SURFACE PLASMON RESONANCE BINDING STUDIES OF EPIGENETIC TARGETS

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Introduction

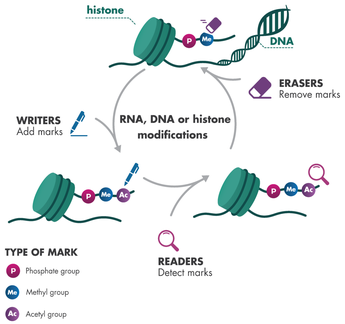
Epigenetic modifications are dynamic and reversible processes that regulate gene expression via chromatin modifications and do not alter the sequence of the DNA.

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.

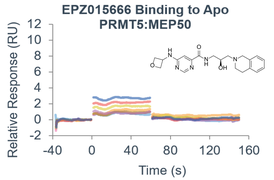


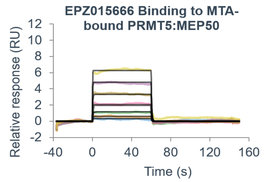
Writers: PRMT5

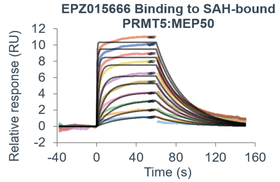
Protein arginine methyltransferase 5 (PRMT5) belongs to a group of enzymes that are responsible for arginine methylation of both histones and other cellular proteins using SAM as the methyl donor. PRMT5 is involved in cell death, cell growth proliferation, and cell cycle progression and has emerged as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

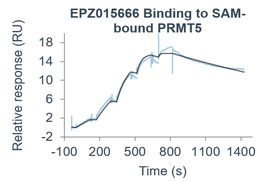
Inhibitors of PRMT5 can be competitive, noncompetitive, uncompetitive with respect to the substrate and SAM (or SAM analogues). Here we show SPR binding data for SAH and three known PRMT5 selective inhibitors:

- **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).



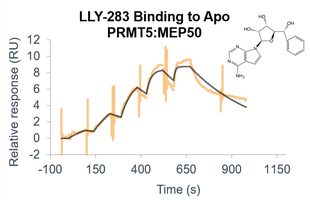


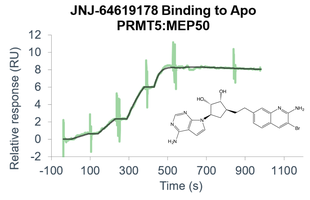




Target	Analyte	ka (1/Ms)	kd (1/s)	KD (M)
Apo PRMT5:MEP50	EPZ015666	Minimal signal changes/binding		
MTA+ PRMT5:MEP50	EPZ015666	6.64e+04	1.03E00	1.60E-05
SAH+ PRMT5:MEP50	EPZ015666	3.46E+04	3.64E-02	1.05E-06
SAM+ PRMT5:MEP50	EPZ015666	1.55E+05	4.76E-04	3.07E-09

Writers: PRMT5



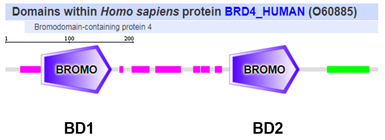


Target	Analyte	ka (1/Ms)	kd (1/s)	KD (M)
Apo PRMT5:MEP50	LLY-283	1.17E+06	2.70E-03	2.31E-09
Apo PRMT5:MEP50	JNJ-64619178	1.69E+06	1.49E-04	8.81E-11

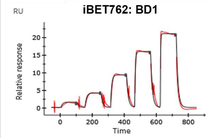
Readers: Bromodomain 4

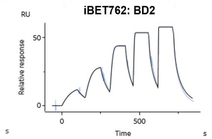
Bromodomains are epigenetic readers that recognize acetylated lysine residues on histones and other proteins. A well-known bromodomain family is the BET protein family, which is characterized by the presence of two tandem bromodomains and an extra terminal domain. Members of the BET family have been 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 Bromodomain 4 (BRD4) screening data from an SPR study using both commercial (iBET762) and Reaction Biology designed compounds (O3 series).

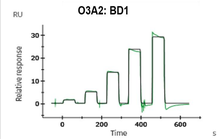


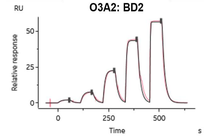
Readers: Bromodomain 4



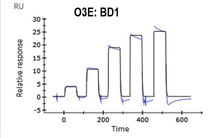


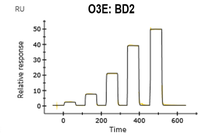
	BD1	BD2
ka (1/Ms)	2.39E+06	1.44E+06
kd (1/s)	9.39E-02	2.56E-02
KD (M)	3.77E-08	1.78E-08





	BD1	BD2
ka (1/Ms)	5.06E+05	3.35E+05
kd (1/s)	8.37E-01	7.40E-02
KD (M)	1.65E-06	2.21E-07





	BD1	BD2
ka (1/Ms)	2.42E+06	3.59E+05
kd (1/s)	5.48E-01	7.28E-01
KD (M)	2.26E-07	2.03E-06

References

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