Highlights

Modelling the assembly and flexibility of antibody structures

Dongjun Guo, Maria Laura De Sciscio, Joseph Chi-Fung Ng, Franca Fraternali

- Conformational flexibility of antibodies affects their function.
- Currently available experimental data pose challenges to the modelling of full-length antibody assemblies.
- Integrative modelling combining experiments and computational methods can address this challenge.

Modelling the assembly and flexibility of antibody structures

Dongjun Guo[a,b], Maria Laura De Sciscio[a,c], Joseph Chi-Fung Ng[a], Franca Fraternali[a]

[a]*Institute of Structural and Molecular Biology, University College London, Darwin Building, Gower Street, London, WC1E 6BT, United Kingdom*
[b]*Randall Centre for Cell & Molecular Biophysics, King's College London, New Hunt's House, Guy's Campus, London, SE1 1UL, United Kingdom*
[c]*Department of Chemistry, Sapienza University of Rome, P.le A. Moro 5, Rome, 00185, Italy*

Abstract

Antibodies are large protein assemblies capable of both specifically recognising antigens and engaging with other proteins and receptors to coordinate immune action. Traditionally, structural studies have been dedicated to antibody variable regions, but efforts to determine and model full-length antibody structures are emerging. Here we review the current knowledge on modelling the structures of antibody assemblies, focusing on their conformational flexibility and the challenge this poses to obtaining and evaluating structural models. Integrative modelling approaches, combining experiments (cryo-electron microscopy, mass spectrometry etc.) and computational methods (molecular dynamics simulations, deep-learning based approaches etc.), hold the promise to map the complex conformational landscape of full-length antibody structures.

Keywords: Antibody structures, Antibody isotypes, Conformational sampling, Antibody flexibility

1. Introduction

Antibodies are large protein assemblies secreted by B cells in response to immune challenges. They are considered as one of the most promising therapeutics, due to their ability to specifically recognise antigens and interact

with receptors on different effector cells in the immune system to orchestrate immune response. Understanding the structural assembly of antibodies is important to dissect the mechanisms of immune response and improve the design of therapeutic biologics. Antibodies are typically depicted as Y-shaped protein assemblies of two heavy (H) and two light (L) chains. Two H-L pairs form the variable (V) region, which contains antigen-binding surfaces specific to an antigen [1] (Figure 1A). The rest of the molecule forms the constant (C) region, harbouring binding sites for receptors and a number of features which govern the flexibility and integrity of this protein assembly: (i) disulphide bonds bind disparate H and L chains together to form the assembly; (ii) some antibody isotypes contain a long, flexible hinge linking the antigen binding fragment (Fab) and the crystalisable fragment (Fc), and allow large movements of the antibody arms; (iii) post-translational modifications (PTM), such as glycosylation, can be found decorating the antibody molecule, adding further complexity to study these protein assemblies. In humans, there are a total of nine isotypes, each with variable hinge lengths (or an extra immunoglobulin domain in place of the hinge), disulphide bond and glycosylation patterns (Figure 1B). An additional tailpiece allows these assemblies to be anchored on cell surfaces in the form of B cell receptors (BCR). B cells can switch between isotypes, in a process called Class-Switch Recombination (CSR), to adapt themselves in different biological contexts (reviewed in [2]).

A large number of structural studies is dedicated to antibodies, but we are only beginning to address and understand the principles of assembly and functional stability of full-length antibody structures. The solution of complex structures covering both Fab and Fc is important to understand the multifunctional properties of antibody assemblies: techniques such as cryo-electron microscopy (cryo-EM) are beginning to fill in the gap for a complete picture of these large macromolecular assemblies (Table 1). Existing full-length antibody structures capture a variety of complex assemblies that antibody can form, at times coupled with other proteins, one example being the joining chain (encoded by the gene *JCHAIN*) which binds monomers of IgM and IgA to form secretory antibodies [27]. These high-order assemblies (e.g. secretory IgM are typically pentameric, comprising a total of 20 antibody protein chains and 1 joining chain) are complex, and pose significant challenges to computational approaches such as AlphaFold-Multimer [28]. Currently, the available 3D structural information is still heavily skewed to-

