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Assessing the *in vitro* Binding Specificity of Histone Modification Reader Proteins Using Histone Peptide Arrays

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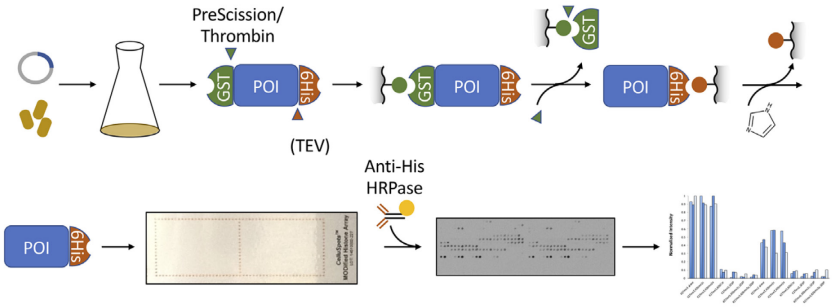
[Abstract] In the field of chromatin biology, a major goal of understanding the roles of histone post-translational modifications is to identify the proteins and domains that recognize these modifications. Synthetic histone peptides containing one or more modifications are a key tool to probe these interactions in pull-down assays with recombinant proteins or cell lysates. Building on these approaches, the binding specificity of a protein of interest can be screened against many histone peptides in parallel using a peptide array. In this protocol, we describe the expression and purification of a recombinant protein of interest in bacteria, followed by an assay for binding to histone post-translational modifications using a commercially available histone peptide array. The purification uses a versatile dual-tagging and cleavage strategy and equipment commonly available in a molecular biology laboratory.

Graphic abstract:



Overview of protocol for purifying recombinant protein and hybridizing to a histone peptide array.

Keywords: Histone peptide array, Recombinant protein, Affinity purification, Chromatin, Histone modifications, Histone tail, Chromodomain, Epigenetics

[Background] The histone proteins that package the eukaryotic genome can exhibit covalent post-translational modifications, including methylation, phosphorylation, and acetylation, at numerous residues. The functions of some of these modifications in chromatin regulation are mediated by interactions with effector proteins containing so-called ‘reader’ domains. To date, many reader domains have been characterized, including the CHROMO (chromatin organization modifier), Tudor, PWWP,

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MBT (Malignant Brain Tumour) repeat, PHD (plant homeodomain), ankyrin repeat, ADD (ATRX-DNMT3-DNMT3L), YEATS (Yaf9, ENL, AF9, Taf14, Sas5), WD40, and bromodomains (reviewed in Musselman *et al*., 2012). These domains are present in diverse proteins with roles in fundamental processes, including gene regulation, DNA repair, recombination, and replication. However, the full catalogue of chromatin reader proteins remains to be uncovered.

Early biochemical and structural studies of histone modification reader proteins revealed modification-dependent interactions with short histone peptides (Dhalluin *et al.*, 1999; Jacobson *et al.*, 2000; Bannister *et al.*, 2001; Jacobs and Khorasanizadeh, 2002; Nielsen *et al.*, 2002). In addition, many of the characterized histone modifications occur on the N- or C-terminal histone 'tail' domains, which are intrinsically disordered and likely to be conformationally dynamic in the absence of interaction partners (reviewed in Ghoneim *et al.*, 2021). Thus, when assaying interactions of reader proteins with histone modifications, a short histone peptide with the modification and its neighbouring sequence is likely to be an effective proxy for the native histone protein.

The introduction of peptide arrays to the chromatin field provided a powerful method to assay the binding of a protein of interest to many different modifications simultaneously in parallel (Nady *et al.*, 2008). Since then, several types of commercially available histone peptide arrays have been developed, and their characteristics have been thoroughly reviewed recently (Mauser and Jeltsch, 2019). In this protocol, we use the MODified™ histone peptide array (Active Motif) (Bock *et al.*, 2011), which contains 384 unique peptide spots in duplicate, synthesized using the CelluSpots method (Winkler *et al.*, 2009). The cellulose-bound 19mer peptides on the array represent sequences from different regions of the core histones (H3 amino acids # 1-19, 7-26, 16-35, 26-45, H4 1-19, 11-30, and H2A, H2B 1-19) as well as five control spots. The peptides are either unmodified, contain a single modification, or a combination of up to 4 modifications on the same peptide. A total of 59 modifications are represented, including acetylation, mono-, di- and tri-methylation of lysine, symmetric and asymmetric dimethylation of arginine, phosphorylation, and citrullination of different residues. A limitation of the array approach is that interaction detection is restricted to the particular modifications, combinations, and peptide sequences present on the array.

Heterologous protein expression in *E. coli* and affinity purification is a widely used strategy to obtain protein for *in vitro* assays. The protocol described here is adapted from standard protocols (for a detailed discussion, see Harper and Speicher, 2011). We designed plasmids for tandem purification with protease-cleavable tags at both the N- and C-termini. The N-terminal glutathione S-transferase (GST) fusion aids in solubilization and is used for the first affinity purification on glutathione-sepharose resin. The GST is then cleaved by PreScission Protease (or thrombin) to elute the recombinant protein, which contains a C-terminal 6×Histidine (His) tag. The 6×His tag is used for a second purification on an immobilized metal resin, as well as for antibody detection on the histone peptide array. We have used this versatile 2-step purification strategy and histone peptide array protocol to examine the binding specificities of several *C. elegans* chromo domain-containing proteins (Saltzman *et al.*, 2018).

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Materials and Reagents

Note: Equivalent materials and reagents can be used.

A. Clone cDNA into GST/His expression vector (pALS099 or pALS343)
   1. Chemically competent *E. coli* cloning strain (*e.g.*, DH5α, Invitrogen, catalog number: 18265017 or equivalent)
   2. High-fidelity DNA polymerase (*e.g.*, Q5, New England Biolabs, catalog number: M0491S; Phusion, New England Biolabs, catalog number: M0530S)
   3. Gel-purification kit (*e.g.*, GeneAid, catalog number: DFH300)
   4. Template for cDNA of interest or a cDNA clone
   5. Reagents for cloning method of choice:
      (Option 1) Reagents for Gibson assembly cloning:
      a. Plasmid pALS343 (pGEX-2TP-cTevHis-Gibson/MCS; Addgene, catalog number: 139788)
      b. FastDigest *Pdi*I restriction enzyme (Fisher Scientific, catalog number: FD1524)
      c. FastDigest *Kpn*I restriction enzyme (Fisher Scientific, catalog number: FD0524)
      d. NEBuilder HiFi DNA Assembly Master Mix (New England Biolabs, catalog number: E2621) or homemade Gibson assembly master mix (Gibson *et al.*, 2009)
      (Option 2) Reagents for Gateway™ cloning
      a. Plasmid pALS099 (pGEX-2TP-cTevHis-DEST; Addgene, catalog number: 139787)
      b. A Gateway™ donor vector (non-ampicillin resistant; *e.g.*, pDONR221, Fisher Scientific, catalog number: 12536017)
      c. LR Clonase™ II enzyme mix (Invitrogen, catalog number: 11791-020)

B. Express tagged protein
   1. Nalgene PPCO centrifuge bottles with sealing closure (500 ml; Fisher Scientific, catalog number: 3141-0500 or equivalent, matched with available rotor – see Equipment)
   2. 0.22 µm filter bottles, 500 ml (Fisher Scientific, catalog number: 09-741-05)
   3. 0.22 µm filter units, 50 ml (Millipore Sigma, catalog number: SCGP00525)
   4. Expression plasmid(s) cloned above
   5. Chemically competent *E. coli* protein expression strain, *e.g.*, BL21(DE3)pLysS (Invitrogen LSC606010) or C41(DE3)pLysS (Novagen 60444-1) (see Notes)
   6. LB-Agar plates containing 100 µg/ml ampicillin and additional antibiotics as required for your expression strain
   7. Dehydrated 2× YT media (Fisher Scientific, BD Difco™, catalog number: DF0440-17-0)
   8. Glucose (Sigma-Aldrich, catalog number: G7021)
   9. Isopropyl-β-D-thiogalactoside (IPTG, MW 238.3 g/mol) (Calbiochem, catalog number: 420322)
   10. 20% (w/v) Glucose (see Recipes)
   11. 2× YTG Media (see Recipes)
   12. 0.5 M IPTG (See Recipes)

