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Design and Evaluation of an Apta-Nano-sensor to Detect Acetamiprid *in vitro* and *in silico*

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Abstract:

Pesticide detection is a main concern of food safety experts. Therefore, it is urgent to design an accurate, rapid and cheap test. Biosensors that detect pesticide residues could replace current methods, such as HPLC or GC-MC. This research designs a biosensor based on aptamer (Oligonucleotide ss-DNA) in the receptor role, silver nanoparticles (AgNPs) as optical sensors and salt (NaCl) as the aggregative inducer of AgNPs to detect the presence of Acetamiprid. After optimization, 0.6 μM of aptamer and 100 mM of salt were employed. The selectivity and sensitivity of the complex were examined by different pesticides and different Acetamiprid concentrations. To simulate *in vitro* experimental conditions, bioinformatics software was used as *in silico* analysis. The results showed the detection of Acetamiprid at the 0.02-ppm (89.8 nM) level in addition to selectivity. Docking outputs introduced two loops as active sites in aptamer and confirmed aptamer-Acetamiprid bonding. Circular Dichroism spectroscopy (CD) confirmed upon Acetamiprid binding, aptamer was folded due to stem-loop formation. Stability of the Apt-Acetamiprid complex in a simulated aqueous media was examined by molecular dynamic studies.

Keywords: Acetamiprid, Aptnanosensor, Docking, Molecular dynamics, Pesticide residues.

Introduction:

Acetamiprid is a new chloronicotine class of systemic broad-spectrum insecticides. It acts as the agonist of the nicotinic acetylcholine receptors (nAChRs) of the postsynaptic membrane of nerve cells and inhibits the normal conduction of central nerves (Marín et al., 2004). Acetamiprid has brilliant properties such as relatively low and chronic mammalian toxicity and no long-term cumulative toxicity, which makes it a replacement for organophosphorous insecticides to control greenhouse, garden and former pests. However, frequent spray increases the cumulative risks in groundwater and humic soil, which causes a serious human health crisis (Shi, Zhao, Liu, Fan, & Cao, 2013). Conventional residual analysis mainly includes high-performance liquid chromatography (HPLC) (Xie et al., 2011), gas chromatography (GC) (Zhang et al.,

2010) and enzyme-linked immunosorbent assay (ELISA) (Kim, Shelver, & Li, 2004). Moreover, HPLC and GC require expensive instruments, time-consuming sample preparation and trained personnel. Therefore, the design of a rapid test to measure Acetamiprid residues with a simple, fast, sensitive and selective method is needed.

In recent years, pesticide sensors have been developed with colorimetric strategies. These sensors are particularly attractive because detection can be easily done by the naked eye and because of other desirable factors. One of the cheapest colorimetric approaches is the use of semiconductor nanoparticles (NPs) (Shang, Zhang, & Dong, 2009; Touceda-Varela, Stevenson, Galve-Gasion, Dryden, & Mareque-Rivas, 2008). Nanoparticle sensors are sophisticated and eliminate several limitations, such as low sensitivity, selectivity and analytical interference (Männel-Croisé & Zelder, 2009). The most important optical characteristic of metal NPs, such as Gold (Au) and Silver (Ag), is their localized surface plasmon resonance (LSPR), which refers to the collective oscillation of the conducting electrons of metal NPs when their frequency matches the incident electromagnetic radiation (Lopatynskyi, Lopatynska, Jay Guo, & Chegel, 2011). LSPR properties exhibit a reliable optical signal upon the aggregation of metal nanoparticles. The red Au NPs solution shifts to blue, and the yellow Ag NPs solution shifts to brown (Vilela, González, & Escarpa, 2012). Although Ag NPs have been used less extensively than Au NPs in colorimetric assays, very interesting research studies have been published based on the color change from yellow to brown corresponding to dispersed and aggregated AgNPs, respectively (Han & Li, 2010). For example, highly stable silver nanoparticles modified with calixarene were used for the detection of pesticides in water; thus, the modified AgNPs can be utilized as a rapid colorimetric probe for Optunal, down to a concentration of 10^{-7} M (Xiong & Li, 2008). To reach ultra-sensitivity and selectivity, artificial receptors for chemical sensors have been developed. The potential of aptamers as artificial receptors has been documented by many researchers (Mirsky & Yatsimirsky, 2011). Aptamers are short nucleic acid sequences (RNA or DNA, typically 15–60 nt) that bind to ligands with high affinity and specificity. Aptamers were discovered in the powerful technique of SELEX (systematic evolution of ligands by exponential enrichment), which made it possible to isolate specific binders out of a combinatorial library of nucleic acid sequences by iterative rounds of selection and section amplification (Ellington & Szostak, 1990; Tuerk & Gold, 1990). Aptamers had been developed to bind several different ligands, including non-nucleic acid targets such as amino acids, peptides, proteins, drugs, vitamins, a large varieties of other small organic molecules and metal ions, or even entire cells (Famulok, 1994; Gebhardt, Shokraei, Babaie, & Lindqvist, 2000; Haller & Sarnow, 1997; Wilson, Keefe, & Szostak, 2001). They form tertiary structures and bind to their targets by complementary shape interaction. Aptamer–target interaction is similar to antibody–antigen interaction. The binding affinity of aptamers to their targets is comparable or even superior to that of antibodies with dissociation constants down to the picomolar range (Jenison, Gill, Pardi, & Polisky, 1994; Win, Klein, & Smolke, 2006). Moreover, aptamers have the advantage of being smaller and showing much greater conformational flexibility than their antibody counterparts. Fan, Zhao, Shi, Liu, & Li (2013) developed a simple aptasensor for

sensitive and selective Acetamiprid detection based on electrochemical impedance spectroscopy (EIS). To optimize the aptasensor, an aptamer platform was immobilized on a gold electrode and electrodeposited by cycle voltammetry (CV). Shi et al., (2013) reported a highly effective colorimetric method employing unmodified Au NPs as optic probes and ABA (aptamer bonding Acetamiprid) as a recognition element for convenient Acetamiprid detection. Before addressing the importance and novelty of the present research, the simulation method as *in silico* analysis played an undeniable role in clarifying the mechanism with no cost. Prediction of the aptamer-target interaction was a suitable method used by many researchers (Li et al., 2014). Prevalent computational docking predicts the interaction between the ligand and macromolecules. Over the past two decades, research studies have achieved many successful protocols for ligand-receptor docking. AutoDock® is known as a dominant software for predicting the optimal conformations of ligands to proteins. Various modes of the binding of ligand-protein or nucleic acid complexes are analyzed by AutoDock and aided by High-Performance Computing to assay massive bimolecular complexes (Cosconati et al., 2010). Molecular Dynamics (MD) is defined as a computational molecular microscope (Reza Kalani & Tajkhorshid, 2014) that calculates the motions of molecules in terms of time. In physiological conditions, molecules are always in motion such that the motion of molecules is a key factor in predicting the structure interaction. Circular dichroism (CD) spectroscopy is very sensitive to distinguish the secondary structure of polypeptides and spectral studies of DNA (Holm, Nielsen, Hoffmann, & Nielsen, 2010). Recently, the advantages of CD spectroscopy, such as low cost and sensitivity, allowed it to be utilized in oligopeptide and oligonucleotide structure studies. Both *in vitro* and *in silico* approaches motivated us to explore an operationally colorimetric sensing platform, which employed a combination of silver nanoparticles (AgNPs) and aptamer for the Acetamiprid recognition in the aqueous solution based on the aggregation. The simulation results (docking and MD) and colorimetric assay are valued when we understand the conformational changes of aptamer upon Acetamiprid binding. To do so, Circular Dichroism spectroscopy (CD) monitored the secondary structure of aptamer in the presence and absence of Acetamiprid. Thus, in the present work, a highly sensitive and selective colorimetric method was designed by assembling unmodified AgNPs as optic signals, aptamer as an artificial receptor of Acetamiprid and salt (NaCl) as an aggregation inducer.

