A Quarter Century of In Vitro Selection

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Nucleic acid research has always been an exciting research field and has produced a myriad of important discoveries in science and technology. However, the time around 1990 was particularly electrifying for many established scientists and young students like myself who was working in this arena. Tomas Cech and Sidney Altman had just won the Nobel Prize in Chemistry in 1989 “for their discovery of catalytic properties of RNA” (i.e., the discovery of ribozymes). And then, two brilliant papers were published in 1990 that demonstrated an elegant technique now widely known as “SELEX” (Tuerk and Gold 1990) or “*In Vitro* Selection (IVS)” (Ellington and Szostak 1990). These studies marked the birth of “aptamers,” new kids on the block of functional biomacromolecules: Ellington and Szostak created RNA aptamers for some synthetic organic dyes (Ellington and Szostak 1990) while Tuerk and Gold discovered an RNA aptamer that binds a protein molecule (Tuerk and Gold 1990). Earlier in the same year, Robertson and Joyce also published a paper where they demonstrated that a similar technique can be used to evolve a novel function from a natural ribozyme: they isolated a mutant form of the celebrated RNA-splicing *Tetrahymena* ribozyme that is capable of cleaving DNA efficiently (Robertson and Joyce 1990). In the next few years, more ground-breaking discoveries were made with this same technique. These include the first DNA-based aptamers (Ellington and Szostak 1992; Bock et al. 1992), first synthetic ribozymes (Bartel and Szostak 1993), and the first

deoxyribozymes or DNazymes—enzymes made of DNA (Breaker and Joyce 1994). By now, a huge collection of aptamers, ribozymes, and DNazymes has been derived by research groups all over the world using this simple yet powerful approach. Equally impressively, the availability of these new functional molecules has motivated researchers from different disciplines to devise unique or useful applications in wide-ranging areas, such as chemical biology, synthetic biology, nanotechnology, diagnostics, and therapeutics, just to list a few.

At a scientific conference in 2014 that featured many wonderful presentations on functional nucleic acids, Niles Lehman (the Editor-In-Chief of this journal) and I realized that 2015 coincides with the 25th anniversary of the SELEX/IVS technique. Our conversation quickly turned into a discussion on an exciting idea of assembling a special issue in the *Journal of Molecular Evolution* to commemorate the development of the SELEX/IVS technique. I was tasked to assemble this issue, with a goal to put together a combination of original research papers, reviews, and commentaries by early pioneers.

I am very pleased to report that the response from the SELEX community to our idea has been overwhelmingly positive. Their support has led to an assemblage of 14 papers for this special issue. These include short commentaries from Professors Larry Gold (Gold 2015), Andrew Ellington (Ellington 2015), and Gerald Joyce (Joyce 2015), some of the pioneers who helped initiate this field. These short pieces are fun to read as these innovators recount aspects of early days of IVS/SELEX and share their views on future directions of the field.

This issue then features seven papers on aptamers. Professor Maria DeRosa and her team conduct a comprehensive analysis of SELEX parameters based on 492 SELEX experiments published between 1990 and 2013

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(McKeague et al. 2015). Professor Weihong Tan and his colleagues provide a review on recent progress made with DNA aptamers as cell-imaging probes (Jin et al. 2015). Professor Zhuo Tang and his associates describe an experimental effort to exploit fluorescence-activated cell sorting technique to isolate RNA aptamers that can induce fluorescence from a fluorogenic organic compound within *E. coli* cells (Zou et al. 2015). Professor Chaoyong Yang and his coworkers provide an account of their experiment to isolate DNA aptamers that bind AIB1, a transcriptional coactivator that is highly expressed in many human cancers (An et al. 2015). Professor Dana Baum and her colleagues investigate the redox abilities of several aptamer-cofactor complexes and their potential as redox catalysts (Emahi et al. 2015). Professor Chris Le and his group look into the impact of SELEX conditions on the outcome of aptamers selected for a couple of bacterial pathogens (Hamula et al. 2015). And lastly, Professor Hiroaki Suga and his team communicate their findings on the utility of a protein-binding peptide aptamer, derived by a SELEX-like process, as a cell-imaging probe (Iwasaki et al. 2015).

The issue also includes four papers on the in vitro selection of deoxyribozymes. Professor Scott Silverman and his colleagues describe an in vitro selection experiment that has led to the isolation of a group of deoxyribozymes with intriguing tyrosine kinase activity (Walsh et al. 2015). Professor Yi Lu and his coworker describe the reoccurrence of a Na(I)-dependent DNAzyme in two separate in vitro selection experiments (Torabi and Lu 2015). Professor Juewen Liu and his group report on their effort in isolating a leadzyme-like DNAzyme (Saran et al. 2015). A paper contributed from my own team demonstrates that in vitro selection can be applied to evolve a noncatalytic DNA sequence into an efficient RNA-cleaving DNAzyme (Chan et al. 2015).

All the papers in this special issue serve well to celebrate a test-tube technique that was invented 25 years ago but is still very pertinent today. I did a quick search in the “Web of Science” on November 1, 2015 using “in vitro selection” or “SELEX” as the topic, and the resulting publication count was 25,321. This translates into an annual publication rate of 1013/year. When the search was restricted to 2015 as the publication year, the result was 1313. I think it is reasonable to assume that by the end of this year, the publication count will surpass 1500. Although this is only a very rudimentary estimation, it does show that the interest in this technique continues to be particularly strong. It is worth making a special note that this technique is not only directly responsible for the discovery of a large number of wonderful synthetic nucleic acid aptamers and enzymes, it also deserves some credit for the discovery of many naturally occurring RNA aptamers in the form of riboswitches (Winkler et al. 2002; Tucker and Breaker

2005)—we thought we humans “invented” something brilliant, but it turns out that Mother Nature got it first. Of course, the biggest winners from the past 25 years of in vitro selection are the nucleic acids themselves, as the scientific world now has a much better appreciation of their functional capability, a revelation that is truly worth celebrating!

I would like to express my sincere gratitude to all the authors for their wonderful contributions, and I especially want to thank all the young contributors for your great effort—I know many of you worked extra hard to speed up your experiments in order to complete the data set for your story. I want to give special thanks to Larry, Andy, and Jerry for sharing your early experience with the SELEX/IVS technique and your thoughts on the future of this field. My sincere appreciation goes to all the referees for your timely reviews and constructive comments—without your help, this special issue would not have been possible. Finally, I want to thank Niles Lehman and the JME editorial staff for all the encouragements and help along the way.

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