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Proximity Dependent Biotin Labelling in Zebrafish for Proteome and Interactome Profiling

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Abstract

Identification of protein interaction networks is key for understanding intricate biological processes, but mapping such networks is challenging with conventional biochemical methods, especially for weak or transient interactions. Proximity-dependent biotin labelling (BioID) using promiscuous biotin ligases and mass spectrometry (MS)-based proteomics has emerged in the past decade as a powerful method for probing local proteomes and protein interactors. Here, we describe the application of an engineered biotin ligase, TurboID, for proteomic mapping and interactor screening *in vivo* in zebrafish. We generated novel transgenic zebrafish lines that express TurboID fused to a conditionally stabilised GFP-binding nanobody, dGBP, which targets TurboID to the GFP-tagged proteins of interest. The TurboID-dGBP zebrafish lines enable proximity-dependent biotin labelling in live zebrafish simply through outcrossing with existing GFP-tagged lines. Here, we outline a detailed protocol of the BLITZ method (Biotin Labelling In Tagged Zebrafish) for utilising TurboID-dGBP fish lines to map local proteomes and screen novel interactors.

Keywords: Proximity-dependent biotin labelling, GFP Nanobody, Zebrafish, TurboID, Proteomics, BioID

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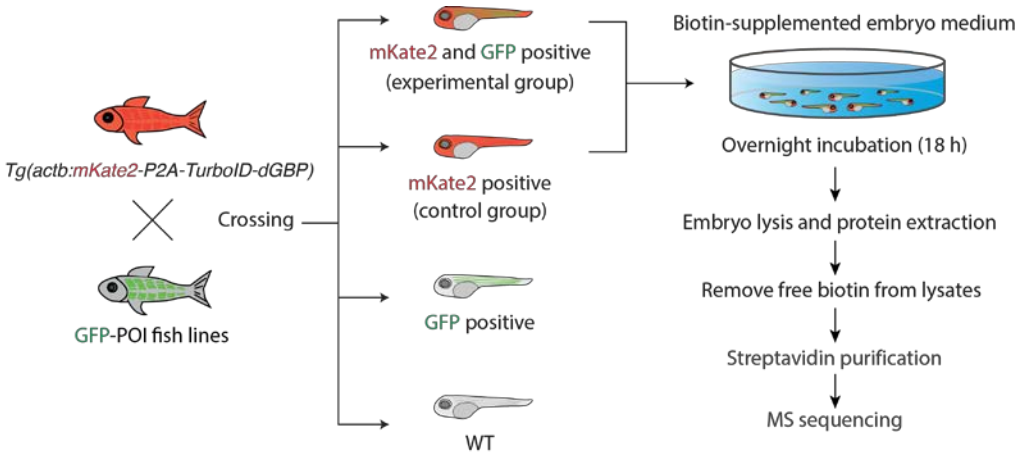
Graphic abstract:



Schematic overview of the BLITZ method.
TurboID-dGBP fish are crossed with GFP-tagged lines to obtain embryos co-expressing TurboID-dGBP (indicated by mKate2) and the GFP-POI (protein of interest). Embryos expressing only TurboID are used as a negative control. Embryos (2 to 7 dpf) are incubated overnight with a 500 µM biotin-supplemented embryo medium. This biotin incubation step allows TurboID to catalyse proximity-dependent biotinylation in live zebrafish embryos. After biotin incubation, embryos are solubilised in lysis buffer, and free biotin is removed using a PD-10 desalting column. The biotinylated proteins are captured by streptavidin affinity purification, and captured proteins are analysed by MS sequencing.

Background

In the past decade, proximity-dependent biotin labelling (BioID) has emerged as a powerful method for studying local proteome and protein interaction networks in living cells. This method utilises a genetically modified biotin ligase to covalently attach biotin molecules to proteins in the immediate vicinity. TurboID is one such biotin ligase that converts ATP and biotin into a highly reactive biotin-AMP that covalently labels proximal proteins (Branon *et al.*, 2018). Compared with other existing biotin ligases, TurboID has the most robust enzymatic kinetics, which enables its application in living organisms such as *Drosophila* and *C. elegans* (Branon *et al.*, 2018; May *et al.*, 2020). However, the applicability of TurboID has been limited by the necessity of genetically tagging the biotin ligase to each protein of interest (POI) and generating transgenic organisms.

