FEATURE HIGHLIGHT: *Replicable Characterization of Functional GPCRs*

Details taken from Application Note: **Reproducible Characterization of GPCR and Small Molecule Compound Interactions Using Agile R100.** Read the full App Note.

Agile™ R100

Difficult targets, *made easy.*

Agile R100 is a label-free personal assay system that makes finicky GPCRs easy to study. Need to use detergents to solubilize your GPCR, or low temperatures to stabilize it? Having difficulty expressing and purifying functional membrane protein, leaving you just a tiny bit of sample? Not a problem. Agile R100 uses a proprietary electrical technique that's revolutionizing GPCR characterization capabilities, giving you the data you need for this critical drug target.

PAPER SUMMARY

- GPCRs are crucial targets for therapeutic intervention in areas such as cancer, immune and inflammatory disorders, and neurological and metabolic diseases. However, this target class is notoriously difficult to characterize due to the instability of the solubilized protein. Prior kinetic binding techniques have substantial roadblocks in studying GPCRs, but in this application note, we present Agile R100's success in analyzing these difficult targets.

- Agile R100 advantages that enable successful GPCR characterization include: the **ability to sense in complex samples** containing solubilizing detergents, a non-microfluidic format that allows the target to be applied directly to the biosensor surface, **reducing protein degradation, temperature versatility** to keep GPCRs within their required stability range, and **low target density requirements**, enabling the study of even a tiny amount of purified GPCR.

- Three example experiments are presented: The first 2 show that a **single-concentration kinetics measurement** performed with Agile R100 **is highly consistent with kinetics found by running a full dose-response curve,** letting you reduce the total number of measurements needed to gain accurate binding data, cutting material costs and experiment time.

- The last example displays the temperature versatility of Agile R100. The system is placed on a bed of ice to keep the temperature-sensitive GPCR target stable throughout the experiment. The **temperature is monitored with built-in thermometers** included in standard Agile biosensor chips, and **displayed in real-time through Agile Plus software**.

MATERIALS AND METHODS

Biosensor Chip Immobilization

Two GPCR His-tagged targets, full-length chemokine receptor (CR) and A_{2A}, were immobilized using Agile NTA biosensor chips and Agile Plus software protocol. The CR experiments were performed at room temperature, and the A_{2A} experiment was performed with Agile R100 on a bed of ice. To immobilize the GPCRs, the NTA biosensor chips were activated with $NiCl_2$. Target immobilization was achieved using 300 nM CR or 300 nM A_{2A} in immobilization buffer (1X PBS pH 7.4, 0.05% DDM, 0.01% CHS for CR and 50 mM HEPES pH 7.4, 0.025% DDM, 0.005% CHS for A_{2A}). Both targets were incubated for 60 minutes.

Biosensor Chip Measurement

The small molecules interacting with GPCRs CR and A_{2A} were Compound A and theophylline (TH), respectively. The assay buffer for each interaction was the respective immobilization buffer with 2% and 0.1% DMSO, respectively. A zero-concentration measurement (i.e. fresh assay buffer) was taken. The CR biosensor chips were first exposed to 100 µM Compound A in assay buffer for a single-concentration kinetics measurement (n = 3). Dose-response curve measurements were then performed with 0.3 to 2000 µM Compound A (n = 2 or 3 for each concentration) in assay buffer in 3-fold dilutions. The A_{2A} biosensor chips were exposed to 100 µM TH in assay buffer for a single-concentration kinetics measurement (n =2). Association was performed for 5 minutes, and fresh assay buffer was then added to initiate dissociation for 5 minutes.

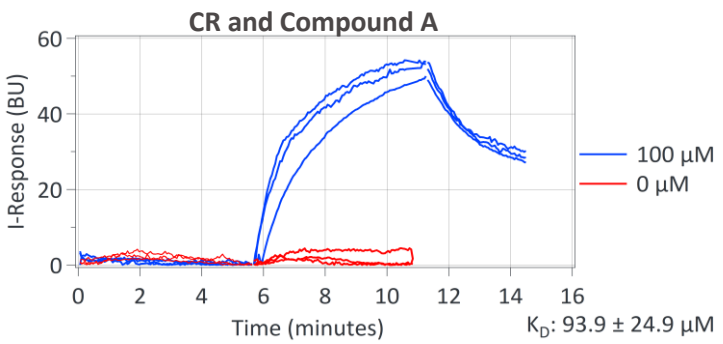
RESULTS AND DISCUSSION

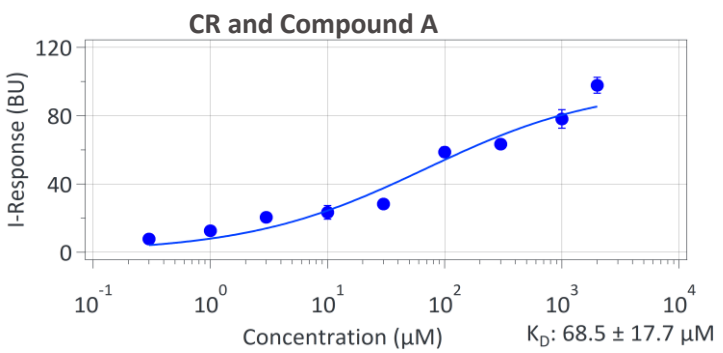
The 1st figure to the right shows the response of each independent replicate of the single-concentration kinetics measurement of 100 µM Compound A binding to GPCR CR. The stacked sensorgrams display low variability. The K_D value from this methodology was 93.9 ± 24.9 µM as calculated by Agile Plus software.

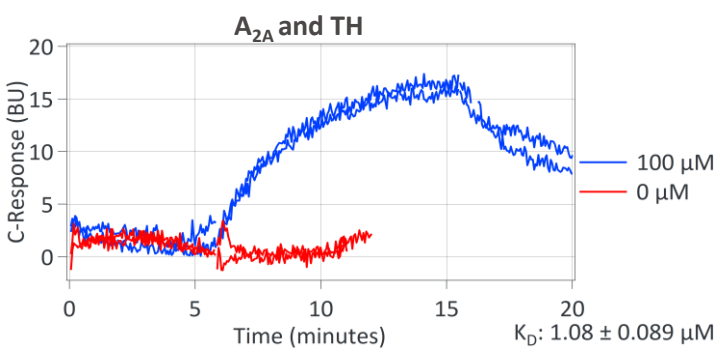
The GPCR CR and Compound A interaction was further characterized using a full dose-response curve, shown in the 2nd figure on the right. The K_D from this methodology was 68.5 ± 17.7 µM, **within a factor of 1.4** from the K_D determined using single-concentration kinetics. With **single-concentration kinetics highly indicative of full dose-response curve results,** researchers can reduce the number of total measurements to save time and resources.

Despite the temperature-sensitivity of GPCR A_{2A}, we were able to perform the TH measurement at 5 to 10°C by placing Agile R100 on a bed of ice. The stacked sensorgrams for the duplicate measurements are shown in the 3rd figure below, and the K_D was 1.08 ± 0.089 µM.

The system's small size and electrical sensing mechanism (that is unaffected by temperature) enables experiments to be performed on a bed of ice to preserve the structural integrity of temperature-sensitive targets. ***Agile R100 delivers advanced quantitative kinetic characterization that is not impeded by detergents and solvents, fluid-flow stress, temperature sensitivity, and low target density*.** These advancements open new doors by providing easier real-time, label-free kinetic characterization of GPCRs.







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