Edited by
Sven Klussmann

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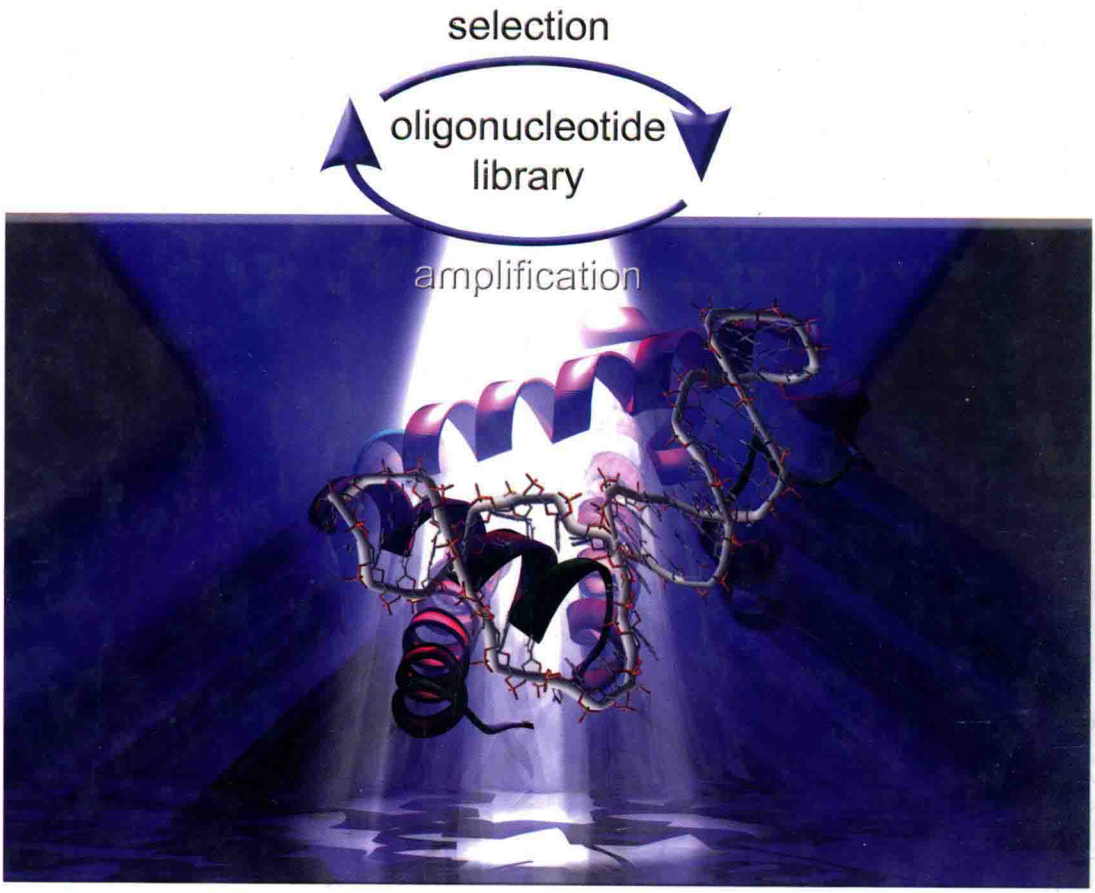
The Aptamer Handbook

Functional Oligonucleotides and Their Applications



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Preface

Why a book about aptamers? This question may be raised by all readers who are already familiar with aptamers, since quite a few reviews of outstanding quality have been published in the scientific literature. But the very same question may be asked by any reader who is – although scientifically interested – not familiar with aptamers at all. When I was asked by the publisher whether I would be interested in editing a book on aptamers I asked myself a different question: Is it really true that there is not a single textbook on aptamers so far – more than 15 years after they had first been described? Indeed, I realized that this was the case! Although the interested reader will find more than 110 000 hits when typing the term "aptamer" into the internet search machine Google, although hundreds and hundreds of publications are listed in the scientific databases, although many patents have been filed and granted, although several companies worked and still work on and with aptamers, and although last but not least a viable aptamer-based drug (Macugen) has been clinically developed, entered the market, and now helps many patients to manage the devastating disease of age-related macular degeneration, to date there has been no textbook that summarizes the great opportunities associated with aptamers.

The idea of *The Aptamer Handbook* is to present a detailed view on the many facets of aptamers and especially their applications. It is conceptually designed for a very broad audience that not only comprises the pure scientific disciplines of molecular biology, biochemistry, and chemistry. It also addresses fields that are usually more application-oriented such as pharmacology and medicine, but it may also be helpful for managers of the pharma and biotech industries who (should) consider new and innovative technologies to be used or established more broadly. The huge amount of work invested by many brilliant scientists and the money that has been spent through either public or commercial funding so far, has helped to create an extensive, broad, and solid basis of knowledge about and around aptamers that should be recognized by an increasing number of people.

