Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Switch-On Near Infrared Emission in Albumin Behind Dark Fabric: Toward Application in Forensic Latent Bloodstain Detection

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Photophysical spectra:

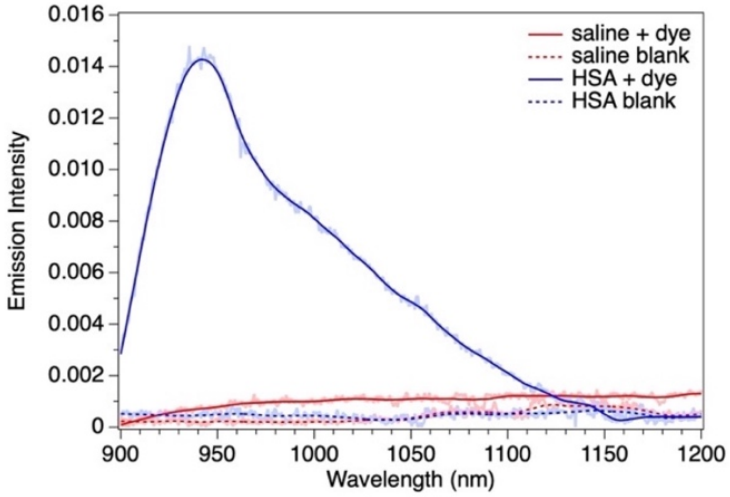

Figure S1. Raw emission spectrum of **SO3C7** in HSA and saline compared to background scan of a blank solution (solvent without dye), showing near background noise level emission from the dye in saline.

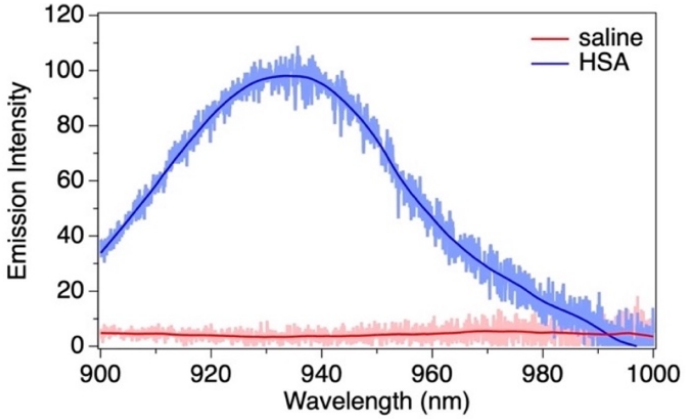

Figure S2. Emission spectrum of **SO3C7** in HSA and saline detected using a silicon CCD detector. Smoothed emission is the darker line in front of the lighter raw emission data.

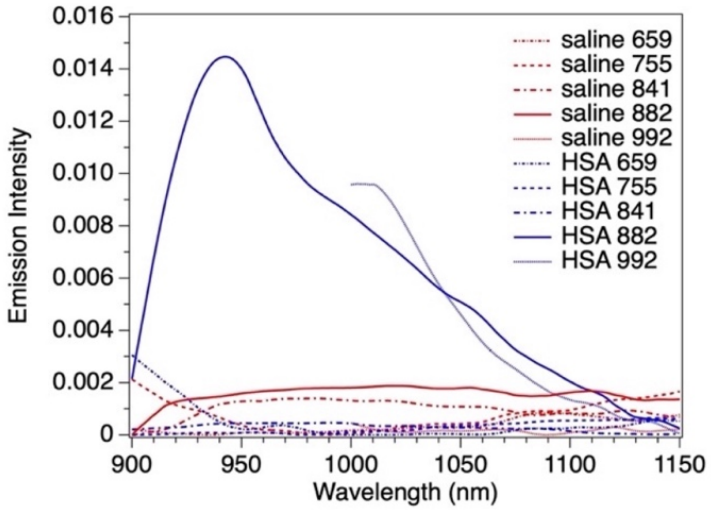

Figure S3. Emission spectrum of **SO3C7** in HSA and saline at different excitation wavelengths, showing no substantial emission from saline while the HSA sample is most emissive at wavelengths ≥ 882 nm.

