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## **Kill three birds with one stone: Mitochondria-localized tea saponin derived carbon dots with AIE properties for stable detection of HSA and extremely acidic pH**

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1 **Kill three birds with one stone: Mitochondria-localized tea saponin derived**  
2 **carbon dots with AIE properties for stable detection of HSA and extremely acidic**  
3 **pH**

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16 **ABSTRACT**

17 In this work, tea saponin (TS) which is indispensable in *Camellia oleifera*  
18 industry was used to synthesize a class of hydrophobic carbon dots (TS-CDs) with  
19 aggregation-induced emission (AIE) properties. A new fluorescent sensing platform  
20 based on AIE and non-modified TS-CDs for the detection of human serum albumin  
21 (HSA) and pH was developed, respectively. Interestingly, the developed platform is  
22 capable of ratiometric detecting extremely acidic pH in the range of 0.2-1.8 linearly  
23 ( $R^2=0.9959$ ) due to protonation-deprotonation. Meanwhile, TS-CDs exhibited well  
24 stability toward HSA detection over a wide linear range (0~180  $\mu\text{M}$ ), long-term (48 h),  
25 and wide pH range (2~13). Furthermore, TS-CDs was utilized to localize to  
26 mitochondria and detect HSA in living cells, demonstrating its promising perspective  
27 in biosensing applications. This work may pave a novel avenue for high value-added  
28 utilization in the extraction process of extracting camellia oil for food woody oil.

29 **Keywords:** Tea saponin; Carbon dots; AIE; HSA; pH; Mitochondria localization; Cell  
30 imaging.

31

## 32 **Introduction**

33 Carbon dots (CDs) are zero-dimensional materials with extremely small size,  
34 generally less than 10 nm in diameter (Xu et al., 2020). A large number of functional  
35 groups on the surface of carbon dots (such as hydroxyl, carboxyl, amino, etc.) enable  
36 CDs to be easy to functionalize (Alas et al., 2020). Due to excellent optical properties,  
37 such as excitation-dependent, up-converted luminescence, high resistance to  
38 photobleaching and photo-blinking, CDs were applied in many fields, such as  
39 biosensing (Zhu et al., 2013), anti-counterfeiting (Qu et al., 2012), drug transport  
40 (Panwar et al., 2019), super capacitor (Guo et al., 2022) etc.. Many fluorescent  
41 sensors based on CDs were reported to detect analytes such as vitamins (Luo et al.,  
42 2018), metal ions (He et al., 2020), amino acids (Lu et al., 2018), selenol (Wang et al.,  
43 2017). Certainly, accurate, timely and stable detection of signal molecules in living  
44 organisms is of great significance for studying activities in living organisms and  
45 diagnosing diseases in early stage. The sensing platform including “turn-off”, “turn-  
46 on”, “on-off-on” and “ratiometric” mode, in which “turn-on” and “ratiometric” probes  
47 can improve accuracy and suffer less background interference. Recently, researchers  
48 construct a ratiometric sensing platform based on neutral red and urea to detect L-  
49 Lysine and pH in living cells (Chang et al., 2022). Although there are many carbon  
50 dots used for biosensing, it’s still an important issue to develop multi-functional  
51 carbon dots with “turn-on” and ratiometric mode. Fluorescence of conventional  
52 probes will be quenched by aggregation due to  $\pi$ - $\pi$  stacking effects which restricted  
53 their application. Therefore, Tang’s group in 2001 reported Aggregation induced  
54 emission (AIE) effect that realized solid state fluorescence through inhibiting  
55 intramolecular rotation or vibration (Luo et al., 2001). CDs with AIE properties were  
56 firstly synthesized by modifying polymer long-chain on the surface of CDs (Gao et al.,  
57 2013). Since then, AIE properties of carbon dots were synthesized by researchers  
58 through surface passivation which requires post-modification and the fluorescence  
59 may be quenched at high concentration. Therefore, it’s still a challenge to synthesis  
60 carbon dots with AIE properties without surface passivation (Arshad et al. 2021; Yang

61 et al., 2019).

62 HSA and pH are two important indicators related to human physiological  
63 activities. Unusual level of HSA is highly relative to diabetes and liver diseases  
64 (Murch et al., 1996). On the other hand, pH is related to various physiological  
65 activities (Shangguan et al., 2016). In recent years, many fluorescent probes for HSA  
66 and pH detection have been reported ( J.-F. Xu et al., 2022; Li et al., 2020; Liu et al.,  
67 2022; Ning et al., 2018). However, most of the conventional dyes need to be ready-to-  
68 assay and cannot be stored for a long time, thus it makes sense to develop probes with  
69 good detection stability. Moreover, to the best of our knowledge, there is no probe that  
70 can detect pH values up to 0.2, and there are few probes that can detect HSA in a wide  
71 pH range and after long-term storage. Therefore, it is still necessary to achieve stable  
72 detection of HSA and detection of extremely acidic pH.

73 *Camellia oleifera* is one of the four major woody oilseeds in the world and well  
74 applied in edible oil industry, however, the utilization rate of oil residue after oil  
75 extraction is very low. The oil residue contains 10-15% tea saponin (TS), which is a  
76 natural surfactant that mixed of oleanane-type pentacyclic triterpenoid saponins was  
77 usually used as soap, ponding agent, etc. (Feng et al., 2015). There is little research  
78 has been demonstrated on the deep application of tea saponin (Kuo et al., 2010).  
79 Herein, we first used TS as raw material to synthesize a new class of carbon dots (TS-  
80 CDs) with the properties of AIE and pH responsive. TS-CDs was prepared by a  
81 solvothermal method without any surface modification or heteroatom doping.  
82 Moreover, TS-CDs has two fluorescence emission peaks at 313 nm and 533 nm with  
83 the properties of excitation-dependent and excitation-independent, respectively.  
84 Surprisingly, TS-CDs can bind to HSA through hydrophobic interaction and realize  
85 the detection of HSA by AIE and solvent effect. TS-CDs can also detect extremely  
86 acidic pH ratiometrically through protonation-deprotonation of TS-CDs. Compared  
87 with previous studies, TS-CDs has the advantages as follows: (1) Simple and  
88 economical synthesis of multifunctional CDs with a high quantum yield of 46.6%. (2)  
89 TS-CDs can stably detect HSA between pH 2~13 and the fluorescence intensity can

90 remain unchanged for 48 hours after adding HSA, label-free HSA imaging at cellular  
91 levels with low cytotoxicity can also be achieved. (3) Ratiometric and rapid detection  
92 of pH in extremely acidic conditions can be achieved.

