Protein-Mediated Analysis of MicroRNA with Capillary Electrophoresis

Honour’s Thesis
Winter 2010
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One of the most exciting fields in modern biochemistry is the study of small interfering RNA (siRNA) and micro RNA (miRNA) and the range of possible applications they have in science and medicine. Discovered in the 1990s, but not fully realized until the twenty first century, micro RNA are fragments of double stranded RNA with a low number of base pairs\(^1\) (~22 nucleotides). Formed by the degradation of double stranded RNA (dsRNA) mediated by DICER, an important regulatory protein\(^2\), these molecules have shown to possess strong post transcriptional control elements with high specificity, which they are able to achieve by associating with complementary messenger RNA (mRNA) through the RISC complex thereby forming double stranded RNA (dsRNA) which is cleaved catalytically by argonaute, an RNase. While these two classes of molecules are very similar siRNA tend to be very specific, binding to a single mRNA and having it be immediately degraded, while miRNA is more general, binding to large assortment of similar mRNA, inactivating a range of related processes but with less strength. In both systems however, this mechanism allows a small number of these small RNA molecules to eliminate a large amount of mRNA from the cytoplasm, reducing translation very effectively and, in sufficient quantities, creating gene knockout conditions, where the gene is inactive or does not produce protein, in organisms still possessing the initial gene\(^3\). Creating knockout conditions was historically a time consuming process involving genetic transformation, creation of transgenic embryos, with the possible requirement for selective breeding afterwards\(^4\). This means that the development of a method to perform this same task requiring only the injection of a few small dsRNA particles would greatly expedite the study of the effects of gene repression. Using siRNA also gives the advantages of making the gene knockout reversible, as well as applicable to post-embryonic organisms – opening up the possibility of therapeutic uses. While actual application of siRNA was not as simple as injection leading directly to knockout conditions, great advancements have been made with its use including the potential treatment of macular degeneration\(^5\), use in developing superior vaccines\(^6\), as well as possible use in combating cancer and the human immunodeficiency virus\(^7\).
Due to their incredible potential it has become essential to develop methods to detect and separate both specific and total siRNA and miRNA from solutions and organisms. These methods are diverse and include the use of northern blotting techniques, utilization of microarrays, processing using RT-PCR, as well as a number of other varied hybridization methods. Each of these methods have advantages and disadvantages depending on the goal and breadth of the experiment, while some are used primarily for their simplicity and historical precedent. One major aspect of any method of separation, however, is whether it requires the isolation of total RNA prior to any steps involving the miRNA separation and analysis. This, while resulting in a cleaner analysis, also can result in the destabilization and degradation of the miRNA, which greatly reduces effectiveness in a study attempting to quantify or compare the amount of miRNA, either specifically or in general. It is also possible to separate total miRNA chemically, which leads again to very clean analysis but with even greater risk of loss of analyte. A number of different systems have been devised to separate total miRNA, but most lead back to separation by the phenol-chloroform extraction followed by filtration, as column systems often do not allow for the recovery of the small RNAs. This extraction works by separating the DNA and
RNA into the organic and aqueous phases respectively, as RNA is of greater polarity than DNA. RNA is often complexed with protein, and is degraded by the ubiquitous RNases, so the addition of a protein denaturing agent such as guanidinium thiocyanate is also required in preparing a stable RNA aqueous phase. After this phase is removed, the total RNA can be precipitated out by the addition of a common alcohol, such as ethanol or isopropanol. To isolate the miRNA specifically a solution of total RNA is filtered through glass fibers, using a low concentration of alcohol to immobilize the large RNAs and a higher concentration to immobilize the smaller RNAs, isolating them after the second filtration. There are many kits that exist with the ability to carry out this transformation, but those using the phenol-chloroform guanidinium thiocyanate extraction and storing the resulting material at -80ºC yields the most stable and complete miRNA\textsuperscript{11}.

