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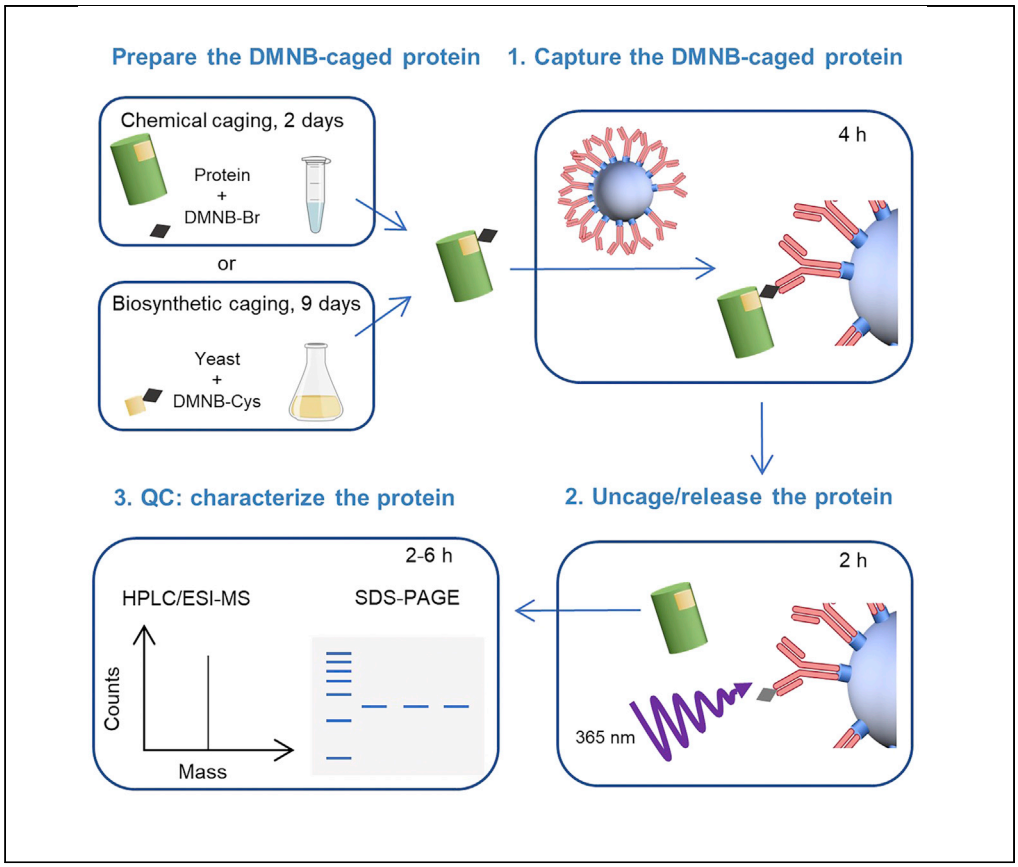
Selective immunocapture and light-controlled traceless release of transiently caged proteins



The 4,5-dimethoxy-2-nitrobenzyl (DMNB) photocaging group introduced into small biomolecules, peptides, oligonucleotides, and proteins is commonly used for spatiotemporal control of chemical and biological processes. Here, we describe the use of a DMNB-selective monoclonal antibody for non-covalent capture of chemically or biosynthetically produced proteins containing surface-exposed DMNB caging groups followed by light-controlled traceless decaging and release of the bound proteins into solution for a variety of downstream applications.

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Highlights

Orthogonal manipulation of proteins by caging with surface-exposed photolabile groups

Antibody-mediated capture of transiently DMNB-caged proteins from complex mixtures

Light-controlled traceless uncaging and release of antibody-bound proteins

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Selective immunocapture and light-controlled traceless release of transiently caged proteins

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SUMMARY

The 4,5-dimethoxy-2-nitrobenzyl (DMNB) photocaging group introduced into small biomolecules, peptides, oligonucleotides, and proteins is commonly used for spatiotemporal control of chemical and biological processes. Here, we describe the use of a DMNB-selective monoclonal antibody for non-covalent capture of chemically or biosynthetically produced proteins containing surface-exposed DMNB caging groups followed by light-controlled traceless decaging and release of the bound proteins into solution for a variety of downstream applications.
For complete details on the use and execution of this protocol, please refer to Rakauskaitė et al. (2020).

BEFORE YOU BEGIN

Photochemical transformations enable exquisite spatiotemporal control over biochemical processes. Here we describe the application of a newly developed monoclonal antibody for selective capture and light-controlled release of proteins tagged with the biocompatible photo-labile DMNB groups under near physiological non-denaturing conditions. The procedure encompasses the following three major steps that can be used as necessary for achieving one's particular goals: selective immunocapture of a DMNB-caged protein on antibody-bound silica beads, photochemical traceless uncaging and quality assessment of the released proteins. Such manipulations are often desirable for laboratory production of designer proteins, or even less accessible selenoproteins, and can be adapted for applications in miniature light-controlled systems involving microfluidic devices, microchips, and nanoparticles. The protocol describes the manipulations of two exemplary DMNB-caged proteins: EGFP D117C-DMNB – obtained via chemical labeling of the native protein with DMNB bromide, and SUMOstar I106C-DMNB – produced via biosynthetic incorporation of DMNB-Cys in genetically expanded yeast cells and selectively enriched from a crude yeast extract. We have also tested this protocol with a number of other DMNB-caged proteins prepared by both methods (Rakauskaitė et al., 2020). The following two sections describe two alternative ways (chemical and biosynthetic) of preparing a DMNB-caged protein, which is the key ingredient of the principle protocol.

Chemical photocaging of the EGFP D117C protein with DMNB bromide

Timing: 2 days

The chemical reaction involving DMNB bromide predominantly targets surface-exposed cysteines (Marriott and Heidecker, 1996) but may also modify other nucleophilic centers present in histidine,

tyrosine, and lysine residues. Here we use a variant of the Enhanced Green Fluorescent Protein (EGFP D117C) containing an engineered Cys residue at the 117 (surface) position (Rakauskaitė et al., 2020).

1. Prepare buffers, keep at 4°C.
2. Make a working solution of the EGFP D117C protein by diluting it in Protein dilution buffer to 1.3–1.5 mg/mL (45–52 μM). Mix gently. Make an aliquot of 100 μL in a vial.
3. Add 1 μL of 10 mM DTT to the vial containing 100 μL of the protein solution. Gently mix and incubate at 20°C–22°C for 20 min.

Note: prepare 1M DTT, make aliquots and store at −20°C for up to 6 months. Use aliquots once, do not refreeze.

4. Prepare a fresh 10 mM DMNB bromide solution.
   a. Weigh 5.52 mg of DMNB bromide and dissolve in 80 μL of DMF (250 mM DMNB bromide solution in DMF).
   b. Make a 1:25 dilution: mix 4 μL of 250 mM DMNB bromide in DMF and 96 μL of DMF.

⚠ CRITICAL: DMNB group is light-sensitive, make DMNB bromide solution and perform all protein bioconjugation steps in dark environment.

⚠ CRITICAL: DMNB bromide and DMF are hazardous materials. When handling, use personal protective equipment.

5. Add 5 μL of 10 mM DMNB bromide (to 500 μM) into the protein vial (from step 3). Gently mix and incubate in the dark at 20°C–22°C for 2 h. Troubleshooting 1
6. Fill a dialysis tube with 14.4 mL of protein dialysis buffer. Transfer the reaction solution into a dialysis cup and place inside the dialysis tube. Incubate in the dark with gentle circular shaking at 4°C for 2 h.
7. Fill a 15 mL conical tube with 14.4 mL of protein dialysis buffer and transfer the dialysis cup into it. Incubate in the dark with gentle circular shaking at 4°C for 2 h.
8. Repeat step 7, incubate as above for 16–18 h.
9. Transfer the dialyzed protein into a new vial, make 50 μL aliquots and keep frozen at −20°C.

Note: Some protein precipitation may occur due to hydrophobic nature of the DMNB conjugates. Troubleshooting 2

10. Determine the conjugation efficiency by HPLC/ESI-MS (see steps 29–31 of the main protocol).

Biosynthetic production of the photocaged SUMOstar I106C-DMNB protein

Timing: 9 days

This procedure describes biosynthesis of an engineered variant of the Small Ubiquitin-like Modifier protein (SUMOstar I106C-DMNB) containing a photocaged DMNB-Cys residue at the genetically encoded 106 position (located near the C terminus of the protein).

Note: Incubate yeast plates at 30°C, incubate yeast liquid cultures at 30°C with 220 rpm shaking.

1. Days 1–5. Co-transform yeast strain LWUPF1Δ with plasmids pRR6 and pRR78 by conventional method (Becker and Lundblad, 1994) and select transformant colonies on standard synthetic drop-out medium lacking tryptophan and uracil (SC-Trp-Ura).

Note: Use yeast nitrogen base (YNB) w/o amino acids and copper to prepare synthetic media.

2. Day 6. Pick one colony and resuspend in 0.6 mL of SC-Trp-Ura medium, incubate for up to 24 h.
3. Day 7. Add 1.2 mL of selective medium to the culture, incubate for 16–18 h.
4. Day 8. Use overnight cells to start 20 mL of new culture in SC-Trp-Ura medium at an initial OD_{595} of ~0.06. Incubate for 16–18 h, until culture reaches an OD_{595} of 0.8–1.2.
5. Day 9. Collect cells by centrifugation at 2000 × *g* for 3 min at 20°C–22°C.
6. Resuspend cells in 4 mL of Induction medium. Incubate in the dark for 5 h. Troubleshooting 3

⚠ CRITICAL: From this point onward, handle cells in the dark: wrap culture flask with aluminum foil, dim the light in the room as much as possible.

