# BioWIRE Objectives

**Motivation-** Currently, the development of nanowires is limited in size and cost by traditional lithography techniques. Research in DNA nanowires is a hot topic right now because of the duplex's unique structure and modifiable bonding sites. However, since the conductivity of DNA was found to be too low to carry a significant signal, the objective of this project is to enhance the conductivity of DNA by inserting silver ions into its structure. If successful, this could have important everyday uses, including but not limited to:

- Electronics:
  - Increasing the number of transistors that fit on a single microprocessing chip, leading to faster and more powerful computers
  - Enhancing the functionality of logic gates
  - Development of nanocircuits
  - Development of more effective solar cells
- Medicine:
  - University of Arkansas researching the use of nanowires to coat titanium implants, improving the anchoring of biological tissue
  - (very far future) development of nanorobotics for patient-personalized internal delivery of medicine
  - Biosensing (?)

**Inspiration:** 2011 and 2012 papers by Ono about the specific binding (N3 cytosine) of Ag+ with C:C mismatched base pairs. We hope to use this relationship to form strands of varying C:C mismatch patterns, and then compare the differing conductivity of the resulting nanowires.

**Desired Technologies/Resources:**

- rtPCR: Ag+ detection, formation of duplexes (testing to see if it duplexes)
- CSAFM: basic current/conductivity data
- Phengreen fluorescence: silver detection
- NMR: bond placement, confirmation of N3 cytosine bonding using COSY-H
- XPS/XANES/X-ray crystallography: structure (see if Ag+ alters overall 3-D form)
- Gel electrophoresis: pattern testing, compare conductivities of different silver distribution patterns
- Nanodrop: duplex, nonspecific
- TEM: visualization of largescale structure
- ICPMS: test Ag+ uptake from solution
- ESI-MS: Molar ratio and efficiency
- UVspec: wavelength/absorbance, melting point test ( note: issue with this is that our machine won't go above 44.8 ˚C. Also, it is important to observe that since our strands don't contain C-G bonds, the melting point of our DNA is lower than normally expected)

**Calendar/Weekly Objectives:**

| Due Date: | Goals: | Results: |
|---|---|---|

| | | |
|---|---|---|
| 6/21 | 1. UV spec<br>2. Phengreen<br>3. NMR email/access<br>4. Longer oligos | 1. UV not possible because of temp limitations<br>2. No Phengreen yet<br>3. Started rtPCR, issue with stock solutions?<br>4. Waiting for confirmation of NMR access |
| 6/28 | 1. Troubleshoot<br>2. rtPCR<br>3. gel<br>4. access to tech<br>5. PhenGreen<br>6. ICPMS prep | 1. MOPS buffer made, showed better results with ssDNA than qH2O<br><br>2. confirmed access to CSAFM<br><br>3. qPCR showed extreme variation in results of identical samples, abandoning this method for now |
| 7/5 | 1. rtPCR<br>2. tie loose ends on pattern proving<br>3. ICPMS | 1. going back to UV spec, this time using MOPS<br><br>2. results from UV show clear differences in structure for solutions with and without Ag+ (see below); however, no consistent change in melting temp to support stability hypothesis<br><br>3. promising results from PhenGreen- looking to replicate in multiple samples next week to get statistically significant differences |
| 7/12 | 1. NMR<br>2. CSAFM(?) | 1. PhenGreen results show it is sensitive to Ag+; still waiting on statistically |

| | | |
|---|---|---|
| | 3. brick | significant differences proving Ag+ uptake<br><br>2. Granted permission for SEM training; looking to schedule for next week<br><br>3. demonstrated that 4% and 7% gels sensitive to 10bp differences in oligos<br><br>4. saw a difference in distance traveled between the 10 C:C mismatch DNA and control DNA on gel, possibly signifying effect of Ag+ added charge |
| 7/19 | 1. reality check<br>2. brick (cont.) | 1. Confirmed that TEM, not SEM, is better visualization method- will train next week<br><br>2. Phen Green shows positive results. Difference in fluorescence between DNA with and without mismatches is confirmed. More mismatches, less fluorescence.<br><br>3. New sequence designed by Kosuke ordered to evaluate hairpin formation via fluorescence quenching |
| 7/26 | 1. Repeat weeks 1&2 in **RNA**<br>2. X-ray methods | 1. Gel electrophoresis confirmed duplex formation and charge alteration in the presence of silver for the 10 C:C mismatches<br><br>2. PhenGreen shows a trend between number of |

| | | |
|---|---|---|
| | | mismatches (and by association, number of Ag+ ions) and fluorescence |
| 8/2 | 1. Make long strands<br>2. SEM | 1. Began research on TEM sample prep<br><br>2. Began FRET analysis<br><br>3. Silver gradient with 10 C:C mismatch strand showed promising results on PAGE gel<br><br>4. dsDNA ligated successfully; need progress on the ssDNA |
| 8/9 | 1. Methods of transport to Brown<br>2. Establish Stanford tasks for remaining time | 1. FRET results must be repeated to obtain consistent pattern<br><br>2. TEM showed that resolution may be as small as 2 nm. Looking to improve methods of sample preparation, including shadowing.<br><br>3. CSAFM not possible due to baseline current limitations |
| 8/16 | 1. Remaining ideas: nanoparticles | 1. TEM successfully imaged the striations of our samples. Resolution is still too coarse to view on an atomic level. |
| Fall 2013 | 1. Emily tests conductivity with Prof. Xu at Brown, works on noise removal<br>2. Tighten up presentation and all iGEM competition related things | 1. Located a lab that will perform x-ray crystallography<br><br>2. Discussing methods of conductivity testing |

**Current Issues:**

9/12: Positive response from lab willing to perform x-ray crystallography on our samples, meaning that the next week should be devoted to sample prep. Conductivity measurements are still in the planning stage. It seems like the microelectrode gap structure will not be suitable due to possible interference from "bundling" of PCRed product. Current plan is to test the impedance (and by imaginary value extraction, the conductance) of samples of varying mismatch content using solution placed on a chip, and looking for a relationship between change in impedance and change in silver content. To ensure accuracy, all remaining aqueous silver must be removed from solution, and the DNA should be resuspended in buffer with low conductivity.

New development: A professor at Brown in the physics department studies the conductivity of dsDNA moving through nanopores. He was very interested in the project when it was first brought up to him, and is currently looking over our data and the Ono references.

9/4: Elucidating ion distribution from TEM images using ImageJ. Looking for paired particles (as this is the distribution in the star sequence). Particle size bounded for silver ion (0.08nm^2 at the high end, and 0.4nm^2 at the low end to account for occlusion). Hoping to see distance between center of particles have local maxima at multiples of 3.4A, or 1bp. Observed with manual counting at 3.4A and 6.8A, but need scientifically sound manner of computing distance between centers of all particles identified.

8/28: ssDNA Brick from Elim has restriction sites, which are palindromic and consequently form a strong hairpin. PCR of this strand may be difficult, though Kosuke says it should still be possible. DNA 2.0 now has pSB1C3 for the synthesis of the second brick, and we're hoping to have it arrive prior to 9/11. May be a rush to the end/ at the end.

8/16: TEM was performed on grids with and without 0.25 ammonium acetate. One grid was prepared with stock plasmid and no ammonium acetate, while the other was with a combination of stock plasmid and 50 bp 6 C:C ligated sample with ammonium acetate. Both were stained in uranyl acetate with 50% ethanol, and subsequently sputtered in the Denton vacuum using a platinum foil. The latter of the two grids successfully showed a striated conglomeration of the ligated product, reflecting that ammonium acetate was crucial in distributing the sample over the grid.

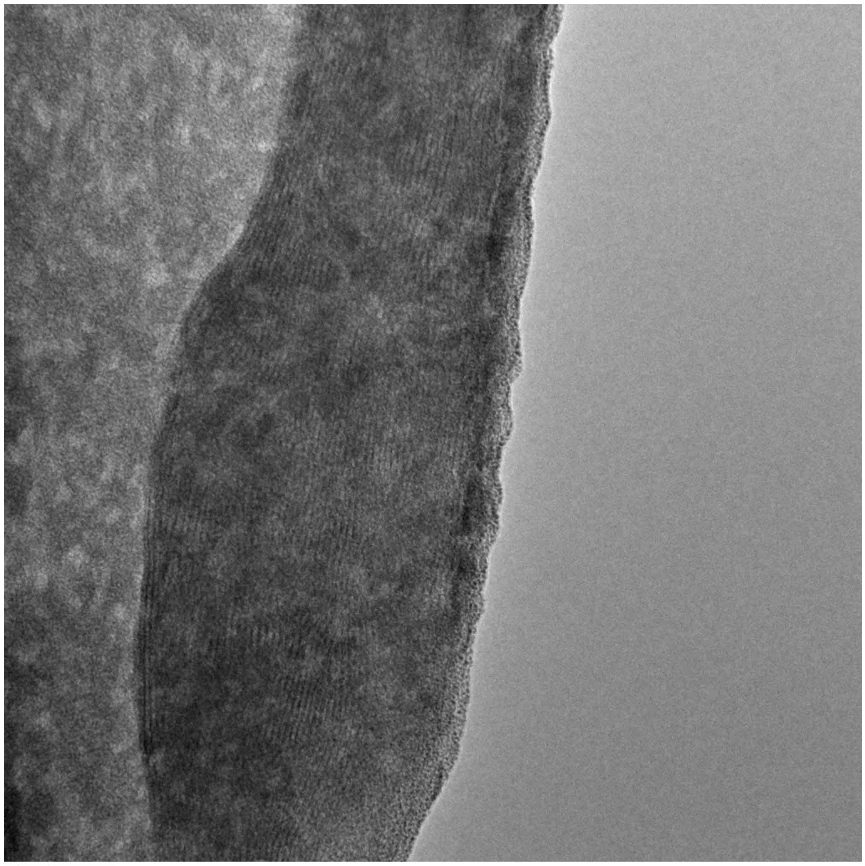

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image2
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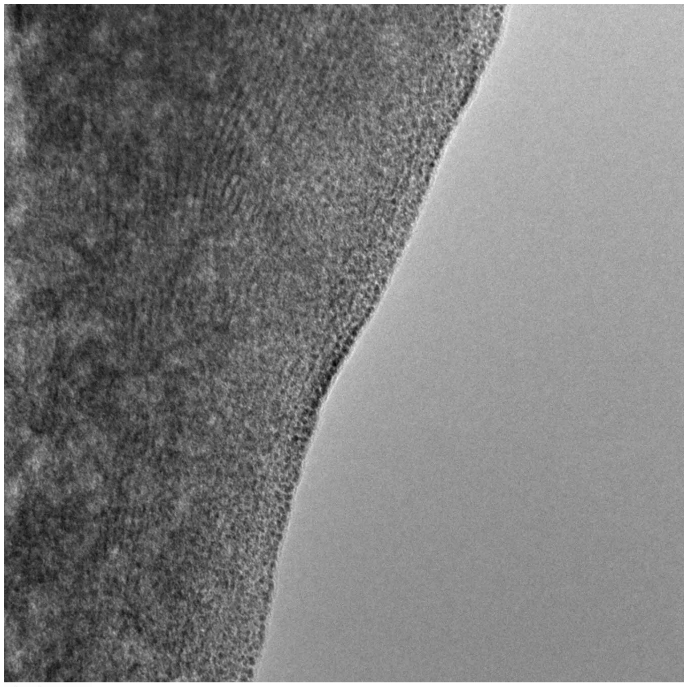
TEM Mode: Imaging

20 nm
HV=300.0kV
Direct Mag: 60000x
 Tilt:
UCSC MACS Facility @ NASA Ames



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20 nm

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Direct Mag: 120000x
Tilt:
UCSC MACS Facility @ NASA Ames

