International Society on Aptamers

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EDITORIAL

Welcome to the second edition of the 2018 INSOAP times, your source to what's happening in the aptamer world presented by the INSOAP team. This year has been progressing so fast. It doesn't seem possible that the end of the year is in less than 3 months! The 5th Aptamer Symposium was held in April in Oxford and we had a great turn out. If you haven't already, check out the full details of the conference proceedings in the Aptamers Journal. And while you're there, have a look at the other articles we have published this year. We are still offering free publication until the end of the year so please submit your articles before the end of the year. We accept research articles and reports, methods, reviews, editorials, and meeting reports.

On that note, we are gearing up for the 6th Aptamer Symposium to be held at Oxford, UK on April 3-4th 2019. Registration is open and we are looking forward to catching up with you all in April. We are very pleased to announce that Professor Dr Günter Mayer is our Symposium Chair for 2019. We are also dedicating a session to flash talks again, given how successful they were this year. We have already secured some speakers and it's exciting to note that we have some new speakers on the agenda. If you've not visited the Symposium website yet, I suggest that you have a look (http://libpubmedia.co.uk/aptamers-2019/). I am also positive that the weather will be better in 2019 than it was this year.

I would like to take this opportunity to thank the entire INSOAP team for their efforts, and generous donation of time and talent. Things wouldn't have been possible without you and I am greatly appreciative of all the assistance of the team for ensuring we produce timely updates for INSOAP. If you would like to contribute to the newsletter, please get in touch! As always, the INSOAP welcomes your ideas and suggestions.

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/. Please don't forget to also follow us on twitter (@Aptamer Society, @Japtamers).

May you all have a great few months, and we'll see you in Oxford in April!

Dr Sarah Shigdar
President

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Dr Muhammad Sohail

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.shiqdar@deakin.edu.au.

Aptamers Journal

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

Aptamers Journal

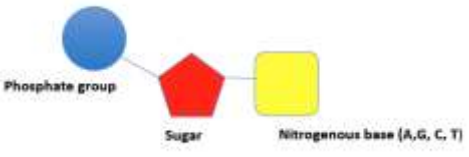
The Aptamers journal is the official journal of the International Society on Aptamers and will publish studies on all aspects of aptamer research. The journal has a strong belief that both positive and negative data can have a large impact on scientific research so we encourage the submission of both. Do you have a troubling troubleshooting issue that you want to share? A protocol that you are proud of? 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. So if you'd like to publish your work in the first Aptamers journal, please follow this link http://www.JAptamers.co.uk.

Techniques to Determine the Structure of Aptamers and Antibodies

Dr Harleen Kaur, Email: harleen2212@gmail.com

Over the last two decades, aptamer have been considered potential alternatives to antibodies in the therapeutic and diagnostic market. With one aptamer US FDA approved, many others in clinical trials, and several advantages over popular antibodies such as easy chemical modification, less batch to batch variation and non-immunogenic nature, this has led to a constant therapeutic battle between these two biomolecules. In order to explore their therapeutic potential, different analytical techniques have been adopted by scientists worldwide to deduce the structure of these biomolecules that are structurally very different from each other and also to understand their binding mechanism with their respective targets.

Aptamers are generally single stranded DNA or RNA molecules composed of monomeric units called nucleotides. Each nucleotide is composed of one of four nitrogen-containing nucleobases (cytosine [C], guanine [G], adenine [A] or thymine [T]), a sugar called deoxyribose or ribose, and a phosphate group (Figure 1A). On the other hand, antibodies are large Y-shaped proteins composed of Fab region with antigen binding site (paratope) on the variable region and fragment crystallizable region (Fc). Fab component is composed of one constant and one variable domain of each of the Heavy and the light chain (Figure 1B).



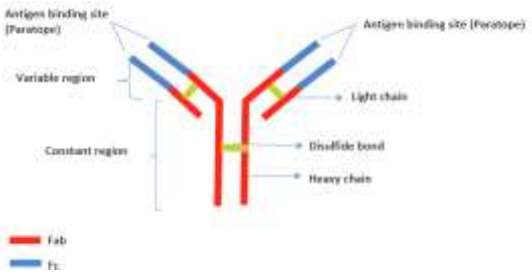


Figure 1A: Schematic Representation of nucleotide structure

Figure 1B: Schematic Representation of antibody structure

Aptamers can fold into a variety of conformations due to their tendency to form loops (stem-loops and pseudoloops), quadruplexes and helices. These conformations allow aptamers to bind with high affinity and high specificity to their cognate target which can be determined by studying the tertiary structure of the aptamer. Techniques such as nuclear magnetic resonance (NMR) has been employed to explore the effect of ions on the aptamer. Mao et al. has shown that quadruplex DNA aptamers such as thrombin binding aptamer (TBA) are

sensitive to the presence of specific metal ions. The results from the work indicated that the inter-tetrad distance of the Sr2+:TBA complex is 3.8 angstroms, or 0.7 angstroms larger than in the K+:TBA complex which could be attributed to different binding sites of Sr2+ and K+ on TBA. Also, software packages such as m-fold and UNA-fold that are based on energy minimization approaches have been used to determine the secondary structures of aptamers and even oligonucleotides. Kaur et al. have determined the stem-loop conformation of anti-VEGF DNA aptamer molecule using m-fold software and successfully probed highest affinity binding domain in a 66-mer aptamer sequence [3]. In addition, the circular dichroism (CD) technique has also widely been used to study the structure of aptamers and understand the change in the conformation under different temperature and ionic concentrations [4,5]

While antibodies share a conserved structural framework, their complementarity-determining region (CDR) loops are highly variable in size and sequence. Thus, monitoring protein integrity is indispensable to ensure batch to batch variations are within acceptable limits. Techniques such as nuclear magnetic resonance (NMR) has been used to study subtle changes in the protein structure and generate highly resolved spectra. However, due to the complexity of the NMR spectra, the use of the technique is limited to small size protein molecules such as antibody domains and some scFv (single chain fragment variable). Arata has used NMR techniques to study antigen-antibody interactions and the obtained NMR spectra of the intact protein and proteolytic fragments from the work suggest the carbonyl carbon chemical shift data could be valuable in understanding the ways in which information is transmitted through different domains on antigen binding site [6]. In addition, the X-ray crystallography technique has been used to determine high-resolution, three-dimensional structures of antibodies and works on the approach of high-quality diffracting crystals. Oyen and colleagues have used X-ray crystallography to study the binding mode of an antibody with the NANP peptide [7]. Two Fab fragments of the anti-malarial antibodies with different light chains that are specific to NANP peptide were used in the study. The results demonstrated that both antibodies use different binding mode to bind NANP and recognize the NANP repeats as well-defined β-turns, modified turns or extended structures. Thus, these structural insights could be useful in future antibody engineering and designing the next malarial vaccine.

To conclude, I would say both aptamers and antibodies have been around for a while now and have demonstrated promising potential with great future prospects ahead. I believe the use of structural based approach for determining and analyzing the structure of these biomolecules could be extremely useful in the basic research, drug design, drug discovery and bringing new therapeutic agents to the market.

References

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INSOAP updated list of recently published aptamers

Dr Maureen McKeague, McGill University, Canada

Continuing from last year, we have provided a list of reported aptamers that have been characterized (includes at least dissociation constant) throughout 2018 (Table 1). We used PubMed to identify newly published aptamers with the keywords “aptamer” and “SELEX”. We identified over 40 publications reporting new DNA, RNA, or modified nucleic acid-based aptamer sequences! If we have missed any newly reported aptamers, please let us know (maureen.mckeague@mcgill.ca). Readers should consult the literature (link provided) for verification and further information.

Table 1: Newly-reported aptamers published in 2018.

Target	**Link**	Nucleic acid type
https://www.ncbi.nlm.nih.gov/pubmed/29361056	diethylthiatricarbocyanine	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29346617	ciprofloxacin	RNA
https://www.ncbi.nlm.nih.gov/pubmed/29501140	florfenicol	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29495282	protein A	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29496467	Staphylococcal enterotoxin A	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29501140	florfenicol	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29499933	skeletal muscle-specific RNA aptamer	RNA
https://www.ncbi.nlm.nih.gov/pubmed/29499932	CI-H460 non-small-cell lung cancer cells	2'-F RNA
https://www.ncbi.nlm.nih.gov/pubmed/29505267	malachite green	RNA
https://www.ncbi.nlm.nih.gov/pubmed/29594592	Mycobacterium tuberculosis Ag85A (FbpA)	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29608400	endothelial cell lines mouse (bEND3), human (hCMEC/D3)	RNA
https://www.ncbi.nlm.nih.gov/pubmed/29609164	H1N1 virus	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29655714	Norovirus	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29666232	prostate cancer cells	RNA
https://www.ncbi.nlm.nih.gov/pubmed/29667819	HIV reverse transcriptase	TNA
https://www.ncbi.nlm.nih.gov/pubmed/29670956	amyloid-β peptide	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29698672	Streptococcus pyogenes	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29708252	Glioblastoma multiforme cells	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29722521	Plasmodium falciparum glutamate dehydrogenase	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29724225	P. falciparum lactate dehydrogenase	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29733244	chemokine (C-C motif) ligand 21	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29756774	E. coli O157:H7	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29749406	anti-HIV-1 integrase	DNA with modifie T
https://www.ncbi.nlm.nih.gov/pubmed/29790932	Zona Pellucida	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29858057	alpha-synuclein	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29858077	mutant huntingtin	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29872833	Cefquinome	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29893086	cervical intraepithelial neoplasia	DNA

https://www.ncbi.nlm.nih.gov/pubmed/29910175	Toll like receptor 4	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29906496	Annexin A2	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29928472	CD19	DNA
https://www.ncbi.nlm.nih.gov/pubmed/29931157	sulforhodamine B and other dyes	RNA
https://www.ncbi.nlm.nih.gov/pubmed/29964028	Streptococcus pneumonia	DNA
https://www.ncbi.nlm.nih.gov/pubmed/30070419	gluten	DNA
https://www.ncbi.nlm.nih.gov/pubmed/30085205	ochratoxin A	XNA/TNA
https://www.ncbi.nlm.nih.gov/pubmed/30098503	metastatic breast cancer	DNA
https://www.ncbi.nlm.nih.gov/pubmed/30141409	Renal cell carcinoma	DNA
https://www.ncbi.nlm.nih.gov/pubmed/30153406	saxitoxin, domoic acid, and tetrodotoxin	DNA
https://www.ncbi.nlm.nih.gov/pubmed/30155822	FokI nuclease domain	DNA
https://www.ncbi.nlm.nih.gov/pubmed/30185972	Anti-Coagulant Dabigatran Etexilate	DNA
https://www.ncbi.nlm.nih.gov/pubmed/30205966	Tuberculous meningitis	DNA
https://www.ncbi.nlm.nih.gov/pubmed/30216975	clenbuterol	DNA
https://www.ncbi.nlm.nih.gov/pubmed/30251354	nonylphenol ethoxylate	DNA

Can aptamers replace antibodies in diagnostic tests?

Dr Sarah Sigdar, Deakin Unoversity, Australia

I recently presented some work at a clinical diagnostics conference and I thought I'd share some of my thoughts regarding the topics of discussion. Geoff Baird wrote a though provoking editorial in 2010 asking "where are all the aptamers?". My opinion at the time, after reading this article was that we would likely start seeing aptamers move into clinical laboratory diagnostics within the next 5 years. This however has not been the case, and candidly I do struggle to fathom why? Quite a number of us are successfully using aptamers to perform functions traditionally only antibiodes could, such as fluorescently tagging live cells to quantify by flow cytometry, fluorescent microscopy, and immunohistochemistry (or aptahistochemistry as we call it). We even have our own ELASAs rather than ELISAs. So why have aptamer based diagnostics not become more prevalent? Gleaning more insights from Geoff's editorial he suggested a number of reasons – lack of knowledge, too comfortable using antibodies, and too much money having been invested in antibodies. On reflection, I'd like to propose an additional reason.

In the age of technological advancements and biosensors, I'd also suggest that we are developing aptamers in diagnostic applications that are different to the needs of clinical diagnostics. Reviewing aptamer literature, our research is progressively making the case for aptamers replacing antibodies in quite a number of clinical applications in diagnostic laboratories in addition to being used as companion diagnostics for therapeutic purposes. My opinion is that this latter application will start promoting aptamers' transition into mainstream clinical applications. This conjecture is purely based on the fact that the exact same aptamer has the duality to be used in both therapeutic and diagnostic applications. The big advantage of aptamer platforms are that different functional molecules can be attached with no or limited loss of specificity and sensitivity. This is an important characteristic when it comes to effective therapy as epitope matching needs to be ensured and the same aptamer can be used in diagnostics and therapeutics. This process is getting easier for antibodies, but there are a number of functional molecules that still require harsh chemical reactions or heat for attachment which are quite likely to denature the antibody, making it non-functional. Being mindful of the ease with which we use aptamers in flow cytometry, we can easily see them transitioning into immunophenotyping. Similarly, aptamers offer a viable replacement for antibodies when staining paraffin embedded tissues for characterisation of tumour tissue, which leads back to the companion diagnostic tests. Even for ELASAs, we see a better limit of detection with aptamers, meaning that smaller or residual amounts of target can be detected. This increased sensitivity is certainly one reason to switch to aptamers instead of antibodies and relates to the size of the aptamer versus a standard diagnostic antibody. Because aptamers are small, more can surround the cell, leading to a greater staining intensity compared to antibodies. In practical terms, this can mean the difference between a patient receiving a targeted

therapeutic or the standard non-specific chemotherapy.

Sensitivity can be increased slightly through modification of binding affinity. To develop reagents with high affinity to their target, the modification of aptamer sequences through base swapping is a relatively simple process. In comparison, modification of antibodies requires the generation of combinatorial phage-display antibody libraries from which antibody variants of higher affinity can be selected. This is a similar 'panning' process to initial aptamer generation and can take substantially more time to find an antibody than the simple process of substituting nucleotides to alter the molecular conformation of an aptamer. Directed *in vitro* evolution is an additional methodology for improving antibody binding affinity. However, as there are six hyper-variable regions, or complementarity determining regions, and a number of amino acid residues in each area, it can be quite a time consuming process to evolve antibodies into high affinity probes. What this equates to is the realization that aptamers are quicker and easier to modify than antibodies.

The achilles heel of antibodies are the issues relating to specificity and this has been suggested to be one of the main reasons for the reproducibility crisis. Currently validation of antibodies (by companies that are investing in this process), is through the use of knockout cell lines to remove the suggested target for the antibody, mass spectrophotometry to confirm binding of antibody to target, Western blotting, histo or cyto chemistry, and protein arrays. While the majority of these tests are designed to confirm the antibody specific binding to the target antigen(s), only the method of protein array analysis would delinate alternate proteins for which the antibody cross reacts, with the notable cavaet however that this method of analysis does not account for post-translational modifications which potentially introduce further cross reactive targets. The myriad of validations required to ensure the specificity of antibodies are only being done now in response to the 'reproducibility crisis'. Building a databse consisting of comprehensive reactivity profiles of commonly used diagnostically used antibody will undoubtake time. In comparison, the majority of these validation tests have already been completed for aptamers during the initial characterisation process. As aptamers bind to a much smaller region of an epitope, it can be argued that it is generally unlikely that aptamers generated to specific proteins will cross-react with others. Granted however, validation of aptamers for cross reactivity using protein arrays should be conducted as well.

Let us assume for a moment that both aptamers and antibodies are created equally . . . why should we still switch to aptamers in clinical diagnostics? Or to be more provactive, why haven't you switched over to aptamers yet? Aptamers are undoubtley cheaper to source than antibodies and routinely less reagent is used equating to significant cost savings of aptamer based assays in comparision to antibody assays. The cost per sample factor should be enough incentive? Couple with this that aptamers are stable at room temperature and have no batch-to-batch variation. In practical terms aptamers don't need to be stored in the fridge and when ordering a new batch, valuable time doesn't need to be spent validating each new batch. Aptamer based assays utilize exactly the same equipment as is currently in place meaning there's no extra capital spend when switching. Generally speaking, the incubation times are much shorter than we see with antibodies, which gives a more rapid turnaround time for the patient. So back to my central question, what is the reason apatmers have not been used?

Awarness and familiarity! Unfortunately there is still a lack of knowledge regarding aptamers and what we can do with them. But from my conversations with companies that have a large catalogue of antibodies, their only interest is investigating the utility of aptamers for applications for which antibodies are unsuitable. If we think about this it makes senses, these companies don't want to lose their 'bread and butter' revenue from antibodies. Knowing that there are better molecules available for use in diagnostics that will benefit patients in the future, but continuing to stick with out-dated reagents because 'it's what we've done for the last 40 years' is not only showing a lack of future proofing, but is also a huge disservice to the patients who need this technological advancement now. Where do we go from here? We need to start moving towards getting aptamers approved by the FDA for in vitro diagnostics (IVDs). A number of us have sufficient data that would take only a little more to add to it to validate our aptamers for the FDA. We have companies that are specialising in aptamers that have catalogues of aptamers validated in assays for R&D. Once we have the first aptamer approved by the FDA for IVDs, I think we'll start seeing a flood of aptamer applications. This isn't the direction I thought this article would go in when I sat down to write it, but these are the thoughts that keep me scratching my head. All of us that work with aptamers know how great they are. We know they're not perfect, but they sure are better than the current gold standard.

Interview with a researcher: Gunter Mayer

Professor Dr Gunter Mayer studied for his Masters in Chemistry (University of Munich) and PhD in biochemistry (University of Bonn) in Germany. His postdoctoral career involved founding a company and moving around the world, travelling to Glasgow (UK) during his academic career. Since 2010, he has been a professor of chemical biology and aptamers at the University of Bonn where he and his group are exploring the broad applicability of aptamers for developing novel therapeutic and diagnostic strategies. Prof. Mayer's overall goal is to develop molecular tools that allow the investigation of biological systems with high precision.

Q1) How did you become interested in the field of aptamers?
I was searching for a suitable project for my Diploma Thesis in 1997 (similar to a Master thesis) and came across Michael Famulok at the Gene Center in Munich. He introduced Selex and aptamers to me and this was it: since then I never left the field.

Q2) From your point of view, what is unique about aptamers?
They can be considered as chemicals and biologicals. This opens a plethora of applications and opportunities. Also, the rapid generation of aptamers cannot be beaten (at least to date) by any other technology yielding binders. These characteristics make aptamers a unique compound class.

Q3) What do you think is the future of aptamers?
The future is bright and I am positive on the success of aptamers in diagnostic, therapeutic applications/approvals as well as on their outstanding contributions as molecular tools helping to unravel and to understand biological and chemical systems.

Q4) What are the major challenges that need to be solved?
Reliability, adaptability, and awareness of the scientific community for installing standards, i.e. characteristics and experiments to be done with aptamers and which they have to meet prior publication, e.g. interaction analysis with at least two different methods and specificity range.

Q5) What we should do for the aptamer science?
Be honest and transparent when publishing aptamers. Do blinded studies and carefully do the statistics.

Q6) Tell us about your research.
Well, my group works all in all on aptamers and their application, novel selection method variants, different library compositions, on the crossroads of biology and chemistry as well as in basic and translational sciences.

Q7) How did you know about the INSOAP?
At the first aptamer meeting 2014 in Oxford.

Q8) How will you support the INSOAP?
I don't know yet.

Q9) What kind of advice can give to the young researchers about aptamers?
Have fun! Don't be afraid of failures as long as you are willing to learn and trust your skills, you will make it.

Q10) What is your personal philosophy on life and science?
It is a privilege to be allowed to do science and also a duty to spend the money consciously and to make sth useful out of it. If you decide to do sth, do it whole heartedly.

Q11) What was your favourite part about research?
There are so many, cannot decide.

Q12) What do you like to do in your free time?
I like running, play guitar and attend concerts.

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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