# HSADab: A Comprehensive Database for Human Serum Albumin

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**Abstract:**

Human Serum Albumin (HSA), the most prevalent protein in human body fluids, is integral to the transportation, absorption, metabolism, distribution, and excretion of drugs. Its influence on a drug's therapeutic efficacy is substantial. Despite the importance of HSA as a drug target, the available data on its interactions with external agents (e.g., drug-like molecules and antibodies) are rather limited, which poses challenges for both molecular modelling investigations and the development of empirical scoring functions or machine learning predictors on this target. Moreover, the reported entries in existing databases often contain major inconsistencies due to varied experiments and conditions, which incurs worries about the data quality. To address these issues, we established a pioneering database through extensively reviewing more than 30000 scientific publications published between 1987 and 2023, encompassing over 5000 affinity data at multiple temperatures and more than 130 crystal structures that involve both the ligand-bound and apo forms. The current HSADab resource (www.hsadab.cn) serves as a reliable foundation for protocol validations of

molecular simulations (e.g., traditional virtual screening workflow using docking, end-point and alchemical free energy techniques) as well as the data source for the implementation of machine learning predictors.

**Key words: Database, Webserver, Binding Affinity, Crystal structure, Human Serum Albumin**

## 1. Introduction.

Human serum albumin (HSA), identified as FDAHT; PRO0883; PRO0903; PRO13411, 2, the most prevalent protein which constitutes 60% of protein mass and is found in plasma, lymph, saliva, cerebrospinal, and interstitial fluid3, is a monomeric multidomain macromolecule. It plays a pivotal role in determining plasma oncotic pressure and is the primary modulator of fluid distribution between body compartments4. Genetically, HSA is located on the long arm of chromosome 4, close to the centromere. It is divided into 15 exons by 14 intervening introns, with the final exon being untranslated 1, 5. Notably, it is readily soluble in salt solutions within the pH range of 4.0 to 8.5 and remains stable in aqueous environments6. HSA has garnered increasing interest (Figure 1a-b) due to its curial role in influencing drug behavior in vivo. HSA is a complex protein composed of 609 amino acids, with a molecular weight of 66.5 kDa and a maximum circulatory half-life of up to 19 days in human.7 Structurally, its secondary formation includes 67% α-helix, 23% stretched chain, 10% β-sheets as well as bends. HSA features a heart-shaped globular conformation, containing three homologous domains typically referred to as I (5–195), II (195–383), and III (384–582). Each domain is further divided in two subdomains, A and B, which are composed of four and six α-helices, respectively.7 HSA contains 9 fatty acid (FA) binding sites.

HSA is recognized as a potent prognostic marker in both the general population and various pathological conditions. Low serum albumin levels are associated with the onset of numerous cardiovascular and metabolic diseases8, 9. HSA possesses catalytic capabilities, enabling it to hydrolyze the carbamate carbaryl10, β-lactam drugs11 and other drugs. The development of recombinant human albumin primarily targets its use as a component in pharmaceutical formulations, often substituting human-derived serum albumin12, 13. This innovation is particularly relevant for enhancing drug efficacy. For instance, researchers have employed HSA to attach or encapsulate drugs14-16 to improve drugs' distribution and metabolism, thereby enhancing its bioactivity and therapeutic impact.

Any drug, regardless of its method of administration—oral, intravenous, sublingual, subcutaneous, or intramuscular—is transported through the blood, where its initial interaction occurs not with cellular components but with plasma proteins17. Therefore, HSA is extremely significant for drug transportation, absorption, metabolism, distribution, excretion and toxity18. Most small-molecule drugs can bind reversibly to plasma proteins, mainly serum albumin and alpha-1-acid glycoprotein. Considered a crucial factor in drug discovery, the interaction between drugs and plasma proteins significantly influences pharmacokinetics and pharmacodynamics (PK/PD). This includes aspects such as clearance, exposure, half-life, and distribution volume2, 19. Only 5.2% clinic trial of small molecules (Figure 1c) can be finally successful after the 3 three phase clinical trials due to the awful absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties20. The binding interaction between drugs and HSA is magnificent in pharmaceutical distribution, metabolism, excretion, and toxicity (DMET). When the drugs absorbed in blood, HSA interacts with medications, impacting their pharmacokinetics by affecting drug concentration levels in the blood (Figure 1d). In binding state phase, the drugs show no bioactivity, no transmembrane ability, no glomerular filtration, and little affected by metabolism. This interaction influences the time it takes for the drug to reach its peak concentration, the peak drug concentration, the drug's half-life, and etc. Consequently, it indirectly affects the DMET of the medication. Therefore, binding affinities on HSA serve as a proactive approach to influence the pharmacodynamics and pharmacokinetics of preclinical drug candidates 21.

