

Supporting Information for

**Albumin-Based NIR-II Fluorescence/MRI Bimodal Imaging
Probe for Precise Diagnosis of Lymph Node Tumor
Metastasis**

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Experimental section

Materials and Instrumentation

Materials. All reagents and solvents were obtained commercially and used without further purification unless otherwise noted. 1 % Intralipid were purchased from Sigma-Aldrich (USA). IR820 was sourced from Beyotime Biotech (China). BSA was bought from Picasso-e (China).

Measurements. UV-vis-NIR absorption spectra were conducted on a UV-3210PC spectrophotometer. NIR-II fluorescence emission spectra were measured on Horiba Fluorolog-QM spectrophotometer equipped with a liquid-nitrogen-cooled InGaAs near-infrared array detector under ambient atmosphere. NIR-II Fluorescence imaging were performed on a NIR-II fluorescence imaging system (Suzhou Yingrui Optics Technology Co., Ltd.). MRI imaging were performed on a MRI experiment with a 3.0 T MR scanner (Ingenia elition, Philips) equipped with a custommade mice coil. The mice were scanned after injection at different time points. Parameters: TE = 9.72 ms, TR = 191.10 ms, FA = 50°, FOV = 25 × 25 mm and 192 × 192 matrix.

Supporting figures

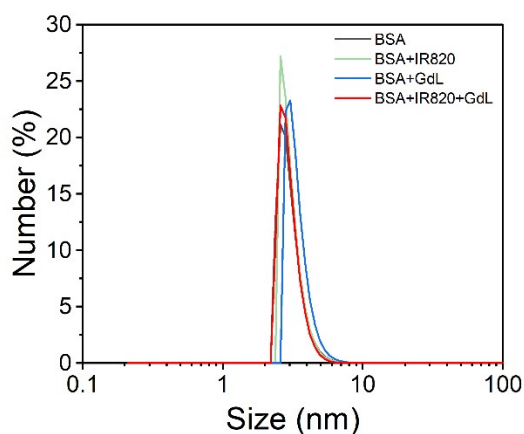


Fig. S1 Hydrodynamic diameters of BSA, BSA-IR820, BSA-GdL, and BSA-IR820-GdL measured by DLS.

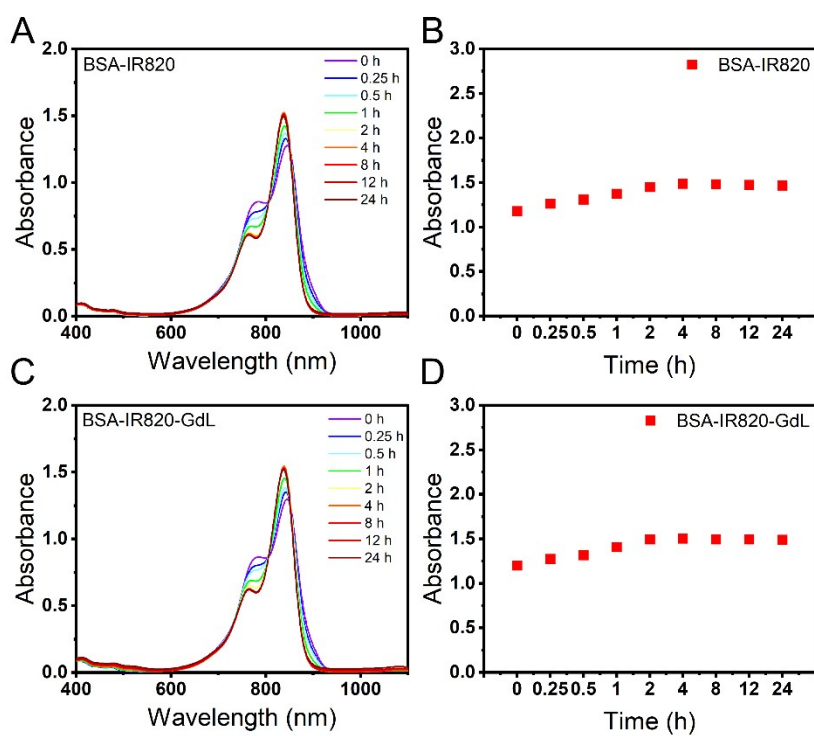


Fig.S2 The stability of IR820 and the IR820 + GdL mixture in BSA solution were assessed by UV-vis-NIR absorption spectra. (A, C) The absorbance change curves of BSA-IR820 and BSA-IR820-GdL at different time points (0-24 h) were measured. (B, D) The trend of absorbance of BSA-IR820 and BSA-IR820-GdL at 833 nm with time.

