Chemistry & Biology
Perspective

Aptamers and SELEX in *Chemistry & Biology*

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Nucleic acid aptamers, or simply aptamers, are oligonucleotides that bind specific ligands that vary from small molecules to proteins. An aptamer for a specific ligand is routinely identified through the process of systematic evolution of ligands by exponential enrichment, although some aptamers are found in nature as ligand-binding sites of special RNA structures called riboswitches. Aptamers have significant value in biotechnology and for the development of aptamer-based therapeutics. This perspective briefly highlights the tight connection between the journal *Chemistry & Biology* and in vitro selection technologies over the past two decades. We then focus our discussion on the summary of the current state of the art of aptamer technologies and provide our view of the future challenges and opportunities for the field.

The launch of *Chemistry & Biology* in 1994 filled an important gap. In the April 15, 1994, prelaunch issue, the founding editors wrote, "It is the explosion of research at the interface of chemistry and biology…that has motivated us to provide a new outlet for research papers that should be read by researchers in both disciplines. At present, authors of such papers have the uncomfortable choice of publication in a chemical journal, which is rarely read by biologists, or the reverse. *Chemistry & Biology* will aim to be accessible to both sets of readers, and we will consider the journal a success if it lowers the language barriers between these two fields, which have so much to say to each other" (Schreiber and Nicolaou, 1994). Now, 20 years later, the journal can definitely be considered a success, as it continues to publish on research topics that are at the intersection of the two fields, and centrally positioned within the scope of chemical biology, a discipline that emerged at about the same time as the journal and has since established itself.

Some of the highly interdisciplinary fields that have been represented in the journal from its inception are aptamer research, the in vitro selection of functional nucleic acids, and systematic evolution of ligands by exponential enrichment (SELEX). Indeed, the journal quickly established itself as one of the most influential places for publishing cutting-edge results in aptamer, SELEX, ribozyme and riboswitch research and in the in vitro selection of novel ribozymes, deoxyribozymes, and aptazymes, as discussed here and in a related perspective included in this issue of *Chemistry & Biology* (Joyce and Breaker, 2014). Some of the breakthroughs that were published in the pages of *Chemistry & Biology* are the report of the first catalytic DNA, DNAzyme (Breaker and Joyce, 1994); one of the first riboswitch papers describing the coenzyme B12 riboswitch (Nahvi et al., 2002); and the first crystal structure of a riboswitch (Serganov et al., 2004). Additional notable early discoveries that remain relevant to this day include a hemin-binding DNA aptamer; a G-quadruplex structure that exhibited peroxidase activity in the presence of hemin (Travascio et al., 1998), which is now widely used as a catalytic G-quadruplex reporter system; a famous in vitro selected Diels-Alderase ribozyme (Seelig and Jäschke, 1999); and other ribozymes, DNAzymes, and aptazymes that expanded the scope of nucleic acid-catalyzed chemical transformations (Carmi et al., 1996; Fusz et al., 2005; Jenne and Famulok, 1998; Kim and Joyce, 2004; Levy and Ellington, 2002; Sengle et al., 2001; Tang and Breaker, 1997; Wilson and Szostak, 1998; Zhang and Cech, 1998). Over the years, many other aptamers, too many to list individually, have appeared in this journal, forming a basis for further research and development, including the development of therapeutic aptamers, such as the 2′-NH_2-modified anti-VEGF aptamer (Green et al., 1995) that was the first of a series of modified VEGF-binding aptamers (Burmeister et al., 2005; Ruckman et al., 1998) leading to the development of Macugen (the first clinically approved therapeutic aptamer) and several aminoglycoside-binding aptamers (Lato et al., 1995; Wallis et al., 1995; Wang and Rando, 1995).