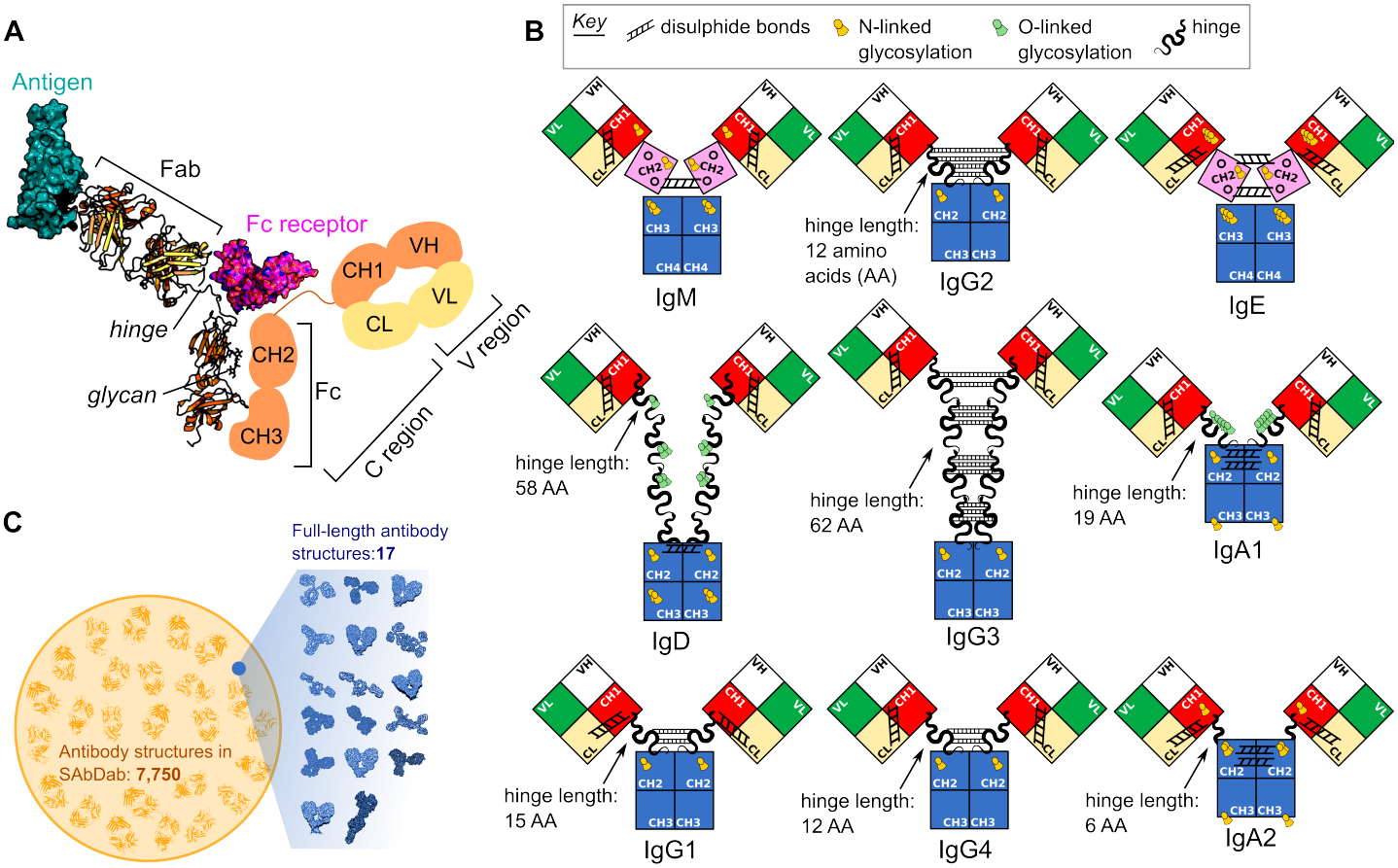

Figure 1: Human antibody structures. (A) Schematic of human IgG1 in complex with the antigen (SARS-CoV-2 Spike Receptor Binding Domain [RBD] [PDB 6zlr] illustrated here as an example) and the Fc receptor FcγRIII-B (PDB 1t83). One half of the full-length IgG1 was depicted with cartoon illustration (PDB 1hzh) and another half was depicted with a simplified domain illustration. (B) Schematic of all nine human isotypes with labels of the hinge length [3], number of inter-chain disulphide bonds [4, 5, 6, 7, 8] and glycosylation sites [9, 10, 11, 12]. (C) The number of human antibody structures available in the SAbDab database [13] (accessed 5 October, 2023) and the number of full-length human antibody structures available on the Protein Data Bank (PDB, accessed 5 October, 2023).

PDB ID	Description	Species	Isotype	Experimental methods	Resolution	Publication
1mco	An antibody harbouring hinge deletion	Human	IgG1	X-ray crystallography	3.20 Å	[14]
1iga	X-ray scattering analysis of serum IgA1	Human	IgA1	Solution scattering	N/A	[15]
1hzh	Antibody recognising HIV-1 gp120	Human	IgG1	X-ray crystallography	2.70 Å	[16]
1r70	Recombinant hapten-binding monomeric IgA2	Human	IgA2	Solution scattering	N/A	[17]
2esg	IgA1 complexed with human serum albumin	Human	IgA1	Solution scattering	N/A	[18]
2qtj	Dimeric IgA1 isolated from myeloma serum	Human	IgA1	Solution scattering	N/A	[19]
3chn	Secretory IgA1 isolated from colostrum samples	Human	IgA1	Solution scattering	N/A	[20]
3cm9	Secretory IgA2 isolated from colostrum samples	Human	IgA2	Solution scattering	N/A	[21]
5dk3	Pembrolizumab structure	Human	IgG4	X-ray crystallography	2.28 Å	[22]
6gfe	Therapeutic anti-NPRA antibody	Human	IgG4	X-ray crystallography	1.80 Å	[23]
7xq8	IgM BCR with Fab from HIV-1 neutralising antibody VRC-01	Human	IgM	cryo-EM	3.30 Å	[24]
8ady, 8adz, 8ae0, 8ae3, 8ae2	Human IgM of different conformations	Human	IgM	cryo-EM	5.20 Å, 6.70 Å, 7.10 Å, 6.80 Å, 8.50 Å	[25]
1igt	Anti-canine lymphoma monoclonal antibody	Mouse	IgG2a	X-ray crystallography	2.80 Å	[26]

Table 1: Current available full-length antibody structures available in the Protein Data Bank (PDB). Human structures are organised in chronological order of the associated publications. HIV-1, human immunodeficiency virus-1; NPRA, natriuretic peptide receptor A; BCR, B cell receptor; cryo-EM, cryo-electron microscopy.

wards Fab structures (Figure 1C). Given the advancement of single-cell sequencing methods to generate antibody sequence data comprising both the V and C regions [29], full-length sequences are increasingly accessible in antibody discovery programs and basic research in B cell immune response *in vivo*. These data motivate antibody structural studies beyond the V region, to investigate the structural and functional implication of biological processes such as CSR in changing the antibody C region.

Nowadays, a wide array of approaches can complement experimental structure determination under the umbrella of integrative structural biology. In this review, we survey existing efforts to apply and adapt such approaches to study full-length antibody structures. The inherent conformational flexibility of the Fab arms is one of the major factors posing challenges to accurate structure determination and modelling of antibody assemblies. Following the rise of deep learning models such as AlphaFold2 [30] in generating accurate models of protein structures, we discuss how these techniques, in combination with molecular simulations, can be integrated with experimental approaches to probe the structural landscape of antibodies and understand the principles of antibody assembly.

2. Conformational flexibility of antibodies

In the last decade, thanks to the increased availability of single particle electron microscopy, the vastness of the possible conformational landscapes of antibodies has been addressed [23, 25, 31]. Relative orientation of the subdomains is guided by the flexibility, which is affected by the hinge length and the number of disulphide bonds in the same region (Figure 1). As these are the main discriminators of IgG subclasses, different isotypes are characterized by varying conformational minima, speculated to correlate with their biological functions [32]. Considering the flexibility of the Fab arms [33], the presence of two additional disulphide bonds in the hinge makes IgG2 the most rigid among IgG isotypes. Although IgG4 has the same number of disulphide bonds, the slightly shorter hinge (12 residues vs 15) with respect to IgG1, leads to reduced inter-domain flexibility [23, 32]. Interestingly, the flexibility order (IgG3 > IgG1 > IgG4 > IgG2) seems to reflect quite well the ability to bind the receptor FcγR [32, 33]. IgG4 and IgG2 are generally less potent [32]; IgG2, in particular, has lost the ability to simultaneously bind two antigens [34, 35]. IgG3, containing the longest hinge, was proposed

