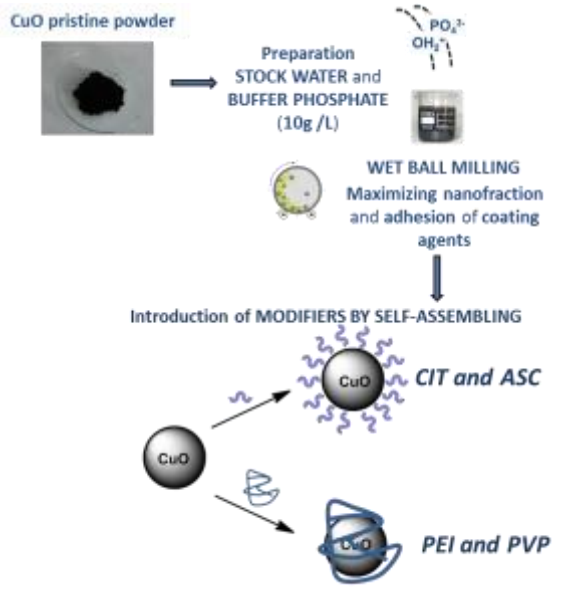

Figure 1: Schematic representation of $S_{by}D$ strategy applied: introduction of modifying agents (i.e. CIT, ASC, PEI and PVP) by self-assembling.

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Gold nanoparticles induce mitochondrial dysfunction in monocytic THP.1 cells: a combined transcriptomics and proteomics approach

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Abstract:
Systems biology is increasingly being applied in the field of nanosafety research for observing and predicting the biological perturbations inflicted by exposure to engineered nanoparticles (NPs). In the present study, we used a combined transcriptomics and proteomics approach to assess the effects on monocytic THP.1 cells of AuNPs of 5 or 20 nm in diameter. The role of surface functional groups was addressed by synthesizing alkylammonium bromide-, alkyl sodium carboxylate- or poly(ethylene glycol) (PEG)-terminated thiolate-AuNPs. These AuNPs were characterized using transmission electron microscopy (TEM), UV-vis. spectroscopy, dynamic light scattering and zeta potential measurements. We also ensured that all the AuNPs were endotoxin-free. Then, we performed a cytotoxicity screening of THP.1 cells exposed to the AuNPs for 24 h at doses up to 100 µg/mL, using the Alamar Blue assay. Cytotoxicity effects were observed only for the ammonium-terminated AuNPs, while no cell death was obtained after exposure to carboxylated or PEGylated AuNPs, regardless of the AuNP size. Using TEM we observed that the ammonium-modified AuNPs were partly located into mitochondrial compartments and that they induced high impact on the cell morphology with appearance of large vacuoles (Figure 1). Next, we performed transcriptomics analysis using highly sensitive Single-cell Tagged Reverse Transcription (STRT)-RNA sequencing in parallel with label-free quantitative mass spectrometry based proteomics analysis, followed by pathway enrichment analysis. The importance of the nanomaterials surface modification, rather than the diameter size, was demonstrated using these approaches. Notably, ammonium-modified AuNPs were found to have the most pronounced effects on the monocytic THP.1 cells with a significant impact on mitochondrial functions. Taken together, these studies have disclosed specific cytotoxic effects of AuNPs as a function of the particle surface properties, in human immune cells.

Keywords: Au nanoparticles; THP.1 cells; STRT-RNA sequencing; label-free quantitative proteomics, mitochondrial dysfunction.

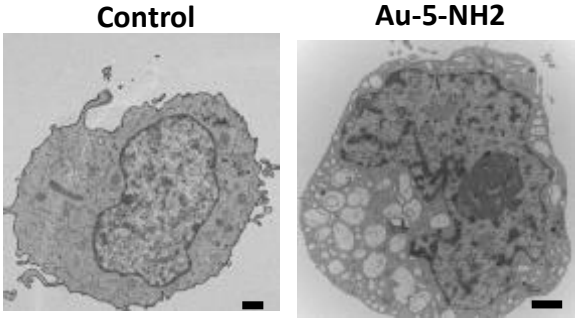

Figure 1: Transmission electron microscopy of THP.1 cells exposed for 4 h to Au-5-NMe_3^+ Br^- nanoparticles at 50 µg/mL. Cytoplasmic vacuolization is noted in NP-exposed cells. Scale bars: 1 µm.

Fabrication of hieratically porous calcium carbonate scaffolds for bone tissue engineering

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Abstract

We report a versatile method for the fabrication of hierarchically porous $CaCO_3$ scaffolds on silicon substrate via a supercritical carbon dioxide nebulization process. This process consists of evaporation of CO_2-enriched water micro-droplets (diameter ~3 μm) deposited from an aerosol onto heated silicon substrates. A variety of porous $CaCO_3$ scaffolds with micron-sized pores (1-3μm) were fabricated by altering the deposition conditions. Post-deposition sintering of the scaffolds resulted in the generation of nano-sized pores around the walls of the porous scaffolds with a dual arrangement of typical pore sizes (~50 nm and 1−3 μm). We observed that our scaffolds formation and micro-droplets evaporations follows typical coffee-ring effect mechanism. Furthermore, $CaCO_3$ scaffolds were exposed to monocytic THP-1 cells. These scaffolds yielded negligible levels of tumor necrosis factor-alpha (TNF-α) and further confirmed the lack of immunogenicity of the scaffolds. These scaffolds were also treated against extracellular matrix (ECM) proteins such as fibronectin (FN), vitronectin (VN) and collagen (CL), respectively. ECM treatment on to $CaCO_3$ scaffolds showed enhanced adsorption in the order of FN > VN > CL as compared to the standard control. Moreover, these investigations demonstrate that our porous $CaCO_3$ scaffolds promoted ECM production and calcium mineralization (which is an important biomarker), in turn beneficial for bone tissue engineering.

Keywords: Supercritical CO_2, $CaCO_3$ scaffolds, Coffee-ring effect and extracellular matrix (ECM) proteins,

Biocompatible scaffolds for tissue regeneration

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Abstract:

The design of scaffolds based on biocompatible materials is a great goal to support tissue regeneration. On this basis, we worked with protein-based and graphene oxide (GO)-based scaffolds to test their properties as supports for neural tissue regeneration. We considered several type of materials (GO, bovine pericardium and alginate) as bases for the development of biocompatible scaffolds, and tested their applicability for growing SHSY5Y cells, as in vitro models of neuronal differentiation.

Our group has previously demonstrated that a protein-based scaffold (1) can be obtained by growing a mutant of the protein Prx (peroxiredoxin from *S. mansoni*) as an array of protein nanotubes. This material can be used to induce the growth and differentiation of SHSY5Y cells in vitro, and can furthermore sustain the growth and development of rat cortical neurons.

We are also studying the ring-shaped monomer (wtPrx) and the mutant Prx, as a polimerization agents to induce graphene oxide gelation into a 3D porous material, to be used as a support for the growth and differentiation of SHSY5Y cells. Cells grow on our scaffold based on graphene-oxyde with or without the presence of the wild-type or a mutant protein(2).

To check the growth and the degree of differentiation we analyzed the surface interaction with SEM and immunofluorescence analysis. We have shown that the GO-based scaffold is biocompatible, in fact the SHSY5Y cells colonize the surface preserving their morphology.

We also know that the mutated protein Prx can induce cell differentiation as well as, a structural reorganization of the GO to get a 3D assembly with more space between the sheets that allows cell growing inside the scaffold.

The goal is to obtain a biocompatible system for 3D neuronal regeneration that allows to culture and manipulate cells in order to study their physiology and characteristics for the possible re-implantation in patients.

We also obtained a decellularized bovine pericardium, a biological tissue widely used as a biomaterial for tissue engineering applications, including the construction of a variety of bioprostheses to repair complex anatomical defects of several tissues such as cardiac defects, abdominal wall defects, and to strengthen the suture line during general surgical procedures and, more frequently, heart valves(3), with good features as a biomaterial that finds application as device adjuvating tissue regeneration after several type of surgery.

In summary, the coating of the scaffolds with GO linked to Prx provide a strong recellularization of these scaffolds. Furthermore the mutated protein can trigger the differentiation of the SHSY5Y cells on all scaffolds and 3D system is very interesting perspective for future purposes.

Keywords: biomaterial,Graphene-oxide,biocompatible scaffolds, Bovine pericardium, Alginate, Prx.

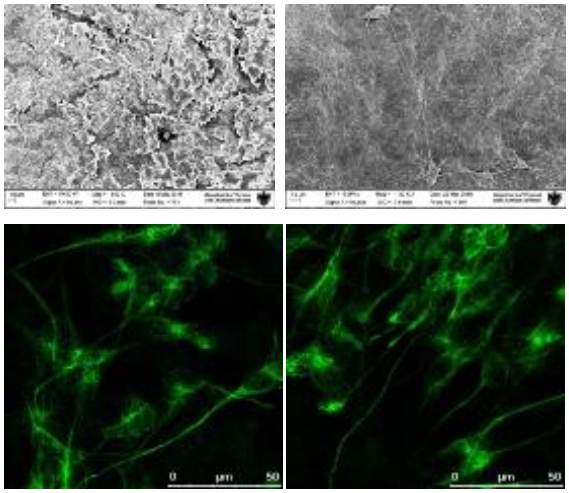

Figure 1: Figure illustrates the differentiation of cells grew on the surface of GO+Prx mutant. On the top is shown SEM investigation of the surface and below IF confocal images stained with N200 antibody.

