Biotechnology Reports 39 (2023) e00803

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Review

Revolutionizing antiviral therapy with nanobodies: Generation and prospects

Mujahed I. Mustafa [a,*], Ahmed Mohammed [b]

[a] Department of Biotechnology, College of Applied and Industrial Sciences, University of Bahri, Khartoum, Sudan
[b] Department of biotechnology, school of life sciences and technology, Omdurman Islamic university, Omdurman, Sudan

ARTICLE INFO

Keywords:
Nanobodies
VHHs
Antibodies
Heavy chain
Half-life

ABSTRACT

As the world continues to grapple with infectious diseases, scientists are constantly searching for effective ways to combat these deadly pathogens. One promising avenue of research is the use of nanobodies as neutralization agents. These small proteins, derived from camelid antibodies, have several unique advantages over traditional antibodies, including their small size. Nanobodies are much smaller than conventional antibodies, typically weighing in at around 15 kDa compared to the 150 kDa of a typical human antibody. This small size allows them to penetrate into tight spaces that larger molecules cannot reach, such as the crevices on the surface of viruses or bacteria. This makes them highly effective at neutralizing viruses by binding to and blocking their key functional sites. In this mini-review we discuss the construction approaches of nanobodies, and some methods to increase the half-life of nanobodies. Moreover, we discuss Nanobodies and their therapeutic potential for infectious agents.

1. Introduction

Engineered antibodies are designed to target specific viral proteins or structures, preventing them from infecting cells or replicating [1]. These antibodies can be produced in large quantities and administered to patients as a treatment or used as a preventative measure in high-risk populations [2].

For example, monoclonal antibodies (mAbs) are essential tools for both research and therapy [3], but their production is costly and challenging, which can strain healthcare and research budgets. Additionally, mAbs have limitations that make them unsuitable for certain applications [4]. For example, their large size (150 kDa) restricts their ability to penetrate tissues and tumors, and they can trigger immune reactions that neutralize their effects, limiting the long-term use of chimeric and humanized Abs [5].

In the early 1990s, Hamers-Casterman and her team unexpectedly discovered a new type of antibody in members of the Camelidae [6]. These "heavy-chain-only antibodies" (HcAbs) lack light chains and consist of two constant domains, a hinge region, and a variable heavy chain domain (VHH) called the nanobody (Nb) [6].

The VHH retains full antigen-binding capacity and is composed of four conserved sequence stretches surrounding three hypervariable complementarity-determining regions (CDR). CDR3 is the main contributor to antigen binding, with at least 60–80% of contact with the antigen occurring through this region [7]. Nbs are small (15 kDa) and have a long protruding CDR3 loop, giving them a prolate shape that exposes a convex paratope. This allows them to access receptor clefts or binding pockets that are inaccessible to conventional antibodies and reach hidden or cryptic epitopes. Ghahroudi et al. described the selection, identification, and production procedure for antigen-specific VHHs, while Muyldermans extensively reviewed other molecular and biochemical properties of Nbs [8].

Nbs, also known as variable domain of heavy chain of IgG (VHH), are single-domain antibodies that have gained significant attention in recent years due to their unique properties and potential therapeutic applications [9]. These tiny proteins, which are derived from the immune systems of camelids such as llamas and camels, are much smaller than conventional antibodies and can bind to specific targets with high affinity and specificity [10].

However, recent research has shown that other animals also have similar strategies to diversify their antibodies. For example, sharks produce a type of antibody called IgNAR (Variable domain of the immunoglobulin new antigen receptor) that is also small and stable like nanobodies [11]. Additionally, some rodents have been found to

* Corresponding author.
E-mail address: mujahedimustafa@gmail.com (M.I. Mustafa).

https://doi.org/10.1016/j.btre.2023.e00803
Received 2 April 2023; Received in revised form 21 May 2023; Accepted 6 June 2023
Available online 7 June 2023

produce single-domain antibodies similar to nanobodies [12].

The production of nanobodies or similar antibodies in different animals involves a complex process of genetic rearrangement and selection. In camels and llamas, for example, the genes encoding the heavy chain of their antibodies undergo a unique process called VHH gene rearrangement that leads to the production of nanobodies [13] In sharks, IgNAR antibodies are produced by a different mechanism involving gene conversion and somatic hypermutation [11]. Similarly, rodents use a process called VHH gene conversion to produce single-domain antibodies [13].

