EUROPEAN SCIENCE FOUNDATION
SETTING SCIENCE AGENDAS FOR EUROPE

Exploratory Workshop Scheme

Standing Committee for the European Medical Research Councils (EMRC)

Standing Committee for Life, Earth and Environmental Sciences (LESC)

Standing Committee for Physical and Engineering Sciences (PESC)

ESF Exploratory Workshop on

BioNanotechnology: Development and Application of Principles of Nano- and Bio-Sciences to Sensing, Diagnostics & Therapy

Sintra (Portugal), August, 31-September, 2 2009

SCIENTIFIC REPORT

Executive summary

Bionanotechnology is an emerging interdisciplinary field at the interface of Nanotechnology and Biotechnology. Advances at this interface are already exerting an influence on the more established fields of Chemistry, Materials Science, Biochemistry, Biosensing, Cell Biology, Molecular Diagnostics and Therapies, and Computer Modelling, and applications in sensing and diagnostics are already being developed, with applications in medicine and therapeutics being predicted for the coming years. Safety aspects are also being assessed in parallel with development of applications.

The Exploratory Workshop took place from August 31st to September 2nd, 2009 in Sintra, Portugal and was attended by 27 scientists from twelve ESF countries and one non-ESF country; over one third of the participants being women. Talks were organized around five positions papers, each with a keynote speaker who introduced and contextualized the sub-topics, followed by short presentations from all other participants grouped under the five sub-topics. At the end of each block of presentations lively discussions were facilitated by the keynote speakers.

Due to the interdisciplinary nature of the theme this Exploratory Workshop was co-sponsored, by the LESC, PESC and EMRC Standing Committees, each of whom had a representative at the workshop. Most of the participating researchers had no previously established collaboration schemes so the Workshop was really "Exploratory" in the sense of giving everyone the opportunity to learn about the ongoing research in the other participants groups, and facilitating the detection of points of common interest and future collaboration.

Three main conclusions emerged regarding priority future directions of Bionanotechnology:

(1) The need for increased investment in basic studies of bio/non-bio interactions from the physico-chemical point-of-view and with consideration of the toxicological implications.

(2) The need for an increased focus on basic studies of bio/non-bio interactions, and in particular, interactions between proteins and metal-based nanoparticles from the physico-chemical point-of-view and to explore the safety and diagnostic implications. Included in this is the need to establish reference *in vivo* models of varying levels of complexity to study nanoparticle-cell interactions.

(3) The potential for development of diagnostic assay kits that are point-of-care, very low-cost, quick to perform and develop, and require minimal manipulation by a non-expert. These assay kits must give a clear answer about a specific health condition (e.g., malaria, tuberculosis), and could be the first really widespread applications of nanotechnology which help to narrow the divide between the first and developing worlds.

The last morning of the Workshop was dedicated to discussion on follow-up activities, such as networking and collaboration opportunities within ESF and EU FP7 Research Programmes. COST and ESF Research Networks were considered especially interesting for this group and two leaders were appointed to start the application processes. In the context of FP7, the "Marie Curie" Initial Training Networks (ITN) was identified by all participants as especially interesting as that program allows for

student training and exchange. Three to four groups of 5-7 participants have shown interest in applying to this Programme. The final purpose of the grouping will be to establish a multi-scale European set of research laboratories to pursue the above mentioned objectives. In order to facilitate the ongoing efforts of the group to develop structure, coherence and critical mass, and to develop sustained activities in this arena, a website has been established for the group, which will host the meeting documentation and outputs, and offer an exchange forum for ongoing discussions. (http://www.cbni.eu/sections/ProjectsFunding/Bionanotechnology-Sintra)

As part of the ongoing efforts to build on the outcomes of the Exploratory Workshop, the 5 keynote speakers are drafting position papers on the 5 main themes of the workshops, which will be published on the Workshop website, and also summarised into one or more peer-reviewed publications on the current state of the field. The 5 position papers are as follows:

Position paper 1: Nanoparticle Synthesis & Bio-Functionalisation.

Position paper 2: Nano-Bio Model Systems: *in vivo* and *in vitro*

Position paper 3: Nano-sensing: biosensor and nanodiagnostics.

Position paper 4: Bio-Nano Compatibility & Nanotherapy

Position paper 5: Theoretical modelling of nano-bio systems,

The position papers are loosely based on the minutes taken at the meeting based on the scientific presentations and the subsequent scientific discussions, and the first drafts have been completed by the keynote presenters, and are now being revised in consultation with the wider group. In particular, the participants are commenting on the position paper on the topic in which they presented, but all participants are invited to comment on all position papers, such that they represent consensus reports on the current state of the art in each of the sub-topics.

