TECHNICAL REPORT

Gel Electrophoresis and Transfer Combination Using Conductive Polymer Cassettes

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1. Introduction

Woodham Biotechnology Holdings commissioned the manufacture of plates with conductive polymers for a precast gel/membrane combination cassette as described in the previous report. This was accomplished with polyaniline (PANI) dispersed in polystyrene (PS) as the conductive matrix. To increase structural integrity for downstream applications, the PANI matrix was placed in an acrylate polymer (acrylic) frame creating a PANI-acrylic hybrid cassette. In this section, we wanted to evaluate the ability of the conductive PANI hybrid cassettes to be used in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transfer to a polyvinylidene fluoride (PVDF) membrane. The combination of these two steps is known more commonly as a western blot, and the goal here was to do both steps in a single cassette unit.

2. Assembly of Polyacrylamide Gel/PVDF Membrane/PANI-acrylic hybrid cassettes

2.1 Overview

A polyacrylamide gel and membrane need to be housed in the same cassette in order to perform both gel electrophoresis and transfer within that cassette. Though different techniques were attempted, the following represents the general and most consistent gel cassette assembly protocol. The final polyacrylamide gel/polyvinylidene fluoride (PVDF) membrane/polyaniline (PANI)-acrylic hybrid cassette will hereafter be referred to as the Woodham PANI hybrid cassette.

2.2 Materials

1. Polyaniline (PANI)/acrylic hybrid cassette (see previous report)
2. Polyvinylidene fluoride (PVDF) membranes (0.2 µm pore-size were used)
3. Tris-HCl buffer (4X ProtoGel Resolving Buffer was used: 1.5 M Tris-HCl, 0.4% sodium dodecyl sulfate (SDS), pH 8.8; National Diagnostics)
4. Acrylamide solution (Ultra Pure ProtoGel was used: 30% w/v acrylamide:0.8% w/v bis-acrylamide stock solution; protein and sequencing electrophoresis grade; National Diagnostics)
5. 10% ammonium persulfate solution (5 g APS in 50 mL H_2O)
6. Tetramethylethylenediamine (TEMED) – CAS number: 110-18-9
7. Methanol – CAS number: 67-56-1
8. Vinyl electrical tape (3M)
9. Deionized H_2O

2.3 Standard Operating Procedure: Assembly of Woodham PANI hybrid cassettes

1. Cut PVDF membrane (BioRad) to 65 mm x 60 mm.
3. Make up 1X gel resolving buffer by diluting 4X ProtoGel Resolving Buffer in H_2O.
4. Presoak PVDF membrane in 50 mL methanol for approximately 10 minutes.
5. Equilibrate PVDF membrane in 50 mL 1X resolving gel buffer for approximately 10 minutes.
6. Premix all of the resolving gel components (resolving gel buffer, acrylamide solution, and H_2O) except for 10% APS and TEMED; typically 15 mL total volume.
7. Place tape over the outer chamber opening on the rear plate.
8. Place the front and rear plates together with a silicone spacer (Invitrogen SureCast system) on the right, left, and lower edges of the plates.
9. Tape the bottom of the cassette together with vinyl electrical tape. The cassette should then open and close at the hinged bottom.

10. Open the cassette and lay the presoaked and equilibrated PVDF membrane on the PANI window of the rear plate (Figure 2-1A).
11. Close the cassette and ensure the PANI windows are aligned.
12. Clip the edges of the cassette together with heavy-duty metal binder clips along the silicon gasket (see Figure 2-1B). Keep the gel at a minimal upward angle to ensure the membrane stays positioned appropriately on the rear plate.
13. Add the appropriate volume of 10% APS (use stock at 1:100) and TEMED (use at 1:1000) to the premixed resolving gel solution (i.e. 150 µL and 15 µL, respectively for a 15 mL resolving gel solution).
14. Fill the cassette with resolving gel and insert an appropriate gel comb. Tap the cassette gently to remove air bubbles. Try to avoid moving the gel to a vertical position to remove bubbles as this can cause the membrane to move within the cassette. A stacking gel can also be added if desired (excluded here for ease). For inclusion of a stacking gel:
    A. Pour resolving gel to the top of the PANI window.
    B. Allow resolving gel to set for 15 minutes.
    C. Prepare stacking gel according to manufacture instructions.
    D. Add stacking gel to the cassette and insert appropriate gel comb.
    E. Allow stacking gel to set for 15 minutes.
15. Allow gel to set for 30 minutes.

