Surface plasmon resonance investigation of DNA hybridization on a sensor surface using gold nanoparticles modified by specific oligonucleotides

M. J. Matsishin\textsuperscript{1,2}, A. I. Kucherov\textsuperscript{3}, Iu. V. Ushenin\textsuperscript{4}, A. M. Lyapin\textsuperscript{4}, A. M. Lopatynskyi\textsuperscript{2,4}, V. I. Chegel\textsuperscript{2,4}, A. E. Rachkov\textsuperscript{1}

\textsuperscript{1} Institute of Molecular Biology and Genetics, NAS of Ukraine
150, Akademika Zabolotnoho Str., Kyiv, Ukraine, 03680

\textsuperscript{2} Institute of High Technologies, Taras Shevchenko National University of Kyiv
2, korp.5, pr. Akademika Hlushkova, Kyiv, Ukraine, 03022

\textsuperscript{3} National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute"
37, Pobedy av., Kyiv, Ukraine, 03056

\textsuperscript{4} V. Ye. Lashkaryov Institute of Semiconductor Physics, NAS of Ukraine
41, pr. Nauki, Kyiv, Ukraine, 03028

oleksandr_rachkov@yahoo.com

\textbf{Aim.} To investigate an influence of the oligonucleotide concentration on their immobilization on the surface of gold nanoparticles (AuNPs), and to study interactions between the AuNPs modified by various oligonucleotides and the oligonucleotides immobilized on the chip of the SPR-based DNA-sensor. \textbf{Methods.} Oligonucleotide immobilization on the surface of AuNPs was investigated by fluorescence spectrometry. The interactions of citrate-stabilized AuNPs modified by oligonucleotides with the oligonucleotides immobilized on the chip of the DNA-sensor were studied by the surface plasmon resonance spectrometry. \textbf{Results.} The initial oligonucleotide concentration influences the level of their immobilization on the surface of citrate-stabilized AuNPs: up to 200 nM the dependence was close to linear, and then saturation was observed at \sim 26 molecules per particle or \sim 0.5\times10^{13} \text{ molecules cm}^{-2}. In contrast, the efficiency of immobilization gradually decreased with an increase in the initial oligonucleotide concentration. Using the SPR-based DNA-sensor, the efficient hybridization between oligonucleotides immobilized on the sensor chip and complementary oligonucleotides of various length (short T2-11m and long T2-18m) immobilized on the surface of AuNPs was demonstrated. In case of AuNPs modified by short oligonucleotides, efficient thermal and chemical regenerations of the bioselective element of the DNA-sensor were achieved. \textbf{Conclusions.} Oligonucleotide immobilization on the surface of AuNPs directly depends on the initial oligonucleotide concentration, whereas the initial oligonucleotide concentration and...
SPR investigation of DNA hybridization using modified AuNPs

the efficiency of their immobilization on the surface of AuNPs demonstrate the inverse relationship. The efficient hybridization of the oligonucleotides of various lengths immobilized on AuNPs with the oligonucleotides immobilized on the sensor surface as well as the possibility of thermal or chemical regeneration allow the sensor reuse and a strong amplification of the sensor signal.

Keywords: gold nanoparticles, oligonucleotides, immobilization, surface plasmon resonance, DNA hybridization, DNA sensor

Introduction

Surface plasmon resonance (SPR) biosensors have become an important tool for characterizing and quantifying biomolecular interactions, because of their ability to measure biomolecular interactions directly, in real time and without the using labels [1–3]. However, this method, despite its many advantages, cannot ensure the detection of DNA sequences in subnanomolar concentrations without molecular label. To improve the detection limit of the SPR biosensors, a sandwich hybridization format can be used. In this format, the procedure consists of two steps: 1) identification of a target DNA by the selective hybridization with DNA probes immobilized on the sensor surface and 2) amplification of the sensor response by the additional hybridization step between a target DNA and so-called detection probe (itself or attached to an amplification agent) [1, 3–5]. A key step to ensure the success of this signal amplification format is an appropriate choice of the amplification agent. In SPR method, a large macromolecule or a nanoobject (e.g., gold nanoparticle) can be used for this purpose.

