

Concentration Sensitivity of Nucleic Acid and Protein Molecule Detection Using Nanowire Biosensors

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Abstract: The concentration detection limit (DL) of biomacromolecules attainable using a nanowire detector has become a topical issue. A DL of 10^{-15} M is required to reveal oncological and infectious diseases at an early stage. This study discusses the DL experimentally attainable in the subfemtomolar concentration range, and possible mechanisms explaining such a low-concentration DL through the cooperative effect of biomacromolecular complexes formed on the surface of the nanowire (NW) chip near the nanowire.

Keywords: nanowire biosensor; ultrasensitive protein detection; detection limit; field-effect nanotransistor



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1. Introduction

Nanowire detectors are usually considered to be nanoelectronic devices containing a matrix of field-effect transistors, in which nanowires (nanoribbons, nanowires) are used as a gate; these devices allow one to detect biomacromolecules [1–8].

Detection of viral particles with nanowire biosensors has been reported [1]; the number of such studies is, however, low, since the detection of single viral particles is difficult due to the following circumstances. After immersion in a suspension of viral particles, a layer of proteins (which are present in unbound form even in highly purified preparations [9]) virtually immediately forms on the functionalized surface of nanostructures—as was demonstrated with the example of a 200 nm AFM probe [10]. This is why nanowire biosensors are, as a rule, used for the detection of proteins and nucleic acids at low (femtomolar and subfemtomolar, i.e., $\leq 10^{-15}$ M) concentrations [5,6,8,11,12].

The sensitive element in such devices is a nanowire, which represents an *n*- or *p*-channel of a field-effect nanotransistor, with the surface facing the analyte solution, and acts as a virtual gate. The operating principle of such devices is as follows. When a charged macromolecule (e.g., a protein or nucleic acid) adsorbs onto the surface of the nanowire, a change in the conductivity between the drain and the source of the field-effect transistor is observed. This change in conductivity is recorded in real time using an electronic system. Protein markers, microRNAs, circular RNAs and other macromolecules associated with socially significant diseases are used as target macromolecules for diagnostic purposes. To provide biospecific detection, the surface of the chip from the nanowire detector is functionalized with molecular probes, which specifically recognize the target macromolecules.

In nanowire biosensors, the nanowires normally have dimensions on a nanometer scale, being commensurable with the size of macromolecules. The nanometer-scale size allows one to increase the sensitivity by increasing the surface area-to-volume ratio [13]. For instance, a nanowire detector has a size of 10 nm to 10 μ m (diameter for a nanowire; thickness for a nanoribbon). Usually, nanowire detectors are fabricated in the form of

an array of field-effect transistors. One chip can accommodate up to 10 or more such transistors. The concentration detection limit experimentally attained with such detectors normally reaches 10^{-17} M to 10^{-15} M [5,11,12,14].

Such a low concentration detection limit is necessary for the detection of oncological and infectious diseases at an early stage [15]. However, the theoretical basis for the experimental achievement of such detection limits using currently existing models, based on macromolecular registration directly on the nanowire's surface, is still being developed. This study discusses a possible mechanism of experimentally attaining femtomolar and subfemtomolar concentration detection limits using a nanowire detector.

2. Concentration Sensitivity Limit of Nanowire Detection

Theoretically, the concentration limit of molecular detection using nanowire devices can reach single molecule [4]. This is not actually true. In analyses of the concentration limit of detection attainable with nanowire detectors, there is an essential issue related to the theoretical substantiation of the experimental results. In order to detect biomacromolecules, an analyte solution containing these macromolecules is placed into the volume of the measuring cell, which is mounted over the nanowire's surface. In this case, biomacromolecules are captured from a large volume of the cell onto the surface of the nanowire, where biospecific complexes are formed. The signal from the nanowire detector is believed to be caused by adsorption of a charged molecule onto the surface of the nanowire. Considering that in the buffer solutions commonly used in such detectors (the buffer concentration is several μ M to mM), the Debye length is shorter than 10 nm, this means that with this mechanism, the distance between the charged molecule and the nanowire should be several nm. On the other hand, the probability of implementing such a process is quite low.

To confirm this, let us present estimation calculations, made for a matrix chip with 10 nanowires (whose dimensions are as follows: width = 3 μ m (taken from the top); length = 10 μ m; wire height = 32 nm), which is used in practice in nanowire biosensors [5]. Firstly, the number of biomacromolecules in the measuring cell, in which the chip serves as the bottom, is estimated. The assumed volume of the measuring cell is $V = 100 \mu$ L. At a concentration of $C = 10^{-17}$ M to 10^{-15} M, such a volume contains:

$$N = N_A CV = 6 \times 10^4 \text{ to } 6 \times 10^2 \text{ biomacromolecules,}$$

where N_A is the Avogadro number. Taking into account that there are 10 nanowires on the chip, it can be concluded that, theoretically, each nanowire can have $1/10 N$ molecules, i.e., no more than 60 to 6×10^3 molecules.

With assumption that the surface of the nanowire chip is modified with a continuous layer of probes, modification of the nanowire can be performed by depositing a probe solution of a certain volume (V) onto the activated area S_d of the nanowire chip's surface. In this case, a wider S_d area (which also includes a much smaller nanowire area, S_W), where a drop with the probes spreads during modification of the nanowire, might be additionally modified. It should be taken into account that not all target biomacromolecules can get onto the nanowire during the fishing (that is, capturing) process, and while they are delivered to the nanowire, losses may occur due to possible adsorption far from the nanowire, particularly onto the S_d area between the nanowires on the nanowire array, since the size of the deposited drop with probes S_d during modification of the nanowire usually exceeds the size of the S_W nanowire. The losses in the simplest case, when only such nonspecific adsorption takes place, need to be estimated.

The nanowire area is around $S_W \sim 3 \times 10^{-30} \mu\text{m}^2$. At the same time, in the process of the nanowire modification, when the probe solution is applied to the nanowire's surface with a robotic dispenser, the area of the droplet can be broader than S_W . Consequently, the proportion of probes immobilized on the nanowire surface is:

$$F = S_d/S_w.$$

Let us suppose that $F \approx 6$. Accordingly, in fact, the number of biomacromolecules that which can get onto the nanowires with a uniform distribution over the area of a drop on the chip's surface at $F = 6$ should be $F = 6$ times lower—namely, 1000 to 10 biomacromolecules at a concentration of $C = 10^{-15}$ M to 10^{-17} M. In other words, the concentration detection limit should be not better than 10^{-15} M if the nanowire detector can detect more than 10 molecules. Concurrently, a signal from macromolecules is experimentally observed at a concentration of 10^{-17} M. This may indicate that the detection mechanism is based on the cooperative influence of all macromolecules, specifically against the probes immobilized on both the nanowire and the rest of the S_d on the area of the applied drop. This cooperative effect can take place due to the spread of the influence of the formation of complexes of biomacromolecules with immobilized probes around the spot of the droplet S_d , and not only on the surface of the nanowire, by means of an aqueous medium. It should be taken into account that water is a heterostructural medium, which, according to the latest literature data, represents a nonequilibrium medium in terms of a spin state, consisting of the para-ortho isomers of water [16–18]. Moreover, the spin-selective interaction of H₂O para-isomers with proteins in aqueous solutions suggests that the hydration shell is formed predominantly by para-isomers, as was noted by Pershin et al. [17]. These authors have recently found that the equilibrium ortho/para ratio of 3:1, which is valid for gases at room temperature, shifts in water towards an increase in the number of H₂O para isomers by more than two times, and any external influence can shift this equilibrium. Thus, it has been demonstrated that water is a nonequilibrium liquid in terms of spin temperature. The stated experimental facts show that the water in the hydration shells of biomolecules and on the chip's surface is structured and has an ice-like structure. The rigidity of the ice-like structure of the shell maintains the shape of the protein and prevents its deformation, turning into a ball with minimum surface energy in accordance with thermodynamics [17]. According to the abovementioned works, hydration layers are formed in water along the surface of the chip and the immobilized probes. A spatial network is formed in the aquatic environment, which includes a surface with the probes. The added biomacromolecules also bear a hydration shell. Taking into account the existence of ice-like structures of I_n type in the water and hydration layers, the formation of complexes can change the ice-like structure around the nanowires and the surrounding area S_d with the probes. In this case, a redistribution of the electron density along the network of these bonds from the site of complex formation to the surface of the nanowire occurs. Due to this fact, charged molecules induce the appearance of an increased electron density around the nanowire through such a structure due to the cooperative effect. An increase in the number of complexes of biomacromolecules around the place of application of a drop with immobilized probes owing to the capture of biomacromolecules leads to the inclusion of additional participants in the cooperative effect of all complexes on the S_d surface on the nanowire conductivity. Thus, it can be concluded that this cooperative mechanism makes it possible to explain the results of registering ultra-low (femtomolar) concentrations of complexes in nanowire nanobiosensor systems. Moreover, consideration of the quantum conversion of para/ortho isomers of water, which mediates the modulation of the network of hydrogen bonds with the transition of ortho-H₂O molecules into the framework voids of the hydration layer network and the so-caused increase in the electrical conductivity [18] during the formation of complexes in the zone of the hydration layer in the S_d area with an increase (owing to complex formation in this region) in the size of macromolecular clusters up to the micron scale (the size of the micron scale that can form for the contact boundary of water with the hydrophilic surface of macromolecules, according to [19,20]), leads to the following conclusion. Such a modulation leads to a corresponding increase in the diffusion rate of H₃O⁺ and OH[−] ions and, accordingly, to an increase in the electrical conductivity (according to [18]), and facilitates the additional long-range (cooperative) influence of charged molecules upon complex formation in the S_d area on the charge state of the nanowire.

