

**NbE201: a Single-Domain Antibody fragment to Inhibit Neutrophil Elastase and reduce inflammation**

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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., the only 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, this VHH better inhibits hNE in sputa from cystic fibrosis patients than alpha-1 antitrypsin. Finally, it 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.