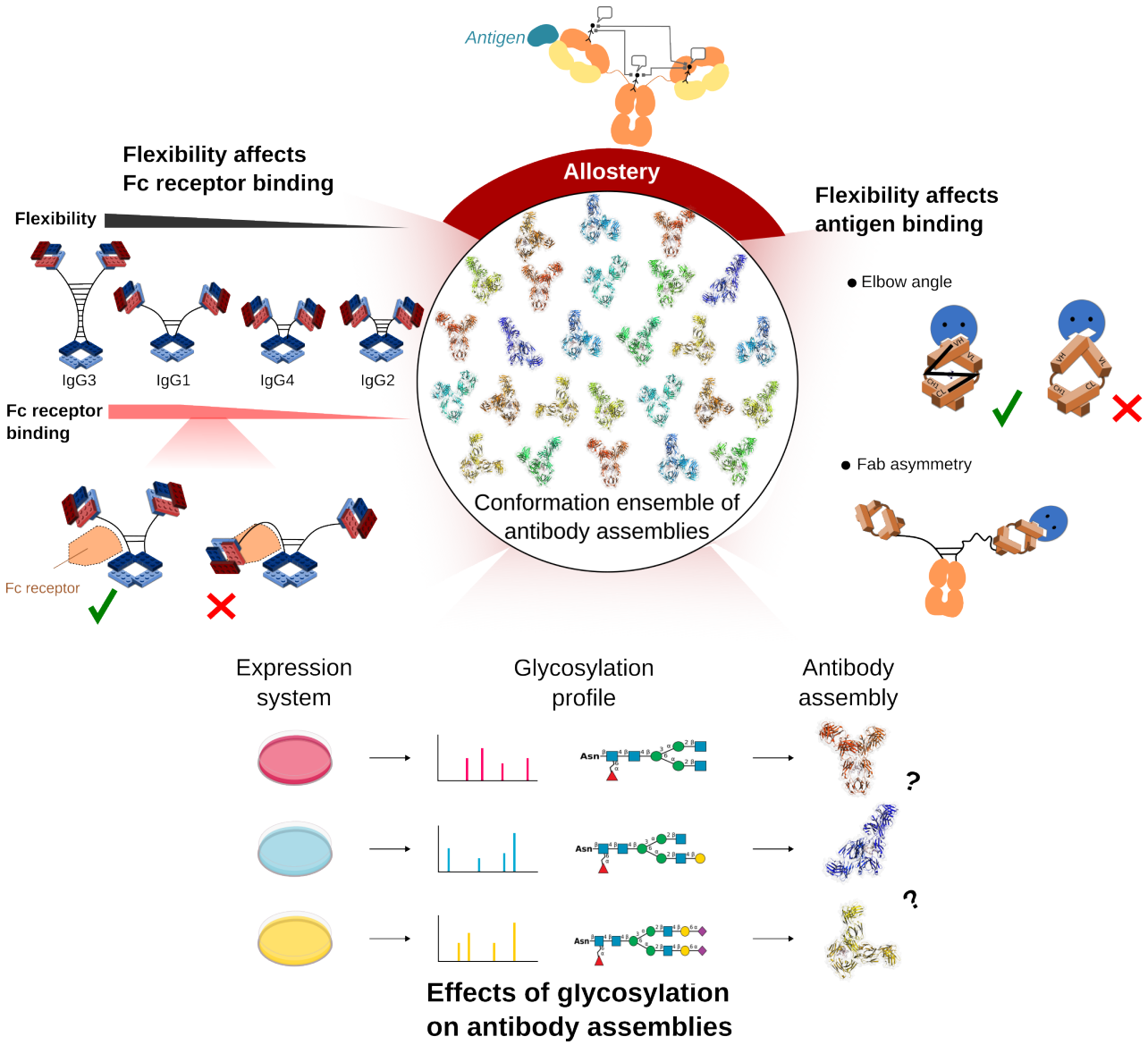

Figure 2: Schematic summary of the role of inherent flexibility and glycosylation in defining conformational ensembles of antibody assemblies.

to allow binding to antigens less targetable by other isotypes [32]. A longer hinge was also shown to improve antibody-mediated effector functions [36]. Furthermore, the amino acid composition in the N-terminal end of the hinge has been shown to affect Fc flexibility, in terms of the extent of CH2-CH2 bending, as well as exhibiting different motions, which may selectively disrupt the binding to FcγR receptor [37].

Binding to Fc receptors is further modulated by post-translational modifications (PTMs) such as glycosylation. Glycans covalently linked to the IgG CH2 domain (position Asn297, EU numbering) are known to affect Fc stability and modulate the interaction with FcγR and FcN receptors, via specific glycan-glycan interactions [34, 38, 39]. Thus, modifications of even a single sugar unit can affect and alter Fc protein binding [40]. Even though N-linked glycosylation sites are fairly conserved among the isotypes, the diversity of glycan structures can lead to 36 functional states (for IgG1), depending on the expression systems used (Figure 2), which differ in terms of enzyme expression patterns [41]. Glycosylation can also appear elsewhere in the antibody: it is worth noting that IgG4 Fabs tend to be more glycosylated while IgG3 shows an unique O-linked glycosylation pattern in the hinge region, a feature shared with human IgD and IgA1 [32, 42]. Differences in glycosylation states were reflected in distinct conformational minima being sampled in molecular dynamics simulations [38, 43]. Whether Fab or hinge glycosylation can alter the structure and biological functions is still under investigation. Fab glycosylation may reduce antibody half-life, due to steric clashes in FcR binding, while hinge O-linked glycosylation could improve resistance towards proteolysis [32, 42].

For the Fab, flexibility can conceivably affect antigen binding. The mutual orientation of the two Fab arms may affect the shape of the paratope, i.e. the antigen binding site. The six hyper-variable loops composing the paratope are characterized by conformational rearrangement in the microsecond-to-millisecond timescale while the elbow angle (angle between variable and constant Fab regions, Figure 2), the VH-VL and CH1-CL motions is in the low nanosecond timescale [44], with a higher degree of freedom in λ rather than κ L chains [45]. Recent *in silico* mutagenesis data highlighted VH-VL angle sensitivity, and the consequential change in solvent-accessible surface area even at distal positions of the structure [46]. Bound and unbound Fab states are characterized by different patterns of interaction. Upon antigen bind-

ing, an allosteric signal propagates across L and H chains and reaches the CH1 domain [47, 48]. Mutations distal to the antigen-binding site (e.g.,in the VH-VL interface) can influence binding by modulating the interdomain conformations as well as by allosterically perturbing the paratope [49]. The hinge adds further to the effects of flexibility on antigen binding: interestingly, it has been recently proposed to act not just a flexible linker but to be able to take part in intradomain/intrachain communication [50], raising the possibility of stabilising antigen binding through mutations beyond the Fab. Hinge disulphide cross-over was shown to enhance receptor binding in agonistic antibodies [51]. Furthermore, due to the nature of the hinge, the two Fabs are now recognised to be asymmetrical in terms of intradomain geometries and motions [52, 53]. A comparison of the dynamics of full-length IgG1 (PDB 1hzh) with one of its Fab in isolation showed that Fabs internal motions are negligible with respect to Fabs moieties fluctuations in the full structure [52], suggesting overall dynamical communication via the hinge and the constant region.