The workshop has generated significant interest and has been featured in the ESF LESC newsletter for October 2009, http://www.esf.org/fileadmin/links/LESC/LESC_newsletter_October09.pdf, as well as being selected as one the ESF workshops that will be highlighted by the ESF communications department as part of a series of short stories.

Feedback from the participants was generally very positive, and good momentum was established, which we are actively working to maintain. One future opportunity that has been identified is to use the planned EpitopeMap Research Networking Programme International conference on NanoTheranostics (Cyprus, April 27-30th 2009) as a focus for the group to meet again, and to further their discussions, plans and proposals.

Scientific content of the event

Nanoparticles offer a wide range of unique properties that makes they very exciting for applications in biotechnology, nanomedicine and nanodiagnostics, including their large surface area and the consequent number of atoms in less than energetically stable conformations, their small size which enables them to access places that larger materials cannot, and their versatility in terms of composition, geometry, shape and structure, which means that they can be tailored to have almost any imaginable properties.

The optical properties of nanoparticles depend strongly on their size and/or shape in a highly predictable way. One of the most interesting optical properties is the presence of a strong plasmon band in the near-infrared spectral region, which makes the nanoparticles potentially useful in cancer hyperthermia, biological assays, and cell imaging. Sometimes a combination of different nanoparticles can generate hybrids with enhanced characteristics, such as in the case of magnetic-fluorescent nanostructures that can be utilized for cell labelling and sorting (Teresa Pellegrino; NNL, Lecce, Italy). One of the most interesting potential applications of nanotechnology in diagnostics is the utilisation of the unique optical properties of nanoparticles for biobarcoding by labelling nanoparticles with spectroscopic labels that are each associated with a specific antibody. This allows a versatile analysis system with a total analysis time of less than three minutes (Ramon Alvarez-Puebla; University of Vigo, Spain).

Nanoparticles have highly interesting optical, electronic, magnetic and catalytic properties which depend strongly on the particle's size and/or shape in a highly predictable way. These nanomaterials with unique structures can be further functionalized with molecules that allow specific immobilization of biomolecules (proteins, carbohydrates, DNA or RNA) at their surface, with potential diagnostic and therapeutic uses. However, this arena of nanoparticle targeting is not as straightforward as all that, due to the fact that nanoparticles (and indeed all surfaces) are coated by a layer of proteins and other biomolecules immediately upon contact with physiological solutions. Thus, the targeting moieties can become coated by other proteins, in a manner that is not yet fully understood and as such cannot yet be controlled or pre-programmed into the nanoparticles (Kenneth Dawson, University College Dublin). However, the indications are that surface curvature and surface morphology play an important role in determining the specificity of proteins that bind to nanoparticles surfaces (Iseult Lynch, University College Dublin). "Controlled morphology" is then a key goal of nanoparticle synthesis research. The presentation in question described a strategy based on multilamellar vesicles that act as "onion-like microreactors" allowing the control of nanoparticle morphology from the structure of the onions (Chrystel Faure; CNRS, Bordeaux, France). The resultant particles have the oval-like shape of the vesicles, as well as a narrow size distribution. Additionally, the synthesis is performed using biological agents with no harsh chemicals needed, and as such is a sort of "green-chemistry" approach to nanoparticles synthesis.

Two novel *in vivo* model systems were discussed: the Hydra model, that has been successfully used in the study of the mechanism of nanoparticle-cell interactions with specific focus on particle internalisation and distribution (Claudia Tortiglione; ICEC, Pozzuoli, Italy); and there was a proposal

of using the well studied mosquito that transmits malaria as a new animal model, able to provide proof-of-concept for, e.g., tissue-specific delivery (Elena Levashina; CNRS, Strasbourg, France).

The dream of label-free detection can become a reality through direct detection of antibody binding. Protein biochips can be created for early stage cancer detection, e.g., prostate and ovarian cancers. The use of dispersed, size-selected gold clusters as binding sites for the antibodies that capture the target marker antigens improves the sensitivity of the chips by an order of magnitude (Richard Palmer; University of Birmingham, UK; and the spin-off company "Inanovate").

