# Development of Peptide Nucleic Acid Assemblies for Application in Drug Delivery and Molecular Sensing

Felice N. Butler,[1,2,3] Oguz Elibol,[2,4] Bridget D. Hines,[1,2,3] Bobby Reddy, Jr.,[2,6] Rashid Bashir,[2,6] Donald E. Bergstrom,[1,2,3,5,*]

*[1]Department of Medicinal Chemistry and Molecular Pharmacology, [2]Birck Nanotechnology Center, [3]Bindley Bioscience Center, [4]School of Electrical and Computer Engineering, [5]Walther Cancer Institute, Purdue University, West Lafayette, IN 47907. [6]Department of Electrical and Computer Engineering & Bioengineering, Micro and Nanotechnology Laboratory, University of Illinois at Urbana-Champaign, Urbana, IL 61801. [*]Corresponding Author, email: bergstrom@purdue.edu, Ph: (765) 494-6275*

## ABSTRACT

We are exploring peptide nucleic acid (PNA) assemblies as interesting scaffolds for the fabrication of nanomaterials relevant to the diagnosis and treatment of disease. The first assembly is a PNA/LNA (locked nucleic acid) duplex employed in the heat-mediated selective functionalization of arrayed nanodevices. The ultimate goal of this arrayed approach is the detection of multiple miRNA sequences simultaneously with high throughput and specificity. Since miRNAs have been linked to certain disease states in various types of cancers, their detection may help lead to early diagnosis in a clinical setting. A second type of assembly has been created from PNA sequences that self-assemble based hybridization in the parallel direction. The resulting PNA nanoparticles traverse breast cancer cell membranes, making them a promising platform for the delivery of oligonucleotide probes.

***Keywords***: pna, lna, nanoparticles, array, MCF-7

## 1 PNA/LNA DUPLEX

PNA is a synthetic DNA analog that contains the nucleic acid bases adenine, cytosine, guanine, and thymine on a neutral peptide-like backbone[1, 2] (Figure 1). Due to its uncharged structure, a PNA strand can hybridize to a complementary DNA or LNA (locked nucleic acid) strand with higher affinity than an analogous DNA sequence.[3] LNA (locked nucleic acid) is a synthetic DNA analog in which the 2'-oxygen is linked to the 4'-carbon via a methylene unit, rendering the monomer more conformationally stable than that of DNA. The PNA/LNA duplex is particularly stable in that a short sequence of only 6 base pairs has a well-defined melting curve with a $T_m$ value well above room temperature.

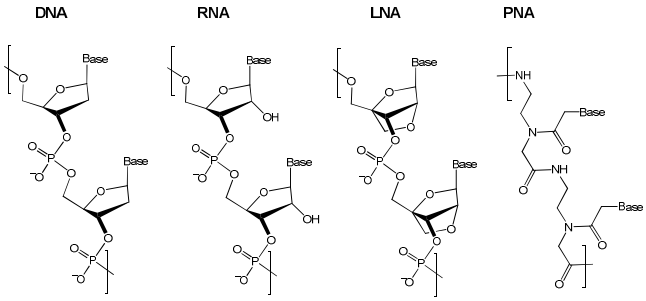


Figure 1: Structures of DNA, RNA, LNA and PNA

### 1.1 Selective Functionalization of Arrayed Nanoplates

We employ this robust PNA/LNA duplex in the temperature-controlled selective functionalization of arrayed nanoplates (Figure2). The ultimate goal of this technology is for the detection of microRNAs (miRNAs), short strands of RNA that combine with and inhibit complementary messenger RNA.[4-6] MicroRNAs are involved in the regulation of many cellular processes, and miRNA expression has been linked to certain disease states in various types of cancers.[7-10] It would therefore be highly desirable to rapidly detect the presence of many different miRNA sequences at one time, in order to investigate the roles of specific miRNAs in disease states and to diagnose early cancerous disease states in a clinical setting.

