Supplementary Information

Multi-color cell imaging under identical excitation with salicylideneaniline analogue based fluorescent nanoparticles

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1. Synthesis and Characterization

1.1 General Procedure for the Preparation of SA Derivatives

The corresponding phenylenediamine (2 mmol) and phenylenealdehyde (4.2 mmol) were added into 20 mL EtOH in a 100 mL flask and stirred for 8 h at room temperature. After EtOH was removed under vacuum, the crude product was washed with cold diethyl ether and dried at 40 °C for 24 h. The characterization data of various SA derivatives are summarized next.

1.2 Characterizations

***N,N′*-Bis(4-hydroxyphenylene)-p-phenylenediamine (BHPpP, 1)**

Yellow solid, yield 98%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm, 298 K): 10.10 (s, 2H), 8.49 (s, 2H), 7.76 (d, J = 8.0 Hz, 4H), 7.24 (s, 4H), 6.87 (d, J = 8.4 Hz, 4H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm, 298 K): 161.23, 159.78, 149.96, 131.29, 128.30, 122.45, 116.33. HRMS: m/z calculated for $[C_{20}H_{17}N_2O_2]^+$: 317.1290, found: 317.1290. IR (KBr): 3273, 1603, 1590, 1517, 1492, 1459, 1443, 1388, 1311, 1285, 1258, 1194, 1168, 1108, 1011, 982, 895, 837, 828, 774, 721, 671, 640, 565, 543, 526, 483 cm^{-1}.

***N,N′*-Bis(salicylidene)-m-phenylenediamine (BSmP, 2)**

Yellow solid, yield 97%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm, 298 K): 12.99 (s, 2H), 9.03 (s, 2H), 7.66 (m, 2H), 7.51 (m, 2H), 7.41 (m, 2H), 7.34 (m, 2H), 6.98 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm, 298 K): 164.83, 161.00, 149.96, 134.14, 133.31, 131.03, 120.85, 119.95, 119.88, 117.33, 114.53. HRMS: m/z calculated for $[C_{20}H_{17}N_2O_2]^+$: 317.1290, found: 317.1299. IR (KBr): 3435, 1618, 1593, 1568, 1500, 1459, 1404, 1356, 1317, 1286, 1195, 1158, 1144, 1132, 1108, 1033, 998, 978, 952, 939, 910, 887, 853, 822, 790, 764, 751, 739, 685, 654, 622, 580, 562, 515, 487, 472 cm^{-1}.

***N,N′*-Bis(salicylidene)-o-phenylenediamine (BSoP, 3)**

Yellow solid, yield 99%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm, 298 K): 12.93 (s, 2H), 8.91 (s, 2H), 7.65 (m, 2H), 7.45 (m, 2H), 7.39 (m, 4H), 6.95 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm, 298 K): 164.71, 161.06, 142.93, 134.10, 133.13, 128.47, 120.41, 120.16, 119.75, 117.35. HRMS: m/z calculated for $[C_{20}H_{17}N_2O_2]^+$: 317.1290, found: 317.1278. IR (KBr): 3447, 1613, 1586, 1562, 1481, 1449, 1403, 1385, 1375, 1362, 1314, 1277, 1237, 1192, 1150, 1115, 1103, 1045, 1029, 998, 977, 944, 934, 910, 886, 855, 831, 809, 787, 760, 746, 640, 582, 530, 502, 472 cm^{-1}.

***N,N′*-Bis(salicylidene)-p-phenylenediamine (BSpP, 4)**

Yellow solid, yield 99%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm, 298 K): 13.05 (s, 2H), 9.01 (s, 2H), 7.53 (m, 8H), 6.97 (t, 4H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm, 298 K): 163.82, 160.96, 147.30, 134.02, 133.23, 123.23, 120.04, 119.87, 117.29. HRMS: m/z calculated for $[C_{20}H_{17}N_2O_2]^+$: 317.1290, found: 317.1300. IR (KBr): 3435, 1611, 1571, 1509, 1492, 1458, 1429, 1409, 1370, 1321, 1281, 1210, 1189, 1161, 1148, 1129, 1035, 969, 905, 860, 827, 775, 761, 750, 734, 618, 561, 526, 488 cm^{-1}.

