Are immunogenicity assay results really “incomparable”?

The critical role of bioanalysis to bring immunogenicity testing to the next level

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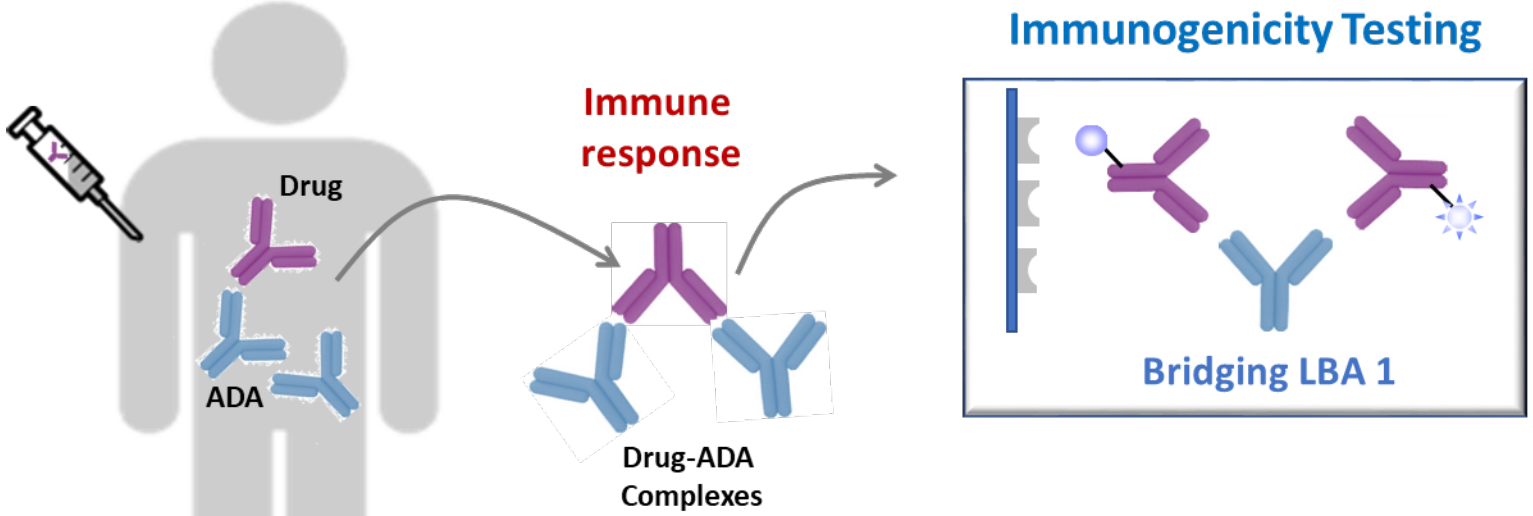
Roche pRED

Development of therapeutic proteins

The immunogenicity challenge

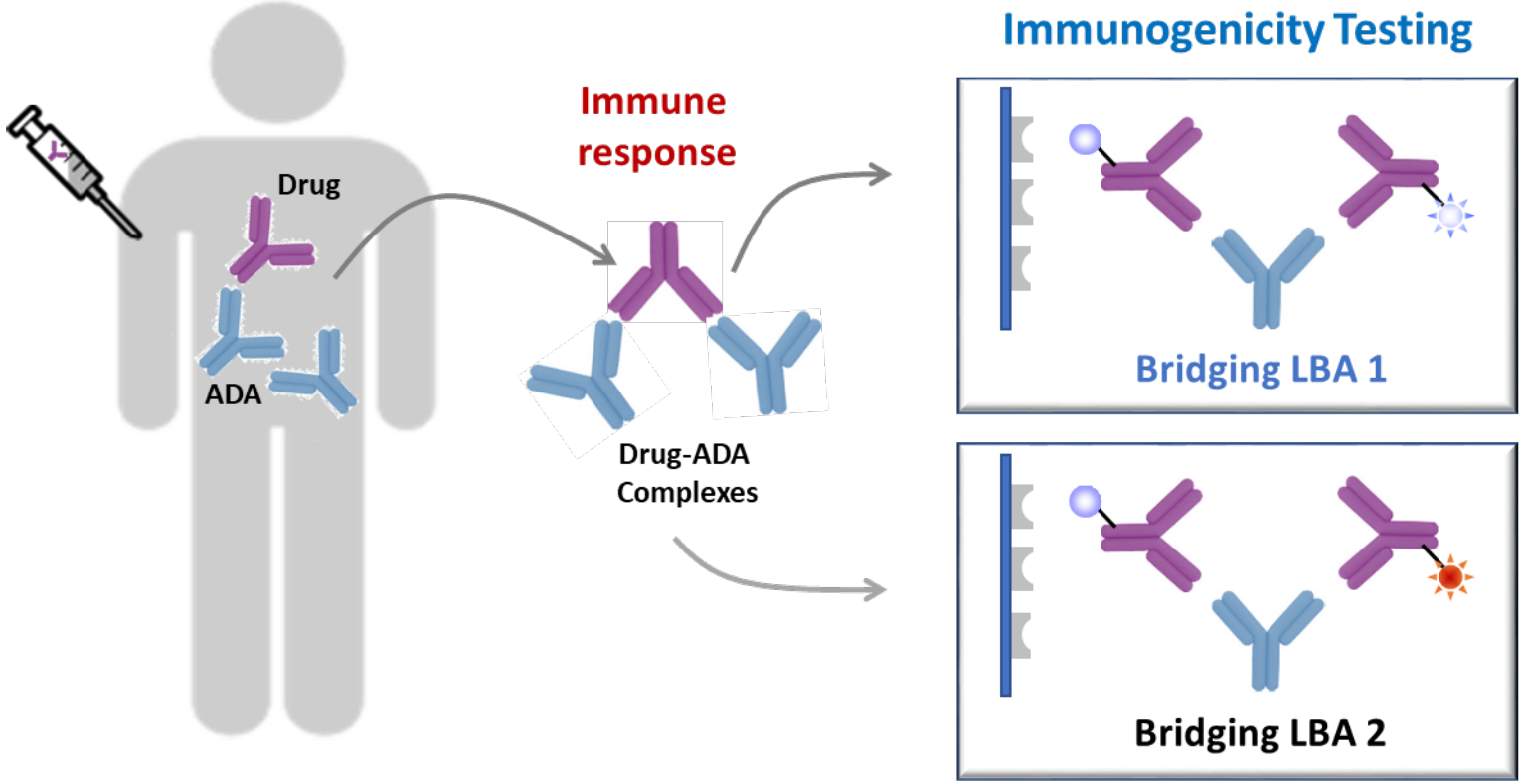


Potential impact on
- Efficacy
- Safety

Evaluation is based on a holistic view on:
- **Immunogenicity/ADA**
- **PK**
- **PD**
- **safety data**

