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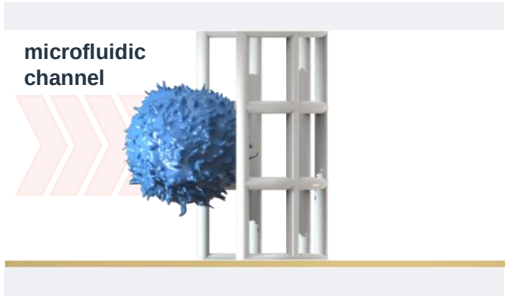
# single-cell Interaction Cytometry (scIC) in drug development and characterisation

hello@rouken.bio

# single-cell Interaction Cytometry (scIC)

**01** LOADING
cell from autosampler




Cell injection and trapping

**02** ASSOCIATION
real-time measurement

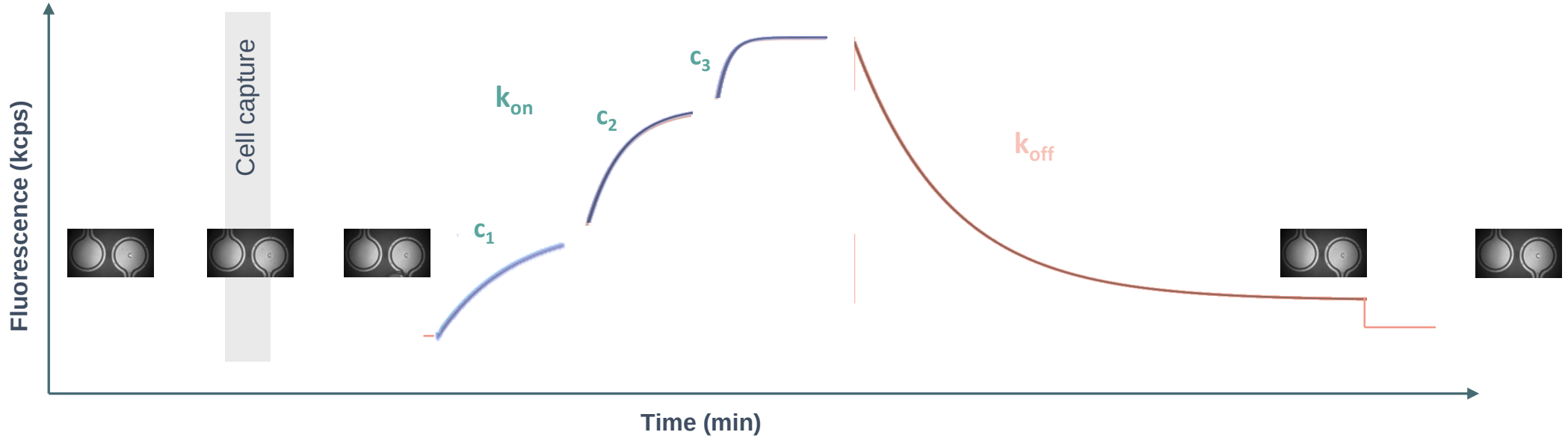

Injection of fluorescent analytes

**03** DISSOCIATION
real-time measurement


Dissociation in buffer flow

**04** UNLOADING
of cell to waste


Trap regeneration

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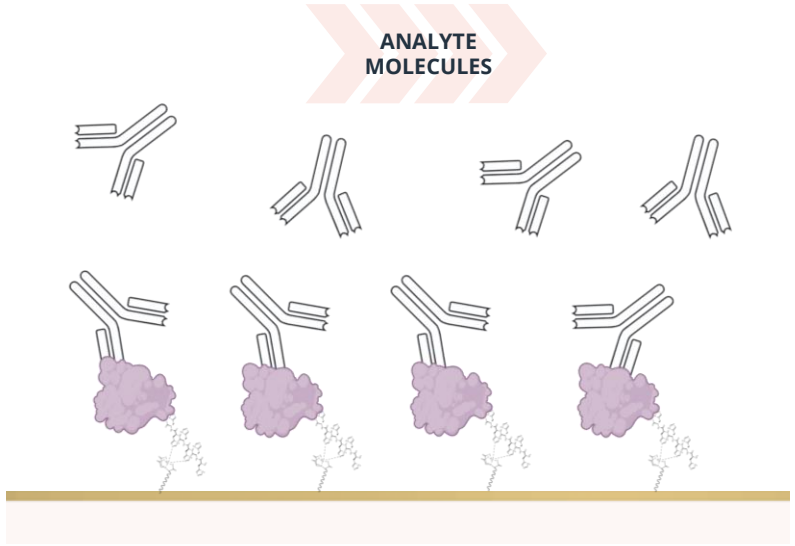
# SPR *vs* sc-IC *vs* FLOW CYTOMETRY

