Chapter 4

Mechanism of protection by Nanoparticles against Environmental Carcinogens induced toxicity.
4.1- Introduction-

The goal of this study is to decipher the intrinsic mechanism behind the protective action of nanoparticles against the environmental carcinogens induced toxicity. In recent published studies, some of the nanoparticles have been employed in scavenging the high molecular weight polycyclic aromatic hydrocarbons (PAHs) from the contaminated soils (Karnchanasest B. et al 2007). Amphiphilic polymer nanoparticles have been used as nano-absorbent for pollutants in aqueous phase. In a recent report, Bochra et al. (2011) have shown the potential application of titanate nanotubes as solid-phase extraction adsorbents for seven PAHs: acenaphthylene, acenaphthene, anthracene, fluorene, phenanthrene, fluoranthene and pyrene, from the environmental water samples. Where the recoveries of PAHs ranged from 90% to 100%.

The scavenging capacities of the nanoparticles for PAH and other pollutants could probably be attributed to their higher affinity towards the xenobiotics (Karnchanasest and Santisukkasaem, 2007). The structural properties and surface chemistry of nanoparticles are the players, further, extremely high surface area to volume ratio results in multiple enhancements of such properties.

Considering this we conducted the experiments in Chapter 3, where we co-exposed the carcinogens and nanoparticles in the biological system (A549 Cell line), after the end point observation of the experiments we concluded that; nanoparticle have protective potential against the deleterious effect of environmental carcinogens. Eventually nanoparticles are also toxic at high concentration as the earlier studies mentioned (Rahman et al 2002), they induce the Reactive oxygen species which lead to genotoxicity and at higher concentration they also act as a cytotoxic agents, which causes cell death. To avoid this incident we used the nanoparticles in very low and safe concentration, and
to our astonishment, at low concentrations the nanoparticles probably act as scavengers of Environmental carcinogens.

Earlier studies showed that PAHs enters the cell via aryl hydrocarbon receptor (AHR) (Yasuhiro S., et al 1999), along with stimulating the AHR to activate transcriptional regulation of xenobiotic response element (XRE) and genes coding for xenobiotic metabolizing enzymes such as Cytochrome P450s (CYPs), UDP glucuronosyltransferase UGT1A6, NAD(P)H: quinine oxidoreductase-1 (NQO1), aldehyde dehydrogenase (ALDH3A1), and various glutathione-S-transferases (Gu Y.Z. et al 2001).

After the enzymatic metabolism, PAHs is converted to PAHs-diol-epoxides, a crucial carcinogenic metabolite of PAHs, that reacts primarily with the residues in DNA (Golomb et al., 1997) to form bulky adducts that block DNA synthesis.

Tobacco specific carcinogen NNK also acts as a site- selective high affinity agonist for the α7 nicotinic acetylcholine receptor, which regulates the growth of a significant SCLC by stimulating the release of autocrine growth factor serotonin. Signaling events are initiated by the binding of NNK to the α7 nicotinic acetylcholine receptor. After binding of NNK to its receptor, activates Raf -1 /MAP Kinase Pathway, resulting in the phosphorylation of c-myc (Jull B.A et al, 2001).

Both of the receptors are responsible for the entry of carcinogens and activation of the deleterious effect of carcinogens inside the cell. These carcinogens cause the DNA damages which are repaired by the DNA repair enzymes, but as already discussed in the previous chapters, these carcinogens and their metabolites may cause the dysfunction of DNA repair enzymes. Also, that the binding of carcinogens to these enzymes may render these enzymes unable to repair the DNA damage. Further, the resulting DNA damage accumulation may lead to cancer.
The current study has been designed in order to explore the possibility of the nanoparticles to scavenge the carcinogens. Considering this we planned to conduct the comparative binding analysis of environmental carcinogens with their bimolecular targets and environmental carcinogens with the nanoparticles, subsequently these experiments will help to explore the intrinsic protection mechanism of nanoparticles against the environmental carcinogens.

4.2 Materials & Methods

Minimum system Requirement for the project is:

4.2.1) Supported Operating Systems

Discovery Studio Visualizer is supported on the following operating systems:

- Microsoft® Windows XP Professional, SP2 and SP3
- Microsoft Windows Vista, Business and Enterprise Editions, SP1
- Red Hat® Enterprise Linux® 4.0, Updates 4-7
- Red Hat Enterprise Linux 5, Retail, Updates 1-2
- SUSE® Linux Enterprise 10 (SP2)

4.2.2) Processor and RAM Requirements

- Processor: An Intel-compatible ≥2 GHz is required.
- RAM: A minimum of 2 GB of memory for the visualizer.

4.2.3) Disk Space Requirements

A standard installation of Discovery Studio Visualizer requires 272 MB of disk space on Windows and 454 MB on Linux.

4.2.4) Software:

- Accelrys discovery studio visualizer 2.5 (Designing of crystal structure, visualizing and manipulating protein and crystal 3D structures)
• PatchDock (Docking server)
• AutoDock MGL Tool
• Open Babel (File converter)
• An Internet Browser and valid internet connection.

After studying of the anatase crystal structure, we found that Accelrys Discovery studio would be the most suitable software for the designing of TiO$_2$ anatase crystal structure.

4.2.5) Method for Designing Crystal Structure of TiO$_2$ Nanoparticle-

After studying the anatase crystal structure, we found that anatase is the thermodynamically favored phase. According to the anatase lattice parameters, the tetragonal crystal have lengths (A, B and C) as; A = B= 3.782 Å, C= 9.502. Å and angles (alpha, beta and gamma) as; Alpha = beta = gamma = 90°. The Accelrys Discovery studio 2.5 program was found to be most suitable software for the designing of TiO$_2$ anatase crystal structure. After the construction of Unit cell of TiO$_2$ anatase by using the anatase lattice parameters, a surface was created and this unit cell was extended in the desired directions (axis) creating a new surface of TiO$_2$ comprising (1,0,1) of 5 unit cells in6direction and 2 unit cells in the Z direction. This gave a surface of dimensions 1.891 x 0.3782 x 1.9004 nm$^3$.

