Fragment-based drug design using Microscale Thermophoresis

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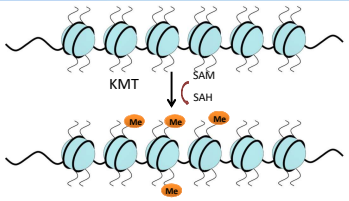
Introduction

- G9a is a lysine methyltransferase (KMT) involved in epigenetic gene regulation by covalent modification of histones
- G9a catalyses the transfer of methyl groups from S-adenosyl methionine (SAM) to lysine residues on histone proteins (Fig A)
- Literature supports the role of G9a in mechanisms of carcinogenesis, making it an attractive oncology target[1-4]
- Domainex has solved the key technical drug discovery challenges associated with KMTs, including generating a number of proprietary crystal structures, assays and a novel screening library of small molecule inhibitors
- In this poster, we report a fragment-based hit-finding approach using Microscale Thermophoresis (MST) for the ternary G9a-SAM-fragment system, hit confirmation using protein X-ray crystallography as well as fragment optimisation.



Microscale Thermophoresis (MST)

- MicroScale Thermophoresis (MST) is a biophysical technique that measures the strength of the interaction between two molecules by detecting variations in fluorescence signal as a result of an IR-laser induced temperature change.
- The range of the variation in the fluorescence signal correlates with the binding of a ligand to the fluorescent target.
- These changes can be used to derive dissociation constants (K_d) within minutes.

Fragment Library

- Our fragment library contains ~1,000 fragments and has been carefully designed to maximise the chances of finding suitable chemical starting points
- The library gives good coverage of 'ideal fragment space' by optimising a number of parameters, is 'Rule of 3' compliant, has good chemical diversity and is SP3 rich
- Additional filters were applied to remove:
  - compounds containing atoms other than H, C, N, O, S, F, Cl and reactive functional groups
- All compounds in the library show > 1 mM aqueous solubility in 1% DMSO

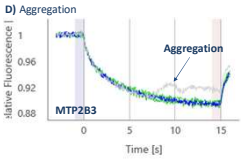
G9a Fragment Screen

- We screened part of our fragment library at 1 mM against a G9a-SAM complex using MST. Fragments were declared as hits if a significant shift in the response compared to the reference was observed (Fig A). The thermophoresis traces allow easy identification of false-positive fragments such as aggregators and compounds effecting the fluorescence signal (Figs D and E, respectively).
- We obtained a 5.3% hit rate. Screening the same fragments using Differential Scanning Fluorimetry (DSF) or the activity-based AlphaScreen both showed only a 0.3% hit rate.
- Hits were taken into secondary screening to determine their binding affinities (K_d) to the G9a-SAM complex using MST (for example Fig. B and Table C).

C) Screening summary

Frag ID	K_d [µM]	LE	STD-NMR Positive binding	X-ray Structure Resolution
MTP3B6	19	0.66	✓	1.5 Å
MTP2C3	56	0.41	X	
MTP4E1	115	0.41	✓	X
MTP2D8	327	0.54	✓	2.0 Å
MTP3G10	411	0.53	✓	1.8 Å
MTP2H9	564	0.44	✓	X
MTP3G1	718	0.36	X	

