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Review Article

Strategies in design of antibodies for cancer treatment

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ABSTRACT

Structural bioinformatics and computational methodologies facilitate antibodies design. The rational design is used to achieve a new function or adopt a new structure. Computer algorithms can systematically search amino acid sequences and select the best one. One of the important points of protein therapeutics is utilizing as drug. The present study reviewed recent studies about antibody design, antibody structure modeling, antibody prediction, and stability, pharmacokinetics and recent algorithms used in antibody design. By advanced technology with computational technologies development of therapeutics antibodies is possible. In early stages, several methods of predicting protein structure and de novo protein design were presented.

***Keywords*:** Antibody design; nano-carriers; pharmacokinetics property; docking; algorithms

INTRODUCTION

Cancer is the main wellbeing issue all around the world, and the relevant statistics show one in four deaths in the United States due to cancer [1]. In 2030, it is predicted that the top three cancers for men consist of prostate, lung, and melanoma, and for women, breast, lung/bronchus, and colorectal [2]. Immune system (innate and acquired immunity) main function is target cancer cells and infectious. The first barrier in the immune system is physical which protects the body from foreign agents. If the innate immunity has not been able to protect the body, the adaptive or

acquired immunity will act using T and B-cells [3].

IgG structure

IgG is a basic format of antibody which is generally utilized in all approved antibodies. Two light chains and two heavy chains altogether construct IgG structure. The most common therapeutic antibodies are IgG1 isotypes since compared to other classes/subclasses; not only has a long half-life in serum, but also the effector function is stronger. Antigen interacts with antibody via VH and VL (Variable) domains; this interaction mostly formed by Complementarity Determining Regions (CDRs); for contacting small targets to antibody two to three CDRs are involved, while for larger proteins, four to six CDRs are involved. In other words, the combination of CDR loops depends on the size of the antigen [4]. Opsonization, cell lysis, mast cells' degranulation, and eosinophils to antigen recognition are some other functions of the Fc" region [5]. These days, plenty of research projects have centered on antibodies to develop drugs and vaccines as particular tools in treating certain malignancies and significant applications in biotechnology [6, 7]. The number of therapeutic antibodies confirmed by the Food and Drug Administration (FDA) is about 25; however, approximately one thousand in development have entered clinical trials within the last decade [8]. Several notable Clinical trials therapeutic antibodies are highlighted (Supplementary data S1). Some successful antibodies to treat patients who suffer from cancer involve the antibodies against CD20 (Ocrelizumab) [9], Her2/neu (Herceptin/ Trastuzumab) [10], Epidermal Growth Factor Receptor (EGFR) (Cetuximab) [11], and Vascular Endothelial Growth Factor (VEGF) (bevacizumab) [12].

Antibodies which are developed by new technologies may have been effective agents for treating various diseases. Various methods are accessible for engineering high-affinity antibody which both biophysical properties and the same biological function are being considered [13].

As an example of drug, Doxil® has been confirmed in the USA and Europe that is the drug doxorubicin encapsulated in PEG-liposomes and suitable for the treatment of AIDS-related Kaposi's sarcoma, ovarian and breast cancer [14].

Strategies in engineering monoclonal antibodies (mAbs)

Improving pharmacokinetics and immunogenicity properties are the goal of the engineering antibody for therapeutic purposes. Antibody properties such as

epitope binding, target affinity, pharmacokinetics, function and immunogenicity level [15] are enhanced by antibody engineering and optimization technologies.

Limitation of natural antibodies

Short half-lives (typically less than 20 h), inefficient immunogenicity, and effector functions are some of the major constraints of native antibodies. Inefficient effector functions can be solved by increasing the efficacy of antibodies, by combining antibodies treatment with chemotherapy, so it would be beneficial for treating patients. Therefore, antibody design strategies emerged to overcome these challenges [16]. Animal immunization is a routine method to discover and optimize antibodies, while this is time-consuming, expensive, and may not produce desirable antibodies. Undoubtedly, antibodies which are present in PDB do not attach to a desirable epitope with high efficiency, but they are practical for crystallography. Therefore, they are beneficial in antibody design to optimize sampling and scoring, raising the improvement of CDR lengths, clusters, and sequences [17].

Antibody design

The antitumor activity of engineering antibodies enhances; hence it improves therapeutic effects by little dosage [18]. To model molecular behaviors, computational antibody designs, algorithms, and molecular mechanics force fields are accessible. Rational design, structure-based design, protein design algorithms, and antibody-specific modeling techniques are some kinds of computational methods [19-21]. These methods have been utilized to improve stability [22,23], optimizing affinity [24,25], and humanizing antibodies [26,27]. Hence, *in silico* molecular modeling techniques emerged [28] with viscosity and phase separation properties [29].

Computer-based strategies

Energy functions which describe the biological system and conformational sampling in computational simulations, are two important factors for binding affinity prediction based on structure. Antibody design based on the structure has been rapidly noted due to development in algorithms and computational power [30]. Maturation of the antibody and design of the antibody with affinity enhancement would be effectively possible with the help of designing strategies. Mutants around CDR loops are the tool for the estimation of binding affinity without contact with an antigen. For estimating binding affinity, Lippow *et al*. in 2007 designed a two-stage procedure so that conformational search is performed by

sampling side-chain rotamers separately applying dead-end elimination with a physics-based energy performance.

The lowest-energy structures is another factor assessed by some more computational models involving poisson-Boltzmann continuum electrostatics, continuum solvent van der Waals, unbound state side-chain conformation search and minimization. Besides, by handling several experiments on free energy in the first design on anti-hen egg-white lysozyme antibody D1.3, Lippow *et al.* confirmed that the computed electrostatic for binding was better to predict affinity compared to total computed binding [24]. Furthermore, by helping computational modeling, Barderas *et al.* in 2008 increased the affinity of human anti-gastrin TA4 scFv from 6 μM to 13.2 nM [25].

For computational docking, understanding interacting partner structures is important; and as monomers structures are not present in all cases, it requires to utilize homology modeled structure for one or all partners [31,32]. The exact prediction of protein-protein complex structures is a major challenge; thus, new docking algorithms obtain the necessary degrees of freedom for fault compensation. It is notable that by homology modeling technology, several successful therapeutic antibodies involving Zenapax (humanized anti-Tac or daclizumab), Herceptin (humanized anti-HER2 or Trastuzumab), and Avastin (humanized anti-VEGF or bevacizumab) arrived in market [33]. High-resolution computational docking is applicable in antibody designing due to the knowledge of complex interactions between antibody and antigen [34].

Although the flexible docking algorithms are not present for antibodies, several relevant modes of internal flexibility through docking approaches were combined [35,36]. Multi-body docking was developed for optimizing assembly of VL, VH and antigen, which targets blind prediction challenge known as Critical Assessment of Prediction of Interactions (CAPRI) [37-39].

