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## Poster

30 YEARS OF EXCELLENCE IN MEDICAL PUBLISHING

# Development of versatile biological models to study nanodevices biomedical potential

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The development of personalized and non-invasive therapies based on new nanoparticles is a major challenge in medicine. In this context, we studied different nanoparticles for cancer therapy.

Firstly, we analyzed the biological efficiency of hollow organosilica nanoparticles. Porous systems are used to be applied to drug adsorption and delivery. In this case, we have loaded two anti-cancer drugs, which have been used to perform in vitro investigations in order to demonstrate their biocompatibility and their potential as drug carrier vehicles to treat cancer.

Moreover, nanoscience has grown considerably in cancer treatment with nanoparticles activated with stimuli as Mn2+-doped Prussian blue nanoparticles. They are many advantages as their flexible structure, porosity and biocompatibility. Indeed, Prussian blue has been approved by the Food and Drug Administration for human. We have demonstrated for the first time that these nanoparticles acted as efficient agents for photothermal therapy under Two-Photon Excitation (TPE) and induce an almost eradication of malignant cells.

Finally, in order to respond to increasing demand for new therapies, the Photo Dynamic Therapy (PDT) has arisen as an alternative to chemo- and radiotherapy for the non-invasive selective destruction of small tumors. PDT is based on photosensitizers activation by irradiation. To enhance the selectivity towards tumor cells and the efficiency of PDT, the photosensitizers are encapsulated in Periodic Mesoporous Organosilica (PMO) nanoparticles. To go further in the biomedical proof of concept of therapeutic nanoparticles, we are currently developing an animal model as Danio rerio (zebrafish) to study cancer. We have implanted fluorescent human cancer cells in zebrafish larvae in order to establish a detectable tumor xenograft. Then, we have intravenously injected PMO for TPE-PDT in zebrafish and irradiated the tumor site with a pulsed laser. The strong decrease in tumor size let us imagine developing such model to test the biomedical potential of different nanoparticles.

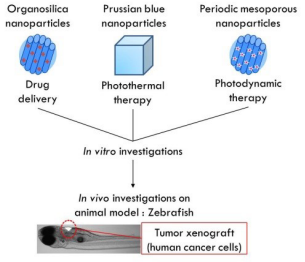


Figure 1: The design of multifunctional nanomaterials with controlled physico-chemical properties thanks to the chemistry expertise allow to the biologists to demonstrate their biomedical potential on *in vitro* and *in vivo* biological models.

## Biography

Morgane Daurat is born in 1991 in Béziers (France). She is a PhD student in third year at Institut des Biomolecules Max Mousseron in Montpellier (France). She works on the development of biological models to study nanoparticles biomedical potential and on lysosomal diseases for the company NanoMedSyn (Montpellier, France). She is co-author of nine articles.

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# Preparation and different sterilization approaches for Nanostructured Lipid Carriers (NLC): Towards industrial production

**Claudia Vairo**
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**Statement of the Problem:** The transition of the production process of lipid nanoparticles from laboratory scale to industry could be difficult. Firstly, when manufacturing lipid nanoparticles, i.e. Nanostructured Lipid Carriers (NLC), it is important to obtain a homogeneous particle size. Secondly, for sterile products, aseptic manufacturing production is required, which implies high costs (class A clean rooms, qualified personnel) and a tedious process validation. To tackle these problems, High Pressure Homogeniser (HPH) could be a feasible equipment suitable for homogeneous industrial production of NLC (1) and, γ-radiation could fulfill sterilisation requirements when carried out as a final sterilisation method (2). The purposes of this study are: (i) to optimise the scaling-up of NLC production by using HPH and (ii) to demonstrate the suitability of γ-radiation as a simple and fast final sterilisation method, maintaining chemical and physical integrity of the loaded drug. Methodology: NLC were produced using the melt-emulsification method (3) including a homogenisation step by using the GEA PANDA HPH (Niro Soavi, USA) at 500 and 1,000 Bar pressure conditions (and 0, 3, 7 and 10 number of cycles. The homogenised emulsions were collected, lyophilised and obtained NLC were irradiated with a dose of 25kGy from 60Co following the European Pharmacopeia recommendations (4). The resulting NLC were then characterised in size, Poly Dispersity Index (PDI) and zeta potential. The drug release profile and chirality were also analysed before and after γ-sterilisation by circular dichroism. Findings: The increase on the number of cycles resulted in a lower particle size and PDI; and 500 Bar were enough to obtain monodisperse samples (Figure 1). Size, PDI, zeta potential, release profile and chirality were not affected by γ-radiation. Conclusion & Significance: HPH and final γ-radiation may be useful to produce NLC in an easy and cost-effective manner at industrial scale.

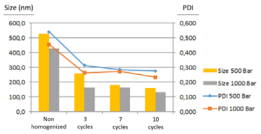


**Figure 1.** Size (nm) and PDI of nanostructured lipid carriers (NLC) at different pressure conditions (500 and 1000 Bar).

## Biography

Claudia Vairo has completed his Degree in Pharmacy (2012) from University of Salerno and a Second level master's in pharmacology from University of the Basque Country (2015). She works as R+D researcher in BioPraxis Research AIE pharmaceutical company and she is completing her PhD studies in Pharmaceutical Technology, in collaboration with NanoBioCel Group from University of the Basque Country. She has published 2 papers in reputed journals concerning medical devices, such as lipid nanoparticles, especially nanostructured lipid carrier (NLC), and gelatin scaffolds, for the treatment of wound healing. Her investigation is recently focused on NLC nanosafety.

