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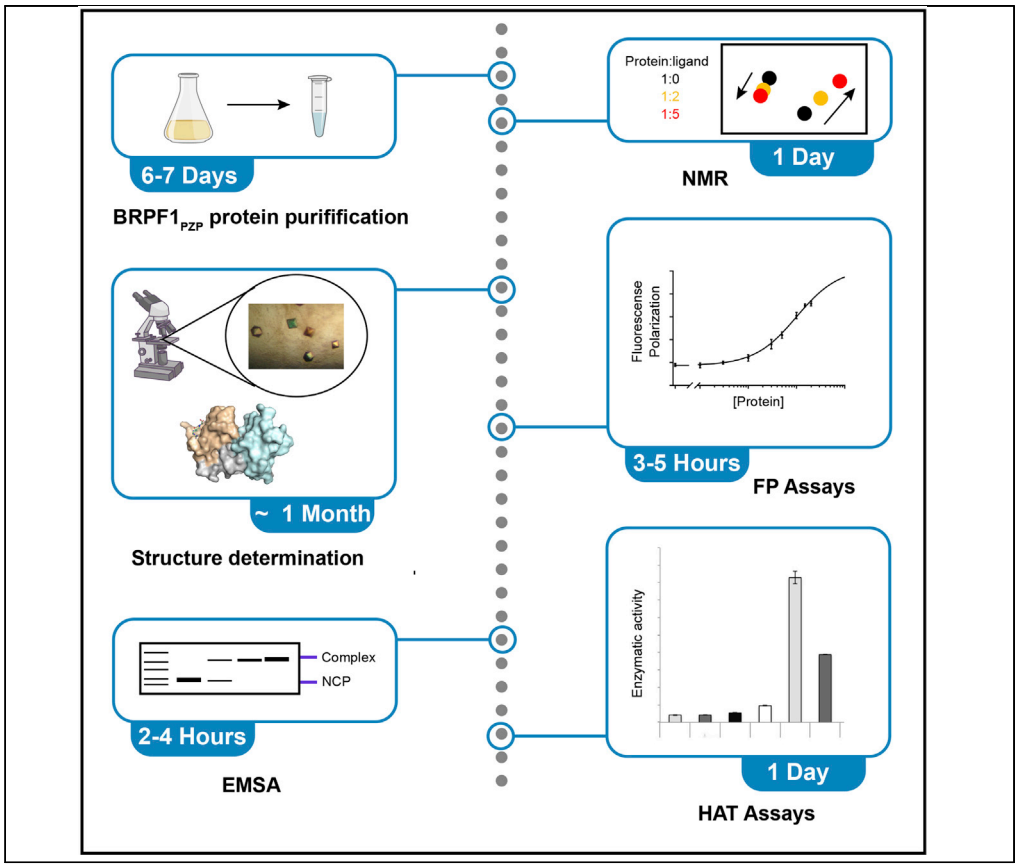
Structural and biophysical characterization of the nucleosome-binding PZP domain



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Highlights

Optimized protocol to purify $BRPF1_{PZP}$ from bacteria cells

Biophysical methods to characterize interactions between PZP, H3, DNA, and NCP

Determination of the crystal structure of the histone H3-PZP complex

Optimized protocol to measure HAT activity of the MORF complex

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Structural and biophysical characterization of the nucleosome-binding PZP domain

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SUMMARY

The core subunit of the MORF acetyltransferase complex BRPF1 contains a unique combination of zinc fingers, including a plant homeodomain (PHD) finger followed by a zinc knuckle and another PHD finger, which together form a PZP domain (BRPF1$_{PZP}$). BRPF1$_{PZP}$ has been shown to bind to the nucleosome and make contacts with both histone H3 tail and DNA. Here, we describe biophysical and structural methods for characterization of the interactions between BRPF1$_{PZP}$, H3 tail, DNA, and the intact nucleosome.

For complete details on the use and execution of this protocol, please refer to Klein et al. (2020).

BEFORE YOU BEGIN

The bromodomain and PHD finger protein 1 (BRPF1) is one of the four core subunits of the monocytic leukemic zinc-finger (MOZ)/MOZ-related factor (MORF) acetyltransferase complex, which acetylates lysine residues in histone H3 tail, H3K23 in particular (Klein et al., 2019). Other core subunits of this complex are the catalytic protein MORF, MEAF6 and ING5. These subunits contain several histone readers, a writer, and DNA-binding domains which contribute to the enzymatic activity of the MORF complex and its association with chromatin (Klein et al., 2014). The PHD-zinc-knuckle-PHD (PZP) domain of BRPF1 (BRPF1$_{PZP}$) has recently been shown to bind both histone H3 tail and DNA within the nucleosome core particle (NCP).

In this protocol, we describe the steps required for characterization of the interactions between BRPF1$_{PZP}$, H3 peptide, DNA and NCP. We detail the procedure for expression and purification of wild type BRPF1$_{PZP}$, mutated BRPF1$_{PZP}$, and H3-linked BRPF1$_{PZP}$. We also describe protocols for determination of the structure of H3-linked BRPF1$_{PZP}$ by X-ray crystallography and biophysical analysis of the association of BRPF1$_{PZP}$ with NCP. Lastly, we report the protocol for measuring histone acetyltransferase (HAT) activity of the BRPF1/MORF/MEAF6/ING5 complex.

Initially, plasmids need to be generated, DNA amplified, histone proteins expressed and purified, nucleosomes assembled, and plasmids transfected into HEK293T cells.

Prepare for cloning, expression, and purification of $BRPF1_{PZP}$

Timing: 1–5 days

1. Prepare the following stocks and store at −20°C.
   a. Weigh out and dissolve 2.5 mg of ampicillin sodium salt (Thermo Sci Fairlawn Chem) powder in Milli-Q water and bring the final volume to 25 mL. Filter the solution with a 0.22 μM filter (Merck Milipore).
   b. Weigh out and dissolve 0.85 mg of chloramphenicol (Fisher BioReagents) in 100% ethanol (Decon Labs, Inc) and bring the final volume to 25 mL.

   ⚠ CRITICAL: Chloramphenicol has the potential to cause an allergic skin reaction, serious eye damage, and may cause cancer. Therefore, avoid direct contact with this reagent and always use appropriate personal protective equipment (PPE).

   c. Weigh out and dissolve 5.95 mg isopropyl β-d-1-thiogalactopyranoside (IPTG) (Goldbio) in Milli-Q water and bring the final volume to 25 mL. Filter the solution with a 0.22 μM filter.

Antibiotics and IPTG stocks	Stock concentration	Solvent	Sterilization
Ampicillin	100 mg/mL	Milli-Q water	Filter sterilize
Chloramphenicol	34 mg/mL	Ethanol	N/A
IPTG	1.0 M	Milli-Q water	Filter sterilize

2. Make several small batches of Luria-Bertani (LB) (1 L contains 10 g tryptone, 5 g yeast extract and 10 g NaCl) media including one for a preculture. Preculture: in a 1 L flask weigh and dissolve 6.25 g of LB (Miller Molecular Genetics Powder) in a final volume of 250 mL Milli-Q water.

   Note: Recipes and tips for preparing media and buffers can be found in the materials and equipment section.

3. Prepare LB agar plates (1 L contains 10 g tryptone, 5 g yeast extract, 10 g NaCl, and 12 g agar) supplemented with ampicillin alone (stock 100 mg/mL; final concentration 100 μg/mL) and both ampicillin (stock 100 mg/mL; final concentration 100 μg/mL) and chloramphenicol (stock 34 mg/mL; final concentration 34 μg/mL).
4. Make a small batch (250 mL) of low salt LB media (1 L contains 10 g tryptone, 5 g yeast extract and 5 g NaCl).
5. Prepare plates for cloning low salt LB agar plate supplemented with zeocin (stock 100 mg/mL; final concentration 50 μg/mL) (Thermo Fisher Sci) following the Gateway® Technology guide. https://tools.thermofisher.com/content/sfs/manuals/gatewayman.pdf
6. Purchase or purify Tobacco Etch Virus (TEV) Protease (Liu et al., 2020b).

   Optional: Prepare 0.1 M phenylmethylsulfonyl fluoride (PMSF) (Sigma-Aldrich) (0.17 g in 10 mL 100% ethanol) and use at a final concentration of 0.1–1 mM. PMSF inhibits serine and cysteine proteases.

   ⚠ CRITICAL: PMSF can be toxic if ingested and can cause severe burns to the skin, and serious eye damage. Therefore, avoid direct contact with this reagent and always use appropriate PPE.

7. Equilibrate glutathione beads (Thermo Fisher Sci) according to the manual (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0011719_Pierce_Glutathione_Agarose_UG.pdf) in PZP Purification Buffer B (50 mM Tris pH 7.5, 500 mM NaCl, and 5 mM DTT).

a. For every 1 L of expressed cells use 0.5 mL glutathione slurry.

Note: $BRPF1_{PZP}$ is expressed with a low yield, therefore do not use excess of beads for purification (40 mg of purified protein per 1 mL resin is adequate), as it is both costly and will reduce purity of the protein. Mix the glutathione agarose slurry before removing from container.

8. Equilibrate HiPrep™ 16/60 Sephacryl® S-100 HR column (Cytiva) in PZP Purification Buffer B.

Alternatives: The HiLoad® 16/60 Superdex® 75 pg (Cytiva) is an alternative column to use and may provide higher purity.

Prepare necessary constructs of wild-type $BRPF1_{PZP}$, mutated $BRPF1_{PZP}$, and H3-linked $BRPF1_{PZP}$

Timing: 1.5–2 weeks

9. Obtain the $BRPF1_{PZP}$ (aa 267–454) construct in pDEST15 plasmid (Klein et al., 2016).

10. Use Gateway® cloning (Thermo Fisher Sci) to generate wild type $BRPF1_{PZP}$ (amino acids 271–454) in pDEST15 plasmid and the H3-GSGSS-BRPF1PZP construct (amino acids 1–12 of histone H3, a GSGSS linker, and amino acids 271-454 of BRPF1) in pDEST15 plasmid.
    a. Obtain pDONR/zeo and pDEST15 vectors from Thermo Fisher Science (cat# 12535035 and cat# 1180214).
    b. Design and order forward and reverse primers following the suggestions outlined in the Gateway® cloning manual (primer sequences can be found in the key resources table). https://tools.thermofisher.com/content/sfs/manuals/gatewayman.pdf
    c. Prepare the following reagents in a flat top PCR tube (USA Sci).

Reagent	Amount
Autoclaved Milli-Q ddH2O	40.2 µL
10× Cloned Pfu Reaction Buffer AD	5 µL
dNTP mix (12.5 mM of each dNTP)	0.8 µL
DNA template (100 ng/µL)	1 µL
10 µM forward primer	1 µL
10 µM reverse primer	1 µL
*Pfu*Turbo DNA Polymerase AD (2.5 U/µL) (Agilent)	1 µL
Total volume	50 µL

    d. Spin down the PCR mixture and run the reaction following the *Pfu*Turbo DNA Polymerase AD guide (https://www.agilent.com/cs/library/usermanuals/Public/600255.pdf) with the below parameters in a T100™ Thermal Cycler (Bio-Rad).

PCR cycling conditions			
A	Temperature	Time	Cycles
Initial Denaturation	95°C	5 min	1
Denaturation	95°C	30 s	30 cycles
Annealing	55°C–56°C	30 s	
Extension	68°C	1 min	
Final extension	68°C	10 min	1
Hold	16°C	Forever	

e. Verify the PCR product on a 1% agarose (Thermo Fisher) gel.
f. Purify the PCR product following the MinElute PCR Purification Kit (QIAGEN) protocol. https://www.qiagen.com/us/resources/download.aspx?id=8f6b09b2-6dcd-4b55-bb4a-255ede40ca3b&lang=en
g. Follow instructions in the Gateway® Technology user guide to set up and perform BP reaction. https://tools.thermofisher.com/content/sfs/manuals/gatewayman.pdf

Pause point: The BP reaction can be stored at −20°C for up to a week prior to transformation.

h. Transform 5–10 μL of the reaction into DH5α competent cells (Thermo Fisher Sci) following the protocol in the DH5α competent cells guide. https://assets.thermofisher.com/TFS-Assets/LSG/manuals/18258012.pdf
    i. Thaw 50 μl of DH10α competent cells on ice.
    ii. Add 5–10 μL of the BP reaction into competent cells.
    iii. Keep cells on ice for 30 min.
    iv. Heat shock at 42°C for 30 s in a water bath.
    v. Put the cells on ice for 5 min.
    vi. Add 200 μL of LB media and incubate the tube at 37°C for 1 h with agitation (200 rpm).
i. Streak out cells evenly on a low salt LB agar plate supplemented with zeocin (50 μg/mL) (Thermo Fisher Sci) and incubate at 37°C for 12–18 h.

⚠ CRITICAL: Keep plates and media with zeocin covered in aluminum foil, as zeocin is light sensitive.

j. Pick a single colony from the plate using a sterile pipette tip and inoculate 5 mL of low salt LB media supplemented with zeocin (50 μg/mL). Incubate at 37°C while shaking at 200 rpm for 15–18 h.
k. Pellet the cells at 3,000 × *g* in an Eppendorf 5810 R Centrifuge with an A-4-62 rotor and remove the supernatant.
l. Purify the plasmid from the cell pellet using a QIAprep Spin Miniprep Kit (QIAGEN) following the manufacturer's protocol. (https://www.qiagen.com/us/resources/download.aspx?id=331740ca-077f-4ddd-9e5a-2083f98eebd5&lang=en).
m. Check the DNA concentration (NanoDrop 2000c, Thermo Fisher Sci).
n. Verify the construct by DNA sequencing using forward and reverse M13 sequencing primers. The sequencing facility provides both the M13 (forward and reverse) and M13/pUC (Invitrogen version; forward) options, either will work for the pDONR/zeo. The primer sequences are provided in the key resources table.
o. Follow instructions in the Gateway® Technology user guide to set up and perform LR reaction with pDEST15 vector. https://tools.thermofisher.com/content/sfs/manuals/gatewayman.pdf

Pause point: The LR reaction can be stored at −20°C for up to a week prior to transformation, if necessary.

Note: The minimum time for both BP and LR reactions is 1 h at 25°C. However, for best results use 4 h to 18 h at 25°C.

p. Transform 5–10 μL of the reaction into DH5α competent cells (Thermo Fisher Sci) following the protocol in the DH5α competent cells guide (and as above). https://assets.thermofisher.com/TFS-Assets/LSG/manuals/18258012.pdf
q. Streak out the cells evenly on LB agar plate supplemented with ampicillin (100 μg/mL) (Thermo Sci Fairlawn Chem) and incubate at 37°C for 12–18 h.

r. Pick a single colony from the plate using a sterile pipette tip and inoculate into 5 mL of LB media supplemented with ampicillin (100 μg/mL) in a disposable 50 mL centrifuge tube (Thermo Fisher Sci). Incubate at 37°C while shaking at 200 rpm for 15–18 h.

