REPORT

NanogeT

Redox-responsive self-immolative nanogels for cellular immunotherapies

Rosemeyre Amaral Cordeiro

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Universidade de Coimbra
Rua Larga, Faculdade de Medicina,
Pólo I, 1º andar
3004-504 Coimbra, Portugal
T+351 239 820 190
F+351 239 822 776

Pólo III – Pólo das Ciências da Saúde
Universidade de Coimbra
Azinhaga de Santa Comba, Celas,
3004-504 Coimbra, Portugal
T+351 239 480 200

UC – Biotech,
Parque Tecnológico de Cantanhede
Núcleo 04, Lote 8
3060-197 Cantanhede, Portugal
T+351 231 249 170

info@cnc.uc.pt
www.cnc.uc.pt

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Rua Larga, Faculdade de Medicina,
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T+351 231 249 170

info@cnc.uc.pt
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Presentation of the subject and objectives of the report

This report was prepared as part of the NanogeT project (reference 2022.15560.UTA), titled *"Redox-Responsive Self-Immolative Nanogels for Cellular Therapies"*, funded by the Portuguese Foundation for Science and Technology (FCT), I.P./MCTES. The research presented in this report was conducted at UT Austin by Rosemeyre Cordeiro, the Principal Investigator of the project, from November 6, 2024 to December 9, 2024.

The primary goal of this report is to provide an overview of the progress made in my research, the professional and personal impact of this research stay on my career and development, and some personal experiences during my time in Austin.

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1. Framework and Task Overview

As outlined in the original NanogeT project plan, the methods and procedures applied in this study are organized into three research tasks, as illustrated in Figure 1:

- **Task 1** – Synthesis and characterization of well-defined cationic glycopolymers with different compositions using controlled/"living" radical polymerization techniques.
- **Task 2** – Development of a polymer-based non-viral vector in the form of a nanogel for plasmid DNA delivery – preparation and characterization.
- **Task 3** – *In vitro* evaluation of nanogel transfection in a T lymphocyte cell line and primary T cells.

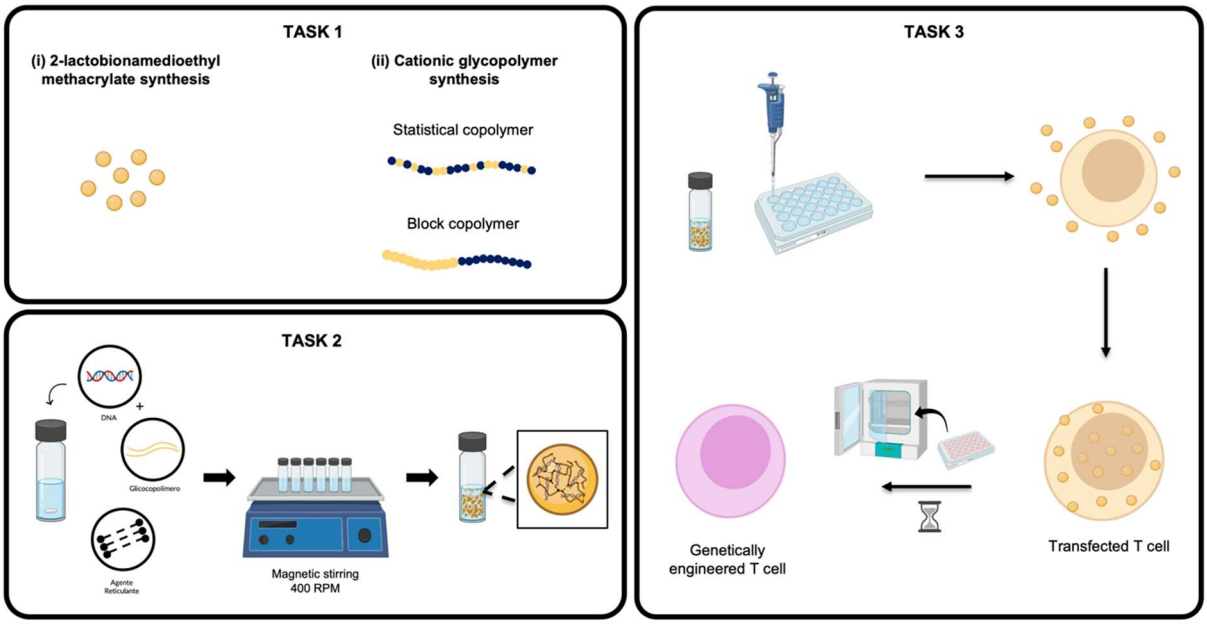

Figure 1. Schematic representation of the tasks planned in the NanogeT project.