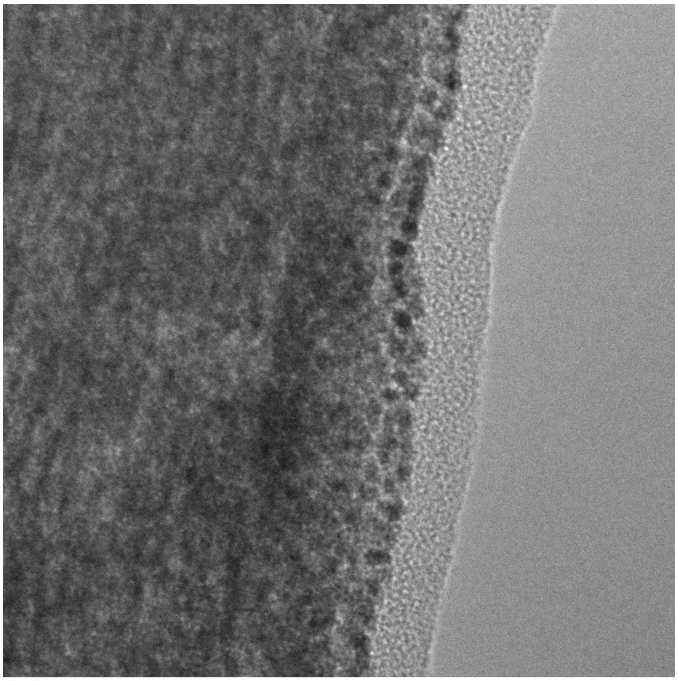

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image2
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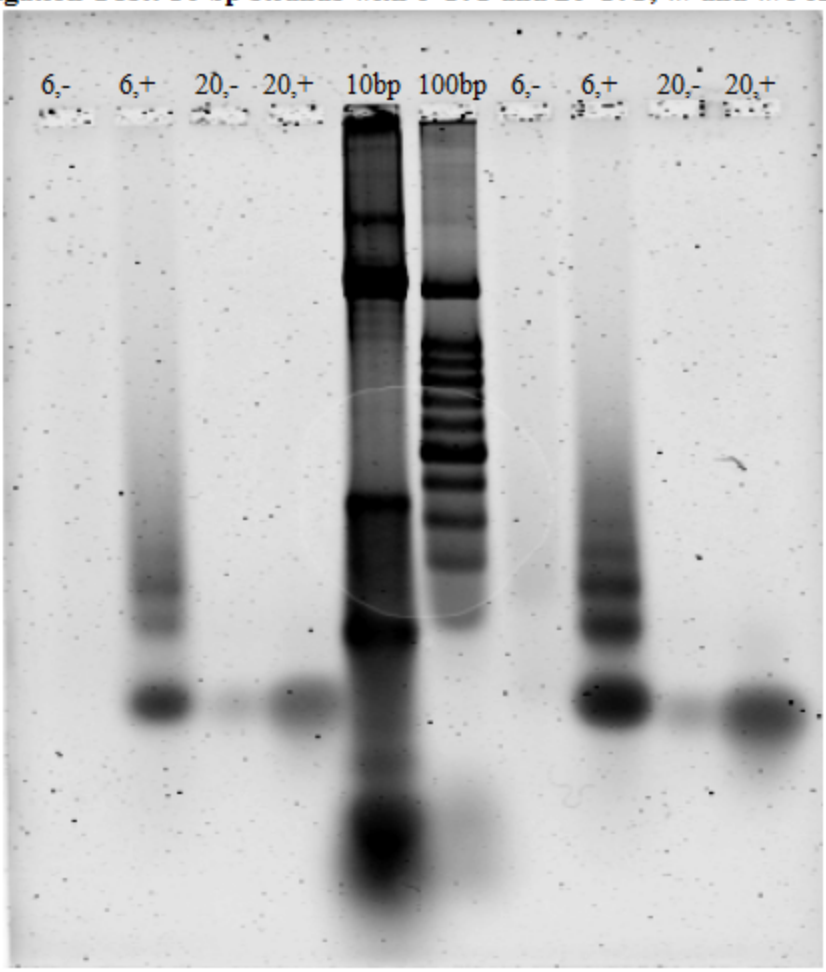
TEM Mode: Imaging

5 nm

HV=300.0kV
Direct Mag: 300000x
Tilt:
UCSC MACS Facility @ NASA Ames

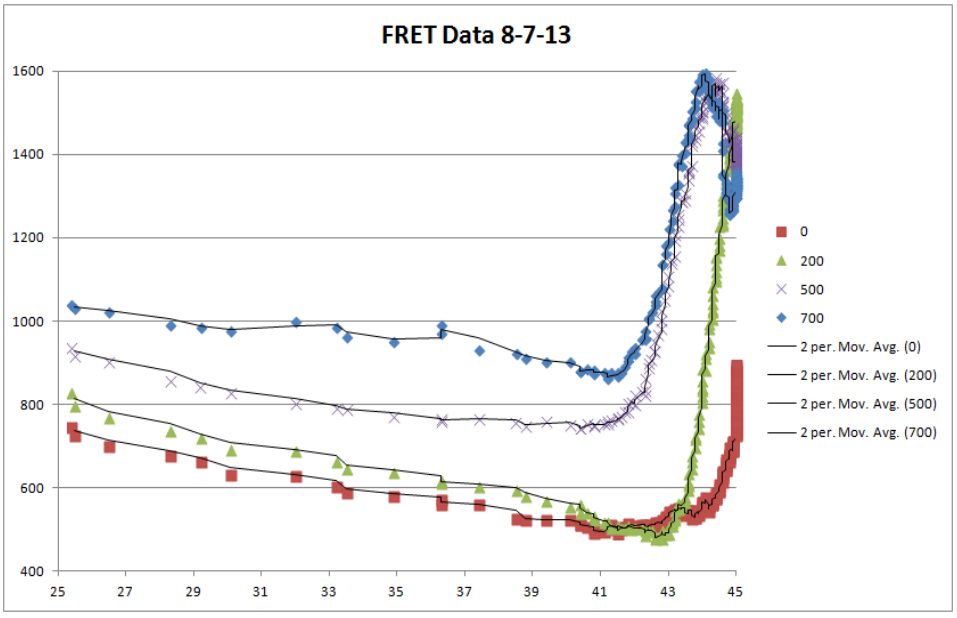
8/12: ESI-MS samples were prepared in anticipation of being sent to Providence for testing. In terms of ligation, 50 bp samples with either 6 or 20 C:C mismatches were annealed with and without silver, and subsequently ligated. As seen on the gel below, the 6 mismatch strand showed a significant increase in ligation efficiency when silver was included, giving credence to the idea that silver enhances duplex stability. The 20 mismatch strand did not show much of a difference with or without silver, revealing that the sequence is probably not optimal for manipulation of mismatches. (*note: in the image below, + or - indicates presence or absence of silver, respectively, while the number indicates the quantity of mismatches in the strand).

**Ligation Test: 50 bp strands with 6 C:C and 20 C:C, w/ and w/o Ag+**

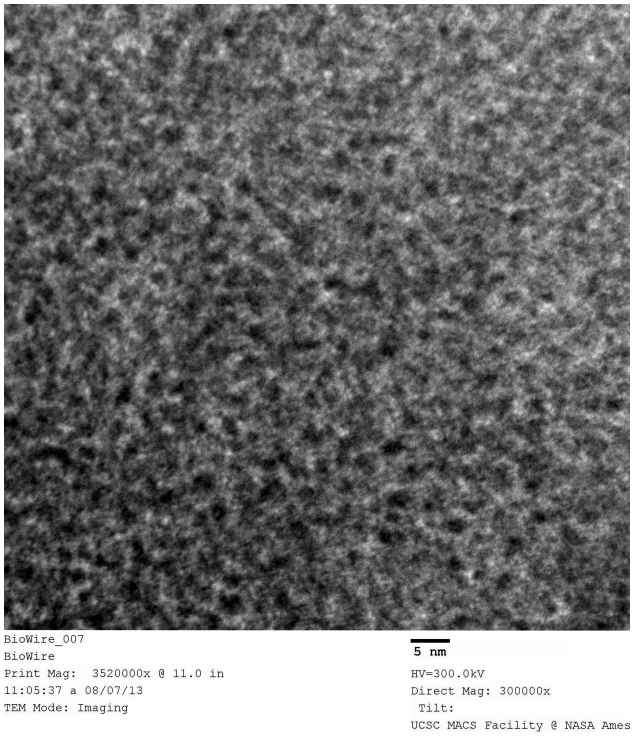


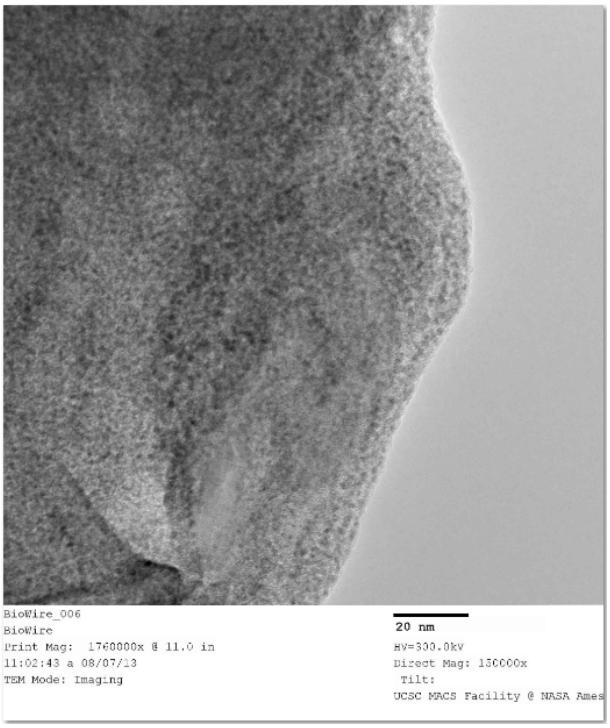

8/8: Recent FRET results seem to contradict the ones previously obtained (see below). Oddly, absolute (**not relative**) fluorescence increases with increasing concentration of silver, which contradicts our expectations. This might signify an interfering effect of silver on the quenching mechanism. It should be noted that although this experiment was based off of Ono's previous work, he graphed curves of intensity versus concentration, and thus did not test kinetics of each concentration as temperature increases. Additionally, the two FRET data sets we have were performed with different concentrations of DNA, which introduces a confounding variable. Thus, we seek to repeat the test a few more times while keeping

concentration stable as a means of reducing interfering effects.



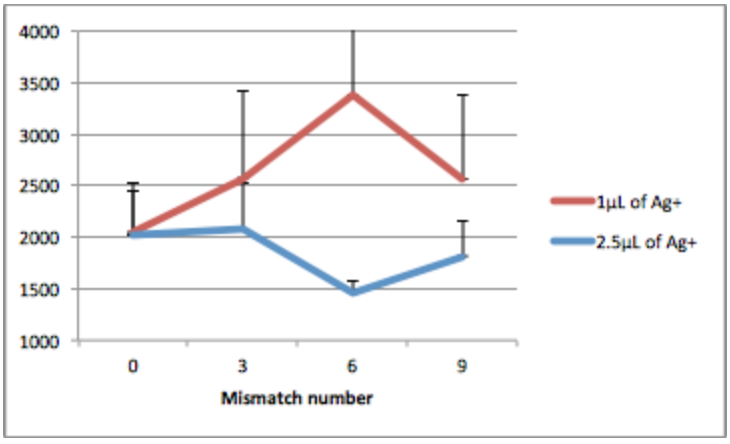

In addition to FRET analysis, yesterday marked the start of our tests with TEM. It immediately became clear that sample preparation was extremely important for the quality of our images.