Since the early days of aptamer research and in vitro selection, the field has matured considerably and hundreds of laboratories worldwide are now engaged in aptamer research in one way or another, or they perform SELEX or in vitro evolution experiments. As such, the development of aptamers and SELEX methodologies has gained tremendous attention within the past 20 years. Because of their unique properties, aptamers are nowadays used in many disciplines and different scientific fields, and for a variety of purposes (Figure 1). For example, aptamers are used as capture molecules (Müller et al., 2011), as genetically encoded reporters (Paige et al., 2011), as building blocks for nanoarchitectures and nanomotors (Zhu et al., 2013), and simply as inhibitors of biological function (Keefe et al., 2010). The latter is the most auspicious application, opening doors to a novel macromolecular therapeutic class.

Aptamers can be synthesized chemically, and their sequence information can be shared digitally as a blueprint for manufacturing them. This allows cheap and fast production with reproducible properties and very low, if any, batch-to-batch variation. The majority of aptamers are currently used for research purposes; hence either no modifications or convenient fluorescent or affinity tags are required. In this way, commercial suppliers synthesize and deliver aptamers upon the provision of their sequences—quickly, cheaply, and at reasonable scales, as simple as primer molecules. These features in particular mark aptamers as unique and clearly set them apart from protein-type macromolecules (e.g., antibodies or their derivatives). Recent developments implement amino acid-like residues into nucleic acid scaffolds (e.g., indole, benzyl, or alkyne moieties)

