International Society on Aptamers

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Inside this issue:

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EDITORIAL

I can't believe we are half way through the year already. I hope you have all had a productive year so far. Since the last newsletter, we have had the Aptamer Symposium, the official conference of INSOAP, and have made a number of announcements. Further details are provided below but we are pleased to announce the official journal of INSOAP, Aptamers. We are currently gearing up for the 5th annual conference, to be held in Oxford on 11-12th April 2018. I have to say, that each year we have been blessed with good weather, as you can see from the pictures at each conference. We even managed to have our lunches outside this year and enjoy the picturesque views. I'm looking forward to seeing you all there and hope that you can spend some time to come and say hello.

You will see a few new additions to this newsletter and we hope to keep these columns as regular features. I'm particularly enjoying the 'interview with a researcher' and if there is someone you would like to see featured, please let us know. We are also going to be providing an update on current literature in various areas of aptamer research: this issue we are focussing on aptamer diagnostics. If you would like to contribute to this section, please get in touch.

As a final note, have you liked our Facebook page? We are currently providing links to new aptamer research papers on a daily basis. Don't have time to keep up to date on current literature? Get our daily updates in your morning newsfeed at https://www.facebook.com/AptaSoc/.

I hope you have a great second half of the year!

Dr Sarah Shigdar
President

INSOAP AGM

We held our second AGM at the 4th Aptamer Symposium and we had good attendance. There were numerous topics up for discussion but the main ones were regarding these newsletters, the conference, and student participation at the conference. We are hoping to start providing reviews of the current state of aptamer research in the newsletter, and I hope you all enjoyed our discussion on the state of aptamer research around the world in the last newsletter. We will be encouraging PhD students to attend and present their research in the format of a 3 minute thesis at the next conference. We will also have specific poster sessions at morning and afternoon breaks to encourage more discussion This year we had 27 posters, and I really enjoyed chatting with all presenters. As the conference continues to grow, having sessions specifically for PhD and ECR researchers will aid in the dissemination of their research and engage attendees in various networking events.

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 31st August 2017

Aptamers Journal

We announced the official journal of INSOAP at Aptamers 2017. Please email us at aptasoc@gmail.com to express your interest in joining the editorial or reviewer team. Please see http://libpubmedia.co.uk/aptamers/ to submit your article.

Past meeting: Aptamers 2017

Aptamers 2017 was well attended for the 4th year in a row and I was happy to see that the British weather held up, with the sun shining on our lunch breaks, allowing us to enjoy the wonderful wildlife while discussing various applications of aptamers. The conference was split into a number of sub-specialities, with the first session focussing on chemical modifications. Kicking off this first session was Professor Jesper Wengel, the co-inventor of locked nucleic acids. Jesper provided an update on the use of Phusion hi fidelity DNA polymerase and engineered polymerases as a way forward for SELEX using LNAs, definitely something that has potential for better bioavailability of aptamers in therapeutic applications. Jesper also discussed the use of LNAs and unlocked nucleic acids as antisense or RNAi drug candidates before presenting results on how to build artificial protein mimics through the combination of oligonucleotides and short peptide sequences. Dr Xianbin Yang, from AM Biotechnologies, then presented information on X-aptamers which are developed using a microbead based single-cycle discovery process and does not rely on PCR amplification allowing a variety of chemical modifications to be incorporated. Dr Dan Schneider from SomaLogic was the third presenter in this session and he talked about enhancing chemical diversity with multiple pyrimidine modifications. These modifications can create novel intramolecular motifs leading to improved aptamer affinities. Finishing off this session was Professor Tom Brown from Oxford University who discussed the synthesis and properties of chemically modified nucleotides.

