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Antibody mimetics: the next generation antibody engineering, a retrospective and prospective analysis

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Abstract

Antibody mimetics is a novel antibody engineering approach after the development of polyclonal, monoclonal antibodies, and genetically engineered antibody fragments. Inspired by the structure and function of natural antibodies, antibody mimetics offer many advantages over conventional antibodies and can be constructed by protein-directed evolution, peptide design and synthesis, or fusion of complementarity-determining regions through intervening framework regions. A series of parent protein/peptide structures and technical roadmaps have been established to induce better recognising properties, superior affinity, stability, penetrability, and cost-effectiveness of the designed mimetics. This article aims to summarise the evolution of antibody mimetics engineering, illustrate the highlights and hotpots in this research field using scientometric analysis, and give an anticipatory analysis on this increasing research topic.

Antibody mimetics: the next generation antibody engineering, a retrospective and prospective analysis

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Abstract

Antibody mimetics is a novel antibody engineering approach after the development of polyclonal, monoclonal antibodies, and genetically engineered antibody fragments. Inspired by the structure and function of natural antibodies, antibody mimetics offer many advantages over conventional antibodies and can be constructed by protein-directed evolution, peptide design and synthesis, or fusion of complementarity-determining regions through intervening framework regions. A series of parent protein/peptide structures and technical roadmaps have been established to induce better recognising properties, superior affinity, stability, penetrability, and cost-effectiveness of the designed mimetics. This article aims to summarise the evolution of antibody mimetics

engineering, illustrate the highlights and hotpots in this research field using scientometric analysis, and give an anticipatory analysis on this increasing research topic.

Keywords: antibody engineering, antibody mimetics, synthetic antibodies, peptidomimetics, protein engineering, peptide design, scientometric analysis

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1 INTRODUCTION

Following polyclonal and full-length monoclonal antibodies as well as genetically engineered antibody fragments (*Shahidian et al., 2020*), antibody mimetics have become a major modality in antibody engineering. It serves as a promising solution to offer more efficient antibody-like molecules, which could be considered as the next generation of antibody engineering, this will be discussed in depth in this article.

Antibody mimetics the property of antibody to recognise and/or neutralise a target molecule, and thus can be used for a wide range of immunoassays and therapeutic purposes. They are designed, modified, or refined from their parent structures, constructed basically by protein directed evolution, peptide design, or complementary determining regions (CDR) fusion through framework region (FR) in different sequences (*Baloch et al., 2016* ;*Van Holsbeeck et al., 2022*). To date, several types of antibody mimetics have been developed, such as affibodies, anticalins, DARPins, nanofitins, fynomers, avimers, adnectin, peptide aptamer, affimer, affitin, and so forth (*Van Holsbeeck et al., 2022* ;*Yu et al., 2017*).

Box 1 | Glossary

Polyclonal antibody (First generation antibody): composed of biochemically distinct paratopes, the antigen-binding

Generation of polyclonal antibodies is relatively simple and cheap as the use of animals (such as chicken, horses, goats, and rabbits) enables the recovery of large quantity of antibodies from serum or egg Yolk (*Yakhkeshi et al., 2022*). Polyclonal antibodies arising from diverse B-cell clones constitute a heterogeneous mixture that manifests varying binding affinities. In contrast, monoclonal antibodies are homogeneous population originated from a single B-cell clone, resulting in well-defined binding specificities and affinities. However, at some point a fresh batch will be sought as the original stock diminishes, which inevitably leads to a batch-to-batch variation in terms of the specificity, sensitivity, and reproducibility (*Bradbury & Plückthun, 2015*). This might include differences in antibody reactivity and titre, and thus polyclonal reagents often lack reproducibility. In comparison, the continuous culture of B cell hybridomas overs a reproducible and potentially inexhaustible supply of antibody with exquisite specificity. Consequently, monoclonal antibodies enable the development of standardised and secure immunoassay system.

Genetically engineered antibody fragments are alternative to full-length antibody in diagnostics and therapeutics for a variety of diseases due to their various advantages (*Holliger & Hudson, 2005*). Firstly, their small size and simpler structure make them ideal for large-scale production in eukaryotic systems, such as mammalian and insect cells. The antibody fragments have known sequences and are reproducible, verifiable, and manufacturable. The single-chain variable fragment (scFv) is a successful example of a genetically engineered antibody fragment. As the variable light chain and variable heavy chain coding sequences are genetically linked in a single transcript, there is no need to balance the expression of the light chain and heavy chain. Engineered single domain antibodies (VHH) enable the rapid generation of antibody fragments at higher yields and lower cost compared to full-size monoclonal antibodies (mAbs) that typically require mammalian expression systems. Their small size also facilitates tissue penetration and access to cryptic epitopes, making them particularly useful for tumour penetration in cancer immunotherapy (*Zinn et al., 2023*). Moreover, the lack of an Fc region removes the risk of bystander immune cell activation and antibody effector functions such as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), or complement-dependent cytotoxicity (CDC), allowing the molecule to bind its target without activating the host's immune system (*Gravina et al., 2023*). However, the absence of an Fc

region is a double-edged sword. The disadvantage is that it not only reduces thermostability and enhances propensity for aggregation, thereby increasing the risk of immunogenicity, but also shortens half-life due to a lack of FcRn-mediated recycling (*Park et al., 2016*). This can lead to the need for higher and more frequent dosing. Fusion of the scFv to albumin or polyethylene glycol (PEG) can be used to improve half-life (*Albrecht et al., 2004* ;*Vazquez-Lombardi et al., 2015*). However, such fusions can offset the advantages that an scFv holds over a mAb due to cost and the increase in size (*Bradbury & Plückthun, 2015*).

The fourth generation of antibody engineering: antibody mimetics is not generated by the body immune system naturally but share a common feature with natural antibody: they shared characteristic of complementary shape at the binding site with the antigen. Similar to enzyme and substrate, the binding interactions between antibody mimetics and antigens can be explained by three models, including the classical lock and key theory, the induced fit mode, and the conformational selection model. Typically, the classical lock and key theory reflects the initial recognition and specifically between antibody mimetics and antigens. The induced fit model suggests that both antibody mimetics and antigens undergo conformational changes upon binding, in which the dynamic interaction allows the active sites of antibody mimetics to adapt to their shape, optimizing the fit with the antigen. Additionally, the conformational selection model proposes a conformational change occurs prior to the binding of antigens, in which the antigen seems to select and stabilize a higher-energy conformation of an antibody mimetic for binding. Of note, these models are not mutually exclusive, but rather represent different aspects of interactions between antibody mimetics and antigens. Antibody mimetics represent a class of engineered molecules mimic the functions and properties of natural antibodies while offering advantages such as enhanced stability, smaller size, improved production yields. These mimetics can manifest in various forms, like smaller antibody fragments synthesized based on the functional regions of antibody fragments such as scFv or Fab. It is possible to design novel proteins with binding properties through site-directed mutagenesis and random mutagenesis. We summarized and compared the features of all the four generations of in terms of stability, specificity, affinity, and so forth (Table 1).

Now it may be the right time to review and analyse the field of antibody mimetics retrospectively and prospectively. Given that scientometric analysis can quantitively evaluate and investigate on all aspects of the literature at different development stages (*Mooghali et al., 2011*), this article aims to provide a vigorous roadmap for antibody mimetics research through this method to identify the major players and their cooperation networks, including countries, academic groups, and individuals; to analyse research status and hotspots, especially the key study findings; to discuss the potentially valuable research directions.

Table 1 Antibody production & diversified antibody generation platforms

	Antibody properties	**Representative source/technology**
Polyclonal antibody	Polyclonal full length	Antiserum; IgY antibody extracted hen egg
Monoclonal antibody	Monoclonal full length	Full length IgG hybridoma technique
Genetically engineered antibody fragments	Monoclonal functional fragments	Fragment antigen-binding (Fab); scFv (sing
Antibody mimetics		

Notes: The relative degree of antibody properties of each generation is displayed using symbol of "+", the larger number of "+" displayed, the better property possessed. The table is based on a summary of literatures (*Ascoli & Aggeler, 2018* ; *Baloch et al., 2016* ;*Ge et al., 2022* ;*Khalili et al., 2013* ;*Mullard, 2021* ;*Wrapp et al., 2020* ;*Yu et al., 2017*).

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2 METHODOLOGY

2.1 Data Collection and Search Strategy

Web of Science (WoS, Clarivate Analytics) is the authoritative database for bibliometric investigation owning to the existence of the Journal Citation Reports (JCR) and the large amount of citation data. Web of Science Core Collection (WoSCC), as the premier resource on the WoS database, was used to collect information on literature about antibody mimetics. The searched time span is 1986-2022 (Data as of 31 Dec. 2022). All key words related to the topic were combined with Boolean operators before starting the search for documents as which serves as an effective search strategy. The search terms and retrieval strategies were developed as follows: TS (Topical Subject) = (antibody mimetics OR antibody mimetic OR affibody OR adnectin OR aptamer OR affimer OR affitin OR affilin OR anticalin OR atrimer OR Avimer OR Knottin) OR TI (Title) = (antibody mimetics OR antibody mimetic OR affibody OR adnectin OR aptamer OR affimer OR affitin OR affilin OR anticalin OR atrimer OR Avimer OR Knottin) OR AK (Author Keywords) = (antibody mimetics OR antibody mimetic OR affibody OR adnectin OR aptamer OR affimer OR affitin OR affilin OR anticalin OR atrimer OR Avimer OR Knottin) OR AB (Abstract) = (antibody mimetics OR antibody mimetic OR affibody OR adnectin OR aptamer OR affimer OR affitin OR affilin OR anticalin OR atrimer OR Avimer OR Knottin); the language type = English; and the document type = articles or reviews or book or book chapter. All data obtained were saved in text format and imported into software for analysis. To ensure the comprehensiveness of our study, we systematically collected and analysed the terms relevant to antibody mimetics from published reviews to define the keywords, aiming to minimize the risk of omitting important information or introduce irrelevant papers.