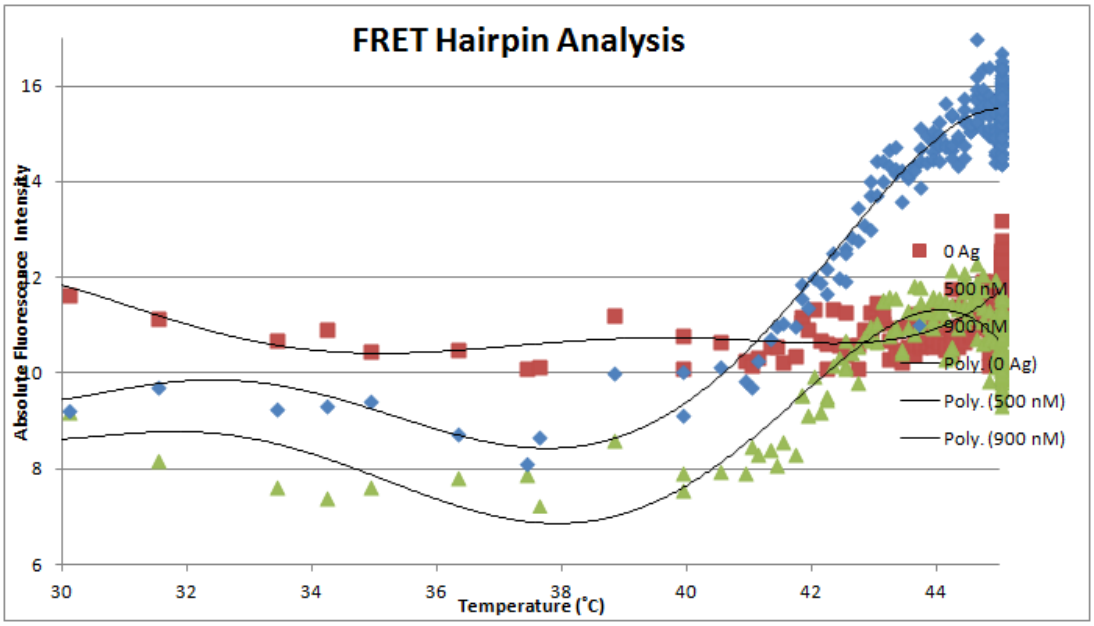

On a positive note, we were able to get the resolution down to 2-5 nm, which ought to be small enough to detect our sample. Currently, our hypothesis is that the sample wasn't dried enough, and that the short strands of DNA were clumped together, making individual strand detection difficult. To improve upon this, our plan is to: 1) use a ligated product longer than 32 bp 2) give the sample a longer drying time 3) try shadowing instead of negative staining.

8/7: Phen Green relaunch. A definitive pattern is .not reflected in the data below, possibly due to pipetting error

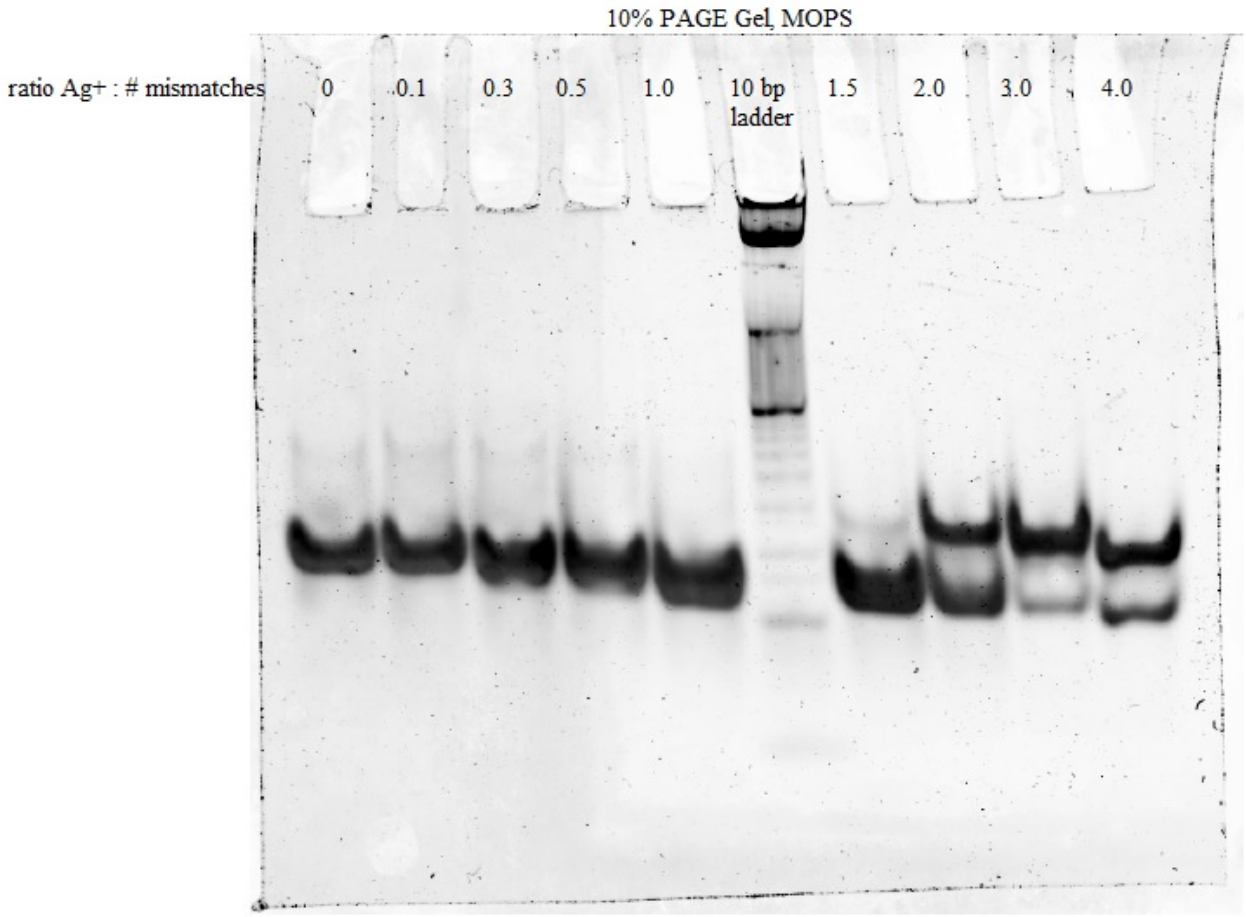



8/6: Results from the most recent FRET analysis are tentatively promising (see below). As shown, the solution with no silver is unable to form a hairpin since the C:C mismatches do not have the capability of bonding. Consequently, fluorescence intensity remains relatively constant as temperature is raised.

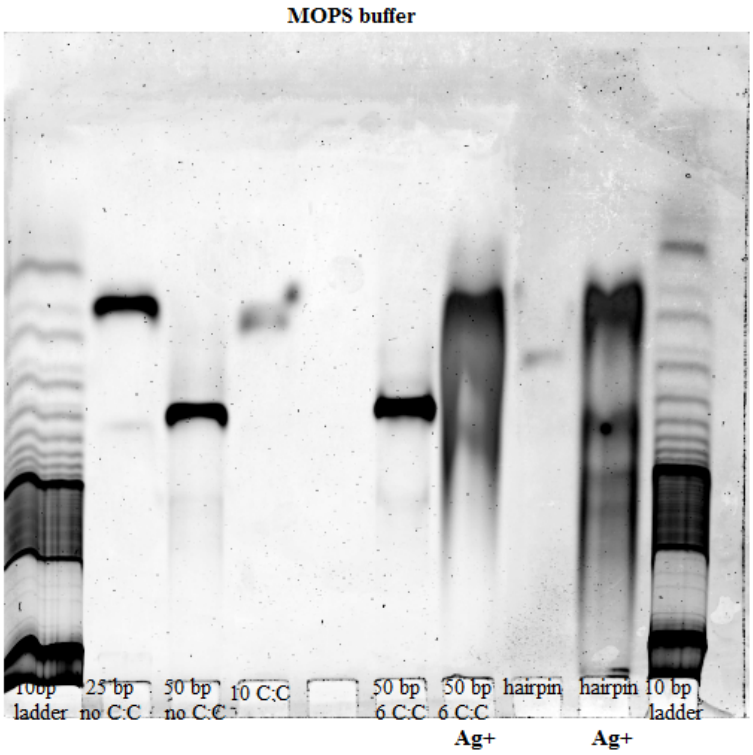
Alternatively, the solution with 500 nM of Ag+ shows a clear melting curve, with an initial intensity lower than that of the hairpin, as expected. The solution with 900 nM of Ag+ also showed a similar melting curve, indicating the formation of a hairpin duplex and its consequential melting as the temperature is increased. Although not practical in terms of quantitative analysis, trendlines of 5th order polynomials were traced over the data points to give a clearer visual representation of the experiment. (*note: experiment was performed at a wavelength of 520 nm).




8/5: PAGE gels performed at the end of last week had poor resolution and inconclusive results. Thus, for the gel we ran today, concentrations of silver were recalculated and the solutions were entirely reannealed with a goal of creating a silver gradient for the 10 C:C mismatch strand. Although good gradient results were already obtained for this strand with regular gel electrophoresis, we hoped to obtain better resolution and as well as a confirmation of previous results. Both of these seemed to be found in the resultant gel (see below):

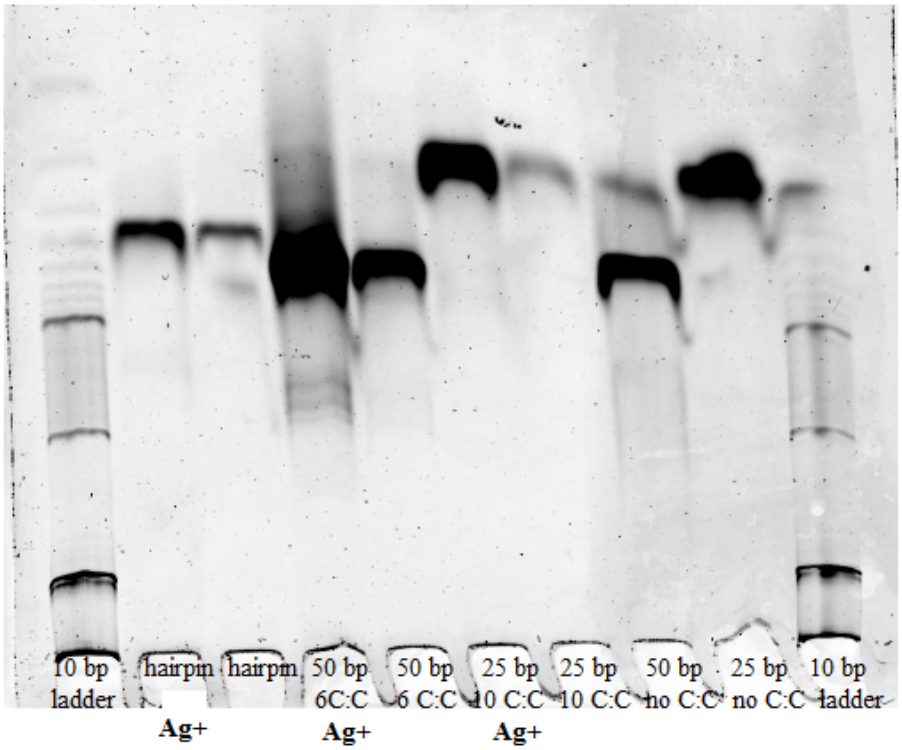



7/31 (part two): The PAGE gel run with MOPS buffer proved to have much better results than the TAE gel. The ladder resolution proved to be far better, as well as the resolution of the other wells. In the two wells containing silver, there is significant drag (see below, note that gel is temporarily upside-down). Interestingly, both solutions have an approximate 25 bp band, which could be an unformed hairpin and the 50 bp strand binding over itself. However, the two lanes containing silver also show the appearance of numerous bands, which could be from varying amounts of silver binding to the structures (for instance, 2, 5, or 7 ions binding to the hairpin of 9 mismatches). Overall, we are satisfied with the MOPS PAGE gel resolution, and will continue using it as a technique.




7/31 (part one): Yesterday we ran a PAGE gel with TAE buffer in an attempt to improve resolution. As seen in the gel below, the new method vastly improved the quality of the ladder resolution, demonstrating that PAGE gels are a superior technique for our solutions. However, the results from the other wells were not consistent with our expectations or previous findings. We think this is due to the TAE buffer and its chelating effects on silver ions. Despite the fact that no significant drag is seen in solutions with silver, except for the 6 C:C sample, those solutions appear significantly darker than their counterparts without silver, leading us to believe that silver is in fact making a difference. However, these results are not definitive or satisfying, so the experiment will be repeated today using MOPS buffer instead of TAE.

