Assay Strategies to Analyse New Antibody Therapeutics in Preclinical and Clinical Studies

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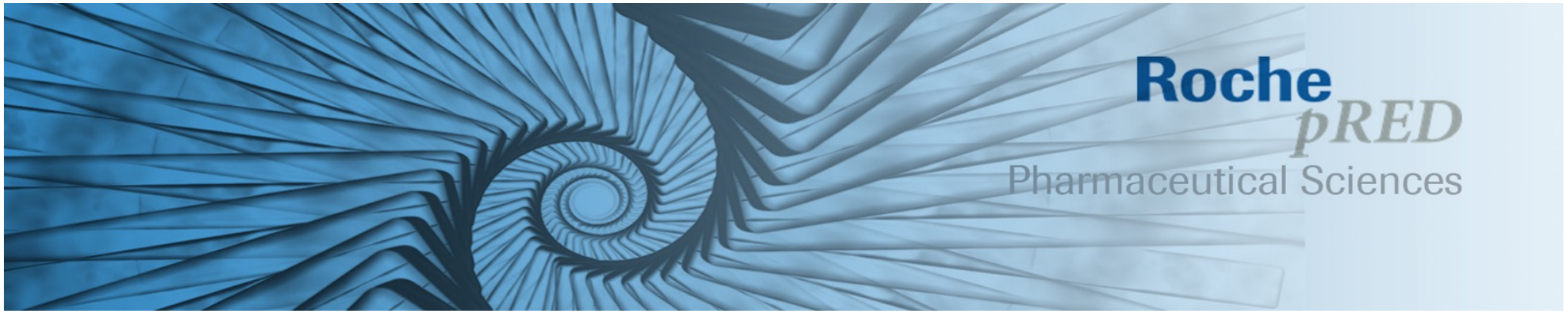
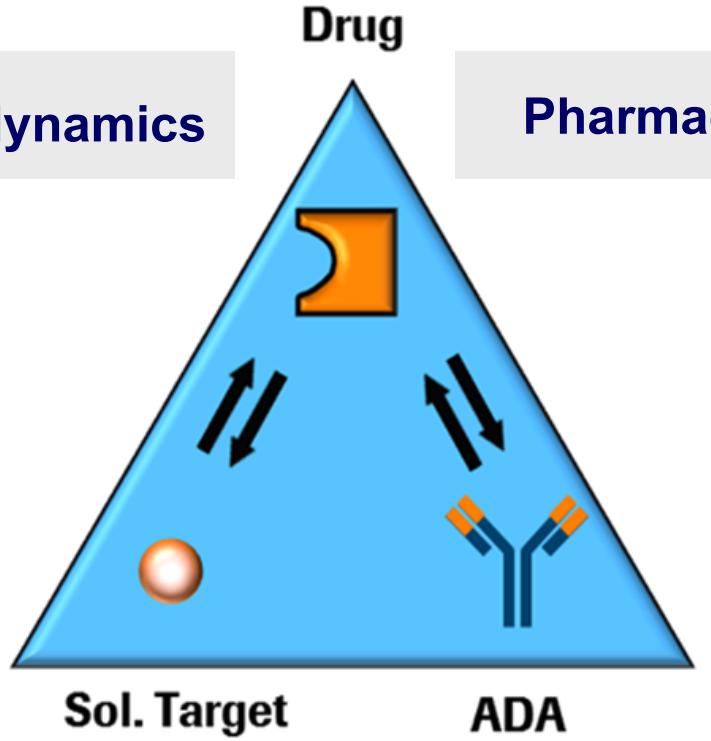


Bioanalytical Assays in Biologics Development
The Interplay between Drug, Target and ADAs



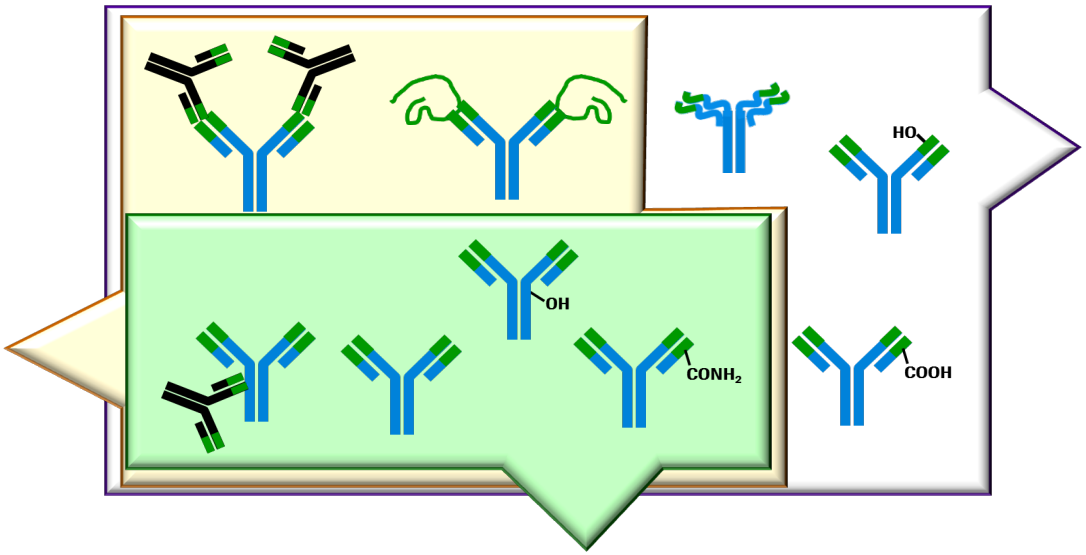
- Analytes are not independent from each other
- Interactions have to be considered for
  - Drug exposure assessment
  - Immunogenicity testing
  - Target engagement
  - … many more

PK-Assays

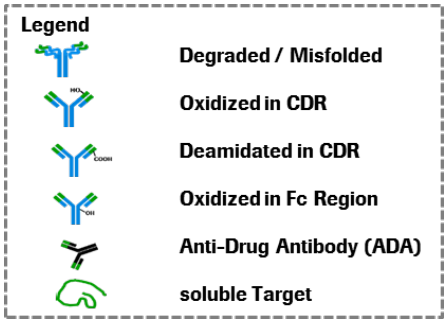
What are we analyzing?

