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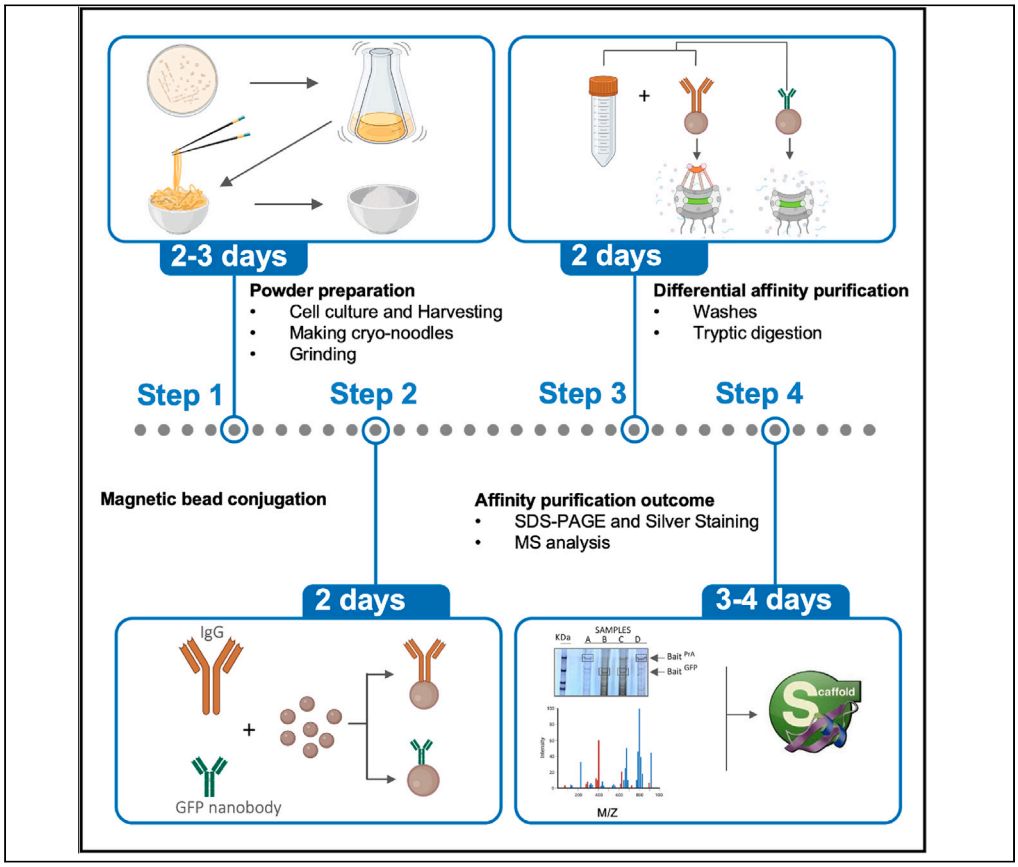
Differential affinity purification and mass spectrometry analysis of two nuclear pore complex isoforms in yeast *S. cerevisiae*



Two isoforms of the nuclear pore complex (NPC) have been identified in the yeast *S. cerevisiae*, which coexist at the periphery of the nucleus and differ by the presence or absence of a nuclear basket. Here, we present a protocol to isolate the two types of NPCs from the same cell extract and dissect their interactomes. We describe steps for powder preparation and magnetic bead conjunction and detail differential affinity purification and outcome evaluation through SDS-PAGE, silver staining, and mass spectrometry analysis.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Cryo-lysis of cells to preserve transient interactors

Purification of partially overlapping subcomplexes from same cell lysate

Sequential affinity purification using IgG and GFP nanobodies

Semi-quantitative mass spectrometry to identify differential subcomplex components

Bensidoun et al., STAR Protocols 4, 102359 September 15, 2023
https://doi.org/10.1016/j.xpro.2023.102359

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Differential affinity purification and mass spectrometry analysis of two nuclear pore complex isoforms in yeast *S. cerevisiae*

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https://doi.org/10.1016/j.xpro.2023.102359

SUMMARY

Two isoforms of the nuclear pore complex (NPC) have been identified in the yeast *S. cerevisiae*, which coexist at the periphery of the nucleus and differ by the presence or absence of a nuclear basket. Here, we present a protocol to isolate the two types of NPCs from the same cell extract and dissect their interactomes. We describe steps for powder preparation and magnetic bead conjunction and detail differential affinity purification and outcome evaluation through SDS-PAGE, silver staining, and mass spectrometry analysis.

For complete details on the use and execution of this protocol, please refer to Bensidoun et al.[1]

BEFORE YOU BEGIN

Nuclear pore complex (NPC) components are conserved across eukaryotes, however, different studies examining NPC composition have demonstrated the differential expression of nuclear pore proteins (nucleoporins, or nups) in distinct cell types and during development.[1–3] The yeast *S. cerevisiae* provides a model to study variations of NPC composition and their function, as it is the only model organism described to date that has two clearly delineated NPC isoforms concurrent within the same nucleus, which could point towards a heterogeneous function of NPCs for accessibility to the nuclear pore and transport of different complexes through its central channel.[4,5]

Cargo access to and release from the NPC is modulated by asymmetrically distributed subcomplexes of the NPC. On the nuclear face, this is accomplished by a large basket-like structure protruding ~100 nm into the nucleoplasm, termed the nuclear basket. In yeast, the basket's main scaffold is assembled by two filamentous protein paralogues, Mlp1 and Mlp2 (myosin-like proteins 1 and 2).[5] One of the proposed functions of the nuclear basket is that of establishing a quality control platform that ensures that only mature messenger ribonucleoparticles (mRNPs) are exported to the cytoplasm[5–7]; however, the exact mechanisms of this process are still elusive.

While the exchange of central framework of the NPC, assembled by various nups, is slow, fluorescence recovery after photobleaching (FRAP) experiments in yeast have shown that exchange of Mlp1 at NPCs is faster when compared to other nups.[8,9] Moreover, in *S. cerevisiae*, NPCs adjacent

to the nucleolus, which occupies about a third of the nuclear volume, are devoid of baskets.[5,10,11] Yet how cells establish these basket-less pores and whether these represent specialized NPCs with functions differing from nucleoplasmic basket-containing pores had not been established.[1,4,5] In order to isolate and characterize cargoes associated with basket-containing and basket-less NPCs, we devised an affinity purification approach that would enable us to separate subcomplexes that in part contain similar components from one another.

The two nucleoporins Nup133 and Nup84, as well as Mlp1 have proven to be efficient baits to isolate the NPCs via affinity purification with good conservation of their structure and composition during the purification steps.[12] Therefore, yeast strains were created in which Nup133 and Mlp1 were C-terminally tagged with GFP and protein A (PrA), respectively, using homologous recombination. To analyze the general NPC interactome, we carried out single-step affinity purification (ssAP) of NPCs followed by mass spectrometry from our Mlp1-PrA/Nup133-GFP double-tagged yeast strain, using Nup133-GFP as bait protein. To ensure the capture of dynamic interactors, we stabilized NPCs and associated proteins using a short in-lysate glutaraldehyde fixation before incubation with antibody-conjugated magnetic resin.[1] To compare the interactomes of NPCs with and without nuclear baskets, we applied a differential affinity purification approach (dAP) that enabled us to separate and isolate the two types of NPCs from the same lysate via two consecutive APs. In the first step, incubation with IgG-conjugated resin allows for the isolation of Mlp1-PrA and its associated complexes including basket-containing NPCs; on the flow-through, a second affinity purification is carried out using Nup133-GFP as bait to isolate the remaining basket-less NPCs.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit IgG	Sigma-Aldrich	I5006
Nanobody Anti-GFP	Rothbauer et al.[8]	GBP
Chemicals, peptides, and recombinant proteins		
Dynabeads M-270 Epoxy	Invitrogen	14302D
RNAsin	Promega	N2111
Pepstatin A	Sigma-Aldrich	P5318
Phenylmethylsulfonyl fluoride (PMSF)	Sigma-Aldrich	P7626
Antifoam	Sigma-Aldrich	A5633
Glutaraldehyde	Sigma-Aldrich	G6257
HEPES	Sigma-Aldrich	H3375
Monosodium phosphate	Sigma-Aldrich	SB0878
Disodium phosphate	Sigma-Aldrich	S9638
Monopotassium phosphate	Sigma-Aldrich	60218
Ammonium sulfate	Biobasic	ABD0060
Triton X-100	Biobasic	TB0198
Tris base	Biobasic	TB0195
Sodium azide	Thermo Fisher	BP9221-500
Triethylamine	Sigma-Aldrich	T0886-500
Glycine	Wisent Bioproducts	800-045-lk
Isopropanol	Thermo Fisher	A451-4
Potassium acetate	Biobasic	PB0438
Dithiothreitol (DTT)	Thermo Fisher	BP172-25
Tween 20	Thermo Fisher	BP337-500
Ammonium acetate	Biobasic	ADB0032
Glycerol	Invitrogen	15504-011
2-Beta-mercaptoethanol	Sigma-Aldrich	M7-154-250
Bromophenol Blue	Bio-Rad	161-0404