## 93 **2. Experimental**

### 94 *2.1. Materials and instruments*

95 All the reagents were of analytical grade, purchased from Sinopharm Group. Tea  
96 saponin, amino acids, simvastatin were obtained from Energy-Chemical.  
97 Bodipy493/503 and mitochondrial red obtained from Tokyo Chemical Industry Co.,  
98 Ltd. and Beyotime Biotechnology, respectively. All proteins were purchased from  
99 Shanghai McLean Biochemical Technology Co., Ltd. Dansyl-L-proline was  
100 purchased from Shanghai Aladdin Biochemical Technology Co., Ltd..

101 Transmission electron microcopy (TEM) and high-resolution TEM images were  
102 acquired from field emission transmission electron microscopy (Talos F200X). X-ray  
103 diffractometer (Bruker, Germany) was utilized to obtain X-ray powder diffraction  
104 (XRD) patterns. Tensor 27 FT-IR spectrometer (Bruker, Germany) was used to obtain  
105 Fourier transform infrared (FT-IR) spectra. X-ray photoelectron spectroscopy (XPS)  
106 was carried out with ESCALAB Xi+ (Thermo Scientific). Hitachi F-7000 was used to  
107 measure fluorescence spectra. UV Absorption Spectroscopy were recorded using a  
108 Shimadzu UV-1800 spectrophotometer. The pH was measured through a Sartorius  
109 PB-10 pH meter. Leica TCS SP8 confocal laser scanning microscope filmed the  
110 fluorescence imaging. Zeta potential was analyzed using a British Malvern Zetasizer  
111 Nano ZS. Toxicity experiments was carried out by Beijing Liuyi's WD-2102A.

### 112 *2.2. Synthesis of TS-CDs*

113 Tea saponin was synthesized with minor adjustments according to the  
114 literature of Kuo's group (Kuo et al., 2010). Tea saponin (0.50 g) was added to H<sub>2</sub>O  
115 (12 mL) containing concentrated sulfuric acid (0.60 mL), then the solution was  
116 transferred into a 50 mL round-bottomed flask. The reaction was carried out at 100°C

117 for 5 h, then the raw materials were removed by filtration, tea sapogenin was  
118 extracted with ethyl acetate, 212 mg mixed sapogenin was obtained after vacuum  
119 drying. Concentrated hydrochloric acid (50  $\mu$ L) was added to the tea sapogenin (55  
120 mg) in ethanol (10 mL). After the solution was heated at 230°C in a 25 mL Teflon  
121 autoclave for 6 h, a red-brown solution was obtained, then silica gel column  
122 chromatography was performed with dichloromethane as eluent to obtain TS-CDs,  
123 finally, 2.7 mg TS-CDs could be obtained after vacuum drying.

### 124 2.3. Calculation of fluorescence quantum yield

125 The method for calculating the fluorescence quantum yield of TS-CD was with  
126 reference to Wu's group (She et al., 2017), and the method is documented in the  
127 Supporting Information.

### 128 2.4. Fluorescence determination of HSA and pH

129 Phosphate buffer solution (PBS) was selected as the stabilizer to perform titration  
130 of HSA and pH. For the detection of HSA, different concentrations of HSA (0~180  
131  $\mu$ M) were added into 200  $\mu$ L of TS-CDs (0.20 mg/mL) acetone solution in 15 mL  
132 colorimetric tube, after adding 500  $\mu$ L of PBS and diluting to 5.0 mL with water for 5  
133 min, the fluorescence spectra were recorded at excitation wavelength of 380 nm.  
134 Under the same condition, selectivity and competition tests were carried out by adding  
135 the following proteins and amino acids including Bovine serum albumin (BSA),  
136 Homocysteine (Hcy), Glutathione (GSH), Cysteine (Cys), Leucine (Leu), Glu,  
137 Ascorbic acid (ASA), Valine (Val), Methionine (Met), Isoleucine (Ile), Serine (Ser),  
138 Arginine (Arg), Tryptophan (Trp), Threonine (Thr), Asparagine (Asn), Tyrosine (Tyr),  
139 Glycine (Gly), Alanine (Ala), Lysine (Lys), HGB (Hemoglobin), Papain, Pepsin,  
140 Chymotrypsin instead of HSA. Furthermore, some ions including  $\text{Na}^+$ ,  $\text{Zn}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  
141  $\text{Hg}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{ClO}^-$ ,  $\text{F}^-$ ,  $\text{I}^-$ ,  $\text{NO}_3^-$ ,  $\text{H}_2\text{O}_2$ ,  $\text{CO}_3^{2-}$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{S}^{2-}$ ,  $\text{SO}_3^{2-}$ ,  
142  $\text{HSO}_3^-$ ,  $\text{S}_2\text{O}_3^{2-}$  were also used to check the selectivity and competition of TS-CDs.

143 For the detection of pH, 2.8 mL of 70% PBS solution with various pH was added  
144 to 200  $\mu$ L of TS-CDs (0.20 mg/mL) acetone solution and the fluorescence spectra

145 were measured at the excitation wavelength of 380 nm.

