SURFACE PLASMON RESONANCE BINDING STUDIES OF EPIGENETIC TARGETS

Rebecca Eells, Qing Chen, Lynn Baker, Haiching Ma

Reaction Biology, 1 Great Valley Pkwy, Malvern, PA, 19355, USA

Introduction

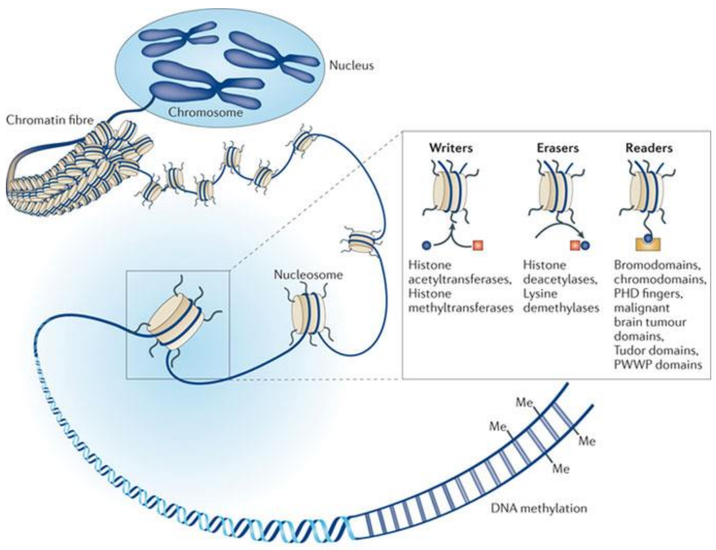
Epigenetic modifications are dynamic and reversible processes that regulate gene expression via chromatin modifications and do not alter the sequence of the DNA.

The proteins that participate in epigenetic modifications can be categorized as:

- Writers = covalently modify the chromatin
- Readers = recognize the modifications
- Erasers = remove the modifications

While essential for normal cellular function, abnormal expression or alterations can lead to disease, which make these epigenetic regulators an attractive target for drug discovery and development.

At Reaction Biology we offer a suite of services and products for drug discovery including the largest panel for epigenetic screening and profiling in the industry.



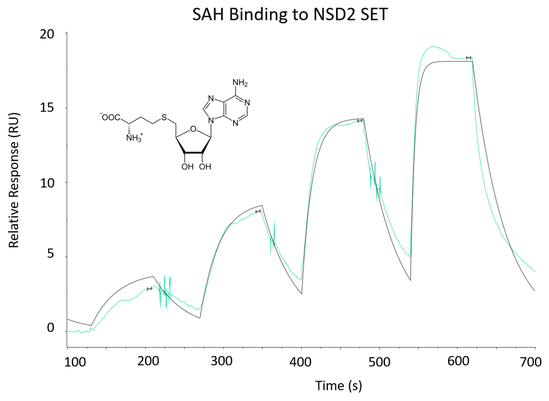
Arrowsmith *et. al.* (2012)
Nature Reviews | Drug Discovery

Writers: NSD2

Nuclear receptor-binding set domain protein 2 (NSD2) is a histone lysine methyltransferase that transfers the methyl group of lysine residues to histones H3 and H4 using S-adenosylmethionine (SAM) as the methyl donor. NSD2 has been implicated in oncogenesis and presents an attractive therapeutic target for a variety of cancers. However, few small molecule inhibitors have been reported.