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Acetic acid	Thermo Fisher	A38-212
Silver nitrate	Sigma-Aldrich	209139
Formaldehyde	Thermo Fisher	BP531-500
Formic acid	Fluka	09676-100
0.22 μm Millex GP filter	Sarstedt	831826.001
Trypsin protease	Promega	V511A
Yeast extract peptone dextrose (YPD)	Biobasic	SD7022
Polyvinylpyrrolidone (PVP-40)	Sigma-Aldrich	9003-39-8
Ultra-pure water	Invitrogen	10977023
NuPAGE Bis-Tris 4–12% gel	Invitrogen	NP0326BOX
C18 ZipTips	MilliporeSigma	ZTC18S096
2-(*N*-morpholino) ethane sulfonic acid - sodium dodecyl sulfate (MES SDS™) NuPAGE (20×)	Invitrogen	NP0002
Deposited data		
All proteomics raw files and spectral searches	This manuscript.	Pride: PXD027872
Experimental models: Organisms/strains		
S. cerevisiae W303-1A	Tollervey Lab	ATCC: 208352
S. cerevisiae W303-1B	Tollervey Lab	ATCC: 201238
S. cerevisiae W303, Nup133-GFP-KanMx6, Mlp1-PrA-His5	Bensidoun et al.[1]	
Software and algorithms		
Proteome Discoverer	Thermo Fisher Scientific	N/A
Mascot 2.5	Matrix Science	N/A
Scaffold™ 4.8.4	Proteome Software, Inc., Portland, OR	https://www.proteomesoftware.com
Other		
Planetary ball Mill PM 100	Retsch	N/A
Dynal MPC-6 magnetic particle concentrator	Invitrogen	Prod. No. 120.02
Stainless-steel grinding jar	Retsch	N/A
Orbitrap Fusion Tribrid™ Mass Spectrometer	Thermo Fisher Scientific	N/A

Note: While a Retsch Planetary Ball Mill PM 100 was used for the cryo-lysis of cells in this protocol, different cryo-mills exits on the market that can be used with equal efficiency. However, the protocol will have to be adjusted to instrument's specifications.

MATERIALS AND EQUIPMENT

Harvesting buffer (100 mL)		
Reagent	Final concentration	Amount
Polyvinylpyrrolidone (PVP-40)	1.2%	1.2 g
HEPES (1 M)	20 mM	2 mL
MilliQ water	N/A	98 mL
Total	**N/A**	**100 mL**

Note: We prepare 1 L of 1 M HEPES buffer pH 7.4 by dissolving 238.3 g of HEPES in 800 mL of MilliQ water stored at room temperature (20°C–25°C). The solution can be kept for two years.

- Adjust the solution to the desired pH with 10 N NaOH.
- Add MilliQ water until the volume is 1 L.
- Harvesting buffer is prepared fresh before harvesting.

Sodium phosphate buffer (0.1 M, 100 mL)		
Reagent	Final concentration	Amount
Monosodium phosphate (NaH_2PO_4)	0.075 M	2.021 g
Disodium phosphate (Na_2HPO_4)	0.025 M	0.339 g
MilliQ water	N/A	To make volume 100 mL
Total	N/A	100 mL

Note: Adjust the solution to pH 7.4 with 10 N NaOH. Sodium phosphate buffer can be stored at room temperature (25°C). The solution can be kept for two years.

PBS -1× (1 L)		
Reagent	Final concentration	Amount
Sodium Chloride (NaCl)	137 mM	8 g
Potassium Chloride (KCl)	2.7 mM	0.2 g
Disodium phosphate (Na_2HPO_4)	10 mM	1.44 g
Monopotassium phosphate (KH_2PO_4)	1.8 mM	0.245 g
MilliQ water	N/A	To make volume 1 L
Total	N/A	1 L

Ammonium Sulfate buffer

- Ammonium Sulfate buffer 3 M is prepared by dissolving 39.6 g of $(NH_4)_2SO_4$ in 50 mL 1× PBS. Then the volume is adjusted to 100 mL with 1× PBS, and the pH is adjusted to 7.4 with 10 N NaOH. Ammonium Sulfate buffer is stored at room temperature (20°C–25°C). The solution can be kept for two years.

PBS - 0.5% Triton X-100

- We prepare 100 mL PBS+ 0.5% Triton X-100 by adding 500 µL of Triton X-100 to 95.5 mL of 1× PBS.
- PBS - 0.5% Triton X-100 buffer is stored at room temperature (20°C–25°C). The solution can be kept for two years.

1 M Tris buffer

- We prepare 1 M Tris buffer by dissolving 121.1 g of Tris base in 800 mL of MilliQ water.
- We adjust the pH to 7.4 with HCl.
- We add MilliQ water until the volume is 1 L.
- 1 M Tris buffer is stored at room temperature (20°C–25°C). The solution can be kept for two years.

PBS-Sodium Azide

- We prepare PBS-Sodium Azide by adding 2 µL of 10% Sodium Azide (final concentration 0.02%) to 10 mL of 1× PBS.
- PBS-Sodium Azide buffer is stored at room temperature (20°C–25°C). The solution can be kept for two years.

Triethylamine buffer

- We add 168 µL of the Triethylamine stock to 11.156 mL of MilliQ water for a concentration of 100 mM.

- Triethylamine buffer should always be prepared fresh before conjugation.

Glycine - HCL (1 L)		
Reagent	Final concentration	Amount
Glycine	0.1 M	7.5 g
Hydrochloric Acid (HCl)	0.02 M	0.832 g
MilliQ water	N/A	To make volume 1 L
Total	**N/A**	**1 L**

- We add 7.5 g of Glycine to 800 mL of MilliQ water.
- We add 0.832 g of Hydrochloric acid to the solution.
- We adjust the solution to pH 2.5 using HCl or NaOH.
- We finally add MilliQ water until the volume is 1 L.
- Glycine-HCl buffer is stored at room temperature (20°C–25°C). The solution can be kept for two years.

Solution P 100× (10 mL)		
Reagent	Final concentration	Amount
Pepstatin A	400 µg/mL	400 µg
Phenylmethylsulfonyl fluoride (PMSF)	18 mg/mL	18 mg
Isopropanol	N/A	10 mL
Total	**N/A**	**10 mL**

- Solution P is stored at −20°C. The solution can be kept for one year.

Extraction Buffer (TBT; 25 mL)		
Reagent	Final concentration	Amount
HEPES 1 M	20 mM	500 µL
Potassium Acetate (KOAc) (5 M)	110 mM	550 µL
Sodium Chloride (NaCl) (5 M)	50 mM	250 µL
Solution P 100×	1×	250 µL
Triton X-100 10%	0.5%	1.25 mL
Dithiothreitol (DTT) (1 M)	1 mM	25 µL
Antifoam B	0.02%	5 µL
RNAsin 40 U/µL	8 U/mL	5 µL
MilliQ water	N/A	To make volume 25 mL
Total	**N/A**	**25 mL**

Note: Extraction buffer is prepared fresh before the affinity purification.