2.2 Statistical analysis

Microsoft Office Excel (v.2016) was conducted for literature quantity analysis. CiteSpace 6.1.R6 software was used to depict network maps of keywords, journals, and countries. Basic elements in the map include labels and lines. Items are represented by circular labels and the size of the label determined by the weight of the item, which demonstrate the importance of the items. Lines between items represent links, which is a connection or a relation between two items (*Wu et al., 2022*). The distance between two keywords in the figure demonstrates the rate of co-occurrence of them on a paper or the relationship between two research areas (*Chen, 2006*).

3 RESULTS

3.1 Publication analysis

A total of 24 356 related papers were retrieved from the database WoS, including 20 981 research articles and 2 420 review articles. Overall, the number of publications reflects an upward trend from 1986 to 2022, with an exponential increase since 2005 (Fig. 1).

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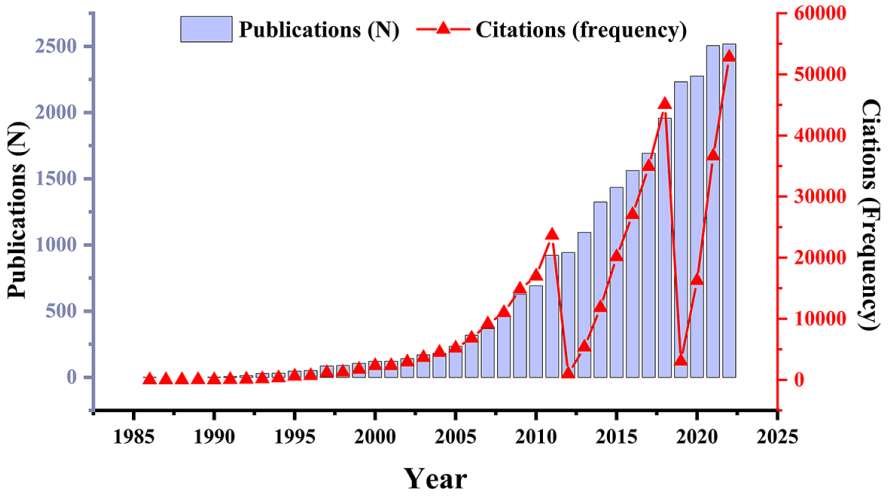

Fig. 1 Publication trend on antibody mimetics between 1986 and 2022

3.2 Keyword Analysis

Keywords demonstrate major research topics in an area with the analysis of burstiness and cluster. Burstiness analysis retrieves keywords with a frequency surge in a particular time period, reflecting stages of rise, bloom and decline of a research topic. Cluster analysis groups keywords into different categories, in which keywords within the same category have high frequency to be co-occurred in an article compared with others. The quality of clusters is determined by the value of silhouette, meaning that clusters with silhouette closest to 1 have the most similarities and homogeneities within cluster and least overlap with other clusters (*Chen, 2004*).

The occurrence of major keywords throughout the development of antibody mimetics is visualized over the timeline. Although the attention of academic community on fundamental research including monoclonal antibody, ligands, gene expression and phage display remain sustained and relatively stable over decades, it is worth noting that research in this field began to enter the stage of therapeutic and diagnostic applications around 2005 (*Chu et al., 2006* ; *Xu et al., 2005*).

Cluster analysis showed 1435 keywords with 6039 links (Fig. 2B), of which 18 keywords appear 100 times or more (aptamer, DNA, nanoparticles, binding, selection, biosensor, gold nanoparticles, *in vitro*selection, assay, protein, aptasensor, ligands, molecules, recognition, sensor, label free, expression, *in vitro*). 1435 keywords were clustered into 11 categories with silhouette vales ranging from 0.641 to 0.982, indicating high cluster credibility and relatively high differentiation of branched research areas within this field. The inherent selectivity of antibody mimetics for their antigen (ligand analyte) confers desirable properties on antibodies to develop technologies that exploit binding events, especially in immunoassays and therapeutics. Clusters #1 "aptasensor", #2 "food safety", #7 "fluorescence anisotropy", Clusters #8 "Alzheimer's disease", #9 "circulating tumor cells" are all focusing on immunoassay. It is notable that the application of immunoassay in various fields has been fully developed as evidenced by the large number of publications in each cluster. Specifically, it acts as important biomarkers for diagnosis of cancers, degenerative diseases such as Alzheimer's' disease and infectious disease such as malaria. Recently, antibody mimetics has been increasingly engineered for detecting bacterial pathogens present in food, water and the environment. This research boom is driven by the development of biosensor technology (*Li et al., 2019*), in which, antibody mimetics that interact with target analyte are combined with physiochemical transducer that transforms the result of the interaction into an optical, piezo-electrical, or electrochemical signal. For example, antibody-nanoparticle bioconjugates have proven to be an ideal vessel for bioconjugation and biosensor purposes due to their unique surface characteristics, optical properties, stability, and consistency. Apart from the wide

application in immunoassay, antibody mimetics also play roles in drug delivery for cancer diseases and neurological disorders. Antibody-conjugated drug-loaded nanoparticles can selectively target cells and release large amounts of drugs to treat diseases like cancers (*Arruebo et al., 2009*). This is because they combine both functions of the nanoparticles such as liposomes, polymeric nanoparticles, dendrimers, and metallic nanoparticles, which control drug release, increase drug solubility, protect drug from degradation, and the antibody mimetics, which can bind to their targets with high affinity and better cell penetration (*Busch et al., 2019*).

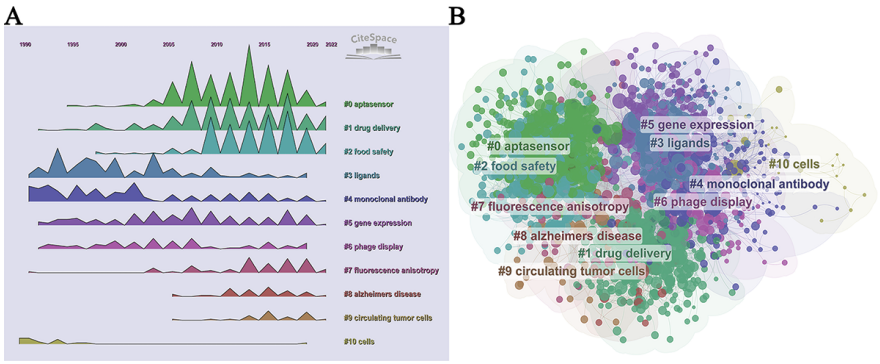

Fig. 2 Keywords burstiness and cluster analysis

Notes: (A) Height of each peak represents the frequency of occurrence of a stated keyword within two years. Different keywords are labelled with different colour. (B) Cluster labels represent the focus themes of each cluster.

One of the most striking information reflected from this visualization is that molecular detection for food safety and increasing application of newly developed immunoassay technology has become the new research trends in recent five years.

3.3 Country and Institution Analysis

A total of 112 countries participated in the study of antibody mimetics (Fig. 3A). Collaborations among countries were relatively small as the centrality of all countries are less than 1.00, and most of which were established after 2007. There were no regular patterns relating to the geographical locations of different countries in this study. The top five countries which have strong networking with others were Brazil, Peru, Netherland, Iraq, and Egypt (centrality value of 0.70, 0.66, 0.53, 0.48, and 0.45). USA, Italy, Japan, Canada, UK, Netherlands, Germany, Austria, Switzerland, and France contributed to the emergence of antibody engineering with the earliest publications averaging between 1990 and 1993. And the number of publications in all these countries has remained consistently high over the past 36 years. Interestingly, collaborations among these leading countries were extremely rare with few links established between nodes. China, USA, Germany, Japan, South Korea were the most productive countries with the highest publication number of 9615, 5811, 1192, 1103, and 1044, respectively.

A total of 921 links are established between 953 institutions which involved in the study of antibody mimetics, interestingly, collaborations among institutions within the same country were much more intensive than international collaboration (Fig. 3B). In addition, compared with country analysis, the proportion of the leading institutions with full colour spectrum was much lower, many institutions like Harvard University reduced their research in recent five years. The top five institutions with the highest publication number were all in China, and many newly emerging institutions were also found in China. The top five institutions with the greatest paper citation number are Laboratory of Molecular Biology, Medical Research Council,

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Cambridge, UK contributing to phage display technology, Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, USA on protein engineering and the design of novel scaffolds, Institute of Biochemistry and Biotechnology, Martin-Luther University Halle-W for development of artificial binding proteins based on non-antibody scaffolds and their applications in biotechnology and medicine, Laboratory of Chemical Biology and Signal Transduction, The Rockefeller University, New York, USA on synthetic small molecules and peptidomimetics for the modulation of protein-protein interactions and therapeutic applications and Department of Biotechnology, Indian Institute of Technology, Madras, India based on novel scaffolds such as spider silk.