11. Pellet the cells at 3,000 × *g* in an Eppendorf 5810 R Centrifuge with an A-4-62 rotor and remove the supernatant.
12. Purify the plasmid from the cell pellet using a QIAprep Spin Miniprep Kit (QIAGEN) following the manufacturer's protocol. (https://www.qiagen.com/us/resources/download.aspx?id=331740ca-077f-4ddd-9e5a-2083f98eebd5&lang=en).
13. Check the DNA concentration (NanoDrop 2000c, Thermo Fisher Sci).
14. Verify the construct by DNA sequencing using pGEX 5' (forward) and T7 reverse sequencing primers (provided by the sequencing facility). The primer sequences are provided in the key resources table.
15. Generate the desired mutant constructs (D294K and K383E/K390E/R392E) using the QuikChange Lighting Site-Directed Mutagenesis Kit (Agilent Technologies).
    a. Use wild type BRPF1$_{PZP}$ (aa 271–454) in pDEST15 generated above for mutagenesis protocol.
    b. Design primers for site directed mutagenesis using the Agilent QuikChange and primer Design website (primer sequences can be found in the key resources table). (https://www.genomics.agilent.com/primerDesignProgram.jsp)
    c. Follow instructions in guide (https://www.agilent.com/cs/library/usermanuals/public/210518.pdf) to set up mixture and perform the PCR reaction in a T100™ Thermal Cycler (Bio-Rad).
    d. Add DpnI from the QuikChange Kit (1 μL for every 25 μL of the reaction mixture) to the PCR reaction and incubate at 37°C for 10 min in T100™ Thermal Cycler (Bio-Rad).
    e. Transform 5–10 μL of the reaction mixture into 50 μL XL 10-Gold Ultracompetent cells (Agilent Technologies) that come with the QuikChange Lighting Site-Directed Mutagenesis Kit XL and follow the protocol in the guide. https://www.agilent.com/cs/library/usermanuals/public/210518.pdf
    f. Streak the cells evenly on a LB plate supplemented with ampicillin (100 μg/mL) and incubate at 37°C for 12–18 h.
16. Pick a single colony from the plate using a sterile pipette tip and inoculate into 5 mL of LB media supplemented with ampicillin (100 μg/mL) in a disposable 50 mL centrifuge tube (Thermo Fisher Sci). Incubate at 37°C while shaking at 200 rpm for 15–18 h.
17. Pellet the cells at 3000 × *g* in an Eppendorf 5810 R Centrifuge with an A-4-62 rotor and remove the supernatant.
18. Purify the plasmid from the cell pellet using a QIAprep Spin Miniprep Kit (QIAGEN) following the manufacturer's protocol. https://www.qiagen.com/us/resources/download.aspx?id=331740ca-077f-4ddd-9e5a-2083f98eebd5&lang=en
19. Check the DNA concentration (NanoDrop 2000c, Thermo Fisher Sci).
20. Verify the construct by DNA sequencing using pGEX 5' (forward) and T7 reverse sequencing primers (provided by the sequencing facility). The primer sequences are provided in the key resources table.

Pause point: Properly sealed agar plates containing transformed colonies can be left at 4°C for up to a month, but it is best to use within a week of transformation to prevent contamination.

Prepare 601 DNA for nucleosome assembly

Timing: 6-8 h

21. Obtain pNucB-LexAL plasmid containing 601 nucleosome positioning sequence (NPS) with LexA binding site (North et al., 2012).
22. Design forward and reverse primers for the PCR reaction.
    a. For the 147 bp 601 DNA sequence or the 601 DNA sequence with 30 bp linkers on both sides (207 bp 601 DNA).
    b. For only the 601 DNA sequence, design the forward primer for labeling the 5′ end with fluorescein.
    c. For the 207 bp 601 DNA sequence, design the forward primer to be labeled with fluorescein 27 bp in from the 5′ end.
23. Use the following reagents to set up a single 1× (100 μL) PCR reaction to amplify the DNA. https://www.agilent.com/cs/library/usermanuals/Public/600255.pdf

Reagent	Amount
10× PFU reaction buffer	10 μL
Template plasmid	60 ng
dNTP mix (10 mM of each dNTP)	2 μL
Forward primer	100 pmol
Reverse primer	100 pmol
PFU polymerase (1U/μL)	2 μL
Milli-Q water	fill to 100 μL

⚠ CRITICAL: Add the enzyme last, just prior to starting the reaction and mix thoroughly using a pipette.

Note: PFU polymerase can be home made and purified following the protocol in (Lu and Erickson, 1997).

24. Spin down the PCR mixture and run the reaction with the below parameters in a T100™ Thermal Cycler (Bio-Rad).

PCR cycling conditions			
Steps	Temperature	Time	Cycles
Initial Denaturation	95°C	2 min	1
Denaturation	95°C	1 min	30 cycles
Annealing	65°C	1 min	
Extension	72°C	1 min	
Final extension	72°C	1 min	1
Hold	4°C	Forever	

Note: When performing a new PCR reaction, it is advisable to first do a test 1× PCR and then run a 2% agarose gel to confirm that the reaction produces dsDNA of appropriate length. Annealing temperature can be adjusted to optimize yield. Once the PCR reaction is optimized, it can be scaled up to 24×, 48×, or even 96×, depending on the amount of DNA needed. When doing larger scale reactions, use plates like the TempPlate 96-well 0.2 mL PCR plate with TempPlate Sealing Film.

25. Prepare TE0 and TE1000 buffers and purify the PCR product by anion exchange chromatography.

a. For a MonoQ 5/50 GL column: set up TE0 as buffer A and TE1000 as buffer B.
b. Equilibrate column with 5 CV of 80% buffer A 20% buffer B at 4°C.
c. Combine the PCR reactions from each well of the plate in a 15 mL tube and use an 30 MWCO Amicon Ultra Centrifugal filter to concentrate down to about 2 mL.
d. Inject PCR product onto column and elute dsDNA using the following protocol:

Steps	Flow rate	Buffer composition	Volume
Isocratic flow	0.75 mL/min	80% A 20% B	3 mL
20%->90% Linear salt gradient	0.75 mL/min	80%->10% A 20%->90% B	13.5 mL
90%->100% Linear salt gradient	0.75 mL/min	10%->0% A 90%->100% B	5 mL
Cleaning	0.75 mL/min	0% A 100% B	2 mL
Return to initial conditions	0.75 mL/min	0%->80% A 100% -> 20% B	1.5 mL
Isocratic flow	0.75 mL/min	80% A 20% B	3 mL

Note: 207 bp DNA should elute at ~77% buffer B and the 147 bp DNA should elute at ~79% buffer B.

26. Collect eluted DNA and concentrate it to ~50–70 µL using a 30 MWCO Amicon Ultra Centrifugal filter while buffer exchanging into 0.5× TE (stock 100× TE diluted by 200×).

Note: For a 48× reaction, ~50 µL of 20 µM dsDNA can be obtained.

27. Store 601 DNA for reconstitution of NCP at −20°C. DNA can be stored for ~2 years.

Expression and purification of histones for nucleosome assembly

Timing: 3–4 weeks

The following steps describe how to express and purify histones following an adapted protocol from (Dyer et al., 2004).

28. Obtain histone containing plasmids: hH2A.1 B/E, hH2B.1 O, hH3.2, H4-pET. (Donovan et al., 2019)
29. Transform histone pET- expression plasmids into Rosetta (DE3) pLysS chemically competent cells (Novagen). Follow the Novagen protocol (https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/SAJ/Brochure/2/TB009.pdf) for transformation and plate on LB agar supplemented with ampicillin (100 µg/mL) and chloramphenicol (34 µg/mL).
30. From single colonies, inoculate using sterile pipette tips, 10–15 tubes (disposable 14 mL sterile polypropylene round-bottom tubes; Corning) containing 5 mL of LB preculture supplemented with ampicillin (100 µg/mL) and chloramphenicol (34 µg/mL).
31. Grow precultures for 3–5 h at 37°C and 200 rpm in a shaker (Forma Scientific- Model 4580 Orbital incubator Shaker) until optical density (OD) of the precultures reaches 0.2–0.5 at 600 nm for 1 cm path length (NanoDrop 2000c, Thermo Fisher Sci).
32. Add precultures to scale-up preculture (2L flask with 100–200 mL LB supplemented with ampicillin (100 µg/mL) and chloramphenicol (34 µg/mL) and continue growing as in previous step.
33. When the OD of the scale-up preculture reaches 0.4 at 600 nm, transfer to the large flasks.
34. In a 6 L Erlenmeyer flask (Thermo Fisher Scientific) containing 1 L of 2xYT or terrific broth (TB) media (Thermo Fisher Sci) supplemented with 0.1% glucose (or glycerol) ampicillin (100 µg/mL) and chloramphenicol (34 µg/mL), evenly distribute the scale-up precultures.

35. When the OD of the culture reaches 0.4 at 600 nm, induce the expression with a 0.4 mM isopropyl β- d-1-thiogalactopyranoside (IPTG) (GoldBio).
36. Continue to grow the cultures for 2–3 h at 37°C and 200 rpm.
37. Harvest the cells by centrifugation in a F10-6 × 500y Fixed-Angle Rotor (Thermo Fisher Sci) in a floor centrifuge (Sorvall) for 10 min at 4,400 × *g* and 25°C.
38. Discard the supernatant (if clear, if not then repeat spin) and wash the cell pellets in Histone Wash Buffer supplemented with 1 mM benzamide and 1mM β-mercaptoethanol (BME).

Note: BME is highly toxic and can be readily absorbed through the skin. Gloves are absolutely essential when handling BME solutions and nitrile rubber gloves are preferred over latex gloves. Handling BME solutions should be done in a chemical hood and wearing full body PPE while doing so is recommended.

39. Flash freeze the pellets in liquid nitrogen then store at −80°C. The cell pellets can be stored up to 2 months at −80°C.

Pause point: The cell pellets can be stored up to 2 months in a −80°C freezer.

40. Thaw cell pellets at 37°C and pour into a glass beaker. Bring up the total volume to 50 mL with Histone Wash Buffer (50 mM Tris-HCl pH7.5 and 100mM NaCl) supplemented with 1 mM benzamide, 1 mM ethylenediamine tetraacetic acid (EDTA) and 5 mM BME.
41. Add 1 mg/mL lysozyme and stir the resuspension on a stir plate with a magnetic stir bar for 15 min at 25°C. If lysis is successful, the resuspension should be very viscous.
42. To reduce the viscosity, pour cells back into 50 mL tube and sonicate the cells on ice (the following steps are optimized for a Branson Sonifier 450 with long tip).
    a. Prewash sonicator probe with ethanol then Milli-Q water.
    b. Put the probe at the bottom of the tube containing lysate then lift back up ~ $^1/_2$ inch.
    c. Set sonicator to amplitude 6 for 15 s. After each 15s cycle, remove the sample, invert to mix.
    d. Check the lysate viscosity with a Pasteur pipette by scraping the side of the tube from bottom to top and looking to see if the lysate is pulled up above the surface when the pipette breaches the surface. If the pipette passes through the surface without pulling up any lysate, the lysate is well sonicated.
    e. If the lysate is not well sonicated repeat 15 s sonication, mix, and check again. It normally takes 4–6 rounds to optimally shear the DNA.
43. Spin the cell suspension in balanced 50 mL Nalgene™ Oak Ridge High-Speed PPCO Centrifuge tubes (Thermo Fisher Sci) at 27,000 × *g* using a SS-34 Fixed Angle Rotor (Thermo Fisher Sci) at 4°C–10°C for 30 min.
44. Gently pour the supernatant into a 50 mL tube, be careful not to disturb the pellet as this contains the histone protein. Keep a small fraction of the supernatant sample on ice for further analysis on a gel.
45. Suspend each pellet in 35 mL of Histone Wash Buffer with 1% (v/v) Triton X-100.
46. Spin cells at 10,000 × *g* at 4°C for 10 min and pour off the supernatant.
47. Repeat previous two steps until top brown layer of cell debris is no longer present, leaving just the bottom white/cream layer containing the inclusion bodies and genomic DNA (typically 6 washes are the maximum needed).
48. Repeat previous two steps again, however when washing pellets, use Histone Wash Buffer without 1% Triton X-100
49. Spin and collect the pellets one more time.
50. Try to drain the pellets before flash freezing them and storing them at −80°C to continue the process another day.

Pause point: The pellets containing the inclusion bodies can be stored for up to 1 week in a −80°C freezer.

51. To each pellet, add 480 μL DMSO (160 μL per starting culture), mix and allow to remain for 30 min at 25°C to break up the inclusion bodies.
52. To extract the histones from the inclusion bodies, add 20 mL of Histone Guanidine Buffer slowly, mix well and stir at 25°C for 1 h with a magnetic stir bar.
53. Spin down sample in Nalgene™ Oak Ridge High-Speed PPCO Centrifuge tubes (Thermo Fisher Sci) at 20,000–27,000 × *g* using a SS-34 Fixed Angle Rotor (Thermo Fisher Sci) at 20°C for 30 min.
54. Observe the clarity of the supernatant;
    a. If supernatant is a light transparent yellow to slightly translucent, carefully decant the supernatant to a fresh 15mL or 50mL tube.
    b. If the supernatant in visibly cloudy with strong gradients in density due to suspended debris, centrifuge again at 20°C, 20,000–27,000 × *g* for 30–60 min longer, then observe the clarity of the supernatant again.
55. After the supernatant and the pellet have been separated, store the supernatant containing the histone proteins on ice.

56. Check purification steps by SDS polyacrylamide gel electrophoresis (PAGE).
    a. A 15% SDS PAGE (10–15 wells) can either be poured (5% stacking, 15% resolving) in a 1.5 mm mini gel cassette (Thermo Fisher Sci) or purchased (gradient 4%–15%).
    b. Prepare gel and 4× SDS loading buffer following standard Bio-Rad protocols. http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6201.pdf
    c. Heat samples at 90°C for 10 min to denature proteins, spin down and then load on gel.
    d. Run until the dye front reaches the bottom of the gel.
    Equilibrate, load, and elute the sample off of the Gel Filtration column (Cytiva HiPrep 26/60 Sephacryl-200 High Resolution column), do all of the following at 20°C–25°C to prevent running buffer precipitation.
    e. Wash column with 2 CV of degassed Milli-Q water at ≤ 2mL/min.
    f. Equilibrate column with 1 CV of Histone Urea buffer 1 at 1.3mL/min.
    g. Inject supernatant sample onto the column.
    h. Elute sample at 1.3 mL/min with Histone Urea buffer 1, collecting 5mL fractions across 1 CV. Typically DNA and remaining debris will elute around 1/3 CV and the sample elutes around $^1/_2$ CV.

57. Measure the optical density of each fraction in the range from 220 nm – 350 nm. If separation between the DNA and protein is good, the OD signal should have a peak at 260 nm for fractions eluted at approximately 1/3 CV corresponding to DNA and then the OD signal should transition to a peak at 280 nm for fractions eluted at approximately $^1/_2$ CV corresponding to protein.

    Note: Depending on resolution of the column and the amount of DNA loaded onto the column, a small amount of DNA (<10%) may cross-contaminate the protein fractions and mask the protein signal at 280 nm. If this is the case, one can at least get an idea of where the bulk of the DNA eluted. Additionally, a small amount of contaminating DNA will not adversely affect subsequent steps. Only if a majority of the DNA is still present will histones tend to aggregate onto the DNA during dialysis.

58. Separate gel filtration fractions containing histone protein into two separate pools: (A) Protein and DNA and (B) Protein only based upon the above quantitation (a 260 nm / 280 nm OD ratio of 0.5–0.6 is considered pure protein).
59. Dialyze each pool using 6–8 kDa MWCO dialysis tubing against a minimum of 5 changes of Milli-Q water and 2mM BME (2L each) at 4°C while stirring with a magnetic stir bar at a low speed (60–100 rpm) for at least 6 h per change. The final dialysis should go at least 18 h. During the dialysis process, DNA will typically aggregate out and the sample may become cloudy. This will not adversely affect the yield unless the DNA is a significant fraction of the sample (>10%–-20%).

This may or may not be the case for Pool A, which could have significantly less protein than Pool (B).