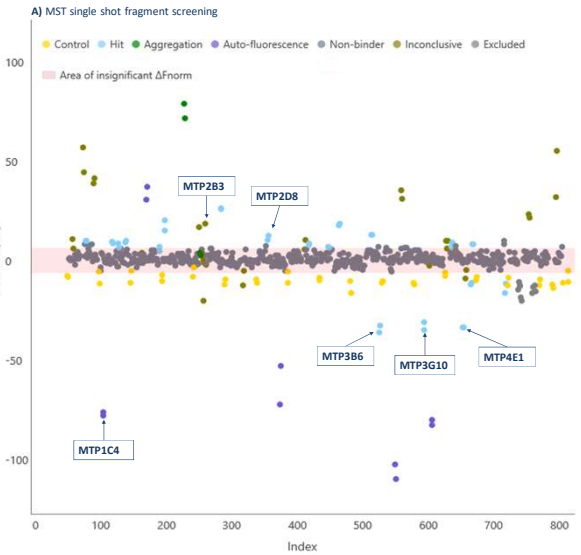
D) Aggregation



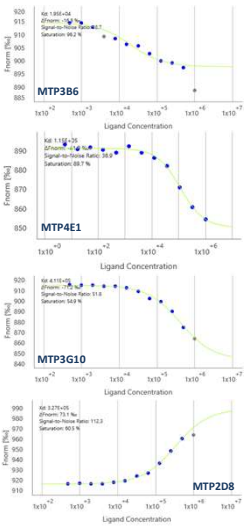
E) Auto-fluorescence

MST raw data (D and E)
Green: DMSO reference; Blue: Test compound

A) MST single shot fragment screening



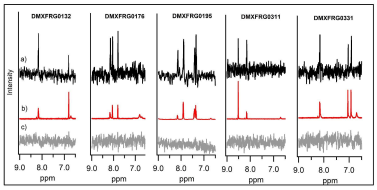
B) Dose-response measurements



Orthogonal Hit Validation

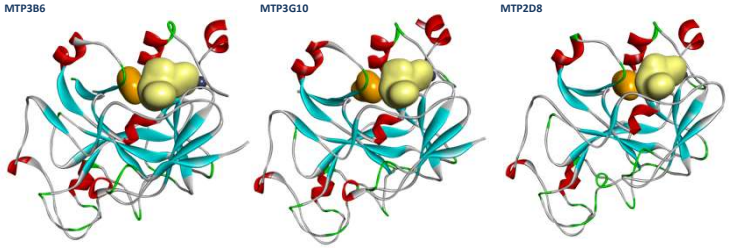
- Orthogonal confirmation of hit binding to G9a was demonstrated by Saturation Transfer Difference (STD) NMR spectroscopy (Fig. F)
- Three G9a-fragment structures were solved in-house in the presence of co-factor SAM with a resolution of 1.5- 2.0Å (Fig G) which revealed different fragment binding modes
- This has lead to several options for FBDD to provide alternative inhibitor chemotypes.

F) STD NMR confirmation

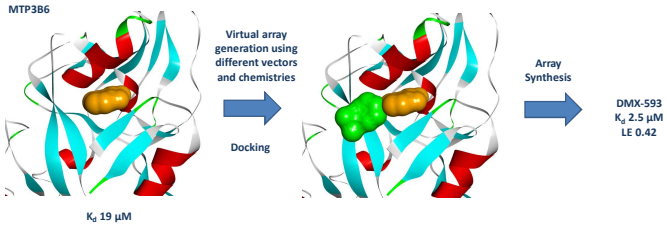


STD-NMR confirmation spectra for five fragments.
Spectrum a (black) shows the NMR-STD confirmation for each fragment, as well as the SAM co-binder.
Spectrum b (red) shows the reference spectrum of each fragment and the SAM co-binder (8.16 ppm) in PBS, pH 8.5 (10 % D2O). Spectrum c (grey) is the false positive control. No signal indicates there was no aggregation of the fragment, and no direct excitation of the fragment or SAM with the on-resonance pulse.

G) In-house G9a-Fragment structures (Orange –Fragment, Yellow –SAM)



Fragment Elaboration



Summary

- We have used MST to successfully screen a sub-set of our fragment library against the KMT G9a with a hit rate of 5.3%, identifying fragments with high ligand efficiencies.
- MST allows easy false-positive identification by highlighting compounds that induce protein aggregation or cause fluorescent effects.
- We were able to identify low and high affinity binders using the same technique.
- Three fragment hits were successfully crystallised bound to G9a, which enabled a SBDD program for this target.
- In one round of fragment elaboration a 10-fold increase in affinity could be achieved.

Services/Contact

Domainex welcomes interest from any potential collaborators, industrial or academic. If you would like to learn more about applying our drug-discovery platform to other targets, please contact:
tom.mander@domainex.co.uk
www.domainex.co.uk

References
[1]Copeland et al., *Nature Reviews*, **2012**, (8), 724-732; [2]Hamamoto et al., *Nature Cell Biology*, **2004**, (6), 731-740; [3]Hamamoto et al., *Cancer Sci.*, **2006**, (97), 113-118; [4]Liu et al. *J. Natl. Cancer. Inst.*, **2013**, *doi: 10.1093/jnci/djt30;* [5]Pons et al. *Eur Heart J* 2009 Feb;30(3):266-77; [6]NanoTemper User Manual Monolith NT.115 2011 July; [7]Casciello et al., *Front Immunol.*, **2015**, (6), 1-12