Video Article

Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) for Analysis of Multiprotein Complexes from Cellular Lysates

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Abstract

Multiprotein complexes (MPCs) play a crucial role in cell signalling, since most proteins can be found in functional or regulatory complexes with other proteins (Sali, Glaeser *et al.* 2003). Thus, the study of protein-protein interaction networks requires the detailed characterization of MPCs to gain an integrative understanding of protein function and regulation. For identification and analysis, MPCs must be separated under native conditions. In this video, we describe the analysis of MPCs by blue native polyacrylamide gel electrophoresis (BN-PAGE). BN-PAGE is a technique that allows separation of MPCs in a native conformation with a higher resolution than offered by gel filtration or sucrose density ultracentrifugation, and is therefore useful to determine MPC size, composition, and relative abundance (Schägger and von Jagow 1991); (Schägger, Cramer *et al.* 1994). By this method, proteins are separated according to their hydrodynamic size and shape in a polyacrylamide matrix. Here, we demonstrate the analysis of MPCs of total cellular lysates, pointing out that lysate dialysis is the crucial step to make BN-PAGE applicable to these biological samples. Using a combination of first dimension BN- and second dimension SDS-PAGE, we show that MPCs separated by BN-PAGE can be further subdivided into their individual constituents by SDS-PAGE. Visualization of the MPC components upon gel separation is performed by standard immunoblotting. As an example for MPC analysis by BN-PAGE, we chose the well-characterized eukaryotic 19S, 20S, and 26S proteasomes.

Video Link

The video component of this article can be found at https://www.jove.com/video/2164/

Protocol

****This video protocol is based on an associated publication [1]:** Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) for the Identification and Analysis of Multiprotein Complexes. Mahima Swamy, Gabrielle M. Siegers, Susana Minguet, Bernd Wollscheid, and Wolfgang W. A. Schamel. Science's STKE 2006 (345): pl2, July 25, 2006, [DOI: 10.1126/stke.3892006pl4]. Please click here to see this publication.

1. Preparation of dialyzed cell lysate

1. Harvest 10x10^6 cells and pellet by centrifugation at 350g for 5 min at 4°C.
2. Wash the cell pellet three times with 1 mL of ice-cold PBS (recipe 1), centrifuge as in step 1.1.
3. Resuspend pellet in 250 µL of ice-cold BN-Lysis Buffer (recipe 2) and incubate on ice for 15 min.
4. Centrifuge at 13,000g for 15 min at 4°C to remove insoluble material.
5. Melt a hole in the cap of a 1.5-mL microcentrifuge tube using the heated large diameter side of a Pasteur pipette, then place the tube on ice to cool down to 4°C.
6. Transfer supernatant from step 1.4 into the chilled tube with the hole in the cap.
7. Place a dialysis membrane (molecular weight cut-off of 10 kD) with forceps on top of the opened tube, close the cap, and cut off excess dialysis membrane that sticks out.
8. Seal the cap on the side carefully with Parafilm.
9. Invert the tubes and centrifuge upside-down at the lowest speed possible in a 50-mL conical tubes in a cell culture centrifuge for 10 sec at 4°C. Remove the inverted tube from the centrifuge using tweezers to avoid turning the tube right side up.
10. Prepare a 100-mL beaker with cold BN-Dialysis Buffer (recipe 3) and a stir plate. Use at least 10 mL of BN-Dialysis Buffer per 100-µL sample.
11. Affix the tube with tape upside-down inside the beaker, and remove air bubbles from the hole beneath the cap using a bent Pasteur pipette.

12. Place beaker on top of a magnet stirrer, switch on the stirrer and leave it for 6 hours or overnight in the cold room. Check occasionally to ensure that stirring is not creating air bubbles at the dialysis membrane.
13. Collect the dialyzed cell lysate in a new chilled microcentrifuge tube.

