



Science For A Better Life



# Chemical Probe BAY-850

ATAD2 Inhibitor

Matyas Gorjanacz, Markus Berger and Amaury Fernández-Montalván

December 8<sup>th</sup>, 2016

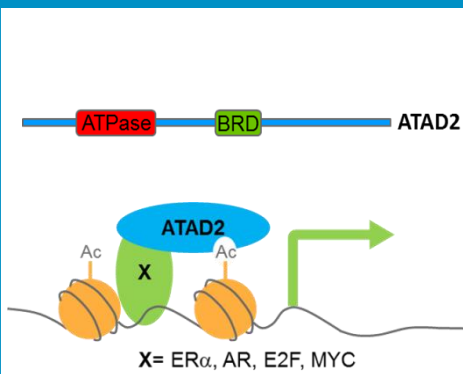
# ATAD2 probe BAY-850



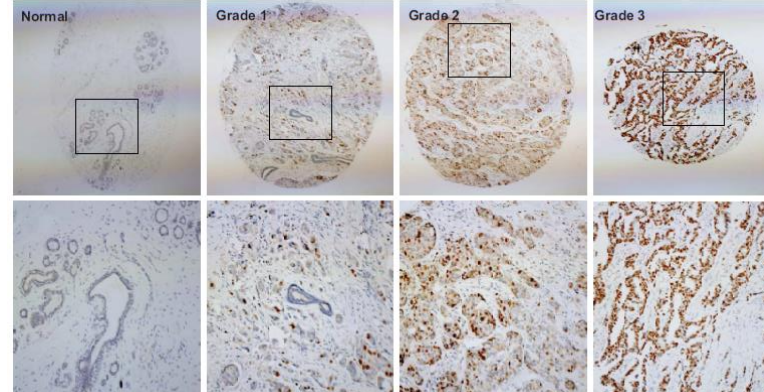
## Scientific rationale - ATAD2 as an anti-cancer target

- 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 ER $\alpha$ , 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

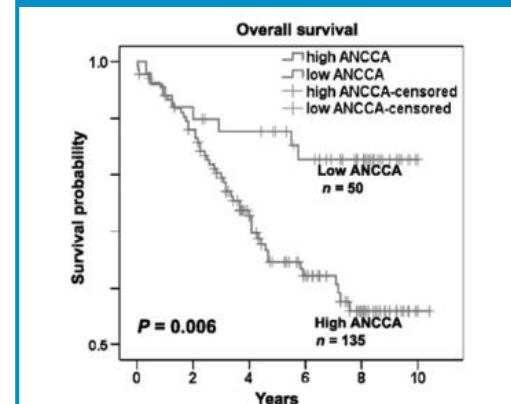


### ATAD2 is highly expressed in high grade breast tumors (breast tissue samples)



Kalashnikova EV et al., 2010; Cancer Res

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



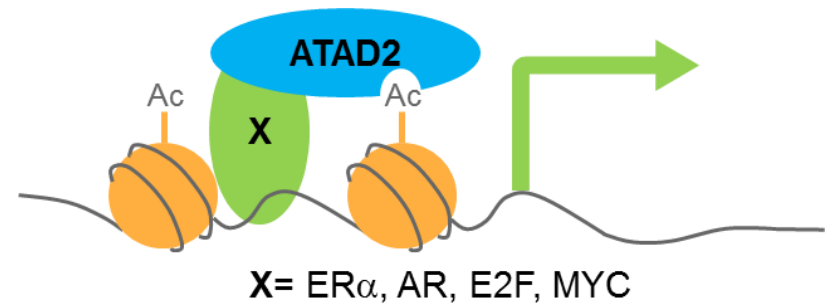
# ATAD2 probe BAY-850



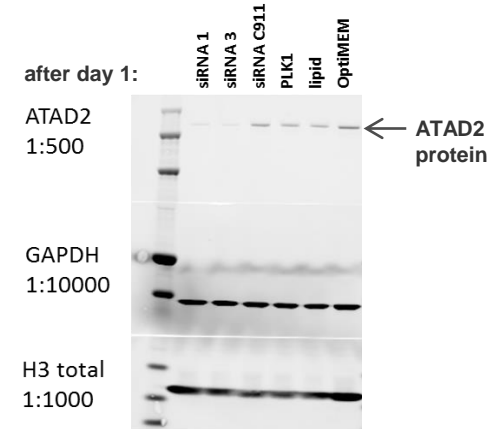
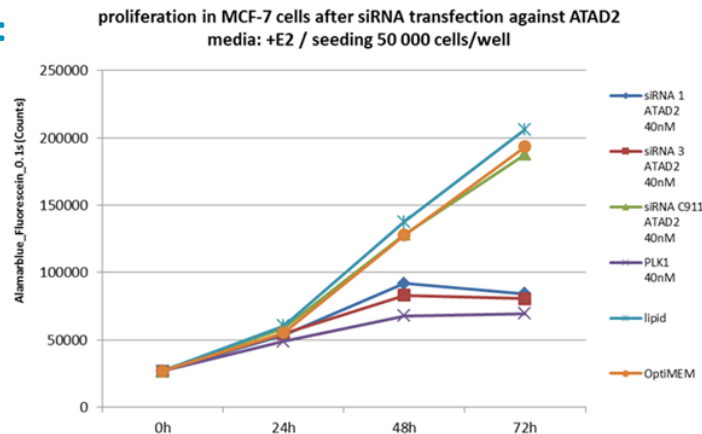
## Disease hypothesis based on literature data

### Published literature:

- ATAD2 functions as a transcriptional co-activator of ER $\alpha$ , 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?

