Science For A Better Life

Chemical Probe BAY-850

ATAD2 Inhibitor

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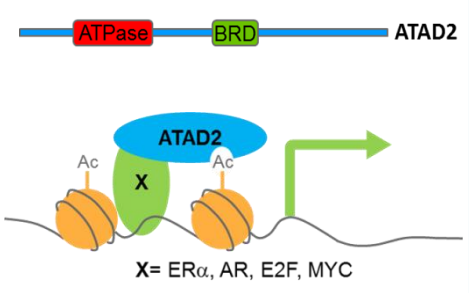
ATAD2 probe BAY-850

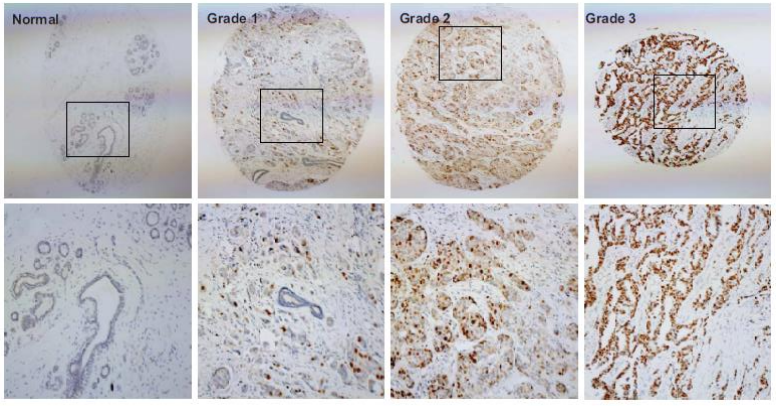
Scientific rationale - ATAD2 as an anti-cancer target

- ATAD2 domain structure: **AAA ATPase** and **bromodomain**
- AAA ATPase and bromodomain are both required for chromatin binding
- ATAD2 was published to function as a **transcriptional co-activator** of ERα, AR, E2F and c-myc
- ATAD2 is not significantly mutated or focally amplified in any cancer types
  → **i.e. there is no clear oncogenomic link**
- In most normal tissues ATAD2 is barely detectable (highly expressed only in testis)
- In several cancer types **ATAD2 is highly expressed**: ALL, CML, Ewing's sarcoma, AML, DLBCL, breast and lung cancer cells, and its expression correlates with the proliferation state of the cancer and with the poor patient prognosis
- ATAD2 is one of the "**70-gene signature**" that predict disease outcome and one of the "**76-gene signature**" that predict disease outcome and distant metastasis in breast cancer

Domain composition and proposed function of ATAD2

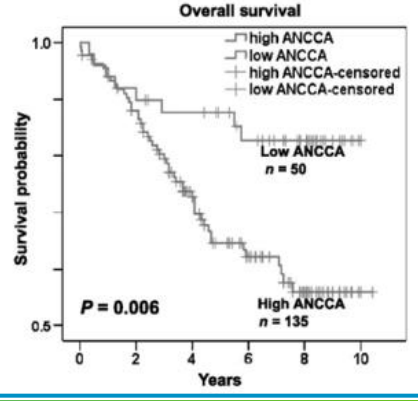


ATAD2 is highly expressed in high grade breast tumors (breast tissue samples)



Kalashnikova EV et al., 2010; Cancer Res

High ATAD2 expression correlates with poor prognosis of breast cancer patients (ATAD2 = ANCCA)



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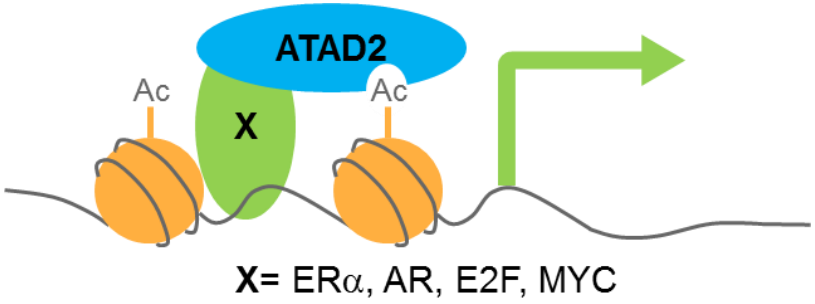
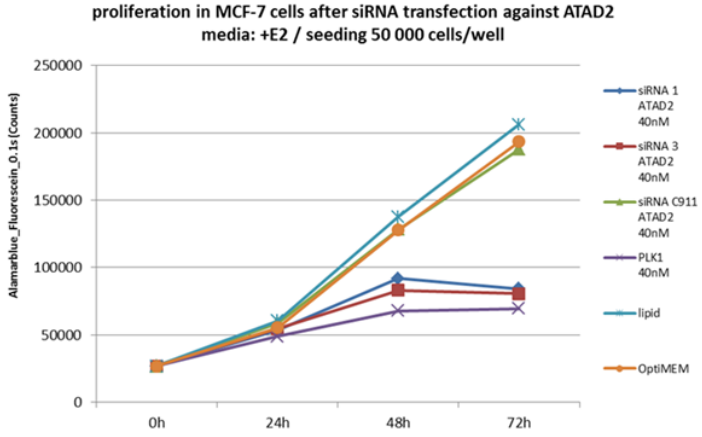
Disease hypothesis based on literature data

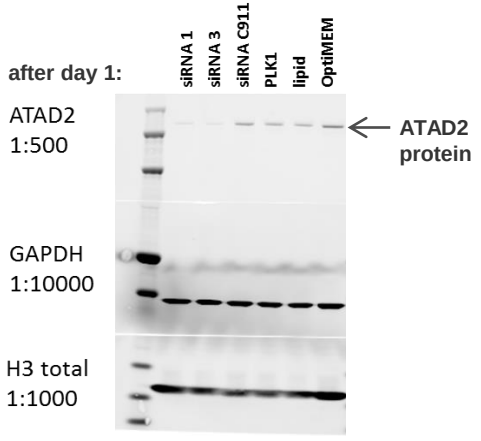
➢ Published literature:

- ATAD2 functions as a transcriptional co-activator of ERα, AR, E2F and C-MYC
- ATAD2 co-regulates estrogen- and androgen-induced gene expression required for cell proliferation
- ER-positive breast cancer cells are particularly sensitive to ATAD2 knockdown by RNAi



➢ Target validation:





Research Question: Can inhibition of ATAD2 bromodomain phenocopy gene knock down?