The main purpose of the research stay at UT Austin was the development of Task 2. In this task, the objective is to establish a protocol to prepare nanogels using the glycopolymers synthesized in Task 1 for nucleic acid delivery. For this purpose, glycopolymers, plasmid DNA, and disulfanediylbis(ethane-2,1-diyl)bis(4-nitrophenyl) carbonate - a crosslinking agent with oxidation-reduction responsive chemical bonds - will be used. These three components will be

mixed at room temperature in phosphate-buffered saline and ultrapure water at different polymer:DNA:crosslinker ratios. The nanogel will form through the reaction between the amine groups present in both the glycopolymer and DNA with the 4-nitrophenyl carbonate groups of the crosslinker, leading to the formation of a self-immolative covalent bond - dithioethyl carbamate. Professor Wang's laboratory has extensive expertise in this chemical reaction and an already well-established protocol. For this reason, their contribution and my research stay at UT Austin could be crucial to the success of the project.

2. Presentation and discussion of the work conducted at UT Austin.

After completing all the initial laboratory safety training at UT Austin and Professor Wang's lab, I began the experimental work. I worked closely with the PhD students Aaron Tasset and Benjamin Marwedel and the post-doc Dr. Wenliang Wang.

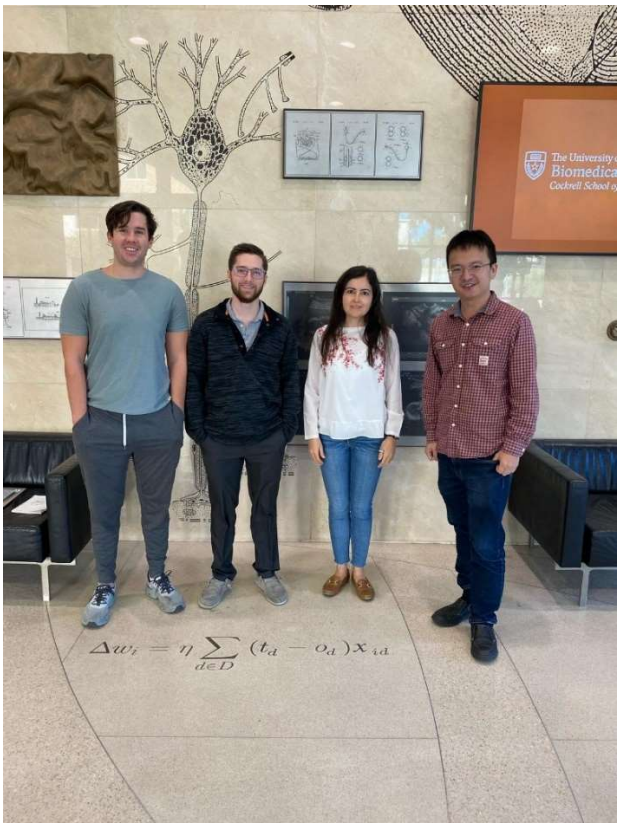

Figure 2. NanogeT team at the atrium of the Department of Biomedical Engineering at UT Austin. From left to right: Aaron, Benjamin, Rosemeyre (PI, UC) and Huiliang (PI, UT Austin).

Initially, I started by synthesizing, purifying (Figure 2) and characterizing the disulfanediylbis(ethane-2,1-diyl)bis(4-nitrophenyl) carbonate (NPC) compound, reproducing the protocol already published by the group.(Wang et al., 2023)

Figure 3. Purification of disulfanediylbis(ethane-2,1-diyl)bis(4-nitrophenyl) carbonate using a chromatographic column.