Nomenclature	Description
Total	The total fraction of the drug
Target-binding competent	Is able to bind to its desired binding partner in an assay
Active	Able to bind its target in vivo (in contrast to total) Can be very challenging (disturbance of equilibrium during sample preparation/analysis)
Free	Is not bound to any other protein

Target-binding competent



Total

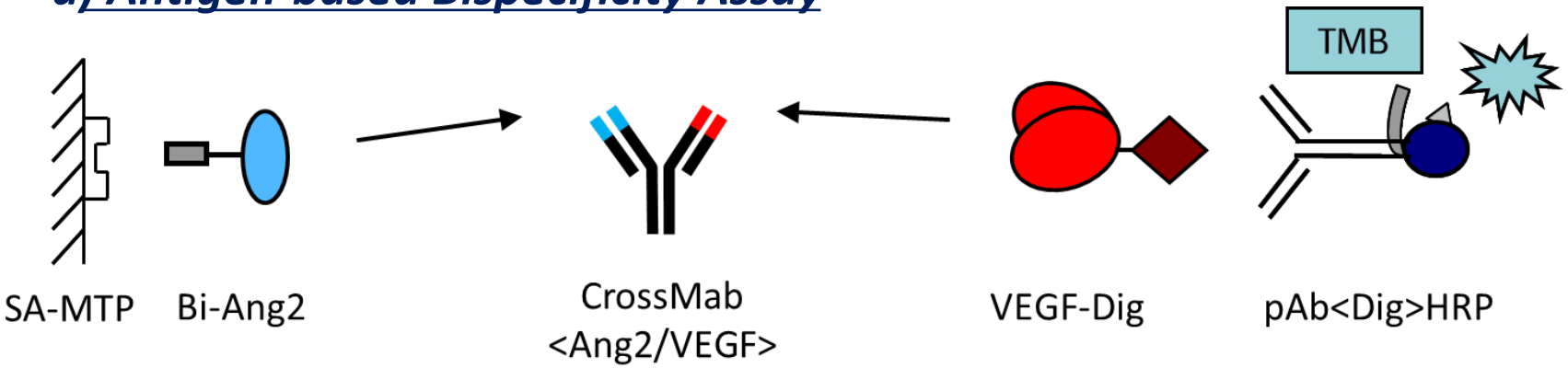


Heinrich et al., Bioanalysis. 7(24):3057-62 (2015)

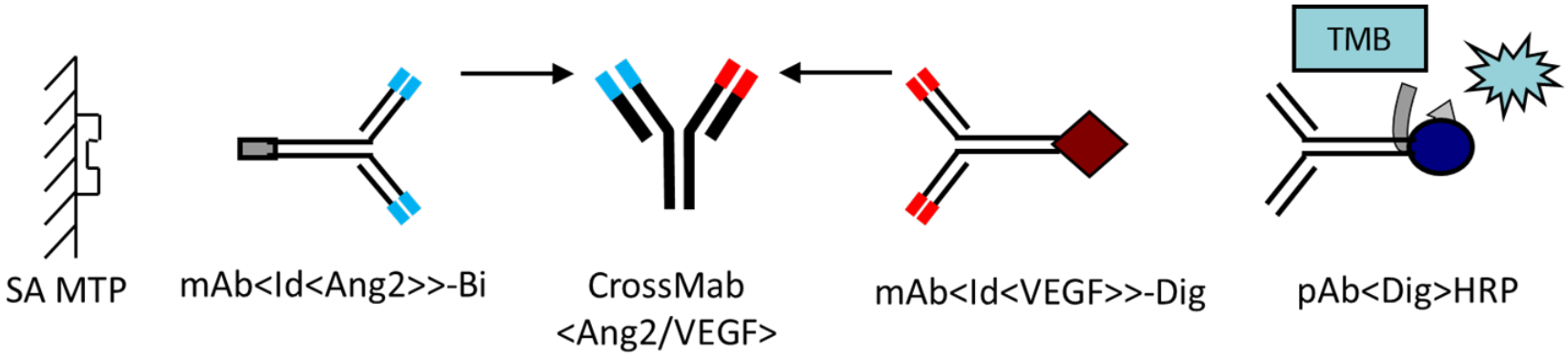
PK-Assay Options for Antibody Therapeutics

Quantification of Active/Target Binding Competent Drug

a) Antigen-based Bispecificity Assay



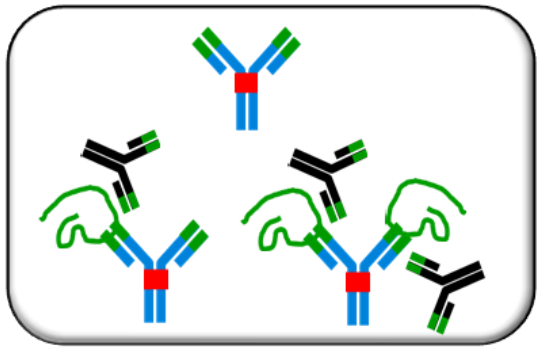
b) Anti-Idiotype Ab-based Bispecificity Assay

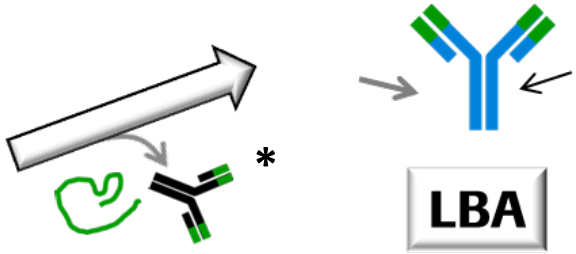


- Disturbance of equilibrium during sample preparation/analysis to be considered for accurate active drug quantification

Staack et. al., Bioanalysis. 6(4), 485-496 (2014)

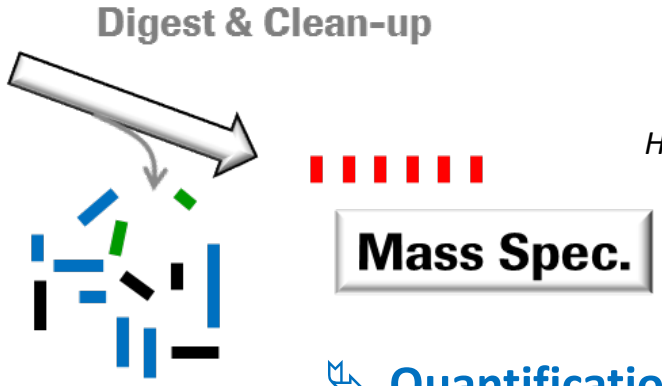
PK-Assay Options for Antibody Therapeutics
Quantification of Total Drug





Stubenrauch et al . JPBA 49(4):1003-8 (2009)
**Kelley M et al. AAPS.J. 15(3), 646-658 (2013)*

