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Split-BioID: a proximity biotinylation assay for dimerization-dependent protein interactions

Sofie De Munter[1], Janina Görnemann[1], Rita Derua[2,3], Bart Lesage[1], Junbin Qian[1], Ewald Heroes[1], Etienne Waelkens[2,3], Aleyde Van Eynde[1], Monique Beullens[1] and Mathieu Bollen[1]

1 Laboratory of Biosignaling & Therapeutics, KU Leuven Department of Cellular and Molecular Medicine, University of Leuven, Belgium
2 Protein Phosphorylation & Proteomics Lab, KU Leuven Department of Cellular and Molecular Medicine, University of Leuven, Belgium
3 SyBioMa, KU Leuven, Belgium

Correspondence
M. Bollen, KU Leuven, Campus Gasthuisberg, Herestraat 49, O&N1, PB901, B-3000 Leuven, Belgium
Fax: +32 16 33 07 35
Tel: +32 16 33 06 44
E-mail: mathieu.bollen@kuleuven.be

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The biotin identification (BioID) protocol uses a mutant of the biotin ligase BirA (BirA*) fused to a protein-of-interest to biotinylate proximate proteins in intact cells. Here, we show that two inactive halves of BirA* separately fused to a catalytic and regulatory subunit of protein phosphatase PP1 reconstitute a functional BirA* enzyme upon heterodimerization of the phosphatase subunits. We also demonstrate that this BirA* fragment complementation approach, termed split-BioID, can be used to screen for substrates and other protein interactors of PP1 holoenzymes. Split-BioID is a novel and versatile tool for the identification of (transient) interactors of protein dimers.

Keywords: biotinylation; phosphatase-substrate mapping; protein–ligand screening; proximity labeling; reporter-fragment complementation

Protein function and regulation is often mediated by associated polypeptides. The identification of interacting proteins therefore represents a key step of protein characterization. Classically, techniques such as affinity purification, protein correlation profiling, chemical cross-linking, reporter-fragment complementation, and two-hybrid screens are used to characterize the interactome of a protein of interest (POI) [1,2]. More recently, enzymatic proximity-labeling protocols, including ascorbate peroxidase (APEX) tagging and proximity-based biotin identification (BioID) have been developed as additional methods to study the interactome of a POI [1]. BioID was developed as a tool to biotinylate proteins that (transiently) associate with or are spatially close to a protein-of-interest (POI) in intact cells [3–5]. It involves the expression of a mutant of the bacterial biotin ligase BirA fused to a POI. The adopted BirA mutant (R118G, further referred to as BirA*) still forms biotinoyl-5′-AMP from biotin and ATP but this 'activated' biotin is only loosely bound at the active site. Hence, the highly reactive biotinoyl-5′-AMP diffuses away and indiscriminately biotinylates proteins at lysine residues within an action radius of about 10 nm [6]. The biotinylated proteins can be recovered by standard biotin-affinity purification approaches (e.g., on NeutrAvidin-coated beads), identified by mass spectrometry and further validated as candidate interactors of the POI. BioID has already been successfully used to explore the interactome of more than 140 distinct proteins [7].

Protein phosphatase PP1 is a very conserved and ubiquitously expressed enzyme [8]. It is estimated to catalyze about half of all protein dephosphorylation events in eukaryotic cells. Nevertheless, PP1 acts in a highly specific and tightly regulated manner *in vivo* due to its association with PP1-interacting proteins

Abbreviations

BioID, proximity-based biotin identification; NIPP1, nuclear inhibitor of PP1; PIP, PP1-interacting protein; PP1, protein phosphatase 1; RepoMan, recruits PP1 onto mitotic chromatin at anaphase.

(PIPs) that determine the activity, substrate specificity, and/or subcellular localization of the phosphatase. Because of the huge diversity of PIPs (~ 200 in mammals) each PP1 holoenzyme catalyzes the dephosphorylation of only a small subset of PP1 substrates. Progress in the functional characterization of PP1 holoenzymes is seriously hampered by the lack of straightforward methods to identify substrates of specific PP1 holoenzymes. A complicating factor is that PP1 only interacts transiently with its substrates and does not form a covalent catalytic intermediate that can be trapped. Also, holoenzyme-specific inhibitors that could be used to induce the selective hyperphosphorylation of their substrates are not available.

Reporter-fragment complementation assays are well suited to study transient protein interactions in a cellular context [2]. These assays are based on the expression of two halves of a reporter protein fused to either of two interacting proteins. The heterodimerization of the latter proteins brings the split-reporter fragments close enough to reconstitute a functional reporter protein. We have designed a fragment complementation version of BioID as a tool to identify protein interactors of specific PP1 holoenzymes. Here, we report that the inactive halves of BirA*, when separately fused to PP1 and a PIP, form a functional biotin ligase that biotinylates substrates and other vicinal proteins upon heterodimerization of the phosphatase subunits (Fig. 1A). We suggest that the split-BioID protocol can be generally applied to map (transient) interactors of protein dimers.

