PUBLICATION HIGHLIGHT: *Characterizing Undruggable Targets*

Details taken from: Jacob, N., et al. **Synthetic Molecules for Disruption of the MYC Protein-Protein Interface**. *Bioorganic & Medicinal Chemistry*. 2018. doi:10.1016/j.bmc.2018.07.019. Read the paper.

Agile® R100

Reduce the noise in your interactions.

Studying "undruggable" targets just got easier. Introducing Agile R100, a novel optics-free assay platform that uses an **electrical sensing technique** to provide sensitive measurements of small molecules and proteins. The **biosensor's graphene surface is nonreactive with biomolecules**, **vastly reducing nonspecific binding**. This means you can achieve higher signal-to-noise ratios and characterize challenging interactions where other methods fail.

PAPER SUMMARY

- Researchers from **Dr. Kim Janda's lab at the Scripps Research Institute** developed a new series of compounds with the potential to down-regulate the transcriptional activities of MYC. Agile R100, described as a "field-effect transistor (Bio-FET) platform," was used to characterize MYC oncoprotein and small molecule compound interactions.
- In the study, Agile R100's graphene surface was found to be significantly less prone to nonspecific interactions than surface plasmon resonance (SPR). The publication lists additional advantages of Agile R100: **A much lower limit of detection than SPR or isothermal calorimetry (ITC)**, and a lower required target density, which reduced the amount of noise caused by aggregates on the chip surface.
- **Binding data from Agile R100 correlated well with cell-based assay data** using chicken embryo fibroblasts (CEF), as opposed to SPR data, which did not.

OVERVIEW

MYC oncoprotein plays a recognized role in the initiation and growth of cancer in humans and is a promising target for anti-cancer drugs. However, an **intrinsically disordered protein**, MYC can bind nonspecifically to and aggregate on dextran surfaces, making small molecule interaction studies for this target difficult to perform on optics-based platforms such as SPR.

In this paper, Agile R100 is incorporated as part of a blind structure-activity-relationship (SAR) study. The system successfully measures the affinity of small molecule interactions with MYC in a label-free manner, while SPR failed.

MATERIALS AND EQUIPMENT

- Agile R100 platform
- Laptop with Agile Plus software
- NTA Agile Biosensor Chips
- Biacore 3000 (SPR measurements)
- Chicken Embryo Fibroblasts (cell-based measurements)
- His-tagged MYC and MYC-MAX by SRI
- MES buffer with 3% DMSO, pH 6.0.

METHODS AND PROCEDURE

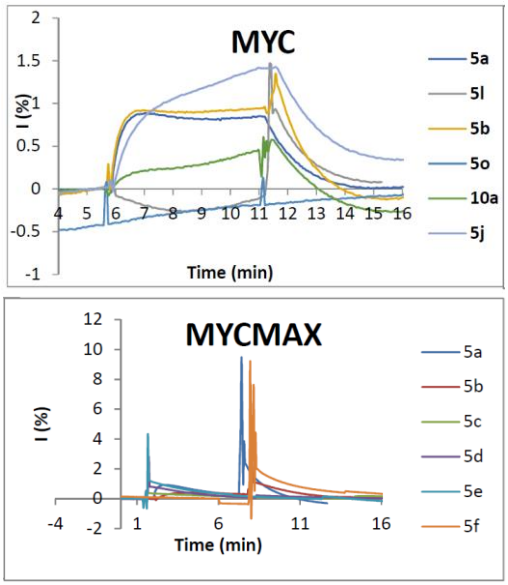
Binding experiments were conducted using Agile R100 at ambient temperature. Biosensor chips were functionalized with Ni-NTA according to standard protocol prescribed by Nanomedical Diagnostics. Surface-bound Ni-NTA sites were then bound to His-tagged protein by 15-minute incubation with ~100 nM protein in 50 mM MES (pH 6.0). Ni-NTA functionalized chips were regenerated via treatment with 250 mM imidazole for 30 mins, followed by extensive washing in MES, then reintroduction of His-tagged protein.

Test compounds were dissolved to desired concentration in MES (pH 6.0) buffer with 3% DMSO. Protein-bound chips were calibrated and rinsed in MES buffer with 3% DMSO. For relative binding studies, 6-8 test compounds were analyzed at 10 µM on a single chip serially, along with one compound common to every chip (compound 5a). In between compounds, biosensor chips were rinsed and re-calibrated. The equilibrium sensor response for each of the test compounds was compared to that of 5a, and 5a was used as a calibrator for any chip-to-chip variation. Compounds were tested a minimum of 3 times each, using different biosensor chips.

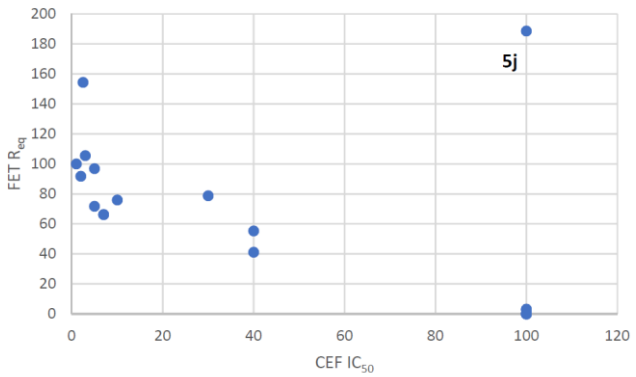
SPR measurements were performed using a Biacore 3000. To prevent aggregation of MYC monomer on the SPR chip surface, additional stabilization of MYC was required. MYC-MAX dimers are known to bind to a particular sequence of double-stranded DNA. Using biotin labeled DNA, a structure was built on a streptavidin-functionalized SPR chip to immobilize MYC-MAX. This approach did not correlate well with cell-based assay data and required additional development and process time.

RESULTS AND DISCUSSION

The figures below display the Agile R100 sensor response for binding of the set of compounds to MYC protein and the control (MYC-MAX) protein.



Calibrated Agile R100 data and cell-based assay data from chicken embryo fibroblasts (CEF) are compared in the figure below. To gather IC50s from CEF, oncogenetic ATG-MYC virus was applied to CEF cells and compounds 5a through 5j were measured. Calibrated Agile R100 sensor responses were calculated by comparing the sensor response for a given compound to the sensor response for compound "5a" on the same chip. If the response of the tested compound equaled that of compound 5a, a value of "100" was assigned. The below figure shows that **data from Agile R100 and cell-based assay data from CEF correlated well.**



Pearson correlation coefficient = -0.68

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