***N,N′*-Bis(4-methoxy-salicylidene)-p-phenylenediamine (BMSpP, 5)**

Yellow solid, yield 96%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm, 298 K): 13.66 (s, 2H), 8.92 (s, 2H), 7.53 (d, J = 8.8 Hz, 2H), 7.46 (s, 4H), 6.55 (dd, 2H), 6.48 (d, J = 2.4 Hz, 2H), 3.79 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm, 298 K): 173.15, 163.80, 162.79, 146.68, 134.79, 122.91, 113.72, 107.58, 101.49, 56.18. HRMS: m/z calculated for $[C_{20}H_{21}N_2O_4]^+$: 377.1501, found:

377.1506. IR (KBr): 3447, 2978, 2945, 2850, 1623, 1612, 1573, 1519, 1498, 1466, 1444, 1399, 1359, 1338, 1290, 1235, 1205, 1194, 1170, 1120, 1030, 980, 966, 887, 853, 840, 810, 654, 589, 558, 541, 504, 477 cm^{-1}.

***N,N'*-Bis(3,5-bis(tertiarybutyl)-salicylidene)-p-phenylenediamine (BTBSpP, 6)**

Yellow solid, yield 96%. ^{1}H NMR (400 MHz, $CDCl_3$, ppm, 298 K): 13.93 (s, 2H), 9.06 (s, 2H), 7.56 (s, 4H), 7.51 (d, J = 2.4 Hz, 2H), 7.39 (d, J = 2.4 Hz, 2H), 1.41 (s, 18H), 1.29 (s, 18H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm, 298 K): 163.51, 158.51, 147.25, 140.87, 137.23, 128.35, 127.06, 122.40, 118.53, 35.34, 34.43, 31.71, 29.66. HRMS: m/z calculated for $[C_{36}H_{49}N_2O_2]^+$: 541.3794, found: 541.3807. IR (KBr): 3459, 2955, 2908, 2868, 1613, 1590, 1502, 1466, 1438, 1390, 1361, 1318, 1273, 1250, 1235, 1198, 1172, 1132, 1028, 984, 970, 932, 877, 856, 833, 802, 771, 729, 666, 643, 525 cm^{-1}.

2. Analytical and spectral characterization data

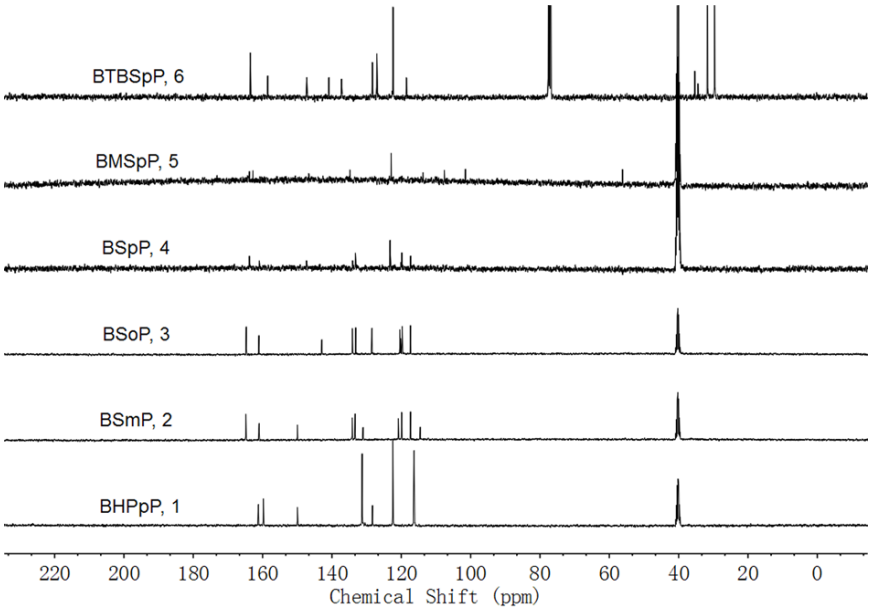

Fig. S1 ^{13}C NMR spectra of salicylideneaniline derivatives BHPpP (1), BSmP (2), BSoP (3), BSpP (4), BMSpP (5) and BTBSpP (6).

