

Development of a bispecific antibody for colorectal cancer: Functional activity evaluation via a biophysical cell-based assay and surface plasmon resonance

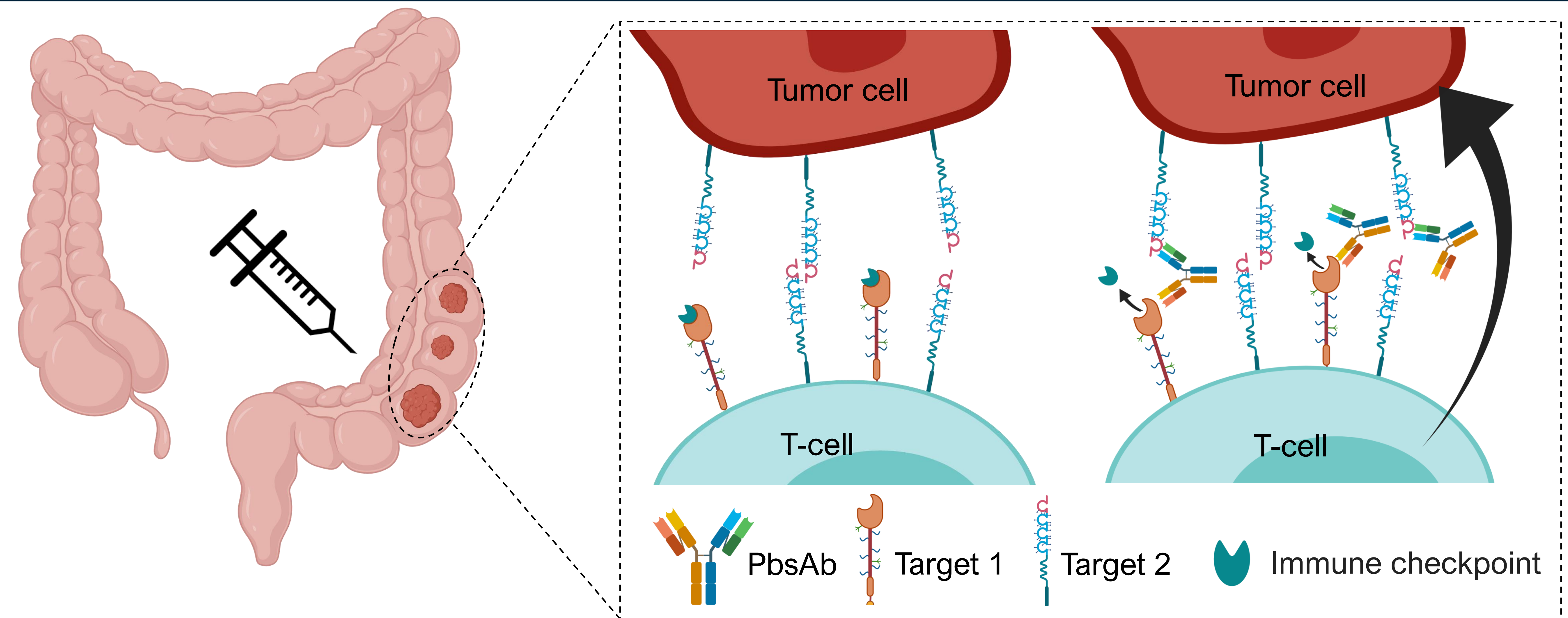
F. Pires¹, M. Skulski¹, A. Dudek¹, P. Dobosz¹, J. Kołodziejski¹, D. Carter¹

