ISIS@MACH ITALIA

Science and Technology Facilities Council
ISIS Neutron and Muon Source

Experiment Proposal

Experiment number GP2024017

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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:**
Access Route	**Previous RB Number:**
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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

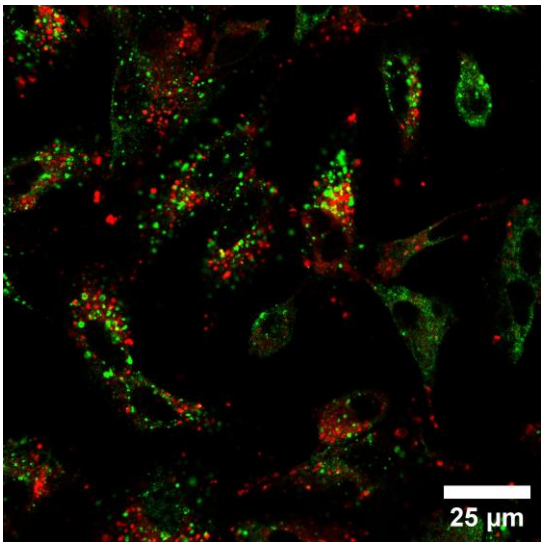
Prep lab needed	No	-	-
Sample Prep Hazards	-	-	-
Special equip. reqs	-	-	-
Sensitivity to air	No	-	-
Sensitivity to vapour	No	-	-
Experiment Hazards	-	-	-
Equipment Hazards	-	-	-
Biological hazards	-	-	-
Radioactive Hazards	-	-	-
Additional Hazards	-	-	-
Additional Details	-	-	-
Sample will be	Removed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

1. Background and Context

The onset of the COVID-19 pandemic, instigated by the novel coronavirus SARS-CoV-2, necessitated a global rush towards developing effective vaccines. Among the various strategies deployed, mRNA vaccines emerged as a pivotal innovation. The European Medicines Agency (EMA) approved several vaccines, including two based on mRNA technology: BioNTech/Pfizer's BNT162b2 and Moderna's mRNA-1273. These vaccines demonstrated approximately 95% efficacy in preventing SARS-CoV-2 infection. They leverage solid lipid nanoparticles (SLNs) as carriers for the mRNA, facilitating its delivery into target cells where the encoded spike protein is synthesized, thereby inducing an immune response.

SLNs play a crucial role in protecting mRNA from degradation, enhancing its stability and delivery efficiency. However, the stability and integrity of these vaccines could be influenced by storage conditions, including temperature, light irradiation and potential interaction with silicon oil from the syringes. Understanding the stability of mRNA vaccines and the efficiency of their delivery systems after exposure to different stressors is crucial for optimizing storage, handling, and administration protocols.

The BNT162b2 mRNA vaccine, developed by BioNTech/Pfizer, has been a critical tool in combating the COVID-19 pandemic. Its effectiveness is partly due to the innovative use of solid lipid nanoparticle (SLN) vectors for mRNA delivery. Previous studies have highlighted the significance of SLNs in ensuring mRNA stability and efficient cellular uptake. However, the stability of these vaccines and the specifics of their intracellular trafficking pathways remain areas requiring further exploration. This study aims to elucidate these aspects, focusing on the behaviour of "stressed" (thermally altered) and “non-stressed” samples of the BNT162b2 vaccine within cellular environments.



Exemplary confocal image of SLNs and vesicles

2. Proposed Experiment

The proposed experiment aims to evaluate the thermal stability of the BNT162b2 mRNA vaccine from BioNTech/Pfizer and its internalization efficiency using adenocarcinomic human alveolar basal epithelial cells (A549) cells as a model. This study will assess the impact of thermal stress on the vaccine nanoparticle vectors and their colocalization with intracellular vesicles, specifically early endosomes and lysosomes, over various timepoints (4h, 6h, 24h, 48h, 72h, 96h).

The core of our experimental design involves a comparative analysis between thermally stressed and non-stressed samples of the BNT162b2 mRNA vaccine. To simulate thermal stress, vaccine samples will undergo a carefully controlled heating process. This process is designed to mimic potential real-world scenarios where the vaccine might be exposed to sub-optimal storage conditions, potentially affecting its stability and efficacy.

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. The thermal stress group will be subjected to a specific temperature regime that exceeds the recommended storage conditions. Based on preliminary data and relevant literature, the stressed samples will be placed in a Stability Test Chamber set at 40°C with 60% relative humidity for a period of 8 days. This condition is chosen to accelerate potential degradation processes, including the breakdown of lipid nanoparticles (LNPs) and mRNA strands, without completely deviating from plausible accidental exposure scenarios.

For the cellular aspect of the study, A549 cells, human lung adenocarcinoma epithelial cells, will be utilized due to their relevance in respiratory virus research and their ample cytoplasm, facilitating the observation of internalization and vesicle interaction. These 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) diluted to a concentration of 2.5 µg/ml. Additionally, we will be marking the nucleus with DAPI and two types of vesicles (early endosomes and lysosomes) with AlexaFluor 488.
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 quantify the interaction between the BNT162b2 vaccine particles and intracellular vesicles, we will employ colocalization analysis. Following the acquisition of confocal images at the designated time points we will analyze these images to measure the colocalization between the DiD-labelled SLNs and the Alexa Fluor 488-labelled vesicles. We will acquire at least ten images at 40X magnification for each timepoint.

This analysis will be facilitated by image analysis software capable of computing Pearson's correlation coefficient and Manders' overlap coefficients. These statistical measures will provide a quantitative assessment of the extent to which vaccine particles co-localize with early endosomes and lysosomes within the cell, offering insights into the internalization,