

## How to Change the Redox Potential of Guanine?

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Due to some important applications of guanine electrode in scientific and technology research such as electro-chemical DNA based biosensors, and a problem of high redox potential of guanine (0.81 V), our research study concentrates on reducing guanine redox potential by substituting Cu, Ag, Au, CH<sub>3</sub>, C<sub>2</sub>H<sub>5</sub> and Cl on sites of 1, 2 and 9 of guanine. A 5.0% reduction of redox potential for 9-Ag-guanine was the sign of favorable result. In order to have further lower potential, silver clusters (Ag<sub>2</sub> and Ag<sub>3</sub>) were used. The obtained percentages of reduction of potentials were 53.6% and 36.8% for 9-Ag<sub>2</sub> and 9-Ag<sub>3</sub>-guanine, respectively. In this research, Gaussian 09 was used and DFT was applied with long-range corrected xc-functional (LC-BLYP).

**Keywords:** Redox Potentials, Guanine, Substitutions, Biosensor

### INTRODUCTION

Electro-chemical DNA based biosensors have a lot of applications today. They can be used to assay pathogenic micro-organisms in water, food, soil and plant samples [1,2]. The ability of these electro-chemical biosensors lies under the electro-activity of their nucleic acids [3]. The binding of small molecules to DNA and damaged DNA [4] in general has been described through the variation of the electrochemical signal of guanine and adenine.

Guanine [5] is an important DNA and RNA base, which shows electrochemical activity on different electrode materials. The achievement of lower redox potential of guanine for better detectivity in DNA based biosensors was the goal of scientists in many experimental approaches, which has led to fabricate the novel improved electrodes, experimentally. Some of them are as follows: On graphene-nafion composite film modified glassy carbon electrode, which decreases redox potential it is possible to determine guanine and adenine in the related physiology process, simultaneously [6]. Electro-deposition of a nano-structured platinum thin film on a glassy carbon electrode was used for sensitive and selective determination of guanine [7].

Electrochemical biosensor based on silver nano-particles poly-dopamine graphene nano-composite has been used for sensitive and simultaneous determination of trace level of guanine and adenine in fish sperm [8]. A graphene sheets (GS), ionic liquid (IL) and chitosan (CS) modified glassy carbon electrode was successfully applied for individual and simultaneous determination of guanine and adenine [9]. The electrochemical behaviors of guanine and adenine were investigated at the TiO<sub>2</sub>-graphene nano-composite modified glassy carbon electrode. The results showed that electro-catalytic activity and voltammetric response towards these species significantly improved comparing with that observed at the graphene film [10]. The kinetics of guanine redox reaction on gold electrode has been studied in detail [11]. So, electrochemical reactions give an electronic signal directly, and there is no need for signal transduction equipment. These mentioned electrochemical approaches have been performed for the analysis of nucleic acids especially guanine which has somewhat direct relation to the extent of damage in DNA.

Our interest was the development of DNA biosensor and electrochemical sensor through guanine; that is how it is possible to the change the redox potential of guanine by focusing on guanine substitutions instead of modification of electrodes, by the aid of quantum theoretical calculations. We studied substitution of transition metals (Cu, Ag and

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Au) and CH<sub>3</sub>, C<sub>2</sub>H<sub>5</sub> and Cl on guanine. This effort was successful on declining of the redox potential of quinone [12]. It has been shown that the copper, silver and gold (Cu, Ag and Au) have interaction with DNA amino acids [13].

Experimental studies of guanine substitutions have been done variously [14-19], which can be a good sign for experimental researchers to study our theoretical results. As examples, series of 2'-substituted derivatives of 9-[2-(phosphonomethoxy) ethyl] guanine were synthesized by Yu *et al.* in 1993 [15]. Study on guanine and cytosine clusters in the gas phase using double resonant laser spectroscopy techniques in 2005 by Abo-Riziq *et al.* is another experimental work on guanine [16].

