

# GTR Interdisciplinary Research Report

## Spanish National Cancer Research Centre (CNIO)

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**Affiliation:** Laboratory of Molecular Biotechnology, Graduate School of Bioagricultural Sciences    **Year:** D1

**Destination:** Spanish National Cancer Research Centre (CNIO)

**Period:** December 2022 – May 2023

**Purpose:** Expression and purification of anti-GPR54 antibody and protein crystallography training

### Summary:

Using the Ecobody technology that our laboratory invented (Ojima-Kato et al., *Scientific Reports*, 2017), I successfully screened some clones of monoclonal antibody against GPR54 receptor protein. For protein characterization, a large amount of protein of both antigen and antibody is required. Besides, for further maximizing the use of anti-GPR54 antibody, I planned to use it for crystallizing the GPR54 protein which until now has not been resolved. Since protein crystallization is not my background, I decided to conduct interdisciplinary research with Prof. Inès Muñoz from Spanish National Cancer Research Centre (CNIO). Thus, from December 2022 to May 2023, I went to Spanish National Cancer Research Centre (CNIO) to learn and conduct protein expression and crystallography.



### Impression:

#### Research:

Here, I learned to do crystallization using a crystallization robot called Mosquito. For the primary screening for crystallization condition, there are more than 100 buffer conditions that need to be tested. Since dispensing the buffer and protein with more than 100 conditions takes time, the Mosquito robot can handle all protein and buffer dispensing in 96 well plate specialized for crystallization screening. After getting the best buffer condition from primary screening,

optimization is done to get more crystal by growing in larger volume of protein. The grown crystal is then sent to synchrotron to be diffracted by X-ray. Another techniques that I learn is Small Angle X-ray Scattering (SAXS) where protein does not need to be crystallized and protein solution is beamed using X-ray. It is much easier compared to standard X-ray crystallography; however, it has size limitation. Until this report is written, I have learnt the crystallization technique and helped to crystallize some proteins from other groups in CNIO, but the anti-GPR54 antibody, my project protein, cannot be crystallized due to difficulty of protein purification. Nevertheless, some conditions to do protein purification has been known, thus improvements of protein purification will be done in Japan.

#### Environment:

Spain in general has lower workload compared to Japan. Here, many people do the jobs (including experiment) slowly, not in a rush, since they usually plan the experiment step by step (sometimes only one step of experiment for one day) until reached their target. Thus, there are some days when they come to work, but do not do anything since they don't have things to do on that day. Meanwhile, during lunch, it is common to have a long lunch time (up to two hours) from 2:00 to 4:00 p.m., and then come back to work until 7:00 or 8:00 p.m. Additionally, dinner in Spain usually starts around 9:00 or 10:00 p.m due to geographical reason where sun sets after 10:00 p.m. Spain has 40 days paid holiday a year, thus it is common to see many people will take paid holiday each month. This kind of workstyle makes Spanish people have high life expectancy and do not have stress in most of their life.

There are many goods Spanish cuisine that I tried when I was in Spain. For example, Paella, Bocadillo, Pollo Asado, etc. It is well known that Spain has the best cuisine in Europe due to their geographical location which has more sun and warmer temperature.

Due to Schengen Agreement, it is possible to go to some of European countries without border restrictions. Flights between Schengen countries is treated as domestic flight and there is no border check in the land border. During my stay in Spain, I went to Portugal and Andorra by Car as well as visited my friend who is now studying in Netherlands.

#### Acknowledgement:

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