Pros and Cons of Different Approaches to ADA Domain Specificity Characterisation

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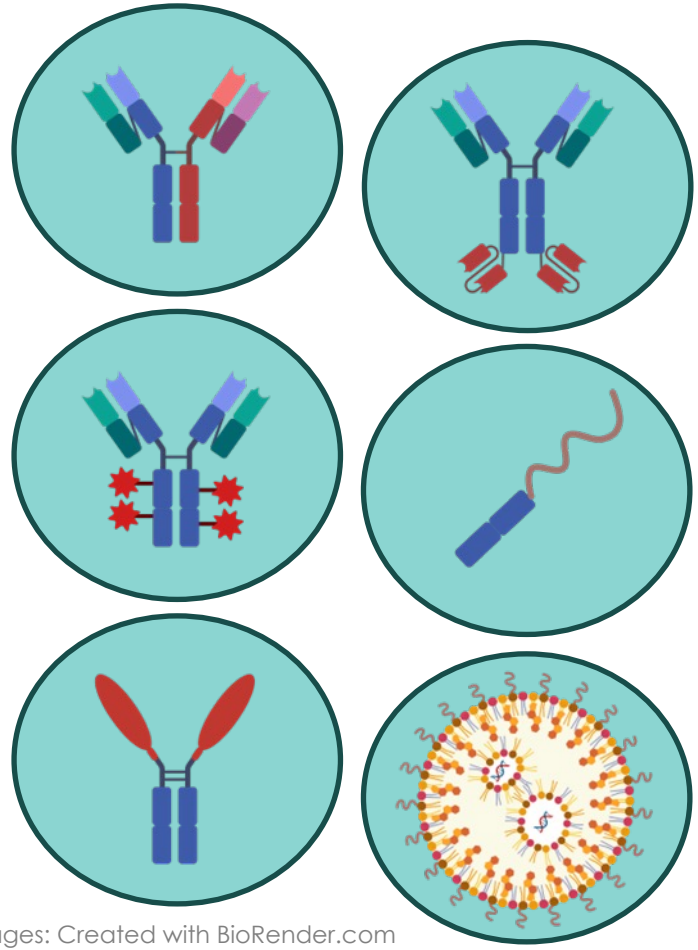
Agenda

- Introduction to multi-domain therapeutics
- Approaches for domain specificity assessment
- Pros & cons of different approaches
- Concluding remarks

Multi-domain therapeutics

What are they?

- Therapeutics which contain 2 or more structural domains or components
- Each with a distinct function relevant to the mechanism of action (MoA)
- Domains linked together through genetic/protein engineering or chemical conjugation
- Examples include:
  - Bi-specific & tri-specific antibodies
  - Antibody-drug conjugates (ADCs)
  - PEGylated proteins/peptides & fusion proteins
  - Lipid nanoparticle (LNP) encapsulated RNA/DNA

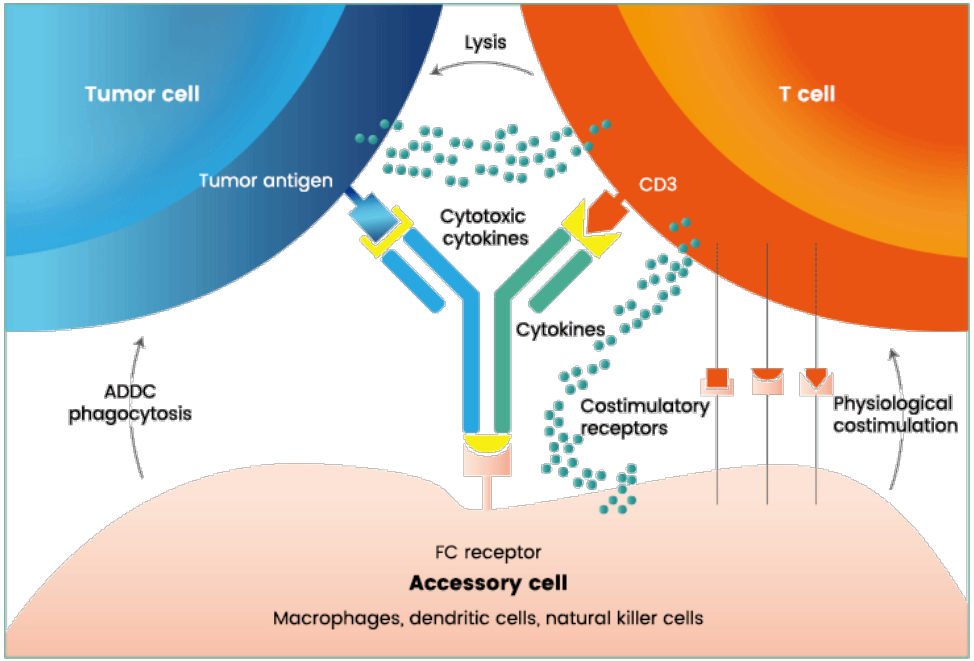


Images: Created with BioRender.com

Multi-domain therapeutics

What are the benefits?

