NANOBIO&MED 2011

INDEX: INVITED CONTRIBUTIONS

INDEX: INVITED CONTRIBUTIONS (SUDOE TRAIN2 SESSION)

INDEX: INVITED CONTRIBUTIONS (COST BIO-INSPIRED SESSION)

INDEX: INVITED CONTRIBUTIONS (CIBER BBN SESSION)

INDEX: KEYNOTE CONTRIBUTIONS

INDEX: KEYNOTE CONTRIBUTIONS (CIC BIOMAGUNE SESSION)

INDEX: KEYNOTE CONTRIBUTIONS (CIBER BBN SESSION)

INDEX: ORAL CONTRIBUTIONS

INDEX: ORAL CONTRIBUTIONS (CIBER BBN SESSION)

INDEX: ORAL CONTRIBUTIONS (SUDOE TRAIN2 SESSION)

INDEX: ORAL CONTRIBUTIONS (COST BIO-INSPIRED SESSION)

ALPHABETICAL ORDER

I: Invited / IS: Invited SUDOE TRAIN2 Session / IC: Invited COST Bio-Inspired Session / IB: Invited CIBER BBN Session / K: Keynote / KG: Keynote CIC biomaGUNE Session / KB: Keynote CIBER BBN Session / O: Oral / OB: Oral CIBER BBN Session / OS: Oral SUDOE TRAIN2 Session / OC: Oral COST Bio-Inspired Session

I: Invited / IS: Invited SUDOE TRAIN2 Session / IC: Invited COST Bio-Inspired Session / IB: Invited CIBER BBN Session / K: Keynote / KG: Keynote CIC biomaGUNE Session / KB: Keynote CIBER BBN Session / O: Oral / OB: Oral CIBER BBN Session / OS: Oral SUDOE TRAIN2 Session / OC: Oral COST Bio-Inspired Session

I: Invited / IS: Invited SUDOE TRAIN2 Session / IC: Invited COST Bio-Inspired Session / IB: Invited CIBER BBN Session / K: Keynote / KG: Keynote CIC biomaGUNE Session / KB: Keynote CIBER BBN Session / O: Oral / OB: Oral CIBER BBN Session / OS: Oral SUDOE TRAIN2 Session / OC: Oral COST Bio-Inspired Session

NANOBIO&MED 2011

ABSTRACTS
ALPHABETICAL ORDER

TO REACH TRANSLATIONAL OBJECTIVES: THE BIOENGINEERING CHALLENGE WITHIN CIBER-BBN

Jordi Aguiló*, Rosa Villa****

*Departament de Microelectrónica i Sistemas Electrónics, Universitat Autònoma de Barcelona
**Instituto de Microelectrónica de Barcelona, Campus UAB, Bellaterra, Spain
Jordi.Aguilo@uab.es

For years, bioengineers have been trying to bring together knowledge and technologies from the engineering world and knowledge and life sciences from the biomedical world with a clear objective: to contribute to the advancement of health through the adaptation and development of technologies

- For easy, accurate and non invasive diagnoses
- To enhance predictive and preventive know-how and capacities
- To develop high technology based devices for local or remote on-line monitoring of relevant vital parameters by having multimodal functionalities suitable for clinical use
- To develop new instrumentation and models allowing scientific progresses and a more in depth knowledge of living mechanisms. Micro-Nano-Bio systems can help to know and interact with high level behavior such as learning mechanisms or psychological diseases up to, on the micro-nano side, to unlock specific aspects of cellular metabolism, membrane structures or gene behaviors.
- To propose and validate new therapies coming from the new knowledge and new available technologies.
- To propose and validate new devices and systems allowing functional recovery and improvement
- To develop technologies and devices to help developing and emerging fields such as biotechnology, cellular and molecular engineering, etc.

Following these main lines, the Biomedical Applications Group, GAB, today within the CIBER-BBN is trying to take advantage of its own technological facilities and its scientific and technical know-how, to transform the innovation possibilities of micro-devices and related technologies into successful commercial biomedical products and advanced applications.

In the recent past the most relevant work of GAB has been the development of microsystem devices to monitor relevant heart parameters during open heart surgery, to measure graft viability on transplants, to contribute to functional recovery of blind people and to set-up the sensoring modules on a telemedicine application.

Concerning technology, a service of micro and nano fabrication including a carbon nanotube growth service has been set-up.

Concerning applications, today and in the near future we are working on the development of microsystem devices and instrumentation to measure the osteointegration level in implants, the degree of liver steatosis, the corneal endothelial permeability through a non-invasive way, to detect and to remove biofilms and the detection of p53-antibodies for early cancer prognostic. We are also taking the first steps in some other domains such as instrumentation to measure osteoporosis, to give objetive measurement of some psychic and somatic diseases and on the development of micro-nano systems for multifunctional stents and catheters.

HOW USE MICRO STRUCTURED SCAFFOLDS AND NANO TAGS TO CONTROL CELL DIFFERENTIATION AND IMPROVE TISSUE REGENERATION

Jose Iñaki Alava, Nerea Garagorri, Patricia Gracía Parra

Tecnalia Research and Innovation Co. Ciber BBN, ITUS-INASMET group, Paseo Mikeletegui 2, San Sebastián –Donostia, 20009, Spain
inaki.alava@tecnalia.com

Furthermore, the extracellular matrix plays a key role in controlling the differentiation of progenitor cells into the cell lines that will be replaced. Depending on the signals that these cells receive from their environment, their capacity for differentiation is enhanced or limited. In last 15 years, many researchers have tried to control neural growth and differentiation by using a wide range of approaches - micropatterned scaffolds [1], conductive biomaterials [2], electrospining [3] and many others [4,7,8,9] The appearance in 2002-2003 of new micro-technologies such as microcontact printing and others in the field of neurobiology, (we will comment on them in the next paragraph) have led to competition aimed at obtaining optimum micro (or nano) patterning of guidance molecules for neural cell growth and differentiation [4].

With the appearance of nanotechnology on the scene, the biocompatibility and special properties of some nanoparticles have led to the use of single wall carbon nanotubes in cell cultures [5] as a way of improving neural signal transfer [6]. In 2006, multiple-channel biodegradable scaffolds were used to promote spinal cord regeneration [7] with Schwann-cells as feeder-cells

The ability to replicate patterns at the micro to nanoscale is crucially important for the progress of micro and nanotechnologies and the study of nanosciences. The planar manufacturing technology used by the semiconductor industry means that integrated circuits are built by stacking one layer of circuit elements on top of another. Each layer is manufactured according to a sequence of well-characterized processes. Lithography is used over and over again to create desired patterns in all these processes. Considerable industrial effort has been devoted to the leading-edge optical methods and the so-called next generation lithography (NGL) techniques, exposing the material to energy beams from UV, electron beam, ion-beam or x-ray sources. However, there are many other non-traditional microelectronic applications that require nanoscale features and demand low-cost nano and micro patterning technologies. One of the main areas is the biological field, with applications in micro and nanofluidic devices for labs-on-a-chip, biosensors, DNA or protein arrays and the alignment of biomaterials for cell patterning or tissue engineering related applications. Surfaces with micro and nanopatterns are being used as cell culture substrates to develop new assays for monitoring cell adhesion and cell proliferation or differentiation on different surfaces [10,11].

Patterned surfaces and their chemical landscape provide cues for cells to attach, migrate and assemble into functioning tissue. Tissue engineering is a multidisciplinary/interdisciplinary field that applies the principles of biology and engineering to develop tissue substitutes that restore, maintain, or improve the function of diseased or damaged human tissues. One approach to tissue engineering involves seeding biodegradable polymeric scaffolds with donor cells and/or growth factors and then culturing and implanting the scaffold to induce and direct the growth of new, healthy tissue. Unfortunately, current polymeric biodegradable scaffolds have several drawbacks: However, most biodegradable polymers being used in clinical practice do not exhibit clearly biocompatible behaviour and their mechanical properties are usually not enough to allow them to be used for load bearing parts of the body. No single material, however, can satisfy all the goals required for creating optimum scaffolding. On the other hand, the extra cellular matrix (ECM) is a complex and strange material. In fact, is impossible for a single material to mimic all ECM properties. A composite multi-material matrix is the natural solution to the different requirements of the wide range of ECMs needed for tissue regeneration [12]. Therefore, the incorporation of nanoparticles such as carbon nanotubes or carbon nanofibers and nanohidroxiapatite to the polymer matrix for increasing the safety of the polymer appears to be very important [13]. We show some examples of micro-structured scaffolds, with nano-tags (molecular signals), that may be used for cell differentiation and tissue repair.

References:

[1] Schmalenberg, K.E., Uhrich, K.E., Biomaterials 26, (2005), 1423-1430
[2] George, P.M., Lyckman A.W., LaVan, D.A., Hegde, A., Leung, Y., Avasare, R., Testa, C., Alexander, P.M., Langer, R., Sur, M., Biomaterials, 26, ,(2005), 3511-3519
[3] Yang, F., Murugan, R., Wang, S., Ramakrishna, S., Biomaterials, 26, (2005), 2603-2610.
[4] Oliva, A.A. Jr, James C.D., Kingman, C.E.,Craighead, H.G.,Banker, G.A., Neurochemical Research, Vol 28, (2003), 11,1639-1648.
[5] Gheih, M.K., Sinani, V.A., Wicksted, J.P., Matts, R.L., Kotov, N.A. Adv. Mater. 17, (2005), 2663-2670.
[6] Lovat, V. Pantarotto, D., Lagostena, L., Cacciari, B., Grandolfo, M., Righi, M., Spalluto, G., Prato, M., Ballerini, L. Nano Letters, Vol 5, 6, (2005), 1107-1110.
[7] Moore, M.J., Friedman J.A., Lewellyn, E.B., Mantila, S.M., Krych, A.J., Ameenuddin, S., Knight, A.M., Lu, L., Currier, B.L., Spinner, R.J., Marsh, R.W., Windebank, A.J., Yaszemski, M.J., Biomaterials, 27, (2006), 419-429.
[8] Recknor, J.B., Sakaguchi, D.S., Mallapragada, S.K., Biomaterials 27. (2006), 4098-4108.
[9] Tsai, E.C., Dalton, P.D., Shoinet, M.S., Tator C.H., Biomaterials.27. (2006), 519-533
[10] Falconnet D. et al., Biomaterials 27, (2006) 3044-3063.
[11] Zhang SG, et al, Biomaterials 20, (1999), 1213-20.
[12] Alava,J.I., Jurado, M.J., García, A., and Murua O. Joint Meeting of the Tissue Engineering Society International and the European Tissue Engineering Society, Congress Abstracts, Lausanne, Switzerland, (2004).
[13] Meike van der Zande, Balaji Sitharaman, X. Frank Walboomers, Lesa Tran, Jeyarama S. Ananta, Andor Veltien, Lon J. Wilson, Jose Inaki Alava, Arend Heerschap, Antonios G. Mikos, and John A. Jansen. Tissue Engineering: Part C Volume 17, Number 1, (2011), 19-26

THERAPEUTIC NANOCONJUGATES

Fernando Albericio, Miriam Royo

CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine Barcelona Science Park, 08028-Barcelona, Spain

Therapeutic Nanoconjugates and Drug Delibery Systems are one of the key strategic lines of the CIBER-BBN.

As it is well known, the number of new chemical and biological entities being accepted by the Food and Drug Administration is stabilized around 20-30 every year. This relatively low number is due to several factors, but one of the main reasons in the poor ADME properties showed by compounds that previously had been good in vitro activity

To improve this situation, several approaches have been used: (i) preparation of therapeutic conjugates that are able to protect the drug until it reaches the target; (ii) preparation of conjugates bearing nanovectors with the objetove of reaching more efficiently the target.

Our laboratories have developed a robust synthetic platform mainly based in peptides able to address the problem mentioned above. Herein, multifunctional polyethylenglycol-based dendrimers for drug delivery; γ-proline based foldamers as cell penetrating peptides; and gold nanoparticles; and multifunctionalized gold nanoparticles with peptides targeted to peptide receptor of a tumour cell line will be discussed.

References:

[1] Marcelo J Kogan, Ivonne Olmedo, Leticia Hosta, Ariel R. Guerrero, Luis J. Cruz, Fernando Albericio Peptides And Metallic Nanoparticles For Biomedical Applications. Nanomedecine, 2, 287-306 (2007).

[2] Leticia Hosta, Mateu Pla-Roca, Jordi Arbiol, Carmen López-Iglesias, Josep Samitier, Luis J. Cruz, Marcelo Kogan, Fernando Albericio. Conjugation of Kahalalide F with gold nanoparticles to enhance in vitro antitumoral activity. Bioconjugate Chem., 20, 138-146 (2009).

[3] Leticia Hosta-Rigau, Ivonne Olmedo, Jordi Arbiol, Luis J. Cruz, Marcelo J. Kogan, andFernando Albericio Multifunctionalized Gold Nanoparticles with Peptides Targeted to Gastrin-releasing Peptide Receptor of a Tumour Cell Line. Bioconjugate Chem, 21, 1070-1078 (2010).

[4] Simon Guerrero, Eyleen Araya, Jenny Fiedler, J. Ignacio Arias, Carolina Adura, Fernando Albericio, Ernest Giralt, Jose Luis Arias, M. Soledad Fernández, and Marcelo J. Kogan. Improving the Brain Delivery of Gold Nanoparticles by Conjugation with an Amphipathic Peptide. Nanomedicine, 5, 897-913 (2010).

TISSUE ENGINEERING FOR LUMBAR SPINAL FUSION IN SHEEP

J. Becerra[1,2], MD Cuenca-López[1,2], S. Gómez[3], P. Zamora-Navas[4,2], E. Guerado[5,2], JA Andrades[1,2]

[1]Dep of Cell Biology, Genetics and Physiology, Faculty of Sciences, Univ of Málaga, Málaga, Spain
[2]Networking Biomedical Research Center in Bioengineering, Biomaterials and Nanomedicine (Ciber-bbn)
[3]Department of Pathological Anatomy, Faculty of Medicine, University of Cádiz, Spain
[4]Service of Orthopaedic Surgery and Traumatology, V. Victoria University Hospital, Málaga, Spain
[5]Division of Orthopaedic Surgery and Traumatology, Hospital Costa del Sol. Marbella, Spain
becerra@uma.es

Posterior lumbar spinal fusion is nowadays a very standardized surgical technique worldwide. The intervention consists of two main steps, a firm fixation by a hardwork instrumentation to provide mechanical stability, and the addition of a biological substance for bone formation enhancement. Grafting enhances bone fusion, and therefore permanent stability, being considered bone autograft as the gold standard. The goals for bone-graft substitutes are to match fusion rates with autologous bone grafting techniques, while avoiding the morbidity of bone graft harvest and extending the quantity of available graft material. So far, there is a long list of bone graft substitutes [1], and most of them need a carrier to be administrated in the surgical field. Anyway, spinal fusion models are particularly unique, compared to other types of bone repair. Spinal fusion requires not to recreate original anatomy but the formation of a heterotopic bone bridge where usually bone does not exist. This may be the cause of the high clinical failure rate, above 35% [2]. Allograft as substitute/carrier is an important osteoinductive and osteoconductive agent but it has been especially claimed that it can provoke disease transmission and immunogenicity [3].

Since osteogenesis is exclusively conducted by bone cells, a very important strategy when dealing with bone substitutes, consists of addressing research projects u-sing osteogenic cells as bone marrow (BM) mesenchymal stem cells (MSCs). Two main lines have been maintained during the last years: molecular stimulation by a growth factor-mediation fashion (BMP-2 and BMP-7) [4,5], and transplantation of cells after amplification and committement [6]. BMPs have demonstrated good fusion rates but questions including high cost, high dose needed and some adverse effects, make them a non-definitive therapeutic tools [7]. Regarding cells, since several types of stem cells are susceptible to in vitro differentiate into multiple skeletal lineages, and are able to form bone, when using with the appropriate scaffold, tissue engineering looks like a good substitute for auto- and allograft in orthopedic surgery [8]. Nevertheless, clinical and animal experimental models research have had very important methodological burdens. On the one hand, most of laboratory work have been made in rodent and lagomorphs, species behaving far away, better than human as far as osteogenesis is concerned; further experimental model did not take into account mechanical situations as in human. Anyway, tissue engineering of bone, by combining osteogenic cells with osteoconductive scaffolds, has not yet yielded any clinically useful applications so far.

We have created an experimental model in a large animal model, the sheep, trying to reproduce what is made in human, a mechanical stabilization by a screwed transpedicular lumbar spinal instrumentation, together with the addition of BM committed MSCs adsorbed on hydroxyapatite (HA).

Surgical procedure. Spinal process from L2-L6, laminae, facet joints, and transverse processes were neatly denudated and prepared for arthrodesis. After that, stainless steel pedicular screws (Xia®, Stryker™) were introduced. Bone decortication was acted upon lateral aspect of articular and transverse processes until some bleeding bone could be seen.

Groups. A group of 20 sheep (female, 3-4 year-old, weighing 50-70 kg) received autograft into the right side (AUTO) and allograft on the left one (ALO). Another 20 sheep received HA with MSCs on the right side (HA+cls), and HA alone on the left one (HA) as control.

Cell product. Twenty milliliters of BM aspirates were harvested. The mononuclear cells fraction were plated at a density of 10x106 cells per 100 mm plate in complete culture medium (DMEM, 10% FBS, 1% Penicillin, 0,5% Amphotericin, 1.25% Glutamine, and 1ng/ml FGF-2) and incubated at 37ºC, 95% humidity, and 5% CO_2. The medium was changed three times a week and the cells at passage 1

were cultured for three weeks. At the end of the culture period, the cells were incubated for 3 days in culture medium supplemented with dexametasone and β-glycerophosphate to help with the osteogenic differentiation.

Results have shown that, although autograft obtained better values for fusion than allograft, there was no statistical significance difference between autograft and allograft. Further, autograft and allograft accomplished a better bone formation rate than committed stem cells. Hydroxyapatite alone produced the worst results both in regard to the amount of bone formed as the rate of spinal fusion. Since *in vitro and in vivo* studies with small animal models have shown that this construct displayed a very strong osteogenic commitment, we aim to improve the implant in sheep, perhaps by increasing its, the number of cells per volume unit, and even introducing certain improvements to the scaffold.

References:

[1] Pneumaticos SG, Triantafyllopoulos GK, Chatziioannou S, Basdra EK, Papavassiliou AG. Trends Mol Med. 2010 Dec 30. [Epub ahead of print]
[2] Reid JJ, Johnson JS, Wang JC. J Biomech. 11;44(2) (2011):213-20
[3] Mroz TE, Joyce MJ, Steinmetz MP, Lieberman IH, Wang JC. J Am Acad Orthop Surg. 16(10) (2008):559-65.
[4] Rihn JA, Makda J, Hong J, Patel R, Hilibrand AS, Anderson DG, Vaccaro AR, Albert TJ. Eur Spine J. 18(11) (2009):1629-36.
[5] Leach J, Bittar RG. J Clin Neurosci. 16(11) (2009):1417-20.
[6] Miyazaki M, Zuk PA, Zou J, Yoon SH, Wei F, Morishita Y, Sintuu C, Wang JC. Spine (Phila Pa 1976). 15;33(8) (2008):863-9.
[7] Hsu WK, Wang JC. Spine J 8 (2008): 419–425.
[8] Bianco P, Robey PG, Simmons PJ. Cell Stem Cell 2 (2008): 313–319.

Figures:

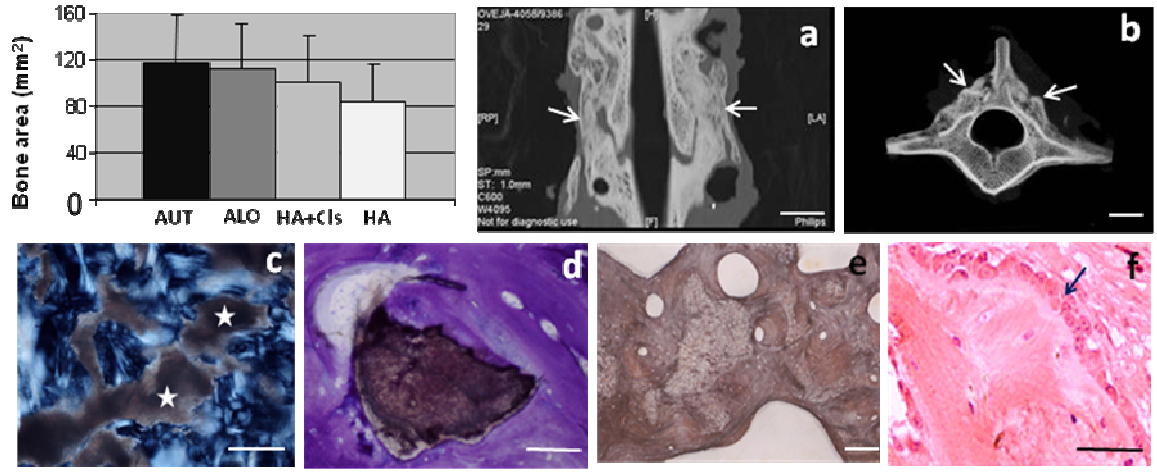


Figure 1: *Graph showing bone area formed in the different types of implants. a) Coronal view (TAC) of the artrodesed segments showing fusion bridges of bone tissue between vertebral bodies (arrows). Bar, 1cm. b) Axial view (TAC) where new bone in the area of the implant can be seen (arrows). Bar, 1cm. c) Tissue section under polarization microscopy showing HA (starts) surrounded by new bone. Bar, 250μm. d) HA fragment (brown color) in resorption surrounded by new bone type woven in an implant with MSCs (section stained with toluidine blue). Bar, 100μm. e) New bone in an implant with MSCs where woven bone types coexist with laminar and haversian types and resorption lacunae (Von Kossa stained). Bar, 200μm. f) Row of osteoblasts (arrow) in active synthesis of new bone (hematoxiline-eosine). Bar, 50μm*

REARRANGING PROTEIN ASSEMBLIES FROM 2D LAYERS TO 1D FIBRES BY ELECTROSPINNING

Alexander Michael Bittner[1,2], Wiwat Nuansing[1], Amaia Rebollo[1], Ulf Baus[3], Thomas Subkowski[4]

[1]CIC Nanogune, Tolosa Hiribidea 76, 20018 Donostia, Spain
[2]Ikerbasque, Alameda Urquijo, 36-5, Plaza Bizkaia, 48011 Bilbo, Spain
[3]BASF SE; Performance Chemicals Division, GVF/C - A030, 67056 Ludwigshafen, Germany
[4]BASF SE; Biotechnology Research, GVF/C - A030, 67056 Ludwigshafen, Germany
a.bittner@nanogune.eu

Natural proteins do usually not assemble to regular structures, which very much impedes their characterisation. However, some of them can be crystallised, i.e. they form regular 3D assemblies. Others assemble in 2D, e.g. membrane proteins, and in 1D, e.g. fibrous proteins and some viruses. Conformational changes inside a protein can change its mode of assembly. Some of the best-known examples are prion proteins, which can change to a conformation with increased beta sheet content, which can result in neuropathogenic fibres. Since Alzheimer disease is based on similar mechanisms, 1D assembly is and will remain a very hot topic in protein science [1,2,3].

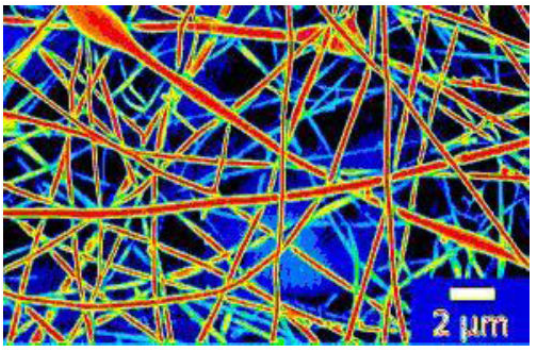


Figure 1: *SEM of electrospun pure hydrophobin. The quasi endless fibre has ca. 300 nm average diameter*

Various proteins, some related to Alzheimer and Parkinson disease, exhibit a surprising in vitro behaviour: Shear forces speed up their assembly to fibres [1,4,5]. We here show that a combination of solvent-induced conformational change and extreme shear induces such severe changes that the assembly mode switches from 2D to 1D. To this end, we dissolved a protein, hydrophobin, at high concentration in an organic solvent, and applied polymer-free (i.e. monomer) electrospinning [6].

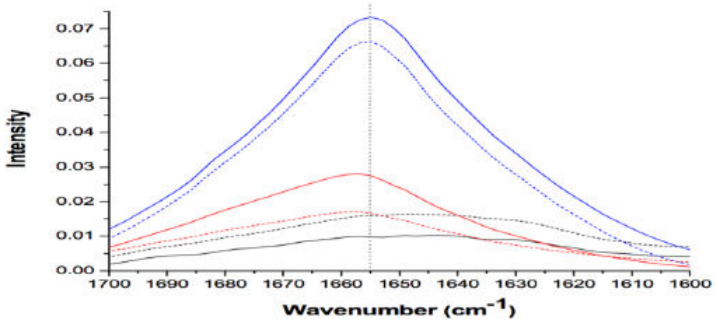


Figure 2: *IR spectra of the amide band region of HP samples. The bottom curves (maxima at 1645 cm^{-1}) correspond to the original state, the top curves with maxima at 1655 cm^{-1} point to an increase in alpha helices in organic solvent.*

Hydrophobins (HPs) exhibit 2D organisation. They are used in the context of "White Biotechnology" as emulsifiers and surface primers (www.hydrophobin.basf.com). HPs are naturally found on fungi, where they determine the very hydrophobic nature of the fungus cap. They can easily be produced in kg amounts by biotechnological fermentation techniques.

Electrospinning requires highly concentrated solutions of HPs in a solvent with high vapour pressure. We showed with electrophoresis (www.hydrophobin.basf.com) and Raman, IR, and circular dichroism spectroscopy that already the dissolution changes the conformation of the HPs, different from contact with water. However, HPs did not assemble in solution. Only during electrospinning the high shear force, together with the evaporation, induced 1D alignment, and we were able to spin extremely long microfibers that consist of pure (polymer-free) proteins. We characterised the fibres with SEM, including environmental SEM in water vapour.

We believe that electrospun fibres of pure proteins can have multiple applications in biotechnology, e.g. as highly biocompatible scaffold materials.

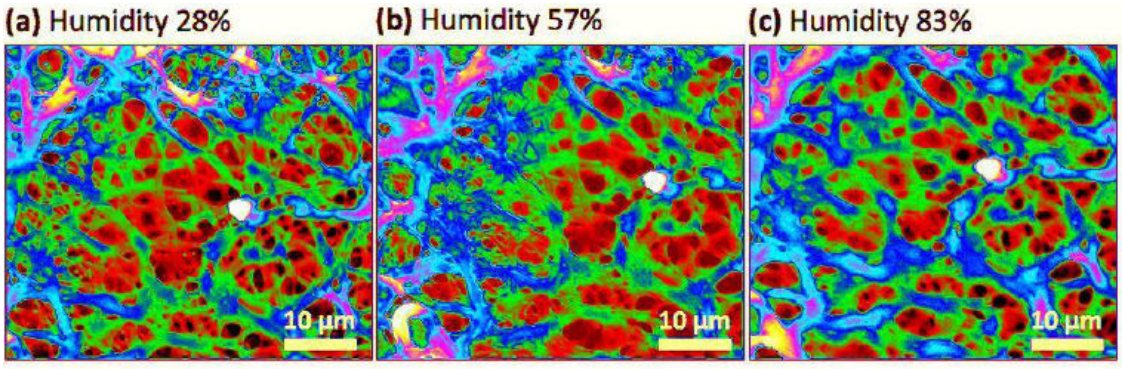


Figure 3: *Environmental SEM of electrospun hydrophobin. The water vapour pressure increases the diameter of the fibres.*

References:

[1] T.P.J. Knowles, C.A. Waudby, G.L. Devlin, S.I.A. Cohen, A. Aguzzi, M. Vendruscolo, E.M. Terentjev, M.E. Welland, C.M. Dobson, Science 326 (2009) 1533
[2] A.M. Bittner, Surf. Sci. Rep. 61 (2006) 383
[3] A.M. Bittner, Naturwissenschaften 92 (2005) 51
[4] G. Bhak, J.-H. Lee, J.-S. Hahn, S.-R. Paik, PLOSone 4 (2009) e4177
[5] P. Hamilton-Brown, I. Bekard, W.A. Ducker, D.E. Dunstan, J. Phys. Chem. B 112 (2008) 16249
[6] G. Singh, A.M. Bittner, S. Loscher, N. Malinowski, K. Kern, Adv. Mater., 20 (2008) 2332

CANTILEVER-LIKE SENSORS FOR USE IN BIO-MEDICAL APPLICATIONS

Anja Boisen

DTU Nanotech, Technical University of Denmark, Lyngby, Denmark
anja.boisen@nanotech.dtu.dk

Small mechanical structures such as cantilevers, bridges and lids can be used as sensitive and label free sensors. A biochemical reaction at the surface of the structure can be monitored as a bending, due to a change in surface stress. Minute temperature changes can be registered by exploring the bimorph effect. Furthermore, mass detection can be achieved by using resonating structures and monitor how the resonant frequency changes as a function of the added mass. In order to obtain high sensitivity the structures need to have micrometer and sometimes nanometer dimensions. They are fabricated by cleanroom processing using either silicon or polymer based materials. We will present examples of our recent advances in the field of sensor development including results on the on-line detection of DNA proteins and nano-particles. Also the strength of combining several independent miniaturized sensors will be discussed and illustrated for the use in explosives detection.

MRI CELL TRACKING USING MAGNETIC NANOPARTICLES

Jeff W.M. Bulte

Russell H. Morgan Department of Radiology and Radiological Science, Division of MR Research, Department of Biomedical Engineering, and Department of Chemical & Biomolecular Engineering, Cellular Imaging Section and Vascular Biology Program, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

jwmbulte@mri.jhu.edu

The clinical use of novel experimental cell therapies calls for suitable methods that can monitor the cellular biodistribution non-invasively following administration. Among the different clinically used imaging techniques, magnetic resonance (MR) imaging has superior spatial resolution with excellent soft tissue contrast. In order for exogenous therapeutic cells to be detected, they need to have a different contrast from endogenous cells. The most sensitive MR label to date are the superparamagnetic iron oxide nanoparticles or SPIOs. SPIOs are clinically approved and create strong local magnetic field disturbances that spoil the MR signal leading to hypo- or hyperintense contrast.