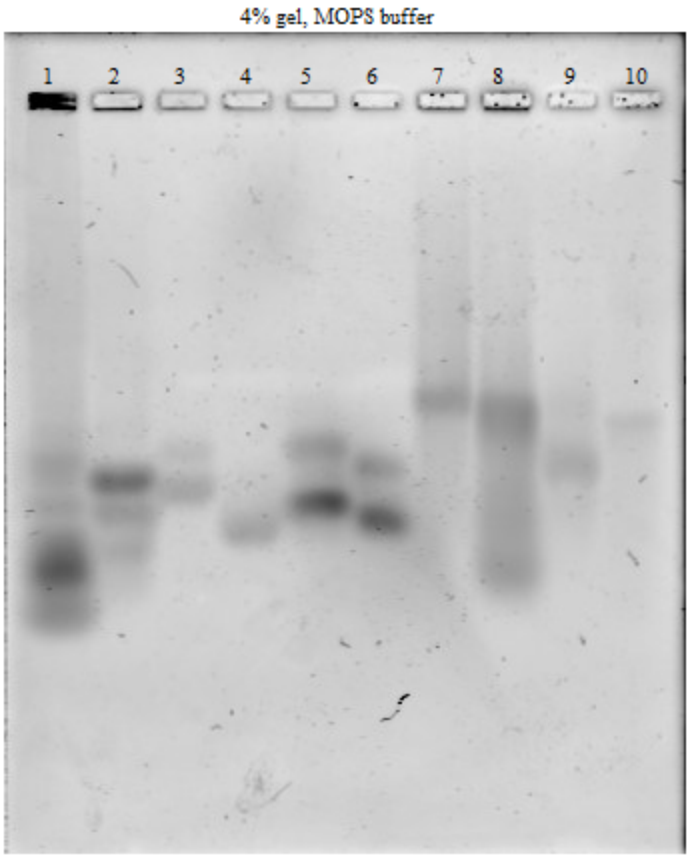
**PAGE gel with TAE buffer**




7/30: The gel from yesterday produced puzzling results, causing us to try a new method today: making our own PAGE gel. As seen below, the 10 bp ladder was significantly blurred, perhaps due to the resolution of the gel itself, and the use of too much ladder in the well. In Well 2, the solution with no mismatches produced multiple bands, leading us to believe that the DNA may have been degraded. The 10 C:C sequence previously tested showed the expected result in Well 3, dragging behind the second well as a result of the positive charge impact. The following two wells were other patterns of 10 mismatches. Well 4 had ten mismatches spaced in an every-other orientation, and didn't show much of a shift. This might be due to the fact that, unlike the other 10C:C solutions, mismatches in this sequence were not paired, making the gap for the Ag+ ion to fit into very small. Well 5 showed a similar shift as Well 3, and was comprised of a cluster of C:C's surrounded by the remaining sequence on either side.

The 50 bp sequences in Well 7-9 were less clear, with the 6 C:C mismatches in Well 8 showing the expected charge drag, while the 20 C:C mismatches failed to show the same pattern. Finally, the second ligation for the ssDNA was clearly unsuccessful, due to the lack of multiple bands in Well 10.

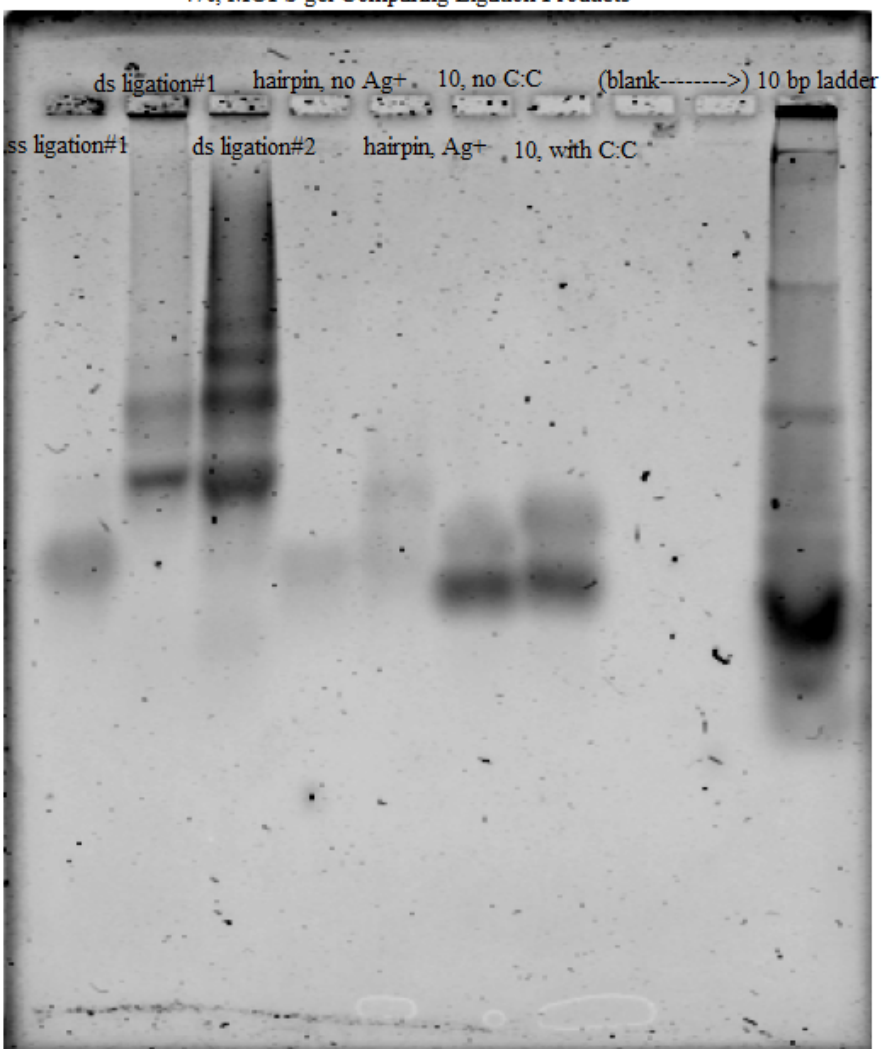
In an attempt to resolve these issues, we reannealed all of the DNA we wish to test, and made our own PAGE gel, using the protocol given to us by Kosuke (see Lab Notebook).




7/26: Currently, we're looking to repeat the promising results obtained from PhenGreen earlier this week. Additionally, the ligation process was started, using the protocol found in the Lab Notebook. Initial results of the ligation products on gel were not conclusive, so the reactions are being run again. We expect the FRET sequence to arrive on Monday.

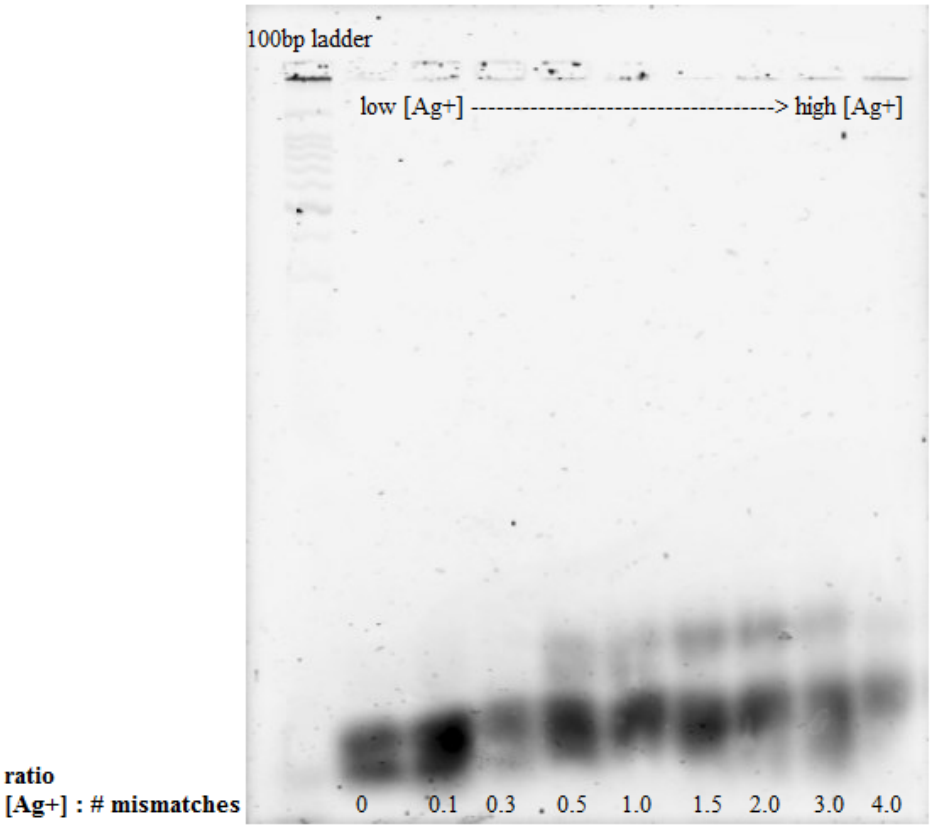
The results from the second gel can be seen below. By comparing lanes 2 and 3, it becomes apparent that both ligations were successful, but the second trial was more efficient. Additionally, by comparing lanes 6 and 7 with the 10 bp ladder in lane 10, one can conclude that the shift in the upper band of lane 7 results from the combination of Ag+ with the C:C mismatches. The hairpin lanes (4,5) show the same result, with lane 5 (with Ag+) exhibiting a significant "drag" that lane 4 does not experience.

4%, MOPS gel Comparing Ligation Products




7/25 (part II):

Great results from the 4% gel with a silver gradient using the 10 C:C mismatch strand (see below).




Samples below the 1:1 ratio clearly show the ssDNA bands. We think that multiple bands may be occurring due to the different conformations of the two oligos. As more silver is added, the band representing dsDNA gradually appears, proving that Ag+ allows for duplex formation. Beyond the 1:1 ratio, increasing amounts of silver produces a charge shift, as shown by the gradual incline in the band paths. The smudges can be explained by the fact that the products won't be definitively single stranded or with exactly 10 silver ions, meaning that some strands may be bound to 1, 2, 3, etc. Ag+ ions instead. In samples beyond the saturation point, we also observed precipitation of silver in the wells, proving Ono's theory of a 1:1 ratio for silver:cytosine mismatches. With these results, we may gather that gel electrophoresis has given us proof of duplex formation via the addition of silver to oligos containing cytosine mismatches, as well as indication of a charge shift from the increasing addition of positive ions.

7/25 (part I):

**RACE TO THE END TO-DO LIST**

1. TEM, sample prep (ET)
2. NMR, sample prep (SV)
3. CSAFM/preliminary conductivity (ET)
4. FRET (NL)
5. RNA/long strand (SV) → need by Sept. 5th
6. **BRICKS** (currently in progress)
7. ESIMS sample prep, mail to Ono (NL)

8. bond energy, modeling (?)

**red** = will need help from Stanford side, especially after August 17th/18th

Goals for today:

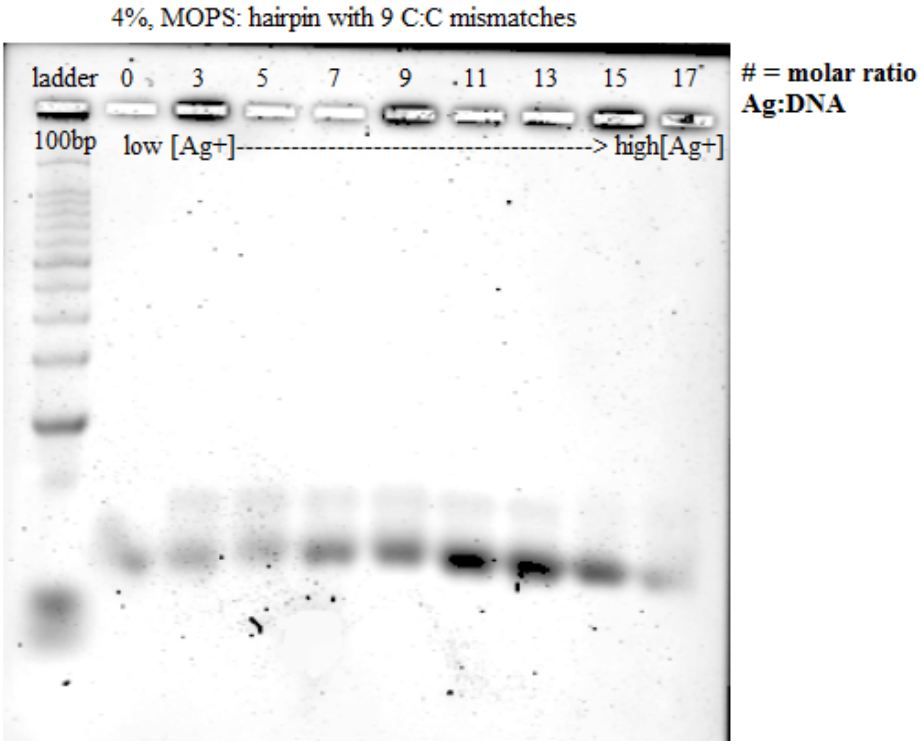
- 1% gel to test ssDNA 50temp (10X dilution) and dsDNA 10C:C (20X dilution)

- 4% gel, Ag+ gradient for 10 C:C mismatches

- PAGE gel of Kosuke hairpin sequence

- (need to wait til Tuesday when machine is available) thermal UV melt of 10 dilutions of Kosuke hairpin, 28 C:C, 10 C:C

7/24: Silver ion gradient tested on the new hairpin structure with 9 C:C mismatches gave interesting results (see below). Increasing the molar ratio of Ag+:DNA from 0-9 gradually slowed the movement of the DNA. However, the ratios 11-17 showed the opposite effect.




An additional problem from today is that the PAGE gel we ran failed to show anything in the scan. We presume that it was not stained for a long enough time (40 min), since PAGE gels are 12% and ought to be stained overnight.

7/23:

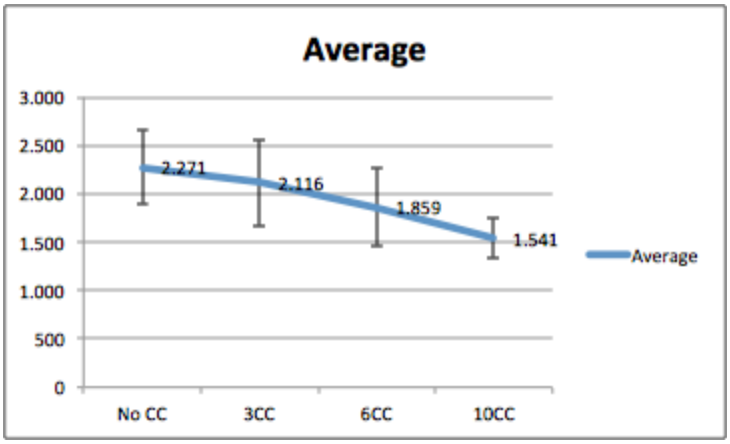
Phen Green test again. This time not only does it show a difference between fluorescence on DNA with

mismatches and without mismatches, but also shows a decrease in the fluorescence as the number of mismatches increases. This indicates that DNA with more mismatches takes up more silver, and gives credence to the hypothesis that Ag+ binds with the mismatches.

Table/ Figure 1 show the fluorescence of Phen Green against the concentration of Ag. This data can be quite conclusive as there is no overlap between the two end points of the graph: No CC vs 10 CC.

(*note: averages were taken from a collective **three samples**)

| 1:2.5 Molar ratio Ag:CC | Average | Standard Dev |
|---|---|---|
| No CC | 2271 | 379 |
| 3CC | 2116 | 448 |
| 6CC | 1859 | 399 |
| 10CC | 1541 | 208 |

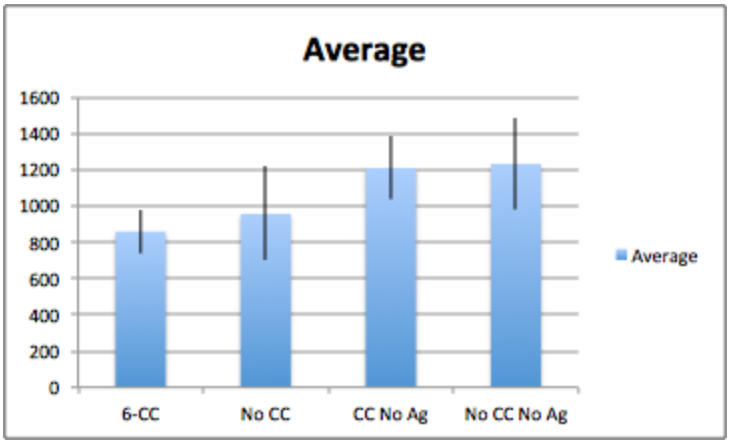



Table/Figure 2 demonstrate the difference in silver uptake between DNA with (6) mismatches and

without mismatch:

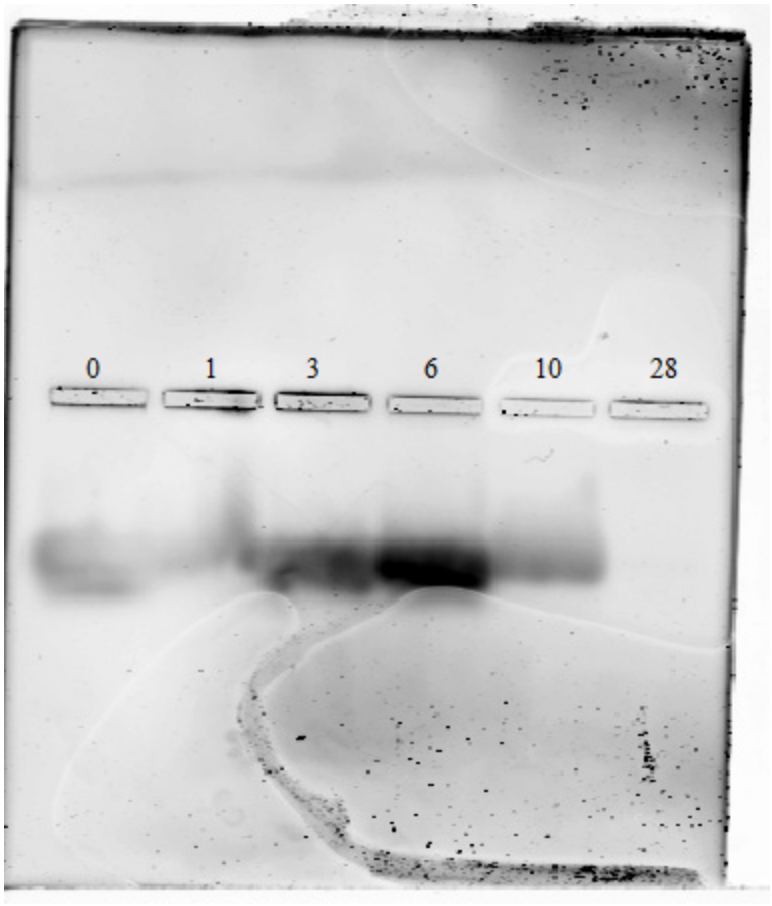
(*note: averages were taken from a collective **six samples)**

| | Average | Standard Dev |
|---|---|---|
| 6-CC | 858,8333333 | 119,8055369 |
| No CC | 956,5116667 | 259,1206275 |
| CC No Ag | 1209,653333 | 172,1027631 |
| No CC No Ag | 1232,333333 | 251,1897025 |

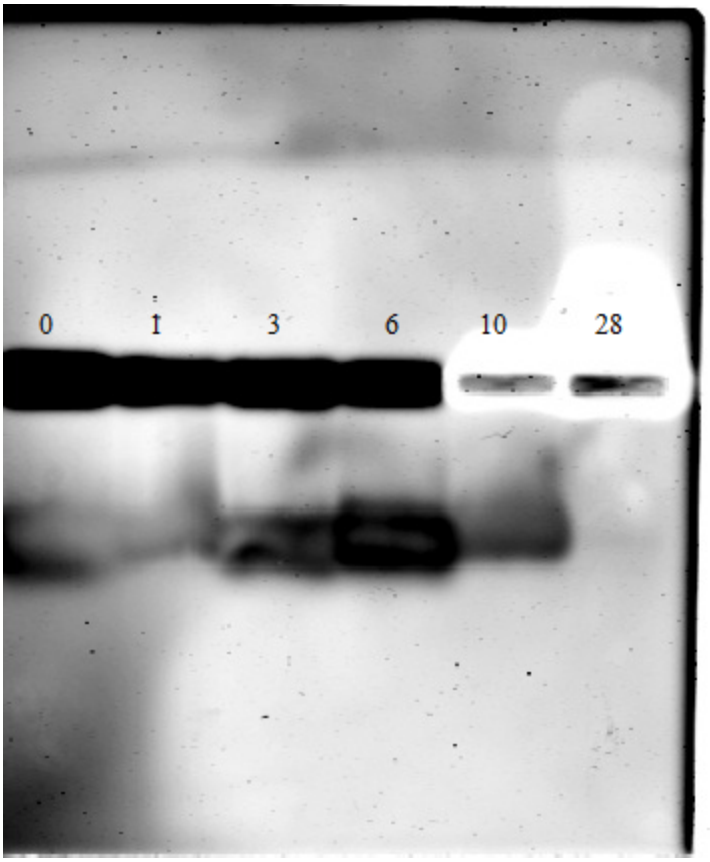



7/19: NL

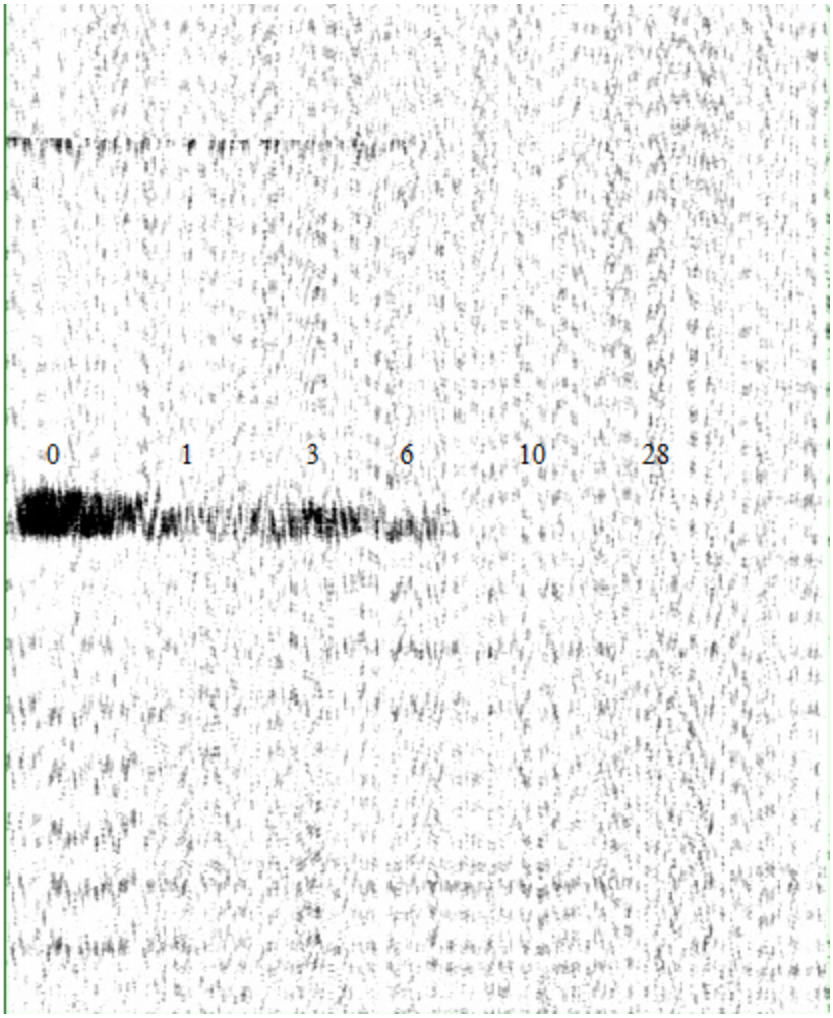
The gel is run again. The picture shows post staining of DNA by SYBR Gold. The SYBR Gold was diluted 10000 times, from 5µL to 50ml. Gel was soaked in a clean lid from a pipet-box. The following picture shows the result:




The gel is then post stained with Phen Green. The result was quite surprising as we didn't really hold out much hope that Phen Green can indeed penetrate the gel. Most of staining is found in the well. We are not sure whether the fluorescence is actually due to the absence of silver or if it is just merely because the Phen Green is stuck there. We did wash the gel after staining with DI water. Following is the result:




When we ran another imaging system (390 BP 100 PHosphor - PMT=507- Laser = Green(532) , it was shown that there was a trend in the fluorescence of Phen Green as the mismatches increase:

