# Experimental report

28/07/2021

**Proposal:** 9-13-856 **Council:** 4/2019

**Title:** Study of DNA-cationic polymer brush interactions for gene delivery

**Research area:** Soft condensed matter

**This proposal is a new proposal**


**Main proposer:** Ali ZARBAKHSH

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**Local contacts:** Philipp GUTFREUND


**Samples:** DNA
poly(dimethylaminoethyl methacrylate
CH2=C(CH3)COOCH2CH2N(CH3)2

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| FIGARO | 0 | 3 | 04/09/2020 | 07/09/2020 |
| D17 | 3 | 0 | | |


**Abstract:**

Structural studies of poly(dimethylaminoethyl methacrylate) cationic polymer brushes and its stable uptake of high levels of oligonucleotides using NR are proposed for Gene therapy. Gene therapy, for the introduction of genetic materials into cells, either to compensate for abnormal genes or to make a desired protein, has gained traction for the treatment of a wide range of diseases. DNA is negatively charged, which is subject to degradation in the bloodstream by endogenous nucleases. In this respect, the entrapment of DNA in cationic vectors such as poly(dimethylaminoethyl methacrylate) polymer brushes protects the DNA from nuclease degradation prior to its release from the matrix.

## 1 PRINCIPAL INVESTIGATOR

Name and institution of the Principal Investigator

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## 2 EXPERIMENT DETAILS

| | |
|---|---|
| Experiment 9-13-856 | |
| Title: Study of DNA-cationic polymer brush interactions for gene delivery | |
| Instrument: FIGARO | |
| Dates scheduled: 4 September 2020 | No. Days allocated: 3 |
| Date of experimental report: 28/07/2021 | |

## 3 EXPERIMENT OBJECTIVES

Gene therapy, for the introduction of genetic materials into cells, either to compensate for abnormal genes or to make a desired protein, has gained traction for the treatment of a wide range of diseases. DNA is negatively charged which is subject to degradation in the bloodstream by endogenous nucleases. In this respect, the entrapment of DNA in cationic vectors such as polymer brushes protects the DNA from nuclease degradation prior to its release from the matrix.

We have studied (Biomacromolecules 2018, 19 (2), 606-615) the preparation of PDMAEMA based polymer brushes on flat surfaces and also silica particles to understand their solution behaviour in different buffer conditions, their interaction with DNA molecules, as well as the transfection efficiency of the SiO2-PDMAEMA/ DNA polyplex. We used surface plasmon resonance (SPR) and in situ ellipsometry to further investigate how DNA interacts with PDMAEMA brushes under those conditions. We found that DNA/RNA binding strongly depends on buffer conditions, brush thickness and density, and correlates with transfection efficiency with epidermal cells. We have proposed a kinetic model of oligonucleotide adsorption and are working with our collaborators in developing a full atomist model for these systems.