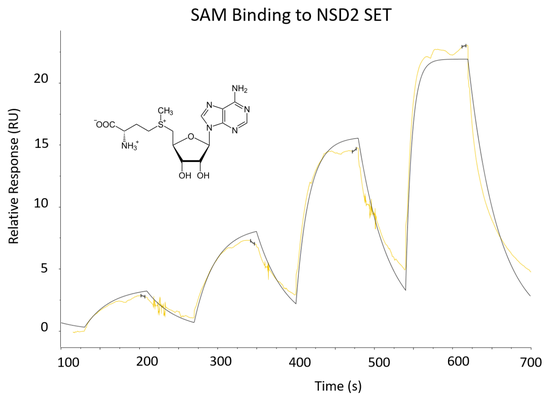
Here we present an example of the NSD2 SPR assay developed at Reaction Biology Corporation (RBC). Here we present the binding kinetics of the co-factor SAM and product SAH (S-adenosylhomocysteine) to the SET domain of NSD2. Using the established conditions, and SAM and SAH as control, the NSD2 Set SPR assay can be used for large scale screens of compound/fragment libraries or to support an on target mechanism of action for inhibitors identified from other screens (1).

SAH Binding to NSD2 SET



Analyte Concentration (µM)
25.00
8.33
2.78
0.93

SAM Binding to NSD2 SET



Analyte	ka (1/Ms)	kd (1/s)	KD (M)	$t_{1/2}$ (s)
SAM	4.33E+03	2.57E-02	5.94E-06	27
SAH	6.83E+03	2.38E-02	3.48E-06	29

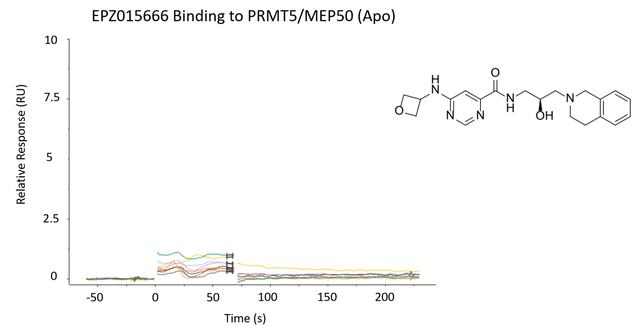
Writers: PRMT5

Protein arginine methyltransferase 5 (PRMT5) belongs to a group of enzymes that are responsible for arginine methylation of both histones and other cellular proteins using SAM as the methyl donor. PRMT5 is involved in cell death, cell growth proliferation, and cell cycle progression and has emerged as an attractive drug target due to its role in tumorigenesis.

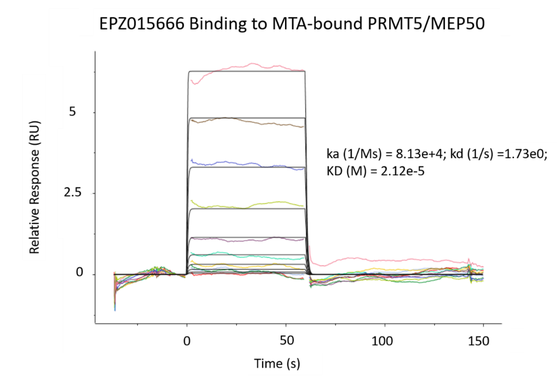
Inhibitors of PRMT5 can be competitive, noncompetitive, uncompetitive with respect to the substrate and SAM (or SAM analogues). Here we show SPR binding data for SAH and three known PRMT5 selective inhibitors:

- **EPZ015666** = a substrate competitive inhibitor that binds only in the presence of SAM or SAM analogue (2).
- **LLY-283** = an inhibitor that binds the SAM-binding pocket but appears to be noncompetitive for both SAM and substrate (3).
- **JNJ-64619178** = a pseudo-irreversible inhibitor that binds the SAM-binding pocket and reaches into the substrate pocket (4).

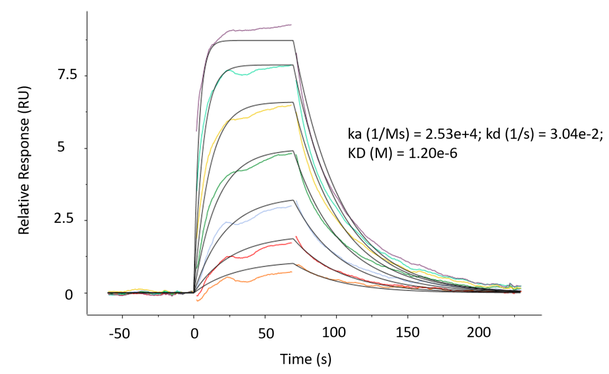
EPZ015666 Binding to PRMT5/MEP50 (Apo)