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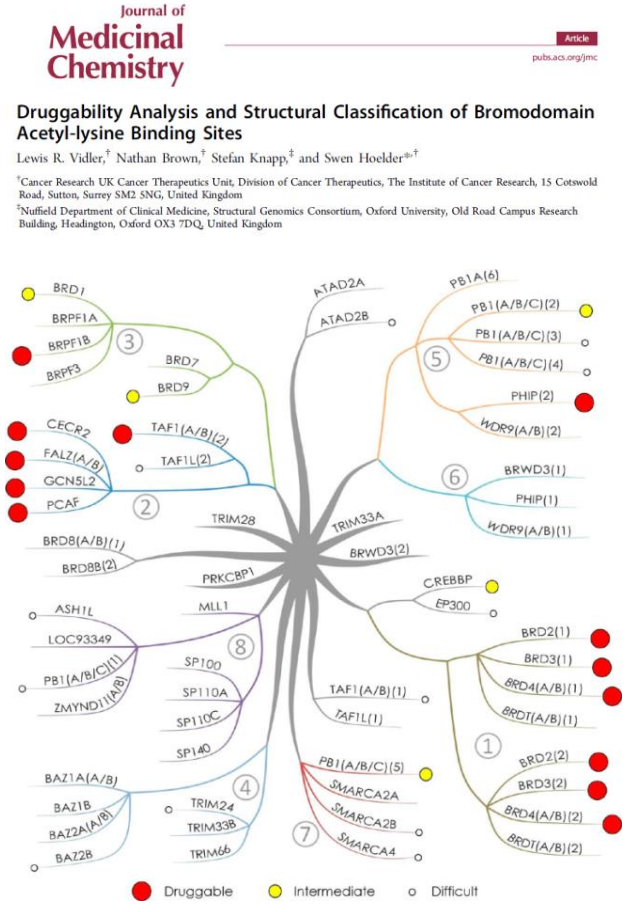
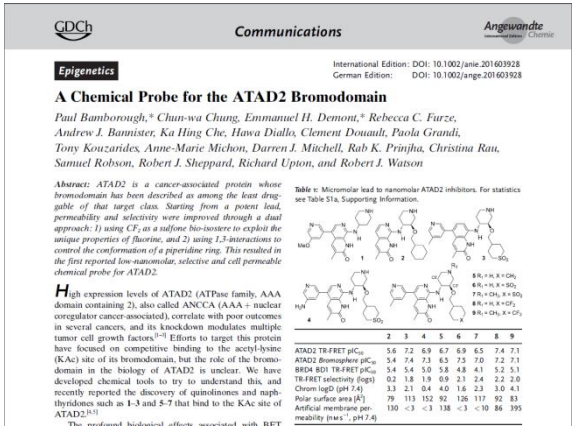
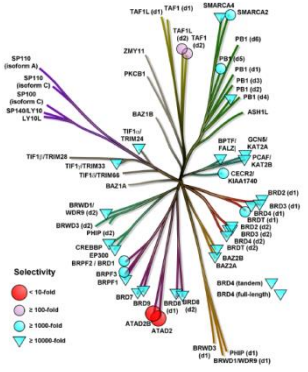
ATAD2 inhibitors: past and present

2012: „Difficult“ *J. Med. Chem.* **2012**, *55*, 7346



2016: Possible *Angew.Chem. Int.Ed.* **2016**, *55*,11382–11386

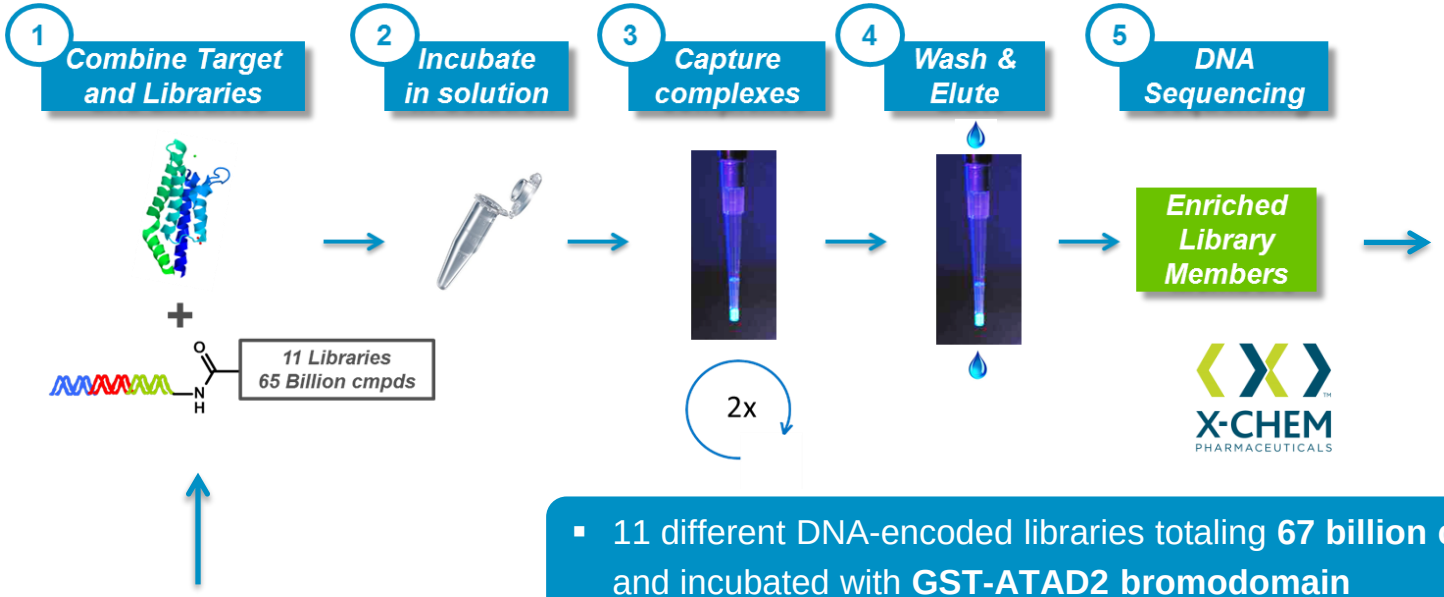




- ATAD2 has been classified as “hard to drug” BD
- Accordingly, literature on ATAD2 inhibitors was scarce until recently, when a series of promising ATAD2 inhibitors was reported by GSK (J Med Chem. 2015)
- The first selective & cell-active ATAD2 chemical probe is an orthosteric “BRD-like” inhibitor of both isoforms (ATAD2A and ATAD2B)

ATAD2 probe BAY-850

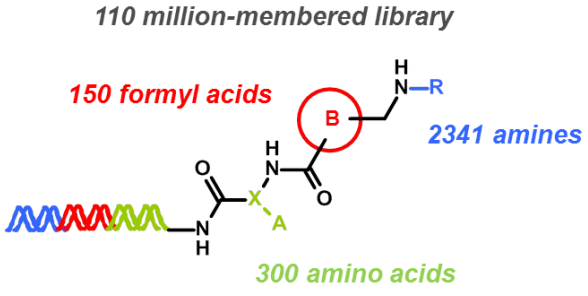
Lead Finding Strategy: DNA-Encoded Library Screen



„off-DNA“ synthesis and confirmation with:

- **TR-FRET**
- **Bromoscan™**
- **SPR**
- *TSA*
- *ITC*
- *MST*
- *NMR*
- *Native MS*

- **Initial hits**
- *Advanced compounds.*

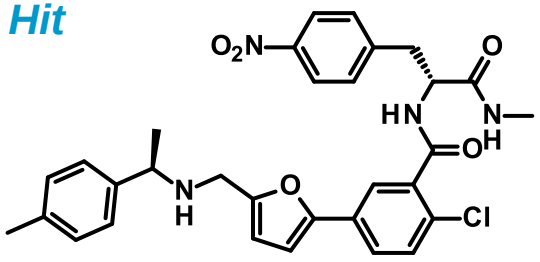


- 11 different DNA-encoded libraries totaling **67 billion compounds** combined and incubated with **GST-ATAD2 bromodomain**
- Affinity-mediated selection by capturing target on glutathione agarose, washing and elution steps followed by 2nd round of selection
- Eluted library members were amplified using PCR and deep-sequenced using the Illumina® platform
- Enriched combinations of building blocks were identified and used to design off-DNA compounds for re-synthesis
- Most promising hits were discovered within a **110-million member library** generated by combining 300 amino acids, 150 formyl acids and 2,341 amines

ATAD2 probe BAY-850

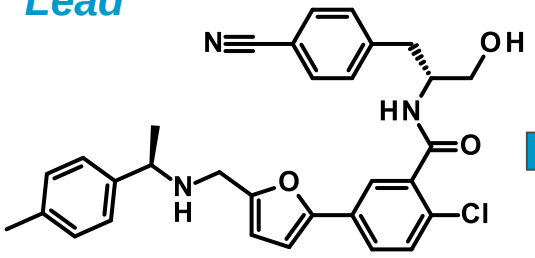
Probe discovery

Hit



- µM hit with an unprecedented structure for a BD inhibitor

Lead



- Sub-µM biochemical activity and cellular target engagement shown

Probe

BAY-850

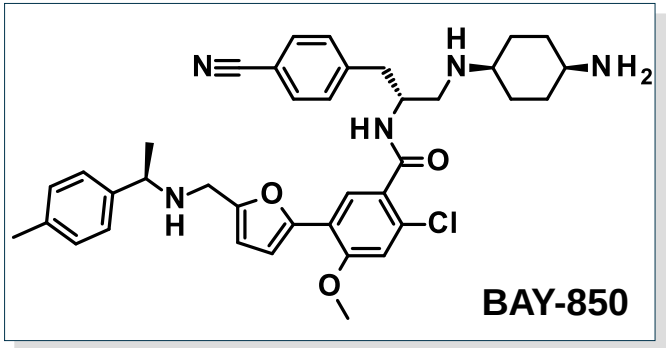
- Result of systematic SAR exploration

	Hit	Lead	BAY-850
ATAD2 HTRF IC_{50}	1.1 µM	370 nM	22-166 nM
ATAD2 TSA ΔT	n.d.	1.2 K	4-14.7 K
BRD4 BD1/BD2 IC_{50} (HRTF)	>20 µM	>20 µM	>20 µM
Cellular target engagement	No	Yes	Yes

Hit-to-Lead optimization started from a DNA encoded library hit to deliver BAY-850

ATAD2 probe BAY-850

Profile



- **Molecular Properties**

MW [g/mol]	654
MWcorr [g/mol]	638
TPSA [Å2]	125
Rotatable bonds	13

- **PhysChem**

Sw $^{pH\ 6.5}$ [mg/L]	>500*
log D (pH 7.5)	2.9

*(measurement of powdered HCl salt

- **Pharmacology**

ATAD2^{H4AcK12} $IC_{50}$$^{(HTRF)}$	0.17 µM
ATAD2$^{H4AcK5/8/12/16}$ $IC_{50}$$^{(HTRF)}$	0.02 µM
LLE^{logD}	2.6
ATAD2 TSA $\Delta T^{(100\mu M)}$	13.9°C
BRD4 ^{BD1} IC_{50} (HRTF)	>20 µM
BRD4^{BD2} IC_{50} (HTRF)	>20 µM
DiscovereX BromoScan™	ATAD2A @ 10 µM
DiscovereX ATAD2A K_D	0.12 µM
MST K_D	0.08 µM
ITC K_D	0.75 µM

