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13. ABSTRACT ***(Maximum 200 Words)***

The goal of this project was to develop a means to separate target DNA oligomers from complex mixtures using a "tag-and-separate" approach that involves the use of surfactants that bind specifically to short sequences of the DNA targets. The surfactants include a peptide nucleic acid (PNA) segment to provide highly specific binding to DNA targets. Separation is achieved using capillary electrophoresis, the standard method of fluid manipulation used in lab-on-a-chip devices. A small unknown sample is first mixed with the PNA surfactants ("PNAA") to tag the DNA targets, and then the sample is flushed with conventional surfactant micelles to pick up the tagged DNA targets. The method has several advantages over other specific DNA separation methods, such as the use of magnetic beads. Binding of PNAA to DNA occurs in solution, rather than on a bead surface, improving binding kinetics and preventing fouling of the bead surface by adsorption of proteins or lipids. The method is also compatible with longer DNA targets possessing overhanging stretches of DNA. The use of surfactants also helps to keep the capillary wall clean and free of adsorbed protein and lipid. The method can also distinguish both sequence and molecular weight of the target DNA.

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July 28, 2006

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Executive Summary

The goal of this project was to develop a means to separate target DNA oligomers from complex mixtures using a "tag-and-separate" approach that involves the use of surfactants that bind specifically to short sequences of the DNA targets. The surfactants have a unique structure that includes a peptide nucleic acid (PNA) segment to allow for binding to DNA targets to tag them for separation. PNAs bind DNA oligomers with superior stability and specificity, minimizing the likelihood of binding non-targets. Separation is achieved using capillary electrophoresis, the standard method of microscale fluid manipulation used in lab-on-a-chip devices and DNA analysis systems. A small unknown sample is first mixed with the PNA surfactants (hereafter referred to as "PNA amphiphiles" or "PNAA") to tag the DNA targets, and then the sample is flushed with conventional surfactant micelles to pick up the tagged DNA targets.

The use of surfactant micelles for separation has several advantages over other specific DNA separation methods, such as the use of magnetic beads. One is that binding of the PNAA to DNA occurs in solution, rather than on a bead surface, so that binding kinetics are fast and not subject to bead fouling by adsorption of proteins or lipids to the bead surface. The method is also compatible with longer DNA targets with overhanging stretches of DNA surrounding the target. These overhanging stretches interfere with binding to oligomers attached to hard surfaces, but are compatible with the soft surface presented by micelles. The use of a large amount of surfactant also helps to keep the capillary wall clean and free of adsorbed protein and lipid. Finally, as we demonstrate below, the tag-and-separate method can distinguish both sequence and molecular weight of the target DNA oligomer.

Methods that purify DNA targets from complex solution will help more reliably analyze cellular and environmental samples for their genomic content, with applications in biowarfare detection, forensic analysis, and medical diagnostics. Pre-enrichment for specific DNA targets will also decrease the incidence of false-positives and maximize the effectiveness of ultrasensitive DNA detection methods such as polymerase chain reaction (PCR), gold nanoparticles, and molecular beacons.

The major accomplishments of the project are summarized below:

- Synthesized PNA amphiphiles that are compatible with capillary electrophoresis in standard UV-vis or fluorescence mode
- Demonstrated sequence-selectivity of the tag-and-separate approach
- Determined the morphology and critical micelle concentration for various PNAA and their mixtures with conventional surfactants
- Created a series of PNA-functionalized liposomes for sensing applications
- Completed a thorough study of the adsorption of PNAA-tagged DNA to micelles in capillary electrophoresis, allowing for determination of target molecular weight
- Observed a strong impact of PNAA chain length on adsorption of tagged DNA to micelles, allowing for multiplexed separations
- Increased the dynamic range of the method to nearly 1000-base PCR products by the use of high micelle concentrations and long PNAA chains
- Demonstrated the feasibility of attachment of more than one PNAA to targets, leading to even greater separation resolution
- Implemented the separation using coated capillaries and demonstrated compatibility with biological fluids (goat serum)
- Synthesized a DNA amphiphile (DNAA) suitable for use as an aptamer-surfactant for protein purification by tag-and-separate methods

Micellar Electrokinetic Chromatography (MEKC) for Separation of PNAA-Tagged DNA

The basic design of PNA amphiphiles consists of the PNA peptide itself, flanked by a 12-18 carbon alkane group on one side and conventional, charged amino acids on the other. The charged groups are required to confer solubility on the otherwise nonpolar PNAA. The typical lysine modification is incompatible with capillary electrophoresis (CE) methods, as it would likely adsorb to the negatively charged silica capillary wall. We synthesized a series of PNAA variants with 12-to-18-carbon alkanes and negatively charged glutamic acid groups as depicted in Figure 1. We found that the glutamic acid modification provided good solubility along with tight binding to DNA in buffers at pH 7 and above. Details for the PNAA synthesis can be found in the literature. [1, 2]

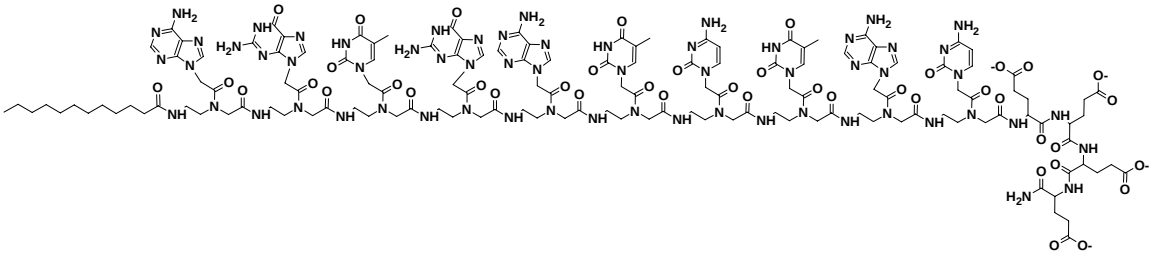

Figure 1. Structure of the PNA Amphiphile C_{12}-agtgatctac-$(Glu)_4$

Binding of the PNAA to DNA was assessed directly in the CE unit (experimental details in Appendix 1). Attachment of the modestly charged PNAA to DNA gave rise to a measurable shift in the elution time (recast here as electrophoretic mobility, Appendix 1) as shown in Figure 2. Similar experiments run with DNA possessing a single-base mismatch showed no shift in mobility, indicating a complete lack of binding. This extreme sensitivity to sequence mismatches is a hallmark of PNA-DNA duplex formation and is preserved in these PNAA implemented in capillary electrophoresis.

Attachment of a single PNAA to DNA oligomers does not provide a significant enough mobility shift to separate tagged DNA from untagged DNA of appreciable length (greater than 20 bases in length). Instead, we rely on the ability of PNAA to adsorb to micelles to provide the requisite mobility shift. Micellar electrokinetic chromatography (MEKC) is a well-established means of separating nonpolar compounds in aqueous solution based on their affinity for surfactant micelles. It involves first introducing a small amount of an unknown sample into the capillary (by pressure injection), then flushing the sample with a solution of micelles (by electrokinetic flow). Generally, the nonpolar compounds are uncharged, and the charged micelles (of an ionic surfactant) are used to sweep the analytes through the capillary.