It was considered that homology modeling combined with knowledge-based and energy-based methods can create more reliable H3 loops [40]. Rosetta antibody combined with homology and *ab initio* modeling can make a primitive homology model by choosing various templates for frameworks and non-H3 CDRs, modeling H3 loop and optimizing the variable domain of heavy and light chains interface using *ab initio* approaches [41,42].

Strategies in the development of fully humanized therapeutic antibodies

Two common molecular engineering processes for therapeutic purposes are the

humanization of mouse antibody [16,43]. Antibody humanization is beneficial for producing a molecule with minimal immunogenicity, while the specificity and affinity of parental non-human antibodies have remained. In 1989 Zenapax® (generic name: Daclizumab), the first FDA approved humanized antibody for therapeutic utilization in the transplantation and treatment of asthma, autoimmunity, inflammation, and multiple sclerosis, was introduced by Queen *et al.* First of all, the human Framework Regions (FRs) were selected to maximize homology with the murine antibody sequence. Then, aiding the computer modeling, some murine amino acids outside the CDRs were identified to interact with the CDRs or antigen to improve the humanized antibody binding [44]. Murine antibodies were originally obtained by mice or rats immunization; then, hybridoma technology was used [45]. A chimeric antibody is achieved by the graft of variable mouse domains of antibody (mAb) to constant domains of human [46,47]. Next phase was emerged by grafting the antigen-binding loops or CDRs from a mouse to a human IgG [43,48]. If some residues from the FRs of mouse parents are conveyed, humanized antibodies would be improved [49]. Human antibodies that are acquired from single-chain variable fragments or Fab phage display libraries have a high affinity [50-52]. Due to the improved affinity of human antibodies, they contain most of human immunoglobulin genes [53,54].

Docking methods

Combining the gold standard of docking with standard Rosetta Dock results in the lowest interface-energy of targets by adding the degrees of freedom to Snug Dock protocol, the chance of successful prediction is dramatically increased. Paratope structural optimization in docking to compensate for the antibody homology model errors is a function of SnugDock. In this algorithm, the position of antibody-antigen rigid-body and the light and heavy chain orientation of antibodies were optimized. The combination of Ensemble Dock and Snug Dock [55] protocol for homology modeling has the same numbers of targets with acceptable predictions as the standard Rosetta Dock using for crystal docking. In general, the results show that the target's flexibility can conquer inaccuracies in homology modeling, leading to higher accuracy in docking [55].

Grafting antibody components together and modeling H3 loop could be done by the Web Antibody Modeling server (WAM) as a source of homology modeling. Snug Dock is responsible for compensating model errors during docking; the results of using Snug

Dock accompanied by WAM models are compared to those of Rosetta antibody models. So, higher accuracy will be obtained by applying Ensemble Dock-plus-SnugDock with Rosetta antibody [55]. By increasing the degree of freedom in local docking, computational algorithms prediction quality will be improved. As a result, by utilizing Ensemble Dock-plus-Snug Dock with homology models, high accuracy can be achieved compared to docking crystal structures with standard Rosetta Dock.

To dock the antibody on the antigen epitope is one issue in antibody modeling. The complementary between antibody and antigen is not determined well due to epitopes and paratopes being typically flat. In comparison to RosettaDock algorithm [56], SnugDock [55] applied alternating rounds of the low-resolution rigid body, high-resolution side-chain, and backbone minimization to make an antibody-antigen model complex. The protocol depends on the random deviation to get minimum energy, with a strong energy funnel and a low RMSD compared to the native one [56].

Affinity and specificity enhancement

Antibody-antigen interactions are significant to design antibodies with high specificity and affinity. Various studies showed the three largest amino acids' distribution in epitope and paratope consists of tyrosine, glycine, and serine [57,58]. These amino acids improve low affinity binding of naive germ line antibodies, enabling them to be affinity maturated [59].

Antibody-Dependent Cell Cytotoxicity (ADCC) and CDC were proposed as the most significant mechanisms of therapeutic antibodies. These functions operate during the interaction of Fc domain with FcγRIIIa for ADCC, C1q for CDC, and neonatal Fc receptor for prolongation of clearance rate [18].

Three methods for *in vitro* affinity maturation and producing antibody variants are accessible that involve: random mutagenesis, targeted mutagenesis, and shuffling approach. *E. coli* mutator bacterial strains, error prone PCR, or saturation mutagenesis are applied in random mutagenesis method. Two instances of this method are Ranibizumab on VEGF by humanized Fab format and L19 on EDB fibronectin domain by humanized diabody format [60-62]. Alanine-scanning or site-directed mutagenesis, like look-through mutagenesis, is utilized in targeted mutagenesis. Synagis on RSV F protein by humanized IgG format, DX-88 on Plasma kallikren and DX-890 on Neutrophil elastase by small recombinant protein format are the instances of site-

directed mutagenesis [63]. The shuffling method involves light chain or CDR shuffling [64-66]. IMC-A12 on IGF-1R by fully human IgG format is an instance of Chain shuffling [67]. With the use of display technologies including phage display, a high affinity antibody is obtained. Furthermore, various sorts of display methods were applied like ribosome display, yeast surface display, *E. coli* surface display and mRNA display [68,69].

Enhancing antibody-antigen binding affinities is feasible by *in silico* mutations on the residues of antibody by the use of the three-dimensional structures of antibody-antigen complexes [70]. Several various methods are used for antibody's affinity enhancement derived from phage antibody libraries, involving error-prone PCR [71], CDR walking [72], hot-spot mutagenesis [73], and parsimonious mutagenesis [74]. To speed up and direct the maturation process, these methods are nowhere near as efficient as rational approaches.

Glycosylation as a strategy to improve therapeutics antibody

The majority of marketed therapeutic antibodies are glycoproteins. Glycosylation is considered as a process in which the oligosaccharide is covalently graft to either side chain of asparagine or serine/threonine. For recognition, signaling, and interaction events, the oligosaccharide part of antibody is required, which is beneficial in folding and defining protein conformation [75]. Producing human therapeutic glycoproteins has some benefits involving faster development and low-cost biopharmaceuticals. For instance, human IgG and rat Erythropoietin (EPO) were completely functional by expression in a glycoengineered *Pichia pastoris* yeast system [76,77]. These improvements arise from N-linked and/or O-linked glycosylation influence activity, pharmacokinetics, clearance, and immunogenicity of drugs [78].

Most current therapeutic antibodies are fucosylated, although de-fucosylated antibodies have some advantages [95].

Variable domain orientations in antigen receptors

The affinity and specificity of an antibody are two significant antigen-binding properties, and by engineering these properties, an improvement in the quality of therapeutic antibodies is achievable. Variable regions of antibody and T-Cell Receptors (TCRs) identify their desired antigens in dissimilar methods. Antibodies bind to a various collection of antigenic shapes, while TCRs binds only to peptide antigens presented by a Major Histocompatibility Complex (MHC). However, they have some similarities in the

structure. Like antibodies, TCR binds utilizing its variable region involving two domains, Vα and Vβ, analogous to antibody VL and VH domains [79,80].