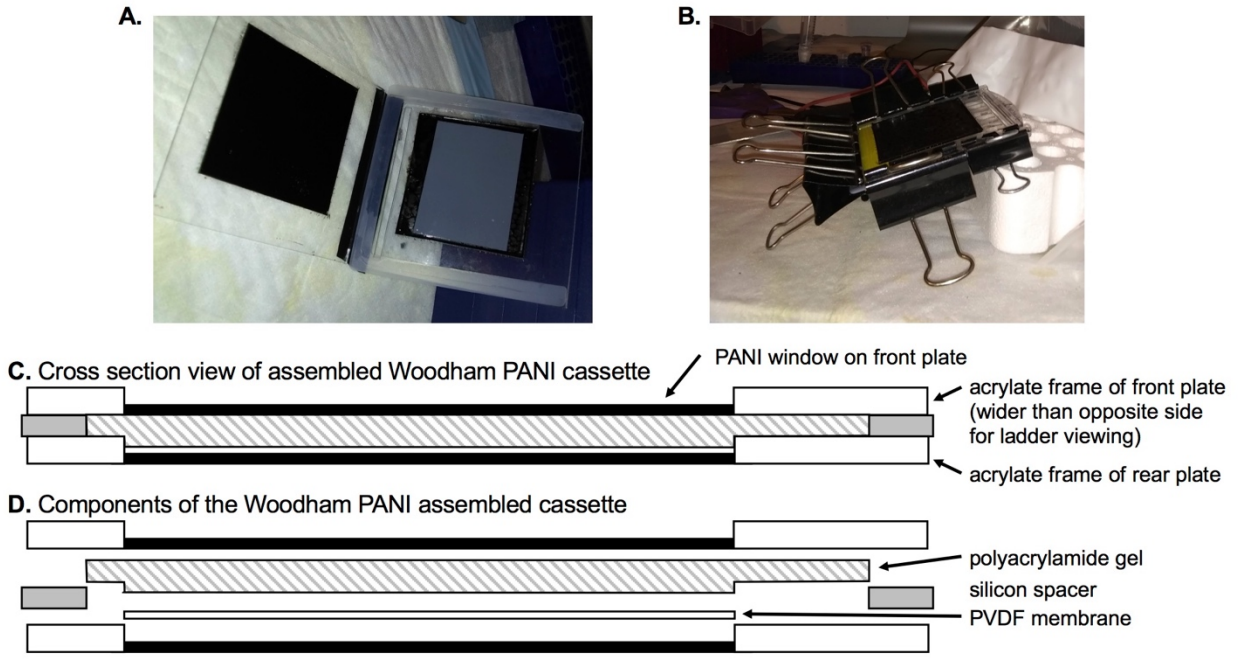

Figure 2-1: Woodham PANI hybrid cassette assemble. A: Cassette with vinyl tape hinge and presoaked membrane in place on the rear plate. B: Clipped cassette with gel poured at a low upward angle to keep the membrane in place. C: Cross-sectional view of the assembled Woodham PANI cassette as if it was cut through the horizontal plane when upright and then viewed from the top. D: The different components have been separated for easier identification.

2.4 Discussion

PVDF was chosen for the test runs because it tends to be more physically durable than nitrocellulose. Additionally, once PVDF is activated by methanol and then equilibrated in an aqueous buffer, methanol is not required for the transfer step whereas methanol is required during the transfer to nitrocellulose membranes. As we desired transfer to occur directly after electrophoresis, in the absence of any additional buffer, the ability to transfer without additional methanol was viewed favorably. The membranes were poured in a fairly horizontal manner, which did create some problems with regards to bubbles not floating to the surface. This could potentially be alleviated through the application of a vacuum after the pour is complete. However, for the purposes of these experiments it was essential to keep the membranes in the appropriate position until the gels were set.