- Enhanced efficacy
- Targeted delivery
- Reduced side effects
- Versatility
- Improved pharmacokinetics
- Cost-effective



Images: https://www.sinobiological.com

Evaluating the immunogenicity of therapeutic proteins

- Clinical immunogenicity assessment typically follows a multi-tiered approach
  - **Screening**
  - **Confirmation**
  - **Characterisation**

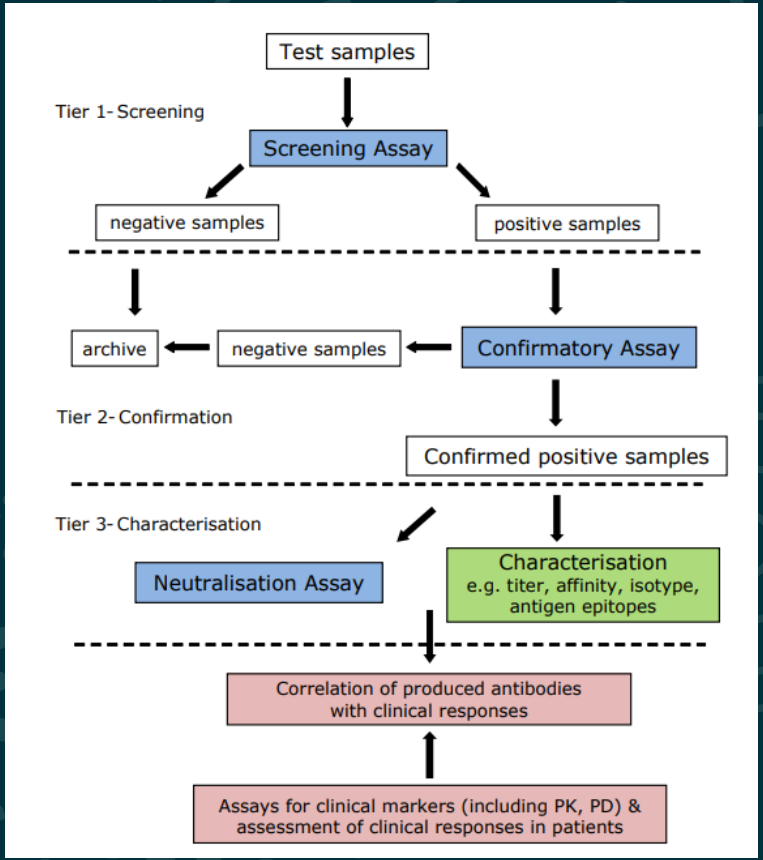


Image: EMA Guideline on Immunogenicity Assessment of Therapeutic Proteins EMEA/CHMP/BMWP/14327/2006 Rev 1

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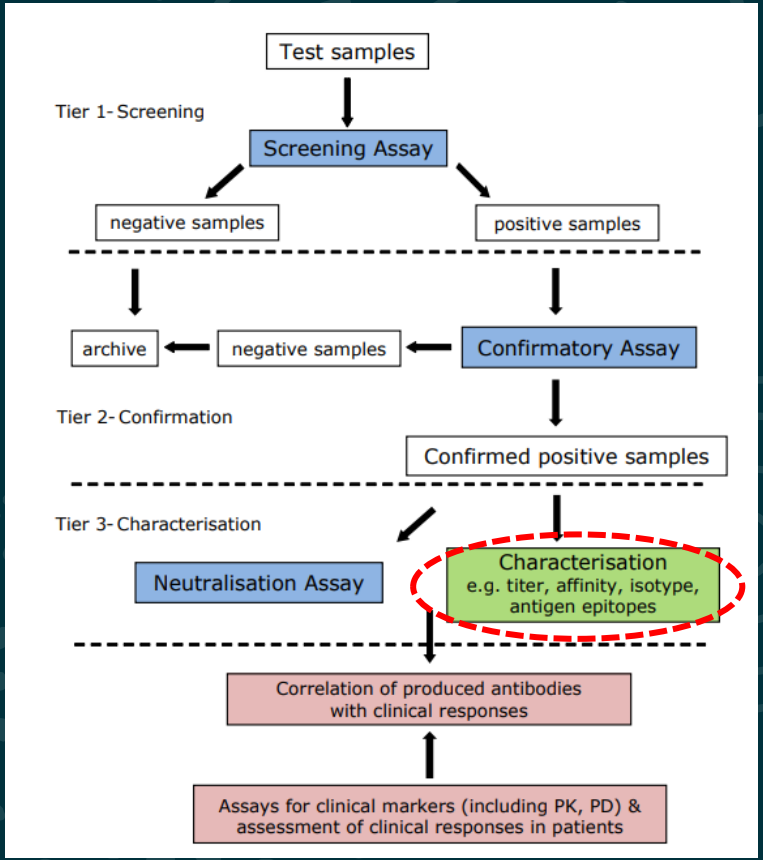
Evaluating the immunogenicity of therapeutic proteins

- For a 'typical' biotherapeutic, characterisation usually consists of:
  - **Titer**
  - **Neutralising antibodies (NAb)** for later clinical studies

- For **multi-domain therapeutics**, characterisation may also require the elucidation of the **domain specificity** of the immune response



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Strategies for assessing ADA domain specificity

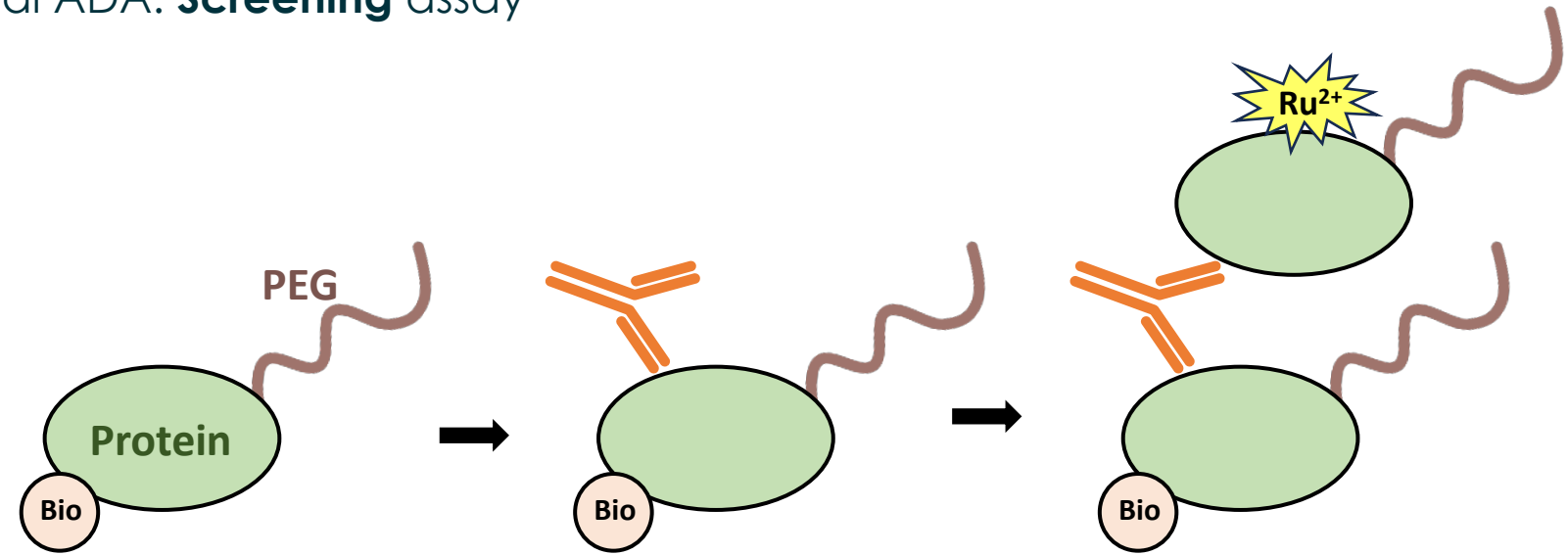
Strategies for ADA domain specificity characterisation

- Characterisation of the ADA domain specificity can be achieved by a number of approaches, typically:
  - **Domain competition** (usually based on the confirmatory assay)
  - **Separate assays** with each domain, and a positive control to that domain

