COST
EUROPEAN COOPERATION
IN SCIENCE & TECHNOLOGY

# Short-Term Scientific Mission Grant
# - APPLICATION FORM[1] -

**Action number: CA20126**

**Applicant name: Chantelle Spiteri**

## Details of the STSM

Title: Fabrication of functionalised porous silicon nanoparticles for mRNA delivery

Start and end date: 05/09/2022 to 30/09/2022

## Goals of the STSM

Purpose and summary of the STSM.

Intracellular delivery mediated by high-intensity laser pulses (known as optoporation) in the presence of photothermal nanomaterials has emerged as a means to control the cell membrane disruption in space and time allowing payloads to access the cell. However, the widely used nanomaterials (gold and carbon-based nanoparticles) are not biodegradable and can be genotoxic, limiting their applicability in advanced therapies and modelling.

The Chiappini lab has demonstrated optoporation with thermally oxidised, biodegradable, biocompatible mesoporous silicon nanoparticles (pSiNPs) coupled with a femtosecond NIR laser, capable of patterned delivery of propidium iodide in MCF-7 cells with a 40% delivery efficiency. With this approach, we demonstrated for the first time the patterned transfection of GFP mRNA leading to GFP protein expression using pSiNPs. Our current limitation is the lower than desirable mRNA transfection efficiency. Thus, we are searching for a means of enhancing the coupling of the nanoparticles with the femtosecond laser to improve transfection efficiency at low laser power.

The Cunin lab is pioneering high-efficiency RNA transfection to MCF-7 through pSiNPs-mediated NIR optoporation, by functionalising the nanoparticles. Thus we propose integrating this functionalisation strategy within our optoporation approach, to improve transfection efficiency and reduce toxicity to a desirable level for patterned gene transfer.

*(max.200 word)*

Applicant enters max. 200 word summary here.

[1] This form is part of the application for a grant to visit a host organisation located in a different country than the country of affiliation. It is submitted to the COST Action MC via-e-COST. The Grant Awarding Coordinator coordinates the evaluation on behalf of the Action MC and informs the Grant Holder of the result of the evaluation for issuing the Grant Letter.

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**Working Plan**

Description of the work to be carried out by the applicant.

Dr Frédérique Cunin and her group at the Institute Charles Gerhardt Montpellier (ICGM), have unique expertise in the functionalisation of pSiNPs for optimal photothermal coupling, and their use for photodynamic therapy and nucleic acid delivery. Presenting their work at the porous semiconductor science and technology (PSST) conference in Italy, we became aware that the Cunin group developed a step-by-step reaction pathway to generate highly-efficient pSiNPs for photostimulation. Henceforth, we agreed with Dr Cunin on the mutual advantage of integrating these particles with our platform to improve its performance. We now aim to use this project to explore this hypothesis by acquiring knowledge of the production process of these novel particles.

Miss Chantelle Spiteri, the PhD student from the Chiappini Lab working on the optoporation project will visit the ICGM and perform the proposed work. The initial work will involve the synthesis of pSiNPs using our established parameters with the desired characteristics. These particles will be characterised through dynamic light scattering and electron-microscopy to ensure reproducible particle size and pore size across different laboratories. The particles will then be functionalised through the step-by-step organic pathways developed by the host institution. Here, Ms Spiteri will become familiar with the synthesis and purification techniques. The particles will be characterised through NMR and FTIR to ensure successful functionalisation. Viability assays will be performed to identify the maximum concentration of pSiNPs that can be applied without impacting the viability of the cells. This can be done through an ATP-viability assay.

The techniques learnt will be transferred to our group at King's College London which will serve to synthesise, analyse and characterise the functionalised nanoparticles on demand. On return to the Chiappini Lab, these particles will be integrated into our protocol to test the efficiency of targeted cell permeability in a 2D system by coupling the laser with these particles. In so doing, this will be the first time that the functionalised pSiNPs (a material developed by the host institution) are used for patterned delivery (approach developed by the applicant's institution). Once successful, the particles are applied to a 3D stem cell system aiming to attain the patterned expression of the protein of interest, a protein that is involved in the very early stages of embryonic development. This will enable analysing the patterned control of cell function as a consequence of the laser-mediated transfection, which we will leverage to shed light on the mechanisms involved in stem cell differentiation.

Shall the results corroborate our current data, we aim to submit our findings for publication within the next year in a multidisciplinary material science journal such as *Advanced Materials* and present the newly acquired information at the *European Society for Biomaterials* conference in 2023.

In summary, the project aims to attain efficient targeted single-cell delivery of nucleic acid. Such a goal is to be achieved in steps including;

Aim 1: Fabricate isocyanatopropyl triethoxysilane (ICPES)-azobenzene-lysine functionalised pSiNPs.

Aim 2: Characterise the particles by SEM, NMR and FTIR and their cytocompatibility by ATP assay.

Aim 3: Assess cell viability and transfection efficiency following GFP-mRNA optoporation.

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Applicant enters max. 500 word summary here.

**Expected outputs and contribution to the Action MoU objectives and deliverables.**

Main expected results and their contribution to the progress towards the Action objectives (either research coordination and/or capacity building objectives) and deliverables.

We have already demonstrated that mesoporous silicon nanomaterial has good biodegradability, couples with the femtosecond laser which as a result provide targeted permeabilization, and the cells maintain long-term viability post-treatment. The novel functionalised pSiNPs should have better interaction with the laser which allows for higher poration efficiency without increasing the laser power. In turn, this would yield higher throughput and efficiency of mRNA transfection and protein expression, enabling patterned control of cell functionality through gene therapy.

The dissemination of the new technology to the COST members will be a vehicle to gather, share and improve access to knowledge, equipment and resources. In the joint research, combining patterned optoporation with the functionalised pSiNPs leads to identifying novel applications to maximise the impact of porous semiconductors in biomedicine. Indeed, the novelty of our project lies in generating new knowledge to address current challenges in biomedicine (as explained above) thereby furthering the COST mission of bridging the gap between fundamental development and technological applications in health. Reflecting on the need for a collaborative nature within international institutions, this collaboration will lead to efficient fund leveraging to achieve tangible, high-gain but low-risk short-term outputs for the COST Action increasing the competence of the European Research Area. Lastly, in line with COST action in promoting the career development of young researchers, mobility will boost our capacities whilst providing us with the opportunity to expand our professional network circle and learn from experienced peers for future healthcare wellbeing.

The collaboration between an internationally leading porous semiconductor group (host institution) and a rapidly expanding biomaterials research group (applicant institution) will bridge the gap between our two institutions leading to the exploration of new ideas that were previously dropped due to the limited resources. Building stronger ties will pave the way for future joint projects to continue expanding our reach in research and rapidly attain set milestones. This translates to increasing both groups' competitiveness in publishing impactful work, including improving the quality of manuscripts in preparation, and hence widening our reach in the scientific community. Joint applications to European projects to translate this technology in the biomedical space are foreseeable.

The budget allocated for this project is as follows;

**Contribution from COST:**

- Accommodation from the 4th September till the 2nd October = €1,800
- Travelling including a return flight from London to Montpellier France and luggage = €200

**Total = €2000**

**Contribution from Applicant:**

- Daily modes of transport (€50) and daily cost of living (€200)
- GFP mRNA (~€430), Label IT - Nucleic Acid Labeling Kit, Cy5 (~€300), silicon wafers (~€15), supplemented cell medium and OptiMEM (~€20), ATP assay (10mL) (~€85), DAPI (~€120), propidium iodide (~€40)

**Total:** €**1260**

**Contribution from Host:**

- Silicon wafers (~€50), chemicals (diamino-azobenzene, Fmoc-Lys-Boc(OH), fluorhydric acid, solvents) (~€500)

- Analysis (NMR, TEM, N2 sorption) (~€200), confocal microscopy at RIO imaging Montpellier Platform (~€200)

**Total = €950**

*(max.500 words)*

Applicant enters max. 500 word summary here.