International Society on Aptamers

DECEMBER 6, 2016 VOLUME 1, NUMBER 2

EDITORIAL

We've reached the end of the year and I hope that you are all celebrating successes in one form or another, whether it is surviving another year, publishing a paper, or receiving a grant. I also hope that you are preparing to attend Aptamers 2017, the 4th symposium to be held in Oxford, UK, on 11th and 12th April 2017, alongside Oligo 2017 on 10th April. I'm very pleased to announce that Professor Ulrich Hahn from University of Hamburg is the Symposium Chair at Aptamers 2017. In this issue, we have articles on the state of Aptamer research around the world, a summary of Aptamers 2016, and an update on what to expect at Aptamers 2017. I wish you all a good end to the year, a great beginning to 2017, and a wish to see you all in Oxford in April!

Dr Sarah Shigdar
President

INSOAP AGM

We will be holding the AGM of INSOAP during the symposium. If you would like to be involved and contribute to the future of our Society, please forward agenda items to sarah.shigdar@deakin.edu.au or AptaSoc@gmail.com by the 24th March 2017.

TRAVEL AWARDS 2016

We would like to thank Base Pair Biotechnologies, Nal Von Minden, and the Library Publishing Group, for their kind sponsorship of student travel grants in 2016. We will be offering travel grants again in 2017 and if you would like to help our future researchers attend this conference, please email AptaSoc@gmail.com or AptamersOxford@gmail.com. If you would like to apply for a travel grant to attend Aptamers 2017, please see the application and eligibility criteria here: http://libpubmedia.co.uk/aptamers-2017/travel-grants/

Inside this issue:

Newsletter Staff:

Dr Sarah Shigdar
Dr Muhammad Sohail
Dr Maureen McKeague
Ms Joanna Macdonald

From the Editor

If you have anything you would like to see in the next issue of the INSOAP newsletter, send it directly to sarah.shigdar@deakin.edu.au. Please ensure that your articles and information are in by close of business on 29th April 2017

Aptamers Journal

We announced the official journal of INSOAP at Aptamers 2016. Please email us at aptasoc@gmail.com to express your interest in joining the editorial or reviewer team.

Past meeting: Aptamers 2016

Aptamers 2016 was a great success, having Professor Beatrix Suess from Technical University of Darmstadt as the Symposium Chair. We had reports from many different areas of research, spanning aptamer selection, improved modifications to nucleotides and the selection process, as well as the use of aptamers as diagnostic and therapeutic agents. Jorg Hartig and Beatrix Suess discussed the use of aptamers in and as riboswitches, while Julian Tanner provided an update on his reagentless disposable diagnostic kit for malaria screening. Staying on the topic of infectious diseases, Ana Maria de Lucas Cerillo talked about her results for the detection of HCV in blood samples, Tony Cass on engineering aptamers for use in biosensors and a simple test for avian flu, SPR imaging for detection of small molecules, and Bill Jackson about a lateral flow test for MRSA. Nicola Stonehouse talked about studying the viral life cycle and using aptamers to inhibit viral activity in FMD. Switching to non-communicable diseases, Yoshi Nakamura, presented some very interesting clinical data from his trials of the FGF2 aptamer for bone diseases. As a cancer researcher, I was particularly excited to hear Ulrich Hahn, Said Ismail, and Sorah Yoon discuss their approaches to treating cancers with aptamer therapeutic conjugates. Gregory Penner also added to the discussion with mention of his HER2 aptamer. Moving back to the area of diagnostics, and keeping with the cancer theme, I presented data on the use of aptamers as sensitive immunohistochemistry agents. As well, Marimutha Citartan discussed EPO doping and detection. Research relevant to all areas of aptamers were the discussions relating to nucleotide modification by Tom Brown, the optimisation strategies for aptamer selection, such as FRELEX from Gregory Penner, and the use of magnetic beads for rapid aptamer selection from Chin-Yih Hong. Finally, Michael Blank discussed the use of NGS sequencing for improved aptamer identification during selection. One very interesting discussion was sparked by the presentation of David Bunka, who presented data on Cell SELEX. Finally, an interested technology, backscatterring interferometry was discussed by Bill Jackson and Darryl Bornhop. Aptamers 2017 looks as though it's going to be just as exciting. We have attendees from around the globe, coming from as far as Australia, Taiwan, Japan, China, Hong Kong, Malaysia, Jordan, the US, and Canada, as well as visitors from Europe and from just around the corner in Oxford. It's also great to see a number of industry representatives attending, and this year we will see the Aptamer Group, RIBOMIC, and AptaIT again, as well as talks from NeoVentures, Horiba Scientific, and Base Pair Biotechnologies. The conference was also punctuated by the conference dinner which provided a great forum for conversation and collaboration, as well as the fantastic food provided by St Hilda's College.

