AURORA®
SERIES

AURORA SERIES® IMPACT PORTFOLIO · PROTEIN–PROTEIN INTERACTIONS

Protein-Protein Interactions: the evidence base for Aurora Series® columns

Comprehensive review of peer-reviewed publications establishes IonOpticks Aurora Series® columns as the chromatographic platform of choice for sensitive, reproducible interaction proteomics. From molecular glue target discovery and native receptor interactomes through to locus-resolved chromatin complexes and residue-level crosslinking.

779

Unique crosslinked residue pairs from a 25 cm Aurora® Ultimate™ 25×75, against 560 from a 50 cm column (Müller et al., 2025). [9]

298

CRBN-recruited targets across 20 IMiD analogs, 270 previously unreported, by diaPASEF on Aurora® Ultimate™ 25×75 (Baek et al., 2025). [2]

124

Putative GLP-1 receptor membrane interactors, 120 previously unreported, on an Aurora® Ultimate™ 25×75 (Dang et al., 2025). [11]

Highlights from the published evidence

Ten studies citing Aurora Series® columns in interaction proteomics workflows have been reviewed.

01 **ENGINEERED PROTEIN–PROTEIN INTERACTIONS**

448 Å² induced interface
69% on the CRBN LON domain

Deep-fractionated 16-plex TMT proteomics on an IonOpticks Aurora Ultimate™ 25×75 C18 UHPLC column (Vanquish Neo – Orbitrap Eclipse) identified G3BP2 as the selective target of the cereblon glue MRT-5702. G3BP2 is recruited by mimicry of a natural protein–protein interaction rather than by any known degron, and cryo-EM later resolved the complex at 2.5 Å.

Annunziato et al., Nat Struct Mol Biol 2026

02 **MAPPING CHEMICALLY INDUCED PROTEIN–PROTEIN INTERACTIONS**

298 CRBN-recruited targets
270 previously unreported

20 IMiD analogs profiled by lysate-based affinity proteomics across two cell lines, read out by diaPASEF on a timsTOF Pro 2 with an Aurora Ultimate 25×75 C18 UHPLC column. The screen widened the molecular glue target space from around 50 known neosubstrates to 298 recruited proteins, 251 of them outside the zinc-finger class.

Baek et al., Nat Commun 2025

03 **MAPPING DEGRADER-INDUCED UBIQUITYLATION SITES**

>2,000 peptides from a single interactome
400 unique high-confidence sites · ~20% previously unreported

diGly enrichment applied to a miniTurbo proximity interactome, separated on an Evosep One 40 SPD Whisper method with an Aurora Elite™ 15×75 C18 UHPLC column and acquired using diaPASEF. Enrichment lifted diGly detection from 30 peptides to over 2,000 and resolved the PWWP1 lysine clusters that UNC8732-driven NSD2 degradation depends on.

Tessier et al., Commun Biol 2026

04 **THE GLP-1 RECEPTOR, AS ITS OWN CELLS SEE IT**

124 putative interactors
120 previously unreported · 3 new regulators of insulin secretion

An Aurora Ultimate 25×75 C18 UHPLC column on a timsTOF Pro 2 carried both the diaPASEF quantification and the biotin-site identification behind the first interactome map of an endogenous membrane receptor, GLP-1R, arguably the decade's most important drug target.

Dang et al., Nat Chem Biol 2025

05 INTERACTOMICS THAT CHANGED A TEXTBOOK MODEL

471 mitochondrial proteins
59 high-confidence interactors · 1 new metabolon

An Aurora Ultimate 25×75 C18 UHPLC column ran the MICU1 immunoprecipitation screen that uncovered a calcium-dependent SDH/GPD2 metabolon. This result revised fifty years of thinking on how calcium regulates mitochondrial metabolism.

Cohen et al., Nat Metab 2026

06 ORTHOGONAL VALIDATION OF PROXIMITY LABELLING HITS

3 orthogonal methods
4 replicates · 1 new regulator, mechanism resolved

In the validation arm, Pol II and Cyclin T1 IP-MS on an Aurora Elite 15×75 C18 UHPLC column (Vanquish Neo into an Orbitrap Exploris 240) independently confirmed dCas9-directed proximity labelling hits and identified FUBP3 as required for heat shock gene activation.

Cenik et al., Mol Cell 2024

07 COMPLEXES, NOT JUST COMPONENTS

Cohesin ring
Pol II holoenzyme · SUMOylated histones

Antibody-free proteo-genomics on an Aurora Ultimate 25×75 C18 UHPLC column (nanoElute2 into a timsTOF SCP, 66-minute dia-PASEF, library-free DIA) recovered intact chromatin complexes and a sub-stoichiometric SUMO-modified histone subproteome in the same workflow.

González-Vinceiro et al., Sci Adv 2025

08 INTERACTOME MAPPING AT NUCLEIC ACID TARGETS

2 million cells
5 of 6 shelterin subunits · 4.3× tighter labelling than HRP

Photocatalytic proximity labelling of DNA loci and lncRNAs on an Aurora Elite 15×75 C18 UHPLC column (90-minute gradients into an Orbitrap Eclipse with FAIMS). The workflow recovered intact complexes and BioPlex-validated interactions from fixed cells without genetic engineering.

Biletch et al., bioRxiv 2026

09 ONE COLUMN, EVERY DATASET

6 datasets
2 baits · 3 cell backgrounds · 60-min gradients

An Aurora Ultimate 25×150 C18 UHPLC column on nanoElute and a timsTOF Pro 2 supported every arm of a study whose entire subject is background: r2 = 0.94–0.96 between control expression and enriched signal across dozens of conditions.

Jiang et al., Mol Cell Proteomics 2025

10 PROTEIN-PROTEIN INTERACTIONS: MORE CROSSLINKS, HALF THE COLUMN

779 vs 560 residue pairs
25 cm IonOpticks column vs 50 cm PepMap column

An Aurora Ultimate 25×75 C18 UHPLC column outperformed a 50 cm PepMap for crosslinked peptide identifications on the Orbitrap Astral. Its 120 Å pores and 1.7 µm particles are matched to an analyte class that linear-peptide columns are not designed for.

Müller et al., Nat Commun 2025

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KEYWORDS

Protein–protein interactions, interactomics, Aurora Series®, Aurora® Elite™, Aurora® Ultimate™, IonOpticks, packed emitter column, nanoZero®, C18, crosslinking mass spectrometry, CLMS, proximity labelling, TurboID, APEX2, BioID, affinity purification, IP-MS, AE-MS, molecular glues, targeted protein degradation, CRBN, diGly, ubiquitylation, O-GlcNAc, dia-PASEF, DIA, TMT, FAIMS, Orbitrap Astral, Orbitrap Eclipse, Orbitrap Fusion Lumos, Orbitrap Exploris 240, Orbitrap Exploris 480, timsTOF Pro 2, timsTOF SCP, Vanquish Neo, nanoElute, Evosep One.

Why Aurora Series® columns work for protein–protein interaction proteomics

The published evidence converges on a consistent explanation. Interaction proteomics is a differential measurement made on scarce, enriched material, and Aurora columns supply the reproducibility, sensitivity and separation that such measurements depend on. Five properties recur across the studies summarised here.

01 Reproducibility is what separates an interactor from background

Every interactome in this summary is defined by comparison: bait against control, compound against vehicle, modified against unmodified. Those comparisons are only as reliable as the separation is consistent. The most direct test measured whether enriched signal scaled with TurboID abundance, and it did, at $R^2 = 0.94$. Across control conditions, up to 97.0% of individual proteins fitted with R2 > 0.5, relationships that can only be measured if peptide-level separation is consistent in every run [7]. GLP-1 receptor interactors were called only where enrichment exceeded 100-fold over the probe-free control and competition by excess GLP-1 exceeded eight-fold, with DIA quantification consistent across biological replicates [11]. Forty-eight of 59 high-confidence MICU1 partners were present in at least two of three replicates [12], and the chromatin studies relied on MS1 label-free quantification, match-between-runs or library-free DIA, each of which assumes consistent retention [10,13,14].

02 Zero post-column dead volume and sensitivity for scarce, enriched material

The Aurora packed-emitter architecture integrates the packed bed directly into the electrospray emitter and uses the nanoZero® fitting, eliminating the pre- and post-column dead volumes that dominate peak broadening in nanoflow LC-MS. In interaction proteomics, where the sample is a pulldown rather than a lysate, this is what turns a scarce eluate into a usable dataset. The studies here recovered five of six shelterin subunits from around 2 million fixed cells [10], 471 verified mitochondrial proteins from an S-Trap digest of co-IP eluate [12], at least 1,000 diGly sites per sample from half of each proximity-enriched digest without fractionation [3], and SETDB1 from a sub-stoichiometric SUMO-modified histone H1 subproteome [14]. On the Orbitrap Astral, the Aurora Ultimate™ 25×75 configuration returned 192 crosslinked residue pairs from just 1 ng of protein [9].

03 Pore and particle geometry matched to large, low-abundance analytes

Not every analyte in interaction proteomics is a linear tryptic peptide. Crosslinked peptides carry the combined mass of two peptides plus the linker and are present at a fraction of the abundance of their linear counterparts. In a direct comparison on the same instrument, gradient and sample, the 25 cm Aurora Ultimate™ 25×75 returned 779 unique residue pairs against 560 for a 50 cm PepMap column, with smaller full-width half-maximum values and higher precursor intensities [9]. Its 120 Å pores (versus 100 Å), 1.7 µm particles (versus 2.0 µm) and pressure tolerance above 1,700 bar are matched to this analyte class. Sharper peaks matter most for the low-abundance crosslinks, which in the same study were enriched for short-distance links spanning the protein core and so carried much of the structural information [9].

04 Depth in the discovery screen defines the target

In chemically induced and native interactome discovery, the proteomics screen is the first step of the programme, and a partner that is not detected cannot be pursued. Twenty-four high-pH fractions per 16-plex TMT set, each run over a 168-minute gradient, detected G3BP2 depletion while its 64%-identical paralogue G3BP1 was unaffected; that dataset defined the target carried through to a 2.5 Å cryo-EM structure [1]. Lysate-based affinity proteomics captured 298 CRBN-recruited proteins, 270 previously unreported and 251 outside the zinc-finger class, and one of them was converted into a selective lead compound [2]. Around 1,200 cell membrane proteins quantified per cell type resolved 124 GLP-1 receptor interactors, 120 of which were absent from BioGRID [11].

05 One chemistry across instruments, acquisition modes and study arms

The strongest interaction datasets hold the separation constant while everything else varies. In one molecular glue study, the Aurora Ultimate™ 25×75 configuration carried label-free DDA on an Orbitrap Exploris 480 and diaPASEF on a timsTOF Pro 2 [2]; in another, the same column carried both diaPASEF quantification and ddaPASEF biotin-site identification [11]. A single column also supported every arm of an Orbitrap Astral versus Orbitrap Eclipse crosslinking benchmark, so that differences could be attributed to the mass spectrometer [9], and in two further studies a single Aurora column generated every proteomic dataset [7,14]. The same Aurora chemistry is available in XT (Thermo EASY-Spray / Nanospray Flex), CSI (Bruker CaptiveSpray), XS (SCIEX OptiFlow) and UNI formats. Because the interaction work summarised here spans Orbitrap Astral, Eclipse, Fusion Lumos, Exploris 480 and 240, timsTOF Pro 2 and timsTOF SCP platforms, laboratories running mixed fleets can standardise on one chemistry for every arm of a study.



