Published in final edited form as:
Methods Enzymol. 2020 January 01; 633: 281–294. doi:10.1016/bs.mie.2019.11.008.

Reversible biotinylation of purified proteins for measuring protein–protein interactions

Hemlata Dwivedi-Agnihotri, **Ashish Srivastava**, **Arun K. Shukla***
Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur, India

Abstract

Measuring protein–protein interactions using purified proteins in vitro is one of the most frequently used approach to understand the biochemical and mechanistic details of cellular signaling pathways. Typically, affinity tags are genetically fused to proteins of interest, and they are used to capture and detect them. However, in some cases, fusion of bulky affinity tags might present a significant limitation in these experiments, especially if the regions in close proximity of tags are involved in protein–protein interactions. Here, we present a step-by-step protocol for an alternative approach that involves reversible biotinylation of purified proteins using a simple chemical-conjugation of cleavable biotin moiety. Biotinylated proteins can be directly used as bait for selective immobilization on solid support for measuring protein–protein interactions. Furthermore, biotinylation of protein of interest also allows specific detection in standard biochemical assays. This simple, straightforward and modular protocol can be directly adapted and applied to facilitate the detection of novel protein–protein interactions as well as measuring apparent affinities of such interactions.

1 Introduction

The process of cellular signaling involves a large number of transient, non-covalent protein–protein interaction networks which are essential for signal-recognition and propagation in cellular context (Braun & Gingras, 2012; De Las Rivas & Fontanillo, 2010; Nooren & Thornton, 2003). Protein–protein interactions are typically tightly regulated with respect to their spatio-temporal patterns and exquisite selectivity, which in turn determine the range and duration of signaling events in cells (De Las Rivas & Fontanillo, 2010; Scott & Pawson, 2009; Yang, Wagner, & Beli, 2015). For example, upon agonist-activation, G protein-coupled receptors (GPCRs) undergo a conformational change followed by coupling to heterotrimeric G proteins (Gilman, 1987; Maguire, Van Arsdale, & Gilman, 1976). This leads to GDP/GTP exchange on Gα sub-unit and dissociation of Gα from Gβγ sub-units followed by activation of their downstream effectors such as adenylyl cyclase and ion channels (Bockaert & Pin, 1999; Gilman, 1987). Subsequently, GPCRs are phosphorylated by GRKs (GPCR kinases) and other kinases which in turn promote the recruitment of multifunctional proteins called arrestins (Inglese, Freedman, Koch, & Lefkowitz, 1993; Ranjan, Dwivedi, Baidya, Kumar, & Shukla, 2017). Arrestins typically block G-protein

*Corresponding author: arshukla@iitk.ac.in.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

coupling through steric hindrance on one hand, and mediate receptor endocytosis on the other via nucleating the assembly of the components of clathrin coated endocytosis machinery such as clathrin and adaptin (Freedman & Lefkowitz, 1996; Goodman et al., 1996; Kang, Tian, & Benovic, 2014).

There are a large number of methods available to measure protein–protein interaction in cellular context such as Bioluminescence Resonance Energy Transfer (BRET), Fluorescence Resonance Energy Transfer (FRET), Proximity Ligation assay (PSA) etc. (Berggard, Linse, & James, 2007; Miura, 2018; Phizicky & Fields, 1995). In vitro detection and characterization of protein–protein interaction can be carried out using label free approaches such as Isothermal Calorimetry (ITC) and Surface Plasmon Resonance (SPR) among others (Lin & Wu, 2019; Nguyen, Park, Kang, & Kim, 2015). While these methods yield immensely useful information on precise affinity of interactions, thermodynamic parameters and interaction stoichiometry, they require sophisticated instrumentation and expertise. As an alternative approach, ELISA and co-immunoprecipitation (co-IP) based assays are more frequently used across a large number of laboratories for qualitative assessment of protein–protein interaction (Lequin, 2005; Lin & Lai, 2017).

Typically, proteins of interest are genetically tagged with affinity tags at either the N- or the C-terminus, followed by their expression and purification. Subsequently, either affinity resins (e.g., Ni-NTA for Histidine tag) or antibody-based approaches (e.g., FLAG M2 antibody agarose for FLAG tag) can be used to capture and detect their interaction using standard ELISA and co-IP assays. In some cases, however, genetic fusion of affinity tags may compromise the activity and functionality of proteins of interest and thereby, limits the utility of this approach. Moreover, this type of approach cannot be employed for proteins isolated from their native sources. Although, using antibodies against proteins of interest provide an alternative strategy in ELISA and co-IP assays, suitable antibodies may not always be available for this purpose. Therefore, a simple, modular and adaptable strategy to capture and detect purified proteins can be of significant interest to many laboratories engaged in protein biochemistry research.

Here, we present a step-by-step protocol for biotinylating purified proteins via chemical conjugation of biotin reagents which can significantly facilitate the detection and characterization of protein–protein interactions in vitro. Considering its small size, biotin-conjugation should not typically interfere with the biological activity of the proteins and it offers a modular approach for labeling the proteins without any genetic modifications. This protocol is based on our previously published proof-of-principle studies using biotinylation of several proteins involved in GPCR signaling and regulatory paradigms (Ghosh et al., 2017, 2019; Kumari et al., 2016, 2017).

2 Materials and methods

2.1 Reagents for biotin labeling of purified proteins

EZ-Link Sulfo-NHS-SS-Biotin (Thermofisher Scientific; cat no. 21331 or A39258)

DMSO (Dimethyl sulfoxide)

Purified protein of interest to be biotinylated

Tris–HCl

HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)

NaCl

PD-10 Desalting Column, with Sephadex G-25 resin, 1.0–2.5 mL samples

Vivaspin protein concentrator spin columns

Slide-A-Lyzer™ Dialysis Cassettes, 3.5K MWCO, 3 mL

Syringe (5 mL)

Regular micro-tips (1000, 200, 20 μL)

Microcentrifuge tubes (1.5, 2 mL)

Centrifuge tubes (15 mL)

Glycerol (for freezing protein sample)

Bradford reagent (Sigma; cat no. B6916).

2.2 Reagents for capture and detection of biotinylated proteins

Streptavidin beads

Magnetic separation rack

DTT (Dithiothreitol)

Pre-cast or manually prepared acrylamide gels for SDS-PAGE

Suitable power supply and SDS-PAGE apparatus

Protein molecular weight marker

Chemicals for different buffers (Tris–HCl, Glycine, Methanol, Tween-20, Hydrochloric acid)

Coomassie Brilliant blue

Suitable power supply and Western blot apparatus for Semi-Dry transfer

PVDF membrane for protein transfer

Bovine Serum Albumin (For membrane blocking)

Streptavidin–horseradish peroxidase antibody (Genscript)

Enhanced chemi-luminescence (ECL) reagents

Chemi-Doc imaging system

96-well Immuno plates

H_2SO_4

3,3,5,5-Tetramethylbenzidine or TMB substrate

Plate reader (Victor 4X multimode plate reader, Perkin Elmer)

2.3 Method

2.3.1 Biotin labeling of purified proteins

2.3.1.1 Preparing the working stock solution of the biotinylation reagent: There are several biotinylation reagents commercially available with or without cleavable biotin group, different spacer arms and functional groups. The protocol described below is based on our previous studies with Sulfo-NHS-SS-Biotin reagent that has a disulfide bond in its spacer arm allowing cleavage by reducing agents (Fig. 1).

2.3.1.1.a Take out the EZ-Link Sulfo-NHS-SS-Biotin from −20°C freezer and equilibrate it to room temperature before opening the vial for weighing. Alternatively, no-weigh vials can also be used to avoid repeated freeze thaw of powder stock of the reagent.

Caution!: Sulfo-NHS-SS-Biotin is hygroscopic in nature. Hence, it is important to bring down the reagent to ambient temperature before opening the vial to avoid moisture retention and tightly cap the vial after usage. It is advisable to store it with desiccant.

2.3.1.1.b Weigh a small amount of reagent, adequate enough for the labeling reaction. Prepare a 10 mM stock in DMSO. An aqueous stock can also be prepared as Sulfo-NHS-SS-Biotin is water soluble.

Caution!: The NHS ester group hydrolyzes readily and therefore, the aqueous solution should always be prepared fresh prior to use and residual reconstituted reagent should be discarded. The DMSO stock, however, can be stored in small aliquots for up to a couple of months at −80°C without a significant loss in labeling efficiency. Repeated freeze–thaw cycle of the stock solution should be avoided.

2.3.2 Preparing the protein sample for biotinylation

2.3.2.1 Determine the concentration of the protein to be labeled using any standard assay. Typically, the concentration should be >1 mg/mL for efficient labeling. If the concentration of the protein sample is low, it can be concentrated using VivaSpin protein concentrator spin columns. Protein of interest should be purified to a reasonable level (e.g., >90%) in order to

avoid labeling of non-specific impurities which may interfere with subsequent experiments.

Caution!: The protein should be resuspended in a buffer that is free of primary amines (Tris or Glycine) as they will react with the biotinylation reagent and therefore, impair the efficiency of the biotinylation reaction. If the protein buffer has amines, then either dialyze in compatible buffer (PBS or HEPES-NaCl) or consider performing size exclusion chromatography using PD-10 desalting columns. The buffer should also be free of any reducing agent to prevent cleavage of disulfide bond. The ester bond is labile at higher pH (10 – 11), and therefore, the optimal pH range is typically between 7 and 9.

2.3.3 Biotinylation of the protein of interest

2.3.3.1 Incubate the protein sample, preferably in a volume of less than 3 mL with 20-fold molar excess of the biotinylation reagent for 30–60 min at room temperature. Alternatively, the labeling reaction can be carried out for longer time periods at 4°C or on ice.

Note: It is advisable that the efficiency of biotinylation is compared and optimized using a range of concentrations of the biotinylation reagent, for example, 1:5, 1:10, 1:20, 1:50 and 1:100 M ratios. In our studies, we have observed that proteins are efficiently biotinylated at a 20-fold molar excess. The labeling efficacy depends on the accessibility of the amine-group at the N-terminus of the protein and the number of exposed Lysine/Arginine residues that harbor free amine-group for biotinylation using NHS reagent.

Note: In addition to NHS-Biotin reagent, a range of other biotinylation reagents are also commercially available which label the proteins of interest through other functional groups such as Iodoacetyl-LC-Biotin (sulfhydryl-reactive groups), Amine-PEG3-Biotin (Carboxyl reactive group) etc.

2.3.3.2 After the completion of the biotinylation reaction, quench the unreacted biotinylation reagent with 50 mM Tris, pH 8.5. Typically, 1/10th of the reaction volume is sufficient to quench the free biotinylation reagent.

2.3.4 Removal of the excess biotinylation reagent—The unreacted biotinylation reagent can be removed either through desalting/gel-filtration column or dialysis.

2.3.4.1 Removal of unreacted biotinylation reagent through buffer exchange on PD-10 desalting columns

2.3.4.1a Remove the bottom seal of the PD-10 column, place it on the column stand and let the storage buffer pass out by gravity flow.

2.3.4.1b Equilibrate the PD-10 column with approximately 25 mL of PD-10 buffer (20 mM HEPES, pH 7.4, 100 mM NaCl) and discard the flow through.

2.3.4.1c Load the biotinylated protein sample on top of the resin bed and collect the flow through. Typically, the sample is loaded in a volume of 2.5 mL, followed by the collection of flow-through and subsequent elution in 3.5 mL with elution buffer in a falcon tube placed below the column. In case, the sample volume is less than 2.5 mL, add protein sample first, let it pass through, and then add buffer to complete the total volume of 2.5 mL. Afterwards, collect seven fractions of 0.5 mL each in microcentrifuge tubes, and assess the presence of protein in the eluted fractions using Bradford dye through colorimetric reaction. Pool the eluted fractions containing the biotinylated protein and store as required for subsequent experiments.

Note: Instead of PD-10 based procedure, a standard size exclusion chromatography may also be used to remove unreacted biotinylation reagent.

2.3.4.2 Removal of unreacted biotinylation reagent through dialysis

2.3.4.2a Take a dialysis cassette and hydrate it by immersing in dialysis buffer (20 mM HEPES, pH 7.5 and 100 mM NaCl) for about 2–5 min.

2.3.4.2b From one end of the cassette, inject the sample using a 24 gauge syringe. Withdraw the air from the dialysis cassette while the needle is still inside so that the sample touches maximum surface of the membrane. Insert the cassette into the groove of the buoy and immerse in dialysis buffer. Dialyze for 12–16 h at 4°C, preferably with replacement of dialysis buffer once at 6–8 h to maximize the dialysis efficiency.

Caution!: It is important to inject or withdraw the sample carefully from the dialysis cassette to avoid membrane rupture. It is advisable to check the cassette for leakage by injecting sterilized water.

2.3.4.2c Collect the sample from dialysis cassette by first inflating it by injecting air into the dialysis bag from one of the side ports using a syringe so that the sample collects at one place and can be sucked into the syringe.

2.3.4.2d Measure the concentration of biotinylated protein using standard assays, prepare small aliquots depending on subsequent experiments, flash-freeze the aliquots in liquid nitrogen and store at −80 °C.

2.3.5 Detection of biotinylated proteins using Western blotting—Biotinylated proteins can be detected using standard Western blotting assay using HRP-coupled streptavidin or anti-biotin antibodies.

2.3.5.1 Separate the biotinylated proteins using standard SDS-PAGE followed by transfer on PVDF/nitrocellulose membrane.

2.3.5.2 After incubating the membrane with 5% BSA in 1 × TBST for 1 h at room temperature to block non-specific sites, incubate it further in HRP-coupled

streptavidin solution (final concentration 0.2 μg/mL) for 2 h at room temperature or overnight at 4°C.

2.3.5.3 Wash the membrane 3 times (5 min each) with 1 × TBST. Add 1–2 mL of ECL (Enhanced Chemiluminescence) substrate sufficient enough to cover the entire membrane and visualize the proteins using a standard CCD camera attached to the ChemiDoc imaging system.

Note: A typical example of detection of biotinylated adaptin (β-appendage of Adaptin2, 592-951 amino acid residues) is presented in Fig. 2A.

2.3.6 Detection of biotinylated proteins using ELISA—Biotinylated proteins can also be detected using standard ELISA assay by means of HRP-coupled streptavidin or anti-biotin antibodies.

2.3.6.1 Immobilize varying amounts of biotinylated proteins on MaxiSorp plates via adsorption. As a negative control, also immobilize non-biotinylated protein at an amount equal to the maximal amount of biotinylated protein. Incubate for 1 h at room temperature.

2.3.6.2 Block the non-specific binding sites in the well using 200 μL of 1% BSA (prepared in 20 mM HEPES, pH 7.5, 100 mM NaCl) in each well for 1 h at room temperature.

2.3.6.3 Incubate the wells with 100 μL of HRP-coupled streptavidin (final concentration of 0.2 μg/mL) for 1 h at room temperature. Subsequently, wash the wells 3–5 times with 100 μL of washing buffer (20 mM HEPES, pH 7.5, 100 mM NaCl, 0.01% MNG). Detect the biotinylated protein by adding 50 μL of TMB-ELISA substrate per well and quench the reaction by adding 50 μL of 2 M H_2SO_4 in each well. Upon addition of TMB-ELISA, a blue color will develop which turns yellow after quenching the reaction with H_2SO_4.

2.3.6.4 Measure the absorbance at 450 nM using a Plate reader (Victor 4X multimode plate reader, Perkin Elmer).

Note

(i) Blocking with BSA or non-fat dry milk reduces signal to noise ratio by blocking non-specific sites. It is advisable to use 200 μL of blocking agent per well to minimize the non-specific binding surface.

(ii) It is important to quench the colorimetric reaction before the signal reaches saturation which can otherwise negatively impact accurate estimation of biotinylated protein.

(iii) It is important to systematically optimize the dilution of HRP-coupled streptavidin to allow the detection of biotinylated protein with suitable signal to noise ratio.

(iv) A typical example of detection of biotinylated Clathrin terminal-domain is presented in Fig. 2B.

(v) In experiments where the bait protein is immobilized on ELISA plate, its interaction with the protein of interested, which is biotinylated, can be detected using HRP-coupled streptavidin following a protocol mentioned above.

2.3.7 Capture and immobilization of biotinylated proteins using magnetic streptavidin beads—Biotinylated proteins can be captured on either standard streptavidin agarose/sepharose beads or magnetic beads coated with streptavidin for subsequent use in protein–protein interaction studies. Here, we present a protocol for capturing biotinylated proteins using magnetic streptavidin beads.

2.3.7.1 Equilibrate magnetic streptavidin beads in equilibration buffer (20 mM HEPES, pH 7.5, 100 mM NaCl) by three cycles of suspension and precipitation using magnetic separation stand.

2.3.7.2 Incubate approximately 5 μg of biotinylated protein in a volume of 100 μL with the pre-equilibrated beads for 10–15 min at room temperature with gentle shaking. Typically, 50 μL of the original magnetic streptavidin bead slurry should be sufficient to capture 5–10 μg of biotinylated proteins.

2.3.7.3 Use the magnetic separation rack to collect the flow-through and take out 20 μL for running on SDS-PAGE.

2.3.7.4 Wash the beads 2–3 times with 100 μL of the washing buffer (20 mM HEPES, pH 7.5, 100 mM NaCl) and collect wash fractions.

2.3.7.5 Elute the bound protein in 100 μL elution buffer (20 mM HEPES, pH 7.5, 100 mM NaCl, 100 mM DTT) by incubating the beads in this buffer for 5–10 min at room temperature with gentle shaking followed by collection of flow through.

2.3.7.6 Analyze the capture and elution of biotinylated proteins by running the samples collected from every step on SDS-PAGE and visualize using Coomassie brilliant blue staining.

Note

(i) Before incubating protein sample with beads in 2.3.7.1, take out 20 μL of the sample and add reducing SDS-PAGE sample running dye to it. Samples should be collected at every step to run SDS-PAGE in order to determine the extent of biotinylation.

(ii) A typical schematic of the capture and elution strategy is presented in Fig. 3A. An example of capture and elution of biotinylated Clathrin terminal-domain is presented in Fig. 3B.

3 Potential problems, troubleshooting guidelines and additional considerations

3.1 Inefficient labeling of protein of interest may arise from a number of reasons including a sub-optimal pH of the buffer, presence of primary-amines and reducing agent in the buffer, or the quality of the biotinylation reagent stock.

3.1.1 The optimal pH range of the buffer is between 7 and 9, and therefore, the purified protein should be prepared in a buffer of this pH range.

3.1.2 Purified protein should not have any contamination of primary amines or reducing agents in the buffer, and therefore, the protein should be purified in appropriate buffer or buffer exchange should be carried out effectively.

3.1.3 Although solution stocks of biotinylated reagents prepared in DMSO can be stored at –80°C, it is advisable to prepare fresh stock every time just before use to avoid any issues with degradation of the biotinylation reagent.

3.2 It should be noted that the protocol presented here is not designed for site-directed biotinylation, and therefore, the functionality and activity of biotinylated proteins should be characterized before using them in subsequent experiments. It is advisable to optimize the biotinylation conditions in such a way that the stoichiometry of biotinylation is typically 1:1, i.e., 1 biotin molecule per molecule of protein.

Acknowledgments

The research program in Dr. Shukla's laboratory is supported by an Intermediate Fellowship of the Wellcome Trust/DBT India Alliance Fellowship (grant number IA/I/14/1/501285) awarded to A.K.S.; the Science and Engineering Research Board (SERB) (EMR/2017/003804); Innovative Young Biotechnologist Award from the Department of Biotechnology (DBT) (BT/08/IYBA/2014-3); and the Indian Institute of Technology, Kanpur. A.K.S. is an Intermediate Fellow of Wellcome Trust/DBT India Alliance, EMBO Young Investigator, and Joy Gill Chair Professor. H.D.-A. and A.S. are supported by National Post-Doctoral Fellowship of SERB (PDF/2016/002930) and Early Career Fellowship of the Wellcome Trust/DBT India Alliance Fellowship (grant number IA/E/17/1/503687), respectively.

References

Berggard T, Linse S, James P. Methods for the detection and analysis of protein-protein interactions. Proteomics. 2007; 7(16):2833–2842. [PubMed: 17640003]

Bockaert J, Pin JP. Molecular tinkering of G protein-coupled receptors: An evolutionary success. The EMBO Journal. 1999; 18(7):1723–1729. [PubMed: 10202136]

Braun P, Gingras AC. History of protein-protein interactions: From egg-white to complex networks. Proteomics. 2012; 12(10):1478–1498. [PubMed: 22711592]

De Las Rivas J, Fontanillo C. Protein-protein interactions essentials: Key concepts to building and analyzing interactome networks. PLoS Computational Biology. 2010; 6(6):e1000807. [PubMed: 20589078]

Freedman NJ, Lefkowitz RJ. Desensitization of G protein-coupled receptors. Recent Progress in Hormone Research. 1996; 51:319–351. [PubMed: 8701085]

Ghosh E, Dwivedi H, Baidya M, Srivastava A, Kumari P, Stepniewski T, et al. Conformational sensors and domain swapping reveal structural and functional differences between beta-arrestin isoforms. Cell Reports. 2019; 28(13):3287–3299 e6. [PubMed: 31553900]

Ghosh E, Srivastava A, Baidya M, Kumari P, Dwivedi H, Nidhi K, et al. A synthetic intrabody-based selective and generic inhibitor of GPCR endocytosis. Nature Nanotechnology. 2017; 12(12):1190–1198.

Gilman AG. G proteins: Transducers of receptor-generated signals. Annual Review of Biochemistry. 1987; 56:615–649.

Goodman OB Jr, Krupnick JG, Santini F, Gurevich VV, Penn RB, Gagnon AW, et al. Beta-arrestin acts as a clathrin adaptor in endocytosis of the beta2-adrenergic receptor. Nature. 1996; 383(6599):447–450. [PubMed: 8837779]

Inglese J, Freedman NJ, Koch WJ, Lefkowitz RJ. Structure and mechanism of the G protein-coupled receptor kinases. The Journal of Biological Chemistry. 1993; 268(32):23735–23738. [PubMed: 8226899]

Kang DS, Tian X, Benovic JL. Role of beta-arrestins and arrestin domain-containing proteins in G protein-coupled receptor trafficking. Current Opinion in Cell Biology. 2014; 27:63–71. [PubMed: 24680432]

Kumari P, Srivastava A, Banerjee R, Ghosh E, Gupta P, Ranjan R, et al. Functional competence of a partially engaged GPCR-beta-arrestin complex. Nature Communications. 2016; 7

Kumari P, Srivastava A, Ghosh E, Ranjan R, Dogra S, Yadav PN, et al. Core engagement with beta-arrestin is dispensable for agonist-induced vasopressin receptor endocytosis and ERK activation. Molecular Biology of the Cell. 2017; 28(8):1003–1010. [PubMed: 28228552]

Lequin RM. Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). Clinical Chemistry. 2005; 51(12):2415–2418. [PubMed: 16179424]

Lin JS, Lai EM. Protein-protein interactions: Co-immunoprecipitation. Methods in Molecular Biology. 2017; 1615:211–219. [PubMed: 28667615]

Lin K, Wu G. Isothermal titration calorimetry assays to measure binding affinities in vitro. Methods in Molecular Biology. 2019; 1893:257–272. [PubMed: 30565139]

Maguire ME, Van Arsdale PM, Gilman AG. An agonist-specific effect of guanine nucleotides on binding to the beta adrenergic receptor. Molecular Pharmacology. 1976; 12(2):335–339. [PubMed: 4726]

Miura K. An overview of current methods to confirm protein-protein interactions. Protein and Peptide Letters. 2018; 25(8):728–733. [PubMed: 30129399]

Nguyen HH, Park J, Kang S, Kim M. Surface plasmon resonance: A versatile technique for biosensor applications. Sensors (Basel). 2015; 15(5):10481–10510. [PubMed: 25951336]

Nooren IM, Thornton JM. Diversity of protein-protein interactions. The EMBO Journal. 2003; 22(14):3486–3492. [PubMed: 12853464]

Phizicky EM, Fields S. Protein-protein interactions: Methods for detection and analysis. Microbiological Reviews. 1995; 59(1):94–123. [PubMed: 7708014]

Ranjan R, Dwivedi H, Baidya M, Kumar M, Shukla AK. Novel structural insights into GPCR-beta-arrestin interaction and signaling. Trends in Cell Biology. 2017; 27(11):851–862. [PubMed: 28651823]

Scott JD, Pawson T. Cell signaling in space and time: Where proteins come together and when they're apart. Science. 2009; 326(5957):1220–1224. [PubMed: 19965465]

Yang J, Wagner SA, Beli P. Illuminating spatial and temporal organization of protein interaction networks by mass spectrometry-based proteomics. Frontiers in Genetics. 2015; 6:344. [PubMed: 26648978]

Europe PMC Funders Author Manuscripts

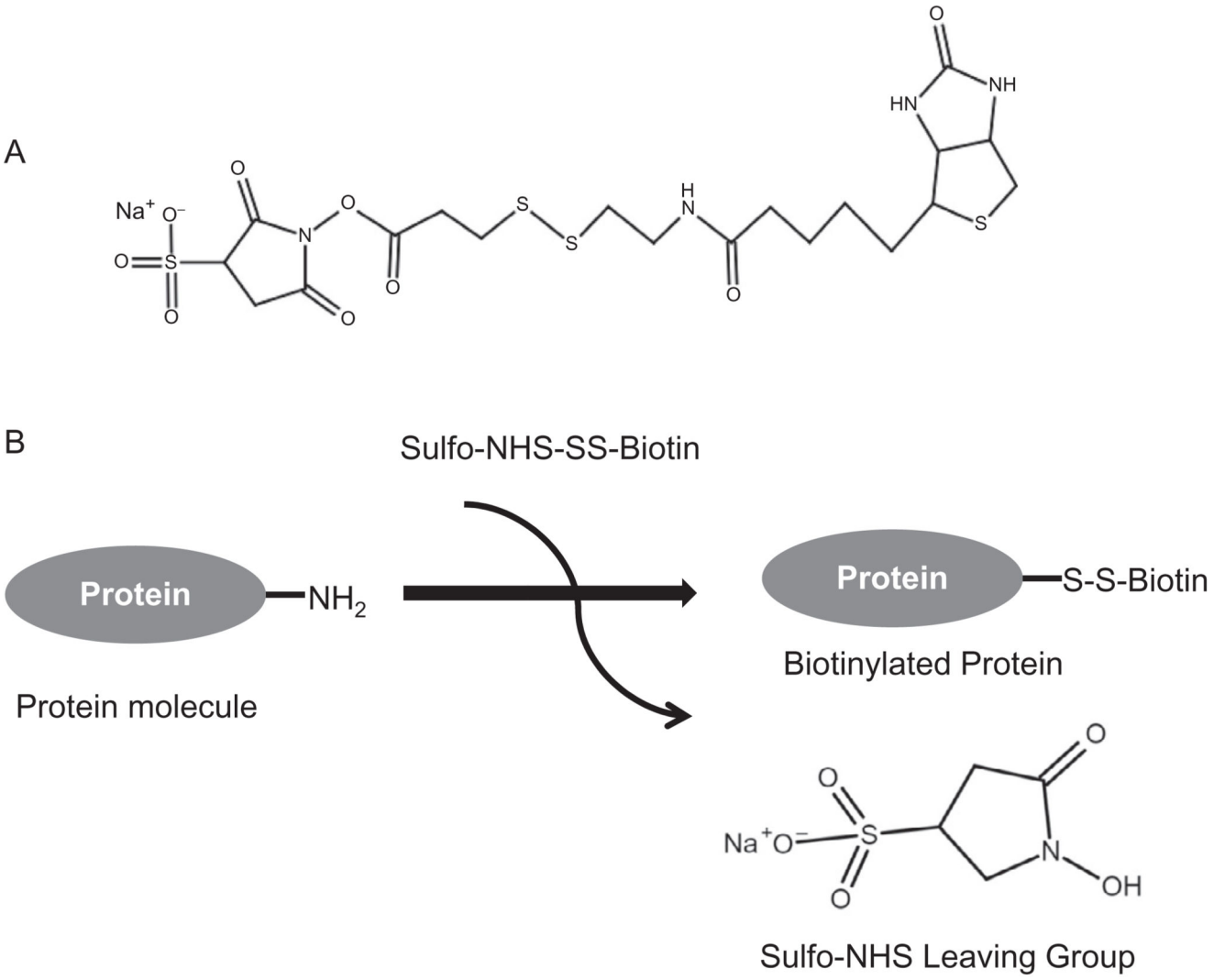

Fig. 1. Chemical structure of Sulfo-NHS-SS-biotin and a schematic of a typical biotinylation reaction.

(A) The Sulfo-NHS-SS-Biotin molecule is a primary amine-reactive, thiol cleavable molecule with long spacer arm of 24.3 Å. (B) The N-terminus primary amine and epsilon amino group of lysine residues react with ester group of Sulfo-NHS-SS-biotin molecule to form an amide bond, which covalently links biotin to the protein of interest. The cleaved Sulfo-NHS group can be subsequently separated though size exclusion chromatography or dialysis.

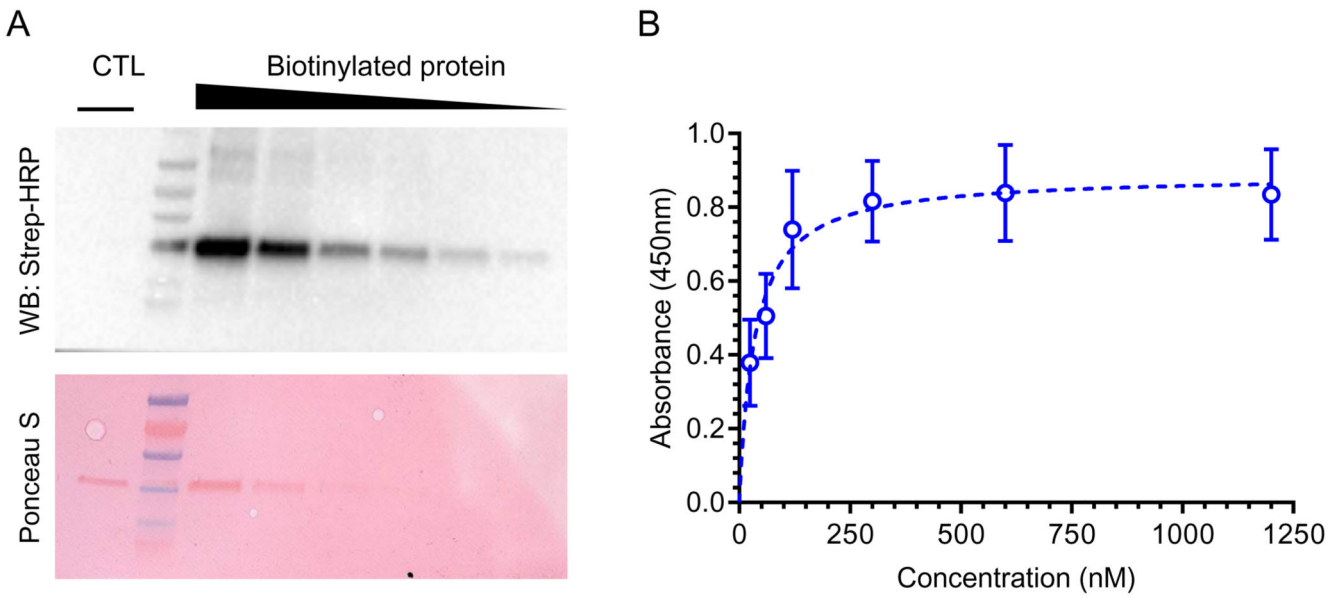

Fig. 2. Detection of biotinylated protein using Western Blotting and ELISA approach.

(A) Varying concentrations of purified and biotinylated β-appendage of adaptin2 (residues from 592 to 951) was separated on SDS-PAGE followed by Western blot based detection using HRP-coupled anti-streptavidin antibody. Non-biotinylated protein was used as negative control (CTL). The upper blot shows the detection of biotinylated protein in a dose-dependent manner (detected up to 1/50th of the highest concentration). Non-biotinylated protein was not detected under the same experimental conditions. The lower panel shows Ponceau S staining of the blot to visualize the total protein loaded on the gel. Due to low sensitivity of Ponceau S, lower amounts of biotinylated adaptin2 are not visible in the lower panel. (B) Biotinylated proteins can also be detected using ELISA based approach as presented here using biotinylated clathrin (terminal-domain) as a case example. Varying amounts of biotinylated protein were immobilized in MaxiSorp 96-well plate followed by blocking of potential non-specific binding sites in the wells with 1% BSA. Subsequently, immobilized protein was detected using HRP-coupled anti-streptavidin antibody as indicated in the main text. Non-biotinylated protein was used as negative control and the signals were normalized.

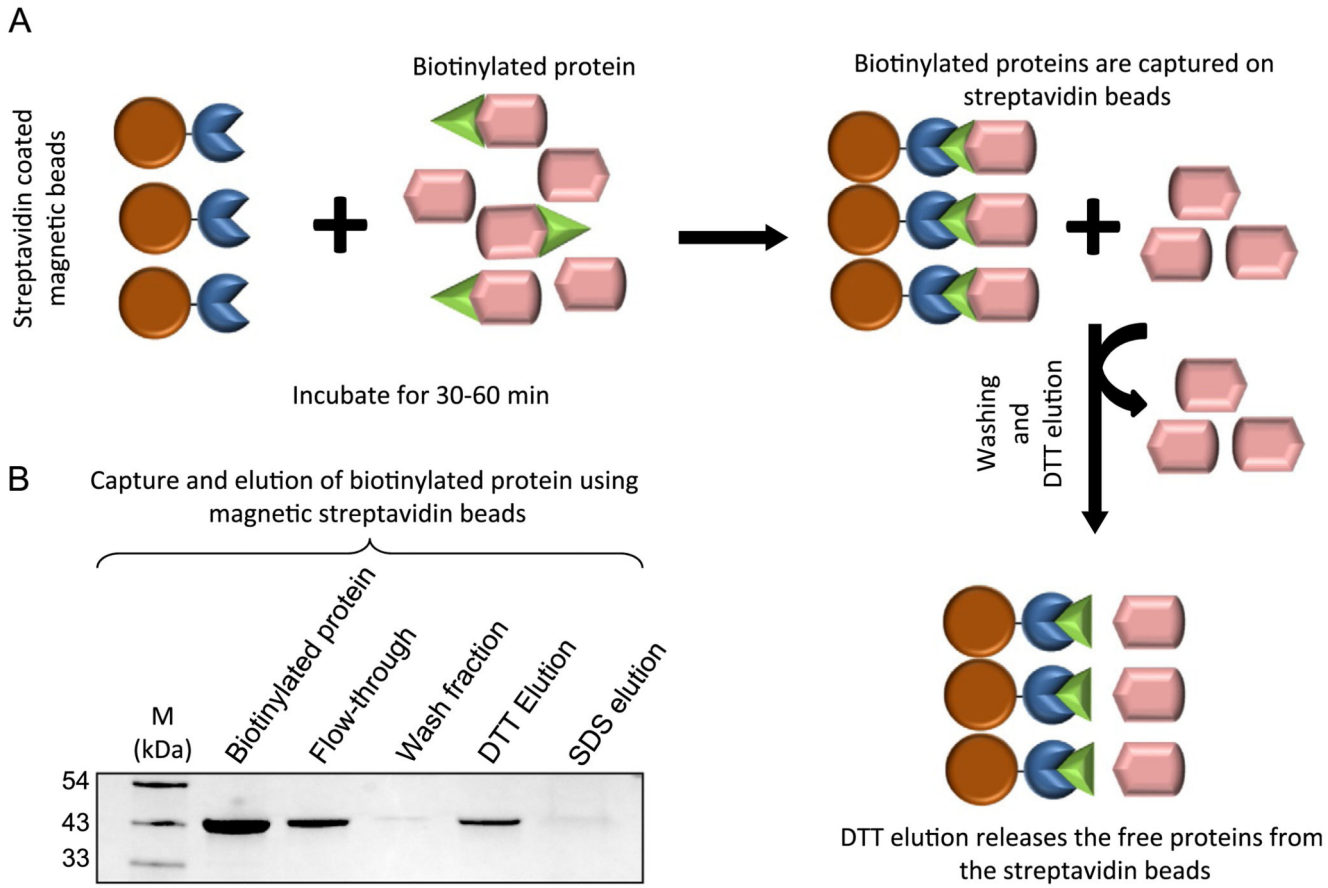

Fig. 3. Capturing biotinylated proteins using magnetic Streptavidin beads.

(A) A schematic flow-diagram describing the process of capturing biotinylated proteins using magnetic streptavidin beads and subsequent elution using a reducing agent. (B) A typical profile of capture and elution experiment using biotinylated clathrin terminal-domain as a case example. Samples from different steps were separated on SDS-PAGE followed by Coomassie staining for visualization. SDS elution is carried out to test whether any biotinylated protein is still present on the beads. Densitometry-based quantification can be performed to measure the apparent capture and elution efficiency.

Europe PMC Funders Author Manuscripts