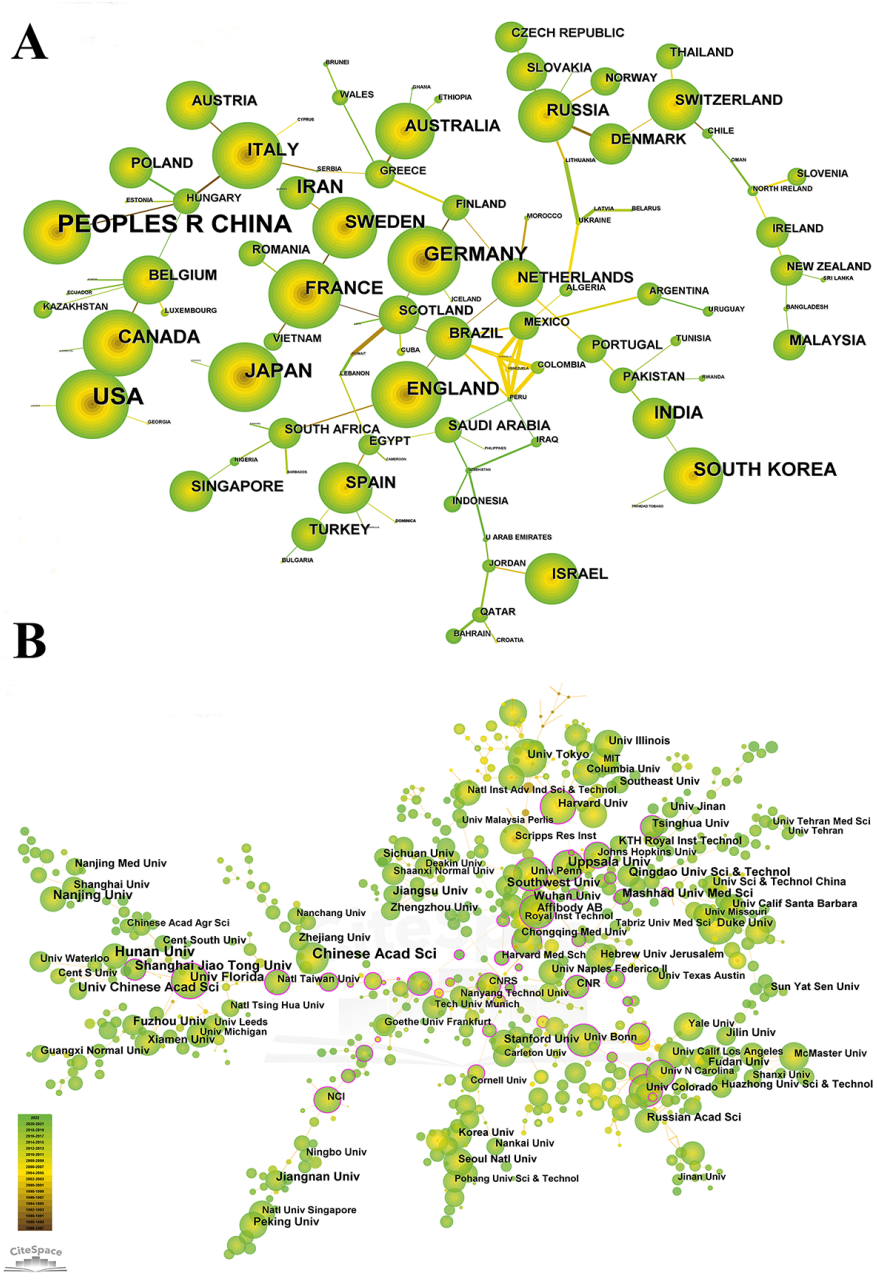

Fig. 3 Country and institution analysis on antibody mimetics between 1986 and 2022

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Notes: Node size represents the number of publications. Node color: average time to appear. The purple circles indicate that these countries have a significant role in the study.

3.4 Journal Analysis

Journal selection is an indicator to reflect the research focus and tendency of antibody mimetics study which is coincide with keywords analysis. A total of 554 journals were retrieved, the most productive journals are Biosensors & Bioelectronics (1146 publications) and Analytical Chemistry (942 publications) (Fig. 4). The most cited journals (*Analytical Chemistry* , *Journal of the American Chemical Society* , *Nature* , *Science* , *PNAS* , *and Biosensors & Bioelectronics*) have been cited more than 10,000 times. In addition, *Journal of Biological Chemistry* ,*Biochemistry-US* , *Journal of Molecular Biology* ,*PNAS* , *Nature* , *Science* , *Acs Sensors* , and*Nature Biotechnology* produced a high citation burst, indicating their critical role in the field (Table 2). Interestingly, increasing emerging papers were published on food related journals like*Foods* , *Food Chemistry* , *Journal of Food Measurement and Characterization* and *Microchemical Journal* , indicating a new application field exploited. Meanwhile, a growing number of interdisciplinary journals relevant to antibody mimetics studies span biology, chemistry, and physics.

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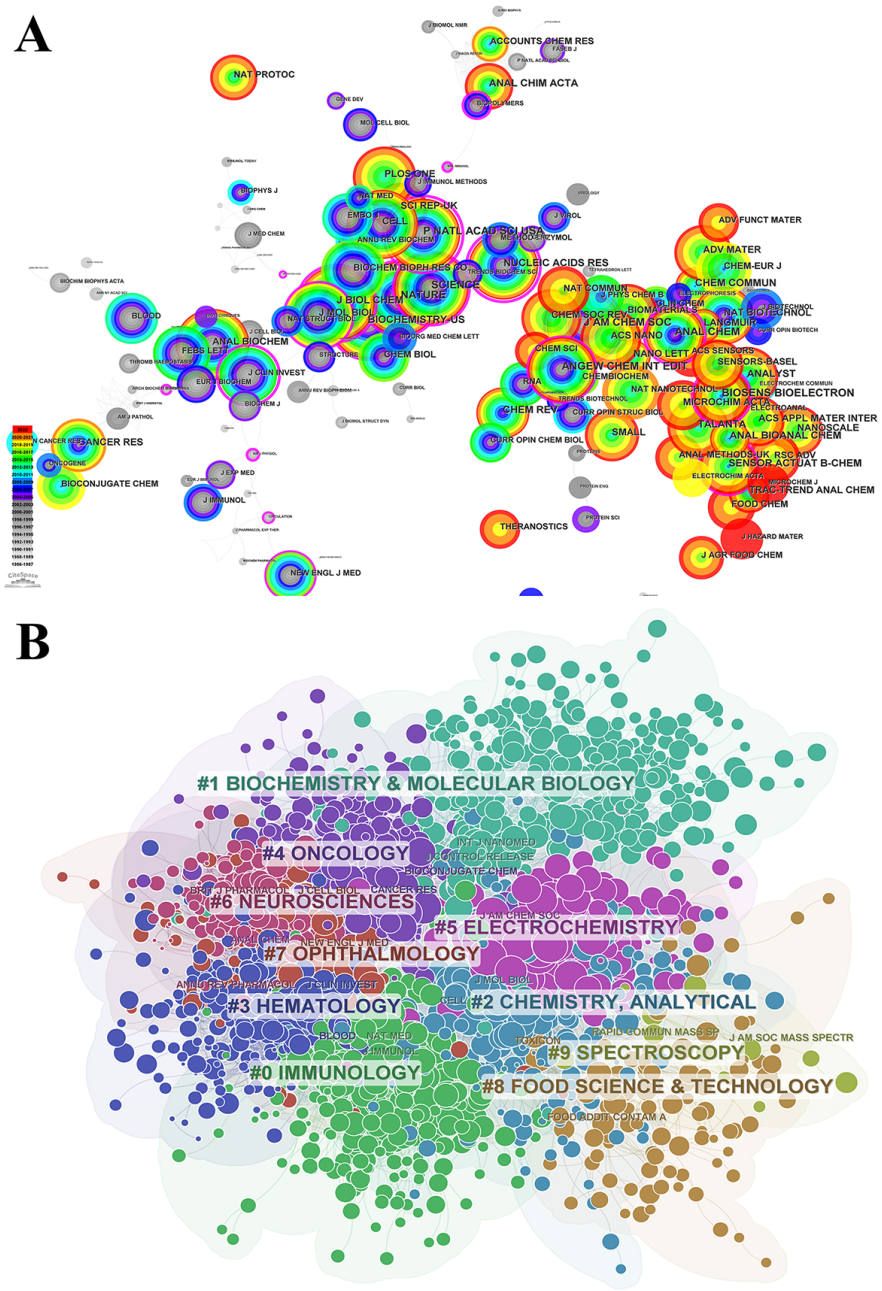

Fig. 4 Journal analysis on antibody mimetics between 1986 and 2022

Notes: (A) Co-cited journals. (B) Co-cited journals clustering. Each node represents a journal; the size of the node is determined by the citation frequency of the journal.

Table 2 The top 20 journals in terms of number of publications and citations

Rank	Citations	Journals	Publications	Journals
1	12254	ANAL CHEM	147	P NATL ACAD SCI USA
2	11601	J AM CHEM SOC	136	CANCER RES

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Rank	Citations	Journals	Publications	Journals
3	10680	NATURE	135	NATURE
4	10612	SCIENCE	132	ANAL CHEM
5	10451	P NATL ACAD SCI USA	131	J AM CHEM SOC
6	10363	BIOSENS BIOELECTRON	130	SCIENCE
7	8646	ANGEW CHEM INT EDIT	126	NUCLEIC ACIDS RES
8	7585	CHEM COMMUN	122	BIOSENS BIOELECTRON
9	7570	NUCLEIC ACIDS RES	115	CHEM COMMUN
10	6202	ANAL CHIM ACTA	111	ANGEW CHEM INT EDIT
11	6166	ANALYST	111	ANAL CHIM ACTA
12	5853	J BIOL CHEM	111	BIOCONJUGATE CHEM
13	5847	SENSOR ACTUAT B-CHEM	109	PLOS ONE
14	5474	TALANTA	107	J BIOL CHEM
15	5332	BIOCHEMISTRY-US	105	CELL
16	4914	CHEM REV	102	CLIN CANCER RES
17	4868	ANAL BIOANAL CHEM	102	J CLIN ONCOL
18	4766	ACS NANO	99	ACS APPL MATER INTER
19	4742	PLOS ONE	99	NAT BIOTECHNOL
20	4621	ACS APPL MATER INTER	98	BIOMATERIALS

4 DISCUSSION

Antibody mimetics is a research heated topic at a rapid development stage witnessed by bibliometrics analysis, and it is predicted that this trend will continue in the following years. Although the first article retrieved from the Web of Science appeared at the year of 1986 according to the keywords, the first ever research "A synthetic peptide mimotope of the hepatitis C virus NS3 protein induces HCV-reactive antibodies and cytokine-secreting T cells in mice" using molecules that mimic the function of antibody was in 1995 (*Wilson et al., 2001*). The year of 2005 was a landmark moment in the development of antibody mimetics, indicating the transition of methodology and basic research into application stage (Figs. 1, 2). Antibody mimetics is applied into more fields such as neuroscience and contamination monitoring in recent years (Fig. 2). The technical focus of the application of antibody mimetics has shifted from basic biological problems such as protein structure prediction and molecular interaction into some physically and chemically based technology such as nanoparticles and quantum technology (Figs. 2, 4). The combination of these newly developed techniques widens the fields of applications by providing antibody mimetics with more desirable properties at various conditions. Therefore, it is predicted that the development of antibody mimetics will be highly influenced by other technologies. Researchers with interdisciplinary backgrounds may be greatly promote the combinations of new technologies into antibody mimetics.