References:

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2. A Peroxiredoxin-based proteinaceous scaffold for growth and differentiation of neuronal cells and tumor stem cells in the absence of pro-differentiation agents. Cimini Annamaria, Ardini Matteo, Gentile Roberta, Giansanti Francesco, Benedetti Elisabetta, Cristiano Loredana,

Fidamore Alessia, Scotti Stefano, Panella Gloria, Angelucci Francesco, Ippoliti Rodolfo University of L'Aquila, Life, Health And Environmental Sciences Journal of Tissue Engineering and Regenerative Medicine 2015

3. Biomimetic acellular detoxified glutaraldehyde cross-linked bovine pericardium for tissue engineering Santosh Mathapati Dillip Kumar Bishi, Soma Guhathakurta, Kotturathu Mammen Cherian, Jayarama Reddy Venugopal , Seeram Ramakrishna c, Rama Shanker Verma Materials Science and Engineering C 33 (2013) 1561–1572

Creating a discovery solution for nanotechnology – Challenges & Prospects

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Abstract:
Vast amount of information and data related to nanotechnology are scattered throughout different journals and patents. Moreover, the lack of standardized nomenclature for nanomaterials is a challenge which makes the finding and transfer of scientific results a difficult task. Here we illustrate a solution under nano.nature.com and explain how these issues can be addressed. Our research solution provides highly indexed and structured information related to nanomaterials and devices derived from peer-reviewed journals and patents. These include composition, synthesis, properties, characterization methods and application information. It aims to provide nanotechnology research communities fast and precise insights into this multi- and interdisciplinary field, and to help them keep up to date with new discoveries and developments.

Keywords: nanotechnology research solution, precise data search, nanomaterials & nanodevices datasheets

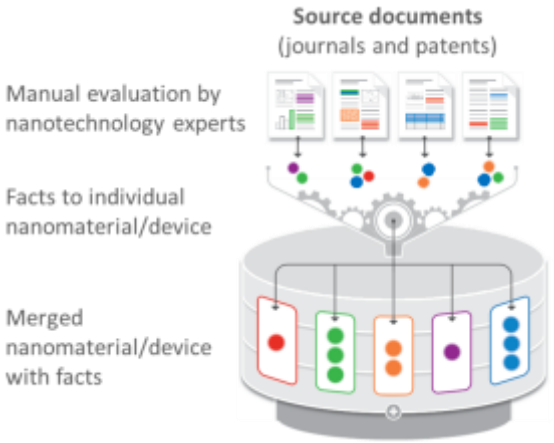

Figure 1: Figure illustrating the creation of curated profiles of nanomaterials/devices

Session V:
Environmental Biotechnology - Green Economy

Sustainability of biobased materials: opportunities and challenges

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Abstract:
Today's linear 'take, make, and dispose' economic model relies on large quantities of cheap, easily accessible materials and energy, and is reaching its ecological limits. In a circular economy, resources are regenerated in the bio-cycle or recovered and restored in the technical cycle. This is an attractive and viable alternative economic model that businesses have already started exploring today[1]. Main drivers for change are societal challenges like climate change, resource scarcity, energy insecurity, waste problems and an increasing population with growing materials demands.
Biobased materials have great potential to boost the transition to a circular economy due to renewable feedstock use and possible waste prevention strategies like biodegradation. However, biobased materials are not intrinsically sustainable.
The Aachen-Maastricht Institute for Biobased Materials (AMIBM) investigates the whole value chain of biobased materials from biological resource to applied biobased materials products. The AMIBM vision is to achieve a paradigm change in the production, application and life cycle management of biobased materials by making use of higher value resources, energy efficient production, novel materials fabrication technologies and (improved) sustainability assessments of biobased materials over the whole value chain.
Life Cycle Assessment studies of biobased materials generally show reduction of greenhouse gas emissions. However, emissions originating from land use change are frequently not accounted for and other environmental impacts are usually increased[2]. Also large differences are observed between LCA studies of biobased materials, partly due to the diversity of methodological choices and assumptions made[2], so the methodology needs has a higher degree of standardization. Finally it is important to also include social and economic aspects to have a full sustainability assessment framework for biobased materials. This contribution will highlight the sustainability opportunities for biobased materials and also the challenges like sustainability issues related to the biobased feedstock and lack of dedicated and integrated assessment methods.

Keywords: sustainability assessments, biobased materials, environmental impact, Life Cycle Assessment, biobased economy, circular economy

Figure 1: Scheme illustrating the life cycle of a bio-based material taking into account all stages from resource extraction to manufacturing of the product, transport, use and end-of life or recycling of the product.

References:

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2. Weiss, M., Haufe, J., Carus, M., Brandão, M., Bringezu, S., Hermann, B. and Patel, M. K. (2012), A Review of the Environmental Impacts of Biobased Materials. *J. Ind. Ecol.*, 16: S169–S181.

Developing biomass producing rhizoremediation technology by symbiosis of duckweed and beneficial bacteria

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Abstract:
Rhizoremediation is a type of bioremediation with less environmental load, which utilizes symbiosis of rhizobacteria and their host plants. Rhizobacteria utilize the plant as a favorable habitat for colonization, because photosyntheic products including sugars and amino acids are supplied from the plant, which results in improvement of bacterial stability and activities for pollutant degradation. This "dormitory mother effect" is also possible for aquatic plants including duckweed. Duckweed is attracting high attention in the decade as a useful biomass for production of energy and starch based chemicals. We have reported possibility of a duckweed biomass production system interlocking with phenol degradation (Yamaga *et al.*, 2010).
It has been a concern that estrogens discharged into river and seawater would cause feminization of aquatic lives resulting to disruption of the ecological balance. It has been reported that chronic exposure of fathead minnow to very low concentrations (5-6 ng/L) of estrogen compound led to feminization of males. Estrogens excreted from human and cattle, living in urban areas, flow into wastewater treatment plants, and most but not all of them are degraded by general activated sludge treatments. On the other hand, in the farmer area, most urine from cattle is discharged directly into the soil, flows into the river without being degraded. Here, we report construction of an effective estrogen degradation system by coupling duckweed with a non-rhizospheric estrogen degrading bacterium strain from a waste watertreatment plant.
Rhodococcus zopfii Y 50158 (Yoshiomoto *et al.*, 2004), an effective estrogen degrading bacterium, was found to be capable of colonizing on the duckweed surfaces including roots and both surfaces of fronds (Figure 1 inset picture). Y 50158/ duckweed system degraded 5 ppm estradiol (E2) in 2 days. After the E2 degradation, the Y 50158/ duckweed system was transferred to a new flask containing 5 ppm E2 in a medium. This transfer of Y 50158/ duckweed system in E2 containig medium was repeated by cycles. It was shown that Y 50158/ duckweed fully functioned for E2 degradation even after 5 cycles experiments (Figure 1). However, it was also observed that duckweed growth as inhibited by some degradation intermediates of E2. Co-inoculation of Y 50158 with plant growth-promoting bacteria, PGPB, to duckweed recovered healthy growth of duckweed. However, the E2 degradation activity was lost because PGPB did not allow coexistence of Y 50158 on the duckweed. Finally, we found that addition of not plant growth-promoting bacteria but active compounds enabled growth recovery of duckweed without impairing excellent E2 degradation activity of Y 50158. To our best knowledge this is the first report for successful application of non-rhizospheric pollutant degrading bacteria to the duckweed rhizoremediation system. This should contribute to expand application range of the aquatic rhizoremediation technology.

Keywords: Symbiosis, plant growth-promotion, rhizoremediation, duckweed biomass, estrogen.

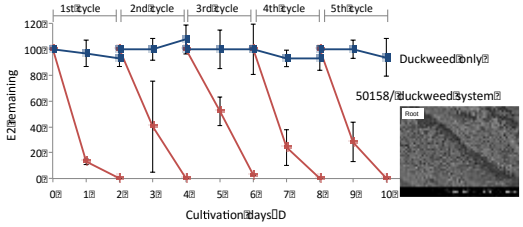

Figure 1: Stable E2 degradation by Y 50158/ duckweed system. E2 at 5 ppm was continuously degraded in two days, demonstrating stability of the system. Inset SEM picture shows significant colonization of Y 50158 on the duckweed roots.

References:

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2. Yoshimoto, T. et al. (2004), Degradation of estrogens by *Rhodococcus zopfii* and *Rhodococcus equi* isolates from activated sludge in wastewater treatment plants. Appl. Environ. Microbiol. 70(9), 5283-5289.