2. Material and methods:

2.1. Reagents and apparatus for colorimetric assay

All the chemicals were commercially available as analytical reagent grade and ultra-pure water (conductivity 0.1-2 μScm^{-1}). All glassware was thoroughly cleaned with freshly prepared 3:1 HCl / HNO₃ and rinsed with deionized water prior to use. To prepare aptamer stock, WFI (water for injection) was used, which was certified by a Meet USP monograph. Silver nitrate (99%) was purchased from Riedel® Company. Glucose,

sodium chloride (DNA-RNase free), and trisodium citrate (99%) were purchased from Merck (Germany). All pesticide references (99.8%) including Acetamiprid, Abamectin, Deltamethrin and Chlorpyrifos were obtained from Sigma-Aldrich (St. Louis, MO, USA). Aptamer was chosen according to the prior reported literature (Fan et al., 2013). The oligonucleotide was purchased from Bioneer® Biotechnology Co (AccuOligo®, Republic of Korea) with the following sequence: 5'-TGTAATTTGTCTGCAGCGGTTCTTGATCGCTGACACCATATTATGAAGA-3', MW: 14957.35 g/mol, Purification: PAGE. The LSPR changes of the complex were monitored with the UV-Vis absorption spectra at room temperature using a double-beam, T80+ UV-Vis spectrophotometer PG (China) with a 1-cm quartz cell. All TEM images were captured by a Phillips EM 208 transmission electron microscope (TEM) operating at 100 kV and 180000 X zoom.

2.2. Preparation of AgNPs

Silver nanoparticles were synthesized via Green Chemistry (the design of products and processes that minimize the use and generation of hazardous substances) by using glucose as a reducing agent by the following method (Gade, Bonde, Meshram, Gupta, & Rai, 2013; Hajizadeh, Farhadi, Forough, & Molaei, 2012). First, 100 ml of 0.85 mM silver nitrate, 1 mM sodium citrate and 1% glucose solution were prepared separately. 50 ml of silver nitrate solution was added to 100 ml of sodium citrate solution and ultrasonicated for 15 minutes. Then, 50 ml volumetric weight of 1% glucose was added to the mixture and placed in a water bath at 80°C for 30 minutes. Illuminating the clear yellow solution illustrated that AgNPs were formed, and the solution was maintained at 4°C under dark conditions.

2.3. Colorimetric detection:

In all experimental stages, the volumes of AgNPs, salt and aptamer were considered to be 300, 150 and 75 µL, respectively. Aptamer (protectant agent) and salt (aggregation inducer) concentrations were optimized with time. The threshold salt concentration was selected among 50, 90, 100, 150, 200 and 500 mM. Then, 0.2, 0.4, 0.6, 0.8 and 1.6 µM aptamer were considered to find the optimal concentration. The optimal aptamer concentration was assessed in the presence of the threshold salt concentration. In selectivity and sensitivity tests, all components were combined according to the following protocol, AgNPs (300 µL), aptamer (75 µL), salt (150 µL) and pesticides (75 µL). All stock solutions of insecticides (Acetamiprid, Abamectin, Deltamethrin and Chlorpyrifos) were prepared with 100 ppm in Methanol-H₂O solution (3:97, v/v). For a selectivity detection test, 30 µL of each stock solution was added to Ag-aptamer-salt and incubated for 10 min by shaking bland at room temperature. To determine the sensitivity of the system, Acetamiprid was titrated into 1, 5, 10, 20, 50 and 100 ppm. Visible color shift and UV spectra were recorded by digital camera and UV-Vis spectra, respectively. The limit of detection (LOD) for the designed biosensor was extracted from the correlation curve. Repeatability and Reproducibility of the

colorimetric aptasensor were calculated by Standard Deviations (99%) with three replications.

2.4. Simulation aptamer-Acetamiprid interaction In Silico

3-D structure of Acetamiprid was downloaded from Chemspider database (ChemSpider ID: 184719). The ligand was optimized with SYBYL-X. Generation of the 3-D structure of aptamer was innovatively conducted by three steps in graphical user interference software. First, an MFOLD server was used to predict secondary structures and select a stable form (Zuker, 2003). One of the brilliant factors of the MFOLD server is that it simulates the experimental conditions such as salt concentration and temperature. Second, the 2-D structure was converted into 3-D by an RNA composer sever and mutated uracil with thymine. Finally, the structures were optimized with a combination of molecular mechanics and molecular dynamics using MOE and a Consistent-Valence Force Field (CVFF), which are suitable for studying peptides, proteins, and a wide variety of organic systems (Bruno, Carrillo, Phillips, Vail, & Hanson, 2008). Aptamer (receptor) and Acetamiprid (ligand) were imported into the AutoDock software configured by the Ricci and Netz (2009) protocol to achieve better results. After the docking procedure, the highest affinity binding and most frequent conformation in the first cluster of docking results were selected to evaluate the complex stability by molecular dynamics. Docking analysis and picture preparation were performed using LigX MOE software to detect the active site and bonding properties.

2.5. Molecular Dynamics

We use the simulation package NANoscale Molecular Dynamics (NAMD) (Phillips et al., 2005) and the visualization package Visual Molecular Dynamics (VMD) (Humphrey, Dalke, & Schulten, 1996). The best-ranked conformation of ligand-receptor interaction delivered from the docking results was introduced into the MD process. We have used the NAMD 2.9-Win32-multicore to carry out the MD simulation for 100 nanoseconds (ns). The all-atom CHARMM force field and potential parameters for nucleic acids (Foloppe & MacKerell Alexander D., 2000) and CGenFF, Parameters for the CHARMM General Force Field, (Vanommeslaeghe et al., 2010) were used for the ss-DNA Aptamer and ligand, receptively. The ss-DNA molecule was immersed in the center of a large cubic box containing equilibrated TIP3P water molecules. After adding 0.09 mM Na⁺, final equilibration and neutralization were performed. The MD simulations were performed using periodic boundary conditions. The cubic boxes were defined with the dimensions of 6 x 6 x 6 nm³. The Ewald was utilized as a reliable method for estimating electrostatic interactions in a spatially limited system (Phillips et al., 2005). The particle-mesh Ewald (PME) algorithm was applied with a grid spacing of 1 Å. The conjugate gradient energy minimization method (5000 steps, 100 ps) was used to eliminate any initial stress. The short-range Lennard-Jones interactions calculated a spherical cut off distance of 13.5 Å with a switch distance of 10 Å and pairlist distance of 15 Å. The complex was composited from 19387 atoms and required significant computational resources. We used

computational resources at the National High-Performance Computing Center - Isfahan University of Technology (<http://nhpcc.iut.ac.ir>). 40 3-Ghz Intel Xeon processors and 27595.561 GHz/hours were used.