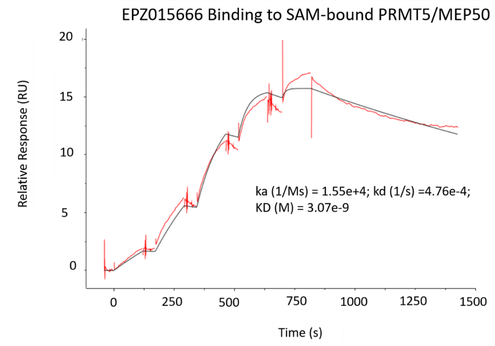
EPZ015666 Binding to MTA-bound PRMT5/MEP50



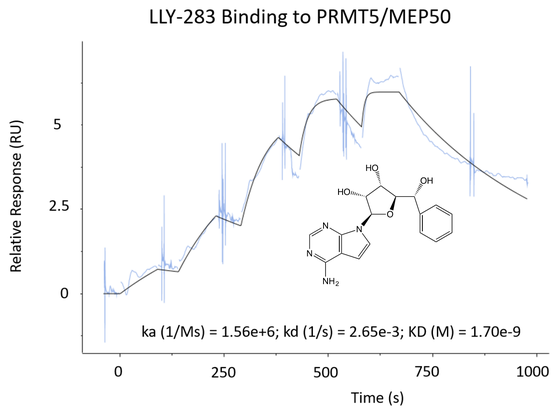
EPZ015666 Binding to SAH-Bound PRMT5/MEP50



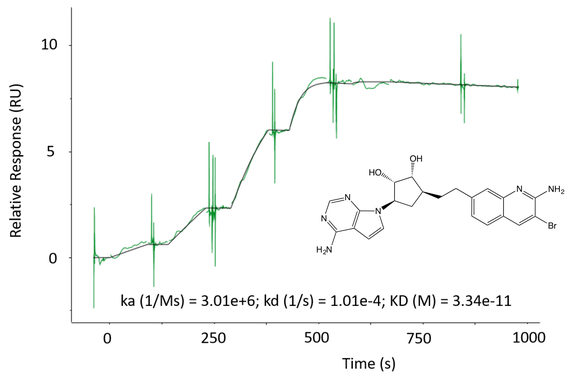
EPZ015666 Binding to SAM-bound PRMT5/MEP50



