BIOLOGY SECTION

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Titulado Superior (Advanced Degree)
**Plan de Empleo Joven* (Youth Employment Plan)

 # Unfortunately, María was killed in a road accident. May she rest in peace.

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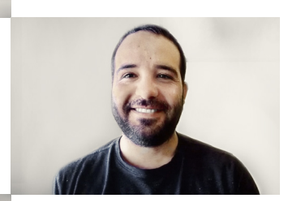



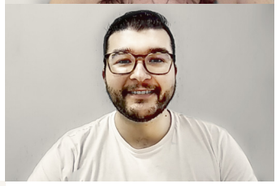

OVERVIEW

Target Engagement assays measure compound binding to a selected target protein inside living cells and enable making correlations with biochemical activity. BRET or bioluminescence resonance energy transfer can be used to study cellular target engagement. NanoBRET assays are based on the fact that NanoLuc luciferase fusion proteins expressed in cells can transfer energy to a proximal fluorophore called tracer. Compound affinity to a given protein can be measured by competitive displacement of the tracer reversibly bound to the corresponding NanoLuc luciferase fusion protein in cells. In the case of PROTACs (Proteolysis Targeting Chimeras), target engagement assays are essential to evaluate the affinity of PROTACs to both the target protein and the E3 ligase, as binding to both is required for the formation of the ternary complex, which triggers the degradation of the target protein. We apply BRET assays routinely in our projects to help in the optimisation of our molecules.

"We successfully established a cellular target engagement assay, based on BRET technology, in our MASTL project; this assay enables us to measure the affinity of compounds for their proteins of interest in cells, as well as to establish correlations with their biochemical activities."

RESEARCH HIGHLIGHTS

During 2020, our Section was involved in several projects:

Cyclin-dependent kinase 8 (CDK8)

Preliminary toxicity studies in rats with our lead compound showed a preclinical toxicity compatible with its potential drug development. Next we want to validate its immunotherapeutic potential in *in vitro* models that evaluate the activation of NK cells.

Microtubule-associated serine/threonine protein kinase-like (MASTL)

This project is undertaken in collaboration with the CNIO Cell Division and Cancer Group. We tested in our biochemical assay using active human full-length MASTL protein around 150 new compounds, both MASTL-i and MASTL PROTAC-like molecules. We measured MASTL engagement in cells (BRET assay) for the most potent biochemical inhibitors and PROTACs molecules. In the case of PROTACs, we also evaluated their cell affinity against the E3 ligases selected in their design. In addition, we started to characterise the ADME-T properties of our more potent MASTL-is, identifying soluble, permeable, and metabolically stable molecules for further characterisation in *in vivo* pharmacokinetic studies.

Telomeric repeat binding factor 1 (TRF1)

This project is carried out in collaboration with the CNIO Telomeres and Telomerase Group. We continued our efforts to decipher the molecular target/mechanism of action (MoA) of our series 2 of TRF1 inhibitors, represented by hit ETP-946. The results of RNAseq experiments with ETP-946 retrieved a potential MoA of ETP-946. After initial validation experiments with orthogonal assays, we are now carrying out an in-depth characterisation that will be reported accordingly. We are also currently working to identify disruptors of TRF1 binding to ds telomeric DNA. In this direction, wet screening has been performed with the hits identified after virtual screening activities. Now, we are validating these hits applying orthogonal assays against TRF1 and the TelDNA probe with freshly prepared and/or resynthesized samples. We anticipate new screening campaigns using a selected library of ETP compounds that bear a privileged structure to disrupt protein-DNA complexes. Finally, it is worth mentioning that we implemented modifications in our initial TRF1 dimerization assay to avoid interferences with the detection system, which will allow the reduction of the false positive rate in future screening campaigns.

SET domain containing lysine methyltransferase 8 (SETD8)

This project was recently internalised and incorporated into the ETP pipeline, and will be carried out in collaboration with the CNIO Genomic Instability Group. Our main objective is to generate and optimise novel SET8 inhibitors as new therapeutic agents. We set up a biochemical assay that has been validated with previously reported SETD8 inhibitors. Next, we will perform screening campaigns with several ETP-libraries in order to identify new chemotypes that inhibit SETD8, for the development and design of novel SETD8-i with intellectual property.