In antibody engineering, a series of antibody features have been highlighted and pursued, which are all demonstrated by the high frequency of related terms retrieved, for example, high affinity (704), high stability (364), high specificity (110), high penetration, good host mediation, simple preparation, high repeatability, and low cost in mass production, in which affinity is the highly emphasized (Fig. 2).

The challenges encountered in establishing appropriate keywords for the bibliometric analysis stem primarily from inherent limitations in the field. Specifically, the inclusion of all articles, reviews, and book chapters related to antibody mimetics proved challenging due to variations in terminology and categorization. Some authors focused on specific aspects of engineered proteins without explicitly labeling them as antibody mimetics. In addition, the broad nature of the keywords employed in the analysis, as highlighted by the editor, may have resulted in the inclusion of irrelevant papers. To avoid of this issue as much as possible, we conducted comprehensive artificial screening process to refine and confine the selection of the papers for analysis. Moreover, it is important to note that the term "antibody mimetics" is not uniformly adopted as a standardized terminology within this research field. Researchers often interpret protein engineering

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techniques from different perspectives, leading to the usage of diverse terms such as "synthetic antibody", "engineered antibody", and "designed drug molecules". Concepts and terminologies in this field are various mainly due to the dynamic and expending understanding on antibody molecules, and novel antibody formats designed, as well as the continuous invention, combination, and modification of different technologies in antibody generation from molecular design to mass manufacturing. For instance, only within the last 20 years, the advent of synthetic diversity libraries provides a 'chemical solution' to the biological constraints on natural repertoire diversity (*Šácha et al., 2016*). Synthetic approaches are particularly attractive to those seeking to understand the fundamentals of antibody–antigen interactions or to engineer desired antibody properties, as they enable precise control over the composition of diversity incorporated into antigen-binding sites (*Miersch & Sidhu, 2012*). This explains why "synthetic antibody" has been used as a general term referring to part of antibody mimetics (Fig. 5). At the same time, with the increase of application scenarios and the diversification of molecular types of the referenced parent structures, the scope of these terms has also taken on a broader meaning. The molecular structures of antibody mimetics generally include peptides and proteins, while aptamer is an exception, it is a short oligonucleotide sequence (DNA or RNA), which can specifically bind to its target on proteins and other molecules, or the entire cells, for further therapeutic or diagnostic interventions (*Yan et al., 2021*). Meanwhile, it is worth noting that the functional repertoire of antibody mimetics has significantly expanded beyond their traditional role as antigen binders. They have evolved into versatile tools capable of recognizing and binding specific targets for the purpose of detecting analytes in biological samples (*Šácha et al., 2016* ;*Šubr et al., 2021* ;*Yu et al., 2017*), as well as in separation methods (*Olson et al., 2012*), cancer therapy (*Guillard et al., 2017* ; *Park et al., 2000*), targeted drug delivery (*Balmforth et al., 2021*), and *in vivo* imaging (*Chomet et al., 2021* ;*Dietrich et al., 2021*). This diversification of functionality showcases the adaptability and potential applications of antibody mimetics in various research fields. Peptide antibody mimetics is a subset of peptidomimetics which provide alternatives to natural biopolymers (Fig. 5). Both antibody mimetics and peptidomimetics are designed to mimic the structure and/or function of antibodies or peptides, respectively. However, there are several differences between them. Antibody mimetics is typically larger in size and more complex in structure than peptidomimetics, as they often incorporate multiple binding domains and/or structural motifs. Peptidomimetics, on the other hand, are usually smaller and simpler in structure, consisting of one or a few modified amino acids. Antibody mimetics is designed to recognize and bind to specific targets, such as proteins or cells, with high affinity and specificity. The development of computational modeling and structure-based design methods, such as conformational constraint and incorporation of non-natural amino acids, has facilitated the rational design and optimization of peptidomimetics, allowing the peptidomimetics to achieve binding specificity on par with antibodies. In addition, given that peptidomimetics offer advantages such as smaller size, easier synthesis, and better stability compared to antibodies. These attributes make them attractive candidates for drug development, where they can target challenging protein-protein interactions or intracellular targets that are not easily accessible to antibodies. Antibody mimetics can have various modes of action, such as blocking protein-protein interactions (*Fernandes et al., 2022*), inhibiting enzymatic activity (*Khalili et al., 2016*), or inducing cell signaling (*Shan et al., 2020*). Peptidomimetics, on the other hand, typically act as ligands or modulators of specific receptors or signaling pathways (*Goodman et al., 2007*). Throughout the development, researchers from different discipline backgrounds and perspectives have been involved, chemists focusing on the design of chemical conjugates for drug delivery may not interpret these structures as antibody mimetics, as this terminology was arisen from immunology research.

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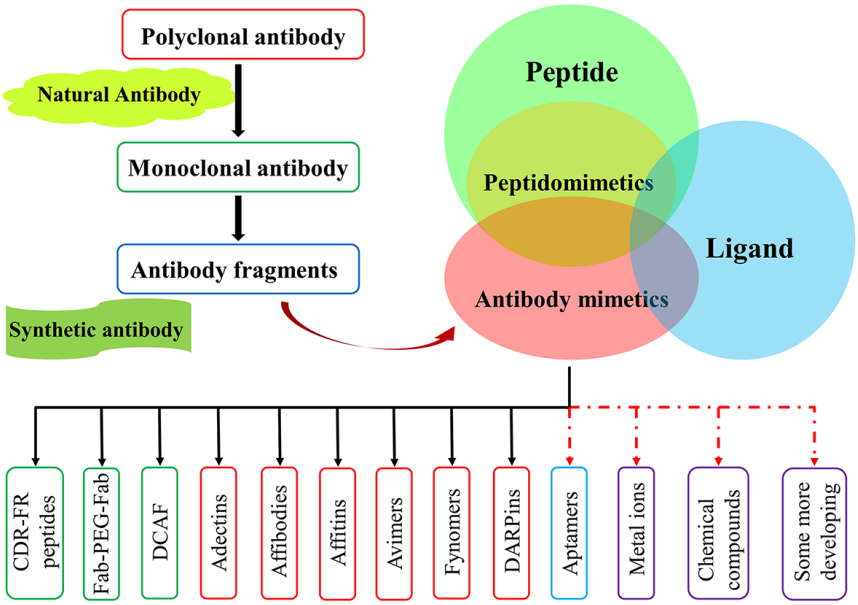

Fig. 5 Evolutionary and integrative perspective and definition of the scope of antibody mimetics

Notes: green, orange, and red ovals represent the assemblies of peptides, peptidomimetics, and ligands, respectively. Boxes are representatives of antibody-derived (deep green) and non-antibody derived (red) protein/peptides, nucleic acids and their mimics (cyan), as well as other components (purple) like ions, chemical molecules, and other developing structures. Antibody mimetics originally evolved from artificially manipulated antibodies, including antibody fragments and synthetic antibodies. Later, this term expanded and focused more on refining the existing parent structures from functional fragments to smaller, more stable, higher affinity, more reproducible and penetrable ones. For instance, natural peptides, peptidomimetics and ligands partially belong to the family of antibody mimetics. As antibody mimetic engineering evolved, different concepts and terminology emerged, but each may better reflect the state of research at that time. Retrospectively, some terms, such as synthetic antibody, are blurry. To certain extent, the term "antibody mimetics" also could not reflect the entire intension and extension of this dynamic concept as increasing non-antibody sourced and non-protein and peptide sourced structures have been introduced into this field. As highlighted by cyan and orange boxes in the figure, aptamer and other substances beyond the scope of biomolecules deserve consideration for inclusion in antibody mimetics repertoire. Therefore, further classification and definition of this field are urgently needed and expected. Here we propose the protein/peptide-based antibody mimetics as the core concept of this field.

Compared to traditional antibodies, the fourth generations of antibody engineering offer antibodies with a range of properties, providing much diverse formats such as small peptides, protein scaffolds, or synthetic polymers, for antibody design which allows for greater flexibility in their application. At the same time, they are smaller in size, which allows for better tissue penetration and target access (*Jiang et al., 2009*). They can be produced using simpler and more cost-effective methods, which enables mass production. Additionally, they have improved stability and can be engineered to have higher specificity and affinity for their target antigen. Through artificial modification and screening, antibody mimetics have demonstrated commendable attributes in comparison to traditional antibodies. These modifications may involve targeted alteration within antigen-binding sites or conjugation of specific proteins/peptides, thereby facilitating the development of more efficacious therapeutic or diagnostic reagents and expediting their market penetration. Such reagents were summarized in the Table 3, and here we only mentioned two compelling representatives. Qiu *et al.* , fused antibody mimetics with the bacterial toxin colicin Ia, resulting in the creation

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of "pheromonicins" that specifically inhibit tumor growth. it displayed superior tumor targeting and penetration capabilities surpassing those of their parent antibodies (*Qiu et al., 2007*). Similarly, Wold *et al.* synthesized antibody mimetics by site-specific binding of small molecules with high affinity and specificity for disease-associated antigens to Fc fragments, enabling to develope antibody-based drugs with enhanced efficacy (*Wold et al., 2015*). By harnessing the unique properties of antibody mimetics, such as their modifiability and specificity, they hold promise for bringing antibody-based drugs to the market. However, there are also some disadvantages to using mimetic antibodies. They may not have the same efficacy as traditional antibodies, particularly in complex biological systems. Additionally, their potential for off-target effects and toxicity may need to be carefully evaluated (*Khatib & Salla, 2022*). Moreover, the antibody mimetic engineering still requires sophisticated design and complicated biopanning process, which also lead to high cost and limits its application and commercialization. The potential for antibody mimetics to replace outdated or obsolete antibody drugs and revive their presence in the market is theoretically possible. However, it must be recognized that reintroducing an antibody taken from the market due to safety concerns entails multifaceted considerations. Safety issues may originate from various factors, encompassing adverse effects, lack of efficacy, or other complications. If the advancements of antibody mimetics could address the specific safety concerns or enable safer alternatives to withdrawn antibodies, it has the potential to usher in novel and improved therapeutic options. Nevertheless, each case requires an extensive reassessment of the safety profile, including careful evaluation, rigorous testing, and regulatory scrutiny to ensure the safety and efficacy of the developed antibody mimetic prior to its contemplation for reintroduction into the market. As depicted in Table 3, we present a summary of antibody mimetics drugs that have entered the clinic or are undergoing clinical validation.

