FACULTY OF MEDICINE AND HEALTH SCIENCES

Nanobody derivatization for molecular study and perturbation of cancer cell invadopodia

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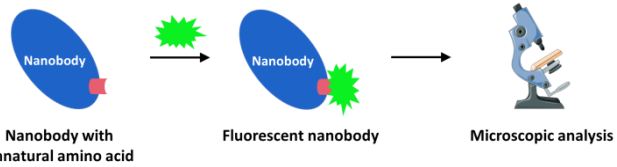
Camelids produce a special type of antibodies lacking a light chain which is always present in conventional antibodies e.g. human antibodies. When taken only the antigen binding domain, it is called a nanobody. Nanobodies are robust, small, easily produced, easily modified, high selective, high specific and applicable as research tool as well as for diagnostic and therapeutic purposes. In this work, nanobodies are used for two goals: to unravel the metastasis and to obtain a useful tool for microscopy.

Initially, nanobodies were used in this study to analyse the influence of the protein N-WASp (neural Wiskott-Aldrich syndrome protein) on invadopodia. Invadopodia are cell structures which act as the "feet" of the cell enabling the spreading throughout the body. They are known to be enhancing the metastasis, which is a lethal process for many cancer patients and which is still difficult to prevent since the working mechanism is not entirely clear. In this study, nanobodies are generated against N-WASp and we were able to conclude that our N-WASp nanobodies reduced the invadopodium formation meaning N-WASp is an important protein during the formation of these invadopodia.

Secondly, nanobodies are modified in two different ways. On the one hand an unnatural amino acid is coupled to the nanobody through an enzymatic manner. On the other hand, an unnatural amino acid (a para-azidophenylalanine) is incorporated into the nanobody during the recombinant nanobody expression. Once such unnatural amino acid is present in the nanobody, a fluorescent molecule is linked to that amino acid via a chemical process. Once the nanobodies are fluorescent, they can be used to stain and visualise specific proteins and structures. In this study, nanobodies are used to visualise cortactin, an invadopodium protein, and β-catenin, an cell-cell interaction protein.



Since proteins (and so also nanobodies) are not able to break through cell membrane, the nanobodies cannot visualise those intracellular proteins. However, a recently developed technique, called photoporation is used to enable this. This technique uses gold or graphene nanoparticles in combination with laser light to introduce small pores in the cell membrane. This allows the entrance of small proteins such as nanobodies. This study succeed to specifically image proteins, in fixed (death) and living cells. The combination of fluorescent labelled nanobodies with the photoporation technique enable the use in further fundamental research and even to study short and long term processes and pathways.

In summary, in this study the nanobody technology was used on the one hand to analyse the role of N-WASp in invadopodium formation and on the other hand to create a tool to allow better imaging of cell structures. This work shows the possibilities of using the nanobodies in different ways to support and enhance fundamental cancer research.

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Publications

Hebbrecht T., *et al.* (2017). VCA nanobodies target N-WASp to reduce invadopodium formation and functioning. PLOS ONE.

Hebbrecht T., *et al.* (2020). Nanobody click chemistry for convenient site specific fluorescent labelling, single step immunocytochemistry and delivery into living cells by photoporation and live cell imaging. NEW BIOTECHNOLOGY.

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A full version of this thesis is available at: https://lib.ugent.be/

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