The cooperative effect described in the present study manifests itself not only in nanowire systems but also during the functioning of enzymatic nano-objects at these concentrations. Thus, in [21], the effect of the cooperative interaction of molecules of the CYP102A1 enzyme in the process of their functioning, accompanied by radiation in the microwave range, was found.

Let us present our considerations regarding the cooperative effect discussed. Firstly, these considerations relate to the model, in which the molecules are considered to be charged spheres. In fact, the molecules represent extended charged structures. Thus, the distribution of charge over the molecule's surface will influence the cooperative effect. In this way, for instance, strongly charged groups of captured protein molecules will cause a local redistribution of the electron density of the ice-like structure along the chip's surface, leading to the influence of these charged groups on the nanowire's conductance.

Another consideration relates to the sensitivity. The intrinsic sensitivity of the nanodetector, which is determined by the number of target molecules registerable with the detector, represents a limiting factor of the entire biosensor system. The sensitivity of the entire nanobiosensor system is determined by both the intrinsic nanodetector's sensitivity and the efficiency of the fishing (capturing of the target molecules onto the nanodetector surface with immobilized molecular probes against the target molecules) technology [22,23]. The fishing efficiency is determined in concentration (mol/L, M) units [22,23].

The sensitivity of nanowire detectors with characteristic nanowire size of the order of 30 nm was shown to be sufficient for the registration of a single-protein molecule [24]. Accordingly, such devices pertain to single-molecule detectors. By increasing the characteristic nanowire size up to 1 micron, the detector's sensitivity can decrease by an order of magnitude [8]. Accordingly, the sensitivity of the nanowire systems considered herein can be sufficient for the registration of single-target molecules to tens of molecules. This is why the amount of 10 molecules has been considered herein as the limit of detection.

As noted above, the efficiency of the biosensor system is determined by several factors. The first factor is the efficiency of the target molecules' delivery towards the sensor's surface, which is determined by hydrodynamic mode in the measuring cell of the biosensor [23]. The second factor is the efficiency of the probe/target (the target is a protein or nucleic acid molecule) complex formation, which is determined by the binding constant K_d [25]. The efficiency of counting of the molecules delivered is another factor. In the present study, the efficiency of the delivery was assumed to be 100%. As regards the efficiency of complex formation, the final K_d of a biorecognition pair on the field-effect transistor-based sensor's surface is determined by not only the intrinsic affinity (K_d) of this pair but also by the influence of the nanotransistor's gate potential. The latter can cause a change in the K_d value by several orders of magnitude [26]. This is why, in our present study, the K_d -dependent efficiency of the fishing technology has not been taken into consideration. Based on these assumptions, an analysis of the sensitivity of the nanowire system has been performed.

3. Conclusions

This work raises the issue of an ultra-low subfemtomolar concentration limit of detection, experimentally measured with nanowire biosensors. It has been discussed that the mechanism of nanowire detection can be based on the cooperative effect of water-protein structures. This mechanism makes it possible to substantiate the sensitivity of nanowire systems with a small nanowire size located on a large chip surface, onto which biomacromolecules are captured from a much larger analyte solution volume within the subfemtomolar concentration range.

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