2.6. Circular Dichroism (CD)

CD spectra were recorded by a JASCO Model 810 spectropolarimeter at room temperature.

The work solution was achieved by a 10-fold protective concentration of aptamer (6 μM) in the stabilizer buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5). The samples' CD spectrums were recorded using the quartz cell, 0.1 cm path length, with a resolution of 1.0 nm over the wavelength range of 200 to 320 nm. To eliminate the buffer effect, the spectra of the buffer were subtracted from the spectra of the samples. Aptamer solutions incubated for 24 h in the different Acetamiprid concentrations at 4°C were used to obtain the spectra while nitrogen gas was blown to remove water condensation.

3. Result:

3.1. Acetamiprid colorimetric detection

3.1.1. Optimization

The mechanism of the detection procedure is illustrated in Scheme 1. In the presence of analyte, AgNPs were transformed from a dispersed state to an aggregated form. The optical signal was quantified by the UV-Vis spectroscopy device. By high-dosage of Acetamiprid (100 ppm) injection, the AgNPs were blurred. This phenomenon was also conducted by adding salt (NaCl) (Fig. 1a). Therefore, optimization of the salt concentration as LSPR degradation and aptamer as an artificial receptor was performed under lab conditions (Nucleotidase-free). The intrinsic optical characterization of the synthesized AgNPs was $\lambda_{\text{max}}=415$ nm, Abs= 1.9. As shown in Figure 1b, when NaCl was titrated, not only did the absorption at 415 of AgNPs remarkably decrease, but a color shift and aggregation state also occurred. The optimized salt concentration was selected as 100 mM. The protected dosage of aptamer was tested in the range of 0.2-1.6 μM . 0.2 and 0.4 μM of aptamer generated low absorption, revealing an insufficient ability to protect the nanoparticles against the optimal salt dosage. However, 0.6-1.6 μM of aptamer was able to protect against aggregation while maintaining the maximum absorption intensity (Fig. 2a). The appropriate amount of aptamer was determined to be 0.6 μM . The dispersive state of AgNPs at the aptamer protection state was confirmed by TEM imaging (Fig. 2b1). On interacting with Acetamiprid in the presence of salt, the particles were immediately aggregated with an intense color change from yellow to gray. In the absence of Acetamiprid, AgNPs formed spherical and granular shapes with an average particle

size of 20–35 nm, while in the presence of Acetamiprid they formed large aggregates of 200–300 nm (Fig. 2b2).

[Figure 1 near here]

[Figure 2 near here]

3.1.2 Assessing the Selectivity and sensitivity of aptamer

The selectivity results showed that Acetamiprid binding aptamer reacted specifically because only the AgNP complex covered by aptamer responded to salt (Fig. 3a). The synthesized peak of AgNPs at 415 nm shifted to the right, forming a second peak at 600 nm by increasing the Acetamiprid concentration (Fig. 3b1). Under the optimized conditions mentioned above, the ratiometric calibration curve, A_{600}/A_{415} as a function of AgNPs-aptamer, had a linear relation with the Acetamiprid concentrations in the range of 1–50 ppm (2.245×10^{-4} M), determination coefficients ($R^2 = 0.994$) and the detection limit of 0.02 ppm (89.8×10^{-9} M) (Fig. 3b2). The Repeatability and Reproducibility of the method were 6.61%, and 1.31%, respectively.

[Figure 3 near here]

3.2. Simulation In Silico

3.2.1. Computational Docking

As noted in the introduction, Acetamiprid acts as the agonist of nAChRs of the postsynaptic membrane. Therefore, interaction of Acetamiprid-nAChRs membranes are considered to recognize the pharmacophore of the ligand. The NMR structures of the $\alpha 7$ nAChR transmembrane domain (PDB code, 2MAW) were downloaded from the PDB database and, after energy minimization, docked to the Acetamiprid as the ligand. The docking results screened the best conformations with more hydrogen bonds as shown in Figure 4b. Acetamiprid recognized the $\alpha 7$ nAChR transmembrane domain by the mean estimated energy binding of 7.813 ± 0.481 kcal/mol ($n=30$) at the active site including phenylalanine (Phe₁₃₄, arene-arene) and glutamate (Glu_{4,6}). In addition, Acetamiprid-aptamer was docked by this protocol by an energy binding of 6.812 ± 0.533 kcal/mol ($n=30$) at T₃₉, A₃₈ and A₄₃ (Fig. 4a). However, due to the different nature of the receptors, the comparison between the aptamer and protein receptor is not accurate, but the comparison reflected that the Acetamiprid consisted of two pharmacophore areas, I: $N \equiv N$ and II: heterocyclic. As shown in Fig. 4a, b, aptamer mimicked the nAChRs membrane receptor to bind with Acetamiprid.

[Figure 4 near here]

3.2.2. MD simulations

3.2.2.1. RMSD and RMSF analysis

The Root Mean Square Deviation (RMSD) suggests the stability of the receptor. Increasing RMSD at the final step of simulation implied that the biomolecule is still looking for a

lower energy level. Thus, the biomolecule is not yet stable (Phillips et al., 2005). During the MD simulations, the stability and flexibility of the conformational biomolecule were monitored by the RMSD and RMSF values for the total atoms and active site residues of the ss-DNA receptor. Figure 5a showed that after 100 ns, the entire complex reached stability, and the average RMSD for all segments of the complex was 38.5 ± 2.61 (Å) (mean $\pm$ SD). The plateau pattern was demonstrated in the RMSD of Acetamiprid and entire ss-DNA after 2 and 0.3 ns by the value 9.28 ± 1.82 (Å) and 14.95 ± 1.33 (Å), respectively (Fig. 5b). Adenine 43, 38 and Thyamin 39 recognized as an active site of aptamer during MD simulation were stable (4.05 ± 0.49 , 3.9 ± 0.7 and 3.6 ± 0.64 Å, respectively) (Fig. 5b). The Root Mean Square Fluctuation (RMSF) was measured based on the residue flexibility over the timescale in MD. However, the essential concern in the current research is the complex fluctuations. RMSF analysis for all atoms of the receptor (0.51 ± 0.07 Å) and Acetamiprid (0.29 ± 0.05 Å) indicated that the mobility complex was significantly low (Figure 6a). Nucleotides composing the active site of aptamer (A43: 0.27 ± 0.05 , A38: 0.25 ± 0.05 and T 390: 0.28 ± 0.05 Å) were significantly fixed during the MD timescale (Fig. 6b).

[Figure 5 near here]

[Figure 6 near here]

3.2.2.2. Radius of Gyration (Rg)

Radius of Gyration (Rg) is the root mean square distance of the collection of atoms from their common center of gravity during monitored aptamer folding simulation. As shown in Figure 7a, the complex with the appropriate compactness rate of 23.56 ± 0.76 (Å) resisted against unfolding phenomena. Nucleotides were placed in the active site after 10 ns trend compactness and fix radius 8.15 ± 0.15 (Å) (Fig. 7.b).

