ISIS@MACH ITALIA

Science and Technology Facilities Council
ISIS Neutron and Muon Source

Experiment Proposal

Experiment number GP2024020

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Experiment title	Investigating Thermal Stability and Cellular Internalization Pathways of SARS-CoV-2 mRNA Vaccine
MRF Instrument	**Confocal Microscope 3** — **Days requested:** 4
Access Route	Direct Access — **Previous GP Number:** -
Science Areas	Biology and Bio-materials, Physics, Technique Development — **DOI:** -
Sponsored Grant	None — **Sponsor:** -
Grant Title	- — **Grant Number:** -
Start Date	- — **Finish Date:** -
Similar Submission?	-
Industrial Links	-

Non-Technical Abstract

This project is about understanding how the COVID-19 vaccine from BioNTech/Pfizer behaves under different conditions. We will look at the efficiency and stability of the mRNA vaccine when heated up.

We are going to use human adenocarcinoma lung cells to study the interaction and the internalization with this vaccine. To do this, we will first expose some vaccine samples to high temperatures to mimic what might happen if they are not stored properly. Then, we will incubate heated samples and normal ones into these cells. We will watch what happens over a few days to assess the internalization of the vaccine.

We will use a confocal microscope to acquire images and analyze them. We will quantify the amount of vaccine internalized inside the cells and the colocalization rate with specific intracellular vesicles.

Overall, this study will help us better understand the bio-nano interactions between mRNA vaccines and target cells and the effect of storage on their efficacy and stability.

Publications -

ISIS neutron and muon source — **E-platform:** No

Instruments	**Days Requested:**
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Sample record sheet

Principal contact Professor Laura Sironi, Università degli Studi di Milano-Bicocca, ITALY
MRF Instrument **Confocal Microscope 3** **Days Requested:** 4
Special requirements:

SAMPLE

Material	Microslide slides with fixed cell samples	-	-
Formula	-	-	-
Forms	Solid		
Volume	cc		
Weight	mg		
Container or substrate	-	-	-
Storage Requirements	-	-	-

SAMPLE ENVIROMENT

Temperature Range	- K	-	-
Pressure Range	- mbar	-	-
Magnetic field range	- T	-	-
Standard equipment	None	-	-
Special equipment	-	-	-

SAFETY

Prep lab needed	No	-	-
Sample Prep Hazards	-	-	-
Special equip. reqs	-	-	-
Sensitivity to air	No	-	-
Sensitivity to vapour	No	-	-
Experiment Hazards	-	-	-
Equipment Hazards	-	-	-
Biological hazards	-	-	-
Radioactive Hazards	-	-	-
Additional Hazards	-	-	-
Additional Details	-	-	-
Sample will be	Removed By User	-	-

1. Background and Context

The onset of the COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, led to a global rush to develop effective vaccines. Among various strategies, mRNA vaccines stood out, with one such vaccine, BNT162b2 developed by BioNTech/Pfizer, showing a 95% efficacy in preventing the infection. This vaccine uses solid lipid nanoparticles (SLNs) to protect the mRNA, thereby enhancing its stability and delivery.

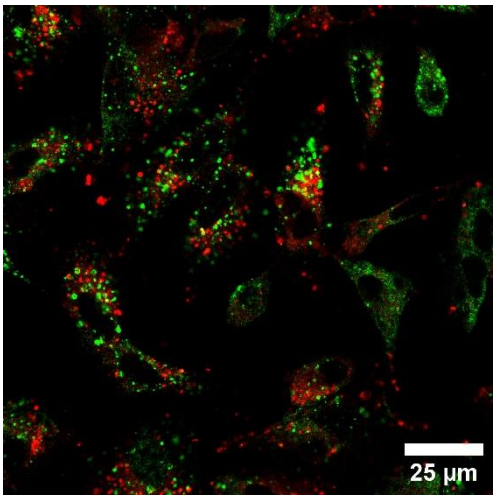
Previous research has highlighted the importance of SLNs in maintaining mRNA stability and promoting efficient cellular uptake. Nonetheless, the detailed stability of these vaccines, particularly under varying conditions, and their specific intracellular trafficking mechanisms are areas that require further investigation. This study aims to shed light on these aspects by examining the behaviour of "stressed" (thermally altered) and "non-stressed" samples of the BNT162b2 vaccine in cellular environments.

2. Proposed Experiment

This experiment aims to evaluate the thermal stability and uptake efficiency of the BioNTech/Pfizer BNT162b2 mRNA vaccine in human lung adenocarcinoma epithelial cells A549 cells, assessing how thermal stress affects its interaction with cellular vesicles over various timepoints.

Initially, aliquots of the BNT162b2 vaccine will be prepared from the same batch to ensure uniformity. These samples will then be divided into two groups: one serving as the control group, which will be stored under recommended conditions to maintain its stability, and the other designated for thermal stress testing (40°C and 60% humidity for 8 days). The thermal stress group will be subjected to a specific temperature regime that exceeds the recommended storage conditions.

A549 cells will be cultured under standard conditions, following the appropriate incubation period to allow for cellular adherence and growth, cells will be treated with the DiD-labeled vaccine samples (both stressed and non-stressed). Additionally, we will be marking the nucleus with DAPI and two types of vesicles (early endosomes and lysosomes) with AlexaFluor 488. An exemplary confocal image is shown in the figure attached.



The treatment will span across the six time points (4h, 6h, 24h, 48h, 72h, 96h) to capture the dynamics of vaccine internalization and its variability with the two types of vesicles.

To assess the interaction of BNT162b2 vaccine particles with intracellular vesicles, we'll use colocalization analysis, capturing at least ten 40X magnification images at each time point. Using software that calculates Pearson's correlation and Manders' overlap coefficients, we'll quantitatively determine the vaccine's co-location with early endosomes and lysosomes. This will reveal insights into the vaccine's cellular uptake, its endosomal escape, and processing, under

both normal and stress conditions. This crucial analysis helps us understand the vaccine's uptake mechanisms and the impact of storage conditions on its effectiveness, aiming to enhance mRNA vaccine stability and storage optimization.

3. Experiment Timeline and justification of experimental time requested

The image acquisition will be performed at the University of Milano-Bicocca, specifically at the Laboratory of Advanced Bio-Spectroscopy, led by Prof. G. Chirico. We will exploit the Leica TCS SP5 fluorescence scanning confocal microscope, which is equipped with Argon and a HeNe laser. These lasers will respectively excite the Alexa Fluor-488-labelled vesicles and the DiD-labelled SLNs. Their fluorescence signals will be detected using a 40X oil-immersion objective to minimize chromatic aberrations while maintaining optimal magnification.

We anticipate spending a total of 4 days on the setup:

- 1 day for exploratory setup and acquisition of reference images.
- 3 days to capture images from at least 10 random regions of each sample (6 timepoints, including control and 2 treatment groups).