DOI: 10.1478/AAPP.97S2A14

AAPP | Atti della Accademia Peloritana dei Pericolanti
Classe di Scienze Fisiche, Matematiche e Naturali
ISSN 1825-1242

Vol. 97, No. S2, A14 (2019)

SMART INTEGRATED NANOSYSTEMS FOR BIOMEDICAL APPLICATIONS: CRITICAL ISSUES AND PERSPECTIVES

Domenico Lombardo [a] *, Luigi Pasqua [b] and Mikhail A. kiselev [c]

Abstract. During the last decades the progresses of nanoscience and nanotechnology for biomedical application stimulated the transition from traditional drug delivery systems to the development of smart integrated nanosystems with stimuli-responsive characteristics. A wide range of smart integrated nanosystems have proven their effectiveness for various types of biomedical tasks, including stimuli-responsive liposomes, polymeric and metal nanoparticles, silica and hybrid (organic/inorganic) nanostructures. Moreover, these nano-platforms include the possibility to develop within the same nano-platform a diagnostic imaging system with the monitoring of the temporal evolution of the molecular response of a disease for each patient. This theranostic approach could enable the selection of the appropriate treatment therapy planning, thus paving the way for the modern approach of the personalized medicine. However, these nano-structured platforms present lack of toxicity assessment tests, and lack of experience between the pre-clinical and clinical studies, thus resulting in the huge difficulties to obtain regulatory and ethics approval. As a result, most of these relatively complex stimulus-sensitive/responsive nano-platforms are not currently approved for clinical use. In this article we review the main breakthroughs for the rational design of theranostic nano-systems for therapeutic treatment in nanomedicine. We also discuss the open questions with the aim of offering possible novel insights to overcame the critical issues which are still present when we want to translate theranostic approaches into the clinical practise.

1. Introduction

Recent achievements in nanoscience has stimulated the development of a ha variety of smart nano-platform for the delivery of therapeutic drugs for applications in biotechnology and nano-medicine (Hruby *et al.* 2015; Liu *et al.* 2016a). Many conventional drug delivery systems present critical issues connected with side effects that limit the their use in the clinical practice (Allen and Cullis 2013; Bozzuto and Molinari 2015). To overcame the non-specific bio-distribution and non-controllable drug release characteristics of traditional nanocarriers, smart integrated nano-scale systems have been developed to achieve the release of drugs at the target sites in a controlled manner (Lee *et al.* 2015). Those medical technologies make use of smaller devices (so called “nano-systems”), as they are less invasive and can possibly be implanted inside some specific part of the body. In particular, nanoscale materials allow a combination of interesting properties in terms of their molecular architecture, size, shape, and surface functionalities for the developing of efficient therapeutic interventions (Bamrungsap *et al.* 2012). The main integrated nanosystems for biomedical and nanomedicine applications employ nanoparticle-based platform that include: polymers based nanocarriers (such as block copolymers (Mikhail and Allen 2009; Hruby *et al.* 2015), dendrimers (Lombardo 2009, 2014; Jain 2017), polymeric hydrogels (Kopeček and Yang 2007)), lipid based nanocarriers (Lombardo *et al.* 2016a; Xing *et al.* 2016), mesoporous silica nanoparticles (MSNPs) (Li *et al.* 2012; Pasqua *et al.* 2019b) and synthesized hybrid (organic/inorganic) nanostructure (Aiello *et al.* 2002; Bonaccorsi *et al.* 2009; Li *et al.* 2017; Watermann and Brieger 2017).

The employment of specific internal or external stimuli allows to manipulate those nanoplatforms in order to increase the drug targeting efficacy and reduce side effects (or toxicities), which are key factors for the improvement of the patient compliance. Moreover, the possibility to develop within the same nano-platform a diagnostic imaging system, allow the temporal evolution monitoring of the disease molecular response for each patient (Liu *et al.* 2016b). The development of smart nanomedicine solutions allow to attain an early and global (diagnostics) profiling of the health/disease of individual patient, thus providing a modern approaches for personalized health monitoring and preventive medicine (Chen and Snyder 2012).

Despite the development of theranostic approaches for the efficient delivery of therapeutic drugs has experienced considerable expansion in recent years, translating nanomedicine discoveries into the clinical practise results in a huge difficulties and requests more innovative strategies and deeper investigation.

In this article we highlight the recent progress made in this rapidly evolving field, while discussing some critical issues and open questions that still limit the clinical applications of those novel technologies.

2. Passive targeting: key role of the EPR effect

The main challenge in drug delivery design is to selectively drive the therapeutic drug molecules (and formulations) to the desired target sites, while avoiding all other potential sites of interaction within the same biological system and improving (both in vitro and in vivo) bio-distribution of therapeutic compounds to target sites. However, most of all drugs can be associated with a number of undesired effects that create limitations to their efficacy and practical use.

Drug delivery strategies have been divided into the two main categories of *passive* and *active* targeting (Bozzuto and Molinari 2015; Hruby *et al.* 2015). The so-called *passive targeting* (i.e. when no specific targeting ligands are used) represents the major mechanism for many intravenously administered formulations and is based on drug accumulation in the areas around the tumors. Pathological tissues and cancer cells exhibit, in fact, a different microenvironment in comparison with the normal cells. In tumour (or inflammatory) tissues the blood vessels have large vascular fenestrations (larger pores with diameters between 50 and 200 nm) that allow most drug-loaded nanocarriers with a smaller size to diffuse outside the blood vessels (extravasation) thus entering the tumor interstitial space and concentrating into the target site (Maeda *et al.* 2000, 2013). The vascular permeability decreases with the increase in the size of the transported particle, a process which is known as *enhanced permeability and retention* (EPR) effect. Furthermore, cationic nanocarriers exhibit higher permeability and preferentially target tumour vessels compared with anionic (or neutral) nanocarriers (Maeda *et al.* 2013) and (Allen and Cullis 2013).

After their administration, most of the nanocarriers are sequestered from the circulation through fenestrations in their microvasculature, and will accumulate in the organs of the *mononuclear phagocyte system (MPS)* (also known as reticuloendothelial system - RES), which causes a decrease in tumor accumulation, and often leads to possible damage to RES-rich organs (such as the liver, spleen, and lungs). Liposomes undergo successive clearing in the MPS by resident macrophages via direct interactions with the phagocytic cells (Allen and Cullis 2013). It is worth pointing that vascular permeability depends both on the properties of the specific nanocarrier and the characteristics of the vasculature. Frequently, only a very small fraction (<5%) of the total administered nanoparticles formulation is delivered to the target site (tumor accumulation) (Bae and Park 2011).