¹Pure Biologics SA, Wrocław, Poland

<https://purebiologics.com>

INTRODUCTION

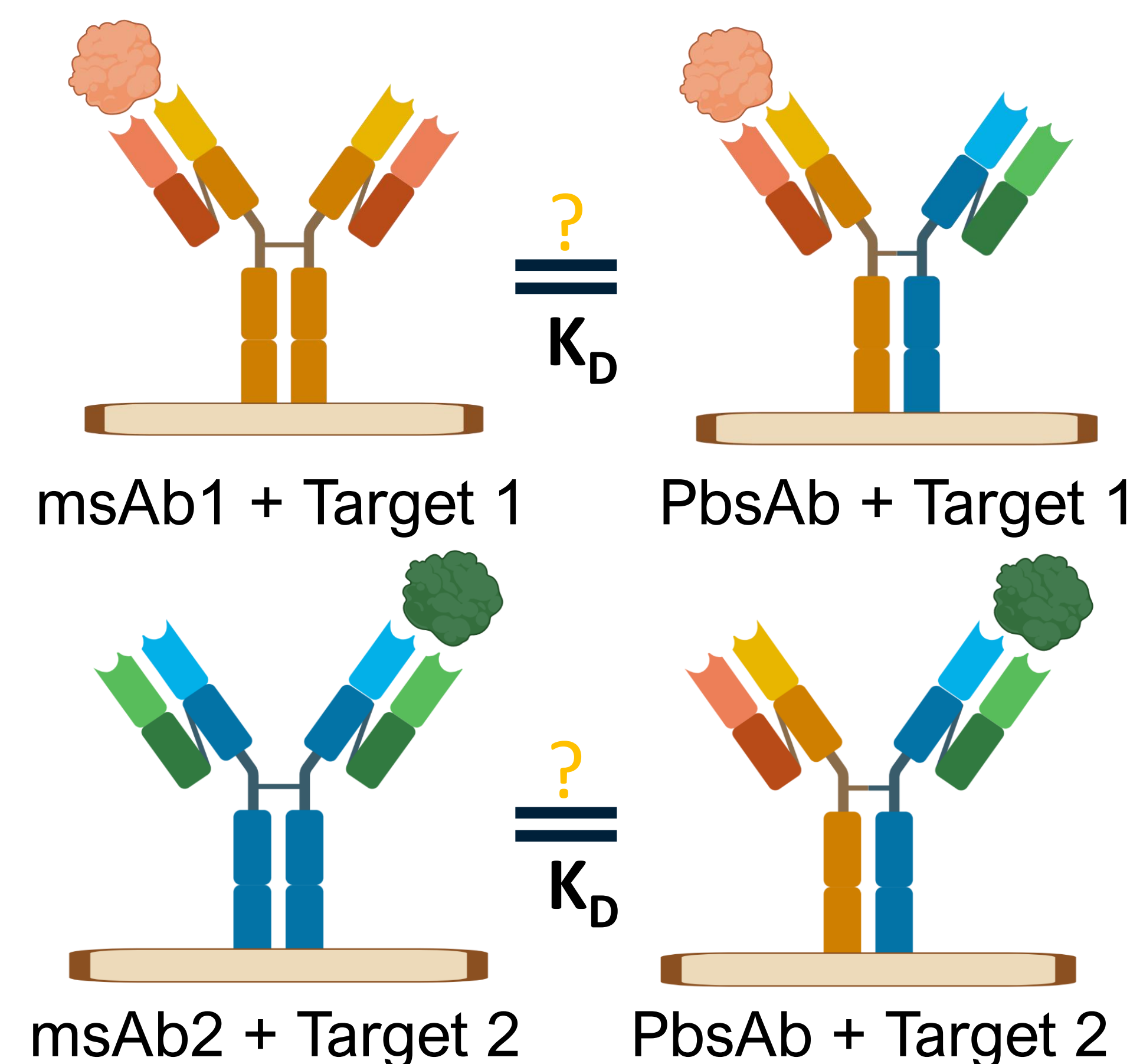
Pure Biologics' PB001 project aims to develop a bispecific antibody (bsAb) to use as a therapeutic agent to treat colorectal cancer (CRC). Mechanistically, the PB001 bsAb is being designed to exert anti-cancer activity via two routes: (1) as an immune checkpoint inhibitor, (2) as a bridge engaging cytotoxic lymphocytes (T-cells) with tumor cells expressing a specific surface antigen. These activities are to be driven by the bsAb's ability to bind its antigens, both individually and simultaneously. Following this concept, we constructed a prototype bsAb (PbsAb) and assessed its binding to two targets selected for the PB001 project. To this end, we leveraged different biophysical technologies (SPR and Ligand Tracer) to measure target engagement of PbsAb, as well as two monospecific antibodies (msAb) from which the PbsAb's arms originate.