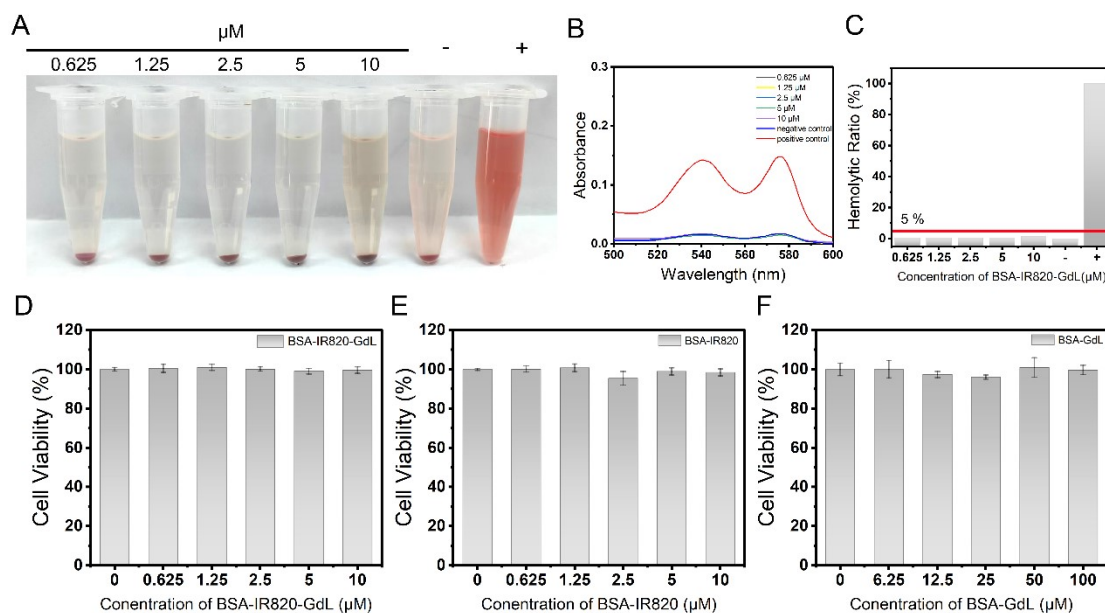


Fig. S3 (A) Optical images of hemolytic phenomenon. (B) UV-vis absorbance spectroscopy of the hemoglobin released from mouse red blood cells (RBCs). (C) The hemolysis rates of different concentrations of BSA-IR820-GdL. (-) and (+) controls are the RBCs in PBS and water, respectively. (D-F) Cell viability of EMT6 cell exposed to the BSA-IR820-GdL and BSA-IR820 at concentrations ranging from 0 to 10 μM for 24 h using MTS assays. (E) Cell viability of EMT6 cell exposed to the BSA-GdL at concentrations ranging from 0 to 100 μM for 24 h using MTS assays.

