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EFFECT OF DNA, RNA AND HSA ON THE SPECTRAL-LUMINESCENT PROPERTIES OF SEVERAL MONOMETHINE CYANINE DYES

Aim. *This work was aimed at investigation of the effect of DNA, RNA, and HSA on the absorption and fluorescence spectra of 8 monomethine cyanine dyes, which are based on different chromophores and contain various affinity-modifying groups.* ***Methods.*** *UV-vis absorption and fluorescence spectroscopy.* ***Results.*** *Several dyes were found to have good fluorescent responses to the presence of DNA, RNA, and HSA. The dye fb128, which contains a charged group, was shown to increase its fluorescence intensity in the presence of DNA and RNA by 160 and 240 times, respectively, while the dye up385 increased its fluorescence intensity by almost 60 times in the presence of human serum albumin.* ***Conclusions.*** *The most promising dye fb128, as well as the dyes fb123, fb124 and fb131, can be further investigated as fluorescent probes sensitive to nucleic acids in various specific applications (PCR, fluorescent microscopy etc.).*

Keywords: *monomethine cyanine dyes, fluorescent probes, nucleic acids, human serum albumin.*

Introduction

Detection and visualization of nucleic acids and proteins are essential for a range of biological and medical techniques for studying biological materials and organisms, such as gel electrophoresis, real-time polymerase chain reaction, fluorescence microscopy, *etc.* [1—8]. Development of new, more sensitive probes that would be efficient for certain applications is ongoing and is constantly receiving new boosts with the emergence of new research methods, as new applications often place new requirements on the properties of probes and their complexes with biological molecules. In ad-

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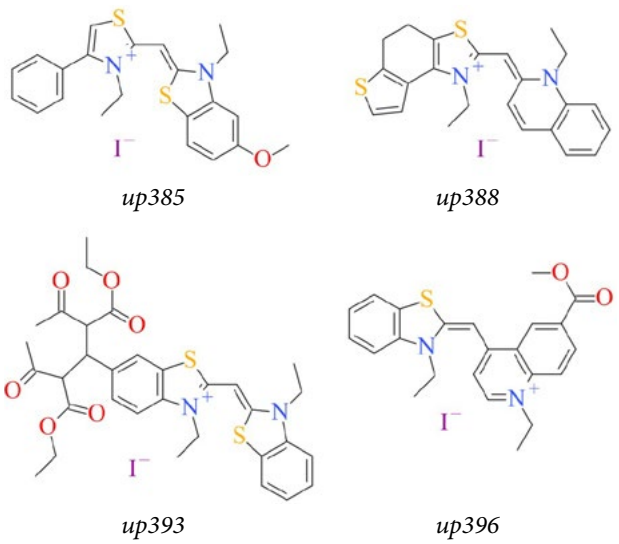


Fig. 1. Structures of monomethine cyanine dyes **up385**, **up388**, **up393** and **up396**

dition to standard requirements, such as high extinction coefficient, the highest possible fluorescence intensity in the presence of a biological molecule and the lowest possible intensity of emission in its absence, as well as high photostability [1, 5], additional ones may include the ability to form a complex with DNA at sufficiently high temperatures (for real-time PCR) [2], the ability or, conversely, the inability to penetrate the cell (for fluorescence microscopy of cells and detection of extracellular DNA, respectively) [9], *etc.* Thus, the search for new fluorescent probes is always relevant; and the first step in such a search is to study the effect of biological molecules on the spectral-luminescent properties of new dye molecules.

The main component of a fluorescent probe is a chromophore — a conjugated π-electron system that absorbs and emits light and interacts with a biological molecule. In addition to the chromophore, fluorescent probes often include additional groups (so-called affinity-modifying groups) that enhance the binding of the probe to the biological molecule [10,11]. Thus, the monomethine cyanine dye thiazole orange, for which an extremely high increase in fluorescence intensity in the presence of DNA was first shown in 1986 [12], became the basis for a number of probes, both through the introduction of affinity-modifying groups (SYBR Green I, SYBR Green II, SYBR Gold, *etc.*) [1], and by combining two identical chromophores using a flexible chain into a bis-cyanine dye (TOTO-1) [13].