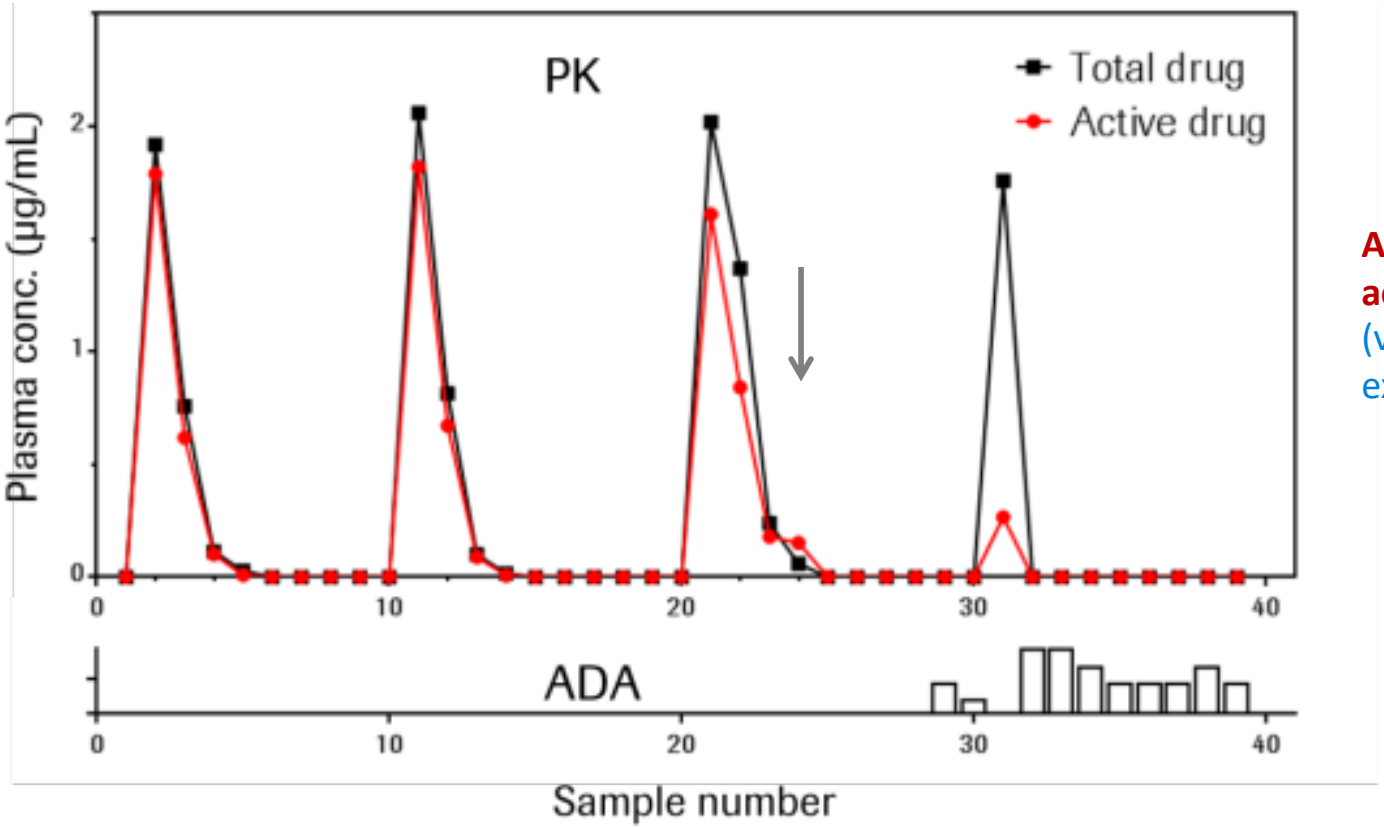
↳ **ADA/Target inference tolerant assays or elimination of interferences**



Heudi et al. Anal Chem. 80(11):4200–7 (2008)

↳ **Quantification of drug-specific peptide**

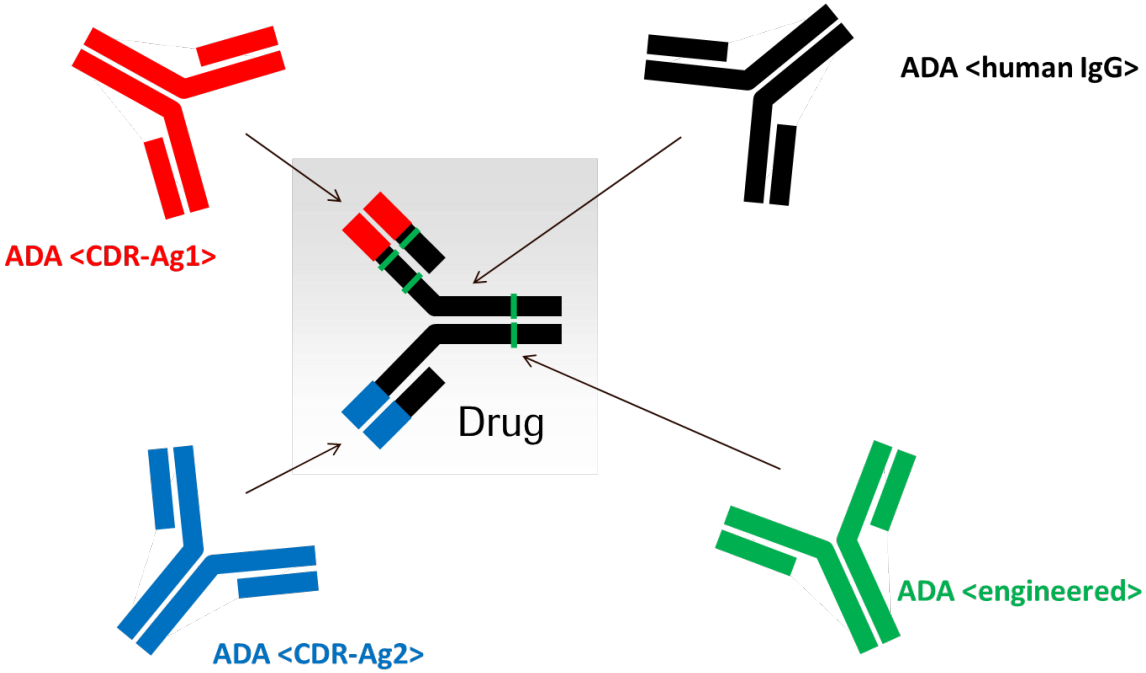
„Active“ vs. „Total“ Drug Exposure
When and why does it matter?



