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## A Simple Elution Strategy for Biotinylated Proteins Bound to Streptavidin Conjugated Beads using Excess Biotin and Heat

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### Abstract

Protein-protein interactions are the molecular basis of cell signaling. Recently, proximity based biotin identification (BioID) has emerged as an alternative approach to traditional co-immunoprecipitation. In this protocol, a mutant biotin ligase promiscuously labels proximal binding partners with biotin, and resulting biotinylated proteins are purified using streptavidin conjugated beads. This approach does not require preservation of protein complexes *in vitro*, making it an ideal approach to identify transient or weak protein complexes. However, due to the high affinity bond between streptavidin and biotin, elution of biotinylated proteins from streptavidin conjugated beads requires harsh denaturing conditions, which are often incompatible with downstream processing. To effectively release biotinylated proteins bound to streptavidin conjugated beads, we designed a series of experiments to determine optimal binding and elution conditions. Interestingly, the concentrations of SDS and IGEPAL-CA630 during the incubation with streptavidin conjugated beads were the key to effective elution of biotinylated proteins using excess biotin and heating. This protocol provides an alternative method to isolate biotinylated proteins from streptavidin conjugated beads that is suitable for further downstream analysis.

### Keywords

$\alpha$ -catenin; BirA; BioID; protein purification; proximal biotinylation; streptavidin

### 1. Introduction

Protein-protein interactions determine how signals are transmitted in and between cells. Rapid changes in cell responses are often initiated by chemical or mechanical means and based on reversible reactions among proteins. Many of these protein interactions are weak and transient, making them difficult to isolate and identify with traditional co-immunoprecipitation techniques, which rely on stable protein interactions during the *in vitro* isolation of bound proteins. This inherent nature of the assay prevents detection of protein interactions that are initiated by mechanical forces. To preserve weak and transient interactions, previous biochemical approaches have used *in situ* chemical cross-linking of

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interacting proteins to stabilize weak protein interactions. Unfortunately, this cross-linking technique is prone to increase insoluble fractions or non-specific cross-linking. Instead, a recent study has demonstrated an alternative method to identify weak protein interactions [1].

Proximity dependent *biotin identification* (BioID) is one of the experimental approaches based on proximal labeling [2–6]. In this technique, the protein of interest is tagged with a promiscuous biotin ligase. Similar to the wild-type BirA from *Escherichia coli*, this mutant BirA\* (R118G) converts soluble biotin to biotinoyl-5'-AMP (bioAMP) using ATP. The wild-type BirA binds tightly to bioAMP and guides a biotinylation reaction of bioAMP to the primary amine of the specific target sequence [7]. Unlike the wild-type BirA, the mutant BirA\* has a reduced affinity toward bioAMP, and consequently, this highly reactive bioAMP is readily released from the mutant BirA\* and reacts with a primary amine of nearby proteins [8]. These biotinylated proteins are extracted and subsequently purified using streptavidin conjugated beads (Fig. 1A). This proximal biotinylation has been successfully used to identify novel protein interactions [see reviews: 4, 9], and has been shown as complementary to traditional affinity purification approaches [10]. Interests in this approach have led to improved variants of BioID proteins: BioID2, a smaller biotin ligase from *Aquifex aeolicus* [11], and a split-BioID as a dimerization dependent biotin ligase [12, 13].

However, the benefit of the strong streptavidin-biotin bond for ease of purification proves to be a disadvantage for elution from streptavidin conjugated beads. The elution of biotinylated proteins requires harsh, denaturing conditions to release the proteins from the beads [14, 15]. These conditions are often incompatible in downstream processing. To avoid such complications, biotinylated proteins can be enzymatically digested and removed from the beads [16, 17], but this leads to undesirable streptavidin peptides in the elution. While streptavidin contamination can be tolerated in some applications, it may interfere with identification of low abundant proteins. To maximize the yield of biotinylated proteins, we sought to identify ideal binding and eluting conditions for promoting elution of biotinylated proteins.

## 2. Materials and Methods

### 2.1 Expression of proximal biotin ligase in MDCK cells

Mutant BirA\* (R118G) promiscuously biotinylates nearby proteins and labels potential binding partners *in situ*, allowing for subsequent purification using streptavidin conjugated beads (Fig. 1A). The BirA\*- $\alpha$ -catenin expressing MDCK cell line was generated and characterized previously and cultured in DMEM with 200  $\mu$ g/ml G418 [18]. The cell line was analyzed with Western blots using  $\alpha$ -catenin (15D9, Alexis Biochemical), GFP (TP401, Torrey Pines Biolabs), and tubulin (DM1 $\alpha$ , Sigma) antibodies (Fig. 1B).

