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Application of Artificial Intelligence and Computational Biology in Protein Drug Development

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Abstract

Protein drugs have evolved into a primary category of biological drugs. Despite the impressive achievements, protein therapeutics still face several challenges, including potential immunogenicity, druggability, and high costs. In recent years, artificial intelligence (AI) and computational biology have emerged as powerful tools to overcome these challenges and reshape the protein drug development pipeline. This review underscores the pivotal role of AI in advancing protein drug development, including the computational analysis of phage libraries, the application of computer-aided techniques for new phage display systems, and the computational optimization and design of novel antibody–drug conjugates, nanobodies, and cytokines. The review delves into the use of AI in predicting the pharmacological properties of these protein therapeutics, providing a comprehensive overview of the transformative impact of computational approaches in these areas.

Keywords
- artificial intelligence
- computational biology
- protein drug
- phage display
- nanobodies
- cytokines

Introduction

Since the first discovery of recombinant protein drug, Humulin, protein therapeutics have become a cornerstone of modern medicine.[1–3] To date, over 200 distinct proteins or peptides have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of various diseases, including diabetes, hemophilia, immunodeficiency, and cancer.[4–7] Compared with small molecule drugs, protein drugs have several advantages. For instance, antibody-based therapies often exhibit greater target specificity, resulting in reduced cytotoxicity and improved safety profiles. The complexity of the three-dimensional structures of proteins and their intricate interactions with biological systems underpin their exceptional specificity and effectiveness. Moreover, protein drugs' development cycle is relatively shorter than that of small molecule drugs, and this can be attributed to advancements in biotechnology, enhanced protein engineering techniques, and streamlined regulatory processes. Moving more quickly from discovery to clinical application allows protein therapeutics to address pressing medical needs with greater speed and agility.[8] These combined advantages highlight the growing importance of protein drugs in contemporary therapeutic strategies and their potential to transform the landscape of medicine. However, despite these advancements, there are significant challenges,

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particularly in improving the pharmacokinetic properties of protein drugs and clinical efficacy.[9,10]

To overcome these challenges, computational biology and artificial intelligence (AI) have emerged as invaluable tools, utilizing a variety of methods to predict protein structures and simulate protein interactions. AI-driven approaches facilitate a deeper understanding of structure–sequence–function relationships, advancing protein drug development and improving success rates.[11,12] A landmark achievement in this domain is the development of AlphaFold2 (AF2) in 2021,[13] which demonstrated protein structure predictions with an accuracy comparable to experimental methods, significantly advancing the field of protein design.

Several methodologies utilize AF2 predictions,[14] whereas others, such as RoseTTAFold Diffusion, are developed independently of AF2 in protein drug design.[15] The advent of pretrained models based on protein sequences and structures has facilitated the predictions of protein properties.[16] By integrating these pretrained models with the mathematical frameworks of neural networks, researchers can refine the protein design process to optimize therapeutic outcomes. This paper reviews recent advances in the application of AI technology in key areas of protein drug development, including phage display, antibody–drug conjugates (ADCs), degrader–antibody conjugates (DACs), nanodrugs, and cytokine therapeutics.

Artificial Intelligence and Phage Display

Introduction of Phage Display and Protein Drug Development

Phages are a group of viruses that host bacteria, fungi, actinomycetes, and other microorganisms.[17] During reproduction, the phage will inject the genetic material into the host bacteria, which will produce the genetic material and capsule proteins of the phage and assemble them into a new phage, thus completing the whole reproduction process.[18] In 1983, Renato proposed the concept of displaying exogenous peptides on the phage surface. In 1985, Smith first inserted exogenous DNA fragments encoding polypeptide sequences into gene III of filamentous phage f1 to produce fusion proteins and display them on the phage surface, leading to the born of the phage display technology era.[19] In 1990, Prof. Winter used this technology to successfully develop the first fully humanized antibody drug, adalimumab,[20–22] adding phage display as a powerful technology in pharmaceutical research and development.

Phage display technology directly links genotype and phenotype.[23] Phage peptide libraries can display a large number of different protein peptides mimicking real epitopes. Many peptides and antibodies screened from phage display libraries have been developed into new drugs, such as romiplostim, a peptidosomal drug for the treatment of thrombocytopenia, and adalimumab for the treatment of rheumatoid arthritis, ankylosing spondylitis, and psoriasis.[24–27] Phage display contributes to the development of new drugs, vaccines, and diagnostic reagents. Its low cost and straightforward operation has received widespread attention from researchers and was awarded the Nobel Prize in Chemistry in 2018.[28] However, the technology faces certain limitations, including a high failure rate and restricted display size. AI may overcome those limitations and provide solutions from initial library design to final analysis.

