5DnanoP Workshop2021 at Petru Poni Institute
Monday October 11th 2021 – 10am -5pm (Romanian time)

BOOK OF ABSTRACTS

5DnanoP Workshop2021 at Petru Poni Institute
Monday October 11th 2021 – 10am -5pm (Romanian time)

Session 1 – Chairman: ***Tudor Vasiliu***.

10.00 – 10.15 Welcome – Academian ***Bodgan Simionescu*** & ***Mariana Pinteala***

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p4. "Conducting Conjugated Polymers as Biomaterials in the frame of 5DnanoP project"

10.35 - 10.50 ***Anca-Dana Bendrea***

p5. "Regarding some of the potential application uses of
unexpected fundamental scientific findings during the research of 5DnanoP"

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12.10 – 13.00 Lunch

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p15. "Multi-arm non-viral vectors for transfection"

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p16. *"In silico studies* of PEI-PEG-squalene-dsDNA polyplex formation: The delicate role of the PEG length to the binding of PEI to DNA"

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Session 5 – Chairman: ***Bodgan Craciun***

15.30 – 15.45 ***Mihaela Turtoi***

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15.45 – 16.00 ***Luminita Marin***

p19. "PEGylated phenothiazine as promising anticancer drugs"

16.00 – 16.15 ***Adrian Fifere***

p20. "Conjugated metal oxides nanoparticles as inorganic antioxidants"

16.15 – 16.45 ***Andrei Neamtu*** – keynote speaker

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16.45 – 17.00 ***Stelian Maier***

p22. "We, all, after 5D-NanoP ends",

17.00 – 17.10 ***Aatto Laaksonen*** – Summary and closing the workshop

Conducting Conjugated Polymers as Biomaterials in the Frame of 5D-nanoP Project

Ioan Cianga[1*], Anca-Dana Bendrea[1], Luminita Cianga[1] and Gabriela-Liliana Ailiesei[2]

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Abstract. Endowed with a unique set of properties that generally matches and facilitates the communication with biological living environments, conjugated polymers (CPs) have made their debut in the field of biomaterials almost three decade ago. Especially in the past decade, the versatile character of these polymers based on their inherent propensity for the self-assembling and their sensitivity to external stimuli, combined with the advent of nanoscience, expanded their horizon of applications, now entering in the fields of nanomedicine and life science.

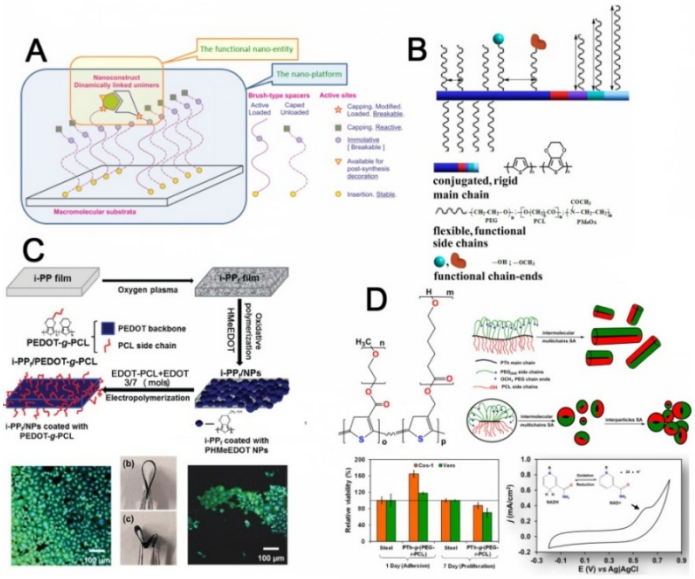

Figure 1.*(**A**)- Nanoplatforms' generic type construction aimed to be developed by the 5Dnano-P project; (**B**)-Design strategy and conjugated polymers (CPs)-based proposed alternatives by the P2 team; The CPs-containing electroactive bioplatforms obtained by following the proposed general construction criteria, engineered at (**C**)-macroscopic level and at (**D**)-supramolecular level*

As the declared main objective of the 5DnanoP project is to develop supramolecular functional entities, able to perform as tools for nanomedicine into a five dimensional space, by mimicking living matter mechanism, the use of CPs for its completion seems to be a suitable choice. Thus, this communication brings a brief overview on the adopted strategy for CPs-based materials construction (Figure 1), as well as on some of the latest advents of their use in the construction of (bio)(nano)platforms with potential for the project's envisioned applications. The experimental results confirm that adopted strategy elicits a way to mediate functions. It allow to modulate the composition and the structural details of CPs-based brunched architectures to obtain new materials having excellent biocompatible and electroactive properties, with potential usefulness in different bioapplications, including biomedicine. Moreover, the results demonstrated that biofunctionalization of CPs is an essential step that can enhance their ultimate properties (biocompatibility, stability, etc) for optimally interfacing with biological media

Regarding some of the potential application uses of the unexpected fundamental scientific findings during the research of 5Dnano-P

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Abstract. Conjugated, conducting graft polymers (*g*-CPs) are rod-coil structures that demonstrated to work as excellent active biointerfaces in various type of bioaplications, especially when their side chains are bicompatible and/or biodegradable ones. In the "side-chains first" strategy for g-CPs synthesis, the use of electroactive macromonomers derived from oligo/polymers already established as biomaterials is a good option, which was adopted in the frame of 5DnanoP project. The experimental results, obtained during usual structural characterization and properties' investigation of thiophene-containing macromonomers presented in **Figure 1**, revealed that these compounds are also interesting materials in their-self.

