Selma technology & camelid phage display for the discovery of anti-SARS-CoV-2 antibodies

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Introduction

The ongoing COVID-19 pandemic situation caused by SARS-CoV-2 and variants of concern such as B.1.1.529 (Omicron) is posing multiple challenges to humanity. The rapid evolution of the virus requires adaptation of diagnostic and therapeutic applications.

Generation of human anti-spike antibodies

In vitro immunization

Human PBMCs were isolated from a Buffy coat and stimulated with the target antigen. Human hybridoma cells were generated by fusing B cells to our transgenic myeloma cell line. By using selma, increasing yields of target specific hybridoma cells could be selected and IgG antibodies were produced and purified.

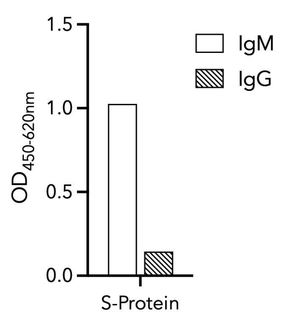
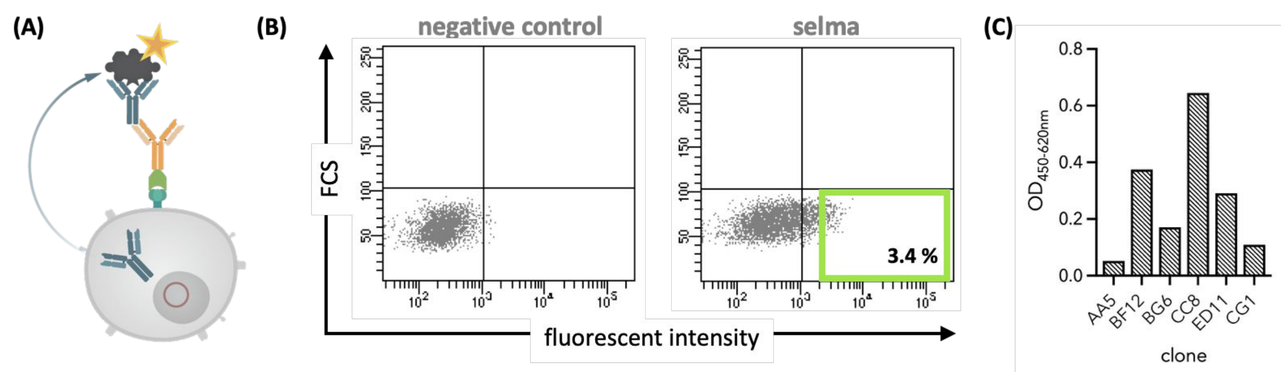


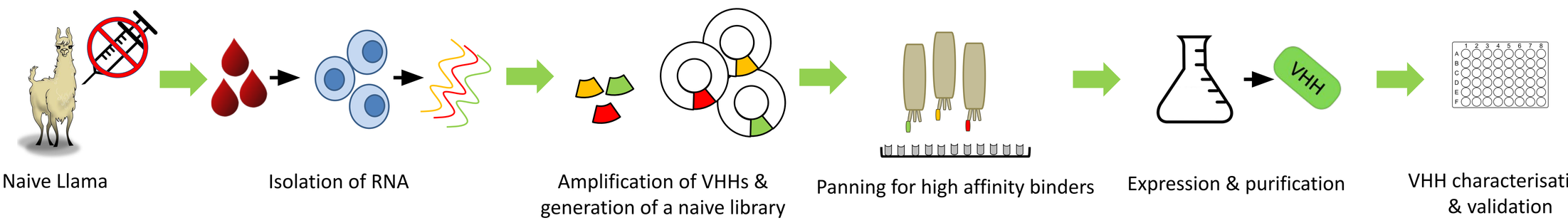
Fig. 1: Indirect ELISA to confirm successful *in vitro* immunization.

selma - selection of monoclonal antibodies

Isotype-specific selection of antibody producing hybridoma cells via FACS. In a final ELISA the target specificity of the selected antibodies was confirmed.