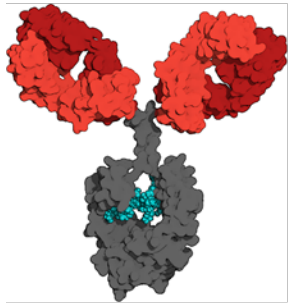
Development of therapeutic proteins

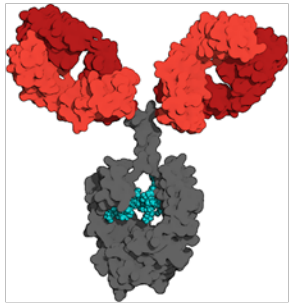
The immunogenicity challenge



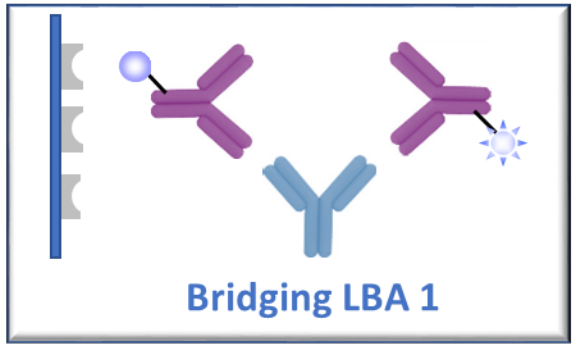
Comparability of ADA Assay results?

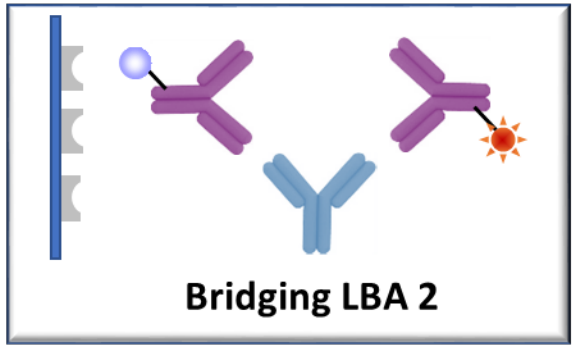
Development of therapeutic proteins



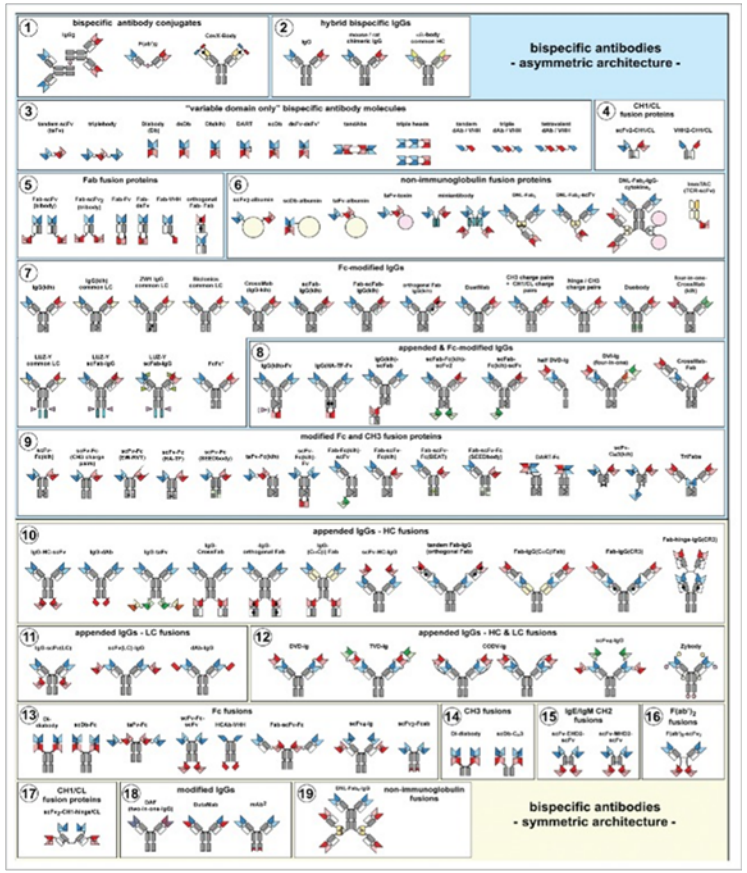


Immunogenicity Testing





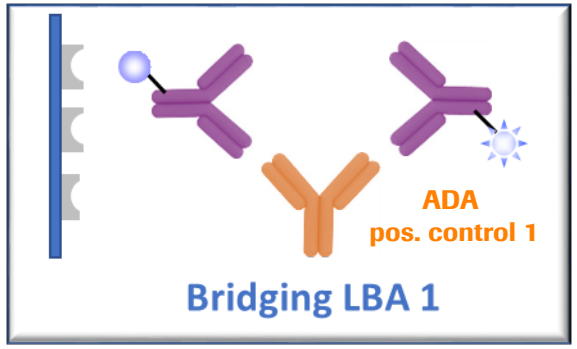
Comparability of ADA Assay results?

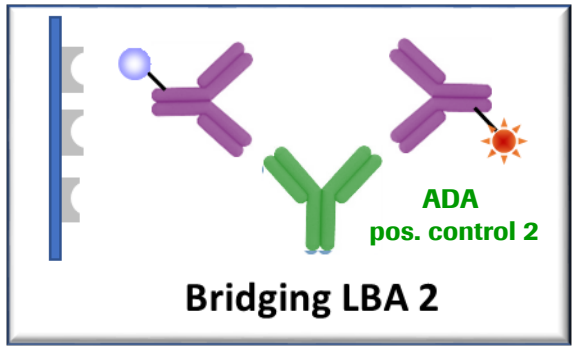


Brinkmann U & Kontermann RE, MAbs. 2017 Feb/Mar;9(2):182-212

Is the performance of the ADA assay the key to comparability?

