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Multi-specifics & multiplexing: An Ideal match!

Sanofi Ghent - TMU

EBF Open Symposium 2025

Cara Smits

01 Bioanalytical strategy

Terminology

Multi-specific compounds: drug compounds binding **more than one therapeutic target**

Active exposure: a regulatory requirement to evaluate **pharmacological activity** of a drug for IND-enabling toxicity studies, demonstrating that the drug can actively bind its target (e.g. via PK/PD read-out)*

Bi/tri-active PK: a PK method that quantifies the **binding competent drug** able to bind all (therapeutic) targets, measuring **active exposure**

Dual bi-active PK: two, bi-active PK methods in a **one-well format** using a multiplexing platform. In this presentation we will focus on the MSD U-plex technology

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*ICH S6(R1) - Preclinical safety evaluation of biotechnology-derived pharmaceuticals

Exposure of biologics in toxicology species

Unexpected changes in exposure in the absence of a PD marker

↓

ADA formation in animals can have variable impact on TK

(*no impact – clearing – neutralizing*)

+

No characterization of non-clinical ADA

(*e.g. domain specificity or NAb assay*)

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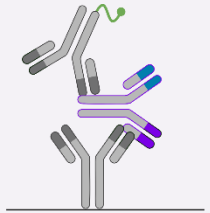
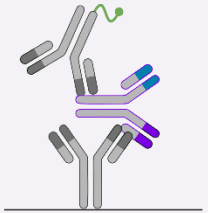
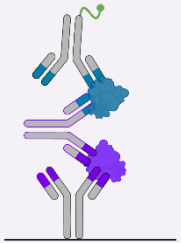
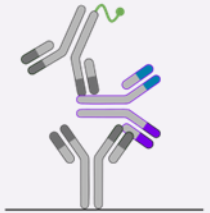
TK evaluation in non-clinical toxicity studies with appropriate combination of assay formats for PK/PD/ADA

PD marker(s)	No PD marker(s)	No PD marker(s)
Sustained PD – active exposure	Total TK method	Active TK method
↓	↓	↓
Total TK method sufficient	Total TK assay + ADA (NAb) assay to show active exposure	**Active PK method** needed to claim active exposure

Bioanalytical strategy

Bi-specifics (simplified)

LBA	Exploratory studies	GLP Toxicity studies		Clinical studies
PK assay	**Total PK** 	**Multiple target PD** *(target engagement, receptor occupancy, etc.)* ↓ **Total PK** 	**No target PD** ↓ **(bi-)active PK** 	**Total PK (or other formats)** 
ADA assay	if needed: Direct binding (or other formats)	Bridging or PandA format		

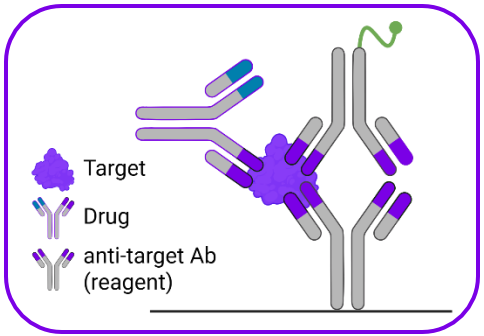
* The bioanalytical strategy is developed in collaboration with the PK scientist (considering modeling, dose selection, etc.) and may be adapted based on project-specific needs.

Need for active exposure in GLP-tox

When target methods are challenging

Target properties that form a challenge:

- Low-expressed target, especially in healthy animals
- Anchored (membrane protein) targets



Non-clinical (NHP) **target methods** can be challenging:

- Commercial kits, often optimized for a human target
  - Not sensitive enough
  - Not drug tolerant
- Homebrew methods
  - Source highly sensitive and non-competitive anti-target reagents
- Relevant and qualitative source for the reference material

When target properties are unfavorable and method development proves to be challenging, choosing an active PK method can be an efficient alternative to demonstrate active exposure

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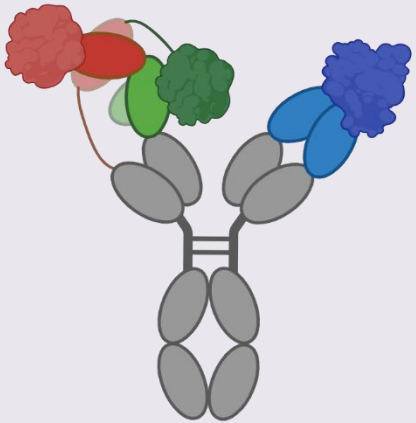
The tri-specific compound