3. Polyacrylamide Gel Electrophoresis in Woodham PANI Hybrid Cassettes

3.1 Overview

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is a standard analytical method to separate proteins, primarily based on their molecular masses, in an electric field. We next evaluated the ability of proteins to effectively separate in a gel housed in a cassette containing conductive polymers i.e. we wanted to test the ability of the Woodham PANI hybrid cassettes to perform SDS-PAGE.

3.2 Materials

1. Woodham PANI hybrid cassette with polyacrylamide gel and PVDF membrane (see assembly above)
2. XCell SureLock Mini-Cell Electrophoresis System (tank, lock, gel dam, etc.; Invitrogen)
3. Tris/glycine/SDS running buffer (Ultra Pure 10X Tris/Glycine/SDS was used: 0.25 M Tris, 1.92 M glycine, 1% SDS; National Diagnostics)
4. Glass plate (or other appropriate spacer)
5. Prestained protein ladder (Precision Plus Protein Dual Color Standards were used; BioRad)
6. Vinyl electrical tape (3M)
7. Deionized H_2O

3.3 Standard Operating Procedure: SDS-PAGE in Woodham PANI hybrid cassettes

1. Remove the binder clips from the filled Woodham PANI hybrid cassette.
2. Remove the tape from the outer chamber opening on the rear plate.
3. Dry the edges of the cassette and use vinyl electrical tape to line the edges (right and left). This is not necessary for electrophoresis but helps during transfer steps described later.
4. Insert the cassette into XCell SureLock Mini-Cell Electrophoresis System.
    A. This is performed in exactly the same manner as standard pre-poured gels (i.e. NuPAGE or Novex gels).
    B. The Woodham PANI hybrid cassette is approximately 1 cm narrower than a standard pre-poured gel, so take care that the right and left edges are lined up with the gasket.
    C. The Woodham PANI hybrid cassette is also thinner than standard pre-poured gels so a glass plate (or other appropriate spacer) can be placed on the side of the gel

dam before locking to ensure a tight seal is made between the inner and outer chambers.

5. Fill the inner chamber of the tank with 1X running buffer (make 1 L from 10X stock). The chamber should be filled so that the level is above the top of the front plate.
6. Remove the comb and gently flush the wells with running buffer. The tank should be filled to a level such that the wells are in contact with the inner chamber fluid.
7. Load protein samples into wells. It is recommended that a prestained protein ladder is used in the well above the wide clear segment of the outer frame to allow tracking of loaded proteins as they run through the gel if using the non-transparent 10% Woodham PANI hybrid cassette (10% PANI) as opposed to the transparent 1% Woodham PANI hybrid cassette through which the ladder can be monitored during the run (Figure 3-1).
8. Fill the outer chamber to a level just below the top of the rear plate.
9. Run the gel at 150 V for 75 minutes (for 10% acrylamide). This requires a standard power supply. Adjust V and time appropriately for different acrylamide concentrations.

3.4 Discussion

SDS-PAGE was successful in all Woodham PANI hybrid cassettes tested (see examples in Figure 3-1). Prestained protein ladders were used exclusively as the protein samples for these experiments for easy visualization of protein separation and electrophoresis efficiency. In particular instances, 1X SDS loading dye (e.g. 0.02% bromophenol blue, 30% glycerol, 10% SDS, 250 mM Tris-Hcl, pH 6.8) was used in lieu of a protein sample to monitor the progression of the dye front (usually for 1% Woodham PANI hybrid cassettes). The ladders ran as expected, and the proteins did not appear to bind to the PVDF membranes during electrophoresis. Moreover, tighter banding could be achieved with the inclusion of a stacking gel. Though it has been suggested that SDS-PAGE cannot be performed between conductive plates, we did not see such limitations. This is likely due to the relatively low conductivity of the PANI in the hybrid cassette compared to the gel within the cassette. This allows for efficient separation of the proteins through the gel despite the surrounding conductive polymers during the electrophoresis step. We also examined the ability of visualizing electrophoresis through the 1% Woodham PANI hybrid cassette (1% PANI in the PANI-PS matrix). Even with the membrane in place, it was possible to visualize the separation of the protein ladder and dye front during electrophoresis (Figure 3-2).