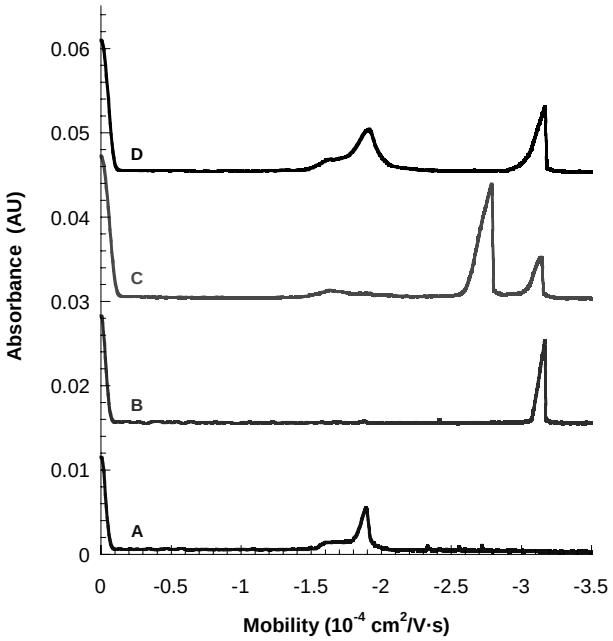

Figure 1. Electropherograms of complementary and non-complementary DNA depicting PNAA/DNA binding detection capabilities. Peak at μ =0 is the neutral marker, benzyl alcohol. (A) 100 uM C_{12}-agtgatctac-E_4 PNAA (B) 100 uM DNA (C) 100 uM C_{12}-agtgatctac-E_4 PNAA + 100 uM complementary DNA (D) 100 uM C_{12}-agtgatctac-E_4PNAA + 100 uM non-complementary DNA.

In our implementation of MEKC, we have a charged analyte (DNA), so nonionic (uncharged) micelles are used. A scheme of the separation is depicted in Figure 3. First, the PNAA are introduced into an unknown DNA sample to allow for PNAA binding to targets. This mixture is hydrodynamically injected into the capillary, then flushed with micelles of Triton X-100 (a nonionic surfactant). Since electrophoresis opposes electro-osmosis in these systems, those components with the highest electrophoretic mobility will take the longest time to emerge from the capillary. The uncharged micelles are quickly swept out by the electro-osmotic flow, and carry PNAA-tagged DNAs with them, displacing them from the untagged DNA.

The effective mobility (μ_{eff}) of the tagged DNA in the presence of micelles at a concentration *[M]*, is given by the following expression (Appendix 2):

$$\mu_{eff} = \frac{\mu^{o}_{Dup} + K[M]\mu^{o}_{mic}}{1 + (K + C_{visc})[M] + KC_{visc}[M]^2} \qquad (1)$$

where C_{visc} is a viscosity correction (accounting for the slight change in solution viscosity with *[M]*, *K* is the partition coefficient of the tagged DNA with the micelles, and μ^{0}_{mic} is the mobility of the micelle with the attached PNAA/DNA duplex. It is important to note that the value of μ^{0}_{mic}will become more negative as the molecular weight of the attached DNA increases. As such, the molecular weight of the tagged DNA can be obtained with a suitable calibration curve.

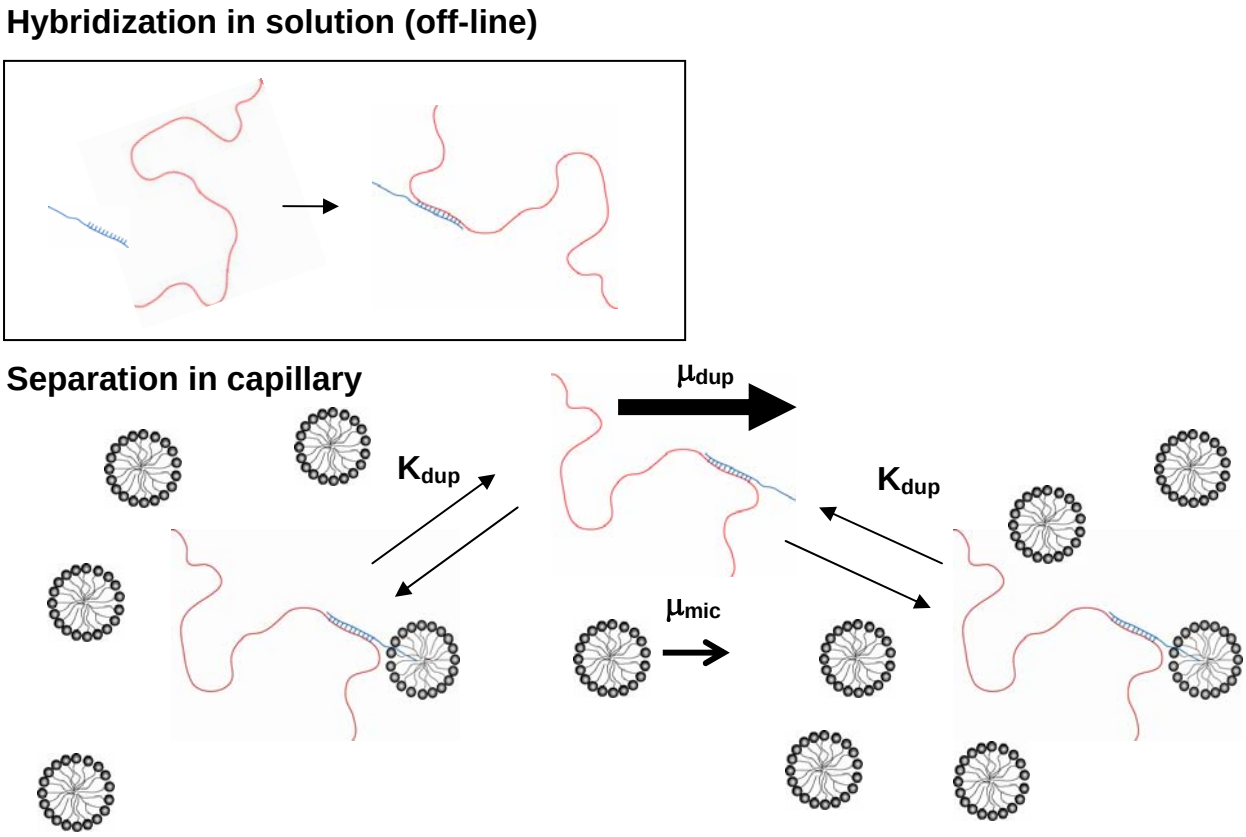

Figure 3. Schematic of "tag-and-separate" method involving attachment of PNAA followed by separation in micellar electrokinetic chromatography (MEKC).

