WHITEPAPER

Mass Transport Limited (MTL) Kinetics Analysis with Alto™ Digital SPR

Overview

Mass transport limitations are a common challenge in obtaining kinetics for fast binding reactions with surface-based biosensors such as those used in a surface plasmon resonance (SPR) assay. If the molecule in solution diffuses from the bulk to the surface at a rate slower than the rate at which it associates with the immobilized ligand, these effects of mass transport can be observed in the data.

In this whitepaper, we discuss the many advantages of using Alto's Digital SPR technology for analyzing interactions that experience mass transport limitations, and demonstrate how Alto's suite of tools and kinetics analysis accommodations are applied to reduce mass transport effects in SPR data.

What is Mass Transport Limitation (MTL) in SPR?

In surface-based biosensor systems, the sample molecules in solution must first diffuse from the bulk solution to the sensor surface to become available for participation in binding interactions. In SPR, this diffusion must occur before the binding of the analyte at the ligand can take place (Figure 1). When measuring interaction kinetics, if the diffusion rate of the analyte from the bulk to the surface is slower than the association rate with the ligand on the surface, these effects of mass transport can be observed in the data. This is generalized using the term "mass transport limitation" and can be described using a two-step binding model (Equation 1). The term k_t represents the mass transfer rate constant of the analyte from the bulk to the surface.

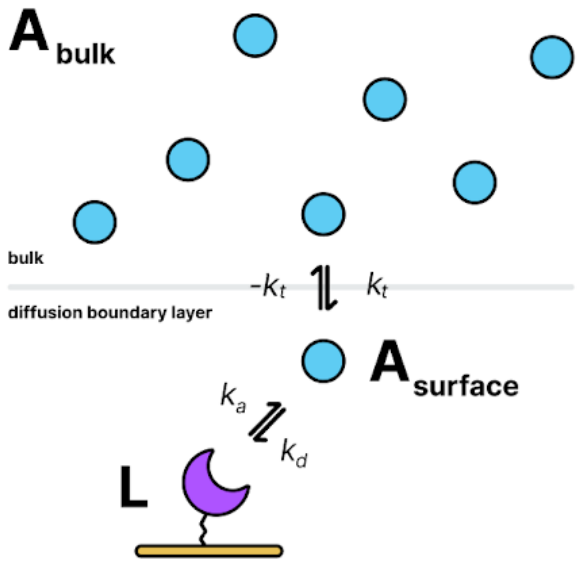

Figure 1: *Schematic representation of mass transport limitation in SPR of the analyte to the ligand immobilized on the sensor surface.*

$$A_{bulk} \underset{k_t}{\overset{k_t}{\rightleftarrows}} A_{surface} + L \underset{k_d}{\overset{k_a}{\rightleftarrows}} LA$$

Equation 1: *Two-step binding model with mass transport limitation. This accounts for partially limited ability of the analyte to reach the surface and make itself available for binding. A_{bulk} represents Analyte in the bulk solution, $A_{surface}$ represents Analyte available at the sensor surface and L represents Ligand.*

Mass transport limitations are most common for fast binding reactions, and would most severely affect the association rate measured. Figure 2 demonstrates how mass transport limitations can impact SPR binding curves of a fast binding system.