• From the start menu, go to the all programs option and select the Accelrys Discovery Studio 2.5 program.
• Now go to file menu >> New >> Molecule Window.
• A black sub window / tab will open up. Goto the Structure menu >> Crystal cell
  >> Create cell.

• A Crystal cell outline will appear in the black window in select mode (yellow
colour).

![Figure 4.1: Crystal Cell Outline](image)

• Now again goto Structure menu >> Crystal cell >> Edit Parameters…. A small
dialog box of Crystal Builder with 4 tabs will pop up. In the first tab, i.e. of Cell
parameters, edit the crystal lengths(A, B and C) and angles(alpha, beta and
gamma) according to the anatase lattice parameters, i.e.,

  • A = B = 3.785.

  • C = 9.514.

  • Alpha = beta = gamma = 90.

• Now under the space group tab select the “/I41/amd” space group and origin as
“origin-1 choice: 1”, and calculate the lattice positions of Ti atoms by the help of
the formulae given under positions in the space group tab.
The calculated positions are:

1) 0, 0, 0
2) 0.5, 0.5, 0.5
3) 1, 0.5, 0.25
4) 0.5, 1, 0.75
5) 0.5, 0, 0.25
6) 0, 0.5, 0.75
7) 0.5, 0.5, 0.5
8) 1, 1, 1
9) 1, 0.5, 0.75
10) 0.5, 0, 0.25
11) 0, 1, 1
12) 0.5, 0.5, 0.5
13) 0.5, 0.5, 0.5
14) 1, 0, 0
15) 0.5, 1, 0.75
16) 0, 0.5, 0.25
17) 0.5, 0.5, 0.5
18) 0, 0, 1
19) 0.5, 1, 0.75
20) 1, 0.5, 1.25
21) 0, 0.5, -0.25
22) 0.5, 0, 0.25
23) 1, 1, 0
24) 0.5, 0.5, 0.5  
25) 0.5, 0.0, 0.25  
26) 1, 0.5, -0.25  
27) 0.5, 0.5, 0.5  
28) 0, 0, 0  
29) 1, 0, 1  
30) 0.5, 0.5, 0.5  
31) 0, 0.5, 1.25  
32) 0.5, 1, 0.75  

These are the 32 Wyckoff positions of the I41/amd space group. These are pre defined positions, which were calculated using the general formulae for different wycoff positions,

1. x, y, z  
2. -x+1/2, -y+1/2, z+1/2  
3. -y, x+1/2, z+1/4  
4. y+1/2, -x, z+3/4  
5. -x+1/2, y, -z+3/4  
6. x, -y+1/2, -z+1/4  
7. y+1/2, x+1/2, -z+1/2  
8. -y, -x, -z  
9. -x, -y+1/2, -z+1/4  
10. x+1/2, y, -z+3/4  
11. y, -x, -z  
12. -y+1/2, x+1/2, -z+1/2
13. $x+1/2, -y+1/2, z+1/2$
14. $-x, y, z$
15. $-y+1/2, -x, z+3/4$
16. $y, x+1/2, z+1/4$
17. $x+1/2, y+1/2, z+1/2$
18. $-x+1, -y+1, z+1$
19. $-y+1/2, x+1, z+3/4$
20. $y+1, -x+1/2, z+1.250000$
21. $-x+1, y+1/2, -z+1.250000$
22. $x+1/2, -y+1, -z+3/4$
23. $y+1, x+1, -z+1$
24. $-y+1/2, -x+1/2, -z+1/2$
25. $-x+1/2, -y+1, -z+3/4$
26. $x+1, y+1/2, -z+1.250000$
27. $y+1/2, -x+1/2, -z+1/2$
28. $-y+1, x+1, -z+1$
29. $x+1, -y+1, z+1$
30. $-x+1/2, y+1/2, z+1/2$
31. $-y+1, -x+1/2, z+1.250000$
32. $y+1/2, x+1, z+3/4$

Considering $x, y, z$ to be complementary with $-x, -y, -z$, i.e., here considering $x = y = z = 0$ and $-x = -y = -z = 1$, here the minus sign indicates the complementary function.
Note: fractional coordinate = Actual length along an axis/

Total length of a unit cell along that axis

- These are the positions (fractional co ordinates) of Titanium. Now according to the bond angle and bond length the corresponding Oxygen co ordinates are calculated. For faster calculation write a program in C to calculate the co ordinates of Oxygen. The program which I have written is:

/*A program for calculation of the fractional co-ordinates of oxygen in anatase*/

/*Author Anupam date:27/05/2013*/

#include<stdio.h>
#include<conio.h>
#include<math.h>

void main()
{

float xa,xb,xc,ya,yb,yc,za,zb,zc,x,y,z=0;
clrscr();

printf("Enter the values of the fractional co-ordinates(x,y,z):");
scanf("%f %f %f",&x,&y,&z);
if(x<1)
{
    xa=x + (1.937*cos(12.308))/3.785;
    xc=z + (1.937*sin(12.308))/9.514;
    xb=0;
}
if(y<1)
{ 
    ya=0;
    yb=y + (1.937*cos(12.308))/3.785;
    yc=z - (1.937*sin(12.308))/9.514;
}
if(z<1)
{
    za=0;
    zb=0;
    zc=z + (1.966/9.514);
}
printf("The fractional co-ordinates for Oxygen atoms are:\nxa,yb,xc,ya,yb,yc,za,zb,zc);  
getch();
}

- Now go back to acelrlys Discovery studio and then goto Structure >> Crystal cell >> create atom... a dialog box will appear. In it, click on the table button, the periodic table will open up. Then choose Ti from it and click the “ok” button. Now give the X, Y, Z fractional co ordinates (Wyckoff positions) of the Ti atoms as calculated above.

