Monolith Protocol MO-P-016

CREBBP – I-CBP112

CREB-binding protein, also known as CREBBP or CBP, is a transcriptional coactivator that carries out its function by activating transcription, where interaction with transcription factors is managed by one or more CREB domains. I-CBP112 is a small molecule inhibitor that binds to the bromodomain domain of CREBBP and has been developed for leukemia therapy.

protein – small molecule interaction | bromodomain | inhibitor

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A1. Target/Fluorescent Molecule

CREB-binding protein (CREBBP), bromodomain
uniprot.org/uniprot/Q92793

A2. Molecule Class/Organism

Transcriptional coactivator
Homo sapiens (Human)

A3. Sequence/Formula

RKKIFKPEEL RQALMPTLEA LYRQDPESLP FRQPVDPQLL GIPDYFDIVK NPMDLSTIKR KLDTGQYQEP WQYVDDVWLM
FNNAWLYNRK TSRVYKFCSK LAEVFEQEID PVMQSLG

A4. Purification Strategy/Source

Crelux GmbH

A5. Stock Concentration/Stock Buffer

10 mg/mL | 700 µM

A6. Molecular Weight/Extinction Coefficient

14 kDa
25,440 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

50 mM HEPES, pH 7.6, 150 mM NaCl, 0.05% TWEEN® 20

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS 2nd Generation (MO-L011, NanoTemper Technologies GmbH)
1* Dye RED-NHS 2nd Generation (10 µg) | 1* B-Column

A9. Labeling Procedure

1. Add 69 µL of dilution buffer[1] to 1 µL of 700 µM CREBBP to obtain 70 µL of a 10 µM solution.
2. Add 12 µL of DMSO to 10 µg Dye RED-NHS 2nd Generation to obtain a ~1.25 mM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Mix 2.8 µL of the 1.5 mM dye solution with 67.2 µL of dilution buffer to obtain 70 µL of a 50 µM dye solution (5x protein concentration).
4. Mix CREBBP and dye in a 1:1 volume ratio (140 µL final volume, 2% final DMSO concentration).
5. Incubate for 30 minutes at room temperature in the dark.
6. In the meantime, remove the top cap of the B-Column and pour off the storage solution. Remove the bottom cap and place with adapter in a 15 mL tube.
7. Fill the column with dilution buffer and allow it to enter the packed resin bed completely by gravity flow. Discard the flow through collected. Repeat this step 3 more times.
8. Add 140 µL of the labeling reaction from step 4 to the center of the column and let sample enter the bed completely.
9. Add 600 µL of dilution buffer after the sample has entered and discard the flow through.
10. Place column in a new collection tube, add 350 µL of dilution buffer and collect the eluate.
11. Keep the labeled CREBBP (~2 µM) on ice in the dark.

A10. Labeling Efficiency

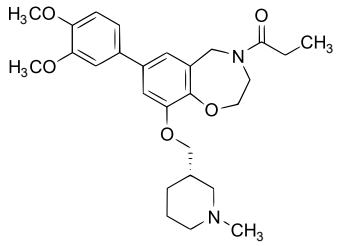
Measurement of protein concentration and degree of labeling (DOL) using a NanoDrop™:
nanotempertech.com/dol-calculator

Absorbance A_{280}	0.061	Protein concentration	2.17 µM
Absorbance A_{650}	0.142	Degree-of-labeling (DOL)	0.33

[1] CREBBP is not stable in the supplied Labeling Buffer NHS from the Protein Labeling kit MO-L011.