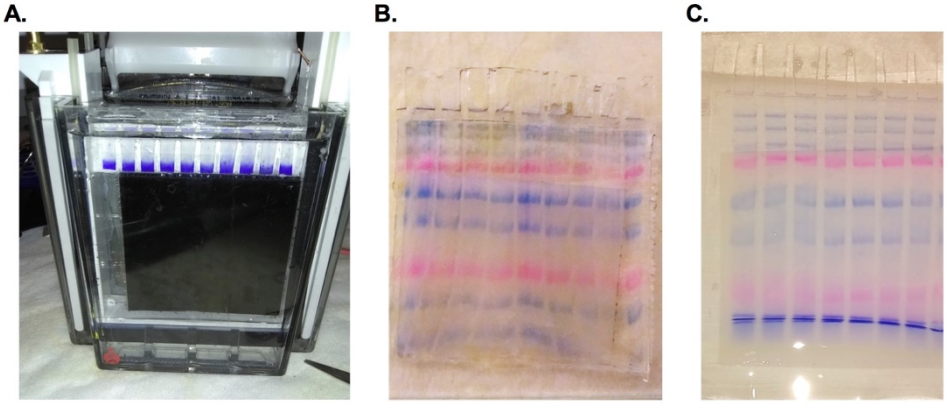

Figure 3-1: Electrophoresis in Woodham PANI hybrid cassettes. A: 10% Woodham PANI hybrid cassette with wells loaded in the XCell SureLock Mini-Cell Electrophoresis System (Invitrogen). B: Gel electrophoresis results from a 15% polyacrylamide gel in a 10% Woodham PANI hybrid cassette (run at 200 V for 60 minutes). C: Gel electrophoresis results from a 10% polyacrylamide gel in a 1% Woodham PANI hybrid cassette (run at 150 V for 75 minutes). Note that the membranes are visible behind the gels in both B and C.

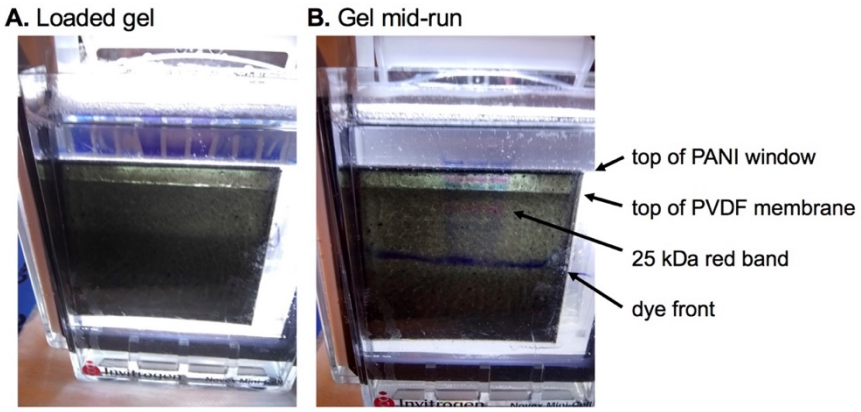

Figure 3-2: Visualization of electrophoresis in the 1% Woodham PANI hybrid cassette. A: The loaded gel has been back-lit to show light passing through both the front and rear plates as well as the PVDF membrane between them. B: Midway through the electrophoretic run, the different bands of the prestained protein ladder (note the red 25 kDa band) and the dye front are clearly visible through the 1% Woodham PANI hybrid cassette.

4. Protein Transfer to PVDF membranes in Woodham PANI Hybrid Cassettes

4.1 Overview

Lastly, we wanted to determine if proteins could be transferred to PVDF membranes following electrophoresis in the Woodham PANI hybrid cassettes by applying an electric field through the plates (perpendicular to the gel) without opening the cassettes. To accomplish this, we first chose a system that would clearly demonstrate that current was traveling through the conductive PANI windows and hence perpendicularly through the gel (Section 4-2). Next, we used a system that is akin to the final embodiment imagined, in which transfer occurs in a tank containing a liquid buffer.

4.2 Protein Transfer to PVDF membranes in Woodham PANI Hybrid Cassettes using solid electrodes

A solid electrode system was used here to demonstrate that when the anode and cathode are applied directly to the PANI windows on the front and rear plates, that an electric field is created through the PANI windows as demonstrated by current traveling from one electrode to the other. This is important in establishing one of the key elements of the system – the ability for electricity to travel perpendicular to the gel within the same cassette that was used for successful electrophoresis.