**Note:** Care should be taken that no two co ordinates are same, i.e., avoid duplicity. Also delete the Ti atoms lying outside the unit cell.
Figure 4.2: Duplicacy of Co-ordinates

- Repeat the above step for oxygen atoms, the co ordinates of which are obtained from the self designed program. (avoid duplicacy of co ordinates here too.)

- Now select a Ti atom and then select adjacent O atom while pressing the “SHIFT” key, so that both Ti and O are selected. Then go to Chemistry >> Bond >> Single. A bond will be created between the two atoms now proceed further keeping the picture of anatase unit cell in mind, obtained from literature.

- Thus finally a unit cell of anatase is formed.

Figure 4.3: Unit Cell of Anatase
Now to create a surface we have to extend this unit cell in the desired directions (axis). Let’s make a (1,0,1) surface comprising of 5 unit cells in X direction and 2 unit cells in the Z direction, this will give us a surface of dimensions $18.925 \times 3.785 \times 19.028 \, \text{Å}^3$.

To implement this go to Structure >> Crystal cell >> Edit Parameters… the Crystal Builder dialog box will open up. Then choose the Preference tab. In this, select Symmetry Style as Positions from the drop down menu, set Special position tolerance to 0.05, uncheck the Proximity Binding check box, in view range set A : 5 , B : 1 , C : 2. Then click on the “apply” button and then the “ok” button. The surface gets generated!!

**Figure 4.4(A&B): 3D structure of Anatase**

- Now save this as “.sd” file. Goto File >> Save As, write “TiO$_2$NP.sd”, select a preferred location to save the file and then click Save button.

- Next step is to convert this file format to “.pdb” format, for this go to Start menu >> All Programs >> Open Babel 2.2.3 >> OpenBabelGUI. The program will open
up. In this select the input file format as “.sd”, then click browse, open the location of the input file “Ligand.sd” and open it. Then choose the output format as “.pdb”. Now enter the address of the output file, i.e., where it has to be saved, give the file name as “TiO₂NP.pdb”. Finally press convert button.

4.2.6) **Procurements of Fullerene and Carbon based Nanotubes**-

**Nanotube Modular** software used for designing of carbon based nanoparticles like -

a) Single-walled carbon nanotubes (SWCNT),
   
   Bond Length – 1.421nm, Tube Length – 25nm

b) Multi-walled carbon nanotubes (MWCNT )
   
   Bond Length – 1.421nm, Tube Length – 25nm, No of wall- 2

c) Fullerene d-1nm

**pdb**. files of CNTs & Fullerene were obtained by using Nanotube modeller.

4.2.7)- **Modelling of 3D structure of Environmental Carcinogens**-

**ChemSketch** - software used for creating 3D structure of environmental carcinogens

a) Benzo-α-Pyrene (BaP)

b) 7,8-Dihydro-7,8 dihydroxybenzo(a)pyrene,9,10-oxide (BPDE)

c) Chrysene

d) Chrysene1,2-diol-3,4-epoxide-2 (CDE)

e) 2,7 Di methyl benzo anthracene (DMBA)

f) 2,7 Di methyl benzo anthracene -3,4 diol-1,2epoxide (DMBAepoxide)

g) 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNK)

h) 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)
The SMILES (Simplified Molecular Input Line Entry Specification) notations of the environmental carcinogens were obtained from the ZINC database. The 3D-structure of environmental carcinogens was generated de novo, via the internet (http://molecular-networks.com/products/corina), by program CORINA on server running in Computer-Chemie- Centrum, Universiy of Erlangen-Nurnburg, Germany. It is a rule and data-base system that automatically generates 3D atomic coordinates from the constitution of a molecule as expressed by connection table or linear code.

4.2.8)- Biomolecules/Proteins Modeling –

DNA Repair Enzymes:

pdb. files of best and most probable targets of environmental carcinogens among DNA repair enzymes were taken from the Chapter 1, as we have earlier discussed in chapter 1 these proteins were procured by RCSB protein data bank and few were generated by modeler.

Aromatic Hydrocarbon Receptor (AHR):

PDB structure of aryl hydrocarbon receptor (AHR Uniprot entry - P35869) is not available in the PDB databank. I-TASSER online server was utilized for the ab-initio modeling to build the 3D structure of AHR as shown in, and validated by the approach of Ramacharndran Plot by using RAMPAGE.

AHR selected as a biomolecular target of PAHs. Previous studies have established that AHR is responsible for the entry and regulation of the enzymatic metabolism of PAHs to PAH dioleopxide, a crucial carcinogenic metabolite of PAHs, which reacts with the DNA, form adduct and resultant DNA damage occurs. (Golomb D. et al., 1997)
**Figure 4.5:** Modeled structures of AHR showing 83.5% of amino acid residues in favored region of Ramachandran plot.

**α7 Nicotinic Acetylcholine Receptor (nAch):**

MODELLER 9.10 used for the modeling of nAch. Tobacco specific carcinogen NNK also acts as a site-selective high affinity agonist for the α7 nicotinic acetylcholine receptor, which regulates the growth of a significant SCLC by stimulating the release of autocrine growth factor serotonin. Signaling events are initiated by the binding of NNK to the α7 nicotinic acetylcholine receptor. After binding of NNK to its receptor, activates Raf-1/MAP Kinase Pathway, resulting in the phosphorylation of c-myc (Jull B.A. *et al*, 2001).
Figure 4.6: DOPE score detail of nAch modeled by Modeler 9.10.

4.2.9) *In Silico* Binding Energy Analysis-

All the binding efficiencies and interaction studies of nanoparticles with environmental carcinogens were performed by using Genetic based algorithm for Fullerene, CNTs & Geometric based algorithm for TiO$_2$NP (due to the absence of force field of Titanium metal in genetic algorithm we used the geometric algorithm for TiO$_2$NP and carcinogens interactions).

Interaction and binding analysis of biomolecules (DNA Repair Enzymes, AHR and nAch) with carcinogens were also performed by the above mentioned algorithms.

4.3) Strategies –

1) Determination of the binding efficiencies of Environmental Carcinogens with nanoparticles.

2) Comparison of binding efficiencies of Environmental Carcinogens with their biomolecular targets, and nanoparticles.
4.4) Results-

Genetic and Geometry based algorithm based software, has been used for the determination of binding energies between Nanoparticles and Environmental carcinogens, Nanoaprticles were in bigger size than Environmental carcinogens so that nanoparticles have been considered as a receptor and Environmental carcinogens considered as a ligands. Whole docking results were divided in two categories one is carbon based Nanoparticles (Fullerenes, Single walled Carbon Nnanotubes and Multi walled carbon Nanotubes) and second one was metal based Nanoparticles (Titanium Dioxide Nanoparticle).

Table No.: 4.1- Binding efficiencies of environmental carcinogens with carbon based nanoparticle Fullerene & CNTs.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Nanoparticle</th>
<th>Carcinogens</th>
<th>Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Energy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Fullerene</td>
<td>BaP</td>
<td>-4.29</td>
</tr>
<tr>
<td></td>
<td>Fullerene</td>
<td>BPDE</td>
<td>-4.31</td>
</tr>
<tr>
<td></td>
<td>Fullerene</td>
<td>Chrysene</td>
<td>-4.45</td>
</tr>
<tr>
<td></td>
<td>Fullerene</td>
<td>CDE</td>
<td>-4.68</td>
</tr>
<tr>
<td></td>
<td>Fullerene</td>
<td>DMBA</td>
<td>-3.82</td>
</tr>
<tr>
<td></td>
<td>Fullerene</td>
<td>DMBAepoxide</td>
<td>-4.05</td>
</tr>
<tr>
<td></td>
<td>Fullerene</td>
<td>NNK</td>
<td>-2.16</td>
</tr>
<tr>
<td></td>
<td>Fullerene</td>
<td>NNAL</td>
<td>-3.41</td>
</tr>
<tr>
<td>2</td>
<td>SWCNT</td>
<td>BaP</td>
<td>-9.94</td>
</tr>
<tr>
<td></td>
<td>SWCNT</td>
<td>BPDE</td>
<td>-9.58</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>-----------------</td>
<td>----------</td>
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<td></td>
</tr>
<tr>
<td>SWCNT</td>
<td>Chrysene</td>
<td>-9.85</td>
<td></td>
</tr>
<tr>
<td>SWCNT</td>
<td>CDE</td>
<td>-9.68</td>
<td></td>
</tr>
<tr>
<td>SWCNT</td>
<td>DMBA</td>
<td>-9.01</td>
<td></td>
</tr>
<tr>
<td>SWCNT</td>
<td>DMBAepoxide</td>
<td>-8.85</td>
<td></td>
</tr>
<tr>
<td>SWCNT</td>
<td>NNK</td>
<td>-17.16</td>
<td></td>
</tr>
<tr>
<td>SWCNT</td>
<td>NNAL</td>
<td>-9.41</td>
<td></td>
</tr>
<tr>
<td>MWCNT</td>
<td>BaP</td>
<td>-11.86</td>
<td></td>
</tr>
<tr>
<td>MWCNT</td>
<td>BPDE</td>
<td>-10.58</td>
<td></td>
</tr>
<tr>
<td>MWCNT</td>
<td>Chrysene</td>
<td>-11.82</td>
<td></td>
</tr>
<tr>
<td>MWCNT</td>
<td>CDE</td>
<td>-9.98</td>
<td></td>
</tr>
<tr>
<td>MWCNT</td>
<td>DMBA</td>
<td>-10.81</td>
<td></td>
</tr>
<tr>
<td>MWCNT</td>
<td>DMBAepoxide</td>
<td>-9.85</td>
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</tr>
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<td>MWCNT</td>
<td>NNK</td>
<td>-15.56</td>
<td></td>
</tr>
<tr>
<td>MWCNT</td>
<td>NNAL</td>
<td>-11.45</td>
<td></td>
</tr>
</tbody>
</table>

After the Binding energy analysis between carbon based Nanoparticles and Environmental Carcinogens; we found that except Fullerene all Nanoparticles were able to bind strongly with the Environmental Carcinogens, both CNTs strongly bind with the NNK and PAHs but the binding affinity for NNK was more than that of PAHs. The highest binding energy was identified between SWCNT and NNK (-17.16) followed by MWCNT with NNK (-15.56), MWCNT with BaP (-11.86), Chrysene (-11.82), MWCNT with NNAL (-11.41), MWCNT with DMBA (-10.81), MWCNT with BPDE (-10.58), MWCNT with CDE (-9.98), SWCNT with BaP (-9.94 Kcal/Mol), SWCNT with Chrysene (-9.85), MWCNT with DMBAepoxide (-9.85), SWCNT with CDE (-9.68),
SWCNT with BPDE (-9.58), SWCNT with NNAL (9.41), SWCNT with DMBA (-9.01) and SWCNT with DMBAepoxide (9.85 Kcal/Mol).

