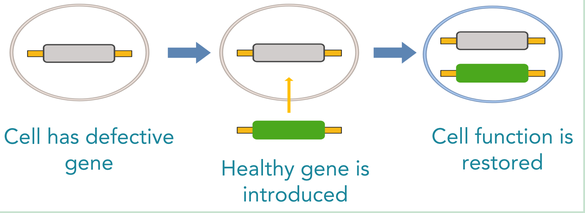
Peptide Nucleic Acid as a Messenger RNA Delivery Vehicle

Megan Garcia[1], I-Che Li[2], Niren Murthy[2]
Irvine Valley College[1], Department of Bioengineering, University of California, Berkeley[2]
2019 Transfer-to-Excellence-Research Experience for Undergraduates (TTE REU) Program

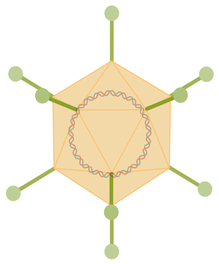
Abstract: Innovation in medicine have been growing exponentially in the recent years, especially in the area of gene therapy. An area of major interest is DNA-based nucleic acid therapeutics and vehicles for delivery. Here we demonstrate peptide nucleic acids (PNA) potential for being a successful delivery vehicle for mRNA through testing the structure's binding and releasing capabilities. Our interest in PNA stems from its neutrality and nontoxicity, making it a stable option for mRNA delivery into cells. This experiment establishes PNA's use as a delivery vehicle for any mRNA, making it a viable gene therapy option.

Background



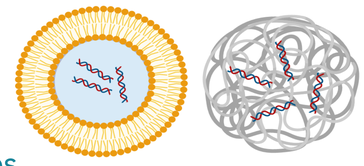
- Goal of DNA-based therapeutics - restore function in cells by introducing the correct functioning gene to produce proteins the body needs.
- Problem - finding safe and effective vehicles to delivery genomic material

Inspiration



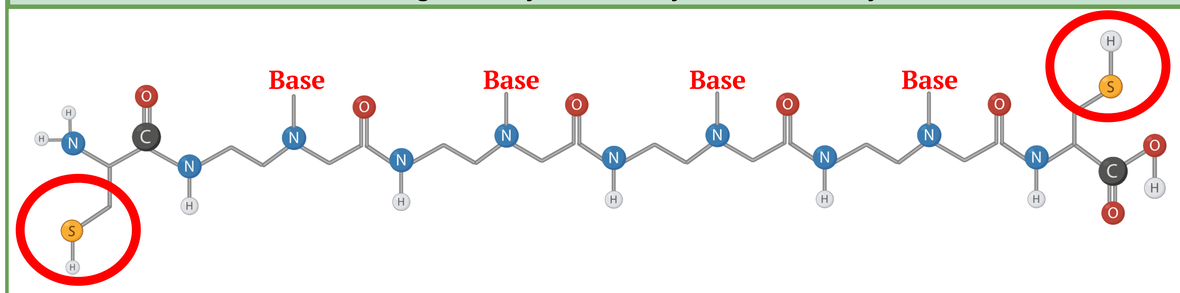
Bacterial plasmids and Viral Vectors

Liposomes and Polymers Nanoparticles

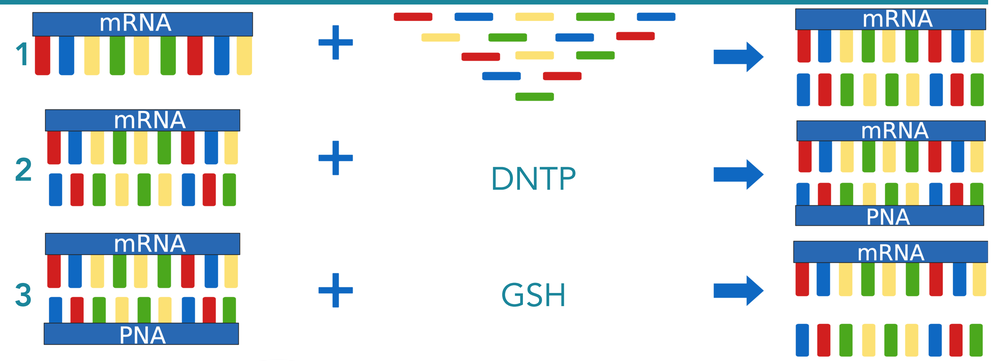


Peptide Nucleic Acid

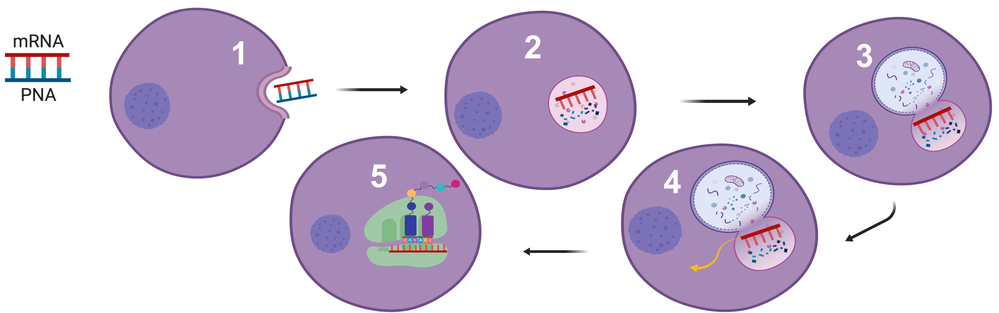
-nontoxic -not recognized by immune system -naturally reduced in cells



Methods

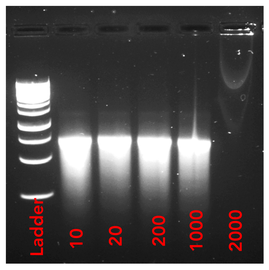


1. mRNA and PNA library pair.
2. DNTP bonds mRNA and PNA.
3. GSH reduces the bonds, releasing mRNA from PNA.



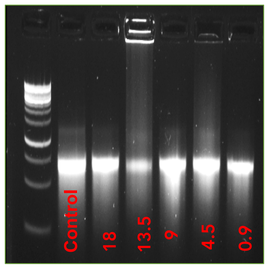
1 & 2. PNA/mRNA taken in by cell in vesicle.
3. Lysosome breaks digests vesicle.
4. Bonds reduced and mRNA escapes from lysosome.
5. mRNA is transcribed into protein.

Bind and Release Assays



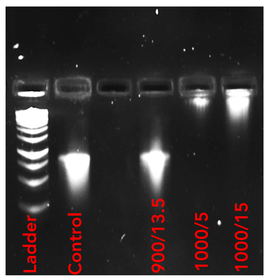
PNA Concentration Assay (uM)

- mRNA and PNA have bound between 1000 uM and 2000 uM of PNA.



DNTP Concentration Assay (mM) w/ PNA 900 uM

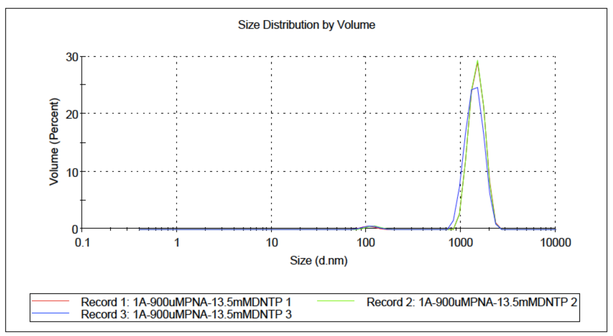
- 13.5 mM of DNTP chemically binds PNA to mRNA.



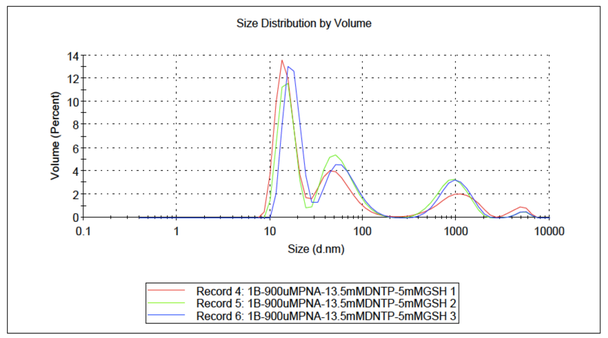
GSH Concentration Assay (mM) w/ PNA 900 uM and 1000 uM

- The condition of 900 uM PNA, 13.5 DNTP, and 5 mM GSH releases mRNA from PNA.

Dynamic Light Scattering Measurement



Before GSH (3353 d.nm)



After GSH (164.4 d.nm)

Conclusion

- Optimal binding and release condition found: 900 uM PNA, 300 ng/mol mRNA, 13.5 mM DNTP, and 5 mM GSH.
- Data shows a PNA delivery vehicle has potential to be an effective DNA-based therapeutic option.

Future Work

- Evaluating the optimal time frames of each reaction.
- Using PNA for green fluorescent protein (GFP) mRNA binding instead of Luciferase mRNA
- In vivo studies including cell transfection of GFP and Luciferase mRNA.

Acknowledgments

I've been supported by many wonderful people throughout this program, but I'd like to especially recognize I-Che Li, Ph.D. and Niren Murthy, Ph.D., Nicole McIntyre, Kimberly Ferry, Victoria Helm, the Transfer to Excellence Program, and the National Science Foundation.

Contact Information
Megan Garcia
1(714)-599-1224
megangarcia@berkeley.edu

Support Information
This work was funded by National Science Foundation Award #1757690

References

[1] Pellestor, F., & Paulasova, P. The peptide nucleic acids (PNAs), powerful tools for molecular genetics and cytogenetics. Eur J Hum Genet. 2004;12(9):694–700. doi: 10.1038/sj.ejhg.5201226 [2] Islam MA, Reesor EKG, Xu Y, et al. Biomaterials for mRNA delivery. Biomater Sci. 2015;3:1519–33. [3] De Temmerman M-L, Dewitte H, Vandenbroucke RE, Lucas B, Libert C, Demeester J, de Smedt SC, Lentacker I, Rejman J. mRNA-Lipoplex loaded microbubble contrast agents for ultrasound-assisted transfection of dendritic cells. Biomaterials. 2011;32:9128–35. [4] De Haes W, van Mol G, Merlin C, de Smedt SC, Vanham G, Rejman J. Internalization of mRNA lipoplexes by dendritic cells. Mol Pharm. 2012; 9:2942–9. [5] Phua KKL, Leong KW, Nair SK. Transfection efficiency and transgene expression kinetics of mRNA delivered in naked and nanoparticle format. JControl Release. 2013;166:227–33. [6.]Malone RW, Felgner PL, Verma IM. Cationic liposome-mediated RNA transfection. Proc Natl Acad Sci USA. 1989;86:6077–81. [7] Lv H, Zhang S, Wang B, Cui S, Yan J. Toxicity of cationic lipids and cationic polymers in gene delivery. J Control Release. 2006;114:100–9.