

High-Throughput Analysis of Epigenetic Targets with Agilent RapidFire/MS Systems: Sirtuin (SIRT) Enzymes

Application Note

Author

Peter Rye, Lauren Frick,
and William LaMarr
Agilent Technologies, Inc.
Wakefield, MA USA

Introduction

Histones and other proteins are subject to a variety of posttranslational modifications that regulate a host of biological processes. One such modification is the deacetylation of specific lysine residues by sirtuins, whose activity has been associated with inflammatory, cardiovascular, proliferative, neurodegenerative, and metabolic disorders.¹ High-throughput bioassays designed to identify sirtuin modulators are therefore of interest, particularly when coupled to the highly-sensitive and specific detection available with mass spectrometry (MS).



Agilent Technologies

Fast and Direct Measurement of Multiple Reaction Products

The RapidFire platform enables mass spectrometric analysis of native molecules by performing high-throughput online desalting. Following enzymatic reactions between SIRT1 enzyme and a multiply-modified substrate, RapidFire/MS/MS was used to monitor every possible acetylation state of the peptide with a sustained throughput of seven seconds per sample. Analysis by RapidFire/MS/MS enabled the fast and direct measurement of multiple acetylated species (Figure 2).

Label-Free Assays with Meaningful Data

The RapidFire/MS system measures native molecules directly, thereby obviating the need for fluorescently-tagged materials or secondary reactions which can be susceptible to compound interference. Label-free analysis enables substrate-to-product conversion measurements to be made directly, facilitating the determination of accurate kinetic parameters for hit identification. Figure 3 shows SIRT1, SIRT2, and SIRT3 inhibition curves with IC_{50} values that concur with those from previous reports.^{5,6}

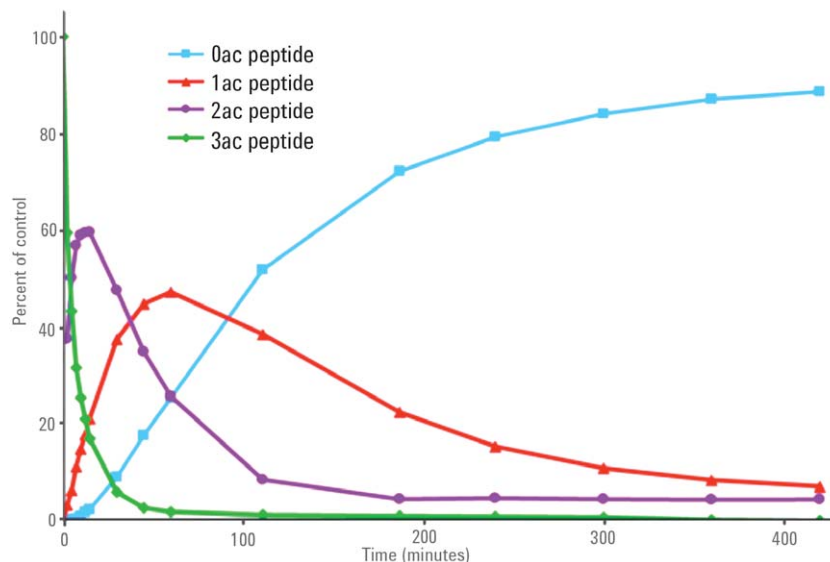


Figure 2. Sequential modification of a triply-acetylated p53 peptide by SIRT1 over time.

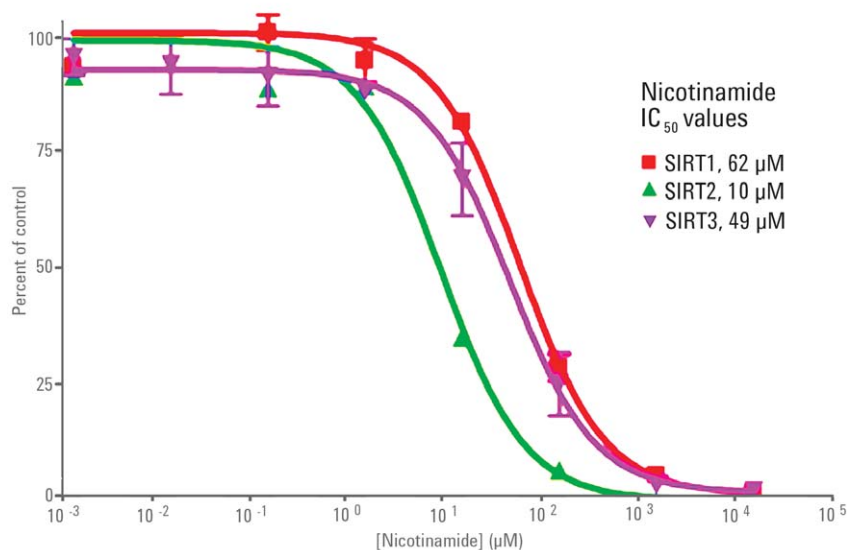


Figure 3. SIRT1, SIRT2, and SIRT3 inhibition curves using the small molecule nicotinamide.

Conclusions

RapidFire/MS/MS was used to analyze SIRT1, SIRT2, and SIRT3 reactions at a sustained rate of approximately seven seconds per sample. The label-free method circumvented the need for non-native surrogate substrates and radioactive methodologies. RapidFire/MS/MS enabled direct detection of peptide reactants and facilitated the identification of kinetic parameters for accurate IC₅₀ determinations. Because RapidFire/MS can measure multiple analytes from each sample quickly and directly, the system is particularly well-suited for studying epigenetic changes which involve sequential modifications.

References

1. Yamamoto, H., Schoonjans, K., Auwerx, J. Sirtuin functions in health and disease. *Mol Endocrinol.*, **2007**, 21(8):1745-55.
2. Rye, PT, *et al.* Advances in Label-Free Screening Approaches for Studying Sirtuin-Mediated Deacetylation. *J Biomol Screen.*, **2011**, Sep 12. [Epub ahead of print].
3. Rye, PT, *et al.* Advances in Label-Free Screening Approaches for Studying Histone Acetyltransferases. *J Biomol Screen.*, **2011**, Sep 9. [Epub ahead of print].
4. Prives, C., and Manley, J.L. Why is p53 acetylated? *Cell*, **2001**, 107(7):815-8.
5. Porcu, M., and Chiarugi, A. The emerging therapeutic potential of sirtuin-interacting drugs: from cell death to lifespan extension. *Trends Pharmacol Sci.*, **2005**, 26(2):94-103.
6. Marcotte, P.A., *et al.* Fluorescence assay of SIRT protein deacetylases using an acetylated peptide substrate and a secondary trypsin reaction. *Anal Biochem.*, **2004**, 332(1):90-9.

www.agilent.com/lifesciences/rapidfire

For Research Use Only. Not for use in diagnostic procedures.

Information, descriptions, and specifications in this publication are subject to change without notice.

©Agilent Technologies Inc., 2011
Published in the USA, November 21, 2011
5990-9345EN



Agilent Technologies