CODV-Ig*



* Cross-Over Dual Variable immunoglobulin-like proteins (CODV-Ig) is a proprietary technology of Sanofi

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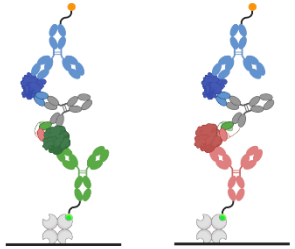
Options for tri-specific active PK



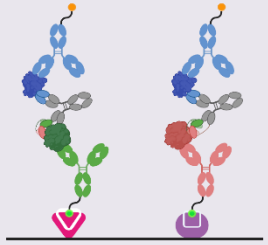
2x bi-active method

2x read-out
ECL

2 methods

+++ Experience

Low development time



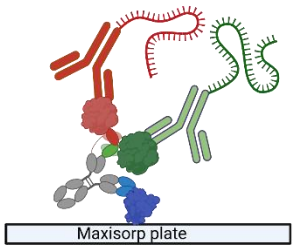
Dual bi-active method

2x read-out
ECL

1 method

+++ Experience

Low development time



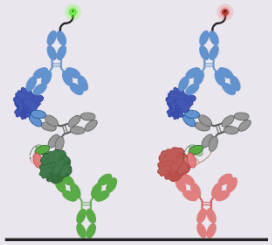
PLA: proximity ligand assay

1x read-out
fluorescent

1 method

Experimental

Extensive development time



Dual bi-active fluoresence

2x read-out
fluorescent

1 method

- Experience

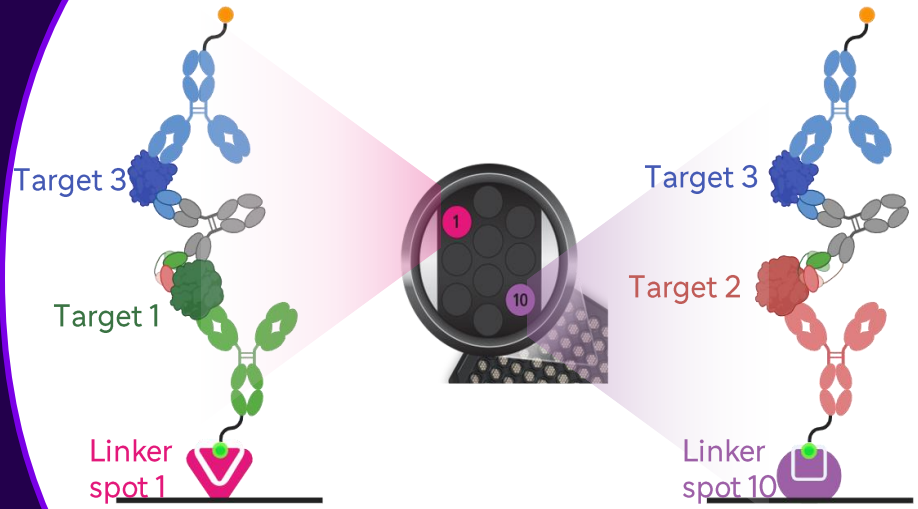
Higher development time

02 Dual bi-active development

Dual bi-active PK method

- 1 well analysis
- MSD U-Plex
- 2x bi-active
- Shared detector reagent
- One dynamic range
- Linked dual read-out
- For serum analysis

Dual bi-active PK



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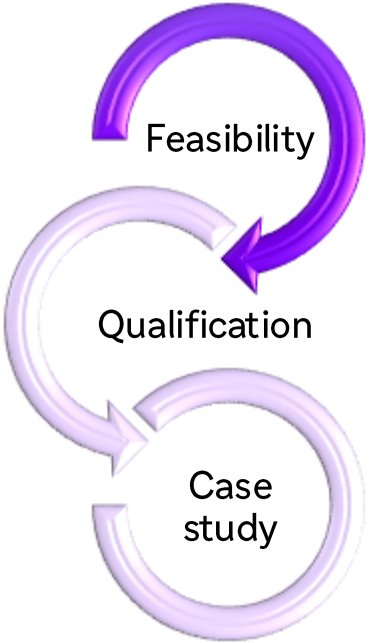
Development challenges

Using a single-well method for multiple PK read-outs adds **complexity** during development

- The same analyte, the drug compound, is shared between reactions in contrast to classic multiplex methods. Shared dynamic range and QC concentrations are preferred.
- The selected anti-target capture antibodies, should be non-competitive for binding the active drug and have comparable affinities to allow similar dynamic ranges.
- Multiplexing requires identical incubations, wash cycles, buffers and MRD between reactions. Compromises may be required during optimization.



