Preface

After the deciphering of the human genome and those of other organisms, the investigation of the function of gene products and their orchestral interplay is now one of the most important challenges in the life sciences. In this regard, specific ligands are required that allow the sensitive detection and functional assignment of gene products, favourably in the native context, which can be a model organism or at least cultured cells. Darwinian-like evolutionary methods, which enable the identification of such ligands, are described in this volume of the Humana Press Methods in Molecular Biology Series, entitled *Nucleic Acid and Peptide Aptamers*. The identified active compounds according to the protocols described in *Nucleic Acid and Peptide Aptamers* harbour information about both their active conformation and the blueprint for their own synthesis. This feature allows the simultaneous screening of up to 10^{16} different molecules in one test tube by the application of appropriate selection schemes and the rapid synthetic access to adapt the ligands for certain purposes. Selection procedures can be performed solely in vitro, allowing the most convenient control of the selection process and thus retaining control of the characteristics of the identified ligands. Target molecules can be either small compounds (or metabolites), proteins, nucleic acids or even complex targets such as living cells.

The present protocol collection covers methods related to the two major classes of molecules employed for in vitro selection procedures: Nucleic acids and peptides/proteins. The 22 chapters of *Nucleic Acid and Peptide Aptamers* highlight important methodologies in the field of evolutionary molecular biology approaches. The collection allows researchers not only to identify ligands for their target molecules but also describes protocols for the application of these ligands in certain research issues. These ligands, unless they are of nucleic acid or of peptidic nature, can act as potent inhibitors and enable the functional investigation and/or the detection of the target molecule. This volume is meant to support students, postdoctoral fellows, and senior scientist in their efforts to investigate biomolecules by using specific nucleic acid and/or peptide aptamers and offers guidelines for their identification and application.

Chapter 1 (by Ellington) describes the synthesis of nucleic acid libraries and methods to investigating their diversity. In the following Chapters 2–6 protocols for the application of different in vitro selection methods targeting distinct molecules are illustrated in detail. These protocols cover the modification of proteins with biotin, enabling access to streptavidin–biotin chemistry for the separation step during the selection process (by Höver and Mayer) and protocols for the identification of aptamers by capillary electrophoresis (by Mosing and Bowser), a method that circumvents any protein modification prior selection. Chapters 4–6 describe the selection of aptamers targeting small molecules (by Piganeau), complex targets (by Franciscis) and ribonucleic acids (by Toulmé). Protocols for the characterization of aptamers by state-of-the-art methods can be found in the Chapters 7 (by Werner and Hahn), describing the application of fluorescence correlation spectroscopy for determining the dissociation constant of an aptamer-target interaction. On the subject of structural investigations Chapters 8 and 9, by Wakeman and Winkler and Batey et al., respectively, give protocols for the application of in-line probing of RNA structures and the growth and analysis of crystals to determine the

secondary and tertiary structure of RNA molecules by X-ray crystallography. Applications of aptamers are highlighted in the Chapters 10–14. These chapters give insight how aptamers can be adapted to different assay formats, covering locked nucleic acids (by Erdmann), the application of aptamers for diagnostic purposes (by Gronewold and by Lu et al.), the use of aptamers to control gene expression (by Weigand and Suess) and as molecular probes for the identification of small molecule inhibitors of protein function with aptamer inherited properties (by Yamazaki and Famulok). The nucleic acid aptamer part is then closed by a Chapter 15 by Tavitian et al. describing protocols allowing the in vivo imaging of aptamers.

The peptide aptamer part commences with protocols that describe different methods for the identification of peptide aptamers. These chapters also include detailed explanations of the construction of suitable peptide libraries for the selection process. Chapter 16 by Arndt et al. describes the use of phage display and complementation assays for the identification of peptides that interfere with protein-protein interactions. Chapter 17 by Takahashi and Roberts introduce the mRNA display methodology and strategies based on the well-known and wide spread used yeast "two hybrid" system for the specific enrichment of peptide aptamers and ligand-regulated peptide aptamers can be found in Chapter 18 (by Miller). Lopez-Ochoa et al. give details for the high-throughput identification and characterization of peptide aptamers in Chapter 19. A recently described variant of peptide aptamers, namely Microbodies, which are embedded in a certain three-dimensional, highly stable scaffold, is introduced in Chapter 20 (by Blind). Chapters 21 and 22 illustrate how peptide aptamers can be used to identify small molecules (by Colas) and for the application of peptides as drug carriers (by Beck-Sickinger).

The protocols given herein represent a state-of-the-art collection of methodologies for the isolation, characterization and application of both peptide and nucleic acid aptamers and will allow researchers to apply these compounds to address distinct research issues.

Günter Mayer, PhD.

http://www.springer.com/978-1-934115-89-3

Nucleic Acid and Peptide Aptamers
Methods and Protocols
Mayer, G. (Ed.)
2009, XI, 408 p., Hardcover
ISBN: 978-1-934115-89-3
A product of Humana Press