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Computational design of antibodies

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Antibody design aims to create new antibodies with biological activity that can be used in therapy and research. Traditional methods for antibody discovery, such as animal immunization and large-scale library screening, generate antibodies that bind to the target of interest, but do not necessarily have the desired functional effect. Computational methods can be utilized as a means to guide the search for biologically relevant antibodies, focusing on specificity and affinity determinants to target a particular region of the antigen. Such an approach would allow for the design of epitope-specific antibodies that will have the desired effect on the function of the targeted protein.

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Introduction

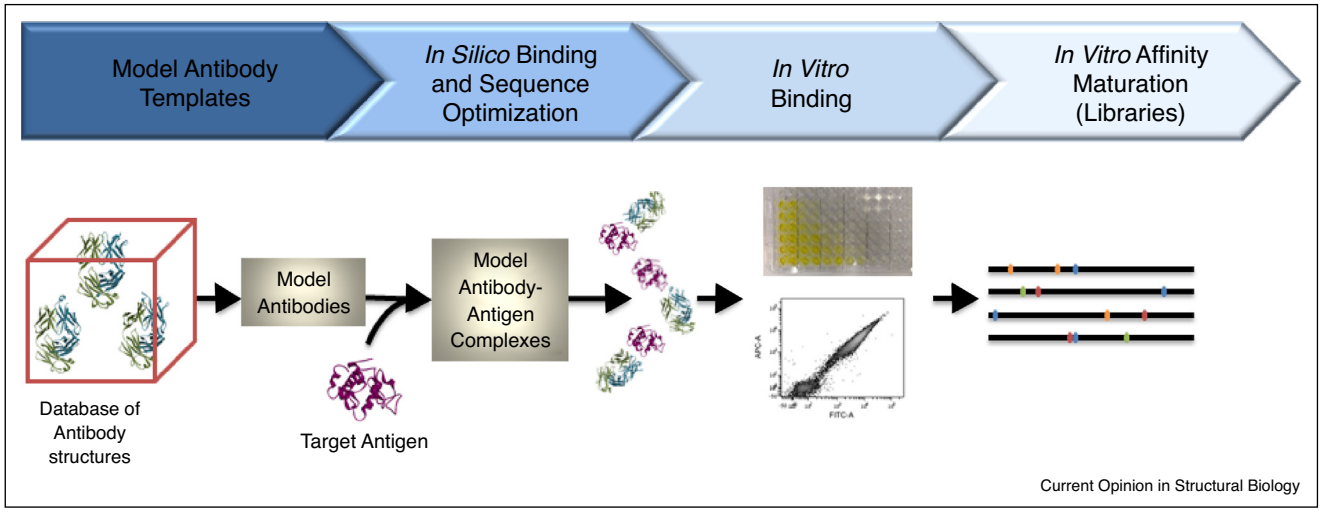
Antibodies are the fastest growing class of therapeutics [1]. However, despite tremendous discovery efforts, existing technologies fail to generate biologically active antibodies against many of the most promising targets. The essential goal of Ab design, particularly in the context of drug design, is to design a novel antibody that has a biological effect. However, most approaches focus getting a specific binder to the target, not on eliciting a desired biological activity. Immunization and screening of large libraries can be employed to obtain binders to a target of interest. Different approaches to the design of such libraries, including restricted codons [2] or combinations of germline H and L chain genes [3,4], have succeeded in producing antibodies with novel binding specificities and in some cases, biological activity [5]. These methods, however, select for the tightest binders, typically to immunodominant epitopes, precluding the discovery of antibodies with lower affinities that may bind to other, functionally relevant sites. Targeting specific sites within a target antigen, for example, those known to agonize or antagonize a biological pathway, remains a challenge in antibody design.

While large-scale, general purpose libraries may yield some functional antibodies, the size of the haystack in which these needles hide makes it difficult to identify them by meticulous functional screening of thousands of binders. Computational approaches offer another route to antibody design. The general scheme of current methods for the computational design of antibodies is presented in Figure 1. A first step toward identifying an antibody that binds the antigen is to model the 3-D structure of candidate antibodies, as well as the structure of the antibody–antigen complex. These antibodies are then tested experimentally for binding, and if necessary, are improved via *in vitro* affinity maturation. Better understanding of the structural basis of antigen binding by antibodies is a key to the success of this approach [6]. Here, we review the current state of computational technologies for antibody design, and suggest how new computational approaches can be applied to design libraries that are more likely to yield biologically active antibodies.