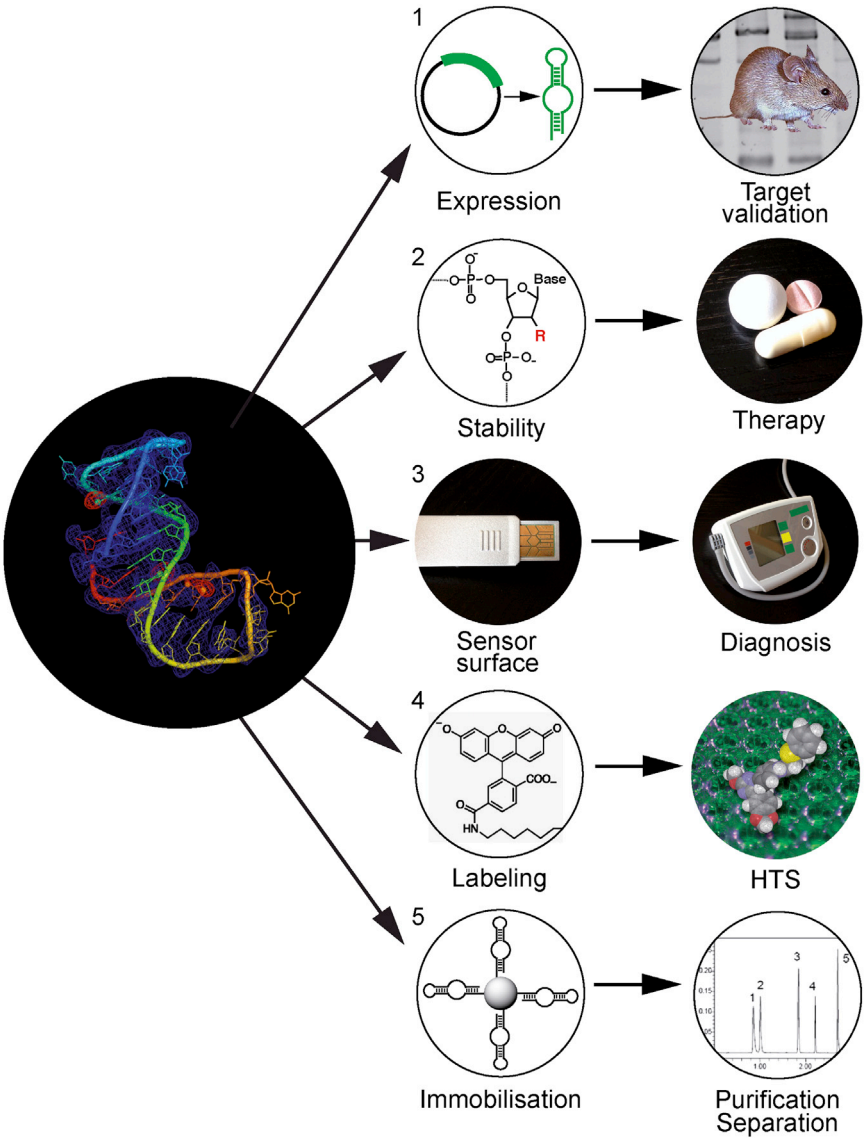

Figure 1. Applications of Aptamers
Aptamers (left) are advantageous to many other molecules because they can be accessed synthetically. This allows their expression in vivo and in cells (middle, 1), their stabilization by chemical modification (middle, 2), the derivatization of sensor surfaces with aptamers (middle, 3), their tagging with a huge variety of probes and anchor molecules (middle, 4), and their immobilization on various carriers and matrices (middle, 5). Consequently, the fields of application of aptamers are very broad, ranging from target validation, therapy, and diagnosis to high-throughput screening (HTS) and purification or separation (right).

(Jäger et al., 2005). These are compatible with enzymatic steps of the selection process (Jäger et al., 2005) and thus allow access to aptamers with chemically modified side chains (Vaught et al., 2010). This approach has led to a tremendous improvement of the success rate of SELEX experiments and to aptamers with an incredibly low off-rate, reflected by the acronym SOMAmers (slow *o*ff-rate *m*odified *apta*mers). More than 1,000 SOMAmers are now embedded in a diagnostic platform, allowing the detection of variations in protein signatures, along with distinct human pathologies (Gold et al., 2010). Whether these aptamers also are valuable as therapeutics has yet not been explored, but because of their sophisticated recognition properties, there is virtually no doubt. However, pharmacokinetics, bioavailability, and toxicity must be explored first. In this regard and besides "normal" aptamers, Spiegelmers are the most developed therapeutic investigational new drugs in the aptamer field (Hoellenriegel et al., 2014; Vater and Klussmann, 2003). These aptamers consist of an L-enantiomeric ribose backbone and hence are almost nuclease resistant. Recent promising data raise the hope that this aptamer class will provide effective therapies in the near future.

Aptamer-based therapeutics are certainly the major long-sought desire to be fulfilled. Besides Macugen, no other aptamer has gained approval during the past 10 years, but some are in advanced stages of clinical trials. Among them the aptamer-antidote pair targeting coagulation factor IXa, identified in Bruce Sullenger's laboratory and now developed further by Regado Biosciences (Rusconi et al., 2004). Likewise, as therapeutics, the field also is awaiting diagnostic assays that advance from the proof-of-concept stage toward routine clinical applications. A first step into this direction has been taken by the groups of Mayer and Pötzsch, providing novel aptamer-based diagnostic assay formats. These enable the sensitive detection of active thrombin and activated protein C (APC) in patient samples (Müller et al., 2011, 2012), whereas the APC assay uses an aptamer that was first published in *Chemistry & Biology* (Müller et al., 2009). Both assays are now commercially available in ready-to-use kit formats provided by American Diagnostica.

Because we expect that the number of commercially available aptamers will continue to grow over the next few years, in no small part due to a steadily increasing number of companies that offer fee-for-service-based identification of aptamers, the number of detectable target molecule-aptamer pairs will become significant. Commercialization of aptamer development will allow access to these molecules by every interested researcher, provided these services are affordable, are transparent, and deliver sequence information and aptamers with reproducible properties. This might form the foundation for widespread aptamer use in a plethora of elaborated approaches. A novel attention-getting branch is the identification of cell- or tissue-specific series of aptamers. These are available by selection procedures using living cells, bacteria, or subcellular preparations thereof as complex target structures. An advanced variant

enables even the use of xenograft mice tumor models to identify tumor-targeting aptamers de novo by an in vivo SELEX approach (Mi et al., 2010). Fortunately, many cell-recognizing aptamers are taken up by the targeted cells or tissues, although the underlying molecular mechanisms are not very well understood as of yet. However, these types of aptamers will provide a novel basis for developing targeted therapies, especially as delivery vehicles to transport macromoleular cargo therapeutics (e.g., small interfering RNA molecules or antagomiR sequences into specific cells (Pastor et al., 2010; Pofahl et al., 2014). Because the molecular targets of such aptamers are unknown, this approach also enables the identification of new biomarkers specifically associated with pathogenic tissue.

