NC3Rs 20 YEARS

Pioneering Better Science

Accelerating the replacement of animal-derived antibodies

Meeting report

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Introduction

1. This report summarises the outcomes from the '*Accelerating the replacement of animal-derived antibodies*' meeting held on 9 June 2023. The meeting brought together stakeholders from academia, industry (including antibody manufacturers), journals, funders and government to discuss the challenges associated with increasing the use of non-animal derived antibodies (NADAs) in scientific research and to develop recommendations to help accelerate their uptake.

Background

2. Antibodies are Y-shaped proteins which are produced by the immune system in response to foreign bodies known as antigens. They are made up of four polypeptide chains, with each chain containing a constant region and a variable region. The variable regions are found at the top of the antibody and are responsible for recognising and binding to antigens. Variable regions contain hypervariable loops, known as complementarity-determining regions, which directly interact with the antigen. The base of the antibody comprises the constant regions and these determine the antibody's class and effector function.

3. Antibodies are widely used as tools in scientific research to detect the presence of specific targets in biological samples. Research antibodies are traditionally produced by inoculating animals (usually mice or rabbits) with an antigen of interest to induce an immune response. The antigen-specific antibody is then recovered from the animal. It is estimated that one million animals per year are used in the EU alone for antibody production [1].

4. There are two types of animal-derived antibodies used in scientific research: polyclonal and monoclonal, which are derived in different ways. Polyclonal antibodies are recovered directly from the blood of inoculated animals. Monoclonal antibodies, however, are produced by isolating antibody-secreting B-lymphocytes from the spleens of inoculated animals and then fusing these with myeloma cells to create immortalised hybridoma cell lines which secrete the specific antibody *in vitro*. Polyclonals therefore consist of a mixture of antibodies produced in the natural immune response to an antigen. This includes multiple antibodies present in the producing animal at the time of serum sampling in addition to the antibody of interest, whereas monoclonal antibodies come from a single B-cell clone. The different production methods give different advantages to each antibody type. Monoclonal antibodies recognise a single epitope on the target antigen, which should give them a high level of specificity and reproducibility, although this is not always the case (see paragraph five). This makes them ideal for use in assays where precise and consistent binding to a particular epitope is required. Polyclonal antibodies recognise multiple epitopes on the target antigen. This gives a broader coverage and increased sensitivity, making them useful for applications where the target antigen is present at a low concentration. Polyclonals are also generally cheaper to produce than monoclonals which can make them a cost-effective option for researchers, particularly when high quantities of antibody are required. A summary of the key features of polyclonal and monoclonal antibodies can be found in Appendix 1.

5. In addition to the use of large numbers of animals in their production, there are also many scientific limitations to animal-derived antibodies. Because they are generated in a biological system, they often recognise additional proteins to the one they are developed to detect and suffer from batch-to-batch variation, impacting specificity and reproducibility between experiments respectively. These limitations are particularly prominent for polyclonal antibodies because of the way they are developed and their undefined composition, but also occur with monoclonals. Reproducibility issues can cause research to be abandoned, and the waste of time, money and resources due to the use of poorly characterised antibodies is estimated to cost $800M per year globally [2].

6. NADAs are made without using animals in the production process. They are selected *in vitro* by cloning genes encoding the variable regions of the selected antibody and inserting these into expression systems such as bacteriophages. In 'phage display' approaches, the antigen-binding part of the antibody is expressed on the surface of the phage particle and its ability to bind to the antigen of interest can be used to immobilise the phage. Antibody diversity can be introduced by cloning native antibody genes into phages that represent the complete human genome. Alternatively, random genetic mutations can be used to diversify the complementarity-determining regions of the antibody variable region. This creates a phage library of billions of different antibody variants that can be exposed to the target antigen. Phages which bind the antigen of interest are captured while those that do not bind are washed away. This selection process is known as 'panning' and can be repeated multiple times to enrich antibodies with the highest affinity. Once the most suitable antibody candidates have been identified from the phage library, they are expressed in recombinant expression systems (e.g. in scFv-Fc or IgG format in mammalian cells) which enable large scale production in a controlled and reproducible manner [3]. Every NADA is defined by its DNA sequence.

7. Phage display is used to produce NADAs but may also be used to create 'immune library' antibodies using genetic material encoding the variable regions of antibodies isolated from animals inoculated with an antigen of interest [4]. It is not always straightforward to create high-affinity binders using a fully synthetic approach and immune libraries can offer a compromise in some circumstances as less animals are used than during traditional antibody production. Researchers should be mindful of this distinction when selecting their antibodies.

