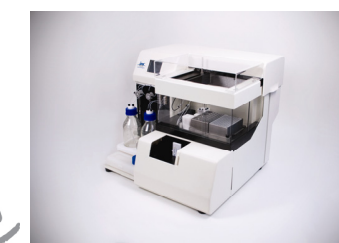


# Analysis of a Small Molecule Inhibitor using AvHiCap Chip

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## Introduction

SensIQ Pioneer is an automated SPR-based instrument for accurate kinetic and affinity analysis. Here we demonstrate a simple method using an AvHiCap chip for monitoring the kinetic binding of Furosemide to Carbonic anhydrase II. Carbonic anhydrase II was conjugated to NeutrAvidin for capture to the biotinylated AvHiCap surface.

## Experimental

The running buffer for all experiments was PBS buffer, pH 7.4, containing 10 mM NaH<sub>2</sub>PO<sub>4</sub> and 150 mM NaCl. The system temperature was 25°C and the sample racks were cooled to 15°C. Prior to use, the buffer was filtered with a 0.2 µm pore vacuum filter.

## CA II Conjugate Reaction

Carbonic anhydrase was dissolved into PBS buffer and incubated with a 5 molar excess of 2-iminothiolane for 15 min at 4°C. Maleimide activated NeutrAvidin powder was measured out so that the mole ratio was 3 moles CA II to 1 mole NeutrAvidin and the reaction was incubated at 4°C overnight.