2014 and Beyond

Aptamers represent an interesting molecule class that is positioned between small molecules and biologicals. They have matured considerably throughout the past two decades and have become true alternative compounds in chemical biology and biomedicine. Nevertheless, acceptance and use of aptamers in industry and by (bio)pharmaceutical companies remains rare and is certainly a gap that needs to be filled in the near future. The question is, why is this the case, and what needs to be done to convince companies to rely on this compound class?

One unquestionable aspect is the low success rate (<30%) of aptamer selection, compared with antibody development. This will be overcome by using modified nucleotides in the selection process or introducing entirely novel base pairs and building blocks (Kimoto et al., 2013; Pinheiro et al., 2012). However, methods to use these approaches are yet not widely available but are limited to a few specialized groups. Advances in sequencing technologies not only will enhance the success rate of SELEX approaches but also will allow deep insight into the governing evolutionary process that takes place during the selection procedure. High-throughput sequencing has the potential to revolutionize SELEX, as it makes it possible to scan millions of sequences in a library simultaneously. From a mathematical point of view, this provides tremendous output and allows the depiction of sequences that are not overrepresented in a library and are rare because of nonoptimized replication behavior but still retain target-binding properties. Conventional sequencing approaches will simply miss these aptamers.

In light of these recent advances, we believe that aptamer selection and application will make as much improvement in the coming 20 years as it did throughout the past 20. However, to convince other researchers, industry, and clinicians of the advantages of aptamers compared with other compound classes, quality standards need to be implemented to certify, secure, and testify to aptamers' properties. This could be realized by establishing independent reference laboratories worldwide, which perform interaction analyses of newly identified aptamers and their cognate targets, essentially prior publication. This endeavor will ensure that identified aptamers are actually doing what they are supposed to do: binding with a certain affinity and specificity to the initial target molecule. Because sequences can be easily shared digitally, this approach will also verify sequence identity and its functional relationship. An adequate certificate can simply be included in every publication of a new aptamer, confirming that this particular molecule showed activity also in the hands of independent scientists. We are convinced that this certification process will enhance confidence in aptamers and avoid conflicts that are known from antibodies, which because of batch-to-batch variation reveal diverse recognition patterns, diverging from the supposed target molecule. This becomes even more important, as several companies now offer aptamer identification on a fee-for-service basis. Including third-party validation of these aptamers will help secure a distinct level of quality and reliability.

Taken together, the future of aptamer and in vitro selection research continues to look bright, and journals such as *Chemistry & Biology* and others focused on chemical biology are well placed to provide an excellent forum for the publication of the most exciting results in this growing research field.

REFERENCES

Breaker, R.R., and Joyce, G.F. (1994). A DNA enzyme that cleaves RNA. Chem. Biol. *1*, 223–229.

Burmeister, P.E., Lewis, S.D., Silva, R.F., Preiss, J.R., Horwitz, L.R., Pendergrast, P.S., McCauley, T.G., Kurz, J.C., Epstein, D.M., Wilson, C., and Keefe, A.D. (2005). Direct in vitro selection of a 2′-O-methyl aptamer to VEGF. Chem. Biol. *12*, 25–33.

Carmi, N., Shultz, L.A., and Breaker, R.R. (1996). In vitro selection of self-cleaving DNAs. Chem. Biol. *3*, 1039–1046.

Fusz, S., Eisenführ, A., Srivatsan, S.G., Heckel, A., and Famulok, M. (2005). A ribozyme for the aldol reaction. Chem. Biol. *12*, 941–950.

Gold, L., Ayers, D., Bertino, J., Bock, C., Bock, A., Brody, E.N., Carter, J., Dalby, A.B., Eaton, B.E., Fitzwater, T., et al. (2010). Aptamer-based multiplexed proteomic technology for biomarker discovery. PLoS ONE *5*, e15004.

