Protein purification of SETD8: a structure and inhibition study

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Background

- SETD8 is the only methyltransferase that is known to target and monomethylate lysine 20 in histone H4 (H4K20). H4K20 methylation by SETD8 hinders DNA replication by blocking acetylation in G1 phase, suppresses p53 activation in cancer cells, and SETD8 overexpression is present in different cancers.
- There are limited known inhibitors that bind to SETD8. Inhibitors may bind to the cofactor, substrate, or possible allosteric sites. Using the "fragment drug discovery" method, we are testing small compounds (fragments) to structurally analyze their binding mode to SETD8. Combining these fragments that bind in different sites of SETD8 could lead to better inhibitors that may be used in cancer treatments.
- The construct of SETD8 containing the catalytic domain was used for the study.

Methods

Our collaborators used Differential Scanning Fluorimetry (DSF) and Surface plasmon resonance (SPR) to screen a library of compounds for possible binding inhibitors of SETD8

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SETD8 overexpression in *E. coli.* Nickel affinity and size exclusion chromatography for purification

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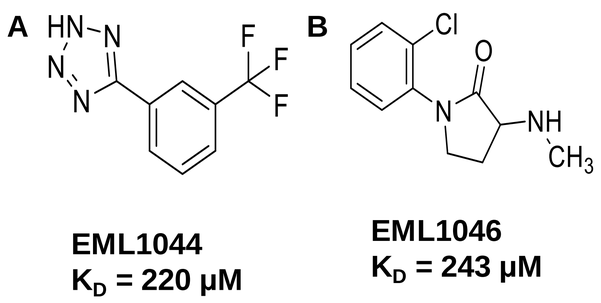
Protein crystallography and X-ray diffraction for structures and find binding location

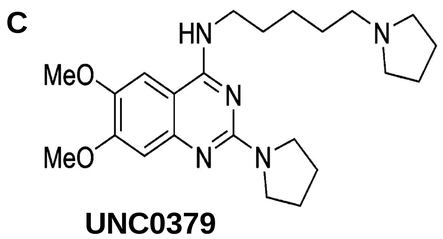
- SETD8 activity was measured using a bioluminescence assay (MTase-Glo™, Promega), in which the by-product SAH is converted into ATP and is detectable by luciferase reaction.

Results



EML1044
K_D = 220 µM

EML1046
K_D = 243 µM



UNC0379
K_D = 36.0 ± 2.3 µM

Figure 1. A and **B** correspond to two of the strongest binding compounds used in this study, these compounds were provided by our collaborators[3]. **C** corresponds to a confirmed binding inhibitor that has been proven to have high binding affinity to SETD8[1,2].

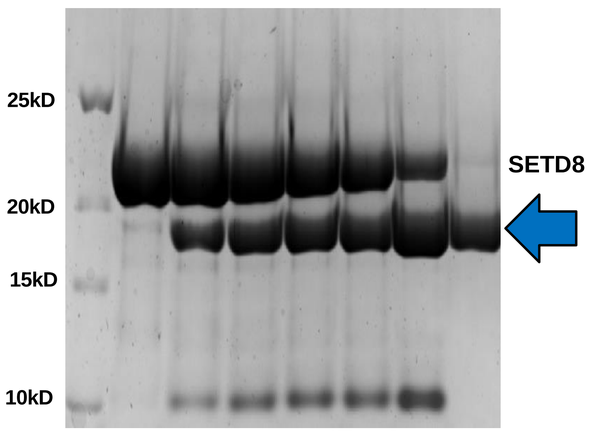


Figure 2. Different time plots were measured after the second Ni column in a Coomassie gel that showed the added Ni His-tag being cut by a protease, leaving only purified SETD8. SETD8 appeared in the last lane between the 20kD-15kD which is accurate to the molecular weight of SETD8 (18743.13). The last band in the gel indicates the cut His-tag that stayed stuck in the column.

- We obtained approximately 150 mg of purified protein from 2 L. After purifying SETD8 using size exclusion chromatography, we tried growing protein crystals with the purified protein under different conditions.