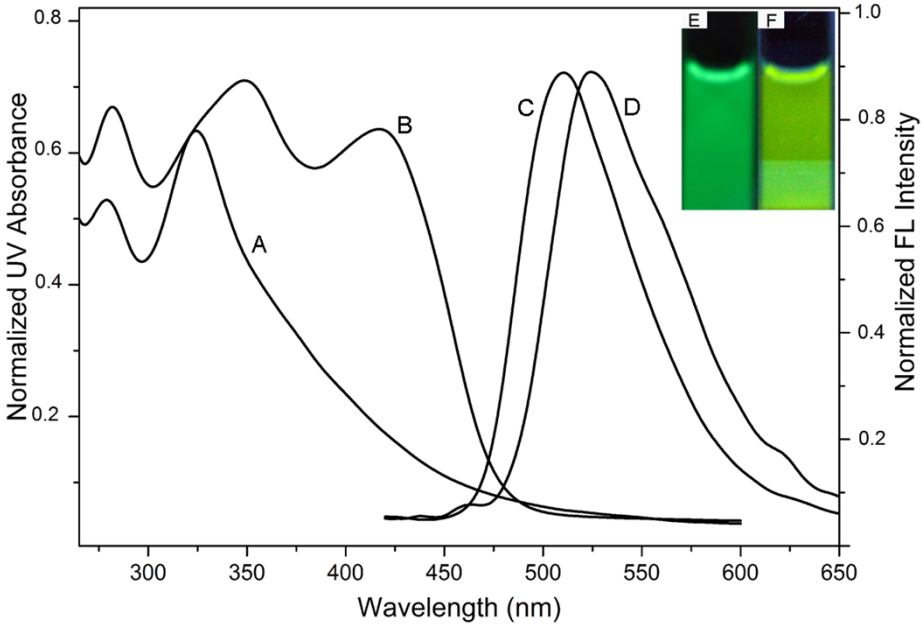

Fig. S2 Multi-color for BMSpP (5): UV (A and B) and FL (C and D) spectra with different water adding speed (slow: A and C), (fast: B and D). The corresponding fluorescence pictures under 365 nm UV lamb (slow: E green), (fast: F green-yellow).

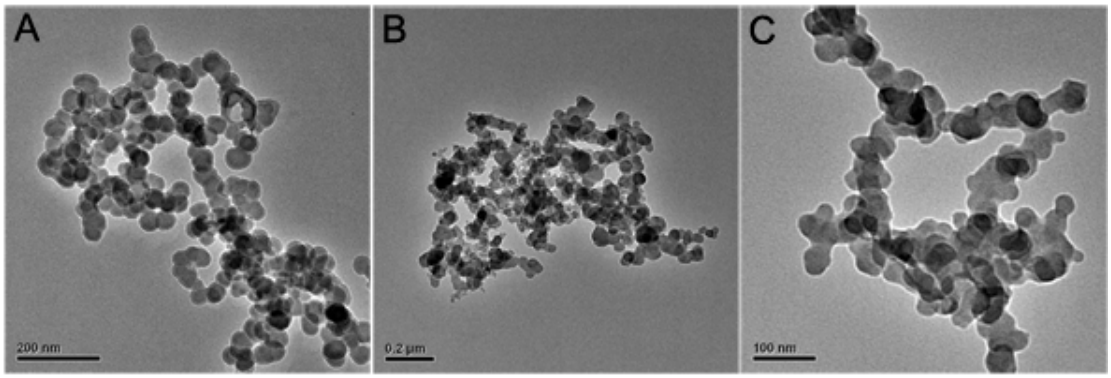

Fig. S3 Transmission electron microscopy (TEM) images of nano-aggregates with highest QYs: BSpP (A), BMSpP (B) and BTBSpP (C).

