A graphene biosensor for measuring the catalytic activity of the Human Topoisomerase I

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Malfunction of enzymes is the origin of various diseases. Monitoring the activity of enzymes is crucial for identifying the severity of the disease and the therapy. Human Topoisomerase I (hTop1) has gained a high clinical interest because it is the unique target of the anti-cancer drugs of the Camptothecin family (CPT) [1]. The efficiency of CPTs is dependent on the intracellular activity of hTop1. HTop1 accomplishes its function by breaking (cleavage) and rejoining (religation) the DNA backbone to solve torsional stresses. Several alterations of the enzyme activity are associated to CPT resistance [2]. For this purpose, numerous methods are being developed as fast, high throughput and simple techniques of analysis for the clinical field [3].

We present a diagnostic tool based on a graphene field-effect transistor biosensor for the detection of the activity of the hTop1. This method is label-free, fast and allows to measure the enzyme activity in real-time. The detection of the enzymatic reaction is carried out by measuring the impedance as a function of the gate voltage, applied through an Ag/AgCl reference electrode in contact with the liquid [4]. The raw data are fitted using a Drude model to extract the Dirac Point (DP). The temporal profile of the DP provides information regarding the reaction kinetics and permits to follow the enzyme activity. The DNA substrates of the hTop1 are coupled to graphene through electrochemical modification. The substrates are designed to detect the cleavage and religation reactions, separately. Control experiments were performed to ensure that the sensor response is due to the specific interaction of the enzyme with the substrate. The detection limit achieved is 300 pM hTop1. While a clear response is registered for the cleavage reaction in real-time, the religation step could be only detected in steady state. The results obtained show promise for the routine use of graphene-based biosensor for the detection of the catalysis of a wide range of enzymes.

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