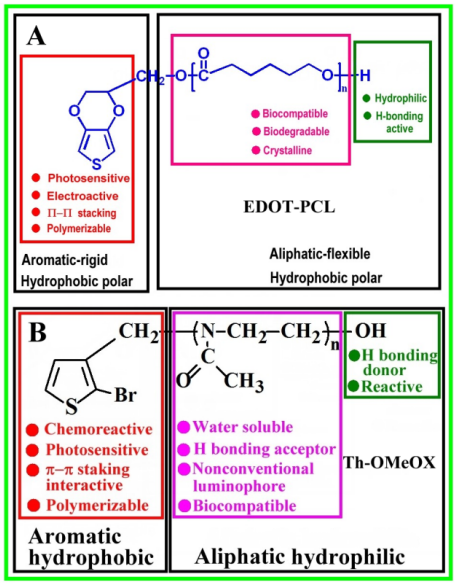

Figure 1. *Design criteria and structural formula of electroactive macromonomers synthesized in the frame of 5Dnano-P project: (**A**)-EDOT-functionalized oligo-e-caprolactone, (**EDOT-PCL**) and (**B**)- Th-functionalized oligo-2-methyl-2 oxazolines, (**Th-OMeOx**)*

Thus, **EDOT-PCL** macromonomer in **Figure 1A**, designed as a shape, hydrophobic amphiphile, besides behaving as a "block-molecule" in organic selective solvents, due to oligo-e-caprolactone presence, also showed crystallization-driven self-assembling capability; it formed in thin film hexagonally-shaped 2D morphology. This unexpected finding is relevant in the context of 5DnanoP, allowing for a future alternative of nanoplatforms construction as free-standing 2D platelets. In fact, in spite of being challenging, the adoption of emerging 2D materials in biomedicine is inevitable, being one of the hottest topics in smart drug delivery systems. On the other hand, **Th-OMeOX** macromonomer, (**Figure 1B**), is derived from poly(2-methyl-2-oxazoline), a recognized water-soluble and peptidomimetic polymer. Interestingly, the photophysical properties measurements of **Th-OMeOX** revealed a clusterization-triggered emission that could be attributable to the abundant presence in the OMeOx structure of the electron rich moieties (carbonyl and N) that, by "through-space" conjugation, ignited an uncommon luminescence from the common MeOx oligomer. This property could be optimized for future use in imaging-guided therapy.

In silico studies of amphiphilic block-copolymers for drug delivery

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Drug delivery copolymers play a very important role in the treatment of various diseases. The current study is focusing on the behavior of a novel copolymer in different organic solvents. The copolymer has π-conjugated polythiophene (PTh) as the main chain and polyethylene glycol (PEG) and poly-ε-caprolactone (PCL) as the side chains. By means of Molecular Dynamics simulations we compared the supra-molecular structures that this copolymer adopts in 4 organic solvents (acetone, acetonitrile, chloroform and toluene) using both implicit and explicit solvent models. Implicit solvation models do not represent the proper behavior of this compound because the "physical" presence of the solvent molecules have an important role in the supramolecular structure. This leads to a behavior similar to vacuum simulations, where the molecule adopts a spherical shape with no structural differences in all 4 solvents. Still, due to its amphiphilic character, a slow microphase separation was observed. On the other hand, explicit solvent models, although requiring a larger calculation power, are considerably better at representing this behavior. In this case, the molecule adopts a more dynamic structure, with the side chains transitioning back and forth from a collapsed state to an elongated one. The phase separations are more pronounced compared to the implicit solvation model and closer to the experimental data and to the expected behavior of these types of amphiphilic copolymers.

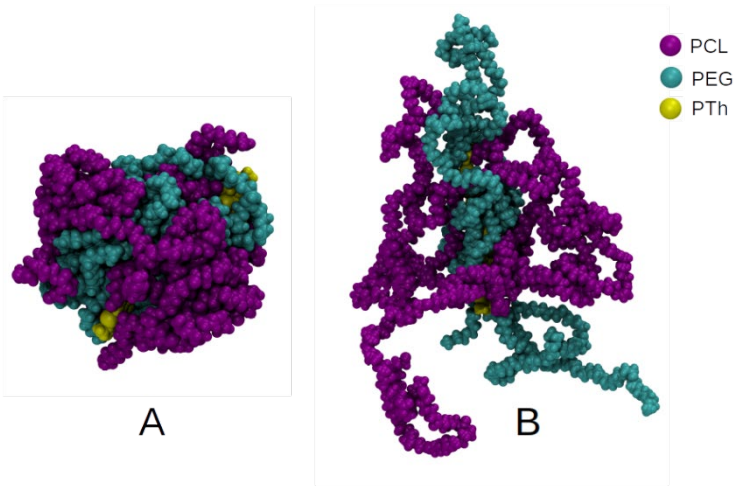

Figure 1: The figure depicts two examples of the supramolecular structure of the molecule in

A) implicit solvent and B) explicit solvent

Experimental and theoretical investigation of the π–π interaction in the di-iminopyrene-di-benzo-18-crown-6-ether molecular system

Dragos-Lucian Isac, Adina Coroaba, Tudor Vasiliu, Cristina Al-Matarneh, Radu Zonda, Andrei Neamtu, Francesca Mocci, Aatto Laaksonen, Mariana Pinteala

Abstract: The pyrene chromophore belongs to a family class of organic compounds that has been used in large numbers of fields, including synthetic chemistry [1], materials [2], supramolecular chemistry [3], theoretical chemistry [4] and excimer formations [5]. In the past decades, there has been a lot of focus on theoretical and experimental investigations regarding the non-covalent interaction of pyrene units. However, the mechanism why it is forming this non-covalent interaction is still unclear and open for debate. A new pyrene derivative, **DPyD-B-C=N-18C6** (Figure 1), was synthesized via –HC=N– bonds connecting a pyrene moiety to each phenyl group of dibenzo-18-crown-6-ether, and studied using molecular dynamic, DFT, and TD-DFT methods. The target compounds can form an intermolecular interaction between di-iminopyrene-dibenzo-18-crown-6-ether units, according to the molecular dynamic simulation, which agrees with WAXD analysis. The electronic structure was studied at the DFT and TD-DFT levels, and the presence of n → π* and π → π* vertical transitions was confirmed. These findings were in line with the experimental excitations data. It was shown that our target compound, **DPyD-B-C=N-18C6**, forms an aggregate with weak π – π interactions, according to both experimental and theoretical techniques.

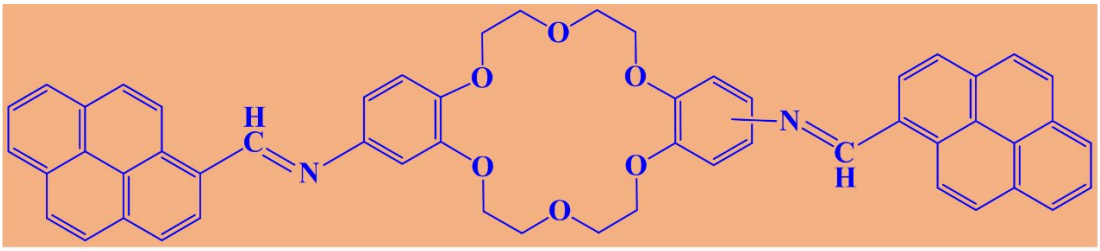

Figure 1. Structural formula of di-iminopyrene-di-benzo-18-crown-6-ether.