Material and methods

Materials

The BirA(R118G)-HA construct, referred to as BirA*, was a gift from Karl Kramer (Addgene plasmid # 53581). For constructs expressing N-terminal BirA* fragments (BirA*-N), a POI and Flag-tag were added to the 5′-end to generate POI/Flag/BirA*-N fusions. Plasmids expressing C-terminal BirA* fragments (BirA*-C) were used to generate BirA*-C/HA/POI fusions. The PP1-binding mutant of NIPP1 (V201A and F203A), referred to as NIPP1 m, was described before [9]. The calculated weight of the BirA*-C/HA/NIPP1 Split 1, 2, and 3 constructs is 68, 62, and 60 kDa, respectively.

The following antibodies were used: anti-HA (Covance Research Products Inc; MMS-101P, Denver, PA, USA), anti-Flag (Sigma, F7425, St. Louis, MO, USA), polyclonal swine anti-rabbit immunoglobulins/HRP (Dako, P0217, Santa Clara, CA, USA), polyclonal rabbit anti-mouse immunoglobulins/HRP (Dako, P0260), Alexa Fluor® 568 donkey anti-(rabbit IgG) (H+L) (Life Technologies, A10042, Carlsbad, CA, USA), Alexa Fluor® 633 goat anti-(mouse IgG) (H+L) (Life Technologies, A21052), Streptavidin Alexa Fluor 488® (Thermo Fisher Scientific, S11223, Waltham, MA, USA), Anti-EGFP (Santa Cruz, 8334, Dallas, TX, USA), anti-CDC5L (homemade rabbit polyclonal) [10], anti-SF3B1/SAP155 (MBL, D221-3, Woburn, MA, USA), anti-PP1 (homemade mouse monoclonal, gift of Dr. J. Vandenheede), anti-SNW1 (Proteintech, 259266-1-AP, Rosemont, IL, USA), and anti-NASP (Proteintech 11323-AP).

Cell culture and sample preparation

HEK293T cells (ATCC, Molsheim, France) were cultured in high-glucose DMEM, supplemented with 10% fetal calf serum. The cells were transfected with Genius (Westburg, Leusden, the Netherlands), jetPRIME (PolyPlus Transfection, Strasbourg, France) or Fugene (Promega, Madison, WI, USA), according to manufacturer's protocol. Twenty-four hours after transfection, 100 μM biotin (Sigma, B4639) was added to the medium for 16 h if indicated. Cells were lysed in a lysis buffer containing 50 mM Tris-HCl at pH 7.5, 100 mM NaCl, 1% NP40, 0.1% SDS, 1.5 mM $CaCl_2$, 0.5 mM phenylmethanesulfonyl fluoride, 0.5 mM benzamidine, and 5 μM leupeptin. Cell lysates were used for immunoblotting. For EGFP immunoprecipitation, the cells were lysed in the same buffer supplemented with protein phosphatase inhibitors (0.5 μM microcystin and 20 mM NaF, and analyzed by immunoblotting and the fusions were precipitated using GFP-Trap® (Chromotek, Planegg-Martinsried, Germany). Flag-tagged proteins were immunoprecipitated (IP) using ANTI-FLAG® M2-Agarose (Sigma, A1205).

BioID and split-BioID

Cell lysates from biotin-treated cells were incubated for 30 min at 37 °C with 60 units micrococcal nuclease (Thermo Fisher Scientific), cleared by centrifugation for 5 min at 4000 ***g***, and then rotated for 1 h at 4 °C with 60 μL Sepharose. Precleared lysates were incubated for 3 h at 4 °C with 60 μL high-capacity NeutrAvidin Agarose Resin beads (Thermo Fisher Scientific). The beads were washed twice with 2% SDS, twice with LiCl buffer (10 mM Tris-HCl at pH 8.0, 0.25 M LiCl, 0.5% NP-40, 0.5% sodium deoxycholate, and 1 mM EDTA), twice with lysis buffer and once with 50 mM Tris-HCl at pH 7.5. For direct analysis by immunoblotting, the beads were boiled with protein loading buffer and loaded on denaturing protein gels. For parallel analysis by mass spectrometry, beads were washed 3× with 50 mM ammonium bicarbonate, resuspended in 200 μL ammonium bicarbonate, and

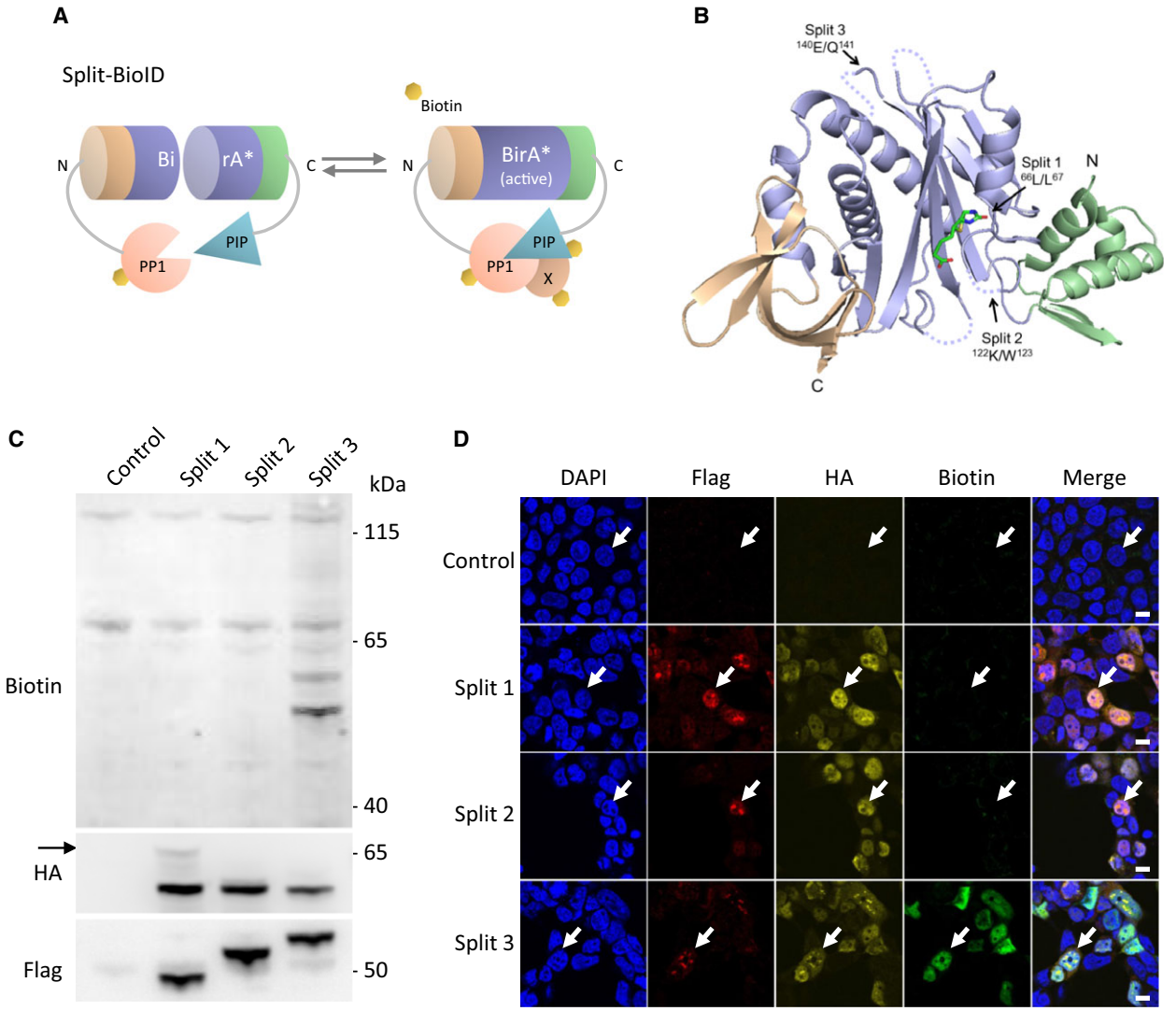


Fig. 1. The principle and design of split-BioID. (A) Scheme illustrating the principle of split-BioID. In the presence of biotin, split-BioID biotinylation depends on BirA* fragment complementation through heterodimerization of fused proteins, as illustrated here for PP1 and a PIP. Both the fusions and vicinal proteins (x) will be biotinylated. (B) Cartoon representation of BirA from *Escherichia coli* (PDB 1BIB). Residues where BirA* was split are indicated with the one-letter code and crystallographically invisible residues are represented as a dotted line. Biotin bound to the active site is displayed as a green stick. Green, N-terminal noncatalytic domain; blue, central catalytic domain; beige, C-terminal noncatalytic domain. This figure was made using PyMol [https://www.pymol.org/]. (C) The fragment complementation pairs were transiently overexpressed and cells without overexpression were used as a negative control (Control). After 16-h incubation with 100 μM biotin, the cells were lysed and analyzed for the presence of biotinylated proteins using Streptavidin Alexa488 (Biotin), PP1 fusions (Flag) and NIPP1 fusions (HA). → denotes the nondegraded form of BirA*$^{67–321}$-HA-NIPP1. (D) Same as in panel C but the cells were fixed and stained for DNA (DAPI), biotinylated proteins (biotin), PP1 fusions (Flag), or NIPP1 fusions (HA). Scale bars, 10 μm.

incubated overnight with 1 μg trypsin (Roche, Basel, Switzerland) at 37 °C. Next, the supernatant was transferred to a fresh tube and the beads were incubated again for 2 h with 0.5 μg trypsin in 150 μL ammonium bicarbonate. The supernatant was pooled with the first one. The beads were washed once more with 100 μL H_2O and the supernatant was pooled with the other eluates.