Aptamers 2017

The Aptamer Symposium to be held in Oxford on the 11th and 12th of April, is already shaping up to be a fantastic conference. Professor Dr Ulrich Hahn from University of Hamburg is Symposium Chair this year, and he will open the conference. We have a number of speakers who will be discussing how effective aptamers are for therapeutic delivery, and we have several speakers who will show how aptamers can be utilised in a number of diagnostic and analytical assays. Also, given that we are constantly improving the selection process for aptamers, there are several talks on modifications to the traditional selection process. Finally, there will be some presentations on the use of modified bases in the selection process, and what we can do to improve the integration of modifications during selection.

Symposium website: http://libpubmedia.co.uk/aptamers-2017
Email: AptamersOxford@gmail.com

Contributions:

North America:

Dr Maureen McKeague

ETH Zurich

(Formerly of Stanford University)

Europe:

Dr Ulrich Hahn

University of Hamburg

Asia:

Dr Julian Tanner

University of Hong Kong

Australia:

Dr Rakesh N Veedu

Murdoch University

Aptamers around the world

North America

Aptamers were first described in 1990 from researchers in North America, and in the last ten years, the reaction of North American scientific audiences to aptamer research has transition from "I've never heard of an aptamer" to "Yes, we all know about aptamers". It is my opinion that about half of the scientific community in North America is still excited about the *potential* of aptamer technology, while the other half feels this is a technology still "searching for a problem to solve". Regardless of this anecdotal evidence, there is some scientific indication that suggests aptamer research continues to be widespread and prolific within North America. While this report covers the state of aptamer research in North America, the majority of the data and opinions used to write this piece focus on the United States. A rapid search in the Web of Science for "aptamer" yields 8653 results, with 32.4% of the results originating from China, 31.9% from the USA, the 6th most prevalent country is Canada (4.3%), and Mexico contributing 12 results (0.14%). Despite my proud Canadian heritage, it is safe to say that the US is the major contributor to aptamer research and likely reflects the overall state of aptamer research in North America.

Year	Funding (Million USD)	
	Aptamer (In project title)	Aptamer (Anywhere in project)
2016	5.78	65.5
2015	4.76	N.C.
2014	2.63	N.C.
2013	5.62	N.C.
2012	6.74	N.C.
2011	3.90	N.C.
2010	4.62	54.81
2008	3.75	N.C.
2005	3.80	N.C.
2001	3.78	14.96

Note: years 2001, 2005, and 2008 were chosen at random.
N.C. = Not calculated (chosen at random).

In terms of publications, the number originating from North America have increased, going from 83 publications in 2005, to 282 publications in 2015 (source: Web of Science). With the top five journals of choice for aptamer research having an average impact factor of 8.434, aptamer research continues to be of impact and importance to the scientific community. As well, two of the top five journals are published by ACS, suggesting the USA is still very much interested in disseminating aptamer-based research. Despite the loss of funding for Biological and Medical Research from the National Institutes of Health (NIH) over the past decade due to budget cuts, etc, funding for aptamer-related research remains steady. In particular, comparison of new aptamer funded projects ("aptamer" appearing in project title) on NIH's RePORTER, shows a very consistent level of funding each year throughout the 2010s and an average increase of 30% more funding provided than the last decade. A broader search shows that aptamer-related funding ("aptamer" used as a broad search term) has increased approximately 5-fold in the past 15 years. Furthermore, the >10-fold increase in funding of aptamer-related projects ("aptamer" used as a broad search term) compared to aptamer-focused projects ("aptamer" appearing in the project title) might suggest that aptamers are more commonly being exploited to help answer research questions or incorporated into research programs in innovative ways.