Across every study the same principle recurs: change the bait, the labelling chemistry or the mass spectrometer, but keep the column.

01 The protein–protein interaction challenge

Almost every cellular process is carried out not by single proteins but by the complexes and transient assemblies they form. Mapping those interactions is how proteomics moves from inventory to mechanism, defining which partners a protein engages, where, when and under what conditions. It is also, increasingly, how drugs are discovered, because molecular glues and degraders work by creating or exploiting protein–protein interfaces [1-3]. Mass spectrometry has become the dominant route to establishing these maps because it identifies interacting partners in their native cellular context, at proteome scale, and without a prior hypothesis about what should be there.

Two large-scale efforts show how far the problem extends. Affinity purification–mass spectrometry of 10,128 human proteins in HEK293T cells produced the BioPlex 3.0 network of 118,162 interactions among 14,586 proteins. A second network, built from 5,522 immunoprecipitations in HCT116 cells, showed that the interactome is extensively remodelled between cell types, with cell-specific interactions rewiring conserved core complexes [4]. Scale is not the only difficulty, and the same literature has shown how hard it is to tell a true interactor from noise. The CRAPome, which launched with 360 negative-control purifications contributed by 12 laboratories, established that nonspecific background is pervasive in affinity purification and that separating bona fide interactors from contaminants is a core analytical problem rather than a clean-up step [5]. Proximity labelling extended interactomics to transient and spatially defined associations, with TurboID and miniTurbo cutting labelling to around ten minutes in living cells [6], but promiscuous labelling brings a background of its own [7]. Crosslinking mass spectrometry adds residue-level structural restraints to interaction maps, at the cost of analysing crosslinked peptides that are rare, large and chromatographically demanding [8, 9].

What these approaches share is the nature of the sample that reaches the mass spectrometer. Interaction proteomics rarely analyses a whole-cell digest. It analyses the eluate of a pulldown, peptides released from streptavidin beads, a diGly-enriched fraction, or crosslinked peptides that make up a small minority of the total. Inputs are small, the signal of interest is outnumbered by what co-purifies with it, and the result is always a differential call: bait against control, compound against vehicle, one condition against another. Every protein on a published interactor list is the product of a fold-change and a statistical threshold applied across replicate injections.

In every one of these workflows, the chromatography defines what can be called an interactor. Peak shape and peak capacity determine whether a low-stoichiometry partner rises above co-purifying background. Retention-time reproducibility determines whether label-free quantification and match-between-runs compare bait and control fairly. Whether large analytes such as crosslinked peptides separate at all depends upon stationary-phase geometry [9]. When control and experimental arms are not separated identically, chromatographic drift becomes indistinguishable from biology [7]. The analytical column is therefore not a neutral consumable but central to the definition of an interactome.

This publication summary sets out the peer-reviewed evidence, and one preprint, that IonOpticks Aurora Series® columns meet that requirement. The studies used the Aurora Ultimate™ 25×75, the Aurora Elite™ 15×75, and in one case, the 150 µm ID Aurora Ultimate™ 25×150, across chemically induced, native, chromatin-anchored and structural interaction proteomics.



02

The evidence base

The studies summarised below span affinity purification–mass spectrometry; enzyme-, ligand- and photocatalyst-directed proximity labelling; lysate-based affinity proteomics of molecular glues; TMT-based degradation profiling; ubiquitin-remnant enrichment; and crosslinking mass spectrometry. They were acquired on Orbitrap Astral, Orbitrap Eclipse, Orbitrap Fusion Lumos, Orbitrap Exploris 480 and 240, timsTOF Pro 2 and timsTOF SCP platforms, interfaced through Vanquish Neo, EASY-nLC, nanoElute, nanoElute 2 and Evosep One systems. Each cites IonOpticks Aurora Series® columns in its methods. Nine have been published in peer-reviewed journals; one, Biletch et al. [10], is a bioRxiv preprint. Citation numbers refer to the reference list in Section 6.

2.1 Chemically induced interactions

2.2 Native interactomes of drug targets and organelles

2.3 Chromatin- and nucleic acid–anchored interactomes

2.4 Background control and residue-level crosslinking

2.1 Chemically induced interactions: molecular glues and targeted protein degradation

Molecular glue degraders and PROTACs act by creating protein–protein interfaces that did not previously exist, recruiting a target to an E3 ligase so that it can be ubiquitylated and degraded. Finding which proteins a compound recruits, how they are recruited, and which lysines are then modified are all interaction proteomics questions. The three studies below address each in turn: the global proteomics screen that identifies a neosubstrate [1], affinity proteomics that maps every protein a glue recruits whether or not it is degraded [2], and site-level mapping of degrader-induced ubiquitylation [3].

PAPER 01 · REFERENCE [1]

Molecular glue discovery starts at the proteomics screen. The IonOpticks Aurora Ultimate 25×75 C18 UHPLC column delivers the proteomic depth needed to uncover G-loop-independent neosubstrates through molecular surface mimicry, turning natural PPI hotspots into druggable glueprints for targeted protein degradation.

G3BP2 hijacks a pre-existing PPI hotspot via surface mimicry: Deep TMT proteomics on an IonOpticks Aurora UltimateTM 25×75 C18 UHPLC column uncovers Cereblon neosubstrate with 69% of the 448 Å2 interface off the canonical CULT site

Annunziato, S., Quan, C., Donckele, E. J., Lamberto, I., Bunker, R. D., Zlotosch, M., Schwander, L., Murthy, A., Wiedmer, L., Staehly, C., Matysik, M., Gilberto, S., Kapsitidou, D., Wible, D., De Donatis, G. M., Trenh, P., SriRamaratnam, R., Strande, V., Lieberherr, R., Lyon, D., Steiner, D., Silva, J., Almeida, R., Dolgikh, E., DeMarco, B., Tsai, J., Sadok, A., Zarayskiy, V., Walter, M., Tiedt, R., Lumb, K. J., Bonenfant, D., Fasching, B., Castle, J. C., Townson, S. A., Gainza, P., & Petzold, G. (2026). Cereblon Induces G3BP2 Neosubstrate Degradation Using Molecular Surface Mimicry. *Nature Structural & Molecular Biology*, 33(3), 479–487. https://doi.org/10.1038/s41594-025-01738-8

> **Study snapshot:** A 16-plex TMT global proteomics screen of CRBN-directed molecular glues in CAL51 cells identified MRT-5702 as a selective degrader of G3BP2. Twenty-four high-pH reversed-phase fractions were each separated on an IonOpticks Aurora UltimateTM 25×75 C18 UHPLC column with 168-minute gradients at 400 nL/min on a Vanquish Neo coupled to an Orbitrap Eclipse. Cryo-EM at 2.5 Å (PDB 9OS2) then showed that cereblon recruits G3BP2 without a G-loop, engaging a pre-existing PPI hotspot on the NTF2L domain by mimicking the FGDF motif of USP10 through LON-domain residues F150–I152, establishing a glueprint strategy for expanding the CRBN target space beyond canonical degrons.

INSTRUMENTATION USED



Orbitrap Eclipse
TMT, 24 fractions



Vanquish Neo
UHPLC



Aurora® Ultimate™ 25×75
25 cm × 75 µm ID, C18

Targeted protein degradation depends on identifying proteins that can be stabilised inside a ternary complex, and until now cereblon (CRBN) was thought to recruit neosubstrates almost exclusively through a β-hairpin G-loop motif binding the CULT domain. overturn that assumption. Profiling a diversity selection of CRBN-centric molecular glue degraders by MS-based global proteomics, the Monte Rosa Therapeutics team identified MRT-5702, a dihydrouracil-containing compound that selectively reduced G3BP2 in CAL51 cells after 6 hours at 10 µM, with a smaller effect on USP10 and no effect on the closely related paralog G3BP1. Neither hit carries a predicted G-loop-like motif. Degradation was cullin-neddylation-, proteasome- and CRBN-dependent, and domain-swap experiments localised recruitment to the N-terminal NTF2L domain, which formed a ternary complex with DDB1–CRBN and MRT-5702 at a TR-FRET EC50 of 13 nM. 5 Å global resolution confirmed that CRBN hijacks the FGDF-binding PPI hotspot on G3BP2 via LON-domain residues F150 to I152.

The resulting interface inverts the established architecture: 69% of the 448 Å2 footprint sits on the LON domain against 13% on CULT, where all nine previously characterised complexes bury 46–84%. For the discovery proteomics, each of the 24 high-pH fractions was separated on an IonOpticks Aurora UltimateTM 25×75 C18 UHPLC column using a 168-minute gradient at 400 nL/min into an Orbitrap Eclipse. This work establishes a glueprint strategy for expanding the CRBN target space beyond G-loop-dependent substrates through surface mimicry of endogenous binding partners.

WHY THIS PAPER MATTERS

- **Breaks the G-loop paradigm.** G3BP2 is recruited by CRBN without a G-loop and without a G-loop surface mimic. Canonical CULT-domain residues (N351, H357, W400) are completely excluded from the interface, and instead uses LON-loop residues F150 to I152, so G-loop motif maps do not fully define CRBN glue space.
- **Establishes 'glueprints' for rational design.** CRBN mimics the USP10 FGDF motif to hijack a high-propensity PPI hotspot on G3BP2. This proves that composite protein–glue surfaces can reproduce natural protein binding footprints prospectively, rather than relying solely on retrospective rationalisation.
- **Demonstrates stereochemical gating of activity.** Only the (S,S)-isomer (MRT-5702D) is active, improving potency 2.7-fold (DC50 234 nM vs 627 nM for racemate) and maximal degradation to 79% (from 67%).
- **Opens therapeutic avenues in cancer and neurodegeneration:** G3BP2 is implicated in breast/lung cancer and stress-granule-driven neurodegeneration. Validation of MRT-5702-mediated degradation (DC50 627 nM, Dmax 67%) provides a first-in-class chemical handle for previously undruggable targets.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **Discovery-grade depth for target identification.** The Aurora Ultimate 25×75 C18 UHPLC column resolved all 24 consolidated high-pH fractions using 168-minute gradients into an Orbitrap Eclipse, enabling detection of G3BP2 depletion (log2 ≈ –3) against the whole-cell proteome background.
- **Paralog-level selectivity in the readout.** G3BP2 was depleted by roughly 8-fold (log2 ≈ –3) at high statistical significance while G3BP1 (64% identical, 76% similar) was unaffected, demonstrating that discriminating these closely related proteins across 16 TMT channels depends on consistent peptide-level separation in every fraction.
- **Delivered quantitative stability across ~67 hours of continuous acquisition.** 24 consolidated fractions × 168-minute gradients required more than two and a half days of continuous nanoflow separation per TMT set, ensuring the run-to-run retention-time consistency that TMT quantitative accuracy requires.
- **Front of the degrader discovery cascade.** The Aurora-separated dataset (ProteomeXchange PXD064243) defined the target that carried the programme through to a deposited 2.5 Å structure, positioning Aurora columns at the target-identification stage of molecular glue discovery rather than in downstream QC.

PAPER 02 · REFERENCE [2]

Deep fractionation on Aurora columns reveals what shallow methods miss, unveiling 270 hidden CRBN targets through high-throughput lysate proteomics and establishing nanoflow chromatography as the critical enabler of systematic molecular glue discovery.