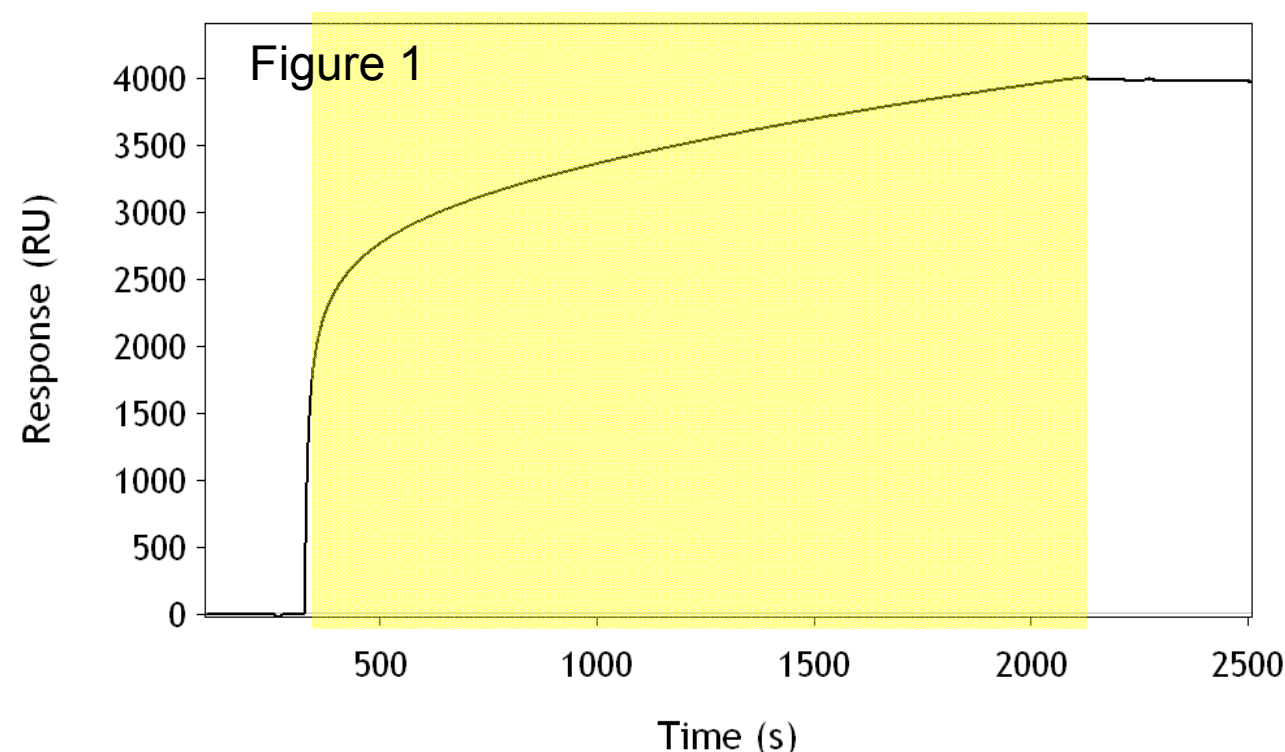
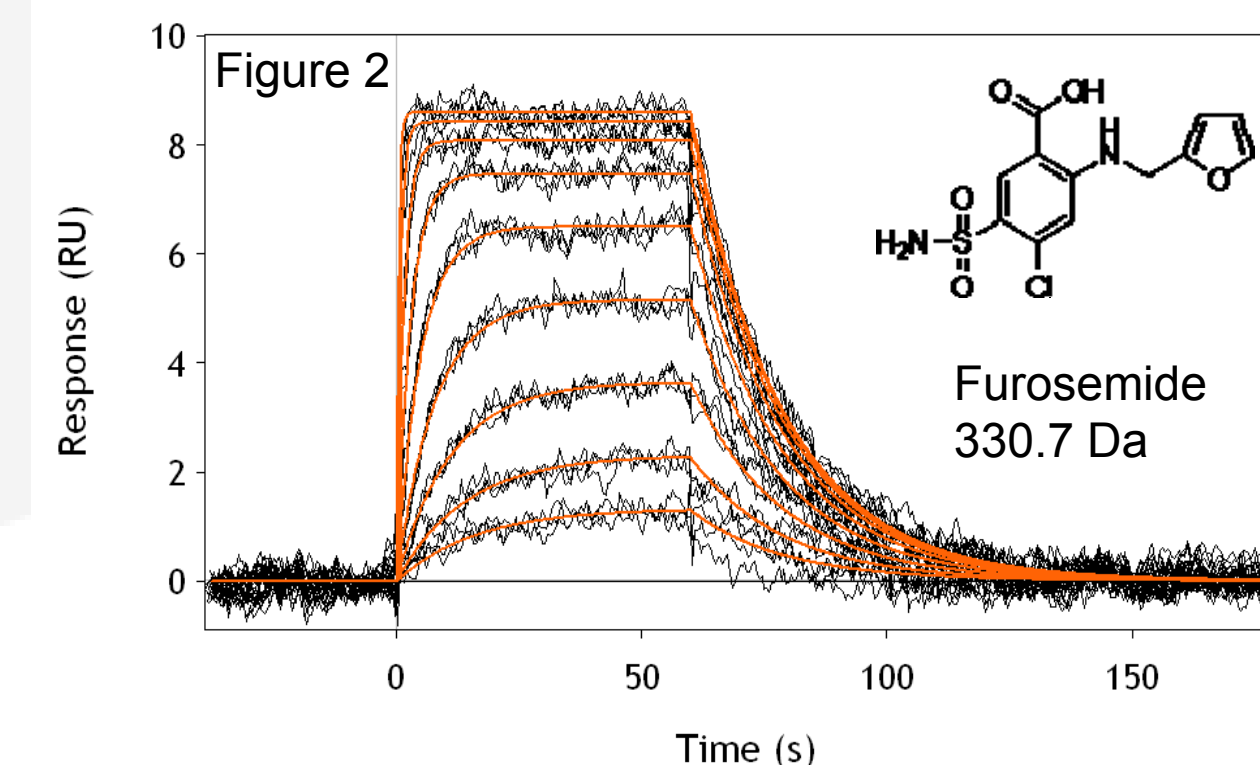


