

A site-specific radiolabelling strategy of Nanobodies for PET imaging

Bridoux, Jessica; Broos, Katrijn; Crauwels, Maxine; Lecocq, Quentin; Martin, Charlotte; Cleeren, Frederik; Bormans, G.; Ballet, Steven; Caveliers, Vicky; Raes, Geert; Breckpot, Karine; Muyldermans, Serge; Devoogdt, Nick; Keyaerts, Marleen; Xavier, Catarina

Publication date:
2018

[Link to publication](#)

Citation for published version (APA):

Bridoux, J., Broos, K., Crauwels, M., Lecocq, Q., Martin, C., Cleeren, F., Bormans, G., Ballet, S., Caveliers, V., Raes, G., Breckpot, K., Muyldermans, S., Devoogdt, N., Keyaerts, M., & Xavier, C. (2018). *A site-specific radiolabelling strategy of Nanobodies for PET imaging*. Poster session presented at Belgian Peptide Group Meeting 2018, Brussels, Belgium.

Copyright

No part of this publication may be reproduced or transmitted in any form, without the prior written permission of the author(s) or other rights holders to whom publication rights have been transferred, unless permitted by a license attached to the publication (a Creative Commons license or other), or unless exceptions to copyright law apply.

Take down policy

If you believe that this document infringes your copyright or other rights, please contact openaccess@vub.be, with details of the nature of the infringement. We will investigate the claim and if justified, we will take the appropriate steps.

A site-specific radiolabelling strategy of Nanobodies for PET imaging

J Bridoux¹, K Broos², M Crauwels^{1,3}, Q Lecocq², C Martin⁶, F Cleeren⁷, G Bormans⁷, S Ballet⁶, V Caveliers⁴, G Raes⁵, K Breckpot², S Muyldermans³, N Devoogdt¹, M Keyaerts⁴, C Xavier¹

¹In Vivo Cellular and Molecular Imaging (ICMI), Vrije Universiteit Brussels (VUB), Belgium

²Laboratory of Molecular and Cellular Therapy (LMCT), VUB

³Cellular and Molecular Immunology (CMIM), VUB

⁴Nuclear Medicine Department, UZ Brussel, Belgium

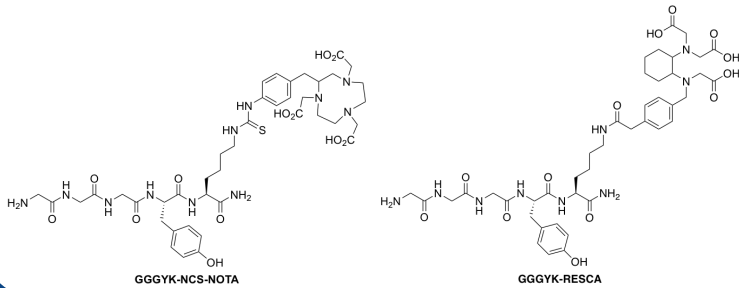
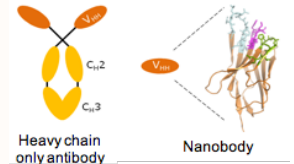
⁵Myeloid Cell Immunology Lab, VIB inflammation Brussel (VUB), VIB Inflammation Research Center, Ghent, Belgium

⁶Department of Organic Chemistry, VUB

⁷Radiopharmaceutical Research, Department of Pharmacy and Pharmacology, University of Leuven, Leuven, Belgium



Introduction: Nanobodies, or single-domain antibody fragments (sdAbs), are antigen-binding fragments derived from *Camelid* heavy-chain-only antibodies (VHH). Their **high affinity** and **specificity**, as well as their **fast clearance** kinetics make them excellent probes for **PET imaging**.

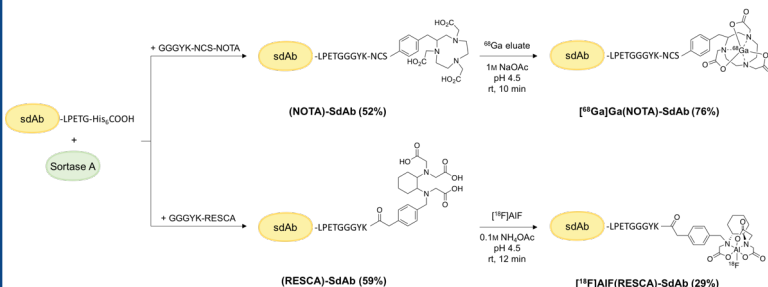
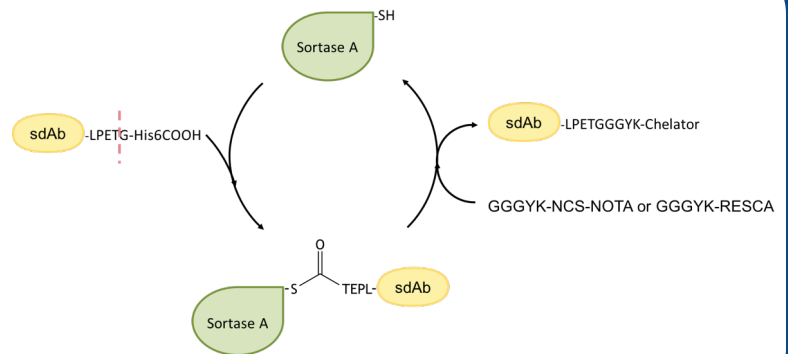


Peptide-based **bifunctional chelators** (BFCs) (GGGYK-NCS-NOTA and GGGYK-RESCA) were used as nucleophilic probes in the Sortase A mediated transpeptidation reaction, allowing **site-specific radiolabelling** of the sdAb with ⁶⁸Ga or Al-¹⁸F respectively.

Methods: The sdAb was engineered to have the recognition motif of the **Sortase A enzyme**, allowing site-specific coupling of the BFCs at its C-terminal.

Characterisation was performed through **Mass Spectrometry** (ESI-Q-TOF), **SDS-PAGE** and **Western Blot**.

NOTA-sdAb was radiolabelled with ⁶⁸Ga and RESCA-sdAb with Al-¹⁸F. Radiochemical purity (RCP) and stability were assayed using **SEC** and **iTLC**.



Results: Site-specific functionalised sdAb with NOTA or RESCA was obtained with **high purity** ($\geq 99\%$) in 52% and 59% yields respectively.

Radiolabelling of NOTA-sdAb with ⁶⁸Ga was performed in a **76%** decay-corrected radiochemical yield (DC-RCY), $\geq 99\%$ RCP with apparent molar specific activity of **63 GBq/ μ mol**. The radiolabelled probe was **stable in vitro**.

Radiolabelling of RESCA-sdAb with Al-¹⁸F was performed in a **29%** DC-RCY and with a RCP $\geq 99\%$.

Conclusion: The Sortase A enzyme coupling allowed to obtain a **site-specifically labelled** probe for ⁶⁸Ga or Al-¹⁸F radiolabelling using NOTA or RESCA chelators. The next step is comparison of *in vivo* stability and *in vivo* tumor targeting studies of both radiolabeled probes, and to select the most suitable probe for **clinical translation**.



This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement N° 675417. Crauwels M. is funded by FWO (G066615N). Broos K. is funded by the Agency of Innovation by Science and Technology. Lecocq Q. is funded by the FWO-SB grant (1S24218N). Keyaerts M is a senior clinical investigator of FWO.