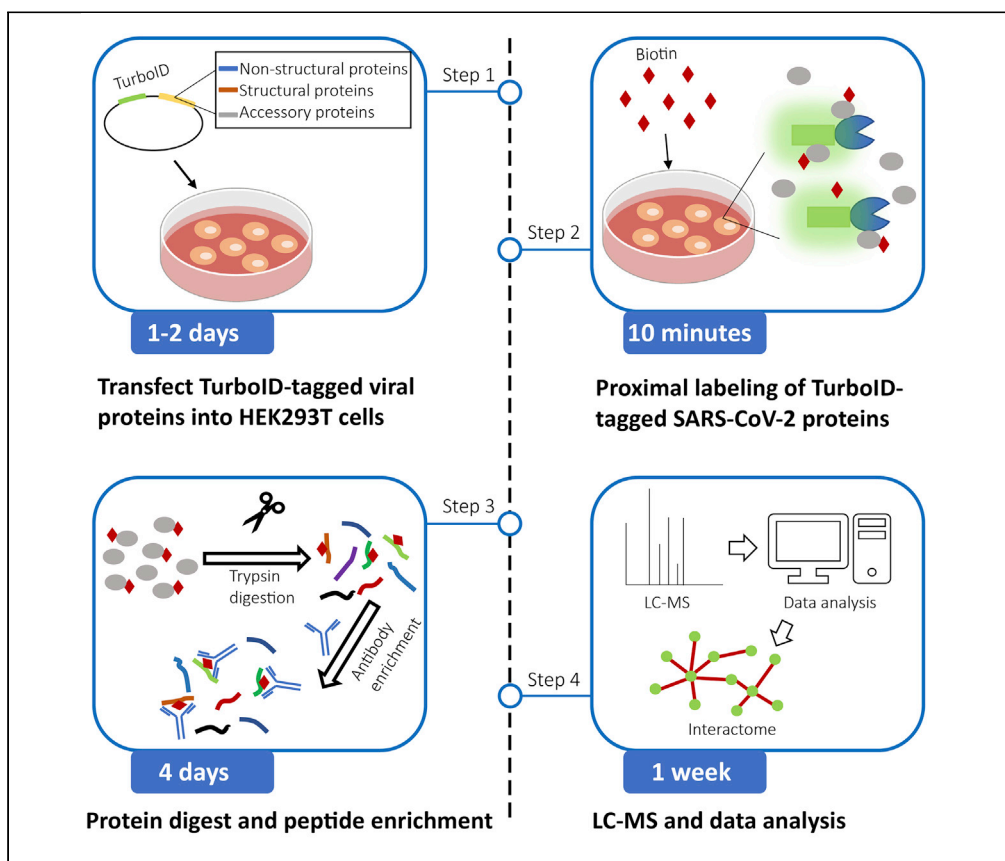# STAR Protocols

Protocol

An antibody-based proximity labeling protocol to identify biotinylated interactors of SARS-CoV-2

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Highlights

Proximity labeling to
identify high-
confidence SARS-
CoV-2 interactors

Enrichment of
biotinylated proteins
by TurboID using
anti-biotin antibody

A stringent
proteomics workflow
to filter non-specific
co-purified proteins

Protocol can be
applied to other viral-
host protein
interaction studies

Elucidating the molecular interactions between virus and host is fundamental to understanding the mechanism of viral pathogenesis. Here, we present a protocol to screen SARS-CoV-2 protein interactors using an antibody-based TurboID proximity labeling approach. This technique directly identifies biotinylated peptides labeled by the TurboID-tagged viral proteins. We describe the steps to prepare biotinylated peptide samples for mass spectrometry analysis and a stringent workflow to identify biotinylated high-confidence interactors of the virus by filtering out non-specific co-purified proteins.

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

STAR Protocols

Protocol

An antibody-based proximity labeling protocol to identify biotinylated interactors of SARS-CoV-2

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https://doi.org/10.1016/j.xpro.2022.101406

SUMMARY

Elucidating the molecular interactions between virus and host is fundamental to understanding the mechanism of viral pathogenesis. Here, we present a protocol to screen SARS-CoV-2 protein interactors using an antibody-based TurboID proximity labeling approach. This technique directly identifies biotinylated peptides labeled by the TurboID-tagged viral proteins. We describe the steps to prepare biotinylated peptide samples for mass spectrometry analysis and a stringent workflow to identify biotinylated high-confidence interactors of the virus by filtering out non-specific co-purified proteins.
For complete details on the use and execution of this protocol, please refer to Zhang et al. (2022).