4.2.1 Materials

1. Woodham PANI hybrid cassette with polyacrylamide gel and PVDF membrane (see assembly above)
2. Electrodes (iBlot transfer electrode stacks were used; Invitrogen)
3. Glass plates (Invitrogen SureCast plates were used)
4. Copper wire (20 gauge)
5. Rubber bands
6. Vinyl electrical tape (3M)
7. Ice pack

4.2.2 Standard Operating Procedure: Protein transfer using solid electrodes

1. Perform electrophoresis as described in Section 3.3.
2. Remove from tank and dry.
3. Cut two iBlot electrodes (other comparable electrodes can be used) to fit over the front and rear PANI windows. These are usually cut a few mm less than the dimensions of the PANI window (e.g. 60 mm by 55 mm).
4. Place cut electrodes over the front and rear PANI windows.
5. Use copper wire to connect electrodes to standard power source leads.
   A. Cut two 12” lengths of copper wire.
   B. Make a small coil at the end of each piece of copper wire length (coil approx. 8” of the 12” to contact the electrodes (Figure 4-1)
   C. Bend the non-coiled end (approx. 1”) back over itself to insert into standard power source leads.
   D. Wrap vinyl electrical tape around the remaining 3” to avoid exposed wires when transferring.
6. Place the copper wire coils on the outside of each electrode (i.e. one on the outside of the electrode on each of the front and rear plates).
7. Place glass plates (approx. 10 cm by 10 cm) on the outside of each Woodham PANI plate and rubber band together (Figure 4-1). As glass is an insulator, this will ensure that the electrical field is contained safely.
8. Place entire transfer unit (Figure 4-1) on an ice pack to keep the unit cold during transfer.
9. Connect the copper wire to the power source leads. Ensure that the wire coming from the front plate is connected to the black lead (negative) and the wire coming from the rear plate is connected to the red lead (positive).
10. Use appropriate high voltage power supply (minimum 1000 V).
11. Run at 1000 V for 6 hours for 10% Woodham PANI hybrid cassette.
12. Turn off power supply.
13. Disassemble Woodham PANI hybrid cassette.
14. Remove gel from membrane.

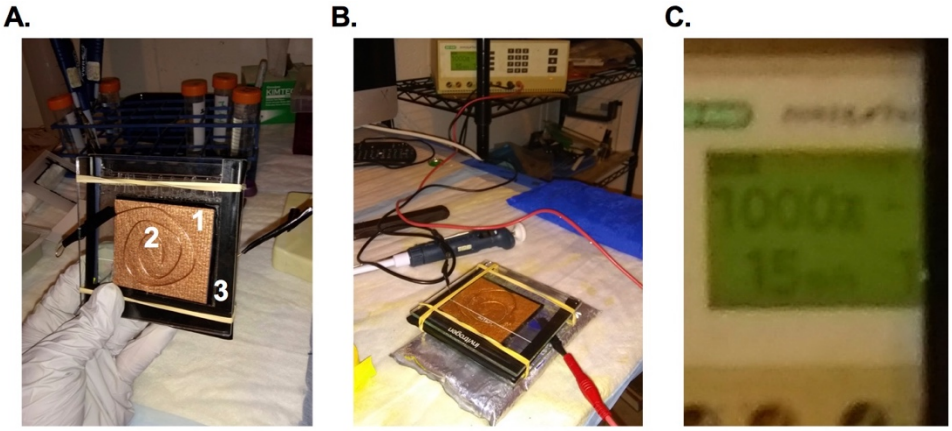

Figure 4-1. Assembly and use of solid electrode transfer unit. A: Electrodes are cut to fit over the front and rear PANI windows (1), copper wire coils are placed over the electrodes to connect to power source leads (2), and glass plates are placed on the outside to insulate the entire unit (3). B: Image during the transfer within a Woodham PANI hybrid cassette. C: Enlargement showing a current of 15 mA at 1000 V approx. 20 min into the transfer.

4.3 Protein transfer to PVDF membranes in Woodham PANI hybrid cassettes in a liquid tank

Next, we wanted to perform the transfer step in a system that would be easier to put into practice, and closer to how it was originally conceived. Thus, we devised a way to use the same XCell SureLock Mini-Cell Electrophoresis System to perform the transfer step.