Figure 4 shows a typical electropherogram for a separation of 20-base DNA in the presence of Triton X-100 micelles. The data can be reduced in the form of plots of the effective PNAA/DNA duplex mobility versus *[M]* (Figure 5) and the partition coefiicient versus [M] (Figure 6). Given the partition coefficient and the micelle concentration, the micelle mobility can be determined from the effective mobility of an arbitrary sample (Figure 7). The length of the DNA can be obtained from the electrophoretic mobility using Figure 7.

A remarkably strong dependence of the PNAA alkane chain length on the partitioning behavior was also observed as shown in Figure 8. Holding all other variables constant, we see that the effective PNAA/DNA duplex mobility shifts dramatically with increasing PNAA alkane length due to a sharp increase in the micelle partition coefficient. Not only does this increase the dynamic range of the method, it also suggests that separations of multiple DNA targets can be accomplished in a single run by attaching different PNA probes to different alkane tails. Results of a proof-of-concept experiment for multiplexed separations are given in Figure 9. Note that, for an unknown sample, a single run could not unambiguously identify both the molecular weight and identity of a bound DNA, but this is easily achieved by performing two or three runs at different micelle concentrations to decouple the effects.

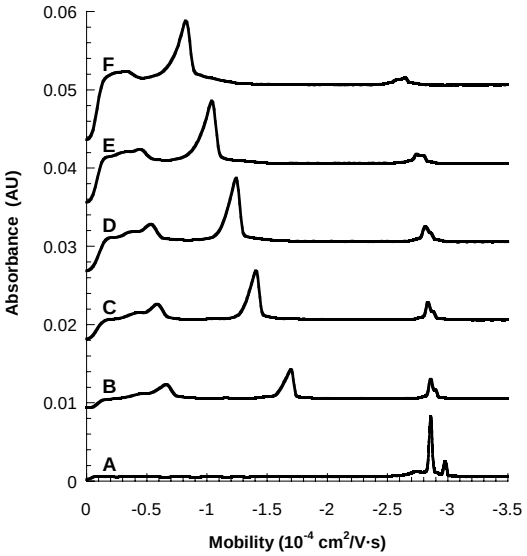

Figure 2. Electropherograms of 50 uM C_{18}-agtgatctac-E_4 PNAA + 50 uM complementary 20 base DNA with varying micellar phase concentrations. (A) 0 mM Triton X-100 (B) 2.4 mM Triton X-100 (C) 4.8 mM Triton X-100 (D) 8 mM Triton X-100 (E) 1 mM Triton X-100 (F) 36 mM Triton X-100.

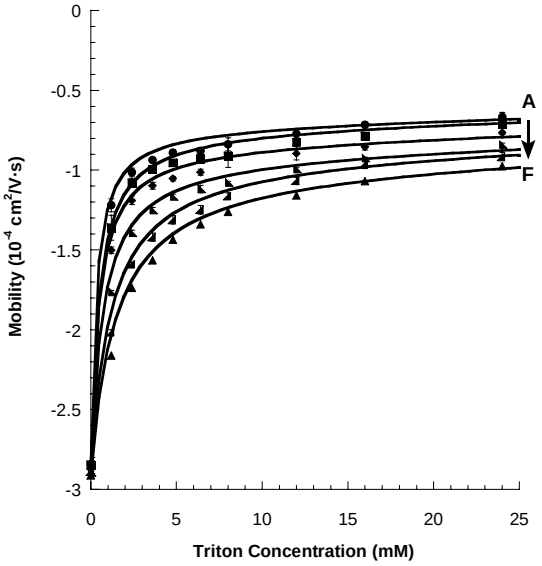

Figure 4. Electrophoretic mobility of the PNAA/DNA duplex as a function of the Triton X100 surfactant concentration for various lengths of complementary DNA (A) 10 base DNA ● (B) 12 base DNA ■ (C) 14 base DNA ◆ (D) 16 base DNA ◣ (E) 18 base DNA ◢ (F) 20 base DNA ▲.

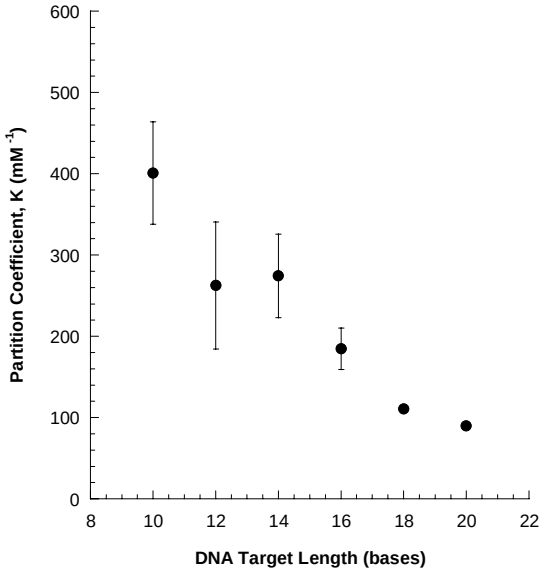

Figure 6. Effective partition coefficient *K* as a function of DNA target length.

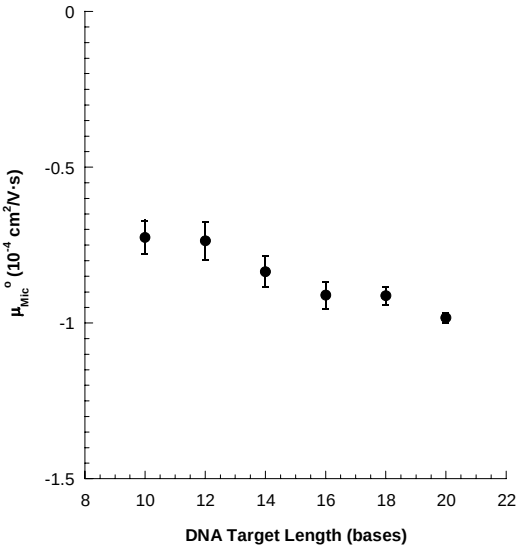

Figure 7. Effective micelle mobility μ_{mic}^{o} as a function of DNA target length.

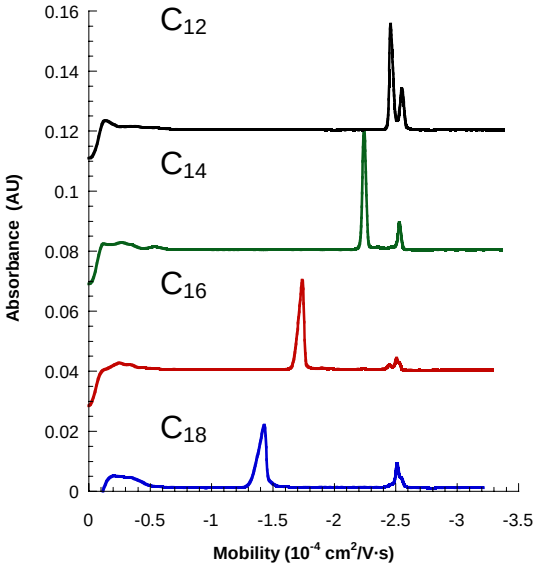

Figure 8. Effect of PNAA tail length on effective mobility in MEKC (48 mM Triton X-100).

