High-Throughput Lead Discovery with Agilent RapidFire/MS Systems: Analysis of UDP-3-O-(R-3-hydroxymyristoyl)-N-Acetylglucosamine Deacetylase (LpxC)

Application Note

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Introduction

The RapidFire High-throughput Mass Spectrometry System provides drug discovery researchers with mass spectrometry-based, high-throughput screening solutions for targets that have proven challenging to screen using conventional approaches. These intractable targets have substrates and products that are either too small to label or undergo modifications that are difficult to detect. RapidFire technology provides the most relevant data, with label-free native analyte detection. Mass spectrometry (MS) detection eliminates the need for cumbersome labeling methods and surrogate substrates that may not accurately represent the native reaction. RapidFire technology enables traditionally low-throughput, intractable assays to be converted into high-throughput assays processed at speeds approaching plate-based optical methods. In this application note, a UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase (LpxC) assay is used to illustrate the power of Agilent RapidFire/MS Systems for screening intractable targets.

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Using RapidFire High-throughput Mass Spectrometry to Analyze LpxC

LpxC, an essential enzyme for lipid A biosynthesis in gram-negative bacteria, represents an attractive target for therapeutics. However, antibacterial drug discovery efforts focused on inhibitors of LpxC have found that the enzyme assay is not suitable for high-throughput screening using conventional technologies due to cumbersome labeling methods and false positive results. The LpxC reaction results in the removal of the N-acetyl group of the substrate to yield a free amine on the deacetylated product (Figure 1). This free amine is difficult to detect in the native product, but can be distinguished when LpxC catalyzes the conversion of a surrogate substrate. The surrogate exhibits substantially different reaction kinetics from the native substrate, further complicating the analysis. The K_m for the native UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine is 2.0 mM, while the K_m for the surrogate substrate, UDP-3-O-(N-hexyl-propionamide)-N-acetylglucosamine, is 367 mM.[1]

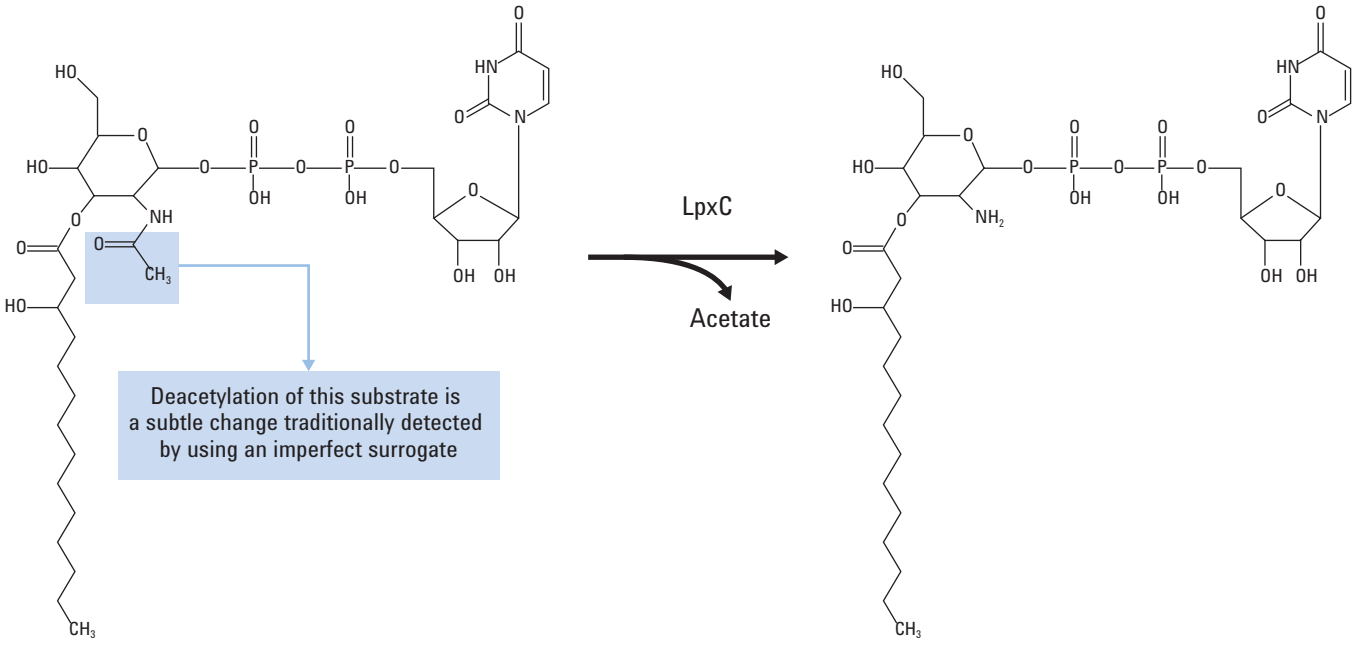

Figure 1. LpxC assay reaction scheme.

