Hybridization biosensor system based on surface plasmon resonance for detection of the sequence of oligonucleotides of the Philadelphia chromosome

Sobolevskyi M.S.[1], Soldatkin O.O.[1], Lopatynskyi A.M.[2], Chegel V.I.[2], Samoylov A.V.[2], Matsishin M.J.[1], Dzyadevych S.V.[1], Soldatkin A.P.[1]

[1]Institute of Molecular Biology and Genetics of the NAS of Ukraine, Akademika Zabolotnoho St, 150, Kyiv-03680, Ukraine
[2]V.E. Lashkaryov Institute of Semiconductor Physics of the NAS of Ukraine, Nauki Avenue, 41, Kyiv-03028, Ukraine

E-mail: maxim.sobolevskiy@gmail.com

Aim

The aim of this research was to show fundamental possibility of enhancement of SPR signal using gold nanoparticles modified with probe oligonucleotides and surface blocking molecules for detection of DNA sequences of the Philadelphia chromosome, which is one of the characteristic genetic markers of chronic myeloid leukemia.

Methods

In accordance with the aim of the study, 80 base pair long oligonucleotide of the site of the e13a2 junction of the Philadelphia chromosome [1] was used as the target molecule; in this study it is referred to as **80-mer BCR-ABL**. The biosensor system (Fig.1) employed for detection of the target molecule consisted of two parts: the SPR sensor surface modified with probe oligonucleotides **mod-Ph** that are complementary to 24 base pair long region of **80-mer BCR-ABL** and gold nanoparticles (AuNPs) modified with the second DNA probe **SH-DP** which is complementary to the free region of the **80-mer BCR-ABL** target with a length of 18 base pairs (Fig. 2). In order to minimize irreversible interactions between individual AuNPs they were also incubated in 2 μM solutions of lipoic acid and 6-mercapto-1-hexanol. This method was determined to provide the AuNPs with the highest colloidal stability and, consequently, to allow for repeated hybridization experiments using the same SPR biosensor chip without loss of sensitivity.

To investigate the processes of hybridization of the oligonucleotides, we used the two-channel SPR spectrometer “Plasmon SPR6” that was developed at the V.Ye.Lashkaryov Institute of Semiconductor Physics of the NAS of Ukraine.