Despite the central importance of HSA, no existing database with comprehensive information of HSA-drug interaction (e.g., binding affinities and bound structures) is available. The absence of a high-quality collection of structural data not only poses a challenge in understanding the interaction patterns of HSA and external agents, but also blocks the possibility of extracting detailed insights into the conformational

landscapes of the apo protein as well as the binding-induced conformational changes through simulation techniques[22-25]. Further, the absence of affinity data serves as a critical bottleneck of AI-driven pharmaceutical research. Although there are some existing data sources[26-30], the number of data entries is very limited (e.g., sample size ~50), the data records are incomplete (only a fraction of information in original experimental references are recorded), and the experimental conditions (e.g., temperature and pH values) pivotal for binding strengths are generally excluded.

To address these shortcomings, we have developed a new database named human serum albumin database (HSADab), which provides a solid foundation for the elucidation the interactions between molecular structures and HSA with molecular modelling technique and serves as a high-quality data source for the development of machine learning models for protein-ligand complexes. This database is designed to overcome the limitations of existing resources in terms of both data diversity and precision, thereby supporting more effective drug development research.

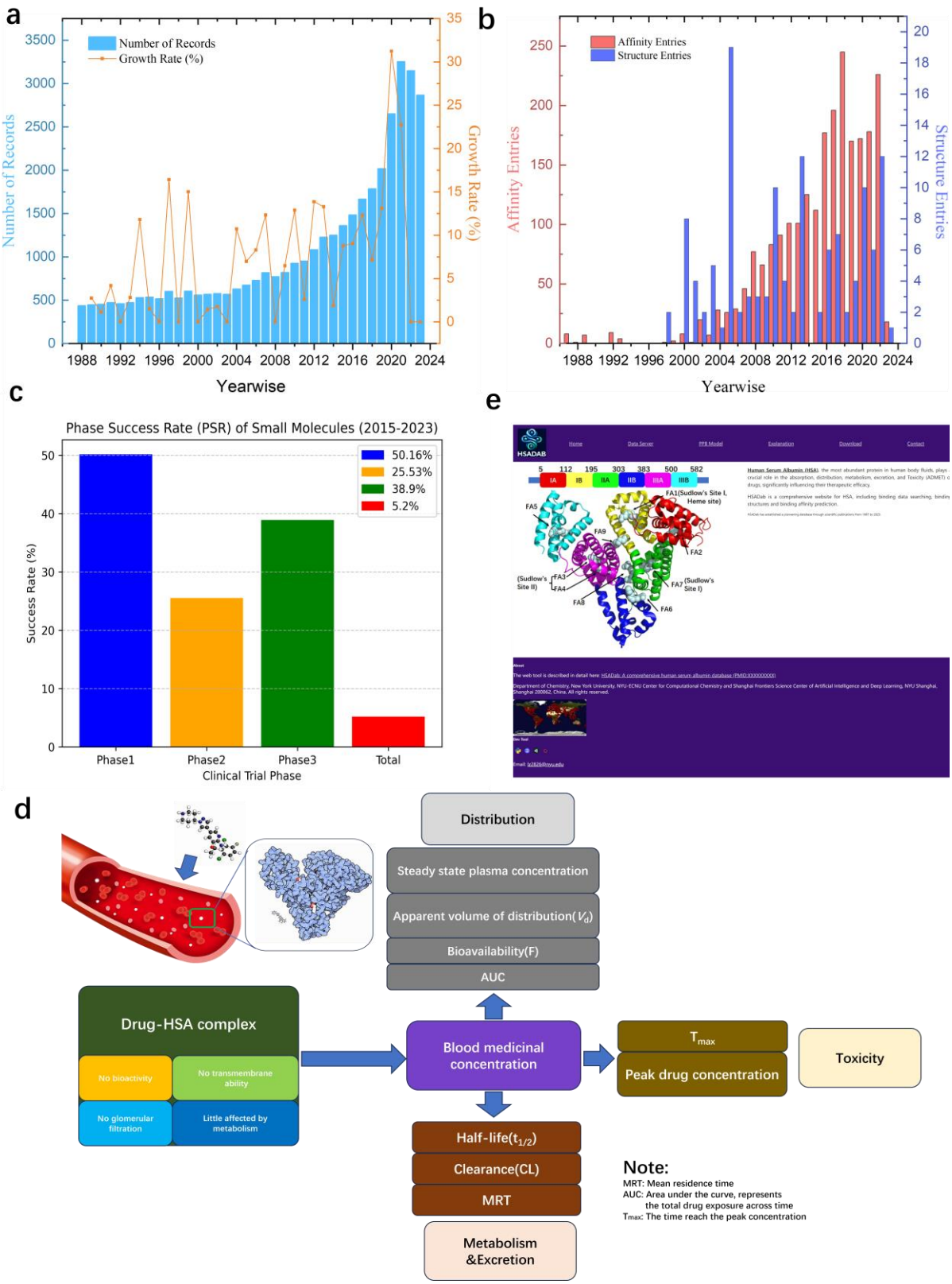


**Figure 1**. a) The number of publications relating to HSA from 1987 to 2023 given by Scifinder, b) the numbers of affinity and structures entries from 1987 to April 2023, c) the phase success rate of small molecules from 2015 to 2023, d) the relationship between HSA-drug interactions and DMET, and e) the webserver of HSADab.

## 2. Principle in data acquisition.

HSADab is a comprehensive database containing affinity and structure data. The data entries are extracted from literatures published before April 2023. The database is freely available in the HSADab webserver (www.hsadab.cn), a glance of which is provided in Figure 1e. The website provides detailed statistics and physiochemical properties of molecules binding to HSA, the visualization of the structures and many other functions. The database maintained by us would be updated periodically to include additional entries deposited recently.