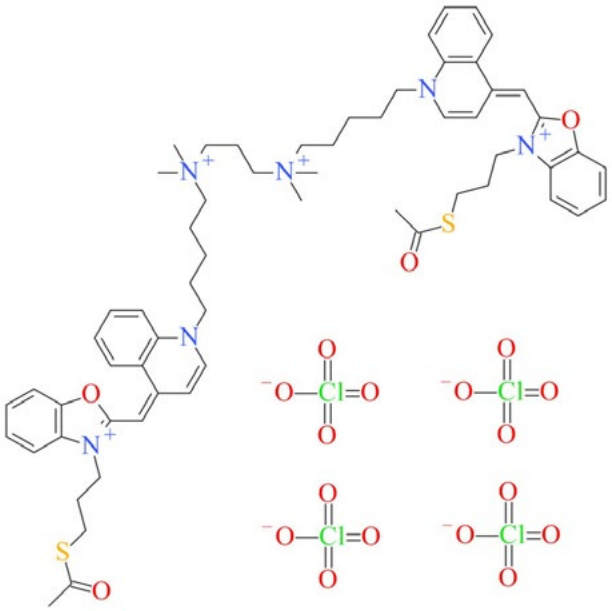
In this work, we investigated the effect of DNA, RNA, and HSA on the absorption and fluorescence spectra of 8 monomethine cyanine dyes, which are based on different chromophores and contain different affinity-modifying groups. It should be noted that 5 of these dyes contain chromophores of the well-known dyes thiazole orange and oxazole yellow [7], and two of them are bis-cyanine dyes. The result of this work is first step for further investigation of the efficiency of the most promising of the investigated dyes as fluorescent probes in various biological and medical methods.

Materials and Methods

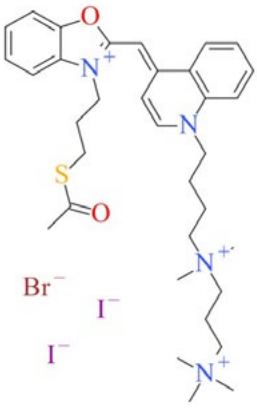
Monomethine cyanine dyes (Fig. 1—3) were kindly provided by Yu. L. Slominskii (Institute of Organic Chemistry of NAS of Ukraine) and Todor Deligeorgiev (Sofia University "St. Kliment Ohridski"). The structure of the studied compounds was confirmed by ^{1}H NMR and elemental analysis. Dimethyl sulfoxide (DMSO) and 0.05 M Tris-HCl buffer (pH 8.0) were used as solvents. Double-stranded DNA (dsDNA) from salmon testes, yeast total RNA and human serum albumin (HSA) were purchased from Sigma-Aldrich Co.

Stock solutions of the studied dyes were prepared in DMSO at a concentration of 2 mM. Stock solutions of DNA and RNA were prepared in 50 mM Tris-HCl buffer (pH 8.0) at a concentration of 6 mM base pairs (b.p.) and 12 mM bases (b.), respectively. Solution of HSA was prepared in 50 mM Tris-HCl buffer pH 8.0 at a concentration of 0.2 mg/ml (that corresponds to 3 μM).

Working solutions of free dyes were prepared by diluting the stock solution of the dye in 50 mM Tris-HCl buffer (pH 8.0) or DMSO. Working solutions of dyes in the presence of nucleic acids were



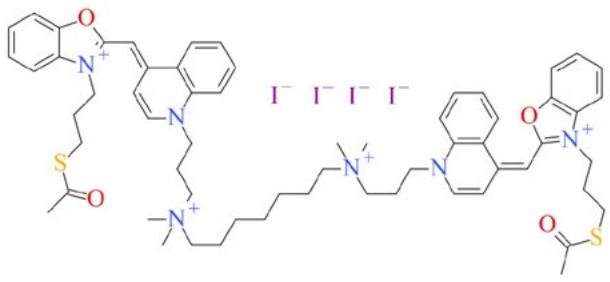
fb123



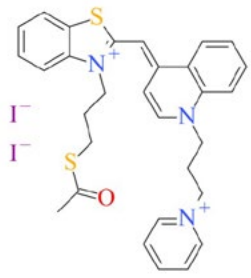
fb128

Fig. 2. Structures of monomethine cyanine dyes **fb123** and **fb128**

prepared by adding an aliquot of the dye stock solution and the nucleic acid stock solution to 50 mM Tris-HCl buffer (pH 8.0). Working solutions of dyes in the presence of HSA were prepared by adding an aliquot of the dye stock solution to HSA solution. In all cases, true solutions were obtained. The concentrations of the dyes, DNA, RNA and HSA in all working solutions were 5 μM, 60 μM b.p., 120 μM b. and 0.2 mg/ml respectively.