Gold nanoparticles (AuNPs) possess many attractive features: they are nontoxic, cheap and can be easily modified by various chemicals or biomacromolecules [4,6]. Therefore, the choice of AuNPs fits well for the development of technologically simple, flexible and cheap method for amplification of the SPR sensor signal. Unfortunately, the use of AuNPs in buffer solutions that support the process of hybridization may be accompanied by the electrolyte-induced aggregation because a rather high ionic strength of the buffer solutions overcomes the electrostatic repulsion between nanoparticles [6, 7]. However, the modification of nanoparticle surface by DNA sequences increases steric hindrance and electrostatic repulsion between AuNPs that increases their stability [8]. It allows us to expand the range of ionic strength of solutions in which the use of nanoparticles becomes possible. If the ionic strength of the solution is high enough to ensure the efficient DNA hybridization, and the AuNPs do not undergo aggregation, it becomes possible to use AuNPs as molecular labels to enhance the sensor signal [3–5, 9].

In suspension of the modified AuNPs, their stabilization is observed when the inter-particle attractive and repulsive forces are balanced [6]. The interface environment alters the existing force balance leading to the surface aggregation of AuNPs. Therefore, adsorption of modified AuNPs is driven by both specific (hybridization of complementary DNA chains) and non-specific adsorption of modified nanoparticles on the sensor surface [10]. If the role of the latter becomes prevalent, an inten-
sive aggregation of the modified AuNPs onto the sensor surface occurs, its regeneration and reuse become impossible. From this point of view, it is crucial to determine the conditions for selective and reversible interactions of the modified AuNPs with a bioselective element of the sensor.

Therefore, this study was aimed at the investigation of an influence of the oligonucleotides concentration on the process of their immobilization on the surface of gold nanoparticles (AuNPs), and revealing the features of interactions of the AuNPs modified by various oligonucleotides with oligonucleotides immobilized on the chip of the SPR-based DNA-sensor.

**Methods and Materials**

1. **Reagents**

Urea, KH$_2$PO$_4$, 6-mercapto-1-hexanol were obtained from Fluka (Buchs, Switzerland). All solutions were made with deionized Milli-Q water.

For immobilization on a gold sensor surface, the single-stranded oligonucleotide $P_2$ functionalized at the 5’-end with a thiol group through hexamethylene spacer (HS-(CH$_2$)$_6$-ACC CAC AAG CGC CGA CTG TTG) was used. Its sequence represents the fragment of the $rpoB$ gene of *Mycobacterium tuberculosis*, the mutations of which lead to the drug resistance of the bacteria.

For hybridization with $P_2$, the thiolated oligonucleotides $T2$-$18$m (HS-(CH$_2$)$_6$-CAA CAG TCG GCG CTT GTG), $T2$-$11$m (HS-(CH$_2$)$_6$-CAA CAG TCG GC) and the unmodified oligonucleotide $T2$ (CAA CAG TCG GCG CTT GTG GGT) were applied. Notably, these three oligonucleotides possess a homological sequence; $T2$-$18$m is shorter than $T2$, but longer than $T2$-$11$m. All listed oligonucleotides hybridize with the complementary oligonucleotide $P2$ in a very similar manner, but the different length of the sequences causes the different stability of the duplexes (Fig. 1).

For testing selectivity of the sensor response, the thiolated oligonucleotide $mp$-$14$ (HS-(CH$_2$)$_6$-GCT GAA GGG CTT TT), which is non-complementary to $P2$, was used.

All oligonucleotides were obtained from Metabion International AG (Germany). The choice of the length and nucleotide sequence of the oligonucleotides was described elsewhere [5].