PNAA: C_x-agtgatctac-$(Glu)_4$;
DNA: T_{25}-TCACTAGATG-T_{25}.

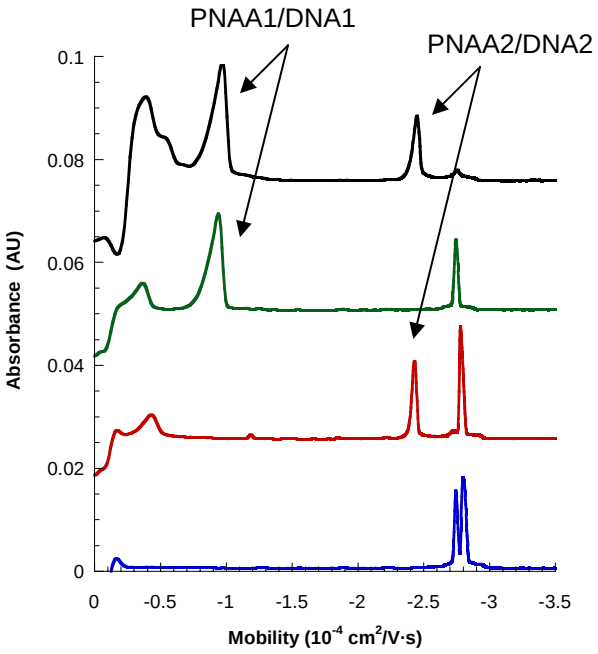

Figure 9. Muliplexed DNA separations using PNAA with differing tail lengths.

PNAA1: C_{12}-agtgatctac-$(Glu)_4$
PNAA2: C_{18}-tgataccgct-$(Glu)_4$
DNA1:CCCCCTCACTAGATGCCCCC
DNA2: CCCCCACTATGGCGACCCCC

Separation of longer DNA oligomers by the tag-and-separate method has also been demonstrated. Since synthetic methods are limited to oligomers about 60-100 bases in length, longer DNA oligomers were prepared by asymmetric PCR (Appendix 3). Briefly, the method uses an unequal amount of forward and reverse primer to obtain a mixture of single- and double-stranded DNA products. Figure 10 shows a separation of 447-base DNA from an untagged fraction, demonstrating a high-resolution separation. PCR-based synthesis of longer DNA oligomers has presented some challenges that we are working to overcome, and in principle, separation of oligomers up to 1000 bases in length can be expected.

Attachment of multiple PNAs to a single DNA target is another option to extend the dynamic range of the method to longer oligomers. Figure 11 shows the separation of a 447-base target with attachment of two PNAA at the same time. Roughly, there is about a doubling of the extent of micelle partitioning observed under these conditions. Note that bind of both PNAAs to the target is not perfect, and some singly-bound DNA is also present. We believe this can be addressed by refinements in the PNAA binding protocol.

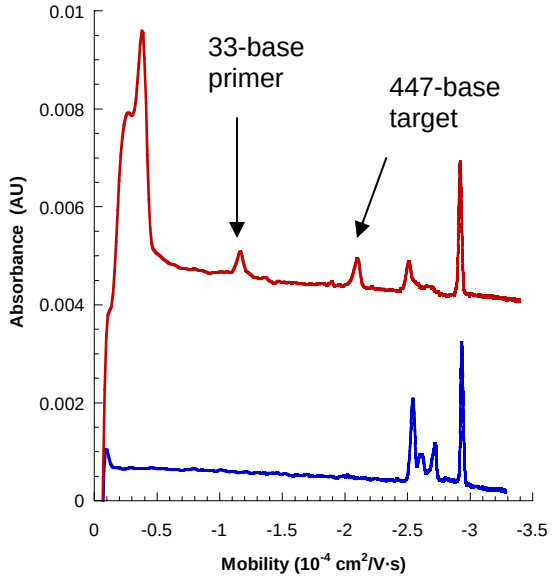

Figure 10. Separation of asymmetric PCR products. 64 mM Triton X-100. Lower trace: PCR product alone; Upper trace: PCR product + PNAA. 33-base primer is also complementary to the PNAA and is shown for comparison.

PNAA: C_{18}-agtgatctac-$(Glu)_4$
DNA: 447-base aPCR product

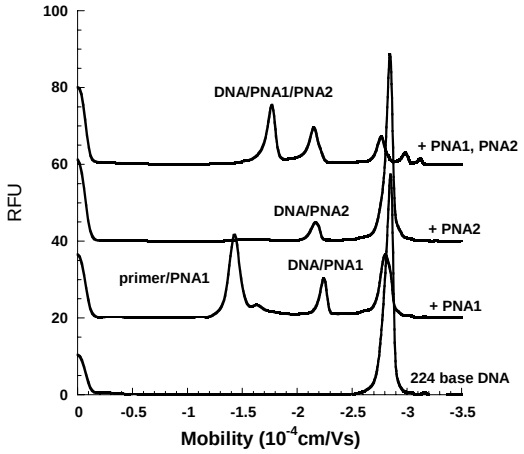

Figure 11. Effect of attachment of multiple PNAA to a single DNA target. 48 mM Triton X-100.

PNAA1: C_{18}-agtgatctac-$(Glu)_4$
PNAA2: C_{18}-tgataccgct-$(Glu)_4$
DNA: 228-base PCR product

Compatibility with Biological Fluids

Of key interest for this project is the compatibility of the protocol with biological fluids, for example, serum. Serum proteins are expected to have a much lower effective mobility than DNA, but may co-elute with PNAA/DNA duplexes if the conditions are right. Eq 1 demonstrates that the effective mobility of the PNAA/DNA can be tuned by adjusting the micelle concentration *[M]* so that the protein peak and PNAA/DNA peaks can be resolved. This also requires that protein contaminants elute in a fairly well-defined band, rather than a smear. While serum proteins do not have a high affinity for the uncoated silica capillary, we found significant adsorption in the presence of the high concentrations of Triton X-100 used in these experiments. Adsorption was also significant using two different coated capillaries. Adsorption is problematic as it can lead to clogging of the capillary and drift of the electric current in the capillary.

The use of mixtures of ionic and non-ionic surfactants solves this problem. Figure 12a shows a separation of PNAA/DNA from unbound DNA in a coated capillary (linear polyacrylamide, eCAP Neutral Capillary, Beckman-Coulter) using a 50/50 mixture of sodium dodecyl sulfate (SDS, an ionic surfactant) and Triton X-100. It should be noted that the composition of the micelles is expected to be predominantly Triton X-100 since the critical micelle concentration of SDS is much higher than that of SDS. Figure 12b shows the same separation in the presence of a 10x dilution of goat serum (Zymed). Note that the peak

positions are not altered by the presence of the serum, but the serum proteins are visible as a low shoulder around the 1-min. migration time. The addition of SDS (mostly in monomer form) is required to denature and surround the serum proteins to prevent adsorption. Note that the elution order is reversed in this case due to the suppression of electro-osmotic flow by the capillary coating.

