Model informed assay development (MIAD). How can we confirm with an orthogonal method the "Signal/Noise" immunogenicity reporting strategy?

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From were we come and were we would like to land

… and which missing pieces we might have to tackle to make assay signals comparable

Assay development → **sample analysis** → **Assay results reporting (→titer or S/B?)**

How can we optimize our assay in regards to the „context of use“…

→ …is it possible to compare different assays/signals

If we understand how our sample behaves in our assay…. We should be able to optimize the assay accordingly…

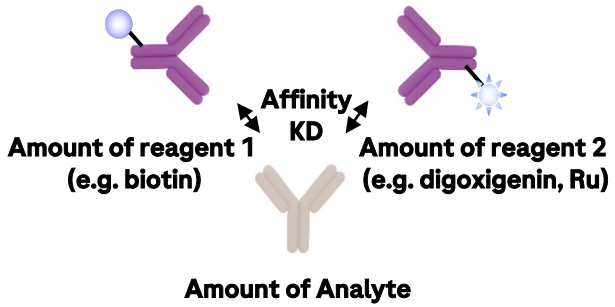
→ Which complexes are formed and which complexes provide an signal

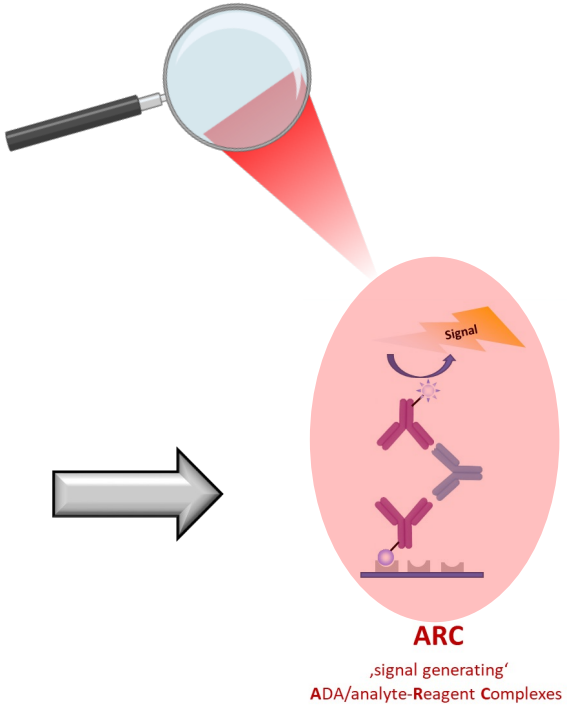
…. Can we even optimize reporting? and provide additional value to drug development?

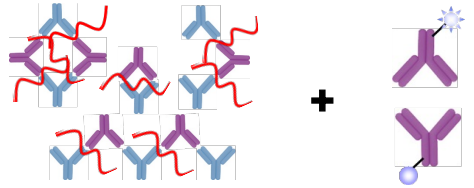
➢ **Where are potential tumbling blocks that potentially hamper comparability**

Central building block of the model

… is the formation and calculation of the Analytical Reagent Complex (ARC)

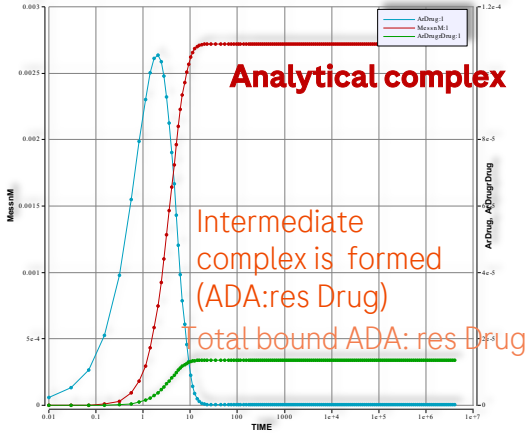






Complex disruption

1:100 of with disruption of complexes



Selected assay concentrations
… equilibrium

Selected assay conditions
… kinetic

The formation of these complexes can be calculated

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

From an equilibrium model to a kinetic model

… using Model Informed Assay Development (MIAD) to optimize and “understand” assay conditions