2. **Synthesis of AuNPs**

The preparation of AuNPs is performed through the reduction of tetrachloroauroate ions (AuCl$_4^-$) by boiling in an aqueous sodium citrate solution [11]. First, 20 ml of 1 mM HAuCl$_4$ are heated to a boil with constant stirring and reflux. Then, 2 ml of 1 % sodium citrate are added. Heating and stirring continued for 10 min. Gold ions are reduced gradually, and the color of the solution becomes saturated red. The solution is cooled to room temperature. AuNPs obtained appear as practically monodispersed spherical structures with a size of about 10–15 nm, which are stabilized by weakly bound citrate ions. AuNPs are characterized by the plasmonic absorption band at approximately 520 nm.

3. **Modification of AuNPs**

For reliable immobilization of thiolated oligonucleotides onto AuNPs, an ability of their SH-groups to form covalent bonds with gold...
surface was used. To determine a level of the oligonucleotide immobilization on the surface of AuNPs, the procedure based on the property of the SYBR Green II fluorescent dye to increase drastically its fluorescence in the presence of a single-stranded oligonucleotide was applied. Namely, a level of fluorescence of SYBR Green II (proportional to the oligonucleotide concentration) in solutions before and after immobilization (initial concentration of oligonucleotides and concentration of unbound oligonucleotides) is measured. The difference is the molar concentration of the bound oligonucleotides. The division of this value to a molar concentration of nanoparticles gives an average number of oligonucleotides immobilized on a single nanoparticle.

The AuNPs modification by the thiolated oligonucleotides is carried out by incubation of AuNPs with the oligonucleotides overnight in 0.1×SSC buffer solution (1.5 mM sodium citrate, 15 mM NaCl, pH 7) at room temperature. After that, nanoparticles are separated from the unbound oligonucleotides by centrifugation at 12,000 rpm for 30 min [12, 13]. The supernatant is used to determine a level of the oligonucleotide immobilization on the surface of AuNPs by SynergyHT (BioTek) Microplate Reader, and the precipitate is analyzed by UV-Vis spectra analysis using spectrophotometer Nanodrop2000.
4. SPR spectrometry of the processes of oligonucleotide immobilization and hybridization on the sensor surface

The two-channel SPR spectrometer “Plasmon 6” was used. This optoelectronic device in the Kretchmann’s optical configuration was developed at V. Ye. Lashkaryov Institute of Semiconductor Physics of National Academy of Sciences of Ukraine. The 45 nm thick gold layer on a glass plate serves as a sensor surface. Prior to modification, gold surface of the glass plate is cleaned with freshly prepared piranha solution (3:1 mixture of concentrated \( \text{H}_2\text{SO}_4 \) and 30 \% \( \text{H}_2\text{O}_2 \); WARNING: piranha solution reacts violently with organic compounds and must be handled with extreme care) at room temperature for 2 min, then rinsed thoroughly with distilled and deionized water, and dried in the air. The cleaned plate is mounted on the spectrometer prism by using immersion liquid of the same refractive index as the prism and the plate. The flow rate (usually 40 µl min\(^{-1}\)) through the measuring flow cell is controlled by the peristaltic pump “Ismatec”. The preparation of bioselective element of DNA-sensor includes two main steps: immobilization of oligonucleotide probe and passivation of the free chip surface. For immobilization of the thiolated probe, 120 µl of 1 µM P2 in 0.5 M KH\(_2\)PO\(_4\) (pH 3.8) are injected into the measuring flow cell and exposed for 1 h. After that, the sites on the gold surface free from immobilized P2, were blocked by 1 mM aqueous solution of 6-mercapto-1-hexanol [14].

At the beginning of the hybridization experiment, the measuring flow cell of SPR spectrometer with the prepared bioselective element of DNA-sensor is thoroughly washed by the running buffer solution, for example, 2×SSC (30 mM sodium citrate, 300 mM NaCl, pH 7) to obtain a stable sensor signal. At the next step, 120 µl of target solution in the running buffer are injected into the measuring flow cell and exposed for 10 min. To reuse the bioselective element of DNA-sensor, it is necessary to disrupt the bonds between hybridized DNA chains. To implement thermal regeneration, a block of thermal regulation of the measuring flow cell, which controls temperature in a range from 20 °C to 60 °C, was developed (Patent of Ukraine O. 110131 issued September 26, 2016, IPC (2015.01) G01N 21/55). After hybridization at a room temperature, and the measuring of the initial biosensor response, the heating to the selected temperature is provided for 5 min at continuous flow of the buffer solution. Dehybridized nucleic acids are removed from the measuring cell. Then, the heating is switched off and the temperature in the cell decreases to room temperature. After that a final determination of the sensor response is performed. By comparing the obtained value with the initial one, the efficiency of the regeneration (or the portion of double-stranded complexes disrupted due to this procedure) can be defined. For chemical regeneration, the injection of 120 µl of 8 M Urea is used.

