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Studying Cell Binding Heterogeneity using SPRM

Dynamic diseases like cancer become more heterogeneous over the course of the disease. This might result in non-uniform distribution of subpopulations of cells in the disease sites (spatial heterogeneity) or variations in the molecular makeup of cells within the same population (temporal heterogeneity).[1]This heterogeneity arises among cells mainly because of genetic change, environmental variations and changes in the cellular properties.[2] A key step in the development of a drug is to screen various candidates by determining their affinities towards a cellular target and the kinetics of the interactions in a heterogenous native environment. Gaining insights into two-dimensional receptor-ligand binding kinetics is of value to understand numerous physiological and pathological processes leading to new strategies in drug design and discovery.[3]

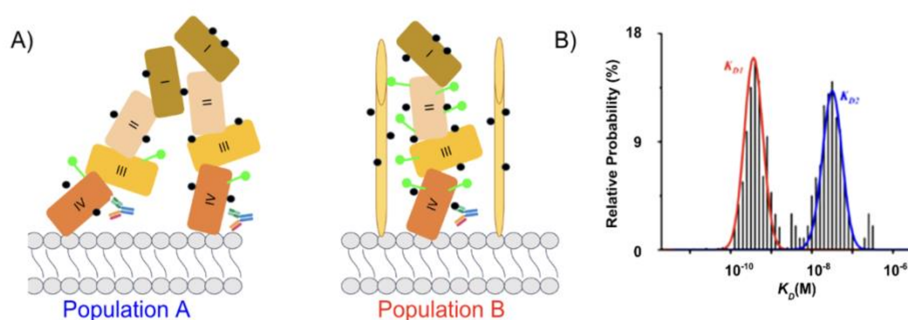

Figure 1: (A) Schematic representations of the anti-HER2 antibody binding to heterogenous HER2 molecules (Population A- non-glycosylated HER2 and Population B- Glycosylated HER2). The residues on HER2 comprising the primary amine groups are depicted as black dots, and the N-linked glycans are shown as strings of green beads. The four domains on the extracellular segment of HER2 are identified. In population B, a mucin molecule is used to represent all HER2-neighboring proteins such as the epidermal and insulin-like growth factor receptors. (B) Binding kinetics measurements of a heterogeneous population using SPRM.

This application note focuses on Dong et al recent work in studying the effect of cell heterogeneity on binding kinetics and the use of Surface Plasmon Resonance Microscopy (SPRM) to gauge the effects of cell heterogeneity in a population.[4] SPRM tackles this issue of cellular heterogeneity with its unique ability to measure label-free the binding kinetics and phenotype of each individual cell. In this way, a statistical analysis of the cell population can be produced to determine the heterogeneous range of binding interactions **(Figure 1B)** and identify the predominant modes of binding interactions.

This study examines the heterogeneity caused by aberrant protein glycosylation occurring in the human epidermal growth factor receptor 2 (HER2) protein, a member of the tyrosine kinase receptor family as seen in **Figure 1A**. Some cancer patients develop Herceptin resistance due to the masking or physical blockade of HER2 receptors by glycans or large glycoproteins like MUC4.[5-7] Although kinetic values of purified proteins serve as good guides for drug development, it is very clear that results obtained with cell-based methods are more biologically meaningful and pharmacologically relevant as they address the cell-cell variations in depth. Dong et al studied the conjugation of an anti-HER2-antibody to the

HER2 receptor on SKBR3 cells to address cell heterogeneity using SPRM **(Figure 2)**. The affinity and kinetic values obtained are listed in **Table 1**.

HER2 and anti-HER2 FITC Interaction	*k*a x 10^4 [1/M*s]	*k*d x 10^{-4}[1/s]	*K*D [nM]
Native in-cell HER2, SKBR3 cells (Population A, non-glycosylated – peak)	8.60	0.25	0.34
Native in-cell HER2, SKBR3 cells (Population B, glycosylated – peak)	4.16	13.4	32.0

Table1: Kinetic parameters measured from anti-HER2 and HER2 binding interaction of the native in-cell forms.

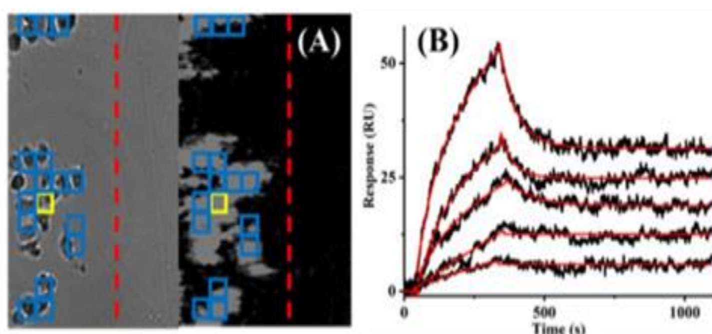

Figure 2. (A) Bright-field (left) and SPRM (right) images of SKBR cells showing the barrier (dashed red line) separating the reference and cell-covered areas and the ROI (thickened yellow box) used to obtain the representative sensorgrams in (B). Injected solutions contained 1.00, 5.00, 10.0, 20.0, and 50.0 nM antibody and the red curve is the simulated sensorgram based on the 1:2 binding model.

The binding interaction in this study follows a typical 1:2-type kinetic model, where interactions are stronger with the native receptor and the weaker binding to the glycosylated receptor were reported **(Table 1).** The association rate constant (*ka*) is faster on non-glycosylated cells, suggesting that the glycans on the glycosylated HER2 hinder the antibody binding to domain IV. Heterogeneity is the fundamental property of cellular systems but there can be loss of information due to ensemble averages at many scales of biology, from single molecules to communities of whole populations. Also, understanding the difference between cellular kinetics due to cell heterogeneity that can affect the fidelity of the kinetic values, is important for guiding the drug discovery process and for evaluating drug efficacy and the associated side effects in clinical settings.

References:

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