Moreover, significant heterogeneity between tumour types (connected with differences in pore dimensions of the vasculature and in vessel structure) may result in heterogeneous extravasation efficiency and delivery and a possible limited impact of drugs nanocarriers. Thus, future investigations will need to systemically evaluate these factors in preclinical models and in patients with various solid tumours and determine whether the models represent all aspects of the EPR effect. Finally, an image-guided diagnostic approach will prove useful to profile and select tumor types and patients, thus facilitating the development of future, effective nanocarrier.

3. Active targeting drug delivery processes

Successful therapeutic application of smart nanocarriers can be achieved through technologies that allow a (locally activated) drug release actions limited to selective sites within the body, such as a tumor or an inflammation tissue. In this respect, the supramolecular self-assembly approaches also offer the opportunity for the control of the active targeting of the diseased tissues, by employing specific ligand–receptor interactions or some specific stimuli for the development of more complex, hierarchical nanostructures. More specifically, the active targeting process can be classified into *ligand–receptor* mediated and *locally activated* drug delivery processes.

Ligand–receptor mediated targeted drug delivery approaches involve the attachment of high affinity ligands to the surface of nanoparticles that targets specific receptors (Bae and Park 2011; Morelli *et al.* 2011). Ligand–receptor interactions are possible only when the two components are in close proximity (<0.5 nm). The ideal targeted drug delivery system (as in cancer therapies) is able to delivers the drug only to the target diseased tissues. However, in real experience the amount of drug delivered to tumor targets is much less than 5% at most. Moreover, a number of previous investigations evidenced that active targeting through the tumor-targeting ligand does not always result in increased accumulation of the nanoparticles in tumors (Kirpotin *et al.* 2006; Pirollo and Chang 2008).

3.1. Locally activated drug delivery: Stimuli-responsive nanocarriers. Locally activated drug delivery processes can occur either by self-triggered targeting (which is based on the presence of specific enzymes or pH changes at the target site) or by externally-activated targeting (based on external factors, such as light, temperature, magnetic field and ultrasound) (Mura *et al.* 2013). It is known that the tumour region presents a complex microenvironment which is quite different from normal tissues, as it is characterized by unevenness of blood flow, hypoxia and acidic pH. Owing to these unique characteristics it is possible to exploit the physiology of diseased tissues for the development of *stimulus-responsive* therapeutic nanoparticles that are able to modulate their therapeutic action in response to an internal (or external) stimulus (Mura *et al.* 2013; Dai *et al.* 2017; Tayo 2017; Tang *et al.* 2018). The design of smart nanocarriers that can specifically respond to the tumour microenvironment is significant to reduce the side effect to healthy tissues.

Various nano-platform have been proposed over the past decade as stimuli-responsive prototypes (Mura *et al.* 2013; Tayo 2017). In light-responsive nanocarriers the presence of photochromic moieties that undergo photochemical changes (such as photoisomerization, photodimerization or photocleavage) upon light exposure, may induce the structural disruption/disaggregation of the nanocarrier and the release of its drug cargo (Tayo 2017; Wang *et al.* 2017). Particularly interesting for biomedical applications are the polymeric nanocarriers which are photo-responsive in the near-infrared (NIR) region, since NIR radiation penetrates deep into tissues and have less destructive effect on the biological tissues than UV light (Mura *et al.* 2013; Tayo 2017; Tang *et al.* 2018). In *pH-responsive* nanocarrier systems the presence of ionizable groups such as either weakly acidic (e.g. carboxylic and sulfonic acids) or basic (e.g. amines, imidazole and pyridine) moieties that are capable of donating or accepting H^+ ions upon a pH changes in the environment, and may cause an electrostatic charge alteration and a perturbation/disruption of the nanocarrier structure (Tayo 2017; Wu *et al.* 2018). For example, this process can be exploited for the controlled drug release at the intrinsic low pH (~ 5.0) level encountered in cancer cells. Various pH-responsive nanosystems have been synthesized using block copolymers, dendrimers, polymer-drug conjugates, nanogels, polymerosomes and even multiple core shell complexes and micellar structures (Tayo 2017; Wu *et al.* 2018). Polymeric nanocarriers containing reducible disulfide bonds offers a special perspective for the development of *redox-responsive* theranostic nanoplatforms for potential intracellular delivery of drug (or functional genes) in targeted tumors and other tissues. Moreover, as glutathione is expressed at relatively higher concentrations in tumors tissues compared with normal ones, glutathione-responsive polymeric micelles has been recently investigated for cancer treatment and nanomedicine applications (McCarley 2012; Tayo 2017). Enhanced permeability of drugs can be obtained by means of *ultrasound-responsive* nanocarriers, consisting on (micro- and nano-) bubbles which can be loaded with anticancer drugs (Zardad *et al.* 2016; Tayo 2017). Under their exposure to intermittent low-frequency ultrasound (US) the bubbles rapidly collapse at inertial cavitation, thus releasing their internal drug cargo. The employment of the complementary effects of thermal induction and mechanical stimulus may help to prevent the problem connected to the short circulation time during the drug delivery process. For example, with the High Intensity Focused Ultrasound (HIFU) approach the tissue is ablated through focusing the high US frequencies directed to malignant tumour tissue with minimal side effects by exploiting the complementary effects of thermal induction, chemical and mechanical stimulus (Zardad *et al.* 2016). Moreover, ultrasound also produces heat as a “secondary stimulus” (caused by energy vibration of acoustic waves through acoustic cavitation) thus

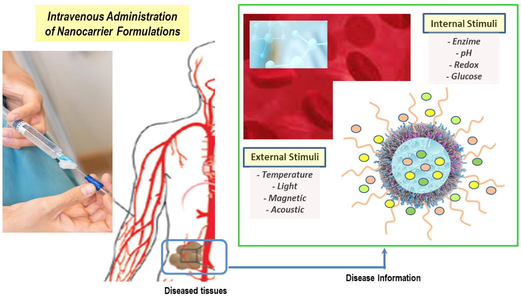

FIGURE 1. Schematic illustration of the stimuli-responsive nanocarriers formulations for intravenous administration.

providing the possibility of a dual-responsive mechanism of action (Zardad *et al.* 2016). Finally, in temperature-responsive nanocarriers the drug loaded cargo is maintained stable in physiological environments at normal body temperature ($\sim$ 37°C), while it is rapidly released when the nanocarrier reach the diseased sites, which generally characterised by local hyperthermia (with T$\sim$ 40-42 °C) (Ward and Georgiou 2011; Lombardo *et al.* 2019). For this purpose, some specific thermo-sensitive polymers are employed, which are fully soluble below a certain temperature, known as lower critical solution temperature (LCST). Above LCST, the disruption of the polymer-water hydrogen bond allows for a controlled destabilization of the nanocarrier structure, thus allowing the controlled triggering of the drug release. (Ward and Georgiou 2011; Lombardo *et al.* 2019). One of the most investigated thermo-responsive polymer is the poly(N-isopropyl acrylamide) (pNIPAAm), which is in water soluble state below the LCST (33°C), and becomes hydrophobic at body temperature.

A large number of optimizations and improvement experiments are needed for the translation of each stimulus from preclinical experimental models to routine clinical practice (Mura *et al.* 2013). Especially, *internal stimuli* (also called "endogenous triggers") are indeed difficult to control because of the complexity of the biological micro-environment encountered and the large variation from one patient to another.

On the other hand, although the *external stimuli* (“exogenous triggers”) responsive systems are much easier to be controlled, they present major problems related to normal tissue damage and tissue-penetration depth. It is worth noticing that exposure to electromagnetic fields may have sensitive influence on cell membrane components (Thakur and Sahu 2016; Calabró and Magazú 2018). For example, even the exposure to extremely low electromagnetic fields influences the vibrations of peptide linkages, (thus modifying the protein secondary structures of α-helix and β-sheet contents) and producing sensitive unfolding process in cell membrane proteins (Calabró *et al.* 2013). It has been demonstrated that shielding action of disaccharides may provide an interesting approach for the development of effective strategies to preserve proteins from electromagnetic fields (Magazú *et al.* 2013, 2016, 2018).

Despite a number of various stimuli-responsive nanocarriers are still at clinical stage, Visudyne still represents the only stimuli-responsive nanoplatform (approved by FDA) to be employed for the photodynamic therapy of tumours (Liu *et al.* 2016b).

3.2. Critical issues and perspectives. The ideal targeted drug delivery system is the one that delivers the drug only to the target tumor tissues (avoiding the healthy ones). However in real experience the amount of drug delivered to tumor targets is much less than 5% at most (Liu *et al.* 2016b). Despite the recent significant progresses in the understanding of the molecular basis of several pathology, molecular targeted therapy still remains a promise of a future innovative approach. Sensitive dynamic changes at the cellular level and biological events that happen in responses to drug delivery processes are very often difficult to describe, to investigate and particularly difficult to predict. Tumor-targeting ligands does not always result in increased accumulation of the nanocarriers in diseased tissues. Furthermore, the tumor-targeting receptor may change in its surface expression over time and the receptors that are overexpressed in a specific disease state are often present also (at lower concentrations) in the membranes of healthy cells. Moreover, receptor overexpression is usually heterogeneous within different cells of a single tumour and also between different patients for the same typology of cancer, posing a fundamental challenge in the detection process of those phenomena. Those critical issues are due to complexities of the diffusional barriers in solid tumors, and to the uncertainties that are connected with the EPR effect. As a result, the mere presence of a ligand-receptor combination on a nanocarrier does not ensure a sure success of the active targeting process. More complex predictive methods are then required in order to improve the response rates to targeted drug delivery therapies (Liu *et al.* 2016b).

It is clear that smart integrated nanostructured systems will constitute an integral part of a therapeutic intervention in the future. Novel designing concepts and versatile control ability offered by smart nanostructures provide ample opportunities in placing any desired combination of functions into a single scaffold (Lombardo *et al.* 2004a; Pasqua *et al.* 2009; Casadonte *et al.* 2010). Those nanostructured system are based on the complex combinations of different (non covalent) supramolecular interactions, that allow the formation of highly functional materials and devices with remarkable properties (Kiselev *et al.* 2001; Longo *et al.* 2006; Lock *et al.* 2013; Calandra *et al.* 2015a). More specifically, supramolecular self-assembly between amphiphilic compounds allows the fabrication of a large variety of nanomaterials with emerging complex properties and various architectures (Mallamace *et al.* 2001; Calandra *et al.* 2010; Lock *et al.* 2013; Turco Liveri *et al.* 2018). These approaches have offered great potential to develop materials with improved therapeutic efficacy including target specificity, controlled drug release, lower therapeutic doses and minimum exposure to normal tissues (Carino *et al.* 2007; Neri *et al.* 2017; Pasqua *et al.* 2019a).

Due to this inherent complexity the development of efficient targeted drug delivery systems will require, then, an improved understanding of multiple factors such as the regulation of distribution in the blood and the dynamic aspects of tumor (including their spatial and temporal heterogeneity). Particularly interesting, in this respect, is the study of model biomembranes during their interaction with nanoparticles, as they can be adopted as simplified models that mimic the relevant processes encountered in real cell membranes

(Sackmann 1995; Kiselev *et al.* 2008; Wanderlingh *et al.* 2014; Lombardo *et al.* 2016b). Those studies has given a strong input to the understanding of the complex processes driven by the interactions that a nanostructured material can develop toward biological systems (Katsaras and Gutberlet 2000; Bourgaux and Couvreur 2014; Kiselev and Lombardo 2017; Lombardo *et al.* 2018). Furthermore, chemical-physics investigation of complex associating properties in nanomaterials (Chen *et al.* 2002; Kiselev *et al.* 2013; Calandra *et al.* 2015b) highlight the prominent role of the interaction patterns (electrostatic, hydrogen bonding arrays, sequences of donor and acceptor groups, ion coordination sites, etc.), in the creation of more and more complex topology, architectures and structural transitions (Micali *et al.* 1998; Lombardo *et al.* 2004b; Calandra *et al.* 2009; Santos *et al.* 2013).

4. Advanced approaches: system biology and personalised nanomedicine

The employment of highly sensitive nano-analytical techniques for molecular diagnostics (such as high-throughput sequencing and mass spectrometry) as well as new smart integrated medical nanosystems (such as biosensors) allow to attain an early diagnostics and a global profiling of the health (and disease) of individual patient, thus providing new approaches for personalized health monitoring and preventive medicine. This approach (called *system biology*) is based on the collection of data from many components in parallel, using the so called "*-omics*" technologies, (like metabolomics, proteomics, genomics) and aims to understand the complex interactions and functioning of the living systems at various levels, by inferring the complex pathways that regulates specific biological (physiological or pathological) processes [21].

