Phase-Appropriate Implementation of a Domain Specificity Strategy

Matthias Reichel, MSc | 23 April 2024

BioAgilytix

Agenda

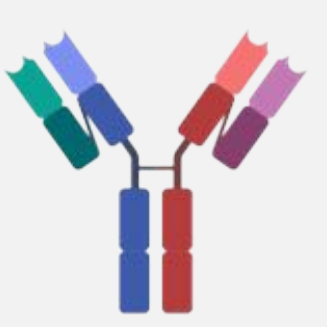
1. Introduction to Multi-Domain Therapeutics
2. Strategies for Assessing Domain Specificity
3. Domain Specificity – a Must-Have for Multi-Domain Therapeutics?

Introduction to Multi-Domain Therapeutics

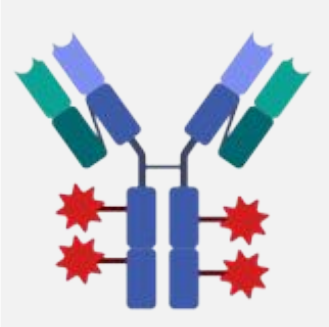
It’s getting more complex

Multi-Domain Therapeutics

- Therapeutics which contain **2 or more** structural domains or components
- Each with distinct function and/or property relevant to the mechanism of action (MoA)
- Domains linked together through genetic/protein engineering, chemical conjugation or by self-assembly

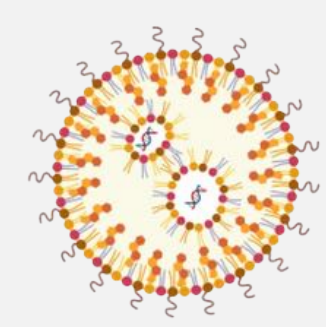


Multi-specific antibodies



Antibody-drug conjugates

PEGylated protein/peptides



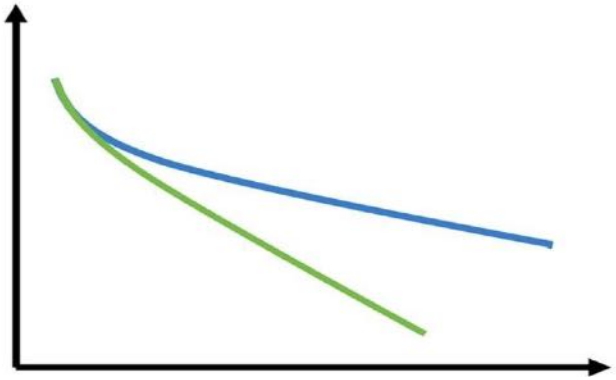
Lipid nanoparticle (LNP) encapsulated RNA/DNA

Images: Created with BioRender.com

Multi Domain Therapeutics

Benefits:

- Improved pharmacokinetics
- Enhanced efficacy
- Targeted delivery
- Delivery of unstable components
- Directing and inducing immune cells
- Induction of protein complexes
- Reduced side effects
- Versatility



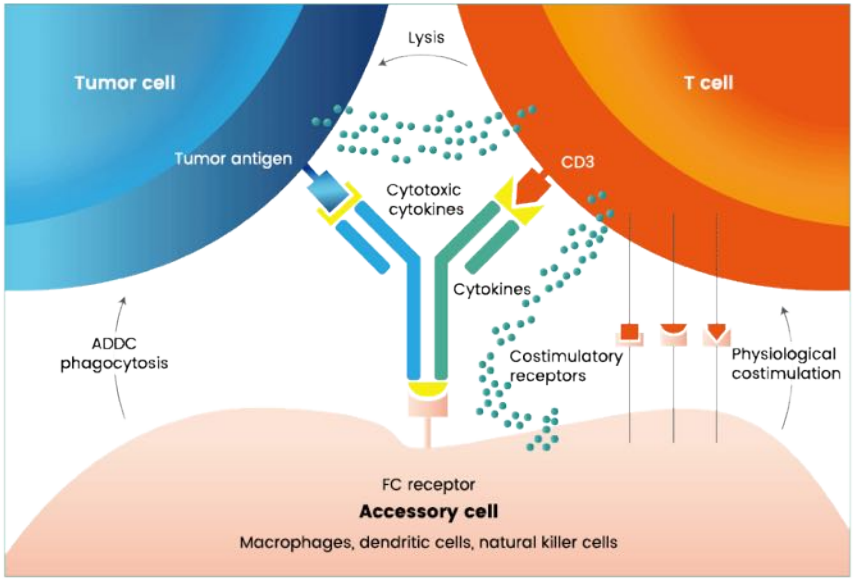


Image: https://www.sinobiological.com

Immunogenicity Testing

- Clinical immunogenicity assessment typically follows a multi-tiered approach
- For a 'typical' biotherapeutic, characterization usually consists of:
  - Titer
  - Neutralizing antibodies (NAb)
- For multi-domain therapeutics, characterization may also require the elucidation of the domain specificity of the immune response