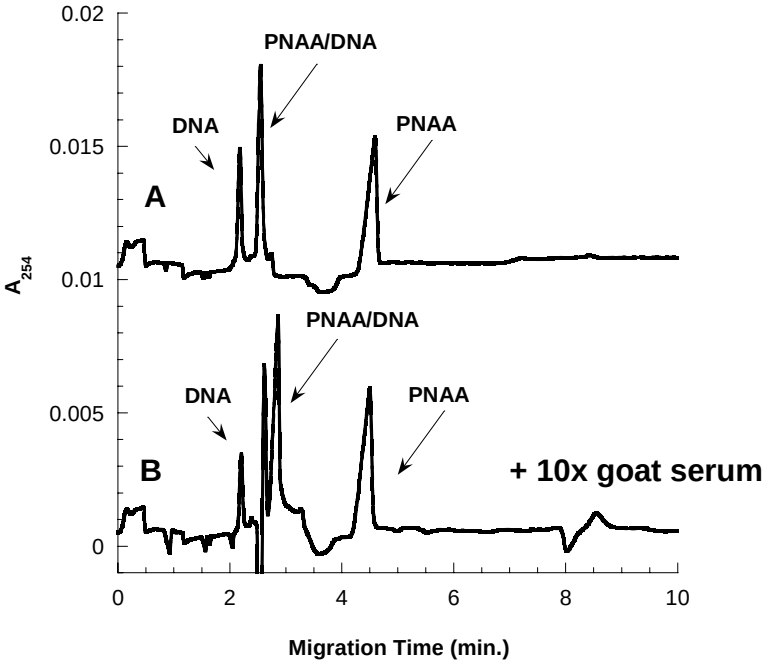

Figure 12. Separations of PNAA-tagged DNA from unbound DNA in the absence (A) and presence (B) of a 10x diluted sample of goat serum. A linear poly acrylamide (LPA)-coated capillary was used to suppress protein adsorption to the capillary wall. The LPA coating also suppressed electro-osmotic flow, leading to a reversal of the order of elution for the three components. 48 mM Triton X-100 and 48 mM SDS. PNAA: C_{18}-agtgatctac-$(Glu)_4$; DNA: 20-mer complement

DNA Amphphiles for Protein Purification

For some applications, it may be desirable to use a DNA tag, rather than PNA. One example is the use of aptamers to target proteins for proteomic analysis. Aptamers are 20-40 base DNA oligomers that are selected by a combinatorial screening technique for their affinity to proteins. These compounds are of great interest since they provide binding constants that compete with antibodies, but are cheaper to produce and can be amplified by PCR methods. We have recently developed a method to synthesize a DNA amphiphile with this goal in mind. Briefly, the synthetic method follows that of Natt and Haner, [3] in which a lipophilic capping reagent is prepared (in this case, containing an 18-carbon tail), and linked to a DNA oligomer during its solid-phase synthesis. Two different versions were prepared; a 20-mer and a 34-mer, and each represents a primer that can be used to extend the

DNAA via PCR. Figure 13 is a plot showing the effective mobility of these DNAAs in the presence of Triton X-100 micelles, and they appear to have partitioning properties similar to DNA bound to PNAA.

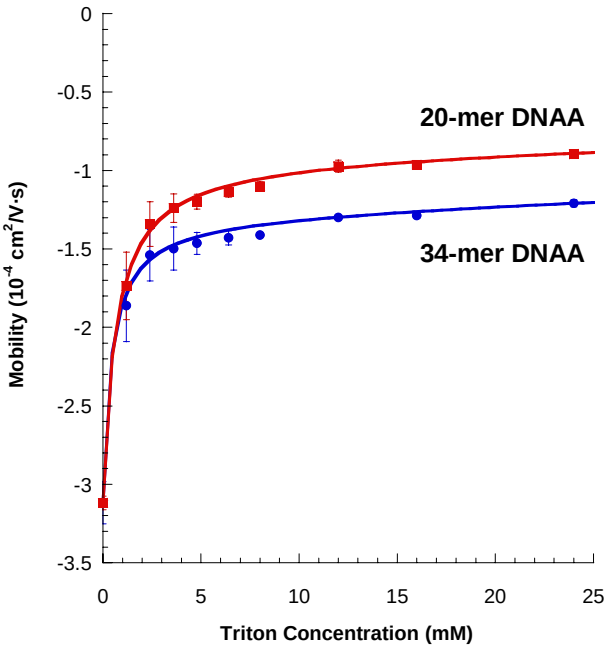

Figure 13. Effective mobility vs. [TX-100] for two 18-carbon DNA amphiphiles (20-mer and 34-mer).

Micellization Properties of PNA Amphiphiles

To better understand the electrophoretic properties of PNAA, we have conducted a thorough study of the micellization properties of PNAA. Using synchrotron x-ray methods, we found that the morphology of the pure PNAA micelles is that of a prolate ellipsoid, and mixed PNAA/SDS micelles form spherical micelles. On addition of complementary DNA, the PNAA/DNA duplexes do not participate in micellization, likely due to the solulibizing power of the attached DNA. Full details of these results are available in the literature.[4]

Liposomes composed of PNAA Amphiphiles

With an eye toward the use of PNA amphiphiles in highly sensitive sensing applications, we have also constructed PNAA with di-alkyl (two-chain) end groups suitable for the preparation of liposomes. PNAA liposomes bind complementary DNA, but with somewhat more stringent limits on the target DNA length owing to electrostatic repulsion between the DNA and the PNAA liposome surface. Generally, 20-mer DNA targets are retained in the PNAA liposomes in 10 mM Tris buffer and 60-mer targets are retained in 1 M Tris buffer. Full details on the PNAA liposomes are available in the literature. [5-7]

Personnel Supported

Faculty:	Dr. James W. Schneider, Associate Professor
Postdoctoral Researcher	Dr. Cheryl Lau
Graduate Research Assistants	Shane Grosser, Bruno Marques, Oxana Selivanova

Publications

1."Purification of PCR Products using PNA Amphiphiles in Micellar Electrokinetic Chromatography," S.T. Grosser, J.M. Savard and J.W. Schneider, submitted to *J. Am. Chem. Soc.* (2006).

2."Specific DNA Oligomer Purification using PNA Amphiphiles in Micellar Electrokinetic Chromatography," S.T. Grosser and J.W. Schneider, submitted to *J. Chrom B.* (2006).

3."Effect of Electrostatic Interactions on Binding and Retention of DNA Oligomers to PNA Liposomes Assessed by FRET Measurements," B.F. Marques and J.W. Schneider, in press for *Coll. Surf. B* (2006).

4. "Sequence-Specific Purification of DNA Oligomers in Hydrophobic Interaction Chromatography using Peptide Nucleic Acid Amphiphiles: Extended Dynamic Range," J.M. Savard and J.W. Schneider, submitted to *Biotech. Bioeng.* (2006).

