Development of selective inhibitors for The human YEATS domains

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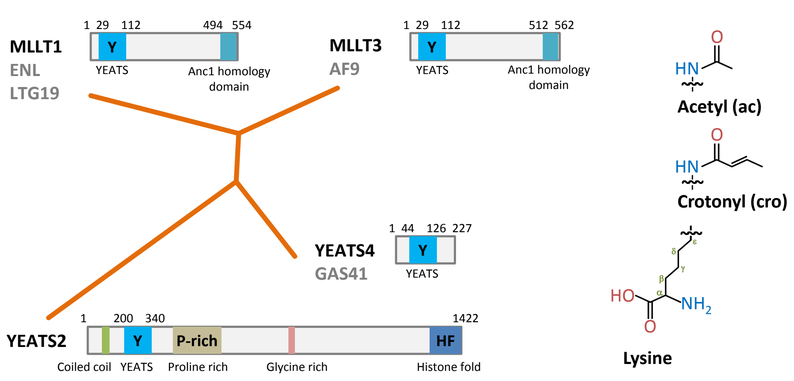
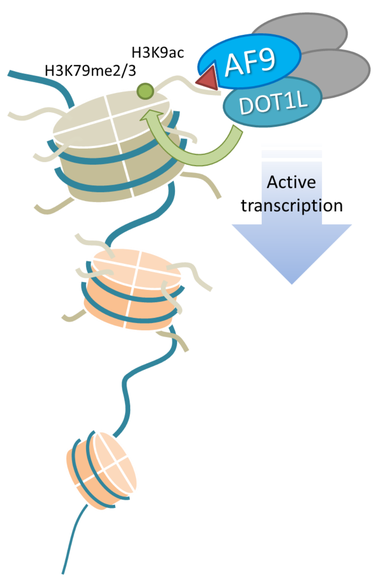
Introduction

In recent years, YEATS domains have emerged as readers of histone post-translational modifications (HPTM) alongside bromodomains, PHD fingers and others. Like bromodomains, they recognise acylation on the ε-carbon atom of lysine and are implicated as actors in a range of cancer types. They appear to favour crotonylation over acetylation and even though they are both associated with active transcription, the significance of crotonylation is poorly understood[1]–[3].



Structurally, YEATS domains form an immunoglobulin like fold that recognises the acyl group of the modified lysines in an aromatic cage at the tip of the β-sheets while the rest of the peptide is in contact with the mostly flat surface of the fold. The aromatic cage is open to both sides and thus allows recognition of larger lysine modifications compared to other acyl-readers (e.g. Bromodomains, which generally only recognise acetylation)[1], [4].



Functionally, YEATS domain containing proteins act as reader and scaffold proteins that direct protein complexes involved in transcription to acylated histones.

Here, we are showing the current progress in developing selective inhibitors for members of the YEATS family with the ultimate goal of developing chemical probes for each member of the family.

Chemical Probes

Since 2008, the SGC has pioneered the development of chemical tools (probes) that inhibit or antagonize proteins of interest. These chemical probes are made available to the research community with no restriction to stimulate research and drug discovery. They are approved by a rigorous progress involving SGC internal and external experts and must meet the following criteria:

- ✓ **in vitro IC_{50} or K_D < 100 nM**
- ✓ **> 30-fold selectivity over proteins in the same family**
- ✓ **Significant on-target cellular activity at 1 µM**

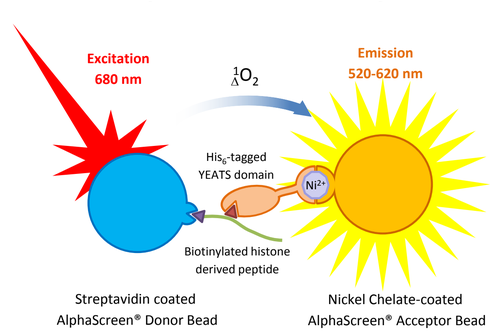
References

1. Li, Y., Zhao, D., Chen, Z. & Li, H. YEATS domain: Linking histone crotonylation to gene regulation. Transcription 0, 1–6 (2016).
2. Zhao, D. et al. YEATS2 is a selective histone crotonylation reader. Cell Res. 1–4 (2016).
3. Rousseaux, S. & Khochbin, S. Histone Acylation beyond Acetylation: Terra Incognita in Chromatin Biology. Cell J. 17, 1–6 (2015).
4. Li, Y. et al. AF9 YEATS domain links histone acetylation to DOT1L-mediated H3K79 methylation. Cell 159, 558–571 (2014).