2. Pouring of BN-gels

1. Gradient gel pouring is done at room temperature with a gradient mixer. Gloves must be worn because polyacrylamide is highly neurotoxic. Avoid any contact with SDS.
2. Place the gradient mixer on a stir plate and attach it to a piece of flexible tubing. Close the channel using the valve and close the tubing with a clamp. Place a magnetic stirrer 15% into the "high" cylinder connected to the tubing.
3. Thread the flexible tubing into a peristalitic pump and attach a syringe needle to its end. Then, place the needle between the two glass plates of the gel apparatus.
4. Prepare 4% (recipe 5) and 15% (recipe 6) separating gel solutions, adding APS and TEMED immediately before use. The combined volumes should be equal to the volume of the separating gel.
5. Pour these gel solutions into the corresponding cylinders of the gradient mixer (4% into the "low" and 15% into the "high" cylinder).
6. Open the valve and force out the air bubble inside the channel connecting the two gel reservoirs by pressing over the left cylinder with your thumb.
7. Switch on the magnetic stirrer, remove the clamp, and switch on the peristalitic pump to 5 ml per minute. Allow the gel to slowly flow between the glass plates. Ensure that the needle is always above the liquid.
8. Allow all liquid to enter the gel apparatus, and then overlay gently with isopropanol. Allow the gel to polymerize for at least 30 min at room temperature.
9. Clean the pouring apparatus immediately with dH_2O (do not use detergent).
10. Remove the isopropanol, wash with dH_2O, and remove the dH_2O with a Whatman paper.
11. Prepare a 3.2% stacking gel (recipe 7), adding APS and TEMED immediately before use.
12. Pour the stacking gel on top of the separating gel and introduce the comb between the glass plates, avoiding bubbles. After the stacking gel has polymerized, cool the gel down to 4°C.
13. Immediately before sample loading, remove the comb slowly, pulling it out at an angle to the plane of the gel. This allows air to enter the pockets rapidly, which improves the quality of the wells.

3. Separation of dialyzed cell lysate by BN-PAGE

1. Load 1 to 40 µL of dialyzed lysate and 10 to 20 µl of Marker Mix (recipe 10) in the dry wells at 4°C. Overlay the samples in each well with cold Cathode Buffer (recipe 8).
2. Fill the inner chamber with cold Cathode Buffer and the outer/lower chamber with cold Anode Buffer (recipe 9).
3. Apply 100 V to a minigel or 150V to a large gel, until the samples have entered the separating gel. Run the gel at 4°C.
4. Increase the voltage to 180 V (minigel) or 400 V (large gel) and run until the dye front reaches the end of the gel. The run takes 3 to 4 hours for a mini-, and 18 to 24 hours for a large gel.

4. Second dimension SDS-PAGE

1. Prepare a standard 10% SDS-gel (recipes 12-15) with a single large lane for the first dimension BN-PAGE lane, one regular lane for the molecular weight marker, and one regular lane for an aliquot of the dialyzed lysate that has been mixed with SDS sample buffer (recipe 11) and boiled for 5 min at 95°C. Use spacers whose thickness was increased by two layers of scotch tape to simplify loading of the BN-PAGE gel slice onto the SDS gel.
2. Remove the BN-PAGE gel in the plates from the electrophoresis apparatus and gently pry up one plate.
3. Remove the stacking gel and cut out the lane of the BN-PAGE gel containing the proteins of interest.
4. Place the BN-PAGE gel slice in 2x SDS Sample Buffer and incubate for 10 min at room temperature.
5. Boil the BN-PAGE gel slice briefly (not more than 20 sec) in a microwave.
6. Incubate the BN-PAGE gel slice in the hot SDS Sample Buffer for another 15 min at room temperature.
7. Load the BN-PAGE gel slice in the large well over the stacking gel of the SDS-PAGE gel avoiding air bubbles, and overlay the slice with SDS Sample Buffer. Load marker and lysate control.
8. Perform electrophoresis according to standard protocols.

5. Detection of MPC subunits by immunoblotting

1. For transfer, prepare six Whatman papers and a PVDF membrane fitting to the size of the SDS-gel.
2. Incubate the PVDF membrane in 100% methanol for 30 s and soak the Whatman papers in transfer buffer (recipe 16).
3. Place three Whatman papers, the PVDF membrane, the SDS-gel (remove stacking gel), and again three Whatman papers in a sandwich-like structure into a semidry transfer cell.
4. Apply 20 V for 25 min.
5. Detect proteins according to standard immunoblotting protocols.

