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Short Communication

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A label-free genetic biosensor for diabetes based on AuNPs decorated with electrochemiluminescent signalling

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Abstract:
The variation of I27L gene associates with increasing risk of type 2 diabetes. It will be greatly significant to develop various methods to identify or monitor I27L genovariation. We report here a novel label-free electrochemiluminescent (ECL) DNA biosensor for simple and effective determination of I27L gene based on Au nanoparticles functionalized ITO electrode. The fabricated electrodes were characterized by scanning electron microscopy, cyclic voltammetry, and electrochemical impedance spectroscopy. The ECL technique was employed to monitor the hybridization of DNA by measuring the changes of its intensity. Here, the ECL signal was quenched since the electrostatic repulsion and space resistance of negatively charged sensor surface toward the probe (luminol anion) to block its access. The quantification of target strand was directly accomplished by calibrating the quenched ECL signals. Under optimal conditions, the decreased ECL intensity had a good linear relation upon the logarithm of target DNA concentration in the range of 1.0 × 10−11 to 1.0 × 10−7 M with a detection limit of 8.06 × 10−12 M. In addition, the biosensor exhibited acceptable stability, excellent reproducibility and outstanding selectivity against one-base mismatched DNA. What's more is that the simple, low-cost, sensitive device could be easily miniaturized, which makes it an attractive candidate for integrating into portable platforms for point-of-care molecular diagnostics.

Introduction:
The annihilation pathway: A reduced specie and an oxidized specie (charged radical ions) are simultaneously generated at the electrode surface by applying alternating pulse potentials. These two species react between them generating an excited form, which in the relaxation process to the ground state emits a photon [3].

Co-reactant pathway: A co-reactant is a chemical specie that is reduced or oxidized at the electrode surface, generating a very reactive intermediates that react with the reduced or oxidized luminophore (specie capable of emit light) present in the solution to produce the excited state. Finally, the excited state returns to the ground state to cause chemiluminescence. Employing a co-reactant is especially useful when either radical charged ions are not stable enough for the ECL annihilation reaction, or radical ions cannot both be formed because of the solvent has a narrow potential window. With a co-reactant ECL can be generated by applying a potential in one direction. There are two reaction paths to produce the excited state of the ECL emitter, reductive-oxidation or oxidative-reduction ECL. For instance, oxalate ion ($C_2O_4^{2-}$) [4,5] and several amines [6,7,8,9] can be used for oxidative-reduction ECL where an oxidative step produces a strong reductant, whereas peroxidisulfate ion ($S_2O_8^{2-}$) [10,11,12] is frequently used for reductive-oxidation ECL.

As described above, ECL reactions require a luminophore. Even though many compounds have been demonstrated to participate in ECL reactions, most of them require aprotic and deoxygenated solution conditions. Therefore, only few compounds and their derivatives are primarily utilized for aqueous-based ECL bioanalytical detection methods. These are luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) and ruthenium (II) chelates $[RuL_3]^{2+}$. Nowadays, new luminophores such as semiconductor nanomaterials are being widely used with great results. This fact is one of the main reasons ECL sensor and biosensor are having a great and successful advance.

Bioanalytical methods based on $[RuL_3]^{2+}$ ECL were not developed until the co-reactant pathway was reported between Tris(bipyridine) ruthenium (II) $[Ru(bpy)_3]^{2+}$ and oxalate [5] in aqueous media and unaffected by the presence of oxygen was reported. $[Ru(bpy)_3]^{2+}$ is a model luminophore that is largely used nowadays; the discovery of its ECL emission in aqueous media with efficient co-reactants such as tri-n-propylamine (TPrA) [8,13] has led to successfully bioassays for clinical diagnosis. Probably one of the most relevant co-reactant pathways is the "oxidative–reductive" system between aliphatic amines and $[RuL_3]^{2+}$ as $[Ru(bpy)_3]^{2+}$. In this mechanism, both $[RuL_3]^{2+}$ and the co-reactant are oxidized (Equations (1) and (2)). The TPrA radical cation is unstable on the time frame of the experiment and quickly deprotonates, forming a free radical, TPrA* (Equation (3)).

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