One of the most promising applications of Nbs is in the treatment of viral diseases [14], chronic inflammations, cancer. Traditional treatments for infectious diseases include antivirals, and vaccines [15]. However, these treatments have limitations such as drug side effects, and lack of efficacy against certain pathogens [16].

Nbs offer a promising new approach to treating viral infectious diseases [12]. Their small size, high specificity, and long half-life make them ideal candidates for use in emergency situations such as pandemics or outbreaks. Further research is needed to fully explore their potential as therapeutics for infectious diseases. In this mini-review we discuss Nbs and their therapeutic potential for viral infectious diseases. Moreover, we discuss the construction and expression approaches of Nbs.

2. Generation of Nbs

Nb libraries are constructed by immunizing animals with antigens of interest, isolating peripheral blood mononuclear cells (PBMCs) from the animal, and extracting the DNA encoding the variable domains of the heavy chain antibodies [17]. These variable domains are then amplified and cloned into phagemid vector to create a library of Nbs. This library can be screened for high-affinity binders to the antigen of interest, allowing for the development of novel therapeutic agents [18].

The process begins with the immunization of a camelid with a target protein. The animal's immune system produces a diverse repertoire of heavy chain-only antibodies, which can be isolated from its blood or lymph nodes. The genes encoding these antibodies are then amplified using PCR (polymerase chain reaction) and cloned into a phage display vector [19].

The phage display library is then screened against the target protein using a process called panning. In this step, the library is incubated with the target protein immobilized on a solid support (such as a microtiter plate). The phages displaying Nbs that bind to the target protein are captured on the support while non-binding phages are washed away [19].

The captured phages are then eluted and used to infect bacteria for amplification. This process is repeated several times to enrich for Nbs that bind strongly to the target protein. Finally, individual clones are selected and sequenced to identify unique Nb sequences [19].

Once identified, these sequences can be further optimized through mutagenesis or affinity maturation techniques to improve their binding affinity or specificity. The resulting Nbs can be produced in large quantities using bacterial expression systems or other recombinant technologies [19].

3. Simpler approach for the production of Nbs

The strategic development of nanobodies utilizing CDRs sourced from pre-existing antibodies would be immensely valuable in circumventing animal immunizations and directly producing binders within the desired framework. However, the creation of nanobody grafts utilizing CDRs derived from conventional antibodies necessitates meticulous consideration, as both heavy and light chain variable domains (VH and VL) form the antigen-binding site and are integral to recognizing the antigenic epitope [20]. Additionally, the framework plays a crucial role in determining CDR conformation and orientation, with specific framework residues often directly contributing to antigen binding. Thus, it is imperative to always take into account framework residues when designing a graft [20].

In order to overcome the challenge of needing extensive libraries and intricate selection procedures, Hanna et al. suggest a two-step method for generating Nbs (Fig. 1) [20]. First, we transplant complementarity-determining regions (CDRs) from traditional antibody formats onto Nb frameworks, resulting in weak antigen binders. Then, these suboptimal binders serve as templates for the construction of targeted synthetic phage libraries to improve affinity [20].

Our opinion is that this methodology serves as a fitting supplement to immune, naïve, and synthetic libraries in the production of protein and hapten binders. It is advantageous that the vast quantity of pre-existing antibodies offers an extensive collection of CDRs that can be utilized in the construction of targeted synthetic VHH libraries. These techniques, which expedite the creation of VHH possessing desired specificities and frameworks, are highly beneficial for advancing nanobody-based research and enhancing the therapeutic potential of VHH-based treatments [20].

4. Methods to increase the half-life of Nbs

The half-life of a drug refers to the time it takes for half of the administered dose to be eliminated from the body. In the case of Nbs, their small size and lack of an Fc region make them vulnerable to rapid renal clearance, resulting in a short circulating half-life of only a few hours [21].

This short half-life poses a challenge for the development of Nbs as therapeutics because it limits their efficacy and requires frequent dosing. In addition, it may also lead to off-target effects due to accumulation in non-target tissues or organs [10].

The short half-life of Nbs is a significant limitation for their use as therapeutic agents. However, several strategies are being explored to overcome this challenge and improve their efficacy and pharmacokinetic properties:

Fusion with Fc region: One of the most common methods to increase the half-life of Nbs is by fusing them with the Fc region of human immunoglobulin G (IgG). The Fc region binds to neonatal Fc receptor (FcRn) present on endothelial cells and prevents degradation and clearance of Nbs by recycling them back into circulation [22].

PEGylation: PEGylation involves covalently attaching polyethylene glycol (PEG) chains to Nbs. This modification increases their hydrodynamic size and reduces renal clearance, resulting in a longer half-life [23].

Albumin binding: Nbs can also be engineered to bind to human serum albumin (HSA), which is a major protein in blood plasma. HSA-bound Nbs have a longer half-life due to reduced renal clearance and increased protection from proteolytic degradation [24].

Site-specific conjugation: Site-specific conjugation involves attaching a linker molecule to a specific amino acid residue on the surface of Nbs, which then binds to a circulating protein such as albumin or transferrin. This method provides controlled conjugation and results in improved pharmacokinetics [25].

Glycosylation: Glycosylation involves adding sugar molecules to specific sites on Nbs, which can improve their stability and reduce clearance by increasing their hydrophilicity [26].

Out of these methods, fusion with Fc region has been shown to be most effective in increasing the half-life of Nbs for therapeutic applications against viruses [27]. This method has been used successfully in developing neutralizing antibodies against SARS-CoV-2, MERS-CoV [28], and Ebola virus [14]. The Fc-fused neutralizing antibodies showed improved efficacy compared to non-fused antibodies due to increased serum persistence and enhanced effector functions such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) [29].

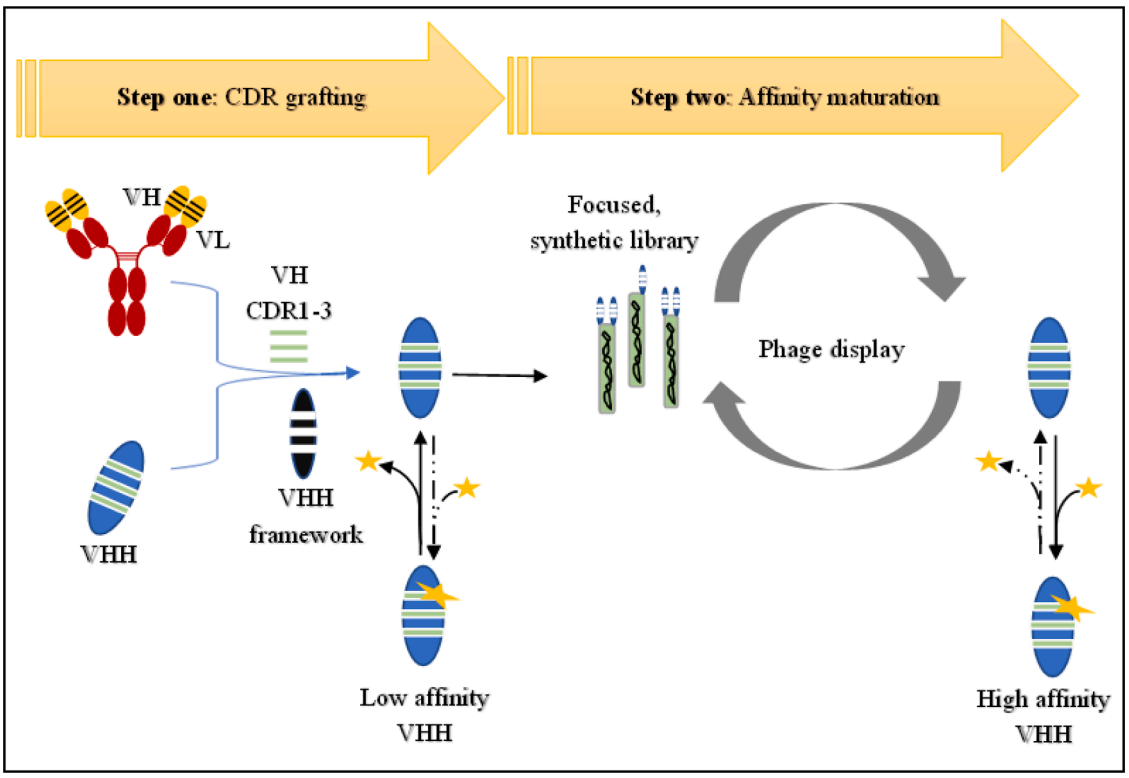

Fig. 1. A two-step approach for generating VHH involves grafting CDRs of VH onto a VHH framework and designing a focused VHH library. The resulting weak binders lacking VL serve as templates for affinity maturation through phage display. The low association rate with the ligand is indicated by a dotted line, while dissociation is indicated by a solid line [20].

