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Small but mighty: the versatility of nanobodies in modern medicine†

Mike Blueggel, [a] Désirée Gül, [b] Roland H. Stauber [b] and Shirley K. Knauer *[a]

Nanotools in biomedicine open up novel applications in research, diagnostics, and clinical care. Here, nanobody technology has emerged as a powerful platform, offering advantages over conventional antibodies due to their small size, high stability, and ability to target cryptic epitopes. Our review summarizes the structural and functional properties of nanobodies, their production and engineering strategies, and explores their expanding role in therapeutic applications. We discuss nanobody-based approaches in oncology, neurodegenerative and infectious diseases, as well as autoimmune disorders, focussing on their integration into molecular imaging, targeted drug delivery, and emerging modalities such as targeted protein degradation (TPD). Advances in nanobody engineering, including bispecific constructs, nanobody–drug conjugates, and intracellular targeting strategies, are accelerating their clinical translation. Despite challenges in manufacturing and regulatory approval, the approval of caplacizumab and ongoing clinical trials underscore the growing impact of nanobody therapeutics. With their versatility and potential for precision and personalized medicine, nanobody-based technologies drive innovation across biomedical research and next-generation therapies.

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1. Introduction

Recently, nanobody technologies and applications have emerged as powerful tools in biotechnology and biomedicine (Fig. 1), offering unique advantages over conventional monoclonal antibodies (mAbs).[1,2] Nanobodies, also known as VHH domains, are derived from the heavy-chain-only antibodies found in camelids (Fig. 2) and also in sharks.[3] Their small size

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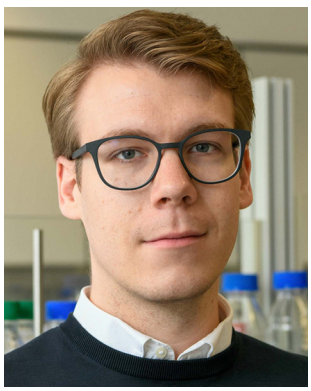


Mike Blueggel

Mike Blüggel obtained his Master's in Medical Biology from the University of Duisburg-Essen – where he also completed his PhD with highest honors under Prof. Peter Bayer, focusing on protein biochemistry and structural biology. As a postdoctoral researcher at the Center of Medical Biotechnology (ZMB), he recently joined Prof. Knauer's group, where he explores nanobody protein engineering, targeted protein degradation, and mechanistic networks for cancer therapy.

Désirée Gül

Désirée Gül earned her doctorate summa cum laude from the University of Mainz, focusing on the functional analysis of the oncologically relevant protease Threonine Aspartase-1. Since 2015, she has been a postdoctoral fellow and junior group leader at the University Medical Center Mainz, heading the "Proteases in Disease" research group, and received her venia legendi in Molecular Medicine in 2024. Her work explores protease (patho)biology, chemical biology, translational oncology, and nanobiomedicine.

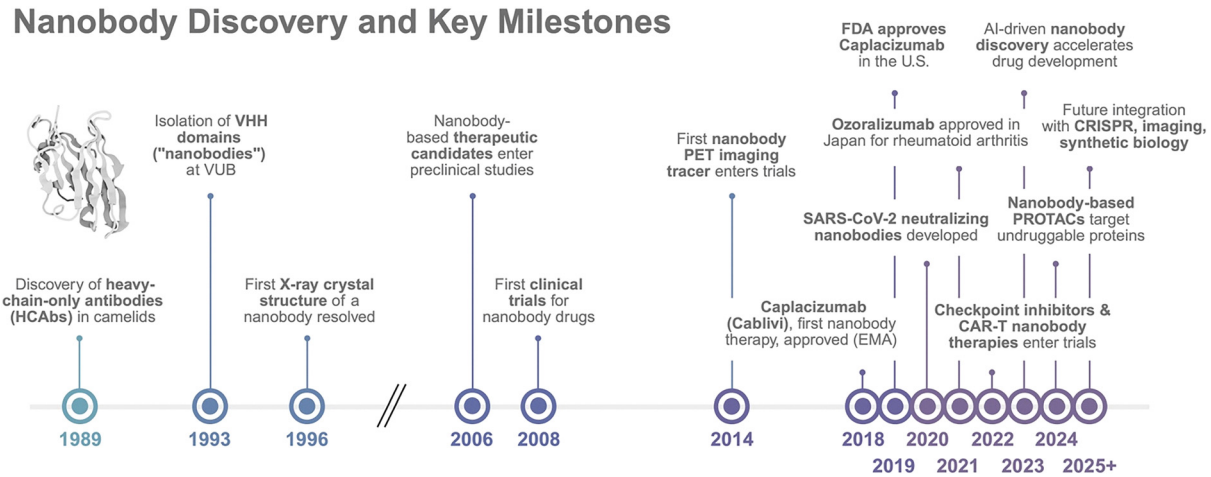
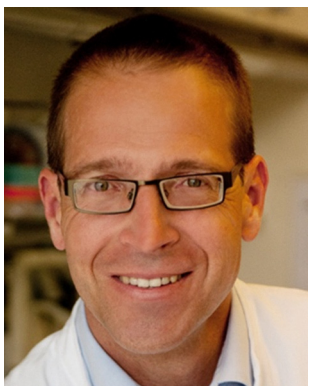


Fig. 1 A chronological overview highlighting major developments in nanobody research, from the initial isolation of camelid-derived nanobodies to the approval of nanobody-based therapeutics by the FDA. The figure includes a structural representation of a nanobody against gelsolin (PDB ID: 2X1P). Created with **https://Biorender.com**.

(~15 kDa), high specificity, and remarkable stability under extreme conditions have made them attractive candidates for diverse therapeutic and diagnostic applications, ranging from oncology and infectious diseases to neurodegenerative disorders.[4–6] One of the key advantages of nanobodies lies in their ability to bind cryptic, three-dimensional epitopes that are often inaccessible to conventional antibodies.[7] Their monomeric nature allows for easier genetic engineering, enabling the development of multivalent or bispecific formats, fusion constructs (Fig. S1, ESI†), and targeted drug delivery systems.[8] Additionally, nanobodies demonstrate excellent tissue penetration, making them valuable for applications such as tumor-targeting and blood–brain barrier (BBB) penetration.[9,10]



Roland H. Stauber

Roland H. Stauber studied Molecular Virology at the University of Würzburg, where he obtained his doctorate. He then conducted postdoctoral research at the National Cancer Institute (USA) before leading research groups at the University of Erlangen and the Georg-Speyer-Haus in Frankfurt. Since 2006, he has been a Professor of Molecular and Cellular Oncology at the Medical University Mainz. His research focuses on cancer cell biology, drug development, and nanobiomedicine. He has led numerous national and international collaborations and has published extensively in high-impact journals.

Shirley K. Knauer

Shirley K. Knauer obtained her PhD with highest honors from the University of Frankfurt. She continued her research as a postdoctoral fellow and as a junior research group leader at the University Medical Center Mainz. In 2010, she joined the University of Duisburg-Essen, where she was later appointed as a full Professor (W2) of Molecular Biology II. Her research focuses on molecular mechanisms of cancer cell biology, with a particular interest in Survivin and Taspase 1 networks. She applies cutting-edge approaches, including live-cell microscopy, to develop novel cancer treatment strategies utilizing supramolecular chemical modulators, nanoparticles, nanobodies, and targeted protein degradation (TPD).

Throughout the years, I've explored nanoscale mechanisms and technologies to understand and manipulate biological systems. I have previously published related work in Nanoscale and Nanoscale Advances and have long appreciated the growing impact of Nanoscale Horizons within the broader nanoscience community. The journal's interdisciplinary scope and commitment to innovation have been a continued source of inspiration. I am honored to contribute to this 10th anniversary collection and look forward to seeing more biology-driven nanoscale research in future issues.

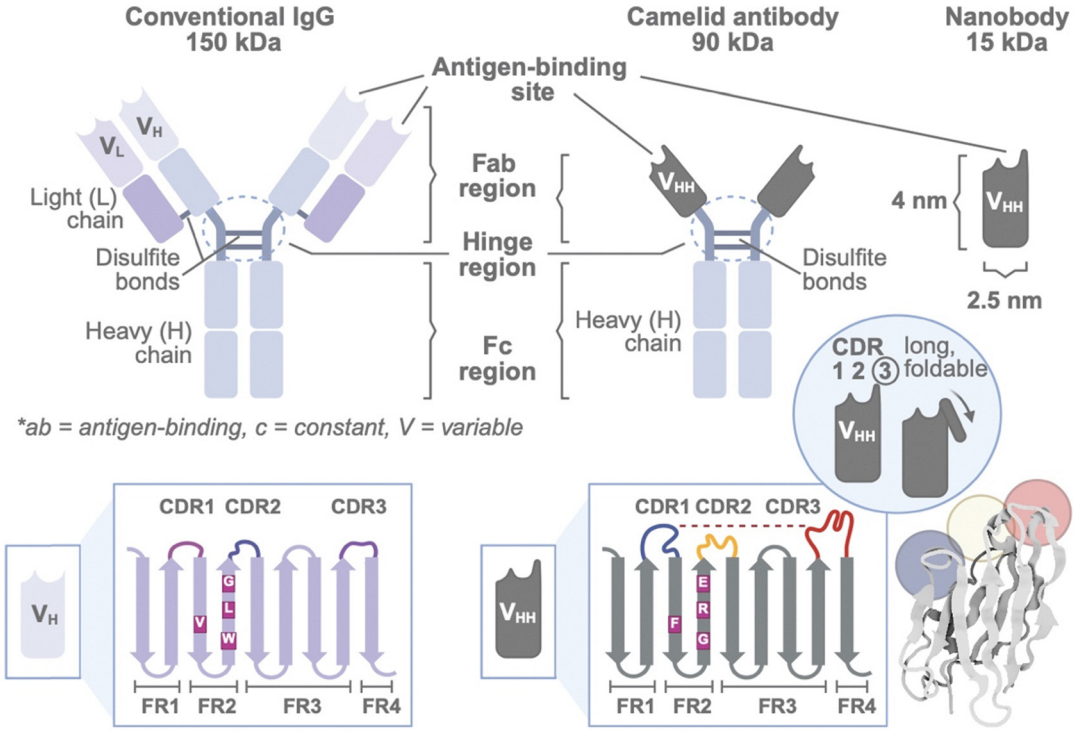


Fig. 2 Schematic of nanobody structure *vs.* conventional antibodies. Side-by-side comparison of a monoclonal antibody (IgG), a camelid heavy chain antibody, and a nanobody (VHH domain), highlighting differences in size, structure, and functional domains (nanobody structure: PDB ID: 2X1P). See main text for details. Created with **https://Biorender.com**.

