Experimental report

29/08/2023

Proposal: **9-11-2092** **Council:** 10/2022

Title: Elucidation of the nanostructure of DNA/RNA complexes with cationic polymer brushes

Research area: Chemistry

This proposal is a new proposal

Main proposer: **Ali ZARBAKHSH**

Experimental team: Ali ZARBAKHSH
Julien GAUTROT
Mario CAMPANA

Local contacts: Philipp GUTFREUND

Samples: poly(dimethylaminoethyl) (PDMAEMA)

Instrument	Requested days	Allocated days	From	To
FIGARO	3	3	17/05/2023	20/05/2023
D17	3	0		

Abstract:

Although the field of gene therapy has matured quickly and is becoming now better established clinically, a number of challenges remain. These include improvement of the stabilisation of complexes and protection of the genetic material to be delivered, as well as the enhancement of their therapeutic efficacy, over prolonged periods of time. We have previously showed that polycationic brushes decorating the surface of nanoparticles display particularly promising properties for the stable capture of RNA oligonucleotides and display high transfection efficiencies. In this proposal, we aim to elucidate the architecture of complexes formed of RNA and DNA double stranded oligonucleotides, mRNA molecules and larger double stranded DNA molecules with dense poly(dimethylaminoethyl) (PDMAEMA) brushes. This understanding will help identify how to best engineer brush architecture for the delivery of different types of RNA/DNA molecules, but also will shed lights onto how such architecture may be expected to evolve in new generations of polymer brush-based vectors, such as block copolymers.

1 PRINCIPAL INVESTIGATOR

Name and institution of the Principal Investigator

Dr A Zarbakhsh
Department of Chemistry
Queen Mary University of London
UNITED KINGDOM

2 EXPERIMENT DETAILS

Experiment: 9-11-2091	
Title: Identification of the collapse mechanism of responsive block copolymer brushes for controlled gene delivery	
Instrument: FIGARO	
Dates scheduled: May 2023	No. Days allocated: 2
Date of experimental report: August 2023	

3 EXPERIMENT OBJECTIVES

Both Experiments (9-11-2091 and 2092) were conducted consecutively. The field of gene therapy has made significant progress, but it still faces challenges in stabilizing complexes, protecting genetic material, and improving therapeutic efficacy over extended periods. Polycationic brushes on nanoparticle surfaces have shown promise in capturing RNA oligonucleotides and achieving high transfection efficiencies. This study aims to investigate the structure of complexes formed by RNA and DNA double-stranded oligonucleotides, mRNA molecules, and larger double-stranded DNA molecules with dense poly(dimethylaminoethyl) (PDMAEMA) brushes. By understanding these interactions, we can optimize brush architecture for delivering various RNA/DNA molecules and gain insights into the evolution of brush architecture in next-generation polymer brush-based vectors, including block copolymers. The proposed research will contribute to refining computational models, elucidating properties, and advancing efficient gene delivery systems.

4 EXPERIMENT REPORT

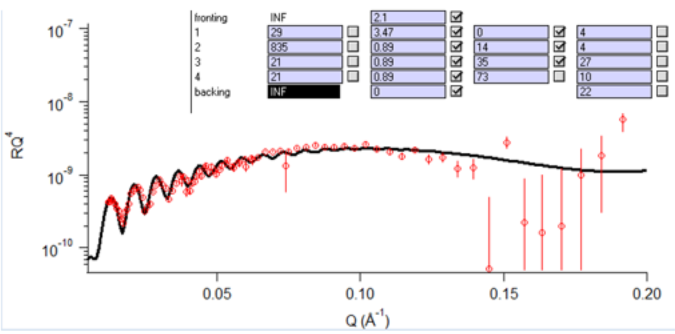
In Experiment 9-11-2091, the objective is to examine the morphological changes (formation of domains or homogeneous barriers) dependent on the thickness of amphiphilic block copolymer brushes grown from solid (Si) substrates. This investigation will help confirm and refine existing computational modeling studies and provide insights into their properties for gene delivery.

