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Improved elution strategy and new monoclonal anti-biotin antibody for LC-MS/MS characterization of protein biotinylation sites

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ABSTRACT

Biotin labeling in combination with mass spectrometry has been widely applied in large-scale biological studies, such as determination of protein partners, protein subcellular localization, and protein post-translational modifications. Previous studies have shown that immunoaffinity enrichment is a better method than streptavidin/avidin purification for site-specific studies of biotinylated molecules. In this study, we made a crucial improvement to the elution phase of the immunoaffinity enrichment step for biotinylated peptides, which involves the addition of a highly organic solvent, and developed a monoclonal anti-biotin antibody that improved the identification number for biotinylated peptides. We then demonstrated its application in the characterization of protein interaction sites for the β2 adrenergic receptor (β2AR) by proximity labeling in living cells. Our research provides an improved and reproducible immunoaffinity enrichment method for site-specific biotin-related research.

1. Introduction

Tagging proteins and peptides with biotin has been a popular method for several years. Biotin-binding proteins, such as streptavidin and avidin, are affinity reagents commonly used for the purification or detection of biotinylated biological molecules. The interaction between streptavidin/avidin and biotin is non-covalent yet strong (Kd = 10^{-15} M) [1], which ensures efficient enrichment and sensitive detection of biotinylated proteins. However, strong binding inhibits the direct characterization of biotinylated peptides. Generally, enriched biotinylated proteins are not necessarily removed from the solid phase. Biotinylated proteins can be digested into peptides, termed on-bead digestion, and then sent for LC-MS/MS for characterization of the biotinylated proteins [2] (Fig. 1).

However, much research requires information on protein biotinylated sites and to accomplish this goal, biotinylated peptides need to be eluted from the solid phase for analysis by LC-MS/MS. The strong interaction between biotin and streptavidin/avidin makes the recovery of biotinylated peptides low; therefore, researchers have been attempting to improve the recovery of biotinylated proteins/peptides by changing the elution strategy, for example, by using harsh conditions or competitive biotin [3], designing cleavable sites between biotin and the tagged proteins [4], and modifying avidin-related affinity reagents, such as neutravidin and monomeric avidin [5]. Udeshi et al. developed a method using an anti-biotin antibody to enrich biotinylated peptides, as published in Nature Methods [6]. Kim et al. evaluated a biotinylated-peptide purification method using antibodies [7]. The anti-biotin antibody is a good capture reagent for biotinylated peptides because of its relatively low binding affinity to biotin, which thereby ensures high recovery via increase in elution efficiency (Fig. 1). Our results (Fig. S2) and previous study findings demonstrate that this method is superior than using neutravidin and other reagents [6,7].

In this study, we aimed to enhance characterization of protein biotinylation sites by focusing on improving peptide recovery for LC-MS/MS analysis. The approach involved developing a monoclonal anti-biotin antibody with better reproducibility as compared to existing polyclonal antibodies (pAbs). We demonstrated the efficiency of the monoclonal antibody (mAb) in enriching and eluting biotinylated peptides, providing a more reliable method for researchers seeking detailed information on protein biotinylation sites. The resulting enrichment kit was commercialized by Cell Signaling Technology, Inc. (#41343) for wider use.