Building on their favorable biophysical properties and molecular simplicity, the term nanobody technology refers not only to the nanobody molecules themselves but also to the broader landscape of engineering strategies, modular formats, and biomedical applications—ranging from precision delivery systems to their integration into diagnostics, targeted therapies, and synthetic biology. The rapid progress in nanobody engineering,[11] including affinity maturation, humanization,[12–14] and conjugation with therapeutic agents,[15] has further accelerated their clinical translation.[16] Caplacizumab (Cablivi), the first FDA-approved nanobody, has paved the way for the development of additional nanobody-based therapeutics.[17] With several candidates currently in clinical trials, this expanding technology pipeline is also poised to help shape the future of personalized and precision medicine.[18–20]

Due to the growing interest of the scientific community in nanobody-based research is clearly reflected by an increasing number of publications (Fig. S2, ESI†). This review provides a comprehensive overview of nanobody technology, highlighting their structural and functional properties, production and engineering strategies, and therapeutic applications. We explore their role in targeted therapies, molecular imaging, drug delivery, and emerging areas such as targeted protein degradation (TPD).[21] Furthermore, we discuss the challenges associated with clinical translation and manufacturing, along with future perspectives on how nanobody-based therapeutics may shape the next generation of biomedicine.

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2. Nanobody technology: fundamentals and engineering

Nanobodies, or single-domain antibodies (sdAbs), are derived from the heavy-chain-only antibodies (HcAbs) found in camelids such as llamas and alpacas (Fig. 2).[3] Unlike conventional IgG antibodies, which require both heavy and light chains for antigen binding, nanobodies rely solely on a single variable

domain (VHH), yet maintain full antigen-binding capacity. Their small size (~15 kDa) and unique structural features, particularly an extended and flexible complementarity-determining region 3 (CDR3), allow them to access epitopes that are often inaccessible to traditional antibodies, making them highly versatile nanotools for therapeutic applications.[7]

2.1 Nanobody discovery and library generation

Nanobody development follows a streamlined process that typically begins with the immunization of camelids (*e.g.*, alpacas, llamas) using an antigen of interest (Fig. 3).[22] After immune stimulation, peripheral blood lymphocytes are isolated, and the VHH gene segments are amplified by polymerase chain reaction (PCR).[23] The resulting sequences are cloned into expression vectors to construct a nanobody library. Traditionally, phage display has been employed for the selection of high-affinity binders through iterative rounds of biopanning.[24]

To address the limitations of this approach, advanced display technologies have been developed. In particular, yeast surface display, combined with flow cytometry (FACS), now enables high-throughput, quantitative screening of large nanobody libraries based on binding affinity, specificity, and expression profiles.[25–27] Innovative *Escherichia coli* display systems now combine the benefits of both phage and yeast display by presenting nanobodies on the bacterial surface *via* the N-terminal domain of intimin.[28] These next-generation platforms not only improve screening efficiency but also enable affinity maturation under physiologically relevant conditions. Downstream validation of selected binders is typically performed using enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance (SPR), or bio-layer interferometry (BLI), providing robust quantification of nanobody–antigen binding kinetics and target engagement. Furthermore, ribosome display and synthetic or naïve libraries have expanded the nanobody discovery toolbox by enabling purely *in vitro* selection, thus eliminating the need for animal immunization.[29–31] Recent advancements have also introduced transgenic 'LamaMice', genetically engineered mice expressing camelid-like heavy-chain-only antibodies (HcAbs), further broadening the accessibility of nanobody discovery.[32]

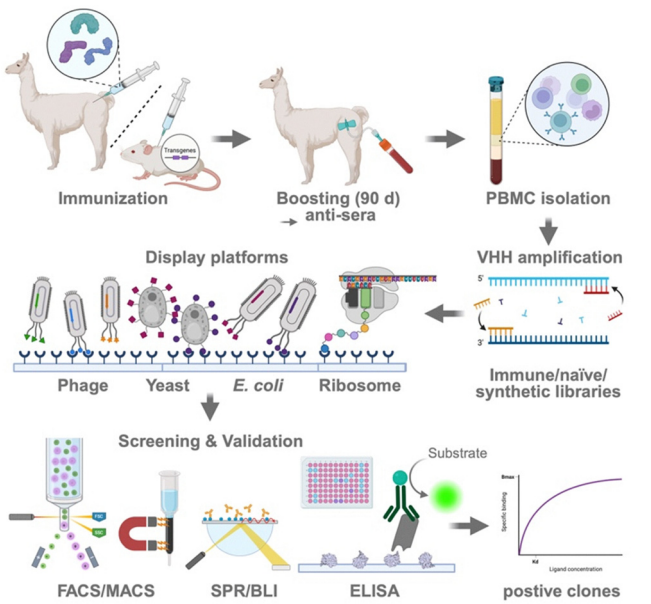

Fig. 3 Generation of nanobodies from camelid immune systems. Workflow illustrating camelid or genetically engineered LamaMice immunization, peripheral blood mononuclear cell (PBMC) isolation, VHH amplification and library construction, followed by nanobody selection through phage display or alternative platforms such as yeast or *E. coli* surface display and ribosome display. Quantitative high- or medium-throughput screening by fluorescence-activated or magnetic cell sorting (FACS/MACS), surface plasmon resonance (SPR)/bio-layer interferometry (BLI) or enzyme-linked immunosorbent assay (ELISA) support downstream validation of antigen-specific binders as positive clones. See main text for details. Created with https://Biorender.com.

2.2 Expression platforms for nanobody production

Nanobody expression is adaptable across multiple host systems, each offering unique advantages (Fig. S3, ESI†).[33] Bacterial systems such as *Escherichia coli* are widely used due to their cost-effectiveness and scalability, making them ideal for research and industrial production.[34] Yeast expression systems, including *Pichia pastoris* and *Saccharomyces cerevisiae*, provide eukaryotic post-translational modifications and higher secretion yields.[35] Mammalian cell lines, such as human embryonic kidney (HEK293) and Chinese hamster ovary (CHO) cells, ensure proper glycosylation and are preferred for therapeutic applications. More recently, *in vivo* expression strategies using viral vectors or mRNA delivery have emerged as promising approaches for direct nanobody administration in patients.[36,37]

2.3 Engineering strategies for therapeutic optimization

To enhance therapeutic efficacy, nanobody engineering strategies focus on improving affinity, specificity, stability, and functionality.[11] Affinity maturation involves introducing mutations in CDR regions (see Fig. 2) through techniques like error-prone PCR or saturation mutagenesis to enhance binding affinity.[38,39] Humanization modifies camelid-derived VHH frameworks to resemble human IgG sequences, reducing potential undesired immunogenicity.[40,41] Multivalent and multispecific nanobodies, including dimeric, trimeric, and bispecific formats, improve avidity and allow simultaneous targeting of multiple antigens (Fig. S1, ESI†).[42,43]

This is particularly beneficial for cancer immunotherapy and immune cell recruitment. Additionally, nanobody–drug and nanobody–enzyme fusions enable targeted drug delivery and enzymatic degradation of disease-associated proteins (Table S1, ESI†).[44–46]

2.4 Functionalization and clinical advantages

Nanobodies offer several distinct advantages over traditional monoclonal antibodies (Table S2, ESI†).[4] Their small size enables superior tissue penetration, allowing better diffusion in solid tumors and across biological barriers, including the blood–brain barrier. Their high thermal and chemical stability makes them suitable for challenging environments, including oral and inhalable formulations. Their simple structure facilitates genetic

manipulation, enabling straightforward conjugation with therapeutic payloads such as toxins, radionuclides, or nanoparticles. Additionally, their production in microbial systems significantly reduces manufacturing costs compared to full-length antibodies. The unique ability of nanobodies to recognize cryptic epitopes, such as enzyme active sites and intracellular protein interactions, further expands their therapeutic potential.[47]

Nanobody engineering has transformed the field of biologics by offering a scalable and adaptable platform for targeted therapeutics. Their unique biophysical properties, combined with advancements in recombinant expression and molecular optimization, have enabled their rapid expansion across diverse biomedical applications.

3. Therapeutic applications of nanobodies

Nanobody technology has rapidly evolved from a promising research tool to a clinically relevant class of therapeutics with applications across multiple disease areas, including cancer, neurological disorders, infectious diseases, and autoimmune conditions.[48,49] Beyond direct therapeutic applications, nanobodies have also been engineered for targeted drug delivery and imaging, further expanding their potential in precision medicine.[50] This chapter provides an overview of the diverse therapeutic applications of nanobody-based strategies, focusing on their role in oncology, neurology, infectious diseases, inflammatory disorders, and other emerging medical fields.