There are relatively few examples of aptamer commercialization; however, the majority of companies that have successfully marketed aptamers for research and development are located in North America. The largest and most established company is Somalogic, Inc. (Boulder, CO, USA). Not only does Somalogic have the largest catalog of high affinity DNA-based reagents (i.e., SOMAmers) available for purchase; they have also coupled all of their SOMAmers to create an assay for rapid protein biomarker discovery and validation (i.e., SOMAscan). Finally, they support several research and development initiatives, including the SOMAscan Assay Biomarker Discovery Grant Program. Other successful examples of aptamer-based companies include NeoVentures Biotechnologies (London, ON, Canada), OTC Biotech (San Antonio, TX, USA), Aptagen (Jacobus, PA, USA) and Base Pair Biotechnologies (Houston, TX, USA). Finally, over the past few years while I was living in the leading hub for high-tech innovation and scientific development (Silicon Valley); I was contacted by numerous VCs, scientists, young inventors, and established companies looking to exploit or develop aptamers for a wide variety of creative technologies. I suspect that we will continue to find new companies that are interested in or that will invest in aptamer-based technology.

Overall, the steady funding, increasing publications, and commercial interest suggest to this author that there continues to be a healthy and expanding interest in aptamer technology. There remains a wide variety of innovative research ideas that can be exploited to expand the utility of aptamers: it is not so much that there is a need to "find their problem to solve" so much as we really have found multifunctional and highly adaptable tool.

Europe

While aptamers were first described by researchers working in North America, a number of European scientists have come back from labs in the US to their home countries, importing aptamers and SELEX to different European countries. Other researchers have become fascinated by the new selection method and the opportunities being offered by this new class of molecule. Thus, there has been and still is, quite a large community performing aptamer research in Europe. This is evidenced by the number of publications associated with each country in Europe – a total of 1322 papers have been published by researchers in Europe, with Germany having the largest number of publications, followed by France, Italy, UK and Spain (Source: PubMed). To compare with the US however, Germany published 77 papers in 2015, a significant increase on the number published in 2005.

It is unsurprising, given the number of papers published by researchers in Germany, that there are a large number of research groups. Michael Famulok was a postdoc in Jack Szostak's lab and brought back his knowledge of SELEX to Germany. Gunter Mayer was a former co-worker of Mitch Famulok and has become a leader in the field, recently inventing click-SELEX and providing an elegant opportunity to broaden the repertoire of aptamer building blocks. Sven Klussmann and Jens Peter Fürste invented Spiegelmers®, becoming the column of the NOXXON company, selecting, optimizing, testing and developing pharmaceuticals against cancer and other diseases. Recently the structure of the first Spiegelmer® has been solved in Christian Betzel's lab by X-ray structure analysis at Hamburg University at atomic resolution.

The labs of Beatrix Süß (riboswitches to be used as genetic regulatory devices) and Ulrich Göringer (aptamers binding to surface components of the trypanosome cell) both at the Technical University of Darmstadt have also the selection of aptamers in their research focus. Further German labs that should be named are those of Meltem Avci-Adali and Hans Peter Wendel (Tübingen), Jörg Hartig (Konstanz), Andres Jäschke (Heidelberg), Sabine Müller (Greifswald) and Beate Strehlitz as well as Mario Mörl (both at Leipzig) and maybe my lab in Hamburg.

Jean-Jacques Toulme, based at the University of Bordeaux, has a long history of working with nucleic acids, having contributed to the development of the antisense strategy before switching to aptamers as a means of interacting with folded RNA hairpins. As well, Jean-Jacques works very closely with Novaptech and has developed automated selection strategies for the development of new aptamers. Italy, UK, and Spain, account for an equivalent number of published papers as Germany and France, and there are several research groups, as well as commercial agencies, working in these countries. Taking Italy and Spain into account immediately the groups of Vittorio de Franciscis and Giovanni Di Fabio, both from Naples, and Ana María de Lucas Cerrillo (Madrid) and Ciara O'Sullivan have to be considered. Many groups engaged in aptamers, both at universities and companies, can be found in the United Kingdom. One just can name some like Tony Cass (Imperial College London), Tom Brown (Oxford), David Bunka (aptamer group) and Nicola Stonehouse or Dejian Zhou (both from Leeds).