## 4 EXPERIMENT REPORT

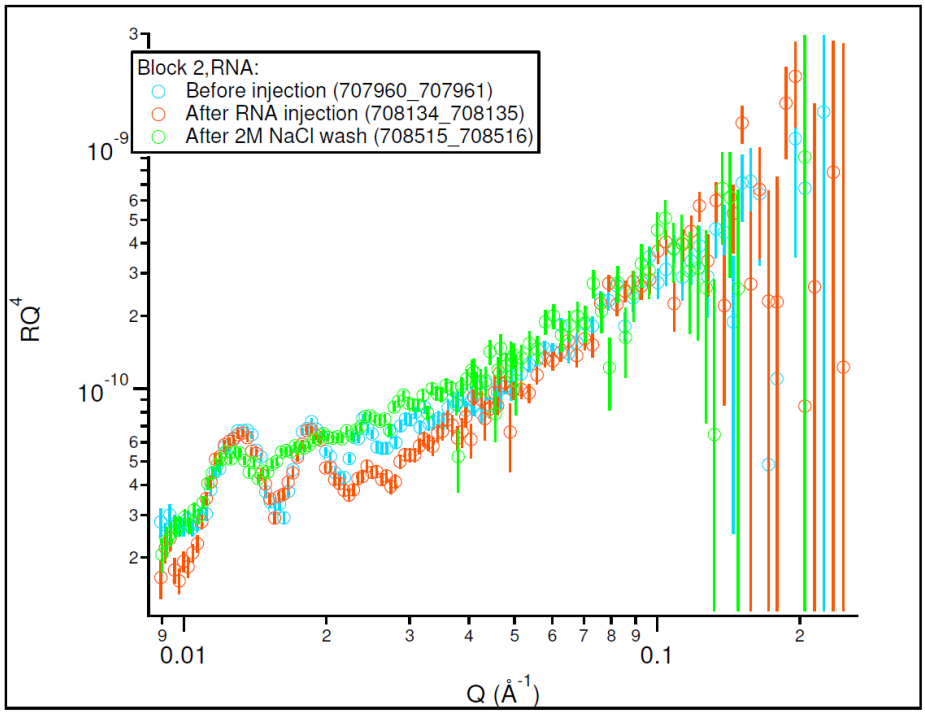
In this proposal, we studied the brush dynamics and their response to oligonucleotides infiltration (collapse, change in morphology), as well as determining the localisation of oligonucleotides (DNA) within brushes (as a function of brush density, thickness and oligonucleotide type and size) using NR. In this first experiment DNA was used. The data analysis is ongoing and will be complemented our in situ ellipsometry and SPR data (already acquired 1). Th extending our existing atomistic modelling and extension to the DNA/RNA interaction obtained from NR is also under way. The experimenatl plan is shown below, including typical data obatined for Block 3.

| | | |
|---|---|---|
| X 3 | Blocks: B1, B2, B3 | PDMAEMA 30 nm/100% density |
| X1 | Block: B4 | PDMAEMA 10 nm/100% density |
| X1 | Block: B5 | PDMAEMA 10 nm/5% density |
| X1 | Block: B6 | PMETAC (30 nm/100%, labelled as 100% 30nm-Q) |

**Proposed series of measurements.**

| Step | **Each step is block specific. Please, <u>do not</u> inject all RNA/DNA solutions on all blocks at the same time. Solutions of RNA/DNA should be made fresh just before injections and the RNA stock solution should be kept in the freezer in between each injection (and diluted solutions prepared fresh).** |
|---|---|

| | |
|---|---|
| 0 | In air, if time permits, to allow the determination of dry thicknesses (to compare to ellipsometry) |
| 1 | Equilibrate **B1-B6** in dPBS-C3. |
| 2 | **B1**: Run in dPBS-C3. |
| 3 | **B2**: Run in dPBS-C3. |
| 4 | **B3**: Run in dPBS-C3. |
| 5 | **B4**: Run in dPBS-C3. |
| 6 | **B5**: Run in dPBS-C3. |
| 7 | **B6**: Run in dPBS-C3. |
| | |
| 8 | Equilibrate **B1-B6** in dPBS-C2. |
| 9 | **B1**: Run in dPBS-C2. |




| **5 LIKELY OUTCOMEs FROM EXPERIMENT**<br>*Please indicate what the experiment is likely to lead to by putting an 'x' next to one or more of the possible outcomes below.* | |
|---|---|
| *Likely outcome* | |
| Journal publication | *x* |
| Data for thesis | *x* |
| Follow-up experiment at ILL | - |
| Follow-up experiment at another facility | *x* |
| Other | *x* |
| No outcome anticipated | - |

**6 SUGGESTIONS FOR IMPROVEMENTS TO YOUR EXPERIMENT, EQUIPMENT OR THE FACILITY**

We are grateful to Figaro Instrument Scientists, in particular Dr philipp gutfreund who organised and enabled this experiment to be conducted remotely.