## SPR

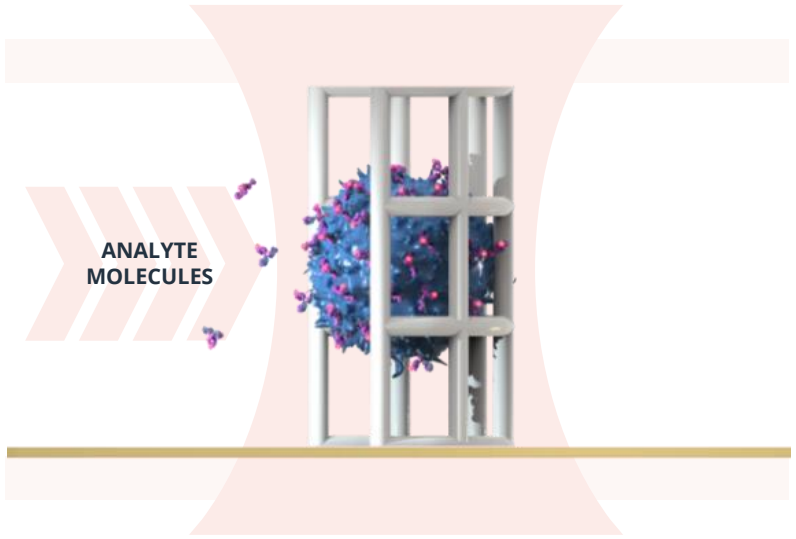
TARGET IMMOBILISED
**REAL-TIME** OBSERVATION OF BINDING KINETICS




CHALLENGES:
Unstable cell immobilisation
Limited detection depth

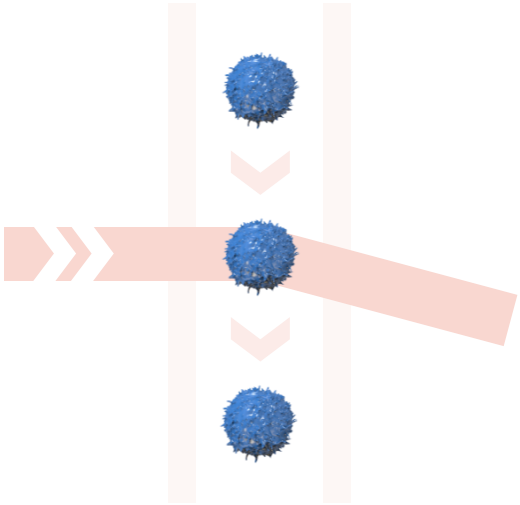
## RT-IC

CELL TRAPPED
**REAL-TIME** OBSERVATION OF BINDING KINETICS



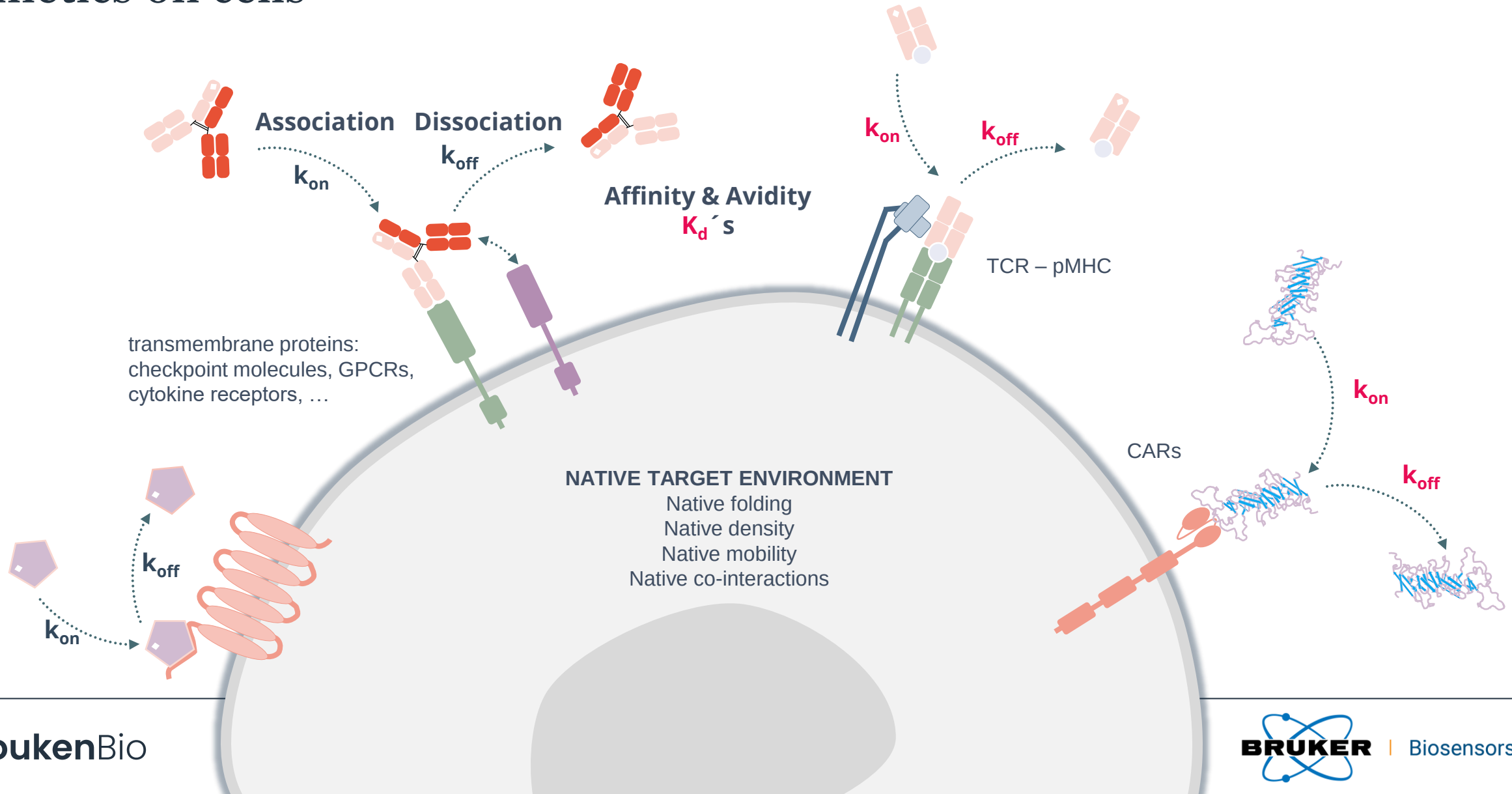

## FLOW CYTOMETRY (FACS)

CELL IN FLOW
SINGLE TIME POINT MEASUREMENT

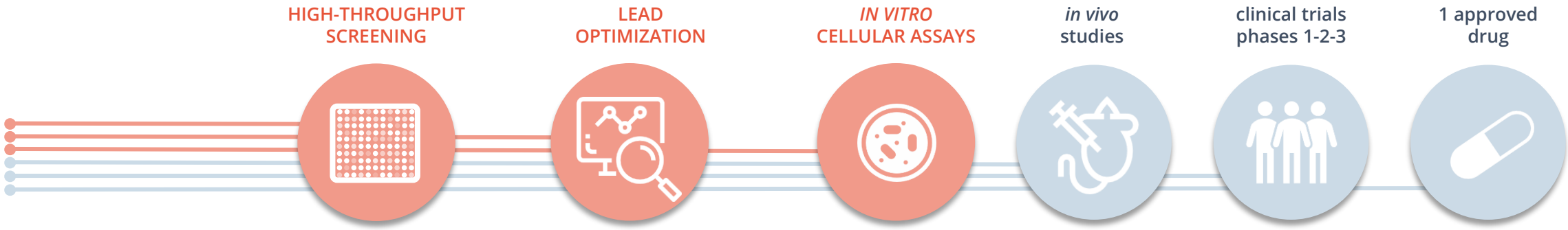


CHALLENGES:
End-point analysis
Tedious workflows

# Kinetics on cells



# ⊕ Biophysical technologies offer decisive information in pivotal drug discovery stages




heliXcyto

better drug candidates through better analytical capabilities → higher success rate

**duration 10-15 years**

**average cost $2 billion +**

## Surface Plasmon Resonance (SPR)

High-throughput antibody screening
*Affinity, specificity, kinetic rates*

## single-cell Interaction Cytometry (scIC)

Cell surface binding
*Native target density, co-receptors*

# scIC Measurements

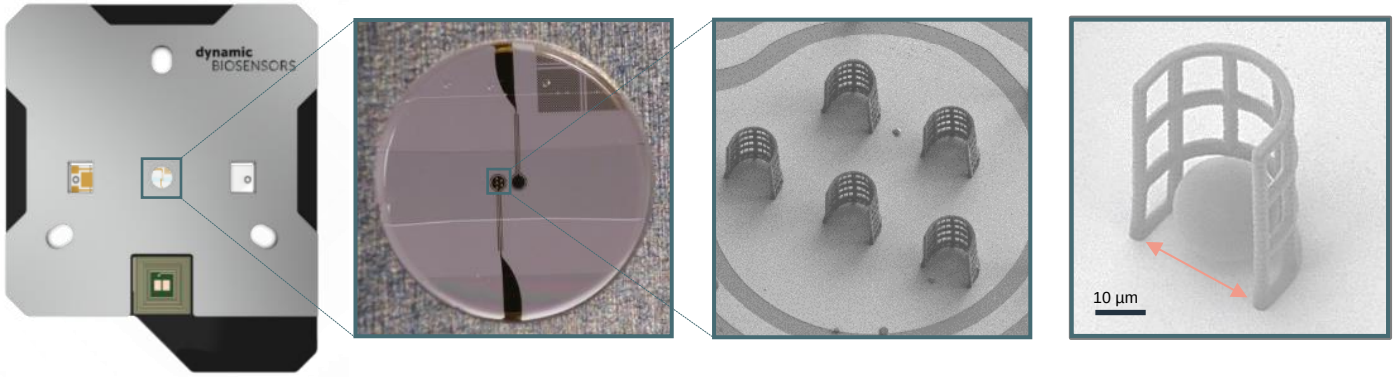
Automated analysis of

- **association kinetics** ($k_{on}$)
- **dissociation kinetics / half-life** ($k_{off}$, $t_{1/2}$)
- **affinities and avidities** ($K_d$)

From cell culture to interaction data in **three simple steps**:

1. Prepare dilution series of fluorescently labeled analyte.
2. Harvest cells (optional: fix cells).
3. Place samples in autosampler and start assay.

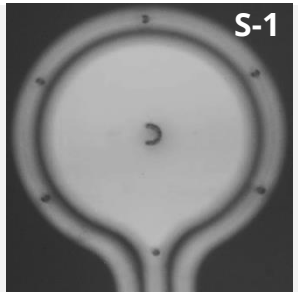
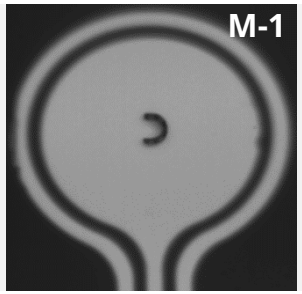
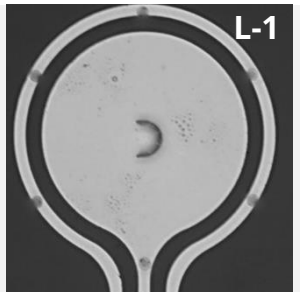
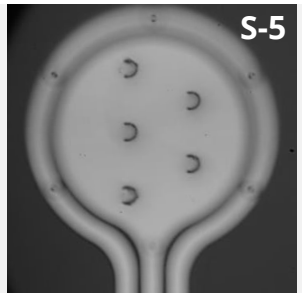
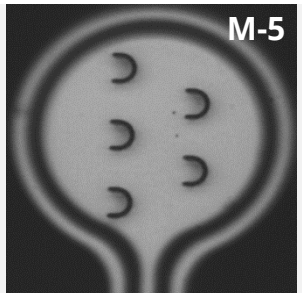
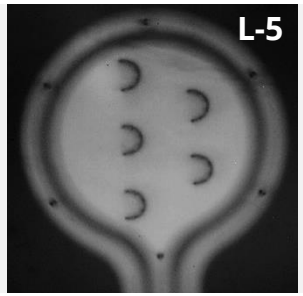

heliX$^{cyto}$ instrument




**heliX**$^{cyto}$ chips feature flow-permeable, bio-compatible polymer cages, which physically retain any type of cells. Trap sizes are tailored to capture single cells.

