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Technology Offer

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New KDAC assay and selection system

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Summary

We present an extremely sensitive, reliable and fast novel Lysine deacetylase (KDAC) assay, which can be performed in a continuous format. This novel KDAC assay is based on Firefly luciferase harboring an acetylation on an essential active site lysine, incorporated by genetic code expansion.

We furthermore offer a bacterial selection system that can help engineer KDACs. The selection system uses a reporter enzyme inactivated by lysine modifications at an essential active site residue, thereby linking deacetylase activity to a selectable output.

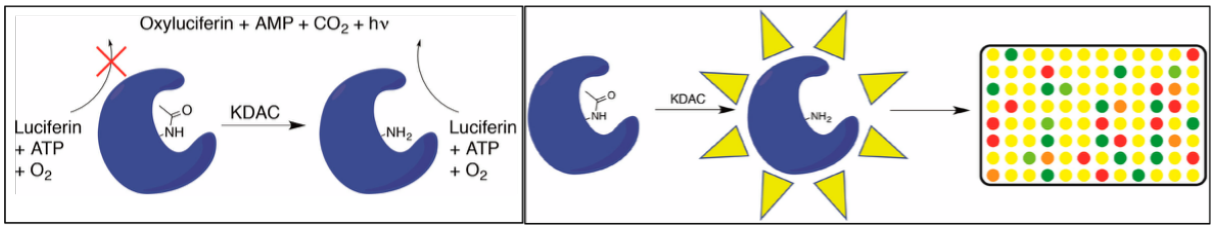
Background

KDACs play a prominent role in many physiological and cellular processes. Defects in KDACs are associated with a variety of diseases such as diabetes [1] or cancer [2], and ageing [3]. KDACs can reverse acetylation of the N(ε)-amino group of lysine residues, a modification that has shown to bear a large variety of functional roles in almost every physiological process. Inhibition of KDACs, for example by the use of chemical inhibitors, has already enlightened their function and generated active leads in pharmaceutical design [4].

The exact mechanisms of how KDAC misregulation contributes to the disease etiology is hard to asses. For future studies, the availability of reliable KDAC assays is key to succeed in identifying and / or characterizing KDACs. Further, engineered KDACs that are for example selective for a certain lysine acylation or for a specific bioorthogonal protecting group, may help to design a strategy for converting a prodrug into an active compound (e.g., to develop cancer therapies).

Technology

Prof. Neumann and his team from the Max Planck Institute of Molecular Physiology have developed a new KDAC assay based on Firefly luciferase harboring an acetylation on an essential active site lysine incorporated by genetic code expansion [5]. Several KDACs can reverse this modification and hence activate luciferase [5]. This new assay is extremely sensitive, reliable, and fast and can be performed in a continuous format [5, 6]. In a recently performed high-throughput screen to identify inhibitors of recombinant human SirT1, 170,000 compounds were tested, about 115 confirmed hits were identified and narrowed down to one SirT1 selective compound class [7].



Left panel: Acetylated Firefly luciferase is activated upon deacetylation by a KDAC. Right panel: Schematic of KDAC screen: Several KDACs can deacetylate and thereby activate the luciferase, creating a specific output suitable for high-throughput screening. (Reprinted with permission from Spinck M, Ecke M, Sievers S, et al. Highly Sensitive Lysine Deacetylase Assay Based on Acetylated Firefly Luciferase, American Chemical Society, July 1 2018. Copyright 2018 American Chemical Society.)

Furthermore, our scientists developed a combined screening and selection system for KDACs with altered substrate specificity or reactivity against bioorthogonal chemical protecting groups [8]. This selection system is based on a bacterial strain that expresses a reporter enzyme inactivated by lysine modifications at an essential active site residue. In this way, KDAC deacetylase activity generates a selectable output [8]. Prof. Neumann and his team used the selection system to identify acyl-type specific KDACs from libraries of >30 million different variants [8]. The enzymes selected from the KDAC library can be used for partial complementation of KDAC deletion strains to reveal the physiological role of particular lysine acylations [8]. This experimental approach represents a promising research tool for mechanistic studies to better understand limited specificity of KDACs for particular protein substrates and types of acylation. Importantly, engineered KDAC variants capable of removing chemical protecting groups may be of interest when developing prodrug strategies for cancer therapy. In this way, the present technology also may have the potential to provide the basis for an important and highly specific tool to study and treat cancer.

We are now looking for a licensing partner. Licensing can also be established regarding partial aspects of the patent application.

Publications

[1] Jing E et al., Proc Natl Acad Sci USA (2011) 108:14608-14613
[2] Haberland M et al., Proc Natl Acad Sci USA (2009) 106:7751-7755
[3] Rogina B et al., Proc Natl Acad Sci USA (2004) 101:15998-16003
[4] Li Y and Seto E, Cold Spring Harb Perspect Med (2016) 6:a026831
[5] Spinck M et al., Biochemistry (2018) 57:3552-3555
[6] Jain N et al., Journal of Biological Chemistry (2021) 296:100078
[7] Spinck M et al., Journal of Medicinal Chemistry (2021) 64(9):5838-5849
[8] Spinck M et al., Angewandte Chemie International Edition (2020) 59:11142-11149

Patent Information

A PCT patent application has been filed in 2018 and the national phase US (US20200240995A1, publication number) and the regional phase EP (EP3684923A0, publication number) have been entered.