It is thus important to understand how hinge flexibility can be controlled to fine-tune antibody function. It has been shown via mutagenesis experiment and treatment with thioredoxin that enhanced flexibility of IgG1, particularly in the hinge region, could lead to reduction of Fc-mediated activity. The authors suggests that the enhanced flexibility of Fabs can limit interactions by steric hinderance of Fc-FcR interactions [54]. In light of the role of Fabs in Fc-FcR interactions, enhanced hinge flexibility could also allow the Fabs to move far away from the Fc, abrogating antibody-dependent cellular cytotoxicity (ADCC). Furthermore, increase in flexibility will also result in an increased exposure of buried proteolysis binding site and PTMs hotspot leading to aggregation and degradation [38, 54].

Recently, biophysical experiments using Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS), Surface Plasmon Resonance (SPR), Hydroxyl Radical Footprint (HRF-MS) and high-speed Atomic Force Microscopy (AFM) proved that ADCC, through FcγIIIR-IgG1 interactions, is stronger when full-length antibodies were used in the experiments, in comparison to Fc-only constructs. 3D structural modelling based on these experimental results corroborated the hypothesis of the need of the Fab to improve binding of FcγR, through which ADCC is exerted [55, 56]. Consequently, this limits the relative orientation between Fc and Fab to accommodate FcγR. Such conforma-

tions seem to be preferred if the antibody is bound to the antigen, suggesting allosteric conformational cooperativity between Fab and Fc [57, 58].

3. Modelling the assembly of antibodies

The sheer complexity and the highly flexible nature of antibodies ~~calls~~ call for specialised methods and considerations in modelling these protein assemblies. ~~AlphaFold2 [30] and AlphaFold-multimer[28] were designed for general-use protein structural modelling. It has been shown that AlphaFold2 is not aware of antibody-specific structural features, such as the conformation of CDR loops [59]. Several~~ Antibody-specific pipelines (ABodyBuilder [60], RosettaAntibody [61], RepertoireBuilder [62], etc.) have made significant progress in improving the prediction of V region using structural modelling. With respect to the more challenging task of modelling CDR3 loops, comparison of those tools against AlphaFold2 [30] and AlphaFold-multimer[28], which were designed for general-use protein structural modelling, suggested that the AlphaFold methods achieved comparable performance for antibodies and nanobodies [59]. The advent of deep learning techniques further improved V region modelling: for example, ABodyBuilder2 [63] was trained with antibody structures using a similar architecture as AlphaFold2. A more comprehensive benchmark will ascertain the differences between these methods in modelling antibodies. Recent advancements in antibody language models represent another attempt to improve antibody structural modelling; specifically, these models often return attention scores, which denote relationships between residues in the input sequence that can be interpreted as spatial proximity in antibody structures. IgFold [59] was built upon the antibody language model AntiBERTy [64] and incorporated structural templates to capture correct location of residues during structural modelling.

These approaches focused nevertheless on modelling the V region, and better methods are still needed to study and model full-length antibody structures, taking into account antibody-specific considerations. A number of recent methods aim to model the structures of large protein complexes, for example by first dividing the complex into subunits, and then re-assemble models of these subcomponents using Monte Carlo tree search methods [65]. Elsewhere, AlphaFold-multimer [28] has made encouraging progresses on multimer assembly, and thus could hold promise in modelling large antibody assemblies, but problems still exist on inter-domain orientation [66]. This is a well-recognised issue in antibody structural modelling,

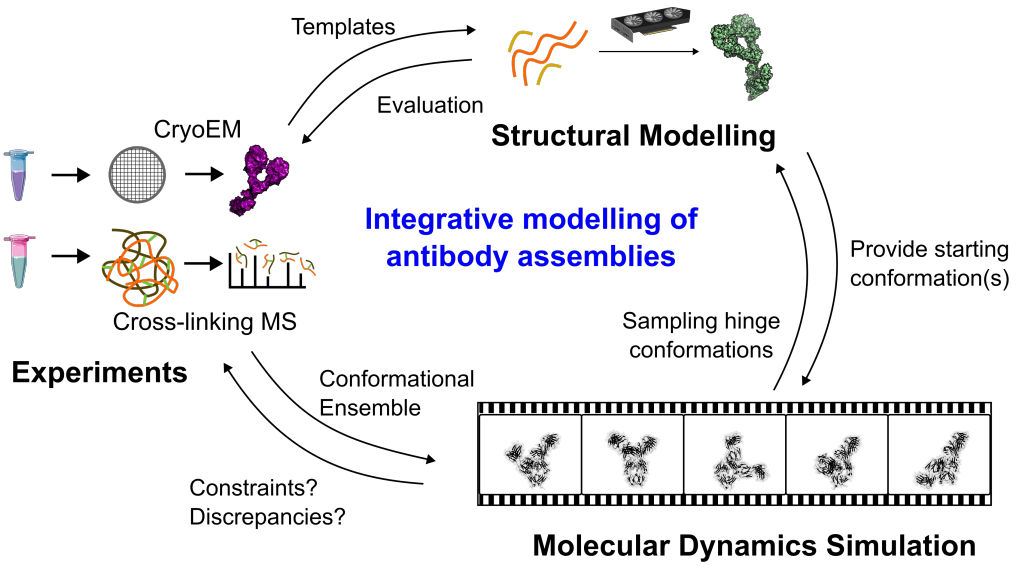

Figure 3: Integrative modelling approach, combining experimental data and computational modelling, can be adopted to model the flexibility of antibody assemblies. See the main text for further details.

mostly on specifying the angle between the VH and VL domains, as this has been shown to be an important factor affecting the geometry of the antigen-binding pocket [67]. Similarly, the positioning and packing angles of the C domains should be considered during the modelling of the complete antibody assembly. Here, the presence of the hinge for some isotypes, and the diversity in its length, further complicates the assembly of immunoglobulin domains during modelling using tools such as AlphaFold. This is most notable for human IgG3, which contains a hinge of over 60 residues, representing a major challenge to predict domain orientation in full-length antibody structures.

