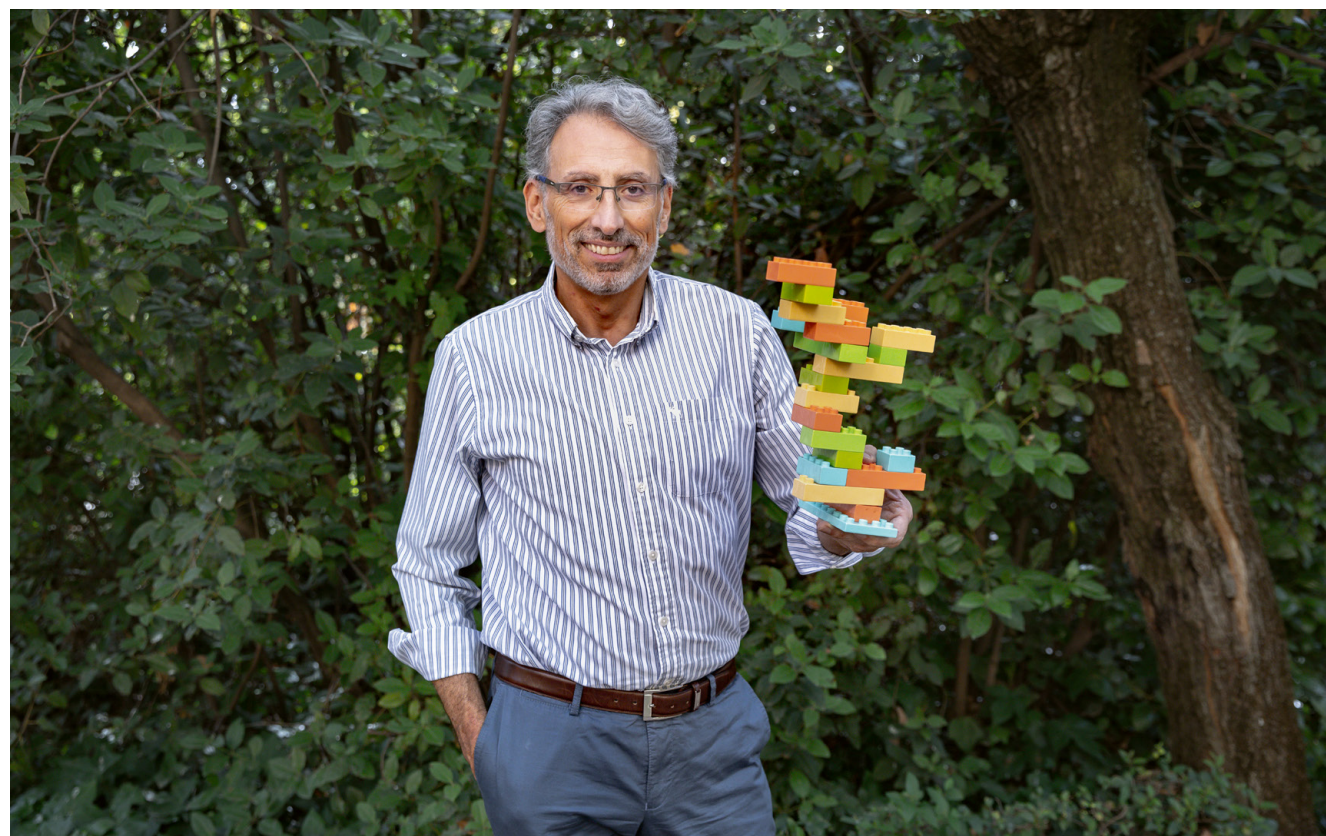


PROTEIN PRODUCTION UNIT

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OVERVIEW

The biological functions of thousands of proteins, especially those involved in cancer development, remain unexplored, and understanding their structures, tissue and cellular distributions and functions is critical for biomedical progress. However, researchers often face the challenge of insufficient supply, since proteins of interest identified in a particular process can be very difficult to produce in high quality and in adequate amounts for analysis, causing a bottleneck in how quickly they can be characterised. The Protein Production Unit is a core service lab that aims to address this bottleneck by offering expertise and state-of-the-art technologies to develop effective processes for producing recombinant proteins. These can be used in diverse downstream applications such as the generation of highly-specific antibodies, biophysical, biochemical or functional analyses, or structural studies, with

“The Protein Production Unit provided many high quality recombinant proteins that were essential for drug discovery projects and for cancer diagnosis through the development of specific antibodies.”

the final goal of accelerating cutting-edge cancer research for CNIO and external research groups.