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# Structure analysis of gold nanorods obtained using selected oligomeric surfactants

**Joanna Maksim**
Adam Mickiewicz University, Poland

The interest in metallic nanoparticles has been growing in recent years. Obtaining different shapes and sizes of nanoparticles allows us to tune their properties and applications. The ability to modify these nanostructures with surfactants is particularly important for wider biomedical applications. It allows attaching macromolecules important for biomedicine and avoiding aggregation of nanostructures. The aim of this study was to synthesize gold nanorods (GNRs) by modified method using seeding growth and analyse them used several methods. To compare the influence on the morphology five oligomeric gemini surfactants (Cl_C6_C8, Cl_C6_C12, imi Cl_C6_12, Cl_C6_C16, imi Cl_C6_16) and two different concentrations of silver nitrate were present during the stage of growth.

Obtained nanoparticles were characterised using microscopic and spectroscopic techniques, including UV-Vis spectroscopy, Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM). Using these methods, the information about shape and size of synthesised nanorods was achieved, which permits to choose the most promising way of synthesis modification. Furthermore, the sample with the best morphology will be modified and tested for biomedical applications.

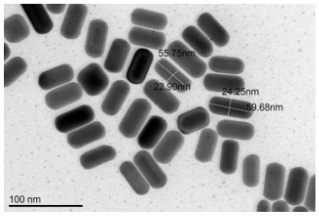


**Figure 1 Transmission Electron Microscopy image of synthesized nanorods.**

## Biography

Joanna Maksim is a fourth-year medical physics student. She studies at Adam Mickiewicz University in Poznan, at Faculty of Physics and works on her master thesis at Department of Macromolecular Physics. In this work, she syntheses rod-shaped nanoparticles which could have biomedical applications and characterizes the microstructures of obtained nanoparticles using spectroscopic and microscopic methods. This research project was supported by programme Best of the Best (Najlepsi z Najlepszych) 3.0 from Ministry of Science and Higher Education (Poland).

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# Gold nanorods – the synthesis by the use of various gemini surfactants

**Karolina Rucińska**
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Nanoparticles of noble metals, especially gold nanoparticles, have fascinated scientists for over a century because of their unique properties which depends on their shape and size. In addition, nanoparticles may have potential application in modern biomedicine, for example in phototherapeutics and drug delivery. The aim of this study was to synthesize rod-shaped nanoparticles using different routes of synthesis, including addition of gemini surfactants. Seed-mediated growth method, with various surfactants, to obtain different size and prevent aggregation of nanorods, has been applied to produce gold nanoparticles. Oligomeric surfactant molecules used in this work consists of three components – hydrophobic, hydrophilic and linker groups. The formation of surfactant bilayers on the nanorods surface allows to electrostatically bind nucleic acid, what may be used in scaffolding for delivery system. Gold nanoparticles synthesized with different surfactants has been studied using Small Angle X-ray Scattering (SAXS) and Transmission Electron Microscopy (TEM). They were also characterized by UV-Vis spectroscopy and Nuclear Magnetic Resonance (NMR) diffusometry to get information about their size, shape and structure. Details of the reactions, such as different number of surfactants and silver nitrate in nanorod growth procedure, were taken into account, discussed and compared in this study.

This research project was supported by the programme Best of the Best (Najlepsi z Najlepszych) 3.0 from Ministry of Science and Higher Education (Poland).

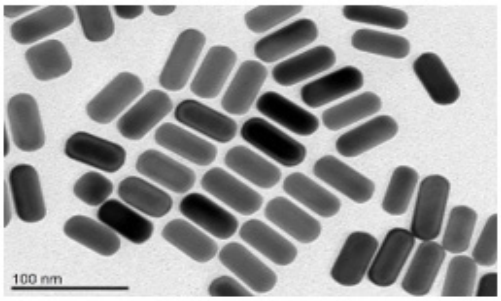


**Figure 1: TEM image of rod-shaped nanoparticles.**

## Biography

Karolina Rucińska is a student of the 4th year of Medical Physics. She studies at Adam Mickiewicz University in Poznan, at Faculty of Physics and works on her master's degree thesis at Department of Macromolecular Physics. In this work, she synthesized rod-shaped nanoparticles, which have potential application in medicine, and characterized by spectroscopic and microscopic methods. This research project was supported by programme Best of the Best (Najlepsi z Najlepszych) 3.0 from Ministry of Science and Higher Education (Poland).

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# Preliminary studies of the synthesis of gold nanoribbons based on the seed size

**Joanna Patalas**
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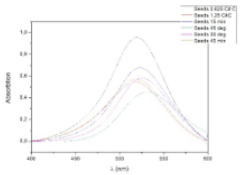
Nanotechnology and medicine gave birth to the new and promising interdisciplinary research field called nanomedicine. Thanks to the improvements in both of the nanotechnology and nanomedicine we are able to help and improve people's health on the nanoscale. One way is using biosensors that can signalize the existence of pathogens, alien DNA, viruses etc. The most important features of the nanomaterial to create a biosensor are their shape and stability. Shapes that are the best fit are elongated and flat nanoparticles such as nanotubes, nanorods, nanowires and nanoribbons. The size of these nanocreations gives an opportunity to later functionalize them with biological and chemical molecules.