ADA domain specificity characterisation

Example: PEGylated peptide/protein

Total ADA: **Screening** assay

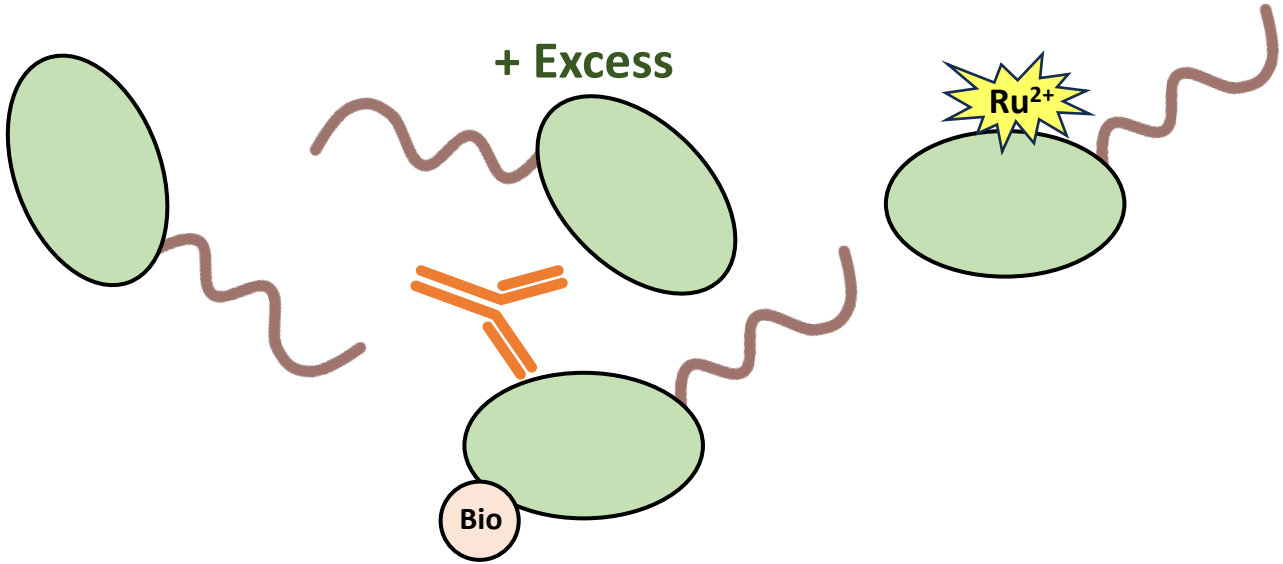


ADA domain specificity characterisation

Example: PEGylated peptide/protein

Total ADA: **Confirmation** assay

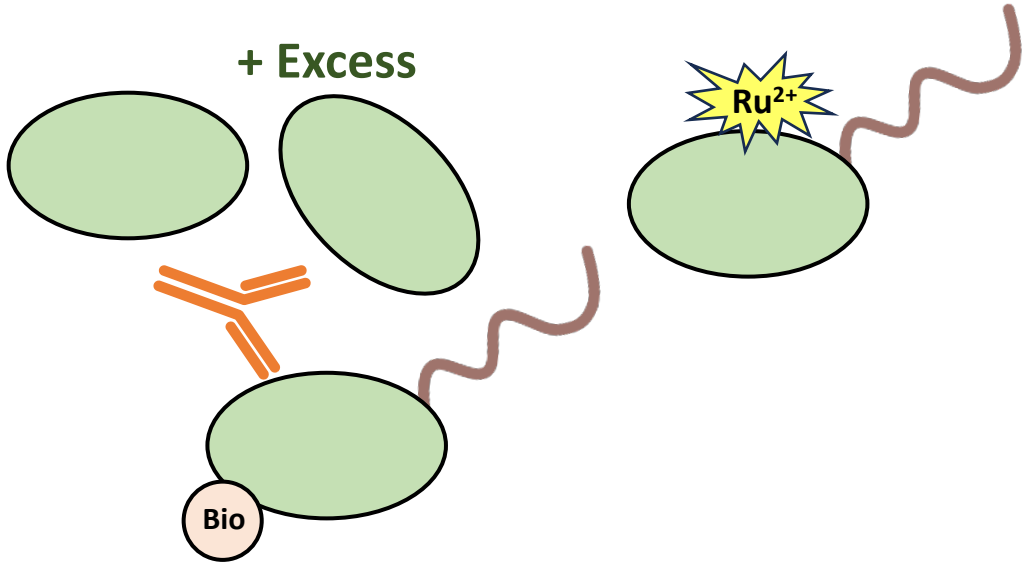
- Compete with whole molecule



ADA specificity determination: Protein domain

Example: PEGylated peptide/protein

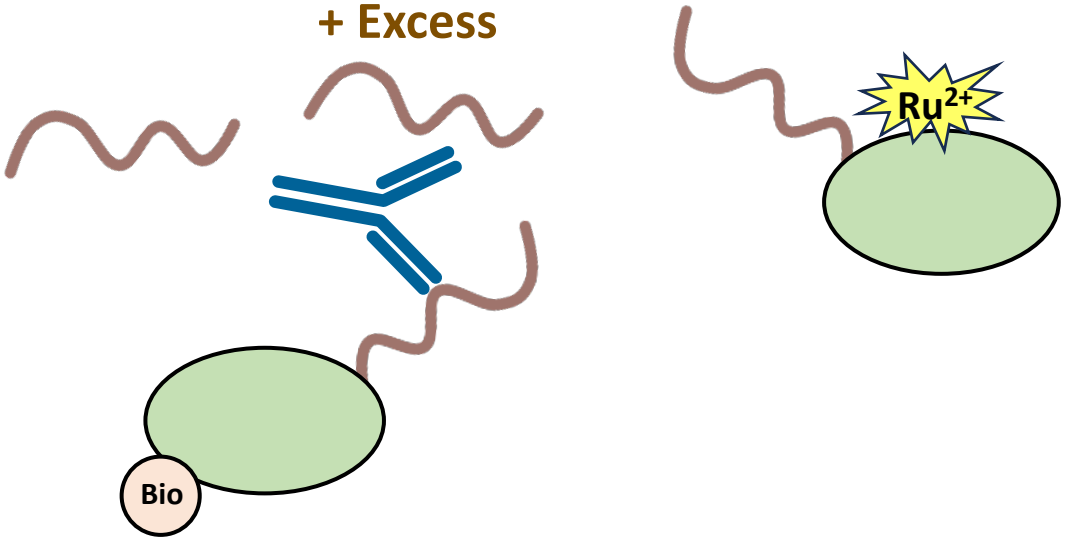