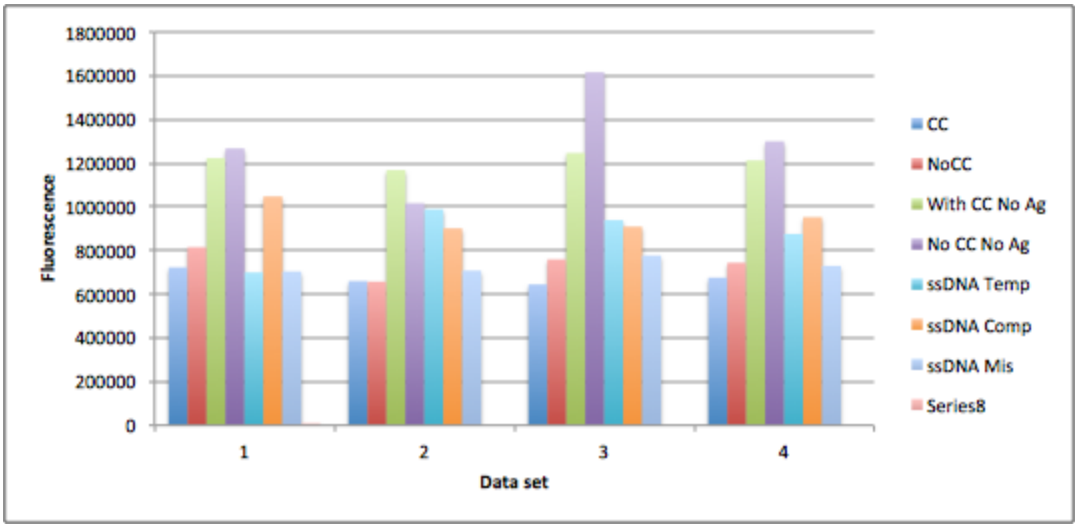



7/19: NL

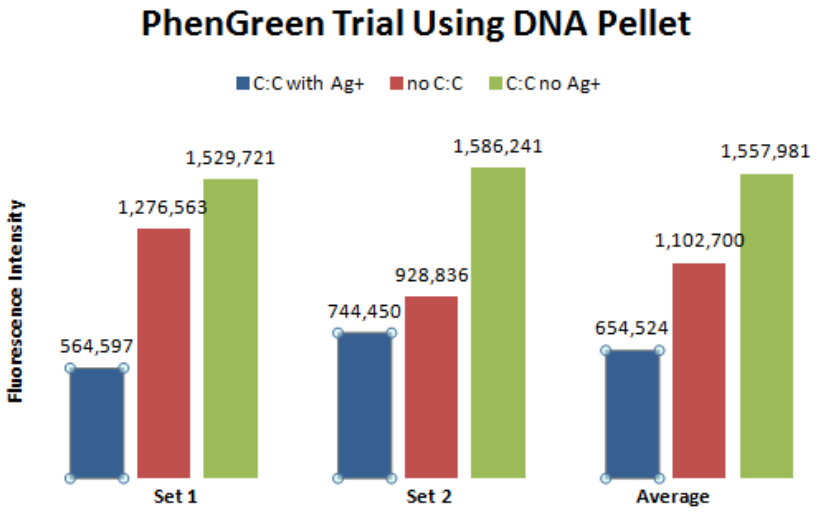
Testing Phen Green directly on the DNA pellet has resulted in quite fair data again. The standard deviation is a little bit larger than it should be, possibly due to issues with the pipet (Phen Green is distributed in only 5μL and a little bubble more or less will result in very different fluorescence). Also, the concentration of silver is much higher than 6 times of DNA this time, as it is possible that Ag+ will also be lost in lattice bonding with phosphate backbone, which is negatively charged. This explains the data may not give a largely significant difference between with and without mismatch. The fourth row indicates average value.

| CC pellet | NoCCpellet | CC-Ag pellet | NoCC-Ag pellet | Tem pellet | Com pellet | Mis pellet |
|---|---|---|---|---|---|---|
| 722.736 | 814.897 | 1.222.619 | 1.267.126 | 699.470 | 1.047.434 | 703.745 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 659.911 | 657.350 | 1.167.794 | 1.016.130 | 988.537 | 901.422 | 707.799 |
| 644.584 | 759.267 | 1.246.101 | 1.615.436 | 938.829 | 909.241 | 776.894 |
| 675.744 | 743.838 | 1.212.171 | 1.299.564 | 875.612 | 952.699 | 729.479 |



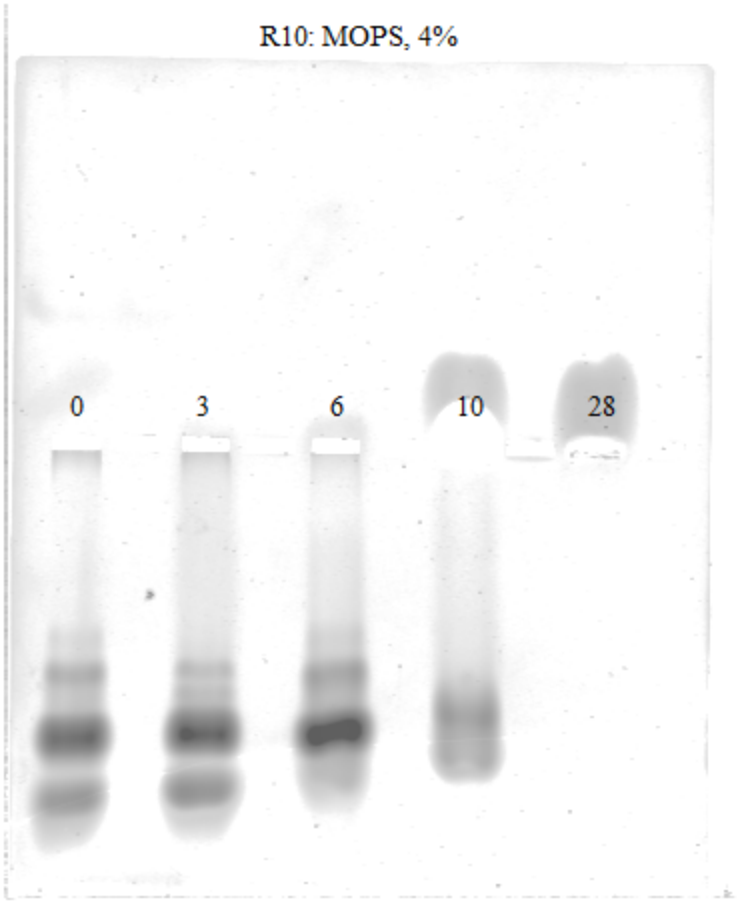

- 7/17 (part II): Data for PhenGreen looks promising. This time the DNA pellets were used, as opposed to supernatant extracted from the samples. See below for results showing that samples with mismatches had greater fluorescence than those without.




- 7/17 (part I): Ran a gel yesterday using a diluted 28 C:C mismatch solution, as well as a 10X dilution of SYBR GOLD, adding 0.5uL of the diluted dye to each sample. Results were puzzling due to the

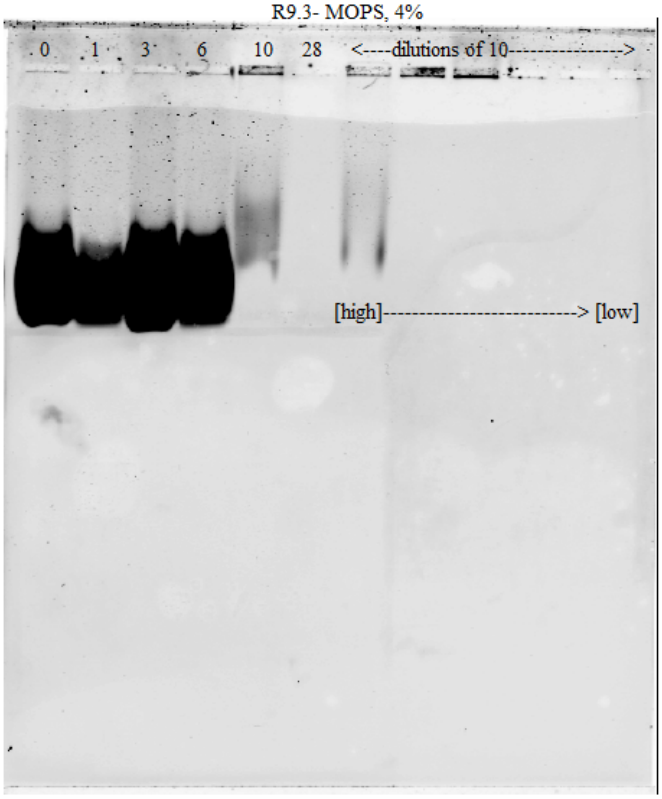
appearance of multiple bands in the control (0) lane. One theory is that running the MOPS gel at 97 V heats the solution above the duplex's $T_m$, leading to the formation of ssDNA. As seen previously, the 10 C:C DNA lagged significantly behind the rest, while the 6 C:C pattern also showed signs of drag this time. However, the appearance of multiple bands makes these results questionable. If we were to run more gels, we would run them at a cooler temperature by setting the apparatus in a refrigerator during its run time. A new idea (by Kosuke, based on Ono's work)is trying a FRET analysis: one end of the strand will have a fluorescent dye, while the other end will have its quencher. With the addition of Ag+, the ssDNA will form a hairpin, quenching the signal that will be measured by a fluorometer. Looking into ordering the desired sequence today:

**5'-(6-Fluorescein)-CTTCTCTCTCTTCTCTTCATTTTTTCAACACAACACACACAAC-(BlackHole Quencher-1**



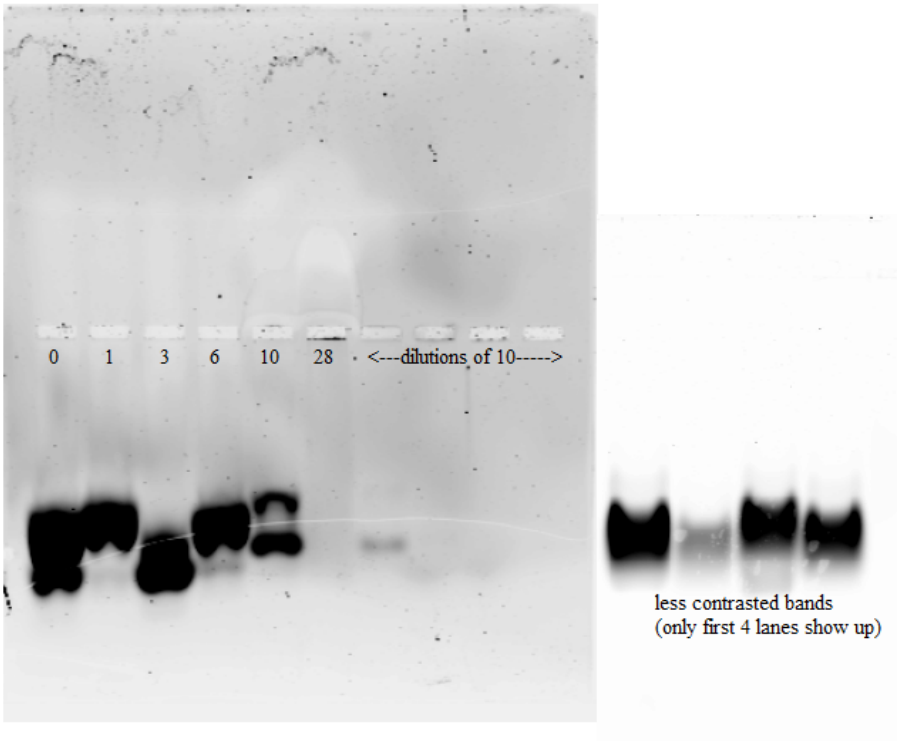

- 7/16: Ran three different gels yesterday with somewhat perplexing results. In R9.2, there is a band difference between 0 mismatches and the entire series of mismatches (1-28). These results were not seen as clearly in R9.3 or R9.1. There is, however, a consistent pattern difference with the 10 C:C mismatches, further supporting the notion that Ag+ is binding to the structure. We tried testing serial dilutions of the 10 C:C mismatch solution, but nothing beyond the most concentrated sample showed up.