5."Morphological Characterization of Self-Assembled Peptide Nucleic Acid Amphiphiles," C. Lau, R. Bitton, H. Bianco-Peled, D.G. Schultz, D.J. Cookson, S.T. Grosser, and J.W. Schneider, *J. Phys. Chem. B* 110:9027-9033 (2006).

6. "Sequence-Specific Binding of DNA to Liposomes Containing di-Alkyl Peptide Nucleic Acid (PNA) Amphiphiles," B.F. Marques and J.W. Schneider, *Langmuir* 21:2488-2494 (2005).

7."Thermodynamic and Structural Characterization of Amino Acid-Linked Dialkyl Lipids," S. Tristam-Nagle, R.N.A.H. Lewis, J.W. Blickenstaff, M. DiPrima, B.F. Marques, R.N. McElhaney, J.F. Nagle, and J.W. Schneider, *Chem. Phys. Lipids* 134:29-39 (2005).

8."Peptide Nucleic Acid (PNA) Amphiphiles: Synthesis, Self-Assembly, and Duplex Stability," J.P. Vernille, L.C. Kovell, and J.W. Schneider, *Bioconj. Chem.* 15:1314-1321 (2004).

9."Sequence-Specific Purification of Oligonucleotides using Peptide Nucleic Acid Amphiphiles in Hydrophobic Interaction Chromatography," J.P. Vernille and J.W. Schneider, *Biotechnol. Prog.* 20:1776-1782 (2004).

Interactions/Transitions

a) Presentations

Invited:

1. "Isolation and Concentration of Biomarkers Using Self-Assembled Nanomaterials," Bionanotechnology Session (Plenary), *AIChE Annual Meeting*, San Francisco CA (Nov. 2006).
2. "Adsorption and Binding of DNA to Functionalized Surfactant Micelles and Liposomes," Department of Physics, Georgetown University (Sept. 2005).
3. "Partitioning of PNA between Aqueous and Nonpolar Phases," Los Alamos National Laboratory, Los Alamos NM (July 2005).
4. "Self-Assembly of Peptide Nucleic Acid Amphiphiles in the Presence of DNA," *229th ACS National Meeting*, San Diego CA (Mar. 2005).
5. "Purification and Concentration of DNA by Surfactant Hybridization," Los Alamos National Laboratory, Los Alamos NM (Jan. 2005).
6. "Purification and Concentration of DNA by Surfactant Hybridization," Dept. Chemical Engineering, Tulane University, New Orleans LA (Nov. 2004).
7. "Purification and Concentration of DNA by Surfactant Hybridization," Dept. Chemical Engineering, McGill University, Montreal QC (Oct. 2004).
8. "Dynamic Mechanical Probing of Soft Surfaces Using AFM," *AIChE Annual Meeting*, San Francisco CA (Nov. 2003).
9. "Peptide Amphiphiles as Biomimetic Materials," *Air Force Cell-Like Entity Biotechnology Symposium*, Dayton OH (June 2003).
10. "Surfactant-Based DNA Extraction Processes," *Transport Phenomena and Separation Processes Session, 3rd Chemical Engineering Conference for Collaborative Research in Eastern Mediterranean*, Thessaloniki Greece (May 2003).
11. "Manipulating and Processing Genetic Material Using Surfactant Systems," Dept. Chemical Engineering, University of Delaware, Newark DE (Mar. 2003).

Contributions:

1. "Surface Diffusion of DNA Oligonucleotides on Patterned Silane Surfaces," T. Crites and J.W. Schneider, *AIChE Annual Meeting*, Cincinnati OH (2005).
2. "Synchrotron X-ray Characterization of PNA-Amphiphile Micelles," S.T. Grosser, C. Lau, and J.W. Schneider, *AIChE Annual Meeting*, Cincinnati OH (2005).
3. "Binding of Target DNA with Overhanging Bases to DNA Probes in Lipid Bilayers and Micelles," B.F. Marques, S.T. Grosser, and J.W. Schneider, *AIChE Annual Meeting*, Cincinnati OH (2005).
4. "Sequence Specific Separation of Target DNA in Micellar Electrokinetic Chromatography," S.T. Grosser and J.W. Schneider, *AIChE Annual Meeting*, Cincinnati OH (2005).
5. "Oligonucleotide Purification by Selective Micelle Partitioning in Capillary Electrophoresis," S.T. Grosser, O. Selivanova, and J.W. Schneider, *13th International Conference on Biopartitioning and Purification*, Amsterdam Netherlands (2005).

6. "DNA Separations using Peptide Nucleic Acid Amphiphiles as Affinity Tags in Hydrophobic Interaction Chromatography," J.M. Savard and J.W. Schneider, *13th International Conference on Biopartitioning and Purification*, Amsterdam Netherlands (2005).
7. "Sequence Specific Separation of Target DNA in Micellar Electrokinetic Chromatography," S.T. Grosser and J.W. Schneider, *229th ACS National Meeting*, San Diego CA (2005).
8. "Electrostatic and Hydrophobic Interactions of DNA Oligomers with Peptide Nucleic Acid (PNA) Liposomes (poster)," B.F. Marques and J.W. Schneider, *229th ACS National Meeting*, San Diego CA (2005).
9. "DNA Detection with Fluorescently Tagged Surfactant Microstructures (poster)," B.F. Marques and J.W. Schneider, *229th ACS National Meeting*, San Diego CA (2005).
10. "Affinity Ligands for Sequence-Specific Duplex DNA Separation in Hydrophobic Interaction Chromatography," J.W. Schneider, J.P. Vernille, and J. Savard, *AIChE Annual Meeting*, Austin TX (2004).
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22. “Sequence-Specific Oligonucleotide Purification using Peptide Surfactants,” J.W. Schneider, J.P. Vernille, B.F. Marques, *225th ACS National Meeting*, New Orleans LA (2003).

b) Consultative and Advisory Functions:

Participated in *Air Force Cell-Like Entity Biotechnology Symposium*, Dayton OH (June 2003).

c) Transitions:

Collaboration initiated with Los Alamos National Laboratory (PI: Steen Rasmussen) on development of synthetic proto-cell system using modified PNA materials.

Collaboration with ANP Technologies to use the PNA-amphiphile system for multiplexed detection of aqueous pathogens.

