

Experimental report

30/11/2023

Proposal: 9-12-694

Council: 4/2023

Title: Investigation of RNA adhesion to functionalized nanoparticles for biomedical applications

Research area: Biology

This proposal is a new proposal

Main proposer: Pavol HRUBOVCAK

Experimental team: Lubos NAGY
Pavol HRUBOVCAK

Local contacts: Nicolo PARACINI

Samples: SiO₂
RNA
Fe₃O₄
APTES

Instrument	Requested days	Allocated days	From	To
FIGARO	2	2	24/11/2023	26/11/2023
D17	0	0		

Abstract:

Functionalized magnetic nanoparticles (NPs) designed to detect viral RNA (e.g. SARS-CoV-2) have been prepared. Magnetic core (Fe₃O₄) of the composite particle is capped by amorphous silica (SiO₂) shell and the surface is modified by specific organic molecules (APTES) that bind to RNA. The system exhibit high application potential for COVID19 test efficiency enhancement or biosensor design. In the proposed experiment, RNA interaction with 3 different samples will be investigated. Flat surface (1) and monolayer of Fe₃O₄@SiO₂ NPs (2.) capped by APTES layer after their deposition on the flat substrate. Sample 3 will contain Fe₃O₄@SiO₂@APTES monolayer, i.e. the NPs have been modified by APTES before their deposition onto substrate. The variation in scattering contrasts and RNA concentration in the chamber will allow for assessing RNA binding, its thickness and density as well as the structure of the three examined systems and their effect on the interaction. The results will help in tailoring of our NP systems by modification of their properties (size, shape, surface) in order to obtain higher RNA affinity and thus enhanced efficiency of potential viral diagnostics tests or biosensors.

Investigation of RNA adhesion to functionalized nanoparticles for biomedical applications

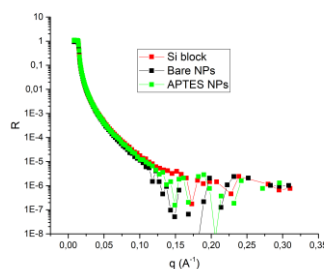
Pavol Hrubovčák, Norbert Kučerka, Adriana Zeleňáková, Ľuboš Nagy, Michael Barutiak, Jaroslava Szűcssová, Eva Beňová, Davide Peddis, Nicolò Paracini

Sample preparation

Langmuir trough was used for the preparation of following samples:

- A) Silicon block (8x5x1.5 cm) + monolayer of NPs (core@shell Fe₃O₄@SiO₂)
- B) Silicon block + monolayer of NPs Fe₃O₄@SiO₂@APTES

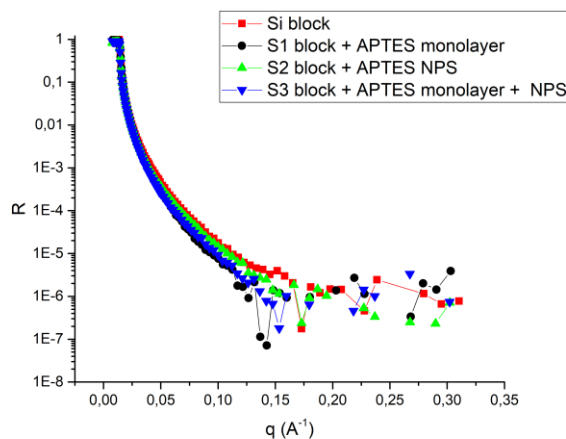
The monolayer of NPs (of the both kinds) was successfully prepared on the water surface in the trough, however, according to the experimental neutron reflectivity (NR) data, none of the NPs bind to the silicon block surface.



Therefor the deposition was repeated again to prepare new samples:

- S1) Silicon block + APTES monolayer
- S2) Silicon block + monolayer of NPs Fe₃O₄@SiO₂@APTES
- S3) Silicon block + APTES monolayer + monolayer of NPs Fe₃O₄@SiO₂@APTES

The geometry of experiment was also changed to reflection down in order to exclude gravity effects. According to the NR data, the NPs did not bind to the surface. On the other hand, the monolayer of APTES attached to the surface of silicon block.



The APTES monolayer on the silicon block surface was prepared following way:

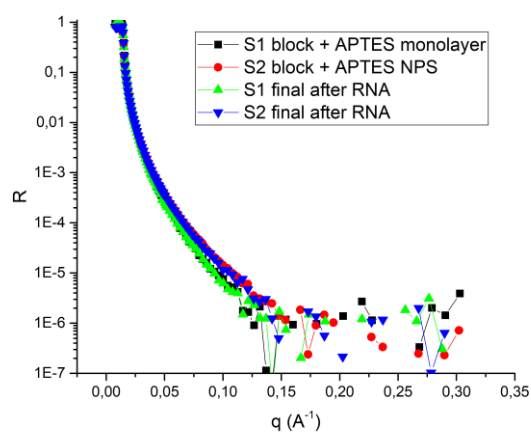
The silicon block was immersed into the solution with volume ratio APTES:toluene(anhydric) 1:100 during 1 hour under desiccator to avoid humid conditions. Afterwards, the block was pulled from the bath while pouring the toluene on its surface to prevent APTES multilayer formation.

In the following, the experiments were performed with two samples:

- S1) silicon block + APTES monolayer
- S2) silicon block (bare)

RNA solution was injected into the experimental chamber (S1, S2). TRIS buffer (20 mM) was used for dilution of torula yeast RNA in concentrations: 1 mg(RNA)/ml(TRIS); 10 mg/ml. The data showed no interaction of RNA with the surfaces S1, S2. Therefor, PEG compound was employed in order to mediate the binding of RNA to the APTES surface. We used 1.25 M NaCl and 10% polyethylene glycol 20 000 and mixed it with solution of 1 mg/ml RNA in TRIS. The ratio was 2.5:1, respectively. However, no changes

in surface structure were observed after comparison with the previous measurement of S1 Block + APTES monolayer.



Conclusions

We found out that:

- Monolayer of our NPs can be prepared employing Langmuir trough, but it does not bind neither to the bare silicon block surface, nor to the surface modified by APTES monolayer
- Under our experimental conditions, the RNA did not bind to any of to the probed surfaces.