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# Insilico Studies on Protein Modified Graphene Oxide Sheets for Potential Biosensing Applications for Curcumin Metabolites

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***Abstract:***
Biosensors are gaining momentum in the field of life science research. Knowing the concentration of the drugs we consume in the form of nutraceuticals, medications, etc is essential for overall well-being of an individual. Curcumin is termed to one of the top selling supplements in the world. But this marvel compound transforms into various metabolites in the systemic circulation. These metabolites hinder the therapeutic activity of curcumin. So, knowing the concentration of metabolites formed in each individual after a particular dosage of curcumin can help monitor the efficacy of this wonder drug. Avidin is a very cost effective and stable protein that has been used in combination with biotin in various bioassays and sensors. But, Avidin as a sole functional compound on graphene oxide has never been attempted before. Hence, before actual experimentation, docking studies were performed to prove the feasibility of the experiment in real time. The current study was performed between avidin and two major metabolites of curcumin i.e. curcuminglucuronide and curcumin sulphate.

***Keywords:*** *Curcuminglucuronide, Curcumin sulphate, Avidin, Discovery studio 4, Graphene oxide*

## I. INTRODUCTION

Graphene, regarded as the "thinnest material in the Universe", is a 2-D one atom carbon sheet with the thickness of one atom. Graphene oxide, used as a nano material, from Graphene family, is regarded due to its unique electronic properties by virtue of having oxygen rich functional groups. These groups may be hydroxyl group, carboxyl group, epoxy group,etc. Now a days, it is being used in biosensor research and application extensively. Modification of Graphene oxide-based biosensor by the addition of small mass organic molecules, metallic or non-metallic oxides enhance the electrochemical properties of Graphene oxide-based biosensors [1]-[8]. Graphene oxidize belongs to the class oxidoreductases, which helps in the oxidation of glucose to D gluon-δ lactones leading to the formation of hydrogen peroxide. A remarkable biopolymer, Chitosan having unique physical/chemical properties with high solubility rate in aqueous acidic solution is mixed with Graphene. This leads to the formation of a hybrid nano composite of graphene chitosan. This nano composite's reaction with glucose oxidase concluded that chitosan has the ability to improve dispersion of graphene and glucose oxidize enzyme molecules along with an excellent sensitivity (37.93 $AmM^{-1}cm^{-2}$) and a great enzyme loading ($1.12 \times 10^{-9}$mol $cm^{-2}$). Also, this biosensor could sustain about 95% enzyme activities after its storage for about 7 days at 4°C [9]-[11].

*January - February 2020*
*ISSN: 0193 - 4120 Page No. 01 - 11*

Biological molecules are also used for the detection of other biological molecules or chemical substances through an analytical device known as Biosensor. Basically, detector molecule and sensor, both are connected to each other and this connection is monitored by a computer which helps in converting the biological systems into certain codes which are being accessed by using certain algorithms. The basic components of a biosensor are (1) biological molecule (e.g. tissue, nucleic acid, cell receptors, enzymes, microorganisms, etc, a bio mimic and they can be produced by bio-engineering, (2) for association of both the components, a transducer is connected in between, (3) an element for detection which can work either of the ways e.g. physiochemical, magnetic, piezoelectric, thermometric, electrochemical, optical, etc). The development of these devices involves the immobilisation of proteins directly on the transducer where maximum of its biochemical activity is sustained and non-specific interactions are minimized [12]. The glycoprotein which is found in egg white is known as Avidin. Biotin is a member of B-vitamin family which is also known as Vitamin-H. Biotin-Avidin system plays a vital role for these biosensor applications as they both are highly specific to each other and shows strong binding affinity. Avidin has four identical sites for binding of biotin which confers binding only to the target of interest. Adsorption of biotin on solid surfaces and fabrication of avidin makes possible for active ligands to get attached to the biosensor surface, henceforth, identification of target species takes place in the sample and the selectivity of biosensor is increased by the reduction of interferences caused by non-specific interactions [13]-[20].

Curcumin, the bioactive diphenol of spice turmeric, when conjugated with glucuronic acid becomes a ubiquitous metabolic pathway for its oral administration.

