DEVELOPMENT OF A MICROFLUIDIC INSTRUMENT FOR PERFORMING ENZYME KINETIC ASSAYS

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ABSTRACT

This paper describes the first version of an integrated microfluidic instrument for performing enzyme kinetic assays for drug discovery. The instrument performs IC50 measurements with good accuracy using 1/100th the reagent per assay compared to conventional plate-based methods. Importantly, the data quality obtained from the microfluidic system is significantly better than data from microtiter plates, resulting in tight fits to kinetic models, improving confidence in the result.

KEYWORDS: High throughput, drug discovery, enzyme kinetics, microfluidic

INTRODUCTION

Enzyme kinetic assays are used extensively in drug discovery to screen for molecules that interact with enzymes. Such assays are used in primary screening (lower resolution screens used to identify molecules of interest) and in secondary screening (higher resolution screens used to measure each molecule's potency and mechanism of interaction with the enzyme). The most commonly used measure of a compound's potency is its IC50 - the concentration at which the compound reduces the activity of the enzyme by 50%. A typical IC50 assay is conducted in a microtiter plate by testing the enzyme against twelve discrete concentrations of the compound, spanning three orders of magnitude. Noise in the data, combined with the small number of data points,

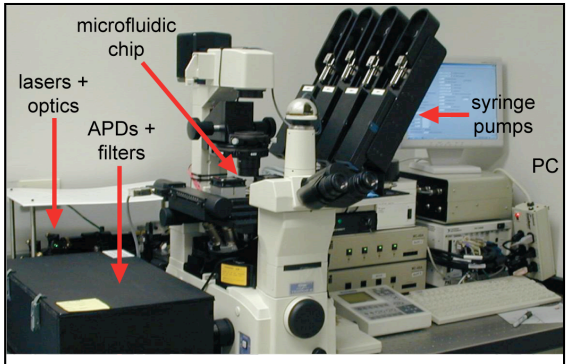

Figure 1. An instrument delivered to an internal GSK customer.

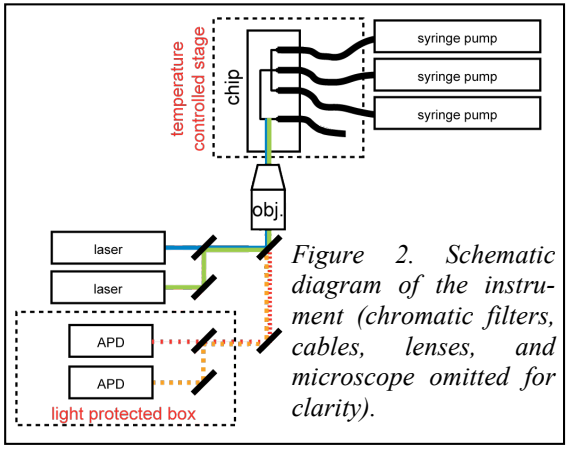

Figure 2. Schematic diagram of the instrument (chromatic filters, cables, lenses, and microscope omitted for clarity).

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frequently makes precise estimates of the IC50 difficult.

EXPERIMENTAL

The system we developed (Fig 1&2) consists of: (1) rigid polymeric microfluidic chips fabricated by laser ablation and hot embossing (Fig 3), (2) Planar interconnects with essentially zero dead volumes, and extremely small (<10fL) unswept volumes (Fig 3) [1], (3) custom-built positive displacement syringe pumps capable of delivering steady flow rates of nanoliters per minute (Fig 4) [2], (4) temperature controlled chambers for both the syringes and chip, (5) laser induced excitation for simultaneous detection of multiple fluorophores, and (6) a custom-designed software package capable of controlling all aspects of the instrument as well as real-time data processing and analysis. Rather than testing a compound at discrete concentrations, the instrument produces continuous concentration gradients that span approximately three orders of magnitude (Fig 4).

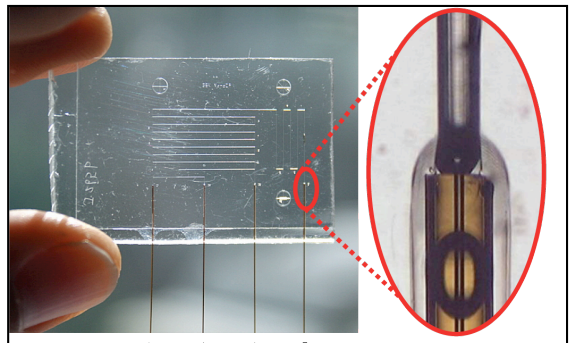

Figure 3. Microfluidic chip and magnified image of fluidic interconnect.