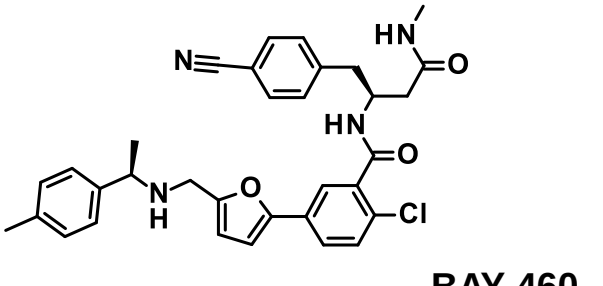
- **In vitro PK**

		Clint [L/h/kg]	Fmax [%]
LM	Human	1.2	53
	Mice	3.5	61
	Rat	1.5	73
Hep	Rat	1.9	69

CaCo2	A-B [nm/s]	B-A [nm/s]	Ratio
	39	11	0.3

ATAD2 negative control BAY-460

Profile



BAY-460

▪ **Molecular Properties**

MW [g/mol]	569
MWcorr [g/mol]	553
TPSA [Å2]	107
Rotatable bonds	11

▪ **PhysChem**

$Sw^{pH\ 6.5}$ [mg/L]	6.0
log D (pH 7.5)	3.2

▪ **Pharmacology**

$ATAD2^{H4AcK12}$ $IC_{50}^{(HTRF)}$	>20 µM
$ATAD2^{H4AcK5/8/12/16}$ $IC_{50}^{(HTRF)}$	16 µM
LLE^{logD}	<1
ATAD2 TSA $\Delta T^{(100\mu M)}$	0.1°C
BRD4 ^{BD1} IC_{50} (HRTF)	>20 µM
$BRD4^{BD2}$ IC_{50} (HTRF)	>20 µM
Discoverex BromoScan™	No hits @ 10 µM
Discoverex ATAD2A K_D	n.d.
MST K_D	n.d.
ITC K_D	n.d.

▪ **In vitro PK**

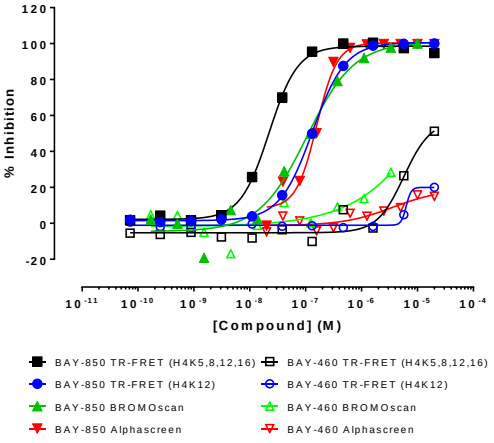
		Clint [L/h/kg]	Fmax [%]
LM	Human	13.2	9
	Mice	76.2	7
	Rat	48.9	8
Hep	Rat	34.5	11

CaCo2	A-B [nm/s]	B-A [nm/s]	Ratio
	37	60	1.6

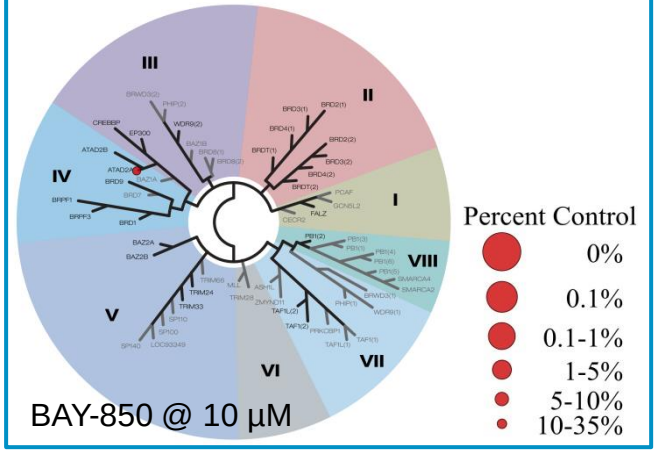
ATAD2 probe BAY-850

Biochemical potency and selectivity

Biochemical Assays (HTRF, Alphascreen, BROMOscan)

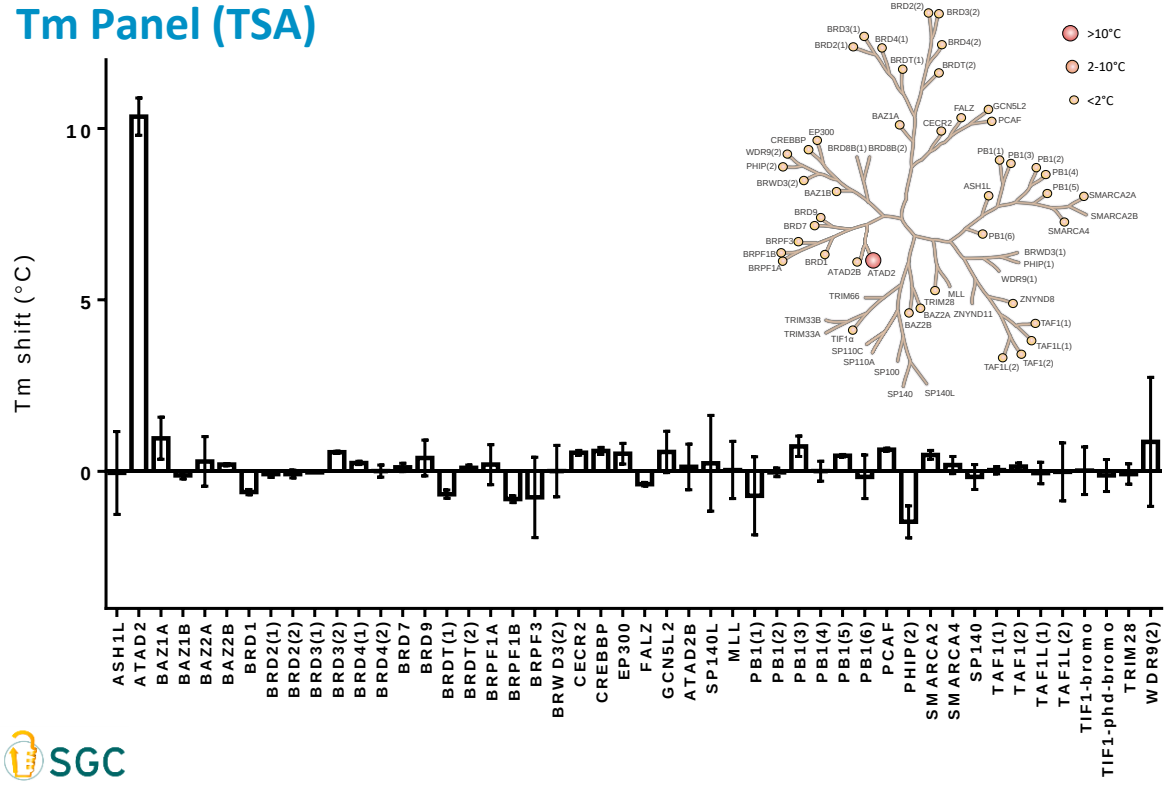


BROMOscan™ (DiscoverX)