The role of the „positive control“





- Bioanalytical Immunogenicity/ADA data
  = crucial piece of information for evaluation of clinical immunogenicity
- Performance of BioA assay the critial parameter, particularly:
  - **Sensitivity**
  - **Drug Tolerance**
- **Comparability** of different assays is **deemed highly difficult/not possible** due to performance differences determined by different positive controls

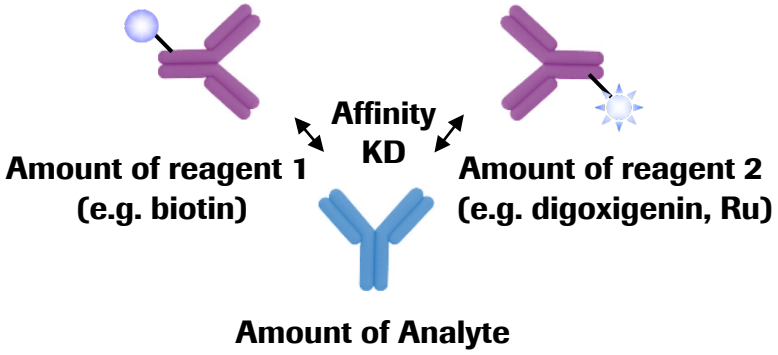
  "Positive control = crucial issue

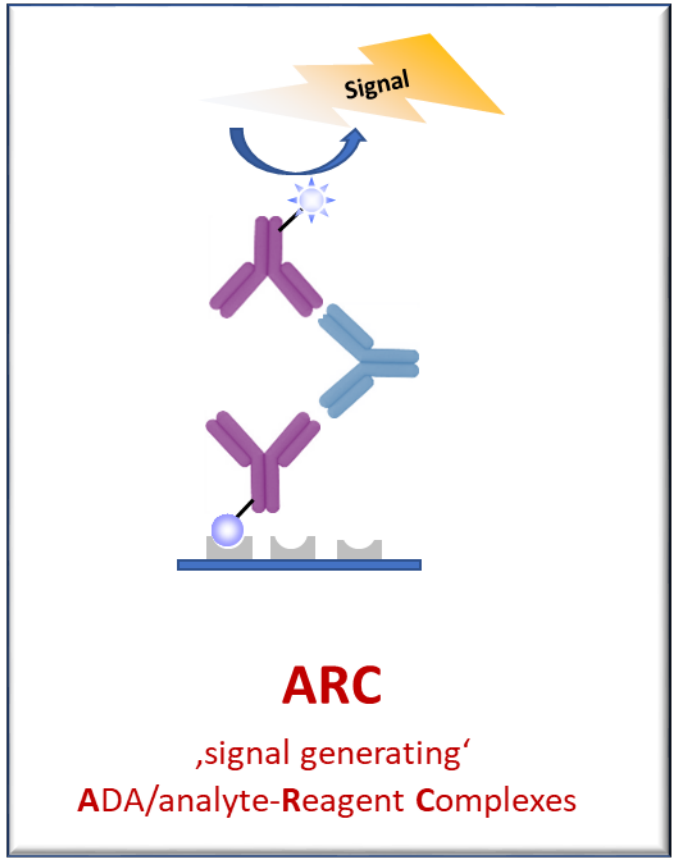
  → FDA proposal: „universal ADA-positive control“

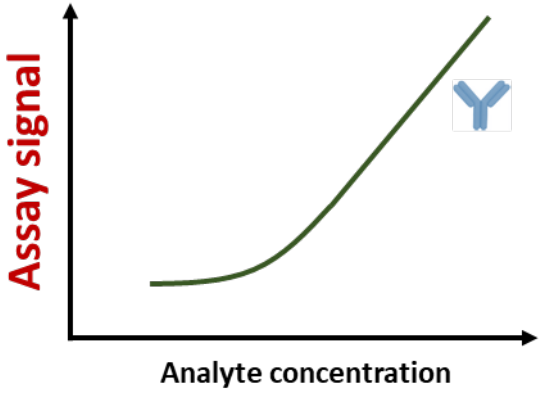
Enabeling comparable ADA assay performance = BioA contribution to achieve scientific sound comparability of the clinical immunogenicty of different drugs/biosimilars/scaffolds

Bioanalytical Assay Performance

What do we actually see?







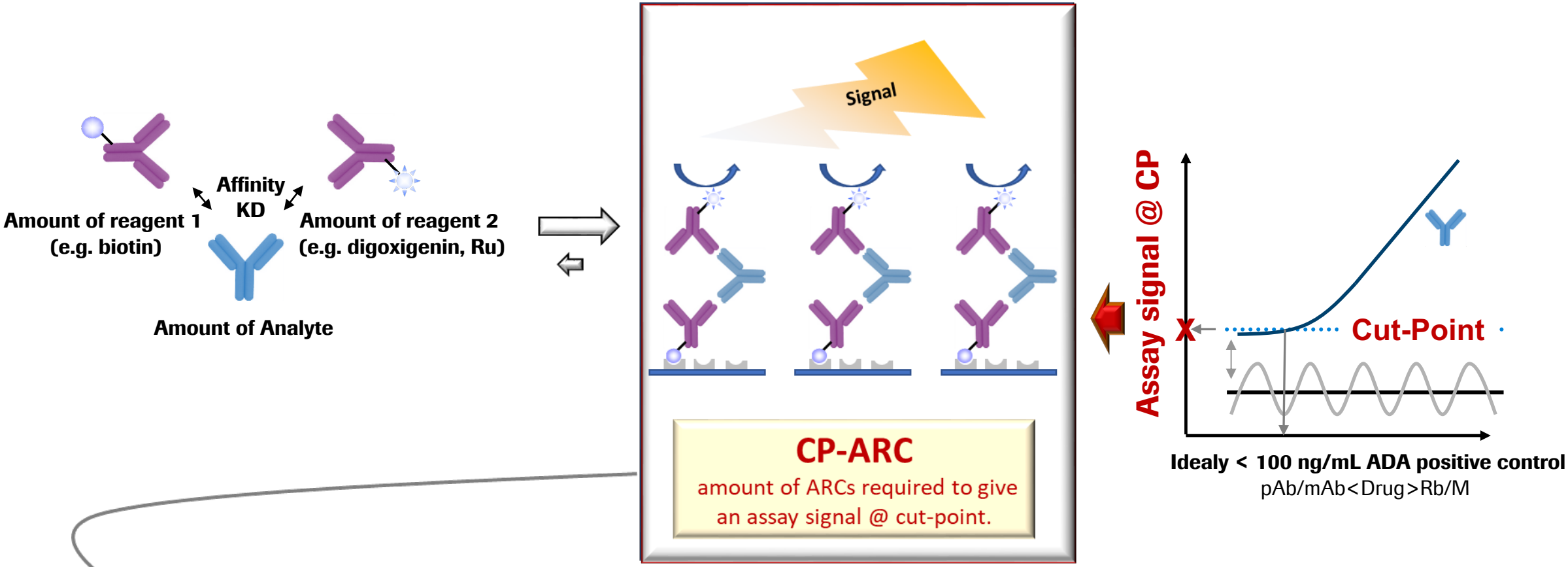
Intensitiy of the assay signal depends on:

- Amount of the analyte/ADA
- Amount of the reagents
- Affinity of analyte-reagent interaction

Jordan, Staack. Bioanalysis. 2020 Jul;12(14):1021-1031

The role of formed ADA/Analyte-Reagent-Complexes at CP (CP-ARC)

… a constant assay performance parameter?



constant assay performance parameter that defines the assay sensitivity & drug tolerance

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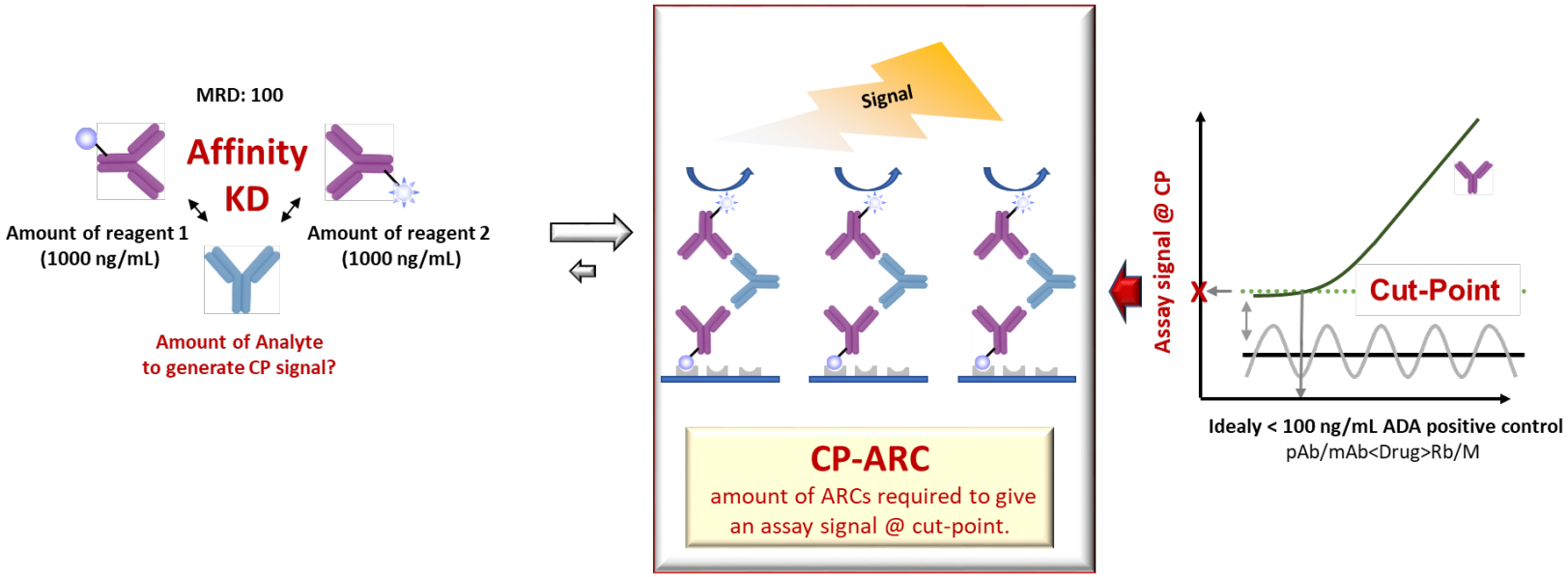
Impact of affinity on CP-ARC and on assay sensitivity

A theoretical consideration



100-fold lower affinity

„*Lower*“ affinity (KD =10 nM) → **230** ng/mL

Positive control Ab (KD = 0.1 nM) → **100 ng/mL** (CP-ARC = 3 pM)

100-fold higher affinity

„*Higher*“ affinitiy (KD = 0.001 nM) → **97** ng/mL

2.3 fold lower sensitivity

~ identical

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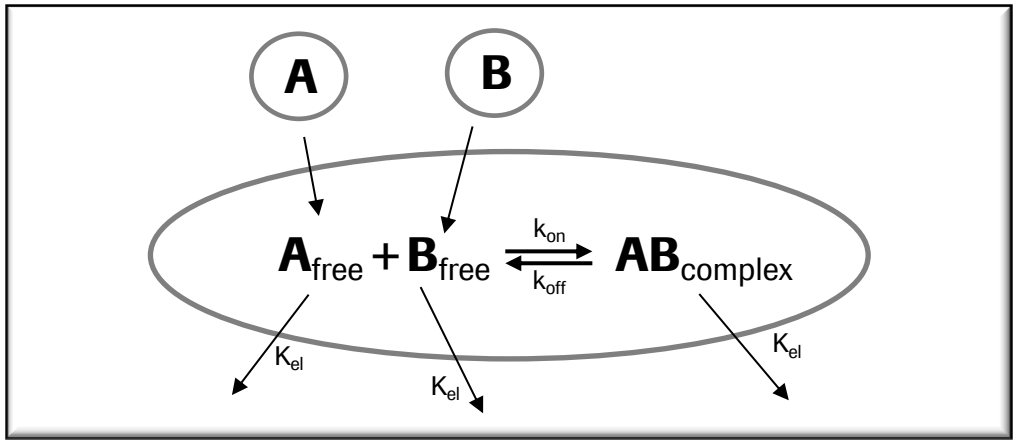
Calculation of binding interactions

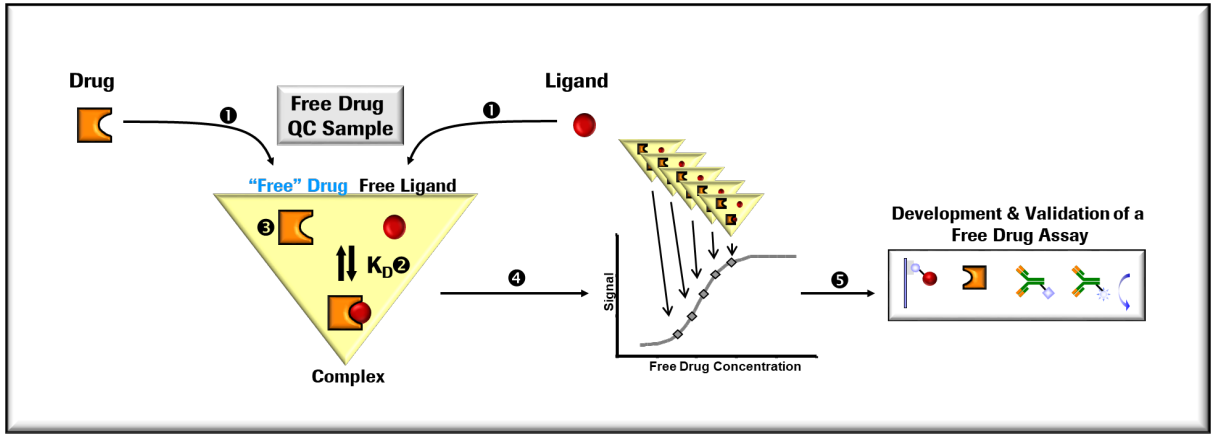
A nice gimmick or valuable for BioA?