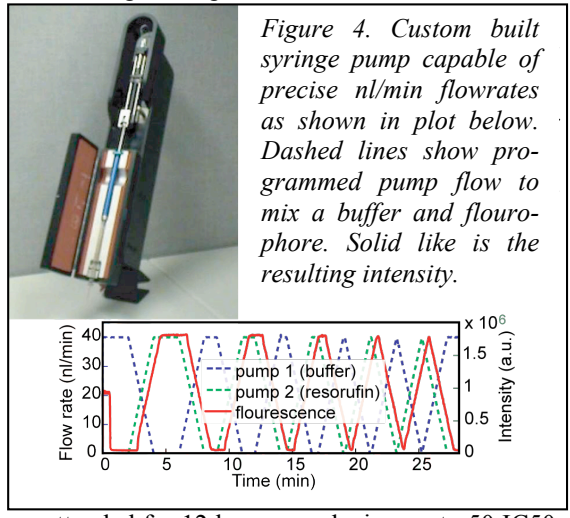

Figure 4. Custom built syringe pump capable of precise nl/min flowrates as shown in plot below. Dashed lines show programmed pump flow to mix a buffer and flourophore. Solid like is the resulting intensity.

RESULTS AND DISCUSSION

Throughput of this new instrument is low, relative to large robotic, automated plate-based systems. The instrument runs three to four IC50s per hour, compared to several thousand per day with microtiter plates using industrial automation. However, the new instrument can run unattended for 12 hours, producing up to 50 IC50s per day. This matches the throughput needed for many secondary screening programs while requiring little operator input. Reduced reagent consumption and greatly improved data quality are both important in secondary screening programs especially when models of the results require precise data. Figure 5 compares results of an inhibition assay (inhibitor = oxalate, enzyme = lactate dehydrogenase, LDH) from a standard plate-based assay and from the microfluidic instrument. A significant increase in the number of data points was achieved while using 1/100th the rea-

gent. Figure 6 compares IC50 results from a plate-based assay and from the instrument. Each large bar represents the results from a single assay. The variation from assay to assay, as measured by the standard deviation, is similar for the plate-based (s.d. = 1.9), and microfluidic (s.d. = 3.5) instruments. However, the fit of the IC50 model to the experimental data is much tighter on the microfluidic instrument than on the plate-based assay. This is seen both in the smaller error bar for each measurement in Figure 6 and, in aggregate, by the smaller average standard error associated with the microfluidic instrument (s.e. = 0.42 for microfluidic instrument vs. s.e. = 2.1 for plate-based instrument).

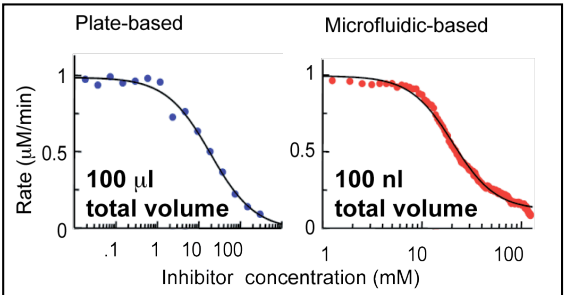

Figure 5. Lactase dehydrogenase inhibition assay. Microfluidic instrument shows obvious increase in the number of data points compared to micro-titer plate-based assay.

Worth noting is the fact that a typical plate-based assay of 384 compounds can require up to 27 plates. A mother plate holds high concentratinons of each compound to be screened. Twelve dilutions of each compound are stamped into 13 compound plates for the initial screen. An additional 13 plates are made for "rework". In contrast, the microfluidic system requires just a single plate for all 384 compounds, since dilutions are not required. Rework is also avoided, since an automated microfluidic system can detect a bad set of data, and re-run the compound "on-the-fly".

CONCLUSIONS

An integrated instrument for performing high throughput enzyme kinetics for drug discovery has been demonstrated. The instrument is capable of generating significantly higher quality data than that obtained from conventional plate-based approaches while using 100 fold lower reagent volumes. A commercial version of this instrument is being produced.

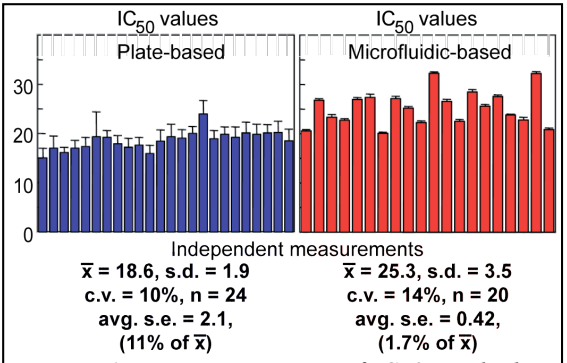

Figure 6. Direct comparison of IC50 results between micro-titer plate and microfluidic instrument. The average standard error (s.e.) suggest a much better fit to the experimental data.

REFERENCES

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