

The Research Group  
**Cellular and Molecular Immunology**

has the honor to invite you to the public defense of the PhD thesis of

## Maxine Crauwels

to obtain the degree of Doctor of Bioengineering Sciences

Title of the PhD thesis:

**Effect of different characteristics of radiometal labelled Nanobodies on the kidney retention.**

Promotors:

**Em. prof. dr. Serge Muyldermans**  
**Prof. dr. ir. Jo Van Ginderachter**  
**Prof. dr. Catarina Xavier**  
**Prof. dr. Nick Devoogdt**

The defense will take place on

**Monday, October 26, 2020 at 15h00**

The defense can be followed through a live stream. Contact [maxine.crauwels@vub.be](mailto:maxine.crauwels@vub.be) for more information

### Members of the jury

Prof. dr. Steven Ballet (VUB, chair)  
Prof. dr. Henri De Greve (VUB, secretary)  
Prof. dr. ir. Tamara Vanhaecke (VUB)  
Prof. dr. Sabrina Santos Oliveira (Utrecht University)  
Prof. dr. Marion Hendriks-de Jong (Erasmus MC, NL)

### Curriculum vitae

Maxine Crauwels (1992) obtained her bachelor and master in Bio-Engineering Sciences, at the Vrije Universiteit Brussel. Her PhD study was supported by an FWO project grant aiming to investigate the kidney retention of radionuclide labelled Nanobodies. Various methods to change the pI, charge density and the Protein A binding site of Nanobodies were developed. This resulted in a Nanobody purification strategy in absence of affinity tags at the C-terminal of Nanobodies.

### Abstract of the PhD research

Nanobodies (Nbs) are the smallest intact antigen-binding fragments derived from heavy-chain only antibodies naturally occurring in Camelidae. Their outstanding characteristics including a small size (MW < 15,000), high stability, high antigen-specificity and low immunogenicity, make them a preferred tool as targeting agent. Coupled to a radioactive nuclide, they can be used in nuclear medicine for diagnostic and/or therapeutic purposes. The excess of radio-labelled Nbs are eliminated from blood circulation via the kidneys. However, this can cause disastrous nephrotoxicity. We aimed to identify the characteristics of Nbs that might lead to reduced kidney retention. Earlier observations indicated that different Nbs carrying the same radionuclide seemed to be retained differentially in kidneys. Hence, we examined *in silico* various Nb properties that might be implicated in this differential kidney retention. A trend was observed between charges and kidney retention. To confirm this observation, we introduced extra charged amino acids at the C-terminal end of the Nb, whereby an increase in kidney retention was observed when either positively or negatively charged amino acids were introduced. Furthermore, the overall charge, or pI, of Nbs was examined, but no significant difference could be identified. We also focused on the possible effects of amino acid sequence imprints of the VHH scaffold located on the solvent exposed sides of the  $\beta$ -sheets of the immunoglobulin fold, i.e. the staphylococcal Protein A (SpA) binding site and the framework region-2 VHH hallmark imprint, on the kidney retention. Although no clear Nb scaffold sequence imprint responsible for kidney retention could be identified, our site directed mutagenesis of Nbs revealed a useful affinity chromatography method to purify Nbs lacking C-terminal purification tags (e.g. C-tag or His-tag) by grafting an SpA binding site into the Nb. The presence or absence of this SpA binding site did not affect the kidney retention of labelled Nbs.