Modelling & Simulation



Chirmule, Jawa, Meibohm. AAPS J. 2012 Jun;14(2):296-302.
Gómez-Mantilla, Trocóniz, Parra-Guillén, Garrido.J Pharmacokinet Pharmacodyn. 2014 Oct;41(5):523-36
Roskos, Schneider, Vainshtein, Schwickart, Lee, Lu, Faggioni, Liang. Bioanalysis. 2011 Mar;3(6):659-75

BioA: „Free Analyte QC Concept“



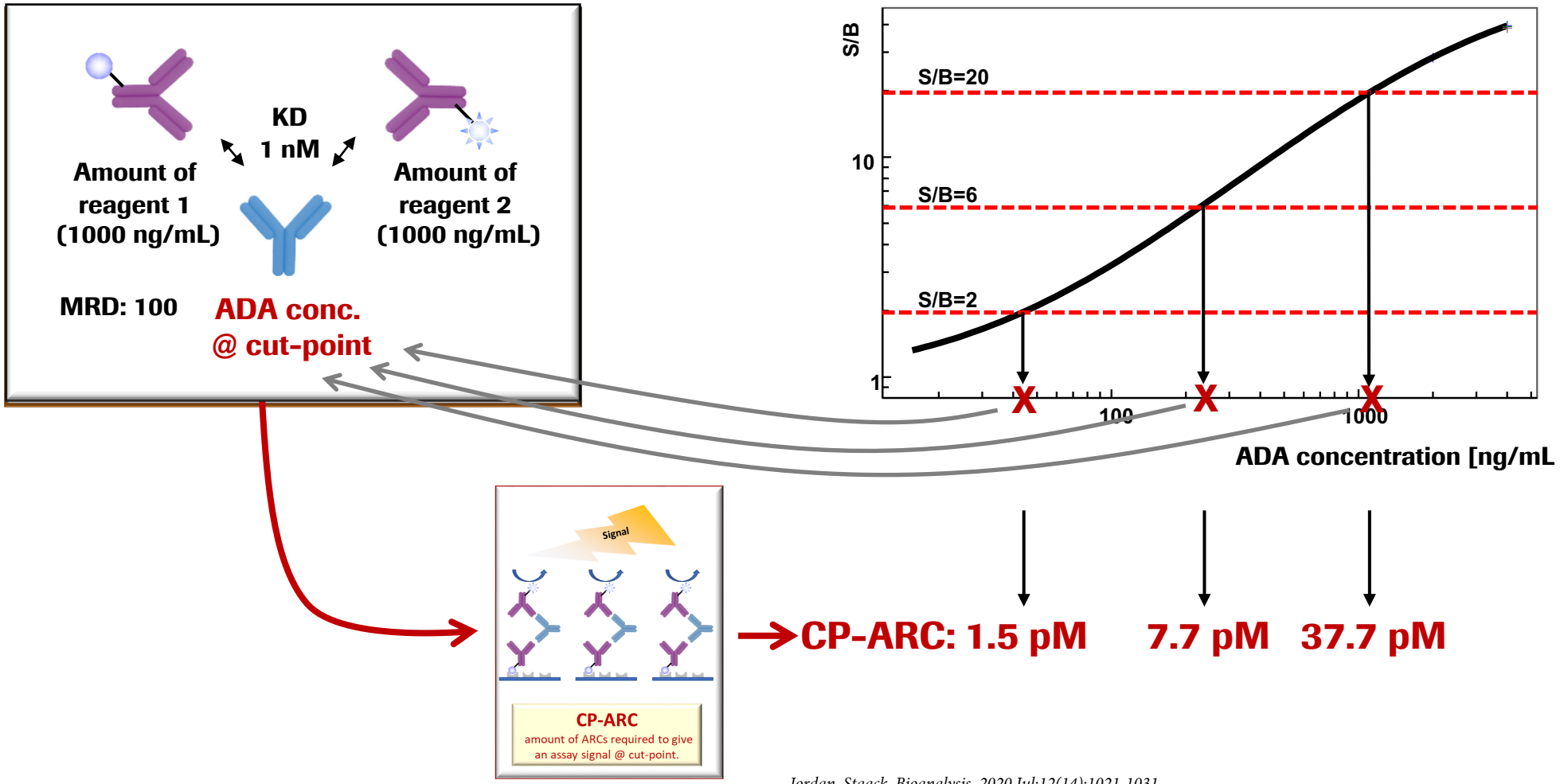
Staack, Jordan, Dahl, Heinrich. Bioanalysis. 2014 Feb;6(4):485-96
Schick, Staack, Haak, Jordan, Dahl, Heinrich, Birnboek, Papadimitriou; Bioanalysis. 2016 Dec;8(24):2537-2549
Jordan, Onami, Heinrich, Staack; Bioanalysis. 2017 Nov;9(21):1705-1717.

