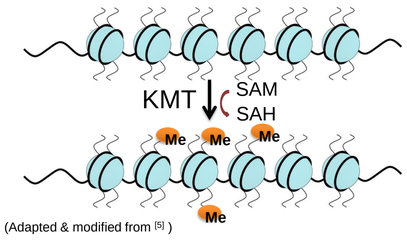
# Fragment screening using Microscale Thermophoresis and X-ray Crystallography against the lysine methyltransferase G9a

## 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 and hit confirmation using protein X-ray crystallography



(Adapted & modified from [5] )

## Microscale Thermophoresis (MST)

- MST detects changes in the size, charge and hydration shell of molecules and enables the measurement of biomolecule interactions or enzyme kinetics
- Fluorescently-labelled molecules are exposed to precise temperature gradients generated by an IR-laser within microliter-volume glass capillaries
- The thermophoretic properties of a molecule change upon ligand binding
- These changes can be used to derive dissociation constants ($K_d$) within minutes

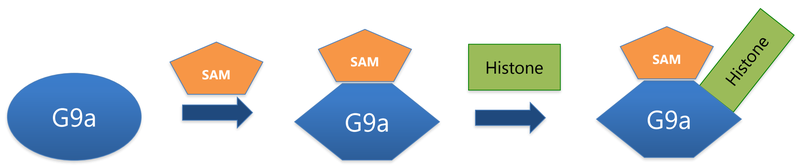
## Fragment Library

- 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

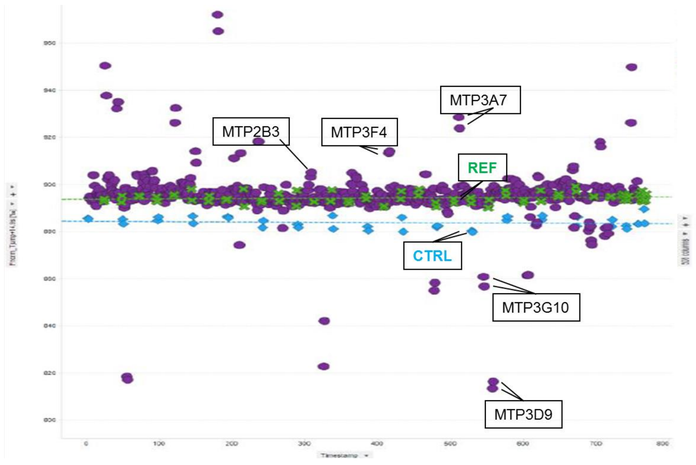
## G9a Fragment Screen

- We screened our fragment library at 1 mM against a G9a-SAM complex using MST. Fragments were declared as hits if a significant shift was observed (Fig B). The thermophoresis traces allow easy identification of false-positive fragments, which caused a shift in amplitude due to protein aggregation (Figs C and D, 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, again using MST (for example, see Fig. E)
- $K_d$ values for 7 hits were also determined without the co-factor, SAM. The $K_d$s for two fragments were essentially unchanged whereas five fragments showed significant reduction in binding, highlighting the importance of being able to study a ternary system (Table F)
- Orthogonal confirmation of hit binding to G9a was demonstrated by Saturation Transfer Difference (STD) NMR spectroscopy (Fig. G)
- Three G9a-fragment structures were solved in-house in the presence of co-factor SAM with a resolution of 1.5- 2.0Å (Fig H) which revealed different fragment binding modes
- This has lead to several options for FBDD to provide alternative inhibitor chemotypes.