8. Several other antibody-like technologies which do not use animals in their production also exist. These are known as non-antibody affinity reagents (ARs) and are produced using molecular engineering techniques. The most widely recognised of these include aptamers, affimers, ankyrons and DARPins, but there are others. Each technology has different advantages (see paragraphs 21 to 23), and they can be selected based on the required application. A summary of the key features of NADAs and ARs can be found in Appendix 1.

9. NADAs/ARs offer significant scientific benefits over traditional antibodies. These include unlimited supply, known chemical structure, minimal batch-to-batch variation, greater sensitivity and specificity for their targets and quicker production times (see paragraphs 14 and 15). Despite these

advantages, uptake of NADAs/ARs by the scientific community has been slow and animal-derived antibodies continue to be widely used.

10. In 2018 the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) convened an expert advisory committee to review the scientific validity of NADAs and ARs used for research, regulatory and diagnostic applications. Results from this were published in 2020 and recommended that animals should no longer be used for the development and production of antibodies for these purposes as well as therapeutic applications [1]. The report highlighted the availability and utility of animal-free technologies to produce reagents with equal or better quality than that offered by antibodies produced using conventional animal-based methods. Since publication of the report, specific action to address barriers to uptake of non-animal derived antibodies has been limited.

Workshop aims

11. The '*Accelerating the replacement of animal-derived antibodies'* workshop brought together stakeholders from across the bioscience sector. The workshop focused on the application of NADAs/ARs for research purposes (not diagnostic or therapeutic) as this is most in line with the NC3Rs remit. Key objectives of the workshop were to:

    - Raise awareness of NADA/AR technologies and the advantages of their applications.
    - Share perspectives on the challenges contributing to the slow uptake of these technologies.
    - Develop a consensus strategy to accelerate the adoption of NADAs/ARs, maximising their scientific, economic and animal welfare benefits.
    - Identify opportunities for how the NC3Rs can best support the research community in delivering this strategy.

Workshop presentations

12. Presentations delivered at the workshop covered the current status of NADAs, established alternative technologies to replace animal-derived antibodies and resources which are available to support their adoption. Dr Kilian Zilkens (Technical University of Braunschweig, Germany) provided an overview of the current research antibody market and emerging applications for NADAs and Dr Alison Grey (University of Nottingham, UK) outlined their scientific benefits. Dr Alejandra Solache (Abcam) expanded on the current and future opportunities in these technologies from a manufacturer's perspective, including the challenges faced in NADA design and production. Dr Darren Tomlinson (University of Leeds, UK) and Dr David Bunka (Aptamer Group, UK) showcased the benefits of novel AR technologies, focusing on affimers and aptamers (particularly Optimers®) respectively. Professor Pierre Cosson (University of Geneva, Switzerland) provided an overview of the ABCD (AntiBodies Chemically Defined) database which gives researchers access to sequencing data for 25,000 antibodies. A series of flash presentations were also given which

provided further detail on methods for generating NADAs and potential applications for these technologies. Key highlights from the workshop presentations are summarised below.

Status of non-animal derived antibodies

13. The current research antibody market is dominated by animal-derived antibodies, particularly polyclonals. Results from a Biocompare search[1] showed that in May 2021 polyclonal antibodies made up 62% and monoclonals 30% of available research antibodies (approximately 2.1M and 1M out of a total of 3.4M respectively). Recombinant antibodies, which are sequence defined but can be of animal or non-animal origin, made up 8% of the market (approximately 270,000) and only eight reagents (out of 3.4M) were defined as non-animal derived antibodies.

14. There are broad ranging benefits and opportunities for scientific advancement which can be achieved through the implementation of NADA phage display approaches. The phage library technique allows complete control over antibody structure, function and diversity, and selection pressures can be applied during the panning process to select antibodies with specific properties. There is the potential to express the world's entire antibody gene repertoire (which consists of over 10 billion human antibody genes) in phage display, and even go beyond this by creating NADAs to previously inaccessible target molecules.

15. A key advantage of NADAs is their speed of production compared to animal-derived antibodies. The phage display process can be used to produce antibodies within weeks, whereas inoculating an animal with antigen and isolating cells to produce antibodies takes months. This speed of generation was demonstrated during the COVID pandemic, where human animal-free neutralising antibodies to SARS-CoV-2 were generated within four weeks of the viral sequence being released and without the need for immunised donors.