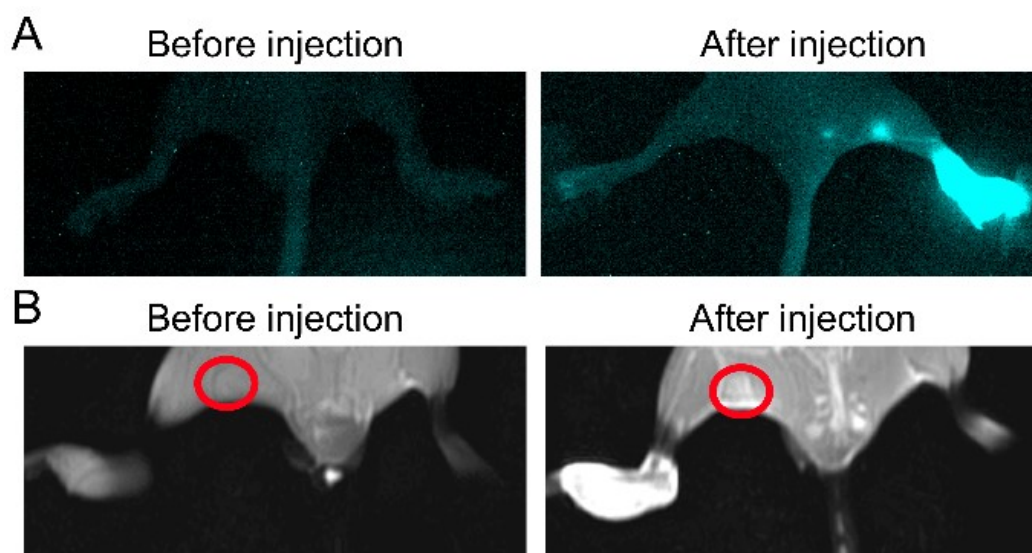


Fig. S4 (A) Comparison of NIR-II fluorescence imaging before and after injection of BSA-IR820-GdL in mice. (B) MRI before and after injection of BSA-IR820-GdL in mice.

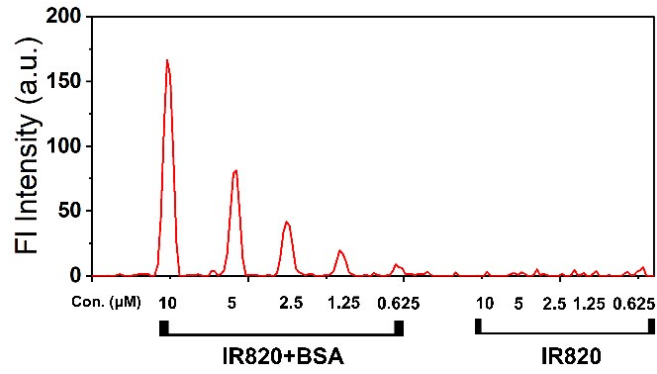


Fig. S5 Capillary streak fluorescence intensity with different concentrations (10, 5, 2.5, 1.25, 0.625 μM) of **BSA-IR820** and **IR820**.

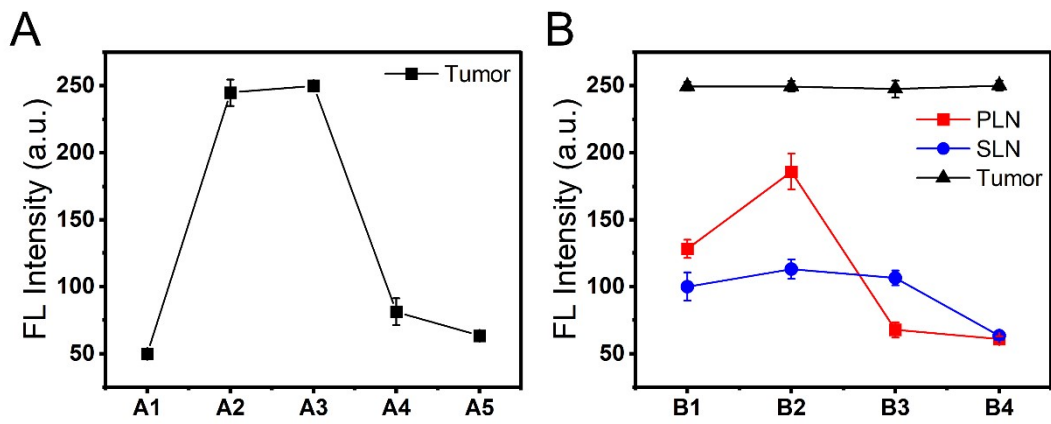


Fig. S6 Fluorescence intensity statistics of mouse back tumors after injection of **BSA-IR820** and fluorescence intensity statistics of popliteal lymph nodes, ischial lymph nodes and foot pad tumors in mouse lymph node metastasis model.