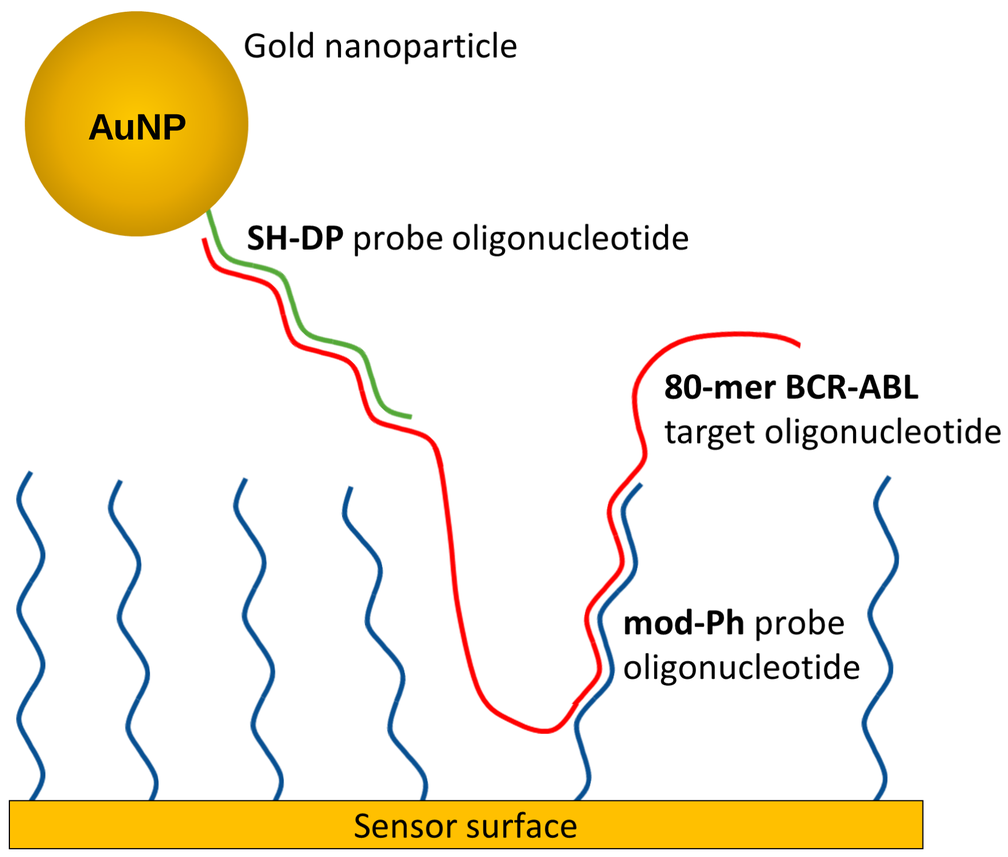

Fig.1 Schematic representation of the hybridization biosensor system

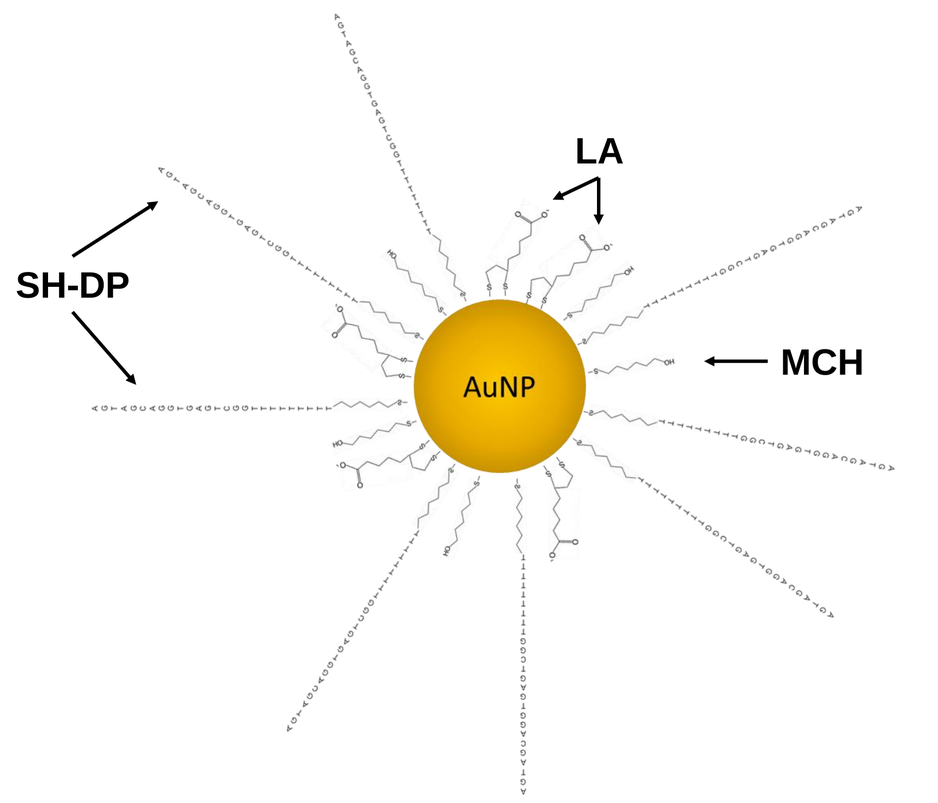

Fig.2 AuNPs used for the SPR signal enhancement were modified with probe molecules **SH-DP** and following surface blocking molecules: 6-mercapto-1-hexanol (**MCH**), lipoic acid (**LA**)

Results

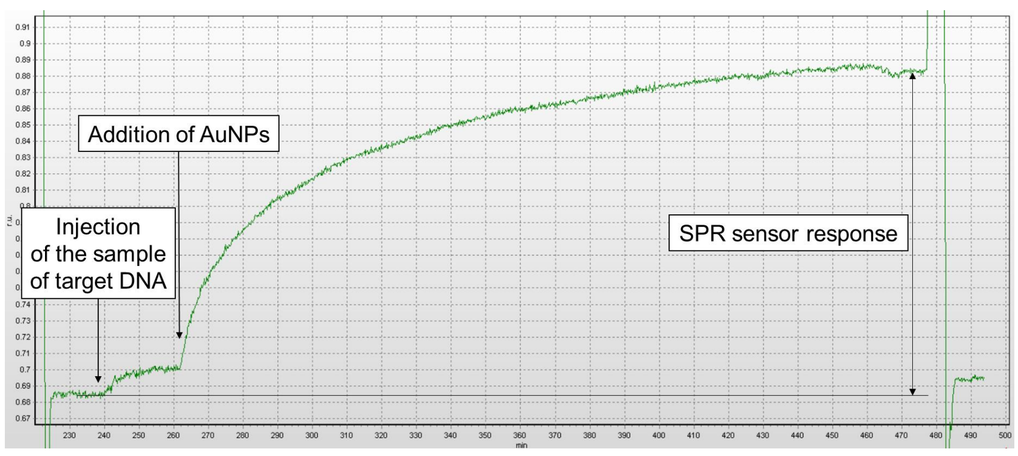

Fig.3 Response of the biosensor system to an increase in the concentration of **80-mer BCR-ABL**

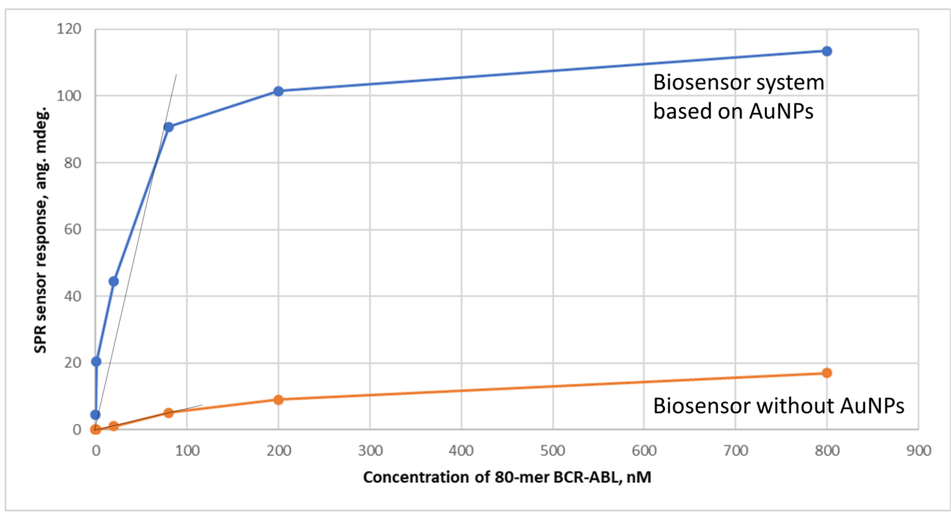

Fig.4 Calibration curve for determining the concentration of 80-mer BCR-ABL in a solution using the biosensor system

Conclusions

We proposed a new DNA hybridization biosensor system which, in comparison with the biosensor without AuNPs [2], made it possible to determine the concentration of 80-mer BCR-ABL in the studied solution with a much higher sensitivity and a much lower detection limit. In the case of measuring the concentration of 80-mer BCR-ABL by the proposed biosensor system, the detection limit was equal to 100 pM, which is 500 times less than that of the known biosensor [2]. The sensitivity of the system is 1201×10^{-6} ang. deg./nM, which is 6 times better than that of the the known biosensor. In addition, the use of AuNPs modified with LA and MCH made it possible to conduct hybridization experiments repeatedly using the same SPR biosensor chip without loss of sensitivity.

References

1. Ross, D., O'Hely, M., Bartley, P. et al. Distribution of genomic breakpoints in chronic myeloid leukemia: analysis of 308 patients // Leukemia.-2013.-N 27,-P. 2105–2107.
2. Matsishin, M. J., Ushenin, I.uV., Rachkov, A. E., & Solatkin, A. P. SPR Detection and Discrimination of the Oligonucleotides Related to the Normal and the Hybrid bcr-abl Genes by Two Stringency Control Strategies // Nanoscale research letters.-2016.-11, N 1.-P. 19