Monolith X Protocol MOX-P-114

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 | bromodomain | inhibitor

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

Batch #PC5585-1
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

20 mM HEPES, pH 7.4, 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. Prepare the Labeling Buffer[1]: 10 mM HEPES, pH 7.6, 300 mM NaCl, 0.05% TWEEN® 20.
2. Add 102 µL of Labeling Buffer to 3 µL of 700 µM CREBBP to obtain 105 µL of a 20 µM solution. Then, transfer 100 µL of 20 µM CREBBP into a fresh vial.
3. 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.
4. Mix 8 µL of the ~1.25 mM dye solution with 92 µL of Labeling Buffer to obtain 100 µL of a 100 µM dye solution (5x protein concentration).
5. Mix CREBBP and dye in a 1:1 volume ratio (200 µL final volume, 4% final DMSO concentration).
6. Incubate for 30 minutes at room temperature in the dark.
7. 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.
8. 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.
9. Add 200 µL of the labeling reaction from step 5 to the center of the column and let sample enter the bed completely.
10. Add 500 µL of dilution buffer after the sample has entered and discard the flow through.
11. Place column in a new collection tube, add 400 µL of dilution buffer and collect the eluate.
12. Centrifuge the eluate at 15,000 rpm and 4°C for 20 minutes to remove aggregates. Then, carefully transfer the supernatant into a fresh tube and mix well by pipetting.
13. Prepare 10 µL aliquots of the labeled CREBBP (~5 µM) and immediately store at -80°C.

A10. Labeling Efficiency

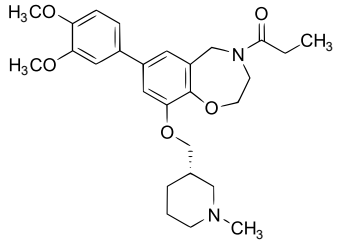
Measurement of protein concentration and degree of labeling (DOL) using a NanoDrop™:
nanotempertech.com/dol-calculator

Absorbance A_{280}	0.132	Protein concentration	4.3 µM
Absorbance A_{650}	0.570	Degree-of-labeling (DOL)	0.68

