

# An hRSV-neutralizing prefusion F-specific single domain antibody that binds to a membrane-proximal site on F

Iebe Rossey<sup>1,2,3</sup>, Ching-Lin Hsieh<sup>4</sup>, Koen Sedeyn<sup>1,2,3</sup>, Marlies Ballegeer<sup>1,2,3</sup>, Bert Schepens<sup>1,2,3</sup>, Jason Mclellan<sup>4</sup>, Xavier Saelens<sup>1,2,3</sup>

<sup>1</sup> VIB-Ugent Center for Medical Biotechnology, Technologiepark-Zwijnaarde 75, 9052 Ghent, Belgium

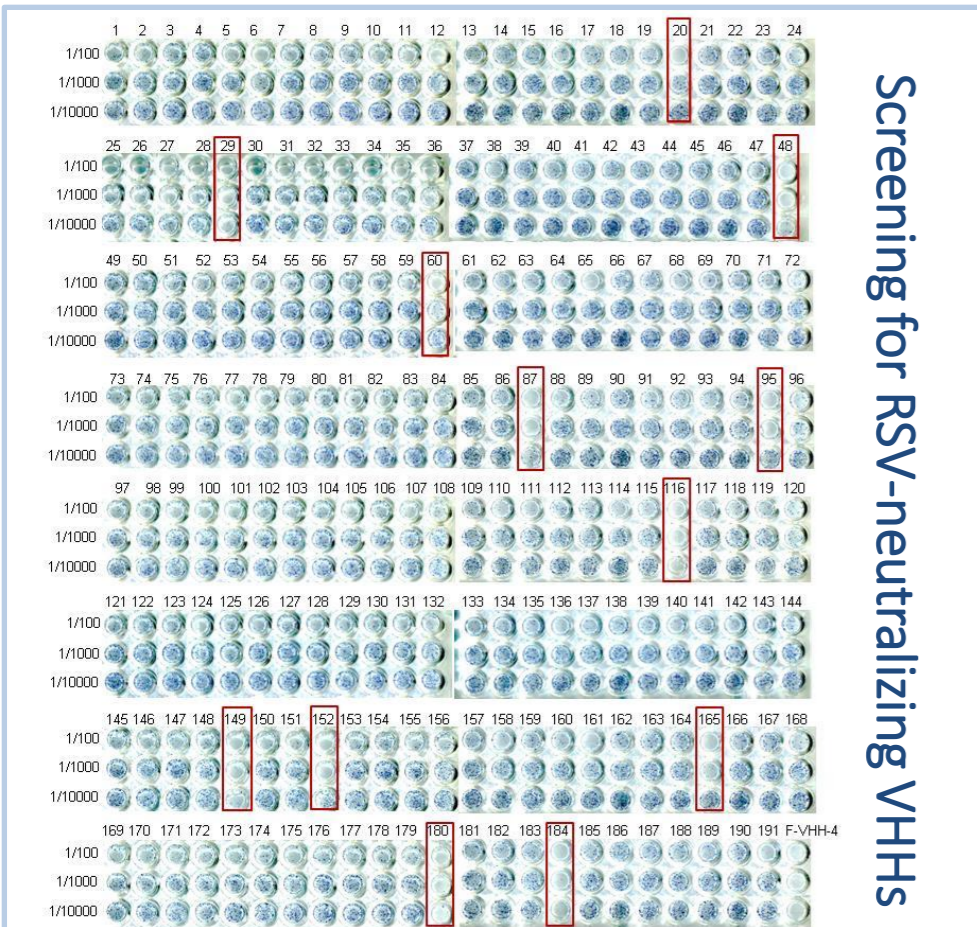
<sup>2</sup> Department of Biomedical Molecular Biology, Ghent University, Technologiepark-Zwijnaarde 71, 9052 Ghent, Belgium

<sup>3</sup> Department of Biochemistry and Microbiology, Ghent University, Technologiepark-Zwijnaarde 75, 9052 Ghent, Belgium

<sup>4</sup> Department of Molecular Biosciences, The University of Texas at Austin, Austin, Texas, USA 78712