Gold nanoribbons are promising metallic support for biosensors in the nanoscale, and thanks to the development of modern technology, we are able to create them using various methods. One of them is using oligomeric and polymeric surfactants – surface active agents, built of hydrophobic and hydrophilic moieties that can wrap around the growing nanoparticles. The properties of surfactants are helping to stabilize the growth of the specific shape of nanoparticle such as nanoribbons. The conducted study has been focused on creation of metallic nanoribbons especially gold nanoribbons with the use of various surfactants. The gold seeds used in the synthesis of nanoribbons have been tested via UV-Vis. Later, properties of synthesized nanoribbons have been tested with Atomic Force Microscopy and Transmission Electron Microscopy. Relying on those spectroscopy and microscopy techniques we were able characterize created nanoparticles.



| Factor | Peak [nm] |
|---|---|
| 0,625 ml NaSCit | 516 |
| 1,25 ml NaSCit | 513 |
| Time 15 min | 520 |
| Time 45 min | 520 |
| Temperature 30°C | 523 |
| Temperature 45°C | 530 |

Figure 1 (left). Impact of the temperature, time and concentration of Na3Cit for the growth of nanoribbons.

Figure 2 (right). Peak value for each factor.

## Biography

Joanna Patalas is a student of the 4th year of Medical Physics, Department of Macromolecular Physics, Faculty of Physics, University of Adam Mickiewicz. She and 3 other students under coordination of prof Maciej Kozak has been working on a project that improves ways of obtaining specific shape of nanoparticles with the use of oligomeric and polymeric surfactants. Her passion is working for the improvements in new methods of helping people such as nanomedicine and gene therapy.

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# Effect of various shapes of gold nanoparticles on growth on cancer and normal cells

**Marika Musielak**
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Recently, particular attention has been paid to the advancements in nanomedicine and its various applications. Special focus is put on the use of nanoparticles (NPs) for cancer treatment. Wide range of morphological structures and the fact that NPs can be prepared from various kinds of metallic materials (e.g. gold or silver), can be a great advantage in nanopharmacy and theranostics. The aim of our study was characterization of the influence of gold nanoparticles, modified by selected surfactants on the cancer and normal cell lines. The cancer MDA-MB-231, MCF-7, PC-3, LNCaP and normal PNT1A cell lines were used to check the cellular response. MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was used as cytotoxicity test of nanoparticles and surfactants used in the synthesis of NPs. Cell cycle arrestation in a given phase can be very important in application for various cancer therapies. An impact of nanoparticles on the cell cycle using flow cytometry was analyzed.

Performed experiment gave information about cytotoxicity of gold nanoparticles. Toxicity of GNPs strongly depends on the amount and type of surfactants used in their synthesis. Both, the cell viability and proliferation decreased with increasing concentration. Gold nanoparticles also affected the cell cycle of chosen cell lines. An experiment confirmed the fact that gold nanoparticles can become a promising tool in the cancer treatment. However, it is still necessary to extend the range of nanoparticle research to animal tests and clinical trials. This research project was supported by the programme Best of the Best (Najlepsi z Najlepszych) 3.0 from Ministry of Science and Higher Education (Poland).

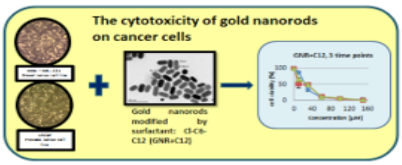


Figure 1: Scheme of the experiment carried out where gold nanorods were added to breast and prostate cancer cells. After a fixed incubation time, a large cytotoxicity effect was observed.

## Biography

Marika Musielak is a student of Medical Physics at the Adam Mickiewicz University in Poznan, faculty of physics, department of macromolecular physics. She takes part in the project under coordination of prof Maciej Kozak, that works on creation of specified shapes of nanoparticles, that can be used for cancer treatment and nanobiosensors. Since her interests lays in cell biology science, she leads the work on the cell culture to determine the effects of nanoparticles on the cancer and normal cell lines. She is keen on innovative methods of oncological treatment.

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# Engineering of new proteinoids and proteinoid nanoparticles of narrow size distribution for anti-fog

**Elisheva Sasson**
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The "fog phenomenon" describes the formation of tiny droplets of water on different surfaces. In day-to-day life, fog affects the light transmission and damages the visibility of different surfaces, such as plastic packaging, lenses, mirrors and windshields. In this study, a new thin coating onto polypropylene films, made of proteionoids and proteinoid nanoparticles for fog prevention, is presented. The proteinoids and proteinoid nanoparticles were synthesized by thermal step-growth polymerization of amino acids and therefore are non-toxic, biodegradable and biocompatible. The anti-fogging ability of proteinoids and proteinoid nanoparticles was discussed in terms of wettability, surface chemistry and morphology, that were measured by contact angle and atomic force microscopy. The efficiency of the anti-fog coatings was also tested by hot and cold fog tests to examine the optical properties of the films under fog formation conditions. The obtained results revealed that the proteinoids and proteinoid nanoparticle coatings perform as a wetting enhancer, mainly due to the low water contact angle (7-40°), that can be attributed to the hydrophilic residues of the proteinoid. Furthermore, proteionoids and proteinoid nanoparticles improved the film roughness by smoothing the surface of films (0.7-1.5 nm). In fog tests, uncoated PP film display many small waters drops on the surface that damaged the transparency of the film. In contrast, PP films coated with proteinoids or proteinoid nanoparticles formed a clear continuous thin layer of water on the surface. Additionally, the coating did not affect the clarity and haze of the films. Therefore, the coated films may be utilized in many applications, such as food packaging, agriculture and esthetic nylon wraps.