Affinity proteomics on Aurora Ultimate 25×75 columns reveals 298 CRBN recruits: a six-fold interactome expansion from ~50 known to 270 novel targets, 251 outside the zinc-finger class

Baek, K., Metivier, R. J., Roy Burman, S. S., Bushman, J. W., Yoon, H., Lumpkin, R. J., Ryan, J. K., Abeja, D. M., Lakshminarayan, M., Yue, H., Ojeda, S., Xiong, Y., Che, J., Verano, A. L., Schmoker, A. M., Gray, N. S., Donovan, K. A., & Fischer, E. S. (2025). Unveiling the Hidden Interactome of CRBN Molecular Glues. *Nature Communications*, 16, 6831. https://doi.org/10.1038/s41467-025-62099-w

Study snapshot: A library of 20 IMiD-based molecular glues was profiled in MOLT4 and Kelly cell lysates using recombinant Flag-CRBN–DDB1ΔB as bait, automated on an Opentrons OT-2 at 96 samples in parallel. The workflow used diaPASEF on a timsTOF Pro 2 through an IonOpticks Aurora UltimateTM CSI 25×75 column at 50ºC with 50-minute gradients at 250 nL/min, and label-free DDA on an Orbitrap Exploris 480 using an Aurora Ultimate of equivalent dimensions but with 60-minute gradients. The study captured 298 proteins recruited to CRBN, 270 of them previously unreported, including 251 non-zinc-finger targets, and carried PPIL4 through to a selective molecular glue with an EC50 of 0.34 µM, creating an open-access proteomics portal for the TPD community.

INSTRUMENTATION USED



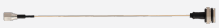
timsTOF Pro 2
diaPASEF, 250 nL/min



Orbitrap Exploris 480
FAIMS, label-free DDA



Aurora® Ultimate™ 25×75
25 cm × 75 µm ID, C18, XT



Aurora® Ultimate™ 25×75
25 cm × 75 µm ID, C18, CSI

Molecular glues are small molecules that create a new protein–protein interface, recruiting a target to an E3 ligase such as CRBN so it can be modified or degraded. Current discovery still leans on global degradation proteomics, which only scores proteins that disappear and therefore misses low-abundance recruits and proteins glued to the ligase without productive turnover. Baek et al. (Dana-Farber) map the induced CRBN interactome by capturing the complex rather than waiting for degradation. Activity-impaired Flag–CRBN–DDB1ΔB is spiked into lysate with compound, pulled on anti-FLAG beads, and co-enriched proteins are quantified by LC-MS. OT-2 automation plus diaPASEF took the method to 20 IMiD-class ligands, including thalidomide, lenalidomide, pomalidomide, CC-220, CC-92480, CC-90009, FPFT-2216 and CC-122, in MOLT4 and Kelly lysates at n = 4. 001 returned 298 enriched proteins, of which only 28 overlapped the roughly 50 neosubstrates reported in the literature. Frequency of enrichment served as a confidence metric: 102 proteins were enriched in at least three independent immunoprecipitations, and PATZ1, ZBED3, WIZ, IKZF2 and ASS1 each in more than 20. ASS1 and ZBED3 recur across the database with no prior report of degradation, examples of targets glued to CRBN without productive turnover. 4 Å (PDB 9DWV and 9DWW). Peptide separation for the enrichment and label-free datasets was performed on an IonOpticks Aurora UltimateTM 25×75 C18 UHPLC column on both a timsTOF Pro 2 and an Orbitrap Exploris 480. This work establishes a robust affinity proteomics workflow for molecular glue target identification and provides an open-access proteomics portal for the TPD community.

WHY THIS PAPER MATTERS

- **Measures recruitment, not degradation.** Using catalytically impaired CRBN–DDB1ΔB to trap ternary complexes, the workflow identifies "non-degrading glue" targets invisible to abundance-based screens. For parental IMiDs, only ~30% of enriched zinc-finger targets were previously reported as degraded, while ~60% were quantified but not degraded, yet remain therapeutically addressable.
- **Establishes lysate-based enrichment as a discovery engine.** The CRBN-DDB1ΔB spike-in workflow captures "non-degrading glue" targets missed by global proteomics, providing a scalable, cost-effective platform for molecular glue target identification using the Opentrons OT-2 automated pipeline.
- **Breaks the zinc-finger monopoly.** 251 of the 298 enriched proteins are non-ZF targets, expanding the CRBN target space beyond the fewer than a dozen non-ZF neosubstrates previously known. RNA-recognition motifs (13% of the top ten superfamilies) and kinase-like domains (6.4%) including CDK7, IRAK1 and TBK1 position molecular glues as kinase degradation alternatives to PROTACs.
- **Closes the loop from target to lead.** Screening ~6,000 IMiD analogs against PPIL4 by TR-FRET identified Z6466608628, a lenalidomide-based phenylpiperazine with improved PPIL4 potency (EC50 of 0.34 µM vs 1.05 µM for FPFT-2216) and selectivity. This compound recruits only PPIL4 and its partner DHX40, demonstrating direct conversion of interactome maps into selective chemical probes.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **Analytical backbone of the entire interactome dataset.** All 21 treatments across MOLT4 and Kelly cells (four replicates each) were separated on the Aurora Ultimate 25×75 column with 50-minute gradients (2–30% B at 250 nL/min, 50 °C) into a timsTOF Pro 2, generating the complete 298-target inventory from a single chromatographic configuration.
- **Sensitivity for low-aundance interactors.** The IP recovers CRBN recruits from whole lysate after a single anti-FLAG capture, including 270 proteins absent from published neosubstrate lists and parental-IMiD zinc-finger hits that were quantified in prior whole-cell degradation studies but never called as degraded. These FLAG eluates were separated on an IonOpticks Aurora Ultimate 25×75 column with integrated emitter into the timsTOF Pro 2.
- **One column format across two vendor platforms.** The same Aurora Ultimate 25×75 geometry served both the DDA proof-of-concept work on an Orbitrap Exploris 480 with FAIMS (60-minute gradients, 5–25% B, 250 nL/min) and diaPASEF interactome screening on the timsTOF Pro 2, enabling cross-platform benchmarking of label-free against TMT quantification.
- **Selectivity readout for lead optimisation.** Distinguishing the promiscuous FPFT-2216 profile from the near-single-target Z6466608628 profile in Kelly lysate rests on chromatographic consistency between immunoprecipitations, placing Aurora columns at the selectivity-assessment step of glue discovery as well as target identification.

PAPER 03 · REFERENCE [3]

When E3 interactomes hide low-abundance diGly peptides from standard BioID, the chromatography has to find them. Aurora Elite 15×75 delivered this sensitivity for PrIUS, mapping hundreds of high-confidence sites including PROTAC-induced modifications.

Mapping degrader-induced ubiquitylation sites at amino acid resolution: Aurora Elite 15×75 chromatography on timsTOF SCP enables the sensitive PrIUS workflow

Tessier, T. M., Maitland, M. E. R., Kutera, M., Dybas, J. M., St-Germain, J., Arrowsmith, C. H., Barsyte-Lovejoy, D., Zhang, W., Raught, B., & Weitzman, M. D. (2026). Identification of E3 ligase substrates and PROTAC-induced ubiquitylation sites using proximity-based identification of ubiquitin sites (PrIUS). *Communications Biology*, 9, 781. https://doi.org/10.1038/s42003-026-09964-6

Study snapshot: PrIUS combines BioID proximity labelling with diGly remnant enrichment to link E3 ligases directly to substrate ubiquitylation sites. Using NEDD4L, Tessier et al. recovered approximately 400 unique high-confidence sites, around 20% unreported in PhosphoSitePlus, against only 30 diGly peptides without enrichment. Applied to the NSD2 degrader UNC8732, PrIUS placed six of eight enriched sites in the PWWP1 domain, with lysine-to-arginine mutants confirming those sites contribute to degradation. SMARCA4 analysis with the PROTAC ACBI1 further identified the K1464 site missed by whole-cell approaches. The diGly-enriched acquisitions ran on an IonOpticks Aurora Elite 15×75 C18 UHPLC column on an Evosep One using a 40 SPD Whisper method on a timsTOF SCP.

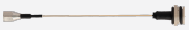
INSTRUMENTATION USED



timsTOF SCP
diGly dia-PASEF



Evosep One
UHPLC



Aurora® Elite™ 15×75
15 cm × 75 µm ID, C18

More than 250,000 human ubiquitylation sites have been catalogued, but most cannot be assigned to the E3 ligase responsible, a gap that limits both ubiquitin biology and the rational design of targeted protein degraders. investigate-this using Proximity-based Identification of Ubiquitin Sites (PrIUS), which splits the tryptic digest of a miniTurbo-tagged E3 interactome into two identical pools: one analysed directly to define the interactome, the other subjected to diGly remnant enrichment to map substrate modification sites. Using the HECT-type ligase NEDD4L as proof of concept, they show that E3 interactomes are intrinsically rich in diGly peptides yet almost entirely invisible without PTM-specific enrichment, with only 30 diGly peptides identified from the non-enriched material versus more than 2,000 unique diGly peptides after enrichment from the identical pool. PrIUS resolved approximately 400 unique high-confidence sites, distinguished substrates from non-substrate interactors including the NDFIP1/2 adapters and the E2 variant UBE2V2, captured both degradative K48 and non-degradative K63 linkages, and identified SCAMP3 K313 as a NEDD4L-regulated site confirmed by K313R mutagenesis. Turning the method on targeted protein degradation enabled mapping of UNC8732-induced ubiquitylation on NSD2 to the PWWP1 domain and validation of two lysine clusters as required for degradation and further identification of SMARCA4 K1464 as a unique site induced by the PROTAC ACBI1. The diGly-enriched acquisitions were separated on an IonOpticks Aurora Elite™ 15×75 C18 UHPLC column using the 40 SPD Whisper method on an Evosep One coupled to a timsTOF SCP, establishing a practical platform for assigning ubiquitylation sites to specific E3 ligases and supporting rational degrader design.

WHY THIS PAPER MATTERS

- **Demonstrates that PTM enrichment is essential for site detection.** Direct analysis of BioID material yielded only 30 diGly peptides, whereas enrichment from the identical peptide pool recovered more than 2,000 unique diGly peptides. Reanalysis of published biotin-based E3 substrate datasets revealed similarly low diGly yields, indicating that proximity-based substrate mapping methods without enrichment systematically miss modification sites.
- **Closes the E3-to-site assignment gap.** PrIUS links specific E3 ligases to specific substrate lysines in a single workflow, identifying approximately 400 high-confidence NEDD4L sites (roughly 20% absent from PhosphoSitePlus). Validation by mutagenesis confirms SCAMP3 K313 as a NEDD4L-regulated site, demonstrating the site-level specificity the ubiquitin field has lacked.
- **Provides mechanistic resolution for degrader programs.** For the NSD2 degrader UNC8732, six of eight enriched ubiquitylation sites mapped to the PWWP1 domain that the compound engages. Mutating the lysine clusters K309/312/317 or K341/343 to arginine shifted the DC50 relative to wild-type, delivering direct evidence of which lysines the degrader uses.
- **Distinguishes proximity from catalytic substrates.** By comparing BioID interactors with diGly-modified proteins, PrIUS differentiates proteins that are merely proximal to the ligase from genuine catalytic substrates.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **Matched platform to the analytical demand.** The Aurora Elite 15×75 C18 UHPLC column on a timsTOF SCP was used for all diGly-enriched acquisitions in the NEDD4L and NSD2 experiments, while the less demanding BioID interactome arm ran on a wider-bore 150 µm column on a timsTOF Pro 2. The narrow-bore Aurora format was selected for the modified peptides where sensitivity was critical.
- **Depth from limited material.** From half of each tryptic digest, the Aurora Elite arm consistently returned at least 1,000 diGly sites per sample and more than 500 high-confidence diGly peptides enriched in NEDD4L-fusion over control. This depth was achieved on proximity-enriched pulldown rather than whole-cell lysates, without fractionation.
- **Resolution of clustered modifications.** The Aurora Elite separated individual ubiquitylation sites within tight lysine clusters (K309/312/317 and K341/343) on NSD2. This provided the site-specific resolution required for the subsequent mutational validation of those clusters.

2.2 Native interactomes of drug targets and organelles

Interactomes are cell-type specific [4], and interactions measured on overexpressed, tagged proteins do not always reflect what an endogenous protein does in its own physiological setting [11]. The two studies below interrogate native interactomes directly: the membrane partners of the endogenous GLP-1 receptor, captured by a ligand-directed proximity probe in pancreatic β cells and neurons [11], and the calcium-dependent partners of the mitochondrial calcium sensor MICU1, captured by immunoprecipitation [12]. In both cases the interaction screen, rather than a prior hypothesis, delivered the biology.

PAPER 04 · REFERENCE [11]

When the target is GLP-1R, billions are at stake and the material is a one-minute labelling reaction on the surface of live cells. An IonOpticks Aurora 25×75 column underpinned both DIA quantification and DDA identification of biotinylated peptides across rat β cells and human neurons, returning 120 membrane interactors absent from BioGRID.

124 putative interactors, 120 of them unreported: mapping cell-type-specific GLP-1R membrane interactomes on an IonOpticks Aurora Ultimate 25×75 column

Dang, T., Yu, J., Cao, Z., Zhang, B., Li, S., Xin, Y., Yang, L., Lou, R., Zhuang, M., & Shui, W. (2025). Endogenous cell membrane interactome mapping for the GLP-1 receptor in different cell types. *Nature Chemical Biology*, 21(2), 256–267. https://doi.org/10.1038/s41589-024-01714-1

> **Study snapshot:** GLP-1R is among the most commercially significant drug targets in medicine, yet its cell membrane partners were largely unmapped because existing methods require an overexpressed, tagged receptor. Dang et al. solve this by turning the agonist itself into the labelling probe, wherein a GLP-1–APEX2 conjugate binds the endogenous receptor and biotinylates proximal proteins. diaPASEF and ddaPASEF analysis on an IonOpticks Aurora Ultimate 25×75 C18 UHPLC column coupled to a timsTOF Pro 2 quantified around 1,200 cell membrane proteins per cell type and resolved 124 putative interactors, with 108 specific to pancreatic β cells and 16 specific to neuronal cells, and with no overlap between them. Only four had been reported previously, and three of the novel interactors, ATP1B3, SLC4A4, and GPC4, proved to be functional negative regulators of GLP-1-induced insulin secretion, establishing ligand-based proximity labelling as a generalisable platform for discovering tissue-specific GPCR interactomes and new therapeutic targets.

INSTRUMENTATION USED



timsTOF Pro 2
diaPASEF and ddaPASEF



nanoElute
UHPLC

Aurora® Ultimate™ 25×75
25 cm × 75 µm ID, C18

Every approach previously used to find GLP-1R binding partners has required either an overexpressed epitope-tagged receptor, a non-mammalian system, or a proximity labelling enzyme fused to the receptor's cytoplasmic tail, which biases discovery towards intracellular partners such as G proteins and β-arrestins. invert this design by conjugating a promiscuous peroxidase to the C-terminus of GLP-1 itself, leaving the N-terminus free to anchor in the orthosteric pocket so that the probe binds and activates the native receptor and then labels proximal proteins on the extracellular face of the membrane. After comparing HRP and APEX2 versions, the authors selected GLP-1–APEX2, which matched native GLP-1 for potency and receptor binding. Applied to rat INS-1E β cells and human SK-N-SH neuronal cells with vehicle and GLP-1-competition controls in triplicate, and using LC-MS/MS with peptide separation on an IonOpticks Aurora Ultimate 25×75 C18 UHPLC column at 300 nL/min, the workflow quantified 1,205 and 1,287 cell membrane proteins respectively.

Proteins both enriched over control and competed by excess GLP-1 were scored as putative interactors, with 108 in β cells and 16 in neuronal cells. Strikingly, the two sets share no overlap, and only four of the 124 appear in BioGRID. Co-immunoprecipitation confirmed eight β cell candidates and three neuronal candidates, and siRNA knockdown identified SLC4A4, ATP1B3 and GPC4 as previously unreported negative regulators of GLP-1-induced insulin secretion, alongside the known interactor GIPR. A four-timepoint temporal experiment tracking 1,196 cell membrane proteins over 60 minutes of receptor activation reveals dynamic interactome remodeling. These findings establish ligand-based proximity labelling as a generalisable platform for mapping endogenous GPCR interactomes with cell-type specificity, revealing distinct regulatory landscapes that offer new therapeutic targets for metabolic disease.

WHY THIS PAPER MATTERS

- **The most commercially important target in metabolic disease.** GLP-1R agonists are the defining drug class of the current decade, and this is the first systematic map of the receptor's native membrane interactome. The three new functional regulators are immediately actionable: SLC4A4 in particular was previously proposed as a β cell therapeutic candidate in type 2 diabetes and is now validated as a negative regulator of GLP-1–stimulated insulin secretion.
- **Cell-type specificity, demonstrated rather than assumed.** The β cell and neuronal interactomes share no proteins at all. Because GLP-1R agonists act on both pancreas and brain with differing therapeutic and side-effect profiles, a receptor with genuinely different membrane partners in each tissue has direct consequences for how tissue-selective drug action might be engineered.
- **Endogenous beats engineered.** Only four of 124 putative interactors were already in BioGRID, whose GLP-1R entries derive mostly from overexpression-based affinity purification and yeast two-hybrid. The gap is the point: interrogating the endogenous receptor in a physiological host returns a substantially different answer from interrogating an engineered one.
- **First endogenous GLP-1R membrane interactome map.** The study pioneers a ligand-based proximity labelling strategy that captures native cell membrane protein networks without requiring receptor overexpression, overcoming a major technical hurdle in GPCR biology.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **One column, both acquisition modes.** The same Aurora Ultimate 25×75 column on a nanoElute coupled to a timsTOF Pro 2 ran the diaPASEF quantification that defined the interactors and the ddaPASEF analysis that identified biotin-phenol-modified peptides. Direct evidence of biotinylation was one of two criteria for selecting candidates for functional validation, making the DDA arm essential to the workflow.
- **Fold-change thresholds that demand quantitative reproducibility.** Putative interactors were defined by enrichment greater than 100-fold over the probe-free control (or detection only in the probe-treated group) and competition greater than than eight-fold by excess GLP-1 (or detection only in the probe-treated group). The authors report that DIA-MS quantification afforded excellent consistency across biological replicates for each group. These stringent, dual-filter criteria are what produced the final 108 (β-cell) and 16 (neuronal) high-confidence interactors.
- **Membrane proteins from an enriched fraction.** Biotinylated proteins were recovered by neutravidin pulldown after a one-minute labelling reaction, then analysed via DIA-MS. This workflow quantified an average of 1,205 cell membrane proteins from INS-1E cells and 1,287 from SK-N-SH cells. Cell-membrane annotation was applied as the primary filter to every protein before interactor calling.
- **Consistent across species, cell types and timepoints.** The same IonOpticks Aurora Ultimate 25x75 column and gradient on the nanoElute-timsTOF Pro 2 system was used for both rat INS-1E and human SK-N-SH single-point experiments (biological triplicates, three conditions each) and the separate four-timepoint kinetic series in INS-1E cells. This allowed direct comparison of the two cell-type-specific interactomes and the temporal profiles under identical chromatographic conditions.

PAPER 05 · REFERENCE [12]

IonOpticks Aurora Ultimate 25×75 delivered not merely a benchmark number, but the trace-level separation required to reveal an unexpected interaction that revised a fifty-year-old model of mitochondrial calcium signalling.

59 high-confidence interactors, one new metabolon: an IonOpticks Aurora Ultimate 25×75 IP/LC-MS screen finds the calcium control point that decades of matrix-calcium research missed

Cohen, H. M., Gottschalk, B., Choya-Foces, C., Chatoff, A., Wilkinson, A., Berezhnaya, E., Garbincius, J. F., Johnson, A., Stevens, T. L., Howe, J. E., Lesniak, H., Schmidt, A., Ngo, J., Megill, E., Tomar, D., Snyder, N. W., Graier, W. F., & Elrod, J. W. (2026). MICU proteins facilitate calcium-dependent mitochondrial metabolon formation to regulate cellular energetics independently of MCU. *Nature Metabolism*, 8(5), 1106–1123. https://doi.org/10.1038/s42255-026-01513-z

Study snapshot: Deletion of the mitochondrial calcium uniporter is largely phenotypically silent, which has left the field without an explanation for how calcium regulates metabolism under normal conditions. Cohen et al. address this gap through quantitative interactome screening, analysing MICU1–HA immunoprecipitations from an equimolar MICU expression system. Separated on an IonOpticks Aurora Ultimate 25×75 C18 UHPLC column coupled to an Orbitrap Fusion Lumos, 471 verified mitochondrial proteins were narrowed to 139 reproducible hits and cross-validated to a 59-protein high-confidence set containing SDHA, SDHB and GPD2. Follow-up mechanistic studies establish that calcium-dependent MICU1/2 heterodimers scaffold SDH and GPD2 into a functional metabolon at the inner boundary membrane, revealing intermembrane space calcium sensing as the principal mechanism driving mitochondrial energetic coupling independent of matrix calcium accumulation.

INSTRUMENTATION USED



Orbitrap Fusion Lumos
Tribrid, 75 min



EASY-nLC
UHPLC

Aurora® Ultimate™ 25×75
25 cm × 75 µm ID, C18

For fifty years, the model of calcium control over mitochondrial metabolism has centred on matrix calcium acting on matrix dehydrogenases. The persistence of calcium-regulated metabolism in uniporter-deficient models, however, has left the field without an explanation for how mitochondria sense calcium under normal conditions. resolve this contradiction by demonstrating that MICU proteins form calcium-dependent heterodimers within the intermembrane space that function completely independently of the mitochondrial calcium uniporter. Using an equimolar MICU expression system and quantitative complexome profiling (IonOpticks Aurora Ultimate 25×75 C18 UHPLC column; 300 nL/min at 60°C over a 75-minute gradient), the authors identify a 59-protein high-confidence interactome containing Complex II subunits SDHA and SDHB and glycerol-3-phosphate dehydrogenase GPD2. Proximity ligation assays, CRISPR-engineered HA/V5 double knock-in models, blue native PAGE, and functional respirometry confirm that MICU1/2 heterodimers scaffold SDH and GPD2 into a calcium-sensitive metabolon at the inner boundary membrane. This metabolon assembles in response to physiological IP3R-mediated calcium release and couples FADH2-driven substrate oxidation directly to oxidative phosphorylation without requiring matrix calcium accumulation. These findings establish MICU-mediated metabolon assembly as the principal mechanism coupling calcium signals to mitochondrial bioenergetics, redefining mitochondrial calcium signaling as an intermembrane space phenomenon orchestrated through direct scaffold-mediated enzyme recruitment.