60. When dialysis is complete, centrifuge each pool at 4°C, 30000 × *g* for 20 min to pellet out DNA.
61. Carefully remove supernatant from DNA pellet, transferring to a 50 mL tube. At this point supernatant from all pools can be combined if desired or kept separate.
62. Flash freeze samples with liquid nitrogen, then lyophilize the samples using a FreeZone 18 L Freeze Dry System (Labconco).
    a. To lyophilize, take the cap off of the 50 mL tube containing the sample, cover each tube with a Kimwipe secured with a rubber band.
    b. Place each 50 mL tube inside one of the drying chamber adapters.
    c. Secure adapter containing tube onto a drying chamber valve and open the valve slowly.
    d. Make sure the vacuum returns to 0.100 mbar or lower before leaving sample 16 h or longer.
    e. Check that the sample has finished (the sample should look like a powder and will not be cold to the touch any more). The larger the sample, the longer it will need, and it is best to lyophilize each sample double the amount of time that it is expect it to take. After the sample is finished, close the valve slowly and remove the adapter from the valve.
    f. Reclaim the lyophilized sample and store it at −20°C.
63. Equilibrate the Ion Exchange column (TSK-GEL SP-5PW 21.5 mm ID × 15 cm, 10 μm particle size Ion Exchange HPLC column with TSKguardgel kit):
    a. Wash column at 1–2 mL/min with 2 CV of degassed dH2O.
    b. Slowly transition column at 1%–2% per minute into 100% Histone Urea buffer 0 at 1–2 mL/min.
    c. Slowly transition column at 1%–2% per minute into 80% Histone Urea buffer 0, 20% Histone Urea buffer 1 at 1–2 mL/min and allow column to equilibrate with 1–2 CV.
64. Dissolve lyophilized protein pellet in just enough Histone Urea buffer 0.2 (80% Histone Urea buffer 0, 20% Histone Urea buffer 1) so that no aggregates are visible.
65. Measure the optical density of the sample using Histone Urea buffer 0.2 as a blank. If the OD at 276 nm, 1 cm path length > 5, add enough Histone Urea buffer 0.2 to bring the OD to ~5 or less. This will ensure that the histones are fully denatured.
66. Allow the protein to denature on ice for 1 h.
67. Centrifuge sample at 4°C, 5000 × *g* for 10 min to precipitate any residual aggregates.
68. Inject no more than 4 mL of sample at OD 276 nm, 1 cm path length ~5 onto the 21.5 mm ID × 15 cm column. These are the limits at above which the column will become overloaded.

69. Use the following program to elute the histones, typically the histones elute in the last 15 min.

Steps	Flow rate	Buffer composition	Time
20%->32% Gradient	2 mL/min	80%->68% A 20%->32% B	3 mins
Isocratic flow	2 mL/min	68% A 32% B	5 mins
32%->60% Gradient	2 mL/min	68%->40% A 32%->60% B	32 mins
Isocratic flow	2 mL/min	40% A 60% B	25 mins
Return to initial conditions	2 mL/min	40%->80% A 60% -> 20% B	3 mins

    a. Collect fractions by hand based on real-time UV-VIS spectra at 280 nm or collect 2 mL fractions across the entire gradient.
    b. Pool fractions containing protein from multiple runs and dialyze in 6–8 kDa MWCO dialysis tubing against at least 5 – 2 L changes of Milli-Q water and 2 mM BME at 4°C for at least 6 h per change, with the last one going a minimum of 16 h.
    c. When dialysis is complete, transfer sample to 50 mL tubes, flash freeze with liquid nitrogen, lyophilize and store at −20°C.

d. Quantify histone yield:
   i. Dissolve lyophilized protein pellet in just enough Milli-Q water so that no aggregates are visible. Thoroughly wash down the sides of the tube to remove protein stuck to the tube wall.
   ii. Measure the optical density of the sample using Milli-Q water as a blank to determine the yield. If the measured concentration of the histone is > 10 mg/mL, add enough Milli-Q water to reduce concentration to ≤ 10 mg/mL. This will ensure that the histone is fully resuspended.

70. Aliquot histones in desired amounts into 1.5 mL centrifuge tubes.
71. Lyophilize histones and store at −80°C. Lyophilized protein can be stored at −80°C for years.

Note: For convenient octamer refolding, aliquot histones using an "x" system to achieve the desired yield of purified octamer later on. Using 1× histone aliquots for refolding produces 125–175 μg of purified histone octamer and 10× aliquots used for refolding produces 1.5–2 mg of purified octamer down the line. For H3 and H4, each x is 6.55 nmol of histone protein, and for H2A and H2B, each x is 7.86 nmol since H2A and H2B are added at a 20% molar excess during octamer refolding. For example, a 2.5× aliquot of H3 would contain 16.4 nmol H3, while a 2.5× aliquot of H2B would contain 19.7 nmol of H2B. Typically 2.5× aliquots of each histone are made.

Refolding the octamer

Timing: 2 days

72. Prepare 100 mL unfolding buffer and 3 × 1.8 L refolding buffer.
73. Determine the scale of octamer refolding required and thaw aliquots of H2A, H2B, H3, and H4 from −80°C storage.
74. Resuspend histone aliquots (H2A, H2B, H3 and H4) in unfolding buffer. To ensure each histone is resuspended at a concentration close to 5 μg/μL, add the following amounts of unfolding buffer to 2.5× aliquots:
    H2A: ~55 μL
    H2B: ~54 μL
    H3: ~50 μL
    H4: ~37 μL

⚠ CRITICAL: It is critical to resuspend the histones at <10 μg/μL concentrations, as concentrations >10 μg/μL lead to precipitation.

75. Let the histones unfold on ice for 1 h.
76. Centrifuge the histone solutions at 20,000 × *g* for 2 min to pellet any aggregates. Transfer supernatants into new tubes.
77. Measure A_{280} to determine concentrations of histones using the table below:

Histone	M.W.	$e_{280}(c^{-1}M^{-1})$
Human H2A	13974	3840
Human H2B	13775	6400
Human H3.2	15271	4040
Human H4	11236	5400

78. Combine histones at a molar ratio of 1:1.2 for H3/H4:H2A/H2B.

Note: The octamer can be separated from aggregates, H2A-H2B dimer, and free histones by size exclusion chromatography. 20% molar excess of H2A and H2B is used to avoid the presence of H3-H4 tetramer, which is more difficult resolve from histone octamer.

79. Once the sample is ready, the microdialysis chamber needs to be prepared. The process to do so is described in the following lines adapted from (Thastrom et al., 2004) and depictions of how to make the microdialysis chamber can be found in this referenced manuscript.
    a. Choose a microdialysis chamber size based on the sample volume. Typical scale octamer refolding is ~5 × −10×, which usually results in a 250 μL sample volume.
    b. Cut a piece of parafilm to use as a sterilized work zone.
    c. Above the parafilm, use a sterilized tube cutter to cut the appropriate tube close to the top or cap of the tube. The cap plus leftover rim will be used to make the chamber, the rest can be discarded.
    d. Cut a small, approximately 1″ × 1″ square out of the 6–8 kDa MWCO dialysis tubing. This will be used as the dialysis membrane for the chamber.

Sample volume	Microdialysis chamber material
50 μL	BrandTech 0.5 mL PCR tube
250 μL	Eppendorf 1.5 mL microcentrifuge tube
> 500 μL	10 mm diameter 6–8 kDa MWCO dialysis tubing

Note: To prevent contamination, always wear gloves cleaned with ethanol and Milli-Q water when handling dialysis membrane.

80. Place the sample inside the tube cap for the microdialysis chamber, then place the dialysis tubing square on top and seal the chamber using the leftover tube rim.
81. Place the microdialysis chamber inside of a 50 mm 6–8 kDa MWCO dialysis bag and add 80 mL of unfolding buffer to the bag.
82. Place the dialysis bag into 1.8 L of refolding buffer with BME to begin double dialysis at 4°C while stirring with a magnetic stirrer at a low speed (60–100 rpm).

⚠ CRITICAL: Remember to add the BME to the refolding buffer once the bag is placed into the buffer.

83. After 3 h, place bag into fresh 1.8 L of refolding buffer with fresh BME and continue dialysis for 3 more h.
84. Place dialysis bag into the third 1.8 L of refolding buffer with BME and finish dialyzing for a minimum of 16 h.
85. Next day, carefully remove sample from microdialysis chamber. Centrifuge sample at 20,000 × *g* for 2 min to pellet aggregates and transfer sample to a new tube.
86. Perform size exclusion chromatography to separate the octamer from aggregates, a dimer, and free histones.
    a. For a Superdex 200 Increase 10/300 GL column (Cytiva), first set up filtered Milli-Q water as buffer A and refolding buffer B without BME.
    b. Equilibrate the column in 2 CV (~50 mL) of refolding buffer at 4°C.
    c. Inject the octamer onto the column and elute with 1 CV of refolding buffer.

Note: Aggregates, an octamer, a dimer, and then free histones should elute at approximately 5.5 mL, 9 mL, 11.75 mL, and 19.5 mL, respectively.

87. Collect the octamer and concentrate it using a 30 kDa MWCO Amicon centrifugal filter.

a. Spin at 2,500 × *g* at 4°C. Mix the octamer every 15–20 min to prevent high local concentration at the bottom of the filter.

Note: For typical refolding of 5×, concentrate to approximately 50 μL of volume. For larger octamer preparations, concentrate to approximately 5 mg/mL.

⚠ CRITICAL: Do not concentrate above 10 mg/mL or the octamer will precipitate.

88. Quantify yield of the octamer and store at −20°C.
    a. Determine the concentration of the octamer using an OD reading of 0.45 at 276 nm with 1 cm path length, equaling 1.0 mg/mL octamer as a standard.
    b. For storage, first measure the final volume of the octamer sample with a pipette, then add an equal volume of cold (stored at 4°C), autoclaved 80% glycerol and store at −20°C for up to 1 year.

Prepare nucleosome core particles (NCPs)

Timing: 3 days

89. Make 100 mL of reconstitution buffer with 2 M NaCl and 2 × 4 L of reconstitution buffer without 2 M NaCl. Store buffers at 4°C until ready to begin dialysis.
90. As was done with the octamer, choose a microdialysis chamber size based on sample volume. Typical scale nucleosome reconstitution involves input of 50 pmol octamer, which usually results in a 50 μL sample volume. Use the appropriate tube with 6–8 kDa MWCO dialysis membrane (Thastrom et al., 2004).

Sample volume	Microdialysis chamber material
50 μL	BrandTech 0.5 mL PCR tube
250 μL	Eppendorf 1.5 mL microcentrifuge tube
> 500 μL	10 mm diameter 6–8 kDa MWCO dialysis tubing

91. To make the microdialysis chamber, refer back to step 49 in the octamer refolding section.
92. Combine the octamer and DNA with 75% molar excess DNA in the chosen sample volume and make sure the sample volume has buffer conditions matching the reconstitution buffer with 2 M NaCl.
93. For a typical 50 pmol scale reconstitution, combine the following in a 50 μL sample volume:

Reagent	Final concentration	Amount
DNA	1.75 μM	87.5 pmol
Octamer	1 μM	50 pmol
5 M NaCl	2 M	20 μL
10× TE + 20 mM BZA	0.5 TE + 1 mM BZA	2.5 μL
Milli-Q water		fill to 50 μL

94. Place the sample inside the tube cap for the microdialysis chamber, then seal it using the dialysis tubing square and leftover rim.
95. Place the microdialysis chamber inside of a 50 mm 6–8 kDa MWCO dialysis bag and add 80 mL of reconstitution buffer with 2 M NaCl to the bag.
96. Place the bag into 4 L of reconstitution buffer without 2 M NaCl to begin double dialysis at 4°C.

97. After 4–6 h, place bag into second 4 L of reconstitution buffer without 2 M NaCl and continue dialysis for 16–18 h.
98. Prepare 5% and 30% Sucrose with 0.5× TE and place them at 4°C for the next day.

99. Make 5–30% sucrose gradients with 0.5× TE in Beckman-Coulter 13.2 mL polypropylene ultracentrifuge tubes using a BioComp Gradient Master. Biocomp Instruments provides a useful instructional video on the process at: http://biocompinstruments.com/documents/type/category/instructional-videos
    a. Fill each tube to the designated level with 5% sucrose with 0.5× TE according to the BioComp SW41 marker block.
    b. Insert 30% sucrose under the 5% sucrose and fill with 30% to the marker block's designated level.

Alternatives: If you do not have access to a BioComp Gradient Master, a Cytiva SG Series Gradient Mixer is a lower cost alternative.

⚠ CRITICAL: Be careful while inserting the 30% sucrose under the 5%; do it slowly and avoid manually mixing the 30% with the 5% sucrose.

    c. Seal the tubes with 10 mm SW41 caps and place them into the Gradient Master tube holder.
    d. Perform a 5%–30% gradient mix program with the Gradient Master.
    e. Gradients are stored at 4°C until nucleosomes are ready to load.

100. Reclaim unpurified nucleosomes from microdialysis chamber and keep them on ice.
101. Remove excess sucrose from the caps, balance the sucrose gradients, and load the nucleosomes on top of the gradients by dispensing against the tube wall close to the top. Do not dispense them directly into the gradient.
102. Gradients are centrifuged for 22 h at 130,000 × *g*, 4°C in a Beckman-Coulter Ti-41 swinging bucket rotor using an Optima L-90K Ultracentrifuge.

Note: During this time, nucleosomes centered on the 601 sequence will be separated from aggregates, improperly-positioned nucleosomes, tetrasomes, hexasomes, and free DNA according to sedimentation velocity.

103. Once the ultracentrifuge has begun its run, make a 5% native gel as done in the EMSA protocol (see below) with 20 wells and pre-run it to prepare it for the next day.

104. Once the ultracentrifuge has finished its run, unload the sucrose gradients and fractionate them using a fraction collector with peristaltic pump like the Bio-Rad Model 2110 fraction collector combined with a Watson Marlow Sci Q 400 peristaltic pump.

Note: The Bio-Rad Model 2110 manual (https://www.bio-rad.com/webroot/web/pdf/lsr/literature/M7318120.pdf) is a great guide for connecting the column to the fraction collector. Additionally, references (Behal et al., 2009; Verdin et al., 2015) provide images for the overall set up of the system.

    a. To fractionate the gradients, first insert a 1-mm glass capillary tube attached to 1 mm I.D. tubing into the bottom of the centrifuge tube.
    b. Connect the tubing to the peristaltic pump and set up the tubing to dispense through the Bio-Rad Model 2110 fractionator arm.
    c. Secure the carousel with fraction 1 directly beneath the fractionator arm and load microcentrifuge tubes into fraction mounts 1–30 in the fractionator.
    d. Set up the fractionator in time mode and set the time interval between carousel rotations to 40 s.

e. Turn on the peristaltic pump at a very low speed initially (set the dial to ~1) and adjust the speed of the peristaltic pump in order to maintain 0.4 mL of sucrose gradient dispensed per fraction.
f. Collect up to 30 of the 0.4 mL fractions and place them on ice, numbering them 1–30 with fraction 1 being the first 0.4 mL fraction dispensed. In this way, the earliest fractions correspond to the molecules with the highest sedimentation velocities.

105. Set up 5% native gel.
    a. prepare a 5% native gel for vertical electrophoresis using the following handbook as a guide: https://assets.thermofisher.com/TFS-Assets/BID/Handbooks/protein-gel-electrophoresis-technical-handbook.pdf

Alternatives: Commercial substitute can be found at the following: https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-gel-electrophoresis/protein-gels/specialized-protein-gels/nativepage-bis-tris-gels.html

    b. Purge wells of unpolymerized acrylamide with 0.5× TBE using a 10 mL syringe with a needle attachment.
    c. Use a syringe with a bent needle to remove air bubbles from the bottom of the gel plates from where the spacer was removed.
    d. Pre-run gel at 300V for 1 h in 0.3× TBE buffer to remove APS from gel.

106. Load 10–15 μL of fractions 1–20 onto the 5% native gel prepared the day before while the gel is running (run gel at 300V for 1.5 h).
107. Image the native gel with a Typhoon FLA9500 phosphor imager via fluorescein excitation to resolve the location of well-position nucleosomes in the fractions that are free of tetrasomes, hexasomes, and free DNA. Alternatively, perform an EtBr bath as described in the EMSA section.