- Compete with protein lacking PEG



ADA specificity determination: PEG domain

Example: PEGylated peptide/protein

- Compete with excess PEG
  - Requires an anti-PEG PC

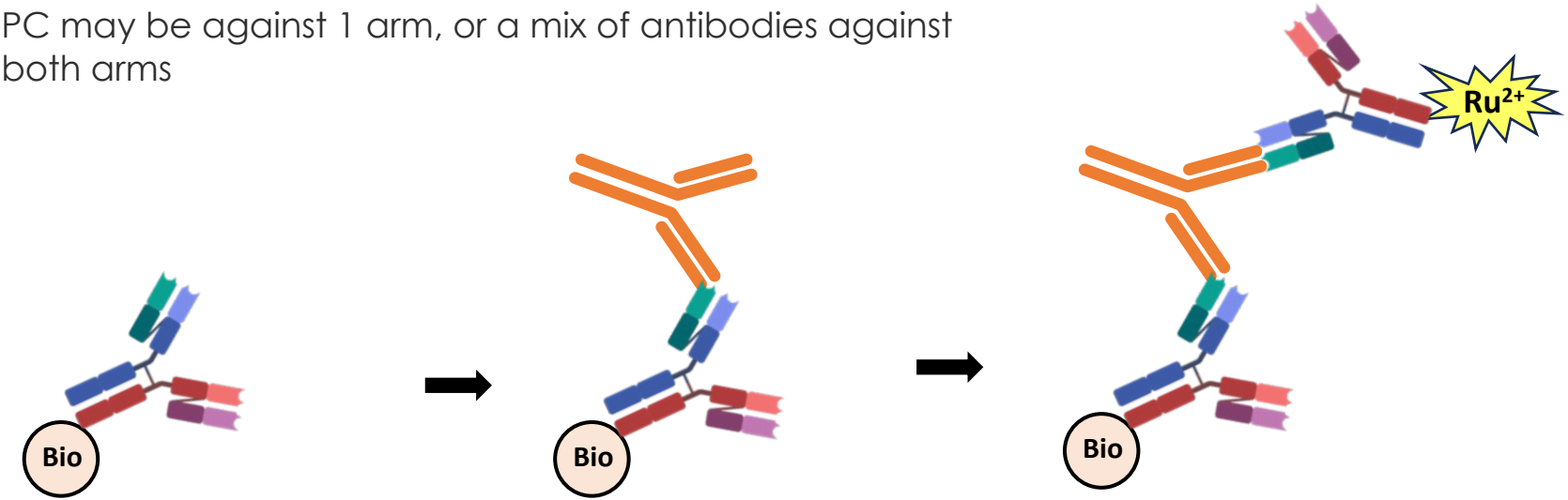


ADA domain specificity characterisation

Example: Bispecific antibody

Total ADA: **Screening** assay

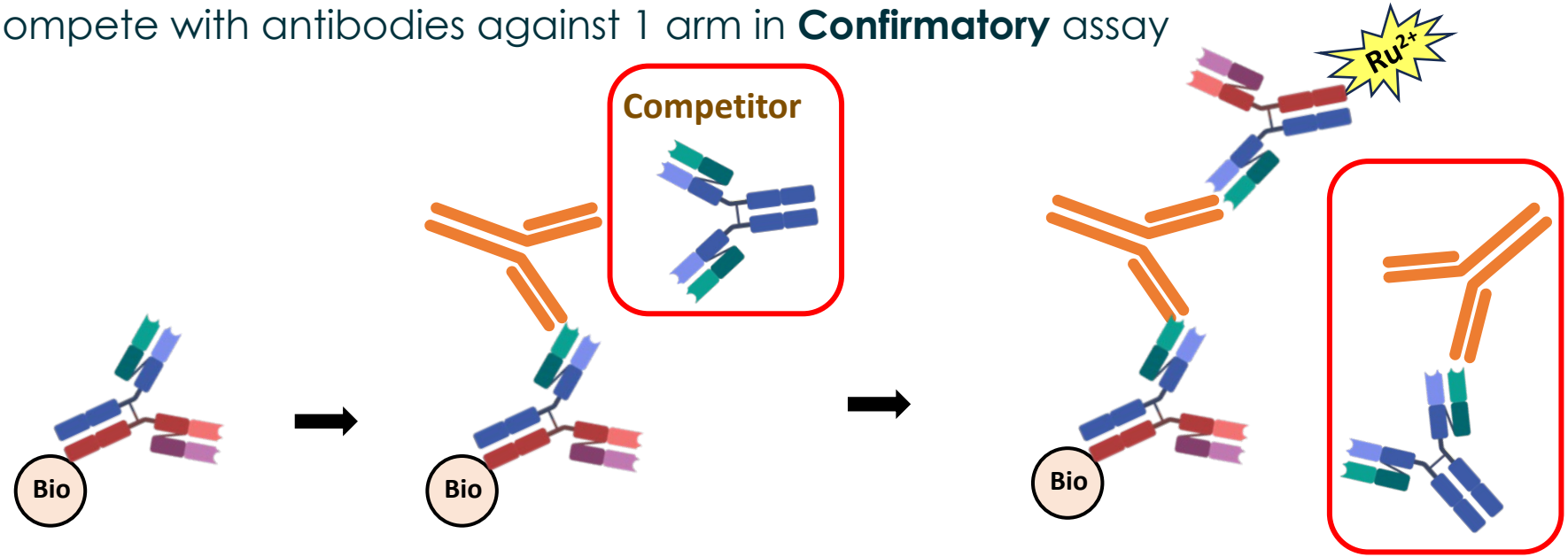
- PC may be against 1 arm, or a mix of antibodies against both arms



ADA domain specificity characterisation

Example: Bispecific antibody

Compete with antibodies against 1 arm in **Confirmatory** assay



Pros & cons of the domain competition approach

Requirements

- Requires 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 confirmation tier

CONs

- 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

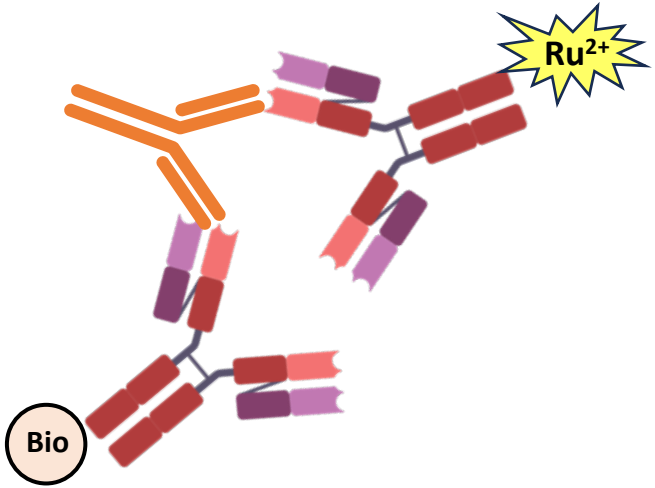
ADA specificity determination: Separate domain assays

Example: Bispecific antibody

- Separate assays with each homodimer can be used for specificity assessment

Heterodimer A-B

Homodimer A



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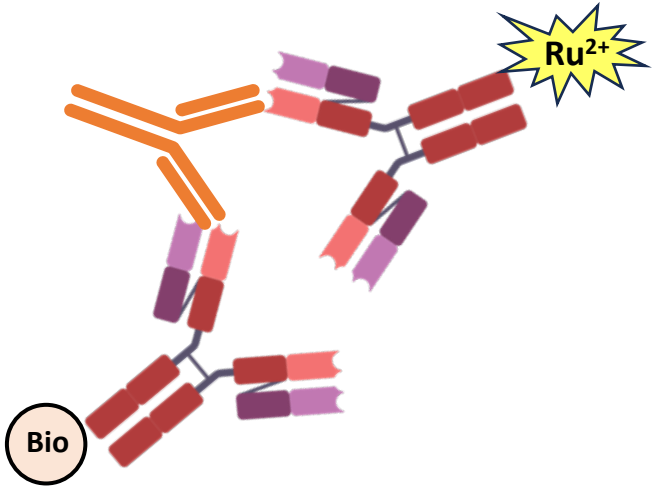
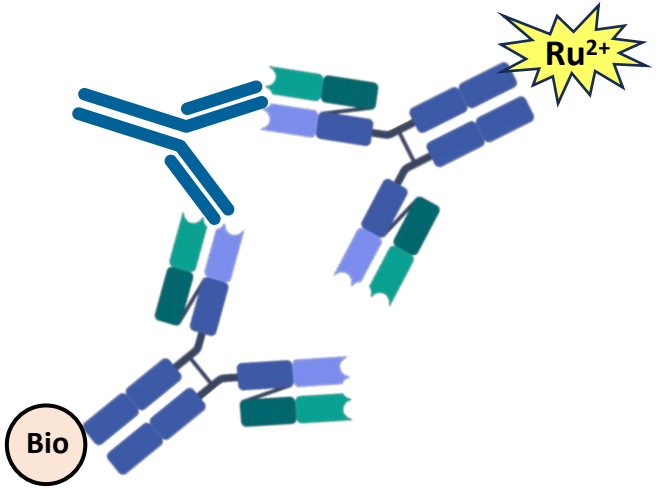


Homodimer B



Pros & cons of the separate assay approach

Requirements

- Requires individual domains for the assay set-up and domain-specific PC
  - 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 optimisation may be required with the different domain capture/ detection and the different PCs
- Usually sensitive to detect domain-specific antibodies

