Luciferase-Based Biobarcode Amplification Assay

Peter I. Belobrov, Ivan A. Denisov, Artur G. Tumanyan, Elena Esimbekova, Ludmila V. Meshajkina, Anton S. Yakimov, Valentina A Kratasyuk

Abstract – **The new biotechnical approach to ecological monitoring and medical devices design using biosensor systems based on luciferase enzyme is described. Main scientific, engineering and technological principles of PCR-free nanoparticles-based biobarcode amplification assay are given.**

Keywords - **Luciferase, Biosensors, Biobarcode, Nanodiamond.**

I. INTRODUCTION

Recent few years are new era of biotechnical and medical devices. They based on ultrahigh-sensitivity biodiagnostic system called the bio-barcode assay that using nanoparticles redefines "undetectable" prostate-specific antigen and biochemical recurrence [1]. The first fabrication of a few nanoparticles complexes with protein [2] induced biobarcoding and luminescent biochips. A nanoparticles-based biobarcode assay (BCA) that has PCR-like sensitivity for both DNA and protein targets have been developed [3, 4]. It has useful clinical application in medicine [1]. The first design of a luminescent biochip with nanodiamond and bacterial luciferase [5] and luciferase-based poly-enzymatic assay for ecological monitoring [6] is basis of our discussion of main engineering and technological principles of luciferase-based biobarcode amplification assay (LBCA) here.

II. TOWARDS LUCIFERASE-BASED BIOBARCODE

Correct and high amplification of biological signals is idea of our approach. The recent review helps us to discuss all amplification strategies: enzymatic - polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA) and luciferase-based assay, and non-enzymatic - optical, e.g. surface-enhanced Raman scattering (SERS) and surface plasmon resonance (SPR); electrochemical, magnetic, mechanical and BCA. Functionalized nanoparticles allow realizing both enzymatic and non-enzymatic strategies of biological signal amplification by BCA technique [7].

Luciferase is enzyme that transforms chemical signal into light, therefore we suppose that high sensitivity of modern photomultipliers (PMT) and avalanche photodiodes (APD) allows detecting in principal a few molecules into probe. But realization of this limit into luciferase biosensor needs more smart electronic amplification. E.g., dB gain of PMT is 160 that is equal to 10^8 times and APD registries of small optical power $\leq 10^{-9}$W, but the limit 1 photon/s is ~ 10^{-18}W. We should take into account internal noise into photon counting mode of PMT or Geiger-mode APD together with chemical and biological noise too. As result our LBCA should have biological and electronic modules with total dB gain ~ 400 for sensing of zeptomolar (10^{-21}M) concentration (1 zmol of bacterial luciferase contains 602 protein molecules).

Flowcharts of a few variants of electronic modules included PMT and/or APD, operational amplifiers with multiple stages in cascade with limited dynamic range to increase gain, companding (**comp**ression + exp**anding**) for better signal-to-noise ratio (SNR), and programmable gain amplifier are described. Main realized idea is to make electronic module with total dB gain ~ 400 using with different biological modules. Biological modules of LBCA are described briefly with accent to gain factor, SNR, specificity and transformation of biological and chemical signals.

The established techniques of PCR and ELISA together with suggested here in the first time LBCA will eventually give way to methods that offer comparable amplification, greater multiplexing capabilities and the prospects of lower cost and portability.

New materials - in particular nanodiamond, noble-metal nanoparticles, quantum dots etc - are now redefining analytical benchmarks for sensitivity, selectivity and versatility. When proteins and other non-nucleic-acid analytes can be detected as robustly as nucleic acids and with a technology as sensitive as PCR, the field of molecular diagnostics and medicine will be revolutionized [1].

III. CONCLUSION

We discussed the technical solution of electronic LBCA module for amplification the luciferase-based biological assay signals for ecological and medical applications.

This research was supported by RFBR (grant 07-04-01340-a), the Ministry of Education and Science of the Russian Federation (grant № 2.2.2.2/5309) and the U.S. CRDF for the Independent States of the Former Soviet Union (grants RUX0-002-KR-06/BP4M02 and RUX0-002-KR-06/BG9202).

REFERENCES

[1] D.A. Giljohann & C.A. Mirkin "Drivers of biodiagnostic development" *Nature* **462**, 461-464, 26 Nov 2009.

[2] A.P. Puzyr, V.S. Bondar, P.I. Belobrov, A.A. Bukaemskii. "Preparation of complexes nanodiamond-protein-δ-aluminum oxide" *Doklady Biochemistry* **373**, pp.139-141, 2000.

[3] J-M. Nam, S-J. Park, C.A. Mirkin. "Bio-Barcodes Based on Oligonucleotide-Modified Nanoparticles" *J. Am. Chem. Soc.*, *124* (15), pp 3820–3821, 2002.

[4] J-M. Nam, S.I. Stoeva, C.A. Mirkin. "Bio-Bar-Code-Based DNA Detection with PCR-like Sensitivity," *J. Am. Chem. Soc.*, **126** (19), pp. 5932-5933, 2004.

[5] A.P. Puzyr, I.O. Pozdnyakova, V. S. Bondar. "Design of a Luminescent Biochip with Nanodiamonds and Bacterial Luciferase," *Phys. Solid State*, **46** (4), pp.761-763, 2004.

[6] E.N. Esimbekova, V.A. Kratasyuk, "Bioluminescent enzymatic tests for ecological monitoring", *Ecology and Safety*, **2** (1). pp. 578-586, 2008.

[7] J. Jeon, D-K. Lim, J-M. Nam. "Functional nanomaterial-based amplified bio-detection strategies" *J. Mater. Chem.*, **19**, pp. 2107 - 2117, 2009.

Peter I Belobrov at. al. – Siberian Federal University, MOLPIT, 79 Svobodny Prospect, Room 13-10, Krasnoyarsk, 660041, Russia, E-mail: peter.belobrov@gmail.com