# ATAD2 probe BAY-850

## ATAD2 inhibitors: past and present



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

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

Journal of  
**Medicinal  
Chemistry**

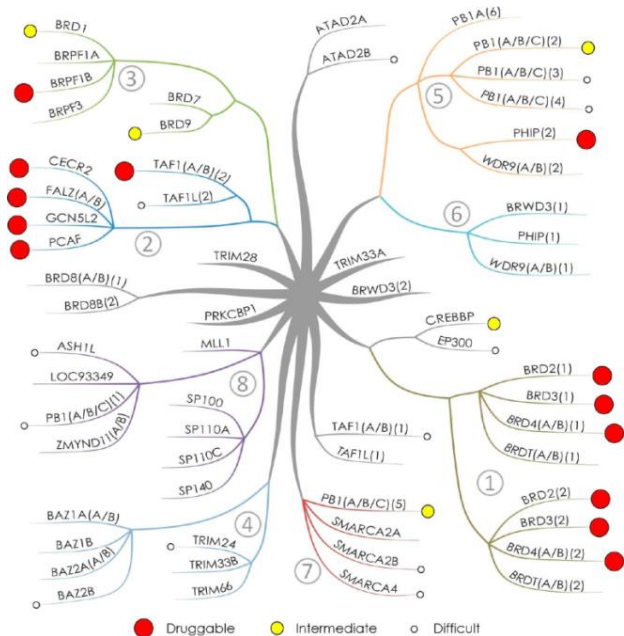
Article  
pubs.acs.org/jmc

### Druggability Analysis and Structural Classification of Bromodomain Acetyl-lysine Binding Sites

Lewis R. Vidler,<sup>†</sup> Nathan Brown,<sup>†</sup> Stefan Knapp,<sup>‡</sup> and Swen Hoelder<sup>\*,†</sup>

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**GDCh** **Communications** **Angewandte Chemie International Edition**

International Edition: DOI: 10.1002/anie.201603928  
German Edition: DOI: 10.1002/ange.201603928

### A Chemical Probe for the ATAD2 Bromodomain

Paul Bamborough,<sup>\*</sup> Chun-wa Chung, Emmanuel H. Demont,<sup>\*</sup> Rebecca C. Furze, Andrew J. Bamister, Ka Hing Che, Hava Djalil, Clement Douault, Paola Grandi, Tony Kouzandis, Anne-Marie Michon, Darren J. Mitchell, Rab K. Prinjha, Christina Rau, Samuel Robson, Robert J. Sheppard, Richard Upton, and Robert J. Watson

**Abstract:** ATAD2 is a cancer-associated protein whose bromodomain has been described as among the least druggable of that target class. Starting from a potent lead, permeability and selectivity were improved through a dual approach: 1) using CF<sub>3</sub> as a surface bio-isostere to exploit the unique properties of fluorine, and 2) using 1,3-interactions to control the conformation of a piperidine ring. This resulted in the first reported low-nanomolar, selective and cell permeable chemical probe for ATAD2.

**High expression levels of ATAD2 (ATPase family, AAA domain containing 2), also called ANCCA (AAA = nuclear coregulator cancer-associated), correlate with poor outcomes in several cancers, and its knockdown modulates multiple tumor cell growth factors.<sup>1–5</sup> Efforts to target this protein have focused on competitive binding to the acetyl-lysine (KAc) site of its bromodomain, but the role of the bromodomain in the biology of ATAD2 is unclear. We have developed chemical tools to try to understand this, and recently reported the discovery of quinolones and naphthylidones such as 1–3 and 5–7 that bind to the KAc site of ATAD2.<sup>6,7</sup>**

**Table 1:** Micromolar lead to nanomolar ATAD2 inhibitors. For statistics see Table S1a, Supporting Information.

	1	2	3	4	5	6	7	8	9
ATAD2 TR-FRET pIC <sub>50</sub>	5.6	7.2	6.9	6.7	6.9	6.5	7.4	7.1	7.1
ATAD2 bromophore pIC <sub>50</sub>	5.4	7.4	7.3	6.5	7.5	7.0	7.2	7.1	7.1
BRD9 BRD TR-FRET pIC <sub>50</sub>	5.4	5.4	5.0	5.8	4.8	4.1	5.2	5.1	5.1
TR-FRET selectivity (log)	0.2	1.8	0.9	2.1	2.4	2.2	2.0	2.0	2.0
Chrom logD (pH 7.4)	3.3	2.1	0.4	4.0	1.6	2.3	3.0	4.1	4.1
Polar surface area (Å <sup>2</sup> )	79	113	152	92	126	112	92	83	83
Artificial membrane permeability (hws <sup>1</sup> ; pH 7.4)	130	< 3	138	< 3	< 10	84	395		

The measured biological effects associated with BET

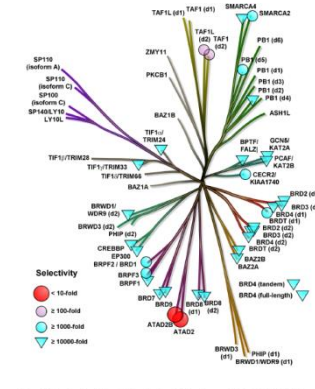


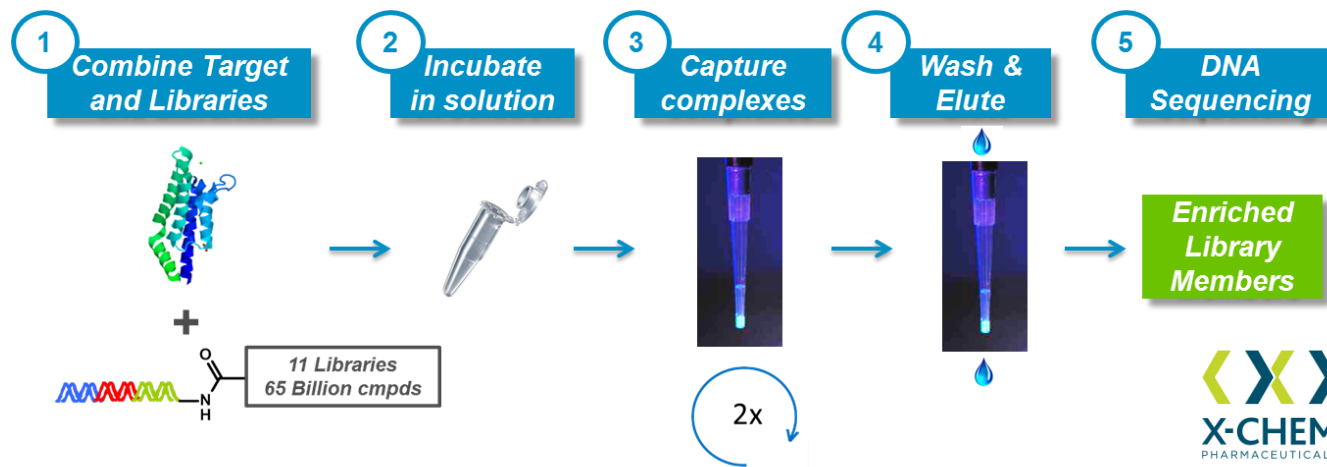
Figure S3. Bromodomain phylogenetic tree showing selectivity of compound 18 for ATAD2 (pKd 8.9) over other bromodomains in the BRDM2con panel.