Recently, we developed a platform for proteomic mapping through proximity-dependent biotin labelling in live zebrafish (Xiong *et al.*, 2021). Compared with conventional proximity-dependent biotin labelling methods, our platform circumvents the necessity of fusing a POI with the biotin ligase and creating transgenic organisms. Instead, the platform uses a modular system for GFP-directed proteomic mapping by combining TurboID with a GFP-binding nanobody (dGBP). By crossing the TurboID-dGBP fish line, *Tg(actb2:mKate2-P2A-TurboID-dGBP)*^{uq23rp}, with existing GFP-tagged fish lines, the dGBP targets TurboID to the GFP-labelled protein/region of interest through the binding of dGBP to GFP, which allows TurboID to capture the interactors of the POI or local proteome (Branon *et al.*, 2018). This system couples the power of the TurboID with the ability to use existing GFP-tagged transgenic zebrafish lines for proteomic mapping *in vivo*. The rapid degradation of unbound TurboID-dGBP, due to the conditionally stabilised nanobody (Tang *et al.*, 2016), improves the signal to noise ratio by preventing unspecific labelling from unbound TurboID-dGBP and achieves tissue specificity by restricting the TurboID-catalysed biotinylation only to tissues expressing GFP-tagged proteins. Our platform allows *in vivo* proteomic studies in specialised cells/tissues such as skeletal muscle, endothelia, and neurons. Furthermore, the method can be extended to study proteome/interactome changes under different physiological conditions and in disease models.

Materials and Reagents

1. Petri dish (Sarstedt, catalog number: 82.1473.001)
2. 24-well plate (Sigma-Aldrich, catalog number: CLS3526-50EA)
3. Eppendorf tube (Eppendorf, catalog number: EP0030108132)
4. PD-10 desalting column (GE Healthcare, catalog number: 17-0851-01)
5. *Tg(actb2:mKate2-P2A-TurboID-dGBP)*^{uq23rp} fish line or similar [this line was generated using the Tol2kit system according to established methods (Kawakami, 2004; Kwan *et al*., 2007)]. The TurboID construct was codon-optimised for zebrafish expression based on the protein sequence from Branon *et al.* (2018). A red fluorescent reporter, mKate2, was indirectly linked to the N-terminus of TurboID-dGBP through a ribosome-skipping sequence, P2A (Shcherbo *et al.*, 2009; Kim *et al.*, 2011). The expression of the construct was driven by the ubiquitous beta actin2 promoter *actb2* (Higashijima *et al.*, 1997; Casadei *et al.*, 2011). The plasmid constructs were co-injected with tol2 mRNA into one-cell-stage WT zebrafish embryos for genomic integration. Injected F_0s were raised and screened for founders producing positive F_1s with Mendelian frequency, indicative of a single genomic integration. The fish line was maintained in a heterozygous state.
6. Streptavidin-HRP (Abcam, catalog number: Ab7403)
7. Biotin (Sigma-Aldrich, catalog number: B4639-1G)
8. Tricaine (Sigma-Aldrich, catalog number: E10521)
9. Pronase (if necessary) (Roche, catalog number: 10165921001)
10. Trypsin/Lys-C Mix, Mass Spec Grade (Promega, catalog number: V5073)
11. InstantBlue™ Coomassie Protein Stain (Expedeon, catalog number: ISB1L)
12. Bolt™ 10% Bis-Tris Plus Gels (Invitrogen, catalog number: NW00100BOX)
13. NeutrAvidin-DyLight 405 (Invitrogen, catalog number: 22831)
14. Sodium deoxycholate (Sigma-Aldrich, catalog number: D6750-10G)
15. NP-40 (Sigma-Aldrich, catalog number: 18896-50ML)
16. EDTA (Astral Scientific, catalog number: BIOEB0185-500G)
17. NaCl (Sigma-Aldrich, catalog number: S9888)
18. Paraformaldehyde (Sigma-Aldrich, catalog number: 158127)
19. Proteinase K (Invitrogen, catalog number: 25530015)
20. Tween 20 (Sigma-Aldrich, catalog number: P1379-500ML)
21. KCl (Sigma-Aldrich, catalog number: P9541-500G)
22. $CaCl_2 \cdot 2H_2O$ (Sigma-Aldrich, catalog number: C5080-500G)
23. $MgCl_2 \cdot 6H_2O$ (Sigma-Aldrich, catalog number: M2670-500G)
24. Complete™ Protease Inhibitor Cocktail (Sigma-Aldrich, catalog number: 11836145001)
25. Pierce™ BCA protein assay kit (Thermo Scientific, catalog number: 23225)
26. PBS tablets (Medicago, catalog number: 09-8912-100)
27. Triton-X100 (Sigma-Aldrich, catalog number: T9284-500ML)
28. Dynabeads™ MyOne™ Streptavidin C1 (Invitrogen, catalog number: 65001)
29. Blot LDS sample buffer (Invitrogen, catalog number: B0008)
30. DAPI (Sigma-Aldrich, catalog number: D9542-5MG)
31. Acetonitrile, LC-MS grade (Thermo Scientific, catalog number: 51101)
32. Formic acid (Thermo Scientific, catalog number: 28905)
33. Trifluoroacetic acid (Thermo Scientific, catalog number: 28904)
34. Embryo medium (see Recipes)
35. Tricaine stock (25×) (see Recipes)
36. Biotin stock solution (see Recipes)
37. Deyolking solution (see Recipes)
38. Embryo blocking solution (see Recipes)
39. DAPI stock and intermediate solutions (see Recipes)
40. Trypsin/Lys C working solution (see Recipes)
41. Formic acid/acetonitrile solution (see Recipes)
42. Trifluoroacetic acid solution (see Recipes)