Computational analysis has emerged as a promising strategy for accelerating the development of novel antibody mimetics, enhancing their capabilities while reducing the cost associated with traditional trial-and-error approaches (*Kadonosono et al., 2020* ;*Raybould et al., 2019*). Notably, prominent computational methods such as RossettaAntibody and AntBo have been employed to achieve various purposes in this context. These methods enable to discern and characterize the crucial attributes of existing antibodies, including affinity, specificity, stability, and immunogenicity. Additionally, they facilitate the determination of key residues responsible for antigen binding. By harnessing computational analysis, it becomes possible to predict and propose modifications that can enhance binding affinity and specificity (*Kuroda et al., 2012* ;*Wang et al., 2021*). Moreover, the integration of machine learning and deep sequencing techniques (*Sloth et al., 2022*) has proven to be efficient in the realm of biopanning and optimization of phage display technology (Fig. 6). These state-of-the-art methodologies provide valuable insights into the discovery and refinement of antibody mimetics.

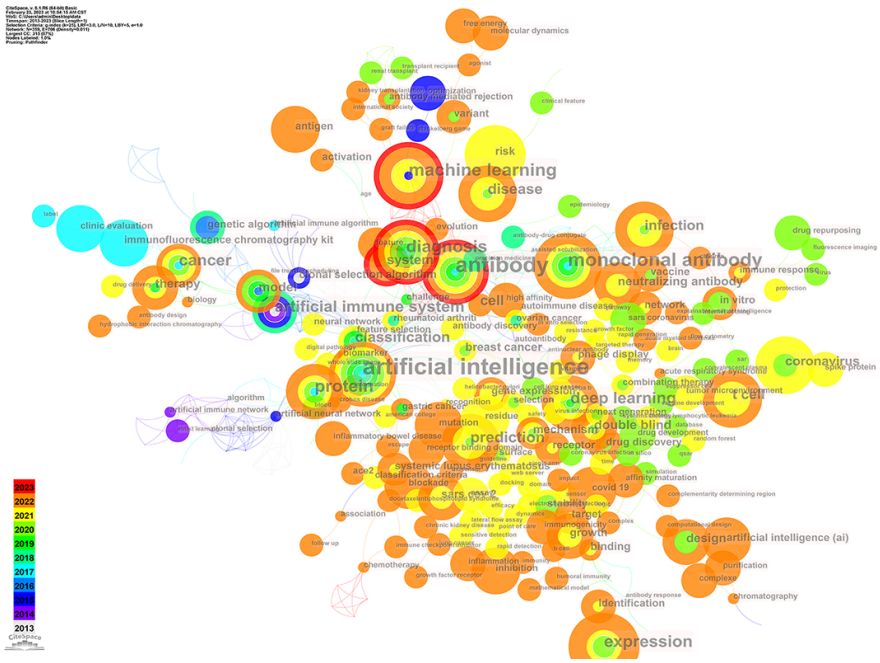

Fig. 6 Application of artificial intelligence in antibody mimetics

Notes: Each node represents a keyword; the size of the keyword is determined by the rate of occurrence.

Furthermore, artificial intelligence technology, such as Alphafold (Fig. 6) (*Tunyasuvunakool et al., 2021*), is applied to accurately predict the structure and conformation of an unknown protein based on regular binding and folding patterns collected from related database such as Protein Data Bank. In particular, the three-dimensional structure of an antibody can already be predicted from its gene. However, there is a big proportion of antibodies without corresponding gene available, furthermore, dynamic conformational change of antibodies when binding with antigens or receptors cannot be predicted yet (*Abanades et al., 2022*). It will greatly diminish time and cost of antibody design if protein structure could be predicted based on the structure of the antigens or receptors, while the conformational change and more limited data of the antibody-antigen complex increase the challenge for this breakthrough. However, there are still a lot of limitation of computational approach restricting its further optimization and application in antibody engineering. For example, computational analysis and AI algorithms require large amounts of data to learn and make accurate predictions. However, there is limited data available on antibody-antigen interactions, which can limit the accuracy of predictions made by these methods. Computational models used for predicting the structure and function of antibody mimetics are based on assumptions and simplifications, which may not accurately represent the complexity of real-world systems. This can lead to inaccuracies in predictions and suboptimal designs, Computational design methods often rely on the availability of templates for the design of new antibody mimetics. However, the diversity of available templates is limited, which can limit the range of possible designs.

Table 3 Antibody mimetics currently approved by the FDA or in clinical trials

Antibody mimetics	**Scaffold**	**Molecular mass (kDa)**	**Example drug**	**Clinical Trials**	**Publications**	**Applications**
Affibody molecules	Z domain of Protein A	6	ABY-025	Phase II (NCT01216033)		Tumor imaging

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Antibody mimetics	**Scaffold**	**Molecular mass (kDa)**	**Example drug**	**Clinical Trials**	**Publications**	**Applications**
Affilins (Anticalins)	Ubiquitin or Lipocalins	10-20	PRS-080	Phase I/II (NCT02754167)		Anemia
Affimers (Adhirons)	Cystatin	12–14	Anti-PD-L1 Affimer		(*Basran & Stanley, 2018*)	Cancer treatment
Alphabodies	Triple helix coiled coil	10	CMPX-1023		(*Desmet et al., 2014*)	Autoimmune disease
Avimers	A domains of various membrane receptors	9-18	C326 (AMG220)	Phase I (NCT00353756)		Crohn's disease
DARPins	Ankyrin repeat motif	14-21	Abicipar	Phase III (NCT02462486, NCT02462928)		Age-related macular degeneration (AMD)
Domain antibodies	Engineered human single-domain Ig scaffold	11-15	CEP37247/PN0621/ ART621	Phase II NCT00928317		Treat autoimmune diseases
Fynomers	Src homology 3 (SH3) domain of Fyn	7	COVA322	Phase I/II (NCT02243787)		Plaque psoriasis
Kunitz domain peptides	Kunitz domains of various protease inhibitors	7	Ecallantide (DX88) Depelstat (DX890)	FDA approved in 2012 Phase II (NCT00455767)		Hereditary angioedema Pulmonary fibrosis
Knottin	Three anti-parallel β-strands connected	4	Ziconotide (Prialt)	FDA approved in 2012		Neuropathic pain
Monobodies (Adnectins)	10th type III domain of fibronectin	10	BMS-986089	Phase II (NCT02515669)		Duchenne muscular dystrophy
β-Hairpin mimetics	β-Hairpin motif	1-2	POL6326	Phase I (NCT01837095)		Tumor suppressor
Monobodies (Adnectins)	10th type III domain of fibronectin	10	BMS-986089	Phase II (NCT02515669)		Duchenne muscular dystrophy

Notes: All the data were derived through thorough investigation of the existing literature, including review articles and research papers, as well as the information available on the Drugbank website regarding antibody mimetic drugs. From these sources, we carefully selected representative compounds from each category, taking into consideration the variations in scaffold structure, and provided an overview of the clinical status for each of the selected drugs.

5 CONCLUSIONS

Antibody mimetics has shown widespread applications in immunoassays and therapeutics, a trend that will continue and have real impact. Antibody mimetic research relies on the advancement of interdisciplinary approach, especially computational analysis, which plays a practical and proficient role in prediction. However, it shows great limitations in predicting the structure of antibody mimetics. Increasing and diversified institutions and countries have been involved in this area with strong collaboration and networking exhibited.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Siran Zhang and Longjiang Wu contribute equally. Siran Zhang: Conceptualization, Methodology, Investigation, Validation, Data curation, Writing – original draft. The endeavors undertaken by Siran Zhang have been duly acknowledged and wholeheartedly supported by her parents. For further details, we kindly direct your attention to the attached informed consent letter, which can be found in a separate file.

Longjiang Wu: Conceptualization, Methodology, Writing – review & editing. Mei Dang: Conceptualization, Writing – review & editing, Supervision.

CONFLICT OF INTEREST

Details of all funding sources should be provided, including grant numbers if applicable. Please ensure to add all necessary funding information, as after publication this is no longer possible.

DATA AVAILABILITY STATEMENT

Data is contained within the manuscript.