- 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

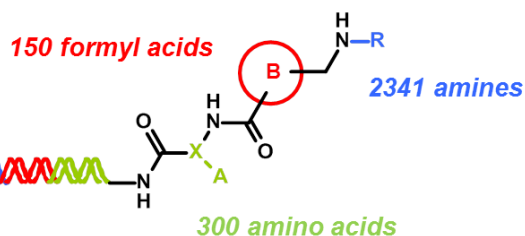


„off-DNA“ synthesis and confirmation with:

- TR-FRET
- Bromoscan™
- SPR
- TSA
- ITC
- MST
- NMR
- Native MS
- Initial hits
- Advanced compounds.



110 million-membered library



- 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

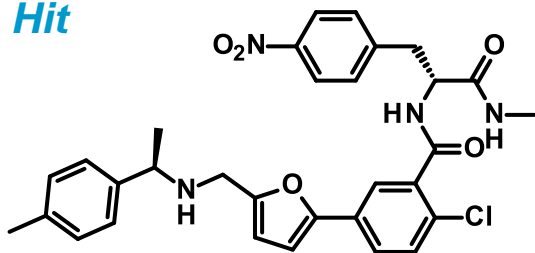


# ATAD2 probe BAY-850

## Probe discovery

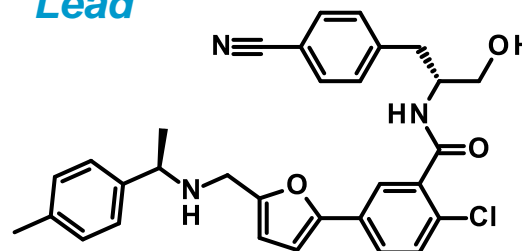


### Hit



- $\mu\text{M}$  hit with an unprecedented structure for a BD inhibitor

### Lead



- Sub- $\mu\text{M}$  biochemical activity and cellular target engagement shown

### Probe

**BAY-850**

- Result of systematic SAR exploration

### Hit

ATAD2 HTRF $\text{IC}_{50}$	1.1 $\mu\text{M}$
ATAD2 TSA $\Delta\text{T}$	n.d.
BRD4 BD1/BD2 $\text{IC}_{50}$ (HRTF)	>20 $\mu\text{M}$
Cellular target engagement	No

### Lead

370 nM
1.2 K
>20 $\mu\text{M}$
Yes

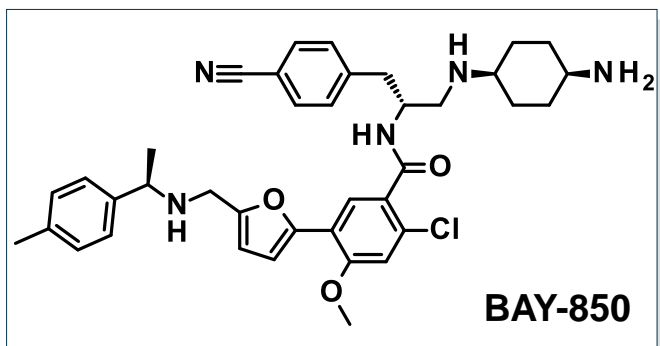
### BAY-850

22-166 nM
4-14.7 K
>20 $\mu\text{M}$
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 [Å <sup>2</sup> ]	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

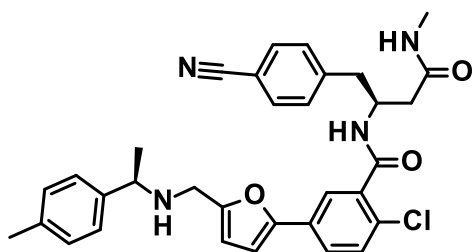
ATAD2 <sup>H4AcK12</sup> IC <sub>50</sub> (HTRF)	0.17 μM
ATAD2 <sup>H4AcK5/8/12/16</sup> IC <sub>50</sub> (HTRF)	0.02 μM
LLE <sup>logD</sup>	2.6
ATAD2 TSA ΔT <sup>(100μM)</sup>	13.9°C
BRD4 <sup>BD1</sup> IC <sub>50</sub> (HRTF)	>20 μM
BRD4 <sup>BD2</sup> IC <sub>50</sub> (HTRF)	>20 μM
DiscovereX BromoScan™	ATAD2A @ 10 μM
DiscovereX ATAD2A K <sub>D</sub>	0.12 μM
MST K <sub>D</sub>	0.08 μM
ITC K <sub>D</sub>	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]	39	B-A [nm/s]	11
			Ratio	
			0.3	