[Figure 7 near here]

3.2.2.3. Distance analysis

In this section, to understand whether the ligand is fixed in its place or is moving far or near, the center ligand to the center aptamer was measured. In Figure 8, the distance between the Acetamiprid and aptamer was 9.03 ± 0.59 (Å) (mean $\pm$ SD). However, the mentioned scaling cannot provide accurate understanding of the critical distance of interactions. Therefore, the distance between the center of gravity of the active site and Acetamiprid was evaluated. As shown in Figure 8, the distance between the active site and Acetamiprid was 2.74 ± 0.33 (Å) and stability bonding occurred.

[Figure 8 near here]

3.2.2.4. Hydrogen bond analysis

The complex structure consisted of interaction between aptamer and Acetamiprid as known intermolecular H bonds and aptamerical hydrogen bonds as known intramolecular bonds (Fig. 9). The main role of the mentioned H bonds is as the primary force to

promote complex stability. H bond fluctuations in MD signal that the complex is verifying or fixing. To assess the stability of the complex, H bonds formed during simulation were calculated between aptamer and Acetamiprid. The average of H bonds for intermolecular was 0.162 ± 0.008 (mean $\pm$ SE). As shown in Figure 9, during the entire length of the MD simulation, sustainable H bonds between the aptamer and Acetamiprid existed. Three nucleotides are active site bonded continuously with Acetamiprid (Supplementary Figure S1a, b, c). For the intramolecular H bond model, average H bond of 21.46 ± 0.04 (mean $\pm$ SD) was observed. Intramolecular H bonds occupied in aptamer indicated hydrogen bonds protected secondary structure not to be broken in water solution during MD.

[Figure 9 near here]

3.3. CD analysis

Figure 10 illustrated the CD spectra of aptamer binding with different concentrations of Acetamiprid. The current control structure of aptamer depicted a positive peak ellipticity at approximately 263 nm and a negative peak at approximately 238 nm, assigned to stem-loop structures and B-form. However, upon the injection of Acetamiprid into the cell (by considering the dilution effect of the Acetamiprid volume), not only did the positive and negative ellipticity at 263 and 238 nm increase gradually, a new peak also emerged at 286 nm.

[Figure 10 near here]

4. Discussion

4.1. Experimental assay

Many researchers have described ion strength as an aggregation agent, which is attributed to the ability of strong electrolytes to gather the aroused electrical double-layer from the capping agent. Chloride ions, replaced by citrate on the Ag⁺ surface, accelerated the particle aggregation while further improving the selectivity and immediately achieved the aggregation threshold (Hormozi-nezhad, Seyedhosseini, & Robatjazi, 2012; Vilela et al., 2012). For example, aptamer shelled AgNPs as a catalytic label-free for the amplified detection of Acetamiprid and acted as a stabilizer to protect AgNPs from salt-induced aggregation (Shi et al., 2013). According to the findings of Li & Rothberg (2004), AuNPs could be stabilized by ss-DNA against salt-induced aggregation. In the absence of analyte, the free aptamer was conformed into a random coil in an aqueous solution (Mirsky & Yatsimirsky, 2011) and wriggled among AgNPs. Aptamer binds to AgNPs by the nitrogen atom of the exterior bases that is cross-linked to the Ag surface (N⁻....Ag⁺). This binding is stronger than low-power electrostatic repulsion, which exists between the negatively charged phosphate backbone and the negatively charged Ag NPs. Thus, the unfolded aptamer (ssDNA) is adsorbed into the AgNPs and enhances the AgNPs stability against salt-induced aggregation. In the presence of a target (Acetamiprid), the active site of aptamer is folded as a rigid structure. Thus,

aptamer could not adsorb on the AgNPs and loses the ability to protect the AgNPs. This process allowed the salt to bridge the nanoparticles and aggregate them together (Wei, Li, Li, Wang, & Dong, 2007). In studies regarding aptamer-target interaction, UV-Vis absorption was considered to be a helpful tool for use worldwide due to its simple and cheap instrument for label-free detection. The photometric characteristic of AgNPs enhanced the photometric features of aptamer. The binding target with subsequent aggregation causes LSPR changes of AgNPs including size and shape (Fig. 2b2).

The main factor for biosensors is achieving minimum LOD. Incubation time was considered to be 10 min to make sure all components interacted efficiently with each other under lab conditions. A gradual decrease was observed in the absorption peak of Ag NPs at 415 nm, while an increase was observed at 600 nm, implicating the gradual aggregation of AgNPs because aptamer was folded by Acetamiprid. Pesticide residues on crops are monitored using the Maximum Residue Limits (MRLs), which are the maximum residue levels of pesticides allowed to remain on food product samples. The Maximum Residue Limit for Acetamiprid has been estimated as 0.06-0.8 (mg/kg: Acetamiprid/crop) based on FAO/WHO for crops and trees (CODEX, 2012). LOD can be considered an index to estimate the presence or absence, but it has more errors in low pesticide concentrations, and therefore the limit of quantification (LOQ) can obviate the abovementioned disadvantage. As a rule of thumb, the LOQ quantities are approximately two times more than the LOD (CODEX, 2012). In our research, LOD ($(STEYX/Slope) \times 3.3$) and LOQ ($(STEYX/Slope) \times 10$) were obtained as 0.02 ppm ($89.8 \times 10^{-9} \text{M}$) and 0.074 ppm, respectively.

By comparing the detection ability of the biosensor with WHO standards, the proposed method could be used as a rapid test in food safety for import gates. Shi et al., (2013) developed an aptamer (ABA)-Au NPs-based colorimetric method that can detect Acetamiprid in the linear range between 75 nM to 7.5 μM with an LOD of 5 nM (Shi et al., 2013). Although AuNPs are the most sensitive among metal nanoparticles, Au nanoparticle colorimetric synthesis is expensive. Hence, we were motivated to create a cheap and practicable method. The limit of detection (LOD) of the current research overlapped the minimum MRL in the WHO protocols. Our current finding is 18-fold more less sensitive than the Shi et al., (2013) method and 90-fold more less sensitive than the (Fan et al., 2013) method. Alternately, the ABA (5'-CTGAC ACCAT ATTAT GAAGA-3' 20 nt.) employed by Shi et al., (2013) formed only one stem-loop as the active site (the current aptamer formed two stem-loops), which tends to exhibit low affinity. Monitoring conformation of ABA by CD spectra showed hairpin formation. With a high concentration of salt, the CD spectrum gradually decreases at the long wavelength area corresponding to variants of B-DNA structures (Hairpin) (Ivanov, Minchenkova, Schyolkina, & Poletayev, 1973). Therefore, decreased ellipticity of ABA at 270 nm, which Shi et al., (2013) claimed as characteristic of the hairpin structure, occurred due to the salt presence, not Acetamiprid. In our research, the fluctuation in CD intensity recommended that aptamer binding Acetamiprid

shifted toward the formation of the stem-loop. Conformation changes induced by the ligand were reported by many researchers and confirmed in the current study (Bikard, Loot, Baharoglu, & Mazel, 2010; Chang, Chen, & Hou, 2012; Kypr, Kejnovska, Renciuk, & Vorlickova, 2009). By inducing a stem-loop in the secondary structure, we should expect a reduction in R_g as a folding index. Compactness conformation, which is the outcome of binding Acetamiprid, occurred in 10 ns. In the mentioned timescale of MD, Acetamiprid initially bonded with aptamer and then immediately induced a small stem-loop (Fig. 10). However, our method is simpler and cheaper with reliable LOD than the abovementioned aptananosensors.