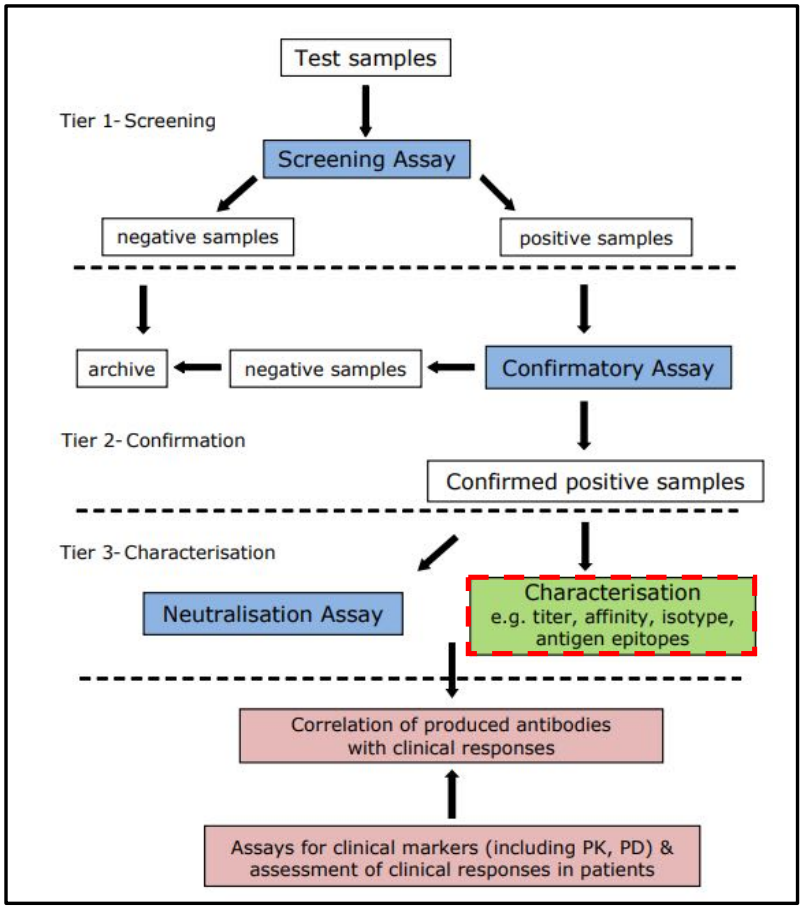


Image: EMA Guideline on Immunogenicity Assessment of Therapeutic Proteins EMEA/CHMP/BMWP/14327/2006 Rev 1

Immunogenicity Considerations

- Considerations for Multi-Domain Therapeutics:
  - immunogenic structural or linear epitopes
  - significant homology of a domain with endogenous protein
  - repetitive antigenic structures
  - neoepitopes or non-natural sequences due to molecule engineering
  - possibility of epitope spreading
  - hapten effect due to conjugation/fusion with larger protein
- No one-size fits all, but assessment based on molecular structure and mechanism(s) of action

Myler, H *et al.*, Anti-drug Antibody Validation Testing and Reporting Harmonization, AAPS J. 2022; 24:4
Gorovits B, Peng K, Kromminga, A. Current Considerations on Characterization of Immune Response to Multi-Domain Biotherapeutics, BioDrugs. 2020 Feb; 34(1):39-54
Gorovits B, Wakshull E, Pillutla R, Xu Y, Manning MS, Goyal J. Recommendations for the characterization of immunogenicity response to multiple domain biotherapeutics. J Immunol Methods. 2014;408:1–12.

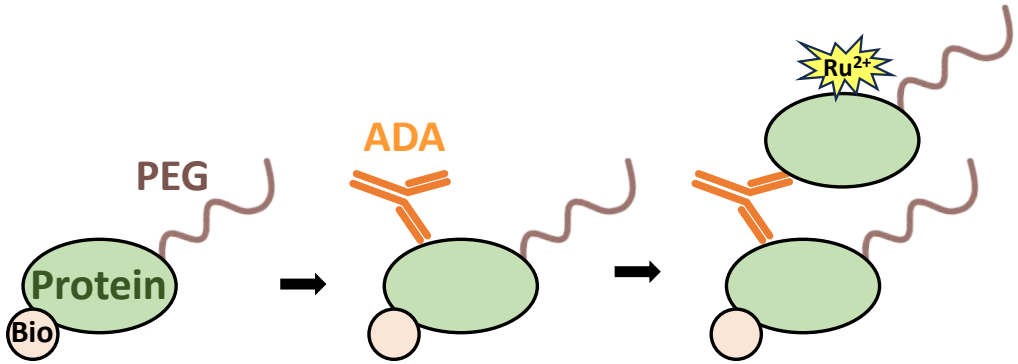
Strategies for Assessing Domain Specificity

