

NANOTHERANOSTICS: Fabrication and Safety Concerns

NANOTHERANOSTICS

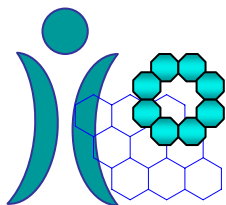
Nanoparticulate systems in therapeutic and diagnostic applications: Fabrication and Safety Concerns

(1) SUMMARY

The “*Nanotheranostics: Fabrication and Safety Concerns*” conference was held in Cyprus as a 4-day event (27th- 30th April 2010), bringing together research teams working on the development of novel nanoparticulate systems (NPs) destined for biomedical applications with teams working on the investigation and understanding of the interactions taking place between NPs and biological systems and the interconnection of the latter to the question of nanosafety. The latter is a key goal of the ESF Networking Program, EpitopeMap, which deals with promotion of the understanding of the nature of the surface-adsorbed protein layer on biomaterials and nanoparticles as a key determining factor of biocompatibility and nanoparticle safety.

The local organising committee of the conference consisted of faculty members of the University of Cyprus and the Cyprus University of Technology. Since nanosafety evaluation is an issue for which the Cyprian governmental authorities are also interested, the Department of Labour Inspection, Ministry of Labour and Social Insurance in Cyprus was also represented by one member in the local organising committee. The international scientific committee consisted of the members of the EpitopeMap Steering Committee and six other members, coming from 13 European countries. 55 delegates participated in the conference including senior investigators as well as young researchers and students involved in the fields of biomaterials, nanomedicine, nanodiagnostics and nanosafety evaluation. Four leading edge plenary speakers with expertise in the fields of nanostructured biomaterials (Prof. R. Bizios), gene-delivery involving nanotechnological approaches (Prof. E. C. I. Smith), nanoimaging (Prof. K. Kostarelos) and nanomedicine (Prof. R. Duncan) participated in the conference, sharing their research expertise and translational experiences. The conference participants came from several different European countries (Portugal, Spain, Greece, Romania, France, Ireland, Sweden, England) as well as from countries outside Europe such as the U.S.A and Japan. Moreover, the conference succeeded in attracting a strong audience from the national academia, students, junior and senior researchers.

Besides the ESF (Network Programme EpitopeMap) support, which provided the majority of the funding for the conference, the meeting was also co-funded by local organisations and local private companies. The registration costs for the conference were kept at minimum (100 euro for non-students and 50 Euro for students) to ensure student participation. Indeed this was accomplished since approximately half of the delegates were students. This conference promoted an effective and friendly communication environment between the conference participants, providing opportunities to further their interactions and develop research collaborations in the near-future.



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(2) DESCRIPTION OF THE SCIENTIFIC CONTENT AND DISCUSSION AT THE EVENT

The fabrication of nanoparticulate systems (NPs) destined for use in therapeutic and diagnostic applications, as well as the necessity to urgently address nanosafety issues related to biomedical applications of nanomaterials, have attracted considerable scientific and societal attention. The applicability of nanotechnology in the medical field involves the development of novel nanomaterials and nanodevices with potential use as therapeutic and diagnostic (theranostic) applications. Such nanomaterials include, among others, nanoparticulate systems (NPs) used as drug delivery and gene transfection agents, nanomaterials employed in tissue engineering applications and nanosystems introduced as diagnostic tools. Hence the conference was divided into 6 scientific sessions, four of which (sessions 1, 3, 4 and 5) were related to the above-mentioned.

- **Session 1:** Fabrication and safety concerns in tissue engineering applications
- **Session 2:** Safety concerns of nanoparticulate systems (NPs)
- **Session 3:** NPs in drug and gene delivery applications
- **Session 4:** Fabrication: magnetic nanosystems in imaging and therapy
- **Session 5:** Fabrication: Gold nanoparticles in imaging and therapy
- **Session 6:** Safety Concerns of NPs

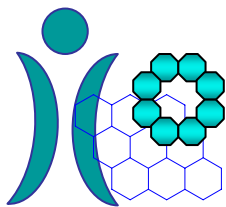
Sessions 2 and 6 were devoted to the understanding of the interactions taking place between biological systems and these NPs and the effect of such interactions on biocompatibility and nanoparticle safety.

In the following, a brief summary of the scientific content at the event corresponding to each session is provided.

