ENL (YEATS Domain) and Acyl-Modified Histone Binding Assay: TR-FRET technology

A protein interaction assay to enable the identification of novel ENL inhibitors

Authors:
Matthew Marunde, Andrea L. Johnstone, Brandon A. Boone, Martis W. Cowles, James R. Bone, Zu-Wen Sun, Michael-Christopher Keogh

EpiCypher® Inc. Research Triangle Park, NC 27713
Correspondence: info@epicypher.com

Overview: YEATS Domain Epigenetic Reader Proteins

Epigenetic reader proteins have gained widespread interest in the scientific community for their essential roles in cellular function and the potential for targeted drug development to treat diseases such as cancer. Recently, a novel family of reader proteins was identified that recognizes acetylated lysine (Kac) residues on histone tails through a highly conserved YEATS domain. In contrast to the widely studied Kac bromodomain reader, the YEATS domain also shows affinity for extended acyl modifications, particularly lysine crotonylation (Kcr) [1]. There are four YEATS domain containing proteins in humans: AF9, ENL, GAS41, and YEATS2. Due to the emerging role of these YEATS:acyl-histone interactions in cellular transcription and human disease [2], there is a compelling need to identify small molecule inhibitors of this novel class of epigenetic readers.

ENL YEATS Domain

Recent studies identify the ENL YEATS domain as a critical driver of oncogenic gene expression in acute myeloid leukemia [3, 4]. ENL is required for leukemic growth *in vitro* and in a mouse xenotransplant model. The role of ENL in RNA polymerase II-mediated transcription at oncogenes is specifically mediated by its YEATS acyl reader domain. These findings underscore the need to develop assays for the identification of novel inhibitors of ENL-YEATS interactions with modified histones.

A biochemical assay to quantify ENL YEATS:acyl-histone interactions

This assay utilizes time-resolved Fluorescence Resonance Energy Transfer (TR-FRET) technology, which enables a quantitative assessment of the ENL YEATS domain interaction with acylated histone peptides (**Figure 1A**). TR-FRET technology provides many advantages over other assay platforms. The time-resolved nature of the fluorescence energy transfer leads to reduced screening artifacts caused by fluorescent interference of small molecules. Further, the assay does not require washes, can be readily miniaturized into microplates, is highly amenable to liquid handling and automation, and robust in nature providing consistent and reproducible data. All of these advantages make this approach ideal for high-throughput screening (HTS) applications.

As previously reported, the LANCE assay shows modest binding of ENL to histone H3 acetylated at lysine 9 (H3K9ac **Figure 1B**) [3, 4]. Moreover, ENL binding is enhanced by histone crotonylation over acetylation at the same sites (**Figure 1B**) [5], offering a variety of acyl-histone peptides that can be used to develop an interaction assay. Here, we show representative HTS assay development data for ENL binding to H3K9cr peptides (**Figure 2-4**).