Find conditions to give a defined start for the assay

Drug

ADA

CIC

Drug

ADA

Reagent A

Reagent B

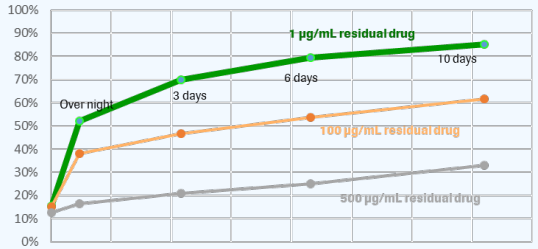
Intermediate complex formation

„1 to 1 signal“

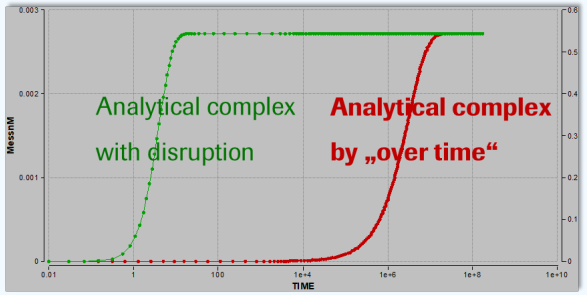
„3 to 1 signal“

Non disruptive conditions

increase of normalized signal with pos. control @375 ng/mL in matrix



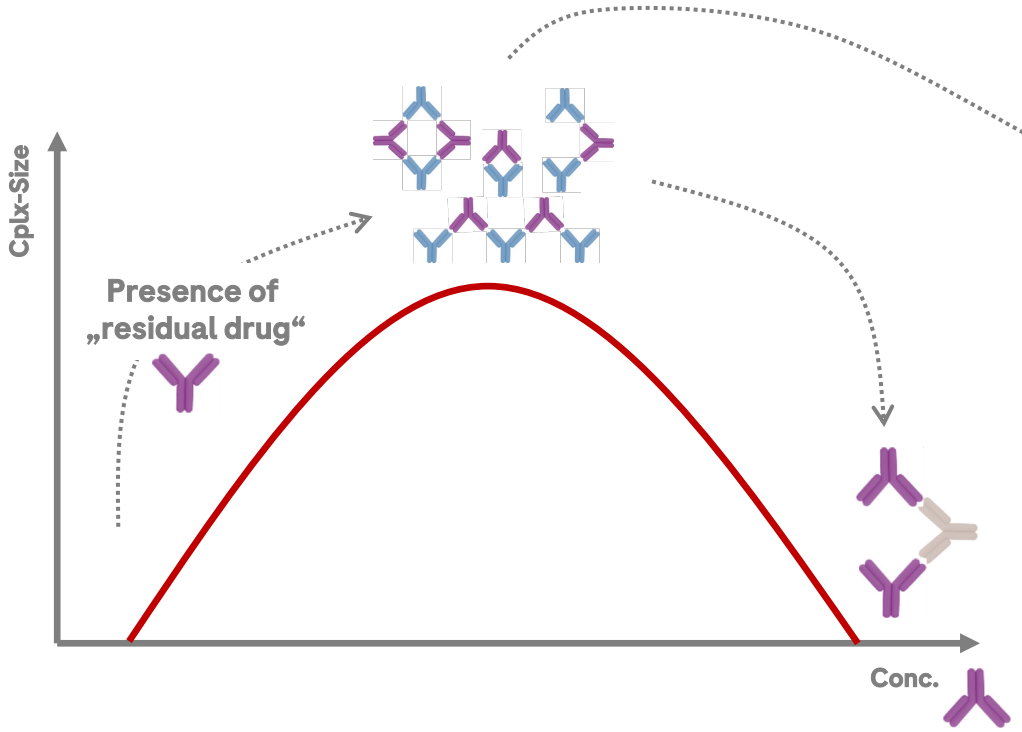
Calculated comparison Disruptive vs. non-disruptive

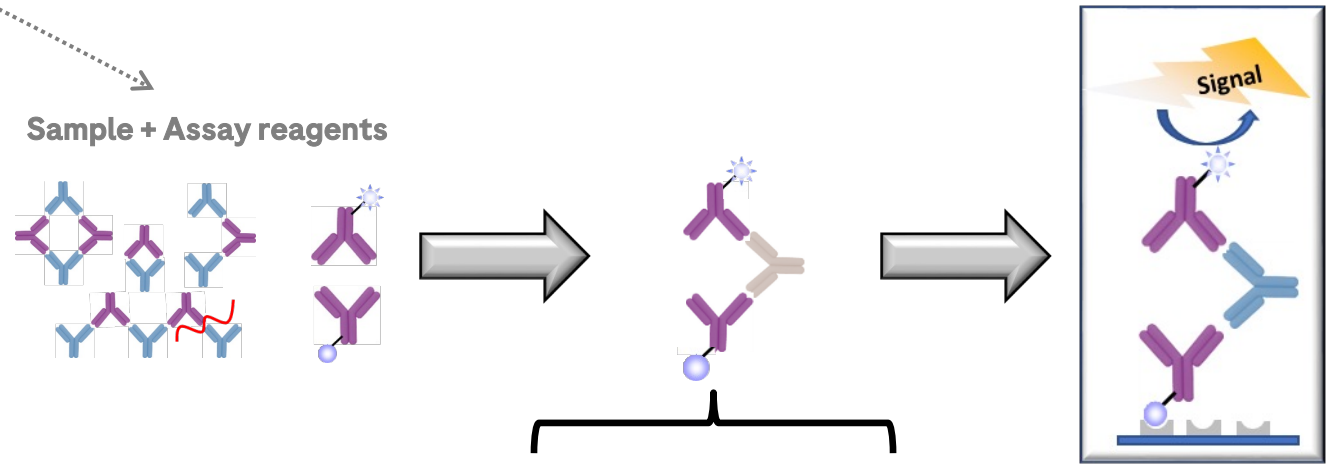


Special thank you to Tim Marchhauser for data generation

From ADA in the sample to an signal in the assay

... where do we have leverage to make an impact?