4.3.1 Materials

1. Woodham PANI hybrid cassette with polyacrylamide gel and PVDF membrane (see assembly above)
2. XCell SureLock Mini-Cell Electrophoresis System (Invitrogen)
3. Vinyl electrical tape (3M)
4. Dry ice (small pellets)

4.3.2 Standard Operating Procedure: Protein transfer in a liquid tank

1. Perform electrophoresis as described in section 3.3.
2. Remove Woodham PANI hybrid cassette from tank and dry.
3. Pour out the running buffer from the tank into a 1 L cylinder or jar and set aside.
4. Use vinyl electrical tape to reseal the outer chamber opening on the rear plate (Figure 4-2). Take care to wipe over the opening many times to ensure that any gel/liquid seeping out of the opening is cleaned off before applying the tape. This will ensure a good seal.
5. Place the Woodham PANI hybrid cassette back into the XCell SureLock Mini-Cell Electrophoresis System in the same manner as to perform electrophoresis. A glass plate or other spacer can be used on the side of the gel dam to ensure a tight seal (see section 3.3 step 4-C above)
6. Refill the inner and outer chambers of the tank to the top of the front and rear PANI windows. This is well below the top of either plate and will ensure that when the electrodes are attached that the current will go through the PANI windows rather than through the gel (Figure 4-2).
7. Attach to an appropriate power source and run at 1000 V for 6 hours for 10% Woodham PANI hybrid cassette or overnight for 1% Woodham PANI hybrid cassette.
8. Add dry ice as needed to the tank to keep the system cool.
9. Turn off power supply.
10. Disassemble Woodham PANI hybrid cassette.
11. Remove gel from membrane.

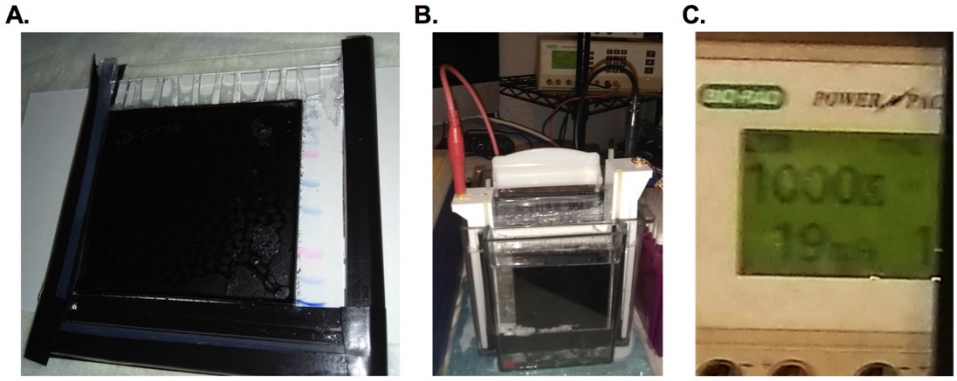

Figure 4-2: Set-up and transfer in a liquid tank. A: 10% Woodham PANI hybrid cassette after electrophoresis with the outer chamber opening resealed with vinyl electrical tape. B: Tank set up for transfer with inner and outer chamber fluid levels filled to the top of the front and rear PANI windows. C: From B, the current for transfer starts at approx. 20 mA (shown at 19 mA) at 1000 V for the 10% Woodham PANI hybrid cassettes.

4.4 Results and Discussion

Both transfer methods, with solid electrodes and in a liquid tank, allowed for current to travel through the PANI windows and thus through the gel in a direction perpendicular to the current created during electrophoresis. This is requisite for an integrated western blot cassette to work, as it allows proteins to migrate towards the membrane placed inside of the rear plate. All transfers were performed at 1000 V, which resulted in initial currents of approx. 20 mA for 10% Woodham PANI hybrid cassettes and 5 mA for 1% Woodham PANI hybrid cassettes. As in any constant voltage electrophoresis or transfer system, the amperage went down over time resulting in a final amperage of 2 mA for the 10% Woodham PANI hybrid cassettes after 6 hours and 1 mA for the 1% Woodham PANI hybrid cassettes, which were run overnight. While these are not ideal amperages for protein transfer, we were able to visualize transferred proteins for both the 1% and 10% Woodham PANI hybrid cassettes (Figure 4-3). It is also possible that the same results could have been achieved with shorter transfer times as the currents at the end of the transfers were not that substantial.