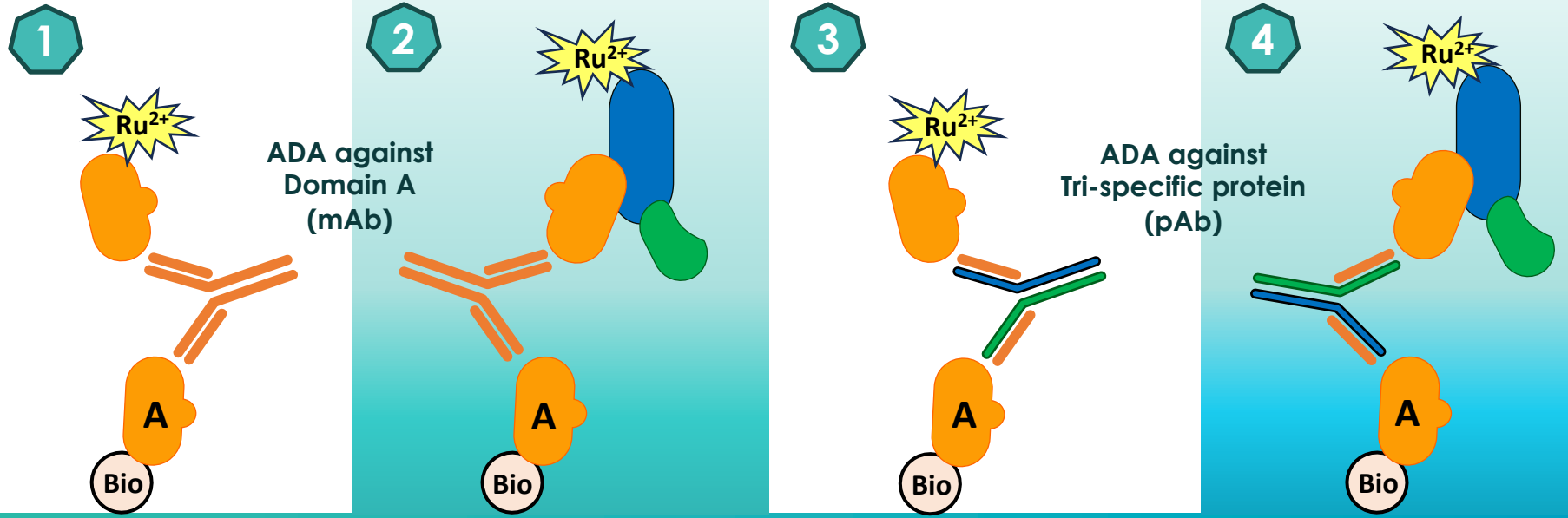
CONs

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

ADA specificity determination: Separate domain assays

Example: Tri-specific protein (e.g. Protein A–B–C)

- Detection may be **single domain** or the whole **tri-specific protein**
- PC may be against **1 domain**, or **antibody against the whole tri-specific protein**



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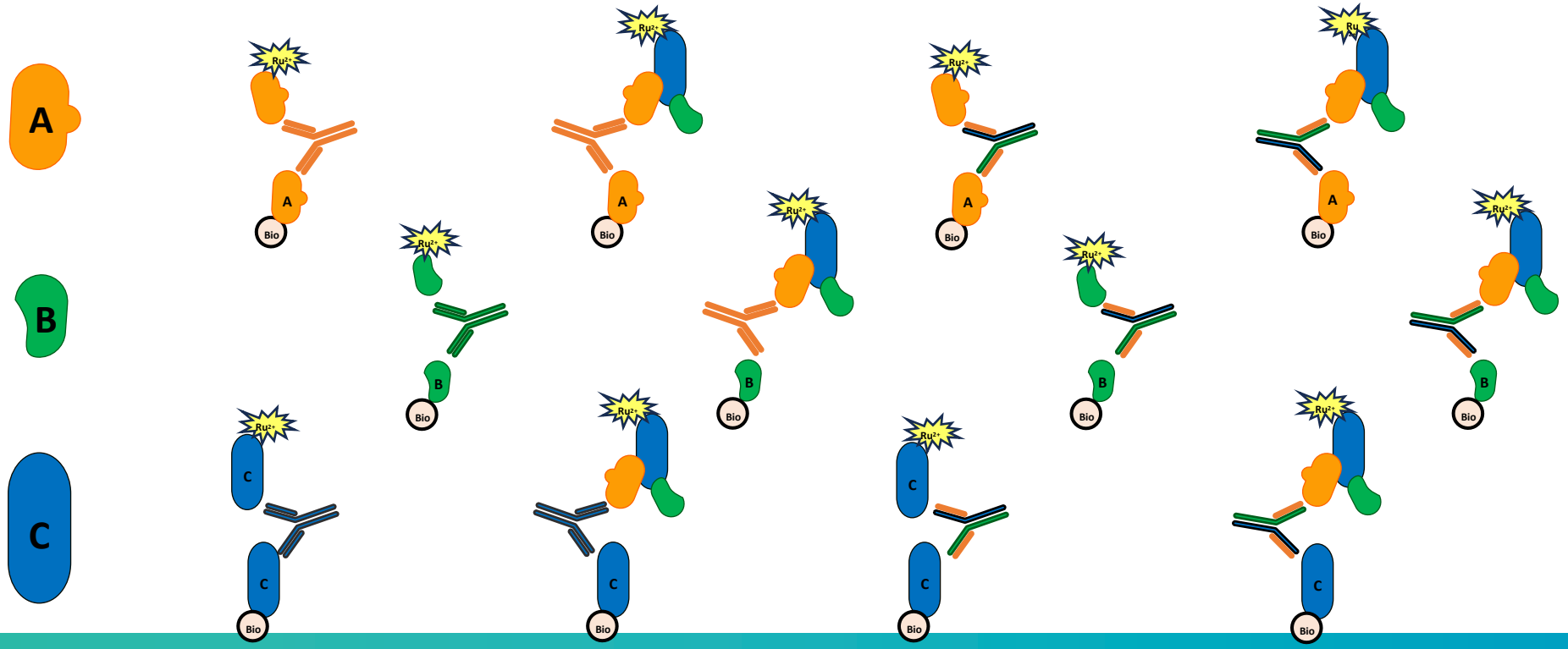