# heliX$^{cyto}$ chips

**heliX$^{cyto}$ chips enable single- and multi-cell measurements**

| S small | M medium | L large | |
|---|---|---|---|
| for 6 - 12 µm diameter | for 12 - 20 µm diameter | for 20 - 25 µm diameter | |
|   |   |   | **single-cell measurements** |
|   |   |   | **multi-cell measurements** |

**Chip options support the capture of individual cells between 6 – 25 µm.**

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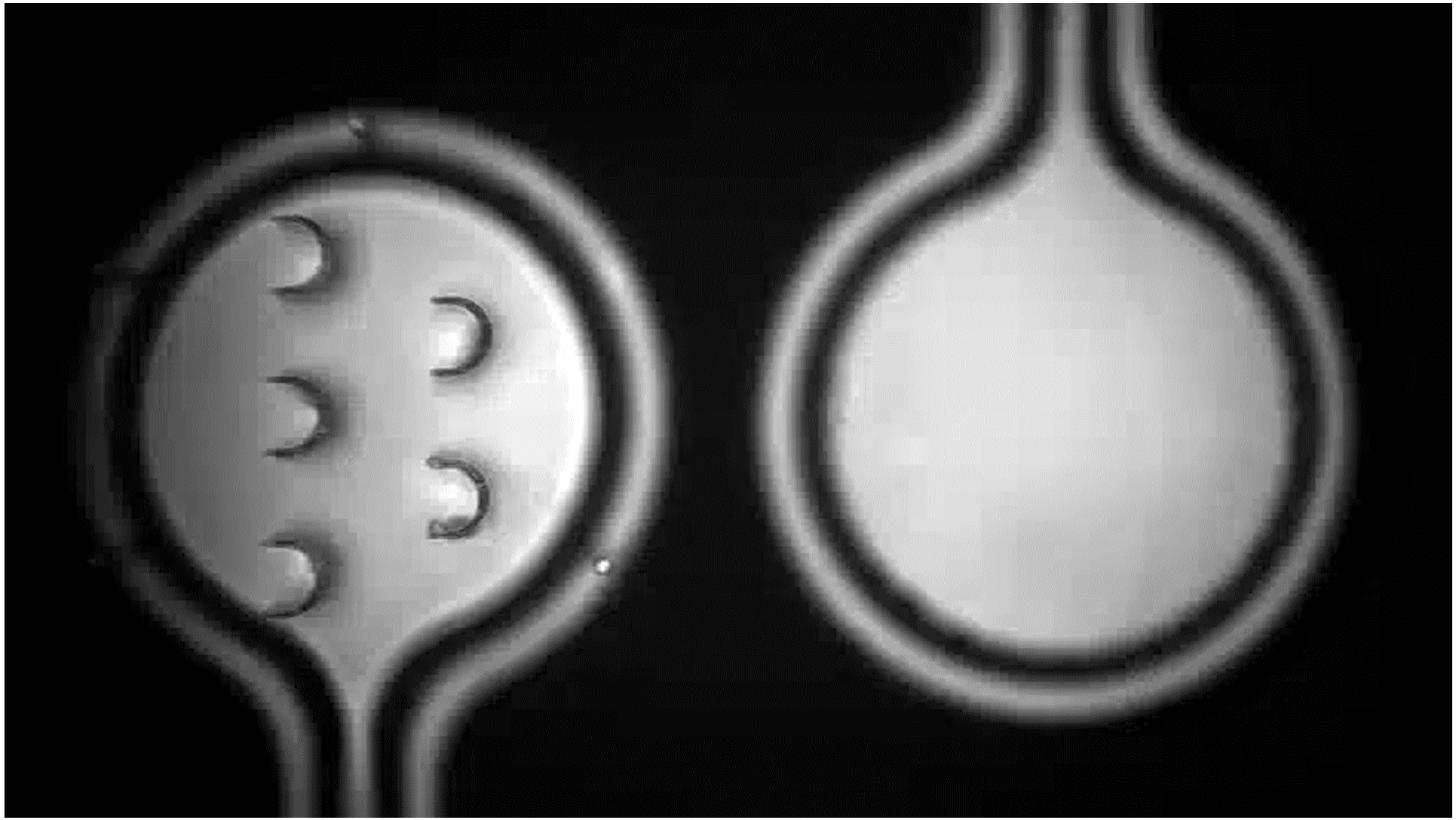
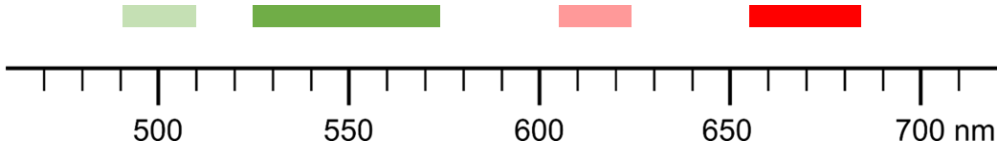
# **REAL-TIME CAPTURE** OF HeLa CELLS



# Fluorescent dyes




- COMMON FLUORESCENT DYES FITTING WITH **heliX**$^{cyto}$ OPTICS

***Green channel*** Ex: 490-510 nm; Em: 525-575 nm

| DYE | SPILLOVER | PHOTOSTABILITY | COMMENTS |
|---|---|---|---|
| FITC | | + | not recommended due to low photostability |
| AlexaFluor488 * | | +++ | |
| Atto488 * | | +++ | |
| 6-FAM * | | ++ | pH sensitive |
| OregonGreen488 | | +++ | |
| TAMRA | to red | ++ | |
| PE | slightly to red | ++ | large protein |

***Red channel*** Ex: 605-625 nm; Em: 655-685 nm

| DYE | SPILLOVER | PHOTOSTABILITY | COMMENTS |
|---|---|---|---|
| AbberiorSTARred * | | +++ | |
| AlexaFluor647 * | | +++ | |
| Cyto LK red 2 * | | +++ | hydrophilic |
| Cy5 * | | ++ | |
| APC * | | ++ | large protein |
| Atto594 * | | +++ | |
| Atto620 * | | +++ | might increase non-specificity |
| Atto633 * | | ++ | |
| Atto643 * | | +++ | |
| Atto647 * | | +++ | pH sensitive |
| Atto647N * | | +++ | might increase non-specificity |
| Atto655 * | | +++ | |
| iFluor647 * | | +++ | |

**Dyes optimally suited for sc-IC are very photostable, small in size, and keep the analyte in solution.**
**In case of DualColor applications ensure no spillover to the other channel.**

** already applied in practical sc-IC experiments.*

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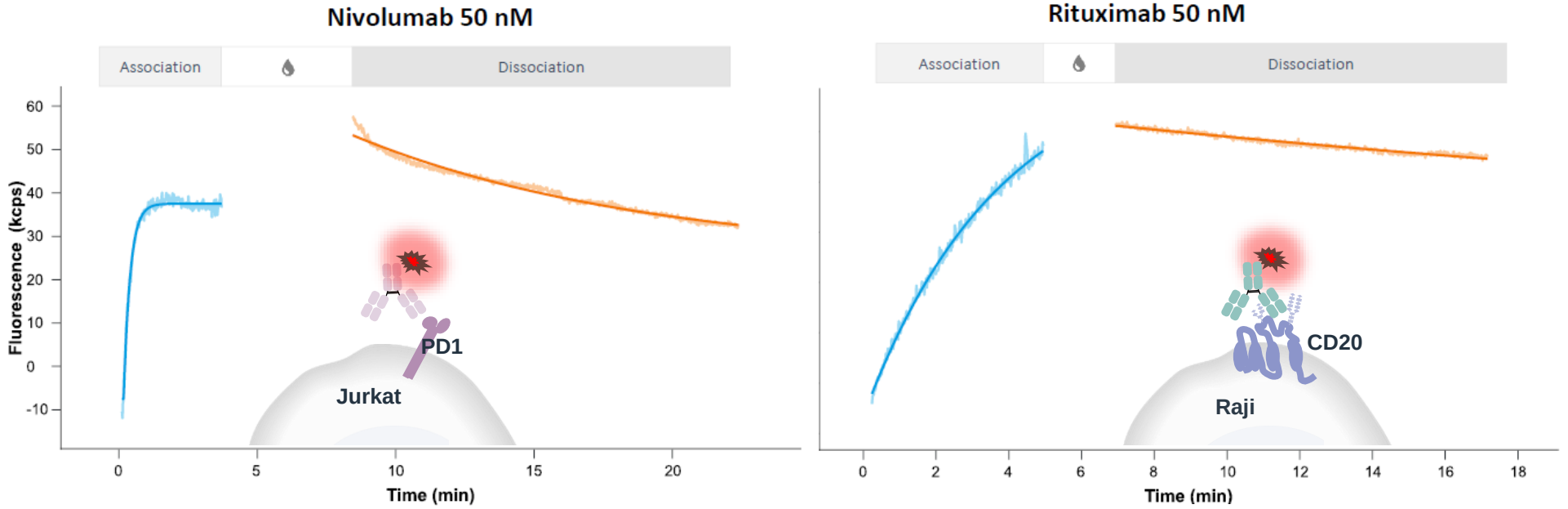
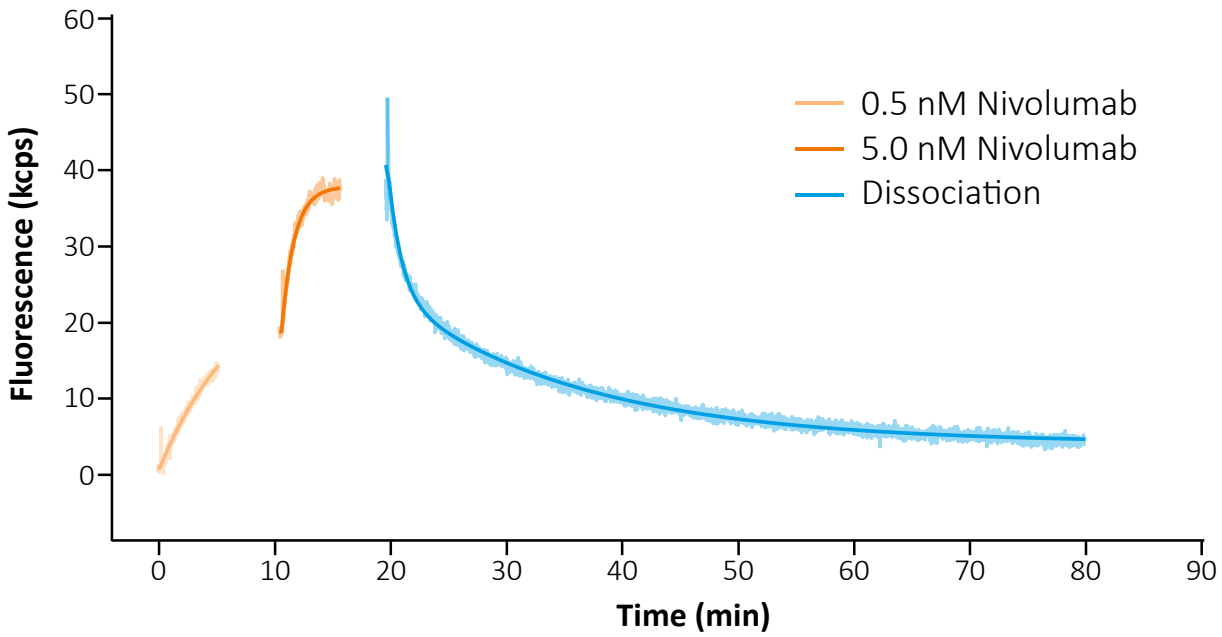
# mAb direct binding to T cells

