Bait 1: PU-bait and associated methods and protocols

Guideline for sample preparation for affinity purification for both brain tissue and iPSC neurons:

Brain tissue is homogenized in 20 mM Tris pH 7.4, 20 mM KCl, 5 mM $MgCl_2$, 0.01% NP40 buffer containing protease and phosphatase inhibitors. iPSC neurons are lysed in the same buffer by three successive freeze (on dry ice) and thaw steps. Next, the samples are incubated at 4 °C for 1h with rotation and centrifuged for 10 min at maximum speed. The supernatants are collected and protein concentration is determined.

Protocol for affinity purification:

PU-immobilized beads or control beads are washed tree times with the lysis buffer. Protein extracts are first pre-cleared by incubation with control beads at 4 °C for 30 min with rotation. For the pull-down, 40 µL bead conjugates are incubated with 250 µg of protein extract (or protein amount determined experimentally) at 4 °C for 3h. The sample volume is adjusted to 200 µl with the lysis buffer. Following the incubation, bead conjugates are washed five times with the lysis buffer. Protein complexes are eluted from the beads by boiling in the loading buffer. Affinity purified protein complexes are resolved using SDS-polyacrylamide gel electrophoresis. The gels are stained by Coomassie blue and analyzed by mass spectrometry.

Protocol for sample analysis by MS:

Separated protein bands are excised from the gels. The number of gel sections per lane averaged to be 14. Gel bands are completely de-stained with 50% methanol and 25 mM NH4HCO3 / 30% acetonitrile and diced into small pieces and dehydrated with acetonitrile and dried using vacuum centrifugation. The gel pieces are rehydrated with 12.5 ng mL−1 trypsin solution (Trypsin Gold, Mass Spectrometry Grade, Promega) in 50 mM NH4HCO3 and incubated at 37 °C overnight. Peptides are extracted twice with 5% formic acid / 50% acetonitrile followed by final extraction with acetonitrile. The tryptic peptides are desalted by using a 2 µL bed volume of Poros 50 R2 reversed-phase beads (Applied Biosystems) packed in Eppendorf gel-loading tips. The purified peptides are diluted to 0.1% formic acid, and each gel section is analyzed separately by LC-MS/MS analysis with an Eksigent 2-D nanoHPLC coupled directly to an Orbitrap XL mass spectrometer (ThermoFisher Scientific) using our published protocols. Additional analyses are performed using a Q Exactive mass spectrometer coupled to a Thermo Scientific EASY-nLC 1000 (Thermo Fisher Scientific, Waltham, MA) equipped with a self-packed 75 µm × 20 cm reverse phase column (Reprosil C18, 3 µm, Dr. Maisch GmbH, Germany) for peptide separation. The mass spectrometer is operated in data-dependent (DDA) mode with survey scans acquired at a resolution of 70,000 over a

scan range of 300–2000 m z−1. Up to ten most abundant precursors from the survey scan are selected with an isolation window of 1.6Th and fragmented by higher-energy collisional dissociation with Normalized Collision Energies (NCE) of 27. The maximum ion injection time for the survey and MS/MS scans is 60 ms and the ion target value for both scan modes was set to 3e6.