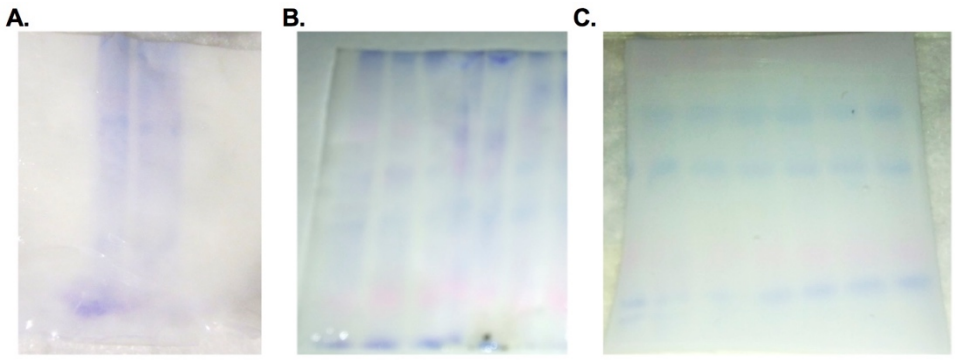

Figure 4-3. Transfer results in Woodham PANI hybrid cassettes. A: Transfer of proteins to a PVDF membrane in a 1% Woodham PANI hybrid cassette using the solid electrode method (1000 V overnight). B: Transfer of proteins to a PVDF membrane in a 10% Woodham PANI hybrid cassette using the solid electrode method (1000 V for 6 hours). C: Transfer of proteins to a PVDF membrane in a 10% Woodham PANI hybrid cassette in a liquid tank (1000 V for 6 hours).

5. Final Comments and Considerations

The liquid tank transfer method resulted in the most even protein deposition on to a membrane, though smaller proteins (bottom of membranes) transferred more efficiently than larger proteins (top of membranes). This is likely due to the ability of the liquid reservoirs to create a uniform electric field across the PANI windows. This may be in contrast to the electrode system, which has the possibility of uneven current travel across the PANI windows. However, it should be noted that the interface between the PANI window and acrylic frame is more vulnerable with the liquid system. As noted in the previous report from Karma Biotechnologies, the LiqPS (the major component in the PANI-PS matrix) dissolves the edge of the acrylate, thus fusing the two together. This results in more fluctuations in the thickness of the PANI window at these sites. These thickness variations can cause shorting events at high voltage. In the electrode system, the electrodes can be placed a couple mm away from these edges. In the liquid tank however, the buffer is in full contact with the edges, thus increasing the potential for adverse electrical shorting. This could likely be resolved by reinforcing the edges between the PANI and acrylate.

It was also found that the polyacrylamide gels adhered to the PVDF membranes when the gels were poured onto the prewet membranes in the cassettes. The gel could be removed by gently applying a gel knife to the membranes. This phenomenon was reduced with lower percentage gels and by keeping the systems cooler during transfer. It was found that a membrane that was presoaked in methanol, and then dried before adding the gel, resulted in minimal gel adherence, but also reduced transfer efficiency. It may also be possible to let the gel set in the cassette, then open the cassette and add the membrane, and finally reseal the cassette. It is predicted that this would work best when done with a vacuum sealer in order to remove any potential air or liquid introduced during that process. We also chose to work with PVDF for these experiments as explained above, but it is possible that other membranes may adhere less to the gels and hence improve results.

It is expected that protein transfer speed and efficiency would improve with increased amperage. One of the most obvious solutions is to use a system that can safely go beyond 1000 V while keeping the system cool. Another potential solution is to increase the salt concentrations in the resolving gel and running buffer, thus increasing available ions for improved transfer efficiency.

Protein electrophoresis and transfer was clearly demonstrated within the Woodham PANI hybrid cassettes. These results are the first of their kind. Though further optimization is required, this groundbreaking technology has the potential to make a significant impact on the western blot and protein analytic fields.