Note: Typically, for the NCP_{207}, well-positioned NCPs begin appearing in fractions 4-5, improperly positioned NCPs begin appearing in fractions 5-6, and free DNA begins appearing in fractions 10-11. For the NCP_{147}, well-positioned NCPs begin appearing in fractions 5-6, free DNA again begins appearing in fractions 10-11, and improperly-positioned nucleosomes should not be observed.

108. Pool fractions containing well-positioned NCPs and concentrate the NCPs to 50–70 μL using a 30k Amicon MWCO centrifugal filter while buffer exchanging into 0.5× TE.

Note: 50%–75% loss of sample can be expected during reconstitution and sucrose gradient purification. Especially in the case of NCP_{207} where much of the sample is converted into improperly-positioned NCPs. For a 50 pmol octamer scale NCP_{207}, expect a yield of ~60 μL NCP_{207} at 90 nM.

109. Add autoclaved 80% glycerol to the nucleosomes for a final glycerol concentration of 20%.
110. Aliquot the NCPs in amounts just needed for one EMSA or fluorescence polarization assay per aliquot. Then flash freeze and store the nucleosomes at −80°C.

Note: The flash frozen NCP_{207} or NCP_{147} can be stored at −80°C for ~1 year.

Prepare for histone acetyltransferase (HAT) assays

Timing: 7–8 days

111. Transform plasmids into DH10α competent cells.

a. Thaw 30 μl of DH10α competent cells on ice.
b. Add 50 ng of plasmids into competent cells.
c. Keep the cells on the ice for 30 min.
d. Heat shock at 42°C for 30 s.
e. Put the cells on the ice for 5 min.
f. Add 1 mL of LB media and incubate the tube at 37°C for 1 h with agitation (225 rpm).
g. Spread the cells on ampicillin plate.
h. Incubate the plate for 12–16 h at 37°C.

112. Amplification of plasmids
    a. Inoculate one colony into 400 mL of LB media.
    b. Incubate at 37°C with shaking at 225 rpm for 12–16 h.
    c. Extract the plasmids by NucleoBond Xtra Maxi kit (Macherey Nagel) according the manufacturer's protocol. https://www.mn-net.com/media/pdf/ed/82/0f/Instruction-NucleoBond-Xtra.pdf
113. Transfection of plasmid in HEK293T cells.

Note: HA-BRPF1 (WT, D294A or PZPΔ359-450), FLAG-MORF$_{N1-716}$, FLAG-ING5 and FLAG-MEAF6 plasmids are in pcDNA3. BRPF1 is tagged with 1xHA while the others proteins are tagged with 3xFLAG.

114. Culture HEK293T cells in DMEM (ThermoFisher) at 37°C in a 5% CO_2 incubator (Panasonic).
    a. Co-transfect (2×10^6) cells with 5 μg of each plasmid following calcium phosphate method.
        i. Mix 5 μg of each expressing plasmids, HA-BRPF1 (WT, D294A or PZPΔ359-450), FLAG-MORF$_{N1-716}$, FLAG-ING5 and FLAG-MEAF6 in 125 μl of 2× Calcium Phosphate buffer and add H_2O to 500 μl.
        ii. Add drop by drop 2× Hepes-buffered saline (HBS) with gentle agitation.
        iii. Let stand for 20 min at 25°C.
        iv. Meantime add fresh complete DMEM medium.
        v. Apply drop by drop the mixture in HEK293T cells (ATCC CRL-11268).
        vi. Following 16–18 h incubation, wash with Hepes 1× buffer.
        vii. Add fresh complete DMEM medium.

Note: It is important to have high-quality plasmids for transfection. The quality of plasmid can be verified by running plasmids on an agarose gel.

115. Purification of HA-BRPF1 (WT, D294A or PZPΔ359-450), FLAG-MORF$_{N1-716}$, FLAG-ING5 and FLAG-MEAF6 complex
    a. Preparation of Nuclear Extracts (Doyon and Cote, 2016)
        i. After 70 h of transfection, remove the DMEM medium by aspiration and wash 2 times with PBS1X (Invitrogen).
        ii. Scrape cells into fresh PBS1X and pellet the cells by centrifuging 5 min at 300 × *g* .
        iii. Remove the supernatant and determine the volume of the cells (using graduated 15 mL tube).

⚠ CRITICAL: The following steps should be done strictly on ice or at 4°C.

        iv. Resuspend rapidly the pellet in 5 volumes of Hypotonic Buffer.
        v. Centrifuge 5 min at 1,850 × *g* and remove the supernatants.
        vi. Resuspend the pellet in 3 volumes of Hypotonic Buffer and keep for 10 min on ice.
        vii. Transfer the cells into Dounce homogenizer with a tight piston (according volume ex: if the volume 10mL, use Dounce Homogenizer 15 mL).
        viii. Homogenize very carefully to avoid bubbles with 15 up and down strokes.
        ix. Transfer the cells into a 15 mL tube and centrifuge 15 min at 3,500 × *g*.

x. Recover the supernatant as Cytoplasmic fraction.
xi. Evaluate the volume of Nuclear pellets using a graduated 15mL tube and resuspend with $^1/_2$ volume of Low Salt Buffer.
xii. Add drop by drop $^1/_2$ volume of High Salt buffer while gently mixing.
xiii. Homogenize with 2 up and down strokes.
xiv. Allow to nuclei extract for 30 min at 4°C with continuous mixing.
xv. Pellet the extract nuclei by centrifuging at 24000 × *g* for 60 min.
xvi. Recover the supernatant as Nuclear Extract (NE).
xvii. Flash freeze and keep in −80°C until purification.

⚠ CRITICAL: Before performing purification, check expression of transfected proteins by Western Blot using FLAG and HA antibodies.

b. Tandem Affinity Purification (TAP) (Avvakumov et al., 2012; Lalonde et al., 2013)
i. Before TAP, prechill rotor, centrifuge tube and ultracentrifuge to 4°C.
ii. Thaw NE on ice.
iii. Add 0.1% Tween™ 20 drop by drop with gentle agitation using vortex.
iv. Ultracentrifuge NE at 30000 × *g* for 30 min (Ti-70 rotor Beckman Coulter).
v. Meantime, wash HA agarose beads with 1 mL of mixture of 3 volumes of Low Salt buffer and 1 volume of High Salt buffer on the wheel during 5 min and centrifuge at 1000 × *g* for 3 min. Remove supernatant by aspiration and resuspend in wash Buffer (3 volumes of Low Salt Buffer and 1 volume of High Salt Buffer).
vi. Recover supernatant from step iv above and add 125 µl of washed HA agarose beads (Anti-HA affinity Matrix; Roche).
vii. Incubate 3 h at 4°C on the wheel (500rpm).
viii. Centrifuge at 1000 × *g* for 3 min and discard the supernatant.
ix. Wash with 1 mL of Wash I buffer for 10 min on the wheel at 4°C.
x. Centrifuge at 1000 × *g* for 3 min and discard the supernatant.
xi. Wash with 1 mL of Wash II buffer for 10 min on the wheel at 4°C.
xii. Centrifuge at 1000 × *g* for 3 min and discard the supernatant.
xiii. Elute with 1.25 volume of HA-beads with Wash II buffer plus 500 µg/mL of 3xHA peptides on the wheel for 60 min.
xiv. Meantime, wash FLAG M2 agarose beads (125 µL for each condition) with 1 mL of Wash II buffer.
xv. Centrifuge at 1000 × *g* for 3 min and recover the supernatant.
xvi. Add 125 µl of FLAG M2 agarose beads.
xvii. Incubation for 2 h on the wheel at 4°C.
xviii. Centrifuge at 1000 × *g* for 3 min and discard the supernatant.
xix. Wash 2 times with 1 mL of Wash II buffer for 10 min on the wheel at 4°C.
xx. Centrifuge at 1000 × *g* and discard the supernatant.
xxi. Elution with 1,25 volume of FLAG-beads with Wash II buffer plus 150 µg/mL of 3xFLAG peptides on the wheel for 60 min.
xxii. Centrifuge at 1000 × *g* and recover the supernatant.
xxiii. Make aliquot and flash freeze and store at −80°C.

Note: FLAG elution can be repeated in order to increase amount of the purified complex.

⚠ CRITICAL: Before achieving *in vitro* HAT assays, the quality of purification should be monitored by Western Blot using anti-HA/FLAG (1/4000 anti-HA HRP (3F10) 12013819001 /FLAG (1/10000 anti-FLAG M2 HRP A8592) antibodies.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-HA HRP High Affinity (3F10)	Roche	Cat. # 12013819001
Anti-FLAG HRP	Sigma-Aldrich	Cat. # A8592-1MG
Bacterial and virus strains		
Escherichia coli Rosetta-2 (DE3) pLysS	Novagen- Thermo Fisher Scientific	Cat. # 71-401-3
Escherichia coli BL21 (De3) RIL	Promega	Cat. # L1195
DH5α Competent Cells	Thermo Fisher Scientific	Cat. # 18265017
XL 10-Gold ultracompetent cells	Agilent Technologies	Cat. #200517-4
Critical commercial assays		
QuikChange Lightning Site-Directed Mutagenesis Kit	Agilent Technologies	Cat. # 210518
QIAprep Spin Miniprep Kit	QIAGEN	Cat # 27106
MinElute PCR Purification Kit	QIAGEN	Cat # 28004
NucleoBond Xtra Maxi Kit	Macherey Nagel	Cat # 740414.100
Other		
Cell filter	Corning	Cat: # 3008
Regenerated Cellulose Dialysis Tubing	Thermo Fisher Scientific	Cat. # 21-152-14
1000 µL Universal Pipetter Tips: wide bore	Axygen	Cat: # 14-222-703
ÄKTA pure 25 M	Cytiva-formerly GE Healthcare	Cat: # 29018226
HiPrep™ 16/60 Sephacryl® S-100 HR column	Cytiva-formerly GE Healthcare	Cat. # 17-1165-01
HiLoad® 16/60 Superdex® 75 pg	Cytiva-formerly GE Healthcare	Cat. # 28-9893-33
Superdex 200 Increase 10/300 GL column	Cytiva	Cat # 28990944
MonoQ 5/50 GL	Cytiva	Cat # 17516601
Cytiva HiPrep 26/60 Sephacryl-200 High Resolution column	Cytiva	Cat. # 17119501
TSK-GEL® SP-5PW 21.5 mm ID x15 cm, 10 µm particle size Ion Exchange HPLC Column with TSKguardgel Kit	Tosoh Bioscience	Cat. # 07575 & 16093
Kimwipes Delicate Task Wipers	Thermo Fisher Scientific	Cat. # 06-666
FreeZone 18 Liter Console Freeze Dry System	Labconco	Cat. # 7755030
Econo-Pac® Chromatography Columns	Bio-Rad	Cat. # 7321010
Amicon Ultra 4 mL 10K NMWL centrifugal filter unit	Millipore	Cat. # UFC801024
Amicon Ultra 15 mL 10K NMWL centrifugal filter unit	Millipore	Cat. # UFC901024
Millex-GV Syringe Filter Unit, 0.22 µm	Millipore	Cat. # SLGU033RS
Sorvall RC-5B Refrigerated Superspeed Centrifuge	Sorvall	N/A
F10-6 × 500y Fixed-Angle Rotor	Thermo Fisher Scientific	Cat. # 78510TS
SS-34 Fixed Angle Rotor	Thermo Fisher Scientific	Cat. # 28020TS
Unico Test Tube Rocker L-TTR200	UNICO	Cat. # 8062520000
Microplate Titer Plate Shaker	Lab-Line Instruments, Inc.	N/A
Eppendorf 5810 R Centrifuge with an A-4-62 rotor	Eppendorf	Cat. # 022625501
Eppendorf Microcentrifuge 5424R with F45-24-11 rotor	Eppendorf	Cat. # 5404000413
Biowave CO8000 Cell Density Meter	Biochrom	Cat. # 80-3000-45
6 L Reusable Glass Narrow-Mouth Erlenmeyer Flasks	Thermo Fisher Scientific	Cat. # FB5006000
MaxQ™ 5000 Floor-Model Shaker	Thermo Fisher Scientific	Cat. # 11-675-208
Innova 4330	New Brunswick Scientific CO., Inc.	INNOVA 4330
Forma Scientific Model 4580 Orbital Shaker Incubator	Forma Scientific	Cat. # 25423
Q500 Sonicator	Qsonica	Cat. # Q500-100
Varian INOVA 600 MHz NMR spectrometer	Agilent Technologies	N/A
Rigaku Micromax 007 high-frequency microfocus X-ray generator	Rigaku	N/A
NanoDrop 2000c	Thermo Fisher Scientific	Cat. # ND-2000C
T100™ Thermal Cycler	Bio-Rad	Cat. # 1861096
Tecan infinite M1000Pro plate reader	Tecan	N/A
Corning® 384-well Black Round Bottom Polypropylene Microplate	Corning	Cat. #3658
Typhoon FLA 9500	GE Healthcare	N/A
Power source (Fisher Scientific FB300)	Fisher Scientific	Cat # S65533Q
Apogee V16 Vertical Electrophoresis Apparatus	Apogee	Cat # 21070010
Apogee V16 Spacer Set, 0.8 mm Thick	Apogee	Cat # 41077017

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Apogee V16 Comb, 20 Well, 0.8 mm Thick	Apogee	Cat # 21076013
Apogee V16 Comb, 12 Well, 0.8 mm Thick	Apogee	Cat # 11074010
Apogee V16 Spacer Blocks	Apogee	Cat # 21070057
Apogee V16 Glass Plates Packaged (3 sets of 2)	Apogee	Cat # 11074010
Binder Clips, Medium, 1-1/4" Wide, 5/8" Capacity, Black	Office Depot	Cat # 561339
Borosilicate glass capillaries, ends cut, 1 mm O.D.	Hilgenberg	Cat # 1408409
Saint-Gobain Versilic Silicone 1mm I.D. Tubing	Fisher Scientific	Cat # 10627555
Long-tip Glass Pipet	Wilmad-LabGlass	Cat. # 803A
Pipette bulb	Sigma-Aldrich	Cat. # Z509035
Shigemi 5 mm Symmetrical NMR microtube	Shigemi, Inc	Cat. # BMS-005B
Falcon™ 50 mL Conical Centrifuge Tubes	Thermo Fisher Scientific	Cat. # 06-443-19
Falcon™ 14 mL Polypropylene Round-Bottom Tubes	Thermo Fisher Scientific	Cat. # 352059
Nalgene™ Oak Ridge High-Speed PPCO Centrifuge Tubes, 50 mL	Thermo Scientific	Cat. # 3119-0050
Additive Screen	Hampton Research	Cat. # HR2-428
Tube rotators	Lab Quake	Cat. # 415110
Magnetic stirrers	Corning	Cat. # PC-410
TempPlate 96-well 0.2 mL PCR Plates	USA Scientific	Cat # 1402-9596
TempPlate Sealing Film	USA Scientific	Cat # 2921-0010
Beckman Coulter 13.2 mL polypropylene ultracentrifuge tubes	Beckman Coulter	Cat # 331372
Optima L-90K Ultracentrifuge	Beckman Coulter	N/A
BioComp Gradient Master	BioComp Instruments	Cat # 108
10 mm Isopycnic (long) caps (Sw40/41)	BioComp Instruments	Cat # 105-514-6
Marker Block for SW41 Tubes	BioComp Instruments	Cat # 105-614A
Bio-Rad Model 2110 fraction collector	Bio-Rad	Cat. # 7318122
Watson-Marlow Sci Q 400A peristaltic pump	Watson-Marlow	Cat. # 040.1S1D.01A
NanoDrop ND-1000 Spectrophotometer	Marshall Scientific	Cat. # ND-1000
Chemicals, peptides, and recombinant proteins		
Dithiothreitol (DTT)	Gold Biotechnology	Cat. # 27565-41-9
β-Mercaptoethanol (BME)	Sigma-Aldrich	Cat. # M6250-100ML
Phenylmethanesulfonyl fluoride (PMSF)	Sigma-Aldrich	Cat. # P7626
Ethylenediamine tetraacetic acid (EDTA)	Fisher Chemical	Cat. # S311-500
Lysozyme	Thermo Fisher Scientific	Cat. # 89833
DNase I	Roche	Cat. # 10104159001
Nonident NP-40	Sigma-Aldrich	Cat. # 127087-87-0
Ampicillin sodium salt	Thermo Sci Fairlawn Chemicals	Cat. # BP176025
Chloramphenicol	Fisher BioReagents	Cat. # C-105
Imidazole	Alfa Aesar	Cat. # A10221
100% Ethanol 200 proof	Decon Labs, Inc	Cat. # V1016
Microbiology media: Luria-Bertani (LB), Miller	Thermo Fisher Scientific	Cat. # BP1426-2
LB agar, Miller	Thermo Fisher Scientific	Cat. # BP9724-500
Terrific broth (TB) media	Thermo Fisher Scientific	Cat. # BP9728-2
TempAssure® 0.2mL PCR Tubes, Flat Caps	USA Scientific	Cat. # 1402-8100
Zeocin™	Thermo Fisher Scientific	Cat. # 45-0430
PfuTurbo DNA Polymerase AD	Agilent Technologies	Cat. # 600255
T4 DNA ligase	New England Biolabs	Cat. # M0202L
Gateway™ BP Clonase™ II Enzyme mix	Thermo Fisher Scientific	Cat. # 11789100
Gateway™ LR Clonase™ II Enzyme mix	Thermo Fisher Scientific	Cat. # 11791-020
Reduced glutathione	Sigma-Aldrich	Cat. # G4251
Ammonium chloride ($^{15}NH_4Cl$)	Sigma-Aldrich	Cat. # 299251
Ammonium chloride ($^{14}NH_4Cl$)	Sigma-Aldrich	Cat. # 21,333-0
Multivitamin tablet	Centrum	Walmart # 573963323
Zinc chloride ($ZnCl_2$)	Fisher Chemical	Cat. # Z53-500
Deuterium oxide (D_2O)	Sigma-Aldrich	Cat. # D4501
Isopropyl β-d-1-thiogalactopyranoside (IPTG)	GoldBio	Cat. # I2481
Sodium dodecyl sulfate (SDS)	Sigma-Aldrich	Cat. # L3771
Quick Start™ Bradford 1× Dye Reagent	Bio-Rad	Cat. # 500-0205