WHY THIS PAPER MATTERS

- **Rewrites calcium control of metabolism.** MICU-mediated metabolon formation in the intermembrane space, not matrix calcium via the uniporter, is the primary mechanism matching energy production to cellular demand. This reframes the textbook model and explains why uniporter knockouts show minimal metabolic phenotypes at baseline.
- **Validated from cells to mouse tissue.** MICU2 loss shifted the SDHA complexes on blue native PAGE, raised succinate/fumarate, reduced state 3 respiration and cut GPD2 activity, all unchanged by MCU deletion. Wild-type MICU2 rescued Complex II activity; the EF-hand mutant did not. Endogenous MICU1/2 co-immunoprecipitated SDHC and GPD2 from hepatic mitochondria.
- **Discovery-driven, not hypothesis-confirming.** The mechanism surfaced from an affinity-purification interactome screen rather than targeted testing. SDHA, SDHB and GPD2 were unexpected partners for a calcium-sensing protein, and the authors note no prior physical or functional relationship between MICU proteins and Complex II or GPD2.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **It ran the discovery IP-MS.** Every proteomic MICU1–HA immunoprecipitation from the equimolar MICU1/2/3 co-expression was separated on an IonOpticks Aurora Ultimate 25×75 C18 UHPLC column at 300 nL/min and 60°C on a Proxeon EASY-nLC into an Orbitrap Fusion Lumos, 75-minute gradient. From 2,209 proteins in those IPs, 471 mapped to mitochondrial databases, and overlap with the prior MICU1–BioID2 set produced the 59-protein list that includes SDHA, SDHB and GPD2.
- **Trace-level input from an affinity pulldown.** The LC-MS input was an S-Trap digest of co-IP eluate, in which genuine partners sit against a large non-specific background. Recovering 471 verified mitrochondrial proteins, then 59 high-confidence hits after BioID2 overlap, is the sensitivity required for this sample type.
- **Reproducibility across a differential design.** The central claim that MICU1/2 and MICU1/3 heterodimers have distinct interactomes rests on comparing enrichment across replicates against a vector control, and this requires consistent retention and peak shape to be achieved across every run. 48 of 59 high-confidence proteins were present in at least two of three MICU1/2 replicates.
- **Recovered partners on both sides of the inner membrane.** The same 75-minute DDA method returned matrix-facing C-II subunits (SDHA, SDHB), the IMM-associated dehydrogenase GPD2, the IMS-facing protease YME1L1, and the matrix protease CLPP. These identifications were what motivated the later PLA, knock-in IP, and activity work.

2.3 Chromatin- and nucleic acid–anchored interactomes

Asking which proteins occupy a specific genomic locus, or associate with a single non-coding RNA, is among the hardest problems in interaction proteomics. The bait is not an abundant protein that can simply be tagged and pulled down, as input is limited, and genuine partners must be distinguished from the general chromatin background. The three studies below take complementary routes: dCas9-directed proximity labelling confirmed by orthogonal IP-MS [13], a single streptavidin pulldown that yields both genomic binding sites and the interacting proteome [14], and photocatalytic labelling delivered through in situ hybridisation and immunofluorescence in fixed cells [10].

PAPER 06 · REFERENCE [13]

Discovery platforms live or die on independent confirmation. The IonOpticks Aurora Elite 15×75 supported both IP-MS arms that confirmed TurboCas candidates, turning proximity labelling hits into a validated heat shock regulator with a mechanistic explanation.

Orthogonal validation on IonOpticks Aurora Elite 15×75 confirms TurboCas hits: single-guide proximity labelling and Pol II/P-TEFb IP-MS converge to identify FUBP3 as a required heat shock regulator

Cenik, B. K., Aoi, Y., Iwanaszko, M., Howard, B. C., Morgan, M. A., Andersen, G. D., Bartom, E. T., & Shilatifard, A. (2024). TurboCas: A method for locus-specific labelling of genomic regions and isolating their associated protein interactome. *Molecular Cell*, 84(24), 4929–4944.e8. https://doi.org/10.1016/j.molcel.2024.11.007

Study snapshot: TurboCas fuses the miniTurbo biotin ligase to catalytically dead Cas9, enabling 30-minute proximity labelling of chromatin-binding proteins at a single non-repetitive locus with one guide RNA per cell line. Applied to the FOS promoter and the MYC promoter, it recovers 498 differentially regulated proteins and identifies the Super Elongation Complex and BRD4 as MYC co-regulators. The orthogonal validation arm, RNA polymerase II and Cyclin T1 immunoprecipitation mass spectrometry in DLD-1 cells across four replicates, was run on an IonOpticks Aurora Elite 15×75 C18 UHPLC column on a Vanquish Neo nano-LC coupled to an Orbitrap Exploris 240, converging with TurboCas on a shared set of heat shock regulators including FUBP3.

INSTRUMENTATION USED



Orbitrap Exploris 240
Label-free IP-MS



Vanquish Neo
UHPLC



Aurora® Elite™ 15×75
15 cm × 75 µm ID, C18

Identifying the full suite of proteins that associate with a specific genomic region has remained an experimentally intractable challenge. Across ChIP-seq and CUT&RUN approaches, only a single known factor can be interrogated at a time, while earlier dCas9-based proximity labelling platforms either recovered only a handful of factors, worked reliably only at repetitive loci, or required the use of hydrogen peroxide which provokes the stress response under study. circumvent these limitations by fusing miniTurbo, the smallest and least biotin-avid of the engineered ligases at 28 kDa, to dCas9, deliberately trading catalytic activity for signal-to-noise and pairing it with a single guide RNA to avoid the steric interference of tiled Cas9. Validated at the FOS promoter across three guide positions with dCas9 ChIP-seq confirming at least three-fold enrichment at the target locus, TurboCas resolved 3,304 proteins and 498 differentially regulated hits on heat shock, from which shRNA knockdown of 29 candidates identified twelve whose depletion attenuated FOS induction, most through an HSF1-independent route. To confirm that these were genuine regulators rather than platform artefacts, an independent validation was performed by chromatin-fraction immunoprecipitation of RNA polymerase II and Cyclin T1.

This used a different cell line, a different bait and a shorter heat shock duration, and was analysed by label-free quantitative mass spectrometry on an IonOpticks Aurora Elite 15×75 C18 UHPLC column coupled to a Vanquish Neo nano-LC and Orbitrap Exploris 240. Despite those differences, the three methods converged on a shared set of heat shock regulators. FUBP3, previously unstudied in this context and the only hit found by all three approaches, was then shown to interact with Pol II on heat shock, to co-occupy heat shock upregulated genes with the SEC scaffold subunits AFF1/AFF4, and to be required for heat shock dependent Pol II Ser2P activation. This work establishes TurboCas as a powerful platform for locus-specific chromatin proteomics, enabling precise mapping of protein interactions at single genomic loci and facilitating discovery of novel transcriptional regulators.

WHY THIS PAPER MATTERS

- **Single-locus, single-guide proximity labelling without peroxide.** MiniTurbo's 30-minute labelling and low biotin affinity provide temporal control without hydrogen peroxide required by APEX2, removing a confound that is particularly acute when the biology under study is itself a stress response.
- **Dynamic rather than static locus proteomics.** As the first locus-targeted labelling method quantifying protein abundance changes at a defined promoter in response to perturbation, TurboCas compares heat shock against control at three guide positions across the FOS promoter and first exon.
- **Cross-validates by orthogonal IP-MS.** TurboCas hits were confirmed by independent RNA Pol II and Cyclin T1 (P-TEFb) immunoprecipitations using different cell lines, baits, and heat shock durations, demonstrating robust concordance between proximity labelling and endogenous complex purification across multiple biological contexts.
- **Sensitivity gain at MYC over the previous benchmark.** TurboCas returned 110 significantly enriched proteins shared between guides, twice as many hits as the published dCas9-APEX MYC dataset with only 11% overlap, newly detecting both SEC and BRD4 CKD9-containing complexes jointly required for MYC expression.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **Analytical platform for the cross-validation arm.** Both Pol II CTD and Cyclin T1 immunoprecipitation proteomes were separated on the Aurora Elite 15×75 C18 UHPLC column on a Vanquish Neo into an Orbitrap Exploris 240 at 200 nL/min over 120 minutes, generating the dataset that converts TurboCas candidates into validated hits.
- **Depth and specificity from in-gel digested IP material.** Across four replicates per bait the configuration resolved differential interactomes at greater than 2-fold change, recovering canonical HSP-family chaperones alongside low-abundance regulators from immunoprecipitated chromatin-fraction protein rather than whole-lysate input.
- **Quantitative reproducibility supporting label-free quantification.** MS1-intensity LFQ was performed in MaxQuant with a two-unique-peptide minimum and Benjamini-Hochberg FDR control, requiring the run-to-run retention and intensity consistency that made the four-replicate comparison viable without isobaric labelling.
- **Evidence base for the paper's central discovery.** FUBP3 was the one candidate identified by all three methodologies and the only one taken forward to full mechanistic validation, with two of those three methods being the Aurora-acquired Pol II and Cyclin T1 interactomes.

PAPER 07 · REFERENCE [14]

One column, every proteomic dataset, three biological replicates, and complexes recovered whole. When a method paper stakes its credibility on the proteomics matching the genomics, the IonOpticks Aurora 25×75 is the column that carried it.

Interactome and genomic binding sites from one pulldown: PLAMseq recovers the cohesin ring at CTCF and the PAF1 complex at Pol II, with all proteomic data acquired on an IonOpticks Aurora Ultimate 25×75 column

González-Vinceiro, L., Espejo-Serrano, C., Soler-Oliva, M. E., Soto-Hidalgo, E., Mateos-Martín, M. L., Rico, D., González-Aguilera, C., & González-Prieto, R. (2025). PLAMseq enables the proteo-genomic characterization of chromatin-associated proteins and protein interactions in a single workflow. *Science Advances*, 11, eady4151. https://doi.org/10.1126/sciadv.ady4151

Study snapshot: PLAMseq exploits the stability of the biotin–streptavidin bond to survive ChIP-style crosslinking and reversal, enabling a single streptavidin pulldown to yield both the sequenced DNA and the interacting proteome of a TurboID-tagged protein without antibody dependency. Validated on CTCF and RNA polymerase II in HeLa cells, the method returned 29,946 CTCF peaks with 74% at annotated ENCODE sites and narrower peaks than conventional ChIP-seq, alongside the full cohesin ring and Pol II holoenzyme as physical STRING subnetworks. Combined with split-TurboID, PLAMseq resolved SETDB1 as a SUMO-preferential interactor of histones H1.2 and H1.4 at repetitive genomic regions. All proteomics in the paper, including CTCF, POLR2I and six SUMO-histone H1 comparisons, was conducted on an IonOpticks Aurora Ultimate 25×75 C18 UHPLC column at 50°C on a nanoElute 2 coupled to a timsTOF SCP in dia-PASEF mode, establishing PLAMseq as an antibody-free platform for comprehensive proteo-genomic characterisation of chromatin-associated proteins.