16. Animal-derived polyclonal antibodies make up the majority of the commercially available antibody market (see paragraph 13), despite having several scientific limitations including batch-to-batch variation and often uncharacterised cross reactivities. However, there are scenarios where polyclonals are useful (such as where multiple epitope recognition or signal amplification is required) and in these instances the recent development of multiclonal NADAs offers an alternative to reagents traditionally derived in animals. Multiclonal NADAs are comprised of a mixture of different, carefully selected monoclonals with complementary epitope binding sites. Sequencing can be used to determine the precise amino acid sequence of the variable regions of antibodies in a polyclonal mix. The polyclonal can then be recreated as a multiclonal using a mix of non-animal

[1]Search conducted on biocompare.com, 4 May 2021.

derived recombinant monoclonals. Multiclonals provide a high level of specificity due to recognition of multiple epitopes on the same antigen, offering many of the advantages of polyclonal antibodies.

17. The availability of recombinant antibody sequences allows them to be easily identified and readily reproduced at scale with limited variability. To facilitate this, they must be unambiguously catalogued and easy to find. Several resources exist to support this (see https://www.antibodysociety.org/web-resources/), but the ABCD database (available at https://web.expasy.org/abcd/) developed by the Geneva Antibody Facility at the University of Geneva is one of the only ones that includes actual sequence information on the antibodies in question. The database is part of a broader project, with the mission of promoting the widespread use of recombinant antibodies by academic researchers and ultimately, the replacement of animal-derived products. It is a manually curated depository providing sequence information for 25,000 antibodies with known targets and can be easily accessed by researchers. Anyone developing their own NADAs are encouraged to submit their antibody sequences to the database.

18. Identifying and applying the most sensitive and specific research antibodies is vital for improving the reproducibility of published research. The Only Good Antibodies (OGA) community is working with stakeholders including research funders, manufacturers, publishers and regulatory authorities to create evidence-based solutions to achieve this and to drive policy change. This includes promoting a shift towards using NADAs/ARs. A partner in this initiative is YCharOS Inc, a Canadian open science company which aims to analyse the performance of antibodies available for every human protein through using knock-out cell lines as controls. This characterisation data is available through the public repository Zenodo (available at https://zenodo.org/communities/ycharos) and can be used by researchers when selecting antibodies. Importantly, they are working closely with antibody manufacturers to ensure that those antibodies which perform poorly can be rederived or removed entirely from production [5].

19. Despite their potential there are challenges to the implementation of NADAs and limitations to their use. Constructing phage display libraries can be challenging and resource intensive. The process typically requires specialised laboratories with substantial experience and although it is well established at commercial providers, the overall capacity is currently too low to meet all research needs. Where *in silico* design is used, this relies on the quality of the computational algorithms and the availability of structural information about the target. Production capacity is currently far from sufficient to replace all research antibodies with animal free methods. However, none of these challenges are insurmountable and the added scientific value offered by using NADAs should be considered carefully for each potential application.

Alternative non-animal technologies

20. Other non-antibody ARs that are derived without using animals are also being developed. Two of the most widely recognised are affimers and aptamers which are emerging as tools that can be

used alongside NADAs and in those applications where the utility of NADAs may be limited. The best reagent to use will depend on the research application.

21. Affimers are synthetic binding proteins derived from the cystatin protein scaffold. Affimers, like NADAs, are isolated from phage display libraries, but have increased stability and increased expression yield in *E. coli* and provide a viable alternative to primary and secondary antibodies in ELISA, Western blot, flow cytometry, immunohistochemistry and lateral flow devices. Their small size of 12 kDa is particularly useful for high-resolution microscopy as improved tissue penetration is beneficial to ensure that the fluorophore is placed in close proximity to the target. Additional advantages include soluble affimer expression in mammalian cells allowing *in vivo* studies, cost-effective protein production in *E. coli* without batch-to-batch variation and high affinity for a wide range of targets including proteins and small molecules.

22. Aptamers differ from antibodies and affimers as they are short single stranded DNA or RNA molecules rather than proteins. This difference in chemical composition allows aptamers to target a different range of molecules to antibodies, including small molecules, peptides and non-immunogenic targets. Like affimers, aptamers are smaller than antibodies enabling better access to their targets, and they can be generated in several weeks. They are also relatively stable and can withstand a range of temperature and pH conditions increasing their longevity and areas of application.