### 2.2 *In situ* biotinylation and cell lysate preparation

For biotinylation analysis, 50  $\mu$ M biotin was added to BirA\*- $\alpha$ -catenin expressing cells for 20–24 hours. Using three different lysis buffers with varying detergent compositions, biotinylated proteins were extracted from cells. Lysis buffer 1 was composed of 50 mM Tris

HCl pH 7.4, 250 mM NaCl, 0.1 % SDS, 0.25 mM DTT, and 1 % Triton X100 [17]. Lysis buffer 2 was composed of 50 mM Tris HCl pH 7.4, 150 mM NaCl, 0.4 % SDS, 1 % IGEPAL-CA630 (Nonidet P-40), 1 mM EGTA, 1.5 mM MgCl<sub>2</sub> [16]. Lysis buffer 3 was composed of 20 mM Tris HCl pH 7.4, 200 mM NaCl, 0.5 % IGEPAL-630, 2.5 mM MgCl<sub>2</sub>. All lysis buffers were supplemented with a protease inhibitor cocktail (Sigma or Millipore) and lysis buffer 2 was also supplemented with 1 µl/ml benzonase (Millipore). Briefly, cells were washed with PBS thrice then scraped with lysis buffer 1 or 3. Alternatively, cells were washed with PBS and centrifuged, then pelleted cells were re-suspended in lysis buffer 2. For lysis buffer 2, cell lysates were incubated for 30 minutes at 4 °C. Lysates were then sonicated at 10–30 % duty ratio for one minute, and centrifuged to separate the soluble fraction from the insoluble pellet. Insoluble pellets were resuspended with 100 µl of 2× sample buffer for subsequent analysis. The supernatants (soluble fraction) were collected, and their concentrations were measured with a RC/DC protein assay kit (Bio-Rad). These procedures were based on previously published protocols [16, 17].

### 2.3 Biotinylated protein purification

To isolate biotinylated proteins from cell lysates, 200 µl of cell lysate at 0.5 mg/ml was incubated with 20 µl of Dynabeads MyOne Streptavidin C1 (Invitrogen), Dynabeads M280 Streptavidin (Invitrogen), or SpeedBead Magnetic Neutravidin Coated Particles (Millipore), and rotated overnight at 4 °C. First, beads were washed with the respective lysis buffer and transferred to new tubes. The beads were washed again with the 2 % SDS in 50 mM Tris HCl pH 7.4, then lysis buffer twice. Biotinylated proteins were eluted from the beads with 30 µl of 25 mM biotin at 95 °C for 5 minutes. Additional 30 µl of 25 mM biotin was added to wash and elute remaining biotinylated proteins. Finally, 80 µl of 4× sample buffer (200 mM Tris HCl pH 6.8, 40 % glycerol, 8% SDS, 8 % β-mercaptoethanol, 0.04 % bromophenol blue) was added to the beads and heated at 95 °C for 5 minutes. The standard elution protocol was followed in all experiments except the analysis shown in Figure 3. Based on Bradford protein quantification analysis (Bio-Rad) of eluted samples, the maximum amount of biotinylated proteins eluted was ~ 0.1 % of cell lysates. In these eluted fraction, biotin concentration was ~ 500 pmole per mg of Dynabead C1 or 50 pmol per mg of total eluted proteins, determined by Fluorescence Biotin Quantification Kit (Pierce Biotech). All reagents were purchased from either Sigma-Aldrich or Fisher Scientific unless noted otherwise.

### 2.4 Western blot and silver stained SDS-PAGE analysis of protein samples

Protein samples were analyzed with Western blots using streptavidin conjugated with Horse Radish Peroxidase, HRP (Invitrogen), and detected with a WesternBright Quantum Chemiluminescence Kit (Advansta). Protein concentrations among the samples were compared based on the lane's total intensity quantified using Image Lab (Bio-Rad). A silver stain kit (Pierce Biotechnology) was used to visualize all proteins in the samples with an Alexa Fluor 568 conjugated streptavidin (Invitrogen) as a streptavidin control.

### 3. Results

#### 3.1 Efficiency of protein solubility and elution depends on lysis buffer

Using MDCK epithelial cells expressing BirA\* tagged  $\alpha$ -catenin [18], we analyzed the biotinylation of proteins surrounding  $\alpha$ -catenin, a component of the E-cadherin complex at cell-cell contacts. The adhesive complexes often associate with the cytoskeleton to regulate the strength and dynamics of cell adhesion. Since cytoskeleton associated proteins are often insoluble, isolation of biotinylated proteins surrounding  $\alpha$ -catenin requires cell lysis conditions capable of solubilizing adhesive protein complexes. Three different lysis buffers were tested to determine which buffer was most favorable to solubilize biotinylated proteins (see Materials and Methods for the compositions of lysis buffers). After sonication and centrifugation of cell lysate to separate the soluble and insoluble pools, the supernatant and pellet samples from each condition were analyzed to detect biotinylated proteins in the soluble (S) and insoluble (I) pools (Fig. 1C). Note that BirA\*-tagged  $\alpha$ -catenin was one of the major biotinylated proteins in these cell lysates (Fig. 1C). As expected, the biotinylated proteins were least soluble in lysis buffer 3 with the least amount of detergent (0.5 % IGEPAL-CA630), while the biotinylated proteins were most soluble in lysis buffer 2 with the highest SDS concentration (0.4 %, Fig. 1C).

Noting that lysis buffer 2 contains both SDS and IGEPAL-CA630, these detergents concentrations in lysis buffer 2 were varied to test protein solubility. The lysates were analyzed for soluble and insoluble pools using Western blots with streptavidin-HRP (Fig. 1D). Since SDS precipitates without IGEPAL-CA630 at 4 °C in this buffer, IGEPAL-CA630 was reduced to 0.2 % from 1 %, but this reduced IGEPAL-CA630 concentration did not change protein solubility (Fig. 1D). In the absence of SDS, the highest amount of insoluble proteins was detected (Fig. 1D, 0% SDS), suggesting that SDS effectively solubilizes biotinylated proteins surrounding  $\alpha$ -catenin.

The solubilized cell lysates from the three different lysis buffers were incubated with streptavidin conjugated magnetic beads (Dynabead C1), then washed with respective lysis buffers and 2 % SDS solution, then eluted using excess biotin (25 mM) and heat (95 °C for 5 minutes – initial, bio), and any remaining bound proteins were eluted again using 4× sample buffer and heat (final, 4×). In both lysis buffer 1 and 3 samples, the bound biotinylated proteins eluted minimally in the initial elution using excess biotin and heat, and most of the bound proteins eluted only in the presence of 4× sample buffer and heat (Fig. 1E). Interestingly, in the lysis buffer 2 sample, the majority of biotinylated proteins eluted with excess biotin and heat, and few remained in the subsequent 4× sample buffer elution (Fig. 1E). Note that the comparable intensities of unbound biotinylated proteins in all samples (see supe in Fig. 1E) suggest that biotinylated proteins bind to streptavidin conjugated beads to a similar extent in all lysate conditions. Yet, biotinylated proteins in lysis buffer 2 eluted at a much higher efficiency than other cell lysates, suggesting that the release of biotinylated proteins from streptavidin conjugated beads strongly depends on the detergent composition of the lysis buffer.

### 3.2 Biotinylated protein elution depends on detergents present during binding

Using soluble fractions isolated with lysis buffer 2 containing different SDS and IGEPAL-CA630 concentrations (see Fig. 1D), these cell lysates were incubated with streptavidin conjugated beads (Dynabead C1), then eluted with excess biotin (25 mM) and heat (95 °C for 5 minutes). In the absence of biotin in growth media (– biotin), three distinct bands, naturally biotinylated carboxylase, eluted in the presence of SDS in lysis buffer (Fig. 2A). Surprisingly, regardless of biotin addition in the growth media, no biotinylated proteins eluted in the absence of SDS (Fig. 2A – 0 % SDS), and most biotinylated proteins eluted with the highest concentrations of detergents (Fig. 2A, – 0.4 % SDS and 1 % IGELPAL-CA630).

To further explore the effects of detergent concentrations on elution efficiency, cells were lysed in the lysis buffer 2 with 0.4 % SDS and 1 % IGEPAL-CA630. This cell lysate was diluted in the same buffer except with different SDS concentrations so that final SDS concentrations were 0.1 %, 0.4 %, and 0.6 % SDS, then the cell lysates were incubated with streptavidin conjugated beads (Dynabead C1). Using excess biotin and heat, very few biotinylated proteins eluted from beads incubated with cell lysate in 0.1 % SDS (Fig. 2B), but biotinylated proteins eluted with 4× sample buffer and heat (Fig. 2B). In contrast, in the presence of 0.4% and 1.6% SDS, biotinylated proteins eluted efficiently with excess biotin and heat (Fig. 2B). These data confirm that SDS concentration during the incubation of biotinylated proteins with streptavidin conjugated beads, not the SDS concentration during cell lysis, is critical for effective biotinylated protein elution (albeit the presence of SDS is required for protein solubility – see Fig. 1C and D). Furthermore, these data suggest that SDS concentration during the incubation of biotinylated proteins with streptavidin should be at least 0.4 % in order to efficiently elute biotinylated proteins using excess biotin and heat.

In addition to SDS concentrations, the effect of IGEPAL-CA630 on elution efficiency was also analyzed (Fig. 2C). As IGEPAL-CA-630 concentration increased, the eluted biotinylated proteins sharply decreased with excess biotin and heat elution (Fig. 2C), while the proteins recovered from the beads using 4× sample buffer increased and the sum of the proteins eluted (sum of excess biotin and 4× SB elution) remained constant (Fig. 2C). Higher IGEPAL-CA630 concentration in the lysis buffer appears to strengthen biotin-streptavidin bonds, thus elution under these conditions require strong denaturants (i.e., 8 % SDS in 4× sample buffer). Overall, these results suggest that biotinylated proteins efficiently bind to streptavidin conjugated beads regardless of the SDS and IGEPAL-CA630 concentration; however, the detergent concentration in the lysis buffer strongly affects the ease of biotinylated protein elution.

### 3.3 Biotinylated protein elution requires both excess biotin and heating

Previous experiments demonstrated that detergent concentrations in lysis buffers alter the efficiency of biotinylated protein elution, but the elution condition was kept the same – excess biotin (25 mM) and heating (95 °C for 5 minutes), then remaining proteins were eluted with denaturing buffer (4× sample buffer) with additional 5 minutes of 95 °C heating. To test whether excess biotin or heat is required for efficient elution of biotinylated proteins from streptavidin conjugated beads, the bound biotinylated proteins were eluted in 0 mM or

25 mM of biotin or 4× sample buffer, and either left at room temperature or at 95 °C for 5 minutes before collecting the elutes. In the absence of biotin in the elution buffer, minimal amount of proteins was detected in both room temperature or 95 °C elutes. In the presence of 25 mM biotin, however, the bound proteins eluted efficiently with heating, but not at room temperature (Fig. 3A, initial, 25 mM). Similar level of bound proteins eluted with 4× sample buffer, but also only with heating (Fig. 3A, initial, 4×).

To elute the remainder of bound proteins from streptavidin conjugated beads, the beads were further incubated with 4× sample buffer and heated at 95 °C for 5 minutes. With 4× sample buffer/heat elution, biotinylated proteins eluted from the room temperature treated beads (Fig. 3A). Interestingly, when the biotinylated proteins bound streptavidin conjugated beads were pre-heated at 95 °C in the absence of biotin, minimal amount of biotinylated proteins eluted (Fig. 3A), suggesting that heating without any biotin deters further elution in 4× sample buffer. In addition, elution buffer with various biotin concentrations (0 – 25 mM) were tested to elute biotinylated proteins off the beads (Fig. 3B). As in the previous streptavidin blot, using 0 mM biotin to elute was completely ineffective at recovering biotinylated proteins in the initial elution as well as in the subsequent 4× sample buffer elution (Fig. 3B). However, 0.025 mM biotin was enough to significantly elute biotinylated proteins off the beads (Fig. 3B).

### 3.4 Other biotin binding beads and potential streptavidin contamination

To test the effectiveness of our protocol with other biotin binding proteins, following commercially available biotin binding beads were compared to Dynabeads streptavidin C1: Dynabeads M280 streptavidin and SpeedBead magnetic neutravidin. Using cell lysates prepared in lysis buffer 2, biotinylated proteins were eluted with excess biotin and heat, followed by 4× sample buffer and heat. Similar levels of proteins were collected from the streptavidin and neutravidin conjugated beads with minimal proteins remaining in the 4× sample buffer (Fig. 4A), suggesting that biotinylated proteins efficiently elute from both streptavidin and neutravidin conjugated beads under these conditions.

To test the degree of streptavidin contamination from Dynabead C1, eluted samples were analyzed with silver stained SDS-PAGE and purified streptavidin (SA) as a marker (Fig. 4B). With excess biotin and heat elution, no detectable streptavidin bands were observed (Fig. 4B). In contrast, subsequent 4× sample buffer and heat elution removed significant amounts of streptavidin from the beads (Fig. 4B), indicating that strong denaturant (8% SDS) introduces unwanted streptavidin contamination.

## 4. Discussion

Due to the strength of biotin-streptavidin bonds, isolation of biotinylated molecules using streptavidin is a simple procedure. Unfortunately, this strong interaction also hinders elution of biotinylated molecules from streptavidin-conjugated beads. A common approach to elute biotinylated proteins from streptavidin conjugated beads for mass spectrometry analysis is to treat the beads with proteases and digest the peptides off the beads. However, this produces undesirable streptavidin peptides in the eluted protein sample that may interfere with downstream processing. In addition, on-bead digest/elution of biotinylated proteins has been

shown to yield a lower number of identified proteins in mass spectrometry analysis compared to non-invasive elution by a cleavable linker [19]. Alternatively, using highly concentrated SDS sample buffer (e.g. 4× sample buffer) and heating, biotinylated proteins are eluted, but this condition also favors removal of streptavidin from the beads (Fig. 4B).

Our results demonstrate that the strength of the biotinylated protein-streptavidin interaction depends on the detergent concentration during the incubation of biotinylated proteins with streptavidin conjugated beads. In the presence of SDS (0.4 %) and IGEPAL-CA630 (1 %), biotinylated proteins can be efficiently eluted from the beads with excess biotin and heat. Interestingly, while high SDS concentration (> 0.4 %) promotes elution of biotinylated proteins with excess biotin and heat (Fig. 2B), high IGEPAL-CA630 concentration (> 2 %) prevents elution with excess biotin and heat (Fig. 2C), suggesting that the bonds between biotinylated proteins and streptavidin increased in strength with higher IGEPAL-CA630 concentration. Furthermore, in the absence of biotin or SDS, heat treatment (95 °C for 5 minutes) alone does not elute biotinylated proteins, and significantly inhibits the release of biotinylated proteins from streptavidin conjugated beads (Fig. 3A). This is contrary to a previous report that biotinylated DNA can be eluted with water at > 70 °C [20]. Similar to high concentration of IGEPAL-CA630, heating in the absence of excess biotin may induce structural changes in biotinylated proteins and/or streptavidin that prevent the release of biotinylated proteins from streptavidin conjugated beads.

Optimizing the yield of eluted biotinylated proteins while minimizing the background of non-biotinylated proteins will improve sample quality for subsequent analysis and ease interpretation of results. By controlling the binding conditions, we are able to achieve efficient elution of biotinylated proteins from streptavidin conjugated beads. However, the elution of biotinylated proteins in our current protocol is not complete (see Fig. 2 and 3). This may be due to protein loss in sample handling and/or irreversible biotin binding to streptavidin-conjugated beads. It is interesting to note that the eluted proteins are typically > 50 kDa despite the presence of lower molecular weight biotinylated proteins in cell lysates. This raises a possibility that some strongly bound proteins may be lower molecular weight proteins. An alternative approach is to pre-digest cell lysates, then purify biotinylated peptides, which can be eluted with organic solvents that are otherwise incompatible with protein samples [21]. Due to the superior strength of biotin-streptavidin bonds, systematic analysis of binding and elution conditions is required for optimal isolation of biotinylated proteins that are compatible to subsequent downstream processing.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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## Abbreviations

<b>BioID</b>	Biotin Identification
<b>bioAMP</b>	biotinoyl-5'-AMP
<b>GFP</b>	Green Fluorescence Protein
<b>MDCK</b>	Madin-Darby Canine Kidney

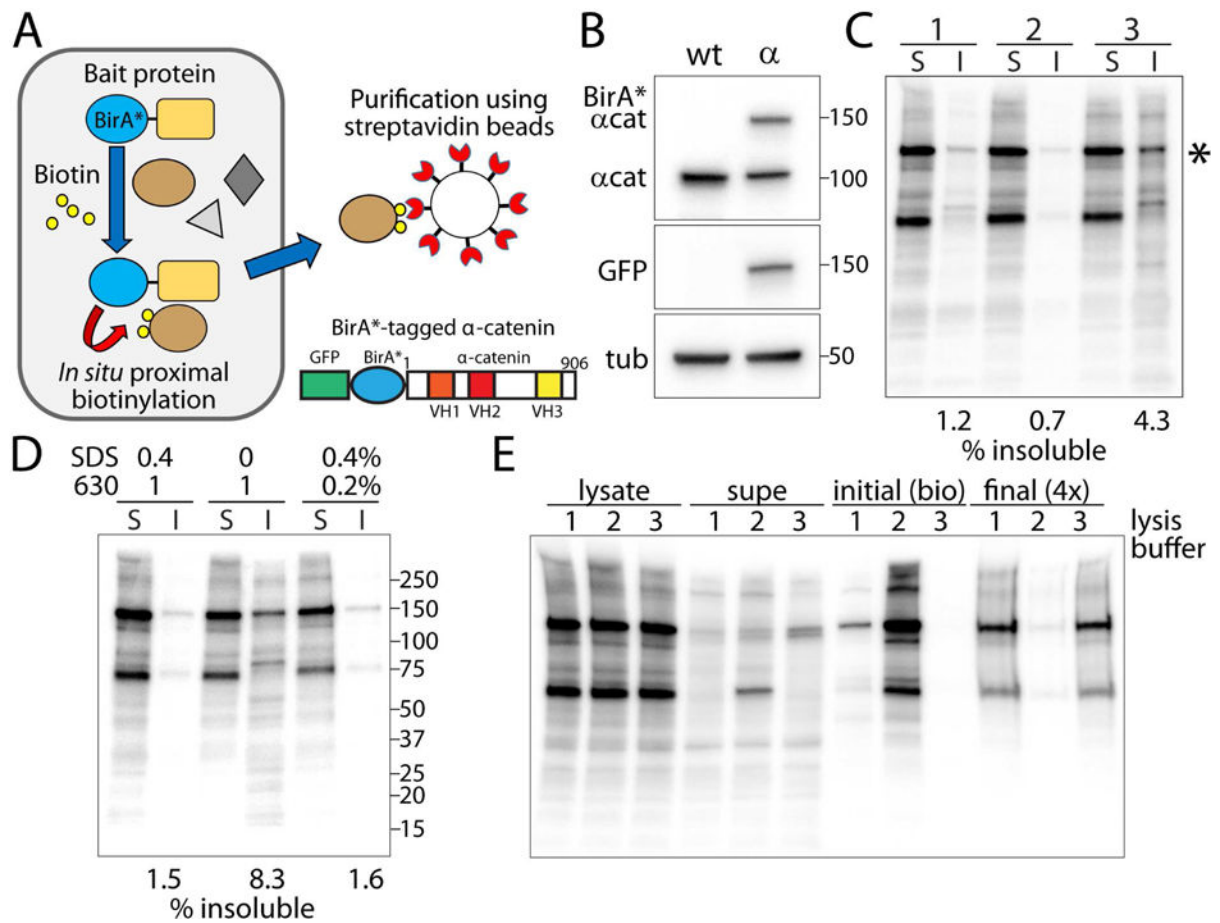
## References

1. Roux KJ, Kim DI, Raida M, Burke B. A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. *J Cell Biol.* 2012; 196:801–810. [PubMed: 22412018]
2. Mehta V, Trinkle-Mulcahy L. Recent advances in large-scale protein interactome mapping. *F1000Res.* 2016; 5
3. Rees JS, Li XW, Perrett S, Lilley KS, Jackson AP. Protein Neighbors and Proximity Proteomics. *Mol Cell Proteomics.* 2015; 14:2848–2856. [PubMed: 26355100]
4. Kim DI, Roux KJ. Filling the Void: Proximity-Based Labeling of Proteins in Living Cells. *Trends in cell biology.* 2016; 26:804–817. [PubMed: 27667171]
5. Li P, Li J, Wang L, Di LJ. Proximity labeling of interacting proteins: Application of BioID as a discovery tool. *Proteomics.* 2017
6. Lonn P, Landegren U. Close Encounters - Probing Proximal Proteins in Live or Fixed Cells. *Trends Biochem Sci.* 2017; 42:504–515. [PubMed: 28566215]
7. Chapman-Smith A, Cronan JE Jr. The enzymatic biotinylation of proteins: a post-translational modification of exceptional specificity. *Trends Biochem Sci.* 1999; 24:359–363. [PubMed: 10470036]
8. Kwon K, Beckett D. Function of a conserved sequence motif in biotin holoenzyme synthetases. *Protein science: a publication of the Protein Society.* 2000; 9:1530–1539. [PubMed: 10975574]
9. Varnaite R, MacNeill SA. Meet the neighbors: Mapping local protein interactomes by proximity-dependent labeling with BioID. *Proteomics.* 2016; 16:2503–2518. [PubMed: 27329485]
10. Lambert JP, Tucholska M, Go C, Knight JD, Gingras AC. Proximity biotinylation and affinity purification are complementary approaches for the interactome mapping of chromatin-associated protein complexes. *J Proteomics.* 2015; 118:81–94. [PubMed: 25281560]
11. Kim DI, Jensen SC, Noble KA, Kc B, Roux KH, Motamedchaboki K, Roux KJ. An improved smaller biotin ligase for BioID proximity labeling. *Molecular biology of the cell.* 2016; 27:1188–1196. [PubMed: 26912792]
12. De Munter S, Gornemann J, Derua R, Lesage B, Qian J, Heroes E, Waelkens E, Van Eynde A, Beullens M, Bollen M. Split-BioID: a proximity biotinylation assay for dimerization-dependent protein interactions. *FEBS Lett.* 2017; 591:415–424. [PubMed: 28032891]
13. Schopp IM, Ramirez CC Amaya, Debeljak J, Kreibich E, Skribbe M, Wild K, Bethune J. Split-BioID a conditional proteomics approach to monitor the composition of spatiotemporally defined protein complexes. *Nature communications.* 2017; 8:15690.
14. Rybak JN, Scheurer SB, Neri D, Elia G. Purification of biotinylated proteins on streptavidin resin: a protocol for quantitative elution. *Proteomics.* 2004; 4:2296–2299. [PubMed: 15274123]
15. Rosli C, Rybak JN, Neri D, Elia G. Quantitative recovery of biotinylated proteins from streptavidin-based affinity chromatography resins. *Methods Mol Biol.* 2008; 418:89–100. [PubMed: 18287652]
16. Hesketh GG, Youn JY, Samavarchi-Tehrani P, Raught B, Gingras AC. Parallel Exploration of Interaction Space by BioID and Affinity Purification Coupled to Mass Spectrometry. *Methods Mol Biol.* 2017; 1550:115–136. [PubMed: 28188527]
17. Roux KJ, Kim DI, Burke B. BioID: a screen for protein-protein interactions. *Current protocols in protein science / editorial board, John E. Coligan ... [et al.].* 2013; 74 Unit 19 23.

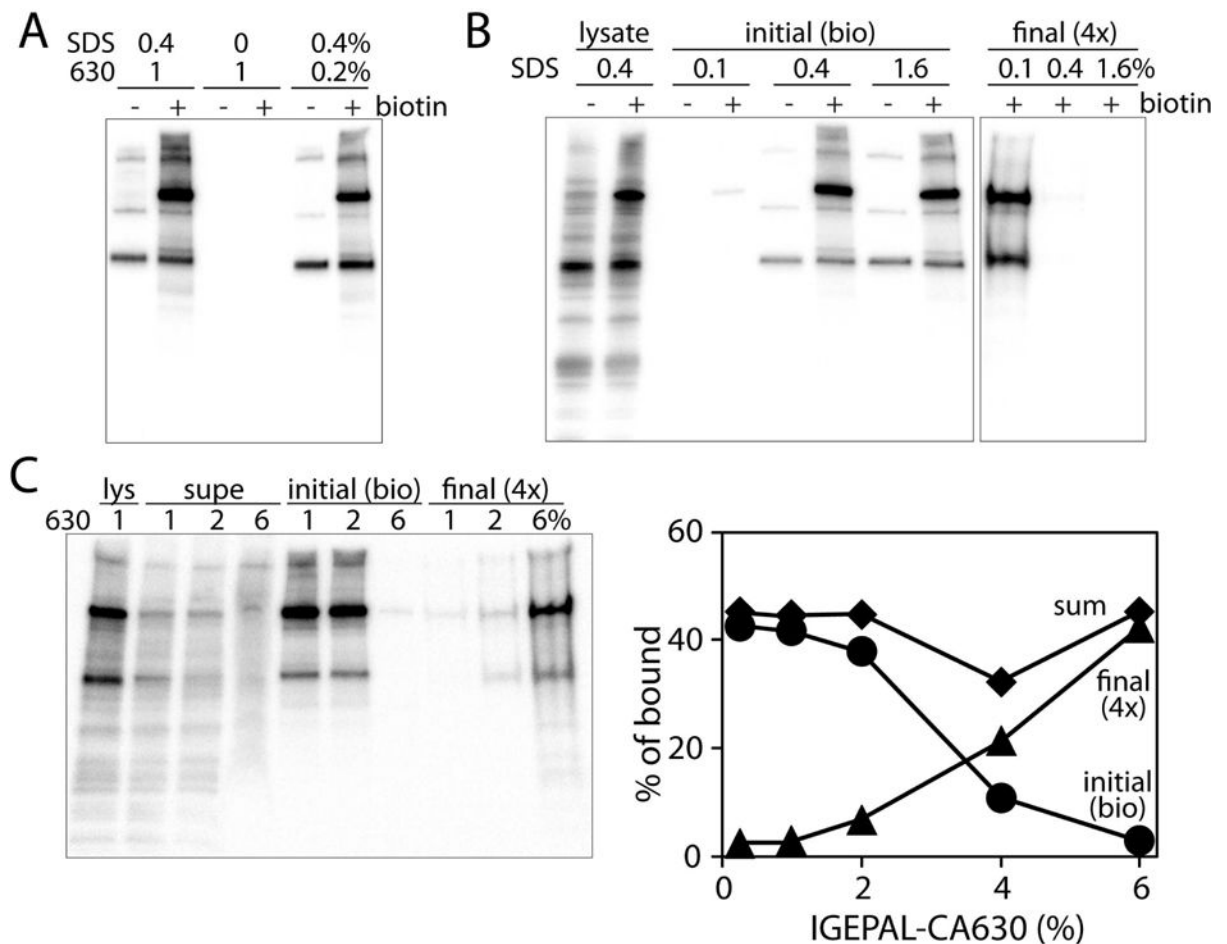
18. Ueda S, Blee AM, Macway KG, Renner DJ, Yamada S. Force dependent biotinylation of myosin IIA by alpha-catenin tagged with a promiscuous biotin ligase. *PLoS one*. 2015; 10:e0122886. [PubMed: 25806963]
19. Scheurer SB, Roesli C, Neri D, Elia G. A comparison of different biotinylation reagents, tryptic digestion procedures, and mass spectrometric techniques for 2-D peptide mapping of membrane proteins. *Proteomics*. 2005; 5:3035–3039. [PubMed: 16003826]
20. Holmberg A, Blomstergren A, Nord O, Lukacs M, Lundeberg J, Uhlen M. The biotin-streptavidin interaction can be reversibly broken using water at elevated temperatures. *Electrophoresis*. 2005; 26:501–510. [PubMed: 15690449]
21. Schiapparelli LM, McClatchy DB, Liu HH, Sharma P, Yates JR 3rd, Cline HT. Direct detection of biotinylated proteins by mass spectrometry. *J Proteome Res*. 2014; 13:3966–3978. [PubMed: 25117199]

**Highlights**

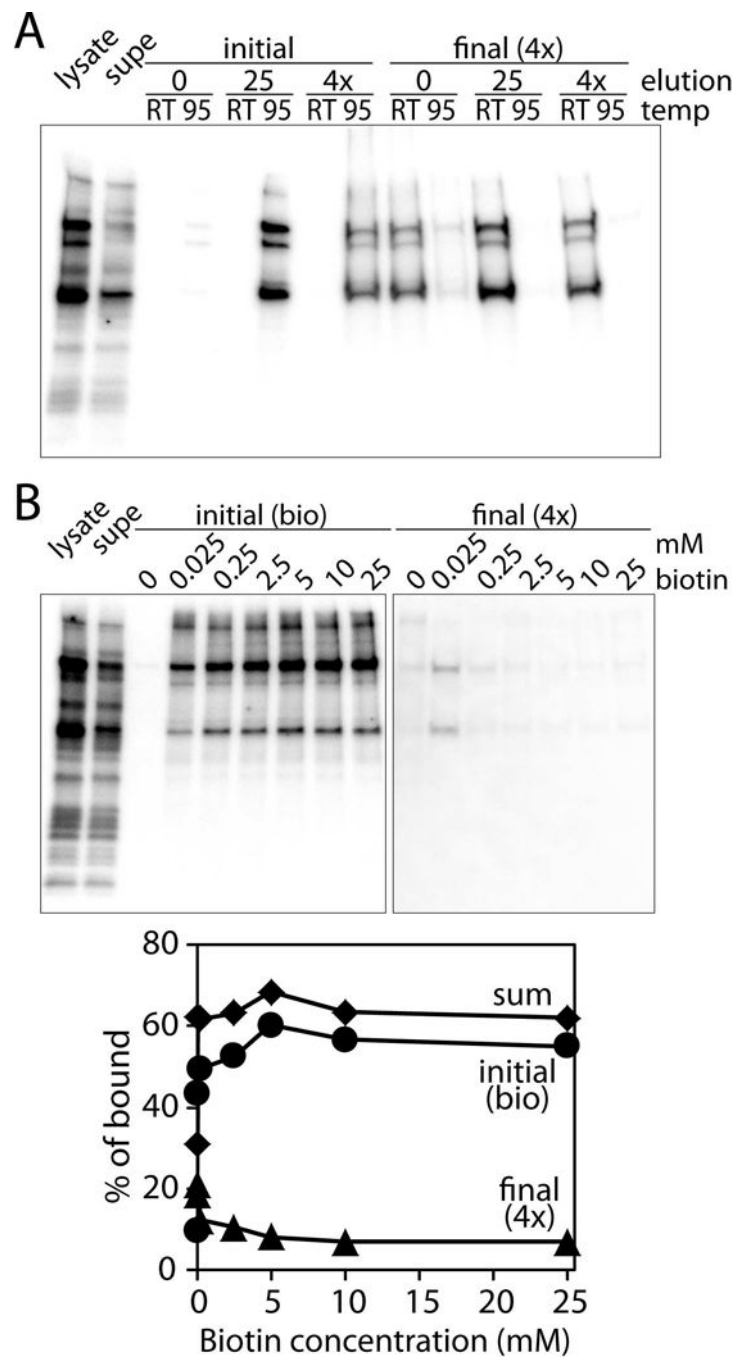
- Streptavidin-biotinylated protein interactions depend on the presence of detergents
- SDS weakens and IGEPAL-CA630 strengthens streptavidin-biotinylated protein bonds
- Excess biotin and heat can elute biotinylated proteins off streptavidin beads



**Fig. 1.** Characterization of cell line and lysis condition. (A) Schematic of proximal biotinylation and GFP-BirA\* tagged  $\alpha$ -catenin. (B) Western blot analysis of wild type (wt) and BirA\*- $\alpha$ -catenin ( $\alpha$ ) cell line expressing chimeric  $\alpha$ -catenin tagged with GFP and BirA\*. Western blots were analyzed using  $\alpha$ -catenin (top), GFP (middle), and tubulin (bottom) antibodies. (C) Streptavidin Western blot of soluble lysate (S) and insoluble pellet (I) fraction of cell lysate from three different lysis buffers (1, 2, 3 – see Materials and Methods for the buffer compositions). \* denotes GFP-BirA\*- $\alpha$ -catenin protein band. (D) Streptavidin Western blot of soluble and insoluble fraction of cell lysate from lysis buffer 2 at varying detergent concentrations. (E) Streptavidin Western blot of the lysate, supernatant (supe), eluted biotinylated proteins using excess biotin and heat (initial, bio), and final 4 $\times$  sample buffer/heat elution (final, 4 $\times$ ) using three different lysis buffers (1, 2, 3).

**Fig. 2.**

Elution efficiency depends on detergent concentration in lysis buffer. (A) Effect of SDS and IGEPAL-CA630 on elution efficiency. Streptavidin Western blot of eluted biotinylated proteins of cultured cells incubated with and without biotin and lysed in buffer 2 containing different detergent compositions. (B) Streptavidin Western blot of lysed cells incubated with and without biotin in lysis buffers 2 of different SDS concentration. The blot shows lysate, eluted biotinylated protein using excess biotin and heat (initial, bio), and final 4× sample buffer/heat elution (final, 4×). (C) Streptavidin blot (left) and quantification (right) of cells incubated with biotin, lysed in lysis buffer 2 with various IGEPAL-CA630 concentrations. Quantification shows the eluted protein with excess biotin/heat (initial, bio) and 4× sample buffer/heat elution (final, 4×), and their sum as a percentage of the bound proteins (calculated based on the difference between lysate and supernatant protein concentrations).



**Fig. 3.** Optimization of biotinylated elution condition. (A) Proteins eluted (initial) from streptavidin conjugated beads using 0 mM biotin, 25 mM biotin or 4× sample buffer, with or without heating were analyzed with a streptavidin Western blot along with the lysate and supernatant (supe). The remaining bound proteins were eluted with subsequent 4× sample buffer elution (final, 4×). (B) Streptavidin Western blots (top) of lysate, supernatant (supe), initial eluted proteins (initial, bio), and subsequent 4× sample buffer elution (final, 4×). Proteins were initially eluted with different concentrations of biotin at 95 °C for 5 minutes. Quantification

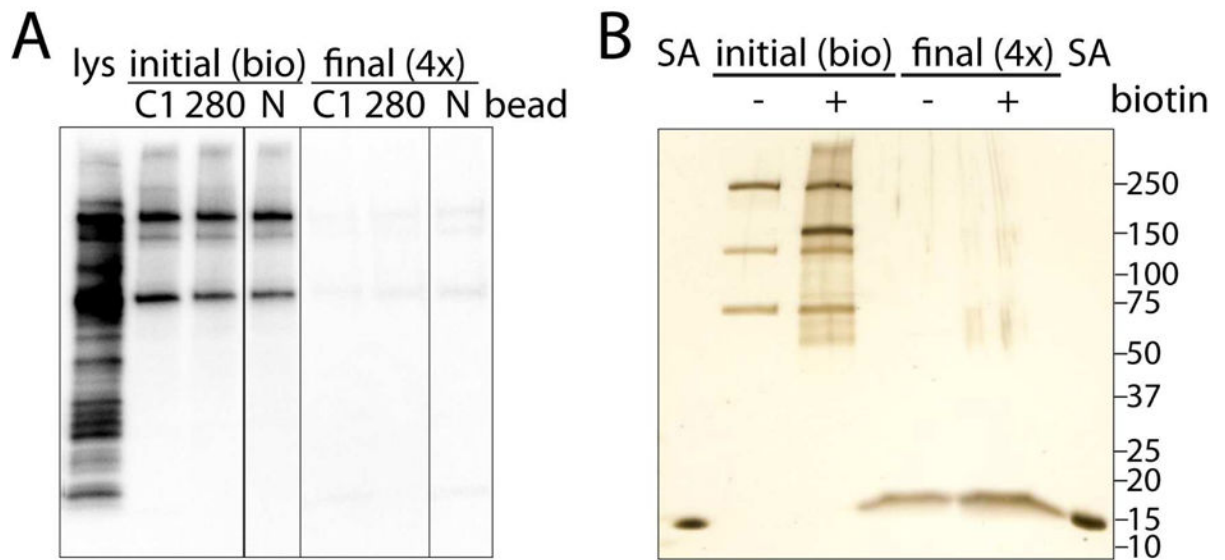
(bottom) shows the eluted protein (initial, bio), 4× sample buffer elution (final, 4×), and their sum as a percentage of bound proteins resulting from initial elution with different biotin concentration. Bound proteins were calculated based on the difference in the concentrations of biotinylated proteins detected in the supernatant and the lysate.

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**Fig. 4.**

Efficacy of different biotin-binding beads and elution of streptavidin. (A) Binding and elution efficiency with different beads. Streptavidin Western blot of cell lysate (lys), eluted proteins with excess biotin/heat (initial, bio) and 4× sample buffer/heat elution (final, 4×) resulting from cell lysate incubation with different beads: Dynabeads MyOne Streptavidin C1 (C1), Dynabeads M280 Streptavidin (280), and SpeedBead Magnetic Neutravidin Coated Particles (N). (B) Silver stained SDS-PAGE of eluted biotinylated proteins from Dynabead C1 with 25 mM biotin (initial, bio) and final 4× sample buffer elution (final, 4×) with heat. Cell lysates were prepared from cells incubated with (+) and without (-) added biotin in the media. Purified streptavidin (SA) were run on either side of the gel as a size marker for streptavidin.