Preface

Antibodies are important tools that are used extensively in basic biomedical research, in diagnostics, and in the treatment of diseases.

Traditionally, the production of antibodies relies on the immunization of an animal. For example, for the generation of monoclonal antibodies by the hybridoma technology, usually mice and rats are preferred. For polyclonal antibody production, larger mammals (e.g., rabbits, sheep, and goats) are used as the relatively huge amount of serum that can be collected from these animals serves as a rich source for antibody purification. These antibodies are all based on an immunoglobulin scaffold and are derived from a genuine in vivo immune response. Despite their widespread applications as detection, diagnostic, and therapeutic agents, in vivo-generated polyclonal and monoclonal antibodies bear some limitations. For example, polyclonal antibodies as detection reagents are not only prone to batch-to-batch variability but also contain significant amounts of nonspecific antibodies. Furthermore, due to their inadequate characterization, it is not surprising that many experimental results that are obtained with polyclonal antibodies are often not reproducible. In contrast, hybridoma-derived monoclonal antibodies are considered to be perfectly defined reagents with unique specificities. Very often, however, they secrete additional light and/or heavy chains, which makes it cumbersome to evaluate if the binding behavior of the hybridoma-derived mAb is intrinsic to the mAb from the target B cell or due to artificial chain combinations caused by the presence of the additional chains derived from the fusion cell line. Furthermore, hybridoma cells can lose expression, are prone to mutations, and thus require frequent retesting.

The restrictions of these traditional in vivo-generated antibodies have been overcome by modern synthetic recombinant in vitro antibody technologies.

One of the most significant difference between naturally occurring and *synthetic immunoglobulins* per se is the way these two groups are generated. Naturally occurring immunoglobulins are generated in vivo by processes of V(D)J recombination and somatic hypermutation of the B cell antigen receptor during B cell development and differentiation and its secretion as soluble immunoglobulin by plasma cells. *Synthetic antibodies* on the other hand can be defined in general as affinity reagents engineered entirely in vitro, thus completely eliminating animals from the production process. (Although this definition might get blurred, e.g., by processes such as antibody humanization, which basically is the replacement of frameworks of a murine antibody generated in vivo with their human counterparts by recombinant genetic engineering in vitro. Therefore, a humanized antibody could be considered as "semisynthetic").

Synthetic affinity reagents include recombinantly produced *immunoglobulin antibodies derived from combinatorial antibody libraries* (i.e., antibody libraries built on in silico-designed and chemically defined diversity on the basis of synthetic oligonucleotides) and so-called *antibody mimetics* that are based on alternative protein/polypeptide scaffolds.

In addition, the term "*synthetic antibody*" is also often used to describe affinity reagents that are different from protein/polypeptides but share typical antibody characteristics such as diversity and specific binding affinities. For example, *aptamers* as a class of small nucleic

acid ligands are composed of RNA or single-stranded DNA oligonucleotides. Like antibodies, *aptamers* interact with their corresponding targets with high specificity and affinity.

An example of synthetic "plastic antibodies" are *molecularly imprinted polymers* (MIPs), which are polymeric matrices obtained by a technique called *molecular imprinting technology* to design artificial receptors with a predetermined selectivity and specificity for a given analyte. MIPs are able to mimic natural recognition entities, such as antibodies and biological receptors.

This volume on Synthetic Antibodies aims to present a set of protocols useful for research in the field of recombinant immunoglobulin and alternative scaffold engineering, aptamer development, and generation of MIPs. Part I includes methods that deal with amino acid-based synthetic antibodies. Brief protocols about the generation of antibody libraries are detailed, as well as techniques for antibody selection, characterization, and validation. This section is completed by a brief description of a bioinformatics platform that supports antibody engineering during Research and Development. Part II contains basic procedures about the selection and characterization of aptamer molecules, and Part III describes fundamental processes of MIP generation and application.

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