

THE CONDUCTIVITY OF THE MONOLAYER OF COMPLEXES OF DNA
WITH QUANTUM DOTS IN THE PRESENCE OF INTERCALATING
LIGANDS

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The noncompetitive DNA hybridization and conductivity in the presence of non-charged ligands have been investigated and the comparison is made with the ligand-free case. It has been shown that the intercalating ligands enhance the sensitivity of the DNA chips as compared with the ligand-free case at the high enough concentration of ligands.

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Introduction. Fast, specific detection of nucleic acid sequences is of great practical importance due to its possible application in various fields, from the detection of pathogens to the diagnosis of genetic diseases [1]. Among the various detection methods, photoelectrochemical methods still attract considerable interest, because of their sensitivity [2–4], simplicity, and cheapness [5]. Moreover, quantum dots (QDs) with their unique fluorescent and photoelectrochemical properties are photoactive materials that are extremely promising for the development of nucleic acid sensors (hereinafter, DNA chips). One of the important directions in the development of DNA chips is to increase their sensitivity by enhancing the electrical signal and the stability of the target hybridization probe. The efficiency of DNA chips, in particular, depends on the accuracy of prediction of the experimental parameters responsible for the thermal stability of nucleic acid duplexes and the time of DNA duplex formation [6].

An increase in the sensitivity of DNA sensors can be achieved by using electrochemically active compounds with a higher affinity for double-stranded DNA (ds-DNA) than for single-stranded (ss-). This type of compounds can significantly increase the stability of double-stranded sections and at the same time, the amplitude of the generated signal, which, in turn, will increase the sensitivity of the DNA

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sensor. Such ligands are, for example, intercalators, molecules with a flat heterocyclic structure, which are placed between nitrogenous bases and change the local structure of ds-DNA [7]. In addition to stabilizing the duplex form of DNA, intercalating ligands affect the conductivity of the sensitive layer of the DNA chip [8]. This approach was implemented *e.g.* when developing a new architecture of ds-DNA immobilized on the substrates of electrodes crosslinked with CdS nanoparticles and structurally controlled by photocurrent generation upon irradiation of these arrays with light [8]. The binding of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ ligands-intercalators to the DNA double helix provides tunneling routes for the electrons of the conduction band and, thus, leads to an increase in the photocurrent. The Fig. 1 shows the assembly of DNA crosslinked with CdS nanoparticles on an Au electrode. The conductivity of DNA in such a system is regulated by the incorporation of redox intercalators into ds-DNA [9].

From our point of view, charge transfer between intercalated ligands due to tunneling seems doubtful, since the existing data more likely indicate that charge transfer through DNA can be rather described as a mechanism of multiple, partially coherent jumps between delocalized regions containing bases with tight stacking [9].

The present work is devoted to investigation of the hybridization of DNA on the surface in presence of the ligands bound to the native DNA, and of the conductivity of monolayer formed in this case.

Hybridization of the Conjugated ss-DNA on the Surface and Photocurrent Development. To recognize complementary target ss-DNA on the surface of a DNA-chip electrode we need a sensitive surface with ss-DNA probes immobilized on it [10]. Here we consider the ss-DNA probes grafted to QDs (*e.g.* CdS). In the process of hybridization of complementary ss-DNAs in presence of intercalating ligands a monolayer is formed consisting of ds-DNA–QD and target-probe complexes (see Fig. 1). During the interaction of light with a monolayer, a photoexcitation site of QDs occurs with the subsequent appearance of free charge carriers (an electron-hole pair) and, as a result, a photocurrent in the monolayer, based on which the concentration of the target DNA in the solution can be determined.