**Results and Discussion**

An influence of the initial concentration of oligonucleotides on the level of their immobilization on the surface of AuNPs was investigated. Fig. 2A shows that the increase in the initial oligonucleotide concentration resulted in higher level of their immobilization. Up to 200 nM the dependence is close to linear, and then the approach to saturation can be ob-
served. In contrast, the efficiency of immobilization (a ratio of the immobilized oligonucleotide concentration to the initial one) gradually decreases along with the increase of the initial oligonucleotide concentration (Fig. 2B).

Given an average diameter of the spherical AuNP (13 nm) and its surface area (530 nm²), the maximum surface density of the immobilized oligonucleotides **T2-18m** was ~26 molecules per particle, or ~0.5×10¹³ molecules cm⁻², or ~8 pmol cm⁻². It is well established that salt concentration determines the loading capacity of thiolated DNA by AuNPs, where a higher salt concentration results in more DNA molecules adsorbed. When the NaCl concentration is close to 1 M, ~110 thiolated DNA strands can be immobilized on each 13 nm AuNP [15–17]. In our previous investigations, the levels of oligonucleotide immobilization on the plain gold surface of the SPR chip were 28 pmol cm⁻² or 1.7×10¹³ molecules cm⁻² for oligonucleotides **P2** [5], and 7.6×10¹² molecules cm⁻² for oligonucleotides **mod-Ph** [18]. When 50 nM **P2-Cy3** in 0.1×SSC was

---

**Fig. 2.** The influence of the initial oligonucleotide (ODN) **T2-18m** concentration on the level of immobilization on the surface of AuNPs (A), and the efficiency of immobilization (B).
used for immobilization on the spherical surface of AuNPs, the surface density was approximately 13.5 oligonucleotides per nanoparticle or $2.5 \times 10^{12}$ molecules/cm$^2$ [12]. On the one hand, electrostatic repulsion between DNA chains on a flat surface is stronger, which reduces the level of immobilization, and, on the other hand, the process of immobilization on the flat surface was held at 0.5 M KH$_2$PO$_4$. It significantly shields the charges of phosphate residues of DNA, and thus contributes to more effective immobilization. Therefore, the obtained values of the surface density of the immobilized oligonucleotides T$_2$-18m are in good agreement with the data presented by other authors as well as with our previously published results for both cases of surface curvature.

Further application of the modified AuNPs is associated with hybridization between the oligonucleotides immobilized on the AuNPs surface and other (complementary) sequences of nucleic acids. The efficient hybridization occurs only under relatively high ionic strength. However, in this case the equilibrium between van der Waals attractive and electrostatic repulsive forces in the AuNPs dispersion is disturbed and electrolyte-induced aggregation occurs. The dispersed AuNPs exhibit only a single peak at ~520 nm, whereas particle aggregates show two light extinction maxima. As the interparticle spacing decreases, the first peak becomes weaker, while the second peak intensifies and shifts to longer wavelengths [6, 7].

As it was observed earlier, the stability of modified AuNPs depends on a charge which is carried by the modifying compounds, and the negatively charged compounds increase the stability of nanoparticle dispersion [19, 20]. Therefore, AuNPs modified by negatively charged oligonucleotides would be more stable against the electrolyte-induced aggregation than unmodified ones. Indeed, AuNPs with a higher surface density of the immobilized oligonucleotides demonstrated a higher stability. Their level of light extinction at 520 nm remained unchanged up to 0.5×SSC [9]. These results allow using the modified AuNPs for DNA hybridization because the concentration of the buffer solution can provide rather stringent and highly selective conditions for the hybridization process [18, 21].