This interdisciplinary approach prelude to the development of the *Personalized Medicine* (figure 2), which is projected to allow improved treatment for a wide range of traditional diseases, employing genomics and proteomics technologies. *Personalized Medicine* aims also to provide (at the clinic stage) the most suitable pharmacological approach and medical therapy based on an individual profiling for each different patient. Such recognition of inter-individual differences in drug response is an essential step toward the development of advanced medical approaches based of the optimized therapy. The *personalized medicine approach*, that will focus its main activity on preventive medicine, will also facilitates earlier disease detection via genomic approach and the employment of specific biomarkers in disease development. This challenge requires a synergistic activity between the cross disciplinary fields of nano- and molecular medicine, biochemistry, bioengineering and biotechnology (Micali *et al.* 1998; Terracciano *et al.* 2006; Preianó *et al.* 2012).

However, the complexity of the human genome due to the numerous genes involved (both in disease origins detection and in drug response) is one of the critical issues that impedes the effective, routine clinical application. Moreover, as a large number of genetic variations may exist, their complete identification within the complex genetic map will require a time-consuming and expensive tasks to perform. Although the biocompatibility, toxicological aspects and ethical implications still represent critical issues that are far to be completely resolved, the *personalized medicine approach* could have an expanding role in the modern approaches and future practice of medicine (Chen and Snyder 2012).

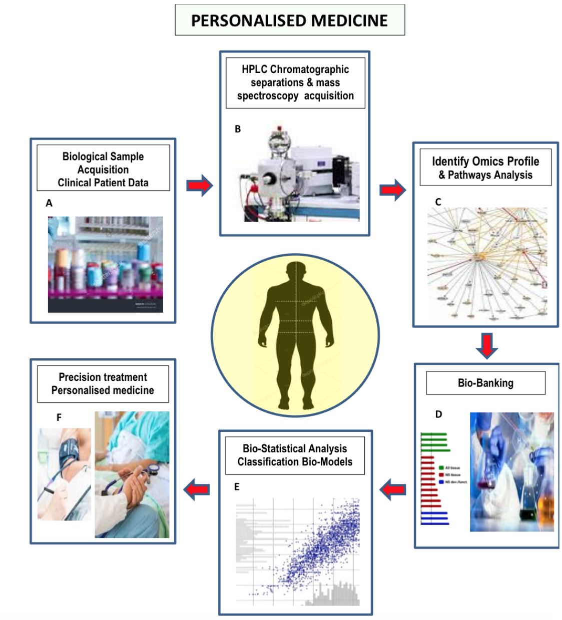

FIGURE 2. Schematic illustration of the basic stages of the personalised medicine approach.

5. The perspectives of the European program for health: the effort of a coordinated action

In this section we shortly analyse the global health agenda-setting process in the European Union (EU) contest. Early initiatives for the development of a European perspective on global health have already been established by means of the development of the Horizon 2020 (research and innovation) founding program (Aluttis *et al.* 2014). More specifically the call for research and innovation project founding in the years 2018-2020 (*H2020-SC1-BHC-2018-2020* - Better Health and care, economic growth and sustainable health systems) aim at reconciling better health and healthy ageing with the need to develop sustainable health and care systems and growth opportunities for the health and care related industries [74, 75].

More specifically the scopes of the program range from prevention, diagnosis, stratified approaches, predictive toxicology. It also includes the development of novel therapeutic approaches including: medical technologies and advanced therapies, cohorts and registries-based research to integration of care and systemic digital solutions for health and ageing well. It also aims to translate new knowledge into innovative applications and accelerate large-scale uptake and deployment in different health and care settings, making health and care systems and services more accessible, responsive and efficient in Europe and beyond. The main aim of the program is to force policy makers in Europe to look beyond their

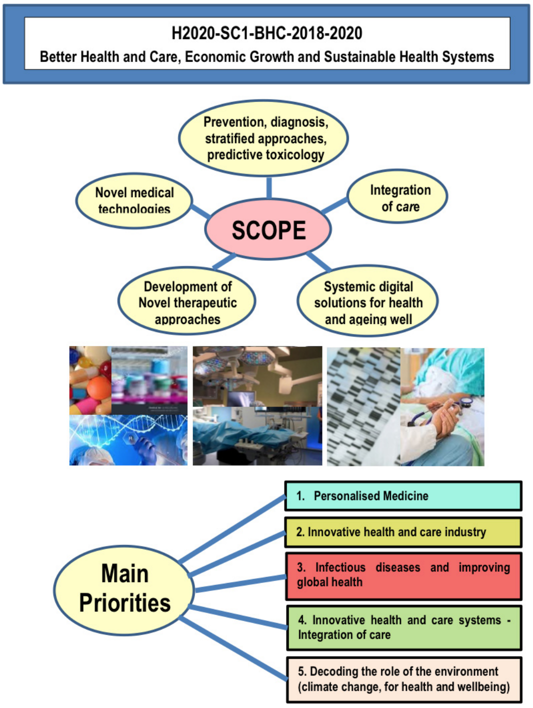

FIGURE 3. Sketch indicating the main scopes and priority of the Horizon 2020 founding initiatives for research and innovation projects in the years 2018-2020 (*H2020-SC1-BHC-2018-2020* - Better Health and care, economic growth and sustainable health systems).

borders in order to protect the health and well-being of European citizen (Aluttis *et al.* 2014).

To this end, the European commission encourage the inclusion of private companies and other innovators in the various dedicated projects. As evidenced in figure 3, this research and innovation program will be implemented through five main priorities including: (1) Personalised medicine, (2) Innovative health and care industry, (3) Infectious diseases and improving global health, (4) Innovative health and care systems - Integration of care, (5) Decoding the role of the environment, including climate change, for health and wellbeing. Research under this priority will also attempt to develop an understanding of the economic impact and the potential of personalised medicine to transform health systems. Additional impacts are to:

(1) establish Europe as a global leader in personalised medicine research;
(2) support the personalised medicine science base through a coordinated approach to research;
(3) provide evidence to policy makers of the benefit of personalised medicine to citizens and healthcare systems.

The International Consortium on Personalised Medicine will be instrumental to achieve these aims.

