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P h y s i c s

EFFECT OF LIGAND BINDING ON FUNCTIONALITY OF DNA FIELD-EFFECT TRANSISTOR

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Motivated by the prospects of developing DNA field-effect transistors, as tools for a variety of application fields such as medical diagnostics, environmental pollutants monitoring, biological weapons defense, and taking into account that the efficiency of DNA-sensors depends on the precise prediction of experimental parameters responsible for thermostability of nucleic acids duplexes and specific times of formation of DNA duplexes, we analyze the factors influencing both the thermodynamics of hybridization and the stability of DNA–DNA and DNA–RNA duplexes. In this work the case of competition-free DNA hybridization is analyzed. It is shown how the intercalating ligands effectively increase the binding constant for the target sequences and thus affects the sensitivity of the DNA-chip.

***Keywords*:** ligand binding, thermostability, hybridization, DNA-chip.

Introduction. A DNA field-effect transistor (DNAFET) is a transistor, which uses the field-effect due to the partial charges of DNA molecules to function as a DNA sensor. The gate structure of DNAFET is realized as a layer of immobilized single-stranded (ss) DNA molecules which act as surface receptors. When complementary DNA strands hybridize to the receptors, the surface charge changes, which in turn modulates current transport through the semiconductor transducer. DNAFETs are one of the promising tools with very diverse areas of application such as medical diagnostics, environmental pollutants monitoring, biological weapons defense etc. [1, 2]. One of the important directions of DNAFETs improvement is the increasing selectivity and sensitivity in expense of enhancement of electric signal and target probe hybridization stability [3]. Some following factors influence on the thermodynamics of hybridization, in particular: the density of ss-DNA assays (the length 25–49 nucleotides) immobilized on the surface; the presence of competing hybridization; and some other factors. The better understanding of physical chemistry behind the DNA and RNA hybridization on the surface of electric transducer is relevant for the improvement of effectiveness and manufacturing of DNA sensors [4]. DNA duplex stability strictly depends on the solvent conditions, including pH, ionic strength, low-molecular compounds (ligands), interacting with nucleic acids and other co-solutes.

The increase of selectivity and sensitivity of DNAFET and other DNA-sensors can be reached by using electro-chemically active compounds with higher affinity to the double-stranded (ds) DNA than to the ss-DNA. This kind of compounds can substantially increase the

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ds-DNA stability and, at the same time, the amplitude of generated signal, which increases the DNA-sensor sensitivity. Among this kind of ligands are intercalators, molecules with flat heterocyclic structure, which fit between nucleic acids and change the local structure of ds-DNA [5–8].

Thermodynamics and kinetics of hybridization both in the bulk [8] and on the surface [4, 9–17] has been extensively studied in recent years. The spectrum of considered problems includes e.g. kinetics of hybridization on surface [13, 15] the effects of salt on the DNA hybridization in the bulk, the isotherms of hybridization on surface [4] etc. At the same time, the DNA-ligand interactions also has been addressed in the huge number of papers devoted to intercalating [5–7] and minor groove binding [18, 19] ligands, their cross-docking [20] etc. However, to the best of our knowledge, the effect of DNA-ligand binding on the hybridization thermodynamics and kinetics has never been addressed before. In the context of development of DNA-biosensors, theoretical analysis of the effect of intercalating ligands on DNA hybridization on surface becomes necessary. The paper is focused on the isotherm of hybridization of DNA on the surface in presence of ligands, binding with ds-regions of DNA. In practice, the DNA chips are immersed in the target solution for a relatively short time and kinetic of hybridization plays a crucial role. However, understanding of the equilibrium state is also necessary to identify the relative importance of kinetic and thermodynamic controls of the performance of DNA chips.

The Competition-Free Hybridization: A. Free Energy. Let us compare the equilibrium hybridization isotherms for the idealized, but experimentally accessible situation, where DNA chip immersed in solution containing intercalating ligands and only one type of ss-DNA target (see Fig. 1). We consider the length N of probes and targets to be equal. Let us consider the spot of N_0 single-stranded probes p, wherein the N_{pt} of them are hybridized with target t. The hybridization of p and t creates a ds-oligonucleotide pt, at the surface. In simplest case of single species of ss-DNA target, surface will be covered only by free probes p and hybridized ones pt. In this case the only reaction is

$$p + t \leftrightharpoons p \tag{1}$$

and no competitive hybridization reactions occur (Fig. 1).

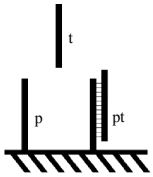

Fig. 1. Scheme of non-competing surface hybridization.

The dependence of the hybridization degree, $x = N_{pt}/N_0$, on the concentration of the target, c_t, is described by hybridization isotherm. For the intercalating ligands ℓ the binding reactions are written

$$pt + \ell \leftrightharpoons pt_1,\ pt_1 + \ell \leftrightharpoons pt_2, \ldots,\ pt_{N-1} + \ell \leftrightharpoons pt_N, \tag{2}$$

where pt is the free duplex, while pt_j is the target-probe duplex bound to i ligands ℓ. Without ligands the free energy of the probe layer is written as [12]

$$G = G_0 + N_{pt}\mu^0_{pt} + (N_0 - N_{pt})\mu^0_p + N_0\Sigma\gamma_{el} + k_BT\left[N_{pt}\ln\frac{N_{pt}}{N_0} + (N_0 - N_{pt})\ln\frac{N_0 - N_{pt}}{N_0}\right], \tag{3}$$

where Σ is the area per probe; G_0 is the free energy density of the bare surface; μ^0_{pt} and μ^0_p are the chemical potentials of the pt and p probes in a reference state and γ_{el} is the electrostatic free energy density of the probe layer. If intercalation is the only