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REFERENCES

Abanades, B., Wong, W. K., Boyles, F., Georges, G., Bujotzek, A., & Deane, C. (2022). *ImmuneBuilder: Deep-Learning models for predicting the structures of immune proteins .https://doi.org/10.1101/2022.11.04.514231*

Adams, J. J., & Sidhu, S. S. (2014, Feb). Synthetic antibody technologies. *Curr Opin Struct Biol, 24* , 1-9.*https://doi.org/10.1016/j.sbi.2013.11.003*

Albrecht, H., Burke, P. A., Natarajan, A., Xiong, C. Y., Kalicinsky, M., DeNardo, G. L., & DeNardo, S. J. (2004, Jan-Feb). Production of soluble ScFvs with C-terminal-free thiol for site-specific conjugation or stable dimeric ScFvs on demand.*Bioconjug Chem, 15* (1), 16-26.*https://doi.org/10.1021/bc030018+*

Arruebo, M., Valladares, M., & González-Fernández, Á. (2009). Antibody-conjugated nanoparticles for biomedical applications. *Journal of Nanomaterials, 2009* , 1-24.*https://doi.org/10.1155/2009/439389*

Ascoli, C. A., & Aggeler, B. (2018, Sep). Overlooked benefits of using polyclonal antibodies.*Biotechniques, 65* (3), 127-136.*https://doi.org/10.2144/btn-2018-0065*

Balmforth, M. R., Haigh, J., Kumar, V., Dai, W., Tiede, C., Tomlinson, D. C., Deuchars, J., Webb, M. E., & Turnbull, W. B. (2021, Oct 20). Piggybacking on the Cholera Toxin: Identification of a CTB-Binding Protein as an Approach for Targeted Delivery of Proteins to Motor Neurons. *Bioconjug Chem, 32* (10), 2205-2212.*https://doi.org/10.1021/acs.bioconjchem.1c00373*

Baloch, A. R., Baloch, A. W., Sutton, B. J., & Zhang, X. (2016). Antibody mimetics: promising complementary agents to animal-sourced antibodies. *Crit Rev Biotechnol, 36* (2), 268-275.*https://doi.org/10.3109/07388551.2014.958431*

Basran, A., & Stanley, E. (2018, 07/01). Abstract 3776: Generation and formatting of an Affimer® biotherapeutic for the inhibition of the PD-L1/PD-1 pathway: Proof of concept in mouse. *Cancer Research, 78* , 3776-3776.*https://doi.org/10.1158/1538-7445.AM2018-3776*

Bradbury, A., & Plückthun, A. (2015, Feb 5). Reproducibility: Standardize antibodies used in research.*Nature, 518* (7537), 27-29.*https://doi.org/10.1038/518027a*

Busch, R. T., Karim, F., Weis, J., Sun, Y., Zhao, C., & Vasquez, E. S. (2019, Sep 17). Optimization and Structural Stability of Gold Nanoparticle-Antibody Bioconjugates.*ACS Omega, 4* (12), 15269-15279.*https://doi.org/10.1021/acsomega.9b02276*

Chen, C. (2004, Apr 6). Searching for intellectual turning points: progressive knowledge domain visualization.*Proc Natl Acad Sci U S A, 101 Suppl 1* (Suppl 1), 5303-5310.*https://doi.org/10.1073/pnas.0307513100*

Chen, C. M. (2006, Feb 1). CiteSpace II: Detecting and visualizing emerging trends and transient patterns in scientific literature [Article]. *Journal of the American Society for Information Science and Technology, 57* (3), 359-377.*https://doi.org/10.1002/asi.20317*

Chomet, M., van Dongen, G., & Vugts, D. J. (2021, Jul 21). State of the Art in Radiolabeling of Antibodies with Common and Uncommon Radiometals for Preclinical and Clinical Immuno-PET. *Bioconjug Chem, 32* (7), 1315-1330.*https://doi.org/10.1021/acs.bioconjchem.1c00136*

Chu, T. C., Marks, J. W., 3rd, Lavery, L. A., Faulkner, S., Rosenblum, M. G., Ellington, A. D., & Levy, M. (2006, Jun 15). Aptamer:toxin conjugates that specifically target prostate tumor cells. *Cancer Res, 66* (12), 5989-5992.*https://doi.org/10.1158/0008-5472.Can-05-4583*

Desmet, J., Verstraete, K., Bloch, Y., Lorent, E., Wen, Y., Devreese, B., Vandenbroucke, K., Loverix, S., Hettmann, T., Deroo, S., Somers, K., Henderikx, P., Lasters, I., & Savvides, S. N. (2014, Oct 30). Structural basis of IL-23 antagonism by an Alphabody protein scaffold. *Nat Commun, 5* , 5237.*https://doi.org/10.1038/ncomms6237*

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Dietrich, A., Andreeff, M., Koi, L., Bergmann, R., Schubert, M., Schreiner, L., Löck, S., Sihver, W., Freudenberg, R., Hering, S., Pietzsch, H. J., Steinbach, J., Kotzerke, J., Baumann, M., & Krause, M. (2021, Feb). Radiotherapy enhances uptake and efficacy of (90)Y-cetuximab: A preclinical trial. *Radiother Oncol, 155* , 285-292.*https://doi.org/10.1016/j.radonc.2020.11.013*

Fernandes, L. A., Gomes, A. A., Guimarães, B. G., de Lourdes Borba Magalhães, M., Ray, P., & da Silva, G. F. (2022, Jun). Engineering defensin α-helix to produce high-affinity SARS-CoV-2 spike protein binding ligands. *Protein Sci, 31* (6), e4355.*https://doi.org/10.1002/pro.4355*

Fosgerau, K., & Hoffmann, T. (2015, Jan). Peptide therapeutics: current status and future directions.*Drug Discov Today, 20* (1), 122-128.*https://doi.org/10.1016/j.drudis.2014.10.003*

Ge, S., Wu, R., Zhou, T., Liu, X., Zhu, J., & Zhang, X. (2022, Feb 12). Specific anti-SARS-CoV-2 S1 IgY-scFv is a promising tool for recognition of the virus. *AMB Express, 12* (1), 18.*https://doi.org/10.1186/s13568-022-01355-4*

Goodman, C. M., Choi, S., Shandler, S., & DeGrado, W. F. (2007, May). Foldamers as versatile frameworks for the design and evolution of function. *Nat Chem Biol, 3* (5), 252-262.*https://doi.org/10.1038/nchembio876*

Gravina, A., Tediashvili, G., Rajalingam, R., Quandt, Z., Deisenroth, C., Schrepfer, S., & Deuse, T. (2023, Jan 2). Protection of cell therapeutics from antibody-mediated killing by CD64 overexpression. *Nat Biotechnol .https://doi.org/10.1038/s41587-022-01540-7*

Guillard, S., Kolasinska-Zwierz, P., Debreczeni, J., Breed, J., Zhang, J., Bery, N., Marwood, R., Tart, J., Overman, R., Stocki, P., Mistry, B., Phillips, C., Rabbitts, T., Jackson, R., & Minter, R. (2017, Jul 14). Structural and functional characterization of a DARPin which inhibits Ras nucleotide exchange.*Nat Commun, 8* , 16111.*https://doi.org/10.1038/ncomms16111*

Holliger, P., & Hudson, P. J. (2005, Sep). Engineered antibody fragments and the rise of single domains.*Nat Biotechnol, 23* (9), 1126-1136.*https://doi.org/10.1038/nbt1142*

Jiang, T., Zhang, L., Wang, J., & Li, X. (2009, Nov). Reasonable design of antibody mimetics for targeting glioma and small cell lung cancer. *Eur J Cancer Prev, 18* (6), 431-435.*https://doi.org/10.1097/CEJ.0b013e32832e0912*

Kadonosono, T., Yimchuen, W., Ota, Y., See, K., Furuta, T., Shiozawa, T., Kitazawa, M., Goto, Y., Patil, A., Kuchimaru, T., & Kizaka-Kondoh, S. (2020, Jan 21). Design Strategy to Create Antibody Mimetics Harbouring Immobilised Complementarity Determining Region Peptides for Practical Use. *Sci Rep, 10* (1), 891.*https://doi.org/10.1038/s41598-020-57713-4*

Khalili, H., Godwin, A., Choi, J. W., Lever, R., Khaw, P. T., & Brocchini, S. (2013, Nov 20). Fab-PEG-Fab as a potential antibody mimetic. *Bioconjug Chem, 24* (11), 1870-1882.*https://doi.org/10.1021/bc400246z*

Khalili, H., Lee, R. W., Khaw, P. T., Brocchini, S., Dick, A. D., & Copland, D. A. (2016, Nov 22). An anti-TNF-α antibody mimetic to treat ocular inflammation. *Sci Rep, 6* , 36905.*https://doi.org/10.1038/srep36905*

Khatib, S. E., & Salla, M. (2022). The mosaic puzzle of the therapeutic monoclonal antibodies and antibody fragments - A modular transition from full-length immunoglobulins to antibody mimetics. *Leuk Res Rep, 18* , 100335.*https://doi.org/10.1016/j.lrr.2022.100335*

Kuroda, D., Shirai, H., Jacobson, M. P., & Nakamura, H. (2012, Oct). Computer-aided antibody design.*Protein Eng Des Sel, 25* (10), 507-521.*https://doi.org/10.1093/protein/gzs024*

Lenci, E., & Trabocchi, A. (2020, Jun 7). Peptidomimetic toolbox for drug discovery. *Chem Soc Rev, 49* (11), 3262-3277.*https://doi.org/10.1039/d0cs00102c*

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Li, Z., Mohamed, M. A., Vinu Mohan, A. M., Zhu, Z., Sharma, V., Mishra, G. K., & Mishra, R. K. (2019, Dec 10). Application of Electrochemical Aptasensors toward Clinical Diagnostics, Food, and Environmental Monitoring: Review. *Sensors (Basel), 19* (24).*https://doi.org/10.3390/s19245435*

Lipman, N. S., Jackson, L. R., Trudel, L. J., & Weis-Garcia, F. (2005). Monoclonal versus polyclonal antibodies: distinguishing characteristics, applications, and information resources. *Ilar j, 46* (3), 258-268.*https://doi.org/10.1093/ilar.46.3.258*

Miersch, S., & Sidhu, S. S. (2012, Aug). Synthetic antibodies: concepts, potential and practical considerations. *Methods, 57* (4), 486-498.*https://doi.org/10.1016/j.ymeth.2012.06.012*

Mooghali, A., Alijani, R., Karami, N., & Khasseh, A. (2011). Scientometric analysis of the scientometric literature. *International Journal of Information Science and Management (IJISM), 9* (1), 19-31.