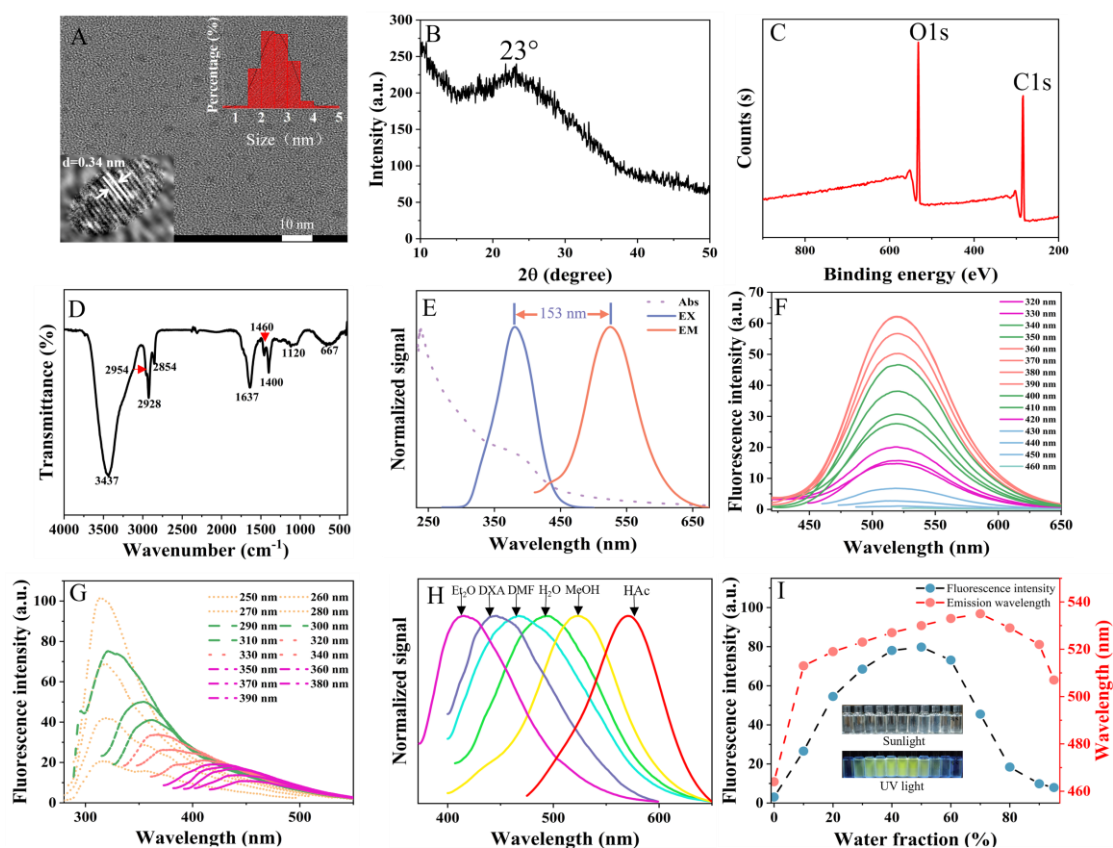
## 146 2.5. Bioimaging

147 Bioimaging experiments in HepG2 cells were with reference to (Ke et al., 2022;  
148 Ning et al., 2018), and the method was recorded completely in the Supporting  
149 Information.

## 150 3. Results and discussion

### 151 3.1. Characterization of TS-CDs

152 The structure and optical properties of TS-CDs were characterized by TEM,  
153 XRD, XPS, FT-IR, UV-Vis, FS. The lattice and size of TS-CDs were performed by  
154 TEM and HR-TEM images. As shown in Fig. 1A, TEM image and HR-TEM image of  
155 TS-CDs demonstrated near spherical structure and particle size of  $2.5 \pm 1$  nm (The  
156 particle size statistical distribution curve was drawn used more than 100 particles).  
157 HR-TEM shows a 0.34 nm lattice spacing, which corresponds to (002) planes of bulk  
158 graphite. Similarly, XRD spectra (Fig. 1B) show a broad peak at  $23^\circ$  corresponding to  
159 the d-spacing value of around 0.34 nm. XPS was recorded to characterize surface  
160 elements of TS-CDs, the full survey scan (Fig. 1C) indicates that TS-CDs is mainly  
161 composed of carbon and oxygen. The HR-XPS C1s spectra (Fig. S1A) displays the  
162 existence of C-C or C=C (284.8 eV), -COOR (285.5 eV) and C-O (286.2 eV). HR-  
163 XPS O1s spectra (Fig. S1B) show the existence of C-O-C (533.8 eV) and C-O/C=O  
164 (532.5 eV). The functional groups of TS-CDs were analyzed by FT-IR, as the Fig. 1D  
165 shows, the peak of TS-CDs at  $3437\text{ cm}^{-1}$  is caused by the stretching vibrations of O-  
166 H/N-H. The peaks at  $2954\text{ cm}^{-1}$  and  $2854\text{ cm}^{-1}$  are caused by symmetric and  
167 asymmetric stretching vibrations of  $-\text{CH}_3$ , respectively. The scissor, symmetric and  
168 asymmetric stretching vibrations of saturated methylene are located at  $1460\text{ cm}^{-1}$ ,  
169  $2854\text{ cm}^{-1}$  and  $2928\text{ cm}^{-1}$ , respectively. The peaks at  $1637\text{ cm}^{-1}$  and  $1460\text{ cm}^{-1}$  are  
170 generated by the stretching vibrations of C=C and C-N.



171

172 **Fig. 1.** (A) TEM image of TS-CDs, inset: particle size distribution image of TS-CDs (top right),  
 173 HR-TEM image of TS-CDs (bottom left). (B) XRD spectra of TS-CDs (C) XPS spectrum of TS-  
 174 CDs. (D) FT-IR spectrum of TS-CDs. (E) UV absorption spectra (purple line), fluorescence  
 175 excitation spectra (blue line,  $\lambda_{em}=533$  nm) and PL emission spectra (red line,  $\lambda_{ex}=380$  nm) of TS-  
 176 CDs. (F). Fluorescence spectra of TS-CDs in acetone (DMK) under different excitation  
 177 wavelength (250 nm to 380 nm). (G). Fluorescence spectra of TS-CDs in MeOH under various  
 178 excitation wavelength (320 nm to 460 nm). (H). Fluorescence emission ( $\lambda_{ex}=380$  nm) spectra of  
 179 TS-CDs in solvents with different polarity (from left to right are Ethyl ether (Et<sub>2</sub>O), 1,4-Dioxane  
 180 (DXA), N,N-Dimethylformamide (DMF), Water (H<sub>2</sub>O), Methanol (MeOH), Acetic acid (HAc). (I)  
 181 Fluorescence emission intensity ( $\lambda_{ex}=380$  nm) and fluorescence wavelength trend graphs of TS-  
 182 CDs at different water fraction (0% to 90%), inset: photographs of TS-CDs with different water  
 183 fraction (0% to 90%) under sunlight (above) and UV light (down).

### 184 3.2. Optical properties of TS-CDs

185 The optical properties of TS-CDs were characterized by UV-Vis and FS, As  
 186 shown in Fig. 1E, the absorption peak at 237 nm belongs to the  $\pi-\pi^*$  transition of C=C,  
 187 and another characteristic band at 380 nm is from n- $\pi^*$  transition of C=N/C=O. The  
 188 maximum excitation and emission wavelengths of TS-CDs in methanol are 380 nm  
 189 and 533 nm, respectively, which corresponding to a large Stokes shift of 153 nm. The



190 maximum emission wavelength under acidic conditions is 593 nm and the Stokes shift  
191 reaches 213 nm (Fig. S2). Additionally, the fluorescence spectra of TS-CDs in acetone  
192 and MeOH were also collected as shown in Fig. 1F and 1G, emission peaks at 313 nm  
193 and 533 nm with excitation-dependent and excitation-independent feature respectively  
194 were investigated. As previously reported in the literature, the maximum emission  
195 peak at 313 nm with excitation-dependent belongs to classical luminescence of  
196 graphitic carbon cores, and the emission peak at 533 nm belongs to the luminescence  
197 of surface states of carbon dots (H. Yang et al., 2019).