5. Therapeutic approaches of Nbs

5.1. Targeting viral glycoproteins

Viral glycoproteins are proteins that are present on the surface of viruses and play a crucial role in the infection process [30]. They interact with host cell receptors and facilitate viral entry into the cell. Therefore, targeting these glycoproteins with Nbs can block viral entry and replication, leading to effective antiviral therapy [30].

One example of Nbs targeting viral glycoproteins is the development of Nbs against the spike protein of SARS-CoV-2 [31]. The spike protein is essential for viral entry into host cells and has been a major target for vaccine development [31]. Several studies have reported the isolation of potent Nbs that bind to different regions of the spike protein and inhibit its function. These Nbs have shown promising results in preclinical studies and could potentially be used as therapeutics against SARS-CoV-2 [32].

In addition to SARS-CoV-2, Nbs targeting viral glycoproteins have also been developed for other viruses such as Monkeypox virus [33], and Ebola virus [34]. These Nbs have shown potent antiviral activity in vitro and in animal models, highlighting their potential as therapeutics against viral infections.

5.2. Nbs prevents host-virus interactions

An alternative strategy to targeting viral proteins with Nbs is to focus on cellular proteins that play a crucial role in virus attachment and entry. This approach is particularly intriguing for viruses that exhibit an extraordinary rate of sequence variation, such as SARS-CoV-2 [35]. Siqi H. et al. developed Nb that could bind to angiotensin I-converting enzyme-2 (ACE2) protein. By binding to ACE2, these Nbs prevented the virus from infecting cells in laboratory experiments [36].

This study [36] demonstrate how Nbs can be used to target specific proteins on the surface of viral hosts, such as human cells infected with SARS-CoV-2. By blocking these proteins, Nbs can prevent viruses from entering or replicating within host cells.

5.3. Nbs act as immunomodulators

Traditional antiviral drugs target specific viral proteins or enzymes, but viruses can quickly mutate and develop resistance to these drugs. In contrast, Nbs target the virus itself by binding to its surface proteins or other structures essential for its replication and infectivity [9]. This approach not only provides a broader spectrum of activity against different strains of viruses but also reduces the risk of drug resistance.

Moreover, Nbs can be engineered to enhance their potency and specificity against a particular virus. These Nbs can be administered as inhalable sprays or injections to prevent or treat SARS-CoV-2 infections [37].

Another way nanobodies can modulate the immune response is by enhancing antibody-dependent cellular cytotoxicity (ADCC). ADCC is a process where antibodies bind to infected cells and recruit immune cells, such as natural killer cells, to kill the infected cells. Nanobodies can be engineered to enhance this process by increasing antibody affinity for infected cells or by recruiting more immune cells to the site of infection [38].

Another advantage of Nbs is their ability to recruit and activate the immune system against viruses. Nbs can act as immunomodulators by binding to immune cells or molecules involved in antiviral responses and enhancing their activity [32]. Furthermore, Nbs can be used in combination with other immunotherapies such as checkpoint inhibitors or cytokines to boost antiviral immunity [6].

Nanobodies can also be used to inhibit viral replication directly. By targeting essential viral proteins, such as polymerases or proteases, nanobodies can prevent viral replication and reduce overall viral load [39].

Finally, nanobodies can be used to modulate cytokine production during a viral infection. Cytokines are signaling molecules that regulate inflammation and immune responses. During a viral infection, excessive cytokine production can lead to a cytokine storm, which can cause tissue damage and organ failure. Nanobodies can be engineered to block specific cytokines or cytokine receptors, preventing excessive inflammation and reducing tissue damage [38].

Overall, nanobodies have great potential for modulating the immune response during a viral infection. By targeting specific aspects of the immune response or directly inhibiting viral replication, nanobodies could provide a powerful tool for combating viral infections [40].