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Tryptone	Sigma-Aldrich	Cat. # T9410
Yeast extract	Sigma-Aldrich	Cat. # Y1625
Mixed bed resin	Sigma-Aldrich	Cat. # M8032
Glacial acetic acid	Sigma-Aldrich	Cat. # 695092
Sodium acetate trihydrate	Sigma-Aldrich	Cat. # 236500
Sucrose	Sigma-Aldrich	Cat. # S0389
Scintillation cocktail	MP Biomedicals	Cat. # 0188247504
NHS-Fluorescein	Thermo Fisher	Cat #46409
Boric acid	Sigma-Aldrich	Cat: # B0394-5KG
EDTA	Sigma-Aldrich	Cat: # E9884-1KG
Acrylamide	Sigma-Aldrich	Cat: # A2792-100ML
Ammonium persulfate (APS)	Sigma-Aldrich	Cat: # A3678-25G
AccuGel 29:1 acrylamide:bis-acrylamide solution (40%)	National Diagnostics	Cat # EC-852
TEMED	Sigma-Aldrich	Cat: # T9281-25ML
Ethidium bromide (EtBr) solution (10 mg/mL)	Sigma-Aldrich	Cat # E1510
Benzamidine hydrochloride hydrate	Sigma-Aldrich	Cat # B6506-100G
UltraPure™ Agarose	Thermo Fisher	Cat: # 16500-100
Triton x 100	Calbiochem	Cat: # 9410-1L
Tris-base	Fisher BioReagent	Cat: # BP1525
Na_2HPO_4-$7H_2O$	MP Biomedicals	Cat: # ICN19144101
Potassium phosphate monobasic (KH_2PO_4)	Fisher Chemical	Cat: #P380-212
Calcium chloride ($CaCl_2$)	Sigma-Aldrich	Cat: # C3881-500G
D(+)-Glucose monohydrate	Acrose	Cat: # 450740010
NaCl	Fisher Chemical	Cat: # S27110
Magnesium sulfate ($MgSO_4$)	Sigma-Aldrich	Cat: # M7506-1KG
DMEM	Thermo Fisher	Cat: # 11995040
HEPES	Fisher	Cat: # BP310-5
Tween-20	Sigma-Aldrich	Cat # P9416
Glycerol	Fisher	Cat: # G334
Potassium chloride (KCl)	Sigma-Aldrich	Cat: # P9333-1kg
Sodium butyrate	Sigma-Aldrich	Cat: # B5887
Na_2HPO_4	Sigma-Aldrich	255793-50G
Guanidine HCl	MP Biomedicals	Cat # 820540
dNTP 10 mM solution	New England Biolabs	Cat # N0447L
Magnesium sulfate heptahydrate ($MgSO_4$*7H2O)	Sigma-Aldrich	Cat # M1880
KCl	Sigma-Aldrich	Cat # P9541
Ammonium sulfate $(NH_4)SO_4$	Sigma-Aldrich	Cat # A4418
Triton-X	Sigma-Aldrich	Cat # A7638
EDTA salt dihydrate	Sigma-Aldrich	Cat # E1644
NaCl	Sigma-Aldrich	Cat # S3014-5KG
Pierce Glutathione Agarose	Thermo Fisher Scientific	Cat. # 16101
Streptavidin magnetic beads	Thermo Fisher Scientific	Cat. # 88816
Anti-HA agarose beads	Roche	Cat. # 118-150-16001
FLAG M2 agarose beads	Sigma Aldrich	Cat. # A2220-5ML
HA agarose beads (Anti-HA affinity Matrix)	Roche	Cat. # 11815016001
$H3_{1-12}$, $H3_{15-34}$, $H3_{1-31}$ peptides	Synpeptide	N/A
3xHA peptides	GenScript	On demand
3xFLAG peptides	Sigma-Aldrich	Cat. # F4799-25mg
Tobacco etch virus (TEV) protease	Liu et al., 2020	Home made
Free histones (purified from HeLa cells)	Côté et al., 1995	Home-made
Short oligonucleosomes (purified from HeLa cells)	Côté et al., 1995	Home-made
0.125 μCi of ^3H labeled Ac-CoA (0.1 mCi/mL)	Perkin Elmer Life Sciences	Cat. # NET290250UC
Deposited data		
Crystal structure of human BRPF1 PZP bound to histone H3 tail	This study	PDB: 6U04
X-ray crystal structure of BRPF1 PZP domain	Klein et al., 2016	PDB ID: 5ERC
Experimental models: Cell lines		

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Human: HEK293T	ATCC	Cat. # CRL-3216
Oligonucleotides		
Primer: D294K Forward: AATGTCATCCTCTTCTGTAAGATG TGCAACCTGGCCGTG	This study - IDT	N/A
Primer: D294K Reverse: CACGGCCAGGTTGCACATCTTAC AGAAGAGGATGACATT	This study - IDT	N/A
Primer: KKR Forward: CACCAGCTCGCTGGGAACTCAC CTGCTAC	This study - IDT	N/A
Primer: KKR Forward: ACCTGCTACATTTGCGAACA AGAGGGCTCAGGGGCCTGCATCCAGTGC	This study - IDT	N/A
Primer: KKR Reverse: GTAGCAGGTGAGTTCCCAGCG AGCTGGTG	This study - IDT	N/A
Primer: KKR Reverse: GCACTGGATGCAGGCCCCT GAGCCCTCTTGTTCGCAAATGTAGCAGGT	This study - IDT	N/A
Primer: TEV_H3_F Forward: GAGAATTTGTATTTTCAGGCGCGTACTAAGCAG	This study - IDT	N/A
Primer: Linker_F Forward: GTTCTGGCTCTTCTGACGAGGATGCTGTTTG	This study - IDT	N/A
Primer: Linker_R Reverse: AGAAGAGCCAGAACCGCCGGTGGATTTC	This study - IDT	N/A
Primer: New_universal_GW_F Forward: GGACA AGTTTGTACAAAAAAGCAGGCTCGGAGAATTTGT ATTTTCAG	This study - IDT	N/A
Primer: RF_TEV_F Forward: GTACAAAAAAGCAGGCTTCGAGAATTTGTATTTTC	This study - IDT	N/A
Primer: RF_linker_R Reverse: CAGATACAGCAAACAGCATCCTCGTCAGAAGAGC CAGAAC	This study - IDT	N/A
Primer: Wt_PZP_TEV_f Forward: GAAAACCTGTATTTTCAGGGCGACGAGGATGCTG TTTGCTGTATC	This study - IDT	N/A
Primer: Univ_Fwd_TEV Forward: GGGGACAAGTTTGTACAAAAAAGCAGGCTT CGAAAACCTGTATTTTCAGGGC	This study - IDT	N/A
Primer: Wt_PZP_r Reverse: GGGGACCACTTTGTACA AGAAAGCTGGGTCTTATTAGCGTGCTGAACC	This study - IDT	N/A
Primer: 207_f Forward: TCATAAGGAGGACACTGGGA CATGCA[Fluorescein dT]CGGC	This study - IDT	N/A
Primer: 207_r Reverse: GTAGCGTCAACTCACTG CCCTATGCATTATACAGGATGTATATATCTGACAC	This study - IDT	N/A
Primer: 147_f Forward: Amino Modifier C6-CTGGA GATACTGTATGAGCATACAGTACAATTGGTCGTAGCA	This study - IDT	N/A
Primer: 147_r Reverse: ACAGGATGTATATAT CTGACACGTGCCTGGAGACTA	This study - IDT	N/A
Primer: 207_f Forward: TCATAAGGAGGACACTGGGA CATGCA[Fluorescein dT]CGGC	This study - IDT	N/A
Primer: 207_r Reverse: GTAGCGTCAACTCACTGCC CTATGCATTATACAGGATGTATATATCTGACAC	This study - IDT	N/A
Primer: 147_f Forward: Amino Modifier C6-CTGGAGATA CTGTATGAGCATACAGTACAATTGGTCGTAGCA	This study - IDT	N/A
Primer: 147_r Reverse: ACAGGATGTATATATCTGACACGTGCCTGGAGACTA	This study - IDT	N/A
M13 (-21) Forward TGTAAAACGACGGCCAGT	Sequencing facility	N/A
M13 (-21) Reverse CAGGAAACAGCTATGAC	Sequencing facility	N/A
M13/pUC Forward: CCCAGTCACGACGTTGTAAAACG (Invitrogen)	Sequencing facility	N/A
pGEX 5′ Forward: GGGCTGGCAAGCCACGTTTGGTG	Sequencing facility	N/A

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
T7 Reverse: GCTAGTTATTGCTCAGCGG	Sequencing facility	N/A
Recombinant DNA		
Plasmid: pDEST15-BRPF1$_{PZP}$ (aa 267-454)	Klein et al., 2016	N/A
Plasmid: Invitrogen™ Gateway™ pDEST™15 Vector	Thermo Fisher Scientific	Cat: # 1180214
Plasmid: Invitrogen™ Gateway™ pDONR™/Zeo Vector	Thermo Fisher Scientific	Cat: # 12535035
Plasmid: pDEST15-BRPF1$_{PZP}$ (aa 271-454)	Klein et al., 2020	N/A
Plasmid: pDEST15-H3-GSGSS- BRPF1$_{PZP}$ construct (aa 1-12 of histone H3, a GSGSS linker, and aa 271-454 of BRPF1)	Klein et al., 2020	N/A
Plasmid: pDEST15-BRPF1$_{PZP}$ (aa 271-454) (D294K)	Klein et al., 2020	N/A
Plasmid: pDEST15-BRPF1$_{PZP}$ (aa 271-454) (K383E/K390E/R392E)	Klein et al., 2020	N/A
Plasmid: hH2A.1 B/E	North et al., 2012	N/A
Plasmid: hH2B.1 O	North et al., 2012	N/A
Plasmid: hH3.2	North et al., 2012	N/A
Plasmid: H4-pET	North et al., 2012	N/A
Plasmid: pNucB3-LexAL	North et al., 2012	N/A
Plasmid: pcDNA3-HA-BRPF1 WT and D294A	Klein et al., 2016	N/A
Plasmid: pcDNA3-HA-BRPF1$_{PZP\Delta 359-450}$	Klein et al., 2016	N/A
Plasmid: pcDNA3-FLAG-MORF$_{N1-716}$	Klein et al., 2016	N/A
Plasmid: pcDNA3-FLAG-ING5	Klein et al., 2016	N/A
Plasmid: pcDNA3-FLAG-MEAF6	Klein et al., 2016	N/A
Software and algorithms		
HKL3000	Minor et al., 2006	https://www.hkl-xray.com/hkl-3000-installation-instructions-0
Phenix	Liebschner et al., 2019	http://www.phenix-online.org/
Coot	Emsley et al., 2010	https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/
MolProbity	Chen et al., 2010	http://molprobity.biochem.duke.edu/
PDB (validation)	Berman et al., 2003	https://validate-rcsb-1.wwpdb.org/
PDB (deposit)	Berman et al., 2003	https://deposit-2.wwpdb.org/deposition/
NMRPipe	Frank et al., 1995	https://www.ibbr.umd.edu/nmrpipe/
Prism	GraphPad	https://www.graphpad.com/scientific-software/prism/

MATERIALS AND EQUIPMENT

M9 minimal media

To prepare 1 L of M9 minimal media, weigh and dissolve the reagents below in 960 mL Milli-Q water and autoclave.

Reagent	Final concentration	Amount
Na_2HPO_4-$7H_2O$	47.7 mM	12.8 g
KH_2PO_4	22 mM	3.0 g
NaCl	8.56 mM	0.5 g
$^{15}NH_4Cl$ or $^{14}NH_4Cl$	18.4 mM	1 g
1 M $MgSO_4$	2 mM	2 mL
1 M $CaCl_2$	200 μM	200 μl
0.1 M $ZnCl_2$	50 μM	0.5 mL

The M9 minimal media can be stored at 25°C for 1 week. This media can be used to generate uniformly ^{15}N-labeled proteins for NMR if $^{15}NH_4Cl$ is used, or unlabeled proteins if $^{14}NH_4Cl$ is used.

Include the following reagents before adding cell culture:

Reagent	Amount
Multivitamin solution (1 multivitamin tablet (commercially available Centrum) dissolved in 50 mL water, mixed and filtered with a 0.22 μm filter)	5 mL
20% (w/v) glucose (filtered)	25 mL

Luria-Bertani LB

To prepare 1 L of LB media, weigh and dissolve the reagents below in 950 mL Milli-Q water.

Reagent	Amount
Tryptone	10 g
NaCl	10 g
Yeast Extract	5 g

Adjust pH to 7.5 at 25°C, bring the volume up to 1 L with Milli-Q water and autoclave. The media can be stored at 25°C for 1 week.

Low salt LB

To prepare 1 L of low salt LB media, weigh and dissolve the reagents below in 950 mL Milli-Q water.

Reagent	Amount
Tryptone	10 g
NaCl	5 g
Yeast Extract	5 g

Adjust pH to 7.5 at 25°C, bring the volume up to 1 L with Milli-Q water and autoclave. The media can be stored at 25°C for 1 week.

PZP purification buffer A

50 mM Tris pH 7.5, 500 mM NaCl, 0.05% (v/v) Nonidet P 40, 5 mM dithiothreitol (DTT), 50 μM $ZnCl_2$, and 5 mM $MgCl_2$

To prepare PZP purification buffer A combine the following reagents in 800 mL of Milli-Q water.