R9.3- MOPS, 4%

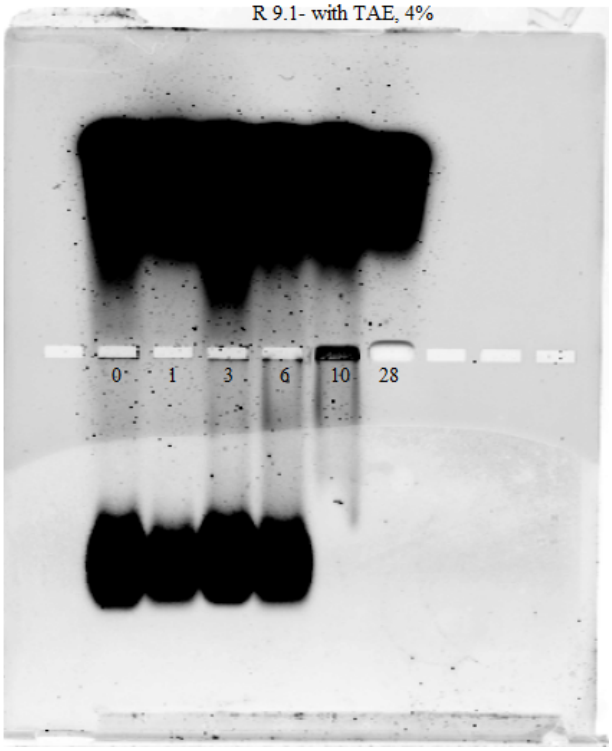



[high]----------> [low]

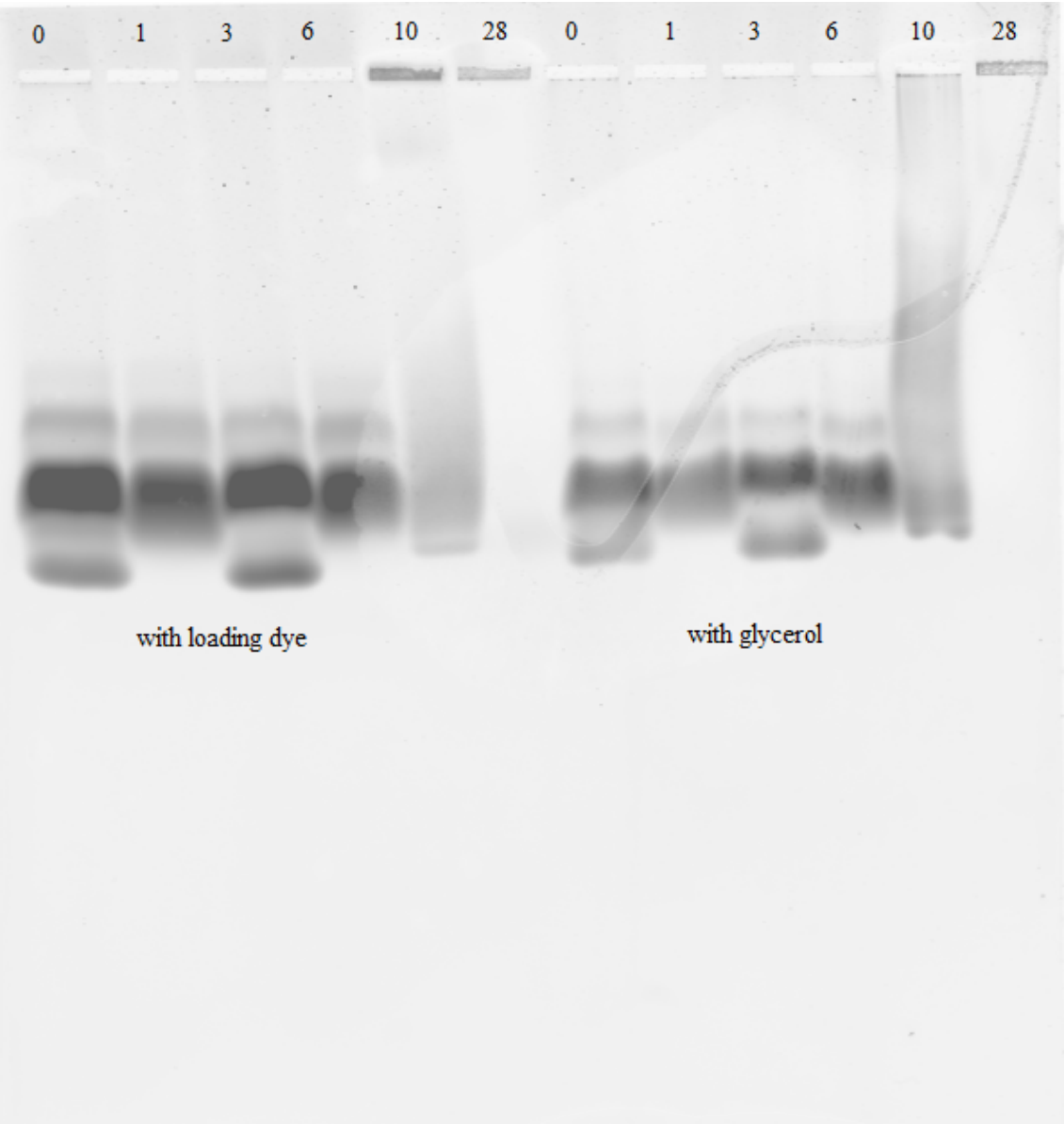
R 9.2 - MOPS, 4%



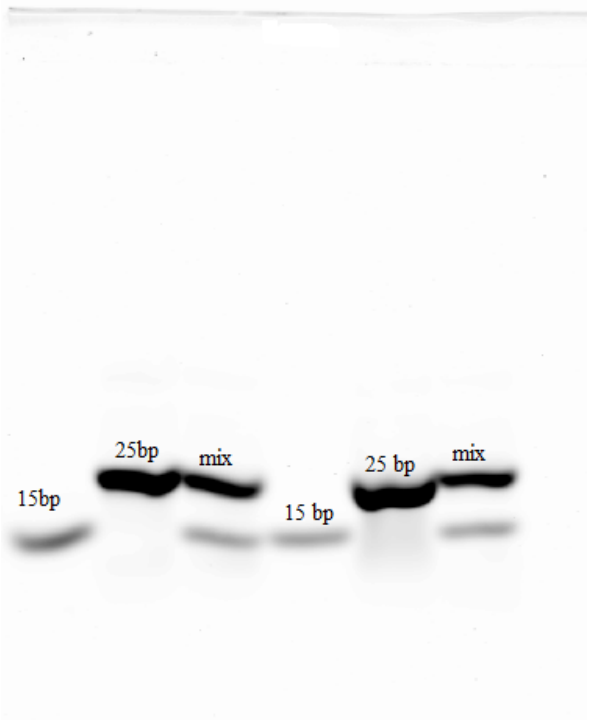

less contrasted bands
(only first 4 lanes show up)




- 7/12 (part II): PAGE gel inconclusive due to possible interference from an air bubble. Comparison of loading dye vs. glycerol showed that glycerol was more effective in imaging and perhaps had less impact on silver precipitation. There was no significant difference in patterns for 0, 1, 3, and 6 mismatch combinations when tested against each other. However, the 10 mismatch showed very noticeable dragging of DNA, possibly due to the binding of silver ions that would make its overall charge less negative (see gel below). The new goal is to run solutions of 10 C:C mismatch oligos against each other with varying dilutions of $AgNO_3$ to see if an optimal amount is capable of being detected. Another test is setting the comb in the center of the gel to test if any solutions are running backwards.




- 7/12 (partI): In the past two days, ran both a 4% and 7% gel to show separation between 15 and 25 bp oligos (see 7% below). When run in MOPS buffer with 100mM NaNO3, instrument overheated from too high of a current due to salt contribution to ion count. Also, lack of loading dye in wells caused DNA to "float" away. Making two changes to compensate for this: 1) running gel with only MOPS at pH=7 (no NaNO3) 2) running tandem solutions with loading gel or glycerol to weigh down the DNA. Gels are viewed by soaking for 15 min in a 10,000X dilution of SYBRGOLD. Also running a PAGE gel in parallel.




- 7/10: Data from PhenGreen has significant deviation between duplicate samples of the same solution (see below). Must reevaluate experimental conditions to determine reason behind variation. Also, the mass spec facility at NASA is incapable of running MS without fragmenting the DNA. Looking into Stanford options now.

PhenGreen Data: Sets 1 and 2

| | | 2 isocntrl | | 2 ethcntrl | | 3 isocntrl | | 3 ethcntrl |
|---|---|---|---|---|---|---|---|---|
| | | 11153.33 | | 10735.62 | | 10971.82 | | 11282.44 |
| | | 10926.11 | | 10664.07 | | 11210.52 | | 11858.2 |
| | average: | 11039.72 | average: | 10699.84 | average: | 11091.17 | average: | 11570.32 |
| | stdev: | 160.6653 | stdev: | 50.59066 | stdev: | 168.7843 | stdev: | 407.1196 |
| 60 uM | | | | | | | | |
| -1040.1 | | 3 iso +CC | | 3 iso - CC | | 2 iso +CC | | 2 iso -CC |
| 20 uM | | 5896.36 | | 12661.02 | | 10981.03 | | 10838.43 |
| -2155.72 | | 10475.15 | | 10610.04 | | 11585.28 | | 10816.68 |
| 2 uM | average: | 8185.757 | average: | 11635.53 | average: | 11283.16 | average: | 10827.55 |
| 561.261 | stdev: | 3237.696 | stdev: | 1450.258 | stdev: | 427.27 | stdev: | 15.37745 |
| 200 nM | | | | | | | | |
| | | | | | | 2 eth | | |
| 366.333 | | 3 eth+CC | | 3 eth-CC | | +CC | | 2 eth -CC |
| | | 12613.09 | | 11217.29 | | 10795.9 | | 11183.49 |
| | | 10946.43 | | 11803.7 | | 11022.44 | | 11210.52 |
| | average: | 11779.76 | average: | 11510.5 | average: | 10909.17 | average: | 11197.01 |
| | stdev: | 1178.503 | stdev: | 414.6517 | stdev: | 160.1851 | stdev: | 19.1131 |

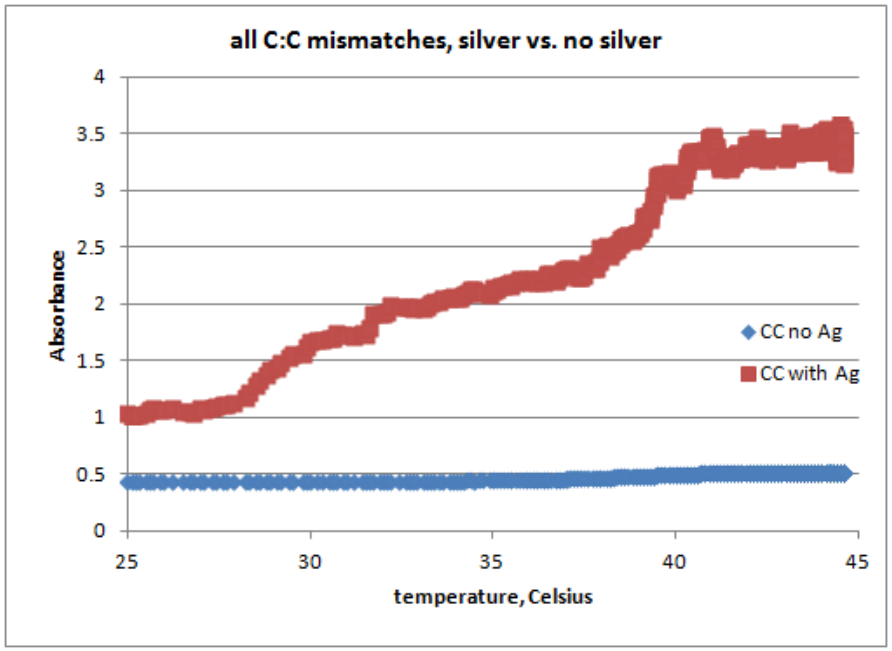
| | 2iso+CC | 2 iso-CC | 3iso+CC | 3iso-CC | | 2eth+CC | 2eth-CC | 3eth+CC | 3eth-CC |
|---|---|---|---|---|---|---|---|---|---|
| | 1470.439 | 1608.514 | 2364.271 | 2250.697 | | 1669.953 | 1691.688 | 1821.468 | 2303.7 |
| | 1454.383 | 1266.291 | 1708.019 | 1872.17 | | 1222.07 | 1411.356 | 1832.418 | 1971.039 |
| | 1504.485 | 1337.481 | 1972.939 | 1868.501 | | 1234.945 | 1364.379 | 1794.124 | 2095.216 |
| | 1494.045 | 1662.829 | 2496.538 | 2257.522 | | 1716.72 | 1772.772 | 1792.141 | 2467.034 |
| | 1473.32 | 1351.425 | 1712.563 | 1864.97 | | 1244.028 | 1398.288 | 1824.233 | 2008.969 |
| | 1514.507 | 1321.693 | 2006.335 | 1866.44 | | 1255.816 | 1426.866 | 1774.396 | 2250.182 |
| average | 1485.197 | 1424.706 | 2043.444 | 1996.717 | average | 1390.589 | 1510.892 | 1806.463 | 2182.69 |
| stdev | 22.88123 | 166.8372 | 327.5864 | 199.402 | stdev | 235.2332 | 174.5748 | 22.8046 | 191.1423 |

