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Detection Methods and Research Progress of Human Serum Albumin

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ABSTRACT

Human serum albumin (HSA) is a biological macromolecule with important physiological functions; abnormal HSA levels are associated with coronary heart disease, multiple myeloma, diabetes, nephropathy, neurometabolic disorders, liver cirrhosis and other diseases. Therefore, accurate and quantitative detection of HAS have extremely important research and application value in biological science, molecular biology, clinical medicine and other fields. As for the detection method of HSA, dye-binding method and immune method are the first to be used, and have been applied in clinical detection. In recent years, many new detection technologies have emerged, such as fluorescent probe detection method, nano-materials for HSA detection, biosensor and so on. Although there are many methods developed recently to detect HSA, comprehensive reviews for HSA detection methods are still rare. Thus, writing this review to fill in the blank is in need. In order to highlight the recent progress in the field of HSA detection, in this review, the methods used to detect HSA are summarized and sorted, the advantages and disadvantages of these detection methods are also listed, then the research progress of small molecular fluorescence probe method is emphatically introduced in this paper. Then, we briefly discussed the challenges and future development directions in this field.

KEYWORDS

Biosensor; binding site; detection methods; fluorescent probe; human serum albumin

Introduction

Serum albumin (SA), included human serum albumin (HSA) and bovine serum albumin (BSA). HSA is a highly homologous primary structure. HSA is the most abundant protein in human blood[1] (accounting for 60% of the total plasma protein), which is synthesized only in the liver, with an average of about 12-25 g per day, accounting for 50% of the liver protein synthesis.[2,3] Serum albumin concentration is about 35-50 g/L, and urine albumin concentration is usually less than 30 mg/L[4] and the serum half-life is about 20 days.[5] Excessive HSA in urine can lead to microalbuminuria, which is an early marker of cardiovascular disease and kidney disease in diabetes mellitus and hypertension.[6] However, low levels of HSA in the blood plasma, known as hypoproteinemia, may be a sign of liver cirrhosis, failure, and chronic hepatitis.[7] Therefore, accurate detection of HSA is of clinical importance.

The history of albumin estimation began with the exploration of various precipitating agents such as phosphotungstic acid, ammonium sulfate, magnesium sulfate, sodium sulfate, trichloroacetic acid, and the separation of albumin by filtration and centrifugal of globulin.[8–10] In the past, precipitation-based methods were popular for estimation of HSA. In1927, Wu and Ling invented the Folin phenol reagent colorimetric method to determine serum protein. Along with precipitation based methods optimization, electrophoretic separation of plasma proteins HSA been developed.[11] At present, for the determination of albumin sensitivity and specificity, more and more analytical tools such as dye binding, immunochemistry, chromatography, infrared spectroscopy, etc. have been improved. Although there are many methods developed recently to detect HSA, comprehensive reviews for HSA detection methods are still rare. Thus, writing this review to fill in the blank is in need. In this review, we briefly introduced the above detection methods, then summarized the recent progress and advances in the development of small-molecule fluorescent probes for the quantitative detection of HSA. We want to conclude the former research and provide our opinions on future study.

Brief introduction of HSA

Human serum albumin is a single-chain protein that HSA a heart shape, and contains 580-585 amino acid residue with a molecular weight of 60 kDa. HSA has extremely important medical value and is commonly used clinically in the symptomatic treatment of hemorrhage, shock, burns, polycythemia and hypoalbuminemia. Because of its low cost, BSA is widely used as an alternative to HSA in many biochemical and pharmacological applications.[12] But actually, BSA has only 75.8% of the biological functions of HSA, so it cannot replace HSA in many applications.[13] Misuse of the two proteins will lead to fatal injury to patients. Thus, it is a great challenge to distinguish HSA from BSA.

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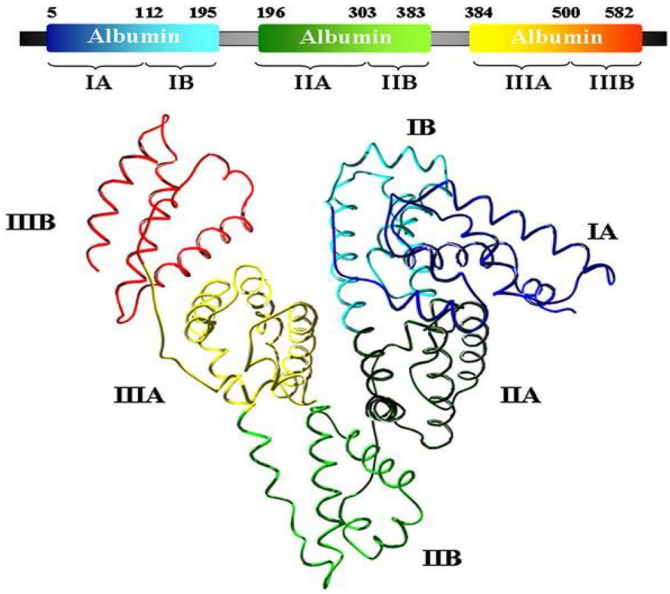


Figure 1. Modular domain organization of HSA.[16]

The structure of HSA

In 1975, the main sequence of HSA was identified for the first time.[14] In 1989, carter and his colleagues first analyzed the three-dimensional structure of HSA.[15] The crystal structure of HSA shows that it is a helical protein, the mature secondary structure of HSA is mainly composed of a series of α-helix (68%), every three α-helix forms a substructure domain, and two pairs of each substructure formed a structure domains, the entire structure of HSA contains following three homologous α-helical domains, I (residues 1–195), II (196–383) and III (384–585) (Figure 1).[16]

The synthesis and metabolic process of HSA

The synthesis of HSA occurs in the liver. HSA begins to be synthesized from 24 amino-terminal amino acid residues, called proalbumin, then split in endoplasmic reticulum.[17] Then the proalbumin is transported to trans-Golgi and 6 amino acid long N-terminal oligopeptide are removed to form mature albumin.[18] The synthesis of HSA is influenced by many factors, including hormone, nutritional status, plasma osmotic pressure, stress state, body temperature and so on. Under normal conditions, the daily degradation of denatured proteins by tissues is 13.3-13.6 g, which is consistent with the amount of albumin daily synthesized in the liver.[19]

The drug binding sites of HSA

HSA can bind to many endogenous substances and exogenous drugs, fatty acids, metal ions, drugs and metabolites, which may be related to their conformational adaptation. Sudlow et al. first used drugs to study the binding sites of albumin, the results showed that most drugs bind to albumin on one or two strong affinity binding sites in the IIA and IIIA subdomains of HSA, namely, site I and site II.[20] Ghuman et al. revealed the precise structure of the two primary drug-binding sites on the protein, and pointed out that residues are the key determinants of binding specificity through crystallographic analysis of 17 different complexes of HSA with a wide variety of drugs and small-molecule toxins.[21] Site I HSA a strong affinity for larger heterocyclic compounds and dicarboxylic acid, such as butazodine, warfarin, apazone, sulfamethoxazole; Site II is more likely to to bind to small molecules, such as ibuprofen, diazepam (Table 1).[22–24]

The physiological function of HSA

HSA is a main carrier for endogenous and exogenous molecules including many drugs, glycolipids, fatty acids and metabolites.[25] HSA is widely used clinically to treat a wide range of diseases, including hypovolemia, shock, burns, surgical blood loss, trauma, hemorrhage, cardiopul-monary bypass, acute respiratory distress syndrome, hemodialysis, acute liver failure, chronic liver disease, nutrition support, resuscitation, and hypoalbuminemia.[16] In 1940s, during the Second World War, clinical use of HSA was successfully made by Surgeon Isidor S. Ravdin who clinically administered purified human serum albumin to seven severely burned patients, all of them survived successfully..[26] In addition, HSA has been successfully used as a stabilizing agent to enhance the potential of therapeutic proteins, such as interferon (IFN),[27,28] interleukin-2 (IL-2),[29] antibody molecules[30,31] and vascular endothelial growth factor (VEGF).[32] HSA exhibits enzymatic activity, but more slowly than other enzymes. Therefore, it is considered to be pseudesterase. However, the degreased HSA showed greater pseudesterase activity.[33]

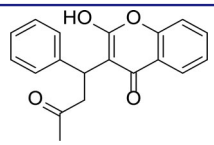
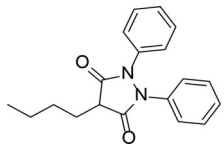
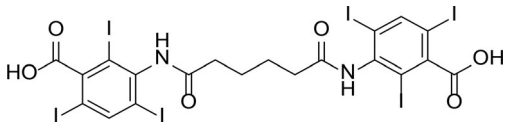
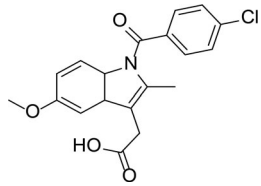
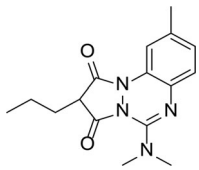
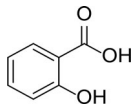
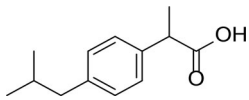
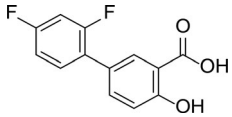
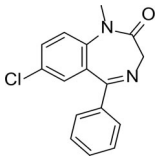
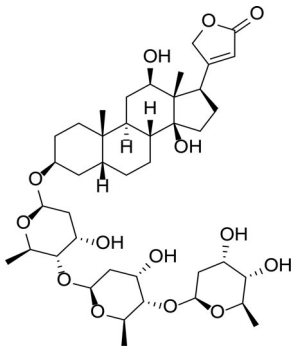
Methods and research progress of HSA detection and estimation

To date, various methods including dye-binding methods, immunochemical methods, electrophoresis-based methods, chromatography based methods, as well as spectroscopy based methods have been used for the detection of HSA and each of these methods has its advantages and disadvantages. Depending on the scope of clinical practice and analytical methods, HSA measurement should meet the following requirements: 1) high selectivity, sensitivity and rapid-response; 2) high accuracy and repeatability; 3) high throughput and low cost.[4] Next, we reviewed the research progress of various methods and compared their advantages and disadvantages.