Acknowledgements This publication received funding from the PN-III-P4-ID-PCCF-2016-0050 grant, funded by the Ministry of Research and Innovation, CNCS/CCCDI–UEFISCDI, within the PNCDI III program.

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Production and characterization of surrogates for biomedical testing

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In order to make drug development less costly and more efficient, one of the strategies is to use model systems that mimic more closely the *in vivo* tumor. These surrogate tissues can measure *in vitro* the drug efficiency during the preclinical stages of drug development or can be used to study tumor biology *in vitro* by monitoring cancer cell proliferation, invasion, matrix remodelling, angiogenesis or metastasis. The two most important systems used today for testing chemotherapeutics are conventional two-dimensional (2D) systems (cell monolayers obtained by culture in plastic flasks) and three-dimensional (3D) tumor culture including spheroids, cellular multilayers, matrix embedding cultures, bioreactors and microfluidic devices.

The 2D cell cultures are not accurate representations of a tumor since they lack stroma and structural architecture, and it is not possible to create a transport gradient. On the other hand, 3D models have well-defined geometry, cellular heterogeneity and mass transport limitations. If 2D models completely neglected the role of stroma compartment, in 3D model after initial aggregation, cells generally started to secrete extracellular matrix components and upregulated protein mediated cell-cell interactions. In addition, in 3D tumor models it is possible to cultivate cancer cells with stroma elements to better mimic the *in vivo* tumor.

3D normal tissue *in vitro* models - like kidney, liver, skin or bone models- can also be used in preclinical drug screening in order to bring information regarding the toxicity and efficiency of the drug prior to their testing on animals or humans.

These substitutes consist of two main components, i.e. cells and their carriers. The cell carrier, often referred to as a "scaffold", can be made of purely biological molecules, such as collagen, elastin, fibronectin, laminin, hyaluronic acid and other extracellular matrix (ECM) molecules, of synthetic and inorganic molecules, e.g. synthetic polymers, carbon-based materials, ceramics, metal-based materials, or of various combinations of these materials. The materials should be biocompatible, i.e. non-toxic, non-mutagenic, non-immunogenic and matching the mechanical properties of the replaced tissue. However, in advanced tissue engineering, these materials should not just be passively tolerated by cells, but they should act as analogues of the native ECM, i.e. they should control the extent and the strength of cell adhesion, cell proliferation, cell differentiation and maturation to the desired phenotype, and to proper cell functioning. Advantages and disadvantages of different origin materials used for the production of scaffold-based 3D in vitro tumor models are also discussed

In-situ forming hydrogels based on thiolated alginate and pluronic diacrylate

Irina Popescu[1], Marieta Constantin[1], Gheorghe Fundueanu[1], Raluca Nicoleta Darie-Nita[1], Mihaela Turtoi[2], Maria Anghelache[2] and Manuela Calin[2]

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In-situ forming hydrogels can be used to encapsulate cells or bioactive ingredients by mixing the precursors followed by the cross-linking. In this study, *in-situ* forming hydrogels were obtained using thiol-acrylate photo-coupling between pluronic diacrylate (PL-DA) and thiolated alginate (Alg-SH) (Figure 1). For optimization purpose, we studied the influence of the reaction conditions, namely irradiation time, photoinitiator concentration, temperature, and the ratio between thiol and acrylate groups on the hydrogel formation

Hydrogels with different ratios between Alg-SH and PL-DA were then characterized from the viewpoint of their swelling, mechanical properties, permeability, and degradability. The oscillatory rheological measurements and the compression tests showed the formation of hydrogels with excellent structure and mechanical properties. With the increase of the ionic component, Alg-SH, the swelling of the hydrogels increased and the mechanical resistance decreased. The porosity of the swollen hydrogels ranged from 20 to 110 μm, being permeable for macromolecules with low molar mass (FITC-dextran Mw=10 kDa). When FITC-dextran with higher molar mass (Mw=150 kDa) was incorporated in the hydrogels, its release was retarded and influenced by the composition.

The biological assays revealed that the hydrogels induced the proliferation of human keratinocytes in a time-dependent manner and had an anti-inflammatory effect reducing the level of tumor necrosis factor TNF-α, a pro-inflammatory cytokine. The anti-inflammatory effect of the hydrogel wasdue to the synergic action of both precursors (Figure 2).

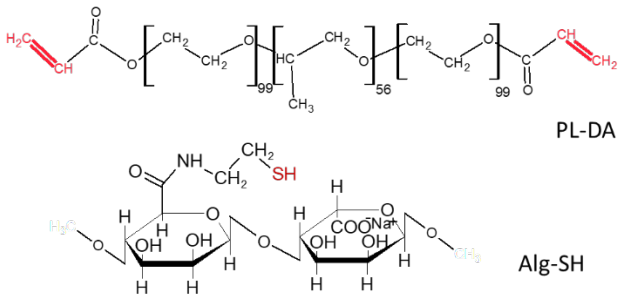

Figure 1. Chemical structure of pluronic diacrylate and thiolated alginate derivatives

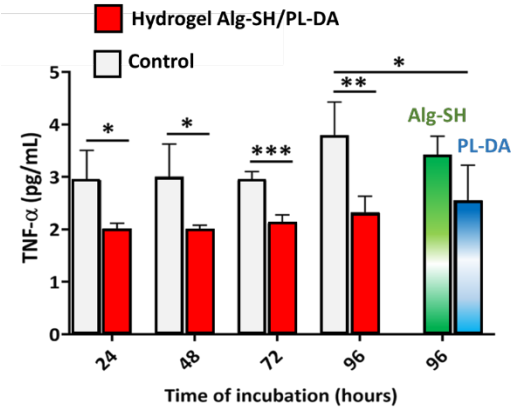

Figure 2. The release profile of TNF-α by HaCaT cells.