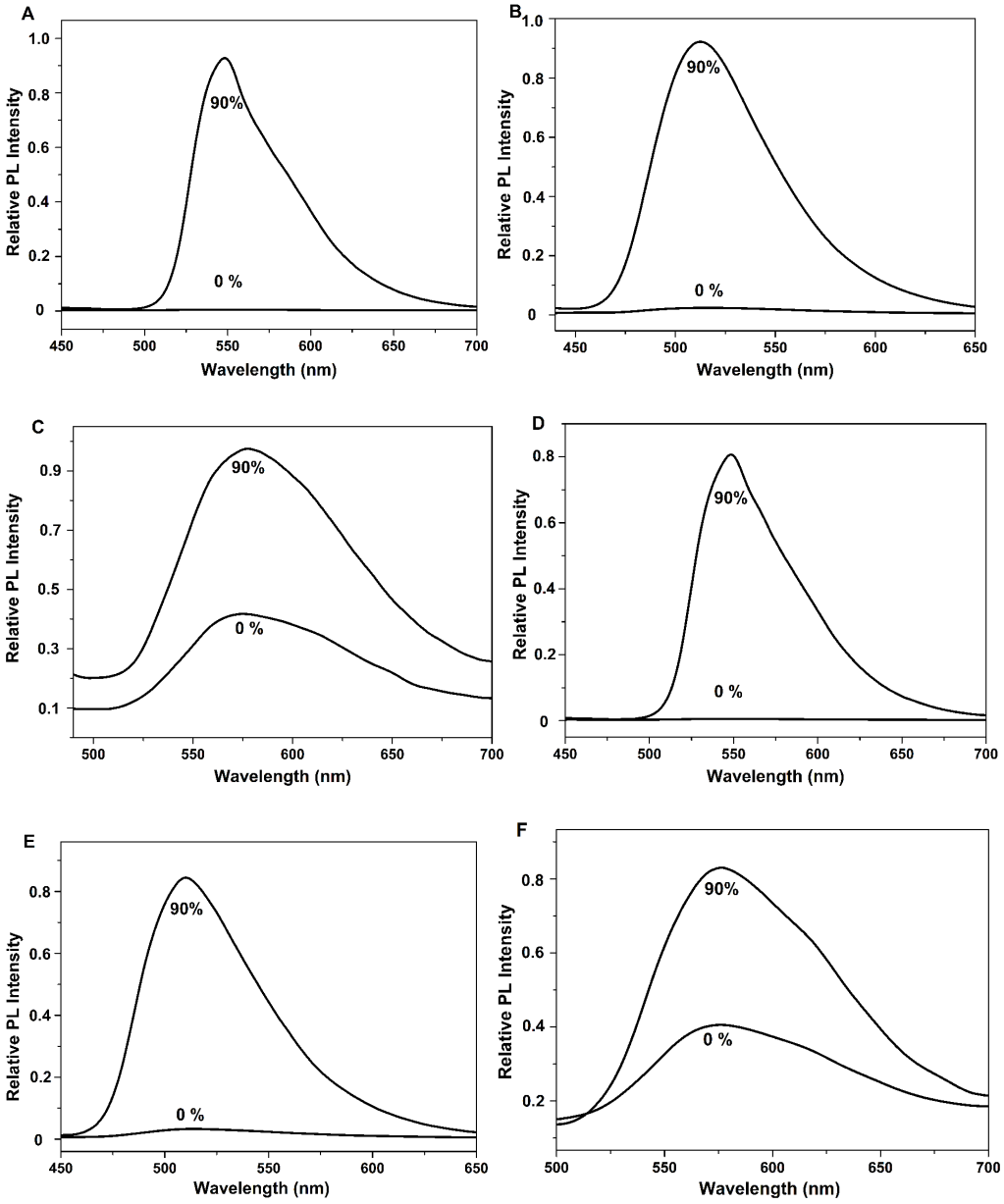

Fig. S4 Relative fluorescence emission spectra of SA derivatives BSpP (A and D), BMSpP (B and E) and BTBSpP (C and F) in THF/H_2O mixtures (A, B and C) and DMSO/H_2O mixtures (D, E and F) with 0 % and 90 % water contents. Concentration = 10 μM, λ_{ex} = 400 nm.