ADA-dependent loss of active exposure
(vs. maintained total drug exposure)

- The **interference** of **soluble targets**, extracellular domain of the target **receptors**, or **ADA** on the PK assay need to be considered
- The validity of safety (and efficacy) studies relies upon the demonstration of **active drug exposure**

ADA Responses are Polyclonal and can be Diverse
e.g. different Ig isotypes, directed against different epitopes



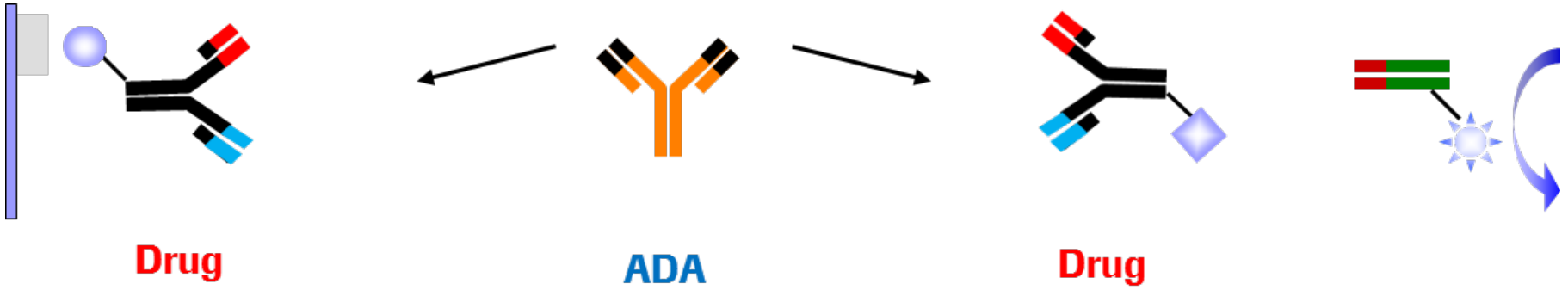
- **Diverse specificities**
- **Different affinity**
- **Several Ig isotypes**
- **Different stability**

Main Goals for Immunogenicity Testing of New Antibody Therapeutics::

- Detection of whole ADA responses
- ADA characterization, e.g. domain specificity

Current ADA Assay Gold Standard: Bridging Assay

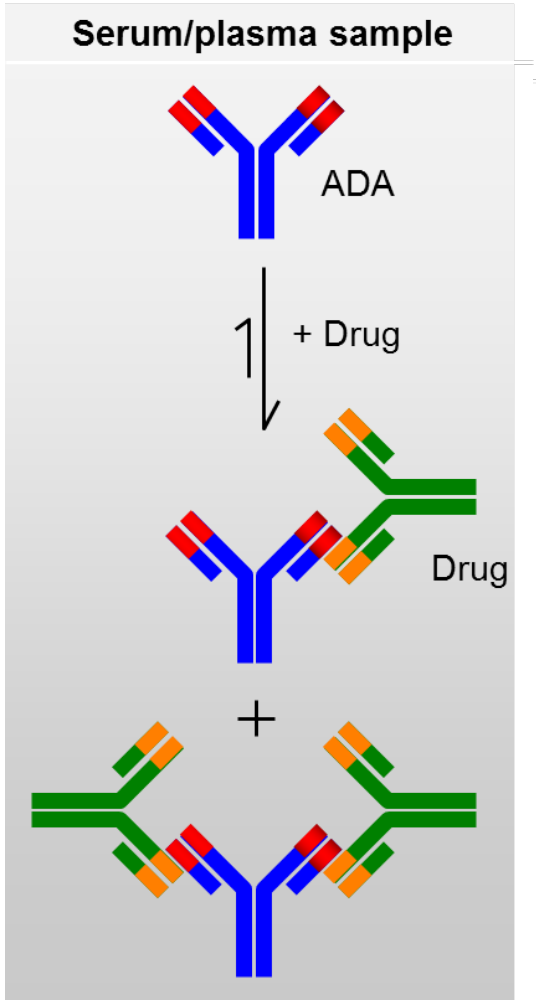
Example: Bispecific Antibody



Appropriate assay to detect nearly all ADA isotypes and any ADA specificity:

- Both sets of CDRs („antigen binging regions“)
- Engineered part
- Human IgG framework

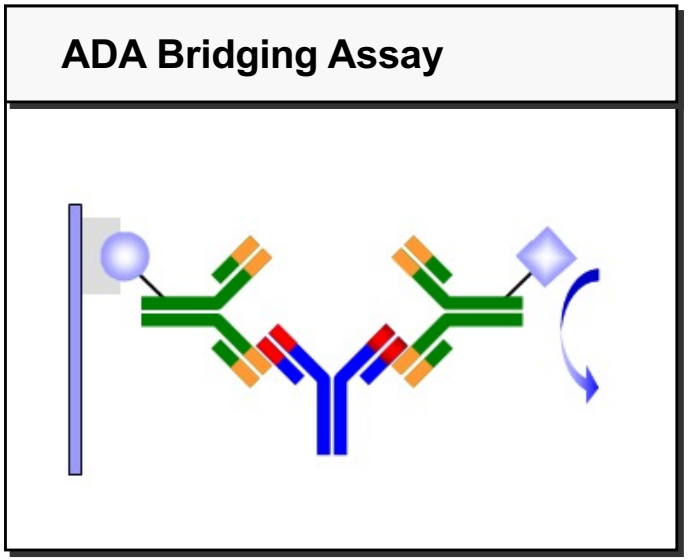
Issue for ADA Bridging Assays: Drug Interference