fb124



fb131

Fig. 3. Structures of monomethine cyanine dyes **fb124** and **fb131**

All spectral measurements were performed immediately after the preparation of corresponding working solutions. Absorption spectra were recorded on a Genesys 20 Visible Spectrophotometer (Thermo Fisher Scientific, USA). Fluorescence spectra were recorded using a Cary Eclipse fluorescence spectrophotometer (Varian, Australia). Measurements were performed in quartz cuvettes (10×10 mm) at room temperature.

Results and Discussion

Spectral-luminescent properties of free dyes

The characteristics of the absorption and fluorescence spectra of dyes in organic solvent (DMSO) and in aqueous medium (TRIS-HCl buffer, pH 8.0) are presented in Table 1. The spectra are presented in the Fig. 4, Fig. 5 and Fig. 6. The absorption spectra of all dyes in DMSO, which correspond to the monomeric form of these dyes,

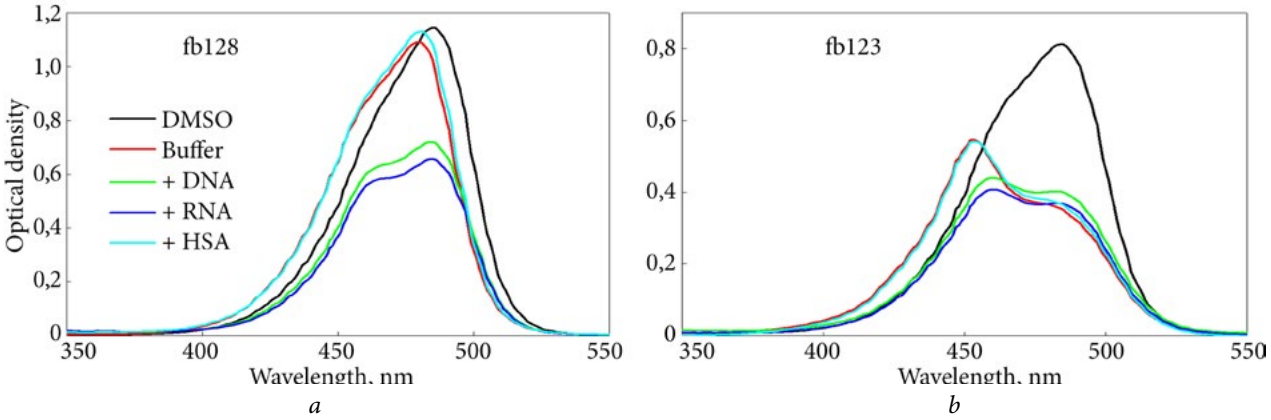


Fig. 4. Absorption spectra of the dyes **fb128** and **fb123** in DMSO, in buffer, and in the presence of DNA, RNA and HSA: *a* — The dye **fb128**; *b* — The dye **fb123**

have maxima in the range of 432—581 nm. For four of them (**up393**, **up396**, **fb128** and **fb131**), the absorption spectra in the buffer retain their monomeric shape, while the maximum shifts by 2—7 nm to the short-wavelength side compared to the corresponding spectra in DMSO (Fig. 4*a*). At the same time, for the remaining dyes (**fb123**, **fb124**, **up385** and **up388**), the shape of the absorption spectra changes significantly, with the position of the main maximum shifted to the short-wavelength region by 27—52 nm compared to the maximum in DMSO, and a less intense long-wavelength maximum is also present (Fig. 4*b*). This form of the absorption spectrum indicates the formation of dimers or H-aggregates by these dyes in buffer, which is a common phenomenon for cyanine dyes in aqueous solutions at given concentrations [14—16]. The mentioned long-

Table 1. **Spectral characteristics of monomethine cyanine dyes in DMSO and buffer**

Dyes	DMSO				0,05M TRIS-HCl buffer (pH 8.0)			
	λ_{abs}, nm	D	λ_{flu}, nm	I_0, a.u.	λ_{abs}, nm	D	λ_{flu}, nm	I_0, a.u.
up385	581	0,299	615	237	632 529	0,128 0,192	680	24
up388	508	0,192	—	—	481	0,158	—*	5
up393	432	0,541	476	35	425	0,55	476	18
up396	523	0,228	—	—	519	0,245	—*	2
fb123	484	0,814	545	39	454	0,548	552	138
fb124	487	0,732	526	19	454	0,555	559	9
fb128	486	1,15	516	31	479	1,09	524	13
fb131	511	0,302	547	5	509	0,336	541	4

$\boldsymbol{\lambda}_{abs}$, absorption maximum wavelength (nm); D, optical density; $\boldsymbol{\lambda}_{flu}$, fluorescence maximum wavelength (nm); I, dye fluorescence intensity at band maximum wavelength (a.u.); * — intensity of noise where it was not possible to record emission spectra.

wavelength maximum in the case of **fb123** and **fb124** is located approximately at the wavelengths of the monomers and therefore may correspond to the monomeric form of the dye, while for the dyes **up385** and **up388** it is located at a longer wavelength and, given the width of the band, probably corresponds to a less intense long-wavelength transition of dimers or H-aggregates in the case of up385 [12] and J-aggregates in the case of up388 [17].

The fluorescence spectra of the studied dyes in DMSO (except for **up388** and **up396**, for which the emission spectrum could not be recorded) correspond to the emission of dye monomers; their maxima are located between 476 and 615 nm, Stokes shifts are from 30 to 61 nm. Solutions of all dyes in DMSO have low values of fluorescence intensity (I_0 < 40 arbitrary units, a.u.), with the exception of the dye **up385** (I_0 = 237 a.u.). When moving from DMSO to an aqueous medium (TRIS-HCl buffer), the maxima of the fluorescence spectra of the dyes **up393**, **fb123**, **fb128** and **fb131** change by no more than 8 nm compared to the spectra in DMSO and, apparently, correspond to the emission of monomers. At the same time, in the case of **up385** and **fb124**, a larger shift is observed (65 and 33 nm, respectively), which may indicate that these spectra belong to aggregates. As for the fluorescence intensity, its value decreases for all the dyes (except for **fb123**) in buffer as compared to DMSO. For dyes **up388** and **up396** in TRIS-HCl buffer, as well as in DMSO, it was not possible to record emission spectra and only the noise emission intensity is indicated in Table 2.

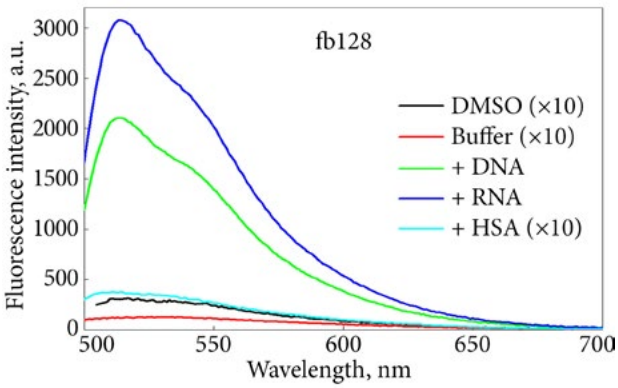


Fig. 5. Fluorescence emission spectra of the dye **fb128** in DMSO, in buffer, and in the presence of DNA, RNA and HSA. The spectra in DMSO, buffer and in the presence of HSA are increased in 10 times for better visibility

Table 2. **Spectral characteristics of the dyes in the presence of DNA, RNA and HSA**

Dyes	DNA					RNA					HSA				
	λ_{abs}, nm	D	λ_{flu}, nm	I_{DNA}, a.u.	ΔQ	λ_{abs}, nm	D	λ_{flu}, nm	I_{RNA}, a.u.	ΔQ	λ_{abs}, nm	D	λ_{flu}, nm	I_{HSA}, a.u.	ΔQ
up385	529–632	0,228–0,216	679	38	1,6	529–632	0,234–0,216	676	58	2,4	528–606	0,240–0,148	618	1409	58,7
up388	482	0,15	543–641	13	2,6	487	0,132	577	23	4,6	480	0,16	619	136	27,2
up393	426	0,557	465	278	15,4	425	0,477	463	660	36,6	425	0,579	482	121	6,72
up396	523	0,329	557	204	102	526	0,365	556	462	231	518	0,365	566	10	5
fb123	459	0,442	537	1545	11,2	461	0,41	530	1500	10,9	454	0,544	547	197	1,4
fb124	461	0,432	520	685	76,1	461	0,449	515	492	54,6	454	0,54	553	88	9,7
fb128	485	0,782	513	2090	160,7	485	0,65	513	3090	237,7	480	1,13	510	38	2,9
fb131	515	0,227	536	590	147,5	515	0,26	538	873	218,3	510	0,27	536	10	2,5

λ_{abs}, absorption maximum wavelength (nm); D, optical density; λ_{flu}, fluorescence maximum wavelength (nm); I, dye fluorescence intensity at band maximum wavelength (a.u.); ΔQ, fluorescence intensity increase.

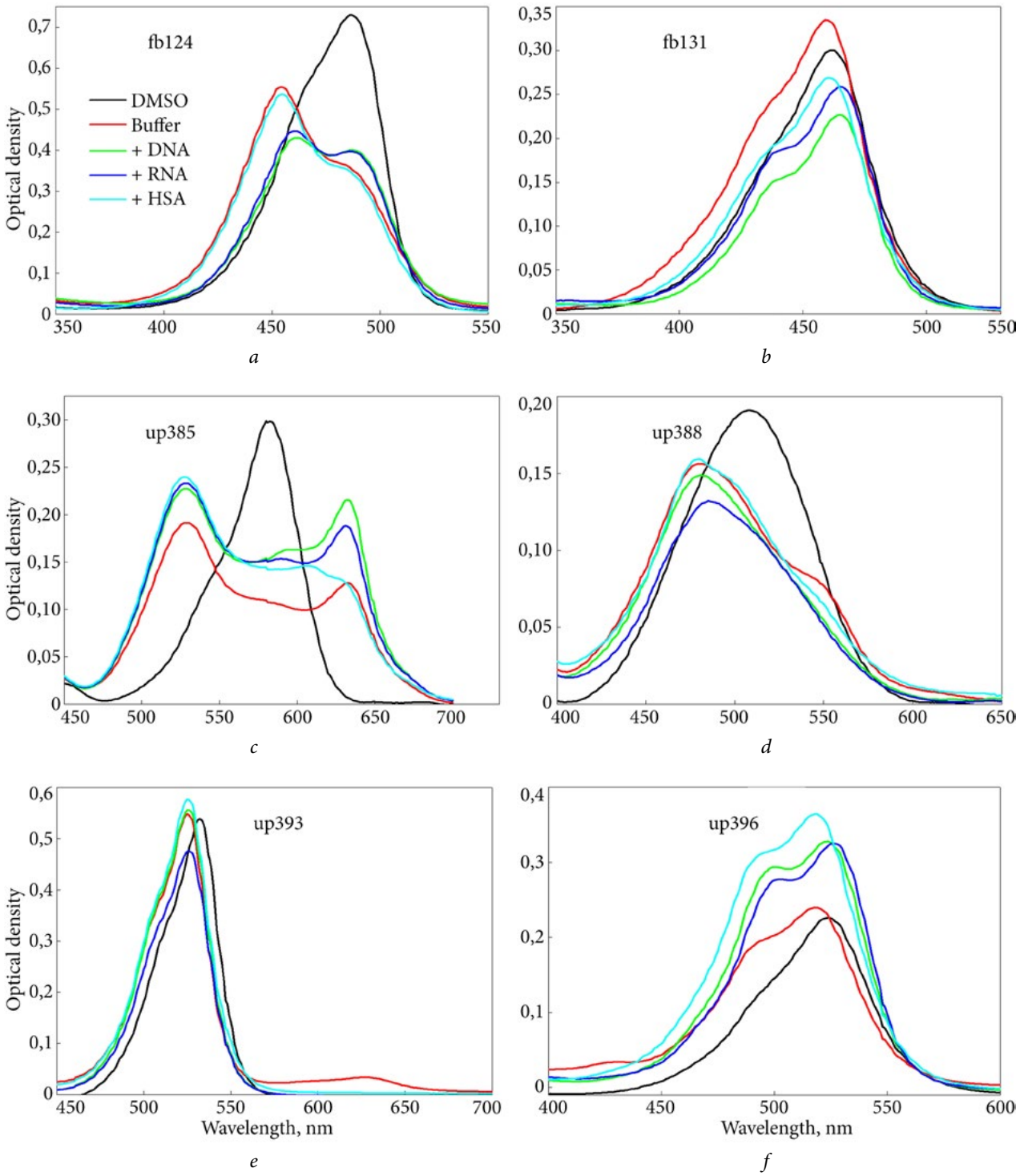


Fig. 6. Absorption spectra of the dyes **fb124**, **fb131**, **up385**, **up388**, **up393** and **up396** in DMSO, in buffer, and in the presence of DNA, RNA and HSA: *a* — the dye **fb124**; *b* — the dye **fb131**; *c* — the dye **up385**; *d* — the dye **up388**; *e* — the dye **up393**; *f* — the dye **up396**

Spectral properties of the dyes in the presence of DNA and RNA

The characteristics of the absorption and fluorescence spectra of the dyes in the presence of DNA and RNA are presented in Table 2. The spectra are presented in the Fig. 4, Fig. 5 and Fig. 6. The table shows that the addition of DNA or RNA either does not noticeably change the positions of the main maxima of the absorption spectra of the dyes (**up385**, **up393**, and **up388** in the presence of DNA), or causes a short-wavelength shift of 4—7 nm (**up388** in the presence of RNA and the remaining dyes). However, in the case of dyes **fb123** and **fb124** (and to much lesser extent in the case of **fb131** and **fb128**), there is an increase in the relative contribution to the spectrum of the long-wavelength band, which corresponds to the absorption of dye monomers; this may be due to the interaction of the dyes with DNA and RNA, the chromophores of these bis-cyanine dyes binding in the form of monomers (Fig. 4).

The fluorescence spectra of most of the studied dyes in the presence of DNA and RNA correspond to the emission of dye monomers, and their maxima are close to the corresponding maxima in DMSO. The exception is **up385**, whose maxima in the presence of DNA and RNA are shifted by 64 and 61 nm, respectively, relative to the monomer maximum in DMSO, but are close to the aggregate maximum in buffer. However, the presence of DNA and RNA leads to an increase in the fluorescence intensity of the studied dyes. The highest values of fluorescence intensity in the presence of nucleic acids were demonstrated by dyes **fb123** and **fb128** (1500—3000 a.u.) (Fig. 5). At the same time, the maximum ratio of the intensity in the presence of DNA/RNA to that in the buffer (ΔQ) was observed for the dyes **up396**, **fb128** and **fb131** (100—240 times). For dyes **up393**, **fb123** and **fb124**, the addition of DNA or RNA resulted in the ΔQ value between 10.9 and 76.1, while for **up385** and **up388** it did not exceed 4.6.

Spectral properties of the dyes in the presence of HSA

The characteristics of the absorption and fluorescence spectra of the dyes in the presence of HSA are presented in Table 3. The spectra are presented in the Fig. 4, Fig. 5 and Fig. 6. The positions of the maxima of the absorption spectra of the dyes in the presence of HSA do not differ from the corresponding maxima in the spectra of the dyes in the buffer by more than 2 nm (Fig. 4). The only exception is **up385**, where the addition of HSA leads to a decrease in the intensity of the maximum at 632 nm and the appearance of a maximum at 606 nm, which can be attributed to monomers of this dye. This is confirmed by the fact that the maximum of the fluorescence spectrum of this dye in the presence of HSA (618 nm) is close to the monomeric maximum in DMSO (615 nm), while the fluorescence intensity in the presence of HSA increases almost 60-fold. Thus, among the dyes studied, **up385** demonstrates both the highest fluorescence intensity in the presence of HSA and its increase, while its response to the presence of DNA and RNA was the lowest. Another dye that demonstrates a significant (27-fold) increase in emission intensity upon addition of protein is **up388**. For the remaining dyes, this increase does not exceed 10-fold.

Discussion

Thus, we studied the effect of DNA, RNA, and HSA on the absorption and fluorescence spectra of 8 monomethine cyanine dyes. It should be noted that 5 of these dyes contain chromophores of the well-known dyes thiazole orange (**up396** and **fb131**) and oxazole yellow (**fb123**, **fb124**, and **fb128**). The same chromophores are a basis of a number of well-known commercially available probes for nucleic acid detection, such as SYBR Green I and II, SYBR Gold, *etc.* [1, 18]. It is therefore not surprising that these 5 dyes demonstrated significant increases in intensity in the presence of nucleic acids. Besides, it should be noted that these

dyes did not demonstrate significant selectivity for DNA or RNA; the highest selectivity was observed for **up396**, for which the intensity in the presence of RNA is 2.3 times higher than in the presence of DNA. In the structures of the studied dyes, the mentioned chromophores are combined with the affinity-modifying groups that can affect the binding of dyes to biological molecules, providing additional stability to the formed complexes. In particular, when interacting with nucleic acids, the phosphate groups of which are negatively charged, the presence of additional groups containing a positive charge in the dye molecule is important. Such groups with additional positive charges are contained in **fb128** and **fb131**, while the dyes **fb123** and **fb124** are bis-cyanines, where 2 chromophores are connected with positively charged chains. The efficiency of conjugation of two probe dyes as a way to obtain a more efficient probe, as well as the role of positive charges in the structure of the connecting chain, have been repeatedly described in the literature [13, 19]. Thus, the most efficient of the studied dyes, **fb128**, which has both the highest fluorescence intensity in the presence of nucleic acids and the highest emission increase, as well as other dyes that showed good results, can be further investigated in various applications where fluorescent probes sensitive to nucleic acids are used.

It is also worth noting the other 3 dyes studied. For example, the **up393** dye, which consists of 2 benzothiazole groups, one of which contains a branched oxygen-containing group, demonstrates good sensitivity to DNA and RNA. In addition, the **up385** and **up388** dyes, which have a very low response to the presence of nucleic acids, were sensitive to HSA (especially **up385**). Therefore, these dyes bind to human serum albumin and can be transported through the body by this transport protein.

Conclusions

The effect of DNA, RNA, and HSA on the absorption and fluorescence spectra of 8 monomethine cyanine dyes of different structures were investigated. Several dyes were found to have good fluorescent responses to the presence of DNA, RNA, and HSA. Particularly, the dye **fb128**, which contains a charged group, increases the fluorescence intensity in the presence of DNA and RNA by 160 and 240 times, respectively, and the dye **up385** increases the intensity by almost 60 times in the presence of human serum albumin. These, as well as other studied dyes, can be further investigated as fluorescent probes in specific applications (e.g., in fluorescence microscopy).

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ВПЛИВ ДНК, РНК І ЛСА НА СПЕКТРАЛЬНО-ЛЮМІНЕСЦЕНТНІ ВЛАСТИВОСТІ КІЛЬКОХ МОНОМЕТИНЦІАНІНОВИХ БАРВНИКІВ

Мета. Метою даної роботи було дослідження впливу ДНК, РНК та ЛСА на спектри поглинання та флуоресценції 8 монометинових ціанінових барвників, які мають в основі різні хромофори та містять різні ефекторні групи. ***Методи.*** Спектроскопія поглинання в УФ-видимому діапазоні та флуоресцентна спектроскопія. ***Результати.*** Було виявлено декілька барвників з високим флуоресцентним відгуком на присутність ДНК, РНК та ЛСА. Зокрема, барвник fb128, який містить заряджену групу, підвищує інтенсивність флуоресценції в присутності ДНК і РНК в 160 та 240 раз відповідно, а барвник up385 підвищує інтенсивність майже в 60 раз в присутності людського сироваткового альбуміну. ***Висновки.*** Найперспективніший барвник fb128, а також барвники fb123, fb124 і fb131 можна рекомендувати для подальших досліджень в якості флуоресцентних зондів, чутливих до нуклеїнових кислот, в конкретних застосуваннях (ПЛР, флуоресцентна мікроскопія тощо).

***Ключові слова*:** монометинові ціанінові барвники, флуоресцентні зонди, нуклеїнові кислоти, людський сироватковий альбумін.