4.2. Computational Docking

Active site recognition and binding affinity are important factors in apt-biosensors (Bini, Mascini, Mascini, & Turner, 2011). The secondary structure of aptamer included two stem-loops with potential binding sites for ligands (Supplementary Figure S2). The stem-loops were documented as active sites for targets based on structural compatibility, aromatic ring stacking, electrostatic and Van der Waals interactions, and hydrogen bonding or a combination of these requirements (Stoltenburg, Reinemann, & Strehlitz, 2007). The SS2-55 and SS4-54 loops of aptamers were recommended as the most likely active sites for binding to the four organophosphorous pesticides (Wang et al., 2012). In docking simulation, Acetamiprid-nAChRs membranes illustrated two regions (N and arene) as pharmacophore of Acetamiprid (Fig. 4b). Acetamiprid interacted with the small stem-loop (A38,43 and T39) of aptamer with the same pattern of binding as to the active sites of nAChRs membranes. All stages were simulated by graphical user interference software on a Windows platform with no need for Linux experience. The current aptamer and ABA (Shi et al., 2013) were compared based on the binding energy in the simulation analysis. The results showed that the current aptamer with binding energy of 6.812 ± 0.533 (kcal/mol) was more stable than ABA with binding energy of 4.935 ± 0.316 (kcal/mol), which could interact with the ligand (Acetamiprid) (Supplementary Figure S3).

4.3. Molecular dynamics

The critical idea in performing MD is to evaluate the complex stability after 100 ns.

Molecular dynamics simulations are used as a tool for distinguishing stability by many researchers when RMSD is depicted as a line-style plot and for confirming the plateau pattern of the current complex (Knapp, Frantal, Cibena, Schreiner, & Bauer, 2011).

Regarding the plateau pattern for RMSF, the active site and ligand implied an overall low variability in the course of MD. Another parametric feature of the complex stability is the R_g (Shukla, Bafna, Sundar, & Thorat, 2014). The consistency of R_g hinted at the stability of tertiary structures in the overall simulation and confirmed the uniform intranucleical H bond rate. In addition, R_g is categorized as the intra-distance of aptamer, but stability has a close relationship with inter-distance (distance of aptamer-Acetamiprid). Distance deviations were established between Acetamiprid-aptamer and affected the complex stability. By monitoring the inter-distance during

simulation, Acetamiprid has been fixed in the active site due to the continuous forming of intramolecular hydrogen bonds during the timescale of MD.

Conclusion

Apta-sensors have long been developed as pioneers for the rapid test of pesticides. Several ss-DNA or ss-RNA were screened, but in spite of immense potential, they were ignored due to the expensive experiments required to demonstrate the mechanism. This research, by computer-aided and AgNPs colorimetric assay, examined Apt-receptor for detecting Acetamiprid and mechanism validation. The high stability of the aptamer-Acetamiprid complex was observed and confirmed by colorimetric assay. In fact, the results of CD spectra and Rg confirmed that the aptamer conformation became folded upon Acetamiprid binding. Therefore, it delineates the possibility of assembling efficient and selective apt-nano-sensors from Acetamiprid.

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Figure Citation:

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Figure 6. The root mean-square fluctuation (RMSF) achieved MD simulation (A) RMSD calculated for aptamer, Ligand during the 13 ns (B) RMSD of three nucleotides composed active site.

Figure 7. Radius of gyration of Aptamer. (a) R_g of all-atoms (b) R_g of active site depicted stem-loop formation after 10 ns.

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Scheme 1. Detection mechanism of colorimetric based on aptamer implant and aptamer denaturation due to binding to Acetamiprid.

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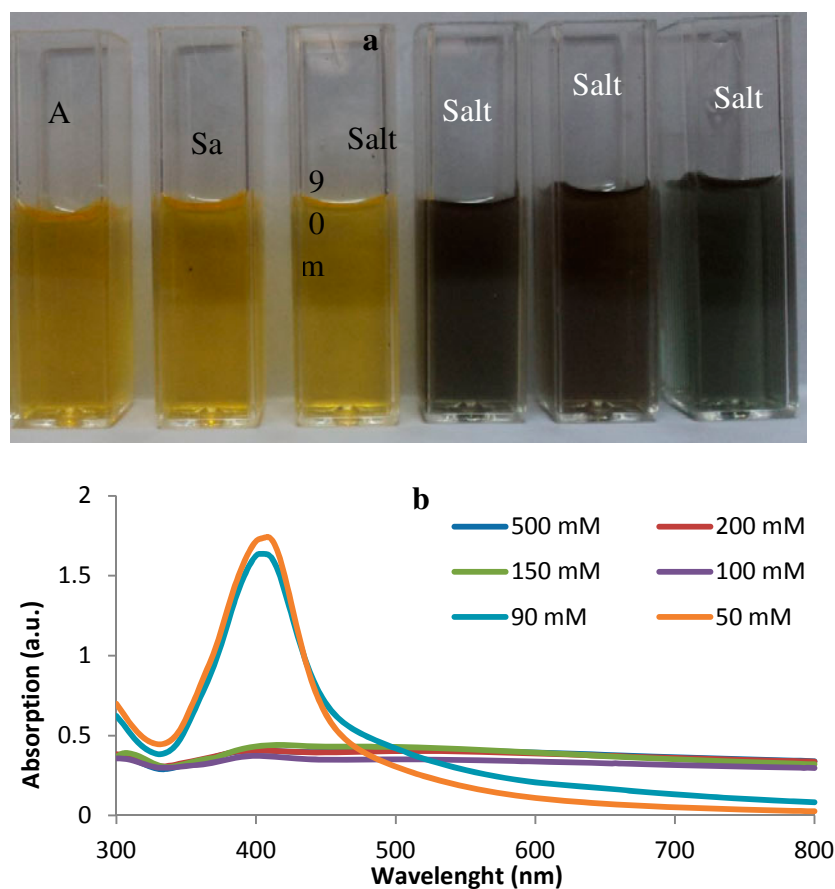