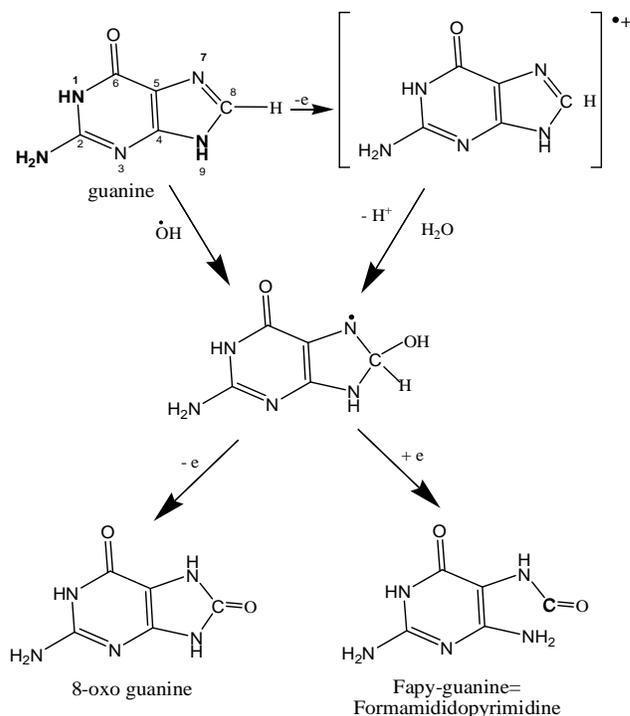
## COMPUTATIONAL METHOD

In the present work the quantum calculations have been carried out by utility of density functional theory (DFT) part of the Gaussian 09 [20], the redox potential calculations have been also performed by the long-range corrected density functional theory (LC-DFT) methods [21-29] using a polarized triple zeta basis set (cc-pVTZ) which was used before for guanine type systems [30]. Mixed basis sets of cc-pVTZ and LANL2DZ were used for the systems containing Au and Ag.

The mechanism of redox reaction of guanine is described in Fig. 1 [31,32]. The formation of radical is the main step which should be mentioned in theoretical calculations as a starting point and the final two-electron product is 8-oxo-guanine.

Two-electron redox potentials of guanine can be calculated from the indirect mechanism, [18], which will be discussed in following. Figure 2 is the whole equation of two-electron redox reaction in which G represents guanine, G-OXO represents 8-oxo guanine, M-G' represents guanine with substitution on one of its active site and M-G'-OXO represents substituted 8-oxo guanine.

The first step is to calculate Gibbs free energy ( $\Delta G$ ) of all the species in Fig. 2. G is our reference system, as its oxidation potential is known and M-G' is a species which its redox potential is wished to be measured. So, by considering the system of G and regarding to its redox potential ( $E_G$ ) which was reported in the literature by others, we can find unknown  $E_{G'}$ . The two-electron redox potential



**Fig. 1.** The mechanism of two electron redox reaction of guanine, with its numbering and three bolded active sites.

of substituted guanine ( $E_{G'}$ ) can be calculated through Eq. (1),

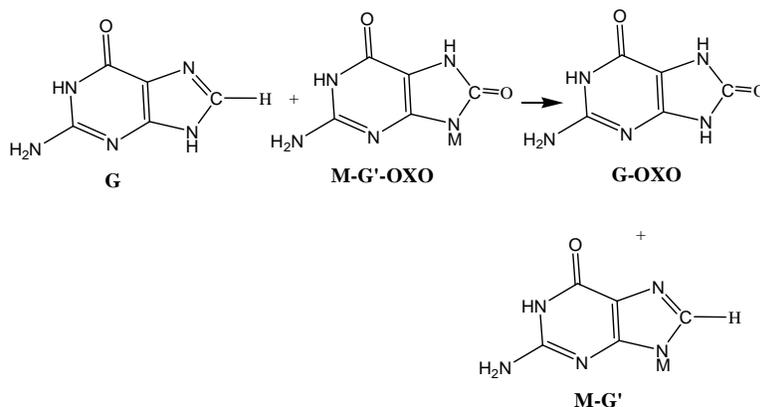
$$E_{G'} - E_G = -\frac{\Delta G_{rxn}}{|Z|F} \quad (1)$$

where  $E_G$  is the experimental redox potential of reference molecule,  $|Z|$  is the number of electrons to be transferred (here equals two),  $F$  is the Faraday constant (96485.3399 C mol<sup>-1</sup>) and  $\Delta G_{rxn}$  is the differences of Gibbs free energies of products and substrates,

$$\Delta G_{rxn} = \Delta G_{products} - \Delta G_{substrates} \quad (2)$$

It is now possible to obtain  $E_{G'}$  from Eq. (1), and calculate redox potential of our substituted guanine ( $E_{G'}$ ) system by Eq. (3).