After approximately a decade of animal studies using MRI cell tracking these nanoprobes entered the clinic for cell tracking in 2004. The first phase I trial demonstrated the feasibility and safety of MRI (dendritic) cell tracking in cancer patients. A surprising finding, only observable by MRI, was that misinjection of cells occurred in half the patients. While the injection procedure was performed under ultrasound guidance, neither radionuclide or US imaging was able to reveal the failure of targeted cell delivery. Therefore, MR-guided targeted cell delivery may have significant advantages for clinical implementation of novel treatment paradigms using cellular therapeutics. If the administration is done in real-time under MR guidance, then verification of accurate cell delivery in, adjacent, or remote from the target site is mandatory. As of the end of 2010, 6 clinical trials using SPIO-based cell tracking have now been published, and some of these will be highlighted.

ADVANCED THERAPIES FOR OCULAR SURFACE RECONSTRUCTION: FROM *IN VITRO* RESEARCH TO CLINICAL TRIALS

Margarita Calonge Cano

IOBA (Instituto Universitario de Oftalmobiología Aplicada)
Universidad de Valladolid, Valladolid, Spain

The corneoscleral junction or limbus is the native niche for corneal stem cells (SC), especifically called limbal epithelial SC (LESC). Dysfunction of these cells (LESC deficiency syndrome) usually results in blindness and chronic pain by causing corneal opacity and persistent ocular surface inflammation, notably decreasing quality-of-life.

In these cases, corneal transplantation was the only option to recover vision, but a poor outcome was usually the rule due to the lack of LESC in the donor corneal graft. For the last 15 years, transplantation of pieces of limbal tissue directly from a cadaveric source or a living relative met good short-term results but only in some partial unilateral cases, failing (mainly due to immune rejection) in the more frequent cases of diffuse and/or bilateral disease. A more successful approach is the transplantation of *in vitro* expanded and cultivated LESC, an approach first described in 1997. Since then, progress has been made and at present transplantation of LESC has become the most successful therapy for LESC deficiency syndromes. This actually represents one of the first and clearest successes of regenerative medicine, as our work team is also demonstrating in an on-going clinical study. This study is being performed following good manufacturing practice (GMP) and good clinical practice (GCP) rules, and subsequently LESC are cultivated in a validated clean room (as mandated by the Spanish legislation, transposition of the European directive) and patients are followed under a strict clinical and surgical protocol. To date, 25 patients have been transplanted (mean follow-up, 9.2 months) with 80% global success, substantial quality-of-life improvement, and visual gain in 63% of patients. The 13 autologous and the 12 allogeneic transplants have presented similar results. The required immunosuppression for allogenic cases has been minimal, no sign of immune rejection has been observed, and additionally we have seen lower costs and less surgical morbidity. However, an important drawback remains, which is the dependency of donations in bilateral cases and the fact that an extra-surgical procedure is required in the only good-eye of a unilateral affected patient, increasing potential morbidity and sanitary costs. Therefore, an extraocular source of SC could be extremely valuable. As it is well known, mesenchymal stem cells (MSC) have a remarkably immunomodulatory capacity, and allogeneic transplantation of bone marrow-derived MSC (BM) has been successfully used for experimental ocular surface reconstruction. For this reason, we set out to demonstrate the hypothesis that the transplantation of BM-MSC cells is equal or superior to LESC transplantation through a randomized, double-masked clinical trial.

VIRUS SCAFFOLDS AS ENZYME NANO-CARRIERS (ENCS) TO ORGANIZE BIO-CATALYTIC ENZYME CASCADES. DESIGN OF A SCANNING ELECTROCHEMICAL NANOREACTOR MICROSCOPY DEVICE

Daniela Cardinale[1], Noëlle Carette[1], Amandine Barra[1], Jocelyne Walter[1], Thierry Delaunay[1], Christophe Demaille[2], Agnés Anne[2], Olivier Courjean[3], Nicolas Mano[3], Jean-Paul Salvetat[3], Jean-Pierre Aime[4], Thierry Michon[1]

[1]INRA, UMR 1090 GDPP, IBVM Virologie, 71 Av. Edouard Bourlaux, B.P. 81, 33883 Villenave d'Ornon, France
[2]Laboratoire d'Electrochimie Moléculaire, UMR CNRS – P7 7591, Université Paris Diderot – Paris 7, Bâtiment Lavoisier, 15 rue Jean-Antoine de Baïf, 75205 Paris cedex 13, France
[3]CRPP, Centre de Recherche Paul Pascal, 115 Av. Schweitzer, 33600 Pessac, France
[4]CPMOH, Centre de Physique Moléculaire Optique et Hertzienne, UMR5798,Université Bordeaux 1, 351 Cours de la Libération, 33405 Talence, France
daniela.cardinale@bordeaux.inra.fr

In cellular systems, the association of collaborating enzymes in supramolecular structures enables metabolic processes to be performed more efficiently, accelerating reactions rates and preventing the diffusion of intermediates in the cell medium. The aim of the Cascade project is to create a new experimental tool at the nanoscale level mimicking in vivo enzymatic cascade reactions. The set up will also offer the opportunity to study enzymatic processes at the level of one single or few molecules. To this purpose two model enzymes will be used to build artificial redox cascades: the lipase B (CalB) from *Candida antarctica* and the glucose oxidase (GOX) from *Penicillum amagasakiense*. The free electrons generated by enzymatic activities will be detected by electrochemistry. To this purpose a new nanoelectrochemical technique will be used to confine the clustered enzymes and to measure the final activity. The confined reaction medium will be permitted by a "nanocavity" microelectrode fabricated at the tip of an AFM probe; the combination of AFM/SECM (Scanning Electro Chemical Microscopy) will enable to measure the electrochemical current generated by a few enzyme molecules. Varying the diameter of the nanocavity from few hundreds to about ten nanometers should eventually permit to follow a single enzyme activity.

In order to study one-single enzyme kinetics the very faint signal of the enzyme needs to be amplified. After CalB hydrolysis of p-aminophenyl acetate (pAPA) an electro-inactive substrate to p-aminophenol (pAP) the electro-active product the red-ox couple pAPA/pAP will be continuously recycled between two electrodes amplifying the single enzymatic initial event. Kinetics parameters of pAPA hydrolysis by CALB determined spectrophotometrically and electrochemically are in good agreement. This supports the suitability of pAPA for AFM/SECM single enzyme studies.

In order to control the distribution of enzymes on the electrode we will use virus capsids as Enzyme Nano-Carriers (ENCs). To this aim, two plant viruses, Tobacco mosaic virus (TMV) and potato virus A (PVA) will be tested. Three different strategies will be attempted for the virus to enzyme interfacing: the fusion of leucine zipper (LZ) pairs to enzymes and capsomers, bi-specific antibodies and peptides obtained from phage display screening.

Regarding the first strategy, three pairs of LZ having different characteristics in term of length, affinity and orientation have been selected and the cloning at the N- and C- terminus of CalB is in progress. One assembly CalB (LZKg-CalB) was expressed in Escherichia coli periplasm and we are currently optimizing its purification.

Monoclonal antibodies for CalB have been produced and fusion with antibodies for TMV and PVA will be attempted.

As third strategy, three peptides were selected that recognize PVA. Cloning at the N-terminus of CalB and GOX is in progress.

NANOTECHNOLOGIES AND "SECURITY AT WORK": A NEW CHALLENGE FOR INDUSTRIAL RELATIONS AND SOCIETY

Patrick Chaskiel

CERTOP, UMR 5044 CNRS, UT2, UT3-UPS, 115 rte de Narbonne, 31077 Toulouse cedex 4, France
patrick.chaskiel@iut-tlse3.fr

Nanotechnologies, considered here as a "security at work" theme, raised a new challenge to industrial relations, i.e. relations between employers, state and workers. Nanotechnologies question the social organization of risks control relations, generally based on a single industry-wide approach when the entire cycle of nanoproduct life: from fabrication to disposal, would suppose an inter/multi-industrial approach of risks control.

Historically industrial relations on "security at work" in most industrialized countries fit the shape of general industrial relations, i.e. collective bargaining. The reason for that is not only historical: it relies on the very fact that "security at work" problems (and also working conditions) are linked - and must be referred - to a well identified type of activity, for instance: chemical or nuclear industries. To that extent and as it has been revealed by previous researches, industrial relations on "security at work" combined "shop-floor" day-to-day negotiations, company-wide talks and more formal agreements, these latter appearing quite infrequent in France. Nevertheless, it can be said that "security at work" is mainly a plant-wide issue, but regularly addressed at a wider level. It does not signify that risks, notably nanotechnologies problems, are not assessed at company level before implementing a nanoactivity in a plant. We only said that controlling "security at work" is eventually a shop-floor topic embedded in a larger framework and we need to know if nanotechnologies fit this model.

In France the main industrial relations institution implied in controlling "security at work" is the CHSCT (*Comité d'Hygiène, Sécurité et Conditions de travail*) [1]. Since a couple of years CHSCTs have played an increasing role in industrial relations, and changes have been noticed in the "security at work" topic: this latter is a more and more addressed issue, by employers, unionists and state and by public opinion either [2]. Growing costs of "repairing" industrial accidents (compensations), internal as well as public claims (from state or public opinion) to decrease accidents explain why the "security at work" topic has become that much important in industries and in society as a whole.

References:

[1] Health, Security, and Working Conditions Committee.
[2] Here public opinion is defined as the flow of discussions aiming to build an universal point of view. So it is not to be sought as results of polls published by mass medias.

PHONONIC METAMATERIALS FOR SHAPING FLUID FLOW

Jonathan Cooper

University of Glasgow, UK

The development of microfluidic systems is often constrained both by difficulties associated with the chip interconnection to other instruments, and by mechanisms that can enable fluid movement and processing. Surface acoustic wave (SAW) devices have previously shown promise in allowing samples to be manipulated, although designing complex fluid manipulations involves the generation of mixed signals at multiple electrode transducers.

We now demonstrate a new and very simple interface between a piezoelectric SAW device and a disposable microfluidic chip, involving the use of phononic structures, Figure 1(a) to shape the acoustic field. The surface wave is coupled from the piezoelectric substrate into the disposable chip to allow fluid actuations, including droplet movement, splitting, jetting, nebulisation and centrifugation to be performed. The phononic structure has a band-gap and by showing that the interaction of the fluid within the chip structure is dependent upon the acoustic frequency, we provide providing a method to programme complex fluidic functions into a microchip.

Further, we demonstrate the application of this new technology in a number of new analytical proceedures, including for example, the implementation of digital microfluids, the interface of microfluidics with mass spectrometry (Figure 1(b)), PCR, and the lysis of cells on-chip. We show initial results demonstrating the integration of these methods. In doing so, we propose to create a "tool-box" of different diagnostic functions (sample processing, cell separation, cell lysis, PCR, nebulization for MS, detection) each of which requires a different phononic structure. Just as in electronics, where discrete components are combined to create a circuit, so we will use different combinations of phononic lattices to create fluidic microcircuits, each of which provides a unique diagnostic function.

Figures:

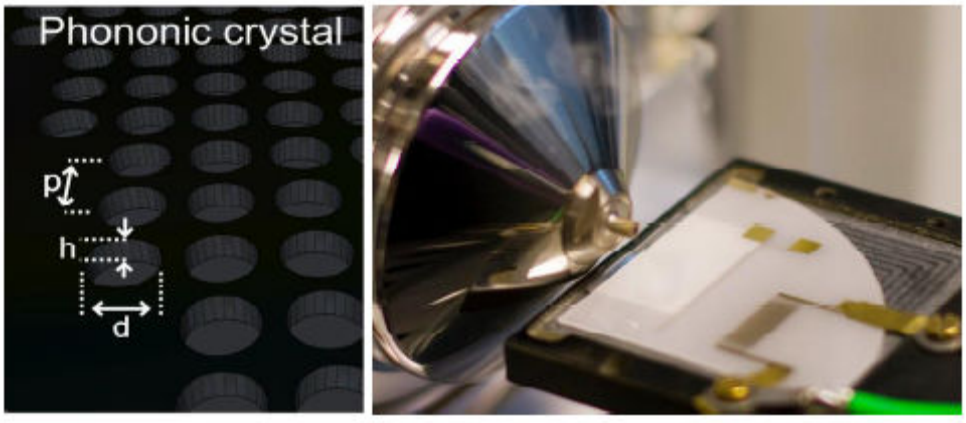


Figure 1: *Left, shows a schematic of a phononic crystal, used for microfluidics whilst, right, shows the interface of a disposable chip with a MS instrument.*

INTERACTIONS OF NANOPARTICLE WITH LIVING ORGANISMS

Kenneth Dawson

University College Dublin, Ireland

Nanoscale materials can interact with living organisms in a qualitatively different manner than small molecules. Crucially, biological phenomena such as immune clearance, cellular uptake and biological barrier crossing are all determined by processes on the nanometer scale. Harnessing these endogeneous biological processes (for example in creation of new nanomedicines or nanodiagnostics) will therefore require us to work on the nanoscale. This ensures that nanoscience, biology and medicine will be intimately connected for generations to come, and may well provide the best hope of tacking currently intractable diseases.

These same scientific observations lead to widespread concern about the potential safety of nanomaterials in general. Early unfocussed concerns have diminished, leaving a more disciplined and balanced scientific dialogue. In particular a growing interest in understanding the fundamental principles of bionaointeractions may offer insight into potential hazard, as well as the basis for therapeutic use.

Whilst nanoparticle size is important, the detailed nature of the nanoparticle interface is key to understanding interactions with living organisms. This interface may be quite complex, involving also adsorbed protein from the biological fluid (blood, or other), leading to a sort of 'protein corona' around the nanomaterial surface. We discuss how this corona is formed, and how it may be a determining feature in biological interactions.

References:

[1] Cedervall T, Lynch I, Lindman S, Berggård T, Thulin E, Nilsson, H, Linse S, Dawson KA. Understanding the nanoparticle protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles, Proc. Natl. Acad. Sci. USA, 104, 2050-2055 (2007)

[2] Linse S., Cabaleiro-Lago, C., Xue W.F., Lynch I., Lindman S., Thulin E., Radford S.E., Dawson, K.A., Proc. Natl. Acad. Sci. USA, 104 (21); 8691-8696 (2007)

[3] Lundqvist M, Stigler J, Cedervall T, Elia G, Lynch I, and Dawson KA. Nanoparticle Size and Surface Properties determine the Protein Corona with possible implications for Biological Impacts. Proc. Natl. Acad. Sci. USA, 105, 14265-14270 (2008)

[4] Cabaleiro-Lago C., Quinlan-Pluck F., Lynch I., Lindman S., Minogue A.M., Thulin E., Walsh, D.M., Dawson K.A., Linse, S., J. Am. Chem. Soc., 130 (46); 15437-15443 (2008)

[5] Lynch I, Salvati A and Dawson KA. Protein-nanoparticle interactions: What does the cell see? Nature Nanotech. 4, 546-547 (2009)

[6] Cabalerio-Lago C., Quinlan-Pluck F., Lynch I., Dawson K.A., Linse S., ACS Chem. Neurosci., 1 (4), 279 (2010)

[7] Walczyk D, Baldelli Bombelli F, Monopoli PM, Lynch I and Dawson KA. What the Cell "Sees" in Bionanoscience, J. Am. Chem. Soc., 132 (16), 5761–5768 (2010)

TARGETING THE IMMUNE SYSTEM WITH BIODEGRADABLE NANO ENGINEERED POLYMERIC MICROCAPSULES

Bruno G. De Geest, Stefaan De Koker, Chris Vervaet, Jean Paul Remon

Laboratory of Pharmaceutical technology, Ghent University, Ghent, Belgium
br.degeest@ugent.be

Vaccines that can elicit strong T-cell responses are undoubtedly one of the major challenges for medicine today. [1] For this purpose, dendritic cells (DCs) have to internalize antigen, process them into peptide fragments and present them to T-cells. Whereas soluble antigen largely fails to induce potent cellular immune responses, formulation of antigen into microparticles has emerged as an attractive alternative. Here, we demonstrate efficient *in vitro* and *in vivo* antigen delivery to DCs using biodegradable polyelectrolyte capsules as antigen carrier.

Figure 1 schematically shows the encapsulation procedure of antigen into hollow polyelectrolyte capsules. [2,3] In a first step, antigen loaded $CaCO_3$ microparticles (3 µm diameter) are fabricated by co-precipitation of $CaCl_2$ and Na_2CO_3 in the presence ovalbumin (OVA) as model antigen. Subsequently these $CaCO_3$ microparticles are coated (2 bilayers) by sequential deposition of dextran sulfate and poly-L-arginine using electrostatic interaction as driving force. Finally hollow polyelectrolyte capsules are obtained after dissolution of the $CaCO_3$ core templates in aqueous EDTA medium.

As demonstrated by transmission electron microscopy (Figure 2A) and confocal microscopy (figure 2B), polyelectrolyte capsules are efficiently taken up by DCs and both the capsule membrane as well as the encapsulated antigen becomes readily processed. [4,5] Antigen presentation to T-cells was assessed by incubating DCs with OVA loaded capsules followed by co-culturing with respectively OT-I and OT-II cells (Figure 2C). OT-I and OT-II cells are transgenic CD8, respectively CD4 T cells that specifically recognizes the OVA CD8 peptide, respectively CD4 peptide. Compared to soluble antigen a dramatic increase in T-cell presentation is observed. Especially cross-presentation to CD8 T-cells, which are crucial to induce cellular immune responses, is strongly promoted. [4]

In vivo studies show mild tissue reactions [6,7] upon subcutaneous injection [5 while potent humoral and cellular immune responses [8] are induced which show protective immunity against viral infection as well as cancer. Moreover in recent studies we have also demonstrated an easy strategy – involving main stream pharmaceutical technology – to scale the production of polyelectrolyte microcapsules using a one-step procedure which encapsulates antigen with extremely high yields while barely hampering its biological activity. [9]

References:

[1] De Koker, S.; Lambrecht, B.N.; Willart, M.A.; Van Kooyk, Y.; Grooten, J.; Vervaet, C.; Remon, J.P.; De Geest, B.G., Chemical Society Reviews, 40 (2011) 320.
[2] De Koker, S.; De Cock, L.J.; Auzély-Velty, R.; Gil, P.R., Parak, W.J.; Grooten, J.; Vervaet, C.; Remon, J.P.; De Geest, B.G. Advanced Drug Delivery Reviews, in press.
[3] De Cock, L.J.; De Koker, S.; De Geest,B.G.; Grooten, J.; Vervaet, C.; Remon, J.P.; Sukhorukov, G.B.; Antipina, M.N. Angewandte Chemie International Edition, 40 (2010) 6954.
[4] S. De Koker, B.G. De Geest,* S.K. Singh, R. De Rijcke, T. Naessens, K. Van Kooyk, J. Demeester, S.C. De Smedt, J. Grooten, Angewandte Chemie International Edition, 48 (2009) 8485.
[5] P. Rivera-Gil, S. De Koker, B.G. De Geest,* W.J. Parak, Nano Letters, 9 (2009) 4398.
[6] De Koker, S.; De Geest, B.G.; Cuvelier, C.; Ferdinande, L.; Deckers, W.; Hennink, W.E.; De Smedt, S.C.; Mertens, N. Advanced Functional Materials, 17 (2007) 3754.
[7] L.J. De Cock, J. Lenoir , V. Vermeersch, S. De Koker, A.G. Skirtach, P. Dubruel, E. Adriaens, C. Vervaet, J.P. Remon, B.G. De Geest, Biomaterials, 32 (2011) 1967
[8] De Koker, S.; Naessens, T.; De Geest, B.G.; Bogaert, P; Demeester, J.; De Smedt, S.C.; Grooten, J., Journal of Immunology, 184 (2010) 203.
[9] M. Dierendonck, S. De Koker, C. Cuvelier, J. Grooten, C. Vervaet, J.P. Remon, B.G. De Geest* Angewandte Chemie International Edition, 49 (2010) 8620.

Figures:

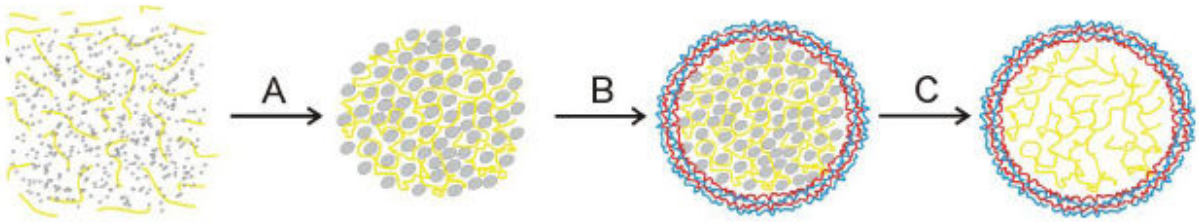


Figure 1: *Polyelectrolyte microcapsule synthesis. (A) Antigen (yellow) is mixed with $CaCl_2$ and Na_2CO_3, resulting in the generation of macromolecule-filled $CaCO_3$ microparticles (gray), which are (B) subsequently coated with alternating layers of dextran sulfate and poly-L-arginine (red, blue). (C) Dissolution of the $CaCO_3$ core by EDTA results in the generation of a hollow microcapsule composed of macromolecules surrounded by the polyelectrolyte shell.*

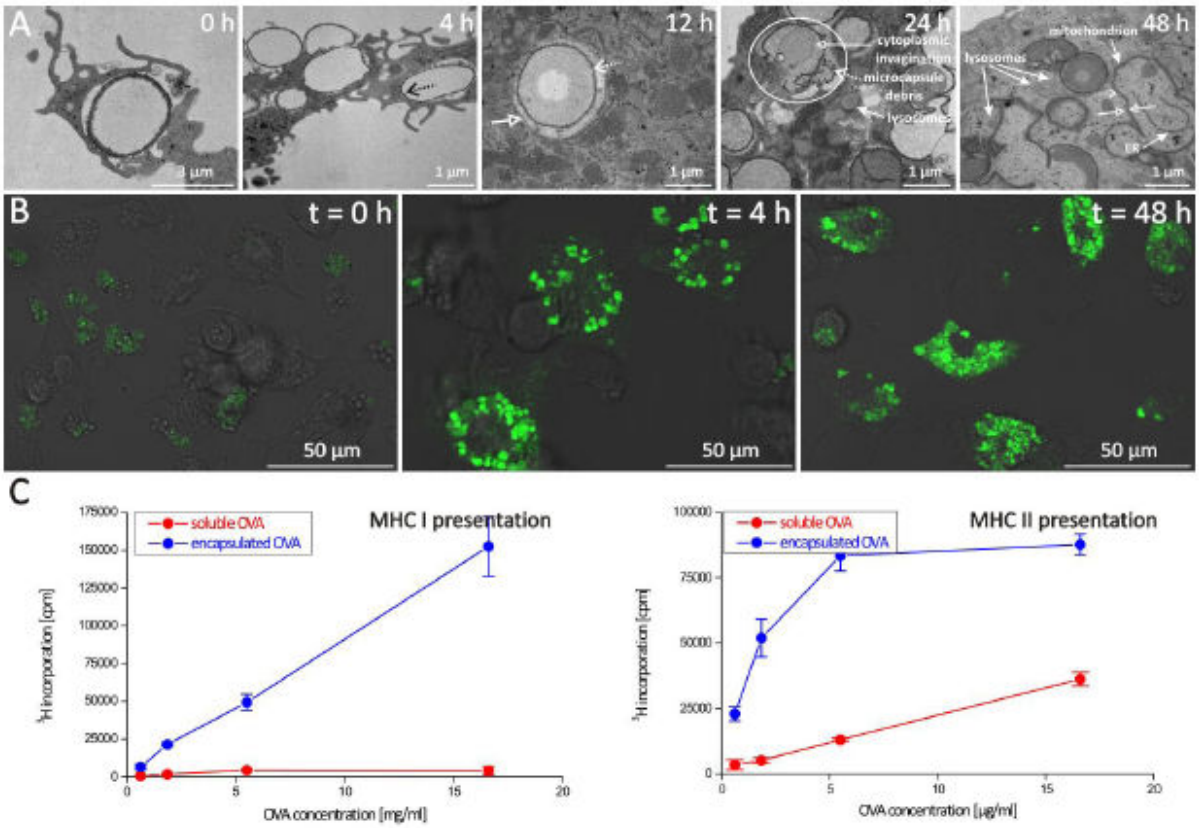


Figure 2: *(A) TEM images of BM-DCs that have internalized dextran sulfate/poly-L-arginine microcapsules at the indicated time intervals. Microcapsule shell: dotted arrows; membranes surrounding the microcapsules: open arrows. In the encircled area, microcapsule rupture and cytoplasmic invagination are clearly distinguishable. Lysosomes, endoplasmatic reticulum (ER), and a mitochondrion are indicated by the solid arrows. (B) Processing of dextran sulfate/poly-larginine microcapsule encapsulated OVA was analyzed using DQ-OVA. Confocal microscopy images of BM-DCs incubated with OVA-DQ microcapsules for 0, 4 and 48 h (overlay of green fluorescence and DIC). (DQ-OVA is ovalbumin oversaturated with BODIPY dyes. Upon proteolytic cleavage, quenching is relieved and green fluorescence appears. (C) Antigen presentation by BM-DCs after uptake of soluble and encapsulated OVA. Proliferation of OT-I cells was used as a measure for MHC-I-mediated cross-presentation of OVA (left graph), proliferation of OT-II cells as a measure for MHC-II mediated presentation (right).*

INTERNALISATION MECHANISMS OF MODIFIED TITANIUM OXIDE NANOPARTICLES IN SKIN CELLS AND MULTICELLULAR LIVING SPECIMENS : RESULTING TOXICITY

M.H. Delville[1], M. Simon[2], A. Girard[1,2], P. Barberet[2], P. Moretto[2], H. Seznec[2], D. Dupuy[3]

[1]CNRS, Univ de Bordeaux, ICMCB, 87 avenue du Dr. A. Schweitzer, Pessac, F-33608 Cedex, France
[2]Université Bordeaux, CNRS/IN2P3, Centre d'Etudes Nucléaires de Bordeaux; France
[3]Gradignan, CENBG, Chemin du Solarium, BP120, 33175 Gradignan, France
Institut Européen de Chimie et Biologie, Univ Bordeaux 1, 2 rue R. Escarpit, 33607 Pessac, France

Nanotechnologies are of great interest from both academic and industrial points of view, with numerous applications in domains such as medicine, catalysis and material sciences. However, their nanotoxicology has also attracted the attention of public and governments' worldwide and established methods of chemical safety assessments have to be modified to address the special characteristics of nanoparticles and more especially to assess the biological effects of these highly reactive materials.

Most of theses manufactured nanoparticles have been produced for several decades on an industrial scale. There is an urgent need to evaluate the risks of these particles to ensure their safe production, handling, use, and disposal. Moreover, a comprehensive study is clearly needed to fully explore the toxicity of manufactured nanoparticles, which may help to better understand their deleterious health effects and create environmentally friendly and biologically relevant nanoparticles. In particular, the behavior of nanoparticles inside living cells is still an enigma, and no metabolic responses induced by these particles are understood so far.

This presentation concerns the potential toxicity due to exposure of TiO_2 NPs used in sunscreens and cosmetics. We propose to apply an original imaging methodology (Ion Beam Analysis, TEM, and Confocal microscopy) to in vitro studies, combining technologies for on one hand, the detection, tracking, and quantification of TiO_2 nanoparticles and on the other one, the use of indicators for ion homeostasis, cell metabolism, or cell fate.

The main goal is to precisely identify the molecular and cellular mechanisms involved in the nanotoxicity of TiO_2 nanoparticles in eukaryotic cells and in multi-cellular organisms such as Caenorhabditis elegans (C. elegans). This study addresses the current knowledge gap of human cells and C. elegans responses to TiO_2 nanoparticles exposure. Since the nematodes feed on bacteria and are considered as particle-ingesting organisms, the present study will offer new perspectives in nanoparticles-related risk assessment and food web accumulation modelling.

REGULATORY ISSUES IN NANOMEDECINE: A FUTURE UNDER CONSTRUCTION

Jean-Marie Devoisselle

Institut Charles Gerhardt Montpellier, UMR CNRS/UM2/ENSCM/UM1 5253 U.F.R. des Sciences Pharmaceutiques et Biologiques 15 ave Ch. Flahault, BP14491, 34093 Montpellier, France
jm.devoisselle@univ-montp1.fr

Research in nanotechnology and specially nanomaterials has gone now to a large commercial undertaking. The scientific literature on environment, health and safety aspects of nanomaterials show clearly how these aspects are now of primary importance. First of all the first question is: how to define nanomaterials and nanomedecine? Several definitions can be found but some initiatives tend to clarify these definitions. The application of nanotechnology to healthcare is already demonstrated by the increasing intensity of research and competition in the pharmaceutical industry. It can be illustrated by the design of new therapeutics and/or diagnostic tools. The field of new drug delivery technologies is also growing rapidly. The research on life sciences applications of nanotechnologies (mainly dedicated to drug delivery and therapeutics) has doubled since 2002. Nanotechnology-based drug delivery systems and devices provide new features and functions that other technologies cannot match. Various nanoscale structures of different size, shapes, texture and chemical compositions has been included as nanopharmaceuticals. Although there are quite a few approved nanopharmaceuticals, several others are under development or close to commercialization. There are currently several challenges and risks concerning the commercialization of nanopharmaceuticals. The most important are environmental, safety, ethical and regulatory issues. These products (drug-loaded nanomaterials or devices) displaying new size-dependent properties and toxicological profiles need new approaches from the regulatory agencies. A global approach is needed. Discussions concern the application of existing regulation and the implementation of new legislation in this field.

References:

[1] Dr. Angela Hullmann, European Commission, Unit “Nano and Converging Sciences and Technologies”, European activities in the field of ethical, legal and social aspects (ELSA) and governance of nanotechnology, october 2008, see http://cordis.europa.eu./nanotechnology
[2] Scientific Committee on Emerging and Newly Identified Health Risks, SCENIHR, 6 July 2010, http://ec.europa.eu/health/ph_risk/risk_en.htm
[3] Office of Pharmaceutical Science, MAPP 5015.9 (6/3/2010)
[4] S.F. Hansen, Nanomed. Nanobiotechnol. (2010) 2 :441-449.
[5] Aida Maria Ponce Del Castillo, CRISP, (2010) n°2065
[6] Bawa, R. Current Drug Delivery, (2011, Feb.3)

IMMUNOLOGICAL PROPERTIES OF ENGINEERED NANOMATERIALS

Marina A. Dobrovolskaia

Nanotechnology Characterization Laboratory, Advanced Technology Program, SAIC-Frederick Inc., NCI-Frederick, Frederick, MD 21702, USA

marina@mail.nih.gov

Nanotechnology is finding growing applications in industry, biology and medicine. The clear benefits of using nanosized products in various biological and medical applications are often challenged by questions regarding toxicity of these materials. One area of interest involves the interactions between nanoparticles and the components of the immune system. Nanoparticles can be engineered to either avoid immune system recognition or to specifically interact with components of the immune responses. This presentation will review data regarding nanoparticle-mediated immunostimulation and immunosuppression; how manipulation of particle physicochemical properties can influence their interaction with components of the immune system (specifically interaction with erythrocytes, effects on blood coagulation system, activation of complement and effects on immune cell function), and discuss challenges with preclinical immunological characterization of engineered nanomaterials (specifically endotoxin contamination, depyrogenation, sterility and sterilization, and nanoparticle interference with traditional immunological tests).

