**Application for Beam Time**

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| **Experiment title** | Interaction of functionalized magnetic nanoparticles with model lipid membrane |
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| **Application details** |

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| **Scientific background** |

We have prepared series of functionalized magnetic nanoparticles designed for the fight with SARS-CoV-2 disease. Magnetic core (Fe3O4) of the composite particle is capped by amorphous silica (SiO2) and the surface is modified by specific molecules according to the desired application. These organic compounds on the surface may bind either virus mRNA (diagnosis) or potential anti-COVID19 drug (therapy). Since the final step of investigated nanoparticles’ development is their introduction into the human body, it is inevitable to address the question of their interaction with cellular plasmatic membrane. A few works devoted to the systems similar to ours have already been published. It has been reported [A,B,C] that nanoparticles may in general exhibit these kind of interactions with phospholipid bilayer (primitive biologic membrane model)

* Binding to the bilayer surface
* Integration into the bilayer
* Destabilization or disruption of the bilayer

However, the authors concluded on multiple factors that substantially affect the interaction (e.g. particle charge or concentration, bilayer composition, temperature etc.). Due to this, any prediction of interaction scenario (even though the nanoparticles and the bilayer are well characterized) is without experimental support only a speculation. In the context of this knowledge we decided to examine the interaction of our specific nanoparticle systems with model lipid bilayer.

A. Tatur, S., Maccarini, M., Barker, R., Nelson, A. & Fragneto, G. Effect of Functionalized Gold Nanoparticles on Floating Lipid Bilayers. *Langmuir* **29**, 6606–6614 (2013).

B. Luchini, A. *et al.* Neutron Reflectometry reveals the interaction between functionalized SPIONs and the surface of lipid bilayers. *Colloids Surfaces B Biointerfaces* **151**, 76–87 (2017).

C. Lolicato, F. *et al.* The Role of Temperature and Lipid Charge on Intake/Uptake of Cationic Gold Nanoparticles into Lipid Bilayers. *Small* **15**, (2019).

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| **Preliminary work already carried out** |

We have ample experiences with composite nanoparticles’ synthesis, characterization and modification for biomedical applications (see our recent works ref. 1,5,7). We also have experiences with model lipid bilayer preparation, modification and its characterization by means of neutron reflectometry at GRAINS facility in FLNP (ref. 2,4). The series of nanoparticle systems that we propose to examine in the experiment have already been characterized by other experimental methods. In general, average particle magnetic core size is 7 nm, silica shell thickness 2 nm. The majority of nanoparticle systems exhibit negative zeta-potential (-30 mV) and good stability in pure water environment. However, the first experiments conducted on live cells have suggested none or only a very modest interaction of selected systems with the cells. This observation is the principal source of our motivation for the submitted proposal.

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| **Aim of proposed experiment** |

* To determine the kind of interaction between selected nanoparticle system and model lipid membrane (none/adhesion/inclusion/destruction/other?)

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| **Details of proposed experimental approach** |

In the proposed experiment, the interaction between magnetic core@shell Fe3O4@SiO2 nanoparticle system and single planar lipid bilayer (1,2-dioleoyl-sn-glycero-3-phosphocholine + 29 mol% of cholesterol) will be investigated by means of neutron reflectometry. In the first step, crystal support will be characterized in D2O environment. Afterwards, model bilayer (DOPC + cholesterol) will be prepared on the crystal surface and the system will be measured in D2O as a reference. In the final step, nanoparticle system (in clinically relevant concentration) will be injected into the chamber. After system stabilization (30 min.), the water environment in the chamber will be exchanged (D2O). The water exchange will facilitate the removal of particles that do not interact with the membrane. Although NSLD of particles’ magnetic cores (6.9x10-6 Å-2) is close to the NSLD of D2O, we expect that contrast between silica shell (NSLD = 2.2x10-6 Å-2) and D2O environment will allow for determination of structural changes on the surface or within the model lipid bilayer.

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| **Instrument and days required** |

We request the access to GRAINS facility for the period of 3 days.

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| **Your publication record** |

1. Magnetic characterization and moderate cytotoxicity of magnetic mesoporous silica nanocomposite for drug delivery of naproxen; Adriana Zeleňáková, Jaroslava Szucsová, Ľuboš Nagy, Vladimír Girman, Vladimír Zeleňák, Veronika Huntošová; Nanomaterials, 2021, 11, 901. https://doi.org/ 10.3390/nano11040901
2. Approaches for a Closer Look at Problems of Liquid Membranes with Amyloid-Beta Peptides. in Soft Matter Systems for Biomedical Applications, edited by Leonid Bulavin and Nikolai Lebovka

Tomáš Kondela, Pavol Hrubovčák, Dmitry Soloviov, Dina Badreeva, Tatiana Murugova, Vadim Skoi, Alexander Kuklin, Olexander Ivankov, Norbert Kučerka

Springer 2021

1. Investigating the competitive effects of cholesterol and melatonin in model lipid membranes

Kondela, T., Dushanov, E., Vorobyeva, M., ...Leonenko, Z., Kučerka, N.

Biochimica et Biophysica Acta - Biomembranes, 2021, 1863(9), 183651

1. Reflectometry and molecular dynamics study of the impact of cholesterol and melatonin on model lipid membranes

Hrubovčák, P., Dushanov, E., Kondela, T., ...Kholmurodov, K., Kučerka, N.

European Biophysics Journal, 2021

1. Particle Size Determination in SBA15 Nanocomposite Using Model Based Analysis of SANS and Magnetization Experimental Data

Hrubovčák, P., Kučerka, N., Zeleňáková, A., Zeleňák, V.

Acta Physica Polonica A, 2020, 137(5), pp. 730–732

1. Structural changes introduced by cholesterol and melatonin to the model membranes mimicking preclinical conformational diseases

Murugova, T., Ivankov, O., Ermakova, E., ...Kuklin, A., Kučerka, N.

General Physiology and Biophysics, 2020, 39(2), pp. 135–144

1. Size and distribution of the iron oxide nanoparticles in SBA-15 nanoporous silica via SANS study; Zeleňáková, A., Hrubovčák, P., Kapusta, O., ...Ivankov, O., Zeleňák, V.; Scientific Reports, 2019, 9(1), 15852

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| **Significance of results for society and/or technological development** |

***What impact can the results of the experiment have on the progress of applied science and/or the development of technologies and/or the solution of topical societal problems?***

The determination of interaction process that takes place when prepared nanoparticles are in contact with model lipid membrane is crucial from both fundamental and, in our case, application point of view. The answer to this question will help in further tailoring of the nanoparticle systems by modification of their size or surface ligands. Although the prepared nanoparticles may be potentially employed for wide spectrum of biomedicine applications, we are focused on their utilization for SARS-CoV-2 disease diagnostics and therapy. Hence, we assume the topic is of high relevance and urgency. We consider the proposed experiment as pioneering in the sense we have not investigated this type of interaction by the selected neutron reflectometry method. In the case of obtaining valid results, we are determined to submit further proposals addressing more specific questions regarding the investigated interactions. Moreover, the experiments would serve as a platform for the establishment of mutual international collaboration between the group of Dr. N. Kučerka from FLNP (expert in the scope of model lipid membranes) and the group of Dr. A. Zeleňáková from P. J. Šafárik University in Slovakia (expert in functionalized magnetic nanoparticles synthesis and characterization).