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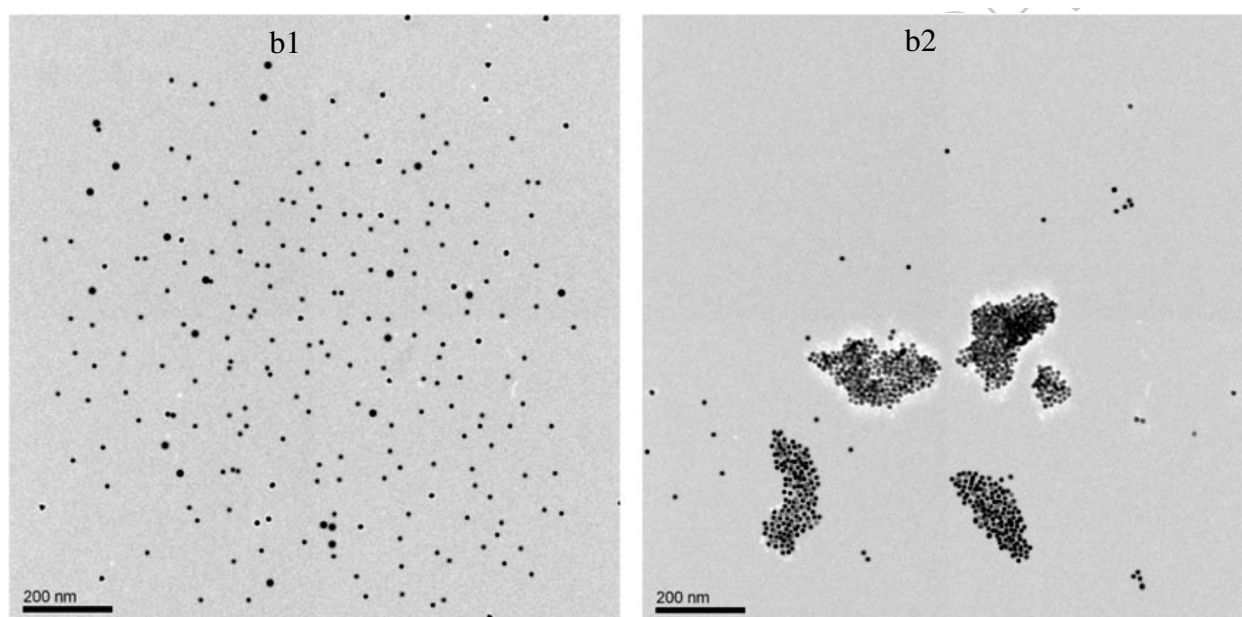
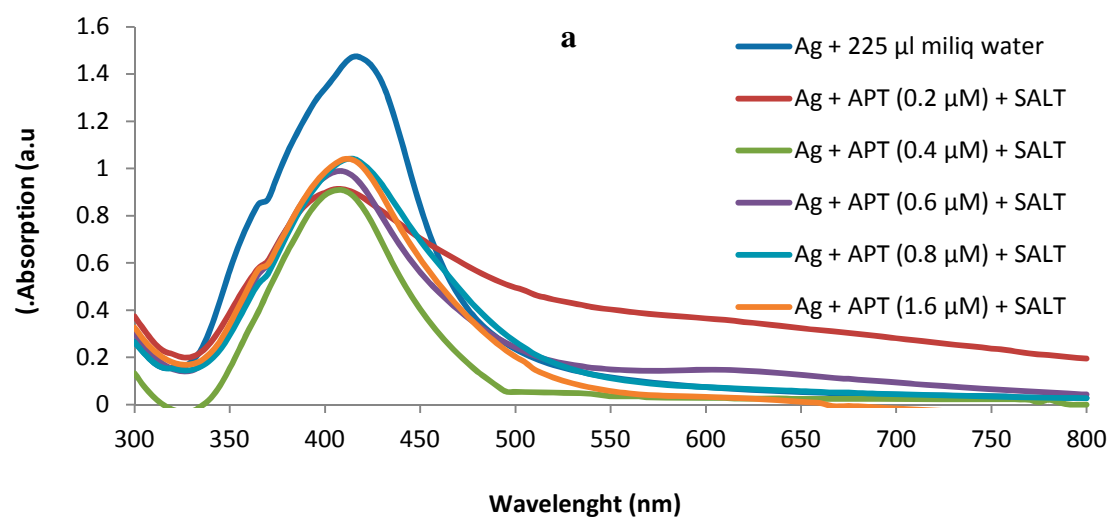


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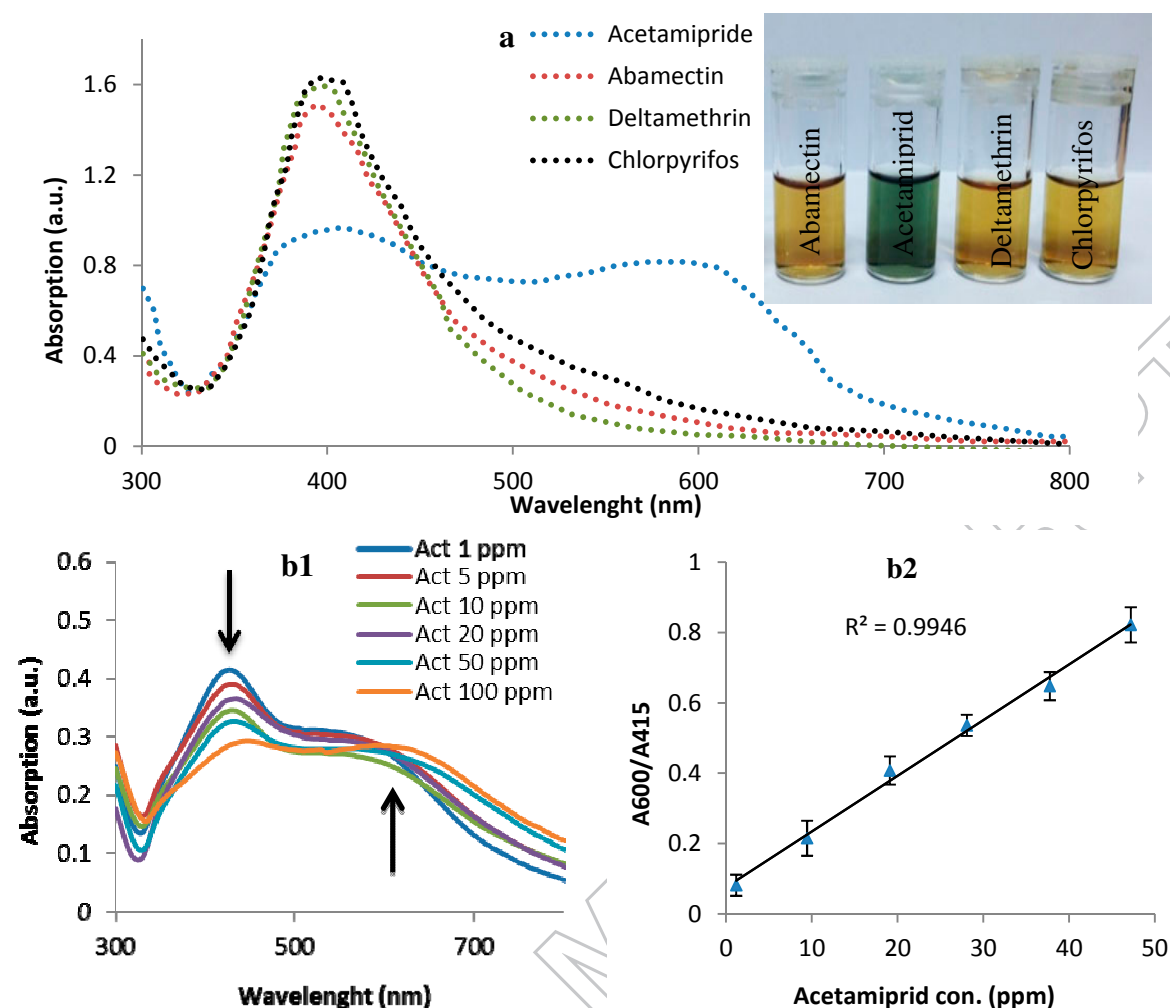