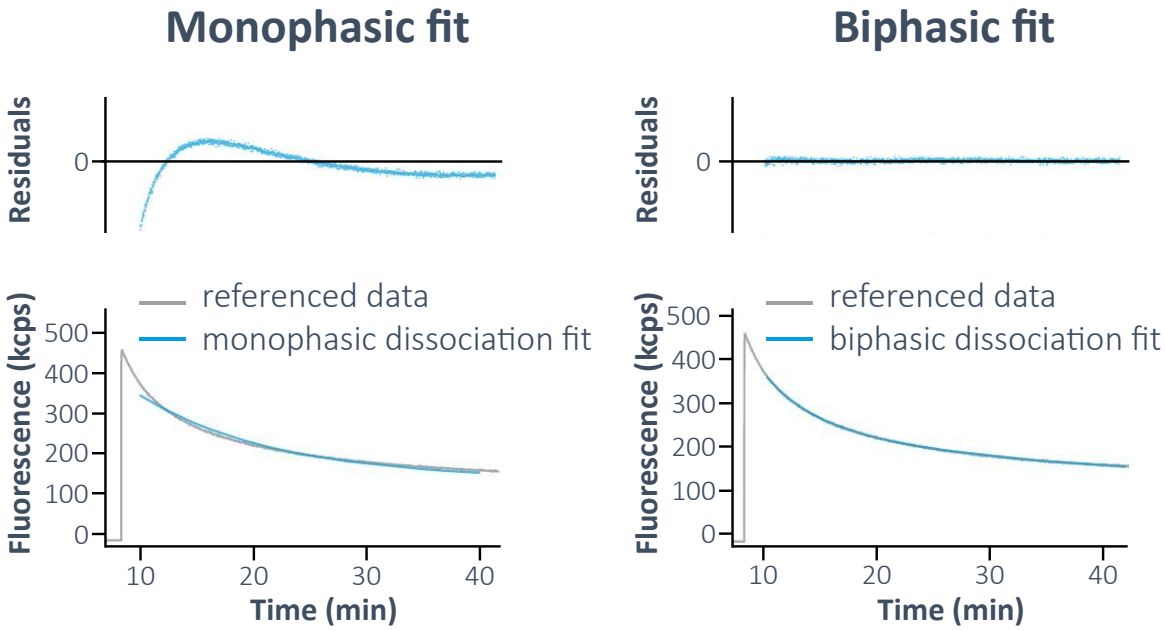



| | # cells | $k_{on}$ (M-1s-1) | $k_{off}$ (s-1) | $K_D$ (nM) | $K_D^{Literature}$ |
|---|---|---|---|---|---|
| Nivolumab – PD1 | 5 | 1.34 ± 0.26 E+6 | 3.53 ± 0.44 E-3 | 2.64 | 2.60 |
| Rituximab – CD20 | 5 | 80.70 ± 4.0 E+3 | 488 ± 180 E-6 | 6.00 | 8.00 |

# Nivolumab binding to T cells

## AVIDITY AS THE BASIS FOR *IN VIVO* EFFICACY: STABLE BINDING ENABLES T CELL ACTIVATION




| | $k_{on}$ (M-1s-1) | $k_{off}$ (s-1) | $K_D$ (nM) |
|---|---|---|---|
| **Affinity** | 2.82 ± 0.09 E6 | 1.46 ± 0.08 E-2 | 5.18 |
| **Avidity** | 2.82 ± 0.09 E6 | 1.00 ± 0.01 E-3 | 0.36 |

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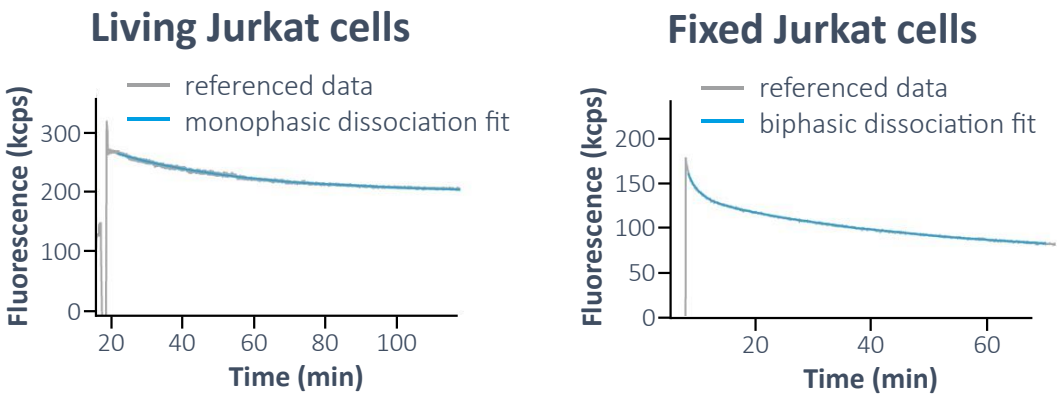
# Influence of target mobility

## LIVING CELLS MOSTLY SHOW AVIDITY BINDING MODE, FIXED CELLS SHOW A MIX OF AVIDITY AND AFFINITY



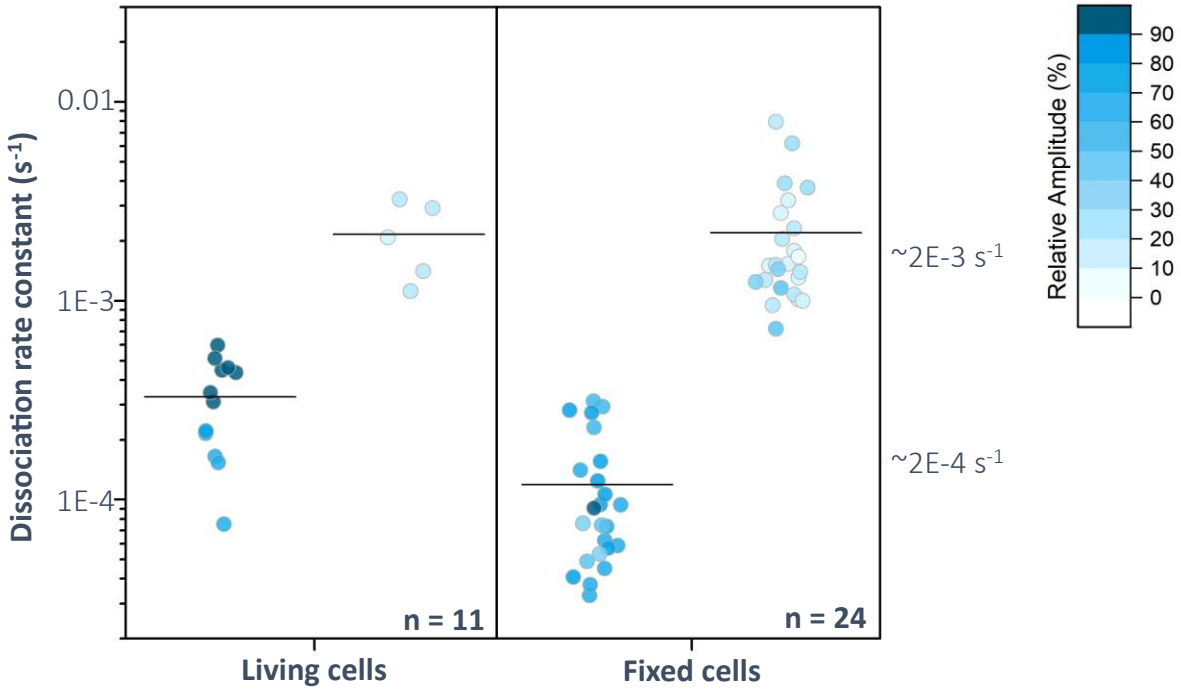

*Exemplary curves for dissociation only runs*

**Living cells:**

30 % displayed mixed modes, 70 % showed only slow dissociating OKT3, representing a 1:2 avidity binding mode. Avidity seems to be promoted by receptor mobility in the fluid membrane of living cells

**Fixed cells:**

Always display mixed binding modes with fast and slow dissociation rates.

