Small molecule interactions

Instrument for Kinetic Analysis

SUMMARY

The inQuiQ® instrument uses a unique optical technology with ring resonators to achieve precise molecular binding measurements. This facilitates real-time, label-free analysis of biomolecular interactions. This white paper displays capability of the instrument in kinetic analysis of biomolecule interactions using Carbonic Anhydrase II (CAII), Acetazolamide and Sulpiride interaction as a model system.

INTRODUCTION

Delta Life Science leverages innovative optical technology featuring ring-resonators to accurately measure and characterize molecular interactions on a reusable sensor chip. The compact and cost-effective inQuiQ® instrument is designed for a wide range of applications, including biophysics, bioanalysis, physical organic chemistry, and medicinal chemistry research. This instrument analyses biomolecular interactions in real-time without the need for labelling.

In this white paper, we show the sensitivity of the instrument in analysing the kinetic interactions between a protein and therapeutic inhibitors. For this purpose, we use the well-known model system with Carbonic Anhydrase II (CAII) as ligand and Acetazolamide and Sulpiride as analytes to explore the protein-ligand interactions involving small molecules.

MATERIALS AND EQUIPMENT

- InQuiQ® instrument
- InQuiQ® biosensor with a hydrogel coated optical chip
- Amine Coupling Kit
- Ligand: Carbonic Anhydrase II
- Analyte: Acetazolamide and Sulpiride
- Running Buffer: 10mM M NaH_2PO_4, 1.8 mM $NaHPO_4$, 137 mM NaCl, 2.7 mM KCl + 0.05% Tween-20, pH 7.4
- Data Analysis: TraceDrawer Software 1.9.2 BioPhysics (Ridgeview Instruments AB)

RESULTS AND DISCUSSION

In this study, we used a medium density polycarboxylated hydrogel coated optical chip with sixteen detection spots. We immobilized one flow channel with CA II (29 kDa) by means of amine coupling and used another flow channel unchanged as reference. 13000 RU of CA II was immobilized and concentration series of the two CA II inhibitors; Acetazolamide (222 Da) and Sulpiride (341 Da) were injected. Replicates of reference subtracted binding curves are shown below.

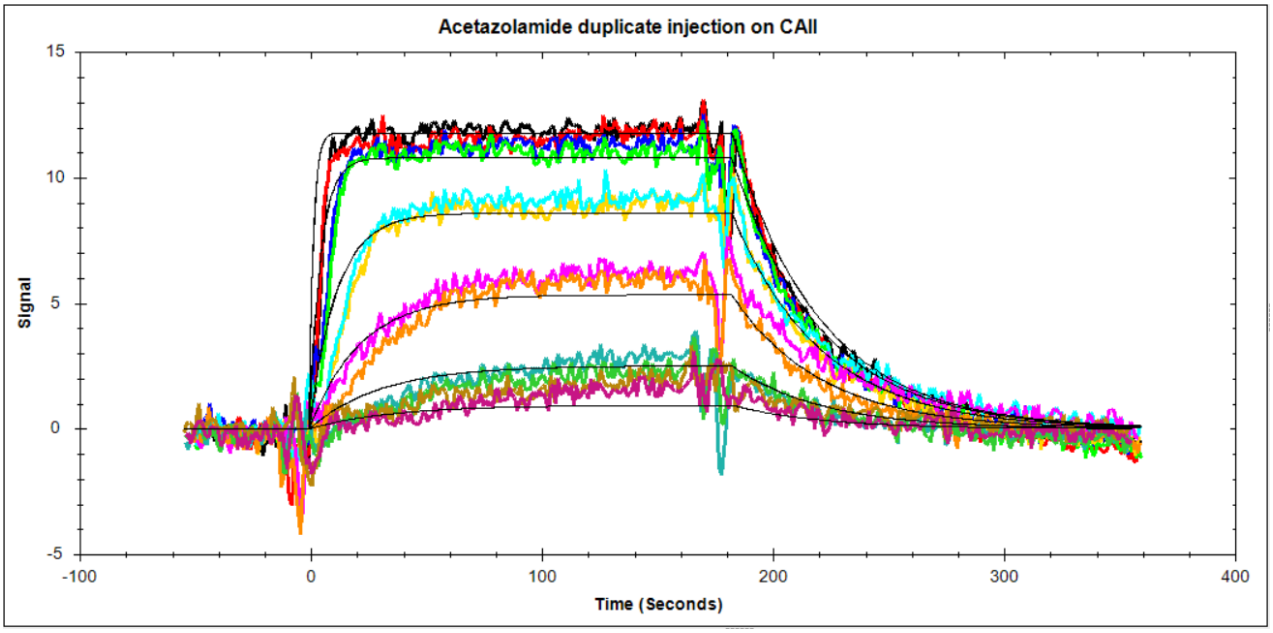

Figure 1: Binding curves of Acetazolamide to CA II. The concentrations were 2, 6.2, 18.5, 55.5, 167 and 500 nM. The association and dissociation time were both 180 seconds at a flow rate of 100 µL/min.