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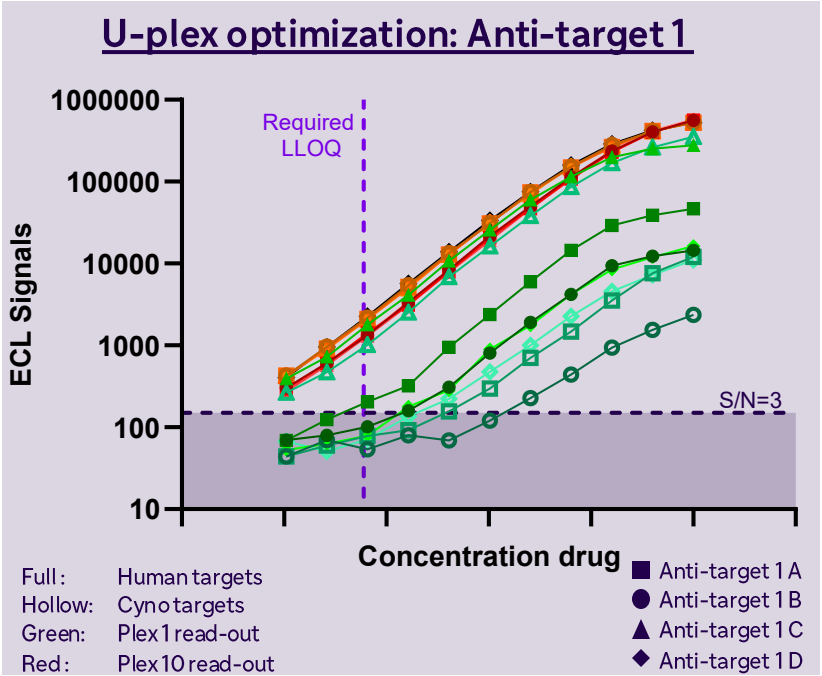
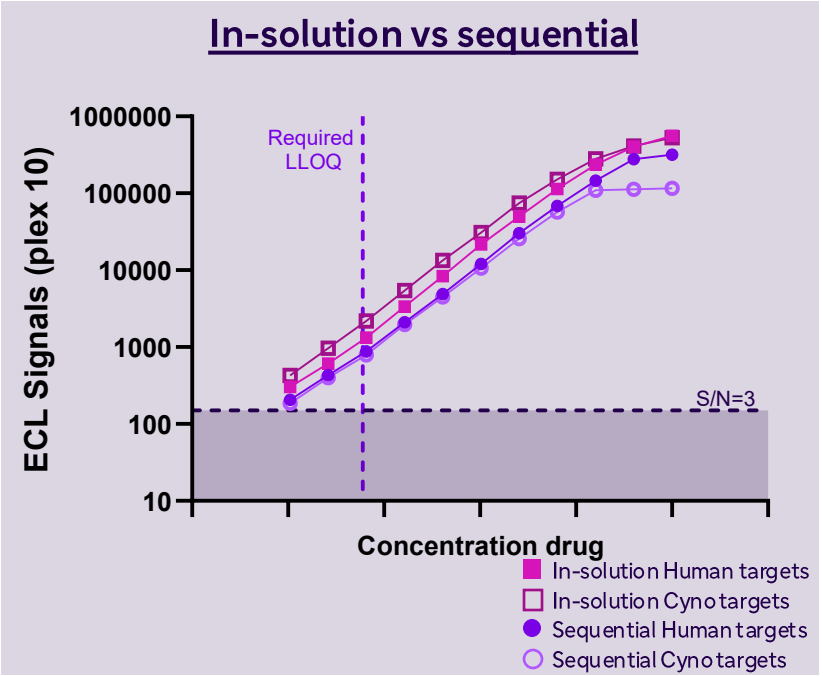
U-plex feasibility: method development





Tool screening (*single well*) → U-plex compatibility → In solution vs sequential → Optimization (sequential)

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Optimized method protocol for the tri-specific compound