7. Collect cells by centrifugation at 4000 × *g* for 5 min at 20°C–22°C. Discard growth medium.

⚠ CRITICAL: Culture medium containing DMNB-Cys should be properly disinfected and treated as hazardous waste.

8. Wash cells with 4 mL of ddH_2O.
9. Repeat step 7. Thoroughly aspirate all supernatant.
10. Determine the weight of the resulting wet biomass.

Pause point: Cell pellets can be frozen for temporary storage at −20°C or −80°C.

11. Resuspend yeast cells in YPER solution with added cOmplete™, EDTA-free Protease Inhibitor Cocktail using equivalent ratio: 50 mg of cells per 125 µL of solution. Incubate with orbital rotation for 20 min at 20°C–22°C.
12. Collect the cell debris by centrifugation at 14,000 × *g* for 10 min, save the supernatant.
13. Repeat steps 11 and 12 with the pellet and combine both lysates.

Note: Other yeast disruption methods (e.g., glass beads, homogenization, enzymatic) can be used to make the lysate.

Pause point: Lysate can be frozen at −20°C for up to two weeks.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal anti-DMNB antibody DMNB12B6	Antibodies are available upon request	N/A
Chemicals, peptides, and recombinant proteins		
4,5-Dimethoxy-2-nitrobenzyl bromide	Sigma-Aldrich	Cat#392855; CAS: 53413-67-5
DMNB-Cys (TFA salt or an equivalent amount of HCl salt)	(Lemke et al., 2007, Kang et al., 2013, Rakauskaitė et al., 2015)	N/A
Yeast nitrogen base without amino acids and without copper	Formedium	Cat#CYN0901
SC Double Drop Out: -Trp -Ura	Formedium	Cat#DSCK2519
Silica Particles, Protein A coated, supplied in 2.5% suspension – 1.0 µm/1 mL	Kisker Biotech	Cat#PSI-1.0PA
Slide-A-Lyzer™ MINI Dialysis Device, 10K MWCO, 0.5 mL	Thermo Scientific	Cat#88401
4× Laemmli sample buffer	Bio-Rad	Cat#161-0747

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
YPER	Thermo Scientific	Cat#78991
cOmplete™, EDTA-free Protease Inhibitor Cocktail	Merck	Cat#11873580001
EGFP D117C	(Rakauskaitė et al., 2020)	N/A
BSA	Sigma-Aldrich	Cat#A7030; CAS: 9048-46-8
Experimental models: cell lines		
Mouse hybridoma cells DMNB12B6	(Rakauskaitė et al., 2020)	N/A
Experimental models: organisms/strains		
Yeast *S. cerevisiae* strain LWUPF1Δ	(Wang and Wang, 2008)	N/A
Recombinant DNA		
pRR6	(Rakauskaitė et al., 2015)	N/A
pRR78	(Rakauskaitė et al., 2020)	N/A
Software and algorithms		
MassHunter Qualitative Analysis Software B.03.01	Agilent	P/N G3336-60038 https://www.agilent.com/en/product/software-informatics/mass-spectrometry-software/data-analysis/quantitative-analysis
ProtParam tool	(Gasteiger et al., 2005)	web.expasy.org/protparam/
Other		
IKA® HS 250 shakers, Model KS 250	Merck	Cat#Z404098
Fisherbrand™ Mini Tube Rotator	Fisher Scientific	Cat#88861052
Nanodrop 2000 spectrophotometer	Thermo Scientific	Cat#ND-2000
New Brunswick™ Excella® E25 incubated shaker	Eppendorf	Cat#M1353-0002
Centrifuge 5424 R	Eppendorf	Cat#5424R
Protein LoBind® Tubes, 0.5 mL	Eppendorf	Cat#0030108094
Mini-PROTEAN Tetra Cell	Bio-Rad	Cat#1658000EDU
Poroshell StableBond 300 C8, 2.1 × 75 mm, 5 μm	Agilent	Cat#660750-906

MATERIALS AND EQUIPMENT

HPLC/ESI-MS equipment

An Integrated HPLC/ESI-MS system comprising an Agilent 1290 Infinity high performance liquid chromatograph equipped with a Poroshell StableBond 300 C8 column (2.1 × 75 mm, 5 μm), G4226A autosampler and an Agilent 6520 Accurate-Mass Q-TOF analyzer fitted with an G3251B electrospray ion source. Analogous HPLC/MS equipment from other vendors permitting adequate protein characterization can be used.

Data analysis

Agilent MassHunter Qualitative Analysis software B.03.01.

UV light source and setup

A custom assembled 365 nm LED light source providing adjustable optical power of 6.2–96 mW and a luminous flux variation of no more than ±2% over a 30 min time period was used. A cap cut from a 0.5 mL Protein LoBind tube was used as a sample vial and closely fitted under the LED (at a distance of approx. 2 mm but avoiding direct physical contact with the LED), such that most of the luminous flux was directed at the sample surface area of 0.8 cm^2. A pre-chilled mirror under the sample ensured consistent UV and temperature conditions (Figure 1).

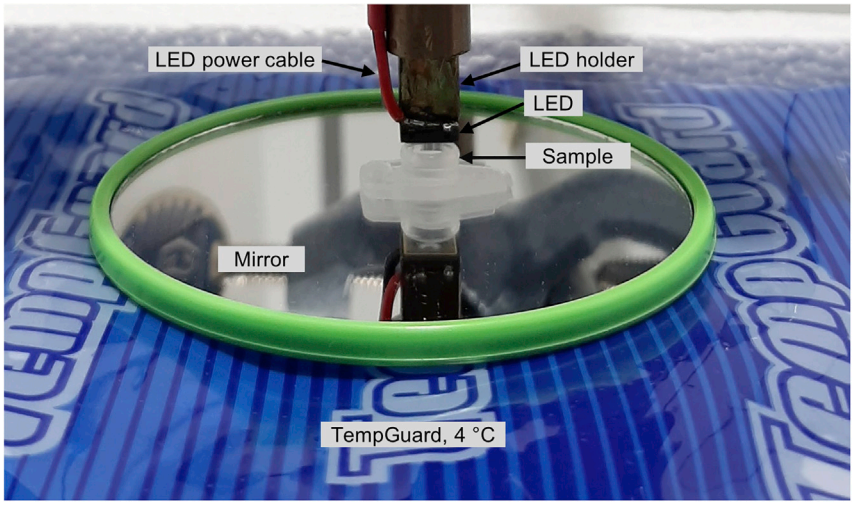

Figure 1. LED assembly for light-induced protein decaging

Alternative light sources (such as Nichia model NCCU033E, https://www.nichia.co.jp/en/about_nichia/2003/2003_120301.html) can be used provided that adequate optical power and distance between the sample and the LED are maintained.

Buffers and media

Protein dilution buffer		
Reagent	Final concentration	Amount
NaH_2PO_4/Na_2HPO_4 pH 7.5 (250 mM)	20 mM	4 mL
KCl (2 M)	200 mM	5 mL
ddH_2O	n/a	41 mL
Total	**n/a**	**50 mL**
Keep at 4°C for up to 30 days.		

Protein dialysis buffer		
Reagent	Final concentration	Amount
NaH_2PO_4/Na_2HPO_4 pH 7.5 (250 mM)	5 mM	2 mL
NaCl (5 M)	200 mM	4 mL
ddH_2O	n/a	94 mL
Total	**n/a**	**100 mL**
Keep at 4°C for up to 30 days.		

Induction medium		
Reagent	Final concentration	Amount
SC-Trp-Ura	n/a	4 mL
$CuSO_4$ (100 mM)	100 μM	4 μL
DMNB-Cys (TFA salt)	2 mM	3.4 mg
Make fresh, do not store.		

⚠ CRITICAL: The DMNB group is light sensitive. Protect from light during handling as much as possible.

⚠ CRITICAL: Use personal protective equipment when handling DMNB-Cys, or Induction medium.

PBS/1% BSA		
Reagent	Final concentration	Amount
PBS (10×)	1×	1 mL
BSA (10%)	1%	1 mL
ddH_2O	n/a	8 mL
Total	**n/a**	**10 mL**
Make fresh, do not store.		

PBS/2 mM DTT		
Reagent	Final concentration	Amount
PBS (10×)	1×	1 mL
DTT (2 M)	2 mM	0.01 mL
ddH_2O	n/a	8.99 mL
Total	**n/a**	**10 mL**
Make fresh, do not store. Prepare 2 M DTT, make aliquots and store at −20°C for up to 6 months. Use aliquots once, do not refreeze.		

⚠ CRITICAL: DTT is hazardous material. When handling, use personal protective equipment.

PBS/10 mM L-Met		
Reagent	Final concentration	Amount
PBS (10×)	1×	0.5 mL
L-Methionine (100 mM)	10 mM	0.5 mL
ddH_2O	n/a	4 mL
Total	**n/a**	**5 mL**
Make fresh immediately before use, since L-methionine readily oxidizes in solution. Do not store or reuse.		

STEP-BY-STEP METHOD DETAILS

Immunocapture of DMNB-caged proteins

Timing: 1–2 days

During this step immunocapture (IC) complexes are formed consisting of Silica Protein A beads, anti-DMNB monoclonal antibodies (MAbs), and DMNB-proteins. Both chemically conjugated or biosynthetic DMNB-proteins can be used with the protocol.

Note: All centrifugations should be performed at 1300 × *g* for 5 min at 20°C–22°C. If beads of different size are used, centrifugation conditions should be adjusted accordingly.

Note: During incubation time the beads must always be in suspension. Avoid beads settlement by periodic vortex mixing or orbital rotation.

1. Transfer 50 µL per tube of well resuspended silica Protein A beads to 6 Eppendorf 1.5 mL tubes.
2. Add 200 µL of PBS to each tube, resuspend by vortex mixing for 1 min.
3. Collect the beads by centrifugation. Remove and discard the supernatant.
4. Repeat steps 2 and 3 one more time.

5. Make 1.3 mL 0.95 mg/mL of anti-DMNB MAbs 12B6 solution in PBS. Determine the initial amount of MAbs in solution by taking A_{280} reading.
6. Add 200 μL per tube of prepared anti-DMNB MAbs 12B6 to the washed beads, resuspend by vortexing and incubate in orbital rotator for 1 h at 20°C–22°C.

Note: Alternatively, anti-DMNB MAbs can be bound by 16–18 h incubation at 4°C.

7. Collect the beads by centrifugation, save the supernatant, take its A_{280} reading and determine the amount of unbound MAbs.
8. Calculate the amount of MAbs bound to the beads. You should expect approximately 30 μg of MAbs bound to the beads equivalent to 50 μL of initial bead suspension. Troubleshooting 4
9. Add 200 μL of PBS to the beads and rinse as above (steps 2 and 3).
10. Add 300 μL of PBS/1% BSA to the beads, incubate in orbital rotator for 1 h at 20°C–22°C.
11. Collect the beads by centrifugation and rinse two times as above (steps 2 and 3).
12. Make 1.3 mL of a DMNB-protein solution.
    a. If working with bioconjugted EGFP D117C-DMNB, make solution in PBS at 25 μg/mL.
    b. If working with cell lysate containing biosynthetic SUMOstar I106C-DMNB, dilute the lysate 1:10 in PBS with added cOmplete™, EDTA-free Protease Inhibitor Cocktail.

⚠ CRITICAL: This and all subsequent steps should be performed in the dark: wrap tubes in aluminum foil, dim the light in the room as much as possible.

13. Add 200 μL of the DMNB-protein solution to each tube containing the beads. Mix thoroughly by vortex mixing and incubate with orbital rotation at 20°C–22°C for 1 h. Troubleshooting 5

Note: Alternatively, DMNB-proteins can be bound by 16–18 h incubation at 4°C.

14. Collect the beads by centrifugation. At his point, the IC complexes are fully assembled.
15. Wash the IC complexes three times with 300 μL PBS/2 mM DTT as above (steps 2 and 3).
16. Use 200 μL of PBS/2 mM DTT to combine and resuspend the IC complexes from each 3 tubes into one aliquot, finally resulting in 2 tubes, each containing 200 μL of IC suspension. One aliquot is required to make samples for HPLC/ESI-MS analysis (steps 17–23), while the other aliquot will be used to make samples for SDS-PAGE analysis (steps 24–28).

Pause point: We recommend to use the IC complexes immediately. Alternatively, they can be stored at 4°C until the next day.

Light-controlled photochemical release of uncaged proteins

Timing: 2 h

During this step, the IC complexes are exposed to 365 nm light (Figure 1) and photocage-free proteins are released into solution (Wang, 2013). Samples for HPLC/ESI-MS (Figure 2 steps 19–22) and SDS-PAGE analyses (Figure 2 steps 19–22, 25, and 26) are prepared.

Note: Prepare the UV light source for work ahead of time.

Note: All centrifugations should be performed at 1300 × *g* for 5 min at 10°C. If different beads are used, centrifugation conditions should be accordingly adjusted.

⚠ CRITICAL: Handle the IC complexes in the dark: keep racks with tubes covered with aluminum foil, dim the light in the room as much as possible.

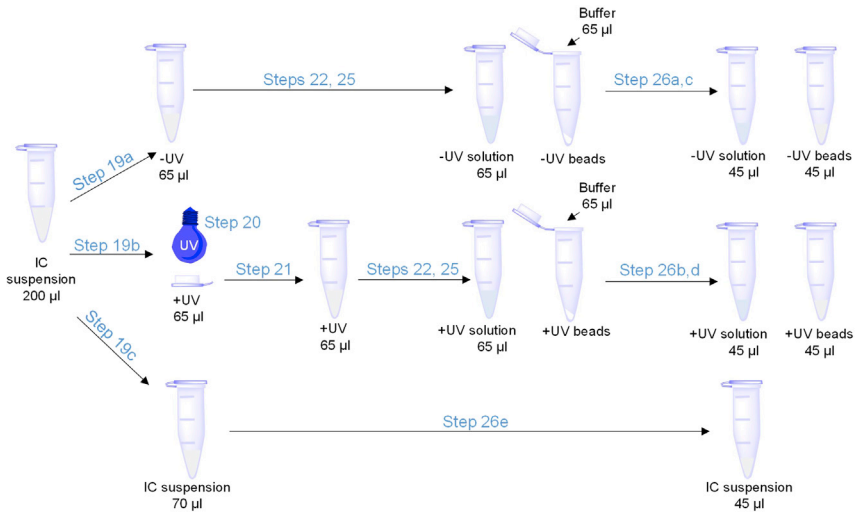

Figure 2. Sample handling scheme

17. Collect the IC complexes from one of the 200 µL preparation (step 16) by centrifuging. Remove and discard the supernatant.
18. Add 200 µL of PBS/10 mM L-Met buffer, resuspend very well by pipetting and vortex mixing.
19. Make two 65 µL aliquots:
    a. -UV. Transfer 65 µL of IC suspension to a new tube. Keep at 4°C.
    b. +UV. Transfer 65 µL of IC suspension to a cap cut from the 0.5 mL Protein LoBind tube.
    c. Keep the remaining IC suspension at 4°C.
20. Place the cap on a pre-chilled mirror and assemble under the LED light source (Figure 1). Turn on the UV light and let run for 10 min.

Note: Our in-house LED light source was operating at an optical power of 6.2 mW directed at a cap surface area of 0.8 cm^2, which afforded a nearly complete removal of DMNB-groups from the target proteins after a 10 min exposure. When other 365 nm light sources are used, optimal exposure time can be determined in control experiments by following the decaging efficiency of a particular DMNB-protein in solution at different time points by HPLC/ESI-MS.

⚠ CRITICAL: It is essential to use shielding against UV illumination.

21. Thoroughly resuspend the beads inside the cap by pipetting and transfer to a new tube.
22. Centrifuge the -UV (step 19a) and +UV (step 21) samples.
23. Transfer 40 µL of each supernatant to HPLC tubes, add 2 µL of 1 M DTT, mix thoroughly and proceed with the HPLC/ESI-MS analysis (steps 29–31).
24. Repeat steps 17–22 with a second aliquot of IC complexes (step 16).
25. Without disturbing beads, carefully aspirate and transfer supernatants to new tubes; save tubes containing packed beads.
26. Prepare samples for SDS-PAGE analysis (Figure 2):
    a. -UV solution. Transfer 45 µL of supernatant to a new tube.
    b. +UV solution. Transfer 45 µL of supernatant to a new tube.
    c. -UV beads. Reconstitute suspension by adding 65 µL of PBS/10 mM L-Met buffer to the packed beads and mixing thoroughly. Transfer 45 µL of suspension to a new tube.
    d. +UV beads. Reconstitute suspension by adding 65 µL of PBS/10 mM L-Met buffer to the packed beads and mixing thoroughly. Transfer 45 µL of suspension to a new tube.

e. IC suspension. Resuspend saved IC complexes (step 19c), transfer 45 μL of suspension to a new tube.

27. Add 15 μL of reducing 4× Laemmli sample buffer to each sample, mix thoroughly and heat at 95°C for 10 min.

Pause point: Samples can be stored at −20°C up to a week. Reheat at 95°C for 10 min prior to SDS-PAGE analysis.

28. Load 8 μL of each sample on a SDS-PA gel (37.5:1). Use 11% and 15% SDS-PA gels to fractionate EGFP and SUMOstar proteins, respectively. Run and visualize by standard procedures.

Optional: Same samples (10 μL) can be analyzed by western blotting.

Characterization of released proteins by HPLC/ESI-MS

Timing: 2–6 h

During this step, protein samples are fractionated on a reversed-phase HPLC column and the molecular masses of eluting major species are determined via ESI/Q-TOF mass spectrometry.

Note: Prepare the HPLC/ESI-MS system for work ahead of time.

29. Place protein samples (from step 23) inside the autosampler chamber, prechilled to 4°C. Load 40 μL of sample onto the HPLC column, apply linear chromatography gradient of solvents A (1% formic acid in water) and B (1% formic acid in acetonitrile) at flow rate of 0.4 mL/min at 30°C as follows: 0–1 min, 2% B; 1–6 min, 2%–98% B; 6–7 min, 98% B; 7–9 min, 98%–2% B; 9–10 min, 2% B.
30. Collect high-resolution mass spectra for protein products at 100–3200 m/z range, positive ionization mode.
31. Analyze the data using Agilent MassHunter Qualitative Analysis B.03.01 software, applying deconvolution parameters for protein mass spectra: Maximum entropy; calculation range of 4,000–80,000 Da. Use ProtParam tool (web.expasy.org/protparam/) to calculate theoretical mass values.

Note: Manual adjustments based on the nature of protein maturation or chemical modifications may be required.

EXPECTED OUTCOMES

Immunocapture of DMNB-caged proteins and subsequent photochemical traceless release of uncaged proteins into solution can be confirmed by two analytical methods.

SDS-PAGE analysis of the collected samples allows to evaluate the quality of the immunoprecipitation complexes, effectiveness of the photochemical reaction and assess the size homogeneity of the protein released into solution (Figure 3A). Regardless of whether the capture complexes were formed using purified DMNB-EGFP, or complex cellular lysate containing single-labeled DMNB-SUMOstar proteins, the main protein species attached to the beads were the heavy and light chains of the MAbs and the DMNB-protein (Figure 3A lane 1). During UV exposure, the uncaged target proteins were released from the beads and appeared in solution (Figure 3A lanes 4 and 5).

HPLC/ESI-MS analysis enables a precise measurement of the molecular mass of the released protein to confirm the removal of the photocaging group (Figure 3B).

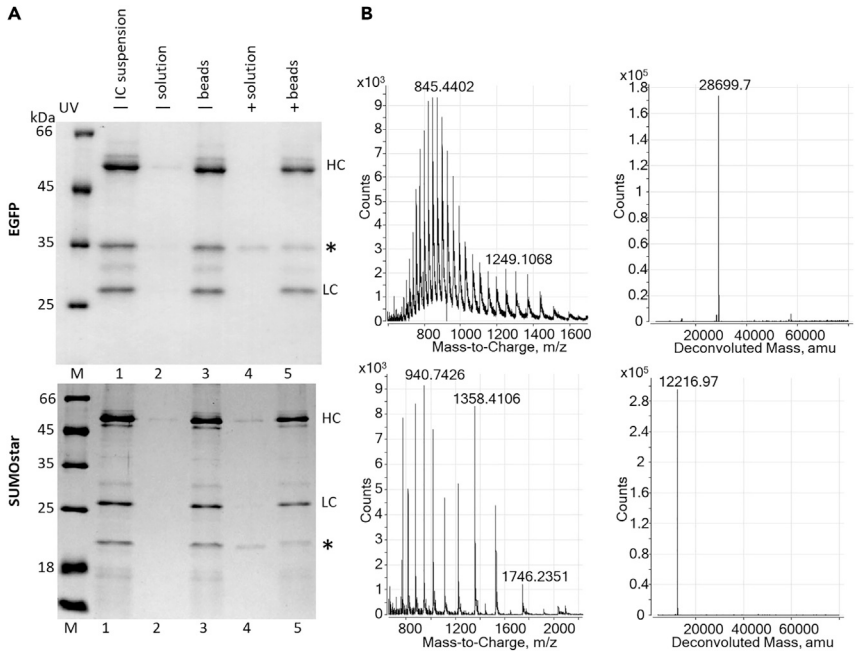

Figure 3. Characterization of the photolytically released proteins

(A) SDS-PAGE analysis of samples (step 28) collected during photodecaging process of the EGFP D117C-DMNB (top) and SUMOstar I106C-DMNB (bottom) proteins. Bands of the target proteins are indicated by asterisks. HC, heavy chain. LC, light chain. M, protein size marker. Figure adapted with permission from Rakauskaitė et al., 2020.

(B) HPLC-ESI/MS analysis of samples (step 23) identifying the decaged EGFP D117C (top) and SUMOstar I106C (bottom) proteins. Mass spectra (left) and deconvoluted masses (right) of the photolysis products corresponding to lane 4 in (A).

LIMITATIONS

At the DMNB-caging step, installation of a largely hydrophobic DMNB group in certain positions of a protein may render it insoluble in physiological buffers and thus unsuitable for further manipulations. The effect of DMNB caging on physical properties of a protein is hard to predict in advance, which apparently depends on the position and number of caging groups incorporated. We have encountered only two such cases out of 25 examined suggesting that it may not be a common problem.

At the immunocapture step, we found that the binding of a DMNB-caged protein was strongly dependent on the position and the number of caging groups accessible on the protein surface. This observation is consistent with the relative bulkiness of the antibody Fab region (Chiu et al., 2019), which may preclude making selective intermolecular contacts with sterically obscured DMNB moieties. However, for both model systems examined, multiple surface positions were identified that permitted selective capture of biosynthetically produced (single DMNB groups) or chemically modified (multiple DMNB groups) proteins. If your target protein appears to lack surface Cys or Sec residues, which are the major targets for chemical DMNB tagging, these need to be engineered by site-specific mutagenesis.

At the photolytic uncaging step, it is essential to protect the released target proteins against the damaging effects of free radicals and reactive oxygen species generated during the photochemical reaction (Kerwin and Remmele, 2007). This is typically achieved by adding radical scavenging compounds such as methionine (or other soluble thioethers).

TROUBLESHOOTING

Any mechanical equipment with the same function could be used. However, beads and anti-DMNB antibody are critical reagents and cannot be changed as no alternatives were tested with this protocol.

Problem 1
Protein degradation during the chemical caging reaction at 20°C–22°C.

Potential solution
For temperature-sensitive proteins perform the reaction at a lower temperature. Monitor the modification efficiency by HPLC/ESI-MS and adjust the incubation time accordingly.

Problem 2
Precipitation of DMNB-protein during chemical photocaging.

Potential solution
Try using lower concentrations of DMNB bromide and/or the reducing agent as well as a different reaction buffer.

Problem 3
Low concentration of the biosynthetic DMNB-protein.

Potential solution
Concentrating cells generally allows to save the precious noncanonical amino acid (Liu et al., 2010), but if this step significantly compromises the yield of the recombinant protein, resuspend the cells in 20 mL of induction medium.

Problem 4
Poor binding of the antibody to the beads.

Potential solution
Double the incubation time at 20°C–22°C or incubate at 4°C for 16–18 h.

Check the pH 7.4 of the PBS buffer.

Problem 5
Poor binding of the DMNB-protein to the beads.

Potential solution
Double the concentration of the DMNB-protein and/or incubation time at 20°C–22°C or incubate at 4°C for 16–18 h.

Make sure not to exceed the concentration of the reducing agent in steps 15-16 as it may have adverse effects on protein binding and downstream applications. See also discussion of Limitations.

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to the lead contact, Saulius Klimašauskas (saulius.klimasauskas@bti.vu.lt).

Materials availability
Plasmids described in this study will be made available upon request.

The antibody generated in this study will be made available on request, but we may require a payment and/or a completed Materials Transfer Agreement.

Data and code availability
This study did not generate datasets or codes.

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AUTHOR CONTRIBUTIONS

Conceptualization, S.K. and R.R.; methodology, investigation, and validation, R.R., G.U., and M.S.; resources, A.Ž. and S.K.; data curation, G.U.; writing – original draft, R.R., G.U., M.S., and S.K.; writing – review & editing, R.R. and S.K.; supervision, S.K. and A.Ž.; project administration, R.R. and S.K.; funding acquisition, S.K.

DECLARATION OF INTERESTS

The authors are inventors on a related patent application.

REFERENCES

Becker, D.M., and Lundblad, V. (1994). Introduction of DNA into yeast cells. Curr. Prot. Mol. Biol. *27*, 13.17.1–13.7.10.

Chiu, M.L., Goulet, D.R., Teplyakov, A., and Gilliland, G.L. (2019). Antibody structure and function: the basis for engineering therapeutics. Antibodies *8*, 55.

Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S.E., Wilkins, M.R., Appel, R.D., and Bairoch, A. (2005). Protein Identification and Analysis Tools on the ExPASy Server. In The Proteomics Protocols Handbook, J.M. Walker, ed. (Humana Press).

Kang, J.Y., Kawaguchi, D., Coin, I., Xiang, Z., O'leary, D.D., Slesinger, P.A., and Wang, L. (2013). In vivo expression of a light-activatable potassium channel using unnatural amino acids. Neuron *80*, 358–370.

Kerwin, B.A., and Remmele, R.L., Jr. (2007). Protect from light: photodegradation and protein biologics. J. Pharm. Sci. *96*, 1468–1479.

Lemke, E.A., Summerer, D., Geierstanger, B.H., Brittain, S.M., and Schultz, P.G. (2007). Control of protein phosphorylation with a genetically encoded photocaged amino acid. Nat. Chem. Biol. *3*, 769–772.

Liu, J., Castañeda, C.A., Wilkins, B.J., Fushman, D., and Cropp, T.A. (2010). Condensed E. coli cultures for highly efficient production of proteins containing unnatural amino acids. Bioorg. Med. Chem. Lett. *20*, 5613–5616.

Marriott, G., and Heidecker, M. (1996). Light-directed generation of the actin-activated ATPase activity of caged heavy meromyosin. Biochemistry *35*, 3170–3174.

Rakauskaitė, R., Urbanavičiūtė, G., Rukšėnaitė, A., Liutkevičiūtė, Z., Juškėnas, R., Masevičius, V., and Klimašauskas, S. (2015). Biosynthetic selenoproteins with genetically-encoded photocaged selenocysteines. Chem. Commun. (Camb.) *51*, 8245–8248.

Rakauskaitė, R., Urbanavičiūtė, G., Simanavičius, M., Lasickienė, R., Vaitiekaitė, A., Petraitytė, G., Masevičius, V., Žvirblienė, A., and Klimašauskas, S. (2020). Photocage-selective capture and light-controlled release of target proteins. iScience *23*, 101833.

Wang, Q., and Wang, L. (2008). New methods enabling efficient incorporation of unnatural amino acids in yeast. J. Am. Chem. Soc. *130*, 6066–6067.

Wang, P. (2013). Photolabile protecting groups: structure and reactivity. Asian J. Org. Chem. *2*, 452–464.