Samples were dried in a speed-vacuum concentrator before being desalted on a C-18 Micro Spin Column (Harvard Apparatus, Holliston, MA, USA). The resulting peptide mixture was submitted to high-resolution LC-MS/MS using an Ultimate 3000 nano UPLC system interfaced with an Orbitrap
Q-Exactive MS via an EASY-spray (C-18, 15 cm) column (Thermo Fisher Scientific). The Q-Exactive MS was operated in data-dependent mode selecting the top ten precursors for MS/MS. Protein identifications were obtained from the MASCOT (Matrix science, version 2.2.2, Boston, MA,

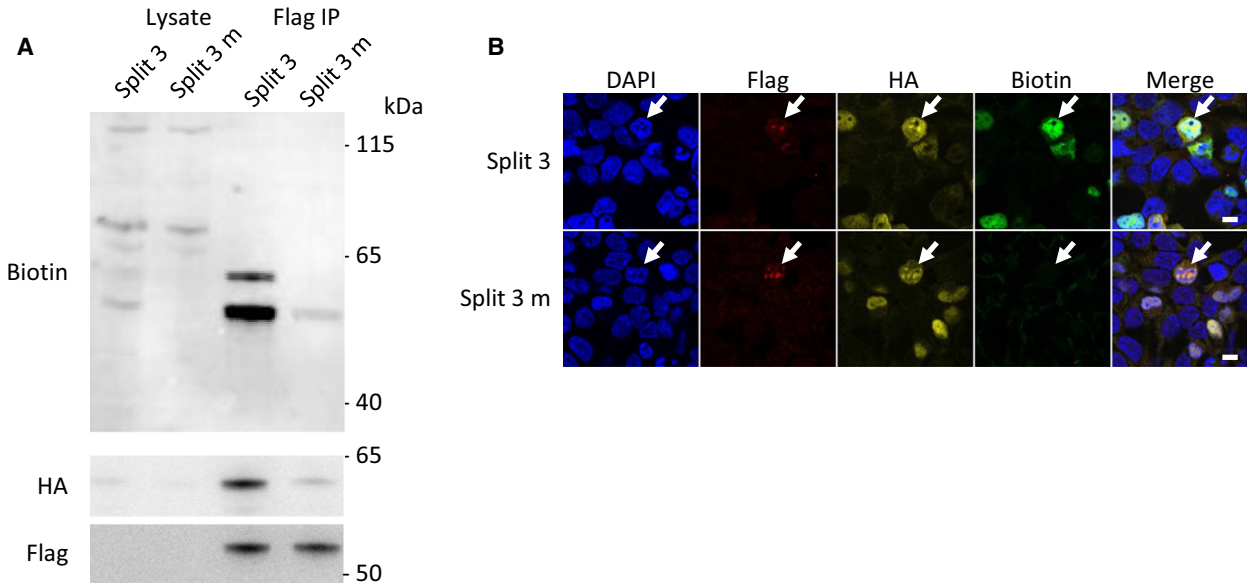


Fig. 2. BirA* fragment complementation depends on the heterodimerization of the fusions. (A) The NIPP1 fusion of the split 3 complementation pair was mutated in an essential PP1-binding site (Split 3 m) [9]. The binding of the wild-type and mutated complementation pairs was examined by Flag immunoprecipitation of the PP1 fusion and HA detection of the NIPP1 fusion. The presence of biotinylated proteins was examined with Streptavidin Alexa488 (Biotin). (B) Same as in panel A but the cells were fixed and stained for DNA (DAPI), biotinylated proteins (biotin), PP1 fusions (Flag), or NIPP1 fusions (HA). Scale bars, 10 μm.

USA) search engine using UniProt/SwissProt (Homo sapiens, 20339 entries) as a database and allowing up to three missed tryptic cleavages, oxidation of Met, and biotinylation of Lys as variable modifications. Only peptides with a score above the significance threshold were taken into account. Relative quantification of proteins from unique peptides after protein grouping was executed with PROGENESIS software (Nonlinear Dynamics, Durham, NC, USA).