The orientation of variable domains, VH and VL in antibodies, can influence the binding site. James Dunbar et al, surveyed the corresponding property for TCRs and Vβ-Vα orientation compared to natural antibodies [19]. The orientation of variable domain affects the position of CDR loops, thereby affecting the geometry of antigen binding site [81,82]. It is shown conformations that are present in TCRs and antibodies are specific. An antibody binds to a pMHC in a similar way a TCR does, and similar conformation with certain amino-acids should be achieved in antibodies as in TCR. Packing long Vα CDR3 in the domain-domain interface is considered to be a factor resulting in the difference between the TCRs and antibodies' orientation. A similar packing effect can be obtained in the antibodies applying a bulky residue at IMGT position 50 on the VH domain. Other situations are recognized, which may aid in enhancing a TCR-like orientation in the antibodies. These situations must be profitable in engineering therapeutic TCR-like antibodies [83]. Considering the study of Narayanan *et al*., the orientation of VL-VH significantly affects antigen binding attributes of an antibody. They suggested that optimizing the orientation of VL-VH and antibody-antigen may result in several intramolecular alterations [84].

Antibody mimetics

As mentioned earlier, the restriction of native antibodies to penetrate solid tumor motivates scientists to make smaller alternatives. To identify antigens by the whole antibody, all six CDR loops in VH and VL are necessary [7,85-88]. Although the derivatives of CDR sequences are able for antigen-recognition [87,89,90], *in vivo* activity of modified CDR antibody mimetics is not reported to be desirable. This could be rooted in unsuitable CDR modification or lack of spacers among CDR derivatives [90,91].

Considering the study of Xiao-Qing Qiu *et al*., the fusion of two CDRs, VHCDR1 and VLCDR3, with their cognate FRs (VHFR2) not only make the mimetics maintain the antigen recognition of their parent antibody, but also increase its capacity for penetrating tumors. It may be suitable to make smaller, high-affinity binders of therapeutic value. The framework of either VH or VL domain orients two CDRs roughly the same as their native condition. The suitable orientation of VH and VL domains conferred by an FR spacer can ensure the retaining partial synergic interactions for VH and VL

domains. For detecting optimal pair of VH and VL domain CDRs with the best keeper synergic interactions, they made four mimetics based on HB-168, a monoclonal IgG against EBV envelope glycoprotein gp 350/220. *In vivo* results, including distribution and targeted tumor growth inhibition, reveal that synthetic VHCDR1-VHFR2-VLCDR3 mimetic and toxin-mimetic fusions penetrated and accumulated in solid tumors more than the parent antibody (this sort of antibody mimetics is displayed in Figure 1). Mimetics with no FRs linkage could also have some disadvantages like VHCDR1-VLCDR3 mimetic and a cyclic peptide containing key residues of all six CDRs, which lack a quasi-physiological linkage among CDR derivatives. This does not allow their interaction with antigenic epitopes to have a suitable interface.

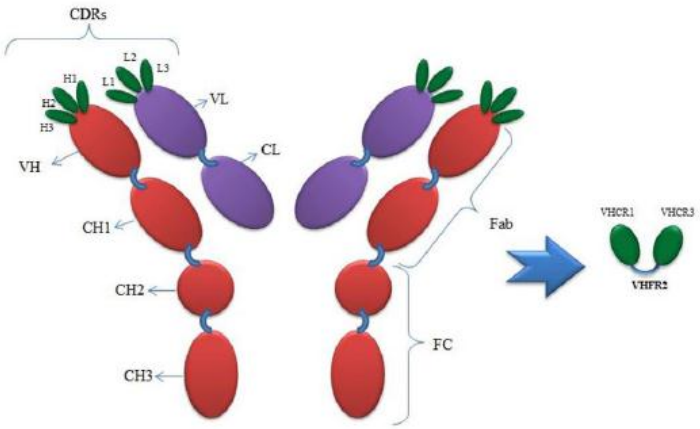

Figure 1. Fusion of two CDRs, VHCDR1 and VLCDR3.

Fusion proteins called pheromonicins, were created by linking the antibody mimetics to the bacterial toxin, enabling targeted tumor growth inhibition. Based on the results, pheromonicins directed against tumor-specific surface markers penetrate tumors more than their parent antibodies [92].

The antibody mimetic design is problematic when the active region of a protein consists of discontinuous segments of the polypeptide chain. Some methods have been suggested to mimic the discontinuous binding surface of a protein [110,111]. Rational strategies to design 'continuous' sequence mimetics of discontinuous regions of protein have not been fruitful so much [112,113]. The reason is that the recognition components of a mimetic structure are constricted compared to conformational and spatial orientations witnessed in the parent molecule [114]. In the study of Florence Casset *et al.*, after synthesizing and testing 446 combinations of cyclization, the ultimate mimic showed great activity. They utilized peptide mapping to define active antigen recognition residues, molecular modeling and a molecular elements trajectory analysis so that they can create a peptide mimic of an anti-CD4 antibody, containing antigen contact residues from multiple CDRs. The design was a 27-residue peptide created by residues from 5 CDR regions. Consequently, the affinity for the antigen (CD4) was 0.9 nM, compared to 2 nM for the parent antibody ST40. However, the mimetic indicates low biological activity in an anti-retroviral assay [107].

The Algorithms in antibody Design

Pantazes and Maranas modeled OptCDR [94] and OptMAVEn (Optimal Method for Antibody Variable region Engineering) [94] methods for computational antibody design, assemble their structure elements to interact with new epitopes. OptCDR samples are due to six CDRs groups in the existence of a fixed antigen situation. It is handled by putting side chains based on sequence priority in every cluster, a rotamer search from a backbone-dependent rotamer library [95], and a CHARMM-based energy function. The structures of an antibody are divided into OptMAVEn with V(D) J recombination: antibody heavy- and light-chain V regions, CDR3s, and post-CDR3 elements from MAPS database [96]. OptMAVEn is beneficial for de novo design of variable regions human antibody for interacting with any desired antigen by assembly of six best-scored Modular Antibody Parts (MAPs) [96]. Considering the template, MAPs modeling is done; the templates are considered initial structures of random Variable (V), Diversity (D), and Joining (J) domains in the database, leading to gene combinations with the fewest amino acids alters from the target [96].

After predicting the antibody structure successfully, its affinity can be matured

using Iterative Protein Redesign and Optimization (IPRO) framework. It can redesign an entire combinatorial protein library in one step utilizing energy-based scoring functions. Framework is optimized by side-chain substitutions in design situations with the use of a mixed-integer optimization model. Backbone of protein is also adjusted by local minimizations to novel side-chains [97].