Choosing an assay format

PEGylated Protein: Total ADA Assay

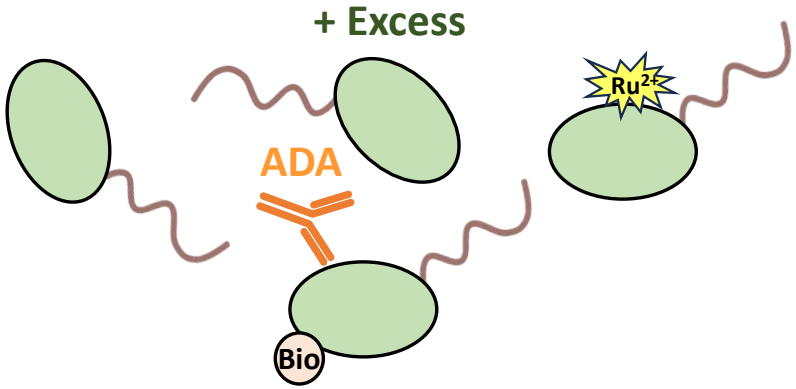
Screening Assay

Whole molecule for bridging assay



Confirmatory Assay

Competition with whole molecule

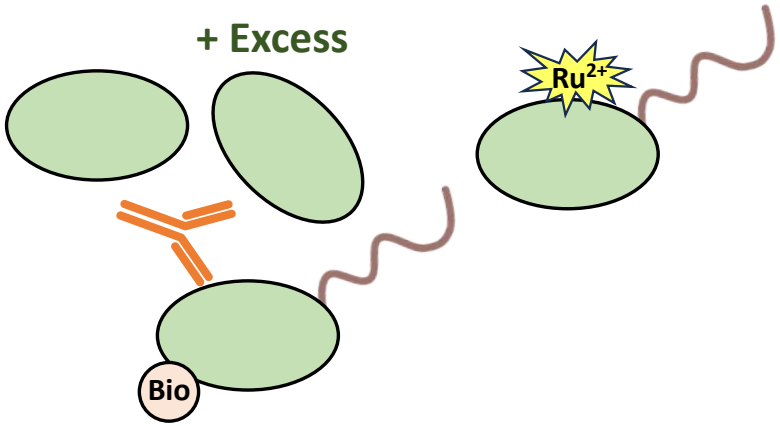


Images: Created with BioRender.com

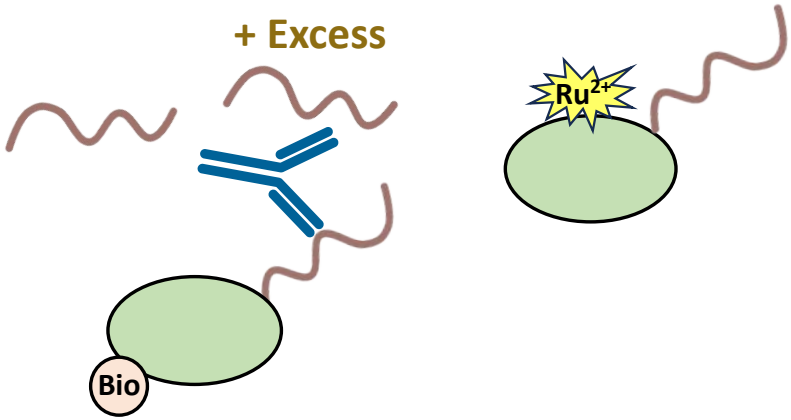
PEGylated Protein: ADA Specificity Assay

Domain Competition Confirmatory Assays

Competition with protein domain



Competition with PEG domain



A specific anti-PEG SPC has to be used.

Images: Created with BioRender.com

Domain Competition Confirmatory Assays

Requirements

- Individual domains for the competition assays and (ideally) domain-specific positive control (PC) antibodies
  - Domain reagents are readily available for some molecule formats, but can be extremely challenging to produce for other formats
  - May be possible to use a polyclonal PC which contains antibodies against each domain

PROs

- Relatively straightforward to set up, as the specificity assay(s) are based on the confirmatory tier (only one development)

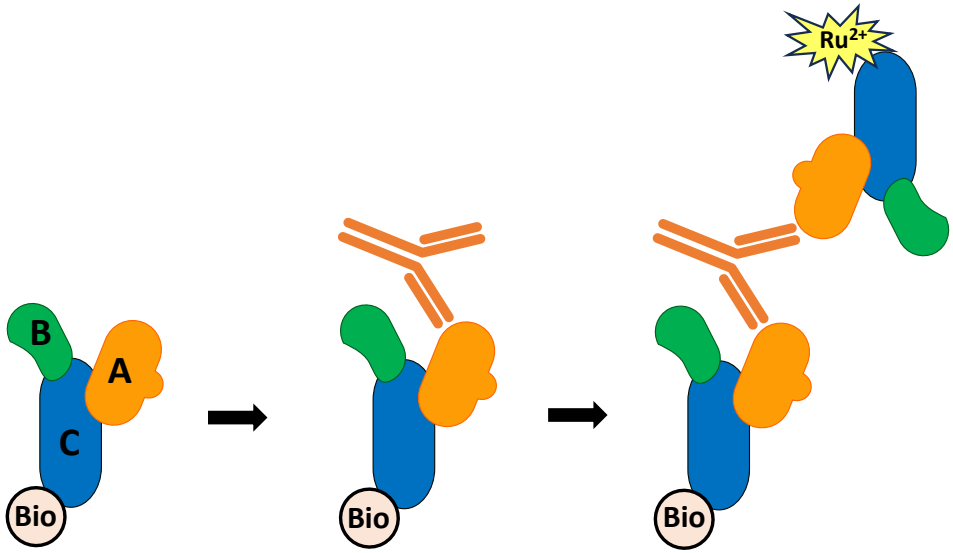
CONs

- Only qualitative results possible (i.e. positive vs. negative for each domain)
- The approach can lack sensitivity to detect low levels of domain specific antibodies, particularly if there is a high prevalence of ADA to the other domain