Step	Target 1/3 bi-active (Spot 1)	Target 2/3 bi-active (Spot 10)	Incubation time
U-PLEX® linking	Spot 1 linker to anti-target 1 mAb-biotin	Spot 10 linker to anti-target 2 mAb-biotin	Per kit instruction – 30 min
Primary capture	Anti-target 1 mAb-biotin-linker	Anti-target 2 mAb-biotin-linker	Solution with both linked mAb's - 1 h
Secondary capture	Target 1	Target 2	Solution with both targets – 1 h
Sampling	Tri-specific compound		1 h
Primary detection	Target 3		1 h
Secondary detection	Anti-target 3 mAb-sulfoTag		1 h

Common incubation steps **reduce assay variability**

Total assay time ~ 6h

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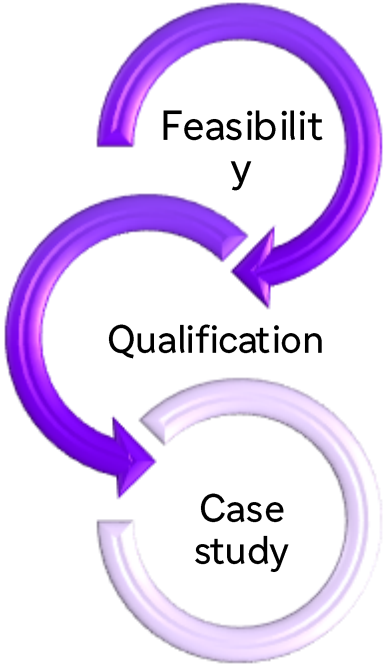
03 Dual bi-active - Qualification

Method qualification

For investigational purposes, the method underwent a **limited qualification** to evaluate robustness of the assay prior to testing samples.

- P&A: *3 independent runs*
- Selectivity: *5 individuals*
- Freeze-thaw stability: *1x FT*
- Target interference: *for capture targets*



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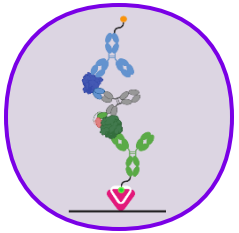
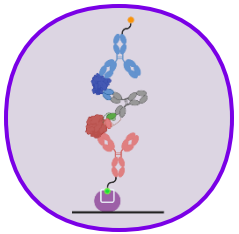
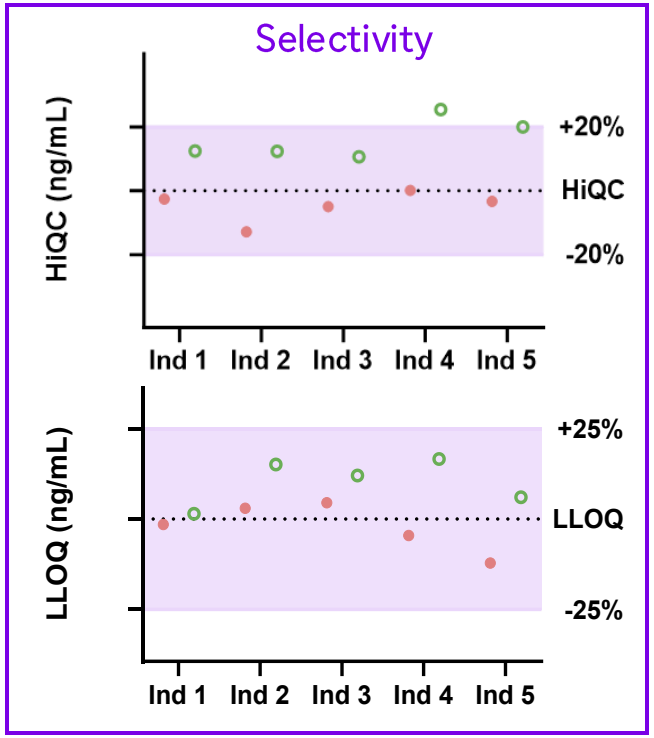
Precision & Accuracy and selectivity

Precision and accuracy					
	Intra-run statistics (Pooled):		Inter-batch statistics (ANOVA):		Total error (%)
Concentration (ng/mL):	% CV	%RE	% CV	%RE	
Target 1/3 bi-active (spot 1)					
LLOQ	5.2	2.3	5.2	2.6	7.8
LoQC	5.8	-3.7	5.8	-4.0	9.8
MeQC	6.8	-7.0	8.2	-6.6	14.8
HiQC	9.1	8.2	13.5	7.3	20.8
ULOQ	10.6	7.2	24.1	4.4	28.5
Target 2/3 bi-active (spot 10)					
LLOQ	6.9	-2.7	6.9	-2.1	9.0
LoQC	6.2	-7.8	6.2	-8.1	14.3
MeQC	3.6	-6.7	3.7	-6.7	10.4
HiQC	7.6	-5.4	7.6	-4.7	12.3
ULOQ	4.8	-6.5	6.6	-7.1	13.7