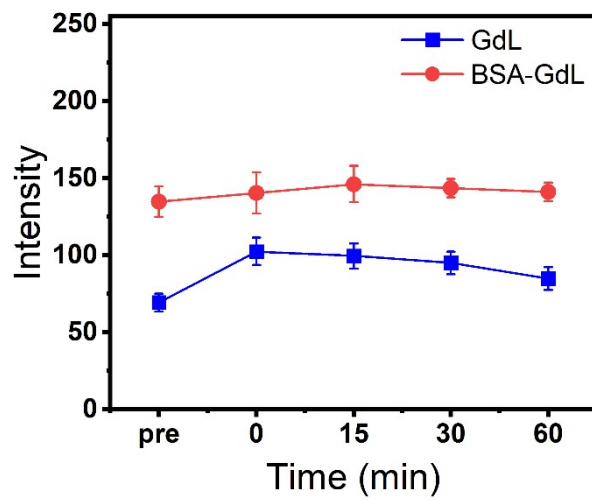


Fig. S7 Statistical analysis of MRI imaging intensity of popliteal lymph nodes in mice after injection of **GdL** and **BSA-GdL**.

Supporting tables

Table S1. Atomic coordinates of free **IR820** from DFT geometry optimization.

Number	Atom	Coordinates		
		X	Y	Z
1	C	1.247476	-0.191948	-0.222110
2	C	0.009282	-0.809116	0.053060
3	C	-1.247970	-0.194722	-0.090973
4	Cl	0.034859	-2.512351	0.571634
5	C	1.213840	1.255398	-0.680427
6	H	1.179143	1.290703	-1.778919
7	H	2.138254	1.757464	-0.380736
8	C	-1.284933	1.283435	-0.439744
9	H	-2.108073	1.759704	0.103647
10	H	-1.511147	1.417670	-1.505539
11	C	0.015587	2.004583	-0.103129
12	H	-0.023115	3.022943	-0.500717
13	H	0.127795	2.076674	0.986012
14	C	-2.445575	-0.914484	0.010165
15	H	-2.374532	-1.964837	0.254079
16	C	2.456360	-0.886170	-0.130564
17	H	2.400559	-1.907742	0.214575
18	C	3.714912	-0.378458	-0.466875
19	H	3.770751	0.637444	-0.836729
20	C	-3.704971	-0.367273	-0.237082
21	H	-3.744671	0.683355	-0.486453
22	C	4.912625	-1.080464	-0.374597
23	C	5.161050	-2.507924	0.135876
24	C	-4.918638	-1.050607	-0.251220
25	C	-5.210631	-2.528625	0.025818
26	C	4.451879	-3.537156	-0.776792
27	H	4.713077	-4.553782	-0.475636
28	H	3.365652	-3.437332	-0.725799
29	H	4.761503	-3.400199	-1.816925
30	C	4.727037	-2.629841	1.616323
31	H	3.647066	-2.514241	1.728442
32	H	5.007202	-3.607421	2.014152
33	H	5.219556	-1.862647	2.220885
34	C	-4.831250	-2.881145	1.483000
35	H	-3.756826	-2.771782	1.647977
36	H	-5.355745	-2.225090	2.183653
37	H	-5.106794	-3.913010	1.710841
38	C	-4.474796	-3.419853	-1.004385
39	H	-3.391478	-3.362238	-0.879204
40	H	-4.776214	-4.462856	-0.886091
41	H	-4.721157	-3.107513	-2.023329
42	C	6.675880	-2.620266	0.009626
43	C	7.560704	-3.682517	0.321446
44	C	7.169249	-1.432940	-0.497949
45	C	7.156724	-4.945519	0.839837