To design antibodies against a hydrophobic heptamer peptide antigen with a repetitive sequence (FYPYPYA) OptMAVEn can be utilized [99]. Lapidoth *et al*. presented Ab Design [99], which is similar to Opt-MAVEn, separating antibodies into V regions and CDR3, and then categorizing V region by the length of CDR1 and CDR2. Ab Design combines and designs the sequence from position-specific scoring matrices of aligned antibody sequences of their length-based categories of V regions and CDR3 regions. Ab Design is recently applied to make antibodies against insulin and mycobacterial acyl-carrier protein [100].

Rosetta Software Suite [30] is one of the reliable antibody designs which applied for a diversity of modeling like loop modeling [101], protein-protein docking [98,102], structure refinement [103-105], de novo protein design [106], enzyme design [107,108], and interface design [109,110]. Rosetta Software could supply the frameworks for sampling and also optimizing protein-protein interaction conformations.

RAbD and Ab Design employ clustering structural antibody fragments and their related sequence to make novel antibodies. Moreover, during the design of antigen-antibody complex, they apply Rosetta docking and side-chain repacking. Furthermore, they have several significant differences; for instance, Ab Design isolates every antibody domain into two fragments, V region up to CDR3 and the leftovers of variable domain up to its C-terminus. In the present design method, V regions merge sequence lengths of CDR1 and CDR2, which cause issues due to several CDR clusters having some residue such as glycine in special sites for organizing correct loop conformation. Thus, Ab Design is not appropriate to optimize present antibodies. In contrast, RAbD handles every CDR separately and samples structures in PyIg classify database. CDRs are mixed and matched together, and they are grafted onto the frameworks of antibody obtained by a user. RAbD and Ab Design are performed in

distinct ways; Ab Design is not appropriate for special issues in antibody design like sampling defined lengths of a CDR or particular germline or CDR cluster while RAbD is a perfect sort of Rosetta application [17].

The loop length and recognition of key residues within or outside CDR regions can be predicted for the humanization antibody [48]. When non-human CDRs are grafted onto human frameworks, antigen-binding site conformation could alter; thus, affinity antibody–antigen-binding decreased [8,111]. When similar sequence motifs are found from Protein Data Bank (PDB), the structures can be defined [112]. Modeling L1, L2, L3, H1 and H2 loops with high accuracy can be achieved by identifying sequence-structure relationships [113]. Having the main role in antigen recognition and affinity maturation, they are put in the antigen's binding site. By decreasing the computational search space for loop modeling, sequence-structure rules for CDR-H3 loops may aid solving the problem [114]. Gray *et al.* suggested Rosetta antibody for modeling antibodies; at first, they modeled CDR-H3 loops, assembled fragments and minimizing them utilizing Rosetta protocol [115]. Their antibody models could apply in computational docking methods utilizing Rosetta dock [116]. Ten antibody homology models for every input sequence are produced in Rosetta antibody, and it is possible to combine simultaneously by Ensemble Dock. Nevertheless, the CDRs (H2 and H3) errors of Rosetta antibody homology models can still invalidate docking [115].

Ab initio approach is a method to enumerate feasible loop conformations that apply generic Ramachandran to make probable backbone conformations. Applying CONGEN program in the earliest antibody modeling approaches, Martin et al. combined database searching and *ab initio* loop prediction [40]. An *ab initio* loop modeling protocol was designed by Jacobson *et al.* They investigate conformational space by backbone torsion-angle sampling by refining energy-based, scoring all-atom optimized potentials for liquid simulations and solvent model [117]. This method can be used for loop modeling in the generic proteins [118] and CDR-H3 modeling [119].

Despite the difficulty of predicting the loop longer than 12 amino acids [120], as the conformation can be different according to its interactions with antigen or artifacts associated with crystal packing, long

surface loops in proteins are often flexible [121]. Tramontano *et al.* designed PIGS webserver to provide several alternatives for light and heavy chains [122]. CDRs were created by grafting structures and CDR-H3 loops of other antibodies onto the modeled framework on this web. Rosetta antibody applies a VL/VH docking due to the Rosetta energy function and rigid-body minimization to refine the orientations following CDR-H3 modeling [41,115].

Utilizing code is hard, since large computational resources are needed and servers for most of Rosetta applications are inaccessible; applying Rosetta molecular modeling software is limited [123]. A Rosetta application called ROSIE is an improved version of Rosetta platform. The main progress in this software is the loop modeling utilizing Kinematic Loop Closure (KLC) algorithm [101]. CDR-H3 is restricted with knowledge-based rules, along with an updated version of the structural database, prepares better templates for VL, VH, and the CDR loops. For light and heavy chain, the inputs are sequences [124]. The output coordinates of FV model antibody, can be applied for modeling antibody-antigen complexes by EnsembleDock [56] or SnugDock [55] (Supplementary data S2).

CONCLUSION

Dramatic advancements in high-throughput technology combined with computational technologies lead to developing therapeutics antibody [125-127]. Correct prediction of antibody structures from their sequences is the main issue; fortunately, recent advancement was made through this purpose. Predicting antibody–antigen-binding modes has also produced high advancement by computational protein-protein docking, especially by using knowledge of antigen-binding sites and experimental data. The exact prediction of epitopes and paratopes requires more advancement accuracy [24,114]. PDB, DIGIT, IEDB, and IMGT are several antibody data resources in terms of their contents and properties. To know the antibody-antigen recognition mechanism, to assess the stability and immunogenicity of antibody, and to predict function/efficacy alter upon modification 3D structures are required. A small section of protein structure space can only solve by experimental techniques like crystallography and NMR. Moreover, developing this space is feasible by applying computational approaches choosing suitable templates, predicting epitope and modeling CDR region by

acceptable deviation. Nonetheless, combining these computational approaches may lead to decrease errors, especially for predicting antibody-antigen complex structure [128].

The prediction of protein structures from a sequence can be successful if the templates can be appropriately determined and aligned. Since conformational sampling and force fields guiding sampling are not perfect, no method is present to predict structures template free [129,130]. No method is present to score those models accurately; it was proposed that all puzzle pieces are required to construct structure. Despite this, no method can assemble them properly in a blind predictive capacity [131, 132]. Thus, the advancement in force fields, the capability for predicting correct residue ability, residue contacts, β-sheet topologies, alignments to non-homologous templates, and efficient conformational sampling methods are the main elements to solve the problem of protein folding [133].

If *de novo* design were completely handled by applying computers, it would be basically impractical. Therapeutics proteins are hindered by proteolytic cleavage, poor solubility, and poor permeability. Applying Post-Translational Modifications (PTMs) and NCAAs can address these issues because altered peptides are less likely to be identified by proteases, and modifications of peptide can be chosen to fine-tune bioavailability. The design of modified peptide sequences adds more complexity because of existing over 400 known PTMs for design [134]. PTMs and NCAAs methods have been at an initial step of advancement, which raises the main issue in protein structure prediction and *de novo* protein design.

REFERENCES

[1]. Siegel R, Naishadham D, Jemal A. Cancer statistics. *CA Cancer J Clin*, 2013; 63(1): 11-30.

[2]. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Adv Cancer Res*, 2014.

[3]. Richman SA, Kranz DM. Display, engineering, and applications of antigen-specific T cell receptors. *Biomol. Eng*, 2007; 24(4): 361-73.

[4]. Presta LG. Molecular engineering and design of therapeutic antibodies. *Curr. Opin. Immunol*, 2008; 20(4): 460-70.

[5]. Heyman B. Complement and Fc-receptors in regulation of the antibody

response. *Immunol. Lett*, 1996; 54(2-3): 195-99.

[6]. Diamandis EP, Christopoulos TK. The biotin-(strept) avidin system: principles and applications in biotechnology. *Clin. Chem*, 1991; 37(5): 625-36.

[7]. Holliger P, Hudson PJ. Engineered antibody fragments and the rise of single domains. *Nat. Biotechnol*, 2005; 23(9): 1126- 36.

[8]. Almagro JC, Fransson J. Humanization of antibodies. *Front Biosci*, 2008; 13(1): 1619-33.

[9]. Hutas G. Ocrelizumab, a humanized monoclonal antibody against CD20 for inflammatory disorders and B-cell malignancies. *Curr Opin Investig Drugs*, 2008; 9(11): 1206-15.

[10]. Baselga J, Norton L, Albanell J, Kim Y-M, Mendelsohn J. Recombinant humanized anti-HER2 antibody (Herceptin™) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. *Cancer Res*, 1998; 58(13): 2825-31.

[11]. Kawaguchi Y, Kono K, Mimura K, Sugai H, Akaike H, Fujii H. Cetuximab induce antibody-dependent cellular cytotoxicity against EGFR-expressing esophageal squamous cell carcinoma. *IJC*, 2007; 120(4): 781-87.

[12]. Yang JC, Haworth L, Sherry RM, Hwu P, Schwartzentruber DJ, Topalian SL, et al. A randomized trial of bevacizumab, an anti–vascular endothelial growth factor antibody, for metastatic renal cancer. *NEJM*, 2003; 349(5): 427-34.

[13]. Hudis CA. Trastuzumab—mechanism of action and use in clinical practice. *NEJM*, 2007; 357(1): 39-51.

[14]. Torchilin V. Antibody-modified liposomes for cancer chemotherapy. *Expert Opin Drug Deliv*, 2008; 5(9): 1003-25.

[15]. Igawa T, Tsunoda H, Kuramochi T, Sampei Z, Ishii S, Hattori K, editors. Engineering the variable region of therapeutic IgG antibodies. *MAbs*, 2011.

[16]. Carter P. Improving the efficacy of antibody-based cancer therapies. *Nat Rev Cancer*, 2001; 1(2): 118- 29.

[17]. Adolf-Bryfogle J, Kalyuzhniy O, Kubitz M, Weitzner BD, Hu X, Adachi Y, *et al*. Rosetta antibody design (RAbD): A general framework for computational antibody design. *PLOS Comput. Biol*, 2018; 14(4): 1006112.

[18]. Kubota T, Niwa R, Satoh M, Akinaga S, Shitara K, Hanai N. Engineered therapeutic antibodies with improved effector functions. *Cancer Sci,* 2009; 100(9): 1566-72.

[19]. Kuroda D, Shirai H, Jacobson MP, Nakamura H. Computer-aided antibody design. *Protein Eng. Des Sel*, 2012; 25(10): 507-22.

[20]. Marshall SA, Lazar GA, Chirino AJ, Desjarlais JR. Rational design and engineering of therapeutic proteins. *Drug Discov Today Drug*, 2003; 8(5): 212-21.

[21]. A Caravella J, Wang D, M Glaser S, Lugovskoy A. Structure-guided design of antibodies. *Curr Comput Aided Drug Des*, 2010; 6(2): 128-38.

[22]. Chennamsetty N, Voynov V, Kayser V, Helk B, Trout BL. Design of therapeutic proteins with enhanced stability. *Proc Natl Acad Sci*, 2009; 106(29): 11937-42.

[23]. Der BS, Kluwe C, Miklos AE, Jacak R, Lyskov S, Gray JJ, et al. Alternative computational protocols for supercharging protein surfaces for reversible unfolding and retention of stability. *PLoS One*. 2013; 8(5): e64363.

[24]. Lippow SM, Wittrup KD, Tidor B. Computational design of antibody-affinity improvement beyond in vivo maturation. *Nat Biotechnol*, 2007; 25(10): 1171.

[25]. Barderas R, Desmet J, Timmerman P, Meloen R, Casal JI. Affinity maturation of antibodies assisted by *in silico* modeling. PNAS, 2008.

[26]. Pulito VL, Roberts VA, Adair JR, Rothermel AL, Collins AM, Varga SS, et al. Humanization and molecular modeling of the anti-CD4 monoclonal antibody, OKT4A. *J Immunol,* 1996; 156(8): 2840-50.

[27]. Lazar GA, Desjarlais JR, Jacinto J, Karki S, Hammond PW. A molecular immunology approach to antibody humanization and functional optimization. *Mol Immunol*, 2007; 44(8): 1986-98.

[28]. Gilliland GL, Luo J, Vafa O, Almagro JC. Leveraging SBDD in protein therapeutic development: antibody engineering. *Structure-Based Drug Discovery*; 2012. 321-49.

[29]. Chow C-K, Allan BW, Chai Q, Atwell S, Lu J. Therapeutic antibody engineering to improve viscosity and phase separation guided by crystal structure. *Mol Pharm*, 2016; 13(3): 915-23.

[30]. Das R, Baker D. Macromolecular modeling with rosetta. *Annu Rev Biochem,* 2008; 77: 363-82.

[31]. Tovchigrechko A, Wells CA, Vakser IA. Docking of protein models. *Protein Sci,* 2002; 11(8): 1888-96.

[32]. Mosca R, Pons C, Fernández-Recio J, Aloy P. Pushing structural information into the yeast interactome by high-throughput protein docking experiments. *PLoS Comput. Biol,* 2009; 5(8): 1000490.

[33]. Schwede T, Sali A, Honig B, Levitt M, Berman HM, Jones D, et al. Outcome of a workshop on applications of protein models in biomedical research. *Structure,* 2009; 17(2): 151-59.

[34]. Dübel S, Reichert JM. Handbook of therapeutic antibodies: John Wiley & Sons; 2014.

[35]. Sandak B, Wolfson HJ, Nussinov R. Flexible docking allowing induced fit in proteins: insights from an open to closed conformational isomers. *Proteins,* 1998; 32(2): 159-74.

[36]. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ. Geometry-based flexible and symmetric protein docking. *Proteins,* 2005; 60(2): 224-31.