BEFORE YOU BEGIN

The interaction of viral and host factors is essential for the viral life cycle during its infection. Owing to the complexity of virus-host interactions, molecular mechanisms of viral pathogenesis are poorly understood. Thus, depicting the virus-host interactome is necessary to reveal viral protein functions during infection. Recently, BioID-based proximal proteins of SARS-CoV-2 have been investigated by a streptavidin enrichment strategy (Laurent et al., 2020; Samavarchi-Tehrani et al., 2020; St-Germain et al., 2020). However, due to the strong binding affinity of streptavidin and biotin, the biotinylated peptides are difficult to be identified, which is critical for evaluating the confidence of proximity interactions. To overcome this limitation, we used biotin specific antibody to enrich proximal proteins with biotinylated peptides labeled by TurboID-tagged viral proteins (Kim et al., 2018; Udeshi et al., 2017). Through a stringent data analysis process, these significantly enriched proteins with biotinylated sites in sample groups are regarded as high-confidence interactors of SARS-CoV-2 proteins.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Myc-HRP	Thermo Fisher Scientific	Cat# R95125
Biotin Antibody Agarose	ImmuneChem	Cat# ICP0615

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
DMEM	Cell World	Cat# C0162-811
Fetal Bovine Serum	Biological Industries	Cat# 04-001-1A
Penicillin-Streptomycin Solution	Cell World	Cat# C0160-611
Tris base	Sigma-Aldrich	Cat# T6687
Sodium deoxycholate	Sigma-Aldrich	Cat# D6750
Protease Inhibitor Cocktail	MedChemExpress	Cat# HY-K0010
Streptavidin-HRP	Thermo Fisher Scientific	Cat# 21130
Bovine Serum Albumin	Sigma-Aldrich	Cat# A1933
Triton X-100	Sigma-Aldrich	Cat# T8787
Dithiothreitol	Sigma-Aldrich	Cat# D9163
Iodoacetamide	Sigma-Aldrich	Cat# I1149
Urea	Sigma-Aldrich	Cat# U5128
Triethylammonium bicarbonate buffer	Sigma-Aldrich	Cat# T7408
Trypsin	Promega	Cat# V5111
MOPS	Sigma-Aldrich	Cat# 69947
Acetonitrile	Sigma-Aldrich	Cat# 34851
Trifluoroacetic acid	Acros Organics	Cat# 139721000
Water	Sigma-Aldrich	Cat# 34877
Formic acid	Sigma-Aldrich	Cat# 695076
Critical commercial assays		
Seamless Cloning Kit	Beyotime Biotechnology	Cat# D7010M
Polyethylenimine	Polysciences	Cat# 23966
Lipofectamine 2000	Thermo Fisher Scientific	Cat# 11668027
TurboFect	Thermo Fisher Scientific	Cat# R0531
Chemiluminescent Substrate	Thermo Fisher Scientific	Cat# 34580
2×SDS-PAGE loading buffer	Solarbio	Cat# P1018
Precast SDS-PAGE Gel	Solarbio	Cat# PG01015-S
BioTrace NT Nitrocellulose Transfer Membrane	PALL	Cat# 66485
BCA Protein Assay Kit	Beyotime	Cat# P0012
Centrifugal Filter Unit	Millipore	Cat# UFC5010BK
Matrix Active Group C18	Sigma-Aldrich	Cat# 66883-U
Deposited data		
A proximity labeling map of SARS-CoV-2 and human	http://www.proteomexchange.org	http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD022086
Experimental models: Cell lines		
HEK293T	ATCC	CRL-11268
Oligonucleotides		
TurboID-F: gggattgataaacagggagcc	General Biol	N/A
T7-F: taatacgactcactatagg	General Biol	N/A
pCAG-F: gctaaccatgttcatgccttct	General Biol	N/A
Recombinant DNA		
pcDNA3.1-myc-TurboID	This study	N/A
pcDNA6B-TurboID-myc	This study	N/A
pCAG-TurboID-myc	This study	N/A
Software and algorithms		
Mascot	Matrix Science	http://www.matrixscience.com/
SAINTexpress	(Teo et al., 2014)	https://sourceforge.net/projects/saint-apms/files/
Pepdistiller	(Li et al., 2012)	http://www.bprc.ac.cn/pepdistiller
PANDA	(Chang et al., 2019)	https://sourceforge.net/projects/panda-tools/

MATERIALS AND EQUIPMENT

RIPA lysis buffer

Reagent	Stock concentration	Final concentration	Amount
Tris pH 8	1 M	50 mM	2.5 mL
NaCl	5 M	150 mM	1.5 mL
EDTA	0.5 M	5 mM	0.1 mL
SDS	20%	0.2%	0.1 mL
Sodium deoxycholate	n/a	0.5%	250 mg
Triton X-100	100%	1%	0.5 mL
Water	n/a	n/a	Up to 50 mL
Total			50 mL

Note: Store at 4°C for up to a year.