Gene stacking of multiple traits for high yield of fermentable sugars in plant biomass

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Abstract:

Second-generation biofuels produced from biomass can help to decrease dependency on fossil fuels, which would have many economical and environmental benefits. To make biomass more suitable for biorefinery use we need a better understanding of plant cell wall biosynthesis. Increasing the ratio of C6 to C5 sugars in the wall is an important target for engineering of plants that are more suitable for downstream processing for second-generation biofuel production. Likewise, decreasing the content of lignin is an important goal. Study of the basic mechanisms of cell wall biosynthesis led to the identification of the GALS1 galactan synthase [1] and the URGT1 UDP-galactose transporter [2] involved in biosynthesis of pectic galactan. We have applied these findings to engineer plants that have a more suitable biomass composition and have used synthetic biology and gene-stacking tools [3] to achieve this goal. Plants were engineered to have up to three-fold increased content of pectic galactan in stems by expressing *GALS1*, *URGT1* and *UGE2*, encoding a UDP-glucose epimerase. Furthermore, the increased galactan was engineered into plants that were already engineered to have low xylan content by restricting xylan biosynthesis to vessels where this polysaccharide is essential [4]. Finally, the high galactan and low xylan traits were stacked with low lignin obtained by expressing the *QsuB* gene encoding dehydroshikimate dehydratase [5]. By targeting the transgene expression to fibers, we could substantially improve saccharification while avoiding adverse effects on plant growth and development.

Keywords: plant cell wall, galactan, Arabidopsis, pectin, jStack, xylan, lignin, plant engineering, biofuels.

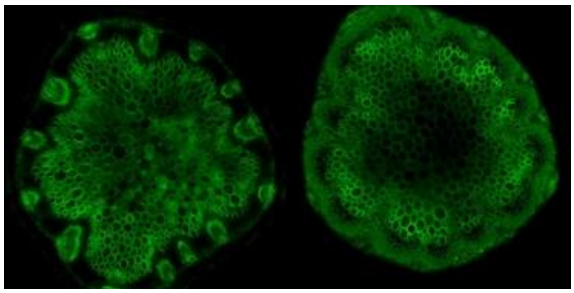

Figure 1: Galactan immuno-detection in stems of xylan-engineered (left) and galactan enriched xylan-engineered plant (right).

References:

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Conceptual design of a self-sufficient hybrid biorefinery for syngas production and fermentation to ethanol

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[2] Delft University of Technology, Department of Biotechnology, Delft, The Netherlands
[3] DSM Biotechnology Center, Delft, The Netherlands

Abstract:
Biorefineries are commonly classified according to conversion route as either biochemical or thermochemical. A third platform, currently at an earlier stage of development, consists of a hybrid route incorporating both conversion types in sequence. The present study explores a possible arrangement of hybrid biorefinery for the production of bioethanol from biomass residues, specifically sugarcane bagasse from Brazilian sugar-mills. The design comprises indirectly-heated biomass gasification followed by syngas fermentation to ethanol and includes units of heat recovery and power generation to ensure energy self-sufficiency in the integrated plant (Figure 1). Syngas fermentation, a relatively novel technology, is accomplished with autotrophic, anaerobic bacteria that metabolize $CO/H_2/CO_2$ via acetogenic pathway. The resulting broth is highly dilute (aprox. 2.0 wt% ethanol), therefore ethanol purification is carried with heat-integrated multiple-effect distillation to reduce energy expenses. The process was designed and simulated using commercial software Aspen Plus, such that mass and energy balances are rigorous and comply with limitations by the Second Law of Thermodynamics. The design achieves 330 liters of hydrous ethanol per dry metric ton of biomass, with overall carbon conversion to ethanol of 30%. Moreover, a financial analysis including Monte Carlo Simulation predicts non-negative Net Present Value for 80% of the cases with ethanol selling price of US$0.78/L, considering uncertainties in fixed capital investment and raw materials costs within ranges of 30% and 70%, respectively.

Keywords: second generation bioethanol, biomass gasification, syngas fermentation, process design and simulation, financial analysis

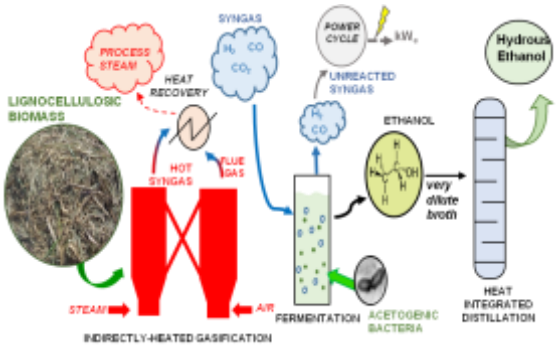

Figure 1: Illustration of a hybrid pathway for production of second generation ethanol in a self-sufficient biorefinery.

References:

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A comparative study on efficacy of bioinoculants and chemical fertilizers on crop and resident microflora

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Abstract:
The usage of bioinoculants to enhance the crop yield is well studied and documented too, but their effects on the resident microbial community (also known as non-target effects) is still at its infancy. Present study aims to get an insight into the effect of mixed consortium containing *Bacillus megaterium, Pseudomonas fluorescens* and *Trichoderma harzianum* with that of the chemical fertilizers not only on the plant (*Cajanus cajan*) growth parameters but also on the resident microflora. The effect was assessed by using both cultivation dependent (enumeration) as well as cultivation-independent methods {Denaturing Gradient Gel Electrophoresis (DGGE) and qPCR for qualitative and quantitative assessment respectively}. The evaluation was carried out to target the structure (16S rRNA) and function (nitrogen cycle) of rhizospheric microbiota, using both DNA and RNA as markers. The results revealed that the mixed consortium surpassed the result of chemical fertilizers with respect to grain yield (by 1.2-fold). An enhanced effect on resident microflora of phosphate solubilizers (2.5- fold), *Pseudomonas* spp. (1.9- fold), nitrogen fixers (1.2- fold) and fungi (1.6- fold) was also observed. Significant changes on qualitative profile of microbial communities has been observed in treated soil as compared to the control through DGGE indicating the effect of bioinoculant's treatment. An enhancement of *nif*H and *amo*A transcripts by 2.7- and 2.0- fold respectively was also found. This shows that there is no adverse effects of bioinoculants in *Cajanus cajan's* rhizosphere, and hence, are safe to release in the agricultural fields for sustainable agriculture.

Keywords: Bioinoculants, *Cajanus cajan*, Denaturing Gradient Gel Electrophoresis, Sustainable agriculture, 16S rRNA

Poster Session

Squalene synthase is a peroxisomal enzyme in the slime mould *Dictyostelium discoideum*

Murtakab Y. Al-Hejjaj[1,2,] Donald J. Watts[1] and Ewald H. Hettema[1]

[1] Department of Molecular Biology and Biotechnology/ University of Sheffield/ Sheffield/ UK
[2] College of Veterinary Medicine/ University of Basrah/ Basrah/ Iraq

Abstract:
All previous investigations of the intracellular location of the first sterol biosynthesis enzyme, squalene synthase (SQS) have found that it locates to the ER membrane in eukaryotic cells. However, we found that *Dictyostelium discoideum* SQS (DdSQS) contains a typical peroxisomal targeting signal type 1 (PTS1) and that this enzyme is indeed localised to peroxisomes. All known PTS1-containing proteins are localised to the peroxisomal matrix whereas membrane proteins use a different pathway. However, we found that DdSQS behaves as a membrane protein. Interestingly, it has an amino acid sequence potentially forming a hydrophobic helix which is located immediately upstream of the PTS1. Deletion of this helix does not affect peroxisomal targeting but does affect its association with the membrane and may therefore serve as a tail anchor. Furthermore, the helix plays an important role forming SQS homodimer. SQS is the first example of a peroxisomal membrane protein that makes use of the PTS1 pathway for its localisation. The topogenic information is transplantable and therefore it could be a new tool for synthetic biology approaches.

Keywords: *Dictyostelium discoideum*, peroxisome, sterol biosynthesis, Squalene synthase, tail anchor protein.

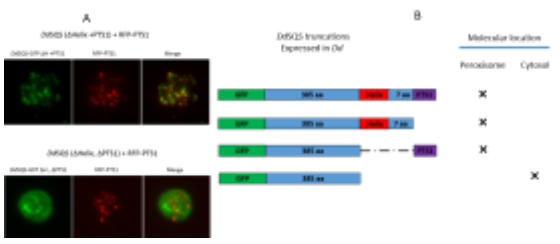

Figure 1) shows the role of the PTS1 import pathway in *D. discoideum*. A) Amoebae expressing GFP-*Dd*SQSΔHelix and mRFP-PTS1 as a peroxisomal marker. The GFP co-localized with mRFP. Amoebae expressing GFP-*Dd*SQSΔHelixΔPTS1 and mRFP-PTS1 as a peroxisomal marker. It is very clear that the GFP-*Dd*SQSΔHelixΔPTS1 was cytosolic in *Dd*. B) microscopy investigations show that the whole lingth *Dd*SQS and both trancations of *Dd*SQS (*Dd*SQSΔH and *Dd*SQSΔPTS1) were guided to the peroxisomes. While GFP-*Dd*SQSΔHΔPTS1 located in the cytosol.