Moore, G. P. (1989, Sep). Genetically engineered antibodies. *Clin Chem, 35* (9), 1849-1853.

Mullard, A. (2021, Jul). FDA approves 100th monoclonal antibody product. *Nat Rev Drug Discov, 20* (7), 491-495.*https://doi.org/10.1038/d41573-021-00079-7*

Nelson, P. N., Reynolds, G. M., Waldron, E. E., Ward, E., Giannopoulos, K., & Murray, P. G. (2000, Jun). Monoclonal antibodies. *Mol Pathol, 53* (3), 111-117.*https://doi.org/10.1136/mp.53.3.111*

Olson, C. A., Nie, J., Diep, J., Al-Shyoukh, I., Takahashi, T. T., Al-Mawsawi, L. Q., Bolin, J. M., Elwell, A. L., Swanson, S., Stewart, R., Thomson, J. A., Soh, H. T., Roberts, R. W., & Sun, R. (2012, Dec 7). Single-round, multiplexed antibody mimetic design through mRNA display. *Angew Chem Int Ed Engl, 51* (50), 12449-12453.*https://doi.org/10.1002/anie.201207005*

Park, B. W., Zhang, H. T., Wu, C., Berezov, A., Zhang, X., Dua, R., Wang, Q., Kao, G., O'Rourke, D. M., Greene, M. I., & Murali, R. (2000, Feb). Rationally designed anti-HER2/neu peptide mimetic disables P185HER2/neu tyrosine kinases in vitro and in vivo. *Nat Biotechnol, 18* (2), 194-198.*https://doi.org/10.1038/72651*

Park, H. I., Yoon, H. W., & Jung, S. T. (2016, Nov). The Highly Evolvable Antibody Fc Domain. *Trends Biotechnol, 34* (11), 895-908.*https://doi.org/10.1016/j.tibtech.2016.04.005*

Qiu, X. Q., Wang, H., Cai, B., Wang, L. L., & Yue, S. T. (2007, Aug). Small antibody mimetics comprising two complementarity-determining regions and a framework region for tumor targeting. *Nat Biotechnol, 25* (8), 921-929.*https://doi.org/10.1038/nbt1320*

Qu, H., Csordas, A. T., Wang, J., Oh, S. S., Eisenstein, M. S., & Soh, H. T. (2016, Aug 23). Rapid and Label-Free Strategy to Isolate Aptamers for Metal Ions. *ACS Nano, 10* (8), 7558-7565.*https://doi.org/10.1021/acsnano.6b02558*

Raybould, M. I. J., Marks, C., Krawczyk, K., Taddese, B., Nowak, J., Lewis, A. P., Bujotzek, A., Shi, J., & Deane, C. M. (2019, Mar 5). Five computational developability guidelines for therapeutic antibody profiling. *Proc Natl Acad Sci U S A, 116* (10), 4025-4030.*https://doi.org/10.1073/pnas.1810576116*

Šácha, P., Knedlík, T., Schimer, J., Tykvart, J., Parolek, J., Navrátil, V., Dvořáková, P., Sedlák, F., Ulbrich, K., Strohalm, J., Majer, P., Šubr, V., & Konvalinka, J. (2016, Feb 12). iBodies: Modular Synthetic Antibody Mimetics Based on Hydrophilic Polymers Decorated with Functional Moieties. *Angew Chem Int Ed Engl, 55* (7), 2356-2360.*https://doi.org/10.1002/anie.201508642*

Shahidian, A., Ghassemi, M., Mohammadi, J., & Hashemi, M. (2020). 4 - Immunotherapy. In A. Shahidian, M. Ghassemi, J. Mohammadi, & M. Hashemi (Eds.),*Bio-Engineering Approaches to Cancer Diagnosis and Treatment* (pp. 69-114). Academic Press.*https://doi.org/https://doi.org/10.1016/B978-0-12-817809-6.00004-2*

Shan, L., Cook, K. M., Haskins, N., Omar, B., Jiang, Y., Garcia, A., Koksal, A., Oganesyan, V., Rosenthal, K., Wu, H., Dall'Acqua, W. F., & Damschroder, M. M. (2020, Oct 14). Long-acting antibody ligand mimetics

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for HER4-selective agonism.*Sci Rep, 10* (1), 17257.*https://doi.org/10.1038/s41598-020-74176-9*

Sloth, A. B., Bakhshinejad, B., Jensen, M., Stavnsbjerg, C., Liisberg, M. B., Rossing, M., & Kjaer, A. (2022, Oct 29). Analysis of Compositional Bias in a Commercial Phage Display Peptide Library by Next-Generation Sequencing. *Viruses, 14* (11).*https://doi.org/10.3390/v14112402*

Ståhl, S., Gräslund, T., Eriksson Karlström, A., Frejd, F. Y., Nygren, P., & Löfblom, J. (2017, Aug). Affibody Molecules in Biotechnological and Medical Applications. *Trends Biotechnol, 35* (8), 691-712.*https://doi.org/10.1016/j.tibtech.2017.04.007*

Stojanovic, M. N., de Prada, P., & Landry, D. W. (2001, May 30). Aptamer-based folding fluorescent sensor for cocaine. *J Am Chem Soc, 123* (21), 4928-4931.*https://doi.org/10.1021/ja0038171*

Šubr, V., Ormsby, T., Šácha, P., Konvalinka, J., Etrych, T., & Kostka, L. (2021). The role of the biotin linker in polymer antibody mimetics, iBodies, in biochemical assays. *Polymer Chemistry, 12* (41), 6009-6021.*https://doi.org/10.1039/d1py00707f*

Tiede, C., Bedford, R., Heseltine, S. J., Smith, G., Wijetunga, I., Ross, R., AlQallaf, D., Roberts, A. P., Balls, A., Curd, A., Hughes, R. E., Martin, H., Needham, S. R., Zanetti-Domingues, L. C., Sadigh, Y., Peacock, T. P., Tang, A. A., Gibson, N., Kyle, H., Platt, G. W., Ingram, N., Taylor, T., Coletta, L. P., Manfield, I., Knowles, M., Bell, S., Esteves, F., Maqbool, A., Prasad, R. K., Drinkhill, M., Bon, R. S., Patel, V., Goodchild, S. A., Martin-Fernandez, M., Owens, R. J., Nettleship, J. E., Webb, M. E., Harrison, M., Lippiat, J. D., Ponnambalam, S., Peckham, M., Smith, A., Ferrigno, P. K., Johnson, M., McPherson, M. J., & Tomlinson, D. C. (2017, Jun 27). Affimer proteins are versatile and renewable affinity reagents. *Elife, 6* .*https://doi.org/10.7554/eLife.24903*

Tunyasuvunakool, K., Adler, J., Wu, Z., Green, T., Zielinski, M., Žídek, A., Bridgland, A., Cowie, A., Meyer, C., Laydon, A., Velankar, S., Kleywegt, G. J., Bateman, A., Evans, R., Pritzel, A., Figurnov, M., Ronneberger, O., Bates, R., Kohl, S. A. A., Potapenko, A., Ballard, A. J., Romera-Paredes, B., Nikolov, S., Jain, R., Clancy, E., Reiman, D., Petersen, S., Senior, A. W., Kavukcuoglu, K., Birney, E., Kohli, P., Jumper, J., & Hassabis, D. (2021, Aug). Highly accurate protein structure prediction for the human proteome. *Nature, 596* (7873), 590-596.*https://doi.org/10.1038/s41586-021-03828-1*

Van Holsbeeck, K., Martins, J. C., & Ballet, S. (2022, Feb). Downsizing antibodies: Towards complementarity-determining region (CDR)-based peptide mimetics. *Bioorg Chem, 119* , 105563.*https://doi.org/10.1016/j.bioorg.2021.105563*

Vazquez-Lombardi, R., Phan, T. G., Zimmermann, C., Lowe, D., Jermutus, L., & Christ, D. (2015, Oct). Challenges and opportunities for non-antibody scaffold drugs. *Drug Discov Today, 20* (10), 1271-1283.*https://doi.org/10.1016/j.drudis.2015.09.004*

Wang, D. D., Chan, M. T., & Yan, H. (2021). Structure-based protein-ligand interaction fingerprints for binding affinity prediction. *Comput Struct Biotechnol J, 19* , 6291-6300.*https://doi.org/10.1016/j.csbj.2021.11.018*

Wilson, D. S., Keefe, A. D., & Szostak, J. W. (2001, Mar 27). The use of mRNA display to select high-affinity protein-binding peptides. *Proc Natl Acad Sci U S A, 98* (7), 3750-3755.*https://doi.org/10.1073/pnas.061028198*

Wold, E. D., Axup, J. Y., Felding, B. H., & Smider, V. V. (2015, Dec 16). Fc-Small Molecule Antibody Mimetics. *Bioconjug Chem, 26* (12), 2311-2314.*https://doi.org/10.1021/acs.bioconjchem.5b00530*

Wrapp, D., De Vlieger, D., Corbett, K. S., Torres, G. M., Wang, N., Van Breedam, W., Roose, K., van Schie, L., Hoffmann, M., Pöhlmann, S., Graham, B. S., Callewaert, N., Schepens, B., Saelens, X., & McLellan, J. S. (2020, May 28). Structural Basis for Potent Neutralization of Betacoronaviruses by Single-Domain Camelid Antibodies. *Cell, 181* (5), 1004-1015.e1015.*https://doi.org/10.1016/j.cell.2020.04.031*

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Wright, A., Shin, S. U., & Morrison, S. L. (1992). Genetically engineered antibodies: progress and prospects.*Crit Rev Immunol, 12* (3-4), 125-168.

Wu, R., Yakhkeshi, S., & Zhang, X. (2022, Apr). Scientometric analysis and perspective of IgY technology study. *Poult Sci, 101* (4), 101713.*https://doi.org/10.1016/j.psj.2022.101713*

Xu, D., Xu, D., Yu, X., Liu, Z., He, W., & Ma, Z. (2005, Aug 15). Label-free electrochemical detection for aptamer-based array electrodes. *Anal Chem, 77* (16), 5107-5113.*https://doi.org/10.1021/ac050192m*

Yakhkeshi, S., Wu, R., Chelliappan, B., & Zhang, X. (2022). Trends in industrialization and commercialization of IgY technology. *Front Immunol, 13* , 991931.*https://doi.org/10.3389/fimmu.2022.991931*

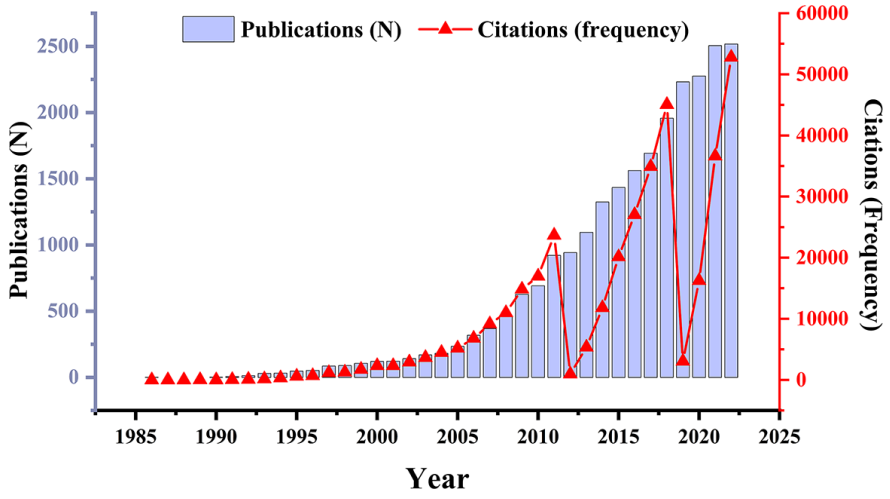
Yan, J., Gao, T., Lu, Z., Yin, J., Zhang, Y., & Pei, R. (2021, Jun 23). Aptamer-Targeted Photodynamic Platforms for Tumor Therapy. *ACS Appl Mater Interfaces, 13* (24), 27749-27773.*https://doi.org/10.1021/acsami.1c06818*

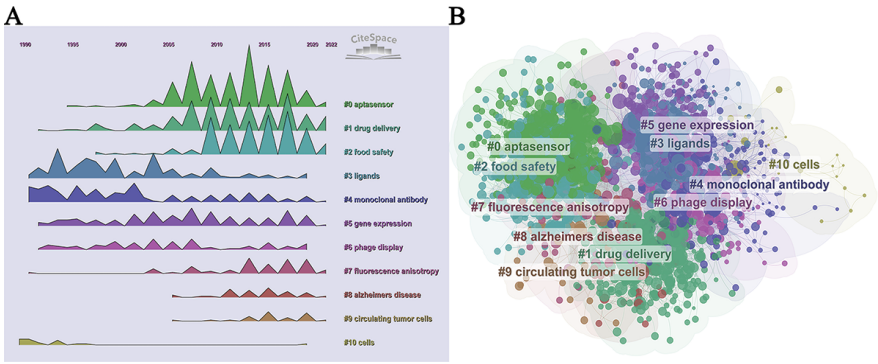
Yang, K. A., Barbu, M., Halim, M., Pallavi, P., Kim, B., Kolpashchikov, D. M., Pecic, S., Taylor, S., Worgall, T. S., & Stojanovic, M. N. (2014, Nov). Recognition and sensing of low-epitope targets via ternary complexes with oligonucleotides and synthetic receptors. *Nat Chem, 6* (11), 1003-1008.*https://doi.org/10.1038/nchem.2058*

Yu, X., Yang, Y. P., Dikici, E., Deo, S. K., & Daunert, S. (2017, Jun 12). Beyond Antibodies as Binding Partners: The Role of Antibody Mimetics in Bioanalysis. *Annu Rev Anal Chem (Palo Alto Calif), 10* (1), 293-320.*https://doi.org/10.1146/annurev-anchem-061516-045205*

Zhang, X., & Diraviyam, T. (2017). Antibody Mimetics, Peptides, and Peptidomimetics. *Methods Mol Biol, 1575* , 3-13.*https://doi.org/10.1007/978-1-4939-6857-2_1*

Zinn, S., Vazquez-Lombardi, R., Zimmermann, C., Sapra, P., Jermutus, L., & Christ, D. (2023, Feb 20). Advances in antibody-based therapy in oncology. *Nat Cancer .https://doi.org/10.1038/s43018-023-00516-z*





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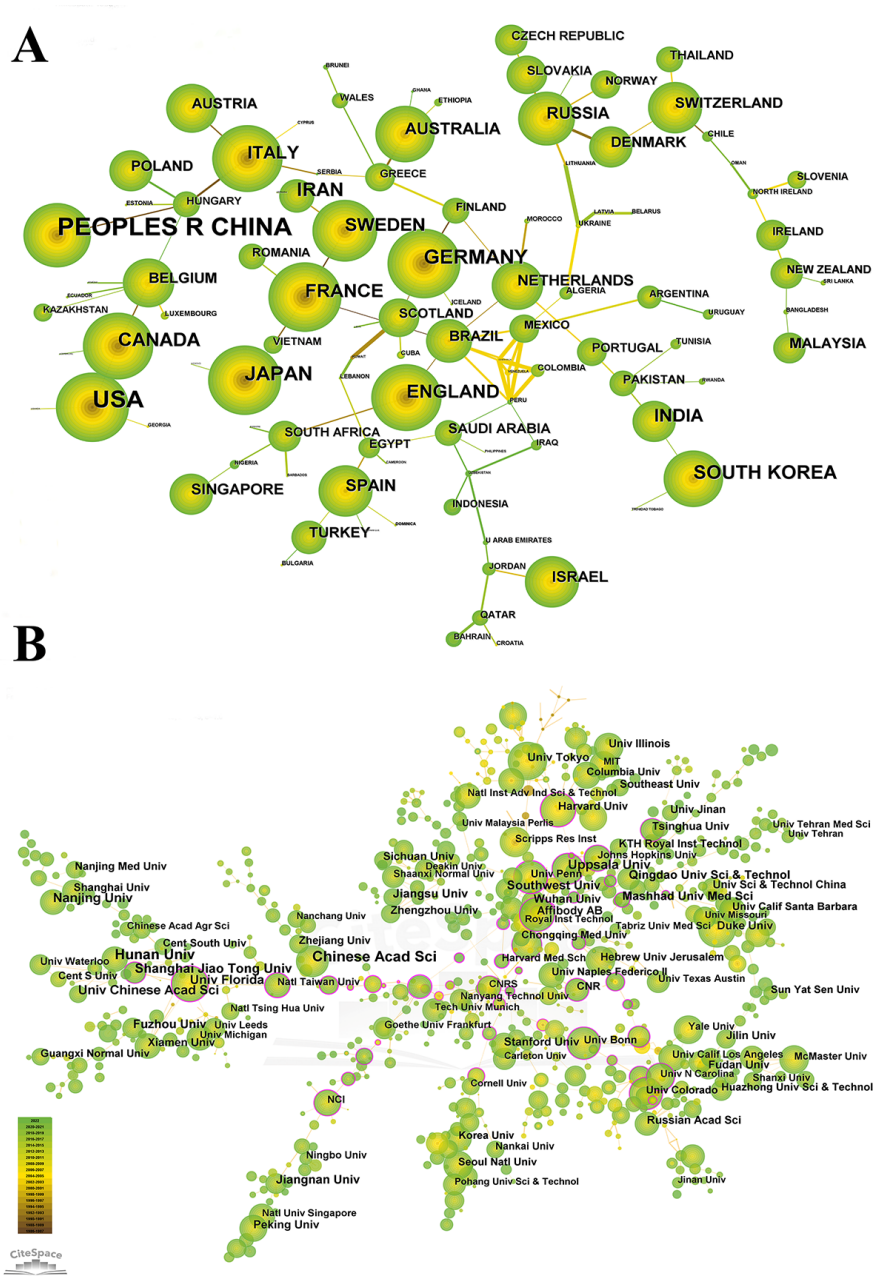

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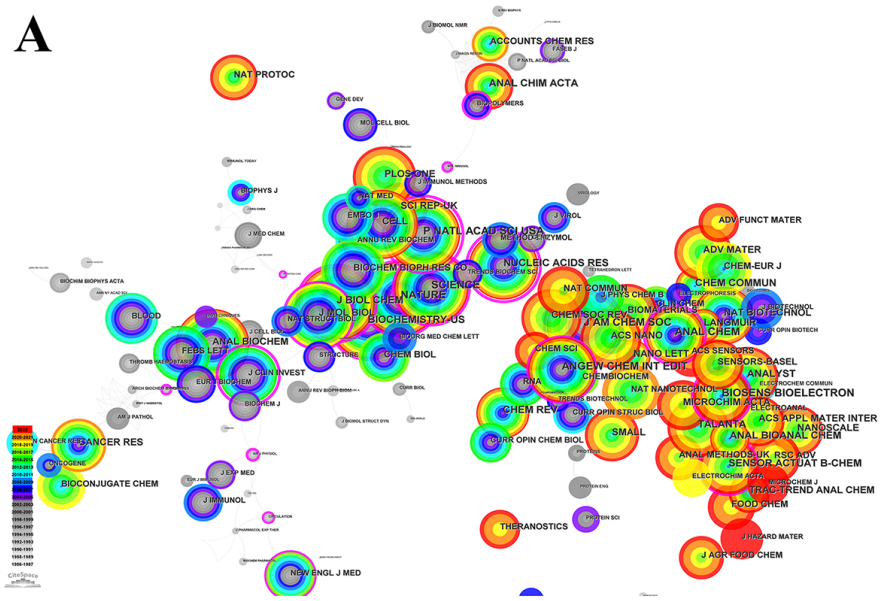

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