Table No. 4.2: Binding efficiencies of environmental carcinogens with Metal based Nanoparticle TiO$_2$ NP.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Nanoparticle</th>
<th>Carcinogens</th>
<th>Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TiO$_2$NP</td>
<td>BaP</td>
<td>-48.10</td>
</tr>
<tr>
<td>2</td>
<td>TiO$_2$NP</td>
<td>BPDE</td>
<td>-49.27</td>
</tr>
<tr>
<td>3</td>
<td>TiO$_2$NP</td>
<td>Chryscene</td>
<td>-46.33</td>
</tr>
<tr>
<td>4</td>
<td>TiO$_2$NP</td>
<td>CDE</td>
<td>-44.29</td>
</tr>
<tr>
<td>5</td>
<td>TiO$_2$NP</td>
<td>DMBA</td>
<td>-46.07</td>
</tr>
<tr>
<td>6</td>
<td>TiO$_2$NP</td>
<td>DMBAepoxide</td>
<td>-45.38</td>
</tr>
<tr>
<td>7</td>
<td>TiO$_2$NP</td>
<td>NNK</td>
<td>-38.48</td>
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<tr>
<td>8</td>
<td>TiO$_2$NP</td>
<td>NNAL</td>
<td>-47.52</td>
</tr>
</tbody>
</table>

After the Binding analysis of Metal based Nanoparticles (TiO$_2$ NP), Environmental Carcinogens and their metabolites we found that highest binding affinity of TiO$_2$ NP with the BPDE (49.27) followed by BaP (-48.10), NNAL (-47.52), DMBA (-46.07), DMBAepoxide (-45.38), CDE (-44.29) and NNK (-38.48).

Table No. 4.3: Comparative binding analysis of best and most probable biomolecular targets among DNA repair enzymes with environmental carcinogens, their metabolites and carbon based nanoparticle Fullerene.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Biomolecular Carcinogenicity</th>
<th>BE</th>
<th>Nanoparticle</th>
<th>Carcinogen</th>
<th>BE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Targets</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
This result clearly indicated that the binding energies of fullerene with environmental carcinogens and their metabolites were not higher than the environmental carcinogens and their metabolites with their best and most probable biomolecular targets among DNA repair enzymes. It’s mean that fullerene have not posses very good protection against the environmental carcinogens which were clearly reflected in the chapter 3.

**Table No. 4.4:** Comparative binding analysis of best and most probable biomolecular targets among DNA repair enzymes with environmental carcinogens, their metabolites and carbon based nanoparticle SWCNT.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Biomolecular</th>
<th>Carcinogen BE</th>
<th>Nanoparticle</th>
<th>Carcinogen</th>
<th>BE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>POLG1</td>
<td>BaP</td>
<td>-8.86</td>
<td>SWCNT</td>
<td>BaP</td>
</tr>
<tr>
<td>2</td>
<td>POLD1</td>
<td>BPDE</td>
<td>-9.66</td>
<td>SWCNT</td>
<td>BPDE</td>
</tr>
<tr>
<td>3</td>
<td>PLOG1</td>
<td>Chrysene</td>
<td>-9.26</td>
<td>SWCNT</td>
<td>Chrysene</td>
</tr>
<tr>
<td>4</td>
<td>POLG1</td>
<td>CDE</td>
<td>-8.98</td>
<td>SWCNT</td>
<td>CDE</td>
</tr>
</tbody>
</table>
This result indicated that SWCNT have higher binding energy with few of environmental carcinogens like NNK and BaP than their best and most probable biomolecular targets among DNA repair enzymes but the other environmental carcinogens and their metabolites were not having good binding energy with the SWCNT as compare to their best and most probable biomolecular targets among DNA repair enzymes so they were unable to provide the very good protective action as mentioned in previous chapter.

Table No. 4.5: Comparative binding analysis of best and most probable biomolecular targets among DNA repair enzymes with environmental carcinogens, their metabolites and carbon based nanoparticle MWCNT.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Biomolecular Targets</th>
<th>Carcinogen</th>
<th>BE</th>
<th>Nanoparticle</th>
<th>Carcinogen</th>
<th>BE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>POLG1</td>
<td>BaP</td>
<td>-8.86</td>
<td>MWCNT</td>
<td>BaP</td>
<td>-11.86</td>
</tr>
<tr>
<td>2</td>
<td>POLD1</td>
<td>BPDE</td>
<td>-9.66</td>
<td>MWCNT</td>
<td>BPDE</td>
<td>-10.58</td>
</tr>
<tr>
<td>3</td>
<td>PLOG1</td>
<td>Chrysene</td>
<td>-9.26</td>
<td>MWCNT</td>
<td>Chrysene</td>
<td>-11.82</td>
</tr>
<tr>
<td>4</td>
<td>POLG1</td>
<td>CDE</td>
<td>-8.98</td>
<td>MWCNT</td>
<td>CDE</td>
<td>-9.98</td>
</tr>
<tr>
<td>5</td>
<td>HUS1</td>
<td>DMBA</td>
<td>-8.78</td>
<td>MWCNT</td>
<td>DMBA</td>
<td>-10.81</td>
</tr>
<tr>
<td>6</td>
<td>RRM2B</td>
<td>DMBAepox</td>
<td>-9.33</td>
<td>MWCNT</td>
<td>DMBAepox</td>
<td>-9.85</td>
</tr>
</tbody>
</table>
This result indicated that MWCNT have higher binding energy with few of environmental carcinogens like NNK, BaP, NNAL and Chrysene, DMBA than their best and most probable biomolecular targets among DNA repair enzymes but the other few of environmental carcinogens and their metabolites like BPDE, CDE and DMBAepoxide were not having very higher binding energy with the MWCNT as compare to their best and most probable biomolecular targets among DNA repair enzymes so they were unable to provide the very good protective action as mentioned in previous chapter.