Funded by NCI Contract No.HHSN261200800001E

STUDY OF THE DISTRIBUTION OF MAGNETITE NANOPARTICLES IN AN EXPERIMENTAL MODEL OF HEPATIC METASTASES

J.J. Echevarria[1], I. Garcia-Alonso[2], J.A. Larena[1], F. Sanz-Sanchez[1], F. Plazaola[3], M. Insausti[3], N. Etxebarria[3], J. Salado[3], B. Fernandez-Ruanova[1]

[1]Radiology Department. Galdakao-Usansolo Hospital, Labeaga s/n, 48960 Galdakao, Spain
[2]Experimental Surgery Laboratory, Medicine Faculty, University of the Basque Country (UPV/EHU), 48940 Leioa, Spain
[3]Faculty of Science and Technology, Univ. of the Basque Country (UPV/EHU), 48940 Leioa, Spain
fernando.plazaola@ehu.es

The purpose of this work is to assess a magnetic fluid for its affinity for liver metastases at different stages of tumor development in laboratory rats, prior to its potential use in antitumor thermal therapy. Iron oxide magnetic nanoparticles ranging between 3.8 and 7.1 nm were synthesized using the polyol method. From this synthesis process, magnetic nanoparticles of Fe_3O_4 capped by oleic acid were obtained. The lipid nature of these ligands allows the particles to be suspended in iodized oil Lipiodol, when the mixture is exposed to ultrasonification. A magnetic fluid consisting of suspensions of 2 mg of iron oxide nanoparticles in 0.2 ml of Lipiodol was prepared for transarterial hepatic infusion in each animal.

The magnetic fluid infusion procedure in the selected animals required midline laparotomy to enable the exposure of visceral arterial vessels. The main branches of the celial trunk were clamped, with the exception of the hepatic artery with the aim of driving towards this artery most of the celiac vascular flow. A direct punction of the celiac trunk was carried out using a needle, connected by an elongated catheter to an infusion pump previously filled with the magnetic fluid, and the suspension was slowly and selectively infused into the liver.

The experimental study was carried out using 33 male WAG/RijCrl rats. In order to induce metastases in the liver of laboratory animals, syngenic cells of colon adenocarcinoma, CC-531, were inoculated into the liver of the rats. The splenic reservoir technique was used as a source for the dissemination of tumor cells. A total of 21 rats developed metastases, but six animals died during subsequent surgical procedures. Of the surviving animals, ten were chosen at random to receive the magnetic fluid, via the hepatic artery. The remaining five rats constituted the control group and did not receive the suspension.

Within the first 12 hours after administration of the magnetic fluid, Multi-Detector Computed Tomography (MDCT) and Magnetic Resonance Imaging (MRI) were performed to the animals to check the effectiveness of the infusion procedure. The observation on MDCT of a hyperdense liver due to the presence of Lipiodol in the arterial tree, and the lack of extravasations in the puncture area or the existence of gross intra-arterial contrast media deposits, were considered to be evidence of the correct transarterial administration of the magnetic fluid. MRI studies were carried out on a 1.5 T Siemens Symphony system, using a standard cranial coil and axial Short Time Inversion Recovery (STIR) and gradient-echo (GRE) weighted sequences were performed. The observation on STIR sequences of smooth hyper intense masses into liver was considered as metastatic lesions. The homogeneous decay of signal intensity of liver and metastases on GRE sequences was attributed to the presence of magnetic nanoparticles in the different tissues, and it was considered to be evidence of proper vascular diffusion of the magnetic fluid.

After the imaging studies animals were sacrificed. Livers were extracted, and the number and size of the metastases were determined by visual analysis. Two categories of tumor growth were considered. On the one hand, early stage neoplastic infiltration corresponding to metastases that were smaller than 3 mm, non-overlapping (separated by healthy parenchyma) and with no more than ten visually identifiable lesions. On the other hand, livers that showed extensive neoplastic infiltrations, in an advanced stage, were characterized by having metastases larger than 3 mm or more than ten visually detectable lesions.

Liver and neoplastic tissue were taken to determine iron concentrations, using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Statistical analysis was performed with non-parametric tests (Wilcoxon test for related samples). Once significant differences between groups had been demonstrated using the Kruskal-Wallis test, comparisons between possible pairs of groups were performed using the U Mann-Whitney test. The minimum level of significance accepted was $P < 0.05$.

Of the ten animals in which surgical procedures and the magnetic fluid infusion were successfully completed, five showed early stage metastatic development while the other five presented advanced stage metastasis. After subtracting the mean endogenous iron values of the control group, from the iron found in animals that had received the magnetic fluid, the mean concentration attributable to exogenous administration in the early stage group was 172.2 mg/g in tumor tissue and 65.2 mg/g in healthy liver tissues, In these animals tumor tissue accumulated 2.6 times more iron than healthy liver tissue. In contrast, exogenous iron values found for the advanced stage group were 22.7 mg/g and 43.1 mg/g in metastatic and healthy tissue, respectively. In this group, metastases of animals with sever tumor infiltrations, accumulated half as much exogenous iron as healthy liver. Moreover, in the comparative study between concentrations of exogenous iron determined in liver and metastases within groups, significant differences were found in the early stage group, but not in the advanced stage group.

Our model of metastatic adenocarcinoma has revealed important differences in the vascularization of metastatic lesions according the stage of development of the disease. Therefore, we should consider the possibility of there being substantial differences in vascular perfusion in neoplastic lesions of similar type, but in different stages of development. The increase in tumor volume does not necessarily lead to a similar development in its vascularization, rather the increase in tumor mass may lead to the appearance of regions with decreased artery supply.

DNA ORIGAMIS: MECHANISMS OF FORMATION AND MELTING, STRUCTURE FLUCTUATIONS

JM. Arbona, **J. Elezgaray** and JP. Aimé

CBMN, UMR5248, 33600 Pessac, France
jm.arbona@iecb.u-bordeaux.fr

In this communication, we focus on the process of DNA-origami formation. In order to do this, several formation-melting experiments have been performed, where we keep track of the UV absorption as a function of the temperature. In parallel, for the melting process, we also image the resulting structures by AFM. These experiments show that, for the usual cooling-heating speed reported in most publications, folding of the origami shows hysteresis. The melting process is characterized by two transition temperatures correlated with the GC content of the staple strands and a third one at a higher temperature that may be correlated with the Origami structure. However, when the origami is prepared under constraint, the third transition disappears. During the formation of the origamis, only two transitions are observed, correlated with the GC content. In order to get further insight in the formation process, we also consider the folding-unfolding properties of a set of three ssDNA as a simplified model of origami (see figure). Using numerical simulations compared to melting experiments, we discuss entropic and defect contributions during the DNA-origami folding and the influence of a cooperative process.

In a separate study, we study the thermal fluctuations of a rectangular origami using a rigid base approximation. To do this, we use a simplified, yet accurate, model of DNA: the Stack of Plates (SOP) model [1], designed to model DNA at the base-pair level. In the SOP model, the right handed DNA structure is the result of the competition between two forces (stacking of the base pairs and a harmonic constraint to mimic sugar-phosphate backbones) and an additional simple geometric constraint. Monte-Carlo simulations show that the equilibrium conformation of the Rothemund origamis is not planar but slightly twisted. The same simulations allow to quantify the predominant elastic modes and the bending elasticity constant. We also discuss how this information can be used to design 'self-constrained' versions of the otherwise 'planar' structures.

References:

[1] Modeling DNA structure, elasticity, and deformations at the base-pair level. Mergell, B.; Ejtehadi, M.R.; Everaers, R

Figures:

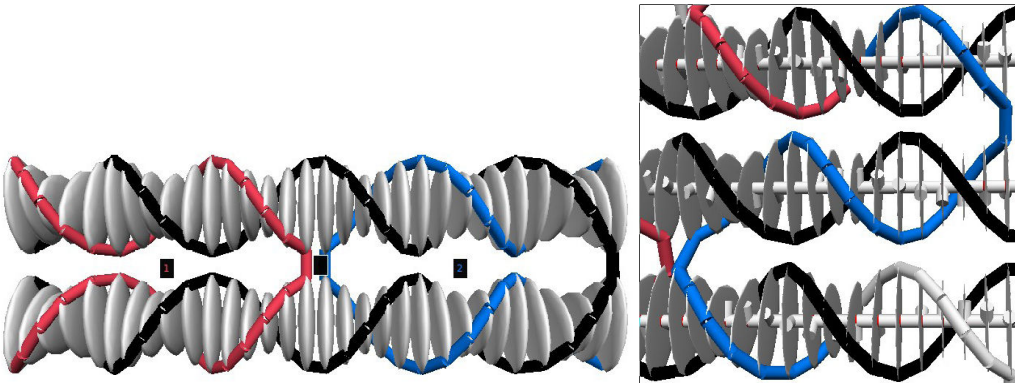


Figure 1: *Left: Simplified Origami made of 64 bases (black line) and two 32 bases long staples (red and blue lines). Right: Rectangular Origami Virus black line, staples red and blue lines.*

SUPRAMOLECULAR STRUCTURAL CHARACTERIZATION OF CHOLESTEROL-RICH VESICLES TO BE USED IN DRUG DELIVERY: INFLUENCE OF THE PREPARATION METHOD

Elisa Elizondo[1,2], Ingrid Cabrera[1,2], Evelyn Moreno-Calvo[1,2], Nora Ventosa[1,2] and Jaume Veciana[1,2]

[1]Department of Molecular Nanoscience and Organic Materials. Institute of Materials Science (ICMAB-CSIC), Campus UAB, 08193 Bellaterra, Spain;
[2]CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN)
ventosa@icmab.es; vecianaj@icmab.es

In the past 30 years, the explosive growth of nanotechnology has promoted challenging innovations in pharmacology, which is currently revolutionizing the delivery of biologically active compounds [1]. Indeed, some existing drugs and new therapeutic compounds emerging from drug discovery processes present special delivery challenges, pushing nanotechnology towards the development of new drug nanocarriers that enhance the bioavailability of drugs [2]. Such nanocarriers or drug delivery systems (DDS) are intended not only to protect drugs from degradation but also to achieve their temporal and spatial site-specific delivery.Vesicles constitute one of the most studied DDS since their discovery in the mid 60s [3]. However, in order to achieve optimal performance of these self-assembled structures as functional materials, a high grade of structural homogeneity is required. For example, the behaviour of vesicles as drug delivery systems (DDS) is highly affected by their homogeneity, not only in size or morphology, but also in their membrane composition and supramolecular organization. Concretely, the vesicular membrane plays an important role in terms of vesicles stability, rigidity, permeability, functionalization or response to external stimuli [4]. In the latter case, the homogeneity in the membrane composition and supramolecular organization between the different vesicles forming a certain system would be a crucial issue in order to have sharp responses that allow homogenous triggering of the drug at the site of action (Figure 1). Attending to this, methods for the preparation of homogeneous vesicular systems, not only in terms of size and morphology, but also regarding the supramolecular organization of the membrane constituents are required for fully exploiting the potential of these self-assembled structures as functional materials.

In the early 90's, compressed fluid (CF)-based processes emerged as an alternative to conventional methods using liquid solvents, attracting enormous interest for the production of micro- and nanoparticulate materials [5]. Our research group has experience in using these novel technologies for the controlled nanostructuration of materials to be used in drug delivery [6,7]. Recently, a CF-based method, DELOS-susp, has been developed for the production of vesicular systems. This one-step process allows the achievement of stable, nanoscopic and unilamellar cholesterol-rich vesicles [8,9], which present higher structural homogeneity regarding size and morphology than those produced by a conventional multi-step hydration method (Figure 2). In this work, by analyzing the membrane composition and supramolecular organization of vesicles prepared by both methodologies, we demonstrate that apart from size and morphology, the superior homogeneity observed for vesicular systems produced by CFs is also present in the molecular assembly of the membrane constituents, which is crucial for an optimum performance of these supramolecular structures as pharmaceutical carriers.

References:

[1] Couvreur, P. and Vauthier, C., Pharmaceutical Research, 23 (2006) 1417.
[2] Peer, D., Karp, J.M., Hong, S., Farokhzad, O.C., Margalit, R. and Langer, R., Nature Nanotechnology, 2 (2007) 751.
[3] Bangham, A.D., Standish, M.M. and Watkins, J.C., Journal of Molecular Biology, 13 (1965) 238.
[4] Guo, X. and Szoka, F.C., Accounts of Chemical Research, 36 (2003) 335.
[5] Davies, O.R., Lewis, A.L., Whitaker, M.J., Tai, H.Y., Shakesheff, K.M. and Howdle, S.M., Advanced Drug Delivery Reviews, 60 (2008) 373.
[6] Elizondo, E., Sala, S., Imbuluzqueta, E., González, D., Blanco-Prieto, M. J., Gamazo, C., Ventosa, N. and Veciana, J, Pharmaceutical Research (2010), doi:10.1007/s11095-010-0248-x.
[7] Imbuluzqueta, E., Elizondo, E., Gamazo, C., Moreno-Calvo, E., Veciana, J. and Ventosa, N., Acta Biomaterialia (2010), doi:10.1016/j.actbio.2010.11.031.

[8] Cano-Sarabia, M., Ventosa, N., Sala, S., Patino, C., Arranz, R. and Veciana, J., Langmuir, 24 (2008) 2433.
[9] Cano-Sarabia, M., Angelova, A., Ventosa, N., Lesieur, S. and Veciana, J., Journal of Colloid and Interface Science, 350 (2010) 10.

Figures:

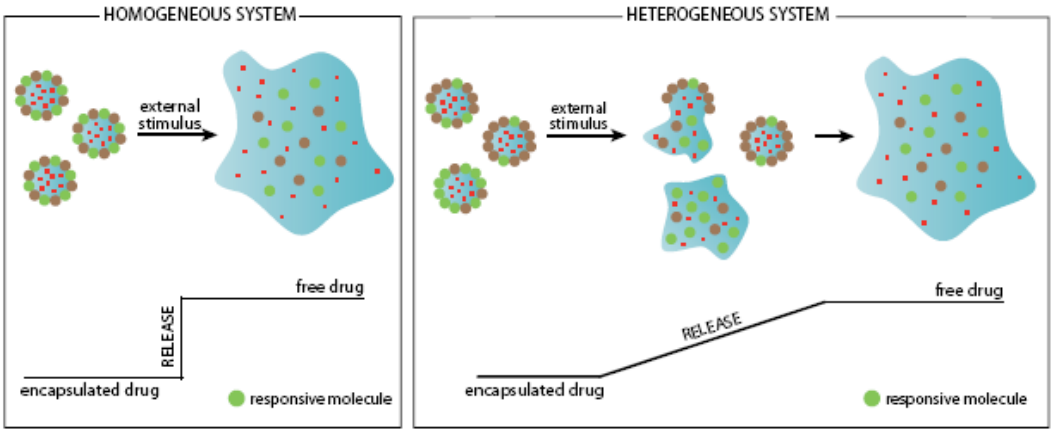


Figure 1: *Schematic illustration of the response to an external stimulus presented by a vesicular DDS with homogeneous (left) and heterogeneous (right) vesicle to vesicle composition and supramolecular arrangement.*

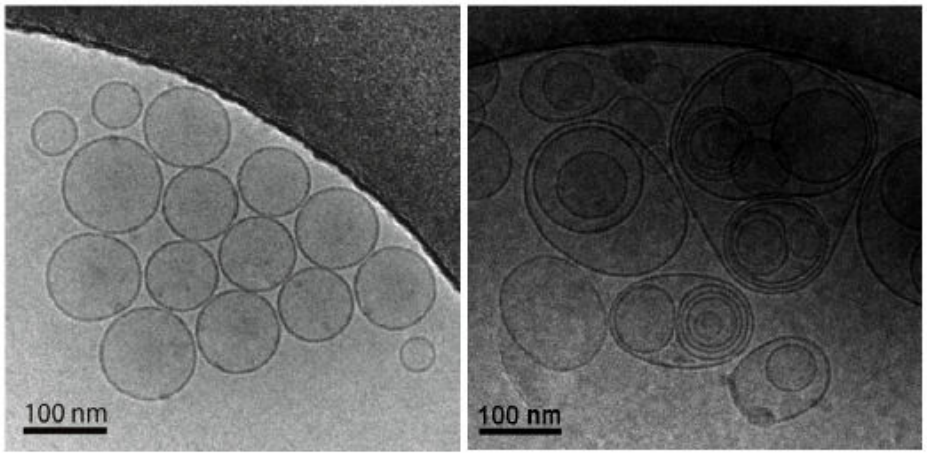


Figure 2: *CryoTEM images corresponding to cholesterol-rich vesicles prepared by DELOS-susp (left) and the hydration method (right).*

QUANTIFICATION OF NANOPARTICLE UPTAKE AND THEIR COLOCALIZATION WITH CELL CONSTITUENTS AT SINGLE CELL LEVEL

I. Estrela-Lopis, J. Fleddermann, M. Dorn, G. Romero, E. Rojas, Sergio Moya, Changyou Gao, Edwin Donath

Institute of Medical Physics & Biophysics, Leipzig University, Härtelstraße 16, 04107 Leipzig, Germany
Irina.Estrela-Lopis@medizin.uni-leipzig.de

The development of nanotechnology in recent years is unprecedented. Understanding the effects of nanoparticles (NPs) on human health is thus of major importance.[1,2] The degree and the mechanism of uptake, localization and distribution of NPs in cells are major issues concerning toxicity and risk assessment, and the effectivity of NPs as delivery devices. A better understanding of the potential effects of nanomaterials on human health is crucial regarding the introduction of nanoproducts into the market and has thus considerable economical importance.

Ion Beam Microscopy (IBM) and Confocal Raman Microspectroscopy (CRM) were therefore employed as label-free techniques capable of detecting and characterizing nanomaterials within single cells. The uptake, intracellular distribution and toxicity of carbon nanotubes (CNTs) and metal oxide nanoparticles in hepatocarcinoma (HepG2) and lung cells (A549) were studied employing these techniques.

By means of IBM the intracellular concentration and distribution of NPs can be established. Overlapping of cell basis element P with CeO_2 NPs can be easily seen in Figure 1. The concentrations of NPs in / or on the cell were calculated in one cell by choosing the mask of cell area. This provides the basis for intracellular dose dependent toxicity studies.

By means of Raman spectra deconvolution and subsequent cross-correlation analysis the colocalization of NPs with different intracellular environments, such as lipid rich regions, cytoplasm and nucleus was quantified. Figure 2 demonstrates the distribution of lipids and poly-(sulfo propyl methacrylate) (PSPM) modified CNTs in HepG2 cell. CRM, furthermore, was capable of detecting nanomaterial induced changes in the secondary nuclear protein structure and nucleobases content. These changes can be used as an indicator of the toxic effect of NPs. This was confirmed with cell proliferation tests. Studies with NPs surface engineered with lipids and polyelectrolytes showed that the nature of the surface of NPs and their modification in biological fluids is crucial for uptake and toxicity.

References:

[1] Lee J, Lilly GD, Doty RC, Podsiadlo P, Kotov NA 2009 Small 5 1213-21.
[2] Hillegass JM, Shukla A, Lathrop SA, MacPherson MB, Fukagawa NK, Mossman BT 2010 Wiley Interdiscip Rev Nanomed Nanobiotechnol 2 219-31.
[3] Romero G, Estrela-Lopis I, Zhou J, Rojas E, Franco A, Espinel CS, Fernandez AG, Gao C, Donath E, Moya SE 2010 Biomacromolecules 11 2993-2999

Figures:

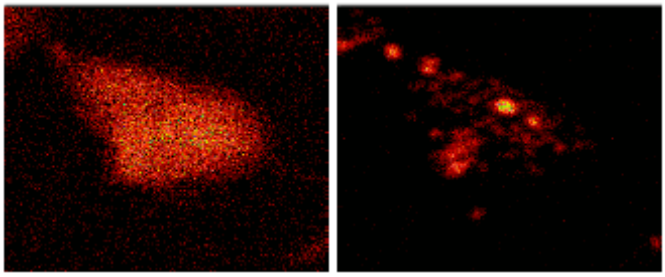


Figure 1: *Proton Induced X-Ray Emission elemental mapping of the cells A549 treated during 72 h with CeO_2 NPs. Left image demonstrate the P distribution and right image – Cerium.*

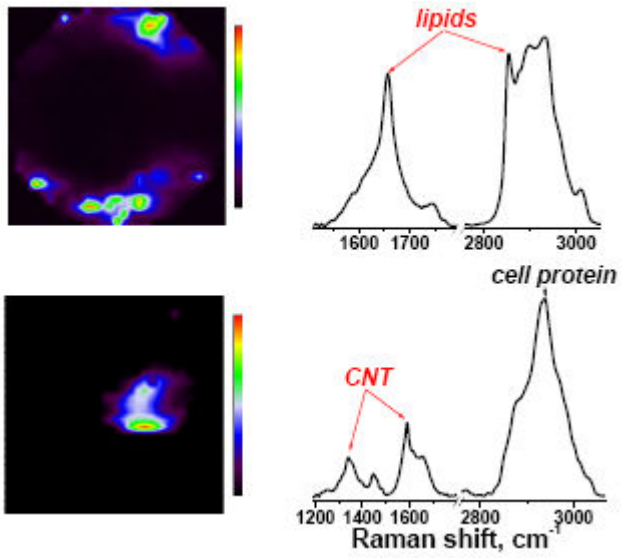


Figure 2: *CRM mappings of a HepG2 cell treated with CNT/PSPM NPs. Top and bottom images show distribution of lipid rich region and CNTs, respectively. The spectra refer to the spot of maximum concentration of both components in corresponding images of the cell.*

UNDERSTANDING INTERACTIONS OF ENGINEERED NANOMATERIALS WITH THE IMMUNE SYSTEM

Bengt Fadeel

Division of Molecular Toxicology, Institute of Environmental Medicine,
Karolinska Institutet, Stockholm, Sweden
bengt.fadeel@ki.se

Engineered nanoparticles may affect the innate or adaptive immune system; such interactions, in turn, could result in adverse outcomes or could potentially be exploited for therapeutic gain. The recognition or non-recognition of engineered nanomaterials by immune-competent cells may determine not only the toxic potential of such materials but also their biodistribution. However, understanding the physico-chemical properties that drive cellular interactions of nanoparticles remains a key challenge. Needless to say, particular attention should be paid to novel, adverse properties arising as a consequence of the nano-scale size. For instance, nanoparticles may escape immune surveillance and translocate to distal sites following entry into the body. In addition to particle size, other aspects including particle shape, and surface charge, may also play an important role for immune recognition and subsequent handling of nanomaterials.

When human subjects are deliberately exposed to engineered nanomaterials, for diagnostic or therapeutic purposes (or both), it becomes critically important to determine the ultimate fate of the nanoparticles: are engineered nanomaterials excreted from the body, or biodegraded by cells of the immune system, or do they bioaccumulate, thereby leading to potentially harmful long-term effects? The surface of nanoparticles can be modified using targeting moieties, etc but as these particles enter into a biological system, for instance via inhalation or injection into the bloodstream, it is likely that the surface of the particles is covered with biomolecules – proteins and lipids – that modify the properties of the nanoparticles and the way in which the particles interact with cells, including immune-competent cells. Moreover, the binding of proteins to nanoparticles may also induce modifications of the proteins. Understanding such nano-bio-interactions is critical for the safe application of nanoparticles in medicine.

Further reading:

[1] Fadeel B, Garcia-Bennett AE. Better safe than sorry: understanding the toxicological properties of inorganic nanoparticles manufactured for biomedical applications. Adv Drug Deliv Rev. 2010;62:362-74.
[2] Feliu N, Fadeel B. Nanotoxicology: no small matter. Nanoscale 2010;2:2514-20.
[3] Shvedova AA, Kagan VE, Fadeel B. Close encounters of the small kind: adverse effects of man-made materials interfacing with the nano-cosmos of biological systems. Annu Rev Pharmacol Toxicol. 2010;50:63-88.

THE USE OF NANOSTRUCTURED MICROPARTICLES IN A SUSPENSION BASED ADSORPTION SYSTEM FOR EXTRACORPOREAL BLOOD PURIFICATION TO TREAT ACUTE LIVER FAILURE AND SEPSIS

Dieter Falkenhagen, M.Brandl, Jens Hartmann and Viktoria Weber

Department of Clinical Medicine and Biotechnology, Danube University Krems
Krems, Austria

Introduction: Acute liver failure and sepsis are severe diseases that are characterized by high morbidity but also mortality. Especially sepsis as the general inflammatory response of a mainly bacterial infection is characterized by high mortality of 20-60% depending of the stage of the disease. Alone in the US about 200.000 patients die annually (N.Engl.J.2002) on this dangerous disease. Interestingly, also the high mortality of acute liver failure is caused by an inflammatory scenario which is caused by the disturbed liver function which in contrast to a normal liver does not inhibit the transfer of the very toxic endotoxins derived from Gram-negative germs of the intestine into the blood circulation. Therefore, sepsis as well as acute liver failure are characterized by many processes which are based on the activation of cellular mechanisms as well as communication pathways between different blood- and tissue cells which are over stimulated in a very dangerous way. We have developed a technology – the so called MDS (Microspheres based Detoxification System, D.Falkenhagen et al: Therapeutic Apheresis and Dialysis 10, 154, 2006)) which combines membrane separation and adsorption processes in an extracorporeal blood purification (EBP) – device in order to influence by separation specific signaling factors such as cytokines but also toxic substances such as endotoxins as well as liver specific metabolites such as bilirubin but also uremic toxins.

Methodology: For our investigations – neutral microadsorbents based on styrene-divinylbenzene-copolymers with different pore size dimension from 15-20 nm (type 1), 30-40 nm (type 2) and 80 to 100 nm (type 3) as an average have been developed in cooperation with the company Dow Chemical characterized all by an average diameter of 5 respectively 10µm. Those nanostructured microparticles have been analysed in batch tests but also in MDS-systems investigations with respect to the removal of

- endotoxins using the microparticles mentioned coated with polymyxin B just by adhesion forces based on hydrophobic interaction,
- bilirubin and cholic acid using the microparticles coated/uncoated with human serum albumin (HSA)
- Cytokines TNF, IL-1ß, IL-8, IL-10 investigating all microparticle mentioned coated/uncoated with human seumalbumin (HSA)

Finally systems investigation using the complete MDS with 1.2 L whole blood were performed. The microspheres containing suspension was based on 100 ml HAS coated neutral resins sized 10µm in average having an inner pore size structure of 30-40 nm.

Results: 1. Endotoxin removal from heparinized plasma was optimal using PS-resins – type 3 having an inner pore size of 80-100 nm in comparison to smaller pore size containing resins.

The immobilization of PM-B by just physical forces demonstrated highest efficiency for endotoxin removal which was not negatively influenced by autoclavation.

HSA – coated PS-resins type 2 having an inner pore size of 30-40 nm were optimal adsorbents for bilirubin being characterized by significant less protein C and S as well as fibrinogen adsorption in comparison to the HSA-non-coated resins interestingly not loosing their efficiency for bilirubin – an HSA – bound liver-specific substance.

HSA-pre-treated PS-resins type 1 characterized by an inner pore size of 10-20 nm showed nearly an untouched adsorption of different cytokines (except TNF) avoiding the adsorption of considerable amounts of protein C and S as well as fibrinogen.
HSA-coating of type 1 and 2 - resins demonstrated a significant improvement of blood compatibility especially related to the adsorption of protein C and also fibrinogen which is essential for the use of microparticles in EBP.

Systems investigations clearly demonstrated the efficiency of MDS especially in case of the removal of strongly bound biirubin but also of many cytokines such as IL 6,8,10 despite the fact that the inner filtration rate in the suspension circuit connected to the plasmafilter (FMC, P1) was by far not optimal.

Conclusion: Optimization of inner pore size structure of hydrophobic resins for adsorption seems to be a useful way to develop efficient microadsorbents for the elimination of different substances being relevant for acute liver failure and sepsis. The extracorporeal blood purification technology MDS applying the microparticles in suspension and not in a column is closed to become clinically used.

Key words: Adsorption, therapy, extracorporeal blood purification, sepsis, liver failure

IN SITU DELIVERY OF TROPHIC FACTORS AND MOLECULES IN THE HUMAN BRAIN BY CONVECTION TECHNOLOGIES

Massimo S. Fiandaca, Adrian P. Kells, Dali Yin, Ernesto Aguilar Salegio, R. Mark Richardson, Piotr Hadaczek, John Bringas, Mitchell S. Berger, John Forsayeth, and Krystof S. Bankiewicz

UCSF Neurosurgery, San Francisco, California, USA
massimo.fiandaca@ucsf.edu

Convection-enhanced delivery (CED) is a direct method of distribution for therapeutic agents within the central nervous system (CNS) that bypasses the blood-brain barrier (BBB). CED allows the homogenous distribution of a variety of molecular species within the CNS extracellular space (ECS) by developing a pressure gradient between the tip of the infusion catheter and the surrounding ECS [1]. The propagation of therapeutic materials within the resulting bulk flow of infusate is not size dependant, as it is for delivery technologies that are based upon diffusion. This fact allows the transmission of macromolecules and viruses in addition to small molecular species within the CNS via CED [2-5].

Our laboratory has been actively involved in developing a delivery platform that combines MRI and CED technologies (real-time convective delivery, RCD) [6]. We currently utilize RCD for our preclinical investigations and stress the importance for its use in clinical applications for neurodegenerative diseases [7], neuro-oncology [8], and inherited metabolic disorders [9] affecting the CNS, since it allows us to directly monitor the infusion of therapeutics within the target site. This direct visualization may not only help improve treatment efficacy for a therapeutic, by standardizing the volumetric distribution, but is also important in preventing reflux and leakage associated with CED [10].

In this presentation, we aim to review the how CED technologies function and how they may impact future treatments of human brain disorders.

References:

[1] Bobo, R. H.; Laske, D. W.; Akbasak, A.; Morrison, P. F.; Dedrick, R. L.; Oldfield, E. H., Proc Natl Acad Sci U S A 91 (1994) 2076-2080.
[2] Bankiewicz, K. S.; Eberling, J. L.; Kohutnicka, M.; Jagust, W.; Pivirotto, P.; Bringas, J.; Cunningham, J.; Budinger, T. F.; Harvey-White, J., Exp Neurol 164 (2000) 2-14.
[3] Lonser, R. R.; Schiffman, R.; Robison, R. A.; Butman, J. A.; Quezado, Z.; Walker, M. L.; Morrison, P. F.; Walbridge, S.; Murray, G. J.; Park, D. M.; Brady, R. O.; Oldfield, E. H., Neurology 68 (2007) 254-261.
[4] Morrison, P. F.; Lonser, R. R.; Oldfield, E. H., . J Neurosurg 107 (2007) 74-83.
[5] Szerlip, N. J.; Walbridge, S.; Yang, L.; Morrison, P. F.; Degen, J. W.; Jarrell, S. T.; Kouri, J.; Kerr, P. B.; Kotin, R.; Oldfield, E. H.; Lonser, R. R., J Neurosurg 107 (2007) 560-567.
[6] Fiandaca, M. S.; Forsayeth, J. R.; Dickinson, P. J.; Bankiewicz, K. S., Neurotherapeutics 5 (2008) 123-127.
[7] Fiandaca, M. S.; Bankiewicz, K. S., Curr Opin Mol Ther 12 (2010) 519-529.
[8] Dickinson, P. J.; LeCouteur, R. A.; Higgins, R. J.; Bringas, J. R.; Roberts, B.; Larson, R. F.; Yamashita, Y.; Krauze, M.; Noble, C. O.; Drummond, D.; Kirpotin, D. B.; Park, J. W.; Berger, M. S.; Bankiewicz, K. S., J Neurosurg 108 (2008) 989-998.
[9] Aguilar-Salegio, E.; Kells, A. P.; Richardson, R. M.; Hadaczek, P.; Forsayeth, J.; Bringas, J.; Sardi, P. S.; Passini, M. A.; Shihabuddin, L. S.; Cheng, S. H.; Fiandaca, M. S.; Bankiewicz, K. S., Hum Gene Ther 21 (2010) 1093-1103.
[10] Varenika, V.; Dickenson, P.; Bringas, J.; LeCouteur, R.; Higgins, R.; Park, J.; Fiandaca, M.; Berger, M.; Sampson, J.; Bankiewicz, K. S., J Neurosurg 109 (2008) 874-880.

Figures:

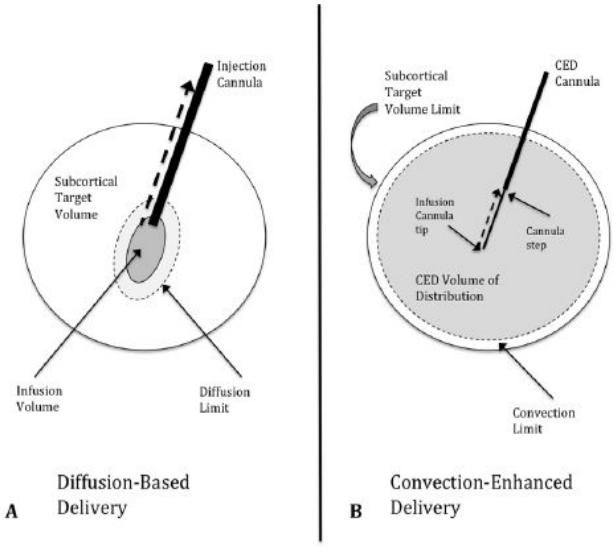


Figure 1: *Cannula-based delivery options utilized with gene therapy. (A) Diffusion-based delivery system: A characteristically large injection cannula is used to deliver the infusion volume within the target region. The infusion volume typically displaces the surrounding parenchyma at the tip of the cannula and forms a small cavity from which diffusion occurs into the surrounding brain, eventually expanding to the diffusion limit, but falling significantly short of filling the subcortical target volume. Multiple factors influence the diffusion limit for infused substances, with molecular size being one of the most significant factors. Macromolecules, proteins and viral particles are limited significantly in their ability to diffuse beyond the infusion volume. Another factor that limits the effectiveness of this technique is the development of backflow or reflux (dashed black arrow) of the infusate out of the target region, along the path of the injection cannula. (B) Convection-enhanced delivery (CED) system: Optimal CED cannulae consist of an outer guide cannula and an inner fused-silica infusion cannula that is attached to the pump mechanism that controls the rate of infusion. The infusion cannula extends beyond the guide cannula, with the transition between the two referred to as the cannula step. The infusate is delivered with a constant flow rate (most commonly 0.5 to 1.0 µl/min) from the infusion cannula tip. This flow rate establishes a pressurized extracellular bulk flow that allows the homogenous distribution of molecules of various sizes, including liposomes, proteins and viral particles, for significant distances (multiple centimeters, if necessary) from the infusion cannula tip. Reflux (dashed black arrow) typically only occurs up to the cannula step, and major backflow along the cannula and out of the target region is prevented by central placement of the step within the target volume. The convection limit can approach the subcortical target volume limit more easily compared with the diffusion-based delivery system. (Adapted with permission from Massimo S Fiandaca. © 2010 Massimo S Fiandaca)*

BIOMEDICAL APPLICATIONS OF DOUBLE-WALLED CARBON NANOTUBES AND QUESTIONS RELATED TO THEIR POTENTIAL IMPACT ON HUMAN HEALTH AND THE ENVIRONMENT

E. Flahaut, B. Pipy, L. De Gabory, L. Bordenave, F. Mouchet, L. Gauthier, A-M. Galibert, B. Soula, P. Lukanov, C. Tilmaciu, S. Benderbous, I. Loubinoux, L. Vaysse, A. Beduer, C. Vieu

Universite Paul Sabatier, France

Carbon nanotubes (CNT), with an annual world production reaching several hundreds of tons, represent a special category of nanomaterials with exceptional characteristics. In particular, they are currently investigated for many biomedical applications, ranging from medical imaging (MRI, Raman) to targeted drug delivery and cancer treatment. Tissue engineering is a field in which CNT could bring many improvements as compared to existing biomaterials, and especially for regeneration of neuronal tissues as they provide a unique combination of useful electrical and mechanical properties at the nanometer scale. Double-walled CNT [1], at the frontier between SWNT and MWNT, are very promising for applications in the biomedical field.

Recent results will be presented, showing that through surface engineering, we can direct and guide the growth and differentiation of neural cells. Topographical grooves obtained by a moulding process against a silicon master turned efficient for both neural lines cells and adult neural stem cells. Double-walled CNT [1] patterns obtained by soft lithography were also found very efficient and have the advantage of possible electrical stimulation due to their metallic electrical behaviour. In both cases, low cost fabrication processes (moulding or soft-lithography) are developed, enabling further large scale applications for biological or medical purposes. Potential application of DWNT for medical imaging (MRI) and gene delivery will also be presented.

Although the toxicity and the environmental impact of CNT have now both been investigated by many different groups (although the latter never focused much attention until very recently), there is yet a controversy about the results and still no answer to the simple question: "are CNT toxic?" The fact is that the large range of kinds of CNT and methods to produce and then (in most cases) process them make any comparison almost impossible. The results presented here were obtained with the same batch of CCVD-produced DWNT [1] investigated for biomedical applications, and concern both the investigation of their potential impact on human health (*in vitro* / *in vivo* models) [2, 3] and the environment (*in vivo* models) [4]. They lead to the conclusion that all the experimental parameters (dealing both with CNT and biological models used) play a very important role and can easily explain the large differences between the results obtained by the different researchers.

References:

[1] E. Flahaut et al., Chem. Comm. (2003) 1442.
[2] E. Flahaut, M.C. Durrieu, *, Ch. Baquey, Carbon, 44, (6), (2006), 1093-1099
[3] J. C. Debouzy, D. Crouzier, E. Flahaut, Env. Toxicol. Pharmacology, 30, (2), (2010), 147-152 [4] E. Flahaut, Nanomedicine, 5, (6), (2010), 949-590

ELECTROCHEMICAL APTASENSORS BASED ON CARBON NANOTUBES AND CONDUCTING POLYMERS

Tibor Hianik[1], Maja Šnejdarková[2], Alexandra Poturnayová[2], Anna Porfireva[3], Gennady Evtugyn[3], Anna Miodek[4], Hafsa Korri-Youssoufi[4], Isabel Neudlinger[5], Ivana Karpisova[1], Christian Rankl[5], Peter Hinterdorfer[5], Andreas Ebner[5]

[1]Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynská dolina F1, 842 48 Bratislava, Slovakia
[2]Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, 900 28 Ivanka pri Dunaji, Slovakia,
[3]Analytical Chemistry Department, Kazan Federal University, 18 Kremlevskaya Street, Kazan, 42008, Russian Federation,
[4]Equipe de Chimie Bioorganique et Bioinorganique, CNRS UMR 8182, Institut de Chimie Moléculaire et de Matériaux d'Orsay, Université Paris-Sud, Bâtiment 420, 91405 Orsay, France,
[5]Biophysics Institute, Johannes Kepler University Linz, Altenbergertrasse 69, 4040 Linz, Austria
tibor.hianik@fmph.uniba.sk

DNA and RNA aptamers are single stranded oligonucleotides with high affinity to proteins or other ligands, comparable to those of antibodies. The aptamers are selected in vitro by the SELEX method [1]. In solution, the aptamers maintain an unique configuration that contains specific binding site to the ligand. Aptamers can be easily modified by biotin, SH or amino- groups, leading to a variety of immobilization strategies on solid supports. Using simple molecular engineering based on DNA hybridization it is possible to create aptamer dimers with two binding sites like that in antibodies [2]. These aptamer dimers (aptabodies) are characterized by improved sensitivity to the analyte, for example to thrombin. We have shown that typical guanine quadruplexes that form binding site for thrombin are stable in aptamer dimers [3]. Currently there is increased interest in development of aptamer based biosensors for detection proteins and other molecules using various methods of detection, such us optical, acoustical and electrochemical [4,5]. These biosensors could be used in fast and low cost medical diagnostics. The sensitivity of detection depends not only on the selectivity of binding site, but also on supporting part of the aptamer that serve for immobilisation onto a solid support. Using multiwalled carbon nanotubes (MWCNTs) as an immobilization matrix we developed high sensitive biosensor for detection human thrombin [2] and cellular prions (PrP^C) [6] in biological liquids. In this work we analyzed in detail the properties of DNA aptasensors sensitive to thrombin, immobilised either on a gold support covered by neutravidin or on a surface of MWCNTs. We have shown that immobilisation of aptamers and aptamer dimers at MWCNTs improved the sensitivity of the sensor for thrombin and allowing detection in a complex matrix such as blood plasma. Using single molecule force spectroscopy (SMFS) we studied in detail the forces between enhanced single stranded aptamers (BFA) and aptamer dimers (BFF) immobilised on an AFM tip and the thrombin immobilised on a mica surface. By varying the pulling velocity in force distance cycles the formed thrombin – aptamer complexes were ruptured at different force loading allowing determination of the energy landscape. It turned out that the BFA aptamer shows a higher binding force at the investigated loading rates and a significant lower dissociation rate constant, k_{off}, compared to BFF. The lower binding strength of BFF in comparison with those of BFA may be due to certain sterical hindrance between two G-quadruplexes of this aptamer dimer. However, the potential of the aptabody BFF to form more stable double bound complex could clearly be shown.

Using thickness shear mode acoustic method (TSM) we analyzed in detail the interaction of thrombin with DNA aptamers of various configurations immobilised at the neutravidin layer chemisorbed on TSM quartz crystal transducer and showed enhanced sensitivity for detection thrombin by aptamer heterodimer (BFH) [8]. The obtained results agree well with those of SMFS studies.

By means of electrochemical quartz crystal microbalance method (EQCM) we performed comparative analysis of the sensitivity of DNA aptamers and antibodies specific to human cellular prions (PrP^C) immobilised on a surface of MWCNTs (Fig. 1). We found that the detection limit (LOD) for both aptamers (50 pM) and antibodies (20 pM) was rather low indicating high, but comparable sensitivity (Fig. 2). The LOD was also much better in comparison with immobilisation of aptamers on a surface of conducting co-polymers and using QCM and surface plasmon resonance (SPR) as detecting

methods. Higher stability of aptamers in comparison with antibodies and possibility to easy regenerate aptasensors make them rather promising candidates for practical applications.

Acknowledgements:

Financial support of Agency for Promotion Research and Development under the projects No. LPP-0341-09 and SK-FR-0025-09, Slovak Academy of Sciences under the project mnt-era.net (proposal No. 2009-50), VEGA 1/0794/10 and COST TD1003 is gratefully acknowledged.

References:

[1] A.D. Ellington, J.W. Szostak, Nature, 346 (1990) 818.
[2] T. Hianik, A. Porfireva, I. Grman, G. Evtugyn, Protein and Peptide Letters, 15 (2008) 799.
[3] S. Ponikova, K. Tlučková, M. Antalík, V. Víglaský, T. Hianik, Biophys. Chem., 2011, in press
[4] T. Hianik, J. Wang, Electroanalysis, 21 (2009) 1223.
[5] M. Mascini (Ed.) Aptamers in Bioanalysis, Wiley, New Jersey, 2009.
[6] T. Hianik, A. Porfireva, I. Grman, G. Evtugyn, Protein and Peptide Letters, 16 (2009) 363.
[7] T. Hianik, I. Grman, I. Karpišová, Chem. Commun. (2009) 6303.

Figures:

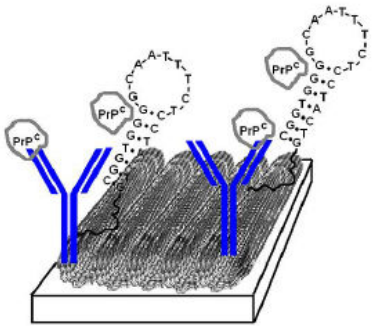


***Figure 1**: Schematic representation of the sensor surface composed of MWNTs and immobilised DNA aptamers and antibodies with bounded PrP^c.*

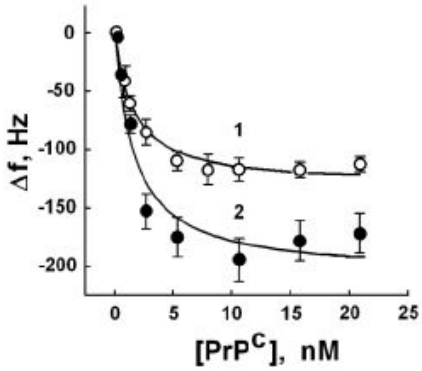


***Figure 2**: The plot of the frequency changes as a function of PrPC concentration for biosensor based on: 1 – single-stranded aptamer and 2 – BAR 223 antibody immobilized on the surface of MWCNTs. The points are experimental results and the lines are fit according to Langmuir isotherm (error bars: SD, N = 3) [7].*

FIELD FLOW FRACTIONATION COUPLED WITH LIGHT SCATTERING AND ICP-MS FOR QUANTITATIVE BIO-NANO MEASUREMENTS

Erik H. Larsen, Katrin Löschner, Bjørn Schmidt

Technical University of Denmark, National Food Institute, 19, Mørkhøj Bygade, DK-2860, Denmark
Ehlar@food.dtu.dk

An instrumental platform based on asymmetric flow field flow fractionation (AFFF) coupled with classical multi angle light scattering (MALS), dynamic light scattering (DLS) and ICP-MS was established and applied to the study of silver, gold and selenium NPs in biological materials.

Using the platform, gold nanoparticles (10-60 nm o.d.) were separated and quantified with respect to their size and mass concentration (Fig. 1), and figures of merit including LOD, recovery and repeatability were established [1]. The bare gold NPs adhered to instrument surfaces, as demonstrated by electron microscopy, and caused incomplete recoveries. Gold NPs were administered by intravenous injection and recovered from rats' livers following alkaline solubilisation of the tissue. The gold NPs were successfully stabilised with bovine serum albumin (BSA) in the alkaline suspension, but TEM imaging showed that the NPs were associated with un-digested tissue residues, which precluded appropriate separation by AFFF.

The platform was used to the study absorption, distribution, metabolism and excretion (ADME) in rats of BSA-stabilised Se^0 NPs (20 nm o.d.) using selenite as positive control. The results (Fig. 2) showed that selenium as nano-Se^0 particles or as selenite both were highly bio-available and mainly were deposited in liver and kidney or excreted via urine and feces. Se^0 was detected in tissues of rats following in situ reaction with sulphite to form the selenosulfate anion, which was determined by HPLC-ICP-MS. The finding of Se^0 both in tissues from nano- Se^0 and selenite dosed animals brings new information to the current knowledge about metabolic pathways of selenium.

Research on dedicated methods for sample preparation of food prior to silver NP detection is underway in the NanoLyse project, funded by the European Commission (www.nanolyse.eu)

The general conclusion of our work on biological research with nanoparticles is that access to a variety of tools and methods, including appropriate sample preparation, separation and atomic spectrometric detection and electron microscopy, are necessary for trouble shooting and acquisition of robust data.

References:

[1] Bjørn Schmidt, Katrin Loeschner, Niels Hadrup, Alicja Mortensen, Jens J. Sloth, Christian Bender Koch and Erik H. Larsen, Quantitative Characterization of Gold Nanoparticles by Field-Flow Fractionation Coupled On-line with Light Scattering Detection and Inductively Coupled Plasma Mass Spectrometry, Anal. Chem., (submitted)

Figures:

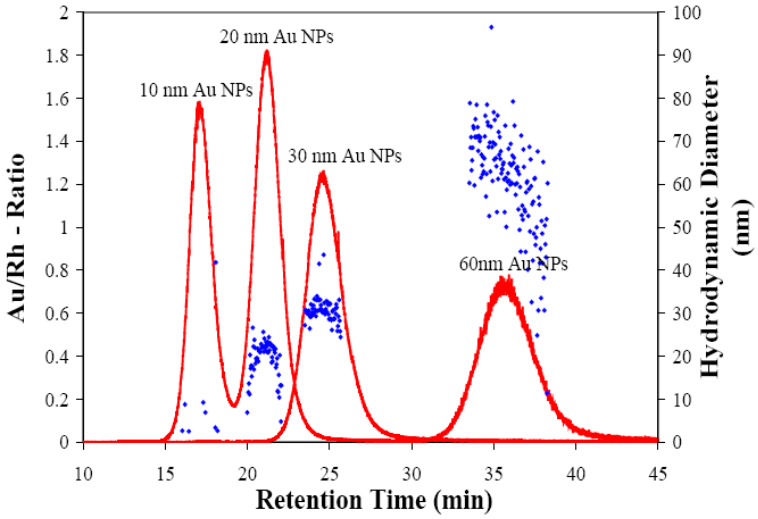


Figure 1: *AFFF-LS-ICP-MS fractogram and hydrodynamic diameter of a mixture of 4 gold nanoparticles*

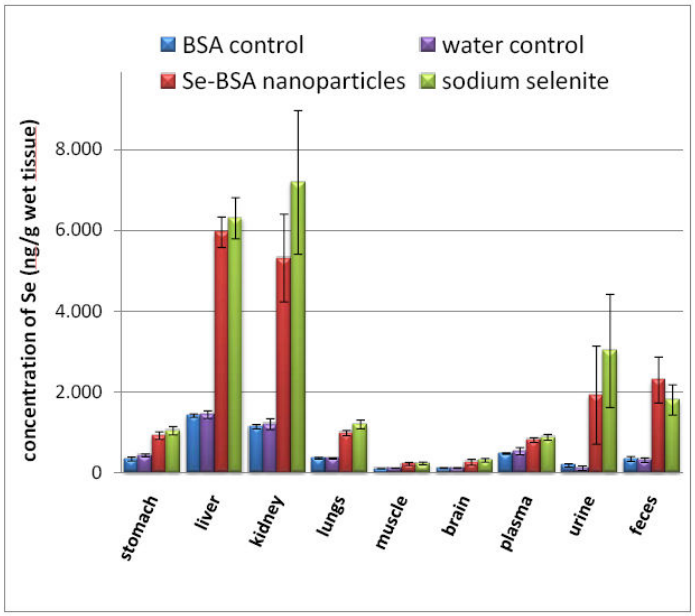


Figure 2: *Distribution of selenium in biological samples from rats administered selenium at 0.4 mg/kg b.w./day for 28 days as BSA-stabilised nano-Se^0 or selenite*

FUNCTIONALIZED NANOPARTICLES FOR BIOMOLECULAR IMAGING AND SENSING

Raphaël Lévy, Yann Cesbron, Chris Shaw, Umbreen Shaheen and Daniel Nieves

Institute of Integrative Biology, Crown Street, L69 7ZB Liverpool, United Kingdom
http://raphazlab.wordpress.com/, rapha@liverpool.ac.uk

Gold nanoparticles have been used for several decades as labels in immuno-electron microscopy. In the last decades, progress in the synthesis, functionalization and optical detection of these particles have opened a range of new applications in imaging and sensing. For most of these applications, the capping layer is a critical feature as it provides colloidal stability and control specific and unspecific interactions.

The presentation will cover three related themes of research. First, I will present the synthesis and characterization of peptide-capped gold nanoparticles [1] and discuss our efforts to generate complex and controlled nanomaterials based on self-assembly of these small molecules at the surface of the particles. Then, I will report on the entry and fate of the particles in live cells, with a particular focus on the fate of the capping layer [2]. Finally. I will discuss our current attempts to break the barrier of endocytotic trapping of the nanoparticles using a variety of methods including toxins, signalling peptides and direct injection.

References:

[1] Rational and combinatorial design of peptide capping Ligands for gold nanoparticles; Lévy, R., Thanh, N.T.K., Doty, R.C., Hussain, I., Nichols, R.J., Schiffrin, D.J., Brust, M. and Fernig, D.G. (2004) . J. Am. Chem. Soc. 126, 10076-10084

[2] Cathepsin L Digestion of Nanobioconjugates upon Endocytosis; Violaine Sée, Paul Free, Yann Cesbron, Paula Nativo, Umbreen Shaheen, Daniel J. Rigden, David G. Spiller, David G. Fernig, Michael R. H. White, Ian A. Prior, Mathias Brust, Brahim Lounis and Raphaël Lévy, ACS Nano, 2009, 3, 2461-2468

DIAGNOSTIC AND THERAPEUTIC CHALLENGES IN NEURODEGENERATIVE DISEASES

Gurutz Linazasoro

INBIOMED Foundation, San Sebastián, Spain

Nanotechnology will play a key role in developing new diagnostic and therapeutic tools. Nanotechnologies use engineered materials with the smallest functional organization on the nanometre scale in at least one dimension. Some aspects of the material can be manipulated resulting in new functional properties.

Neurodegenerative diseases of the elderly currently represent a major challenge to the health care system. With the increasing longevity of the general population, the predicted figures for the prevalence of these disorders and their global financial impact are truly staggering. For example, it has been estimated that by 2030 as many as 7.7 million people in the US will have Alzheimer's disease, and by 2050 this number will reach approximately 13.5 million, with total annual costs for care rising from $172 billion in 2010 to $1.08 trillion in 2050. This analysis does not take into account the value of unpaid care provided by families and others, estimated to have been $144 billion in 2009. In the UK, the cost of dementia is now estimated to exceed the combined cost of cancer, heart disease and stroke. The prevalence of Parkinson's disease is expected to double by the year 2030, and, given the greater risk of Parkinson's disease dementia in elderly individuals with Parkinson's disease, it is expected that the health care costs for this disorder will increase even more relative to the prevalence of the disease. These concerns highlight the urgent need for the development of effective disease-modifying therapies. Effective treatment would have a major influence on the economic and social burden of these age-related disorders. For example, in the case of Alzheimer's disease, it has been estimated that a delay in onset by 5 years would translate into a 50% decrease in disease prevalence, and a delay of 10 years would result in a virtual disappearance of the disease. Unfortunately, despite considerable investment, to date all attempts at developing such treatments have failed. In summary, there is an urgent need of developing reliable biomarkers of very early phases of the neurodegenerative process and to discover effective therapies directed at the core of the biological process in order to stop disease progression.

Besides this general and urgent need, there are many other key points in the diagnosis and tretatment of neurodegenerative diseases that can be targeted by nanotechnologies. Among them it is worth mentioning:

1. - Early diagnosis:
   a) To monitor the rate of cell loss in brain nuclei and alert the defence system and the compensatory mechanisms systems (neurogenesis included) when the rate is exceeding the normal expected decline.
   b) To monitor the state of defence and compensatory mechanisms.
   c) To create biosensors to monitor neurotransmitter levels in precise locations within the brain which can be reduced long before the appearance of symptoms.
   d) To improve image technologies (Fluorophores and quantum dots) which may also facilitate surgical approaches.
   e) Systems to detect abnormalities in plasma, thus avoiding the need of invasive tools.

2. - Study of pathogenetic mechanisms. To increase our knowledge about the mechanisms of cell death, thereby opening the doors to new drug targets.

3. - Treatment
   - To direct drugs to their target in a very specific way: Smart targeted drug delivery systems. Drugs can act on signalling pathways required for neurotransmission (symptomatic effect) or in signalling pathways involved in neurodegeneration (neuroprotection and neurorescue). (eg. Nanoparticles, drug encapsulation strategies, multifunctional nanotherapeutics, ablation of areas with nanoparticles, DNAbots to identify and destroy pathogenic proteins,...)
   - To develop systems able to overcome the blood brain barrier (eg, biobar codes)
   - To create biosensors to monitor neurotransmitter levels in precise locations within the brain.

- Intracellular manipulations and interventions: To repair DNA and other damages, cleaning of deposits of aggregated abnormal proteins,…
- To introduce genes and proteins required for normal functioning in a highly controlled way (durable and controlled expression of the gene) avoiding the needs of viral vectors and complex control systems or of infusion pumps (Eg organic silica particles)
- To create bridges between different nuclei affected by the degenerative process and to favour their development by blocking the expression of antireparative signals (no-go, etc)
- Creation of media to push the development of functional specific type of neurons from stem cells.

All these points can be considered as opportunities for nanotechnologies. Indeed, the NEURONANO era is here.

LAYER-BY-LAYER ASSEMBLY OF BIOMOLECULE-SILICA NANOPARTICLE HYBRIDS FOR ELECTROCHEMICAL BIOSENSORS

Sven Christian Feifel and **Fred Lisdat**

Biosystems Technology, Technical University Wildau Bahnhofstrasse1, 15745 Wildau, Germany
flisdat@th-wildau.de, feifel@th-wildau.de

Biomolecule-nanoparticle hybrid systems have excellent prospects for interfacing biological recognition events with electronic signal transduction so as to design a new generation of bioelectronic devices with high sensitivity. Direct electron transfer between redox proteins and electrodes is of practical and theoretical interest and can be improved by electrode or protein modification. Communication between proteins immobilized in multiple layers on the electrode can be achieved by in situ generation of small shuttle molecules or more advantageously by direct interprotein electron transfer. This allows the construction of new sensing electrodes.[1,2] As a new approach we have tested the use of modified silica nanoparticles (SiNPs) for the built up of fully electro-active cytochrome c (CytC) multilayer assemblies. For this purpose silica nanoparticles of different size are synthesized by adjustment of the Stöber method[3] and the SiNPs were modified by silan-based chemistry[4], to be applied for assembly formation by the layer-by-layer deposition technique.

In this study we use carboxy-modified SiNPs to provide an artificial environment - similar to that of the redox protein in the native system - to construct fully electro-active CytC multilayer architectures. The particles are characterized by dynamic-light-scattering (DLS), zeta-potential and FT-IR. The conditions of assembly formation and stability are determined by QCM. The electrochemical properties of the multilayer assemblies are analyzed by cyclic voltammetry (CV). Special focus is on the size influence of the SiNPs and the electron transfer ability of the multilayer assembly, in dependence on the deposited protein layers. This novel approach may provide a general way to fabricate enzyme multilayers useful in practical applications for biosensors. A future aim is the embedment of specific enzymes into these assemblies to obtain sensorial signal chains.

References:

[1] F. Lisdat, R. Dronov, H. Möhwald, F. W. Scheller, D. G. Kurth,; Chem. Commun. 2009, 274-283.
[2] S. M. Bonk, F. Lisdat; Biosens. Bioelectron. 2009, 25, 739-744.
[3] W. Stöber, A. Fink, E. Bohn; J. Colloid Interface Sci. 1968, 26, 62-69.
[4] R. D. Badley, W. T. Ford, F. J. McEnroe, R. A. Assink; Langmuir 2006, 22, 4357-4362.

Figures:

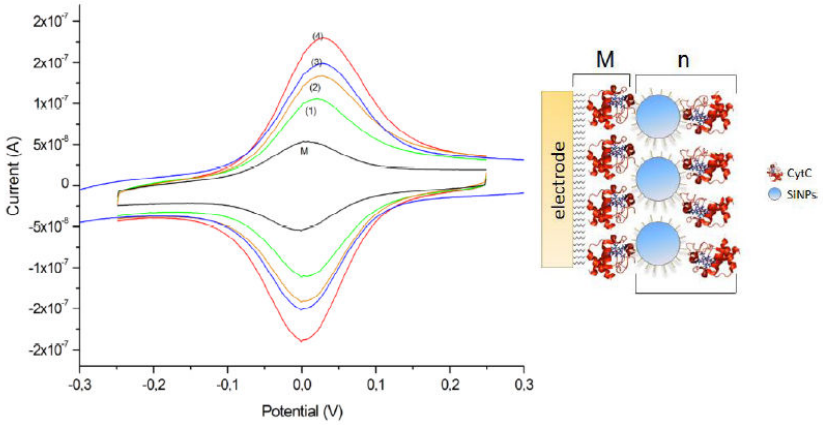


Figure 1: *Cyclic voltammetry of SiNPs/CytC multilayer assemblies, (M) Au-MU/MUA-CytC, (1) M-[SiNPs/CytC]$_1$, (2) M-[SiNPs/CytC]$_2$, (3) M-[SiNPs/CytC]$_3$, (4) M-[SiNPs/CytC]$_4$ for comparison (scan rate100 mV/s, KPP pH7).*

SYNTHESIS OF POSITRON EMITTER LABELED METAL OXIDE NANOPARTICLES FOR BIODISTRIBUTION STUDIES BY DIRECT ACTIVATION WITH HIGH ENERGY PROTONS

Jordi Llop[1], Carlos Pérez Campaña[1], Vanessa Gómez-Vallejo[1], Eneko San Sebastián[1], Abraham Martín[1], Torsten Reese[1], R.Ziolo[3], Sergio E. Moya[2]

[1]Molecular Imaging Unit, CIC biomaGUNE, Paseo Miramon 182, San Sebastian, Spain
[2]Biosurfaces Unit, CIC biomaGUNE, Paseo Miramon 182, San Sebastian, Spain
[3]Advanced Materials Dept. CIQA, Blvd. Enrique Reyna Hermosillo 140, Saltillo, Mexico
jllop@cicbiomagune.es

Nanoparticles (NPs) are widely used and have potential applications in different areas like health care, electronics, manufacturing, food industry, etc. The widespread use of NPs raises several issues regarding their possible toxicological end points. A key issue regarding the study of the possible toxicological effects of NPs is to determine their biological fate and biodistribution. For this, the use of animal models and the application of techniques such as Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) become highly necessary. There is, therefore, an increasing interest for the development of radiolabeling strategies of such NPs, either to determine their pharmacokinetic properties or to assess potential toxicological effects related to long term exposure.