Reagent	Final concentration	Amount
Tris-base	50 mM	6.06 g
NaCl	500 mM	29.22 g
Nonident® P-40	0.05% (v/v)	0.5 mL
0.1 M $ZnCl_2$	50 μM	0.5 mL
1 M $MgCl_2$	5 mM	5 mL
DTT	5 mM	0.77 g

Adjust pH of the buffer to 7.5 at 25°C. Add Milli-Q water up to 1 L and filter the buffer using a 0.22 μm filter. The buffer can be stored at 4°C for 14 days.

⚠ CRITICAL: DTT, 10 μg/mL Dnase I, 0.1–1 mM phenylmethanesulfonyl fluoride (PMSF) and 0.1–0.5 mg/mL lysozyme should be added to the buffer just prior to use.

Note: Avoid contact, ingestion and inhalation when handling Nonidet® NP-40 and PMSF.

PZP purification buffer B
50 mM Tris pH 7.5, 500 mM NaCl, and 5 mM DTT

To prepare PZP Purification Buffer B combine the following reagents in 800 mL of Milli-Q water.

Reagent	Final concentration	Amount
Tris-base	50 mM	6.06 g
NaCl	500 mM	29.22 g
DTT	5 mM	0.77 g

Adjust pH of the buffer to 7.5 at 25°C. Add Milli-Q water up to 1 L and filter the buffer using a 0.22 μm filter. The buffer can be stored at 4°C for 14 days.

⚠ CRITICAL: DTT should be added to the buffer just prior to use.

PZP purification buffer C
50 mM Tris pH 7.5, and 5 mM DTT

To prepare PZP Purification Buffer C combine the following reagents in 800 mL of Milli-Q water.

Reagent	Final concentration	Amount
Tris-base	50 mM	6.06 g
DTT	5 mM	0.77 g

Adjust pH of the buffer to 7.5 at 25°C. Add Milli-Q water up to 1 L and filter the buffer using a 0.22 μm filter. The buffer can be stored at 4°C for 14 days.

⚠ CRITICAL: DTT should be added to the buffer just prior to use.

PZP purification buffer D
50 mM Tris pH 7.5, 150 mM NaCl, and 5 mM DTT

To prepare PZP Purification Buffer D combine the following reagents in 800 mL of Milli-Q water.

Reagent	Final concentration	Amount
Tris-base	50 mM	6.06 g
NaCl	150 mM	8.77 g
DTT	5 mM	0.77 g

Adjust pH of the buffer to 7.5 at 25°C. Add Milli-Q water up to 1 L and filter the buffer using a 0.22 μm filter. The buffer can be stored at 4°C for 14 days.

⚠ CRITICAL: DTT should be added to the buffer just prior to use.

2xYT
For 3 L of media, combine:

Reagent	Final concentration	Amount
Tryptone	50 mM	48 g
Yeast Extract	500 mM	30 g
NaCl	5 mM	15 g

Add Milli-Q water to bring up to 500 mL and autoclave. This media can be stored at 25°C for up to a day or 4°C for up to 3 days, but should be used only if still sterile.

5× TBE buffer		
Reagents	Final concentration	Amount
Tris-base	0.45 M	54 g
Boric acid	0.45 M	27.5 g
EDTA (0.5 M pH 8.0)	0.01 M	20 mL

Note: Filter 5× solution through a 0.22 μm filter, which may prevent (delay) formation of precipitates. However, in drier climates precipitation may occur after 1–2 months even with filtration. Store at 25°C for up to 1 year.

Histone wash buffer		
Reagents	Final concentration	Amount
2 M Tris-HCl	50 mM	12.5 mL
NaCl	100 mM	2.9 g

Adjust pH to 7.5 at 25°C and bring the volume up to 500 mL with Milli-Q water. Filter sterilize with a 0.22 μm filter. The buffer can be stored at 4°C for up to a month. Just prior to use add the protease inhibitors and reducing agents to the buffer, as per the protocol.

Histone guanidine buffer		
Reagents	Final concentration	Amount
Guanidine hydrochloride	6 M	22.9 g
1 M Tris	20 mM	0.8 mL
0.5 M TCEP	0.5 mM	40 μl

Adjust pH to 7.5 at 25°C and bring the volume up to 40 mL with Milli-Q water. Filter sterilize with a 0.22 μm filter. Store at 4°C for up to a month.

Histone urea buffer 0		
Reagents	Final concentration	Amount
Urea	8 M	960 g
3 M Sodium Acetate	20 mM	13 mL
0.5 M EDTA pH 8.0	1 mM	4 mL
Mixed Bed Resin	N/A	20 g
14 M BME	5 mM	714 μl

Combine all ingredients except BME. Allow to dissolve with mild heat (70°C–90°C for around 20 min). Monitor the color of the mixed bed resin. If all beads turn yellow, then add more resin (slowly add up to 1 g at a time during mixing until beads no longer turn yellow). When done mixing, add BME. Filter and degas through a 0.22 μm filter. Store at 25°C. The mixed bed resin acts to deionize the urea. Histone Urea Buffer 0 lasts for about 24 h until the urea needs to be combined with more of the mixed bed resin.

Histone urea buffer 1		
Reagents	Final concentration	Amount
Urea	8 M	960 g
3 M Sodium Acetate	20 mM	13 mL
0.5 M EDTA pH 8.0	1 mM	4 mL
Mixed Bed Resin	N/A	20 g
14 M BME	5 mM	714 μl
NaCl	1 M	116.88 g

Combine all ingredients except BME. Allow to dissolve with mild heat (70°C–90°C for around 20 min). Monitor the color of the mixed bed resin. If all beads turn yellow, then add more resin (slowly add up to 1 g at a time during mixing until beads no longer turn yellow). When done mixing, add BME. Filter and degas through a 0.22 μm filter. Store at 25°C. The mixed bed resin acts to deionize the urea. Histone Urea Buffer 1 lasts for about 24 h until the urea needs to be combined with more of the mixed bed resin.

10× PFU reaction buffer

Reagent	Final concentration	Amount
2 M Tris-HCl pH 8.8	200 mM	10 mL
1 M $MgSO_4$*7H2O	20 mM	2 mL
1 M KCl	100 mM	10 mL
1 M $(NH_4)SO_4$	100 mM	10 mL
100% (v/v) Triton X-100	1%	1 mL
Milli-Q		fill to 100 mL

Filter sterilize with a 0.22 μm filter and store at −20°C.

2 M Tris-HCl pH X

Reagent	Final concentration	Amount
Tris-base	2 M	242.28 g

Bring volume to 800 mL with Milli-Q water. Set the desired pH using HCL. Bring volume to 1 L. Filter with a 0.22 μm filter.

0.5 M EDTA pH 8.0

Reagent	Final concentration	Amount
EDTA Salt Dihydrate	0.5 M	186.1 g

Bring volume to 800 mL with Milli-Q water. Stir vigorously and pH to 8.0 with NaOH. EDTA may not

100× TE

Reagent	Final concentration	Amount
Tris-base	1 M	121.1 g
EDTA Salt Dihydrate	100 mM	37.22 g

dissolve completely until pH reaches 8.0. Bring volume to 1 L. Filter with a 0.22 μm filter. Store at 25°C for up to 1 year.

TE0

Reagent	Final concentration	Amount
2 M Tris-HCl pH 8.0	25 mM	12.5 mL
0.5 M EDTA pH 8.0	1 mM	2 mL

Bring volume to 700 mL with Milli-Q water. pH to 8.0 with HCl (~42 mL). Bring volume to 1 L. Filter with a 0.22 μm filter. Store at 25°C for up to 1 year.

TE1000

Reagent	Final concentration	Amount
2 M Tris-HCl pH 8.0	25 mM	12.5 mL
0.5 M EDTA pH 8.0	1 mM	2 mL
NaCl	1 M	58.44 g

Bring the volume up to 1 L with Milli-Q water and filter with a 0.22 μm filter. Store at 25°C for up to 1 year.

Unfolding buffer		
Reagent	Final concentration	Amount
Guanidine HCl	7 M	66.85 g
2 M Tris-HCl pH 7.5	20 mM	1 mL
1 M DTT	10 mM	1 mL

Bring the volume up to 1 L with Milli-Q water and filter with a 0.22 μm filter. Store at 25°C for up to 1 year.

Refolding buffer		
Reagent	Final concentration	Amount
NaCl	2 M	210 g
2 M Tris-HCl pH 7.5	10 mM	9 mL
0.5 M EDTA pH 8.0	1 mM	3.6 mL
14 M BME	5 mM	643 μL

Bring the volume up to 100 mL with Milli-Q water. Filter with a 0.22 μm filter. Store at 4°C for up to 1 week.

Refolding buffer without BME		
Reagent	Final concentration	Amount
NaCl	2 M	117 g
2 M Tris-HCl pH 7.5	10 mM	5 mL
0.5 M EDTA pH 8.0	1 mM	2 mL

Bring the volume up to 1.8 L with Milli-Q water. Store at 4°C for up to 1 week.

0.5 M BZA		
Reagents	Final concentration	Amount
Benzamidine hydrochloride hydrate	0.5 M	19.58 g

⚠ CRITICAL: Reducing agents should be added to the buffer just prior to use.

Bring the volume up to 1 L with Milli-Q water. Filter with a 0.22 μm filter. Store at 25°C for up to 1 year.

Reconstitution buffer with 2 M NaCl		
Reagents	Final concentration	Amount
100× TE	0.5×	500 μl
0.5 M BZA	1 mM	200 μl
5 M NaCl	2 M	40 mL

Reconstitution buffer without 2 M NaCl		
Reagents	Final concentration	Amount
100× TE	0.5×	20 mL
0.5 M BZA	1 mM	8 mL

Bring the volume up to 250 mL with Milli-Q water. Filter through 0.22 μm filter. Store at 4°C for up to 1 year.

5% sucrose with 0.5× TE		
Reagents	Final concentration	Amount
100× TE	0.5×	500 μl
Sucrose	5%	5 g

30% sucrose with 0.5× TE		
Reagents	Final concentration	Amount
100× TE	0.5×	500 μl
Sucrose	5%	30 g

Bring the volume up to 100 mL with chilled Milli-Q water. Store at 4°C for up to 1 week.

Bring the volume up to 4L with chilled Milli-Q water. Store at 4°C for up to 1 week.

Bring the volume up to 100 mL with chilled Milli-Q water. Filter with a 0.22 μm filter and store at 4°C for up to 3 months.

Bring the volume up to 100 mL with chilled Milli-Q water. Filter with a 0.22 μm filter and store at 4°C for up to 3 monts.

Reagent	Final concentration	Amount
2 M Tris-HCl	30 mM	75 μl
5 M NaCl	90 mM	90 μl
1% (v/v) Tween-20	0.0075% (v/v)	37.5 μl
80% (v/v) Glycerol	12% (v/v)	750 μl
1 M DTT	6 mM	30 μl

Electrophoretic mobility shift assay (EMSA) reaction buffer

25 mM Tris-HCl pH 7.5, 75 mM NaCl, 0.00625% (v/v) Tween-20, 10% (v/v) glycerol and 5 mM DTT

To prepare 5 mL EMSA Reaction Buffer, add the reagents below to 4017.5 μl Milli-Q water. (For a final volume of 5000 ul)

Store at 4°C for up to 1 week.

Reagent	Final concentration	Amount
2 M Tris-HCl	27.8 mM	69.4 μL
5 M NaCl	83.3 mM	83.3 μL
1% (v/v) Tween-20	0.0069% (v/v)	34.7 μL
1 M DTT	5.6 mM	27.8 μL

Fluorescence polarization reaction buffer

25 mM Tris-HCl pH 7.5, 75 mM NaCl, 0.00625% (v/v) Tween-20, and 5 mM DTT

To prepare 5 mL Fluorescence Polarization Reaction Buffer, add the reagents below to 4784.7 μL

1 M $CaCl_2$		
Reagent	Final concentration	Amount
$CaCl_2$	1 M	14.7 g

Milli-Q water (For a final volume of 5000 ul)

280 mM NaCl, 50 mM Hepes, and 1.5 mM Na_2HPO_4		
Reagent	Final concentration	Amount
5 M NaCl	280 mM	5.6 mL
Hepes	50 mM	1.19 g
100 mM Na_2HPO_4	1.5 mM	1.5 mL

⚠ CRITICAL: DTT should be added to the buffer just prior to use.

Calcium phosphate buffer ($CaCl_2$)

Add Milli-Q water up to a final volume of 100 mL and filter using 0.22 μm filter. The buffer can be

Reagent	Amount
KCl	5 g
NaCl	83 g
Hepes	24 g

stored at 20°C–25°C.

2× HEPES-buffered saline (HBS) buffer

Adjust pH to 7.0 with NaOH. Add Milli-Q water up to 100 mL and filter using 0.22 μm filter. The buffer can be stored at 4°C for 1 month.

Reagent	Final concentration	Amount
1 M Hepes pH 7.9	10 mM	1 mL
$MgCl_2$	1.5 mM	150 μl
1 M KCl	10 mM	1 mL

HEPES 10× buffer

Adjust pH to 7.3 with 5 N KOH. Add Milli-Q water up to 1 L and filter using 0.22 μm filter. The buffer should be protected from neon light and can be stored at 4°C.

Hypotonic buffer (nuclear extraction)

10 mM HEPES pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM DTT, 1 mM PMSF, and cOmplete protease inhibitor cocktail Roche

Add Milli-Q water up to 100 mL and filter using 0.22 μm filter. The buffer can be stored at 4°C for up to 1 month.

Reagent	Final concentration	Amount
1 M Hepes pH 7.9	20 mM	2 mL
50% (v/v) Glycerol	10% (v/v)	20 mL
$MgCl_2$	1.5 mM	150 μl
1 M KCl	20 mM	2 mL
0.5 M EDTA	0.2 mM	40 μl

Note: Add 1 mM of DTT, 1 mM PMSF and 1 tablet of protease inhibitor (cOmplete protease inhibitor cocktail Roche) per 1 L buffer just prior to use.

Low salt buffer (nuclear extraction)

20 mM HEPES pH 7.9, 10% (v/v) Glycerol, 1.5 mM $MgCl_2$, 20 mM KCl, 0.2 mM EDTA, 1 mM DTT, 1 mM PMSF, and cOmplete protease inhibitor cocktail (Roche)

Reagent	Final concentration	Amount
1 M Hepes pH 7.9	20 mM	2 mL
50% (v/v) Glycerol	10% (v/v)	20 mL
$MgCl_2$	1.5 mM	150 µL
3 M KCl	1.2 M	40 mL
0.5 M EDTA	0.2 mM	40 µL

Add Milli-Q water up to 100 mL and filter using 0.22 µm filter. The buffer can be stored at 4°C for up to 1 month.

Note: Add 1 mM of DTT, 1 mM PMSF and 1 tablet of protease inhibitor (cOmplete protease inhibitor cocktail Roche) per 1 L buffer just prior to use.

High salt buffer (nuclear extraction)

20 mM HEPES pH 7.9, 10% (v/v) glycerol, 1.5 mM $MgCl_2$, 1.2 M KCl, 0.2 mM EDTA, 1 mM DTT, 1 mM

Reagent	Final concentration	Amount
1 M Hepes pH 7.9	20 mM	2 mL
50% (v/v) Glycerol	10% (v/v)	20 mL
10% (v/v) Tween-20	0.1% (v/v)	1 mL
3 M KCl	300 mM	10 mL

PMSF, and cOmplete protease inhibitor cocktail (Roche)

Add Milli-Q water up to 100 mL and filter using 0.22 µm filter. The buffer can be stored at 4°C for up to 1 month.

Note: Add 1 mM of DTT, 1 mM PMSF and 1 tablet of protease inhibitor (cOmplete protease inhibitor cocktail Roche) per 1 L buffer just prior to use.