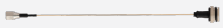
INSTRUMENTATION USED



timsTOF SCP
dia-PASEF



nanoElute 2
UHPLC



Aurora® Ultimate™ 25×75
25 cm × 75 µm ID, C18

Mapping where a chromatin protein binds and identifying what it binds to have remained two separate experiments, each with its own bottleneck. While ChIP-seq, CUT&RUN and CUT&Tag all depend on a ChIP-grade antibody, co-immunoprecipitation struggles to retain transient interactions. unify both into one protocol by reasoning that biotin–streptavidin, the strongest non-covalent interaction in biology, should survive the formaldehyde crosslinking and reversal steps of a ChIP-seq workflow. A ten-minute biotin pulse on a TurboID-tagged protein of interest is followed by crosslinking, sonication and a single streptavidin capture. The DNA is then released by decrosslinking and sequenced, while the biotinylated proteins remain on the beads for on-bead trypsin digestion and mass spectrometry. Validation on CTCF and the POLR2I subunit of RNA polymerase II, both expressed at near-endogenous levels from a doxycycline-inducible construct, showed the genomic output to be at least equivalent to ChIP-seq, with sharper CTCF peaks than the ENCODE reference and better signal-to-noise for Pol II, while the proteomic output recovered the interaction architecture of each bait as high-confidence physical complexes. 4 over their unmodified counterparts. Realignment to the T2T-CHM13 genome placed SUMOylated H1 at rDNA and pericentromeric repeats in the same chromatin states as H3K9me3. Every proteomic measurement in the study was acquired on an IonOpticks Aurora Ultimate 25×75 C18 UHPLC column held at 50°C, on a nanoElute 2 coupled to a timsTOF SCP running dia-PASEF, with library-free directDIA+ processing. This established PLAMseq as a generalisable antibody-free platform for comprehensive proteo-genomic characterisation of chromatin-associated proteins.

WHY THIS PAPER MATTERS

- **Two datasets from one pulldown.** Genomic localisation and interacting proteome come from the same streptavidin beads in the same experiment, removing both the antibody dependency of ChIP-seq and the separate co-immunoprecipitation needed to get interactors. This represents a genuine reduction in experimental burden rather than an incremental sensitivity gain.
- **Genomics that beats the antibody-based reference.** 29,946 significant CTCF peaks, 74% overlapping previously annotated ENCODE CTCF sites with measurably narrower peak widths than ChIP-seq. For POLR2I, PLAMseq achieves better signal-to-noise ratio than ENCODE Pol II ChIP-seq, with 96.85% of peaks falling within 500 bp upstream of annotated transcription start sites.
- **Physical complexes recovered intact.** The CTCF proximal proteome returned the cohesin complex and SMARCA5. The POLR2I proteome captured Pol II subunits alongside PAF1 and LEO1, the Integrator complex, the NELF complex, and PHF3. Both interaction networks resolve as STRING physical subnetworks at the highest confidence threshold (0.9).
- **Novel mechanistic insight into histone H1 SUMOylation.** Applied to previously uncharacterised SUMOylated histone H1, PLAMseq revealed that SETDB1 binds preferentially to SUMOylated H1.2 and H1.4 at repetitive genomic regions, linking SUMOylation to heterochromatin formation via H3K9me3.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **Sole chromatographic platform for the entire study.** A single Aurora Ultimate 25×75 column was used in the generation of every proteomic dataset. The CTCF and POLR2I proximal proteomes and all SUMO-histone H1 versus total H1 comparisons were run at 50ºC on a nanoElute 2 coupled to a timsTOF SCP, using a 66-minute gradient from 2 to 37% acetonitrile.
- **Depth sufficient to resolve complexes rather than components.** The Aurora Ultimate 25×75 column delivered the chromatographic resolution needed to recover the complete cohesin ring at CTCF and the assembled Pol II holoenzyme together with its elongation and processing partners (PAF1, LEO1, Integrator, NELF, PHF3) at POLR2I. This depth of analysis is what allowed the study to interrogate high-confidence (interaction score 0.9) STRING physical complexes, and make complex-level claims about each interactome.
- **Sensitivity for a sub-stoichiometric modified subproteome.** SUMOylated histone H1 is a low-abundance species relative to total H1, so split-TurboID material is limited by design. Despite this, SETDB1 and the SUMO E3 machinery were resolved as significantly enriched against total-H1 controls, turning a scarce, PTM-selective sample into a clear mechanistic finding.
- **Ion mobility compatibility for library-free DIA.** The column supported dia-PASEF acquisition across a 0.64–1.45 V·s/cm2 mobility range with directDIA+ processing in Spectronaut without a project-specific spectral library. This approach requires the retention-time consistency that library-free searching depends on.

PAPER 08 · REFERENCE [10] PREPRINT

When the bait is a genomic locus or a single lncRNA rather than an abundant protein, sensitivity is the whole experiment. With the IonOpticks Aurora 15×75 C18 UHPLC column, POCA delivers imaging-confirmed DNA and RNA proximal proteomes from just 2 million cells.

One photosensitiser, three biomolecule classes: POCA resolves DNA, RNA and protein interactomes from 2 million fixed cells without genetic engineering, using an IonOpticks Aurora Elite 15×75 column

Biletch, E. B., Herlihy, C. P., Li, L., Krebs, M., Kelly, C. J., Longhi, N. J., Weissenfels, O., Goldberg, H. G., Brandt, K., Grimm, J. B., Lavis, L. D., Huttlin, E. L., Schweppe, D. K., Backus, K. M., & Beliveau, B. J. (2026). A unified photosensitizer platform for in situ DNA-, RNA-, and protein-directed proximity labelling. bioRxiv 2026.04.30.721698 (preprint, posted 1 May 2026). https://doi.org/10.64898/2026.04.30.721698

Study snapshot: A single organic photosensitiser, dibromofluorescein, delivered through standard in situ hybridisation and immunofluorescence workflows, maps the proximal proteomes of genomic loci, long non-coding RNAs and proteins in fixed cells without genetic engineering, from as few as 2 million cells and under $3 of labelling reagent per replicate. Nucleic acid–targeted (ISH-POCA) proteomics was acquired on an IonOpticks Aurora Elite 15×75 C18 UHPLC column with FAIMS on an Orbitrap Eclipse using 90-minute gradients, recovering five of the six shelterin subunits at telomeres and 70 nucleolar and 119 nuclear speckle proximal proteins at ITS1 and MALAT1, with BioPlex-validated enrichment of known protein–protein interactions.

INSTRUMENTATION USED



Orbitrap Eclipse
FAIMS, label-free MS1



Aurora® Elite™ 15×75
15 cm × 75 µm ID, C18

Proximity labelling has become the primary route to unbiased interactome mapping in situ, but the established platforms are constrained on two fronts: enzyme-based catalysts such as BioID, TurboID and APEX2 require genetic engineering to deliver a catalyst–bait fusion, and peroxidase-based approaches generate diffusible tyramide radicals that blur the labelling radius. address both of these limitations by extending POCA (photosensitiser-dependent oxidation and capture by amine) into fixed cells, conjugating the photosensitiser to secondary antibodies and secondary oligonucleotides so that a single validated reagent can deliver labelling to any target with a validated primary antibody or hybridisation probe. Because the photosensitiser is also a fluorophore, on-target delivery is confirmed by imaging before any sample is committed to mass spectrometry. 3-fold improvement and within 26 nm of the 371 nm optical floor of the microscope. For the nucleic acid–targeted arm, streptavidin-enriched, on-bead digested peptides were separated on an IonOpticks Aurora Elite 15×75 C18 UHPLC column over 90-minute gradients and electrosprayed at 2,700 V into an Orbitrap Eclipse operating with FAIMS (compensation voltages −40/−60/−80 V) and label-free MS1 quantification from just 2 million cells at under $3 per replicate. Pairing protein- and RNA-anchored labelling in the same compartment provides reciprocal cross-validation analogous to a reciprocal co-immunoprecipitation, yielding high-confidence proximal proteomes for nucleoli, nuclear speckles and centromeres. This work establishes POCA as a unified platform for spatial proteomics of DNA, RNA, and protein targets, enabling subcellular proteome mapping from fixed cells without genetic engineering.

WHY THIS PAPER MATTERS

- **One platform across three biomolecule classes.** DNA, RNA, and protein targets are labelled with the same DBF photosensitiser chemistry and read out with identical proteomic workflows, eliminating genetic engineering, catalyst-bait fusions, and cell line generation to make proximity interactomics accessible in primary and engineering-intolerant systems.
- **Achieves sharper resolution than peroxidase labelling.** FRC analysis places POCA biotinylation at 397 ± 10 nm (direct) and 393 ± 38 nm (click), within 26 nm of the optical limit versus 1,699 ± 44 nm for HRP, with short-lived singlet oxygen confining labeling better than diffusible tyramide radicals.
- **Enables cross-modality proteome comparison.** By targeting both protein (IF-POCA) and RNA (ISH-POCA) baits within the same nuclear compartment (e.g., SON vs. MALAT1 at nuclear speckles), POCA resolves shared and distinct proximal proteomes from orthogonal molecular perspectives.
- **Generates new candidate interactors for follow-up.** RBM4B and TPP2 at telomeres, RNMT, KIF22, MPHOSPH8, RPL23A and SET at pericentromeres, and BRD4 and RNF20 at nuclear speckles, all from ISH-POCA datasets, demonstrating discovery value beyond recapitulation of canonical annotations.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **Chromatographic platform for the nucleic acid–targeted arm.** All four ISH-POCA proximal proteomes (Pan Alpha, telomeres, ITS1, MALAT1) were separated on the Aurora Elite 15×75 column over 90-minute gradients at 2,700 V into an Orbitrap Eclipse with FAIMS, making it the analytical foundation for every DNA- and RNA-directed dataset.
- **Depth from minimal, fixed-cell input.** From roughly 2 million fixed cells per replicate and low-microgram on-bead digests, the configuration resolved 42 differentially enriched proteins between Pan Alpha and telomeres, including five of six shelterin subunits, representing an explicit improvement on the same authors' earlier HRP-based DNA O-MAP results at these loci.
- **Retention time stability supporting match-between-runs quantification.** The ISH-POCA data were processed with IonQuant label-free quantification with match-between-runs enabled across replicate sets, a requirement that depends on consistent chromatographic behaviour, delivering 70 ITS1-enriched and 119 MALAT1-enriched proteins at 85.7% and 65.5% compartment-annotation precision respectively.

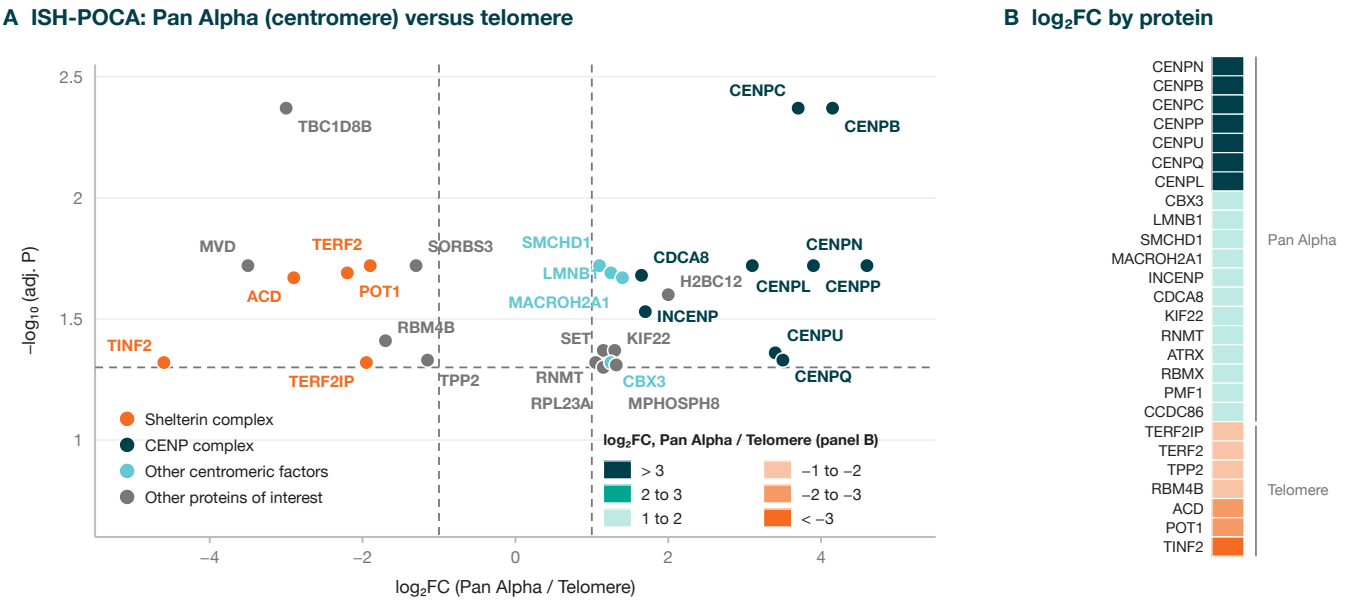


Figure 1. Aurora Elite 15×75 column enables locus-specific interactome recovery from minimal input. ISH-POCA enriches five of six shelterin subunits at telomeres and CENP family members at Pan Alpha satellites from 2 million fixed cells per replicate at under $3 labeling cost. Panel A - Volcano plot showing significant enrichment of shelterin complex proteins (tan) and CENP complex members (cyan) at their respective loci (FDR < 0.05, >2-fold change). Panel B - Heatmap of log2 fold-changes for pairwise comparisons between Pan Alpha and telomere targets.
Adapted from Biletch et al., bioRxiv, 2026; https://doi.org/10.64898/2026.04.30.721698

2.4 Background control and residue-level crosslinking

Two studies address the measurement itself rather than a particular biological system. The first quantifies how the choice of control determines what a proximity labelling experiment reports as an interactome [7]. The second benchmarks instrument, fragmentation and column choices for crosslinking mass spectrometry, the method that resolves interactions to the level of contacting residues [9]. Both used a single Aurora configuration as the fixed reference against which every other variable was measured.