**Table No. 4.6: Comparative binding analysis of best and most probable biomolecular targets among DNA repair enzymes with environmental carcinogens, their metabolites and Metal based nanoparticle TiO$_2$ NP.**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Biomolecular</th>
<th>Carcinogen</th>
<th>BE</th>
<th>Nanoparticle</th>
<th>Carcinogen</th>
<th>BE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>POLG1</td>
<td>BaP</td>
<td>-46.93</td>
<td>TiO2NP</td>
<td>BaP</td>
<td>-48.1</td>
</tr>
<tr>
<td>2</td>
<td>POLD1</td>
<td>BPDE</td>
<td>-47.54</td>
<td>TiO2NP</td>
<td>BPDE</td>
<td>-49.27</td>
</tr>
<tr>
<td>3</td>
<td>PLOG1</td>
<td>Chrysene</td>
<td>-51.97</td>
<td>TiO2NP</td>
<td>Chrysene</td>
<td>-46.33</td>
</tr>
<tr>
<td>4</td>
<td>POLG1</td>
<td>CDE</td>
<td>-52.23</td>
<td>TiO2NP</td>
<td>CDE</td>
<td>-44.29</td>
</tr>
<tr>
<td>5</td>
<td>HUS1</td>
<td>DMBA</td>
<td>-48.78</td>
<td>TiO2NP</td>
<td>DMBA</td>
<td>-46.07</td>
</tr>
<tr>
<td>6</td>
<td>RRM2B</td>
<td>DMBAepox</td>
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<td>TiO2NP</td>
<td>DMBAepox</td>
<td>-45.38</td>
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<tr>
<td>7</td>
<td>UDG</td>
<td>NNK</td>
<td>-37.82</td>
<td>TiO2NP</td>
<td>NNK</td>
<td>-38.48</td>
</tr>
<tr>
<td>8</td>
<td>SMUG1</td>
<td>NNAL</td>
<td>-44.81</td>
<td>TiO2NP</td>
<td>NNAL</td>
<td>-47.52</td>
</tr>
</tbody>
</table>
This result indicated that TiO₂ NP have higher binding energy with few of environmental carcinogens like BaP, BPDE, NNK and NNAL than their best and most probable biomolecular targets among DNA repair enzymes but the other few of environmental carcinogens and their metabolites like Chrysene, CDE, DMBA and DMBAepoxide were not having very higher binding energy with the TiO₂ NP as compare to their best and most probable biomolecular targets among DNA repair enzymes so they were unable to provide the very good protective action as mentioned in previous chapter.

Table No. 4.7: Comparative binding analysis of biomolecular target, environmental carcinogens and carbon based nanoparticle Fullerene.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Biomolecular Targets</th>
<th>Carcinogen</th>
<th>BE</th>
<th>Nanoparticle</th>
<th>Carcinogen</th>
<th>BE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AHR</td>
<td>BaP</td>
<td>-5.95</td>
<td>Fullerene</td>
<td>BaP</td>
<td>-4.29</td>
</tr>
<tr>
<td>2</td>
<td>AHR</td>
<td>Chrysene</td>
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<td>Fullerene</td>
<td>Chrysene</td>
<td>-4.45</td>
</tr>
<tr>
<td>3</td>
<td>AHR</td>
<td>DMBA</td>
<td>-5.55</td>
<td>Fullerene</td>
<td>DMBA</td>
<td>-3.82</td>
</tr>
<tr>
<td>4</td>
<td>nACh</td>
<td>NNK</td>
<td>-5.47</td>
<td>Fullerene</td>
<td>NNK</td>
<td>-2.16</td>
</tr>
</tbody>
</table>

Table No. 4.8: Comparative binding analysis of biomolecular target, environmental carcinogens and carbon based nanoparticle SWCNT.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Biomolecular Targets</th>
<th>Carcinogen</th>
<th>BE</th>
<th>Nanoparticle</th>
<th>Carcinogen</th>
<th>BE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AHR</td>
<td>BaP</td>
<td>-5.95</td>
<td>SWCNT</td>
<td>BaP</td>
<td>-9.94</td>
</tr>
<tr>
<td>2</td>
<td>AHR</td>
<td>Chrysene</td>
<td>-5.67</td>
<td>SWCNT</td>
<td>Chrysene</td>
<td>-9.85</td>
</tr>
<tr>
<td>S.No</td>
<td>Biomolecular</td>
<td>Carcinogen</td>
<td>BE</td>
<td>Nanoparticle</td>
<td>Carcinogen</td>
<td>BE</td>
</tr>
<tr>
<td>------</td>
<td>--------------</td>
<td>------------</td>
<td>-----</td>
<td>--------------</td>
<td>------------</td>
<td>------</td>
</tr>
<tr>
<td>1</td>
<td>AHR</td>
<td>BaP</td>
<td>-5.95</td>
<td>MWCNT</td>
<td>BaP</td>
<td>-11.86</td>
</tr>
<tr>
<td>2</td>
<td>AHR</td>
<td>Chrysene</td>
<td>-5.67</td>
<td>MWCNT</td>
<td>Chrysene</td>
<td>-11.82</td>
</tr>
<tr>
<td>3</td>
<td>AHR</td>
<td>DMBA</td>
<td>-5.55</td>
<td>MWCNT</td>
<td>DMBA</td>
<td>-10.81</td>
</tr>
<tr>
<td>4</td>
<td>nAch</td>
<td>NNK</td>
<td>-5.47</td>
<td>MWCNT</td>
<td>NNK</td>
<td>-15.56</td>
</tr>
</tbody>
</table>

Table No. 4.9: Comparative binding analysis of biomolecular target, environmental carcinogens and carbon based nanoparticle MWCNT.

After the Binding energy comparison between Carbon based Nanoparticles with Environmental Carcinogens and Biomolecules with Environmental Carcinogens, we explore that, expect Fullerene all Nanoparticles having higher binding capacity with the Environmental Carcinogens than theirs biomolecular targets, both SWCNT and MWCNT are very strongly bind with the PAHs and TSNAs than with theirs biomolecular targets. The highest bind energy was observed between SWCNT and NNK -17.16 after that MWCNT with NNK -15.56, MWCNT with Chrysene -11.82, MWCNT with DMBA -10.81, SWCNT with BaP -9.94, SWCNT Chrysene with -9.85 and SWCNT with DMBA -9.01 Kcal/Mol, whereas NNK and nAch was only -5.47, on other side BaP and AHR -5.95, Chrysene and AHR -5.67 and DMBA and AHR were -5.55 Kcal/Mol, these all
binding energies of Environmental Carcinogens and theirs biomolecular targets were very less than Nanoparticles and Environmental Carcinogens.