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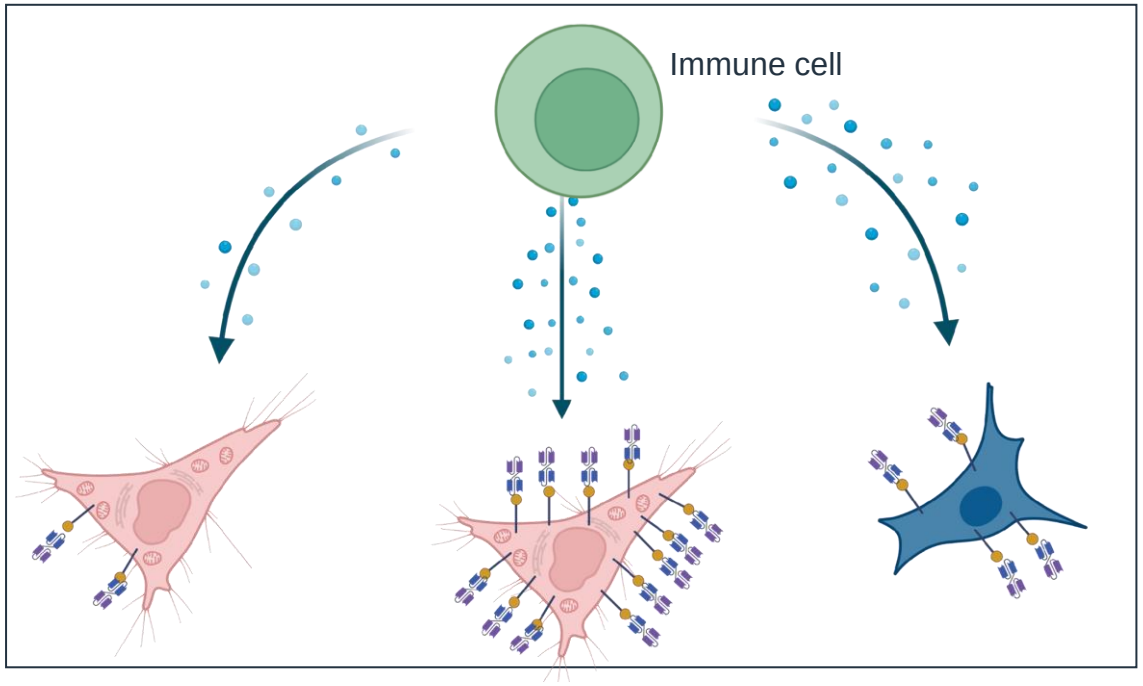
# The importance of target antigen density

**Immune cell therapies, Immune cell engagers, Antibody-Drug Conjugates (ADCs), Conventional mAbs**




**B. Preclinical Considerations for the Vector Component of CAR T Cells**

The design of the CAR vector and the process by which the transgene is delivered to the T cells are critical in determining product safety and activity. Genetic material encoding the CAR has been delivered to T cells using multiple vector types, including gammaretroviral and lentiviral vectors, transposons, and naked mRNA (Ref. 10).

A major determinant of CAR T cell safety and efficacy is the antigen recognition domain used to confer target specificity. The antigen recognition domain may originate from monoclonal antibodies (mAbs), endogenous ligand/receptor pairs, or from other sources. Preclinical evaluation of the antigen recognition domain should include assessment of the specificity and affinity for the target antigen to evaluate the potential for on-target/off-tumor and off-target toxicities. Undesired targeting of healthy/normal tissue that express the intended target antigen (on-target/off-tumor), as well as unintended targeting of other antigens expressed on healthy/normal tissue is a safety concern that may be evaluated using both in vitro and in vivo studies. Examples include: (1) tissue

- Cancer cell **– low expression**: Immune escape, loss of therapeutic function
- Cancer cell – **high expression**: Therapeutic effect
- Healthy cells **– low expression**: toxicity, on-target off-tumour effects

# Titratable expression with a large dynamic range – IndEx-2: a dual inducible, customisable cell line platform

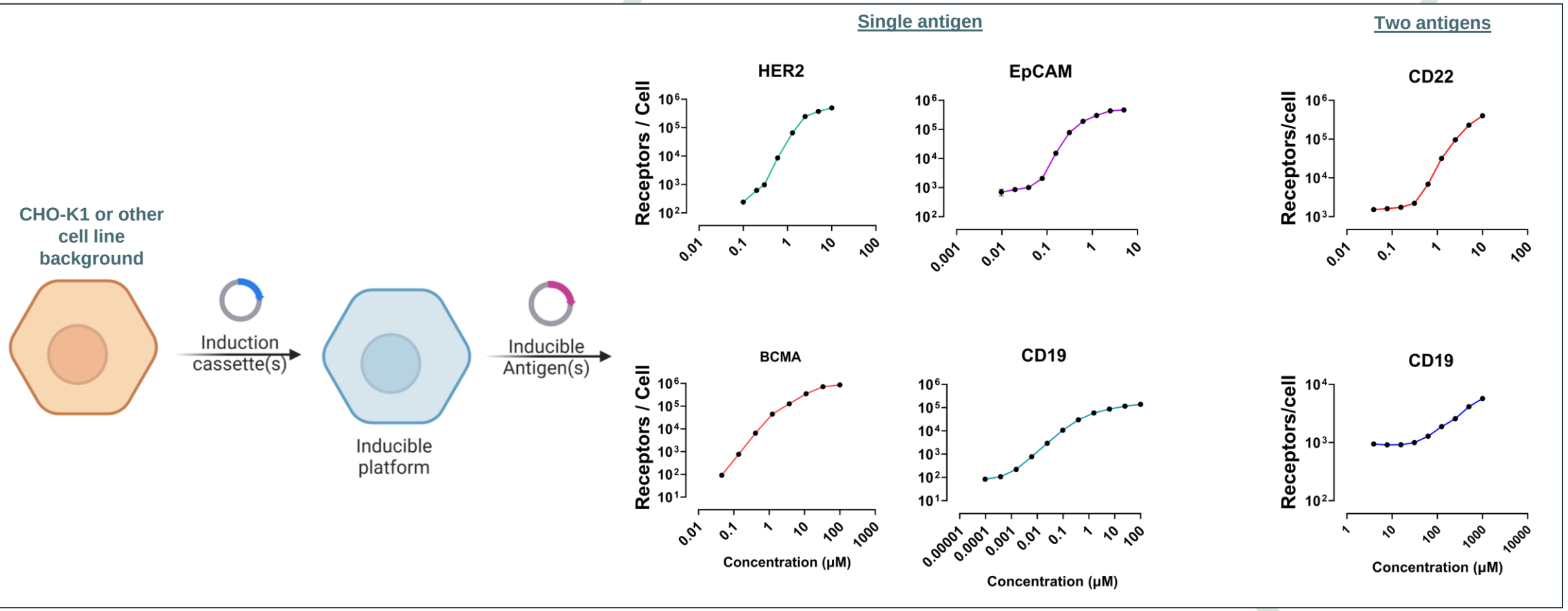
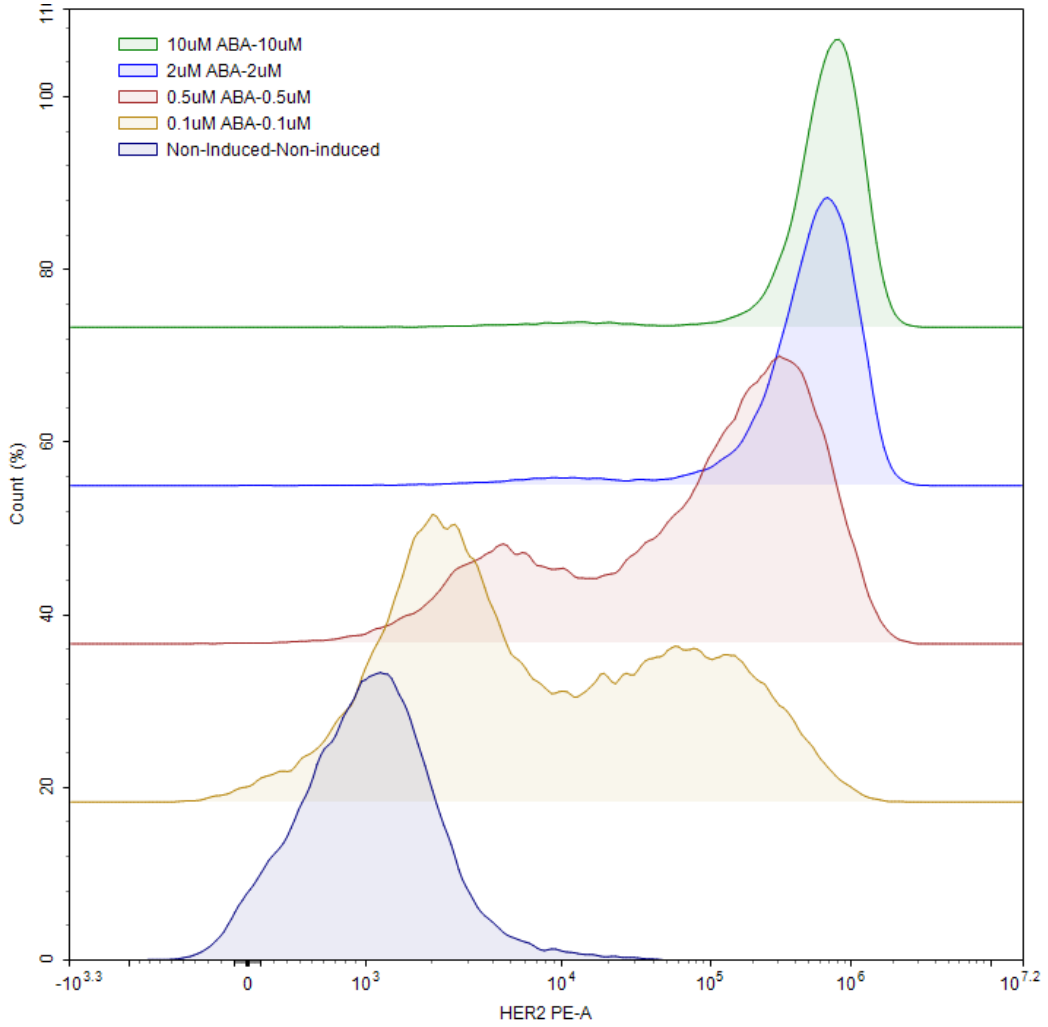
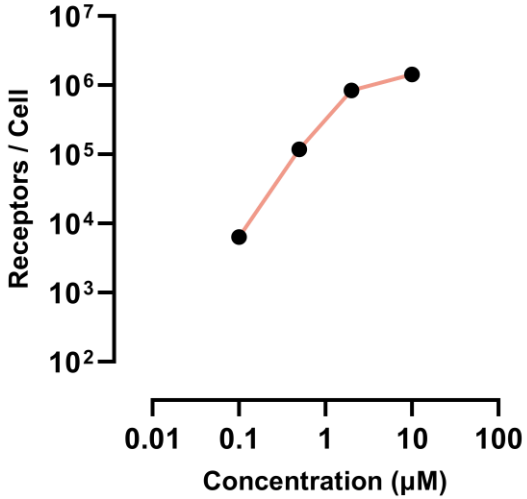



Figure generated with Biorender

# Titratable expression of HER2 (1,255aa)




Expression of HER2 increases with increasing concentration of inducer. Receptor numbers per cell quantified using Bangs beads.

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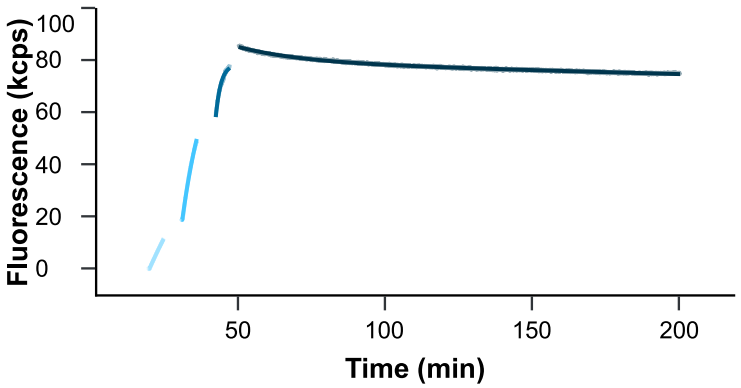
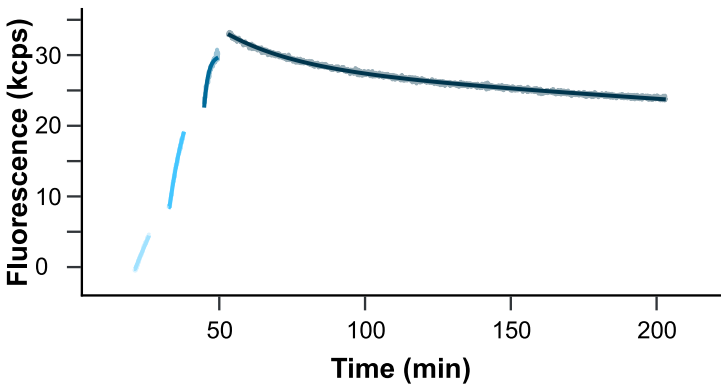
# **sc-IC** measurements on cells with differential HER2 levels