$$E_{G'} = \frac{\Delta G_{rxn} - |Z|FE_G}{-|Z|F} \quad (3)$$



**Fig. 2.** The equation of two-electron redox reaction of guanine and its substitutions.

**Table 1.** Calculated Potentials for Substituted Guanine and Percentage of Deviation

Species	$E_G (E_{G_{ref}} = + 0.81 \text{ V})$					
	9-M guanine	Deviation (%)	2-M amino-guanine	Deviation (%)	1-M guanine	Deviation (%)
Au	+0.804	-0.6	+0.801	-0.9	+0.796	-1.4
Ag	+0.761	-5.0	+0.788	-2.2	+0.787	-2.3
Cu	+0.850	+4.0	+0.797	-1.3	+0.786	-2.4
CH <sub>3</sub>	-	-	+0.807	-0.3	-	-
C <sub>2</sub> H <sub>5</sub>	-	-	+0.808	-0.2	+0.799	-1.1
Cl	+0.817	+ 0.7	+0.817	+ 0.7	+0.816	+0.7

Effect of Silver Clusters Ag <sub>n</sub> (n = 1-3) on Redox Potentials of 9-Substituted Guanine						
9-Ag <sub>n</sub> guanine	Monomer	Deviation (%)	Dimer	Deviation (%)	Trimer	Deviation (%)
	+0.761	-5.0	+0.274	-53.6	+0.442 (triangular)	-36.8

M can be Au, Ag, Cu, CH<sub>3</sub>, C<sub>2</sub>H<sub>5</sub> and Cl.

The redox potential of 0.81 V versus normal hydrogen electrode [33] was reported for guanine reference system ( $E_G$ ) under physiological conditions. It is notable to remind that the unit of Gibbs free energy ( $\Delta G$ ) should be in joule, to get redox potential in volt.

## RESULTS AND DISCUSSION

We have carried out calculations in the hope of finding better redox potentials for the substituted guanine

applicable in DNA biosensors. Au, Ag, Cu, CH<sub>3</sub> and C<sub>2</sub>H<sub>5</sub> were selected as substitutions that can be used for biosensors due to low toxicity, low cost, availability and their acceptable physiological conditions.

Three available sites of guanine were selected as proper candidates for substitutions. As shown bolded in Fig. 1, H that centered on N<sub>(1)</sub>, called 1-substituted, H that centered on N<sub>(9)</sub>, called 9-substituted and there is one NH<sub>2</sub> on C<sub>(2)</sub> that its hydrogen atoms can be good substitution site and called 2-substituted amino-guanine. In Table 1 the

calculated potentials for guanine substitutions and the percentage of deviations are listed.

At first, each substitution is studied alone, and the results are reported accordingly. In Au substitution, all three sites decreased the redox potentials, the best of them was 1-Au-guanine with 1.4% decrease. In Ag substitution, all three sites decreased the redox potentials and the best of them was 9-Ag-guanine which decreased the redox potential about 5.0%. 1-Cu-guanine was the best site for Cu which decreased the redox potential of guanine 2.4%. Its 9-substituted site was one of the unfavorable results that increased the redox potential of guanine and not obey our desire. For CH<sub>3</sub> substitution, only 2-substituted site was acceptable and decreased the potential about 0.3% but the other two sites gave unreasonable results and did not applicable in biosensor modeling. 1-C<sub>2</sub>H<sub>5</sub>-guanine decreased the redox potential 1.1% and its 9-substituted site, as in the CH<sub>3</sub> case, was one of the unreasonable results which was overlooked. We also studied the effect of Cl as electron withdrawing substituent. In all three sites, the increase in redox potentials was 0.7% which did not meet our target.

So, among all of the selected guanine substitutions, 9-Ag-guanine with about 5.0% decrease in redox potential, showed the best performance. By putting Ag<sub>2</sub> and triangular Ag<sub>3</sub> clusters in 9-substituted site of guanine, the decrease in redox potential was about 53.6% and 36.8%, respectively, which are reported in Table 1. It seems that 9-substitution is the favorable site for guanine-type calculations. We encouraged to study 9-OH-guanine, 9-NH<sub>2</sub>-guanine and 9-NO<sub>2</sub>-guanine, too.  $E_G$ , for OH and NH<sub>2</sub> were as follows: 0.794 V and 0.809 V, respectively which showed 1.57% and 0.13% deviation. The result for 9-NO<sub>2</sub>-guanine showed high increase in the redox potential and did not satisfy our desire and was overlooked.

## CONCLUSIONS

Study of the guanine substitutions instead of designing modified electrodes, to obtain better guanine redox potentials, with density functional calculations have been carried out. In achieving the better detectivity and lower redox potential of guanine, if one prefers to have 9-substituted of guanine, Ag and its clusters are the best

choice. This case for 2-substituted site is also Ag substitution and for 1-substituted of guanine are Ag and Cu. As whole Cl substitution provides undesirable high redox potential and Ag is the best for biosensor modeling.

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