Machine Learning and Phage Display Application

Machine learning plays a pivotal role in advancing bacteriophage research, particularly in identifying both existing and newly discovered bacteriophages. Nami et al provided a comprehensive review of the application of machine learning methods across various aspects of bacteriophage research. These applications include automated data curation, identification, and classification of bacteriophages, host species recognition, virion protein identification, and life cycle prediction.[29]

Design of the library, including the cDNA library, random peptide library, human peptide library, etc., is critical for the successful application of phage display. Thus, many libraries with special professional functions have been designed. For example, Ito et al constructed a "weakly enriched" library by judiciously selecting deep sequencing data and applying machine learning methods to process sequence data, aiming to identify sequence space where functional variants are enriched.[30] "Weakly enriched" library is functional variants that are weakly enriched in phage display pools displaying human RNA-binding protein.[30] Bayesian optimization was used with a variety of amino acid descriptors based on physicochemical properties or structural topology to train and rank the probability-of-improvement score. A Bayesian machine learning model generates nine clusters with distinct sequence patterns, which were used to design phage libraries, and four improved variants were identified by the subsequent biopanning.[30]

Combined with next-generation sequencing technology, AI can help skip time-consuming, labor-intensive, and expensive titer assays.[31,32] For example, Hogan et al trained a set of machine learning models by using two libraries called F and S, which can be used to predict antibody-displaying phage to be observed after the 5th round of panning.[33] Saksena et al reported a computational counterselection method combining deep sequencing and machine learning to identify nonspecific candidate antibodies and demonstrated advantages over more established molecular counterselection methods.[34]

More importantly, the integration of AI algorithms with phage display technology could lead to the development of novel methods and advancements. In 2011, Esvelt and colleagues introduced phage-assisted continuous evolution (PACE), a system designed for the directed evolution of proteins using a continuous coculture strategy of phages and their host, *Escherichia coli*. In this approach, the coding nucleic acid sequence of the protein or enzyme of interest (POI) is inserted into the phage genome. Upon infecting *E. coli*, the phage utilizes the host's error-prone replication system to generate a library of POI mutants.[35,36] To ensure the evolution proceeds in the desired direction, only mutants that exhibit the target properties are allowed to propagate by infecting new *E. coli* hosts. This process is further automated

by a liquid circuit control system, enabling the steps to repeat uninterrupted until the ideal mutant is obtained. By coupling the evolution of the protein to the phage lifecycle, PACE achieves up to 60 evolutionary rounds every 24 hours—an efficiency 100 times greater than traditional directed evolution methods. Moreover, PACE eliminates much of the manual intervention required in conventional approaches, enabling "spontaneous" and continuous directed evolution with significant advantages. By combining molecular biology, bioinformatics, and structural biology, phage display technology has been redefined and will play a pivotal role in modern biotechnological applications.

Philpott et al proposed a rapid cellular phage display platform: μCellect.[37] It reproduces the complex *in vivo* binding environment and can produce high-performance human antibodies in a short period. The original phage library was first incubated with a heterogeneous mixture consisting of a few cells expressing the target antigen and cells lacking the target antigen. Target cells were then labeled with magnetic nanoparticles that specifically captured the probe and sorted by microfluidic cell sorters. The sorted products were sequenced by next-generation sequencing. Candidate clones were selected for validation through a bioinformatics analysis. Thus, the problems of time-consuming and high failure rates that still exist in traditional phage display technology will be solved. This approach is low cost, high throughput, and compatible with multiple cell types, enabling wide application of antibody development.

Through a previous phage display-enriched dodecapeptides that bind anti-Aβ antibodies,[38] the interaction between anti-Aβ antibodies and various peptides can be investigated by training a transformer-based model ABTrans.[39] Antibody and phage display enriches are encoded by the sum of one hot sequence and positional embedding, which are processed by two different encoder modules. The ABTrans can predict a peptide binding to anti-Aβ antibodies with negative, low binding, intermediate binding, and high affinity, corresponding to enrichment rounds in phage display (►**Fig. 1**).

In summary, phage display is a technique used to study protein–protein, protein–peptide, and protein–DNA interactions by displaying peptides, proteins, or antibodies on the surface of bacteriophages. The applications of AI in phage display can significantly accelerate and enhance the process of screening, analyzing, and designing biomolecules, and may help peptide/antibody library design, binding affinity prediction, high-throughput screening optimization, and evolutionary design, with increased efficiency and enhanced accuracy.

Artificial Intelligence and Antibody–Drug Conjugates

Composition of Antibody–Drug Conjugates

ADCs are a kind of large molecule drug with high targeting, high specificity, high activity, and low toxicity. They are mainly composed of monoclonal antibodies (mAbs) capable of targeting tumor-specific antigens or tumor-associated antigens and a specific number of small molecule cytotoxins, coupled through linkers.[40] After the drug is localized to the tumor cells, the cytotoxin molecule exerts cytotoxicity and efficiently kills the tumor cells.[41] The off-target effect of small molecule cytotoxin is effectively reduced due to the coupling between mAb and small molecule cytotoxin via a linker.[42] Cytotoxic payloads should be highly stable during blood circulation to avoid large loss of drug during delivery, while also exerting an effective tumor-killing effect at a lower dose.[43] In the clinic, the small molecule cytotoxic payload commonly used in ADC drug development may include microtubule protein inhibitors, DNA synthesis inhibitors, topoisomerase inhibitors, and RNA polymerase II inhibitors.[44]

The characteristic of the linker between the antibody and the payload also plays a crucial role in the functioning of the ADC. First, the linker needs to be stable to ensure that the ADC is structurally intact and can be successfully delivered to the surface of the tumor cell. When the ADC is delivered to the surface of the tumor cell, the linker needs to be able to be hydrolyzed so that the small molecule drug can be successfully released into the tumor cell. In addition, when the linker is attached to the antibody, the appropriate site needs to be selected to minimize the interfering effect on the antibody's action.[45,46]

The target antigens of ADCs should be tumor-specific and stably expressed to minimize off-target effects.[47] These antigens should be nonsecretory to prevent their secretion outside the tumor site. Once a suitable target antigen is identified, humanized or fully human mAbs with high affinity and specificity for the same antigenic determinant cluster are selected to reduce immunogenicity concerns.[48] As a result, these antibodies not only have strong affinity and specificity for the target antigen but also have a long half-life and excellent stability *in vivo*.

Degrader–Antibody Conjugates

DACs, as an emerging class of therapeutic agents, have attracted much attention.[49–52] DACs, with the same composition of ADC, link mAbs to the payload of small molecule protein degraders, e.g., molecular glue or PROTACs (Proteolysis Targeting Chimeras). DACs and ADCs share similar mechanisms. DACs not only provide selective delivery but also improve the therapeutic index of toxins. By leveraging the targeting and internalization capabilities of antibodies, the use of antibodies as a delivery mechanism effectively overcomes the bioavailability issues encountered by PROTAC payloads with poor physicochemical and/or drug metabolism and pharmacokinetics properties.[51,52] DACs also avoided specific formulations to enable unconjugated PROTACs to achieve meaningful *in vivo* exposures.[49] The additional targeting of PROTACs mitigates the lack of biological selectivity of a drug in the case of the DAC antibody's off-target binding compared with ADCs where off-target binding may cause toxicity.[52]

DACs have been used for different antibody targets like CLL1, STEAP1, HER2, and CD22, combined with PROTAC targets of BRD4, Erα, TGFßR2, and BRM.[49–52] For example, bromodomain and extra-terminal protein degrader were linked to an antibody targeting CEACAM6/CD66c to treat