C. Purify tagged protein

1. Cell pellet from above
2. Nalgene Oak Ridge Style Round Bottom Centrifuge Tube (Fisher Scientific, catalog number: 3114-0050 or equivalent, matched to available rotor – see Equipment)
3. Empty small chromatography columns (*e.g.*, Bio-Rad Biospin chromatography columns, catalog number: 732-6008)
4. Glutathione Sepharose 4B (Cytiva – formerly GE Life Sciences, catalog number: 17075601) (store at 4°C)
5. GST-tagged PreScission Protease (Cytiva – formerly GE Life Sciences, catalog number: 27084301) (store at -20°C)
6. TALON Metal Affinity Resin (Cobalt Sepharose 6B-CL, Takara, catalog number: 635501) (store at 4°C)
7. Fast Macro DispoDialyzer, cutoff > 10kDa (Harvard Apparatus, catalog number: 74-0802)
8. NaCl (Sigma-Aldrich, catalog number: S3014)
9. KCl (Sigma-Aldrich, catalog number: P9541)
10. Tween-20 (Sigma-Aldrich, catalog number: P9416)
11. Triton X-100 (Sigma-Aldrich, catalog number: T8787)
12. Phenylmethanesulfonyl fluoride (PMSF) (Roche, catalog number: 10837091001)
13. cOmplete™ mini EDTA-free Protease inhibitor cocktail tablets (Roche, catalog number: 4693159001) (store at 4°C)
14. NaH_2PO_4 (119.98 g/mol) (Sigma-Aldrich, catalog number: S0751)
15. Na_2HPO_4 (141.96 g/mol) (Sigma-Aldrich, catalog number: S7907)
16. Imidazole (68.08 g/mol) (Sigma-Aldrich, catalog number: I5513)
17. 37% Hydrochloric Acid (HCl) (Sigma-Aldrich, catalog number: 258148)
18. 1 M HEPES-NaOH pH 7.5 (Tekno, catalog number: H1035)
19. 0.5 M EDTA pH 8.0 (Invitrogen, catalog number: LS15575020)
20. 2-mercaptoethanol (2-ME) (14.3 M) (Sigma-Aldrich, catalog number: M3148) (store at 4°C)
21. 1 M DTT solution (Sigma-Aldrich, catalog number: 43816) (aliquot and store at -20°C)
22. Glycerol (Sigma-Aldrich, catalog number: G5516)
23. Reagents for SDS-PAGE (homemade gels or other suppliers can be substituted):
    a. 4× Laemmli protein sample buffer (Bio-Rad, catalog number: 161-0747)
    b. Precast Protein Gels (*e.g.*, Bio-Rad Mini-PROTEAN® TGX™ of appropriate percentage for your protein)
    c. 10× Tris-Glycine-SDS running buffer (Bio-Rad, catalog number: 161-0732)
    d. Precision Plus Protein Dual Xtra Prestained Protein Standards (Bio-Rad, catalog number: 161-0377)
24. Coomassie Brilliant Blue R-250 (Fisher Scientific, catalog number: BP101)
25. Protein quantification reagent (*e.g.*, Bradford assay; Bio-Rad, catalog number: 500-0006)
26. Stock Solutions (see Recipes)

a. 5 M NaCl
b. 2 M KCl
c. 10% (v/v) Triton X-100
d. 10% (v/v) Tween-20
e. 100 mM PMSF
f. 1 M NaH_2PO_4
g. 0.5 M Na_2HPO_4
h. 100 mM Sodium Phosphate buffer, pH 8.0
i. (5× Reduced Glutathione Buffer – optional – only required if eluting from the GSH-sepharose rather than cleaving off the GST)
j. 3 M Imidazole pH ~7.5
k. 5× Lysis Buffer

27. Buffers (see Recipes)
    a. 1× Lysis Buffer
    b. GST High Salt Wash Buffer
    c. GST Low Salt Wash Buffer
    d. PreScission Protease Buffer
    e. Talon binding/wash buffer
    f. Talon Elution Buffer
    g. Dialysis Buffer
    h. (Tris pre-elution buffer – optional – only required if eluting from the GSH-sepharose rather than cleaving off the GST)
    i. (Glutathione elution buffer – optional – only required if eluting protein from the GSH-sepharose rather than cleaving off the GST)
    j. Coomassie Staining Solution
    k. Coomassie Destain Solution

D. Histone Peptide Array assay
1. Plastic food wrap
2. Autoradiography ruler (Diversified Biotech ARS-150, Sigma-Aldrich, catalog number: R8133)
3. Film (Carestream® BioMax® XAR Film, catalog number: 1651454; Sigma-Aldrich, catalog number: F5513)
4. Film cassette (*e.g.*, Cytiva – formerly GE Life Sciences, catalog number: GERPN11649)
5. MODified™ histone peptide array (Active Motif, catalog number: 13001) (store at -20°C)
6. Container to incubate the 1 inch × 3 inch array slide (*e.g.*, small plastic boxes used for blot strips – Genhunter B123, Genhunter B130, Nunc 267061, or a slide hybridization chamber)
7. Purified protein of interest (aliquots stored at -80°C)
8. Anti-His(C-term)-HRP (Invitrogen, catalog number: R931-25) (store at 4°C)
9. 10× phosphate-buffered saline (PBS) (Fisher Scientific, catalog number: BP399-1)

10. Skim milk powder (EMD Millipore, catalog number: 115363)
11. Bovine serum albumin (BSA; Sigma-Aldrich, catalog number: A7906)
12. Enhanced chemiluminescence reagents (ECL) (*e.g.*, Western Lightning Plus-ECL, Perkin-Elmer NEL103E001EA) (store at 4°C) (see Notes)
13. Buffers (see Recipes)
    a. Wash buffer (PBS-T)
    b. Blocking Buffer
    c. Pre-binding buffer
    d. Peptide Array binding buffer

Equipment

Note: Equivalent equipment can be used.

1. Sonicator (*e.g.*, Branson Sonifier 250/450, with appropriate tip size) or similar ultrasonic homogenizer, alternatively a French Press for cell lysis
2. Refrigerated high-speed centrifuge with rotors for 4,000-7,000 × *g* for 500 ml bottles and 20,000 × *g* for Oakridge 30-50 ml tubes (*e.g.*, Beckman Avanti-JE with JA-10 and JA-25.50 rotors; or Sorvall RC-6 with SLA-3000 and SS-34 rotors)
3. Refrigerated low-speed centrifuge (*e.g.*, as above or Beckman Allegra X-14R with swinging bucket rotor SX4750)
4. Refrigerated microcentrifuge (*e.g.*, Eppendorf 5424R, or microcentrifuge in a cold room)
5. Shaker incubator (*e.g.*, New Brunswick™ I24, with refrigeration (I24R) if low induction temperatures required – *e.g.*, 16-18°C)
6. Cold room (4°C) or glass door refrigerator
7. Platform shaker (*e.g.*, VWR, model: 3500 Standard Shaker, catalog number: 89032-092)
8. End-over-end tube rotator/rotisserie (*e.g.*, Fisher Scientific, catalog number: 11676341)
9. Magnetic stir plate (*e.g.*, Fisher Scientific, catalog number: 1152016S)
10. Vertical electrophoresis system for SDS-PAGE and Western blotting (*e.g.*, Bio-Rad Mini-PROTEAN tetra cell 1658000)
11. Spectrophotometer (OD_{600} and OD_{595} for protein assay)
12. Film developer and darkroom (or alternate detection method, *e.g.*, a Bio-Rad Chemidoc 12003153)
13. Scanner (*e.g.*, Epson Perfection V850 Pro)

Software

1. Array Analyze Software (Active Motif, https://www.activemotif.com/documents/Array_Analyze_Software_v16.zip)

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Procedure

A. Clone cDNA into expression vector

To maximize the chance of generating a soluble, stable, and active protein, it is advisable to test several constructs, including full-length protein as well as fragments truncated based on predicted domain or secondary structure boundaries (Graslund *et al.*, 2008). Histone modification binding domain constructs are frequently used successfully in histone peptide binding assays. However, it is possible that the binding characteristics of the full-length protein may differ slightly from the isolated domain.

1. Two plasmids are provided for dual affinity purification. Select the appropriate plasmid to clone using Gibson assembly (a) or Gateway™ cloning (b). To enable dual tagging, the cDNAs should not have a start or stop codon:
   a. Clone cDNA of interest into plasmid pALS343 (pGEX-2TP-cTevHis-Gibson/MCS; Addgene，catalog number: 139788) using Gibson Assembly (standard restriction enzyme cloning may also be used):
      i. Digest plasmid with *Pdi*I and *Kpn*I. Purify the fragment from an agarose gel using a standard kit.
      ii. Design primers as shown below (Figure 1), where the N's are 21-24 bases of homology to the target cDNA. In the FOR primer, ensure your sequence is in-frame with the AAA AAA (KK) codons. In the REV primer, ensure your sequence is in-frame with the TAC (Y, sense strand) within the *Kpn*I site. To preserve the *Kpn*I site, the final codon of your insert must end in a G. Example primers are shown below.