2. Materials and methods

2.1. Materials

Cell culture reagents were purchased from Invitrogen. Materials used

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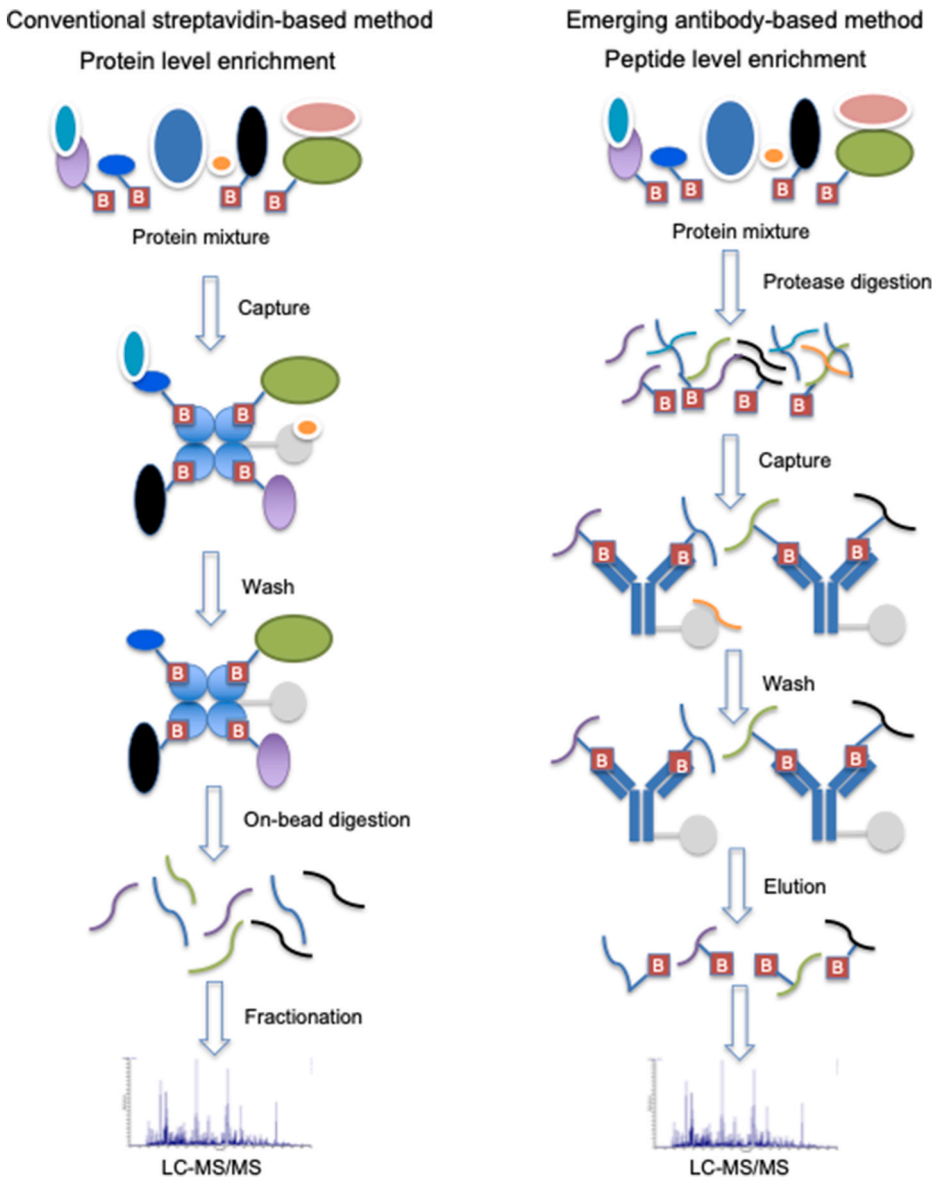


[Figure 1]. Illustration of conventional protein-level and emerging peptide-level methods for biotinylation identification. The graph was modified from the one shown in the publication [6].

for western blotting analysis were obtained from Cell Signaling Technology (CST). All other chemicals and enzymes were purchased from Sigma-Aldrich or Thermo Scientific, unless otherwise indicated.

2.2. Antibody development

A monoclonal anti-biotin antibody was developed at CST using a standard antibody development pipeline [8,9]. First, the biotinylated protein was utilized as an antibody-antigen to immunize New Zealand White rabbits. The antibodies were then purified by protein A column chromatography to isolate the IgG antibody fraction and further purified using a biotin-peptide-containing affinity column. Finally, the rabbit with good reactivity was identified and used to generate a single monoclonal antibody (A7C2A) using the Trade-Secret monoclonal antibody development pipeline.

2.3. Biotin labeling and spiked-in experiments

Trypsin-digested mouse liver peptides (CST, #12291) were labeled with biotin by reacting with EZ-link NHS-biotin. First, 1 mg of a lyophilized mouse liver peptide sample was reconstituted in 1 mL of 50 mM 4-hydroxyethylpiperazine ethanesulfonic acid (HEPES) (pH 8.5). Then, 410 μL of DMSO was added to 8 mg of biotin reagent and immediately added directly to the mouse liver peptide. The biotin reagent reacted with the peptide for 30 min, followed by 480 μL of 5 % hydroxylamine shaken for 15 min at room temperature to stop the reaction. The labeled peptide was dried using a vacuum concentrator, resuspended in a 5 % acetonitrile (ACN)/0.1 % formic acid (FA) solvent. Desalting was performed by using a SepPak column (Waters, WAT036945). The column was activated with 50 % methanol and water, and then prewet with 5 % ACN/0.1 % FA solution. After sample loading, the salts were washed twice with 5 % ACN/0.1 % FA solution. Peptides were eluted with 50 % ACN/0.1 % FA. Finally, the biotinylated mouse liver peptide was dried, redissolved, and mixed with mouse liver peptides at a mass ratio of 1:1000 to prepare a low-concentration biotinylated-peptide sample that mimicked natural organisms (Fig. S1). Aliquots (1 mg) were placed in 1.5 mL centrifuge tubes and dried.

