FEATURE HIGHLIGHT: *Sense in Complex Samples: Solvents & Detergents*

Agile® R100

Complex samples finally have a simple solution.

Agile R100 is a label-free personal assay system that lets you measure in buffers with high concentrations of solvents and detergents. The proprietary Field Effect Biosensing (FEB) technology on which Agile R100 is based is an electrical technique that is unaffected by the optical properties of complex samples, enabling easy, efficient kinetic binding analysis without time-consuming and error-prone solvent correction.

PAPER SUMMARY

- DMSO is often needed to keep high concentrations of small molecule compounds soluble during early stage drug discovery. This is a problem for common optical kinetic binding platforms, as **DMSO negatively impacts optical properties.**
- Agile R100 leverages an orthogonal technology called Field Effect Biosensing (FEB), an electrical technique that is unaffected by changes in optical properties. The platform **easily senses in optically challenging samples** such as buffer containing 10% DMSO.
- In this application note, we share Agile R100 kinetic binding data (kon, koff, and KD) from the interaction of 3 small molecule compounds dissolved in 10% DMSO binding with their respective targets - **2 GPCRs solubilized in additional detergents** and a cytokine protein.

OVERVIEW

The ability to test compounds in high concentrations of DMSO is crucial during early stage drug discovery. However, with many common optical kinetic binding platforms, **DMSO causes a high level of background noise**, making it difficult to accurately characterize compound interactions. In contrast, FEB is an electrical, orthogonal technique that lets you easily gain accurate real-time data in optically challenging samples such as buffer with high concentrations of DMSO. In this application note, we present kinetic binding data for the interactions of 3 small molecule compounds dissolved in 10% DMSO and their respective targets - 2 GPCRs and 1 cytokine protein. In addition, we show that Agile R100 is also unaffected by buffer additives used to stabilize GPCRs, such as detergents. Agile R100 is a **versatile sensing platform capable of testing high concentrations of compounds in high concentrations of solvents and detergents** for early stage drug discovery.

MATERIALS AND METHODS

Biosensor Chip Immobilization

A_{2A} and CR GPCRs

Both His-tagged GPCR targets were immobilized using NTA biosensor chips and Agile Plus software protocol. The A_{2A} experiments were performed with Agile R100 in an ice bath maintaining 5 to 10°C, and the chemokine receptor (CR) experiments were performed at room temperature. To immobilize the GPCRs, the NTA biosensor chips were activated with NiCl2. Target immobilization was achieved using 300 nM A_{2A} or 15 µg/mL CR in immobilization buffer (50 mM HEPES pH 7.4, 500 mM NaCl, 0.025% DDM, 0.005% CHS for A_{2A} and 1X PBS pH 7.4, 0.05% DDM, 0.01% CHS for CR). Both targets were incubated for 60 minutes.

TNFα Protein

TNFα was immobilized using COOH biosensor chips and Agile Plus software protocol. Target immobilization was achieved at room temperature using 10.2 nM TNFα in immobilization buffer (1X PBS pH 7.4) incubated on the biosensor chip for 15 minutes. Quench 1 (PEG-amine) and Quench 2 (ethanolamine) were added to block exposed graphene to nonspecific binding and deactivate any remaining surface binding sites.

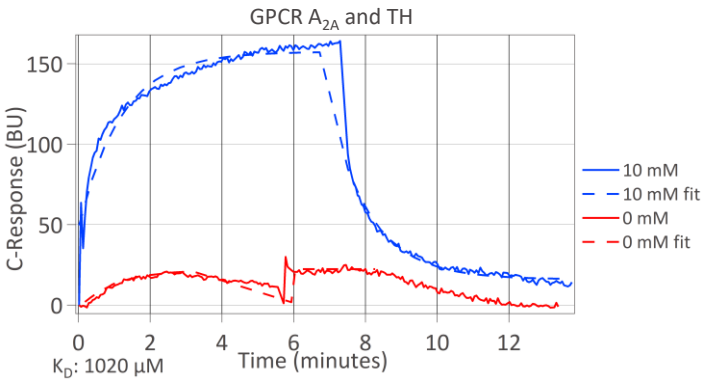
Biosensor Chip Measurement

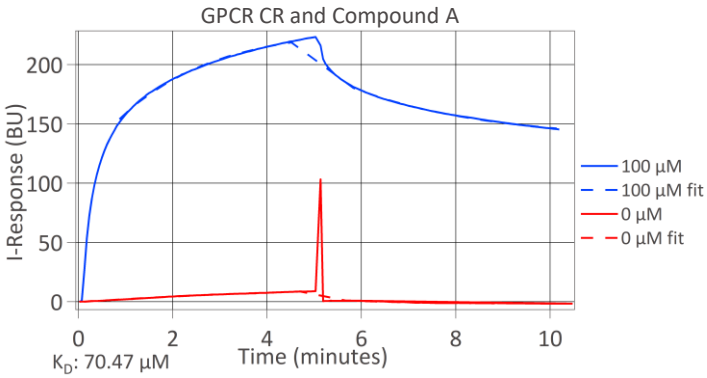
The small molecules interacting with the A_{2A}, CR, and TNFα are theophylline (TH), Compound A, and SPD304, respectively. The assay buffer for each interaction was the respective immobilization buffer with 10% DMSO. A zero-concentration measurement (i.e. fresh assay buffer) was taken. The A_{2A}, CR, and TNFα biosensor chips were exposed to the respective small molecule at 10 mM TH, 100 µM Compound A, or 30 µM SPD304 in the respective assay buffer for 5 minutes to measure association. Fresh assay buffer was then added to initiate dissociation for 5 minutes.

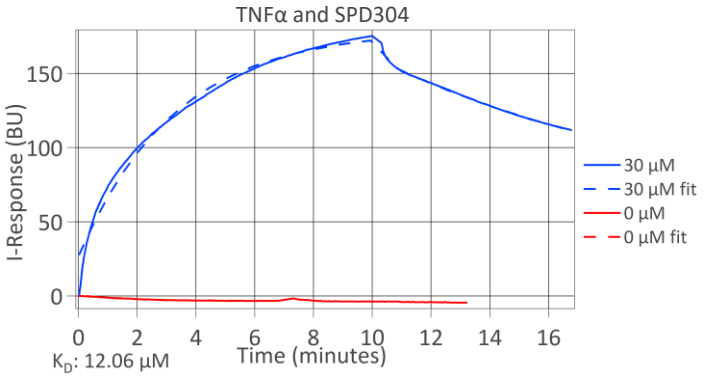
RESULTS AND DISCUSSION

The sensorgrams to the right show interactions of compounds in 10% DMSO. The first figure depicts 10mM TH added to target GPCR A_{2A}. The second figure shows 100 µM Compound A added to immobilized GPCR CR, and the third figure shows 30 µM SPD304 added to target protein TNFα. K_D values range from low micromolar to low millimolar.

All interactions resulted in large sensor responses (~150 to 250 BU) that were significantly greater than their corresponding zero-concentration curves, showing that 10% DMSO causes negligible noise on Agile R100. The range of KD values and concentrations depicted show that the platform can detect lower concentrations of compound as affinity improves. Agile R100 is a single personal assay platform to characterize a wide range of crucial drug targets such as GPCRs and cytokine proteins, in buffers containing solvents and detergents, making it a highly versatile label-free kinetic binding tool.







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