3.1 Oncology applications

Cancer remains a leading cause of mortality worldwide, necessitating the development of highly selective and potent therapeutics.[51] Nanobody-based strategies have shown promise in multiple aspects of cancer therapy, notably in immune checkpoint inhibition, targeted drug delivery, and cellular immunotherapy (Fig. 4).[42,52]

3.1.1 Immune checkpoint inhibition. The immunosuppressive tumor microenvironment frequently inhibits T-cell activity through checkpoint molecules such as PD-1, PD-L1, CTLA-4, and TIGIT. Nanobody-based immune checkpoint inhibitors restore T-cell-mediated antitumor responses by blocking these pathways. Due to their small size and favorable pharmacokinetics, nanobodies penetrate solid tumors more efficiently and may exhibit reduced off-target toxicity compared to conventional monoclonal antibodies.[53] Nanobodies targeting PD-1 and PD-L1 have been shown to reinvigorate exhausted T cells, while those against CTLA-4 and TIGIT address alternative immune resistance mechanisms.[54]

3.1.2 Targeted drug delivery *via* nanobody–drug conjugates (NDCs). NDCs leverage nanobody scaffolds to selectively deliver cytotoxic agents to tumor cells while sparing healthy tissues.[55–57] These conjugates typically incorporate cleavable linkers that release the drug payload upon internalization or within the tumor microenvironment. HER2-specific nanobody conjugates have demonstrated efficacy in preclinical breast cancer models,

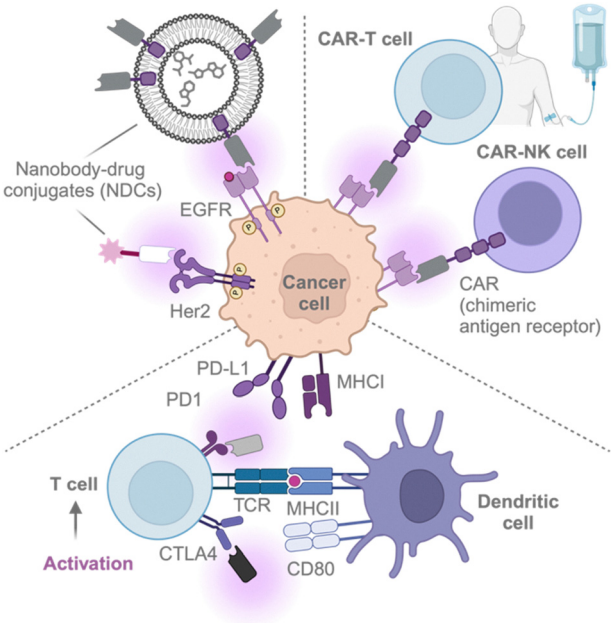

Fig. 4 Nanobody applications in cancer immunotherapy. Nanobody–drug conjugates (NDCs) for tumor targeting (upper left), nanobody-based CAR-T therapy (nanobody-directed CARs, upper right) and nanobody-based immune checkpoint inhibitors (anti-PD1 and anti-CTLA4, bottom). See main text for details. Created with https://Biorender.com.

while EGFR- and PD-L1-targeting NDCs are being investigated for non-small cell lung cancer and or head and neck tumours.[58–61]

3.1.3 Nanobody-based cellular immunotherapy (CAR-T/CAR-NK). Chimeric antigen receptor (CAR) T-cell (CAR-T) and natural killer (CAR-NK) cell therapies rely on engineered surface receptors to redirect immune effector cells against cancer. Nanobodies are increasingly used as the antigen recognition domains in CAR constructs, offering enhanced engineering flexibility and reduced immunogenicity.[52,60,62] Nanobody-CAR-T cells have been developed for hematological malignancies (*e.g.*, $CD19^+$ leukemias), $HER2^+$ breast cancer, and EGFR-mutated glioblastomas.[63–65] CAR-NK cells incorporating nanobody recognition domains provide a potentially safer alternative, with lower risks of cytokine release syndrome and graft-*versus*-host disease. In addition, nanobody-CARs allow rapid affinity tuning and multi-specificity designs, supporting broader tumor targeting strategies.

3.1.4 Emerging non-classical CAR approaches. Beyond classical genetic engineering, non-classical nanobody-CAR strategies are emerging, particularly those leveraging non-genetic receptor engagement on NK cells. A recently described CAR-like nanobody platform co-opts endogenous activating receptors on NK cells to achieve tumor recognition and cytotoxicity without the need for gene modification.[66] In parallel, recent literature has highlighted the potential of these approaches in providing modular, regulatory-simplified, and scalable solutions for safer, off-the-shelf cell therapies.[67] These non-classical strategies complement conventional CAR technologies and broaden the translational landscape of nanobody-guided cellular immunotherapy.

3.2 Neurological disorders and brain delivery

The ability of nanobodies to cross the blood–brain barrier (BBB) makes them valuable for treating central nervous system (CNS) diseases (Fig. 5).[68,69] Targeted nanobody-based approaches are being explored for the delivery of therapeutic agents to the brain, addressing neurodegenerative diseases such as Alzheimer's and Parkinson's disease, which are characterized by the accumulation of misfolded proteins.[69,70] Nanobody-based strategies have been investigated for selectively targeting and neutralizing these toxic protein aggregates. anti-Tau and anti-amyloid-β nanobodies are under development for reducing Tau tangles and amyloid-β plaques in Alzheimer's disease,[71] while α-synuclein-targeting nanobodies are being explored as a means to prevent fibril formation in Parkinson's disease.[72]

Beyond extracellular targeting, recent studies have demonstrated that intracellularly expressed nanobodies, "intrabodies", can disrupt aggregate seeding and propagation within neurons.[73,74] For example, intrabodies directed against Tau, α-synuclein, and TDP-43 have shown potent inhibition of pathological spread in cell models of neurodegeneration.[75]

To enhance BBB transport, receptor-mediated transcytosis (RMT) strategies have been implemented, where nanobodies are engineered to engage endogenous receptors such as transferrin receptor (TfR), insulin-like growth factor 1 receptor (IGF1R), or low-density lipoprotein receptor-related protein 1 (LRP1).[76,77] VHHs such as FC5 and FC44 have demonstrated efficient transcytosis and brain delivery *via* the TfR and IGF1R pathways, with improved CNS uptake *in vivo*.[78] pH-sensitive anti-TfR nanobody variants, such as the M1 mutant, further enhance brain delivery while avoiding receptor degradation and saturation.[79] Bispecific nanobody constructs combining BBB-shuttling domains with anti-aggregate nanobodies have achieved dual functionality in preclinical models.[80,81] For instance, anti-TfR/BACE1 bispecific nanobodies reduced brain amyloid-β levels by approximately 40% in transgenic mouse models, while TfR-amyloid-β conjugates (*e.g.*, Bapi-TXB2) achieved over a threefold increase in brain penetration compared to conventional monoclonal antibodies.[82]

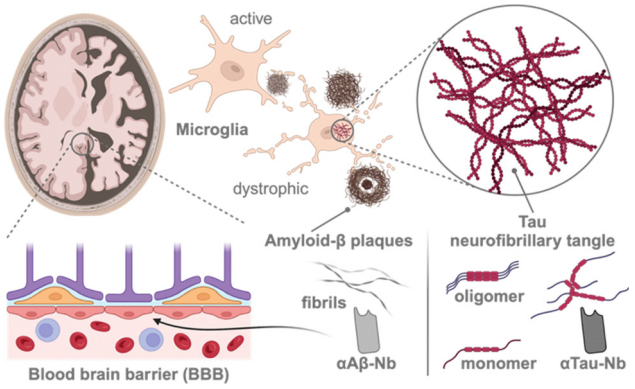

Fig. 5 Nanobody-based therapies for neurological disorders. Graphical representation of nanobody drugs targeting neurodegenerative diseases (*e.g.*, anti-Tau, anti-Aβ nanobodies for Alzheimer's disease) and potential delivery enabling blood–brain barrier (BBB) crossing. See main text for details. Created with **https://Biorender.com**.

Moreover, nanobody-based imaging agents are being developed for real-time, non-invasive visualization of pathology.[83] PET- and MRI-compatible anti-amyloid-β and anti-α-synuclein nanobodies fused with TfR-shuttling domains might thus be used to image aggregate distribution in living animals, enabling longitudinal disease monitoring. Emerging delivery technologies such as nanobody–nanoparticle conjugates and mRNA-encoded nanobody expression systems hold promise for systemic administration, sustained brain exposure, and payload versatility.[84–86] These approaches offer scalable and modular formats for both therapy and diagnosis.

Together, these innovations position nanobody platforms as versatile tools for both therapeutic intervention and diagnostic imaging in complex CNS disorders.

3.3 Infectious disease therapeutics

Nanobodies offer a promising approach for combating viral, bacterial, and fungal infections due to their ability to recognize conserved epitopes and neutralize pathogens effectively.[72,87]

Several nanobody candidates targeting SARS-CoV-2 have been developed to neutralize the virus by binding to the spike protein, with some progressing to clinical trials.[18,88] Nanobody therapies for HIV and influenza have also shown potential by blocking viral entry through interactions with key glycoproteins.[87,89] In bacterial infections, nanobody-based neutralizing agents have been developed against toxins from *Clostridium difficile*, anthrax, and botulinum, providing a novel strategy for treating sepsis and toxin-mediated diseases.[90,91]

3.4 Other and emerging therapeutic areas

Nanobody-based inhibitors of proprotein convertase subtilisin/kexin type 9 (PCSK9) have shown promise for lowering cholesterol levels in patients with hypercholesterolemia.[92] Additionally, nanobody therapies are being investigated for their potential in treating lysosomal storage diseases, such as Gaucher's disease, by targeting defective enzymes.[93] As research into nanobody applications continues, their potential for addressing rare diseases and novel therapeutic targets is expanding though it will require 'smart' innovative strategies, such as nanobody-/drug-/nanomaterial combinations.

3.5 Perspectives

Nanobody-based therapeutics have demonstrated remarkable potential across a broad spectrum of diseases, offering advantages such as enhanced tissue penetration, high specificity, and reduced immunogenicity. Their application in cancer immunotherapy, infectious disease treatment, neurodegenerative disorders, and autoimmune diseases highlights their versatility and clinical relevance. Ongoing development efforts continue to expand the scope of nanobody-based interventions, paving the way for their integration into precision/personalized medicine and next-generation therapeutics. Indeed, several of the described applications increasingly leverage advanced delivery and imaging strategies, as highlighted in the following section on nanobody-based technologies for targeted delivery, molecular imaging, and intracellular modulation.

4. Nanobody-based technologies in drug delivery and molecular imaging

Complementing their direct therapeutic applications, nanobodies also serve as powerful platforms for precision delivery and molecular imaging.[94] Their ease of genetic and chemical modification enables the creation of multifunctional constructs that improve tissue-specific drug targeting, enhance biodistribution, and facilitate real-time visualization of disease processes. This section outlines the technological advances that underpin these applications and illustrates how nanobody-based formats support the broader goals of personalized and systems-level medicine.