46	C	8.966942	-3.459543	0.095878
47	C	8.540662	-1.193879	-0.718766
48	C	8.081953	-5.926647	1.117624
49	H	6.107480	-5.143799	1.019116
50	C	9.889548	-4.495737	0.395672
51	C	9.416047	-2.212243	-0.417929
52	H	8.901544	-0.247092	-1.103092
53	C	9.462019	-5.704640	0.895554
54	H	7.748646	-6.882085	1.511759
55	H	10.946655	-4.313960	0.221092
56	H	10.481604	-2.069200	-0.571330
57	H	10.178257	-6.488997	1.120150
58	C	-6.717922	-2.587142	-0.194837
59	C	-7.630265	-3.667556	-0.104853
60	C	-7.165405	-1.328201	-0.555040
61	C	-7.273727	-4.996998	0.258718
62	C	-9.012949	-3.392820	-0.405178
63	C	-8.511539	-1.042832	-0.859116
64	C	-8.222501	-5.992764	0.323004
65	H	-6.242778	-5.233774	0.489948
66	C	-9.961040	-4.446127	-0.327315
67	C	-9.412157	-2.079987	-0.778111
68	H	-8.833266	-0.050636	-1.152806
69	C	-9.579699	-5.719876	0.028502
70	H	-7.925901	-6.999161	0.603129
71	H	-11.000265	-4.225257	-0.555939
72	H	-10.459204	-1.902597	-1.005325
73	H	-10.315150	-6.516552	0.084248
74	C	-6.166180	0.983567	-0.943953
75	H	-5.253929	1.243796	-1.478850
76	H	-6.985955	1.079682	-1.662435
77	C	-6.396535	1.901211	0.276760
78	H	-7.334566	1.610213	0.761969
79	H	-5.602578	1.743315	1.014588
80	C	-6.484995	3.377793	-0.121557
81	H	-5.581850	3.691967	-0.656129
82	H	-7.324981	3.514929	-0.813504
83	C	-6.712404	4.284472	1.082950
84	H	-5.826452	4.340719	1.722943
85	H	-7.548613	3.922679	1.690616
86	C	6.300777	0.816936	-1.237976
87	H	7.191471	0.814719	-1.869079
88	H	5.459743	1.072123	-1.886451
89	C	6.445289	1.825471	-0.093706
90	H	5.554223	1.779218	0.542891
91	H	7.300861	1.539826	0.529316
92	C	6.634804	3.246009	-0.631367
93	H	7.518984	3.288633	-1.276695
94	H	5.769837	3.533908	-1.239314
95	C	6.801187	4.239630	0.518346

96	H	7.687770	4.027151	1.121244
97	H	5.923334	4.265719	1.169426
98	S	7.014667	5.914423	-0.107463
99	S	-7.136792	5.983402	0.608387
100	O	5.760931	6.381443	-0.705853
101	O	8.264327	6.000468	-0.859661
102	O	-8.410734	5.873699	-0.169483
103	O	-5.988101	6.473760	-0.211592
104	N	6.104382	-0.542175	-0.740995
105	N	-6.081197	-0.428355	-0.566403
106	O	7.284840	6.762420	1.265634
107	H	6.429340	7.077814	1.618255
108	O	-7.298823	6.712586	1.901660

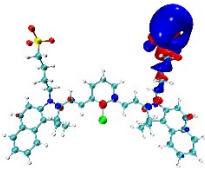
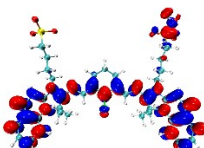
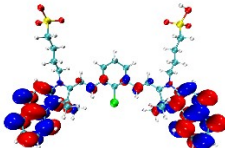
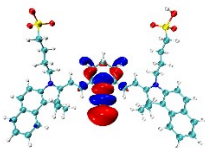
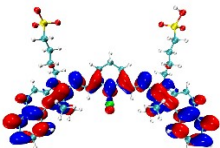
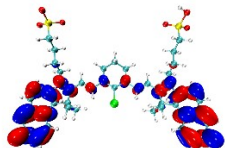
Table S2. Atomic coordinates of the **IR820-BSA** geometry obtained from molecular docking simulations. (BSA omitted).