Signal in ADA assay

No signal in ADA assay

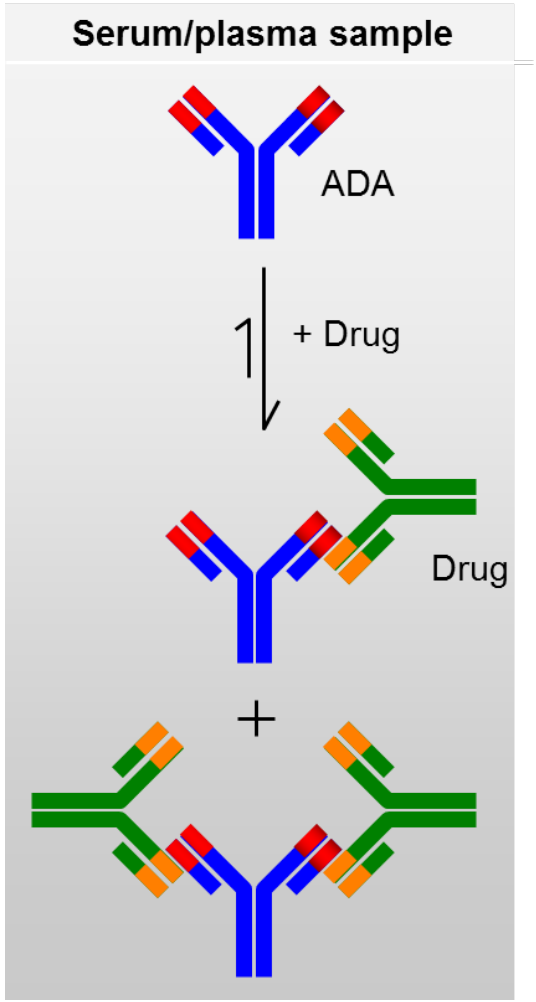
No signal in ADA assay

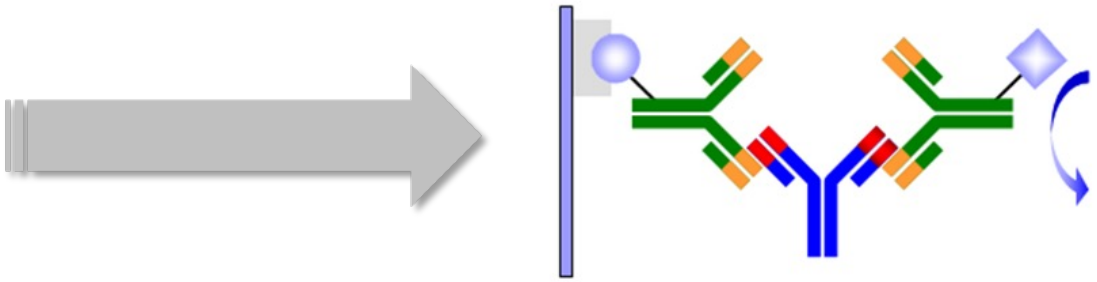


Analytical consequence

Masking of ADA by Drug can result in ADA False-Negatives

Principles to Overcome Drug Interference Issues





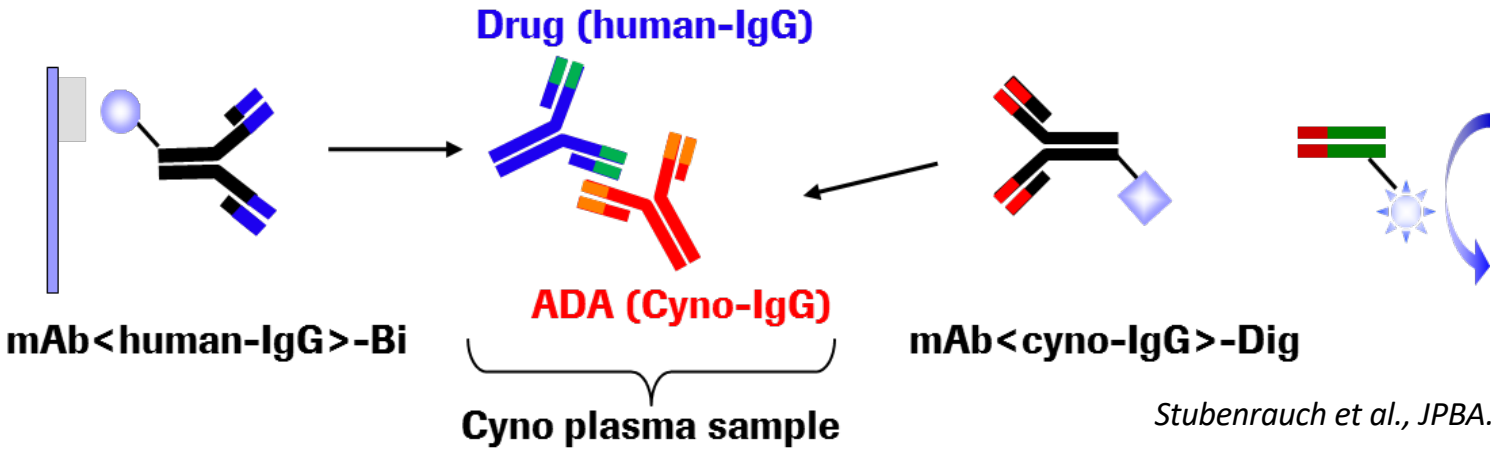
- High assay sensitivity for free ADA
- Shift of the equilibrium towards free ADA
- Dissociation of ADA-drug complexes by sample pre-treatment
- ADA enrichment//separation/purification

and

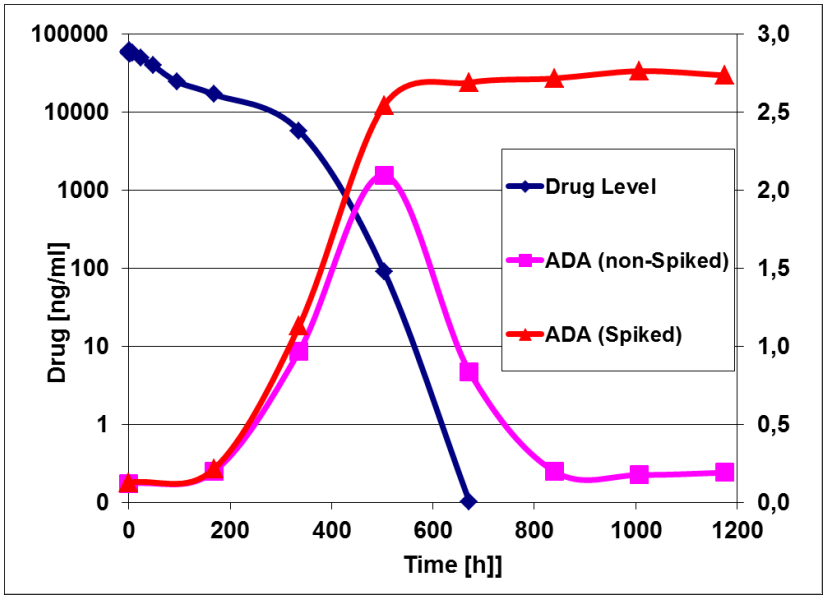
- Alternative Assay Formats

Drug-ADA Immune Complex Assays

Inherently resistant to drug and target interference



Stubenrauch et al., JPBA. 52(2):249-54 (2010)