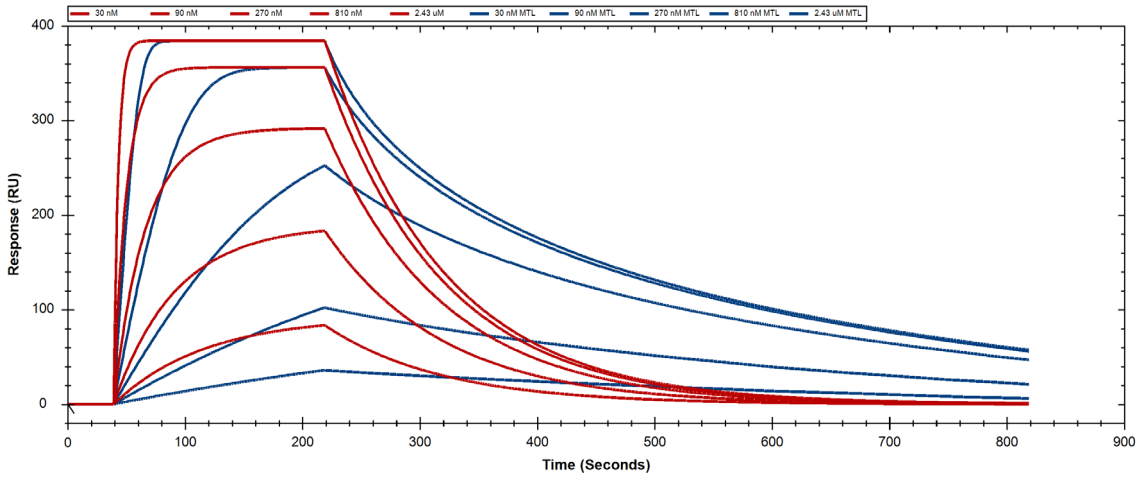

Figure 2: *Simulated example data demonstrating the impact of MTL on SPR kinetic binding curves. Red curves = data with no MTL contribution, Blue curves = same data affected by MTL. Simulated data inputs: Rmax = 400 RU, k_a = 1.00E+5 M^{-1} s^{-1}, k_d = 1.00E-2 s^{-1}, k_t = 1.00E+7 RU M^{-1} s^{-1}.*

Ways to Reduce Mass Transport Effects in SPR with Alto

To reduce mass transport limitation, SPR systems are designed with flow or mixing of the solution over the surface, however, even with rapid movement of the solution a small diffusion boundary layer will always be present as shown in Figure 1. There are 3 common methods used to limit mass transport effects in SPR data and specific recommendations are provided for use with Alto below:

1) Higher Flow Rates

In traditional flow-cell based SPR systems, increasing the flow rate will effectively help reduce the diffusion boundary layer that the analyte needs to overcome to interact with the ligand on the sensor surface. However increasing the flow rate comes with the tradeoff of either being limited to a shorter sample interaction time, or requiring a larger sample volume. In Alto, which is empowered by digital microfluidics (DMF) technology, sample mixing is induced using droplet oscillation as opposed to traditional flow. Alto's droplet oscillation rate has been optimized to reduce the diffusion boundary layer to be equivalent to fast flow rates, and has the advantage of having an unlimited sample interaction time without any sample dispersion occurring.

2) Lowering Surface Density of the Ligand

The more ligand you have immobilized on your sensor, the more possible interaction sites for the analyte to bind. Reducing the amount of ligand immobilized to the sensor will help reduce mass transport limited effects as less analyte needs to diffuse for the interaction to occur. The trade-off with lowering the surface density is a decrease in the Rmax (max signal at saturation), so the data could be noisier. Using Alto sensors, a lower amount of ligand than what is typically required in traditional SPR can be used due to the confined nature of the localized surface plasmon resonance (LSPR) detection range, as well as the extremely small spot size of the sensor substrate on the tip of an optic fibre. Users also have the flexibility to adjust the concentration and contact time of ligands used in the Alto experiment protocol design to control the immobilized density.

3) MTL Corrected Analysis Model

The best practice for accounting for mass transport limited effects in SPR data is to use a fitting model that includes mass transport in the overall reaction equations. This is a common practice in the SPR field, and does not impart a negative impact on the quality of the data analysis results[1,2]. Alto's analysis software offers an MTL correction accommodation, which is further detailed in the proceeding section.

Analyzing MTL Kinetic Results with Alto

In Alto's Nicosystem Analysis software suite, the user has the option to analyze kinetic results using a 1:1 Langmuir model with or without an MTL accommodation applied. With the MTL accommodation applied, an additional term, the mass transfer rate constant (k_t), is included in the fit model and reported in the analysis results. Alto's analysis software calculates the kinetics results with the MTL accommodation based off of the following differential equations, with terms defined in Table 1:

$$\frac{dR}{dt} = k_a \cdot C_s \cdot \left(R_{\max} - R\right) - k_d \cdot R$$

$$\frac{dC_s}{dt} = k_t \left(C_{bulk} - C_s\right) - k_a \cdot C_s \cdot \left(R_{\max} - R\right) + k_d \cdot R$$

Equation 2: *Differential equation model including MTL accommodation[1,3].*

Table 1: Kinetics analysis with MTL accommodation parameter definitions.

Parameter	Definition	Units
t	Time	s
R	Sensor binding response at time t, equivalent to the concentration of ligand-analyte complex formed (LA)	RU
R_{max}	Maximum sensor binding response, when analyte-ligand complex on the surface is saturated	RU
C_s	Concentration of analyte present at the surface ($A_{surface}$)	M
C_{bulk}	Concentration of analyte present in bulk (A_{bulk})	M
k_a	Association rate constant	M^{-1} s^{-1}
k_d	Dissociation rate constant	s^{-1}
k_t	Mass transfer rate constant	RU M^{-1} s^{-1}

MTL Model Performance

The MTL model implemented was verified by comparing the analysis output of the Alto software to simulated datasets as well as real datasets obtained with the Alto instrument with a system known to exhibit MTL. A wide range of simulated data produced from a third party commercial software was analyzed, focusing on fast association rate data where MTL has the largest impact to the results (k_a on the order of 1E+04 - 1E+06 M^{-1} s^{-1}). Table 2 provides a summary of the Alto analysis software results for several example simulated interactions in both multi-cycle kinetics (MCK) and single-cycle kinetics (SCK) assay styles. An example output of the data fitted from the Alto analysis software to the simulated data is provided in Figure 3.

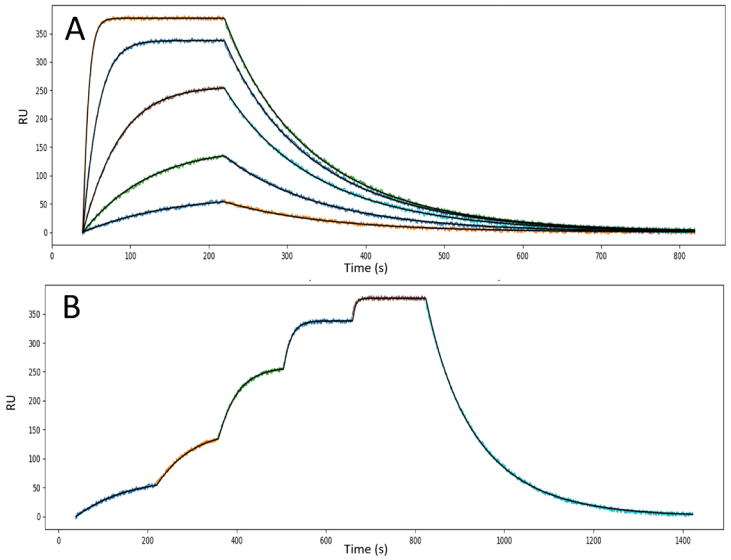

Figure 3: *Example fitting results of Alto analysis software (black lines) to simulated data (colored lines) for A) MCK and B) SCK assay styles for the following simulated data inputs: Rmax = 400 RU, k_a = 1.00E+4 M^{-1} s^{-1}, k_d = 1.00E-2 s^{-1}, k_t = 1.00E+7 RU M^{-1} s^{-1}.*

Table 2: Example results of analysis of MTL simulated data with Alto's analysis software.

	Simulated Data Inputs					Alto Analysis Software Result					
Style	Rmax (RU)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	k_t (RU M^{-1} s^{-1})	Rmax (RU)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	k_t (RU M^{-1} s^{-1})	Chi^2
MCK	400	1.00E+04	1.00E-02	1.00E-06	1.00E+07	400	1.02E+04	1.01E-02	9.96E-07	1.06E+07	6.24
	400	3.00E+04	3.00E-02	1.00E-06	1.00E+07	399	3.03E+04	3.02E-02	9.96E-07	1.07E+07	8.37
	400	1.00E+05	1.00E-02	1.00E-07	1.00E+07	401	9.85E+04	1.02E-02	1.03E-07	9.46E+06	6.74
	400	1.00E+05	1.00E-03	1.00E-08	1.00E+07	401	1.16E+05	1.15E-03	9.95E-09	8.59E+06	5.79
	200	1.00E+06	1.00E-02	1.00E-08	7.00E+07	197	8.42E+05	8.27E-03	9.82E-09	7.73E+07	2.77
SCK	400	1.00E+04	1.00E-02	1.00E-06	1.00E+07	400	1.06E+04	1.05E-02	9.90E-07	9.45E+06	6.98
	400	3.00E+04	3.00E-02	1.00E-06	1.00E+07	399	3.61E+04	3.56E-02	9.86E-07	8.88E+06	9.74
	200	1.00E+06	1.00E-02	1.00E-08	7.00E+07	200	8.73E+05	8.86E-03	1.01E-08	7.36E+07	3.80

The kinetics results of a real dataset were compared to commercially available TraceDrawer (Ridgeview Instruments AB) SPR analysis software as a control. Using the high-throughput nature of Alto, the interaction was measured with 30 repeats in the same experiment. Table 3 provides a summary of the fitted kinetic results, and example outputs of the fit data is provided in Figure 4. Alto analysis software results differed from the control software by less than 5% for all kinetic parameters (k_a, k_d, K_D) and resulted in a lower Chi^2 value, indicating an overall better fit to the data.

