new/era/mabs

WHITE PAPER

HOW NANOBODIES OPEN NEW GATES

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NANOBODY FACTS

The appearance of antibodies consisting only of the heavy chain (VHH) was published two decades ago by Serge Muylderman, whose group discovered these antibodies by chance during a student internship. Since then, his group has done fundamental work in establishing protocols for the generation and characterization of this type of antibody[1]. Nowadays, such antibodies are obtained by research groups worldwide from Llama phage display libraries through conventional phage display and antigen-specific hit selection (Fig. 1).

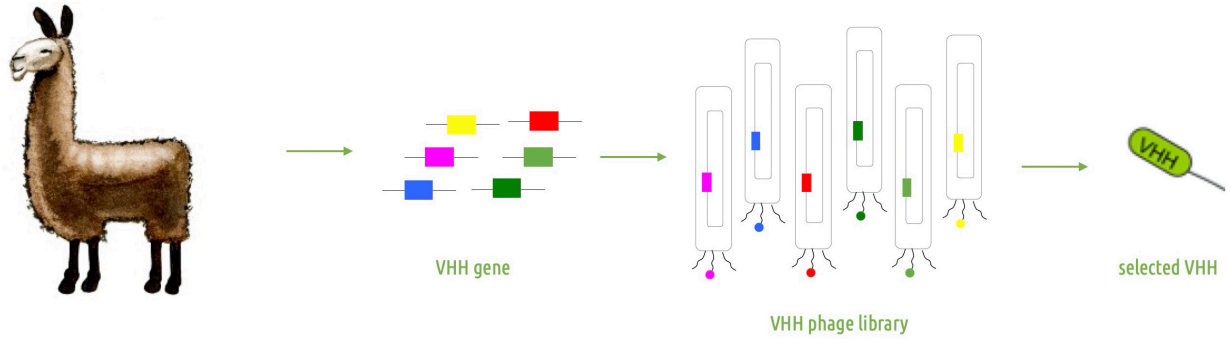

Figure 1: Schematic presentation of camelid phage display and nanobody selection.

The use of single domain camelid antibodies as a complement to conventional heavy and light chain antibodies, e.g. from mice, has several important advantages.

Camelid nanobodies are only one-tenth the size of a conventional antibody (12-15 kDa versus 150 kDa), making them the smallest antigen-binding moiety found in nature. Because they efficiently bind antigens with only one chain, these binders are also characterized by good manufacturability and easy-to-use modification options, such as the generation of multispecific versions or antibody-drug conjugates (ADCs).

[1]Muyldermans S. Nanobodies: Natural Single-Domain Antibodies. Annual Review of Biochemistry 2013 82:1, 775-797

NANOBODY BENEFITS

Conventional monoclonal antibodies (mAbs) can only bind to exposed and overtly accessible target epitopes. Particularly in viral infections where the pathogen mutates frequently, as in the recent SARS-CoV-2 pandemic, these mutations cluster in exposed regions of the viral surface that are readily accessible to conventional human immunoglobulins. These "mutation hotspots" lead to the quick emergence of new pandemic virus variants that are resistant to antibodies generated against their predecessors.

Nanobodies can be used in the same directions as conventional antibodies, but in addition have very valuable advantages that conventional antibodies cannot offer.

01 recognition of buried and hidden epitopes

02 high stability and solubility

03 good manufacturability

04 small size and low immunogenicity

05 easy humanization and high penetration

Based on their single antigen binding site, they can adopt a specific finger-like structure to recognize and bind hidden or buried target epitopes. These buried,

inaccessible epitopes are consequently subject to low selection pressures and generally represent evolutionary conserved parts of the pathogen. Nanobodies that bind to these conserved regions are therefore able to recognize different variants of the pathogen and successfully neutralize a great variety of different mutants.

The single heavy chain structure of nanobodies brings not only excellent solubility, high stability, ease of humanization, low immunogenicity, deep tissue penetration, combined with superior binding affinities. Nanobodies are also easier and cheaper to manufacture. These properties can be translated into novel forms of antibody-based diagnostic products and drug therapies.

As demonstrated by the first nanobody-based drug approved by the FDA in 2019 and licensed by Sanofi[2] for the treatment of acquired thrombotic thrombocytopenic purpura, this class of antibodies will play a fundamental role in the future. More than 20 nanobodies are now being investigated in clinical trials worldwide.