2.4. Engineered ascorbate peroxidase (APEX) proximity-labeling

HEK 293T cells stably expressing fused β2AR, a prototype for heterotrimeric guanine nucleotide–binding protein–coupled receptors (GPCRs), and APEX were given by the Kruse lab (Harvard Medical School). These were generated as described previously [2]. Specifically, β2AR -APEX contains a glycine-serine linker (GGSSGGSS) and APEX2, a protein C affinity tag (EDQVDPRLIDGK). APEX2 DNA was amplified from pcDNA3 APEX2-NES. DNA constructs were cloned into the pcDNA5/FRT/TO vector. Transfection was performed using Lipofectamine 2000 with pOG44 Flp-Recombinase. Proximity-labeling experiments were conducted as follows: cell line stocks were recovered and passaged into 10 cm culture plates, with five plates for each condition. The HEK 293T cell medium was supplied with 1 μg/mL doxycycline hyclate for 48 h, then labeling medium (DMEM supplemented with 10 % FBS, 10 μg/mL gentamicin, and 500 μM biotinyl phenol) was added and incubated for 1 h. BI167107 was added to a final concentration of 100 nM for 10 min. Next, freshly diluted H_2O_2 in Dulbecco's phosphate-buffered saline (DPBS) was added at 1 mM final concentration for 1 min. Subsequently, the labeling was quenched by the solution (DPBS, 10 mM sodium ascorbate, 5 mM Trolox, and 10 mM sodium azide) and the same buffer containing 5 mM EDTA. The cells were pelleted and stored at −80 °C.

2.5. Cell peptide preparation

The cell pellets were resuspended in lysis buffer (9 M Urea, 100 mM HEPES, pH 8.5) and sonicated. After centrifugation at 10,000×*g* for 15 min at room temperature, supernatant containing soluble proteins was obtained. After protein concentration measurement using a Pierce BCA kit, proteins were reacted with 4.5 mM dithiothreitol for 1 h, and 10 mM iodoacetamide for 0.5 h at room temperature. This was diluted four-fold with 20 mM HEPES, (pH 8.0), and trypsin (CST, #72969) was added at a 1:50 ratio and incubated overnight. Peptides were purified at room temperature using a Sep-Pak column (Waters, WAT051910). Subsequently, 20 % Trifluoroacetic Acid (TFA) was added to the digest to achieve a final concentration of 1 % TFA. After acidification, the samples were incubated on ice for 15 min to form pellets. Precipitate was removed, and the resulting supernatant was prepared for the column. The column was prewetted with 5 mL of 100 % methanol and washed with 0.1 % trifluoroacetic acid (TFA) in water. After loading, the peptide was rinsed with 0.1 % TFA, eluted with 40 % acetonitrile and a 0.1 % TFA solution, freeze-dried, and lyophilized to obtain the lyophilized peptides.

2.6. Immunoaffinity enrichment

CST protocol immunoaffinity enrichment was done in the following steps: The resulting sample peptides (1 mg) were resuspended in IAP buffer (50 mM MOPS/NaOH, 10 mM Na_2HPO_4, 50 mM NaCl pH 7.2). The antibody beads were washed with 1 mL of 1X PBS four times. The peptide solution (1.4 mL) was transferred to a vial containing 40 μL of antibody beads and incubated for 2 h at 4 °C on an end-over-end rotator. After peptide binding, antibody beads were washed by 1 mL of IAP buffer four times and 1 mL of cooling water (1 mL) twice. The beads were eluted with 55 μL of 80 % ACN, 0.2 % TFA for 10 min twice. The eluates were mixed in the same 1.7 mL tube and dried. After redissolved in 100 μl of 0.15 % TFA. If a white pellet was observed, sonicated for 1 min, centrifuged at 20,000×*g* for 10 min, and the pellet was carefully removed by taking the supernatant into a new Eppendorf tube. The resulting peptides were dried and redissolved in 100 μL of 0.15 % TFA, and then further purified by C18 stage tips. Each C18 tip was equilibrated by passing through 50 μL of 0.1 % TFA and 80 % ACN. Samples were then loaded to the C18 tips and washed with 55 μL of 0.1 % TFA twice. Finally, the peptides were eluted from the C18 tip twice using 10 μL of 0.1 % TFA and 80 % ACN solvent and dried in a vacuum concentrator.

The protocol published in Nature Methods utilized a pAb from ImmuneChem [6]. Briefly, 1 mg of the spiked-in peptide was redissolved in 1 mL of IAP buffer. Samples were incubated with anti-biotin antibody beads for 1 h at 4 °C. After enrichment of biotinylated peptides, beads were washed with 1X PBS four times, followed by peptide elution using 50 μL of 0.15 % TFA twice. The eluents were combined, and purified by C18 stage tips. The samples were washed with 100 μL of 0.1 % FA twice, and eluted from the C18 stage tips using 50 μL of 50 % MeCN and 0.1 % FA and dried in a vacuum concentrator.

2.7. Mass spectrometry analysis

Dried peptides were resuspended in 0.15 % FA and analyzed by LC-MS/MS. An EASYnLC 1200 UPLC was coupled to Orbitrap Fusion Lumos Tribrid MS (Thermo Fisher Scientific). Mobile phase solvent A was 0.1 % FA, whereas solvent B was 0.1 % FA, 80 % ACN. A gradient from 0 % to 40 % B at 300 nL/min over 300 min was applied. The analytical column was self-made, at a length of 75 μm × 50 cm, using C18 beads (2 μm, 100 Å). MS parameters were set as follows: for full MS, a mass range of 300–1500, a mass resolution of 120,000 (*m/z* 200), an AGC target value of 4.0E5, and a maximum injection time of 50 ms, were set to cover most peptides; dynamic exclusion was 60 s at a top speed of 3 s, whereas collision-induced dissociation energy was set at 35; for MS/MS spectra, AGC target value was set at 3.0E3, and maximum injection time was set at 350 ms.

The collected raw data were evaluated using SEQUEST as follows: The database was *Homo sapiens* FASTA From Swissprot (September 2018). The mass accuracy was set at ±5 ppm for mother ions and at 0.02 Da for daughter ions. Enzyme was defined as trypsin, allowing 4 missed cleavages. Modifications were set as carbamidomethylation at Cysteine (static mod), oxidation at methionine residues, and biotin labeling at lysine/arginine or tyrosine residues in individual cases. False discovery rates were set at 1 % with inclusion of reverse decoy databases. After identification of biotinylated peptides, Skyline (version 3.1) was used for more precise peptide quantification, via manual validation of peak areas of biotinylated peptides. Student's T-test was performed to determine significant changes in the biotinylated peptides. Motifs were analyzed by https://www.phosphosite.org/staticMotifAnalysis, utilizing the data from β2AR-APEX HEK 293T cells and spiked-in mouse liver peptide samples.

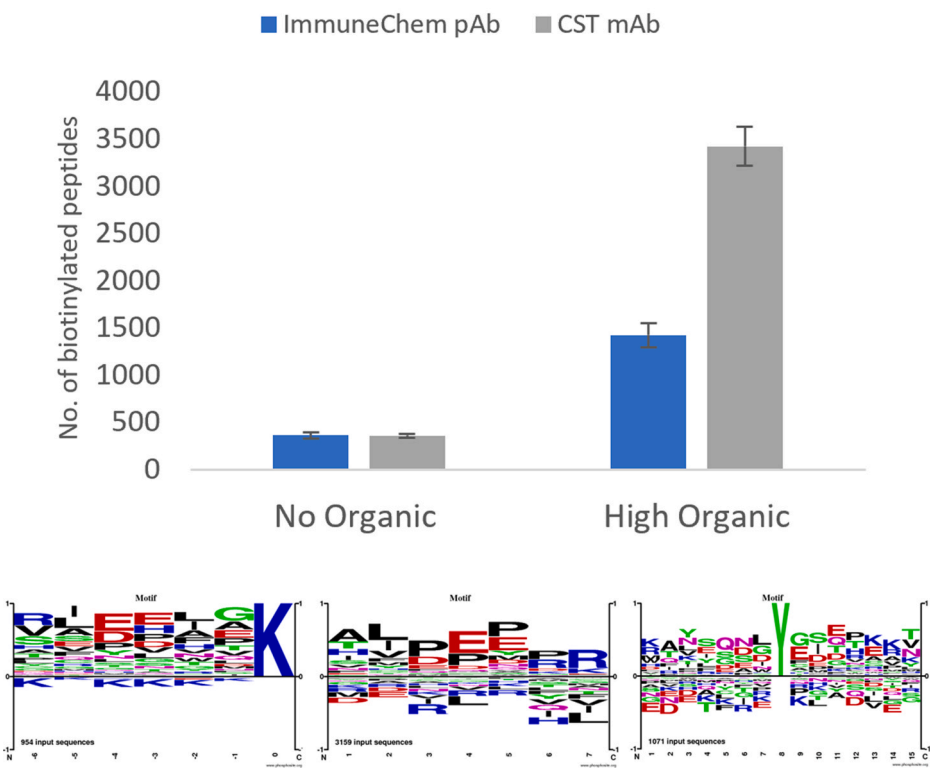
2.8. Western blotting

Aliquots of urea buffer lysed cell samples were subjected to western blotting analysis. Protein concentrations were determined using the protein bicinchoninic acid (BCA) assay. Aliquots were separated by SDS-PAGE, incubated with anti-biotin antibody and then anti-rabbit monoclonal secondary antibody (CST) at a ratio of 1:1000 for 1 h, and finally detected using the ChemiDoc imaging system (BioRad).

3. Results

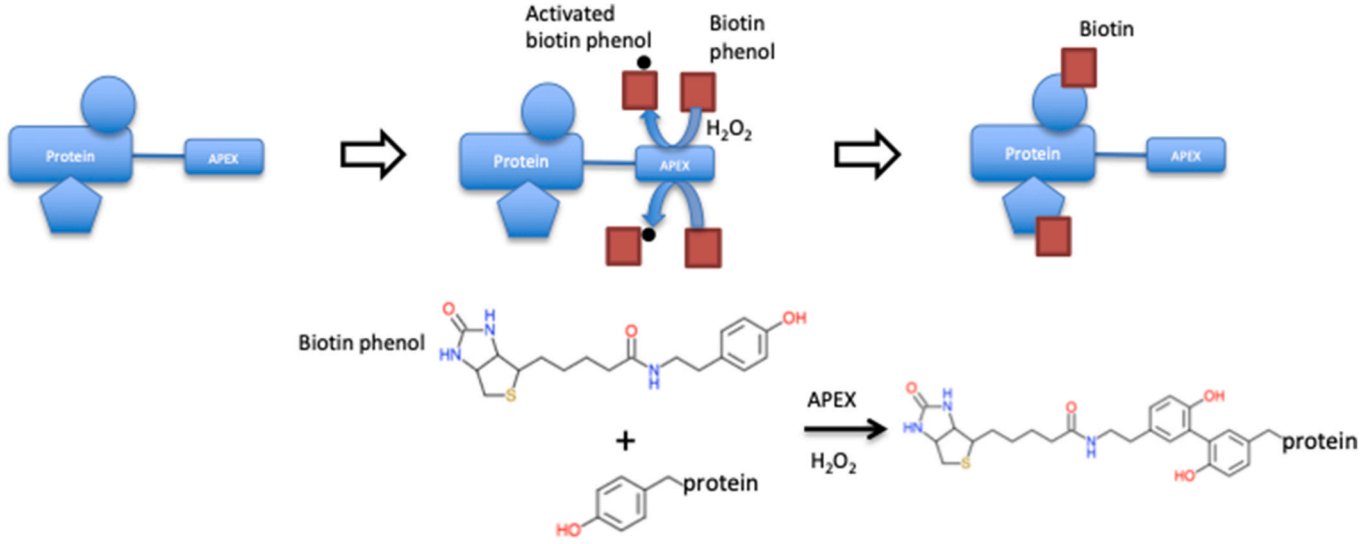
3.1. Improved elution strategy for immunoenrichment

We found that a high concentration of ACN in the eluent was critical for the elution of biotinylated peptides. The published protocol results demonstrate poor recovery of biotinylated peptides and poor reproducibility [6]. Using the exact sample preparation steps, two replicates yielded hundreds of biotinylated peptides from the spiked-in mouse liver peptide samples (Fig. S1). Further research revealed that the key to eluting biotinylated peptides was to add ACN to the eluent buffer. The existing method uses a common elution buffer composed of 0.15 % TFA. Under acidic conditions, antibodies lose affinity to protein A/G and the biotinylated peptides. The biotinylated peptides were solubilized in an eluent buffer and further purified. However, when the antibody beads

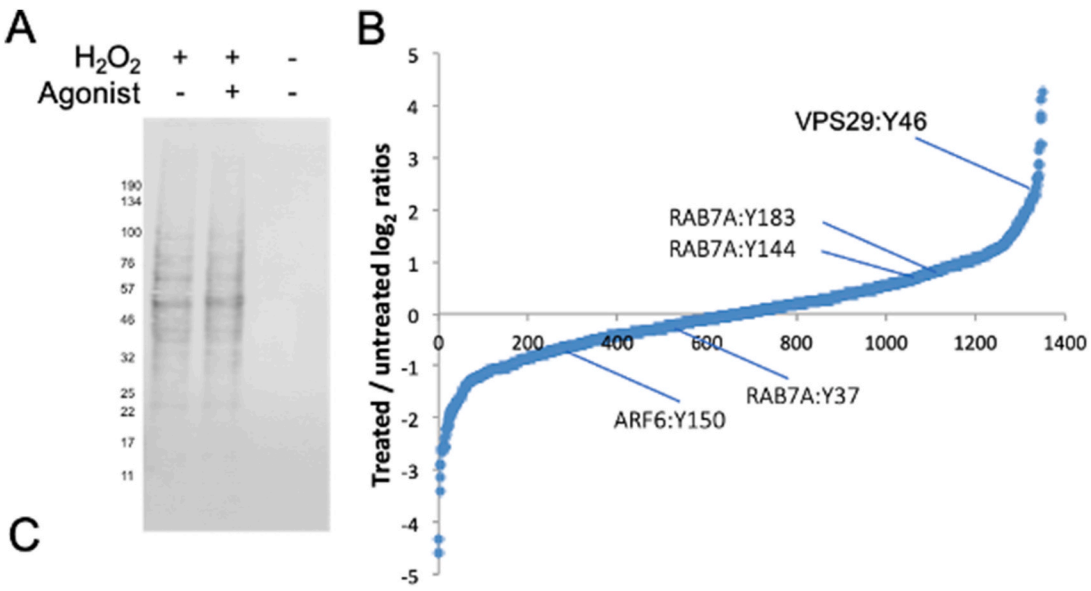


[Figure 2]. (Top) Number of identified biotinylated peptides from spiked-in biotinylated mouse liver peptides sample. High-organic eluent buffer (80 % ACN/0.2 % TFA) significantly increases recovery of the biotinylated peptides compared to non-organic eluent buffer (0.15 % TFA). CST mAb with CST protocol performs better than ImmuneChem's pAb using reported approaches in the literature. (Bottom) Motif analysis for CST mAb, which was formed by applying all biotinylated peptides identified in this study.

were eluted with 0.15 % TFA, only 365 biotinylated peptides were identified in the spiked-in sample. When the beads were further incubated with 80 % ACN/0.2 % TFA after 0.15 % TFA, 1349 biotinylated peptides were identified. We compared different biotin antibodies, including ImmuneChem pAb and CST mAb and non-organic (0.15 % TFA) and highly organic (80 % ACN/0.2 % TFA) elution buffers (Fig. 2). An increase from 361 ± 5 % with non-organic to 1425 ± 9.0 % in highly organic elution buffers occurred for pAb, and from 361 ± 3 % in non-organic to 3425 ± 6.6 % in highly organic elution buffers for mAb occurred. The improvement clearly shows that elution strategy is the key for biotinylated-peptide identification. We believe that this may be caused by the poor solubility of the biotinylated peptides in aqueous solutions.



[Figure 3]. Overview of engineered ascorbate peroxidase (APEX) proximity-dependent labeling technology. Protein of interest was co-expressed with APEX in cells, biotin phenol and H_2O_2 were added to activate biotinylation reaction, and then nearby interacting proteins were labeled with biotin. The graph also shows the chemical mechanism involved in this process.



Proteins	Biotinylated Peptides	Biotinylated Site	Treated/Untreated
VPS29	ESY*DYLK	46	5.6
ARF6	NWY*VQPSCATSGDGLYEGLTWLTSNYK	150	-2.3
RAB7A	NALKQETEVELY*NEFPEPIK 183	183	2.1
RAB7A	FSNQY*K 37	37	-1.1
RAB7A	AQAWCY*SK 144	144	1.9

[Figure 4]. (A) Western blotting of biotinylated proteins from the β2 adrenergic receptor (β2AR)-APEX proximity labeling. Dot plot (B) and examples (C) show relative abundances of identified biotinylated peptides after agonist BI167017 perturbation, indicating potential interacting proteins and sites of β2AR.

3.2. *Excellent performance of CST biotin mAb*

We then compared our new biotin mAb with a pAb from a previous publication (Fig. 2). mAbs represent a single population of antibodies, whereas pAbs represent a mixed population of antibodies. mAbs bind only to a single specific site, whereas pAbs may bind off-target epitopes. mAb is a renewable resource, whereas pAb is a limited resource that will not last. In general, mAbs are uniform in performance, whereas lots of pAbs differ in performance. In this case, our mAb discovered more biotin peptides than the pAb, which added a determining factor advantage over the pAb at 1425 ± 9.0 % vs 3425 ± 6.6 % (n = 2). The motif logos for internal tyrosine, internal lysine, and peptide N-terminals showed that this CST mAb is a general biotin antibody that only recognizes biotin, not the amino acid residues surrounding the biotin modification.

3.3. *Applications*

Proximity labeling, such as APEX or biotin ligase (BioID, TurboID), is an advanced technology that differentiates protein partners and their subcellular localization [10,11]. Fig. 3 shows an overview of APEX proximity-dependent labeling technology. The demonstration for the application of the methodology is APEX proximity-labeling strategy for determining interacting proteins and sites of β2AR. Western blotting revealed that protein biotinylation was directly caused by proximity labeling (Fig. 4A). LC-MS/MS experiments identified 1354 biotinylated peptides (858 proteins). One hundred and seventy-five biotinylated peptides (145 proteins) responded to BI167107 agonist significantly (fold change ≥1.5, $p \leq 0.05$). Figures and tables show examples of the identified biotinylated peptides and their corresponding biotinylation sites and proteins (Fig. 4B/C).

We further compared the results from our anti-biotin antibody enrichment method and those from Paek et al. [2] who applied the streptavidin enrichment for the same samples (schematics are shown in Fig. 1). Biotinylated sites were identified much more frequently in our data than in Paek et al.'s data, because of the different foci of the methodologies (Fig. S3). In total, 582 proteins were identified in the data from both studies originating from β2AR-APEX cells with BI167017 treatment. Our study quantified the biotinylated peptides based on MS1 peak areas. Paek et al. quantified biotinylated proteins based on the TMT labeling of non-biotinylated peptides. The Excel table in the supplementary describes the proteins characterized using both methods. This shows that the peptide-level analysis can provide site-specific information, and can also be used as a complementary and confirmation method for the protein-level analysis of biotinylation. Our method identified an additional 276 proteins and provided biotinylation site information for all identified biotinylated proteins.

4. Discussion

The immunoaffinity enrichment method to enrich biotinylated peptides has become increasingly popular and has seen application in multiple large-scale site-specific protein biotinylation studies, in addition to proximity labeling, characterization of protein post-translational modifications, such as protein carbonylation and glycosylation, and profiling of cell surface proteins [12–16]. However, poor reproducibility was observed when we attempted to repeat the method described by Udeshi et al. published in Nature Methods [6]: The number of identified biotinylated peptides in the spike-in sample was considerably lower than what was reported in the paper. This inconsistency has been independently verified by multiple labs. We found the key to this issue is the composition of the elution buffer. In addition, published methods are based on pAbs for biotin, which have limited batch-to-batch reproducibility. It is critical to optimize this method to achieve the best performance; therefore, we improved the elution strategy and further

developed a new monoclonal biotin antibody. We demonstrated that adding a high concentration of the organic solvent ACN to the elution buffer increased the identification number of biotinylated peptides. It may be caused by the general hydrophobicity of the biotinylated peptides. Whether the percentage of ACN in the solvent or the type of organic solvent will impact the recovery of biotinylated peptides, and whether other anti-post-translational modified peptides will benefit from adding a high-organic solvent in the immunoaffinity enrichment step, still requires further research, but current improvements have already been inspiring for the characterization of all biotinylated molecules. We further utilized the method to characterize protein interaction sites in β2AR-APEX cells. In this study, we developed a monoclonal anti-biotin antibody to ensure the reproducibility and performance of the method. The enrichment kit coupling the presented biotin mAb with the entire updated protocol was commercialized at CST (#41343), which will be a useful tool for researchers in this area.

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CRediT authorship contribution statement

Yiying Zhu: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Yiying Zhu reports financial support was provided by Cell Signaling Technology Inc. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2024.101711.

References

[1] O.H. Laitinen, V.P. Hytönen, H.R. Nordlund, et al., Genetically engineered avidins and streptavidins, Cell Mol, Life Sci. 63 (24) (2006) 2992–3017, https://doi.org/10.1007/s00018-006-6288-z.
[2] J. Paek, M. Kalocsay, D.P. Staus, et al., Multidimensional tracking of GPCR signaling via peroxidase-catalyzed proximity labeling, Cell 169 (2) (2017) 338–349.e11, https://doi.org/10.1016/j.cell.2017.03.028.
[3] J.S. Cheah, S. Yamada, A simple elution strategy for biotinylated proteins bound to streptavidin conjugated beads using excess biotin and heat, Biochem. Biophys. Res. Commun. 493 (4) (2017) 1522–1527, https://doi.org/10.1016/j.bbrc.2017.09.168.
[4] L. Nierves, P.F. Lange, Detectability of biotin tags by LC-MS/MS, J. Proteome Res. 20 (5) (2021) 3002–3008, https://doi.org/10.1021/acs.jproteome.0c01049.
[5] L.M. Schiapparelli, D.B. McClatchy, H.H. Liu, et al., Direct detection of biotinylated proteins by mass spectrometry, J. Proteome Res. 13 (9) (2014) 3966–3978, https://doi.org/10.1021/pr5002862.
[6] N.D. Udeshi, K. Pedram, T. Svinkina, et al., Antibodies to biotin enable large-scale detection of biotinylation sites on proteins, Nat. Methods 14 (12) (2017) 1167–1170, https://doi.org/10.1038/nmeth.4465.
[7] D.I. Kim, J.A. Cutler, C.H. Na, et al., BioSITe: a method for direct detection and quantitation of site-specific biotinylation, J. Proteome Res. 17 (2) (2018) 759–769, https://doi.org/10.1021/acs.jproteome.7b00775.
[8] A. Guo, H. Gu, J. Zhou, et al., Immunoaffinity enrichment and mass spectrometry analysis of protein methylation, Mol. CellINTRODUCED_TEXT Proteomics 13 (1) (2014) 372–387, https://doi.org/10.1074/mcp.O113.027870.
[9] H. Zhang, et al., Phosphoprotein analysis using antibodies broadly reactive against phosphorylated motifs, J. Biol. Chem. 277 (42) (2002) 39379–39387, https://doi.org/10.1074/jbc.M206399200.
[10] W. Qin, K.F. Cho, P.E. Cavanagh, et al., Deciphering molecular interactions by proximity labeling, Nat. Methods 18 (2) (2021) 133–143, https://doi.org/10.1038/s41592-020-01010-5.
[11] K.J. Roux, D.I. Kim, B. Burke, et al., BioID: a screen for protein-protein interactions, Curr. Protoc. Protein Sci. 91 (2018) 19.23.1–19.23.15, https://doi.org/10.1002/cpps.51.
[12] S. Yamanaka, Y. Horiuchi, S. Matsuoka, et al., A proximity biotinylation-based approach to identify protein-E3 ligase interactions induced by PROTACs and molecular glues, Nat. Commun. 13 (1) (2022) 183, https://doi.org/10.1038/s41467-021-27818-z.
[13] Y. Xu, X. Fan, Y. Hu, In vivo interactome profiling by enzyme-catalyzed proximity labeling, Cell Biosci. 11 (1) (2021) 27, https://doi.org/10.1186/s13578-021-00542-3.
[14] C.T. Pfeiffer, J.A. Paulo, S.P. Gygi, et al., Proximity labeling for investigating protein-protein interactions, Methods Cell Biol. 169 (2022) 237–266, https://doi.org/10.1016/bs.mcb.2021.12.006.
[15] K. Garapati, H. Ding, M.C. Charlesworth, et al., sBioSITe enables sensitive identification of the cell surface proteome through direct enrichment of biotinylated peptides, Clin. Proteonomics 20 (1) (2023) 56, https://doi.org/10.1186/s12014-023-09445-6.
[16] S.L. Xu, R. Shrestha, S.S. Karunadasa, et al., Proximity labeling in plants, Annu. Rev. Plant Biol. 74 (2023) 285–312, https://doi.org/10.1146/annurev-arplant-070522-052132.