- To prepare 5 M KOAc we dissolve 294.42 g KOAc in 400 mL of MilliQ water and add glacial acetic acid until a pH of 4.6 is reached (this requires about 40–50% of the final volume). We adjust the volume to 1 L and keep the solution at room temperature (20°C–25°C). The solution can be kept for two years.
- To prepare 5 M NaCl we dissolve 292.2 g NaCl in 800 mL of MilliQ water and adjust the volume to 1 L and keep the solution at room temperature (20°C–25°C). The solution can be kept for two years.
- We prepare 1 M DTT by dissolving 3.09 g DTT in 20 mL of 0.01 M sodium acetate. We dispense the solution in 1 mL aliquot stored at −20°C. The solution can be kept for one year.

Last wash buffer (10 mL)		
Reagent	Final concentration	Amount
NH_4OAc (3 M)	0.1 M	333 μL
$MgCl_2$	0.1 mM	1 μL
Tween 20	0.02%	2 μL Tween
MilliQ water	N/A	To make volume 10 mL
Total	**N/A**	**10 mL**

- To prepare 3 M NH_4OAc we dissolve 23.1 g in 80 mL in MilliQ water and adjust the volume to 100 mL and keep the solution at room temperature (25°C). The solution can be kept for two years.
- To prepare 1 M $MgCl_2$ we dissolve 20.33 g of $MgCl_2$ in 80 mL in MilliQ water and adjust the volume to 100 mL and keep the solution at room temperature (20°C–25°C). The solution can be kept for two years.

Detergent-free Last wash buffer (10 mL)		
Reagent	Final concentration	Amount
NH_4OAc (3 M)	0.1 M	333 μL
$MgCl_2$	0.1 mM	1 μL
MilliQ water	N/A	To make volume 10 mL
Total	**N/A**	**10 mL**

Note: Last wash buffers with and without Tween are prepared fresh before the affinity purification.

Laemmli buffer (10 mL)		
Reagent	Final concentration	Amount
Tris base 1 M pH 6.8	0.25 M	2.5 mL
SDS	10% w/v	0.4 g
Glycerol	50% w/v	5 mL
2-Beta-mercaptoethanol	10% v/v	1 mL
Bromophenol Blue	–	20 mg
MilliQ water	N/A	To make volume 10 mL
Total	**N/A**	**10 mL**

Note: Laemmli buffer is stored at room temperature (20°C–25°C). The solution can be kept for two years. Add the 2-Beta-mercaptoethanol just before use.

Fixing solution (100 mL)		
Reagent	Final concentration	Amount
Ethanol	30%	30 mL
Acetic acid	10%	10 mL
MilliQ water	N/A	To make volume 100 mL
Total	**N/A**	**100 mL**

Rehydration solution

- We prepare the rehydration solution by adding 20 mL 100% Ethanol to 80 mL MilliQ water.

Reduction solution

- We prepare the reduction solution by adding 6 μL of 1 M DTT in 100 mL of MilliQ water to a final concentration of 6 μM.

Silver nitrate solution

- To prepare the 0.1% silver nitrate solution we dissolve 0.1 g of $AgNO_3$ in 100 mL MilliQ water.

Developer solution

- Developer solution is prepared by mixing 50 μL of Formaldehyde 37% with 100 mL of MilliQ water and dissolving 3 g of Na_2CO_3 for a final concentration of 200 mM.

Stop solution

- Stop Solution is made with 5% of acetic acid by adding 5 mL of acetic acid to 95 mL of MilliQ water (in this specific order).

Note: All buffers listed above for silver staining of protein gels are prepared fresh while the gel is running.

STEP-BY-STEP METHOD DETAILS

Harvesting, making cryo-noodles, cryo-griding cells, and magnetic bead conjugation

Timing: 4 days (Handling time: 1 h for step 1; 2 h for step 2; 2 h for step 3; 1 h for step 4; 2 h for step 5)

Our cells are grown in large volumes of YPD to generate enough material to do multiple affinity purification experiments. We start with a small pre-culture from which we then inoculate the large- scale culture. Typically, 6 L of YPD harvested at 0.6 OD 600 nm produce ~10 g of yeast powder. Our yeast strains typically have a doubling time of 90 min at 30°C.[13]

Day 1

1. Inoculate and grow culture
   a. To start a liquid pre-culture in Yeast Extract-Peptone-Dextrose (YPD), inoculate one colony grown on selective solid media.
   b. Grow 10 mL of preculture during the day (~8 h).
   c. Inoculate the preculture to harvest the large culture at ~0.6 OD 600 nm on Day 2. We typically let the culture grow overnight to harvest on day 2 after ~12 h.

Day 2

2. Harvest culture and freeze cells
   a. Once the culture reached the desired OD, spin down the culture at 4000 × *g*, for 10 min, at 4°C.
   b. Wash and pool the cell pellets in 50 mL MilliQ water on ice and transfer the resuspended cells into two 50 mL-Falcon tubes.
   c. Spin cells down at 2600 × *g*, for 5 min, at 4°C and repeat this wash once.
   d. Resuspend the pellet on ice with an equal volume of harvesting buffer.
   e. Spin down the cells at 2600 × *g*, for 15 min and make sure that you aspirate all the buffer from the pellet.

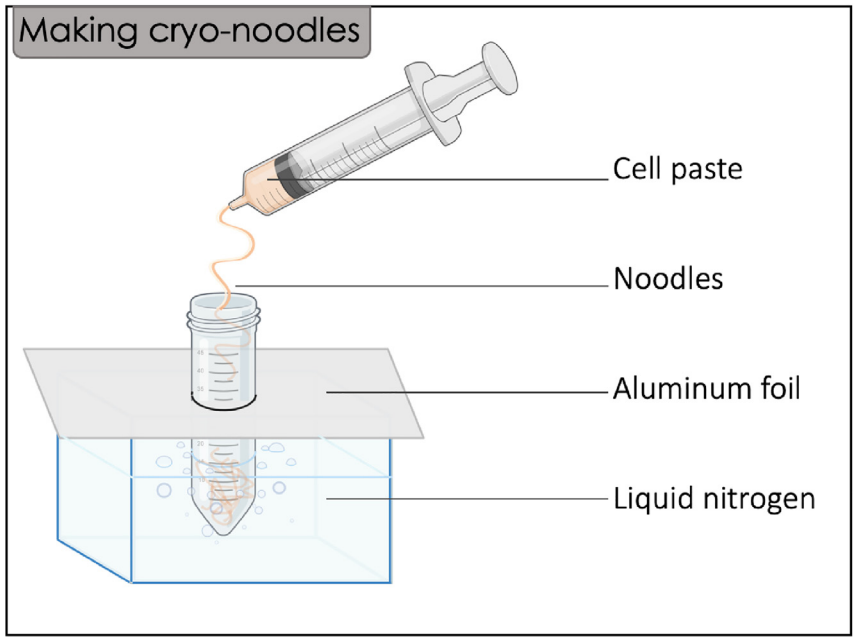

Figure 1. Making cryo-noodles

The cell paste is injected directly in a 50 mL-Falcon tube containing liquid nitrogen which has been placed into a Styrofoam box filled with liquid nitrogen, using a large syringe. A rack can be placed inside the Styrofoam box first to hold the falcon tube upright. If the liquid nitrogen evaporates, the falcon tube can be refilled carefully using a ladle.

f. To ensure that all the buffer is removed spin the pellet at 2600 × *g* for 5 min and remove any remaining buffer with a 1-mL pipette (at this point, the pellet should be fairly dry and resemble a thick paste).
g. Place a Falcon tube rack and some liquid nitrogen into a Styrofoam container, top it with aluminum foil, and place a 50 mL-Falcon tube through a hole in the foil into the rack. Allow the tube to cool down (Figure 1).
h. Fill the cooled 50 mL-falcon tube with liquid nitrogen to the very top.
i. With a spatula, scoop out the cell paste and place it into a 10 mL or 20 mL syringe. Press the cell paste through the syringe into the liquid nitrogen in the Falcon tube.
j. Once all the cell paste has been transferred into the Falcon tube, decant the liquid nitrogen from the tube (you can pour off the liquid nitrogen by poking holes into the cap of the Falcon tube).
k. Do not tighten the Falcon tube completely, to allow liquid nitrogen vapors to escape.
l. Store the tubes at −80°C. The protocol can be stopped at this point. Noodles can be kept at −80°C for at least two years.