6. Representative Results

We present the analysis of the eukaryotic 19S, 20S, and 26S proteasomes as an example for MPC characterization by 2D BN/SDS-PAGE (Figure 1A). HEK293 cells were lysed with a buffer containing 0.1% Triton X-100 as a detergent to disrupt the membranes and solubilize membrane protein complexes. These lysates were dialyzed against BN-Dialysis buffer to remove salts and small metabolites. Then, MPCs were

separated by 4-15% gradient BN-PAGE followed by a second dimension SDS-PAGE. Proteins were visualized by immunoblotting with antibodies against the subunits β2 and Mcp21 of the 20S proteasome.

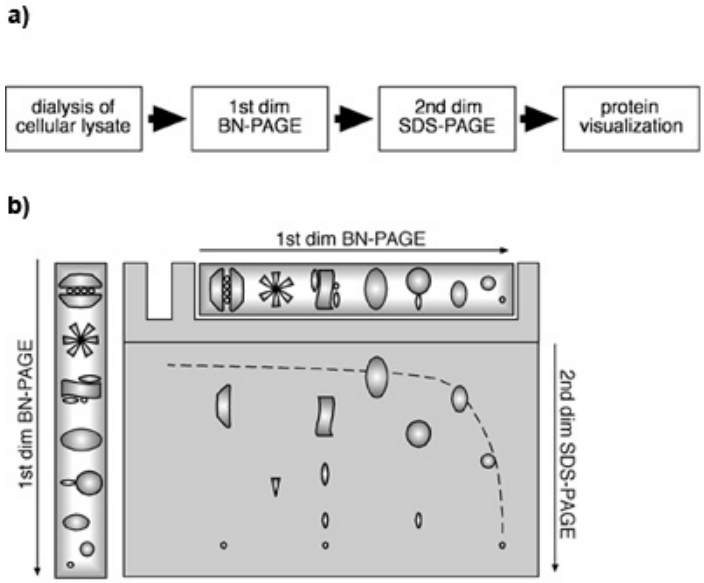

Figure 1. A two-dimensional BN-PAGE/SDS-PAGE approach using cellular lysates. (A) Flow diagram of a 2D BN-PAGE/SDS-PAGE approach from cellular lysates. (B) Schematic scheme of a 2D BN-PAGE/SDS-PAGE. Proteins and MPCs are separated under native conditions by BN-PAGE in a first dimension. For the second dimension, proteins and/or MPCs are denatured by SDS in the gel strip after separation by BN-PAGE and subsequently subjected to SDS-PAGE. Monomeric proteins will migrate in a hyperbolic diagonal due to the gradient gel in the first and a linear gel in the second dimension. Components of one concrete MPC will be found below the diagonal, located on a vertical line.

It has been shown that by combination of first dimension BN- and second dimension SDS-PAGE, monomeric proteins migrate within a hyperbolic diagonal due to the gradient gel in the first and the linear gel in the second dimension ((Camacho-Carvajal, Wollscheid *et al.* 2004); Figure 1B). Components of MPCs are located below this diagonal. Proteins that represent subunits of the same MPC can be found in one vertical line in the second dimension, whereas several spots of the same protein in a horizontal line indicate the presence of the protein in several distinct MPCs. Figure 2 shows that in our experiment immunoblotting against β2 and Mcp21 revealed the presence of specific protein complexes containing these proteasomal subunits. Both proteins were detectable as individual spots arranged in a horizontal line, indicating that β2 and Mcp21 represent constituents of several distinct MPCs. These MPCs could be clearly identified as the 26S proteasome (20S plus 19S cap), the 20S proteasome together with the regulatory subunit PA28, and the 20S proteasomes alone, on the basis of their size and composition. Taken together, these results demonstrate that endogenous MPCs can be identified and characterized by a two-dimensional BN-PAGE/SDS-PAGE approach using cellular lysate. This method is applicable for determination of size, composition, and relative abundance of MPCs.

