Medical Sciences

Summarizing Solvation Effects on Antibody Structure and Function

Ramachandran Murali[1], Somdutta Saha[2], Anastas Pashov[3], and Thomas Kieber-Emmons[2,*]

[1] Department of Biological Sciences, Research Division of Immunology, Cedars-Sinai Medical Center, Los Angeles, CA, USA. [2] Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, Little Rock, AR, USA. [3] Stephan Angelov Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria.

Protein interactions at the atomic-scale are influenced by the solvent microenvironment, playing a role in molecular recognition by modulating a direct relationship between biological (macroscopic solvent) function and effects on protein binding mechanisms. The antibody molecule is a prototypical protein for understanding molecular recognition, binding and evolution in function. The first order biological function of antibodies is to bind to antigens. While a wealth of knowledge accumulated in understanding solvent mediated antibody structure stability, the role of solvent and its relationship to evolution (i.e., affinity maturation or antigen mimicry) and antibody polyspecificity is poorly understood. Recently, we examined how the solvent microenvironment affects antibody flexibility and the molecular interactions that define the recognition of the neolactoseries antigen Lewis Y (LeY), designated tumor-antigen associated with several solid cancers. In particular, we were interested in how antibodies that are close in sequence to antibody germline genes can distinguish glycans considering the effect of solvent microenvironment on the recognition process. Here, we place our results in context of a broader viewpoint of antibody interactions and significance in developing antibody-based therapeutics.

Lewis Y | Antibody Structure | Solvation Effects | Flexibility | Germline

Introduction

Among proteins, antibodies have a unique position. They play a prominent role in defense mechanisms against infection and are now considered and used in front line therapies of cancer (1-4). Their role in the early response against pathogens requires antibodies to first recognize a vast array of antigens followed by the emergence of antibody clones that display increase specificity. The first targets of antibodies in the host defense are carbohydrate antigens. Carbohydrates are critical for numerous biological processes such as cell–cell adhesion, protein folding, protein trafficking, and cell signaling.

Setting the Stage

Carbohydrates are valuable targets for diagnostics and therapeutics. Yet, it can be difficult to determine if a particular antibody has an appropriate, desired, specificity. Typically, anti-carbohydrate antibodies recognize a glycan antigen that is two to six residues long (5). This is most interesting since many carbohydrates are large and heterogeneous with branches that define the most salient recognition elements. Not surprisingly, there are tens of thousands of glycan recognition elements expressed among pathogens and tumor cells (5, 6)

A fundamental question is how an antibody can selectively recognize a chemically and structurally complex antigen? It is typically thought that affinity maturation evolves polyspecific antibodies into highly specific antibodies (Figure 1). One biophysical property that distinguishes polyspecificity from specificity is protein flexibility, which can be mediated by microenvironment solvent affects depending on the size and nature of antigen. The necessity to recognize a vast array of antigens from their three-dimensional disposition is manifested in the flexibility of germline antibody genes to adapt to structures leading to antigen recognition (7).

A flexible combining site is therefore able to adopt different conformations that recognize different antigens, while a rigid combining site is locked into a conformation that is specific for a given antigen's structural configuration. This suggests that nature solves the problem of recognizing an infinite number of potential foreign molecules, without recognizing any self molecules (which would cause autoimmunity), by evolving flexible, polyspecific germline antibodies, which are kept at low concentration (to prevent autoimmunity), into more rigid and specific mature antibodies.

Flexibility is therefore a hallmark of germline antibodies. The practical affect of flexibility is the polyspecific nature of an antibody. The maturation process allows for increased affinity to be achieved in antigen/ligand binding (8). But flexibility is modulated by several factors at different stages of maturation. Among factors, primary sequence is assumed to encompass all the information necessary for an antibody function which include antibody backbone atoms that has an intrinsic mobility because of folding requirements. The affect of amino acid sequence on recognition and its relationship to flexibility can be approached from *in silico* analyses. Consequently, much of the research in the field has focused on the conformational changes induced by antigen binding. But another important factor with little attention affecting flexibility in the recognition process is the solvent microenvironment of the binding region. Unfortunately, little is understood to the effect of the solvent microenvironment on antibodies close in sequence (9-11).