Even though considerable preliminary work had been carried out earlier, the starting shot was set in 1990 when *in vitro* evolutionary selection techniques were used in the groups of Joyce, Gold, and Szostak to identify unique RNA-based structures that displayed new or altered functionalities: binding to a target

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molecule and enzymatic activity, respectively. In the *Nature* publication of Ellington and Szostak the target-binding RNA molecules were named "aptamers", while in their *Science* publication Tuerck and Gold termed the process used to identify aptamers "SELEX" (Systematic Evolution of Ligands by EXponential enrichment). In subsequent years aptamers were raised against almost any type of target imaginable: small molecules, peptides, proteins or even ribosomal and viral particles. While many people believed that only antibodies could bind to targets with high affinity and specificity it had to be recognized that aptamers – oligonucleotide structures – could do these jobs as well, sometimes even better.

The first chapter by Carothers and Szostak gives a general overview of aptamers and places their discovery into the context of discussion about the origins of life and the RNA world. The following two chapters provide the reader insight into (*in vitro*) evolution and fitness landscapes from a theoretical point of view. The second part of the book comprises five chapters, each dealing with aptamers that bind to certain types of targets such as small molecules, antibiotics, proteins, and nucleic acid structures. The last in the row introduces aptamer motifs called "riboswitches" that were evolved by nature itself. These "natural aptamers" are embedded in messenger RNAs and can directly sense small molecules and are therefore able to serve as regulatory elements in gene control. In the smaller, third part of the book two chapters describe the catalytic functionalities of RNA and DNA oligonucleotides. Although these so-called ribozymes or deoxyribozymes are not classified as aptamers, they can be obtained by *in vitro* evolutionary selection methods and exert their functions through the selected three-dimensional structures.

The fourth part of the book presents an overview of the many applications of aptamers. These range from aptamers as *in vitro* tools for target validation outside the cell and within the cell (intramers) over so-called aptazymes, which can be used as biosensors and are made of a combination of aptamers and (deoxy)ribozymes, to aptamers as lead structures for small molecule development, and as ligands in affinity chromatography applications. Furthermore, these versatile molecules can exert their function *in vivo* as well. Due to their ability to bind and thereby block important disease targets, aptamers can be and are used as *in vivo* imaging agents and therapeutics. For these applications aptamers usually have to be chemically modified in order to render them biostable; aptamers that are built from mirror-image nucleotides (so-called spiegelmers) already display a native biostability and do not need further stabilization. The last chapter introduces Macugen, the antivascular endothelial growth factor aptamer that was approved by the US regulatory authorities for the treatment of age-related macular degeneration in December 2004. Finally, Larry Gold, who was the first to transfer SELEX from an academic lab into the environment of a biotech company, summarizes his personal view on aptamers in an epilogue.

I hope that this book will help the interested reader to get a comprehensive impression of the fascinating field of aptamers. The different topics were selected to light up as many different areas of aptamer research as possible, knowing that

completeness is very difficult to achieve if possible at all. Further, I hope to attract the best and brightest to join the field to push the limits further.

I am very grateful to all who made this book possible. Many thanks to the authors for their excellent chapters, to Jerry Joyce for his superb foreword, to Christian Mihm for the cover artwork, and last but not least thank you to the staff at Wiley-VCH, especially Frank Otmar Weinreich and Steffen Pauly, for their continuous support.

Sven Klussmann
Berlin, December 2005

Foreword

It has been 15 years since the term "aptamer" and the acronym "SELEX" were coined. With the field of directed molecular evolution now transitioning from its adolescence to young adulthood, it is an appropriate time to take stock of what aptamer science has to offer, both now and for the future. In this monograph, the first ever completely devoted to the subject of aptamers, you will find a well-chosen set of contributions from leading investigators in the field, describing the methods and applications of aptamer technology. This is not a laboratory manual, but neither is it a collection of review articles; it is a handbook that is meant to give you an appreciation for the principles and practice of *in vitro* selection as applied to functional nucleic acids. Whether you already are or will be a practitioner yourself, or simply want to know what all the fuss is about, this book is something that you will want to attack with a highlighter pen and scratch paper on the side. Evolution is a very powerful process, but it is surprisingly easy to carry out in a modern laboratory. You too can evolve molecules for fun and profit.