The objectives of the present work are:

1. To develop a new strategy for the introduction of a radioactive atom (positron emitter) in the core of metal oxide NPs.
2. To characterize NPs before and after irradiation to evaluate the effects of activation in the physic-chemical properties.
3. To perform preliminary in vivo biodistribution studies in rodents.

Aluminum oxide NPs incorporating Oxygen-18 in their crystalline structure (which can be activated to the positron emitter Fluorine-18, half-life = 110 min) were synthesized by dissolving an aluminum salt ($AlCl_3$, $Al_2(SO_4)_3$) in [^{18}O]H_2O in the presence of a base (NH_4OH, Urea). NPs were irradiated with 18 MeV protons in an IBA cyclone 18/9 cyclotron. The activated NPs were introduced in a specifically designed phantom and measured with a PET camera (eXplore Vista-CT, GE Healthcare) for 6 hours in 1-20 minutes frames. Time-Activity Curves were obtained and the percentage of the activity as ^{18}F was determined. In the best scenario ($AlCl_3$ / NH_4OH) up to 570 MBq/g of ^{18}F were produced in 6 minutes (Beam intensity on target: 5 μA, beam current: 0.5 μAh). Characterization by means of TEM, light Scattering and X-Ray showed no significant changes in crystal structure, crystal size and NP size after irradiation. In vivo studies were performed using PET after administration of the radioactive NPs to mice and rats (I.V. and oral). Images were co-registered with MRI to localize anatomically regions of interest (ROIs) and Time-Activity curves were determined for liver, kidneys, brain, lungs, stomach, intestine and bladder.

In conclusion, metal oxide NPs containing ^{18}O could be synthesized and activated by bombardment with high energy protons. The irradiation process did not introduce significant changes in particle size and crystal structure. Final amount of radioactivity was sufficient to perform whole body in vivo biodistribution studies in rodents.

ANIMAL MODELS OF DISSEMINATED DISEASE FOR THE DEVELOPMENT OF NANOPARTICLE-DIRECTED DELIVERY OF CANCER THERAPY

R Mangues[1,2], P Álamo[1,2], R Bosch[1,2], R Diéguez[1,2], MJ Moreno[1,2], U Unzueta[1,2], Neus Ferrer-Miralles[1,3], A Villaverde[1,3], M Trias[1,4], J Pavía[1,5], S Rojas[1,5], R Herranz[1,5], L Mondragón[1,6], I Abasolo[1,7], M Royo[1,8], R Martinez[1,6], S Schwartz[1,7], F Albericio[1,8], E Vázquez[1,3], I Casanova[1,2], MV Céspedes[1,2]

[1]CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN) Bellaterra, Barcelona, Spain
[2]Grup d'Oncogènesi i Antitumorals, Institut de Recerca, Hospital de Sant Pau, Barcelona, Spain
[3]Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, Bellaterra, Spain
[4]Departament de Surgery, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain
[5]Grupo de Imagen Biomédica, Universidad de Barcelona, Spain
[6]Grupo de Química Molecular Aplicada, Universidad de Valencia, Spain
[7]CIBBIM Nanomedicine, Vall d'Hebron University Hospital, Spain
[8]Grupo de Química de Péptidos y Nanopartículas del Parc Cientific de Barcelona-UB, Spain
rmangues@santpau.cat

Nanomedicine offers an unprecedented opportunity for targeted drug delivery. The ability of nanometric size particles to enter cells through receptor-mediated endocytosis opens the possibility of designing vehicles for the specific delivery of therapy to disease cells. This directed delivery will highly increase the therapeutic window, by reducing the toxicity on normal cells whereas achieving an enhanced therapeutic response. Even rational therapies (e.g. monoclonal antibodies (mAb)) have limiting toxicities because they are biodistributed to both normal and diseased cells [1]. Thus, the combined effect of a specific delivery vector and a targeted drug, loaded into it, promises to improve current therapeutic approaches.

Most cancer patients have macroscopic or subclinical metastases at diagnosis. Moreover, therapy for metastatic cancer is faced with high level of recurrence, acquired resistance and systemic toxicity [2]; thus, most patients nowadays still die because of metastases [3]. Novel targeted therapies, including mAbs have not significantly improved outcome in this disease [4]. Existing cancer therapies, including mAbs have been developed to block the primary tumor rather than the metastatic process. However, it is known that the same tumor displays a differential regulation of the cell cycle and cell death pathways [5] and a dramatically different response to the same drug [6] depending on the organ where it is growing. The fact that currently used preclinical drug development models (mainly subcutaneous xenografts) do not predict clinical response to antitumor drugs [7] could be in part due to these differences.

Animal models of disseminated disease can be used to assess the efficacy of targeted delivery systems as well as antimetastatic effect. We have developed orthotopic xenograft models of human colorectal and pancreatic tumors, as well as human lymphoma and leukemia, which closely reproduce the metastatic pattern observed in humans [8-11]. We are characterizing tumor cells for their mutational spectrum, to define targets for therapy, as well as the over-expression of membrane receptors, to identify target receptors for the selective delivery of therapy. The design of targeted vectors able to undergo receptor-mediated endocytosis in tumor cells and their ability to selectively deliver targeted therapy, against the oncogenes driving the tumor, inside tumor cells is being tested in such models. This approach may have a significant impact on cancer therapy [12]. Our capacity of using non-invasive radioactive, fluorescent or optical methods, to assess nanoparticle biodistribution and antimetastatic effect, will greatly facilitate our ability to evaluate the effectiveness of these novel therapies.

References:

[1] Adams GP, Weiner LM. Monoclonal antibody therapy of cancer. Nat Biotechnol 2005, 23(9):1147-57

[2] Guyot F, Faivre J, Manfredi S, Meny B, Bonithon-Kopp C, Bouvier AM. Time trends in the treatment and survival of recurrences from colorectal cancer. Ann Oncol. 2005, 16(5):756-61

[3] Coia LR. Cancer Management, 4th ed. Huntington, NY: PRR.Inc, 2000:273-99;

[4] VanCutsem E et al. Cetuximab and Chemotherapy as Initial Treatment for Metastatic Colorectal Cancer. N Engl J Med 2009, 360(14):1408-17.

[5] Farre L, Casanova I, Guerrero S, Trias M, Capella G, Mangues R. Heterotopic implantation alters the regulation of apoptosis and the cell cycle and generates a new metastatic site in a human pancreatic tumor xenograft model. FASEB J. 2002 Jul;16(9):975-82. (IF: 6,820) PMID: 12087058

[6] Teicher BA. Xenografts of EMT-6 mouse mammary tumor cells. Mol Cancer Ther 5(10):2435, 2006

[7] Johnson JI et al. Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials. Br J Cancer. 2001 May 18;84(10):1424-31.

[8] Guerrero S, Figueras A, Casanova I, Farre L, Lloveras B, Capella G, Trias M, Mangues R. Codon 12 and codon 13 mutations at the K-ras gene induce different soft tissue sarcoma types in nude mice. FASEB J. 2002 Oct;16(12):1642-4.

[9] Céspedes MV, Espina C, García-Cabezas MA, Trias M, Boluda A, Gómez del Pulgar MT, Sancho FJ, Nistal M, Lacal JC, Mangues R. Orthotopic microinjection of human colon cancer cells in nude mice induces tumor foci in all clinically relevant metastatic sites. Am J Pathol 170 (3):1077-1085, 2007.

[10] Cespedes MV, Casanova I, Parreño role in M, Mangues R. Mouse models in oncogenesis and cancer therapy. Clin Transl Oncol. 2006 May;8(5):318-29.

[11] Céspedes MV, Larriba MJ, Alamo P, Casanova I, Parreño M, Feliu A, Sancho FJ, Muñoz A and Mangues R. Site-dependent E-cadherin cleavage and nuclear translocation in a metastatic colorectal cancer model. Am J Pathol. 177(4):2067-79, 2010.

[12] Vazquez E, Ferrer-Miralles N, R. Mangues, J. L. Corchero, Schwartz S Jr, and A. Villaverde, Modular protein engineering in emerging cancer therapies, Curr. Pharm. Des, 15 (2009) 893-916.

MICROFLUIDICS & MINIATURIZATION - THE TEENAGE YEARS ARE OVER...

Andreas Manz

KIST Europe, Saarbruecken, Germany
manz@kist-europe.de

After a brief overview of historic micro- and nanotechnology for “lab on chip” devices, I will focus on 2 examples of near market applications: Bacteria causing infections can be identified by detecting specific DNA sequences. This method is widely used and has a high degree of specificity. However, for this purpose, the micro organisms will have to be captured, and it is not clear whether they are dead or alive. Metabolite studies have the advantage that remote evidence for live cells can be identified.

I will present portable equipment for volatile metabolite quantification, based on multi-capillary GC and ion-mobility detection [1]. The raw data is mathematically treated to receive best discrimination of patient groups. Applications for diagnostics of lung infections and lung cancer will be shown. In addition, a chip-based hand held real-time PCR instrument will be presented. A "virtual reactor" approach, i.e., a free droplet in oil, is used for thermal cycling and subsequent melting temperature profiling of the PCR product [2].

References:

[1] Perl, T., Vautz, W., Nolte, J., Baumbach, J.I. & Quintel, M. Ion mobility spectrometry of human pathologic bacteria - metabolic profiling by volatile organic compounds. Infection 37, 24-24 (2009).

[2] Pavel Neuzil, Chunyan Zhang, Juergen Pipper, Sharon Oh, Lang Zhuo, Ultra fast miniaturized real-time PCR: 40 cycles in less than six minutes. Nucleic Acids Research, 2006, Vol. 34, doi:10.1093/nar/gkl416

New Nanobiotechnological Approaches For The Diagnostic Of Infectious And Cardiovascular Diseases

M.-Pilar Marco, Carme Pastells, Pablo Salvador, Gloria Colom, Nuria Sanvicens and Nuria Pascual

Applied Molecular Receptors Group (AMRg). Institute for Advanced Chemistry of Catalonia (IQAC) of the Spanish Council for Scientific Research (CSIC)
CIBER de Bioingeniería, Biomateriales y Nanomedicina, Spain
pilar.marco@cid.csic.es

Future trends in medicine demand for rapid, reliable diagnostic technologies able to assist doctors on a more personalized and efficient medicine. At the same time, the so-called “omic” technologies have accelerated the number of candidate biomarkers discovered pointing to a future in which the heath status or disease of an individual will be established based on molecular signatures or footprints showing if there is any alteration on the biomarker expression profiles. Biotechnology, microtechnology and more recently nanotechnology are the most promising emerging technologies of the last decades. At the interface of these sciences lies the nanobiotechnology (or the micro/nanobiotechnology) which makes use of the knowledge from these fields to create biological micro/nanosystems and biofunctional devices. The Nanobiotechnology and Biomolecular Diagnostics research line of the CIBER-BBN supports research in this direction with a clear aim to translate the results into the clinical arena. With this final goal, several ongoing projects are addressing the possibility to develop a new generation of improved diagnostic devices and biosensing systems based on novel nanobiotechnological approaches.

Examples illustrating the use of nanoparticles, nanostructured materials and microelectronic devices to create functional biohybrid materials for the detection of bioactive substances will be presented in this oral communication. Thus, reliable methods for rapid, selective detection of pathogens for diagnosing infectious diseases, being necessary single-cell detection for certain types of body fluids (ex. blood or cerebrospinal fluid) and microorganisms (Pseudomonas aeruginosa, Staphylococcus aureus, etc), causing nosocomial diseases. On the other hand, cardiovascular diseases (CVD) are a major cause of death in Europe. The disease develops through a series of consecutive steps in which several validated and candidate biomarkers have been identified. Development of diagnostic methods able to simultaneously detect a panel of representative biomarkers of each of these stages would allow early-diagnosis before heart failure occurs. Regarding multiplexation, an interesting approach is the use of nanoparticles with distinct optical or electrical properties, such nobel metal nanoparticles, quantum dots (QDs) or other types of nanoparticles that show great potential for multiplexation. As an example, a fluorescent quantum dot (QD)-based antibody array has been developed to detect Escherichia coli in serum samples. The microarray reaches a detectability of 1CFU mL^{-1}, three order of magnitude lower than the ELISA (enzyme-linked immunosorbent assay) using the same immunoreagents [1,2]. On the other hand, the localized surface plasmon resonance of noble metal nanorods with different aspect ratio allows envisaging the possibility to develop cost-effective multiplexed devices [3]. Similarly, in respect to electrochemical nanoprobes made of metal sulfides with distinct redox potentials. Finally, strategies for creating universal diagnostic devices based on DNA-directed immobilization approaches will also be discussed.

References:

[1] Sanvicens, N.; Pascual, N.; Fernandez-Arguelles, M. T.; Adrian, J.; Costa-Fernandez, J. M.; Sanchez-Baeza, F.; Sanz-Medel, A.; Marco, M. P. Analytical and Bioanalytical Chemistry 399, 2011, , 2755.
[2] Sanvicens, N.; Pastells, C.; Pascual, N.; Marco, M. P. TrAC - Trends in Analytical Chemistry 28, 2009, 1243.
[3] Mannelli, I.; Marco, M. P. Analytical and Bioanalytical Chemistry 399, 2011, 1923.

Figures:

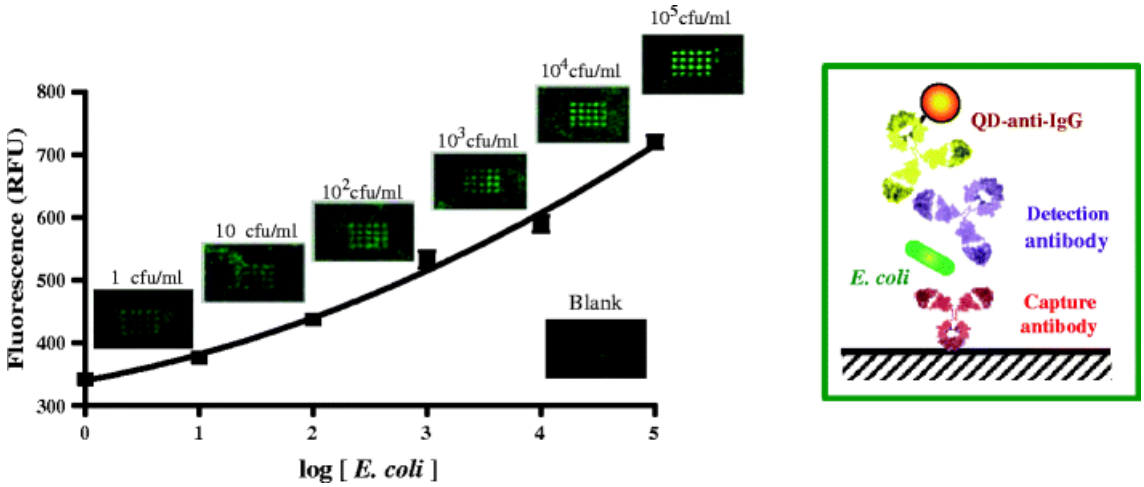


Figure 1: *Dose-response curve for E. coli O157:H7 in a sandwich array-based assay. The standard curve was fitted to a quadratic polynomial equation as indicated by Herman et al [20] and the LOD was calculated using the statistical approach reported by Long and Winefordner [21]. Results correspond to the average and standard deviation of four assays run on 4 different days in duplicate (n = 12)*

TARGETING CANCER AND ACTIVATION OF THE IMMUNE SYSTEM WITH QUANTUM DOTS

Carlos Juan C. Mareque-Rivas[1,2,3], Nina Gomez-Blanco[1], Malou Henriksen-Lacey[1], Carmen Rodriguez-Maldonado[3], Martin Krembuszewski[3], Tom Barr[4] and David Gray[4]

[1]CIC biomaGUNE, Biofunctional Nanomaterials Unit, Paseo Miramón 182, 20009 San Sebastian, Spain
[2]Ikerbasque, Basque Foundation for Science, 48011 Bilbao, Spain
[3]School of Chemistry, University of Edinburgh, Edinburgh, United Kingdom EH9 3JJ
[4]Institute of Immunology & Infection Research, School of Biological Science, University of Edinburgh, Edinburgh, United Kingdom EH9 3JT
jmareque@cicbiomagune.es

The use of inorganic nanoparticles for molecular imaging applications has attracted considerable interest in the last few years. Semiconductor nanocrystals (quantum dots, QDs) in particular have become important tools in biology as alternatives to traditional organic and genetically-encoded fluorophores [1]. For optical imaging, QDs offer high molar extinction coefficients and high quantum yields, strong resistance to photobleaching and degradation, continuous absorption spectra spanning the UV to the near infrared (depending on the size and elements forming the semiconductor core), narrow emission and large Stokes shifts. In addition, QDs have large two-photon cross-sectional efficiency (2-3 orders of magnitude that of organic dyes), which makes them ideal also for *in vivo* deep-tissue imaging using two-photon excitation [2].

However, QDs could potentially become more than passive bio-probes. New risks and new opportunities may arise when QDs are combined and interact with other molecules. We are particularly interested in the interactions between QDs and components of the immune system, and between QDs and photoactive molecules which can target/damage DNA.

The immune system has the essential task of controlling host defenses against infections and can be used to recognize and kill cancer cells. For these functions activation of a family of highly conserved and recently discovered receptors termed Toll-like receptors (TLRs) appears to be critically important [3]. The recognition/activation of each TLR is a complex event, and it is mediated by a specific pathogen-associated molecular pattern (PAMP) –a conserved molecular motif which is present in a bacteria and/or a virus and is absent in mammalian cells. We have found that QDs biofunctionalised with PAMPs provide strong stimulation of the mammalian immune system via TLR activation in both *in vitro* and *in vivo* experiments [4]. Moreover, when the nanoparticle carries both an antigen and a TLR ligand the magnitude and quality of the immune response is significantly improved. These results suggest that QDs and other traceable nanoscopic materials can, as pathogen-mimetic materials, make important contributions in fundamental and applied research pertinent to the development of safer and more effective vaccines.

The site-directed generation of cytotoxic effects upon targeted light irradiation to convert photosensitive inert chemical compounds into toxic tumour-killing agents is, on the other hand, becoming an increasingly successful treatment for some forms of cancer. Currently this new anticancer technique – photodynamic therapy (PDT) – requires an organic dye to absorb the radiation and use it to generate cytotoxic singlet oxygen, which has considerable limitations [5]. Our recent studies suggest that it may be possible to develop alternative PDT methodologies by combining suitable QDs with suitable photoactive metal complexes and light of a specific wavelength [6]. Moreover, we have found that the same type of combination can significantly enhance DNA damage [7]. Thus, in the context of targeting cancer there could be an opportunity for the interaction between QDs and small photoactive molecules to be exploited to generate or release in a controlled fashion anticancer drugs or other cytotoxic species using light.

References:

[1] Michalet, X. et al. Science, 307 (2005) 538; Bruchez, M. J et al Science, 281 (1998) 2013; Gao, X. et al. Curr. Opin Biotech.,16 (2005) 63; Medintz, I. L et al Nat. Mater., 4 (2005) 435.
[2] Larson, D. R et al. Science, 300 (2003) 1434.
[3] Hennessy E. J. et al. Nat Rev. Drug Discovery, 9 (2010) 293.
[4] Barr, T. A. et al. Molecular Biosystems, 6 (2010) 1568.
[5] Dougherty T. J. et al. J. Natl. Cancer Inst.,90 (1998) 889; Brown S. B. et al. Lancet Oncol., 5 (2004) 497; Moore C. M. et al. Nat. Clin Pract. Urol., 6 (2009) 18.
[6] Gomez-Blanco, N et al. Chem. Commun., 35 (2009) 5257.
[7] Hernandez-Gil, J. et al. Chem. Commun., (2011), Advance article, DOI: 10.1039/C0CC04163G

MICRO-ORDERING OF LUMINESCENT NANOPARTICLES BY TARGETING OF BIOMOLECULES

Emma Martin Rodriguez[1], Nicoleta Bogdan[1], Jorge Lamela[2], Daniel Jaque[2], Francisco Jaque[2], José García Solé[2] and John A. Capobianco[1]

[1]Department of Chemistry and Biochemistry, Concordia University, 7141 Sherbrooke St. W, H4B 1R6 Montreal, Canada
[2]Departamento de Física de Materiales, C-IV, Universidad Autonoma de Madrid, C/Francisco Tomas y Valiente 7, 28049 Madrid, Spain.
emma.martin@uam.es

Nowadays a great effort has been directed towards the early detection of pathogenic agents or cancer cells by optical methods. One of the most investigated approaches is the use of inorganic luminescent nanoparticles (quantum dots, gold nanorods and lanthanide doped nanoparticles), which have been used to obtain high resolution images of cancer cells in in-vitro and in-vivo assays. The specificy of bioassays can be improved by using the interactions between ligands found on cell surfaces and protein receptors as they are involved in several biological processes such as bacteria and virus adhesion, cancer diseases, inflammation, immunology, and cell-cell communications. Thus, it is of vital importance to develop nanoparticles exposing multiple copies of these ligands to be potentially used in highly sensitive assays for the detection of bacteria or virus infections, or cancer tumors.

As natural polysaccharide, chitosan (containing glucosamines groups) is used in many biological applications as it is nontoxic, biocompatible and biodegradable. It display multiple biochemical functions at its surface chitosan so could serve as support to bind luminescent nanoparticles with the above mentioned ligands, so it would be possible to develop luminescent markers for the detection of specific biomolecules. Thus in this study chitosan molecules were patterned at the surface of epoxy-silane modified slides and, it was demonstrated that the chitosan labeled at the surface of these modified-slides is able to strongly interact with water dispersible $NaGdF_4:Er^{3+},Yb^{3+}$ up-converting luminescent nanoparticles.

Chitosan molecules have been directly patterned on modified slides using microcontact printing, a method that has been very successfully adopted for the precise and gentle transfer of biomolecules (proteins, DNA and lipid bilayers) from stamp a to a substrate. This occurs in a few seconds and without loss of biological activity. The stamps were fabricated using Polydimethylsiloxane (PDMS) (Sylgard 184). PDMS was molded using a prefabricated master, consisting in a grating with 250 lines/mm. The stamp was then inked with a chitosan /acetic acid solution and pressed after drying in the silane coated slide allowing for a conformal contact between the stamp and the surface of the glass-slide. The transfer of the chitosan molecules from the stamp to the glass-slide could be observed by using an optical microscope under reflection illumination (see Figure 1(a)). The fabricated chitosan patterns were then submerged in a water solution of $NaGdF_4:Er^{3+},Yb^{3+}$ (1mg/mL) nanoparticles. These nanoparticles were synthesized by acidic treatment of the nanoparticles previously synthesized via the thermal decomposition procedure.

After removing the sample from the solution, it was abundantly washed with distilled water. To ensure the presence of the nanoparticles in the sample after the washing process, the sample was illuminated by a laser diode with 980 nm wavelength. A strong green signal was observed with the naked eye. The emission spectrum of the sample was then characterized, corresponding to the Er^{3+} emission spectrum. To ensure the binding of the nanoparticles to the patterns, the washing procedure was repeated 10 times. The intensity emitted by the nanoparticles was not observed to significantly decrease after the washing process, indicating a strong binding chitosan-nanoparticles.

The characterization of the samples has been carried out by two different optical techniques. By Near-field Scanning Optical Microscopy (NSOM) optical contrast could be observed between the lines with chitosan and the surrounding regions, the higher brightness corresponding to the chitosan lines. Then we use fluorescence microscopy to detect the distribution of the nanoparticles in the sample. For this purpose we focused a 980 nm wavelength laser beam by means of a 100X objective, and the variation of the luminescence intensity of the sample was mapped. Although we could detect Er^{3+} emission from

the nanoparticles along the entire sample (see Figure 1(b)), the intensity of emission was higher along the chitosan lines, showing that the nanoparticles bind preferably to the chitosan molecules.

In conclusion, we have demonstrated that luminescent nanoparticles can bind to chitosan molecules. We have demonstrated this preference by the fabrication of chitosan micropatterns where the nanoparticles are preferently bound. In addition to the biological applications of the nanoparticles to targeting of specific biomolecules, the work describes a fast and easy technique for the fabrication of luminescent micro arrays, with applications in other fields of science and technology, as for instance biosensing, chromatography, diagnostic immunoassays, cell culturing, DNA microarrays, and other analytical procedures.

Figures:

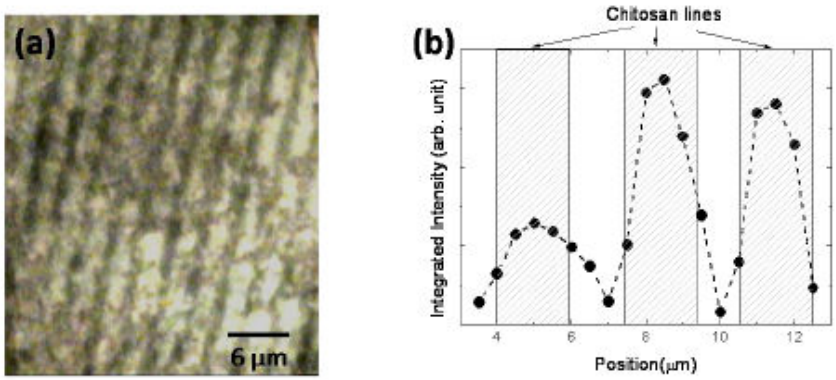


Figure 1: *(a) Optical microscope image of the micropatterned sample, white ones correspond to chitosan; (b) Er^{3+} luminescence intensity across three chitosan lines showing the preferent binding of $NaGdF_4:Er^{3+},Yb^{3+}$ nanoparticles to chitosan. Dotted lines are guides for the eyes.*

SYNTHETIC STRUCTURES TO AID REGENERATION IN THE CENTRAL NERVOUS SYSTEM

Manuel Monleón Pradas

Centro de Biomateriales e Ingeniería Tisular, Universidad Politécnica de Valencia, Spain
Centro de Investigación Príncipe Felipe, Valencia, Spain
CIBER-Biomateriales, Bioingeniería, Nanomedicina
mmonleon@upvnet.upv.es

Trauma brain injury, stroke and several neurodegenerative processes entail the loss of neuronal populations and of tissue structures. The very limited regeneration ability of the central nervous system has represented an unsurmountable obstacle for the overcoming of the ensuing disabilities. In this context, the discovery of the pluripotency of various cell types has opened wider possibilities for new therapeutic strategies. Nonetheless, cells supplied to injured or degenerated sites, and even cells migrating to those sites from nearby proliferative regions have proved to be short-lived and have failed to achieve significant improvements. This may be due to an impossibility for these cells to rebuild or restore lost neuronal circuitry connections, and also to the loss of viability of those cells in an aggressive microglial environment at the lesion site. It is in this situation where synthetic biomaterial structures may be of help, in that they may *(i)* host and supply in a neuroprotective environment cell populations for transplant; *(ii)* deliver neurotrophic factors; *(iii)* sustain and stimulate neural progenenitor cell differentiation and axonal growth; *(iv)* provide targeted guidance to axon outgrowth. Success of these synthetic structures has as a prerequisite their biocompatibilty, their integration in the host tissue without eliciting a glial scar that would invalidate their functions, and, possibly, their ability to be vascularized in order to maintain the viability of the biohybrid construct. These demands condition the choices of chemistries and the development of inner architectures and morphologies of the synthetic materials. Our group has been working in the identification of brain-compatible biomaterials and has developed different types of structures suited for implantation in the cortex and in the nigrostriatal tract [1]. The presentation discusses some of the results obtained *in vitro* and *in vivo* in rat model, with special reference to cell differentiation and migration, glial scar formation and angiogenesis.

References:

[1] Martínez-Ramos et al. Tissue Engineering A 14 (2008) 1365; Soria J M et al. J Biomed Mater Res Part A 97 A (2011) 85; Martínez-Ramos et al. (2011, to appear)

Figures:

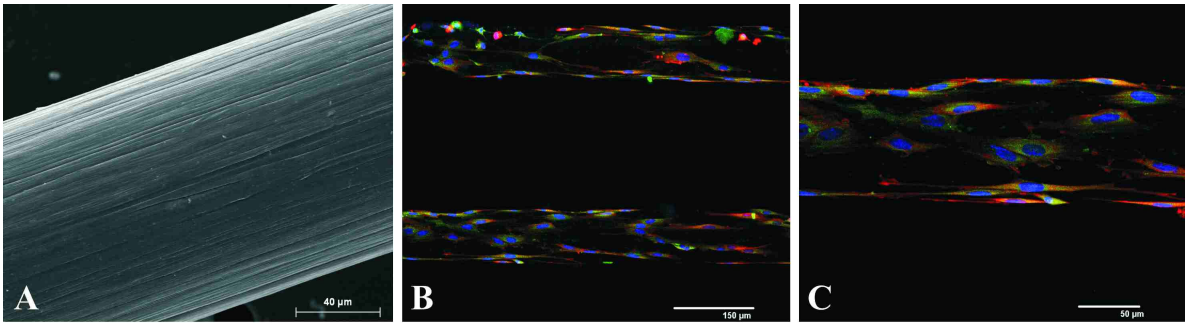


Figure 1: *Textured PCL microfilament (A) and olfactory ensheathing glial cells grown on them (B and C)*

GLYCONANOTECHNOLOGY TO DEVELOP MULTIFUNCTIONAL AND MULTIMODAL NANOMATERIALS FOR BIOMEDICAL APPLICATIONS

Soledad Penadés

Laboratory of GlycoNanotechnology, Biofunctional Nanomaterials Unit, CICbiomaGUNE and CIBERBBN, Parque Tecnológico, Pº de Miramon 182, 20009 San Sebastian, Spain
spenades@cicbiomagune.es

During the last years our laboratory has developed a new technology (*Glyconanotechnology*) for tailoring - in a simple and versatile way – bio-functional gold nanoclusters (*glyconanoparticles*). [1,2,3] The manipulation of the metallic cluster to obtain magnetic nanoparticles for in vivo application in cellular labeling and imaging by magnetic resonance (MRI), is comprised within the potential of this novel technology. [4, 5]

Glyconanoparticles (GNPs) bearing biological significant carbohydrates (antigens) in varying density have been prepared to study biological mechanisms [6, 7] and to intervene in cell adhesion processes. [8] The methodology includes the preparation of *hybrid* GNPs incorporating carbohydrates and other molecules such as fluorescent probes, biotin as well as biological molecules such as peptides, DNA and RNA.