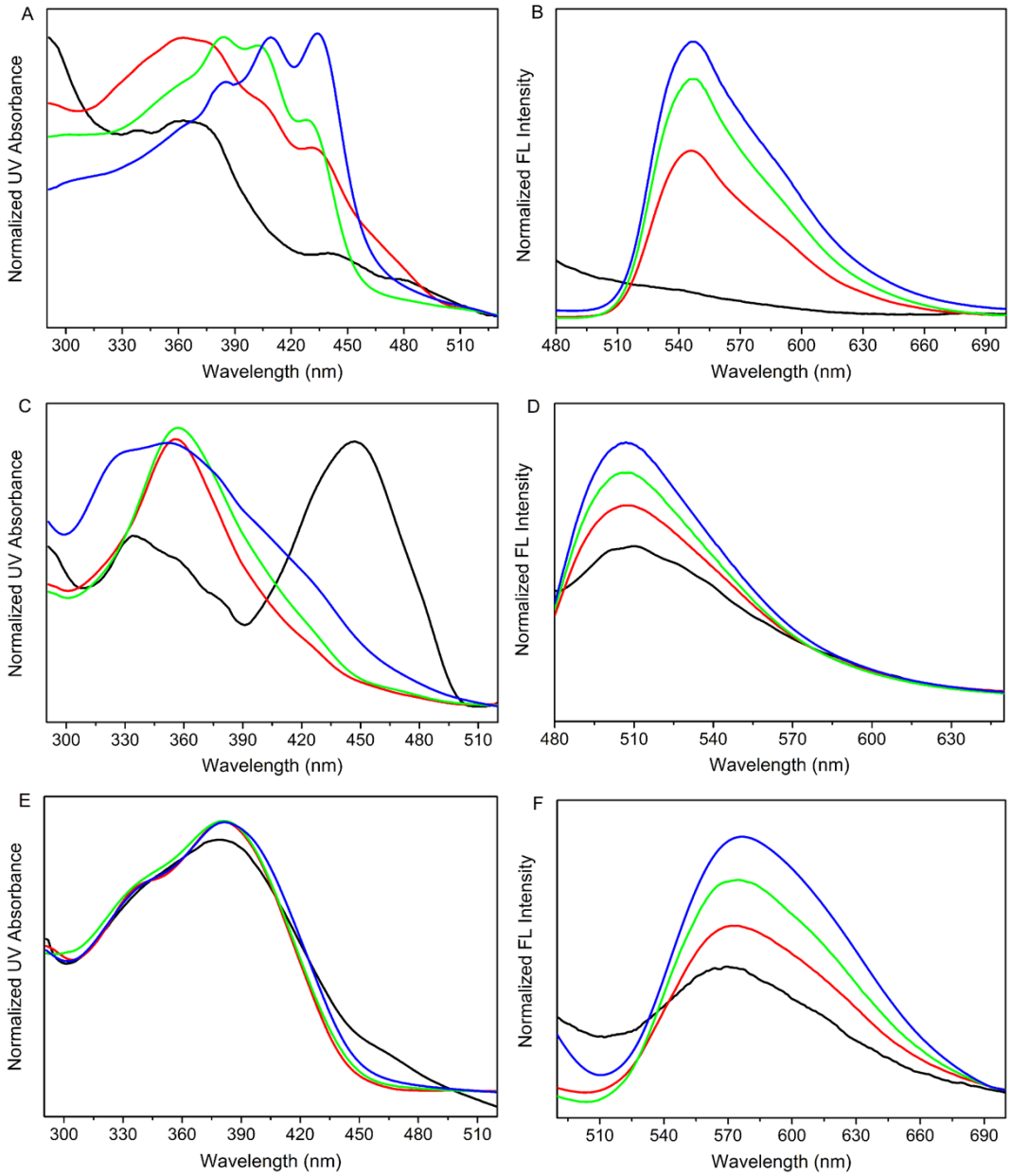

Fig. S5 Normalized concentration-dependent UV-Vis absorption (A, C, E) and steady state fluorescence (B, D, F) of BSpP (A and B), BMSpP (C and D) and BTBSpP (E and F) in DMF/H_2O mixed solvents. Concentrations: 1.0×10^{-7} M (black), 1.0×10^{-6} M (red), 1.0×10^{-5} M (green), 1.0×10^{-4} M (blue). Water content 85%. λ_{ex} = 400 nm.