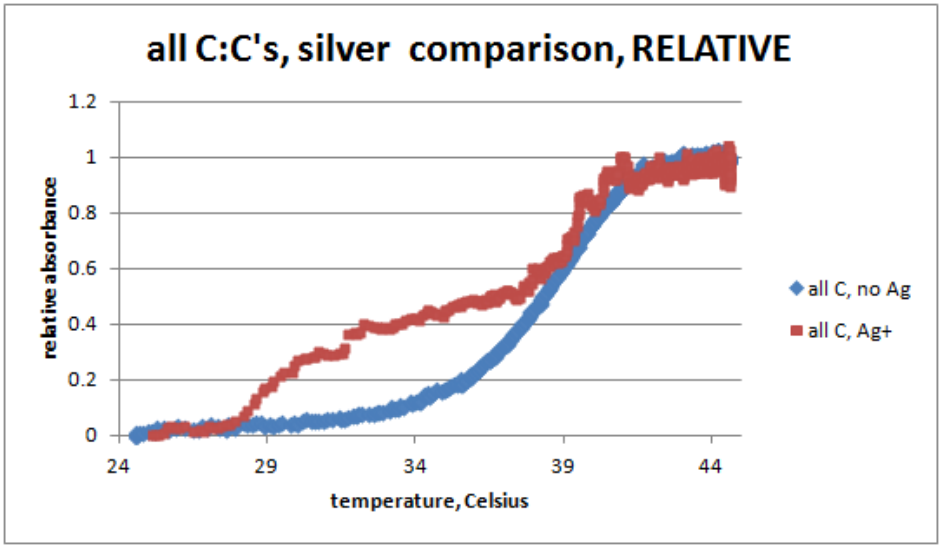
| | 2ethcntrl | 2isocntrl | 3ethcntrl | 3isocntrl | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1779.578 | 1656.319 | 1804.738 | 1681.796 | | | | | |
| | 2007.046 | 1729.247 | 1831.987 | 1866.877 | | | | | |
| | 1802.547 | 1646.691 | 1869.18 | 1703.531 | | | | | |
| | 2067.601 | 1739.408 | 1882.571 | 1852.496 | | 60 uM | 20uM | 2uM | 200nM |
| | 1349.462 | 1746.101 | 1842.882 | 1771.025 | | 280.987 | 152.319 | 236.5 | 350.628 |
| | 1261.222 | 1745.371 | 1884.945 | 1789.08 | | 276.059 | 181.364 | 250.949 | 332.98 |
| average | 1711.243 | 1710.523 | 1852.717 | 1777.468 | average | 278.523 | 166.8415 | 243.7245 | 341.804 |
| stdev | 334.9331 | 46.21192 | 31.71555 | 75.39142 | stdev | 3.484622 | 20.53792 | 10.21699 | 12.47902 |

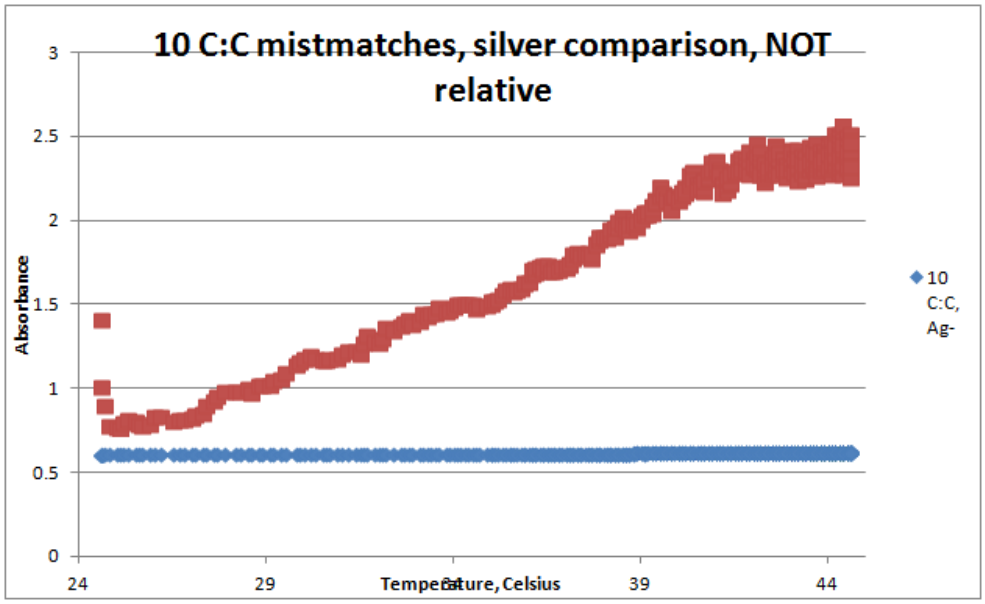
- 7/8: Data from UV spec showed differences in structure when comparing non-relative absorbance intensities. However, relative intensities show little change in melting point, even with control strands, bringing into question whether or not the structures are actually stabilized. Yet, evidence is still there that new structures are being formed in the presence of Ag+. In the paper " Specific and Nonspecific Dimer Formation in the Electrospray Ionization Mass Spectrometry of Oligonucleotides" by Ding and Anderegg, UV melts were performed on oligos and those with lack of significant increase in UV absorbance were interpreted as having no duplexes present, thereby supporting our data.
    - Results from PhenGreen show an overall increase in fluorescence with the introduction of DNA into the silver solution, meaning that the silver ions are being taken up by the DNA.

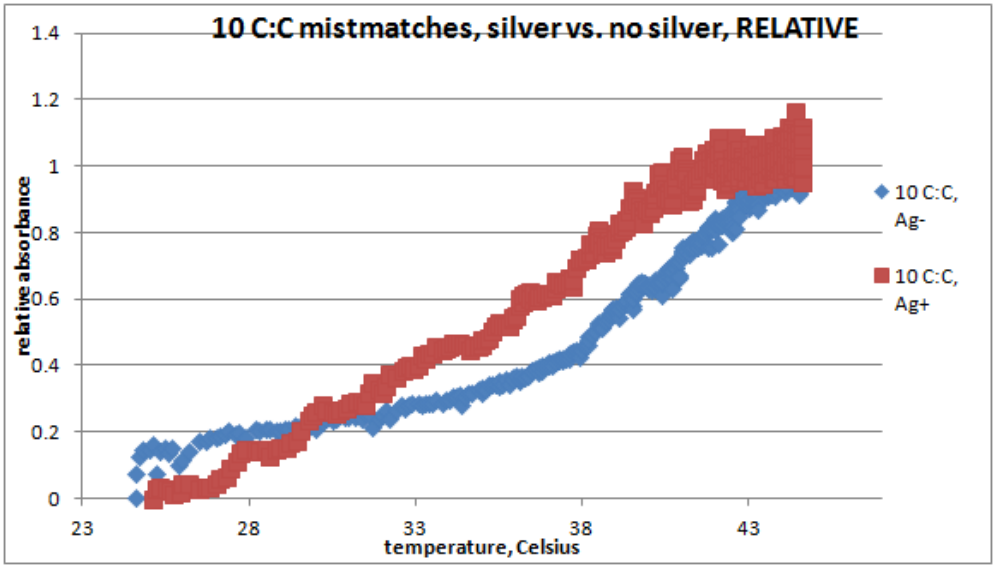
However, differences between solutions were quantitatively small, and need to be replicated to reach a definite conclusion. Hoping to get access to ICPMS, NMR, and mass spec within the next couple of weeks.

UV Spec Graphs:



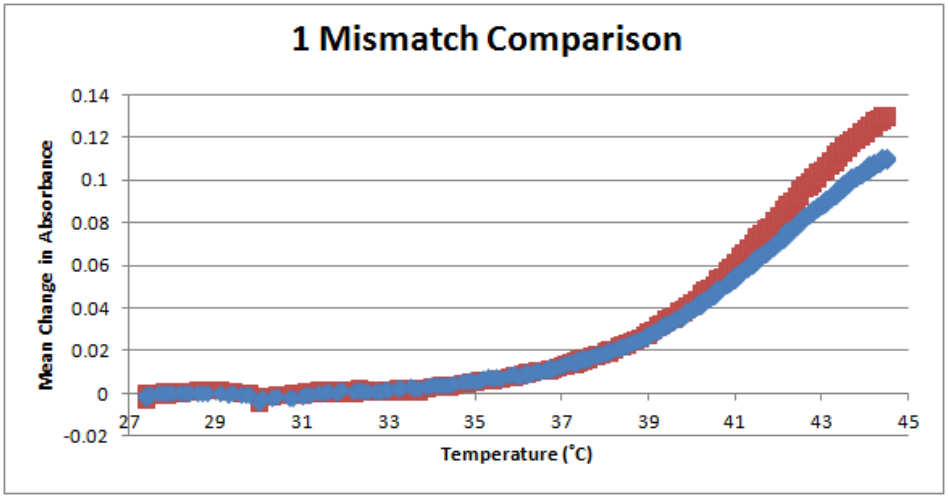


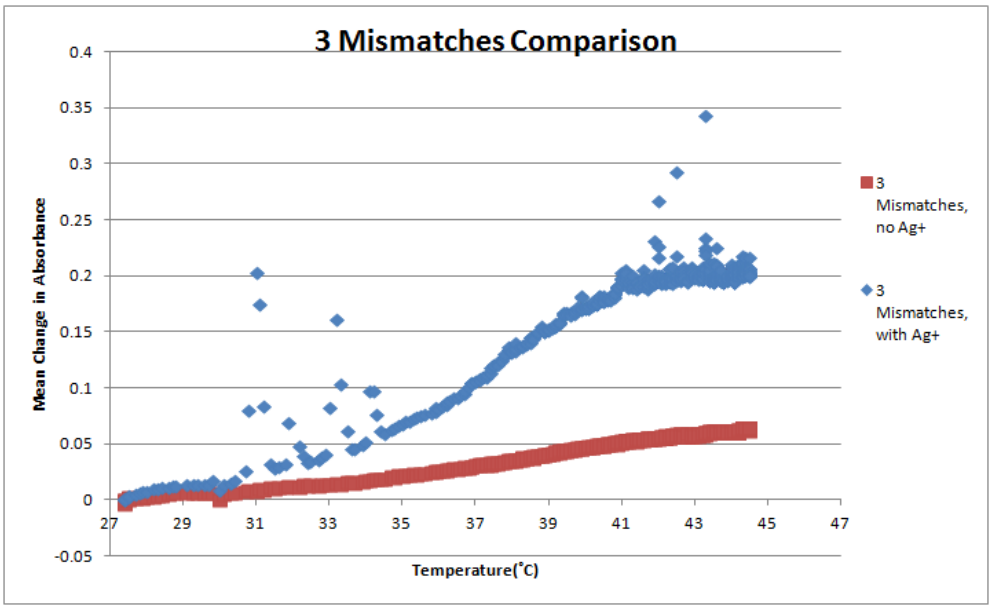




**note: Arel = (A,given temp - A, start temp)/(A, end temp - A, start temp)**

- 6/24: rtPCR and UVspec were inconclusive; in fact, UVspec tended to show the opposite of what we wanted, namely that the solutions containing Ag+ showed a **lower melting point** than solutions without Ag+. Issues could include buffer, formation of stock solutions, or improper formation of duplexes.

  <u>Example UV Spec Data:</u> Comparison of Mean Change in Absorbance


1 Mismatch Comparison
Mean Change in Absorbance
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
27
29
31
33
35
37
39
41
43
45
Temperature (˚C)


3 Mismatches Comparison
Mean Change in Absorbance
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
27
29
31
33
35
37
39
41
43
45
47
Temperature(˚C)
3 Mismatches, no Ag+
3 Mismatches, with Ag+