While it is not known how biological solutions ultimately regulate the polyspecific nature of an antibody, a glimpse of potential regulation is observed under various in vitro conditions, both experimentally and *in silico*. *In vitro* notably all factors able to destabilize an antibody mediate polyspecificity. For example, mild treatment with protein destabilizing agents like low pH, Fe^{2+} ions, ROS, etc, are known to induce polyspecificity in some antibodies (12, 13). The underlying mechanism lies in the fact variable regions that are sensitive to destabilizing agents seem to be unable to return to their native conformation and thereby expanding the reactive conformational space. This expansion seems to be very discrete and not associated with aggregation or other type of non-specific phenomenon that can be ascribed to antibody stickiness. In contrast to factors that reveal hidden specificity by denaturing mechanisms, polyspecificity can be induced by structure forming factors such as heme (12, 13). In

Conflict of Interest: No conflicts declared.

* Corresponding Author. Email: TKE@uams.edu

this case it seems that such factors rigidifies antibody structures. This ability is counterintuitive (13). It is intriguing, that some of the tested destabilizing agents can also change the solvation of the proteins. On theater hand, local inflammation produces sufficient amount of these agents to alter the spectrum of reactivity's of polyclonal human immunoglobulin (IgG) (14).

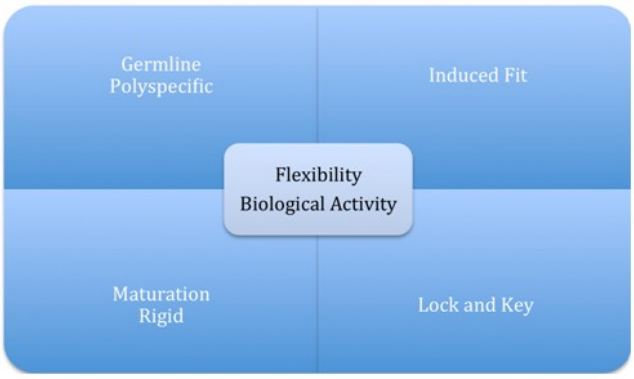

Figure 1. Molecular Characteristics of Antigen Recognition underlying antibody biological activity.

From an *in silico* perspective our recent studies along with other *in silico* studies we get a glimpse into maturation of germ line antibodies; from molecular dynamics calculations affinity maturation appears to occur through reorganization of the combining site geometry in a manner that optimizes the balance of gaining favorable electrostatic interactions with the antigen and losing those with the solvent microenvironment during the binding process (15, 16). Importantly, antibody dynamics is tailored during affinity maturation to mediate functional molecular recognition; and this provides the key to determining which, if any, of the observed dynamics represent adaptations due to antigen driven selection. The detailed characterization of changes of biologically relevant motions as a function of evolution can provide insight into immune function, evolution and design principles that can lead to enhanced therapeutics.

Our *in silico* studies suggest that even though the maturation of antibodies leads to optimization of enthalpic contributions in the binding process, solvent microenvironment affects entropic aspects and can modulate flexibility required for some of the mature antibodies. Such entropic factors might allow for better recognition and binding of internal structures of extended carbohydrate structures on tumor cells, which are likely to display some level of heterogeneity. But the downside of the entropic contribution is that it can increase flexibility, in which case an antibody may display unexpected polyspecificity. Here, we discuss several observations of these relationships that influence antibody structure, recognition and functionality from considering solvent effects from *in silico* studies.

Antibodies at a Glance

Antibodies belong to a family of proteins called immunoglobulins, comprising the largest structural fold in the immune system. Briefly, the overall structure of an antibody consists of two chains (Light and heavy) two arms of Fab (contains variable light chain (VL) and heavy chain (VH)) linked to Fc receptor. Fab domains of antibody are responsible for molecular recognition. Six regions, termed complimentary determining regions (CDR) from light chains (3 CDRs) and heavy chain (3 CDRs) are responsible for antigen recognition. Amino acids in the binding site can determine specificity either by contacting the antigenic determinant or by influencing a contacting residue. Because of insertions and deletions, residues frequent in CDR, contacting residues or conformationally important residues would also often be located at varying distances from fixed residues in the structural segments. So while spacing of particular residues can be quite different, the same residue type can lend to a particular specificity.

The same residue types are also evident when considering single chain molecules reactive with carbohydrates. Specificity for carbohydrates can be achieved by lectins, which in this context can mimic antibodies. Antiglycan antibodies and lectins have been widely used to understand carbohydrate expression levels on cancer cells. For example, the lectin Griffonia simplicifolia (GSA) can bind to the neolactoseries antigen Lewis Y as does the antibody BR55-2. Residues types interacting with the antigen are shared between these anti-glycan proteins (17). Likewise, Camel antibodies, that are principally single chain dimers, are also reactive with carbohydrate antigens and can mimic carbohydrate antigens (18).

The specificity and affinity of antibody-antigen interactions are fundamental for understanding their biological activity (Figure 1). Antibody germline genes are known to sculpt antibody-combining sites containing innate, key side-chain contacts that define the antigen recognition step (19, 20). It is often concluded that for a high affinity antibody the three-dimensional structure of an antigen and its chemical composition is key (21). However, in germline antibodies-low affinity antibodies- due to flexibility the antibody might discriminate based on chemical composition (i.e. nature of the amino acid residues comprising the antibody-combining site) and less on shape complementarity.

The typical germline antibody paratope has evolved to accommodate diverse epitopes relying on flexibility of these antibodies to mold themselves around antigens forming a template for affinity maturation to occur (9, 19). Generally, antibodies recognize the shape of an antigen that includes small molecules (i.e. haptens) and a small region on the surface of antigen (22-24). The association between an antigen and antibody has been extensively characterized (25) and studies show it involves networks of hydrogen bonds, hydrophobic interactions and electrostatic forces which are all weak and non-covalent in nature between the antigenic determinant (epitope) of the antigen and the variable-region (V_H/V_L) domain of the antibody molecule (26). So a large number of such weak interactions are required to form a strong antigen-antibody interaction. These interactions can only take place if the antigen and antibody molecules are close enough for some of the individual atoms to fit into complementary recesses or binding site or pocket.

A strong antigen-antibody interaction depends on a very close fit between the antigen and antibody, which translates into antibody displaying a high degree of specificity for the antigen. During the process of affinity maturation antibodies become more rigid and that impacts on both the recognition process lending to ligand specificity and the organization or sculpting of the ligand-binding site. The T cell independent antibody response to carbohydrate antigens is considered independent of germinal centers since it is characteristic of the typically extrafollicular marginal zone B cells (27) and they are supposed to stay closer to germline. Nevertheless, anticarbohydrate antibodies also accumulate mutations although to a lesser extent than the products of the typical follicular B cells.

Genes responsible for host defense can exhibit fast evolutionary rates. Evolutionary rates are directly linked to the distribution of amino acids at individual sites within proteins (28) and such distributions have long ago defined antibody CDRs (29) and Idiotype Determining Regions (IDR) (30, 31). For the immune system these sites are related to immunogenicity and

antigen recognition (32). Residue substitutions can change the intrinsic flexibility of proteins, which in turn is modulated by solvent, which in turn mediates molecular recognition. On average, mutations introduced at solvent-exposed sites are less likely to disrupt protein structure and function than mutations introduced at buried (33).

Antibodies display a varying degree of flexibility and exhibit a large degree of structural asymmetry (34, 35) as assessed from high-resolution crystallography, CryoEM Tomography and Electron Microscopy (34, 36, 37). The structural flexibility in different pH are of direct relevance to understanding the effects of solvents on protein structural dynamics and stability and their functional significance in antigen recognition (34, 35, 37). Even IgGs with over 90% sequence identity exhibit a broad range of solution behaviors and challenges that are affected depending on the solution environment (37, 38). For example, homology models of two antibodies displaying 90% homology with most sequence differences restricted to the CDRs (37). Small angle x-ray scattering (SAXS) analysis suggested that the two antibodies have very similar solution conformations under these solution conditions. Solution SAXS analysis of form factors, benefiting particularly from Ensemble Optimization Method analysis, provided independent evidence of molecular flexibility primarily about the hinge region of antibodies (37).

Role of Solvent in Microenvironment of the Binding Site

The inclusion of solvent microenvironment effects on molecular recognition is of fundamental importance to fully understand binding mechanisms because deducing binding mechanisms from structural models alone can be misleading. Generally, protein solvation has several functions. 1) Assist in proper folding, (2) stabilize tertiary structures by modulating polar interactions, and configurational entropy of molecules 3) mediate the recognition process, and 4) impart dynamic feature such as enabling flexibility in molecules.

The exact nature of solvents and its role in protein function can only be elucidated by X-ray crystallography. The consideration of solvent effect, however on molecular recognition processes, is exceptionally challenging theoretically and computationally; bulk solvent effects are often used in the absence of any information from crystallographic studies. Nonetheless, rigorous models represent solvent atomically and therefore positionally, while more efficient models represent solvent implicitly by replacing individual molecules with a linearly polarizable continuum (39).

Structural studies of several antigen-antibody complex has provided a strong basis for developing a robust computer-aided drug design (CADD) techniques, which have been used to explore antigen binding and to develop de novo antigens/ligands for antibodies in vaccine development (40, 41). Since crystallographic studies are time consuming and expensive, CADD can be very effective in reducing costs and speeding up the drug discovery process (42). However, the determination of binding and solvation free energies is pivotal for this process and is therefore, the subject of many studies (43, 44). In estimating the contribution of solvents to binding profiles, the solvation free energy of a small molecule provides a surrogate to the desolvation of the ligand in the thermodynamic process of protein-ligand binding.

Every ligand also has its own solvation characteristics. Experimental data on the transfer of small molecules between vacuum and water are relatively sparse. This makes it difficult to assess whether computational methods are truly predictive of this important quantity or merely good at explaining what is observed (43). To explore this behavior typically a prospective test is performed of two different methods for estimating solvation free energies: an implicit solvent approach based on the Poisson–Boltzmann equation and an explicit solvent approach using free energy calculations (39). In simple terms, docking of ligands or antigens to a receptor/antibody-combining site typically treats the target protein/antibody as rigid. In practice, a high level of accuracy in predicting ligand-binding modes depends on carefully accounting for the presence of multiple potential ligand-bound orientations and the possibility of protein conformational changes on ligand binding (45).

Solvents have been implicated in molecular mimicry. Molecular mimicry at the atomic level is represented by network of hydrogen bonds between parent-antigen and the mimicry-antigen. Polyspecificity of an antibody is one such manifestation where solvents can transiently mediate critical hydrogen bonds and exhibit binding to antigen's conformational heterogeneity. The atomic basis for this observation comes from two seminal studies. Herron et al (46) studied the effect of solvents on antigen binding. In their study with Flurorescein-4-4-20 antibody complex, they found that charge-shielding effects (i.e. electrostatic effects on polar atoms and their ability to mediate salt links) by solvents affected the binding affinity of the antibody without any significant conformation changes in the binding site. Similarly, Quiocho et al have demonstrated that a peptide mimic of carbohydrates is through a network of hydrogen bonds via water molecules (47). These observations suggest that solvents play a critical role in sculpting recognition (i.e. binding affinity) of antigens.

Lessons from LeY Reactivity

Despite several studies on antibody-antigen complexes (9-11), the functional role and the structural basis of conformational change in antibody binding, in particular anti-carbohydrate antibodies (19), remains unpredictable, and the structural and thermos-dynamic determinants of antibody specificity and affinity as they relate to affinity maturation of antibodies from germline genes are still not completely understood. Much like lectin–glycan interactions antibody-glycan binding is typically stabilized in two ways: by hydrogen bonding between amino acids in the carbohydrate-recognition domain and the glycan hydroxyl groups, and by Van der Waals packing of the hydrophobic glycan face against aromatic amino acid side chains (15, 16). Water, the predominant solvent in antibody-antigen interactions, affects the stability and specificity of recognition by mediating direct hydrogen bond interactions. Solvent also weakens interactions by modulating electrostatic interactions that can influence the flexibility of molecules (15). The inclusion of a solvent in our calculations reaffirms that low levels of electrostatic interactions are associated with conformational flexibility (15).

Our molecular simulation studies of crystallographically determined and modeled antibody-LeY complexes suggests that the heavy-chain germline gene and the light-chain germline gene are sufficient to account for the recognition of the trisaccharide-H determinant Types 1-4, while the specificity for LeY is driven by the CDR3 backbone conformation of the heavy chain and not the side chain interactions. These results confirm that these monoclonals use germline-encoded amino acids to recognize simple carbohydrate determinants like trisaccharide-H, but relies on somatic mutations in the periphery of the combining site to modify affinity for LeY through electrostatic interactions that leads to their optimized binding. These observations bring further attention to the role of mutations in T-cell independent antibodies to distinguish self from non-self carbohydrate antigens. Comparison of the structural characteristics resulting from molecular dynamic simulations suggest that the mature antibody

complexes undergo more restrained fluctuations than the germline complex suggesting that with maturity enthalpic contributions increase along with a decrease in entropic contributions. In some respect, the increased enthalpic contribution is facilitated by electrostatic component optimization.

Binding energy analysis of the antibody-LeY complexes reveals that van der Waals interactions and nonpolar contributions to solvation are similar and drive the formations of both the germline and mature antibody-LeY complexes. Among the various components of molecular interactions that define specific binding of ligands/antigens by antibodies, electrostatics is of special importance because of their long-range nature and their influence on polar or charged molecules. In our calculations we found that solvent increases the flexibility of some anti-LeY antibodies relative to the germline. This expands antigen antibody reactivity patterns and participates in further stabilizing the LeY core recognition through the addition of several hydrogen bonds. A comparison of *in vacuo* and solvation dynamics calculations strongly suggest that polar contacts including hydrogen bonds and salt bridges are important features required for specificity of the LeY antigen. This finding has practical consequences. First, it suggests that not all mature antibodies are equivalent. Some have high affinity for an antigen and some have moderate affinities, which can be related to solvent affects and not just residue substitutions that alter antigen contact. For some antibodies, an increase in flexibility can be detrimental lending to promiscuous feature. This view of nonspecific binding is at odds with the exquisite specificity with which antibodies are known to interact with antigens or ligands. It is imperative that all antigens are differentiated specifically to mount an effective and targeted immune response. How then do binding sites accomplish being both specific and cross-reactive?

We observe that anti-LeY antibody complexes can display a large degree of flexibility despite being of high affinity for their respective ligand (15). One mechanistic basis for this behavior is that each cross-reactant is bound specifically, forming different hydrogen bonds dependent upon its particular chemistry and the availability of complementary antibody residues. The inherent flexibility can provide an opportunity for hydrogen bonding partners to form. This aspect might render the antibody to be polyspecific contributing to unwanted cross-reactivity with cell surface molecules on normal tissue for example. From a practical perspective this finding is of particular importance when considering humanized antibodies. While humanization is focused on increasing the enthalpic contributions (to achieve high affinity), an important contribution from entropy through solvents is often ignored.

Alteration of glycan recognition

Altered glycosylation is a universal feature of cancer cells, and certain glycan structures are well-known markers for tumor progression. Engineering humanization of murine monoclonal antibodies, which is considered necessary for developing therapeutics, are not often subjected to energetic and solvent analysis relative to antigen binding. Humanization which involves creating a high affinity antibody might contribute to toxicities associated with immune pathology; high affinity antibodies may bind prematurely to antigens expressed on normal tissues. For example, Ahmed et al (48) have compared affinities of two anti-GD2 antibodies, (ME36.1, 14G2a) showing a reasonable affinity of 19nM and 77nM, respectively, and engineering a high affinity (Kd=5nM) 3F8 antibody. To accomplish high affinity, Ahmed et al mutated surface exposed glycine residues (located in the solvent environment) in the antibody to more hydrophobic residues such as isoleucine or tryptophan that increased enthalpy, reduced entropy and stabilized the VL-VH interface. Interestingly, high affinity 3F8 in clinical trials for the treatment of neuroblastoma have been suggested to lend to unwanted side effect mostly likely the result of recognition of GD2 on peripheral pain fibers and complement depositions (49).

Previously, Adams et al (50) demonstrated that low affinity anti-Her2 scFv efficiently penetrated tumor, while high affinity scFv found at the peripheral of tumor. Based on the observations, Adams et al concluded that the affinity of an antibody does not correlate with tumor response. This observation suggests consideration of a cautious approach in humanization that often ignores the role of solvent or deliberately replaces residues with hydrophobic moiety to displace solvents so that the affinity can be enhanced. On the flip-side, it is unclear whether, considering that there can be a large number of residue substitutions in the variable domain of engineered/humanized antibodies, the impact of solvent models associated with engineering might affect the specificity patterns of the therapeutic antibodies.

Our studies indicated that BR55-2 might bind to various forms of LeY (15). While we have not performed any mutational studies to understand how residue substitutions might affect LeY binding, some studies have been performed on another LeY reactive antibody (51). In these studies an increase of avidity for LeY was observed expressed on cancer cells by 65 fold using M13 phage libraries (51). Two mutants in particular with improved the affinity were identified by screening the libraries on carcinoma cell lines. One mutant, M1, at position 97 (Asp to Ala) in CDR3 of the heavy chain, resulted in an 8- to 10-fold improvement in Ag binding. A second mutant, M2, at position 53 (Gly to Asp) in CDR2 of VH increased binding three- to five fold. When these mutations were combined, the resulting Fab M3 was improved approximately 30-fold. An additional library was constructed in CDR1 of M1. Screening the library with an enzyme conjugate of synthetic LeY tetrasaccharide. isolated M4, a mutation with three amino acid substitutions in CDR1. This mutant improved BR96 Fab affinity to the moiety by an estimated 15- to 20-fold by ELISA, and 14-fold as measured by surface plasmon resonance. The M4 IgG had 65-fold improved avidity to LeY relative to the BR96 IgG. In these cases both the final mutant had residues that affect the solution interactions.

Summary

In summary, our studies suggest that solvents not only play a role in stabilizing antigen-antibody complex, but also contribute sculpting the antigen binding site during evolution (i.e. maturation). More interestingly, the solution microenvironment may be responsible for polyspecificity in particular for antigens like carbohydrates. Polyspecificity, by itself can be a boon or bane depending on the context in which antibody response is elicited. On the flip side, engineered antibodies for high affinity, in particular ubiquitous antigens like carbohydrates, might lend to undesirable biological reactions when solvent is ignored. Where experimentally, understanding the role of solvents remains a challenge, computational consideration might fill the gap in antibody engineering for the development of immunotherapies.

1. Lee JJ, Chu E. First-line use of anti-epidermal growth factor receptor monoclonal antibodies in metastatic colorectal cancer. Clin Colorectal Cancer. 2007;6:S42-6.
2. Sharkey RM, Goldenberg DM. Targeted therapy of cancer: new prospects for antibodies and immunoconjugates. CA Cancer J Clin. 2006;56:226-43.
3. Heimann DM, Weiner LM. Monoclonal antibodies in therapy of solid tumors. Surg Oncol Clin N Am. 2007;2007 Oct;16:775-92.
4. Polakis P. Arming antibodies for cancer therapy. Curr Opin Pharmacol. 2005;5:382-7.
5. Sterner E, Flanagan N, Gildersleeve JC. Perspectives on Anti-Glycan Antibodies Gleaned from Development of a Community Resource Database. ACS Chem Biol. 2016;11:1773-83. doi: 10.021/acschembio.6b00244. Epub 2016 May 25.
6. Cummings RD. The repertoire of glycan determinants in the human glycome. Mol Biosyst. 2009;5:1087-104. doi: 10.39/b907931a. Epub 2009 Jul 28.
7. Nguyen HP, Seto NO, MacKenzie CR, Brade L, Kosma P, Brade H, et al. Germline antibody recognition of distinct carbohydrate epitopes. Nat Struct Biol. 2003;10:1019-25. Epub 2003 Nov 16.
8. Willis JR, Briney BS, DeLuca SL, Crowe JE, Jr., Meiler J. Human germline antibody gene segments encode polyspecific antibodies. PLoS Comput Biol. 2013;9:e1003045. doi: 10.1371/journal.pcbi.. Epub 2013 Apr 25.
9. Chong LT, Duan Y, Wang L, Massova I, Kollman PA. Molecular dynamics and free-energy calculations applied to affinity maturation in antibody 48G7. Proc Natl Acad Sci U S A. 1999;96:14330-5.
10. Thorpe IF, Brooks CL, 3rd. Molecular evolution of affinity and flexibility in the immune system. Proc Natl Acad Sci U S A. 2007;104:8821-6. Epub 2007 May 8.
11. Yin J, Beuscher AEt, Andryski SE, Stevens RC, Schultz PG. Structural plasticity and the evolution of antibody affinity and specificity. J Mol Biol. 2003;330:651-6.
12. Dimitrov JD, Pashov AD, Vassilev TL. Antibody polyspecificity: what does it matter? Adv Exp Med Biol. 2012;750:213-26.:10.1007/978-1-4614-3461-0_16.
13. Dimitrov JD, Kaveri SV, Lacroix-Desmazes S. Gain of function of immunoglobulins after partial unfolding or cofactor binding. Mol Immunol. 2013;55:195-6. doi: 10.1016/j.molimm.2013.03.009. Epub Apr 9.
14. Mihaylova NM, Dimitrov JD, Djoumerska-Alexieva IK, Vassilev TL. Inflammation-induced enhancement of IgG immunoreactivity. Inflamm Res. 2008;57:1-3.
15. Saha S, Murali R, Pashov A, Kieber-Emmons T. The Potential Role of Solvation in Antibody Recognition of the Lewis Y Antigen. Monoclon Antib Immunodiagn Immunother. 2015;34:295-302. doi: 10.1089/mab.2015.0037.
16. Saha S, Pashov A, Siegel ER, Murali R, Kieber-Emmons T. Defining the recognition elements of Lewis Y-reactive antibodies. PLoS One. 2014;9:e104208. doi: 10.1371/journal.pone.0104208. eCollection 2014.
17. Blaszczyk-Thurin M, Murali R, Westerink MA, Steplewski Z, Co MS, Kieber-Emmons T. Molecular recognition of the Lewis Y antigen by monoclonal antibodies. Protein Eng. 1996;9:447-59.
18. Transue TR, De Genst E, Ghahroudi MA, Wyns L, Muyldermans S. Camel single-domain antibody inhibits enzyme by mimicking carbohydrate substrate. Proteins. 1998;32:515-22.
19. Evans DW, Muller-Loennies S, Brooks CL, Brade L, Kosma P, Brade H, et al. Structural insights into parallel strategies for germline antibody recognition of lipopolysaccharide from Chlamydia. Glycobiology. 2011;21:1049-59. Epub 2011 May 4.
20. Gerstenbruch S, Brooks CL, Kosma P, Brade L, Mackenzie CR, Evans SV, et al. Analysis of cross-reactive and specific anti-carbohydrate antibodies against lipopolysaccharide from Chlamydophila psittaci. Glycobiology. 2010;20:461-72.
21. Mariuzza RA, Phillips SE, Poljak RJ. The structural basis of antigen-antibody recognition. Annu Rev Biophys Biophys Chem. 1987;16:139-59.
22. Mian IS, Bradwell AR, Olson AJ. Structure, function and properties of antibody binding sites. J Mol Biol. 1991;217:133-51.
23. Kabat EA. Dimensions and specificities of recognition sites on lectins and antibodies. J Supramol Struct. 1978;8:79-88.
24. Haji-Ghassemi O, Blackler RJ, Martin Young N, Evans SV. Antibody recognition of carbohydrate epitopesdagger. Glycobiology. 2015;25:920-52. doi: 10.1093/glycob/cwv037. Epub 2015 Jun 1.
25. Mohan S, Kourentzi K, Schick KA, Uehara C, Lipschultz CA, Acchione M, et al. Association energetics of cross-reactive and specific antibodies. Biochemistry. 2009;48:1390-8. doi: 10.021/bi801901d.
26. Sinha N, Mohan S, Lipschultz CA, Smith-Gill SJ. Differences in electrostatic properties at antibody-antigen binding sites: implications for specificity and cross-reactivity. Biophys J. 2002;83:2946-68.
27. Cerutti A, Cols M, Puga I. Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes. Nat Rev Immunol. 2013;13:118-32. doi: 10.1038/nri3383.
28. Ramsey DC, Scherrer MP, Zhou T, Wilke CO. The relationship between relative solvent accessibility and evolutionary rate in protein evolution. Genetics. 2011;188:479-88. doi: 10.1534/genetics.111.128025. Epub 2011 Apr 5.
29. Wu TT, Kabat EA. An analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light chains and their implications for antibody complementarity. J Exp Med. 1970;132:211-50.
30. Kieber-Emmons T, Kohler H. Towards a unified theory of immunoglobulin structure-function relations. Immunol Rev. 1986;90:29-48.
31. Kieber-Emmons T, Getzoff E, Kohler H. Perspectives on antigenicity and idiotypy. Int Rev Immunol. 1987;2:339-56.
32. Kieber-Emmons T, Kohler H. Evolutionary origin of autoreactive determinants (autogens). Proc Natl Acad Sci U S A. 1986;83:2521-5.
33. Vitkup D, Sander C, Church GM. The amino-acid mutational spectrum of human genetic disease. Genome Biol. 2003;4:R72.
34. Sandin S, Ofverstedt LG, Wikstrom AC, Wrange O, Skoglund U. Structure and flexibility of individual immunoglobulin G molecules in solution. Structure. 2004;12:409-15.
35. Saphire EO, Stanfield RL, Crispin MD, Parren PW, Rudd PM, Dwek RA, et al. Contrasting IgG structures reveal extreme asymmetry and flexibility. J Mol Biol. 2002;319:9-18.
36. Valentine RC, Green NM. Electron microscopy of an antibody-hapten complex. J Mol Biol. 1967;27:615-7.
37. Lilyestrom WG, Shire SJ, Scherer TM. Influence of the cosolute environment on IgG solution structure analyzed by small-angle X-ray scattering. J Phys Chem B. 2012;116:9611-8. doi: 10.1021/jp303839t. Epub 2012 Aug 3.
38. Shire SJ. Formulation and manufacturability of biologics. Curr Opin Biotechnol. 2009;20:708-14. doi: 10.1016/j.copbio.2009.10.006. Epub Oct 31.
39. Shivakumar D, Deng Y, Roux B. Computations of Absolute Solvation Free Energies of Small Molecules Using Explicit and Implicit Solvent Model. J Chem Theory Comput. 2009;5:919-30. doi: 10.1021/ct800445x. Epub 2009 Mar 24.
40. Luo P, Canziani G, Cunto-Amesty G, Kieber-Emmons T. A molecular basis for functional peptide mimicry of a carbohydrate antigen. J Biol Chem. 2000;275:16146-54.
41. Cunto-Amesty G, Dam TK, Luo P, Monzavi-Karbassi B, Brewer CF, Van Cott TC, et al. Directing the immune response to carbohydrate antigens. J Biol Chem. 2001;276:30490-8. Epub 2001 May 30.
42. Sevy AM, Meiler J. Antibodies: Computer-Aided Prediction of Structure and Design of Function. Microbiol Spectr. 2014;2(6).10.1128/microbiolspec.AID-0024-2014.
43. Martins SA, Sousa SF, Ramos MJ, Fernandes PA. Prediction of Solvation Free Energies with Thermodynamic Integration Using the General Amber Force Field. J Chem Theory Comput. 2014;10:3570-7. doi: 10.1021/ct500346y.
44. Shivakumar D, Williams J, Wu Y, Damm W, Shelley J, Sherman W. Prediction of Absolute Solvation Free Energies using Molecular Dynamics Free Energy Perturbation and the OPLS Force Field. J Chem Theory Comput. 2010;6:1509-19. doi: 10.021/ct900587b. Epub 2010 Apr 14.
45. Mobley DL, Graves AP, Chodera JD, McReynolds AC, Shoichet BK, Dill KA. Predicting absolute ligand binding free energies to a simple model site. J Mol Biol. 2007;371:1118-34. Epub 2007 Jun 8.
46. Herron JN, Terry AH, Johnston S, He XM, Guddat LW, Voss EW, Jr., et al. High resolution structures of the 4-4-20 Fab-fluorescein

complex in two solvent systems: effects of solvent on structure and antigen-binding affinity. Biophys J. 1994;67:2167-83.
47. Vyas NK, Vyas MN, Chervenak MC, Bundle DR, Pinto BM, Quiocho FA. Structural basis of peptide-carbohydrate mimicry in an antibody-combining site. Proc Natl Acad Sci U S A. 2003;100:15023-8. Epub 2003 Nov 25.
48. Ahmed M, Goldgur Y, Hu J, Guo HF, Cheung NK. In silico driven redesign of a clinically relevant antibody for the treatment of GD2 positive tumors. PLoS One. 2013;8:e63359. doi: 10.1371/journal.pone.0063359. Print 2013.
49. Yang RK, Sondel PM. Anti-GD2 Strategy in the Treatment of Neuroblastoma. Drugs Future. 2010;35:665.
50. Adams GP, Schier R, McCall AM, Simmons HH, Horak EM, Alpaugh RK, et al. High affinity restricts the localization and tumor penetration of single-chain fv antibody molecules. Cancer Res. 2001;61:4750-5.
51. Yelton DE, Rosok MJ, Cruz G, Cosand WL, Bajorath J, Hellstrom I, et al. Affinity maturation of the BR96 anti-carcinoma antibody by codon-based mutagenesis. J Immunol. 1995;155:1994-2004.