[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

5.7 mg/mL | 10 mM
DMSO

B6. Molecular Weight/Extinction Coefficient

468.59 Da

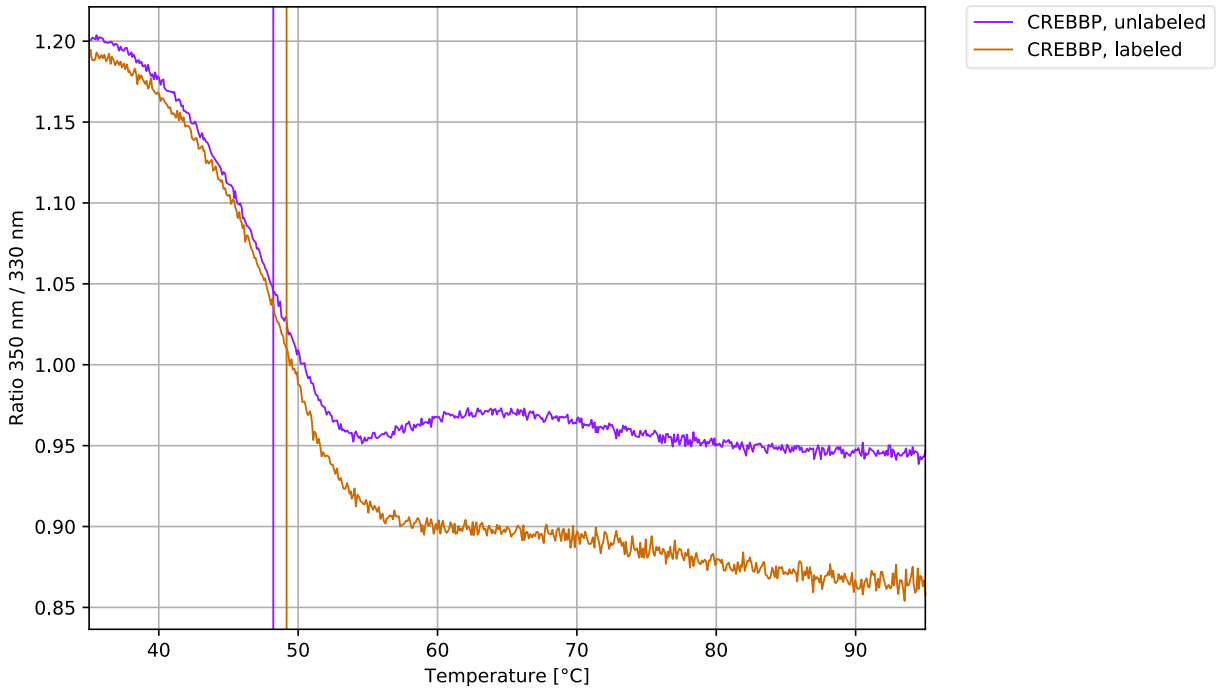
B7. Serial Dilution Preparation

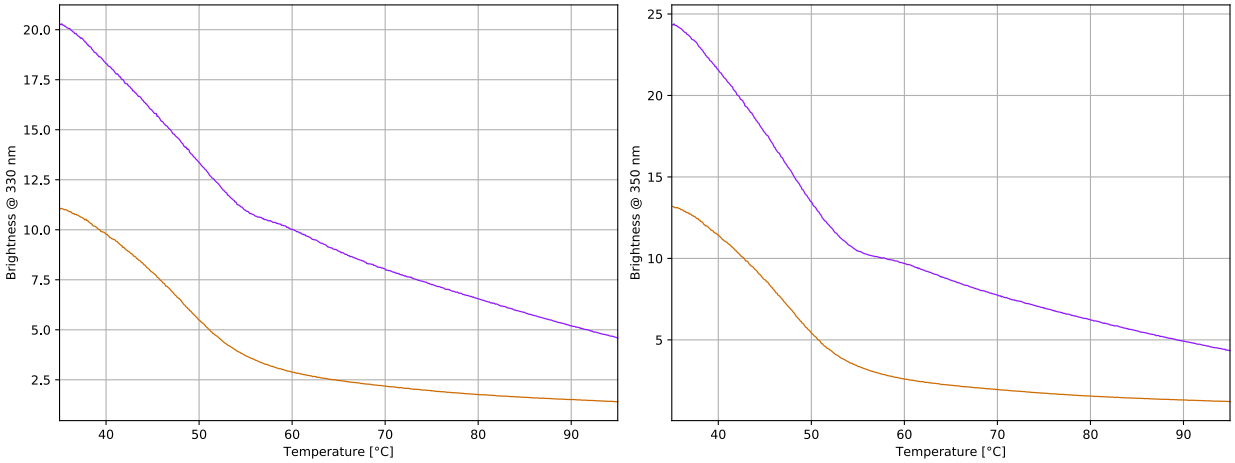
1. Mix 2 µL of 10 mM I-CBP112 with 998 µL of dilution buffer to obtain 1 mL of 20 µM I-CBP112.
2. Mix 2 µL of DMSO with 998 µL of dilution buffer to obtain 1 mL of a 0.2% DMSO solution.
3. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 20 µM I-CBP112 solution into tube **1**. Then, transfer 10 µL of the 0.2% DMSO solution into tubes **2** to **16**.
4. 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.
5. Mix 4 µL of labeled CREBBP (~5 µM) with 196 µL of dilution buffer to obtain 200 µL of ~100 nM CREBBP.
6. Add 10 µL of CREBBP (~100 nM) to each tube from **16** to **1** and mix by pipetting.
7. Centrifuge PCR tubes at 15,000 rpm and 4°C for 30 minutes to remove remaining aggregates.
8. Carefully load capillaries from the top of the tubes without touching the bottom.

C. Tycho

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

CREBBP, unlabeled	5 µL of 5 µM CREBBP + 5 µL of dilution buffer with 0.2% DMSO	T_i = 48.2°C
CREBBP, labeled	5 µL of B-Column eluate (~4 µM) + 5 µL of dilution buffer with 0.2% DMSO	T_i = 49.2°C