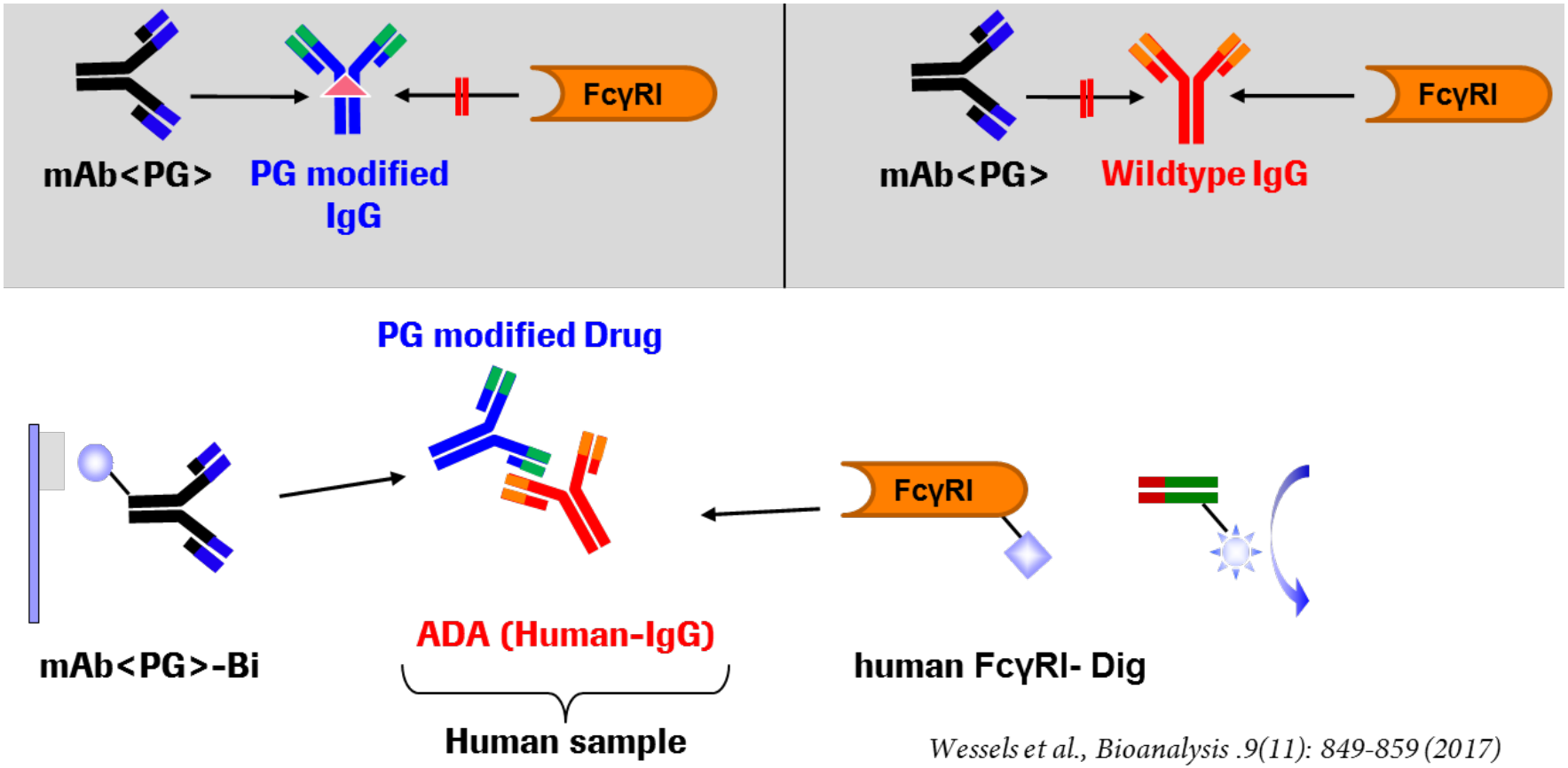


Two assay variants allow:

1. Detection of ***in vivo* formed immune complexes** (drug bound ADA only, magenta) and
2. By *ex vivo* spiking of drug into samples, **all ADA detected** (*in vitro* formed complexes, red)

Drug-ADA Immune Complex Assays

For drugs with PG modified Fc – also applicable in clinical studies



Wessels et al., Bioanalysis .9(11): 849-859 (2017)

- Inherent resistance against residual drug
- FcγRI detection provides proof, that signal is indeed caused by an antibody

Drug-ADA Immune Complex Assays

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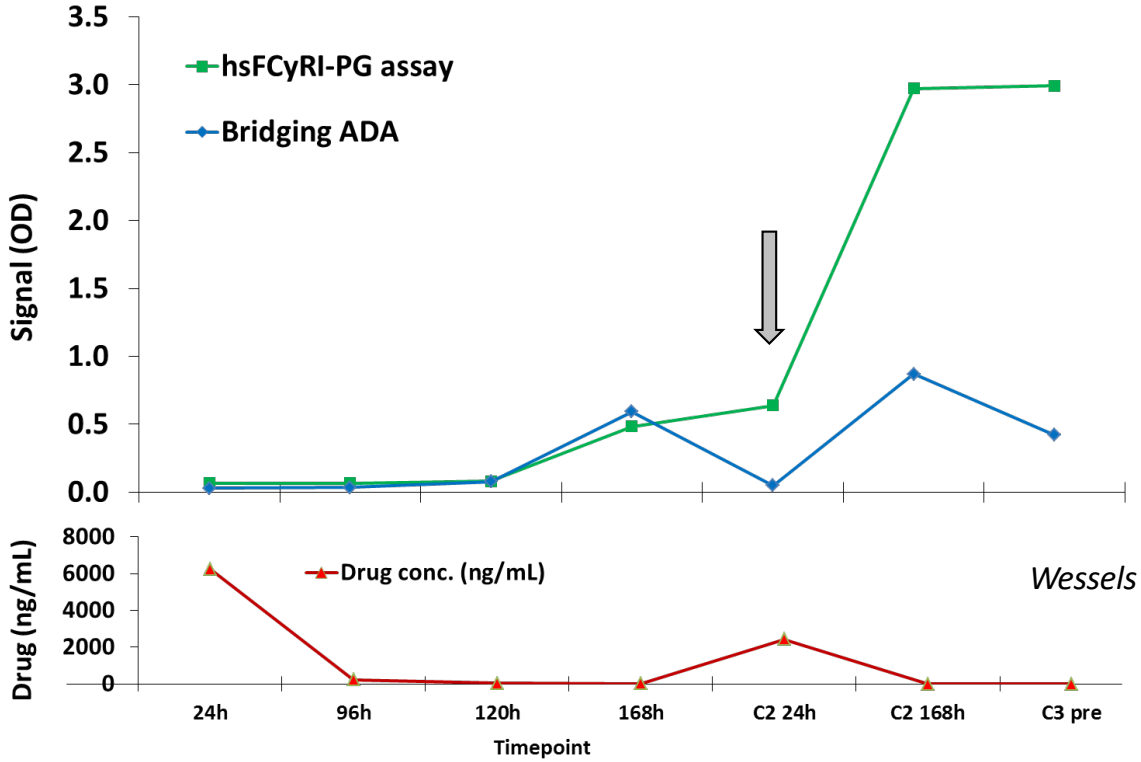