Dye binding methods

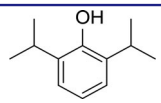
Until the early 1950s, dye-binding methods for albumin replaced the previous method. The method previously described is cumbersome and time consuming. The development of dye binding methods is a milestone in albumin estimation. It makes albumin estimation accurate and fast.[34]

Table 1. Summary of the site-specific binding drugs of HSA.

Site-specific drugs	Structure	Main binding site
Warfarin		IIA subdomain
Phenylbutazone		IIA subdomain
Iodipamide		IIA subdomain
Indomethacin		IIA & IB subdomains
Azapropazone		IIA & IB subdomains
Salicylic acid		IIA & IB subdomains
Ibuprofen		IIIA subdomain
Diflunisal		IIIA subdomain
Dizazepam		IIIA subdomain
Digitoxin		IIIA subdomain

(*continued*)

Table 1. Continued.

Site-specific drugs	Structure	Main binding site
Propofol		IIIA & IIIB subdomains

Methyl orange

Methyl orange is the first dye used to detect HSA.[35] It is an acid dye which binds to site 1 of HSA by the electrostatic interaction between the sulfonic group of MO and the protonated groups of Lys444 and Arg218, The proton exchange reaction between the diazenyl group in the dye and the carboxyl group of albumin Glu292 causes a color change, which can be used for HSA measurement.[36,37] However, due to its poor specificity, it has never been used in laboratory testing.[38]

HABA

Like methyl orange, HABA (2-(4′-hydroxyazobenzene) benzoic acid) was also the first dye introduced in the 1950s for HSA detection. It is an anionic azo dye. The detection of albumin by HABA was first proposed by Karush, who first used it for the detection of bovine serum albumin. Inspired by this, David D. Rutstein et al. successfully applied it to the detection of human serum albumin.[35] It is worth noting that Substances like sulfonamides, salicylates, and free FA interferes with HABA for estimating albumin interferes with the estimation of albumin using HABA.[39]

Phenol red

Phenol red was first used to estimate HSA in 1938.[40] By binding to HSA site 1, the absorbance of the complex at 560 nm changes, thereby achieving the purpose of measurement. Bilirubin, fatty acid FA and anions such as naphthalene on sulfonate interfere with phenolic red to estimate albumin.[41–43]

Albumin blue (AB) dyes

Albumin blue dyes can specifically target albumin by forming a strong fluorescent complex. After binding to albumin, the fluorescence of AB complex will increase by more than an order of magnitude, and will not be interfered by other urine proteins.[44] Among two of its derivatives, AB633 and AB670 have been used for HSA detection, but their applications are limited due to poor reagent stability. So AB580 was developed, which has both good stability and good performance in detecting HSA. It has been reported that the interference from Bence Jones protein, transferrin, FA, etc. is small, and the interference can be further reduced by using excessive dyes.[45] In addition, AB580 can also be used in the design of nanoprobes to make them specific, which will be mentioned later.

BCG

In 1965, Rodkey[46] first introduced BCG (Bromo cresol green) binding as a method to measure HSA, but one of the problems was that the absorbance of the blank reagent was high at pH = 7.1. In 1971, P.J.N. Howorth[47] applied it to the detection of serum albumin. Again, in 1971, Doumas et al.[48] purified the reagent concentration, added surfactants to prevent precipitation of the albumin-dye complex, and improved the calibration method to form the classic BCG detection method. That is, BCG shows a characteristic discoloration by binding to albumin site I near pH 4, and then the formed albumin-dye complex was measured at 625 nm by spectrophotometry to estimate albumin levels. But they also reported a flaw in the method, which lacked specificity for albumin, especially at low albumin and high globulin concentrations. This is due to the reaction of BCG with acute pHSAe reactants and α- and β-globulin serum fractions. In 1976, Gustafsson[49] greatly improved the specificity of the BCG method by performing absorbance measurements immediately after mixing serum and reagents, thereby preventing the binding of other serum proteins.

BCP

In 1968, Louderback[50] first proposed the BCP method to measure HSA. Subsequently, Carter, Pinnell, and Northam et al.[51,52] improved the method. Compared with the lack of specificity of the above BCG method, BCP is specific for HSA and is not affected by precipitation.[52] This method shows a change in color by combining with the HSA site 1 through hydrophobic interactions, and estimates HSA at 590 nm by spectrophotometry.[53] However, this method has a big disadvantage. In hemodialysis and peritoneal dialysis patients, the HSA results measured by the BCP method will be low. This is due to the presence of 3-carboxy-4-methyl-5-propyl-2-propylfuranpropionic acid (CMPF), the main endogenous ligand substance present in serum of uremia.[54–56] In addition, studies have shown that heparin can interfere with BCG and BCP methods.[57,58]

BCB

R.Flores first introduced the BCB method for measuring HSA. BCB method estimates HSA by binding to the HSA site I under acidic conditions and then measuring the absorbance at 610 nm which can be stable for eight hours.[41,59] This method is interfered by globulin and is almost unaffected by bilirubin, uric acid and creatinine (Table 2).[60]

Table 2. Properties of various dyeing methods.

Dye	Binding site	Estimation methods and characteristics	References
MO	site I,	proton exchange reactions cause changes in characteristic color; interfered by globulins	[36, 37]
HABA	No date	Interfered by Substances like sulfonamides, salicylates, and free FA	[39]
PR	site I,	absorbance of HSA-PR complex at 560 nm; interfered by bilirubin, FA	[41–43]
AB580	No date	excitation at 590 nm; emission at 616 nm isopropanol is used as a solvent. Less interfered	[45]
BCG	site I,	absorbance of HSA-BCG complex at 625 nm; interfered by globulins	[48]
BCP	site I,	absorbance of HSA-BCP complex at 590 nm ; uninterfered by globulins, transferrin, and salicylate	[53–56]
BPB	site I,	absorbance of HSA-BPB complex at 610 nm ; interfered by globulins, unaffected by bilirubin, uric acid, and creatinine	[41,59,60]

Fluorescence probes

Fluorescent probes refer to a class of fluorescence with characteristic fluorescence in the ultraviolet-visible-near infrared region[61,62]whose fluorescent properties can be sensitively changed according to the nature of the environment, such as polarity, refractive index, viscosity, etc.[63–67] Fluorescent molecules are molecular measurement devices based on spectral chemistry and optical waveguides and measurement technology that specifically convert the chemical information of the analysis object into easily measurable fluorescent signals. Czarnik first explicitly proposed the basic composition of fluorescent probes in 1994, that is, fluorescent probes consisted of a recognition group, a fluorescent group, and a linking arm.[61] The recognition group determines the selectivity and specificity of the probe molecule. It is mainly responsible for selectively reacting with a specific analyte. The fluorescent group is responsible for converting the recognition signal into an optical signal. The linking arm is mainly responsible for connecting the recognition group and the fluorescent group.[68,69] Common fluorophores are coumarin, fluorescein, rhodamine, anthocyanin derivatives, BODIPY, etc. In recent years, some fluorescent sensors that detect HSA have been reported and we mainly outline the design of HSA probes from the perspective of various fluorophores.

Dicyanomethylene-4H-chromene based fluorescent probes

Dicyanomethylene-4*H*-chromene and its derivatives are very important chromophores. Its emission wavelength is more than 600 nm and it HSA near-infrared characteristics. It also has good light stability. Due to these excellent optical properties, more and more researchers are applying it to the design of fluorescent probes[70,71]. In 2014, J-l Fan et al.[72] designed a fluorescent turn-on HSA probe **1** by introducing aromatic amine into the dicyanomethylene-4Hchromene framework via double bonds to form a conjugated p-electron system. It shows a dramatic fluorescence enhancement toward HSA without interference from other proteins .Then in 2017, K. Rajasekhar et al.[73] also designed a dicyanomethylene-4H-chromene-based red-NIR fluorescence probe **2** for the selective detection of HSA. In their work Dicyanomethylene-4*H*-chromene was conjugated with an electron-donating moiety (*N*,*N*-diethyl amino-salicylaldehyde) through an alkenyl-double bond to derive the probe 2 with a built-in, push–pull-based π-electron system. It is capable of performing multiple applications involving detection, quantification and monitoring of HSA concentration levels, imaging of the SA in live cells and selective staining of albumin in PAGE (gel imaging). Both of the two dicyanomethylene-4H-chromene-derived fluorescence probe bind to HSA mainly through site 1.