B1. Ligand/Non-Fluorescent Binding Partner

I-CBP112



B2. Molecule Class/Organism

Bromodomain inhibitor

B3. Sequence/Formula

$C_{27}H_{36}N_2O_5$

B4. Purification Strategy/Source

Sigma-Aldrich GmbH
SML1134

B5. Stock Concentration/Stock Buffer

23.4 mg/mL | 50 mM
DMSO

B6. Molecular Weight/Extinction Coefficient

468.59 Da

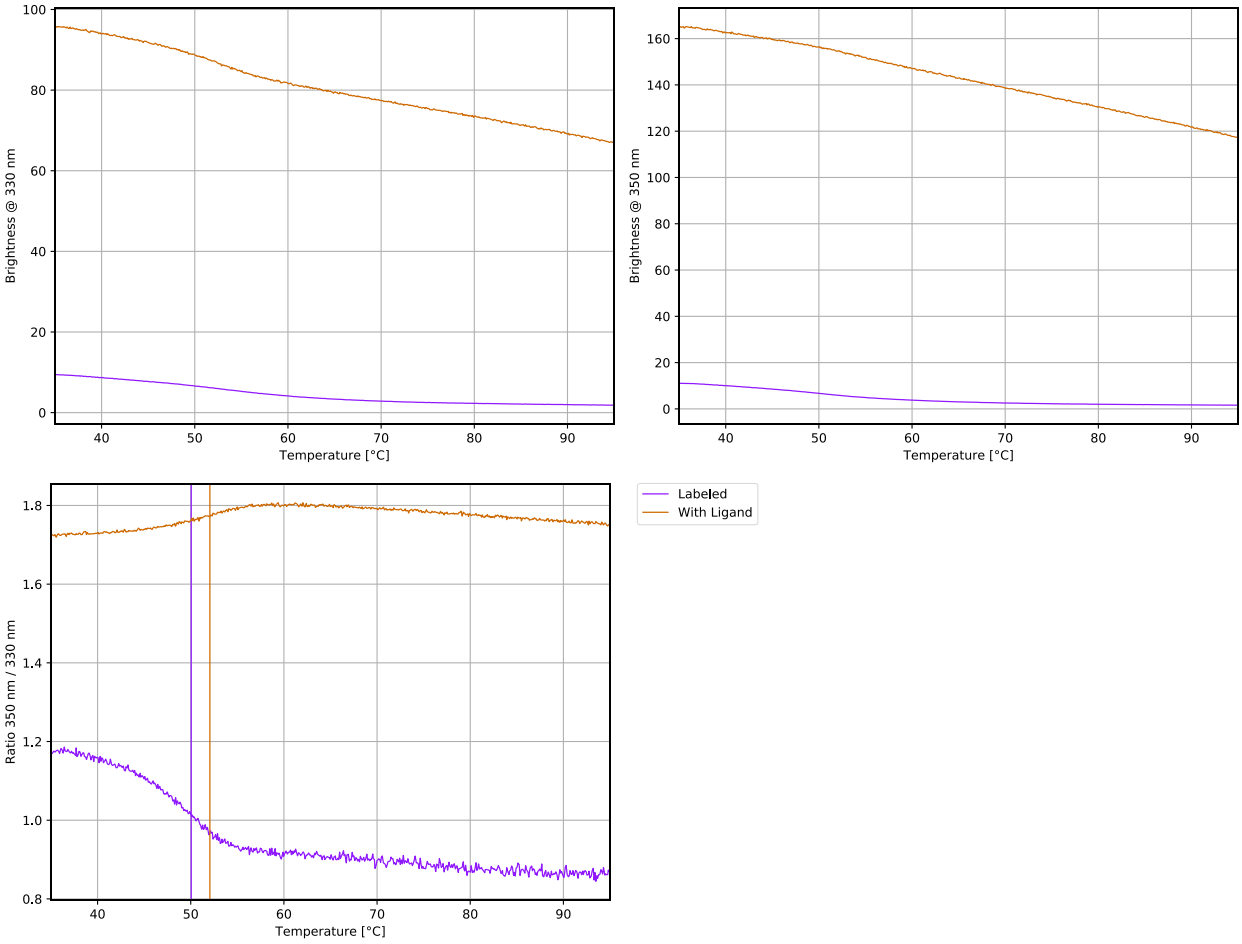
B7. Serial Dilution Preparation

1. Mix 2 µL of 50 mM I-CBP112 with 18 µL of DMSO to obtain 20 µL of a 5 mM solution.
2. Mix 2 µL of the 5 mM I-CBP112 solution with 998 µL of dilution buffer to obtain 1 mL of a 10 µM I-CBP112 solution.
3. Mix 2 µL of DMSO with 998 µL of dilution buffer to obtain 1 mL of a 0.2% DMSO solution.
4. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 10 µM I-CBP112 solution into tube **1**. Then, transfer 10 µL of the 0.2% DMSO solution into tubes **2** to **16**.
5. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
6. Mix 10 µL of labeled CREBBP with 190 µL of dilution buffer to obtain 200 µL of ~100 nM CREBBP.
7. Add 10 µL of CREBBP (~100 nM) to each tube from **16** to **1** and mix by pipetting.
8. Incubate for 20 minutes on ice before loading capillaries.