NANOBODY GENERATION

The best method to obtain potent nanobodies with high affinity is to immunize llamas or alpacas with the desired antigen and create an antigen-specific immune library. By isolating the antibody sequences from peripheral B lymphocytes and cloning them into phagemid vectors, a specific library can be created and used for phage display (Fig. 1). Specific hits are selected by ELISA-based screening or next-generation sequencing (NGS). Potent binders can then be recombinantly produced in various prokaryotic or eukaryotic expression hosts with high yield and purity.

Further modifications such as combining different nanobodies into multispecific binders or to create bivalent, biparatopic or bispecific versions can be carried out very reliably.

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[2] https://media.nature.com/original/magazine-assets/d41573-019-00104-w/d41573-019-00104-w.pdf

NEW/ERA/MABS PORTFOLIO

new/era/mabs has developed an antibody/nanobody platform with naïve and immunized libraries that successfully has been used for the generation of binders against various antigens such as SARS-CoV-2 spike protein, human glycoprotein 2, human neuroplastin, double-stranded nucleic acids and miRNAs, ESAT-6 for visualization of *M. tuberculosis* infection in lung sections – and several more.

For the selection of specific hits, which is normally done via excessive, labor- and cost-intensive and time-consuming ELISA screenings, new/era/mabs has developed its own proprietary selection platform technology ***selma***[3].

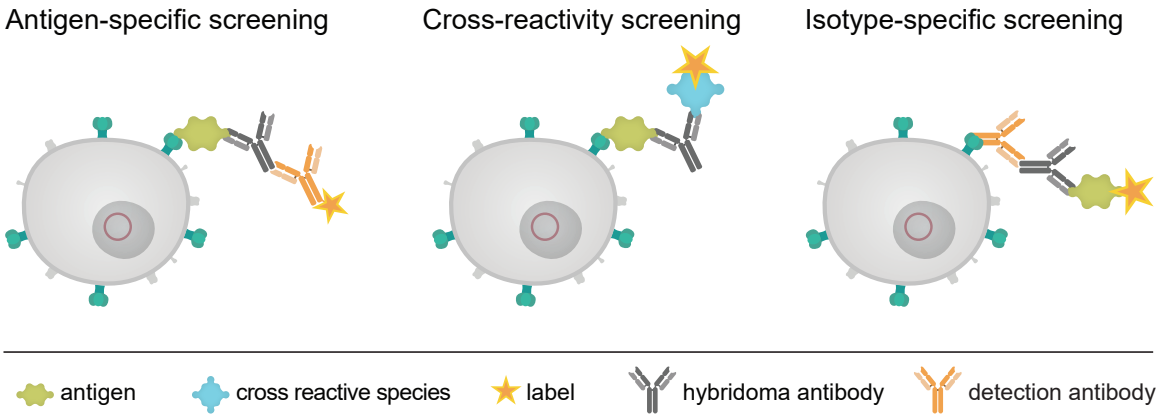

Figure 2: Schematic presentation of different selection options using NEM´s selma platform

selma is based on transgenic cell lines expressing an artificial cell surface anchor that allows the naturally produced and released antibody to be retained on the cell surface of the secreting cell (Fig. 2). By using fluorescence-labeled antigen as an option, the specificity and binding performance of the released antibody can be determined at the earliest possible time (Fig. 2, right).

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[3] Listek M, Hönow A, Gossen M, Hanack K. A novel selection strategy for antibody producing hybridoma cells based on a new transgenic fusion cell line. Sci Rep. 2020 Feb 3;10(1):1664. doi: 10.1038/s41598-020-58571-w.

selma enables rapid high-throughput screening of antibody candidates, four times faster than conventional ELISA-based screening. The system is suitable for hybridoma antibodies as well as recombinantly expressed antibodies or antibody fragments, e.g. nanobodies (Fig. 3).

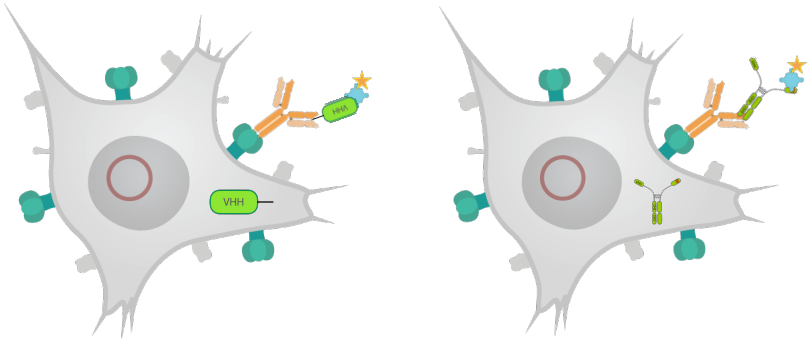

Figure 3: Schematics for the selection and production of VHH fragments (left) and recombinant antibodies (right).