Modeling antibodies and antibody–antigen complexes

Structure-based computational protein design in general, and antibody design in particular, relies heavily on quality three-dimensional structural data for both the template for design (in this case, the antibody), the desired target (in this case, the antigen), and their complex. Antibody modeling has advanced to the state where the majority of the antibody variable domain can be modeled reliably. The success in modeling is in part due to structurally canonical conformations of most CDRs [7]. However obtaining accurate models of the variable CDR H3 and the relative orientation of the H and L chains, arguably the most important elements in determining binding, remains a challenge [8•] (for a review of antibody modeling and challenges see [9]). Among other reasons, this is due to the unique conformation of H3 in different Abs [10]. As H3 comprises part of the H-L interface, modeling both of these regions is interdependent. H3 modeling can be improved by implementing geometric constraints that describe a conserved structural kink [11] (For a review of H3 modeling see [12]). Addressing both H3 modeling and VH-VL orientation, Marze *et al.* [13] demonstrate improvements to antibody modeling accuracy by utilizing multiple templates of VH-VL orientation in addition to CDR grafting with RosettaAntibody [14]. Deane and colleagues implement a Random Forest classifier to

Figure 1



Computational design of antibodies — general scheme. Current methods for computational antibody design begin with modeling an antibody and an antibody–antigen complex. Selected antibody sequences are tested experimentally for antigen binding, for example, either with a soluble antigen or a cell-expressed antigen, and binders are further optimized by affinity maturation methods.

identify specific sequence positions that characterize the VH-VL orientation as a series of torsion and bend angles affecting the possible degrees of freedom, to improve orientation prediction [15,16].

However, even when a reliable model for the antibody is obtained, modeling the Ab-Ag complex is a difficult task. The community wide critical assessment of protein interactions (CAPRI), which assesses the performance of computational tools for modeling complexes, demonstrates this difficulty. In its recent experiment [17], 67 research teams using state-of-the-art methods attempted to model 20 complexes. The teams submitted >20 000 models (i.e. an average of 1000 models per complex), and yet for six out of the 20 complexes there was not a single model that was deemed 'acceptable' in its quality (e.g. identifying correctly 50% or more of the interface contacts). The success of docking that is based on models of the subunits, is even poorer [18]. These difficulties are encountered in antibody–antigen docking as well [19].

Importantly, even when complex modeling successfully generates a correct model among its best models, there is no straightforward way of telling which one it is. Consequently, attempts to design an antibody that are based on modeling the 3-D structure of the complex, cannot rely on a single model, and hence require the synthesis of dozens, sometimes even hundreds, of different sequences, hoping that one of them binds.

Selecting models as a basis for computational design, however, is only the first step. Methods to predict changes in the free energy of mutants are then used to improve antibodies or to introduce cross-reactivity. These methods use either crystal structures or models of the antibody–antigen complexes [20–22] as their starting point. A study by Sirin *et al.* [23] highlights the limited performance of these methods. This study used a large dataset of mutants to compare the experimentally determined and the computationally predicted effects of mutations on binding free energies of antibody–antigen complexes. The computational methods tested included those based on statistical potentials as well as all-atom force-fields. They conclude that some of the computational methods perform reasonably well in identifying mutations with a large effect on binding, but the problem of identifying mutations with moderate or small effects is still unresolved. Another study [24] found that using consensus scoring of some of these programs can improve the identification of mutations that weaken binding. However, the study did not distinguish between mutations that improve affinity and mutations that were neutral. A recent study by Clark *et al.* [25] on a small number of antibodies shows that predictions of binding energy changes correlate with experimental alanine scanning data. However, the authors conclude that their tool is not yet a "robust, automated protocol . . . suitable for application to an arbitrary protein–protein interaction." Taken together, the results of these studies demonstrate challenges that still exist for computational design of antibodies: predicting whether, and how, the designed proteins are going to interact is a major challenge and predicting which mutations can improve affinity is not easier. This is why existing approaches require many experimental attempts and large libraries for improving preliminary binders.