3. Cryo-lysis of cells.

Note: For this specific step, we wear cryo-gloves and safety goggles as the cryogrinding requires handling a relatively large amount of liquid nitrogen (~5 L for grinding of one strain). For 20–50 mL of frozen cell pellet noodles, we use a 125 mL stainless-steel jar and 12 × 20 mm stainless-steel balls. For less than 15 mL of noodles, we use a 50 mL stainless-steel jar and 4 × 20 mm stainless-steel balls.[13]

a. Fill a rectangular ice bucket with liquid nitrogen and pre-chill the stainless-steel grinding jar, the stainless-steel lid, the grinding balls, and the tube containing the frozen yeast noodles in the liquid nitrogen (Figure 2).
b. The pre-cooling step is finished when the liquid nitrogen has stopped bubbling vigorously.
c. Once everything is chilled, pour the noodles into the grinding jar.

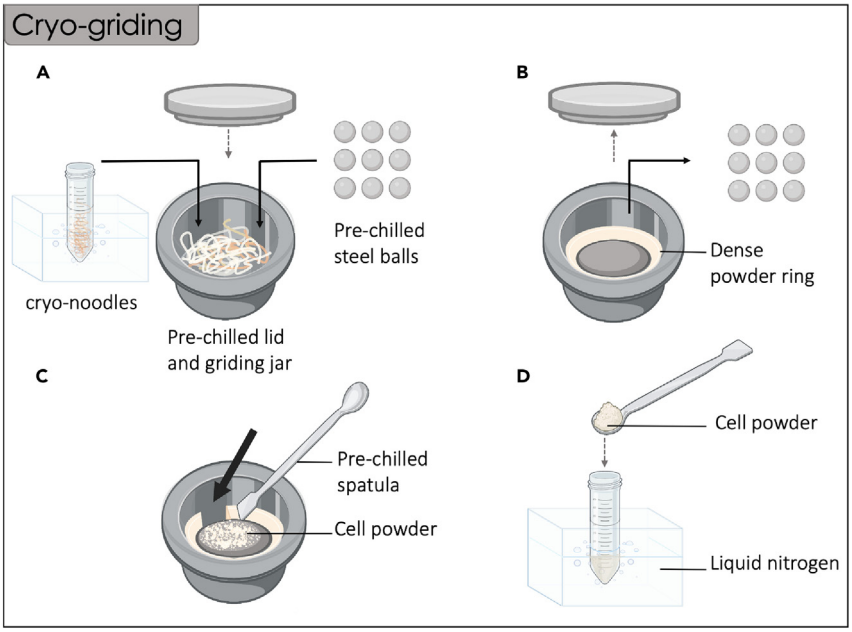

Figure 2. Grinding the noodles

(A) The pre-chilled grinding jar, lid, and the grinding balls and the noodles are placed into the jar.
(B) After the elapsed grinding cycles, the grinding balls are removed from the jar using large tweezers.
(C) The dense powder ring along the inside walls of the jar is broken up using a prechilled metal spatula (C).
(D) Finally, the powder is transferred into a pre-chilled 50 mL-Falcon tube placed inside a Styrofoam box filled with liquid nitrogen (D).

d. Weigh the grinding jar with the noodles and then adjust the counterbalance weight on the ball mill (we use a planetary ball mill from Retsch, model PM 100).
e. With a 125 mL-grinding jar, we use 7–11 × 20 mm stainless steel grinding balls, and the number of grinding balls should be adjusted based on the amount of noodles:
   i. 75–50 mL of noodles require 7–9 grinding balls.
   ii. 25–50 mL of noodles requires 9–11 grinding balls.
   iii. 15–25 mL of noodles requires 11 grinding balls.
   iv. For <15 mL of noodles, use a 50 mL-grinding jar with 3 grinding balls.

Note: Grinding with a 50 mL jar and 20 mm stainless balls does not allow grinding less than 2 mL of noodles. For smaller volumes of frozen cell pellets, the cell lysis is done in microcentrifuge tubes by sonication.[14]

⚠ CRITICAL: Make sure that there is no liquid nitrogen left in the grinding jar before starting your cell grinding.

f. Grinding is performed in eight cycles; each cycle is set in the following manner:
   i. 125 mL-jar: 400 RPM; 3 min; 1 min reverse rotation with no breaks between rotations.
   ii. 50 mL jar: 500 rpm; 3 min; 1 min reverse rotation with no breaks between rotations.

Note: During the grinding, you must hear the balls rattling around in the jar; if the rattling stops, see troubleshooting, problem 1.

g. Between each cycle, the jar is removed and cooled in liquid nitrogen for 5 min.

Note: To ensure the lid is chilled we use an empty Falcon tube to pour liquid nitrogen over the top of the grinding jar. The jar should not be opened or submerged during this time.

h. When eight cycles have been completed, remove the powder with a pre-chilled spatula – if some powder is stuck to the side of the jar, we repeat 1 grinding cycle at 350 rpm, 2 min, 1 min reverse rotation no breaks between rotations (See troubleshooting, problem 1).
i. Transfer the ground cell powder to a 50 mL-Falcon tube and clean jar and grinding balls with warm water and Windex™ solution, then dry them in a 30°C incubator.
j. The yeast powder should be stored at −80°C.

Note: This is a STOPPING point, and the powder can be stored for at least two years.

Day 3

4. Conjugation of magnetic beads with antibodies.

Note: For the differential affinity purification, we conjugated our magnetic beads with commercial IgG or with GFP nanobodies expressed and purified in our lab following the protocol detailed here.[15] Once the beads are conjugated with IgG or GFP nanobodies, they last ~1 month when stored at 4°C.

a. Resuspend the entire vial of Dynabeads (300 mg beads) in 16 mL of 0.1 M $NaPO_4$ buffer, pH 7.4.
b. Vortex the bottle for 30 s.
c. To make handling easier, we divide the bead suspension into four 15 mL-Falcon tubes (4 mL of suspension in each tube).

Note: The number of tubes will vary based on the number of samples and conditions being tested. Wash any remaining beads in the glass vial with an additional 2 mL of 0.1 M $NaPO_4$ buffer and divide the 2 mL equally between the four Falcon tubes.

d. Shake the bead suspension slowly for 10 min at room temperature on a Nutator or rocking platform.
e. While the bead suspension is on Nutator, prepare the antibody mix by adding the solutions to a 50 mL-Falcon tube, in this specific order:
   i. 40 mg of IgG or nanobody (2 mL), previously aliquoted in MilliQ water at a concentration of 20 μg/μL and stored at −80°C.
   ii. 9.850 mL 0.1 M $NaPO_4$ buffer.
   iii. Slowly, while gently shaking the tube, add 6.65 mL of 3 M Ammonium Sulfate.
   iv. Filter the solution using a .22 μm Millex GP filter.
f. Place the Falcon tubes with the bead suspension onto a magnetic holder and wait until all beads are attached to the magnetic rack. Once the bead solution becomes clear, aspirate the buffer (be careful to not aspirate off the beads).
g. Wash again with 4 mL of 0.1 M $NaPO_4$.
h. Vortex for 15 s, put on the magnet, and aspirate off the buffer.
i. Add 5 mL of antibody mix to each tube and vortex for 15 s.
j. Wrap the tops of each Falcon tube with parafilm and place on a rotating wheel at 30°C overnight (incubation should be done for at least 18 h and should not exceed 24 h).