4.1 Targeted drug delivery platforms

The ability to guide therapeutic agents precisely to diseased cells or tissues while minimizing off-target effects is a major goal in modern drug development. Nanobody-based drug delivery systems leverage their unique properties to enhance targeting efficiency (Fig. 6).[95] Cell-penetrating peptides (CPPs) like TAT and arginine-rich R9 help deliver otherwise impermeable therapeutics into cells by interacting with negatively charged membranes, mainly *via* endocytosis or direct translocation.[96] Similar charged regions can also be engineered into nanobodies, creating cell-penetrating domain antibodies.[97] Nanobody–drug conjugates (NDCs) function similarly to antibody–drug conjugates (ADCs) but with improved tumor penetration and reduced immunogenicity.[16,45,98–100] These conjugates typically involve a cytotoxic payload attached to a nanobody *via* a cleavable linker, as seen in HER2-targeted NDCs for breast cancer, which selectively eliminate HER2-positive cancer cells,[58] and EGFR-targeted NDCs for colorectal cancers,[101] which enable precise targeting of EGFR-mutated tumors. Nanobodies have also been used to functionalize and improve nanoparticles, enhancing their ability to deliver drugs specifically to target cells. Various nano-particle/-material platforms have been developed, including liposomes that encapsulate chemotherapeutic drugs for enhanced tumor targeting, polymeric nanoparticles designed for controlled drug release in chronic diseases, and gold or magnetic nanoparticles explored for theranostic applications, where they serve as both therapeutic carriers and imaging agents.[102,103] Exosomes – nanoscale extracellular vesicles naturally secreted by most cell types – have gained significant attention as biocompatible drug delivery systems due to their inherent stability, low immunogenicity, and natural ability to traverse biological barriers.[104] Recent advances have enabled the functionalization of exosomes with nanobodies to enhance targeting precision.[105,106] In this strategy, nanobodies are typically displayed on the exosome surface *via* genetic fusion to endogenous exosomal membrane proteins such as Lamp2b or CD63, enabling selective binding to target antigens on recipient cells. This approach allows the targeted delivery of therapeutic payloads, including siRNAs, small-molecule drugs, or gene-editing systems (*e.g.*, CRISPR-Cas components), while minimizing off-target effects.[107] Nanobody-modified exosomes have shown promising results in preclinical models of cancer and other diseases, offering a modular, customizable, and cell-compatible platform for next-generation targeted therapies.[108] Nanobody-based targeting moieties are increasingly employed in future gene therapy and mRNA delivery strategies, with lipid nanoparticle-based micro-/-mRNA delivery systems enabling tissue-specific RNA transport as well as adeno-associated virus (AAV) targeting approaches improving the specificity of viral vectors for gene therapy.[109]

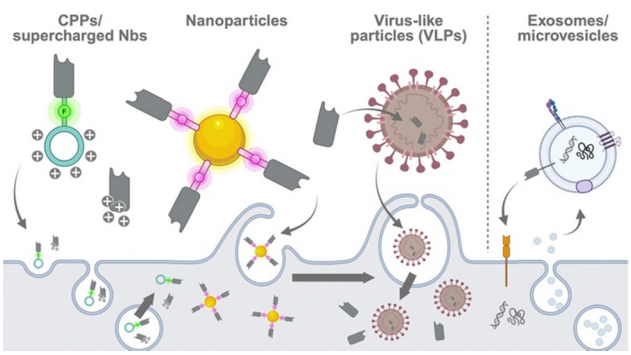

Fig. 6 Intracellular nanobody delivery strategies. Illustration of methods for delivering nanobodies inside cells, including (cyclic) cell-penetrating peptides (CPPs) or cell-penetrating, supercharged nanobodies, nanobody-functionalized nanoparticles and virus-like particles (VLPs) for nanobody packaging (left). Moreover, exosomes might be genetically engineered for cell-specific cargo delivery (right). See main text for details. Created with https://Biorender.com.

4.2 Nanobody-based diagnostic and imaging tools

Likewise, nanobody-based imaging agents offer significant advantages in diagnostic imaging, including rapid clearance from circulation, high specificity, and improved contrast (Table S3, ESI†).[110] Indeed, their small size allows for deep tissue penetration, making them valuable tools in both preclinical and clinical settings.

Nanobody conjugates labeled with radioactive isotopes have been developed for positron emission tomography (PET) and single-photon emission computed tomography (SPECT) imaging, such as HER2-specific nanobody radiotracers for early-stage breast cancer detection and PD-L1 imaging tools that assess immunotherapy responses in real time (Fig. S4, ESI†).[111,112] Nanobody-based fluorescent probes enable real-time imaging in both *in vitro* and *in vivo* settings, including applications such as electrochemical sensing and live-cell imaging of intracellular targets using nanobody-GFP fusions (Fig. 7) to study protein–protein interactions, and fluorescently labeled nanobodies for tumor margin detection during surgical resection.[113–115] More universally applicable tagging systems, such as the ALFA-tag, that consists of a small, rationally designed α-helical epitope, can even function independently of their position on the target protein.[116]

When combined with high-affinity nanobodies, these systems enable a broad array of applications – including super-resolution and live-cell microscopy, immunoblotting, immunoprecipitation, and gentle purification of native protein complexes or whole cells – while their compact size further supports high-precision imaging of subcellular structures.[117] Additionally, nanobody-conjugated contrast agents have been designed to enhance magnetic resonance imaging (MRI) and ultrasound imaging,

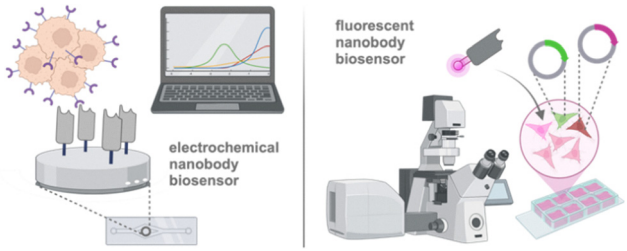

Fig. 7 Nanobody-based biosensors and molecular probes. Schematic of a nanobody-functionalized electrochemical biosensor for disease biomarkers and fluorescent nanobody biosensors for live-cell imaging. See main text for details. Created with https://Biorender.com.

with nanobody-functionalized iron oxide nanoparticles used for imaging of brain tumors and microbubble-based ultrasound imaging improving vascular and tumor imaging precision.[118–120]

4.3 Targeted protein degradation (TPD)

One of the most exciting developments in nanobody technology is its application in targeted protein degradation (TPD) strategies (Fig. 8).[121,122] By leveraging nanobody-based approaches, researchers are designing novel methods to selectively degrade disease-associated proteins. Nanobody-PROTAC conjugates represent a new frontier in targeted degradation, offering higher specificity than small-molecule PROTACs and enabling the degradation of traditionally undruggable targets.[123] E3 ligase-recruiting nanobody-PROTACs bring target proteins into proximity with E3 ligases, leading to ubiquitination and subsequent degradation,[122] while intracellular protein degradation strategies have been explored for oncogenic transcription factors, misfolded proteins in neurodegenerative diseases, and inherited blood disorders like sickle cell disease and β-thalassemia.[124] Molecular glues facilitate protein–protein interactions to induce targeted degradation,

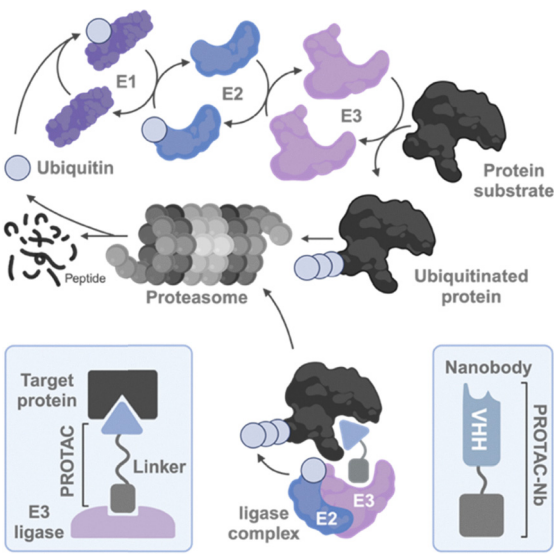

Fig. 8 Nanobody-based PROTACs and targeted protein degradation. Illustration of a nanobody-PROTAC mechanism where nanobody recruitment leads to ubiquitination and degradation of a target protein. See main text for details. Created with https://Biorender.com.

and nanobody-based molecular glues are being developed to enhance degradation pathways for proteins lacking suitable PROTAC binding sites.[125–127]

4.4 Nanobody-based vaccine platforms

The awarding of the 2023 Nobel Prize in Physiology or Medicine for the development of mRNA vaccine technologies underscores the transformative potential of modular biologics. In this context, nanobodies are emerging as promising components for next-generation vaccine platforms. For example, mRNA-encoded nanobody-Fc fusions delivered *via* lipid nanoparticles (LNPs) have demonstrated protective efficacy against lethal botulinum neurotoxin A in preclinical models.[128] Similarly, lung-selective LNPs encoding anti-MERS-CoV nanobodies achieved robust *in vivo* expression and potent viral neutralization.[129] The potential of nanobodies in antiviral immunity has also been shown in the context of SARS-CoV-2, where an alpaca-derived nanobody effectively neutralized the virus by blocking spike protein–receptor interactions.[130] These findings suggest that future vaccine strategies may leverage nanobodies to create multivalent or multiepitope constructs, improve thermostability, or enable rapid *in vivo* expression—positioning nanobody platforms at the forefront of vaccine innovation.[6]

4.5 Challenges and perspectives

Despite the significant progress in nanobody-based drug delivery and imaging, various challenges remain. Their short circulatory half-life, due to rapid renal clearance, necessitates strategies such as albumin fusion or PEGylation to improve stability.[131,132] Limited intracellular delivery efficiency remains a hurdle, as nanobodies primarily target extracellular antigens, prompting ongoing efforts to enhance cell-penetrating nanobody strategies.[97,133] Scalability and manufacturing issues must also be optimized, as bacterial expression systems provide cost-effective production, but large-scale manufacturing requires optimized downstream processing.[132]

Future perspectives in nanobody-based technologies include expanding into CRISPR-based gene editing with nanobody-fused CRISPR effectors for targeted gene modulation,[134] developing supramolecular nanobody conjugates for biomolecular assembly and synthetic biology applications,[135–137] and leveraging AI-driven approaches to design patient-specific nanobody therapeutics (Fig. S5, ESI†).[138] As advances in engineering, conjugation chemistry, and intracellular targeting continue, nanobody-driven innovations are foreseen to play a critical role in shaping the future of personalized medicine.