Cyanine dyes based fluorescent probes

The history of cyanine dyes can be traced back to more than 160 years ago. At that time, soybean powder blue cyanine dyes were first synthesized and reported by researchers, and cyanine dyes with different structures were continuously enriched and developed. The outstanding photophysical properties of cyanine dyes and the rapid development of fluorescent labeling technology have made it increasingly active in the field of life sciences (genetic diagnosis, expression and disease diagnosis). This type of dye structure is easy to control and modify, and its spectral range covers the entire visible light region, and can even reach the near infrared light region, which has laid a solid foundation for its various applications.[74,75] In 2015, S. I Reja et al.[76] designed and synthesized the first Indole hemicyanine dye NIR-probe **3** with a donor–π-conjugation–acceptor system to detect HSA. It loses its fluorescence through a non-radiative pathway due to TICT, once it interacts with HSA, the TICT will be blocked, and the probe stabilizes itself by adopting a planar geometry and becomes fluorescent. Then in 2016, X.J Peng group[77] reported a similar structure for HSA detection. They replaced the heterocycle with benzothiazole and synthesized the probe **4**. Compared with probe 3, its emission wavelength is redshifted and its response is fast (it only takes 5 seconds to respond completely).In the next year, given that probe 3 was not stable because of protonation or deprotonation in the physiological pH range, Wei Yang et al.[78] synthesized four red-emitting (> 600 nm) benzindocyanine dye by ethylene bridging of N-substituted indolium and aminobenzaldehydes. All the four fluorescent dyes have TICT characteristics, and two of them (probe **5** and probe **6**) showed high sensitivity and remarkable selectivity for HSA. The difference is that probe 5 binds to HSA through site I and probe 6 binds to HSA through site II. In 2018, they reported two more benzindocyanine dye NIR-fluorescent probes that can distinguish HSA/BSA.[79] Where probe **7** showed high sensitivity toward HSA over BSA, and it located within site I of HSA, which mainly depended on the hydrophobic residues and π-π interaction. These probes are all introduced by conjugated vinyl to increase their emission wavelength to the NIR region. Another idea based on the design of cyanine dye probes is the supramolecular aggregates of cyanine dye, which are rich in color and show good absorption and emission range, far from the background of spontaneous fluorescence of

biological species. And they are highly sensitive to specific biomolecules, these properties make cyanine to cluster together and have the potential to be supramolecular probes. In 2013, H.X. Sun et al.[80] reported a cyanine dye supramolecular aggregate (Probe **8**).When it interacts with the biological macromolecule HSA, it will cause the supramolecular aggregates to transform. The aggregates cause a strong discoloration or redshift in the absorption band, and make the aggregates to fall into different light absorption ranges, thereby presenting different colors and fluorescence intensity .The probe combined with HSA shows significant fluorescence enhancement and short response time, which can be well used for HSA detection.

Dimethylaminobenzal rhodanine based fluorescent probes

Five-membered ring rhodanine and its derivatives play an important role in medicinal chemistry and pharmaceutical research due to its wide pharmacological activity. Many rhodanine derivatives have entered the clinical trial stage, showing antibacterial, antiviral, antimalarial and antitumor activities.[81,82] In addition to pharmacological activity, rhodanine is also a very good electron acceptor unit. Due to its good electron-withdrawing properties and it can maintain excellent electron injection efficiency, which HSA attracted widespread attention from researchers. By using dimethylamino as the electron donor (D), rhodanine as the electron acceptor (A), carbon-carbon double bond (-C = C-) or benzene or triazole with carbon-carbon double bond, etc as connecting bridges (π conjugates) to form a D-π-A system with TICT characteristics is an important idea for HSA fluorescent probe design.

X-j Peng's group[83] first designed such a probe **13**. Due to the TICT mechanism, HSA was lighted up with a yellow emission for its recognition in urine and serum samples, and it HSA good selectivity and sensitivity to HSA. Its detection limit reached 0.13 mg/L. It is important to note that probe **13** is located at the FA1 site that is differently from many other probes. Before that, in 2015, their research group also synthesized four environment-sensitive fluorescent dye (probe **9**, probe **10**, probe **11**, probe **12**) on the platform of classical D-π- A structure. All the four probes could induce great fluorescence enhancements with various emissions from 500 nm to 700 nm after locating in HSA cavities.[84]

Based on previous research, in 2018, Y-J Xu et al.[85] screened a highly selective HSA sensor probe **14** through two rounds of selective evolution by replacing different electron donors and electron acceptors. Its selectivity (~6 fold for HSA: BSA = 1:10), sensitivity (LOD ~ 5 nM, over 700-fold enhancement), and stability (over 24 h) are better than the former probes. It is worth noting that due to its very long response time, it may have more than one HSA binding site. In the same year, their research group developed another HSA probe **15** with a similar structure.[86] The difference is that they replaced the benzene ring with quinoline. And with this change, while maintaining its high selectivity and strong stability, the response time is greatly shortened, and it takes only 5 s to fully respond .This gives us a lot of inspiration, and we can improve its properties by replacing different connecting bridges (π conjugates) (such as naphthalene ring, coumarin, etc.)

Bodipy based fluorescent probes

BODIPY[87](4,4-difluoro-4-bora-3a,4a-diaza-s-indacene)-based fluorescent molecules HSA been widely used in the development of fluorescent probes due to its excellent characteristics such as high light stability, extinction coefficient and quantum yield, adjustable excitation and emission spectra, etc. In 2013, Jun Cheng Er et al.[88] synthesized a novel HSA drug site 2-specific fluorescent probe 16 based on BODIPY. More important, they found that emission of the probe 16 does not overlap with that of the Dan sulfonyl fluorophor, so they combined probe **16** and dansylamide (DNSA), a site 1-specific fluorescent probe, and developed a simple, high-throughput methodology for the examination of large collections of therapeutic drugs at HSA binding sites. However, an important drawback of BODIPY dyes is their relatively small Stokes shish, which may lead to experimental limitations. So in the same year, Jun Cheng Er et al.[89] doped triazole and piperidine in the 3 and 5 positions of the dual-function BODIPY nucleus, resulting in a very large Stokes shift in the dye. And through the screening of composite libraries, they screen a highly sensitive and species-selective HSA chemosensor probe **17** which binding site specificity at the FA1 site (heme site) of HSA. These results prove that BODIPY dye is a novel fluorophore for the development of fluorescent probes to detect HSA.

Flavonoid based fluorescent probes

It is well known that flavonoids have excellent anticancer properties as a wide range of natural products, but recent studies have shown that the specific affinity between the chromone ring of flavonoids and the central hydrophobic chamber of HSA may lead to the strong binding of flavonoids to HSA.[90–92] This provides a good inspiration for the design of HSA fluorescent probe. Given that, in 2016, B. Liu et al.[93] modified the chromone skeleton of flavonoids with an amide group to enhance the hydrogen bond interaction between the probe and HSA, and introduced the donor-π-acceptor (D-π-A) structure into the fluorophore to provide intramolecular charge transfer (ICT) characteristics. As a result, they synthesized three flavonoid-based D-π-A fluorescent dyes with different electron donors. Two of them (probe **18** and probe **19**) shows selectivity to HSA. Probe **18** showed high sensitivity, high selectivity and quantitative response to HSA in aqueous solution, as well as high tolerance to ambient pH, anti-interference and time insensitivity, indicating a promising sensing method for HSA.

Naphthalene based fluorescent probes

Due to its excellent optical properties, naphthalene and its derivatives are used as fluorescent modification units to synthesize a large number of different probe molecules with good structural properties through different routes to form

fluorescent sensors.[94] In 2017, Y.-R. Wang et al.[95] synthesized a naphthalene ring based ultra-sensitive and conformational sensitive fluorescent probe **20** (2-(((6-hydroxynaphthalene−2-group) methylene) malononitrile). It can distinguish natural albumin from denatuated albumin under physiological conditions, and it is worth noting that this is the first report to detect natural human albumin in complex biological samples using a practical dye-binding method.

Lophine based fluorescent probes

2, 4, 5-Triphenylimidazole is also known as Lophine. When it is oxidized by hydrogen peroxide in alkaline media, it can emit green light, which is the earliest chemiluminescence system in literature. These compounds not only have good properties of nonlinear optics (NLO), transparency and thermal stability, but also are novel two-photon absorption compounds.[96] Due to its excellent optical properties, many fluorescent probes based on Lophine derivatives have been developed. In 2013, N. Kishikawa et al.[97] synthesized a Lophine derivative probe **21** (4-[4-(4-dimethylaminophenyl)-5-phenyl 1*H* imidazole-2-yl] methyl benzoate). By studying its binding constants and binding sites with HSA under physiological conditions, it can be used for HSA detection. The probe binds to HSA through site two and emits strong fluorescence at 510 nm.

Rosamines based fluorescent probes

Rosamines[98] are derivatives of rhodamine dyes. Compared with rhodamine compounds, rosamine compounds do not have carboxyl group in the 2 positions, so they cannot form the inner lipid structure of five-membered ring like rhodamine compounds. However, the spectral properties of rosamine dye are similar to that of rodamine dye. The maximum absorption and fluorescence emission are close to the red light region (520-600 nm), which has a high fluorescence quantum yield, generally good light stability, and is not sensitive to pH. The applications of rosamines in fluorescent probes have been extensively reported.

In 2008, Ahn et al.[99] reported a screening of rosamine libraries for different macromolecules that led to the discovery of a potential HSA sensor (probe **22**). After combining with HSA, the fluorescence intensity increased by about 36 times, and showed high selectivity and sensitivity to HSA.