To investigate the influence of the sequence and length of the oligonucleotides, which were used for the modification of AuNPs, on the level of their interactions with the oligonucleotides P$_2$ immobilized on the chip surface of the SPR-based DNA-sensor, three different oligonucleotides were applied (T$_2$-18m, T$_2$-11m and mp-14). After overnight incubation of 1 μM solution of each oligonucleotide with 5 nM AuNPs and the separation of nanoparticles from the unbound oligonucleotides by two-step centrifugation, the concentration of the obtained AuNPs was determined spectrophotometrically at 520 nm. Losses at the cleaning procedure constituted 15-30%. Finally, 0.25 nM solution of AuNPs modified by either mp-14, T$_2$-18m or T$_2$-11m was injected into the measuring flow cell of the DNA-sensor, and the sensor response was recorded.

To reuse a bioselective element of the DNA-sensor after hybridization step, thermal or chemical regeneration could be applied. The strength of bonding between two hybridized oligonucleotides is easy to predict, because it depends on the number of comple-
mentary base pairs. This strength can be expressed as thermodynamic parameters of hybridization or melting temperature ($T_m$) of a double-stranded complex. Using the DINAMelt web server http://mfold.rna.albany.edu/?q=DINAMelt [22] the values of the melting temperature ($T_m$) of double-stranded oligonucleotides of varying degree of complementarity were calculated for homogeneous state (in solution) for the next oligonucleotides. $P_2$ and $T_2$ are fully complementary 21-mers, $T_2$-18 and $T_2$-11 are, respectively, 18- and 11-mer shorter fragments of $T_2$. So, $T_2$-18 and $T_2$-11 – partially complementary to $P_2$, and therefore the strength of their double-stranded complexes with $P_2$ is smaller and $T_m$ is lower than that of completely complementary pair $P_2$-$T_2$ (Table 1). And the difference in 3 nucleotides between $T_2$ and $T_2$-18 leads to the decrease in $T_m$ value of their duplex with $P_2$ by approximately 5 °C, whereas the difference in 10 nucleotides between $T_2$ $\tau a$ $T_2$-11 – by ~25 °C. Table 1 also shows the direct dependence of $T_m$ on ionic strength of the medium (multiplicity of SSC buffer solution).

Oligonucleotides $T_2$-11m and $T_2$-18m have the same nucleotide sequence as oligonucleotides $T_2$-11 and $T_2$-18, respectively, but they were additionally modified at the 5’-end by SH-group for reliable immobilization on gold surfaces. AuNPs modified by the oligonucleotides mp14, which are not complementary to $P_2$, were used as a control.

For thermal regeneration, a block of thermal regulation of the measuring flow cell was used. As expected, the injection of the non-complementary oligonucleotides mp14 as a part of the modified nanoparticles into the measuring cell of the SPR biosensor did not cause any sensor response (data not shown). It indicates the absence of non-specific adsorption of the nanoparticles modified by non-complementary oligonucleotides. As shown in Fig. 3 and 4, the injection of both complementary oligonucleotides as parts of the modified nanoparticles into the measuring cell of the DNA-sensor at room temperature caused significant and approximately the same sensor response, indicating a successful hybridization with $P_2$ immobilized on the sensor chip. However, the elevation of temperature in the thermo-regulated measuring cell to 45°C and subsequent cooling to the initial temperature affected the stability of double-stranded complexes formed with $T_2$-18m or $T_2$-11m in different way. In case of a longer oligonucleotide (their duplexes are characterized by a higher $T_m$), the sensor signal returned almost to the level observed before heating. That is, the temperature elevation to 45 °C was

---

**Table 1.** The values of melting temperature of the pairs of studied oligonucleotides calculated for homogeneous state (in solution) for different ionic strength (multiplicity of SSC buffer solution).

<table>
<thead>
<tr>
<th>Pairs of oligonucleotides</th>
<th>$T_m$ in 0.5×SSC, °C</th>
<th>$T_m$ in 1×SSC, °C</th>
<th>$T_m$ in 2×SSC, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_2$ $\tau a$ $T_2$</td>
<td>62.5</td>
<td>65.7</td>
<td>69.1</td>
</tr>
<tr>
<td>$P_2$ $\tau a$ $T_2$-18</td>
<td>57.6</td>
<td>60.8</td>
<td>64.1</td>
</tr>
<tr>
<td>$P_2$ $\tau a$ $T_2$-11</td>
<td>37.8</td>
<td>40.6</td>
<td>43.6</td>
</tr>
</tbody>
</table>

All calculations are performed for 22 °C, 0 M Mg$^{2+}$, and 100 nM concentration of the oligonucleotides.
insufficient to melt duplex $P2/T2-18m$ and to regenerate the bioselective element of the DNA-sensor (Fig. 3). The thermal regeneration after hybridization of $P2$ with $T2-18m$ immobilized on the surface of AuNPs requires heating above 60 °C, and was not applied because of technical limitations of block thermal regulation of the measuring flow cell.

In case of the short oligonucleotides $T2-11m$ (their duplexes are characterized by $T_m$ below 45 °C), after the temperature elevation to 45 °C and subsequent cooling to the initial temperature the sensor signal returned to the level observed before hybridization. That is, heating to 45 °C caused the efficient regeneration of the bioselective element by thermal destruction of the duplexes $P2/T2-11m$ (Fig. 4).

Fig. 5 shows the sensograms representing the hybridization between oligonucleotides $P2$ immobilized on the sensor chip and oligonucleotides $T2-11m$, which are a part of the modified AuNPs, as well as the impact of heating to 45 °C and subsequent cooling to the initial temperature on the stability of the double-stranded complexes formed. The reaction medium is 0.5×SSC.
cal regeneration of the bioselective element of the DNA-sensor. The injection of the non-complementary oligonucleotides mp14 as a part of the modified nanoparticles, as expected, did not cause any sensor response (curve 1). The injection of both complementary oligonucleotides as a part of the modified AuNPs into the measuring cell of the DNA-sensor caused significant and approximately the same sensor response, indicating a successful hybridization with P2. In case of AuNPs modified by the short oligonucleotides T2-11m (curve 2), the injection of 8 M Urea resulted in rather efficient regeneration, which is comparable with that at the hybridization of free standing (without AuNPs) completely complementary oligonucleotides T2 [5, 21, 23]. Although in case of AuNPs modified by longer oligonucleotides T2-18m (curve 3) a significant part of the double-stranded complexes (~70%) was destroyed using 8 M Urea, a less efficient regeneration was observed. This relatively low efficiency of regeneration cannot be explained only by stronger specific interactions of P2 with 18-mer (compared with 11-mer), because the duplex of P2 with completely complementary T2 (21-mer without AuNPs) is easily disrupted by 8 M Urea. Probably, it can be explained by the contribution of non-specific interactions of AuNPs with the bioselective element of the DNA-sensor (in addition to P2/T2-18m hybridization). Therefore, a search for conditions of more efficient chemical regeneration after hybridization of P2 with T2-18m immobilized on the surface of AuNPs should be continued.

Taking into account that in the field of SPR sensors, the limit of detection (LOD) is usually defined as the concentration of analyte that produces sensor response corresponding to 3 standard deviations of sensor response measured for a blank sample [1, 24], LOD for
AuNPs modified by \textbf{T2-11m} or \textbf{T2-18m} was calculated to be \(~10\) pM. Previously, the detection of 20 nM \textbf{T2} (free standing) by the same \textbf{P2}-based DNA-sensor was shown [9]. Therefore, the calculated LOD is in a good agreement with our earlier prediction that at using AuNPs modified by specific oligonucleotides, a response of the DNA hybridization sensor based on the SPR spectrometer “Plasmon 6” can be amplified by over 1000 times [9]. Additionally, this LOD value is 2 orders of magnitude smaller, than the level of detection of \textbf{T2}, which was achieved using the impedimetric \textbf{P2}-based DNA-sensor [25].