The earliest method capable of analyzing a specific miRNA was through application of the northern blot. This blotting procedure involves the separation of RNA molecules based on size using gel electrophoresis, with larger RNA molecules travelling a shorter distance along the gel. The gel is then transferred, or “blotted” onto a nylon sheet to which the fluorescent probe is applied, incubated, and analyzed, providing a specific location and retention factor of the target RNA\textsuperscript{12}. As the most widely used method for detecting miRNA it certainly possesses advantages, it is a simple process to identify for isolation and analysis a single miRNA that corresponds to your hybridization probe. It also only requires the isolation of total RNA as the procedure results in a specific band corresponding to siRNA and miRNA, which is significantly further down the gel than the other major types of RNA due to their sizes. Also of note is the ability of the northern blot to detect degraded and damaged materials, which although not as applicable to analysis of small miRNAs, can show by unexpected retention factors if the RNA has been reduced in size by degradation, giving a depiction of quality. Disadvantageously however, it is very low throughput-put, as only one RNA, or possibly a small number of RNAs, are capable of being analyzed on a single blot, depending on the availability of different fluorescent materials attached to the probes\textsuperscript{13}. The
process itself is also a slow one, often taking an entire day for the blot to be completed; it is also difficult to determine the amount of material that is present, often determined based on an almost qualitative comparison of band darkness.

A method that increases the thorough-put of miRNA analysis is the use of DNA microarrays. A microarray is a piece of silicon that has thousands of individual small hybridization probes bound covalently to its surface\(^4\). A selection of cDNA that one wishes to analyze is then fixed with a fluorescent probe and applied to this surface, or “chip”, and incubated to afford hybridization. The fluorescence of each well can then be determined, and, as each probe is known, it is possible to determine if a wide variety of cDNA is present in the analyzed sample. While this provides a major advantage in the sheer number of different miRNAs that can be screened for in one experiment, there are a number of important drawbacks. First of all, notably the procedure is carried out using cDNA. This requires that this cDNA is produced using RT-PCR from the miRNA that is to be analyzed, requiring that siRNA be isolated prior to RT-PCR to avoid a large amount of excess cDNA being created from the larger RNA molecules\(^5\). While this introduces a number of steps in the procedure, this is not really an issue because of the time that microarrays save in general; it does however increase the chance for RNAs to be damaged, reducing the accuracy of using this mechanism for quantitative analysis. Additionally, and perhaps the most limiting factor in using microarrays more commonly, is that the production of a desired microarray chip is very expensive and is a difficult task in its own right, making their use only viable if a number of experiments are to be run using the same conditions.

On the other end of the spectrum, one way to improve specificity for a desired miRNA is the use of RT-PCR analytically. Using the aforementioned procedure to generate cDNA from the miRNA bank, the amplification is then undergone using a forward primer specific for the miRNA that is being analyzed\(^6\). Using the principles of real time PCR (QPCR), it is possible to monitor exactly the initial quantity of the target by the increase in fluorescence. This is accomplished through use of a fluorescent
probe that is cleaved by polymerase when reached in the elongation process, releasing its fluorescent material. This can then be monitored, and, when the fluorescence reaches a detectable limit, used to back calculate to the initial concentration. A similar method of detection involves using a dye that binds specifically to double stranded DNA, however this is not as specific, binding to combinations of the primers as well as any contamination dsDNA\(^\text{17}\). The use of RT-QPCR has the advantages of being very specific and quantitative, giving an excellent idea of the initial concentration of a single miRNA in a sample. Also, because of the use of specific primers in amplifying the cDNA this can be performed on a cell lysate or other messy sample containing mRNAs and other contaminants. The disadvantages are simply the other side of the advantages; it is very low thorough-put as only one miRNA can be detected at a time.