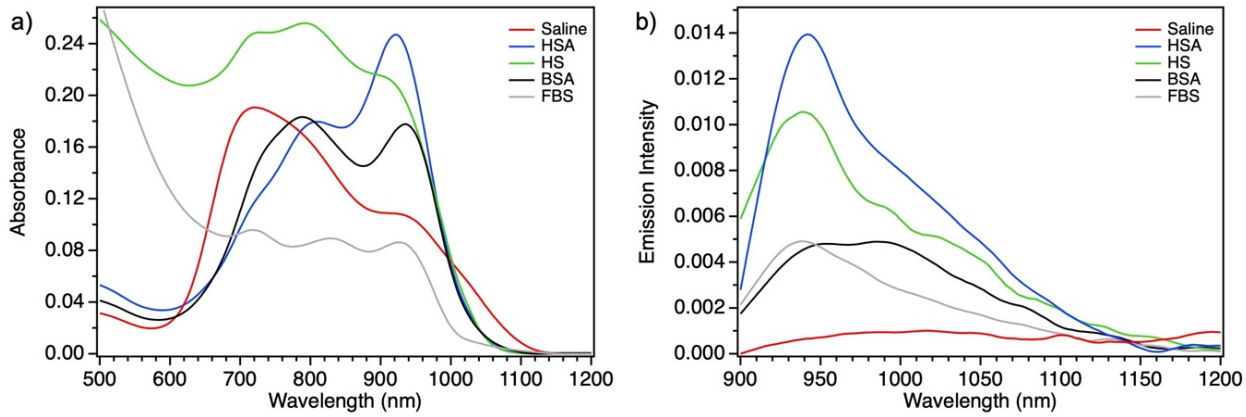

Figure S4. Absorption (a) and emission (b) of **SO3C7** in different albumin-containing solutions. HSA = human serum albumin, HS = human serum, BSA = bovine serum albumin, FBS = fetal bovine serum. All albumin sources diluted with 0.85% $NaCl_{(aq)}$ to similar albumin concentrations (~6×10^{-5} M) based on average albumin content of commercial serums. FBS was used as purchased due to the albumin content being ~4×10^{-5} M.

