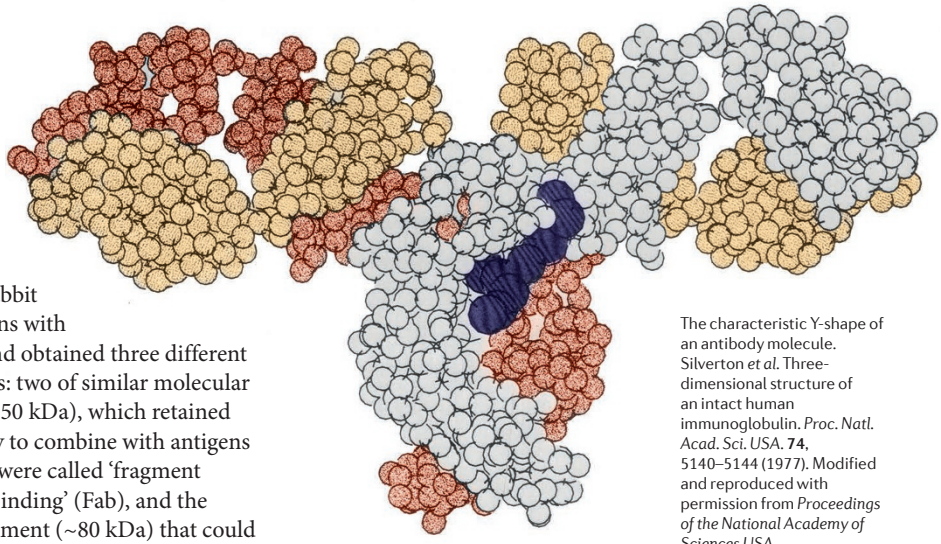
MILESTONE 7

Putting antibodies into shape



The characteristic Y-shape of an antibody molecule. Silverton *et al.* Three-dimensional structure of an intact human immunoglobulin. *Proc. Natl. Acad. Sci. USA.* **74**, 5140–5144 (1977). Modified and reproduced with permission from *Proceedings of the National Academy of Sciences USA.*

The characteristic Y shape of antibodies is arguably the most easily recognizable protein structure. Since the 1970s, X-ray crystallography and electron-microscopy studies have provided a wealth of structural details on immunoglobulins. However, the basic chemical structure of antibodies had been already determined during the previous decade, in particular thanks to the works of Rodney Porter and Gerald Edelman.

Understanding the structural basis of how antibodies could recognize millions of different antigens was the goal of immunologists since before World War II (MILESTONES 2, 10). Does each antibody have a unique three-dimensional structure, or do all antibodies share the same architecture? To tackle this problem, Edelman and Porter applied a divide-and-conquer approach: they broke immunoglobulins into smaller fragments by means of chemical or enzymatic treatment and then analyzed the physicochemical and biological properties of each fragment.

By treating immunoglobulins with reducing, alkylating and denaturing chemicals, Edelman identified two distinct chains of 20–24 kDa (light chain (L)) and 50–60 kDa (heavy chain (H)). Edelman determined that each immunoglobulin contains two identical copies each of L and H, connected by disulfide bridges to form two symmetrical L-H pairs. Each L-H pair contains one antigen-binding site formed by amino acids contributed by both L chains and H chains.

For his studies, Edelman used γ-globulins as well as Bence-Jones proteins. First described by Henry Bence Jones in 1847, the eponymous Bence-Jones proteins are immunoglobulins (dimers of L chains, as Edelman showed) found in the urine of patients with multiple myeloma that are particularly suitable for structural studies because of their high homogeneity and availability.

Porter treated rabbit γ-globulins with papain and obtained three different fragments: two of similar molecular weight (~50 kDa), which retained the ability to combine with antigens and thus were called 'fragment antigen-binding' (Fab), and the third fragment (~80 kDa) that could not bind to antigens but readily formed crystals at low temperatures and thus was named the 'fragment crystallizable' (Fc.)

Porter found that the two Fab fragments share similar chemical and biological properties and that each includes an entire L chain and the amino-terminal portion of an H chain; he thus confirmed that the antigen-binding site is formed by the pairing of an L chain and an H chain. The structurally different Fc fragment did not seem to have a particular function at first. Only later did it become clear that the Fc fragment bears the effector function of the immunoglobulin.

Notably, the antibody topologies independently proposed by Porter and Edelman were very similar and remarkably accurate. In 1969, Edelman and colleagues obtained the complete primary sequence of a human γG1 immunoglobulin. Combined with the wealth of studies published over the course of the previous decade, the sequence of γG1 allowed Edelman to provide a comprehensive depiction of the chemical structure of antibodies. Edelman's model would soon be fully validated by crystallographic analyses beginning with Fab fragments by the David R. Davies and Roberto J. Poljak laboratories and culminating in 1977 in the three-dimensional structure of an intact immunoglobulin by E.W. Silverton, Manuel Navia and Davies.

The structural basis of antibody diversity and specificity still awaited clarification. In 1970, Tai Te Wu and Elvin Kabat compared most of the amino acid sequences of L and H chains then available. It had been previously observed that the amino-terminal portions of L and H chains of different antibodies are highly variable in sequence. Wu and Kabat used statistical criteria to analyze variable and constant regions of 77 amino acid sequences of L chains. They identified three constant stretches of high variability, corresponding to amino acids 24–34, 50–56 and 89–97: the complementarity-determining regions. H chains would have corresponding highly variable regions of amino acids 31–35, 50–65 and 95–102 that also participate in forming the antigen-binding site.

Thus, the works of Edelman, Porter and others elucidated many of the key structural aspects of antibodies before crystal structures of immunoglobulins were determined. In 1972, Edelman and Porter shared the Nobel Prize in physiology or medicine "for their discoveries concerning the chemical structure of antibodies."

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