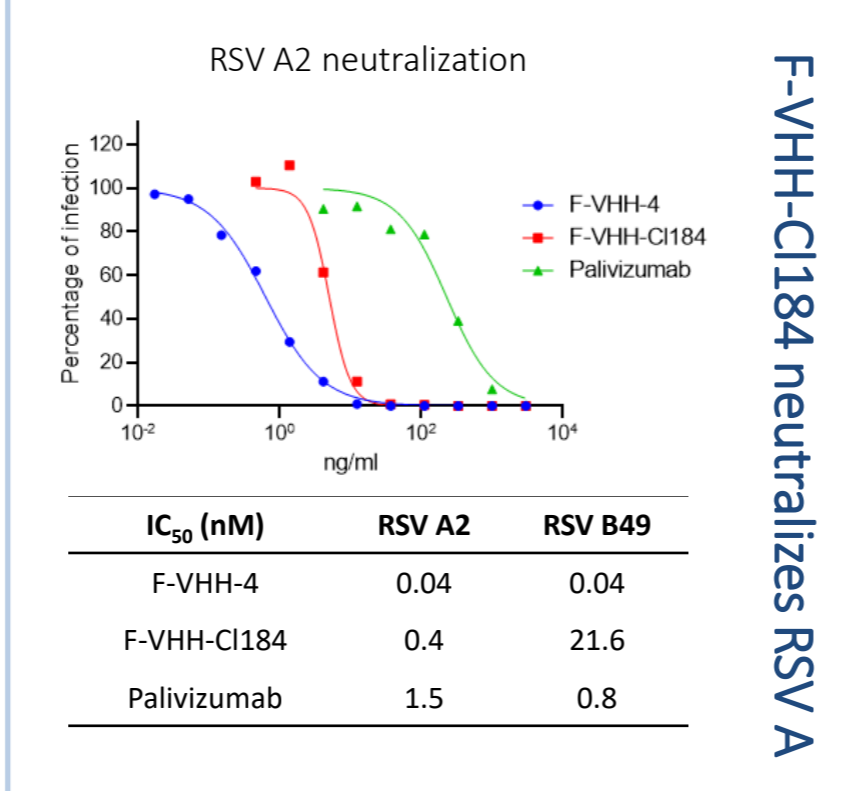
iebe.rossey@vib-ugent.be

HRSV is an important respiratory pathogen, for which still no vaccine nor specific therapy is available. The **fusion protein (F)** is highly conserved and transforms during viral fusion from a metastable prefusion conformation to a postfusion conformation. Previously we identified two RSV-neutralizing prefusion F-specific **single domain antibodies (VHHs)**, F-VHH-4 and F-VHH-L66. In this study, a new screen for RSV-neutralizing VHHs was performed. F-VHH-C184 was selected based on its **high RSV A-neutralizing activity** and its ability to bind RSV prefusion F at a **different site than F-VHH-4**. Crystal structures of the VHH in complex with prefusion F show that it primarily binds to **antigenic site I**, a site which is typically associated with poorly or non-neutralizing antibodies.