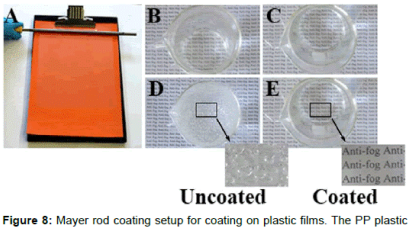


**Figure 8:** Mayer rod coating setup for coating on plastic films. The PP plastic film is put on a flat surface and the Mayer rod is pulled over the mixture of proteinoid or proteinoid NPs with A131-X in water, which leaves a uniform layer with thicknesses of 6 µm (A), images of uncoated PP film (B) and P(EI)-coated PP film (C) at time zero, uncoated PP film (D) and P(EI)-coated PP film (E) after 3 h in 60°C.

## Biography

Elisheva Sasson is PhD Candidate from The Institute of Nanotechnology and Advanced Materials, Department of Chemistry, Bar-Ilan University. Her research deals with the design of coatings on plastic films by proteinoisd and proteinoid nanoparticles for anti-fog applications. This work is carried out under the supervision of Prof. Shlomo Margel.

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# Graft polymerization of styryl bisphosphonate monomer onto polypropylene films for inhibition of biofilm formation

**Hanna P Steinmetz**
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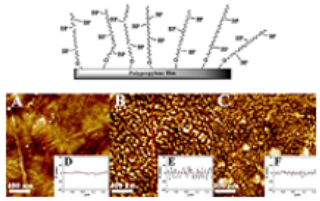
There has been increased concern during the past few decades over the role bacterial biofilms play in causing a variety of health problems, especially since they exhibit a high degree of resistance to antibiotics and are able to survive in hostile environments. Biofilms consist of bacterial aggregates enveloped by a self-produced matrix attached to the surface. Ca2+ ions promote the formation of biofilms, and enhance their stability, viscosity, and strength. Bisphosphonates exhibit a high affinity for Ca2+ ions and may inhibit the formation of biofilms by acting as sequestering agents for Ca2+ ions. Although the antibacterial activity of bisphosphonates is well known, research into their anti-biofilm behavior is still in its early stages. In this study, we describe the synthesis of a new thin coating composed of poly (styryl bisphosphonate) grafted onto oxidized polypropylene films for anti-biofilm applications. This grafting process was performed by graft polymerization of styryl bisphosphonate vinylic monomer onto O2 plasma-treated polypropylene films. The surface modification of the polypropylene films was confirmed using surface measurements, including X-ray photoelectron spectroscopy, atomic force microscopy, and water contact angle goniometry. Significant inhibition of biofilm formation was achieved for both Gram-negative and Gram-positive bacteria.




Poly(styryl bisphosphonate) (BP) grafted onto oxidized polypropylene film for anti-biofilm applications. AFM images (A, B, C) and the cross-section profiles (D, E, F) of the PP, oxidized PP, and PP/PStBP films, respectively.

## Biography

Hanna P Steinmetz is PhD Candidate from The Institute of Nanotechnology and Advanced Materials, Department of Chemistry, Bar-Ilan University. Her research focuses on the design of bisphosphonate coating and polymeric bisphosphonate nanoparticles grafted onto polymeric films for biomedical applications. This work is carried out under the supervision of Prof. Shlomo Margel.

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# Development of galactosamine decorated andrographolide loaded nanocochleates for liver cancer targeting

**Bothiraja Chellampillai**
Poona College of Pharmacy, India

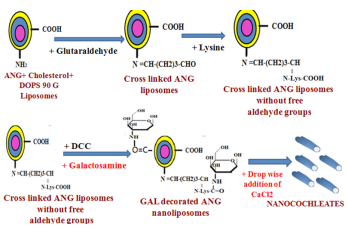
**Statement of the Problem:** Andrographolide (ANG), an anticancer chemotherapeutic phytoconstituent has been used in the treatment of various tumours and produced 39% inhibition cancer cell due to lack of specific affinity for site of action or to its limited biopharmaceutical properties. This juncture demands an effective, controlled release and safe formulation of AND would be a significant advance for the treatment of cancer. Nanocochleates are unique lipid-based supramolecular assemblies composed of a negatively charged phospholipid and a divalent cation. Aim of the study was to develop galactosamine (GA) decorated andrographolide (ANG) loaded Nano-Cochleates (NC) for liver targeting.

**Methodology:** GA was attached to ANG-loaded 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS) nanoliposomes (GA-ANG-NL) by aldehyde chemistry. GA-ANG-NL was converted into nanocochleates (GA-ANG-NC) by addition of Ca2+ ions and evaluated in terms of in-vitro and in-vivo and compared with ANG and ANG-NC.

**Findings:** ANG-NC and GA-ANG-NC showed particle size of 149 and 835 nm and zeta potential of -0.308 and -2.08 mV, respectively. ANG-NC and GA-ANG-NC showed higher release in pH 5.3 as compared to pH 7.4. GA-ANG-NC demonstrated higher in-vitro anticancer activity in Human hepatoma cell line Hep-G2. The targeting effect for the GA-ANG-NC was also demonstrated in which fourfold improved GI50 as compared ANG. Moreover, bioavailability of AGN from GA-ANG-NC increased by 3-fold with long circulation time and slower plasma elimination. Furthermore, GA-ANG-NC showed 2.1-fold increases in liver drug concentration ANG.