Immunofluorescence

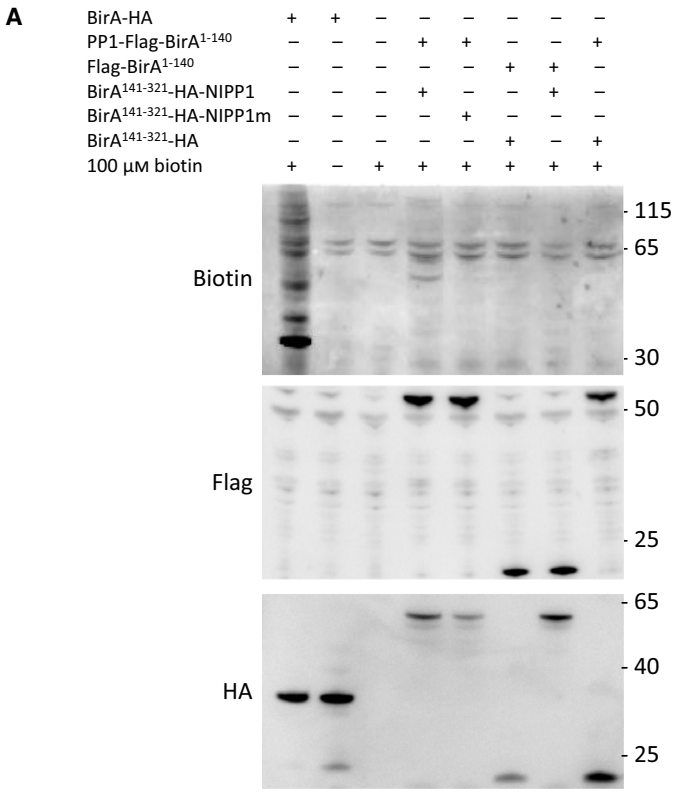
For immunofluorescence imaging, cells were consecutively grown on polylysine-coated coverslips in a 24-well chamber, fixed with 4% paraformaldehyde, permeabilized with 0.5% Triton X-100, blocked in 3% BSA/PBS and incubated in 1% BSA/PBS with the primary antibodies for 3 h at room temperature and with secondary antibodies for 1 h at room temperature. DNA was stained with 4',6-diamidino-2-phenylindole (DAPI). Confocal images were acquired with a Leica TCS SPE laser-scanning confocal system mounted on a Leica DMI 4000B microscope and equipped with a Leica ACS APO 40 × 1.15NA oil objective (Leica, Wetzlar, Germany).

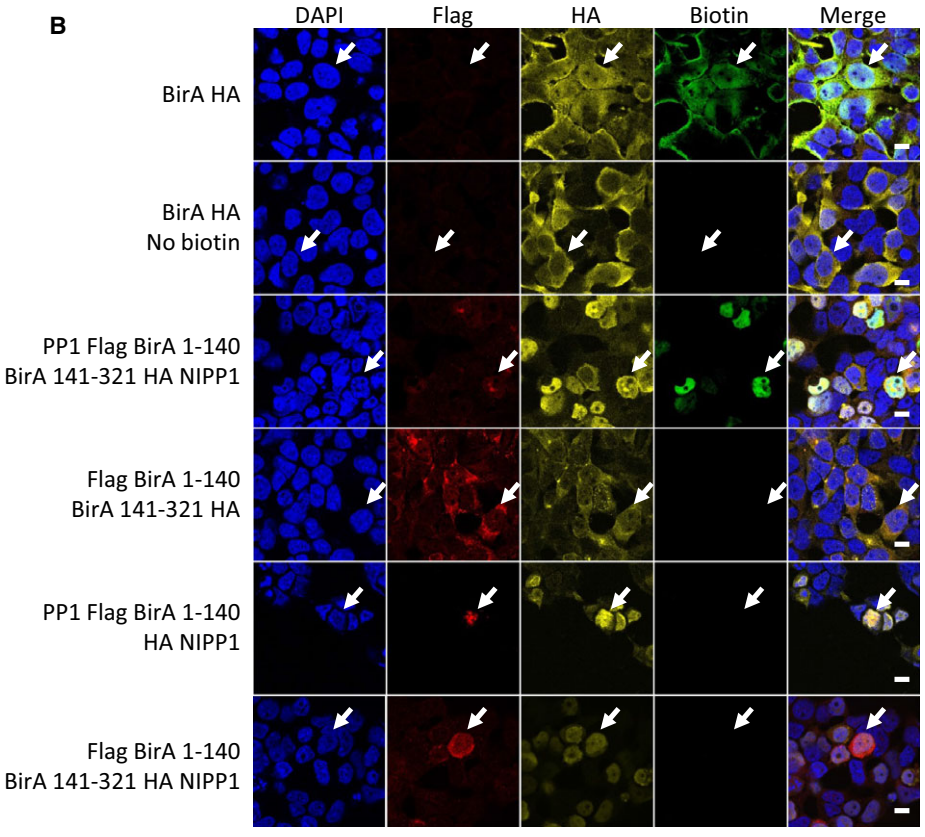
Results and Discussion

The design and validation of BirA*-fragment complementation pairs

The crystal structure of BirA from *Escherichia coli* was used to rationally design three pairs of possible complementation fragments [11]. BirA-R118G (BirA*) was split into two fragments within surface loops of the catalytic domain so as not to disrupt secondary structure elements: N-terminal to (a) L67 between the β2 strand and αD helix (Split 1), (b) W123 between the β4 and β5 strands (Split 2), or (c) Q141 between the β5 strand and the αF helix (Split 3) (Fig. 1B). The N-terminal fragments of BirA* were fused to the C terminus of Flag-tagged PP1γ. We reasoned that fusions with the C terminus of PP1 were unlikely to interfere with phosphatase function because the C terminus of PP1 is unstructured, not part of the catalytic domain and not required for the binding of most PIPs [8]. The C-terminal fragments of BirA* were N-terminally fused to HA-tagged NIPP1, a ubiquitously expressed nuclear PIP. The choice for a fusion

Fig. 3. Split-BioID is critically dependent on the expression of both BirA* complementation fragments. (A) BirA* or the indicated BirA* fragments and their fusions were transiently transfected in HEK293T cells. Expression of these proteins was examined using Flag and HA antibodies and biotinylation was checked using Streptavidin Alexa488. (B) Same as in panel A but the cells were fixed and stained for DNA (DAPI), biotinylated proteins (biotin), PP1 fusions (Flag), or NIPP1 fusions (HA). Scale bars, 10 μm.


A
BirA-HA + + – – – – – –
PP1-Flag-BirA1-140 – – – + + – – +
Flag-BirA1-140 – – – – – + + –
BirA141-321-HA-NIPP1 – – – + – – + –
BirA141-321-HA-NIPP1m – – – – + – – –
BirA141-321-HA – – – – – + – +
100 µM biotin + – + + + + + +
Biotin
115
65
30
Flag
50
25
HA
65
40
25


B
DAPI
Flag
HA
Biotin
Merge
BirA HA
BirA HA
No biotin
PP1 Flag BirA 1-140
BirA 141-321 HA NIPP1
Flag BirA 1-140
BirA 141-321 HA
PP1 Flag BirA 1-140
HA NIPP1
Flag BirA 1-140
BirA 141-321 HA NIPP1

with the N terminus of NIPP1 was based on previous observations that N-terminal tagging of NIPP1 does not affect its binding to PP1 and substrates [12].

HEK293T cells were transiently transfected with the PP1/Flag/BirA*-N and BirA*-C/HA/NIPP1 fusion pairs for 24 h, followed by an overnight incubation with 100 μM biotin. Subsequently, the cell lysates were examined for expression of the fusions and the presence of biotinylated proteins by immunoblotting (Fig. 1C). Strikingly, biotinylated proteins were only detected after expression of the fusions with the Split 3 BirA* fragments (Fig. 1C). Selective fragment complementation with the Split 3 pair was confirmed by immunostaining for biotin in fixed cells (Fig. 1D). The Split 3 complementation fragments (PP1/Flag/BirA*-N and BirA*-C/HA/NIPP1) each comprise about half of the catalytic domain of BirA* and also split the active site (Fig. 1B). The absence of complementation with the Split 1 and 2 pairs is possibly due to the proteolytic degradation of the corresponding BirA*-C/HA/NIPP1 fusions, as suggested by their migration with a smaller than calculated molecular mass during SDS/PAGE (the arrow in Fig. 1C denotes the intact Split 1 fusion). It should also be pointed out that the fusion with the BirA* fragments did not affect the well-established nuclear targeting of NIPP1 and PP1γ (Fig. 1D) [10,13].

Both immunoblotting (Fig. 2A) and immunostaining (Fig. 2B) revealed that virtually no protein biotinylation was detected with the Split 3 complementation pair when NIPP1 was mutated (Split 3 m) in an essential PP1-binding site, known as the RVxF-motif (mutation of Val201 and Phe203 to Ala). It was verified that this mutation strongly reduced the binding of NIPP1 to PP1 (Fig. 2A), consistent with previous data [9]. This shows that the BirA* fragments alone do not interact with each other with sufficient affinity to reconstitute a functional enzyme and that the complementation was dependent on the heterodimerization of PP1 and NIPP1. Consistent with this notion, no protein biotinylation was detected after transfection with the Split 3 BirA* fragments alone or when only one of the two BirA* fragments was fused to PP1 or NIPP1 (Fig. 3A,B). In contrast, the expression of full-length BirA* alone resulted in a considerable background biotinylation (Fig. 3A).

Mapping of the interactome of PP1-NIPP1 and PP1-RepoMan by split-BioID

We have subsequently used split-BioID to map the putative protein interactome of heterodimers of PP1 and two nuclear PIPs, namely NIPP1 or RepoMan [8]. Biotinylated proteins were purified by affinity purification using NeutrAvidin-agarose and identified by mass spectrometry after trypsinolysis (Table S1). Four selection criteria were used to generate a stringent list of candidate proximal interactors of PP1-NIPP1 (NIPP1 split) (Fig. 4A) and PP1-RepoMan (RM split) (Fig. S1). First, the signals for putative interactors of PP1-NIPP1 and PP1-RepoMan were normalized for the same amount of PP1 in both complexes. Subsequently, a ratio of the signals in the PP1-NIPP1 versus the PP1-RepoMan candidates list, and vice versa, was calculated. All ratios above 1.5 were considered to reflect selective association with one holoenzyme. Second, we compared the signals for candidate interactors of PP1-NIPP1 and PP1-RepoMan with signals of background biotinylation (negative control) using full-length BirA*; the signals were normalized for total abundance and only ratios larger than 1.5 were retained. Third, we only kept candidates for which the spectral counts, reflecting the number of associated MS/MS spectra, were at least 1. Fourth, typical background contaminants were removed. More specifically, we removed proteins from the candidate list if they showed up with average spectral counts of ≥ 20 and/or were identified in ≥ 50% of the screens analyzed by the CRAPome [14]. Figure 4A contains candidate interactors with ratio ≥ 1.5 in both comparisons (NIPP1 split versus RM split and NIPP1 split versus
control).

The remaining stringent list of biotinylated proteins after expression of the PP1/Flag/BirA*-N and BirA*-C/HA/NIPP1 fusions included PP1 (PPP1CC) and NIPP1 (PPP1R8) themselves, which represents additional independent and direct evidence for successful BirA*-fragment complementation. Other proteins that were specifically biotinylated with this complementation pair included CDC5L and SF3B1/SAP155 (SF3B1), two established interactors of the ForkHead-Associated (FHA) domain of NIPP1 and likely substrates of PP1-NIPP1 [10,15,16]. The next top-candidate interactors of PP1-NIPP1 were the splicing factor SNW1 [17], the histone chaperone NASP [18], the cytokine and mitotic phosphatase regulator IK [19], and the signaling adaptor protein CRK [20]. PP1 and the top five candidates of the PP1-NIPP1 split-BioID could all be independently validated as interactors of ectopically expressed EGFP-tagged NIPP1 in HEK293T cells in coimmunoprecipitation (IP)experiments (Fig. 4B).

Protein phosphatase 1 and RepoMan were identified as biotinylated proteins following the expression of the PP1/Flag/BirA*-N and BirA*-C/HA/RepoMan fusions, attesting to their heterodimerization and successful BirA* complementation (Fig S1). Split-BioID also identified importin α (KPNA2) as a PP1-RepoMan

A

Gene name	NIPP1 split / RM split	NIPP1 split / Control
PPP1R8	193	135
PPP1CC	1*	23.8
CDC5L	78.9	10.8
NASP	26.2	2.3
SF3B1	24.6	5.8
SNW1	24.1	5.4
IK	14.9	5.0
CRK	10.4	2.2
HAT1	2.6	2.1
NPEPPS	1.6	1.9
RNH1	1.6	1.8

B

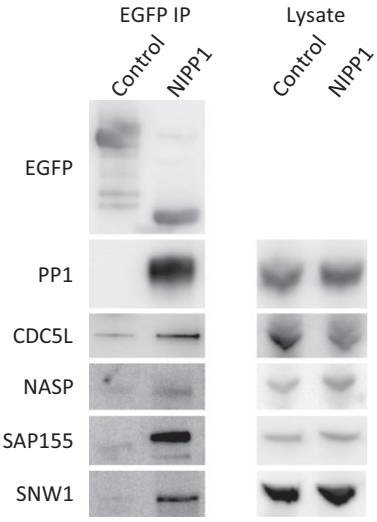


Fig. 4. Split-BioID analysis of the PP1-NIPP1 interactome. (A) Top candidates of associated proteins with PP1/NIPP1. The middle column shows the split-BioID signal ratios for PP1/NIPP1 (NIPP1 split) versus PP1/RepoMan (RM split) that were ≥ 1.5. All signals for putative interactors of PP1/NIPP1 and PP1/RepoMan were first normalized for the same amount of PP1, resulting in a PP1 ratio of 1 (*). The right column shows the split-BioID signal ratios for PP1-NIPP1 (NIPP1 split) versus BirA* (control) that were ≥ 1.5. Panel A only shows candidate-associated proteins with signal ratios in both the middle and right column ≥ 1.5, with spectral counts for each protein in the considered condition ≥ 1 and that are not typical background contaminants according to the CRAPome [14]. (B). HEK293T cells were transiently transfected with EGFP-β-galactosidase (Control) or EGFP-NIPP1 (NIPP1). The fusions were immunoprecipitated (IP) and the lysates and IPs were examined for the indicated proteins by immunoblotting.

interactor, in accordance with SILAC data from two independent studies [21,22]. The major substrates of RepoMan-associated PP1, aside from RepoMan itself, are histones. Although several histones were in the candidate list of interactors (Table S1), they were removed as likely contaminants by the CRAPome analysis [14].

Comparison of BioID and split-BioID

Finally, we compared putative protein interactors of RepoMan and PP1-RepoMan, as identified by BioID and split-BioID, respectively (Table 1). The list of candidate interactors was restricted using the same criteria as detailed for Figs 4A and S1, except that signal ratios with another PIP or PP1 holoenzyme were not taken into consideration. BioID validated several previously described direct interactors of RepoMan, including PP1 (PPP1CC), two subunits of PP2A (PPP2R2A and PPP2R5D), and Importin 7 (IPO7) [21–23]. Consistent with the association of RepoMan with chromatin, numerous transcription factors and DNA repair proteins were also identified as potential vicinal proteins. The RepoMan/PP1 split-BioID identified much less biotinylated proteins compared to RepoMan BioID (Table 1). This was expected because split-BioID only causes the biotinylation of proteins in the vicinity of the PP1-RepoMan heterodimer and not all RepoMan is associated with PP1 [24]. In addition to PP1 and RepoMan themselves, the DNA-replication inhibitor MCM3AP [25] was identified as a common neighboring protein of PP1-RepoMan and RepoMan. Importin α (KPNA2) and α1-antitrypsin (SERPINA1) were identified as solely associated with PP1-RepoMan but not with RepoMan. The selective binding of Importin α to the PP1-RepoMan holoenzyme can be rationalized by a previous report showing that the binding of importins to RepoMan depends on the dephosphorylation of RepoMan by associated PP1 [24].

Conclusions

Split-BioID is a novel tool that combines reporter-fragment complementation and proximity-labeling, two strategies that are commonly used to study protein–protein interactions [1,2]. Compared to BioID, split-BioID suffers much less from background biotinylation because a functional BirA* is only generated upon forced heterodimerization of the BirA* fragments. We have used split-BioID to identify known and novel

Table 1. Comparison of BioID and split-BioID. Top-candidate interactors of RepoMan BioID (A) and PP1-RepoMan split-BioID (B). Shown are the signal ratios for RepoMan BioID (RM BioID) and PP1-RepoMan split-BioID (RM split) versus BirA* (control) that were ≥ 1.5. Only candidate-associated proteins with a spectral count ≥ 1 were retained. Typical background contaminants according to the CRAPome were removed [14]

A

Gene name	RM control
CDCA2	353.2
PSIP1	71.6
CDCA8	33.1
MECP2	19.8
WIZ	15.0
HDGF	14.6
IPO4	14.2
PPP1CC	9.8
TPX2	8.7
TOX4	7.6
RAD21	6.7
UBQNL2	5.9
DLGAP5	5.9
ARID3B	5.9
ZNF280C	5.8
BAZ2A	5.4
FEN1	5.4
PHF6	5.3
KIF23	5.1
PPP2R2A	4.1
NAP1L4	4.0
TMPO	3.7
SUGP1	3.5
MRE11A	3.3
CBX3	3.3
SNW1	3.3
ZNF148	3.2
PABPN1	3.2
CDC73	3.1
YWHAQ	3.1
RBBP7	2.9
IK	2.8
MTA1	2.5
PPP2R5D	2.5
GTF2A1	2.5
SERBP1	2.4
RIF1	2.4
ALKBH5	2.3
PDAP1	2.1
ZC3H15	2.0
SPDL1	1.9
IPO7	1.8
RANBP3	1.8
TNPO1	1.7
ATG3	1.7
RANGAP1	1.7
MCM3AP	1.7
PPIL4	1.6

Table 1. (Continued).

A

Gene name	RM control
CWC27	1.6
DDX42	1.5
PSPC1	1.5

B

Gene name	RM split control
CDCA2	64.8
PPP1CC	31.8
MCM3AP	10.3
SERPINA1	8.2
PGK1	2.2
KPNA2	1.7

interactors of two heterodimeric PP1 holoenzymes but the technique can in theory be used to identify vicinal proteins of any protein dimer. To our knowledge, this is the first available tool that enables the specific tagging of (transient) interactors of protein dimers in a cellular context. The general strengths and weaknesses of split-BioID are similar to those of BioID [5]. The main advantage of (split-) BioID is that it detects protein–protein associations in a cellular context, including transient and/or weak interactions such as enzyme–protein substrate interactions. Also, the loss of associated proteins during cell fractionation does not affect the outcome thanks to the *in cellulo* labeling with biotin. A limitation of (split-)BioID stems from the duration of the labeling with biotin (16 h) and the possible interference of the BirA* tags with the localization or function of the POIs. Also, biotinylated proteins are not necessarily present in the same complex as the POI (heterodimer). Therefore, additional tests are needed to distinguish between proximal proteins and (in)direct interactors. One specific disadvantage of the split-BioID protocol is the need for co-overexpression of the two fusions, resulting in a lower transfection efficiency. However, this limitation can probably be overcome by using cell lines that stably overexpress at least one of the fusions. We only performed (split-)BioID in untreated cells but proximity-dependent biotinylation can also be performed after treatments (e.g., addition of cell-cycle inhibitors, stress-inducing or DNA-damaging agents) that are expected to interfere with protein–protein interactions of interest. In conclusion, we have presented here a novel and versatile fragment complementation variant of BioID, termed split-BioID, that can in principle be used to specifically map (transient) interactors of any protein dimer.

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Author contributions

SD, JG, JQ, RD, and MBe performed the experiments and analyzed the data. BL developed the concept of split-BioID. EH generated Fig. 1B. AV, MBe, EW, and MBo supervised the study. SD, MBe, and MBo wrote the manuscript. MBo coordinated the project.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Split-BioID analysis of the PP1/RepoMan interactome.

Table S1. (Split-)BioID data for PP1-NIPP1, PP1-RepoMan, and RepoMan analyzed with Progenesis software.