A number of other methods of using hybridization to detect miRNA are possible; studies have been done using bioluminescence, where a luciferase containing DNA sequence competes with the target for probe hybridization\(^\text{18}\). This leads to a decrease in luminosity with increasing target concentration. In a similar vein, it has been shown that you can detect miRNA by an increase in current. By covalently linking oxidative moieties to complete miRNA and then incubating this with an immobilized probe, one is able to wash the unbound oxidative material away and then measure the change in current of the solution containing the immobilized probe\(^\text{19}\). Both of these models allow for a high degree of sensitivity, but require the addition of a specialized detectable group to either the probe or the total miRNA. A large number of similar detection ideas could be developed along these lines, where a highly visible group is added to the miRNA, which is then incubated with a probe bound to a vessel in such a way so that it will not be removed upon washing. The vessel would then be washed, removing all free material and resulting in only probe and bound miRNA remaining. Then the method which makes visualization of the group possible is utilized and the target miRNA can be proven to be present and the concentration determined.
An additional method for isolating miRNA is through the use of capillary electrophoresis (CE). Capillary electrophoresis is an excellent method for separating molecules, doing so based on their charge and molecular mass\textsuperscript{20}. It proceeds by the injection of a small amount of analyte into a small capillary. After the injected material has passed through a large portion of the capillary it reaches the “window”, a clear portion of the capillary where detection of the desired compound occurs. This detection can occur using a number of different methods, but when using a fluorescent marker on the hybridization probe, the miRNA is detected through the monitoring of emission after laser excitation. This method is highly sensitive and can detect concentrations in the realm of hundreds of molecules, making it ideal in the use of quantitative analysis of the miRNA. It does, however, possess a drawback, due to their similarities in retention time it is difficult to resolve the bound complex of siRNA and hybridization probe from that of the unbound probe. The most common method for increasing resolution in CE is through the use of gel CE\textsuperscript{21}. This method works much like a combination of regular capillary electrophoresis with gel electrophoresis, causing longer strands to be held back even more readily creating more separation. With the increased resolution, however, there is also a decrease in flexibility – gel CE does not allow for the range of injectable materials of which regular CE is capable. Since this would restrict the use of CE for the analysis of cell lysates an alternative method of resolving the peaks was investigated. Since CE separates materials by their charge and size, a method of altering these qualities of one of the materials would result in greater separation. The first method used to accomplish this was through the use of single stranded binding protein (SSB)\textsuperscript{22}. This protein, as suggested by the name, binds selectively to single stranded DNA, which usefully means it binds to the unbound probe used to detect miRNA, but not to the probe/miRNA complex. This addition of a largely neutral protein dilutes the acidity of the unbound DNA probe, causing it to migrate faster than complex, greatly increasing the resolution.

\textit{Figure 2:} Use of SSB to Improve Resolution of miRNA-Probe Complex
A second method, and the method used in this study, is through the use of p19 siRNA inhibition protein. This protein, with similar specificity as SSB, binds to small double stranded RNA molecules. When used in the cell this protein stops the miRNA from entering the RISC and beginning the RNAi process, however when used analytically it binds specifically to the probe/miRNA complex causing it to be eluted earlier, resulting in an elution pattern opposite that of using SSB.

The first step in the study on the detection of siRNA utilizing p19 mediated electrophoresis was the standardization of the capillary electrophoresis systems. This was done by running a number of standard dilutions of fluoresceine, a common reagent that emits light when excited by a laser. This fluorophore standardization accomplishes two things, it allows for the determination of the limit of detection (LOD), as well as establishing a curve which can be used for internal standardization.

The limit of detection gives a value to sensitivity of the device, showing the lowest value that can be detected of a certain fluorophore. It is wasteful to use large concentrations of the biological reagents, as they are labour intensive to obtain, expensive in regards to both time and money. It is therefore important to determine the lower limit of detection on these devices, as the knowledge allows one to decide on acceptable levels of dilution. The LOD also helps to determine what experiments are
possible, such as detection of a miRNA, which may be so dilute \textit{in vivo} that detection is limited by the device.

The second benefit of fluoresceine is the use of it as an internal standard. As known concentrations can be prepared readily, the internal standard can be added very precisely to a sample to be injected. This allows one to compare the area of the fluoresceine peak, a known value, with the area of the peak to be analyzed\textsuperscript{24}. If the fluorophore of the peak to be analyzed is related to fluoresceine then a comparison can be drawn that would allow one to determine the area of the unknown peak. This method of injecting both the standard and the sample in the same run is more powerful than simple comparison to a standard curve, as it accounts for differences between each runs, such as new capillaries and any sort of anomaly in the buffers. One disadvantage, however, is the possibility of interactions between the fluoresceine and the analyte, reducing the stringency of the assay.

Two devices were used in experiments in our lab, the PACE/MDQ system and the PA 800. While all experiments in a related project would ideally be run on one machine, it is still useful to compare the values obtained from LOD between the two devices, which were detected for fluoresceine.
Figure 3: LOD of Fluoresceine of PACE/MDQ System – Area of fluoresceine was measured at concentrations of 20, 50, and 200 fM. This was then plotted and the line of regression was determined and the LOD was found to be 15.23 fM. Capillary length 48.7, window 38.5.
Figure 4: LOD of Fluoresceine of PACE/MDQ System – Area of fluorescine was measured at concentrations of 15, 20, and 200. This was then plotted and the line of regression was determined and the LOD was found to be 13.19. Capillary length 50.2, window 40.0.

Based on these values it can be shown that the PA 800 is more sensitive for the laser mediated detection of fluoresceine, and was the device utilized for the remainder of the work. This difference could be due to a number of things, such as laser intensities or injection issues, as only the detector was shared between the machines.

An important difference to account for when performing capillary electrophoresis, is if the binding being measured is through the non-equilibrium capillary electrophoresis of equilibrium mixtures (NECEEM) or equilibrium capillary electrophoresis of equilibrium mixtures (ECEEM) process. While both of these processes allow for the measurement of the binding affinities of the protein to dsRNA, they
differ in one very important way. In ECEEM the running buffer contains an identical concentration of protein as the injection mixture, while in NECEEM the running buffer does not contain protein. This lack of protein in NECEEM disturbs the bound to unbound equilibrium, which is maintained in ECEEM, resulting in a number of different outcomes in the CE profile. The most notable of these changes in our analysis is the ability to more directly correlate the amount of protein in the injection sample with the intensity of the peak representing the complex. In NECEEM an increase in the concentration of protein should be visible as an increase in the peak area for the complex as well as a decrease in the peak size of the free RNA. In ECEEM these peaks should be more stable.

In our studies the NECEEM experiments did not yield viable results. Only one peak was visible, and its intensity increased with increasing protein concentration, however it did not possess a free RNA peak, and the peak was at an unusual migration time for a bound complex. This problem could have a number of sources, a likely one being contamination of the protein sample with a fluorescent reagent. The ECEEM results however, were able to show a promising difference between the CE profile of p19 with the RNA as opposed to merely RNA alone.

**Experimental:**

**Formation of the Complex**
The initial mixtures were formed by 10 uL of p19 (p19-III-16) with 10 uL of siRN (mixture of CSK2 21-nt siRNA and analogous probes, sequence: 5’ C U A C C G C A U C A U G U A C C A U dT dT 3’), regardless of concentration. These mixtures were then incubated at 20 °C, in the Eppendorf Thermocycler, for 20 minute periods before being set up for injection. In ECEEM studies, 100 mL of running buffer containing the same concentration of protein as the running buffer, is utilized. So as not to waste protein, this sample is used instead of two separate buffer samples as shown in the basic CE-LIF procedure.
Experiments were performed on a PA 800 capillary electrophoresis system using a laser-mediated fluorescence detector with an excitatory wavelength of 488nm. Polymer coated capillaries were used, with the distance to the window equalling 40.0 cm, while the total length was 50.2 cm. The running buffer consisted of 25 mM tris acetate, with a pH of 8.3, containing an equivalent concentration of p19 as the injection sample. Prior to each run the capillary is rinsed with 100mM HCl, 100mM NaOH, ddH₂O, and 25 mM tris. Post injection the capillary is then briefly placed in a second solution of 25 mM tris, and finally in the running buffer. A similar procedure was also attempted using 25 mM of borate buffer in place of the tris, although these results were not as useful.

Results and Discussion:

The first step in the ECEEM analysis of p19 binding of complexed siRNA is the injection of the desired concentration of siRNA without protein. This gives a reference with which to monitor any migration time related changes.

Figure 5: Injection of 100 nM siRNA in Tris-Acetate Buffer. A 100 nM mixture of siRNA and probe was injected into the PA 800 CE system. This profile shows a peak close to four minutes (0.1 RFU) and a larger peak (0.35 RFU) at eleven minutes. The small peak is present in injections of pure tris.
After the negative control was established injections were performed with samples where p19 was allowed to complex with the siRNA mixture through incubation. This was attempted using a variety of different concentrations of p19. The following graphs are all measured using the same scale as Figure 5.

**Figure 6:** Injection of 100 nM siRNA + 100 nM p19 in Tris-Acetate Buffer. Peaks are present at 4 minutes (0.05 RFU), 7.5 minutes (0.03 RFU), and 9.7 minutes (0.05 RFU).

The combination with 100 nM of the binding protein results in some observable separation, however both the free probe and complex peaks are very short, much below the peak size observed by the CE analysis of pure miRNA.
Figure 6: Injection of 100 nM siRNA + 200 nM p19 in Tris-Acetate Buffer. Peaks are present at 4 minutes (0.05 RFU), 7.2 minutes (0.04 RFU), and 10 minutes (0.04 RFU).

Injection of 200 nM of the binding protein produced a similar profile, with a slightly more pronounced peak for the separated region, but still much shorter than that of the pure miRNA analysis.

Figure 6: Injection of 100 nM siRNA + 300 nM p19 in Tris-Acetate Buffer. Peaks are present at 4 minutes (0.05 RFU), 6-8 minutes (0.20 RFU) with a side peak at 7.5 minutes (0.13 RFU), and 10 minutes (0.04 RFU).
The mixture of 300 nM p19 with the miRNA, however, resulted in a much larger and broader peak, with an observable side peak. As this concentration showed clear and expected results it was repeated to ensure reproducibility.