A possible alternative approach to antibody design that may mitigate these challenges should focus on predicting specific functional determinants, rather than modeling the full complex. It has been shown that specific contacts between antibodies and antigens can be predicted, sometimes even without modeling the full complex [26,27]. Furthermore, a functional antibody has been successfully designed by grafting a few specific contacts from a known complex onto an antibody template [28••].

Specificity and affinity determinants — contribution of specific positions and CDRs

The challenge of designing a new antibody can be thus reduced to forming a handful of residue–residue contacts between the designed antibody and the target protein. Predicting which positions to select for variation, and what variation to introduce there in order to elicit an antibody with novel function, may hold the key to the design of relatively small, focused libraries. It is well known that certain amino acid residues, for example, tyrosines, are prevalent in CDRs and contribute to their ability to bind diverse ligands [29,30•,31••,32]. Several studies, using both knowledge-based and physico-chemical-based methods, describe the identification and prediction of specificity determinant positions of antibodies as well as their composition. One such study has shown the computational identification of antigen binding fragments based on structural analysis [27]. Using MD simulation of 20 Ab-Ag complexes and MM/GBSA calculations of binding free energies, Osajima and Hoshino [33] show the significant role the H chain CDRs, and Tyr in particular, contribute to the free energy of binding. These observations are supported by results of another study on a much larger dataset of 403 Ab-Ag complexes [34]. This and other studies identified the prevalence of Tyr, in addition to other amino acids (Trp, Ser, Asn, Asp, Thr, Arg, Gly) in Ab-Ag interfaces (see [35] and references therein). Interestingly, Tyr, Ser, and Trp are over-represented in germline residues that contact the antigen but not in antigen-contacting residues that are introduced into the Ab during somatic hypermutation [31••]. The prevalence, thus, is encoded in the germline rather than selected for during *in vivo* affinity maturation.

Further characterization of the specificity determinants of paratopes shows that while almost all antibody binding regions (ABRs) within the six variable loops, contribute to the binding free energy of the complex, each ABR has distinct amino acid compositions as well as preferences for binding different amino acids on the antigen [30•]. For example, H chain ABRs (particularly H2) are largely shown to mediate charged interactions, while L chain ABRs (L1 and L3) contribute to polar interactions. Another study that compared natural antibodies to synthetic ones has found that in natural antibodies each CDR tends to specialize in specific types of contacts [36]. This information is useful when considering library design for antibody engineering, particularly for narrowing the variation down to incorporate only residues shown in nature to contribute to specificity.

When designing libraries it is crucial to not only consider the variability which will be introduced, but where to introduce it as well. Figure 2 dissects an antibody–antigen complex into the different determinants that contribute to specificity and affinity. A large scale structural and statistical analysis of 196 Ab-Ag complexes [31••] identified the positions in germline sequences that most likely undergo somatic hypermutation and their predicted contribution to binding affinity. The contribution of positions to affinity, it shows, depends on the structural region of the antibody in which they occur. Importantly, these favored positions are not only in the Ag contacting residues, but also in the H-L interface, and in CDR positions that do not mediate direct contact with the antigen. The study also characterizes the contribution of germline residues to binding affinity, relative to positions that underwent somatic hypermutation. It has been shown that some positions in the framework contribute to binding specificity, via long range and allosteric effects [31••,37,38,39••,40]. When engineering antibodies, it is important to consider the effects of all these positions. As seen in Figure 2, while most of the contacts come from the CDRs, projecting positions that tend to be altered *in vivo* during SHM onto the structure of the antibody reveals that only a small fraction of the CDR positions are in direct contact with the antigen. Moreover, some positions that are highlighted by SHM are not in the paratope, revealing indirect effects. In addition, some CDR positions contribute to stabilizing the H-L interface.