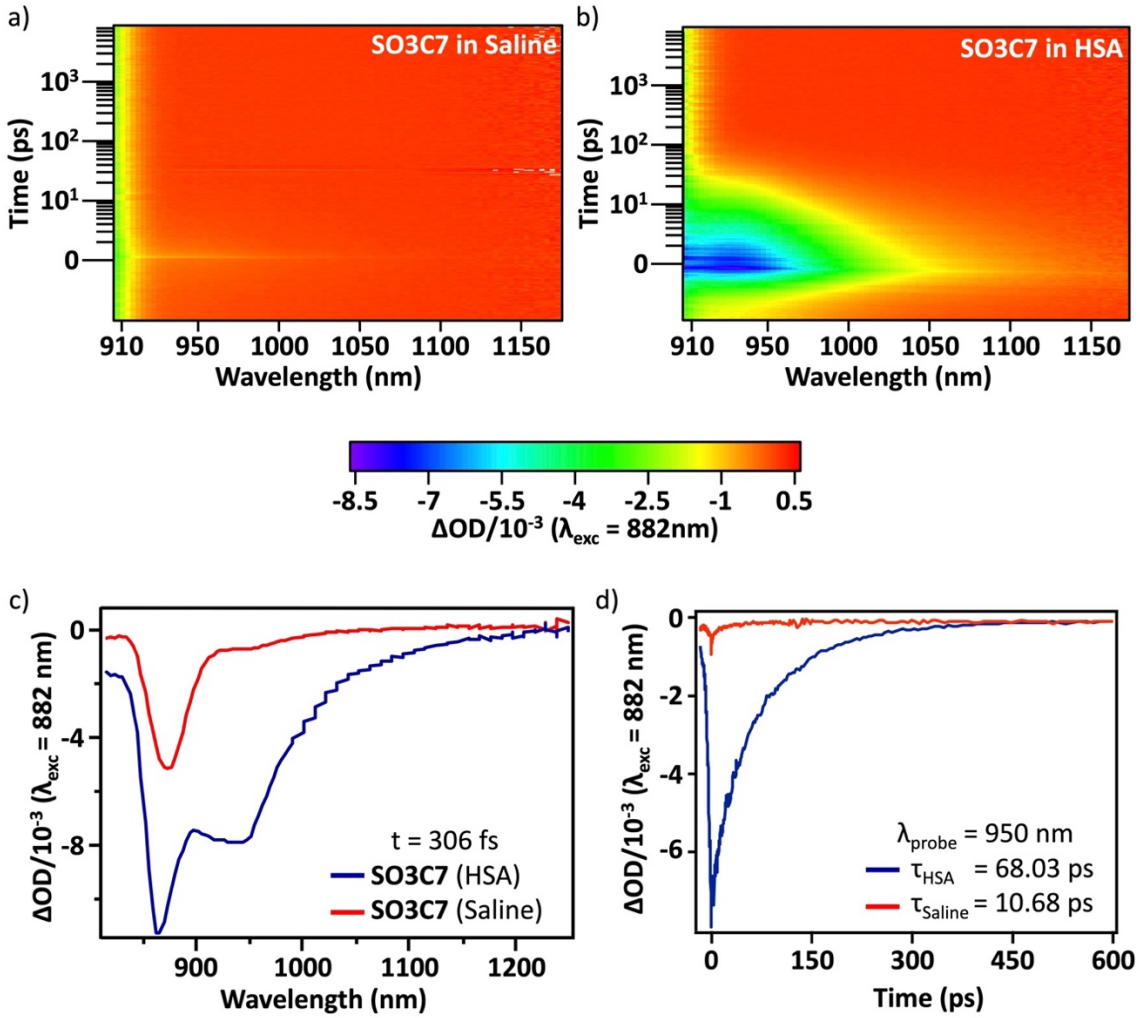

Figure S5. (a,b) Three-dimensional differential absorption spectra for **SO3C7** in saline and HSA. The excitation (pump) pulse was at 882 nm. (c,d) Comparisons between the stimulated emission of **SO3C7** in HSA and saline, isolated at the maximum signal in time (c) and wavelength (d).

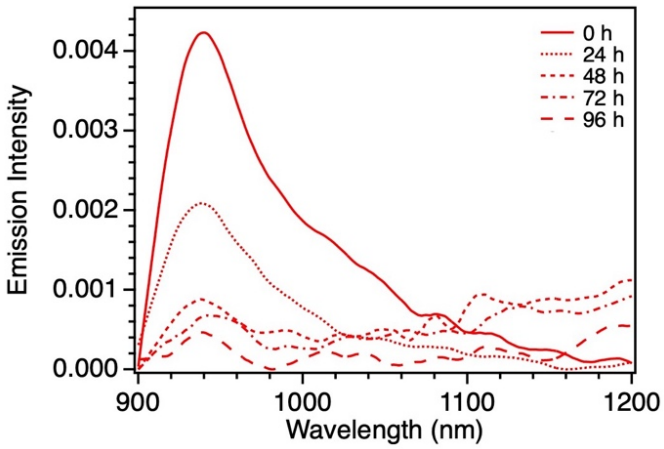

Figure S6. Emission spectra of **SO3C7** in HSA kept in ambient light over 96 h.

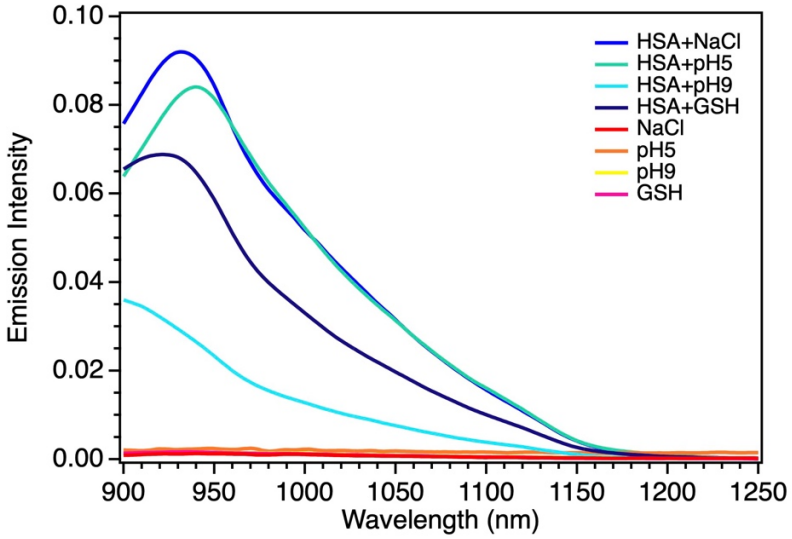

Figure S7. Emission spectra of **SO3C7** with several biologically relevant environments (0.85% NaCl, 0.1 M pH 5, 0.1 M pH 9, and 1×10^{-4} M GSH) both in the presence of HSA and without HSA (used in Figure 3a). Samples irradiated with 850 nm excitation.

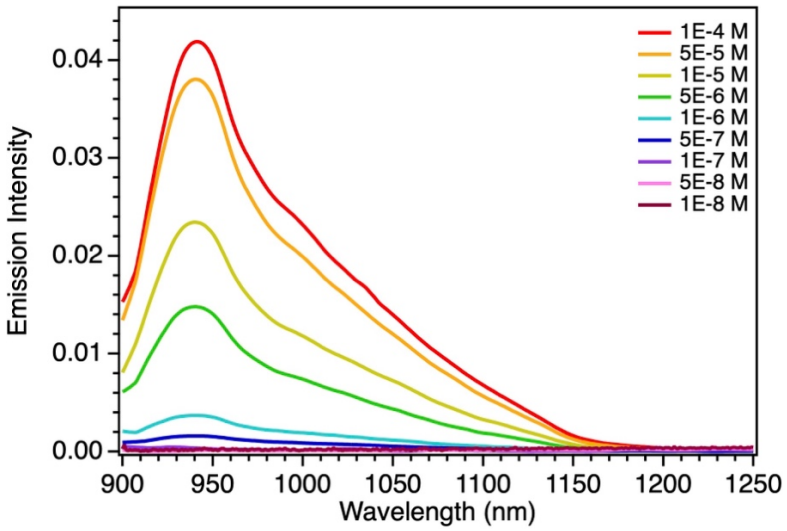

Figure S8. Emission spectra of **SO3C7** for Hill plot (Figure 3b) with different concentrations of HSA.