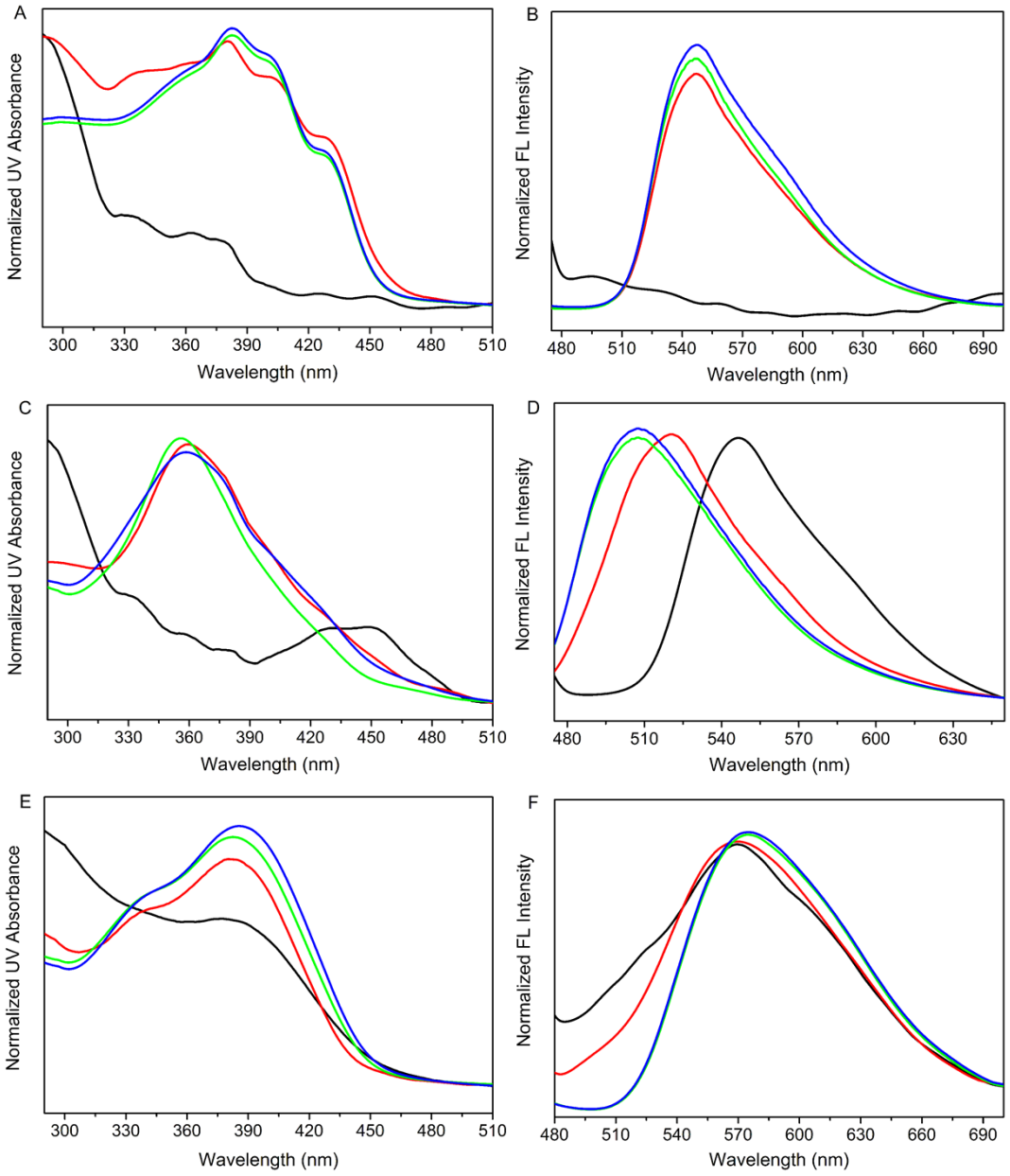

Fig. S6 Normalized concentration-dependent UV-Vis absorption (A, C, E) and steady state fluorescence (B, D, F) of BSpP (A and B), BMSpP (C and D) and BTBSpP (E and F) in DMSO/H_2O mixed solvents. Concentrations: 1.0×10^{-7} M (black), 1.0×10^{-6} M (red), 1.0×10^{-5} M (green), 1.0×10^{-4} M (blue). Water content 85%. λ_{ex} = 400 nm.