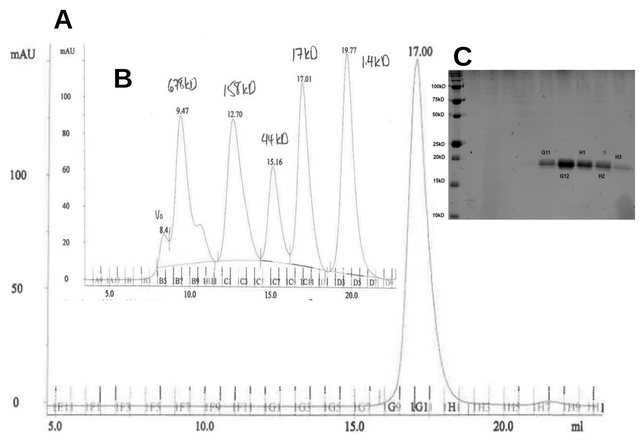


Figure 3. A- To get a more accurate reading on our purified protein, we decided to take fractions that came off a preparative sizing column and put through an analytical column. **B-** Known markers including Myoglobin (17kD), γ-globulin (158kD), and Thyroglobulin (670kD). **C-** Gel of purified SETD8 determined to be a monomer with a size of 18kD.

- No apo-crystals were formed using the long protein construct of SETD8. We were successful in producing crystals with protein and SAM cofactor using a citrate salt after screening hundreds of conditions that included several salts, buffers, and other precipitating agents.

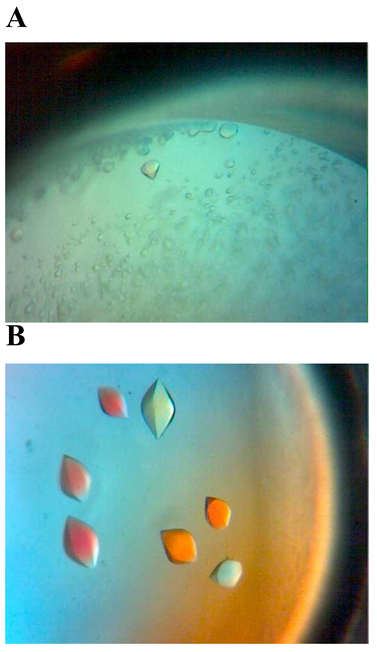


Figure 3. **A**- Using the sitting drop method, we obtained a small crystal using SETD8 (16mg/mL) and SAM with a screen condition of 1.4M sodium citrate tribasic and .1M HEPES pH7.5. **B-** Improved protein crystal using SETD8(16mg/mL) and SAM in a screen condition of 1.2M sodium citrate tribasic and .1M HEPES pH7.6.

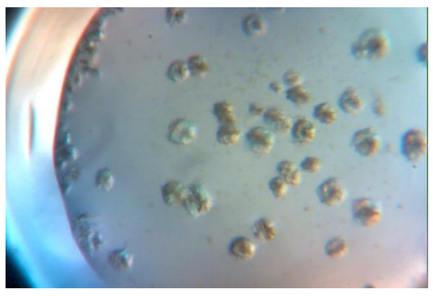


Figure 4. Crystallization using condition referred in Figure 3.B was used along compound EML1046 (Figure 1.B). Results were inconclusive because crystals did not appear like the previous crystals. Produced crystals were not useful in the present form, we cannot confirm what the crystals are, but they might contain the binding compound tested.

Conclusions

- Further testing is required to know the exact binding location of the binding compounds.
- Using x-ray diffraction data from SETD8 crystals, we can better understand the binding location of possible inhibitors. We can use this data to modify the compounds in the hopes that someday this information may lead to cancer treatment drugs.

Acknowledgements

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References

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3. Milite, C., Feoli, A., Viviano, M. et al. The emerging role of lysine methyltransferase SETD8 in human diseases. Clin Epigenet 8, 102 (2016).