

Visualization of proteins in SDS-PAGE nUView system.

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Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) is likely the most prevalent and long standing protein chemistry tools used in laboratories world-wide. Although many buffer recipe derivatives and acrylamide to bis acrylamide mixtures are in use, the primary principle has not changed since its introduction in the now highly cited contribution of Laemmli [1]. The method follows the general idea of denaturing of a protein mixture in the presence of SDS followed by electrophoretic migration through the polyacrylamide matrix, which leads to separation of the proteins based on a charge to mass ratio and a simple Ferguson plot allows for one to extract molecular weight or Stokes radius information on unknown proteins from selection of standards.

One of the most popular uses of the SDS-PAGE technique is following protein purifications. However, the excitement of a protein preparation experiment is sometimes lost in the wait in the staining-destaining step of ones SDS-PAGE in order to illuminate the presence of the protein bands. How often does one spend time around the light box waiting to see the protein bands? This frustration has been recognized by researchers for decades, which has inspired many alternative protein visualization technologies to replace the standard Coomassie Brilliant Blue (CBB) and the laborious silver staining approaches. However, most of these methods utilize either expensive dyes or specialized visualization systems.

The nUView technology reduces the wait required for protein band visualization to minutes. The nUView technology is based on an excited-state reaction of the indole ring of the tryptophan amino acid with a halogen derivative of ethanol, trichlorethanol (TCE) [2,3]. The sensitivity being dependent on the content of tryptophan residues in the protein. The addition of TCE into the gel during polymerization has been shown to have no affect on the migration properties of the gel [3,4]. With the TCE already incorporated into the gel there is no need for post run soaking or fixing. To eliminate the bands it only requires that the gel is placed on a typical UV transilluminator routinely used in molecular biology laboratories for the visualization of DNA by DNA interchelating dyes (e.g., ethidium).

The reaction between the tryptophan and TCE occurs under the illumination by UV light. The result is the proteins become fluorescent in the visible range of the spectrum under the excitation of the UV light box. The time of exposure for best results, however, is very dependent on the transilluminator available, the filters in

place and the camera system used to document the fluorescence. One can see in Figure 1 two different exposure times leading to increased fluorescence intensity from the bands when allowed to react longer. Very long exposure times however will result in the bands dimming due to the chemical transition of the tryptophan residues and loss of UV visualisation. Therefore, one needs to optimize the conditions for any given gel documentation system and UV transilluminator.

Optimization protocol.

Use a UV trans-illuminator light box with light bulbs that provide a wavelength as in the range 260-320nm. A wavelength of 300nm works best (faster) but the others work fine. To determine the optimal illumination time, run a gel with molecular weight standards at different concentrations of loading (suggest 0.25x, 0.5x, 1x, 2x your normal protein load concentration). Remove gel from electrophoresis apparatus and place directly onto the transilluminator. Turn the UV light on and expose gel for 2 min. If possible with your system monitor the gel development in real time and optimize your camera parameters (F stop, focus, etc). At the 2 min mark take a picture, and then leaving the camera settings fixed, take pictures at 2 min intervals up to 10-15 min (at the longer time you may notice a decrease in intensity). Compare the pictures and select the optimal detection time. This exposure condition becomes the illumination time for your particular transilluminator and camera settings. There is very little variability over the lifetime of the UV bulbs in the transilluminator, but it is useful to recalibrate upon bulb replacement.

Applications.

Apart from following protein purification, SDS-PAGE is used for other protein biochemistry applications including: autoradiography, Western blotting, and proteomics approaches by 2D-PAGE. The nUView method provides excellent results to evaluate a gel prior to autoradiography aimed at examining ³⁵S-Methionine incorporated proteins (results not shown). The gel was 'developed' to verify the gel ran well and to get a full view of all proteins in the extract. The gel was then treated with 10%acetic acid, 10% isopropanol to fix for 30 min and then was placed in contact with the negative film and exposed for radiation evaluation. Similarly, the nUView also worked well for evaluating extracts prior to development for phosphorylation reactions.

Figure 2 shows the success in using the nUView for examining a gel prior to Western blotting. The presence of the TCE had no affect on the ability of the proteins to transfer to the membrane (PVDF is shown and transfer to nitrocellulose is also successful). Thus one has a fast and efficient way to document the gel prior to transfer. After transfer the proteins on the membrane are also amenable to Ponceau S staining to verify transfer. Furthermore the original gel can be placed back onto the transilluminator and documented to evaluate the degree of protein transfer to the membrane.

An important application of consideration for the nUView method is the use of these gels as the second dimension for 2D-PAGE analysis of proteins for proteomic questions. Figure 3 shows an example of a 2D-PAGE experiment from Bacterial soluble cell extract. An outcome of this experiment was the somewhat different detectability of proteins from the standard CBB to the nUView. This is seen in panels C through F where some spots are more easily identified by nUView. Several spots of interest were successfully excised and compared by mass spectroscopy. An issue with CBB staining is that for most MS methods the stain must be extracted before use. The bands visualized with the nUView method could be digested and applied to MALDI-TOF plates directly [4].

Concluding remarks

Overall there is no difference in the migration properties of proteins or in down stream processing of proteins between traditional PAGE and nUView. Many different gel documentation systems have been utilized with the nUView method using protein extracts from plants, animals and bacteria and visualization has always been achieved in less than 10 min after placement on the gel illuminator. Additionally, it has been routinely noted that proteins could be visualized with this approach that were not well stained by CBB staining. An exciting aspect of this technology is that one can easily stain the gel post nUView visualization, by any other protein visualization method. Thus the nUView method provides a quick look, which then can be followed by standard imagings. Furthermore, this combination provides a more comprehensive picture of all proteins in the sample as not all proteins are detectible by a single visualization method. The nUView method provides convenience and speed with compatibility to protein biochemistry approaches.