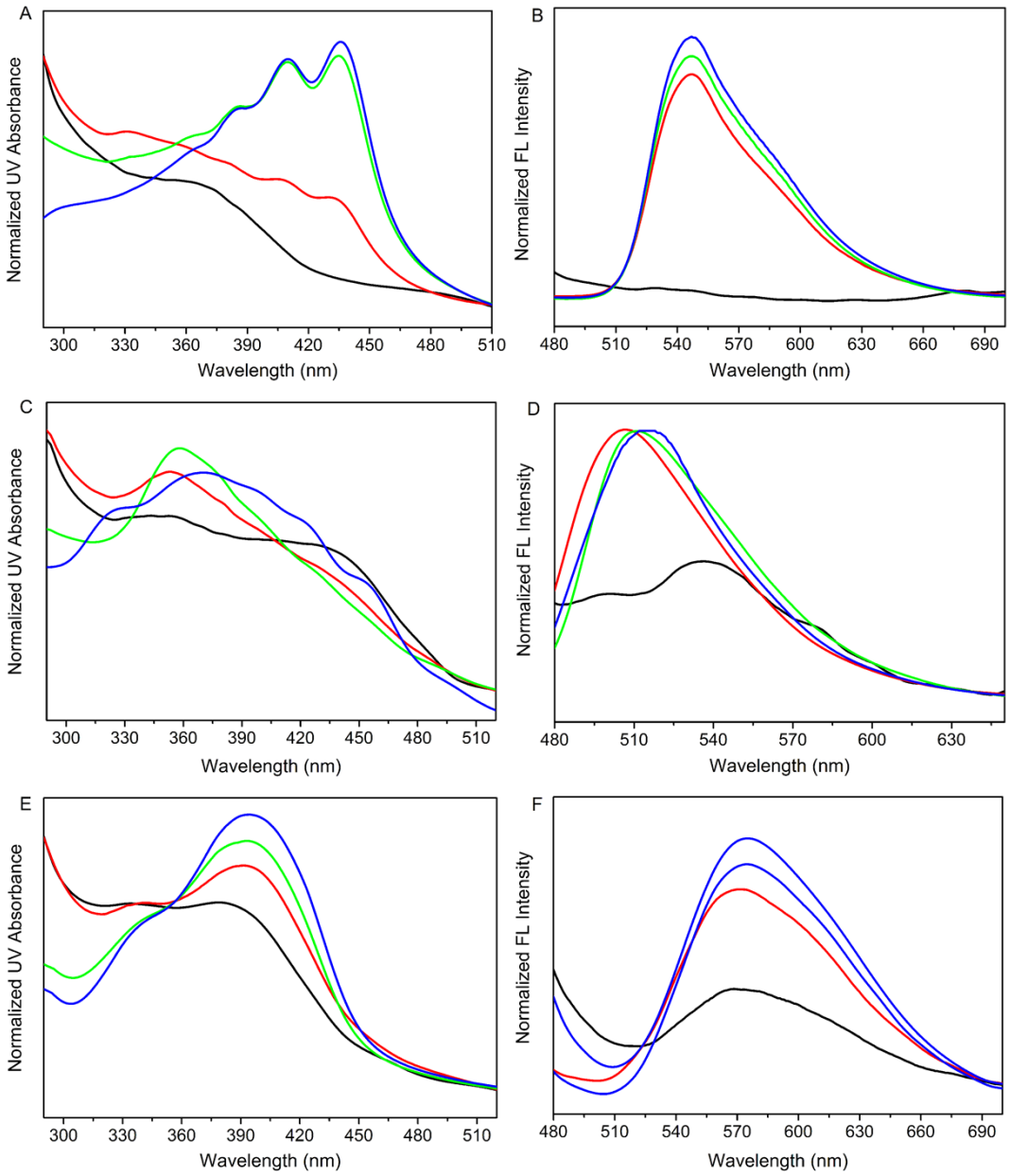

Fig. S7 Normalized concentration-dependent UV-Vis absorption (A, C, E) and steady state fluorescence (B, D, F) of BSpP (A and B), BMSpP (C and D) and BTBSpP (E and F) in THF/H_2O mixed solvents. Concentrations: 1.0×10^{-7} M (black), 1.0×10^{-6} M (red), 1.0×10^{-5} M (green), 1.0×10^{-4} M (blue).Water content 85%. λ_{ex} = 400 nm.

Table S1. UV-Vis absorption maxima, fluorescence emission maxima, fluorescence quantum yields, size of nano-aggregates and two-photon absorption cross-sections

	$\lambda_{max,ab}$(nm)[a]	$\lambda_{max,ab}$(nm)[b]	$\lambda_{max,em}$(nm)[a]	$\lambda_{max,em}$(nm)[b]	Φ_f[c]	Size (nm)[d]	δ (GM)[e]
BSpP, 4	371	413, 444	--	547	0.26	57	38
BMSpP, 5	378	279, 323	511	517	0.10	52	7
BTBSpP, 6	381	392	575	575	0.06	48	27

[a] Determined from absorption spectra in DMF solutions.

[b] Acquired from absorption spectra after assembling into nano-aggregates with 90% water content.

[c] Quantum yields were measured for the corresponding nano-aggregates using Rhodamine 6G as the standard.

[d] Determined from the results of TEM.

[e] Two-photon absorption cross-sections with Rhodamine B as the standard.

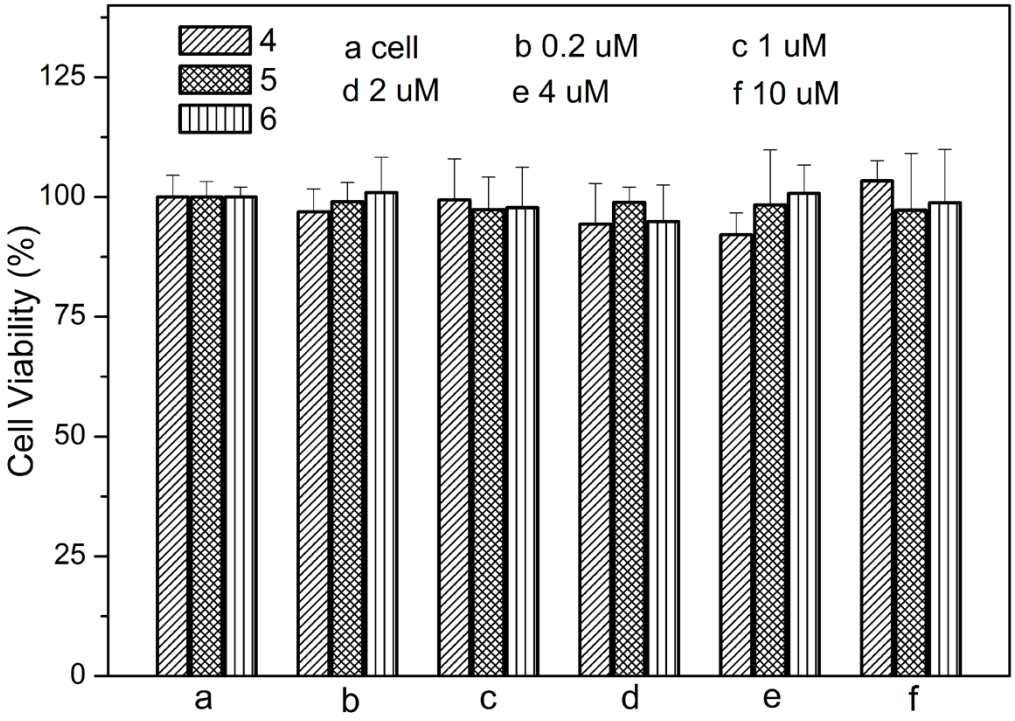

Fig. S8 Cell viability of L929 cells against salicylideneaniline derivatives BSpP (4), BMSpP (5) and BTBSpP (6) after cultured for 48 h with different concentrations: 0 (a), 0.2 μM (b), 1 μM (c), 2 μM (d), 4 μM (e) and 10 μM (f).