Despite the relevant resources identified for the implementation of these programs, some critical issues for the development of a common agenda, still remain. More specifically, the main barriers for creating a synergistic European global health program are the fragmentation of the policy community and the lack of a common definition (common agenda) for global health in Europe. More efficient and coordinated efforts could best allow the achievement of the principal objectives of the EU programs, that aims to deliver solutions for a better health for all by [68]:

- Moving towards the effective integration of personalised medicine approaches into healthcare services and systems to the benefit of patients and citizens in the European contest;
- Exploring the digital potential for health innovation and healthcare, including the building of a "European health research and innovation cloud";
- Stimulating innovation in the European healthcare domain and industry by exploring the application of advanced technologies, improve the health of the workforce and promote regulatory science.

In this respect, the stakeholders for global health need to engage in much more intensive dialogue on the definition and priority areas of a European approach to global health to align their position within a more general program that include a world perspective on global health. Furthermore, an additional difficulty is connected with the actual times of economic austerity with its obstacles and hardships to the identification of the appropriate resources to be allocated for initiatives of common interest in the development of a sustainable health system. Finally, it is important to be aware of the fact that the European Union still represent an important actor on health matters on a world global perspective.

6. Conclusions and future Outlook

The recent development of integrated nano-systems platform for biomedical applications has led to significant progress in biotechnology and nanomedicine applications. These materials have offered great potential to develop nano-platforms with improved therapeutic efficacy including target specificity, controlled drug release, lower therapeutic doses and minimum exposure to normal tissues. The introduction of stimuli responsive (assembly/disassembly) nanostructured systems in combination with the ligand–receptor recognition processes furnish a large variety of theranostic solutions for the definition of specific bio-medical tasks such as the controlled release of drugs, imaging capabilities and multi-component (multi-functional) therapeutics.

While modern approach of supra-molecular (and hierarchical) self-assembly allows the construction of nanostructured materials with a large variety of chemical composition, architectures, and surface properties, the development of integrated (smart) medical nano-systems allow to attain an early diagnostics and a global profiling of the health (and disease) of individual patient, thus providing new approaches for personalized health monitoring and preventative medicine.

Although these nano-platform evidence good performance against a large number of specific diseases, a number of inherent drawbacks and critical issues, limit their translation in the clinic experience. Much efforts are currently being directed at bridging the gap to put these smart nano-platform into practice, by a deeper investigation of their safety, therapeutic efficacy, and a detailed understanding of their physico-chemical behaviour. Future efforts should move towards the effective integration of personalized medicine approaches into healthcare services and systems to the benefit of patients and citizens. Moreover the exploration of the digital potential for health innovation and healthcare, should include the contraction of an 'European health research and innovation cloud' by exploring the application of advanced technologies, and the promotion of regulatory science.

Acknowledgments

D.L. acknowledges funding from Marie Curie Actions under EU FP7 Initial Training Network SNAL 608184. The work of M.A.K. was financed by the Russian Scientific Foundation (Project no. 14-12-00516).

References

Aiello, R., Cavallaro, G., Giammona, G., Pasqua, L., Pierro, P., and Testa, F. (2002). "Mesoporous silicate as matrix for drug delivery systems of non-steroidal antinflammatory drugs". *Studies in Surface Science and Catalysis* **142 B**, 1165–1172.

Allen, T. and Cullis, P. (2013). "Liposomal drug delivery systems: From concept to clinical applications". *Advanced Drug Delivery Reviews* **65**(1), 36–48. DOI: 10.1016/j.addr.2012.09.037.

Aluttis, C., Krafft, T., and Brand, H. (2014). "Global health in the European Union - a review from an agenda-setting perspective". *Glob Health Action* **7**(SUPP.1), 23610. DOI: 10.3402/gha.v7.23610.

Bae, Y. and Park, K. (2011). "Targeted drug delivery to tumors: Myths, reality and possibility". *Journal of Controlled Release* **153**(3), 198–205. DOI: 10.1016/j.jconrel.2011.06.001.

Bamrungsap, S., Zhao, Z., Chen, T., Wang, L., Li, C., Fu, T., and Tan, W. (2012). "Nanotechnology in therapeutics: A focus on nanoparticles as a drug delivery system". *Nanomedicine* **7**(8), 1253–1271. DOI: 10.2217/nnm.12.87.

Bonaccorsi, L., Lombardo, D., Longo, A., Proverbio, E., and Triolo, A. (2009). "Dendrimer template directed self-assembly during zeolite formation". *Macromolecules* **42**(4), 1239–1243. DOI: 10.1021/ma802393e.

Bourgaux, C. and Couvreur, P. (2014). "Interactions of anticancer drugs with biomembranes: What can we learn from model membranes?" *Journal of Controlled Release* **190**, 127–138. DOI: 10.1016/j.jconrel.2014.05.012.

Bozzuto, G. and Molinari, A. (2015). "Liposomes as nanomedical devices". *International journaltitle of Nanomedicine* **10**, 975–999. DOI: 10.2147/IJN.S68861.

Calabró, E., Condello, S., Curró, M., Ferlazzo, N., Vecchio, M., Caccamo, D., Magazú, S., and Ientile, R. (2013). "50 Hz electromagnetic field produced changes in FTIR spectroscopy associated with mitochondrial transmembrane potential reduction in neuronal-like SH-SY5Y cells". *Oxidative Medicine and Cellular Longevity*, 414393 [8 pages]. DOI: 10.1155/2013/414393.

Calabró, E. and Magazú, S. (2018). "Resonant interaction between electromagnetic fields and proteins: A possible starting point for the treatment of cancer". *Electromagnetic Biology and Medicine* **37**(3), 155–168. DOI: 10.1080/15368378.2018.1499031.

Calandra, P., Caschera, D., Turco Liveri, V., and Lombardo, D. (2015a). "How self-assembly of amphiphilic molecules can generate complexity in the nanoscale". *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **484**, 164–183. DOI: 10.1016/j.colsurfa.2015.07.058.

Calandra, P., Di Marco, G., Ruggirello, A., and Liveri, V. (2009). "Physico-chemical investigation of nanostructures in liquid phases: Nickel chloride ionic clusters confined in sodium bis(2-ethylhexyl) sulfosuccinate reverse micelles". *Journal of Colloid and Interface Science* **336**(1), 176–182. DOI: 10.1016/j.jcis.2009.03.066.

Calandra, P., Ruggirello, A., Pistone, A., and Liveri, V. (2010). "Structural and Optical Properties of Novel Surfactant Coated TiO2-Ag Based Nanoparticles". *Journal of Cluster Science* **21**(4), 767–778. DOI: 10.1007/s10876-010-0330-x.