➢ **Calculation of protein interactions is a routine task**

…but needs broader implementation into BioA

Determination of CP-ARC

→ "real" example



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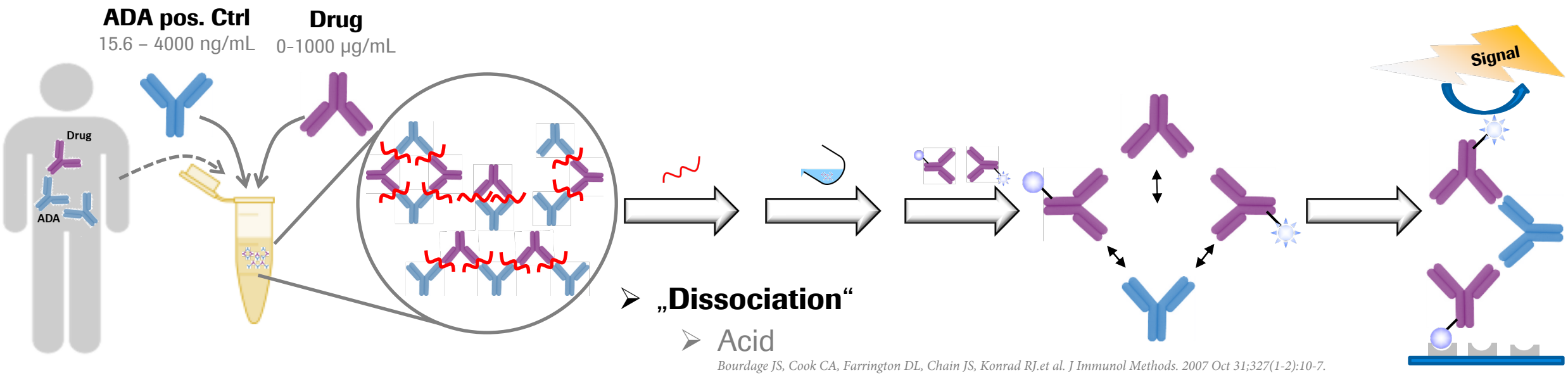
Challenge of „real life“ samples

→ *Presence of residual drug*



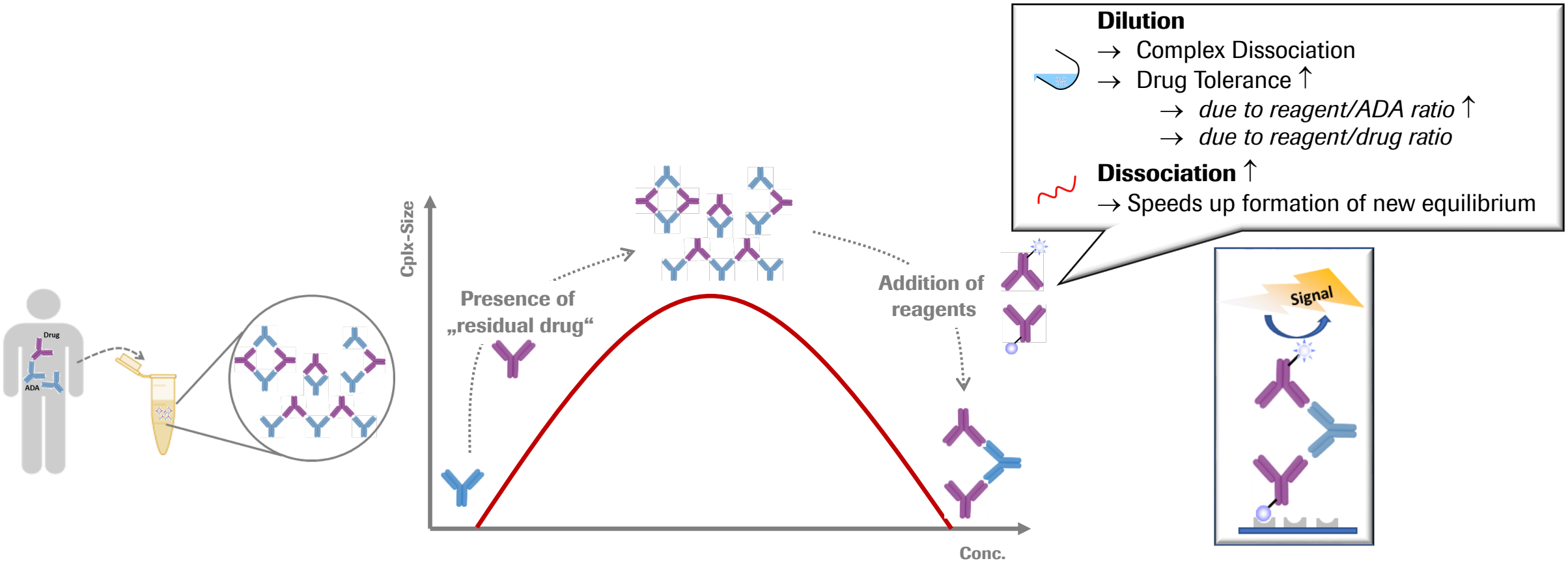
- **„Dissociation“**
  - Acid

    Bourdage JS, Cook CA, Farrington DL, Chain JS, Konrad RJ.et al. J Immunol Methods. 2007 Oct 31;327(1-2):10-7.
    Smith HW, Butterfield A, Sun D. Regul Toxicol Pharmacol. 2007 Dec;49(3):230-7
  - HISDA

    Jordan, Pöhler, Guilhot, Zaspel, Staack. Bioanalysis. 2020 Jun;12(12):857-866 → ***Presentation Gregor Jordan @ EBF20***
- **Dilution** (MRD 100)
- **Reagents** = new binding partners (1000ng/mL, each)

ADA-Analysis – from the ADA in the sample to an assay signal

…nothing than equilibrium shifts?



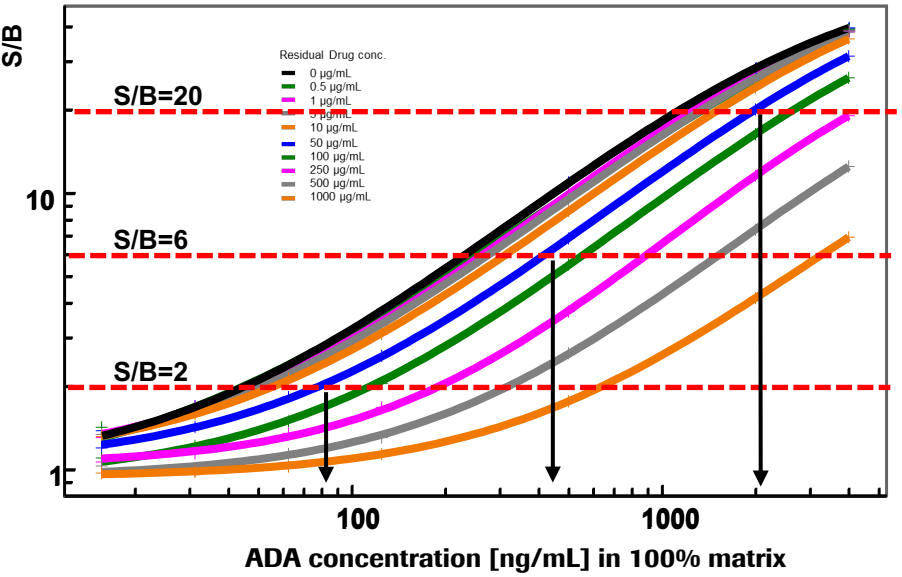
Binding Partner A = **Constant** concentration → ADA