Some other important focus points that were raised by some of the presentations included:

(i) toxicity issues and the importance to address them, especially when nanoparticles are intended for therapeutic uses (Kenneth Dawson; University College Dublin, Ireland);

(ii) technological utilization of nanoparticles in bioremediation with a "sense and shoot" approach to pollution treatment (Piet Lens; Wageningen University, The Netherlands);

(iii) environmentally benign and "green" nanoparticle synthesis (Eulalia Pereira; University of Porto, Portugal);

and (iv) metal oxides as alternatives to the widespread use of silver nanoparticles as antibacterial agents (now limited by FDA) (Aharon Gedanken; Bar-Ilan University, Ramat Gan, Israel).

Assessment of the results, contribution to the future direction of the field, outcome

Three main conclusions emerged regarding priority future directions of Bionanotechnology:

(1) The need for increased investment in basic studies of bio/non-bio interactions from the physico-chemical point-of-view and with consideration of the toxicological implications.

(2) The need for an increased focus on basic studies of bio/non-bio interactions, and in particular, interactions between proteins and metal-based nanoparticles from the physico-chemical point-of-view and to explore the safety and diagnostic implications. Included in this is the need to establish reference *in vivo* models of varying levels of complexity to study nanoparticle-cell interactions.

(3) The potential for development of diagnostic assay kits that are point-of-care, very low-cost, quick to perform and develop, and require minimal manipulation by a non-expert. These assay kits must give a clear answer about a specific health condition (e.g., malaria, tuberculosis), and could be the first really widespread applications of nanotechnology which help to narrow the divide between the first and developing worlds.

Summary statements on the position papers:

Position paper 1: Nanoparticle Synthesis & Bio-Functionalisation.

1. Well defined / controlled particles needed to assess interactions with living systems
2. Controlled size, shape, structure & composition of particles: inorganic nanoparticles are interesting because of their functionality.
3. Variety of surface coatings available to provide excellent colloidal properties in aqueous solution.
4. Particles must be purified well and well characterised
5. In contact with biological media, properties of particles change
6. Bio-conjugation of particles is still in its infancy and generalised protocols for defined conjugates are still needed.

Position paper 2: Nano-Bio Model Systems: *in vivo* and *in vitro*

1. Functionalisation of nanoparticles and micro-pattered and nano-pattered surfaces.
2. Design a nanomaterial for biological applications – need to first find a real problem (therapy, diagnosis, basic research)– biomoleucles and in biological interactions involved (proteins, carbohydrate, antibodies…)
3. NP vectorisation – np will go specifically to target – need right design of the nanoparticles
4. Stability: stable in physiological conditions, avoid non-specific interactions, and avoid macrophage uptake.
5. Patterning of surface to control cell adhesion, stem cell differentiation etc.
6. Many applications but need a good design of the NP and good model systems to study in an ethical way.

Position paper 3: Nano-sensing: biosensor and nanodiagnostics

1. DNA hybrisization sensors – Screening for genetic or infectious diseases etc.
2. Electrochemical DNA biosensors. Label detection – enzymes, nanoparticles
3. Immunosensors and cell sensors
4. Nanoparticles more stable than enzymes, and allow detection of several analytes simultaneously.
5. Nanoparticle optical sensing – Au absorption changes on aggregation induced by binding. SPR – nanoparticles tethered to surface.
6. Interference due to nanoparticles needs to be considered – e.g. Quenchers of fluorescence.

Position paper 4: Bio-Nano Compatibility & Nanotherapy

1. New fundamental length-scales: Less than 100nm enter cell, less than 40nm enter nucleus, less than 35nm pass Blood Brain Barrier. What are the fundamental control parameters? Uptake mechanisms / energy dependence?

2. Nanoparticles utilise existing biological pathways possibly in new ways. Nanoparticles are trafficked unlike conventional drugs which diffuse across membranes. Role of the nanoparticle-protein corona? What are the trafficking signals – epitopes?
3. Labelling for live cell imaging. Conventional routes leak dye. Fluorescence penetration depth not sufficient for *in vivo* work. Can we develop new approaches?
4. Bioaccumulation of nanoparticles in cells. Important for regulation and for therapy – seems that nanoparticles do not pick up an exit signal from surroundings. Can such a signal be engineered in?
5. Nanoparticle reproducibility – can we achieve it? Batch-to-batch variability of nanoparticles – differences in surface charge have dramatic differences on biological impacts.
6. Nanoparticle dispersion in biofluids. Quantitative reproducibility in nanobiology – can we achieve it? Minor differences in serum / medium can have dramatic effects.