[37]. Hendrix DK, Klein TE, Kuntz ID. Macromolecular docking of a three-body system: The recognition of human growth hormone by its receptor. *Protein Sci*, 1999; 8(5):1010-22.

[38]. Ben-Zeev E, Kowalsman N, Ben-Shimon A, Segal D, Atarot T, Noivirt O, et al. Docking to single-domain and multiple-domain proteins: old and new challenges. *Proteins,* 2005; 60(2): 195-201.

[39]. Vajda S, Vakser IA, Sternberg MJ, Janin J. Modeling of protein interactions in genomes. *Proteins,* 2002; 47(4): 444-46.

[40]. Martin A, Cheetham JC, Rees AR. Modeling antibody hypervariable loops: a combined algorithm. *PNAS,* 1989; 86(23): 9268-72.

[41]. Sircar A, Kim ET, Gray JJ. RosettaAntibody: antibody variable region homology modeling server. *Nucleic Acids Res,* 2009; 37; 474-79.

[42]. Weitzner BD, Kuroda D, Marze N, Xu J, Gray JJ. Blind prediction performance of Rosetta antibody 3.0: grafting, relaxation, kinematic loop modeling, and full CDR optimization. *Proteins,* 2014; 82(8): 1611-23.

[43]. Jones PT, Dear PH, Foote J, Neuberger MS, Winter G. Replacing the

complementarity-determining regions in a human antibody with those from a mouse. *Nature*, 1986; 321(6069): 522.

[44]. Queen C, Schneider WP, Selick HE, Payne PW, Landolfi NF, Duncan JF, et al. A humanized antibody that binds to the interleukin 2 receptor. *PNAS*, 1989; 86(24): 10029-33.

[45]. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*, 1975; 256(5517): 495.

[46]. Boulianne GL, Hozumi N, Shulman MJ. Production of functional chimaeric mouse/human antibody. *Nature*, 1984; 312(5995): 643.

[47]. Morrison SL, Johnson MJ, Herzenberg LA, Oi VT. Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *PNAS*, 1984; 81(21): 6851-55.

[48]. Riechmann L, Clark M, Waldmann H, Winter G. Reshaping human antibodies for therapy. *Nature*, 1988; 332(6162): 323-27.

[49]. Vaughan TJ, Osbourn JK, Tempest PR. Human antibodies by design. *Nat. Biotechnol*, 1998; 16(6): 535-39.

[50]. de Haard HJ, van Neer N, Reurs A, Hufton SE, Roovers RC, Henderikx P, et al. A large non-immunized human Fab fragment phage library that permits rapid isolation and kinetic analysis of high affinity antibodies. *J Biol Chem*, 1999; 274(26): 18218-30.

[51]. Knappik A, Ge L, Honegger A, Pack P, Fischer M, Wellnhofer G, et al. Fully synthetic human combinatorial antibody libraries (HuCAL) based on modular consensus frameworks and CDRs randomized with trinucleotides1. *J. Mol. Biol*, 2000; 296(1): 57-86.

[52]. Vaughan TJ, Williams AJ, Pritchard K, Osbourn JK, Pope AR, Earnshaw JC, *et al*. Human antibodies with sub-nanomolar affinities isolated from a large non-immunized phage display library. *Nat. Biotechnol*, 1996; 14(3): 309-14.

[53]. Tsutsumi Y, Onda M, Nagata S, Lee B, Kreitman RJ, Pastan I. Site-specific chemical modification with polyethylene glycol of recombinant immunotoxin anti-Tac (Fv)-PE38 (LMB-2) improves antitumor activity and reduces animal toxicity and immunogenicity. *PNAS*, 2000; 97(15): 8548-53.

[54]. Fishwild DM, O'Donnell SL, Bengoechea T, Hudson DV, Harding F, Bernhard SL, et al. High-avidity human IgGκ monoclonal antibodies from a novel strain of minilocus transgenic mice. *Nat. Biotechnol*, 1996; 14(7): 845-51.

[55]. Sircar A, Gray JJ. SnugDock: paratope structural optimization during antibody-antigen docking compensates for errors in antibody homology models. *PLoS Comput. Biol*, 2010; 6(1): 1000644.

[56]. Chaudhury S, Gray JJ. Conformer selection and induced fit in flexible backbone protein–protein docking using computational and NMR ensembles. *J. Mol. Biol*, 2008; 381(4): 1068-87.

[57]. Conte LL, Chothia C, Janin J. The atomic structure of protein-protein recognition sites1. *J. Mol. Biol*, 1999; 285(5): 2177-98.

[58]. Clark LA, Ganesan S, Papp S, van Vlijmen HW. Trends in antibody sequence changes during the somatic hypermutation process. *J. Immunol*, 2006; 177(1): 333-40.

[59]. Rubinstein ND, Mayrose I, Halperin D, Yekutieli D, Gershoni JM, Pupko T. Computational characterization of B-cell epitopes. *Mol. Immunol*, 2008; 45(12): 3477-89.

[60]. Chowdhury PS, Pastan I. Improving antibody affinity by mimicking somatic hypermutation *in vitro*. *Nat. Biotechnol*, 1999; 17(6): 568.

[61]. Martineau P. Error-prone polymerase chain reaction for modification of scFvs. *Antibody Phage Display*, 2002; 287-94.

[62]. Yang W-P, Green K, Pinz-Sweeney S, Briones AT, Burton DR, Barbas III CF. CDR walking mutagenesis for the affinity maturation of a potent human anti-HIV-1 antibody into the picomolar range. *J. Mol. Bio*l, 1995; 254(3): 392-403.

[63]. Rajpal A, Beyaz N, Haber L, Cappuccilli G, Yee H, Bhatt RR, et al. A general method for greatly improving the affinity of antibodies by using combinatorial libraries. *PNAS*, 2005; 102(24): 8466-71.

[64]. Chiang C-J, Lo W-C, Yang Y-W, You S-L, Chen C-J, Lai M-S. Incidence and survival of adult cancer patients in Taiwan, 2002–2012. *JFMA*, 2016; 115(12): 1076-88.

[65]. Jermutus L, Honegger A, Schwesinger F, Hanes J, Plückthun A. Tailoring *in vitro* evolution for protein affinity or stability. *PNAS*, 2001; 98(1): 75-80.

[66]. Bendtsen JD, Nielsen H, von Heijne G, Brunak S. Improved prediction of signal peptides: SignalP 3.0. *Jmb*, 2004; 340(4): 783-95.

[67]. Shi L, Wheeler JC, Sweet RW, Lu J, Luo J, Tornetta M, *et al.* De novo selection of high-affinity antibodies from synthetic fab libraries displayed on phage as pIX fusion proteins. *J. Mol. Biol*, 2010; 397(2): 385-96.

[68]. Daugherty PS, Chen G, Iverson BL, Georgiou G. Quantitative analysis of the effect of the mutation frequency on the affinity maturation of single chain Fv antibodies. *PNAS*, 2000; 97(5): 2029-34.