Binding Partner A = **Constant** concentration

Binding Partner B = **Increasing** concentration

Dilution

→ Complex Dissociation

→ Drug Tolerance ↑

→ *due to reagent/ADA ratio* ↑

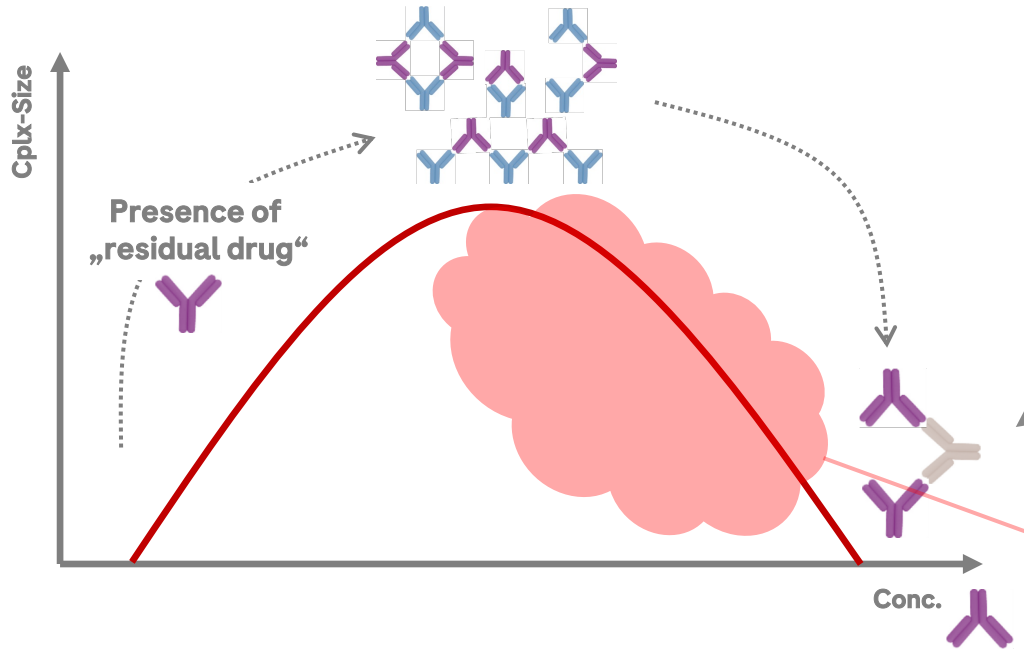
→ *due to reagent/drug ratio*

→ Forming new equilibria between ADA and reagent

→ Dissociation speeds up formation of new equilibrium

The model can only be as good as we understand the assay conditions

…. what is predicted to what we see/observe?



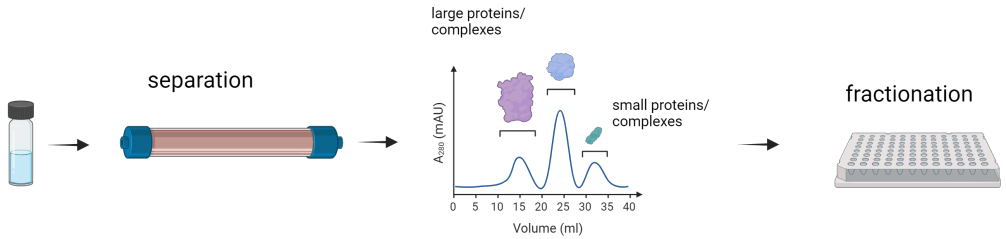
We would like to “land here”

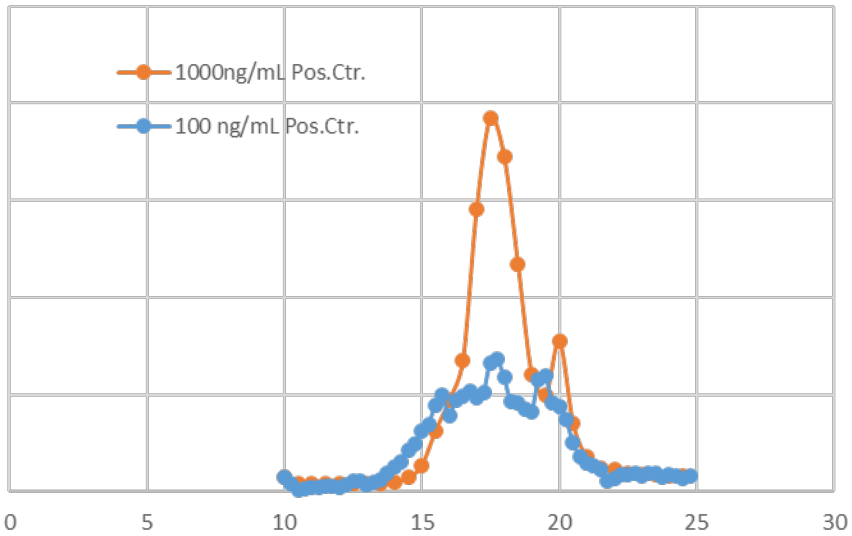
→ **final sample condition in the assay to enable comparability of different samples**

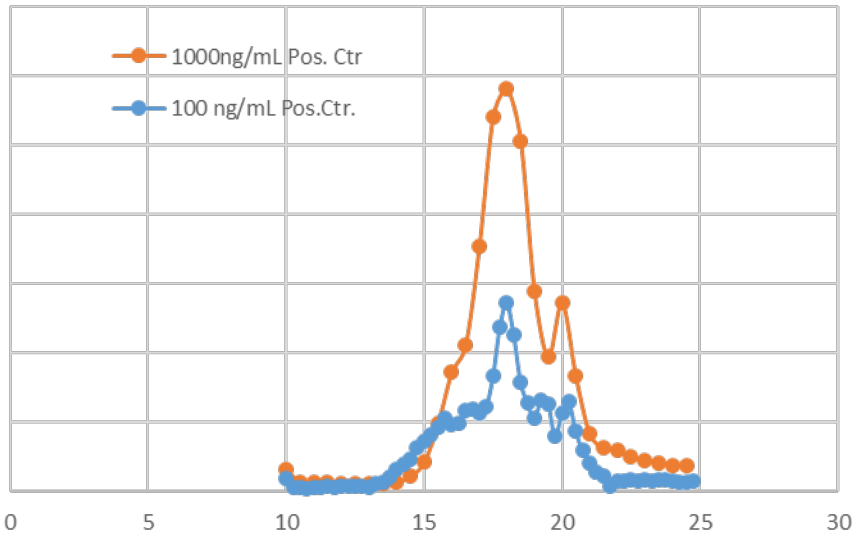
Although we have the „correct“ reagent concentrations… might it be possible to form complexes of higher order „ab initio“?
Impact of mAb versus pAb on ARC formation?