GOALS



SPR

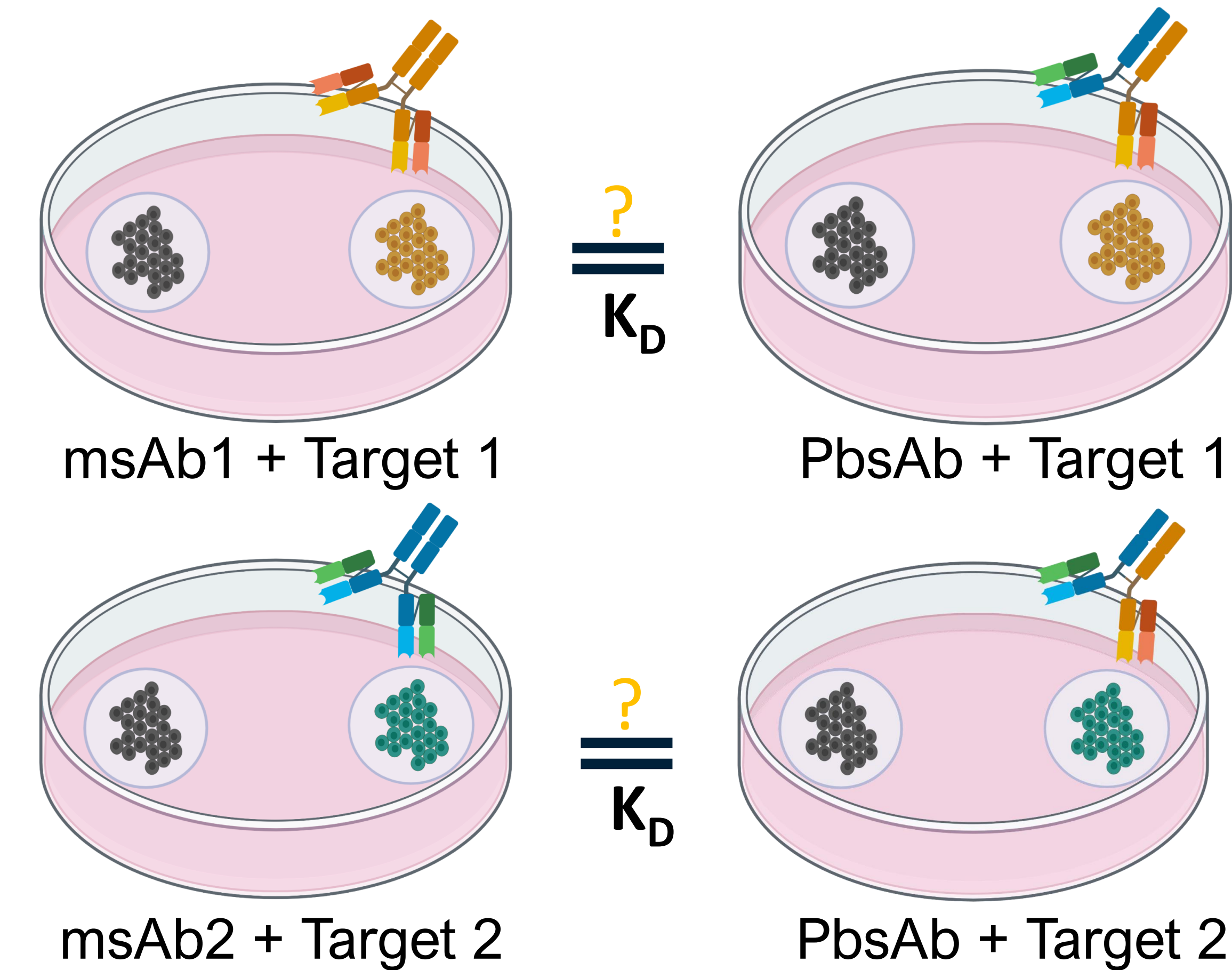


Measure affinities (K_D values) of PbsAb binding to each target and compare these values to those measured using the two monospecific antibodies from which the PbsAb derived.