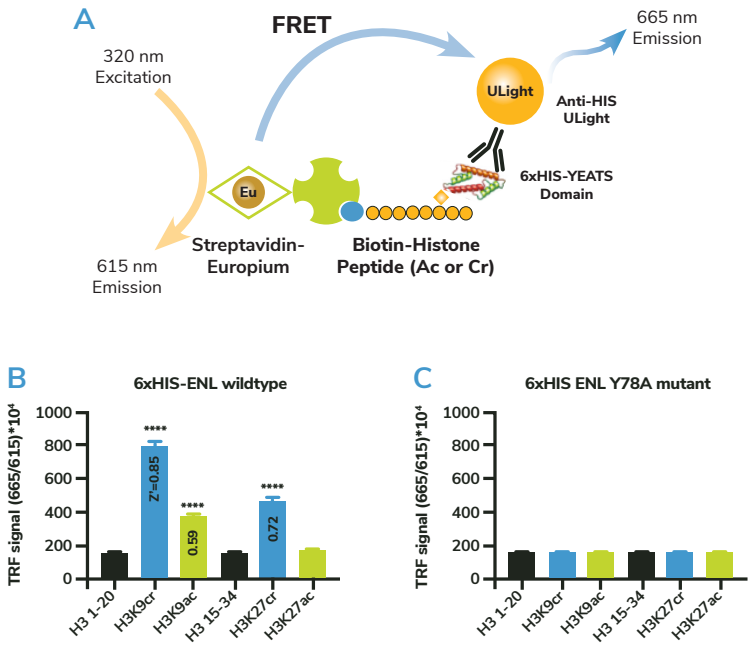

FIGURE 1: ASSAY PRINCIPLE. *TR-FRET technology can be used to quantify YEATS reader domain binding to the histone in a homogenous fashion (no wash).* (A) *Streptavidin-Europium (Eu) chelate captures the biotinylated histone peptide, while Anti-6xHIS ULight™ binds 6xHIS-ENL. When the Eu chelate is excited at 320 nm, fluorescence resonance energy transfer (FRET) occurs between Eu and ULight if they are brought within proximity via ENL binding to acyl-peptides. ULight emission (FRET) is measured at 665 nm and normalized to the 615 nm (Eu) emission to reduce variability between wells.* (B) *This assay was optimized to quantify the interaction of the ENL YEATS domain with acetylated (ac) or crotonylated (cr) histone peptides. ENL binding to H3K9ac, H3K9cr, and H3K27cr histone peptides is enriched over unmodified H3 peptide. Binding to H3K27ac was not distinguishable from background. Values within the bars indicate Z' values compared to unmodified H3 (****p<0.0001 compared to unmodified H3 using One-way ANOVA with Bonferroni post-test, n=9).* (C) *Point mutation of a conserved aromatic residue in the ENL YEATS domain (Y78A) previously identified to abolish binding to acyl lysines [3, 4] also abolishes the TRF signal.*

Continued...

EpiCypher®
Bringing Epigenetics to Life

ENL (YEATS Domain) and Acyl-Modified Histone Binding Assay: TR-FRET technology

TECHNICAL NOTE #110

A protein interaction assay to enable the identification of novel ENL inhibitors

Continued from page 1

Reagents and Materials Required for the Assay:
NOTE: store protein aliquots @ -80°C, avoid repeated freeze/thaw

REAGENTS/MATERIALS	VENDOR	CAT#
6xHIS ENL YEATS Domain (Wild type)	EpiCypher®	15-0069
6xHIS ENL YEATS domain (F59A)	EpiCypher®	15-0070
Biotin H3 1-20aa	EpiCypher®	12-0001
Biotin H3K9ac 1-20aa	EpiCypher®	12-0003
Biotin H3K9cr 1-20aa	EpiCypher®	12-0099
Biotin H3 15-34aa	EpiCypher®	12-0016
Biotin H3K27ac 15-34aa	EpiCypher®	12-0042
Biotin H3K27cr 15-34aa	EpiCypher®	12-0100
non-Biotin H3 1-20aa	EpiCypher®	12-8001
non-Biotin H3K9ac 1-20aa	EpiCypher®	12-8103
non-Biotin H3K9cr 1-20aa	EpiCypher®	12-8099
non-Biotin H3 15-34aa	EpiCypher®	12-8104
non-Biotin H3K27ac 15-34aa	EpiCypher®	12-8042
non-Biotin H3K27cr 15-34aa	EpiCypher®	12-8100
LANCE Ultra Ulight anti-6xHIS (5 µm)	PerkinElmer	TRF0105
LANCE Eu-SA (8.3 µm)	PerkinElmer	AD0060
AlphaPlate-384™, Light Gray	PerkinElmer	6005350
TopSeal™-A Plus Film	PerkinElmer	6050185

Assay Buffer

50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.5% casein;
add 0.1% NP-40 fresh *DO NOT ADD A REDUCING AGENT

Standard Protocol

NOTE: Briefly centrifuge plate (≤ 800 xg for ~10 seconds) after the addition of each reagent to maximize reproducibility.

1. Add 4 µL of 5X test inhibitors, competitor peptides, or vehicle control
2. Add 4 µL of 5X 6xHIS-ENL protein (125 nM final)
3. Incubate 15 minutes at RT
4. Add 4 µL of 5X Biotin-H3 peptides (5 nM final)
5. Incubate 30 minutes at RT
6. Add 8 µL 2.5X Anti-HIS ULight (15 nM final) + Streptavidin-Eu chelate (0.5 nM final)
7. Incubate 60 minutes at RT
8. Read using TR-FRET capable Plate Reader: Ex 320 nm,Em 615 nm and 665 nm

References

1. Sabari BR, Zhang D, Allis CD, Zhao Y. Metabolic regulation of gene expression through histone acylations. Nat Rev Mol Cell Biol. 2017 Feb;18(2):90-101.
2. Schulze JM, Wang AY, Kobor MS. YEATS domain proteins: a diverse family with many links to chromatin modification and transcription. Biochem Cell Biol. 2009 Feb;87(1):65-75.
3. Wan L, Wen H, Li Y, Lyu J, Xi Y, Hoshii T, Joseph JK, Wang X, Loh YE, Erb MA, Souza AL, Bradner JE, Shen L, Li W, Li H, Allis CD, Armstrong SA, Shi X. ENL links histone acetylation to oncogenic gene expression in acute myeloid leukaemia. Nature. 2017 Mar 9;543(7644):265-269.
4. Erb MA, Scott TG, Li BE, Xie H, Paulk J, Seo HS, Souza A, Roberts JM, Dastjerdi S, Buckley DL, Sanjana NE, Shalem O, Nabet B, Zeid R, Offei-Addo NK, Dhe-Paganon S, Zhang F, Orkin SH, Winter GE, Bradner JE. Transcription control by the ENL YEATS domain in acute leukaemia.Nature. 2017 Mar 9;543(7644):270-274.
5. Li Y, Sabari BR, Panchenko T, Wen H, Zhao D, Guan H, Wan L, Huang H, Tang Z, Zhao Y, Roeder RG, Shi X, Allis CD, Li H. Molecular Coupling of Histone Crotonylation and Active Transcription by AF9 YEATS Domain. Mol Cell. 2016 Apr 21;62(2):181-93.
6. Zhang, J.H., T.D. Chung, and K.R. Oldenburg, A Simple Statistical Parameter for Use in Evaluation and Validation ofHigh Throughput Screening Assays. J Biomol Screen. 1999. 4(2): p. 67-73

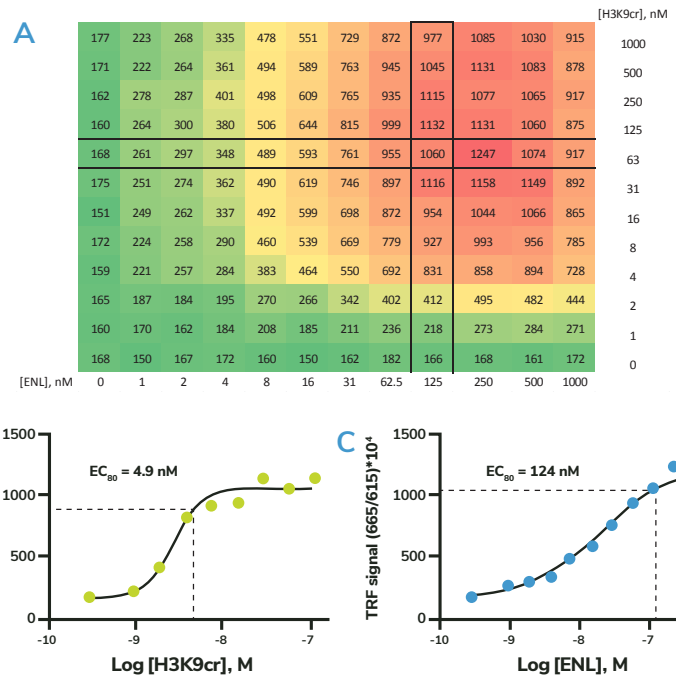

FIGURE 2: PROTEIN/PEPTIDE TITRATIONS. *(A) 6xHIS-ENL (horizontal axis) was titrated against Biotin-H3K9cr peptide (vertical axis). Titrations of each binding partner from 1000 nM down to 1 nM (1:2 dilutions) determined the optimal concentrations for assay development. TRF signal is shown and colored by relative intensity. The boxed regions outline the ENL/histone titrations graphed below. Nonlinear regression analysis identified the concentration at which 80% response was achieved (EC80) for ENL titrated against 63 nM H3K9cr (B) and H3K9cr titrated against 125 nM ENL (C). Based on these findings, 125 nM ENL and 5 nM H3K9cr was selected for subsequent experiments.*

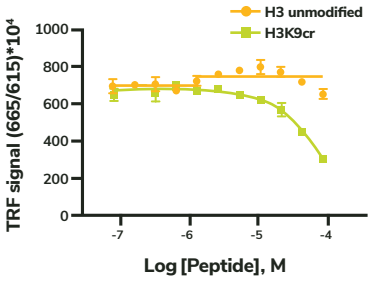

FIGURE 3: COMPETITION ASSAY.
Non-biotinylated histone competitor peptides (12-point dose-response in 1:2 titrations) and 6xHIS ENL were pre-incubated for 15 minutes. Biotinylated peptides were added and incubated for 30 minutes. Finally, Streptavidin-Eu and anti-HIS ULight were added and incubated for 60 minutes. After the incubation, TRF signal was read with 320 nm excitation, and measuring 665/615 nm emission sequentially. Competition with the non-biotinylated H3K9cr peptide inhibited the TRF signal in the low-to-mid micromolar range, consistent with the binding affinity of ENL to acyl-modified histones [5].

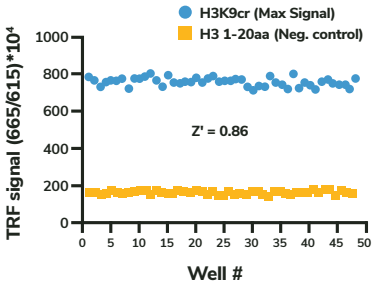

FIGURE 4: Z' FACTOR DETERMINATION.
6xHIS-ENL (125 nM) was incubated with Biotin-unmodified H3 peptide (5 nM) as a negative control. Incubation with Biotin-H3K9cr (5 nM) determined the maximum signal. A Z' factor of 0.86 was calculated as described [6], indicating readiness for high throughput screening (Z'>0.5).

EpiCypher®
Bringing Epigenetics to Life
855-374-2461 • info@epicypher.com • www.epicypher.com