The RapidFire/MS method offers a fast, sensitive, and reliable alternative to the typical LpxC assay. It employs mass spectrometry to directly detect product with high sensitivity, eliminating the need for the surrogate substrate. In addition, RapidFire/MS employs a solid phase extraction (SPE) sample cleanup step directly coupled to MS detection, further streamlining the process. Using this system, data were collected that illustrate enzyme concentration linearity (Figure 2) and standard kinetics (Figure 3) for conditions used in screening for LpxC inhibitors.[2] Since native substrate can be used, RapidFire/MS technology also reduces assay development time for the determination of standard enzyme kinetics.

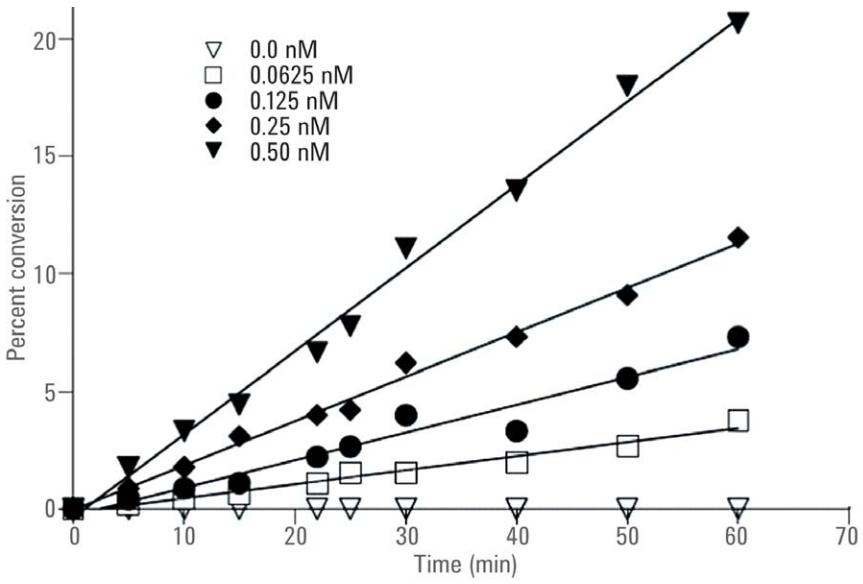

Figure 2. Linear dependence of product formation on LpxC enzyme concentration.[2]

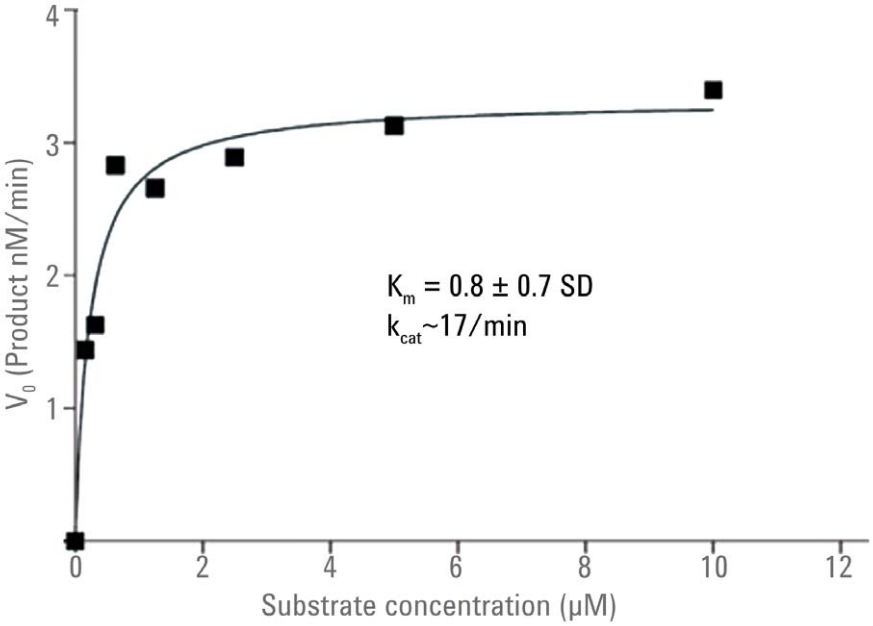

Figure 3. Demonstration of standard enzyme kinetics and reaction rate dependence on LpxC substrate concentration.[2]