**Conclusion & Significance:** The proposed strategy is advantageous in terms of targeted drug delivery and has high potential to address the current challenges in drug delivery. Thus, the prepared nanocarrier offers a novel formulation that combines the unique properties of a biodegradable material, galactosamine and nanocochleates for biomedical applications.

**Acknowledgement:** The authors sincerely acknowledge Lipoid GmbH, Ludwigshafen, Germany for providing gift sample of1,2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS).




## Biography

Bothiraja has expertise in the field of novel and targeted drug delivery systems. His rigorous research work has been dedicated in various research projects like nanoparticulate systems drug delivery, tumor targeting, solid dispersion and crystal engineering. He has 50 research papers published in various international and national journals depict quality, innovativeness and expertise achieved by him in mentioned research fields. He would also like to use his enthusiasm for science to involve students and help them to become successful and contributing members of the scientific community.

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# Zooplankton-bacterial interactions: A hindrance to aquatic health

**Sujoy Midya**
Raja N L Khan Women's College, India

The present study has tried to claim the first approach towards zooplankton – bacterial interactions among different wetlands. The bacterial isolates which were obtained from copepods from the freshwater study sites showed higher Colony Forming Unit (CFU). The abundance of bacteria at freshwater study site in Panskura (FW-I) was 7.51 CFU/ml. In context of brackish water study site at New Digha (BW-II), Colony Forming Units (CFU) as well as isolates tended to diminish in number that is 3.56 CFU/ml. Such change in bacterial population with respect to different water bodies are supposed to have been determined by varying salt gradient, nutrient availability and some other physicochemical parameters of water. This study tries to unearth information on ecology and trophic relationships focusing on zooplankton-microbe's interactions with biotechnological approach like Vitek 2 characterization, FESEM visualization. A vast array of antibiotics based on their efficacies towards cell wall breakdown, DNA synthesis inhibition, and protein synthesis inhibition were considered and differential susceptibilities against wide spectra of antibiotics were observed. Among the total of fourteen antibiotics, Oxacillin and Tazobactam (penicillin group) showed resistivity to all the gram-negative isolates. Among cephalosporins group, especially Cefixime, Ceftizoxime and Cefepime exhibited resistivity for Enterobacter cloacae complex, and Aeromonashydrophila. Several other bacterial isolates of Pseudomonas sp. displayed resistivity to all the cephalosporins and sulfamethoxazole with a high MIC value. Among Gram positive isolates Staphylococcus epidermidis and Staphylococcus auricularis exhibited resistance at low MIC value to penicillin group antibiotics (Benzylpenicillin and Oxacillin) in contrast to eight other groups of antibiotics, where Sulfamethoxazole, a miscellaneous antibiotic group showed resistance at a high MIC value. Other groups of antibiotics (Aminoglycosides, Fluoroquinolone, Macrolide, Lipopeptide, and Glycopeptide) have shown their sensitivities against these bacteria. So, the present study tries to unravel the mystery of the root causes of different human diseases (gastrointestinal, typhoid etc.) and also justify zooplankton-bacteria hitchhiking.

## Biography

Midya S has an expertise over microbial ecology, antibiotic analysis, zooplankton population dynamics which enabled him to publish a good number of articles in some reputed international journals. Recently, he has joined as an Assistant Professor in a prestigious college Raja N.L. Khan Women's College of West Bengal, India.

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# Insect mediated dry fish spoilage: An insight to bacterial hitchhiking

**Abdullah Al-Helal**
Vidyasagar University, India

Insect, the largest group in the animal kingdom successfully dwells among different ecological niche and habitat of the world. However, very least information's are available on the roles of insects in developing indigenous and innovative biotechnological tools for the sustainable ecosystem management and biodiversity conservation. In such context the present research studies have elucidated the functional harmful roles in causing spoilage of a diversity of fin and shell fishes having the potential for developing dried fish related industry. A yearlong ecological survey throughout the length and breadth of Sundarbans Mangroves ecosystem have generated some interesting research information pertaining to diversity, mode of seasonal occurrence respectively, with drying fish potential in some selected eco-zones of Mangroves estuaries complex Sundarbans, India. Ten different species of insects belonging to order Coleoptera, Diptera, Hymenoptera, Isoptera, Acarina and Diplura were collected from different research sites. In order to recognize the root cause of spoilage, several anatomical appendages of insects like antennae, legs, abdomen etc. were used to culture any attached microorganisms. Four different species of Bacillus sp. were isolated and identified using 16srRNA sequencing. These species were also subjected to Vitek2 analysis to understand their biochemical properties which help to design a bio-pesticide. Somnolently, attempt had been made to identify three different plant species with bioactive substance which appear to be a cause of combating or relating the spoilage of fish protein by its anti-bacterial activities.

## Biography

Al-Helal M. A. has completed his Master of Science in Zoology from National University, Dhaka, Bangladesh. He had received prestigious international fellowship named Bangabandhu Science and Technology Fellowship (Ministry of Science and Technology, Government of the People's Republic of Bangladesh) an International Fellowship for pursuing Ph. D. at Vidyasagar University, Department of Zoology, West Midnapore-721102, West Bengal, India. He has developed an expertise in field of insect biology and microbial ecology which enabled him to publish a good number of research articles in esteemed journals.