5. Clinical translation and commercialization of nanobody-based therapeutics

Nanobody-based therapeutics are advancing from preclinical research to clinical applications, with several candidates achieving regulatory approval or progressing through trials. Their transition into widespread clinical use requires overcoming

regulatory, manufacturing, and commercialization challenges (Fig. S6, ESI†).[139]

Caplacizumab (Cablivi) is the first approved nanobody drug, targeting von Willebrand factor for treating acquired thrombotic thrombocytopenic purpura (aTTP).[17] Other nanobody-based therapies, including TNFα inhibitors (Ozoralizumab)[140] and anti-IL-6 agents (Vobarilizumab),[141] are in development for autoimmune diseases. Nanobody-based imaging agents for HER2 and PD-L1 are also in clinical trials.[142,143] Investigations extend to cancer immunotherapy (nanobody-based checkpoint inhibitors, CAR-T),[142,144] neurological diseases (anti-Tau and anti-Aβ nanobodies for Alzheimer's),[71,145] and infectious diseases (SARS-CoV-2 neutralizers).[146] A list of clinical studies and examples for FDA-approved and clinically investigated nanobodies can be found in Tables S4 and S5 (ESI†). As mentioned, challenges include rapid clearance requiring half-life extension, limited intracellular targeting, and potential immunogenicity. Regulatory approval follows biologics guidelines, but nanobody-specific regulations are still evolving. Market entry requires novel rigorous preclinical, clinical, and post-market assessments. Nanobody production relies on scalable microbial expression systems, such as *E. coli* (cost-effective but requires refolding), the yeast *Pichia pastoris* (proper protein folding), and mammalian cells (*e.g.*, CHO) (Fig. S3, ESI†). Manufacturing is generally more cost-effective than monoclonal antibodies, but commercialization depends on pricing, reimbursement, and patent strategies.[147,148] Key industry players include Ablynx (Sanofi), Merck, Novartis, and emerging biotech firms developing nanobody-PROTACs and intracellular degraders. To sum up: future developments focus on nanobody-enhanced CAR-T therapies,[113] CRISPR-based gene editing,[134] and AI-driven nanobody design for personalized medicine.[138]

Conclusions and outlook

Nanobody technology has emerged as a transformative platform in biotechnology and medicine, offering distinct advantages over conventional antibody-based therapeutics. Their small size, high specificity, and engineering flexibility have enabled applications ranging from molecular imaging and targeted therapy to intracellular protein modulation and immune regulation. Over the past three decades, nanobody-based therapies have advanced from experimental research to clinical translation, with caplacizumab marking a milestone as the first approved nanobody therapeutic. Ongoing clinical trials further highlight their promise in oncology, autoimmune diseases, and infectious diseases. In this context, this review bridges the gap between the biophysical design of nanobodies and their translational integration into advanced therapeutic platforms such as TPD, CAR-T, and gene editing tools. We highlight emerging display and screening strategies, including yeast and *E. coli* display, FACS/MACS, SPR/BLI, and ribosome display, that enable quantitative, high-throughput selection of nanobody binders with therapeutic potential.

AI-driven discovery and optimization strategies are accelerating the development of next-generation nanobody therapeutics with improved affinity, stability, and bioavailability. The convergence of nanobody technology with gene editing (*e.g.*, CRISPR-Cas), CAR-T cell therapy, and nanomaterial-based drug delivery is expanding its therapeutic landscape, unlocking new opportunities for precision medicine.

Despite these advances, challenges remain, including intracellular delivery barriers, regulatory complexities, and large-scale manufacturing hurdles (Table S5, ESI†). However, rapid progress in nanobody engineering, AI-guided affinity maturation, and *in vitro* library technologies, combined with growing industrial investment is likely to overcome these obstacles in the coming years. As these technologies mature, nanobody-based therapeutics are poised to revolutionize disease treatment, enabling minimally invasive diagnostics, patient-specific therapies, and interventions targeting previously "undruggable" proteins. With continued innovation, nanobody-driven approaches will play a crucial role in shaping the future of precision medicine and next-generation biologics, offering new hope for patients with complex and currently untreatable diseases.

As extensively demonstrated by many colleagues and us, nanoscale biological and synthetic structures can be characterized with high precision under idealized conditions.[149–151] However, this precision is compromised in complex physiological or natural environments, particularly in (bio)medical applications. In such settings, proteins and other biomolecules rapidly adsorb onto nano-sized structures, leading to the formation of the so-called "protein/biomolecule corona" (not related to the SARS coronavirus), which critically influences the nanomaterial's (patho)biological behaviour, technical performance, and biomedical efficacy.[152–156] Given the corona's impact on both *in vitro* and *in vivo* applications in humans and ecosystems, a mechanistic understanding of its significance and the biophysical forces governing its formation is essential.[157–160] Notably, such investigations have yet to be conducted for nanobodies preceding their therapeutic use, where they come into contact with *e.g.* blood serum or other biofluids.[84]

Nanobodies, within the context of 'Nanoscale Horizons', represent a promising yet challenging frontier at the intersection of biological and synthetic nanostructures. Beyond current applications, nanobody-based biologics are increasingly viewed as potential successors to conventional monoclonal antibodies in next-generation medicine. Their compact size, modularity, and microbial producibility offer distinct advantages for targeting dense tissue environments, enabling intracellular modulation, and facilitating real-time diagnostics. Nevertheless, the path to widespread translational adoption still requires overcoming major hurdles, most notably, the development of reliable endosomal escape strategies for intracellular targets, scalable and globally accessible production platforms, and computationally guided engineering workflows that leverage AI to enhance nanobody properties. Ongoing convergence with gene and cell therapies, as well as smart delivery technologies, will likely define the next wave of innovation in this rapidly evolving field. We hope that our review contributes to stimulating experimental and scientific dialogue in this domain. The complexity of these challenges not only necessitates but may

also inspire interdisciplinary research efforts. Finally, we would like to thank *Nanoscale Horizons* for offering a high-quality platform that supports scientific inquiry and fosters meaningful innovation.

Author contributions

Each named author has substantially contributed to drafting and revising this manuscript.

Conflicts of interest

There are no conflicts to declare.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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References

1 P. C. Fridy, M. P. Rout and N. E. Ketaren, *Mol. Cell. Proteomics*, 2024, **23**, 100865.
2 J. Gettemans and B. De Dobbelaer, *Am. J. Physiol.: Cell Physiol.*, 2021, **320**, C195–C215.
3 E. Alexander and K. W. Leong, *J. Nanobiotechnol.*, 2024, **22**, 661.
4 Y. Wu, *Discover Nano*, 2025, **20**, 23.
5 V. M. Minatel, C. R. Prudencio, B. Barraviera and R. S. Ferreira, *Front. Immunol.*, 2024, **14**, 1303353.
6 S. S. Rizk, D. M. Moustafa, S. A. ElBanna, H. T. Nour El-Din and A. S. Attia, *World J. Microbiol. Biotechnol.*, 2024, **40**, 209.
7 S. Ma, D. Zhang, Q. Wang, L. Zhu, X. Wu, S. Ye and Y. Wang, *PLoS Pathog.*, 2024, **20**, e1012438.
8 Y. Xiang, J. Xu, B. L. McGovern, A. Ranzenigo, W. Huang, Z. Sang, J. Shen, R. Diaz-tapia, N. D. Pham, A. J. P. Teunissen, M. L. Rodriguez, J. Benjamin, D. J. Taylor, M. M. T. van Leent, K. M. White, A. García-Sastre, P. Zhang and Y. Shi, *Cell*, 2024, **187**, 6966–6980.e6923.
9 G. Bao, M. Tang, J. Zhao and X. Zhu, *EJNMMI Res.*, 2021, **11**, 6.
10 E. Ruiz-López and A. J. Schuhmacher, *Biomolecules*, 2021, **11**, 1131.
11 J. Liu, L. Wu, A. Xie, W. Liu, Z. He, Y. Wan and W. Mao, *J. Nanobiotechnol.*, 2025, **23**, 87.
12 Z. Sang, Y. Xiang, I. Bahar and Y. Shi, *Structure*, 2022, **30**, 418–429.e413.
13 G. L. Gordon, M. I. J. Raybould, A. Wong and C. M. Deane, *Front. Immunol.*, 2024, **15**, 1399438.
14 M. Tabasinezhad, Y. Talebkhan, W. Wenzel, H. Rahimi, E. Omidinia and F. Mahboudi, *Immunol. Lett.*, 2019, **212**, 106–113.
15 R. K. Tripathy and A. H. Pande, *Pharm. Res.*, 2025, **42**, 219–236.
16 P. C. Fridy, M. P. Rout and N. E. Ketaren, *Mol. Cell. Proteomics*, 2024, **23**, 100865.
17 M. le Besnerais, V. Agnès, B. Ygal and P. Coppo, *Expert Opin. Biol. Ther.*, 2019, **19**, 1127–1134.
18 Y. Yang, F. Li and L. Du, *J. Nanobiotechnol.*, 2024, **22**, 304.
19 M. Hosseininejad-Chafi, Z. Eftekhari, A. Oghalaie, M. Behdani, N. Sotoudeh and F. Kazemi-Lomedasht, *Med. Oncol.*, 2024, **42**, 36.
20 L. Wang, R. Luo, W. Zhang, H. Jiang, Y. Yu, W. Zhou, F. Zhang, J. Ma and L. Mei, *Smart Mater. Med.*, 2024, **5**, 501–513.
21 M. Békés, D. R. Langley and C. M. Crews, *Nat. Rev. Drug Discovery*, 2022, **21**, 181–200.
22 L. D. J. Schiffelers, Y. M. Tesfamariam, L.-M. Jenster, S. Diehl, S. C. Binder, S. Normann, J. Mayr, S. Pritzl, E. Hagelauer, A. Kopp, A. Alon, M. Geyer, H. L. Ploegh and F. I. Schmidt, *Nat. Commun.*, 2024, **15**, 8266.
23 M. Ganji, P. Safarzadeh Kozani and F. Rahbarizadeh, *J. Transl. Med.*, 2023, **21**, 891.
24 S. Muyldermans, *Annu. Rev. Biochem.*, 2013, **82**, 775–797.
25 J. Zahradník, D. Dey, S. Marciano, L. Kolářová, C. I. Charendoff, A. Subtil and G. Schreiber, *ACS Synth. Biol.*, 2021, **10**, 3445–3460.
26 C. McMahon, A. S. Baier, R. Pascolutti, M. Wegrecki, S. Zheng, J. X. Ong, S. C. Erlandson, D. Hilger, S. G. F. Rasmussen, A. M. Ring, A. Manglik and A. C. Kruse, *Nat. Struct. Mol. Biol.*, 2018, **25**, 289–296.
27 T. Uchański, T. Zögg, J. Yin, D. Yuan, A. Wohlkönig, B. Fischer, D. M. Rosenbaum, B. K. Kobilka, E. Pardon and J. Steyaert, *Sci. Rep.*, 2019, **9**, 382.
28 V. Salema and L. Á. Fernández, *Microb. Biotechnol.*, 2017, **10**, 1468–1484.
29 K. Kajiwara, W. Aoki and M. Ueda, *AMB Express*, 2020, **10**, 51.
30 A. Guilbaud and F. Pecorari, in *Genotype Phenotype Coupling: Methods and Protocols*, ed. S. Zielonka and S. Krah, Springer US, New York, NY, 2023, pp. 19–31, DOI: **10.1007/978-1-0716-3279-6_2**.
31 A. Guilbaud and F. Pecorari, *Methods Mol. Biol.*, 2023, **2681**, 19–31.
32 T. Eden, A. Z. Schaffrath, J. Wesolowski, T. Stahler, N. Tode, N. Richter, W. Schafer, J. Hambach, I. Hermans-Borgmeyer, J. Woens, C. M. Le Gall, S. Wendler, C. Linke-Winnebeck, M. Stobbe, I. Budnicki, A. Wanney, Y. Heitz, L. Schimmelpfennig, L. Schweitzer, D. Zimmer, E. Stahl,