TRASTUZUMAB BINDS TO FIXED CHO CELLS EXPRESSING HER2 BUT NOT CONTROLS

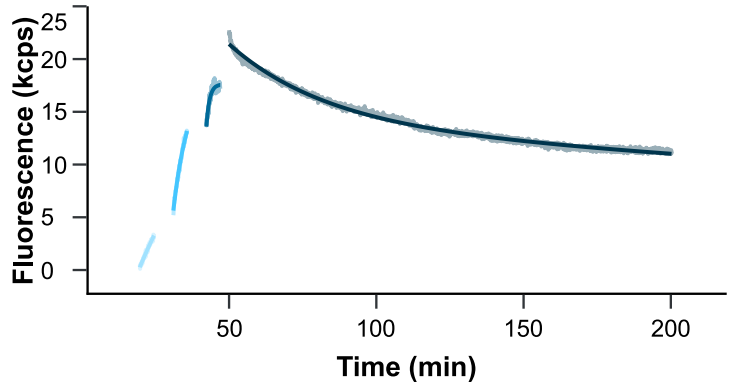
1 433 528 receptors / cell



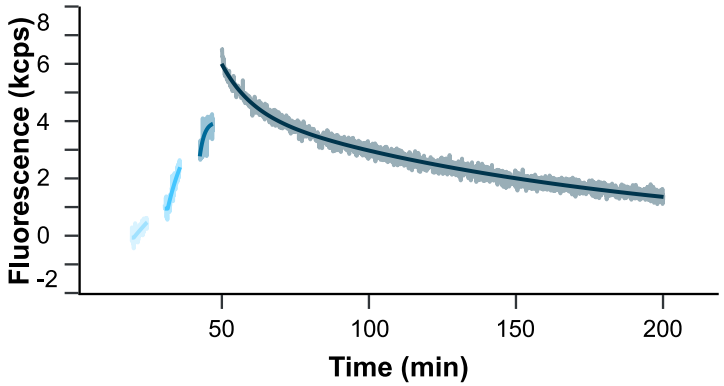

835 582 receptors / cell



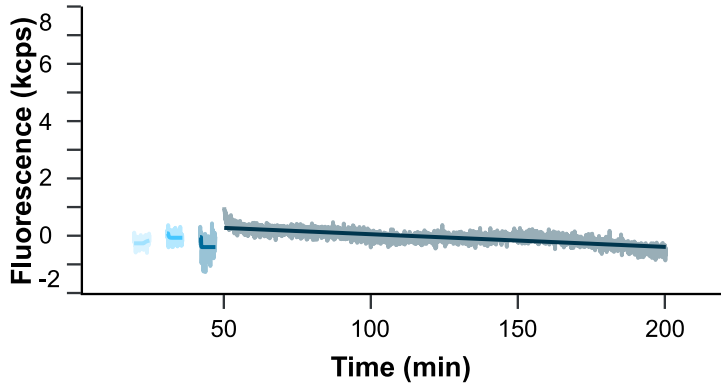

118 532 receptors / cell



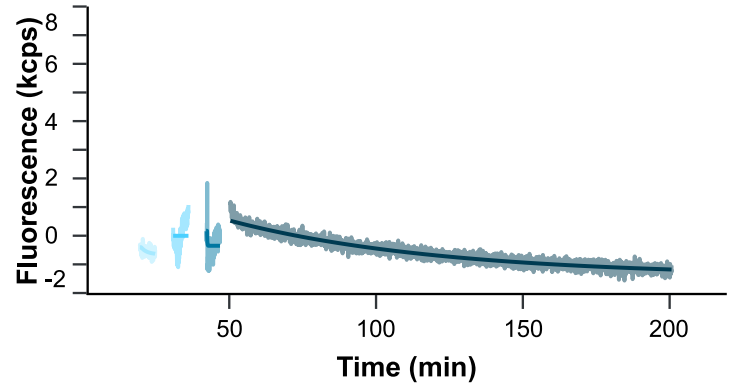

6 377 receptors/cell




WT control




Non-induced control

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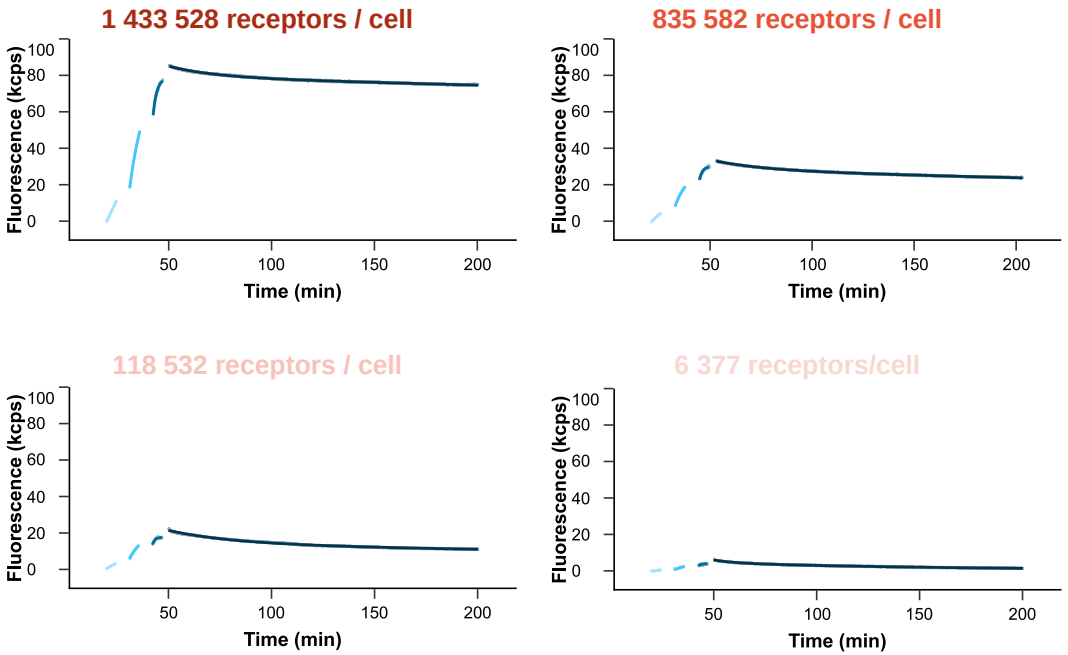
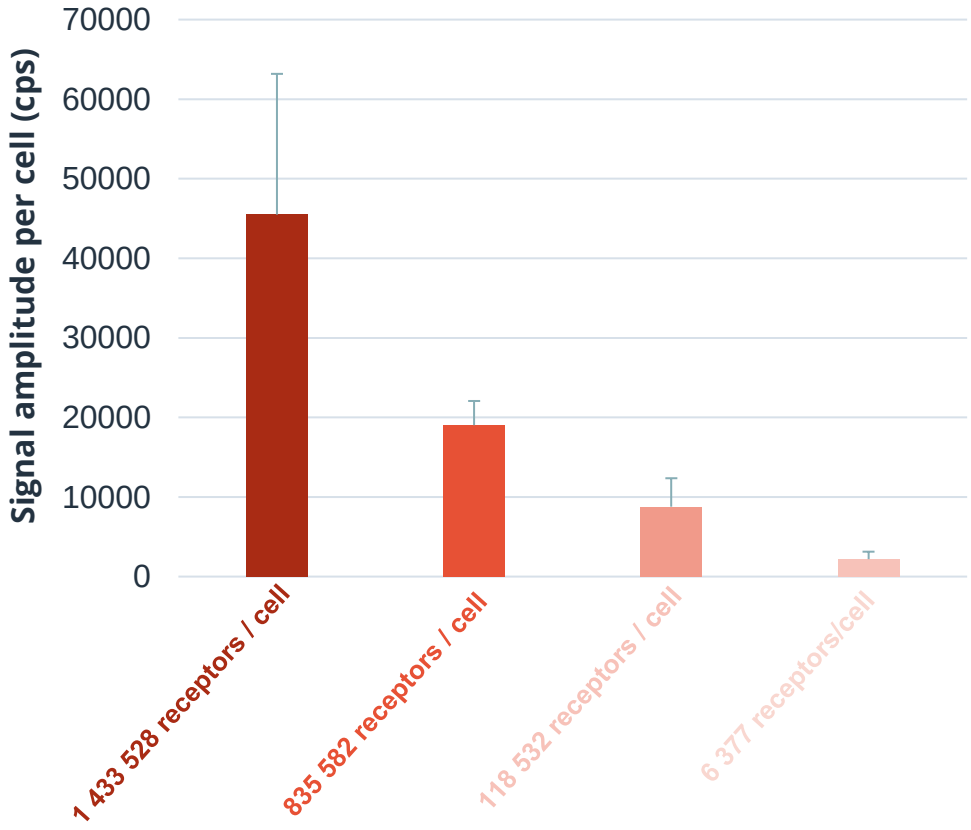
| | |
|---|---|
| Cells | Fixed CHO (receptor numbers / cell as indicated) |
| Analyte | Trastuzumab |
| Concentration | 1, 5, 25 nM |

# HER2 expression level affects signal amplitude per cell

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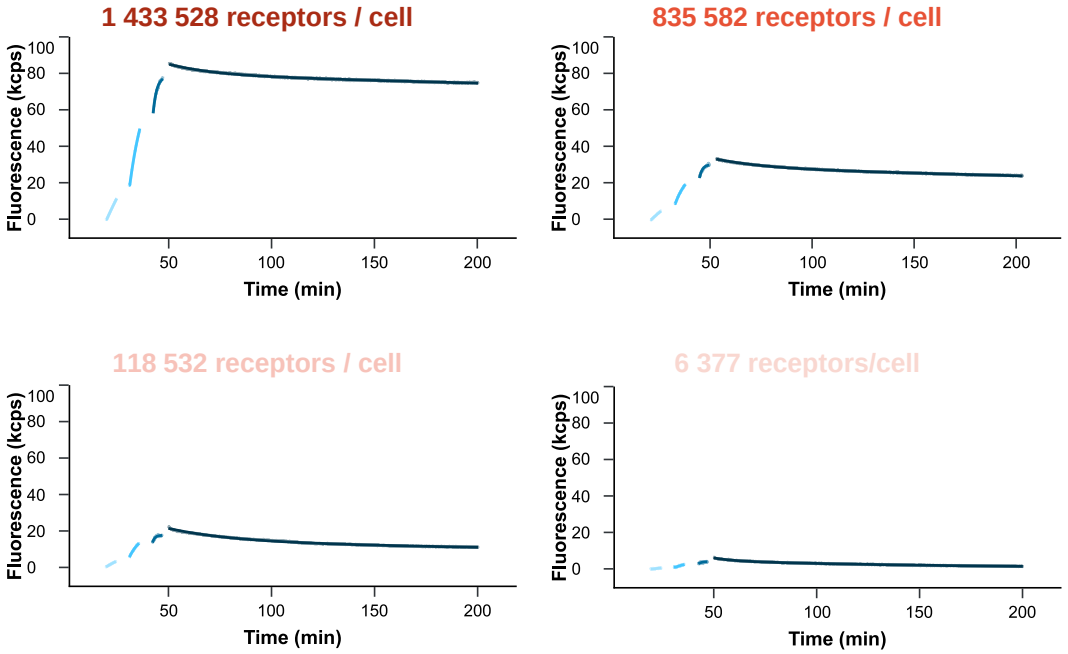
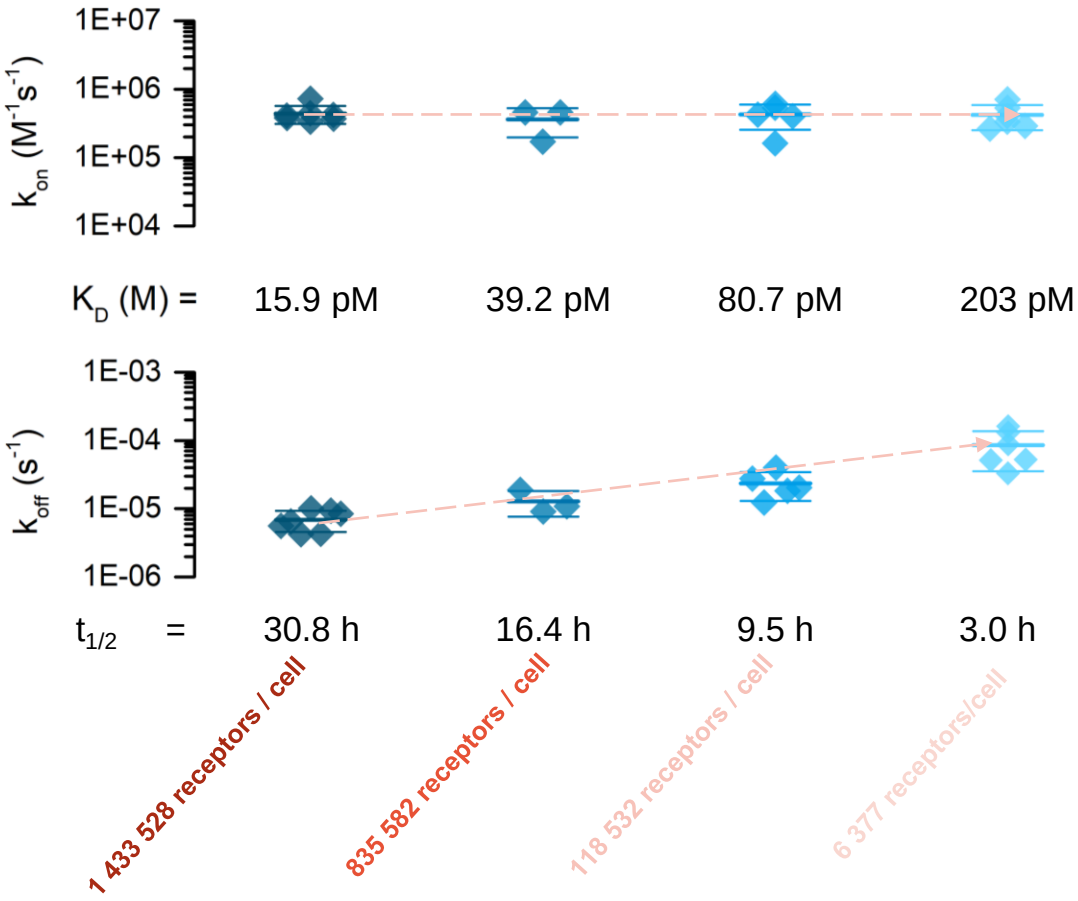
| | |
|---|---|
| Cells | Fixed CHO (receptor numbers / cell as indicated) |
| Analyte | Trastuzumab |
| Concentration | 1, 5, 25 nM |