New Discoveries, Inventions, or Patent Disclosures

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James Vernille, Best Student Presentation, 12th International Conference on Biopartitioning and Purification (2003)

Appendix 1. Micellar Electrokinetic Chromatography (MEKC) Methodology

PNAA Synthesis. PNAA was synthesized following fmoc protected solid phase synthesis techniques described elsewhere. [1, 2] The 10 base PNAA sequence used was (N-C) C_{18}-agtgtacatc-$(Glu)_4$. DNA oligomers were obtained from Integrated DNA Technologies (Coralville, Iowa) and used as received. DNA stock solutions were prepared in 50 mM Tris MES buffer, pH 8.0 with an approximate concentration of 2.5mM. The 10-20 base DNA sequence complementary to the PNAA of interest were 5'-GTAGATCACT-3', CGTAGATCACTC, CCGTAGATCACTCC, CCCGTAGATCACTCCC, CCCCGTAGATCACTCCCC and CCCCCGTAGATCACTCCCCC. The non-complementary 20 base DNA sequence contained a single-base mismatch, 5'-CCCCCGTAGA**G**CACTCCCCC-3'. Triton X-100 was purchased from Fluka and used as received. Stock solutions of Triton X-100 were prepared daily by vortexing a suitable amount of Triton X-100 in Tris MES to arrive at a stock concentration of 48 mM. Aliquots were prepared at concentrations ranging from 1.2 to 48 mM, vortexed, and centrifuged to remove any air bubbles. The Tris MES buffering system was chosen in an effort to minimize fronting of the DNA peak caused by electrodispersion. Tris HCl and Tris acetate buffering systems were also investigated but produced significant peak distortion for high DNA concentrations (data not shown).

Capillary Zone Electrophoresis. Capillary Electrophoresis was performed on a P/ACE MDQ (Beckman Coulter, Fullerton CA) equipped with a UV absorbance detector. The capillary used was a 50 um I.D. fused silica capillary (Beckman Coulter), 31 cm total length, 21 cm length to detector. Hydrodynamic injection (0.5 psi for 5 sec) was used to introduce sample into the capillary. Electrophoretic separation was conducted under normal polarity (from anode to cathode) with an electric field strength of 700 V/cm. UV detection was performed at 254 nm and capillary coolant temperature was maintained at 22ºC. 10% methanol was added to the sample buffer to determine the electroosmotic velocity of the running buffer in instances where a decrease in the Triton X-100 baseline was not sufficient.

Data collection and analysis was performed using 32 Karat software (Beckman Coulter). Although the migration time of the electroosmotic flow velocity marker rarely shifts more than 15 sec, the slightest shift in EOF velocity can have a large effect on the calculated electrophoretic velocity of a migrating species. These minor changes in EOF are most likely due to changes in the running buffer storage temperature which imparts a small change in the degree of capillary wall ionization and viscosity of the running buffer. It is for this reason that the migration time was normalized with respect to the EOF velocity through the conversion from migration time to effective mobility. The following equation was used to convert from migration time, t, to apparent electrophoretic mobility of a migrating species:

$$\mu = \frac{\upsilon}{E} = \frac{lL}{tV} \qquad \text{(I.1)}$$

where, l is the length to the detector, L is the total capillary length and V is the applied voltage. With the above equation it is possible to convert the x-axis of a standard electropherogram from migration time to apparent mobility. It is possible to go one step

further and normalize with respect to the apparent electrophoretic mobility of the neutral marker through the following relation:

$$\mu_{Eff} = \mu_{App} - \mu_{EOF} \quad \text{(I.2)}$$

where μ_{eff} is the effective mobility, μ_{app} is the apparent mobility and μ_{EOF} is the electrophoretic mobility of the neutral marker traveling with the EOF.

Viscosity Constant Determination. In order to correct for the change in viscosity imparted by the high micelle concentration used in the separations, the standard MEKC model equation is extended (Appendix 2). This constant is determined using capillary viscometry inside the P/ACE MDQ. Briefly, Triton-containing running buffer is pumped into the capillary via hydrodynamic pressure and allowed to equilibrate. A small plug of UV-transparent Tris MES is injected for 5 s at 5 psi followed by a pressure separation of Triton X-100 at 2.5 psi. The migration time of the sample plug is noted and the viscosity of the Triton solution is calculated according to Poiseuille's law:

$$\eta = \frac{\Delta P r^2 t}{8 l L} \quad \text{(I.3)}$$

where ΔP is the applied pressure and r is the capillary radius. This process is repeated for each Triton concentration and the viscosity is plotted versus micelle concentration. For simplicity, the micelle concentration is calculated as

$$[M] = \frac{[S] - cmc}{N} \quad \text{(I.4)}$$

The CMC and aggregation number N were determined using the solvochromatic fluorescent probe Nile Red as discussed previously. [4] The CMC and aggregation number were found to be 0.33 mM and 128 respectively. The slope and intercept of this curve can be used to determine the viscosity constant, C_{visc}, and the micelle free buffer viscosity, η_o. The two values were calculated to be 0.55 ± 0.02 and 0.951 ± 0.005 respectively.

Appendix 2. Calculation of Partition Coefficients and Micelle Mobility from Electropherograms

If we write the duplex/micelle interaction process as an equilibrium reaction,

$$Dup_{aq} + M \leftrightarrow Dup_M \quad \text{(II.1)}$$

We can define a partition coefficient K as:

$$K = \frac{[Dup_M]}{[Dup_{aq}][M]} \quad \text{(II.2)}$$

where $[Dup_m]$ and $[Dup_{aq}]$ are the concentrations of duplex in the micellar phase and aqueous phases respectively, and $[M]$ is the concentration of micelles. From the partition coefficient we see that:

$$\left[Dup_M\right] = \frac{K\left[M\right]\left[Dup_{Total}\right]}{1+K\left[M\right]} \text{ and } \left[Dup_{aq}\right] = \frac{\left[Dup_{Total}\right]}{1+K\left[M\right]} \tag{II.3}$$

Combining we have:

$$\frac{\left[Dup_M\right]}{\left[Dup_M\right]+\left[Dup_{aq}\right]} = \frac{K\left[M\right]}{1+K\left[M\right]} \equiv f_{Mic} \tag{II.4}$$

The above quantity is simply the fraction of duplex found within a micelle, f_{mic}. Similarly, the fraction of duplex found in the aqueous phase can be written:

$$f_{aq} \equiv \frac{1}{1+K\left[M\right]} \tag{II.5}$$

Therefore the effective mobility, μ_{eff}, of the duplex population is simply a weighted average of the intrinsic mobility of the duplex, μ_{Dup}, and mobility of a duplex containing micelle, μ_{mic}, according to

$$\mu_{eff} = f_{aq} \cdot \mu_{Dup} + f_{mic} \cdot \mu_{mic} \tag{II.6}$$

Substituting in,

$$\mu_{eff} = \frac{1}{1+K\left[M\right]} \cdot \mu_{Dup} + \frac{K\left[M\right]}{1+K\left[M\right]} \cdot \mu_{mic} \tag{II.7}$$

Since it is not possible to directly measure μ_{dup} for any non-zero surfactant concentration, it is assumed to be directly proportional to μ_{dup} in a surfactant free running buffer, μ_{dup}^{o}. Recalling,

$$\mu_{Dup} = \frac{C\,\varepsilon\zeta_{Dup}}{\eta} \tag{II.8}$$

where C is a constant, ε is the permittivity of the running buffer, ζ_{Dup} is the zeta potential of the duplex and η is the viscosity of the running buffer. Since only η is a function of $[M]$,

$$\frac{\mu_{Dup}}{\mu_{Dup}^{o}} = \frac{\eta_o}{\eta} \tag{II.9}$$

where η_o is the viscosity of the surfactant free running buffer. A similar argument applies for μ_{mic} and substitute in,

$$\mu_{eff} = \frac{1}{1+K[M]} \cdot \frac{\eta_o}{\eta} \cdot \mu_{Dup}^o + \frac{K[M]}{1+K[M]} \cdot \frac{\eta_o}{\eta} \cdot \mu_{mic}^o \tag{II.10}$$