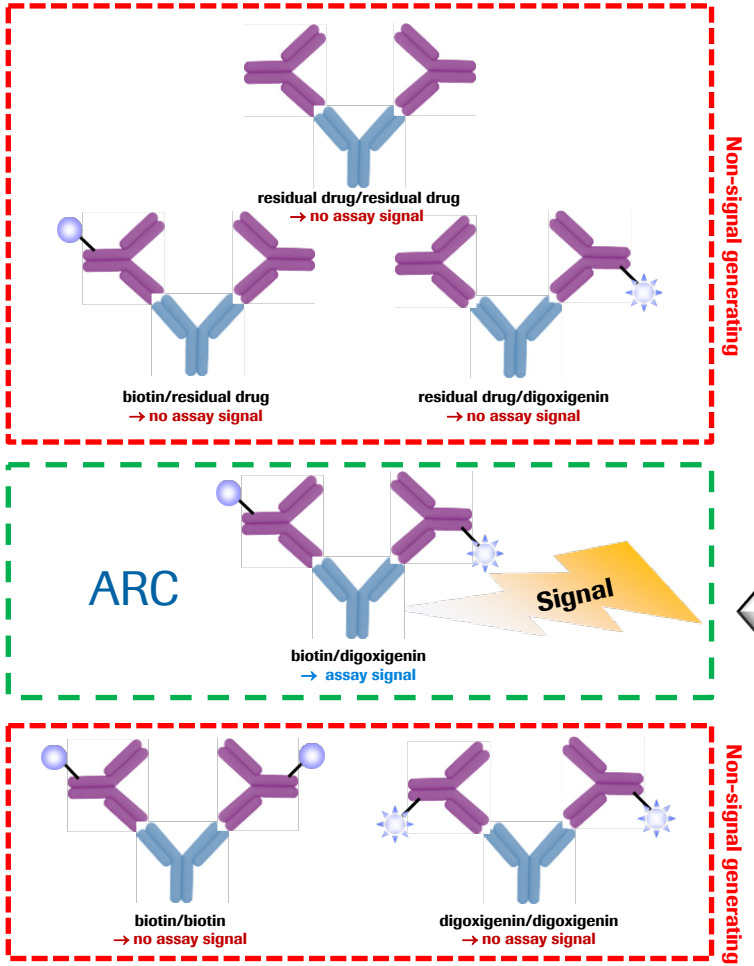
Binding Partner B = **Increasing** concentration → Drug → & Reagents

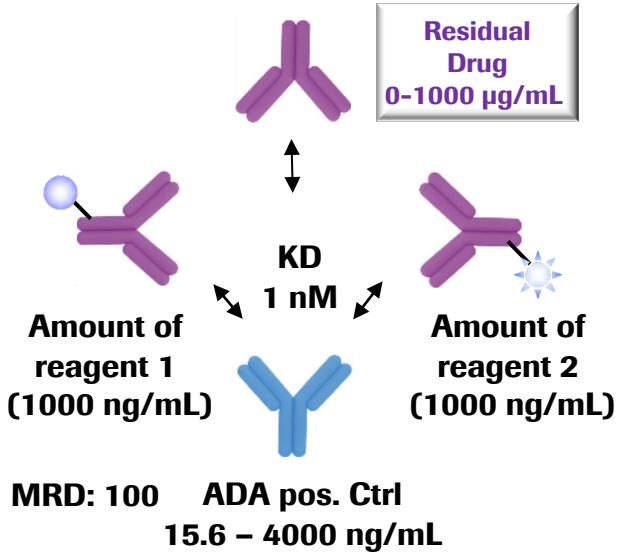
ADA Analysis @ presence of residual drug

„Reduced Drug Tolerance-Effect“

- **Formation of additional non-signal generating complexes**







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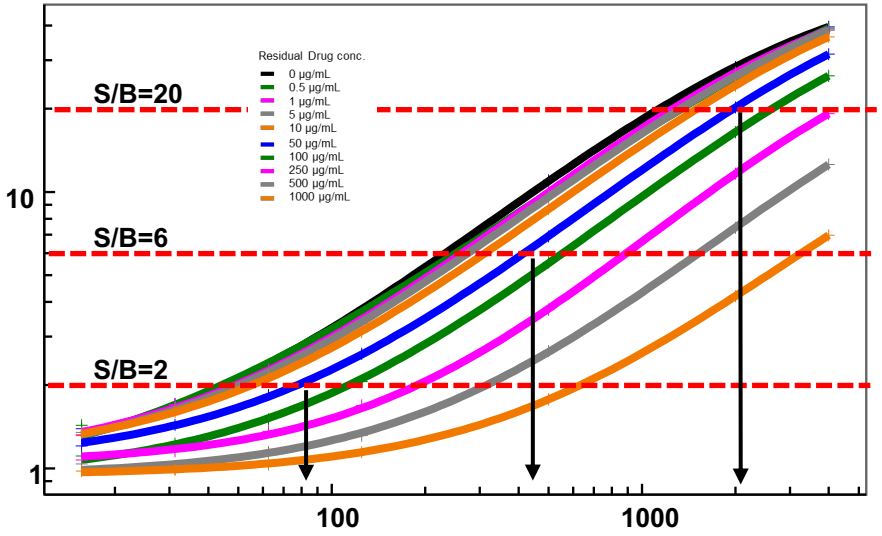
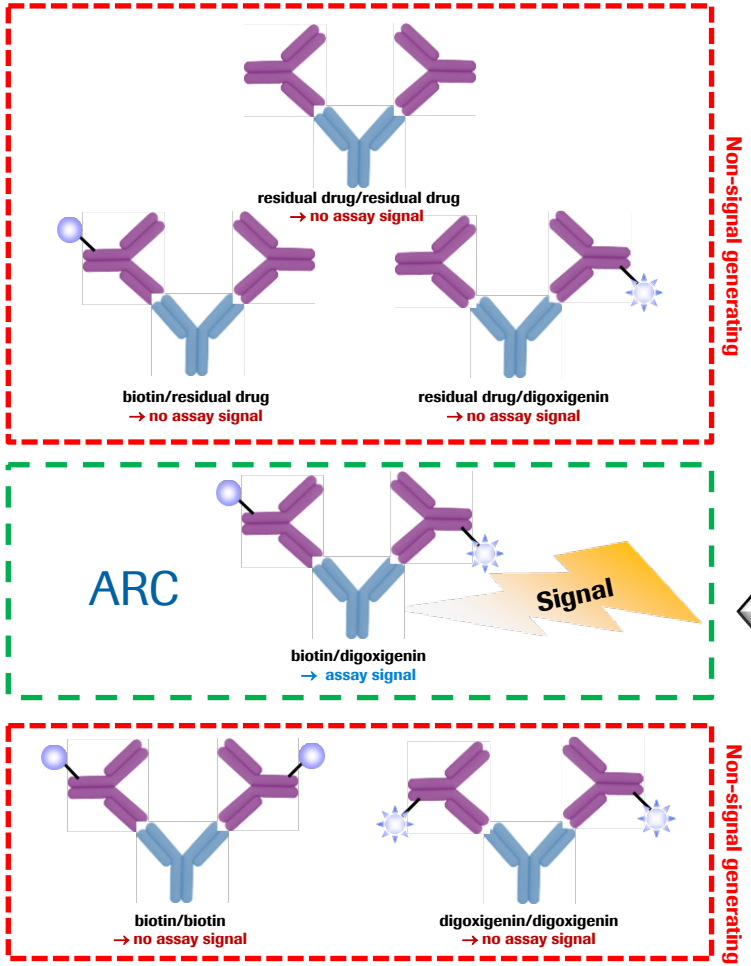
ADA Analysis @ presence of residual drug