In our work we perform a heat-mediated exchange reaction on the device surface in which a competing LNA strand invades an existing PNA/LNA duplex to form a more robust product. This exchange will also introduce new functionality to the device surface, allowing it to detect the presence of a specific miRNA sequence. We have successfully performed this exchange reaction on a single nanoplate device in the presence of other nanoplates by the selective heating of the device.

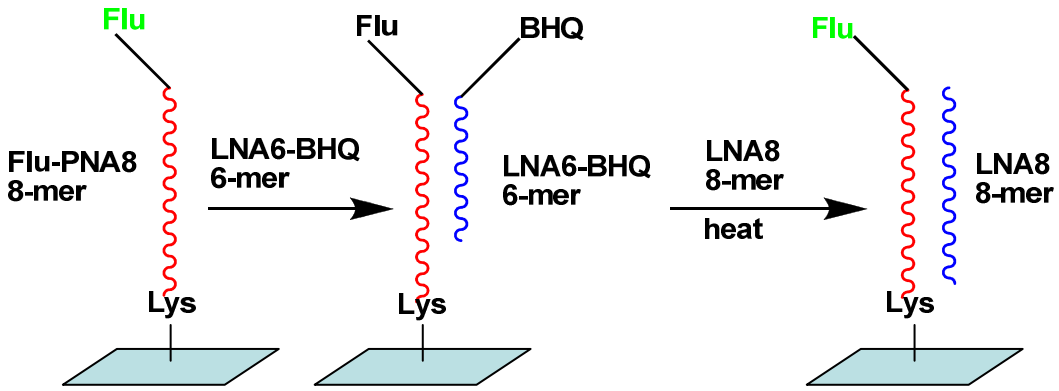


Figure 2: Temperature-controlled selective functionalization of arrayed nanoplates through LNA exchange on attached PNA

## 2 PNA NANOPARTICLES

Another interesting property of PNA is its ability to hybridize to other PNA strands in a parallel (as well as antiparallel) fashion. We have designed a PNA sequence (gcatcg) that exploits this parallel hybridization by self-assembling into nanoparticles via "tiling" (Figure 3a). The UV thermal profile of the PNA sequence alone in solution suggests the assembly of higher order PNA structures.

The robust association of PNA with DNA and its resistance to degradation by natural enzymes makes PNA a good candidate as a carrier for oligonucleotide probes. An important limitation to this idea is PNA's inability to penetrate the cell membrane and enter the cytosol. We report the formation of self-assembled PNA nanoparticles with the ability to traverse cellular membranes. In preliminary studies, we showed the internalization of fluorescein-labeled PNA nanoparticles into MCF-7 breast cancer cells, as determined with confocal microscopy (Figure 3c and d).

### 2.1 Internalization of PNA Nanoparticles into MCF-7 Cells

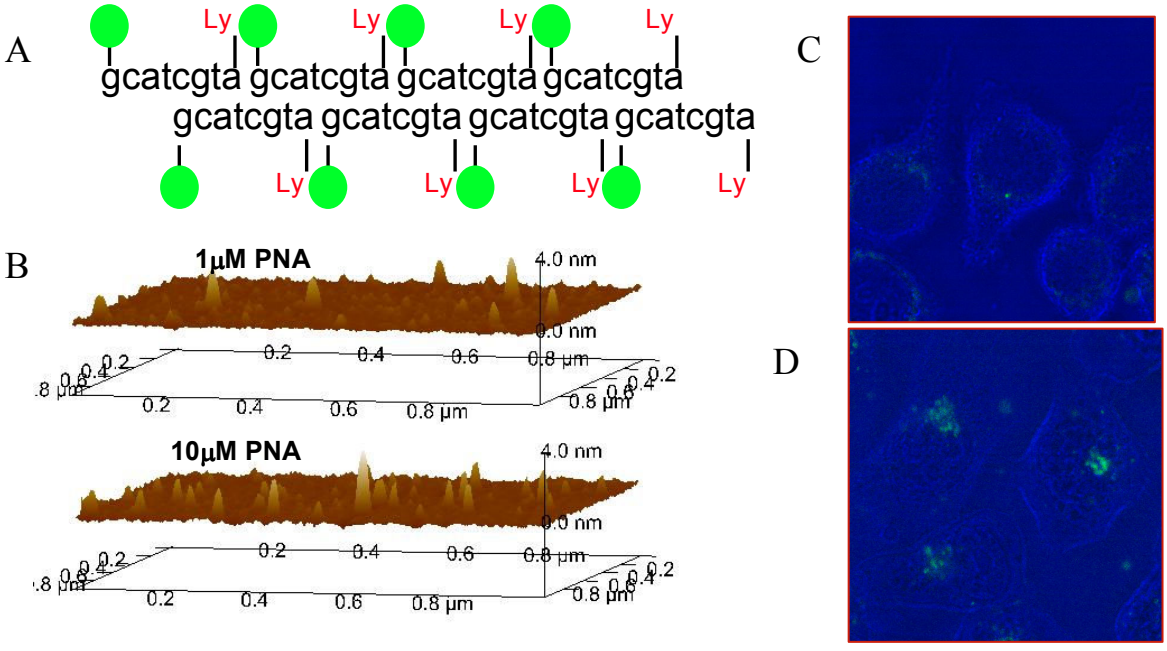


Figure 3: A) PNA tiling; B) AFM images of PNA nanoparticles; C) Confocal microscopy of MCF-7 cells alone; D) Confocal microscopy of MCF-7 cells with fluorescein-labeled PNA nanoparticles


NSTI-Nanotech 2008, www.nsti.org, ISBN 978-1-4200-8504-4 Vol. 2

## 2.2 Correlation Between PNA Concentration and Particle Size

Experiments in our lab have discovered a correlation between PNA nanoparticle size and the concentration of PNA during particle formation (Figure3b). The effects of pH, salt concentration, and annealing rate on the particle size distribution will be studied, as well as the effect of these factors on the kinetics of particle formation and denaturation, and the apparent melting/annealing temperatures ($T_{1/2}$) of the assemblies.

## CONCLUSIONS

The interesting properties of PNA make it a unique platform for the fabrication of biologically relevant nanomaterials. We are currently exploring various avenues to exploit the characteristics of PNA in the diagnostics and treatment of disease such as cancer.

## REFERENCES


[1] Hyrup, B. & Nielsen, P. E. (1996) *Bioorgan Med Chem* **4,** 5-23.

[2] Nielsen, P. E. (2001) *Current Opinion In Biotechnology* **12,** 16-20.

[3] Ng, P. S. & Bergstrom, D. E. (2005) *Nano Lett* **5,** 107-11.

[4] Ambros, V. (2001) *Cell* **107,** 823-826.

[5] Bartel, D. P. (2004) *Cell* **116,** 281-297.

[6] Lim, L. P., Glasner, M. E., Yekta, S., Burge, C. B. & Bartel, D. P. (2003) *Science* **299,** 1540-1540.

[7] Calin, G. A., Dumitru, C. D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Aldler, H., Rattan, S., Keating, M., Rai, K., Rassenti, L., Kipps, T., Negrini, M., Bullrich, F. & Croce, C. M. (2002) *P Natl Acad Sci USA* **99,** 15524-15529.

[8] Calin, G. A., Sevignani, C., Dan Dumitru, C., Hyslop, T., Noch, E., Yendamuri, S., Shimizu, M., Rattan, S., Bullrich, F., Negrini, M. & Croce, C. M. (2004) *P Natl Acad Sci USA* **101,** 2999-3004.

[9] Lu, J., Getz, G., Miska, E. A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebet, B. L., Mak, R. H., Ferrando, A. A., Downing, J. R., Jacks, T., Horvitz, H. R. & Golub, T. R. (2005) *Nature* **435,** 834-838.

[10] Xie, X. H., Lu, J., Kulbokas, E. J., Golub, T. R., Mootha, V., Lindblad-Toh, K., Lander, E. S. & Kellis, M. (2005) *Nature* **434,** 338-345.