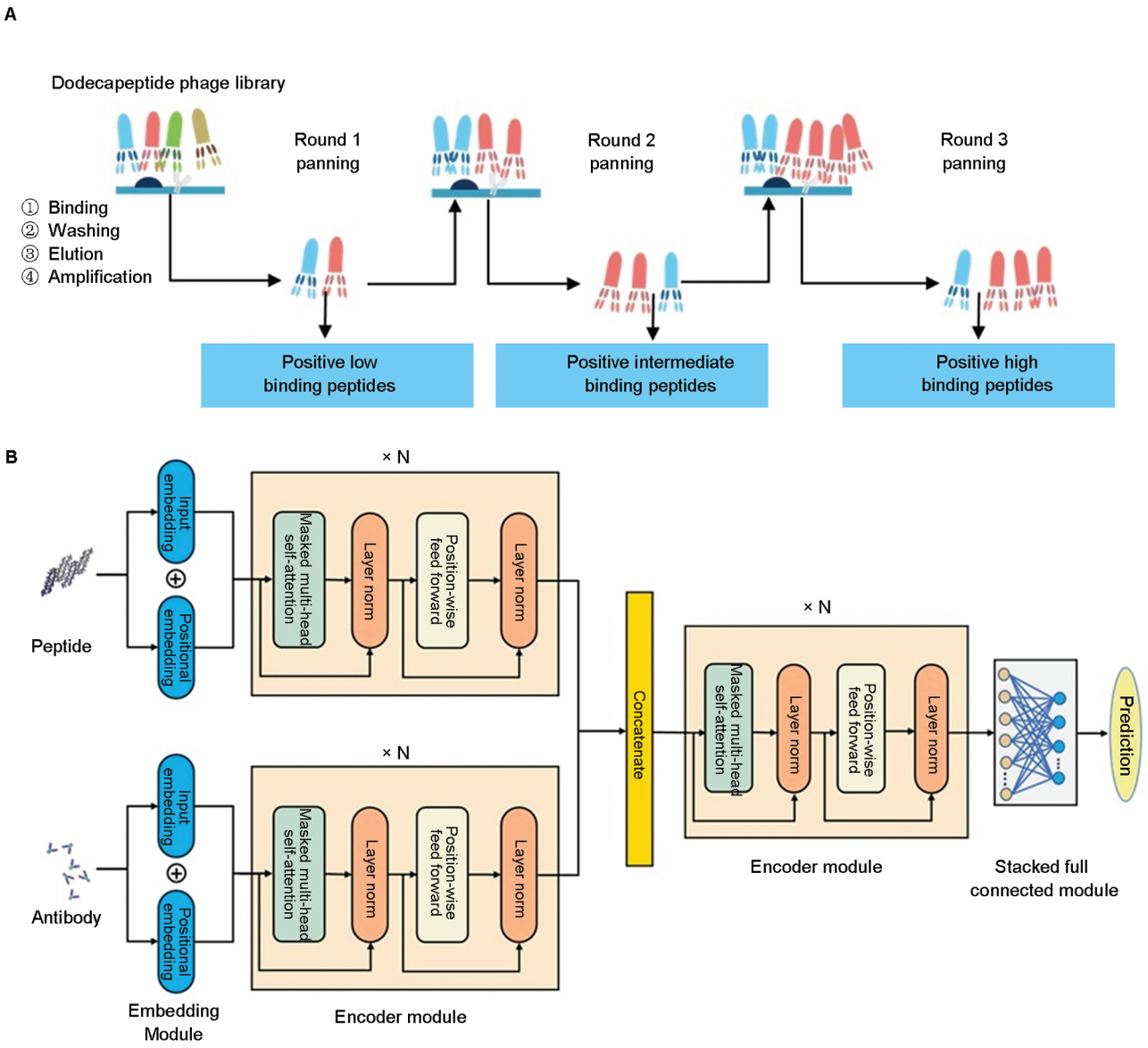


Fig. 1 ABTrans model predicting the interaction of anti-Aβ antibodies with dodecapeptide. (**A**) The experimental workflow generates dodecapeptide. Panning and amplification were performed over three rounds. (**B**) Transformer model framework predicting the binding of antibodies against Aβ to dodecapeptide. Reproduced with permission from Su et al.[39]

pancreatic ductal adenocarcinoma.[53] A CD79b mAb is linked with a PROTAC degrading Bruton's tyrosine kinase, which has been associated with several diseases, including systemic lupus, mantle cell lymphoma, chronic lymphocytic leukemia, and arthritis.[54]

Artificial Intelligence and Antibody–Drug Conjugates Design

During the circulation into the bloodstream, ADCs are exposed to different conditions,[55] and optimizing the components of ADCs for different challenges can provide new insights into the design of novel immune couplings with desirable pharmacokinetic and pharmacodynamic properties. Computational approaches are effective tools for selecting the best target for ADCs,[56] as well as modeling ADC pharmacokinetics and pharmacodynamics to assess the stability, persistence, and efficacy of ADCs in the human body.[57] AI can be used to predict the structure of mAbs in ADC drugs, mainly focusing on modeling the FV region of the antibody and predicting the structure of the complementarity-determining region (CDR) ring of antibodies by establishing suitable models and algorithms, which will play an important role in the development of more effective ADC drugs in future studies.[58]

The target antigens expressed on tumor cells serve as the "navigation" for ADCs to recognize tumor cells and determine the mechanisms of payload delivery including endocytosis. Therefore, selecting appropriate target antigens is a primary consideration for the development of ADCs. The target antigens of marketed ADCs are typically overexpressed specific proteins in cancer cells, such as HER2 and EGFR in solid tumors, and CD19, CD22, and CD33 in hematologic malignancies.[47] In-depth exploration of reported

biomedical data helps drive the discovery of new targets. There are two commonly used methods for target data mining: (1) text mining and (2) multiomics data mining. The former relies on various databases to retrieve and extract information from a large amount of literature through statistical and machine learning methods, obtaining candidate targets and ranking their importance in the literature mining network.[59] The latter primarily involves reanalyzing genomic/transcriptomic/proteomic data of target diseases or samples from databases such as GEO and PRIDE and adopting bioinformatics methods to identify candidate target genes or biological pathways. Fauteux et al reported a machine learning strategy to identify ADC targets against breast cancer.[60] They identified 50 cell membrane targets based on microarray data from public datasets and incorporating differential expression and subcellular localization information, with HER2 being the most likely target. Among them, HER2 is already on the market, with six targets in clinical trials, and many more in various stages of preclinical research and development, indicating the usefulness of this strategy in discovery of target.[60]