(1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadi ene-3,5-dione),also known as Diferuloy methane, is found in the rhizome of Curcuma longa(turmeric). It serves as a medical herb due to its antioxidant, anti-mutagenic, anti- inflammatory and anti-cancer properties. This polyphenol has been shown to target multiple signalling molecules while demonstrating activity at cellular level which has helped to support its multiple health benefits. One of the major problems with ingesting curcumin by itself is its "poor bioavailability" due to poor absorption, rapid metabolism and elimination [21]-[25]. The degradation of this polyphenol in the body involves the autoxidation in which heptadienedione undergoes oxygen moiety addition and cyclization in order to form cyclopentadione derivatives. As the degradation of Curcuminglucuronide is slower in regards of two orders of magnitude as compared to Curcumin, itself, hence it proves that our desired compound is much more stable than its active precursor - Curcumin. When the oxidation of curcuminglucuronide was catalyzed with Horseradish peroxidase it was observed that oxidation occurred at around eighty percent rate with curcumin leading to good transformation. The major products of oxidative transformation were analyzed and recognized as glucuronidatedbicyclopentadione distereomers.10% of the products were accounted by virtue of their cleavage into vanillin-glucuronide [26]-[28]. When specific species bind to the avid in/biotin surface, an increase in the thickness of deposited film takes place which helps in measuring extent of binding of species to the sensor surface. Discovery Studio is a comprehensive suite software. It is a suite for small and macromolecule interaction studies. It is a product from Accelrys.

The aim of our present study is to investigate the experimental binding feasibility of avidin to GO for potential sensing application. In the field of life science, it is well known that Biotin- avidin have the strongest non-specific binding. Many sensing assays are devised in immunology using this interaction as a major binding scenario. We also have literature evidence of biotin binding with curcumin for detection of human breast cancer cell lines. Hence,

TEST Engineering & Management

*January - February 2020*
*ISSN: 0193 - 4120 Page No. 01 - 11*

we thought of replacing the biotin candidate with avidin for sensing curcumin. Avidin seems to be cheaper alternative to biotin there is no evidence as to why it has not been used so far. Hence, the present study was undertaken to investigate the experimental binding possibility between avidin and curcumin.

## II. MATERIAL AND METHODS

The PDB file for avidin was loaded into the software and processed in the ligand receptor binding tool. After processing the software gives information about the different amino acids attached in each parent protein chain, its hydrophobicity, acidic coefficient and average isotropic values. The A chain are represented using colours from red to orange to yellow to green to cyan with a total of 119 amino acids. The B chain continues from 120$^{th}$ amino acid (lysine 3) depicted as cyan to blue to Indigo to purple to pink with a total of 113 amino acids. The curcumin information was obtained for PubChem. The file number for curcuminglucuronide and curcuminsulphate was 227466-72-0 and 339286-19-0 respectively. GO data was neither found in PubChem or in Chemspider, hence the structure of GO was drawn using Chemdraw and loaded into the software. The software generated a 3D structure for the same which was used to check its interaction with avidin.

## III. RESULT AND DISCUSSION

The Insilco studies were performed for curcuminglucuronide and sulphate with avidin and then compared with the binding scores of Biotin and avidin complex. Figure 1 shows the 3D image of curcuminglucuronide and curcuminsulphate binding with avidin (from left to right) respectively. The red colour long chemical structure in the left corner of each image represents curcuminglucuronide (left) and curcuminsulphate (right) respectively.

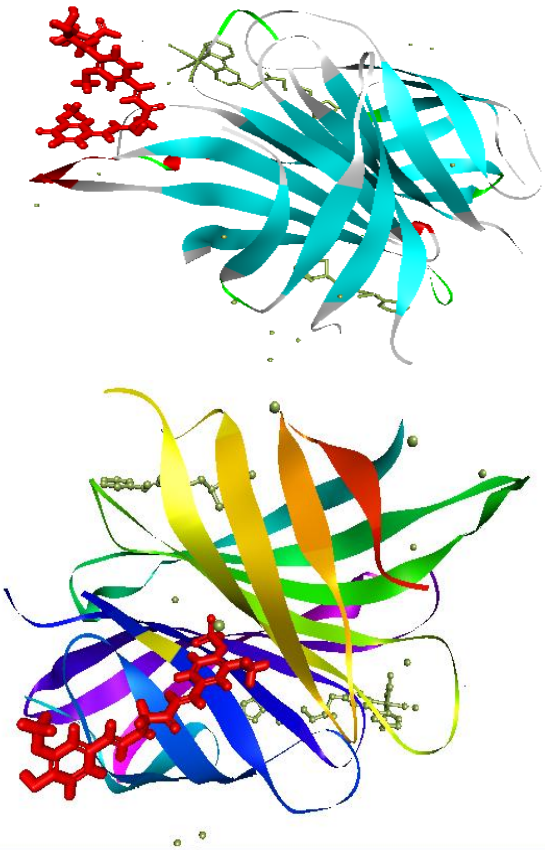


Fig 1 (left and right) 3D images of Curcumin Glucuronide + Avidin and Curcumin sulfate + Avidin binding.