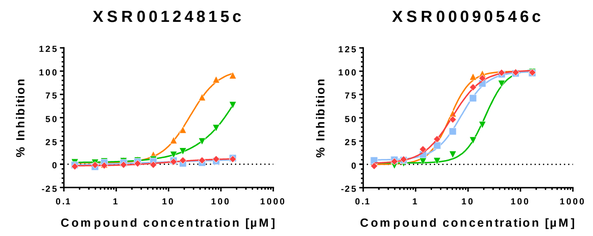
Peptide Displacement Assay

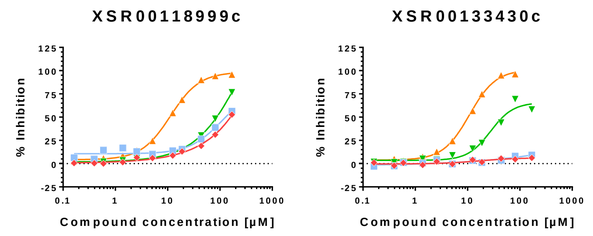
Initially, several libraries (such as internal bromodomain inhibitors and the 16k and 24k libraries of the Ontario Institute for Cancer Research) were screened in an AlphaScreen® assay at single concentrations against MLLT1 in the first instance. Compounds that inhibited at the first concentration (100 µM) were then re-tested at lower concentrations to establish potency range. A counter-screen with a [biotin]-His_6 peptide instead of a [biotin]-peptide:YEATS-His_6 pair was also performed to exclude compounds that disrupt the chemistry of the assay (e.g. metal chelators or fluorescence quenchers).
Of the hits re-tested at different concentrations, a number were followed up with full dose-response curves in AlphaScreen®.

AlphaScreen® Chemistry



Dose response curves

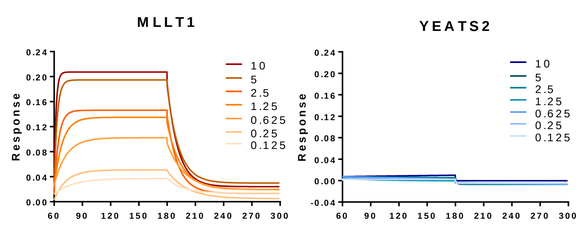




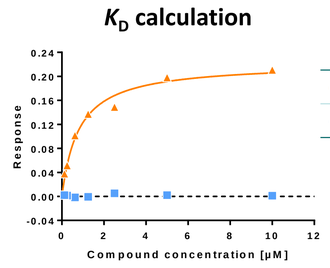
Single concentration screen (and counter screen) → Structure based selection → Full dose response curve (IC_{50} determination)

Biolayer Interferometry

Response curves



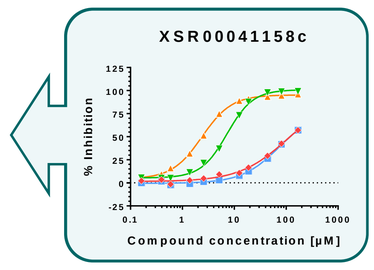
K_D calculation



Protein	KD
MLLT1	0.53 µM
YEATS2	*N.D.*

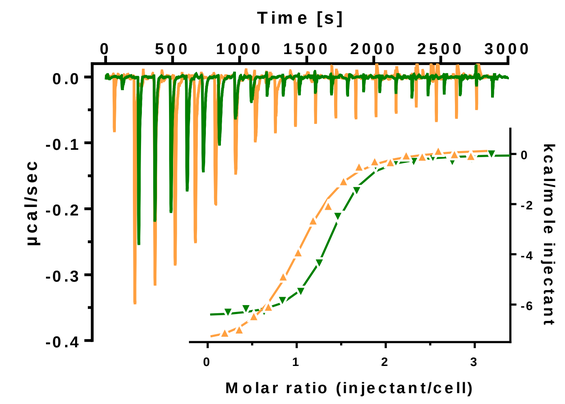
Protein:Peptide	IC_{50} [µM]
MLLT1:H3K18ac	2.4
MLLT3:H3K9ac	7.3
YEATS2:H3K27cro	*N.D.*
YEATS4:H3K9ac	*N.D.*