Collaborations with other CNIO Groups

ETP-Biology provided ongoing support in screening activities performed by the Brain Metastasis Group. We also supported *in vivo* studies of selected compounds and drugs, such as pharmacokinetics, distribution and/or antitumour efficacy, performed by the Microenvironment and Metastasis and the Metabolism and Cell Signalling Groups. Furthermore, we provided analogues of the hits identified in a screening campaign for the validation of PrimPol inhibitors, in collaboration with the DNA Replication Group. Finally, we collaborated with the Experimental Oncology Group, validating the hits obtained after a screen with the ETP-antitumour library to identify novel treatments of mutant KRas NSCLC mouse cell lines that regrow after knocking down mutant KRas.

Collaborations with other institutions

Target X. ETP-Biology performed pharmacokinetic studies of more advanced inhibitors developed against target X in a previous collaboration with *Vlaams Instituut voor Biotechnologie VIB* (the Flanders Institute for Biotechnology). ■

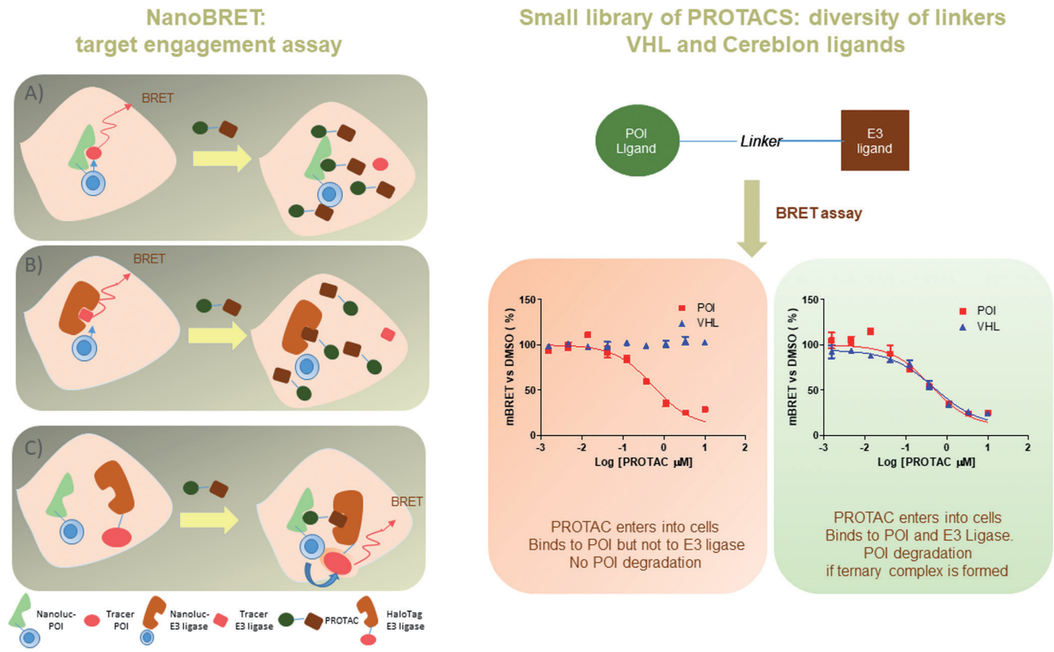

FIGURE Target engagement assays based on BRET technology (Promega). Nanoluc POI or E3 ligase excite a tracer that emits BRET; competition with the tracer with PROTACs will decrease BRET signal. Ternary complex formation is measured by BRET emission brought about by the proximity of Nanoluc POI and Halotag E3 ligase mediated by the PROTAC molecule. (**A**) BRET binding to POI. (**B**) BRET binding to E3 ligase. (**C**) BRET mediated by formation of the ternary complex. These 3 assays can be used to characterise a library of PROTACs to identify those that bind to POI and E3 ligase and select them for ternary complex formation.

PUBLICATIONS

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