The payload of ADCs, acting as the "warhead," is crucial in determining the pharmacokinetics–pharmacodynamics (-PK/PD) of ADCs. The bioavailability can be predicted by absorption, distribution, metabolism, excretion, and toxicity. PK/PD models can be established by mathematical modeling and simulation.[58] Shah et al built the first PK/PD model[61] to accurately predict the biological distribution of ADC payloads in tumors and plasma and successfully predicted the clinical response of trastuzumab-emtansine. The drug-to-antibody ratio (DAR) is associated with the biological distribution of the payloads and influences ADCs' pharmacokinetics and off-target toxicity. Kim et al developed a Poisson model that correlates the average DAR value of ado-trastuzumab emtansine (Kadcyla) determined by ultraviolet spectrophotometry with the distribution of drug payloads, guiding producing uniform and stable ADCs and controlling the distribution of drug payloads.[62] Srivastava studied the mechanisms of action of nine marketed ADCs using various computational methods and tools,[63] optimizing the structure of payloads and predicting other potential targets, contributing to a better understanding of key attributes of ADCs and improving ADC design.

AI-based strategies analyzing and mining large datasets of known ADC structures and activity profiles can be effective in screening and evaluating numerous candidate drug structures. These strategies help in designing novel antibodies, linkers, drug payloads, and suitable targets, thereby accelerating the ADC design and optimization process. This also improves the efficiency and success of ADC development. By constructing PK/PD models *in vivo*, clinical studies can be optimized, with clinical efficacy predicted more accurately. As computational power continues to increase and the algorithm improves, we expect to see the emergence of more accurate and efficient methods for ADC design. Furthermore, the integration of big data and deep learning techniques is expected to uncover more structure–activity relationships and drug design principles, providing valuable insights and guidance for the continued development of ADCs.

Overall, ADCs combine the specificity of mAbs with the potency of cytotoxic drugs, enabling targeted delivery of therapeutics to specific cells, and minimizing off-target effects. An emerging class of therapeutic agents DACs expanded the applications of ADC, whereas AI offers innovative solutions to accelerate ADC discovery, improve design, and optimize manufacturing processes, and its applications include antibody design and optimization, target selection and validation, linker design, toxicity prediction, and payload optimization.

Artificial Intelligence and Nanobodies Design

Nanobodies are characterized by smaller size and simplified structure, and the molecular weight is only 15 kDa, which is 10 times smaller than traditional full antibodies,[64] that is, it can overcome the limitations of traditional antibodies in terms of large size and complex structure. Meantime, nanobodies exhibit higher affinity and stability, allowing them to bind more effectively to target molecules.[65] They can be easily expressed in microbial systems, enabling cost-effective production. Nanobodies have low immunogenicity, good water solubility, and strong tissue penetration capabilities and emerged as a valuable tool in the diagnosis and treatment of diseases. Their unique properties have expanded the possibilities of antibody-based therapeutics and opened up new avenues for improving patient care.[66] They are included in the treatment of a myriad of diseases, including viral infections and cancer,[67–69] confirming their significance in the biopharmaceutical domain. AI plays a key role in advancing the development of nanobody drugs, with its specific applications encompassing the design of nanobody libraries and structural elucidations.

Design of Nanobody Library and Humanization of Nanobodies

Candidate antibody libraries are related to the quality of antibody discovery. *De novo* production of most existing proteins is based on multiple sequence alignment (MSA); however, MSA-producing is not robust for antibodies with extremely high variability in the CDR region. Shin et al[70] trained a generative autoregressive model based on an extended convolutional neural network based on a publicly available database of alpaca nanobodies. The result shows that the SeqDesign nanobody library has similar biochemical properties to natural nanobodies, but with higher diversity. Compared with the MSA-based method, SeqDesign shows better robustness, suggesting that AI can design antibody sequences in the designated domain of antibodies, such as specifying CDR3 or all CDR, for accurate synthesis according to different needs.