What can we expect in the future? My feeling is that new strategies for the delivery of different kinds of drugs, nano particles and miRNAs, etc, as well as new "artificial" aptamer derivatives (click-SELEX) and therapeutic Spiegelmers® will soon be reported about - and hopefully a method making the selection of aptamers as easy as pie.

Asia

Aptamer research across Asia is expanding rapidly as scientists from multiple disciplines have come to see the many advantages of aptamers in molecular recognition for a plethora of applications. As a first very rough estimate of aptamer activity, a simple Pubmed "aptamer" keyword count provides some useful indicators, showing China (1759 papers), Japan (410 papers) and South Korea (320 papers) clearly acting as the main powerhouses of aptamer research activity in Asia. Significant research output has also emerged from Iran (130 papers), Taiwan (101 papers), India (92 papers), Singapore (49 papers) with smaller contributions from several other countries including Israel and Jordan. Perhaps most dramatic is the rapid increase just over the last recent few years as aptamers come to the fore.

Of course, Asia does have a few labs that clearly have led at the highest international levels in aptamer research over many years. In Japan, Ichiro Hirao has pioneered many new approaches in aptamer science including the development of aptamers using an expanded genetic alphabet with an unnatural hydrophobic base pair. Ichiro Hirao has recently moved his research group to Singapore so it will be interesting to see how this further develops interest in aptamers in Singapore. In China, Hunan University has developed as a major world-leading centre for aptamer research through many investigators including Weihong Tan (also based in Florida). Weihong Tan has had a major impact on the interface between aptamer science and nanotechnology with a highly prolific output of important publications over many years including impact on cancer theranostics as well as many imaging

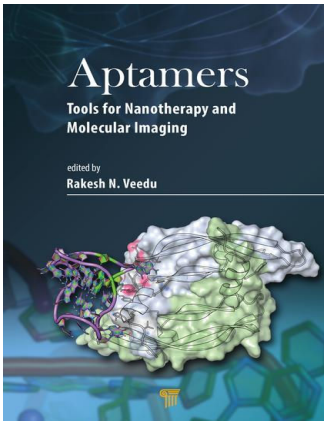
applications of aptamers. In South Korea, Man Bock Gu has had a major impact on linking aptamers with biosensors and in the development of graphene oxide based aptamer selections against small molecules. In Israel, Itamar Willner amongst his wide-ranging contributions has had significant impact on aptamer research over many years, including most recently the development of nucleoapzymes.

There have also been success stories on the commercial side of aptamer research in Asia. Japan has perhaps the most successful aptamer therapeutics company in Asia, Ribomic Inc., developed by Yoshikazu Nakamura, Emeritus Professor of the University of Tokyo. Ribomic completed its IPO in 2014. Michael Donovan, a colleague of Weihong Tan, founded Veraptus in Hunan, also aiming at therapeutic applications of aptamers. Aptamer Sciences is a privately held biotech company based in Pohang, South Korea with particular interest in rapid diagnostic testing. No doubt, there are many other aptamer-related companies at early stages of development across Asia.

What is particularly exciting now is the number of up and coming research groups across Asia who have published significant aptamer-related articles in recent years. Chaoyong James Yang at Xiamen University, Kazunori Ikebukuro at Tokyo University of Agriculture and Technology, Osamu Nureki at the University of Tokyo, Julian Tanner at the University of Hong Kong, Ge Zhang at Hong Kong Baptist University, Changill Ban at POSTECH are making significant contributions amongst many others too many to name.

It is exciting to be part of a fast-growing community of aptamer scientists in a part of the world with rich scientific opportunity. The interdisciplinary overlap of aptamer science, nanotechnology, chemistry and directed evolution will no doubt deliver a great deal from Asia over the coming years.