VS



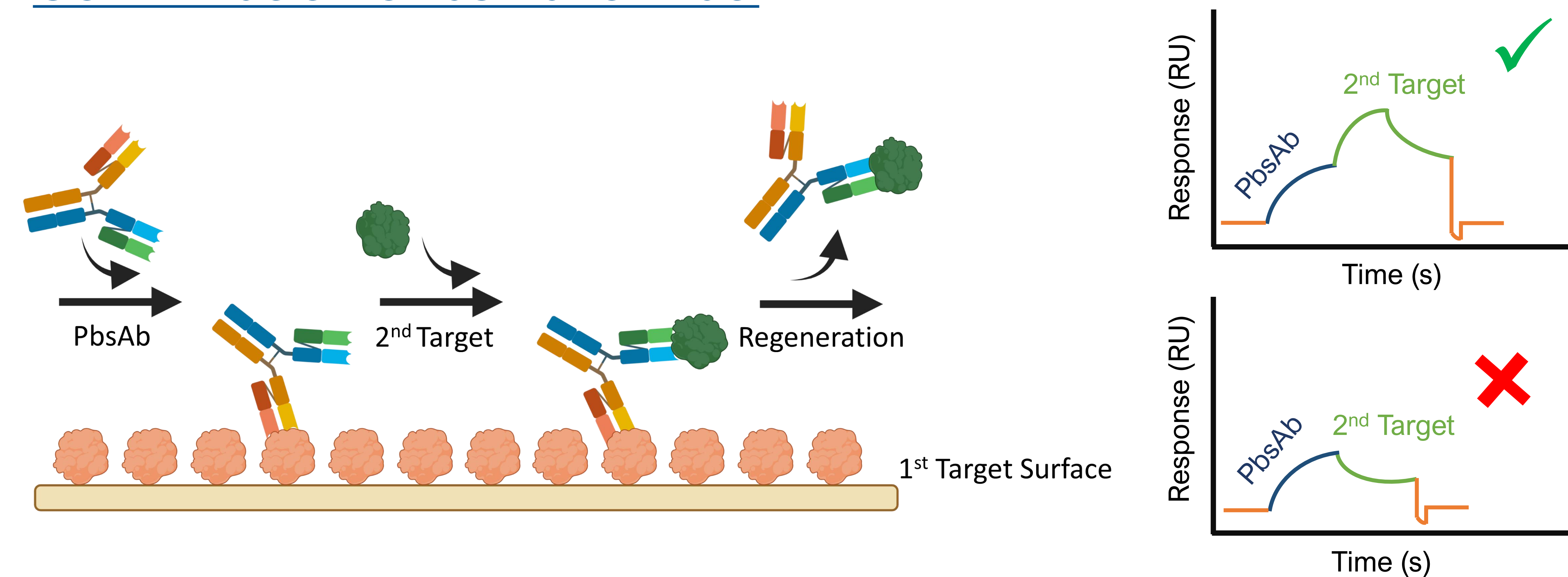
Ligand Tracer



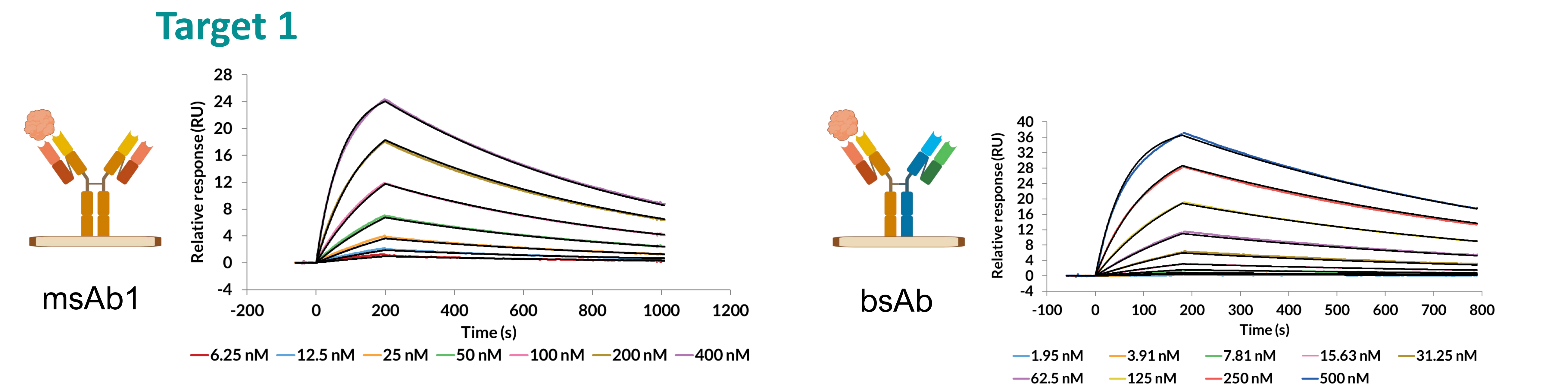
Demonstrate that the PbsAb binds to model cell lines expressing each target and measure the cell surface binding kinetics of these interactions.

RESULTS – SPR

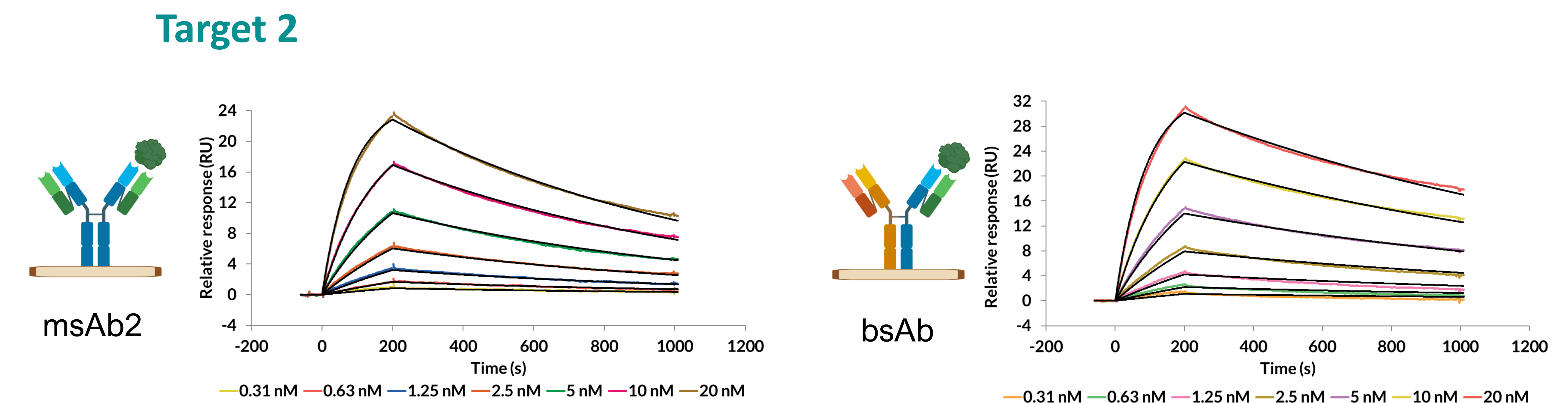
Confirmation of bsAb format:



Affinity determination:



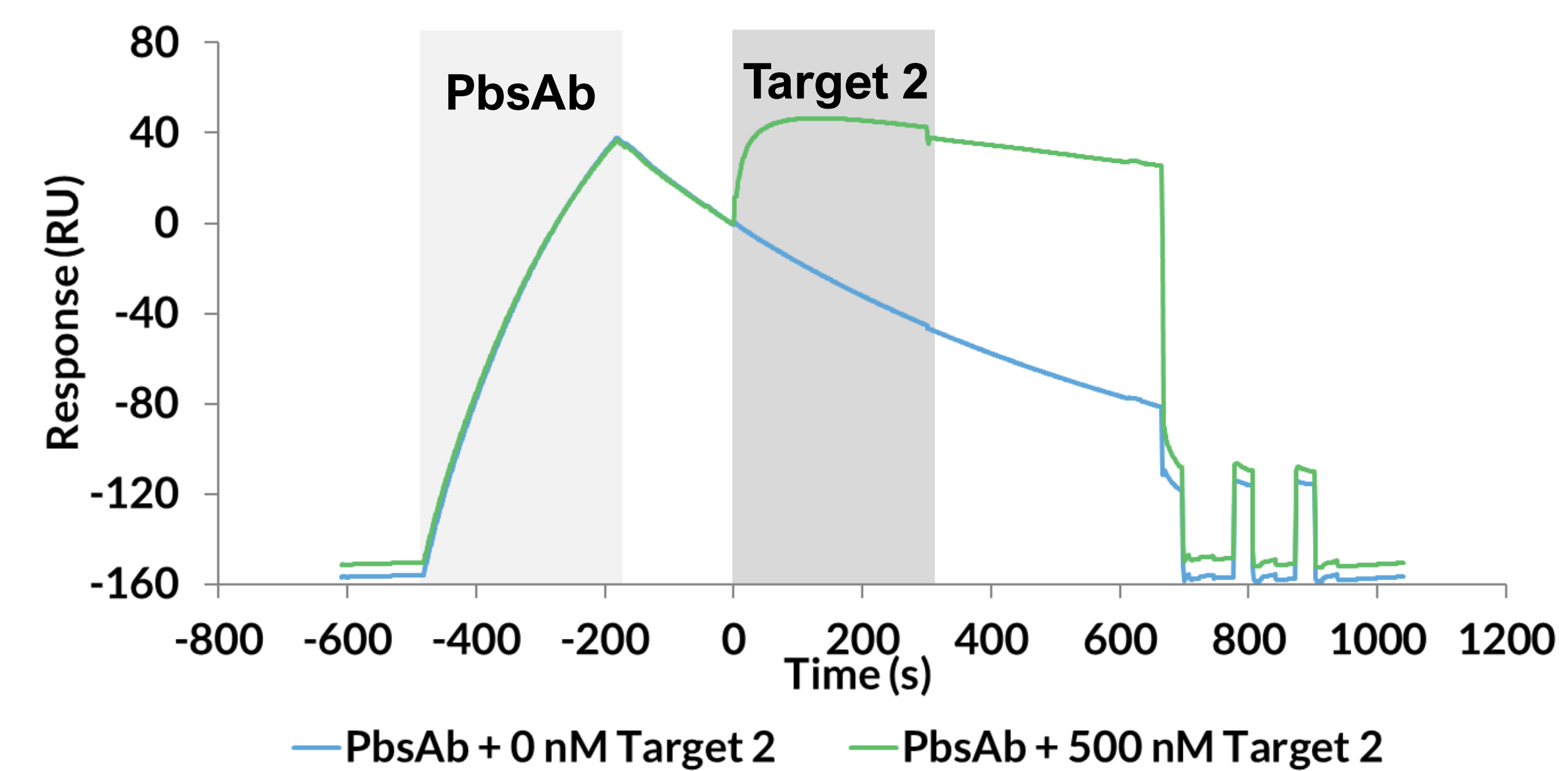
Ligand	Analyte	k_{on} (1/Ms)	k_{off} (1/s)	K_D (M)	N
msAb1	Target 1	$(3.06 \pm 0.09) \times 10^4$	$(1.31 \pm 0.04) \times 10^{-3}$	$(4.29 \pm 0.27) \times 10^{-8}$	2
PbsAb	Target 1	$(3.22 \pm 0.08) \times 10^4$	$(1.32 \pm 0.04) \times 10^{-3}$	$(4.16 \pm 0.08) \times 10^{-8}$	2