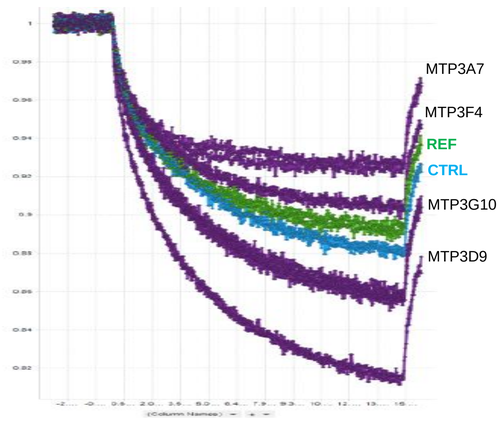
**A)** Schematic of G9a function



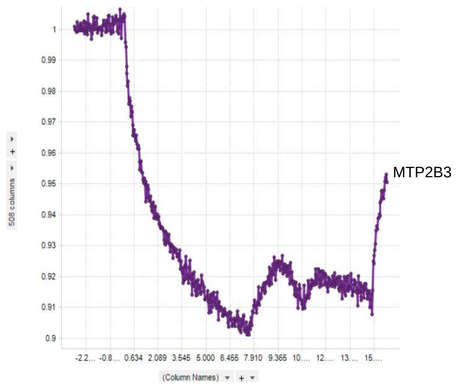

**B)** MST single shot fragment screening, MTP#s given are hits



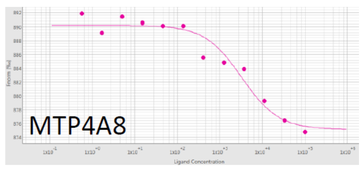

**C)** Thermophoresis traces

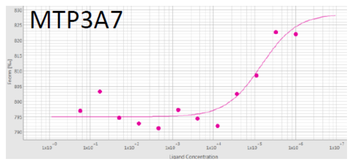



**D)** Aggregation




**E)** Dose-response measurements

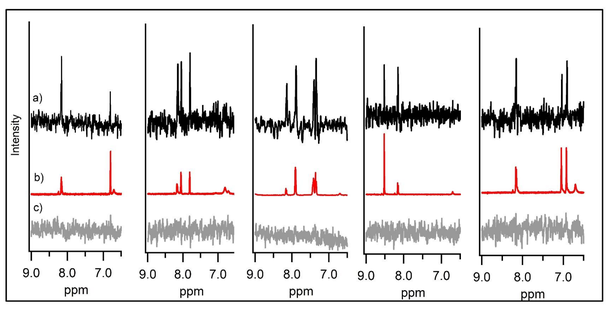



**F)** Screening summary

| | $K_d$ + SAM | LE (+SAM) | $K_d$ - SAM | Comment | STD-NMR | Crystal trials | X-ray Structure |
|---|---|---|---|---|---|---|---|
| **Frag ID** | **$K_d$ [µM]** | | **$K_d$ [µM]** | **MOA** | **Positive binding** | **X-ray Structure** | **Resolution** |
| MTP4E1 | 117 | 0.41 | 94 | SAM independent | ✓ | X | |
| MTP3G1 | 718 | 0.36 | 518 | SAM independent | X | | |
| MTP3B6 | 17 | 0.65 | 109 | SAM dependent | ✓ | ✓ | 1.5 Å |
| MTP2C3 | 56 | 0.41 | >1 mM | SAM dependent | X | | |
| MTP3G10 | 195 | 0.56 | >1 mM | SAM dependent | ✓ | ✓ | 1.8 Å |
| MTP2D8 | 534 | 0.50 | Non Binder | SAM dependent | ✓ | ✓ | 2.0 Å |
| MTP2H9 | 564 | 0.44 | Non Binder | SAM dependent | ✓ | X | |

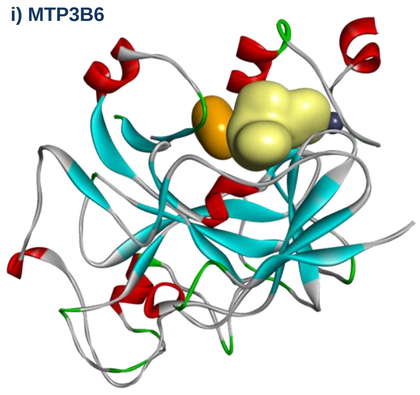
**G) STD NMR confirmation**



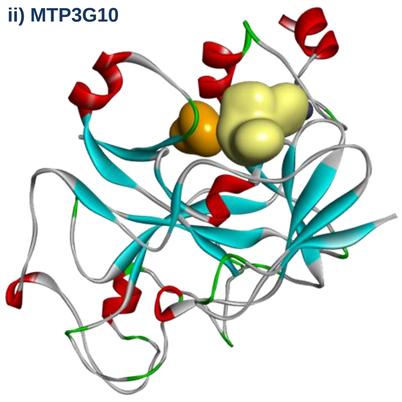

**STD-NMR confirmation spectra for five fragments.**
Spectrum A (black) shows the NMR-STD confirmation for each fragment, as well as the SAM co-binder.
Spectrum B (red) shows the reference spectrum of each fragment and the SAM co-binder (8.16 ppm) in PBS, pH 8.5 (10 % D2O).
Spectrum C (grey) is the false 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.

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

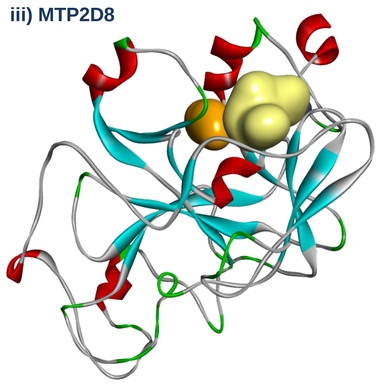
**i) MTP3B6**



**ii) MTP3G10**



**iii) MTP2D8**



## 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.

## 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