Position paper 5: Theoretical modelling of nano-bio systems

1. Mechanisms of reactions can be modelled leading to tranition state structures
2. Mutatued enzyymes with desired new catalytic activities can be designed using catalytic fields
3. Transition state analogs can be designed to form molecular imprinted polymers with desired catalytic activity
4. Using DTSS approach, mutated enzymes with eliminated catalytic activity can be designed to act as sorbents or sensors.
5. Training in molecular modelling.

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(http://www.cbni.eu/sections/ProjectsFunding/Bionanotechnology-Sintra)

ESF EMRC/LESC/PESC Exploratory Workshop:

BioNanotechnology: Development and Application of Principles of Nano- and Bio-Sciences to Sensing, Diagnostics & Therapy

Sintra (Portugal), August, 31-September, 2 2009

Final programme

Monday 31 August 2009

Afternoon	*Arrival*
13.00-14.30	*Lunch*
15.00-15.15	**Welcome by Convenor**
15.15-15.35	**Presentation of the European Science Foundation (ESF)** **Salim Belouettar** (ESF Standing Committee for Physical and Engineering Sciences - PESC), **Constantin Doukas** (ESF Standing Committee for Life, Earth and Environmental Sciences - LESC) **Martin Röllinghoff** (ESF Standing Committee for the European Medical Research Councils - EMRC)
15.35-16.20	**Position Paper WG1** **Wolfgang Parak** (University of Marburg, Germany) *Nanoparticle Synthesis & Bio-Functionalization*
16.20-17.30	**Presentation by Participants from WG1** (9 minutes presentation of each Group/Institution) **Eulália Pereira** (REQUIMTE, Porto, Portugal) *New methodologies for the synthesis of anisotropic gold nanoparticles* **Maria Grazia Rimoli** (University of Naples, Italy) *Synthesis and functionalization for drug targeting* **Ladislau Vékás** (Romanian Academy, Timisoara, Romania) *Magnetic nanoparticles and biocompatible magnetic nanofluids: synthesis and structure* **Rodica Turcu** (National Institute for Research and Development of Isotopic and Molecular technologies, Cluj-Napoca, Romania) *Biocompatible nanosystems based on magnetic nanoparticles and functionalized polymers* **Ramon Alvarez-Puebla** (University of Vigo, Vigo, Spain) *Synthesis and preparation of new nanostructured materials with optical activity for ultra-sensitive analyses* **Teresa Pellegrino** (National Nanotechnology Laboratory, Lecce, Italy) *Magnetic-Fluorescent Nanostructures based on Superparamagnetic Iron Oxide Nanoparticles and Oligothiophenes for Cell Labeling and Sorting*

Chrystel Faure (CNRS, Bordeaux, France)
Synthesis of inorganic nanoparticles with controlled morphology using lipid-based multilamellar vesicles

17.30-18.15 **Discussion**

18.15 *Get-together, social event, informal (Sintra)*

19.00 *Dinner*

Tuesday 1 September 2009

09.00-09.45 **Position Paper WG2**

Jesus de la Fuente (Instituto de Nanociencia de Aragón, Zaragoza, Spain)
Nano-Bio Model Systems: in vivo and in vitro

09.45-10.30 **Presentation of Participants from WG2**

(9 minutes presentation of each Group/Institution)

Claudia Tortiglione (Instituto di Cibernetica "Eduardo Caianiello", Pozzuoli, Italy)
An in vivo model system to test functional nanoparticles

Hélène Feracci (CNRS- Bordeaux, Bordeaux, France)
Cell adhesion: from cadherin nanomechanics to cellular biosensing

Aharon Gedanken (Bar-Ilan University, Ramat Gan, Israel)
Antibacterial, Antiviral, and Antibiofilms Nanoparticles

Markus Linder (Technical Research Centre of Finland, Espoo, Finland)
Genetically engineered proteins for functionalizing and making nanomaterials

Piet Lens (Wageningen University, Wageningen, The Netherlands)
Microbial manufacture of chalcogen quantum dots

10.30-11.00 **Discussion**

11.00-11.25 *Coffee / Tea Break*

11.25-12.10 **Position Paper WG5**

W. Andrzej Sokalski (Wroclaw University of Technology, Poland)
Theoretical Modelling of Nano-Bio Systems

12.10-12.20 **Presentation of Participants from WG5**
(9 minutes presentation of each Group/Institution)

Borys Szefczyk (Wroclaw University of Technology, Poland)
Application of molecular modelling and ab initio methods to study interactions and processes at the solid phase surface and self-assembled monolayers