Acknowledgment: This work was supported by a grant of Ministry of Research and Innovation, CNCS - UEFISCDI, project number PN-III-P4-ID-PCCF-2016-0050, within PNCDI III

In silico studies of thermoresponsive polymers

E. S. Băcăiță, F. Mocci, A. Laaksonen

Poly(n-isopropylacrylamide), PNIPAM, is, by far, the most intensively studied temperature triggered polymer, since its transition temperature (305K) is close to the physiological temperature of the human body (310K), thus existing the possibility that the drug release to be modulated by the human body temperature. Insights on this phase transition are still not unequivocally established, despite the plethora of studies in this regard, through both experimental and computational methods. Among the last ones, molecular dynamics (MD) simulations were intensively used due to their capacity to offer important insights on the microscopic structures and of the interactions within, being also able to determine certain system properties by tracking the time-dependent positions of the atoms. But, MD results are debatable because they are strongly influenced by the methods and parameters used, such as the starting structure, the force field, the solvent model and, most important, the simulation time.

Through this work we intend a step forward in bringing together MD simulations and experiments by mitigating two of the identified shortcomings: the viability of the starting structure and the lenght of the simulation. For this purpose, we plan: i) to assess the influence of the starting structure on the MD simulations results, ii) to prolong the MD simulation time longer than the ones existing so far, for three different water models.

In order to understand the conformational changes of PNIPAM and changes in its hydration state, the following properties were evaluated as essential molecular descriptors: radius of gyration, the end-to-end distance, number of hydrogen bonds among the polymer and water, radial distribution function of the water oxygen towards polymer backbone, number of water molecules in the first hydration shell and in the second hydration shell of the polymer.

FUNCTIONALIZED SILICA AS DOXORUBICIN CARRIERS

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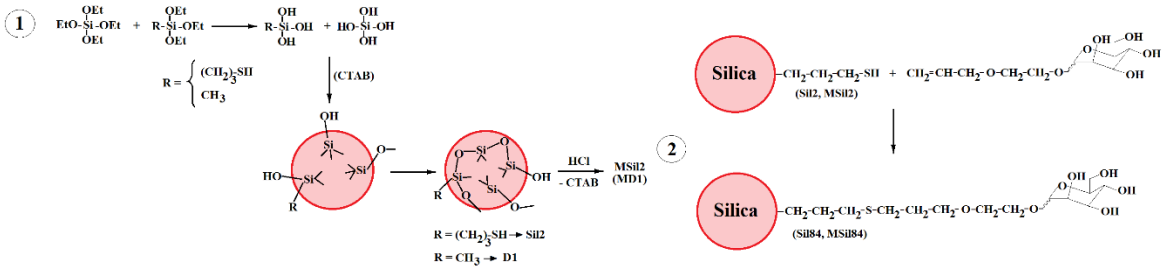
In this study, five silica materials have been prepared and investigated for loading and release of doxorubicin hydrochloride. Their chemical structures are presented in Scheme 1, i.e. a silica with ca. 10% mercaptopropyl groups (Sil 2), a mesoporous silica derived from this one (MSil2), two silicas carrying glucoside-modified functional groups (Sil84 and MSil84), and a mesoporous silica with ca. 20% CH_3 groups (MD1). The chemical modification affects the hydrophilicity of the silica, the mesopores influence the encapsulation efficiency, while the glucose moieties were sought for possible easing of the doxorubicin conjugate docking to the cancer cells, contributing to pH and metabolism modification in solid tumors.



Scheme 1: Synthesis of the silica materials

High encapsulation efficiency (>90%) and loading degrees were obtained. The mesoporous silica MSil2 and MSil84, presented the highest loading efficiency. The cumulative release at 24h varied within large intervals depending on the silica structure, that is 25-75% from the encapsulated amount in pH 7.4, 57-80% in pH 5, and 29-60% in pH 2.6, the release mechanism being generally a two-step diffusion.

The cell viability was evaluated by Celltier-Glo assay, and the effect of Dox-loaded silica samples was compared with that of "free" Dox solutions. The results on three cell lines (NHDF, Mewo and HeLa) are discussed and correlated with compositional and release behavior aspects. Possible applications are proposed for future in-depth (*in vivo*) investigations. ¨

New silatranes possesing biodegradable and bioactive functionalities

Mirela-Fernanda Zaltariov[1], Maria Cazacu[1], Dragos Peptanariu[2], Corneliu Cojocaru[1], Lilia Clima[2], Bianca-Iulia Ciubotaru[1], Alexandra Bargan[1]

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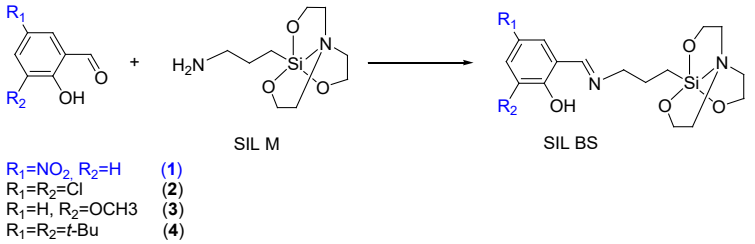
Silatranes and their derivatives are a class of neutral pentacoordinated silicon compounds with a particular transannular interaction between the silicon and nitrogen atoms in the structure (N→Si bond). They are known as biologic active agents being used as antiviral, anti-inflammatory and anticancer agents. Our ongoing studies aimed to explore the chemical modification of aminopropyltrietoxy silatrane in a simple way leading to N-derivatives silatranes (Schiff bases) possessing 5-nitro-(1), 3,5-dichloro- (2), 3-methoxy- (3) and 3,5-di-tert-butyl- (4) substituents (Scheme 1).



Scheme 1. Synthetic pathway of different substituted silatrane derivatives (Schiff bases)

The structure of the compounds was confirmed by analytical, spectroscopic and X-Ray diffraction analyses. Their hydrolytic stability was studied in environments that mimic the conditions in the body at different pH values. The interaction with the biological environment was studied *in vitro* on normal cells (NHDF) and tumor cells (MCF-7 and HEPG2). The most promising antitumor agent (5-nitro derivative) was also tested for the ability to bind proteins in human/bovine serum by UV-vis, fluorescence, circular dichroism and molecular docking methods. The nitro, imine and phenol groups were verified to be important for mucoadhesive properties and antimicrobial activity (*Aspergillus fumigatus*, *Penicillium frequentans*, *Fusarium*, *Bacillus sp*. and *Pseudomonas sp*.). Given the water solubility and very good antimicrobial activity of the aminopropyltrietoxy silatrane, its antiviral capacity was also tested by molecular docking simulations on MPRO (COVID-19 main virus protease).