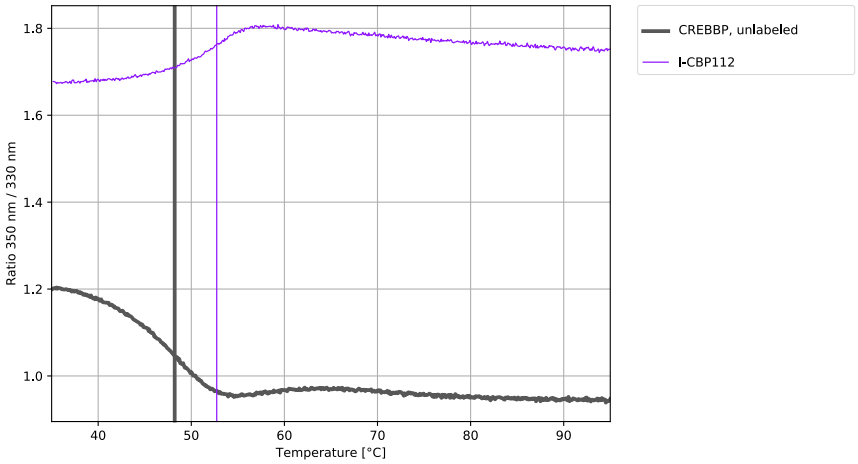


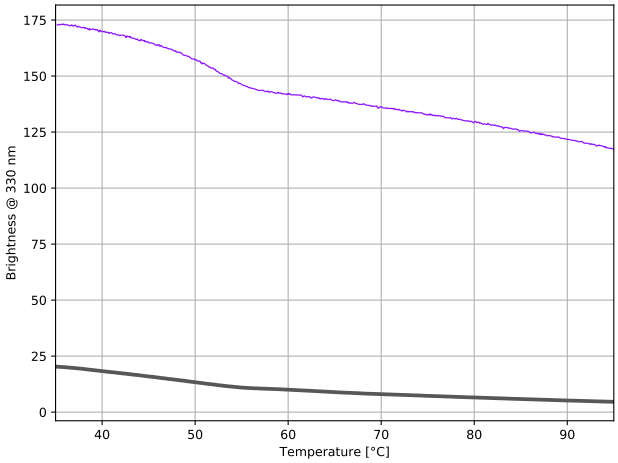


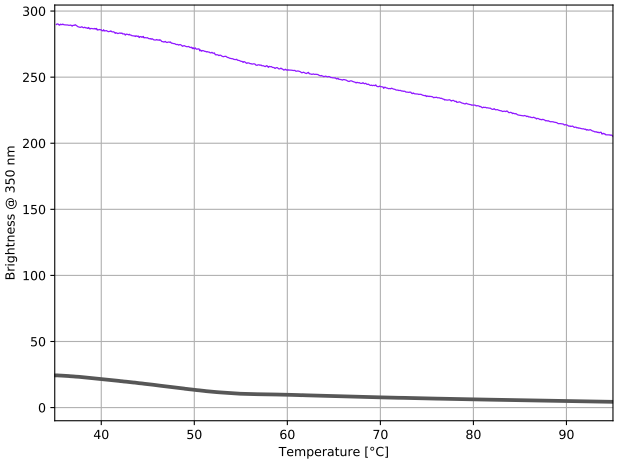
Validation of functionality of CREBBP using Tycho NT.6:

nanotempertech.com/tycho

I-CBP112	5 µL of 5 µM CREBBP + 5 µL of 20 µM I-CBP112	T_i = 52.7°C







D1. Monolith System/Capillaries

Monolith X (NanoTemper Technologies GmbH)
Monolith Premium Capillaries (MO-K025, NanoTemper Technologies GmbH)

D2. Monolith Software

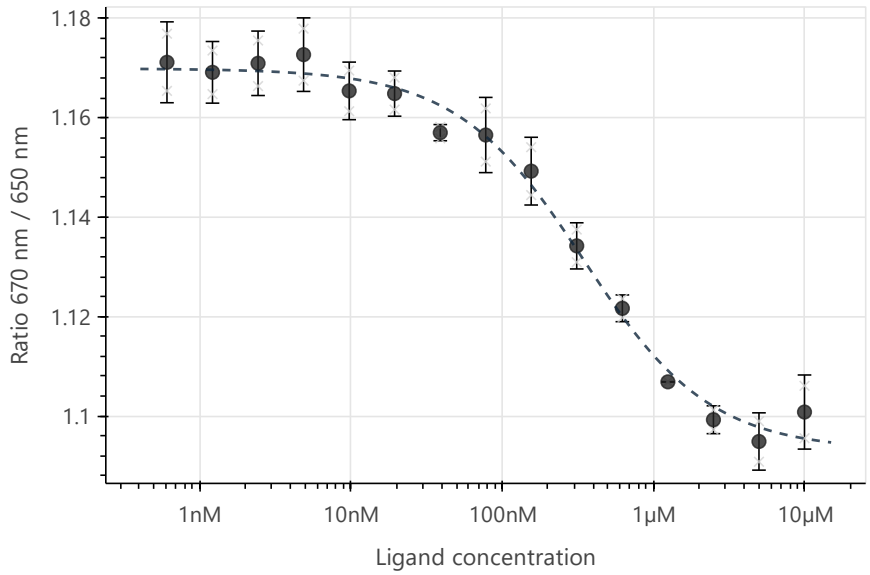
MO.Control v2.4.2 (NanoTemper Technologies GmbH)
https://nanotempertech.com/monolith-mo-control-software/

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

20 mM HEPES, pH 7.4, 150 mM NaCl, 0.1% DMSO[2], 0.05% TWEEN® 20
50 nM CREBBP | 10 µM – 306 pM I-CBP112 | 20°C | 100% excitation power

D4. Monolith Results (Dose Response)

K_d = 320 ± 45 nM (S/N = 25.8)



D5. Reference Results/Supporting Results

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

E. Contributors

Andreas Langer[3]

[2] 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).

[3] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com