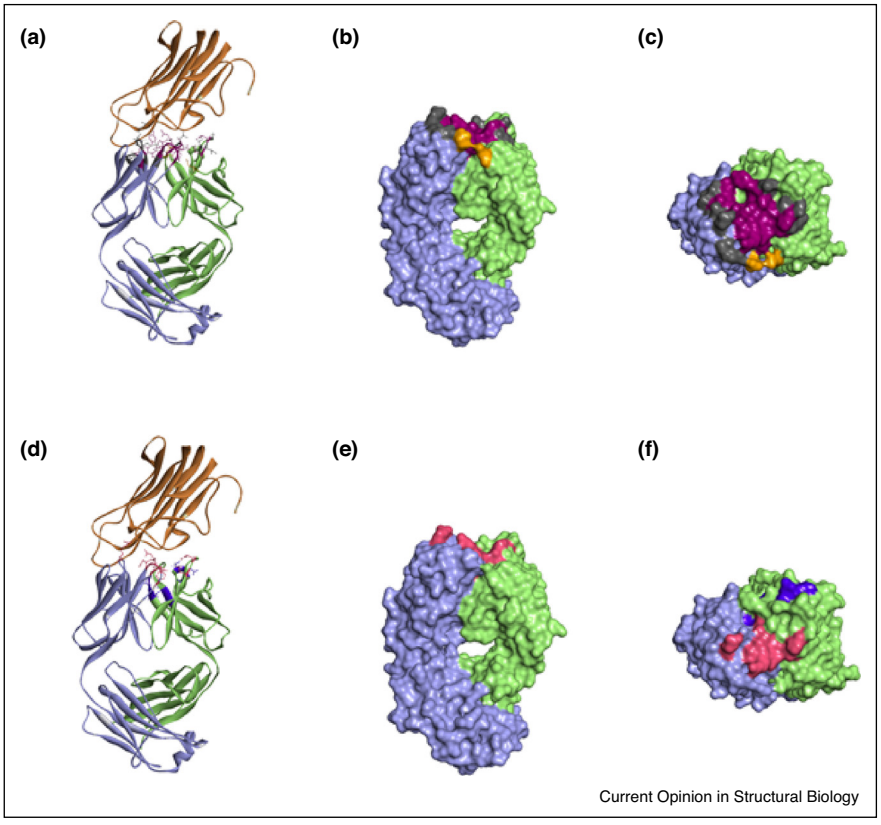
De novo antibody engineering

While some computational tools have been proposed for improving the affinity of existing antibodies [41], the bigger challenge is to design an antibody with a new function. Several recent examples demonstrate success in this task. Each employs different computational methods for modeling the antibody–antigen complex and predicting beneficial mutations to introduce in the antibody to improve specificity and affinity. A main goal of the computational tools is to minimize the number of variants that need to be experimentally screened.

Engineering an antibody that will have a functional effect on a target requires a detailed understanding of the target. The epitope within the target that should be bound to affect the function must be identified. Antibody-specific epitope prediction provides a method to overcome this difficulty, sometimes even in the absence of a 3-D structure of the antibody [26,42–44].

Several computational approaches for engineering functional antibodies *de novo* have been designed to mimic

Figure 2



Specificity and affinity determinants. (a,d) Crystal structure of the antibody Fab Adalimumab (Humira) bound to its antigen TNF-alpha (PDB 3WD5). The H chain is in green, the L chain is in blue, the antigen is in orange. The paratope of antibodies may be comprised of residues located in different structural regions of the antibody. The paratope residues (6 Å from the antigen) are shown in stick in **(a)** and on the surface of the antibody in **(b)** and **(c)**. The antigen has been removed in (b) and (c) for clarity. (c) is rotated ~90° about the *x*-axis relative to (b). CDR (ABR as defined by Paratome [50]) residues that participate in the antigen interface only, are in grey; CDR residues that comprise both the antigen interface and H-L (6 Å) interface are in purple; framework residues that comprise both the antigen interface and H-L interface are in light-orange. **(d)** Antibody positions with high frequencies of SHM *in vivo* [31••] are mapped onto the structure of Adalimumab and shown in stick, and on the surface of the antibody in **(e)** and **(f)**. The antigen has been removed in (e) and (f) for clarity. (f) is rotated ~90° about the *x*-axis relative to (e). Those residues that contact the antigen (6 Å) are shown in pink; residues that do not contact the antigen are shown in dark blue. As seen, some of the residues that are commonly altered during SHM are not surface residues, suggesting that engineering and design of affinity should not be focused exclusively on interface residues. The different colors of the structural and functional elements suggest guidance for design focusing at specificity and affinity determinants.