In Experiment 9-11-2092, the goal is to understand the architecture of complexes formed by RNA and DNA double-stranded oligonucleotides, mRNA molecules, and larger double-stranded DNA molecules with dense poly(dimethylaminoethyl) (PDMAEMA) brushes. This understanding will aid in optimizing brush architecture for delivering different types of RNA/DNA molecules and provide insights into the expected evolution of such architecture in future generations of polymer brush-based vectors, including block copolymers.

B1	PDMAEMA-PNIPAM 30-30	B6	PDMAEMA 30 nm
B2	PDMAEMA-PNIPAM 30-10	B7	PDMAEMA 30 nm
B3	PNIPAM 30 nm	B8	PDMAEMA 30 nm
B4	PMETAC	B9	PDMAEMA 30 nm
B5	PDMAEMA-PNIPAM 30-60	B10	PDMAEMA 30 nm
		B11	PDMAEMA 30 nm (low density)

Table 1.

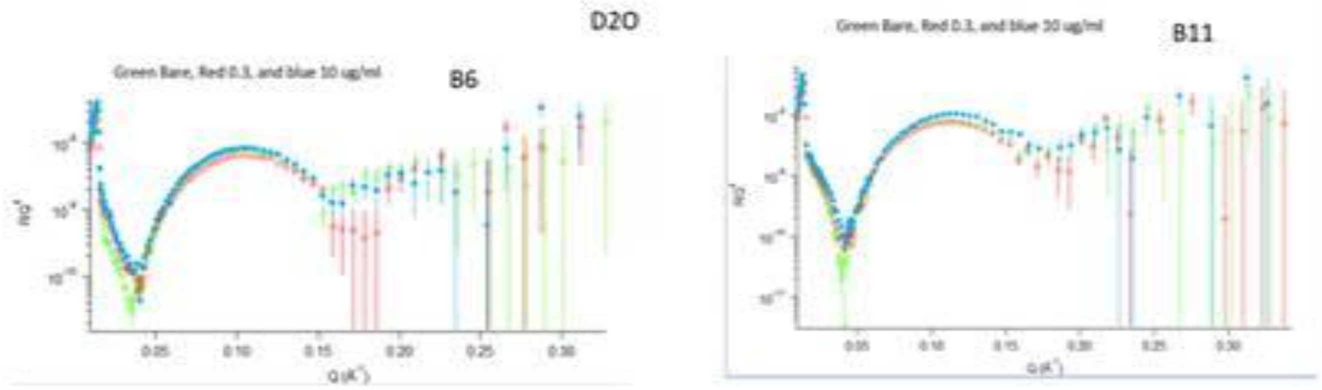
Figure 1.



In Experiment 9-11-2092, the goal is to understand the architecture of complexes formed by RNA and DNA double-stranded oligonucleotides, mRNA molecules, and larger double-stranded DNA molecules with dense poly(dimethylaminoethyl) (PDMAEMA) brushes. This understanding will aid in optimizing brush architecture for delivering different types of RNA/DNA molecules and provide insights into the expected evolution of such architecture in future generations of polymer brush-based vectors, including block copolymers.

The initial part of the experiment involved the dry characterization of all blocks, as shown in Table 1, The si/brush/air Block 1 NR profile and fit are depicted in Figure 1. Subsequently, a comprehensive characterization was conducted using three contrasts. The thermal responsiveness and reversibility of PNIPAM brushes were also investigated. In the second part of the experiment, the confirmation of PDMAEMA brush structures was achieved by utilizing three contrasts: D2O, PDMAEMA-matched SLD medium (SLD 0.91 10-6 A-2), and H2O. Fringes observed in D2O allowed for fine-tuning fits to identify a realistic set of parameters that would also fit the expected low feature reflectometry spectra observed in PDMAEMA-matching. The adsorption of five different types of DNA/RNA molecules was studied: 22 bp double-stranded RNA, 22 bp double-stranded DNA, 100 bp double-stranded DNA, 1000 bp double-stranded plasmid DNA, and 1000 bp mRNA. The data for a single injection at concentrations of 2 and 10 µg/mL were collected, with a typical dataset shown in Figure 2.

Figure 2.



5 LIKELY OUTCOMEs FROM EXPERIMENT

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

NA