https://doi.org/10.17952/37EPS.2024.P2238

Nanobody CDR3 loop peptidomimetic improves binding on living *Acinetobacter baumannii*

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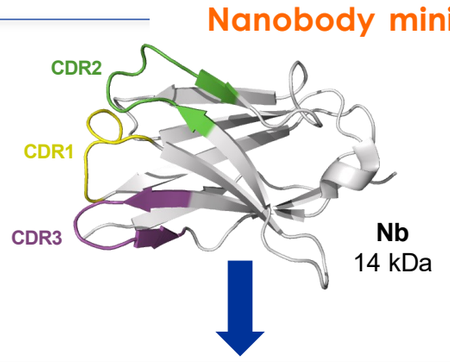
INTRODUCTION

Acinetobacter baumannii represent one of the **most persistent, highly virulent and multidrug-resistant bacterial species** that threaten human life. These are Gram-negative capsulated and nonflagellated bacteria producing multiple types of lipooligosaccharides which **widely vary between clinical isolates** due to its highly dynamic genome.

Nanobodies (ca. 15 kDa) are interesting, downsized variants of monoclonal antibodies (ca. 150 kDa) displaying remarkable specificity and high affinity binding capabilities. Nevertheless, only a ***limited number* of nanobodies able to bind or neutralize bacteria have been reported to date.** **Recently, we discovered a nanobody able to bind *Acetinobacter baumannii* bacterial cells**, but only upon formaldehyde fixation. To overcome this fixation step, **peptides** (ca. 1-2 kDa) representing the nanobody complementarity-determining regions (CDR) were considered as **downsized alternatives enabling increased membrane access**.[1,2]

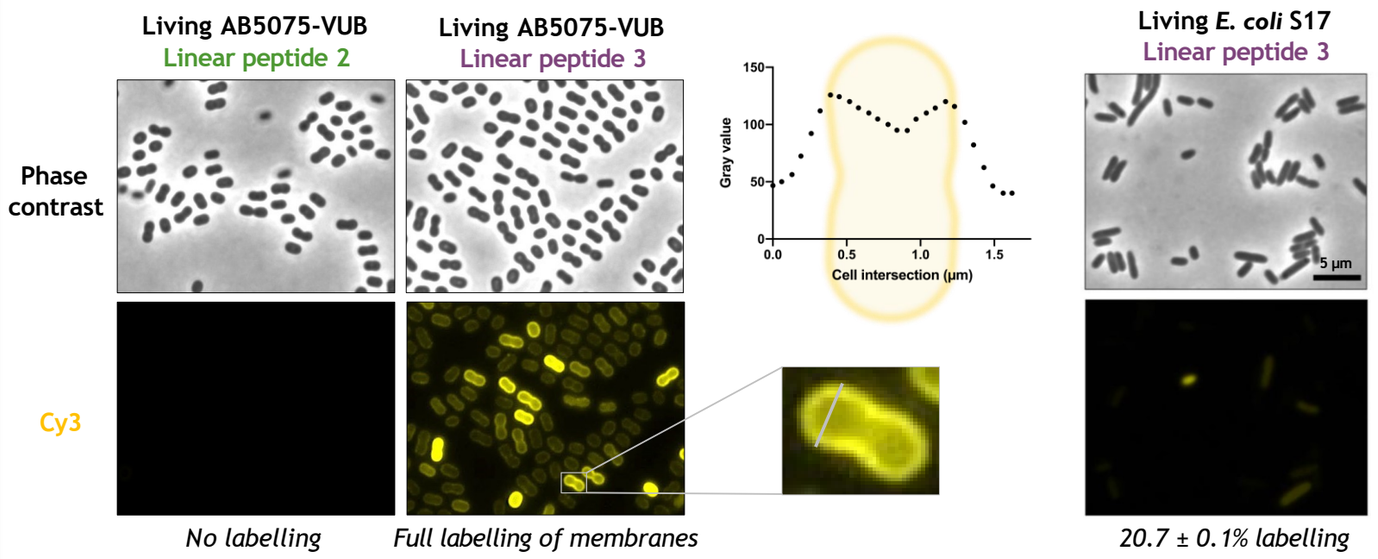
Nanobody miniaturization through its CDR loops



- Target not identified: no structural data available
- **Nb CDR definitions: classical Ab numbering schemes insufficient**
- Structural comparison to overlapping experimental structures (extended IMGT CDR definitions)