The 2D binding image of the interaction taking place between curcuminglucuronide and avidin is shown in figure 2. Electrostatic bonds are formed between A chains glutamine 61 and arginine 69 with the oxygen attached to the aliphatic chain of curcumin. The distance of the bonds from the aliphatic chain was around 3.4 Å and 5.7 Å for glutamine 61 and arginine 69 respectively. Weak Van der Waals attraction was seen between curcuminglucuronide and A chains asparagine 86, phenyl alanine 84 and B chains isoleucine 106. The binding score was found to be 9.1 which proves binding between curcuminglucuronide and avidin is not feasible. This might be due to big sterically inhibiting structure of the compound that doesn't fit well with avidin

January - February 2020
ISSN: 0193 - 4120 Page No. 01 - 11

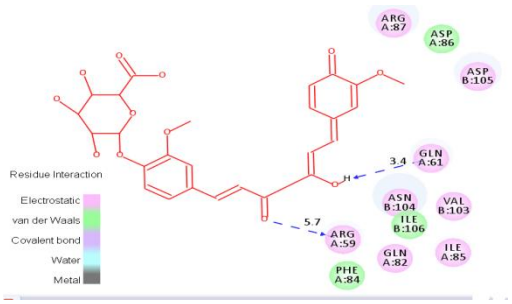



Fig 2 2D interaction between CurcuminGlucuronide + Avidin = score 9.1

The 2D binding image of the interaction taking place between curcumin sulfate and avidin is shown in figure 3. Electrostatic bonds are formed between B chains threonine 52 and A chains histidine 52 and threonine 67 with the oxygen attached to the sulfate group of the curcumin sulfate. The distance of the bonds from the aliphatic chain was around 3.6 Å, 2.8 Å and 3.2 Å for threonine 52, histidine 52 and threonine 67 respectively. Weak Van der Waals attraction was seen between curcumin sulfate and B chains glutamine 28 threonine 30 and B chains asparagine 24, glutamine 28 and serine 25. The binding score was found to be 117.4 which proves binding between curcuminsulfate and avidin is feasible. Since, sulfate group is attached to curcumin the redox potential will definitely vary while using cyclic voltammetry mode for sensing.

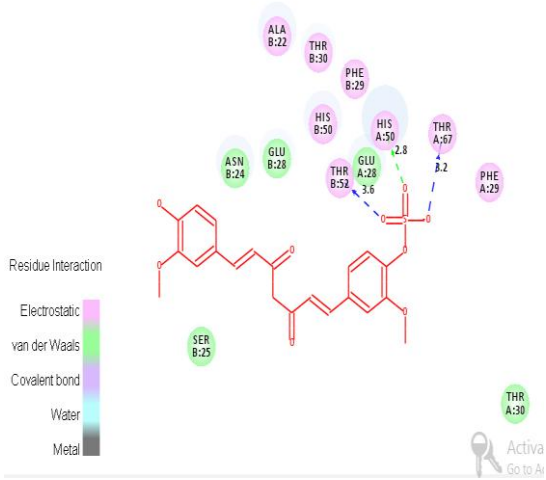



Fig 3 2D interaction between Curcumin sulfate + Avidin = score 117.4

Figure 4 shows the 3D image of biotin binding with avidin. The red colour long chemical structure in the left corner of the image represents biotin

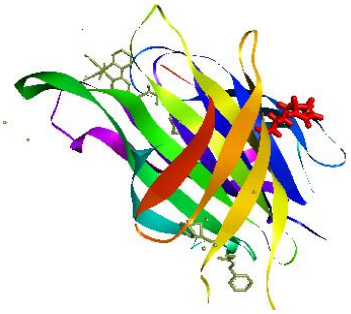
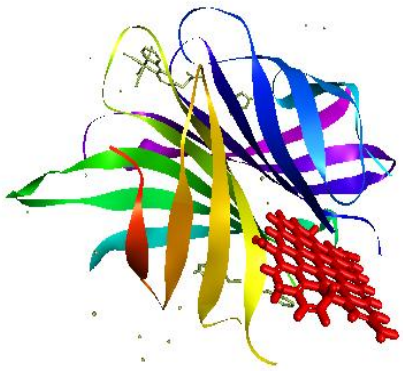


Fig 4 3D image of Biotin + Avidin binding

The 2D binding image of the interaction taking place between biotin and avidin is shown in figure 5. Electrostatic bonds are formed between B chains threonine 52 and A chains threonine 30 and threonine 67 of avidin with biotin. The distance of the bonds was around 4.4 Å, 4.4 Å and 3.5 Å for threonine 52, threonine 30 and threonine 67 respectively. Water bond were also speculated during simulation. Weak Van der Waals attraction was seen between biotin and B Chains Phenyl alanine 29. The binding score was found to be 85 which proves binding between biotin and avidin is feasible. This data is very much in sync with previously reported data on nonspecific binding between biotin avidin. This binding is extensively used now a day to form immunological assays for sensing specific targets. When we compare the binding scores of biotin avidin with curcumin and avidin there is a difference of 25. So, we would like to infer although the binding won't be as specific as biotin and avidin but interaction is feasible. And sulfate form shows score more than biotin avidin which makes us curious if it would be experimentally as proficient as it claims to be theoretically



TEST Engineering & Management

*January - February 2020*
*ISSN: 0193 - 4120 Page No. 01 - 11*

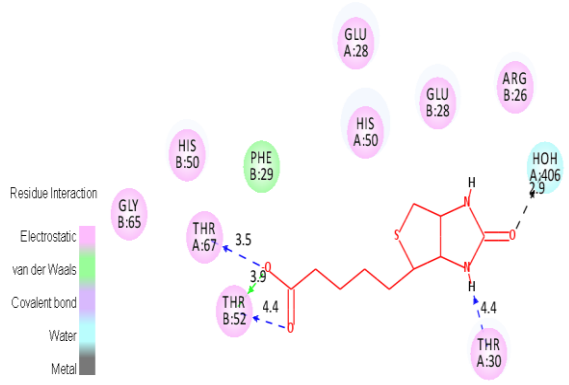



Fig 5 2D interaction between Biotin + Avidin = score 85

Figure 6 shows the 3D image of GO and rGO binding with avidin (left to right) respectively. The red colour flat chemical structure in the corner of the image represents GO and rGO from left to right respectively

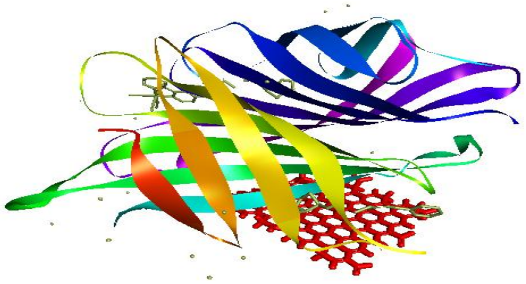


Fig 6 (left to right) 3D image of Graphene oxide +avidinand rGraphene oxide +avidin binding respectively

The 2D binding image of the interaction taking place between GO and avidin is shown in figure 7. As the carbon skeleton of both the materials is the same with a variation in number of oxygen atoms, the 2D interaction is similar for both the materials. The only difference is GO has a greater number of oxygen groups in comparison to rGO. Electrostatic bonds are formed between B chains threonine 55 and A chains lysine 71 and GO oxygen groups. The distance of the bonds was around 3.4 Å and 5.4 Å threonine 55 and A chains lysine 71 respectively. Pi bonds are formed between B chains arginine 26 and the carbon skeleton of GO. Weak Van der Waals interaction is seen between A chains histidine 50, proline 48 and GO. The binding score was found to be 106.66 for GO. More oxygen groups provide more binding sites for the protein to bind and have proper anchorage. Hence, we infer that avidin can possibility bind to GO providing experimental feasibility. But, based on our data the binding would be more efficient in GO. The binding score between avidin and GO is higher than the benchmark molecule also (biotin and avidin). Hence showing very high experimental feasibility.

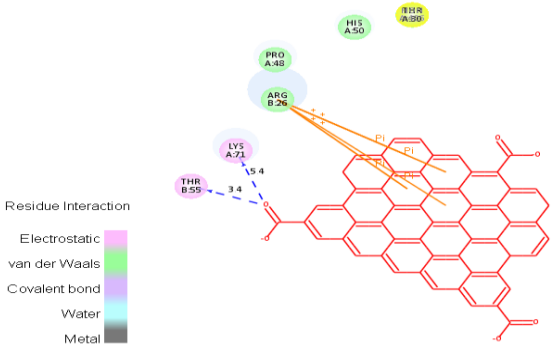



Fig 7 2D interaction between GO with avidin

Figure 8 shows the 3D image of GO binding with avidin and curcuminglucuronide. The red colour flat chemical structure in the corner of the image represents GO. The green long chemical structure is curcuminglucuronide. The binding score was found to be 9.16. This score is similar to the score for curcuminglucuronide and avidin. The 2D interactions obtained for this complex was the same as obtained for GO or rGO with avidin. Hence, we would like to speculate that binding won't be feasible for this set of complexes

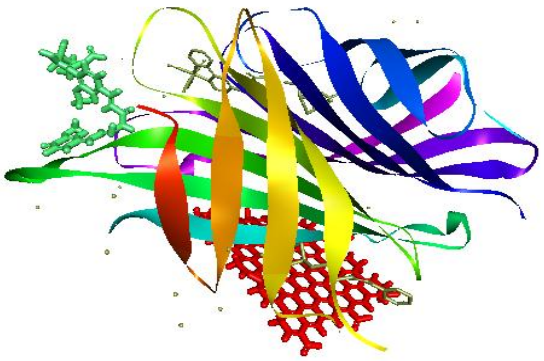


Fig 8 Graphene oxide +avidin + CurcuminGlucuronide.

*January - February 2020*
*ISSN: 0193 - 4120 Page No. 01 - 11*

Figure 9 shows the 3D image of GO binding with avidin and curcumin sulfate. The red colour flat chemical structure in the corner of the image represents GO. The green long chemical structure is curcuminsulphate. The binding score was found to be 117. This score is similar to the score for curcuminsulphate and avidin. The 2D interactions obtained for this complex was the same as obtained for GO with avidin. Hence, we would like to speculate that binding is feasible for this set of complexes. But if we use cyclic voltammtery for experimental investigation of this complex conjugate the redox potential would be different from the complex of curcumin.

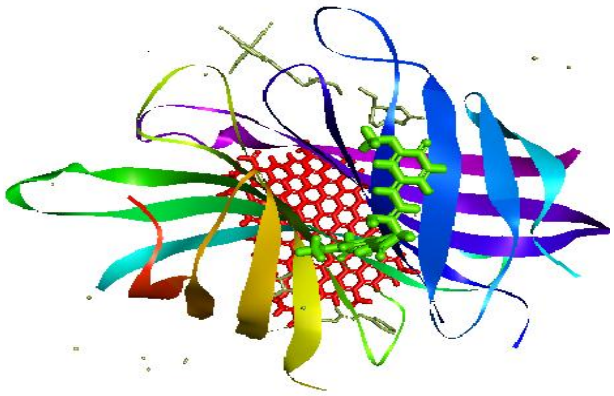


Fig 9 Graphene oxide +avidin + Curcumin sulfate

## IV. CONCLUSION

Hence, we would like to conclude that there is an experimental feasibility between curcumin, avidin and GO. Discovery studio 4 has provided us with a general visualization of the possible interaction between our major reacting candidates.Binding score between avidin and curcuminsulphate is above 50. So, we infer experimental feasibility. The binding score between avidin and GO is higher than the benchmark molecule (biotin – avidin). Hence showing very high experimental feasibility. With GO as an anchor material avidin can be used to bind to curcumin theoretically. Hence, we can try having a sensing system using avidin as a detector molecule for curcuminsulphate. For curcuminglucuronide the binding score seems to be very less. This might be due to steric hindrance of the bulk glucuronide moiety. Therefore, a sensing system is possible with the same theoretically. We have to check its experimental feasibility by running wet lab reactions in future.

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*January - February 2020*
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Dr. Nibedita Deyhas completed her PhD in bio nanotechnolgy from Anna University. She has 9 publications in many peer reviewed journal. She was DST inspire fellow till March 2019 and has received 'Dr A P J Abdul Kalam young scientist award' – 2019 from Marina Labs Chennai. At present she a faculty in the Department of Biotechnology in Saveetha School of Engineering.

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