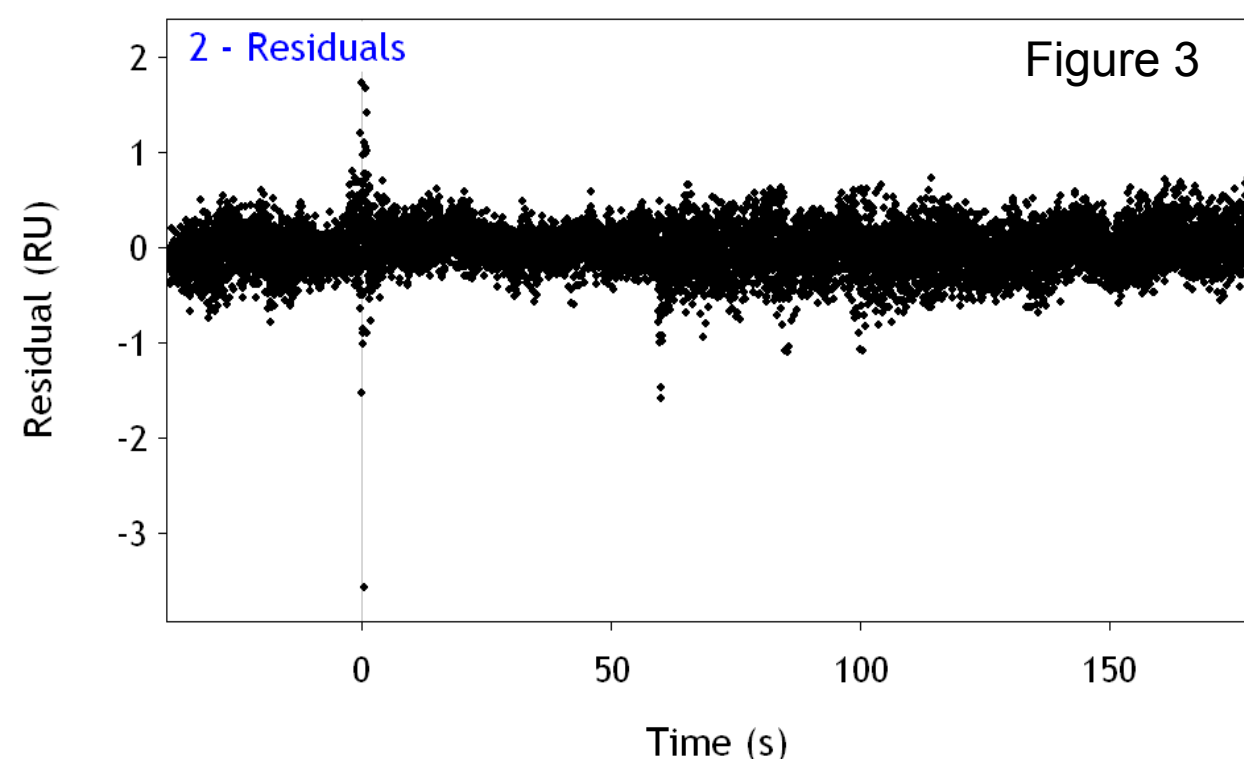
Figure 1 shows the response curve of immobilization of carbonic anhydrase conjugate to the AvHiCap chip. The highlighted region illustrates the sample injection. After the injection, all the captured conjugate is immobilized with negligible loss over time. No purification of the conjugate was performed but an equivalent of 1000 RU of CA II is estimated to be bound in this example.

## Kinetic Assay Protocol

Furosemide was dissolved in PBS at a stock concentration of 1 mM. An assay protocol was designed for the kinetic binding of furosemide to immobilized carbonic anhydrase. The protocol included a sample injection for 1 min followed by dissociation of 3 min. The buffer flow rate was constant at 50 µL/min and the system was purged in between cycles. SPR data was collected at a rate of 2 Hz. A serial doubling dilution set was prepared of furosemide from top concentration of 30 µM to 0.117 µM. Each analyte sample and a buffer blank were analyzed randomly in **triplicate**. The following figure shows the resulting data set of the kinetic assay.



The figure below shows the plot of residuals for each of the 27 response curves fit by the 1:1 kinetic model.



## Kinetic Interaction Analysis

A kinetic 1:1 model was fit to the overlaid and double referenced responses. The rate constants of association and dissociation ( $k_a$  &  $k_d$ ) and the  $R_{max}$  were fit globally. The table below presents the calculated kinetic parameters.

$k_a$	$8.09 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$
$k_d$	$0.0535 \text{ s}^{-1}$
$K_D$	661 nM
$R_{max}$	8.78 RU
Res SD	0.24 RU

The fitted kinetic constants above are within 1.2 fold of those previously published<sup>1</sup>.

## Conclusion

The goodness of fit was measured as the average residual standard deviation of the regression from the actual curves. A value of 0.24 RU was observed for Figure 2 and Figure 3 shows random distribution of residuals. This confirms that the data does follow the pseudo-first-order binding interaction model. Also the data performed in triplicate are clearly super-imposed due to the high sampling reproducibility of the SensIQ Pioneer system. We conclude that the capture of NeutrAvidin conjugated enzyme to a biotinylated chip surface is a simple method for the kinetic analysis of inhibitor binding.