References:

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Metabolite profiling of root-knot nematode (*Meloidogyne incognita*) at different growth stages

Bum-Soo Hahn, Parthiban Subramanian, Chang-Muk Lee and Joon-Soo Sim

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Abstract:
Root-knot nematode (*Meloidogyne incognita*) is a soil pest causing major agricultural losses in several crop plants. The life cycle of this plant-parasitic nematode consists of five major stages including egg, juvenile J2, J3, J4 and female of which the egg and J2 stages occur outside the plant tissues whereas the J3, J4 and adult stages are found in association with plant roots. Metabolome analysis was performed at egg, J2, J3, J4 and female stages of the root-knot nematode. Among 245 metabolites detected, 110 metabolites with standards were quantified. From results, metabolite expression could be grouped based on stages where J3 and J4 stages had distinct metabolic profiles indicating more translation, polyamine synthesis, antioxidants and urea cycle related compounds. Egg, J2 and female stage metabolites indicated high energy metabolism, glycolysis, TCA cycle, branched chain and aromatic amino acid synthesis. Overall, amino acids formed the dominant group with other highly expressed metabolites including osmolytes proline, betaine, hydroxyproline; antioxidant molecules putrescine and glutathione; sensory regulators GABA and β alanine; organic acids lactic and malic acids, RNA synthesis molecules GMP, AMP and inosine. In this study, metabolite profiling expressed at various stages of the root-knot nematode life cycle can be a useful resource for expanding the strategies currently employed to control the pathogen.

Keywords *Meloidogyne incognita*, metabolites, stages, KEGG pathway, quantitative analysis

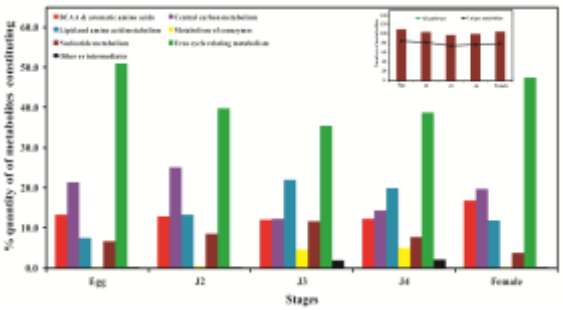

Figure 1: Quantitative estimation of metabolites and involved metabolic pathways. Diversity of metabolites contributing to cellular metabolic pathways. Total sum of quantity of the metabolites expressed in each stage of the nematode life cycle that contributed to metabolic pathways.

References:

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2. Schroeder, F. C., (2015), Modular assembly of primary metabolic building blocks: a chemical language in *C. elegans*, *Chem*. *Biol*., 22, 7-16.

Investigation of neutral lipid production by a new strain of the green alga *Desmodesmus armatus*

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Abstract:
Eukaryotic microalgae are considered as promising sources of alternative energy that can replace the use of conventional fossil fuels and limit their contribution to climate change. *Desmodesmus armatus* was isolated as a new strain from the Weston Park pond (Sheffield, UK). The strain was identified using different molecular markers including 18S rDNA, ITS1, ITS2, and 5.8S rDNA. In general, microalgae accumulate high amounts of neutral lipid under stress conditions like high salinity and nitrogen starvation or depletion. Neutral lipids produced by microalgae can form the basis of biodiesel production by transesterification reactions. Therefore, *D. armatus* was studied to investigate its neutral lipid content, when grown in normal BG11 medium and under stress conditions including high salinity (0.2, 0.4 M NaCl), different concentrations of sodium nitrate in the BG11 media (10% $NaNO_3$, N-free), and different sources of nitrogen (NH_4Cl, urea) to evaluate the effect of these stress conditions on the lipid accumulation. Nile Red fluorescence was used to determine the neutral lipid content of *D. armatus* after optimising the procedure by determining the best cell concentration, the best concentration of Nile Red dye and the optimal length of time after adding the dye to exhibit the highest fluorescent intensity. The results showed that N-free BG11 medium was the best stress condition to use to increase the amount of neutral lipid after 28 days incubation *(Figure 1)*. A calibration curve, using triolein as the standard neutral lipid, was produced to estimate the amount of neutral lipid in terms of dry weight of biomass. Fatty Acid Methyl Esters (FAME) conversion yield was examined using a direct transesterification method and the composition of fatty acids was investigated using GC-MS. *Desmodesmus armatus* grown in N-free BG11 medium showed the highest yield and the contents of C16 and C18 fatty acids increased significantly when compared with *D. armatus* cells grown under normal culture conditions. Random mutation of *D. armatus* cells (Ultra Violet light at 254 nm) was performed using different exposure times to generate new strains with high lipid content based on the conjunction between Nile Red fluorescent dye and automated fluorescence assisted cell sorting (FACS) technique. The results showed the selection of a new mutant isolate with 5 times greater yield of neutral lipid than the wild type strain.

Keywords: *Desmodesmus armatus*, Neutral lipid, FACS, Nile Red, UV mutation

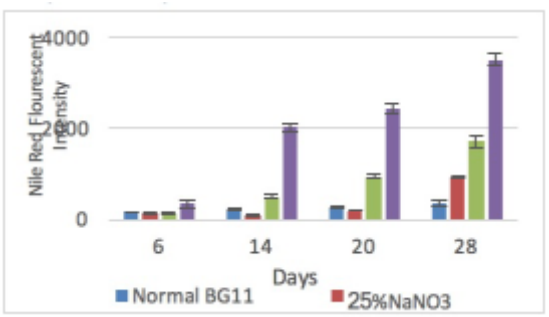

Figure 1: The Nile Red fluorescence intensity of *Desmodesmus armatus* cells grown in Normal BG11, 25% $NaNO_3$, 10% $NaNO_3$ and N Free BG11 media. Each column represents the mean difference between the average of four stained readings from the average of four unstained readings. Error bars represent technical repeats (n=3)

References:

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Non-targeted metabolomics-based screening of functional biomakers with antioxidant activity from diverse hot peppers

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Abstract:
Hot pepper, belongs to the Solanaceae family, is one of popular and important crops as food spices and vegetables. It has many functional metabolites including capsaicinoids, carotenoids, flavonoids, and vitamins. Various usages of hot pepper metabolites have been reported as potential therapeutic agents for the treatment of cancer, obesity, inflammation, and pain. In this research, we performed non-targeted metabolite profiling of 256 diverse accessions of *Capsicum* species including *Capsicum annuum*, *Capsicum chinense*, *Capsicum baccatum*, *Capsicum frutescens*, *Capsicum chacoense*, and *Capsicum eximium*.We have developed and optimized Ultra-high performance liquid chromatography coupled with diode array detector and electrospray ionization quadrupole time-of-flight tandem mass spectrometry (UHPLC-DAD-ESI-Q-TOF/MS) for the analysis of 70% MeOH extracts of hot pepper ripe fruits and multivariate analysis was performed to investigate the differences among groups. In addition, antioxidant activies of the same extracts were determined by ABTS assay and the correlation map between 435 metabolites and antioxidant activities was produced. Resulting biomarker candidates related to the antioxidant activity were selected by Pearson's correlation coefficients and annotated by comparing retention time, UV λ_{max} (nm), exact mass, isotopic pattern, and MS/MS fragmentation pattern from literatures, in-house libraries and on-line databases.

Keywords: hot pepper, non-targeted metabolomics, functional biomarker, antioxidant activity, UHPLC-Q-TOF/MS.

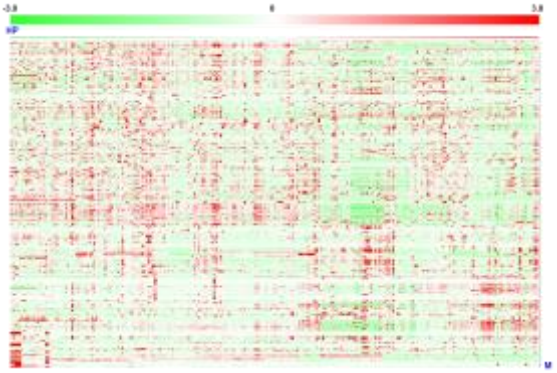

Figure 1: Diversity of metabolites from ripe fruits of hot pepper accessions. Heat-map between normalized peak areas of 435 metabolites and diverse 256 hot pepper fruits was created.