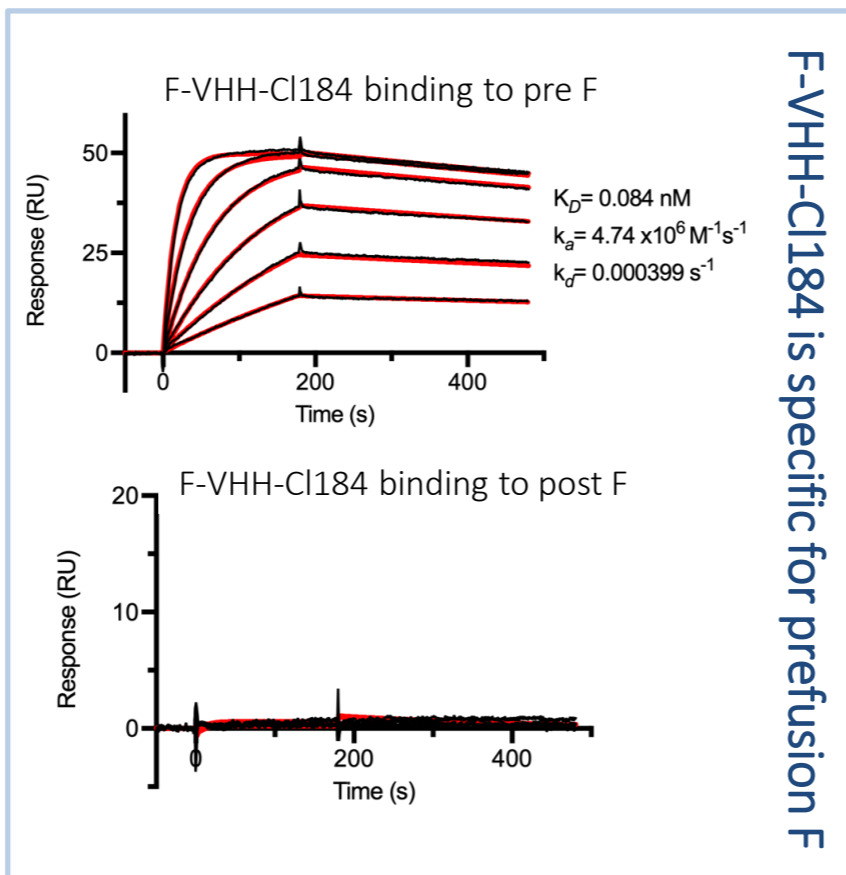


Screening for RSV-neutralizing VHHs

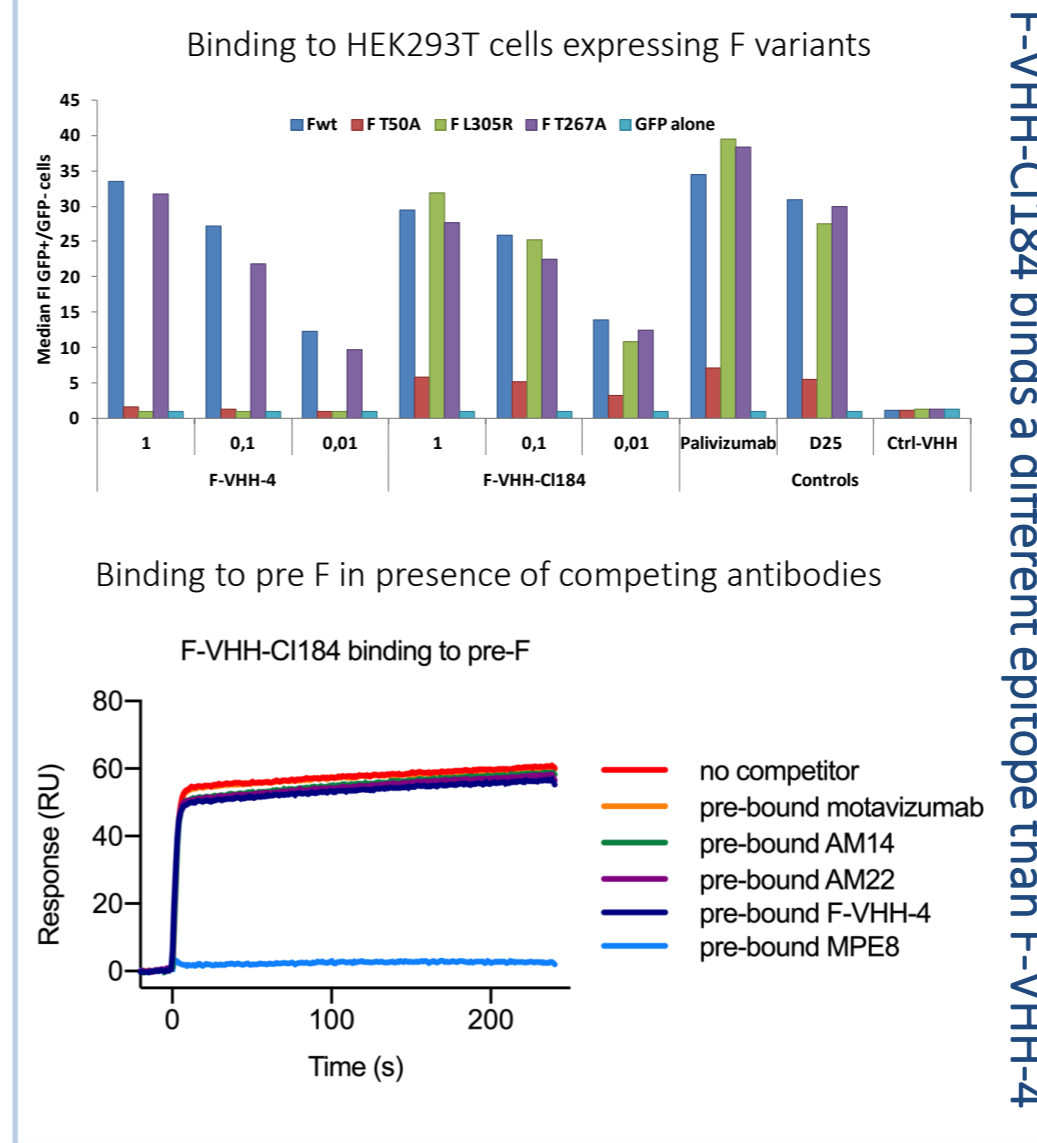
Transform *Pichia pastoris* with VHH-library obtained after two panning rounds on prefusion F in presence of F-VHH-4. Test neutralizing activity in crude supernatans of VHH-producing *P. pastoris*.