[69]. Siegel RW. Antibody affinity optimization using yeast cell surface display. *Biosensors Biodetection*, 2009. 351-83.

[70]. Zhao J, Nussinov R, Wu W-J, Ma B. In silico methods in antibody design. *Antibodies*, 2018; 7(3): 22.

[71]. Luginbühl B, Kanyo Z, Jones RM, Fletterick RJ, Prusiner SB, Cohen FE, et al. Directed evolution of an anti-prion protein scFv fragment to an affinity of 1 pM and its structural interpretation. *J. Mol. Biol*, 2006; 363(1): 75-97.

[72]. Barbas CF, Hu D, Dunlop N, Sawyer L, Cababa D, Hendry RM, *et al. In vitro* evolution of a neutralizing human antibody to human immunodeficiency virus type 1 to enhance affinity and broaden strain cross-reactivity. *PNAS*, 1994; 91(9): 3809-13.

[73]. Ho M, Kreitman RJ, Onda M, Pastan I. *In vitro* antibody evolution targeting germline hot spots to increase activity of an anti-CD22 immunotoxin. *J. Biol. Chem*, 2005; 280(1): 607-17.

[74]. Schier R, Balint RF, McCall A, Apell G, Larrick JW, Marks JD. Identification of functional and structural amino-acid residues by parsimonious mutagenesis. *Gene*, 1996; 169(2): 147-55.

[75]. Helenius A, Aebi M. Intracellular functions of N-linked glycans. *Science,* 2001; 291(5512): 2364-69.

[76]. Li H, Sethuraman N, Stadheim TA, Zha D, Prinz B, Ballew N, et al. Optimization of humanized IgGs in glycoengineered Pichia pastoris. *Nat. Biotechnol,* 2006; 24(2): 210.

[77]. Hamilton SR, Davidson RC, Sethuraman N, Nett JH, Jiang Y, Rios S, et al. Humanization of yeast to produce complex terminally sialylated

glycoproteins. *Science*, 2006; 313(5792): 1441-43.

[78]. Li H, d'Anjou M. Pharmacological significance of glycosylation in therapeutic proteins. *Curr. Opin. Biotechnol*, 2009; 20(6): 678-84.

[79]. Chothia C, Lesk AM. Canonical structures for the hypervariable regions of immunoglobulins. *J. Mol. Biol*, 1987; 196(4): 901-17.

[80]. Abhinandan K, Martin AC. Analysis and improvements to Kabat and structurally correct numbering of antibody variable domains. *Mol. Immuno*l, 2008; 45(14): 3832-39.

[81]. Chothia C, Novotný J, Bruccoleri R, Karplus M. Domain association in immunoglobulin molecules: the packing of variable domains. *J. Mol. Biol*, 1985; 186(3): 651-63.

[82]. Vargas-Madrazo E, Paz-García E. An improved model of association for VH–VL immunoglobulin domains: asymmetries between VH and VL in the packing of some interface residues. *J. Mol. Recognit*, 2003; 16(3): 113-20.

[83]. Dunbar J, Knapp B, Fuchs A, Shi J, Deane CM. Examining variable domain orientations in antigen receptors gives insight into TCR-like antibody design. *PLoS Comput. Biol*, 2014; 10(9); 1003852.

[84]. Narayanan A, Sellers BD, Jacobson MP. Energy-based analysis and prediction of the orientation between light-and heavy-chain antibody variable domains. *J. Mol. Biol*, 2009; 388(5): 941-53.

[85]. Hoogenboom HR. Selecting and screening recombinant antibody libraries. *Nat. Biotechnol*, 2005; 23(9): 1105-16.

[86]. Borg NA, Ely LK, Beddoe T, Macdonald WA, Reid HH, Clements CS, et al. The CDR3 regions of an immunodominant T cell receptor dictate the'energetic landscape'of peptide-MHC recognition. *Nat. Immunol*, 2005; 6(2): 171-80.

[87]. Laune D, Molina F, Ferrieres G, Mani J-C, Cohen P, Simon D, et al. Systematic exploration of the antigen binding activity of synthetic peptides isolated from the variable regions of immunoglobulins. *J. Biol. Chem*, 1997; 272(49): 30937-44.

[88]. Ewert S, Huber T, Honegger A, Plückthun A. Biophysical properties of human antibody variable domains. *J. Mol. Biol*, 2003; 325(3): 531-53.

[89]. Heap CJ, Wang Y, Pinheiro TJ, Reading SA, Jennings KR, Dimmock NJ. Analysis of a 17-amino acid residue, virus-neutralizing microantibody. *J. Gen. Virol*, 2005; 86(6): 1791-1800.

[90]. Casset F, Roux F, Mouchet P, Bes C, Chardes T, Granier C, *et al.* A peptide mimetic of an anti-CD4 monoclonal antibody by rational design. *Biochem. Biophys. Res. Commun*, 2003; 307(1): 198-205.

[91]. Qin W, Feng J, Li Y, Lin Z, Shen B. Fusion protein of CDR mimetic peptide with Fc inhibit TNF-α induced cytotoxicity. *Mol. Immunol*, 2006; 43(6): 660-66.

[92]. Qiu X-Q, Wang H, Cai B, Wang L-L, Yue S-T. Small antibody mimetics comprising two complementarity-determining regions and a framework region for tumor targeting. *Nat. Biotechnol*, 2007; 25(8): 921.

[93]. Pantazes R, Maranas C. OptCDR: a general computational method for the design of antibody complementarity determining regions for targeted epitope binding. *Protein Eng. Des. Sel*, 2010; 23(11): 849-58.

[94]. Li T, Pantazes RJ, Maranas CD. OptMAVEn–a new framework for the de novo design of antibody variable region models targeting specific antigen epitopes. *PLoS One*, 2014; 9(8): 105954.

[95]. Dunbrack Jr RL, Cohen FE. Bayesian statistical analysis of protein side-chain rotamer preferences. *Protein Sci*, 1997; 6(8): 1661-81.

[96]. Pantazes RJ, Maranas CD. MAPs: a database of modular antibody parts for predicting tertiary structures and designing affinity matured antibodies. *BMC Bioinformatics*, 2013; 14(1): 168.

[97]. Saraf MC, Moore GL, Goodey NM, Cao VY, Benkovic SJ, Maranas CD. IPRO: an iterative computational protein library redesign and optimization procedure. *Biophys J*, 2006; 90(11): 4167-80.

[98]. Gray JJ, Moughon S, Wang C, Schueler-Furman O, Kuhlman B, Rohl CA, et al. Protein–protein docking with simultaneous optimization of rigid-body displacement and side-chain conformations. *J. Mol. Biol*, 2003; 331(1): 281-99.

[99]. Lapidoth GD, Baran D, Pszolla GM, Norn C, Alon A, Tyka MD, et al. AbDesign: A n algorithm for combinatorial backbone design guided by natural conformations and sequences. *Proteins*, 2015; 83(8):1385-406.