BAY-850 @ 10 µM

Tm Panel (TSA)



SGC

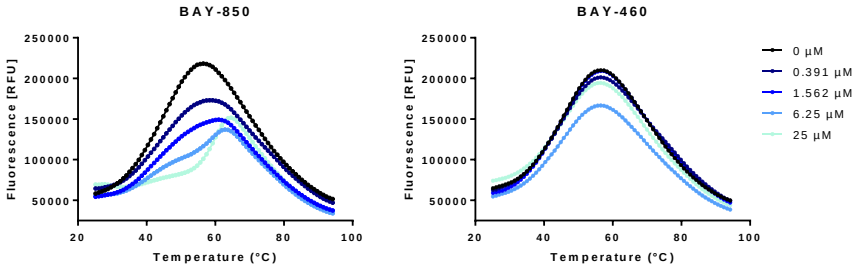
BAY-850 is a potent and selective ATAD2A inhibitor in:

- In-house and external panels
- Orthogonal biochemical and biophysical assays

ATAD2 probe BAY-850

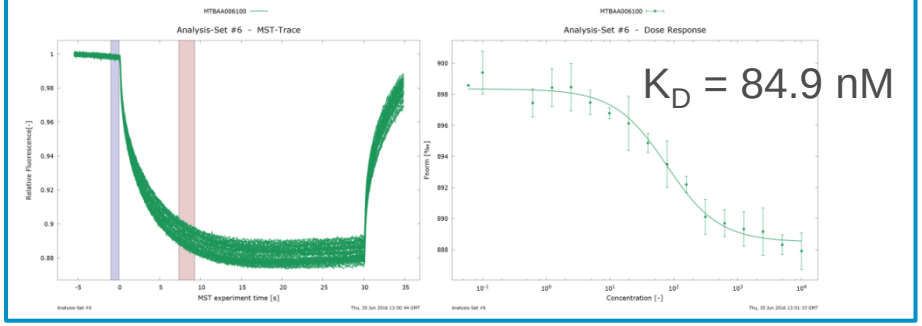
Biophysical validation and MoA studies

TSA: Dose-dependent thermal stabilization

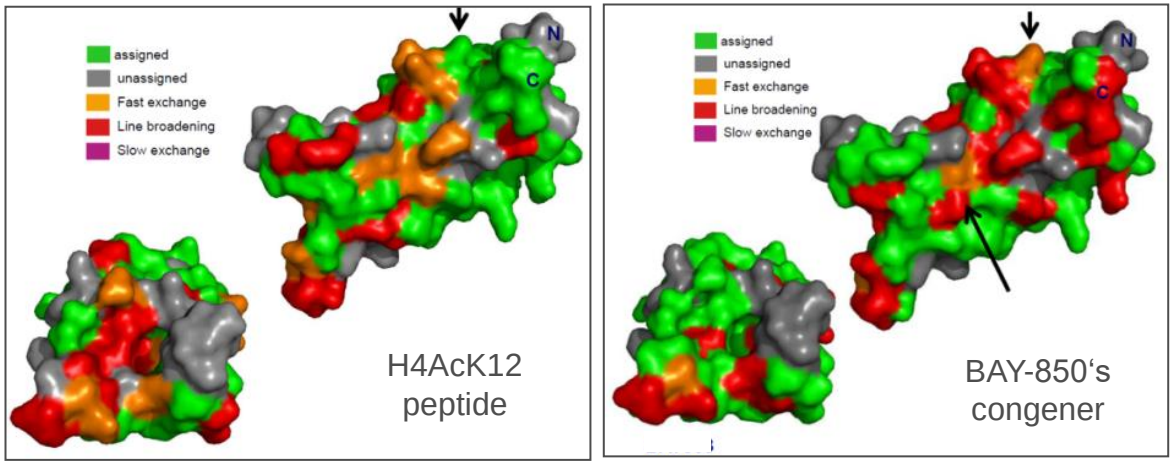


MST: Nanomolar affinity

SGC



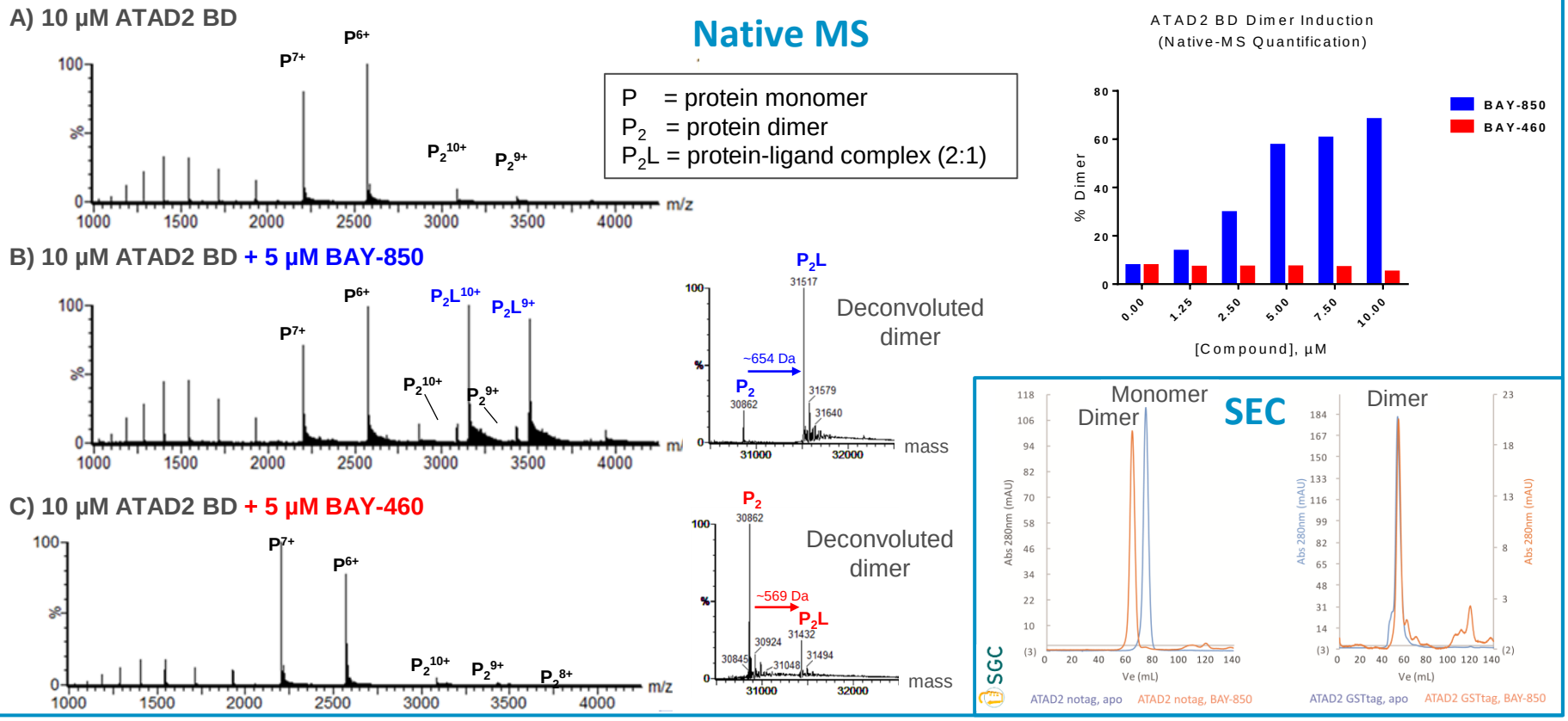
NMR: Specific binding to new sites



- Dose-dependent thermal stabilization of tag-free ATAD2 bromodomain
- Saturatable, dose-dependent thermophoresis with K_D<100 nM
- Dose-dependent NMR chemical shift induction (=target engagement)

ATAD2 probe BAY-850

Biophysical validation and MoA studies

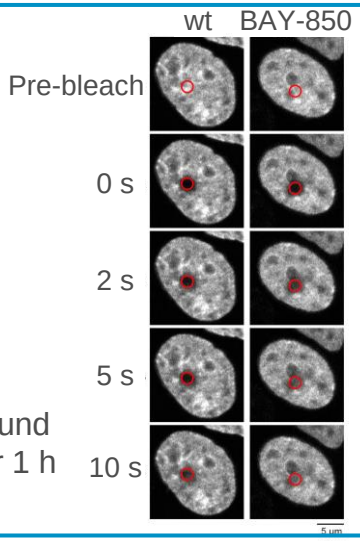


BAY-850 induces dose-dependent dimerization upon binding to ATAD2, whilst acetylated histone peptides and AcK mimetic ligands do not induce dimerization (not shown)

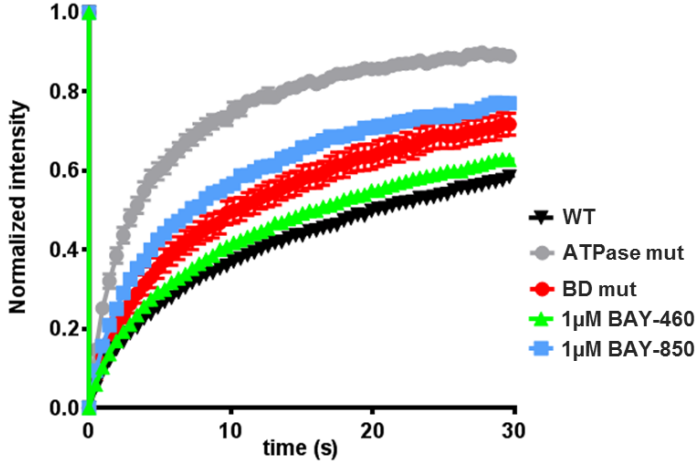
ATAD2 probe BAY-850

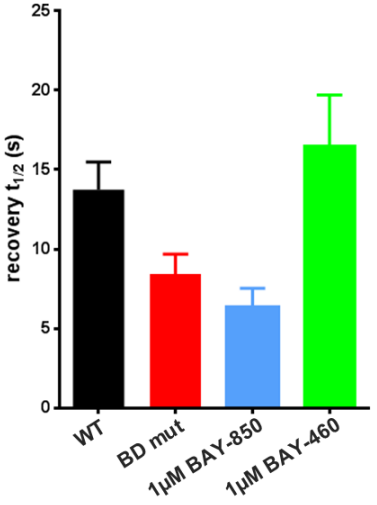
Cellular target engagement & pharmacology

FRAP



- **MCF7** cells
- **1µM** Compound treatment for 1 h at 37 °C





Proliferation inhibition

	H4 tetraAc pep	H4K12Ac pep	MCF7		NCI-H526		MDA-MB-231	
cpd	IC_{50} biochem	IC_{50} biochem	IC_{50}	MaxInh(%)	IC_{50}	MaxInh(%)	IC_{50}	MaxInh(%)
BAY850	22 nM	170 nM	3,2 µM	100 %	2,40 µM	100 %	1,84 µM	99,9 %
BAY460	16 µM	> 40 µM	>30 µM	0,5 %	>30 µM	0 %	24,7 µM	60,8 %

