Graphene Patterned Microchip For Colorectal Cancer Detection

Kavya Hemmanur[1], Sanjay Robin[1], Isaac Macwan[1,], Prabir Patra[1,2], Juan Liu[3] and Manju Hingorani[3]
[1]Department of Biomedical Engineering, University of Bridgeport
[2]Department of Mechanical Engineering, University of Bridgeport
[3] Department of Molecular Biology and Biochemistry, Wesleyan University

ABSTRACT

Cancer currently stands as the second-leading cause of death worldwide. Studies reveal colorectal cancer (CRC) to be the 4th leading cause of mortality due to cancer. It is estimated that about 30% of CRC cases are hereditary, of which 5% are attributed by known syndromes, particularly Lynch Syndrome. This pilot study aims to fabricate a DNA-graphene-polypyrrole (DGP) based biosensor to diagnose deficiency of functional MMR proteins present in patients at a scale of less than ng/ ml. We have followed LAB-on-CHIP method. We find that the interactive forces between avidin and graphene are mainly hydrophobic, along with some van der Waals, electrostatic and hydrogen bonding interactions. Different mismatch combinations were performed, to prove the activity of each component on the chip. 30 such combinations were done. Electrochemical impedance spectroscopy was done to confirm the working of the bio sensor by corresponding change in electrical impedance. To assist this real-world system, we have carried out simulation studies as well. In the simulation studies from 0-200ns, we present the progression structures of human MutS protein to biotinylated DNA that has been fixed to simulate the manner of a biosensor, furthermore the mismatch within the DNA has been manually introduced with the aid of computational tools to reveal the interactions of the DNA and the protein. This research additionally permits us in early detection of colorectal cancer and the mapping and expertise of the method related to the area of the mismatch repair.

EXPERIMENTAL METHODS AND RESULTS

- Chromium based nickel plated chips were used to fabricate DNA-Graphene-Polypyrrole (DGP) based biosensor.
- Gppy substrate was formed using cyclic voltammetry set up with initial conditions 800mV final 900mV at a scan rate of 20mV/s for 100 cycles.
- Avidin was deposited and impedance was measured using Electrochemical spectroscopy.
- DNA dilutions were performed, around 27-28nanomoles of DNA was poured on the dry chip and EIS was measured.
- Similar step was repeated with MutS protein.
- Concentration of DNA and MutS was decreased with each trail and the system was measured was measured for least possible concentration.
- Impedance of different combinations of the five components involved in fabrication of biosensor was measured. Around 40 combinations were performed.

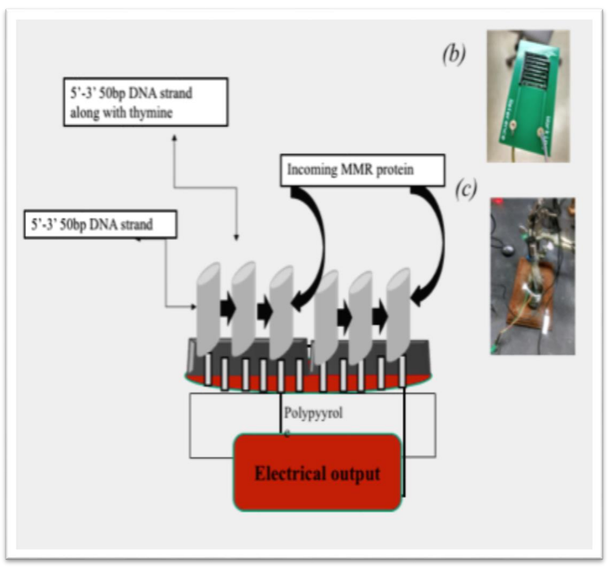


Figure 1.*a Schematic representation of final assembled device; b) Finely deposited grapheme-polypyrole chip c) Electropolymerization of grapheme and pyrole on chip*

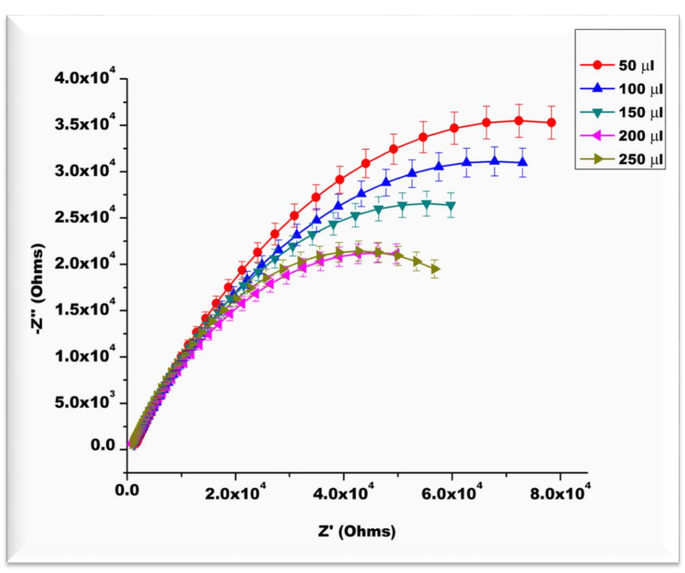


Figure 2*: The Nyquist plot demonstrates the change in impedance with different concentrations of MutS Substrate*

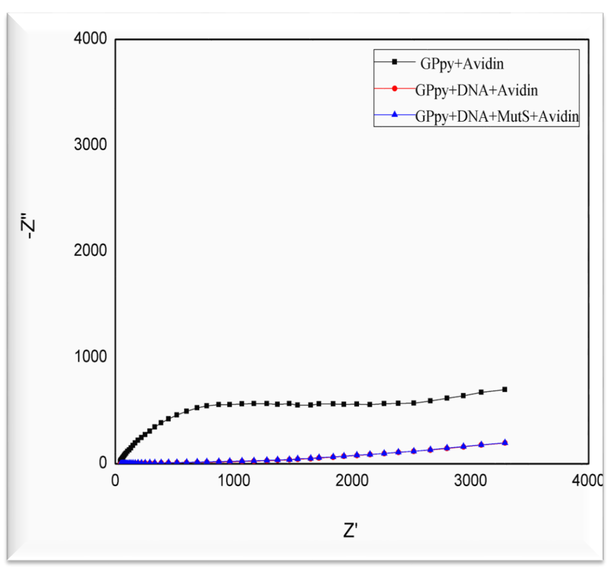


Figure 3. *The Nyquist plot demonstrates the change in impedance for the different combination which include avidin deposition.*

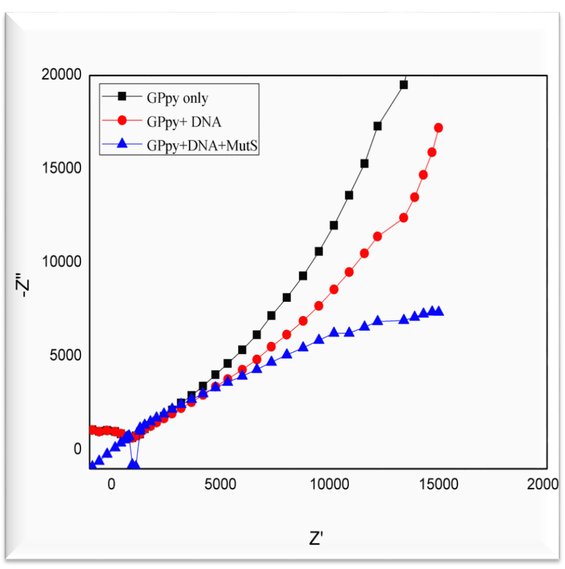
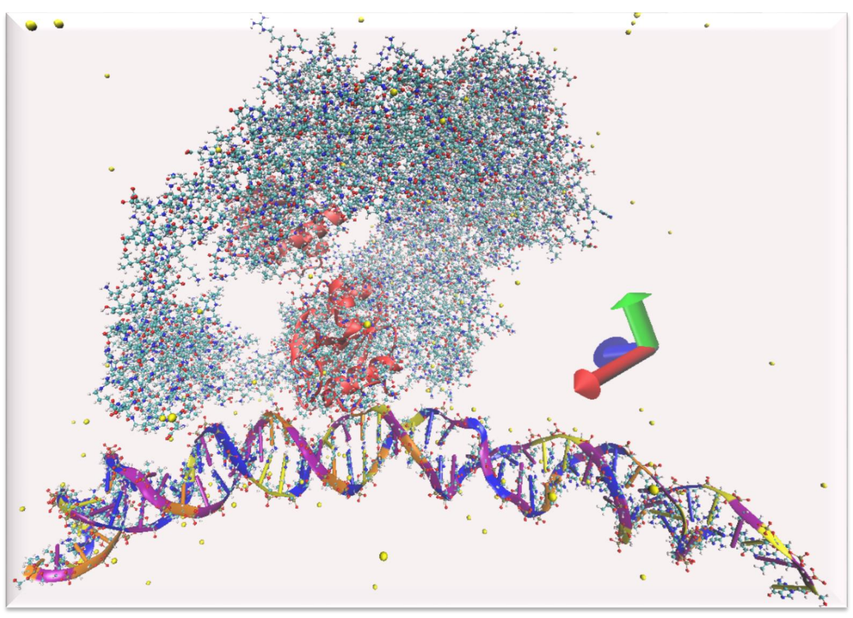


Figure 4: *The Nyquist plot demonstrates the change in impedance with absence of avidin.*

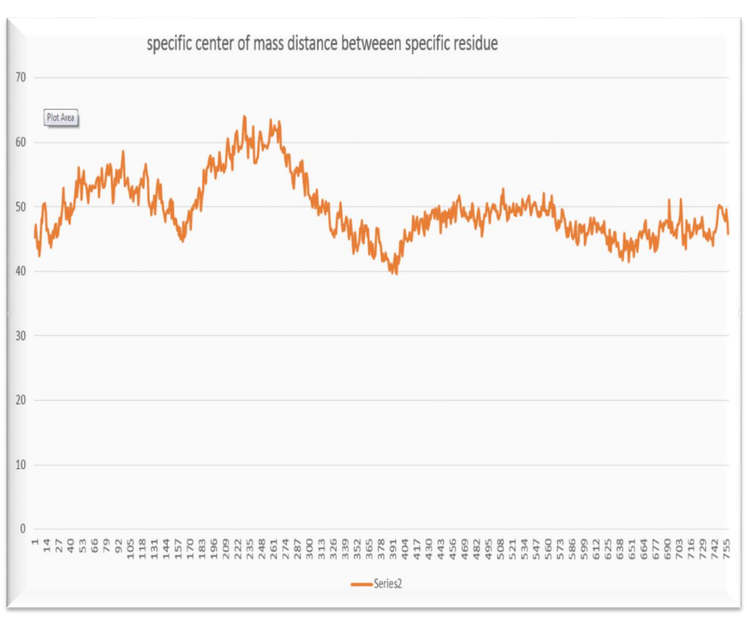
SIMULATION STUDIES

- The system was modeled using VMD[3] and the simulations were carried out foe 200ns using NAMD[4].
- CHARMM force field was used for NAMD simulations.
- TIP3 water model was used for solvation and further ionization of the system.
- The mismatched DNA segment (50/51 bp) was generated using "Random DNA sequence generator" tool.
- The DNA was fixed at the CYT at the 5' end specifically at the atoms 1641 to 1668.
- VMD plugins for RMSD, H2 bonds and Interaction energy are used along with the TCL scripts for Center of Mass variation and Number of atoms.



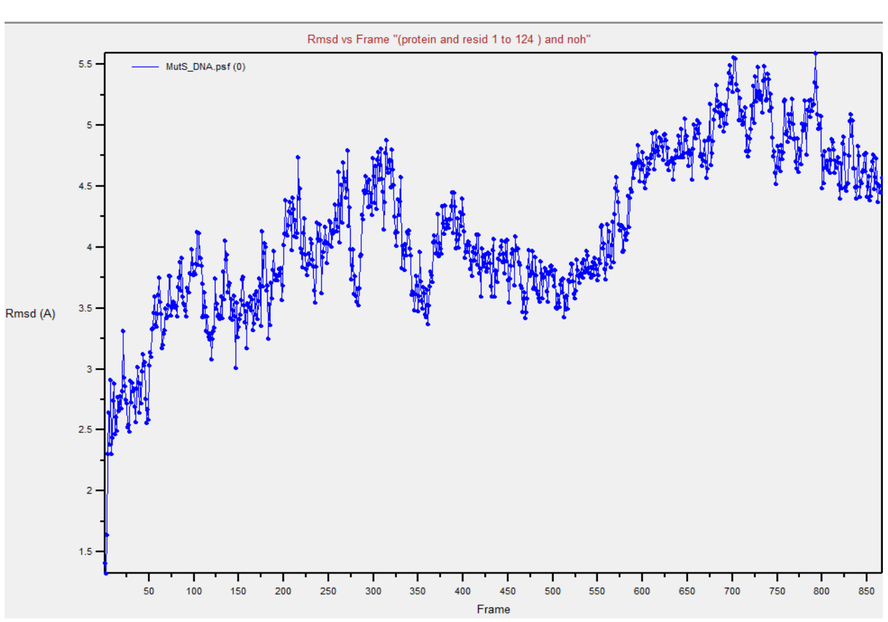
(a)

Figure a*. Visual view of the 200ns molicula dynamic interaction between the MutS Protien and the DNA , the highlighted segment represent the 1to 124 residues,*



(b)

Figure(b) *Distance between the center of mass of specific residue and mismatch*



(c)

Figure (c) *RMSD of specific residue form the center of mass*

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