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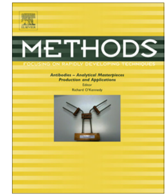

Antibodies: From novel repertoires to defining and refining the structure of biologically important targets

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ABSTRACT

Antibodies represent a highly successful class of molecules that bind a wide-range of targets in therapeutic-, diagnostic- and research-based applications. The antibody repertoire is composed of the building blocks required to develop an effective adaptive immune response against foreign insults. A number of species have developed novel genetic and structural mechanisms from which they derive these antibody repertoires, however, traditionally antibodies are isolated from human, and rodent sources. Due to their high-value therapeutic, diagnostic, biotechnological and research applications, much innovation has resulted in techniques and approaches to isolate novel antibodies. These approaches are bolstered by advances in our understanding of species immune repertoires, next generation sequencing capacity, combinatorial antibody discovery and high-throughput screening. Structural determination of antibodies and antibody-antigen complexes has proven to be pivotal to our current understanding of the immune repertoire for a range of species leading to advances in man-made libraries and fine tuning approaches to develop antibodies from immune-repertoires. Furthermore, the isolation of antibodies directed against antigens of importance in health, disease and developmental processes, has yielded a plethora of structural and functional insights. This review highlights the significant contribution of antibody-based crystallography to our understanding of adaptive immunity and its application to providing critical information on a range of human-health related indications.

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1. Introduction

Due to their exquisite specificity, tunability, stability and manufacturability, antibodies are high value therapeutic, diagnostic, biotechnological and research tools. Worldwide the pharmaceutical-, diagnostic- and research-based markets are worth US$40bn, US$8bn and US$2bn respectively [1] and are the fastest growing segment of the biologics market [2,3]. Our increased understanding of the origin, function and specificity of antibodies has resulted in a enhanced collective knowledgebase that draws observations from across multiple disciplines encompassing immuno-genetics, cellular immunology, molecular biology, structural biology and bioinformatics [1,2,4].

The IgG isotype is the most abundant form of circulating antibody in humans (Fig. 1A) and mice, it is a heterotetramer composed of two heavy and two light chains and within this molecule the antibody binding diversity is presented within the complementarity determining regions (CDR), which are 'housed' in the fragment variable (Fv) of the antibody. The light chain are primarily of λ and κ isotype, which are structurally distinct but functionally indistinguishable. In addition, two light chain isotypes have been identified all cold-blooded vertebrates (σ) and restricted to elasmobranchs (σ-cart/2) [5,6]. The frequency of light chain isotype usage differs between species with the murine repertoire composed of approximately 95% κ, while the human repertoire is composed of a 60:40 ratio of λ:κ [2]. The heavy chain gives rise to the various classes of antibody which have distinct roles in the immune system (IgG, IgD, IgE, IgA and IgM) [2]. The fragment crystallisable (Fc) region is responsible for effector functions such as interactions will cell surface receptors and proteins involved in the complement pathway. While traditional monoclonal technologies remain the mainstay of antibody generation, combinatorial antibody technologies offer avenues for more rational-approaches to isolate unique antibodies with tailored properties. More recently the use of immune repertoires, disease affected individual repertoires and man-made libraries combined with efficient *in vitro* selection methods such as phage-, ribosome-, yeast- and *E. coli*-display [1,2,7–9] have facilitated the generation of a wide range of novel antibodies with superior attributes in comparison to traditional monoclonal antibody approaches.

2. Antibody and antibody-based crystallography

The first antibody crystal structures solved in the 1970's demonstrated that the variable and constant domain structure was conserved and the term 'Ig-fold' was coined [10–12]. Subsequently, this Ig-fold has appeared as a fundamental structural building block in a wide range of proteins with diverse and distinct functions. The capacity to crystallise full-length immunoglobulins is a fortuitous process. Accordingly, the majority of structures are of the Fab fragment which can be generated by enzymatic and recombinant methods. However, the small number of available full-length Ig structures provide details on the subclasses of Ig and identify implications subtle differences have in terms of structure. These represent a snapshot of the broad range of conformations adopted by Ig and reinforce the structural flexibility of the molecule (Fig. 1B) [13]. This is particularly prudent for example, as structural information taken from the crystal structures of isolated Fc fragments may not truly reflect the scenario in the context of the full Ig molecule [14].

Traditional monoclonal technologies have predominated, however, combinatorial antibody technologies offer avenues for more rational-approaches to isolate unique antibodies with tailored properties from virtually any species [15–17]. The capacity of the immune repertoire to create molecules that possess the capability to recognise their cognate antigens and subsequently proliferate those that bind with superior characteristics to remove or neutralise the target, is a critical feature of the adaptive immune system. While antibodies are the main topic of this review, a number of alternative binding scaffolds, for example Darpins [18], have had success in crystallisation of novel proteins and are reviewed elsewhere [19].

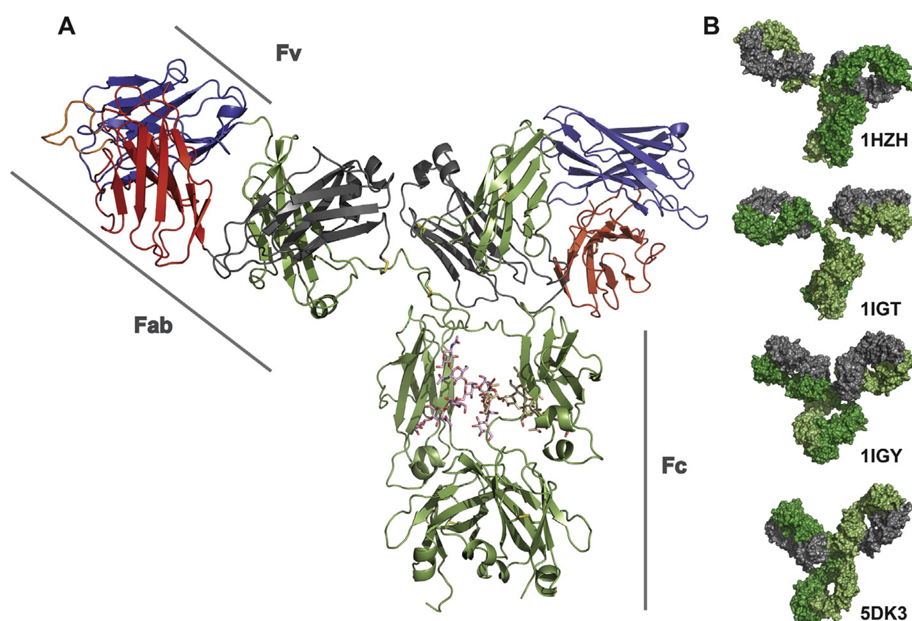

Fig. 1. Structure of a human IgG. (A) Cartoon representation of a human IgG (PDB: 1 HZH) coloured by the variable (heavy: blue, light: red) and constant (heavy: green, light: grey) domains with glycosylation shown as coloured sticks. Disulfide bonds are represented by yellow sticks. The various domains are indicated by grey bars: Fc, fragment crystallisable; Fab, Fragment antigen binding; Fv, Fragment variable. (B) Surface representation of a number of full-length Ig structures illustrating structural and conformational flexibility. The heavy chains are coloured in shades of green and the light chains in grey. Image was generated using MacPymol version 1.8.2.3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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