The sensitive layer of the DNA-chip is formed by ss-DNAs and ds-DNAs, immobilized on the surface of the electrode. At the same time, ss-DNA has a much greater resistance (R_{ss}) than ds-DNA (R_{ds}). It is assumed that ds-DNAs contain a certain amount of intercalating ligands that enhance the photocurrent in the sensitive layer of the DNA chip. The fraction of ss-DNA hybridized with target DNA and the number of ligands bound to ds-DNA on the surface were estimated in [11] for noncompetitive hybridization as

$$\begin{aligned} \frac{x(1-r)^N}{1-x} &= c_t K_t \exp(-\Gamma(1+x-zrx)), \\ \frac{r}{1-r} &= c_l K_l \exp\left(\frac{\Gamma}{N}(1+x-zrx)\right), \end{aligned} \quad (1)$$

where x is the fraction of hybridized ss-DNA targets on the electrode surface; r is the degree of filling of the ds-DNAs by ligand-intercalator; N is the number of base

pairs of the single ds-DNA on the surface; z is the ligand charge; c_t and c_l are the concentration of ss-DNA targets and ligands in solution, respectively; K_t and K_l are the binding constants of ss-DNA targets and ligands, respectively; $\Gamma = 8\pi N\sigma_0\ell_B\frac{r_D^2}{H}$ and $\sigma_0 = \frac{NN_0}{A}$, where N_0 is the total number of ss-DNA probes on the surface; A is the electrode surface area; ℓ_B is the Bjorrum length; r_D is the Debye screening radius; H is the thickness of the surface layer. The dependence of the fraction x on the concentration c_t of ss-DNA targets in solution is called the hybridization isotherm.

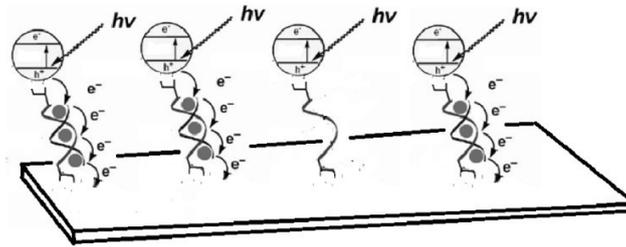


Fig. 1. The sketch of the sensitive layer, formed by single- and double stranded DNAs with conjugated QDs and bound ligands.

The resistance of the ds-DNA monolayer shown in Fig. 1 corresponds to the parallel connection of xN_0 resistances R_{ds} (or R_{ds}^0 , in ligand-free case). Therefore, the total resistance of the monolayer is estimated as

$$R = \frac{R_{ds}}{xN_0}, \quad R^0 = \frac{R_{ds}^0}{xN_0}. \quad (2)$$

According to the results obtained in [12], DNA resistance usually increases linearly with length, which is characteristic of the mechanism of incoherent jumps. However, for DNA sequences with GC-pair stacking, a periodic oscillation is superimposed on the linear dependence of length, indicating partial transport coherence. Here, for simplicity, we will assume a low content of GC-pairs and estimate the resistance of the subchain from base pairs between the two nearest intercalators as [12]

$$R(n) = \frac{2}{e^2\rho(E_F)k_l} \exp\left(\frac{E_a}{k_B T}\right) + \frac{n-1}{e^2\rho(E_F)k} \exp\left(\frac{E_a}{k_B T}\right), \quad (3)$$

where $\rho(E_F)$ is the density of states at the Fermi level; k_l is the charge transfer rate from the ligand to an adjacent base pair; k is the charge transfer rate between adjacent base pairs and E_a is the activation energy. It is supposed that k_l is of the same order as the charge transfer rate for the electrode; k_{el} . If r is the degree of filling of ds-DNA with intercalating ligands, then the average number of ligands in duplex DNA is estimated as $m = Nr$, and the average chain length of ds-DNA between adjacent intercalated ligands as $n = \frac{1}{r}$. Then the resistance of one ds-DNA-QD complex can be estimated as

$$R_{ds} = \frac{\exp\left(\frac{E_a}{k_B T}\right)}{e^2\rho(E_F)} \left\{ \frac{2Nr+1}{k_l} + \frac{1}{k_{QD}} + \frac{N(1-r)+1/r-1}{k} \right\}, \quad (4)$$

while without ligands the resistance of the dsDNA–QD complex is written [12]

$$R_{ds}^0 = \frac{\exp\left(\frac{E_a}{k_B T}\right)}{e^2 \rho(E_F)} \left\{ \frac{1}{k_{el}} + \frac{1}{k_{QD}} + \frac{N-1}{k} \right\}. \quad (5)$$

For simplicity, we assume $k_l = k_{el}$.