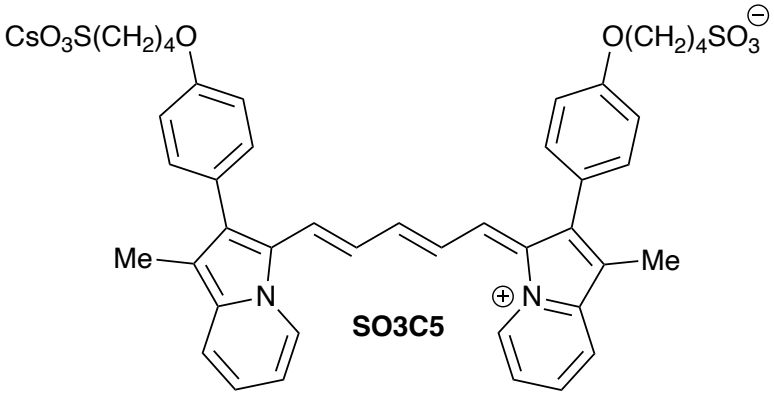

Figure S9. Structure of **SO3C5**.

NIR photographs:

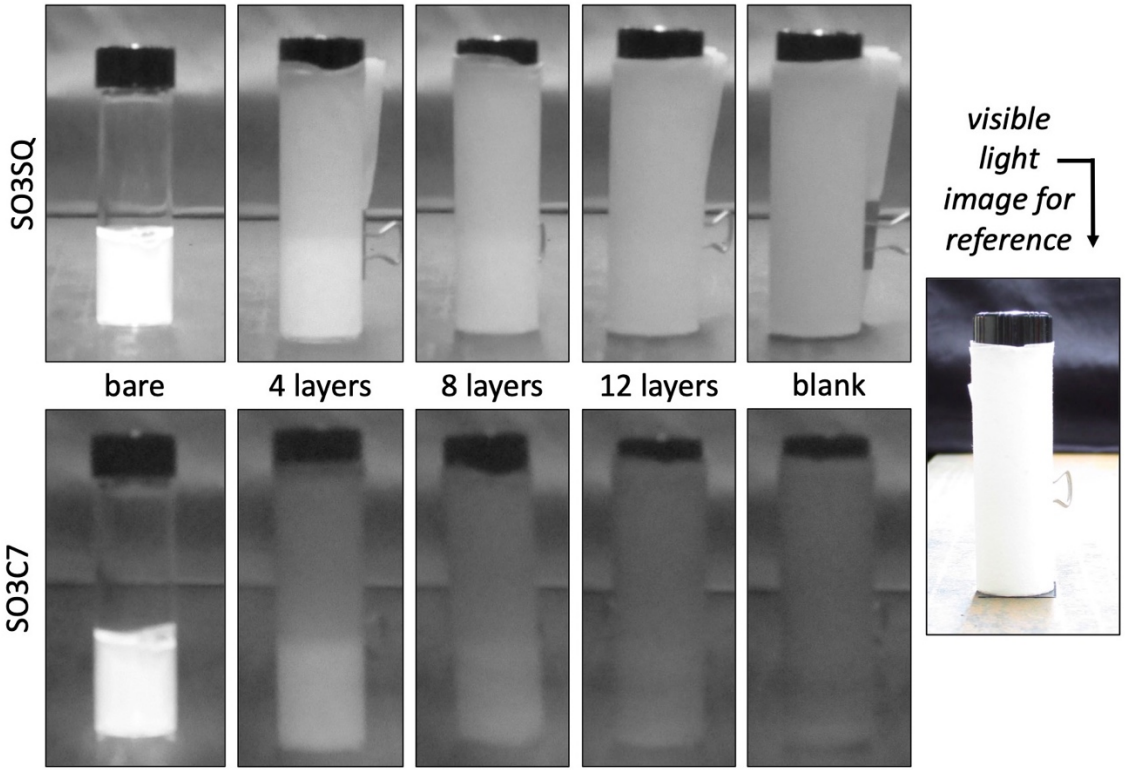

Figure S10. Grayscale NIR photographs of **SO3SQ** in HSA and **SO3C7** in HSA with layers of Kimwipes wrapped around the vial. Blank = water with no dye with the maximum number of layers around the vial to display a non-emissive reference.