The design and preparation of complex bio-functional GNPs and their application as polyvalent tools to study and intervene in carbohydrate mediated biological interactions will be highlighted. As examples of application in Nanomedicine, the preparation and study of GNPs as anti-adhesion agents in inhibition of metastasis, [8] as potential microbicides for blocking HIV-1 infection, [9] or as magnetic probes for in vivo labeling and tracking specifically cells by means of magnetic resonance imaging (MRI) will be also reviewed.

References:

[1] J. M. de la Fuente et al., Gold Glyconanoparticles as Water-Soluble Polyvalent Models To Study Carbohydrate Interactions, Angew. Chem. Int. Ed., 40 (2001) 2258-2261.
[2] A. G. Barrientos et al., Gold Glyconanoparticles: Synthetic Polyvalent Ligands Mimicking Glycocalyx- Like Surfaces as Tools for Glycobiological Studies, Chem. Eur. J. 9 (2003), 1909-1921.
[3] I. García et al., Glyconanoparticles: multifunctional nanomaterials for biomedical applications. Nanomedicine, 5, (2010) 777-792
[4] J. Gallo et al., Water-soluble magnetic glyconanoparticles based on metal-doped ferrites coated with gold: Synthesis and characterization. J. Mat. Chem.20 (2010) 10010-10020
[5] I. García et al., Magnetic Glyconanoparticles as a Versatile Platform for Selective Immunolabeling and Imaging of Cells. Bioconjugate Chem. doi.org/10.1021/bc1003923
[6] M.-J. Hernáiz et al., A Model System Mimicking Glycosphingolipid Clusters to Quantify Carbohydrate Self-Interaction by Surface Plasmon Resonance, Angew. Chem. Int. Ed. 41 (2002) 1554-1557.
[7] J. M. de la Fuente et al., Thermodynamic Evidence for Ca2+ mediated self-aggregation of Lewis X gold glyconanoparticles. A model for cell adhesion via carbohydrate-carbohydrate interaction, J. Am. Chem. Soc. 127 (2005) 6192-6197.
[8] J. Rojo et al., Gold Glyconanoparticles as New Tools in Anti-adhesive Therapy, ChemBioChem. 5 (2004) 291-297.
[9] O. Martínez-Ávila et al., Multivalent Manno-Glyconanoparticles Inhibit DC-SIGN Mediated HIV-1 trans-Infection of Human T-cells. ChemBioChem. 10 (2009) 1806-1809.

A NOVEL DNA CHIP FOR SINGLE MOLECULE ANALYSIS

T.Plénat[1,2], C.Tardin[1,2], , C.Vieu[3,2], C.Thibault[3,2], E.Trévisiol[3,4], and L.Salomé[1,2]

[1]CNRS; IPBS (Institute of Pharmacology and Structural Biology); 205 route de Narbonne, F-31077 Toulouse, France
[2]University of Toulouse; UPS; IPBS; F-31077 Toulouse, France
[3]LAAS, Nanobiosystems, 7, Avenue du Colonel Roche, F-31077 Toulouse, France
[4]UMR 5504, UMR 792, Ingénierie des Systèmes Biologiques et des Procédés & Plate-forme Biopuces, CNRS, INRA, INSA, 135, avenue de Rangueil, 31077 Toulouse, France
plenat@ipbs.fr, salome@ipbs.fr

The last two decades have seen the emergence of single-molecule experiments [1]. By avoiding the ensemble averaging inherent to traditionnal bulk-phase biochemistry, the study of molecular machineries at the single-molecule level permits a better understanding of the behavior of living systems. Indeed the dynamics of the machineries processes can be characterized and rare subpopulations can be identified [2].

One of the main shortcoming of single molecule experiments is that the acquisition of statistically solid data is very time consuming, which explains the fact that they are still not widely used in laboratories.

We will present the development of a new single DNA chip, allowing the simultaneous analysis of hundreds of single DNA molecules by the Tethered Particle Motion (TPM) technique. Our single DNA chip gives high-throughput capabilities to this approach of valuable interest for multiple applications.

The principle of a TPM experiment consists in tracking a bead tethered at the free end of a DNA molecule which is immobilized by the other end to a coverslip thanks to optical videomicroscopy. The amplitude of the Brownian motion of the bead is related to the effective length of the DNA molecule [3]. Any conformational change of the DNA molecule due to external factors (proteins, ions, temperature), that induces a variation of the effective length of the DNA tether, can be thus monitored by TPM [4].

References:

[1] Cornish, P. V.; Ha, T., Acs Chemical Biology 1 (2007) 53.
[2] van Oijen, A. M., Nature Chemical Biology, 8 (2008) 440.
[3] Yin, H.; Landick, R.; Gelles, J., Biophys J, 6 (1994) 2468.
[4] Pouget, N. et al.; Nucleic Acid Res., 9 (2004) e73

BIO-TEMPLATED SYSTEMS FOR NANOELECTRONICS

Danny Porath

Institute of Chemistry and Center for Nanoscience and Nanotechnology,
The Hebrew University of Jerusalem, Israel
porath@chem.ch.huji.ac.il

In our research we use bio-templated systems to realize one-dimensional conducting nanowires and nanodevices for scientific investigation of electrical charge transport in these systems, for nanoelectronics and for nanotechnology applications. One example is dsDNA and its synthetic derivatives. Within this frame we measured electrical charge transport in dsDNA, measured the energy level spectrum of dsDNA, showed polarizability of DNA derivatives and more. I will briefly review our previous results and our ongoing activity in this direction. Another example for bio-templated systems is the SP1 protein which is hybridized with various nanoparticles to form memory units and protein-particles conducting chains. We demonstrate the construction of various building blocks, acquiring specific attachment to gold or Si surfaces, array formation and finally charging and logic operations in hybrid SP1-nanoparticle systems. I will review this activity in more details. The research is conducted by my group in close collaboration with several groups from complementary fields.

References:

[1] “Direct Measurement of Electrical Transport Through Single DNA Molecules of Complex Sequence”, Hezy Cohen, Claude Nogues, Ron Naaman and Danny Porath, PNAS 102, 11589 (2005).
[2] “Electrical characterization of self-assembled single- and double-stranded DNA monolayers using conductive AFM”, Hezy Cohen et al., Faraday Discussions 131, 367 (2006).
[3] “Long Monomolecular G4-DNA Nanowires”, Alexander Kotlyar, Nataly Borovok, Tatiana Molotsky, Hezy Cohen, Errez Shapir and Danny Porath, Advanced Materials 17, 1901 (2005).
[4] "Polarizability of G4-DNA Observed by Electrostatic Force Microscopy Measurements", Hezy Cohen et al., Nano Letters 7(4), 981 (2007).
[5] “High-Resolution STM Imaging of Novel Poly(G)-Poly(C)DNA Molecules”, Errez Shapir, Hezy Cohen, Natalia Borovok, Alexander B. Kotlyar and Danny Porath, J. Phys. Chem. B 110, 4430 (2006).
[6] “Electronic structure of single DNA molecules resolved by transverse scanning tunneling spectroscopy”, Errez Shapir et al., Nature Materials 7, 68 (2008).
[7] “SP1 Protein Based Nanostructures and Arrays”, Izhar Medalsy et. al., Nano Letters 8, 473 (2008).
[8] "Float And Compress: A Honeycomb Like Array Of A Highly Stable Protein Scaffold", Arnon Heyman et al., Langmuir 25, 5226 (2009).
[9] “Protein Scaffold Engineering Towards Tunable Surface Attachment”, Arnon Heyman et al., Angewandte Chemie Int. Ed., 48, 9290 (2009).
[10] “A DNA sequence scanned”, Danny Porath, Nature Nanotechnology 4, 476 (2009).
[11] "Logic implementations using a single nanoparticle-protein hybrid", Izhar Medalsy et. al., Nature Nanotechnology 5, 451 (2010).

Figures:

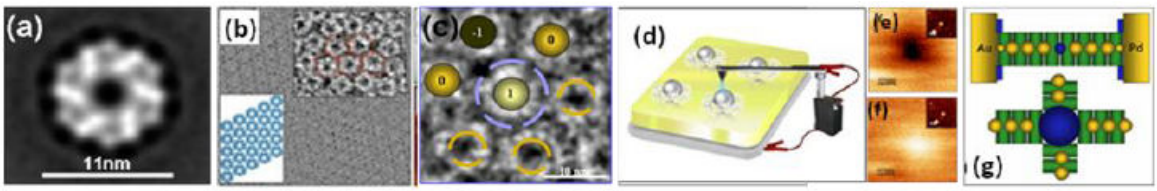


Figure 1: *SP1 protein as a basis for nanoelectronic applications: (a) TEM of a single SP1 protein. (b) A large packed ordered array of SP1 molecules. Lower inset: a scheme of the array, upper inset: enlargement of part of the array, where the hexagonal packing is marked. (c) Overlaid scheme of the suggested memory array. (d) Scheme of the suggested implementation, where the writing is by charging individual particles with AFM and reading by EFM. (e-f) Two charged states of the hybrid and topography (inset). (g) Scheme of chain structures.*

SURFACE CELL GROWTH ENGINEERING ASSISTED BY A NOVEL BACTERIAL NANOMATERIAL AND THE IMPACT OF GENETIC TAILORING ON THEIR PROPERTIES

Imma Ratera[1,2], César Díez-Gil[1,2], Sven Krabbenborg[1,2], Elena García-Fruitós[2,3], Esther Vázquez[2,3,4], Escarlata Rodríguez-Carmona[2,3], Rosa M. Ferraz[2,3,4], Nora Ventosa[1,2], Joaquín Seras[2,3,4], Olivia Cano[2,3], Neus Ferrer-Miralles[2,3,4], Antonio Villaverde[2,3,4], and Jaume Veciana[1,2]

[1] Department of Molecular Nanoscience and Organic Materials. Institut de Ciencia de Materials de Barcelona (CSIC) Bellaterra, Barcelona, Spain
[2]CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN) Bellaterra, Barcelona, Spain
[3]Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, Bellaterra, Spain
[4]Department of Genetics and Microbiology Universitat Autònoma de Barcelona, Bellaterra, Spain
iratera@icmab.es

Bacterial inclusion bodies (IBs) are highly pure protein deposits in the size range of a few hundred nanometers produced by recombinant bacteria.[1] Although IBs were supposed to be undesirable side products on protein transcription processes it has been recently probed that those intriguing nanoparticulate proteic materials retain part of their original functionality and further more that it is possible to tailor its properties during biological production. The polypeptide chains that form IBs fold into an unusual amyloid-like molecular architecture compatible with their native structure, thus supporting the biological activities of the embedded polypeptides (eg fluorescence or enzymatic activity).[2] Therefore, a wide spectrum of uses as functional and biocompatible materials might arise upon convenient engineering.[3] Although theoretically feasible through adjusting genetic and production conditions, the biophysical features of these proteinaceus nanoparticles, such as activity and size, have been never engineered and very few is known about their physicochemical properties.

As IBs are biofunctional by nature, engineering of IBs might have wide applications in different nanomedical scenarios. In this study (Figure 1), we have characterized the relevant nanoscale properties of IBs as particulate materials using Scanning Electron Microscopy (SEM), Dynamic Light Scattering (DLS), Atomic Force Microscopy (AFM) and Confocal Microscopy (CM). We have demonstrated that these particles are mechanically stable and fully biocompatible, and that their size and biological activities can be tailored by appropriate genetic and process engineering.[4-6] Moreover, wettability and nanomechanical studies developed on IBs produced in different Escherichia coli genetic backgrounds depict distinguishable characteristics within the proteinaceous nanoparticles. (Figure 2 and 3).[5] As a proof of concept of the biomedical potential of IBs we have modified the topology of surfaces suitable for mammalian cell culture by adsorbing these particles, resulting in a dramatic stimulation of cell proliferation.[6] Furthermore, IB-grafted surfaces have been produced by using the microcontact printing (mCP), on which cell growth has been driven in desired patterns (Figure 1 g-i). Furthermore it was possible to observe how cultured mammalian cells respond differentially to inclusion body variants when used as particulate materials to engineer the nanoscale topography, proving that the actual range of referred mechanical properties is sensed and discriminated by biological systems.[5] The unique properties of this proteinaceous material including biocompatibility, manipulability and functionality make it especially appealing for regenerative medicine.

References:

[1] A. Villaverde, M. M. Carrio, Biotechnol. Lett., 25 (2003) 1385-1395
[2] (a) N. Gonzàlez-Montalbán , E. García-Fruitós, A. Villaverde, Nat. Biotechnol. 25(7) (2007) 718-720 (b) E. Garcia-Fruitos, E. Aris, A. Villaverde, Appl. Environ. Microbiol., 73 (2007) 289-294
[3] E.Garcia-Fruitos, M. Martínez-Alonso, N. Gonzàlez-Montalbán, M. Valli, D. Mattanovich, A. Villaverde, J. Mol. Biol. 374 (2007) 195-205
[4] (a) Garcia-Fruitos E, Martinez-Alonso M, Gonzalez-Montalban N, Valli M, Mattanovich D, Villaverde A. Divergent Genetic Control of Protein Solubility and Conformational Quality in Escherichia coli. J.Mol.Biol. 2007;374:195-205. (b) E. Vazquez, Mónica Roldán, C. Diez-Gil, U. Unzueta, J. Domingo-Espín, J. Cedano, O. Conchillo, I. Ratera, J. Veciana, Xavier Daura, N. Ferrer-Miralles & A. Villaverde, Nanomedicine, 5(2) (2010), 259–268

[5] C. Díez-Gil, S. Krabbenborg, Elena García-Fruitós, Esther Vázquez, Escarlata Rodríguez-Carmona, Rosa M. Ferraz, Nora Ventosa, I. Ratera Joaquín Seras, Olivia Cano, Neus Ferrer-Miralles, Antonio Villaverde, Jaume Veciana, Biomaterials, 31 (2010) 5805-5812

[6] (a) Patent ES- P200900045 (b) E. García-Fruitós, E. Rodríguez-Carmona, C. Diez-Gil, R. M. Ferraz, E. Vázquez, J. L. Corchero, M. Cano-Sarabia, I. Ratera, N. Ventosa, J. Veciana, A. Villaverde, Adv. Mater., 21 (2009) 4249–4253

Figures:

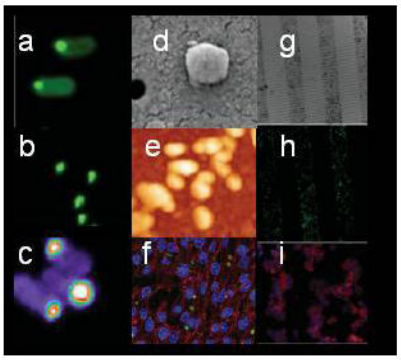


Figure 1: *IBs formed by the green fluorescence protein (GFP) when produced in bacteria (a), after purification (b), and under confocal analysis for florescence mapping (c). In (d) and (e), purified inclusion bodies observed by SEM and AFM respectively. In (f), BHK21 cells growing on polystyrene plates decorated with GFP IBs. In (g), a silica surface patterned with pure GFP IBs, that still being fluorescent (h), drive the growth of BHK 21 cells under the same lineal pattern (i).*

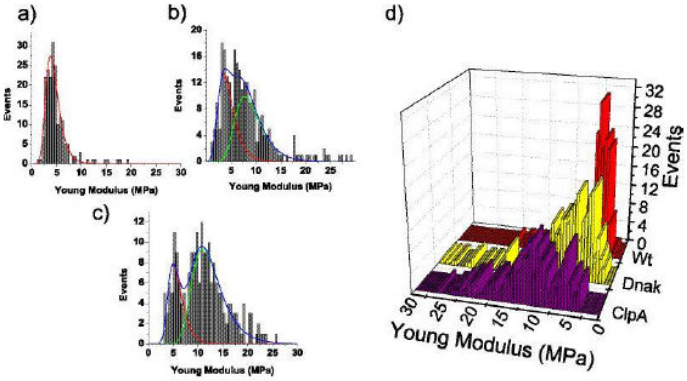


Figure 2: *Histogram representation of the number of events vs. Young modulus for IBs produced in bacterial mutants. a) Wt IBs showing only one peak at 3.73 MPa; b) DnaK- IBs show two overlapped Young modulus distributions which centered at 3.56 and 7.75 MPa; c) ClpA- IB show the presence of two different young modulus distributions, at 5.01 and 10.99 MPa; d) 3D representation of the later histograms.*

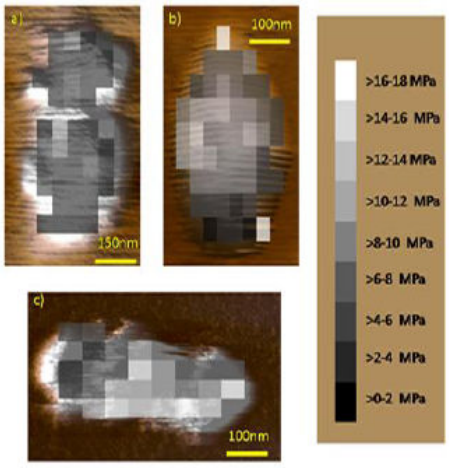


Figure 3: *2D software developed reconstruction of the elasticity maps of selected IBs from the three genetic backgrounds. a) wt IBs, b) DnaK- IBs, c) ClpA- IBs. Observations infer the existence of a homogeneously spread distribution of Young modulus values over the wt IBs. On the other hand, maps obtained for DnaK- and ClpA- IBs indicate the existence of two elasticity populations, with the harder areas segregated and localised on the centre of the DnaK- IBs and on the right side of ClpA- particles.*

STUDYING SURFACE-ADSORBED COLLOIDAL PARTICLES WITH QUARTZ CRYSTAL MICROBALANCE: HYDRODYNAMIC EFFECTS

I. Reviakine

Centro de Investigación Cooperativa en Biomateriales, Parque Tecnológico de San Sebastián, E-20009 San Sebastián, Spain
Department of Biochemistry and Molecular Biology, University of the Basque Country, 48940 Leioa, Spain

Quartz crystal microbalance (QCM) is widely used for studying soft interfaces in liquid environments. Many of these interfaces are heterogeneous in nature, in the sense that they are comprised of isolated entities adsorbed at a surface. When characterizing such interfaces, one is interested in determining parameters such as surface coverage and size of the surface-adsorbed entities. Currently this information is obtained by fitting QCM data—shifts in resonance frequency, ΔF, and bandwidth $\Delta\Gamma$ (also referred to as dissipation shifts)—with the model derived for smooth, homogeneous films using the film acoustic thickness and shear elastic moduli as fitting parameters. This is the so-called continuum approach. Experimentally, we investigated adsorption of proteins, liposomes, and icosahedral virus particles on inorganic surfaces with QCM and with a combined atomic force microscopy (AFM)-QCM setup. Theoretically, we modeled the QCM response with finite element method (FEM) calculations based on the incompressible Navier-Stokes equation to incorporate hydrodynamic effects.

The results of our studies can be summarized as follows. Experimentally, the relationship between the surface coverage and the frequency shift is not linear. This non-linearity can be modeled with the FEM calculations clarifying the contribution of the solvent to the frequency shift and the origin of dissipation in these heterogeneous films. In particular, our results show that the dissipation in these heterogeneous layers occurs mostly in the solvent and arises due to the motion of the adsorbed particles. In other words, it is not a measure of the elastic properties of the adsorbed particles, but rather of the particle-surface contact.

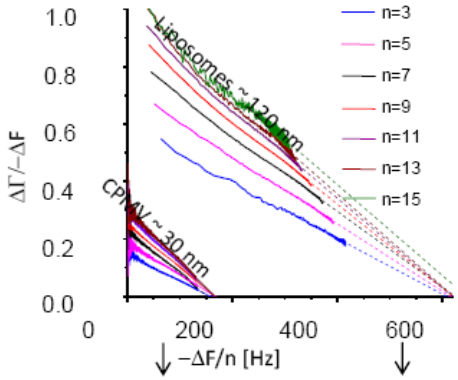
To directly compare the predictions of the continuum approach with the experimental results, we focus on the ratio between the bandwidth and frequency shifts, $\Delta\Gamma/\Delta F$ (the *Df* ratio). The continuum model predicts that this should increase both with surface coverage and particle size. Instead, we observe that this ratio increases with increasing particle size, but decreases with increasing surface coverage. This behavior is again consistently modeled with the FEM calculations: in other words, this is a hydrodynamic effect.

Finally, we find that the size of the adsorbed colloidal particles can be recovered from a model-independent analysis of the plot of the $\Delta\Gamma/\Delta F$ ratio vs. the frequency shift on many overtones (Figure1).

References:

[1] Tellechea, E.; Johannsmann, D.; Steinmetz, N. F.; Richter, R.; Reviakine, I. Langmuir 2009, 25, 5177 - 5184.
[2] Johannsmann, D.; Reviakine, I.; Richter, R. P. Anal.Chem. 2009, 81, 8167-8176.
[3] Johannsmann, D.; Reviakine, I.; Rojas, E.; Gallego, M., Effect of sample heterogeneity on the interpretation of qcm(-d) data: Comparison of combined quartz crystal microbalance/atomic force microscopy measurements with finite element method modeling. Analytical Chemistry 2008, 80, (23), 8891-8899.
[4] Rojas, E.; Gallego, M.; Reviakine, I. Analytical Chemistry 2008, 80, (23), 8982-8990.
[5] Bingen, P.; Wang, G.; Steinmetz, N. F.; Rodahl, M.; Richter, R. P. Analytical Chemistry 2008, 80, 8880-8890.
[6] Carton, I.; Brisson, A. R.; Richter, R. P. Analytical Chemistry 2010, 82, (22), 9275-9281.

Figures:



-ΔF/n [Hz]	175 ± 5	448 ± 32	616 ± 61
Calculated height, nm	29 ± 0.8	81 ± 6	111 ± 11
Particle diameter, nm	28	83 ± 4	114 ± 8

Figure 1: *The plot of the ΔΓ/-ΔF ratio vs. the frequency shift, -ΔF /n, for liposomes and cowpea mosaic virus (CPMV) particles adsorbing on inorganic surfaces. Different overtones are represented by different colors. The ratio decreases with the frequency shift (~ surface coverage), but is greater for larger objects. The curves are nearly linear and extrapolate to frequency shifts that can be converted into object heights via the Sauerbrey relationship. These are found to correlate with the crystallographic dimensions of the CPMV particles and liposome sizes measured by dynamic light scattering.*

NANOSCALE FILMS OF BIOMOLECULAR HYDROGELS – A NOVEL PLATFORM TO INTERROGATE THE RELATIONSHIP BETWEEN SUPRAMOLECULAR ORGANIZATION AND DYNAMICS, AND BIOLOGICAL FUNCTION

Nico B. Eisele[1,2], Patricia M. Wolny[1,3] and **Ralf P. Richter**[1,3]

[1]CIC biomaGUNE, Biosurfaces Unit, Paseo Miramon 182, 20009 San Sebastian, Spain
[2]Max-Planck-Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany
[3]Max-Planck-Institute for Metals Research, Heisenbergstrasse 3, 70569 Stuttgart, Germany
rrichter@cicbiomagune.es

Nature has evolved complex materials that are exquisitely designed to perform specific functions. Certain proteins and glycans self-organize in vivo into soft and dynamic, strongly hydrated gel-like matrices. Illustrative examples of such biomolecular hydrogels are cartilage or mucus. Even though biomolecular hydrogels are ubiquitous in living organisms and fulfill fundamental biological tasks, we have today a very limited understanding of their internal organization, and how they function. The main reason is that this type of assemblies is difficult to study with conventional biochemical methods.

In order to study biomolecular hydrogels directly on the supramolecular level, we have developed an unconventional approach that draws on knowledge from several scientific disciplines. Exploiting surface science tools, we tailor-make model films with thicknesses in the nanometer range by directed selfassembly of purified components on solid supports. With a toolbox of biophysical characterization techniques, these model systems can be investigated quantitatively and in great detail down to the nanometer scale. The experimental data, combined with polymer theory, allow us to develop a better understanding of the relationship between the supramolecular organization and dynamics of biomolecular hydrogels, their physico-chemical properties and their biological function. To illustrate this concept, I will present two examples of our recent research. They relate to (i) a nanoscopic protein hydrogel that is responsible for the regulation of all macromolecular transport between the nucleus and the cytosol of eukaryotic cells [1] (Figure 1), and (ii) a microscopic glycoconjugate hydrogel that is involved in fertilization as well as cancer progression [2, 3].

References:

[1] N. B. Eisele, S. Frey, J. Piehler, D. Görlich and R. P. Richter, Ultrathin nucleoporin FG repeat films and their interaction with nuclear transport receptors. EMBO Rep., 11 (2010), 366-372.
[2] R. P. Richter, K. K. Hock, J. Burkhartsmeyer, H. Boehm, P. Bingen, G. Wang, N. F. Steinmetz, D. J. Evans and J. P. Spatz, Membrane-Grafted Hyaluronan Films: a Well-Defined Model System of Glycoconjugate Cell Coats. J. Am. Chem. Soc., 127 (2007), 5306-5307.
[3] P. M. Wolny, S. Banerji, C. Gounou, A. R. Brisson, A. J. Day, D. G. Jackson and R. P. Richter, Analysis of CD44-hyaluronan interactions in an artificial membrane system: insights into the distinct binding properties of high and low molecular weight hyaluronan. J. Biol. Chem., 285 (2010), 30170-30180.

Figures:

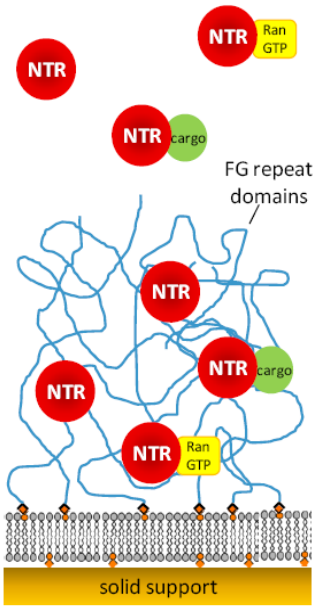


Figure 1: *Macromolecular transport between the cell's nucleus and the cytosol occurs through nuclear pore complexes (NPCs). The transport is selective: objects (cargo) beyond a certain size (30 kDa) need to attach to soluble nuclear transport receptors (NTRs) in order to be channeled efficiently through the pore. A supramolecular assembly of specialized and natively unfolded protein domains within the NPC is thought to be the key component of the NPC´s permeability barrier. The mechanism behind transport selectivity is at present only poorly understood. We have developed ultrathin FG repeat domain films as a surface-confined model system of the permeability barrier. In this contribution, we will present how such model systems can provide insight into the mechanism of transport across the permeability barrier.*

NANOSCALE PROPERTIES OF IMPLANTABLE BIOMATERIALS

Federico Rosei

INRS, Univ. of Quebec, Canada

Modifying the nanoscale structure/chemistry of materials allows to tailor and optimize their properties [1]. Our strategy rests on creating nanopatterns that act as surface cues [2,3] and affect cell behavior. We developed a chemical treatment of Ti-based materials that produces a unique nanostructured topography [4], showing that chemical oxidation is a general strategy that affects biocompatibility [5]. Our treatment generates multifunctional surfaces that promotes the growth of certain cells while inhibiting that of others, without using any growth factors. Nanostructured Ti surfaces selectively inhibit fibroblastic cell growth [4] and promote osteogenic cell activity [6] in vitro. Controling nanoscale features and functionalizing surfaces with molecular overlayers [7] will lead to a new generation of intelligent biomaterials that selectively influence cell behavior at the tissue-biomaterial interface, for example by controlling the adsorption of proteins [9]. Further enhancement of mechano-biocompatibility may be provided by coating with spider silk, whose structural/functional properties are currently being studied [10, 11].

References:

[1] F Rosei, J Phys Cond Matt 16, 1373 (2004).
[2] F Cicoira, F Rosei, Surf Sci 600, 1 (2006).
[3] F Variola et al, Small 5, 996 (2009).
[4] F Variola et al, Biomaterials 29, 1285 (2008).
[5] F Vetrone et al, Nanolett 9, 659 (2009).
[6] L Richert et al, Adv. Mater. 20, 1488 (2008).
[7] S Clair et al, J. Chem. Phys. 128, 144705 (2008).
[8] F. Variola et al., Adv. Eng. Mater. 11, B227 (2009).
[9] L. Richert, F. Variola, F. Rosei, J.D. Wuest, A. Nanci, Surf. Sci. 604, 1445 (2010).
[10] C. Brown, F. Rosei, E. Traversa, S. Licoccia, Nanoscale in press (2011).
[11] C Brown et al, in preparation.

NANOBIOTECHNOLOGIES: DISRUPTING THE LOGIC OF THE DRUG INDUSTRY

Valérie Sabatier, Vincent Mangematin

Grenoble Ecole de Management, 12 rue Pierre Sémard - BP 127 F-38003 Grenoble cedex 01, France
Valerie.sabatier@grenoble-em.com

The emergence of nanobiotechnologies in the drug industry is not only a scientific challenge, it is also challenging corporate strategy and business models. Based on an analysis of recent changes in the drug industry, we identify which are the triggers of change in an industry's dominant logic, defined as the general scheme of value creation and capture shared by its actors. Breakthrough innovations leading to technological discontinuities are a necessary, but not sufficient, condition for the disruption of such dominant logics. The emergence of new business models questions an industry's existing value chain and the relations between its actors.

We argue that in the drug industry, experiencing strong discontinuities and with high technological uncertainties, business models will tend to fit into the dominant logic of the industry and value chains will remain unchanged. But, as the new technologies evolve and uncertainty decreases, disruptive business models will emerge and challenge dominant industry logics and reshape established value chains, especially if they involve new and diversifying players joining the industry.

As technologies emerge and converge, they are leading us towards a more holistic healthcare industry. New approaches to healthcare – such as personalized medicine, nanobiotechnology, theranostics, or systems biology – are opening new business opportunities that build on new ways to address patients' needs and that provide physicians and hospitals with new therapeutic principles. Nanobiotechnologies, defined as the applications of nanotechnologies in the life sciences [1], promise improvements in diagnostics and drug delivery, particularly in the quantity and toxicity of drugs injected into patients. It offers the promise of a convergent approach that can merge diagnosis, treatment and monitoring.

We observe here the beginnings of a new industry that will emerge from the upheaval of the established drug industry and its merging with the diagnostics and other industries. We will discuss the new business opportunities and what firms are doing today in order to prepare tomorrow, in a new drug industry being transformed by nanobiotechnologies.

References:

[1] Briquet-Laugier V, Ott M.O., Biofutur, 265 (2006) 57-62.

NANOBIOPLATFORM BASED ON OLFACTORY RECEPTORS FOR DIAGNOSTIC PURPOSES

Josep Samitier

UCSF Institute for Bioengineering of Catalonia (IBEC)
CIBER of Bioengineering, biomaterials and nanomedicine
University of Barcelona, Barcelona, Spain
jsamitier@ibecbarcelona.eu

The use of smell in different fields has been rediscovered due to major advances in odour sensing technology and artificial intelligence. However, current electronic noses, based on electronic sensors, have significant limitations relating sensitivity, reliability and selectivity, amongst others. These limitations are at the basis of recurrent troubles of the electronic nose technology to reach essential applications in different areas, such as food safety, disease diagnosis, security, environment.

An electronic nose (E-nose) can be regarded as a modular system comprising: a set of active materials that detect the odour; associated sensors that transduce the chemical response into electrical signals, and an appropriate signal conditioning and processing to classify known odours or identify unknown odours, through the processing system (done by the brain in the human nose).

Similar concepts as in the case of the E-nose, but for analysis in liquids have been described recently. These systems are related in similar ways to the sense of taste, so for them the terms 'electronic tongue' or 'taste sensor' have been created. Such systems use as sensors lipid/polymer membranes on a multichannel electrode, resonant sensors, sensor beads or the principle of voltammetry.

Recently, a number of cell-based biosensors have been designed, using yeasts, or more specialized taste or olfactory sensory neurons, or even cells recombinantly expressing olfactory receptors (ORs), to measure changes of electrical parameters in the presence of volatile chemical compounds. These findings will allow the development of a new generation of nanobiosensors to detect chemical volatiles.

The BOND project proposes a new bioelectronic nose based in olfactory receptors in order to mimic the human nose. For this aim, micro/nano, bio and information technologies will converge to develop an integrated bioelectronic analytical nanoplatform platform based on olfactory receptors for odour detection. The general objective of the BOND project is to develop an array of smart nanobiosensor based on olfactory receptors for the detection of odorant signatures.

Briefly, the basis of the nanobioplatform will be the olfactory receptors (OR), proteins specifically sensitive to the odorants belonging to the odorant disease signature prepared in the form of small vesicles immobilized onto the nanotransducers. An array of smart nanotransducers will acquire and process electronically the odour detected. Such an easy-to-use nanobioplatform, with user-friendly interface and odorant identification algorithm, will detect and discriminate the production profiles of odour compounds.

DOXORUBICIN LOADED MAGNETIC POLYMERSOMES: THERANOSTIC NANOCARRIERS FOR MR IMAGING AND MAGNETO-CHEMOTHERAPY

Olivier Sandre, Charles Sanson, Odile Diou, Julie Thévenot, Emmanuel Ibarboure, Alain Soum, Annie Brûlet, Sylvain Miraux, Eric Thiaudière, Sisareuth Tan, Alain Brisson and Sébastien Lecommandoux

Laboratoire de Chimie des Polymères Organiques (LCPO)
UMR5629 Université de Bordeaux / CNRS / Institut Polytechnique de Bordeaux,
ENSCBP – 16 avenue Pey Berland, 33600 Pessac, France
olivier.sandre@ipb.fr

Hydrophobically modified maghemite (γ-Fe_2O_3) nanoparticles were encapsulated within the membrane of poly(trimethylene carbonate)-b-poly(L-glutamic acid) (PTMC-*b*-PGA) block copolymer vesicles using a nanoprecipitation process. This formation method gives a simple access to highly magnetic nanoparticles (MNPs) (loaded up to 70 wt %) together with a good control over the vesicles size (100 to 400 nm). The simultaneous loading of maghemite nanoparticles and doxorubicin was also achieved by nanoprecipitation. The deformation of the vesicle membrane under an applied magnetic field has been evidenced by small angle neutron scattering. These superparamagnetic hybrid self-assemblies display enhanced contrast properties that open potential applications for Magnetic Resonance Imaging. They can also be guided in a magnetic field gradient. The feasibility of controlled drug release by radio-frequency magnetic hyperthermia was demonstrated in the case of encapsulated doxorubicin molecules, showing the viability of the concept of magneto-chemotherapy. These magnetic polymersomes can be used as efficient multifunctional nano-carriers for combined therapy and imaging.

References:

[1] S. Lecommandoux, O. Sandre, F. Chécot, J. Rodriguez-Hernandez, R. Perzynski, Adv. Mat, 17 (2005), 712-718.
[2] S. Lecommandoux, O. Sandre, F. Chécot, J. Rodriguez-Hernandez, R. Perzynski, Journal of Magnetism and Magnetic Materials 300 (2006), 71-74.
[3] S. Lecommandoux, O. Sandre, F. Chécot, R. Perzynski, Progress in Solid State Chemistry, 34 (2006), 171-179.
[4] C. Sanson, J-F. Le Meins, C. Schatz, A. Soum, S. Lecommandoux, Soft Matter, 6 (2010), 1722-1730
[5] C. Sanson, C. Schatz, J-F. Le Meins, A. Brûlet, A. Soum, S. Lecommandoux, Langmuir 26 (2010), 2751-2760.
[6] C. Sanson, C. Schatz, J-F. Meins, A. Soum, E. Garanger, J. Thévenot, S. Lecommandoux. Journal of Controlled Release, 147 (2010), 428-435.
[7] C. Sanson, O. Diou, J. Thévenot, E. Ibarboure, A. Soum, A. Brûlet, S. Miraux, E. Thiaudière, S. Tan, A. Brisson, V. Dupuis, O. Sandre, S. Lecommandoux, ACS Nano, Published online (2011), doi: 10.1021/nn102762f.

Figures:

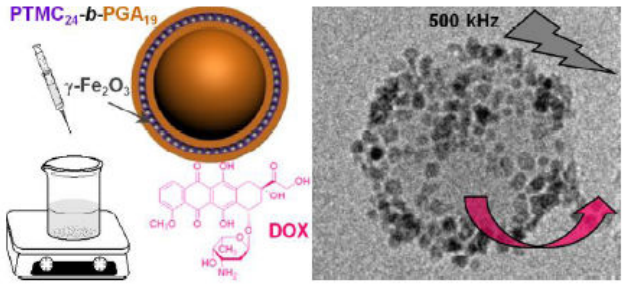


Figure 1: *Left: Sketch of dually-loaded vesicles prepared by addition of an aqueous buffer into a mixture of PTMC-b-PGA copolymer, hydrophobically coated magnetic nanoparticles and doxorucin drug. Right: Cryo-TEM image of vesicle showing the dense mantle of MNPs, which excitation by a radiofrequency magnetic field transmits heat locally to membrane and accelerates the DOX release.*

SPECIFIC IMMOBILIZATION AND PURIFICATION OF RECOMBINANT PROTEINS USING DIETHYLAMINOETHYL-FUNCTIONALIZED MAGNETIC NANOPARTICLES

Jesús M. Sanz[1], Gracia Retamosa-Hernández[1], Beatriz Maestro[2], María J. Rodes-Martínez[1], M. Luisa Ferrer[3] and M. Puerto Morales[3]

[1]Instituto de Biología Molecular y Celular. Universidad Miguel Hernández. Avda. Universidad, s/n. Elche-03202, Spain
[2]Instituto de Electroquímica. Universidad de Alicante. 03690 - San Vicente del Raspeig, Spain
[3]Instituto de Ciencia de Materiales de Madrid - CSIC, 28049-Madrid, Spain
jmsanz@umh.es

The affinity of proteins for their ligands is usually employed in several biotechnological processes that involve their immobilization on solid surfaces, among which affinity chromatography is perhaps the best known application. In this sense, the choline-binding modules (CBMs) such as the CLytA protein, have been used in these tasks with appreciable success [1-4]. CBMs constitute a family of polypeptides that are part of several enzymes such as the murein hydrolases from *Streptococcus pneumoniae* (pneumococcus) [5-6] and that present a high affinity for choline and other tertiary and quaternary amines [7], as well as for supports that contain these groups, such as diethylaminoethyl (DEAE)-cellulose. Fusion proteins containing one of these choline-binding modules as affinity tags can be immobilized and/or purified in a single chromatographic step by means of a simple, gentle, noncovalent procedure [1-4,8].

With the aim of checking the performance of the CBM system on the immobilization of proteins in nanostructures, we have evaluate the binding of the C-LytA and C-LytA fusion proteins on several classes of magnetic nanoparticles. Firstly, we carried out the synthesis of Fe_2O_3 magnetic nanoparticles by coprecipitation methods which were subsequently coated with DEAE-dextran, with a resulting diameter of ~ 10 nm. On the other hand, we also assayed commercial magnetite nanoparticles coated with DEAE-starch (Fig. 1) (200 nm diameter) or with DEAE-silica (750 nm diameter). In all cases we observed a very efficient binding of the C-LytA module, either isolated or fused to the green fluorescent protein (C-LytA-GFP) or to the β-galactosidase (C-LytA- β -gal). Bound proteins could withstand strong washes with up to 1 M NaCl, conserved their structure and activity, and could be specifically eluted upon addition of 150 mM choline as a competitor ligand (Fig. 2). With this in mind, we set up a simple purification procedure of recombinant proteins from a bacterial extract using the 200-nm particles and the protocol depicted in Fig. 3. Proteins could be purified this way to electrophoretic homogeneity (Fig. 4), with a yield of 50 mg per gram of nanoparticles, and the whole procedure could be accomplished in as low as 20 minutes.

The procedure described here displays a series of advantages with respect to other current methods: DEAE nanoparticles are easy to synthetize and the protein immobilization is fast, efficient and strong although, due to its non-covalent nature, the regeneration of the particles can be easily carried out. Moreover, buffers are simple and do not contain components that may potentially inactivate enzymes or be harmful to the human body. Besides the rapid purification of proteins, DEAE-containing magnetic nanoparticles may act as protein carriers to be used in a wide panoply of applications, such as the construction of enzymatic electrodes, recyclable enzymatic bioreactors, or *in vivo* delivery of proteins to specific tissue targets by means of the application of an external magnet.

References:

[1] Sánchez-Puelles, J.M. et al. Eur. J. Biochem. 203, (1992),153-159.
[2] Ortega, S. et al Bio/Technology 10 (1992), 795-798.
[3] Akerstrom, B. et al. Biochim. biophys. Acta 1482 (2000),172-184.
[4] Caubin, J.et al. Biotechnol. Bioeng. 74 (2001), 164-171.
[5] García,P.et al. J. Appl. Biomed. 8, (2010), 131–140.
[6] CW_binding_1 family: Pfam code PF01473; http://pfam.sanger.ac.uk/family?PF01473
[7] Sanz,J.M. et al. FEBS Lett. 232, (1988), 308-312.
[8] LYTAG and LYTAG-Two-Phase user manuals. Biomedal, S.L. http://www.biomedal.es

Figures:

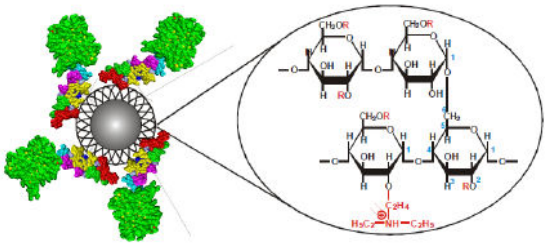


Figure 1: *Scheme of magnetic nanoparticles derivatized with DEAEstarch (taken from Chemicell GmbH) coated with C-LytA-GFP.*

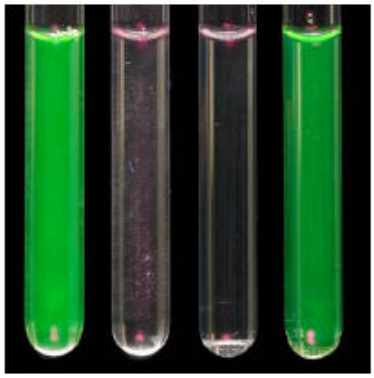


Figure 2: *Left to right: C-LytA-GFP solution prior to binding to magnetic nanoparticles; two washes with 1M NaCl; elution of protein from the particles with choline.*

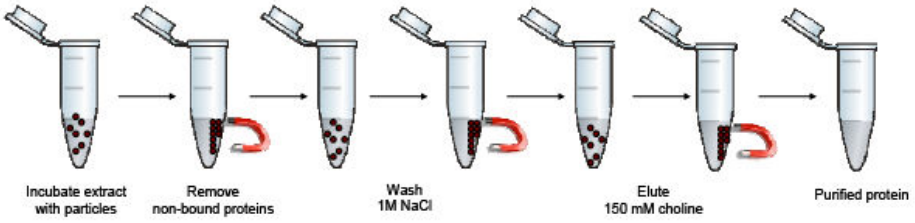


Figure 3: Scheme *of the purification method of CBM-tagged proteins*

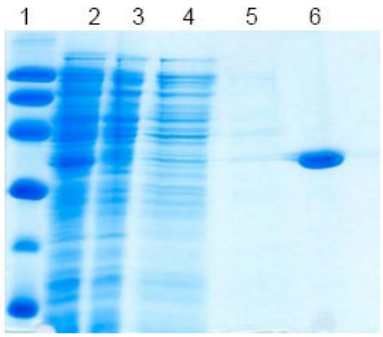


Figure 4: *Polyacrylamide gel electrophoresis in the presence of SDS showing the purification of C-LytA-GFP. Lane 1, protein molecular weight markers; Lane 2, total extract of Escherichia coli RB791 overproducing CLytA-GFP; Lane 3, non-bound proteins upon incubation with magnetic nanoparticles; Lane 4, first wash with NaCl; Lane 5, last wash with NaCl; Lane 6, protein eluted with choline.*

APTAMERS FOR NANOSTRUCTURED BIOSENSORS

Beate Strehlitz, Christine Reinemann, Regina Stoltenburg

Helmholtz-Centre for Environmental Research – UFZ, Permoserstr 15, D-04318 Leipzig, Germany
beate.strehlitz@ufz.de

Aptamers are ssDNA or RNA oligonucleotides featured by a very high affinity of their target binding, offer new possibilities for nanotechnological applications. Aptamers are suitable as molecular recognition elements in a wide range of analytical systems with the goal of separation and purification of molecules from complex mixtures or of detection of molecules in complex matrices. In this context, aptamers will play an important role as new receptors in biosensors [1, 2]. Moreover, aptamers have a great potential for their use in the field of medical and pharmaceutical basic research as well as clinical diagnostic and therapy (e.g. inhibition of enzyme activities, blocking of receptor binding sites).

Aptamers as nucleic acids are very attractive compounds for combinatorial chemistry. They are able to fold into defined secondary and tertiary structures, and they can easily be amplified by PCR. Very complex libraries of random sequence oligonucleotides with about 10^{15} different molecules can be produced by chemical synthesis and screened in parallel for a particular functionality, such as recognition and high affinity binding to a given target or catalytic activity. In 1990, three laboratories independently described a method for the identification of nucleic acid sequences, exhibiting predetermined properties, within large pools of randomised synthetic oligonucleotides [6-8]. This method is known as SELEX (Systematic Evolution of Ligands by Exponential enrichment) and is now widely used for the selection of aptamers, which bind their target with high affinity and specificity.

The functionality of aptamers is based on their stable 3D-structure, which depends on the primary sequence, the length of the nucleic acid molecule (smaller than 100 nt) and the ambient conditions. Typical structural motives are stems, internal loops, bulges, hairpin structures, tetra loops, pseudoknots, triplicates, kissing complexes, or G-quadruplex structures. In presence of the target, the aptamers undergo adaptive conformational changes and their three-dimensional folding creates a specific binding site for the target. The intermolecular interactions between aptamer and target are characterised by a combination of complementarity in shape, stacking interactions between aromatic compounds and the nucleobases of the aptamers, electrostatic interactions between charged groups, and hydrogen bondings [6-8].

The aptamer selection process (SELEX) is an iterative process (Fig. 1). Starting point is a chemically synthesised random DNA oligonucleotide library consisting of about 10^{13} to 10^{15} different sequence motifs. Several rounds (typically 6 to 20 rounds) of in vitro selection (binding, partitioning) and enzymatic amplification of oligonucleotide variants result in the enrichment of relatively few sequence motifs with the highest affinity and specificity for the target. This evolution is driven by the selection conditions (target features and concentration, buffer, temperature, incubation time, efficiency of the partition method, negative selection steps ...). The stringency strongly affects the affinity and specificity of the aptamers to be selected, and is typically progressively increased in the course of a SELEX process.

The SELEX technology has been applied to different classes of targets. Inorganic and small organic molecules, peptides, proteins, carbohydrates, antibiotics as well as complex targets like target mixtures or whole cells and organisms were used for an aptamer selection [9-13]. Aptamers can also be selected for toxic or non-immunogenic targets. Once selected, they can be produced by chemical synthesis in high amount and with high reproducibility. Multiple modifications are possible e.g. to enhance their stability or to permit the quantification and immobilisation of the aptamers. Denatured aptamers can be regenerated. Because of these properties aptamers represent an alternative to antibodies regarding analytical applications.

With this presentation we will show several results of our aptamer selections and some examples of their application in biosensors and assays.

References:

[1] O'Sullivan, C. K., Anal. Bioanal. Chem. 372 (2002) 44-48.
[2] Tombelli, S., Minunni, A.Mascini, A., Biosens. Bioelectron. 20 (2005) 2424-2434.
[3] Ellington, A. D., Szostak, J. W., Nature 346 (1990) 818-822.
[4] Tuerk, C., Gold, L., Science 249 (1990) 505-510.
[5] Robertson, D. L., Joyce, G. F., Nature 344 (1990) 467-468.
[6] Hermann, T., Patel, D. J., Science 287 (2000) 820-825.
[7] Patel, D. J., Curr. Opin. Chem. Biol. 1 (1997) 32-46.
[8] Patel, D. J., Suri, A. K., Jiang, F., Jiang, L. C., Fan, P., Kumar, R. A.Nonin, S., J. Mol. Biol. 272 (1997) 645-664.
[9] Famulok, M. Curr. Opin. Struct. Biol. 9 (1999) 324-329.
[10] Göringer, H. U., Homann, M.Lorger, M., Int. J. Parasitol. 33 (2003) 1309-1317.
[11] Klussmann, S. (2006) The Aptamer Handbook. Functional Oligonucleotides and Their Applications, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.
[12] Stoltenburg, R., Reinemann, C., Strehlitz, B., Biomol. Eng. 24 (2007) 381-403.
[13] Wilson, D. S., Szostak, J. W., Annu. Rev. Biochem. 68 (1999) 611-647.

Figures:

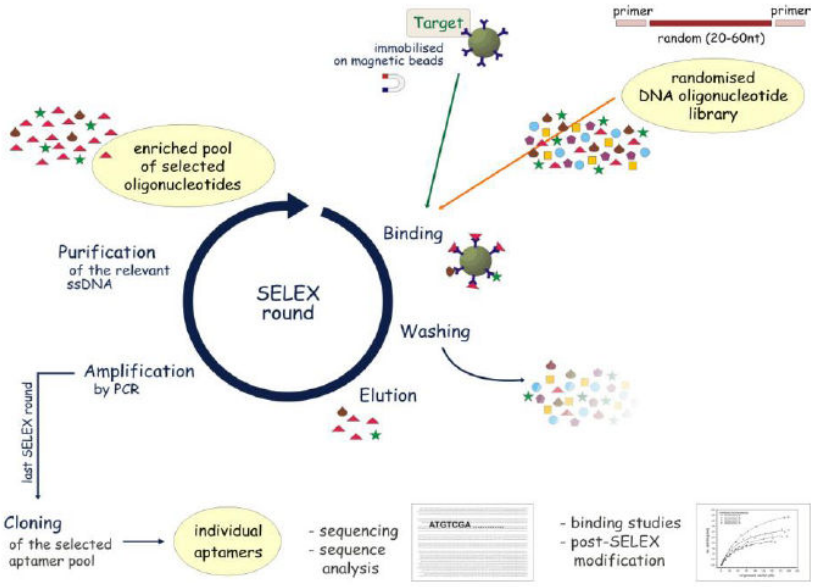


Figure: 1: *SELEX process for the selection of target-specific DNA aptamers. The starting randomised oligonucleotide library is directly used for binding with the target molecules (in this case immobilised on magnetic beads) in the first SELEX round. Unbound oligonucleotides are removed by several stringent washing steps of the binding complexes. The target-bound oligonucleotides are eluted and subsequently amplified by PCR. A new enriched pool of selected oligonucleotides is generated by purification of the relevant ssDNA from the PCR products. This selected new oligonucleotide pool is then used for the next selection round. If an enrichment of target-specific oligonucleotides is observed the last SELEX round is finished after the amplification step. The enriched aptamer pool is cloned and several individual aptamers have to be characterised.*

BISPHOSPHONATE-SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES CONJUGATES FOR DUAL-MODALITY PET/SPECT-MR MEDICAL IMAGING

Rafael Torres Martin de Rosales, Richard Tavaré, Arnaud Glaria, Maite Jauregui-Osoro, Rowena L. Paul, Gopal Varma, Andrea Protti, Istvan Szanda and Philip J. Blower

Department of Imaging Chemistry and Biology - Division of Imaging Sciences & Biomedical Engineering - King's College London - London, United Kingdom

rafael.torres@kcl.ac.uk

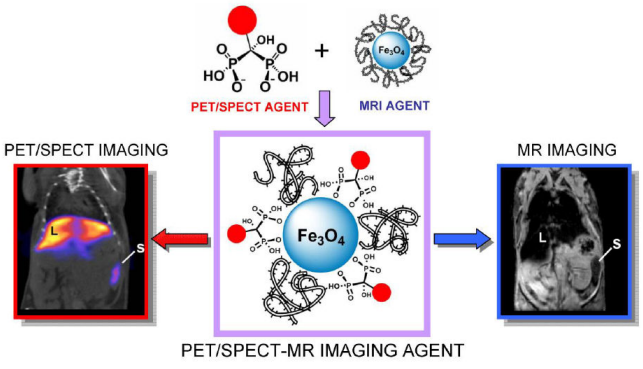
The combination of radionuclide-based imaging modalities such as single photon emission computed tomography (SPECT) and positron emission tomography (PET) with magnetic resonance imaging (MRI) is likely to become the next generation of clinical scanners. Hence, there is a growing interest in the development of SPECT- and PET-MRI agents [1]. These dual-modality agents may allow users to make the most of the high sensitivity of PET/SPECT and the high anatomical resolution and soft tissue contrast of MRI in a synergistic fashion. To this end, we have developed a new class of SPECT/PET-MR imaging agents based on the conjugation of radiolabeled bisphosphonates (BP) directly to the surface of superparamagnetic iron oxide (SPIO) nanoparticles (Figure 1) [2]. We demonstrate the high potential of the BP-iron oxide conjugation using radiolabeled BPs (^{64}Cu for PET or ^{99m}Tc for SPECT), and Endorem/Feridex, a liver MRI contrast agent based on SPIO. The labeling of SPIOs with BPs can be performed in one step at room temperature if the SPIO is not coated with an organic polymer. Heating is needed if the nanoparticles are coated, as long as the coating is weakly bound as in the case of dextran in Endorem. The size of radiolabeled Endorem was characterised by TEM (5 nm, Fe_3O_4 core) and DLS (106 ± 60 nm, Fe_3O_4 core + dextran). EDX, Dittmer-Lester and radiolabeling studies demonstrate that the BP is bound to the nanoparticles and that it binds to the Fe_3O_4 cores of Endorem, and not its dextran coating. The bimodal imaging capabilities and excellent stability of these nanoparticles were confirmed *in vivo* using MRI and nanoSPECT-CT or nanoPET-CT imaging, showing that the radionuclides and Endorem co-localise in the reticuloendothelial system (liver and spleen) and the lymph nodes, as expected for particles of the composition and size of Endorem. To the best of our knowledge, these are the first examples of radiolabeling SPIOs with BP conjugates and the first examples of radiolabeling SPIO nanoparticles directly onto the surface of the iron oxide core, and not its coating. This work lays down the basis for a new generation of SPECT/PET-MR imaging agents in which the BP group could be used to attach functionality to provide targeting, stealth/stability and radionuclides to Fe_3O_4 nanoparticles and other inorganic materials of biomedical interest using very simple methodology readily amenable to GMP.

References:

[1] a) A. Louie, Chem. Rev. 110, (2010), 3146; b) L. E. Jennings, N. J. Long, Chem. Commun. (2009), 3511; c) S. Lee, X. Chen, 8, Mol. Imaging (2009), 87; d) J. Kim, Y. Piao, et al., Chem. Soc. Rev. 38, (2009), 372; e) J. Cheon, J. H. Lee, Acc. Chem. Res. 41, (2008), 1630.

[2] a) R. Torres Martin de Rosales, R. Tavaré, et al., Submitted, (2010); b) R. Torres Martin de Rosales, C. Finucane, et al. Chem. Commun. (2009), 4847.; c) R. Torres Martin de Rosales, C. Finucane, et al., Bioconjugate Chem. 21, (2010), 811.

Figures:



APTAMERS FOR DIAGNOSTIC

Jean-Jacques Toulmé[1], Eric Dausse[1], Carmelo Di Primo[1], Michèle Allard[2], Julie Miguel[2] and Sonia Da Rocha[3]

[1]Inserm, European Institute of Chemistry and Biology, University of Bordeaux, Bordeaux, France
[2]General Hospital, Bordeaux, France
[3]Novaptech, 2 rue R. Escarpit, Pessac, France
jean-jacques.toulme@inserm.fr

Aptamers are oligonucleotides identified through a combinatorial process known as SELEX (Systematic Evolution of Ligands by EXponantial enrichment). They exhibit both high affinity and specificity for a pre-determined taget of interest. They can be raised against a wide range of molecules -even living cells- including marker proteins for major diseases. Following association to various devices aptamers can signal the presence of their cognate ligand (fluorescence, SPR, ...).

We developped an automated platform that speeds up the selection of apatmers and designed a high throughput screening method for the identification of aptamers to various proteins, including human matrix metalloproteases and viral components. Aptamer-based tools were subsequently synthesized for imaging human brain tumors by scintigraphy and detecting viral proteins on micro-arrays.

NANOSCIENCE NANOTECHNOLOGIES: A NEW IMAGINERY IN SCIENCE AND PHILOSOPHY

Daniel Truong Loï

France

The buzz about nanosciences and technologies can be explained in reference to the imaginery they invite and disrupt; by changing the scheme of visibility, by producing objets, they activate some mythical characters of mastery (Faust, Prometheus, Frankenstein for example), and their fate are thought of as misfortunes. In the same mind the NBIC give rise to a dissenting picture of an enclosed science, to plot against and destabilize society. To objectivize this imaginary can help to assess the link between science and society.

DEVELOPMENT OF AN ELECTROCHEMICAL IMMUNOSENSOR BASED ON SPECIFIC ANTIBODIES LABELLED WITH CDS NANOPARTICLES DEVOTED TO THE PARAQUAT RESIDUES DETECTION IN SPIKED POTATO SAMPLES

Enrique Valera[a,b], Raül García-Febrero[a,b], Isabel Pividori[c], Francisco Sánchez-Baeza[a,b], M.-P. Marco[a,b]

[a]Applied Molecular Receptors Group (AMRg), IQAC-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain
[b]CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Barcelona, Spain
[c]Unitat de Química Analítica, Grup de Sensors i Biosensors. Edifici C - Campus de la UAB 08193 Bellaterra (Cerdanyola del Vallès), Barcelona, Spain
enrique.valera@iqac.csic.es

The analysis of the presence of potentially hazardous chemicals (e.g. pesticides, antibiotics) in food remains a major concern in the European Community. However, to ensure quality and traceability, there is a great need to increase the continuous control and monitoring of foodstuff at critical steps in the food chain, such as for example after the recollection of the raw materials, after the food processing (monitoring of storage and logistics), as well as of final products.

Fast, reliable and cost-effective analytical methods are necessary to ensure the safety of the food products. Following the flexibility, sensitivity, specificity and efficiency of analysis demonstrated by the numerous immunochemical and biological tests today available, research is now intending to go forward by developing devices capable of working out of laboratory, i.e. in the different steps of the food chain. With this idea the concept arises of biosensor as a miniaturized analytical devices, consisting of an immobilized biological component (antiody, enzyme, receptor, DNA, etc.) in intimate contact with a transducer (optic, electrochemical, piezoelectric, etc.) that may convert the biorecognition process into a quantifiable electrical signal.

With regards to the use of biosensors as a method to verify compliance of legislation, most of the devices reported until now, rely on the use of labels to reach the necessary detection limits required. Likewise, the use of inorganic nanocrystals tracers as labels for electrochemical immunoassays have recently received great attention because the possibility of obtain simultaneous detection/measurements of DNA targets and proteins [1,2].

In this work, the potential of a new electrochemical immunosensor to detect residual amounts of paraquat (PQ) in a complex matrix, such as potatoes, is evaluated. The immunosensor presented is based on graphite composite electrodes (GECs), immunoreagents specifically developed to detect paraquat, magnetic µ-particles, and CdS nanoparticles labelled to the specific antibodies. The assay relies on the immunochemical competitive reaction between the pesticide residues and a fixed amount of the immobilized antigen on the magnetic beads for a small amount of the specific antibody. At the end of the reaction the amount of antibody captured by the free antigen is evacuated (Figure 1). By means of the well-known anodic stripping techniques, CdS nanoparticles are read, and the amounts of its metal ions are expressed as a signal of current or charge. Due to the amplification of the amperometric/coulombimetric signal, produced by the presence of the CdS nanoparticles, PQ can be detected in spiked potato samples. The results obtained showed that after the extraction and dilution of the matrix, PQ can be determined in potato samples with limits of detection of 0.64 µg L^{-1} and 0.39 µg L^{-1}, depending of the chosen parameter for the detection (current or charge), and taking into account the dilution used. Hence, the LODs obtained are far below the Maximum Residue Level (0.02 mg Kg^{-1}) established by EU for most crops.

