Preface

Antibodies play such a central role in research and development due to their high versatility and universal applicability that research without them would be inconceivable. Their use ranges from protein localization, cell separation, and screening to functional assays being applied in many formats including high-throughput assays. With the breakthrough in the generation of monoclonal antibodies, the practical potential of antibodies due to their almost-designer specificities was immediately recognized, especially regarding their applications in diagnostics and therapeutics. However, surprisingly it still took until the 1990s for antibodies to begin to make a substantial impact in drug development and to be applied as effective therapeutics. The industry has since grown into a multibillion-dollar market, and 34 therapeutic antibodies have been FDA approved, most of them still being on the market in 2012. Additionally, literally hundreds of antibodies are now in the development pipeline. These are being generated by a variety of platforms incorporating many technical enhancements, such as improved half-life and effector functions. This rapidly growing field is the result of many advancing technologies allowing current developments to take advantage of molecular engineering to create tailor-made antibodies. New antibody formats and scaffolds are being explored, exemplified by bispecifics and antibody drug conjugates.

This volume, *Antibody Methods and Protocols*, attempts to provide insight into the generation of antibodies using in vitro and in vivo approaches, as well as technical aspects for screening, analysis, and modification of antibodies and antibody fragments. Even though this volume covers subjects as diverse as classical methods, such as hybridoma technology and phage display, to the more recent developments including Fc engineering, it is still beyond the scope of any single volume to present the multitude of techniques now available for antibody isolation, screening, and modification. Instead, we have focused on basic protocols for isolating antibodies and, at the same time, selected a range of specific areas with the aim of providing guides for the overall process of antibody isolation and characterization as well as protocols for enhancing classical antibodies and antibody fragments. The antibody process begins with antigen generation and presentation; this is discussed in the first chapter. An overview of in vitro approaches is presented in the chapters by Ron Geyer, Dev Sidhu, Konstantin Petropoulos, Christoph Rader, Georg Thom, and Elisabetta Traggiai. These cover phage display, ribosome display, as well the use of human B cells for antibody isolation. Chapters by E-Chiang Lee, Michel Cogné, and Chonghui Zhang discuss the usefulness of mice in the development of antibodies, in particular genetically engineered mice to develop human and humanized antibodies directly in the mouse. We touch upon biophysical and biochemical characterization, affinity measurements by surface plasmon resonance, and glycosylation analysis with chapters by Michael Schräml and Christiane Jäger. Further, we have included a description of antibody fragments, cloning approaches, and modification by pegylation presented in chapters by Christoph Rader and Simona Jevševar. More recent developments in the field of antibody engineering addressing half-life extension, effector function modulation, and the rising field of bispecific antibodies, as well as approaches for antibody decoration including antibody drug conjugates, are covered in

chapters by Ulrich Brinkmann, Gloria Meng, and Michel Cogné. While we cannot address all the new and exciting developments in this fast-developing field, we believe that this volume provides a broad and useful background to support ongoing efforts and encourages the development of new imaginative approaches.

The assembly of this volume would not have happened without the commitment of all contributors, their discussions and rapid responses, making this a valuable and relevant contribution to antibody methods and protocols. We are also grateful to Dr. Michael V. Wiles for his help in editing of this volume and providing many dinners.

We like to thank Dr. John Walker for the opportunity to assemble this work and his encouragement and help throughout the process. We wish to thank also the team from Humana Press, especially David Casey, for continuous support.

We hope that this volume will provide useful insights for both experts and novices and that it will stimulate further development of antibody approaches and encourage the community to continuously share ideas and protocols.

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