4. Future direction

Recently, successes in the solution of intact antibody structures have been reported [24, 25], though some of them still miss parts of the full-length antibody assembly [68], illustrating existing experimental difficulties in acquiring the intact antibody structure. Computational routes offer an alternative which complements experimental studies. Integrative modelling combining experimental data and computational methods hold the promise of consid-

ering the conformational flexibility of antibodies whilst incorporating experimental measurements to constrain computational predictions (Figure 3). For example, cross-linking mass spectrometry provides distance restraints between protein domains [69], and methods such as cryo-EM captures diverse structural snapshots indicating various conformations of large protein assemblies [70]. Such experimental information can serve either as structural templates or as ground-truths for evaluating the predicted models. Here, considering the inherent flexibility of antibodies, it remains a question on how best to evaluate models of antibody structures. Traditionally, models are compared against X-ray crystal structures. Modelling antibody structures as an ensemble of possible conformations will perhaps reflect better the diversity of conformations that antibodies can adopt.

3D structural modelling, using either (or a combination of) homology modelling or deep-learning approaches, can model full-length antibody structures. Here, different conformations provided by these modelling pipelines can serve as different starting conformations for molecular dynamics simulations. Recent works have been taken to explore multiple conformational states of predicted protein models by modifying the multiple sequence alignment (MSA) input of AlphaFold [71]. Simulations using different starting conformations allow a more extensive sampling of possible conformational states, as done in a number of simulation studies [52, 57, 72]. In return, the inter-domain conformation sampled in MD simulation facilitates the modelling of the flexible linker between domains (such as hinge) in antibody structural modelling process. Again, experimental data provide constraints to restrict the sampling of conformations to reflect *in vitro* behaviours of the antibody; on the other hand, insights from simulations can inform further experimental investigations to deeply characterise the dynamical behaviours of antibodies (Figure 3). Such integrative modelling paradigm shows promise in modelling other large protein complexes [73]; on modelling antibodies, collaborative efforts between computational modellers and various experimental communities will facilitate the adoption of these combinations of techniques to map the complexity of the antibody structural landscape. Not only will this enable rational design of therapeutic antibodies to optimise their function, efficient modelling of antibody structures beyond the V region will enable studies on the structural implication of biological processes such as CSR in naturally occurring antibody response *in vivo*.

References

[1] P. B. P. S. Reis, G. P. Barletta, L. Gagliardi, S. Fortuna, M. A. Soler, W. Rocchia, Antibody-Antigen Binding Interface Analysis in the Big Data Era, Frontiers in Molecular Biosciences 9 (2022) 945808. doi:10.3389/fmolb.2022.945808.

[2] Z. Xu, H. Zan, E. J. Pone, T. Mai, P. Casali, Immunoglobulin class-switch DNA recombination: induction, targeting and beyond, Nature Reviews. Immunology 12 (2012) 517–531. doi:10.1038/nri3216.

[3] M.-P. Lefranc, G. Lefranc, The immunoglobulin factsbook, Factsbook series, Academic Press, San Diego, 2001.

[4] H. Liu, K. May, Disulfide bond structures of IgG molecules, mAbs 4 (2012) 17–23. doi:10.4161/mabs.4.1.18347.

[5] F. Samsudin, J. Y. Yeo, S. K.-E. Gan, P. J. Bond, The structural basis for distinct binding avidity of Pertuzumab and Trastuzumab IgM towards HER2, 2019. doi:10.1101/709923.

[6] B. A. Helm, Y. Ling, C. Teale, E. A. Padlan, M. Brüggemann, The nature and importance of the inter-ϵ chain disulfide bonds in human IgE, European Journal of Immunology 21 (1991) 1543–1548. doi:10.1002/eji.1830210631.

[7] P. de Sousa-Pereira, J. M. Woof, IgA: Structure, Function, and Developability, Antibodies 8 (2019) 57. doi:10.3390/antib8040057.

[8] L. C. Lin, F. W. Putnam, Structural studies of human IgD: isolation by a two-step purification procedure and characterization by chemical and enzymatic fragmentation, Proceedings of the National Academy of Sciences of the United States of America 76 (1979) 6572–6576. doi:10.1073/pnas.76.12.6572.

[9] J. M. Woof, M. W. Russell, Structure and function relationships in IgA, Mucosal Immunology 4 (2011) 590–597. doi:10.1038/mi.2011.39, number: 6 Publisher: Nature Publishing Group.

[10] J. N. Arnold, C. M. Radcliffe, M. R. Wormald, L. Royle, D. J. Harvey, M. Crispin, R. A. Dwek, R. B. Sim, P. M. Rudd, The

Glycosylation of Human Serum IgD and IgE and the Accessibility of Identified Oligomannose Structures for Interaction with Mannan-Binding Lectin, The Journal of Immunology 173 (2004) 6831–6840. doi:10.4049/jimmunol.173.11.6831.

[11] J. N. Arnold, M. R. Wormald, D. M. Suter, C. M. Radcliffe, D. J. Harvey, R. A. Dwek, P. M. Rudd, R. B. Sim, Human Serum IgM Glycosylation: Identification of Glycoforms that can Bind to Mannan-binding Lectin*, Journal of Biological Chemistry 280 (2005) 29080–29087. doi:10.1074/jbc.M504528200.

[12] K.-T. Shade, M. E. Conroy, R. M. Anthony, IgE Glycosylation in Health and Disease, Current topics in microbiology and immunology 423 (2019) 77–93. doi:10.1007/82_2019_151.

[13] J. Dunbar, K. Krawczyk, J. Leem, T. Baker, A. Fuchs, G. Georges, J. Shi, C. M. Deane, SAbDab: the structural antibody database, Nucleic Acids Research 42 (2014) D1140–1146. doi:10.1093/nar/gkt1043.

[14] L. W. Guddat, J. N. Herron, A. B. Edmundson, Three-dimensional structure of a human immunoglobulin with a hinge deletion., Proceedings of the National Academy of Sciences 90 (1993). doi:10.1073/pnas.90.9.4271.

[15] M. K. Boehm, J. M. Woof, M. A. Kerr, S. J. Perkins, The Fab and Fc fragments of IgA1 exhibit a different arrangement from that in IgG: a study by X-ray and neutron solution scattering and homology modelling, Journal of Molecular Biology 286 (1999) 1421–1447. doi:10.1006/jmbi.1998.2556.

[16] E. O. Saphire, P. W. H. I. Parren, R. Pantophlet, M. B. Zwick, G. M. Morris, P. M. Rudd, R. A. Dwek, R. L. Stanfield, D. R. Burton, I. A. Wilson, Crystal Structure of a Neutralizing Human IgG Against HIV-1: A Template for Vaccine Design, Science 293 (2001). doi:10.1126/science.1061692.

[17] P. B. Furtado, P. W. Whitty, A. Robertson, J. T. Eaton, A. Almogren, M. A. Kerr, J. M. Woof, S. J. Perkins, Solution Structure Determination of Monomeric Human IgA2 by X-ray and Neutron Scattering,

Analytical Ultracentrifugation and Constrained Modelling: A Comparison with Monomeric Human IgA1, Journal of Molecular Biology 338 (2004) 921–941. doi:10.1016/j.jmb.2004.03.007.

[18] A. Almogren, P. B. Furtado, Z. Sun, S. J. Perkins, M. A. Kerr, Purification, Properties and Extended Solution Structure of the Complex Formed between Human Immunoglobulin A1 and Human Serum Albumin by Scattering and Ultracentrifugation, Journal of Molecular Biology 356 (2006) 413–431. doi:10.1016/j.jmb.2005.11.060.

[19] A. Bonner, P. B. Furtado, A. Almogren, M. A. Kerr, S. J. Perkins, Implications of the Near-Planar Solution Structure of Human Myeloma Dimeric IgA1 for Mucosal Immunity and IgA Nephropathy1, The Journal of Immunology 180 (2008) 1008–1018. doi:10.4049/jimmunol.180.2.1008.

[20] A. Bonner, A. Almogren, P. B. Furtado, M. A. Kerr, S. J. Perkins, Location of secretory component on the Fc edge of dimeric IgA1 reveals insight into the role of secretory IgA1 in mucosal immunity, Mucosal Immunology 2 (2009). doi:10.1038/mi.2008.68.

[21] A. Bonner, A. Almogren, P. B. Furtado, M. A. Kerr, S. J. Perkins, The Nonplanar Secretory IgA2 and Near Planar Secretory IgA1 Solution Structures Rationalize Their Different Mucosal Immune Responses, Journal of Biological Chemistry 284 (2009) 5077–5087. doi:10.1074/jbc.M807529200.

[22] G. Scapin, X. Yang, W. W. Prosise, M. McCoy, P. Reichert, J. M. Johnston, R. S. Kashi, C. Strickland, Structure of full-length human anti-PD1 therapeutic IgG4 antibody pembrolizumab, Nature Structural & Molecular Biology 22 (2015) 953–958. doi:10.1038/nsmb.3129.

[23] M. Blech, S. Hörer, A. B. Kuhn, S. Kube, H. Göddeke, H. Kiefer, Y. Zang, Y. Alber, S. M. Kast, M. Westermann, M. D. Tully, L. V. Schäfer, P. Garidel, Structure of a Therapeutic Full-Length Anti-NPRA IgG4 Antibody: Dissecting Conformational Diversity, Biophysical Journal 116 (2019). doi:10.1016/j.bpj.2019.03.036.

[24] Q. Su, M. Chen, Y. Shi, X. Zhang, G. Huang, B. Huang, D. Liu, Z. Liu, Y. Shi, Cryo-EM structure of the human IgM B cell receptor, Science 377 (2022). doi:10.1126/science.abo3923.

[25] Q. Chen, R. Menon, L. J. Calder, P. Tolar, P. B. Rosenthal, Cryomicroscopy reveals the structural basis for a flexible hinge motion in the immunoglobulin M pentamer, Nature Communications 13 (2022) 6314. doi:10.1038/s41467-022-34090-2,
, **A cryo-EM study describing various conformations of the full-length IgM structure.

[26] L. J. Harris, S. B. Larson, K. W. Hasel, A. McPherson, Refined Structure of an Intact IgG2a Monoclonal Antibody,, Biochemistry 36 (1997). doi:10.1021/bi962514+.

[27] V. Sørensen, I. B. Rasmussen, V. Sundvold, T. E. Michaelsen, I. Sandlie, Structural requirements for incorporation of J chain into human IgM and IgA, International Immunology 12 (2000) 19–27. doi:10.1093/intimm/12.1.19.

[28] R. Evans, M. O'Neill, A. Pritzel, N. Antropova, A. Senior, T. Green, A. Žídek, R. Bates, S. Blackwell, J. Yim, O. Ronneberger, S. Bodenstein, M. Zielinski, A. Bridgland, A. Potapenko, A. Cowie, K. Tunyasuvunakool, R. Jain, E. Clancy, P. Kohli, J. Jumper, D. Hassabis, Protein complex prediction with AlphaFold-Multimer, 2022. doi:10.1101/2021.10.04.463034.

[29] A. Agrafiotis, D. Neumeier, K.-L. Hong, T. Chowdhury, R. Ehling, R. Kuhn, I. Sandu, V. Kreiner, T.-S. Cotet, D. Shlesinger, D. Laslo, S. Anzböck, D. Starkie, D. J. Lightwood, A. Oxenius, S. T. Reddy, A. Yermanos, Generation of a single-cell B cell atlas of antibody repertoires and transcriptomes to identify signatures associated with antigen specificity, iScience 26 (2023) 106055. doi:10.1016/j.isci.2023.106055.

[30] J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Žídek, A. Potapenko, A. Bridgland, C. Meyer, S. A. A. Kohl, A. J. Ballard, A. Cowie, B. Romera-Paredes, S. Nikolov, R. Jain, J. Adler, T. Back, S. Petersen, D. Reiman, E. Clancy, M. Zielinski, M. Steinegger, M. Pacholska, T. Berghammer, S. Bodenstein, D. Silver, O. Vinyals, A. W. Senior, K. Kavukcuoglu, P. Kohli, D. Hassabis, Highly accurate protein structure prediction with AlphaFold, Nature 596 (2021). doi:10.1038/s41586-021-03819-2.

[31] R. K. Jensen, F. Jabs, M. Miehe, B. Mølgaard, W. Pfützner, C. Möbs, E. Spillner, G. R. Andersen, Structure of intact IgE and the mechanism of ligelizumab revealed by electron microscopy, Allergy 75 (2020) 1956–1965. doi:10.1111/all.14222.

[32] T. H. Chu, E. F. Patz, M. E. Ackerman, Coming together at the hinges: Therapeutic prospects of IgG3, mAbs 13 (2021) 1882028. doi:10.1080/19420862.2021.1882028.

[33] K. Hansen, A. M. Lau, K. Giles, J. M. McDonnell, W. B. Struwe, B. J. Sutton, A. Politis, A Mass-Spectrometry-Based Modelling Workflow for Accurate Prediction of IgG Antibody Conformations in the Gas Phase, Angewandte Chemie 57 (2018) 17194–17199. doi:10.1002/anie.201812018.