43. 4% paraformaldehyde (see Recipes)
44. Fish embryo lysis buffer (see Recipes)
45. SDS Washing buffer (see Recipes)
46. Urea Washing buffer (see Recipes)
47. Destaining solution (see Recipes)

Equipment

1. TripleTOF 6600 quadrupole time-of-flight mass analyser or similar (SCIEX)
2. Eksigent ekspert™ nanoLC 400 system or similar (SCIEX)
3. Dynabeads magnetic particle concentrator or similar (Applied Biosystems, catalog number: A13346)
4. Nikon SMZ18 stereo microscope with Nikon Intensilight Mercury White Light source or similar (Nikon)
5. Virtis Virsonic 100 ultrasonic cell disruptor or similar (Virtis Virsonic)
6. Zeiss LSM 710 meta upright confocal microscopes or similar (Zeiss)
7. Vortex
8. Thermo Fisher Pico™ 17 Microcentrifuge or similar (Thermo Fisher Scientific)
9. VWR® Tube Rotator or similar (VWR)
10. PowerPac™ HC High-Current Power Supply or similar (Bio-Rad)
11. Mini-PROTEAN Tetra Vertical Electrophoresis Cell or similar (Bio-Rad)
12. Vacufuge plus centrifuge concentrator or similar (Eppendorf)
13. Elmasonic S150 ultrasonic sonicator bath or similar (Elma)

Software

1. Analyst® TF 1.7 (SCIEX, https://sciex.com/support/software-support/software-downloads; require purchasing a licence or request a free trial)
2. ProteinPilot™ 5.0.1 (SCIEX, https://sciex.com/support/software-support/software-downloads; require purchasing a licence or request a free trial)
3. Microsoft Excel (Microsoft, https://www.microsoft.com/en-au/microsoft-365/excel)

Procedure

A. Outcross TurboID-dGBP lines with GFP-tagged lines of interest (*e.g.*, zebrafish lines expressing GFP-labelled proteins or regions of interest)

1. Set up zebrafish pairs in mating tanks. It is preferable to use males from the TurboID-dGBP line and females from the GFP-tagged lines for protein extraction from embryos younger than 3 dpf (see Note 1).
2. Collect embryos the next morning and incubate them in the embryo medium at 28.5°C.
3. Sort embryos at 3 dpf according to fluorescent marker expression under a fluorescence microscope. The transgenic fish lines are normally maintained as heterozygote carrying a single copy of the transgene. Therefore, an outcross will produce offspring with all four genotypes: WT embryos with no fluorescent marker, POI-GFP embryos with GFP fluorescent marker, TurboID-dGBP embryos with mKate2 fluorescent marker, POI-GFP and TurboID-dGBP co-expressing embryos with both GFP, and mKate2 fluorescent markers (Figure 1). For protein extraction from 3 dpf embryos or younger, sort the embryos after biotin incubation.