F. Seyfried, A. J. Gebhardt, L. Dieckow, K. Riecken, B. Fehse, P. Bannas, T. Magnus, M. Verdoes, C. G. Figdor, K. F. Hartlepp, H. Schleer, J. Funer, N. M. Tomas, F. Haag, B. Rissiek, A. M. Mann, S. Menzel and F. Koch-Nolte, *Nat. Commun.*, 2024, **15**, 4728.

33 I. Legastelois, S. Buffin, I. Peubez, C. Mignon, R. Sodoyer and B. Werle, *Hum. Vaccines Immunother.*, 2017, **13**, 947–961.

34 A. de Marco, *Protein Expression Purif.*, 2020, **172**, 105645.

35 Y. Wang, X. Li, X. Chen, J. Nielsen, D. Petranovic and V. Siewers, *Microb. Cell Fact.*, 2021, **20**, 134.

36 N. Silva-Pilipich, C. Smerdou and L. Vanrell, *Microorganisms*, 2021, **9**, 1956.

37 Y. Zhang, C. Tian, X. Yu, G. Yu, X. Han, Y. Wang, H. Zhou, S. Zhang, M. Li, T. Yang, Y. Sun, W. Tai, Q. Yin and G. Zhao, *Vaccines*, 2024, **12**, 1315.

38 N. Langreder, D. Schäckermann, T. Unkauf, M. Schubert, A. Frenzel, F. Bertoglio and M. Hust, in *Phage Display: Methods and Protocols*, ed. M. Hust and T. S. Lim, Springer US, New York, NY, 2023, pp. 395–410, DOI: **10.1007/978-1-0716-3381-6_20**.

39 R. Gopal, E. Fitzpatrick, N. Pentakota, A. Jayaraman, K. Tharakaraman and I. Capila, *Viruses*, 2022, **14**, 2694.

40 T. Sulea, in *Single-Domain Antibodies: Methods and Protocols*, ed. G. Hussack and K. A. Henry, Springer US, New York, NY, 2022, pp. 299–312, DOI: **10.1007/978-1-0716-2075-5_14**.

41 C. Vincke, R. Loris, D. Saerens, S. Martinez-Rodriguez, S. Muyldermans and K. Conrath, *J. Biol. Chem.*, 2009, **284**, 3273–3284.

42 M. I. Mustafa and A. Mohammed, *SLAS Discovery*, 2023, **28**, 358–364.

43 M. Compte, L. Sanz and L. Álvarez-Vallina, in *International Review of Cell and Molecular Biology*, ed. F. Aranda, P. Berraondo and L. Galluzzi, Academic Press, 2022, vol. 369, pp. 71–87.

44 H. Huang, T. Wu, H. Shi, Y. Wu, H. Yang, K. Zhong, Y. Wang and Y. Liu, *Chem. Commun.*, 2019, **55**, 5175–5178.

45 L. Liu, B. Tu, Y. Sun, L. Liao, X. Lu, E. Liu and Y. Huang, *J. Controlled Release*, 2025, **381**, 113562.

46 M. S. Abdelhamid, A.-H. S. Wadan, H. A. Saad, W. A. El-Dakroury, A. W. Hageen, D. H. Mohammed, S. Mourad, O. A. Mohammed, M. A. Abdel-Reheim and A. S. Doghish, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 2025, **398**, 6369–6389.

47 U. Zavrtanik, J. Lukan, R. Loris, J. Lah and S. Hadži, *J. Mol. Biol.*, 2018, **430**, 4369–4386.

48 I. Jovcevska and S. Muyldermans, *BioDrugs*, 2020, **34**, 11–26.

49 D. I. Frecot, T. Froehlich and U. Rothbauer, *J. Cell Sci.*, 2023, **136**, jcs261395.

50 M. Erreni, T. Schorn, F. D'Autilia and A. Doni, *Biomolecules*, 2020, **10**, 1695.

51 S. Paul, M. F. Konig, D. M. Pardoll, C. Bettegowda, N. Papadopoulos, K. M. Wright, S. B. Gabelli, M. Ho, A. van Elsas and S. Zhou, *Nat. Rev. Cancer*, 2024, **24**, 399–426.

52 A. Maali, M. Gholizadeh, S. Feghhi-Najafabadi, A. Noei, S. S. Seyed-Motahari, S. Mansoori and Z. Sharifzadeh, *Front. Immunol.*, 2023, **14**, 1012841.

53 R. Ghemrawi, L. Abuamer, S. Kremesh, G. Hussien, R. Ahmed, W. Mousa, G. Khoder and M. Khair, *Biomedicines*, 2024, **12**, 2158.

54 L. Ma, J. Gai, P. Qiao, Y. Li, X. Li, M. Zhu, L. Guanghui and Y. Wan, *Biochem. Biophys. Res. Commun.*, 2020, **531**, 144–151.

55 M. H. Abdolvahab, P. Karimi, N. Mohajeri, M. Abedini and H. Zare, *Cancer Cell Int.*, 2024, **24**, 67.

56 V. M. Medina Pérez, M. Baselga and A. J. Schuhmacher, *Cancers*, 2024, **16**, 2681.

57 M. Wang, T. Ying and Y. Wu, *Acta Pharm. Sin. B*, 2024, **14**, 2854–2868.

58 A. H. Hansen, K. I. H. Andersen, L. Xin, O. Krigslund, N. Behrendt, L. H. Engelholm, C. H. Bang-Bertelsen, S. Schoffelen and K. Qvortrup, *Molecules*, 2025, **30**, 391.

59 J. Fan, X. Zhuang, X. Yang, Y. Xu, Z. Zhou, L. Pan and S. Chen, *Signal Transduction Targeted Ther.*, 2021, **6**, 320.

60 Q. Zhang, L. Wu, S. Liu, Q. Chen, L. Zeng, X. Chen and Q. Zhang, *Cancer Cell Int.*, 2020, **20**, 570.

61 T. Saha, M. Fojtů, A. V. Nagar, L. Thurakkal, B. B. Srinivasan, M. Mukherjee, A. Sibiyon, H. Aggarwal, A. Samuel, C. Dash, H. L. Jang and S. Sengupta, *Sci. Adv.*, 2024, **10**, eadi2046.

62 E. R. Verhaar, W. J. C. van Keizerswaard, A. Knoflook, T. Balligand and H. L. Ploegh, *PNAS Nexus*, 2024, **3**, pgae184.

63 A. Page, N. Chuvin, J. Valladeau-Guilemond and S. Depil, *Cell. Mol. Immunol.*, 2024, **21**, 315–331.

64 H. E. Marei, K. Bedair, A. Hasan, L. Al-Mansoori, S. Caratelli, G. Sconocchia, A. Gaiba and C. Cenciarelli, *Cancer Cell Int.*, 2025, **25**, 3.

65 B. Bhutani, V. Sharma, N. K. Ganguly and R. Rana, *Biomed. Pharmacother.*, 2025, **186**, 117987.

66 L. Gong, Y. Li, K. Cui, Y. Chen, H. Hong, J. Li, D. Li, Y. Yin, Z. Wu and Z. Huang, *Small*, 2021, **17**, e2103463.

67 L. Coënon, E. Rigal, H. Courot, C. Multrier, S. Zemiti, J. Lambour, M. Pugnière, M. de Toledo, G. Bossis, G. Cartron, B. Robert, P. Martineau, B. Fauvel, J. Presumey and M. Villalba, *J. Immunother. Cancer*, 2024, **12**, e009070.

68 M. E. Tsitokana, P.-A. Lafon, L. Prézeau, J.-P. Pin and P. Rondard, *Int. J. Mol. Sci.*, 2023, **24**, 2632.

69 F. Zheng, Y. Pang, L. Li, Y. Pang, J. Zhang, X. Wang and G. Raes, *Front. Immunol.*, 2022, **13**, 978513.

70 D. M. Wilson, M. R. Cookson, L. Van Den Bosch, H. Zetterberg, D. M. Holtzman and I. Dewachter, *Cell*, 2023, **186**, 693–714.

71 Y. Zhang, H. Chen, R. Li, K. Sterling and W. Song, *Signal Transduction Targeted Ther.*, 2023, **8**, 248.

72 Y. R. Butler, Y. Liu, R. Kumbhar, P. Zhao, K. Gadhave, N. Wang, Y. Li, X. Mao and W. Wang, *Nat. Commun.*, 2022, **13**, 4060.