The second session of the conference focussed on disease, with Dr Greg Penner of NeoNeuro kicking off the session with a discussion of aptamer theranostics for Alzheimer's disease. Greg's team preselected the randomised library by injecting the library into the tail vein of mice and then collecting only the aptamers that has passed into the brain. They then performed aptamer selection on human blood from Alzheimer's patients to pick out targets that could be used as a diagnostic panel. Their panel was effective in stratifying patients into early, middle and late stage Alzheimer's disease and they are now investigating this Aptamarker AD fingerprint for earlier diagnosis of disease. Next up was Professor Dr Ulrich Hahn from Hamburg University, and our Symposium Chair. Uli presented data on selectin and integrin aptamers as a means of preventing metastasis. Both of these aptamers were shown to have an anti-adhesive effect on cancer cells. Interestingly, the truncation of the integrin binding aptamer had a detrimental effect on the function of the aptamer. Next up was Professor Yoshikazu Nakamura from Ribomic Inc who has been developing a fibroblast growth factor 2 (FGF-2) binding aptamer that can restore bone growth in Achondroplasia (ACH) and is now gearing up for clinical trials. This aptamer has restored body weight and length in ACH transgenic mice and has an excellent profile in rat and monkey plasma. They are now looking at it in age-related macular degeneration. Yoshi was followed by Professor Fernando Pastor, from CIMA in Spain, who was presenting data on generating a co-stimulatory agonistic aptamer. This aptamer, which bound to both CD28 and multidrug resistant protein 1 (MRP-1), was shown to inhibit growth of tumours in mice. Last up in this session was Dr Sarah Shigdar, our very own President, from Deakin University, who was presenting data on a bifunctional aptamer capable of not only crossing the blood brain barrier from systemic circulation, but also capable of specifically targeting cancer cells in the brain. This aptamer has been tested in vitro and in vivo and shows promise for future targeted drug delivery.

Session 3 focussed on aptamer analysis and diagnostics and started with some exciting work being presented by Professor Anthony Cass from Imperial College London. Tony discussed his diagnostics tool, dual recognition element lateral flow assay (DREFLA), to differentiate specific influenza strains from other similar strains. Next in this session was Dr Julian Tanner, one of our executive editors from the Aptamers Journal, who was presenting data on using aptamers for malaria diagnostics, and has developed the APTEC test for Plasmodium falciparum. He has also been developing 'origami boxes' which 'opens' when the target is present, allowing for better point of care testing. Last in this session was Professor Victoria Calzada from Uruguay, who has linked technetium-99m and gallium-67 to an aptamer, sgc-8, through the chelators HYNIC and DOTA, and showed good biodistribution and pharmacokinetics.

The fourth session completed day 1 and also extended into day 2, and given the topic, it is hardly surprising we had a number of presenters in this area. Dr David Bunka of the Aptamer Group, kicked off the session on Tools/Selections/Design off with a discussion on 'Aptabind' and their industrial application of protein purification. Their patented technology can be 'tuned' to the process to ensure that the process is not only efficient, but is also capable of purifying intact functional proteins. Next up was Dr Duncan Borthwick from Dynamic Biosensors who has developed an electroswitchable biosurface to measure accurate binding kinetics of aptamers. Last of the session on day 1 was Dr Sean Dembowski from Minneapolis who has focussed on capillary electrophoresis as a means of enhancing the SELEX process for membrane proteins.

Starting off day 2 and continuing the Tools/Selection/Design session was Dr Terry Steele from Nanyang Technological University, Singapore, who was discussing the use of multiple use fluorescent aptasensors, which could be used for rapid detection in real time field samples and effluents. Dr Meltem Avci-Adali from University Hospital Tuebingen, Germany, who discussed the use of aptamer functionalised hydrogels loaded with VEGF aptamers to capture endothelial cells to provide nutrients and oxygen delivery to cells. Last in this session was Professor Philip Johnson from York University in Toronto, who discussed the complexities of two-site binding of the cocaine-binding aptamer, as well as the ATP-binding aptamer.

The penultimate session focused on riboswitches and was began by Professor Dr Beatrix Suess from Technical University of Darmstadt, Germany. Beatrix discussed how aptamers can be applied as engineered riboswitches, providing several exciting recent examples from her research group. For example, the Suess group has developed strategies to control exon skipping for splicing in cells, as well as tuneable regulation of cell apoptosis. Next, Dr Florian Groher from the Suess group presented his work in the de novo selection and subsequent in vivo screening of a new synthetic riboswitch responsive to the antibiotic ciprofloxacin. Both of these presentations emphasized the challenges in developing riboswitches from in vitro selected aptamers, and highlighted the need for improved in vivo screening systems following conventional SELEX. Next, Dr Carlos Penedo, the Principal Investigator from the Laboratory for Biophysics and Biomolecular Dynamics at the University of St. Andrews presented the elegant and insightful studies using single-molecule FRET for the structural analysis of the aptamer-expression platforms in riboswitch sequences. Completing the session on riboswitches, Professor Dr Mario Mörl from Leipzig University, discussed some of the benefits and challenges of the modular composition of a standard riboswitch.