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## Notes:

# Delivery method of the silencers RNA by PBCA nanoparticles for the treatment of multiform glioblastoma

**Cyro von Zuben V Negrão**
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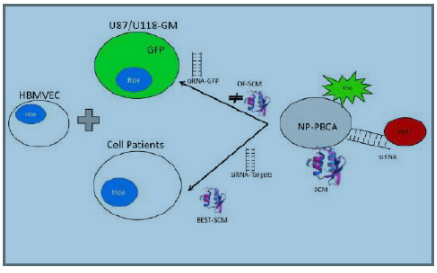
Glioblastoma multiform (GBM) has been reported as the most severe and the deadliest brain cancer, with a prognosis of only 14 months. Treatment is executed by the extraction of the tumor, followed by a radiation therapy and chemotherapy. Nowadays, brain therapy has found great obstacles to become effective due to factors such as the drug resistance and the difficulty to cross the blood-brain barrier. One of the great promises for the diagnosis, treatment and precaution of several diseases has been the use of nanoparticles (NPs). Among the different types of NPs, the PBCA nanoparticles have been gaining space in medicine because of its unique properties and its multiples employability. PBCA nanoparticles also have the ability to cross the blood-brain barrier (BBB). Another great promise in the treatment of diseases is the use of interfering RNA technique by presenting a specific and unique gene silencing capacity through molecules denominated in this project a silencers RNA. Several researches have been conducted focusing on several categories of cancers, which have been employed different NPs, as well as the use of diverse silencers RNA. The aim of this project is to create a delivery method of RNA silencers by nanoparticles for the treatment of GBM. So, to achieve it, different mechanisms will be used in the topography of PBCA-NPs to make them specific tumor tissue, besides using the silencers RNA focused on genes that brings resistance to most important drug and also genes responsible for the maintaining of tumor cells in different lines cell: U87/U118-GM and patients cells. Therefore, the main purpose of this project is to create an alternative method of treating one of the deadliest cancers from two scientific pillars of major biotechnological fields of modern medicine: nanoparticles and interfering RNA.




## Biography

Cyro von Zuben, PhD student has his expertise in interfering RNAs (RNAi). He worked with the use of RNAi tool for the control of pests in the agriculture, by which he awarded as the first place in Congress Open for Biology Students (2013) and he was selected for the oral presentation in 10th Latin Congress of Entomology (2018th). Actually, he has a team in the best University of Latin America with the professor Henrique Marques-Souza to found news genetics molecules for the most important Brazilian pests. This technology was also awarded as the best technology by the companies that were present in Open Innovation Week (2018). Last year, Cyro was approved on the program “New Talents” of one of the most prestigious and famous Institute of Brazil: Institute for Technological Research. By now, he is developing the nanoparticles with the RNAi technology for the treatment of diseases as cancer.

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# Accepted Abstracts

# Personalized cancer-specific protein corona affects the therapeutic impact of nanoparticles

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Despite recent progress in nanomedicine, there is still a lack of efficient nanotherapeutics for cancer treatment.

When nanoparticles (NPs) are injected, they circulate the bloodstream and interact with biomolecules, creating a biomolecular shell. This shell of biomolecules surrounding the NPs consists mostly of proteins and is referred to as the Protein Corona (PC). The PC composition depends on various factors, including, but not limited to i) surface properties of NPs (e.g. surface chemistry and charge), and ii) experimental parameters (e.g. incubation time, pH, temperature). The impact of these factors on the formation of the PC has been widely investigated and is nowadays well known. However, the effects of the biological milieu (e.g. patient's health status, plasma vs serum) on the PC formation and composition have been underexamined and still need to be deeply clarified.

Recently, it has been demonstrated that the protein pattern constituting the PC of NPs exposed to plasma of cancer patients is different than that of the PC formed by exposure of NPs to healthy plasma. These variations can be miniscule but crucial in the fate of NPs. In fact, NPs surrounded by a PC lose their synthetic identity and acquire a new biological identity responsible for their biological destiny (cell targeting, accumulation, immune response).

In this work, we have studied the PCs formed around NPs using plasmas of patients with one of eight cancers to gain insights into the cancer-related variations of the PC composition and into their potential effects on the therapeutic efficacy of NPs. Our results confirmed that the same NPs incubated with plasmas of patients affected by different tumors have distinct PCs. Overall, this is the first wide study unveiling the specific PCs formed around NPs using plasmas of eight cancers. This comprehensive report acts as a tool for researchers in nanomedicine to design personalized nanotherapeutics in a cancer-specific manner for clinical applications.

**Methods:** Silica NPs were incubated with plasma of healthy subjects and cancer patients. NPs in the study were 100 nm in size and the PCs in each subject's plasma were characterized and compared using SDS-PAGE and LC-MS/MS. Plasmas of patients with one of 8 cancers have been employed: breast, rectum, lung, kidney, thyroid, uterine, bladder, ovary.