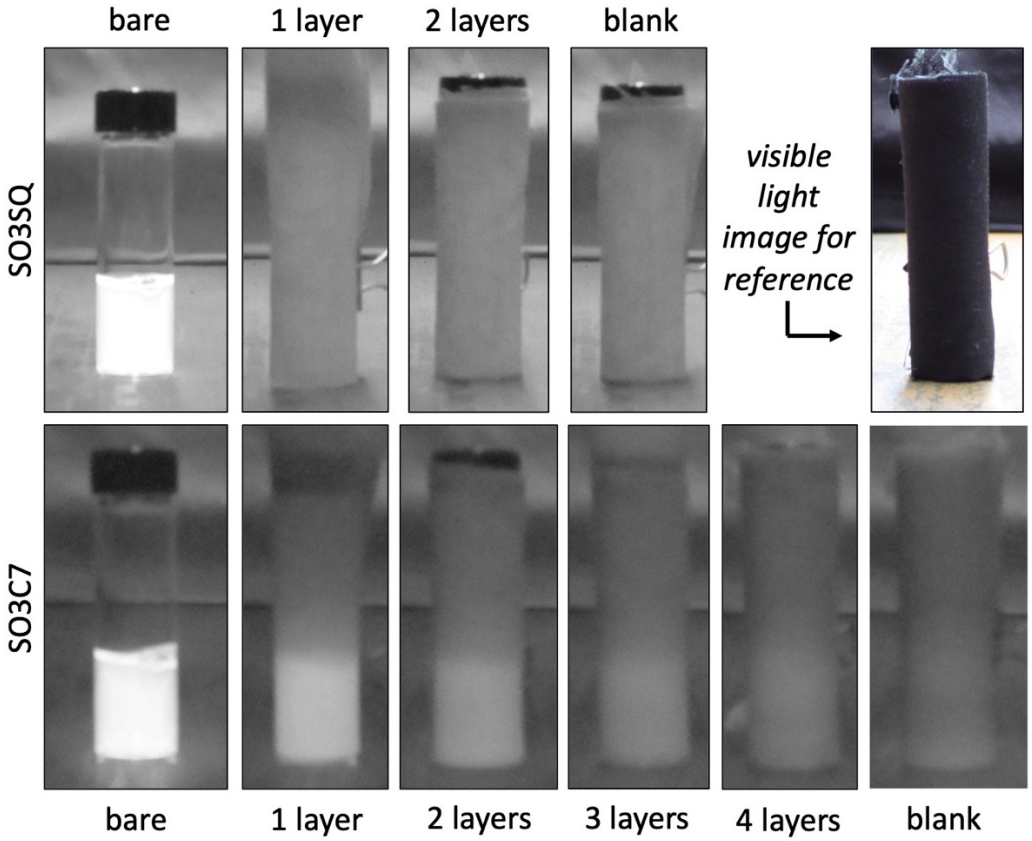

Figure S11. Grayscale NIR photographs of **SO3SQ** in HSA and **SO3C7** in HSA with layers of black fabric wrapped around the vial. Blank = water with no dye with the maximum number of layers around the vial to display a non-emissive reference.

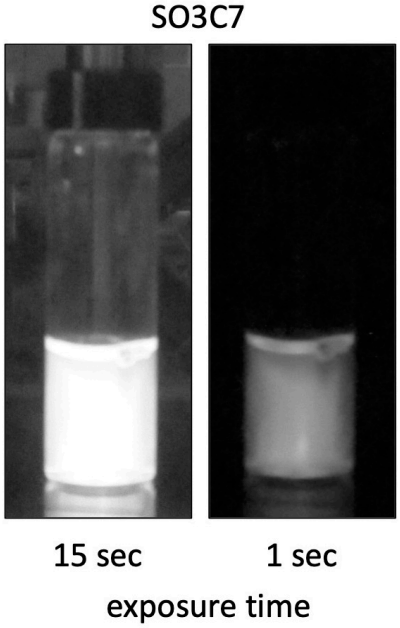

Figure S12. Grayscale NIR photographs of **SO3C7** in HSA with 15 seconds vs. 1 second exposure time. While dimmer, the emission is still captured with a short exposure time.