23. Optimers are a type of next generation aptamer that have been optimised according to the target, the desired end-use and the best manufacturing profile. They are created by analysing an aptamer at a sequence and structural level and testing multiple fragments for functionality. The best performing fragment becomes the Optimer. These tend to be 20% to 80% of the size of the parent aptamer, increasing tissue penetration and the binder's stability.

Breakout group discussions

24. Two breakout group sessions were held as part of the meeting. The aim of these sessions was for delegates to work together to (i) establish the challenges associated with the adoption of NADAs within the scientific community and (ii) to develop a strategy to best overcome these and accelerate their uptake to replace animal-derived antibodies.

Challenges to increasing the adoption of NADAs

25. Several challenges to increasing the adoption of NADAs were identified during the breakout group discussions, with many of these being highlighted consistently across the different groups. These included:

    - A lack of awareness of NADA technology, the products available and the scientific, commercial and animal welfare benefits of their application.

- Inertia amongst scientists to deviate from tried and tested reagents that have been published in the peer-reviewed literature.
- Concerns over the validation status of NADA/ARs, and the costs and time required to adopt these new methods.

26. There was general agreement amongst delegates that the wider scientific community was not aware of the broad availability of NADA/ARs or the state of readiness of these reagents for diverse applications where traditional animal-derived products are currently used. This may be being fuelled by researchers accepting the *status quo* and not recognising the wider scientific, commercial and animal welfare benefits these technologies offer. This extended to a lack of clarity over the identification of NADAs within antibody catalogues. Manufacturers will often use "recombinant" as an umbrella term to describe both animal- and non-animal derived antibodies that have been created through recombinant technologies without being explicit of their origin.

27. The pressure that scientists are under to generate and publish results quickly to obtain further grant funding was considered a contributing factor in the slow adoption of NADA/ARs. The "publish or perish" culture makes researchers more likely to select an antibody which has previously been used successfully within their lab or within established literature than they are to test an alternative. The current peer review process compounds this as reviewers are less likely to challenge accepted practice and reagents compared to novel tools and technologies. This results in a lack of drivers within scientific communities to encourage change. While animal-derived antibodies are available for purchase and funders and journals do not insist on the use of NADAs, scientists are unlikely to change practice.

28. Costs, both financial and time, were highlighted as barriers to uptake. NADAs are often more expensive to develop initially when compared to traditionally derived antibodies. However, once the sequence has been defined, reproducing NADAs is relatively cheap. Together with the cost savings from less variable results and limited batch-to-batch variation the initial outlay to develop the NADA can be offset fairly quickly. Adopting new affinity reagents can be a lengthy process because of the time needed to test and validate new antibodies, to ensure their reproducibility and to benchmark results against previous experiments and to adapt existing protocols. The lack of data available from suppliers to assess the quality of individual products was considered to contribute to the time it takes to adopt NADAs and was not helpful in building confidence in the technology.

Recommendations for increasing the use of NADAs

29. Breakout groups devised a list of recommendations for increasing the use of NADAs within the scientific community. These fit into key themes of raising awareness of NADAs, improving accessibility of reagents and resources, consistent characterisation of reagents, funding for work involving NADAs and adopting policy to phase out the use of animal-derived antibodies.

30. Greater education within the research community to raise awareness of both the availability and the scientific and 3Rs benefits of NADAs will foster a culture more accepting of these reagents. This will have a knock-on effect of driving demand for NADA/AR products, improving their accessibility and reducing production costs. Several initiatives to facilitate this were proposed and included:

    - Development of a central online platform or resource containing information on NADAs and ARs that include the scientific, commercial and 3Rs benefits of their application. This platform could provide links to existing repositories of sequenced antibodies, such as the ABCD database.

    - Suppliers should be encouraged to include easily accessible high level information on the breadth of NADA/ARs offered within their catalogues.

    - The development of relevant educational materials, including technology and application focused webinars and case studies championing NADA/AR uptake within specific contexts of use. This material could be hosted on the platform described above and showcased within scientific communities to help increase awareness and drive a change in end-user behaviour.

    - Journals promoting the inclusion of Research Resource Identifiers for all NADAs used in published research. This will highlight NADAs to scientists and support them in adopting published methods and reagents.

    - Publication of invited review articles in a high-profile journal to introduce NADAs to new areas of the research community.

31. To increase the uptake of NADAs amongst scientists who are already aware of their existence, financial barriers will need to be addressed. NADA/AR manufacturers and funding agencies were considered key stakeholders in this space. Some manufacturers already offer limited incentives to support the adoption of non-animal derived recombinant antibodies, but more opportunities to provide end-users with trial sizes of these products should be explored to facilitate their wider uptake. Abcam already offers such a scheme and is seeing the benefit of this, with their portfolio switching to offer a higher proportion of non-animal derived products reflecting preferential sales.