Moreover, once the recombinant lead candidates have been selected, their cells of origin can be used for recombinant eukaryotic expression of the corresponding nanobodies. Re-cloning into suitable expression hosts is not required. Modifications such as Fc tagging or labeling with biotin can also be involved in this process.

> With our antibody discovery platform we are 400% faster than anybody else

USE CASE #1

GENERATION OF NEUTRALIZING SARS-COV-2 NANOBODIES

With its ***selma*** platform, new/era/mabs has developed potent SARS-CoV-2-specific nanobodies with high neutralizing capacity for multiple viral variants, including the recent Omicron strains (Fig. 4)[4]. Epitope mapping and *in silico* docking studies revealed a unique binding site in a cleft of the N-terminal and receptor-binding domain that is inaccessible to conventional heavy and light chain antibody formats.

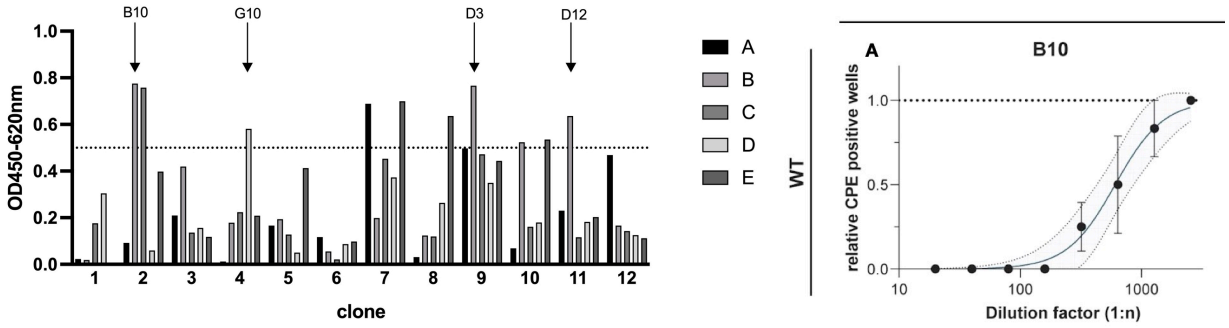

Figure 4: Phage display hit selection of SARS-CoV-2 Spike protein specific VHHs and neutralisation experiments with SARS-CoV-2 wildtype virus.

Additional modifications were made by preparing an Fc-labeled version of the nanobody with two identical binding sites and a molecular weight of 90 kDa. The presence of duplicate binding sites instead of a single one resulted in increased avidity and thus improved the binding performance by a factor of 18 (0.5 nM vs. 9.0 nM, Fig. 3B). Another modification under development focuses on the preparation of a bivalent nanobody construct for preclinical studies.

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[4] Schlör A, Hirschberg S, Amor GB, Meister TL, Arora P, Pöhlmann S, Hoffmann M, Pfaender S, Eddin OK, Kamhieh-Milz J, Hanack K. SARS-CoV-2 neutralizing camelid heavy-chain-only antibodies as powerful tools for diagnostic and therapeutic applications. Front Immunol. 2022 Sep 14;13:930975. doi: 10.3389/fimmu.2022.930975.

USE CASE #2

GENERATION OF AN ESAT-6 NANOBODY

Infections with M. tuberculosis remain among the top 10 causes of death worldwide. Although treatments are available and effective, diagnosis of the early onset of infection is critical for survival. ESAT-6 is considered an important secreted virulence factor of the pathogen that can be detected at an early stage in blood samples and biopsies of lung tissue.