Generation of camelid anti-spike antibodies



Prokaryotic expression and purification of VHH B10

Following overnight expression, the periplasmic fraction was isolated and purified via Ni-NTA (Fig. 2A). Elution fraction 1 was then used for further purification by size exclusion chromatography (Fig. 2B). SDS-PAGE was performed after each purification step. The result of the purification process was a highly purified VHH.

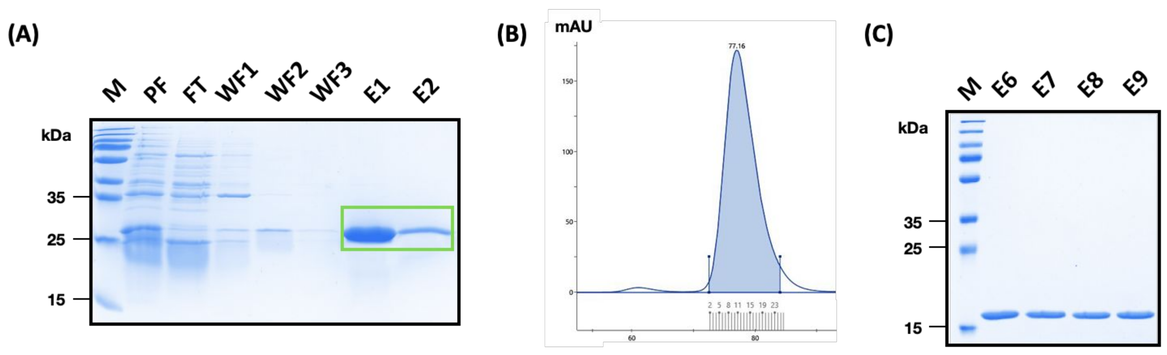


Fig. 2: Two step purification process of VHH B10 via (A) Ni-NTA (B) size exclusion chromatography. Protein fraction in Coomassie-stained 15 % PAA gel under reducing conditions. VHH B10 with calculated MW of 15 kDa. PF - Periplasmic fraction, FT - Flow through, WF - Wash fraction, E - Elution fraction, M - Protein standard PageRuler Prestained Plus (ThermoFisher Scientific).

hcAb-upgrade for VHH B10

The VHH B10 was cloned into our in-house vector pNEM_hcAb to generate a VHH with a camelid IgG2 Fc domain (= hcAb; Fig.4A). The camelid Fc tag does not affect the specificity of the VHH (Fig. 4C). The hcAb is detected using secondary in-house antibodies.

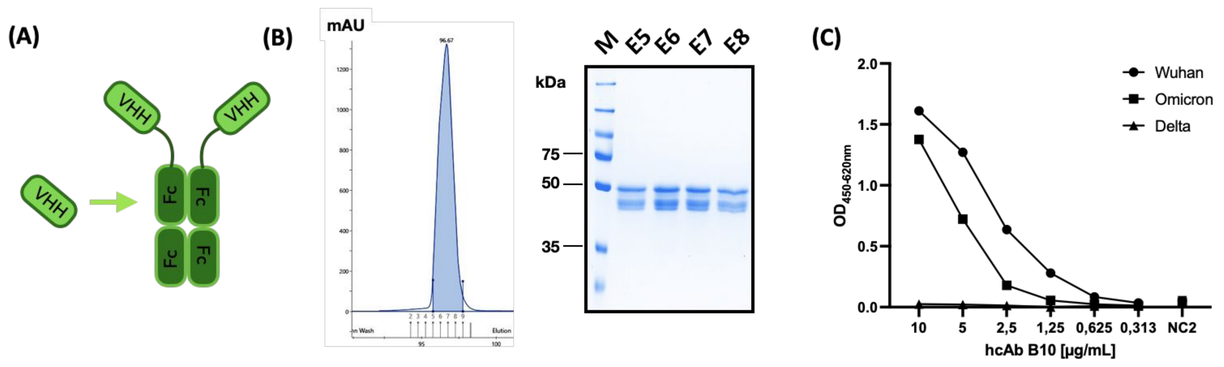


Fig. 4: (A) Schematic illustration of VHH updated to hcAb (B) Affinity purification via protein A (ÄKTA). Elution fraction (E) 5-8 (fraction 4, 14-15 not shown) in Coomassie-stained 10 % PAA gel under reducing conditions. HcAb B10 with calculated MW of 42,5 kDa as monomer, non-glycosylated. E - Elution fraction, M - Protein standard PageRuler Prestained Plus (ThermoFisher Scientific).(C) Coated spike protein was recognized by hcAb B10 in an indirect ELISA. The spike protein of Wuhan and Omicron could be detected by the hcAb. No signals were detected for Delta sublinage. In-house HRP-labeled anti-camelid IgG2 antibody used as detector.