32. Funders should direct resources towards projects to test and characterise NADAs. This should include specific funding within a grant application to enable researchers to purchase NADA/ARs and characterise these alongside their regular products, and new funding to support the development of NADA/ARs where they do not already exist. This was considered by many participants to offer the lowest barrier to change, rather than expecting researchers to replace commonly used existing reagents. Specific funding to support the development or expansion of resources such as databases to collate existing NADA/ARs was also considered necessary.

33. Manufacturers should consider using a term other than “recombinant” in their antibody catalogues, as it is unclear if this refers to non-animal or animal-derived products. Specific and clear labelling of products developed without the use of animals will help customers to make informed purchases. Labelling should be fully transparent and describe whether animals have been used in both product development and production.

34. Scientists are highly data-driven. Greater effort should be made by manufacturers to characterise NADA/ARs for specific applications and compare these to the animal-derived antibodies more commonly used. Access to this information was considered as likely to drive uptake. Workshop participants recognised the efforts of the YCharOS group in characterising commercially available antibody reagents in immunoblot, immunoprecipitation and immunofluorescence applications, but highlighted the need to also provide validation for other applications and scale-up this approach to deliver the project in a timely manner.

35. Early career researchers are a key group to drive the uptake of NADAs. The next generation of scientists are generally more considerate of the 3Rs than generations before them and better informed on the issues of reproducibility and research integrity as most receive training in these as part of their PhD programmes. Focused effort should be made to target early career researchers with information on NADAs to drive change within their own practices and throughout wider scientific communities as they establish their own independent programmes of research.

36. Accelerating the use of NADAs and a shift from animal-derived products will require collective effort from the research community, manufacturers, funders and journals. There was general willingness amongst workshop delegates to remain involved in efforts to accelerate the adoption of NADA/ARs and suggestion that a ‘community of practitioners’ could be formed to facilitate sharing of best practice and to create new knowledge to encourage uptake of these reagents within the wider research community.

Next steps

37. Based on the recommendations from the breakout group discussions, the NC3Rs will take the following steps to support the research community adopt NADA/ARs and maximise their scientific and 3Rs impacts:

    - We will develop a diverse range of resources and materials to raise awareness of NADA/ARs amongst the research community. This will include, but is not limited to, an online platform that will host contemporary information about NADA/ARs, a webinar series and real-world case studies describing novel non-animal derived reagents and their application and signposting to available NADA/ARs and how to access these. These resources and materials will be freely and easily accessible through the NC3Rs website to enable all communities, including ethical review board members, researchers, policy makers and funders to benefit from them and facilitate change at a community level.

- We will work with NC3Rs-funded researchers and early career scientists so that they are supported in adopting antibody best practice and form a cohort of NADA/AR champions well-placed to influence research practices of their peers.

- We will support access to NADA/ARs so that any researcher, irrespective of career stage, can apply these novel tools in their own research. We will explore innovative funding mechanisms and partnerships with antibody manufacturers to facilitate this and enable characterisation/validation studies of these tools alongside existing products. Results from these studies will be made available to the scientific community to increase confidence in the technology.

- We will review our policies and application processes as a research funder on the use of NADA/AR in the work we sponsor. Applicants to our funding schemes will be encouraged to consider NADA/ARs and expected to justify their continued use of animal-derived antibodies. We will work with our funding panels on effectively assessing this. We will also explore opportunities for NC3Rs-funded researchers to apply Research Resource Identifiers to all antibodies they use (animal-derived or NADA/ARs) in publications, as is already required for the NC3Rs Gateway. We will engage other funders in developing similar policies/practices to ensure a harmonised approach.

- We will support efforts to validate both animal-derived antibodies and NADA/ARs to generate the evidence base necessary for wider adoption of the most reproducible reagents. We have established a partnership with the Only Good Antibodies initiative and YCharOS, leaders in this space, to move these aspirations forward. We will host a joint workshop in 2024 focused on improving the integrity and reproducibility of research involving antibodies and other affinity reagents.

38. We will continue to engage with antibody users, commercial suppliers and technology developers to deliver these actions. If you would like to contribute to this work please contact Dr Rachel Eyre, NC3Rs Programme Manager (Rachel.eyre@nc3rs.org.uk).

