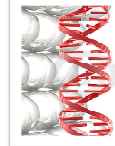


ANALYZING STRUCTURAL DNA BINDING WITH NANOPARTICLES FOR GENE THERAPY



Kshama Rane ¹, Ashish Aphale ², Shrinivas Bhosale ¹, Prabir K Patra ^{1,4}, Kathleen Engelmann ³
¹Department of Biomedical Engineering, University of Bridgeport, CT, USA
²Department of Computer Science and Engineering, University of Bridgeport, CT, USA
³Department of Biology, University of Bridgeport, CT, USA
⁴Department of Mechanical Engineering, University of Bridgeport, CT, USA



Abstract

The study of DNA binding with nanoparticles and their packaging assembly is an essential requirement for gene therapy. The structural features of DNA and nanoparticles would be very useful in development of novel gene therapy in genes. Plants being good test system as they are tolerant of being bombarded with small particulates and usually grow normally even after such treatments. We used primers from plant named "Frigida".

Introduction

Gene expression is currently detected by homogenizing tissue of interest. Disrupting the cells and immediately treating with reagents to stabilize the RNA. The mRNA is reverse transcribed, amplified, fluorescently labelled. Hybridized to cDNA probes for the gene of interest. Two nanostructures each with cDNA probes bound to the ends. The probes would hybridize with adjoining segments of an mRNA. Resulting mRNA would act like a piece of velcro binding the two nanostructures together. nanostructure design should be such that we can send a detectable signal or record event in a way that could be retrieved and monitor gene expression in real time. RNA being chemically unstable compared to DNA we worked with DNA. Structural binding of DNA with nanoparticles will help us understand better.

Research Methods

Graphene Dispersion:

Graphene which can be described as honey comb lattice of carbon only a single atom thick has attracted considerable interest in research community as a result of its outstanding properties (Alexander A. Green & Hersam, 2010)

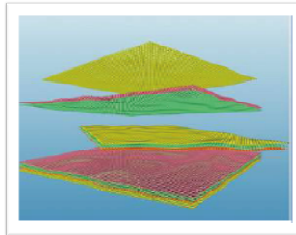


Fig 1 : Graphene dispersion with tailored thickness, lateral area and shape (Alexander A. Green & Hersam, 2010)

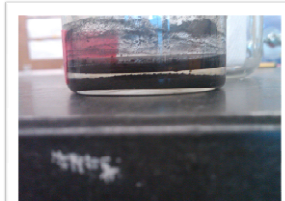


Fig 2 : Graphene dispersion in 1X TE buffer

Graphene being hydrophobic in nature could not be dispersed in water. Tris-EDTA (TE) Buffer was used for graphene dispersion. Different dilutions of Tris-EDTA buffer were used like 1X, 5X, 7X and 10X to see the best results of graphene dispersion. Each batch of dilution was dispersed with graphene till the best results of dispersion were seen. "Sonication" which is the action of sound energy used to agitate particles for various purposes was used to disperse nanoparticles in the solution.

Binding of Primers :

Primers were sonicated with the different dilutions of graphene dispersed TE solutions. The sonication was carried out at maintained temperature 0-2°C. The oligonucleotide sequence used

5-GAA ACT TGT CTC GCA GAG
CTC ATA G-3

Centrifugation:

The binding of graphene with the Primers in TE Buffer solution was done.

Assuming that some primers would not bind to graphene we needed only binding structures in the solutions for further Scanning electron microscopic studies. We washed the solution using Centrifugation technique. Different pellets were collected and will be further studied for SEM studies.

A centrifuge uses a centrifugal force (g-force) to isolate suspended particles from surrounding medium in a continuous-flow basis. Centrifugal force used $g \sim 14,000$. The Calculation with standard formula :

$$g = (1.118 \times 10^{-5}) R (S)^2$$

R- radius of the rotor in cms

S- Speed of the centrifuge in rpm



Fig 3 : Primer + 5X TE after centrifugation

Further Studies

The different pellets for each dilution will be further studied using Scanning electron microscope, FTIR, PCR. Simulations can be carried out for better understanding graphically.

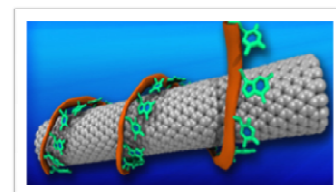


Figure 4: DNA and SWNT binding (Robert R Johnson, A. T. Charlie Johnson, & Klein, 2010)

Single walled nanotube graphical understanding above we can interpret graphene sheet binding with further studies.

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- Alexander A. Green, & Hersam, M. C. (2010). Emerging Methods for Producing Monodisperse Graphene Dispersions *The Journal of Physical Chemical Letters*, 1(2), 544-549.
 Robert R Johnson, A. T. Charlie Johnson, & Klein, M. L. (2010). "The Nature of DNA-Base-Carbon-Nanotube Interactions". *Small*, 6(1), 31-34.