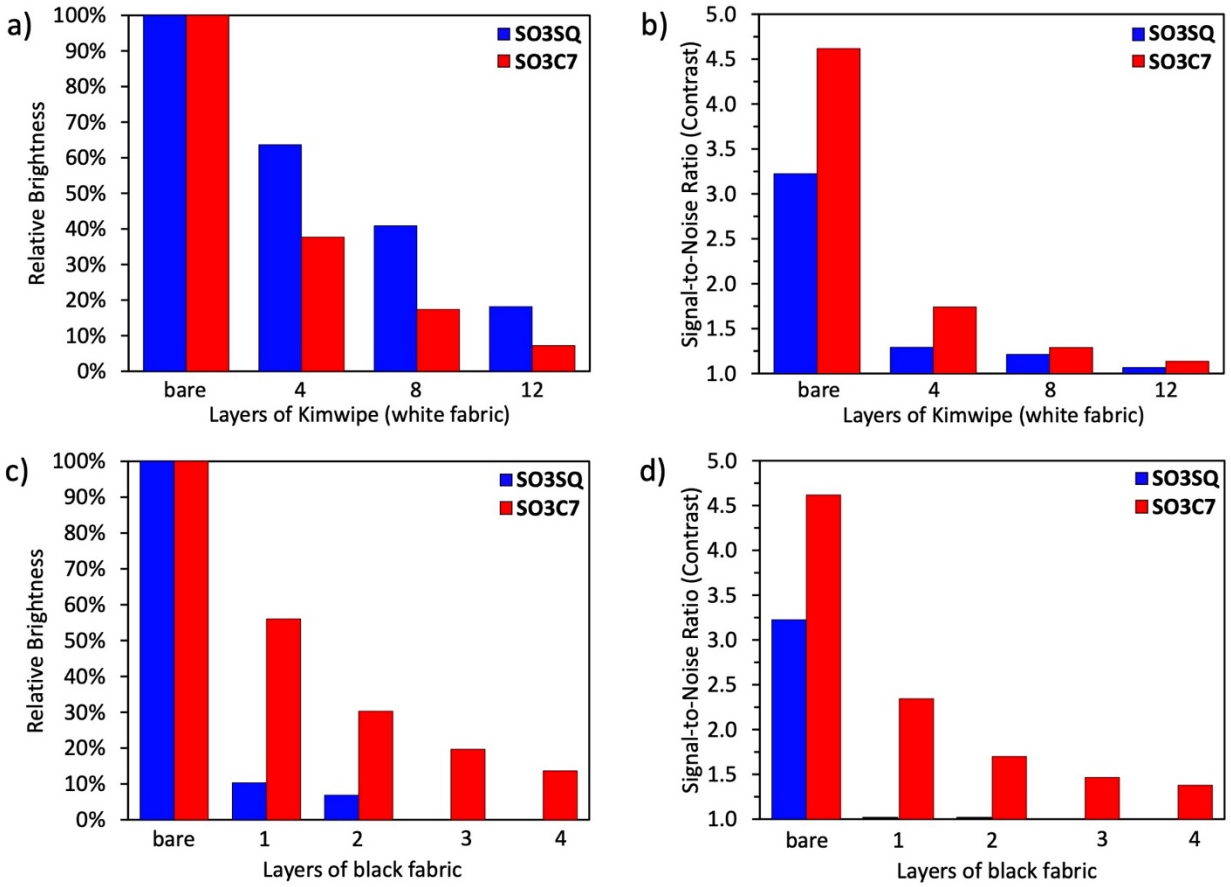

Figure S13. Relative brightness of **SO3SQ** in HSA versus **SO3C7** in HSA with a) white fabric (Kimwipe) or c) black fabric layers wrapped around the vial. The bare vial brightness value was set to 100% and the blank vial set to 0%. Note that the brightness was not determined for 3 and 4 layers for **SO3SQ** since those samples were not photographed. To determine contrast, that brightness value was compared to the background noise (darkest point above the emissive area of each vial) to produce a signal-to-noise ratio (brightness of emissive area divided by the brightness of the background) for **SO3SQ** in HSA versus **SO3C7** in HSA with b) white fabric (Kimwipe) or d) black fabric layers wrapped around the vial (see Figure S14). A signal-to-noise value of 1.00 equates to no difference in brightness of the emissive area compared to the background (entire vial is uniform in brightness). The blank sample in each case was very near this value. See Table S1 for full data table.

Table S1. Grayscale brightness values for the NIR photographs of **SO3SQ** and **SO3C7** used in the relative brightness (% bright.) and signal-to-noise ratio (SNR) graphs in Figure S13. Brightness was determined by taking the eyedropper tool in Microsoft PowerPoint at a point in the middle of the emissive area for each image (see Figure S14), then the grayscale slider was used to obtain the brightness value. Note that the brightness was not determined for 3 and 4 layers for **SO3SQ** since those samples were not photographed.

		layers of Kimwipe				layers of black fabric				
	bare	4	8	12	blank	1	2	3	4	blank
SO3SQ (signal)	100	84	74	64	56	48	46	–	–	42
SO3SQ (noise)	31	65	61	60	58	48	46	–	–	43
% bright.	100%	64%	41%	18%	0%	10%	7%	–	–	0%
SNR	3.23	1.29	1.21	1.07	0.97	1.00	1.00	–	–	0.97
SO3C7 (signal)	97	54	40	33	28	68	51	44	40	34
SO3C7 (noise)	21	31	31	29	29	29	30	30	29	28
% bright.	100%	38%	17%	7%	0%	56%	30%	20%	14%	0%
SNR	4.62	1.74	1.29	1.14	0.97	4.62	2.34	1.70	1.47	1.38