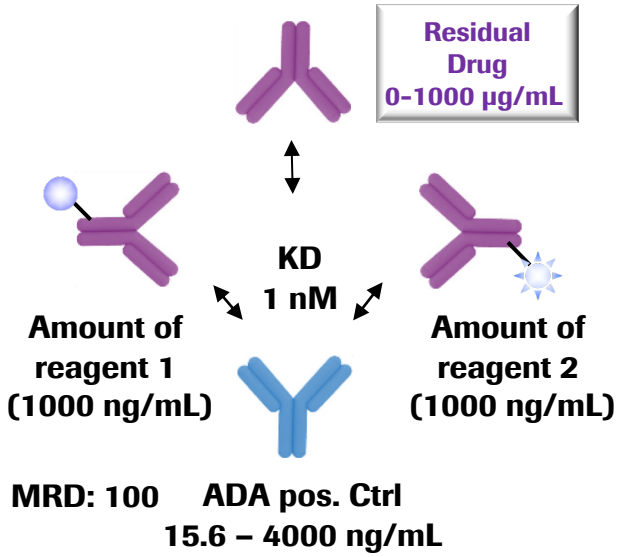
Better understanding of drug tolerance performance thanks to CP-ARC?

Analytical experiment:
Determination of ADA conc. that generated a
pos. assay signal @ S/B 2, 6 & 20
in the presence of increasing residual drug concentrations





Calculation:
ADA conc. required to form sufficient amounts of
CP-ARCs @ S/B 2, 6 & 20
(1.5, 7.7, 37 pM)
in the presence of increasing residual drug concentrations



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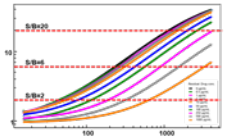
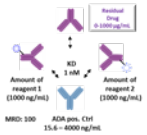
Prediction of „real“ assay performance by CP-ARC?

Analytical experiment:





Calculation:

Residual Drug →	0 µg/mL			0.5 µg/mL			1 µg/mL			5 µg/mL			10 µg/mL		
ADA concentration at different CP → [ng/mL]	ADA exp.	ADA calc	Prec. [%]	ADA exp.	ADA calc	Prec. [%]	ADA exp.	ADA calc	Prec. [%]	ADA exp.	ADA calc	Prec. [%]	ADA exp.	ADA calc	Prec. [%]
S/B=2	0.45	0.45	**100%**	0.44	0.45	**98%**	0.49	0.45	**108%**	0.50	0.47	**107%**	0.55	0.50	**112%**
S/B=6	2.32	2.32	**100%**	2.51	2.33	**108%**	2.63	2.35	**112%**	2.74	2.44	**112%**	3.11	2.56	**122%**
S/B=20	11.38	11.38	**100%**	13.36	11.44	**117%**	12.59	11.50	**109%**	13.30	11.96	**111%**	15.05	12.55	**120%**

Residual Drug →	50 µg/mL			100 µg/mL			250 µg/mL			500 µg/mL			1000 µg/mL		
ADA concentration at different CP → [ng/mL]	ADA exp.	ADA calc	Prec. [%]	ADA exp.	ADA calc	Prec. [%]	ADA exp.	ADA calc	Prec. [%]	ADA exp.	ADA calc	Prec. [%]	ADA exp.	ADA calc	Prec. [%]
S/B=2	0.77	0.70	**110%**	1.13	1.01	**112%**	1.89	2.28	**83%**	3.15	5.49	**57%**	6.33	16.13	**39%**
S/B=6	4.16	3.62	**115%**	5.55	5.21	**106%**	8.89	11.72	**76%**	15.23	28.33	**54%**	32.58	83.19	**39%**
S/B=20	19.69	17.76	**111%**	26.24	25.56	**103%**	OOR	57.45	--	OOR	138.89	--	OOR	407.78	---

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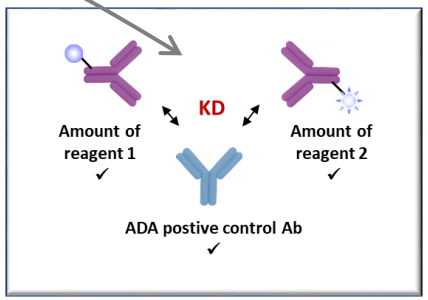
Summary & Conclusion

- Example **confirms the CP-ARC hypothesis**
  → knowledge of assay CP-ARC can predict assay performance
- **CP-ARC = constant assay parameter that defines the sensitivity and drug tolerance of a <u>given</u> assay**
  - Sensitivity: clear understaning of assay sensitivity independent of the positive control
    - *FDA 2019 : „..*high affinity positive controls may overestimate the sensitivity of the assay…"

What do we need to reach goal?

- **A joint effort of the entire BioA community from assay development to regulators!**

- **Characterisation of binding properties** of ADA positive control
- Expansion of the concept on other assay formats
- Better understanding of the binding properties of „real ADAs"



Benefit for the BA communitiy

- Application of CP-ARC concept enables **comparison of ADA-Assay performance/results**

- **Improved ADA reporting**
  - No „inconclusive“ results, since the assay performance is fully understood
    - **Clear statement what ADA concentrations are detectable**
      - *Example:* <100 ng/ml ADA with an affinity of xyz nM

- **BioA contribution to enable valid comparison of „clinical immunogenicity“ of different compounds**

Acknowledgement

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@ Roche Innovation Center Munich & Roche Innovation Center Basel

Doing now what patients need next