Journal of Physical Chemistry & Biophysics

Butusov et al., J Phys Chem Biophys 2015, 5:6
DOI: 10.4172/2161-0398.1000189

Research Article Open Access

# Interaction of DNA with Globular Proteins of Different Structures in Thin Films on Substrates of Monocrystalline Silicon

**Butusov LA[1*], Nagovitsyn IA[2,3], Kurilkin VV[1] and Chudinova GK[2,4]**

[1]*Peoples' Friendship University of Russia, Russia*
[2]*Natural Science Center of General Physics Institute RAS, Russia*
[3]*NN Semenov Institute of Chemical Physics RAS, Russia*
[4]*National Research Nuclear University MEPhI (Moscow Engineering Physics Institute), Russia*

**Abstract**

The two-component films of mixtures of DNA (from calf thymus) with different proteins: rabbit immunoglobulin (IgG), methemoglobin (MHB) and human serum albumin (HSA) were studied on single-crystal silicon substrates by the method of fluorescence spectroscopy. Registration of fluorescence spectra was performed by λex=260 and 280 nm in the range 340-380 nm. To prepare films the spincoating method was used. Solutions deposited on the substrate contained the small concentrations of proteins $10^{-9}$ - $10^{-15}$ M at the same quantity of DNA.

Shape dependencies of the fluorescence intensity versus concentration differ markedly for each of the used proteins. The decreasing concentration of protein in the film is accompanied by a significant increase of the integrated fluorescence intensity (in comparison with the concentration of $10^{-9}$ M) for films of DNA-HSA in 2.5 and 5 times ($10^{-13}$ and $10^{-15}$ M HSA), for films of DNA-IgG in 4.6 and 15.9 times ($10^{-11}$ and $10^{-13}$ M IgG), for films of DNA-MHB in 3.4 times ($10^{-11}$ M MHB). The single-component films of proteins was studied as control samples whose properties noticeably differ from the properties of DNA-protein systems. The specificity of the fluorescent characteristics of DNA-protein films for proteins of different structure and their different concentrations could be used as the basis for developing biosensor systems.

**Keywords:** DNA; Photoluminescence; Immunoglobulin; Methemoglobin; Albumin; Single-crystal silicon

## Introduction

The interaction of DNA with proteins is the most important fundamental problem and one of the main factors determining the specificity of the interaction is a three-dimensional structure of both compounds. The vast majority of modern researches in this area are aimed at studying the mechanisms of specific recognition of various natural and synthetic regulatory model proteins of individual nucleotides and their sequences [1].

At the same time the directions of artificial, not found in nature, supramolecular systems, including nanoscale, on the basis of DNA are developed [2,3]. The last direction is important as from the point of view of clarifying the fundamental aspects of the interaction of biomacromolecules [4], and also for medical applications [5].

It should be noted that the design of systems of molecular-scale with programmable physico-chemical and structural properties is a key task of the modern applied science [6]. Quality advantages of the DNA-systems can be considered relatively easy projecting of predictability and geometry, while, unfortunately, only in relation to objects of small size, for example, DNA nanostructures.

It is possible the creation of materials as a result of self-organization (self-assembly) that do not lose their working properties at the scale changes, where the preparation conditions define the properties and potentials for practical applications, [7-14].

The possibility of structure-function complication of the currently available DNA-structures, the programmability of self-assembly processes, the sensitivity of the DNA structures for the processes of molecular recognition allows effective use of them in fundamental and applied researches as effective optical and structural probes and also as biochemical transport system [15-17].

This article considers the interaction of DNA and physiologically important proteins (hemoglobin, serum albumin and immunoglobulin) in thin films on substrates of monocrystalline silicon. The aim of this work was to study changes in fluorescence systems "DNA-protein" and to study the possibilities of applying DNA-protein interaction for its use with small quantities of protein.

Silicon in its various forms (crystalline, polycrystalline, amorphous) is frequently used not only in microelectronics, etc., but also, especially in recent years, for the study of physico-chemical processes involving biological macromolecules. Such properties of the silicon substrates as homogeneity and a weak influence on photoluminescence of organic systems allows effectively to use them for studies of luminescent characteristics of thin films of biological macromolecules, including for the creation of biosensors [18-22].