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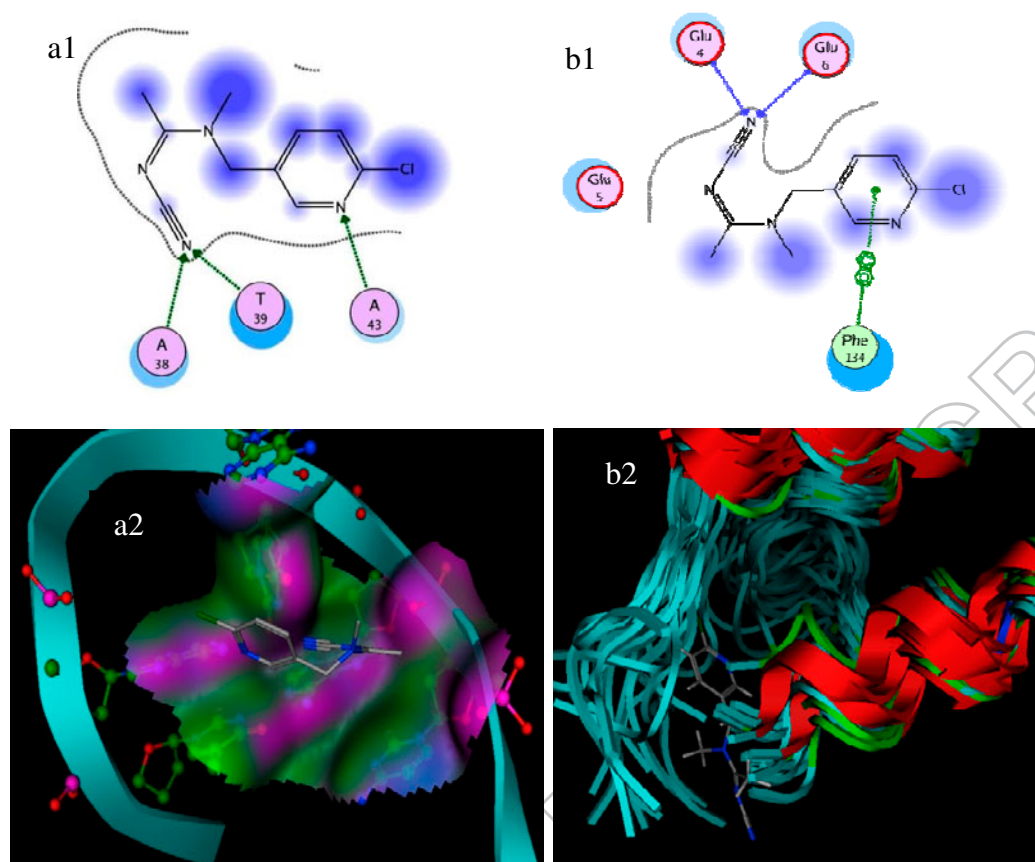


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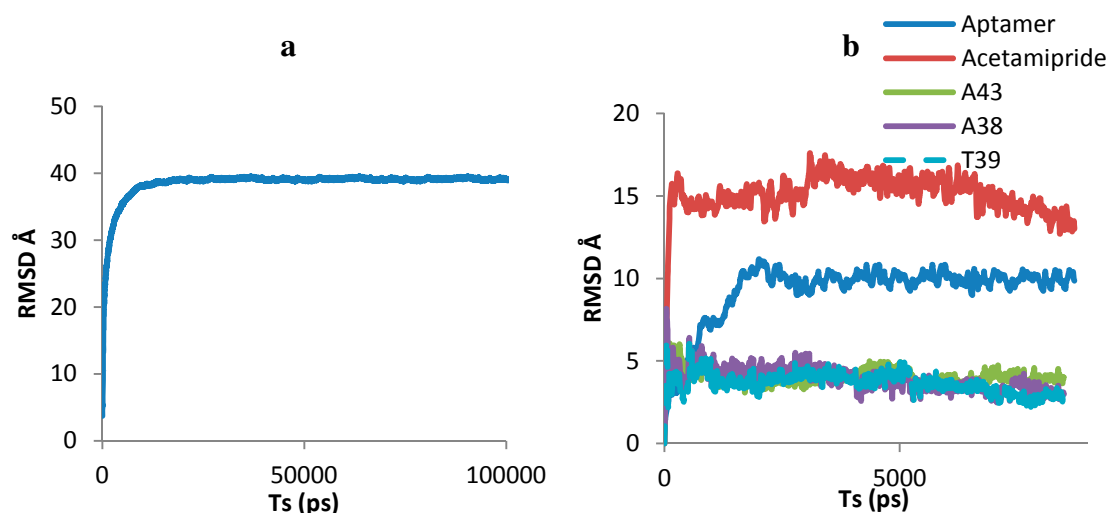


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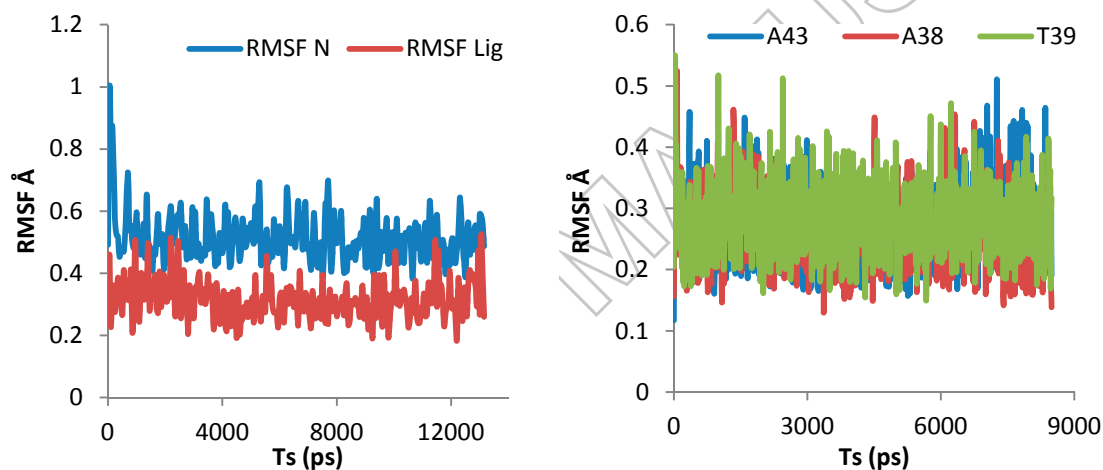


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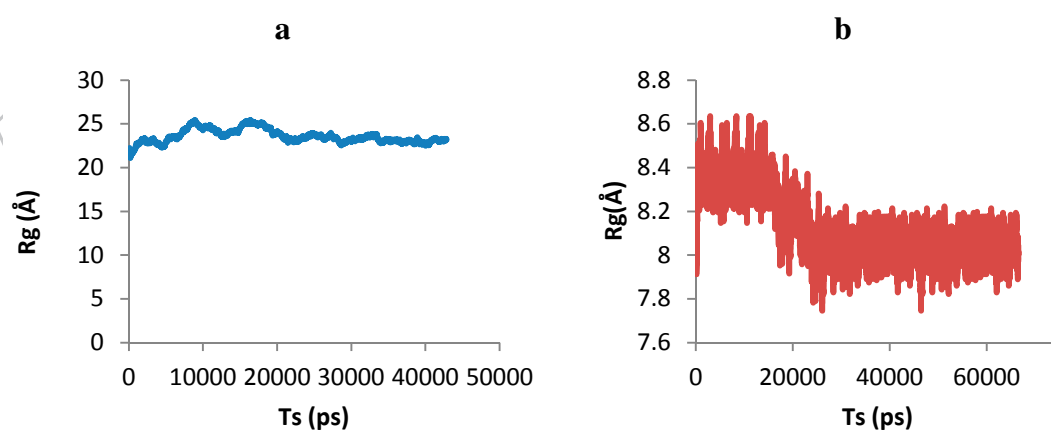