\textbf{Conclusions}

The results of the present study show that a level of the oligonucleotide immobilization on the AuNPs surface directly depends on the initial oligonucleotide concentration, whereas the initial oligonucleotides concentration and the efficiency of their immobilization on the surface of AuNPs demonstrate the inverse relationship. The obtained values of the surface density of the oligonucleotides immobilized on the surface of AuNPs are in good agreement with the data presented by other authors as well as with our previously published results.

Using SPR-based DNA-sensor, the efficient hybridization between oligonucleotides immobilized on the sensor chip and complementary oligonucleotides of various lengths immobilized on AuNPs was demonstrated. In case of the AuNPs modified by the short oligonucleotides (\textbf{T2-11m}), the efficient thermal and chemical regenerations of the bioselective element of the DNA-sensor were achieved. In case of AuNPs modified by longer oligonucleotides (\textbf{T2-18m}), chemical regeneration of the bioselective element of the DNA-sensor by 8 M Urea was not so efficient, probably due to a contribution of nonspecific interactions of AuNPs with the bioselective element of the biosensor.

Additionally, our prediction that at use of AuNPs modified by specific oligonucleotides a response of the DNA hybridization sensor based on the SPR spectrometer “Plasmon 6” can be amplified by over 1000 times [9] was confirmed also. The efficient hybridization of the oligonucleotides of various lengths immobilized on AuNPs with the oligonucleotides immobilized on the sensor surface as well as the possibility of thermal and chemical regenerations allow multi-use of the sensor along with a strong amplification of the sensor signal.

\textbf{Funding}

This work was partially supported by STCU project N 6044; 2015.

\textbf{REFERENCES}

6. \textit{Ghosh SK, Pal T.} Interparticle coupling effect on the surface Plasmon resonance of gold nanoparti-


25. Matsishin M, Rachkov A, Errachid A, Dzyadevych S, Soldatkin A. Development of impedimetric DNA biosensor for selective detection and discrimination of oligonucleotide sequences of the rpoB gene of
Дослідження гібридизації ДНК на поверхні сенсора поверхневого плазмонного резонансу з використанням наночастинок золота, модифікованих специфічними олігонуклеотидами

М. Й. Мацшин, А. І. Кучеров, Ю. В. Ушенін, О. М. Ляпін, А. М. Лопатинський, В. І. Чегель, О. Е. Рачков

Мета. Вивчення впливу концентрації олігонуклеотидів на їх іммобілізацію на поверхні наночастинок золота (AuNPs) та виявлення деяких особливостей взаємодії AuNPs, модифікованих різними олігонуклеотидами, з олігонуклеотидами, іммобілізованими на чипі ДНК-сенсора поверхневого плазмонного резонансу. Методи. Рівень іммобілізації олігонуклеотидів на поверхні AuNPs досліджували флуоресцентною спектрометрією. Взаємодія стабілізованих цитратом AuNPs, модифікованих олігонуклеотидами, іммобілізованими на чипі ДНК-сенсора, вивчали за допомогою спектрометрії поверхневого плазмонного резонансу. Результати. При іммобілізації олігонуклеотидів на поверхні стабілізованих цитратом AuNPs початкова концентрація олігонуклеотидів впливає на рівень їх іммобілізації: до 200 нМ залежність була близькою до лінійної, а потім спостерігали наближення до насичення (~26 молекул на одну частинку або ~0,5 × 10^{13} молекул см^{-2}). Відмінно від цього, ефективність іммобілізації постійно зменшується разом із збільшенням початкової концентрації олігонуклеотидів. Використовуючи ДНК-сенсор поверхневого плазмонного резонансу, продемонстрували ефективну гібридизацію між олігонуклеотидами, іммобілізованими на сенсорному чипі, та комплементарними олігонуклеотидами різної довжини (короткі T2-11m і довгі T2-18m), іммобілізованими на поверхні AuNPs. У випадку AuNPs, модифікованих короткими олігонуклеотидами, були досягнуті ефективні термічна та хімічна регенерація біоселективного елемента ДНК-сенсора. Висновки. Рівень іммобілізації олігонуклеотидів на поверхні AuNPs прямо пропорційно залежить від виходної концентрації олігонуклеотидів та ефективність їх іммобілізації на поверхні AuNPs демонструють зворотний зв’язок. Ефективна гібридизація олігонуклеотидів різної довжини, іммобілізованих на AuNPs, з олігонуклеотидами, іммобілізованими на поверхні сенсора, а також можливість термічної або хімічної регенерації дозволяють багаторазово використовувати сенсор та досягати величезного підсилення сенсорного сигналу. Ключові слова: наночастинки золота, олігонуклеотиди, іммобілізація, поверхневий плазмонний резонанс, гібридизація ДНК, ДНК-сенсор.