Day 4

5. Washing of conjugated beads

Note: We do all washes described below in 15 mL-Falcon tubes and we aspirate the supernatant using a vacuum aspirator while the tubes are on a magnetic rack.

a. Wash once with 3 mL of 100 mM Glycine HCl pH 2.5. Put the solution on, resuspend the beads quickly and take the solution off as fast as possible.
b. Wash once with 3 mL of 10 mM Tris pH 8.8.
c. Wash once with 3 mL of 100 mM Triethylamine (make a fresh solution of 100 mM Triethylamine by adding 168 μL stock to 11.156 mL of MilliQ water). Put the solution on and take it off as fast as possible.
d. Wash the coated beads by adding 1× PBS to the beads and placing on a rocker/nutator for 5 min – repeat this wash 4 times.
e. Wash once with PBS + 0.5% Triton X-100 for 5 min.
f. Wash again with PBS + 0.5% Triton X-100 for 15 min on rocker/nutator.
g. Finally, resuspend all beads in a total of 2 mL of 1× PBS + 0.02% Sodium Azide.
h. Store the conjugated beads at 4°C. Conjugated beads can be kept at 4°C for three months.

Differential affinity purification of NPC isoforms

Timing: 2 days (handling time: 30 min for step 6; 2 h for steps 7 and 8, overnight for step 9; 30 min for step 10)

To purify nuclear pore complexes, we typically use a ratio of 50 μL beads per 1 g of cell powder and carry out the binding in 9 mL of extraction (TBT) buffer. However, depending on the abundance of the bait protein and the affinity of the antibody, this ratio can be adjusted, and the efficiency of the affinity purification (AP) monitored with Western blot and/or silver staining. As an indication, for an abundance of 3000 molecules/cell, we use 2 g of yeast powder, for an abundancy of less than 1000 molecules/cell we use 5–7 g of powder; everything that is more abundant than 5000 molecules/cell we generally use 0.5–1 g.[13] If you need to use a lot of powder (e.g., 3 g and more) you can split the powder between two tubes to do the AP then pool the beads by combining them after the binding step during the first wash.

Note: We generally control for the specificity of our affinity purification by western blot, silver staining, or directly by mass spectrometry, comparing our samples against negative or background controls. To obtain these controls, we simply incubate and wash our beads as described above with an identical ratio of untagged cell extract. Alternatively, a cell extract can be used that expresses the epitope tag alone from a promoter. This allows the identification of non-specific binding (proteins cross-reacting with the antibody or epitope tag or sticking to the beads) in our sample.

Day 1

Optional: We generally test the efficiency of our affinity purification on non-crosslinked samples in parallel. To do so, we include one sample that is not treated with glutaraldehyde and does not undergo the crosslinking steps 6f. and 6g. We then take samples for SDS-PAGE followed by western blot analysis and/or silver- staining throughout the procedure. For analysis of these samples (Figure 3).

6. Preparation of resin and stabilization of dynamic complexes
   a. Pre-wash your IgG- and GFP- nanobody conjugated Dynabeads three times with 5 mL of extraction buffer by pipetting up and down and divide them up into 15 mL-Falcon tubes. Keep the beads at 4°C until you reach the binding steps.
   b. Weigh the yeast powder into a 50 mL-Falcon tube pre-cooled in liquid nitrogen. On ice, let cell powder thaw very slightly to look like soft ice cream.
   c. Add 9 mL of room temperature extraction buffer per 1 g of cell powder and vortex for 30 s.
   d. Polytron for 30 s with a hand polytron homogenizer (7-mm probe)

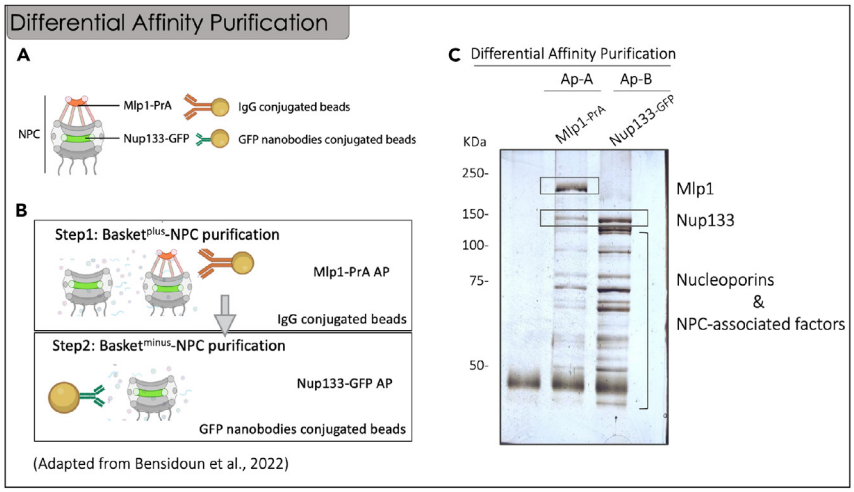

Figure 3. Differential affinity purification

(A) In the strain used for the differential affinity purification, Mlp1 is tagged C-terminally with a PrA and Nup133 is C-terminally tagged with GFP.

(B) Mlp1-PrA associated complexes are purified via IgG- conjugated beads, followed by the isolation of Nup133-GFP associated complexes via GFP-nanobodies from the flow-through allowing for the separation of basket-containing and basket-less NPCs from the same sample.

(C) In (C), a silver-stained gel is shown on which 10% of a differential affinity purification using 1 g of cell powder has been separated (see more on ratio beads/powder in the differential affinity purification section above). While Mlp1-PrA is identified in the basket-containing AP (AP-A), no bands corresponding to Mlp1-PrA can be identified in the basket-less (i.e., Nup133-GFP) AP (AP-B), suggesting a high degree of depletion of the basket-containing NPCs and Mlp1 in the first round of AP. However, as expected, a band corresponding to Nup133 as well as other nucleoporins and associated factors can be identified in both AP samples.

Note: A different type of homogenizer could be used here, however, we recommend the use of a similar probe as it could otherwise result in greater dispersion of cell material or breaking of complexes.

e. Spin at 2600 × *g* for 10 min and transfer the supernatant to a fresh tube.
f. Add 10 mM glutaraldehyde for 5 min and gently agitate on ice on a rocker/nutator.[16]
g. Quench the reaction by adding 1 mL of Tris buffer 1 M, pH 8, to a final concentration of 100 mM.

7. First affinity-purification of Mlp1-PrA complexes, including basket-containing NPCs (IgG AP)
   a. Pour the supernatant from step 6g onto the magnetic beads - the binding time starts at this point.
   b. Place the tubes on a rotating wheel and incubate for 30 min at 4°C.
   c. After 30 min, immediately place the tubes on a magnetic rack until the solution is clear.

⚠ CRITICAL: take off the supernatant very carefully using a pipette and transfer to a fresh 15 mL-Falcon tube. Do not aspirate the supernatant or the IgG beads, which should remain in the tube on the magnetic rack!

   d. Add 1 mL extraction buffer to the IgG beads and keep them on ice. Label this sample "IgG AP".

8. Second affinity purification of Nup133 (basket-less) NPC complexes (GFP AP)
   a. Add the anti-GFP-conjugated beads to the supernatant from step 7c in the 15 mL-Falcon tubes, place the tubes on a rotating wheel and incubate for 30 min at 4°C.
   b. While the GFP AP is incubating for the isolation of Nup133, resuspend the IgG beads in the 1 mL extraction buffer, mix by pipetting (never vortex at this point), and transfer to a microcentrifuge tube on a magnet.

c. Wash the IgG AP beads three more times with 1 mL of extraction buffer by pipetting up and down, then place the tube on a magnetic rack and aspirate off the supernatant each time.
d. Wash the IgG AP beads with 1 mL of Last Wash buffer for 5 min on a nutator/rotator.
e. Place the tube on a magnetic bar and keep them on ice while you are proceeding to the washes of the second affinity purification.
f. Once the second incubation with anti-GFP-conjugated beads (GFP AP) is completed, place the tubes on a magnetic rack until the solution is clear.
g. Move the GFP AP supernatant to a tube on ice for further processing for SDS PAGE analysis if needed.
h. Wash the GFP AP beads three more times with 1 mL of extraction buffer by pipetting up and down, then place the tube on a magnetic rack and aspirate off the supernatant each time.
i. Wash the GFP AP beads with 1 mL of Last Wash buffer for 5 min on a nutator/rotator.
j. Place the tube on a magnetic bar.