- BAY-850 (1 µM) displaces ATAD2 from chromatin to the same extend as BD mutation
- BAY-850 inhibits the proliferation of three different cancer cell lines (in line with KD results)

ATAD2 probe BAY-850

Summary / Conclusion

- BAY-850 is an ATAD2 inhibitor fulfilling all chemical probe criteria:
  - Nanomolar biochemical activity (IC_{50}/K_D in 20-150 nM range)
  - Favorable membrane permeability and maximal on-target cellular activity at 1 µM (FRAP assay in MCF7 cells)
  - Selective against all family members (ΔTm and BromoScan™ panels). No additional/off target activities are expected from this structure class

- Additionally, a structurally related compound with no relevant ATAD2 activity was identified and will be provided as negative probe (BAY-460)

- ***BAY-850 is a potent, cellularly active and exquisitely selective ATAD2 BD inhibitor, which will allow to further study the biology of ATAD2 in vitro***

- ***BAY-850 represents a novel and unprecedented chemotype for a BRD inhibitor***

We ask for acceptance of ATAD2 inhibitor BAY-850 as chemical probe, accompanied by BAY-460 as negative control

ATAD2 probe BAY-850

Acknowledgements

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Marcus Bauser
Ursula Egner

Cora Scholten
Marion Hitchcock

Apirat Chaikuad‡
James Bennett‡
Kilian Huber‡
Laura Díaz-Sáez‡
Oleg Fedorov‡

X-Chem Pharmaceuticals

EDELRIS

**Bayer Core Team*
‡SGC

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Thank you!