73 J. X. Dong, Y. Lee, M. Kirmiz, S. Palacio, C. Dumitras, C. M. Moreno, R. Sando, L. F. Santana, T. C. Südhof,

B. Gong, K. D. Murray and J. S. Trimmer, *eLife*, 2019, **8**, e48750.
74 B. Leonard, V. Danna, L. Gorham, M. Davison, W. Chrisler, D. N. Kim and V. R. Gerbasi, *Anal. Chem.*, 2023, **95**, 8747–8751.
75 D. Rodrigues Martins, F. Sha, W. Van der Elst, P. Y. Shih, J. Devoght, K. Van Kolen, M. Mercken, B. Van Broeck, P. Declerck and C. Theunis, *Mol. Ther.–Methods Clin. Dev.*, 2023, **31**, 101158.
76 R. Faresjö, E. O. Sjöström, T. Dallas, M. M. Berglund, J. Eriksson, D. Sehlin and S. Syvänen, *mAbs*, 2024, **16**, 2410968.
77 E. Ruiz-López and A. J. Schuhmacher, *Biomolecules*, 2021, **11**, 1131.
78 E. Pothin, D. Lesuisse and P. Lafaye, *Pharmaceutics*, 2020, **12**, 937.
79 B. J. Tillotson, L. I. Goulatis, I. Parenti, E. Duxbury and E. V. Shusta, *PLoS One*, 2016, **10**, e0145820.
80 L. Rué, T. Jaspers, I. M. S. Degors, S. Noppen, D. Schols, B. De Strooper and M. Dewilde, *Pharmaceutics*, 2023, **15**, 1748.
81 T. M. Do, C. Capdevila, L. Pradier, V. Blanchard, M. Lopez-Grancha, N. Schussler, A. Steinmetz, J. Beninga, D. Boulay, P. Dugay, P. Verdier, N. Aubin, G. Dargazanli, C. Chaves, E. Genet, Y. Lossouarn, C. Loux, F. Michoux, N. Moindrot, F. Chanut, T. Gury, S. Eyquem, D. Valente, O. Bergis, E. Rao and D. Lesuisse, *Mol. Ther.–Methods Clin. Dev.*, 2020, **19**, 58–77.
82 Y. Wouters, T. Jaspers, L. Rué, L. Serneels, B. De Strooper and M. Dewilde, *Fluids Barriers CNS*, 2022, **19**, 79.
83 Á. G. de Lucas, U. Lamminmäki and F. R. López-Picón, *Biomolecules*, 2023, **13**, 164.
84 P. Stahl, S. Kollenda, J. Sager, L. Schmidt, M. A. Schroer, R. H. Stauber, M. Epple and S. K. Knauer, *Small*, 2023, **19**, e2300871.
85 Y. Xue, C. Wang, H. Li, S. Du, Y. Zhong, Y. Zhang, S. Wang, K. Guo, X. Hou, D. D. Kang, Z. Liu, M. Tian, D. Cao, B. Deng, D. W. McComb, T. Markovic, J. Pan, M. Borna, E. J. Nestler, P. C. Peng and Y. Dong, *Adv. Mater.*, 2025, e2417097, DOI: **10.1002/adma.202417097**.
86 M. Yuan, Z. Han, Y. Liang, Y. Sun, B. He, W. Chen and F. Li, *Biomater. Res.*, 2023, **27**, 90.
87 L. Zhu, B. Huang, X. Wang, F. Ni, M. Ao, R. Wang, B. Zheng, C. Chen, J. Xue, L. Zhu, C. Yang, L. Shi, S. Geng, J. Hu, M. Yang, D. Zhang, P. Yang, M. Li, Y. Li, Q. Hu, S. Ye, P. Zheng, H. Wei, Z. Wu, L. Zhang, Y. Wang, Y. Liu and X. Wu, *Nat. Commun.*, 2024, **15**, 6961.
88 Q. Liu, Y. Lu, C. Cai, Y. Huang, L. Zhou, Y. Guan, S. Fu, Y. Lin, H. Yan, Z. Zhang, X. Li, X. Yang, H. Yang, H. Guo, K. Lan, Y. Chen, S.-C. Hou and Y. Xiong, *Cell Death Dis.*, 2024, **15**, 458.
89 Z. S. Chen, H. C. Huang, X. Wang, K. Schön, Y. Jia, M. Lebens, D. F. Besavilla, J. R. Murti, Y. Ji, A. A. Sarshad, G. Deng, Q. Zhu and D. Angeletti, *Nat. Commun.*, 2025, **16**, 432.
90 J. Schmidt Diane, G. Beamer, M. Tremblay Jacqueline, A. Steele Jennifer, B. Kim Hyeun, Y. Wang, M. Debatis, X. Sun, A. Kashentseva Elena, P. Dmitriev Igor, T. Curiel David, B. Shoemaker Charles and S. Tzipori, *Clin. Vaccine Immunol.*, 2016, **23**, 774–784.
91 S. L. Kordus, H. K. Kroh, R. C. Rodríguez, R. A. Shrem, F. C. Peritore-Galve, J. A. Shupe, B. E. Wadzinski, D. B. Lacy and B. W. Spiller, *PLoS Pathog.*, 2023, **19**, e1011496.
92 A. Thaiprayoon, Y. Chantarasorn, W. Oonanant, A. Kasorn, P. Longsompurana, S. Tapaneeyakorn, P. Riangrungroj, F. Loison, A. C. Kruse, M. P. DeLisa and D. Waraho-Zhmayev, *Sci. Rep.*, 2025, **15**, 3594.
93 S. Muro, *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.*, 2010, **2**, 189–204.
94 S. Muyldermans, *Annu. Rev. Anim. Biosci.*, 2021, **9**, 401–421.
95 M. Kijanka, B. Dorresteijn, S. Oliveira and P. M. van Bergen en Henegouwen, *Nanomedicine*, 2015, **10**, 161–174.
96 D. M. Copolovici, K. Langel, E. Eriste and Ü. Langel, *ACS Nano*, 2014, **8**, 1972–1994.
97 J. D. Rabb, L. E. Kruse and Q. Lin, *Angew. Chem., Int. Ed.*, 2025, **64**, e202424076.
98 M. Grairi and M. Le Borgne, *Drug Discovery Today*, 2024, **29**, 104241.
99 S. S. Panikar, N. Banu, J. Haramati, S. del Toro-Arreola, A. Riera Leal and P. Salas, *J. Controlled Release*, 2021, **334**, 389–412.
100 L. Maksymova, Y. A. Pilger, L. Nuhn and J. A. Van Ginderachter, *Mol. Cancer*, 2025, **24**, 65.
101 J. Narbona, L. Hernández-Baraza, R. G. Gordo, L. Sanz and J. Lacadena, *Biomolecules*, 2023, **13**, 1042.
102 L. Sun, H. Liu, Y. Ye, Y. Lei, R. Islam, S. Tan, R. Tong, Y.-B. Miao and L. Cai, *Signal Transduction Targeted Ther.*, 2023, **8**, 418.
103 A. N. Al-Thani, A. G. Jan, M. Abbas, M. Geetha and K. K. Sadasivuni, *Life Sci.*, 2024, **352**, 122899.
104 S. El Andaloussi, I. Mäger, X. O. Breakefield and M. J. A. Wood, *Nat. Rev. Drug Discovery*, 2013, **12**, 347–357.
105 J. J. J. Liu, D. Liu, S. K. Y. To and A. S. T. Wong, *Mol. Cancer*, 2025, **24**, 166.
106 Z. Chen, M. Xiong, J. Tian, D. Song, S. Duan and L. Zhang, *J. Nanobiotechnol.*, 2024, **22**, 18.
107 R. Zhang, D. Li, Z. Zhou, H. Hong, J. Shi and Z. Wu, *ChemBioChem*, 2024, **25**, e202400512.
108 B. Önal Acet, D. Gül, R. H. Stauber, M. Odabaşı and Ö. Acet, *Nanomaterials*, 2024, **14**, 823.
109 A. M. Eichhoff, K. Börner, B. Albrecht, W. Schäfer, N. Baum, F. Haag, J. Körbelin, M. Trepel, I. Braren, D. Grimm, S. Adriouch and F. Koch-Nolte, *Mol. Ther.–Methods Clin. Dev.*, 2019, **15**, 211–220.
110 M. Rashidian and H. Ploegh, *Immuno-Oncol. Technol.*, 2020, **7**, 2–14.
111 J. Bridoux, K. Broos, Q. Lecocq, P. Debie, C. Martin, S. Ballet, G. Raes, S. Neyt, C. Vanhove, K. Breckpot, N. Devoogdt, V. Caveliers, M. Keyaerts and C. Xavier, *Biomolecules*, 2020, **10**, 1388.
112 L. Berland, L. Kim, O. Abousaway, A. Mines, S. Mishra, L. Clark, P. Hofman and M. Rashidian, *Biomolecules*, 2021, **11**, 637.