**Figure 7**: 4 Trial Injections of 100 nM siRNA + 300 nM p19 in Tris-Acetate Buffer. Peaks are present at 4 minutes, 6-8 minutes with a side peak at 7.5 minutes, and 10 minutes. One trial has peaks shifted slightly to the right and an anomalous starting RFU.

These trials showed that the peaks maintain a reasonable amount of consistency across multiple trials, with migration time, at least, being highly maintained.

The initial injection just siRNA shows two peaks, with one of these peaks being present in all injections using the tris buffer. This implies that the larger peak consists of the miRNA-probe complex as well as the unbound probe; this is reasonable as it consists mainly of nucleotides and should have a relatively long elution time.

The initial two injections, with p19 concentrations of 100 and 200 nM, show a dramatic change in the CE profile, with the large peak being shortened considerably and an additional peak appearing. This is promising as it suggests the formation of a miRNA-protein complex, which is what the second
peak consists of, as well as the separation of this complex from the free probe, which remains at the original migration time. This agrees with predicted results, as the formation of a protein complex should reduce the migration time due to an increase in the charge-mass ratio.

The injection with p19 concentration at 300 nM, however, shows a much better CE profile. In this case the protein complex peak is much more pronounced. This likely speaks to the stabilization of the complex by the excess of binding protein. With this concentration it is also possible to cleanly see a side peak, which likely corresponds to a degradation peak\textsuperscript{27}, where the protein and RNA are only partially associated, resulting in less reduction in the charge-mass ratio and a smaller reduction in migration time. The presence of a degradation peak at 300 nM hints heavily at the reason of the poor quality of the peaks at the lower concentrations. It is likely that the majority of these dsRNAs are only partially associated with protein, so, while they separate easily from the free RNA, they do not form a clean peak.

**Future Work:**
There are a great deal of more fields that the use of p19 in detecting miRNA can be applied to. Most urgently is the use of the remaining p19 mutants, comparing their ability to bind and separate the siRNA/probe complex from unbound probe. Experimentation in this area would optimize the assay, resulting in more clear peaks for bound and unbound probe, with potentially less degradation slope. In addition to these mutants, we would also be able to study the binding capability of the monomeric protein in addition to the active dimer, which may provide additional insights into the binding affinity of p19. Outside of optimization of our assay, this work would also advance research on the binding capabilities of the p19 mutants, which is of particular interest to our collaborators at the NRCC, who are researching p19 itself.

**Table 1: Testable p19 Mutants**

<table>
<thead>
<tr>
<th>p19 Cys Mutants</th>
<th>Information</th>
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Similar to the diversification of p19 mutants, it would also be beneficial to investigate the use of other sequences of dsRNA. Only one sequence was used in our study, and while p19 binds generally to miRNA in vivo it could be useful to determine if binding is effected by sequence, a large quantity of G-C, versus A-U interactions, and sequence length. Sequence length may be particularly important; as it is suggested by our collaborators that p19 may be used as a molecular ruler for fragments of RNAs 21 nucleotides in length.α

An additional field that must eventually be explored is the use of p19 in detecting these miRNAs directly from a cell lysate environment. This is really the true test of the procedure, as it allows for a number of important analytical experiments to be done, detecting the presence and concentration of virtually any miRNA in the cell. This procedure has previously been accomplished successfully using the similar procedure of the formation of a protein-DNA complex with unbound probe using SSB as a starting point from which a number of important modifications to the procedure were made. The biggest concern in detecting miRNAs from the cell lysate, is that after cells are lysed they become a

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**Table 2: Available siRNA sequences**

<table>
<thead>
<tr>
<th>RNA</th>
<th>Sequence</th>
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</thead>
<tbody>
<tr>
<td>siRNAs</td>
<td></td>
</tr>
<tr>
<td>CSK2 21-ntα</td>
<td>Sense: 5’ C U A C C G C A U C A U G U A C C A U dT dT 3’</td>
</tr>
<tr>
<td>GL2 21-nt</td>
<td>Sense: 5’ C G U A C G C G G A A U A C U U C G A dT dT 3’</td>
</tr>
<tr>
<td>GL2 19-nt</td>
<td>Sense: 5’ U A C G C G G A A U A C U U C G A dT dT 3’</td>
</tr>
<tr>
<td>GL2 21-nt ssβ</td>
<td>5’ C G U A C G C G G A A U A C U U C G A dT dT 3’</td>
</tr>
<tr>
<td>miRNAs</td>
<td></td>
</tr>
<tr>
<td>miR-122</td>
<td>Sense: 5’ A A C G C C A U U A U C A C A C U A A A U A 3’</td>
</tr>
<tr>
<td>miR-155</td>
<td>Sense: 5’ C U C C U A C A U U A U A G C A U U A A C A 3’</td>
</tr>
</tbody>
</table>