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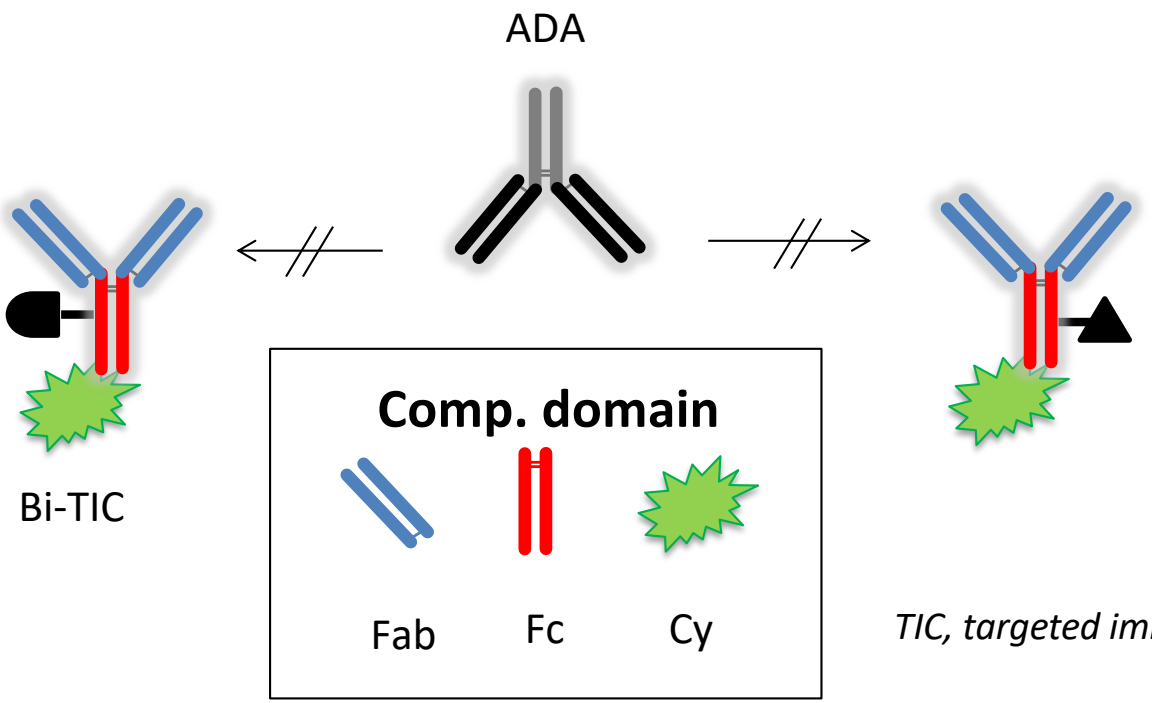
Pro: Highly drug-tolerant, appropriate assay format to detect ADA against FcR-effector function suppressed antibody drugs (with engineered Fc part)

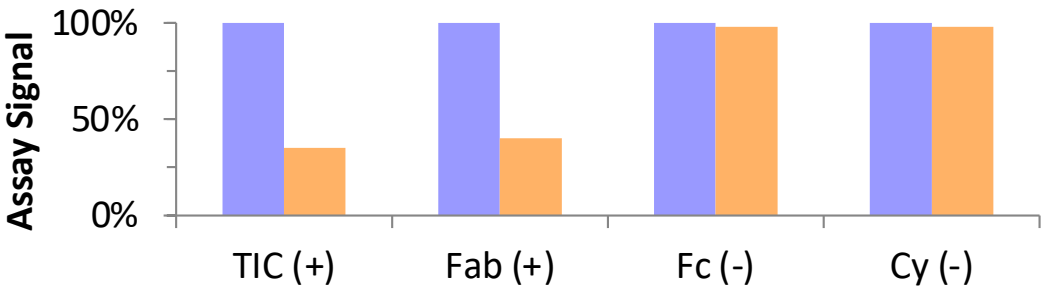
Con: ADA of other isotypes than IgG 1 (e.g. IgM are not detected and require additional assays

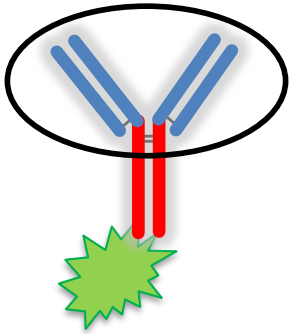
ADA Characterization: Domain Competition Assays



TIC, targeted immunocytokine; Cy, cytokine

ADA predominantly directed against Fab





Stubenrauch et al., JPBA. 114:296-304 (2015)