М. Й. Мацшин, А. І. Кучеров, Ю. В. Ушенін, А. М. Ляпін, А. Н. Лопатинський, В. І. Чегель, А. Э. Рачков

Цель. Исследование гибридизации ДНК на поверхности сенсора поверхностного плазмонного резонанса с использованием наночастицы золота, модифицированных специфическими олігонуклеотидами

Исследование гибридизации ДНК на поверхности сенсора поверхностного плазмонного резонанса с использованием наночастицы золота, модифицированных специфическими олігонуклеотидами

М. Й. Мацшин, А. І. Кучеров, Ю. В. Ушенін, А. М. Ляпін, А. Н. Лопатинський, В. І. Чегель, А. Э. Рачков

Цель. Изучение влияния концентрации олігонуклеотидов на их иммобилизацию на поверхности наночастицы золота (AuNPs) и выявление некоторых особенностей взаимодействия AuNPs, модифицированных различными олігонуклеотидами, с олігонуклеотидами, иммобилизованными на чипе ДНК-сенсора поверхностного плазмонного резонанса. Методы. Уровень иммобилизации олігонуклеотидов на поверхности AuNPs исследовали флуоресцентной спектрометрией. Взаимодействие стабилизированных цитратом AuNPs, модифицированных олігонуклеотидами, с олігонуклеотидами, иммобилизованными на чипе ДНК-сенсора поверхностного плазмонного резонанса. Результаты. При иммобилизации олігонуклеотидов на поверхности стабилизированных цитратом AuNPs начальная концентрация олігонуклеотидов влияет на уровень их иммобилизации: до 200 нМ зависимость была близка к линейной, а затем наблюдался приближен к насыщению (~26 молекул на одну частицу или ~0,5 × 10^{13} молекул см^{-2}). В отличие от этого, эффективность иммобилизации постепенно уменьшается вместе с увеличением начальной концентрации олігонуклеотидов. Используя ДНК-сенсор поверхностного плаз-
монного резонанса, продемонстрировали эффективную гибридизацию между олигонуклеотидами, иммобилизованными на сенсорном чипе, и комплементарными олигонуклеотидами различной длины (короткие Т2-11м и длинные Т2-18м), иммобилизованными на поверхности AuNPs. В случае AuNPs, модифицированных короткими олигонуклеотидами, были достигнуты эффективные термическая и химическая регенерация биоселективного элемента ДНК-сенсора.

Выводы. Уровень иммобилизации олигонуклеотидов на поверхности AuNPs прямо пропорционально зависит от исходной концентрации олигонуклеотидов, тогда как исходная концентрация олигонуклеотидов и эффективность их иммобилизации на поверхности AuNPs демонстрируют противоположную связь. Эффективная гибридизация олигонуклеотидов различной длины, иммобилизованных на AuNPs, с олигонуклеотидами, иммобилизованными на поверхности сенсора, а также возможность термической или химической регенерации позволяют многократно использовать сенсор и достигать огромного усиления сенсорного сигнала.

Ключевые слова: наночастицы золота, олигонуклеотиды, иммобилизация, поверхностный плазмонный резонанс, гибридизация ДНК, ДНК-сенсор.

Received 04.07.2017