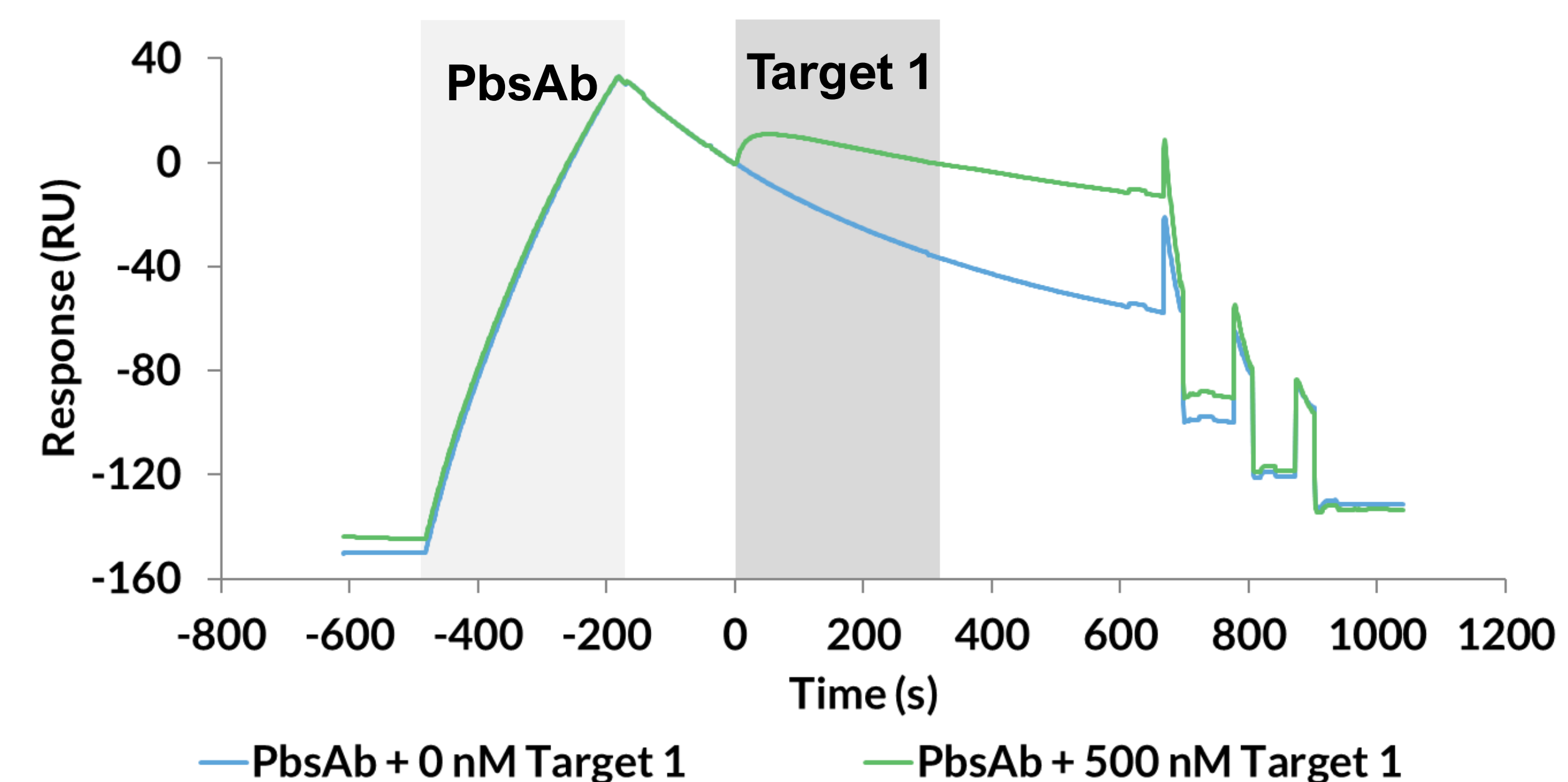
Ligand	Analyte	k_{on} (1/Ms)	k_{off} (1/s)	K_D (M)	N
msAb2	Target 2	$(5.37 \pm 0.30) \times 10^5$	$(8.64 \pm 2.92) \times 10^{-4}$	$(1.60 \pm 0.46) \times 10^{-9}$	2
PbsAb	Target 2	$(5.50 \pm 0.10) \times 10^5$	$(7.73 \pm 0.91) \times 10^{-4}$	$(1.41 \pm 0.13) \times 10^{-9}$	2