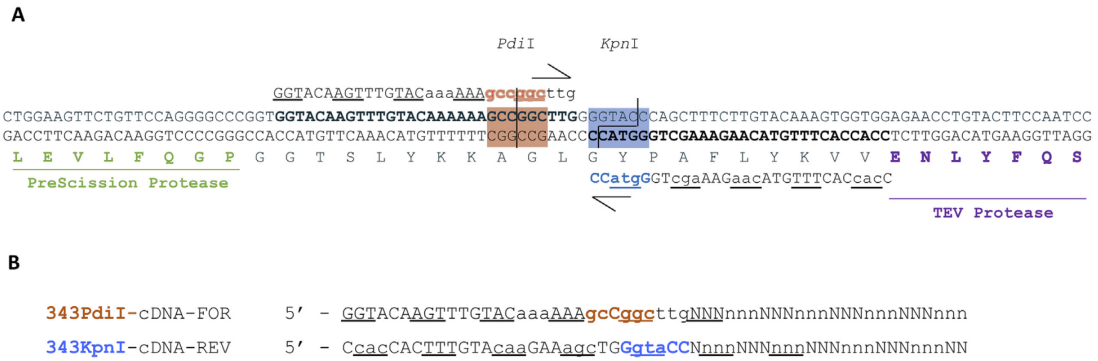

Figure 1. Scheme of the pALS343 vector insertion site and primer sequences used for cloning. A. Cloning insertion site of pALS343 (pGEX-2TP-cTevHis-Gibson/MCS) with key features indicated. B. Example primers indicating reading frame and restriction sites. N's represents ~18-24 bases of template-specific sequence.

   iii. PCR-amplify the cDNA of interest using a high-fidelity polymerase (*e.g.*, Q5 or Phusion polymerase). Purify the fragment from an agarose gel using a standard kit.

iv. Combine the gel-purified digested plasmid and the gel-purified PCR amplicon with NEBuilder HiFi DNA Assembly Master Mix, incubate, and transform competent cells (*e.g.*, DH5α) according to the manufacturer's protocols.

v. Screen colonies and confirm the reading frame and full sequence of your insert by Sanger sequencing.

b. Alternatively, clone cDNA of interest into destination vector pALS099 (pGEX-2TP-cTevHis-DEST; Addgene #139787) using Gateway™ cloning:

i. Design primers to amplify the cDNA flanked by *att*B sites as shown (Figure 2), where the N's are 21-24 bases of homology to the target cDNA. In the FOR primer, ensure your sequence is in-frame with the AAA AAA (KK) codons. The first codon of the insert must begin with a T. In the REV primer, ensure your sequence is in the frame indicated. The last codon of the insert is NAC (in the sense direction).

attB1-cDNA-FOR 5′ - GGGGACAAGTTTGTACaaaAAAgcaGGCtnnNNNnnnNNNnnnNNNnnn

attB2-cDNA-REV 5′ - GGGGACCACTTTGTACAAGAAAGCTGGGtnNNNnnnNNNnnnNNNnnnNNN

Figure 2. **Example primers to amplify an *att*B-flanked insert for Gateway™ cloning**. The N's represents ~18-24 bases of template-specific sequence.

ii. PCR-amplify the cDNA of interest using a high-fidelity polymerase (*e.g.*, Q5 or Phusion polymerase). Purify the fragment from an agarose gel using a standard kit.

iii. Create an entry clone and then an expression clone with sequential BP Clonase™ and LR Clonase™ steps according to the manufacturer's protocols. Alternatively, in a 1-step reaction (Liang *et al.*, 2013), combine the gel-purified *att*B-flanked PCR amplicon with the destination vector, donor vector, and LR Clonase™. Transform competent cells (*e.g.*, DH5α) according to the manufacturer's protocols.

iv. Screen colonies and confirm the reading frame and full sequence of your insert by Sanger sequencing.

B. Express tagged protein

Part I. Pilot expression to test induction and solubility

Before proceeding to a larger scale growth, test induction conditions and the solubility of your protein as described in this section. Process a known well-expressed and soluble protein (*e.g.*, GST-His from empty pALS343 vector) in parallel as a positive control.

Day 1. Transform expression plasmid into *E. coli* expression strain

1. Prepare sterile 2× YTG media, sterile glassware, and 5× lysis buffer (see Recipes) for the next day.
2. Transform *E. coli* BL21(DE3)pLysS [Note 1] with expression plasmid miniprep DNA according

to the manufacturer's instructions but scaled down by half.

3. Plate on LB-Agar plates containing 100 µg/ml ampicillin and 34 µg/ml chloramphenicol.
4. Incubate at 37°C overnight.

Days 2-3. Culture *E. coli*, induce expression, harvest cell pellet

5. In the early morning, pick a single colony into a 3 ml starter culture of 2× YTG containing 100 µg/ml ampicillin and 34 µg/ml chloramphenicol (2× YTG/Amp/Cam) [Note 2].
6. Grow with vigorous shaking (~250-300 RPM) at 37°C for ~4 h, or until the culture is visibly turbid.
7. To test three induction conditions, prepare a sterile Erlenmeyer flask with 150 ml 2× YTG/Amp/Cam [Note 3]. For aeration, ensure that the flask is ~ 4× the volume of the culture.
8. Dilute the starter culture 1:50 into the 150 ml culture.
9. Grow with vigorous shaking at 37°C until the OD_{600} reaches 0.6-0.8.
10. Save a 0.5 ml aliquot in a 1.5 ml tube on ice.
    a. This will be the 'uninduced' control for SDS-PAGE analysis.
    b. Keep this sample briefly on ice.
    c. When ready, pellet the cells by centrifuging at maximum speed for 1 min in a microcentrifuge at 4°C. Remove the supernatant. Freeze the pellet at -20°C until ready to process for SDS-PAGE.
11. To induce expression, add IPTG to a final concentration of 0.5 mM [Note 4].
12. Mix and split the culture into three sterile Erlenmeyer flasks with 40 ml each.
13. Test several induction temperatures and durations. Suggested conditions are:
    a. 30°C for 4-5 h.
    b. 37°C for 3 h.
    c. 14-16°C overnight (requires a refrigerated shaker).
14. At the end of each induction, remove 0.5 ml of culture.
    a. These will be the 'induced' samples. Keep sample briefly on ice.
    b. When ready, pellet the cells by centrifuging at maximum speed for 1 min in a microcentrifuge at 4°C. Remove the supernatant. Freeze the pellet at -20°C until ready to process for SDS-PAGE.
15. Transfer the remaining 40 ml cultures to centrifuge tubes. Centrifugation can be done in a low-speed centrifuge in disposable conical tubes (3,000 × *g* for 15 min at 4°C in SX4750 rotor) or in a high-speed centrifuge in Oakridge type tubes (6,000 × *g* for 10 min at 4°C).
16. Discard the supernatant.
17. Continue to prepare lysates or freeze the pellets at -20°C or -80°C for longer-term storage.

Days 2-3 continued. Lyse *E. coli* and separate soluble and insoluble fractions

Note: Keep samples at 4°C or on ice throughout.

18. Resuspend the cell pellet in 1/40th volume (1 ml) of 1× lysis buffer containing reducing agent and protease inhibitors (see Recipes) [Note 5].

19. Transfer to 1.5 ml tubes on ice.
20. Sonicate with the tubes in an ice-water bath.
    a. Suggested starting point for Branson 250/450 with microtip. Optimize for your system.
    b. Ensure tip is well-submerged but does not contact tube walls:
    c. Set Output = 4.5 and Duty cycle = 50%.
    d. Pulse 12 times. Rest on ice for 30 s.
    e. Repeat (12 pulses with 30 s rest) up to a maximum of 8 times. Monitor the sample – upon lysis, the sample will become slightly less opaque and slightly darker in colour. This should be evident for a control protein such as GST-His. Lysis can also be monitored by taking small aliquots over time. Centrifuge these aliquots to remove insoluble material and determine the total protein concentration (*e.g.*, using a Bradford protein assay). Determine when the concentration reaches a plateau.
    f. Clean the tip well by rinsing and a brief sonication in 70% ethanol.
21. Take two 60 µl aliquots to new tubes to prepare the 'whole' and 'pellet' samples for SDS-PAGE analysis.

    Keep the 'whole' lysate 60 µl aliquot on ice for SDS-PAGE analysis (do not centrifuge).
22. Centrifuge the bulk of the sonicated lysate and the 'pellet' aliquot at ~20,000 × *g* at 4°C for 30 min.

    For the 60 µl 'Pellet' aliquot for SDS-PAGE analysis – remove and discard the supernatant. Resuspend the pellet in Lysis buffer or PBS to a final volume of 60 µl.
23. For the bulk of the sonicated lysate – transfer the supernatant to a new tube and discard the pellet.
24. Take a 60 µll aliquot of the supernatant to a new tube – this is the 'soluble' sample for SDS-PAGE analysis.
25. Freeze the remaining supernatant at -20°C or -80°C for longer-term storage. This supernatant could be used for a small-scale test purification if desired.

<u>Days 2-3 continued. Analyze samples on SDS-PAGE</u>

26. Prepare an SDS-PAGE gel (or use a precast gel) as required to resolve your fusion protein of interest.
27. Prepare an aliquot of 4× Laemmli sample buffer with 2-ME as needed (add 100 µl 2-ME per 900 µl 4× sample buffer).
28. 'Uninduced' and 'induced samples:
    a. Thaw the pellets.
    b. Add 1× lysis buffer or PBS to bring volume to 50 µl. Pipette or vortex to resuspend.
    c. Add 16.7 µl 4× Laemmli sample buffer with 2-ME.
    d. Heat at 95°C for 5 min with intermittent vortexing. Repeat for another 5 min.
    e. Spin for 5 min in a microcentrifuge (maximum speed) and take the supernatant to a new tube.

f. Samples are ready to load on an SDS-PAGE gel (load 20-25 µl).

29. 'Whole,' 'pellet' and 'soluble' samples (60 µl):
    a. Add 20 µl 4× Laemmli sample buffer with 2-ME.
    b. Heat at 95°C for 5 min.
    c. Spin for 5 min in a microcentrifuge (maximum speed).
    d. Samples are ready to load on an SDS-PAGE gel (load 20-25 µl).
30. Run SDS-PAGE and perform Coomassie staining. Note that the 'whole'/'pellet'/'soluble' samples have been concentrated by 40-fold, whereas the 'induced'/'uninduced' have been concentrated 10-fold.
31. Analysis – inspect the stained gel for an induction-dependent band that is of approximately the correct molecular weight for your fusion protein of interest.
32. If there is not an obvious induced band of expected size, repeat the SDS-PAGE and perform a Western blot using the Anti-His(C-term)-HRP conjugated antibody. Because it is specific to a C-terminal 6×His tag, this antibody performs well in detecting fusion proteins in *E. coli* whole cell lysate samples.
33. Assess the proportion of soluble fusion protein and select the best conditions for a larger preparation. Note that a modest level of expression and solubility may be sufficient for a histone peptide array experiment, which may typically be achieved with tens of µg quantities of protein (see Figure 3 below).

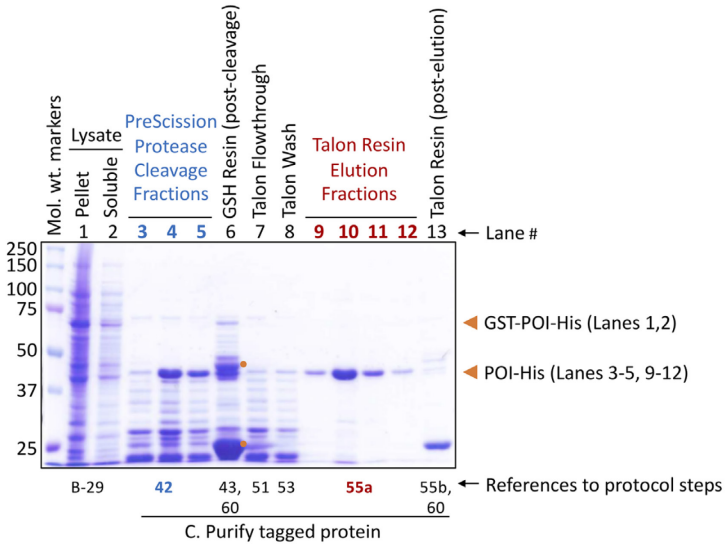

Figure 3. Selected fractions from a representative preparative protein purification (Coomassie-stained SDS-PAGE). Sizes of molecular weight markers (in kDa) are shown to the left of the gel. Positions of bands corresponding to the protein of interest (POI) before and after cleavage of the GST are shown to the right of the gel (orange arrowheads). References to relevant protocol steps are shown below the gel. Note that, for this POI fusion, it is difficult to

detect induction by Coomassie stain in the lysate (Lanes 1-2). Nevertheless, sufficient protein was enriched by the 2-step purification (Lanes 9-12). In Lane 6, orange dots mark the positions of cleaved GST (~26 kDa) and the GST-tagged PreScission Protease (~46 kDa), which remain bound to the GSH-sepharose resin.

Part II. Preparative protein expression

The yield of fusion protein can vary dramatically, in the range of 0.1-10 mg of purified protein per L of culture. For the histone peptide array conditions described in section D, 100 µg would be sufficient for several experiments for a protein that is <70 kDa.

Day 1. Transform expression plasmid into *E. coli* expression strain

Note: We have found that freshly-transformed cells generally perform better and therefore do not freeze glycerol stocks.

1. Prepare sterile 2× YTG media and sterile glassware for the next day.
2. Transform *E. coli* BL21(DE3)pLysS with expression plasmid miniprep DNA according to the manufacturer's instructions but scaled down by half.
3. Plate on LB-Agar plates containing 100 µg/ml ampicillin and 34 µg/ml chloramphenicol.
4. Incubate at 37°C overnight.

Day 2. Culture *E. coli*, Induce Expression

Note: Use flask sizes at least 4× the culture volume to allow sufficient aeration.

5. In the early morning, inoculate a 25 ml 2× YTG/Amp/CAM starter culture with a single colony.
6. Grow for ~5-6 h at 37°C with vigorous shaking.
7. Dilute starter 1:50-1:100 into 1 L of 2× YTG/Amp/CAM.
8. Grow 37°C with vigorous shaking until OD_{600} reaches 0.6-0.8.
9. (optional) For low temp induction, briefly cool culture flask.
   Remove 0.5 ml uninduced control culture sample. Store on ice.
10. Induce by addition of IPTG to a final concentration of 0.5 mM.
11. Induce for 4 h at 30°C, or as determined in your pilot expression.
12. During this time, pre-weigh and pre-cool centrifuge bottles, rotor, and centrifuge to 4°C.
13. Transfer the culture to centrifuge bottles.
    Remove 0.5 ml induced control culture. Store on ice and process with uninduced as described in the pilot expression section above.
14. Balance culture bottles and centrifuge at ~ 4,000 × *g* for 20 min or 7,700 × *g* for 10 min at 4°C.
15. Decant supernatant. Weigh bottle and calculate the pellet weight.
16. Store at -80°C.
17. Prepare buffers for the next day:
    a. Make 1.8 L dialysis buffer without DTT. Store at 4°C.
    b. Make purification buffers without reducing agents (2-ME or DTT) or protease inhibitors

(PMSF, protease inhibitor tablets), which will be added fresh (see Recipes). Store at 4°C.

C. Purify tagged protein

Note: All steps should be done on ice or at 4°C to minimize protein degradation. Aliquots are saved throughout the process to monitor afterwards by SDS-PAGE.

Day 3. Part I – Lyse *E. coli*

18. In the case of overnight induction, centrifuge culture and proceed with pellet.
19. Add reducing agents and protease inhibitors to the buffers for glutathione-sepharose purification (see Recipes). Store on ice.
20. Thaw the frozen cell pellet on ice.
21. Pre-chill the centrifuge tubes on ice.
22. Resuspend the bacterial pellet in ice-cold 1× Lysis Buffer containing protease inhibitors and DTT at ~5 ml per gram of pellet. For a 1 L preparative culture, this is usually ~25 ml.
23. Transfer to pre-chilled tubes for sonication.
24. Sonicate while keeping the samples cooled in an ice-water bath.
    Note: See the sonication conditions and notes in the optimization section above. If a larger tip is not available for your sonicator, split the resuspended culture into an appropriate number of tubes with smaller volumes and re-pool (combine) after sonication.
25. Transfer the lysate to pre-chilled 30-50 ml Oakridge style centrifuge tubes.
    Remove two 60 µl aliquots for the 'whole' and 'pellet' fractions. Process in a microcentrifuge as described in the pilot experiment above.
26. Ensure that accurately balanced tubes are prepared. Centrifuge the lysate to pellet insoluble material at 20,000 × *g* × 30 min at 4°C.
    a. During the centrifugation, prepare the GSH-Sepharose resin as described below.
    b. Pre-chill a tube for the supernatant on ice.
27. Transfer the supernatant (clarified lysate) to the clean, pre-chilled tube on ice.

Day 3. Part II – Purify on Glutathione-Sepharose 4B

Note: All steps should be done on ice or at 4°C to minimize protein degradation. The glutathione-sepharose resin should be kept with buffer throughout the procedure. Take care not to lose resin during pipetting steps.

28. Prepare GSH-Sepharose resin:
    a. Resuspend the resin by hand (inversion and shaking).
    b. For a 1 L preparative culture, use a 500 µl bed volume (BV).
    a. The resin is provided as a 75% slurry. Therefore, for 500 µl BV, transfer 666 µl (1.33× BV) to an appropriate tube (*e.g.*, 15 ml sterile conical disposable tube).
    b. Wash the resin 2-3× with cold 1× Lysis Buffer (or cold PBS and then cold 1× Lysis buffer).
    c. For each wash, spin 500 × *g* for 5 min at 4°C (*e.g.*, in Beckman Allegra swinging bucket

rotor) and carefully remove the buffer without disturbing the resin.

d. Use the last wash to split the resin into two 15 ml tubes (or as needed for the sonicate volume).

e. Store the resin in 1× lysis buffer until ready to use.

29. Remove the buffer from the resin and immediately add the clarified lysate prepared above.

30. Bind to GSH-Sepharose rotating at 4°C × 2.5-3 h.

a. During the incubation, add reducing agents and protease inhibitors to other purification buffers as needed.

b. Prepare labelled tubes on ice to collect aliquots for SDS-PAGE analysis: Flowthrough, high salt wash, low salt wash, PreScission buffer wash, PreScission cleavage.

31. Centrifuge at 500 × *g* for 5 min at 4°C.

Save 10 µl of the supernatant ('Flowthrough' or unbound fraction).

32. Wash GSH-Sepharose with 10× BV (5 ml) High Salt Wash Buffer. Incubate at 4°C with rotation for 5 min and centrifuging the resin at 500 g for 5 min at 4°C.

Save 10 µl of the supernatant ('High salt wash')

33. Wash GSH-Sepharose with 10× BV (5 ml) Low Salt Wash Buffer. Incubate at 4°C with rotation for 5 min and centrifuging the resin at 500 × *g* for 5 min at 4°C.

Save 10 µl of the supernatant ('Low salt wash').

34. Proceed to elute the protein from the resin by cleaving the GST.

Alternatively, the GST-fusion protein can be eluted from the resin using glutathione [Note 6].

35. Wash with 10× BV (5 ml) of PreScission Cleavage Buffer.

36. Remove the wash leaving a volume of ~1 ml.

37. Use the remaining buffer to resuspend the resin and transfer it to a 1.5 ml tube.

38. Wash the original tube(s) used for the binding using an additional ~0.5 ml of PreScission Cleavage buffer and add this to the 1.5 ml tube.

39. Centrifuge and remove the supernatant.

Save 10 µl aliquot of supernatant ('PreScission buffer wash').

40. Add 460 µl PreScission Buffer and 40 µl of PreScission Protease (2,000 units/ml).

If the resin bed volume used was not 500 µl, scale accordingly.

41. Incubate for 5 h at 4°C on a rotator to cleave and elute the 6×His-tagged protein. The PreScission protease is GST-tagged and will bind to the resin.

During the incubation, prepare the TALON resin (see below) and buffers for TALON purification

42. Collect the cleaved protein in the supernatant.

a. Centrifuge the resin and transfer the supernatant (500 µl) containing the cleaved protein to a new tube on ice.

Save 10 µl aliquot ('PreScission Cleavage').

b. Add 1× BV (500 µl) Talon Binding/wash buffer to the GSH-sepharose resin. Rotate for 30 min at 4°C.

c. Centrifuge and pool the supernatant with the previous fraction.

d. Add another 1× BV (500 µl) Talon Binding/wash buffer to the GSH-sepharose resin. Rotate 15 min at 4°C.
e. Centrifuge and pool the supernatant with the previous fractions for a total of 1.5 ml.
f. Proceed to the 2nd purification of the 6×His-tagged protein in the next section.

43. Aside – To check if there is protein remaining on the GSH-sepharose resin, add 1× BV (500 µl) of leftover PreScission cleavage buffer to the remaining GSH-sepharose resin. Store at 4°C to take a sample later for SDS-PAGE analysis as described on Day 4.
44. Store the aliquots collected during the purification at -20°C for SDS-PAGE analysis.

Day 3. Part III – Purify on TALON beads (Cobalt Sepharose 6B-CL)

45. Prepare the Talon Resin in a 2 ml tube.
    a. Thoroughly resuspend the resin.
    b. For 1 L purification, use a bed volume (BV) of 200 µl.
    c. The resin is provided as a 50% slurry, so transfer 2× BV (400 µl) to a 2 ml tube.
    d. All centrifugation steps with Talon resin below are performed at 700 × *g* for 2 min at 4°C.
    e. Wash 2-3 × with cold 1× PBS or cold Talon Binding/Wash buffer.
    f. Keep in Talon Binding/Wash buffer until ready to proceed.
46. To the 1.5 ml pooled supernatants from the first purification step, add $MgCl_2$ to a final concentration of 0.2 mM (3 µl of 100 mM).
47. Centrifuge the Talon resin, remove the buffer and add the 1.5 ml of supernatant from the first purification.
48. Incubate with rotation at 4°C for 1 h.
    a. During this incubation, add DTT to the cold dialysis buffer and split it into 3 large beakers with stir bars (600 ml each) at 4°C.
    b. Prepare a Dispo-dialyzer chamber by snapping one membrane between the plastic holder and the plastic ring as described in the manual. Add 1 ml of dialysis buffer in the chamber. Leave the chamber open (do not add the 2nd membrane).
    c. Place the Dispo-dialyzer in a beaker of dialysis buffer at 4°C on a magnetic stir plate with gentle stirring.
    d. Label 1.5 ml tubes to collect fractions and place at 4°C (‘Talon Flowthrough,’ ‘Talon Wash 1’, ‘Talon Wash 2’, ‘Talon Elution 1’, ‘Talon Elution 2’, ‘Talon Elution 3’, and ‘Talon Elution 4’).
49. Set up an empty Bio-Rad mini column over a 1.5 ml collection tube (‘Talon Flowthrough’) at 4°C. If a rack or holder for the mini column is not available, use a clean pipette tip as a wedge to brace the column vertically with its outlet inserted at the top of the collection tube.
50. Transfer the Talon resin and buffer to the empty Bio-Rad mini column at 4°C.
51. Allow the column to drain by gravity flow into the ‘Talon Flowthrough’ tube.
52. When nearly all the buffer is through, change to the ‘Talon Wash 1’ collection tube.
53. Add 10× BV (2 ml) Talon Binding/Wash Buffer to the column and allow to drain.
54. Repeat the above steps for a 2nd wash.

55. Elute 4× using 1× BV (200 µl) of Talon Elution Buffer for each elution. Collect these fractions in the 'Talon Elution' 1-4 tubes.
    a. Save 10 µl aliquots of each wash and elution fractions for SDS-PAGE analysis.
    b. Cap the column and add an equal volume of Talon Elution Buffer.
56. Pool (combine) the 4 elution fractions.
57. Remove the buffer from inside the Dispo-Dialyzer chamber and add the pooled protein elutions. Seal the top with a 2nd membrane and plastic ring.
58. Allow to dialyze overnight with gentle stirring at 4 °C.

Day 4. Finish dialysis and quantify protein. Analyze purification fractions by SDS-PAGE.

59. The next day, transfer the Dispo-Dialyzer to the 2nd and 3rd dialysis beakers for 2-3 h each.
60. During the dialysis:
    a. Prepare samples of the used remaining GSH-sepharose and Talon resins ('post elution') for SDS-PAGE:
        i. Resuspend the resins in an equal volume of buffer as indicated above.
        ii. Transfer 30 µl to a 1.5 ml tube.
        iii. Add 10 µl of 4× Laemmli sample buffer and heat at 95°C for 5 min.
        iv. Centrifuge for 1 min at maximum speed.
        v. Transfer the supernatant (~20 µl) to a new tube.
        vi. Store at -20°C for SDS-PAGE.
    b. Prepare the aliquots saved during the purification by adding Laemmli sample buffer to a final concentration of 1× and heating at 95°C for 5 min. Briefly centrifuge and resolve the samples on an SDS-PAGE gel of appropriate percentage for the size of your protein.
    c. Stain with Coomassie and evaluate the purification as described below in data analysis.
61. Pipette the sample from the Dispo-dialyzer chamber into a pre-chilled tube on ice.
62. Measure concentration with a Bradford or other protein quantification assay, comparing to a standard curve (*e.g.*, of BSA).

    Note that the sample must be diluted in a buffer without detergents or reducing agents for a Bradford assay (*e.g.*, PBS). Check an equivalent dilution of the dialysis buffer to ensure that there is no interference with the assay. Dilutions in the range of 1:3 to 1:5 should generally fall in the assay range [Note 7].
63. Aliquot the protein to pre-chilled, pre-labelled tubes.
    a. Calculate the amount required for the peptide array assay (see the next section) to estimate the aliquot sizes. Freeze-thaw cycles should be avoided.
    b. Also freeze a 2-4 µg aliquot to examine the composition of the final product by SDS-PAGE
64. Flash freeze aliquots in liquid nitrogen and store at -80°C.

D. Histone peptide array assay

Note: The optimal query protein concentration may be protein-dependent. However, we have found

satisfactory results for several proteins using a final concentration of 175 nM. The range of concentrations recommended by the manufacturer is 10 nM to 1 µM. To minimize the amount of protein required, use a dish close to the size of the slide or a specialized slide hybridization chamber. The volumes here were used for a plastic container measuring 34 mm × 88 mm × 28 mm (L × W × H).

Day 1. Block and bind protein to histone peptide array

All incubations are done on a flat platform shaker with gentle agitation (~80 RPM). A vacuum aspirator may be used to help remove buffer between wash steps. Once wetted with solution, the array should be kept with buffer throughout.

1. Prepare PBS-T, blocking buffer, and pre-binding buffer (see Recipes) [Note 8].
2. Thaw an aliquot of the purified protein on ice.
3. Centrifuge at 15,000 × *g* for 2 min at 4°C to pellet any insoluble material.
4. Transfer the supernatant to a clean pre-chilled tube.
5. Using clean forceps and handling the array at the ends, place the peptide array slide into an appropriate chamber (see Materials and Reagents, section **D**).
6. Wash briefly 2× with PBS-T.
7. Incubate in 10 ml of blocking buffer for 1 h 20 min at room temperature with agitation.
8. Wash briefly with PBS-T.
9. Incubate in 10 ml pre-binding buffer for 10 min 4°C with agitation.
10. During the incubation on ice, dilute the protein to the desired concentration in a final volume of 4 ml of cold binding buffer.

    For example, a 4 ml solution containing 175 nM protein requires 700 pmol of protein. For a 25 kDa protein, this is 17.5 µg, and for a 75 kDa protein, this is 52.5 µg:

    (pmol protein = volume × concentration

    $= 4 \times 10^{-3}$ L × 175 × 10^{-9} mol/L × 1 × 10^{12} pmol/mol)

    (µg protein = molecular weight × mol

    = kDa × 1000 Da/kDa × 700 pmol × 1 × 10^{-12} mol/pmol × 1 × 10^{6} µg/g)
11. Remove the pre-binding buffer and add the protein solution. Seal the container with Parafilm.
12. Incubate overnight (~12 h) at 4°C with agitation.
13. Store the remaining blocking solution at 4°C for use the next day.

Day 2. Part I – Wash and detect protein on array

All incubations are done on a flat platform shaker set to ~80 RPM.

14. Wash the array briefly in PBS-T.
15. Wash 3× in ~18 ml PBS-T, 5 min each, at room temperature with agitation.
16. During the washes, prepare the HRPase-conjugated anti-His(Cterm) antibody in 10 ml of blocking buffer at a final dilution of 1:3,500 (v/v) [Note 9].
17. Remove the last wash and add the antibody solution.
18. Incubate for 1 h 20 min at room temperature with agitation.

19. Wash briefly in PBS-T.
20. Wash 3 × in ~18 ml PBS-T, 5 min each, at room temperature with agitation.
21. When ready to proceed to imaging, remove the last wash and add 5 ml of each ECL reagent [Note 10].
22. Agitate gently by hand for 1min or as directed for your ECL.
23. Blot excess liquid on absorbent paper and wrap the array carefully in plastic wrap.
24. Place in a film cassette along with an autoradiography ladder.
25. Expose to film for 5 min.
26. Expose to another film for 1 min.
27. Develop the films and optimize the exposure time if required.
    a. An ideal exposure will have a strong signal-to-background ratio but will not have saturated or over-exposed peptide spots.
    b. Examine the two duplicate arrays and if the signals are similar to each other, as expected.
    c. Examine the negative control spots (positions P20-P24 in the bottom right corner of the array). It is expected that these would have no or low signal.

    For representative film images, please see Figure 1B and Figure S2A in Saltzman *et al.* (2018). If a satisfactory image is not achieved by varying the exposure time, it may be necessary to vary the protein concentration or buffer conditions. For additional troubleshooting, see [Note 8] and [Note 10].
28. Align the developed films with the autoradiography ladder.
29. Carefully mark the slide corners and the positions of alignment grid dots surrounding the array with a Sharpie marker. Take care not to mark on the array area.

Day 2. Part II – Scan array and analyze results

30. Scan the film(s) at 600 dpi in grayscale. Turn off any automatic adjustments of the scanner software.
31. If required, use image editing software to rotate the image so that the rows of peptide spots are straight and the array label is on the right-hand side.
32. Crop the image to just outside the array spots and save as .tif.
33. Open the .tif file in ActiveMotif's 'Array Analyze' software.
34. Following the Array Analyze software manual, select spots to allow the software to overlay the grid of peptide spots on your image. If required, repeat this procedure to ensure good alignment of the image.
35. Enter a sample '*name*' for your sample. This is appended at the beginning of the filenames of the saved spreadsheets and figures.
36. Select '*analyze array,*' and the software will calculate the intensity of each spot and open a results window.
37. In the '*Spot Statistics*' tab, examine the concordance of the left and right array duplicates. A histogram of the error between the duplicates and a scatterplot of the intensity on the left vs.

right duplicate can be saved as 'name_fig1.png' and 'name_figXY.png,' respectively.

38. In the '*Background Settings*' tab, examine the graph of intensity values. By default, the background threshold is set at the 67th percentile of the spot intensities. However, the background threshold can be manually raised or lowered (by dragging the orange line) to change the stringency with which spots are considered in further analysis. A graph of the ranked intensities can be saved as 'name_fig2.png'.
39. Examine the additional tabs and save the data tables and graphs as described in 'Data Analysis' below.

Data analysis

A. Analysis of protein purification by SDS-PAGE

To monitor the purification, examine the aliquots saved at each step by SDS-PAGE with Coomassie staining. The composition of the buffers or amount of resin can be adjusted if protein of the expected size is seen in the flowthrough or wash fractions or if there are co-purifying proteins. The PreScission cleavage or imidazole elutions can also be optimized if the protein is not efficiently removed from the resins. Note that following PreScission cleavage, the PreScission protease and GST will remain bound to the GSH-sepharose. These proteins will likely be visible in the GSH-sepharose 'post-elution' sample at ~46 kDa and ~26 kDa, respectively. An aliquot of the final protein preparation after dialysis should also be examined by SDS-PAGE to confirm purity and concentration.

B. Analysis of histone peptide array data

The approach to array analysis will depend on the modification(s) recognized by the protein and the peptides present on the array with this modification. For most modifications represented on the array, there are peptides containing the modification alone ('single' modification) as well as in combination with other modifications ('multiple' modifications). Signal intensity difference in the multiply-modified peptides may be biologically meaningful. For example, binding of some chromatin reader domains to methylated H3K9 or H3K27 is abrogated by phosphorylation of the adjacent serine residue.

The '*Specificity*' tab and associated figure (name_fig3.png) can display either the 'multiple' modification peptides with the top specificity score (see below), or the 'single' modification peptides with the highest background-subtracted intensities. The '*Reactivity*' tab (name_fig6.png) displays the background-subtracted average intensities for all peptides with only single modifications. Once the top modifications of interest are identified, the '*Modification analysis*' (name_fig5.png) tab can be used to plot the background-subtracted intensities for all peptides that contain that modification. Examples of the graphs listed above are included in the software manual: (https://www.activemotif.com/documents/1700.pdf).

For custom analysis and plotting, three spreadsheets can be saved from the Array Analyze software in comma-separated value (.csv) format:

1. The raw data is saved in the 'name_data.csv' file. Each of the 384 peptide spots is shown in

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order of their position on the array. The intensity on each duplicate sub-array, the average intensity, and the error between the duplicates are shown. In our analysis, we used these raw data to calculate a normalized, background-subtracted intensity, where the background was defined as the 60th percentile of the negative control spot intensity, and spots with intensities lower than the background were assigned an intensity of zero:

$$norm.intensity_i = \frac{intensity_i - background}{intensity_{max} - background}$$

2. The 'name_ranked_list.csv' shows the 384 peptides ranked by 'activity,' which is the average intensity found in the raw data file. The spreadsheet also indicates which individual modifications are present on each peptide. In addition, a 'normalized activity' is reported for each peptide, scaled from 0 to 1:

$$norm.act_i = \frac{activity_i - activity_{min}}{activity_{max} - activity_{min}}$$

3. The 'name_ranked_modifications.csv' lists the 59 modifications present on the array, ranked by 'specificity.' For each individual modification, the number of peptides on the array containing the modification ("No pos") and their average intensity ("Av Int pos"), and the number of peptides without the modification ("No neg") and their average intensity ("Av Int neg") are indicated. A 'specificity' score (plotted in the 'multiple modification' figure described above) is then reported:

$$Specificity_{\ i} = \frac{(average\ intensity\ of\ spots\ with\ modification._i)}{(average\ intensity\ of\ spots\ without\ modification._i)}$$

Notes

A. Pilot expression notes

[Note 1] We have expressed several proteins successfully in *E. coli* BL21(DE3)pLysS. However, if your protein is not induced well or is insoluble in this strain at any tested induction temperature, it is recommended to test other expression strains. Suggestions are C41(DE3)pLysS (Lucigen), or the Rosetta, Origami, or Rosetta-Gami strains (Novagen).

[Note 2] This protocol avoids overnight culture growth, which can be problematic for some plasmids.

[Note 3] The culture volume here is determined by the desired lysis buffer resuspension ratio and minimum volume that can be sonicated with a Branson microtip. If you are able to sonicate a smaller volume with your system, the culture volumes can be reduced accordingly.

[Note 4] The IPTG concentration can also be optimized, usually in the range of 0.1-1 mM.

[Note 5] Lysozyme can also be added to help with cell lysis. The concentration and type of detergents in the lysis buffer can also be optimized to help solubilize your protein. Test

concentrations up to 1% or substituting Tween-20 with Triton X-100.

B. Preparative protein expression and purification notes

[Note 6] It is also possible to elute the GST-fusion protein from the GSH-Sepharose resin using free reduced glutathione (GSH) if you do not want to cleave off the GST. In this case, after the step "Wash GSH-Sepharose with 10× BV (5 ml) Low Salt Wash Buffer":

a. Wash GSH-Sepharose with ~2-5× BV Tris Pre-elution buffer.
b. Elute with 1-2× BV GSH elution buffer for 2 h rotating at 4°C. Store the eluate on ice.
c. Elute again with additional 1× BV GSH elution buffer rotating overnight at 4°C.
d. Pool the two eluates and dilute in Talon Binding buffer (≥2.5× dilution).
e. Proceed to the His-purification by binding to the Talon beads according to main protocol. Alternatively, go directly to the dialysis.

[Note 7] Alternative protein quantification reagents may be less sensitive to detergents or reducing agents (*e.g.*, RC DC Protein Assay, Bio-Rad, catalog number: 5000121. If the protein is too dilute, it can also be concentrated (*e.g.*, Amicon Ultra-2 Centrifugal Filter Unit, Sigma-Aldrich, catalog number: UFC201024).

C. Histone peptide array notes

[Note 8] The stringency of the blocking and washes can also be optimized. Several conditions have been tested (Petell *et al.*, 2019); however, this study used a different peptide array format.

[Note 9] As an alternative to the chemiluminescence-based detection described here, an antibody conjugated to a fluorescent dye (*e.g.*, AlexaFluor) or infrared dye can be used together with an appropriate scanner.

[Note 10] To troubleshoot weak signals, a more sensitive ECL reagent can be tested (*e.g.*, Pierce SuperSignal™ West Femto Maximum Sensitivity Substrate, Fisher Scientific, catalog number: 34094).

Recipes

Note: All solutions are prepared with ultrapure water (e.g., Milli-Q® water with resistivity of 18.2 MΩ.cm).

A. Stock solutions and media

1. 2× YTG media (2× YT with 2% glucose) (1 L)
   a. In ~ 700 ml ddH_2O, dissolve:
      16 g tryptone
      10 g yeast extract
      5 g NaCl
   b. Adjust the volume to ~850 ml with ddH_2O.
   c. If necessary, adjust pH to 7.0.
   d. Adjust volume to 900 ml with ddH_2O.

e. Loosen cap and cover in foil w/ autoclave tape.
f. Autoclave at 121°C for 30 min on a liquid cycle with a loosened cap.
g. Allow to cool.
h. Before use, add 100 ml of filter-sterilized 20% (w/v) glucose using sterile technique.

2. 20% (w/v) glucose (500 ml)
   a. Dissolve 100 g of glucose in ~400 ml of ddH_2O.
   b. Adjust final volume to 500 ml with ddH_2O.
   c. Filter-sterilize with a 0.22 µm vacuum filter.
   d. Store at room temperature and open under sterile conditions.
3. 5 M NaCl (1 L)
   a. Dissolve 292.2 g of NaCl in ~800 ml of ddH_2O.
   b. Adjust final volume to 1 L with ddH_2O.
   c. Autoclave at 121°C for 30 min on a liquid cycle with a loosened cap.
   d. Store at room temperature.
4. 2 M KCl (1 L)
   a. Dissolve 149.1 g of KCl in ~800 ml of ddH_2O.
   b. Adjust final volume to 1 L with ddH_2O.
   c. Autoclave at 121°C for 30 min.
   d. Store at room temperature.
5. 0.5 M IPTG (MW 238.3 g/mol)
   a. Dissolve 4.76 g of IPTG in ~30 ml of ddH_2O.
   b. Adjust final volume to 40 ml with ddH_2O.
   c. Filter-sterilize with a 0.22 µm vacuum or syringe filter.
   d. Store small aliquots at -20 °C; avoid freeze-thaw cycles.
6. 10% (v/v) Triton X-100
   a. Add 4 ml of Triton X-100 to 36 ml of ddH_2O.
   b. Mix gently and thoroughly.
   c. Store at room temperature, protected from light.
7. 10% (v/v) Tween-20
   a. Add 4 ml of Tween-20 to 36 ml of ddH_2O.
   b. Mix gently and thoroughly.
   c. Store at room temperature, protected from light.
8. 100 mM PMSF
   a. Weigh 0.87 g of PMSF.
   b. Dissolve in isopropanol and adjust the final volume to 50 ml.
   c. Store aliquots at -20°C or short-term at 4°C.
9. 1 M NaH_2PO4 (MW 119.98 g/mol)
   a. Dissolve 59.99 g NaH_2PO4 in ~350 ml ddH_2O.
   b. Adjust the final volume to 500 ml with ddH_2O.

c. Autoclave at 121°C for 30 min (or filter-sterilize).
d. Store at room temperature.

10. 0.5 M Na_2HPO_4 (MW 141.96 g/mol)
    a. Dissolve 70.98 g Na_2HPO_4 in ~800 ml ddH_2O.
    b. Adjust the final volume to 1 L with ddH_2O.
    c. Autoclave at 121°C for 30 min (or filter-sterilize).
    d. Store at room temperature.
11. 100 mM Sodium Phosphate buffer, pH 8.0
    a. To a sterile container (*e.g.*, 50 ml tube), add:
       9.32 ml of 0.5 M Na_2HPO4 (see Recipe above)
       0.34 ml of 1 M NaH_2PO_4 (see Recipe above)
       40.34 ml of ddH_2O (to 50 ml)
    b. Store at room temperature.
12. (Optional – only required if eluting from the GSH-sepharose rather than cleaving off the GST): 5× Reduced Glutathione Buffer (0.3 M Tris-Cl pH 8.8, 0.15 M reduced glutathione)
    a. Dilute 1.5 M Tris-Cl pH 8.8 5× to 0.3 M (*e.g.*, 10 ml 1.5 M Tris-Cl pH 8.8 + 40 ml ddH_2O).
    b. Dissolve 0.46 g of reduced glutathione (MW 307.32) in 10 ml of 0.3 M Tris-Cl pH 8.8.
    c. Take a small aliquot, dilute 1:10 and check that the final pH is ≥8.
    d. Store small aliquots at -20°C; avoid freeze-thaw cycles.
13. 3 M Imidazole pH ~7.5 (4°C)
    a. Dissolve 4.08 g of imidazole in ~ 12 ml ddH_2O.
    b. Adjust the volume to 18.5 ml with ddH_2O.
    c. Add 1.5 ml of concentrated HCl.
    d. Take an aliquot and dilute it to $1/10^{th}$ in ddH_2O. Check that the pH is ~7.5.
    e. Store at 4°C.
14. 5× Lysis Buffer (100 mM HEPES-Na pH 7.5, 1.5 M NaCl, 0.5 mM EDTA, without DTT or protease inhibitors) (25 ml)
    a. To a sterile container (*e.g.*, 50 ml tube), add:
       14.98 ml ddH_2O
       2.5 ml 1M HEPES-Na pH 7.5
       7.5 ml 5M NaCl
       25 µl 0.5 M EDTA pH 8
    b. Store at 4°C.

B. Buffers for protein purification

Note: buffers without reducing agents (DTT, 2-ME) or protease inhibitors can be stored at 4°C. Quantities below are sufficient for 2 × 1 L purifications.

1. 1× Lysis Buffer (20 mM HEPES-Na pH 7.5, 300 mM NaCl, 0.1 mM EDTA, 1 mM PMSF, 10 mM 2-ME, ±0.5% Tween-20**)

*** Note detergent is optional. See Notes. Detergent can be added after sonication.*

a. To a sterile container (*e.g.*, 50 ml tube), add:

Component	For 30 ml
ddH_2O	22.2 ml
5× Lysis Buffer (see Recipe above)	6 ml
10% Tween-20 (see Recipe and Note above)	1.5 ml
(add fresh before use) 100 mM PMSF (see Recipe above)	300 µl
(add fresh before use) 2-ME	21 µl
(add fresh before use): Complete mini protease inhibitor tablet	1 tab per 10 ml

b. Keep on ice or at 4°C; do not store.

2. GST High Salt Wash Buffer (20 mM HEPES-Na pH 7.5, 500 mM NaCl, 0.1 mM EDTA, 10 mM 2-ME)

a. To a sterile container (*e.g.*, 50 ml tube), add:

Component	For 5 ml
ddH_2O	4.4 ml
1 M HEPES-Na pH 7.5	100 µl
5 M NaCl	500 µl
0.5 M EDTA pH 8	1 µl
(add fresh before use) 2-ME	3.5 µl

b. Keep on ice or at 4°C; do not store.

3. GST Low Salt Wash Buffer (20 mM HEPES-Na pH 7.5, 100 mM NaCl, 0.1 mM EDTA, 10 mM 2-ME)

a. To a sterile container (*e.g.*, 50 ml tube), add:

Component	For 5 ml
ddH_2O	4.8 ml
1 M HEPES-Na pH 7.5	100 µl
5 M NaCl	100 µl
0.5 M EDTA pH 8	1 µl
(add fresh before use) 2-ME	3.5 µl

b. Keep on ice or at 4°C; do not store.

4. PreScission Protease Buffer (20 mM HEPES-Na, 150 mM NaCl, 1 mM EDTA, 2.5 mM 2-ME, 0.01% Tween-20)

a. To a sterile container (*e.g.*, 50 ml tube), add:

Component	For 10 ml
ddH_2O	9.47 ml
1 M HEPES-Na pH 7.5	200 µl
5 M NaCl	300 µl
0.5 M EDTA pH 8	20 µl
10% Tween-20	10 µl

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(add fresh before use) 2-ME 1.75 µl

b. Keep on ice or at 4°C; do not store.

5. Talon binding/wash buffer (50 mM Sodium phosphate pH 8, 300 mM NaCl, 0.25% Tween-20, 5 mM imidazole, 2.5 mM 2-ME, 1 mM PMSF)
   a. To a sterile container (*e.g.*, 50 ml tube), add:

Component	For 10 ml
ddH_2O	4.03 ml
100 mM Sodium phosphate buffer, pH 8 (see Recipe above)	5 ml
5 M NaCl	600 µl
10% Tween-20	250 µl
3 M Imidazole (see Recipe above)	16.7 µl
(add fresh before use) 2-ME	1.75 µl
(optional; add fresh before use) 100 mM PMSF	100 µl

   b. Keep on ice or at 4°C; do not store.
6. Talon Elution Buffer (composition of wash buffer except 300 mM imidazole)
   a. To a sterile container (*e.g.*, 50 ml tube), add:

Component	For 5 ml
ddH_2O	1.53 ml
100 mM Sodium phosphate buffer, pH 8 (see Recipe above)	2.5 ml
5 M NaCl	300 µl
10% Tween-20	125 µl
3 M Imidazole (see Recipe above)	500 µl
(add fresh before use) 2-ME	0.88 µl
(optional; add fresh before use) 100 mM PMSF	50 µl

   b. Keep on ice or at 4°C; do not store.
7. Dialysis Buffer (25 mM HEPES-Na, 100 mM KCl, 0.2 mM EDTA, 1 mM DTT, 20% glycerol)
   a. To a 2 L graduated cylinder, add 360 ml of glycerol.
   b. Add ddH_2O to approximately 1.2 L.
   c. Add 45 ml of 1 M HEPES-Na pH 7.5.
   d. Add 90 ml of 2 M KCl.
   e. Add 720 µl of 0.5 M EDTA pH 8.
   f. Add ddH_2O to a final volume of 1.8 L.
   g. Seal tightly with 2 layers of parafilm.
   h. Invert several times until thoroughly mixed.
   i. Chill to 4°C at least overnight.
   j. (fresh before use) Add 1.8 ml of 1 M DTT and repeat mixing.
   k. Divide 600 ml into each of 3 beakers for dialysis.
   l. Keep at 4°C; do not store.
8. (Optional – only required if eluting from the GSH-sepharose rather than cleaving off the GST)

Tris pre-elution buffer (60 mM Tris-Cl pH 8.8, 10 mM 2-ME)

a. Combine the following in a sterile tube:

Component	For 5 ml
ddH_2O	4.8 ml
1.5 M Tris-Cl pH 8.8 (or pH 8)	0.2 ml
(add fresh before use) 2-ME	3.5 µl

b. Keep on ice or at 4°C; do not store.

9. (Optional – only required if eluting protein from the GSH-sepharose rather than cleaving off the GST) Glutathione elution buffer (60 mM Tris-Cl pH 8.8, 30 mM reduced glutathione, 200 mM NaCl, 20% glycerol, 10 mM 2-ME)

   a. Combine the following in a sterile tube:

Component	For 5 ml
ddH_2O	2.8 ml
5× Reduced glutathione (see Recipes)	1 ml
5 M NaCl	0.2 ml
Glycerol	1 ml
(add fresh before use) 2-ME	3.5 µl

   b. Keep on ice or at 4°C; do not store.

10. Coomassie Staining Solution (0.2% Coomassie Brilliant Blue R-250, 50% methanol, 10% acetic acid)

    a. In a 500 ml glass bottle, combine:
       200 ml ddH_2O
       250 ml methanol
       50 ml glacial acetic acid
       1 g Coomassie Brilliant Blue R250
    b. Excess staining solution can be recovered and re-used.
    c. Store at room temperature.

11. Coomassie Destain Solution (40% methanol, 10% acetic acid)

    a. In a 1 L glass bottle, combine:
       500 ml of ddH_2O
       400 ml of methanol
       100 ml of glacial acetic acid
    b. Store at room temperature.

C. Buffers for histone peptide array

1. Wash buffer (PBS-T – phosphate buffered saline with 0.05% (v/v) Tween-20)

   a. Dilute 1 part 10× PBS with 9 parts ddH_2O.
   b. Add Tween-20 using a wide-bore tip to a final concentration of 0.05% (v/v) (0.5 parts per 1000).

2. Blocking buffer (5% (w/v) milk in PBS-T)
   a. Dissolve 1 g of skim milk powder in ~18 ml of PBS-T.
   b. Adjust the final volume to 20 ml with PBS-T.
3. Pre-binding buffer (3% (w/v) BSA in PBS-T)
   a. Dissolve 0.6 g of BSA in ~18 ml of PBS-T.
   b. Adjust the final volume to 20 ml with PBS-T.
4. Peptide Array Binding Buffer (1× PBS-T containing 0.45% (w/v) BSA, 0.5 mM EDTA, 0.1 mM DTT, 10% glycerol)
   a. To a sterile tube, add the following components:

Component	For 5 ml
PBS-T	3.75 ml
3% BSA in PBS-T (see above)	750 µl
Glycerol	500 µl
0.5 M EDTA pH 8	5 µl
(add fresh before use) 1 M DTT	0.5 µl

   b. Make fresh before use and keep on ice. Do not store.

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Competing interests

No competing interests to declare.

Ethics

This protocol does not use human or animal subjects.

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