Number	Atom	Coordinates		
		X	Y	Z
1	N	-5.039704	-1.330944	-0.119607
2	N	5.336088	-0.431992	0.472996
3	C	-9.057544	-0.933344	0.909226
4	C	-8.551828	-0.560264	-0.356583
5	C	-7.150836	-0.730795	-0.608169
6	C	-6.343871	-1.258500	0.387971
7	C	-6.363066	-0.418985	-1.882792
8	C	-4.954621	-0.850506	-1.443391
9	C	-1.464173	-0.195944	-1.917040
10	C	-0.090342	-0.679399	-1.681891
11	C	0.934525	0.166359	-1.363047
12	C	4.214849	-1.140495	0.293635
13	C	6.446759	-1.359129	0.609346
14	C	5.995693	-2.653891	0.517256
15	C	4.494614	-2.647852	0.336984
16	C	6.916559	-3.746159	0.612921
17	C	8.283688	-3.443028	0.797302
18	O	-4.868059	3.622655	3.257775
19	O	-6.639074	1.832189	2.956595
20	O	-5.297680	2.727933	1.009026
21	O	7.066183	6.784709	-1.196592
22	O	8.527713	5.322426	0.128535
23	O	6.239249	6.129978	1.017249
24	S	-5.198747	2.474256	2.434866
25	S	7.177356	5.671189	-0.274825
26	H	6.684951	5.884484	1.807934
27	Cl	0.231143	-2.383839	-1.793247
28	C	0.667559	1.653553	-1.293085
29	H	1.407060	2.366740	-1.592088
30	H	0.521322	1.713747	-0.234835

31	C	-0.427085	2.061781	-2.282367
32	H	-0.002722	1.861275	-3.243936
33	H	-0.714899	3.090861	-2.227083
34	C	-1.715517	1.294102	-1.985460
35	H	-2.079787	1.693085	-1.061869
36	H	-2.459843	1.415205	-2.744547
37	C	3.786020	-3.281625	1.548523
38	H	3.829324	-4.347822	1.469470
39	H	4.272790	-2.970698	2.449234
40	H	2.763611	-2.966704	1.568648
41	C	4.066716	-3.274693	-1.003006
42	H	2.998532	-3.300987	-1.059506
43	H	4.452258	-2.687978	-1.810486
44	H	4.452051	-4.270645	-1.070058
45	C	-6.367315	1.088801	-2.196099
46	H	-6.630927	1.239136	-3.222163
47	H	-7.080508	1.581436	-1.568750
48	H	-5.393462	1.493449	-2.015071
49	C	-6.823771	-1.294675	-3.062842
50	H	-6.900340	-2.312833	-2.742855
51	H	-7.778769	-0.956099	-3.406709
52	H	-6.112305	-1.223527	-3.858865
53	C	2.300549	-0.337526	-1.125992
54	H	2.940072	-0.480002	-1.971931
55	C	2.848620	-0.651974	0.073100
56	H	2.217078	-0.514479	0.925830
57	C	-3.900902	-0.743643	-2.303490
58	H	-4.175372	-0.404034	-3.280338
59	C	-2.495714	-1.074817	-2.082495
60	H	-2.193831	-2.101249	-2.096817
61	C	-6.853896	-1.633233	1.662480
62	H	-6.248852	-1.992465	2.468567
63	C	-8.190520	-1.465941	1.913730
64	H	-8.609811	-1.730761	2.861867
65	C	-10.447633	-0.787812	1.201924
66	H	-10.871655	-1.001509	2.160798
67	C	-11.311941	-0.287577	0.258823
68	H	-12.358100	-0.173668	0.452405
69	C	-10.816887	0.091152	-1.014757
70	H	-11.415486	0.488960	-1.807429
71	C	-9.480851	-0.039451	-1.309556
72	H	-9.104228	0.249129	-2.268606
73	C	7.820775	-1.053316	0.810291
74	H	8.145331	-0.043170	0.948737
75	C	8.720658	-2.083906	0.898774
76	H	9.763437	-1.892338	1.043040
77	C	9.243498	-4.494068	0.887908
78	H	10.269720	-4.255428	1.074510
79	C	8.850818	-5.808014	0.799947
80	H	9.553097	-6.613063	0.859949