msAb K_D values = PbsAb K_D values

Surface with Target 1



Surface with Target 2



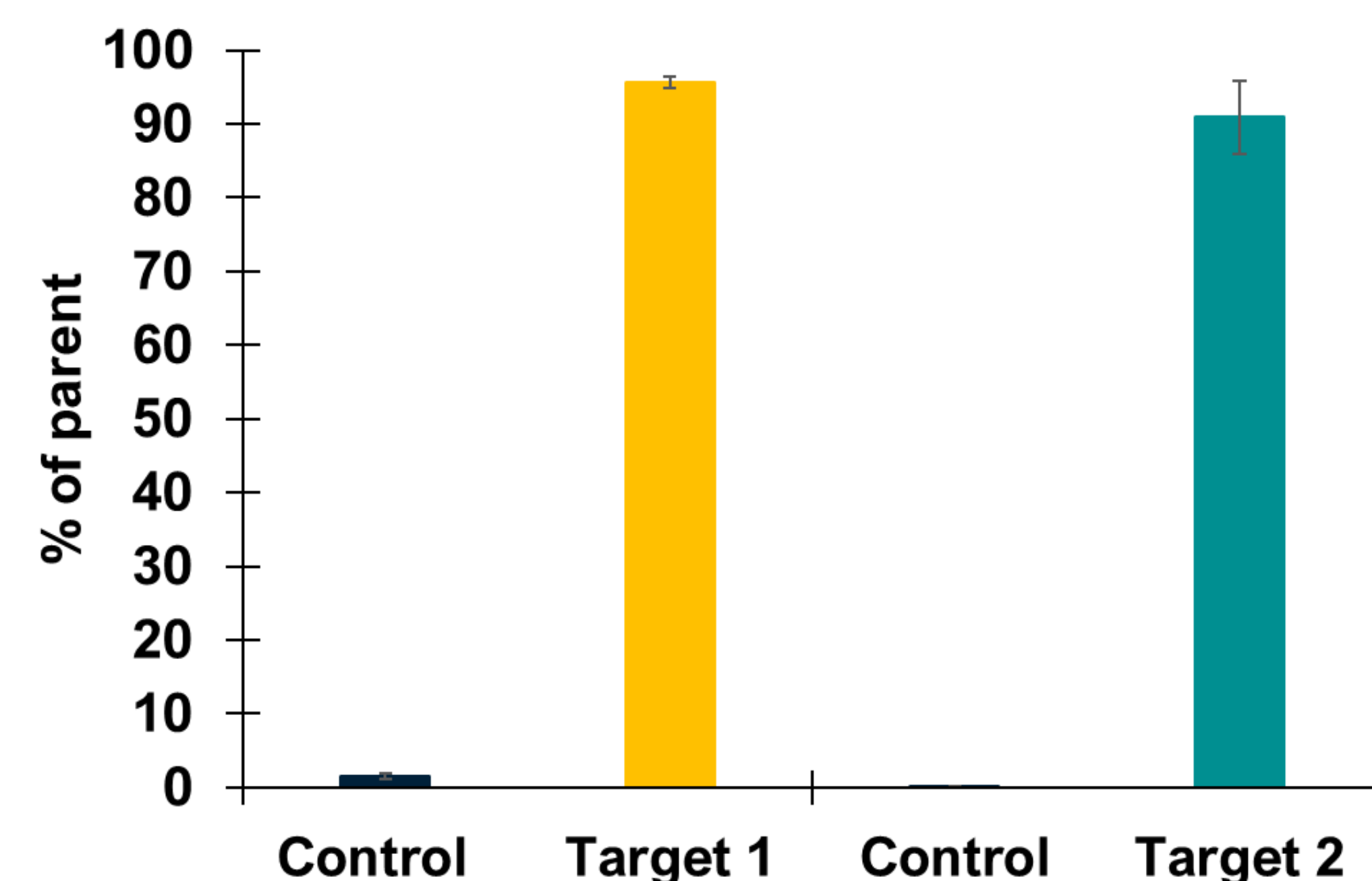
PbsAb binds its two targets independently and simultaneously



Confirmed presence of bsAb format

RESULTS – Ligand Tracer

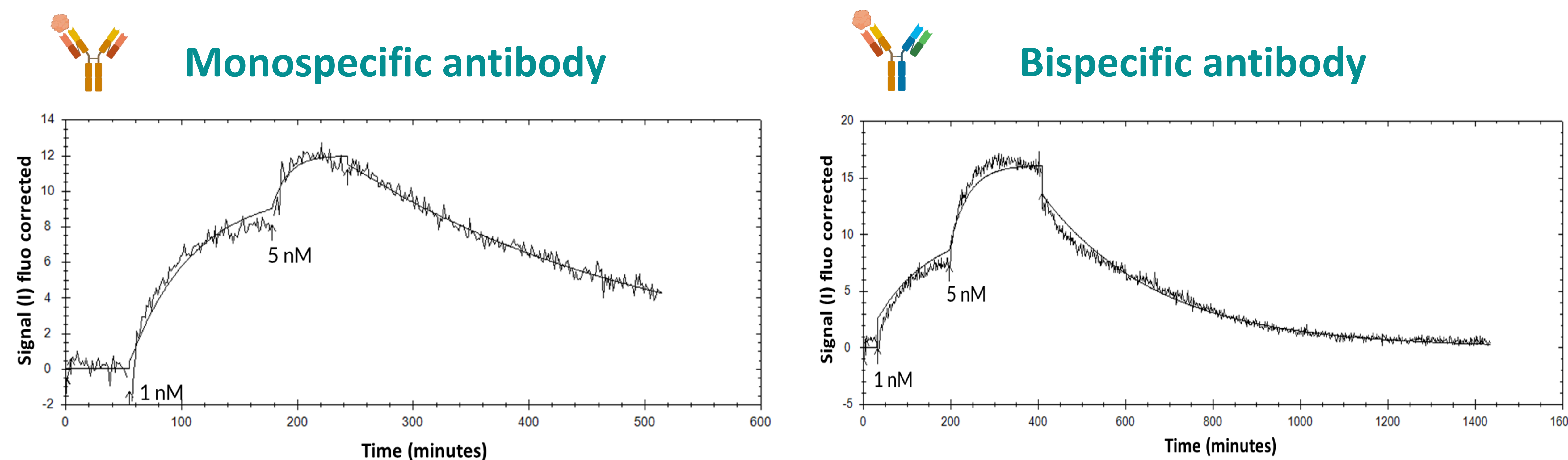
Confirmation of targets expression on cells