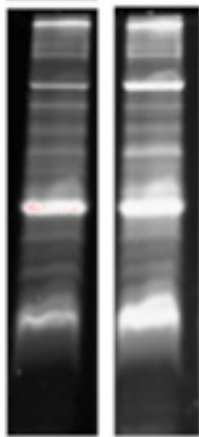


Figure 1. Fluorescence visualization of a membrane protein extract from a purification preparation from *Escherichia coli*.

Samples were separated with a 10% SDS-PAGE and exposed on a UVP transilluminator. The first lane is a 3 min irradiation on the UV box and the second lane is 9 min. The lower band is the protein EmrE, which stains very poorly by Coomassie Brilliant Blue, but is easily visualized by the nUView method.

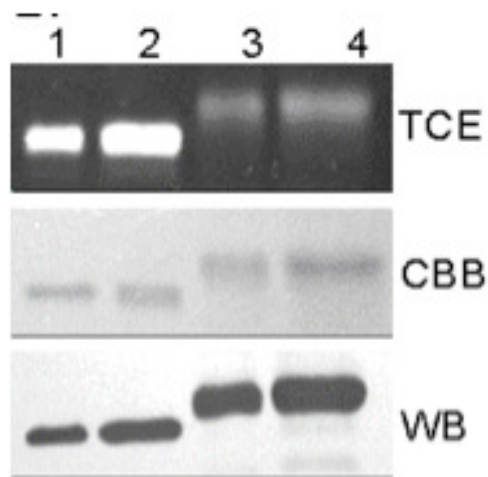


Figure 2. Evaluation of nUView technique for visualization of proteins in the SDS-PAGE gel prior to Western blotting.

SDS-PAGE of DmsD:His₆, 0.25 and 0.5 μ g (lanes 1 and 2) and TehB:His₆ (lanes 3 and 4). The top gel was visualized with the TCE in-gel method (TCE panel) and then this gel was blotted to PCDF membranes using standard Western blot protocols and probed with antiHis₆-horseradish peroxidase conjugate and developed calorimetrically (WB panel). A duplicate set of lanes were run on the same gel which was then separated and stained under standard conditions by Coomassie Brilliant Blue (CBB panel).

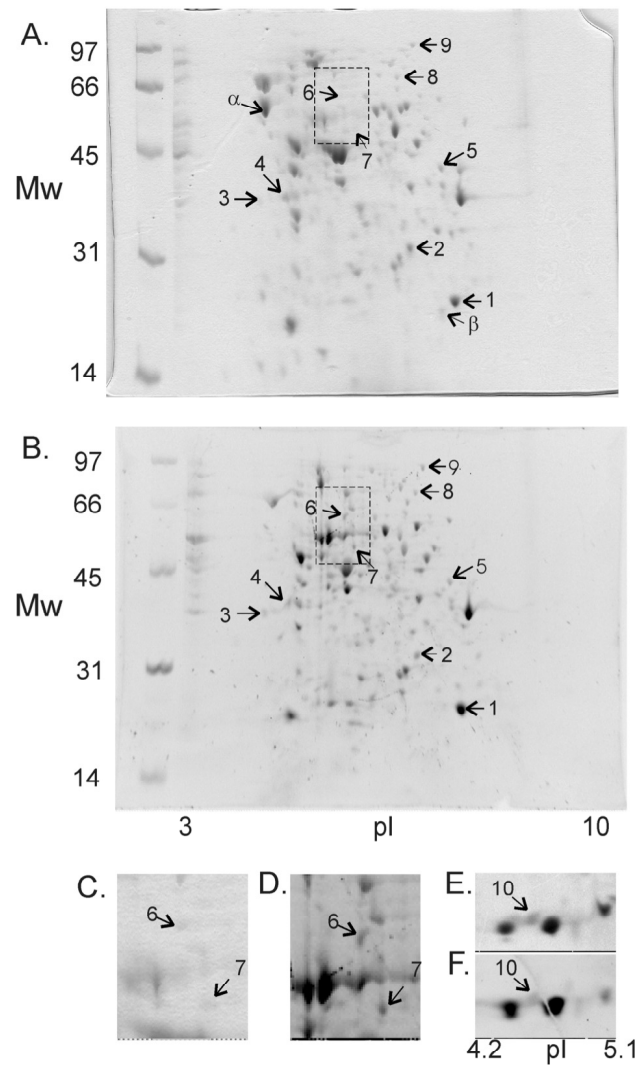


Figure 3. Two-dimensional electrophoresis (2-DE) gel of an *Escherichia coli* extract.

Panel A visualized with Coomassie Brilliant Blue BB staining.

Panel B visualized by the nUView technology with the image contrast inverted for direct comparison.

The first dimension is a pH 3-10 non-linear gradient, while the second is a 12% SDS-PAGE gel. The indicated area on the CBB and NuView gel is shown in panel C and D, respectively. Panel E and F show a region from a pH 4-7 15% SDS-PAGE gel for nUView and CBB, respectively. Spots indicated were cut out and the proteins successfully analyzed by MALDI-TOF mass spectrometry.

References.

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