α- Used in ECEEM experiments
β- Single stranded
much less controlled environment, with all of the components of the cell basically being placed in one solution. This results in degradation proteins, most worryingly RNases and DNases, being present in the same mixture as the desired miRNAs and applied probes. To prevent this degradation, additional nucleotides, called “masking” DNA or RNA, can be added to the mixture, tying up the degradation enzymes\textsuperscript{28}. These masking nucleotides are merely large concentrations, (100x-1000x) in SSB studies, of DNA or RNA sequences that are of similar size to the probe and miRNA respectively, with sequence not being of importance as tRNA was used to prevent the degradation of miRNA. This saturation of the degradation enzymes with added nucleotides allows the desired analytes to remain unscathed, allowing for their detection in cell lysated injected into the CE.

While these methods allow for the quick, precise, and sensitive analysis of miRNA, it is still very low throughput, with only one sequence being detected at a time. In an attempt to expand the application of this method, it would be desirable to be able to analyze a sample for multiple varieties of miRNA at once\textsuperscript{22}. The specificity in this mechanism, as in the case of most miRNA analysis methods, is with the probe. Therefore, any mechanism that attempts to expand the number of miRNAs that are analyzed at once must contain multiple probes. The issue with this, however, is that changes in the sequence of the probe will not alter its mass charge ratio, as it will still bind to a similarly sized miRNA, and therefore the peaks will be indistinguishable after a CE run. To correct this, therefore, each miRNA that is to be detected simultaneously must be bound be a uniquely modified probe.

Two conceivable ways to go about this are the use of probes with different excitation dyes, and the use of probes that have mass-charge ratio altering additions. As the CE detector is capable of receiving multiple emission wavelengths, as shown by the difference between the probe and fluoresceine, it would be possible to use two probes containing different fluorophores. This way each complex could be analyzed separately, on different channels, even though they possess similar charge-mass ratios. While this would allow for effective separation, it carries the disadvantage of each peak not
being directly comparable to the other, as different emission wavelengths may be detected with different intensities and result in different peaks even at identical concentrations. This means the mechanism that allows for separation is also the mechanism that limits the analysis. To keep the peaks analogous to each other, therefore, the same fluorophore must be maintained and separation must be obtained by changing the mass-charge ratio of the probe. Probes are highly modifiable, the 5’ end is already bound to the fluorophore, therefore the simplest way to alter the charge-mass ratio is to modify the probe at the 3’ end. One mechanism that makes this possible is the addition of extra nucleotides to the end of one of the probes. In SSB studies, this was incorporated as a hairpin loop, which allows for the addition of nucleotides without modifying the binding region of the molecule. This increases the acidity of the probe, elongating the migration time, and shifting the peak to the right. To shift to the left, however, a different mechanism is required. Just as the binding of the bulky neutral p19 protein shifts the bound probe to the left, the addition of a large neutral molecule to the probe would shift the unbound probe to the left. As an added bonus, it is possible for agents to be added that interact with this neutral add-on, altering the migration time based on their concentration. In SSB studies cholesteryl was added to the molecule, which interacts with Triton to increase the hydrophobic nature of the entire complex. This means that not only can the migration time be decreased, but it can be manually controlled by raising or lowering the concentration of Triton in the buffer. These methods are tested and give results, but a number of different molecules could easily be utilized so long as they alter the charge-mass ratio.

Conclusions:

Through comparing the two capillary electrophoresis systems in the laboratory, it was found that the PA 800 system was the most sensitive instrument with an LOD of 13.19 fM of fluoroscience, compared to the LOD of 15.23 fM for the PACE/MDQ system. It was also found that, through use of
ECEEM conditions, p19 allows for the separation of probe/miRNA complex from unbound probe at multiple concentrations, with the clearest peaks at concentrations of 300 nM p19 and 100 nM RNA.
References:


30. - Chen, Yong; Cheng, Guofeng; Mahato, Ram Pharm. Res. (2008) 25, 1, 72