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A two-step process for improving of refractory sulfide concentrate biooxidation

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Abstract:
Biooxidation of sulfide raw materials by acidophilic chemolithotrophic microorganisms is widely and successfully used in the industrial processes to extract non-ferrous and precious metals [1,2]. Acidophilic microorganisms that are able to oxidize sulfide minerals are phylogenetically heterogeneous and include representatives of several bacterial and archaeal phyla, such as mesophilic (*Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*), thermotolerant (*Leptospirillum ferriphilum*, *Ferroplasma acidiphilum*, *Acidiferrobacter thiooxydans*), and moderately thermophilic species (*At. caldus*, *Sulfobacillus* spp., *Acidimicrobium* spp., and *Acidiplasma* spp.) [3]. In this study a method for improving the treatment efficiency of a refractory gold-bearing sulfide concentrate is proposed. This method consist of the oxidation of the concentrate through a two-step process. The first step is leaching with biogenic ferric iron at elevated temperature using the ferric iron-containing solution produced during the second step. The second step is biooxidation of the products of the first step by acidophilic chemolithotrophic microorganisms. A flotation concentrate, which contained pyrrhotite, arsenopyrite, pyrite, and antimonite, was used in the study. The concentrate contained 27.0% Fe, 8.21% As, 5.59% Sb, 20.32% S and 108 g/t Au. Comparison of the two-step and traditional (one-step) technologies was carried out and the flow sheet for the new process was proposed (Figure 1). Gold recovery from the sulfide concentrate by carbon-in-pulp cyanidation was 67.8% in the one-step process and 93.0% in the two-step process at 4 days of biooxidation, while it reached 82.4% and 94.1% in the one-step and two-step processes at 8 days of biooxidation, respectively. The proposed two-step process is a promising approach for enhancing the efficiency of gold recovery from sulfide concentrates by means of biooxidataion.

Keywords: biooxidation, acidophilic chemolitothrophic microorganisms, gold recovery, sulfide concentrate, ferric leaching.

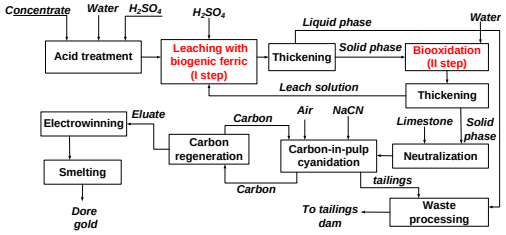

Figure 1: Gold recovery from sulfide concentrate using a two-step process.

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Structural studies of proteins of Red pathway system in Lambda phage

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Abstract

Homologous recombination in λ bacteriophage is performed via the Red-pathway system, the main function of which is to repair DNA double-stranded breaks, due to exposure to ionizing radiation, collapse of replication forks, restriction endonuclease incision or cleavage by terminase protein.

Amongst the genes associated with Red pathway system is the gene *ea10*, which encodes a small 14 kDa protein Ea10. Unpublished data from Gary Sharples lab (University of Durham, UK) suggests that it is a DNA binding protein, and its gene is under the control of the same promoter as other Red pathway proteins. Highly diffracting heavy-atom derivatized crystals were obtained for Ea10, from which its structure was solved to 2.7 Å using MAD phasing. The protein has a dimeric structure. As yet, no homologues have been found for Ea10 to help predict its main function. Structural comparisons for Ea10 via the Dali Server suggest a notable similarity with part of the Q-beta replicase core complex, indicating a possible association with RNA. Interaction mapping has suggested possible *E. coli* host protein partners. Experiments are ongoing to unravel the mystery of the Ea10 function (figure 1).

Recently, experiments revealed λ DNA carries a structure specific endonuclease called Rap, which targets recombination intermediates generated by the Red system. Rap possesses the ability to cleave three stranded junctions such as "D-loop", "flaps" and "Y- junctions". Therefore Rap may act as a general debranching endonuclease as well as a Holliday junction resolvase during phage recombination. We are currently working to determine the structure of the Rap protein via X-ray crystallography. Crystal trials have been set up for the purified full-length Rap endonuclease and two truncated versions of the Rap endonuclease, which removed the first 30 and 70 residues of a possibly disordered N-terminus.

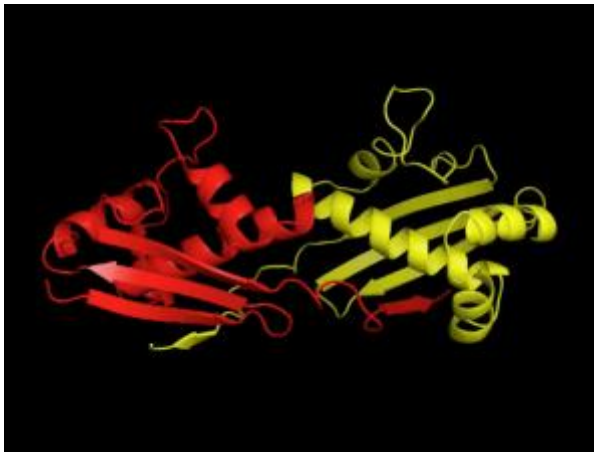

Figure 1: A cartoon form of solved Ea10 structure, colored by chain via pymol software. It is an interlocked dimer with the C-terminal part of the polypeptide from each monomer forming the fourth strand in a beta sheet with strands from the other monomer. We are tempting to discover the E10 precise function and more evidence about DNA binding relationship.

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The Constituents of *Betula platyphylla* that have Cytotoxic Effects on A549 Adenocarcinomic Human Alveolar Basal Epithelial Cell Line

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Abstract:
Betula platyphylla (BP, Betulaceae) has been used as traditional medicine of inflammatory disorders(1). However, there are few studies about BP for the inhibition of lung cancer. We tried to investigate the active compounds for anti-lung cancer from BP. The dried stems of BP (10.0 kg) were refluxed with methanol for 6 hs. The methanolic extract (321.4 g) was suspended in distilled water to be consecutively partitioned by using *n*-hexane, dichloromethane (CH_2Cl_2), ethyl acetate, and *n*-butanol. The CH_2Cl_2 soluble fraction (49.6 g) was applied to C18 medium pressure liquid chromatography. As results, lupeon (51.4 mg), betulinic acid (76.0 mg), oleanolic acid-3-acetate (281.7 mg), rhododendrol (118.0 mg), 1,7-bis[4-hydroxyphenyl]-3-hepten-5-one (27.5 mg), hannokinin (20.0 mg), centrolobol (7.0 mg), oleanolic acid (17.0 mg), and tetradecane (64.0 mg), were isolated. In MTT cell viability assay, oleanolic acid-3-acetate and 1,7-bis[4-hydroxyphenyl]-3-hepten-5-one significantly decreased A549 cell viability to 39.31 ± 1.05% and 18.93 ± 0.81%, respectively, at 50 μM. Moreover, they significantly induced the production of intracellular reactive oxygen species (ROS) in A549 cells. These data indicate that the active compounds oleanolic acid-3-acetate and 1,7-bis[4-hydroxyphenyl]-3-hepten-5-one from natural product could be helpful for the development of anti-lung cancer drugs.

Keywords: *Betula platyphylla*, lung cancer, oleanolic acid-3-acetate, 1,7-bis[4-hydroxyphenyl]-3-hepten-5-one

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The Improved Protective Effects of Phase I Metabolites of Isoliquiritigenin on Glutamate-Induced Cell Death of Mouse Hippocampal Neurons

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Abstract:
The investigation of drug metabolites is important to evaluate their toxic or preventive effects after administration(1,2). In the previous study, a chalcone compound isoliquiritigenin (ISOLIQ) from Glycyrrhizae Radix (licorice roots) significantly protected the mouse hippocampal neuronal cell line (HT22) against 5 mM glutamate toxicity(3). However, there are little reports about the protective effects of metabolites derived from ISOLIQ on HT22 cells. Therefore, ISOLIQ and its Phase I metabolites were prepared through isolation or organic synthesis, and their HT22 protective effects against glutamate-induced cell death were examined. As ISOLIQ metabolites, liquiritigenin, 2',4,4',5'-tetrahydroxychalcone, sulfuretin, butein, davidigenin, and *cis*-6,4'-dihydroxyaurone were prepared. Amon these six metabolites, 2',4,4',5'-tetrahydroxychalcone, sulfuretin, and butein showed significantly higher HT22 protective activities than the parent compound, via inhibition of intracellular reactive oxygen species (ROS) production. These results suggest that the neuroprotective effect of ISOLIQ could be improved by its active metabolites involving 2',4,4',5'-tetrahydroxychalcone, sulfuretin, and butein that contain the catechol moieties in their structures.