Immunogenicity Testing for Bifunctional Molecules
Domain Competition Assay

Advantages

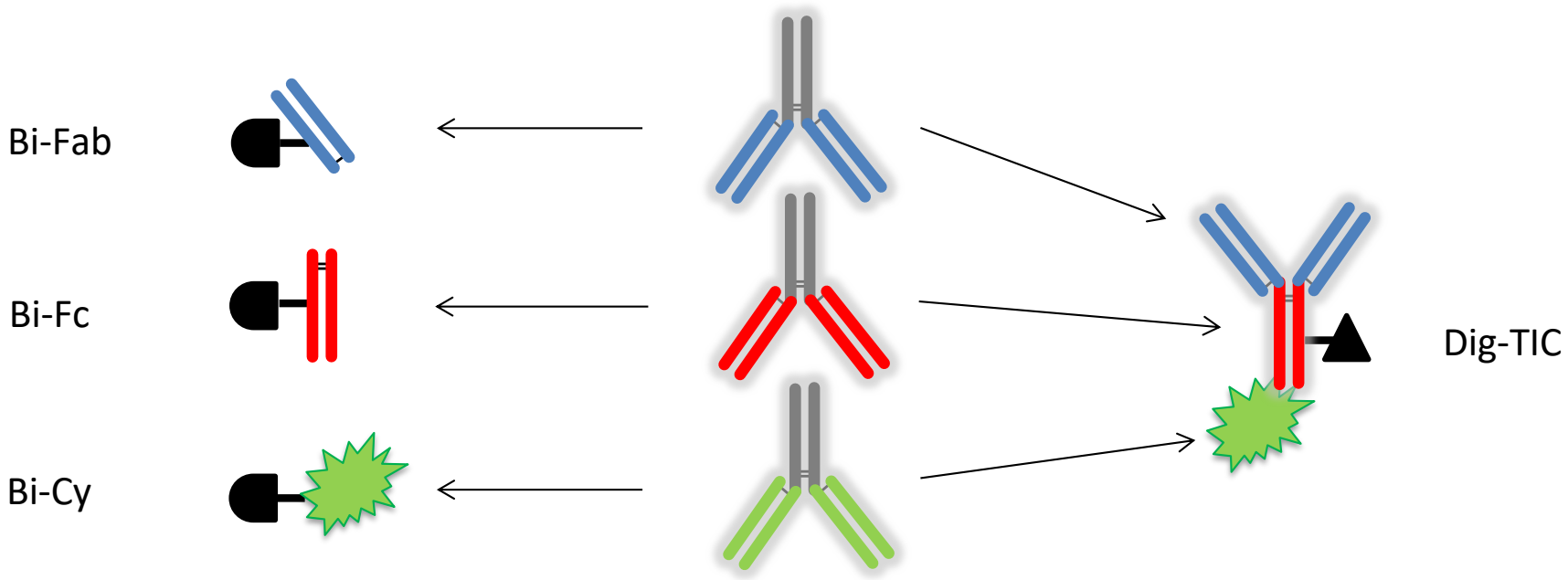
- Method similar to standard confirmation assay
- Require single drug domains only (not conjugated with label)
- Less effort for reagent and assay development
- Relatively fast and easy to implement

Challenges

- Production of single drug domains
- Determination of specific confirmation CPs for each domain required
- Higher intrinsic variability (result generated from 2 data points)
- In case of multi-specific ADA responses: Risk to miss low levels of ADAs

↳ May be the approach of choice for early development

ADA Characterization: Domain Detection Assays



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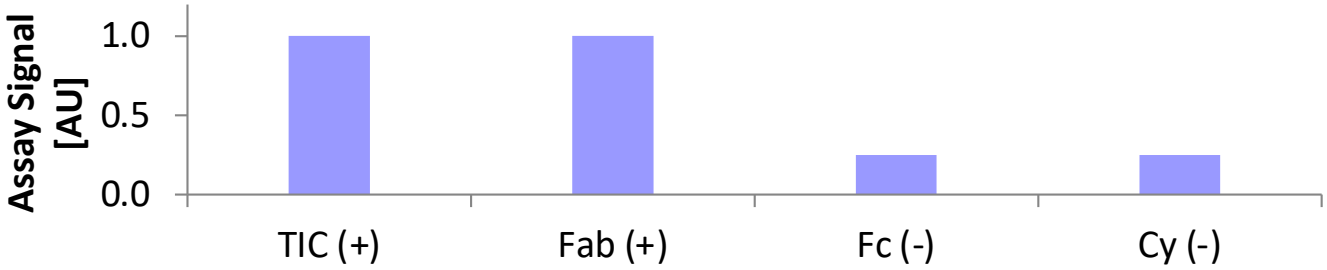

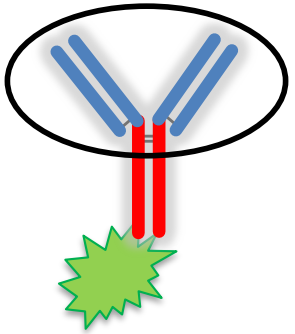

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Immunogenicity Testing for Bifunctional Molecules
Domain Detection Assay

Advantages

- May be superior for detecting minor ADA fractions in multi-specific ADA responses
- Allows titer assessment of each ADA specificity (each domain)
- Better data quality since analytical result is based on a single readout instead of two for DCAs

Challenges

- More extensive reagent development
- Labelling of drug fragments may increase risk of conformational changes or epitope masking
- Limited accessibility of small domains immobilized on solid phases
- Separate assays to be established for each domain, incl. assay validation

May be the approach of choice for later development

Bioanalysis of New Antibody Therapeutics
Conclusions

- **Understand your drug and targets to develop assay strategies that result in relevant data**
- **Invest the time to generate appropriate reagents well in advance of clinical studies**
- **Depending on molecule design, new antibody therapeutics require a bit or significantly more bioanalytical investment**
- **New antibody therapeutics enable new options for disease treatment – and also new chances for bioanalysis**

Acknowledgement

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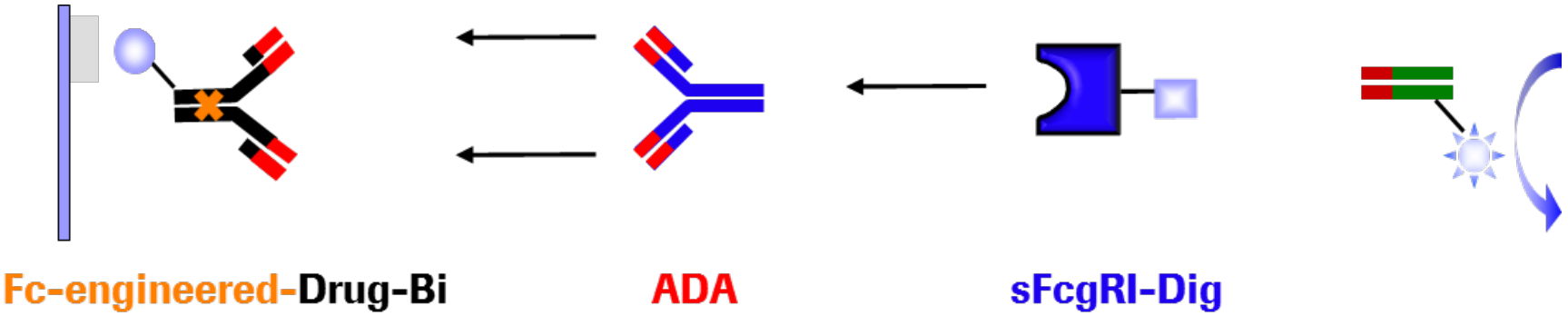
Several LMR, PS, RPD and DTA-Teams
…. and many more

Doing now what patients need next

ADA Assay Formats Different to Bridging Assays
e.g. suitable for Fc engineered antibody drugs



Wessels et al., Bioanalysis. 8(20):2135-45 (2016)

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Doing now what patients need next