[34] D. Yang, R. Kroe-Barrett, S. Singh, C. J. Roberts, T. M. Laue, IgG cooperativity - Is there allostery? Implications for antibody functions and therapeutic antibody development, mAbs 9 (2017) 1231–1252. doi:10.1080/19420862.2017.1367074.

[35] C. D. Michele, P. D. L. Rios, G. Foffi, F. Piazza, Simulation and Theory of Antibody Binding to Crowded Antigen-Covered Surfaces, PLOS Computational Biology 12 (2016). doi:10.1371/journal.pcbi.1004752.

[36] T. H. Chu, A. R. Crowley, I. Backes, C. Chang, M. Tay, T. Broge, M. Tuyishime, G. Ferrari, M. S. Seaman, S. I. Richardson, G. D. Tomaras, G. Alter, D. Leib, M. E. Ackerman, Hinge length contributes to the phagocytic activity of HIV-specific IgG1 and IgG3 antibodies, PLoS pathogens 16 (2020) e1008083. doi:10.1371/journal.ppat.1008083.

[37] S. G. Remesh, A. A. Armstrong, A. D. Mahan, J. Luo, M. Hammel, Conformational Plasticity of the Immunoglobulin Fc Domain in Solution, Structure 26 (2018) 1007–1014.e2. doi:10.1016/j.str.2018.03.017.

[38] D. S. Tomar, G. Licari, J. Bauer, S. K. Singh, L. Li, S. Kumar, Stress-dependent Flexibility of a Full-length Human Monoclonal Antibody: Insights from Molecular Dynamics to Support Biopharmaceutical Development, Journal of Pharmaceutical Sciences 111 (2022) 628–637. doi:10.1016/j.xphs.2021.10.039.

[39] S. Yanaka, R. Yogo, K. Kato, Biophysical characterization of dynamic structures of immunoglobulin G, Biophysical Reviews 12 (2020) 637–645. doi:10.1007/s12551-020-00698-1.

[40] M. Aoyama, N. Hashii, W. Tsukimura, K. Osumi, A. Harazono, M. Tada, M. Kiyoshi, A. Matsuda, A. Ishii-Watabe, Effects of terminal galactose residues in mannose α1-6 arm of Fc-glycan on the effector functions of therapeutic monoclonal antibodies, mAbs 11 (2019) 826–836. doi:10.1080/19420862.2019.1608143.

[41] C. Reily, T. J. Stewart, M. B. Renfrow, J. Novak, Glycosylation in health and disease, Nature Reviews Nephrology 15 (2019). doi:10.1038/s41581-019-0129-4.

[42] F. Nimmerjahn, G. Vidarsson, M. S. Cragg, Effect of posttranslational modifications and subclass on IgG activity: from immunity to immunotherapy, Nature Immunology (2023). doi:10.1038/s41590-023-01544-8.

[43] S. Saporiti, C. Parravicini, C. Pergola, U. Guerrini, M. Rossi, F. Centola, I. Eberini, IgG1 conformational behavior: elucidation of the N-glycosylation role via molecular dynamics, Biophysical Journal 120 (2021) 5355–5370. doi:10.1016/j.bpj.2021.10.026.

[44] M. L. Fernández-Quintero, V. J. Hoerschinger, L. M. Lamp, A. Bujotzek, G. Georges, K. R. Liedl, VH -VL interdomain dynamics observed by computer simulations and NMR, Proteins 88 (2020) 830–839. doi:10.1002/prot.25872.

[45] M. L. Fernández-Quintero, K. B. Kroell, M. C. Heiss, J. R. Loeffler, P. K. Quoika, F. Waibl, A. Bujotzek, E. Moessner, G. Georges, K. R. Liedl, Surprisingly Fast Interface and Elbow Angle Dynamics of Antigen-Binding Fragments, Frontiers in Molecular Biosciences 7 (2020) 609088. doi:10.3389/fmolb.2020.609088.

[46] E. R. Rhodes, J. G. Faris, B. M. Petersen, K. G. Sprenger, Common framework mutations impact antibody interfacial dynamics and flexibility, Frontiers in Immunology 14 (2023) 1120582. doi:10.3389/fimmu.2023.1120582.

[47] M. M. Al Qaraghuli, K. Kubiak-Ossowska, V. A. Ferro, P. A. Mulheran, Antibody-protein binding and conformational changes: identifying allosteric signalling pathways to engineer a better effector response, Scientific Reports 10 (2020) 13696. doi:10.1038/s41598-020-70680-0.

[48] D. Corrada, G. Morra, G. Colombo, Investigating Allostery in Molecular Recognition: Insights from a Computational Study of Multiple Antibody–Antigen Complexes, The Journal of Physical Chemistry B 117 (2013) 535–552. doi:10.1021/jp310753z.

[49] P. Koenig, C. V. Lee, B. T. Walters, V. Janakiraman, J. Stinson, T. W. Patapoff, G. Fuh, Mutational landscape of antibody variable domains reveals a switch modulating the interdomain conformational dynamics and antigen binding, Proceedings of the National Academy of Sciences 114 (2017) E486–E495. doi:10.1073/pnas.1613231114.

[50] T. Tarenzi, M. Rigoli, R. Potestio, Communication pathways bridge local and global conformations in an IgG4 antibody, Scientific Reports 11 (2021). doi:10.1038/s41598-021-02323-x.

[51] C. M. Orr, H. Fisher, X. Yu, C. H.-T. Chan, Y. Gao, P. J. Duriez, S. G. Booth, I. Elliott, T. Inzhelevskaya, I. Mockridge, C. A. Penfold, A. Wagner, M. J. Glennie, A. L. White, J. W. Essex, A. R. Pearson, M. S. Cragg, I. Tews, Hinge disulfides in human IgG2 CD40 antibodies modulate receptor signaling by regulation of conformation and flexibility, Science Immunology 7 (2022) eabm3723. doi:10.1126/sciimmunol.abm3723,
***, This paper presented cellular and crystallographic data to highlight the engineering potential of cross-over hinge disulphide, to improve receptor binding in agonistic mAbs.**

[52] R. Natesan, N. J. Agrawal, Non-covalent Fc-Fab interactions significantly alter internal dynamics of an IgG1 antibody, Scientific Reports 12 (2022). doi:10.1038/s41598-022-13370-3.

[53] B. D. Belviso, G. F. Mangiatordi, D. Alberga, V. Mangini, B. Carrozzini, R. Caliandro, Structural Characterization of the Full-Length Anti-CD20 Antibody Rituximab, Frontiers in Molecular Biosciences 9 (2022) 823174. doi:10.3389/fmolb.2022.823174.