Equally performant plexes







Target 1/3 bi-active (spot 1)

Target 2/3 bi-active (spot 10)

Intra-run %CV / Inter-batch %CV — QC ≤ 20.0%, except for LLOQ and ULOQ: %CV ≤ 25.0%
Intra-run |%RE| / Inter-batch |%RE| — QC ≤ 20.0%, except for LLOQ and ULOQ: |%RE| ≤ 25.0%
Total error: |%RE| + %CV — QC ≤ 30.0%, except for LLOQ and ULOQ: ≤ 40.0%

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Target interference in an active PK

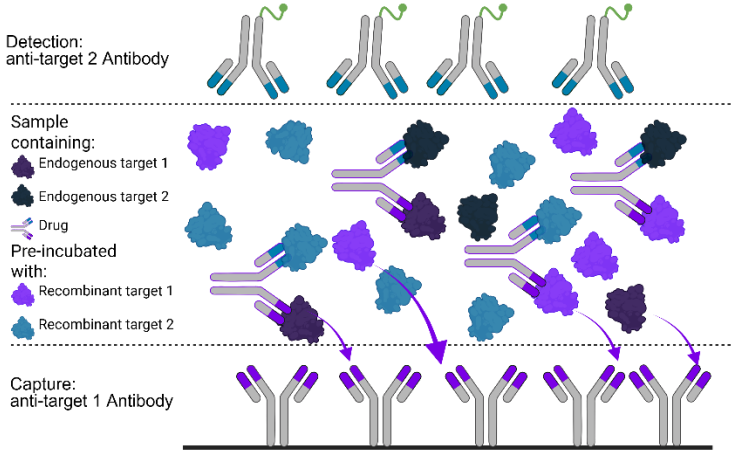
How to deal with possible interference of endogenous target protein on capturing of the compound

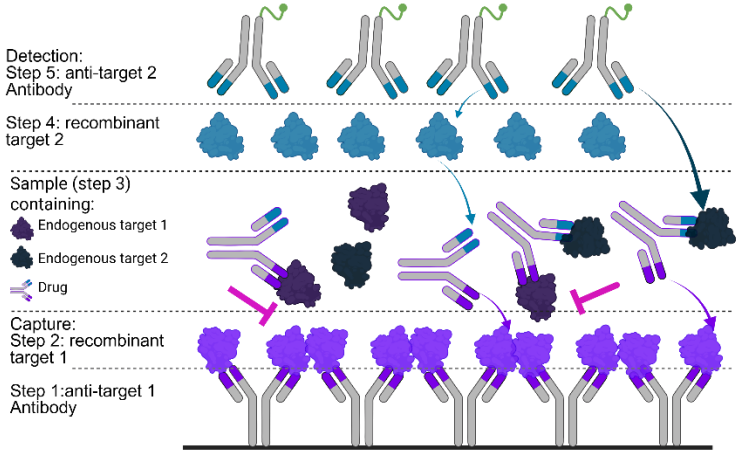
In-solution method (*targets + drug compound*)

- ⊕ TI: all target-bound compound is captured
- ⊖ Capturing of free target in solution → lose sensitivity

Sequential method

- ⊖ TI: only free compound is captured
- ⊕ High sensitivity for low endogenous target concentrations





Next step: based on literature data, predicted or previously measured target concentrations

↓

Simulate **expected target interference** in the method based on expected drug occupancy

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Target interference: simulated vs empirical

Target interference only observed for **high target** concentrations

QC →	ULOQ		HiQC		MeQC		LoQC		LLOQ		% simulated drug ↓ occupancy
Target conc ↓	Plex 1	Plex 10	Plex 1	Plex 10	Plex 1	Plex 10	Plex 1	Plex 10	Plex 1	Plex 10	
Very high	1.7%	1.3%	1.5%	1.5%	11%	11%	35%	79%	38%	89%	Target 1 Target 2
High	0.4%	0.3%	0.5%	0.4%	2.9%	2.7%	11%	26%	13%	51%	Target 1 Target 2
Medium	0.3%	0.2%	0.3%	0.2%	1.7%	1.6%	6.9%	15%	8.3%	33%	Target 1 Target 2
Low	0.1%	0.1%	0.1%	0.1%	0.6%	0.5%	2.4%	5.4%	2.5%	12%	Target 1 Target 2
Very low	0.1%	0.1%	0.1%	0.1%	0.5%	0.4%	1.9%	4.3%	2.3%	9.8%	Target 1 Target 2