Tri-functional Protein: Total ADA Assay

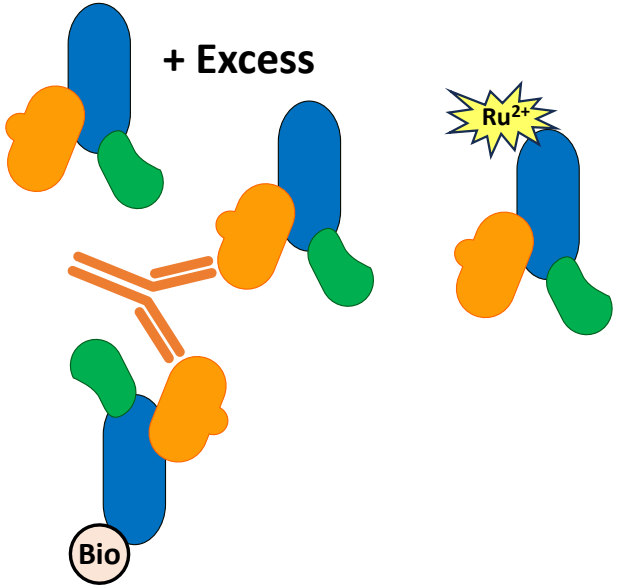
Screening Assay

Whole molecule for bridging assay



Confirmatory Assay

Competition with whole molecule

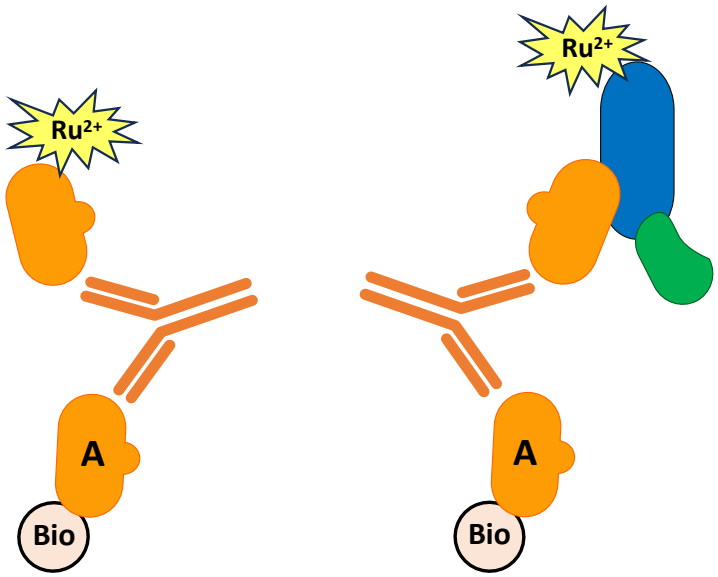


Images: Created with BioRender.com

Tri-functional Protein: ADA Specificity Assay

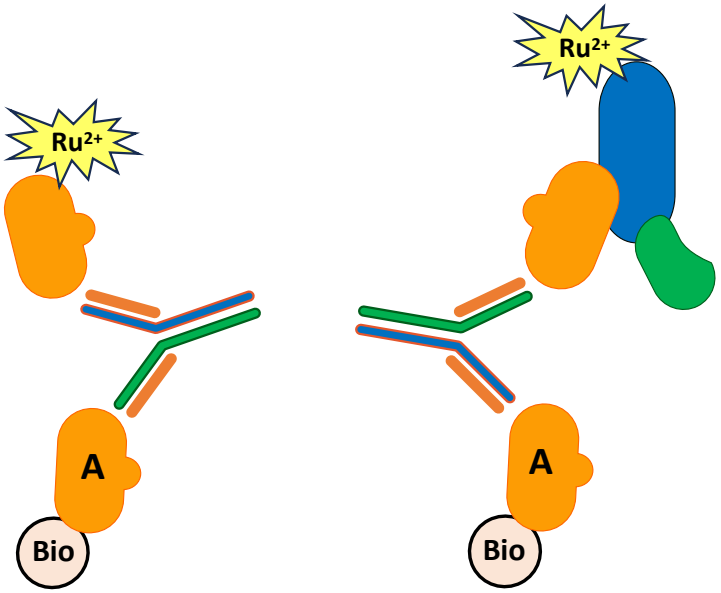
Separate Screening Assays

Monoclonal SPC against Domain A



Option 1 Option 2

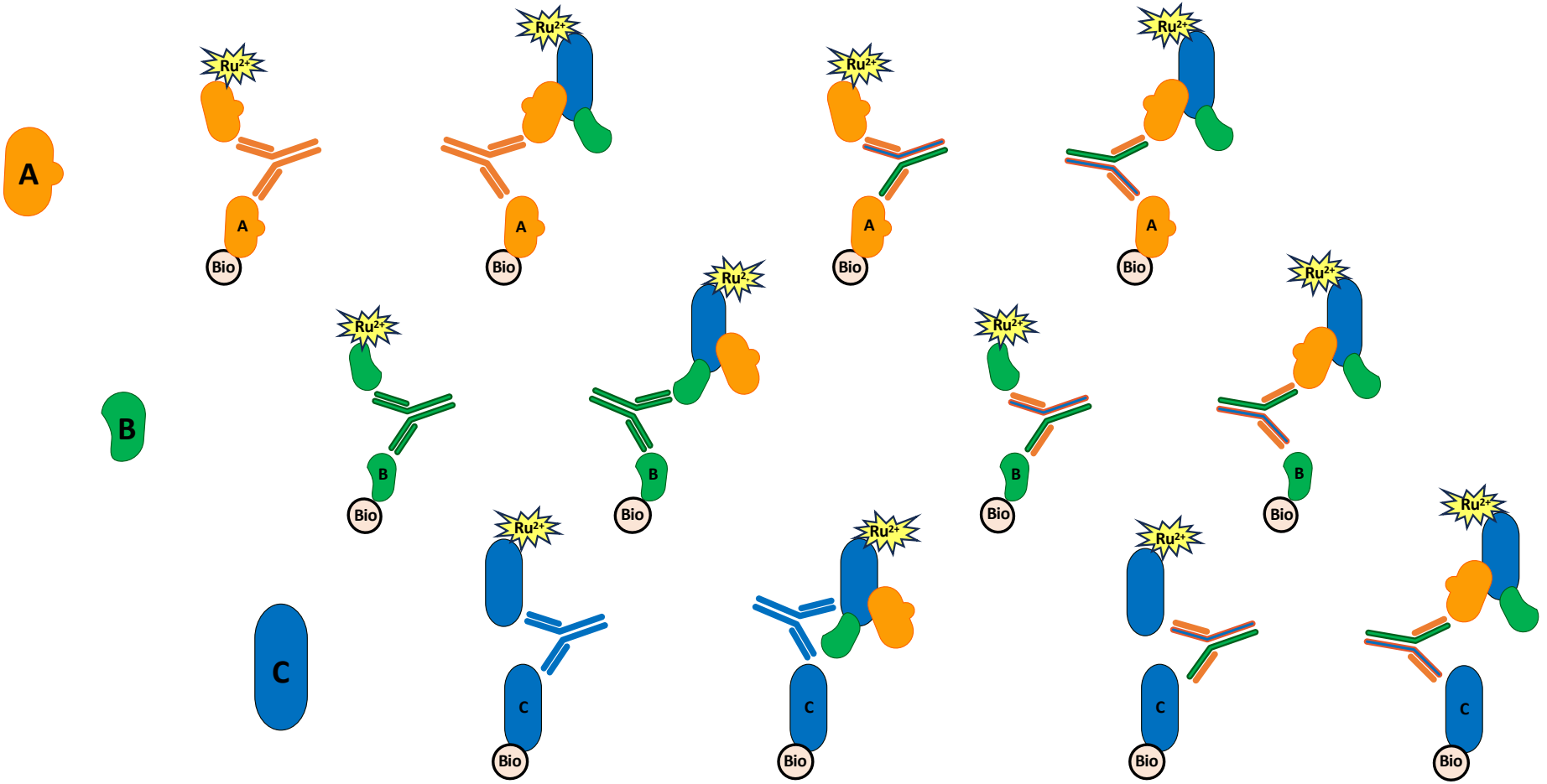
Polyclonal SPC against Tri-functional Protein



Option 3 Option 4

Images: Created with BioRender.com

Tri-functional Protein: ADA Specificity Assay



Images: Created with BioRender.com

Separate Domain Screening Assays

Requirements

- Individual domains for the assay set-up and domain-specific or polyclonal SPC
  - Domain reagents are readily available for some molecule formats, but can be extremely challenging to produce for other formats

PROs

- For simpler molecules like bispecifics, it can be relatively straightforward to set up, as the specificity assays are based on the screening assay conditions
  - Some optimization may be required with the different domain capture/ detection and the different PCs
- Usually sensitive to detect domain-specific antibodies
- Semi-quantitative readout possible (by titer or S/NC ratio)

CONs

- For more complex multi-domain proteins, extensive assay set-up is required for each domain-specific assay

Fusion Protein with Endogenous Counterparts

Therapeutic Fusion Protein

Domain A (sequence-optimized)

Domain B (sequence-optimized)