Afterward, the synthesized compound was used to bundle DNA, following a procedure previously developed in Prof. Wang's lab.(Wang et al., 2023) The bundled DNA was complexed with some polymers synthesized in Task 1 of the project and developed at the University of Coimbra (Portugal), namely poly[(2-aminoethyl methacrylate hydrochloride ($PAMA_{102}$), poly(2-aminoethyl methacrylate hydrochloride)-*block*-poly(2-lactobionamidoethyl methacrylate) ($PAMA_{90}$-*b*-$PLAMA_{113}$) and poly[(2-aminoethyl methacrylate hydrochloride)-*co*-(2-lactobionamidoethyl methacrylate) (P[(AMA_{92})-*co*-($LAMA_{95}$)]). Three different polymer/DNA ratios were tested: 2/1, 5/1, and 10/1. The hydrodynamic diameter of the complexes was measured using dynamic light scattering (Figure 4).

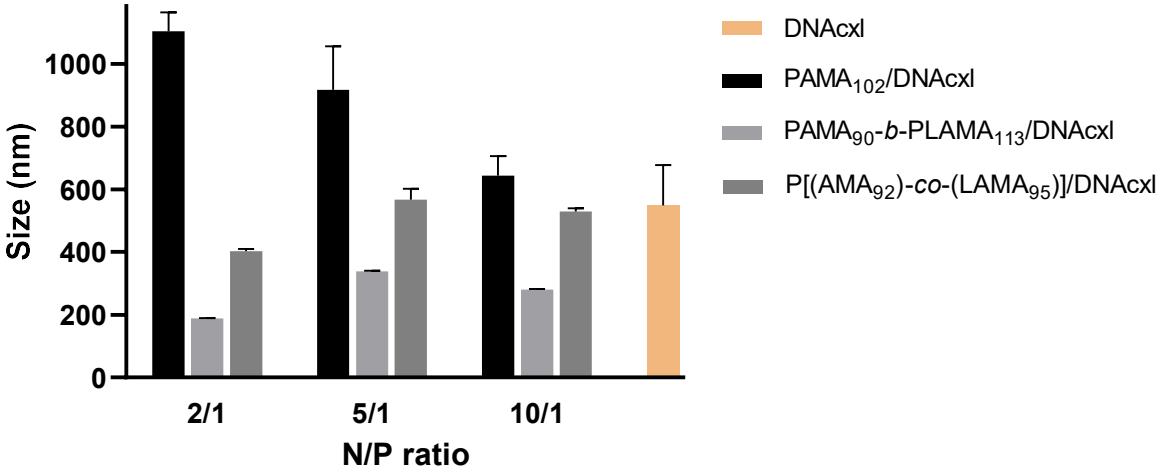

Figure 4. Hydrodynamic diameter of DNA bundled (DNAcxl), and $PAMA_{102}$-, $PAMA_{90}$-b-$PLAMA_{113}$-, and P[(AMA_{92})-co-($LAMA_{95}$)]-based complexes prepared at 2/1, 5/1 and 10/1 N/P ratio.

Overall, the formed complexes are relatively large, with those prepared using the block copolymer $PAMA_{90}$-*b*-$PLAMA_{113}$ standing out, as they achieved smaller sizes, ranging from 200 to 300 nm across the different tested ratios. After these initial positive results, we proceeded with the preparation of the nanogels. For this, we followed a similar procedure to that used for bundling DNA, but with the polymer added simultaneously with the DNA and the crosslinking agent. The size of these initial nanogels was too large (>1000 nm), requiring refinements to the procedure. Various factors were tested, including the order of component addition, reaction time, and the nanogel washing/purification process. Despite multiple experiments, the nanogel size remained high (>500 nm).

Following a productive discussion on the results, we agreed that changing the crosslinking agent could facilitate the bundling process and lead to smaller particles. Given Prof. Wang's extensive experience in this area, he suggested using a PEGylated disulfanediylbis(ethane-2,1-diyl)bis(4-nitrophenyl) carbonate. Since my research stay was coming to an end, he proposed producing the compound and shipping it to Portugal to continue the work there. Thus, my stay at UT Austin was crucial for the successful development of the NanogeT project and helped strengthen the relationship between the two institutions.