In the present work we study the characteristics of DNA films on substrates of monocrystalline silicon with the addition of hemoglobin, serum albumin and immunoglobulin to show the possibility to detect

***Corresponding author:** Leonid Butusov, Peoples' Friendship University of Russia, Russian Federation, Tel: +79851694226; E-mail: leonid.butusov@ya.ru

**Received** October 20, 2015; **Accepted** November 05, 2015; **Published** November 10, 2015

**Citation:** Butusov LA, Nagovitsyn IA, Kurilkin VV, Chudinova GK (2015) Interaction of DNA with Globular Proteins of Different Structures in Thin Films on Substrates of Monocrystalline Silicon. J Phys Chem Biophys 5: 189. doi:10.4172/2161-0398.1000189

changes caused by the nature and concentration of added protein by the method of fluorescent spectroscopy.

## Materials and Methods

Used materials were obtained from Sigma-Aldrich Co.: DNA from calf thymus, rabbit IgG, human serum albumin (HSA), human hemoglobin (MHB). The DNA suspension was exposed by ultrasound in a Branson 1510 set for ultrasonic treatment (42 kHz) of 40 minutes in a 0.1 M NaCl solution. The DNA solution (0.17 mg/ml) was mixed with protein ones of various concentrations in 0.1 M NaCl at a ratio of 9:1, so that the concentration of proteins in solutions for application to substrates ranged from $10^{-9}$ to $10^{-15}$ M.

Thin films were obtained by the spincoating method on substrates of monocrystalline silicon (18 × 18 mm) using the set created in the laboratory on the basis of centrifuges "Elekon" CLMN-P10-02 (Russia) [23]; the rotation speed of substrate was 2000 rpm, the volume of deposited solution - 20 μl.

The samples of the silicon with surface orientation (100), thickness of 380 ± 20 μm, the roughness of the working surface ≤ 0.06 were used.

The fluorescence of the samples was measured on a spectrofluorometer RF-5300pc (Shimadzu). Registration of fluorescence intensity was performed with an interval of 0.2 nm with slits of excitation and registration of 3 and 5 nm, respectively. The software Origin 6.0 for data processing was used. For the calculation of the maxima of the fluorescent bands used spectra, processed using the "Adjacent Averaging" method smoothing of the curves (number of pixels for averaging is 20). The integral intensity of the fluorescence was calculated as the area under the curve the fluorescence intensity versus the wavelength in the wavelength region of 340-380 nm.

The use of integrated intensity it is convenient to unify the results, including the use different devices with different optical characteristics.

## Results

The fluorescence of mixtures of DNA with proteins films was investigated on substrates of monocrystalline silicon. Samples of single-crystal silicon absorb in the ultraviolet region of the spectrum [24,25], including in the area of the maxima of the absorption bands of DNA and proteins, however, possess its own luminescence in the infrared region with energies ≈ of 1.1 eV with a low quantum yield of the order of 10-4 % at room temperature [26].

Observed luminescence of samples of single crystal silicon without the addition of DNA or protein (Figure 1, spectrum 1) is likely determined by the luminescence of the oxide film on the surface of a single crystal sample associated with radiation of single and aggregate color centers (F-centers) in the oxide matrix [27,28]; thus, when the excitation light wavelength of 260 nm (the absorption band of DNA), the maximum band fluorescence of the substrate was observed at 364 nm (Figure 1, spectrum 1). Shoulders in areas of 355-360 and 370-375 nm indicate, apparently, the different size of clusters in $SiO_2$ oxide film.

When applied to the substrate single-component films of DNA (0.17 mg/ml), the change of the maximum position of the fluorescence band only slightly, while the intensity of the fluorescence increased almost 4 times (Figure 1, spectrum 2).

In the used samples of single-crystal silicon its own fluorescence with maxima at 688 and 722 nm was detected. There is a model explaining the origin of the photoluminescence by properties of the boundary of Si–SiOx saturated with defects [29,30]. On the samples of porous silicon it was shown that the position of the photoluminescence bands can significantly (1,75-2 eV) change with aging of the samples [31]. In this article we don't consider the influence of biomacromolecules on the fluorescence of substrates in the visible range.

DNA has its own fluorescence in solutions with a maximum at 358 nm [32-34]. Thus, the resulting increase in the fluorescence intensity when DNA applied (Figure 1) due to apparently the presence of DNA, although we cannot exclude the possibility of increased ultraviolet fluorescence of the substrate oxide film under the action of DNA, as was shown for substrates of transition metals oxides in the $SiO_2$ matrix [35,36].