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12.20-12.30	**Discussion**
12.30-14.30	*Lunch*
14.30-15.15	**Position Paper WG4**
	Kenneth Dawson (University College Dublin, Dublin, Ireland) *Bio-Nano Compatibility & Nanotherapy*
15.15-16.00	**Presentation of Participants from WG4**
	(9 minutes presentation of each Group/Institution)
	Richard Palmer (University of Birmingham, Birmingham, UK) *Size-selected metal clusters to immobilize proteins: deposition, atomic imaging, biochips*
	África Gonzalez (University of Vigo, Vigo, Spain) *Biocompatibility and immunogenicity of nanoparticles*
	Iseult Lynch (University College Dublin, Dublin, Ireland) *Designing nanoparticles for cellular targeting and delivery*
	Nicola Tirelli (University of Manchester, Manchester, UK) *Oxidation-responsive nanoparticles and other nanomaterials: Molecular design and interactions with (inflammatory) cells.*
16.00-16.20	*Coffee / Tea Break*
16.20-16.50	**Discussion**
16.50-17.35	**Position Paper WG3**
	Alfredo de la Escosura Muñiz (Institut Català de Nanotecnologia (ICN), Bellaterra, Spain) *Nano-sensing: Biosensor and Nanodiagnosis*
17.35-18.30	**Presentation of Participants from WG3**
	(9 minutes presentation of each Group/Institution)
	Ricardo Franco (FCT/UNL, Lisbon, Portugal) *Bionanoconjugates of DNA or proteins and gold nanoparticles as biosensors*
	Ivo Safarik (Academy of Sciences, Ceske Budejovice, Czeck Repulbic) *Magnetically responsive nano(bio)composite materials for bioapplications*
	Pawel Kafarski (Wrocław University of Technology/EIT+, Poland) *An Approach to Construct Diagnostic Chips for Thyroid Cancer*
	João Cortez (REQUIMTE, Caparica, Portugal) *Nanobiosensors based on nanoparticle-protein interactions*

Elena LEVASHINA (CNRS, Strasbourg, France)
Potential for development of bionanotech-based immune-sensors for malaria parasites.

18.30-19.00 **Discussion**

19.30 *Conference Dinner*

Wednesday 2 September 2009

09.00-09.30 **Introduction to upcoming calls on ESF and FP7**
RNP; EUROCORES; FP7 - NMP/KBBE

09.30-12.30 **Discussion on follow-up activities/networking/collaboration**

12.30 *End of Workshop and Lunch*

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Final list of participants

Name	First name	Affiliation	Country
ALVAREZ-PUEBLA	Ramón	Universidade de Vigo	Spain
CORTEZ	João	Universidade Nova de Lisboa	Portugal
DAWSON	Kenneth	University College Dublin	Ireland
DE LA ESCOSURA-MUÑIZ	Alfredo	Institut Catala de Nanotecnologia	Spain
DE LA FUENTE	Jesus	Instituto de Nanociencia de Aragón	Spain
FAURÉ	Chrystel	Centre National de la Recherche Scientifique	France
FERACCI	Hélène	Centre National de la Recherche Scientifique	France
FRANCO	Ricardo	Universidade Nova de Lisboa	Portugal
GEDANKEN	Aharon	Bar-Ilan University	Israel
GONZÁLEZ-FERNÁNDEZ	África	Universidade de Vigo	Spain
KAFARSKI	Pawel	Wrocław University of Technology	Poland
LENS	Piet	Wageningen University	Netherlands
LEVASHINA	Elena	CNRS Institut de Biologie Moléculaire et Cellulaire	France
LINDER	Markus	VTT Technical Research Centre of Finland	Finland
LYNCH	Iseult	University College Dublin	Ireland
PALMER	Richard	University of Birmingham	United Kingdom
PARAK	Wolfgang	University of Marburg	Germany
PELLEGRINO	Teresa	University of Brescia	Italy
PEREIRA	Eulália	Universidade do Porto	Portugal
Grazia RIMOLI	Maria	University of Naples	Italy

SAFARIK	Ivo	Academy of Sciences	Czech Republic
SOKALSKI	Andrzej	Wrocław University of Technology	Poland
SZEFCZYK	Borys	University of Porto	Portugal
TIRELLI	Nicola	University of Manchester	United Kingdom
TORTIGLIONE	Claudia	University of Naples	Italy
TURCU	Rodica	National Institute R&D for Isotopic and Molecular Technologies	Romania
VÉKÁS	Ladislau	University Politehnica Timisoara	Romania

Statistical information on participants (age bracket, countries of origin, etc.)

Average age	45.4
% Female	37
ESF countries	12
Non-ESF country	1