For a Newtonian fluid,

$$\frac{\eta}{\eta_o} = 1 + \nu\phi = 1 + C_{visc}[M] \tag{II.11}$$

Where ν is a shape factor, ϕ is the "particle" volume fraction. We define a new constant,

$$C_{visc} \equiv \frac{\nu\phi}{[M]} \tag{II.12}$$

Substituting in and simplifying ,

$$\mu_{eff} = \frac{\mu_{Dup}^o + K[M]\mu_{mic}^o}{1 + (K + C_{visc})[M] + KC_{visc}[M]^2} \tag{II.13}$$

The above equation results in an expression that directly relates the effective mobility of the duplex to the total concentration of micelles in the running buffer through the partition coefficient *K*, and the micelle mobility μ_{mic}. In an effort to determine these two coefficients, equation (II.13) is linearized to allow for significantly more straight forward parameter estimation. Equation (II.6) can be rewritten as:

$$\mu_{eff} = (1 - f_{mic}) \cdot \mu_{Dup} + f_{mic} \cdot \mu_{mic} \tag{II.14}$$

when combined with (II.4), (II.9), and (II.11):

$$\mu_{eff}(1 + C_{visc}) - \mu_{Dup}^o = \frac{K[M](\mu_{mic}^o - \mu_{Dup}^o)}{1 + K[M]} \tag{II.15}$$

If we take the reciprocal of each side, we arrive at:

$$\frac{1}{\mu_{eff}(1 + C_{visc}) - \mu_{Dup}^o} = \frac{1 + K[M]}{K[M](\mu_{mic}^o - \mu_{Dup}^o)} = \frac{1}{(\mu_{mic}^o - \mu_{Dup}^o)} + \frac{1}{K(\mu_{mic}^o - \mu_{Dup}^o)} \frac{1}{[M]} \tag{II.16}$$

which can readily be solved by linear regression.

Appendix 3. PCR methodology

ssDNA Generation. To provide a rigorous test of the separation capability of PNA amphiphiles in conjunction with TX-100 micelles in MEKC, it was necessary to generate single-stranded DNA over 100 bases in length, which represents the upper limit of chemical DNA oligomer synthesis. Consequently, alternative ssDNA generation techniques were investigated in relation to three essential elements. First, the technique must produce sufficient amounts of product to allow for UV-Vis detection; second, it must produce a homogeneous ssDNA population; finally, it must allow for flexibility in regards to the location and number of PNA recognition sequences. In light of these requirements, we determined that asymmetric PCR was the most logical choice for ssDNA generation. Asymmetric PCR utilizes unequal primer concentrations to generate both single and double stranded DNA of equal length in a single reaction. Single stranded DNA of lengths 88, 13, 216, and 447 bases were used in this study.

Primer Design. Design criteria was largely taken from two recent studies aimed at improving ssDNA production in asymmetric PCR, termed linear-after-the-exponential PCR, or LATE-PCR. [8, 9] A thorough discussion on successful primer design for LATE-PCR can be found in these references. Briefly, primers were chosen such that the concentration-corrected melting temperature of the reverse (limiting) primer was greater than that of the forward (excess) primer. By using primers deliberately designed for asymmetric PCR, ssDNA formation is large, predictable, and reproducible. The concentration-dependent melting temperature of each primer were found using MELTING, an online nearest-neighbor nucleic acid melting temperature calculator available at <http://bioweb.pasteur.fr/seqanal/interfaces/melting.html.> [10]

The Allawi et al. nearest-neighbor set and the Santalucia salt correction factor were used. [11, 12] The primers used in this study are featured in Table 1. The PNA recognition sequence, denoted by the underlined portion of the forward primer pSP64 EH13, was appended to the 5' end of all ssDNA products by including it in the forward primer design, rather than cloning it into the PCR template itself.

PCR Conditions. All PCR reactions were conducted in a Smart Cycler (Cepheid) at a total volume of 100 μl. PCR reactions included the following reagents: 1000 nM excess primer, 75 nM limiting primer, 0.2 mM dNTP mixture, 1.5 mM MgCl2, 1x PCR reaction buffer [500 mM KCl, 100 mM Tris-HCl (pH 9), 1% TX-100 (Promega)], 0.04 ng/μl plasmid pSP64 template (Promega), and 0.04 Units/μl Taq DNA Polymerase (Promega). Plasmid pSP64 was cloned in Subcloning Efficiency DH5α Competent *Escherichia coli* cells (Invitrogen). For the generation of 88, 134, and 216 base DNA strands, an initial denaturation step of 120 seconds at 95ºC was followed by 50 cycles of 95ºC for 10 seconds, 60ºC for 15 seconds, and 72ºC for 15 seconds. 447 base DNA strand generation required elongation of the denaturation, annealing, and extension steps to 15, 30, and 30 seconds, respectively. The purity of each PCR reaction was assessed on 8% non-denaturing polyacrylamide gel electrophoresis (BioRad). The chosen annealing temperature (60ºC) represents an optimal

balance of a low cycle threshold value as judged by the onset of SYBR green fluorescence, and a homogenous product as judged by PAGE (data not shown).

Sample Preparation. Between 2 and 4 100 μl PCR reactions were diluted with 50 mM Tris MES (pH 8) to a total volume of 500 μl. Samples then underwent 3 buffer exhanges with 50 mM Tris MES (pH 8) in conjunction with a 30,000 Dalton cutoff Microcon spin filter (Millipore). Next, each was centrifuged for 13,400 g for 10 minutes or until a final volume of approximately 25 μl was reached. Final nucleic acid concentration was between 1 and 10 μM (strand basis) for all samples. 25 μM PNAA (C_{18}-agtgatctac-$(Glu)_4$) was then added to each DNA sample and the mixture was incubated at room temperature for 5-10 minutes to ensure maximum hybridization. Each sample was subsequently loaded and stored at 10ºC for the duration of the experiment.

Table 1. Forward and reverse primers used in this study, and A PNA binding sequence, underlined in the excess primer pSP64 EH13, was added to the resulting ssDNA strand during PCR by incorporation of the italicized bases.

Excess primer	**Sequence**	T_m **(ºC)**
pSP64EH13	*CGCGGTAGATCACT*CCGAATTCGTAATCATGTCATAGC	55.8 (*66.5*)
Limiting primers	**Sequence**	T_m **(ºC)**
pSP64 rp1	AGCTCACTCATTAGGCACCCCAGG	60.0
pSP64 rp2	TCGTATGTTGTGTGGAATTGTGAGCGGA	60.6
pSP64 rp3	TGGCCGATTCATTAATGCAGCTGGCA	61.5
pSP64 rp4	CGGAGCCTATGGAAAAACGCCAGCA	61.7

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