A novel Electrochemical Immunosensor for miRNAs Detection using Reduced Graphene Oxide Electrodes.

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Micro-RNAs (miRNAs) are a class of small, non-coding RNAs which play important role in various regulatory functions and disorders as cancers and heart diseases.

Current standard methods for identification and quantification of miRNAs are based on traditional molecular biology techniques (Northern blot, microarray, qRT-PCR). These approaches although very sensitive and reliable are often expensive, time consuming. That's why a real challenge is to develop devices able to detect and quantify easily and simultaneously different miRNA sequences at sub-picomolar level.

We have recently designed a label-free and reagentless microRNA sensor based on an interpenetrated network of carbon nanotubes and electroactive polymer , with application to Prostate Cancer Biomarker miR-141 [1] .

Another extremely original method has been described by D. Stollar [2-3], and developed later [4]. The breakthrough consists in using antibodies specifically directed to RNA/DNA hybrids, which can thereafter be exploited for RNA detection through classical ELISA.

A recent work based on this approach used SPR biosensor and can detect RNA at concentration down to 2 pM [5]. Another system using silicon micro-ring resonators detects also directly miRNA at 10 pM [6].

Here, we designed an electrochemical immunosensor for miRNA detection. DNA capture probes were grafted on reduced graphene oxide (RGO)-modified electrode and then complementary miRNA were added. Following hybridization, specific RNA/DNA - antibody was introduced and shown to bind selectively to the hybrid.

Two different architectures will be discussed. The first uses a quinone-based conducting polymer which generates current increase upon hybridization, followed by a negative current change upon complexation with the specific antibody. This allows double-checking of mi-RNA detection and improves selectivity. The second proposes an enzymatic electrochemical amplification step which significantly lowers the LOD down to 10 fM. These approaches have never been described in the literature, and can be advantageously compared to classical ELISA assays in terms of selectivity and sensitivity.

References

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