Calandra, P., Turco Liveri, V., Ruggirello, A., Licciardi, M., Lombardo, D., and Mandanici, A. (2015b). "Anti-Arrhenian behaviour of conductivity in octanoic acid-bis(2-ethylhexyl)amine systems: A physico-chemical study". *Journal of Materials Chemistry C* **3**(13), 3198–3210. DOI: 10.1039/c4tc02500h.

Carino, I., Pasqua, L., Testa, F., Aiello, R., Puoci, F., Iemma, F., and Picci, N. (2007). "Silica-based mesoporous materials as drug delivery system for methotrexate release". *Drug Delivery* **14**(8), 491–495. DOI: 10.1080/10717540701606244.

Casadonte, F., Pasqua, L., Savino, R., and Terracciano, R. (2010). "Smart trypsin adsorption into N-(2-aminoethyl)-3-aminopropyl-modified mesoporous silica for ultra fast protein digestion". *Chemistry - A European Journal* **16**(30), 8998–9001. DOI: 10.1002/chem.201000120.

Chen, R. and Snyder, M. A. (2012). “Systems biology: personalized medicine for the future?” *Current Opinion in Pharmacology* **12**(5), 623–628. DOI: 10.1016/j.coph.2012.07.011.

Chen, S., Mallamace, F., Faraone, A., Gambadauro, P., Lombardo, D., and Chen, W. (2002). “Observation of a re-entrant kinetic glass transition in a micellar system with temperature-dependent attractive interaction”. *The European Physical Journal E* **9**(3), 283–286. DOI: 10.1140/epje/i2002-10081-5.

Dai, Y., Xu, C., Sun, X., and Chen, X. (2017). “Nanoparticle design strategies for enhanced anticancer therapy by exploiting the tumour microenvironment”. *Chemical Society Reviews* **46**(12), 3830–3852. DOI: 10.1039/c6cs00592f.

Hruby, M., Filippov, S. K., and Štěpánek, P. (2015). “Smart polymers in drug delivery systems on crossroads: Which way deserves following?” *European Polymer journaltitle* **65**, 82–97. DOI: 10.1016/j.eurpolymj.2015.01.016.

Jain, K. (2017). “7 - Dendrimers: Smart nanoengineered polymers for bioinspired applications in drug delivery”. In: *Biopolymer-Based Composites*. Ed. by S. J., S. M., and S. J. Woodhead Publishing, pp. 169–220. DOI: https://doi.org/10.1016/B978-0-08-101914-6.00007-7.

Katsaras, J. and Gutberlet, T. (2000). *Lipid bilayers. Structure and Interactions*. Springer-Verlag Berlin Heidelberg. DOI: 10.1007/3-540-27076-0_9.

Kirpotin, D., Drummond, D., Shao, Y., Shalaby, M., Hong, K., Nielsen, U., Marks, J., Benz, C., and Park, J. (2006). “Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models”. *Cancer Research* **66**(13), 6732–6740. DOI: 10.1158/0008-5472.CAN-05-4199.

Kiselev, M., Janich, M., Hildebrand, A., Strunz, P., Neubert, R., and Lombardo, D. (2013). “Structural transition in aqueous lipid/bile salt [DPPC/NaDC] supramolecular aggregates: SANS and DLS study”. *Chemical Physics* **424**, 93–99. DOI: 10.1016/j.chemphys.2013.05.014.

Kiselev, M., Lesieur, P., Kisselev, A., Lombardo, D., Killany, M., Lesieur, S., and Ollivon, M. (2001). “A sucrose solutions application to the study of model biological membranes”. *Nuclear Instruments and Methods in Physics Research, Section A: Accelerators, Spectrometers, Detectors and Associated Equipment* **470**(1-2), 409–416. DOI: 10.1016/S0168-9002(01)01087-7.

Kiselev, M. and Lombardo, D. (2017). “Structural characterization in mixed lipid membrane systems by neutron and X-ray scattering”. *Biochimica et BiophysicaActa (BBA) - General Subjects* **1861**(1), 3700–3717. DOI: 10.1016/j.bbagen.2016.04.022.

Kiselev, M., Lombardo, D., Lesieur, P., Kisselev, A., Borbely, S., Simonova, T., and Barsukov, L. (2008). “Membrane self assembly in mixed DMPC/NaC systems by SANS”. *Chemical Physics* **345**(2–3), 173–180. DOI: 10.1016/j.chemphys.2007.09.034.

Kopeček, J. and Yang, J. (2007). “Hydrogels as smart biomaterials”. *Polymer International* **56**(9), 1078–1098. DOI: 10.1002/pi.2253.

Lee, B., Yun, Y., and Park, K. (2015). “Smart nanoparticles for drug delivery: Boundaries and opportunities”. *Chemical Engineering Science* **125**, 158–164. DOI: 10.1016/j.ces.2014.06.042.

Li, F., Zeyu, L., and Ling, D. (2017). “Smart Organic-Inorganic Nanogels for Activatable Theranostics”. *Current Medicinal Chemistry* **24**(1), 1366–1376. DOI: 10.2174/0929867324666170920164614.

Li, Z., Barnes, J. C., Bosoy, A., Stoddart, J. F., and Zink, J. I. (2012). “Mesoporous silica nanoparticles in biomedical applications”. *Chemical Society Reviews* **41**(7), 2590–2605. DOI: 10.1039/C1CS15246G.

Liu, D., Yang, F., Xiong, F., and Gu, N. (2016a). “The smart drug delivery system and its clinical potential”. *Theranostics* **6**(9), 1306–1323. DOI: 10.7150/thno.14858.

Liu, D., Yang, F., Xiong, F., and Gu, N. (2016b). “The smart drug delivery system and its clinical potential”. *Theranostics* **6**(9), 1306–1323. DOI: 10.7150/thno.14858.

Lock, L., Lacomb, M., Schwarz, K., Cheetham, A., Lin, Y.-A., Zhang, P., and Cui, H. (2013). "Self-assembly of natural and synthetic drug amphiphiles into discrete supramolecular nanostructures". *Faraday Discussions* **166**, 285–301. DOI: 10.1039/c3fd00099k.

Lombardo, D. (2009). "Liquid-like ordering of negatively charged poly(amidoamine) (PAMAM) dendrimers in solution". *Langmuir* **25**(5), 3271–3275. DOI: 10.1021/la804234p.