To construct the database, we grab over 30000 references from Scifinder, after which an extensive review is performed. The database is designed to facilitate research on ligand-HSA interactions, in order to enable deeper insights into drug transportation. Therefore, the affinity bank is constructed primarily on small molecules (i.e., drug-like molecules). In the structure bank, due to the small number of experimentally deposited structural data, we collect all HSA-involved structures available so far, including not only the apo form of the protein but also the bound form with either drug-like ligands or antibody chains as the external agent. In total, we accumulate a substantial collection of ~2600 original reference entries with multi-temperature data and various details about experimental conditions, encompassing ~5000 affinity points, and ~130 structures that contain both the apo and the ligand-bound forms of HSA. The structure of the database is shown in Figure 2.

**Some details about data accumulation.** Modified HSA forms, such as those altered through glycation, S-nitrosylation, or recombination, are not included in the database. We ensure that the pH of HSA experiments is approximately 7.4, within a range of 6.8 to 8.0, as the experimental pH significantly impacts the value of binding interactions [31].

HSADab does not include data on organic-metal complexes that bind to HSA. This exclusion is due to limitations in the SMILES (Simplified Molecular Input Line Entry System) notation, which inaccurately represents coordination bonds between metal elements and organic compounds. In SMILES, these bonds are often mistakenly depicted as salt bonds, leading to incorrect structural representations of organic-metal complexes. In instances where small salt molecules dissociate into anions and ions in solution, our focus is solely on the organic component that predominantly interacts with HSA. Additionally, we exclude data on covalent binding as it is irrelevant in our context. The molecular structures in our database are meticulously recorded using isomeric SMILES notation.

The primary data are accumulated in experimental reference published between 1987 and 2023. Significant improvements and evolution are experienced in this long time period. As a result, our data entries incorporate various experimental methods, including Fluorescence Resonance Energy Transfer (FRET), Isothermal Titration Calorimetry (ITC), and UV-Vis spectroscopy, and many others. A direct result is the difference between reported properties (e.g., kinetics) aside from the binding thermodynamics. Further, different methods have different weaknesses and accuracies. We therefore include many details of experimental measurements into the data entries, in order to make the records as specific as possible. In section S1, we briefly review the experimental methods used by literatures to secure the binding thermodynamics.

While the binding thermodynamics are of central importance in HSA-ligand interactions, other properties such as the binding site description and binding kinetics are also of relevance. Therefore, we additionally include many other information either from literatures or from in-house calculations, e.g., the binding site information, the solvent-accessible surface area and so son. In section S2-S4, these additional recorded data are briefly explained.