Excitation to the absorption band of the protein (λex=280 nm) does not change significantly the shape of the spectrum and the silicon substrate, and a single-component film of DNA on it, leading only to the decrease of the fluorescence intensity in both cases as compared with the intensity observed when excited by light with a wavelength of 260 nm (spectra not shown).

In Figure 2 the fluorescence spectra of two-component films of HSA - DNA on a silicon substrate with an excitation wavelength of 260 nm were presented. The dependence of the fluorescence intensity on the concentration of HSA is non-linear. The integral intensity (area under the curve of the spectrum in the region of 340-380 nm) is 45.4, 38.7, 113.8, 234 a.u. for concentrations of HSA - $10^{-9}$, $10^{-11}$, $10^{-13}$, $10^{-15}$ M, respectively. Thus, with reducing the concentration of HSA in the film we has seen an appreciable enhancement of fluorescence in 2.5-5 times.

It should be noted that the addition of protein and change in its concentration does not significantly shift the maximum of the fluorescence band, so when λex=260 nm, the maxima of the bands observed in region 363, 360, 362, 361 nm, and when λex=280 nm region 362, 359, 361, 360 nm for concentrations of HSA $10^{-9}$, $10^{-11}$, $10^{-13}$, $10^{-15}$ M, respectively.

When excited by light with a wavelength of 280 nm (absorption band of the protein, spectra not shown) integrated fluorescence intensity is higher than in λex=260 nm at 13-19 %, which is obviously due to a significantly lower quantum yield of fluorescence of nucleic acid bases in comparison with tryptophan [37]. The shape of the spectra and the dependence of intensity on concentration of HSA for λex=280 nm are similar to those in Figure 2. Enhancing of the integrated intensity of fluorescence at concentrations of HSA $10^{-13}$ and $10^{-15}$ M is 2.5 and 5.1 times. The research results of influence of the MHB and IgG are shown in Table 1.

In the spectra of two-component films with addition of IgG and MHB, the shoulders are more pronounced in the area of about 370-375 nm compared to films of DNA- HSA. In the case of adding IgG ($10^{-9}$ M) and MHB ($10^{-15}$ M) we observed the peak occurrence, and in the case of the MHB, its intensity was comparable to the intensity of the peaks in the region 356-360 nm (Figure 3).

Figure 4 shows the change in the relative integrated fluorescence intensity ($S/S_0$) when we excited by light with a wavelength of 280 nm for films of DNA-HSA, DNA–MHB, and DNA-IgG. When λex=260 nm the shape of the dependencies remains. The most interesting cases of HSA and IgG; the first is a monotonous dependence, the second is a significant increase of fluorescence (almost in 16 times), that makes them promising for development of sensitive biosensor systems.

The fluorescence intensity of single-component films of proteins differ from one of the mixture films, and the difference depends on the nature of protein and its concentration (Table 2). The position of the

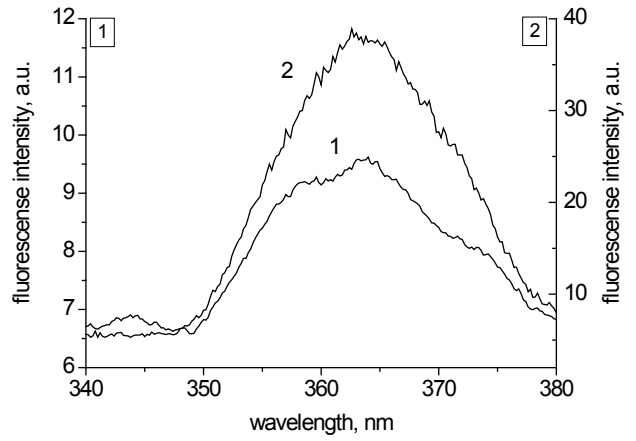


**Figure 1:** Fluorescence spectra (λex=260 nm) of a monocrystalline silicon substrate (1) and DNA films on monocrystalline silicon (2).

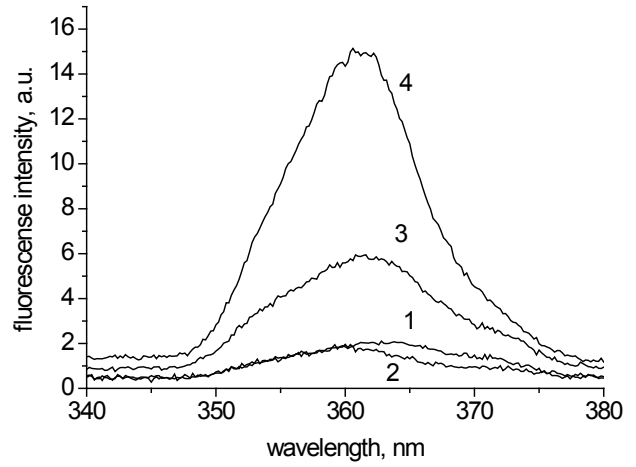


**Figure 2:** The fluorescence spectra of two-component films of DNA –NAV when λ=260 nm. Concentrations of HSA (mol/l): 1 – $10^{-9}$, 2 – $10^{-11}$, 3 – $10^{-13}$, 4 – $10^{-15}$.

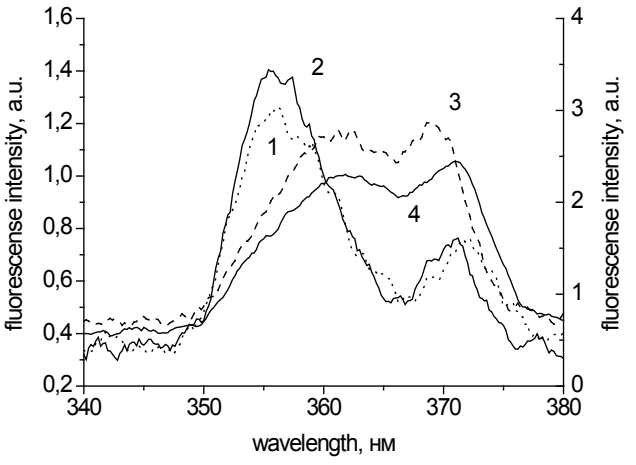


**Figure 3:** The fluorescence spectra of two-component films of DNA with the addition of IgG $10^{-9}$ M (1,2; the left y-axis) and the MHB $10^{-15}$ M (3,4; right ordinate). 1,4 - λex=260 nm; 2,3 - λex=280 nm.

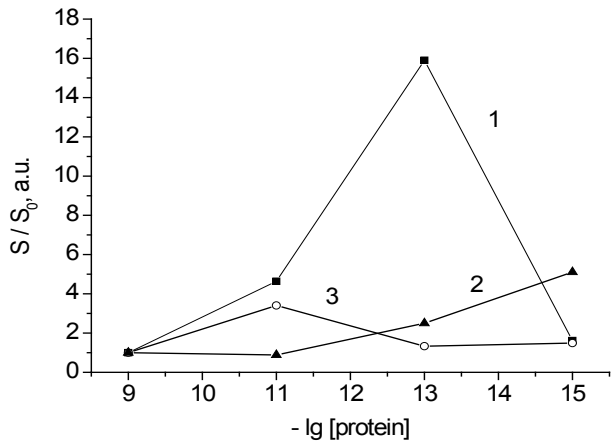


**Figure 4:** The relative integrated fluorescence intensity (S/S0) while adding protein to the DNA film, λex=280 nm. S – integral intensity of the fluorescence, S0 is the integrated intensity of fluorescence protein at a concentration of $10^{-9}$ M. 1 – IgG (-■-), 2 – HSA (-▲-) ,3 – MHB (-○-).

band maxima of the protein films are given in Table 3.

In Figure 5 the change in the relative integrated fluorescence intensity ($S/S_0$) are shown by light excitation with a wavelength of 280 nm for single-component films of HSA, MHB and IgG. Comparison of the data Figure 4 and Figure 5 shows that the interaction of DNA with MHB slightly changes the behavior of the system, as indicated by the similar shape of the dependency of the intensity on concentration, and similar values of the intensity for films of DNA-MHB and the MHB. However, the interaction of DNA with IgG and HSA leads to qualitative changes, significantly enhancing the fluorescence of the system at extremely low concentrations of protein.

Since in the present supramolecular systems a specific intermolecular binding (like, for example, by the reaction antigen-antibody or complementary nucleotides binding) are absent, apparently, the tertiary structure of protein is a major factor of influence on the general structure of the films and, consequently, on their fluorescence.

The proteins, selected for study, vary in size, structure and physiological functions. Albumin is a transport protein of molecular weight 69 kDa has a size 8 × 6 × 3 nm [38], and it is the main transport protein of blood plasma. Immunoglobulin – one of the most important proteins of the humoral immune system with a molecular weight of 150 kDa, it has an elongated ellipsoid with an area of up to 75 $nm^2$ and a height of 15 nm [39], it works in blood plasma and in cell membranes.

Hemoglobin is located in erythrocytes and is an allosteric enzyme with a molecular mass of 67 kDa and dimensions 5 × 5 × 7.1 nm [40], has the four prosthetic groups of heme (a complex of $Fe^{2+}$ and protoporphyrin IX) in its structure. All the studied proteins have important physiological functions, and their qualitative and quantitative definition of registration in medical laboratory analysis, and study of their physico-chemical characteristics in multi-component biological model systems because this is an important problem.

In the literature there is a wide range of works devoted to the interaction of DNA with various biological macromolecules. In ref. [41] authors describe the interactions of different amino acids with DNA, and their specificity is noted.

The interaction of DNA with antibodies in autoimmune process

| The concentration of protein in solution for making films, mol/l | DNA-IgG | | | | DNA-MHB | | | |
|---|---|---|---|---|---|---|---|---|
| | The position of the band maxima of fluorescence, nm | | Integrated fluorescence intensity, S, 340-380 nm, arb. units | | The position of the band maxima of fluorescence, nm | | Integrated fluorescence intensity, S, 340-380 nm, arb. units | |
| | The excitation wavelength, nm | | | | | | | |
| | 260 | 280 | 260 | 280 | 260 | 280 | 260 | 280 |
| $10^{-9}$ | 356; 372 | 356; 370 | 26,1 | 26,4 | 370 | 369 | 40,0 | 43,7 |
| $10^{-11}$ | 358 | 358 | 110,7 | 122,5 | 357 | 357 | 134,9 | 148,9 |
| $10^{-13}$ | 359 | 358 | 374,7 | 419,8 | 358 | 357 | 52,5 | 58,3 |
| $10^{-15}$ | 361 | 369 | 36,2 | 42,6 | 362; 370 | 361; 369 | 58,2 | 65,1 |

**Table 1:** Properties off DNA films with the addition of immunoglobulin and methemoglobin.

| Protein concentration in the initial solution, mol/l | Integrated fluorescence intensity, S, 340-380 nm, arb. units | | | | | |
|---|---|---|---|---|---|---|
| | IgG | | MHB | | HSA | |
| | The excitation wavelength, nm | | | | | |
| | 260 | 280 | 260 | 280 | 260 | 280 |
| $10^{-9}$ | 34,5 | 35 | 99,5 | 108,5 | 678,8 | 754,9 |
| $10^{-11}$ | 179,1 | 203,7 | 90,7 | 100,2 | 60 | 69 |
| $10^{-13}$ | 32,5 | 35,2 | 88,1 | 96,8 | 35 | 40,7 |
| $10^{-15}$ | 93,6 | 106,1 | 126 | 143,6 | 29,6 | 33 |

**Table 2:** The position of the fluorescence band maxima of single-component protein films.

| Protein concentration in the initial solution, mol/l | The position of the band maxima of fluorescence, nm | | | | | |
|---|---|---|---|---|---|---|
| | IgG | | MHB | | HSA | |
| | The excitation wavelength, nm | | | | | |
| | 260 | 280 | 260 | 280 | 260 | 280 |
| $10^{-9}$ | 362; 371 | 361; 369 | 361 | 360 | 360 | 359 |
| $10^{-11}$ | 362 | 361 | 358 | 357 | 362 | 361 |
| $10^{-13}$ | 364 | 362 | 363 | 362 | 360 | 359 |
| $10^{-15}$ | 361 | 361 | 363 | 362 | 359 | 360 |

**Table 3:** The position of the fluorescence band maxima of single-component protein films.

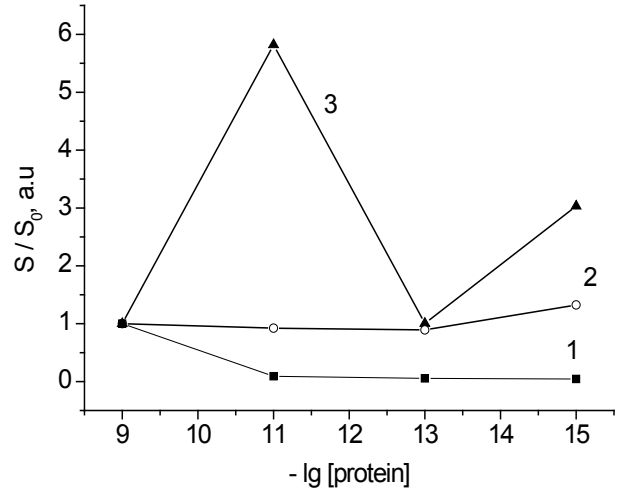


**Figure 5:** The relative integrated fluorescence intensity (S/S0) of the single-component protein films, λex=280 nm. S – integral intensity of the fluorescence, S0 is the integrated intensity of fluorescence protein at a concentration of $10^{-9}$ M. 1 – IgG (-■-), 2 – HSA (-▲-) ,3 – MHB (-○-).

was observed in ref. [42]. The formed complexes consist of DNA-protein do not contain covalent bonds. Interaction DNA with protein is carried out by means of hydrogen bonds directly with DNA or through interaction with water, ionic bonds, such as formation of salt bridges, or interact directly with the DNA frame, and van der Waals interactions, including hydrophobic interactions. The study of interactions in such systems opens prospects for creation of new materials for biosensors, pharmacology [24].

## Conclusions

The obtained results are important for the study of physico-chemical aspects of interaction of components in biomacromolecular complex systems in vitro, and possibly, for the development of biosensors for identification of these proteins and registration their quantity in model and physiological environ.

### References

1. Rohs R, Jin X, West SM, Joshi R, Honig B, et al. (2010) Origins of specificity in protein-DNA recognition. Annu Rev Biochem 79: 233-269.
2. Pippig DA, Baumann F, Strackharn M, Aschenbrenner D, Gaub HE (2014) Protein-DNA chimeras for nano assembly. ACS Nano 8: 6551-6555.
3. Kuniharu I, Ringsdorf H (1998) Protein-DNA Double and Triple Layers: Interaction of Biotinylated DNAFragments with Solid Supported Streptavidin Layers. Langmuir 14: 2796–2800.
4. Morán MC, Jorge AF, Vinardell MP (2014) Sustainable DNA release from chitosan/protein based-DNA gel particles. Biomacromolecules 15: 3953-3964.
5. Oupicky D, Konak C, Dash P, Seymour L, Ulbrich K (1999) Effect of albumin and polyanion on the structure of dna complexes with polycation containing hydrophilic nonionic block. Bioconjugate Chem 10: 764–772.
6. Andersen ES, Dong M, Nielsen MM, Jahn K, Subramani R, et al. (2009) Self-assembly of a nanoscale DNA box with a controllable lid. Nature 459: 73-76.
7. Amin R, Kim S, Park S H, LaBean T (2009) Artificially Designed DNA Nanostructures. NANO: Brief Reports and Reviews 4: 119–139.
8. Adar R, Benenson Y, Linshiz G, Rosner A, Tishby N, et al. (2004) Stochastic computing with biomolecular automata. Proc Natl Acad Sci U S A 101: 9960-9965.

9. Adleman L (1998) Computing with DNA. Scientific American 279: 54–61.
10. Barish RD, Rothemund PW, Winfree E (2005) Two computational primitives for algorithmic self-assembly: copying and counting. Nano Lett 5: 2586-2592.
11. Bath J, Turberfield AJ (2007) DNA nanomachines. Nat Nanotechnol 2: 275-284.
12. Benenson Y, Gil B, Ben-Dor U, Adar R, Shapiro E (2004) An autonomous molecular computer for logical control of gene expression. Nature 429: 423-429.
13. Chen HL, Goel A (2004) Error Free Self-assembly Using Error Prone Tiles. DNA Computing 62–75.
14. Chen HL, Schulman R, Goel A, Winfree E (2007) Reducing facet nucleation during algorithmic self-assembly. Nano Lett 7: 2913-2919.
15. Majumder U, LaBean T, Reif J (2007) Activatable Tiles: Compact, Robust Programmable Assembly and Other Applications. DNA Computing 4848: 15-25.
16. Tian Y, He Y, Mao C (2006) Cascade signal amplification for DNA detection. Chembiochem 7: 1862-1864.
17. Seeman NC (2010) Nanomaterials based on DNA. Annu Rev Biochem 79: 65-87.
18. Green MA, Keevers MJ (1995) Optical properties of intrinsic silicon at 300 K. Progress in Photovoltaics: Research and Applications 3: 189-192.
19. Green MA (2008) Self-consistent optical parameters of intrinsic silicon at 300 K including temperature coefficients. Solar Energy Materials and Solar Cells [web] 92: 1305–1310.
20. Batalov RI (2004) The structure and optical properties of semiconductor thin-film silicon-based compounds synthesized by pulsing energy actions. PhD thesis, Kazan, Russia.
21. Evans BD, Pogatshnik GJ, Chen Y (1994) Optical properties of lattice defects in a-Al2O3. Nuclear Instruments and Methods in Physics Research B 91: 258–262.
22. Kostyuk AB, Belov AI, Larks IY, et al. (2010) Light-emitting ion-synthesized structures based on silicon nanocrystals in oxide matrices. Bulletin of the NI Lobachevsky Nizhny Novgorod University 5: 264-270.
23. Hajiyev TT, Nagovicyn IA, Chudinova GK (2012) Photovoltaic and optical properties of the composite films 5,10,15,20-tetraphenylporphyrin and grapheme. Applied Physics 6: 29-33.
24. Wilson KA, Kellie JL, Wetmore SD (2014) DNA-protein Π-interactions in nature: abundance, structure, composition and strength of contacts between aromatic amino acids and DNA nucleobases or deoxyribose sugar. Nucleic Acids Res 42: 6726-6741.
25. Malhotra S, Sowdhamini R (2012) Re-visiting protein-centric two-tier classification of existing DNA-protein complexes. BMC Bioinformatics 13: 165.
26. Brand LH, Henneges C, Schüssler A, Kolukisaoglu HÜ, Koch G, et al. (2013) Screening for protein-DNA interactions by automatable DNA-protein interaction ELISA. PLoS One 8: e75177.
27. Siggers T, Gordân R (2014) Protein-DNA binding: complexities and multi-protein codes. Nucleic Acids Res 42: 2099-2111.
28. Strauch MA (2001) Protein–DNA Complexes: Specific. Encyclopedia of life sciences.
29. Bao XM, He X, Gao T, Yan F, Chen HL. (1999) Oxygen-related surface states and their role in photoluminescence from porous Si. Solid State Communications 109: 169-172.
30. Domashevskaya EP, Terekhov VA, Turishchev SY, Khoviv DA, Parinova EV, et al. (2007) Peculiarities of electron-energy structure of surface layers of porous silicon formed on p-type substrates. J Electron Spectrosc 48: 445–451.
31. Lenshin AS, Makarov VM, Turishchev SY, Smirnov MS, Domashevskaya EP (2012) The influence of natural aging on the photoluminescence of porous silicon. Technical Physics 82: 150-152.
32. Ivanov S, Kirpichenok LN (2008) Using fluorescence techniques in medicine. Medical News 11: 56-61.
33. Chernitskii EA, Slobozhanina EI (1989) Spectral luminescent analysis in medicine. Minsk: Science and Technology: 140
34. Paston SV, Tarasov AE (2011) The Effects of caffeine on DNA conformational changes y-irradiation in vitro. Structural Chemistry 52: 1246-1251.
35. Chudinova GK, Nagovitsyn IA, Hadzhiev TT,et al. (2014) Fluorescence of ZnO:SiO2 and SnO2:SiO2 Nanosized Composite Films under the Action of Human Serum Albumin. Physical Chemistry Reports 456: 74–76.
36. Fort E, Gresillon S (2008) Surface enhanced fluorescence. Journal of Physics D: Applied Physics 45: 1-5.
37. Umarov MF (2014) Optical spectroscopy of bioactive agents. Monograph, Vologda, Russia: 147.
38. Carter DC, Ho J (1994) Structure of serum albumin. Advances in Prot. Chem 45: 159-196.
39. Chudinova G, Savransky V, Prokhorov A (1997) The study of antigen-antibody reaction by fluorescence method in LB films for immunosensor. Thin Solid Films 307: 294-297.
40. Perutz M (1976) Structure of haemoglobin. Br Med Bull 32: 196-208.
41. Shtaller DV, Morozova IL, Zenkov MA, Vlasov VV (2006) Fundamentals of the specificity of the interaction of proteins and nucleic acids. Bulletin VOGiS 10: 285-297.
42. Gololobov GV (1992) Interaction of DNA + protein in autoimmune process. PhD Dissertation, Moscow, Russia.