Session 1

The use of *nanomaterials in tissue engineering applications* is directed towards efforts to restore, maintain or improve damaged tissues. Nanomaterials offer considerable advantages for tissue engineering as their nanoscale dimensions are capable of mimicking the surface properties of natural tissues. Applications include the use of nanomaterials in bone, cartilage, vascular, neural and bladder tissue engineering.

The first plenary lecture, which was on this topic, was given by Professor Rena Bizios (The University of Texas at San Antonio, U.S.A) and emphasized the necessity for identification and elucidation of materials structures and properties, and of the interaction mechanisms involved between biomolecules and the surfaces of nanostructured materials used as implant biomaterials. The above-mentioned are very crucial for design and fabrication of the next-generation of nanostructured biomaterials presenting the potential of major clinical impact towards this direction. Moreover, it has been concluded that the establishment of the biocompatibility and safety of nanomaterials destined for implant applications is of paramount importance. This lecture was very inspirational and it was followed by a very lively discussion.



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Four follow-on talks in this session were presented providing both, experimental and computational insights into this topic.

Session 3

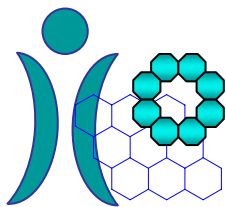
The development of novel *nanomaterials destined for use as drug delivery systems or gene transfection agents* has attracted great attention during recent years. The introduction of nanostructures aims to improve various aspects related to the above-mentioned applications including stability, drug targeting and effectiveness, reduction of drug toxicity and improved pharmacokinetics. A key requisite in the use of NPs for medical applications is that the NPs themselves must do no harm to the body or to the environment, and thus, safety considerations must be taken into account at the design stage.

The session “*Nanoparticulate systems in drug and gene delivery applications*” was opened by an excellent presentation by Prof. Edvard Smith (Clinical Research Center, Department of Laboratory Medicine, Karolinska Institutet, Sweden). In Prof. Smith’s lecture novel nanotechnology approaches in gene delivery applications were presented. More precisely, the fabrication of lipid-spermine derivatives used as a means for transferring genes carried in a plasmid was described, as well as the development of short, single-stranded antisense oligonucleotides capable of simultaneously binding to both strands of a DNA double helix were discussed, among other recent developments.

The follow-on talks covered issues such as the use of polymeric nanocarriers as gene transfection reagents, drug delivery nanocapsule design *via* the use of multiphysics and robotic virtual reality platform and navigable in blood vessels drug delivery nanocapsules. Issues related to the use of NPs in drug delivery applications were also discussed in sessions 4 and 5.

Sessions 4 and 5

In recent years, the development of *nanodiagnostic* tools has seen great progress. The driving force for this development is the necessity to understand biological and medical processes carried out at a molecular level in order to be able to detect diseases earlier in their progression, and thereby to implement treatment earlier, and potentially reverse disease progression and reduce disease severity. The development of state-of-the-art instrumentation in combination with advances in Nanotechnology may provide the means for addressing this great challenge. Nanomaterials for diagnostic use include nanoparticulate systems exhibiting magnetic or fluorescent properties which can report back on their environment. Multimodal nanoparticulate systems bearing complementary imaging moieties, which allow for the investigation of the particle localization across a number of platforms, such as magnetic, optical or nuclear imaging offer really exciting potential. Imaging agents which combine luminescent and magnetic properties are considered advantageous in multimodal imaging. Using a variety of nanomaterials for multiplex diagnostics and imaging applications may offer sensitive, rapid and cost-effective solutions for the modern clinical laboratory.



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Professor Kostas Kostarelos (School of Pharmacy, University of London, U.K.) opened Session 4 with an excellent plenary lecture focused on the design of carbon nanotube-based imaging agents. Carbon nanotubes are a new class of nanomaterials attracting great scientific attention directed towards their use in a variety of therapeutic and diagnostic applications. The lecture was focused on the design and biological investigation of different carbon nanotube-based nanomaterials, in which CNTs are combined with imaging probes for *in vitro* and *in vivo* detection and monitoring. For each proposed strategy, the advantages and limitations of using CNTs as imaging agents have been highlighted.

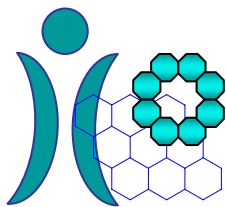
Session 5 was opened by Professor Ruth Duncan (Cardiff University, U.K.) who gave an excellent overview of the design and development of nano-medicine and nano-imaging agents for clinical use, and presented the background ideas and examples from her own and other state of the art research, in her presentation “*Nano-Medicines and Nano-Imaging Agents: Design and Development for Clinical Use – A practical Reality?*”. In her presentation Prof. Duncan prompted the audience to take lessons from both the design and development of the “first generation” nanomedicines and imaging agents that have already entered clinical practise as well as from the nanotechnologies that failed to progress into clinical trials, prior to their beginning the process of designing and developing their own new nanomaterials destined for biomedical applications. She also emphasized the key steps required for a successful clinical use of nanotechnologies in medicine including disease focus, interdisciplinary collaboration and nanomaterial safety assessment.

The presentations following the plenary talks in sessions 4 and 5 gave further insights into the area of nanomedicine and nanoimaging.

Sessions 2 and 6

Since nanotechnology probably comprises the most important inter-disciplinary research area of the future, and will likely underpin many technological applications in the future, in addition to the efforts which are carried out towards the development of novel bionanosystems, it is equally important to understand the biological effects of these materials and their impact on human health and the environment. Currently, there is only limited knowledge regarding the potential risks of nanoparticulate systems used in the biomedical field. Efforts directed towards modulation of pharmacokinetic properties (e.g. blood half-life, elimination, biodegradation), toxicity and immunogenicity involve rational surface modifications and coating of NPs.

The necessity to address nanosafety issues related to nanomaterials and nanobiotechnologies led to the devotion of two sessions of this conference to *Safety Concerns of NPs*. In these two sessions the work of the people involved in the activities of the ESF Epitopemap Program was represented by several oral presentations, emphasizing the expertise and on-going activities within this programme: Dr. Lynch from University College Dublin gave a talk entitled “*Designer nanoparticles for Quantitative Bionano Interactions*”. Dr. Cedervall from Lund University, Sweden gave a talk on “*Surface dependent structural changes in proteins after binding to nanoparticles*”. Ms. Fiona Quinlan-Pluck presented work related to the investigation of protein fibrillation in the presence of nanoparticles and the correlation of the fibrillation mechanism to toxicity. Moreover, Dr. Lynch in her



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introductory presentation entitled “*EpitopeMap Research Networking Programme*” given on the first day of the meeting, presented the scientific activities and funding opportunities of the European Science Foundation, focusing mainly on the activities of the ESF EpitopeMap programme. She invited all conference participants to participate in EpitopeMap through exchange and short visits to the partner laboratories and other laboratories to conduct projects related to the topic of understanding the role of proteins in mediating nanoparticle interactions with living systems. Brochures of the EpitopeMap programme were also distributed to all participants on the 1st day of the conference during registration. Thus, an additional outcome of the meeting was an increased awareness of the ESF and the EpitopeMap research networking programme among the participants.

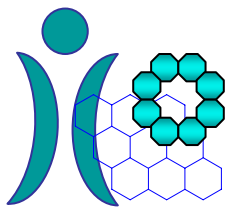
Besides the oral contributions, 18 posters were presented at the conference. The poster presenters (mostly students) were provided the opportunity to highlight their work in the 2-hour Poster Session held on the 2nd day of the conference. The posters were evaluated by a 5-member committee (Prof. Bizios, Prof. Kostarellos, Prof. Smith, Dr. Lynch and Dr. Krasia-Christoforou) and 3 poster prizes were announced at the conference:

Best Poster Award: Stylianou Panayiota for the poster entitled: “*Imaging morphogenesis, in Xenopus with Quantum Dot nanocrystals*”.

2nd Poster Award: Terzaki Konstantina for the poster entitled: “*Fabrication of three-dimensional metal-binding structures by non-linear lithography and their functionalisation with amyloid peptides*”

3rd Poster Award: To: Tamamis Phanourios for the poster entitled: “*Species specificity of the complement inhibitor compstatin: An interpretation by all-atom molecular dynamics simulations of the human and rat C3c: Compstatin complexes*”

The conference ended with a panel discussion chaired by Dr. Iseult Lynch that was carried out in a friendly and highly communicative way between the participants. During this discussion each of the 4 keynote speakers were asked to give short closing remarks on the topic of what is nanotheranostics, and what advice they have to give to the younger researchers present at the workshop.



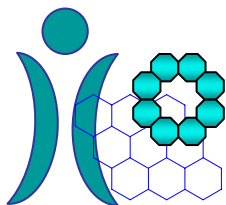
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(3) ASSESSMENT OF THE RESULTS AND IMPACT OF THE EVENT ON THE FUTURE DIRECTION OF THE FIELD

The oral and poster presentations were of high quality and the conference participants interacted effectively with each other, not only during the discussions following the presentations but also in between, during lunch, coffee breaks and dinners. The presentations given by the four plenary speakers were excellent, highly educational and inspiring, especially for the postgraduate students and the postdoctoral researchers who gained a wider perspective on their research. Many participants took the time to write to the organisers following the meeting to express their thanks: *“Thank you for organizing such a splendid meeting. The science was great and the organization excellent! I liked it very much in all respects.”* - Prof. E. Smith. *“I thought it was vibrant and constructive and just of the right size to get to know everyone participating. Thank you for everything”* – Prof. K. Kostarelos. *“The venue, was most successful not only because of its scientific content but also because it gave opportunities for interactions among the participants.”* – Prof. R. Bizios. *“Thank you again for organizing an exciting meeting and your warm hospitality - I was also very pleased to see the high quality of science in Cyprus”* – Dr. A. Mitraki. *“It was a really good meeting on possible applications of nanostructured materials in biomedicine. What I wish to evidence even more is the excellent organization in a beautiful place...It was a hard work for you, congratulations”* – Prof. L. Vekas.

During the conference, the participants had the opportunity to develop new research linkages and received information regarding the EpitepeMap programme, its goals and visions. The conference was highly valuable to Cypriot researchers working in this area who had the opportunity to discuss with the other participants and in some cases find common research lines for future collaborations. Increased awareness of the EpitepeMap Research Training Network, and the European Science Foundation in general was also developed. This is evidenced by the fact that people participating at this meeting have already expressed their interest in joining the EpitepeMap RNP activities (for example Dr. Ricardo Franco who has recently applied for a short visit grant).

The panel discussion chaired by Dr. Lynch was opened by Prof. Rena Bizios, who mentioned that Theranostics/nanotheranostics is a new field, just starting out, and as it develops it will experience some growing pains. It will borrow from applied, basic, physical, medical sciences, but it will need to develop a unique set of theories and hypotheses, including participating in the discussion of *“what Nano is”*. Prof. Bizios predicted that in the future we will look back and see that we were at least asking the right questions, and identifying the important issues. An important aspect of the theranostics research theme is the need to bring materials from the laboratory to the clinic, and that this process starts from a biochemical / medical problem. It is also important to take account of the fact that proteins mediate the interaction between materials and patients, and thus interaction issues are of paramount importance. She summarised by suggesting that more work is required regarding how proteins interact with materials (which is a key goal for EpitepeMap), and she cautioned in terms of choosing / selecting proteins for *in vitro* and *in vivo* applications, saying that the behaviour as isolated proteins is often not typical of their behaviour in the more complex biological arena.

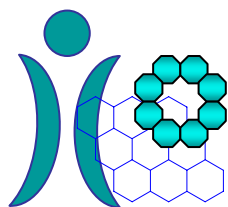


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Prof. Ruth Duncan continued the discussion by asking the audience to raise their hands if they considered that they worked in Theranostics. Nobody raised their hand since the work presented at the conference was focused on either the Therapeutic or the Diagnostic use of novel nanomaterials (not both) as well as on the investigation of nanosafety issues related to such systems. This highlighted the newness of the area, and that the younger people will be the ones to establish groups dedicated to this topic upon developing inter-disciplinary collaborations. She also mentioned that ESF is also in the process of trying to define nanomedicine and that it is important to avoid hype and overuse of buzz words. She highlighted the fact that when she established her research group, she called it “*polymer controlled drug delivery*”, but later changed it to “*polymer therapeutics*” as regulatory agencies see them as a drug not a container that simply releases drugs. Nanomedicine is still growing and evolving. *Theranostics = diagnosis and therapy combined.* Prof. Duncan also highlighted again that the 1st theranostic devices do exist: antibodies labelled with a radioisotope to enable their tracking. She highlighted the need to enjoy the science, and the fact that there is a lack of drug scientists / pharmacologists integrated into this type of research. She suggested that a new education & training programme is needed to train a new generation of theranostic scientists who also understand both the process of drug development and translational medicine. Schemes such as the EU Marie Curie and Erasmus-Mundus were mentioned as possibilities here. There was some discussion on this point, and all senior scientists present converged on the idea that multi- and cross-disciplinary training should come at a PhD level, and that a solid education in a core discipline (chemistry, physics, medicine, pharmacology etc.) was essential. Disciplines are the bricks in the educational wall, with the interdisciplinarity being the cement that holds it together. Thus, it is necessary to be competent in the basic sciences first. She ended by quoting a great hero in the field of tumour science, Judah Folkman (Moses Judah Folkman: 1933 – 2008) “*if you die before your hypothesis is proven, you are called stubborn*”. Her message to the younger researchers was thus: “*Believe in your science and yourself, listen to your supervisor, but remember that science does not go forward unless people question the assumptions and the current understanding!*”

Prof. Kostas Kostarelos, building on the theme of how we identify the research area, emphasized that the important issue is to ensure that we do not oversell the brand of theranostics too early, and that we need to back up the claims for the arena of theranostics with hard science. He defined nanotheranostics as the application of nanotechnology to medicine, and that at the end of the day, success or failure will be judged on the basis of delivery of new functional products, and that longevity will be judged on the basis of outputs. He also recognised the need for more workshops on the scale of the present one, to really tease out the issues and the important questions. Advice for the younger researchers present was to not hype, to build up scientific knowledge, to have a scientific vision and to persevere in the pursuit of it.

Finally, Prof. Edvard Smith suggested that the tools to address the questions and challenges for which nanotheranostics offer such great promise do not yet exist. He suggested that significant basic research is still needed before focusing on applications and translation to the clinic.



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(4) FINAL PROGRAMME OF THE MEETING

NOTE: Due to the volcano eruption in Iceland, and the subsequent disruption of flights across Europe in the following weeks, three of the speakers were unable to attend. However, we were lucky that almost all participants were able to travel to and from the meeting with minimal disruption.

Tuesday 27 April

14.00-19.00	Registration
19.30-21.30	Welcoming Reception

Wednesday 28 April

8.30-9.00	Registration	
9.00-9.10	Welcome and Opening	T. Krasia-Christoforou
	Remarks	
9.10-9.30	RTDI Activities in Cyprus	C. Makri
9.30-9.50	EpitopeMap Research	I. Lynch
	Networking Programme	
		Chairman: A. Odysseos
9.50-10.30	Plenary Lecture (PL-1)	R. Bizios Nanostructured biomaterials for implant applications: Potential, challenges and future directions

10.30-11.00 Coffee Break – Poster exhibition

Session 1. Fabrication and safety concerns in tissue engineering applications
Chairman: A. Odysseos

Oral Contributions

11.00-11.20	Oral Contribution 1.1	T. K. Georgiou	Inject-able gels for tissue engineering applications
11.20-11.40	Oral Contribution 1.2	K. Kapnisis	In-vitro biomechanical studies of endovascular devices
11.40-12.00	Oral Contribution 1.3	A. Mitraki	Self-assembling peptides as templates for the formation of inorganic nanomaterials and cell attachment
12.00-12.20	Oral Contribution 1.4	G. Archontis	Oligopeptide self-assembly: Insights from experiments and simulations

12.30-14.00 Lunch Break – Poster exhibition

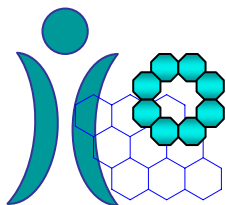
Chairman: H. J. Byrne

14.00-14.40	Plenary Lecture (PL-2)	T. J. Webster	In situ nanotechnology-derived sensors for ensuring implant success - CANCELLED
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Session 2. Safety concerns of nanoparticulate systems
Chairman: H. J. Byrne

Oral Contributions

14.40-15.00	Oral Contribution 2.1	S. Vazquez-Campos	NANOPOLYTOX project: Toxicological impact of nanomaterials derived
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15.00-15.20	Oral Contribution 2.2	I. Lynch	from processing, weathering and recycling of polymer nanocomposites used in various industrial applications
15.20-15.40	Oral Contribution 2.3	T. Cedervall	Designer nanoparticles for quantitative bionano interactions
15.40-16.00	Oral Contribution 2.4	F. Quinlan-Pluck	Surface dependent structural changes in proteins after binding to nanoparticles
16.00-16.20	Oral Contribution 2.5	K. Hadjigeorgiou	Investigating protein fibrillation in the presence of nanoparticles: from the mechanism of fibrillation to toxicity <i>in vitro</i>
16.20-16.50	Coffee Break – Poster exhibition		

17.00-19.00 POSTER SESSION

Thursday 29 April

8.30-9.30	Registration	Chairman: T.K. Georgiou	
9.30-10.10	Plenary Lecture (PL-3)	E.C.I. Smith	Nanotechnology approaches in gene delivery applications

10.10-10.30 Coffee Break – Poster exhibition

Session 3. Nanoparticulate systems in drug and gene delivery applications
Chairman: T.K. Georgiou

Oral Contributions

10.30-10.50	Oral Contribution 3.1	K. Pafiti	Star polymer nanocarriers for siRNA therapeutics: Development of hydrophilic cationic star polymer transfection reagents
10.50-11.10	Oral Contribution 3.2	A. Ferreira	Drug delivery nanocapsule design using multiphysics and robotic virtual reality platform
11.10-11.30	Oral Contribution 3.3	A. Ferreira	Navigable drug delivery nanocapsules in blood vessels

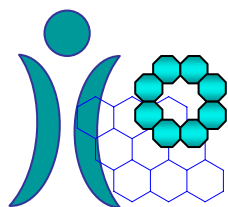
11.30-13.30 Lunch Break – Poster exhibition

13.30-14.10	Plenary Lecture (PL-4)	Chairman: A. Ferreira	
		K. Kostarelos	The design of carbon nanotube-based imaging agents

Session 4. Fabrication: Magnetic nanosystems in imaging and therapy
Chairman: A. Ferreira

Oral Contributions

14.10-14.30	Oral Contribution 4.1	L. Vekas	Water based magnetic nanofluids: synthesis procedures, colloidal stability and properties
14.30-14.50	Oral Contribution 4.2	R. Turcu	Design of magnetic nanostructures using different polymers or block copolymers for magnetic nanoparticles encapsulation



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14.50-15.10 Oral Contribution 4.3 T. Georgelin Coupling bleomycin to multifunctionalized magnetic nanoparticles, toward an efficient cancer treatment

15.10-17.30 Visit: "Thalassa", Municipal Museum of the Sea, Ayia Napa

19.00 - Conference Dinner – Poster Prize announcement

Friday, 30 April

9.30-10.10 Plenary Lecture (PL-5) **Chairman: I. Lynch**
R. Duncan Nano-Medicines and Nano-Imaging agents: Design and development for clinical use – A practical reality?

Session 5. Fabrication: Gold nanoparticles in therapy and imaging
Chairman: L. Vekas

Oral Contributions

10.10-10.30 Oral Contribution 5.1 S. Roux Gold nanoparticles for multimodal imaging and therapy - **CANCELLED**

10.30-10.50 Oral Contribution 5.2 K. Rahme Synthesis and stabilisation of gold nanoparticles using poly(ethylene-oxide)-based polymers: stability studies, grafting density and cellular uptake

10.50-11.10 Oral Contribution 5.3 M. Angelidou Design of novel, bioinspired, gold nanostructures for diagnostic applications

11.10-11.40 Coffee Break

Session 6. Safety Concerns of NPs
Chairman: I. Lynch

11.40-12.00 Oral Contribution 6.1 H.J. Byrne In vitro mammalian cytotoxicological study of PAMAM dendrimers

12.00-12.20 Oral Contribution 6.2 D. Sheehan Identification of protein oxidation in biological systems in response to nanomaterials - **CANCELLED**

12.20-12.40 Oral Contribution 6.3 S. Ramírez-García TiO₂ suspensions for toxicological studies

12.40-13.00 Oral Contribution 6.4 M. P. Monopoli Protein corona associated with nanoparticles

13.00-14.30 Panel Discussion - Concluding Remarks and Poster Prize Announcement
14.30-

Lunch Break