Optional: If samples to test the efficiency of the affinity purification have been included, take 10% of each IgG and GFP AP samples for a Western blot or silver staining and resuspend in 20 μL 1× Laemmli buffer.

k. To prepare your samples for mass spectrometry analysis, wash all your samples/resin four more times in the Last Wash buffer without Tween:
   i. three short washes,
   ii. one 5-min wash

⚠ CRITICAL: As Tween could damage the mass spectrometry instrument, we want to remove it at this point.

l. After the last wash, add 50 μL of 20 mM Tris-HCl, pH 8.0 to each tube and resuspend the resin.

9. Trypsin digestion of samples
   a. The proteins are digested on beads at 37°C using 1 μg of trypsin and incubating for 16 h (our trypsin stock is kept at −80°C and resuspended at 500 ng/μL in 20 mM Tris-HCl).

Day 2

10. Trypsin digest continued and collection of peptides
   a. The digestion is quenched by the addition of 2 μL of 50% formic acid.
   b. At this step, we recommend injecting the samples for a mass spectrometry analysis as soon as possible, waiting no longer than a few days.
   c. Peptides are stored at −80°C in 20 mM Tris-HCl for short periods of time or can be lyophilized in a speed vacuum centrifuge or by other method and stored at −80°C if the injection for the mass spectrometry analysis has to be delayed for more than a few days.

SDS PAGE analysis of samples and silver-staining

Timing: 1–2 days (handling time: 2 h for step 11; 2 h 30 minutes for step 12)

We generally test the efficiency of affinity purification by western blot or silver-staining on non- cross-linked samples. The silver staining provides a good indication of the AP efficiency as it shows the bait as well as the proteins co-affinity purified (Figure 3C and 4) and can therefore be a good approach to test different salt concentrations (See troubleshooting, problem 2). The amount of material loaded on a gel depends on the quantity of powder used in your sample input as well as the cellular abundance of the complex of interest. Both Mlp1 and Nup133 are believed to have an abundance of >2000 molecules/cells and we loaded 10% of the sample on gel starting from 1 g of powder for affinity purifications of both Mlp1-PrA and Nup133-GFP.

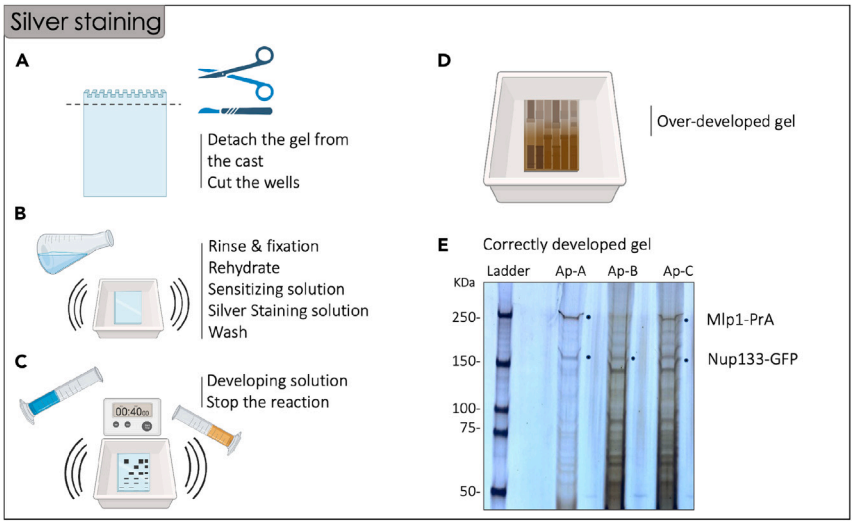

Figure 4. Silver staining overview

(A) The gel is removed from its cast and the upper part of the gel is cut.

(B) The first part of the silver staining protocol (B) consists of four main steps carried out on a shaking platform: fixation, re- hydration, sensitizing, and staining, separated by washes.

(C) The developing step has to be monitored carefully on the shaking platform and stopped as soon as the desired bands appear.

(D) Over-developed gels appear yellow/brown and individual bands, which can turn silver- black in coloring, become harder to distinguish. On a correctly developed gel, the co-affinity purified bait and prey proteins are easily distinguishable.

(E) In the example shown in (E), AP-A is a basket- containing NPC AP using Mlp1-PrA as bait; AP-B is a basket-less NPC AP using Nup133-PrA as bait; and AP-C is an AP of both types of NPCs using Nup133-GFP as bait.

Day 1

Note: We use here pre-cast 4–12% gels and MES buffer to improve the protein resolution all along the gel regardless of the protein sizes. However here, homemade polyacrylamide gels run with classical electrophoresis buffers (e.g., Tris-Glycine-SDS buffer) can also be used.

11. SDS PAGE analysis of samples.
    a. Take 10% of the beads after the last wash of the affinity purification (from step 8g, above).
    b. Resuspend the beads in 20 µL of Laemmli buffer.
    c. Boil the beads at 70°C for 5 min to elute the complexes from the beads.
    d. After the elution step, place the tubes on a magnetic rack and place the supernatant in a new tube (samples can be kept at −20°C).
    e. Prepare the NuPAGE Bis-Tris 4%–12% gel and fill the electrophoresis chamber with 1× MES buffer.

Note: We are using NuPage 4–12% Bis-Tris gradient gels which provider publication-grade images. For these gels, we recommend using MES over MOPS buffer (the two system-compatible buffers) as MES provides a better separation across a wide range of molecular weight. Samples can be separated on any other gel system.

    f. Add 1 mL of NuPAGE antioxidant directly into the running buffer (use 1 mL of antioxidant for 600 mL of 1× MES buffer).
    g. Keep the samples on a magnetic rack to make sure that no beads will be loaded in the wells and load 15 µL of the samples.
    h. Run the gels for 35 min at 200 V.

12. Silver staining the gel (Figure 4).
    a. Carefully remove the gel from the cast.
    b. Wash the gel quickly with MilliQ water in a clean glass dish.
    c. Fix the gel in the fixing solution for 1 h to overnight on a rocker platform.
    d. Wash twice for 10 min with 200 mL of 20% ethanol.
    e. Rehydrate twice for 10 min with MilliQ water.
    f. Incubate for 30 min with 400 mL of reduction solution.
    g. Wash twice for 10 min with 200 mL of 20% ethanol.
    h. Incubate for 30 min with the silver nitrate solution.
    i. Wash for 30 s with MilliQ water.
    j. Incubate the gel with the developing solution and wait until desired darkness of bands is achieved.
    k. Stop the reaction by adding 10 mL of stop solution for 5 min and scan the gel.
    l. The gel can be stored at 4°C in liquid (e.g., MilliQ water)

Mass spectrometry analysis

Timing: 3–4 days, not including follow-up and validation experiments

After affinity purification and trypsin digestion, peptides were cleaned using C18 ZipTips following the supplier's recommendations. Because our samples are relatively complex and are generated from the digestion of numerous proteins at very different abundances, each sample was injected near signal saturation to be able to detect even low abundant peptides. Information about the liquid chromatography method, as well as the settings and references of the MS instrument, are detailed in Bensidoun et al., 2022.[1] We inject each sample near the saturation of the signal. We also inject a blank between each sample to limit carry-over between samples (see troubleshooting, problem 3 and Figure 5). For protein identification, we used Proteome Discoverer to generate the peak list. Protein database searching was performed with Mascot 2.5 (Matrix Science) against the NCBI - *S. cerevisiae* protein database (20160802).