Table No. 4.10: Comparative binding analysis of biomolecular target, environmental carcinogens and Metal based nanoparticle TiO$_2$ NP.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Biomolecular Targets</th>
<th>Carcinogen</th>
<th>BE</th>
<th>Nanoparticle</th>
<th>Carcinogen</th>
<th>BE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AHR</td>
<td>BaP</td>
<td>-43.71</td>
<td>TiO$_2$NP</td>
<td>BaP</td>
<td>-48.10</td>
</tr>
<tr>
<td>2</td>
<td>AHR</td>
<td>Chrysene</td>
<td>-42.51</td>
<td>TiO$_2$NP</td>
<td>Chrysene</td>
<td>-46.33</td>
</tr>
<tr>
<td>3</td>
<td>AHR</td>
<td>DMBA</td>
<td>-36.48</td>
<td>TiO$_2$NP</td>
<td>DMBA</td>
<td>-46.07</td>
</tr>
<tr>
<td>4</td>
<td>nAch</td>
<td>NNK</td>
<td>-35.27</td>
<td>TiO$_2$NP</td>
<td>NNK</td>
<td>-38.48</td>
</tr>
</tbody>
</table>

Binding energy comparison between Metal based Nanoparticles (TiO$_2$NP) with Environmental Carcinogens and Biomolecules with Environmental Carcinogens, we explore that, TiO$_2$NP have higher binding capacity with the Environmental Carcinogens than theirs biomolecular targets, TiO$_2$NP is very strongly bind with the PAHs and TSNAs than with theirs biomolecular target AHR and nAch. The highest bind energy was observed between TiO$_2$NP and BaP -48.10 after that TiO$_2$NP with Chrysene -45.33, TiO$_2$NP with DMBA -46.07, TiO$_2$NP with NNK -36.48, whereas BaP and AHR -43.71 and NNK with nAch were -35.27, these all Environmental Carcinogens and Biomolecular targets binding energies were very less than Nanoparticles and Environmental Carcinogens.
4.5) Discussion-

Nanomaterials are very small particles that have at least one dimension less than 100-nm being evaluated in many fields in material sciences. Now a day, nanomaterials have gained a lot of attention in various scientific endeavors due to their distinguished properties in comparison to the bulk form. These unique properties are contributed for high surface to volume ratio and very small size. Recently, nanomaterials have been used for the environmental remediation processes (Khin M.M. et al 2012). These materials adsorb various pollutants and used for the removal of toxic gases, (CO, NOx, SO2, etc), organic pesticides, heavy metals, biological substances (bacteria, virus, amino acids, antibiotics) from a range of biological matrices (Vaseashta A., et al 2007). Organic pollutant adsorption on carbon nanomaterials (CNMs) are commercially used for purifying drinking water, for example, in the removal of arsenic (Onyango M.S., et al 2003).

As described in the previous chapter, the co-treated A549 cells with nanoparticles and carcinogens showed the significant reduction in environmental carcinogens induced toxicity. In order to explore the probable protection mechanism, we analyzed the scavenging potential of nanoaprticles against carcinogens.

To analyze the scavenging potential, we have compared the binding energy between the environmental carcinogens and their most probable biomolecular targets among DNA repair proteome; with the binding energy between the environmental carcinogens and nanoparticles. Each nanoparticle interacted with carcinogens with a docking score slightly higher than the docking scores of respective carcinogens with their molecular targets, except fullerenes, which gave lesser docking score with carcinogens as compared to the docking score of carcinogens with their molecular targets (Table 4.3, 4.4, 4.5 &
4.6. This indicated that presence of nanoparticles in the biological system may provide a mild/weak competition to the target binding of carcinogens, and therefore may provide only slight protection against the damaging effects of carcinogens. Whereas, the wet lab data (Chapter 3) showed a much higher protection by nanoparticles against the carcinogens, indicating that some other mechanism/s must also be involved in this protection along with the scavenging action of these nanoparticles.

Therefore, we extended our study to explore whether nanoparticles somehow affect the entry of these carcinogens in the cellular system? In order to get answer to this, we performed the in silico study by docking the carcinogens PAHs and TSNA with their respective receptors (AHR and nAch), and also with the nanoparticles. The comparison of the docking scores revealed a clear cut difference in the binding affinities. Carcinogens interacted with each nanoparticle, except fullerene, with much higher docking score than the carcinogen binding to their corresponding receptors (Table 4.7, 4.8, 4.9 & 4.10). This helped us to conclude that, if carcinogens and nanoparticles are both present in cell vicinity, the carcinogen will very efficiently and readily bind and therefore be adsorbed on the nanoparticles, instead of their receptors. In other words, nanoparticles may trap the carcinogens by adsorbing them, preventing/reducing their entry in to the cell via their receptors.

Finally, the overall strong protective potential of the nanoparticles (except fullerenes) could be a result of both the actions of nanoparticles. Firstly and more importantly, nanoparticles may trap the carcinogens outside the cells, thereby trimming down their entry into the cells. Secondly, although less strongly, nanoparticle may scavenge these carcinogens by adsorbing them and probably expelling them out of the cells via transcytosis.
The protection of cells by nanoparticles against carcinogens as a result of strong adsorption is explainable as we know when bulk substances are reduced to the nano-size, their physical and chemical properties change due to the low quantum confinement. After this conversion from bulk to nano-sized; particles become very good adsorbent for the Aromatic or high electron rich organic species (Gotovac S. et al 2007). Both classes of carcinogens PAHs and TSNAs, posses Aromatic properties as well as high electron rich configurations, ensuring easy and strong adsorption on the surface of Nanoparticles. The reason of this binding could be different with the different natured nanoparticles as follows:

4.5.1) Metal Based Nanoaprticles (TiO$_2$ NP)-

Once a bulk TiO$_2$ is brought to nano-size, it loses its surface atomic coordinates thereby increasing free surface energy. The stronger binding of the TiO$_2$ NP on PAHs and TSNAs may be due to Titanium dioxide nanoparticles (TiO$_2$-x) containing oxygen vacant sites. These high electron rich species on TiO$_2$ surface could be an effect of nanosized TiO$_2$ in order to minimize high free surface energy, to accomplish the atomic coordination at the surface and to establish electronic neutrality (Synowczynski J., et al 2007).