[100]. Le Grand SM, Merz Jr KM. Rapid approximation to molecular surface area via the use of Boolean logic and look-up tables. *J. Comput. Chem*, 1993; 14(3): 349-52.

[101]. Mandell DJ, Coutsias EA, Kortemme T. Sub-angstrom accuracy in protein loop reconstruction by robotics-inspired conformational sampling. *Nat. Methods,* 2009; 6(8): 551.

[102]. Wang C, Bradley P, Baker D. Protein–protein docking with backbone flexibility. *J. Mol. Biol*, 2007; 373(2): 503-19.

[103]. Tyka MD, Keedy DA, André I, DiMaio F, Song Y, Richardson DC, et al. Alternate states of proteins revealed by detailed energy landscape mapping. *J. Mol. Biol*, 2011; 405(2): 607-18.

[104]. Conway P, Tyka MD, DiMaio F, Konerding DE, Baker D. Relaxation of backbone bond geometry improves protein energy landscape modeling. *Protein Sci*, 2014; 23(1): 47-55.

[105]. Nivón LG, Moretti R, Baker D. A Pareto-optimal refinement method for protein design scaffolds. *PLoS One*, 2013; 8(4) :59004.

[106]. Kuhlman B, Dantas G, Ireton GC, Varani G, Stoddard BL, Baker D. Design of a novel globular protein fold with atomic-level accuracy. *Science.* 2003; 302(5649): 1364-68.

[107]. Röthlisberger D, Khersonsky O, Wollacott AM, Jiang L, DeChancie J, Betker J, *et al.* Kemp elimination catalysts by computational enzyme design. *Nature*, 2008; 453(7192): 190.

[108]. Jiang L, Althoff EA, Clemente FR, Doyle L, Röthlisberger D, Zanghellini A, et al. De novo computational design of retro-aldol enzymes. *Science*, 2008; 319(5868): 1387-91.

[109]. Guntas G, Purbeck C, Kuhlman B. Engineering a protein–protein interface using a computationally designed library. *PNAS*, 2010.

[110]. Fleishman SJ, Corn JE, Strauch E-M, Whitehead TA, Karanicolas J, Baker D. Hotspot-centric de novo design of protein binders. *J. Mol. Biol*, 2011; 413(5): 1047-62.

[111]. Morea V, Lesk AM, Tramontano A. Antibody modeling: implications for engineering and design. *Methods*, 2000; 20(3): 267-79.

[112]. Kuroda D, Shirai H, Kobori M, Nakamura H. Systematic classification of CDR-L3 in antibodies: Implications of the light chain subtypes and the VL–VH interface. *Proteins,* 2009; 75(1): 139-46.

[113]. Morea V, Tramontano A, Rustici M, Chothia C, Lesk A. Antibody structure, prediction and redesign. *Biophys. Chem*, 1997; 68(1-3): 9-16.

[114]. Clark LA, Boriack-Sjodin PA, Eldredge J, Fitch C, Friedman B, Hanf KJ, et al. Affinity enhancement of an in vivo matured therapeutic antibody using structure-based computational design. *Protein Sci,* 2006; 15(5): 949-60.

[115]. Sivasubramanian A, Sircar A, Chaudhury S, Gray JJ. Toward high-resolution homology modeling of antibody Fv regions and application to antibody–antigen docking. *Proteins,* 2009; 74(2): 497-514.

[116]. Gray JJ, Moughon SE, Kortemme T, Schueler-Furman O, Misura KM, Morozov AV, *et al.* Protein–protein docking predictions for the CAPRI experiment. *Proteins*, 2003; 52(1): 118-22.

[117]. Sherman W, Beard HS, Farid R. Use of an induced fit receptor structure in virtual screening. *Chem Biol Drug Des*, 2006; 67(1): 83-84.

[118]. Rossi KA, Weigelt CA, Nayeem A, Krystek Jr SR. Loopholes and missing links in protein modeling. *Protein Sci,* 2007; 16(9): 1999-2012.

[119]. Sellers BD, Nilmeier JP, Jacobson MP. Antibodies as a model system for comparative model refinement. *Proteins,* 2010; 78(11): 2490-505.

[120]. Zhu K, Pincus DL, Zhao S, Friesner RA. Long loop prediction using the protein local optimization program. *Proteins,* 2006; 65(2): 438-52.

[121]. Rapp CS, Pollack RM. Crystal packing effects on protein loops. *Proteins,* 2005; 60(1): 103-109.

[122]. Marcatili P, Rosi A, Tramontano A. PIGS: automatic prediction of antibody structures. *Bioinformatics,* 2008; 24(17): 1953-54.

[123]. Lyskov S, Chou F-C, Conchúir SÓ, Der BS, Drew K, Kuroda D, *et al.* Serverification of molecular modeling applications: the Rosetta Online Server that Includes Everyone (ROSIE). *PLoS One*, 2013; 8(5): 63906.

[124]. Kuroda D, Shirai H, Kobori M, Nakamura H. Structural classification of CDR-H3 revisited: a lesson in antibody modeling. *Proteins,* 2008; 73(3): 608-20.

[125]. Reichert JM. Monoclonal antibodies as innovative therapeutics. *Curr. Pharm. Biotechnol.,* 2008; 9(6): 423-30.

[126]. Carter PJ. Potent antibody therapeutics by design. *Nat. Rev. Immunol,* 2006; 6(5): 343.

[127]. Nelson AL, Reichert JM. Development trends for therapeutic antibody fragments. *Nat. Biotechnol,* 2009; 27(4): 331.

[128]. Fischman S, Ofran Y. Computational design of antibodies. *Curr. Opin. Struct. Biol,* 2018; 51: 156-62.

[129]. Bradley P, Misura KM, Baker D. Toward high-resolution de novo structure prediction for small proteins. *Science,* 2005; 309(5742): 1868-71.

[130]. Raval A, Piana S, Eastwood MP, Dror RO, Shaw DE. Refinement of protein structure homology models via long, all-atom molecular dynamics simulations. *Proteins,* 2012; 80(8): 2071-79.

[131]. Zhang Y, Skolnick J. The protein structure prediction problem could be solved using the current PDB library. *PNAS,* 2005; 102(4): 1029-34.

[132]. Skolnick J, Zhou H, Brylinski M. Further evidence for the likely completeness of the library of solved single domain protein structures. *J Phys Chem,* 2012; 116(23): 6654-64.

[133]. Khoury GA, Smadbeck J, Kieslich CA, Floudas CA. Protein folding and de novo protein design for biotechnological applications. *Trends Biotechnol,* 2014; 32(2): 99-109.

[134]. Khoury GA, Baliban RC, Floudas CA. Proteome-wide post-translational modification statistics: frequency analysis and curation of the swiss-prot database. *Sci. Rep,* 2011; 1: 90.