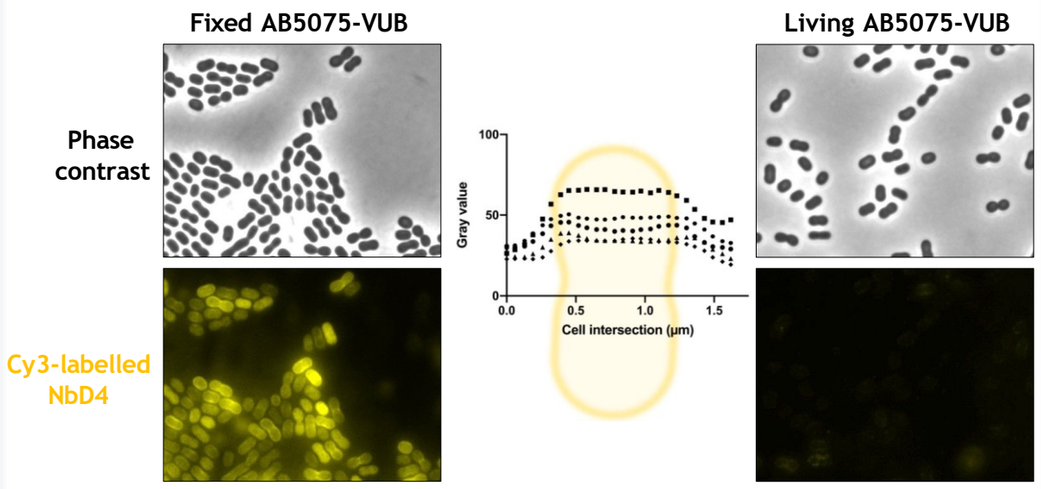
Code	Nb CDR	Sequence
1	CDR1	SulfoCy3-β-Ala-Gly-Ile-Ser-Lys-Ser-Ile-Thr-Ile-NH_2
2	CDR2	SulfoCy3-β-Ala-Thr-Ile-Thr-Ser-Gly-Gly-Thr-Thr-Asn-NH_2
3	CDR3	SulfoCy3-β-Ala-Asn-Ala-Arg-Arg-Leu-Arg-Glu-Tyr-Trp-NH_2

- SPPS of linear peptides
- In solution labelling using NHS ester of SulfoCy3



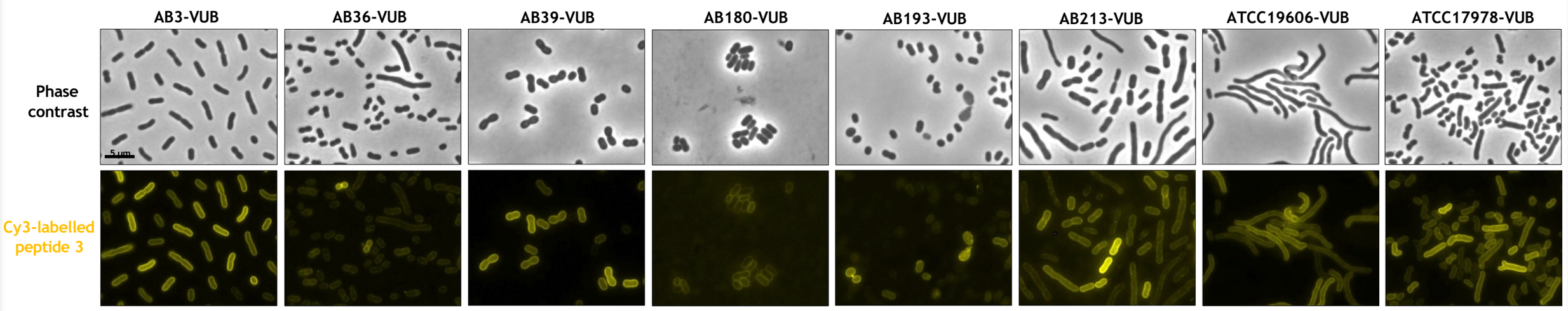
- **CDR3 as minimal required element** for membrane binding
- Peptide size allows **enhanced accessibility** on living capsulated *A. baumannii* bacteria