Color code: empirical result

Green = Pass *|%RE| QC <20% (<25% for ULOQ and LLOQ)*

Red = Fail *|%RE| QC >20% (>25% for ULOQ and LLOQ)*

Grey = No Result

Value%: simulated % drug occupancy

Fit-for-purpose for non-GLP tox:

- The "medium" concentration = the highest empirically measured accumulated concentration for a related anti-target 1 compound.
- The "very low" concentration = the upper range of predicted accumulated target concentrations for this drug compound.

All QC samples were spiked with both targets → target tolerance for drug compound in **complex with both capture targets**

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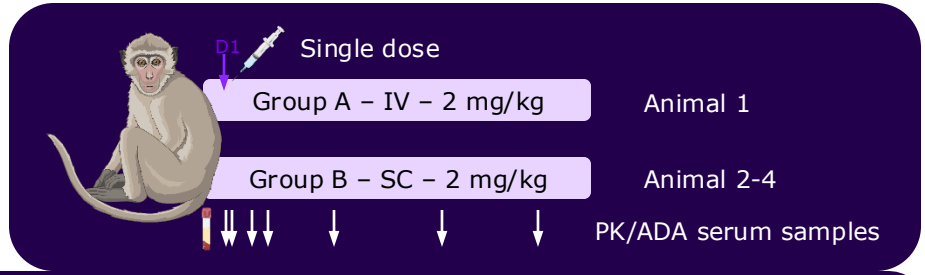
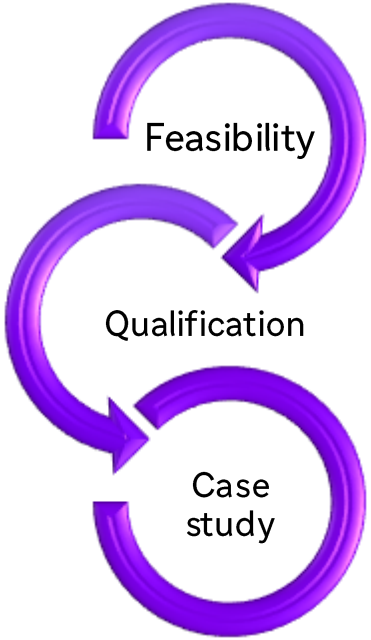
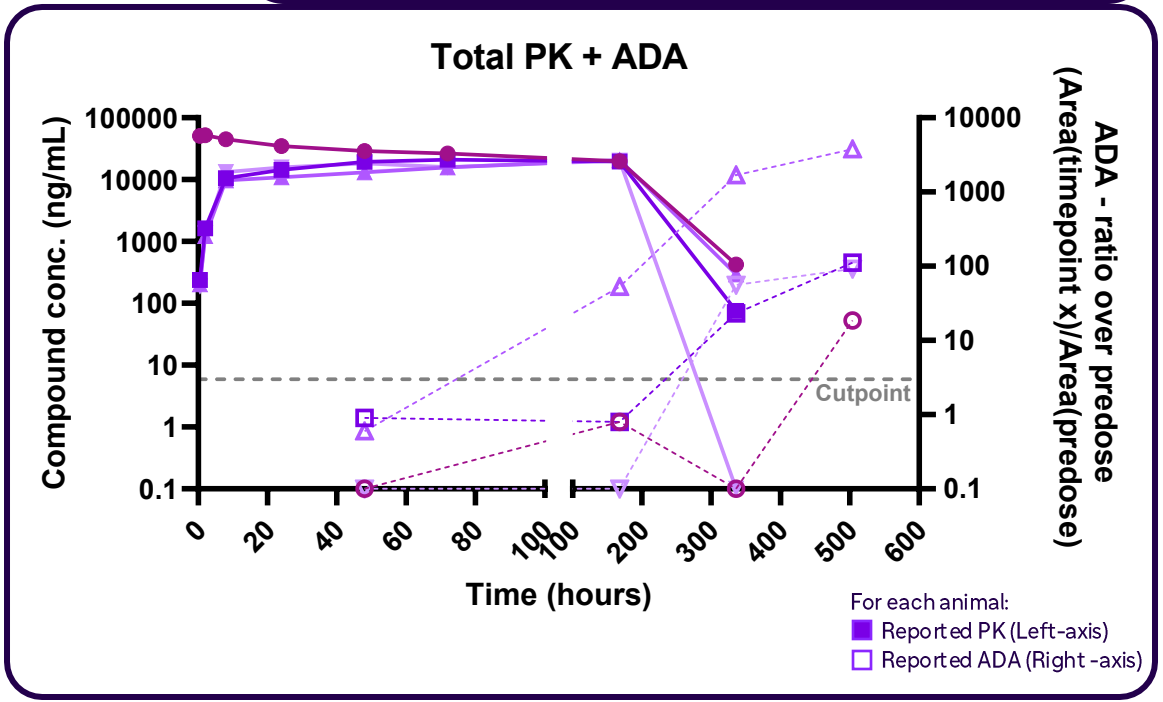
04 Dual bi-active: Case study