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The final session, focused on computational methods for analysing or selecting aptamers. First, Dr Muslum Ilgu from Aptalogic Inc discussed his combined approach, using computational models and biochemical interrogation to determine aptamer 3D structure. Specifically, MC-Sym was used to predict a number of structures in silico. Next, 2-aminopurine (2-AP)-substituted aptamers were generated and analysed. With this modification, changes in fluorescence represented regions of structural flexibility and facilitated a rapid, and relatively inexpensive method for determining aptamer structure. Next, Dr Greg Penner presented again, this time representing NeoVentures Biotechnology. At NeoVentures, they have developed an analysis of SELEX progression using next generation sequencing at each round of selection. Greg spoke of SELEX as being a combination of math and chemistry. For example, as expected, as a selection experiment proceeds, the complexity of the pool decreases; however, NeoVentures tends to observe similar sequences beginning at rounds 7-10, and it is the trajectory throughout the selection experiments that is important when isolating/finding the best aptamer sequences. In the final talk of the conference, Dr. Maureen McKeague from ETH Zürich, discussed her analysis of the past 23 years of SELEX experiments. Her analysis revealed that the best selection experiments (e.g., those with the highest affinity aptamers) were done at 37 degrees Celsius, with low magnesium concentrations, and using efficient methods for separating PCR double-stranded products.

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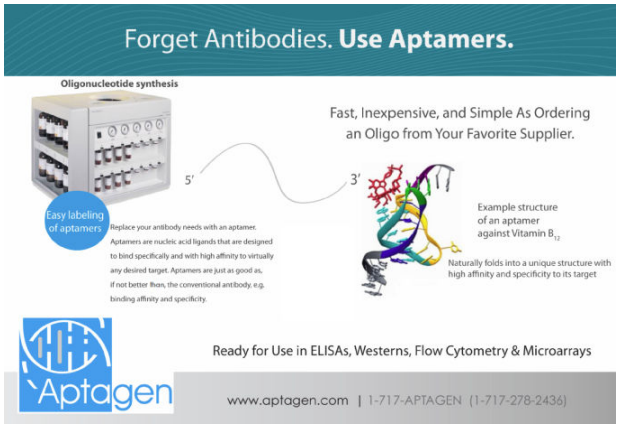
In addition to these fantastic presentations, we also had close to 30 abstracts submitted for poster presentations which covered a great range of topics. We had presenters from Europe (Spain, Germany, Austria, and UK), North and South America, Asia, and Australasia presenting on a diverse range of topics, from enhancing SELEX, to diagnostic applications such as lateral flow devices or medical imaging, to therapeutic applications such as drug delivery. We can't wait to see who will be attending the 5th Aptamers Symposium on 11th and 12th April 2018 and presenting their exciting data!

Aptamers as the next tool for more reproducible diagnostics?



Whilst concerns regarding reproducibility are nothing new, and the causes are most certainly multifactorial, concern over the role that research-grade antibodies play in the reproducibility crisis is gaining momentum [1-5]. Biological reagents account for an estimated 36% of total irreproducibility [6], and antibodies represent perhaps the most ubiquitously used biological reagents [5]. Antibodies are big business. There are currently around 2 million research antibodies marketed by over 300 different companies [1]. Indeed, as expected, there is great variability between vendors when it comes to reliability. When investigators from the Human Protein Atlas validated more than 5,000 commercial antibodies from 51 different vendors by both WB and histochemistry on fixed-tissue microarrays, only 49% made the grade. Furthermore, when stratified by vendor, it became apparent that success rates ranged from 0 to 100% [7]. Similarly, according to reagents portal antibodies-online.com, less than 50% of antibodies make the grade when subjected to independent validation [1]. As a result, some experts estimate that around half of the US$1.6billion spent globally on research antibodies each year is money down the drain [2]. The batch-to-batch inconsistencies seen with antibody reagents add a further layer of complexity in terms of reproducibility. The potential for cross-reactivity and lack of consistency between batches of polyclonal antisera is well-known. This is largely due to the fact that only around 0.5% to 5% of total immunoglobulins are actually specific for the cognate target [2]. And affinity purification is not always sufficient to remove all cross-reactive clones from polyclonal antisera [8]. Moreover, there is significant batch-to-batch variation – even when the very same animal is re-immunised [8]. Batches originating from a new generation of animal are less consistent still. Yet, even in this case, some vendors are not compelled to assign a new batch number to the existing reagent [1, 3].

Long-term research projects often rely heavily on an uninterrupted supply of affinity reagents of constant quality [9]. This precariousness can have disastrous results and waste years of hard work – not only when a new batch fails to recapitulate past results but also when supply may be interrupted or halted without warning [2]. Furthermore, not all companies are immediately upfront in confirming the discontinuation of an antibody [4], which may prolong the pain and result in more wasted time. Recently, this has sparked a call from Bradbury and Pluckthun (along with more than 100 co-signatories), to initiate a transition to 'renewable' affinity reagents that are sequenced at the molecular level - such as recombinant antibodies, protein scaffolds (affibodies) and aptamers [2, 9].

Sequence-defined recombinant antibodies may circumvent much of the batch variability introduced by production in animals [1] and may represent a comfortingly familiar option for companies that have invested millions in the marketing and humanisation of monoclonal antibodies [10]. But these do not yet represent a cost-effective alternative to conventional monoclonals [10]. There also remains subtle batch-to-batch variabilities associated with cell-based cloning of recombinant antibodies [2]. In contrast, aptamers represent a cost-effective, highly consistent and easily modifiable alternative [11]. They also possess unique properties which are beyond the capabilities on protein-based reagents [10].

As a result of their functional versatility and design flexibility, aptamers can do virtually everything that antibodies can and more, with easier synthesis and less lot-to-lot variability [10, 11]. Properties unique to aptamers which assist in the development of affinity binding assays include enhanced stability, reversible folding and ease of chemical modification [12]. Ease of modification means that 'design-for-purpose' aptamer modifications may afford better performance in existing applications. Conversely, better characterisation - based on a known sequence - allows for the

modification of working protocols to accommodate the best available affinity reagents. Ultimately, sequence definition allows reagents to be identified unambiguously, so that researchers may accrue and compare data which is consistent over time and between research groups. In this way, sequenced reagents are effectively immortalized and both tradeable and upgradable via email or publication.

It is important to note that monoclonal antibody reagents required almost 40 years of research and development to attain commercial success [10]. Furthermore, the considerable financial investments made by many of the major pharmaceutical and biotech companies in the humanisation of monoclonal antibodies is likely driving much of the resistance towards aptamers from many of the larger commercial players [10]. As a result, even after almost three decades in the development phase, aptamers seem to be perpetually stuck as the reagents of the future. However, it is clear that support is growing for a switch to renewable, sequence-defined affinity reagents. And momentum is gathering, with antibody validation standards attracting the attention of publishers and funding bodies [3, 13]. As a result, the future may finally catch up to aptamer technology. Perhaps even faster than is currently expected. We had a number of talks (see conference 2017 update) as well as posters presented at Aptamers 2017 detailing some very unique uses of aptamers as diagnostic agents. It is likely that aptamers will gain their foothold in the diagnostic arena due to their uniqueness, as well as their superior specificity and sensitivity, before invading other areas that have been reliant on antibodies for the past twenty years or more.

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Interview with a researcher: Ulrich Hahn

One of the suggestions to come out of the AGM this year was a request for interviews with leaders in the field of aptamer research. We have chosen, as our inaugural leader in the field of aptamers, Professor Dr Ulrich Hahn. We have grown to appreciate Uli's sense of humour at the Aptamers conference over the years and we hope that he will continue to be a regular attendee, even though he has hung up his lab coat.

Prof. Dr. Ulrich Hahn is originally from Kassel, Germany. He received his PhD (Mikrobiologie, Diplom, Dr. rer. nat.) from the Max-Planck-Institute of Experimental Medicine and the University of Göttingen in 1980. He worked for ten years with Wolfram Saenger studying protein design with ribonuclease T1. In 1993, he began his independent career as a university lecturer at the Medical University of Lübeck and worked as full professor at the University of Leipzig (from 1994) and at the University of Hamburg (from 2002). His lab studied RNA biochemistry, in vitro-evolution, and protein design. After a very successful career, Prof. Dr. Ulrich Hahn, retired in 2016. He enjoys music, art, bicycling and football. We were honoured to have Prof. Dr. Ulrich Hahn as our Symposium chair in 2017.

Q1) How did you become interested in the field of aptamers?

I had been working together with my former boss, Wolfram Saenger, for about a decade on protein design with ribonuclease T1. Towards the end of 1990, I was interested in starting my own new project. At the time, I remembered the legendary and well-cited SELEX publication of Craig Tuerk and Larry Gold which I understood only after reading it for the third time. It took my quite a while to understand the crucial point, that the "gene" and "the gene product" were identical.

Q2) From your point of view, what is unique about aptamers?

Unlike proteins, the fact that the gene is the product and vice versa.

Q3) What do you think is the future of aptamers?

They will become more and more important as fundamental tools, both in basic and applied sciences (for example analytical methods and medicine).

Q4) What are the major challenges that need to be solved?

We need an expansion of the reservoir of aptamer building blocks. For example, we need more new unnatural bases and new polymerases to accept those building blocks as substrates.

Q5) What we should do for the aptamer science?

We just need some spectacular results or a generally accepted breakthrough; an ideal option would be to develop an important and well-recognized therapeutic agent.

Q6) Tell us about your research.

In the field of aptamers, we were mainly working on aptamers specific for cell surface proteins. We were interested in aptamers that were internalized with their target and thus serving as vehicles for targeted drug delivery; and we studied aptamers that interfered with cell-cell contacts especially in case of metastasis.

Q7) How did you know about the INSOAP?

My former PhD student Kati Redder (née Berg), drew my attention to the "Aptamers 2015" meeting in Oxford which we then attended together with Florian Mittelberger, another PhD student. Kati there had the chance to present her results in a short oral presentation.

Q8) How will you support the INSOAP?

I accepted the invitation to join the editorial board of "Aptamers – the official open-access journal of INSOAP", the International Society on Aptamers. However, after my retirement I should fade into the background as this is a very good springboard for a younger scientist at the beginning of her/his carrier.

Q9) What kind of advice can you give to the young researchers about aptamers?

Don't give up after initial failures. If you work honestly and diligently, you will have success in science. Sometimes, however, you might need a longer time window to yield first successes.

Q10) What is your personal philosophy on life and science?

Same as the previous question: If you have just only a little bit of luck, as a scientist you will be a privileged person, as you will have the possibility to work on a topic you have chosen yourself, which you like and which brings personal success. However, the most exciting part is the pleasure to work with motivated young people.

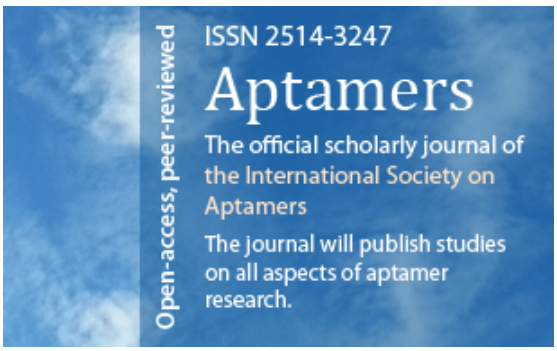
Q11) What was your favourite part about research?

PhD student at Max-Planck-Institute of Experimental Medicine in Göttingen

Q12) Will you miss about running a lab (if anything!)

I retired on October, 1st 2016. My original lab at Hamburg University is just being renovated for my successor and because of private reasons I moved from Hamburg to the island Amrum in the German North Sea. Towards the end of this year, our new house in the Hamburg area will be finished and my wife and I will move back. So my scientific activities have fallen considerably. I have just entered a new phase of my life where I do not do research anymore.

Aptamers Journal



The Aptamers journal is the official journal of the International Society on Aptamers and will publish studies on all aspects of aptamer research. The journal has a strong belief that both positive and negative data can have a large impact on scientific research so we encourage the submission of both. Do you have a troubling troubleshooting issue that you want to share? A protocol that you are proud of and want to share? Or even some R & D news or an Editorial you want to contribute? We would like to hear from you. We will also be accepting full research articles, research reports, reviews and mini reviews, as well as meeting reports. We're hoping to publish the first meeting report of 4th Aptamers Symposium soon. So if you'd like to publish your work in the first Aptamers journal, please follow this link http://libpubmedia.co.uk/aptamers/. We will be publishing free of charge until 15th December 2017. Have you seen our first articles?

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.

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.

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