Acknowledgment: This work was supported by a grant of Ministry of Research and Innovation, CNCS - UEFISCDI, project number PN-III-P4-ID-PCCF-2016-0050, within PNCDI III.

Polymer assisted ultrafiltration: Experimental design, modeling and process optimization

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Ultrafiltration (UF) deals with pressure-driven filtration of liquid systems through a porous membrane [1]. Therefore, UF is frequently employed as a separation process in chemical environmental engineering, biotechnology, and the food industry. In this study, the *polymer-assisted ultrafiltration* (PAUF) process was investigated aiming to remove the anionic dye (Acid-Orange 7, AO7) from aqueous solutions using polyethyleneimine (PEI) as a chelating agent. To explore the effects of factors on the main responses (rejection efficiency and permeate flux), a central-composite design of experiments was adopted. The data-driven modeling and optimization of the PAUF process were done with the aid of multiple regression analysis and genetic algorithms, respectively. Under established optimal conditions, a composite membrane (made of Polysulfone and nanoclay) was tested, unveiling the highest rejection efficiency equal to 99.62%. Details about molecular interactions were disclosed by molecular docking (**Fig.1**) that was performed by the Autodock-VINA algorithm included in the YASARA program.

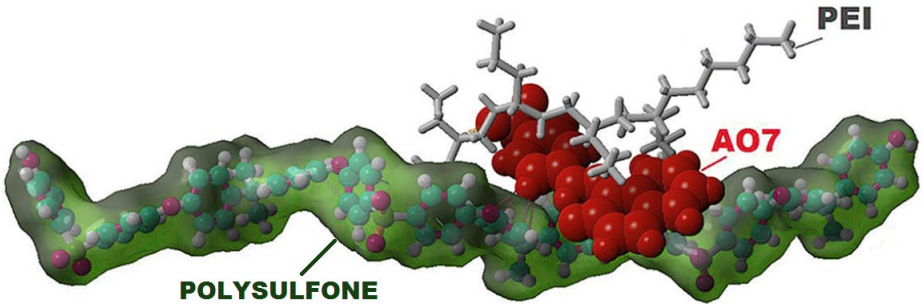

Fig.1. Docking complex between Polysulfone (receptor) and PEI@AO7 aggregate (ligand).

The results of computational molecular docking suggested that PEI@AO7 aggregate may interact with Polysulfone macromolecule (representing the basic membrane component) through hydrophobic, H-bonding, π-π, and cation-π contacts [2].

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5DnanoP & Covid-19 – why *in silico* studies are useful

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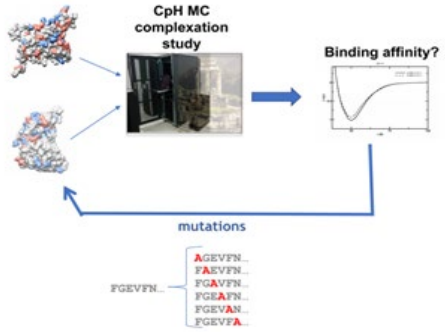
Abstract. Our lives have changed drastically since the beginning of 2020 when Covid-19 pandemic swept over the world. Behind it is the coronavirus SARS-CoV-2 which was more severe threat than other viruses in past. Also, its capability to mutate is worrying. The idea here is to understand the mechanisms of infection and find efficient antibodies to prevent the infection. In a way this project is "inverted" 5DnanoP as we deal with a virus, not a synthetic antiviral carrier. In 5DnanoP we try to find a way to deliver the cargo inside the cell. Here we want to find means to prevent it. But we think that we learn from the wicked virus. Also, we learn from using *in silico* methods what are the key mechanisms and how to inhibit them while in 5DnanoP they could be exploited more. We use here constant pH Monte Carlo, pKproceed and umbrella sampling to find the most efficient monoclonal antibodies to several mutations of SARS-CoV-2 virus. Electrostatic interactions are highly important in design of antibodies. Several experimental studies have appeared confirming our calculations. *In silico* studies are useful!



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Multi-arm non-viral vectors for transfection

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Gene therapy brings into question the possibility of curative treatments for genetic pathologies, as well as the possibility of generating modern vaccines. The SAR-COV2 pandemic has accelerated the introduction of gene therapy vaccines and a large number of those in circulation are non-viral.

In recent years, our research group has designed several non-viral vectors structured on various cores, with several polyethyleneimine arms. The multi-arm core combination brings several advantages: it increases the density of electrical charges and the ability to bind to nucleic acids, it offers the possibility to include elements with other functions such as targeting.

In an effort to find solutions for the greatest possible biocompatibility of vectors, our recent studies have begun to explore the use of polypeptides as arms of vectors. Polylysine is known to be one of the oldest non-viral vectors. Its positive electrical charge ensures the binding of nucleic acid molecules, but the transfection is inefficient due to the lack of buffering and proton sponge effect. This inconvenience can be compensated by the introduction of histidine which is an amino acid with proton buffering effect. There was also a need for a core with multiple functionalized points that could hold several functional arms. Polyethylene glycol (PEG) is a polyether compound with large applications in industry, including the medical sector due to its highly biocompatible profile. This makes it a very good candidate for the core function of the vector. Thus, we present here the concept and preliminary experimental data of a highly biocompatible non-viral vector, which is composed of a PEG core and peptide arms (Figure 1).