References:

[1] Wang, J.; Liu, G.; Merkoçi, A., Journal of the American Chemical Society, 125 (2003) 3214-3215.
[2] Liu, G.; Wang, J.; Kim, J.; Jan, M.-R., Analytical Chemistry 76 (2004) 7126-7130.

Figures:

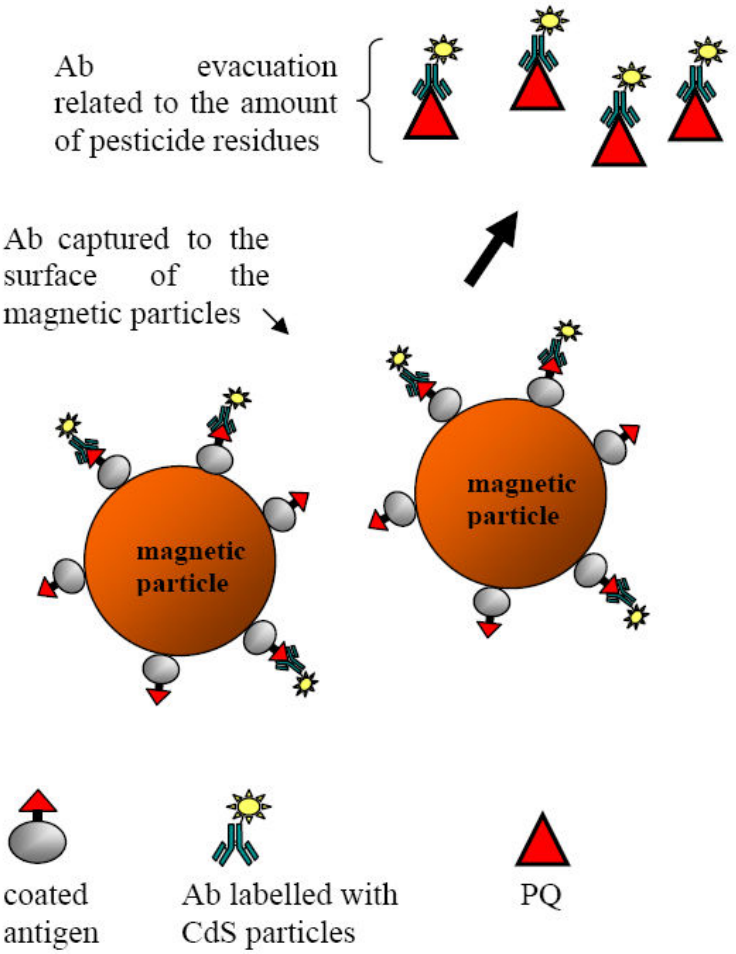


Figure 1: *Electrochemical immunosensor reaction. An amount of the specific antibody is captured by the coated antigen. Likewise, the amount of Ab bound to the pesticide was evacuated. The amount of CdS particles is indirectly related to the PQ residues concentration.*

POLYMERSOME NANOREACTORS AS ARTIFICIAL ORGANELLES

J.C.M. van Hest, S.F.M. van Dongen, W.P.R. Verdurmen, R.J.R.W. Peters, R.J.M. Nolte, R. Brock

Radboud University Nijmegen,Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands
j.vanhest@science.ru.nl

Compartmentalisation is one of the techniques that cells adopt to enable a high level of control over (bio-)chemical processes, for instance the order in which enzymes react. In many cases, the compartment also serves to protect the cell from the action of its degrading contents, as is the case with lysosomes. It furthermore serves as a scaffold for the precise positional assembly of enzymes that work together in a multistep cascade reaction.

In an effort to mimic these complex enzyme systems, many studies concerning enzyme encapsulation or assembly have been reported in the literature. The focus of this research initially was on phospholipid liposomes. However, the relative fragility of liposomes limits their potential applicability. Like liposomes, polymersomes are spherical aggregates that contain a bilayer architecture. They are formed by the self-assembly of amphiphilic block copolymers in an aqueous environment and their polymeric bilayer shows a greater stability, mainly due to the lower critical aggregation concentration of amphiphilic macromolecules. A drawback of polymersome membranes is their low permeability even to water, which hampers application as nanoreactors.

To overcome this problem, block copolymers have to be used that give an intrinsically porous bilayer when self assembled. One such copolymer is PS-PIAT. On dispersal in water it forms porous polymersomes that possess a relatively high degree of Small molecules can move across their membranes while larger molecules, such as proteins, cannot.

In a first line of research, we have positioned enzymes at three different locations on these polymersomes, namely, in their lumen (glucose oxidase, GOx), in their bilayer membrane (Candida antarctica lipase B, CalB) and on their surface (horseradish peroxidase, HRP, see figure 1). The surface coupling was achieved by ‘click’ chemistry between acetylene-functionalised anchors on the surface of the polymersomes and azido functions of HRP, which were introduced by using a direct diazo transfer reaction to lysine residues of the enzyme [1].

To determine the encapsulation and conjugation efficiency of the enzymes, they were decorated with metal-ion labels and analysed by mass spectrometry. This revealed an almost quantitative immobilisation efficiency of HRP on the surface of the polymersomes and a more than statistical incorporation efficiency for CalB in the membrane and for GOx in the aqueous compartment. The enzyme-decorated polymersomes were studied as nanoreactors in which glucose acetate was converted by CalB to glucose, which was oxidised by GOx to gluconolactone in a second step. The hydrogen peroxide produced was used by HRP to ABTS to $ABTS^{\cdot+}$ (figure 2). Kinetic analysis revealed that the reaction step catalysed by HRP is the fastest in the cascade reaction.

In a second line of research, artificial organelles were created. For this purpose we modified the outer surface of the polymersome nanoreactors with cell-penetrating peptides, in particular the tat sequence. As a result, the polymersomes obtained the property to enter cells. Enzymes which were encapsulated in the polymersomes could be transported into mammalian cells and perform their biological activity in a living system. This was demonstrated via the introduction of HRP, which could neutralize hydrogen peroxide and therefore protect the cell against oxidative stress (figure 3) [2].

References:

[1] S. F. M. van Dongen, M. Nallani, J.J. L. M. Cornelissen, R. J. M. Nolte, J. C. M. van Hest Chemistry - a European Journal 15 (2009) 1107-1114.

[2] S. F. M. van Dongen, W. P. Verdurmen, R. J.R.W. Peters, R. J.M. Nolte, R. Brock, and J. C.M. van Hest. Angew. Chem. Int. Ed Engl 49 (2010) 7213-16

Figures:

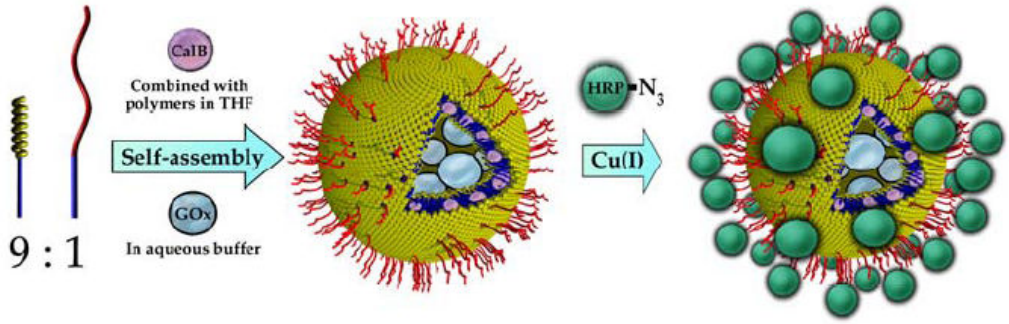


Figure 1: *Positional assembly of enzymes in a polymersome.*

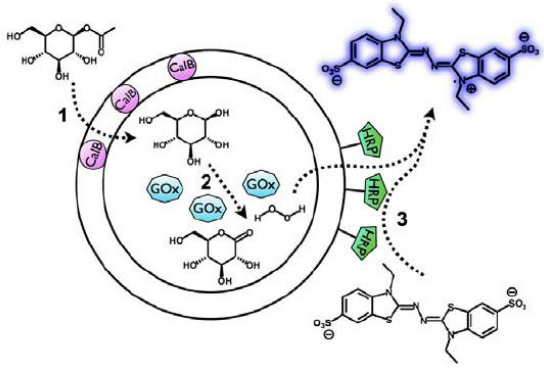


Figure 2: *Schematic representation of the multistep reaction. 1) Monoacetylated Glucose is deprotected by CalB, which is embedded in the polymersome membrane. 2) In the inner aqueous compartment, GOx oxidises glucose to gluconolactone, providing a molecule of hydrogen peroxide. 3) Hydrogen peroxide is used by HRP to convert ABTS to $ABTS^{\cdot+}$. HRP is tethered to the polymersome surface.*

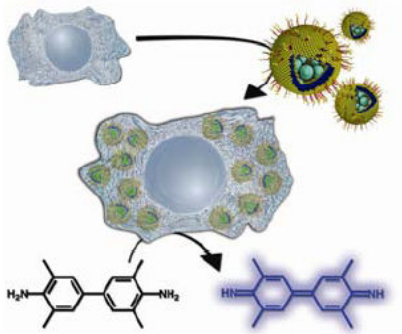


Figure 3: *Schematic representation of the introduction of polymersomes into cells. Polymersomes filled with HRP and functionalized on the periphery with cell penetrating peptides are taken up by mammalian cells. They display their activity as artificial organelles by the oxidation of TMB with hydrogen peroxide.*

PARTICULATE MOLECULAR MATERIALS FOR DRUG DELIVERY: CHALLENGES IN ITS LARGE-SCALE PREPARATION

Jaume Veciana, Nora Ventosa, Santi Sala, Maria Muntó, Mary Cano-Sarabia, Elisa Elizondo, Ingrid Cabrera, Alba Córdoba

Institut de Ciència de Materials de Barcelona (CSIC) and Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Campus Universitari de Bellaterra, E-08193 Cerdanyola, Spain

vecianaj@icmab.es

The obtaining of particulate micro and nanostructured molecular materials and the understanding of how to manipulate them at nanoscopic and supramolecular level are currently playing a crucial role in drug delivery and clinical diagnostics [1-3]. It has been observed that polymeric nanoparticles, micelles, microemulsions, nanosuspensions, nanovesicles, and nanocapsules are efficient drug carriers that can significantly help to develop new drug delivery routes, more selective and efficient disease-detection systems, drugs with a higher permeability to biological membranes with controlled released profiles, and to enhance their targeting towards particular tissues, cells or intracellular compartments.

The potential of «bottom-up» strategies, based on molecular self-assembling, is much larger than that of «top-down» approaches for the preparation of such micro- and nanostructures. For instance, by precipitation from conventional liquid solutions it should be possible to control particle formation, and hence particle size and size distribution, morphology and particle supramolecular structure. However, this is still a dream up to now when conventional liquids are used.

The solvent power of compressed fluids (CFs), either in the liquid or supercritical state, can be tuned by pressure changes, which propagate much more quickly than temperature and composition solvent changes. Therefore, using compressed solvent media, it is possible to obtain supramolecular materials with unique physicochemical characteristics (size, porosity, polymorphic nature morphology, molecular self-assembling, etc.) unachievable with classical liquid media [4,5]. Small changes in temperature and pressure of CFs result in large but homogenous changes in the fluid's density, and hence in its solvent power. This tunable range in density (solvation ability) cannot be achieved so easily with any conventional solvent. The most widely used CF is compressed CO_2 (cCO_2), which is non-toxic, non-flammable, cheap and easy recyclable. It has gained considerable attention, during the past few years as a «green substitute» to organic solvents and even to water in industrial processing. During the past few years, CFs based technologies, in particular precipitation procedures, are attracting increasing interest for the preparation of particulate molecular materials with application in the field of drug-delivery and nanomedicine [6-8].

In this presentation a few examples of particulate drugs and encapsulated medicines inside vesicles, prepared in a large scale, with CFs will be presented.

References:

[1] M. E. Davis, Z. Chen, D. M. Shin, Nature Reviews-Drug Discovery 2008, 7, 771-782.
[2] P. Couvreur, C. Vauthier, Pharmaceutical Research 2006, 23, 1417-1450.
[3] D. Peer, J. M. Karp, S. Hong, O. C. Farokhzad, R. Margalit, R. Langer, Nature Nanotechnology 2007, 2, 751-760.
[4] E. Reverchon, R. Adami, Journal of Supercritical Fluids 2006, 37, 1-22.
[5] H.M. Woods, M.M.C.G. Silva, C. Nouvel, K.M. Shakesheff, S.M. Howdle, Journal of Material Chemistry 2004, 14, 1663-1678.
[6] M. Cano-Sarabia, N. Ventosa, S. Sala, C. Patiño, R. Arranz, J. Veciana, Langmuir 2008, 24, 2433-2437.
[7] K. Mishima, Advanced Drug Delivery Reviews 2008, 60, 411–432
[8] E. Elizondo, S. Sala, E. Imbuluzqueta, D. González, M. J. Blanco-Prieto, C. Gamazo, N. Ventosa, J. Veciana Pharmaceutical Research 2010, DOI 10.1007/s11095-010-0248-x

LANTHANIDE-DOPED UPCONVERTING NANOPARTICLES: FROM SYNTHESIS TO APPLICATIONS IN BIOLOGY

Rafik Naccache[1], Laura Martinez Maestro[2], Emma Martin Rodriguez[1,2], Daniel Jaque[2], José García Solé[2], John A. Capobianco[1], **Fiorenzo Vetrone**[3]

[1]Concordia University, Department of Chemistry and Biochemistry, 7141 Sherbrooke St. W., Montréal, Québec, Canada, H4B 1R6
[2]Universidad Autónoma de Madrid, Fluorescence Imaging Group, Departamento de Física de Materiales, Madrid, 28049, Spain
[3]Université du Québec, Institut National de la Recherche Scientifique – Énergie, Matériaux et Télécommunications, 1650 Boul. Lionel-Boulet, Varennes, Québec, Canada, J3X 1S2
vetrone@emt.inrs.ca

The recent development of novel highly sensitive and specific luminescent probes with optical properties superior to organic dyes and fluorescent proteins have attracted a diverse group of researchers in a number of key areas. Luminescent nanoparticles such as semiconductor quantum dots (QDs) are emerging as useful tools in diagnostic medicine and therapeutics and are finding widespread applications [1]. However, the majority of these probes require high-energy (UV or blue) light as the excitation source. This has considerable disadvantages resulting in increased background fluorescence (auto-fluorescence), decreased penetration depths in biological tissues as well as photo-induced damage to the specimen under study [2]. To alleviate such issues, multi-photon excited biolabels, including nanoparticles, being more widely used [3]. In particular QDs and gold nanorods (GNRs) are being extensively studied. These nanoparticles are excited with near-infrared (NIR) light, and thus have considerable advantages. For example, NIR light is silent to tissues thus minimizing autofluorescence, possesses greater tissue penetration capabilities and does not cause damage to the sample. Moreover, the nanoparticles require ultrafast (femtosecond) excitation light to induce the multi-photon excited luminescence, which results in increased spatial resolution.

An exciting recent development is the adaption of upconversion for biological applications including biosensing and multi-photon imaging [4-6]. Upconversion is a process inherent to lanthanide-doped materials whereby two (or more) low energy NIR photons are absorbed and in turn, emit higher energy radiation (in the UV-visible-NIR region). This is a very common phenomenon in Ln^{3+}-doped insulating materials because their energy level scheme is favorable for serial addition of multiple isoenergetic photons. Due to the multiphoton nature of the process where “real” long-lived electronic energy states participate, intense upconversion can be observed with low-power commercial cw laser diodes. As a result, inorganic Ln^{3+}-doped nanoparticles are very promising materials and can be used to develop new biocompatible ultra-highly sensitive fluorescent upconverting probes for advanced biomedical applications. Ln^{3+}-doped nanoparticles offer high output, stability with respect to photobleaching, reasonably small size, and flexibility in surface chemistry, which should facilitate their delivery and targeting in biological applications.

Here, we present the synthesis of lanthanide-doped fluoride nanoparticles and subsequent strategies to impart biological functionality. Finally, we show relevant biological applications of these nanoparticles including their ability to be used as imaging probes for malignant cancer cells.

References:

[1] W. C. W. Chan, D. J. Maxwell, X. Gao, R. E. Bailey, M. Han, S. Nie, Curr. Opin. Biotechnol., 13 (2002) 40.
[2] nig. J. Microsc., 200 (2000) 83.
[3] D. Larson, W. Zipfel, R. Williams, S. Clark, M. Bruchez, F. Wise, W. Webb. Science, 300 (2003) 1434.
[4] F. Wang, Y. Han, C. S. Lim, Y. Lu, J. Wang, J. Xu, H. Chen, C. Zhang, M. Hong, X. Liu, Nature, 463 (2010) 1061.
[5] F. Vetrone, R. Naccache, A. Zamarrón, A. Juarranz de la Fuente, F. Sanz-Rodríguez, L. Martinez Maestro, E. Martín Rodriguez, D. Jaque, J. García Solé, J. A. Capobianco, ACS Nano, 4 (2010) 3254.
[6] F. Vetrone, R. Naccache, A. Juarranz de la Fuente, F. Sanz-Rodríguez, A. Blazquez-Castro, E. Martín Rodriguez, D. Jaque, J. García Solé, J. A. Capobianco, Nanoscale, 2 (2010) 495.

Figures:

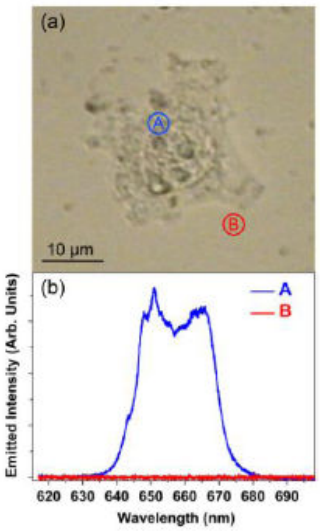


Figure 1: *(a) Optical transmission image of a single HeLa cancer cell after 1.5 h incubation in a water solution containing $NaYF_4:Er^{3+}$, Yb^{3+} nanoparticles. (b) Visible two-photon micro-fluorescence spectra obtained when the fs NIR laser is focused inside and outside the HeLa cell (points A and B in the optical transmission image, respectively).*

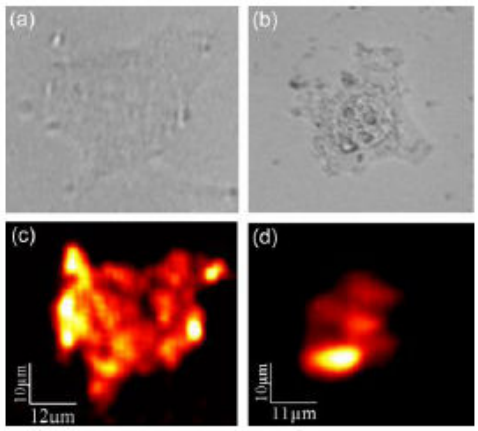


Figure 2: *Top – optical transmission images of two HeLa cells after incubation with $NaYF_4:Er^{3+}$, Yb^{3+} nanoparticles during (a) 1.5 and (b) 3 h. Bottom – confocal fluorescence images of the same HeLa cells ((c) and (d) for 1.5 and 3 h incubations, respectively).*

TOWARDS A TUNABLE ARCHITECTURE OF PROTEIN-ONLY ARTIFICIAL VIRUSES

A. Villaverde[1,2,3], U. Unzueta[1,2,3], J. Cedano[1,4], J. Domingo-Espín[1,2,3], R. Cubarsí[5,3], M. Roldán [6], Valérie Petegnief[7], A. Planas[7], I. Ratera[8,3], O Conchillo[1], E García-Fruitós[1,2,3], C. Diez-Gil[8,3], X. Daura[1,9], P. Saccardo[1,2,3], N. Gonzalez-Montalbán[10], J Ganz 11,12, R. Mangues[3,13], J. Veciana[8,3], H. Peluffo[11,12], N. Ferrer-Miralles[1,2,3], E. Vázquez[1,2,3]

[1]Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, Bellaterra, Spain
[2]Department of Genetics and Microbiology Universitat Autònoma de Barcelona, Bellaterra, Spain
[3]CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN) Bellaterra, Barcelona, Spain
[4]Departament de Bioquímica i Biologia Molecular, Univ Autònoma de Barcelona. Bellaterra, Spain
[5]Departament de Matemàtica Aplicada IV. Universitat Politècnica de Catalunya. Barcelona, Spain
[6]Servei de Microscòpia, Universitat Autònoma de Barcelona, Bellaterra, Spain
[7]Dep d'Isquèmia Cerebral i Neurodegeneració. Inst d'Investigacions Biomèdiques de Barcelona (IIBB), CSIC-Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
[8]Department of Molecular Nanoscience and Organic Materials. Institut de Ciencia de Materials de Barcelona, CSIC, Bellaterra, Barcelona, Spain
[9]Catalan Institution for Research and Advanced Studies (ICREA), 08010 Barcelona, Spain
[10]Center for Biomedical Engineering and Technology (BioMET), University of Maryland Baltimore (UMB), Baltimore, USA
[11]Neurodegeneration Laboratory, Institut Pasteur de Montevideo, CP 11400 Montevideo, Uruguay
[12]Dept of Histology and Embryology, Faculty of Medicine, UDELAR, CP 11800 Montevideo, Uruguay
[13]Grup d'Oncogènesi i Antitumorals, Institut de Recerca, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain
antoni.villaverde@uab.cat

Nucleic Arginine-rich peptides are well known in molecular medicine by their abilities to condense DNA, to cross cell membranes and as efficient nuclear localization signals [1-4]. Altogether, these properties make them very useful functional elements of artificial viruses [5] for gene therapy and drug delivery. Very recently, we have shown that the homogeneous peptide R9 acts, in addition, as an unexpected architectonic agent at the nanoscale, promoting the self-assembling of a multifunctional protein that contains it, as protein-only, planar nanoparticles of 20 nm in diameter [6]. These particles, that show a strong nuclear avidity and accumulate in the cell nucleus a few minutes after exposition [7], are able to bind, condense and deliver expressible DNA [6].

As the self-organizing properties of R9 seemed to result in much more regular nanoparticles that those offered by conventional self-assembling amyloidogenic peptides, that render either fibers [8] or amorphous aggregates [9], we explored if cationic peptides other than R9 could also promote the self-assembling of holding building blocks. A series of unrelated peptides with diverse amino acid sequences and structures were tested as architectonic tags by using an EGFP as convenient building block. Interestingly, all these peptides were able, at different extents, to promote the spontaneous formation of protein nanoparticles of different sizes, ranging from 20 to 100 nm, in a process in which the arginine residues are critical for the final geometry of the resulting particles. On the contrary, Lysine-rich peptides, although very useful as DNA condensers, do not show any architectonic ability when incorporated to artificial viruses [10].

The use of arginine-rich peptides as structural agents of protein-only nanoparticles opens intriguing possibilities to the tailoring particle geometry through conventional protein engineering, a possibility so far unapproachable in bionanomedicine [11].

References:

[1] E. Vazquez, N. Ferrer-Miralles, R. Mangues, J. L. Corchero, Schwartz S Jr, and A. Villaverde, Modular protein engineering in emerging cancer therapies, Curr. Pharm. Des, 15 (2009) 893-916.

[2] P. Saccardo, A. Villaverde, and N. Gonzalez-Montalban, Peptide-mediated DNA condensation for non-viral gene therapy, Biotechnol. Adv., 27 (2009) 432-438.

[3] E. Vazquez, N. Ferrer-Miralles, and A. Villaverde, Peptide-assisted traffic engineering for nonviral gene therapy, Drug Discov. Today, 13 (2008) 1067-1074.

[4] N. Ferrer-Miralles, E. Vazquez, and A. Villaverde, Membrane-active peptides for non-viral gene therapy: making the safest easier, Trends Biotechnol., 26 (2008) 267-275.

[5] E. Mastrobattista, M. A. van der Aa, W. E. Hennink, and D. J. Crommelin, Artificial viruses: a nanotechnological approach to gene delivery, Nat. Rev. Drug Discov., 5 (2006) 115-121.

[6] Vazquez, E., Roldán, M, Diez-Gil, C, Unzueta, U., Domingo-Espin, J., Cedano, J, Conchillo-Sole, O., Ratera, I, Veciana, J, Ferrer-Miralles, N, and Villaverde, A. Protein nanodisk assembling and intracellular trafficking powered by an arginine-rich (R9) peptide. Nanomedicine 5 (2010).259-268.

[7] E. Vazquez, R. Cubarsi, U. Unzueta, M. Roldan, J. Domingo-Espin, N. Ferrer-Miralles, and A. Villaverde, Internalization and kinetics of nuclear migration of protein-only, arginine-rich nanoparticles, Biomaterials, 31 (2010) 9333-9339.

[8] Y. Zhao, M. Tanaka, T. Kinoshita, M. Higuchi, and T. Tan, Self-assembling peptide nanofiber scaffolds for controlled release governed by gelator design and guest size, J. Control Release, 147 (2010) 392-399.

[9] W. Wu, L. Xing, B. Zhou, and Z. Lin, Active protein aggregates induced by terminally attached self-assembling peptide ELK16 in Escherichia coli, Microb. Cell Fact., 10 (2011) 9.

[10] J. Domingo-Espin, E. Vazquez, J. Ganz, O. Conchillo-Sole, E. García-Fruitós, J. Cedano, U. Unzueta, V. Petegnief, N. Gonzalez-Montalban, A. M. Planas, X. Daura, H. Peluffo, N. Ferrer-Miralles, and A. Villaverde, The nanoparticulate architecture of protein-based artificial viruses is supported by protein-DNA interactions, Nanomedicine, In press, DOI 10.2217/NNM.11.28 (2011).

[11] E. Vazquez and A. Villaverde. Engineering building blocks for self-assembling protein nanoparticles, Microb. Cell Fact., 9 (2010) 101.

USING PARALLEL-STRANDED DUPLEXES TO DIRECT CONTROLLED FORMATION OF PARALLEL-STRANDED G-QUADRUPLEX STRUCTURE

Liliya A. Yatsunyk[1,2] and Jean-Louis Mergny[2]

[1]Swarthmore College, Swarthmore PA 19081, INSERM U869, Bordeaux, France
[2]InstitutEuropéen de Chimie et Biologie, INSERM U869, Bordeaux, France

Nucleic acids are gaining quick popularity and utility for creating new nanomaterials. G-quadruplexes are an attractive alternative to regular B-DNA to assemble nucleic acids, but suffer from a fatal flaw: the rules of pairing, i.e. formation of a G-quartet, in which four identical bases are paired, prevent the controlled assembly between different strands. Complex mixtures are obtained instead of well-defined objects.

In this report, we propose a solution to this problem.Three carefully designed parallel stranded duplexes were used to direct the formation of all parallel G-quadruplex DNA from four different strands. G-quadruplex core can serve as a 'knot' due to its known unusual stability. The presence of multiple points of attachments allows for additions of DNA sequences that are prone to formation of desired specific structures: Watson-Crick duplexes, i-motifs, other G-quadruplexes, etc adding to the versatility of the structure. The correct formation of the overall structure was assessed using gel electrophoresis; the presence of four strands within the structure was demonstrated using fluorescent labels. The presence of the G-quadruplex core was demonstrated through Uv-vis and fluorescent titrations with G-quadruplex specific ligands. The thermal properties of the target structure as well as its duplex components were thoroughly analyzed. The structure obtained displayed unusual but expected stability under denaturing conditions. Attempts to extend the design to one and two dimensional materials are underway in our laboratory.

NANOENGINEERED SELF-ASSEMBLED MONOLAYERS FOR SENSING BIOINSPIRED RECOGNITION

Jing-jiang Yu

Nanotechnology Measurement Division, Agilent Technologies, Inc., Chandler, AZ 85226, USA
Jing-jiang_yu@agilent.com

Self-assembled monolayers (SAMs) have attracted tremendous attention due to their highly ordered structure, stability and rich terminal group chemistry and they offer very promising applications in development of biocompatible materials, solid-phase bioanalytical techniques and biosensors. Particularly, nanometer-scaled mixed self-assembled monolayers (SAMs) are better systems than pure SAMs in mimicking biomembranes because of the presence of segregated domain structures and variety of surface functionalities. In nature, the collective properties and biofunctionalities of these ensembles depend not only on the individual molecular unit but also on the organization at the molecular or nanoscopic level. It has been demonstrated that high-resolution nanofabrication of mixed SAMs with sub-10 nm precision can be achieved readily using either lithography or natural growth approaches [1]. These artificially engineered organic thin films with both desired surface chemistry and designed spatial distribution provide a unique scaffold to investigate bioinspired molecular recognition in the fields of biosensors and immunoassays. For example, HIV infection of CD4 negative cells is initiated by the binding of the viral envelope glycoprotein gp120 to galactosylceramide (GalCer), a glycosphingolipid that serves as the cellular receptor for viral adhesion. By constructing a series of GalCer nanostructures with various geometries via AFM-based lithography and using high-resolution AFM imaging as an in situ, real-time and label-free detection approach to directly monitor the subsequent binding of recombinant gp120 molecules to those engineered carbohydrate ligand nanostructures, the polyvalent interactions between HIV-gp120 protein and GalCer nanostructures are revealed both qualitatively and quantitatively and a better understanding of HIV viral infection process at single molecular level is gained [2]. In addition, our recent studies on advanced strategies to generate thiol-exposing SAMs [3,4] that can serve as highly selective bio-platforms for development of biosensors will be discussed.

References:

[1] Yu et al. A Nanoengineering Approach to Regulate the Lateral Heterogeneity in Mixed Self-Assembled Monolayers. J. Am. Chem. Soc. 2006, 128, 11574-11581.
[2] Yu et al. Polyvalent Interactions of HIV-gp120 Protein and Nanostructures of Carbohydrate Ligands. NanoBiotechnology 2005, 1,201-210.
[3] Yu et al. Nanografting versus Solution Self-Assembly of α,ω-Alkanedithiols on Au(111) Investigated by AFM. Langmuir 2008, 24, 11661-11668.
[4] Li et al. Engineering the Spatial Selectivity of Surfaces at the Nanoscale Using Particle Lithography Combined with Vapor Deposition of Organosilanes. ACS Nano 2009, 7, 2023- 2035.