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# Constitutive and induced resistance to pathogens in conifers: An integrated view of molecular, anatomical and chemical responses

**Adriana Arango-Velez**
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Conifers defenses against herbivores have been challenged by climate change, allowing the expansion in range and frequency of bark beetles and their associated pathogens, into previously unexplored areas. Two of the most aggressive bark beetles (the mountain – MPB- and southern pine beetle -SPB-) are attacking naive hosts in forested areas of Canada and the U.S, where prior colonization by these pests were unforeseen. As these beetles use pathogenic fungi to modify host tissues to favor brood development and overcome tree defenses, fungal associations are crucial for beetle success. To evaluate constitutive (pre-attack) and induced (post-attack) responses of conifer trees, two independent studies were conducted using the MPB and SPB associated pathogenic fungi Grosmania clavigera and Ophiosoma minus at 1-, 7- and 28- and 62-days post inoculation respectively. To understand the complex interactions that modulate tree defense responses, we performed (i) microarray and targeted gene expression analyses (chitinases and terpene synthases), under well-watered and water deficit conditions (ii) UHPLC and GC-MS chemical profiling of phenols and terpenoids, (iii) phyohormones and iv) histochemical analyses, in coevolved and naive pine trees. Results show that upon pathogen attack, jasmonic and salicylic acid were implicated in local and systemic response to fungal inoculation; in addition, an increased transcript expression of chitinases, pathogenesis-related genes, as well as genes associated with jasmonate and ethylene signaling were observed. Differences in expression patterns due to fungal inoculation were observed between naïve and coevolved species (Fig. 1). Chemical analyses showed induction of epi/catechin, three unknown phenolic compounds and several phloem terpenoids (α-pinene, β-myrcene, limonene, terpinolene and α-pinene) indicating an elevated tree response against pathogen attack. Lastly, histochemical analyses demonstrated the capacity of naïve pine trees to induce traumatic resin ducts production and lesion development to confine fungal development. Taken together these responses, naïve and coevolved conifers respond differently to fungal attack.

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## Notes:

# Development of a novel multifunctional bioglass-based coating for the next generation of prostheses

**Angela Bejarano-Villafuerte**
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Promethean Particles is a UK-based SME that designs and develops inorganic nanomaterials in liquid dispersions. The company's technology is based on a patented reactor design that allows truly continuous hydrothermal (or solvothermal) synthesis of inorganic nanoparticles [1]. Promethean use small scale reactor systems for rapid prototyping to tune the optimum product for each application and backs this up with pilot-scale production facilities, as well as a multi-ton scale nanoparticle manufacturing plant (capacity more than 1000 tons per year), see figure 1.a. Our unique production process allows us to tailor the nanoparticles to get the best functionality for an end use application. Innovation is a large focus of our business, as a direct outcome of our R&D activities. We are currently active partners in three Innovate UKfunded projects and four EU-funded Horizon 2020 projects. One of our Innovate UK projects aims to develop a novel multifunctional bioglassbased coating for the next generation of prostheses. The cost of implant failure can be massive, both financially for medical services (€ 800 m/year in Europe alone in 2010 for dental implants) and personally to the patient where amputations can be life altering. Bioglass-based coatings can help bone integration of the implant and reduce these costly infections from occurring.

Due to the versatility of our lab-scale technology, we are able to tailor bioglass-based materials using different components and with dopants such as ZnO and Cu. We have achieved excellent control over the ratio of components, the particle size within an amorphous matrix (see figure 1.b-c) and the solid phase. Further tests have been performed by our partners Johnson Matthey (JM) and Queen's Mary University London (QMUL). Our bioglass-based materials are used by JM to coat substrates which are then tested by QMUL to investigate their biocompatibility and antimicrobial properties. Within this project, our Bioglass-based materials are demonstrating promising antimicrobial activities.


**Figure 1. (A) Promethean's multi-ton scale nanoparticle manufacturing plant (capacity more than 1000 tons per year).(B) SEM image of a bioglass-based material.**

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# Design, synthesis and validation of nano-drug delivery systems in fluorescent cancer stem cell models

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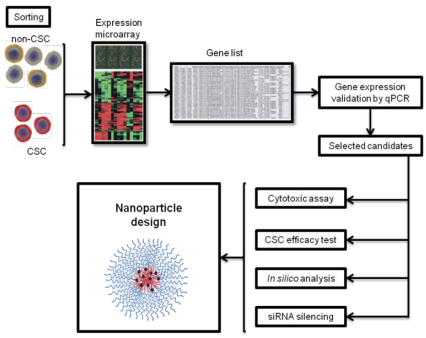
Metastatic spread to distal organs and progressive gain of chemo-resistance of advanced cancers are sustained by the presence of Cancer Stem Cells (CSCs) within the tumor. Therefore, many advanced therapies in development, aim to eliminate CSCs and thus prevent tumor growth and the appearance of metastases. Likewise, we developed polymeric nanoparticles loaded with ZileutonTM, a potent inhibitor of CSCs. ZileutonTM as anti-CSC drug was chosen based on specific target selection using various CSC fluorescent models, previously developed in our laboratory.

Drug delivery through nanoparticles has great potential to increase efficacy and reduce toxicity and adverse effects. In this context, ZileutonTM nanoparticles effectively target CSCs, block their ability to form mammospheres and to invade in vitro, and do not cause any toxicity in vivo, in healthy animals. Besides, our nanoparticles reduce number of CSCs within the tumor and effectively block the Circulating Tumor Cells (CTCs) in the blood stream.

However, therapies seeking for elimination of CSCs in order to prevent tumor growth and the appearance of metastases have an important limitation, because the eliminated CSCs are constantly replaced by new cells with "stem" phenotype, thanks to their considerable plasticity. Indeed, eliminated CSCs are being constantly replaced by a process of de-differentiation (reversion) of "bulk" tumor cells in order to ensure the propagation of the tumor after treatment. As a result, CSCs specific treatments have just limited clinical success. We have thus successfully evaluated, the combination of polymeric nanoparticles with ZileutonTM and the AbraxaneTM (Nab-PTX) nanoparticles used in clinic, that eliminate "bulk" tumor. This cocktail represents an ideal option to abrogate tumor and metastatic growth, avoiding CSC reversion (dynamic phenotype).