Lombardo, D. (2014). "Modeling dendrimers charge interaction in solution: Relevance in biosystems". *Biochemistry Research International* **2014**, 837651 [10 pages]. DOI: 10.1155/2014/837651.

Lombardo, D., Calandra, P., Barreca, D., Magazú, S., and Kiselev, M. (2016a). "Soft interaction in liposome nanocarriers for therapeutic drug delivery". *Nanomaterials* **6**(7), 125 [26 pages]. DOI: 10.3390/nano6070125.

Lombardo, D., Calandra, P., Bellocco, E., Laganá, G., Barreca, D., Magazú, S., Wanderlingh, U., and Kiselev, M. (2016b). "Effect of anionic and cationic polyamidoamine (PAMAM) dendrimers on a model lipid membrane". *Biochimica et BiophysicaActa (BBA) – Biomembranes* **1858**(11), 2769–2777. DOI: 10.1016/j.bbamem.2016.08.001.

Lombardo, D., Calandra, P., Magazú, S., Wanderlingh, U., Barreca, D., Pasqua, L., and Kiselev, M. (2018). "Soft nanoparticles charge expression within lipid membranes: The case of amino terminated dendrimers in bilayers vesicles". *Colloids and Surfaces B: Biointerfaces* **170**, 609–616. DOI: 10.1016/j.colsurfb.2018.06.031.

Lombardo, D., Longo, A., Darcy, R., and Mazzaglia, A. (2004a). "Structural Properties of Nonionic Cyclodextrin Colloids in Water". *Langmuir* **20**(4), 1057–1064. DOI: 10.1021/la035370q.

Lombardo, D., Micali, N., Villari, V., and Kiselev, M. (2004b). "Large structures in diblock copolymer micellar solution". *Physical Review E* **70**(21), 021402 [8 pages]. DOI: 10.1103/PhysRevE.70.021402.

Lombardo, D., Munaò, G., Calandra, P., and Caccamo, M. (2019). "Evidence of pre-micellar aggregates in aqueous solution of amphiphilic PDMS–PEO block copolymer". *Physical Chemistry Chemical Physics* **22**(23), 11983–11991. DOI: 10.1039/C9CP02195G.

Longo, A., Calandra, P., Casaletto, M., Giordano, C., Venezia, A., and Liveri, V. (2006). "Synthesis and physico-chemical characterization of gold nanoparticles softly coated by AOT". *Materials Chemistry and Physics* **96**(1), 66–72. DOI: 10.1016/j.matchemphys.2005.06.043.

Maeda, H., Nakamura, H., and Fang, J. (2013). "The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo". *Advanced Drug Delivery Reviews* **65**(1), 71–79. DOI: 10.1016/j.addr.2012.10.002.

Maeda, H., Wu, J., Sawa, T., Matsumura, Y., and Hori, K. (2000). "Tumor vascular permeability and the EPR effect in macromolecular therapeutics: A review". *Journal of Controlled Release* **65**(1-2), 271–284. DOI: 10.1016/S0168-3659(99)00248-5.

Magazú, S., Calabró, E., and Caccamo, M. (2018). "Experimental study of thermal restraint in bio-protectant disaccharides by FTIR spectroscopy". *The Open Biotechnology Journal* **12**, 123–133. DOI: 10.2174/1874070701812010123.

Magazú, S., Calabró, E., Caccamo, M., and Cannuli, A. (2016). "The shielding action of disaccharides for typical proteins in aqueous solution against static, 50 Hz and 1800 MHz frequencies electromagnetic fields". *Current Chemical Biology* **10**(1), 57–64. DOI: 10.2174/221279681066616041953722.

Magazú, S., Migliardo, F., Vertessy, B., and Caccamo, M. (2013). "Investigations of homologous disaccharides by elastic incoherent neutron scattering and wavelet multiresolution analysis". *Chemical Physics* **424**, 56–61. DOI: 10.1016/j.chemphys.2013.05.004.

Mallamace, F., Beneduci, R., Gambadauro, P., Lombardo, D., and Chen, S. (2001). "Glass and percolation transitions in dense attractive micellar system". *Physica A: Statistical Mechanics and its Application* **302**(1–4), 202–219. DOI: 10.1016/S0378-4371(01)00465-4.

McCarley, R. (2012). “Redox-responsive delivery systems”. *Annual Review of Analytical Chemistry* **5**, 391–411. DOI: 10.1146/annurev-anchem-062011-143157.

Micali, N., Scolaro, L., Romeo, A., Lombardo, D., Lesieur, P., and Mallamace, F. (1998). “Structural properties of methanol-polyamidoamine dendrimer solutions”. *Physical Review E* **58**(5), 6229–6235. DOI: 10.1103/PhysRevE.58.6229.

Mikhail, A. and Allen, C. (2009). “Block copolymer micelles for delivery of cancer therapy: Transport at the whole body, tissue and cellular levels”. *journaltitle of Controlled Release* **138**(3), 214–223. DOI: 10.1016/j.jconrel.2009.04.010.

Morelli, C., Maris, P., Sisci, D., Perrotta, E., Brunelli, E., Perrotta, I., Panno, M., Tagarelli, A., Versace, C., Casula, M., Testa, F., Andò, S., Nagy, J., and Pasqua, L. (2011). “PEG-templated mesoporous silica nanoparticles exclusively target cancer cells”. *Nanoscale* **3**(8), 3198–3207. DOI: 10.1039/c1nr10253b.

Mura, S., Nicolas, J., and Couvreur, P. (2013). “Stimuli-responsive nanocarriers for drug delivery”. *Nature Materials* **12**(11), 991–1003. DOI: 10.1038/nmat3776.

Neri, G., Micale, N., Scala, A., Fazio, E., Mazzaglia, A., Mineo, P., Montesi, M., Panseri, S., Tampieri, A., Grassi, G., and Piperno, A. (2017). “Silibinin-conjugated graphene nanoplatform: Synthesis, characterization and biological evaluation”. *FlatChem - Chemistry of Flat Materials* **1**, 34–41. DOI: 10.1016/j.flatc.2016.10.002.

Pasqua, L., Cundari, S., Ceresa, C., and Cavaletti, G. (2009). “Recent development, applications, and perspectives of mesoporous silica particles in medicine and biotechnology”. *Current Medicinal Chemistry* **16**(23), 3054–3063. DOI: 10.2174/092986709788803079.

Pasqua, L., De Napoli, I., De Santo, M., Greco, M., Catizzone, E., Lombardo, D., Montera, G., Comandè, A., Nigro, A., Morelli, C., and Leggio, A. (2019a). “Mesoporous silica-based hybrid materials for bone-specific drug delivery”. *Nanoscale Advances* **1**(8), 3269–3278. DOI: 10.1039/C9NA00249A.

Pasqua, L., De Napoli, I., De Santo, M., Greco, M., Catizzone, E., Lombardo, D., Montera, G., Comandè, A., Nigro, A., Morelli, C., and Leggio, A. (2019b). “Mesoporous sili ca nanoparticles in cancer therapy: Relevance of the targeting function”. *Nanoscale Advances* **1**(8), 3269–3278. DOI: 10.1039/C9NA00249A.

Pirollo, K. and Chang, E. (2008). “Does a targeting ligand influence nanoparticle tumor localization or uptake?” *Trends in Biotechnology* **26**(10), 552–558. DOI: 10.1016/j.tibtech.2008.06.007.

Preianó, M., Pasqua, L., Gallelli, L., Galasso, O., Gasparini, G., Savino, R., and Terracciano, R. (2012). “Simultaneous extraction and rapid visualization of peptidomic and lipidomic body fluids fingerprints using mesoporous aluminosilicate and MALDI-TOF MS”. *Proteomics* **12**(22), 3286–3294. DOI: 10.1002/pmic.201200204.

Sackmann, E. (1995). “Physical basis of self-organization and function of membranes: Physics of vesicles”. *Handbook of Biological Physics* **1**(C), 213–304. DOI: 10.1016/S1383-8121(06)80022-9.

Santos, S., Medronho, B., and Santos T. Antunes, F. E. (2013). “Amphiphilic Molecules in Drug Delivery Systems”. In: *Drug Delivery Systems: Advanced Technologies Potentially Applicable in Personalised Treatment*. Ed. by J. Coelho. Vol. 4. Dordrecht: Springer Netherlands, pp. 35–85. DOI: 10.1007/978-94-007-6010-3_2.

Tang, Q., Yu, B., Gao, L., Cong, H., Song, N., and Lu, C. (2018). “Stimuli responsive nanoparticles for controlled anti-cancer drug release”. *Current Medicinal Chemistry* **25**(16), 1837–1866. DOI: 10.2174/0929867325666180111095913.

Tayo, L. (2017). “Stimuli-responsive nanocarriers for intracellular delivery”. *Biophysical Reviews* **9**(6), 931–940. DOI: 10.1007/s12551-017-0341-z.

Terracciano, R., Gaspari, M., Testa, F., Pasqua, L., Tagliaferri, P., Cheng, M.-C., Nijdam, A., Petricoin, E., Liotta, L., Cuda, G., Ferrari, M., and Venuta, S. (2006). “Selective binding and enrichment for low-molecular weight biomarker molecules in human plasma after exposure to nanoporous silica particles". *Proteomics,* **6**(11), 3243–3250. DOI: 10.1002/pmic.200500614.

Thakur, H. and Sahu, D. (2016). "Biological Effects of Electromagnetic Waves: Case Studies and Safety Standards". *Indian Journal of Science and Technology* **9**(47), 1–7. DOI: 110.17485/ijst/2016/v9i47/106851.

Turco Liveri, V., Lombardo, D., Pochylski, M., and Calandra, P. (2018). "Molecular association of small amphiphiles: Origin of ionic liquid properties in dibutyl phosphate/propylamine binary mixtures". *Journal of Molecular Liquids* **263**, 274–281. DOI: 10.1016/j.molliq.2018.05.003.

Wanderlingh, U., D'Angelo, G., Branca, C., Conti Nibali, V., Trimarchi, A., Rifici, S., Finocchiaro, D., Crupi, C., Ollivier, J., and Middendorf, H. (2014). "Multi-component modeling of quasielastic neutron scattering from phospholipid membranes". *The Journal of chemical physics* **140**(17), 174901 [10 pages]. DOI: 10.1063/1.4872167.

Wang, Y., Deng, Y., Luo, H., Zhu, A., Ke, H., Yang, H., and Chen, H. (2017). "Light-Responsive Nanoparticles for Highly Efficient Cytoplasmic Delivery of Anticancer Agents". *ACS Nano* **11**(12), 12134–12144. DOI: 10.1021/acsnano.7b05214.

Ward, M. and Georgiou, T. (2011). "Thermoresponsive polymers for biomedical applications". *Polymers* **3**(3), 1215–1242. DOI: 10.3390/polym3031215.

Watermann, A. and Brieger, J. (2017). "Mesoporous silica nanoparticles as drug delivery vehicles in cancer". *Nanomaterials (Basel)* **7**(7), 189 [17 pages]. DOI: 10.3390/nano7070189.

Wu, W., Luo, L., Wang, Y., Wu, Q., Dai, H.-B., Li, J.-S., Durkan, C., Wang, N., and Wang, G.-X. (2018). "Endogenous pH-responsive nanoparticles with programmable size changes for targeted tumor therapy and imaging applications". *Theranostics* **8**(11), 3038–3058. DOI: 10.7150/thno.23459.

Xing, H., Hwang, K., and Lu, Y. (2016). "Recent Developments of Liposomes as Nanocarriers for Theranostic Applications". *Theranostics* **6**(9), 1336–1352. DOI: 10.7150/thno.15464.

Zardad, A.-Z., Choonara, Y., Toit, L. du, Kumar, P., Mabrouk, M., Demarco Kondiah, P., and Pillay, V. (2016). "A review of thermo- and ultrasound-responsive polymeric systems for delivery of chemotherapeutic agents". *Polymers* **8**(10), 359. DOI: 10.3390/polym8100359.

---

[a] Consiglio Nazionale delle Ricerche, Istituto Processi Chimico-Fisici, (IPCF)
Viale Ferdinando stagno D'Alcontres,37
98158 Messina, Italy

[b] Università della Calabria
Dipartimento di Ingegneria per l'Ambiente e il Territorio e Ingegneria Chimica
Via Pietro Bucci, 87036 - Arcavacata di Rende (CS)

[c] Frank Laboratory of Neutron Physics
Joint Institute for Nuclear Research
Dubna, Moscow 141980, Russia

* To whom correspondence should be addressed | email: lombardo@its.me.cnr.it

Paper contributed to the international workshop entitled "New approaches to study complex systems",
which was held in Messina, Italy (27–28 november 2017), under the patronage of the *Accademia Peloritana dei Pericolanti*
Manuscript received 31 August 2018; published online 20 December 2019