

# Small Molecule and Drug Applications

## Summary

Label-free measurement of drug concentration and binding kinetics for its specific target interaction is demonstrated.

## Background

One of the challenges of label-free technologies which detect changes in mass near a surface, has been the ability to measure the direct attachment of small molecules including drugs, toxins and antibiotics among others to immobilized molecules (targets) attached to a substrate surface. With enhanced sensitivity for the detection of molecules in the range 150 Da – 500 Da, LFIRE™ permits a wider range of applications which include high throughput screening of drugs to thousands of specific targets including protein, antibodies and receptors.

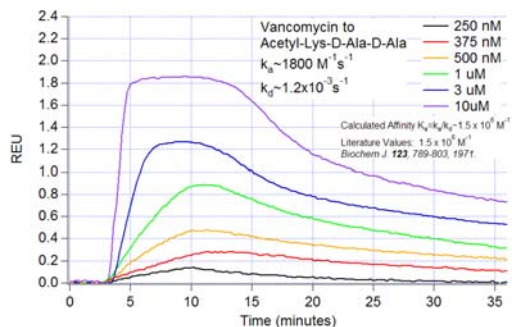
In this example, the data demonstrates the quantitative measurement of antibiotic drug interactions with a specific target peptide moiety. Antibiotics are becoming less effective in general due to fast mutation rates of numerous strains of bacteria. Vancomycin is an antibiotic which kills gram-positive bacteria by binding to cell wall precursors terminating in Acetyl-Lys-D-Ala-D-Ala (ALDADA), a short peptide sequence. Vancomycin is the drug of choice for treating Methicillin-Resistant Staphylococcus Aureus (MRSA) and the measurement of drug concentration and kinetics of its target interaction is relevant for determining drug efficacy.

## Method and Protocol

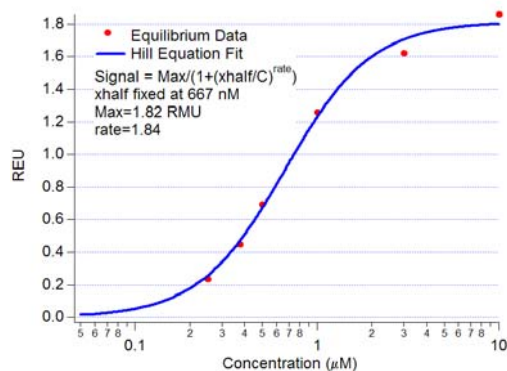
Microarray spots of Acetyl-Lys-D-Ala-D-Ala (MW: 330.4 Da) were spotted on our LFIRE™ slides and a flow cell configuration was used to measure kinetics. Vancomycin (MW: 1449.2 Da) was introduced into the multichannel flow cell at various concentrations from 250 nM to 10 μM and flow rates of 25 μl/min.

## Results

The graph below illustrates the binding kinetics of the Vancomycin attachment to ALDADA. Exponential curve fits to the increasing signal yielded values of the association constant,  $k_a \sim 1800 \text{ M}^{-1}\text{s}^{-1}$ .



The same was done to the dissociation part of the curve at 11 minutes when the Vancomycin was replaced by buffer in the flow cells. Exponential decay fits gave  $k_d \sim 1.2 \times 10^{-3} \text{ s}^{-1}$ . The affinity,  $K_a = k_a/k_d \sim 1.5 \times 10^6 \text{ M}^{-1}$ , value is consistent with earlier references in the literature (Nieto, M. and Perkins, H.R., Biochem. J., **123**, 789-803, 1971).



The second figure above is a dose response curve calculated from curve fits of the kinetic data. The maximum response is plotted as a function of concentration and the Hill Equation fit was good with only the max response and rate allowed to vary. Assay sensitivity is estimated to be 30 nM for Vancomycin based on the theoretical curve fit and detection limit of 2 standards of deviation above the noise.