Wash I buffer

20 mM Hepes pH 7.9, 10% (v/v) glycerol, 300 mM KCl, 0.1% (v/v) Tween-20, 1 mM DTT, 1 mM PMSF

Reagent	Final concentration	Amount
1 M Hepes pH 7.9	20 mM	2 mL
50% (v/v) Glycerol	10% (v/v)	20 mL
10% (v/v) Tween-20	0.1% (v/v)	1 mL
3 M KCl	150 mM	5 mL

and cOmplete protease inhibitor cocktail (Roche)

Add Milli-Q water up to 100 mL and filter using 0.22 µm filter. The buffer can be stored at 4°C for up to 1 month.

Note: Add 1 mM of DTT, 1 mM PMSF and 1 tablet of protease inhibitor (cOmplete protease inhibitor cocktail Roche) per 1 L buffer just prior to use.

Wash II buffer

20 mM Hepes pH 7.9, 10% (v/v) glycerol, 150 mM KCl, 0.1% (v/v) Tween-20, 1 mM DTT, 1 mM PMSF, and cOmplete protease inhibitor cocktail (Roche)

Reagent	Final concentration	Amount
1M Tris pH 8.0	250 mM	250 μl
50% (v/v) Glycerol	25% (v/v)	500 μl
0.5 M EDTA	0.5 mM	1 μl
1M DTT	5 mM	5 μl
0.1 M PMSF	5 mM	50 μl

Add Milli-Q water up to 100 mL and filter using 0.22 μm filter. The buffer can be stored at 4°C for up to 1 month.

Note: Add 1 mM of DTT, 1 mM PMSF and 1 tablet of protease inhibitor (cOmplete protease inhibitor cocktail Roche) per 1 L buffer just prior to use.

50mM carbonate buffer ($NaHCO_3$–Na_2CO_3, pH 9.2)		
Reagent	Final concentration	Amount
Na_2CO_3	0.5 M	70 mL
$NaHCO_3$	0.5 M	5 mL

5× HAT buffer
250 mM Tris pH 8.0, 25% (v/v) glycerol, 0.5 mM EDTA, 5 mM DTT, and 5 mM PMSF

Add Milli-Q water up to 1 mL and filter using 0.22 μm filter. The buffer should be aliquoted and can be stored at −20°C.

Note: Avoid freeze/thaw cycles and flash freeze in liquid nitrogen or ethanol/dry ice within small aliquots (100μl aliquots).

Carbonate buffer (10×)
The buffer should be diluted to 50 mM for use with Milli-Q water.

STEP-BY-STEP METHOD DETAILS

$BRPF1_{PZP}$ expression and purification

Timing: 6–7 days

Prepare $BRPF1_{PZP}$ proteins for NMR, crystallography, fluorescence polarization, and EMSA experiments.

1. Transform plasmids into Rosetta-2 (DE3) pLysS chemically competent cells (Novagen). Follow the Novagen protocol (https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/SAJ/Brochure/2/TB009.pdf) for transformation and plate on LB agar supplemented with ampicillin (100 μg/mL) and chloramphenicol (34 μg/mL). Details describing how to make media and buffers are in the materials and equipment section.

Note: The Rosetta-2 (DE3) pLysS chemically competent cells have an inherent chloramphenicol resistance. Therefore, to maintain the rare codons supplied with this *E. coli* strain, chloramphenicol must be present.

2. From a single colony, using a sterile pipette tip, inoculate 250 mL of LB preculture supplemented with ampicillin (100 μg/mL) and chloramphenicol (34 μg/mL) in a 1 L flask.
3. Grow preculture for 14–18 h at 37°C and 180–200 rpm in a shaker (Forma Scientific- Model 4580 Orbital incubator Shaker).
4. In 6 L Erlenmeyer flask (Thermo Fisher Scientific) containing M9 Minimal Media with antibiotics ampicillin (100 μg/mL) and chloramphenicol (34 μg/mL), glucose, and vitamin (commercially available Centrum) add preculture (1:100 dilution).

⚠ CRITICAL: If the protein is to be used for NMR experiments, $^{15}NH_4Cl$ should be used in the media instead of $^{14}NH_4Cl$, to produce uniformly ^{15}N-labeled protein.

5. Transfer 6 L flasks to an InnOva 4330 floor shaker (New Brunswick Scientific CO., Inc.) prewarmed to 37°C and shake at 200 rpm. When the optical density (test in a Biowave CO8000 Cell Density Meter, Biochrom) reaches 0.6–0.8 at an absorbance of 600, turn down the temperature and speed to 16°C and 180 rpm, respectively.
6. Once the temperature reaches 16°C–19°C, add isopropyl β-d-1-thiogalactopyranoside (IPTG) (GoldBio) to a final concentration of 0.5 mM. Express protein at 16°C for 18 h.

Note: Zinc ions are essential for the expression of the $BRPF1_{PZP}$ domain. At this point, to increase yield of the protein it helps to add (20–30 μM $ZnCl_2$) along with the IPTG during induction. Additionally, more IPTG added during expression of $BRPF1_{PZP}$, may cause protein production to increase, however we have observed a delicate equilibrium that exists between well folded protein, and protein that is not. Therefore, we found a final concentration of 0.5 mM (to less than 1mM) IPTG in combination with zinc to be the best for expression of folded protein.

7. Harvest the cultures and collect cell pellets by centrifugation in a F10-6 × 500y Fixed-Angle Rotor (Thermo Fisher Sci) in a floor centrifuge (Sorvall) for 10 min at 4,400 × *g* and 10°C.

Pause point: Cell pellets can be stored for several months at −80°C.

Note: If using more than 2 L of expressed cells at a time during cell lysis, it is best to freeze the cells in liquid nitrogen and then thaw them before starting next step. If the cells are to be stored, they can be frozen again in liquid nitrogen before storing in −80°C.

8. Resuspend cells in a beaker using PZP Purification Buffer A (50 mM Tris pH 7.5, 500 mM NaCl, 0.05% (v/v) Nonidet P 40, 5 mM dithiothreitol (DTT), 50 μM $ZnCl_2$, and 5 mM $MgCl_2$). Best to stir the cells for complete resuspension for 10–30 min at 4°C on a stir plate with a stir bar.

Note: Lysozyme (Thermo Fisher Sci) and Dnase I (Roche) will promote cell lysis and enhance solubility of the protein. If protein degradation during purification is visible, 1 mM phenylmethlysulfonyl fluoride (PMSF) (Sigma-Aldrich) can be added during cell lysis.

9. Sonicate the suspended cells in a beaker on ice for 5 min.
   a. Settings for the sonicator (Qsonica): 60% power with intervals of 5 s on and 10 s off.
10. Mix cells and repeat previous step. Always keep cell suspension on ice.
11. Spin down the cell suspension in balanced 50 mL Nalgene™ Oak Ridge High-Speed PPCO Centrifuge tubes (Thermo Fisher Sci) at 27,000 × *g* using a SS-34 Fixed Angle Rotor (Thermo Fisher Sci) at 4°C–10°C for 30 min.
12. Gently collect the supernatant, be careful not to disturb the pellet containing the cell debris and insoluble fraction.
13. Combine the supernatant with equilibrated glutathione beads in a 50 mL centrifuge tube and place on a rocker (Unico) (at low-medium speed) for 1 h at 4°C.

14. Wash the beads using PZP Purification Buffer B (50 mM Tris pH 7.5, 500 mM NaCl, and 5 mM DTT) following the glutathione bead protocol (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0011719_Pierce_Glutathione_Agarose_UG.pdf) using either batch or gravity flow column method (Econo-Pac® Chromatography Column Bio-Rad).
15. After washing out the unbound impurities, take a 15 μL sample of the beads for analysis by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE).
16. In the tube containing the beads, bring the volume up to 15–20 mL with PZP Purification Buffer B. To cleave the GST-tag, add TEV enzyme and follow protocol from the manufacture or use home purified enzyme (14 mg/mL, 30 μL) (Liu et al., 2020a) and gently rock on a platform shaker set to level 2-3, (Microplate Titer Plate Shaker, LAB-LINE INSTRUMENTS, Inc.) for 15–20 h at 4°C.

Note: Fast or rough agitation at this stage in the purification may not be good for the protein stability.

17. Verify cleavage and purification steps by SDS PAGE.
    a. The 10–15 well 15% SDS polyacrylamide gels can either be poured (5% stacking, 15% resolving) in a 1.5 mm mini gel cassette (Thermo Fisher Sci) or purchased (gradient 4%–15%).http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6201.pdf
    b. Prepare gel and 4× SDS loading buffer (200 mM Tris-HCl (pH 6.8), 400 mM DTT, 8% (w/v) SDS, 0.4% (w/v) bromophenol blue, and 40% (v/v) glycerol).
    c. Heat samples at 90°C for 10 min to denature proteins, spin down for 5–10 s at 5,000 × *g* in an Eppendorf Microcentrifuge 5424R (Eppendorf) and then load on gel.
    d. Run until the dye front reaches the bottom of the gel.
18. After desired cleavage is obtained (visually inspect that 75%–90% of cleavage has occurred), elute the protein contained in the supernatant. Using a 10,000 Da MWCO centrifugal filter (Millipore) concentrate the protein to 2 mL at 3,000 × *g* in an Eppendorf 5810 R Centrifuge with an A-4-62 rotor (Eppendorf).
19. To further purify the $BRPF1_{PZP}$ protein, inject concentrated protein into a HiPrep™ 16/60 Sephacryl® S-100 HR (Cytiva) equilibrated with Purification Buffer B. Run column at 0.2 mL/minute in 4°C. Protein elutes at approx. 66 mL on this column.
20. Run SDS PAGE as in step 16 to verify and select pure fractions. Pool fractions and concentrate to 20–40 mg/mL using a 10,000 Da MWCO centrifugal filter (Millipore) at 3,000 × *g* in an Eppendorf 5810 R Centrifuge with an A-4-62 rotor (Eppendorf).
21. $BRPF1_{PZP}$ proteins can be used immediately or flash frozen in liquid nitrogen and stored at −80°C.

⚠ CRITICAL: Prior to freezing and storage of protein, aliquot protein into multiple tubes (10–50 μL each) to prevent unnecessary repeated freezing and thawing.

Nuclear magnetic resonance (NMR) titration experiments

Timing: approx. 1 day for each ligand

Evaluate the interaction between $BRPF1_{PZP}$ and its ligands by NMR spectroscopy.

22. Gently thaw purified ^{15}N-labeled $BRPF1_{PZP}$ on ice. Centrifuge at 15,000 × *g* in an Eppendorf Microcentrifuge 5424R (Eppendorf) and separate supernatant from any debris or precipitation that has formed during freeze thaw process.
23. Check the protein concentration (NanoDrop 2000c, Thermo Fisher Sci). The first time the protein concentration is checked it should be calculated using Bradford solution and a standard curve using BSA at known concentrations. With the standard curve, it is possible to determine the unknown concentration of the $BRPF1_{PZP}$ protein. The calculated protein concentration by Bradford solution can be compared to that found using the extinction coefficient (extinction coefficient

determined from the protein sequence). If using the extinction coefficient is an accurate method to determine BRPF1$_{PZP}$ concentration, future protein concentration calculations can be completed using this method.

24. Dilute protein to 0.1–0.2 mM using Purification Buffer C (50 mM Tris pH 7.5, and 5 mM DTT) and Purification Buffer D (50 mM Tris pH 7.5, 150 mM NaCl, and 5 mM DTT), bringing the salt concentration of the buffer to 150 mM NaCl. Final buffer for NMR sample is 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM DTT.
    a. Combine 198 μL sample with 22 μL of D_2O (final concentration of 10%), mix gently up and down with a pipette.
    b. Using a glass pipette (Wilmad Labglass) gently pipette into a shigemi tube (Shigemi, Inc.). Once all the sample is added, insert the plunger, remove all bubbles by gently rotating the plunger up and down and seal with parafilm.
25. Record $^{1}H,^{15}N$ heteronuclear single quantum coherence (HSQC) spectra of the apo-state of the protein at 298K on a Varian INOVA 600 MHz NMR spectrometer (Figure 1).
26. For titration, add increasing amounts of the histone H3 (aa 1-12, 15-34, or 1-31) peptide (synthesized by Synpeptide) to the NMR sample and record $^{1}H,^{15}N$ HSQC spectra after each addition of the ligand.
27. Continue to repeat the previous step until saturation is reached.
28. Use NMRPipe software (Delaglio et al., 1995) to process the spectra. To analyze chemical shift perturbations induced in BRPF1$_{PZP}$, overlay the spectra.

X-ray crystallization and structure determination of H3-linked BRPF1$_{PZP}$

Timing: approx. 1 month

Generate crystals of H3-linked BRPF1$_{PZP}$, collect crystallization data sets, and determine the structure of the complex.

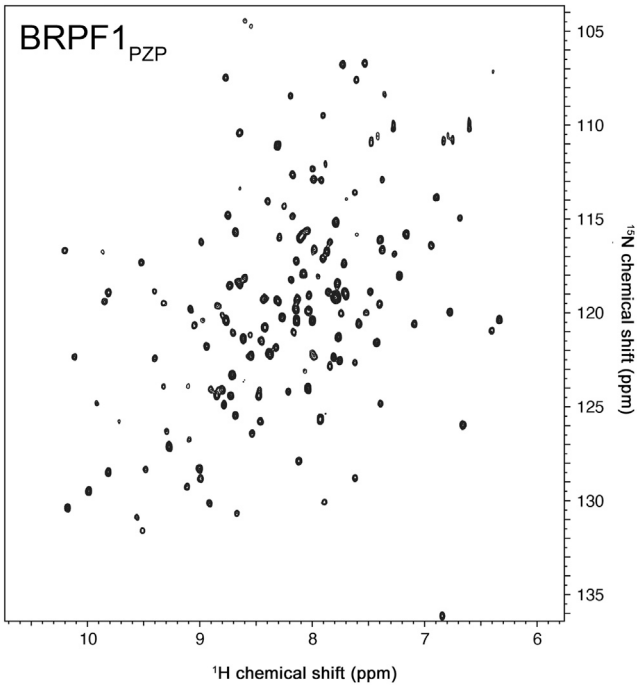

Figure 1. $^{1}H,^{15}N$ HSQC spectrum of the apo-state of BRPF1$_{PZP}$
Good dispersion of resonances indicates that the protein is folded.

29. Prepare and concentrate the H3-GSGSS-BRPF1$_{PZP}$ protein as above (> 20 mg/mL) in Purification Buffer B (50 mM Tris pH 7.5, 500 mM NaCl, and 5 mM DTT).
30. Dilute protein with Purification Buffer C (50 mM Tris pH 7.5, and 5 mM DTT) to approx. 6 mg/mL to final buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM DTT). Spin down the protein at 15,000 × *g* in an Eppendorf Microcentrifuge 5424R (Eppendorf) to remove any precipitation before setting up crystal drops.
31. Grow crystals at 18°C using the sitting-drop diffusion method.
    a. Set up crystal screening trays using commercially available kits (Hampton Research).
        i. Each well is composed of 90% of the well condition (0.2 M calcium acetate, PEG8000 10% (w/v), and 0.1 M imidazole pH 8) and 10% additive from the commercially available additive screen (Hampton Research).
        ii. Set up drops by combing 0.6 μL of protein with 0.6 μL of well solution.
32. Add 1.2 μL of a 60% (v/v) glycerol solution directly to the drop (final concentration of 30% (v/v)). With an appropriate loop size, loop the crystal and mount directly on the homesource (Anschutz Medical Campus X-ray crystallography core facility Rigaku Micromax 007 high frequency microfocus X-ray generator equipped with a Pilatus 200K 2D area detector).

    Optional: Additional well and glycerol solution can be added if the drop dries out slightly, however maintain cryoprotectant percentage of 30% (v/v) glycerol. Crystals can be frozen in liquid nitrogen for storage or shipping to a synchrotron.