VHH B10 binds specific Wuhan and Omicron spike protein

VHH B10 recognizes its target spike protein Wuhan and also Omicron (B.1.1.529). It does not recognize Delta (B.1.617.2) in an indirect ELISA.

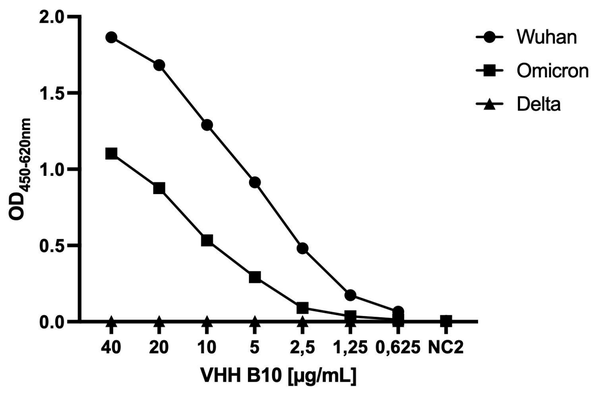


Fig. 3: Coated spike protein was recognized by VHH B10 after purification in an indirect ELISA. The spike protein of Wuhan and Omicron could be detected by the VHH. No signals were detected for Delta (B.1.617.2). Detection of VHH was performed by using an HRP-labeled anti-His Tag antibody (Sigma).

Affinity measurements with Monolith

The calculated Kd for binding of hcAb B10 to Wuhan was 0.92 nM ± 0.69 and for the Omicron trimer was 9.03 nM ± 0.30.

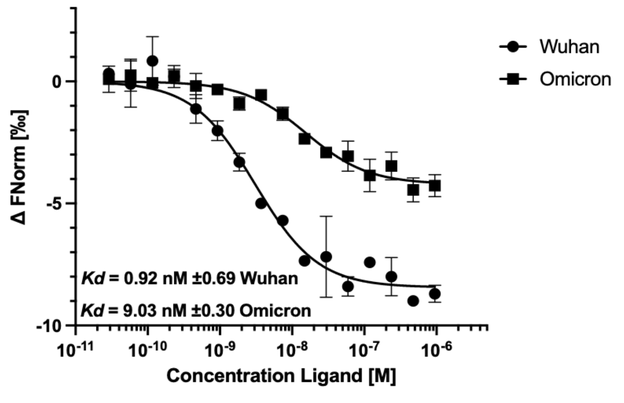


Fig. 5: Binding affinity analysis with a fixed concentration of NT-647 conjugated hcAb B10 (20 nM) and variable concentrations of Wuhan or Omicron as unlabeled binding partner varied from 0.95 µM to 2.9E-05 µM. An MST on time of 1.5 s was used for analysis. For Kd determination, the resulting dose-response curves were fitted to a single-site binding model. MST measurements were performed in duplicate (n=2) for Wuhan and in triplicate (n=3) for Omicron. Error bars represent standard deviation.

References:

[1] Listek, M., Micheel, B., Hanack, K. (2015) EP3134435A1 „Biomolecule releasing cell and selection thereof by means of a surface protein“ patent owner: new/era/mabs GmbH

[2] Listek, M., Hönow, A., Gossen, M., Hanack, K. (2020) „A novel selection strategy for antibody producing hybridoma cells based on a new transgenic fusion cell line“ Sci Rep. 10(1):1664

[3] Schlör A, Hirschberg S, Amor GB, et al. (2022) „SARS-CoV-2 neutralizing camelid heavy-chain-only antibodies as powerful tools for diagnostic and therapeutic applications“ Front Immunol. 13:930975.

Acknowledgement:

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