Label-Free Kinetic Analysis of Anti-TCR Binding to Jurkat T Cells Using Surface Plasmon Resonance Microscopy

Understanding how antibodies interact with cell surface receptors is fundamental to the development of immunotherapies, T-cell engagers, and other biologic drugs. Flow cytometry is widely used to characterize antibody binding to receptors on suspension cells and can provide quantitative measures of binding or functional responses, such as EC_{50} or IC_{50} values. However, these endpoint measurements do not directly resolve the association and dissociation kinetics underlying the observed interaction. Surface Plasmon Resonance Microscopy (SPRm) complements these measurements by enabling real-time, label-free analysis of antibody–receptor interactions directly on whole cells, providing association (ka) and dissociation (kd) rate constants and equilibrium binding affinity (KD), while simultaneously imaging the cells being measured.[1,2]

Jurkat T cells are among the most widely used cellular models for studying T-cell biology and immune signaling.[3,4] Derived from human acute T-cell leukemia, Jurkat cells express the complete T-cell receptor (TCR) complex and many of the downstream signaling molecules found in primary T lymphocytes. Jurkat cells grow in suspension; however, once they are firmly immobilized on the sensor surface, they provide an ideal system for demonstrating the ability of SPRm to perform quantitative kinetic measurements on non-adherent immune cells.

The T-cell receptor is the primary recognition molecule of adaptive immunity. Expressed on the surface of T lymphocytes, the TCR recognizes antigenic peptides presented by major histocompatibility complex (MHC) molecules on antigen-presenting cells. Engagement of the TCR initiates a cascade of intracellular signaling events that regulate T-cell activation, proliferation, cytokine secretion, and cytotoxic responses. Consequently, the TCR has become an important therapeutic target for cancer immunotherapy, autoimmune disease research, and the development of T-cell engaging antibodies. [5]

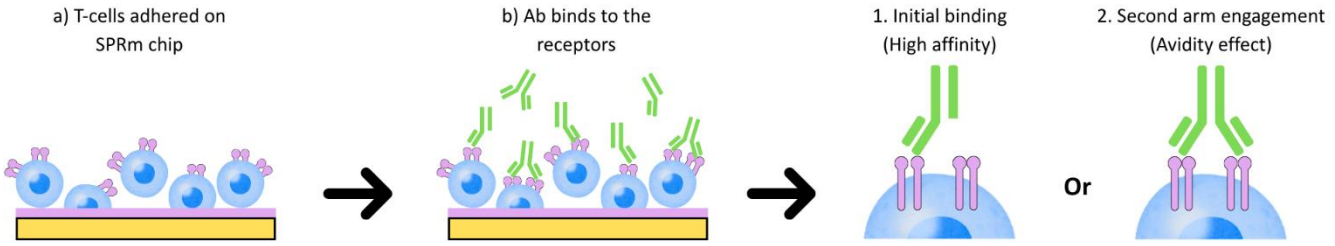

Figure 1: Schematic of antibody binding to receptors on T cells immobilized on a SPRm chip. a) *T cells with surface receptors adhered on senor chips.* ***b)*** *Injected antibody binds receptors on the cell surface. Binding may occur through a single antibody arm (1), representing the initial high-affinity interaction followed by engagement of a second arm with a nearby receptor (2), producing an avidity effect and increasing apparent binding stability.*

An anti-TCR monoclonal antibody was injected over the immobilized Jurkat T cells at 8 concentrations ranging from 25 to 0.19 nM to measure antibody binding **(Figure 1)**. The measured kinetics revealed two binding populations **(Figure 2)**. The fitted association rate constants were $ka_1 = 1.15 \times 10^5\ M^{-1}s^{-1}$ and $ka_2 = 2.38 \times 10^5\ M^{-1}s^{-1}$; however, the association phases were substantially overlapping and were not interpreted as evidence of distinct association behaviors. In contrast, the dissociation phase and the equilibrium dissociation clearly resolved two kinetic modes, with $kd_1 = 4.33 \times 10^{-4}\ s^{-1}$ and $kd_2 = 1.31 \times 10^{-2}\ s^{-1}$ and $KD_1 = 5.41$ nM and $KD_2 = 171$ nM. Together, these results are consistent with heterogeneous affinity/avidity interactions characterized primarily by distinct dissociation behaviors rather than substantially different association kinetics.

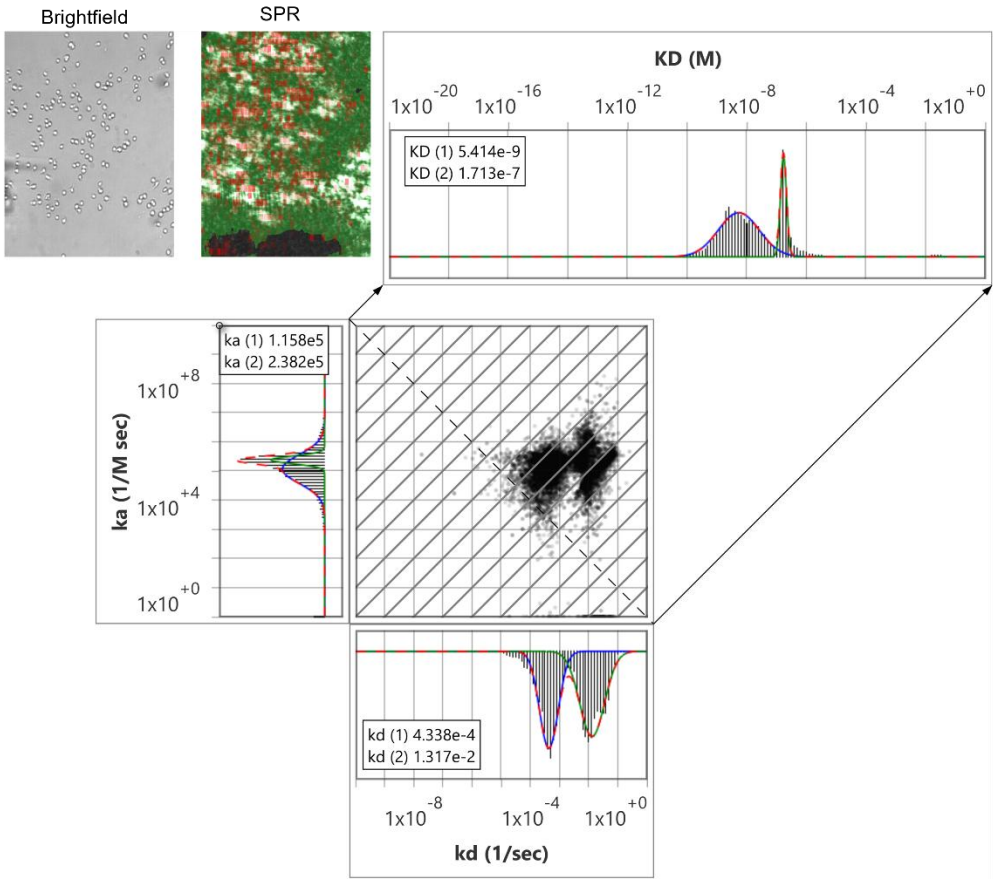

Figure 2: SPRm analysis of antibody binding kinetics on Jurkat T cells. *Brightfield and SPRm images show individual Jurkat T cells selected for kinetic analysis. The kinetic distributions summarize the equilibrium dissociation constant (KD), association rate constant (ka), and dissociation rate constant (kd). Histograms and fitted curves reveal two distinct binding populations, while the central ka versus kd scatter plot illustrates cell-to-cell heterogeneity in antibody receptor binding kinetics.*

This behavior is expected because the anti-TCR antibody is an IgG molecule containing two identical antigen-binding Fab arms. Following the initial engagement of one Fab arm with a TCR molecule, the second Fab arm can bind an adjacent receptor on the same cell surface. This bivalent interaction increases the overall stability of the complex through avidity, effectively slowing dissociation and producing multiple apparent kinetic states. Such multivalent binding behavior is difficult to observe using conventional biochemical assays but becomes readily apparent when receptors are measured directly on intact cell membranes.

SPRm provides direct access to association and dissociation kinetics while preserving the natural organization of receptors within the plasma membrane. Furthermore, analysis can be performed at the single-cell level, enabling researchers to quantify cell-to-cell heterogeneity that is often masked in bulk measurements. By combining quantitative kinetic analysis with label-free imaging of intact cells, SPRm offers an attractive solution for characterizing therapeutic antibodies against membrane receptors under physiologically relevant conditions, making it a valuable tool for immunology research and biologic drug development.

References:

1) *Mauriz E et al, Sensors.* 2020; 20:4745.
2) Wang W, et al. *Scientific Reports*. 2014; 4:6609.
3) Jing W, et al. *Membranes*. 2020; 10(9):247.
4) Lin P, et al. *ACS Sensors*. 2025 ; 15-32.
5) Courtney AH et al, *Trends in Biochemical Sciences*. 2018; 43(2):108–123.