Pressure test of the formed analytical reagent complexes

… two different **mAb** used as ADA positive surrogate molecules





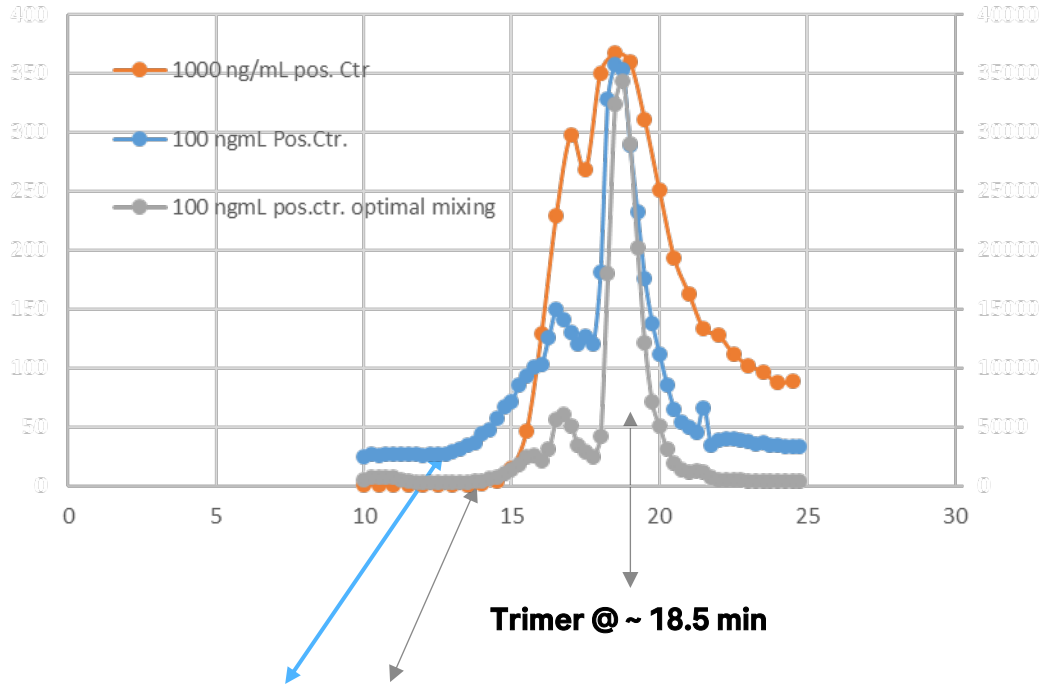


➢ **Pre-dominant Trimers and Tetramers were formed … when the assay is running at optimal conditions**

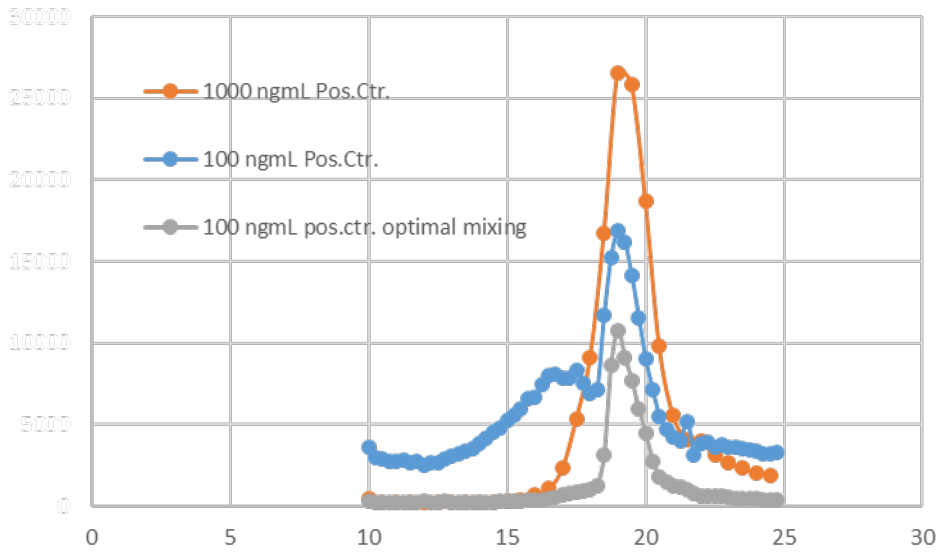
Special thank you to Thomas Bach for data generation

Pressure test of the formed analytical reagent complexes

... with pAb preparations of two species



	Normal 100 ng/mL	optimal 100 ng/mL
non Trimer	14%	8%
Trimer	**86%**	**92%**



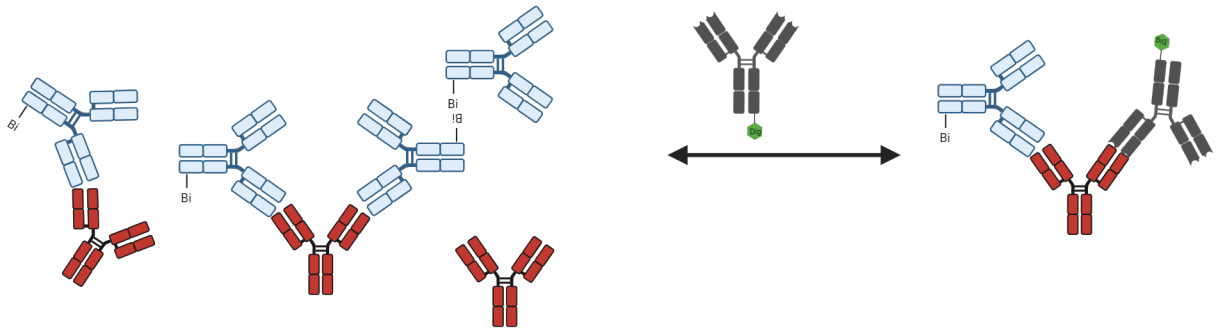
	Normal 100 ng/mL	optimal 100 ng/mL
non Trimer	16%	4%
Trimer	**84%**	**96%**

- **Predominant Trimer followed by tetramer were formed**
- **For one pos. control slight pentamer formation**

KD impact on ARC formation

… can we optimize the condition to reduce KD dependency on ARC formation?