Nanobody, as a natural antibody found in animal serum, needs to be humanized to be a therapeutic antibody. However, simultaneous humanization of both the CDR and FR of natural nanobodies is a challenging task. AI has emerged as a

potential tool in the field of antibody humanization. Humanized antibodies can be predicted by several training models, including the Tabhu tool, AntiBERTy developed by the Gray Laboratory team in 2021, and BioPhi automated antibody design launched by Danny A Bitton's team in 2022.[71–73] In addition, the potential impact of humanization on the pharmacokinetics of nanobodies and other properties can also be analyzed by AI.[74]

Currently, antibody humanization is commonly achieved through CDR transplantation and random mutations. However, this approach often faces challenges in maintaining the original CDR conformation and the degree of humanization, leading to reduced antibody affinity. Prihoda et al[73] developed an AI model to automate the human-derived transformation of antibodies by learning from human-derived antibody sequences from the OAS public dataset. This model was validated with 25 publicly known humanized antibody sequences. Building upon this, Mason et al[75] optimized the algorithm through multiobjective optimization to maintain or improve antibody affinity during antibody engineering transformation. The results showed a significant increase in the number of candidate-optimized antibody molecules while ensuring 100% binding. This demonstrates the potential of AI technology in purposefully optimizing lead antibodies and providing more screening possibilities in the early stages of antibody discovery.

Structure Design of Antibody and Structural Elucidation of Antigen–Antibody Complexes

Structure determines function. Based on antibody structure data from AbDb, Eguchi et al[76] trained an Ig-VAE model that simultaneously detect the torsion and distance information of antibodies to create a high-precision antibody database. NanoNet trains an end-to-end deep learning structure model using variable domains that have common folds but differ in their CDRs. When given a specific sequence, NanoNet is able to generate the 3D coordinates of the backbone and Cβ atoms for the entire VH domain.[77]

Lozzo et al[78] developed a protein complex structure elucidation workflow that includes cryo-EM structure elucidation, computer modeling, and molecular dynamics simulation. This workflow allows cryo-EM antigen–antibody complex structures to be obtained within days to weeks. AF2, docking, and residue mutation were employed to study the structure and mechanism of action. These findings provide insights into how nanobodies neutralize SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) and its variants, as well as potential therapeutic targets for coronavirus disease 2019 treatment.[79]

Computational Biology for Other Aspects of Nanobody Development

Yang et al[80] studied the interaction between nanobodies and ochratoxin A (OTA) peptidomimetics through computer-aided simulation and developed two novel immunoassay platforms (PNELISA and APN-ELISA). The proposed new methods have high reliability in OTA detection in food. Using parathion Nb as a model, Li et al[81] described a method based on X-ray crystallography, molecular docking, and rational site-directed saturation mutation to construct a rapid and effective nano-body evolution platform. A research team at Tsinghua University[82] has discovered a nanobody with high neutralizing activity against a variety of new coronavirus variants such as BF.7, BQ.1.1, and XBB that have emerged at home and abroad. In the future, the application of AI in nanobodies research and development is yet to be further expanded, especially in downstream clinical studies.

In short conclusion, nanobodies, or single-domain antibodies derived from camelid immunoglobulins, are gaining prominence in therapeutic and diagnostic fields due to their small size, stability, and high affinity. AI is revolutionizing the development of nanobodies by addressing key challenges in target selection, design, and optimization. Key areas include target identification and validation, nanobody sequence design, affinity and specificity optimization, and epitope mapping.

Artificial Intelligence and Cytokine Drug Development

Challenges in Pharmaceutical Use of Cytokine

Cytokines are proteins that convey intracellular signaling between immune cells, alter enzyme activity or transcriptional programs, and thereby modulate effector functions, which is crucial for health and disease. Cytokines, including interleukins, interferons, tumor necrosis factor, colony-stimulating factors, chemokines, and growth factors, play pivotal regulatory effects in the human body mainly by interacting with cytokine receptors on the cell surface and activating downstream signaling pathways,[16] making them promising entities for the development of biopharmaceuticals. For example, IL-2 and IL-15 have shown antitumor efficacy by stimulating the induction of cytotoxic T lymphocytes and fostering the generation, proliferation, and activation of natural killer cells.[83] However, the development and application of cytokines or cytokine receptor agonists face multifaceted challenges. Although IFN-α-2B (for hairy cell leukemia), IFN-γ (for chronic granulomatous disease), and IL-2 (for melanoma) were approved for clinical usage by the U.S. FDA in the late 20th century, their widespread use has been limited due to severe adverse reactions and constrained efficacy.[84] This is largely attributed to the inherent characteristics of natural cytokines, e.g., cell pleiotropy and a short half-life, which make it challenging to effectively and precisely target specific cytokine receptor subunits and signaling pathways after *in vivo* administration.