113 M. A. de Beer and B. N. G. Giepmans, *Front. Cell. Neurosci.*, 2020, **14**, 573278.
114 T. M. Lwin, M. A. Turner, H. Nishino, S. Amirfakhri, S. Hernot, R. M. Hoffman and M. Bouvet, *Biomolecules*, 2022, **12**, 711.
115 R. Fan, Y. Li, K. W. Park, J. Du, L. H. Chang, E. R. Strieter and T. L. Andrew, *ECS Sens. Plus*, 2022, **1**, 010601.
116 H. Götzke, M. Kilisch, M. Martínez-Carranza, S. Sograte-Idrissi, A. Rajavel, T. Schlichthaerle, N. Engels, R. Jungmann, P. Stenmark, F. Opazo and S. Frey, *Nat. Commun.*, 2019, **10**, 4403.
117 L. Teodori, M. Omer, A. Märcher, M. K. Skaanning, V. L. Andersen, J. S. Nielsen, E. Oldenburg, Y. Lin, K. V. Gothelf and J. Kjems, *J. Biol. Methods*, 2022, **9**, e159.
118 Z. Liu, T. Lammers, J. Ehling, S. Fokong, J. Bornemann, F. Kiessling and J. Gätjens, *Biomaterials*, 2011, **32**, 6155–6163.
119 S. Hernot, S. Unnikrishnan, Z. Du, T. Shevchenko, B. Cosyns, A. Broisat, J. Toczek, V. Caveliers, S. Muyldermans, T. Lahoutte, A. L. Klibanov and N. Devoogdt, *J. Controlled Release*, 2012, **158**, 346–353.
120 D. Wegierak, P. Nittayacharn, M. B. Cooley, F. M. Berg, T. Kosmides, D. Durig, M. C. Kolios and A. A. Exner, *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.*, 2024, **16**, e2007.
121 S. Ma, J. Ji, Y. Tong, Y. Zhu, J. Dou, X. Zhang, S. Xu, T. Zhu, X. Xu, Q. You and Z. Jiang, *Acta Pharm. Sin. B*, 2022, **12**, 2990–3005.
122 H. Zhang, Y. Han, Y. Yang, F. Lin, K. Li, L. Kong, H. Liu, Y. Dang, J. Lin and P. R. Chen, *J. Am. Chem. Soc.*, 2021, **143**, 16377–16382.
123 F. Shen and L. M. K. Dassama, *Chem. Sci.*, 2023, **14**, 8433–8447.
124 F. Shen, G. Zheng, M. Setegne, K. Tenglin, M. Izadi, H. Xie, L. Zhai, S. H. Orkin and L. M. K. Dassama, *ACS Cent. Sci.*, 2022, **8**, 1695–1703.
125 J. Wang, G. Chistov, J. Zhang, B. Huntington, I. Salem, A. Sandholu and S. T. Arold, *Heliyon*, 2024, **10**, e34487.
126 S. Cao, S. Kang, H. Mao, J. Yao, L. Gu and N. Zheng, *Nat. Commun.*, 2022, **13**, 815.
127 M. Konstantinidou and M. R. Arkin, *Cell Chem. Biol.*, 2024, **31**, 1064–1088.
128 E. A. Panova, D. A. Kleymenov, D. V. Shcheblyakov, E. N. Bykonia, E. P. Mazunina, A. S. Dzharullaeva, A. N. Zolotar, A. A. Derkaev, I. B. Esmagambetov, I. I. Sorokin, E. V. Usachev, A. N. Noskov, I. A. Ivanov, T. S. Zatsepin, S. E. Dmitriev, V. A. Gushchin, B. S. Naroditsky, D. Y. Logunov and A. L. Gintsburg, *Front. Immunol.*, 2023, **14**, 1098302.
129 Y. Zhang, C. Tian, X. Yu, G. Yu, X. Han, Y. Wang, H. Zhou, S. Zhang, M. Li, T. Yang, Y. Sun, W. Tai, Q. Yin and G. Zhao, *Vaccines*, 2024, **12**, 1315.
130 L. Hanke, L. Vidakovics Perez, D. J. Sheward, H. Das, T. Schulte, A. Moliner-Morro, M. Corcoran, A. Achour, G. B. Karlsson Hedestam, B. M. Hällberg, B. Murrell and G. M. McInerney, *Nat. Commun.*, 2020, **11**, 4420.
131 W. Song, W. Wei, X. Lan and W. Cai, *Eur. J. Nucl. Med. Mol. Imaging*, 2023, **50**, 2591–2594.
132 J. Vanderstraeten, P. Marchal, J. Moray, T. Leal, S. Muyldermans, M. Dumoulin and R. Vanbever, *Rev. Mal. Respir.*, 2023, **40**, 139.
133 D. Schumacher, J. Helma, A. F. L. Schneider, H. Leonhardt and C. P. R. Hackenberger, *Angew. Chem., Int. Ed.*, 2018, **57**, 2314–2333.
134 M. V. Van, T. Fujimori and L. Bintu, *Nat. Commun.*, 2021, **12**, 537.
135 L. Sánchez-García, E. Voltà-Durán, E. Parladé, E. Mazzega, A. Sánchez-Chardi, N. Serna, H. López-Laguna, M. Mitstorfer, U. Unzueta, E. Vázquez, A. Villaverde and A. de Marco, *ACS Appl. Mater. Interfaces*, 2021, **13**, 29406–29415.
136 P. Bitsch, E. S. Baum, I. Beltrán Hernández, S. Bitsch, J. Harwood, S. Oliveira and H. Kolmar, *Pharmaceutics*, 2023, **15**, 2374.
137 K. J. Metcalf, B. R. Kimmel, D. J. Sykora, J. A. Modica, K. A. Parker, E. Berens, R. Dai, V. P. Dravid, Z. Werb and M. Mrksich, *Bioconjugate Chem.*, 2021, **32**, 143–152.
138 J. T. Hadsund, T. Satława, B. Janusz, L. Shan, L. Zhou, R. Röttger and K. Krawczyk, *Bioinf. Adv.*, 2024, **4**, vbae033.
139 Y. Wu, *Discover Nano*, 2025, **20**, 23.
140 C. Ishiwatari-Ogata, M. Kyuuma, H. Ogata, M. Yamakawa, K. Iwata, M. Ochi, M. Hori, N. Miyata and Y. Fujii, *Front. Immunol.*, 2022, **13**, 853008.
141 M. Van Roy, C. Ververken, E. Beirnaert, S. Hoefman, J. Kolkman, M. Vierboom, E. Breedveld, B. t Hart, S. Poelmans, L. Bontinck, A. Hemeryck, S. Jacobs, J. Baumeister and H. Ulrichts, *Arthritis Res. Ther.*, 2015, **17**, 135.
142 I. Hrynchak, L. Santos, A. Falcão, C. M. Gomes and A. J. Abrunhosa, *Int. J. Mol. Sci.*, 2021, **22**, 10745.
143 X. Ma, B. Hu, X. Zhou, L. Wang, H. Chen, F. Xie, H. Zhu, B. Jia and Z. Yang, *Bioorg. Chem.*, 2025, **156**, 108222.
144 P. Safarzadeh Kozani, A. Naseri, S. M. J. Mirarefin, F. Salem, M. Nikbakht, S. Evazi Bakhshi and P. Safarzadeh Kozani, *Biomarker Res.*, 2022, **10**, 24.
145 R. Abskharon, H. Pan, M. R. Sawaya, P. M. Seidler, E. J. Olivares, Y. Chen, K. A. Murray, J. Zhang, C. Lantz, M. Bentzel, D. R. Boyer, D. Cascio, B. A. Nguyen, K. Hou, X. Cheng, E. Pardon, C. K. Williams, A. L. Nana, H. V. Vinters, S. Spina, L. T. Grinberg, W. W. Seeley, J. Steyaert, C. G. Glabe, R. R. Ogorzalek Loo, J. A. Loo and D. S. Eisenberg, *Proc. Natl. Acad. Sci. U. S. A.*, 2023, **120**, e2300258120.
146 M. Schoof, B. Faust, R. A. Saunders, S. Sangwan, V. Rezelj, N. Hoppe, M. Boone, C. B. Billesbølle, C. Puchades, C. M. Azumaya, H. T. Kratochvil, M. Zimanyi, I. Deshpande, J. Liang, S. Dickinson, H. C. Nguyen, C. M. Chio, G. E. Merz, M. C. Thompson, D. Diwanji, K. Schaefer, A. A. Anand, N. Dobzinski, B. S. Zha, C. R. Simoneau, K. Leon, K. M. White, U. S. Chio, M. Gupta, M. Jin, F. Li, Y. Liu, K. Zhang, D. Bulkley, M. Sun, A. M. Smith, A. N. Rizo, F. Moss, A. F. Brilot, S. Pourmal, R. Trenker, T. Pospiech, S. Gupta, B. Barsi-Rhyne, V. Belyy, A. W. Barile-Hill, S. Nock, Y. Liu, N. J. Krogan, C. Y. Ralston, D. L. Swaney, A. García-Sastre, M. Ott, M. Vignuzzi, P. Walter and A. Manglik, *Science*, 2020, **370**, 1473–1479.
147 B. Partopour and D. Pollard, *Trends Biotechnol.*, 2025, **43**, 462–475.

148 U. Storz, *mAbs*, 2011, **3**, 310–317.
149 D. Westmeier, S. K. Knauer, R. H. Stauber and D. Docter, *Adverse Effects of Engineered Nanomaterials: Exposure, Toxicology, and Impact on Human Health*, 2nd edn, 2017, pp. 1–12, DOI: 10.1016/B978-0-12-809199-9.00001-X.
150 D. Westmeier, A. Hahlbrock, C. Reinhardt, J. Fröhlich-Nowoisky, S. Wessler, C. Vallet, U. Pöschl, S. K. Knauer and R. H. Stauber, *Chem. Soc. Rev.*, 2018, **47**, 5312–5337.
151 A. Gribko, J. Kunzel, D. Wunsch, Q. Lu, S. M. Nagel, S. K. Knauer, R. H. Stauber and G. B. Ding, *Int. J. Nanomed.*, 2019, **14**, 4187–4209.
152 S. Tenzer, D. Docter, S. Rosfa, A. Wlodarski, J. Kuharev, A. Rekik, S. K. Knauer, C. Bantz, T. Nawroth, C. Bier, J. Sirirattanapan, W. Mann, L. Treuel, R. Zellner, M. Maskos, H. Schild and R. H. Stauber, *ACS Nano*, 2011, **5**, 7155–7167.
153 D. Docter, U. Distler, W. Storck, J. Kuharev, D. Wunsch, A. Hahlbrock, S. K. Knauer, S. Tenzer and R. H. Stauber, *Nat. Protoc.*, 2014, **9**, 2030–2044.
154 O. Vilanova, J. J. Mittag, P. M. Kelly, S. Milani, K. A. Dawson, J. O. Radler and G. Franzese, *ACS Nano*, 2016, **10**, 10842–10850.
155 M. Lundqvist, J. Stigler, T. Cedervall, T. Berggard, M. B. Flanagan, I. Lynch, G. Elia and K. Dawson, *ACS Nano*, 2011, **5**, 7503–7509.
156 M. Hadjidemetriou, Z. Al-Ahmady, M. Mazza, R. F. Collins, K. Dawson and K. Kostarelos, *ACS Nano*, 2015, **9**, 8142–8156.
157 S. Tenzer, D. Docter, J. Kuharev, A. Musyanovych, V. Fetz, R. Hecht, F. Schlenk, D. Fischer, K. Kiouptsi, C. Reinhardt, K. Landfester, H. Schild, M. Maskos, S. K. Knauer and R. H. Stauber, *Nat. Nanotechnol.*, 2013, **8**, 772–781.
158 Y. F. Wang, Y. Zhou, J. Sun, X. Wang, Y. Jia, K. Ge, Y. Yan, K. A. Dawson, S. Guo, J. Zhang and X. J. Liang, *Nano Res.*, 2023, **16**, 715–734.
159 D. Docter, D. Westmeier, M. Markiewicz, S. Stolte, S. K. Knauer and R. H. Stauber, *Chem. Soc. Rev.*, 2015, **44**, 6094–6121.
160 D. Docter, S. Strieth, D. Westmeier, O. Hayden, M. Gao, S. K. Knauer and R. H. Stauber, *Nanomedicine*, 2015, **10**, 503–519.