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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 μM), 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 π - π 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 H₂O
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 μ 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 μ M) were added into 200 μ L of TS-CDs (0.20 mg/mL) acetone solution in 15 mL
132 colorimetric tube, after adding 500 μ 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 Na^+ , Zn^{2+} , Mg^{2+} , K^+ ,
141 Hg^{2+} , Fe^{3+} , Fe^{2+} , Cu^{2+} , Ca^{2+} , Al^{3+} , ClO^- , F^- , I^- , NO_3^- , H_2O_2 , CO_3^{2-} , Cl^- , SO_4^{2-} , S^{2-} , SO_3^{2-} ,
142 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 μ L of TS-CDs (0.20 mg/mL) acetone solution and the fluorescence spectra

145 were measured at the excitation wavelength of 380 nm.

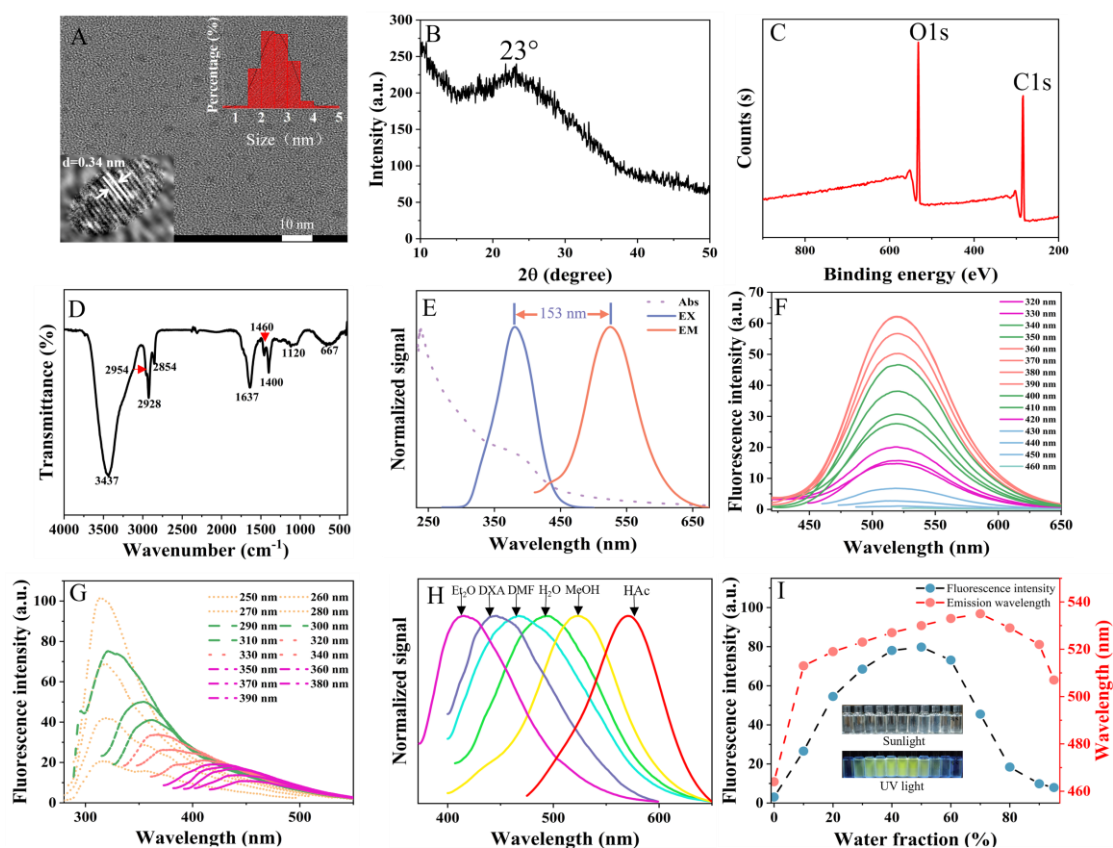
146 2.5. Bioimaging

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 ± 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° 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 cm^{-1} is caused by the stretching vibrations of O-
166 H/N-H. The peaks at 2954 cm^{-1} and 2854 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 cm^{-1} ,
169 2854 cm^{-1} and 2928 cm^{-1} , respectively. The peaks at 1637 cm^{-1} and 1460 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 (Et₂O), 1,4-Dioxane
 180 (DXA), N,N-Dimethylformamide (DMF), Water (H₂O), 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- π^* 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).

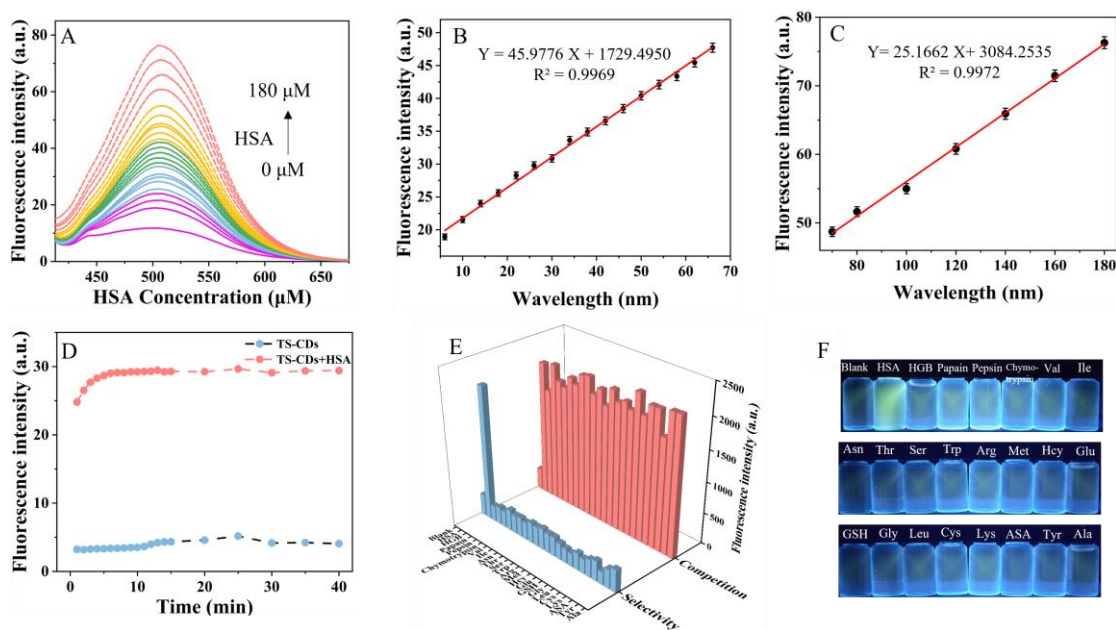
212 The fluorescence spectra of TS-CDs in solvents with different water fraction
213 were recorded to study its AIE properties (Fig. S3C and Fig. 1I), TS-CDs emits blue
214 fluorescence in good solvent and gradually decreases accompanied by gradually
215 increases of the yellow-green fluorescence at 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 π - π 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 μ M, the fluorescence intensity of TS-CDs increased by 6.5 times with good
244 linearity in both detection ranges of 6~70 μ M and 80~180 μ 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 (λ_{ex} =380 nm) of TS-CDs (8.0 µg/mL) upon addition of
 261 HSA (0~180 µM) in PBS (pH=7.4, containing 4% acetone) (B) Linear relationship of fluorecence
 262 emission (λ_{ex} =380 nm, λ_{em} =505 nm) and concentration of HSA (6~70 µM). (C) Linear
 263 relationship of fluorecence emission (λ_{ex} =380 nm, λ_{em} =505 nm) and concentration of HSA
 264 (70~180 µM). (D) Time course of fluorecence emission (λ_{ex} =380 nm, λ_{em} =505 nm) intensity
 265 graphs of TS-CDs in the absence (black line) and presence (red line) of HSA (70 µM) during 40
 266 min. (E) Fluorecence emission intensity (λ_{ex} =380 nm, λ_{em} =505 nm) of TS-CDs (2.0 µg/mL, blue
 267 strips) and TS-CDs (2.0 µg/mL) + HSA (70 µM, 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 Na^+ , Zn^{2+} , Mg^{2+} , K^+ , Hg^{2+} , Fe^{3+} ,
279 Fe^{2+} , Cu^{2+} , Ca^{2+} , Al^{3+} , ClO^- , F^- , I^- , NO_3^- , H_2O_2 , CO_3^{2-} , Cl^- , SO_4^{2-} , S^{2-} , SO_3^{2-} , 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.

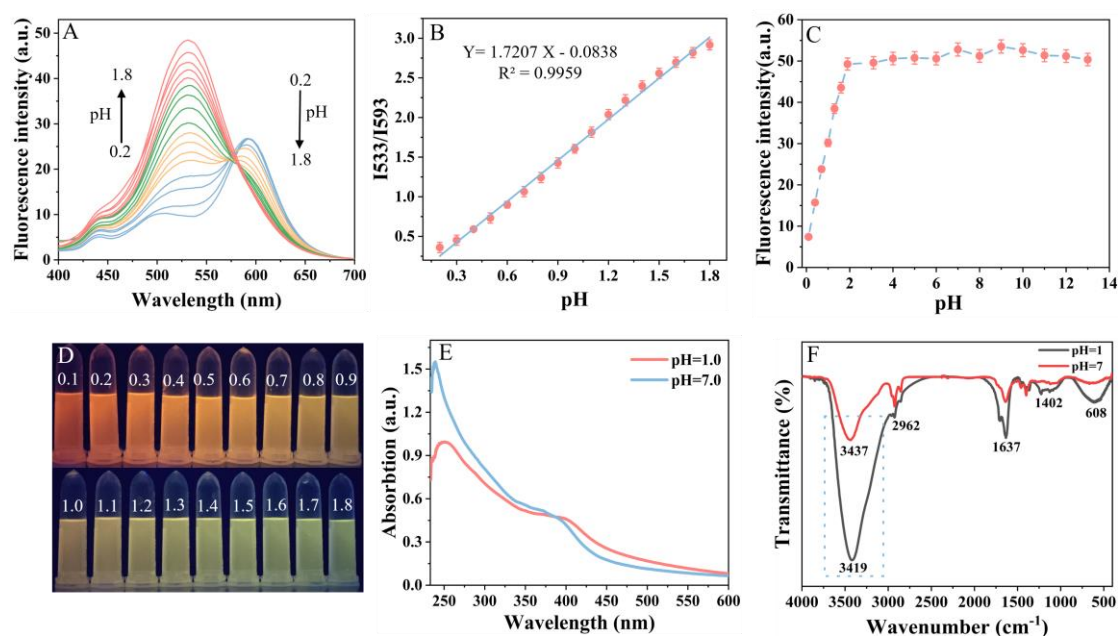
287 3.4. Ratiometric detection of pH

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 ≥ 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 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/-NH₂ peak is red-
325 shifted from 3419 cm^{-1} to 3437 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 -OH/O=C-NH₂/-NH₂ of TS-CDs were depleted by
 334 deprotonation (Dan et al., 2021). Moreover, corresponding to the infrared absorption
 335 spectra, the stretching vibration of -OH/-NH₂ 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.

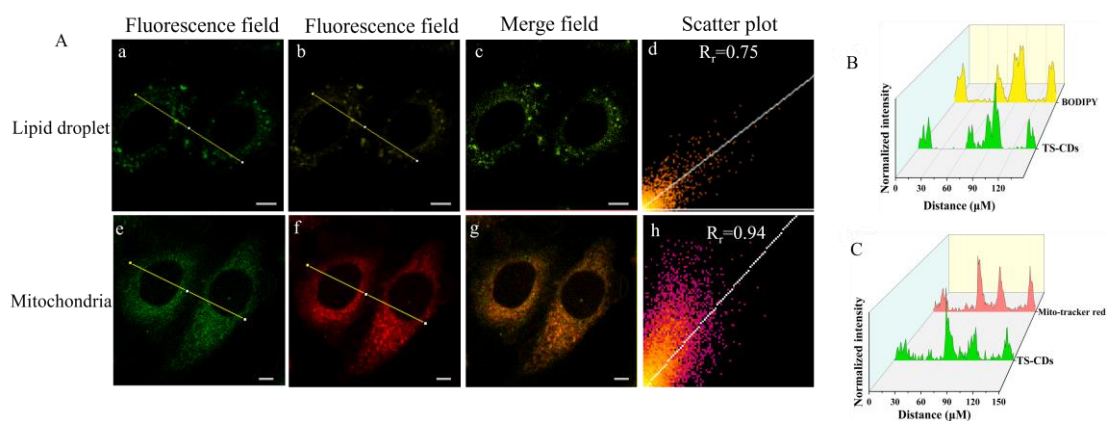


338
 339 **Fig. 3.** (A) Fluorescence emission spectra of TS-CDs (8.0 µg/mL) (30% acetone and 70% water)
 340 at different pH (0.2~1.8) (B) Linear relationship of the fluorescence emission intensity (8.0 µg/mL)
 341 ratio of TS-CDs between 533 nm and 593 nm. (C) Changes in fluorescence emission intensity of
 342 TS-CDs (8.0 µg/mL, λ_{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.



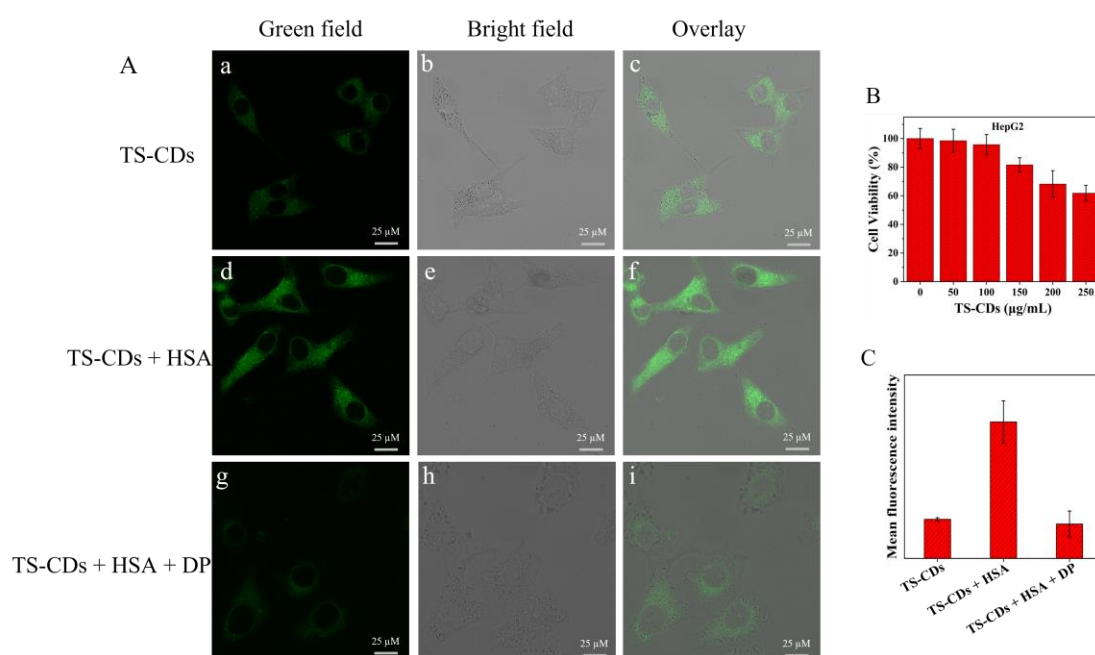
355

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 μM) for 30 min. (d) Co-localization
 358 scatter plot of TS-CDs (8.0 $\mu\text{g/mL}$) and BODIPY 493/503 (1.0 μM). (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 μM) 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 (λ_{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.

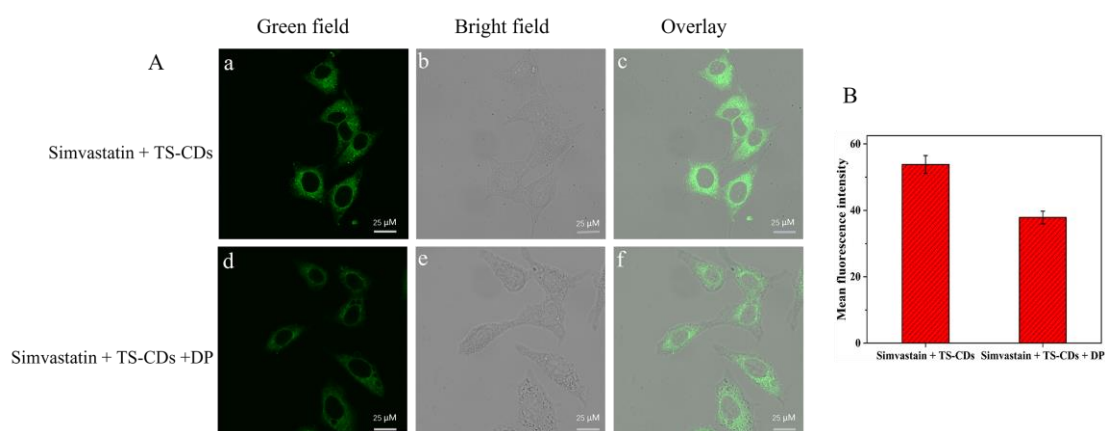


382 **Fig. 5.** (A) Confocal images of HepG2 cells after incubated with TS-CDs ($8.0 \mu\text{g/mL}$) for 30
 383 min(a-c), TS-CDs ($8.0 \mu\text{g/mL}$) for 30 min and HSA ($70 \mu\text{M}$) for 24 h (d-f). HSA ($70 \mu\text{M}$) for 24 h,
 385 DP (3.0 mM) for 30 min and TS-CDs ($8.0 \mu\text{g/mL}$)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

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 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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424 **References**

- 425 Alas, M. O., Alkas, F. B., Aktas Sukuroglu, A., Genc Alturk, R., & Battal, D. (2020). Fluorescent
426 carbon dots are the new quantum dots: an overview of their potential in emerging technologies and
427 nanosafety. *Journal of Materials Science*, *55*(31), 15074-15105. doi:10.1007/s10853-020-05054-y
- 428 Arshad, F., Pal, A., & Sk, M. P. (2021). Review—aggregation-induced emission in carbon dots for
429 potential Applications. *ECS Journal of Solid State Science and Technology*, *10*(2).
430 doi:10.1149/2162-8777/abdfb8
- 431 Chakrabarty, A., Mallick, A., Haldar, B., Das, P., & Chattopadhyay, N. (2007). Binding interaction of a
432 biological photosensitizer with serum albumins: a biophysical study. *Biomacromolecules*, *8*(3),
433 920-927. doi:10.1021/bm061084s
- 434 Chang, D., Zhao, Z., Shi, H., Feng, J., Yang, Y., & Shi, L. (2022). Ratiometric fluorescent carbon dots
435 for enantioselective sensing of L-lysine and pH discrimination in vivo and in vitro. *Sensors and*
436 *Actuators B: Chemical*, *362*, 131792. doi:10.1016/j.snb.2022.131792
- 437 Dan, C., Zhao, Z., Feng, J., Xin, Y., Yang, Y., & Shi, L. (2021). Lysosome-targeted red-fluorescent
438 carbon dots for turn-on detection of permanganate and pH in vivo and in vitro. *Sensors and*
439 *Actuators B: Chemical*, *349*, 130774. doi:10.1016/j.snb.2021.130774
- 440 Feng, J., Chen, Y., Liu, X., & Liu, S. (2015). Efficient improvement of surface activity of tea saponin
441 through Gemini-like modification by straightforward esterification. *Food Chemistry*, *171*, 272-279.
442 doi:10.1016/j.foodchem.2014.08.125
- 443 Gao, M. X., Liu, C. F., Wu, Z. L., Zeng, Q. L., Yang, X. X., Wu, W. B., . . . Huang, C. Z. (2013). A
444 surfactant-assisted redox hydrothermal route to prepare highly photoluminescent carbon quantum
445 dots with aggregation-induced emission enhancement properties. *Chemical Communications*
446 *(Camb)*, *49*(73), 8015-8017. doi:10.1039/c3cc44624g
- 447 Guo, J., Lu, Y., Xie, A. Q., Li, G., Liang, Z. B., Wang, C. F., . . . Chen, S. (2022). Yellow-emissive
448 carbon dots with high solid-state photoluminescence. *Advanced Functional Materials*, *32*(20).
449 doi:10.1002/adfm.202110393
- 450 Ha, C. E., Ha, J. S., Theriault, A. G., & Bhagavan, N. V. (2009). Effects of statins on the secretion of
451 human serum albumin in cultured HepG2 cells. *Journal of Biomedical Science*, *16*, 32.
452 doi:10.1186/1423-0127-16-32
- 453 He, X., Han, Y., Luo, X., Yang, W., Li, C., Tang, W., . . . Li, Z. (2020). Terbium (III)-referenced N-
454 doped carbon dots for ratiometric fluorescent sensing of mercury (II) in seafood. *Food Chemistry*,
455 *320*, 126624. doi:10.1016/j.foodchem.2020.126624
- 456 Ke, Y., Cao, J., Gong, J., & Fu, N. (2022). A near-infrared naphthalimide fluorescent probe for targeting

457 the lysosomes of liver cancer cells and specifically selecting HSA. *Sensors and Actuators B: Chemical*, 352, 131015. doi:10.1016/j.snb.2021.131015

458

459 Kuo, P. C., Lin, T. C., Yang, C. W., Lin, C. L., Chen, G. F., & Huang, J. W. (2010). Bioactive saponin
460 from tea seed pomace with inhibitory effects against *Rhizoctonia solani*. *Journal of Agricultural
461 and Food Chemistry*, 58(15), 8618-8622. doi:10.1021/jf1017115

462 Li, X., Feng, Q., Qu, L., Zhao, T., Li, X., Bai, T., . . . Li, J. (2020). A water-soluble and incubate-free
463 fluorescent environment-sensitive probe for ultrafast visualization of protein thiols within living
464 cells. *Analytica Chimica Acta*, 1126, 72-81. doi:10.1016/j.aca.2020.06.026

465 Liu, Y., Yu, Y., Meng, Q., Wei, Q., He, W., Zhao, Q., . . . Zhang, J. (2022). A fluorescent pH probe for
466 evaluating the freshness of chicken breast meat. *Food Chemistry*, 384, 132554.
467 doi:10.1016/j.foodchem.2022.132554

468 Lu, M., Duan, Y., Song, Y., Tan, J., & Zhou, L. (2018). Green preparation of versatile nitrogen-doped
469 carbon quantum dots from watermelon juice for cell imaging, detection of Fe³⁺ ions and cysteine,
470 and optical thermometry. *Journal of Molecular Liquids*, 269, 766-774.
471 doi:10.1016/j.molliq.2018.08.101

472 Luo, J., Xie, Z., Lam, J. W., Cheng, L., Chen, H., Qiu, C., . . . Tang, B. Z. (2001). Aggregation-induced
473 emission of 1-methyl-1,2,3,4,5-pentaphenylsilole. *Chemical Communications (Camb)*(18), 1740-
474 1741. doi:10.1039/b105159h

475 Luo, X., Zhang, W., Han, Y., Chen, X., Zhu, L., Tang, W., . . . Li, Z. (2018). N,S co-doped carbon dots
476 based fluorescent "on-off-on" sensor for determination of ascorbic acid in common fruits. *Food
477 Chemistry*, 258, 214-221. doi:10.1016/j.foodchem.2018.03.032

478 Murch, S. H., Phillips, D., Walker-Smith, J. A., Winyard, P. J. D., Meadows, N., Koletzko, S., . . . Klein,
479 J. (1996). Congenital enterocyte heparan sulphate deficiency with massive albumin loss, secretory
480 diarrhoea, and malnutrition. *The Lancet*, 347(9011), 1299-1301. doi:https://doi.org/10.1016/S0140-
481 6736(96)90941-1

482 Ning, Y., Cui, J., Lu, Y., Wang, X., Xiao, C., Wu, S., . . . Zhang, Y. (2018). De novo design and
483 synthesis of a novel colorimetric fluorescent probe based on naphthalenone scaffold for selective
484 detection of hypochlorite and its application in living cells. *Sensors and Actuators B: Chemical*,
485 269, 322-330. doi:10.1016/j.snb.2018.04.156

486 Ning, Y., Wang, X., Sheng, K., Yang, L., Han, W., Xiao, C., . . . Wu, S. (2018). A novel colorimetric
487 and fluorescence turn-on pH sensor with a notably large Stokes shift for its application. *New
488 Journal of Chemistry*, 42(17), 14510-14516. doi:10.1039/c8nj02860e

489 Panwar, N., Soehartono, A. M., Chan, K. K., Zeng, S., Xu, G., Qu, J., . . . Chen, X. (2019).
490 Nanocarbons for biology and medicine: sensing, imaging, and drug delivery. *Chemical Reviews*,
491 119(16), 9559-9656. doi:10.1021/acs.chemrev.9b00099

492 Qian, L., Tong, B., Shen, J., Shi, J., Zhi, J., Dong, Y., . . . Tang, B. Z. (2009). Crystallization-induced
493 emission enhancement in a phosphorus-containing heterocyclic luminogen. *The Journal of
494 Physical Chemistry B*, 113(27), 9098-9103. doi:10.1021/jp900665x

495 Qu, S., Wang, X., Lu, Q., Liu, X., & Wang, L. (2012). A biocompatible fluorescent ink based on water-
496 soluble luminescent carbon nanodots. *Angewandte Chemie International Edition*, 51(49), 12215-
497 12218. doi:10.1002/anie.201206791

498 Shangguan, J., He, D., He, X., Wang, K., Xu, F., Liu, J., . . . Huang, J. (2016). Label-free carbon-dots-
499 based ratiometric fluorescence pH nanoprobe for intracellular pH sensing. *Analytical Chemistry*,
500 88(15), 7837-7843. doi:10.1021/acs.analchem.6b01932

501 She, M., Wu, S., Wang, Z., Ma, S., Yang, Z., Yin, B., . . . Li, J. (2017). Exploration of congeneric
502 Hg(II)-mediated chemosensors driven by S-Hg affinity, and their application in living system.
503 *Sensors and Actuators B: Chemical*, *247*, 129-138. doi:10.1016/j.snb.2017.03.015

504 Song, Z., Quan, F., Xu, Y., Liu, M., Cui, L., & Liu, J. (2016). Multifunctional N,S co-doped carbon
505 quantum dots with pH- and thermo-dependent switchable fluorescent properties and highly
506 selective detection of glutathione. *Carbon*, *104*, 169-178. doi:10.1016/j.carbon.2016.04.003

507 Vijayakumar, C., Kartha, K. K., Balan, B., Poullose, S., & Takeuchi, M. (2019). Protein-assisted
508 supramolecular control over fluorescence resonance energytransfer in aqueous medium. *The*
509 *Journal of Physical Chemistry C*, *123*(20), 13141-13146. doi:10.1021/acs.jpcc.9b02002

510 Wang, G., Xu, W., Guo, Y., & Fu, N. (2017). Near-infrared squaraine dye as a selective protein sensor
511 based on self-assembly. *Sensors and Actuators B: Chemical*, *245*, 932-937.
512 doi:10.1016/j.snb.2017.01.172

513 Wang, Q., Zhang, S., Zhong, Y., Yang, X. F., Li, Z., & Li, H. (2017). Preparation of yellow-green-
514 emissive carbon dots and their application in constructing a fluorescent turn-on nanoprobe for
515 imaging of selenol in living cells. *Analytical Chemistry*, *89*(3), 1734-1741.
516 doi:10.1021/acs.analchem.6b03983

517 Xia, C., Cao, M., Xia, J., Zhou, G., Jiang, D., Zhang, D., . . . Li, H. (2019). An ultrafast responsive and
518 sensitive ratiometric fluorescent pH nanoprobe based on label-free dual-emission carbon dots.
519 *Journal of Materials Chemistry C*, *7*(9), 2563-2569. doi:10.1039/c8tc05693e

520 Xu, D., Lin, Q., & Chang, H. T. (2020). Recent advances and sensing applications of carbon dots.
521 *Small Methods*, *4*(4). doi:10.1002/smt.201900387

522 Xu, J.-F., Yang, Y.-S., Jiang, A.-Q., & Zhu, H.-L. (2022). Detection methods and research progress of
523 human serum albumin. *Critical Reviews in Analytical Chemistry*, *52*(1), 72-92.
524 doi:10.1080/10408347.2020.1789835

525 Xu, Z.-Q., Yang, Q.-Q., Lan, J.-Y., Zhang, J.-Q., Peng, W., Jin, J.-C., . . . Liu, Y. (2016). Interactions
526 between carbon nanodots with human serum albumin and γ -globulins: The effects on the
527 transportation function. *Journal of Hazardous Materials*, *301*, 242-249.
528 doi:https://doi.org/10.1016/j.jhazmat.2015.08.062

529 Yang, H., Liu, Y., Guo, Z., Lei, B., Zhuang, J., Zhang, X., . . . Hu, C. (2019). Hydrophobic carbon dots
530 with blue dispersed emission and red aggregation-induced emission. *Nature Communnications*,
531 *10*(1), 1789. doi:10.1038/s41467-019-09830-6

532 Yang, M., Song, Y., Zhang, M., Lin, S., Hao, Z., Liang, Y., . . . Chen, P. R. (2012). Converting a
533 solvatochromic fluorophore into a protein-based pH indicator for extreme acidity. *Angewandte*
534 *Chemie International Edition*, *51*(31), 7674-7679. doi:10.1002/anie.201204029

535 Yang, Y. Z., Xiao, N., Liu, S. G., Han, L., Li, N. B., & Luo, H. Q. (2020). pH-induced aggregation of
536 hydrophilic carbon dots for fluorescence detection of acidic amino acid and intracellular pH
537 imaging. *Materials Science & Engineering C-Materials for Biological Applications*, *108*, 110401.
538 doi:10.1016/j.msec.2019.110401

539 Yuan, F., Ding, L., Li, Y., Li, X., Fan, L., Zhou, S., . . . Yang, S. (2015). Multicolor fluorescent
540 graphene quantum dots colorimetrically responsive to all-pH and a wide temperature range.
541 *Nanoscale*, *7*(27), 11727-11733. doi:10.1039/c5nr02007g

542 Zhang, F., Du, T., Jiang, L., Zhu, L., & Tian, D. (2022). A combined "AIE + ESIPT" fluorescent probe
543 for detection of lipase activity. *Bioorganic Chemistry*, *128*, 106026.
544 doi:10.1016/j.bioorg.2022.106026

545 Zhu, S., Meng, Q., Wang, L., Zhang, J., Song, Y., Jin, H., . . . Yang, B. (2013). Highly
546 photoluminescent carbon dots for multicolor patterning, sensors, and bioimaging. *Angewandte*
547 *Chemie International Edition*, 52(14), 3953-3957. doi:10.1002/anie.201300519

548