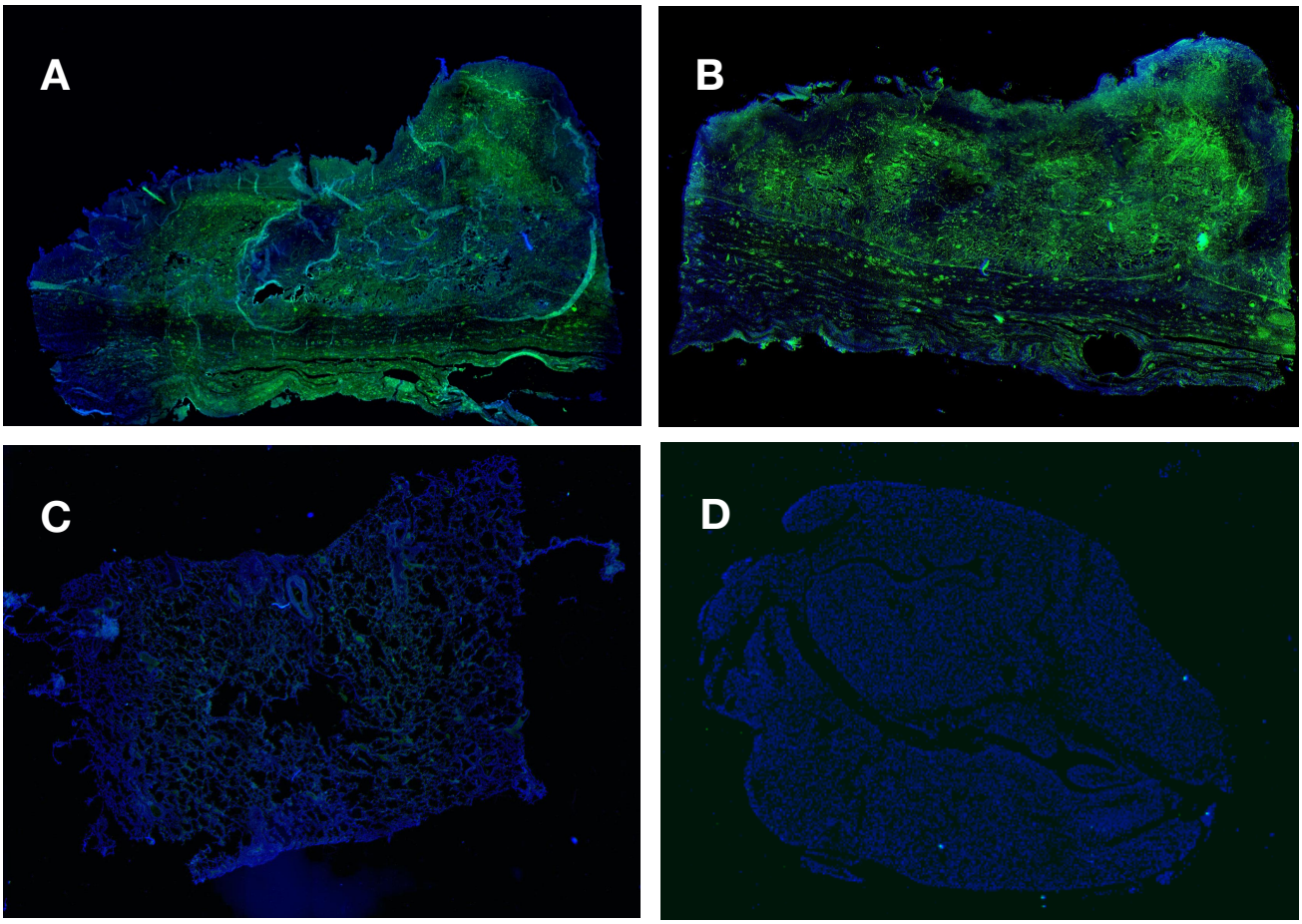

Figure 5: IHC with anti-ESAT6 nanobody. The formalin-fixed paraffin lung sections and HEp2 fake tissue were stained with the ESAT6-specific nanobody (green) and a commercial murine antibody (green). Staining of cell nucleus was done with DAPI (blue). (A) TB-positive lung section: anti-ESAT6 nanobody, (B) TB-positive lung section: commercial murine anti-ESAT6 antibody, (C) healthy lung section: anti-ESAT6 nanobody, (D) ESAT6-negative HEp2 cell „fake tissue“: anti-ESAT6 nanobody. Pictures have a 10x magnification.

After immunization of a llama new/era/mabs created an ESAT-6 immune library and selected potential candidates via phage display. After recombinant production, the nanobody candidates were purified and used for immunhistochemistry in lung biopsies from tuberculosis patients and healthy controls (Fig. 5). The photographic results demonstrate the perfect suitability of nanobodies as highly selective and specific immunofluorescence agents in immunohistochemistry.

ABOUT NEW/ERA/MABS

new/era/mabs performs human, murine or camelid antibody and downsized nanobody discovery and development services at minimized cost and superior quality:

01 in unsurpassed short time

02 with maximum avoidance of animal use

03 with unmatched yields of specific clones

04 against otherwise inaccessible epitopes

The company offers strategic partners the transfer and use of its proprietary technologies including its ***selma*** discovery platform under license.

Please inquire with our President & CEO Katja Hanack, PhD
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