PAPER 09 · REFERENCE [7]

False positives plague proximity labeling when controls don't match expression levels. The IonOpticks Aurora Ultimate 25×150 column delivers the chromatographic consistency needed for expression-matched TurboID controls, turning noisy background into confident, reproducible interactome assignments.

When background is the experiment, chromatographic consistency is essential: an IonOpticks Aurora 25×150 C18 UHPLC column supports proximity-labelling controls from 953 to 7,109 proteins per streptavidin enrichment

Jiang, F., Ge, X., & Bennett, E. J. (2025). Employing Expression-Matched Controls Enables High-Confidence Proximity-Based Interactome Classification. *Molecular & Cellular Proteomics*, 24(7), 101001. https://doi.org/10.1016/j.mcpro.2025.101001

> **Study snapshot:** A systematic evaluation of TurboID control strategies for proximity-labelling proteomics, using the ubiquitin ligases RNF10 and HUWE1 as baits. Conventional unmatched controls produced large swings in reported interactors as a function of control TurboID expression alone, while common normalisation approaches corrected the mismatch unevenly across abundance classes. Expression-matched TurboID-eGFP controls reduced background interference and recovered known partners for both baits. All six datasets were acquired on a single chromatographic platform consisting of an IonOpticks Aurora Ultimate 25×150 C18 UHPLC column on a nanoElute coupled to a timsTOF Pro 2 running dia-PASEF.

INSTRUMENTATION USED



timsTOF Pro 2
dia-PASEF



nanoElute
UHPLC



Aurora® Ultimate™ 25×150
25 cm × 150 µm ID, C18

Aurora® Ultimate™ 25×150
25 cm × 150 µm ID, C18, CSI

Proximity labelling with TurboID is promiscuous by design, which makes the choice of control the single largest determinant of what a study reports as an interactome, and the field has no standard. systematically evaluate control strategies, first demonstrating that background arises from two independent sources: nonspecific adsorption to streptavidin agarose, which alone yielded more than 1,600 protein identifications from cells expressing no TurboID at all, and TurboID self-labelling, which is invisible to a TurboID-negative control. The study shows that the control's TurboID expression level, not just its presence, drives the result. Sorting TurboID-eGFP cells into four expression bins produced protein counts that scaled with TurboID abundance. 5. 3-fold, so no single scaling factor can correct the mismatch. Expression-matched controls resolved these issues, recovering known RNF10 partners UBE2D2/3, USP10, RPS2 and RPS3 and HUWE1 interactors VCPIP1, VCP and ATXN3 while correctly rejecting the endogenously biotinylated background protein ACACA. Every measurement was separated on an IonOpticks Aurora Ultimate 25×150 C18 UHPLC column over 60-minute gradients at 400 nL/min on a nanoElute coupled to atimsTOF Pro 2 system, delivering the chromatographic consistency required for robust differential analysis.

These findings establish expression-matched controls as a critical requirement for proximity labelling proteomics, providing a framework that minimies false discoveries and enables high-confidence interactome mapping.

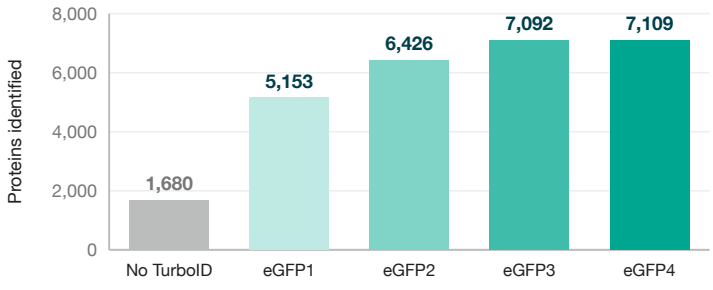
WHY THIS PAPER MATTERS

- **Reframes control selection as a primary experimental variable.** The number of RNF10 interactors called varied substantially with control TurboID expression alone, under identical bait, labelling, and processing conditions. Any proximity labelling dataset published without a matched-expression control carries an unquantified risk of that magnitude.
- **Explains precisely why normalisation cannot rescue a mismatch.** Enrichment responds non-uniformly to TurboID expression across abundance classes (60.2-fold for bottom-quartile proteins versus 20.3-fold for the top 1%). Total-intensity and RT-dependent normalisation perform best on abundant proteins and worst on the low-abundance proteins that matter most, while MaxLFQ produces more uniform mean changes but still leaves residual distortion at both extremes.
- **Separates the two background sources cleanly.** Bead adsorption accounted for more than1,600 identifications with no TurboID present, and those proteins were biased toward high abundance and high isoelectric point. TurboID-eGFP controls, by contrast, sampled the full abundance distribution, demonstrating why a TurboID-negative control is structurally insufficient.
- **Delivers a practical workflow.** MS-based quantification of TurboID expression serves as an explicit matching step before the labelling experiment. For large bait libraries, researchers can bin baits against a small panel of low, medium and high expression controls rather than matching one to one.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **Sole chromatographic platform for the entire study.** The Aurora Ultimate 25×150 C18 UHPLC column, coupled to a Bruker nanoElute with CaptiveSpray into a timsTOF Pro 2, generated all datasets, both bait proteins, three cell backgrounds and every control condition, each in biological triplicate.
- **The right column for an experiment whose subject is background.** This study measures the composition of nonspecific signal, so any chromatographic drift between arms would be indistinguishable from the effect being characterised. The correlations the paper's central argument rests on (TurboID expression tracking total enriched intensity, and up to 97.0% of individual proteins fitting at R2 > 0.5) are only obtainable if peptide-level separation is reproducible across every run in the series.
- **Dynamic range across a wide span of sample complexity.** The same method resolved enrichments from the low hundreds of proteins (no-TurboID or no-biotin controls) to several thousand (high-expressing TurboID-eGFP) without changing gradient, flow rate or column.
- **Throughput matched to a large conditional design.** 60-minute gradients at 400 nL/min on the 150 µm ID format column, paired with dia-PASEF at a 2.6 s cycle over 25 scans, made it practical to run dozens of control conditions in triplicate. This scale was essential, as a slower method would have forced the authors to sample fewer expression levels and weakened the conclusion.

A Proximity labelling proteomics



B TurboID expression vs enriched signal

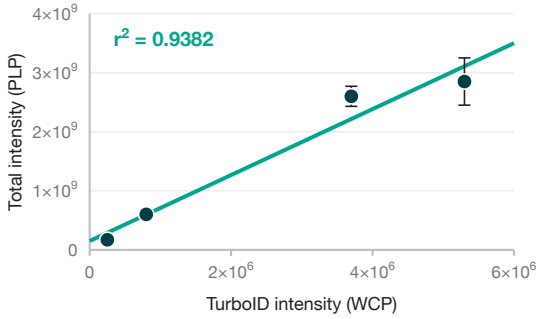


Figure 2. IonOpticks Aurora 25×150 powers TurboID-eGFP to capture background labelling across the full expression range with signal intensity scaling linearly with TurboID abundance. Panel A - Streptavidin enrichment from TurboID-eGFP cells identifies 5,153 to 7,109 proteins across four expression levels, compared to 1,680 proteins without TurboID, demonstrating that TurboID-eGFP captures promiscuous background independent of expression level. Panel B - Total enriched signal correlates linearly with TurboID abundance (R^2 = 0.9382), establishing the quantitative relationship required for expression-matched control normalisation.

Adapted from Jiang et al., Molecular & Cellular Proteomics, 2025; 24(7):101001, https://doi.org/10.1016/j.mcpro.2025.101001

PAPER 10 · REFERENCE [9]

For crosslinking mass spectrometry, peak sharpness determines what interactions you detect. The IonOpticks Aurora Ultimate 25×75 C18 UHPLC column delivered 28% more unique crosslinks than a 50 cm competitor, with superior peak shape and intensity that maximised detection of large, low-abundance crosslinked species.

28% more crosslinks from half the column: Aurora Ultimate 25×75 identifies 779 residue pairs versus 560 for a 50 cm PepMap

Müller, F., Birklbauer, M. J., Bubis, J., Stejskal, K., Dorfer, V., & Mechtler, K. (2025). Breaking Barriers in Crosslinking Mass Spectrometry with Enhanced Throughput and Sensitivity Using Orbitrap Astral. *Nature Communications*, 16, 9877. https://doi.org/10.1038/s41467-025-64844-7

Study snapshot: Head-to-head benchmarking of the Orbitrap Astral against the Orbitrap Eclipse for crosslinking mass spectrometry, standardised throughout on an IonOpticks Aurora Ultimate 25×75 C18 UHPLC column. The Astral identified over 40% more unique residue pairs than the Eclipse, FAIMS added a further 30%, single HCD outperformed stepped HCD on the Astral. In a direct column comparison the Aurora Ultimate 25×75 delivered 779 unique residue pairs against 560 for a 50 cm PepMap, with sharper peaks and higher precursor intensities. Using PhoX and DSSO on crosslinked Cas9-Halo, the Aurora Ultimate column enabled superior detection of large, low-abundance crosslinked peptides, establishing optimised conditions for high-confidence structural proteomics.