Keywords: licorice root, isoliquiritigenin, neuroprotection, metabolites

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Development of a Cell-based High-Throughput Assay for Evaluating PTBP1 Alternative Splicing Inhibition in Live Cells

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Abstract:
Lineage-specific alternative splicing (LS-AS) helps establish tissue identity; in humans, aberrant LS-AS is linked to neurological diseases (*1*, *2*). The role of LS-AS in malignant transformation remains undefined. Polypyrimidine tract binding protein 1 (PTBP1) is a RNA binding protein that regulates LS-AS in the brain (*3*). PTBP1 is amplified and/or overexpressed in glioblastoma (GBM); this alters LS-AS and promotes tumorigenicity. Our data demonstrate that inhibition of PTBP1 may be useful for the treatment of GBM (*2*). To identify small molecule modulators of PTBP1-mediated splicing, we have developed a cell-based high-throughput screen assay (CBHTA) that allows us to assess the regulation of PTBP1 in living cells. The PTBP1-CBHTA uses a mini-gene reporter approach to quantitatively and reliably measure the specific activity of PTBP1 in live cells. The mini-gene reporter approach exploits the interactions between PTBP1 and the *α-actinin* pre-mRNA, a direct splicing target of PTBP1 **(Figure 1)**. A fragment of the *α-actinin* gene harboring the PTBP1 binding sites was cloned upstream of luciferase (ATN-Luc) and used to make stable GBM cells (SNB19-MG and U251-MG) that contain ATN-Luc. In cells that express PTBP1, the pre-mRNA is spliced out of frame and luciferase expression is minimal. In the absence of PTBP1, pre-mRNA is spliced in frame and luciferase is expressed. The PTBP1-CBHTA measures the increased luciferase signal if PTBP1 is inhibited. These cells also express tetracyclin-inducible shRNA specific for PTBP1, which allows us to use the absence or presence of tetracycline as negative and positive controls, respectively. We are adapting this for the robotics platform of the HTS group at Southern Research (SR, Birmingham, AL) in either a 384 or 1536-well format to interrogate the impact of 2,000,000 different compounds on PTBP1 splicing. Our approach will identify small molecule compounds that inhibit PTBP1, and which can be evaluated for therapeutic value in the treatment of GBM.

Keywords: alternative splicing, ANXA7, glioblastoma, lineage-specific, neural precursor cells, PTBP1, targeted small molecule-based therapy.

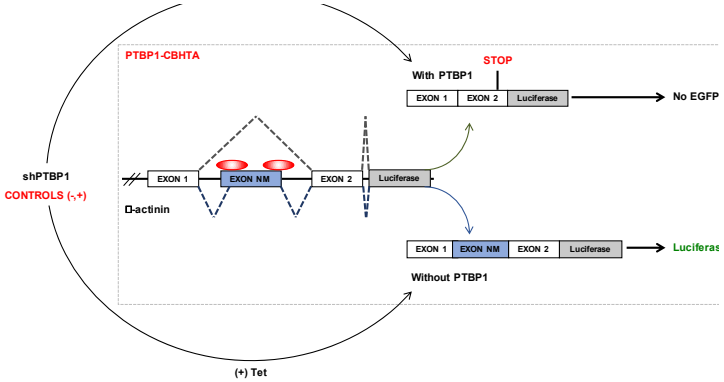

Figure 1: Model of Cell Based High-throughput Assay (CBHTA) to Assess PTBP1 Inhibition. Modified GBM cells (*inside dashed box*) are used to screen the Southern Research (SR) large-scale chemical library for inhibitors of PTBP1. When PTBP1 is present, the mini-gene is spliced out of frame and a premature stop codon prevents luciferase expression. However, if PTBP1 activity is inhibited, exon NM is included and luciferase is expressed. The administration of tetracycline (Tet) (*entire Figure*) will be used as a positive control. When grown in the presence of Tet, *PTBP1* shRNA is expressed and luciferase levels increase; the absence of Tet will be used as a negative control. Luciferase levels are minimal in these cells and not influenced by known PTBP1 inhibitors.

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Acknowledgement: This research is funded by a Pilot Grant in Drug Discovery and Development of the Alabama Drug Discovery Alliance (ADDA), a collaboration between the University of Alabama at Birmingham (UAB) School of Medicine (UAB Center for Clinical and Translational Science [CCTS] and UAB Comprehensive Cancer Center [CCC]) and Southern Research (SR).

Therapeutic effects of recombinant *Salmonella typhimurium* harboring CCL17 miRNA on atopic dermatitis-like skin in mice

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Abstract:
Atopic dermatitis (AD) is an inflammatory, chronically relapsing, puritic skin disorder. These syndromes result from multifactorial inheritance, with interaction between genetic and environmental factors. In particular, the chemokine CCL17 is directly implicated in skin inflammatory reactions and its levels are significantly elevated in serum and correlated with disease severity in AD. We tested the suppression of the CCL17 gene by microRNA (miRNA) and observed the effects in mice with inflammation similar to AD. We used Salmonella as a vector to deliver miRNA. The recombinant strain of *Salmonella typhimurium* expressing CCL17 miRNA (ST-miRCCL17) was prepared for in vivo knockdown of CCL17. ST-miRCCL17 was orally inoculated into mice and the CCL17 gene suppressed with CCL17 miRNA in the activated lymphocytes. IgE were inhibited and interferon-γ was induced after treatments with ST-miRCCL17 and CCL17 was suppressed. Further, Th17 cells were suppressed in the atopic mice treated with ST-miRCCL17. These results suggest that ST-miRCCL17 may be an effective genetic agent for treating atopic dermatitis.

Keywords: biological therapy; chemokine CCL17; dermatitis, atopic; immunotherapy; RNA interference; Salmonella typhimurium

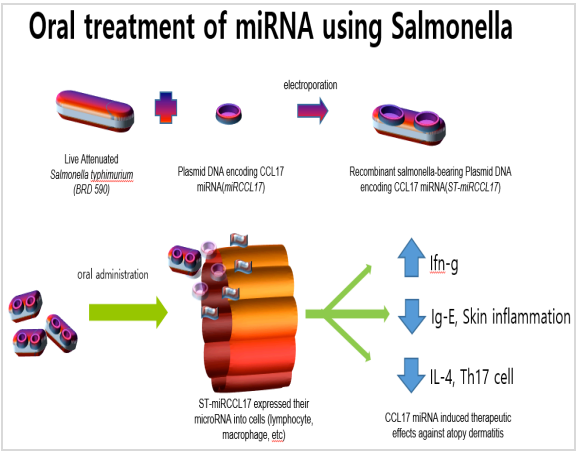

Figure 1: Oral treatment of miRNA using Salmonella. Recombinant *S. typhimurium* expressing CCL17 miRNA(ST-miRCCL17) were constructed by pcDNATM6.2-GW/EmGFP-miR expression vector using electrophoration. In atopic like animal model, Changes in IL-4, IFN-γ and IgE levels in serum were tested by elisa, Th 17 cells were tested by FACS analysis (R&D system). Samples were collected seven days after oral inoculation.

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Investigating The Mechanical Properties of The Hydrogels Using The Atomic Force Microscopy (AFM)

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Abstract:
The study of biological systems as structures began in the early part of the 20th. It is known that the biological science is a mature field; however, the study of biological and biomedical materials and their applications is still nascent and evolving. The field of tissue engineering is in its early stages and the structural relationships in many biological materials remain difficult to understand. The assessment of the mechanical properties of some soft tissues is limited by the complex nature of their structure and physiological environment. Therefore, the aim of this study to investigate the mechanical properties of a type of tissue engineering materials or biomaterials which are the hydrogel products. The hydrogels comprise two groups: either they are natural or synthetic, based on their origin. A hydrogel is defined as a water-swollen cross-linked polymeric network which is produced by one or more monomer by a simple reaction. In this research, three different consentrations of the hydrogels were studied to calculate their mechanical properties such as the Young's modulus, stiffness and hardness using atomic force microscopy (AFM) with a tipped cantilever as an indenter to acquire force-indentation (F-δ) curves as presented in Figure 1.

Keywords: AFM, Hydrogels, Yung's modulus, stiffness, Hardness, Tissue engineering.

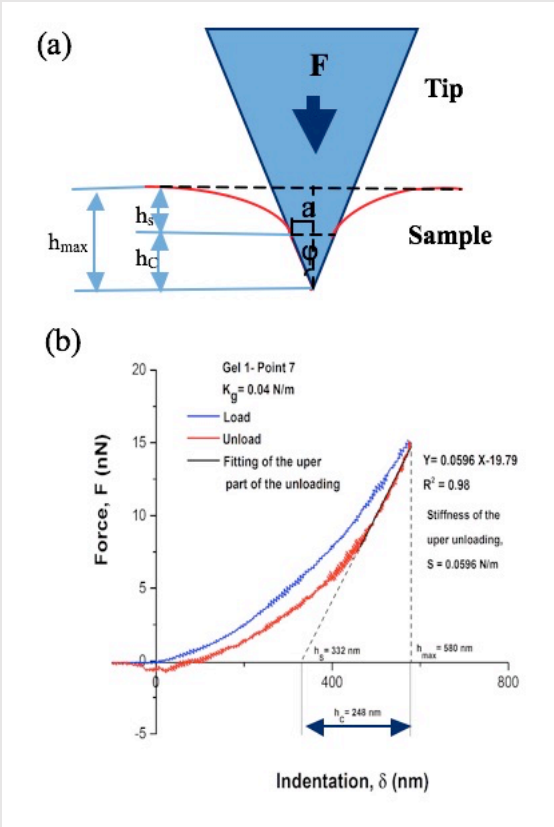

Figure 1: (a) Schematic drawing showing indentation form of the conical tip at maximum force, F_{max}. (b) The force-indentation (F-δ) curve of the hydrogel with stiffness 0.04 N/m, where h_s, h_{max} and h_c are shown on the curve and the contact depth, h_c = 248 nm.

3D-printed magnetically induced fluidized-bed reactor for electrochemical applications

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Abstract:
Electrochemical processes proceed at the boundary between the electrode and the surrounding solution. In order to achieve high conversion rates, the ratio of electrode surface area to solution volume must therefore be maximized. Due to their high specific surface area, fluidized-bed electrodes are particularly suitable for this purpose. However, in practice they are rarely used because the controlled fluidization as well as the electrical contacting of these fluidized-beds are major challenges and so far only partially solved. One approach for better contacting of the conductive particle electrodes is the magnetically induced fluidized-bed reactor. Here, the fluidized bed is intended to be under the influence of a magnetic field and at the same time serve as an electrode for the electrochemical reaction.
To meet these challenges a scaleable magnetically induced fluidized-bed reactor was designed (Figure 1) and afterwards fabricated by 3D-printing. The fluidized-bed reactor consists of three elements and can be closed by means of two clamps. The reactor offers standard connectors, wich allow e.g. the connection of analytical equipment like flow-through spectrometers or pH-electrodes, Overall, the reactor is intended to be compatible to standard laboratory equipment.
We propose this new kind of fluidized-bed reactor as a tool for the optimization of electrochemical prozesses at low concentrations, as they can be found e.g. in electrochemical recycling or water treatment processes.

Keywords: magnetically induced fluidized-bed reactor, electrochemical reaction, 3D-printed reactor system, scale-up, economic process development.

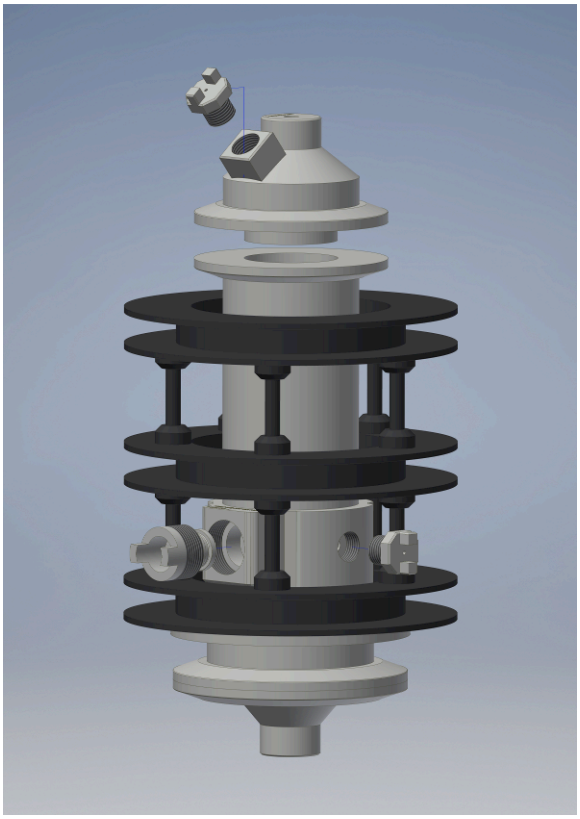

Figure 1: Layout of a electrochemical fluidized-bed reactor with three inputs for connecting the electrodes. A coil is placed around the reactor to influence the electrode particles inside the system.

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Biosorption of Chromium and Cadmium from tannery effluent by free and immobilized cyanobacterial and bacterial consortium: Equilibrium and structural studies

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Abstract:
Biosorption of heavy metals from industrial effluents can be considered as an alternative technology in industrial wastewaters treatment. The ways of the creation of cyanobacterial-bacterial communities with a high remediation potential for cleaning of water reservoirs contaminated with pollutants are in demand. The efficiency of cyanobacteria-bacteria consortium for remediation of tannery effluent was studied. The process of biosorption of chromium and cadmium by *Spirulina spp* and *Halomonas nitritophilus* consortium and the sorption potential in both free and immobilized states were investigated (Figure 1). Several factors like variation in pH, contact time and biomass concentration were studied in order to understand the effect of biosorption on heavy metal removal. The Langmuir, Freundlich adsorption models were applied to describe biosorption process. The present data fit into isotherm models with very high regression coefficient indicating good biosorption efficiency for removal of heavy metals. FTIR studies reveled interactions of numerous chemical groups present on the cell surface of immobilized cyanobacterium-Bacteria consortium with Cr and Cd. The finding of the study showed that cyanobacterium-Bacteria consortium has much potential as a biosorbent for the sorption of Cr and Cd in both free and immobilized state from tannery effluent.

Keywords: adsorption isotherm, *Halomonas nitritophilus*, biosorption, immobilization, *Spirulina* spp.

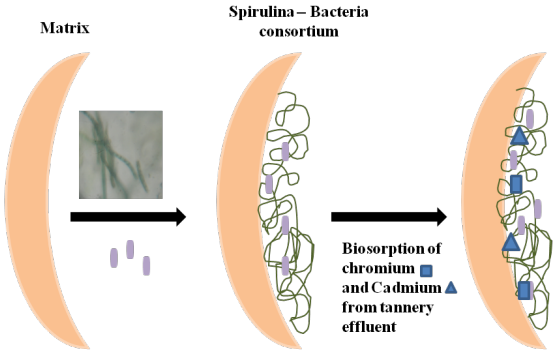

Figure 1 : Figure illustrating the consortia of cyanobacteria and bacteria can work in a synergistic way for the degradation of heavy metals from tannery effluent. The main concern of this study was to achieve maximum heavy metals removal from tannery effluent using immobilized blue green algae (cyanobacteria) - bacteria consortium system which provide an alternative method for waste water treatment.

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Screening of plant endophytes to control pine wood nematode

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Abstract:
Pine wilt disease is caused by the pine wood nematode (PWN) *Bursaphelenchus xylophilus*. Recent outbreak of pine wilt disease in many countries threatens the vegetation of *Pinus* species seriously. Furthermore, *Pinus densiflora* and *Pinus thunbergii* are predominant species in Korea forest are highly susceptible to the pine wood nematode. Therefore, there is an urgent demand for the development of a new chemical method for controlling the pinewood nematode. To find novel sources for nematocidal agents, we isolated more than 120 isolates endophytic bacteria from tissues of *Taxus* species, such as leaves and flowers. And we also isolated 100 endophytic fungi from reported plants which have nematocidal activity. The extracts of endophytes can be a useful resource for the nematocidal development. The endophytes are also tested for increasing the efficacy of the nematocidal effects of avermectins. The avermectin precursors and avermectins in *Streptomyces avermitilis* are added to the incubating media or the protein extract of endophytes, and then tested for the nematocidal efficacy. As the final frontier of finding out novel compounds, endophytes can be a useful resource for nematode control by themselves or by converting old nematocidal agents to brand-new effective agents.

Keywords: Pine wood nematode, plant endophytes, nematocidal activity, Avermectin

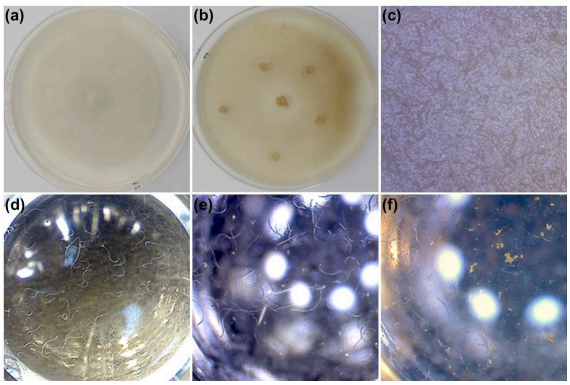

Figure 1: Culture of pine wilt nematode (PWN) on *Botrytis cinerea* and assay for the nematocidal effect. Growth of *Botrytis* (a). Plugs of PWN on the *Botrytis* media (b). Propagation of PWN on the *Botrytis* media (c). The grown PWN were harvested and tested for nematocidal effect. The swimming PWN in distilled water in a well of 96 well plate as the control (d). Addition of various concentration of methanol into the control. Ten percentage of methanol (v/v) did not have nematocidal effect (e). Addition of various concentrations of crude extract of *S. avermitilis* (f). PWN did not swim and have a relatively straight thread shape.

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Reaction Characteristics of Catalytic Triglyceride Hydroconversion over Noble Metal-Supported Zeolite Catalyst for Production of Bio-Jet Fuel from Palm Oil

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Abstract:
Bioenergy can be produced from a variety of raw materials and can be used in the form of fuels for heat, electricity and transportation. Biofuels for transportation can be produced not only from grains but also from a variety of biomass raw materials such as non-edible raw materials such as organic wastes, woody algae and seaweeds. Biofuels are also applied to transportation vehicles as well as vehicles. Recently, as the need for CO_2 reduction in the aviation sector has increased, research on biofuel oil technology development is under way. Deoxygenation and isomerization are key challenges in the development of bio-jet fuel production technology from oil-based materials.[1]
In this study, a zeolite catalyst supported on metal catalyst was synthesized as a catalyst applicable to the process of producing palm oil-derived bio-jet fuel and the reaction characteristics were investigated as a result of applying it to various reaction conditions. Figure 1 shows the process by which carbon oil is produced by the combination of hydrodeoxygenation and isomerization reaction from palm oil having fatty acid composition with a carbon number distribution.