Day 1

13. Analysis of mass spectrometry data
    a. Set the protein and peptide identification thresholds in Scaffold™ to 95%. This results in a decoy false discovery rate (FDR) of 6% (Figure 5A; the FDR can be set between 1%–6% depending on the sample and coverage).

Note: Figure 5 shows the first ten proteins in our scaffold file for basket-containing and basketless NPC APs. As suggested by the differential AP validation tests (silver staining in Figure 3C), basket proteins are identified specifically in basket-containing pores APs (in triplicate), while other nucleoporins are identified in similar ratios in both APs.

    b. Using the Scaffold™ menu, select 'Exclusive Spectrum Counts' to analyze the data (Figure 5B).
    c. Extract the data from Scaffold™ as shown in (Figure 5C) and export it as an Excel file.
    d. Average non-specific interactors identified in a non-tagged and/or epitope-tag-only-expressing cellular extract (as described in the introductory note of the "Differential affinity purification of NPC isoforms" section). This represents the background.
    e. Subtract the background exclusive spectrum count values for each protein from the actual protein value measured in the bait sample affinity purification.
    f. For semi-quantitative analyses across samples, retain only the proteins with an Exclusive Spectral Counts (ESC) value $\geq$ 10 (See troubleshooting, problem 2, problem 3).

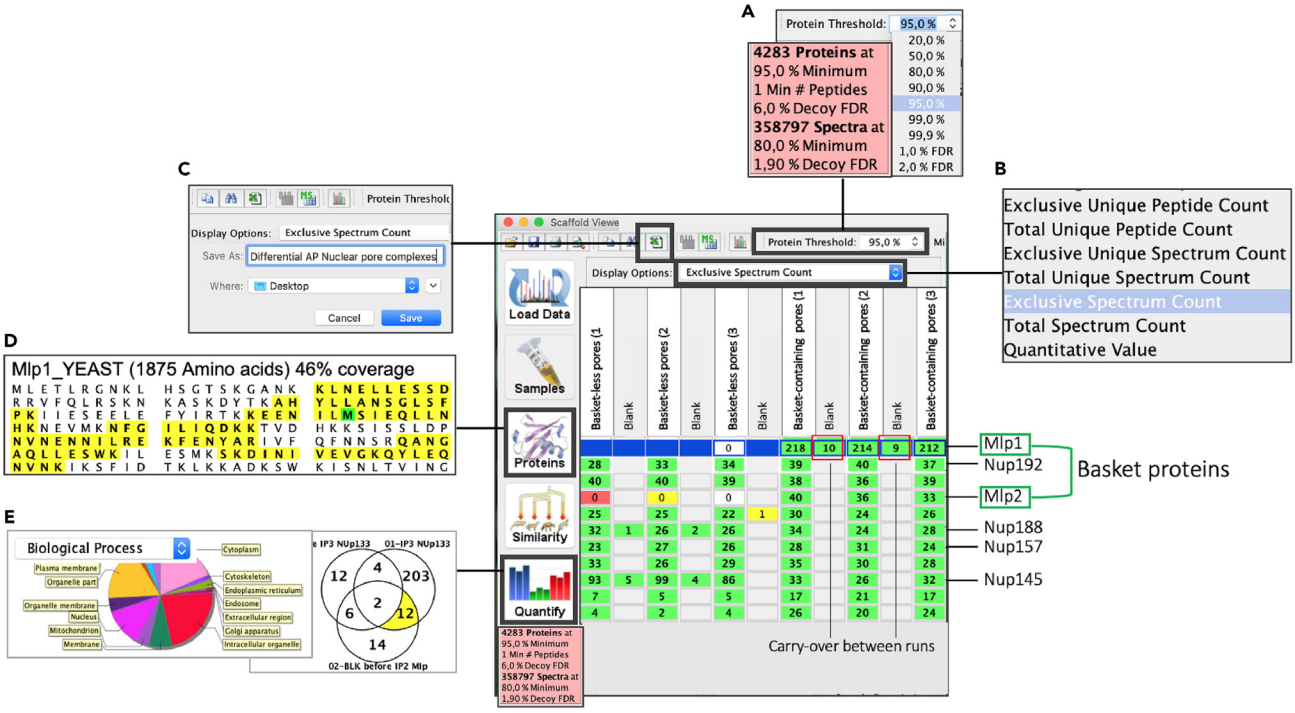

Figure 5. Scaffold data overview

(A) The protein threshold is set to 95%, giving a false discovery rate (FDR) of 6%.

(B) The display option is set to 'Exclusive Spectrum Counts'.

(C) Scaffold™ data can be exported into Excel for further analysis.

(D) Scaffold™ has different options allowing to view peptide coverage (in yellow) alongside the identified proteins (here, Mlp1).

(E) Scaffold™ has also different tools that allow to do a quick search for GO term enrichment and to compare the quantities of factors differentially identified in each sample (Venn diagram).

g. Normalize values against the average values of proteins associated with the bait proteins in the different APs:
   i. Mlp2 for Mlp1-PrA
   ii. Y-complex nucleoporins (Nup84, Nup85, Nup120, and Nup145C)[11] for Nup133-GFP

Note: See troubleshooting, problem 4.

Note: This allows normalization of the data sets against proteins of similar size, stoichiometry, and segregation behavior. Curating mass spectrometry data is very specific to the purified complex and different cut-offs, FDRs, normalization strategies, and possibilities to represent the data can be applied depending on the experimental design and objectives. However, some online tools such as CRAPome2.0 have been developed to facilitate and standardize the background identification in the proteomics field.

EXPECTED OUTCOMES

This method allows for semi-quantitative measurement of the components of the nuclear pore complex and NPC-associated proteins. Furthermore, we have been able to identify factors associated specifically with one type of NPC, basket-containing or basket-less, and to compare the relative quantities of factors associated with the two types of NPCs. We found that the preferential partner of the bait protein Mlp1- PrA, which forms the scaffold of the nuclear basket and used to purify basket-containing NPCs, namely Mlp2 was identified by mass-spectrometry only in the Mlp1-PrA AP (Figure 5).

On the other hand, the NPC components expected to be associated with both types of NPCs were identified in similar ratios in the differential affinity purifications. These results provided a good validation of our approach, indicating that the purification and the separation of basket-containing and basket-less NPCs was effective. Therefore, as shown in the original study, we could identify a specific basket-containing NPC interactome, which included the components of the TREX2 complexes and the pre-mRNA processing factor Pml39, while other factors involved in mRNA maturation and/or mRNA export, such as the export receptors and the poly(A) binding proteins, were found associated with both types of NPCs. This dissection of the two respective NPC interactomes allowed us to suggest preferential roles for basket- containing and basket-less NPCs.[1] We expect that our strategy will enable the isolation and interactome identification of different isoforms or subunits of other macromolecular complex from cells, beyond the NPC and budding yeast.

LIMITATIONS

We expect that proteins interacting with and cargos passing through the nuclear pore may have very rapid interaction kinetics or only transient interactions with the NPC. Therefore, the NPC interactomes identified here by mass spectrometry may not represent the complete *in vivo* NPC interactome. Furthermore, while some proteins remain stably associated with the NPC during affinity purification, others may dissociate during the incubation with the IgG or GFP nanobody-conjugated beads (see troubleshooting).

To avoid this last issue, we used mild glutaraldehyde crosslinking to stabilize the complexes prior to the incubation with the magnetic beads. However, despite the stabilization with a cross-linker, some proteins expected or known to interact with NPCs were not detected by mass spectrometry. For example, the Ubiquitin-like modifier protein Ulp1 was not detected in any of our APs, while we have shown by microscopy that this protein colocalizes specifically with basket-containing NPCs. Whether this is due to a stabilization problem or because Ulp1 peptides generated by trypsin digest have a composition preventing their detection by mass spectrometry is unknown.

In addition, we can predict that comparing the relative compositions of two complexes with drastically different cellular abundancies may lead to some problems in interpretation of the mass spectrometry data. Indeed, components of the less-abundant isoform may be below the mass spectrometry detection threshold and therefore be characterized as 'not associated' with the less-abundant isoforms. In our case, basket-containing and basket-less NPCs seemed to have similar abundance in cells; however, we still corrected for the abundance of the two complexes in the other sample to be able to compare the relative abundance of the proteins purified with the two types of NPCs (See troubleshooting, problem 4).