KD determination is quite challenging. Possible solution → generation of Fab fragments, determine the KD and then assume the KD will be maintained in case of full IgG? Here the KD is determined with the ADA assay reagents and different positive controls. In solution approach was chosen.



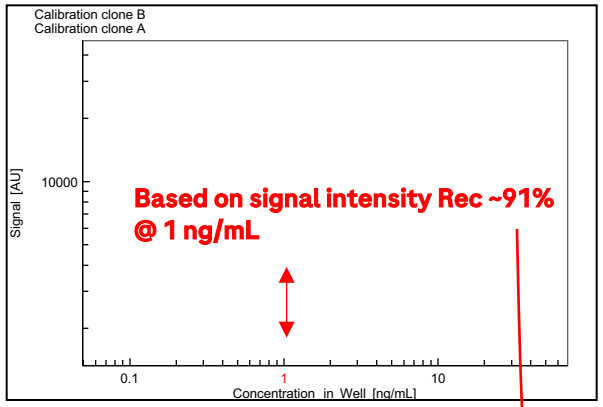
Clone A ~ 1.5 nM (“screening-”SPR ~0.17nM)
Clone B ~ 0.5 nM (“screening-”SPR ~ 0.19nM)

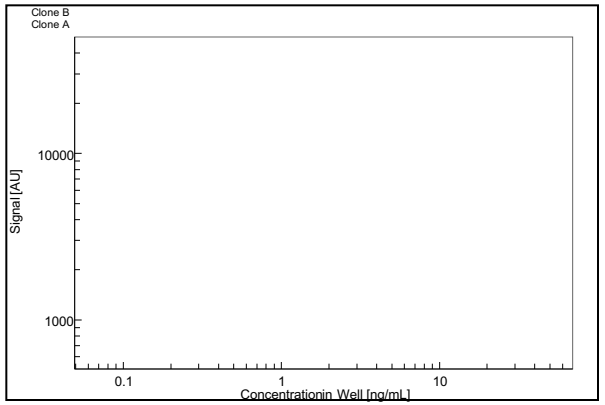
Differences observed in SPR and in-solution KD values
For these experiments two clones were selected with “stable k_{off} rate” $t_{1/2}$~2h

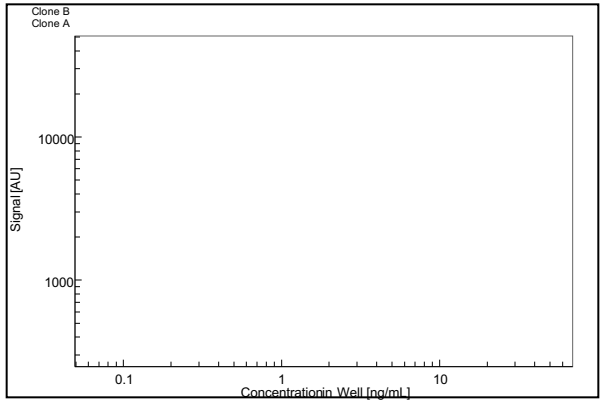
Does the KD value matter?

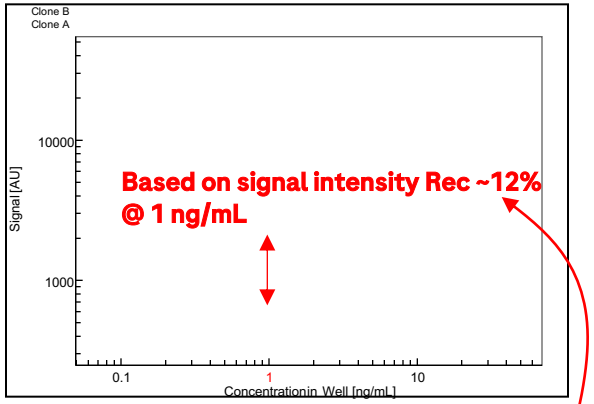
…or can we normalize differences in the KD value? …where to optimize?

Reagent concentration in well:
1000 ng/mL → **100 ng/mL** → **50 ng/mL** → **10 ng/mL**

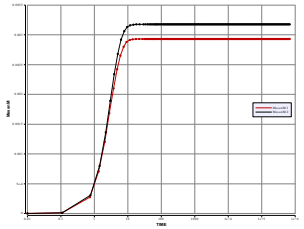








Calculated ARC differences



- **Recovery ~ 92%**

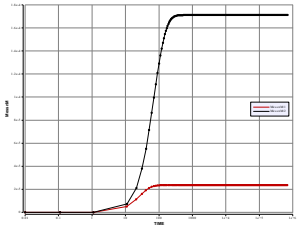
- **Clear dependency of reagent concentration on comparability of the two clones**

- **Model fits with experimental results**

Reagent	1000 ng/mL	10 ng/mL
Assay	91 %	12 %
Model	92 %	8 %

- **Differences in the assay observed, but we can optimize accordingly**

Calculated ARC differences



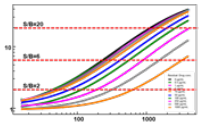
- **Recovery 8 %**

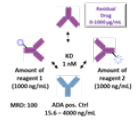
Special thank you to Franziska Endt for data generation

Model valid only @ Cut point level and what about res. drug impact?