mechanism of ligands binding, DNA–ligand complex formation will be restricted only by ds-regions and the free energy of probe layer yields

$$G_L = G + N_{pt}\left\{\mu_b^0 + k_BT\left[m\ln\frac{m}{N} + (N-m)\ln\frac{N-m}{N}\right]\right\}, \tag{4}$$

where m is the number of bound ligands per hybridized probe pt and μ_b^0 is the chemical potential of the bound ligand in a reference state. It is supposed that the available number of binding sites on the pt duplex is coincides to the length N. Thus, the free energy of probe layer is written as a function of independent variables N_{pt}, the number of hybridized probes and $N_b = mN_{pt}$, the number of bound ligands as

$$G_L(N_{pt}, N_b) = G(N_{pt}) + \mu_b^0 N_b + k_BT\left[N_b\ln\frac{N_b}{NN_{pt}} + (NN_{pt} - N_b)\ln\frac{NN_{pt} - N_b}{NN_{pt}}\right]. \tag{5}$$

Isotherms of Absorption and Hybridization. The equilibrium state for the reactions (1) and (2) are determined by conditions:

$$\mu_{pt} = \mu_p + \mu_t, \qquad \mu_\ell = \mu_b, \tag{6}$$

where μ_{pt} is the chemical potential of hybridized probe pt; μ_t is the chemical potential of target; μ_p is the chemical potential of probe; μ_b and μ_ℓ are the chemical potentials of the bound and free ligand, correspondingly [21].

The exchange chemical potential of the hybridized probe, $\Delta\mu_{pt} = \mu_p - \mu_t$ is written:

$$\Delta\mu_{pt} = \frac{\partial G_L}{\partial N_{pt}} = \Delta\mu_{pt}^0 + N\frac{\partial\gamma_{el}}{\partial\sigma} + k_BT\ln\frac{x}{1-x} + k_BTN\ln(1-r), \tag{7}$$

where $r = \dfrac{N_b}{NN_{pt}}$ is the degree of the ligand ℓ adsorption on the ds-DNA. The electrostatic free energy density γ_{el} is considered as a function on the number charge density of the surface σ. At the same time, the chemical potential of the bound ligand yields [22]

$$\mu_b = \partial G_L/\partial N_b = \mu_b^0 - k_BT\ln(1-r). \tag{8}$$

In the approximation of weak solution, the chemical potential of the target in the bulk is written

$$\mu_t = \mu_t^0 + k_BT\ln c_t \tag{9}$$

and the chemical potential of the ligand in the bulk yields

$$\mu_\ell = \mu_\ell^0 + k_BT\ln c_\ell, \tag{10}$$

where c_t and c_ℓ are the bulk concentrations of targets and ligands correspondingly. Taking into account Eqs. (6)–(10), the isotherm of hybridization is written as:

$$\frac{x(1-r)^N}{c_t(1-x)} = K_t\exp\left(-\frac{N}{k_bT}\cdot\frac{\partial\gamma_{el}}{\partial\sigma}\right), \tag{11}$$

where $K_t = \exp\left(-\dfrac{\Delta G^0}{k_bT}\right)$ and $\Delta G^0 = \mu_{pt}^0 - \mu_t^0$. The equilibrium distribution of ℓ between bound and free states is described by the isotherm of adsorption

$$r/c_\ell(1-r) = K_\ell = e^{\left(-\Delta g^0/k_bT\right)}, \tag{12}$$

where $\Delta g^0 = \mu_b - \mu_\ell^0$.

Results and Discussion. The hybridization isotherm for targets in the ligand-free case $x_0(c_t)$ [13] is reproduced by setting $r = 0$ in the Eq. (11). To compare the hybridization degree with the ligand-free case let us consider the shift of the hybridization isotherm, caused by ligands, $\delta x = x - x_0$. The equilibrium degree of adsorption r^* is written

$$r^* = c_\ell K_\ell/(c_\ell K_\ell + 1). \tag{13}$$

Thus, the effect of the intercalating ligand adsorption is reduced to the renormalization of the binding constant K_t such as

$$\tilde{K}_t = K_t \exp\left(N \ln(1 - r^*)\right). \tag{14}$$

The electrostatic free energy density of the probe layer γ_{el} was estimated in [12] in the two-component box approximation [22–25]. In this approximation, the assumption of a step-function monomer profile allows the polyelectrolyte brush to be treated as a continuum region of uniform, smeared-out charge density. In the high salt regime, the screening of the charged layer is dominated by the contribution of the salt and

$$\frac{\gamma_{el}}{k_B T} = 4\pi\sigma^2 \ell_B \frac{r_D^2}{H}, \tag{15}$$

where $\ell_B = \dfrac{e^2}{\varepsilon k_B T}$ is the Bjerrum length; ε is the dielectric constant; r_D is the Debye screening length; H is the thickness of the layer, where the charges are uniformly smeared. Since each chain carries a charge of $-eN$, the number charge density σ depends on the degree of hybridization x as

$$\sigma = \sigma_0(1 + x). \tag{16}$$

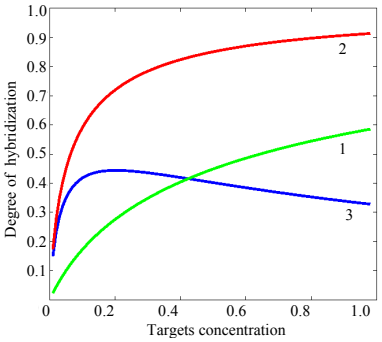

Fig. 2. Isotherm of hybridization in:
1. Ligand-free case;
2. Presence of ligands;
3. Shift of hybridization isotherm δx.

Taking into account Eqs. (11), (13),(14) and (16), the isotherms of hybridization were obtained, which are presented in the Fig. 2.

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