Australia



Nucleic acid aptamer technology has captured tremendous attention since their discovery in early 90's, and the enormous potential of aptamers has been utilised across various disciplines for targeted molecular recognition. However, the widespread use of antibodies have overshadowed the potential utility of aptamer technologies in Australia compared to the rest of the world, though the field has increasingly gained in popularity 'down-under' since 2010 based on recent contributions. Although there are not many research groups focused exclusively on aptamer development and their synthesis chemistries as such, there are several laboratories using the technology for drug delivery, therapeutic, diagnostic and bio-sensing applications.

One important Australian contribution regarding the aptamer selection procedures is the development of a rapid one-step selection methodologies by an active aptamer research group led by Rakesh N. Veedu of Murdoch University and Western Australian Neuroscience Research Institute. The advantage of this approach is that selection can be performed in under two days and in addition, the method can also be used for libraries containing some chemical modifications with only one-enzymatic step. Another solid Australian contribution is the development of an aptamer against epithelial cell adhesion molecule (EpCAM), the first cancer stem-cell targeting aptamer developed by the research team of Sarah Shigdar and Wei Duan of Deakin University, Victoria. Since publication, this aptamer has been applied extensively by various researchers for specific targeting of cancer cells, and this aptamer is even selling commercially by some aptamer companies (eg. Aptagen). This very active aptamer research group contributed several articles in this field. Towards aptamer synthesis chemistries (incorporation of various modified nucleotides, linkers and labelling chemistries), the research laboratory of Rakesh Veedu is well established with two oligonucleotide synthesisers (Expedite 8909 and GE AKTA Oligopilot systems) at Murdoch University, Australia. As it is the only such research facility in Australia, this group has extensive research collaboration across Australia. To highlight the field, Veedu edited a special issue in 2015 entitled "Medicinal Chemistry of Aptamers" in the journal *Current Topics in Medicinal Chemistry*, and in 2016 he published an edited book entitled "Aptamers: Tools for targeted Nanotherapy and Molecular Imaging" (Panstanford Publishing, CRC Press) including contribution from other Australian authors.

In summary, based on the rise in number publications involving the applications of aptamers recently, I firmly believe that the future of aptamer technologies in Australia appears quite promising, and gaining popularity particularly in medical research and agricultural research. I admit that this field is still relatively new compared to the well-established antibody market in Australia. However, there are obvious advantages in using aptamers over antibodies, and a small aptamer focused research community is continually trying to highlight this field through innovative research, collaborations and

conferences. Towards this goal, in 2015, the first international conference on functional nucleic acids was organised at Murdoch University (www.fnaperth.org) with excellent participation from Australian researchers, and this conference will be held again in 2018.

Nominations for INSOAP committee

We are currently asking for expressions of interest for membership of the management committee of INSOAP. If you would like to be an integral part of our Society as it moves forward, please contact me at sarah.shigdar@deakin.edu.au before the 31st of March 2017.

Updates to the website

We have been working on updating the website for INSOAP and you will now see that we have a listing of all aptamer companies throughout the world, as well as a listing of all the aptamer laboratories to date. If we haven't got you listed, please get in touch and we will add you to our growing list. We are also providing a careers page so please get in touch with any vacancies you wish to be listed. Finally, if there are any suggestions for improvements to the website, please contact us and we will make the changes.

Keep in touch

http://aptamersociety.org
http://www.linkedin.com/groups/8282517
www.facebook.com/AptaSoc
https://twitter.com/AptamerSociety
https://twitter.com/aptamer_connect.