In addition to streamlining high-throughput screening, the LpxC method developed for RapidFire/MS can uncover novel inhibitors that may not be discoverable when using surrogate substrates. Figure 4 includes the results of an LpxC MS screening campaign. The optimized LpxC assay was used to evaluate a random compound library for enzyme inhibitors and analyze the activity exhibited by each compound mixture. Each hit compound was subjected to confirmation testing using 5-point dose-response curves starting at half-log above the screening concentration.[2]

Conclusions

The Agilent RapidFire High-throughput Mass Spectrometry System demonstrated a number of key benefits for the high-throughput screening of LpxC as an example of an intractable target traditionally requiring an imperfect surrogate substrate. RapidFire provides sample processing speeds of 6-10 seconds while delivering more reliable data for challenging drug targets.

RapidFire/MS employs direct detection of native enzyme substrates and products, facilitating the discovery of novel inhibitors that may not be found by conventional screening methods. As a result, incorporation of RapidFire/MS systems into the lead discovery phase of the drug discovery process enables efficiency and productivity advances unrivaled by other technologies.

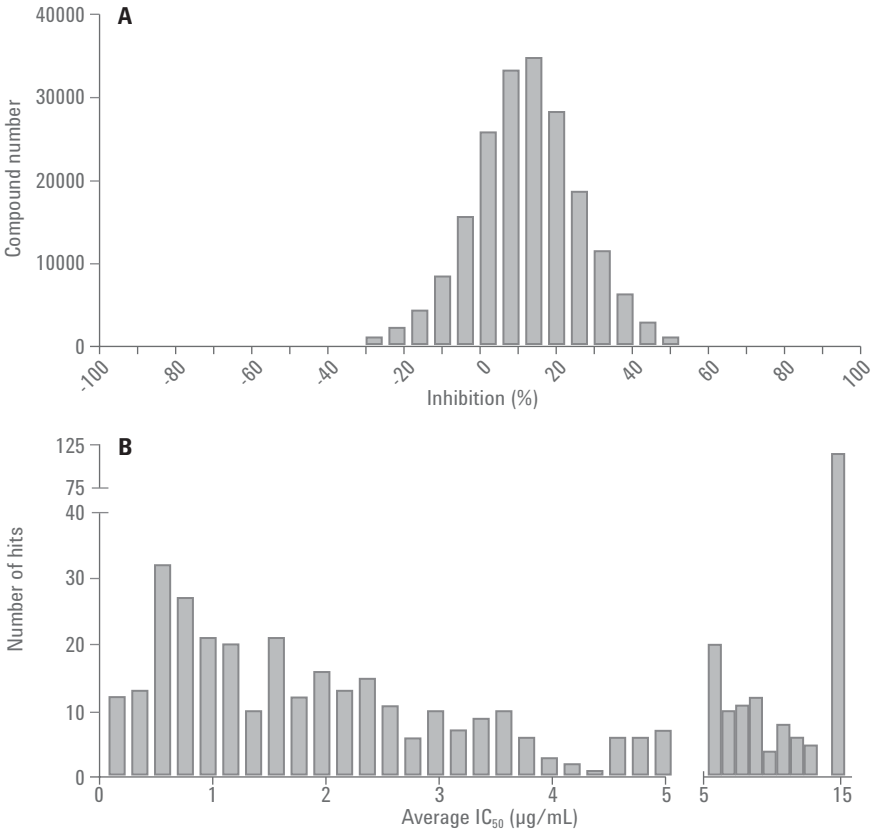

Figure 4. Results of LpxC MS screening campaign showing activity for each compound (A) and the results of confirmation testing using 5-point dose-response curves (B).

References

1. Wang, W., *et al*. A Fluorescence-Based Homogeneous Assay for Measuring Activity of UDP-3-*O*-(*R*-3-Hydroxymyristoyl)-*N*-acetylglucosamine Deacetylase. *Analytical Biochemistry*, **2001**, 290:338-346.
2. Langsdorf, E., *et al*. Screening for Antibacterial Inhibitors of the UDP-3-O-(R-3-Hydroxymyristoyl)-N-Acetylglucosamine Deacetylase (LpxC) Using a High-Throughput Mass Spectrometry Assay. *J Biomol Screen.,* **2010**, 15(1):52-61.

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