REALIZATION OF A BIOSENSOR FOR THE SPECIFIC AND QUANTITATIVE MEASUREMENT OF TRANSCRIPTIONAL ACTIVATORS (SUCH AS NFKB) TO BE APPLIED TO THE MONITORING OF THE PHARMACOLOGICAL ACTIVITY OF THERAPEUTIC AGENTS IN THE FIELD OF CARDIOVASCULAR DISEASES

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This abstract describes the research activity on the development of a DNA based biosensor, at the moment in progress in the PhD course as a natural evolution of the work carried out during the degree thesis.
This work was focalised on the preparation of sensing biosurfaces which, after the activation of opportune bioreceptors properly immobilized, are able to interact with the environmental parameter of interest, giving a result proportional to it concentration.
The main aim of the present work is the application of the above-mentioned knowledge to the realization of a biosensor for diagnostic use, capable of supplying a quantitative determination of transcriptional activators, associated to specific pathologies.
In particular the monitoring of early events in cellular stresses of acute and/or chronic type, such as those caused by inflammatory processes, by ischemic damage, by oxidative processes or by other pathologies, is an objective of great interest, especially in pharmacological studies where a direct verification of the effectiveness of therapeutic agents, also of new generation, is necessary.

- For example, in the case of atherosclerosis or of other diseases of the cardiovascular system, the damaging pathological conditions of tissue (endothelium, smooth musculature, cardiomyocytes, etc.) is strictly dependent on the activation of genes whose expression gives the cells an altered phenotype related for example to an increased proliferative activity, to an unusual migratory ability (chemotaxis), to a different adhesion to the basal matrix, etc. This tissue remodelling is mediated by (and gives rise itself to the production of) growth factors, cytokines, lymphokines, chemokines, stress proteins.
- In this situation, *in vitro* and *in situ* studies evidenced the importance of factors such as the NFkB or other transcriptional activators that are available in the various stress situations. As an example, the NFkB factor activation is correlated with the vascular cell dysfunctions during the atherogenesis. The NFkB activity, in fact, causes the stimulation of expression of genes like those of: cytokines (IL1, IL6, TNFα...), iNOS, COX-2, and adhesion molecules (ICAM-1, VCAM-2), as well as of the major complex of histocompatibility MHC-I, proteins of acute phase and other.

The target of the project is the design and realisation of a prototype of biosensor, specific for transcriptional activators supplied with DNA-binding activity, more specifically a biosensor capable of discriminating the active shape of the activator from that inactive, taking advantage from the binding affinity of the protein with an oligonucleotide probe. At the beginning, the biosensor, based on optical principles, will be planned for the specific quantitative measurement of NFkB factor in biological reports, like blood cells, endothelial tissue, muscular smooth tissue, etc. Then, the prototype will be used in the measurement of transcriptional activators related to particular pathological events.
The sensitive part of the device will be constituted by a bioreceptor formed by a synthetic double strand-DNA, labelled with a fluorophore that mimes the specific binding site of the transcriptional activator to be measured. Therefore, the biosensor will be developed in order to transduce the binding with the unknown factor in an easily measurable and quantifiable physical phenomenon like the fluorescence variation induced by the probe-analyte complex formation. The achievement of direct and quantitative measurements of these activators will be strongly advantageous with respect to the traditional analytic techniques (EMSA, western blot, immunoprecipitation...) that are not only extremely laborious and expensive but are also not well-reproducible and only capable of giving semi-quantitative results.