[54] S. A. Gurjar, J. X. Wheeler, M. Wadhwa, R. Thorpe, I. Kimber, J. P. Derrick, R. J. Dearman, C. Metcalfe, The impact of thioredoxin reduction of allosteric disulfide bonds on the therapeutic potential of monoclonal antibodies, Journal of Biological Chemistry 294 (2019) 19616–19634. doi:10.1074/jbc.RA119.010637.

[55] H. Kosuge, S. Nagatoishi, M. Kiyoshi, A. Ishii-Watabe, Y. Terao, T. Ide, K. Tsumoto, Biophysical characterization of the contribution of the fab region to the IgG-FcγRIIIa interaction, Biochemistry 62 (2023) 262–269. doi:10.1021/acs.biochem.1c00832,
***, Thermodynamics and kinetics characterization of antibody and FcγR interaction, probing that the presence of Fab domains stabilised the interaction.**

[56] Y. Sun, S. Izadi, M. Callahan, G. Deperalta, A. T. Wecksler, Antibody–receptor interactions mediate antibody-dependent cellular cytotoxicity, Journal of Biological Chemistry 297 (2021). doi:10.1016/j.jbc.2021.100826,
****, Full-length IgG1-FcγR structure-function dissection through HRF-MS and MD simulations, demonstrating the importance of Fab in FcR binding.**

[57] J. Zhao, R. Nussinov, B. Ma, Antigen binding allosterically promotes Fc receptor recognition, mAbs 11 (2019) 58–74. doi:10.1080/19420862.2018.1522178,
***, Experimentally validated MD simulations probing allosteric activation of IgG1 towards FcR binding upon antigen binding.**

[58] C. Orlandi, D. Deredge, K. Ray, N. Gohain, W. Tolbert, A. L. DeVico, P. Wintrode, M. Pazgier, G. K. Lewis, Antigen-Induced Allosteric Changes in a Human IgG1 Fc Increase Low-Affinity Fcγ Receptor Binding, Structure 28 (2020) 516–527.e5. doi:10.1016/j.str.2020.03.001.

[59] J. A. Ruffolo, L.-S. Chu, S. P. Mahajan, J. J. Gray, Fast, accurate antibody structure prediction from deep learning on massive set of natural antibodies, Nature Communications 14 (2023). doi:10.1038/s41467-023-38063-x,
****, This paper combined antibody language model, graph**

transformer, antibody structure templates and structural refinement for accurate prediction of the V region structure of antibodies.

[60] J. Leem, J. Dunbar, G. Georges, J. Shi, C. M. Deane, ABodyBuilder: Automated antibody structure prediction with data-driven accuracy estimation, mAbs 8 (2016) 1259–1268. doi:10.1080/19420862.2016.1205773.

[61] B. D. Weitzner, J. R. Jeliazkov, S. Lyskov, N. Marze, D. Kuroda, R. Frick, J. Adolf-Bryfogle, N. Biswas, R. L. Dunbrack, J. J. Gray, Modeling and docking of antibody structures with Rosetta, Nature Protocols 12 (2017) 401–416. doi:10.1038/nprot.2016.180.

[62] D. Schritt, S. Li, J. Rozewicki, K. Katoh, K. Yamashita, W. Volkmuth, G. Cavet, D. M. Standley, Repertoire Builder: high-throughput structural modeling of B and T cell receptors, Molecular Systems Design & Engineering 4 (2019). doi:10.1039/C9ME00020H.

[63] B. Abanades, W. K. Wong, F. Boyles, G. Georges, A. Bujotzek, C. M. Deane, ImmuneBuilder: Deep-Learning models for predicting the structures of immune proteins, Communications Biology 6 (2023) 575. doi:10.1038/s42003-023-04927-7.

[64] J. A. Ruffolo, J. J. Gray, J. Sulam, Deciphering antibody affinity maturation with language models and weakly supervised learning, 2021. doi:10.48550/arXiv.2112.07782.

[65] P. Bryant, G. Pozzati, W. Zhu, A. Shenoy, P. Kundrotas, A. Elofsson, Predicting the structure of large protein complexes using AlphaFold and Monte Carlo tree search, Nature Communications 13 (2022). doi:10.1038/s41467-022-33729-4,
***, This paper proposed to model large protein complexes by first modelling their sub-components, and assemble them using a Monte Carlo tree search implementation.**

[66] W. Zhu, A. Shenoy, P. Kundrotas, A. Elofsson, Evaluation of AlphaFold-Multimer prediction on multi-chain protein complexes, Bioinformatics 39 (2023) btad424. doi:10.1093/bioinformatics/btad424.

[67] J. Dunbar, A. Fuchs, J. Shi, C. M. Deane, ABangle: characterising the VH-VL orientation in antibodies, Protein engineering, design & selection: PEDS 26 (2013) 611–620. doi:10.1093/protein/gzt020.

[68] X. Ma, Y. Zhu, D. Dong, Y. Chen, S. Wang, D. Yang, Z. Ma, A. Zhang, F. Zhang, C. Guo, Z. Huang, Cryo-EM structures of two human B cell receptor isotypes, Science 377 (2022) 880–885. doi:10.1126/science.abo3828.

[69] F. J. O'Reilly, J. Rappsilber, Cross-linking mass spectrometry: methods and applications in structural, molecular and systems biology, Nature Structural & Molecular Biology 25 (2018). doi:10.1038/s41594-018-0147-0.

[70] P. Fontana, Y. Dong, X. Pi, A. B. Tong, C. W. Hecksel, L. Wang, T.-M. Fu, C. Bustamante, H. Wu, Structure of cytoplasmic ring of nuclear pore complex by integrative cryo-EM and AlphaFold, Science 376 (2022). doi:10.1126/science.abm9326.

[71] D. Sala, F. Engelberger, H. S. Mchaourab, J. Meiler, Modeling conformational states of proteins with AlphaFold, Current Opinion in Structural Biology 81 (2023) 102645. doi:10.1016/j.sbi.2023.102645.

[72] R. Natesan, N. J. Agrawal, IgG1 and IgG4 antibodies sample initial structure dependent local conformational states and exhibit non-identical Fab dynamics, Scientific Reports 13 (2023). doi:10.1038/s41598-023-32067-9.

[73] P. I. Koukos, A. M. J. J. Bonvin, Integrative Modelling of Biomolecular Complexes, Journal of Molecular Biology 432 (2020) 2861–2881. doi:10.1016/j.jmb.2019.11.009.