References: Famulok M, *et al*. (2007) Functional aptamers and aptazymes in biotechnology, diagnostics, and therapy. *Chem Rev* 107: 3715.; Stoltenburg R, *et al.* (2007) SELEX–A (r)evolutionary method to generate nucleic acid ligands. *Biomol Eng* 24: 381.; Lipi F, Chen S, Chakravarthy M, Rakesh S and Veedu RN (2016) In vitro evolution of chemically modified aptamers: Pros and Cons, and comprehensive selection strategies. RNA Biology, DOI:10.1080/15476286.2016.1236173; Lauridsen LH, Shamaileh HA, Edwards SL, Taran E, Veedu RN (2012) Rapid One-Step Selection Method for Generating Nucleic Acid Aptamers: Development of a DNA Aptamer against α-Bungarotoxin. *PLOS ONE* 7: e41702.; Shigdar S, Lin J, Yu Y, Pastuovic M, Wei M, et al. (2011) RNA aptamer against a cancer stem cell marker epithelial cell adhesion molecule. *Cancer Science* 102: 991-998. Veedu RN (2015) Editorial: medicinal chemistry of aptamers. *Current Topics in Medicinal Chemistry* 15(12):1065. R. N. Veedu, Aptamers: Tools for Targeted Nanotherapy and Molecular Imaging, Pan Stanford Publishing Pte Ltd, CRC Press, Singapore, 2016.; Kimoto, M., R. Yamashige, K. Matsunaga, S. Yokoyama, and I. Hirao, Generation of high-affinity DNA aptamers using an expanded genetic alphabet. Nat Biotechnol, 2013. 31(5): p. 453-7.; Zhu, G., J. Zheng, E. Song, M. Donovan, K. Zhang, C. Liu, and W. Tan, Self-assembled, aptamer-tethered DNA nanotrains for targeted transport of molecular drugs in cancer theranostics. Proc Natl Acad Sci U S A, 2013. 110(20): p. 7998-8003.; Nguyen, V.T., Y.S. Kwon, J.H. Kim, and M.B. Gu, Multiple GO-SELEX for efficient screening of flexible aptamers. Chem Commun (Camb), 2014. 50(72): p. 10513-6.; Golub, E., H.B. Albada, W.C. Liao, Y. Biniuri, and I. Willner, Nucleoapzymes: Hemin/G-Quadruplex DNAzyme-Aptamer Binding Site Conjugates with Superior Enzyme-like Catalytic Functions. J Am Chem Soc, 2016. 138(1): p. 164-72.; Ma, Y., Y. Mao, D. Huang, Z. He, J. Yan, T. Tian, Y. Shi, Y. Song, X. Li, Z. Zhu, L. Zhou, and C.J. Yang, Portable visual quantitative detection of aflatoxin B1 using a target-responsive hydrogel and a distance-readout microfluidic chip. Lab Chip, 2016. 16(16): p. 3097-104.; Tsukakoshi, K., Y. Ikuta, K. Abe, W. Yoshida, K. Iida, Y. Ma, K. Nagasawa, K. Sode, and K. Ikebukuro, Structural regulation by a G-quadruplex ligand increases binding abilities of G-quadruplex-forming aptamers. Chem Commun (Camb), 2016. 52(85): p. 12646-12649.; Kato, K., H. Ikeda, S. Miyakawa, S. Futakawa, Y. Nonaka, M. Fujiwara, S. Okudaira, K. Kano, J. Aoki, J. Morita, R. Ishitani, H. Nishimasu, Y. Nakamura, and O. Nureki, Structural basis for specific inhibition of Autotaxin by a DNA aptamer. Nat Struct Mol Biol, 2016. 23(5): p. 395-401.; Cheung, Y.W., J. Kwok, A.W. Law, R.M. Watt, M. Kotaka, and J.A. Tanner, Structural basis for discriminatory recognition of Plasmodium lactate dehydrogenase by a DNA aptamer. Proc Natl Acad Sci U S A, 2013. 110(40): p. 15967-72.; Liang, C., B. Guo, H. Wu, N. Shao, D. Li, J. Liu, L. Dang, C. Wang, H. Li, S. Li, W.K. Lau, Y. Cao, Z. Yang, C. Lu, X. He, D.W. Au, X. Pan, B.T. Zhang, C. Lu, H. Zhang, K. Yue, A. Qian, P. Shang, J. Xu, L. Xiao, Z. Bian, W. Tan, Z. Liang, F. He, L. Zhang, A. Lu, and G. Zhang, Aptamer-functionalized lipid nanoparticles targeting osteoblasts as a novel RNA interference-based bone anabolic strategy. Nat Med, 2015. 21(3): p. 288-94.; Jo, H., S.K. Kim, H. Youn, H. Lee, K. Lee, J. Jeong, J. Mok, S.H. Kim, H.S. Park, and C. Ban, A highly sensitive and selective impedimetric aptasensor for interleukin-17 receptor A. Biosens Bioelectron, 2016. 81: p. 80-6.