The stay at UT Austin was a highly productive period for the UC–UT Austin collaboration. In addition to the progress made on the NanogeT project, new ideas for joint projects emerged. One of these ideas has already been submitted to the "*2024 Call for*

Exploratory Research Projects under the UT Austin Portugal Program" with the title *"Mechanoluminescent nanotransducers for deep brain sono-optogenetics in Parkinson's disease treatment"* (reference 2024.13446.UTA). This proposal includes a longer research stay (six months) for the Portuguese team at UT Austin to further maximize the benefits of these exchanges and collaborations. Additionally, we are preparing a review article for submission in the near future.

3. Austin life

Despite the short duration of the stay and the intensity of the experiments, I tried to immerse myself in the local culture - and what better way than by enjoying some authentic Texas barbecue!

Figure 5. Barbecue experience at the Michelin-starred Interstellar Barbecue Restaurant.

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I also had the opportunity to visit some of Austin's main landmarks, such as the Texas Capitol and the Lady Bird Lake. The pleasant weather was an invitation for some sightseeing tours.

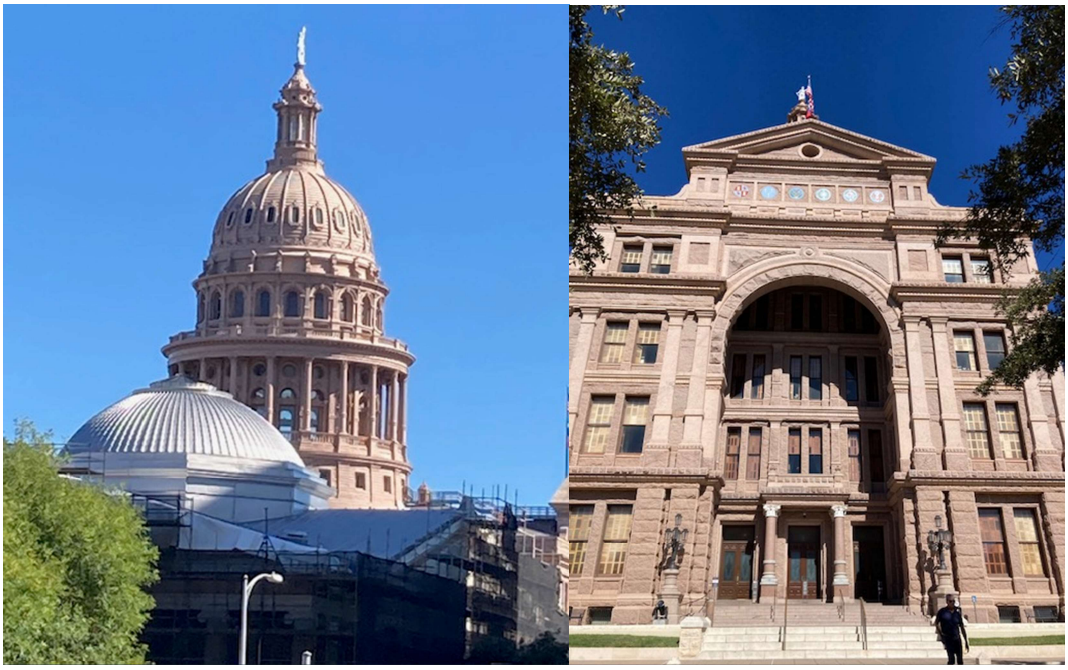

Figure 6. Texas Capitol.

Figure 7. Allens Boots store.

4. Conclusions and considerations

The research stay at UT Austin was highly positive, with a beneficial impact not only on the development of the NanogeT project but also on strengthening the collaborative relationship with Prof. Wang and his team. It was also important for my career, as international experiences are highly valued in academic selection processes.

However, I would like to highlight that, to make the most of this type of research stay, I would recommend a minimum duration of two months. A significant amount of time is spent on administrative procedures and training for laboratory access, which leaves a rather limited timeframe for experimental work.

5. Reference

Wang, W., Tasset, A., Pyatnitskiy, I., Lin, P., Bellamkonda, A., Mehta, R., Gabbert, C., Yuan, F., Mohamed, H. G., Peppas, N. A., & Wang, H. (2023). Reversible, Covalent DNA Condensation Approach Using Chemical Linkers for Enhanced Gene Delivery. *Nano Letters*, *23*(20), 9310–9318. https://doi.org/10.1021/acs.nanolett.3c02429