198 The fluorescence spectra of TS-CDs in different polar solvents were also studied  
199 to explore its optical properties. As shown in Fig. 1H, the fluorescence peaks among  
200 410 nm to 593 nm of TS-CDs were recorded in various solvents, and the fluorescence  
201 emission wavelength of TS-CDs gradually red-shifted with the increasing of polarity.  
202 Correspondingly, the red-shift trend of the UV absorption of TS-CDs in different  
203 solvents is consistent with the trend of the fluorescence spectrum was recorded in Fig.  
204 S3A. Fig. S3B is the photo of TS-CDs in different solvents under UV light and sun  
205 light, which shows the same trend as the fluorescence plot. TS-CDs shows blue  
206 fluorescence in solvents with less polar like DMK and yellow fluorescence in MeOH,  
207 while when the polarity continues to increase to that of acetonitrile and water, the  
208 fluorescence turns green due to the strong hydrophobicity of TS-CDs resulting in poor  
209 solubility and aggregated in water. The fluorescence peak of TS-CDs in acetic acid is  
210 only one at 593 nm, which is caused by the doping nitrogen protonation of TS-CDs  
211 carbon nuclei under acidic conditions (Xia et al., 2019).

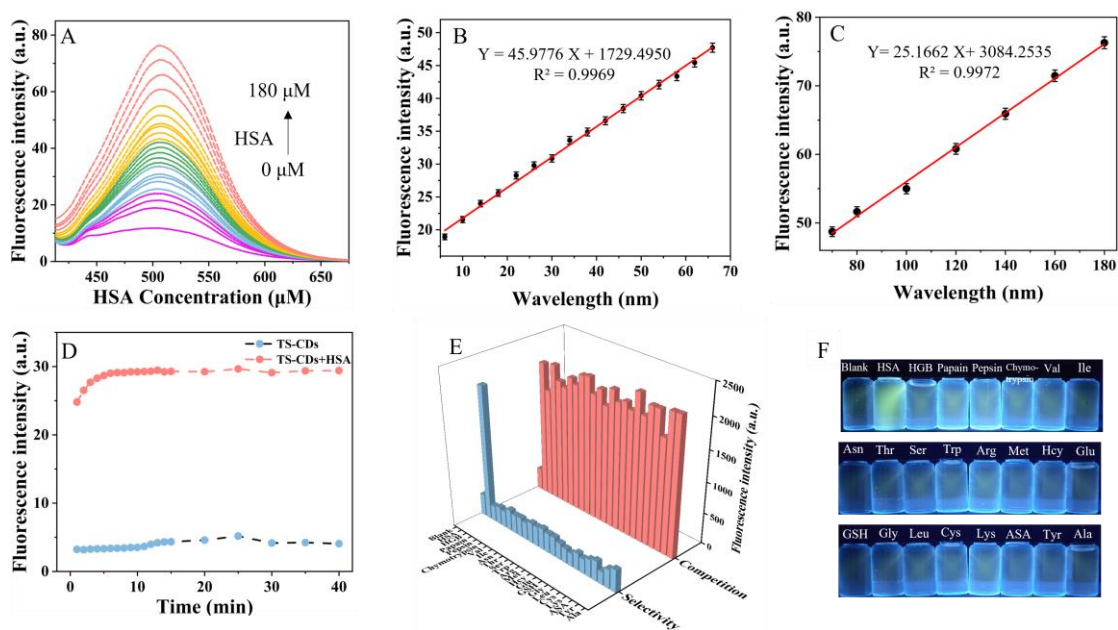
212 The fluorescence spectra of TS-CDs in solvents with different water fraction  
213 were recorded to study its AIE properties (Fig. S3C and Fig. 1I), TS-CDs emits blue  
214 fluorescence in good solvent and gradually decreases accompanied by gradually  
215 increases of the yellow-green fluorescence at 533 nm as the gradual addition of poor  
216 solvent water from 0% to 60%. Equally, the UV absorption longer than 380 nm of TS-  
217 CDs also red-shifted with the increase of water fraction (Fig. S3D). As the water  
218 fraction increased to 90%, the strong hydrophobicity of TS-CDs leads to the

219 formation of larger aggregates, which enhanced the  $\pi$ - $\pi$  stacking effect and gradually  
220 decreased the fluorescence intensity (Qian et al., 2009; Zhang et al., 2022). Such  
221 experiment was performed as shown in Fig. S4A, it was seen that high concentrations  
222 of TS-CDs (1 mg/mL) at 90% water fraction resulted in a turbid state with weak  
223 fluorescence, while the control group in methanol showed no precipitation and bright  
224 fluorescence. The fluorescence spectra of TS-CDs in different MeOH fraction were  
225 recorded to verify this hypothesis. It can be seen from Fig. S4B, the fluorescence  
226 intensity of TS-CDs increased as the gradual addition of poor solvent MeOH from 0%  
227 to 99%. The differentiation with Fig. S3C may be caused by the good solubility of  
228 TS-CDs in MeOH, making it difficult to form large aggregates.

### 229 3.3. Detection of HSA

230 Since TS-CDs is highly hydrophobic and possesses aggregation-induced  
231 emission property, solvent effect, we speculate that TS-CDs can response to HSA and  
232 emission enhanced. Researchers had proved that the addition of HSA can reduces the  
233 polarity of the system and will inhibit the rotation or vibration of the fluorophore  
234 (Chakrabarty et al., 2007; Vijayakumar et al., 2019; Xu et al., 2016). The  
235 fluorescence of TS-CDs increases with the addition of HSA due to the decreased of  
236 solvent polarity, which is consistent with the optical properties of TS-CDs that  
237 fluorescence intensity increased as the water fraction decreased from 90% to 60%. As  
238 designed, we performed experiments of TS-CDs in response to different proteins,  
239 amino acids, and ions, and found that the fluorescence intensity was enhanced only  
240 when HSA and bovine serum albumin (BSA) were added. TS-CDs is a sensitive  
241 fluorescence nanoprobe to detect HSA, and the limit of detection of HSA achieved  
242 140 nM (Table. S1). As shown in Fig. 2A, with the concentration of HSA increased to  
243 180  $\mu$ M, the fluorescence intensity of TS-CDs increased by 6.5 times with good  
244 linearity in both detection ranges of 6~70  $\mu$ M and 80~180  $\mu$ M, which  $R^2$  reaches  
245 0.9969 and 0.9972, respectively (Fig. 2B and C). We also studied the kinetic curve of  
246 TS-CDs-HSA (Fig. 2D). The fluorescence intensity of TS-CDs increased 7.7-fold at  
247 10 s and reached a stable value at 6 min.

248 The stability of HSA detection was also studied. Initially, TS-CDs can stably  
 249 detect HSA in the pH range of 2~13 (Fig. S5A). In addition, we investigated the  
 250 ability of TS-CDs to resist ionic strength (demonstrated with NaCl). The fluorescence  
 251 intensity of TS-CDs changed only very slightly at a concentration of 0.50 M and the  
 252 intensity remained 83% at 1.0 M (Fig. S5B). Moreover, it was found in Fig. S5C that  
 253 fluorescence intensity of TS-CDs-HSA remained after storage 48 h at room  
 254 temperature which overcomes the disadvantages of traditional fluorescent dyes with a  
 255 short storage time, which proved that TS-CDs could be used to detect HSA stably. TS-  
 256 CDs could stably detect HSA Compared with Moreover, some comparisons in terms  
 257 of synthesis, biocompatibility and responsiveness between TS-CDs and previously  
 258 probes used to detect HSA were shown in Table. S2.



259  
 260 **Fig. 2.** (A) Fluorescence emission spectra ( $\lambda_{ex}=380$  nm) of TS-CDs (8.0  $\mu\text{g/mL}$ ) upon addition of  
 261 HSA (0~180  $\mu\text{M}$ ) in PBS (pH=7.4, containing 4% acetone) (B) Linear relationship of fluorescence  
 262 emission ( $\lambda_{ex}=380$  nm,  $\lambda_{em}=505$  nm) and concentration of HSA (6~70  $\mu\text{M}$ ). (C) Linear  
 263 relationship of fluorescence emission ( $\lambda_{ex}=380$  nm,  $\lambda_{em}=505$  nm) and concentration of HSA  
 264 (70~180  $\mu\text{M}$ ). (D) Time course of fluorescence emission ( $\lambda_{ex}=380$  nm,  $\lambda_{em}=505$  nm) intensity  
 265 graphs of TS-CDs in the absence (black line) and presence (red line) of HSA (70  $\mu\text{M}$ ) during 40  
 266 min. (E) Fluorescence emission intensity ( $\lambda_{ex}=380$  nm,  $\lambda_{em}=505$  nm) of TS-CDs (2.0  $\mu\text{g/mL}$ , blue  
 267 strips) and TS-CDs (2.0  $\mu\text{g/mL}$ ) + HSA (70  $\mu\text{M}$ , red trips) in the presence of other proteins and  
 268 amino acids. (F) Images of TS-CDs in the addition of other proteins and amino acids under 365  
 269 nm UV lamp.

270 Selective and competitive experiments were performed with proteins, amino

271 acids, anions, and cations against TS-CDs in PBS (pH 7.4). As shown in Fig. 2E,  
272 Under the same test conditions, Hcy, GSH, Cys, Leu, Glu, ASA, Val, Met, Ile, Ser,  
273 Arg, Trp, Thr, Asn, Tyr, Gly, Ala, Lys, BSA, HGB, Papain, Pepsin and Chymotrypsin  
274 that may co-exist with protein were used for selective and competitive experiments,  
275 only HSA among proteins, amino acids, anions, and cations had a largely enhanced  
276 fluorescence on making TS-CDs, probably because HSA can reduce the polarity of  
277 the system, while the other analytes cannot, the relative fluorescence spectrograms  
278 were shown in Fig. S6. Some anions and cations that  $\text{Na}^+$ ,  $\text{Zn}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Hg}^{2+}$ ,  $\text{Fe}^{3+}$ ,  
279  $\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{ClO}^-$ ,  $\text{F}^-$ ,  $\text{I}^-$ ,  $\text{NO}_3^-$ ,  $\text{H}_2\text{O}_2$ ,  $\text{CO}_3^{2-}$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{S}^{2-}$ ,  $\text{SO}_3^{2-}$ ,  $\text{HSO}_3^-$ ,  
280  $\text{S}_2\text{O}_3^{2-}$  also does not affect the detect of HSA (Fig. S7A). Competition experiments  
281 were also done for TS-CDs, the presence of other ions did not affect the response of  
282 TS-CDs to HSA (Fig. S7B). We also measured the response of TS-CDs to BSA,  
283 which is highly homologous to HSA, TS-CDs also respond to BSA (Fig. S8).  
284 However, BSA is not present in the human body, the response of TS-CDs to BSA does  
285 not affect the detection of HSA. These data indicate that TS-CDs have good  
286 selectivity for the detection of HSA.

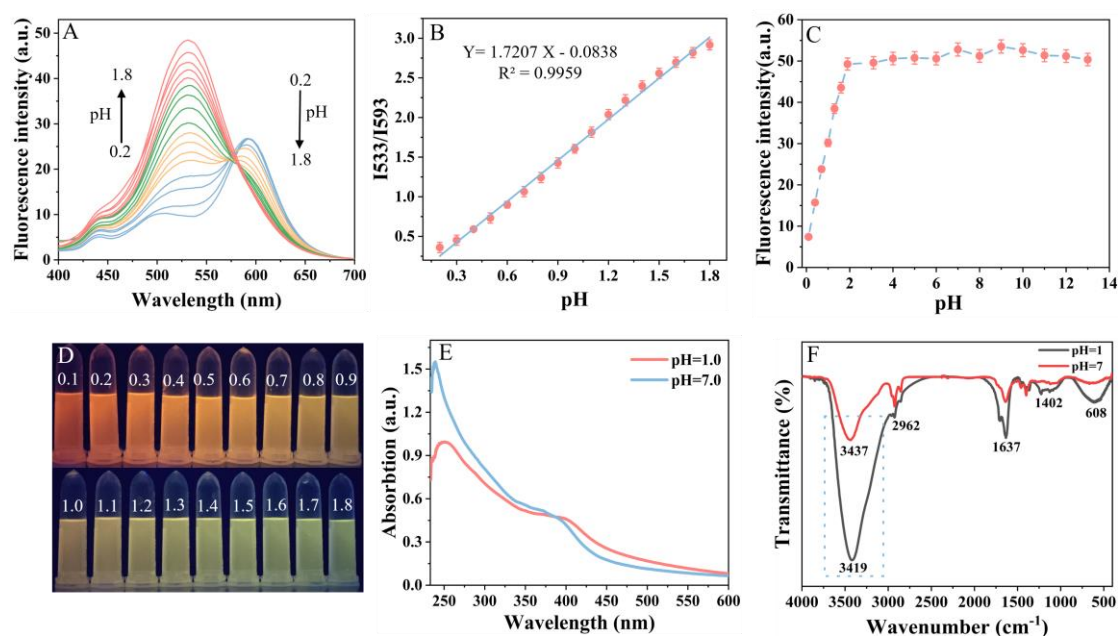
### 287 3.4. Ratiometric detection of pH

288 Monitoring pH at different locations in human body is important to predict  
289 diseases and to understand the impact of pH on human health. Nowadays, there are  
290 many proposing probes for detecting pH (Yang et al., 2012), although only few places  
291 in human body in extremely acidic conditions, such as helicobacter pylori, microbiota  
292 in gastric juice, etc., highly sensitive probes that can stably detect extremely acidic pH  
293 are needed. The fluorescence intensity of TS-CDs upon the excitation wavelength of  
294 380 nm varies greatly under extremely acidic conditions, but changes slowly when pH  
295  $\geq 2$  which indicated that TS-CDs possess excellent stability that false positives could  
296 be avoided when detecting pH under extremely acidic conditions. The detection of pH  
297 on fluorescence emission intensity under an extremely acidic condition of 0.2~1.8 was  
298 recorded. It can be seen intuitively that the fluorescence intensity of TS-CDs at 593

299 nm decreased as pH increased from 0.2 to 1.8, on the contrary, the fluorescence  
300 intensity at 533 nm increased simultaneously (Fig. 3A). More importantly, a good  
301 linear relationship ( $R^2=0.9959$ ) of  $I_{533\text{ nm}}/I_{593\text{ nm}}$  could be observed from Fig. 3B,  
302 indicating that TS-CDs is highly pH sensitive. Moreover, the responses of TS-CDs to  
303 pH from 0.2-1.8 were reversible during 5 cycle times as shown in Fig. S10. Therefore,  
304 TS-CDs can be used to detect pH under extremely acidic conditions. From Fig. 3D,  
305 the orange fluorescence of TS-CDs was gradually weakened, and the yellow-green  
306 fluorescence increased with the pH increasing, which is consistent with the changes in  
307 the fluorescence picture.

308 To clarify the mechanism of TS-CDs responds to pH, the UV-Vis, FT-IR and zeta  
309 potential under different pH were studied. Fig. 3E is the UV absorption spectra of TS-  
310 CDs at pH 1.0 and pH 7.0, the UV absorption at 380 nm decreased slightly with the  
311 decrease of pH accompanied by a broadening of the absorption peak, which is the  
312 result of the widening of the particle size distribution due to the smaller particle size  
313 of the TS-CDs fraction under acidic conditions. Moreover, it combined with a red-  
314 shift of the UV absorption peak at 380 nm, corresponding to fluorescence spectra, the  
315 nitrogen is protonated due to both the edge/surface groups and the rigid carbon core  
316 structure under acidic conditions (Yuan et al., 2015). With the enhancement of  
317 alkalinity, the fluorescence at 593 nm decreases due to the doped nitrogen being  
318 deprotonated first, while the fluorescence enhancement at 533 nm is caused by the  
319 enhancement of van der Waals forces between carbon dots because of the  
320 deprotonation of edge/surface groups, followed by the aggregation of carbon dots.  
321 (Yang et al., 2020). The FT-IR absorption maps of TS-CDs at different pH were also  
322 investigated, as shown in Fig. 3F, the peak near  $3400\text{ cm}^{-1}$  becomes weaker as the pH  
323 increases from 1.0 to 7.0, which is due to the weakening of the intensity of N-H and  
324 O-H caused by the deprotonation of carbon sites, and the -OH/-NH<sub>2</sub> peak is red-  
325 shifted from  $3419\text{ cm}^{-1}$  to  $3437\text{ cm}^{-1}$  as the pH increases from 1.0 to 7.0, which is a  
326 result of the enhanced of hydrogen bonds between TS-CDs hydroxyl groups (Song et  
327 al., 2016). Zeta potential of TS-CDs was investigated to explore the reason for the

328 aggregation of TS-CDs, as shown in Table S3, zeta potential value of TS-CDs was -  
 329 4.43 mV at pH 7.0, which demonstrated that the surface of TS-CDs is negatively  
 330 charged. As pH of TS-CDs decreased to 0.5, the zeta potential value achieved 2.97  
 331 mV by protonation of the surface groups of TS-CDs as the pH decreased. The zeta  
 332 potential of TS-CDs increased sharply as the pH of TS-CDs increased from 7.0 to  
 333 12.0, which indicated that the  $-\text{OH}/\text{O}=\text{C}-\text{NH}_2/-\text{NH}_2$  of TS-CDs were depleted by  
 334 deprotonation (Dan et al., 2021). Moreover, corresponding to the infrared absorption  
 335 spectra, the stretching vibration of  $-\text{OH}/-\text{NH}_2$  sharply decreased when the pH  
 336 increased to 7.0. Based on the above observations, we concluded that TS-CDs has  
 337 better solubility at acidic solutions and poor solubility in alkaline solutions.

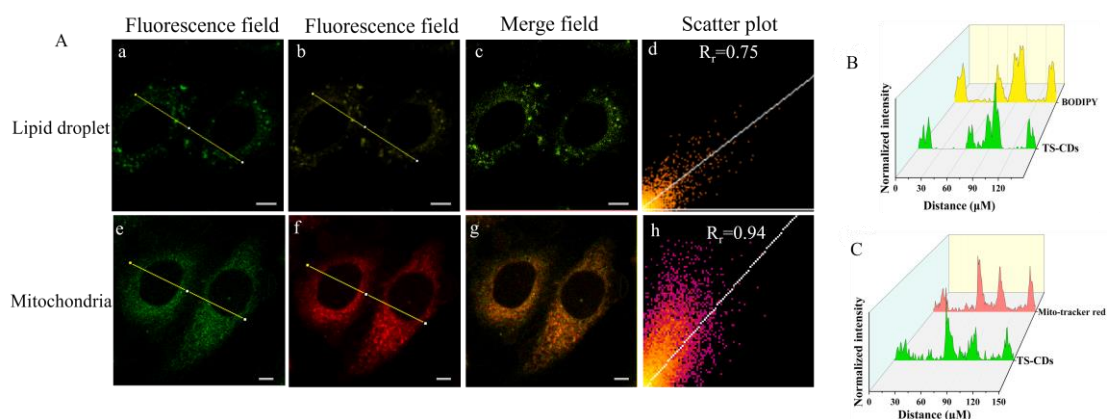


338  
 339 **Fig. 3.** (A) Fluorescence emission spectra of TS-CDs (8.0 µg/mL) (30% acetone and 70% water)  
 340 at different pH (0.2~1.8) (B) Linear relationship of the fluorescence emission intensity (8.0 µg/mL)  
 341 ratio of TS-CDs between 533 nm and 593 nm. (C) Changes in fluorescence emission intensity of  
 342 TS-CDs (8.0 µg/mL,  $\lambda_{em}=533$  nm) at different pH (0.1~13). (D) Images of TS-CDs at different pH  
 343 (0.2~1.8) under UV-light (365 nm). (E) UV absorption diagram of TS-CDs at pH=1.0 (red line)  
 344 and pH=7.0 (blue line). (F) FT-IR spectra of TS-CDs at pH=1.0 (black line) and pH=7.0 (red line).

### 345 3.5.1. Mitochondrial targeting assay of TS-CDs

346 To determine the intracellular localization of TS-CDs, we performed  
 347 fluorescence co-localization experiments by using commercial Mito-tracker red and  
 348 TS-CDs in HepG2 cells, as shown in Fig. 4A. There is a large overlap between the

349 green channel of TS-CDs fluorescence and the red channel of Mito-tracker red  
 350 (Pearson correlation coefficient of 0.94), and by intensity cross-sectional analysis can  
 351 be seen that the two peaks overlap well (Fig. 4B). In addition, the Pearson correlation  
 352 coefficient was only 0.75 when using the lipid titration dye BODIPY 493/505 for co-  
 353 localization experiments with TS-CDs (Fig. 4C), which clearly indicates that TS-CDs  
 354 can target mitochondria.



355

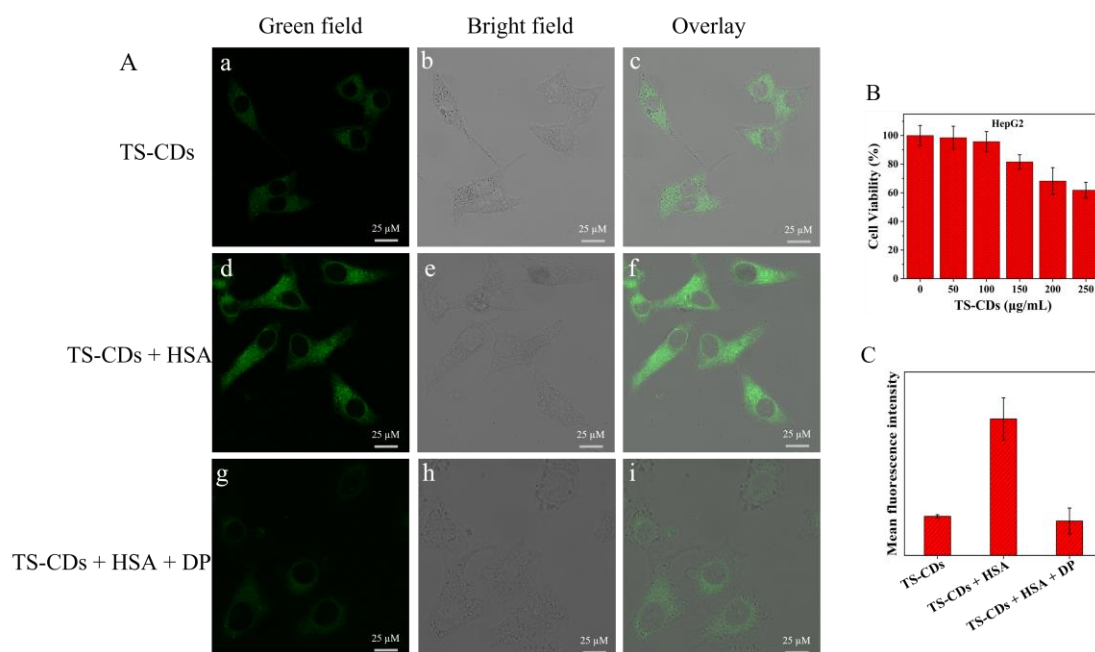
356 **Fig. 4.** (A) Confocal images of HepG2 cells. (a-c) Co-localization images of HepG2 cells after  
 357 adding TS-CDs (8.0  $\mu\text{g/mL}$ ) and BODIPY 493/503 (0.5  $\mu\text{M}$ ) for 30 min. (d) Co-localization  
 358 scatter plot of TS-CDs (8.0  $\mu\text{g/mL}$ ) and BODIPY 493/503 (1.0  $\mu\text{M}$ ). (e-g) Co-localization images  
 359 of HepG2 cells incubated with TS-CDs (8.0  $\mu\text{g/mL}$ ) and Mito-tracker red (1.0  $\mu\text{M}$ ) for 30 min. (h)  
 360 Co-localization scatter plot of TS-CDs (8.0  $\mu\text{g/mL}$ ) and Mito-tracker red. (B) Fluorescence  
 361 intensity distribution of selected areas of a and b channels. (C) Fluorescence intensity distribution  
 362 of selected areas of e and f channels. (a) and (e) The wavelength setting range is 480 nm to 530  
 363 nm for TS-CDs ( $\lambda_{\text{ex}} = 380$  nm). (b) The wavelength setting range is 530 nm to 560 nm for  
 364 BODIPY 493/503. (f) The wavelength setting range is 560 nm to 630 nm for Mito-tracker red ( $\lambda_{\text{ex}}$   
 365 = 579 nm).

### 366 3.5.2. Cell imaging of exogenous HSA

367 For probes used for biological imaging, good biocompatibility is acquired,  
 368 especially for intracellular detection. Therefore, we first performed MTT experiments  
 369 using HepG2 to examine the cytotoxicity of TS-CD. Fig. 5B shows the viability of  
 370 HepG2 cells with TS-CDs concentration of 0~250  $\mu\text{g/mL}$ , this test concentration is  
 371 much higher than the fluorescence test concentration of 8.0  $\mu\text{g/mL}$ , the viability of  
 372 150  $\mu\text{g/mL}$  in TS-CDs can be seen higher than 90%, indicating that TS-CDs has less  
 373 cytotoxicity. Fig. 5A (a-c) shows that HepG2 cells co-incubated with TS-CDs (8.0



374  $\mu\text{g/mL}$ ) for 30 minutes in the green channel showed only weak fluorescence. In  
 375 contrast, bright green fluorescence was observed after incubation with HSA ( $70.0 \mu\text{M}$ )  
 376 for 24 hours followed by TS-CD ( $8.0 \mu\text{g/mL}$ ) for 30 minutes (Fig. 5A, d-f). Fig. 5A,  
 377 (g-i) are the imaging pictures after adding HSA inhibitor Dansyl-L-proline (DP),  
 378 whose fluorescence intensity was weaker than d-f. Fig. 5C from left to right shows the  
 379 mean fluorescence intensity of HepG2 cells after incubation with TS-CDs, TS-CDs +  
 380 HSA and TS-CDs + HSA + DP, respectively. These results indicated that TS-CDs can  
 381 be used to image intracellular HSA.



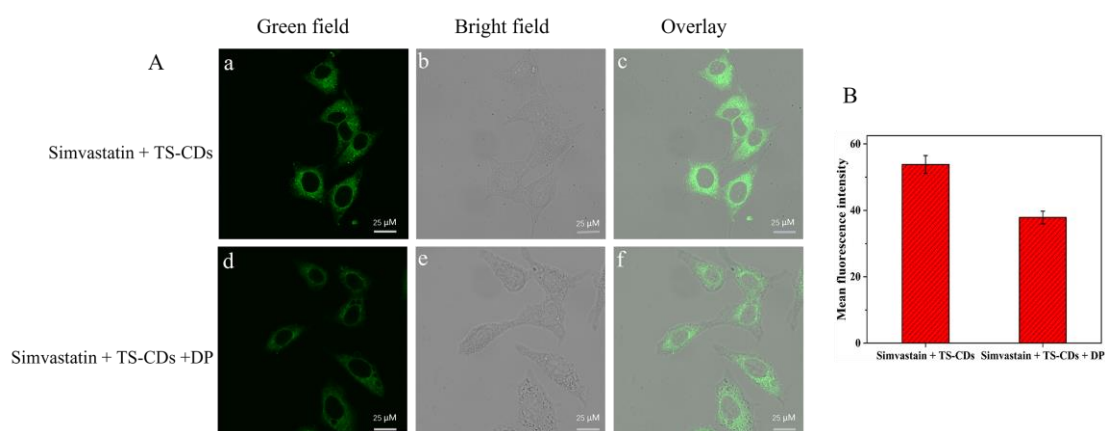
382 **Fig. 5.** (A) Confocal images of HepG2 cells after incubated with TS-CDs ( $8.0 \mu\text{g/mL}$ ) for 30  
 383 min(a-c), TS-CDs ( $8.0 \mu\text{g/mL}$ ) for 30 min and HSA ( $70 \mu\text{M}$ ) for 24 h (d-f). HSA ( $70 \mu\text{M}$ ) for 24 h,  
 384 DP ( $3.0 \text{ mM}$ ) for 30 min and TS-CDs ( $8.0 \mu\text{g/mL}$ ) for 30 min (g-i). (B) Cell viability of HepG2  
 385 cells after incubated with TS-CDs ( $0\sim 250 \mu\text{g/mL}$ ). (C) Mean fluorescence intensity of TS-CDs,  
 386 TS-CDs + HSA and TS-CDs + HSA + DP.  
 387

### 388 3.5.3. Drug-induced cell imaging of HSA

389 HSA levels are often associated with drug and toxic substance assessments. We  
 390 used simvastatin (a drug used for rising content of HSA) to stimulate HepG2 cells to  
 391 produce HSA (Ha et al., 2009). As shown in Fig. 6A (a-c), after incubation with  
 392 simvastatin ( $1.0 \mu\text{M}$ ) for 24 hours and then with TS-CD for 30 minutes, the  
 393 fluorescence intensity was significantly enhanced. However, the control group to  
 394 which the simvastatin inhibitor DP ( $3.0 \text{ mM}$ ) was added showed weaker fluorescence



395 Fig. 6A (d-f) (Wang et al., 2017). The above experiments show that TS-CDs can  
396 penetrate cell membranes and have the ability to detect intracellular HSA levels.



397

398 **Fig. 6.** (A) Confocal images of HepG2 cells after incubated with simvastatin (1.0 μM) for 24 h  
399 and TS-CDs (8.0 μg/mL) for 30 min (a-c). Simvastatin (1 μM) for 24 h and TS-CDs (8.0 μg/mL)  
400 for 30 min, DP (3.0 mM) for 30 min (d-f) (B) Mean fluorescence intensity of simvastatin +TS-  
401 CDs and simvastatin + TS-CDs + DP.

#### 402 4. Conclusion

403 In summary, we synthesized a multifunctional hydrophobic TS-CDs by a simple  
404 solvothermal method using tea saponin as raw material. The synthesized TS-CDs has  
405 strong hydrophobicity, AIE properties, solvent effect and double emission at 313 nm  
406 and 533 nm, respectively. Results indicated that TS-CDs can be applied as a sensing  
407 platform for the detection of HSA due to AIE property and pH under extremely acidic  
408 condition (0.2-1.8). Due to the use of natural product as carbon source, the  
409 synthesized TS-CDs has good biocompatibility that can stably detect HSA in a wide  
410 pH range (2-13) and a long time (48 h), and this probe could be applied to visualize  
411 the concentration of HSA in living cells. Moreover, the ratiometric detection of pH  
412 under extremely acidic conditions (0.2-1.8) was also realized due to protonation –  
413 deprotonation of TS-CDs. The current work demonstrates that the synthesis and  
414 biological applications of TS-CDs may pave a novel avenue for high value-added  
415 utilization in the extraction process of extracting camellia oil for food woody oil. This  
416 strategy could be easily extended to detection other disease-related biomarker proteins.  
417

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