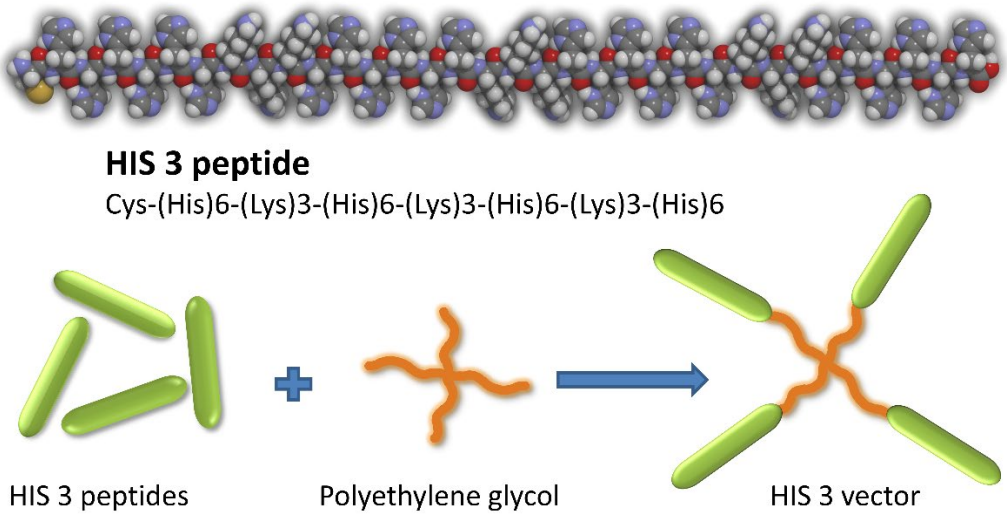

Figure 1. The concept of a non-viral vector with several arms of peptides rich in lysine and histidine

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In silico analysis of the mechanism of interaction between PEG PEI and DNA at an atomistic level

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Poly ethylene glycol is an FDA approved polymer widely used in biomedical applications due to its biocompatibility and ability to protect bioactive compound during blood circulation. Recent studies have observed that PEG can weaken polycation-polyanion interactions, especially in gene carriers that utilize branched polyethyleneimine (bPEI), but the exact mechanism of interactions at the atomic scale are not known. To elucidate these mechanisms of interaction that appear in the specific case of formation of polyplexes between b-PEI-PEG based carriers and DNA, we used in silico simulations on three carriers with different PEG MW. It was observed that the binding between DNA and the gene carriers are highly influenced by the size of PEG. In order to explain the mechanism of interaction between PEG PEI and DNA we used a two-step MD simulation protocol, that showed, that the key mechanism is the hydrogen bond formation between PEI and PEG. Although computationally demanding we find it important to study the behaviour of the carrier prior to the addition of bioactive molecules to understand the molecular mechanisms involved in the forming of the polyplex.

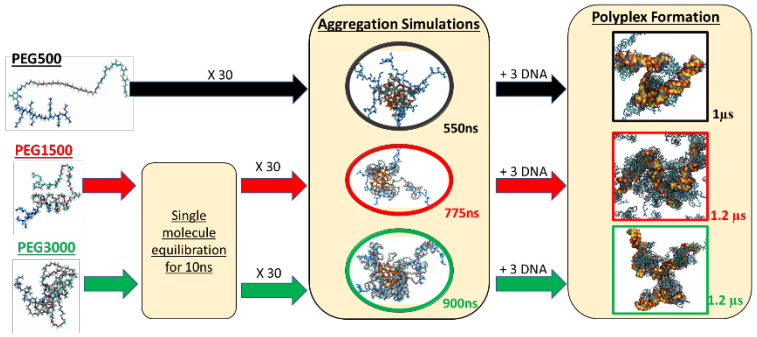

Figure 1. Schematic representation of the simulation protocol of the polyplex formation, for the three studied vectors represented on the left from top to bottom: PEG500, PEG1500, PEG3000.

The first step of the simulations for PEG aimed at reproducing the aggregation of 30 vector molecules, while for PEG1500 and PEG3000 a single vector molecule was equilibrated in water for 10 ns before putting together 30 vector moleculs. Stable aggregates were obtained after 550 – 900 ns of MD simulation, depending on the size of the vector. 3 DNA molecules were added to the equilibrated vector, and MD simulations of in the μs timescale were performed

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Vasiliu, T., Craciun, B., Neamtu, A., Clima, L., Isac, D. L., Maier, S., MocciF. & Laaksonen, A. (2021). In silico study of PEI-PEG-squalene-dsDNA polyplex formation: The delicate role of PEG length to the binding of PEI to DNA. *Biomaterials Science*.

Evaluation of the interaction of potential anticancer drugs with G-Quadruplex DNA: insights from spectroscopic studies, in silico docking and Molecular Dynamics simulation

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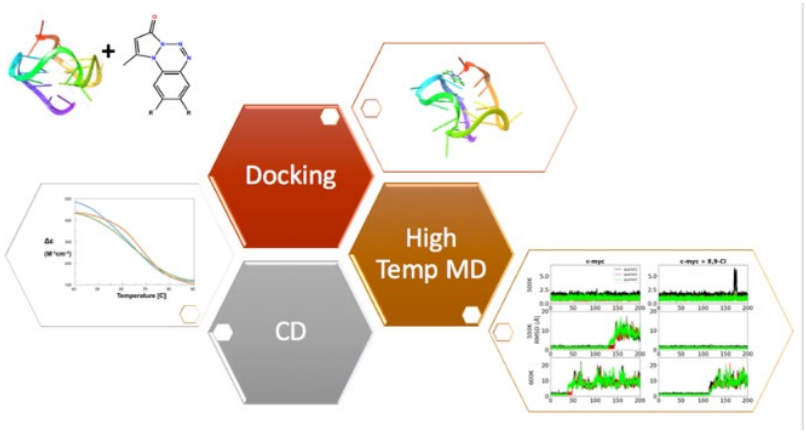
I will describe our recent collaborative effort to study the interaction between a series of pyrazolo[1,2-a]benzo[1,2,3,4]tetrazin-3-one derivatives (PBTs) molecules with parallel G-quadruplex (GQ) DNA aimed at correlating their previously reported anticancer activities and the stabilizing effects observed on c-myc oncogene promoter GQ structure. Circular dichroism (CD) melting experiments were performed to characterize the effect of the studied PBTs on the GQ thermal stability. CD measurements indicate that two out of the eight compounds under investigation induced a slight stabilizing effect (2–4 °C) on GQ depending on the nature and position of the substituents. Molecular docking results allowed us to verify the modes of interaction of the ligands with the GQ and estimate the binding affinities. The highest binding affinity was observed for ligands with the experimental melting temperatures (T_ms). However, both stabilizing and destabilizing ligands showed similar scores, whilst Molecular Dynamics (MD) simulations, performed across a wide range of temperatures on the GQ in water solution, either unliganded or complexed with two model PBT ligands with the opposite effect on the T_ms, consistently confirmed their stabilizing or destabilizing ability ascertained by CD. Clues about a relation between the reported anticancer activity of some PBTs and their ability to stabilize the GQ structure of c-myc emerged from our study. Furthermore, Molecular Dynamics simulations at high temperatures are herein proposed for the first time to verify the stabilizing or destabilizing effect of ligands on the GQ, also disclosing predictive potential in GQ-targeting drug discovery.



