COMMENT
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Cyanine-tagged albumin facilitates high-performance NIR-II biomedical imaging

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The emerging NIR-II imaging modality is promising for real-time visualization of living systems and multiple disease diagnoses, particularly through its enhanced tissue penetration and superior spatial resolution [1, 2]. Cyanine dyes possess the advantages of low administration dosage, high biocompatibility, and strong fluorescence emission intensity [3]. Cyanine dyes can be easily structurally modified through molecular engineering methods, while the large hydrophobic conjugation systems hinder further in vivo imaging application. The traditional surfactant encapsulation strategy cannot effectively solve the aggregation caused-quenching issue of the dyes in aqueous solutions and may even mask their reaction active sites [4]. Due to the special hydrophobic pocket to act as a probe carrier and the ability to serve as the pan-marker for multiple diseases, albumin is chosen as the preferred binding protein for cyanine dyes [5]. Albumin interacts with cyanine dyes through a “hydrophobic pocket” and further improves its properties such as water solubility, biosafety, and fluorescence quantum yield. Cyanine dyes can modify the binding efficiency with albumin through structural design and regulate the targeting performance. This means that cyanine-tagged albumin can respond to pathological changes caused by different diseases and monitor the disease process in real time with high contrast through NIR-II fluorescence signals. Further development of cyanine-tagged albumin probes is helpful for in-depth comprehension of the binding mechanism and guiding the directed synthesis of cyanine molecules with specific protein binding behaviors and optical properties, thereby achieving precise targeting and high-performance NIR-II bioimaging of specific diseases. This point is essential for the design, preparation, and even clinical translation of NIR-II targeting dye molecules in precise diagnosis and treatment.

The interaction between cyanine dyes and albumin can be simply determined by the changes in spectra. Due to H-aggregation, the absorption spectrum of cyanine dyes in aqueous solutions (such as phosphate buffer solution) will exhibit a remarkable blue shift compared to that in organic solvents like dimethyl sulfoxide [6]. The binding of cyanine to albumin can inhibit H-aggregation to a certain extent and restore its absorption spectrum to the sharp absorption in dimethyl sulfoxide. Furthermore, the fluorescence intensity after the binding of cyanine with albumin will also be remarkably improved. Nevertheless, the covalent binding of the dyes to albumin cannot be judged merely through above-mentioned methods. Cyanine dyes with reactive activity typically have a meso-Cl structure. To determine whether cyanine dyes are covalently bound to albumin and to determine the binding efficiency, gel electrophoresis of the mixed solution is necessary and a preliminary judgment is made based on the relative fluorescence intensities of the protein bands and the free dye bands (Figure 1A) [7]. The binding efficiency is ultimately determined through high-resolution mass spectrometry of the solution.

The binding mode and binding sites of cyanine dyes and albumin have also been extensively reported. The key binding sites of different cyanine dyes in human serum albumin are not entirely identical but are mainly located in domain III of the albumin (Figure 1A) [8–10]. The fluorescence enhancement can be attributed to the formation of either non-covalent interaction or covalent binding, yet covalent bonds can assist in fixing the dye in the hydrophobic pocket. Molecular docking and molecular dynamics simulations are employed to determine the optimal binding conformation of cyanine dyes in the hydrophobic pocket [9]. Cyanine molecules are highly likely to achieve fluorescence enhancement by interacting with multiple amino acid residues. The twisted conformation fixed in the hydrophobic pocket can realize emission at higher wavelengths through enhancing the twisted intramolecular charge transfer process [7]. Thus, the binding with albumin endows the ability upon NIR-I cyanine dyes to achieve NIR-II fluorescence imaging through off-peak emission [11].

The cyanine-tagged albumin complex pre-prepared in vitro has been widely applied in the real-time visualization of life activities such as high-contrast vascular and lymphatic imaging because of its ultra-high brightness [12, 13]. Albumin is a pan-cancer biomarker, which is mainly attributed to the high expression of albumin-binding proteins in cancer and the enhanced permeability and retention effect effect of tumors (Figure 1B). In certain studies, cyanine dyes are referred to as tumor-seeking dyes [14, 15]. This is because cyanine dyes with a meso-Cl structure can realize effective tumor targeting by binding to albumin [16]. In addition, albumin has a relatively long half-life, facilitating the maximal accumulation and persistence of cyanine dyes in tumors. However, it should be noted that albumin also has a relatively high accumulation signal in metabolic organs such as the liver. Interestingly, the cyanine dyes with blocked meso-Cl also possess pan-cancer targeting ability, and there is no evidence that albumin plays a crucial role in this process [17]. This series of cyanine dyes also shows a faster metabolism, a lower skin signal, and a better signal-to-noise ratio in tumor-targeted imaging. This indicates that the interaction rules among the molecular structure of cyanine dyes, tumor targeting, and albumin tagging require deeper exploration.

The concept of in vivo in situ albumin-tagged cyanine dyes has been gradually clarified. Different from the dyes@albumin pre-prepared in vitro, in situ albumin-tagged dyes mean that the dye molecules bind with endogenous albumin rapidly and efficiently after being injected into the body [18]. The high serum concentration of albumin ensures its ability to in situ capture cyanine dyes, while its large protein size limits its distribution in normal organ tissues. Therefore, these molecules have been widely used in the high-contrast dynamic monitoring of various diseases, such as blood-testis barrier disruption, ischemic stroke, glomerular filtration breakdown, and inflammatory bowel disease, etc [10, 19–24] (Figure 1C). The targeting principle mainly relies on the increased permeability of the vascular endothelial barrier after injury, resulting in the leakage of serum macromolecules in the disruption area.

Although albumin-tagged cyanine dyes have a remarkable brightness advantage, they also have unavoidable background signals. The dyes are clearly not applicable to disease processes that require long-term monitoring because the signal decline resulting from metabolism cannot support observations over a period of weeks or even months. In addition, if the dyes are injected repeatedly, they will cause an increased background signal, leading to a poorer signal-to-noise ratio. Therefore, a albumin-escaping dye whose meso-Cl is blocked but still retains certain non-covalent interactions with albumin was specifically designed [25]. This dye rapidly illuminates the blood vessels after intravenous administration and maintains a high brightness for several minutes, and then is almost completely metabolized by the liver within three hours without any

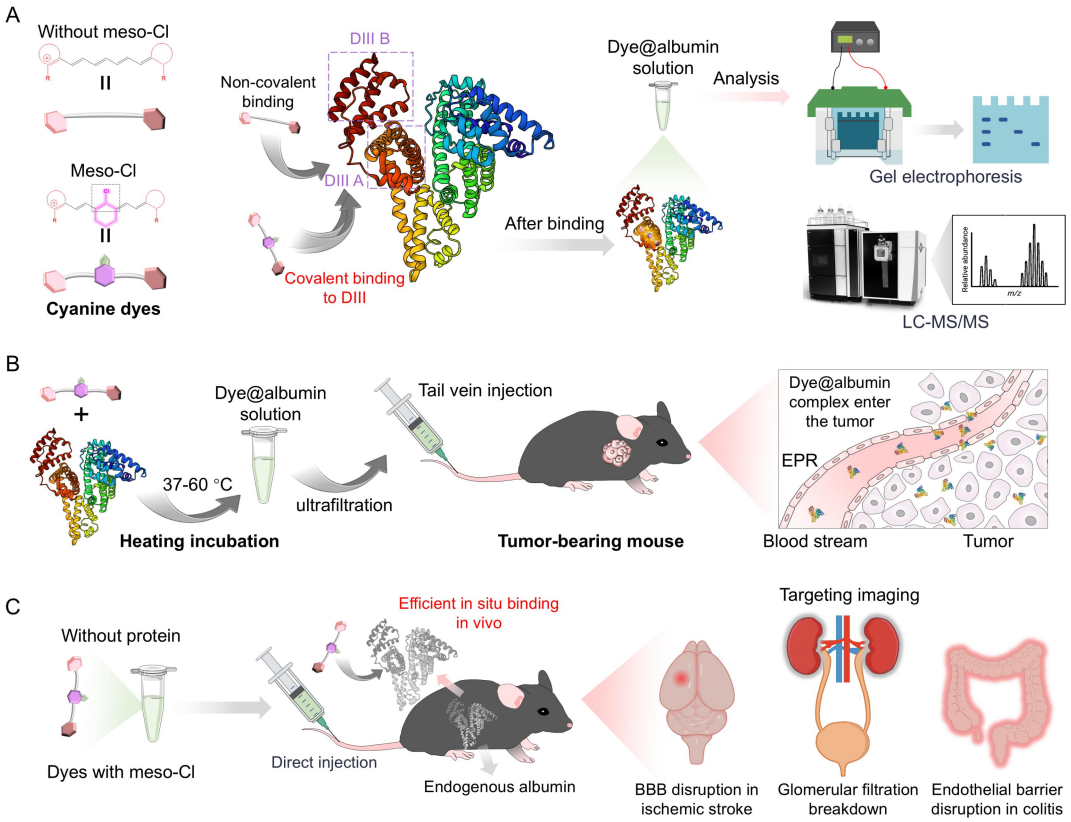

Figure 1 The binding between cyanine dyes and albumin and the targeting principle. (A) The interaction and covalent binding determination of different cyanine dyes with albumin. (B) The principle of dye@albumin complex targeting tumors. (C) In situ albumin tagging for targeted imaging of barrier disruption. Albumin structure was generated by the Protein Data Bank. Some schematic diagrams were designed using BioRender software. EPR, enhanced permeability and retention effect; LC-MS, liquid chromatography-mass spectrometry; MS, mass spectrometry; DIII, domain III; BBB, blood-brain barrier.

background signals left. This dye has been applied in the long-term monitoring of vascular reconstruction in the flap transplantation model and the characterization of the spatiotemporal features of long-term vascular pathological changes in ischemic stroke [26].

In conclusion, cyanine-tagged albumin has been extensively utilized in in vivo high spatiotemporal resolution imaging and high-contrast dynamic monitoring of multiple diseases. The targeting capacity of cyanine dyes with meso-Cl structure mainly originates from the efficient binding with albumin. Nevertheless, the targeting design of the molecular structure still largely depends on the trial-and-error approach, and the inherent laws between the structure and the targeting ability have not been clearly investigated as of now. This demands further in-depth exploration, which is essential for realizing intelligent targeted dye design and precise diagnosis of diseases in the future.

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Author contributions

Dang ZT collected relevant information and wrote the draft. Zhu SJ revised and edited the manuscript. All authors have reviewed and agreed to the final draft.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

NIR, near infrared.

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