# HER2 expression affects kinetic rates | high expression might increase avidity




| | |
|---|---|
| Cells | Fixed CHO (receptor numbers / cell as indicated) |
| Analyte | Trastuzumab |
| Concentration | 1, 5, 25 nM |

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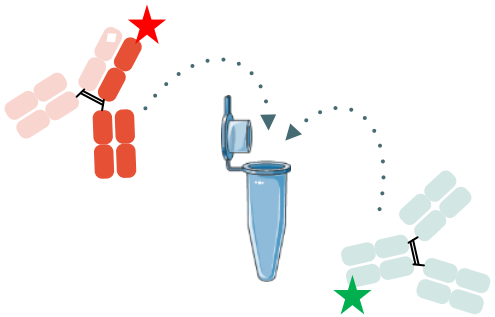
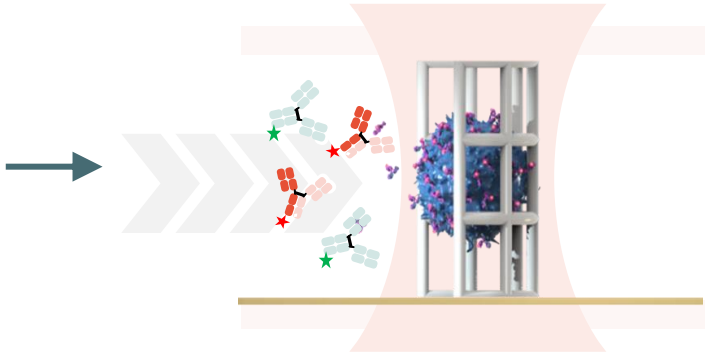
# Dual-Colour Assays

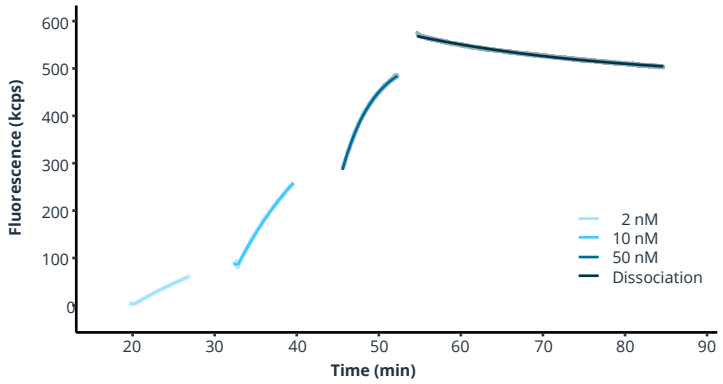
## Measure two interactions on cells simultaneously

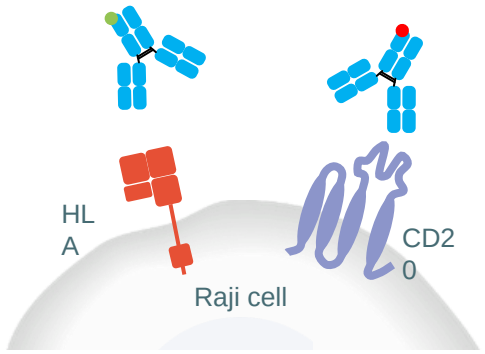





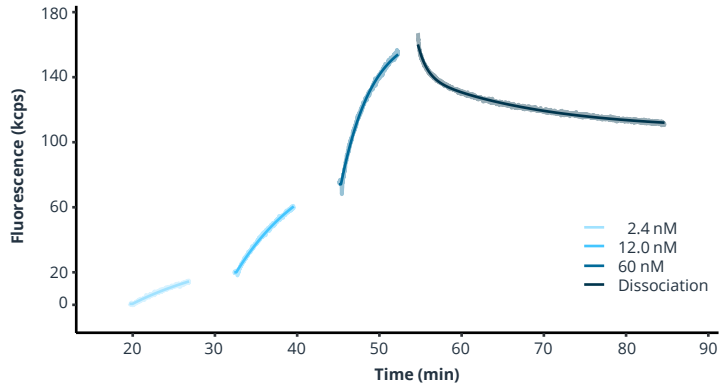
**Two-color detection** for monitoring two events

Measurement of **Interaction 1 in green:** anti-HLA antibody




Measurement of **Interaction 2 in red:** Rituximab



# SC-IC in Summary

**sc-IC** measures direct binding to targets in their **native environment** on cells.

Measure **kinetics** AND **avidity.**

We provide **knowledge-guided assay design** and access to **experts in the field** of biophysical characterisation.

# Contact our experts to accelerate your drug discovery project with our sc-IC services:

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# **VARIOUS** CELL TYPES

## SUSPENSION AND ADHERENT CELLS CAN BE TRAPPED IN helix$^{cyto}$ CHIPS

### SUSPENSION CELLS

- Jurkat (human T-cell line)
- CAR-T (modified Jurkat)
- HPB-All (human T-cell)
- T2 (hybrid human B/T-lymphoblast)
- Raji (human B lymphocyte, Burkitt lymphoma)
- Reh cells (human B cell precursor leukemia)
- OCI-Ly18 (human large B cell lymphoma)
- NALM-6 (human lymphocyte-like, acute lymphoblastic leukemia)
- Ba/F3 (murine pro-B cell)
- MEC-1 (human chronic B cell leukemia)
- H929 (human B lymphocytes, plasmacytoma myeloma)
- Daudi (human lymphoblasts, Burkitt lymphoma)
- Raw264.7 (murine macrophage)
- MOLM-13 (human acute monocytic leukemia)
- DH82 (canine macrophage-like from malignant histiocytosis)
- Monocytes
- THP-1 (human monocyte cell line)
- KATO-III (human gastric carcinoma)
- SHP-77 (human epithelial cells from lung carcinoma)
- Expi293 (HEK293-based expression system)
- K-562 (human chronic myeloid leukemia)
- EOL-1 (human eosinophilic leukemia)
- RBL (rat basophilic leukemia)
- KHYG-1 (human natural killer cell leukemia)
- HMC (human mast cell line)
- primary T-cells (sorted into CD4+, CD8+)
- primary NK cells (human lymphocytes)
- primary Th17 cells (human lymphocytes)

### ADHERENT CELLS

- HeLa (human cervical cancer)
- CHO (chinese hamster ovary)
- HEK293T cells (Human embryonic kidney 293 cells)
- Huh7 (human hepatoma)
- HepG2 (human hepatocarcinoma)
- Ovcar8 (human ovarian carcinoma)
- SKOV3 (human ovarian adenocarcinoma)
- PC-3 (human prostatic adenocarcinoma metastasis)
- LNCap (human prostate carcinoma)
- Caco-2 (human epithelial colorectal adenocarcinoma)
- HCT 116 (human colon cancer)
- A431 (human epithelial derived from epidermoid carcinoma)
- A549 (human lung carcinoma)
- HCC-78 (human lung carcinoma)
- H2009 (human lung adenocarcinoma)
- A375 (human epithelial derived from melanoma)
- UMUC3 (human urinary bladder, epithelial-like cells)
- SKBR3 (human breast adenocarcinoma)
- MCF7 (human breast adenocarcinoma)
- T-47D (human breast cancer)
- MDA-MB-231 (human breast adenocarcinoma)
- SK-N-MC (human neuroblastoma)
- EA.hy926 (human endothelial)

**T cells**

**B cells**

**Monocytes/ Macrophages**

**Solid tumors**

**Primary human cells**



dynamic BIOSENSORS