RESEARCH HIGHLIGHTS

The Protein Production Unit was created at the beginning of 2022, with the aim of providing high-quality recombinant proteins to meet the needs of CNIO Research Groups and external collaborators. During the year, the laboratory was refurbished with state-of-the-art technologies for heterologous recombinant protein expression and purification, to implement efficient production protocols for each particular protein. With its portfolio of services ranging from cDNA cloning in expression vectors to purification in milligrams of purified protein, the Unit contributed to the research projects of several CNIO Groups. It is worth mentioning the elucidation of the structure of the RAF1-HSP90-CDC37 complex, in collaboration with the Experimental Oncology Group; the production of active MIDKINE protein for functional assays and the generation of specific monoclonal antibodies for the Melanoma Group; and the production of exosome-secreted micropeptides to develop antibody-based detection tools in collaboration with the Microenvironment and Metastasis Group; among others. In addition, we worked closely with the Monoclonal Antibodies Unit, providing purified proteins to generate highly-specific monoclonal antibodies, such as CSF3R, IL4I1, TACI and PILRA; and with the Biology Section of the

Experimental Therapeutics Programme, producing active full-length human MASTL for functional assays. Other tool proteins for in-house use (i.e. sortases and AG-MNase) were also produced in the Unit.

Apart from providing such services, the Unit carries out research activities focused on the development of specific recombinant antibodies and antibody fragments for diagnostic and therapeutic purposes. In 2022, llama-derived nanobodies against the metalloprotease MT1-MMP were developed and have shown their great potential as radiotracers in PET imaging for the detection of triple-negative breast cancer in mouse models. This project was a joint CNIO-*CIEMAT* collaboration and was supported by a grant from the BBVA Foundation. ■

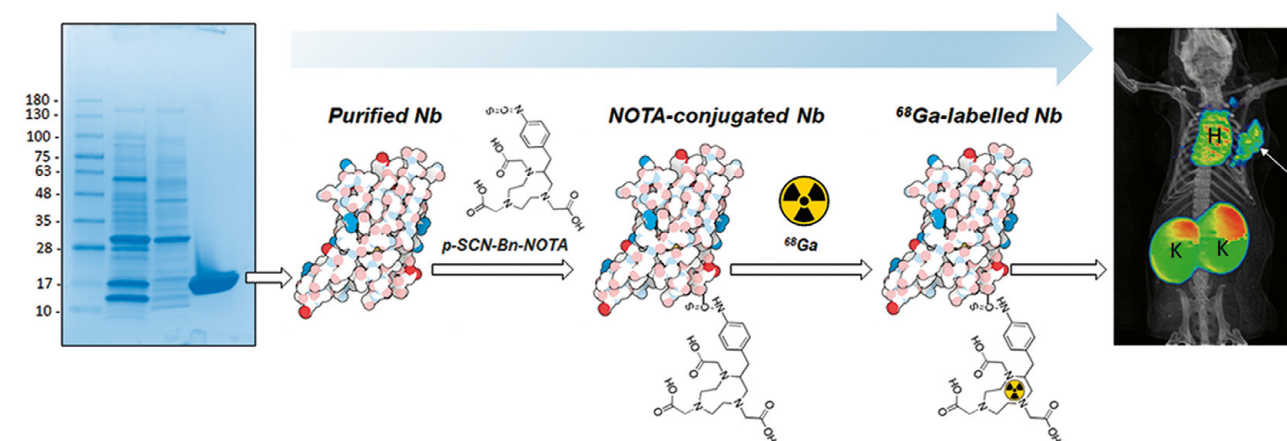


FIGURE 1 Strategy to obtain nanobody-based radiotracers for tumour detection by PET imaging. After nanobody expression and

purification, conjugation with NOTA, and labelling with ^{68}Ga , the biodistribution of the ^{68}Ga -labelled nanobodies was monitored by PET.

Arrow indicates tumour position, K: kidney, H: heart.

PUBLICATIONS

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