F-VHH-C184 neutralizes RSV A



F-VHH-C184 is specific for prefusion F

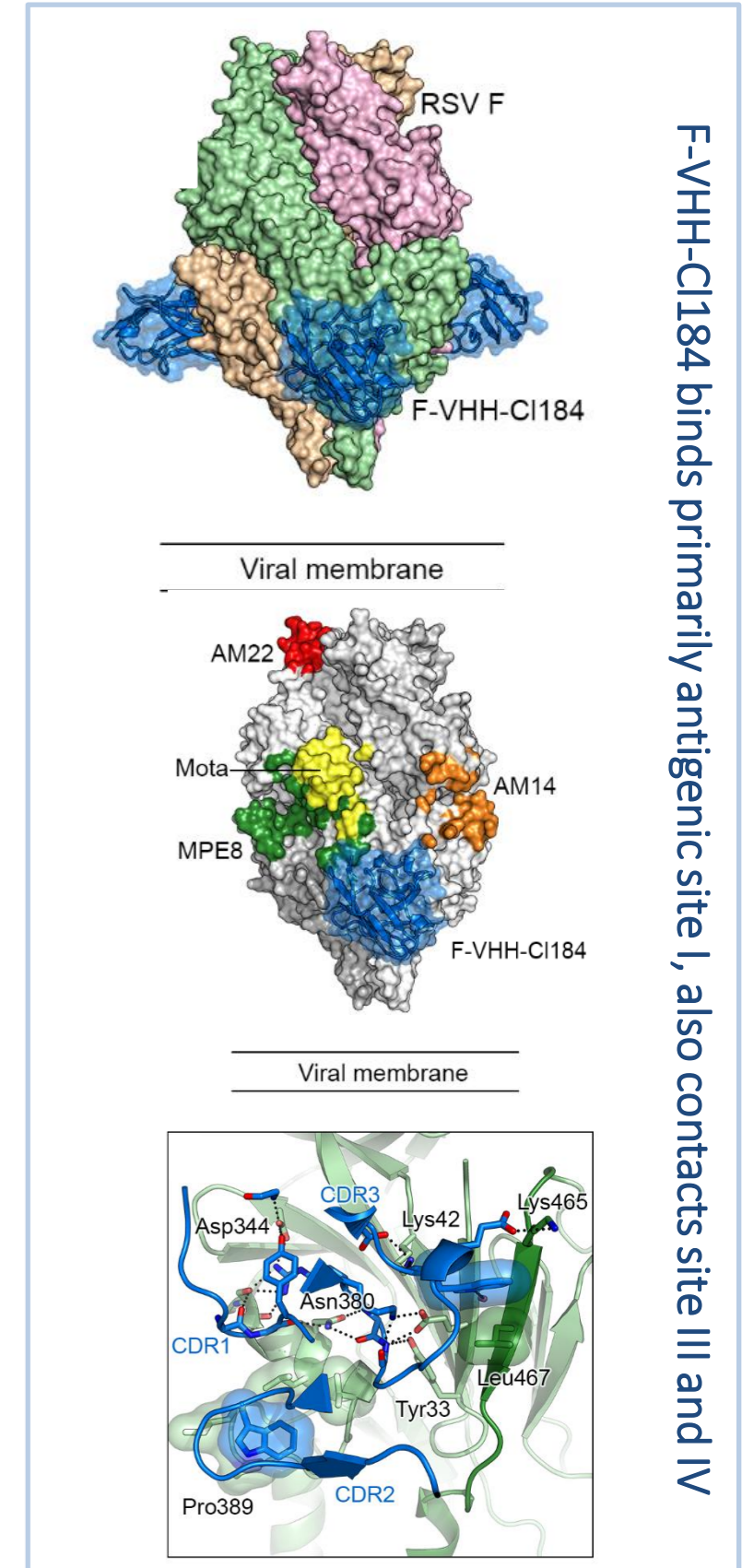


F-VHH-C184 binds a different epitope than F-VHH-4

We isolated and characterized an RSV-neutralizing VHH that binds a unique prefusion F-specific epitope. The single-domain antibody has high neutralizing activity against RSV A, and limited neutralizing activity against RSV B. The VHH binds specifically and with high affinity to prefusion F. The epitope of F-VHH-C184 has not been described before and mainly overlaps with antigenic site I, with additional interactions with sites III and IV.

**Conclusion**

The authors declare that no competing interests exist.



F-VHH-C184 binds primarily antigenic site I, also contacts site III and IV