Target density matters: Binding kinetics of antibody-based therapeutics are highly influenced by target expression levels

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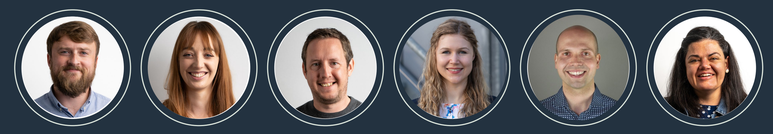
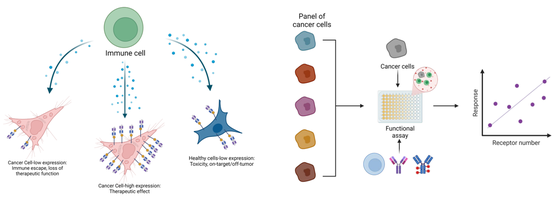


Introduction

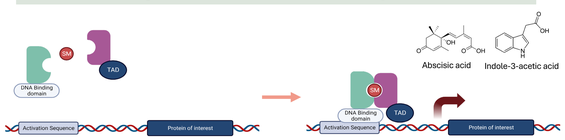
Understanding how antibody-based therapeutics interact with their targets at varying expression levels is vital for optimising tissue and cell-specific drug efficacy and safety. Traditional methods, such as SPR, BLI and QCM, face limitations when assessing binding kinetics in cellular environments due to challenges with sensitivity, throughput and physiological relevance. To address these gaps, this study explores the impact of target density on therapeutic binding kinetics using the novel IndEx-2 system and single-cell Interaction Cytometry (scIC). This integrated approach provides real-time kinetic data in a cellular context, offering critical insights into drug performance under conditions that closely mimic tumour heterogeneity.

The importance of target density

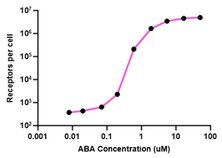


Limitations of traditional methods are **logistical** (access & cost) and **methodological** (expression range & cell line background).

CIP-based systems for titratable expression



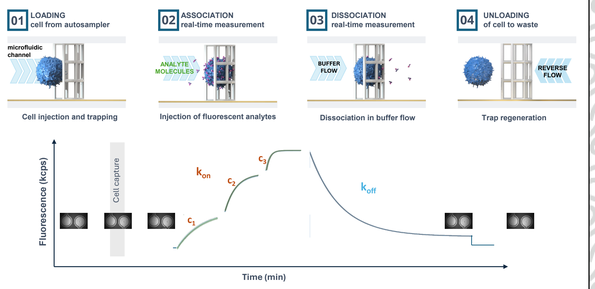
Controlled expression of HER2



Induction of HER2 expression was achieved by the addition of varying concentrations of ABA for approximately 24 hours. Cells were stained with labelled antibody and expression levels quantified using a bead-based quantification kit.

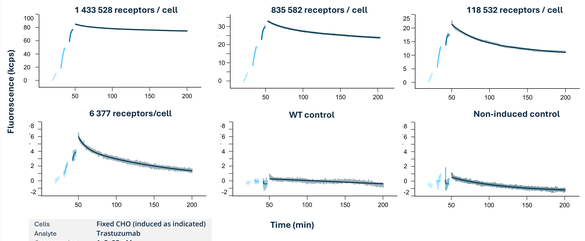
Figures were created by using BioRender.

single-cell Interaction Cytometry (sc-IC)



Trastuzumab binds to HER2 on cells

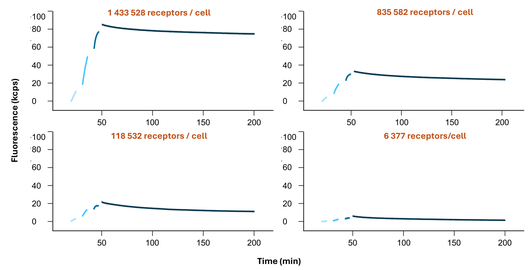
Fluorescently labelled trastuzumab was passed over captured cells at three different concentrations followed by an extended injection of buffer to follow dissociation. Trastuzumab bound all four HER2 expressing cells. Controls included a wild-type CHO-K1 cell line as well as an un-induced control.

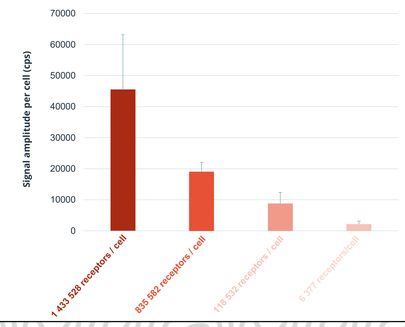


Cells	Fixed CHO (induced as indicated)
Analyte	Trastuzumab
Concentration	1, 5, 25 nM

Expression level affects signal amplitude

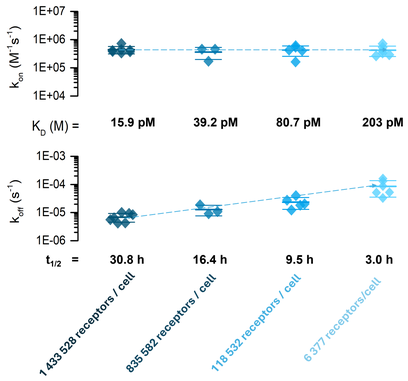
When normalising the fluorescence signal to the number of cells, the amplitude of the signal observed directly relates to the number of receptors per cell. More receptors present more binding sites for trastuzumab and therefore a higher signal is measured.





Expression level affects kinetics

Cells with the lowest receptor number have the highest K_D and therefore lowest affinity whereas cells that have the highest receptor numbers have the lowest K_D and therefore highest affinity. The on-rates are very similar for all four cell densities. Off-rates drive the differences – the lower the receptor number, the faster trastuzumab dissociates from the surface. Residence time is 10X higher for cells with the highest number of receptors strongly suggesting avidity plays a role in the binding at higher receptor numbers



Graphical representation of calculated kinetic values. Affinity values are shown in pM with the half life values calculated from the fit model for each interaction.

Summary

We have investigated the effect of target density on the kinetics of trastuzumab binding to HER2 on the surface of cells. By utilising the IndEx-2 system, which allows controlled expression of HER2 in CHO-K1 cells, with scIC we have measured the real-time binding kinetic parameters. Our analysis has shown that at higher levels of HER2 expression, therapeutic residence time increased due to avidity effects. In addition, differences in binding affinity across HER2 densities were mainly attributed to changes in dissociation rate rather than association rate. This approach has provided new insights into how target density influences therapeutic binding, offering a more physiologically relevant framework for drug development.

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