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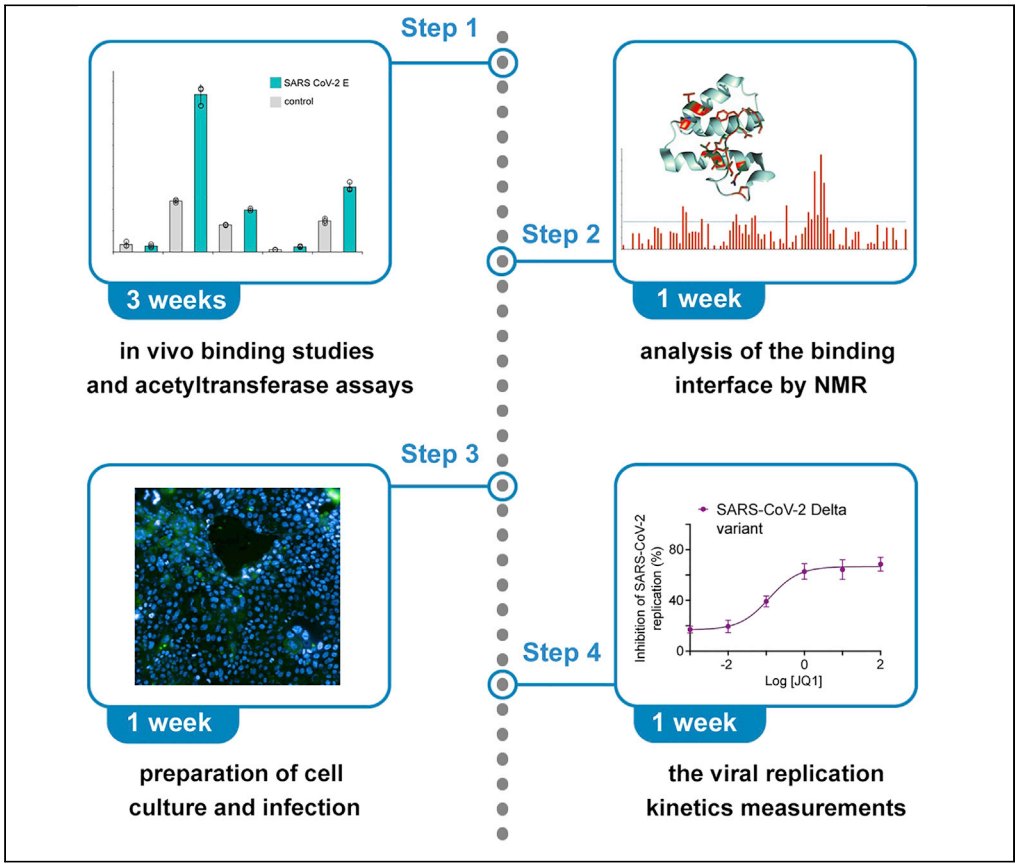
Characterization of multiple interactions between the envelope E protein of SARS-CoV-2 and human BRD4



The SARS-CoV-2 envelope (E) protein hijacks human BRD4 (bromodomain and extra-terminal domain protein 4). Here, we describe a protocol to characterize the interaction of the acetylated E protein with BRD4 *in vivo*. We detail steps to use NMR spectroscopy to map the binding interface and include steps to monitor the effect of BRD4 inhibitors in SARS-CoV-2-infected human lung bronchial epithelial cells. This approach could be applied to study interactions involving other viral and human proteins.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Mohamad Zandian, Suk Min Jang, Catherine Lachance, Arpan Acharya, Siddappa N. Byrareddy, Jacques Côté, Tatiana G. Kutateladze

sid.byrareddy@unmc.edu (S.N.B.)
jacques.cote@crchudequebec.ulaval.ca (J.C.)
tatiana.kutateladze@cuanschutz.edu (T.G.K.)

Highlights

Optimized protocol to monitor acetylation of the viral E protein

Characterization of the interaction of the acetylated E protein with BRD4

NMR chemical shift perturbation analysis to map the binding interphase

Protocol to study the effect of BRD4 inhibitors in SARS-CoV-2-infected cells

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Protocol

Characterization of multiple interactions between the envelope E protein of SARS-CoV-2 and human BRD4

Mohamad Zandian,[1,4] Suk Min Jang,[2,4] Catherine Lachance,[2,4] Arpan Acharya,[3,4] Siddappa N. Byrareddy,[3,5,*] Jacques Côté,[2,5,*] and Tatiana G. Kutateladze[1,5,6,*]

[1]Department of Pharmacology, University of Colorado School of Medicine, Aurora, CO 80045, USA

[2]Laval University Cancer Research Center, CHU de Québec-UL Research Center-Oncology Division, Quebec City, QC G1R 3S3, Canada

[3]Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68131, USA

[4]These authors contributed equally

[5]Technical contact

[6]Lead contact

*Correspondence: sid.byrareddy@unmc.edu (S.N.B.), jacques.cote@crchudequebec.ulaval.ca (J.C.), tatiana.kutateladze@cuanschutz.edu (T.G.K.)
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SUMMARY

The SARS-CoV-2 envelope (E) protein hijacks human BRD4 (bromodomain and extra-terminal domain protein 4). Here, we describe a protocol to characterize the interaction of the acetylated E protein with BRD4 *in vivo*. We detail steps to use NMR spectroscopy to map the binding interface and include steps to monitor the effect of BRD4 inhibitors in SARS-CoV-2-infected human lung bronchial epithelial cells. This approach could be applied to study interactions involving other viral and human proteins.

For complete details on the use and execution of this protocol, please refer to Vann et al. (2022).[1]

BEFORE YOU BEGIN

This protocol integrates complementary biochemical and cell and molecular biology approaches, enabling a rapid and straightforward evaluation of the interaction between the viral protein E from SARS-CoV-2 and the human protein BRD4. Generic protocols for protein purification, NMR titration experiments, and crystal structure determination applicable to this study are described in previous Star Protocols.[2,3] In the present work, we detail procedures for immunoprecipitation and western blotting and measuring acetyltransferase activity of histone acetyltransferases (HATs) and native HAT complexes. We then describe the methodology for NMR-based characterization of the interaction between the ET domain of BRD4 and SARS-CoV-2 E peptide. Lastly, we report procedures for measuring the kinetics of viral replication using qRT-PCR and immunofluorescence. To begin, we need to generate the expression plasmids, prepare nuclear extracts for immunoprecipitation and HAT assays, and culture human bronchial epithelial Calu-3 cells for SARS-CoV-2 infection.

Preparation for immunoprecipitation and western blotting of acetylated SARS-CoV-2 E 3×FLAG

Timing: 13–14 days (for step 1)

Aim: To assess whether SARS-CoV-2-E-3×FLAG can be acetylated *in vivo* by human acetyltransferases (KATs) and whether acetylated SARS-CoV-2-E-3FLAG interacts with BRD4 proteins.

1. Subclone SARS-CoV-2 E protein in pcDNA3.1-3×Flag:
   a. Resuspend the SARS-CoV-2 E peptide gBlock ordered from IDT in TE at 10 ng/μL.
   b. Heat at 50°C for 20 min and keep the resuspended SARS-CoV-2 E peptide gBlock on ice.
   c. PCR amplification of the SARS-CoV-2E peptide gBlock using KOD polymerase in a T100 thermal cycler (BioRad) with For BamHI E peptide and Rev XhoI E peptide.

PCR reaction mix

Reagent	Final concentration	Amount
10× KOD buffer	1×	5 μL
25 mM $MgSO_4$	1.5 mM	3 μL
2 mM dNTPs	0.5 mM	5 μL
50 μM For BamHI E peptide	0.5 mM	0.5 μL
50 μM Rev XhoI E peptide	0.5 mM	0.5 μL
SARS-CoV-2 E peptide gBlock	0.2 ng/μL	1 μL
2.5 U/μL KOD HS	1 Unit	1 μL
Ultrapure water	N/A	34 μL (up to 50 μL)

PCR cycling conditions

Steps	Temperature	Time	Cycles
Initial Denaturation	98°C	3 min	1
Denaturation	98°C	15 s	30 cycles
Annealing	60°C	15 s	
Extension	70°C	15 s	
Final extension	70°C	30 s	1
Hold	12°C	forever	

   d. Purify 100 μL of PCR reactions with Monarch DNA gel extraction purification kit using manufacturer instructions and eluted with 20 μL elution buffer provide in the kit (https://www.nebiolabs.com.au/protocols/2015/11/23/monarch-dna-gel-extraction-kit-protocol-t1020).
   e. Digest the SARS-CoV-2 E peptide PCR amplicons and pcDNA3 3× FLAG plasmid with BamH1 and XhoI restriction enzymes in Cutsmart buffer for 1 h at 37°C.

Restriction enzyme digestion reaction mix

Reagent	Final concentration	Amount
Purified PCR product or pcDNA3 3×FLAG	0.1 μg/μL	10 μL (3 μg)
10× cutsmart buffer	1 ×	3 μL
XhoI 20 U/μL	1 U/μL	1.5 μL
BamHI-HF 20 U/μL	1 U/μL	1.5 μL
Ultrapure water	N/A	14 μL (up to 30 μL)

   f. Add 1 μL of calf intestine phosphatase (CIP) to the digest pcDNA3 3× FLAG and incubate 1 h at 37°C.
   g. Migrate digested SARS-CoV-2 E peptide and pcDNA3 3× FLAG on 0.8% agarose gel and purify extracted bands with the Monarch gel extraction kit using manufacturer instructions (https://www.nebiolabs.com.au/protocols/2015/11/23/monarch-dna-gel-extraction-kit-protocol-t1020).
   h. Ligate 15.2 ng of the SARS-CoV-2 E peptide with 100 ng of the pcDNA3 3×FLAG (molar ratio 3:1) using T4 DNA Ligase. Incubate 2 h at room temperature.
   i. Transform 50 μL of DH10α Home-made competent cells with 5 μL of ligation product (cf step 6 for transformation).

j. Inoculate one colony into 3 mL LB media supplemented with 100 μg/mL ampicillin. Incubate at 37°C with shaking at 250 RPM for 12–16 h.
k. Extract plasmid with Favorprep Plasmid Extraction Mini Kit according to the manufacturer's protocol (http://www.favorgen.com/favorgen/serv_1/mem_t1/h_1/pdf/plasmid/FAPDE%20000_Mini%20001%20001-1.pdf).

Note: pcDNA3 SARS-CoV-2 E 3×FLAG need to be sequenced to check in-frame integration of SARS-CoV-2 E peptide with FLAG Tag.

2. Transform pcDNA3.1-3×Flag plasmid containing SARS-CoV-2 E protein peptide 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 at 250 RPM.
   g. Spread the cells on ampicillin plate.
   h. Incubate the plate for 12–16 h at 37°C.
3. Amplify pcDNA3.1-3×Flag plasmid containing SARS-CoV-2 E protein peptide:
   a. Inoculate one colony into 5 mL of LB media and incubate at 37°C with shaking at 250 RPMfor 7–9 h (Pre-culture).
   b. Add pre-culture into 400 mL of LB media.
   c. Incubate at 37°C with shaking at 250 RPM for 16–18 h.
   d. Extract the plasmids by NucleoBond Xtra Maxi kit (Macherey Nagel) according to the manufacturer's protocol (https://www.mn-net.com/media/pdf/ed/82/0f/Instruction-NucleoBond-Xtra.pdf).

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. Transfection was achieved according to the manufacturer's protocol.

4. Transfect Human kidney 293T (HEK293T) cells with LipofectamineTM 3000:

Note: Other transfection reagents can be used instead of Lipofectamine TM 3000 (e.g., PEI transfection reagent).

Note: SARS-CoV-2 E DNA construct is in pcDNA3 plasmid and tagged with 3×FLAG. Culture HEK293T cells in DMEM (ThermoFisher) at 37°C in a 5% CO_2 incubator (Panasonic).

   a. Split HEK293T cells 12 h before transfection in order to get 80%–90% confluency in 150 mm plate (~1.6 × 107 cells).
   b. 20 μg of pcDNA3 (negative control) and pcDNA3 SARS-CoV-2E 3×FLAG were used for transient transfection.
   c. Dilute Lipofectamine™ 3000 reagent in 750 μL of Opti-MEM™.
   d. Vortex 3 s.
   e. In another tube, dilute 20 μg of pcDNA3 and 40 μL of P3000 reagent in 750 μL of Opti-MEM™.
   f. Mix well with up and down pipetting.
   g. Mix dilute pcDNA3 and P3000 reagent in dilute Lipofectamine™ 3000 reagent.
   h. Let 5 min at RT.
   i. Add drop by drop in D150 mm plate.
   j. Change the medium after 12 h of transfection.

Preparation for histone acetyltransferase (HAT) assays (tandem affinity purification [TAP] of native HBO1 and MYST acetyltransferase).

Timing: 7–8 days

⚠ CRITICAL: The following steps should be done strictly on ice or at 4°C. K562 cells in RPMI (ThermoFisher) supplemented with 25 mM HEPES-NaOH pH 7.4 at 37°C in a 5% CO2 incubator (Panasonic).

5. Large-scale expansion of K562 native HBO1 and MYST acetyltransferase:
   a. Grow 3 L of K562 cells in spinner flasks with gentle agitation.
   b. Harvest cells at or slightly below 8×10^5 cells/mL.
   c. Pellet the cells by centrifuging at 700 × *g*, 10 min, 4°C. Resuspend and pool the pellets in 50 mL of cold PBS 1×.
   d. Centrifuge at 700 × *g*, 10 min, 4°C, place the pellet on ice, and immediately prepare the NE.
6. Preparation of Nuclear Extracts:[4]
   a. Wash the cell pellets by adding four packed cell volumes of Hypotonic Buffer (Nuclear Extraction).
   b. Centrifuge at 1,900 × *g*, 5 min, 4°C, and remove the supernatant quickly.
   c. Resuspend well with three packed cell volumes of Hypotonic Buffer.
   d. Incubate on ice 10 min.
   e. Transfer the cells to a glass Dounce homogenizer with a type B pestle. Homogenize by douncing 15 times, then centrifuge at 3,500 × *g*, 10 min, 4°C.
   f. Collect the supernatant (cytoplasmic extract) and estimate the packed nuclear volume of the pellet using the gradations on the conical tube.
   g. Add half the packed nuclear volume of Low Salt Buffer and resuspend well with gentle vortexing.
   h. Extract the soluble proteins by adding half the packed nuclear volume of High Salt Buffer dropwise with gentle vortexing.
   i. Dounce twice using a Dounce homogenizer with a type B pestle and incubate for 30 min at 4°C with agitation.
   j. Pellet the extracted nuclei by ultracentrifugation at 100,000 × *g*, 1 h, 4°C.
   k. Quickly transfer the supernatant (i.e., the NE) to a new Falcon tube.
   l. Snap freeze the NE in liquid nitrogen and store at −80°C.
7. Tandem Affinity Purification (TAP)[5,6]:
   a. Before TAP, prechill rotor, centrifuge tube and ultracentrifuge to 4°C.
   b. Thaw the NE on ice, adjust to 0.1% Tween-20, and centrifuge at 40,000 × *g*, 1 h, 4°C (Ti-70 rotor Beckman Coulter).
   c. Preclear the NE using 250 µL Sepharose CL-6B resin prewashed with PBS 1× and equilibrated with TAP buffer in a 10 mL Poly-Prep chromatography column.
   d. Collect the precleared NE in a 15 mL tube.
   e. Add 250 µL anti-FLAG M2 (Sigma-Aldrich, Cat#A2220) affinity beads to the precleared NE and incubate for 2 h at 4°C with rotation.
   f. Transfer to a 10 mL Poly-Prep chromatography column, harvest the flowthrough (FLAG-FT), and pass it through the column again.
   g. Wash the beads with 40 column volumes (CVs) of TAP buffer.
   h. Wash with 40 CVs of Wash I Buffer.
   i. Wash with 40 CVs of Wash II Buffer.
   j. Transfer the beads in a 1.5 mL Eppendorf tube. Use Wash II Buffer to rinse the column and collect all the beads.
   k. Centrifuge at 300 × *g*, 5 min, 4°C and carefully remove the supernatant.
   l. Elute the complex with 2.5 CVs of Wash II supplemented with 200 µg/mL 3×FLAG peptide for 1 h at 4°C on a rotator.

m. Centrifuge at 300 × *g*, 5 min, 4°C and carefully transfer the supernatant into a Micro Bio-Spin column in a 2 mL microcentrifuge tube.
n. Centrifuge at 300 × *g*, 1 min, 4°C to collect the eluate.
o. Collect a 15 μL sample to resolve by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).
p. Repeat steps l and o.
q. Pool the FLAG elutions and add 125 μL Strep-Tactin Superflow Sepharose affinity matrix prewashed with 1 mL PBS 1× followed by 1 mL Wash II Buffer.
r. Incubate for 1 h at 4°C on a rotator.
s. Centrifuge at 300 × *g*, 5 min, 4°C and remove the flowthrough.
t. 3× Wash the beads with 1 mL Wash II Buffer.
u. Elute the complex with 1 CV of Wash II Buffer supplemented with 5 mM D-biotin for 1 h at 4°C on a rotator.
v. Centrifuge at 300 × *g*, 5 min, 4°C and carefully transfer the supernatant into a Micro Bio-Spin column in a 2 mL microcentrifuge tube.
w. Centrifuge at 300 × *g*, 5 min, 4°C to collect the eluate.
x. Aliquot a 15 μL sample for SDS-PAGE.
y. Repeat steps u and x.
z. Aliquot the purified complex. Snap freeze in liquid nitrogen and keep at −80°C.

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

Preparation for analysis of the binding interface by NMR

Timing: 1 day

8. Prepare ^{15}N-labeled protein of interest as described previously.[2,7] Here, ^{15}N-labeled ET domain of BRD4 (amino acids 601–683 of BRD4) was prepared. NMR sample contained 250 μL of 0.15 mM ^{15}N-labeled ET domain.
9. Record ^{1}H,^{15}N heteronuclear single quantum coherence (HSQC) spectrum of 0.15 mM ^{15}N-labeled ET domain in the apo state.
10. Add increasing amounts of the E peptide to the NMR sample stepwise and record HSQC spectra of the sample after each addition of the peptide until saturation is reached. Here, the HSQC spectra were recorded at the protein:peptide molar ratios of 1:1, 1:2, 1:5, and 1:10.
11. Use NMRPipe[8] to process the acquired HSQC data. Please refer to (https://www.ibbr.umd.edu/nmrpipe/install.html) for more information about installation and usage of NMRPipe.

Preparation of Calu-3 cell culture

Timing: 3 to 5 days

12. Culture human bronchial epithelial Calu-3 cells (ATCC, Manassas, VA, USA; catalog # HTB-55) in complete medium [Eagle's Minimum Essential Medium (EMEM) ATCC; catalog # 30-2003] supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin (P/S) in a humidified incubator at 37°C with 5% CO_2.

SARS-CoV-2 (Delta variant) infection of Calu-3 cells and BRD4 inhibitor studies

Timing: 72 h

13. Perform all live virus experiments in a BSL3 laboratory.

14. Seed Calu-3 cells at 15,000 cells/well in a 96 well transparent bottom black color plate and incubate cells at 37°C with 5% CO_2 for 48 h, as described previously.[9]
15. On the day of infection, treat the cells with different concentrations of JQ1 (Cayman Chemical, Cat # 11187) and OTX015 (Cayman Chemical, Cat # 15947). The drug concentration varies from 0.1 mM to 0.00001 mM.
16. Two hours post drug addition, infect the cells with SARS-CoV-2 (Delta variant of concern (VOC); pangolin lineage: B.1.617.2, obtained from BEI Resources, NIAID, NIH; cat # 55672) with 0.5 MOI of viral titer or left uninfected (negative control).
17. One-hour post-infection, remove the virus inoculum from the cells, wash the cells 3 times with 1× PBS (at room temperature), and add fresh media replenished with the same concentration of JQ1 and OTX015 respectively.
18. Return the cells to a humidified incubator at 37°C with 5% CO_2.
19. 24 h post-infection, collect the culture supernatant for measuring the viral replication kinetics.
20. Wash the cells with 1× PBS (at room temperature) three times and fix them with 4% PFA for immunofluorescence analysis.

Measurement of SARS-CoV-2 titer using plaque assay

Timing: 5 days

21. Seed Vero E6 cells in 6-well plates.
22. After 24 h of seeding, wash the cells with 1× PBS at room temperature for three times.
23. Prepare a serial dilution of the SARS-CoV-2 stock in Opti-MEM (Cat # 31985070, Gibco, Thermo Scientific) (dilution series: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8}).
24. Add the different dilutions of viral stock to the Vero E6 cells in triplicate.
25. Incubate the cells in a humidified incubator at 37°C with 5% CO_2 for 1 h.
26. Then add 2 mL of 0.5% agarose prepared in MEM medium (with 5% FBS) in each well of the plates.
27. Return the plates in the incubator at 37°C with 5% CO_2 for 72 h.
28. Add 1 mL of 4% PFA to each well of the plates and keep at 4°C for 24 h.
29. Then remove the agarose from the plates and add 1 mL of 0.5% crystal violet to each well.
30. After 15 min, remove the crystal violate solution from the plates and count the number of plaques in each well. Calculate the mean of number of plaques for each viral stock dilution and find the titer.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-acetyl-lysine	ImmuneChem	Cat # ICP0380
Anti-FLAG M2 affinity beads	Sigma-Aldrich	Cat # A2220
Strep-Tactin Superflow Sepharose affinity matrix	IBA	Cat # 2-1206-010
Anti-BRD4	Abcam	Cat # ab46199
Anti-FLAG HRP	Sigma Aldrich	Cat # A8592-1MG
Bacterial and virus strains		
DH5α Competent Cells	Thermo Fisher Sci	Cat # 18265017
Critical commercial assays		
NucloBond Xtra Maxi Kit	Macherey Nagel	Cat # 740414.100
Recombinant p300 catalytic domain	Active Motif	Cat # 81093
KOD polymerase	EMD Millipore	Cat # 71086-4
Monarch DNA gel extraction	NEB	Cat # T1020L
BamHI	NEB	Cat # R3136
XhoI	NEB	Cat # R0146

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
CIP (Calf Intestin Phosphatase)	NEB	Cat # M0290
T4 DNA Ligase	NEB	Cat # M0202L
Favorprep Plasmid Extraction Mini Kit	FAVORGEN	Cat # FAPDE 300
Other		
NanoDrop ND-1000 Spectrophotometer	Marshall Scientific	Cat # ND-1000
Dounce homogenizer with a type B pestle	Thomas Scientific	Cat # 1229H80
Poly-Prep chromatography columns	Bio-Rad	Cat # 7311550
Micro Bio-spin column	Bio-Rad	Cat # 732-6204
Scintillation vials	Fisher	Cat # 03-337-20
Spinner Flasks	Fisher	Cat # 10203E
P81 phosphocellulose filter paper	St Vincent's Institute of Medical Research	On demands
Chemicals, peptides, and recombinant proteins		
Lipofectamine™ 3000	Invitrogen	Cat # L3000001
Sepharose CL-6B resin	Sigma Aldrich	Cat # CL-6B-200
G-Sepharose beads Dynabeads	Invitrogen	Cat # 10002D
Dithiothreitol (DTT)	Gold Biotechnology	Cat # 27565-41-9
Phenylmethanesulfonyl fluoride (PMSF)	Sigma-Aldrich	Cat # P7626
Ethylenediamine tetraacetic acid (EDTA)	Fisher Chemical	Cat # S311-500
Ampicillin Sodium Salt	Thermo Sci Fairlawn Chem	Cat # BP176025
Microbiology Media: Luria-Bertani (LB), Miller	Thermo Fisher Sci	Cat # BP1426-2
LB Agar, Miller	Thermo Fisher Sci	Cat # BP9724-500
Zinc Chloride ($ZnCl_2$)	Fisher Chemical	Cat # Z53-500
Sodium dodecyl sulfate (SDS)	Sigma-Aldrich	Cat # L3771
Scintillation cocktail	MP Biomedicals	Cat # 0188247504
EDTA	Sigma-Aldrich	Cat # E9884-1KG
Acrylamide	Sigma-Aldrich	Cat # A2792-100ML
Ammonium persulfate (APS)	Sigma-Aldrich	Cat # A3678-25G
TEMED	Sigma-Aldrich	Cat # T9281-25ML
Ultra Pure™ agarose	Thermo Fisher	Cat # 16500-100
Triton × 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
NaCl	Fisher Chemical	Cat # S27110
Magnesium sulfate ($MgSO_4$)	Sigma-Aldrich	Cat # M7506-1KG
DMEM	ThermoFisher	Cat # 11995-065
RPMI 1640	ThermoFisher	Cat # 21870-092
PBS 1×	ThermoFisher	Cat # 10010023
Hepes	Fisher	Cat # BP310-5
Tween20	Sigma-Aldrich	Cat # P9416
Glycerol	Fisher	Cat # G334
Sodium butyrate	Sigma-Aldrich	Cat # B5887
Na_2HPO_4	Sigma-Aldrich	Cat # 255793-50G
KCl	Sigma-Aldrich	Cat # P9541
Ammonium sulfate $(NH_4)_2SO_4$	Sigma-Aldrich	Cat # A4418
Triton-X	Sigma-Aldrich	Cat # A7638
EDTA salt dihydrate	Sigma-Aldrich	Cat # E1644
FLAG M2 agarose beads	Sigma-Aldrich	Cat # A2220-5ML
3×FLAG peptides	Sigma-Aldrich	Cat # F4799-25mg
D-Biotin	Thermo Fisher	Cat # B20656
Short oligonucleosomes (purified from HeLa cells)	Côté et al., 1995[10]	Home-made
0.125 µCi of ^3H labeled Ac-CoA (0.1 mCi/mL)	Perkin Elmer Life Sciences	Cat # NET290250UC

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Human: HEK293T	ATCC	Cat # CRL-3216
Human K562	ATCC	Cat # CCL-243
Oligonucleotides		
For BamHI E peptide	IDT	atataggatccTACAGCTTC GTATCAGAAGA
Rev XhoI E peptide	IDT	atatactcgagTCATTCGAG AACGAGGAGATC
SARS-CoV-2E peptide gBlock	IDT	TACAGCTTCGTATCAGAAG AAACCGGGACACTGATCGT AAATTCTGTGCTCTTGTTTC TGGCATTCGTCGTATTTCTC CTCGTCACACTGGCAATTCT GACTGCATTGAGGCTTTGCG CCTACTGTTGTAACATTGTC AATGTATCTCTCGTGAAACC CTCATTCTACGTTTACAGCA GGGTGAAGAATCTCAATTCT AGCAGGGTGCCGGATCT CCTCGTTCTCGAA
Recombinant DNA		
Plasmid: Invitrogen™ Gateway™ pDEST™15 Vector	Thermo Fisher Sci	Cat # 1180214
Plasmid: Invitrogen™ Gateway™ pDONR™/Zeo Vector	Thermo Fisher Sci	Cat # 12535035
Software and algorithms		
Prism	GraphPad	https://www.graphpad.com/scientific-software/prism/
CCP4	Winn et al., 2011[11]	https://www.ccp4.ac.uk

MATERIALS AND EQUIPMENT

TE buffer		
Reagent	Final concentration	Amount
Tris pH 7.5 (1 M)	10 mM	1 mL
EDTA (0.5 M)	0.1 mM	20 µL
Milli-Q H_2O	N/A	Up to 100 mL

Note: Filter using 0.22 µm filter. The buffer can be stored at room temperature for up to 1 month.

WCE buffer (whole cell extract)		
Reagent	Final concentration	Amount
Tris pH 8.0 (1 M)	50 mM	5 mL
NaCl (5 M)	450 mM	9 mL
Triton X-100 (20% v/v)	0.1% v/v	0.5 mL
$MgCl_2$ (1 M)	2 mM	0.2 mL
$ZnCl_2$ (1 M)	2 mM	0.2 mL
EDTA (0.5 M)	0.2 mM	0.4 mL
Glycerol (50% v/v)	10% v/v	20 mL
DTT (1 M)	1 mM	100 µL
PMSF (0.1 M)	1 mM	1 mL
cOmplete™ protease inhibitor cocktail	N/A	1 tablet
Milli-Q H_2O	N/A	Up to 100 mL

Note: Filter using 0.22 μm filter. Add 1 mM of DTT, 1 mM PMSF and 1 tablet of protease inhibitor (cOmplete protease inhibitor cocktail Roche) per 100 mL buffer just prior to use. The buffer can be stored at 4°C for up to 1 month.

Hypotonic buffer (nuclear extraction)		
Reagent	Final concentration	Amount
Hepes pH 7.9 (1 M)	10 mM	1 mL
$MgCl_2$ (1 M)	1.5 mM	150 μL
KCl (1 M)	10 mM	1 mL
DTT (1 M)	1 mM	100 μL
PMSF (0.1 M)	1 mM	1 mL
cOmplete™ protease inhibitor cocktail	N/A	1 tablet
Milli-Q H_2O	N/A	Up to 100 mL

Note: Filter using 0.22 μm filter. Add 1 mM of DTT, 1 mM PMSF and 1 tablet of protease inhibitor (cOmplete protease inhibitor cocktail Roche) per 100 mL buffer just prior to use. The buffer can be stored at 4°C for up to 1 month.

Low salt buffer (nuclear extraction)		
Reagent	Final concentration	Amount
Hepes pH 7.9 (1 M)	20 mM	2 mL
Glycerol (50% v/v)	10% v/v	20 mL
$MgCl_2$ (1 M)	1.5 mM	150 μL
KCl (1 M)	20 mM	2 mL
EDTA (0.5 M)	0.2 mM	40 μL
DTT (1 M)	1 mM	100 μL
PMSF (0.1 M)	1 mM	1 mL
cOmplete™ protease inhibitor cocktail	N/A	1 tablet
Milli-Q H_2O	N/A	Up to 100 mL

Note: Filter using 0.22 μm filter. Add 1 mM of DTT, 1 mM PMSF and 1 tablet of protease inhibitor (cOmplete protease inhibitor cocktail Roche) per 100 mL buffer just prior to use. The buffer can be stored at 4°C for up to 1 month.

High salt buffer (nuclear extraction)		
Reagent	Final concentration	Amount
Hepes pH 7.9 (1 M)	20 mM	2 mL
Glycerol (50% v/v)	10% v/v	20 mL
$MgCl_2$ (1 M)	1.5 mM	150 μL
KCl (3 M)	1.2 M	40 mL
EDTA (0.5 M)	0.2 mM	40 μL
DTT (1 M)	1 mM	100 μL
PMSF (0.1 M)	1 mM	1 mL
cOmplete™ protease inhibitor cocktail	N/A	1 tablet
Milli-Q H_2O	N/A	Up to 100 mL

Note: Filter using 0.22 μm filter. Add 1 mM of DTT, 1 mM PMSF and 1 tablet of protease inhibitor (cOmplete protease inhibitor cocktail Roche) per 100 mL buffer just prior to use. The buffer can be stored at 4°C for up to 1 month.

TAP buffer		
Reagent	Final concentration	Amount
Hepes pH 7.9 (1 M)	20 mM	2 mL
Glycerol (50% v/v)	10% v/v	20 mL
$MgCl_2$ (1 M)	1.5 mM	150 μL
KCl (3 M)	300 mM	10 mL
EDTA (0.5 M)	0.2 mM	40 μL
DTT (1 M)	1 mM	100 μL
PMSF (0.1 M)	1 mM	1 mL
cOmplete™ protease inhibitor cocktail	N/A	1 tablet
Milli-Q H_2O	N/A	Up to 100 mL

Note: Filter using 0.22 μm filter. Add 1 mM of DTT, 1 mM PMSF and 1 tablet of protease inhibitor (cOmplete protease inhibitor cocktail Roche) per 100 mL buffer just prior to use. The buffer can be stored at 4°C for up to 1 month.

Wash I buffer		
Reagent	Final concentration	Amount
Hepes pH 7.9 (1 M)	20 mM	2 mL
Glycerol (50% v/v)	10% v/v	20 mL
Tween-20 (10% v/v)	0.1% v/v	1 mL
KCl (3 M)	300 mM	10 mL
DTT (1 M)	1 mM	100 μL
PMSF (0.1 M)	1 mM	1 mL
cOmplete™ protease inhibitor cocktail	N/A	1 tablet
Milli-Q H_2O	N/A	Up to 100 mL

Note: Filter using 0.22 μm filter. Add 1 mM of DTT, 1 mM PMSF and 1 tablet of protease inhibitor (cOmplete protease inhibitor cocktail Roche) per 100 mL buffer just prior to use. The buffer can be stored at 4°C for up to 1 month.

Wash II buffer		
Reagent	Final concentration	Amount
Hepes pH 7.9 (1 M)	20 mM	2 mL
Glycerol (50% v/v)	10% v/v	20 mL
Tween-20 (10% v/v)	0.1% v/v	1 mL
KCl (3 M)	150 mM	5 mL
DTT (1 M)	1 mM	100 μL
PMSF (0.1 M)	1 mM	1 mL
cOmplete™ protease inhibitor cocktail	N/A	1 tablet
Milli-Q H_2O	N/A	Up to 100 mL

Note: Filter using 0.22 μm filter. Add 1 mM of DTT, 1 mM PMSF and 1 tablet of protease inhibitor (cOmplete protease inhibitor cocktail Roche) per 100 mL buffer just prior to use. The buffer can be stored at 4°C for up to 1 month.

10× TBS buffer		
NaCl	1.5 M	88 g
Tris	1 mM	24 g
Milli-Q H_2O	N/A	Up to 1 L

Note: pH should be adjusted to 7.2 with HCL. The buffer can be stored at 4°C for up to 1 year.

5× HAT buffer		
Reagent	Final concentration	Amount
Tris pH 8 (1 M)	250 mM	250 µL
Glycerol (50% v/v)	25% v/v	500 mL
EDTA (0.5 M)	0.5 mM	1 µL
DTT (1 M)	1 mM	100 µL
PMSF (0.1 M)	1 mM	1 mL
Milli-Q H_2O	N/A	Up to 1 mL

Note: Avoid freeze/thaw cycles. Flash freeze in liquid nitrogen or ethanol/dry ice within small aliquots (100 µL aliquots). The buffer should be aliquoted and can be stored at −20°C.

Carbonate buffer (10×)		
Reagent	Final concentration	Amount
Na_2CO_3 (0.5 M)	N/A	70 mL
$NaHCO_3$ (0.5 M)	N/A	5 mL

Note: The buffer should be diluted to 50 mM (total carbonate concentration) with Milli-Q water before use.

NMR buffer		
Reagent	Final concentration	Amount
Tris pH 7.0 (1 M)	20 mM	2 mL
NaCl (5 M)	100 mM	2 mL
DTT (1 M)	3 mM	0.3 mL
Milli-Q H_2O	N/A	Up to 100 mL

Note: Filter using 0.22 µm filter. Add D_2O to a final concentration of ~10% (v/v) to the final NMR sample. The buffer can be stored at 4°C for up to 1 month.

Phosphate buffered saline (PBS, 1×, pH 7.4)		
Reagent	Final concentration	Amount
Sodium chloride (mw: 58.44 g/mol)	137.0 mM	8.0 g
Potassium Chloride (mw: 74.55 g/mol)	2.7 mM	0.2 g
Sodium Phosphate Dibasic (mw: 141.96 g/mol)	10.0 mM	1.44 g
Potassium Phosphate Monobasic (mw: 136.09 g/mol)	1.8 mM	0.245 g
Milli-Q H_2O	Up to 1,000 mL	N/A

Note: The buffer can be stored at 4°C for up to 1 year.

Paraformaldehyde (4%)		
Reagent	Final concentration	Amount
Paraformaldehyde	4%	4.0 g
1× PBS	1×	100 mL

Note: The procedure for preparing 4% Paraformaldehyde:

- In a beaker add 80 mL of 1× PBS.
- Add 4 g paraformaldehyde in the beaker.
- Place the beaker in a heating stirrer plate inside a chemical fume hood.
- Heat the mixture to 60°C, while stirring.
- Raise the pH of the mixture by adding 5 N NaOH dropwise to dissolve the paraformaldehyde completely.
- Cool the solution and filter using a 0.22 μm syringe.
- Adjust the volume up to 100 mL with 1× PBS.

STEP-BY-STEP METHOD DETAILS

Immunoprecipitation of acetylated SARS-CoV-2 E 3×FLAG

Timing: 1–2 days

1. Preparation of Whole Cell Extract (WCE):
   a. After 76 h of transfection, remove the DMEM medium by aspiration and wash 2 times with cold phosphate-buffered saline (PBS 1×).
   b. Scrape cells into fresh PBS 1× and pellet the cells by centrifuging 5 min at 300 × *g* at 4°C.
   c. Remove the supernatant, resuspend, and incubate in 500 μL of WCE buffer for 30 min on the wheel at 4°C (50 × *g*).
   d. Centrifuge WCE at 30,000 × *g* for 30 min at 4°C.
   e. Recover supernatant from step d above and add 500 μL of WCE buffer without NaCl in order to reduce NaCl concentration to 225 mM.
2. FLAG immunoprecipitations:
   a. Meantime, wash 50 μL FLAG M2 affinity agarose beads with 1 mL WCE (225 mM NaCl) on the wheel for 5 min, then:
      i. centrifuge at 1,000 × *g* for 3 min.
      ii. Remove supernatant by aspiration.
      iii. Resuspend in WCE (225 mM NaCl) buffer.
   b. Add 50 μL FLAG M2 beads and incubate 2 h at 4°C on a rotating wheel (LabRoller II, cat. H-5100) at 7 rpm.
   c. Centrifuge at 1,000 × *g* for 3 min and discard the supernatant.
   d. 3× Wash with 1 mL of WCE (225 mM NaCl) for 10 min on the wheel at 4°C.
   e. Centrifuge at 1,000 × *g* for 3 min and discard the supernatant.
   f. The immunoprecipitation was eluted in 45 μL of 1× Home-made Laemmli Buffer at 95°C for 10 min.
3. Anti-acetyl-lysine immunoprecipitations:
   a. Add 6 μg of anti-acetyl-lysine antibody and incubate 3 h at 4°C on a rotating wheel at 7 rpm.
   b. Add 50 μL of protein G-Sepharose beads and incubate 4 h at 4°C on a rotating wheel at 7 rpm.
   c. 3× Wash with 1 mL of WCE (225 mM NaCl) for 10 min on the wheel at 4°C.
   d. Centrifuge at 1,000 × *g* for 3 min and discard the supernatant.
   e. The immunoprecipitation was eluted in 45 μL of 1× Laemmli Buffer at 95°C for 10 min.

Western blot of FLAG and acetyl-lysine immunoprecipitations

Timing: 1–2 days

Note: 1% Input and 15 μL of immunoprecipitations was used for Western-blotting.

4. Western-Blot anti-BRD4:

a. Western-Blot of FLAG immunoprecipitations were incubated with anti-BRD4 antibody (Abcam ab46199) at a 1/1000 dilution at 4°C in 1% milk-TBS1×-Tween 0.1% with gentle agitation O/N.
b. 3× Wash with 1× TBS-Tween 0.1% during 10 min with agitation.
c. 1 h incubation with 2nd antibody conjugated to horseradish peroxidase in 1% milk-TBS1×-Tween 0.1%.
d. 3× Wash with 1× TBS-Tween 0.1% during 10 min with agitation.
e. The immunoblots were visualized using a Western Lightning plus ECL reagent.

5. Western-Blot anti-FLAG:
   a. Western-Blot of acetyl-lysine immunoprecipitations were incubated with anti-FLAG M2 conjugated to horseradish antibody (Sigma-Aldrich, Cat#A8592) at a 1/10000 dilution at 4°C in 1% milk-TBS1×-Tween 0.1% with gentle agitation O/N.
   b. 3× Wash with 1× TBS-Tween 0.1% during 10 min with agitation.
   c. The immunoblots were visualized using a Western Lightning plus ECL reagent.

Liquid *in vitro* HAT assay

Timing: 1 day

6. Liquid *in vitro* HAT assays were performed in a final volume of 15 µL using 0.5 µg of E peptide (aa 48–72 of E protein) or H1-depleted short oligonucleosomes (SON) from HeLa cells as substrate:

⚠ CRITICAL: The quality of E Peptide or short oligonucleosomes (SON) should be monitored by Coomassie staining before HAT assays.

   a. Mix free E peptide or short oligonucleosomes (SON) with recombinant p300 (0.1 µg for E peptide and 0.2 µg for SON) or 3 µL of purified native HBO1 or MYST complex, 3 µL of HAT buffer, 1 mM Na Butyrate. The concentration of this mix is adjusted between 50 mM and 100 mM of salt (KCL or NaCl).

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

   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 ($NaHCO_3$–Na_2CO_3, pH 9.2).
   g. Rinse rapidly with acetone and air dry the filter paper.
   h. Place the filter paper in scintillation tube.
   i. Add scintillation cocktail and measure counts in the scintillation counter (Beckman Coulter LS6500) for 30 min.

Mapping the binding interface by NMR chemical shift perturbations

Timing: 1 day

NMR chemical shifts are very sensitive to the chemical environment, which is altered upon the binding of a ligand to a protein. Depending on the binding strength, chemical shift changes or perturbations (CSPs) induced by the ligand fall into three regimes on the NMR time scale: slow exchange (strong interaction), fast exchange (weak interaction), or intermediate exchange (intermediate-strength interaction). Analysis of CSPs and mapping of the binding interfaces work best for the weak interactions, where chemical shifts can be tracked during titration of the ligand.

7. Open the Assign module of CCPN (version 3. For download and installation refer to https://ccpn.ac.uk/software/downloads/).
8. Open the recorded HSQC spectra of BRD4 ET (apo state) and BRD4 ET – E peptide complex by dragging and dropping the related processed HSQC "spectrum_name.ft2" files in the "Drop Area" of CCPN program. In this example, the spectra's names are "apo.ft2" and "complex.ft2" for the apo state and bound state, respectively.

   Note: After dropping the spectra in the "Dropping Area", spectra names are listed under "Spectrum" menu (on the left side of the program window) as "SP:spectrum_name".

   Note: If a spectrum is not displayed after dropping, drag the spectrum name ("SP:spectrum_name" under "Spectra" menu on the left side of the program window) and drop it in the "Dropping Area".

   Note: The spectrum display can be toggled on or off by clicking on the spectrum name in "SpectrumDisplay" module.

   Note: Spectra can be zoomed in or out by scrolling the middle click.

9. Spectrum properties including spectrum name, spectrum type, peak color, and first contour level can be adjusted by opening the "Spectrum Properties" (to open this module, double click on "SP:spectrum_name" under "Spectra" menu on the left side of the program window).
10. Download the chemical shift list for BRD4 ET (aa 601–683) from the Biological Magnetic Resonance Data Bank website (https://bmrb.io) in the NMR-STAR v3 file format. Here, the shift list with bmrb code bmr26041 was used.
11. Import the shift list into the CCPN program and simulate HSQC peaks:
    a. Click on "Project" in the menu bar, hover on "Import" until the pop-up menu appears then select "NMRStar File".
    b. In the "Import NMRStar File" pop-up window, navigate to the location where the NMR-STAR v3 file is saved, select the file, then click on "open".
    c. Check the "Simulate Peaks From Atoms" box to simulate the HSQC peaks and click on "Import". The imported chemical shift list is added under "ChemicalShiftLists" in the left side menu. The simulated.
12. Add the simulated HSQC peaks to the HSQC spectra:
    a. Open the "Peak Table" module by clicking on "View" from the main menu, then click on "Peak Table".
    b. In the "Peak Table" module select "PL:NH.1" from the "PeakList" drop-down menu.
    c. Select all peaks by simultaneously pressing ctrl+A (command+A in Mac).
    d. Right click anywhere on the selected peaks and click on "Copy Peaks…".
    e. In the "Copy peaks to PeakLists" pop-up window, select the desired peak lists from the "Select Destination PeakLists" section. Here, "PL:apo.1" and "PL:complex.1" peak lists were selected by pressing ctrl (command in Mac) and clicking on each desired peak list names.
    f. After selecting the desired peak lists, click "Copy" and then "Close" to close the pop-up window. Now the synthesized peaks can be seen in the related spectrum in "SpectrumDisplay" module.

    Note: Alternatively, peaks can be added to each spectrum by dragging the peak list from the sidebar (default → Spectra → SP:NH → PeakLists → PL:NH.1) and dropping it on the related spectrum in the "SpectrumDisplay" module. For this method, only the desired spectrum should be displayed (toggled on), and other spectra displays should be toggled off (cf. Notes in step 8).

13. Move each peak assignment to the relative peak center in each spectrum:

a. Toggle on one spectrum and toggle off the rest of the spectra.
b. Select one peak assignment by clicking on the peak assignment. Right click on the selected peak assignment and select "Snap Peak(s) to extrema" or use the "S,E" shortcut (press S then press E). If a peak cannot be assigned unequivocally, select the peak and delete it using Delete button (clear or fn+delete in Mac).
c. Repeat the process for all peaks in all spectra.

Note: If "S,E" fails to move the peak assignment peak extrema, drag the assignment by right-clicking (or middle-clicking) on the assignment and drop it near the peak center, then press "S,E".

Note: Shortcut "R,P" refits the selected peak with a gaussian fit. "R,G" refits a group of selected peaks.

14. Export the peak lists:
    a. Open the peak table by clicking on "View" in the main menu, then "Peak Table" (or use shortcut "P,T".
    b. Select the peak list of the apo spectrum (PL.apo.1) from the "PeakList" drop-down menu. If PL.apo.1 is empty, select peak list PL.apo.2.
    c. Right-click on one the column titles and select "Column Settings…"
    d. Check or uncheck to add or remove the columns that need be exported. At least "Assign F1" (^{1}H assignment), "Pos F1" (^{1}H chemical shift), and "Pos F2" (^{15}N chemical shift) are needed for calculating chemical shift perturbations. Click on "Close" after selecting the desired columns.
    e. Right-click anywhere on the peak table and select "Export Visible Table" to merely export the desired columns.
    f. Select the desired location, name, and format to export the peak table, then click "Save". Here, format xlsx was selected.

    Note: Reassure the name is accompanied by an extension such as .xlsx, otherwise table export may not be done properly.

    g. Select the peak list of the complex (PL:complex.1) and repeat steps 14.c through 14.f.
15. Calculate the chemical shift perturbations:
    a. Open the exported peak lists by a spreadsheet program such as Microsoft Office Excel.
    b. Copy columns containing ^{1}H and ^{15}N chemical shifts from complex peak list file and paste them in the apo peak list file, so that each assignment in each row contains the relative ^{1}H and ^{15}N chemical shifts for both apo state and the complex.
    c. Use the following equation to calculate chemical shift perturbations (CSP): $\Delta\delta = \sqrt{(\Delta\delta H)^2 + (\Delta\delta N/5)^2}$, where $\Delta\delta$ is the change in chemical shift in parts per million (ppm).
16. Calculate the average and standard deviation of the chemical shift perturbations.
17. Plot chemical shift perturbations against residue number as a bar plot.
18. Represent the sum of average and standard deviation line on the bar graph.
19. Residues with chemical shift perturbations of greater than the sum of average and standard deviation are the most significantly perturbed residues.

Note: Depending on the data, multiples or fractions of standard deviation can be used to determine the most perturbed residues.

Measurement of viral replication kinetics using qRT-PCR

Timing: 1 day

This step allows the quantification of SARS-CoV-2 genomic RNA present in the culture supernatant collected during the termination of the reaction. The SARS-CoV-2 viral load in the culture supernatant is determined using qRT-PCR. In this method, the culture supernatant is heat-inactivated and directly added to the qRT-PCR reaction mix to quantify viral load.

20. The composition of the reaction mix:

PCR reaction mix		
Reagent	Final concentration	Amount (μL)
PrimeDirect Probe RT-qPCR Mix (2×, Takara RR650A)	1×	12.5
P087 (Forward Primer)	20 picoMol	0.5
P088 (Reverse Primer)	20 picoMol	0.5
P089 (Probe)	10 picoMol	0.5
Reference dye (ROX)	0.2 ng/μL	0.25
Nuclease free water	N/A	8.25
Template (culture supernatant)	N/A	2.5
Total volume	N/A	25

21. The thermal cycling parameter of the reaction:

PCR cycling conditions			
Steps	Temperature	Time	Cycles
Initial Denaturation	95°C	5 min	1
Revers Transcription	60°C	5 min	1
Denaturation	95°C	5 s	40 cycles
Annealing/Extension	55°C	30 s	

22. Heat inactivation of culture supernatant:
    a. Centrifuge the tubes having culture supernatant for a brief spin to ensure all contents are at bottom of the tube.
    b. Pre-heat a heat block to 65°C.
    c. Once temperature is reached, incubate the samples for 30 min at 65°C.
23. The qRT-PCR is performed using a set of primers/probes targeting the E gene of SARS-CoV-2 (P087, P088 and P089). The primer sequences are included in the reagents table.
24. Add the master mix and culture supernatant in a 96 well reaction plate and load the plate in Applied Bio- systems QuantStudio3 real-time PCR system (Applied Biosystems, Waltham, MA, USA).
25. The SARS-CoV-2 genome equivalent copies are calculated using quantitative PCR control RNA from heat-inactivated SARS-CoV-2, isolate USA-WA1/2020 (BEI, Catalog# NR-52347).
26. The percent inhibition of SARS-CoV-2 replication by JQ1 and OTX015 treatment is measured based on viral concentration in positive control wells (considered 0% inhibition) and negative control wells (uninfected cells).
27. IC_{50} values were calculated using four-parameter variable slope sigmoidal dose-response models using Graph Pad Prism 9.0 software.

Immunofluorescence

Timing: 2 days

28. Seed the Calu-3 cells in black clear bottom 96 well plates, infect them with SARS-CoV-2 and terminate the assay as described above.
29. At the end of the viral infection reaction, fix the infected Calu-3 cells with the 4% PFA.
30. Then wash the cells three times with 1× PBS.
31. Permeabilize the cells by adding 50 μL of 0.1% Triton X-100 and block them using 3% bovine serum albumin in phosphate-buffered saline for 2 h.
32. Next, add 50 μL of the primary antibody against the Spike glycoprotein of SARS-CoV-2 (Sino biological, Cat # MA14AP0204) at a 1:1,000 dilution in the fixed cells and incubate them overnight at 4°C on an orbital shaker at 80 RPM.
33. Next day, wash the cells three times with 1× PBS, and add 50 μL of secondary antibody Alexa Fluor 488 Goat anti-rabbit (Thermo Fisher, Cat # A-11034) at a 1:2,000 dilution.
34. Then incubate the cells for 1 h in the dark at room temperature on a shaker.
35. After that, wash them once with 1× PBS, and stain them with Hoechst 33258 (Invitrogen, Cat #H3570) and Cell Mask (Invitrogen, Cat #C10046) to visualize the nucleus and plasma membrane.
36. Capture the Images using a high content analysis system at 20× air on an Operetta CLS and analyze the data using the Harmony analysis software integrated within the Operetta CLS analyzer.

EXPECTED OUTCOMES

The recognition of human transcriptional co-activator BRD4 by either unmodified or acetylated SARS-CoV-2 E protein provides an example of how fundamental host cell signaling programs can be hijacked by viral proteins. While this methodology was developed to study the BRD4-E complex formation, it can be broadly used to examine molecular mechanisms underlying the association of other viral proteins with human proteins.

QUANTIFICATION AND STATISTICAL ANALYSIS

The HAT assays were performed as three independent replicates. Percent inhibition of SARS-CoV-2 infection in Calu-3 cells treated with inhibitors, cell viability, and inhibition of SARS-CoV-2 replication assays were performed as three independent replicates. IC_{50} values were calculated using four-parameter variable slope sigmoidal dose-response models using Graph Pad Prism 9.0 software.

LIMITATIONS

While the application of biochemical and molecular biology methods, including immunoprecipitation, Western blotting and NMR CSPs analysis are suitable for the characterization of soluble proteins, this protocol may not work well for unstable or insoluble proteins. An E peptide was used as a substrate in HAT assays; however, these assays cannot provide information on which lysine residue (K53 and/or K63) is acetylated. Immunoprecipitation with anti-acetyl-lysine antibodies on whole cell extracts from cells expressing FLAG-tagged SARS-CoV-2 E protein also does not identify which lysine residue, or both are acetylated. Likewise, Western blot with anti BRD4 antibodies does not specify by which mechanism BRD4 and the SARS-CoV-2 E protein interact. NMR titration experiments and CSPs analysis can only be applied to relatively small proteins (MW <35 KDa). Due to chemical shift overlap, it is also challenging to analyze NMR spectra of unfolded proteins.

TROUBLESHOOTING

Problem 1

Cells are over-fixed in immunofluorescence.

Potential solution

Lower the time of fixation of the cells gradually using positive controls to optimize the fixation time.

Problem 2
No amplification in real-time PCR samples.

Potential solution
Check in the instrument setting that proper thermal cycling conditions and data collection stage is selected or not. Also include a positive control in the run, which should be shown as an amplification curve. If no amplification is obtained in the positive control sample, change all the reagents including master mix and primer/probes, and repeat the reaction.

Problem 3
PCR on gBlock did not work due to the nicks in sequence.

Potential solution
Clone first gBlock directly into the vector.

Problem 4
The small SARS-CoV-2E 3×FLAG protein is not observed in WCE after transfection.

Potential solution
Use a high percentage gel to observe low molecular weight proteins before transfer.

Problem 5
CPM observed in liquid HAT assays is lower than 1000 CPM.

Potential solution
The salt concentration might be too high. Adjust salt concentration to 50–100 mM or increase amount of the HAT complex up to 20% (v/v). Incubation time can be increased to 60 min.

Problem 6
CPM observed in liquid HAT assays is higher than 5000 CPM. High CPM level is measured on the control (autoacetylation).

Potential solution
Decrease amount of the acetyltransferase or the HAT complex to ensure counts are linked to acetylation of the substrate.

Problem 7
The signal-to-noise ratio in NMR spectra is low.

Potential solution
Increase concentration of the ^{15}N-labelled protein or increase time of the experiment. Produce the protein of interest in a deuterated form. Record ^{1}H,^{15}N TROSY experiments instead of ^{1}H,^{15}N HSQC and use Shigemi tubes, which require small volume (200–250 μL) of the sample, for data collection.

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
The software used in this study has been published and is detailed in the key resources table.

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AUTHOR CONTRIBUTIONS

All authors contributed to writing the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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