

A Single-Domain Antibody Fragment (VHH) Inhibiting Neutrophil Elastase for the Treatment of Inflammatory Lung Diseases

Paola Redeghieri¹, Joël Moray¹, Frédéric Kerff², Angélique Mottais^{3,4}, Cécile Vincke^{5,6}, Teresinha Leal⁷, Sophie Gohy^{3,8}, Serge Muyldermans⁵, Rita Vanbever⁹, Francisco Javier Morales-Yáñez^{1,10,#}, **Mireille Dumoulin**^{1,10,#}

¹Nano-antibodies to Explore Protein Structure and Functions (NEPTUNS), Centre of Protein Engineering, InBios, University of Liège, Belgium; ²Biological Macromolecule Crystallography, Centre of Protein Engineering, InBios, University of Liège, Belgium; ³Department of Pneumology, ENT and Dermatology, Institute of Experimental and Clinical Research (IREC), University Catholique of Louvain, Belgium; ⁴Louvain Center for Toxicology and Applied Pharmacology (LTAP), Institute of Experimental and Clinical Research (IREC), University Catholique of Louvain, Belgium; ⁵Laboratory of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Belgium; ⁶Myeloid Cell Immunology Lab, VIB Center for Inflammation Research, Brussels, Belgium; ⁷Louvain Center for Toxicology and Applied Pharmacology (LTAP), Institute de Recherche Expérimentale et Clinique (IREC), University Catholique of Louvain (UCLouvain), Belgium; ⁸Cystic Fibrosis Reference Centre, Cliniques Universitaires Saint-Luc, Brussels, Belgium; ⁹Advanced Drug Delivery & Biomaterials, Louvain Drug Research Institute; University Catholique of Louvain, Belgium; ¹⁰ALPANANO, Centre of Protein Engineering, InBios, Department of Life Sciences, University of Liège, Belgium.

Equally contributed to this work

Neutrophil elastase (NE) is a serine protease released by neutrophils during inflammation. It plays a major role in a series of inflammatory lung diseases, characterized by a chronic inflammatory state that causes detrimental effects such as progressive tissue destruction. NE inhibition constitutes therefore a promising therapeutic target. From a llama immune library of VHH genes, we selected, by phage display, a VHH referred to as NbE201, able to inhibit tightly, specifically and competitively both human (hNE) and murine (mNE) elastase. Its in vitro inhibitory power is similar to that of alpha-1 antitrypsin (i.e., the main endogenous inhibitor of hNE) and better than that of Sivelestat (i.e., a clinically approved hNE inhibitor). The X-rays crystal structure of the VHH-hNE complex shows that the CDR1 and CDR3 of NbE201 bind into the active site of the enzyme obstructing its access to both small and macromolecular substrates. They, however, do not bind deep enough into the substrate binding pocket to be hydrolyzed. NbE201 is highly stable towards chemical and thermal denaturation, oxidation and deamidation. Moreover, NbE201 prevents the development of acute respiratory distress syndrome induced by the instillation of NE in the lungs of mice. All together, these results highlight the high therapeutical potential of NbE201 to treat pulmonary inflammatory diseases.