A Novel Nano-biosensor for Colorectal Cancer Diagnostics by Detecting DNA Mismatch Repair Proteins

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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. Lynch Syndrome (LS) is caused by loss or malfunction of proteins responsible for DNA mismatch repair proteins (MMR), mostly MLH1 and MSH2, causing increased risks of developing CRC. Despite the small percentage accounted with the disease, the severity of the illness still remains immense since 80% of these patients eventually develop CRC and an overwhelming 40-60 % of female patients develop endometrial cancer, the major form of cancer in women in the developing nations. This pilot study aims to fabricate a DNA-graphene-polypyrole (DGP) based biosensor to diagnose deficiency of functional MMR proteins present in patients at a scale of less than ng/ ml. Fundamental understanding of interactions at the interface of biological molecules, such as proteins, and nanomaterials is therefore crucial for developing such biocompatible hybrid materials and biosensing platforms. Conductive nanomaterials-based biosensors offer the advantage of higher sensitivity and reliable diagnosis mainly due to their superior specific surface area and ballistic conductivity. Such films that immobilize proteins can synergize the properties of transducers and molecular recognition elements in order to improve biosensor performance and diversity. Here we report for the first time, using a combined molecular dynamics simulations and experimental approach, the interactions between avidin and a graphene surface, which is being developed as a sensing platform for early detection of DNA mismatch repair proteins. We find that the interactive forces between avidin and graphene are mainly hydrophobic, along with some van der Waals, electrostatic and hydrogen bonding interactions. Notably, the structure and function of the avidin molecule is preserved after its adsorption on the graphene surface. The MD results agree well with scanning electron microscopy (SEM) and electrochemical impedance spectroscopy (EIS) analysis of avidin immobilized on a graphenated polypyrrole (G-PPy) conductive substrate, which confirm adsorption of avidin on graphene nanoplatelets and corresponding changes in electrical impedance, respectively. A final analysis is being conducted to confirm our hypothesis.

1. Method

1.1 Graphene-Ppy film formation

For this step, we used cyclic voltammetry to co-electropolymerize pyrole and graphene and deposit it on the gold-coated circuit.[1]
CV parameters:

- V_{high}= 900mV
- V_{low} = 800mV
- Scan rate = 20 mV/s
- Number of cycles = 100

1.2 Deposition of Avidin

Avidin molecules were dissolved in a buffer solution of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid solution (HEPES, 10 mM, pH 7.0). The graphene-Ppy chip was then soaked in the resultant solution for 30 minutes.[2]

1.3 Molecular Dynamics

- Interactive forces between graphene and avidin were setup using VMD and simulations were carried out using NAMD.
- An inbuilt graphene builder was used to create a graphene sheet of 88Å X 121Å.
- All simulations were carried out for a time period of 100ns.
- All simulations used CHARMM force field and TIP3 water model with a neutralizing salt concentration.
- Periodic boundary conditions were assumed using a constant temperature of 300K and a pressure of 1atm.
- A 10,000 step energy minimization was performed first to stabilize the system.
- RMSD, RMSF and energy cut-offs were analyzed using Timeline tool in VMD and TCL scripts.
- Particle Mesh Ewald algorithm was used to calculate the short-range and long-range forces.

2. Results

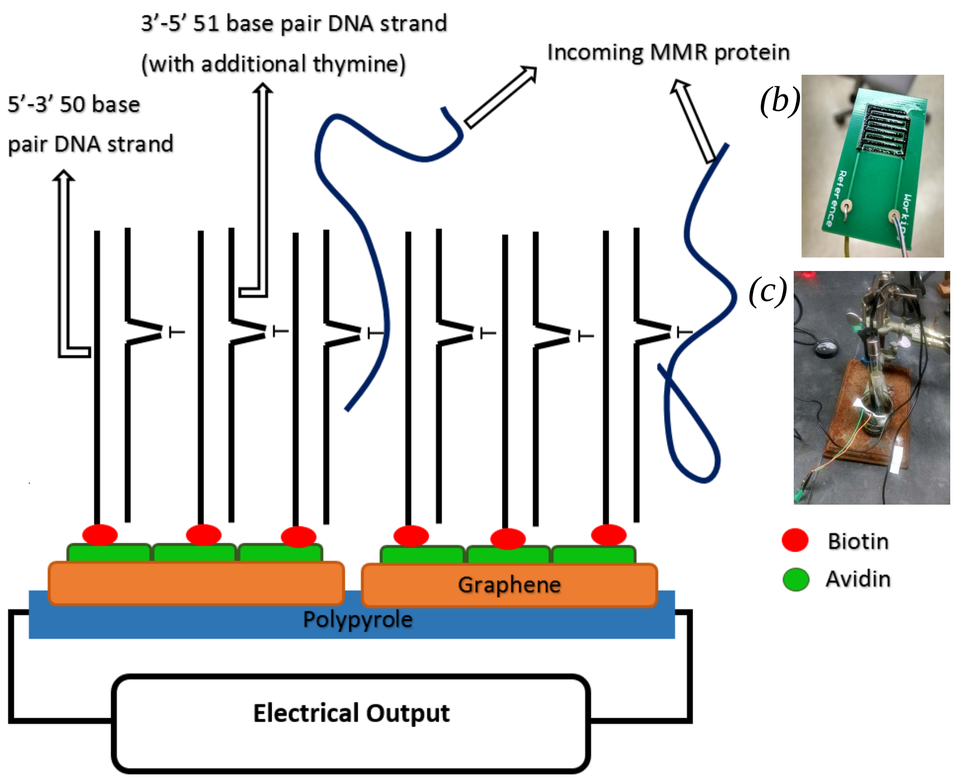

Figure 1: *Schematic of final assembled device; (b) Finely deposited graphene-polypyrole chip; (c) Electropolymerization of graphene and pyrole on chip;*

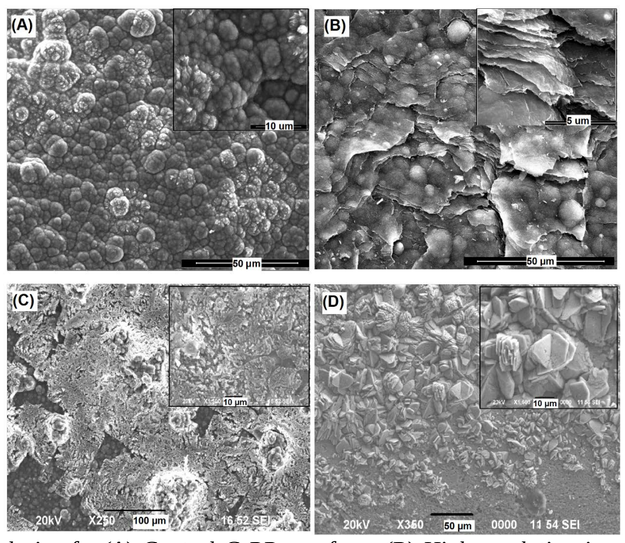

Figure 2: *SEM analysis of : (A) Control G-PPy surface; (B) High resolution image revealing only embedded graphene flakes; (C) Experimental G-PPy-avidin surface showing greater levels of exfoliated graphene; (D) High resolution image revealing graphene-flakes completely engulfed by avidin molecules.*

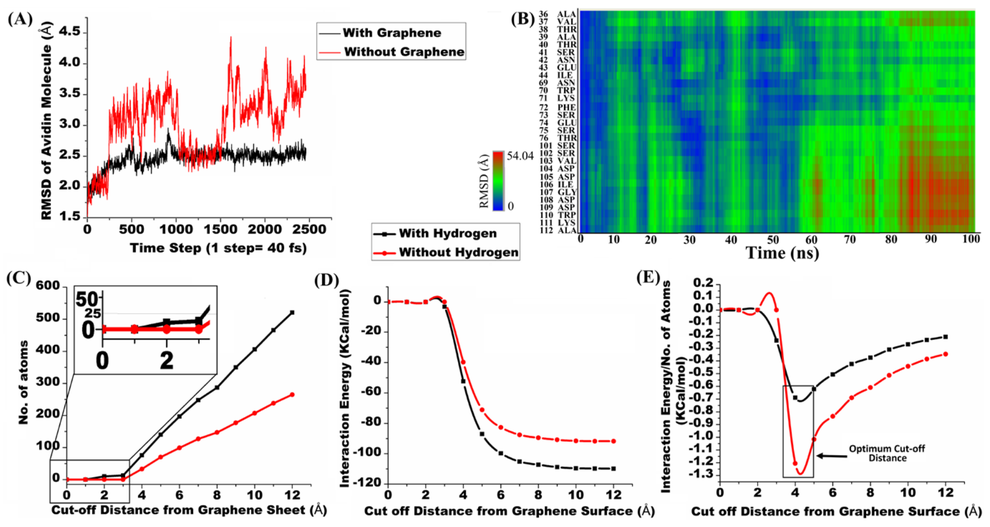

Figure 3: *Analysis of interaction energy between graphene and avidin using MD simulations.*

Residue Name	Residue ID	Type of Residue
Threonine	40	Polar, un-charged
Serine	41, 73, 101, 102, 75	Polar, un-charged
Asparagine	42, 104	Polar, un-charged
Glutamic Acid	43, 74	Acidic, negatively charged side chain
Isoleucine	44	Hydrophobic side chain
Alanine	39	Hydrophobic side chain
Lysine	71, 111, 45	Basic, polar, positively charged chain
Phenylalanine	72	Hydrophobic side chain
Valine	103	Hydrophobic side chain
Aspartic Acid	105, 108	Acidic, negatively charged side chain

TABLE 1: *Residues of avidin within 5Å of the graphene surface.*

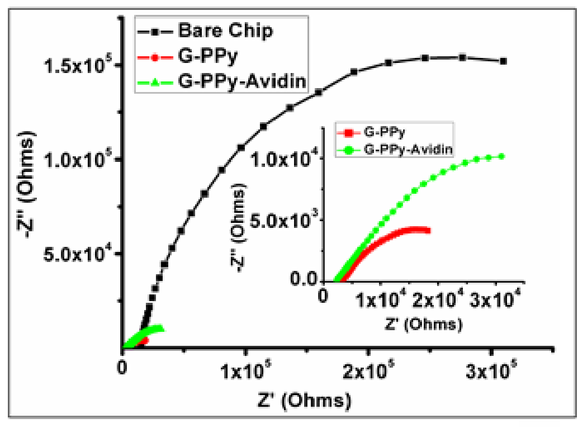

Figure 4: *EIS analysis of the G/Ppy nanocomposite substrate*

3. Conclusion

We have been successful in depositing avidin onto the graphene-polypyrole substrate, which also shows different conductivity compared to graphene-Ppy only chips. Immobilization of the biotinylated DNA probes has be conducted with optimized concentrations of graphene/ Avidin. Detection of MMR proteins is underway.

4. Acknowledgements

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5. References

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