… ACR, with residual drug, at S/B rations covering the calibration curve can be calculated

Analytical experiment:



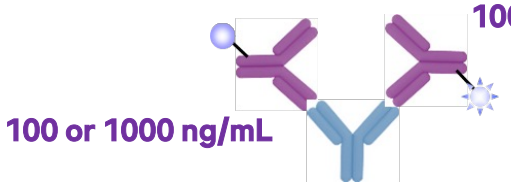


Calculation:

Residual Drug →	50 µg/mL			100 µg/mL			250 µg/mL		
ADA concentration at different CP → [ng/mL]	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%
S/B=6	4.16	3.62	115%	5.55	5.21	106%	8.89	11.72	76%
S/B=20	19.69	17.76	111%	26.24	25.56	103%	OOR	57.45	--

ARC can be calculated covering the entire calibration curve

100 µg/mL res. drug



Bi / Dig [ng/mL]	Analytical [ng/mL]	Calculated [ng/mL]	Precision [%]
1000 / 1000	49.3	ARC=1.63 pM (KD=0.1 nM)	
1000 / 1000	102.4	110.6	108%
100 /100	2385.9	1783.5	75%
100 / 1000	755.0	543.3	72%
1000 / 100	529.4	543.3	103%

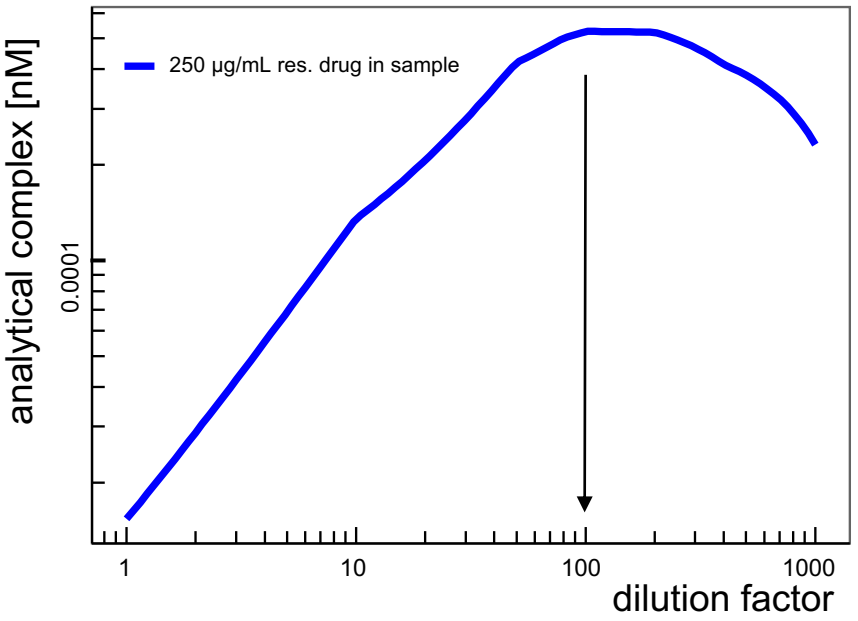
+ 100 µg/mL residual drug

At MRD=100 low impact in case of high reagent concentration → "pressure" to form ARC

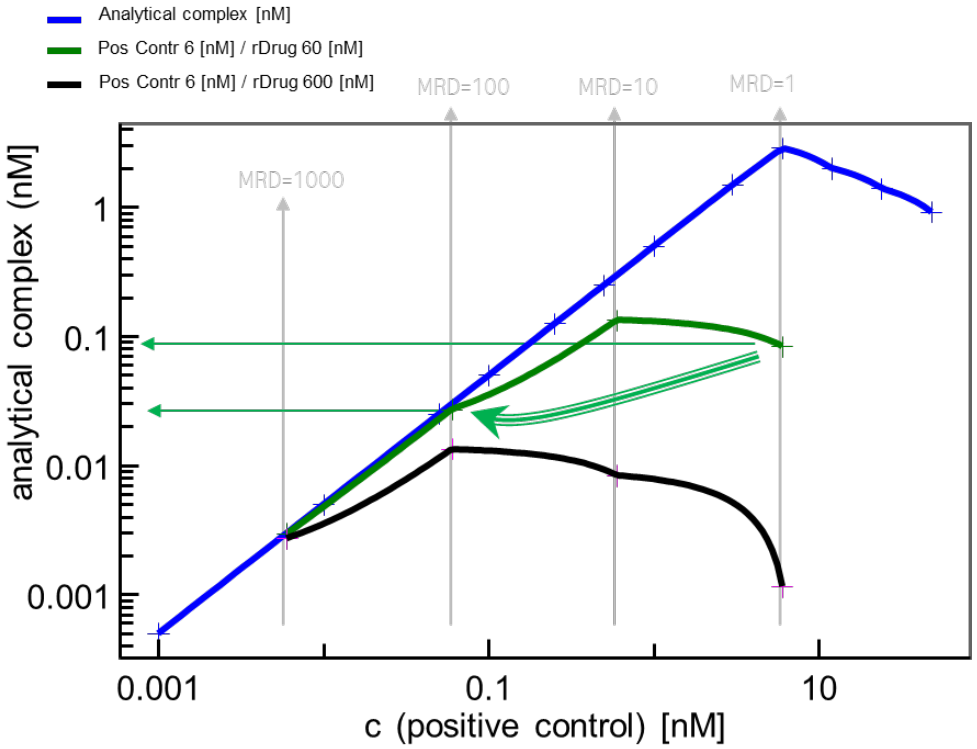
Jordan, Staack, Bioanalysis, 2020, Jul;12(14):1021-1031

Impact of the dilution on the assay readout

… when do loose sensitivity and when do we “limit” res. drug interference

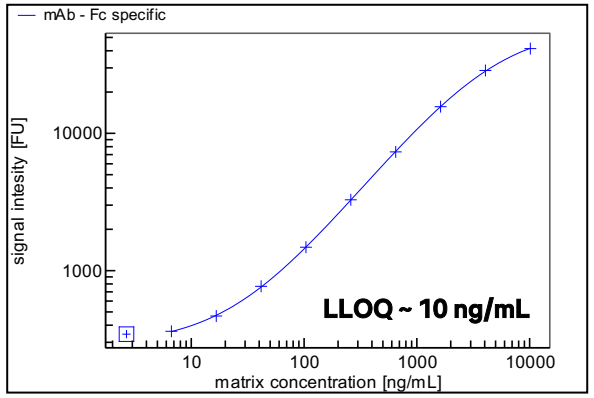


- **In the presence of high res. drug concentration a high sample dilution is beneficial in terms of ADA detectability**



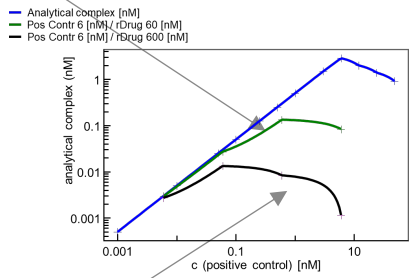
- **In the presence of “moderate” res. drug concentration a MRD of 100 brings the sample close to the calibration**
- **At higher concentration a dilution of 1000 seems to “level” the res. drug impact**

Recovery of res. drug samples after dilution…



samples	FU n=2	measured conc. [ng/ml]	Dilution After MRD MRD=100	Calc. conc. [ng/mL]	Recovery to nominal
4(PC 500 ng/ml)	7024	595.2	1	595.2	**119%**
4.1	1684	117.5	5	587.6	**118%**
4.2	512	19.3	25	481.3	**96%**
4.3	322	#N/A	125		
4.4	289	#N/A	625		
4.5	308	#N/A	3125		
5(PC 2500 ng/ml)	21905	2535.5	1	2536	**101%**
5.1	5772	476.6	5	2383	**95%**
5.2	1344	89.1	25	2226	**89%**
5.3	452	14.2	125	1771	**71%**
5.4	315	#N/A	625		
5.5	290	#N/A	3125		
6(PC 8000 ng/ml)	37999	7358.5	1	7359	**92%**
6.1	15970	1615.6	5	8078	**101%**
6.2	3857	303.7	25	7592	**95%**
6.3	920	53.6	125	6701	**84%**
6.4	397	#N/A	625		
6.5	319	#N/A	3125		
1(PC 500 ng/ml)	2446	181.9	1	182	36%
1.1	1321	87.1	5	436	**87%**
1.2	524	20.3	25	507	**101%**
1.3	361	#N/A	125		
1.4	320	#N/A	625		
1.5	303	#N/A	3125		
2(PC 2500 ng/ml)	7575	648.9	1	649	26%
2.1	4186	332.8	5	1663.8	67%
2.2	1223	78.9	25	1973.5	**79%**
2.3	461	14.9	125	1867.3	**75%**
2.4	327	#N/A	625		
2.5	333	#N/A	3125		
3(PC 8000 ng/ml)	18847	2031	1	2030.3	25%
3.1	10971	1004	5	5018.0	63%
3.2	3139	241	25	6029.9	**75%**
3.3	840	47	125	5868.7	**73%**
3.4	384	#N/A	625		
3.5	320	#N/A	3125		

66 nM res. Drug



4000 nM res. Drug
(600µg/mL IgG)

Model would predict:

1	6 %
5	50 %
25	**80%**

…which is inline with experimental results…
by using a default KD of 0.1nM for calculation

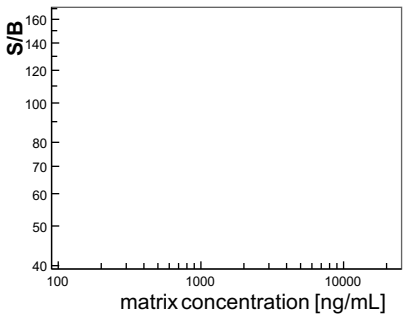
Special thank you to Ahmad Hallak for data generation

Lucky shot? … data from an other project..

… no calculations… comparison of assay signals with and without res. Drug … slightly higher reagent concentrations used

Recovery of assay signal to identical dilution of sample without res. drug

Signals @ ULOQ



ADA [ng/mL]	**20000**	**2000**	**200**	20000	2000	200	20000	2000
res drug [µg/mL]	**600**	**600**	**600**	200	200	200	50	50
1	77%	25% (16%)	16%	111%	**108%**	**103%**	108%	**106%**
2	90%	43% (33 %)	46%	116%	**110%**	**152%**	114%	**95%**
4	93%	58% (53%)	50%	107%	**102%**	**93%**	103%	**107%**
8	**96%**	**75% (71 %)**	69%	**107%**	**106%**	**89%**	**99%**	**117%**
16	**101%**	**90% (84%)**	**94%**	**110%**	**106%**	**97%**	**105%**	**111%**
32	**104%**	**94%**	**103%**	**105%**	**103%**	**100%**	**91%**	**114%**
64	**104%**	**108%**	**105%**	**111%**	**113%**	**102%**	**86%**	**118%**
128	**100%**	**93%**	**101%**	**93%**	**98%**	**104%**	**81%**	**103%**

in brackets calculated (MIAD) recovery

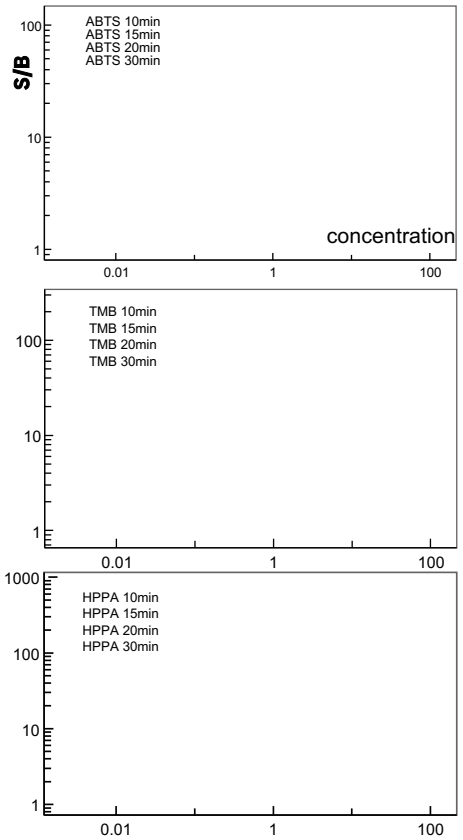
Assay is currently under validation and sensitivity is targeted to be ~ 3 ng/mL (with MRD=100)

- **Recovery data, based on signal intensity, confirms previous results**
- **Calculation meets experimental results**
- **@ dilutions of ~ 800 assay seems to be res. drug tolerant**
- **Switch to MRD=800 (instead of 100) would result in an assay sensitivity of ~ 24 ng/mL**
- **“S/B reporting” would be possible up ~ 120**

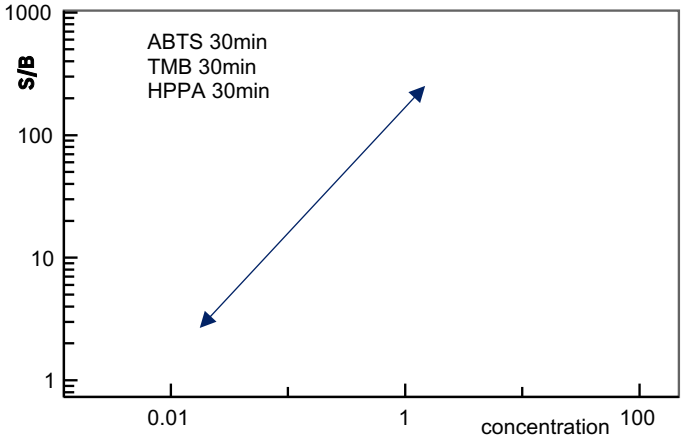
Special thank you to Thomas Bach for data generation

Dynamic ranges of LBA substrates

...under optimal conditions. Investigation of ABTS, TMB and HPPA*

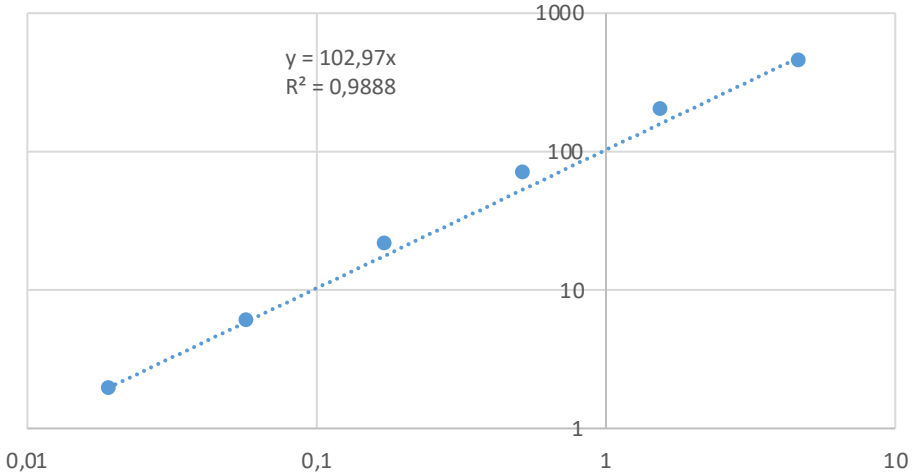


Development time with highest sensitivity selected



Substrate	HPPA	TMB	ABTS
Dynamic range (log10)	3.8	3.3	2.8

linear relation plot for HPPA with origin linear fit



- **High dynamic ranges with fluorogenic substrates achievable**
- **Chemiluminescent substrates not tested**

*Jordan et al: Bioanalysis. 2017 Feb;9(4):407-418.

Summary and conclusion

- **Model Informed Assay Development (MIAD)** is a valuable tool for:
  - **Assay development** and selection of optimal assay conditions in terms of
    - Optimize **drug tolerance**
    - **Level ADA KD differences**
    - Reduce assay **development time** (e.g. no checkerboard for assay development needed)
    - MRD selection
  - **Deeper understanding of ADA assay response** and opens potential **ADA quantification** (is a ADA assay a PK assay?)

- **Higher dilutions** would **reduce impact of residual drug** on assay signal and even enable detectability of ADA in high res. drug containing samples
  - Assay “moves” toward a PK assay with dilution linearity of residual drug samples

- S/B reporting approach: **MRD of 400-500** would cover res. drug concentrations up to 600 µg/mL (IgG) if we would accept an error/deviation of F=2 (comparable to titer approach) and would enable a res. drug concentration independent sample condition reporting

- Experiments with 2 pAb and 2 mAb preparation show a **ARC formation** of **mainly Trimers/Tetramers**. Sample processing might have an influence on ARC formation

- The use of HPPA would be advantageous (for enzyme-based assays) to obtain high dynamic ranges that best cover broad S/B reporting

Acknowledgement

- **Roland Staack**

- **Thomas Bach**
- **Tim Marchhauser**
- **Franziska Endt**
- **Ahmad Hallak**

Doing now what patients need next