# ATAD2 negative control BAY-460

## Profile



BAY-460

### ▪ Molecular Properties

MW [g/mol]	569
MWcorr [g/mol]	553
TPSA [Å <sup>2</sup> ]	107
Rotatable bonds	11

### ▪ PhysChem

Sw <sup>pH 6.5</sup> [mg/L]	6.0
log D (pH 7.5)	3.2

### ▪ Pharmacology

ATAD2 <sup>H4AcK12</sup> IC <sub>50</sub> (HTRF)	>20 μM
ATAD2 <sup>H4AcK5/8/12/16</sup> IC <sub>50</sub> (HTRF)	16 μM
LLE <sup>logD</sup>	<1
ATAD2 TSA ΔT <sup>(100μM)</sup>	0.1°C
BRD4 <sup>BD1</sup> IC <sub>50</sub> (HRTF)	>20 μM
BRD4 <sup>BD2</sup> IC <sub>50</sub> (HTRF)	>20 μM
Discoverex BromoScan™	No hits @ 10 μM
Discoverex ATAD2A K <sub>D</sub>	n.d.
MST K <sub>D</sub>	n.d.
ITC K <sub>D</sub>	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]	37	B-A [nm/s]	60	Ratio	1.6

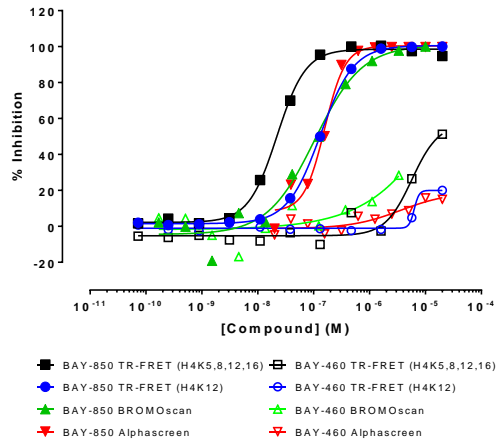


# ATAD2 probe BAY-850

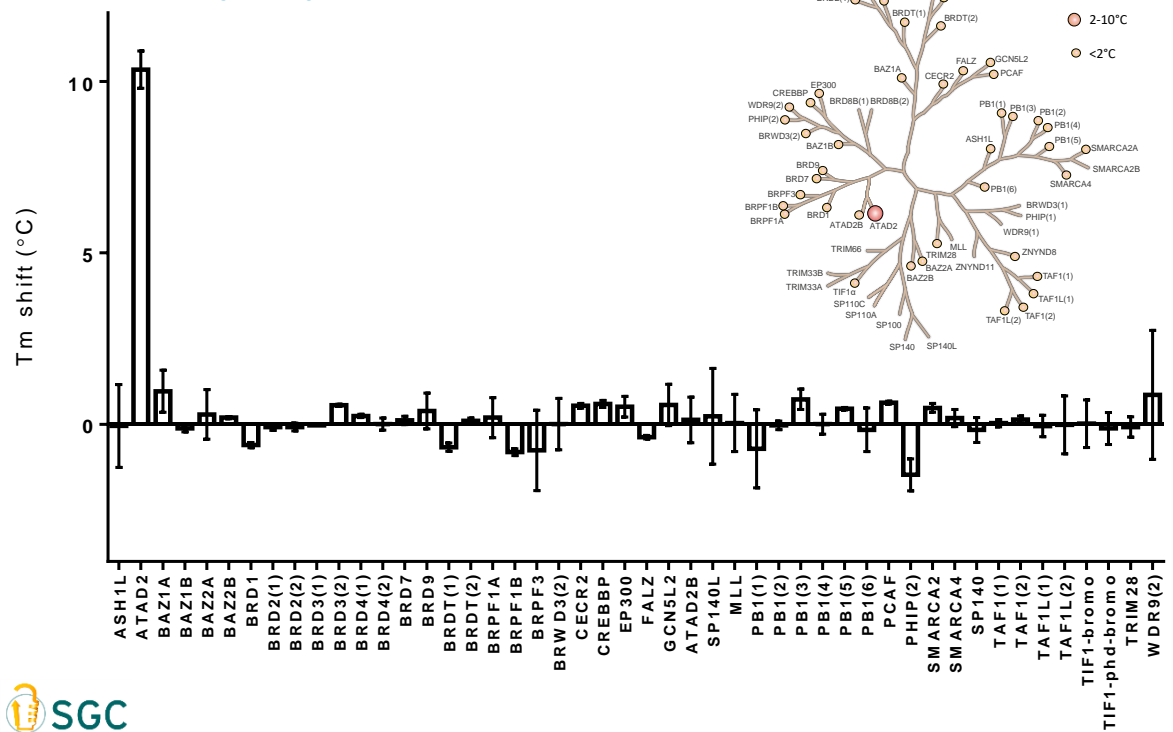
## Biochemical potency and selectivity



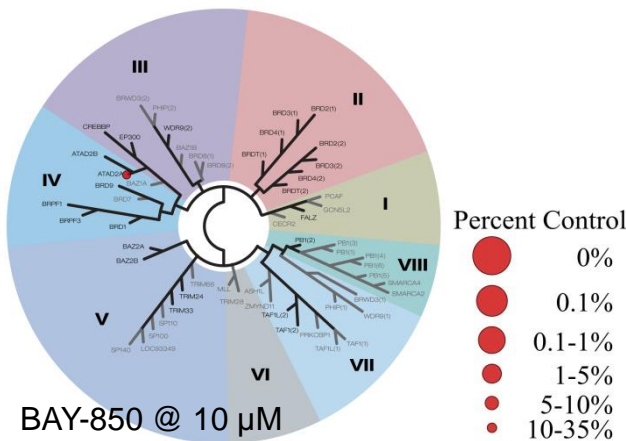
### Biochemical Assays (HTRF, Alphascreen, BROMOscan)



### Tm Panel (TSA)



### BROMOscan™ (DiscoverX)



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

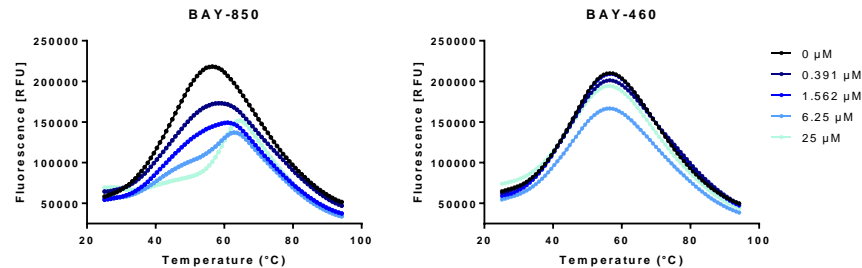
- In-house and external panels
- Orthogonal biochemical and biophysical assays