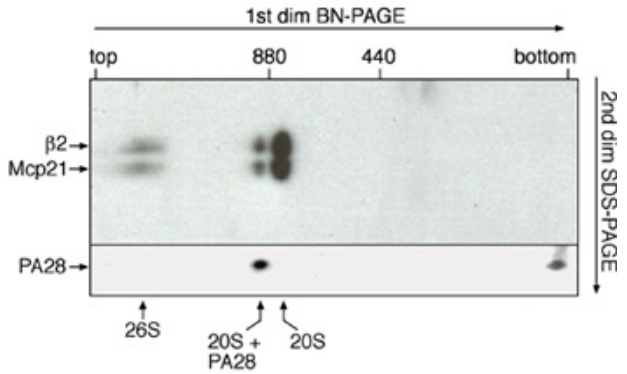

Figure 2. Detection of different forms of the eukaryotic proteasome by immunoblotting after two-dimensional BN-PAGE/SDS-PAGE. For identification and analysis of eukaryotic proteasomes, HEK293 cells were lysed with 0.1% Triton X-100. Cellular lysates were dialyzed and subsequently subjected to BN-PAGE (4-15%) to separate MPCs. Afterwards, a second dimension SDS-PAGE (10%) was run for size separation of individual subcomponents. Immunoblotting was performed with specific antibodies recognizing the Mcp21 and β2 subunit of the 20S core complex, and the regulatory subunit PA28.

I. Table of specific reagents (alphabetical order):

Reagent	Company	Comments
6-aminohexanoic acid (ε-aminocaproic acid)	Sigma-Aldrich, Taufkirchen, Germany	This chemical is an irritant and should be handled with gloves.

Acrylamide-bisacrylamide solution (40%), Mix 32:1	Applichem, Darmstadt, Germany	This solution is neurotoxic and should be handled with gloves.
Bis-tris	Roth, Karlsruhe, Germa-ny	
Brij 96	Sigma-Aldrich, Taufkirchen, Germany	
Coomassie blue G250	Serva, Heidelberg, Ger-many	Do not substitute other types of Coomassie dye such as Coomassie blue R250 or colloidal Coomassie blues.
Digitonin	Sigma-Aldrich, Taufkirchen, Germany	Digitonin is toxic. Gloves should be worn when handling buffers or samples containing this deter-gent.
Dodecylmaltoside	Applichem, Darmstadt, Germany	
Triton X-100	Roth, Karlsruhe, Germa-ny	Triton X-100 is toxic. Gloves should be worn when handling buffers or samples containing this detergent.

II. Table of specific material and equipment:

Equipment	Company
Dialysis membranes (molecular weight cut-off 10 to 50 kD)	Roth, Karlsruhe, Germany
Gel electrophoresis system	For example from Bio-Rad, Munich, Germany
Gradient mixer	Self-made or commercially available from Bio-Rad, Munich, Germany
Peristaltic pump	Amersham Pharmacia Biotech, Freiburg, Germany
Polyvinylidene difluoride (PVDF) membrane	Immobilon-P, Millipore, Eschborn, Germany
Semi-dry transfer equipment	For example from Bio-Rad, Munich, Germany
Silicon tubing (3 to 5 mm diameter, 1 m length)	NeoLab, Heidelberg, Germany