INSTRUMENTATION USED



Orbitrap Astral
CLMS, single HCD



Orbitrap Eclipse
CLMS benchmark



Vanquish Neo
UHPLC



Aurora® Ultimate™ 25×75
25 cm × 75 µm ID, C18

Crosslinking mass spectrometry (CLMS) maps protein-protein interactions and three-dimensional protein architecture, but it depends on detecting crosslinked peptides that are larger, lower in abundance, and chromatographically more demanding than linear tryptic peptides. address this by benchmarking the Orbitrap Astral against the Orbitrap Eclipse under standardised chromatographic conditions, using Cas9-Halo crosslinked with either the non-cleavable reagent PhoX (DSPP) or the MS-cleavable reagent DSSO as a quality-control sample across the entire experimental series. The Astral identified over 40% more unique residue pairs (URPs) than the Eclipse for both crosslinkers, driven primarily by higher MS1 sensitivity and more efficient detection of low-abundance precursors. FAIMS contributed a further 30% through charge-state filtering and noise reduction. On the Astral, single HCD consistently outperformed stepped HCD (909 versus 726 URPs at 250 ng for PhoX), reversing the conventional preference for stepped fragmentation with cleavable reagents. Gradient extension raised identifications from 45 URPs at 10 minutes to 436 URPs at 180 minutes in purified samples, but the benefit plateaued in complex lysate backgrounds, pointing to enrichment rather than longer separations as the path to greater depth. All separations were performed on an IonOpticks Aurora Ultimate 25×75 C18 UHPLC column on a Vanquish Neo UHPLC system with a FAIMS Pro Duo interface. The Aurora Ultimate delivered sharper peaks and higher precursor intensities than a 50 cm PepMap column (779 versus 560 URPs at 100 ng). This chromatographic performance establishes the Aurora Ultimate as the reference platform for the study, enabling robust detection of low-abundance crosslinked peptides while minimising sample requirements.

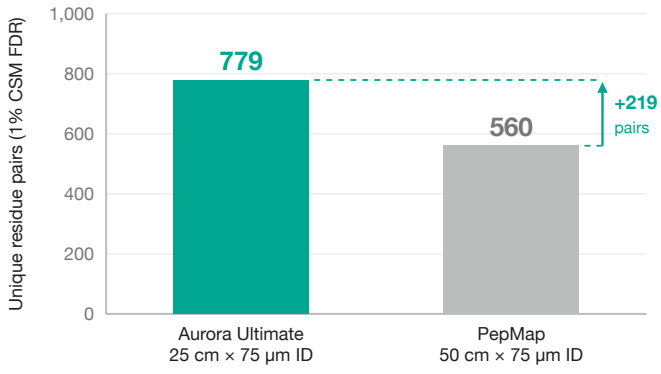
WHY THIS PAPER MATTERS

- **A new sensitivity ceiling for CLMS.** The Astral identified over 40% more unique residue pairs than the Eclipse for both a non-cleavable and an MS-cleavable crosslinker. The Astral returned 192 URPs (PhoX) and 121 URPs (DSSO) from just 1 ng of crosslinked protein, bringing sample-limited and low-stoichiometry interaction studies into range.
- **Low-abundance crosslinks carry the structural information.** Crosslinks were mapped onto an AlphaFold3-predicted Cas9 structure and stratified by MS1 apex intensity. Low-abundance crosslinks were enriched for short-distance (<20 Å) links spanning the protein core, while high-abundance crosslinks localised to surface-exposed regions, demonstrating that sensitivity gains translate into genuine structural coverage rather than redundant identifications.
- **Method optimisation is instrument-specific.** Single HCD outperformed stepped HCD by 25% at 250 ng on the Astral but made no meaningful difference on the Eclipse (1.5% for PhoX at 100 ng). An optimised FAIMS CV triplet (–48 V/–60 V/–75 V) yielded 1,272 URPs, 20% above a high-overlap combination, demonstrating that fragmentation and ion-mobility settings cannot be ported between platforms.

HOW IONOPTICKS COLUMNS CONTRIBUTED

- **More crosslinks from half the column.** 779 unique residue pairs from 100 ng of PhoX-crosslinked Cas9 on the 25 cm Aurora Ultimate 25×75 C18 UHPLC column versus 560 on a 50 cm PepMap, on the same instrument, gradient and sample. The Aurora Ultimate delivered 219 additional residue pairs from half the separation length.
- **Sharper peaks, higher precursor intensity.** Skyline quantitation of five selected crosslinked peptides across a 70-minute gradient returned smaller full-width half-maximum values on the Aurora Ultimate compared with the PepMap column, and the authors identify this intensity gain as. This is identified as the key driver of the performance difference,noting that sharper peaks and higher precursor intensities on the shorter Aurora column enabled superior peptide separation.
- **Pore and particle geometry suited to crosslinked analytes.** Crosslinked peptides carry the combined mass of two peptides plus the crosslinker, so they require larger pores than linear peptides for effective separation. The Aurora Ultimate's 120 Å pores (versus 100 Å for the PepMap), are matched to this analyte class, while 1.7 µm particles (versus 2.0 µm) and a pressure tolerance above 1700 bar support the separation efficiency and robustness the workflow demands.
- **The chromatographic constant across the whole study.** Every arm of the study (Astral versus Eclipse comparison, FAIMS CV optimisation, single versus stepped HCD experiments, and the 10 to 180 minute gradient series) ran on the same Aurora Ultimate 25×75 C18 UHPLC column. This ensured that instrument-level differences were attributable to the mass spectrometer rather than to chromatographic variability.

A Crosslink identifications
100 ng PhoX-crosslinked Cas9, 70 min gradient



B Column format

	Aurora Ultimate	PepMap
Length	**25 cm**	50 cm
Particle size	**1.7 µm**	2.0 µm
Pore size	**120 Å**	100 Å
Pressure limit	**>1,700 bar**	1,500 bar
Emitter	**~6 µm integrated**	10 µm silica

Sharper peaks (smaller FWHM in Skyline) and higher precursor intensities on the Aurora Ultimate, despite half the separation length.

Figure 3. Aurora Ultimate 25×75 identifies 28% more crosslinks than 50 cm PepMap despite half the column length. From 100 ng of PhoX-crosslinked Cas9, the Aurora Ultimate 25×75 resolved 779 unique residue pairs versus 560 for the 50 cm PepMap, with sharper peaks (smaller FWHM) and higher precursor intensities enabling deeper structural coverage.
Data recreated from Müller et al., Nature Communications, 2025; 16:9877, https://doi.org/10.1038/s41467-025-64844-7

PLATFORMS REPRESENTED ACROSS THIS EVIDENCE BASE

MASS SPECTROMETERS


Orbitrap Astral


Orbitrap Eclipse


Orbitrap Fusion Lumos


Orbitrap Exploris 240


Orbitrap Exploris 480

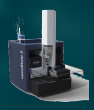

timsTOF Pro 2


timsTOF SCP

UHPLC SYSTEMS


Vanquish Neo


nanoElute


nanoElute 2


Evosep One


EASY-nLC

IONOPTICKS PRODUCTS FEATURED IN THIS NOTE



Aurora® Elite™ 15×75

15 cm × 75 µm ID, 1.7 µm C18, in XT and CSI formats. Sample-limited pulldowns and enriched sub-proteomes: locus- and RNA-anchored proximity labelling, IP-MS validation of chromatin complexes and diGly site mapping. Refs [3, 10, 13]



Aurora® Ultimate™ 25×75

25 cm × 75 µm ID, 1.7 µm C18, in XT and CSI formats. Deep discovery interactomics: molecular glue affinity proteomics, fractionated TMT degradation screens, AP-MS, ligand-directed proximity labelling, chromatin pulldowns and crosslinking MS. Refs [1, 2, 9, 11, 12, 14]



Aurora® Ultimate™ 25×150

25 cm × 150 µm ID. Large conditional designs with many control conditions in replicate, such as expression-matched proximity labelling controls. Ref [7]

DECISION GUIDE

03 Choosing the right Aurora column for your interaction proteomics workflow

The Aurora Series includes several formats suited to different interaction proteomics workflows. The table below summarises the formats used in the published studies in this summary.

AURORA COLUMN	FORMAT	TYPICAL INTERACTION WORKFLOW	THROUGHPUT WINDOW
Aurora Elite™ 15×75	15 cm × 75 µm ID, 1.7 µm	Sample-limited pulldowns and enriched sub-proteomes: locus- and RNA-anchored proximity labelling from fixed cells, IP-MS validation of chromatin complexes, diGly site mapping of E3 ligase and degrader substrates. **[3, 10, 13]**	**40 SPD (Evosep Whisper) to 90–120 min gradients**
Aurora Ultimate™ 25×75	25 cm × 75 µm ID, 1.7 µm	Deep discovery interactomics: molecular glue affinity proteomics and fractionated TMT degradation screens, AP-MS and ligand-directed proximity labelling, proteo-genomic chromatin pulldowns, crosslinking MS. **[1, 2, 9, 11, 12, 14]**	**50–180 min gradients**
Aurora Ultimate™ 25×150	25 cm × 150 µm ID, 1.7 µm	Large conditional designs with many control conditions in replicate, such as expression-matched proximity labelling controls, where robustness and run rate matter. **[7]**	**60 min gradients at 400 nL/min**

Practical guidance. Laboratories running interaction proteomics should keep a single Aurora chemistry across every arm of a study: baits and controls, discovery and validation, and where possible, instruments. Where the experiment is a discovery screen that must reach low-abundance or unexpected partners, Aurora Ultimate™ 25×75 is the format used in the molecular glue, native interactome and crosslinking studies summarised here [1, 2, 9, 11, 12, 14]. Where input is limited by design, as in locus-anchored labelling from fixed cells or PTM-enriched fractions of a pulldown, Aurora Elite™ 15×75 delivered the published results [3, 10, 13]. Where the design calls for many control conditions in replicate, the 150 µm ID Aurora Ultimate™ 25×150 combines 60-minute gradients at 400 nL/min with the consistency needed to compare them [7].



In every one of these workflows, the chromatography defines what can be called an interactor.

04

Summary

Interaction proteomics succeeds or fails on whether a true partner can be distinguished from everything else that co-purifies with it. That distinction is made by comparing enriched samples against controls across replicate injections, usually from small amounts of material, and the separation is what makes those comparisons fair. The studies in this summary converge on the same finding from different directions: the Aurora Series® architecture provides the reproducibility, sensitivity and separation that confident interactome assignment requires.

For drug discovery groups working on molecular glues and degraders, Aurora Ultimate™ 25×75 supported the proteomics screen that identified a G-loop-independent CRBN neosubstrate [1] and the affinity proteomics that expanded the CRBN glue interactome to 298 recruited proteins [2], while Aurora Elite™ 15×75 mapped degrader-induced ubiquitylation to individual lysine clusters [3]. For groups mapping native biology, the same columns revealed the first endogenous membrane interactome of the GLP-1 receptor [11], a calcium-dependent mitochondrial metabolon [12], and the protein complexes present at single genomic loci and long non-coding RNAs [10, 13, 14]. For method developers, Aurora columns served as the fixed reference against which proximity labelling controls [7] and crosslinking workflows [9] were benchmarked, including a direct comparison in which a 25 cm Aurora column outperformed a 50 cm alternative [9].

Across every study the same principle recurs: change the bait, the labelling chemistry or the mass spectrometer, but keep the column. That principle is what makes interaction data comparable between arms, between conditions and between instruments.

IonOpticks works directly with interaction proteomics, chemical biology and targeted protein degradation groups worldwide at academic institutes, pharmaceutical and biotechnology discovery laboratories and CROs to configure the right column, cassette, heating and source interface for each instrument ecosystem. Contact your IonOpticks representative to discuss method development, study design, or evaluation columns for your protein–protein interaction workflow.



Every interactome in this summary is defined by comparison: bait against control, compound against vehicle, modified against unmodified.

05 References

References 4, 5, 6 and 8 establish foundational context for the protein–protein interaction workflows described. References 1, 2, 3, 7, 9, 10, 11, 12, 13 and 14 explicitly cite IonOpticks Aurora Series® columns in their methods sections; references 1, 2, 3, 9, 11, 12, 13 and 14 are peer-reviewed publications and reference 10 is a preprint.

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