Table 3: Example results of analysis of MTL from data obtained on Alto.

Parameter	TraceDrawer Result	Alto Analysis Software Result
Rmax (RU)	379	382
k_a (M^{-1} s^{-1})	6.26E+05	6.02E+05
k_d (s^{-1})	3.50E-03	3.54E-03
K_D (M)	5.78E-09	6.07E-09
k_t (RU M^{-1} s^{-1})	1.04E+08	9.71E+07
Chi^2	34.2	32.5

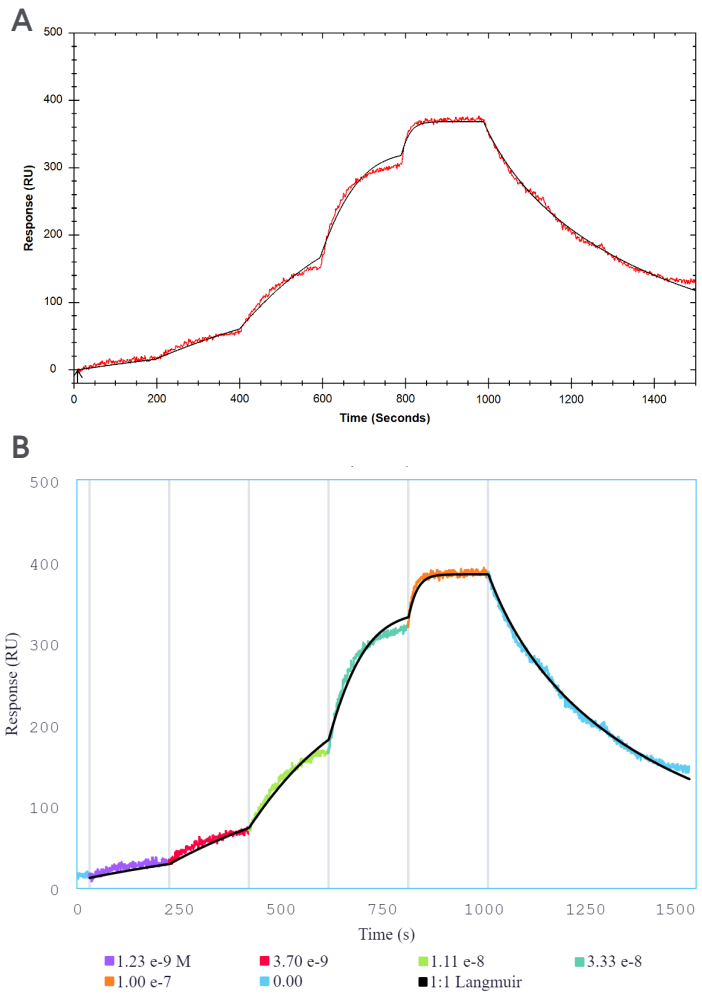

Figure 3: *Example fitting results to real data affected by MTL from A) TraceDrawer and B) Alto analysis software.*

Conclusion

Alto's Digital SPR technology platform is designed to minimize and accommodate for mass transport effects in SPR data. When compared to commercially available SPR analysis software, the Alto platform demonstrates equivalent accuracy for obtaining kinetic measurements for k_a, k_d, K_D and k_t using an MTL model. Alto's analysis software automatically calculates the kinetics results upon completion of a test, significantly decreasing the burden of analyzing large sets of data and accelerating time to discovery. The resulting data highlights Alto's ability to provide high-quality data while reducing time to answer, proving its ability to accelerate the characterization of various binding interactions.

References

1. Myszka DG, He X, Dembo M, Morton TA, Goldstein B. Extending the range of rate constants available from BIACORE: interpreting mass transport-influenced binding data. Biophys J. 1998 Aug;75(2):583-94. doi: 10.1016/S0006-3495(98)77549-6. PMID: 9675161; PMCID: PMC1299734.
2. Myszka DG, Morton TA, Doyle ML, Chaiken IM. Kinetic analysis of a protein antigen-antibody interaction limited by mass transport on an optical biosensor. Biophys Chem. 1997 Feb 28;64(1-3):127-37. doi: 10.1016/s0301-4622(96)02230-2. PMID: 9127943.
3. Marquart JA. Surface Plasmon Resonance and Biomolecular Interaction Analysis Theory and Practice. 2016;4(14):163-166.