Target	Cells	% of parent	SD	N
1	Control	1.57	0.42	3
	Target	95.67	0.06	3
2	Control	0.13	0.13	3
	Target	90.90	5.04	3

Targets are expressed on cells

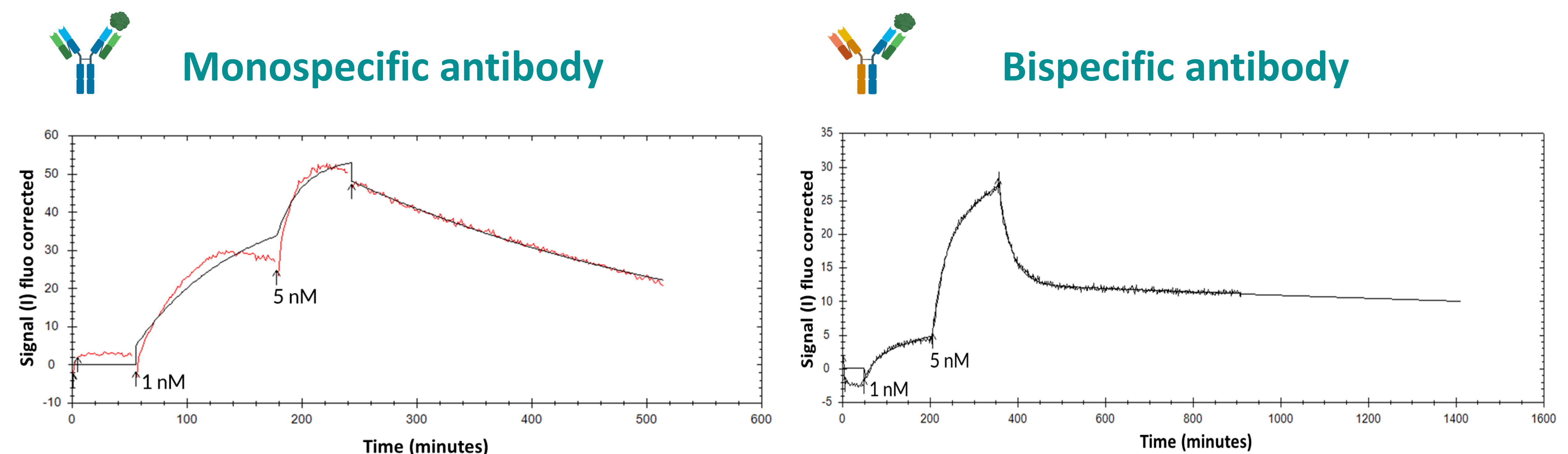
Affinity determination (Target 1):



Ligand	Binding partner	Cell line	Interaction	Ligand Tracer K_D (M)	N
Target 1	msAb1	CHO	Y	$(1.88 \pm 0.99) \times 10^{-10}$	2
Target 1	PbsAb	CHO	Y	$(6.71 \pm 3.90) \times 10^{-10}$	2

msAb1 kinetics \neq PbsAb kinetics
msAb1 K_D values = PbsAb K_D values

Affinity determination (Target 2):



Ligand	Binding partner	Cell line	Interaction	Ligand Tracer K_D (M)	N
Target 2	msAb2	CHO	Y	$(1.10 \pm 1.05) \times 10^{-9}$	2
Target 2	PbsAb	CHO	Y	$(1.06 \pm 0.42) \times 10^{-8}$	2

msAb2 kinetics \neq PbsAb Kinetics
msAb2 K_D values \neq PbsAb K_D values

Conclusions

Target	Antibody	SPR K_D	Ligand Tracer K_D (M)
1	msAb1	$(4.29 \pm 0.27) \times 10^{-8}$	$(1.88 \pm 0.99) \times 10^{-10}$
1	PbsAb	$(4.16 \pm 0.08) \times 10^{-8}$	$(6.71 \pm 3.90) \times 10^{-10}$
2	msAb2	$(1.60 \pm 0.46) \times 10^{-9}$	$(1.10 \pm 1.05) \times 10^{-9}$
2	PbsAb	$(1.41 \pm 0.13) \times 10^{-9}$	$(1.06 \pm 0.42) \times 10^{-8}$

- ✓ Both biophysical techniques confirmed binding of PbsAb to Target 1 and Target 2
- ✓ With SPR, both monospecific antibodies present the same K_D and kinetics as bispecific antibody
- ✓ With Ligand Tracer, msAb1 and PbsAb present similar K_D , but different kinetics
- ✓ With Ligand Tracer, msAb2 and PbsAb present different K_D and different kinetics
- ✓ Binding profiles with both biophysical techniques were qualitatively in agreement