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International Journal of Molecular Sciences, 2021, ***22****, 6028.*
https://doi.org/10.3390/ijms22116028

Hyaluronic acid-based 3D cell model of human hepatocarcinoma for chemotherapeutic drug testing

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Hepatocellular carcinoma is one of the most widespread lethal cancer worldwide that requires urgent therapies and thus reliable models for testing anti-cancer drugs. Our study aims to establish a 3D model of human hepatocarcinoma for drug testing and comparatively assess the cytotoxicity and apoptotic response induced by the anti-tumoral agent, cisplatin, in 3D and 2D culture models. The 3D model was established by seeding human hepatocarcinoma (HepG2) cells in a hyaluronic acid (HA)/poly(methylvinylether-alt-maleic acid)-based scaffold. Cells cultured in the HA-based 3D scaffold proliferate into large cellular aggregates and gain liver-like functions proved by controlling the synthesis and release of hepatocyte-specific parameters (albumin, urea, bile acids, transaminases, and human-relevant cytochrome P450 (CYP)7A1 enzyme), compared to 2D culture. Also, the 3D tumor model enhances the response of HepG2 cells to cytotoxic and apoptotic effects of cisplatin compared to 2D support by a mechanism involving the activation of ERK/p38α-MAPK pathway and dysregulation of NF-kB/STAT3/Bcl-2 expression. Taken together, the results encourage the use of HA-based scaffold as an *in vitro* 3D model for chemotherapeutic drug screening.

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PEGylated phenothiazine as promising anticancer drugs

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The paper reports new PEGylated phenothiazine derivatives with antitumor activity. Their design was conceived considering the potential synergistic effect of the two structural building blocks towards antitumor activity improvement, i.e. the antitumor activity of phenothiazine and PEG ability to increase its bioavailability by protecting it into bodily fluids.

To this aim, PEGylated phenothiazine derivatives were synthetized by attaching PEG at the nitrogen atom of phenothiazine *via* an ether, ester or amide linkage units. The structure of the new compounds was confirmed by 1H-NMR and FTIR spectroscopy, and their behavior in solution was assessed by DLS, UV-vis, and 1H-NMR. The antitumor activity was investigated (i) *in vitro* on five human tumor lines and a mouse tumor line by determination of IC50, and (ii) *in vivo* by the tumors growth test. The results showed IC50 values against mouse colon carcinoma cell line comparable with that of traditional antitumor drugs, 5-Fluorouracil and doxorubicin. *In vivo* tests demonstrated that the phenothiazine PEGylation resulted in a toxicity diminishing, and a significant inhibition of the tumor growth (Figure 1). The investigation of the possible tumor inhibition mechanism suggested the nanoaggregate formation and the cleavage of linkage unit as key factors for the inhibition of cancer cell proliferation and biocompatibility improvement.

It was concluded that phenothiazine and PEG building blocks have a synergetic effect working for both tumor growth inhibition and biocompatibility improvement. All these findings recommend the PEGylated phenothiazine derivatives as a valuable workbench for investigation of a next generation of antitumor drugs.

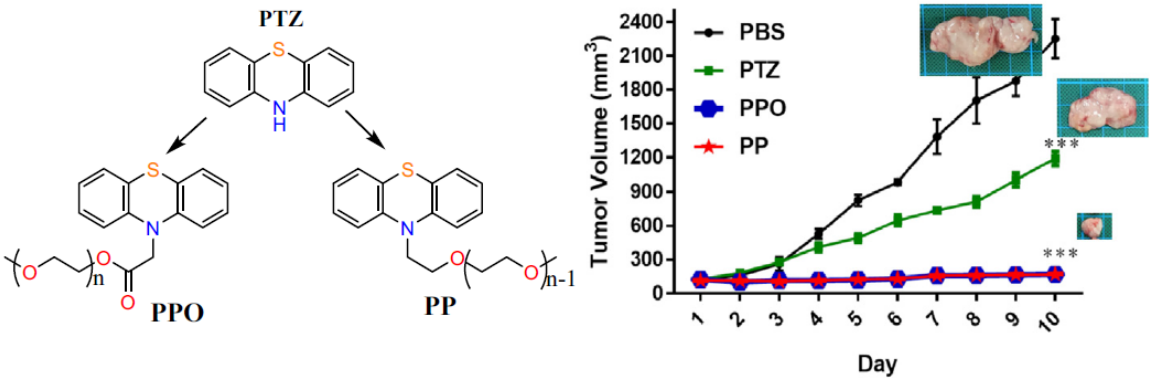

Figure 1. Synthesis and tumor growth inhibition of the PEGylated phenothizine derivatives

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Conjugated metal oxides nanoparticles as inorganic antioxidants

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Conjugation of nanoparticles with drugs is a safe way of attaching bioactive molecules to delivery agents and avoiding the release of the drug during its transport to the target. Some of the carried drugs are antioxidants, which have been shown to be particularly important in regulating redox balance along with endogenous antioxidants. The intake of exogenous antioxidants contributes to the decrease of the excess of reactive oxygen species (ROS) and nitrogen (RNS) that determine the oxidative stress in the cellular tissues. Excess reactive species is correlated with multiple pathologies, such as cardiovascular disease, primarily diabetes aging, and more, so establishing an oxidative balance has overall beneficial consequences in the human body. Natural antioxidants are susceptible to oxidation during storage or during participation in chemical reactions. Therefore, the conjugation of antioxidants with drug delivery agents is limited due to their fragility.

Cerium oxide nanoparticles (CeNP) have become notorious in recent years because they have radical scavenging activity playing an important role in mimicking the role of superoxide dismutase (SOD) in the human body. The conjugation of CeNP with magnetic nanoparticles (MNP) could allow a theranostic approach, where CeNP fulfills its function as an antioxidant in the targeted place due to the transportation by MNP in the magnetic field, while MNPs can also play the role of contrast agents in MRI. This paper presents the synthesis and characterization of nanostructured entities consisting of two types of metal oxides based on cerium (CeNP) and iron (MNP). The conjugation of MNP with CeNP is done by means of polyethyleneimine which, by crosslinking with glutaraldehyde, incorporates both types of metal oxides. Hybrid nanoparticles are obtained by this method, having the characteristics of both types of particles in a manner modified by the interaction between them. Nanoparticles are biocompatible and have low magnetic properties compared to unconjugated MNPs.