6. Challenges and opportunities of Nbs and their therapeutic potential for infectious diseases

One of the most significant challenges in developing Nb-based therapies for viral infectious diseases is identifying suitable targets [10].

While many pathogens have been well-characterized at the molecular level, identifying specific epitopes that can be targeted by nanobodies can be challenging. However, advances in structural biology techniques such as X-ray crystallography and cryo-electron microscopy have enabled researchers to visualize the structures of pathogen proteins at high resolution, providing valuable insights into potential targets for nanobodies.

Another challenge is optimizing the pharmacokinetics and biodistribution of Nbs in vivo [41]. While Nbs are highly stable and can be produced at large scale using recombinant DNA technology, they can be rapidly cleared from circulation by the kidneys. Strategies such as PEGylation (attaching polyethylene glycol molecules) or fusing Nbs with other proteins can improve their half-life and tissue distribution [23].

Despite these challenges, there are several examples of successful applications of Nbs in infectious disease research. For example, researchers have developed Nbs that target the spike protein on the surface of SARS-CoV-2. These Nbs have been shown to neutralize the virus in vitro and in animal models [42].

Another example, researchers have developed Nb-based therapies [43] against respiratory syncytial virus (RSV), a common cause of respiratory infections in infants and young children. These therapies have shown promising results in preclinical studies and are currently being evaluated in clinical trials.

In order for drug candidates to proceed to clinical trials, three key factors must be considered: (a) whether the drug effectively reaches the target organ in adequate quantities to produce the desired outcome, (b) whether the drug's binding to the target is specific enough to elicit biological activity, and (c) whether the compound can effectively modulate the target's function [40].

7. Conclusion and future perspectives

Nbs possess advantageous properties and are adaptable molecules that have been assessed for research tools, diagnostic and therapeutic purposes. The potential of Nbs is limitless, and there is an opportunity to utilize them as pharmaceuticals in daily clinical practice.

Due to their ease of production, we anticipate that more compact and stable Nbs will take the place of mAbs and their fragments, such as Fab and scFv Abs, in various fields. The use of bacterial or yeast systems for Nb expression during the discovery and developmental stages allows for cost-effective in-house protein purification, particularly when compared to traditional Abs. Upstream process development (USP) optimizes the fermentation process, resulting in a consistent and scalable process that produces high-quality end products. However, once Nbs enter clinical trials, the manufacturing process must be scaled up and comply with strict good manufacturing practices (GMP) guidelines.

Their limited target range, short half-life, must also be considered when evaluating their potential as therapeutic agents. Nonetheless, it is clear that Nbs represent an exciting new frontier in drug development with enormous potential for improving human health. Their low immunogenicity is just one piece of this puzzle – but it could be a crucial one in ensuring that these small proteins can be used safely and effectively for years to come.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] P.J. Carter, A. Rajpal, Designing antibodies as therapeutics, Cell 185 (2022) 2789–2805.
[2] J.E. Crowe Jr, Human antibodies for viral infections, Annu. Rev. Immunol. 40 (2022) 349–386.
[3] D. Focosi, S. McConnell, A. Casadevall, E. Cappello, G. Valdiserra, M. Tuccori, Monoclonal antibody therapies against SARS-CoV-2, Lancet Infect. Dis. (2022).
[4] B. Spanov, N. Govorukhina, N.C. van de Merbel, R. Bischoff, Analytical tools for the characterization of deamidation in monoclonal antibodies, J. Chromatogr. Open 2 (2022), 100025.
[5] N. Zambrano, G. Froechlich, D. Lazarevic, M. Passariello, A. Nicosia, C. De Lorenzo, et al., High-Throughput monoclonal antibody discovery from phage libraries: challenging the current preclinical pipeline to keep the pace with the increasing mAb demand, Cancers (Basel) 14 (2022) 1325.
[6] G. Van Heeke, K. Allosery, V. De Brabandere, T. De Smedt, L. Detalle, A. de Fougerolles, Nanobodies® as inhaled biotherapeutics for lung diseases, Pharmacol. Ther. 169 (2017) 47–56.
[7] T.T.T. Truong, V.Q. Huynh, N.T. Vo, H.D. Nguyen, Studying the characteristics of nanobody CDR regions based on sequence analysis in combination with 3D structures, J. Genetic Eng. Biotechnol. 20 (2022) 157.
[8] S. Muyldermans, Nanobodies: natural single-domain antibodies, Annu. Rev. Biochem. 82 (2013) 775–797.
[9] J.Y.J. Kim, Z. Sang, Y. Xiang, Z. Shen, Y. Shi, Nanobodies: robust miniprotein binders in biomedicine, Adv. Drug Deliv. Rev. (2023), 114726.
[10] M. Arbabi-Ghahroudi, Camelid single-domain antibodies: promises and challenges as lifesaving treatments, Int. J. Mol. Sci. 23 (2022) 5009.
[11] W.H. Chen, A. Hajduczki, E.J. Martinez, H. Bai, H. Matz, T.M. Hill, et al., Shark nanobodies with potent SARS-CoV-2 neutralizing activity and broad sarbecovirus reactivity, Nat. Commun. 14 (2023) 580.
[12] A. Moliner-Morro, G.M. McInerney, L. Hanke, Nanobodies in the limelight: multifunctional tools in the fight against viruses, J. Gen. Virol. 103 (2022). May.
[13] E. Pardon, T. Laeremans, S. Triest, S.G. Rasmussen, A. Wohlkönig, A. Ruf, et al., A general protocol for the generation of nanobodies for structural biology, Nat. Protoc. 9 (2014) 674–693. Mar.
[14] G. Pantaleo, B. Correia, C. Fenwick, V.S. Joo, L. Perez, Antibodies to combat viral infections: development strategies and progress, Nat. Rev. Drug Discovery 21 (2022) 676–696.
[15] D. Buonsenso, L.D. Gennaro, C.D. Rose, R. Morello, F. D'Ilario, G. Zampino, et al., Long-term outcomes of pediatric infections: from traditional infectious diseases to long Covid, Future Microbiol. 17 (2022) 551–571.
[16] F. Sakurai, M. Tachibana, H. Mizuguchi, Adenovirus vector-based vaccine for infectious diseases, Drug Metab. Pharmacokinet. 42 (2022), 100432.
[17] B. Liu, D. Yang, Easily established and multifunctional synthetic nanobody libraries as research tools, Int. J. Mol. Sci. 23 (2022) 1482.
[18] M.S. Valdés-Tresanco, A. Molina-Zapata, A.G. Pose, E. Moreno, Structural insights into the design of synthetic nanobody libraries, Molecules 27 (2022) 2198.
[19] Y. Mei, Y. Chen, J.P. Sivaccumar, Z. An, N. Xia, W. Luo, Research progress and applications of nanobody in human infectious diseases, Front. Pharmacol. 13 (2022), 963978.
[20] H.J. Wagner, S. Wehrle, E. Weiss, M. Cavallari, W. Weber, A two-step approach for the design and generation of nanobodies, Int. J. Mol. Sci. 19 (2018). Nov 2.
[21] C. Morrison, Nanobody approval gives domain antibodies a boost, Nat. Rev. Drug Discov. 18 (2019) 485–487.
[22] B. Hu, T. Liu, L. Li, L. Shi, M. Yao, C. Li, et al., IgG-Binding nanobody capable of prolonging nanobody-based radiotracer plasma half-life and enhancing the efficacy of tumor-targeted radionuclide therapy, Bioconjug. Chem. 33 (2022) 1328–1339.
[23] M. Panda, E. Kalita, S. Singh, K. Kumar, V.K. Prajapati, Nanobody-peptide-conjugate (NPC) for passive immunotherapy against SARS-CoV-2 variants of concern (VoC): a prospective pan-coronavirus therapeutics, Mol. Divers. (2022) 1–27.
[24] Y. Zhang, D. Zhang, S. An, Q. Liu, C. Liang, J. Li, et al., Development and characterization of nanobody-derived CD47 theranostic pairs in solid tumors, Research (Wash D C) 6 (2023) 0077.
[25] H. Hong, Z. Zhou, K. Zhou, S. Liu, Z. Guo, Z. Wu, Site-specific C-terminal dinitrophenylation to reconstitute the antibody Fc functions for nanobodies, Chem. Sci. 10 (2019) 9331–9338.
[26] R.E. Kontermann, Half-life extended biotherapeutics, Expert Opin. Biol. Ther. 16 (2016) 903–915.

[27] X. Chi, X. Zhang, S. Pan, Y. Yu, Y. Shi, T. Lin, et al., An ultrapotent RBD-targeted biparatopic nanobody neutralizes broad SARS-CoV-2 variants, Signal Transduct. Target. Ther. 7 (2022) 44.
[28] G. Zhaoa, L. Hea, S. Suna, H. Qiua, W. Taia, J. Chenb, et al., "A novel nanobody targeting middle east respiratory syndrome coronavirus (MERS-CoV) 1 receptor-binding domain has potent cross-neutralizing activity and protective efficacy 2 against MERS-CoV 3," 2018.
[29] V.A. Brentville, M. Vankemmelbeke, R. Metheringham, P. Symonds, K. Cook, R. Urbanowicz, et al., A novel bivalent DNA vaccine encoding both spike protein receptor-binding domain and nucleocapsid protein of SARS-CoV-2 to elicit T cell and neutralising antibody responses that cross react with variants, bioRxiv (2021), p. 2021.06. 18.448932.
[30] Y. Huang, Y. Song, J. Li, C. Lv, Z.S. Chen, Z. Liu, Receptors and ligands for herpes simplex viruses: novel insights for drug targeting, Drug Discov. Today 27 (2022) 185–195.
[31] A.C.A. Aristiguieta, Nanobodies targeting SARS-CoV-2. Biomedical Innovations to Combat COVID-19, Elsevier, 2022, pp. 231–240.
[32] M. Bhattacharya, S. Chatterjee, S.S. Lee, C. Chakraborty, Therapeutic applications of nanobodies against SARS-CoV-2 and other viral infections: current update, Int. J. Biol. Macromol. (2022).
[33] N. Akhtar, V. Kaushik, R.K. Grewal, A.K. Wani, C. Suwattanasophon, K. Choowongkomon, et al., Immunoinformatics-aided design of a peptide based multiepitope vaccine targeting glycoproteins and membrane proteins against monkeypox virus, Viruses 14 (2022) 2374.
[34] M. Walser, J. Mayor, S. Rothenberger, Designed ankyrin repeat proteins: a new class of viral entry inhibitors, Viruses 14 (2022) 2242.
[35] D.B. Naidoo, A.A. Chuturgoon, The potential of nanobodies for COVID-19 diagnostics and therapeutics, Mol. Diagn. Ther. (2023) 1–34.
[36] S. He, J. Wang, H. Chen, Z. Qian, K. Hu, B. Shi, et al., A competitive panning method reveals an anti-SARS-CoV-2 nanobody specific for an RBD-ACE2 binding site, Vaccines (Basel) 11 (2023) 371.
[37] L. Du, Y. Yang, X. Zhang, F. Li, Recent advances in nanotechnology-based COVID-19 vaccines and therapeutic antibodies, Nanoscale 14 (2022) 1054–1074.
[38] I. Jovčevska, S. Muyldermans, The therapeutic potential of nanobodies, BioDrugs 34 (2020) 11–26.
[39] P. Sroga, D. Safronetz, D.R. Stein, Nanobodies: a new approach for the diagnosis and treatment of viral infectious diseases, Future Virol. 15 (2020) 195–205.
[40] X. Xu, Y. Vugmeyster, Challenges and opportunities in absorption, distribution, metabolism, and excretion studies of therapeutic biologics, Aaps J. 14 (2012) 781–791. Dec.
[41] Y. Vugmeyster, C.A. Entrican, A.P. Joyce, R.F. Lawrence-Henderson, B.A. Leary, C. S. Mahoney, et al., Pharmacokinetic, biodistribution, and biophysical profiles of TNF nanobodies conjugated to linear or branched poly (ethylene glycol), Bioconjug. Chem. 23 (2012) 1452–1462.
[42] M.A. Rossotti, H. van Faassen, A.T. Tran, J. Sheff, J.K. Sandhu, D. Duque, et al., Arsenal of nanobodies shows broad-spectrum neutralization against SARS-CoV-2 variants of concern in vitro and in vivo in hamster models, Commun. Biol. 5 (2022) 933.
[43] L. Wilken, A. McPherson, Application of camelid heavy-chain variable domains (VHHs) in prevention and treatment of bacterial and viral infections, Int. Rev. Immunol. 37 (2 2018) 69–76. Jan.