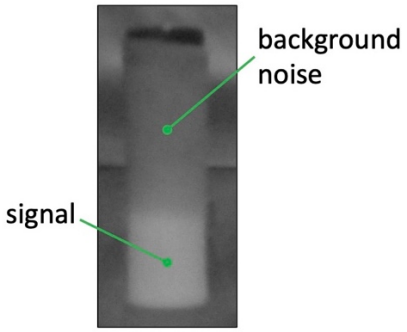

Figure S14. Visual explanation of an example NIR image noting where the source of signal and background noise comes from.

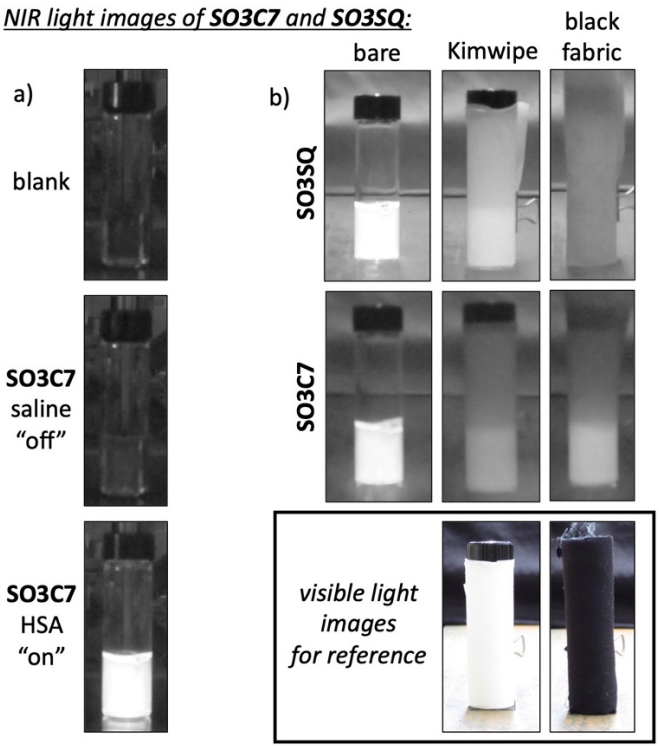

Figure S15. Grayscale version of Figure 4 from the main text.

Table S2. Comparison of **SO3C7** versus previously published dye-albumin complexes with emission enhancement. See main text for reference numbers.

dye	albumin source	λ_{max}^{emis} (nm)	comparison to **SO3C7**	ref.
SO3C7	human & bovine	942	–	*this work*
SO3SQ	human & bovine	722	outside of forensic window	11,12
SO3C5	human & bovine	830	outside of forensic window	11,12
ICG	human & bovine	827	outside of forensic window, emission does not switch-off when not in presence of albumin, no selectivity of human albumin (HSA) over bovine (BSA)	11,12, 19,20
FD-1080	bovine	1080	outside of forensic window, did not use human albumin (HSA)	14
TPE-MI	human	470	outside of forensic window	9
Cm-Np-B	human & bovine	460	outside of forensic window, no discussion of human/bovine selectivity	22
3	bovine	538	outside of forensic window, did not use human albumin (HSA)	15
CH-4T	human & bovine	1055	outside of forensic window, more selective for bovine albumin (BSA), requires 70 ˚C heating for optimal emission response	16
CF1065	bovine	1040	outside of forensic window, did not use human albumin (HSA)	17
FM1210	bovine	1100	outside of forensic window, did not use human albumin (HSA)	17
KSQ-4	bovine	817	outside of forensic window, did not use human albumin (HSA)	23
SQ-1	bovine	669	outside of forensic window, did not use human albumin (HSA)	24
SQ-2	bovine	684	outside of forensic window, did not use human albumin (HSA)	24
VG1-C8	bovine	661	outside of forensic window, did not use human albumin (HSA)	25
VG10-C8	bovine	686	outside of forensic window, did not use human albumin (HSA)	25

Characterization (Nuclear Magnetic Resonance) spectra:

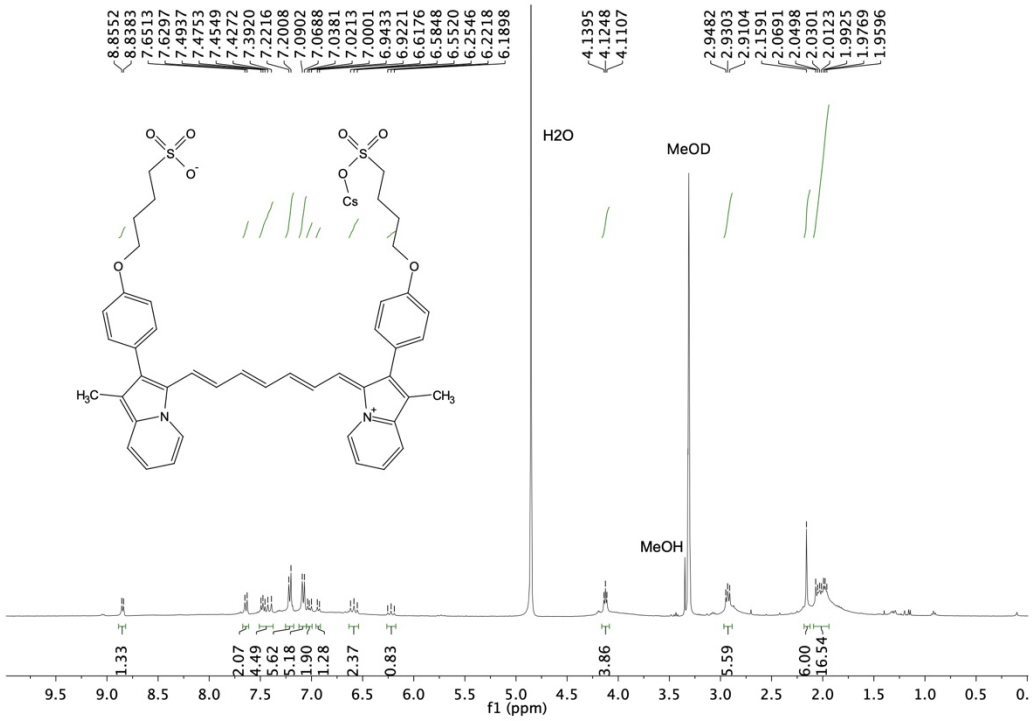

Figure S16. ^{1}H NMR of **SO3C7** in CD_3OD (400 MHz).

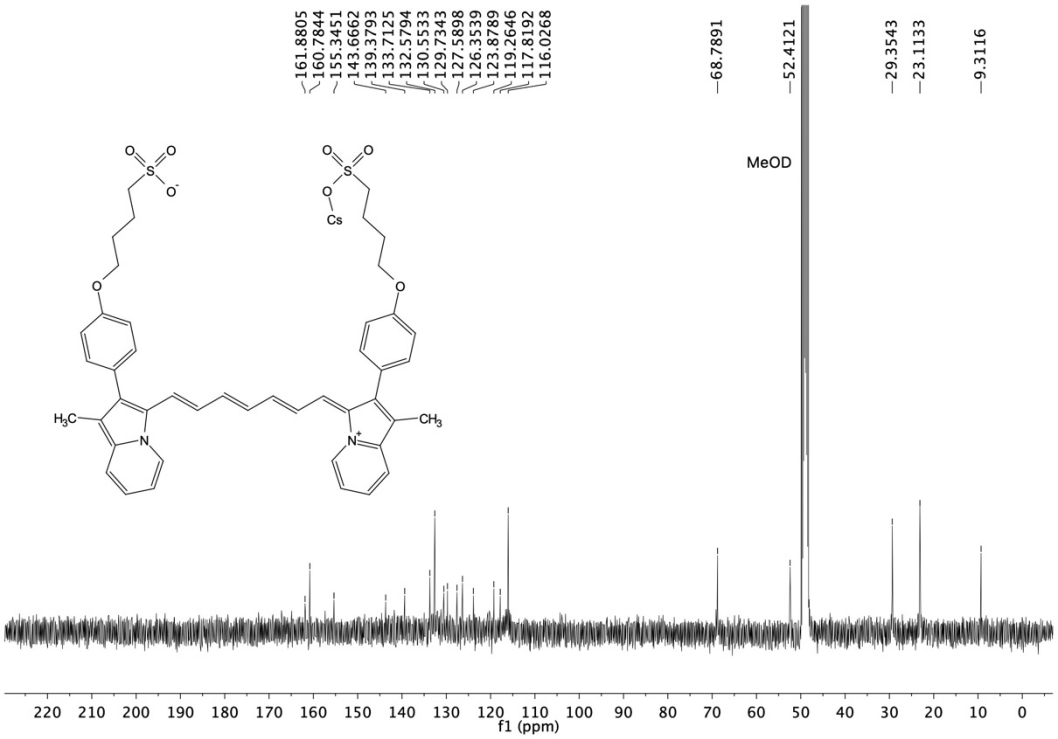

Figure S17. ^{13}C NMR of **SO3C7** in CD_3OD (400 MHz).