BSA blocking buffer

Reagent	Stock concentration	Final concentration	Amount
Bovine Serum Albumin	n/a	1% (w/v)	500 mg
Triton X-100	100%	0.2%(v/v)	0.1 mL
PBS	n/a	n/a	Up to 50 mL
Total			50 mL

Note: Prepare the buffer before use.

Urea buffer

Reagent	Stock concentration	Final concentration	Amount
Urea	n/a	8 M	24 g
Tris-HCl	1 M	0.1 M	Up to 50 mL
Total			50 mL

Note: Store at 4°C for up to a year.

Iodoacetamide solution

Reagent	Stock concentration	Final concentration	Amount
Iodoacetamide	n/a	50 mM	18.5 mg
Urea buffer	n/a	n/a	Up to 2 mL
Total			2 mL

Note: Prepare the buffer before use.

IAP buffer

Reagent	Stock concentration	Final concentration	Amount
MOPS pH 7.2	n/a	50 mM	314 mg
Na$_3$PO$_4$	n/a	10 mM	49.2 mg
NaCl	n/a	50 mM	87.7 mg
Water	n/a	n/a	Up to 30 mL
Total			30 mL

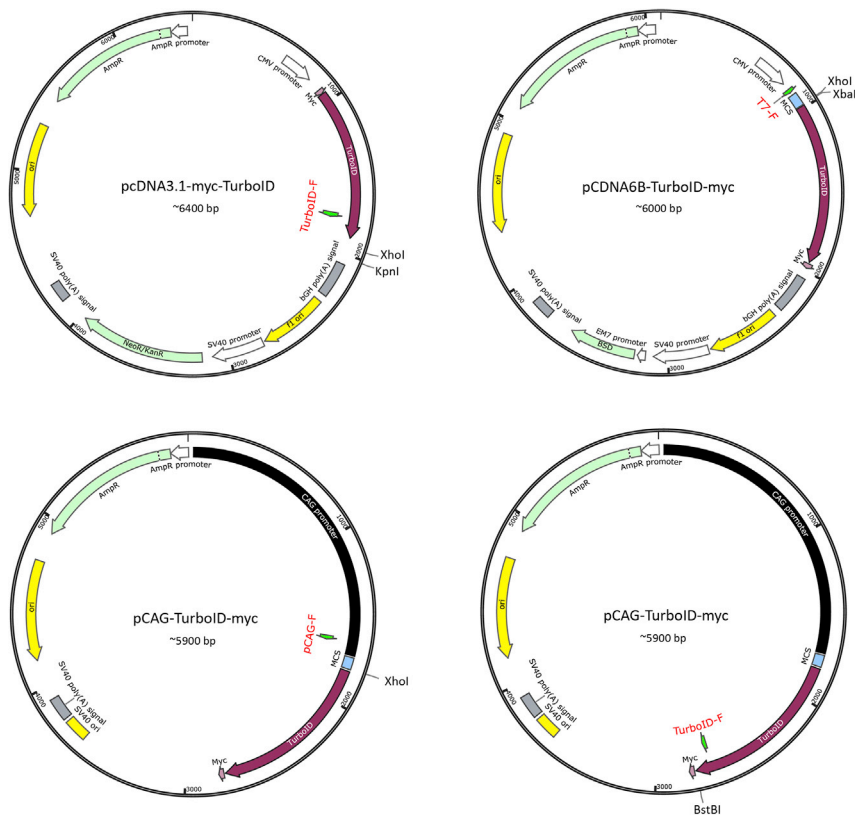*Note:* Store at 4°C for up to a year.

Figure 1. Construction of TurboID-tagged SARS-CoV-2 expression vectors
Schematic illustration of TurboID-tagged viral proteins with different promoters and fusion patterns. The sequencing primers are indicated as red font. The SARS-CoV-2 protein encoding sequencings were homologous recombined with the linearized vectors digested by the corresponding endonucleases listed in Table 1.

STEP-BY-STEP METHOD DETAILS

Construction of viral protein expression vectors

⏲ Timing: 6 weeks

To express TurboID-tagged viral proteins, SARS-CoV-2 protein coding sequences were integrated into TurboID expression vectors and their expressions were validated.

1. Introduce the synthesized ORF encoding sequences of SARS-CoV-2 (GenBank: NC_045512.2) into pcDNA3.1-myc-TurboID, pcDNA6B-TurboID-myc or pCAG-TurboID-myc vector by homologous recombination.
   a. Linearize the vectors by the indicated restriction endonucleases, and SARS-CoV-2 ORFs are integrated into the vectors using a Seamless Cloning Kit (Beyotime Biotechnology, China) (Figure 1).
   b. For the details of designing primers, follow the protocol provided by the manufacture (https://www.beyotime.com/product/D7010M.htm).
2. Verify the cDNA sequences of viral genes are in frame with the TurboID tag without any mutations by DNA sequencing.
   a. The details of vector construction were listed in Table 1.
   b. Use the sequencing primers listed in the key resources table for sequencing the integration of viral ORFs.

Table 1. Construction of TurboID-tagged SARS-CoV-2 expression vectors

Vector	Digested endonucleases	Protein fusion pattern
pcDNA3.1-myc-TurboID	Xho I/Kpn I	Myc-TurboID-SARS-CoV-2
pcDNA6B-TurboID-myc	Xho I/Xba I	SARS-CoV-2-TurboID-Myc
pCAG-TurboID-myc	Xho I	SARS-CoV-2-TurboID-Myc
pCAG-TurboID-myc	BstB I	TurboID- SARS-CoV-2-Myc

Note: All of the constructs are available from the lead contact upon request.

3. Transfect the constructs into HEK293T cells to validate their expression before proximity labeling (Figure 2A).
   a. HEK293T cells were cultured in DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin and maintained in a humidified incubator at 37°C with 5% CO_2.
   b. Approximately 2×10^5 HEK293T cells were seeded into each well of 24-well plate in 1 mL culture medium the day before transfection.
   c. For each well, mix 1 μg plasmid with 5 μL Polyetherimide (PEI, 1 μg/mL) at a 1:5 (M/V) ratio in 100 μL serum-free DMEM medium, incubate the mixture for 20 min at room temperature (RT; 18°C–23°C). Troubleshooting 1.

   Alternatives: Transfection reagent can be replaced by Lipofectamine 2000 or Turbofect listed in the key resources table.

   d. Add the transfection mixture into each well and incubate the plate at 37°C with 5% CO_2 incubator.
   e. 24–48 h after transfection, cell lysis was analyzed by western blot as described below. For the detection of plasmids expression, the nitrocellulose membrane was blocked by 5% milk, followed by incubation with Myc-HRP (with a dilution ratio of 1:1000) antibody and visualized with ECL. Troubleshooting 2.

   Note: For the specificity of proximal labeling interactors of viral proteins, an appropriate control is required. In this protocol, we used TurboID protein expressed by pcDNA3.1-myc-TurboID vector to enrich non-specific binding partners of proximity proteome, which facilitates the identification of high-confident interactors of the virus.

Proximity labeling by TurboID-tagged viral proteins

⏱ Timing: 1 week

TurboID-tagged viral expression vectors were transfected into HEK293T cells to biotinylate proximal proteins.

4. Approximately 5×10^6 HEK293T cells were seeded on each of two 10 cm plates in 10 mL DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin the day before transfection.
5. For each labeling assay, mix 14 μg TurboID tagged viral protein vectors with 70 μL PEI in 1,400 μL serum-free DMEM medium, followed by incubation for 20 min at RT and introduce the mixture into two plates of HEK293T cells at the confluence of 80%.
6. 24 h after transfection, remove the culture medium and change for DMEM with 10% FBS and 50 μM biotin and incubate the plates at 37°C with 5% CO_2 incubator for 10 min.

   ⚠ CRITICAL: To prevent cell loss, pay extra cautions when changing culture medium, since HEK293T cells are easily detached from plates.
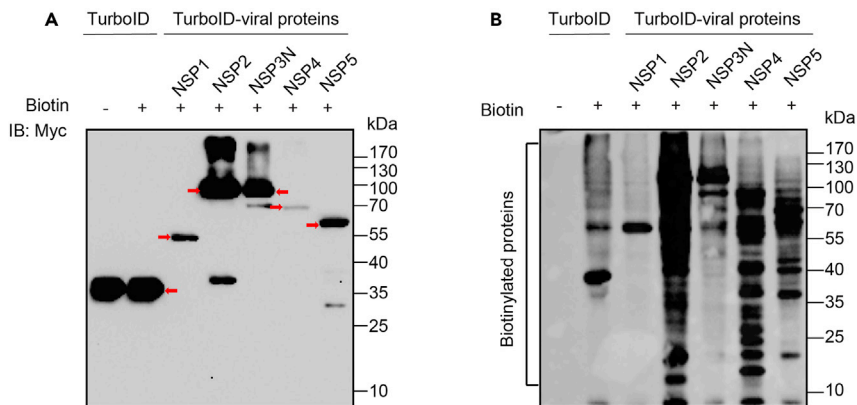
Figure 2. Proximity labeling by TurboID-tagged SARS-CoV-2 proteins
Representative detection of 5 out 29 TurboID-tagged viral proteins and their proximity labeling proteins.
(A) The expression of TurboID-tagged viral proteins was detected by anti-Myc-HRP antibody. The target proteins were indicated by the red arrow.
(B) The biotinylated proteins by TurboID or TurboID-tagged viral proteins were detected by streptavidin-HRP (Strep) after incubation with biotin.

7. Remove the medium and gently resuspend the cells with 5 mL cold PBS for each plate and centrifuge the cells with Beckman Allegra X-15R by using a SX4750A rotor at 200 × g for 3 min at 4°C. Wash the cell pellets with 1 mL cold PBS twice.
8. Resuspend the cells with 600 μL RIPA lysis buffer supplemented with protease inhibitor cocktail for each plate.
9. Sonicate the resuspended cells on ice (30 W, 3 min, with a 4 s pulse on, 4 s pulse off) using ultrasonic homogenizer (Scientz-IID).

    Note: The power capacity and pulse time of the sonication may be adjusted until the cell lysis becomes clear. Keep the cell lysis on ice during sonication.

    ⌷⌷ **Pause point:** The cell lysis can stay up to a month at −80°C.

10. Centrifuge the cell lysis from two plates with Eppendorf centrifuge 5417R by using a F45-30-11 rotor at 16,500 × g for 10 min at 4°C, combine the supernatant into a new 1.5 mL Eppendorf tube.
11. Combine 30 μL cell lysis with 30 μL of 2 × SDS-PAGE loading buffer and boil it at 95°C for 10 min. Load 5 μL sample on a 10% SDS-PAGE gel for protein separation.
12. Transfer the proteins to the nitrocellulose transfer membrane, block the membrane by using BSA blocking buffer on a shaker at 40 rpm for 30 min at RT.
13. Incubate the membrane with streptavidin-HRP diluted in BSA blocking buffer (with a dilution ratio of 1:40000) for 40 min at RT on a shaker.

    ⚠ CRITICAL: The BSA blocking buffer and streptavidin-HRP should be prepared freshly.

14. Wash the membrane with PBST (PBS with 0.1% Tween 20) on a shaker at 70 rpm for 10 min, repeat this step twice to remove unbound streptavidin-HRP.
15. Add the chemiluminescent substrate (ECL) to the membrane for the detection of biotinylated proteins (Figure 2B). Troubleshooting 3 and 4.
16. Determine the protein concentration by the BCA assay. Generally, a 10 cm plate of cells can produce about 3 mg protein after lysis.

    Note: Three biological replicates were carried out by using 2 mg protein from each replicate.

Protein digestion by filter aided sample preparation (FASP)

Ⓣ Timing: 2 days

Cell lysis proteins were reduced, alkylated and digested in Centrifugal Filter Units. The digested peptide mixtures were collected and dried.

17. For each sample, reduction was carried out by adding 12 μL dithiothreitol (DTT, 1 M) into 600 μL cell lysis that contains 2 mg protein with a final concentration of 20 mM, then incubating for 30 min at 56°C.

    Note: The dithiothreitol solution should be prepared freshly.

18. Transfer 600 μL lysis buffer into 2 Amicon Ultra-0.5 Centrifugal Filter Units equally.
19. Add 200 μL urea buffer into the filter, centrifuge it by Eppendorf centrifuge 5417R with a F45-30-11 rotor at 14,000 × g for 15 min at RT, discard the flow-through. Repeat this step.
20. Add 100 μL iodoacetamide solution (IAA) into the filter, gently pipetting the mixture and keep away from light for 30 min and centrifuge at 14,000 × g for 10 min.

    Note: The iodoacetamide solution should be prepared freshly and avoided from light.

21. Add 200 μL urea buffer into the filter, centrifuge at 14,000 × g for 15 min. Repeat this step and discard the flow-through.
22. Add 200 μL 50 mM of triethylammonium bicarbonate buffer (TEABC) into the filter, centrifuge at 14,000 × g for 15 min. Repeat this step.
23. Transfer the filter into a new 1.5 mL Eppendorf tube.
24. Add 100 μL 50 mM of TEABC and 20 μg trypsin into the filter, gently mixed and incubate at 37°C overnight (16 h). Wrap the filter unit with parafilm to avoid sample evaporation.
25. After trypsin digestion, add 100 μL water and centrifuge the tube at 14,000 × g for 15 min. Repeat this step and save the elution.
26. Dry the elution in a vacuum centrifuge (Eppendorf Concentrator plus) at 45°C for 2 h.

    ⫿⫿ Pause point: The peptide samples can stay up to 24 hours at 4°C or at −80°C within a month.

    Note: A schematic overview was illustrated for the clarity of the FASP steps (Figure 3).

    ⚠ CRITICAL: Owing to the high concentration of TEABC, do not dry up the elution, leave about 20 μL sample in the tube to prevent sample spurting out.

Peptide enrichment and desalt

Ⓣ Timing: 2 days

Digested peptides were enriched by anti-biotin antibody, the eluted peptides were desalted by StageTips and dried by vacuum centrifugation.

27. Use 1 mL IAP buffer to pipette the dried peptides up and down at RT until they dissolved.
28. Wash 100 μg biotin antibody conjugated beads with 500 μL IAP buffer, centrifuge at 1,000 × g for 1 min at 4°C, discard the supernatant. Repeat this step twice.

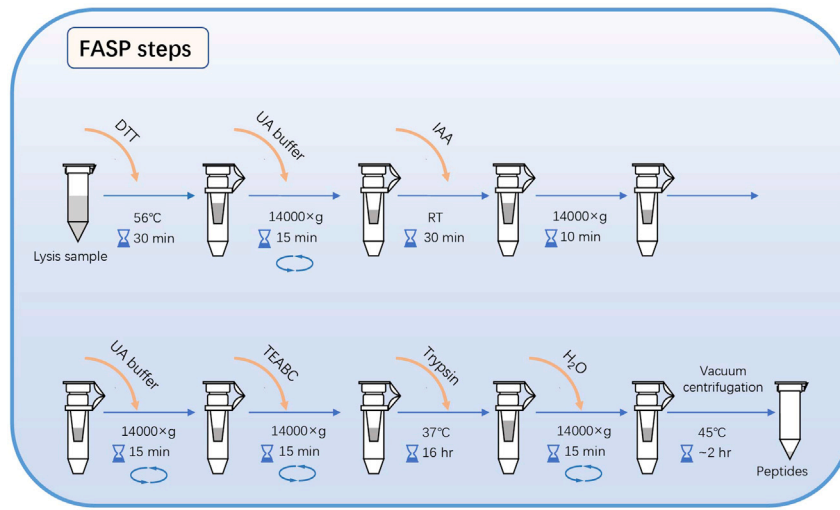   *Note:* The ratio of digested proteins with biotin antibody was set to 20:1.

Figure 3. A step-by-step illustration of protein digestion by filter aided sample preparation (FASP)

29. Combine the dissolved peptides with the washed beads and rotate the mixture at 4°C overnight (16 h) to enrich the biotinylated peptides.
30. Centrifuge the tubes at 1,000 × g for 1 min at 4°C, discard the supernatant.
31. Wash the beads with PBS at 4°C with rotation for 8 min and centrifuge the tubes at 1,000 × g for 1 min, discard the supernatant. Repeat this step three times.
32. Wash the beads with 50 μL 0.15% trifluoroacetic acid and centrifuge at 1,000 × g for 1 min at 4°C, collect the elution into a new tube. Repeat this step.

   ⧗⧗ **Pause point:** The samples can stay up to 24 hours at 4°C or at −80°C within a month.

   ⚠ CRITICAL: When using trifluoroacetic acid, work under a chemical fume hood with protective gloves and eye protection.

33. Load the two-layer C18 StageTip (AXYT400 pipette tips filled with C18) onto a 1.5 mL Eppendorf tube, add 100 μL acetonitrile into the C18 StageTip, centrifuge the tube at 200 × g for 3 min at RT, discard the flow-through.
34. Add 100 μL 50% acetonitrile into the C18 StageTip, centrifuge at 200 × g for 3 min at RT, discard the flow-through.
35. Add 100 μL 0.1% trifluoroacetic acid into the C18 StageTip, centrifuge at 200 × g for 3 min at RT, discard the flow-through. Repeat this step.
36. Load the elution from step 32 onto the C18 StageTip, centrifuge at 200 × g for 3 min, discard the flow-through.
37. Add 100 μL 0.1% trifluoroacetic acid into the C18 StageTip, centrifuge at 200 × g for 3 min at RT, discard the flow-through. Repeat this step.
38. Transfer the C18 StageTip into a new tube, add 50 μL 50% acetonitrile with 0.1% trifluoroacetic acid, centrifuge at 200 × g for 3 min at RT. Repeat this step and save the elution.
39. Dry the elution in a vacuum centrifuge at 45°C.

   ⧗⧗ **Pause point:** The samples can stay up to a week at 4°C or at −80°C within a month.

Mass spectrometry and data analysis

   ⏱ **Timing: 1 week**

Table 2. Mascot parameter settings for analysis of MS data

Parameter	Value
Fixed modification	Carbamidomethyl (C)
Variable modifications	Biotinylation of lysine
	Acetyl (N-terminus)
Maximum of missed cleavages	3
The peptide charge	2+, 3+ and 4+
Peptide error tolerance	10 ppm
MS/MS error tolerance	0.02 Da

40. Dissolve the peptide with 8 μL 0.1% formic acid (FA) and analyze by liquid chromatography-tandem mass spectrometry (LC-MS/MS). In our experiments, the peptides were analyzed by a Q-Exactive HF-X coupled to an UltiMat 3000 RSLCnano system using an 88 min method with a linear gradient of solvent A (0.1% FA) and solvent B (0.1% FA and 80% acetonitrile). For the separation of the peptide, the flow rate for the first 10 min was 300 nL/min and 600 nL/min for the rest 78 min, gradient elution was set by increasing solvent B from 6% to 40% in 80 min and maintained 95% for 5 min, then move back to 6% for 3 min.

41. Search the MS data by Mascot software, using the reviewed Uniprot human database, including SARS-CoV-2 proteins with TurboID tag. The parameters are listed in Table 2. The qualification and the quantification of peptides and proteins were analyzed by Pepdistiller and PANDA with a false discovery rate of 0.01, respectively. Troubleshooting 5.

42. To identify high-confidence SARS-CoV-2 protein proximal interactors, we used SAINTexpress to score the identified interactors, these with a cut-off value $\geq$ 0.6 were further ranked by occurrence, and the top 5th percentile proteins that regarded as non-specific contaminations were removed. The proteins from sample were compared with control by using biotinylated site numbers with a ratio > 2 as the cutoff value. Filtered proteins were regarded as proximity interactors of SARS-CoV-2 proteins.

EXPECTED OUTCOMES

The expression of TurboID-tagged SARS-Cov-2 proteins and their abilities to label proximal interactors enable the comprehensive interactome study of viral proteins and host factors. This protocol used an anti-biotin antibody to capture proximal proteins with biotinylated sites, which facilitates identification of 1388 high-confidence proximal interactors of SARS-CoV-2 proteins (Figure 4), among them 1092 proteins were not covered by streptavidin-based BioID assay in SARS-CoV-2 interactome research, indicating the advantages of antibody-based TurboID technique in identifying proximal interactors. The generated dataset is useful in revealing viral pathogenesis and drug development against SARS-CoV-2.

QUANTIFICATION AND STATISTICAL ANALYSIS

1. The spectral count of proximal proteins identified by each bait derived from the protein group data of Mascot were imported into ".dat" file according to the format of SAINTexpress. After SAINTexpress calculation, AvgP (SAINT Score) of each proximal protein was extracted from the result file "list.txt", and the cut-off value was set as 0.6.

2. Then we perform a further removal for three possible non-specific binding protein groups: top 5% of all identified proteins, top 5% of all biotinylated proteins and top 5% CRAPOME (https://reprint-apms.org/) curated proteins ranked by occurrence. We strongly recommend using this artificial exclusion step, because these three protein groups have a certain overlap ratio and are mainly enriched in histones, heat shock proteins and ribosomal proteins which always were regarded as non-specific co-purified proteins.

3. Then the biotinylated site numbers from sample and control groups were compared, and these proteins with a cutoff value greater than 2 are considered to be the reliable interactors.
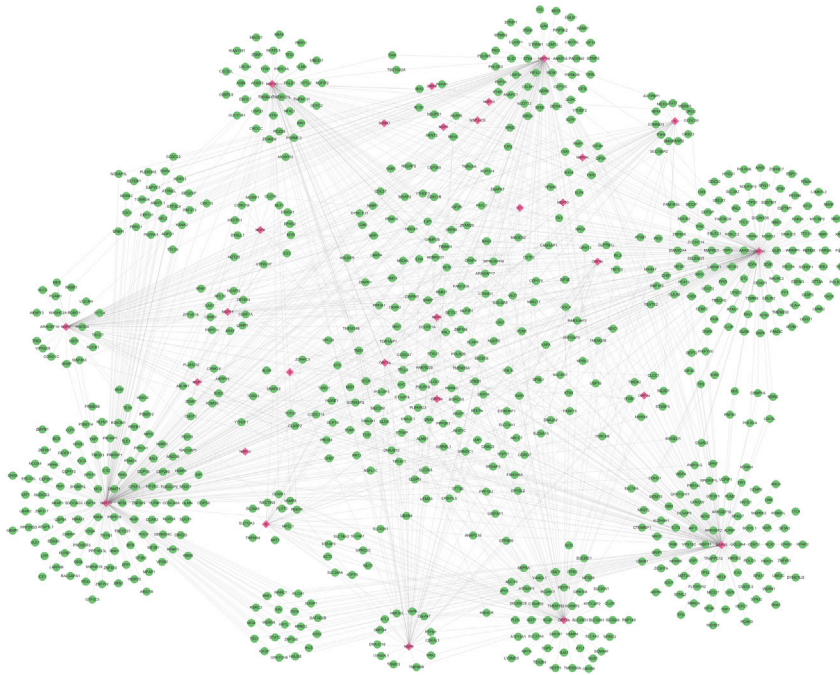
Figure 4. Proximity labeling map of SARS-CoV-2 protein with host factors
1388 high-confidence interactions were identified between viral proteins and host factors using TurboID-based proximity labeling. Green circle nodes represent host factors and red diamond nodes represent viral proteins.

LIMITATIONS

The viral-host interactome generated by overexpression of TurboID-tagged SARS-CoV-2 proteins in mammalian cells might not represent authentic virus infections, and the TurboID tag may hamper viral proteins interact with host factors (Firat-Karalar et al., 2014; Van Itallie et al., 2013). Proximal labeled proteins identified using antibody-based TurboID need to be further validated by orthogonal methods to prove their direct physical interactions with the viral proteins.

TROUBLESHOOTING

Problem 1
Low transfection efficiency of TurboID-fusion expression vectors (step 3).

Potential solution
The aliquoted PEI stored at −20°C should be fully dissolved at RT, insoluble matter can be heated at 65°C for about 30 min until the solution becomes clear, avoid freeze-thaw cycles of PEI.

Problem 2
The expression of TurboID-tagged viral proteins cannot be detected by Western blot (step 3).

Potential solution
Replace CMV promoter of the vector with CAG to enhance their expression. Optimize viral ORF cDNA codons for the eukaryotic expression vector. Alternate the orientations of TurboID and viral proteins.

Problem 3
Poor expression of biotinylated proteins detected by western blot (step 15).

Potential solution

For the high-molecular-weight viral proteins, such as NSP3C and S, harvest cells 48 h after transfection for their higher expression level so that their proximal proteins can be biotinylated and detected.

Problem 4

Some biotinylated proteins were too weak to be identified with streptavidin-HRP by western blot (step 15).

Potential solution

Some TurboID-tagged viral proteins interact with less proximal proteins than others and their biotinylation signals are also weaker. Therefore, always load positive (TurboID with biotin) and negative (TurboID without biotin) controls with samples on the same membrane to justify sample biotinylation as shown in Figure 2.

Problem 5

Biotinylated proteins were detected well, but few proteins were identified by LC-MS/MS (step 42).

Potential solution

This may due to the protein loss during the beads wash in steps 30–32. Avoid pipetting up and down many times to prevent beads binding to the pipette tips. Carefully aspirate the supernatant without aspirating beads.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Jian Wang (wangjian@bmi.ac.cn).

Materials availability

This study did not generate new unique reagents. Published plasmids are available upon request.

Data and code availability

The LC-MS/MS raw data (Zhang et al., 2022) have been deposited to the iProX repository with the dataset identifier (iProX: IPX0002410000) (https://www.iprox.org) or partner repository with the dataset identifier (ProteomeXchange: PXD022086) (http://www.proteomexchange.org).

AUTHOR CONTRIBUTIONS

Conceptualization, P.-H.W. and J.W.; methodology, L.S., Y.Z., Y.L., C.J., Y.Z., and J.Z.; Writing – original draft, L.S.; writing – review & editing, L.S., P.-H.W., and J.W.; funding acquisition, P.-H.W. and J.W.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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