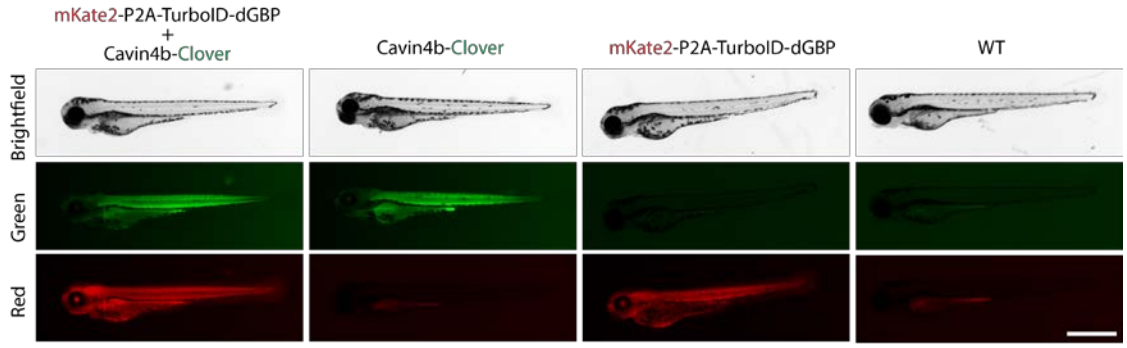


Figure 1. Representative embryos (3 dpf) from outcrossing the mKate2-P2A-TurboID-dGBP with Cavin4a-Clover line.
The transgenic fish lines are maintained as heterozygotes carrying a single copy of the transgene. Therefore, the crossing produces four combinations of genotypes indicated by corresponding fluorescent markers. Scale bar = 1 mm.

B. *In vivo* biotinylation

1. Prepare fresh biotin-supplemented embryo medium by diluting biotin stock solution into embryo medium to reach a concentration of 500 µM biotin.
2. Incubate sorted embryos (~350 each group) in ~ 25 ml biotin-supplemented embryo medium in a 10 cm Petri dish at 28.5°C for 18 h.
3. Remove unincorporated biotin by three changes of fresh embryo medium (20 min each change) after the biotin incubation. Added ~1 ml tricaine to the medium to anesthetise embryos before changing the medium.
4. Check the biotin labelling in the embryos by immunostaining with NeutrAvidin-DyLight 405 and immunofluorescence imaging (see next section).

C. Examining *in vivo* biotinylation by NeutrAvidin immunostaining

Note: This should be performed prior to streptavidin affinity pulldown and mass spectrometry (MS) analysis. A trial experiment of NeutrAvidin immunostaining with a small number of embryos is recommended before conducting a large-scale pulldown.

1. Sample three embryos for each group after *in vivo* biotinylation step and anaesthetise them in 500 ml ice-cold tricaine solution in a 24 well plate for 20 min.
2. Replace the tricaine solution with 500 µl of 4% paraformaldehyde (PFA) by carefully pipetting, and fix the embryos overnight at 4°C.
3. After fixation, wash embryos 3 times with PBS and permeabilise the embryos with 300 µl of 10 µg/ml proteinase K (10 min for embryos at 2 dpf; 15 min for embryos at 3 dpf; or 20 min for embryos at 4 dpf). Fix embryos again with 4% PFA for 15 min.
4. Wash embryos twice in 1 ml of 0.1% Tween 20 in PBS by gently swirling on a shaker for 5 min.
5. Transfer embryos into embryo blocking solution (see Recipes) for 3 h at room temperature.
   Note: Embryos can be kept in blocking solution overnight at 4°C.
6. Stain the embryos with NeutrAvidin-DyLight 405 (1:500 dilution in blocking solution) overnight at 4°C followed by four washes with 0.3% Triton X-100 in PBS, 30 min each wash on a shaker. For nuclear staining (if required), stain embryos with DAPI (see Recipes) for 10 min followed by 3 washes with PBS 0.3% Triton X-100, 20 min each wash on a shaker.
7. Mount embryos on a slide with coverslip; alternatively, the embryos can be imaged directly with a water dipping lens. Fluorophore DyLight 405 can be visualised using a blue filter on a fluorescent microscope or use laser 405 nm for excitation and detect emission between 415-460 nm on a confocal microscope.
   Note: Embryos co-expressing mKate2-P2A-TurboID-dGBP and GFP-POI should show a clear

colocalization of NeutrAvidin and GFP signals, whereas embryos carrying only one of the transgenes should give no or minimum NeutrAvidin staining. An example is provided in Figure 2.

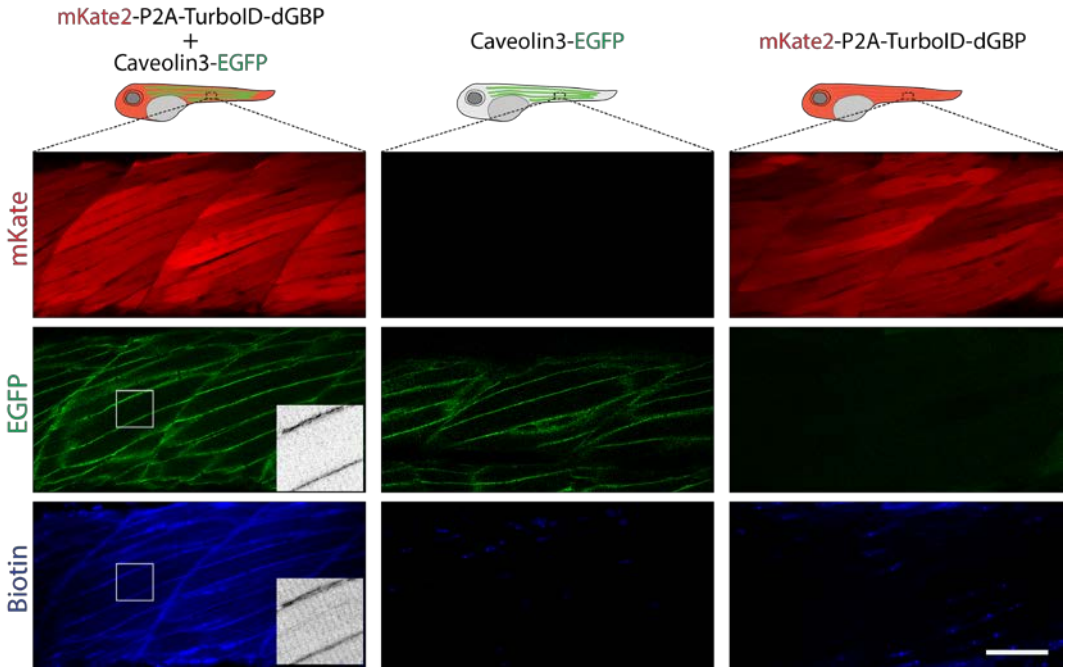


Figure 2. Representative confocal images showing TurboID-catalysed biotinylation in zebrafish embryos.
Embryos were obtained from outcrossing the TurboID-dGBP line with a Caveolin3-EGFP line. They were subjected to overnight biotin incubation at day 4 and fixed at day 5. TurboID-catalysed biotin labelling was visualised by NeutrAvidin-DyLight 405 staining. Strong biotin labelling was observed at the plasma membrane of muscle cells in the embryos co-expressing TurboID-dGBP and Cavoelin3-EGFP. This staining pattern corresponds to the subcellular localisation of membrane protein Caveolin3, and it is absent in the siblings expressing either TurboID-dGBP or Caveolin3-EGFP. The inserts show boxed area in inverted grayscale. Scale bar = 40 μm.

D. Yolk removal (strongly recommended for embryos younger than 5 dpf; otherwise, skip to section E)

1. Anesthetise embryos by adding tricaine solution and incubate on ice. Transfer the embryos into 2 ml Eppendorf tubes.
2. Replace the medium with ice-cold deyolking solution and disrupt yolk sac by pipetting embryos through a 200 μl pipette tip (Figure 3).
3. Pellet the embryos by brief centrifugation and remove supernatant, which contains yolk proteins. Rinse embryos twice with deyolking solution on ice.

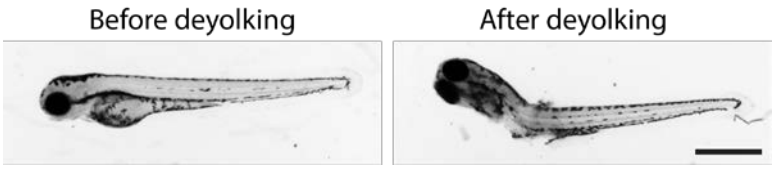


Figure 3. Representative images showing the 3 dpf embryos before and after deyolking.
The disrupted yolk sac was resulted from pipetting through a 200 μl pipette tip.

E. Protein extraction and biotin affinity purification

1. Homogenise deyolked zebrafish embryos in 1 ml of embryo lysis buffer (with freshly added protease inhibitors) by brief pulse sonication (1s sonication at intensity 5 with 1s interval for 1 min). Add another 1 ml of embryo lysis buffer and vortex for 30 min at 4°C. Centrifuge the lysates at 14,000 × *g* for 10 min at 4°C to remove insoluble tissue debris.
   Note: Zebrafish embryo lysates are prone to protein degradation. Add protease inhibitors and keep lysates on ice to minimise protein breakdown.
2. Transfer the supernatant to a new tube and measure the protein concentration using the Pierce™ BCA protein assay kit. For each sample, we used 4 mg of crude fish proteins for biotin affinity purification. However, the amount of crude protein required depends on the GFP-POI expression level and tissue types. For low expressing GFP lines or expression in low abundance cell types, a larger amount of crude protein lysates may be required.
3. Pass the fish lysates through a PD-10 desalting column to remove free biotin. This step is important for subsequent streptavidin pulldown because it increases the efficiency of capturing biotinylated proteins by streptavidin-conjugated beads.
4. Prewash Streptavidin-conjugated Dynabeads™ in embryo lysis buffer three times. Add 200 µl of washed Dynabeads into each lysate sample and constantly mix the lysate/beads on a rotor wheel at 4°C overnight.
5. After the incubation, separate the beads from the fish lysates using a magnetic particle concentrator and transfer to a new Eppendorf tube. Wash the beads twice with fish embryo lysis buffer, once with SDS washing buffer, once with urea washing buffer, and twice in embryo lysis buffer again. Note that the magnetic beads tend to clump together after capturing biotinylated proteins. Vortex the beads in the washing buffer to break down the aggregate and ensure a thorough wash.

F. Protein electrophoresis and protein gel staining (skip this step if on-bead digestion is used)

1. To elute captured proteins, incubate beads in 60 µl of 2× Blot LDS sample buffer with 2 mM biotin and 20 mM DTT at 95°C for 10 min. Briefly vortex the beads after the first 5 min of incubation. Note that the combination of free biotin and excess heat is able to efficiently elute the biotinylated proteins from streptavidin-conjugated beads (Cheah and Yamada, 2017).
2. Load the eluted protein sample to a Bolt™ 4-12% Bis-Tris Plus Gel (or similar) and separate the proteins at 100 V for 30 min.
3. Stain the protein gel with InstantBlue™ Coomassie Protein Stain (or Colloidal Coomassie Blue) (Figure 4). Note that the incubation time varies depending on the amount of protein. For maximal detection sensitivity, incubate the gel overnight in the staining solution.
4. Cut the protein gel into slices of 1-2 mm thickness. Avoid any blank gel in an excised gel slice since blank gel will reduce in-gel digestion efficiency. The excised gel slice can be stored in the fridge for one week.

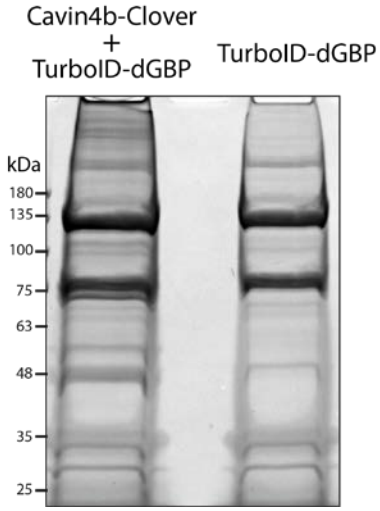


Figure 4. Protein gel stained by InstantBlue™ Coomassie Protein Stain.
The left lane contains a streptavidin pulldown from embryos co-expressing TurboID-dGBP and Cavin4b-Clover, while the right lane contains a streptavidin pulldown from control embryos expressing only TurboID-dGBP.

G. Sample preparation for mass spectrometry

1. For in-gel digestion, destain the gel slices with 500 µl of destaining solution and incubate for 30 min with occasional vortexing.
2. Replace the destaining solution with 200 µl of 100% acetonitrile and leave for 15 min. Replace the acetonitrile again with another 200 µl of 100% acetonitrile for 15-30 min to ensure dehydration of the protein gel. Acetonitrile is removed in preparation for trypsin digestion.
3. Add 200 µl of sequencing grade Trypsin/Lys-C (20 ng/µl in 50 mM ammonium bicarbonate pH 8 buffer). Note that the Trypsin/Lys-C solution should cover the gel pieces, and if required, a further 100 µl of Trypsin/Lys-C solution can be added. Incubate the samples at 37°C overnight.
4. Add 200 µl of 5% formic acid/acetonitrile (3:1, vol/vol) to each sample and incubate for 15 min at room temperature in a shaker. Transfer the supernatant into clean Eppendorf tubes and dry down in a vacuum centrifuge.
5. Add 12 µl of 1.0% (vol/vol) trifluoroacetic acid in H_2O to the tube and vortex. Incubate in a sonication bath for 2 min and then centrifuge for 1 min at 6,700 × *g*. Transfer the solution to an autosampler vial for MS analysis.

Data analysis

MS data were acquired and processed using Analyst TF 1.7 software (SCIEX). Protein identification was carried out using ProteinPilot™ software v5.0 (SCIEX) with Paragon™ database search algorithm (Figure 5). MS/MS spectra were searched against the zebrafish proteome in the UniProt database. The search parameter was set to thorough with False Discovery Rate (FDR) analysis. The cut-off for identified proteins was set to 1% global FDR. Endogenous biotinylated proteins, common contaminants and background proteins present in control embryos were subtracted. The MS2Count of each protein was used to compare the relative abundance of identified proteins.

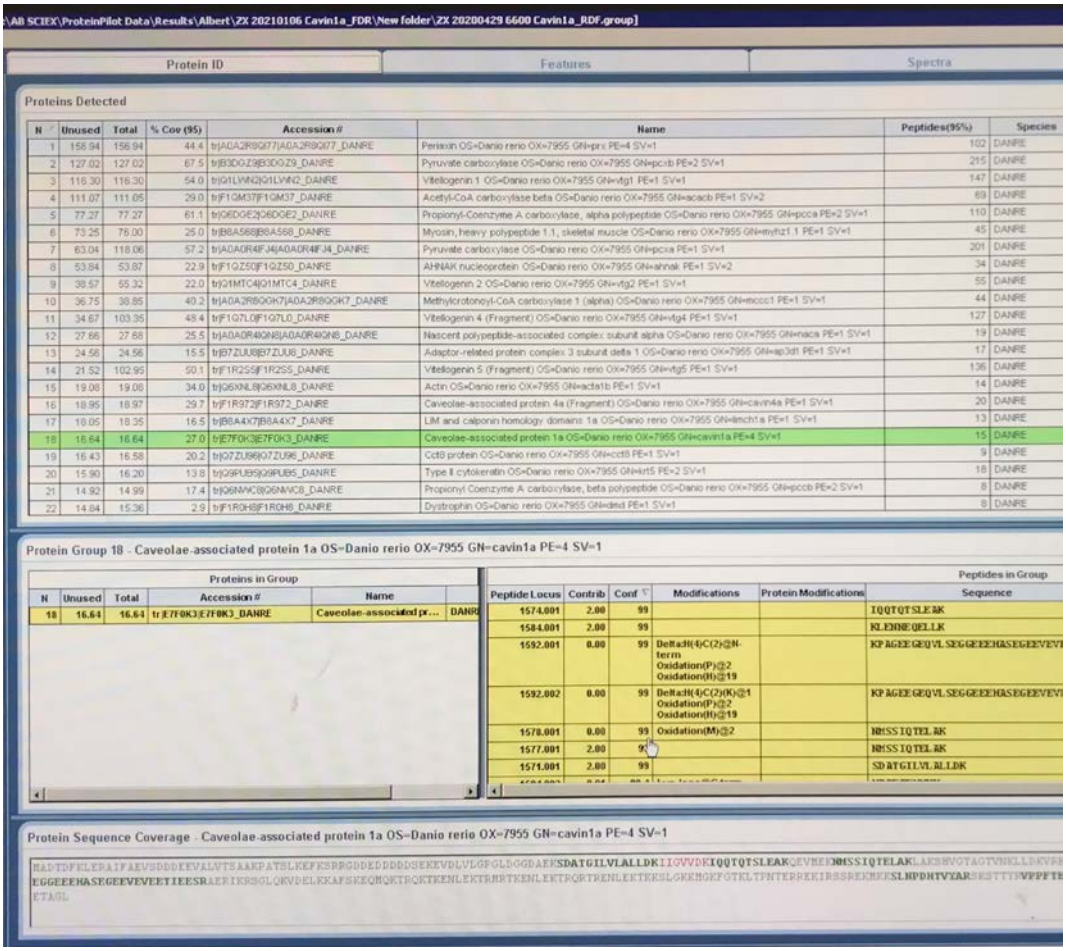


Figure 5. A screenshot of ProteinPilot™ software showing the proteins identified from the embryos co-expressing TurboID-dGBP and Cavin1a-Clover

Notes

1. Select males from the TurboID-dGBP line to cross with females from the GFP line if the proteomic analysis is conducted on embryos that are 3 dpf or younger. This prevents the presence of TurboID-dGBP in the yolk due to maternal inheritance (Pelegri, 2003) and reduces potential interference caused by yolk proteins.
2. The delivery of biotin to target cells/tissues is a prerequisite for TurboID to catalyse proximity-dependent biotinylation in zebrafish. The overnight incubation of 3 dpf zebrafish embryos in 500 µM biotin-supplemented embryo medium is sufficient for biotin to reach the musculature, endovascular system, and motor neurons in which TurboID-catalysed biotinylation was observed (Xiong *et al.*, 2021). The overnight incubation method was also tested with embryos from 2 to 8 dpf, and TurboID-catalysed biotinylation was observed in the musculature of all developmental stages tested (unpublished data).
3. The amount of protein biotinylated by TurboID is highly dependent on the expression level of the GFP-POI. For low GFP-POI expressing lines, older embryos or juvenile zebrafish may be required for producing detectable biotinylated proteins by TurboID. For GFP-POI expressed in low abundance cell types, specific tissue isolation after *in vivo* biotinylation may be required to improve the detection of TurboID-biotinylated proteins over background proteins.
4. For MS analysis, the in-gel digestion method is described in this protocol. Alternatively, on-bead digestion may also be possible for downstream MS analysis. Note that zebrafish embryos have a considerable amount of

endogenous biotinylated proteins (carboxylases). The presence of these endogenous biotinylated proteins might compromise the detection of low-abundance proteins in the sample. The SDS-PAGE step in the in-gel digestion method separates protein samples based on their size, which allows MS analysis on each individual protein band at different molecular weights. Therefore, in-gel digestion allows separate detection of endogenous biotinylated protein (around 70 and 135 kDa) from the proteins biotinylated by TurboID. This might improve MS detection for low-abundance proteins present in samples.

Recipes

1. **Embryo medium**
   5 mM NaCl
   0.17 mM KCl
   0.33 mM $CaCl_2$
   0.33 mM $MgSO_4$

2. **Tricaine stock (25×)**
   Dissolve 400 mg of Tricaine powder in 98 ml of H_2O and add 2 ml of 1 M Tris base (pH 9).
   Adjust pH to 7.
   Store at -20°C.

3. **Biotin stock solution (100 mM biotin in DMSO)**
   Dissolve 244.31 mg biotin power in 10 ml of DMSO with vortexing.
   Keep stock solution in an airtight amber bottle (or a Falcon tube wrapped with aluminium foil).

4. **Deyolking solution**
   55 mM NaCl
   1.8 mM KCl
   1.25 mM $NaHCO_3$
   Add cOmplete™ Protease Inhibitor Cocktail (2 tablets per 50 ml) before use.

5. **Embryo blocking solution**
   0.3% (vol/vol) Triton X-100 and 4% (wt/vol) bovine serum albumin in 1× PBS solution.
   Store solution at 4°C up to 2 weeks.

6. **DAPI stock and intermediate solutions**
   To make 14.3 mM DAPI stock solution, dissolve 5 mg of DAPI in 1 ml of deionised water (sonication might be required to facilitate dissolution). The stock solution can be aliquoted and stored at -20°C.
   Add 2.1 µl of the 14.3 mM DAPI stock solution to 100 µl PBS to make a 300 µM DAPI intermediate dilution.
   Dilute the intermediate solution 1:1,000 in PBS to make a 300 nM DAPI stain solution.

7. **Trypsin/Lys C working solution**
   Rehydrate lyophilised Trypsin/Lys-C Mix in Reconstitution Buffer to 1 µg/µl.
   Aliquot and store the rehydrated Trypsin/Lys-C. Mix in -20°C.
   Dilute an aliquot of the 1 µg/µl Trypsin/Lys-C. Mix in 50 mM ammonium bicarbonate pH 8 to a final concentration of 20 ng/µl.

8. **Formic acid/acetonitrile solution**
   Combine 5% (vol/vol) formic acid in ultrapure H_2O with acetonitrile at a ratio of 3:1 (vol/vol).

9. **Trifluoroacetic acid solution**
   1.0% (vol/vol) Trifluoroacetic acid in ultrapure H_2O

10. **4% paraformaldehyde**
    Dissolve 40 g of paraformaldehyde powder in 800 ml of heated 1× PBS solution (~ 60°C) in a glass beaker on a stir plate (in a ventilated hood).
    Slowly add 1 N NaOH dropwise until the solution clears.
    Adjust the volume of the solution to 1 L with 1× PBS.
    Adjust the pH to approximately 6.9.
    Store the solution at 4°C.

11. **Fish embryo lysis buffer**
    50 mM Tris-HCl, pH 7.5
    150 mM NaCl
    1% NP-40
    0.1% SDS
    5 mM EDTA
    0.5% Na-deoxycholate

12. **SDS Washing buffer**
    2% SDS in 50 mM Tris-HCl pH 7

13. **Urea Washing buffer**
    2 M urea in 10 mM Tris-HCl pH 8

14. **Destaining solution**
    100 mM ammonium bicarbonate/acetonitrile (1:1, vol/vol).
    Dissolve 1.58 g ammonium bicarbonate in 100 ml acetonitrile and add 100 ml ultrapure water.
    Store this solution at 4°C for up to 2 months.

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The TurboID is based on the engineered biotin ligase by Branon *et al.* (2018), and the destabilised nanobody (dGBP) is based on the studies of Tang *et al.* (2016). This protocol describes the methodology used in the research paper (doi: 10.7554/eLife.64631) by Xiong *et al.* (2021).

Competing interests

The authors declare that they have no conflicts of interest and no competing interests.

Ethics

All experiments were approved by the University of Queensland Animal Ethics Committee. Approval ID IMB/271/19/BREED. Approval valid 01/01/2019 – 01/01/2022.

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