# ATAD2 probe BAY-850

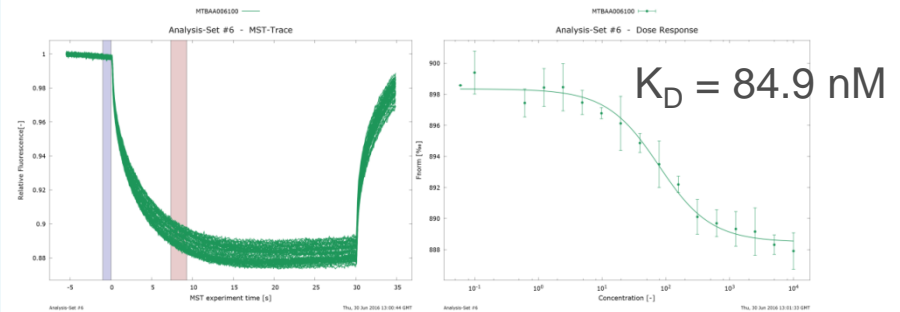
## Biophysical validation and MoA studies



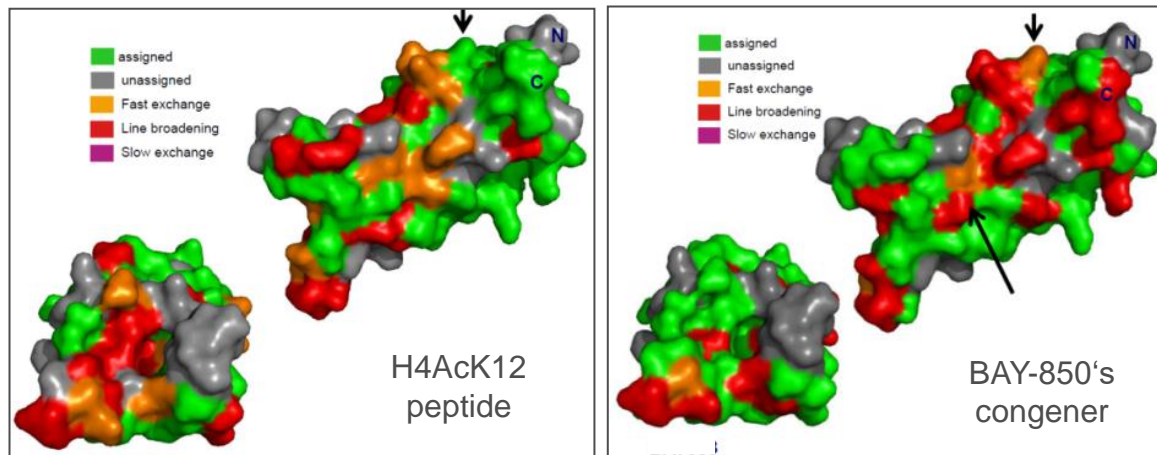
### TSA: Dose-dependent thermal stabilization



### MST: Nanomolar affinity



### NMR: Specific binding to new sites



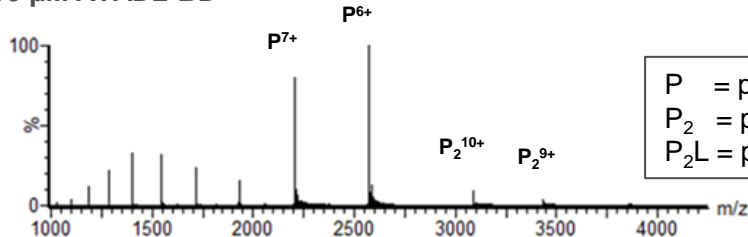
- Dose-dependent thermal stabilization of tag-free ATAD2 bromodomain
- Saturable, dose-dependent thermophoresis with  $K_D < 100$  nM
- Dose-dependent NMR chemical shift induction (=target engagement)

# ATAD2 probe BAY-850

## Biophysical validation and MoA studies



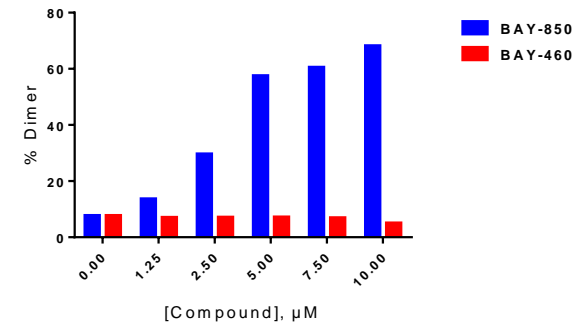
A) 10  $\mu$ M ATAD2 BD



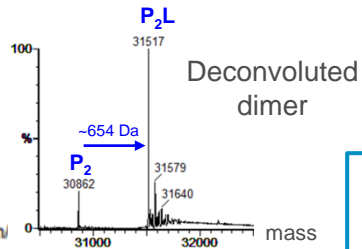
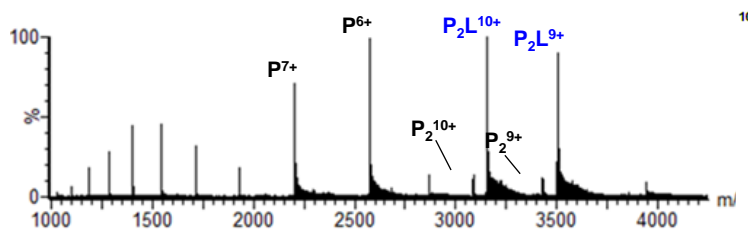
### Native MS

P = protein monomer  
P<sub>2</sub> = protein dimer  
P<sub>2</sub>L = protein-ligand complex (2:1)

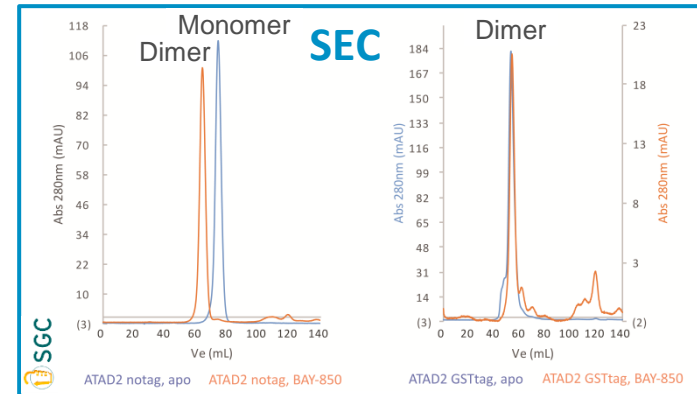
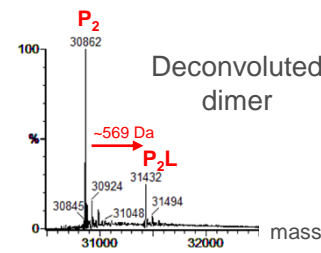
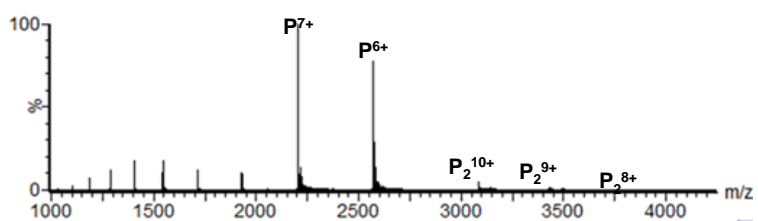
ATAD2 BD Dimer Induction  
(Native-MS Quantification)



B) 10  $\mu$ M ATAD2 BD + 5  $\mu$ M BAY-850



C) 10  $\mu$ M ATAD2 BD + 5  $\mu$ M BAY-460



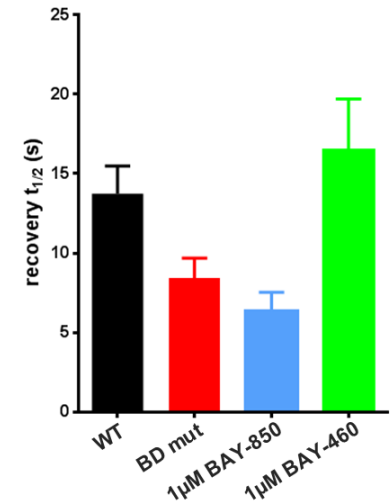
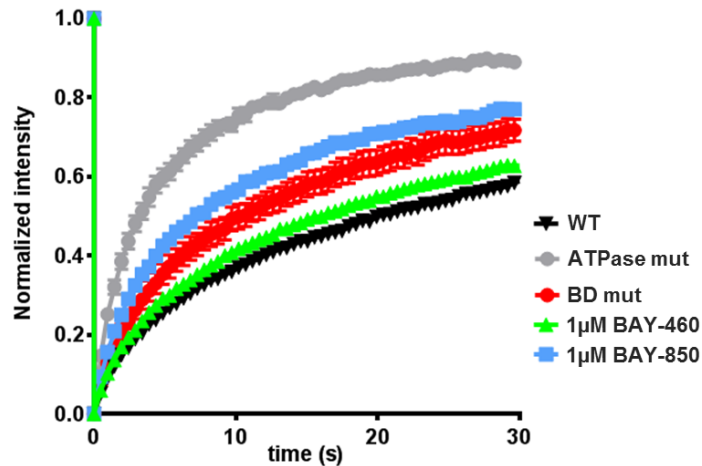
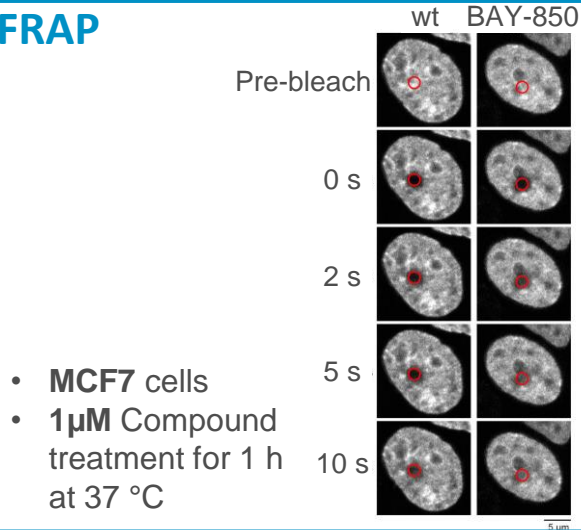
BAY-850 induces dose-dependent dimerization upon binding to ATAD2, whilst acetylated histone peptides and AcK mimetic ligands do not induce dimerization (not shown)

# ATAD2 probe BAY-850

## Cellular target engagement & pharmacology



### FRAP



### Proliferation inhibition

cpd	H4 tetraAc pep	H4K12Ac pep	MCF7		NCI-H526		MDA-MB-231	
	IC <sub>50</sub> biochem	IC <sub>50</sub> biochem	IC <sub>50</sub>	MaxInh(%)	IC <sub>50</sub>	MaxInh(%)	IC <sub>50</sub>	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 extent 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  $\mu$ M (FRAP assay in MCF7 cells)
  - Selective against all family members ( $\Delta T_m$  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



*Amaury E. Fernández-Montalván\**

Anita Krüger

*Antonius ter Laak\**

Benno Kuroпка

Christian Stegmann

Clara Christ

*Detlef Stoeckigt\**

Jan Hübner

Jörg Weiske

*Markus Berger\**

*Matyas Gorjanacz\**

Oliver Schenk

Seong Joo Koo

*Simon Holton\**

Stephan Siegel

Thomas Brumby

*Volker Badock\**

Vera Pütter

Andrea Haegebarth

Anke Mueller-Fahrnow

Ingo Hartung

Marcus Bauser

Ursula Egner

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**X-Chem Pharmaceuticals**

**EDELRIIS**



\*Bayer Core Team

‡SGC





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Thank you!