* amino acid composition different to endogenous proteins

Cross-reactivity of potential immune response
- ADA
- NAb

Full-length endogenous protein A

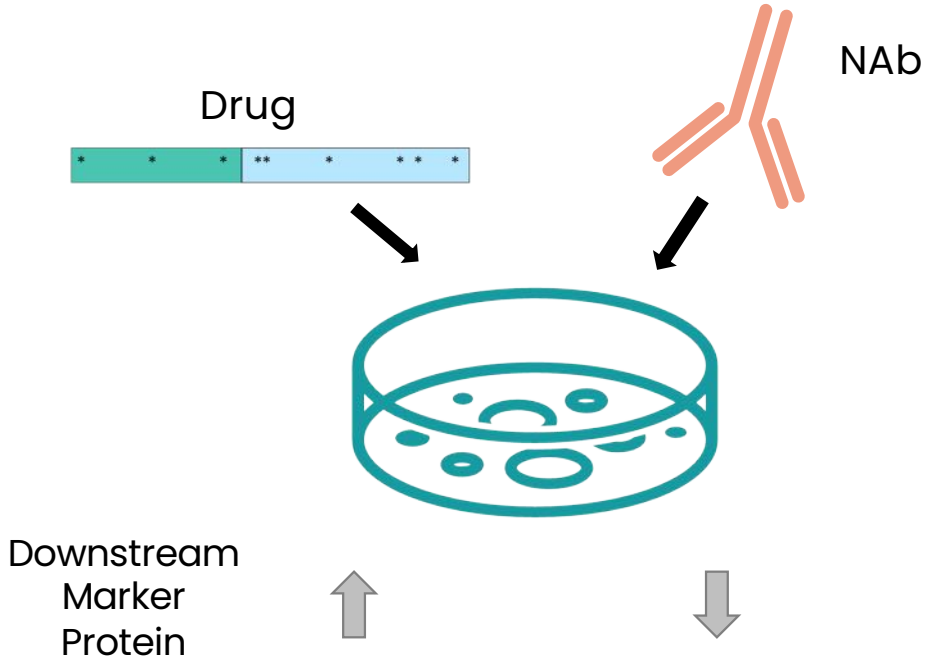
Full-length endogenous protein B

→ Domain Assays use the full-length endogenous proteins but not the sequence-optimized domains of the therapeutic