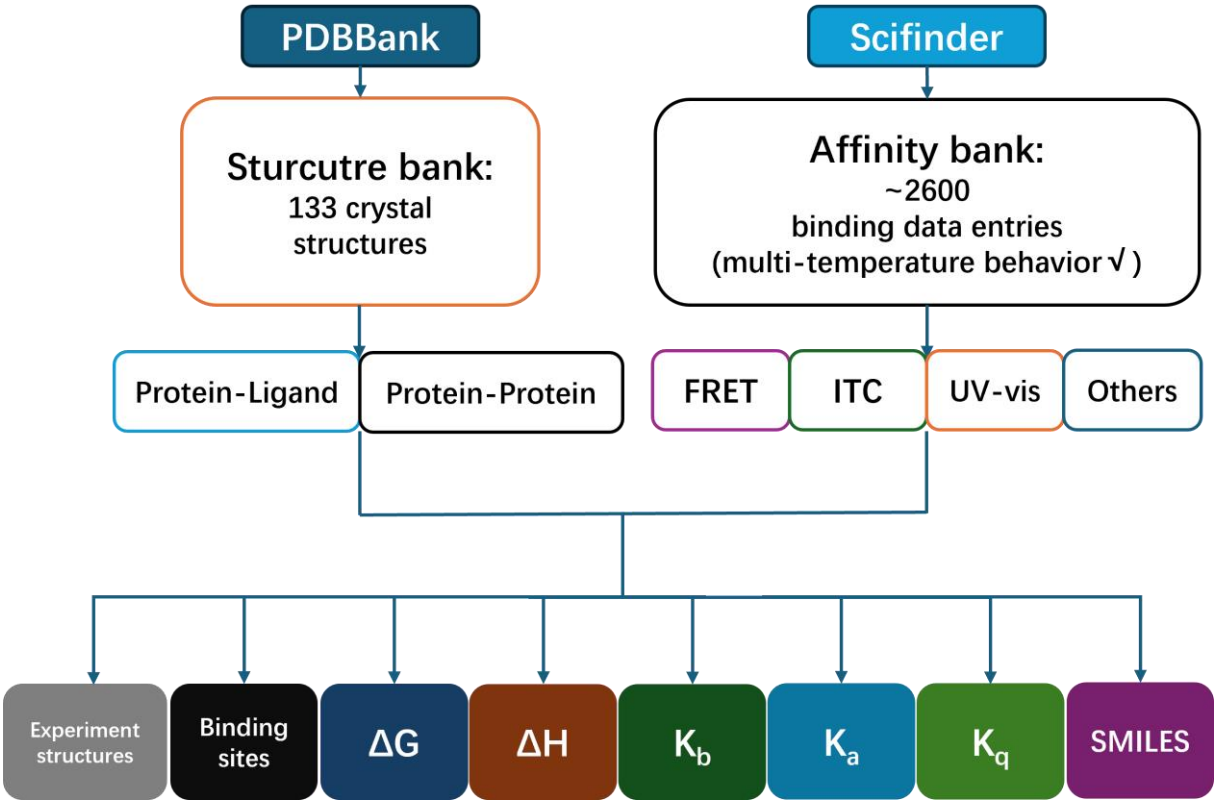


**Figure 2.** The structure of HSADab. The data entries are extracted from existing literatures searched with Scifinder and RCSB PDB. The structure bank contains all HSA-involved structures of biomolecular assemblies involving both the apo protein and the bound forms (with either small molecules/ligands or antibodies/proteins), and the affinity bank deposits ~2600 binding data entries with a multi-temperature behavior measured with multiple experimental techniques.

## 3. Overview of HSADab.

### 3.1. Affinity bank of HSA-ligand complexes.

The data entries in the affinity bank contains not only the binding thermodynamics and the environmental information (e.g., temperature and pH), but also includes relevant details and parameters measured experimentally (such as rate constants and binding stoichiometry). The raw data are summarized in a format defined by Table S1 (general information) and Table S2 (experimental measurements). The distribution of the records number is presented in Figure 3a. The type with the highest population (37.78%) features more than 18 records in each entry and corresponds to ~1000 entries in the affinity bank, which is demonstrating the coverage of our dataset. Among those HSA-bound ligands deposited in our database, there are 747 entries available in Drugbank, some details of which are available in Table S3.

With the SMILES formula of each molecule provided in the affinity bank, the binding thermodynamics and the affinity values could be paired exactly. To illustrate the diversity of the features of the drug-like molecules included in the affinity bank, we compute the Shannon diversity index of integer molecular features from SMILES in our dataset. A Shannon Diversity Index over 1 in the dataset indicates a good level of diversity with a relatively balanced representation of different categories in the feature being analyzed. The large entropies in Figure 3b obviously suggest a considerable level of diversity within the molecular structures under study.

To obtain some insights into the intrinsic behavior of different experimental measurements, we compute the distributions of binding free energies across different methods, including FRET or Fluorescence spectroscopy, ITC, and UV-Vis spectroscopy, and other methods as a collection of the other experimental measurements with small sample sizes, as shown in Figure 3c. Interestingly, while the FRET and ITC distributions exhibit a high consistency in both the shape and range and a close-to-Gaussian behavior, UV-Vis and other methods seems less regular. A note to add is that there are several entries with unreasonable affinities values (e.g., ~-200 kJ/mol), which are excluded in this analysis and should be used with caution.

Finally, we expect to grab some insights into the correlation between molecular features and their binding strengths towards the HSA target. To this aim, we compute the molecular features used in Figure 3b, Morgan fingerprints and MACCSKeys with their SMILES formula, perform a dimension reduction analysis with the t-SNE regime and color the data points with the corresponding binding affinities. The 2D projection is presented in Figure 3d, where the gradient of colors within some clusters may indicate a relationship between structures and affinities. Interestingly, there is no clear separation between the high- and low-affinity regions, indicating that the molecular descriptors may be insufficient to distinguish between stronger and weak binders.

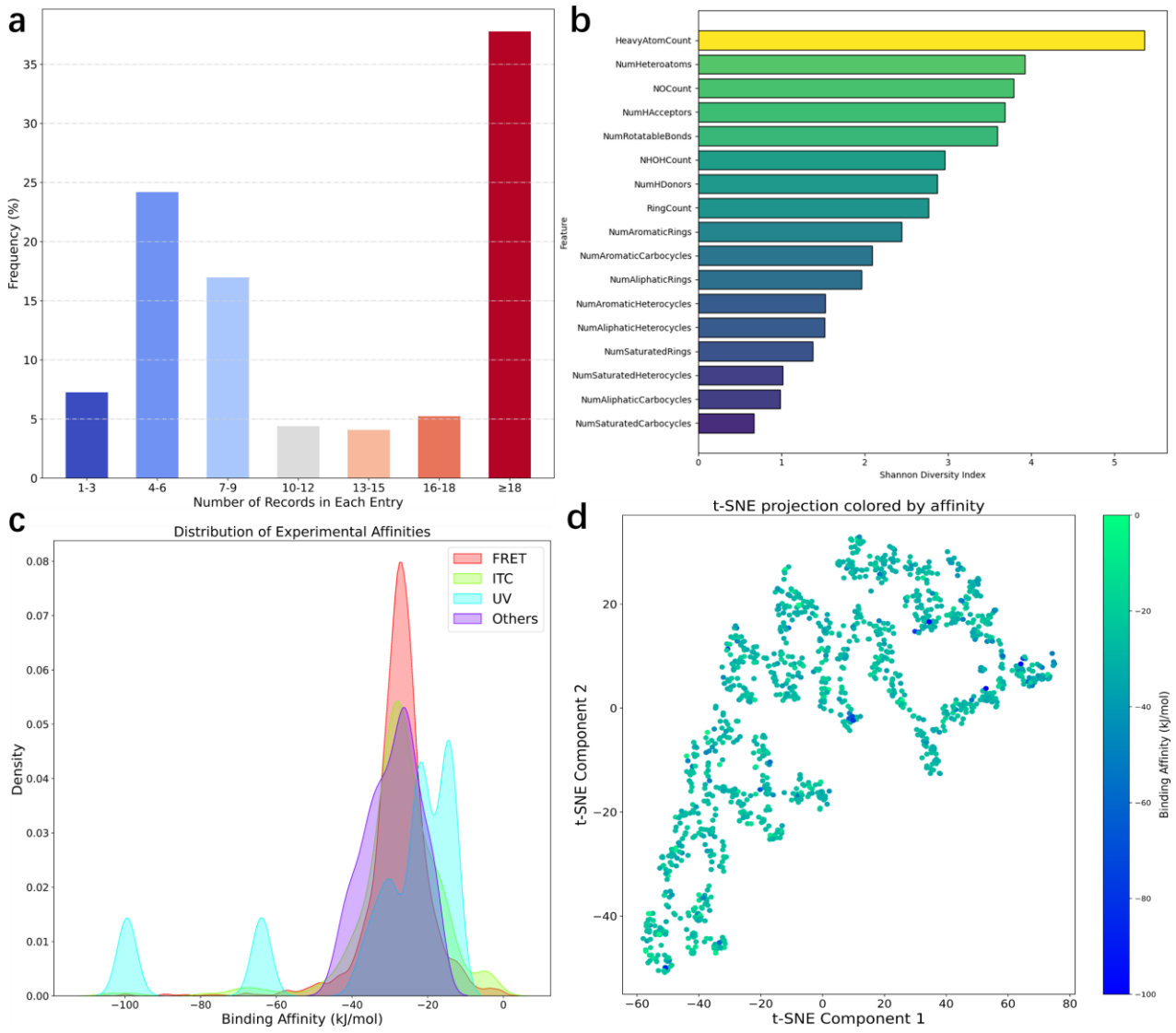


**Figure 3.** a) The distribution of total data quantity in each entry of original binding data, b) The Shannon

diversity index of 18 molecular features, c) the $\Delta G$ distribution of thermodynamic parameters from different experimental measurements, d) 2D Embedding with t-SNE based on physical properties, Morgan fingerprints and MACCSKeys fingerprints of small molecules.

### 3.2. Structure bank.

The structures of HSA-involved complexes and its apo form are limited in number, compared with the affinity data. Even with extensive literature review, we secure only ~130 structures from various sources. Sequence alignment and comparison are performed to verify the consistency between different protein structures. A list of the bound ligands is provided in the supporting xlsx file of the database.

A unique feature of HSA is the presence of multiple FA binding sites.[32-34] Sub-domain IB accommodates FA1, while the junction between sub-domains IA and IIA holds FA2. Sub-domain IIIA, which includes Sudlow's drug-binding site I, comprises FA3 and FA4. FA5 resides in the cavity of Sub-domain IIIB. The boundary between sub-domains IIA and IIB is the location for FA6. FA7 is positioned in sub-domain IIA, close to Sudlow's drug-binding site I. Meanwhile, FA8 and FA9 are located in the niche formed by sub-domains IB, IIA, and IIIA, adjacent to both Sudlow's drug-binding sites I and II. These intricate structural details, including the FA sites, are depicted in Figure 4. Sudlow's drug-binding sites I and II exhibit similar chemical properties[6] and are the most frequent location of binding for most drugs.[35] Many drugs can bind a large hydrophobic cavity that located in subdomain IIA. Furthermore, some published studies have indicated the existence of an additional drug binding site characterized by high affinity (site III, FA1 also called Heme site in subdomain IB)[36-39]. Interestingly, we identify several ligand-binding sites that are not explored/discussed in previous literatures during our examination of the crystal complex structures. Specifically, these are four non-FA binding sites (Figure 5a), which are IA/IIA (PDB ID: 5Z0B), IB/IIA (PDB ID: 4LB9), IIIA/IIIB (PDB ID: 6R7S), and IA/IIA (back) (PDB ID: 7WLF), respectively.

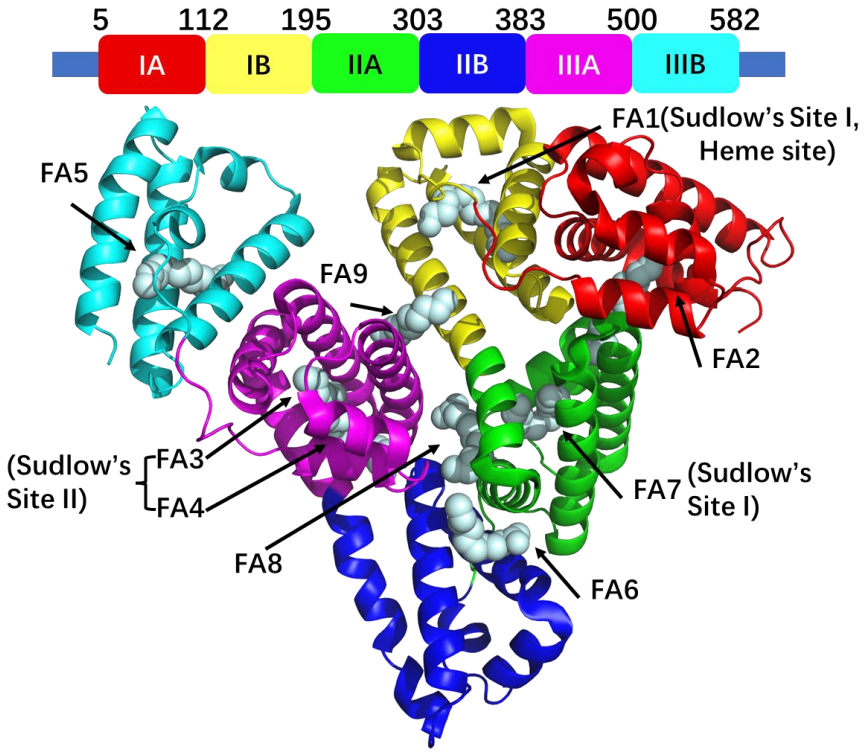


**Figure 4.** Modular domain organization of HSA and FA binding sites. PDB ID:6WUW and 1E7E.

External agents are not equally populated in different pockets, but exhibit certain preferences in the locations of binding. This behavior is caused by the intrinsic properties (e.g., sizes and chemical properties) of these pockets. According to the occupancy of the ligand-binding pockets shown in Figure 5b, the ligands show a great preference to FA7 (33.7%) and FA1 (25.0%), which are also known as Sudlow's Site I and III. The next highly populated pocket is Sudlow's Site II (FA3+FA4+FA3-4) with 19.9%. While the Sudlow's sites I and II (33.7% and 19.9%) are found highly populated also in molecular docking studies, the population of Sudlow's site III is unexpectedly high (25.0%). Such a major inconsistency could imply the potential problems in docking studies, and thus the unreliability of the docking outcomes.

Only FA1, FA3+FA4+FA3-4, FA7, and FA5 are deep cavities formed by one subdomain, whose cavity volume all are over 1000 $Å^3$ (we treated FA3-FA4 as an entirety). As shown in the pocket-forming residues of all sites in Table S4, the compositions of all pockets are generally similar, or at least do not exhibit significant differences. However, FA5 encapsulates the smallest numbers of non-straight-chain-saturated-fatty-acids (5.6%) among the four pockets. According to structural analysis (Figure 5c), we identify that the small entrance of FA5 could be the reason that repulsively reject the entrance of the external ligands.

Aside from the properties of protein pockets, we also analyze the nature of protein-ligand interactions with experimental structures available in the structure bank. As shown in Figure S1a, common interaction patterns such as hydrogen bonding, pi-pi stacking and halogen bonding could be observed in HSA-ligand complexes. In accordance with many previous literatures, FA7, FA1, and FA3-4 are among the pockets with the largest numbers of interactions, and the typical (most prevalent) interaction type is hydrogen bonding. Interestingly, the FA8 pocket that lies at the niche formed by sub-domains IIA, IIB, and IIIA also has a large number of critical pocket-ligand interactions. Further, FA8 is not a pocket dominated by hydrogen bonds but favors many hydrophobic interactions, which compensates the shortage of hydrophobically inclined pockets. The residue-specific information of hydrogen bonds presented in Figure S1b highlights the diversity of the types of such interactions.

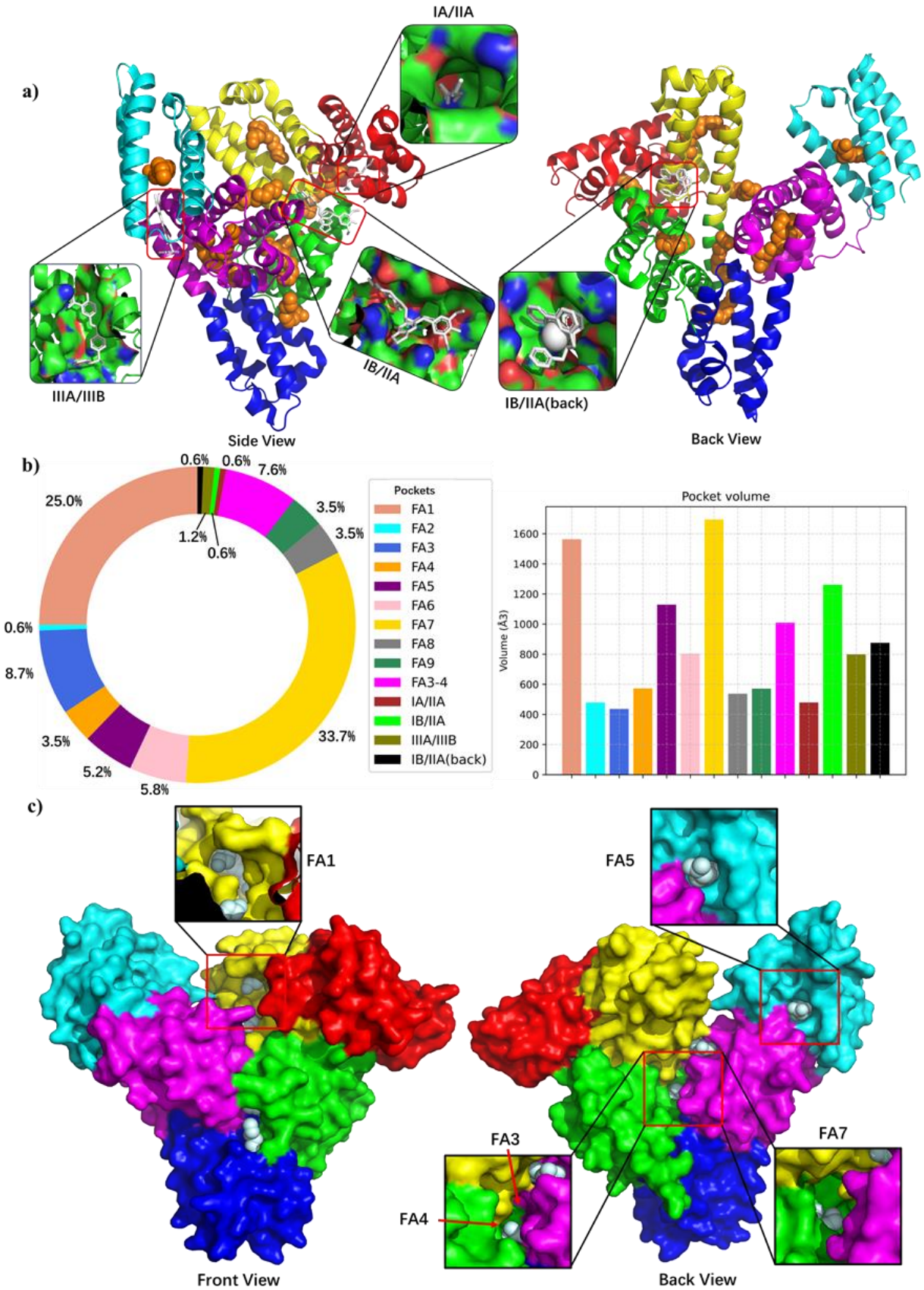


**Figure 5.** a) The locations of the three non-FA binding sites, with FAs in orange and ligands in the sites in white. b) The doughnut diagram of all the ligands in crystal structure excluding straight chain saturated fatty acids along with the pocket volume computed with GROMACS 40. c) The pocket entrances of FA1, FA3-4,

FA5, FA7. The pockets FA3-4 and FA7 share the same large entrance.

## 4. Concluding Remarks.

In the current work, we establish a largest and most comprehensive database for HSA. Through extensive literature review (more than 30000 publications), we summarize more than 5000 affinity data from ~2600 original references and ~130 crystal structures involving HSA. Detailed analyses are performed on both the affinity and structure banks.

Concerning the affinity bank, experimental methods for affinity measurements are diverse, and there are three methods employed most frequently, i.e., fluorescence spectroscopy, ITC, UV–vis. The data entries in our database not only contain the binding strength, the SMILES representation and the temperature label, but also include other factors/parameters (e.g., rate constant and binding stoichiometry) that are often overlook by other databases. The accumulated data entries are cleaned and summarized in a machine-readable format. These high-quality datasets would, obviously, pave the way for AI drug design and open new avenues for exploring drug efficacy and safety.

The development of the HSADab provides an essential resource for understanding how small molecules interact with HSA. In the structure bank, the experimentally deposited structures cover both the bound and unbound species. A unique behavior of HSA is the existence of multiple pockets (>10) for drug binding. Different pockets have different structural features. For example, the volume of pockets ranges from 400 $Å^3$ to 1700 $Å^3$, which obviously could coordinate external agents of different sizes. As a result, there would be some energetic preference of a given ligand binding to different pockets. For many small-sized ligands that could fit multiple binding pockets, multiple identical ligands could be found occupying the internal pockets of the protein target in some bound structures. These findings enrich the understanding of HSA-ligand interactions.

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**Data availability.**

The database is freely available at www.hsadab.cn.

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