Keywords: triglyceride, catalytic hydroconversion, zeolite catalyst, bio-jet fuel, palm oil, reaction characteristics, deoxygenation, isomerization

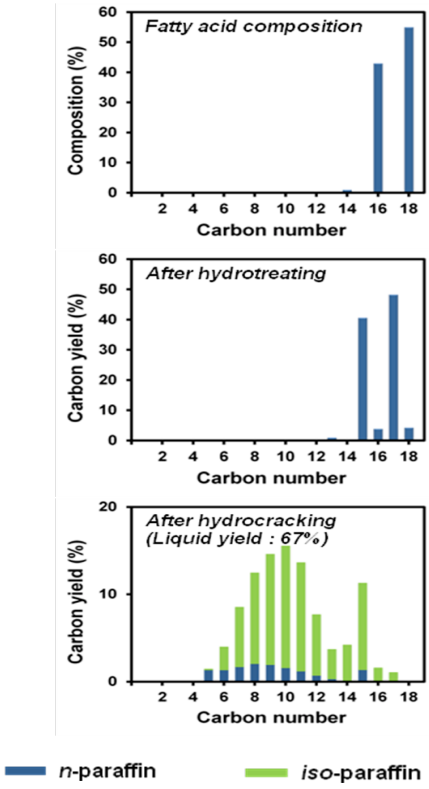

Figure 1: Figure illustrating the process by which carbon oil is produced by the combination of hydrodeoxygenation and isomerization reaction from palm oil having fatty acid composition with a carbon number.

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Catalytic Hydrodeoxygenation over the Metal-Supported Catalysts for Transformation of Oxygenated Hydrocarbon Compounds into Iso-Alkanes

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Abstract:
Recently, researches on the catalytic upgrading of oxygenated hydrocarbon compounds have been carried out to meet the fuel properties and to manufacture related chemicals[1]. The process of converting an oxygenated hydrocarbon compound into a transport fuel in the gasoline range is as follows. First, in order to obtain a high calorific value, it is necessary to manufacture a liquid hydrocarbon compound having a low O/C ratio as well as a high H/C ratio. This is possible by increasing the number of C atoms and reducing the number of O atoms simultaneously during the conversion process. However, since oxygen is completely removed from the fuel as a fuel, the additive such as MTBE, ETBE, methanol, and ethanol must partially exist in the synthetic fuel. Second, it is related to the degree of isomerization by high octane number. Thus, it is essential to produce highly branched hydrocarbons with larger fractions. Catalytic conversion of small oxygen compounds to fuel grade compounds is possible through C-C coupling and hydrogenation reactions.
In this study, the transformation into iso-alkanes from oxygenatied hydrocarbon over metal-supported metal oxide catalysts were conducted by the product distribution derived under different conditions. From these results, the overall reactions involved in the conversion of oxygenated hydrocarbon compounds was depicted, where the main reaction routes were classified. The effect of catalyst characteristics on each route was further elucidated. Consequently, an explanation was formulated for the efficient transformation of oxygenated hydrocarbon to iso-alkanes over metel-supported metal oxide catalysts(Figure 1).

Keywords: catalytic hydrodeoxygenation, metal-supported catalysts, transformation, oxygenated hydrocarbon compounds, iso-alkanes

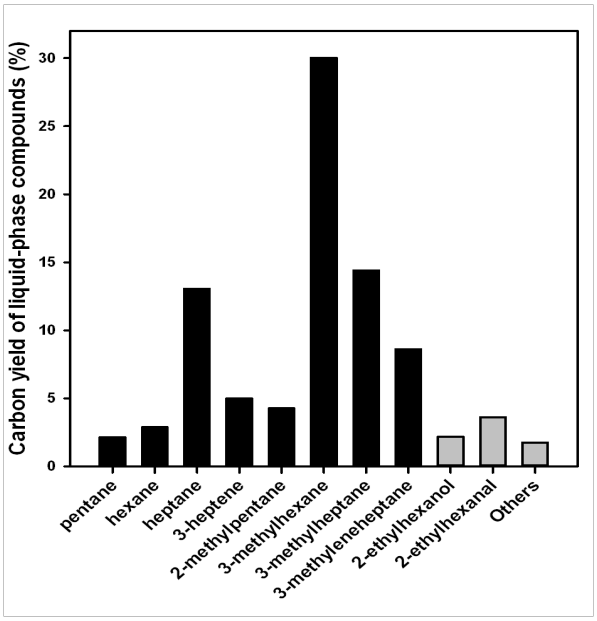

Figure 1: Figure illustrating the carbon yield of O-free (black) and oxygenated (gray) hydrocarbon in liquid-phase products obtained from the conversion of oxygenated hydrocarbon compounds over metal-supported metal oxide catalyst..

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Surface Plasmon Resonance Based stratagies for extraction of tyrosinase inhibitors from natural sources

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Abstract:
Tyrosinase inhibitors have been used as whitening or antihyperpigment agents because of their ability to suppress dermal-melanin production. It may therefore have good potential as antibrowning agents to be applied in food industries as well as in cosmetics. In the present study, extraction of small molecules and peptides from natural sources followed by screening and kinetic evaluation on mushroom tyrosinase (MT) as well as on Human tyrosinase (HT) using surface plasmon resonance has been carried out. Few molecules that showed significant binding were further eluted by SPR and characterized by mass spectroscopy. The study provides information about binding affinity pattern and differences between mushroom and human tyrosinase in terms of KD. Moreover both tyrosinases showed considerable changes in the secondary structure in the presence of inhibitors. Biacore/SPR sensor's ability in the rapid identification and characterization of inhibitors (Figure 1). The inhibitor screening will help to calculate the exact binding time for enzyme inhibitor interaction for long term effect in cosmetics and food industries. The sensors can be used for high-throughput screening of potential pharmaceutical drug candidates. The data generated from this study will be critically analyzed for their utility in developing a commercial product. A dialogue with a private company related to cosmetics and food products will be initiated for technology transfer.

Keywords: enzyme inhibition, surface plasmon resonance, affinity, kinetics, circular dichroism spectroscopy, modeling, mass spectroscopy, mushroom tyrosinase, human tyrosinase, biomedical applications.

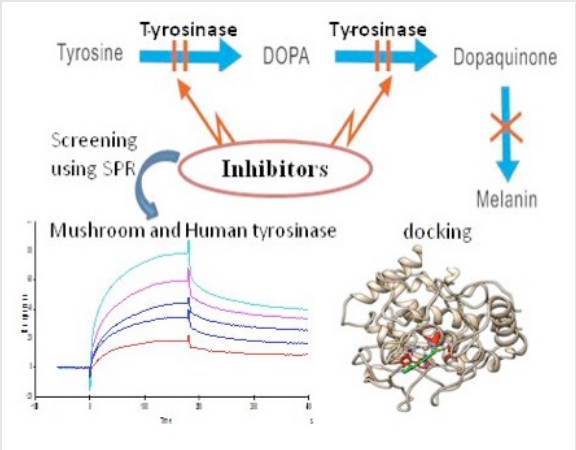

Figure 1: Figure illustrating Graphical representation of the work

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Synthesis, characterization, DFT calculations of copper(II) complex with paracetamol drug. Biological and antioxidant activities.

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Abstract:
Paracetamol or acetaminophen is a widely used over the counter analgesic (pain reliever) and antipyretic (fever reducer). It is commonly used for the relief of fever, headaches, and other minor aches and pains, and is a major ingredient in numerous cold and flu remedies. In combination with non-steroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics, paracetamol is used also in the management of more severe pain (such as postoperative pain).

Copper complexes have attracted great deal of attention the last years due to their therapeutic applications as antimicrobial and antioxidant.

The present work reports the study of the interactions between paracetamol drug and the copper(II) metal. The elemental analysis, conductivity, IR and UV-Vis spectra, thermal (TG/DTG), ^{1}H NMR, ^{13}C NMR, electronic spectral studies of this complex was discussed and deduced the suggested structure which suggested that the paracetamol behaves as bidentate coordinated ligand to the copper ion via the oxygen and carbonyl-O atoms of the amide group. The antimicrobial activity of ligand and their complex was evaluated in vitro against different bacteria and fungi using agar-diffusion method. A significant activity was observed with copper complex against Gram-negative and Gram-positive bacteria.

The antioxidant activity of the ligand and Cu(II) complex was measured in terms of their hydrogen donating or radical scavenging ability by UV–Vis spectrophotometer using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). The Density Functional Theory (DFT) calculations were used to optimize the geometric structure of the ligand and its metal complex. It was done in order to confirm the experimental results.

Key words: paracetamol, copper (II) complex, DFT calculations, antimicrobial and antioxidant activities.

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