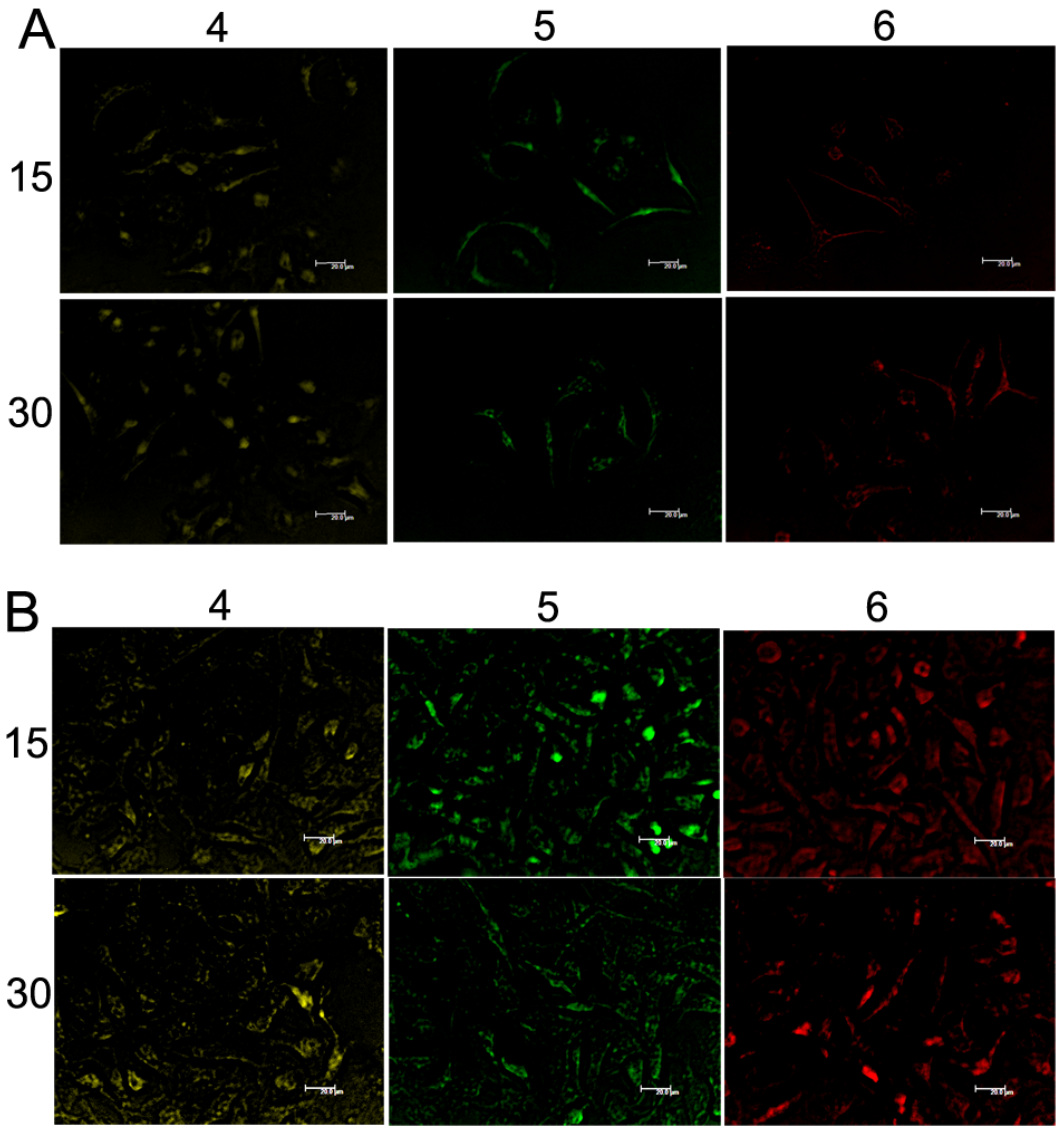

Fig. S9 Cell imaging with different phospholipid-conjugated single-emissive nanoparticles for A549 (A) and MCF-7 (B) cells after 15 min and 30 min cell culture, respectively. Scale bar = 20 μm. Concentration = 2 μM, λ_{ex} = 405 nm. Left tags for cell culture time and middle tags for BSpP (4), BMSpP (5) and BTBSpP (6) FNPs.