III. Table of recipes:

No.	Buffers and solutions	Content	Comments
1	Phosphate-Buffered Saline (PBS)	Na_2HPO_4 8.1 mMKH_2 PO_4 1.5 mM NaCl 138 mM KCl 2.7 mM	Solution should be pH 7.4 if prepared properly.
2	BN-Lysis Buffer	*Base buffer* Bis-tris 20 mM ε-aminocaproic acid 500 mM NaCl 20 mM EDTA, pH 8.0 2 mM Glycerol 10% Adjust pH to 7.0 with HCl. Store at 4°C. *Detergent* Digitonin 0.5 to 1.0% or Brij 96 0.1 to 0.5% or Triton X-100 0.1 to 0.5% or Dodecylmaltoside 0.1 to 0.5% *Protease and phosphatase inhibitors* Aprotinin 10 µg/ mL Leupeptin 10 µg/ mL PMSF 1 mM Sodium fluoride 0.5 mM Sodium orthovanadate 0.5 mM	The appropriate detergent must be determined empirically and should be the same as that used in the other lysis buffer recipes. Digitonin must be added just before use from a 2% stock solution in dH_2O (store in 5-ml aliquots at -20°C). Protease and phophatase inhibitors should be added immediately before use. Upon addition of sodium orthovanadate, the buffer will become yellowish in color.
3	BN-Dialysis Buffer	*Base buffer* Bis-tris 20 mM ε-aminocaproic acid 500 mM NaCl 20 mM EDTA, pH 8.0 2 mM Glycerol 10% Adjust pH to 7.0 with HCl. Store at 4°C.	The appropriate detergent must be determined empirically and should be the same as that used in the other lysis buffers, but at the indicated lower concentra-tions. Detergent must be added to prevent aggregation at the stacking step of gel electrophoresis.

		Detergent Digitonin 0.3 to 0.5% or Triton X-100 0.1% or Brij 96 0.1% or Dodecylmaltoside 0.1% *Protease and phosphatase inhibitors* PMSF 1 mM Sodium orthovanadate 0.5 mM	Protease and phophatase inhibitors should be added immediately before use.
4	3x BN-Gel Buffer	Bis-tris 150 mM ε-aminocaproic acid 200 mM Adjust pH to 7.0 with HCl. Store at 4°C.	
5	4% Separating Gel	3x BN-Gel Buffer (recipe 4) 5.00 mL Acrylamide/Bisacrylamide 1.50 mL dH_2O 8.50 mL APS, 10% in dH_2O 54 µL TEMED 5.4 µL	Add APS and TEMED immediately before pouring gel, as these reagents promote polymerization. This recipe is sufficient to cast a 30-ml gel. Adjust volumes for the number and size of the gels being poured.
6	15% Separating Gel	3x BN-Gel Buffer (recipe 4) 5.00 mL Acrylamide/Bisacrylamide 5.63 mL Glycerol 70% 4.38 mL APS, 10% in dH_2O 42 µL TEMED 4.2 µL	Add APS and TEMED immediately before pouring gel, as these reagents promote polymerization. This recipe is sufficient to cast a 30-ml gel. Adjust volumes for the number and size of the gels being poured. The concentration of acrylamide-bisacrylamide may also be varied as necessary from 10 to 18%.
7	3.2% Stacking Gel	3x BN-gel Buffer (recipe 4) 3.00 mL Acrylamide/Bisacrylamide 0.72 mL dH_2O 5.28 mL APS, 10% in dH_2O 120 µL TEMED 12 µL	Add APS and TEMED immediately before pouring gel, as these reagents promote polymerization. This recipe is sufficient to cast a 30-ml gel. Adjust volumes for the number and size of the gels being poured.
8	Cathode Buffer	Bis-tris 15 mM Tricine 50 mM Coomassie blue G250 0.02% Prepare 1 liter as a 10x stock, adjust pH to 7.0 with HCl, and store at 4°C. Dilute 1:10 with dH_2O before use.	Do not substitute other types of Coomassie dye such as Coomassie blue R250 or colloidal Coomassie blues.
9	Anode Buffer	Bis-tris 50 mM Prepare 1 liter as a 10x stock, adjust pH to 7.0 with HCl, and store at 4°C. Dilute 1:10 with dH_2O before use.	
10	Marker Mix	Aldolase (158 kD) 10 mg/ mL Catalase (232 kD) 10 mg/ mL Ferritin (440 and 880 kD) 10 mg/ mL Thyroglobulin (670 kD) 10 mg/ mL BSA (66 and 132 kD) 10 mg/ mL Bis-tris 20 mM NaCl 20 mM Glycerol 10% Adjust pH to 7.0 with HCl. Store at 4°C.	Molecular weight markers are also commercially available from several sources, including Invitrogen or Pharmacia.
11	SDS Sample Buffer	Tris 12.5 mM SDS 4% Glycerol 20% Bromophenol blue 0.02%	SDS as a powder and β-mercaptoethanol are toxic. Therefore, use gloves and work under a hood.