LLY-283 Binding to PRMT5/MEP50



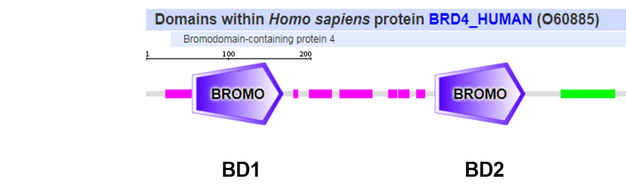
JNJ-64619178 Binding to PRMT5/MEP50

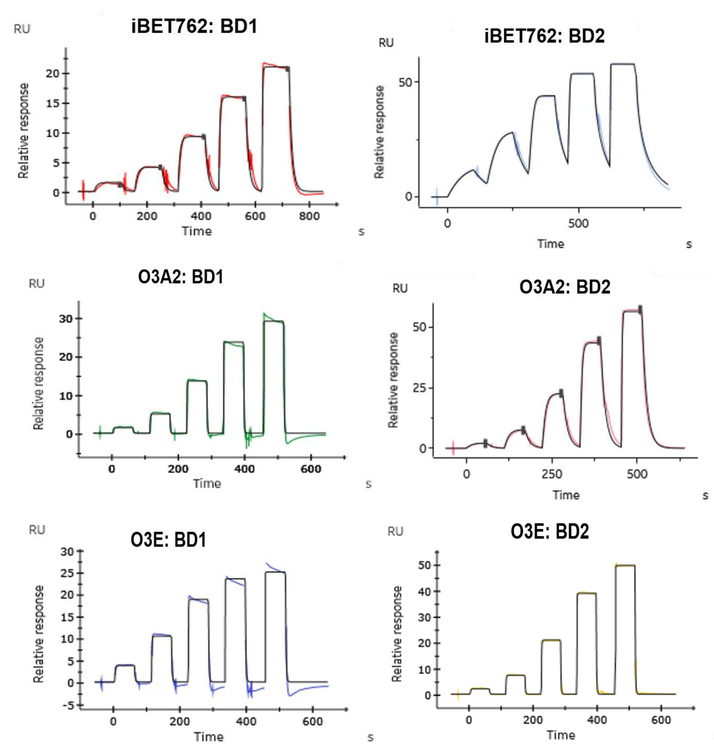


Readers: Bromodomain 4

Bromodomains are epigenetic readers that recognize acetylated lysine residues on histones and other proteins. A well-known bromodomain family is the BET protein family, which is characterized by the presence of two tandem bromodomains and an extra terminal domain. Members of the BET family have been linked to diseases including: multiple sclerosis, inflammation, and viral replication (5) – making them an attractive drug target.

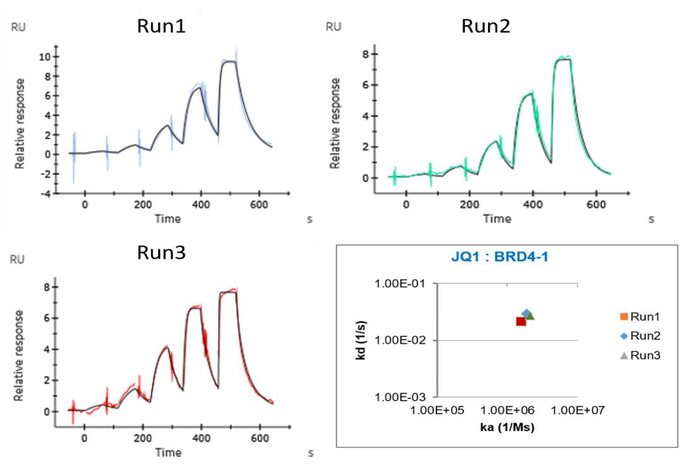
The similarity in sequence and domain organization across members of the mammalian BET family presents a challenge for drug development since domain or isoform specific inhibitors are necessary to avoid adverse effects in non-target tissues. Here we present BRD4 selectivity data from an SPR study using both commercial (iBET762) and RBC designed compounds (O3 series).





Compound: Domain	ka (1/Ms)	kd (1/s)	KD (M)
iBET762:BD1	2.39e6	9.39e-2	3.77e-8
iBET762:BD2	1.44e6	2.56e-2	1.78e-8
O3A2:BD1	5.06e5	8.37e-1	1.65e-6
O3A2:BD2	3.35e5	7.4e-2	2.21e-7
O3E:BD1	2.42e6	5.48e-1	2.26e-7
O3E:BD2	3.59e5	7.28e-1	2.03e-6

Data Reproducibility: JQ1 Binding to BRD4-1



References

1. Coussens *et. al.,* (2018) J. Biol. Chem. 293(35): 13750-13765.
2. Chan-Penebre *et. al.* (2015) Nat. Chem. Biol. 11(6): 432-437.
3. Bonday *et. al.* (2018) ACS Med. Chem. Lett. 9: 612-617.
4. Brehmer *et. al.* (2017) AACR Annual Meeting, Abstract DDT02-04.
5. Belkina and Denis (2014) Nat. Rev. Cancer 12(7): 465-477/

Contact Information

Rebecca Eells

Reaction Biology
1 Great Valley Pkwy #18
Malvern, PA 19355

Tel: +1 610-722-0247 (ext. 215)
Email: Rebecca.Eells@reactionbiology.com
Web: www.reactionbiology.com