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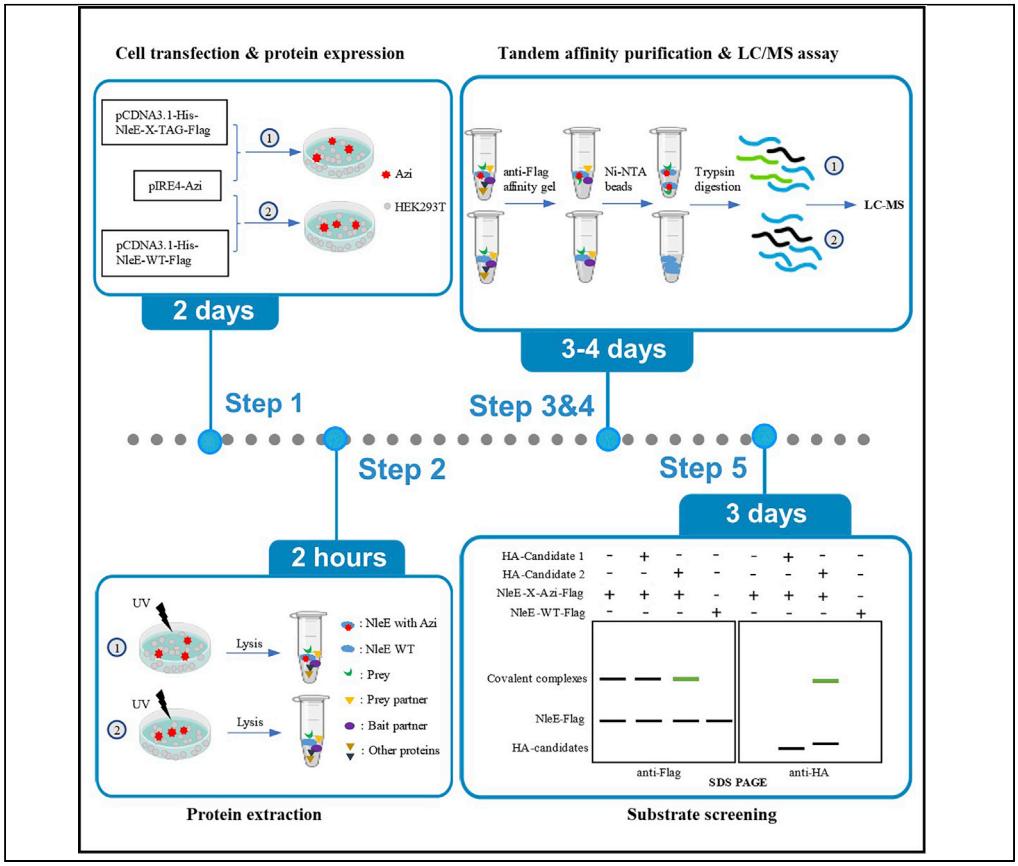
Protocol

Capture and mass spectrometry analysis of effector-substrate complexes using genetically incorporated photo-crosslinkers in host cells



Interactions between effectors and their host targets are often weak or transient, making them difficult to identify. We describe a protocol for covalent capture of effector substrates in living cells using genetic code expansion technology. The effector-substrate complexes are captured by the crosslinker and subsequently purified with tandem chromatography. We detail steps for mass spectrum analysis and substrate verification. While the steps here are specific for substrates of enteropathogenic *E. coli* in HEK293T cells, the protocol has broader applications.

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

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Highlights

Covalent capture using site-specific, incorporated photo-crosslinker

Identify weak or transient interactions of pathogens in living host cells

Steps for mass spectrometry analysis and substrate verification

Applicable for identifying interaction partners of proteins other than effectors

Li et al., STAR Protocols 3, 101882
December 16, 2022
https://doi.org/10.1016/j.xpro.2022.101882

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Capture and mass spectrometry analysis of effector-substrate complexes using genetically incorporated photo-crosslinkers in host cells

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SUMMARY

Interactions between effectors and their host targets are often weak or transient, making them difficult to identify. We describe a protocol for covalent capture of effector substrates in living cells using genetic code expansion technology. The effector-substrate complexes are captured by the crosslinker and subsequently purified with tandem chromatography. We detail steps for mass spectrum analysis and substrate verification. While the steps here are specific for substrates of enteropathogenic *E. coli* in HEK293T cells, the protocol has broader applications.

For complete details on the use and execution of this protocol, please refer to Li et al. (2021).[1]

BEFORE YOU BEGIN

The protocol below describes specific steps for detecting the substrates of *Enteropathogenic Escherichia coli* non-LEE-encoded effector, NleE, using the photo-crosslinking unnatural amino acid (Uaa) 4-azido-L-phenylalanine (Azi, Figure 1A) in HEK293T cells.[2] However, this protocol could be adapted to use other crosslinking Uaas in other cells for substrate screening of other proteins. We describe a protocol for identifying host substrates of effectors. The substrate-effector interactions are usually difficult to be identified because of their weak and transient character.[2–5] Pathogens regulate host physiological processes through effector interactions with host proteins to achieve infection and pathogenicity. The discovery of specific substrates of effectors could help us defend against bacterial infections in the future.

Uaas can be inserted into specific positions of proteins by engineered aminoacyl-tRNA synthetase and $tRNA_{CUA}$ pairs. The engineered aminoacyl-tRNA synthetase specifically recognizes and charges the Uaa onto the orthogonal $tRNA_{CUA}$, which recognizes the antisense codon TAG to incorporate the Uaa into corresponding sites of protein during translation[6–12] (Figure 1B). When the photo-crosslinkers replaced the residues on the interaction surface of protein complexes, they react with adjacent amino acids to form covalent bond under ultraviolet treatment. Thus, the non-covalent interactions between proteins are converted to covalent interactions[13–15] (Figure 1C). Therefore, an effector library with codons replaced with TAG stop codons was constructed to screen covalent complexes (see plasmids and screening library construction for details) (Figure 1D). Expression of effectors with Azi incorporated at specific positions is achieved by applying steps 1–3. The covalent complexes could be easily detected by corresponding to the adduct molecular weight (MW) on denaturing SDS-PAGE gels (Figure 1D). Following covalent

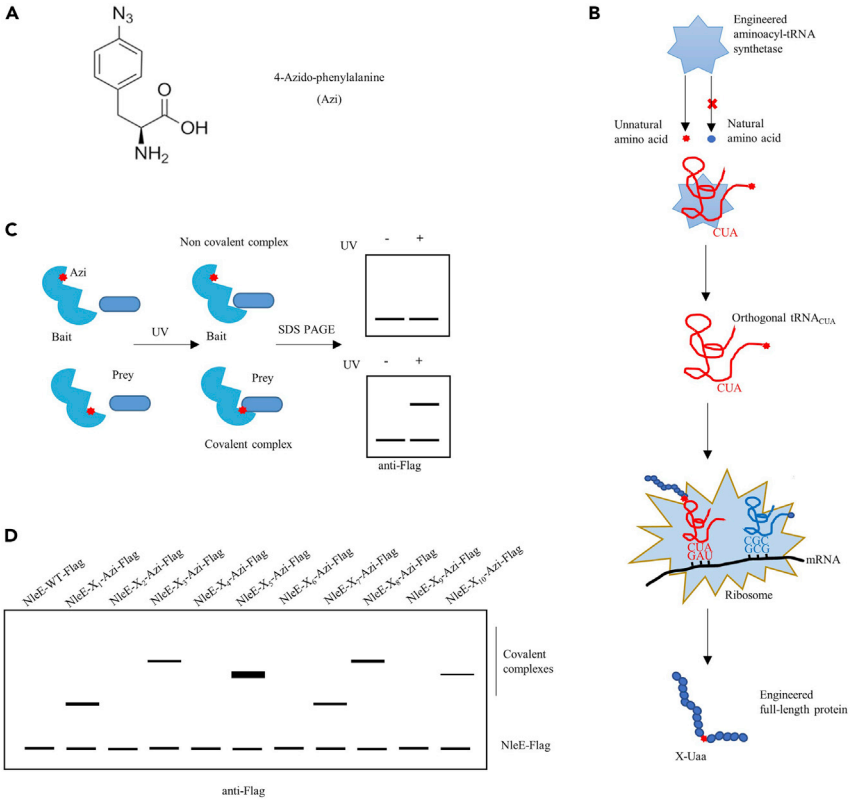

Figure 1. Schematic diagram of covalent capture of effector-substrate complexes

(A) The 4-Azido-L-phenylalanine (Azi) structural formula.

(B) Schematic diagram of genetic code expansion.

(C) Schematic diagram of capturing protein-protein interactions with Azi.

(D) Diagram of immunoblotting results for screening NleE-Xn-Azi-Flag mutants that are involved in covalent capture of interaction partners. Xn represents different residues of NleE.

complex tandem purification, mass spectrometric analysis, and candidate verification to identify the interaction partner.

Plasmids and screening library construction

Timing: 5 days

Here, we take the incorporation of Azi into NleE (protein of interest) in mammalian cells as an example. The engineered aminoacyl-tRNA synthetase and $tRNA_{CUA}$ pairs for Azi are encoded by the pIRE4-Azi plasmid.[1,16] For NleE expression in mammalian cells, clone the *NleE* gene with the required tags into the pCDNA3.1 vector (pCDNA3.1-NleE-WT-Flag plasmid for covalent complexes screening and pCDNA3.1-His-NleE-WT-Flag plasmid for covalent complexes purification). Select the codons (Xn) encoding NleE residues that potentially interact with their substrates and replace them with the TAG stop codons individually (pCDNA3.1-NleE-Xn-TAG-Flag) to get the screening library (Figure 1D). Please note that when decoding TAG stop codons by engineered aminoacyl-tRNA synthetase and $tRNA_{CUA}$ pairs is not complete, truncated NleE proteins are obtained frequently. Therefore, at least one tag should be added at the C-terminal of NleE to avoid interference from the truncations.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
DYKDDDDK-Tag (3B9) Mouse monoclonal antibody (1:2000)	Abmart	RRID: AB_2713960; Cat # M20008
HA-Tag(26D11) Mouse monoclonal antibody (1:2000)	Abmart	RRID: AB_2864345; Cat # M20003
Goat Anti-Mouse IgG H&L (HRP) (1:2000)	Abcam	Cat # ab6789
Chemicals, peptides, and recombinant proteins		
p-azido-phenylalanine	MuJin Biotech Inc	Cat # MJ210715-15
Triton X-100	Sangon Biotech	Cat # A110694
Tris (hydroxymethyl)aminoethane	Sangon Biotech	Cat # A600194
Sodium chloride (NaCl)	Sangon Biotech	Cat # A610476
Sodium dodecyl sulfate (SDS)	Sangon Biotech	Cat # A600485
β-Mercaptoethanol (β-ME)	TCI chemicals	Cat # M1948
Methanol	Sangon Biotech	Cat # A601617
Glycine	Sangon Biotech	Cat # A610235
Polyethylenimine Max (PEI)	Polysciences	Cat # 24765-1
Opti-MEM	Thermo Fisher Scientific	Cat # 31985070
Imidazole	Sigma	Cat # V900153
Urea	Sangon Biotech	Cat # A600148-0002
Protease Inhibitor Cocktail	MCE	Cat # HY-K0010
Dulbecco's Modified Eagle Medium (DMEM)	Gibco	Cat # C11995500BT
Fetal Bovine Serum (FBS)	Ausgenex	Cat # FBSSA 500-s
Experimental models: Cell lines		
HEK293T	National Collection of Authenticated Cell Cultures	Cat # GNHu17
Recombinant DNA		
pIRE4-Azi	Addgene	Plasmid # 105829
pcDNA3.1-His-NleE-WT-Flag	This paper	N/A
pcDNA3.1-NleE-Flag	This paper	N/A
pcDNA3.1-His-NleE-K219TAG-Flag	This paper	N/A
pcDNA3.1-NleE-K219TAG-Flag	This paper	N/A
pCMV-HA-NIP	This paper	N/A
Other		
Anti-DYKDDDDK-Tag Mouse Antibody (Agarose Conjugated)/Anti-Flag affinity gel	Abmart	Cat # M20018L
Ni-NTA Agarose	QIAGEN	Cat # 30210
6-Well Cell Culture Plates	JET	Cat # TCP011006
10 cm Cell Culture Dishes	JET	Cat # TCD010100

MATERIALS AND EQUIPMENT

Azi solution

The Azi (206.2 g/mol) solution must be prepared right before use. The working concentration of Azi is 1 mM for HEK293T cells. For a 6-well plate, each well has 2 mL cell culture medium. For 12 mL culture medium, we need 206.2 g/mol × 1 mmol/L × 12 mL = 2.5 mg of Azi. Put the Azi in a tube, add sterilized water and appropriate amount of 1 M NaOH solution to dissolve it to final volume of 60 μL. Keep the solution in dark. Add 10 μL of Azi solution to each well when needed.

PEI solution (1 mg/mL)

Weigh 10 mg of PEI powder and dissolve in an appropriate amount of sterilized water. Adjust the pH to 7.0 with concentrated hydrochloric acid (w/v:38%), and then adjust final volume to 10 mL. Filter the solution with a 0.22 μm sterile filter, and store one-use aliquots at −80°C (Suggested maximum time for storage: 1 year).

5 M imidazole solution

Dissolve 34.05 g imidazole powder with sterilized water and make up final volume to 100 mL. Store at 4°C in dark (Suggested maximum time for storage: 6 months).

1 M Tris-HCl, pH 7.4/6.8

Weigh 121.1 g of Tris into a 1 L beaker and then add about 800 mL of sterilized water to dissolve it. Adjust the pH to 7.4/6.8 with concentrated hydrochloric acid (w/v:38%), and then adjust the final volume to 1 L (Suggested maximum time for storage: 1 year).

5 × SDS loading buffer

Reagent	Final concentration	Amount
Tris-HCl (1 M), pH 6.8	125 mM	2.5 mL
SDS	5% (w/v)	1 g
Bromophenol blue	0.25% (w/v)	50 mg
β-Me	5% (v/v)	1 mL
Glycerol	25% (v/v)	5 mL
ddH_2O	N/A	11.5 mL
Total	**N/A**	**20 mL**

Store at 4°C; suggested maximum time for storage: 1 year.
Add β-Me and SDS under a fuming hood before use.

Lysis buffer/Flag wash buffer

Reagent	Final concentration	Amount
Tris-HCl (1 M), pH 7.4	50 mM	5 mL
NaCl	150 mM	0.8766 g
Triton X-100	0.5%	0.5 mL
ddH_2O	N/A	94.5 mL
Total	**N/A**	**100 mL**

Store at 4°C; suggested maximum time for storage: 3 months.

Flag elution buffer

Reagent	Final concentration	Amount
Tris-HCl (1 M), pH 7.4	25 mM	2.5 mL
NaCl	500 mM	2.922 g
Urea	8 M	48.048 g
β-Me	5 mM	35 μL
Imidazole (5 M)	10 mM	0.2 mL
ddH_2O	N/A	97.2 mL
Total	**N/A**	**100 mL**

Store at 4°C in dark; suggested maximum time for storage: 3 months.

His wash buffer A

Reagent	Final concentration	Amount
Tris-HCl (1 M), pH 7.4	25 mM	2.5 mL
NaCl	500 mM	2.922 g
Urea	8 M	48.048 g
β-Me	5 mM	35 μL
Imidazole (5 M)	10 mM	0.2 mL
ddH_2O	N/A	97.2 mL
Total	**N/A**	**100 mL**

Store at 4°C in dark; suggested maximum time for storage: 3 months.

His wash buffer B		
Reagent	Final concentration	Amount
Tris-HCl (1 M), pH 7.4	25 mM	1.25 mL
NaCl	500 mM	1.461 g
Imidazole (5 M)	10 mM	0.1 mL
ddH_2O	N/A	48.65 mL
Total	**N/A**	**50 mL**

Store at 4°C in dark; suggested maximum time for storage: 3 months.

UVP crosslinker

The CL-1000L Ultraviolet Crosslinker was purchased from Analytik Jena. It has five UV lamps (8 W, 365 nm) on top of the box. Remove the lid of the plate and put it on ice to keep the distance between the plate and the lamp at 6–8 cm during irradiation.

STEP-BY-STEP METHOD DETAILS

Screening of covalent complexes

Timing: 4 days

Screen covalent complexes using the mutant library of NleE. You may get multiple covalent complexes corresponding to different molecular weight (Figure 1D).

1. Seed HEK293T cells (0.5 million per well) into 6-well plates one day before transfection and grown in DMEM with 10% FBS to 70%–80% confluency.

   Note: The transfection procedure should be completed within 24 h after plating the cells, preferably within 16 h.

2. Taking the Azi as an example of a photo-crosslinking Uaa, the following operations were performed to express the NleE-Xn-Azi-Flag proteins.
   a. Add the plasmids pCDNA3.1-NleE-Xn-TAG-Flag/pCDNA3.1-NleE-WT-Flag (1 μg) and pIRE4-Azi (1.2 μg) into 200 μL Opti-MEM or serum-free DMEM and mixed by pipetting.
   b. Add 6.6 μL of PEI solution (1 μg/μL) into the diluted DNA.
      i. Mix the DNA-PEI solution gently.
      ii. Leave it at 20°C–25°C for 15–20 min (no more than 20 min).

   Note: The volume of PEI used is based on a 3:1 ratio of PEI (μg) to total DNA (μg).

   c. Add the DNA-PEI mixture to the cell culture medium.
   d. Replace the medium with DMEM medium supplemented with 10% FBS and 1 mM Azi at 6–8 h and 24 h after transfection.
3. After 48 h, irradiate the living cells in 6-well plates with UV light (365 nm) for 20 min on ice.
4. Scrape cells off with a scraper and collect into a centrifuge tube, then centrifuge at 1,000 × *g* for 3 min.
   a. Remove the supernatant and resuspend the cells with 1 mL of pre-chilled PBS.
   b. Centrifuge again to collect cells.
5. Resuspend cells with 40 μL of lysis buffer supplemented with protease inhibitor cocktail and incubate it on ice for 30 min.
6. Centrifuge at 17,000 × *g* for 10–15 min at 4°C, collect the supernatants and add 10 μL 5 × SDS loading buffer into 40 μL supernatant and boil at 95°C for 10 min.

7. Add appropriate amount of each sample on SDS-PAGE gel, following by immunoblotting analysis with anti-Flag antibody.

⚠ CRITICAL: Bands corresponding to the adduct molecular weight (MW) on SDS-PAGE gels indicates formation of covalent complexes (Figure 1D).

Covalent complex expression and extraction

Timing: 2 days

Choose the strongest one of each covalent complex for the subsequent experiments. Here, we take the NleE-K219Azi captured complex as an example. In this step, we will perform protein expression and extraction of covalent complex.

8. For one biological replicate, seed HEK293T cells into four 10 cm dishes (4 million cells per dish) one day before transfection. Transfect pCDNA3.1-His-NleE-K219TAG-Flag (7 μg) or pCDNA3.1-His-NleE-WT-Flag (4 μg) and pIRE4-Azi plasmid (8 μg) into HEK293T cells. The experiment could be carried out according to the step two of step-by-step method details.

Note: The His-NleE-WT-Flag was used as a control. Each sample needs three biological replicates. We recommend using endotoxin-free plasmids in this step, which can reduce the cytotoxicity and increase expression efficiency.

9. Add the Uaa (Azi) into culture medium at a final concentration of 1 mM. After 48 h, irradiate the living cells in dishes with UV light (365 nm) for 20 min on ice.

Note: Both the His-NleE-K219Azi-Flag and His-NleE-WT-Flag-expressing cells should be treated with UV light.

10. Harvest cells for each biological replicate and lyse with 2 mL of lysis buffer for 30 min on ice.
    a. Collect the supernatants by centrifuging at 17,000 × *g* for 10–15 min at 4°C.
    b. Transfer them into new tubes.

Note: If your target protein is membrane protein or nuclear protein, please choose an appropriate protein extraction protocol.

⚠ CRITICAL: To avoid protein degradation, all steps for protein extraction and purification must be performed at 4°C.

Flag tag affinity purification

Timing: 7 h

In this step, we will perform non-denaturing affinity purification of the extracted covalent complex by anti-Flag affinity gels (Figure 2).

11. Add 40–60 μL anti-Flag affinity gels of total slurry into the supernatants and invert tubes several times to suspend anti-Flag affinity gels. Place the tubes on a rotator for 6 h at 4°C to bind the protein complexes.
12. Centrifuge the samples at 1,000 × *g* for 3 min at 4°C and remove the supernatants with a pipette. Be careful to remove the supernatants without disturbing the anti-Flag affinity gels.
13. Add 1 mL Flag wash buffer into samples to wash the beads. Centrifuge at 1,000 × *g* for 3 min at 4°C and remove the supernatants. Repeat the wash step three to four times.

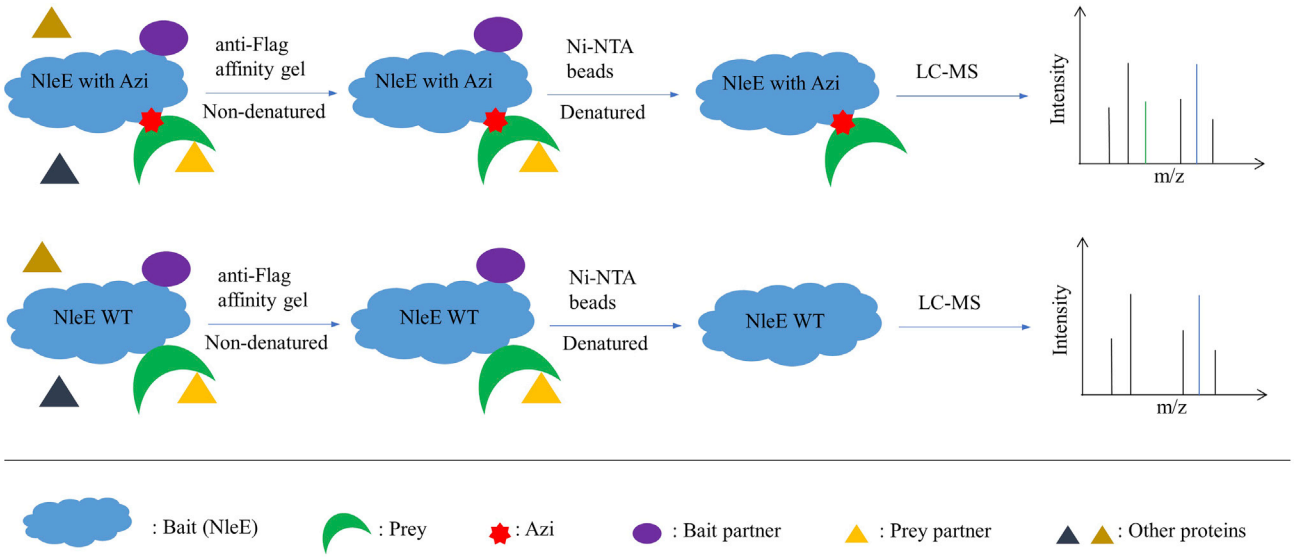

Figure 2. Schematic diagram of purification and LC-MS analysis of effector-substrate complexes

By non-denatured (anti-Flag affinity gel) and denatured (Ni-NTA beads) tandem purification, the enriched effector-substrate complexes were further subjected to mass spectrometry analysis to obtain a series of candidate proteins. The NleE WT sample was taken as a control.

14. Elute the anti-Flag affinity gels with 400 μL Flag elution buffer A for 10 min on a rotator at 4°C.
15. Centrifuge the samples at 1,000 × *g* for 3 min at 4°C and transfer the supernatants to new tubes.
16. Repeat the steps 14 and 15 twice. In total, 1.2 mL elution was obtained.

⚠ CRITICAL: We recommend multiple elution steps rather than one large volume elution to increase elution yields.

His tag purification

Timing: 3 h

After the previous non-denatured purification, we will perform denatured purification by Ni-NTA beads to remove more non-specific binding proteins (Figure 2).

17. Before adding Ni-NTA beads, centrifuge the supernatants in step 16 at 1,000 × *g* for 3 min at 4°C to remove the anti-Flag affinity gels that may have been left.
18. Add 80 μL Ni-NTA beads to the elution of each sample and invert tubes several times to suspend the beads. Put samples on a rotator at 4°C for 2 h.
19. Centrifuge samples at 1,000 × *g* for 3 min (4°C) and then remove the supernatant.
20. Wash the Ni-NTA beads with 1 mL His wash buffer A for 10 min on a rotator. Centrifuge at 1,000 × *g* for 3 min and remove the supernatants. Repeat this wash step once.
21. Wash the Ni-NTA beads with 1 mL His wash buffer B for 10 min on a rotator. After 10 min, centrifuge the samples at 1,000 × *g* for 3 min and remove the supernatants. Repeat this step once.
22. Add 100 μL 1 × SDS loading buffer to the Ni-NTA beads and boil at 95°C for 15 min.
23. Centrifuge the boiled samples at 5,000 × *g* for 3 min and transfer the supernatants to new tubes for further analysis.

Pause point: the boiled samples can be stored at −80°C for one week.

Liquid chromatography-mass spectrometry assay (LC-MS)

Timing: 2 days

The purified covalent complex will be analyzed by mass spectrometry. After data analysis, a series of candidate proteins will be obtained.

24. Separate the samples on SDS-PAGE gel.
    a. Stop the run when the samples completely enter the stacking gel as indicated by bromophenol blue of loading buffer.
    b. Stain the gel with Coomassie blue and carefully cut the bands with samples.
25. The gel with samples can be handed over to commercial companies for subsequent Liquid Chromatograph Mass Spectrum (LC-MS/MS) analysis.[17]
    a. Digest samples with 1 μg trypsin for 16–18 h at 37°C.
    b. The digested peptides were loaded onto a LC instrument coupled to a Q Exactive quadrupole-Orbitrap mass spectrometer.
26. For the data analysis, raw files were analyzed against the Swiss-Prot human protein sequence database (20413 entries, 2017/01/14) in Maxquant (version 1.6) with reverse decoy database with a false discovery rate (FDR) <1%.
    a. Two missed trypsin cleavages were allowed, and cysteine carbamidomethylation was set as a fixed modification.
    b. Oxidation of methionine, acetylation on lysine, and protein N-terminal acetylation were set as variable modifications.
    c. You will get a list of identified proteins with abundances for each sample.
27. By comparing the differences of proteins in wild-type and Uaa-engineered samples in three biological replicates, a series of candidates were obtained which appeared in the His-NleE-K219Azi-Flag samples, but were absent or present at lower levels in the His-NleE-WT-Flag samples.
28. According to the molecular weight of the covalent complex and NleE protein, the range of candidates could be further narrowed down.

Validation of the interaction partners

Timing: 3 days

In this step, we will validate the above series of candidate proteins to finally confirm the substrate of NleE.

29. Clone the candidate NleE interaction partners (NIP) into pCMV-HA plasmid (pCMV-HA-NIP).
30. pCDNA3.1-NleE-K219TAG-Flag, pIRE4-Azi and pCMV-HA-NIP plasmids were co-transfected into HEK293T. For details, refer to the steps of screening of covalent complexes.
    a. Cells expressing pCDNA3.1-NleE-Flag or pCMV-HA-NIP alone were used as controls.
    b. All cells were cultured in DMEM medium supplemented with 10% FBS and 1 mM Azi for 48 h.
    c. Cells expressing NleE-K219Azi-Flag and HA-NIP that were not treated with UV were used as a control.
31. Samples were separated on SDS-PAGE gels and analyzed by immunoblotting with anti-Flag and anti-HA antibodies individually.
    a. Detection of crosslinking bands on anti-Flag immunoblotting indicates successful crosslinking.
    b. The presence of a crosslinking band on anti-HA immunoblotting in UV-treated cells expressing NleE-K219Azi and HA-NIP, but not in WT and non-UV-treated NleE-K219Azi controls, suggests that NIP is a NleE substrate.
32. Other traditional methods, such as immunoprecipitation and pull down, are used to further verify the interactions between the effectors and substrates.

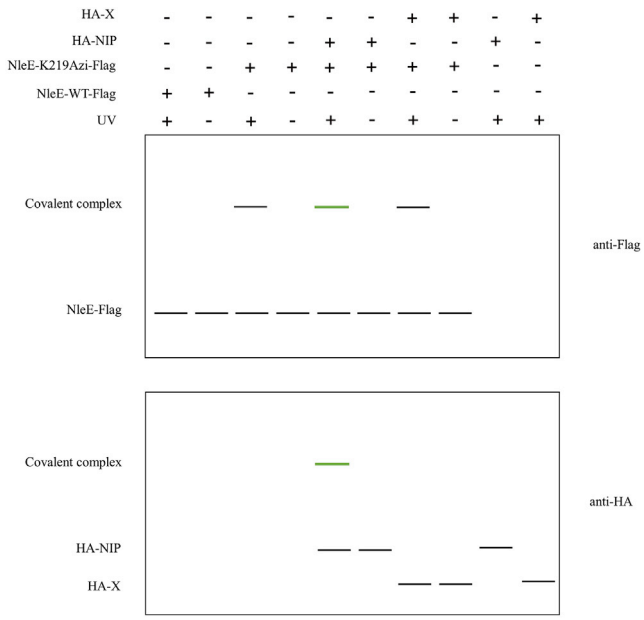

Figure 3. Screening of NleE interaction partners

pCDNA3.1-NleE-K219Azi-Flag and pCMV-HA-NIP were co-expressed in HEK293T. Anti-Flag and anti-HA antibodies individually detect the covalent complex in a UV-dependent manner. Schematic diagrams (not real blots) are shown. The green bands indicate the covalent complexes that could be detected by anti-HA antibodies.

EXPECTED OUTCOMES

You should see that NleE-K219Azi-Flag produces a crosslinking band in anti-Flag immunoblotting (Figure 3). It suggests that our system works and endogenous NIP is captured. NleE-K219Azi-Flag captures exogenous HA-NIP and produces a crosslinking band that could be detected by anti-HA immunoblotting (Figure 3). No crosslinking bands are found in the WT NleE sample or cells co-expressing other HA candidates (Figure 3). Thus, these results indicate that NIP is an interaction partner of NleE in living cells.

LIMITATIONS

The genetically incorporated photo-crosslinkers react with target amino acids when the cells are irradiated with UV light. If the effector interacts with its substrates under certain conditions, such as specific infection stage and different cell cycle, it is essential to figure out the conditions. In addition, the UV treatment causes a series of cell responses, which may affect interaction between the effector and substrate. Therefore, verifying the interaction and its function are suggested.

It is also important to notice that failure of crosslinking only means the capture failed, but does not necessarily mean no interaction.[18] The failure may be caused by unsuitable distance, inappropriate orientation, unusual transient interaction, and so on. The side chain lengths of different Uaas are different. The phenylalanine-based Uaas, such as 4-Azido-L-phenylalanine (Figure 4A), have rigid, short side-chains (distance between Cα of the Uaa to its crosslinking-center: 6–9 Å).[19] The lysine-based (3-(3-methyl-3H-diazirine-3-yl)-propaminocarbonyl-N$^{\varepsilon}$-L-lysine (DiZPK) (Figure 4B) has longer and flexible linkers (distance between Cα and crosslinking-center: 14–15 Å).[14] Therefore, you may need to try different Uaas according to your experiment.

TROUBLESHOOTING

Problem 1

Low expression level of some NleE-X-TAG-Flag protein (step 2 of step-by-step method details).

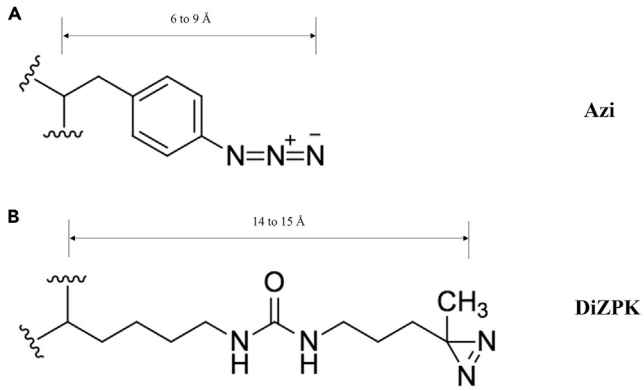

Figure 4. The side chain lengths of different Uaas

The structural formula of Azi (A),[19] and DiZPK (B)[14] and the distance between Cα of the Uaas to its crosslinking-center.

Potential solution

The Uaas are genetically incorporated into proteins of interest by suppressing in-frame amber stop codons (TAG) with the help of orthogonal synthetase/$tRNA_{CUA}$ pair. The mRNA context of the UAG codon results in variation of Uaa incorporation rates in both bacteria and mammalian cells.[8,20,21] Therefore, the Uaa incorporation rates are variable when replacing different residues of NleE. We recommend adjusting the amounts of transfected plasmids according to the expression level.

Problem 2

Cells are in poor condition or even die during addition of Uaa into the cell culture medium (step 2, 9 of step-by-step method details).

Potential solution

For Azi, cells normally grow well with high Uaa-incorporation efficiency at 1 mM Azi in the medium. If the cells are in poor condition, we recommend reducing the amounts of transfected plasmids or de-endotoxin the plasmids you extracted. Different Uaa has various working concentration. If the Uaa is toxic, you may try to reduce the working concentration.

Problem 3

Low protein expression, insufficient lysis and poor binding of beads.

Potential solution

To ensure sufficient protein for analysis, the number of cells can be further increased. The expression pattern of the protein of interest needs to be confirmed. The lysis buffer can be appropriately replaced according to the localization of the protein to ensure that the target protein can be fully extracted. Before using anti-Flag gels or Ni-NTA beads, you need to replace the storage solution with the corresponding buffer in advance to avoid denaturation and other effects on the protein. During binding at 4°C, protease inhibitors should be supplemented to avoid protein degradation, and the binding time can be appropriately extended if the binding is not sufficient.

Problem 4

Covalent complexes failed to be purified (step 11, 18 of step-by-step method details).

Potential solution

The unsuitable position of tags may hinder its interaction with beads, or block bait protein interaction with prey proteins. In this case, trying different tags at different terminus of the bait may solve the problem.

Problem 5

UV-dependent covalent capture of substrates is low efficient (step 3, 9 of step-by-step method details).

Potential solution

The UVP crosslinker which stimulate the azide groups to form active intermediates is critical for the efficient crosslinking. Record the using time of the UV lamps (max using time: 500 h) and replace them on time.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Haiyan Ren (hyren@scu.edu.cn).

Materials availability

Plasmids and any other reagents used in this study are available from our laboratory upon request to other research investigators under a material transfer agreement.

Data and code availability

Source data in this paper is available upon request. This study did not generate new dataset or code.

ACKNOWLEDGMENTS

This work was supported by a grant to H.Y. from the National Key Research and Development Program of China (2018YFA0109200), the National Natural Science Foundation of China (31872727), and 1.3.5 project for disciplines of excellence, West China Hospital, Sichuan University (ZYYC20020).

AUTHOR CONTRIBUTIONS

P.L. and J.X. wrote the protocol. H.Y. edited and revised the protocol.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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