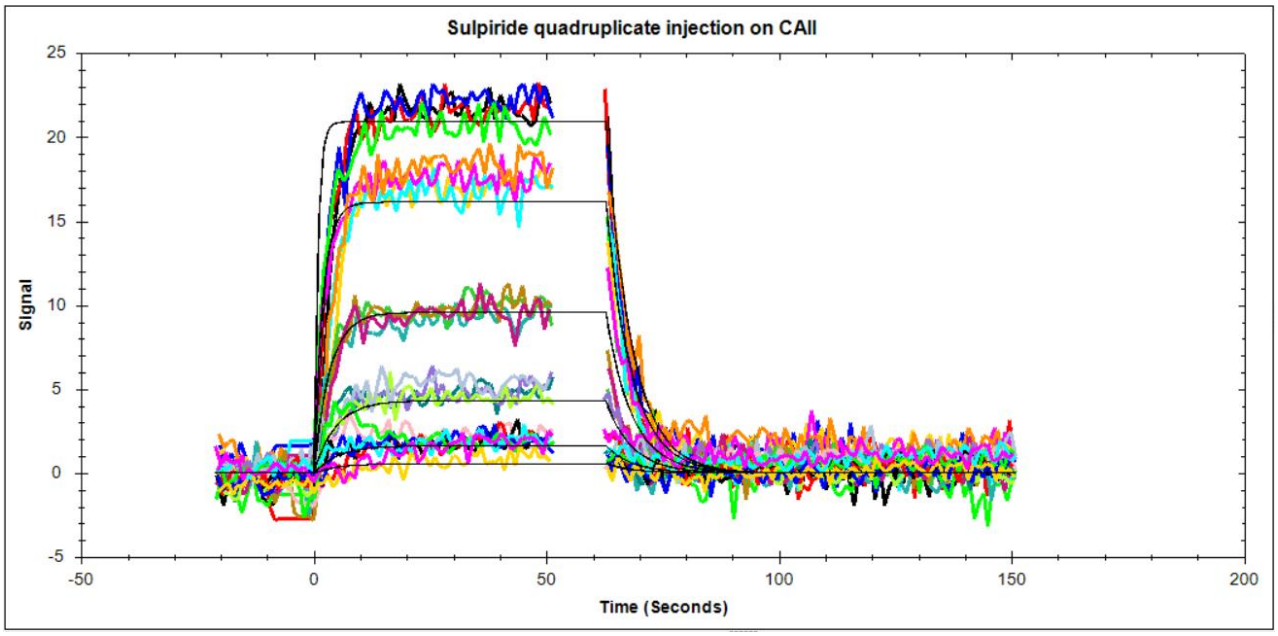

Figure 2: Binding curves of Sulpiride to CA II. The used concentrations were 4.1, 12.4, 37, 111, 333 and 1000 in tripl. The association was 60 seconds, and dissociation was 120 seconds at a flow rate of 100 µL/min.

The results were analysed by fitting a 1:1 binding model and resulting values are shown below.

Table 1: Summary of the kinetic fitting results

	Mw	k_a	k_d	K_D	Rmax	Chi^2
	(Da)	**($M^{-1}s^{-1}$)**	**(S^{-1})**	**(M)**	**(RU)**	
Acetazolamide	222.2	1.07e6	2.60e-2	2.44e-8	12	0.54
Sulpiride	341.1	1.04e3	1.81e-1	1.74e-4	25	1.62

The kinetic constants are shown in **Table 1** with the corresponding fits illustrated through solid lines in the figures above. The kinetic values are similar to those reported in comparable studies [1].

CONCLUSIONS

The study shows that the instrument can adequately measure the interaction between CA II (29 KDa) and the compounds Acetazolamide (222 Da) and Sulpiride (341 Da). Although both inhibitors displayed a 1000-fold difference in association rate constant (or on-rate) and even stronger difference in affinity, the interactions could easily be measured with high reproducibility and with resulting values that are highly similar to those reported in comparable studies. This study shows that we can match the performance of more elaborate and expensive instruments.

FUNDING STATEMENT

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References:

[1] Myszka, David G. "Analysis of small-molecule interactions using Biacore S51 technology." Analytical biochemistry 329.2 (2004): 316-323.