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Appendix 1

Reagent	Source	Features	Pros and Cons
Polyclonal antibody	Animal	Recovered from the blood of animals inoculated with an antigen of interest. Consists of a mixture of antibodies produced in the natural immune response to an antigen.	**Pros** ▪ Broad specificity and high sensitivity – recognise multiple epitopes on the target antigen. ▪ Generally cheaper to produce than monoclonals so can be a cost-effective option for researchers. **Cons** ▪ Batch-to-batch variation. ▪ Cross-reactivity. May recognise additional proteins to those the reagent has been designed to detect. ▪ Require animals for production.

Monoclonal antibody	Animal	Produced by isolating antibody-secreting B-lymphocytes from the spleens of inoculated animals. These are then fused with myeloma cells to create immortalised hybridoma cell lines which secrete the specific antibody *in vitro*. Isolated from a single B cell clone.	**Pros** ▪ High specificity for a single epitope on the target antigen – limits cross-reactivity. ▪ Increased reproducibility compared to polyclonals. **Cons** ▪ Batch-to-batch variation. ▪ May recognise additional proteins to those the reagent has been designed to detect (although to a lesser extent than polyclonals). ▪ Require animals for production.
Recombinant antibody	Can be animal or non-animal	Generated in phage display – genes encoding the variable region of the selected antibody are cloned and inserted into expression systems. Recombinant antibodies are defined by their DNA sequence. Phage display is used to produce NADAs but may also be used to create antibodies using genetic material encoding the variable regions of	**Pros** ▪ Known chemical structure. ▪ Minimal batch-to-batch variation. ▪ High sensitivity and specificity for targets. ▪ Control over structure and function. ▪ Non-animal derived recombinant antibodies do not use animals in the production process. **Cons** ▪ It can be challenging to produce NADAs against complex antigens. ▪ Increased initial production costs compared to polyclonal or monoclonal antibodies. Can affect affordability for researchers.

		antibodies isolated from animals inoculated with an antigen of interest. Care should be taken when selecting recombinant antibodies to ensure they are NADAs.	
Multi-clonal NADA	Non-animal	Created using a mix of non-animal derived monoclonals with complimentary epitope binding sites. Can give benefits of traditional polyclonal antibodies such as multiple epitope recognition or signal amplification.	**Pros** ▪ Broad specificity and high sensitivity. ▪ Pan targets – each monoclonal can target a different form of a protein to give reactivity across the family. ▪ Can be created to allow detection across a range of species by mixing monoclonals. ▪ Do not use animals in production. **Cons** ▪ It can be challenging to produce NADAs against complex antigens. ▪ Increased initial production costs compared to polyclonal antibodies. Can affect affordability for researchers.
Affimer	Non-animal	Synthetic binding protein derived from a cystatin protein scaffold. Isolated from phage display library.	**Pros** ▪ Known chemical structure. ▪ Minimal batch-to-batch variation. ▪ Can be engineered to bind a wide range of targets including proteins, peptides and small molecules.

			▪ Small size (12kDa) – gives improved tissue penetration for applications such as high-resolution microscopy. ▪ High stability – can withstand a range of temperature and pH fluctuations. ▪ Do not use animals in production. **Cons** ▪ Limited commercial selection and availability. ▪ A lack of historical performance data compared to antibodies.
Aptamer	Non-animal	Short single stranded DNA or RNA molecules.	**Pros** ▪ Known chemical structure. ▪ High affinity and specificity to a wide range of target molecules including peptides, small molecules and non-immunogenic targets. ▪ Small size (20-100 nucleotides) – gives improved tissue penetration for applications such as high-resolution microscopy. ▪ High stability – can withstand a range of temperature and pH fluctuations. ▪ Do not use animals in production. **Cons** ▪ Nucleic acids are susceptible to degradation by nucleases. May result in a limited half-life in biological systems. ▪ Limited commercial selection and availability.

			▪ A lack of historical performance data compared to antibodies
Ankyron	Non-animal	Direct *in vitro* selection by ribosome display. Single, highly stable, small 15kD binding proteins, based on ankyrin repeat scaffold. Recombinant, monoclonal, sequence defined by default.	**Pros** ▪ Known chemical structure. ▪ Small size (15kDa) - gives improved tissue penetration for applications such as high-resolution microscopy. ▪ High stability – can withstand a range of temperature and pH fluctuations. ▪ Do not use animals in production. **Cons** ▪ Limited commercial selection and availability. ▪ A lack of historical performance data compared to antibodies.