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PNA-FUNCTIONALIZED MAGNETIC MICROBEADS AS SUBSTRATES FOR ENZYME-LABELLED AMPEROMETRIC GENOASSAY FOR GENETICALLY MODIFIED ORGANISM DNA SENSING

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Nucleic acid-based biosensors (genosensors) have received great attention in the last decade, being nucleic acids promising molecular probes due to the specificity for base pairing.
In a research program dealing with the development of innovative sensors as analytical tools for food safety assessment [1,2], we combined the performance of Peptide Nucleic Acid (PNA) probes with the efficiency of carboxyl-functionalized magnetic microbeads (mMBs) for the development of high performance genoassays with amperometric readout. The latter was carried out on glassy carbon screen printed electrodes (GC-SPEs) and on analogous electrodes based on embedded single-walled carbon nanotubes (SWCNT-SPEs).
The developed genoassays were applied to the determination of non-amplified DNA from genetically modified (GM) soy at trace levels.
A PNA-based Capture Probe (CP), with sequence complimentary to a 20-mer portion of "Roundup Ready" transgenic Soy DNA, was covalently immobilized on the active surface of mMBs, exploiting the reactivity of the carboxylic functionalities.
A signalling PNA probe (SP), complementary to a different portion of the target DNA and bearing a biotin tag, was used in combination with a streptavidin-alkaline phosphatase conjugate (ALP-Strp) to generate a three-probe sandwich leading to signals increasing with the target DNA concentration.

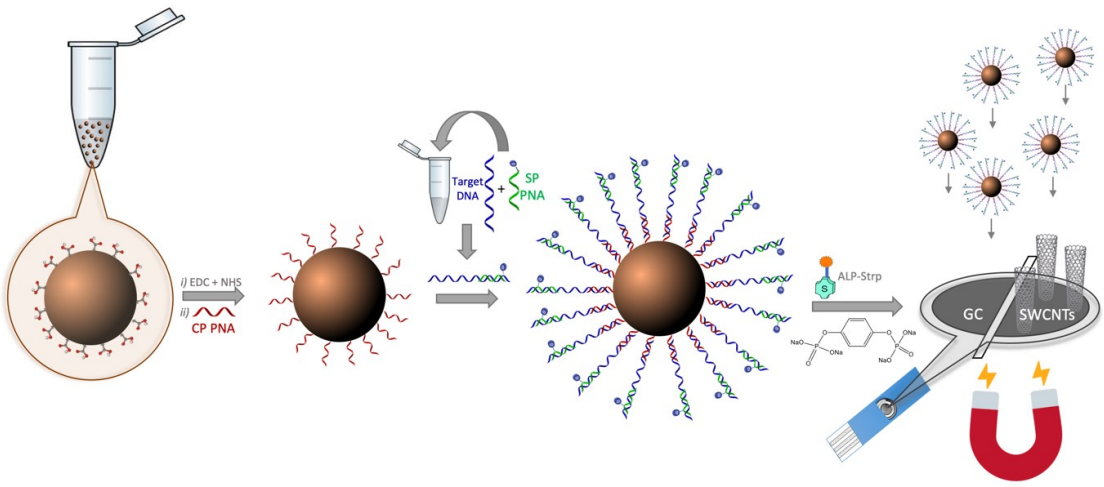

Figure 1. Working principle of the mMBs genoassay

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The performance of the developed genosensors was investigated by comparing the readout on GC-SPEs and SWCNT-SPEs. The intrinsic properties of the mMBs, which were exploited in terms of high reactive surface for the immobilization and hybridization of PNA probes, ensured in both cases an enhancement of sensitivity with respect to the performance exhibited by the corresponding newly developed and validated amperometric genosensors based on covalent immobilization of the same PNA probes on GC-SPEs or SWCNT-SPEs [3].
The best performance was achieved using SWCNT-SPEs for the readout, reaching limits of detection in the femtomolar range.
Finally, the methods based on the use of PNA-functionalized mMBs was validated on genomic DNA extracts from soy flour containing variable percentages of GM Soy, proving the discrimination capability of the genosensor based on SWCNT-SPEs towards GM material at trace levels.

References

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[2] Fortunati S., Rozzi A., Curti F., Giannetto M., Corradini R. and Careri M., Biosensors and Bioelectronics, 2019, 129, 7.
[3] Fortunati S., Rozzi A., Curti F., Giannetto M., Corradini R. and Careri M., Sensors, 2019, 19, 588.