The first aptamer, although it was not referred to as such, actually was created almost 40 years ago, before the advent of recombinant DNA technology ("B.C., before cloning", as Sydney Brenner likes to say). In the late 1960s, Sol Spiegelman realized that the three fundamental processes of Darwinian evolution – amplification, mutation, and selection – could be applied to a population of RNA molecules *in vitro*. Amplification of RNA was achieved by employing an RNA-dependent RNA polymerase, the replicase protein of Qβ bacteriophage. Mutation occurred as a result of the intrinsic error rate of the polymerase in copying variants of Qβ genomic RNA. Selection was based on the ability of particular RNAs to serve as efficient templates for the production of complementary RNAs and, in turn, for the production of additional copies of themselves. "(Go forth and) multiply, with the biological proviso that (you) do so as rapidly as possible," Spiegelman famously declared. The result, following multiple rounds of selective amplification and mutation, was a population of evolved RNA molecules that were amplified much more efficiently by the replicase compared with their ancestors.

Discussion of Spiegelman's pioneering work usually focuses on the perhaps unsurprising result that the evolved RNAs were truncated variants of Qβ genomic

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RNA that, by virtue of their smaller size, could be copied more rapidly than the wild type. A more subtle point, however, is that the evolved RNAs also were selected to be efficient ligands for the replicase protein, which recognizes particular features of RNA secondary and tertiary structure in both the positive- and negative-stranded RNA. Thus the evolved RNAs were both an aptamer for the replicase protein and a substrate for the protein, leading to the production of progeny RNAs.

One of the great advances in the history of life on Earth was the transition from an "RNA world," in which both genetic and functional properties resided within RNA, to a DNA and protein world, in which genotype and phenotype were relegated to separate macromolecules. Another critical advance in directed molecular evolution was the development of techniques that decoupled amplification of nucleic acid molecules from selection based on their functional properties. This made it possible to select RNAs that are a ligand for any protein, for example, T4 DNA polymerase, as demonstrated by Craig Tuerk and Larry Gold. RNAs could even be selected that bound to small molecules, as shown by Andrew Ellington and Jack Szostak.

In the early 1980s, following the discovery of catalytic RNA by Thomas Cech and Sidney Altman, one wondered what it would take to coax Qβ replicase to amplify RNA molecules that included a ribozyme or some other functional motif. Fred Kramer and colleagues had shown that it was possible to sneak exogenous nucleotides into variants of Qβ genomic RNA that could be amplified *in vitro*. Those familiar with the details of the system knew, however, that it was only a matter a time – and usually not much time – before the insert would be trimmed or spit out entirely, resulting in a more efficient amplicon. What was needed was a general-purpose RNA amplification method that would be indifferent to the sequence being amplified.

Then came polymerase chain reaction (PCR), soon followed by reverse transcriptase PCR (RT-PCR), and everything changed. A population of nucleic acid molecules could be asked to do anything the investigator had the nerve to ask them to do: bind a target molecule, bind a target molecule but not some closely related molecule, catalyze a reaction, catalyze a reaction only after binding to some other target molecule, and so on. In retrospect, most of the early efforts were rather timid, but soon the gloves came off and it seemed that nearly everything was fair game. Literally, of course, the gloves were kept on a bit longer because RNA molecules are highly susceptible to degradation by biological nucleases, limiting their potential applications. This limitation was overcome by carrying out directed evolution with RNA analogs that are nuclease resistant, yet can be amplified by RT-PCR. Particularly intriguing in this regard are "Spiegelmers," which first are selected as natural RNAs that bind the enantiomer of the desired target, then are prepared as the corresponding non-natural enantiomer of RNA for binding to the actual target. These reverse aptamers are aptly named because they are the mirror (*Spiegel*) of their biological counterparts, and in recognition of Spiegelman's contributions to initiating the practice of *in vitro* Darwinian evolution.

Aptamer science has now reached maturity, not just as a result of its longevity and accumulated knowledge, but through its growing impact on biology and medicine. In December 2004 the first aptamer compound was approved for clinical use. As discussed in the chapter by Anthony Adamis and colleagues, Macugen (pegaptanib) is a chemically modified RNA aptamer that binds tightly and specifically to vascular endothelial growth factor. It has become a preferred treatment for the neovascular form of age-related macular degeneration. Other chapters describe aptamers that are being developed for various therapeutic applications, medical imaging, clinical diagnostics, drug target validation, biosensor applications, and process chemistry. All this and more awaits you on the pages that follow.

Darwinian evolution in nature has provided a bounty of functional macromolecules. However, just as synthetic organic chemistry has taken us beyond the small molecules that can be harvested as natural products, directed evolution has expanded upon the set of macromolecules to include compounds that have been tailored for our own purposes. This is not intelligent design – quite the opposite in fact – but in this book you will see how the vision and skill of the experimenter, combined with the power of an evolutionary search, can lead to some remarkable discoveries.

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