Modification of natural cytokines may be able to address these issues. These strategies include (1) fusing cytokines to IgG fragments to extend serum half-life; (2) connecting cytokines to specific peptides or proteins to inactive them and leveraging prodrug structures to overcome off-target effects; (3) redesigning cytokines to selectively activate specific signaling pathways within particular cell types by analyzing crystal structures and interactions between cytokines and receptor subunits.[85] However, unlocking the full potential of cytokine-based therapeutic approaches may

require innovative combinations of all these strategies. Achieving this endeavor depending solely on try-and-error effort and experience proves to be time-consuming, resource-intensive, and challenging.[86]

Artificial Intelligence and Cytokine Drug Design

Protein design using computational biology and AI is a promising avenue for the future development of cytokine receptor agonists. This involves two main approaches: utilizing computer-aided protein design to remodel proteins based on natural cytokines and *de novo* protein design from scratch.[87]

Computational protein design encompasses a variety of methodologies, including those that rely on free energy prediction changes, MSAs, backbone redesign, or deep learning. However, relying on natural cytokines as a foundation for remodeling can only tolerate a limited number of modifications. The products of such modifications may exhibit challenges such as diminished stability and enhanced immunogenicity.

Hence, computational tools initially designed for protein structure prediction, such as Rosetta or AF2, may be enhancement tools for the development of cytokine therapeutics. Current research endeavors explore the feasibility of *de novo* protein design based on protein structure predictions to create cytokine mimetics, achieving selective activation of cytokine receptors. Interestingly, preliminary successes have been observed in this approach. For instance, Quijano-Rubio et al developed the Neoleukins method based on Rosetta, which is able to construct entirely novel proteins that mimic the functions of cytokines (and general signaling proteins).[88] IL-2 and Il-15, as potent lymphocyte activators, have received much attention in the research and development of new drugs. Their heterotrimeric receptors include the cytokine-specific private receptors IL-2Rα and IL-15Rα, and two receptor elements they share, IL-2Rβ and γc. However, the current IL-2 engineering strategies with no ligand selectiveness exhibit evidence of peripheral cytotoxicity. A Rosetta-based *de novo* design method through Neoleukins has designed IL-2 and IL-15 analog independent of IL-2Rα/IL-15Rα, which have demonstrated enhanced stability and efficacy in murine tumor models. These variants are currently undergoing testing in phase I clinical trials for various types of cancer.[89]

Artificial Intelligence and Cytokine Prodrug Development

Prodrug, defined by the International Union of Pure and Applied Chemistry, is a chemical that is transformed before it has pharmacological effects.[90,91] Based on how to convert in the body, they are divided into two types: (1) Type I prodrugs turn into the active forms inside the cells; (2) Type II prodrugs turn into the active forms outside the cells including blood or other fluids. Prodrugs contain nontoxic chemicals that change or hide certain features of the active medications. When prodrugs enter the body, chemical reactions or enzymes activate them. Prodrugs are designed in two different ways, carrier-linked and bioprecursor prodrugs.[92]

The prodrug form helps medications reach the site of action and impacts how medications are distributed to specific locations in the body. Based on these features, one of the main benefits of the prodrug is improving a medication's effectiveness, and another is reducing a medication's toxicity or side effects. Therefore, prodrug serves as a potential method to meet the current challenges of cytokine application in medicine.

Nowadays, cytokine prodrugs have been engineered for therapeutical results. IL-2 prodrug (ProIL2), which masks the activity of a CD8 T cell-preferential IL-2 mutein/Fc fusion protein with IL2 receptor β, which is linked to a tumor-associated protease substrate, can be activated within the tumor to expand antigen-specific CD8 T cells. This significantly reduces IL-2 toxicity and mortality without compromising antitumor efficacy and overcomes the resistance of cancers to immune checkpoint blockade.[91] Another study designed TransCon IL-2 β/γ, which permanently attached a small methoxy polyethylene glycol (mPEG) moiety in the IL-2Rα binding site of IL-2 β/γ and transiently attached it to a 40 kDa mPEG carrier via a TransCon (transient conjugation) linker. In monkeys, TransCon IL-2 β/γ induced robust activation and expansion of CD8+ T cells, natural killer cells, and γδ T cells, relative to CD4+ T cells, Tregs, and eosinophils, with no evidence of cytokine storm or VLS.[93]

Although there is no report on the direct use of computational approaches for cytokine prodrug design, computational protein engineering in general may help predict the most effective prodrug structures, optimize drug delivery systems, and predict the biological responses and therapeutic outcomes of cytokine prodrug therapy. Based on the detailed structure of cytokines and their receptors, AI facilitates the *de novo* design or improvement of Neoleukins,[89,94] which could be used as the key components of cytokine prodrugs. AI-enhanced molecular docking and molecular dynamics simulation could help design the most potency combination of cytokine with other immune molecules, such as cell surface makers and immune checkpoints, and give the most appropriate linker. Moreover, structure-based drug design methods allow us to predict the structure and activity of active and inactive states of prodrugs. High-throughput virtual drug screening facilitates the optimal formation of prodrugs. Machine learning and deep learning models can also be used to predict the stability of different formulations. In all, the benefits of next-generation cytokine prodrugs-derived could be not only reaching better functional bias toward target cells or organs, thereby achieving better drug safety, efficacy, and fewer side effects, but also, being affordable by more people all over the world to reduce cost and limitation in drug storage and delivery.

In summary, AI is accelerating cytokine-based drug discovery and optimization by enabling rapid target identification, prediction of cytokine–receptor interactions, and optimization of cytokine properties for therapeutic use. Protein design can directly help cytokine engineering and

cytokine–receptor interaction prediction, and in the future, AI can simulate cytokine dynamics and pharmacokinetics.

Future Direction, Challenges, and Solutions

Computational biology and AI have penetrated all aspects of pharmaceutical sciences. Based on large-scale patient disease and pharmaceutical data, AI is expected to help predict patient responsiveness. Supervised ML models have been used to analyze structured and unstructured nucleic acid, protein, glycan, and cellular phenotypic datasets to identify critical features and molecular networks involved in host–pathogen or tumor–immune cell interactions and immune responses.[95] Thus, it allows prediction of drug efficacy, proper drug components and linkers, and coadministration that determines optimal therapeutic strategies. In recent years, AI methods, such as data processing after field deployment, neural networks and classifiers, and few-shot and multitask models, have been applied to disease diagnosis and MRI 3D reconstruction to assist surgeons in committing operations.[96] Researchers could also learn more about possible drug interactions from clinical models to avoid contraindications to medical use. Drug treatment strategies that conjecture optimal dosages and combinations could improve prognosis. Thus, when we move into an era of innovative AI medicine, more personalized and precise treatment can extend and improve health care for all human beings.

Despite significant advances in the application of AI and computational biology in developing antibody and protein drugs, several challenges remain. First, the accuracy of models is highly dependent on the quality and diversity of the data used. In drug development, data acquisition and processing may be limited by factors such as cost, ethical considerations, and technological constraints, hindering the model's generalization ability. As such, ensuring the availability of high-quality, diverse, and reliable data becomes a key priority for future advancements.

In addition, the key also lies in fostering stronger interdisciplinary collaboration. The development of antibody and protein drugs spans multiple fields, including biology, chemistry, and computer science. Insufficient interdisciplinary cooperation may limit the effective application of new technologies. To overcome this challenge, it is important to establish closer collaborative frameworks that promote effective communication between biologists, computer scientists, and clinical practitioners. Integrating expertise from different fields is an issue that should be addressed moving forward.

Furthermore, careful consideration of ethical and regulatory issues is essential to ensure ethical standards in the application of new technologies. As technology evolves rapidly, increasing attention must be paid to ethical concerns, such as patient privacy, data security, and gene editing in the context of drug development. These issues require thoughtful and informed resolution. Simultaneously, continuous improvement of relevant regulatory frameworks is essential to ensure compliance with societal and legal norms, safeguarding the rights of patients.

The black-box nature of AI algorithms presents a challenge in explaining their decision-making processes, particularly in the medical field. Understanding how these models make decisions is crucial for both scientists and clinical practitioners involved in drug development. Consequently, a future research direction will focus on enhancing the interpretability of AI algorithms, ensuring that their decision-making processes are transparent, understandable, and trustworthy.

Lastly, addressing individual differences remains a persistent challenge in drug development. While AI offers great potential for personalized medicine, the complex biological systems of the human body contribute to substantial variations in patient responses to the same drug. To overcome this challenge, it is required to develop more refined models that incorporate individual factors such as genetic background and environmental influences.

In conclusion, the challenges facing AI and computational biology in antibody and protein drug development span both technical and ethical–social dimensions. By systematically addressing these challenges, we can drive the continued development of this field, ultimately making a significant contribution to the advancement of medicine and the improvement of patient health.

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Conflict of Interest
None declared.

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