81	C	7.479411	-6.123998	0.622384
82	H	7.088250	-7.117100	0.547268
83	C	6.540190	-5.120949	0.534009
84	H	5.502859	-5.348755	0.403818
85	C	5.517919	1.065718	0.621993
86	H	6.064995	1.030692	1.540896
87	H	4.688691	1.733922	0.725837
88	C	6.095247	1.675897	-0.662603
89	H	5.162005	1.727240	-1.183490
90	H	6.813175	1.161774	-1.266886
91	C	6.966257	2.917290	-0.412509
92	H	7.948776	2.756699	-0.804640
93	H	7.020245	3.028891	0.650285
94	C	6.460698	4.223748	-1.053111
95	H	5.409569	4.411502	-0.983995
96	H	6.707740	3.943243	-2.055701
97	C	-3.948009	-1.911298	0.678849
98	H	-3.607693	-2.504338	1.501885
99	H	-4.049341	-2.529644	-0.188492
100	C	-3.170664	-0.898264	1.535581
101	H	-2.284411	-0.329659	1.725700
102	H	-3.158407	-1.787489	2.130584
103	C	-3.623267	0.553661	1.317577
104	H	-4.461875	0.435189	0.663665
105	H	-2.907655	1.207816	0.864940
106	C	-3.902798	1.251798	2.656659
107	H	-2.937306	1.629891	2.920801
108	H	-4.219301	0.538923	3.389145

Table S3. TD-DFT-computed frontier molecular orbitals of free IR820.

