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In vivo visualization of albumin nanoparticles loaded with cyanine dyes

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Abstract. Protein nanoparticles (NPs) based on endogenous biopolymers are promising platform for bioimaging and advanced therapy since they are biocompatible, biodegradable and have low systematic toxicity with high loading capacity. In this work we studied albumin NPs loaded with three cyanine dyes: ICG, IR-806 and IR-820 for colloidal and optical properties. We demonstrated that cross-linked albumin nanoparticles functionalized with IR dyes were promising for optical bioimaging in biotissue transparency window (700-1700 nm). The proposed dye-loaded NPs were of low toxicity *in vitro* and could be promising for *in vivo* applications.

Keywords: bioimaging, albumin nanoparticles, cyanine dyes, IR-806 dye

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In vivo визуализация альбуминовых наночастиц, загруженных цианиновыми красителями

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Аннотация: Белковые наночастицы (НЧ) на основе эндогенных биополимеров являются перспективной платформой для развития тераностики, поскольку они характеризуются биосовместимостью, биоразлагаемостью и низкой системной токсичностью при высокой загрузочной емкости. В данной работе мы исследовали коллоидные и люминесцентные свойства НЧ на основе альбумина, загруженных тремя цианиновыми красителями - ICG, IR-806 и IR-820. Мы показали, что сшитые наночастицы альбумина, функционализированные ИК-красителями, перспективны для оптического биоимиджинга в окне прозрачности биоткани (700-1700 нм). Предлагаемые нагруженные красителем НЧ не демонстрировали in vitro токсичность и могут быть использованы в условиях *in vivo*.

Ключевые слова: биовизуализация, альбуминовые наночастицы, цианиновые красители, краситель IR 806

Финансирование: Исследование подготовлено в рамках выполнения работ по теме «Лазерные технологии для биомедицинских приложений» (№ 2-122122600055) по государственному заказу Министерства образования Российской Федерации. Р.А. Акасов, Т.В. Егорова, М.Е. Степанов и Е.В. Хайдуков являются членами ведущей научной школы Российской Федерации «Оптико-спектральная наноскопия квантовых объектов и диагностика перспективных материалов» (грант Президента РФ НШ776.2022.1.2-).

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Introduction

Protein nanoparticles based on endogenous biopolymers are promising platform for bioimaging and advanced therapy since they are biocompatible, biodegradable and have low systematic toxicity with high loading capacity [1]. In particular, albumin nanoparticles (ANs) represent one of the most promising carriers due to the cost-effectiveness of fabrication and versatility for delivering both hydrophilic and hydrophobic therapeutics and diagnostic agents. Moreover, albumin-based nanoparticles inherite most of the useful properties of albumins themselves. Being the main protein component of the blood (~40%), albumins are non-toxic, biodegradable, easy-to-functionalize, water-soluble and long-circulating molecules [2], so they perfectly fit as binding agents. Still, one of the main challenges of ANs based theranostics is precise luminescent control of

nanoparticles in vitro and in vivo. The desired degree of control can be achieved by using optical methods, namely techniques of optical bioimaging.

Of particular interest today is the optical bioimaging based on near infrared (NIR) light, because light from this spectral region can penetrate deep into the biotissue [3, 4] matching the so-called biological tissue transparency window (~750–1700 nm). The approach with excitation and luminesce detection in biological transparency window provides visualization up to several centimeters deep in the tissue, which is in many cases sufficient for in vivo control purposes.

At this point the idea is to join carrier properties of albumin nanoparticles and imaging properties of NIR dyes by noticing that they can be easily mixed together giving nanocomplexes both friendly to the organism and able to be controlled by NIR light emitted by loaded fluorescent dyes. The problem is then to obtain the brightest nanocomplexes with stable fluorescence, which are still hydrophilic for high solubility in blood plasma and can effectively hide dye molecules from environment and provide their transport to a target area.

In present work we have used two different types of bovine serum albumin (BSA) nanoparticles, loaded with three different cyanine dyes (ICG, IR-806, IR-820) aiming to evaluate visualization ability. Indocyanine green (ICG) dye approved to use in clinical practice [5] was used as a reference dye. Physicochemical and photoluminescent properties of BSA nanocomplexes of all cross-variants were studied. The proposed dye-loaded NPs were found to be of low toxicity *in vitro* and could be promising biovisualizing agents for *in vivo* applications.

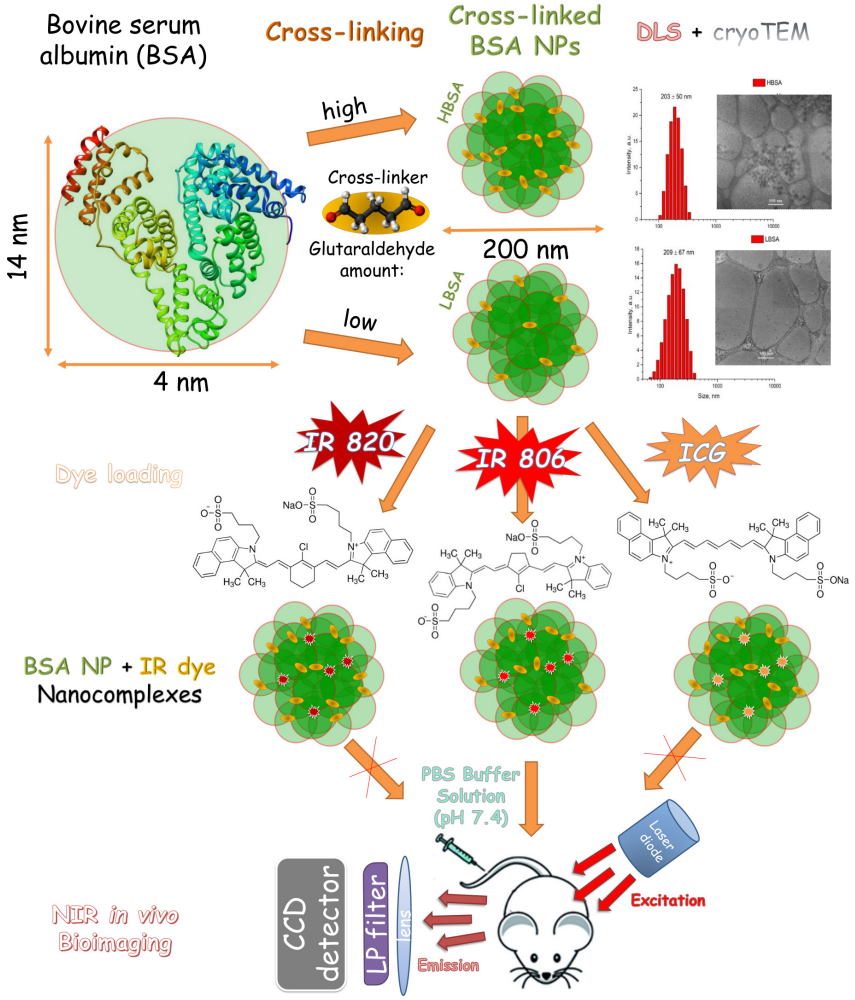

Fig. 1. Scheme illustrating BSA protein molecule, its cross-linking to HBSA and LBSA nanoparticles, size analysis, dye loading and NIR *in vivo* bioimaging. LP filter – long-pass filter used to cut off excitation light, CCD detector is charge-coupled device used to detect the fluorescence emission signal from nanocomplexes *in vivo*

Materials and Methods

The BSA nanoparticles were obtained by precipitation in a non-solvent followed by cross-linking using glutaraldehyde (cross-linking agent binding separate BSA molecules togehter) of high and low concentration, resulting in two types of organic nanoparticles called HBSA (letter "H" stands for high degree of cross-linking) and LBSA ("L" means low degree), correspondingly. The IR dye were incorporated into BSA NPs due to non-covalent interactions.

The scheme illustrating nanoparticles preparation is presented in Fig. 1. The loading capacity and optical properties were studied by spectrometric methods. The luminescence spectra were studied with Fluorolog 3 (HJY, France). The size distribution was measured with dynamic light scattering (DLS) and cryo-TEM microscopy (Tecnai G212 SPIRIT, FEI, USA). *In vivo* studies were done on home-build bioimaging system [6]. The resulting *post mortem* images of mice organs were analyzed with ImageJ software to obtain background-corrected pharmacokinetics data according to brightness of captured NIR fluorescence.

Results and Discussion

HBSA and LBSA had the same size distribution with maximum at 200 nm. Based on DLS study and TEM analysis we found that albumin NPs loading with IR dye dramatically changed the size distribution in the case of LBSA, but not int the case of HBSA. Dye loading capacities were the same for both types of NPs, and were ~ 85%.

Nanoparticles loaded with three different cyanine dyes ICG, IR-806, IR-820 were synthesized, and among them the BSA@IR-806 nanocomplex was the most stable and had the brightest fluorescence, which was substantially greater than in the case of the clinically approved ICG dye. The phenomenon of fluorescence change under entrapment in albumin was reported earlier [5] and confirmed in our experiments for IR-806 and IR 820 dyes (see Fig. 2).

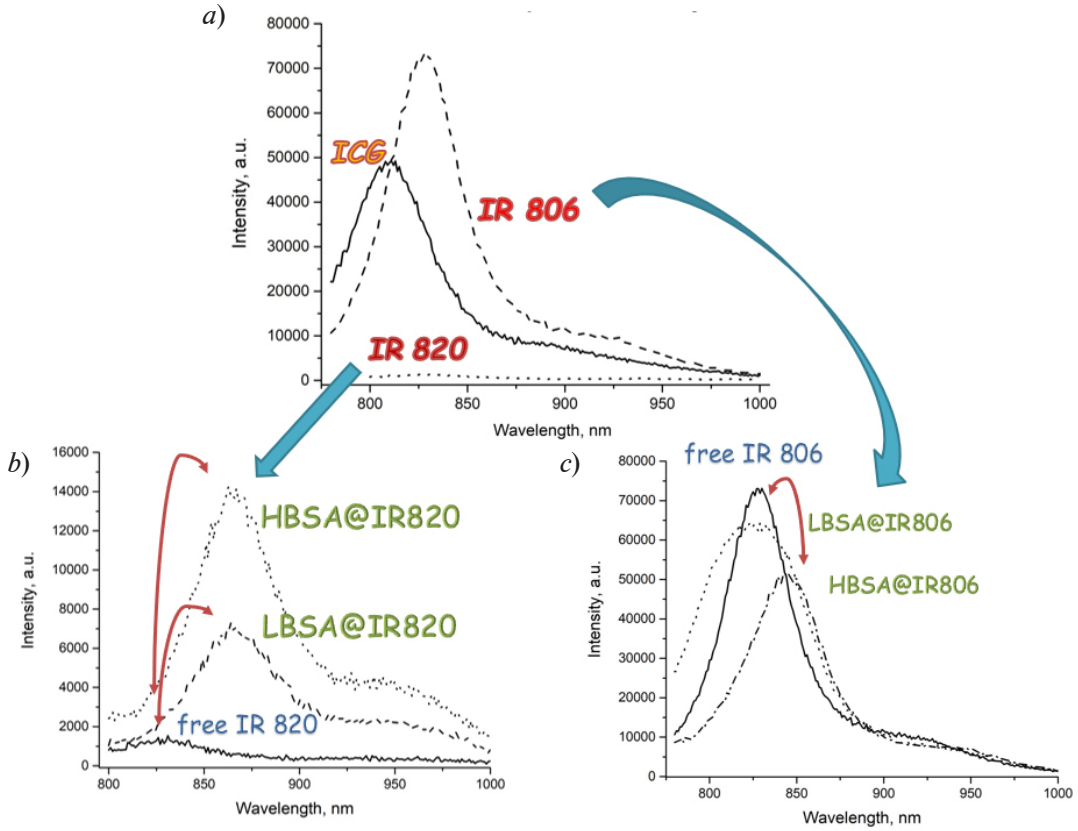

Fig. 2. Fluorescence of free ICG, IR 806 and IR 820 dyes under 750 nm excitation in solution (*a*); fluorescence of BSA@IR-820 nanocomplexes (*b*) and BSA@IR-806 nanocomplexes (*c*). Red arrows show the red shifts. Data corresponding to the free dye in solution is shown here to demonstrate the effect of interaction with albumin nanoparticles on the luminescent properties of the dyes

Additionally, we determined correlation between cross-linking degree and luminescent properties of nanocomplexes (Fig. 2,*b* and 2,*c*). In particular IR 806 and IR 820 demonstrate red shift of luminescence after intercalation in HBSA complexes. At the same time, red shift after intercalation in LBSA nanocomplex is demonstrated only by IR 820 dye. The highest quantum efficiency was observed for BSA@IR-806 (Fig. 2,*a*). The BSA@IR-806 nanocomplex solution was also found to be colloidally stable. Considering all the results, BSA@IR-806 was chosen for subsequent *in vitro* and *in vivo* experiments (as shown at Fig. 1).

The *in vitro* studies demonstrated the absence of cytotoxicity in MTT assay at the concentrations up to 0.05 mg/ml in MCF-7 breast adenocarcinoma and fibroblasts cell culture.

In vivo pharmacokinetics study of BSA@IR806 was made in comparison with free IR 806 dye (Fig. 3) to determine the effect of albumin nanoparticles. We can conclude that 5 min after intravenous injection the similar distribution for free and albumin encapsulated dye was observed. The most intensive signal was observed in liver, gall-bladder and intestines, which makes gall duct the main excretion pathway for the IR 806 dye. It can be attributed to cyanine dyes in general [2] (sulfur-containing groups being responsible). Their appearance can in principle be modified to switch the excretion pathway to renal. In 24 hours the biodistribution was dramatically changed and nanocomplexes were accumulated in liver and spleen in contrary to free dye.

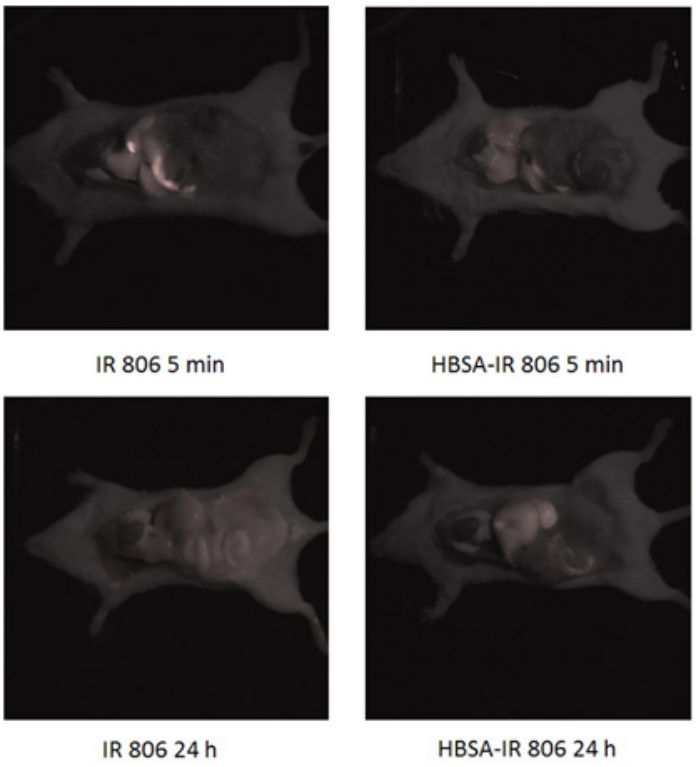

Fig. 3. *In vivo* pharmacokinetics study (5 min and 24 hours after injection) of free IR 806 in comparison with HBSA@IR806 nanocomplexes based on *post mortem* NIR photoluminescent imaging. Each image is an overlap of reference image of mouse and NIR image of dye emission (pictured with light red)

Conclusion

In present work the synthesis of BSA nanoparticles loaded with cyanine dyes (ICG, IR 806, IR 820) was performed for *in vivo* bioimaging. BSA@IR806 dye nanocomplexes were found to demonstrate high colloidal stability and flexible spectral behavior. The *in vitro* studies demonstrated the absence of cytotoxicity in MTT assay at the concentrations up to 0.05 mg/ml in cell cultures. *In vivo* studies showed pronounced time-stable accumulation of BSA@IR806 nanocomplexes mainly in gastrointestinal tract in contrary to free IR 806.

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