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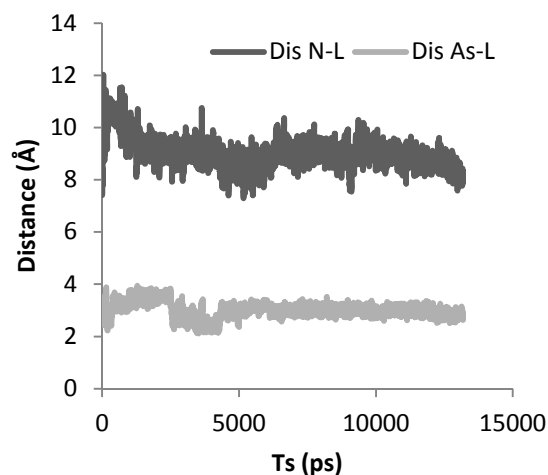


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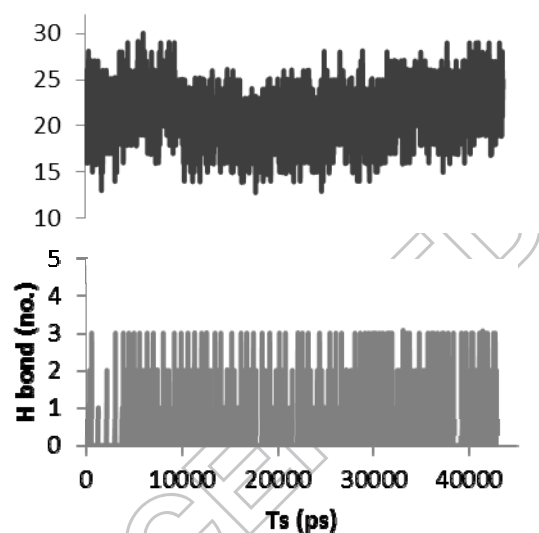


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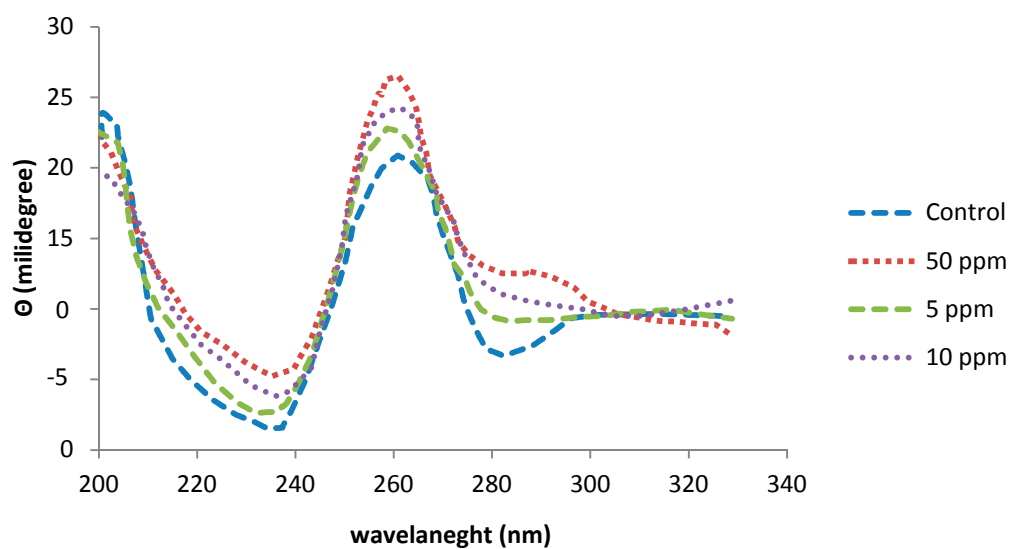
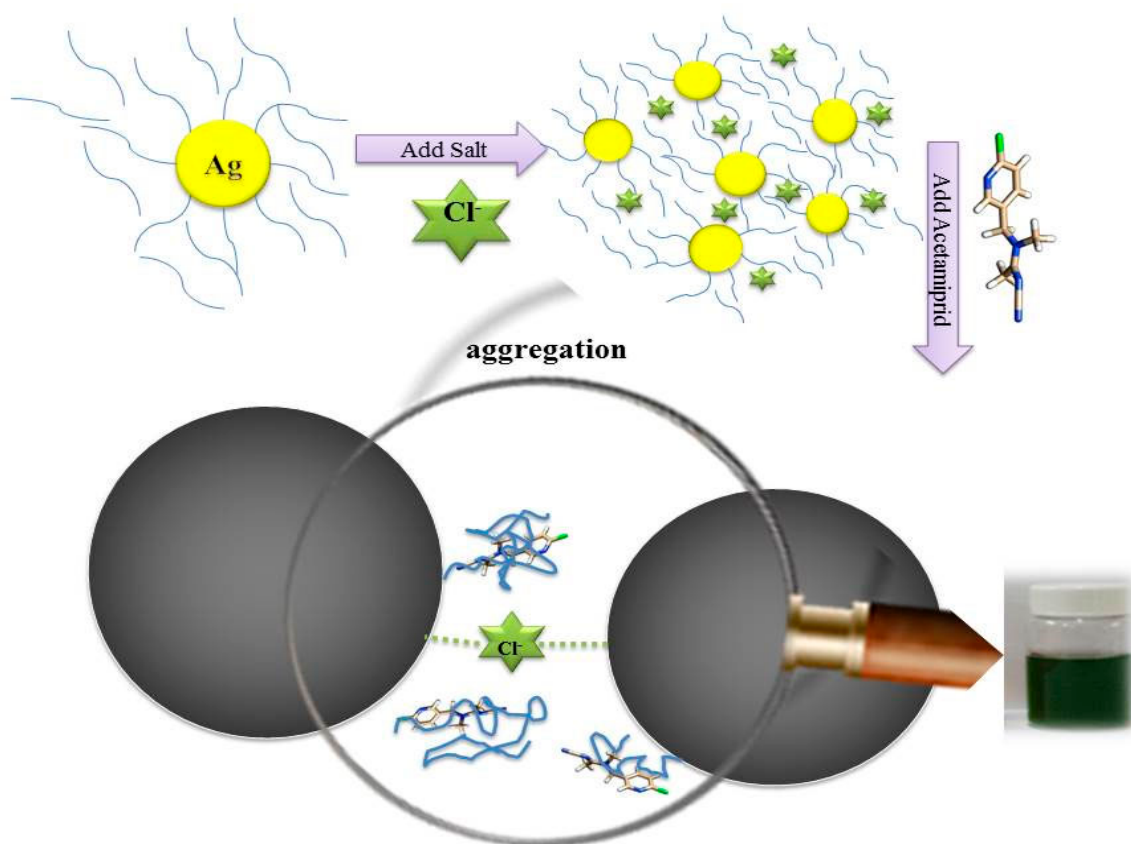


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