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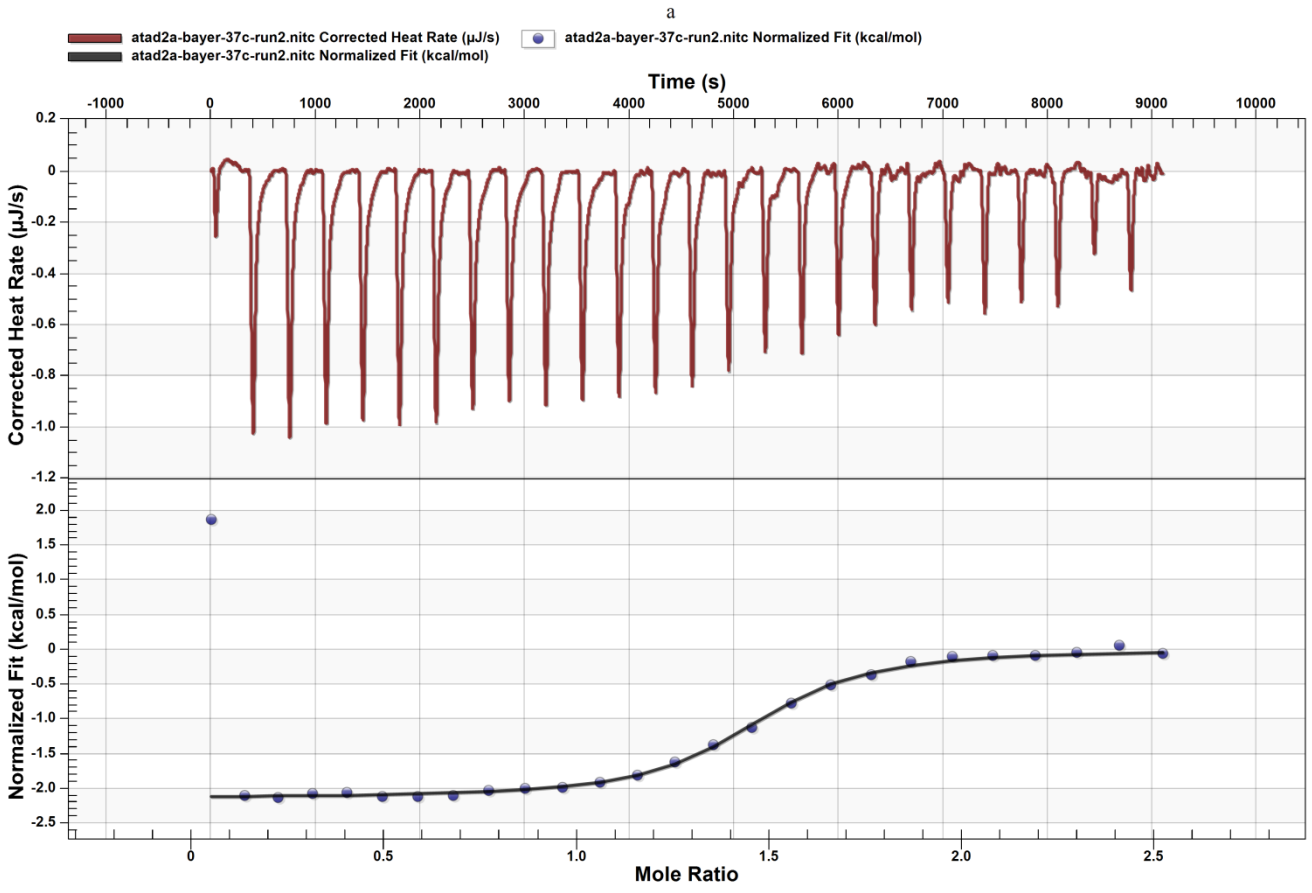
Backup

ATAD2 probe BAY-850

Biophysical validation and MoA studies

ITC*: Sub-micromolar affinity and atypical stoichiometry (> 1)

Kd=753.3 nM
N=1.45
DH=-2.16 kcal/mole
DS= 21.11 cal/mole◦K



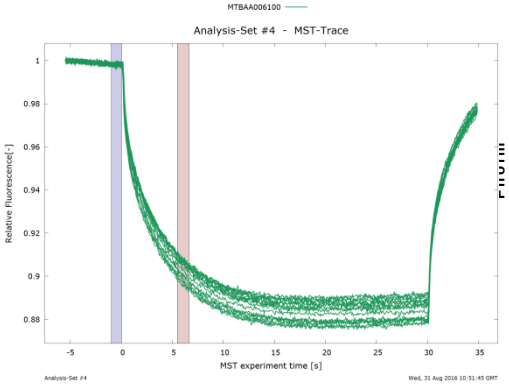
*: Experiments were performed at 37 °C (no binding at RT)