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Backup

# ATAD2 probe BAY-850

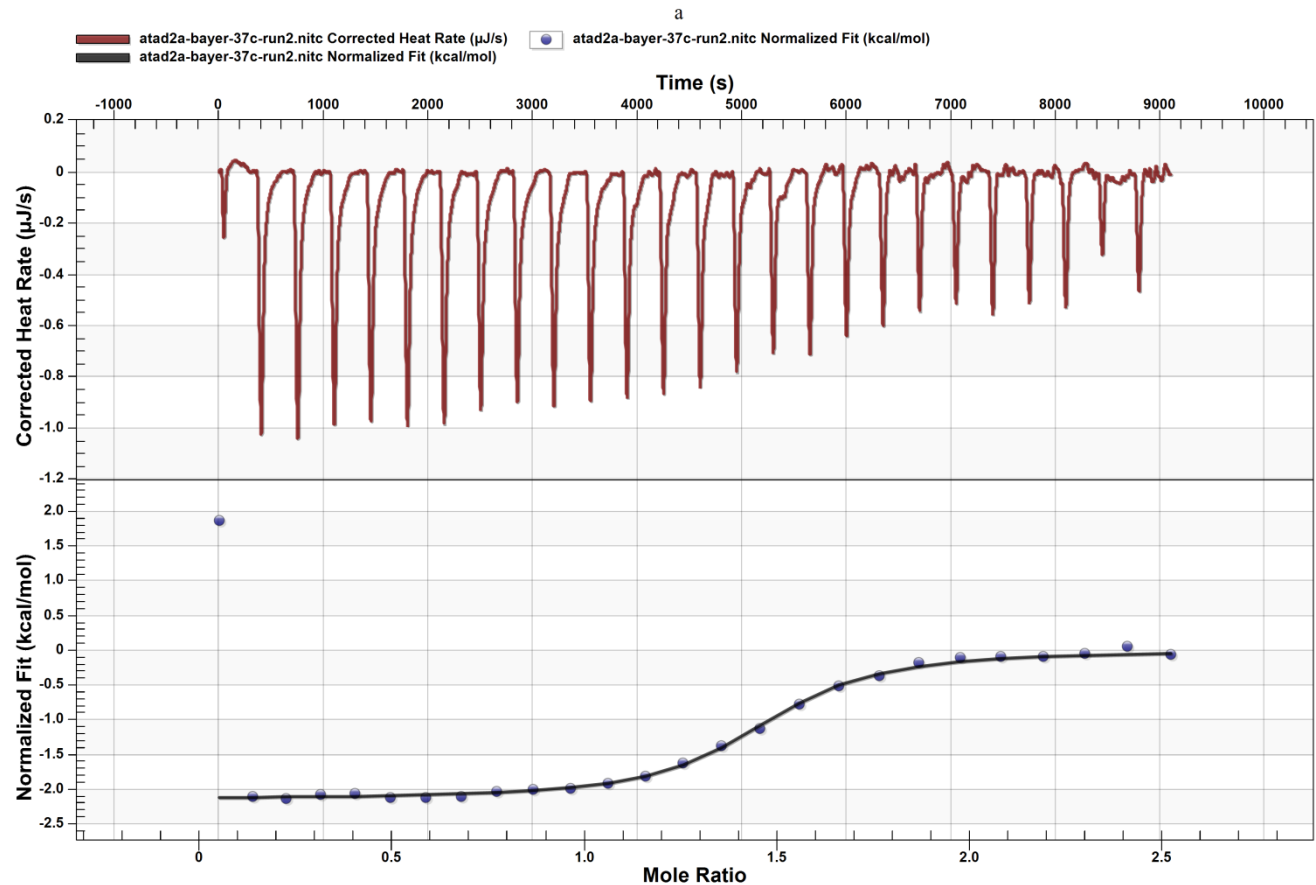
## Biophysical validation and MoA studies



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

$K_d=753.3$  nM  
 $N=1.45$   
 $DH=-2.16$  kcal/mole  
 $DS= 21.11$  cal/mole $^{\circ}K$

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



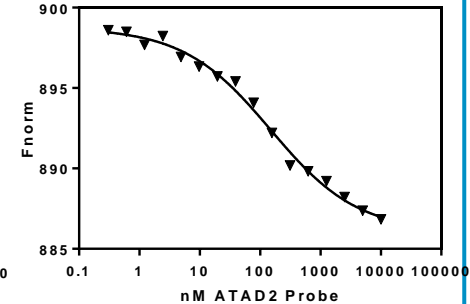
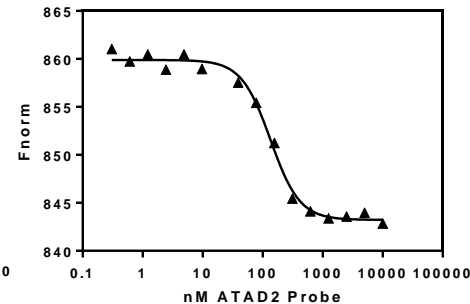
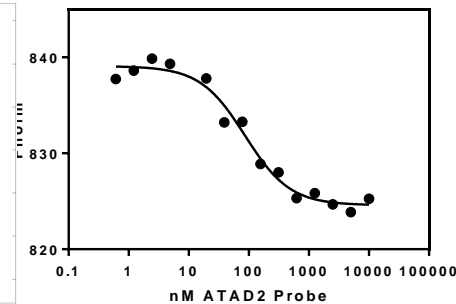
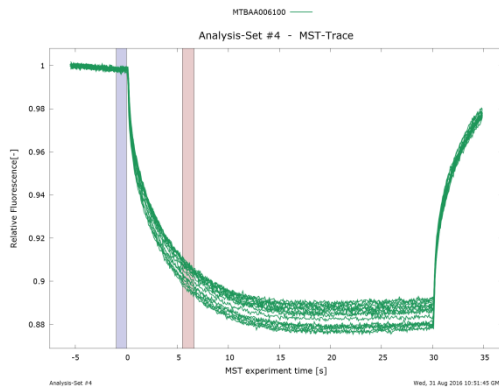
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## Biophysical validation and MoA studies