Case study

Exploratory single dose safety study in cynomolgus monkeys. Total PK concentrations and ADA were measured and reported.







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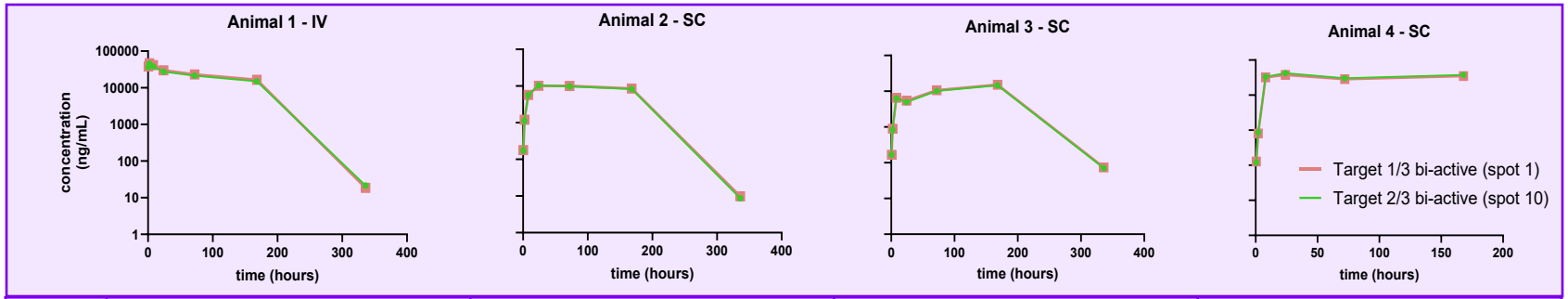
Case study: re-analysis with dual bi-active PK method



	An 1 IV			An 2 SC			An 3 SC			An 4 SC		
Time (h)	Target 1/3 bi-active (spot 1)	Target 2/3 bi-active (spot 10)	|%Diff|	Target 1/3 bi-active (spot 1)	Target 2/3 bi-active (spot 10)	|%Diff|	Target 1/3 bi-active (spot 1)	Target 2/3 bi-active (spot 10)	|%Diff|	Target 1/3 bi-active (spot 1)	Target 2/3 bi-active (spot 10)	|%Diff|
0.5	36658	38147	4.0%	171	178	3.8%	165	167	1.5%	119	127	7.1%
2	45213	46431	2.7%	1132	1202	6.0%	827	897	8.1%	868	805	7.5%
8	38409	41409	7.5%	5627	5713	1.5%	6333	6538	3.2%	33359	32129	3.8%
24	27506	29987	8.6%	10378	10189	1.8%	4926	5413	9.4%	42170	37730	11.1%
72	21375	23089	7.7%	9797	10068	2.7%	10136	10608	4.5%	30375	28467	6.5%
168	14973	16480	9.6%	8298	8518	2.6%	14573	15122	3.7%	37726	34923	7.7%
336	21	18	15.0%	8	9	13.0%	70	73	4.4%	NS	NS	-

Concentrations shown in ng/mL

Low variability between read outs: below 15% difference,
→ e.g. 30% difference allowed for ISR

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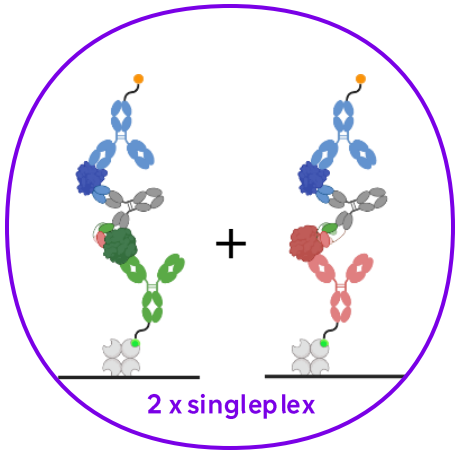
05 Opportunities and considerations