		Adjust pH to 6.8. To reduce disulfide bonds, add 9 mL β-mercaptoethanol.	
12	4x lower buffer	Tris 1.5 M SDS 0.4% Adjust pH to 8.8.	SDS as a powder is toxic. Therefore, use gloves and work under a hood.
13	4x upper buffer	Tris 0.5 M SDS 0.4% Adjust pH to 6.8.	SDS as a powder is toxic. Therefore, use gloves and work under a hood.
14	10% Separating Gel	Acrylamide (30%) 2.0 mL 4x lower buffer 1.5 mL dH_2O 2.454 mL APS, 10% in dH_2O 40 µL TEMED 6 µL	Add APS and TEMED immediately before pouring gel, as these reagents promote polymerization. This recipe is sufficient to cast a 30-ml gel. Adjust volumes for the number and size of the gels being poured.
15	4.8% Stacking Gel	Acylamide (30%) 320 µL 4x upper buffer 500 µL dH_2O 1.16 mL APS, 10% in dH_2O 20 µL TEMED 2 µL	Add APS and TEMED immediately before pouring gel, as these reagents promote polymerization. This recipe is sufficient to cast a 30-ml gel. Adjust volumes for the number and size of the gels being poured.
16	Semidry Transfer Buffer	Tris 48 mM Glycine 39 mM Methanol 20% SDS 0.1% Adjust volume to 1 liter with dH_2O. Store at room temperature.	SDS as a powder and methanol are toxic. Therefore, use gloves and work under a hood.

Discussion

In this study, we describe the analysis of MPCs by BN-PAGE. A 2D approach is used to first separate MPCs under native conditions, and then to further subdivide them into their individual components by a second dimension SDS-PAGE.

Samples are prepared from cell lysates. For the solubilization of many MPCs, an appropriate detergent is needed, which preserves the structure of the protein complexes. Here, we use 0.1% Triton X-100. However, the optimal detergent and its suitable concentration have to be determined empirically for every MPC. In case of Triton X-100, for example, it has been reported that low detergent concentrations allow the identification of a dimeric form of the F_1F_0-ATPase complex (Arnold, Pfeiffer *et al.* 1998). Higher Triton X-100 concentrations, however, lead to the dissociation of the dimer and to a corresponding increase of the monomeric F_1F_0-ATPase complex. This is in line with one of our former studies, were we show that the multivalent T-cell receptor complex (TCR) is preserved when extracted with low concentrations of Brij 96, whereas the usage of higher concentration or of another detergent called digitonin results in the extraction of monomeric TCR (Schamel, Arechaga *et al.* 2005). Commonly used detergents that can be tested include digitonin (0.5 to 1%), Triton X-100 (0.1 to 0.5%), Brij 96 (0.1 to 0.5%), or dodecylmaltoside (0.1 to 0.5%). These reagents are nonionic detergents, which tend to be best for MPC stability. Be aware that contact with SDS and other strong detergents should be avoided (Camacho-Carvajal, Wollscheid *et al.* 2004).

Dialysis of the lysates is required to achieve MPC separation in a BN-gel (Camacho-Carvajal, Wollscheid *et al.* 2004); (Heiss, Junkes *et al.* 2005). It seems that the adjustment of salt concentration or the removal of low molecular weight impurities is crucial for high resolution. It is noteworthy that also membrane preparations and MPCs, which have been immunopurified and later on eluted from the antibody, are suitable for BN-PAGE (Swamy, Siegers *et al.* 2006). In both cases, the samples do not have to be dialyzed for BN-PAGE separation, if membrane lysis or elution is carried out in BN-lysis buffer.