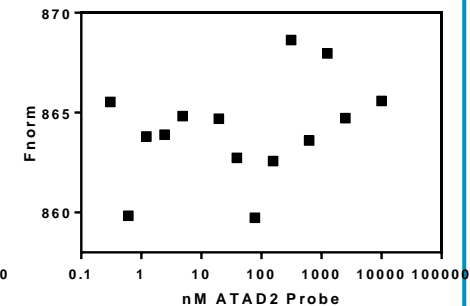
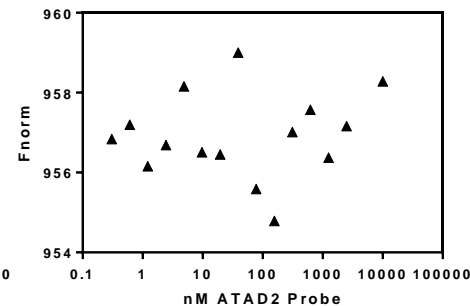
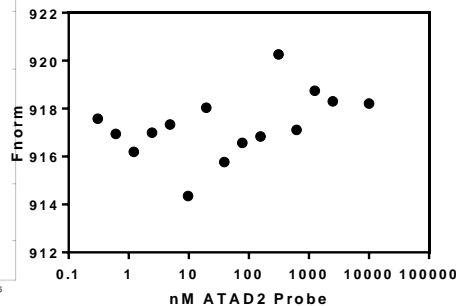
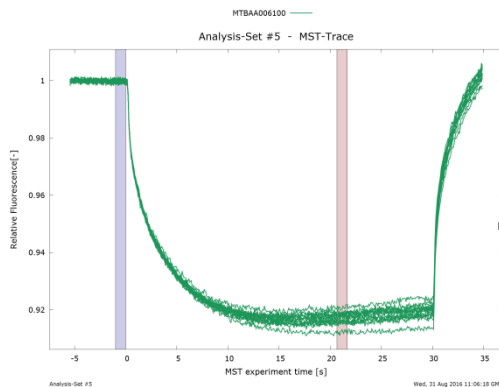


### MST: Binding is independent from GST tag

GST-ATAD2: Binding with  $K_d = 84.9$  nM



### GST-BRD9: No binding

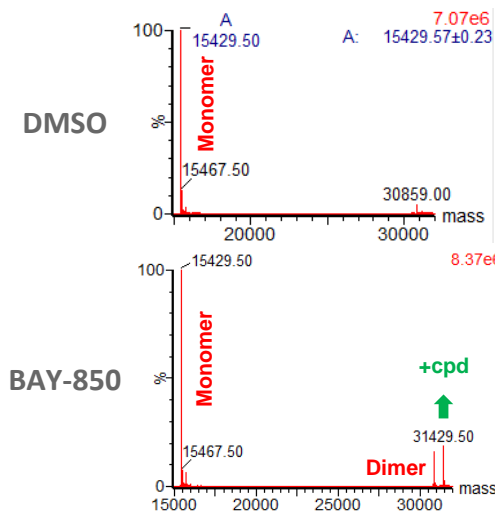


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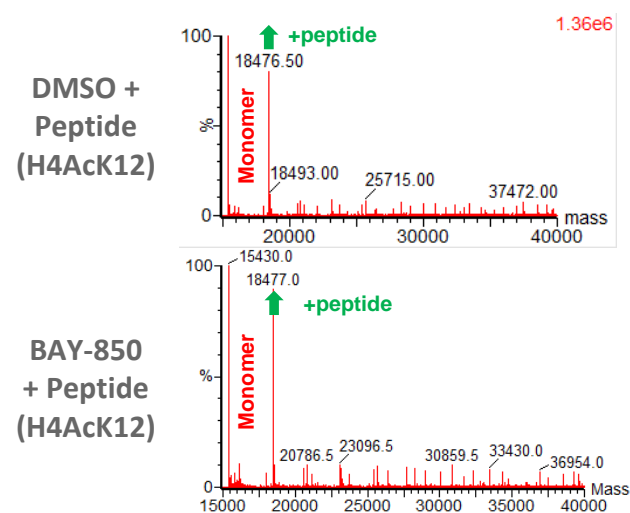
## Biophysical validation and MoA studies



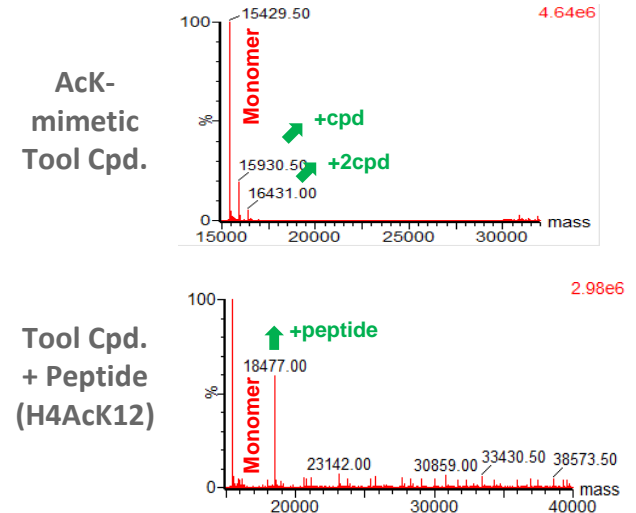
### Native MS: specific binding to dimerized ATAD2



- 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