ADA specificity determination: Separate domain assays

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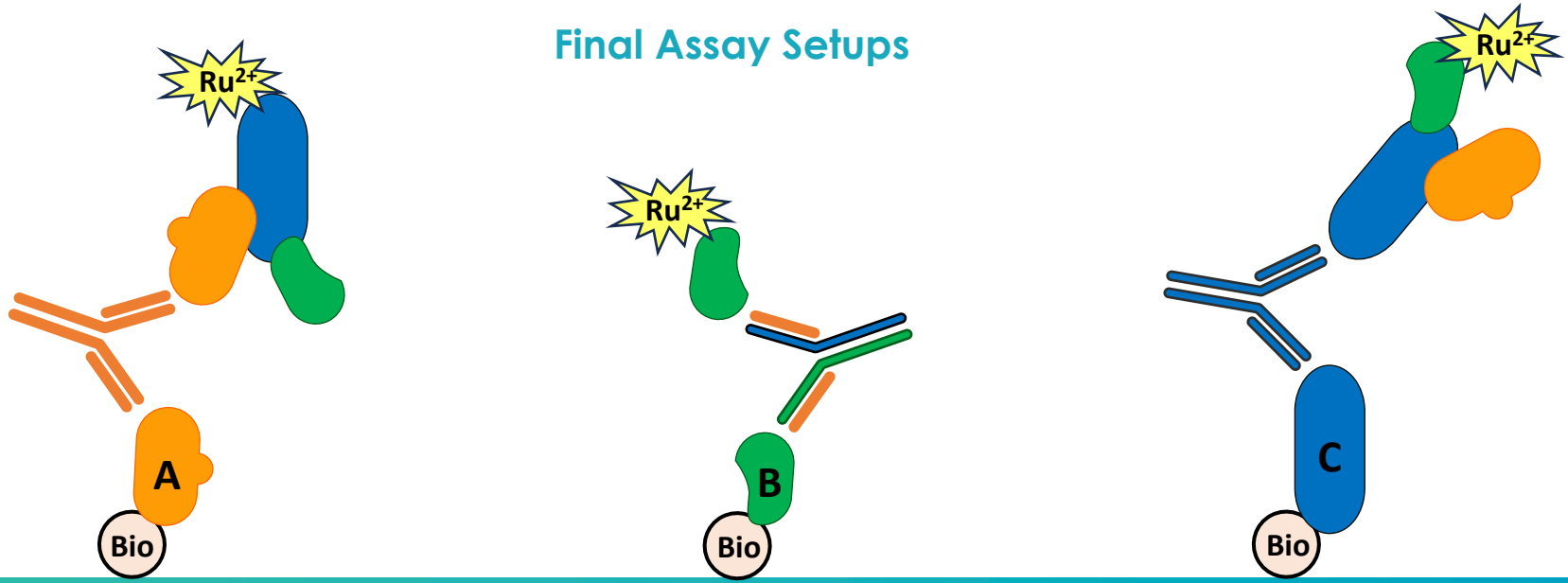
Final Assay Setups



Summary

Advantages of domain specificity characterisation

- Comprehensive understanding:
  - Crucial for understanding the overall immunogenicity of the therapeutic
  - Can design / modify to reduce immunogenicity
- Safety and efficacy: Help in predicting potential cross-reactivity with endogenous proteins
- Regulatory approval: Can provide comprehensive data that can support regulatory submissions

Summary

Challenges of domain specificity characterisation

- Technically challenging: due to the **complexity of the biotherapeutic** and the potential for **cross-reactivity** between domains
- Cost and time: **time-consuming** and **expensive**
- Interpretation challenges: The presence of ADAs against one domain might influence the detection or binding of ADAs against another domain
- Bioanalytical challenges: **Require** highly **specific** and **sensitive** assays

Concluding remarks

When is characterisation of ADA specificity required for multi-domain therapeutics?

- For each multi-domain therapeutic program, the timing and extent of ADA domain specificity assessment needs to be considered

- **Preclinical**
  - Domain specificity assessment is unusual in preclinical studies
  - Where specificity is assessed, it is **driven by project-specific reasons**, e.g., de-risking domains within a molecule platform which could lead to loss of exposure in IND-enabling studies

Concluding remarks

When is characterisation of ADA specificity required for multi-domain therapeutics?

Clinical

- Early clinical studies may not require detailed assessment of domain specificity
  - Generation of reagents/tools that will be required for specificity assessments
  - Developing prototype specificity assays to prepare for later clinical stages

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

Acknowledgements

Thank you to…

- **BioAgilytix colleagues** in Hamburg, USA and Australia
- BioAgilytix **@Scientific Office**
- **EBF** for the opportunity to share our science

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Thank you

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