It is noteworthy that conjugated hybrid nanoparticles have superior antioxidant activity to simple CeNP or the physical mixture of metal oxides. The obtained results highlight the synergistic action of the components that make up the nano-aggregate formed by the conjugation of CeNP and MNP.

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Caught in the act: supramolecular interactions magnified at atomic scale by molecular simulations

Andrei Neamtu, *Tudor Vasiliu, Dragos Isac, Aatto Laaksonen, Francesca Mocci and Mariana Pinteala*

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Molecular self-assembly is a universal phenomenon in biology that underlies many crucial processes in the living world to sustain life, from structural development to cellular signaling[1]. Self-assembly is a process that is driven mainly by hydrophobic forces in watery environment but seconded by non-covalent interactions like long range electrostatic interactions, salt-bridges formation, van der Waals interactions and hydrogen bonding, to give rise to specificity. The impressive increase in computational power in the last decade has made it possible the use of *in silico* resources to complement the experimental data to a level of detail that is unattainable to the latter techniques. Here we present the usefulness and future perspectives of computer simulations in the field of self-assembly, starting with a brief presentation of main methodological aspects. We will further focus on our recent computational studies on self-assembly using molecular dynamics method and how simulation can be related to experiment. These include a combined experimental and theoretical study regarding the ability of ESI-MS technique to characterize higher-order structures of bio-macromolecules by mass spectrometry. Interesting information at atomic level could be withdrawn about the non-covalent gas phase forces with important roles to keep non-covalent protein complexes together during desolvation[2]. More data will be presented about molecular dynamics studies of micellar structures of azo-polysiloxanes modified with quaternary ammonium groups like triethylammonium or dimethyldodecilammonium chloride[3]. Previous experimental results have shown a very particular type of aggregation of long hydrocarbon chain amine-based micelles while with low size tertiary amines the aggregation process of micelles is no longer present. Molecular dynamics simulations revealed the formation of an outer amphiphilic layer mainly composed of solvent accessible long hydrocarbon chains of the ammonium groups, together with chlorobenzyl and azobenzene moieties (**Fig. 1A**). This particular self-assembly opposes to the idea of a continuous electrical layer of low molecular weight molecules on the surface of the micelle. A special attention will be paid to self-assembly and target recognition in transcription factor - DNA interaction. This is an appealing field of research as engineering of transcription factors has drawn substantial attention for artificial gene regulation and genome editing. Phosphorylation of transcription factors plays a crucial role in switching-off or -on gene transcription. It is well known that phosphorylation of EGR-1 transcription factor at a specific Thr residue switches off its gene silencing action by highly reducing its DNA binding affinity. However, the exact molecular mechanism of this loss of affinity is not yet fully understood. By large scale simulations we demonstrated that the phosphorylation of Thr in position 130, which behaves as a protein hot-spot, largely modify the relative orientation of the two neighboring zinc finger mains (which are rich in basic residues for DNA binding) impairing thus the DNA binding and target sequence search (**Fig. 1 B**).

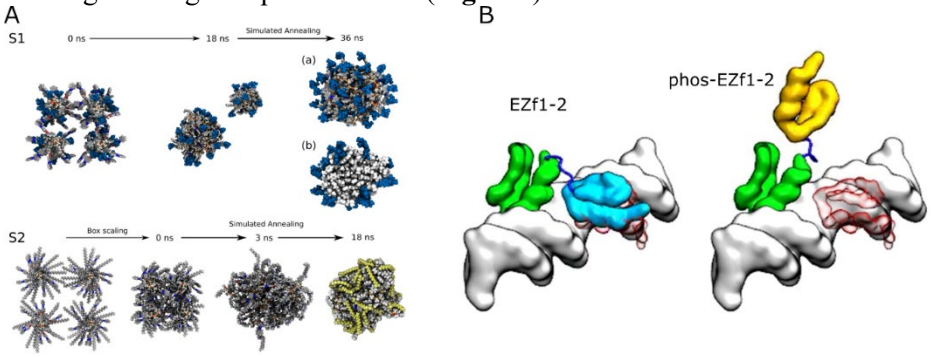

Fig 1. Self-assembly of azo-polysiloxane micelles (**A**) and the effect of phosphorylation on EGR-1 transcription factor conformation (**B**).

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We, all, after 5D-nanoP ends

Stelian S. Maier, Mariana Pinteală

Abstract

In an optimistic and prospective spirit, both the premises and promises of one or several near future projects are analyzed, starting from the achievements gained by 5D-nanoP project, which will sun arrive at end. The main discussed topics extend over the related fields of nanomedicine, embracing the applications at the interface with the classical bioengineering and biotechnology, but also with the advanced cell-, tissue-, and organoid-engineering. Supramolecular chemistry offers the frame of coherently mimicking biological structures in their functionality and energetics (of biophysical origin), and in their sensitivity and responsivity (in biochemical descriptors). The engineering flavor of such applications is unavoidable for the modern scientific projects of great scope, coming from the constraint to ensure the reproducibility of actions and the predictability of behavior of the emulated systems.

If 5D-nanoP project has started from the requirement of the bio-pharma entrepreneurs synthesized in the phrase "*We have the molecules, give us the tools!*", the future proposed themes would mirror the motto "*Tailorable functional entities for personalized nanomedicine applications, capable of being produced extemporaneously (namely with no advance preparation, but based on prior know-how)*", coined by the physicians facing the growing demands of patients. Under such a perspective, no major project will be able to avoid interference with intensive engineering applications, like microfluidics and artificial intelligence, picograms manipulation and nanowatts energizing, long-range molecular sensing and nanoscale mechanical work. All these applicative endeavors, but not only ones, may be sustained or mediated by nano-entities of nanomedicine class. Therefore, a possible future project promoted by all of us could be named using the acronym "*nanoMime*", being dedicated to "***Nanoscale chemo-mimicking of biochemical and immune cues and effectors***".