NbD4 binding properties

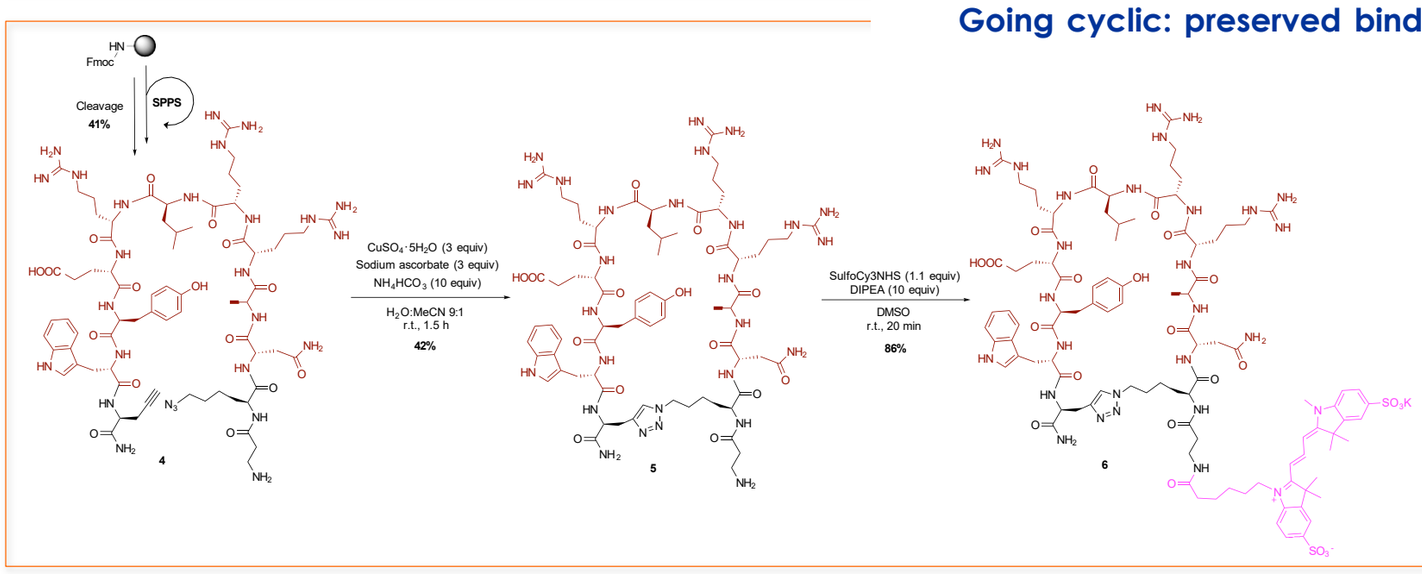


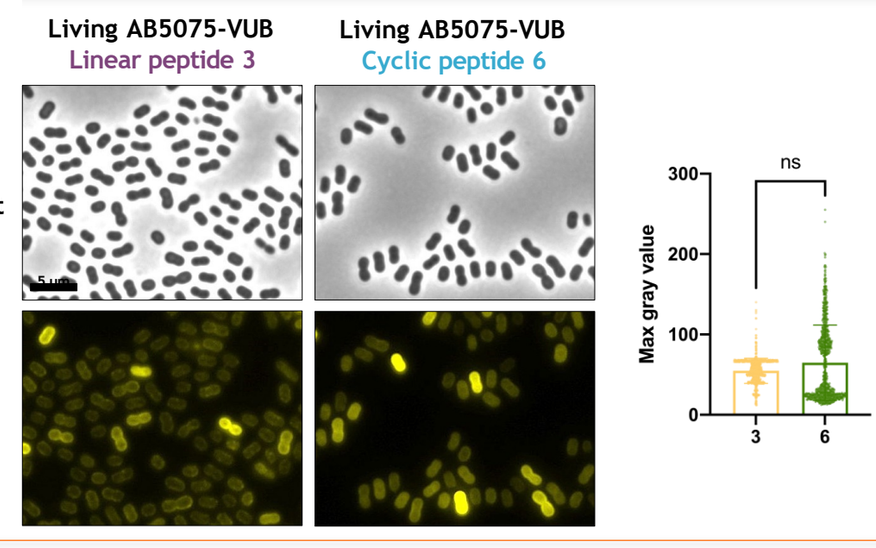
- **Paraformaldehyde alters bacterial surface** facilitating access of NbD4 to target
- Target unknown: inconclusive pull-down and MS experiments

Linear CDR3 peptide binds a variety of A. baumannii isolates



Going cyclic: preserved binding

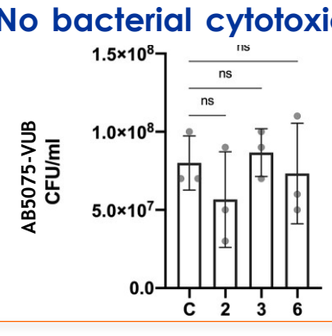




Proteolytic stability

- *In vitro* human plasma assay by HPLC analysis
- Acetylated variants of:
  - Linear peptide **3**: $t_{1/2}$ **36.3 ± 0.5 min**
  - Cyclic peptide **6**: $t_{1/2}$ **55.7 ± 0.3 min**

No bacterial cytotoxicity



CONCLUSION & PERSPECTIVES

- **Necessity of cell fixation for Nb membrane labelling**
- **Downsized CDR3 peptide enables full membrane labelling of unaltered living capsulated *A. baumannii***
- **CDR3 peptide binds variety of living MDR *A. baumannii* isolates, despite high genetic diversity**
- **Peptide cyclization increases proteolytic stability, while preserving labelling potential**
- **Benefit of smaller peptide (1-2 kDa) size vs Nb (14 kDa)**
- **Peptides as targeting tags (visualization, peptide-drug conjugates)**

ACKNOWLEDGEMENTS

K.V.h. and S.B. thank the Research Foundation–Flanders (FWO Vlaanderen) for providing a PhD fellowship to K.V.h. K.V.h., M.M., and S.B. thank the Research Council of the Vrije Universiteit Brussel for financial support through the spearhead (SRP50) program. A.B., C.V.d.H., and H.R. thank the FWO for providing a PhD SB fellowship to A.B. C.V.d.H. acknowledges the Flemish Institute of Biotechnology (VIB) for their support. E.P. and J.S. acknowledge the support and the use of resources of Instruct-ERIC, part of the European Strategy Forum on Research Infrastructures (ESFRI), and the FWO for their support to the nanobody discovery.

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