Selected for further biophysical screening



Determination of K_D via biolayer interferometry was performed on a FortéBio® Octet® RED384 System with biotinylated constructs of the MLLT1 and YEATS2 YEATS domains on streptavidin coated sensors.

Isothermal Titration Calorimetry



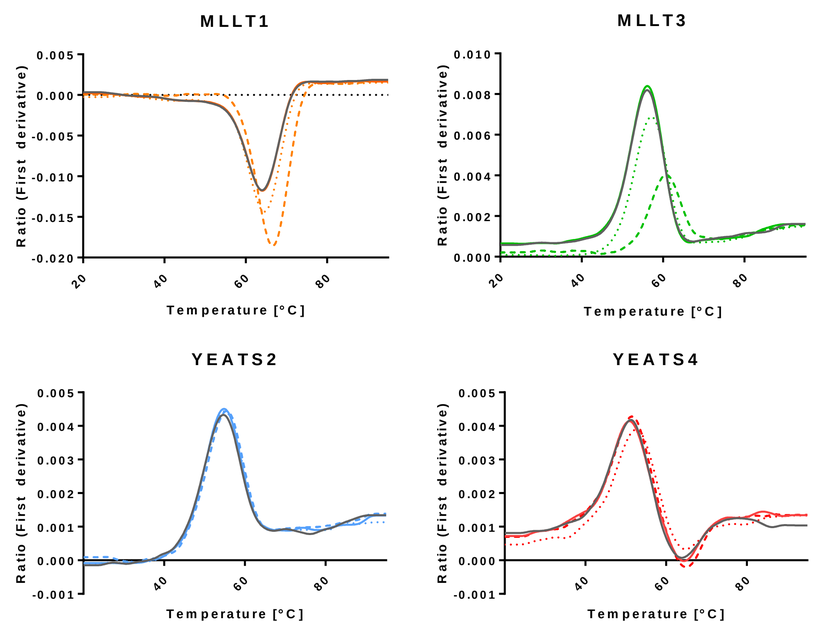
ITC was performed with ligand in cell.

	MLLT1	MLLT3
Protein	288 µM	284 µM
Compound	20 µM	10 µM
N	$1.03 \pm 1.6x10^{-2}$	$1.31 \pm 1.3x10^{-2}$
K_D	1.21 ± 0.171 µM	266 ± 33.6 nM
ΔH*	-8.05 ± 0.234	$-6.53 \pm 8.9x10^{-2}$
ΔG*	-7.80	-8.67
-TΔS*	0.248	-2.14

* kcal/mole

Label-free Differential Scanning Fluorimetry

First derivative of fluorescence ratio (330:350 nm)



Grey line: DMSO reference
Solid line: Buffer control
Dashed line: 50 µM compound
Dotted line: 10 µM compound

	Shift in onset of unfolding [°C] at 50 µM	Shift in inflection of unfolding [°C] at 50 µM
MLLT1	2.1 ± 0.2	2.6 ± 0.1
MLLT3	7.7 ± 1.1	4.7 ± 0.2
YEATS2	1.4 ± 0.3	0.8 ± 0.1
YEATS4	-2.6 ± 0.9	0.3 ± 0.1

Label-free DSF was performed using a Nanotemper Prometheus NT.48, measuring the ratio of tryptophan fluorescence at 330 and 350 nm during thermal un-folding of protein (10 µM) in the presence of compound at different concentrations (50, 25, 10, 5, 2.5 µM; 50 and 10 µM are shown).