For protein separation by BN-PAGE, the dye Coomassie blue is needed, which binds unspecifically to proteins and covers them with negative charges. Thereby, Coomassie blue enables the electrophoretic mobility of proteins towards the cathode at neutral pH (Schägger and von Jagow 1991); (Schägger, Cramer *et al.* 1994). Furthermore, Coomassie blue prevents protein aggregation in the stacking gel during electrophoresis. For BN-PAGE, Coomassie G250 has to be used instead of Coomassie blue R250 or colloidal Coomassie blues.

Before running a BN-gel, it is necessary to ensure that the percentage of the gel fits to the expected size of the MPC of interest. Precast BN-gels with different gradients and suitable buffers are commercially available from Invitrogen (NativePAGE Novex Bis-Tris Gel System). But BN-gels can also be prepared using a gradient mixer together with a persistaltic pump. To guarantee an intact gradient, the liquid should flow constantly during pouring and bubbles should be avoided. We recommend the loading of different sample dilutions onto the gel because overloading can lead to protein precipitation during the electrophoresis process. In addition, BN-gels should be run at 4°C to prevent protein degradation and to keep the MPCs intact.

After BN-PAGE, visualization of MPCs can be achieved by Coomassie brilliant blue staining, silver staining or immunoblotting. Protein bands visualized by Coomassie or silver staining are suitable for further analysis by mass spectrometry (Camacho-Carvajal, Wollscheid *et al.* 2004). In case of immunoblotting, the optimal transfer conditions for the MPCs of interest have to be determined empirically. Be aware that Coomassie

blue is also transferred during blotting of a BN-gel. Therefore, the gel will be colourless after the successful transfer, whereas the membrane will exert a blue colour. Further, it is important to mention that not every primary antibody, which works for detection after SDS-PAGE, is applicable to immunoblotting upon BN-PAGE. It can happen that antibodies do not recognize the MPC of interest because their epitope is hidden in the native conformation of the proteins. To overcome this problem, it is possible to denature the proteins within the BN-gel prior to the transfer by boiling the gel shortly in 1x SDS sample buffer.

In our example, we did not subject the BN-gel directly to detection of protein bands. Instead, we further divided the BN-PAGE-separated lysate by a second dimension SDS-PAGE. In the second dimension SDS-gel, monomeric proteins migrate within a hyperbolic diagonal due to the gradient gel in the first and the linear gel in the second dimension (Camacho-Carvajal, Wollscheid *et al.* 2004). This allows the easy identification of MPCs, since they are localized below this hyperbolic diagonal. Subcomponents of one distinct MPC are separated in a vertical line in the second dimension SDS-PAGE. Components that are constituents of several dinstinct MPCs can be identified on a horizontal line according to the size of the MPC. However, it has to be considered that several protein spots appearing in one vertical line could also be part of separate complexes that migrate at the same position in BN-PAGE. The final proof that they are present in the same MPC can be obtained by an antibody-based gel shift assay. In this assay, cellular lysate is incubated with an antibody against a protein represented by one of the identified spots prior to BN-PAGE. This results in a shift of all MPCs that contain this protein towards a higher molecular mass in the first dimension. Other proteins that are also a part of these MPCs will undergo this complex-specific shift and are therefore easy to identify in the second dimension SDS-gel.

Not only the composition of MPCs can be analyzed by BN-PAGE but also the determination of their stoichiometry is possible (Schamel and Reth 2000); (Schamel 2001), (Swamy, Minguet *et al.* 2007). For this purpose, a NAMOS assay (native antibody-based mobility-shift assay) can be performed. As in the antibody-based gel shift assay, the cellular lysates are incubated with monoclonal subunit-specific antibodies. This leads to the induction of electrophoretic immunoshifts in the BN-gels, which allow the inference from the extent of the shift on the stoichiometry of MPCs

In conlusion, BN-PAGE is suitable for the identification of MPCs and the determination of their size, composition, as well as relative abundance. Performed as a NAMOS assay, it also offers the possibility to determine the stoichiometry of a certain MPC. Given its general applicability, this technique is a very useful tool for the characterization of MPCs (Dekker, Müller *et al.* 1996); (Wittig and Schagger 2008); (Wagner, Rehling *et al.* 2009); (Wittig and Schägger 2009).

Disclosures

No conflicts of interest declared.

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