Green, L.S., Jellinek, D., Bell, C., Beebe, L.A., Feistner, B.D., Gill, S.C., Jucker, F.M., and Janjić, N. (1995). Nuclease-resistant nucleic acid ligands to vascular permeability factor/vascular endothelial growth factor. Chem. Biol. *2*, 683–695.

Hoellenriegel, J., Zboralski, D., Maasch, C., Rosin, N.Y., Wierda, W.G., Keating, M.J., Kruschinski, A., and Burger, J.A. (2014). The Spiegelmer NOX-A12, a novel CXCL12 inhibitor, interferes with chronic lymphocytic leukemia cell motility and causes chemosensitization. Blood *123*, 1032–1039.

Jäger, S., Rasched, G., Kornreich-Leshem, H., Engeser, M., Thum, O., and Famulok, M. (2005). A versatile toolbox for variable DNA functionalization at high density. J. Am. Chem. Soc. *127*, 15071–15082.

Jenne, A., and Famulok, M. (1998). A novel ribozyme with ester transferase activity. Chem. Biol. *5*, 23–34.

Joyce, G.F., and Breaker, R.R. (2014). The expanding view of RNA and DNA function. Chem. Biol. *21*, this issue, 1059–1065.

Keefe, A.D., Pai, S., and Ellington, A. (2010). Aptamers as therapeutics. Nat. Rev. Drug Discov. *9*, 537–550.

Kim, D.E., and Joyce, G.F. (2004). Cross-catalytic replication of an RNA ligase ribozyme. Chem. Biol. *11*, 1505–1512.

Kimoto, M., Yamashige, R., Matsunaga, K., Yokoyama, S., and Hirao, I. (2013). Generation of high-affinity DNA aptamers using an expanded genetic alphabet. Nat. Biotechnol. *31*, 453–457.

Lato, S.M., Boles, A.R., and Ellington, A.D. (1995). In vitro selection of RNA lectins: using combinatorial chemistry to interpret ribozyme evolution. Chem. Biol. *2*, 291–303.

Levy, M., and Ellington, A.D. (2002). ATP-dependent allosteric DNA enzymes. Chem. Biol. *9*, 417–426.

Mi, J., Liu, Y., Rabbani, Z.N., Yang, Z., Urban, J.H., Sullenger, B.A., and Clary, B.M. (2010). In vivo selection of tumor-targeting RNA motifs. Nat. Chem. Biol. *6*, 22–24.

Müller, J., Isermann, B., Dücker, C., Salehi, M., Meyer, M., Friedrich, M., Madhusudhan, T., Oldenburg, J., Mayer, G., and Pötzsch, B. (2009). An exosite-specific ssDNA aptamer inhibits the anticoagulant functions of activated protein C and enhances inhibition by protein C inhibitor. Chem. Biol. *16*, 442–451.

Müller, J., Becher, T., Braunstein, J., Berdel, P., Gravius, S., Rohrbach, F., Oldenburg, J., Mayer, G., and Pötzsch, B. (2011). Profiling of active thrombin in human blood by supramolecular complexes. Angew. Chem. Int. Ed. Engl. *50*, 6075–6078.

Müller, J., Friedrich, M., Becher, T., Braunstein, J., Kupper, T., Berdel, P., Gravius, S., Rohrbach, F., Oldenburg, J., Mayer, G., and Pötzsch, B. (2012). Monitoring of plasma levels of activated protein C using a clinically applicable oligonucleotide-based enzyme capture assay. J. Thromb. Haemost. *10*, 390–398.

Nahvi, A., Sudarsan, N., Ebert, M.S., Zou, X., Brown, K.L., and Breaker, R.R. (2002). Genetic control by a metabolite binding mRNA. Chem. Biol. *9*, 1043.

Paige, J.S., Wu, K.Y., and Jaffrey, S.R. (2011). RNA mimics of green fluorescent protein. Science *333*, 642–646.

Pastor, F., Kolonias, D., Giangrande, P.H., and Gilboa, E. (2010). Induction of tumour immunity by targeted inhibition of nonsense-mediated mRNA decay. Nature *465*, 227–230.

Pinheiro, V.B., Taylor, A.I., Cozens, C., Abramov, M., Renders, M., Zhang, S., Chaput, J.C., Wengel, J., Peak-Chew, S.Y., McLaughlin, S.H., et al. (2012). Synthetic genetic polymers capable of heredity and evolution. Science *336*, 341–344.

Pofahl, M., Wengel, J., and Mayer, G. (2014). Multifunctional nucleic acids for tumor cell treatment. Nucleic Acid Ther. *24*, 171–177.

Ruckman, J., Green, L.S., Beeson, J., Waugh, S., Gillette, W.L., Henninger, D.D., Claesson-Welsh, L., and Janjić, N. (1998). 2′-Fluoropyrimidine RNA-based aptamers to the 165-amino acid form of vascular endothelial growth factor (VEGF165). Inhibition of receptor binding and VEGF-induced vascular permeability through interactions requiring the exon 7-encoded domain. J. Biol. Chem. *273*, 20556–20567.

Rusconi, C.P., Roberts, J.D., Pitoc, G.A., Nimjee, S.M., White, R.R., Quick, G., Jr., Scardino, E., Fay, W.P., and Sullenger, B.A. (2004). Antidote-mediated control of an anticoagulant aptamer in vivo. Nat. Biotechnol. *22*, 1423–1428.

Schreiber, S.L., and Nicolaou, K. (1994). A new journal for an expanding field. Chem. Biol. (*Suppl. 1*), 1.

Seelig, B., and Jäschke, A. (1999). A small catalytic RNA motif with Diels-Alderase activity. Chem. Biol. *6*, 167–176.

Sengle, G., Eisenführ, A., Arora, P.S., Nowick, J.S., and Famulok, M. (2001). Novel RNA catalysts for the Michael reaction. Chem. Biol. *8*, 459–473.

Serganov, A., Yuan, Y.R., Pikovskaya, O., Polonskaia, A., Malinina, L., Phan, A.T., Hobartner, C., Micura, R., Breaker, R.R., and Patel, D.J. (2004). Structural basis for discriminative regulation of gene expression by adenine- and guanine-sensing mRNAs. Chem. Biol. *11*, 1729–1741.

Tang, J., and Breaker, R.R. (1997). Rational design of allosteric ribozymes. Chem. Biol. *4*, 453–459.

Travascio, P., Li, Y., and Sen, D. (1998). DNA-enhanced peroxidase activity of a DNA-aptamer-hemin complex. Chem. Biol. *5*, 505–517.

Vater, A., and Klussmann, S. (2003). Toward third-generation aptamers: Spiegelmers and their therapeutic prospects. Curr. Opin. Drug Discov. Devel. *6*, 253–261.

Vaught, J.D., Bock, C., Carter, J., Fitzwater, T., Otis, M., Schneider, D., Rolando, J., Waugh, S., Wilcox, S.K., and Eaton, B.E. (2010). Expanding the chemistry of DNA for in vitro selection. J. Am. Chem. Soc. *132*, 4141–4151.

Wallis, M.G., von Ahsen, U., Schroeder, R., and Famulok, M. (1995). A novel RNA motif for neomycin recognition. Chem. Biol. *2*, 543–552.

Wang, Y., and Rando, R.R. (1995). Specific binding of aminoglycoside antibiotics to RNA. Chem. Biol. *2*, 281–290.

Wilson, C., and Szostak, J.W. (1998). Isolation of a fluorophore-specific DNA aptamer with weak redox activity. Chem. Biol. *5*, 609–617.

Zhang, B., and Cech, T.R. (1998). Peptidyl-transferase ribozymes: trans reactions, structural characterization and ribosomal RNA-like features. Chem. Biol. *5*, 539–553.

Zhu, G., Zheng, J., Song, E., Donovan, M., Zhang, K., Liu, C., and Tan, W. (2013). Self-assembled, aptamer-tethered DNA nanotrains for targeted transport of molecular drugs in cancer theranostics. Proc. Natl. Acad. Sci. U S A *110*, 7998–8003.