Julolidine based fluorescent probes

8-Hydroxy-galonidin-9-formaldehyde is an excellent fluorophile group, and the fluorescent probes containing the Julolidine group usually have good water solubility, which is conducive to the development of fluorescent probes that can be detected in water system.[100] In recent years, more and more fluorescent probes containing jilonidine have been developed, and HSA probes are no exception. In 2014, Wu et al.[101] made a fluorescent probe (probe **23**) by combining Julolidine with methyl cyanoacetate. In the presence of albumin, the probe showed a significant 400-fold fluorescence enhancement with high selectivity and sensitivity. The probe has been successfully applied to the quantitative determination of urinary albumin.

Carbazole based fluorescent probes

Carbazole derivatives are a class of diphenylamine molecules with isoelectronic structure. They have perfect rigid conjugate planes with blue fluorescence, they also have good delocalization, high stability, strong electron-donating ability and good Hole conductivity. They are a new class of organic optoelectronic functional materials with great potential, and their applications in the fields of organic photoluminescence, electroluminescence, and nonlinear optical materials have attracted increasing interest.[102,103] In 2016, Starting from the *N*-alkylation of carbazole, Dey et al.[104] performed a three-step reaction to synthesize a series of molecular probes with different molecular structures with a common carbazole nucleus to form a small chemical library. Through the study of the structure-optical signal relationship of the system, a carbazole derivative probe **24** with excellent performance was screened. This probe can not only sensitively and accurately detect HSA in biological fluids, but more importantly, it does not cause protein denaturation.

Fluorescein based fluorescent probes

Fluorescein[105] and its derivatives are important fluorescent probe materials. Because the two benzene rings are fixed on a plane by oxygen bridge bonds, the molecule HSA a rigid coplanar structure and can generate strong fluorescence under the action of excitation light. In the past 20 years, research on fluorescein has been a hot topic in the field of chemical and biological analysis. Researchers have modified the structure of fluorescein to improve its application performance. Modified fluorescein derivatives can be more sensitive and highly selective for the detection of specific organisms. In 2014, Smith et al.[106] began to modify fluorescein for use in HSA detection. They modified a fluorescent molecule 2′,7′ -dichlorofluorescein derivative that had been reported, and synthesized five fluorescent molecules of 2′,7′ -dichlorofluorescein derivative, then they selected the probe **25** that best bound to HSA through structural property screening.This probe allows for fluorescence measurements over 15 minutes, thus greatly improving the simplicity of detection.

Pyridinium cations based fluorescent probes

Pyridinium cations have been widely studied as electron-deficient groups with good biocompatibility and membrane permeability. Like the above-mentioned rhodamin, it is often introduced as part of the strong electron acceptance (A) of the D-π-A model of typical TICT molecules. In 2019, Zhang et al.[107] introduced pyridinium cation to the design of the HSA probe and successfully synthesized probe **26**. They used *N*, *N*-dimethylamino group as the strong electron donor (D) and introduced pyridinium cations as the strong electron acceptor (A). They also introduced a double bond

together with the benzene moiety as a bridging unit, giving the probe freedom Intramolecular rotation and the resulting TICT program. and they introduced hydroxyl groups into the diethylaniline skeleton to enhance the ability to donate electrons to promote the fluorescence intensity of the response. The probe has essentially no intrinsic fluorescence. When it enters the HSA hydrophobic cavity, the TICT procedure is inhibited, resulting in strong fluorescence and its detection limit reaches 4.8 nM.

Schiff based fluorescent probes

Schiff[108] base compounds are a class of compounds with large conjugated structures. Since Schiff first reported Schiff bases and their complexes, these compounds have been widely used in medicine and nonlinear optical materials. Due to their π-conjugated systems, they often have good photoelectric performance and exhibit excellent photosensitive effects, so they have been widely concerned in recent years. In 2017, P. Shen et al.[109] combine trianiline with benzo-15-crown-5moieties to synthesize a schiff base derivative probe **27** which detects HSA through the TICT mechanism. The probe showed weak fluorescence or no fluorescence in the physiological environment. After adding HSA, the crown ester core of the probe bound to HSA site 1, and the triphenylamine group of the probe bound to HSA site 2. Then the TICT process is suppressed and the fluorescence is enhanced. Its detection limit reaches 1.7 nM. The terminal 15-crown −5 ether group in probe 27 is important for HSA specific recognition because trianiline does not show the selective behavior of the protein. This also points the way for the design of HSA probes in the future.

GFP based fluorescent probes

GFP[110] has many broad applications in molecular biology, for example, as a powerful biological imaging tool. As a fluorescent label, GFP has been designed to be used as a fluorescent sensor for a variety of targets, including pH values, metal ions and small biomolecules. Over the past two decades, the synthetic analogue of the chromophore of GFP *p*-hydroxybenzylideneimidazolone (*p*-HBI) has been the subject of extensive research by many research groups. D. Xiao group successfully applied the GFP chromophore to the design and development of HSA probes. In 2016, they combined the p-HBI and GSH to synthesize a HSA probe **28**[111] that has significantly enhanced fluorescence by binding to HSA. It binds to HSA site 2 through the FRET process, showing high selectivity and sensitivity. In the next year, in order to further study the properties of fluorescence and expand practical applications based on p-HBI fluorophores, they combined guanine riboside (a basic part of the biological system, which is involved in many important cellular processes, such as protein synthesis, promotion of antibody formation, etc.) with the fluorophore p-HBI to synthesize the probe **29** with significant fluorescence enhancement to HSA. Probe **29**[110] is almost non-fluorescent, which is due to the key twist in its discolored state. When combined with the hydrophobic capsule of HSA, the flexible chromophore HBI exhibits a significant fluorescence enhancement due to its curing effect in a hydrophobic environment. GFP-based chromophore modification is also an important direction in the study of HSA fluorescent probes.

Dapoxyl dye based fluorescent probes

Dapoxyl dyes are highly sensitive fluorescent compound to the environment. Their fluorescence in water is very low, but the fluorescence intensity, stokes shift and extinction coefficient will change greatly depending on the pH and polarity of the solvent. The characteristic dyes of dapoxyl that are sensitive to the environment have been widely used to detect different organelles, including lysosomal and ER, as well as protein conformational and phosphorylation changes. In 2007, Min et al applied the combinational library method to the Dapoxyl scaffold, through solution and solid pHSAe synthesis, to prepare 80 Dapoxyl dye structures to form the Dapoxyl library. Through high-throughput screening, a probe **30** that is highly sensitive to HSA was screened out, which binds to HSA through site 1 and shows more than 55-fold fluorescence enhancement after binding to HSA.[112]

Sulfonyl based fluorescent probes

The sulfonyl fluorescent group is an attractive signal group for fluorescent chemical sensors because of its high emission quantum yield, large stokes displacement, high sensitivity to changes in the surrounding environment and easy derivation. Because of its excellent fluorescence properties, more and more probes based on sulfonyl fluorophore have been developed. In 2013, S.-J. Kim et al used dansyl as the fluorescent reporter group and 4-nitrophenylcarbamate as the dansyl group as the fluorescence quenching group to synthesize five compounds. Because HSA has pseudoesterase activity, it can hydrolyze the reactive acyl portion of the probe, cleave the urethane bond to release 4-nitroaniline and carbon dioxide, and eventually produce quinone methane as a temporarily unstable intermediate. Subsequently, a covalent bond is formed between the methylate of the quinone and the nucleophile near the active site of the enzyme, resulting in the formation of a probe-protein complex to achieve fluorescence enhancement. Through a series of experimental screenings, the probe **31** was screened to have a good response to HSA.[113]

Tricyano dihydrofuran based fluorescent probes

Tricyano dihydrofuran derivative is an optically non-linear luminescent material. This material is currently mainly used in optical technologies such as optical switches, communications, lasers, and optical storage, and semiconductors with a fast development speed. According to the structure of tricyano dihydrofuran, it is known that the cyano group has a strong electron-withdrawing effect, and also has a conjugated double bond in the structure, which has strong

stability. Due to its excellent optical properties, more and more researchers apply it to the design of a probe. In 2019, R. Choudhury, et al coupled a strong electron acceptor tricyanofuran with an ionizable phenol derivative (electron donor moiety) via an alkenyl-double bond to obtain two types of fluorescent probes based on a push-pull π electron system (probe **32** and probe **33**), Both probes bind to HSA via site 1 and both show significant fluorescence enhancement after binding. The difference is that probe **32** can effectively distinguish HSA/BSA, while probe **33** cannot distinguish HSA/BSA.[114]

Squaric acid dyes based fluorescent probes

Squaric acid dyes refer to substituted derivatives obtained by the condensation reaction of squanoic acid or its ester with an electron-rich group. In general, the structure of a typical squarylium dye contains an electron-deficient quaternary acid four-membered ring center as an electron acceptor and two electron-rich groups as electron donors, forming a strong intramolecular "Donor-acceptor-donor "(DAD) electronic structure. These compounds generally have special photophysical properties, such as excellent optical properties, good light and thermal stability, and strong ultraviolet absorption and strong fluorescence emission properties in the visible and near-infrared regions, which has expanded their applications to many technologies. Related fields have gradually attracted the attention of researchers. In 2010, Jisha et al synthesized three Squaric acid dyes with different substituents (probes **34**, **35**, **36**), and they all showed site-diselectivity and enhanced fluorescence. Unfortunately, they could not effectively distinguish HSA/BSA.[115]

AIE based fluorescent probes

From the discussion above, we can see that the design of HSA fluorescent probes is mainly based on the TICT mechanism, but there is another feature that is widely used in the design of HSA fluorescent probes, the AIE phenomenon.[116] In 2001, Tang and colleagues first reported the aggregation induced emission (AIE) phenomenon, in which molecules exhibit weak fluorescence in solution but strong fluorescence in aggregation .Since then, many AIE active molecules have been successfully used as fluorescent chemical sensor systems for metal ions, temperature, pH and biosensors. In 2010, Y.-n. Hong et al. first described a tetrstyrene derivative (probe **37**)[117] that HSA a aggregation induction effect to visualize and monitor human serum albumin. Then in 2017, Jianzuo Li et al. designed and synthesized AIE active amphiphilic molecules with dual functions, *N*, *N*′-bis [4- (3- sulfonyl propoxy)] salicylalkyl sodium (probe **38**).[118] In the molecule, the fluorophore and the hydrophilic sulfate group are connected by a flexible alkyl chain.Also, in the salicylic section Fluorophores are connected by rotatable N-N single bonds, and intramolecular hydrogen bonds ensure that only N-N bonds allow rotation, so their rotation can be restricted in the aggregation state .In particular, due to its amphiphilicity and negative charge on the sulfate base group.

Discussion

From the above, the design of the HSA fluorescent probe is mainly based on the following aspects:

1: Based on TICT mechanism The design of the sensor generally conforms to the D-π-A system. In the selection of fluorophores, fluorophores with excellent fluorescent properties, such as the benzoindole salts and 4-dimethylaminophenyl introduced above, can be used as fluorescent cores. And most sensors contain dimethylamino or diethylamino and other similar groups to enhance the electron donating ability. Most probes use carbon-carbon double bonds or benzene rings as π bonds, and connect strong electron-withdrawing structures such as rhodanine and pyridinium cations as electron acceptors to form D-π-A systems. The future focus of HSA probe design will be on the development of fluorescent cores with excellent properties in all aspects.

2: Based on the AIE effect: a certain type of probe molecule has fluorescence in the monomer state. When the molecule is aggregated into a cluster, due to the π-π stacking between the molecules, the fluorescence quenching effect of aggregation is caused, which makes the fluorescence quench after the presence of HSA in the system. Due to the secondary bond interaction of the hydrophobic cavity of HSA, the aggregated probe molecules are de-aggregated, the original fluorescence is re-opened, and the purpose of quantitative detection of HSA is achieved. Therefore, designing and developing molecules with AIE effect is another idea for HSA probe design.

3: Based on enzymatic reaction: HSA has pseudoesterase activity. The substrate undergoes a hydrolysis reaction with HSA, which breaks at the ester site to form a hydroxyl group and an acyl group, causing the hydroxyl end to become a free fluorescent molecule, and the acyl end is connected to Amino acid residues of HSA (e.g., lysine, tyrosine). So, a variety of specific fluorescent probes can be designed through enzymatic reactions (Figures 2 and 3; Table 3).

Nanoparticle-based methods

Nanomaterials refer to materials with at least one dimension in nanometer size (1-100 nm) in three-dimensional space. Nanomaterials with a size between 1 and 100 nm have surface effects, small size effects, and macro quantum tunneling effects. It has important applications in many areas such as optoelectronics, information processing, catalysis, energy conversion and storage, advanced defense technology, biomedicine, and environmental science. Since nanomaterials entered the field of sensing, their adjustable physicochemical properties have made them a strong candidate for sensing platforms. Researchers use different physical and chemical properties of different nanoparticles (shape, size, color, etc.) to design different types of sensors; they use different functions of different nanomaterials and combine them to build composite nanomaterials to achieve biological transmission of complex systems sense and enable the development of real-time, sensitive portable systems.[119,120]

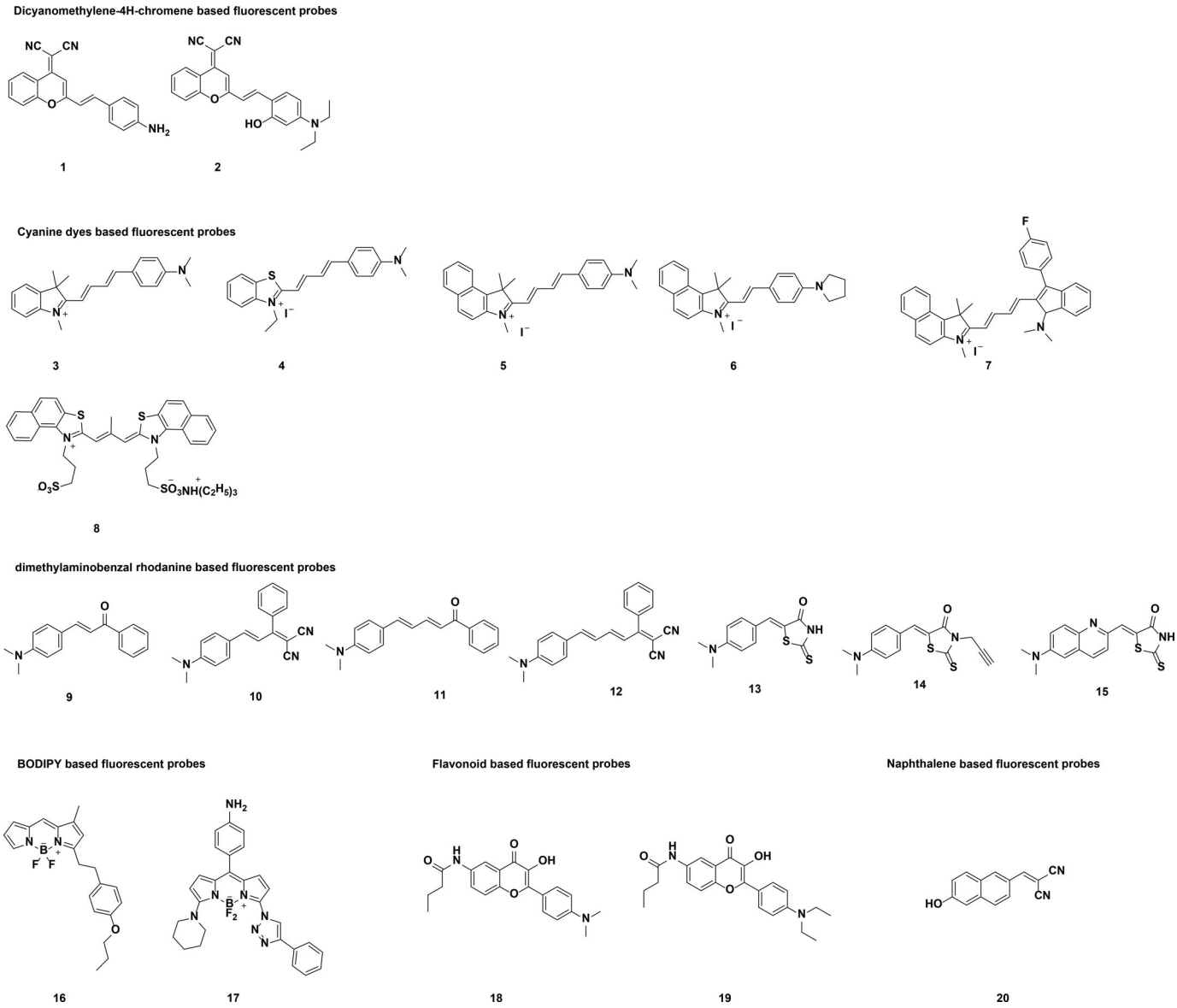


Figure 2. The structure of probe 1–20.

Gold-nanoparticle

Gold nanoparticles have been widely used in biochemical analysis and biomedical detection technology in recent years due to their rich and simple synthetic methods, high electrical conductivity, good water solubility, and biocompatibility. And it continuously developed into a method with high selectivity and sensitivity for analysis and detection. As a new type of light-emitting material, its excellent performance in optical sensing and light-emitting devices makes it widely used in scientific research and practical production. Here we summarize examples of researchers applying gold nanomaterials to HSA detection.[121–123]

A broad use of gold nanoparticles is to use it as substrates for surface enhanced Raman scattering (SERS) spectroscopy. On the rough surface of metal nanomaterials or aggregates, the SERS signal intensity is greatly enhanced. Therefore, more and more studies use SERS to detect various biological molecules. Among them, gold is a widely used metal substrate with SERS activity. Because it provides a highly enhanced SERS signal, it is easy to prepare and shows long-term stability. Many research groups have designed and optimized gold nanomaterials for HSA detection. For example, In 2011, Z-H Lin et al.[121] used gold nanoparticles as substrates and albumin blue 580 (AB 580 has a strong SERS signal and specific interaction with HSA) as reporting molecules to detect HSA based on surface-enhanced Raman scattering effect. This was the first SERS detection method using AB 580 as the report molecule and recognition element, which had high sensitivity and repeatability, and the detection limit was up to 70 pM. Then in 2019, F. Scaglione et al.[124] prepared a porous nano-gold material by de-alloying the amorphous melt-spun ribbon precursor (Au20Cu48Ag7Pd5Si20).Anti-HSA rabbit antibody (Ab anti-HSA) was then covalently attached to it through 4-aminothiophenol (4ATP) to give it functionalization. A nano-probe with SERS effect was developed to detect HSA, and its detection limit reached 0.1 ng/L.

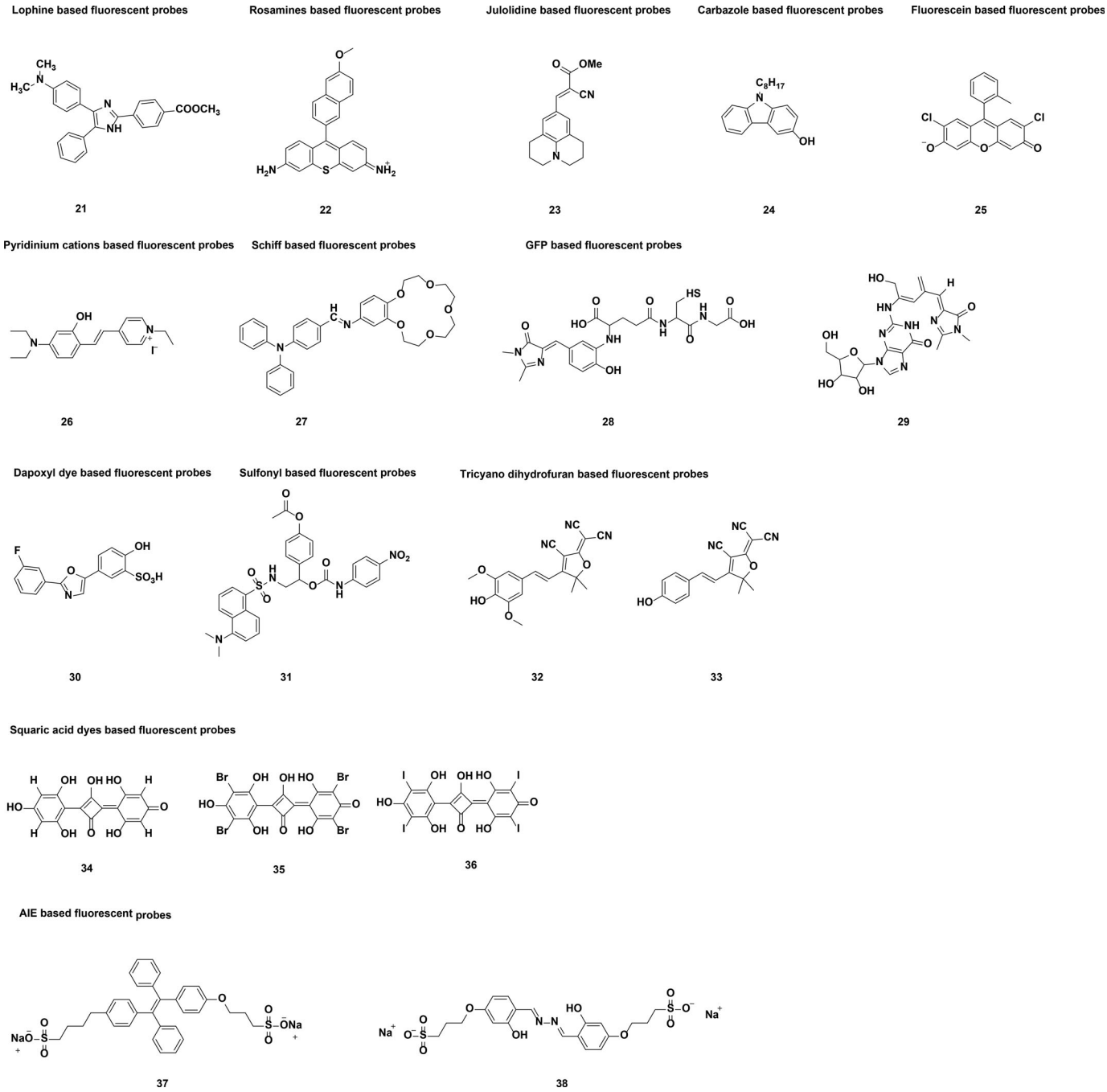


Figure 3. The structure of probe 21–38.

Different from the above that the gold nanoprobe is targeted by ligand modification, R.-D. Jean et al.[125] came up with a new concept for HSA detection of gold nanoparticles, that is, by adsorbing naked gold nanoparticles to a positively-charged nanocarrier of a self-assembled amphiphilic carboxymethylhexanoic acid chitosan (CHC), Electrostatic interaction between CHC nanocarrier, HSA and deposited gold nanoparticles can be adjusted by adjusting pH. So that HSA can be detected without the use of any ligand. Studies have shown that when the ratio of gold nanoparticles/CHC is 2, the detection limit reaches the lowest, which is 1.5 pM.

The other use of Au nanoparticles is for the preparation of biosensors because it is a highly conductive material with a large surface area and volume ratio, and can stably immobilize biomolecules that retain biological activity, which is a great advantage. In 2014, E. Arkan et al.[122] grew gold nanoparticles on the surface of multi-walled carbon nanotubes by electrodeposition, and then coated colloidal gold nanoparticles with thiol groups using 1.6-hexanedithiol (HDT) as a crosslinking agent. Finally, the prepared modified electrode was used as an immobilization matrix for HSA antibodies, and a gold nanoparticle-modified multiwall carbon nanotube impedance immunosensor was formed, which showed high sensitivity and stability, and the detection limit reached 15.4 ng/mL

Gold nanoparticles are also widely used as colorimetric probes due to their good water solubility and excellent biocompatibility. Well-dispersed gold nanoparticles appear red

Table 3. Summary of the properties of each probe.

Probe	Binding site/ Mechanism	$\lambda_{ex}/\lambda_{em}$ (nm)	LOD	Selectivity over BSA	Application	References
1	site I	520/620	5.51 mg/L	Yes	human urine	[72]
2	site I	530/650	1.26 mg/L	No	human urine and cell imaging	[73]
3	site I/ TICT	550/680	11 nM	Yes	human urine	[76]
4	site I/ TICT	580/680	26.16 nM	No date	human urine	[77]
5	site I/ TICT	580/708	8.91 mg/L	Yes	human urine	[78]
6	site II/ TICT	580/620	1.5 mg/L	Yes	human urine	[78]
7	site I/ TICT	540/676	0.66 mg/L	Yes	PBS buffer	[79]
8	Supramolecular aggregate	570/610	No date	No date	human urine	[80]
9	site I/ TICT	428/541,535	No date	No	cell imaging	[84]
10	TICT	428/647,590	No date	No	cell imaging	[84]
11	TICT	428/620,585	No date	No	cell imaging	[84]
12	site I&site II/ TICT	428/770,685	No date	Yes	human urine and cell imaging	[84]
13	FAI/ TICT	474/574,547	0.13 mg/L	Yes	human plasma and urine	[83]
14	More than 1 site/ TICT	436/508	5 nM	Yes	human urine and cell imaging	[85]
15	site I/ TICT	460/574	18.1 nM	Yes	human urine and blood	[86]
16	site II	365/585	No date	No date	No date	[88]
17	FAI	460/585,575	0.3 mg/L	Yes	human urine	[89]
18	site I	400/508	94 nM	No date	PBS buffer	[93]
19	site I	400/517	380 nM	No date	PBS buffer	[93]
20	site I&site II	482/562	0.05 mg/L	Yes	human plasma and urine	[95]
21	site II	370/510	6.7 mg/L	No date	human serum	[97]
22	site I&site II	490/554	No date	Yes	human urine	[99]
23	site I	440/490	0.4 mg/L	Yes	human urine	[101]
24	site I/ FRET	295/400	4 nM	No	human urine and blood	[104]
25	No date	470/520	No date	No date -	No date	[106]
26	site I/ TICT	480/600	4.8 nM	No	human serum and cell imaging	[107]
27	site I&site II/ TICT	360/500	1.7 nM	Yes	human urine	[109]
28	site I/ FRET	425/584	0.198 mg/L	Yes	human urine and serum	[111]
29	site I	485/585	0.015 mg/L	Yes	human urine and serum	[110]
30	site I	360/520,473	No date	No date	No date	[112]
31	No date	340/477	No date	No date	blood-contaminated tissue	[113]
32	site I	-/670	1.1 mg/L	Yes	aqueous solution	[114]
33	site I	-/630	3.4 mg/L	No	aqueous solution	[114]
34	site II	-/600,623	No date	No date	No date	[115]
35	site II	-/625,640	No date	No date	No date	[115]
36	site II	-/634,646	No date	No date	No date	[115]
37	site I&site II/ AIE	350/390,475	0.06 mg/L	No	Gel electrophoresis	[117]
38	AIE	355/508	6.11 mg/L	No date	No date	[118]

in solution, and the aggregated gold nanoparticles appear blue or purple in solution. The purpose of detection can be achieved through color changes. In 2015, Z-Z. Huang et al.[123] developed a simple colorimetric method for the quantitative detection of HSA through the anti-aggregation effect of gold nanoparticles in the presence of HSA. Melamine can induce the aggregation of gold nanoparticles, which can be suppressed when HSA is adsorbed on its surface. Based on this anti-aggregation effect, they used HSA and melamine as inputs, and the color change of gold nanoparticles as an output to form a logic gate for quantitative detection of HSA. This method shows good selectivity and its detection limit reaches 1.4 nM.

Gold nanoparticles have other applications, for example, In 2015, T. Wang et al.[126]used gold nanoparticles to enhance ELSD (evaporative light scattering detection), and developed a new method for detecting HSA. The HSA-gold nano conjugate showed a much stronger ELSD intensity than that of HSA alone. This method is simple, fast, sensitive, and HSA a detection limit of 21 ng.

Sliver-nanoparticle

Compared with the variable color of AuNPs with different particle sizes, silver nanoparticles are generally light yellow and the color does not follow the change in particle size. This limits its application in some aspects, but one of its advantages over gold nanoparticles are that the LSPR sensing of silver nanoparticles is sharper and stronger than the LSPR band of gold nanoparticles. This makes it widely used in the development of LSPR biosensors.[127]

LSPR is an optical phenomenon caused by the collective oscillation of electron gas in metal nanostructures surrounded by a dielectric environment. Local refractive index changes (such as refractive index changes caused by the interaction of biomolecules on the surface of the nanostructure) Monitoring by LSPR peak shift. The extinction and scattering spectra of nanoparticles and the peak wavelength (lmax) of the LSPR extinction maximum spectrum depend on the composition, size, shape, orientation, and local dielectric environment of the nanoparticles. In recent years, the application of nanoparticle-based LSPR has becomes a research hotspot.

For example, in 2010, T-Lai et al.[128] developed a biosensor based on the LSPR effect of silver nanoparticles. They immobilized anti-human albumin antibodies on the prepared silver nanoparticles by the amine coupling method and determined the HSA concentration based on the LSPR spectrum. The sensor detection limit is as low as 1 ng/mL.

Copper-nanoparticle

Compared with gold and sliver nanoparticles, copper nanoparticles have received less attention. However, compared to

Table 4. Comparison of various nanoparticles.

Type of nanoparticle	Advantage	Disadvantage
Gold nanoparticles	Rich and simple synthesis method, high conductivity, good water solubility, good biocompatibility	Easy to enrich in the body
Sliver-Nanoparticle	The LSPR induction of silver nanoparticles is clearer and stronger than that of gold nanoparticles	Its color does not change with the change of particle size Easy to cluster
Copper-Nanoparticle	High conductivity, low price, and rich source of precursors	Preparation technology of copper nanoparticles with controllable size and shape is immature
Self-assembled nanoparticles	Good light stability and biocompatibility, flexible design, durable optical properties and functions	The output is small, and the self-assembly mechanism needs further study

the precious metals gold and silver, metallic copper has high electrical conductivity, similar properties to gold and silver, and it is inexpensive, so it is widely used in industry. In addition, compared to the expensive gold nanoparticle and silver nanoparticle synthetic precursors, the precursors for the preparation of copper nanoparticles are relatively abundant, it is inexpensive and easily obtained from commercial sources, so copper nanoparticles are more suitable for a variety of other precious metal materials application. In terms of copper nanomaterials' sensing detection and biological imaging, the fluorescent properties of copper nanomaterials are mainly used. Copper nanomaterials synthesized by different ligands have different maximum emission wavelengths and different fluorescence quantum yields. Researchers also found that copper nanomaterials with different particle sizes (metal nanomaterials with different numbers of atoms) emit different colors of fluorescence.[129,130]

In 2017, M-J Chen et al.[128] developed a fluorescent method for HSA detection of poly(thymine)-template copper nanoparticles. Under normal circumstances, CuNP using poly(thymine) as a template can show strong fluorescence. In the presence of HSA, it will absorb copper ions, prevent the formation of copper nanoparticles, and cause a significant decrease in fluorescence intensity, thereby achieving the purpose of detection.

Self-assembled nanoparticles

For many years, molecular self-assembly HSA become a powerful tool for building soft functional materials, which have a wide range of applications in materials science and biology. Compared with individual molecules, many of these adjustable size soft structures have powerful functions. Small molecule organic dye nanoparticles are directly assembled into nanoparticles without nano-carriers, showing good light stability and good biological Compatibility and specific response to many biologically important analytes. More importantly, small molecule self-assembling nanoparticles have a variety of flexibility in molecular design, and are durable in optical properties and functions.[131]

We mentioned earlier that squaraine dyes are used in the design of HSA fluorescent probes because of their excellent properties. However, due to its poor water solubility, squaraine dyes generally tend to form aggregates so that they hardly emit fluorescence in aqueous solution. Based on this characteristic, in 2014, Palapuravan Anees et al.[132] developed a novel squaraine dye nanoparticle for selective detection of HSA. Squaraine dyes self-assemble to form non-fluorescent nanoparticles. The nanoparticles selectively respond to human serum albumin by triggering green fluorescence in the presence of other thiol-containing molecules and proteins, and its detection limit reaches 3 nM. However, the above-mentioned nanoprobes have not been used for imaging in vivo to prove stability and reliability under complex biological conditions, and there are no successful examples to demonstrate that nanoparticles assembled from squanoic acid can effectively penetrate cell membranes and image specific proteins in living cells. Therefore, in 2016, X-P Fan et al.[131] designed an assembly/disassembly non-covalent method based on novel cubic acid dye nanoparticles for the selective detection of human serum albumin in solutions and human blood. The probe can self-assemble into nanoparticles in aqueous solution through a variety of interactions (including p-p, C-Hp and hydrophobic interactions), which can effectively detect HSA, and this nanoprobe shows the ability to penetrate cells and successfully applied to fluorescent imaging in living cells (Table 4).

Biosensor-based method

In the recent decades of scientific development, biosensors have shown excellent performance in related detection, and have gradually become the object of extensive research by researchers in the world. Biosensors use immobilized biological components or the organism itself as sensitive materials, combined with appropriate chemical, physical, and biological detection materials to enable rapid detection of various physical, chemical, and biomass devices. Its main function is to use various detectors to detect the reaction between the target and the sensitive material. Then, the degree of the reaction is discernable, and then the size of the detection amount we need is calculated based on the identified signal results. Based on the transduction mechanism, the detection of biosensors can be classified according to different transduction mechanisms of the sensor: quality detection method (QCM), electrochemical and optical methods (colorimetric method, surface plasmon resonance SPR, surface enhanced scattering SERS, fluorescence method)).[133–135]

Quality detection method

This method is based on changes in substrate quality during hybridization. Many researchers have widely used quartz crystals and piezoelectric crystals made of metal electrodes such as gold and silver for the construction of

immunosensors. The combination of the molecule to be detected with the immobilized ligand molecule on the surface of the crystal causes a mass change, and this mass change causes a change in the crystal resonance frequency. For example, in 2002, Reza Saber et al.[136] developed an immunosensor by immobilizing anti-HSA antibodies on quartz crystals to detect HSA in aqueous media. Quartz crystal microbalance (QCM) is a label-free, real-time and label-free technology that is considered an effective detection tool for biological and chemical research.

Electrochemical method

This is one of the most widely used detection methods. It quantifies measurable electrical signals caused by ions or electrons produced by a chemical reaction between a biorecognizing element and a target analyte.[137,138] In 2011, K. Omidfar et al.[139] constructed an electrochemical immunosensor with a gold nanoparticle-labeled antibody and polyvinyl alcohol (PVA) modified screen-printed carbon electrode (SPCE) surface. It has high specificity and selectivity for HSA, and the detection limit is as low as 25 ng/ml. Then in 2016, J-Z Tsai et al.[140] developed a carbon electrode-based immunosensor based on screen printing. The electrode is made of a carbon paste of calcium carbonate and stearic acid, and the anti-human albumin antibody is fixed on the surface of the screen-printed sensor by a covalent fixing method. Quantitative detection of albumin was then performed using timed amperometric (CA) electrochemical measurement technology. The sensor also has HSA high specificity and selectivity, with a detection limit of 9.7 mg/ml.

Optical method

Optical technology is simple and can be easily automated. Optical technology is based on ultraviolet, visible, infrared absorption, fluorescence, and surface plasmon resonance (SPR). The main components of optical biosensors are lighting supplies, optical sensors and detection systems. The output conversion signals of optical biosensors are based on fluorescence, absorption, luminescence, surface plasmon resonance (SPR), reflection, and other optical phenomena. The optical sensor may be based on a measurement of the inherent optical properties of the analyte, the optical properties of the indicator dye or the labeled biomolecule.[141,142]

Surface plasma resonance (SPR) is an optical phenomenon that can be used to track biomolecular interactions in natural states in real time. This method has no damage to biomolecules and does not require any markers. First, a biomolecule (target molecule) is bonded to the surface of the biosensor. Then a solution containing another biomolecule (analyte) that interacts with the target molecule is injected and flows through the biosensor surface. The binding of biomolecules leads to an increase in the surface mass of the biosensor, resulting in an increase in the refractive index at the same rate, and changes in the biomolecular reaction are observed.

Surface enhanced Raman scattering (SERS) effect refers to the phenomenon that the Raman scattering signal of adsorbed molecules is greatly enhanced than that of ordinary Raman scattering (NRS) signal in the excitation region of some specially prepared metal good conductor surface or sol due to the enhancement of electromagnetic field on the sample surface or near the surface. SERS has attracted extensive attention in the biomedical field in recent years due to its excellent properties such as high sensitivity, fingerprint recognition ability, narrower spectral peak (fluorescence band of 1-2 vs 20-50 nm) and light resistant bleaching. By using the metal nanostructure to greatly enhance the electromagnetic field around the Raman reporter, the Raman signal can be significantly amplified and the sensitivity can reach the fetomolality concentration.These advantages made SERS imaging an ideal substitute for fluorescent molecular markers, which were used for ultra-sensitive, accurate and multi-channel biomarker detection

The fluorescent probes, SERS effect and SPR effect of nanoparticles, colorimetry, and so on that we introduced above all belong to optical methods to build biosensors.

In addition to these, there are many biosensors based on optical methods used in HSA detection. For example, quantum dot-based optical immunosensors.[143] Quantum dots (QD) have received a lot of attention due to their unique optoelectronic properties including high quantum yield, large extinction coefficient, long fluorescence lifetime, wide absorption spectrum and narrow PL emission spectrum. QD-based detection methods have been successfully applied to biological imaging, fluorescence analysis, and sensing systems. In 2012, M-C Tu et al.[144] developed a new type of optical immunosensor that uses CdSe/ZnS quantum dots to enhance the detection signal of HSA, and detects HSA through the change of photocurrent caused by fluorescence intensity. Then in 2016, W-y Gui et al.[145] developed a biosensor based on CuInZnS quantum dots (CIZS QD), which has better sensitivity and lower detection limit.

Of course, optical methods are not just the above. For example, R.E. Wang et al.[146] developed a homogeneous sensor that relies on time-resolved (TR) fluorescence resonance energy transfer (FRET) to generate robust sensitivity signals. A. Semeradtova et al.[147] developed an optical microchip based on high-affinity recombinant protein binding agent for HSA detection, C.-Y. Liao et al.[148] reported a fluorescence detection method based on AlGaAs/GaAs phototransistor, and so on. These methods provide different ideas for the development of fluorescence sensors (Table 5).

Immunochemical methods

The formation of the albumin/anti-albumin antibody complex is the mechanism by which immunochemistry detects albumin. Immunochemical methods can quantitatively measure albumin concentrations in serum and urine. Immunochemical methods are mainly sensitive to the detection of microalbuminuria and have high specificity. However, because of its time-consuming process, expensive equipment, and lack of accuracy, immunochemical methods

Table 5. Comparison of various Biosensor-based method.

Conduction type	Advantage	Disadvantage
QCM	Real-time, unmarked	Not suitable for smaller analytes, requires careful control of temperature and pressure
Electrochemical method	High sensitivity, simple and fast	Surfaces need to be functional, not real-time
colorimetric method	Simple and low cost	Higher detection limit
SPR	Real-time, label-free, high sensitivity	Vulnerable
SERS	High sensitivity and selectivity	Expensive equipment
Fluorescence method	High sensitivity	Need to label

are rarely used in routine analysis. In recent years, with the development of instruments and technology, its application in routine analysis has become simple and accurate. Immunochemical methods can be divided into following categories, Radial immunodiffusion, radioimmunoassay, enzyme-linked immunosorbent assay, fluorescence immunoassay, immuneturbidimetry, immunonephelometry, Particle Enhanced Turbidimetric Inhibition Immunoassay. Immunoturbidimetry and immunonephelometry are currently the most frequently applied immunochemical methods for the estimation of albumin. We summarize these immunological methods and compare their advantages and disadvantages as shown in the table below.[149,150]

Radial immunodiffusion

In this method, anti-HSA antibody is added to the agar gel, and the prepared agar gel plate is punched with a gel cutter, and a sample to be analyzed (including a standard solution, a test solution, and a control solution) is added to the well. The sample diffuses into the agar and forms a precipitation complex where albumin-antibody interactions occur. By staining with Coomassie blue, and then distinguishing the rings with dilute acetic acid, the diameter of the rings is measured, and the diameter of this precipitated protein ring is related to the albumin concentration in the sample.[151]

Radioimmunoassay (RIA)

This is a saturation assay performed in the liquid pHSAe in the presence of excess antigen. That is, the use of dual antibody technology in the liquid pHSAe, in which a known amount of radiolabeled albumin (125I-albumin) and albumin compete for antibody binding sites in the test sample. After the separation of free albumin, the albumin concentration in the sample was determined by the comparison with a calibration curve.[151]

Enzyme-linked immunosorbent assay (ELISA)

The ELISA method is a solid-pHSAe enzyme immunoassay technique that uses an enzyme-labeled antigen or antibody to quantitatively or specifically identify specific antibodies, soluble antigens, and cell surface antigens on a solid-pHSAe carrier. This method was first reported by Engrall and Perlmann in 1971. Competitive and sandwich ELISA are mainly used in HSA detection. The sandwich ELISA method refers to coating an anti-protein antibody on a plate, diluting the sample, adding it to the well of the plate coated with the antibody, and washing it three times. Since the antibodies capture albumin, they are not removed during the washing process. An enzyme-labeled secondary antibody is then added, and the labeled antibody binds the immobilized antigen (albumin). By adding an enzyme substrate to form a complex, and then adding a colorant, the color produced is positively related to the albumin concentration. The competitive ELISA method refers to coating an anti-protein antibody on a plate, adding a diluted sample with a certain amount of enzyme-labeled antigen, adding a substrate and a colorant, and the color produced is negatively related to the albumin concentration.[151]

Fluorescence immunoassay (FIA)

Immunofluorescence assay is a detection technology based on immunology, biochemistry and fluorescence imaging systems. The technique is based is based on antibody-antigen specific binding, in which fluorescently labeled antibodies can track antigens in tissue or cells. Immunofluorescence assay of albumin concentration involves the binding of fluorescently labeled antialbumin antibodies to the antigen (albumin) in the sample to form a complex, which is then analyzed by flow cytometry or confocal microscopy to detect HSA.[149,152]

Immunoturbidimetry

The main operating procedure of immunoturbidimetric method is to dilute the standard, reference, test sample and anti-human albumin antibody and add it to the reaction rate analyzer, then detect the absorbance change at 340 nm, and draw a calibration curve to determine the peak height and concentration. The method involves interacting an albumin sample to be tested with an anti-albumin antibody to form an insoluble albumin-anti-albumin complex, which causes the reaction mixture to become cloudy, and then measures HSA by measuring the absorbance at 340 nm.[151]

Immunonephelometry

A certain wavelength of light is irradiated along the horizontal axis, the light encounters the albumin-antibody complex through the solution and is scattered to different places. The intensity of the scattered light is proportional to the content of the complex, that is, the more antigen (albumin) to be tested, the more complexes are formed, the stronger the scattered light.[153]

Table 6. Comparison of various immunochemical methods.

Immunochemical methods	Advantages	Disadvantages
Radial immunodiffusion	less expensive and reliable;	time-consuming, and lack of automation
RIA	good precision;	albumin needs to be labeled with expensive radioactive reagents, radiation may cause potential hazards;
ELISA	faster,uses a small amount of anti-albumin antibodies;	competitive ELISA is less specific for albumin compared to sandwich ELISA;
FIA	the operation is simple;	limited in clinical settings;
Immunoturbidimetry Immunonephelometry	simple, less expensive, and can rapidly analyze a large number of samples;	requires an enormous amount of anti-albumin antibodies,High albumin concentration in the sample causes an underestimation of albumin levels.Appropriate sample dilution is required before measurement;
PETINIA	Not inhibited by high antigen concentration in the sample	Not suitable for marked albuminuria without properly diluted samples

Particle enhanced turbidimetric inhibition immunoassay (PETINIA)

This method inhibits particle agglutination by the competitive binding of polyclonal antibodies to antigen (albumin) fixed columns and antigens in the sample. The increasing rate of absorbance is inversely proportional to the concentration of analyte in the sample. This method successfully maintains the sensitivity of the method using latex beads without affecting the front zone phenomenon (Table 6).[154,155]

Chromatography based methods

Chromatography is a common separation and analysis technique, early in 1977, it was used for the purification of HSA. Recently, High-performance liquid chromatography, liquid chromatography coupled with tandem mass spectrometry (LC/MS) has been described as a promising technique for the determination of microalbuminuria in human urine. This method is proved to be more accurate than immunochemical methods. However, the high cost of instruments limits their application in routine clinical estimation.[156,157]

Electrophoresis-based methods

Electrophoresis means that the charged test product (protein, nucleotides, etc.) is in an inert support medium (such as paper, cellulose acetate, agarose gel, polyacrylamide gel, etc.). Under the action of the electric field, each component swims at its own speed in the direction of its corresponding electrode, so that the components are separated into narrow bands, and the electrophoretic band spectrum is recorded or the content(%) is calculated by a suitable detection method.[158]

Early in 1935, Tiselius developed an electrophoresis apparatus and realized the separation of HSA. In order to make electrophoretic separation cheaper, faster and more reliable, different types of support matrixes such as starch gel, polyacrylamide gel, cellulose acetate gel, and agarose gel are used for better electrophoretic separation. In 1966, an anti-albumin antibody electrophoresis technique was used for the rapid quantitative estimation of albumin in agarose gel. E.B. Duly, S. Grimason used a capillary zone electrophoresis (CZE) technique for the determination of serum albumin, this technique provides more specific results than bromocresol green(BCG).[159–161]

Conclusion and outlooks

The concentration of HSA is related to many diseases and is a diagnostic marker of chronic kidney disease, hepatitis, diabetes, and hypoproteinemia, so its abnormal levels in different body fluids are also a potential parameter for preclinical diagnosis. At present, the quantitative detection of HSA in biological fluids has become an urgent means of preventive medicine and treatment. The detection technology that distinguishes HSA from BSA with low selectivity and low detection limit detection HSA to quickly and accurately detect HSA in different body fluids has very important clinical significance. In addition, through the study of interaction with HSA, many near-infrared probes have also been developed for biological imaging.[162] This article summarizes the various methods of HSA concentration detection in recent years, including dye-binding methods, immunochemical methods, chromatography, fluorescent probe methods, biosensor-based detection methods, etc. Among them, the fluorescent probe method and the method based on biosensor detection are hot topics in recent years. More and more fluorescent molecules based on different characteristics and biosensors based on different materials have been developed for detecting HSA. However, the methods currently used for clinical detection are still classical dye-binding methods, immunological methods, etc. The methods based on fluorescent probes and biosensors have a long way to go before they can be used for clinical detection. We believe that with the continuous improvement of technology and the deepening of research, more and more researchers will develop more comprehensive methods for clinical HSA detection.

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