Advantages and challenges



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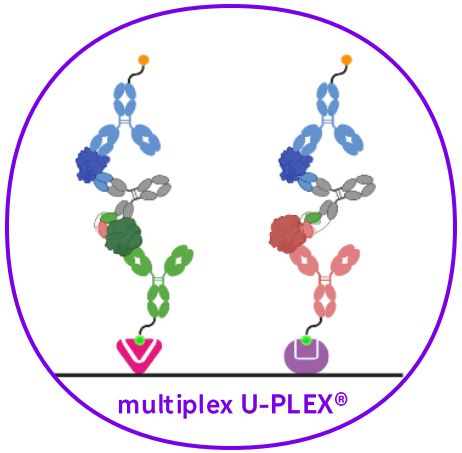

Simpler method development

2 methods to validate
2 PK datasets
Higher assay & technical variability
Higher sample volume

1 method to validate
Reduced inter-assay & technical variability
Lower sample volume
Reduced time & cost

2 PK datasets
More complex method development (common range and assay conditions)

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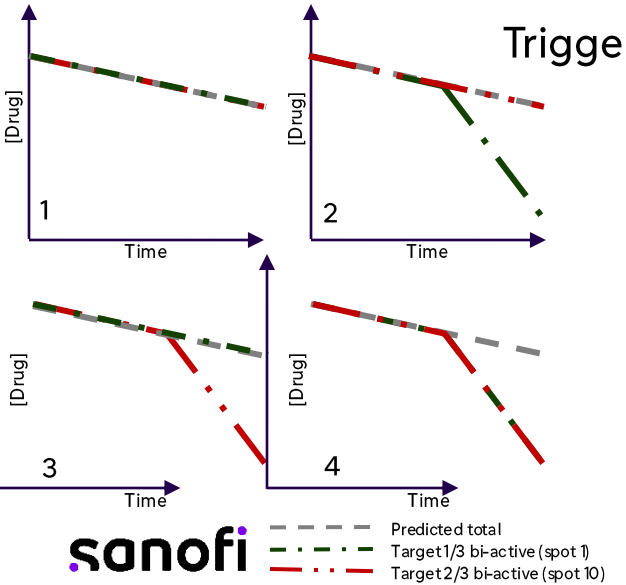
In vivo integrity and functionality of complex proteins

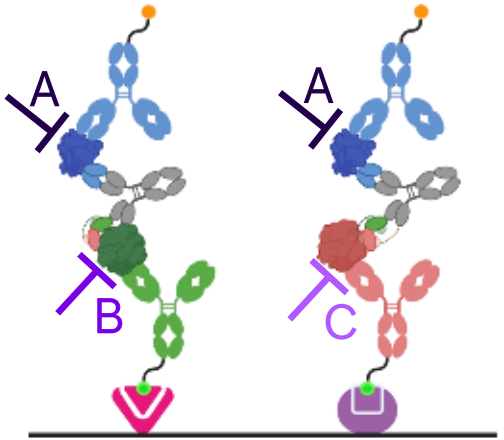
Dual bi-active PK readouts distinguish the activity against individual targets

Dual bi-active PK profiles may give more **granular information** on the nature of ADA or integrity of each anti-target moiety

Trigger additional ADA or drug integrity analysis



Graph	Impact on exposure	NAb identity
1	No loss of exposure	No NAb
2	Loss of exposure via T1	B
3	Loss of exposure via T2	C
4	Loss of exposure via T3, T1&T2, T1&T3 or clearing ADA	A, B+C, A+B+C



06 Conclusion

Conclusions and considerations

A novel approach:

- Active exposure to multiple targets through bi-active PK methods
- P&A, fit-for-purpose target tolerance and selectivity, stable for at least one F/T cycle
- Demonstrated inter-assay variability between both reactions below 15% → lower dataset variability
- Dual bi-active PK method for IND-enabling toxicity studies → *a priori* discussion with the study team to ensure the interpretation of two datasets for TK evaluation.
- Comparing both read-outs can not only provide proof of active exposure but also be indicative of reduced exposure due to (potentially) neutralizing ADA or compound instability.

Acknowledgements

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Marie-Paule Bouche

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