TROUBLESHOOTING

Problem 1

Cryo-lysis of cells, step h: the grinding balls stop rotating in the jar during the grinding cycles.

Potential solution

During the grinding, you must hear the grinding balls rattling around in the jar. Sometimes the rattling stops during a cycle. If so, you can open the jar and remove one or a few balls from the jar until you hear it rattling again. It is not considered a grinding cycle unless there is rattling. If you have to remove a few grinding balls from the jar, you may have to quickly re-weigh the jar afterward and adjust the grinder's counterbalance weight accordingly.

Often, the powder forms a solid circular block along the bottom of the jar. In such a situation, you can open the jar in a Styrofoam box containing liquid nitrogen and remove the grinding balls which would be kept in liquid nitrogen as well. Then, you would be able to break the powder block using a pre-chilled spatula or a pick which to fragment it into a few blocks. Then you can replace the cold

grinding balls in the jar and re-start your grinding. If you have to open the jar to remove some balls or to break the powder block, make sure that there is no liquid nitrogen in the jar when you close it to re-start the grinding.

To avoid the formation of the powder block, you have to make sure that most of the buffer used during the harvesting and the cryo-noodles preparation has been removed from the cellular paste used to prepare the noodles.

Problem 2

Analysis of mass spectrometry data; step f: mass spectrometry, silver staining, or western blotting reveals poor recovery of the expected components of the complex.

Potential solution

This issue may be caused by the fact that the bait protein does not remain associated with the complex during the affinity purification steps. Indeed, it is hard to predict how strongly the protein chosen to be the bait for an AP is associated with a complex. Alternatively, this issue may be caused by the fact that some components of the complex are lost during the APs or the subsequent washes. In both cases, the read-out for the APs will show a good purification of the bait protein (high exclusive spectral counts in MS, and/or a strong band on western blot /silver staining) and low abundance of the proteins that co- affinity-purify.

To limit this problem, we usually optimize the salt concentration (NaCl) in our extraction buffer to adjust the stringency of the AP as well as the number of washes. Our study uses a low salt concentration (50 mM NaCl) which allowed good recovery of NPC-associated factors. However, low salt concentrations may not be efficient to eliminate non-specific binding of proteins traditionally considered background proteins in some instances. Depending on the complex to be purified, higher salt concentration may increase co-AP efficiency and, therefore, we recommend testing several salt concentrations to achieve a good signal-to-noise ratio.

In our approach, once the salt concentration for our affinity purification was optimized, we used a gentle crosslink with glutaraldehyde to stabilize the complex and to be able to purify the proteins believed to have a labile or dynamic interaction with NPCs. While the crosslink was suitable to identify a larger NPC interactome, it is important to note that crosslinked complexes cannot be separated on a gel as glutaraldehyde crosslinks are not reversible and large crosslinked complexes will not enter the gel. Other types of crosslinkers, which can be reversed, may alternatively be used for the stabilization of the complexes under study.[16,17,18] Crosslinking may also increase the background as non-specific association with proteins may be stabilized and will not be flushed out efficiently during the washes. Finally, while crosslinking may enhance the number of proteins identified in mass spectrometry, it can also decrease the number of peptides detected for each protein as the crosslinked peptides are not readily mapped to the reference proteome during the analysis.

Problem 3

Analysis of mass spectrometry data, step f: components of one complex isoform are identified in the affinity purification of the second isoform.

Potential solution

Our method is a subtractive approach where one isoform of the complex is purified from the cellular extract leaving the second isoform in the extract. Therefore, if the second bait protein is present on both isoforms, as in our case, the first isoform can be purified in the second round of AP if its depletion from the extract is not very efficient. Thus, we recommend using the more efficient bait/antibody or epitope-tag for the first affinity purification. In our situation, the affinity of PrA to IgG is stronger than the affinity of GFP to GFP-nanobody. Therefore, we used the PrA-tagged bait for the first AP to achieve high levels of depletion of the basket-containing NPCs (Figure 3C). While the absence of

basket-proteins in basket-less NPC sample was initially monitored by silver staining, mass spectrometry analysis also confirmed the efficiency of the basket-containing NPC AP, as neither Mlp1 nor Mlp2 were identified in basket-less NPC APs (Figure 5). The depletion of one complex can also be tested by western blot by loading a fraction of the sample collected before and after the AP ("Input" vs. "Flow through") on a gel next to the AP samples to compare the amount of bait.

In MS samples, traces of the first bait may also be simply artefactual and be caused by a carry-over of peptides between injections on the mass spectrometry instrument. Indeed, if the first bait is large and produces numerous peptides and/or if it is very abundant, it is very likely that some peptides will contaminate the instrument and be detected in the next sample (Figure 5, blank column between sample runs). To avoid this artifact, we usually inject a blank sample between samples which serves as a wash. In this blank sample, we often detect peptides from the previous injection, indicating that one or several steps of washes may be essential between each run. In addition to limiting this issue, the order of sample injection may be key, and we usually inject the less concentrated samples first as it is expected to result in less carryover. The bait used in our study to purify the basket-containing NPCs, Mlp1-PrA, is a large and highly abundant protein; therefore, basket-containing NPC samples were injected after the basket-less NPC ones.

Problem 4

Analysis of mass spectrometry data, step g: the protein used as bait for affinity purification also possess a "free fraction" not associated with the complex of interest.

Potential solution

In our study, the bait used to purify basket-containing NPCs, Mlp1-PrA, has a large "free fraction" which is probably as abundant, if not more, than the pool of the protein binding to NPCs, while our second bait, Nup133-GFP, has only a small "free fraction" in cells. To be able to compare basket-containing and basket-less NPC interactomes and obtain relative differences in the enrichments of individual associated proteins, we needed to normalize the MS data based on the efficiency of the affinity purifications.

In many experiments in the field, the spectral counts of proteins identified in MS are normalized against the relative abundance of the bait in each AP. Such a strategy would have led us to underestimate the abundance of basket-containing NPC interactome as the Mlp1-PrA "free fraction" is higher than that of Nup133-GFP. Instead, we normalized our data over the relative abundance of proteins interacting directly with our respective baits at the NPC only and having a similar stoichiometry. For example, basket-containing NPC data was normalized over the abundance of another basket-specific protein, the Mlp1 paralog Mlp2. On the other hand, basket-less NPC samples were normalized against nucleoporins assembling with our bait Nup133-GFP, the NPC Y-submodule,[19] whose components at present at a similar stoichiometry than Nup133 per NPC. After normalization, the abundance of core nucleoporins was similar across all samples, and prey proteins with significant differences in basket-containing over basket-less NPCs were classified as differential interactome. Therefore, we suggest that sets of APs isolating differential isoforms of the same complex should be normalized against proteins that are found in each complex at similar levels or stoichiometry than the respective baits, if such information is available.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Marlene Oeffinger (marlene.oeffinger@ircm.qc.ca).

Materials availability

Yeast strains generated in this study are available upon request with a completed material transfer agreement.

Data and code availability

The published article Bensidoun et al. (2022)[1] includes all datasets/codes generated or analyzed during this study.

ACKNOWLEDGMENTS

We thank LC. Aguilar, C. Trahan, T. Reiter, and B. Montpetit for discussions and help and thank D. Faubert (IRCM Proteomics Platform), the IRCM Molecular Biology Platform. This work was supported by the Natural Sciences and Engineering Research Council (RGPIN-2015-05922 to D.Z.; RGPIN-2015-06568 to M.O.), Canadian Institute for Health Research (PJT-425798 to D.Z. and M.O.), and Canadian Foundation for Innovation (D.Z. and M.O.).

AUTHOR CONTRIBUTIONS

P.B., M.O., and D.Z. participated in the conceptualization, optimization, and data collection of the approach and wrote the original draft. M.O. edited and completed the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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