Cell-based NAb Assay For Fusion Protein

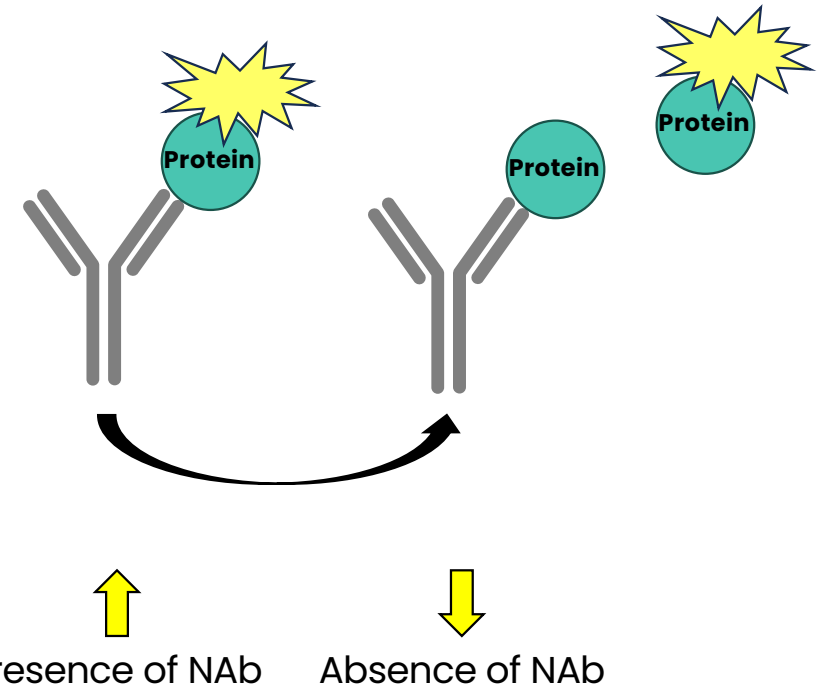
Cell-based Assay

Based on Mechanism-of-Action



Quantification of Marker Protein

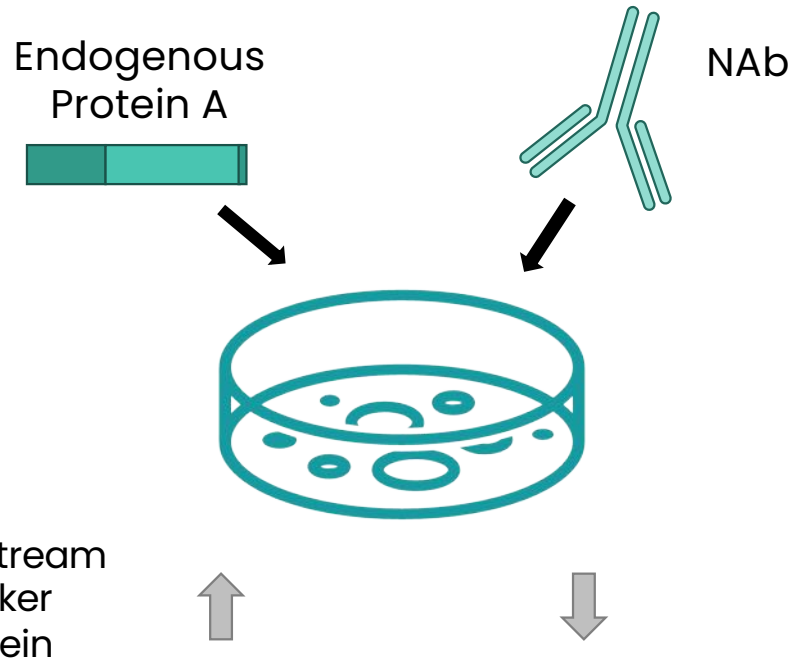
Commercial ELISA



Cell-based NAb Assay For Endogenous Protein A

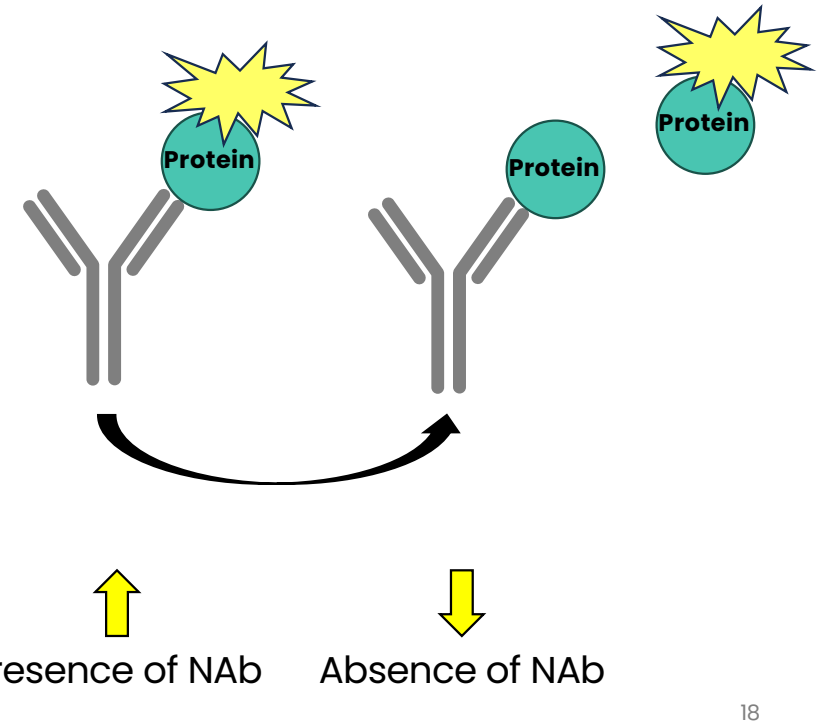
Cell-based Assay

Based on Mechanism-of-Action



