## Contents

**Preface** ........................................................................................................... vi
**Contributors** ................................................................................................... xi

### PART I  OVERVIEW OF SINGLE DOMAIN ANTIBODIES

1. From Whole Monoclonal Antibodies to Single Domain Antibodies:  
   Think Small ........................................................................................................ 3  
   **Jean-Luc Teillaud**

2. Introduction to Heavy Chain Antibodies and Derived Nanobodies .......... 15  
   **Cécile Vincke and Serge Muyldeermans**

3. Overview and Discovery of IgNARs and Generation of VNARs. .......... 27  
   **Stewart D. Nuttall**

### PART II  SINGLE DOMAIN ANTIBODY LIBRARY CONSTRUCTION

4. Creation of the Large and Highly Functional Synthetic Repertoire  
   of Human VH and Vc Domain Antibodies ...................................................... 39  
   **Olga Ignatovich, Laurent Jespers, Ian M. Tomlinson,**  
   **and Ruud M.T. de Wildt**

5. Preparation of a Naive Library of Camelid Single Domain Antibodies  
   ......................................................................................................................... 65  
   **Aurelien Olichon and Ario de Marco**

### PART III  SELECTION OF SINGLE DOMAIN ANTIBODIES

6. Selection by Phage Display of Single Domain Antibodies Specific  
   to Antigens in Their Native Conformation .................................................. 81  
   **Peter Verheesen and Toon Laeremans**

7. Semiautomated Panning of Naive Camelidae Libraries and Selection  
   of Single-Domain Antibodies Against Peptide Antigens.  
   ......................................................................................................................... 105  
   **Jyothi Kumaran, C. Roger MacKenzie, and Mehdi Arbab-Ghabroudi**

8. Pichia Surface Display: A Tool for Screening Single Domain Antibodies  
   ......................................................................................................................... 125  
   **Kristof De Schutter and Nico Callewaert**

9. Bacterial Two Hybrid: A Versatile One-Step  
   Intracellular Selection Method ....................................................................... 135  
   **Mireille Pellis, Serge Muyldeermans, and Cécile Vincke**

10. Intracellular Antibody Capture (IAC) Methods  
    for Single Domain Antibodies ...................................................................... 151  
    **Tomoyuki Tanaka and Terence H. Rabbitts**
11 Selection of Functional Single Domain Antibody Fragments for Interfering with Protein-Protein Interactions Inside Cells: A “One Plasmid” Mammalian Two Hybrid System .............................. 175
Tomoyuki Tanaka and Terence H. Rabbitts

12 Cell-Free Selection of Domain Antibodies by In Vitro Compartamentalization . 183
Armin Sepp and Andrew Griffiths

13 Selection of VHVs Under Application Conditions .......................... 199
Edward Dolk, Theo Verrips, and Hans de Haard

14 Isolation and Characterization of Clostridium difficile Toxin-Specific Single Domain Antibodies .......................... 211
Greg Hussack, Mehdi Arbabi-Ghahroudi, C. Roger MacKenzie, and Jamshid Tanha

15 Selection of VH antibody Fragments That Recognize Different Aβ Depositions Using Complex Immune Libraries .......................... 241
Rinse Klooster, Kim S. Rutgers, and Silvère M. van der Maarel

PART IV EXPRESSION OF SINGLE DOMAIN ANTIBODIES AND DERIVATIVES

16 Expression of Single-Domain Antibodies in Bacterial Systems . 257
Toya Nath Baral and Mehdi Arbabi-Ghahroudi

17 Expression of VHVs in Saccharomyces cerevisiae .......................... 277
Andrea Gorlani, Hans de Haard, and Theo Verrips

18 Stable Expression of Chimeric Heavy Chain Antibodies in CHO Cells .......................... 287
Vishal Agrawal, Igor Slivac, Sylvie Perret, Louis Bisson, Gilles St-Laurent, Yanal Murad, Jianbing Zhang, and Yves Durocher

19 Production of Camel Like Antibodies in Plants .......................... 305
Sylvie De Buck, Vikram Virdi, Thomas De Meyer, Kirsten De Wilde, Robin Piron, Jonah Nolf, Els Van Lerberge, Annelies De Paepe, and Ann Depicker

PART V IMPROVEMENT AND APPLICATIONS OF SINGLE DOMAIN ANTIBODIES

20 Selecting and Purifying Autonomous Human Variable Heavy (VH) Domains .......................... 327
Raffi Tonikian and Sachdev S. Sidhu

21 Solubility and Stability Engineering of Human VH Domains .......................... 355
Dae Young Kim, Wen Ding, and Jamshid Tanha

22 Improvement of Proteolytic Stability Through In Silico Engineering .......................... 373
Lucy Rutten, Hans de Haard, and Theo Verrips

23 Selection of Human VH Single Domains with Improved Biophysical Properties by Phage Display .......................... 383
Kip Dudgeon, Romain Rouet, Kristoffer Famm, and Daniel Christ

24 Improvement of Single Domain Antibody Stability by Disulfide Bond Introduction .......................... 399
Yoshihisa Hagihara and Dirk Saeren