33. Collect X-ray diffraction data sets using a single crystal using a Rigaku Micromax 007 high-frequency microfocus X-ray generator.

    Alternatives: Collecting data at the Advanced Light Source (ALS) beamline 4.2.2, or alternative synchrotron is another option.

34. Use HKL3000 program (Minor et al., 2006) to complete the indexing, integration and scaling of the data set.

    Alternatives: X-ray Detector Software (XDS) (Kabsch, 2010) can be an alternative for processing.

35. Determine the initial structure by molecular replacement using the apo BRPF1$_{pzp}$ (PDB ID: 5ERC) as a model in the Phenix Phaser program (Adams et al., 2010).
36. Carry out manual model building and refinement with Coot (Emsley et al., 2010) and Phenix (Liebschner et al., 2019).
37. Validate the structure with MolProbity (Chen et al., 2010) and Worldwide Protein Data Bank OneDep SYSTEM (https://validate-rcsb-1.wwpdb.org/) (Berman et al., 2003), and fix any issues that arise. These servers provide a report that have details about the quality of model and experimental data. For example, information contained in these reports can be used to determine if a ligand needs work or protein side chains are in an incorrect conformation.

    Note: Additional validation servers are available depending on need and issues. All structures that are deposited with the PDB will go through the validation server, thus a preliminary check is helpful before depositing. Currently, many journals are requesting these reports as part of the manuscript submission process for anyone who submits a structure, so developing a good understanding of the validation system will help authors.

38. Deposit data with the Worldwide Protein Data Bank OneDep SYSTEM (https://deposit-2.wwpdb.org/deposition/) and record PDB ID (Berman et al., 2003).

Electrophoretic mobility shift assay (EMSA)

Timing: 2–4 h

Electrophoretic Mobility Shift Assays (EMSAs) are used to detect binding of two molecules through an observed change in electrophoretic mobility.

Note: To save time, reactions can be started while gel is pre-running.

39. Using EMSA reaction buffer prepare $BRPF1_{PZP}$ running samples. Separately, dilute NCPs in 0.5× TE, and store everything on ice.
    i. For $BRPF1_{PZP}$, make 150, 100, 50, 30, 10, 3, and 1 μM running samples from the protein stock. Make 12 μL of each sample.
    ii. Prepare 20 μL of 30 nM NCPs.

Note: Concentration of $BRPF1_{PZP}$ in the running samples should cover at least 2 orders of magnitude of the dissociation constant for the protein-NCP interaction in order to reach saturation.

40. Mix $BRPF1_{PZP}$ samples with NCP and incubate at 4°C for 15 min.
    a. Combine 2 μL of NCPs with 10 μL of each $BRPF1_{PZP}$ sample to start the reactions. For zero $BRPF1_{PZP}$ concentration, 10 μL of Reaction Buffer is added instead.
41. Load reaction mixtures onto a gel and run electrophoresis at 300V for 1.5 h in 0.3× TBE.
    a. Start the gel running and then quickly load the reaction mixtures.
42. Image the gel using a Typhoon phosphorimager with fluorescence imaging to detect fluorescein emission around 470 nm (Figure 2).

Note: If a phosphorimager is unavailable, it is possible to stain the gel with Ethidium Bromide and image it on a UV transilluminator. To do so, add 10 μL initially of 10 mg/mL EtBr to 1 L of 0.3× TBE and incubate the gel in the EtBr + 0.3× TBE bath for 2 h. Then image the gel via 365 nm UV light. If no bands are observed, then add 5 μL more 10 mg/mL EtBr to the TBE bath and incubate the gel longer.

⚠ CRITICAL: As with BME, EtBr is highly toxic and can be readily absorbed through the skin. Gloves are absolutely essential when handling EtBr solutions and nitrile rubber gloves are preferred over latex gloves. Handling EtBr solutions should be done in a chemical hood and wearing full body PPE while doing so is recommended.

Fluorescence polarization (FP)

Timing: 3–5 h

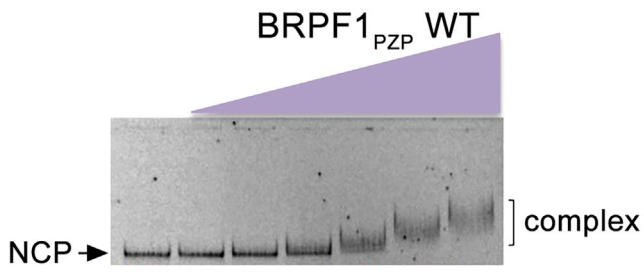

Figure 2. EMSA with NCP_{207} incubated with increasing amounts of wild-type $BRPF1_{PZP}$

Fluorescence polarization (FP) is used to measure binding of a fluorescently labeled molecule to another molecule while exciting the fluorescently labeled molecule with polarized light.

43. Using fluorescence polarization reaction buffer, prepare $BRPF1_{PZP}$ samples (also prepare one set of samples for background measurements). Separately, dilute NCPs in 0.5× TE, and store everything on ice.
    a. Prepare 30 μL of 150, 100, 50, 30, 10, 3, and 1 μM of $BRPF1_{PZP}$ running samples from the protein stock.
    b. Prepare 70 μL of 50 nM NCPs.

Parameter	Setting
Bandwidth	5 nm
Gain	optimal
G-Factor	uncalibrated
Lag time	0 μs
Number of flashes	50
Settle time	0 ms
Z-position	calculated from well

Note: Concentration of $BRPF1_{PZP}$ in the running samples should cover at least 2 orders of magnitude of the dissociation constant for the protein-NCP interaction in order to reach saturation.

44. Mix $BRPF1_{PZP}$ samples with NCP and incubate at 4°C for 30 min
    a. Mix 3 μL of NCP with 27 μL of $BRPF1_{PZP}$ samples to start the reactions. For zero $BRPF1_{PZP}$ concentration, 27 μL of reaction buffer is used instead.
    b. For background measurements use 3 μL of 0.5× TE instead of the NCP sample.
45. Place reaction mixtures into wells of the Costar black plate.
46. Load plate into the Tecan plate reader.
47. Initialize i-control and use the fluorescence module to design a protocol that will measure fluorescence polarization of each reaction by exciting the samples in the wells with 470 nm polarized light and measuring emission at 520 nm (for fluorescein) using the following settings:

Note: Since the determination of dissociation constants in a fluorescence polarization binding assay is insensitive to the absolute polarization values measured, calibrating the G-factor is not important for the purpose of this method.

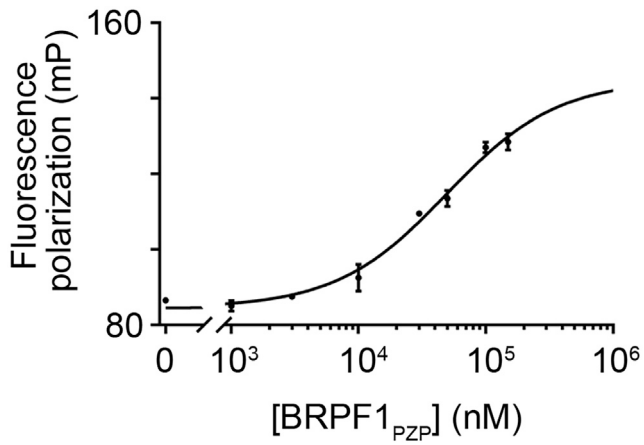

Figure 3. Binding curve obtained for the interaction of wild-type $BRPF1_{PZP}$ with NCP_{207}

48. Make sure the protocol outputs raw data of the polarized emission light intensity parallel and perpendicular to the plane of excitation. Then run the protocol.

Note: If the fluorescence polarization binding assay is outputting data with large deviations between data points, consider increasing the reaction volume. Stirring the reaction mixtures in wells with a pipette tip to make sure they are sitting evenly in the wells may help as well.

49. Export the data.
50. Analyze the data using preferred graphing software (GraphPad Prism, Origin, Gnuplot, etc) (Figure 3).

Liquid *in vitro* HAT assay

Timing: 1 day

51. Liquid *in vitro* HAT assays were performed in a final volume of 15 μl using 0.5 μg of purified free histones or H1-depleted short oligonucleosomes from HeLa cells as substrate.

⚠ CRITICAL: The quality of free histone or short oligonucleosomes should be monitored by Coomassie staining before HAT assays.

a. Mix free histone or short oligonucleosome with 3 μl of purified BRPF1 complex, 3 μl of HAT buffer 5×, 1 mM Na butyrate and 50 mM of KCl or NaCl.

Note: For the best results, the total salt concentration (KCl +NaCl) should be between 50 and 100 mM.

b. Keep the reaction mixture on ice for 15 min.
c. Add 0.125 μCi of ^{3}H labeled Ac-CoA (0.1 mCi/mL).
d. Incubate the mixture at 30°C for 30 min.
e. Spot the reactions on P81 filter paper (St Vincent's Institute of Medical Research).
f. Air dry the filter paper and wash 3 times in 50 mM carbonate buffer ($NaHCO_3$–$NaCO_3$, pH 9.2).
g. Rinse rapidly with acetone and air dry the filter paper.
h. Place the filter paper in scintillation tube (Fisher).
i. Add scintillation cocktail (MP Biomedicals) and measure counts in scintillation counter (Beckman Coulter) for 30 min (Figure 4).

⚠ CRITICAL: In HAT assays, we used FLAG-MORF$_{N1-716}$ instead of full-length MORF because transfection of full-length protein leads to substantial degradation.

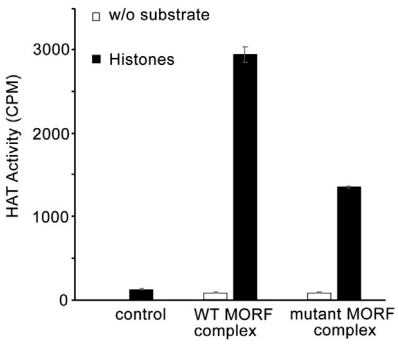

Figure 4. HAT activity of the native wild-type and mutant MORF complexes from K562 cells on histones

Liquid HAT assays, in which reactions were spotted on P81 filters and counted by scintillation as counts per minute (CPM).

⚠ CRITICAL: It is critical to purify the MORF enzyme with its associated subunits, BRPF1, ING5 and MEAF6, because the catalytic activity and substrate selectivity depend on these subunits.

EXPECTED OUTCOMES

This method was developed to study $BRPF1_{PZP}$, but with some variations it can be used to characterize other PZP domain containing proteins (Andrews et al., 2016; Klein et al., 2014). The described set of structural and biophysical experiments, including X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, electrophoretic mobility shift assays (EMSA), and fluorescence polarization (FP) are invaluable in gaining insight into the molecular mechanisms underlying binding of proteins to NCPs and chromatin. A yield of 2–2.5 mg $BRPF1_{PZP}$ could be achieved from 8 L of M9 Minimal Media cell culture. Note, that the wild type, mutated, and H3-linked $BRPF1_{PZP}$ proteins all elute around the elution volume (66 mL) on a HiPrep™ 16/60 Sephacryl® S-100 HR (Cytiva). In liquid HAT assays, Count per minute (CPM) values should be between 1000 to 5000 CPM and less than 300 CPM for negative control (without HAT complex or without histones).

QUANTIFICATION AND STATISTICAL ANALYSIS

The crystal structure of H3-linked $BRPF1_{PZP}$ was determined using materials and software listed in the key resources table. Crystallographic statistics generated for the H3-linked $BRPF1_{PZP}$ structure are described in (Klein et al., 2020).

Polarization values for each reaction were calculated using the exported raw data of polarized emission light intensity parallel and perpendicular to the plane of excitation. Perpendicular and parallel intensities of the background measurements were first subtracted from the reactions for each protein concentration. Polarization values then were calculated from:

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

Where P equals polarization, $I_{\parallel}$ equals parallel intensity and $I_{\perp}$ equals perpendicular intensity. The calculated values are then multiplied by 1000 as polarization values are typically reported in mP.

Polarization values were analyzed using plotting software to quantify binding affinities. Values were analyzed using Graphpad Prism (GraphPad) and fit to a one site specific binding model.

LIMITATIONS

Expression, purification and characterization of multiple zinc fingers that bind multiple ligands, for example histone tails and DNA in the case of $BRPF1_{PZP}$, are substantially more challenging compared to characterization of a single domain that binds to a single ligand. Production of $BRPF1_{PZP}$ can be a particularly difficult task because this protein tends to easily aggregate. Furthermore, the purified sample could be contaminated with bacterial DNA. To increase the yield of $BRPF1_{PZP}$, we had to optimize the construct size. Additional optimization of expression conditions, purification buffers, fusion-tags used, cleavage enzymes, and the column chromatography protocol may be required for improving yield of the protein.

Fluorescence polarization with NCPs is not sensitive to the binding of some PZP domains. This is likely due the fact that these PZP domains do not interact with nucleosomal DNA. Instead, an EMSA or other alternative binding assays will be needed. For PZPs that can bind DNA, but the interaction with DNA is relatively weak (50 uM binding affinity or weaker), the fluorescence polarization binding assay may result in a small polarization shift of only 40–60 mP maximum from the PZP binding, instead of the more typical 100–150 mP. This can result in larger deviations among the runs in a triplicate titration. In this case, more runs will be needed to reduce the uncertainty and allow for an accurate determination of the apparent dissociation constant.

We used histone H3, H4, H2A and H2B as substrates in HAT assays, however, these assays cannot provide information on which sites in the histones are acetylated.

TROUBLESHOOTING

Problem 1

Unable to obtain protein crystals or diffraction quality crystals.

Potential solution

There are many commercial screens available, use these to set up and screen for crystals. Some suggested options are Index (Hampton Research), Crystal Screen (Hampton Research), Natrix (Hampton Research), JCSG + (Molecular Dimensions), Morpheus (Molecular Dimensions), and PACT Premier (Molecular Dimensions). Use a freshly purified protein to set up crystallization screens and add purification steps (ion exchange) to increase purity. Crystal screening may require altering a variety of conditions to obtain diffraction quality crystals, including but not limited to, the concentration of the protein, additives, the size of the drops, hanging vs. sitting vapor diffusion, seeding, temperature, drop ratio, slight changes to pH, reducing agents.

Problem 2

Other components (hexasomes, tetrasomes) are present in the same fractions as well-positioned NCPs that appear to have similar sedimentation velocities but lower electrophoretic mobility.

Potential solution

Make fresh reconstitution buffers.

Problem 3

The NCP:BRPF1$_{PZP}$ complex is difficult to detect in EMSA.

Potential solution

Lower percentage TBE gels or replace TBE with TTE (Tris-taurine/EDTA). Composite gels made of 2% polyacrylamide + 1% agarose can also be used.

Problem 4

Fluorescence polarization assay is outputting data with large deviations between data points.

Potential solution

Consider increasing the reaction volume. Stirring the reaction mixtures in wells with a pipette tip to make sure they are dispersed evenly in the wells may also help.

Problem 5

CPM observed in liquid HAT assays is higher than 5000 CPM.

Potential solution

Decrease the amount of the HAT complex.

Problem 6

CPM observed in liquid HAT assays is lower than 1000 CPM.

Potential solution

Salt concentration of the samples might be above 100 mM. Adjust salt concentration to 50–100 mM or increase the amount of the HAT complex up to 20% (v/v).

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Tatiana Kutateladze (tatiana.kutateladze@cuanschutz.edu).

Materials availability

Reagents generated in this study will be made available on reasonable request.

Data and code availability

Coordinates and structure factors for the H3-Linked BRPF1$_{PZP}$ structure have been deposited in the Protein Data Bank under ID: 6U04.

Software used in this this study have been previously published and are detailed in the key resources table.

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AUTHOR CONTRIBUTIONS

All authors contributed to the writing of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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