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# Artificial RNA editing in mutated BFP (derivative of GFP) by using AID deaminase for restoration of genetic code

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Editing of mutated gene can be a possible means of treatment for genetic diseases caused by mutations in the normal codons. Here, I have tried to engineer deaminase domain of AID (Activated Induced Deaminase, a family member of APOBEC) and MS2 system to target specific Cytosine (C) to restore Thymine (T) that has been caused by the T to C mutations. For this catalytic domain of AID deaminase has been fused with RNA binding protein MS2, which binds to MS2-RNA. Guide RNA was designed complementary to target RNAs. Thus AID deaminase domain was carried out to desired editing site to convert C to U. As a target, Blue Fluorescence Protein (BFP) gene was prepared by mutating at 199 nucleotide of GFP. MS2 system has the ability to Convert Mutated Codon (CCA) to normal codon (CTA) in cellular system (e.g., HEK 293). The system converted CCA to CTA (conversion of cytidine to uridine transforms the BFP gene into the GFP gene) and turned on green fluorescence. cDNA was synthesized from positive cells followed by RNA extraction and PCR-RFLP was done by using BtgI restriction enzyme. The unedited BFP gave the fluorescence in the microscopic observation, remained uncut and edited were cut, also provide the green fluorescence expression by microscopic observation. Final confirmation was done by the Sanger's sequencing analysis where the restored one also gave the peak as the wild type "CTACGG" which was "CCACGG" in case of the mutated one (BFP).

Successful artificial editing of RNA in vivo by MS2 system can pioneer genetic code restoration therapy including stop-codon read through therapy for various genetic diseases.

Key words: RNA editing. AID, BFP, Guide RNA, Genetic code.

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## Notes:

# Magnetic iron oxide alginate beads for removal of dye from aqueous solutions

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In the present study, a novel green and highly efficient magnetic adsorbent alginate beads were synthesized by instantaneous gelation of magnetic iron oxide nanoparticles (IoNPs) and sodium alginate (SAlg) mixture in calcium chloride solution. Magnetic iron oxide nanoparticles were prepared by co-precipitation method by using Gallnut extract as capping and stabilizing agent. The synthesized IoNPs were characterized by transmission electron microscopy and X-ray photoelectron spectroscopy. Highly pours magnetic IoNPs-SAlg beads were fully characterized with scanning electron microscopy, transmission electron microscopy, Fourier transform infrared spectroscopy, and X-ray diffraction. Elemental content of the IoNPs and IoNPs-SAlg beads was analyzed using energy dispersive spectroscopy. The magnetization properties were investigated by vibrating sample magnetometer technique. The synthesized IoNPs-SAlg beads were used as eco-friendly adsorbent for the adsorption of Congo red, Nile blue A, and Rose Bengal from water. Moreover, the maximum capacity of adsorption was compared with that of non-encapsulated IoNPs and SAlg beads. The results showed that addition of IoNPs in sodium alginate enhanced the adsorption capacity. The influence of important factors such as pH, temperature, adsorbent dosage, and initial dye concentration on adsorption capacity of magnetic IoNPs-SAlg beads was investigated. The maximum adsorption was found to be 92, 91, and 78 % for Congo red, Nile blue, and Rose Bengal, respectively. These results reveal that IoNPs-SAlg beads can be an eco-friendly, efficient, and low-cost adsorbent for the removal of dyes from water.

Keywords: Iron oxide nanoparticles, magnetic, alginate, beads, adsorption, dye.

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## Notes:

# Establishment of TaqMan probe qRT-PCR for detecting bovine viral diarrhea virus and clinical applications

**Wei Suocheng**
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**Objective:** The present study aimed to establish a novel TaqMan quantitative real-time PCR (qRT-PCR) for detecting and typing Bovine Viral Diarrhea Virus (BVDV), also to develop a diagnostic protocol which simplifies sample collection and processing.

**Method:** Universal primers and TaqMan-MGB probes were designed from the known sequences of conserved 5' - and 3'-untranslated regions (5'UTR, 3'UTR) of the NADL strain of BVDV. Prior to optimizing the assay, cDNAs were transcribed in vitro to make standard curves. The sensitivity, specificity and stability (reproducibility) were evaluated, respectively. The qRT-PCR was tested on the feces specimens collected from persistently infected (PI) calves.

**Results:** The optimum conditions for qRT-PCR were 17.0 μmol/L primer, 7.5 μmol/L probe and 51.4 annealing temperature. The established qRT-PCR assay could only specially detect BVDV without detecting any other viruses; its detection limit was 1.55×100 copies/μL for viral RNA. It was 100000-fold higher than conventional PCR with excellent specificity and reproducibility. 312 samples of feces were tested using this method and universal PCR from six dairy farms, respectively. Positive detections were found in 49 and 44 feces samples for both assays. The occurrence rate was 89.80%.

**Conclusion:** The established qRT-PCR could rapidly detect BVDV and effectively identify PI cattle. The detection limit of TaqMan qRT-PCR was 1.55 copies/μL. It will be beneficial for enhancing diagnosis and therapy efficacy and reduce losses of cattle farms.

**Keywords:** Bovine viral diarrhea virus; Quantitative real time PCR; TaqMan probe.

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## Notes: