Supplemental Document

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Human serum albumin gradient in serous ovarian cancer cryosections measured by fluorescence lifetime: supplement

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Supplementary Information

Human serum albumin gradient in serous ovarian cancer cryosections measured by fluorescence lifetime

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1. Absorption and fluorescence response of SD to HSA.

A UV-VIS spectrophotometer (Cintra 2020, GBC, Australia) recorded the absorption of SD with different protein additions and the spectral data were recorded in the range of a 450-800 nm wavelength using quartz cuvettes with a 10 mm path length at 298 K. The single-photon emission spectra of the SD solution with 585 nm excitation were recorded using a fluorescence spectrophotometer (iHR-830, HORIBA, USA).

We evaluated the specificity of SD to HSA by investigating the absorption and fluorescence emission spectra of SD in PBS by adding HSA and different proteins existing in tissue generally, including hemoglobin, transferrin, myoglobin, collagen, fibronectin, laminin, elastin, and proteoglycan, respectively. Fig. S1(A) shows that only the addition of HSA decreased the absorption at 689 nm, whereas the absorption peak generated an increase and bathochromic shift from 620 to 633 nm. Such changes in the absorption of SD depended on the concentration of HSA [Fig. S1(B)]. The fluorescence emission of the SD solution was significantly enhanced, and the emission peak generated a bathochromic shift from 632 to 646 nm upon the addition of HSA [Figs. S1(C and D)], which can be attributed to the formation of hydrophobic environment surrounding the SD chromophore [1-3]. The results show that SD molecules converted from aggregate to monomer upon the addition of HSA and that SD bound with HSA specifically because of hydrophobic and electrostatic interactions.

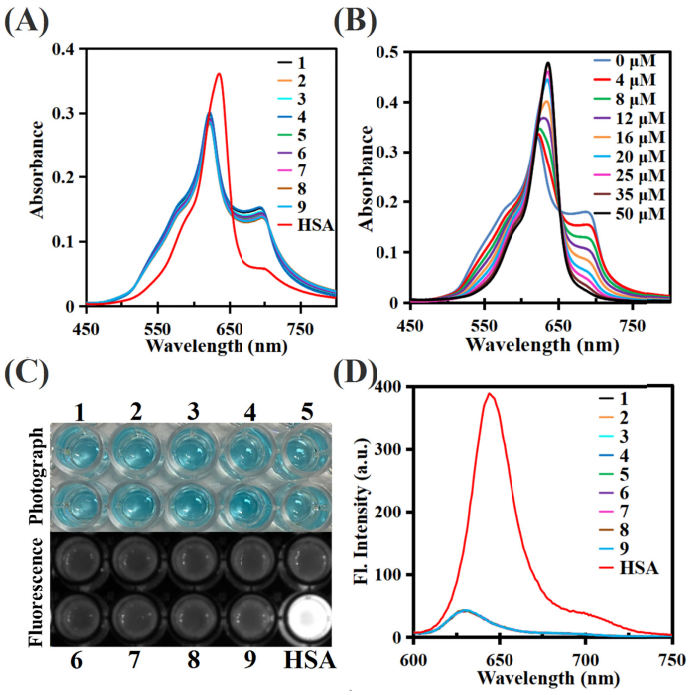

Fig. S1. Absorption and fluorescence response of SD with the addition of different proteins in PBS. (A) Absorption of 40 μM SD alone (1) and with addition of 20 μM hemoglobin (2), transferrin (3), myoglobin (4), collagen (5), fibronectin (6), laminin (7), elastin (8), proteoglycan (9), and HSA. (B) Absorption of SD with titration of HSA. (C) Photograph of SD solution at the same situation as (A). Fluorescence was collected from 640 to 680 nm with 585 nm excitation. (D) Corresponding emission spectra of (C). Since the change is small, the curves 1-9 are overlapping.

2. Two-photon emission spectra of SD solution with different [HSA] addition.

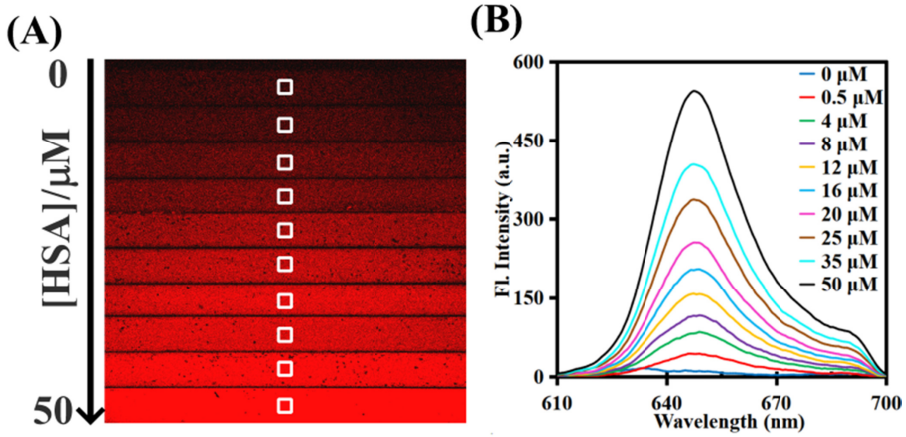

Fig. S2. (A) TPEF imaging of SD solution with different [HSA] addition; White square: ROI; (B) Corresponding spectra in ROI with 840 nm excitation.

3. The fluorescence lifetime (τ) intervals correlated with [HSA] ranges in the cryosections of LGSOC and HGSOC.

With fixing the long lifetime [2439 ps, indicated by the blue circle in Fig. 3 (F)] and short lifetime [214 ps, indicated by the red circle in Fig. 3 (F)], we fitted the fluorescence decay curves of SD to a double exponential model to obtain the τ intervals (Table S1) for different [HSA] ranges in Fig. 3 and Fig. 4, just as the descriptions in previous study [4].

Table S1. The correlation between [HSA] and τ intervals.

LGSOC		HGSOC	
[HSA] / μM	τ / ps	[HSA] / μM	τ / ps
9-15	921-1237	23-44	1728-2214
3-9	674-921	14-23	1392-1728
1-3	317-674	5-14	836-1392

4. Two-photon emission spectra of ROI in cryosections.

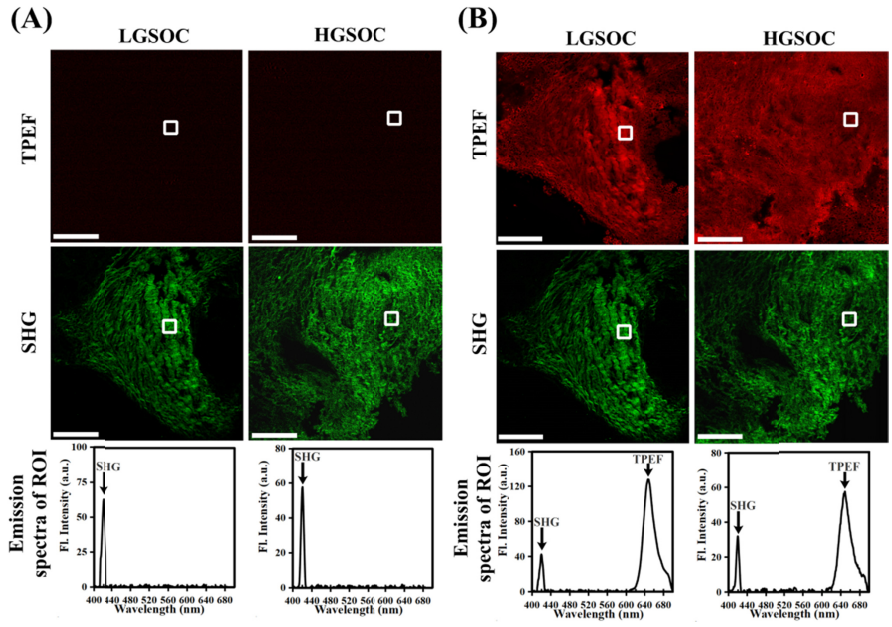

Fig. S3. TPEF/SHG images, and TP emission spectra of ROI for the cryosections before (A) and after (B) being stained with SD. The LGSOC

sample was the same as Fig. 3; The HGSOC sample was the same as Fig. 4; White open square: ROI; Scale bar: 150 μm; The corresponding emission spectra were under 840 nm excitation.

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