Quantification of Marker Protein

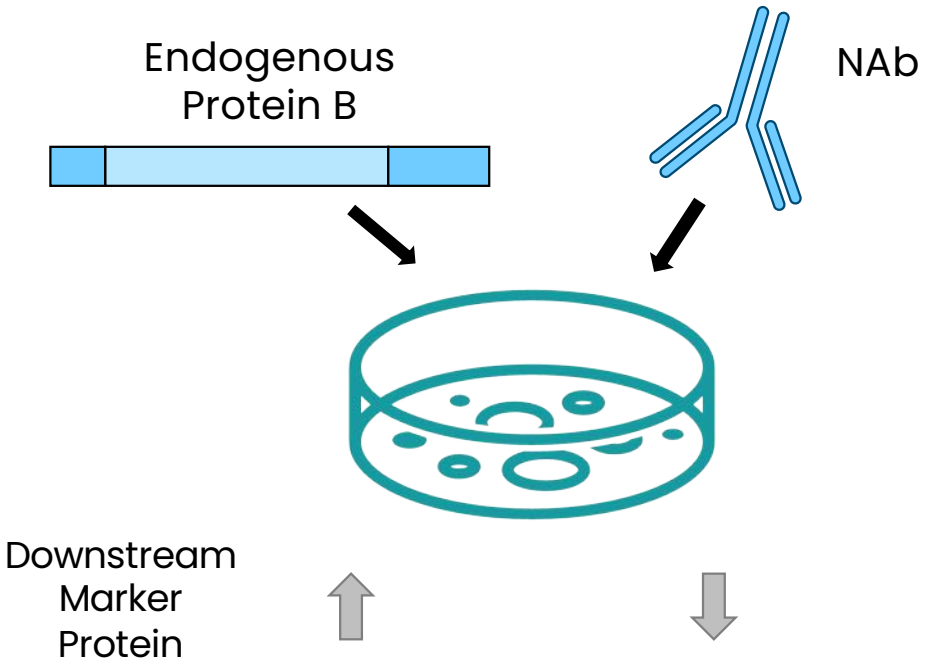
Commercial ELISA



Cell-based NAb Assay For Endogenous Protein B

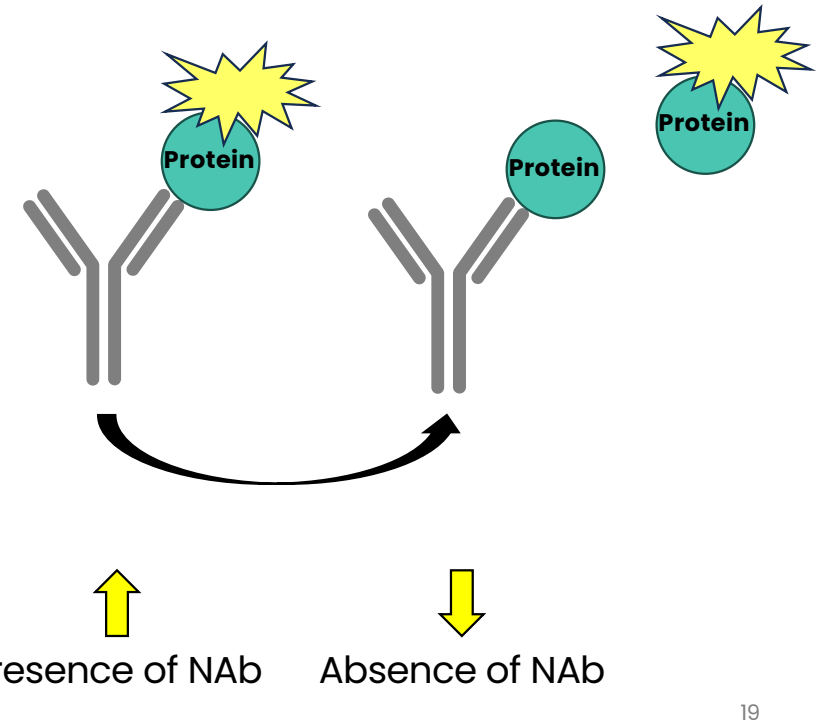
Cell-based Assay

Based on Mechanism-of-Action



Quantification of Marker Protein

Commercial ELISA



Domain Specificity – A Must-Have for Multi-Domain Therapeutics?