C. Applied Quality Checks

Validation of structural integrity and functionality of labeled CREBBP using Tycho NT.6:
nanotempertech.com/tycho

Labeled	5 µL of B-Column eluate (~2 µM) + 5 µL of dilution buffer containing 0.2% DMSO	T_i = 50.1°C[2]
With ligand	5 µL of B-Column eluate (~2 µM) + 5 µL of 10 µM I-CBP112	T_i = 52.1°C



D1. MST System/Capillaries

Monolith NT.115 Red (NanoTemper Technologies GmbH)
Premium Capillaries Monolith NT.115 (MO-K025, NanoTemper Technologies GmbH)

D2. MST Software

MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

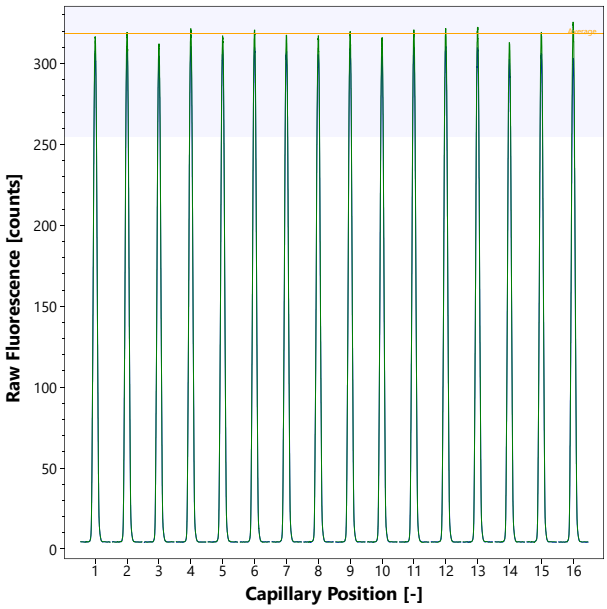
[2] Due to the low thermal stability, always keep on ice when not used.

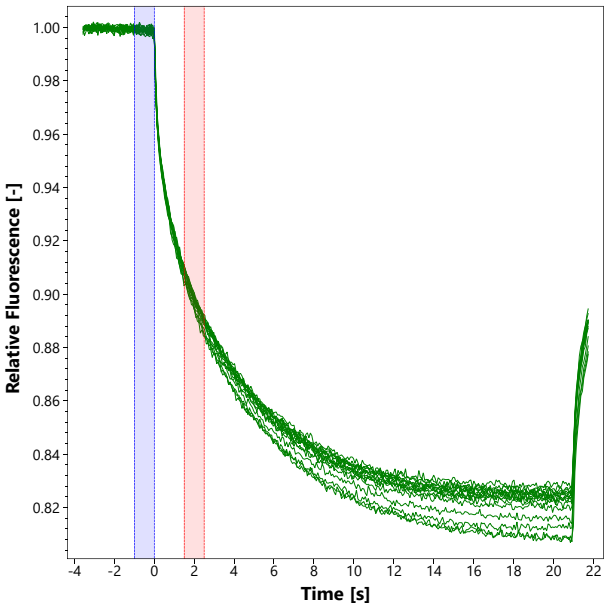
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

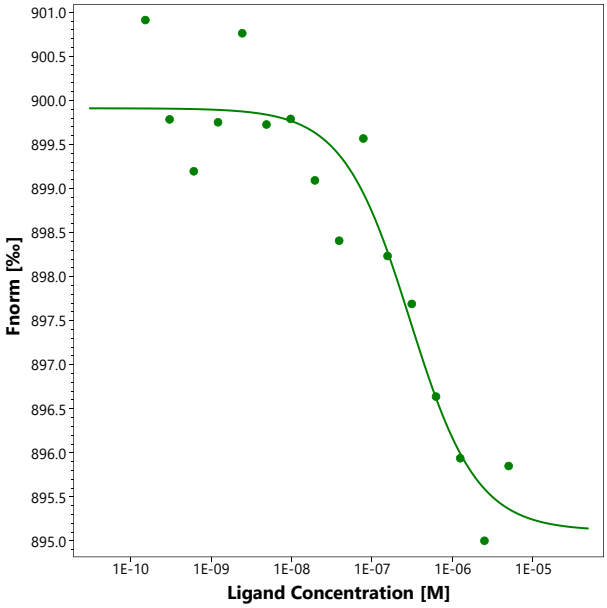
50 mM HEPES, pH 7.6, 150 mM NaCl, 0.1% DMSO[3], 0.05% TWEEN® 20
50 nM CRIBBP | 5 µM – 153 pM I-CBP112 | 22°C | medium MST power | 40% excitation power

D4. MST Results (Capillary Scan/Time Traces/Dose Response)

K_d = 272 nM







[3] Dimethyl sulfoxide (DMSO) itself is an inhibitor of bromodomains at the concentrations commonly used for compound solubilization as it mimics the acetylated lysine motif that is the canonical ligand recognized by bromodomains. Hence, DMSO concentrations > 0.1% can interfere with the assay (Philpott, 2011).

D5. Reference Results/Supporting Results

K_d = 151 nM	Isothermal Titration Calorimetry (ITC) Picaud et al., Cancer Res 75 (23) 2015
K_d = 142 nM	BioLayer Interferometry (BLI) Picaud et al., Cancer Res 75 (23) 2015

E. Contributors

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