antibody development in nature, specifically, simulating V(D)J gene recombination, by assembling an antibody model from structural building blocks [28••,39••,45]. Using modular Ab parts (MAP), in combination with *in silico* affinity maturation, the OptMAVEn program has been used to generate human Abs for different antigens [45]. A recent study by Poosarla *et al.* [46], offers the first experimental validation of this methodology by engineering an antibody with novel peptide binding. Beginning with 31 designs, and further predictions of stability from molecular dynamics simulations, 27 designs were subjected to *in silico* affinity maturation. Five of these designs were tested experimentally and shown to be folded and stable in solution; three showed nM binding affinity to the peptide antigen. A similar study employed the OptCDR [47] tool to predict and model scFvs targeted towards a linear epitope, which resulted in identification of a *de novo* antibody specific for that target [48]. While both these studies demonstrate successful epitope-specific antibody engineering generated by computational predictions, it is important to keep in mind that the antigen in each case comprises a linear epitope, and modeling conformational epitopes (ie. non-sequential epitopes) will likely be more difficult.

Baran *et al.* [39••] utilize AbDesign [49] to generate *de novo* antibodies to two different protein antigens. By combining framework and CDR segments from antibody structures, docking the antigen, and modeling the optimal sequence for the docked complex, they succeed in

generating three antibodies that bind to novel targets, after experimentally testing ~200 designs for stability and binding. Random mutagenesis was then employed, yielding two antibodies with improvements of affinity of an order of magnitude. Interestingly the randomly introduced mutations responsible for increased affinity were not located in the CDRs, but rather in the framework, and are proposed to mediate improved affinity via long-range electrostatic interactions. This observation highlights an additional challenge for predicting specific variations for affinity improvements by focusing on *in silico* affinity maturation of CDR residues in a model of the antibody–antigen complex. Notably, while computational modeling allowed for the discovery of *de novo* antibodies to a given antigen, the design was not epitope-specific.

Going beyond the attempt to design an Ag-specific binder, Liu *et al.* [28••], attempted to design an epitope-specific antibody to cross-block the natural ligand of the target. Their approach combines hot-spot grafting with computational modeling and sequence optimization. This method yielded low affinity initial binders, that were then improved by computational re-design of CDR H3 via *in silico* swapping of CDR H3s from other template structures. The fact that the hotspots were grafted onto H2 and not CDR H3, allowed for the introduction of variation into H3 with minimal concern for impairing function. The crystal structure of the designed antibody in complex with the antigen, while largely consistent with the model, shows differences in CDR loop tilt and VH-VL interface relative to the model. This observation emphasizes the potential of an approach to antibody engineering that focuses on modeling specific functional interactions, rather than a single accurate 3-D model of the Ab-Ag complex.

The examples highlighted here, show how predictions can be used to design antibodies that target a specific epitope or antibodies with a specific function. However, in these cases, a rationally designed library was not implemented. Future directions for this field may include utilizing computational predictions, such as those described above, to guide the design of focused, epitope-specific libraries, predicted to elicit functional antibodies.

Conclusion

Most existing approaches for Ab design start with attempting to model the full complex. However, as demonstrated in the case of Liu *et al.* [28••], success can achieved even when the model was not found to be in full agreement with the crystal structure, as long as the functional interactions were modeled correctly. These results are consistent with the successful modeling of antibody–antigen interfaces in the absence of a model for the entire complex [26] Thus, focusing on modeling specific contacts suggests new avenues for Ab design and engineering. Given the challenges in modeling antibody–antigen complexes, the prediction of functionally important residues, which can be applied to designing focused, epitope-specific libraries, will advance the engineering of biologically active antibodies.

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