Regulatory expectations, timing, advantages, and challenges

Regulatory Expectations

Immunogenicity Testing of Therapeutic Protein Products, FDA

IV 3. Domain Specificity

- For multi-domain therapeutic protein products, the sponsor may need to investigate whether the ADA binds to specific **clinically relevant domains** in the protein.
- For example, to adequately understand the risk of ADA to subjects for therapeutic protein products with modifications such as pegylation, sponsors should **develop assays to determine the specificity of ADA for the protein component as well as the modification** to the therapeutic protein product (Gorovits *et al*. 2014).

Guideline on Immunogenicity Assessment of Therapeutic Proteins, EMEA

7.5. Immunogenicity assessment of conjugated proteins and fusion proteins

- Elicitation of an antibody response with multiple specificities and variable affinity towards different epitopes resulting in **varying degrees of clinical impact** is expected for novel biotherapeutic molecules such as engineered fusion proteins and chemically conjugated proteins.
- The evaluation of this response, in particular, the characterization of the specificity of the induced antibodies is challenging and **may require multiple assays for measuring immune responses to various moieties**.

Regulatory Expectations/ White Paper Recommendation

Bispecific Antibody Development Programs, FDA

C 1. Clinical Pharmacology Studies

- When examining immune responses to bispecific antibodies, **it may be appropriate to develop multiple assays to measure responses to different domains** of bispecific antibodies.

ADA Validation Testing and Reporting Harmonization, Myler *et al.* 2022

- The immunogenicity assessment strategy is driven by the **molecule's risk assessment** and is a regulatory expectation
- For MDB, the regulatory expectations are clear; a sponsor should consider a **determination of immune response to** the entire molecule, **each of the domains** as well as to any neo epitopes to provide a thorough assessment of immunogenicity.
- Current industry practice is to do this evaluation in the standard tiered fashion in the **clinical phases, in particular earlier phases for MDB with limited clinical experience.**

MDB: multi-domain biologic

Timing of Domain Specificity Assessment

Preclinical

- Usually, **no domain specificity assessment**
- Preclinical immunogenicity observations can rarely be translated to humans
- When performed, information might be feed back into future programs

Phase I

- May not require **detailed assessment** of domain specificity for all multi-domain therapeutics
- Would help to **scientifically understand** potential immune responses against the therapeutic
- Developing domain specificity assays and assessing samples **will help determine the level of characterization** required in later clinical stages

Phase II

- May require **some level of ADA domain specificity**
- Extent can be guided by **risk of the molecule** and observation of both **incidence and consequence seen in earlier studies** (e.g. changes in PK/PD)

Phase III

- May require **some level of ADA domain specificity**
- Extent can be guided by **risk of the molecule** and observation of both **incidence and consequence seen in earlier studies** (e.g. changes in PK/PD)

Advantages of Domain Specificity Assessment

Scientific Understanding of Therapeutic's Immunogenicity

- Identify the immunogenic domains of the therapeutic
- Allows for optimization/modification of therapeutic to reduce immunogenicity
- Might only apply to future programs again using the same domain

Advantages of Domain Specificity Assessment

Patient Safety and Therapeutic Efficacy

- Standard ADA assay detects the totality of ADA

  → allows correlation with safety and efficacy-related aspects

- In case any of the domains has an **endogenous counterpart with high homology**, it might be critical to understand if the immune response is directed against this domain, as it might lead to a **loss of the endogenous activity**

- Some components are known to **activate specific immune responses** (e.g. Anti-PEG antibodies trigger complement activation by PEGylated lipid-based nanoparticles. *Senti ME et al. J Controlled Release (2022)*)

Challenges of Domain Specificity Assessment

- **Technically challenging**: due to the **complexity of the biotherapeutic** and the potential for **cross-reactivity** between domains
- **Bioanalytical challenges**: require highly **specific** and **sensitive** assays as well as appropriate **critical reagents**
- **Effort vs. value**: **time-consuming** and **expensive** vs. **deeper understanding of immune response** and **additional correlations** with **PK/safety/efficacy**
- **Interpretation challenges**: presence of ADAs against one domain might influence the detection or binding of ADAs against another domain and **sensitivity** of each of the assays might be different

Acknowledgements

THANK YOU…

BioAgilytix colleagues

GREAT COLLABORATION AROUND THE WORLD

SUPPORTING THIS PRESENTATION

Sponsors

INTERESTING PROJECTS

GREAT SCIENTIFIC DISCUSSIONS

EIP

ORGANIZING THE SYMPOSIUM

PRESENTING OUR WORK

Thank You

Matthias Reichel

Principal Investigator

matthias.reichel@bioagilytix.de

Linkedin.com/company/bioagilytix Twitter.com/bioagilytix YouTube bioagilytix4402

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