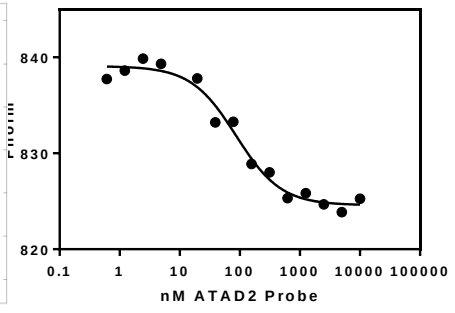
ATAD2 probe BAY-850

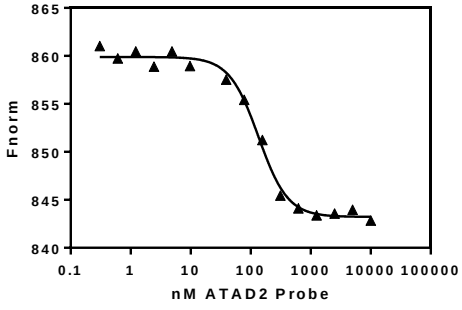
Biophysical validation and MoA studies

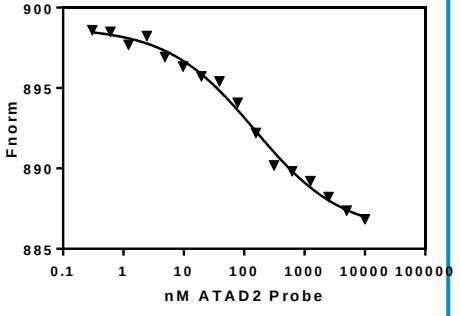
MST: Binding is independent from GST tag

GST-ATAD2: Binding with Kd = 84.9 nM

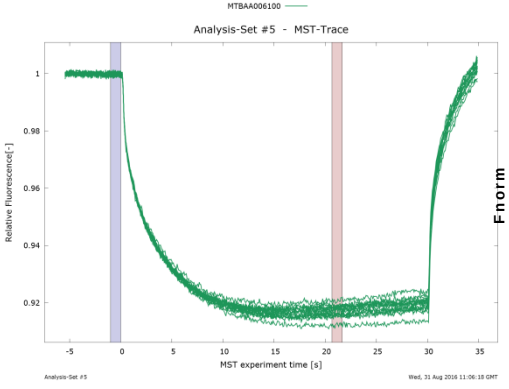


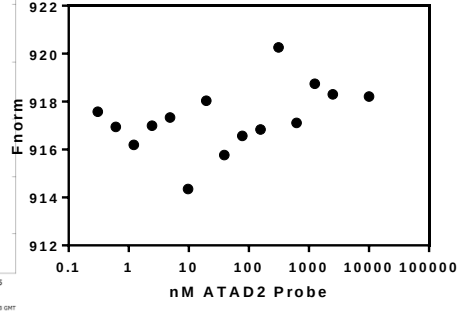


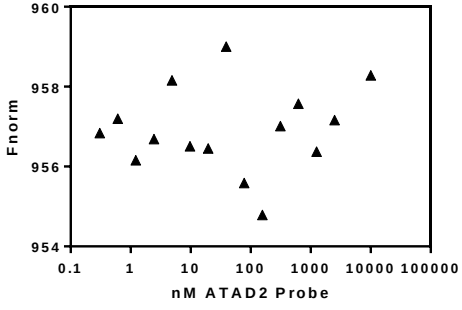


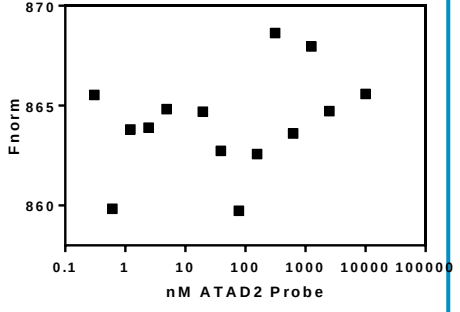


GST-BRD9: No binding







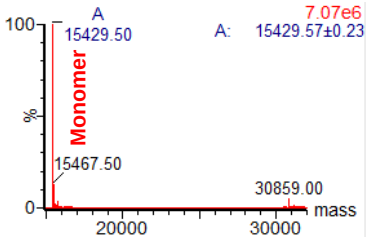


ATAD2 probe BAY-850

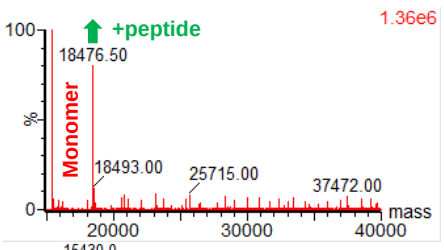
Biophysical validation and MoA studies

Native MS: specific binding to dimerized ATAD2

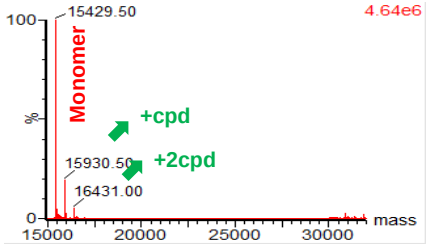
DMSO



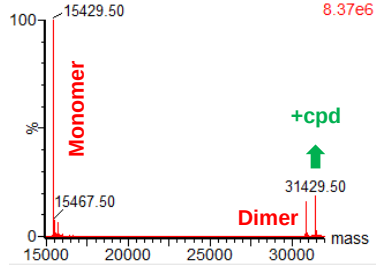
DMSO + Peptide (H4AcK12)



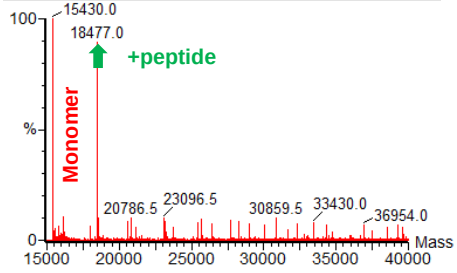
AcK-mimetic Tool Cpd.



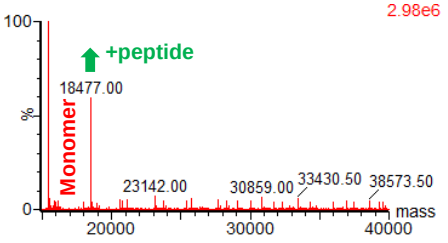
BAY-850



BAY-850 + Peptide (H4AcK12)



Tool Cpd. + Peptide (H4AcK12)



- BAY-850 binds to ATAD2 dimers not present in DMSO treated samples
- H4AcK12 peptide binds to ATAD2 monomers and prevents binding of BAY-850
- AcK mimetic compounds bind to ATAD2 monomers and H4AcK12 peptide prevents their binding