		
LUMO+10	LUMO+9	LUMO+8
		
LUMO+7	LUMO+6	LUMO+5

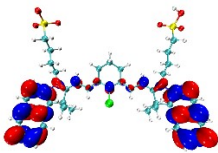
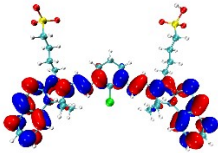
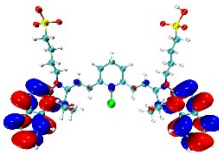
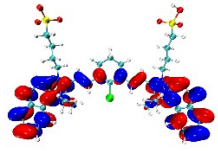
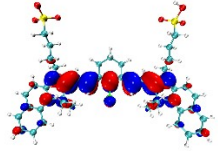
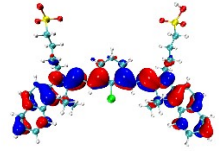
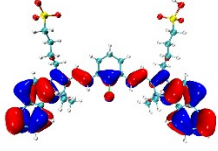
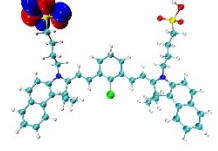
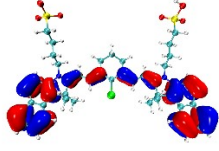
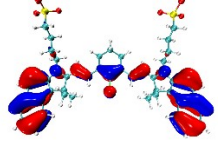
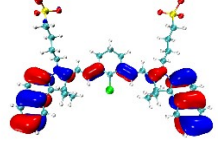
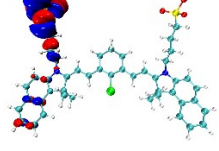
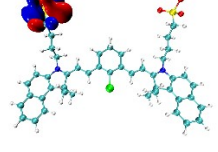
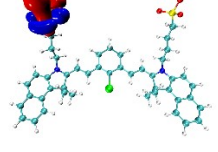
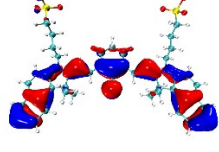
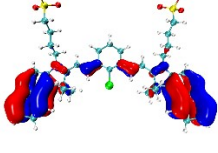
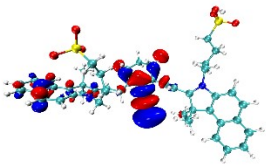
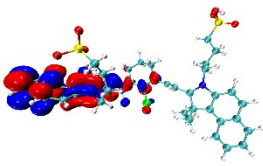
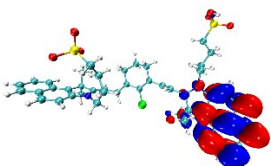
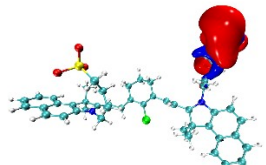
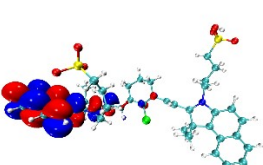
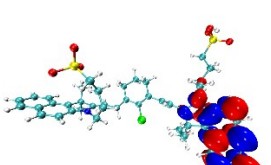
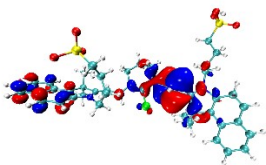
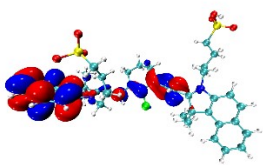
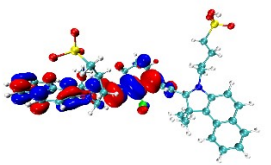
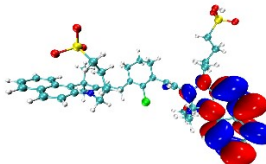
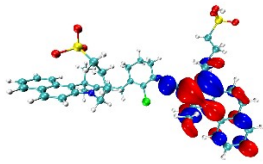
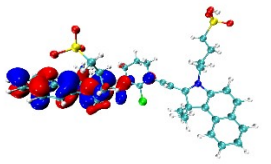
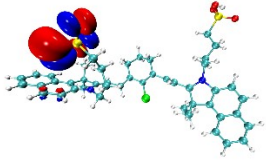
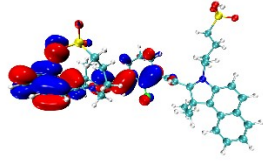
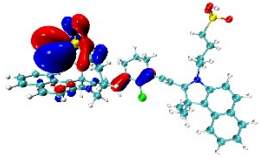
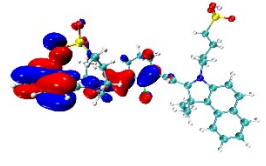
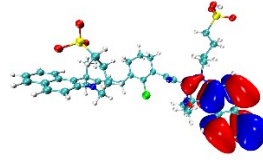
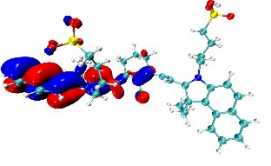
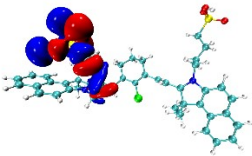
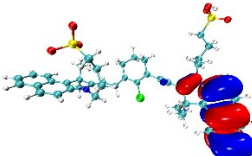
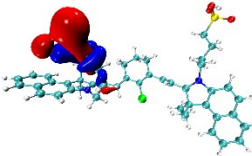
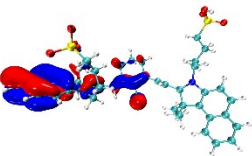
		
LUMO+4	LUMO+3	LUMO+2
		
LUMO+1	LUMO	HOMO
		
HOMO-1	HOMO-2	HOMO-3
		
HOMO-4	HOMO-5	HOMO-6
		
HOMO-7	HOMO-8	HOMO-9
		
HOMO-10		

Table S4. TD-DFT-computed frontier molecular orbitals of **IR820** bound to BSA.

		
LUMO+10	LUMO+9	LUMO+8
		
LUMO+7	LUMO+6	LUMO+5
		
LUMO+4	LUMO+3	LUMO+2
		
LUMO+1	LUMO	HOMO
		
HOMO-1	HOMO-2	HOMO-3
		

HOMO-4	HOMO-5	HOMO-6
		
HOMO-7	HOMO-8	HOMO-9
		
HOMO-10		