4.5.2) Carbon Based Nanoaprticles (Fullerene, SWCNT & MWCNT)-

In a work by Vergari D. 2008, fullerene was found to be bad adsorbent for analytical application in contrast to convenience adsorbents. Carbon nanotubes instead are good adsorbents. There is evidence, that in our in silico experiments also Fullerene is not showing very good binding energy than CNTs. At a given condition by genetic algorithm based software, adsorbed binding energy of environmental carcinogens was highest on
Multi Walled Nanotubes followed by Single Walled Nanotubes and then Fullerene. This results also authenticated by our wet lab data.

In the fullerenes family the Buckminster Fullerene (C60) is the most investigated and experimental. (Nowack B. et al 2007). Buckminster fullerene has a spherical shape and buildup of 60 carbon atoms with the molar weight of 720 g/mol and a diameter is approximately 1nm (Yang K. et al 2006). The shape and size of carbon nanotubes can vary in diameter, length, number of atoms and in molar weight. Carbon nanotubes can either be single walled (SWCNT) or multi walled (MWCNT) and the end can be closed or open.

Most Environmental carcinogens like dioxin and PAH’s are satirically prevented to enter the inner space of fullerenes and therefore the only place for interaction is the outer surface of the Fullerene which is very limited and short as compare to CNTs.

CNTs can vary in diameter, shape and size, that's why the interface with Environmental carcinogens might occur in the inner surface and between the walls if the tubes are open and on the surface.

The mechanism of adsorption of PAHs on CNTs is a greater degree of $\pi$-orbital overlap with the curved surface of CNTs adsorb more strongly.

Gotovac S. et al 2007, studied the liquid-phase adsorption of PAHs to SWCNTs; this study verified that the amount of adsorption depends on the area of overlap between the aromatic pie bond system of adsorbing molecule and the nanotube surface.

For example tetracene (four fused rings) showing six time greater adsorption tendency over the SWCNT surface than phenantherene (three fused rings) because tetracene have very good aligment and much greater coverage area on the axis of SWCNT.
This study is appropriate with theoretical calculations by Grimme, who state that the 
pie-pie stacking effect resultant from favorable orbital-dependent dispersion interaction 
increases nonlinearly with the molecular size of the aromatic $\pi$- ring system. The 
favorable interaction energies can be certified to –

1) A minimization of the electrostatic repulsion between $\pi$- systems by 
parallel displacement of aromatic system from an eclipsed relationship.

2) Correlations between delocalized $\pi$- electrons of two closely stacked 
Polycyclic aromatic system. (Lena K. 2007.)

Figure 4.7: $\pi$-$\pi$ overlapping geometry causes the PHAs adsorption on the surface of 
CNTs, which help to minimize the electrostatic repulsions by the parallel 
displacement of aromatic ring of both PAHs and CNTs.

CNTs are also known for the adsorption of amines contacting groups like Tobacco 
specific Nitrosamine (TSNAs), and its adsorption likely that at equilibrium deprotonated 
by the Lewis-basic oxygen of TSNAs, revealing the free amine base for the adsorption to 
SWCNTs. Even at high concentrations of TSNAs, it is likely that there will be always be 
free CNT surface available for the non-specific amine-CNT adsorption. (Lena K., 2007.)
An article by (Liao Q. et al. 2007) addresses the adsorption of resorcinol (1,3-benzenediol, as a representative for phenolic derivatives) on multi walled carbon nanotubes. In this study, it was the first time that MWCNT adsorbing phenolic derivatives was investigated. By following this evidence, our study concluded that MWCNT also have very good protective potential against the Environmental Carcinogens, as following the above intrinsic mechanism while followed by the SWCNTs. Nanoparticles were showing the protective potential against the Environmental Carcinogens because once a bulk substance is brought to nano-size, it loses its surface atomic coordinates thereby increasing free surface energy. Carcinogens on nanoparticles surface could be an effect of nanosized TiO₂ & CNTs in order to minimize high free surface energy, to accomplish the atomic coordination at the surface and to establish electronic neutrality. Probable reason for the reduced toxic effect of co-exposure could be the direct adsorption of carcinogens onto nanoparticles itself, rendering all carcinogens unavailable to its target molecules, which requires further in depth analysis.

4.6) Conclusion
The scavenging capacities of the nanoparticles for PAH and TSNAs could probably be attributed to their higher affinity towards the Environmental Carcinogens. The structural properties and surface chemistry of nanoparticles are the players, further, extremely high surface area to volume ratio results in multiple enhancements of such properties. Our study is conformity of study of Deng Q. et al., 2011 & 13 who reported the use of titanate nanosheets and nanotubes are significantly reduces the harmful compounds in cigarette smoke like PAHs & TSNAs. Our study confirmed this action in Biological
system by using of Animal Tissue Culture and Bioinformatics tools. We have done the co-exposure of Environmental Carcinogens and Nanoparticles in A549 cells and also done the comparative docking study between Nanoparticles with Environmental Carcinogens and Environmental Carcinogens with Biomolecules, we concluded that except Fullerens, all other Nanoparticles like TiO$_2$NP, SWCNT and MWCNT shown the protective potential by lowering the toxic effects of Environmental Carcinogens.

**Figure 4.8: Interaction Analysis of MWCNT with NNK**
Figure 4.9: Interaction Analysis of SWCNT with NNK

Figure 4.10: Interaction Analysis of SWCNT with DMBA
Figure 4.11: Interaction Analysis of MWCNT with Chrysene

Figure 4.12: Interaction Analysis of Fullerene with BaP
Figure 4.13: Interaction Analysis of TiO$_2$NP with BaP
4.7) References-


