Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: **CA17103**

Grantee name: **José García Coll**

Details of the STSM

Title: Dynamic Covalent Polymers for mRNA complexation

Start and end date: 19/09/2022 to 30/09/2022

Description of the work carried out during the STSM

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section.

In a recent work we have shown the potential of glycosylated dynamic covalent polymers (DCPs) for the delivery of siRNA onto live cells (Ulrich, S. et al., *Angew. Chem. Int. Ed.* **2021**, 60, 5783). Interestingly we evidenced that these vectors are self-assembled in a siRNA-templated manner. After these promising results, we have developed a new generation of DCPs which have shown an improvement for siRNA complexation. This new generation of DCPs is yet to be tested for siRNA transfection in live cells to prove that the better complexation improves cell transfection.

As agreed with the host institution, the *in situ* formation of polyacylhydrazone dynamic covalent polymers (DCPs) by mRNA template effect leading to polyplexes was tested. In that context, 12 different combinations of complementary monomers were studied.

The complexation of mRNA was tested by gel electrophoresis at different N/P ratios. As request when manipulating mRNA, short incubation times were tested. Once the polyplex formation was confirmed, EGPF-mRNA transfection in HCT-116 cells was assessed using the optimal N/P ratio found. The transfection efficacy and cell viability were measured by flow cytometry. Additional cell viability data was estimated by MTT assay.

During the STSM I was able to meet the host institution researchers and learn about their research topics. In addition, I attended a symposium on catalysis and gene delivery by Joost Reek (Invited professor).

[1] This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

Description of the STSM main achievements and planned follow-up activities

Description and assessment of whether the STSM achieved its planned goals and expected outcomes, including specific contribution to Action objective and deliverables, or publications resulting from the STSM. Agreed plans for future follow-up collaborations shall also be described in this section.

On one hand, we confirmed the capability of our polyacylhdyrazones polymers to be self-assembled *in situ* by mRNA template effect leading to mRNA complexation. The different DCPs tested showed an improved ability to complex mRNA in comparison to siRNA, forming all the different polyplexes at lower N/P ratios and way shorter incubation times (30 minutes instead of 16 hours) showing that mRNA acts as a better template than siRNA for DCPs formation.

On the other hand, no efficient transfection of mRNA was detected yet. Different conditions were tested, mostly changing the incubation time of transfection, however no significant change was observed for the different polyplexes. The only significant change was an increase of transfection efficacy for the positive control assessed with lipofectamine. No important cytotoxicity was observed either. Further studies regarding the physicochemical analysis will now be performed by DLS and ζ-potential in order to better understand the transfection problems.

Personally, during the STSM period I learned the basics on mRNA manipulation, gel electrophoresis for mRNA complexation and I was also able to initiate myself in cell transfection, achieving autonomy in complexation assays by gel electrophoresis and assessing the cell transfection under the supervision of the host's technician.

Even if transfection remained not possible, positive results were obtained regarding mRNA complexation with improved results comparing siRNA complexation. We consider overall that it has been a fruitful STSM, with positive results that have opened us a lead into the studies of versatile DCPs allowing the complexation of different lengths of RNA.

Currently, we are working with the same generation of DCPs for siRNA delivery. The ongoing studies will allow us to better understand the potential of our compounds as delivery vectors for gene delivery.

Both institutions will keep in touch for future collaborations in the field of mRNA delivery.