The Effect of Intercalating Ligands in Term of Isotherm of Hybridization.

The dependence of the total resistance of the monolayer on the reduced concentration of ss-DNA targets in solution was calculated on the basis of the equations (2), (4) and (5) and is shown in Fig. 2.

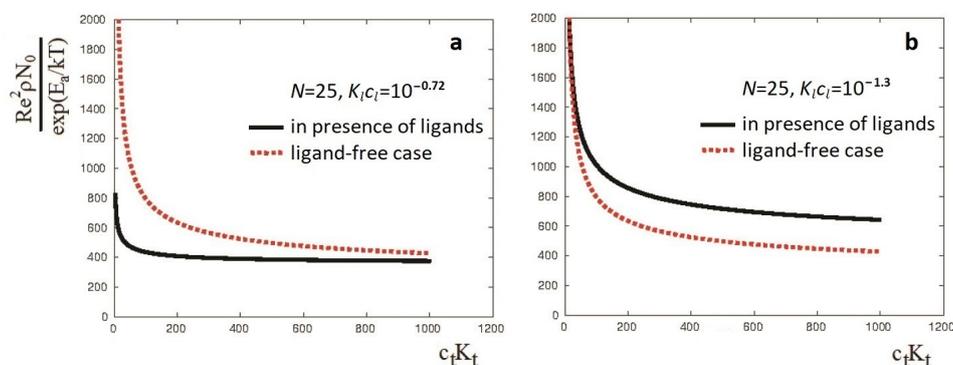


Fig. 2. The dependence of the sensitive layer resistance on the concentrations of targets in solution.

In conclusion, we have shown that the ligands binding results in a decrease in the total resistance of the sensitive layer of the DNA chip at the high enough concentration of ligands (see Fig. 2,a). This, in turn, enhances the intensity of the photocurrent at a given degree of hybridization of ss-DNA probes immobilized on the surface. At the same time, the resistance of the sensitive layer of the DNA chip substantially depends on the concentration of ss-DNA targets in solution, which allows the use of photocurrent measurements to estimate their concentration. With that, at low concentrations of ligands the resistance of the sensitive layer is lower in the ligand-free case (see Fig. 2,b).

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ԴՆԹ-ի ԿՈՄՊԼԵԶՍԵՐԻ ՔՎԱՆՏԱՑԻՆ ԿԵՏԵՐԻ ՆԵՏ ՄՈՆՈԼԵՅԵՐՆԵՐԻ
ՆԱԴՈՐԴՈՒՆԱԿՈՒԹՅՈՒՆԸ ՓՈԽԿԱՊԱԿՅՎՈՂ ԼԻԳԱՆԴՆԵՐԻ
ՆԵՐԿԱՅՈՒԹՅԱՐԲ

Նոդվածում հետազոտվել են ԴՆԹ-ի ոչ մրցակցային հիբրիդացումը և հաղորդունակությունը ԴՆԹ չիպի զգայուն շերտում լիցկավորված լիգանդների առկայության պայմաններում, և համեմատություն է արվել առանց լիգանդների դեպքի հետ: Ցույց է արվել, որ փոխկապակցվող լիգանդները մեծացնում են ԴՆԹ չիպերի զգայունությունը լիգանդների բավականաչափ բաժր կոնցենտրացիայի դեպքում՝ համեմատաբար այն դեպքի հետ, երբ լիգանդներ չկան: