Supporting Information

DNA Hairpin-Templated Silver Nanoclusters: A Study on Stem Sequence

Yahui Guo,[†‡] Fumiao Shen,[†] Yuliang Cheng,[†] Hang Yu,[†] Yunfei Xie,[†] Weirong Yao,[†] Renjun Pei,[§] He Qian[†] and Hung-Wing Li*[,‡]

[†] State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China.

[‡] Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China.

§ CAS Key Laboratory of Nano-Bio Interface, Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences, Ruoshui Road, Suzhou 215123, China.

Corresponding author: H.W. Li (*hwli@hkbu.edu.hk*)

Table S1. Names and sequences of the oligonucleotides used in this work.

No.	Name	Sequence (5'-3')
1	H0	AAGCATGCAACGTTCGATCGCCCCCCCGATCGAACGTTGCATGCTT
2	6T-loop	AAGCATGCAACGTTCGATCGTTTTTTCGATCGAACGTTGCATGCTT
3	0%GC	AATTATTTAAATTTTAATAACCCCCCTTATTAAAATTTAAATAATT
4	10%GC	AATTATTTAAATTTTAATGCCCCCCCGCATTAAAATTTAAATAATT
5	20%GC	AATTATTTAAATTTTAGCGCCCCCCCGCGCTAAAATTTAAATAATT
6	30%GC	AATTATTTAAATTTCCGCGCCCCCCCGCGCGGAAATTTAAATAATT
7	40%GC	AATTATTTAAATGGCCGCGCCCCCCCGCGCGGCCATTTAAATAATT
8	50%GC	AATTATTTAACGGGCCGCGCCCCCCCGCGCGGCCCGTTAAATAATT
9	60%GC	AATTATTTCGCGGGCCGCGCCCCCCCGCGCGGCCCGCGAAATAATT
10	70%GC	AATTATCCCGCGGGCCGCGCCCCCCCGCGCGGCCCGCGGGATAATT
11	80%GC	AATTCCCCCGCGGGCCGCGCCCCCCCGCGCGGCCCGCGGGGGAATT
12	90%GC	AAGGCCCCCGCGGGCCGCGCCCCCCCGCGCGGCCCGCGGGGGCCTT
13	100%GC	GCGGCCCCCGCGGGCCGCGCCCCCCCGCGCGGCCCGCGGGGGCCGC
14	20AT	AAAAAAAAAAAAAAAAAAAACCCCCCTTTTTTTTTTTTTTTTTTT
15	20AT-ds1	AAAAAAAAAAAAAAAAAAAACCCCCCAAAAAAAAAAAAAAAAAAAA
16	20AT-ds2	TTTTTTTTTTTTTTTTTTTTCCCCCCTTTTTTTTTTTTTTTTTTT
17	H0-ds1	AAGCATGCAACGTTCGATCGCCCCCCGCTAGCTTGCAACGTACGAA
18	H0-ds2	TTCGTACGTTGCAAGCTAGCCCCCCCGATCGAACGTTGCATGCTT
19	10%GC	AATTATTTAAATTTTAATGCCCCCCGCATTAAAATTTAAATAATT
20	10%GC-1	AATTATTTAAATTTTAAGCACCCCCCTGCTTAAAATTTAAATAATT
21	10%GC-2	AATTATTTAAATTTTAGCAACCCCCCTTGCTAAAATTTAAATAATT
22	10%GC-3	AATTATTTAAATTTTGCTAACCCCCCTTAGCAAAATTTAAATAATT
23	10%GC-4	AATTATTTAAATTTGCATAACCCCCCTTATGCAAATTTAAATAATT
24	10%GC-9	AATTATTTAGCTTTTAATAACCCCCCTTATTAAAAGCTAAATAATT
25	10%GC-18	GCTTATTTAAATTTTAATAACCCCCCTTATTAAAATTTAAATAAGC
26	50%GC	AATTATTTAACGGGCCGCGCCCCCCCGCGCGGCCCGTTAAATAATT
27	50%GC-1	AATTATTTACGGGCCGCGCACCCCCCTGCGCGGCCCGTAAATAATT
28	50%GC-5	AATTACGGGCCGCGCAATAACCCCCCTTATTGCGCGGCCCGTAATT
29	50%GC-10	CGGGCCGCGCATTTTAATAACCCCCCTTATTAAAATGCGCGGCCCG
30	H0	AAGCATGCAACGTTCGATCGCCCCCCCGATCGAACGTTGCATGCTT
31	H0-1	AGCATGCAACGTTCGATCGACCCCCCTCGATCGAACGTTGCATGCT
32	100%GC	GCGGCCCCCGCGGGCCGCGCCCCCCCGCGCGGCCCGCGGGGGCCGC
33	100%GC-1	GCGGCCCCCGCGGGCCGCGACCCCCCTCGCGGCCCGCGGGGGCCGC
34	2AT-2GC×2	AATTATTTAAATTTCGATGCCCCCCGCATCGAAATTTAAATAATT
35	3AT-2GC×2	AATTATTTAAATTCGAATGCCCCCCGCATTCGAATTTAAATAATT
36	4AT-2GC×2	AATTATTTAAATCGTAATGCCCCCCGCATTACGATTTAAATAATT

37	2AT-2GC×3	AATTATTTAACGTTCGATGCCCCCCCGCATCGAACGTTAAATAATT
38	3AT-2GC×3	AATTATTTCGATTCGAATGCCCCCCCGCATTCGAATCGAAATAATT
39	4AT-2GC×3	AATTATCGAAATCGTAATGCCCCCCCGCATTACGATTTCGATAATT
40	2AT-2GC×4	AATTATGCAACGTTCGATGCCCCCCCGCATCGAACGTTGCATAATT
41	3AT-2GC×4	AATGCTTTCGATTCGAATGCCCCCCCGCATTCGAATCGAAAGCATT
42	4AT-2GC×4	GCTTATCGAAATCGTAATGCCCCCCCGCATTACGATTTCGATAAGC
43	2AT-3GC×2	AATTATTTAAATGGCAACGCCCCCCCGCGTTGCCATTTAAATAATT
44	3AT-3GC×2	AATTATTTAAAGGCTAACGCCCCCCCGCGTTAGCCTTTAAATAATT
45	4AT-3GC×2	AATTATTTAAGGCTTAACGCCCCCCCGCGTTAAGCCTTAAATAATT
46	4GC×1	AATTATTTAAATTTTAGCGCCCCCCCGCGCTAAAATTTAAATAATT
47	2GC×2	AATTATTTAAATTCGAATGCCCCCCCGCATTCGAATTTAAATAATT
48	2GC×3	AATTATTTAACGTTCGATGCCCCCCCGCATCGAACGTTAAATAATT
49	3GC×2	AATTATTTAAATGGCAACGCCCCCCCGCGTTGCCATTTAAATAATT
50	2GC×4	AATTATGCAACGTTCGATGCCCCCCCGCATCGAACGTTGCATAATT
51	4GC×2	AATTATTTAAGCCCTAGCGCCCCCCCGCGCTAGGGCTTAAATAATT
52	3GC×4	AACGCTTCGCATGGCAACGCCCCCCCGCGTTGCCATGCGAAGCGTT
53	4GC×3	AATTGCGCAAGCCCTAGCGCCCCCCCGCGCTAGGGCTTGCGCAATT
54	0%GC-stem5	AATAACCCCCCTTATT
55	0%GC-stem10	ATTTTAATAACCCCCCTTATTAAAAT
56	0%GC-stem15	TTTAAATTTTAATAACCCCCCTTATTAAAATTTAAA
57	0%GC-stem20	AATTATTTAAATTTTAATAACCCCCCTTATTAAAATTTAAATAATT
58	0%GC-stem25	TTATTAATTATTTAAATTTTAATAACCCCCCTTATTAAAATTTAAATAATTAATAA
59	0%GC-stem30	AATAATTATTAATTATTTAAATTTTAATAACCCCCCTTATTAAAATTTAAATAATTAATAATTATT
60	2GC-stem5	AATGCCCCCCCGCATT
61	2GC-stem10	ATTTTAATGCCCCCCCGCATTAAAAT
62	2GC-stem15	TTTAAATTTTAATGCCCCCCCGCATTAAAATTTAAA
63	2GC(10%GC)-stem20	AATTATTTAAATTTTAATGCCCCCCCGCATTAAAATTTAAATAATT
64	2GC-stem25	TTATTAATTATTTAAATTTTAATGCCCCCCCGCATTAAAATTTAAATAATTAATAA
65	2GC-stem30	AATAATTATTAATTATTTAAATTTTAATGCCCCCCCGCATTAAAATTTAAATAATTAATAATTATT
66	50%GC-stem8	TTCGATCGCCCCCCCGATCGAA
67	50%GC-stem12	AACGTTCGATCGCCCCCCCGATCGAACGTT
68	50%GC-stem16	ATGCAACGTTCGATCGCCCCCCCGATCGAACGTTGCAT
69	50%GC-stem20	AAGCATGCAACGTTCGATCGCCCCCCCGATCGAACGTTGCATGCTT
70	50%GC-stem24	AAGCAAGCATGCAACGTTCGATCGCCCCCCCGATCGAACGTTGCATGCTTGCTT
71	50%GC-stem28	ATCGAAGCAAGCATGCAACGTTCGATCGCCCCCCCGATCGAACGTTGCATGCTTGCTTCGAT
72	6GC-stem16	ATTTAACGTTCGATGCCCCCCCGCATCGAACGTTAAAT
73	8GC-stem20	AATTATGCAACGTTCGATGCCCCCCCGCATCGAACGTTGCATAATT
74	10GC-stem24	TAATAACGATGCAACGTTCGATGCCCCCCCGCATCGAACGTTGCATCGTTATTA
75	12GC-stem28	TAATAACGAACGATGCAACGTTCGATGCCCCCCCGCATCGAACGTTGCATCGTTCGTTATTA

76	14GC-stem32	TAATATGCAACGAACGATGCAACGTTCGATGCCCCCCCGCATCGAACGTTGCATCGTTCGTTGCATATTA
77	16GC-stem36	TAATTTCGATGCAACGAACGATGCAACGTTCGATGCCCCCCCGCATCGAACGTTGCATCGTTCGTTGCATCGAAATTA

The secondary structures of DNAs listed in Table S1 were analyzed by an online software OligoAnalyzer (*https://sg.idtdna.com/calc/analyzer*), the specific parameters were set as follows: target type, DNA; sequence type, linear; oligonucleotide concentration, 5 μM; [Na^+] concentration, 10 mM; [Mg^{2+}] concentration, 0 mM; dNTPs concentration, 0 mM; temperature, 25℃; maximum foldings, 20.

Table S2. Reported hairpin-AgNCs synthesized in different pH buffers.

No.	Buffer	pH	λex (nm)	λem (nm)	References
1	NH_4Ac-HAc buffer	6.8	420	525 (green)	*Microchim. Acta 2019, 186, 519.*
2	Tris-acetate buffer	7.0	480	580 (yellow)	*Nucleic Acids Res. 2016, 44, e57.*
3	Phosphate buffer	7.0	490	590 (yellow)	*Biosens. Bioelectron. 2014, 60, 351.*
4	Ammonium acetate	7.0	470 590	560 (yellow) 660 (red)	*J. Phys. Chem. C. 2009, 113, 4229.*
5	Tris-acetate buffer	7.0	480 580	590 (yellow) 660 (red)	*Nanomaterials 2019, 9, 667.*
6	Phosphate buffer	7.0	514 561	575 (yellow) 635 (red)	*Langmuir 2013, 29, 13066.*
7	Phosphate buffer	7.4	490	576 (yellow)	*Biosens. Bioelectron. 2016, 79, 411.*
8	HEPES buffer	7.6	520	620 (red)	*Nanoscale 2013, 5, 2840.*
9	Tris buffer	8.0	560	650 (red)	*Analyst 2017, 142, 1765.*

Table S3. The wavelengths of fluorescence excitation and emission peaks, and corresponding intensities (FI) of the hairpin-AgNCs synthesized using stems of different GC content under pH 6.6/7.4, respectively.

	pH 6.6				pH 7.4			
	Peak 1 (yellow)		Peak 2 (red)		Peak 1 (yellow)		Peak 2 (red)	
	Ex/Em (nm)	FI	Ex/Em (nm)	FI	Ex/Em (nm)	FI	Ex/Em (nm)	FI
0%GC	485/595	88031.3			485/595	38653.5		
10%GC	530/595	143030			530/595	19849.4		
20%GC	530/595	72792.4			535/595	7254.5		
30%GC	530/595	56973.8	625/710	6546.7	565/660	6416		
40%GC	530/595	95487.2	600/685	10634.1			600/680	12500.5
50%GC	530/595	87248.9	600/680	11629.1	540/630	7535.9	595/680	7159.3
60%GC	530/595	94385.4	595/680	10537.7	525/600	7719.9	595/680	7216.3
70%GC	530/595	77718.2	595/680	10227.1	525/600	5589	595/680	5746.9
80%GC	530/595	51667.3	600/680	12306.7	525/600	4725.7	595/680	5001.7
90%GC	530/595	27598.2	590/675	12387.2			595/680	5383.2
100%GC	530/595	43448.3	605/685	15108.5			600.680	5967.3

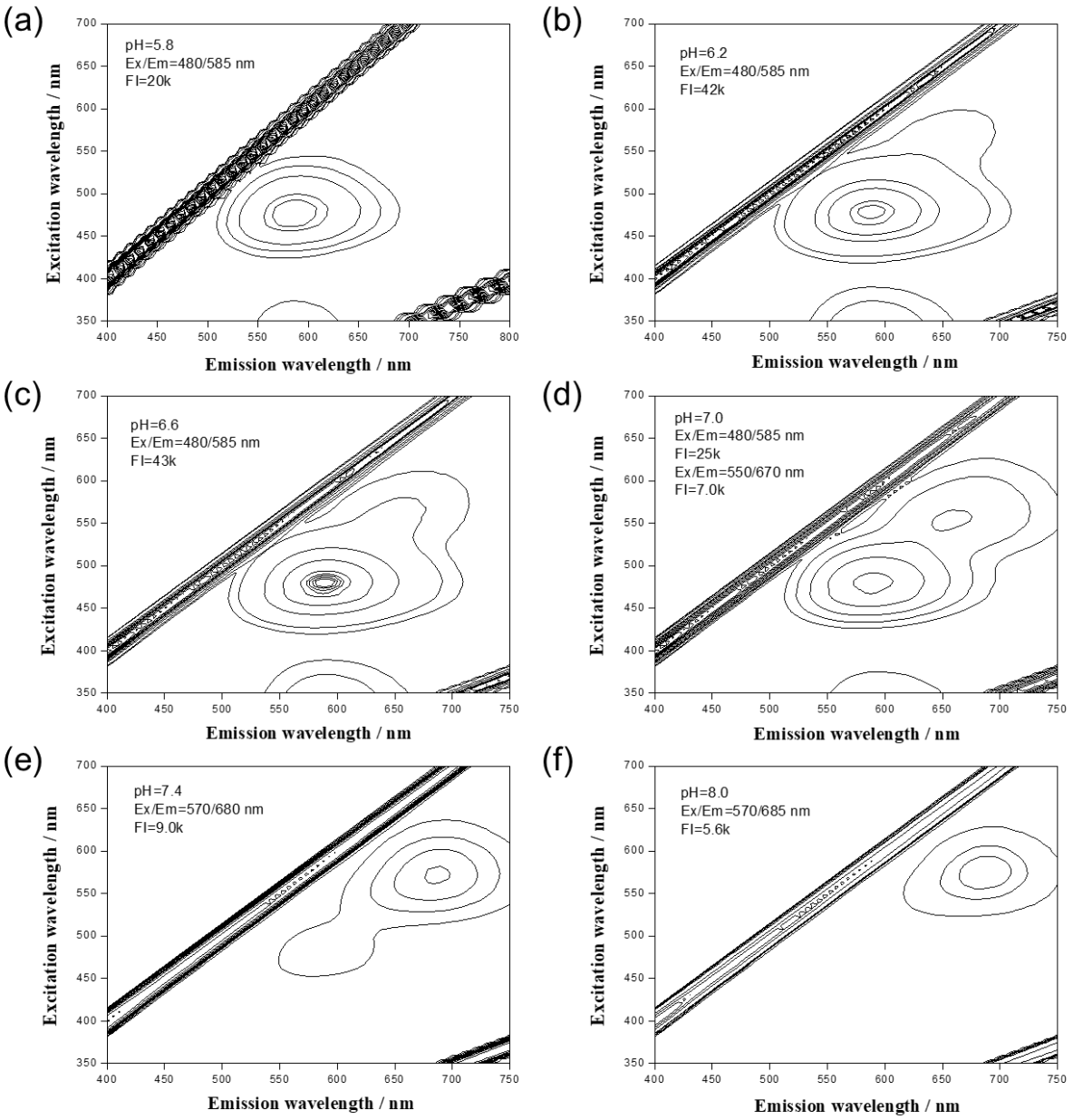

Fig. S1. Three-dimensional fluorescence spectra of H0-AgNCs synthesized at different pH in sodium phosphate buffer (PBS).

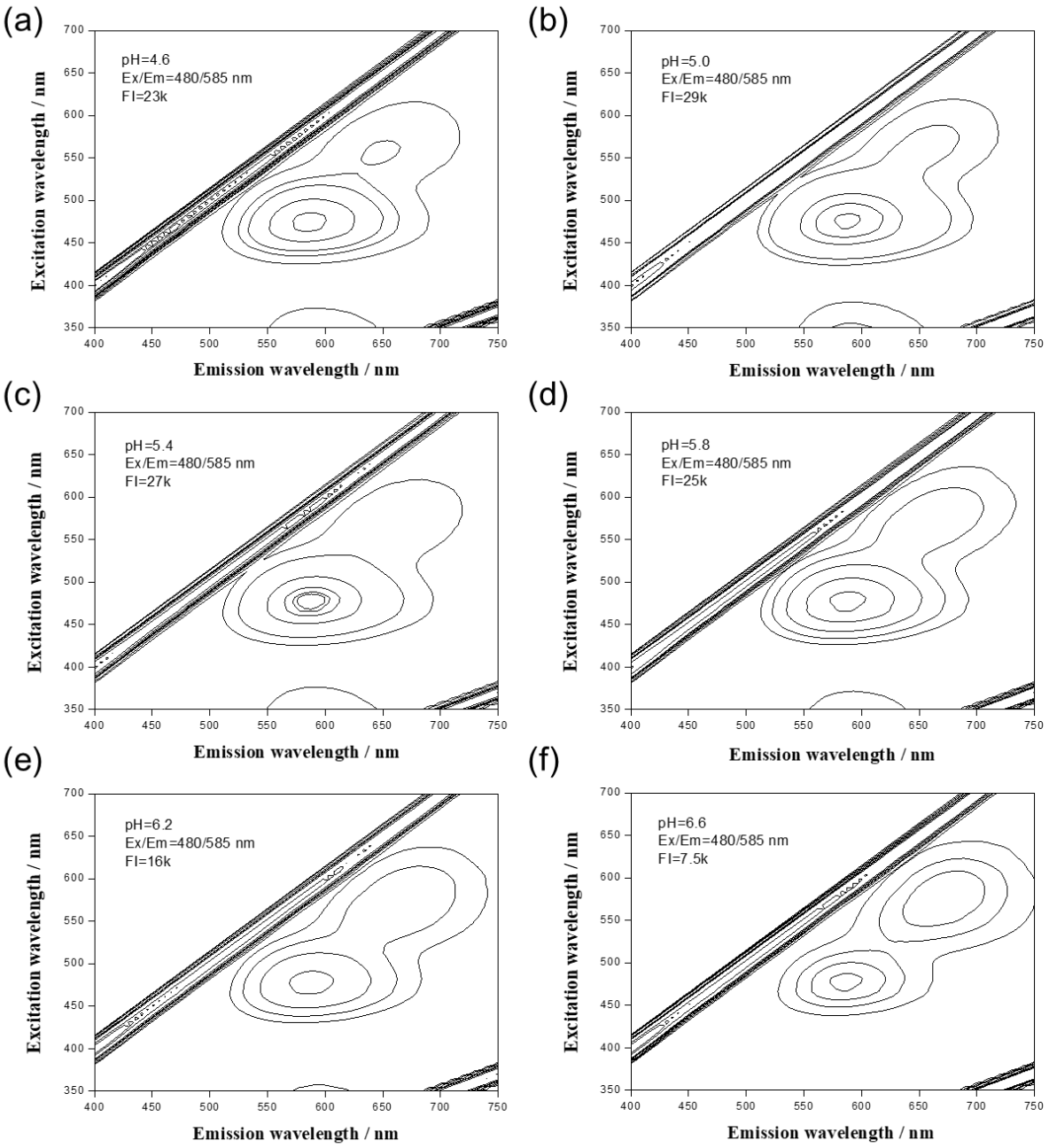

Fig. S2. Three-dimensional fluorescence spectra of H0-AgNCs synthesized at different pH in sodium citrate buffer (SCB).

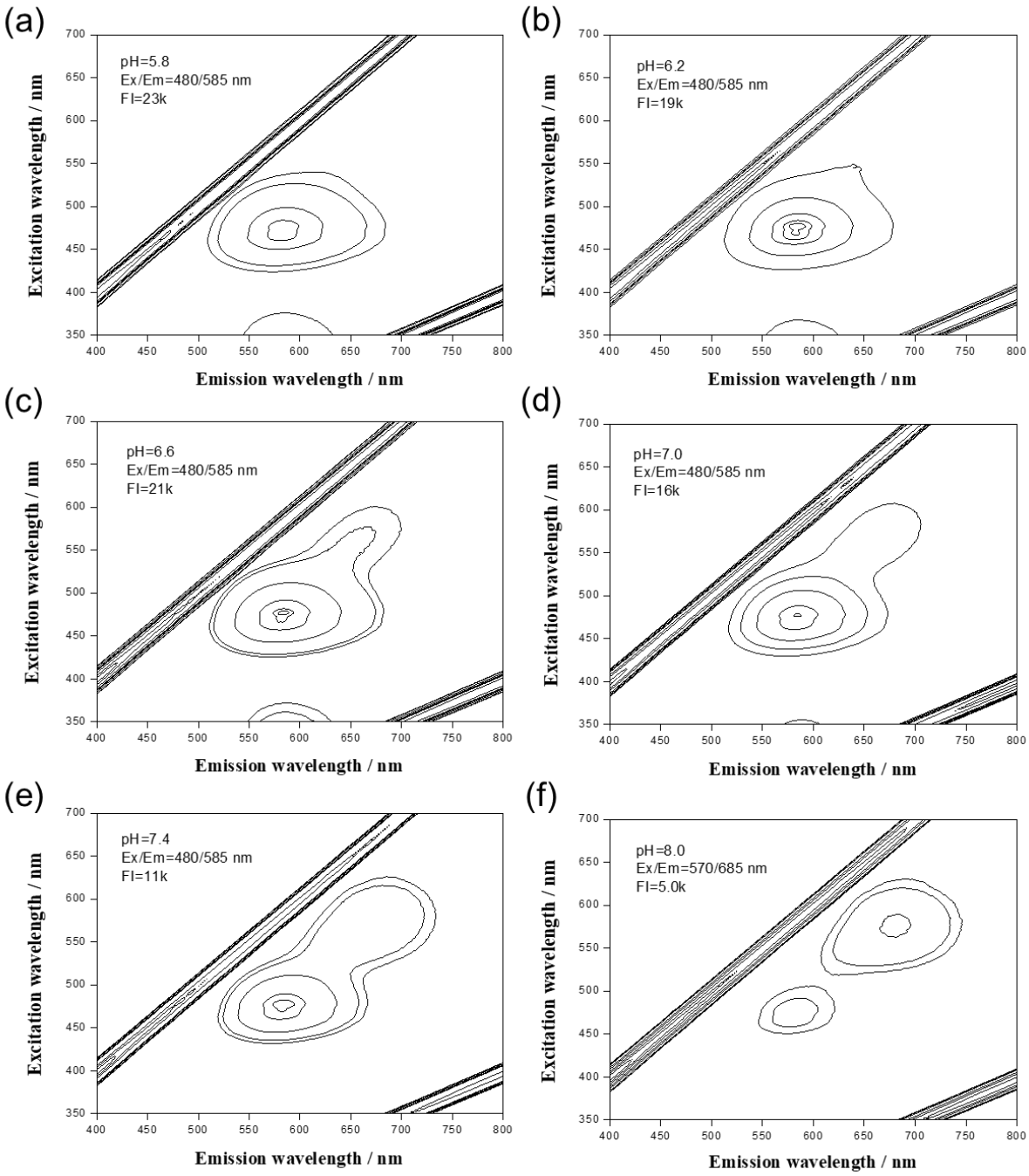

Fig. S3. Three-dimensional fluorescence spectra of H0-AgNCs synthesized at different pH in ammonium acetate buffer (AAB).

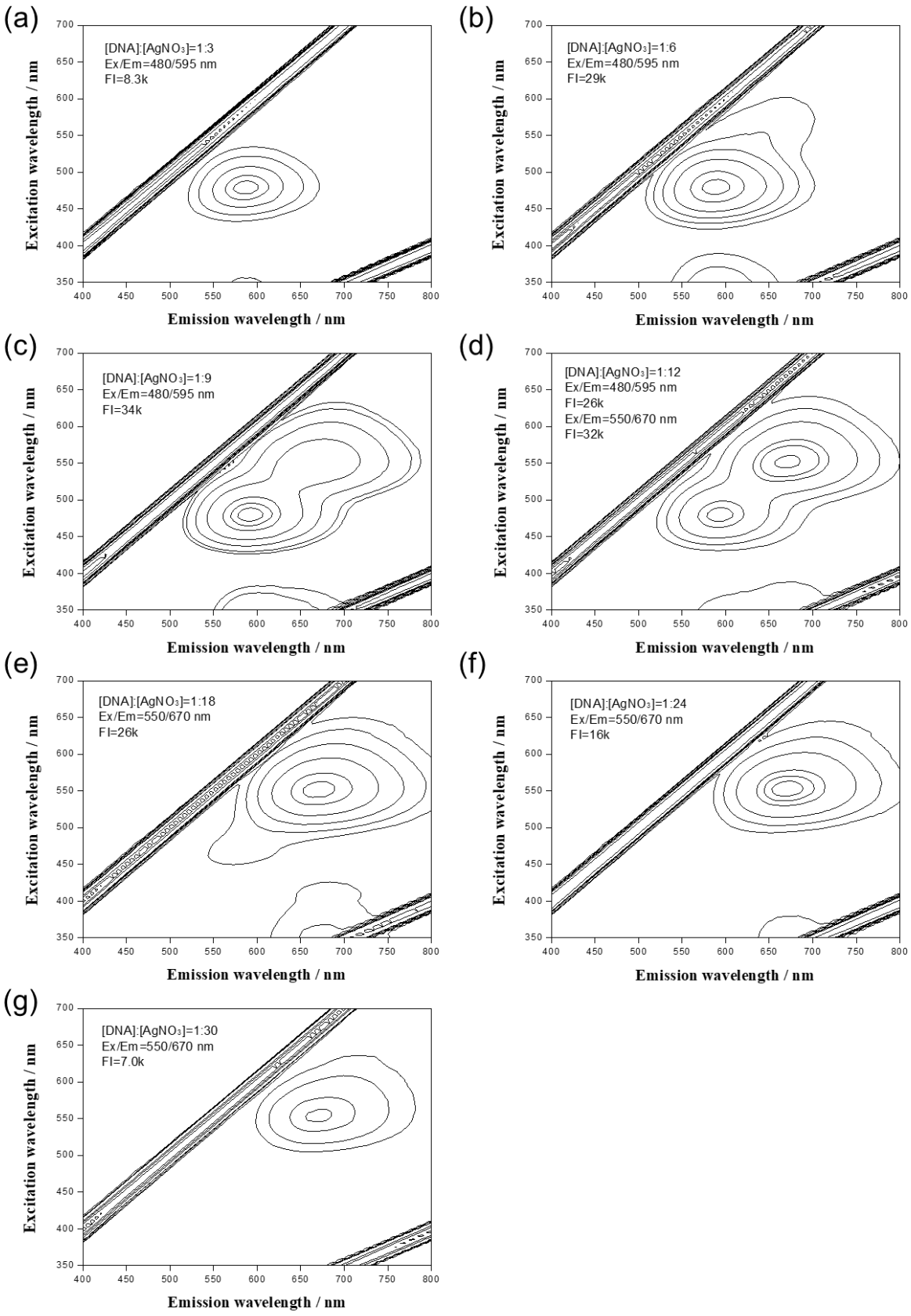

Fig. S4. Three-dimensional fluorescence spectra of H0-AgNCs synthesized with different concentration ratios of [DNA]:[$AgNO_3$]. The ratios of [$AgNO_3$]:[$NaBH_4$] remained constant (1:1) in all these experiments.

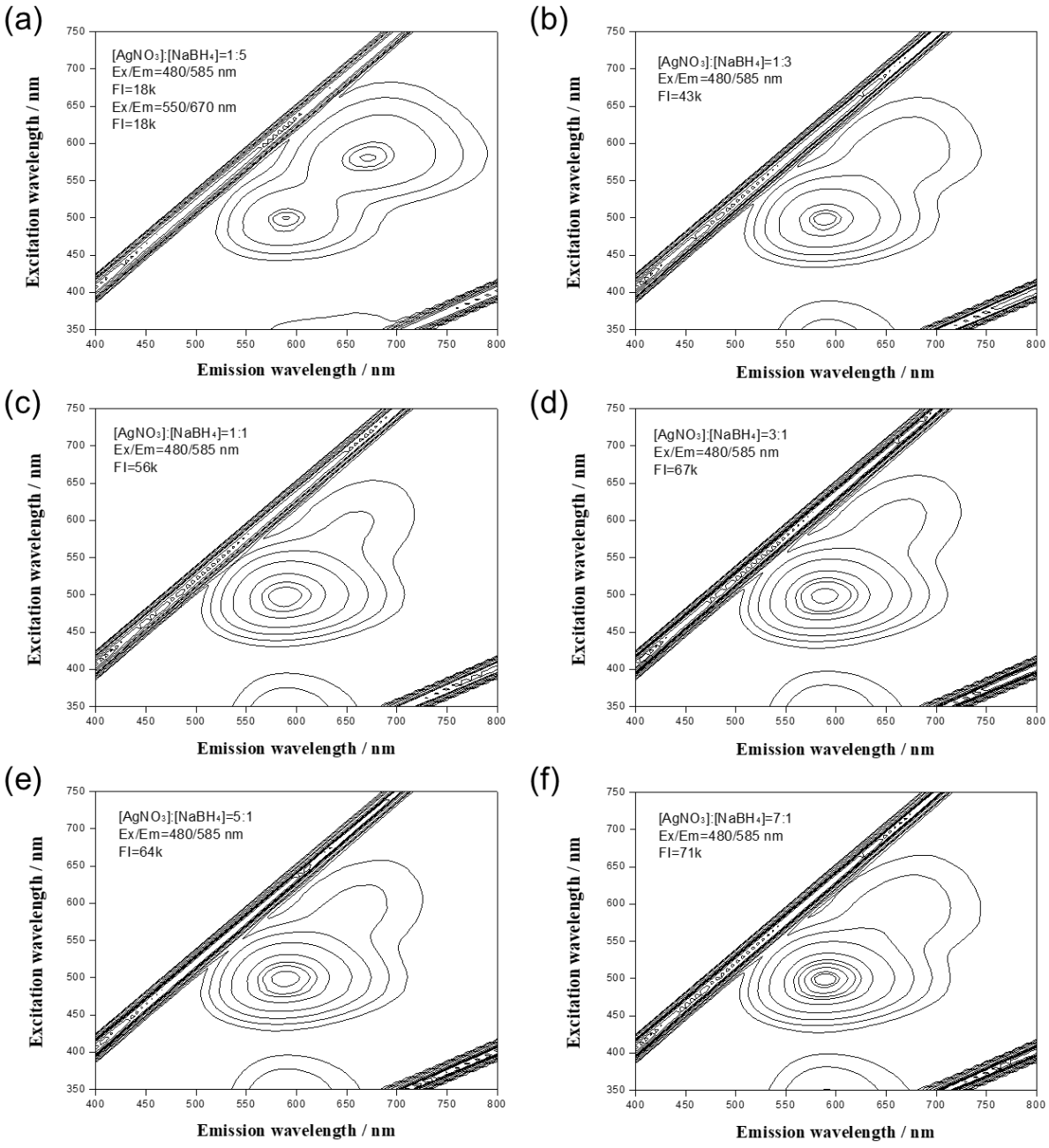

Fig. S5. Three-dimensional fluorescence spectra of H0-AgNCs synthesized with different concentration ratio of [$AgNO_3$]:[$NaBH_4$]. The ratios of [DNA]:[$AgNO_3$] remained constant (1:9) in all these experiments.

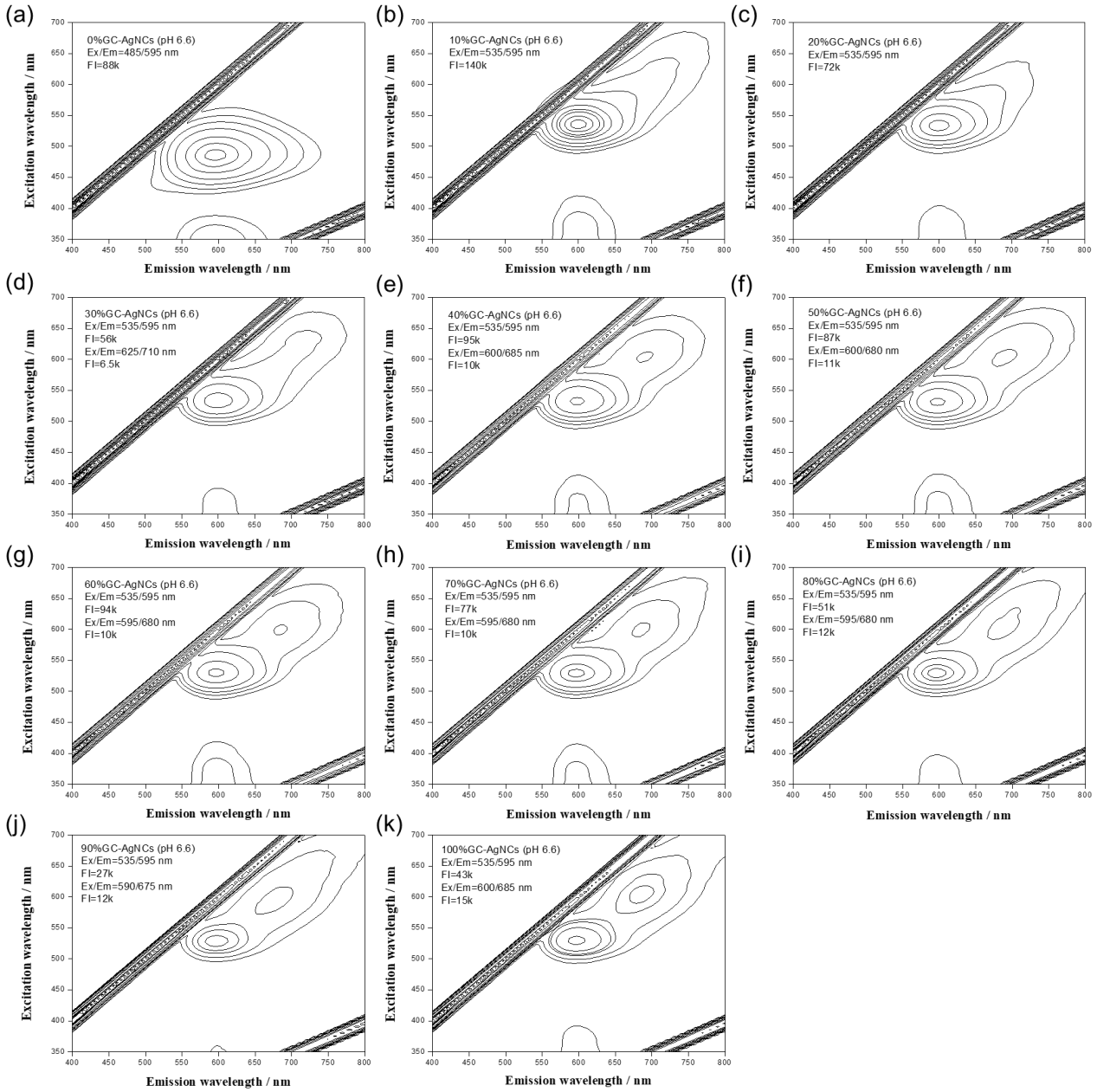

Fig. S6. Three-dimensional fluorescence spectra of hairpin-AgNCs synthesized with different GC content in stem sequence (0%-100%GC, Table S1. No. 3-13). Buffer pH=6.6.

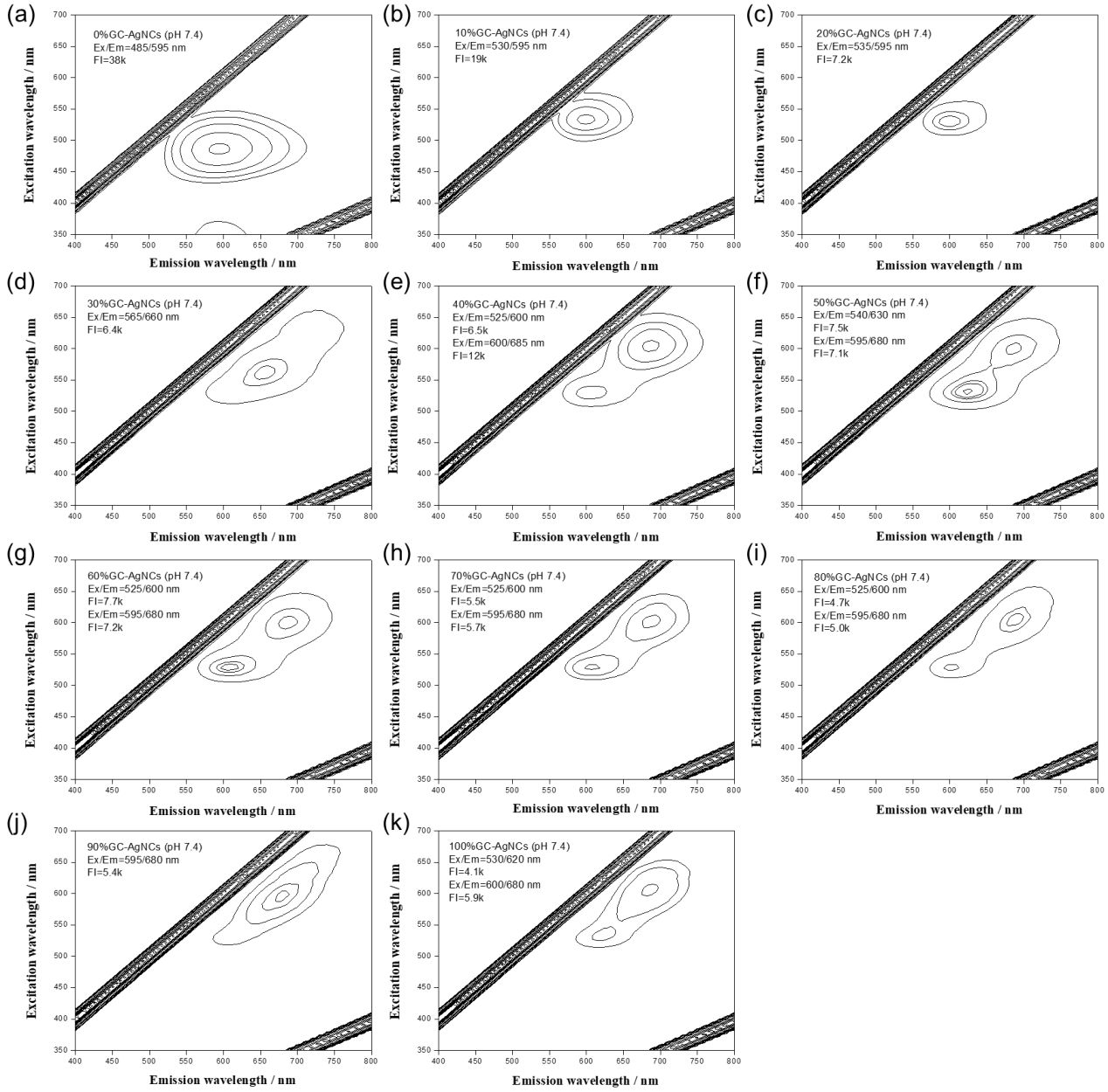

Fig. S7. Three-dimensional fluorescence spectra of hairpin-AgNCs synthesized with different GC content in stem sequence (0%-100%GC, Table S1. No. 3-13). Buffer pH=7.4.

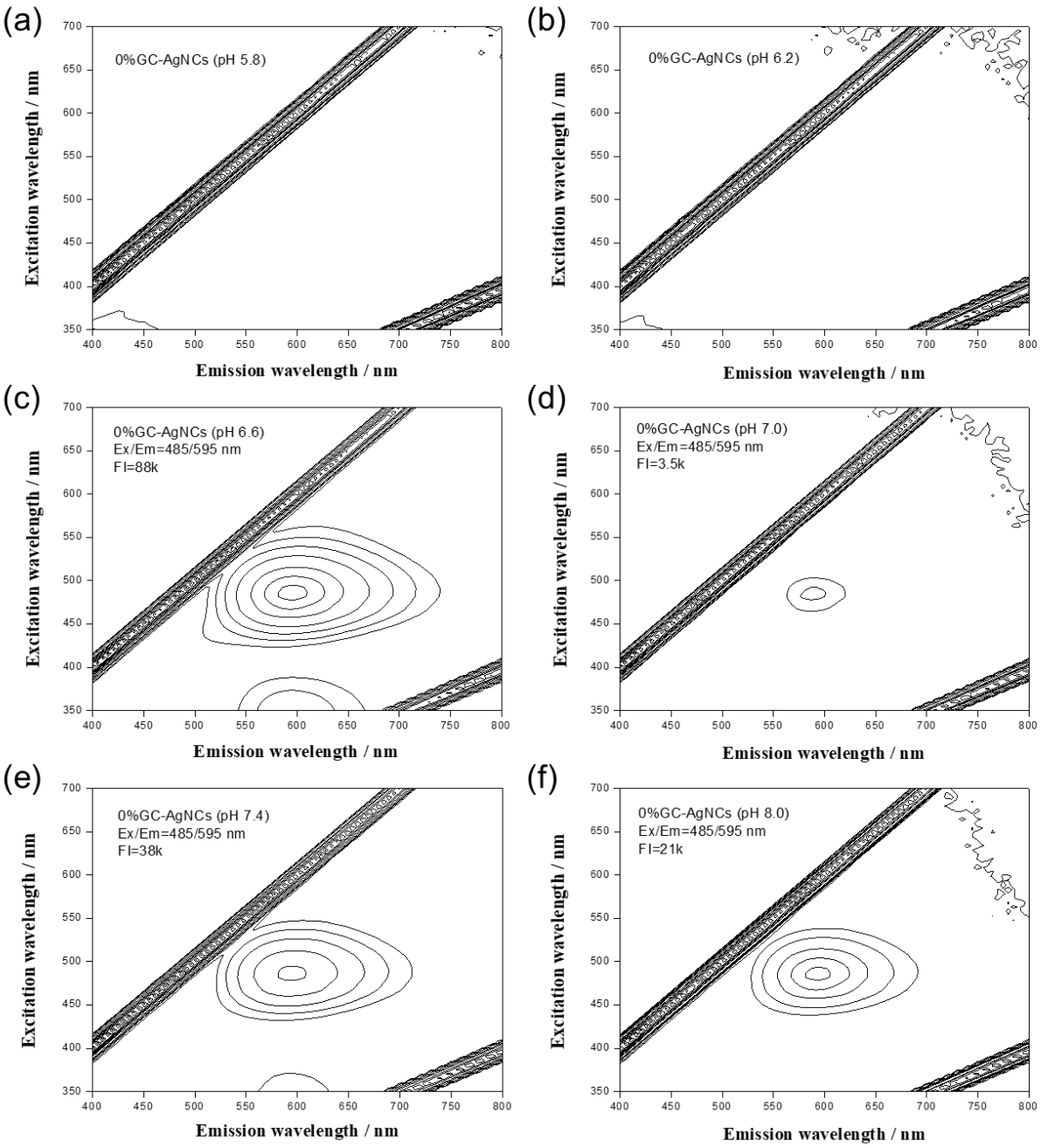

Fig. S8. Three-dimensional fluorescence spectra of the hairpin-AgNCs synthesized with 0% GC content (sequence No.3, Table S1.) at different pH (5.8, 6.2, 6.6, 7.0, 7.4 and 8.0).

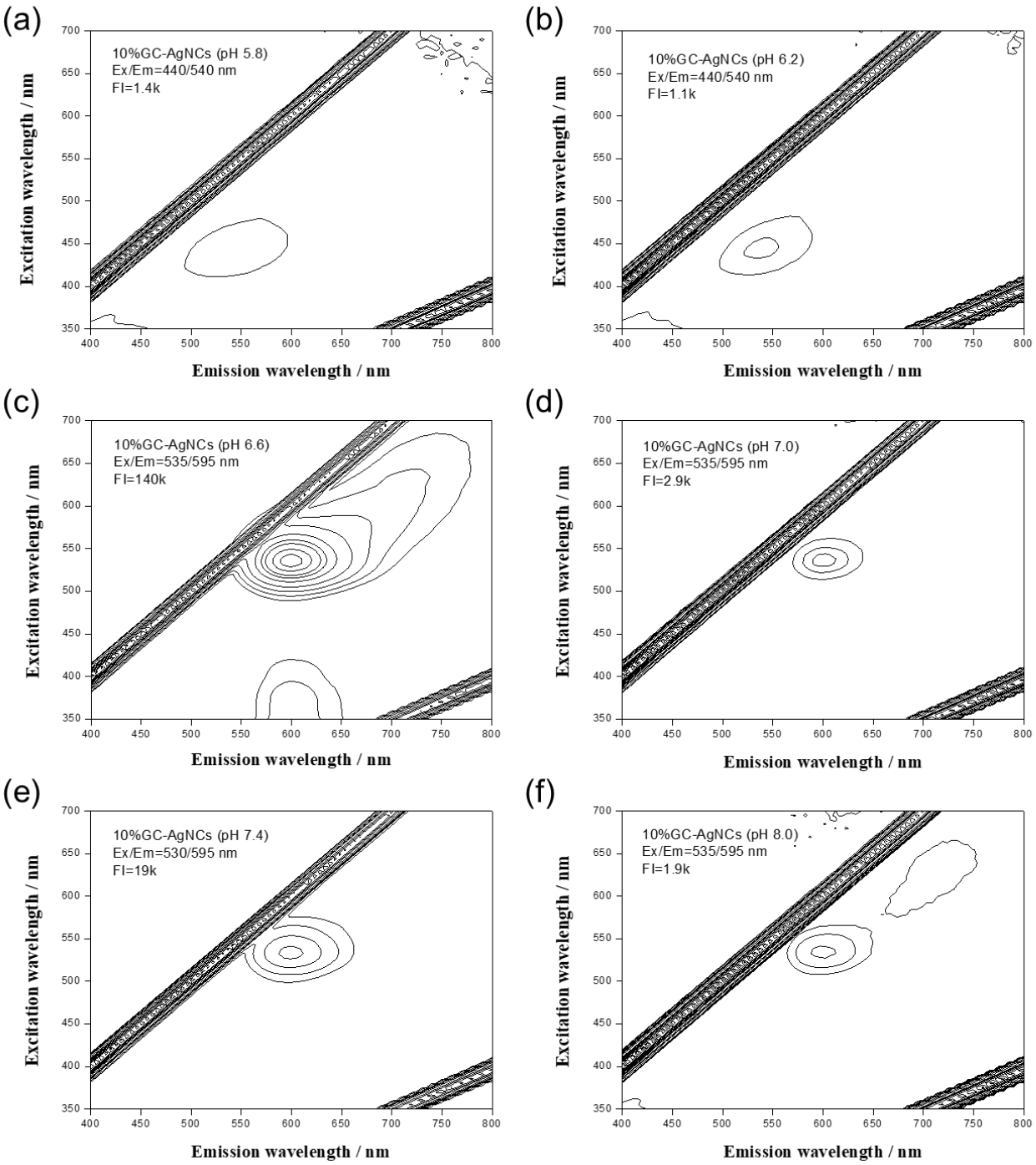

Fig. S9. Three-dimensional fluorescence spectra of the hairpin-AgNCs synthesized with 10% GC content (sequence No.4, Table S1.) at different pH (5.8, 6.2, 6.6, 7.0, 7.4 and 8.0).

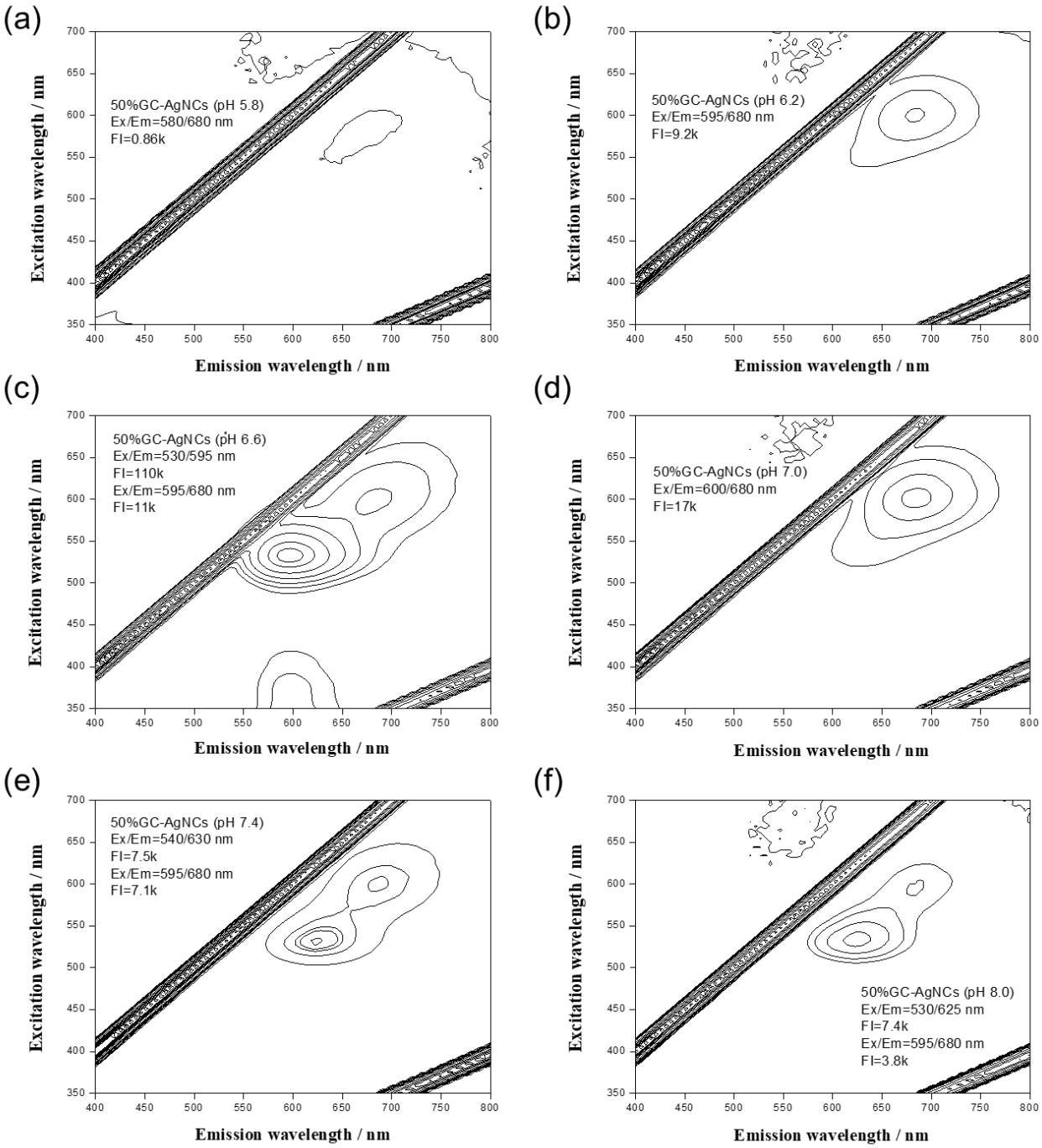

Fig. S10. Three-dimensional fluorescence spectra of the hairpin-AgNCs synthesized with 50% GC content (sequence No.8, Table S1.) at different pH (5.8, 6.2, 6.6, 7.0, 7.4 and 8.0).

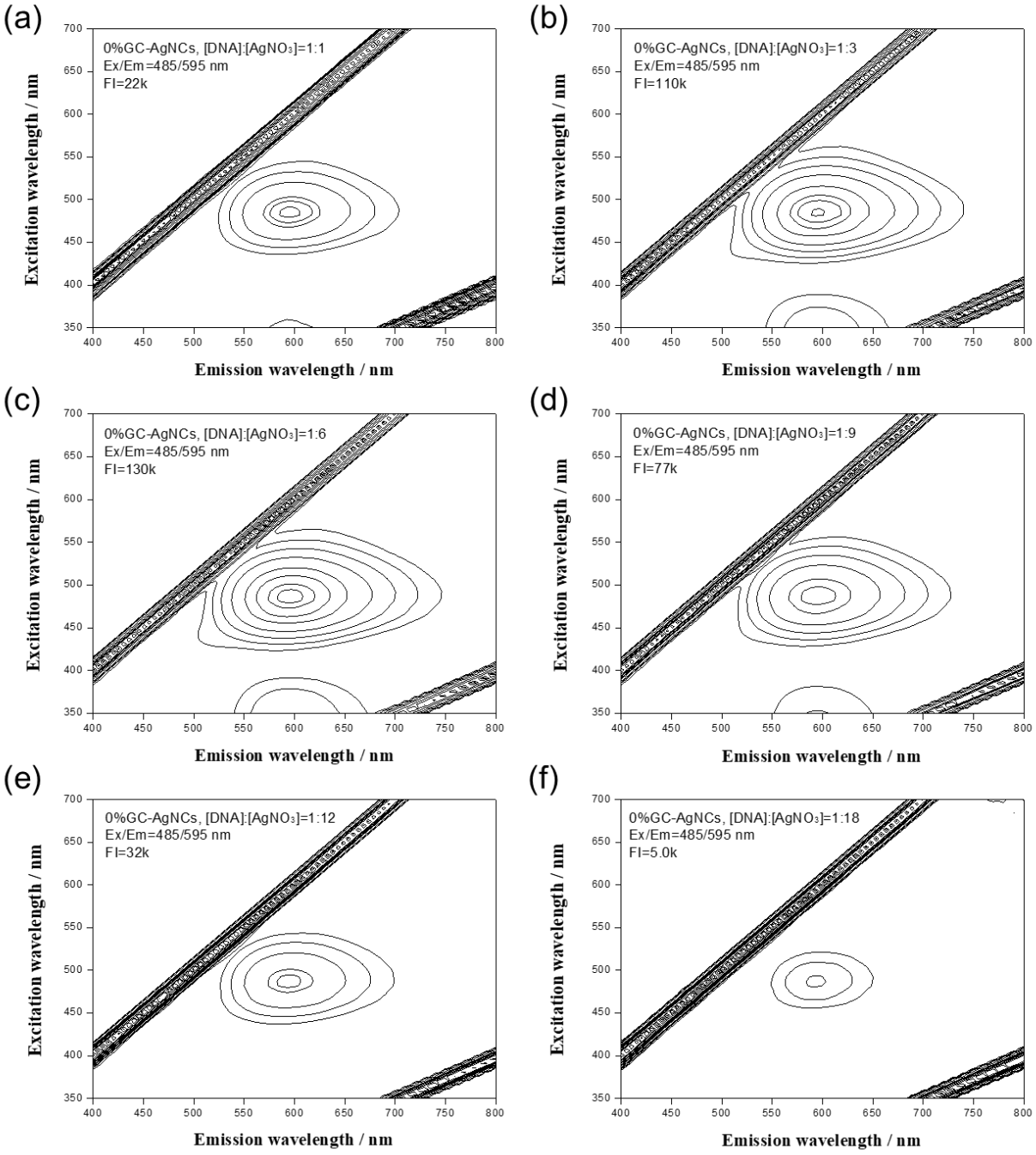

Fig. S11. Three-dimensional fluorescence spectra of the hairpin-AgNCs synthesized with 0% GC stem sequence (No.3, Table S1) at different ratios of [DNA]: [$AgNO_3$].

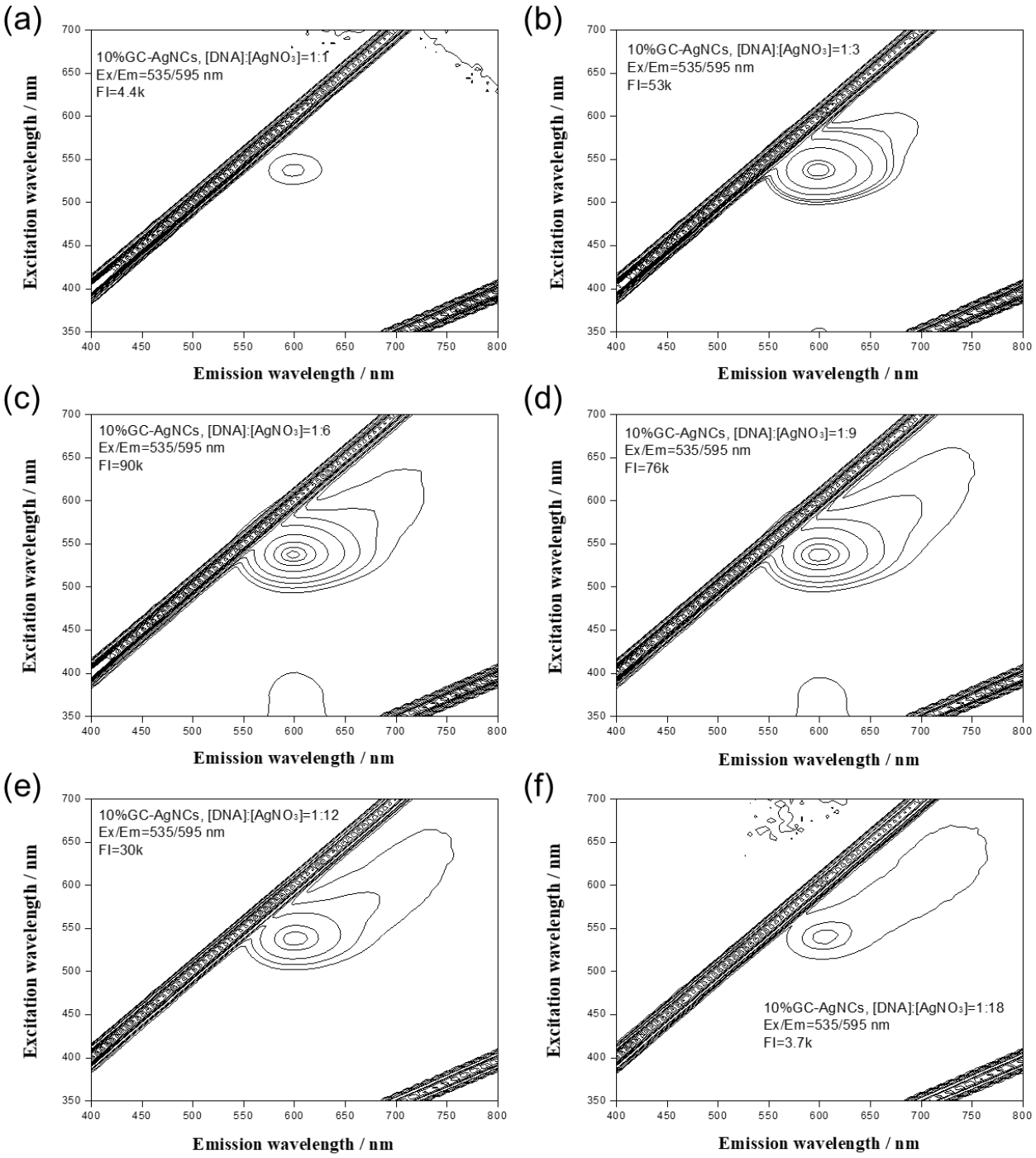

Fig. S12. Three-dimensional fluorescence spectra of the hairpin-AgNCs synthesized with 10%GC stem sequence (No.4, Table S1) at different ratios of [DNA]:[$AgNO_3$].

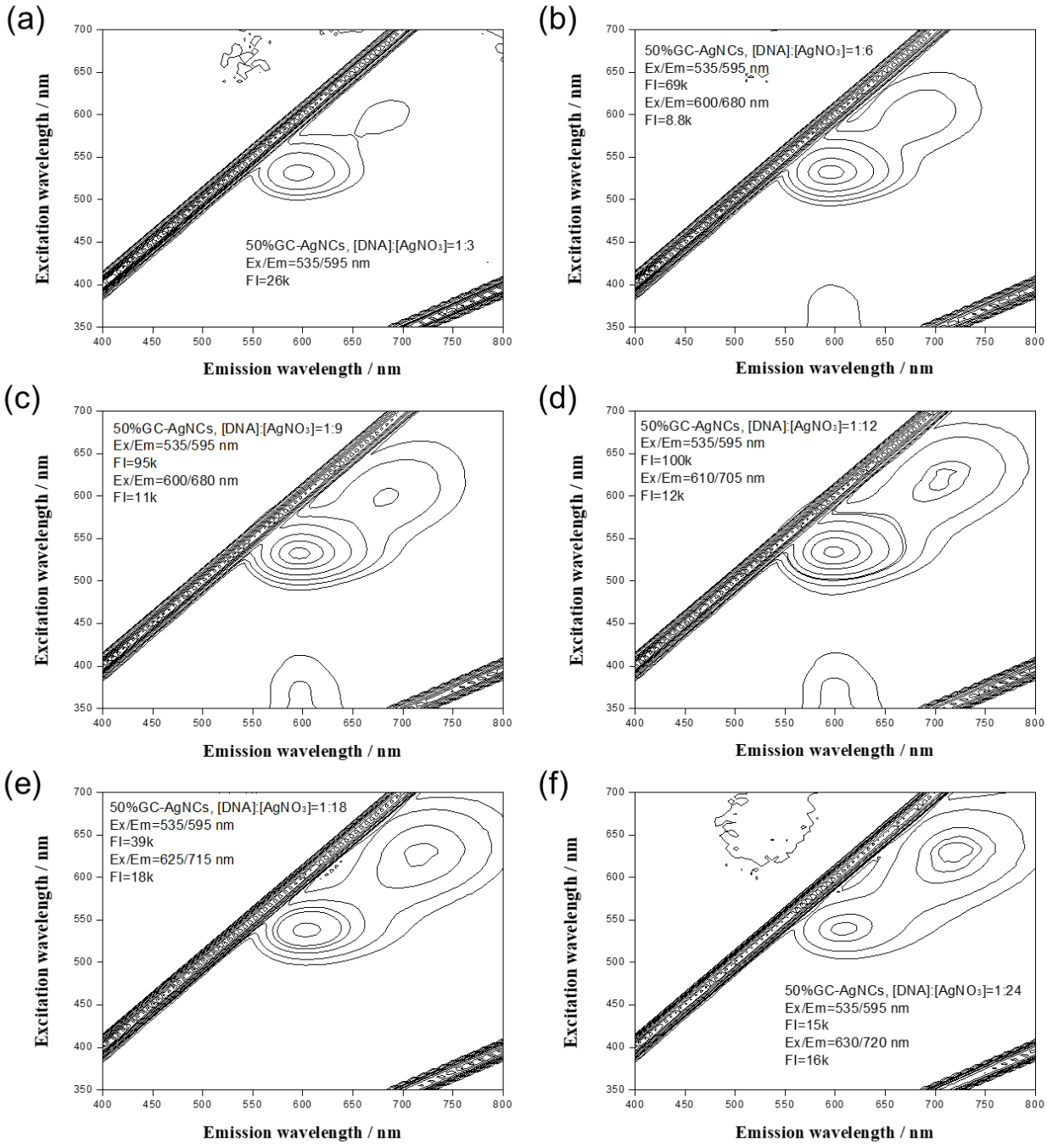

Fig. S13. Three-dimensional fluorescence spectra of the hairpin-AgNCs synthesized with 50% GC stem sequence (No.8, Table S1) at different ratios of [DNA]:[$AgNO_3$].

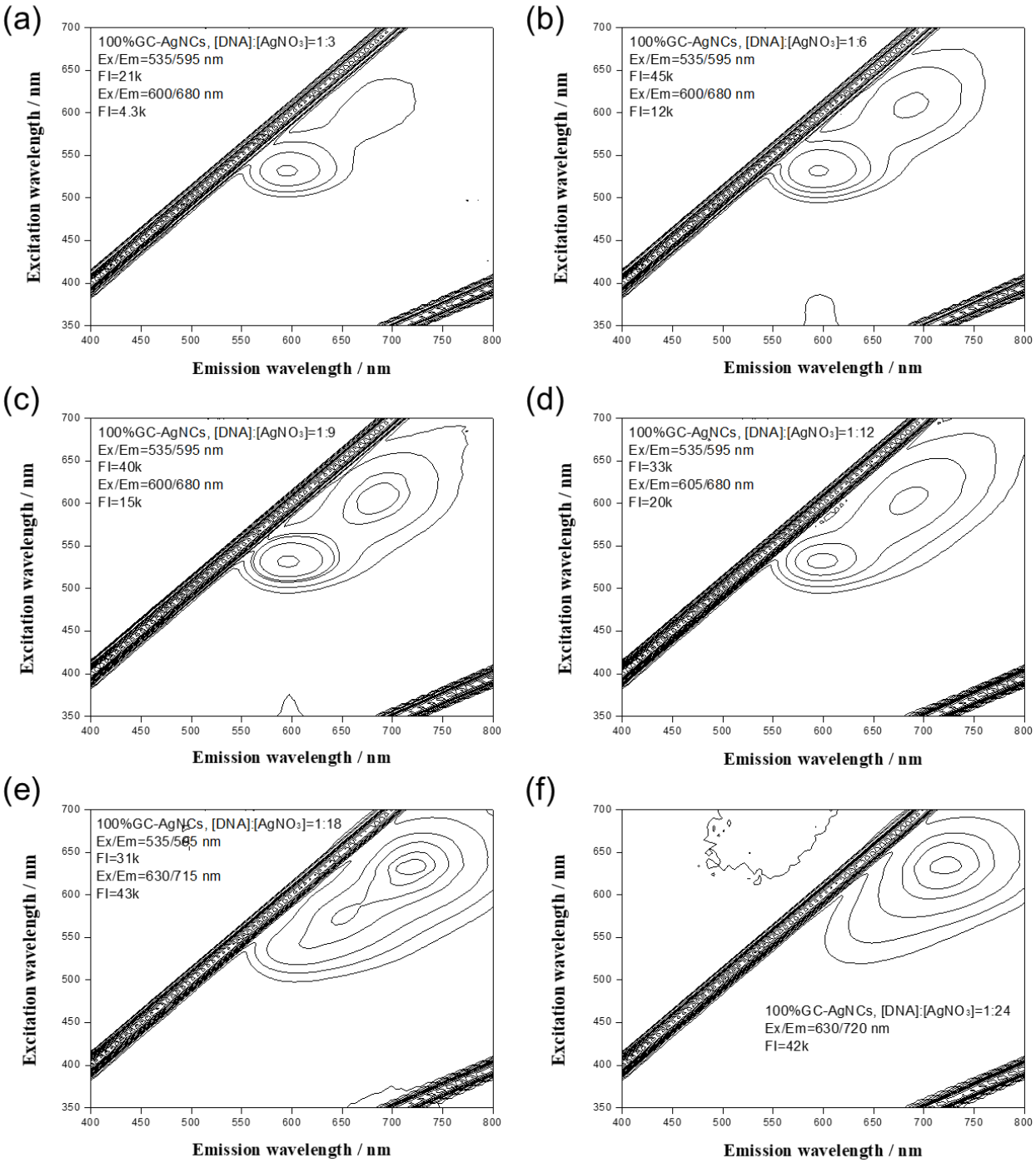

Fig. S14. Three-dimensional fluorescence spectra of the hairpin-AgNCs synthesized with 100%GC stem sequence (No.13, Table S1) at different ratios of [DNA]:[$AgNO_3$].

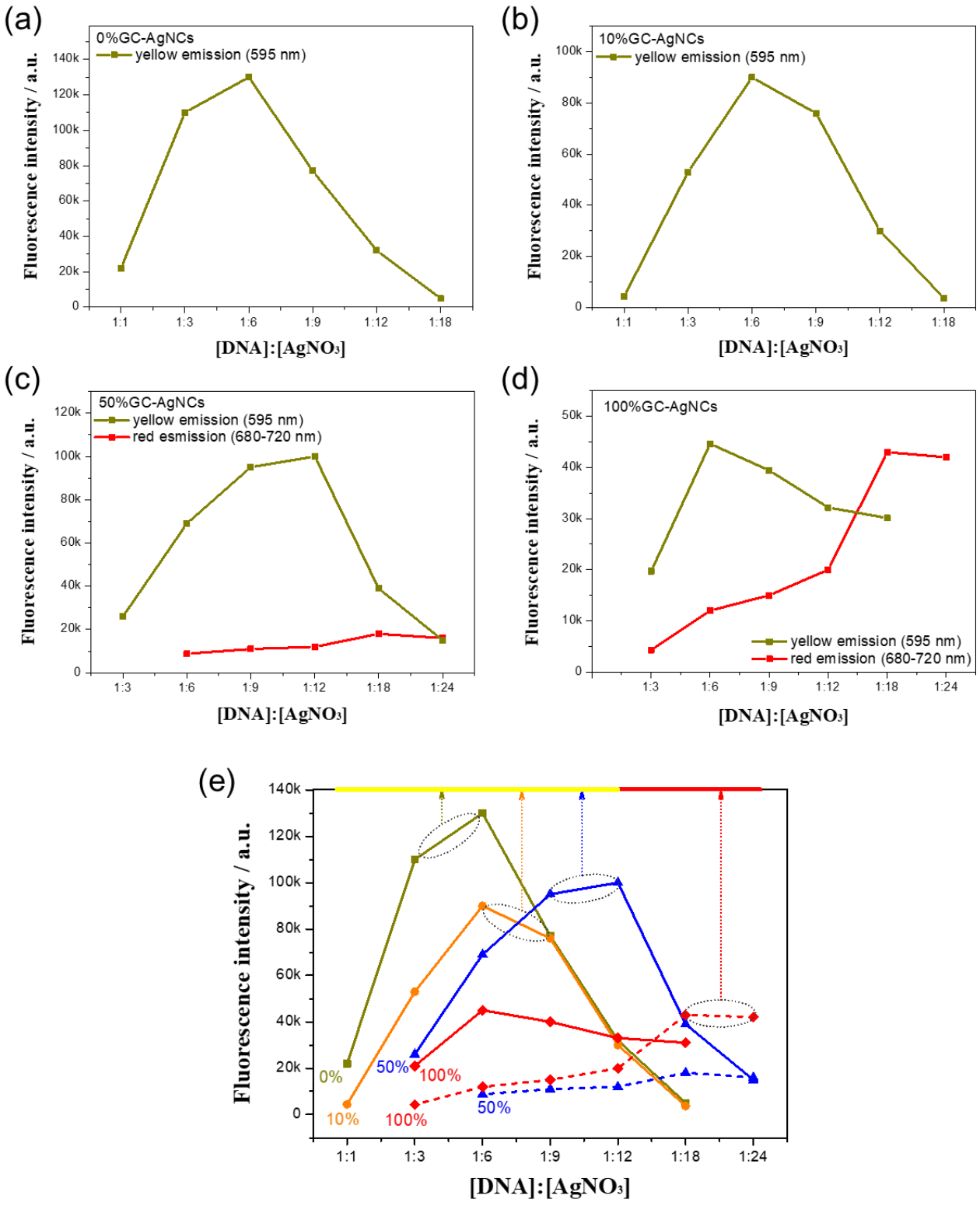

Fig. S15. The fluorescence intensities of hairpin-AgNCs (with different GC content in stem) synthesized using different ratios of [DNA]:[$AgNO_3$].

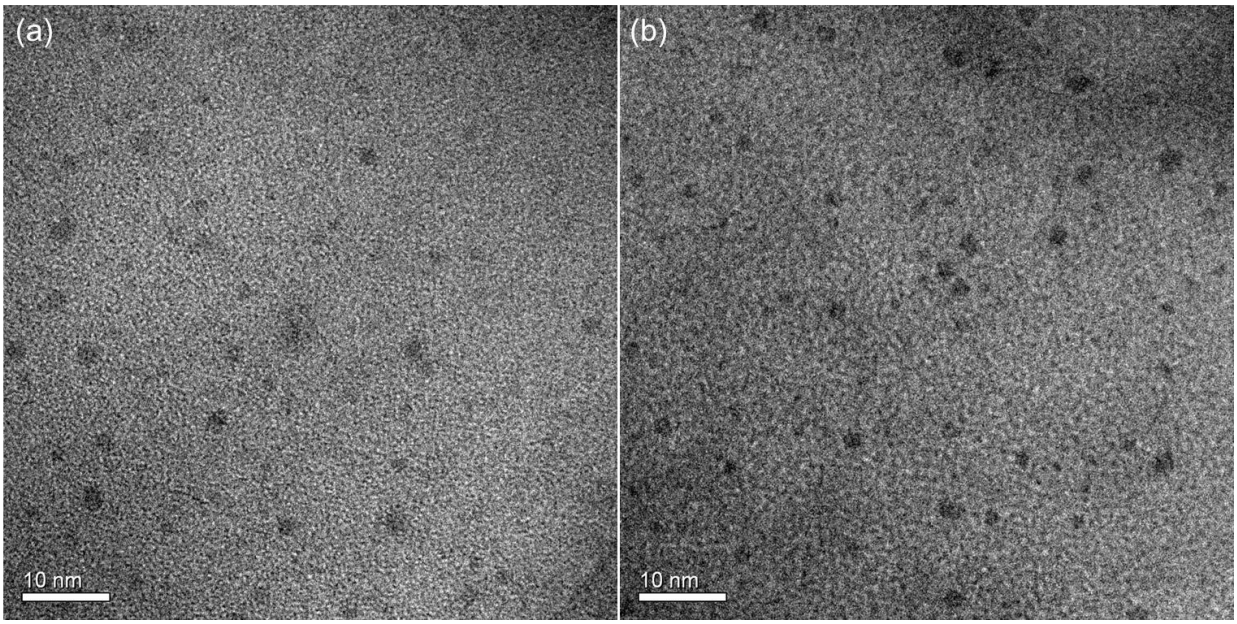

Fig. S16. Respective TEM images of (a) 0%GC-AgNCs and (b) 100%GC-AgNCs.

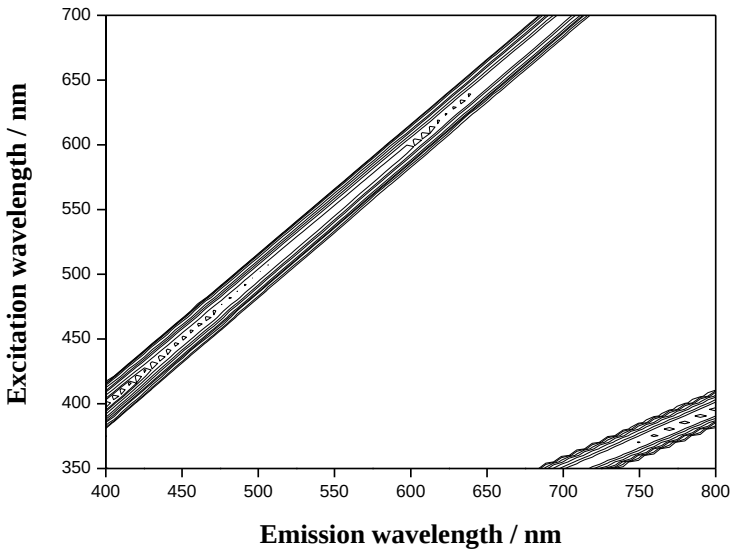

Fig. S17. The collected three-dimensional fluorescence spectrum of the AgNCs synthesized with the stem dsDNA of H0-dsDNA (without loop structure).

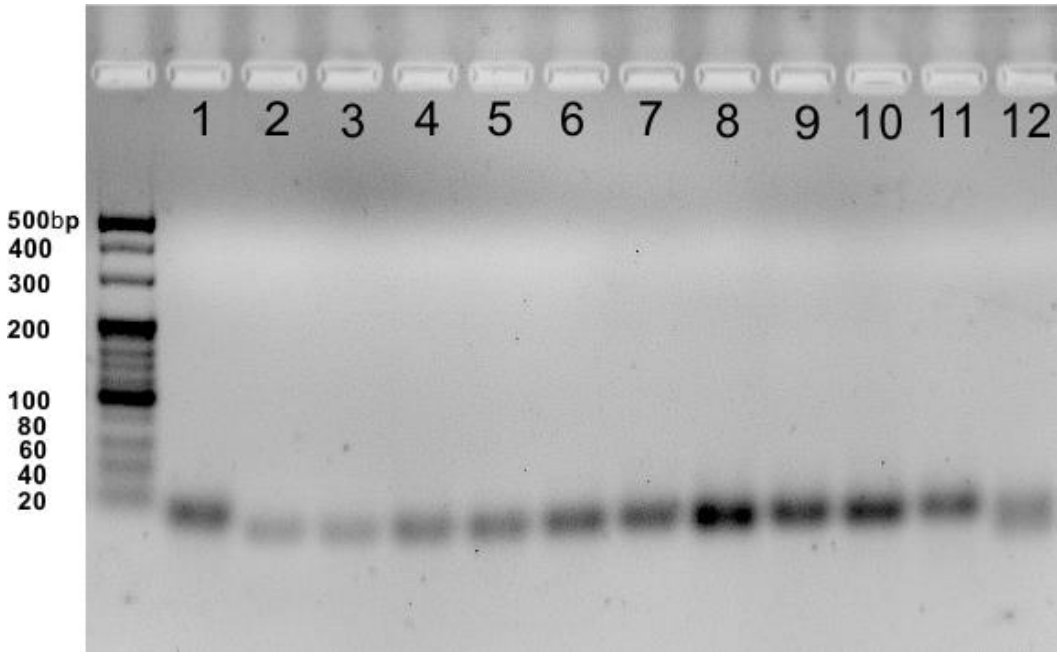

Fig. S18. Agarose gel electrophoresis of the DNAs with sequences (No. 1&3-13) listed in Table S1. Lane1: H0-DNA; Lane2-12: hairpin DNAs with stems containing 0%GC-100%GC base pairs.

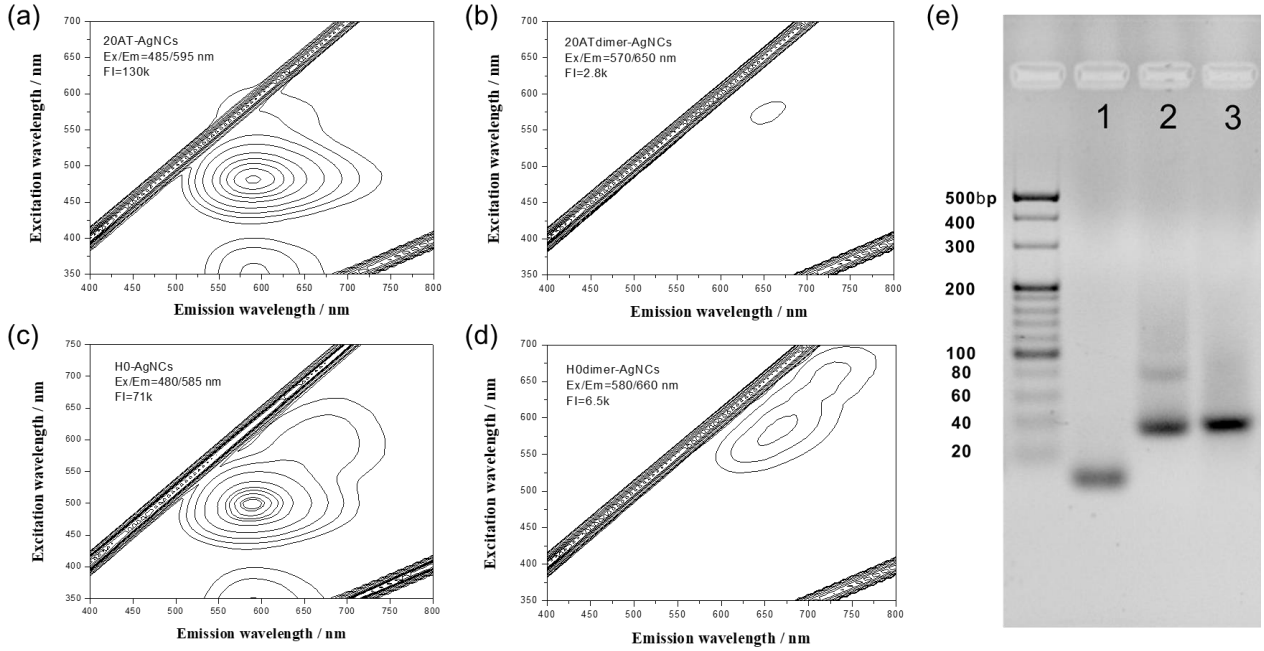

Fig. S19. (a) Three-dimensional fluorescence spectrum of 20AT-AgNCs. (b) Three-dimensional fluorescence spectrum of 20ATdimer-AgNCs (20ATdimer = 20AT-ds1 & 20AT-ds2). (c) Three-dimensional fluorescence spectrum of H0-AgNCs. (d) Three-dimensional fluorescence spectrum of H0dimer-AgNCs (H0dimer = H0-ds1 & H0-ds2). (e) Image obtained from the agarose gel electrophoresis. Lane1: 20AT; Lane2: 20ATdimer; Lane3: H0dimer.

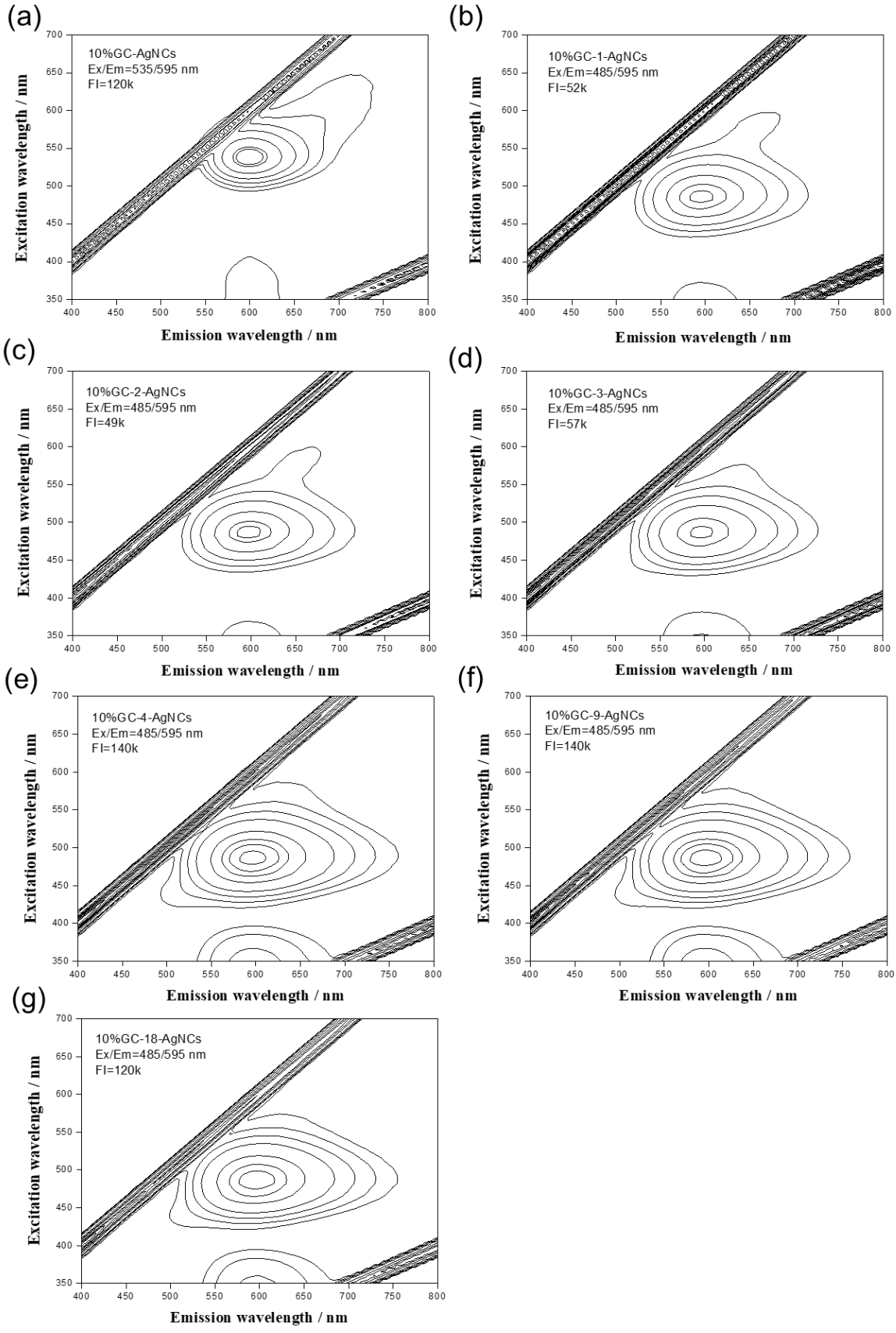

Fig. S20. Three-dimensional fluorescence spectra of the 10%GC-hairpin-AgNCs synthesized using stems with two GC base pairs distributed in different distances towards loop. Sequences were listed in Table S1 (No. 19-25).

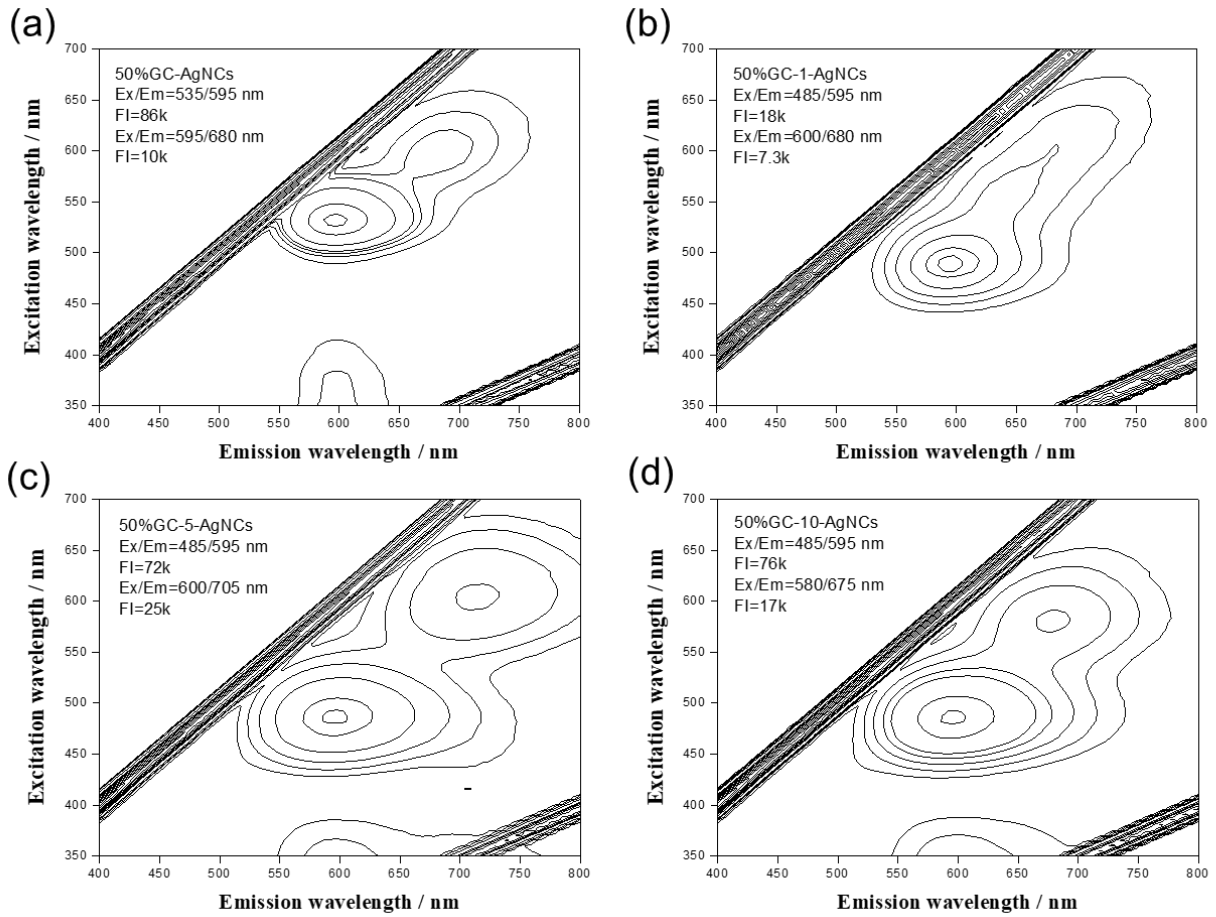

Fig. S21. Three-dimensional fluorescence spectra of the 50%GC-hairpin-AgNCs synthesized using stems with ten GC base pairs distributed in different distances towards loop. Sequences were listed in Table S1 (No. 26-29).

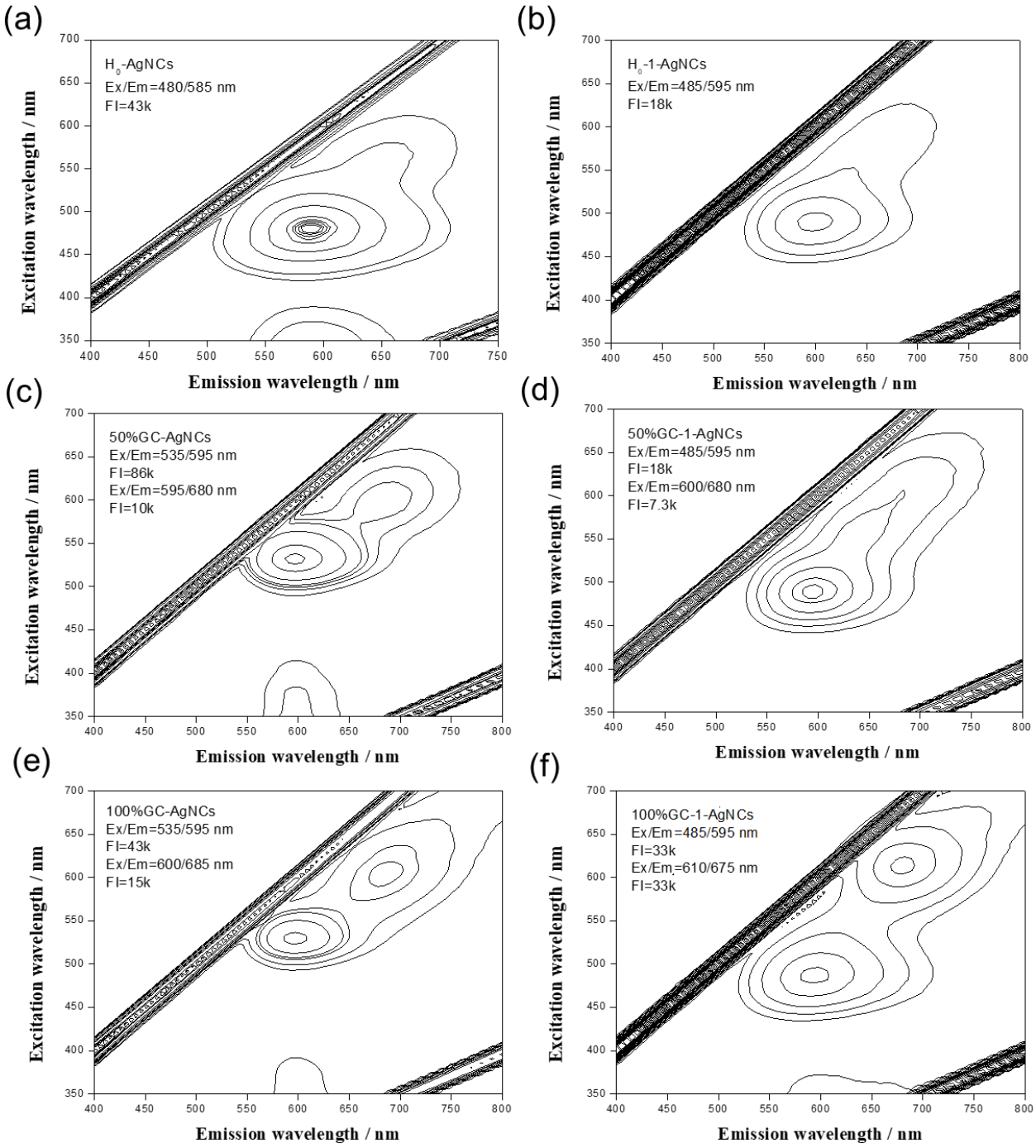

Fig. S22. (a/b) Three-dimensional fluorescence spectra of the H0-AgNCs synthesized using stems without/with 1 AT base pair between loop and GC base pairs. (c/d) Three-dimensional fluorescence spectra of the 50%GC-hairpin-AgNCs synthesized using stems without/with 1 AT base pair between loop and GC base pairs. (e/f) Three-dimensional fluorescence spectra of the 100%GC-hairpin-AgNCs synthesized using stems without/with 1 AT base pair between loop and GC base pairs. Sequences were listed in Table S1 (No. 26-27, 30-33).

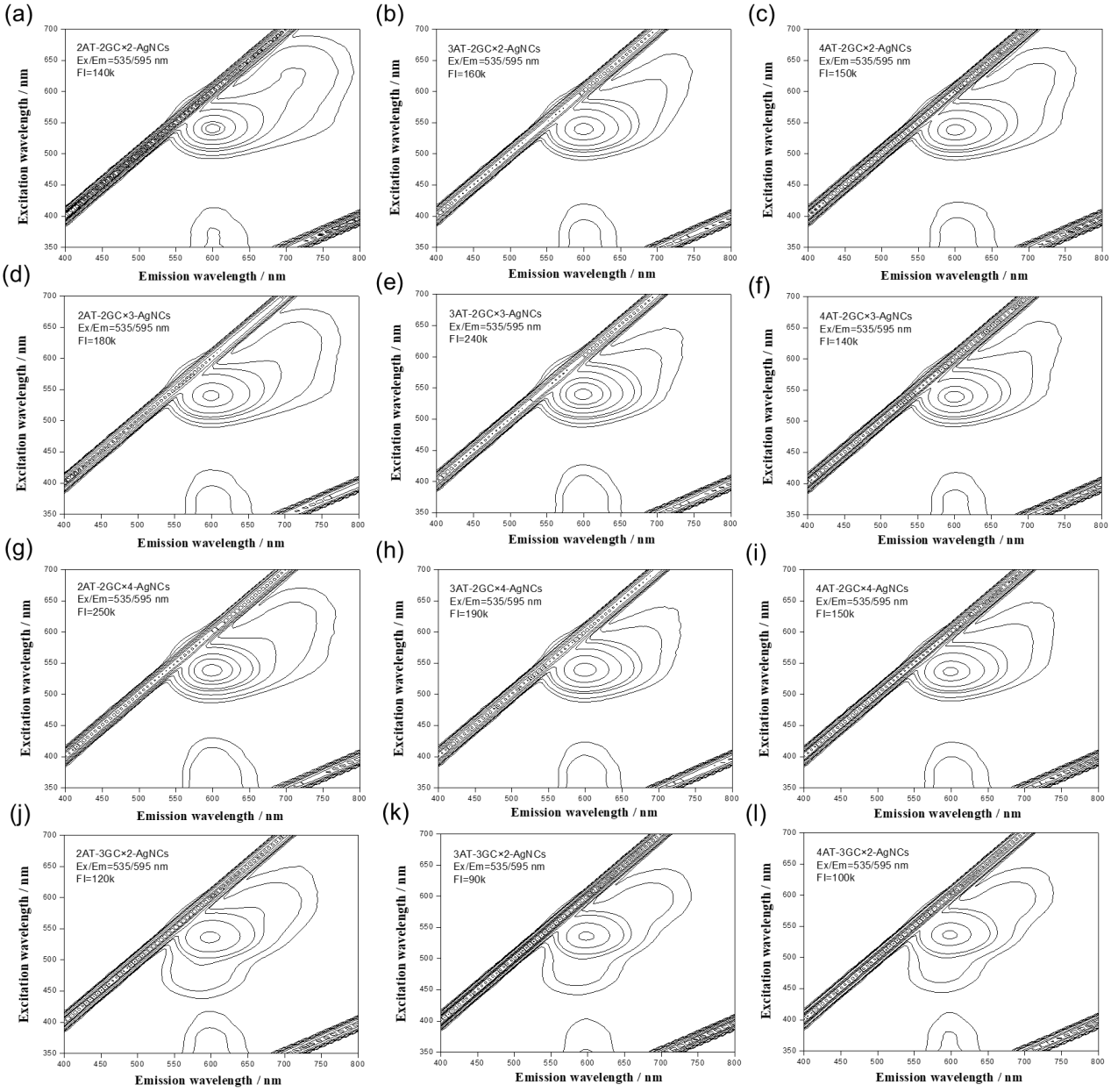

Fig. S23. (a/b/c) Three-dimensional fluorescence spectra of the hairpin-AgNCs with two GC units in stem separated by 2/3/4 AT base pairs respectively (the sequence No. 34-36 in Table S1). (d/e/f) Three-dimensional fluorescence spectra of the hairpin-AgNCs with three GC units in stem separated by 2/3/4 AT base pairs respectively (the sequence No. 37-39 in Table S1). (g/h/i) Three-dimensional fluorescence spectra of the hairpin-AgNCs with four GC units in stem separated by 2/3/4 AT base pairs respectively (the sequence No. 40-42 in Table S1). (j/k/l) Three-dimensional fluorescence spectra of the hairpin-AgNCs with two GCG units in stem separated by 2/3/4 AT base pairs respectively (the sequence No. 43-45 in Table S1).

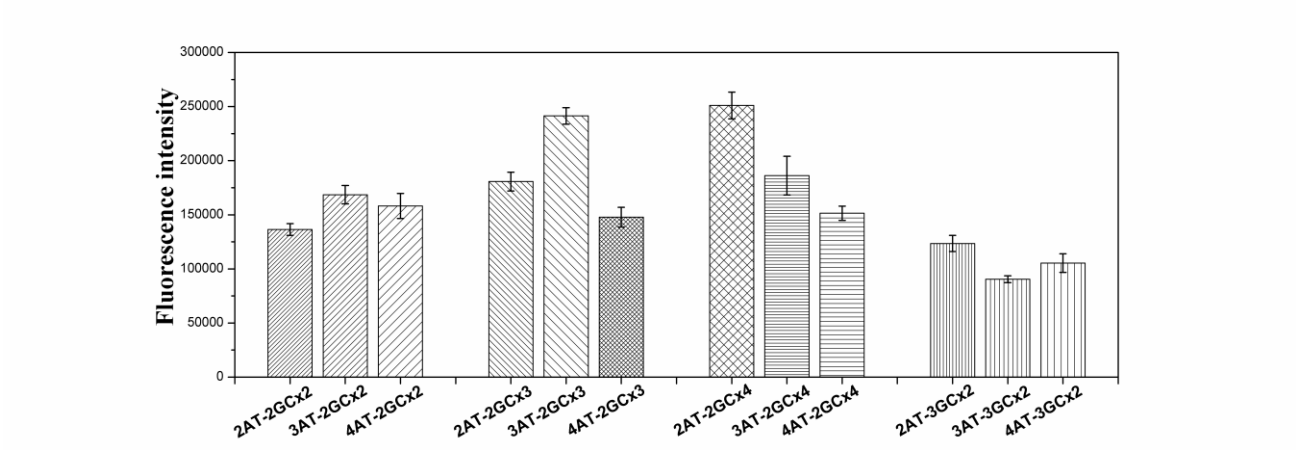

Fig. S24. The fluorescence intensities of the hairpin-AgNCs with different GC units in stem separated by 2/3/4 AT base pairs respectively (the sequence No. 34-45 in Table S1).

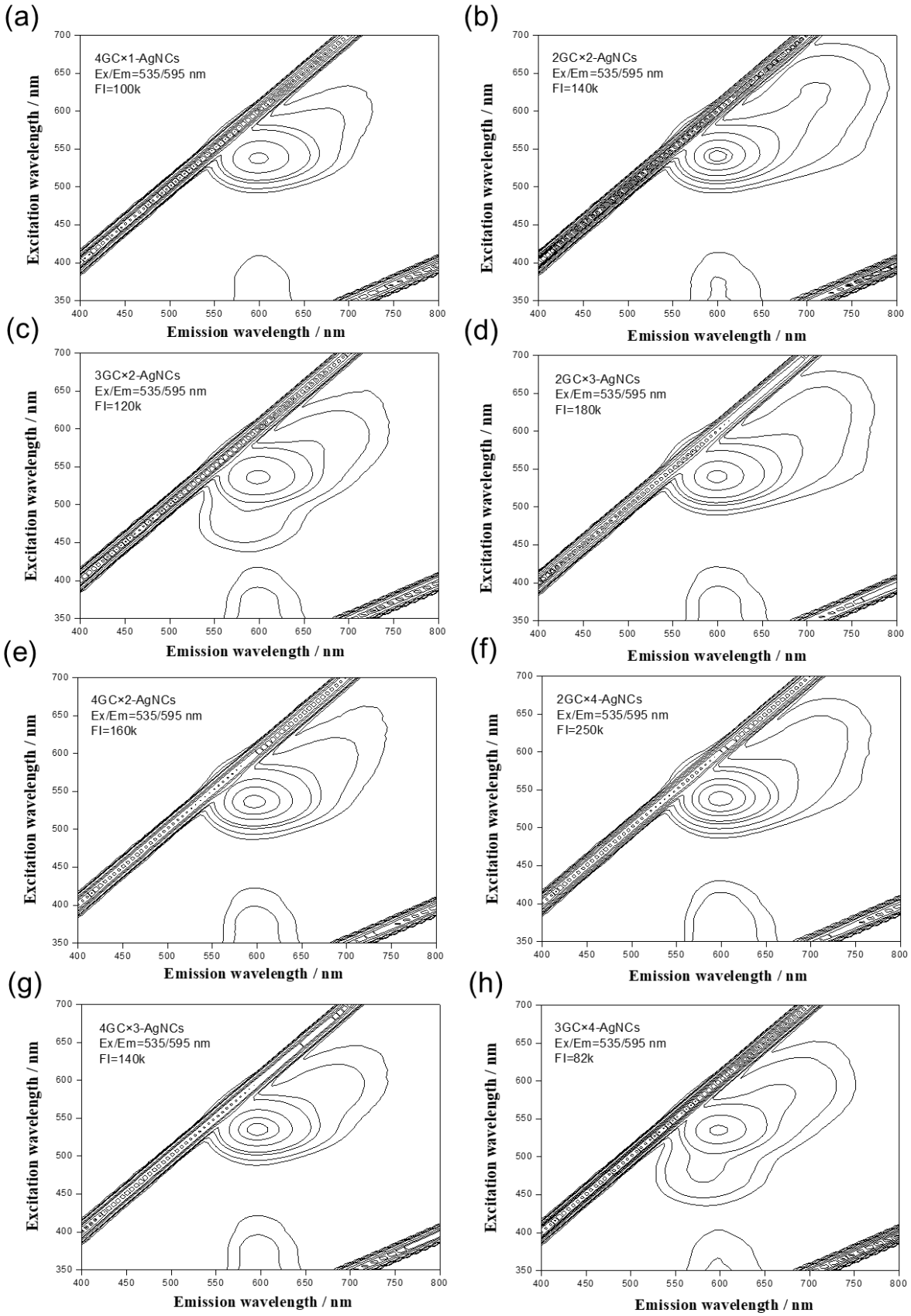

Fig. S25. Three-dimensional fluorescence spectra of hairpin-AgNCs using different stem designed based on different GC unit combinations.

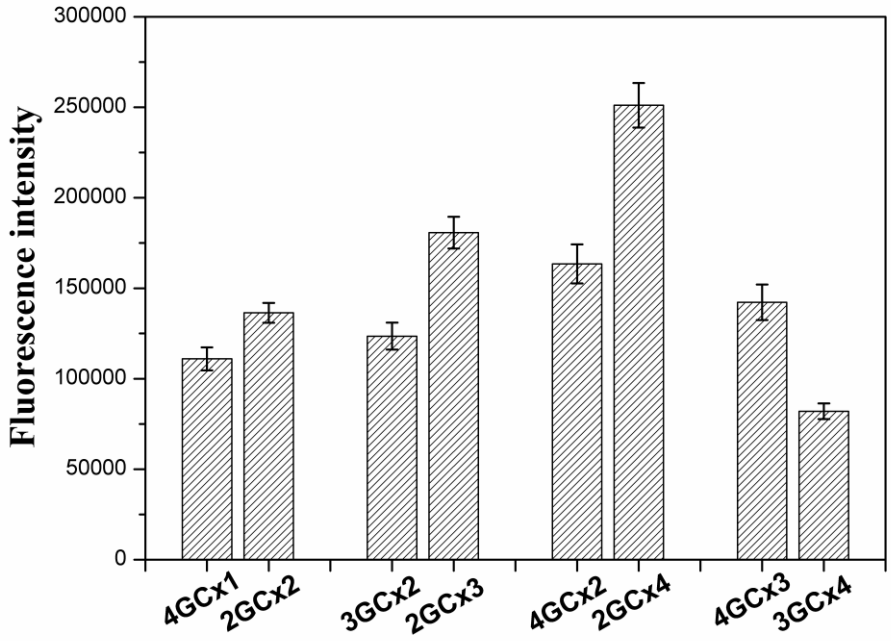

Fig. S26. The fluorescence intensities of the hairpin-AgNCs using different stem designed based on different GC unit combinations.

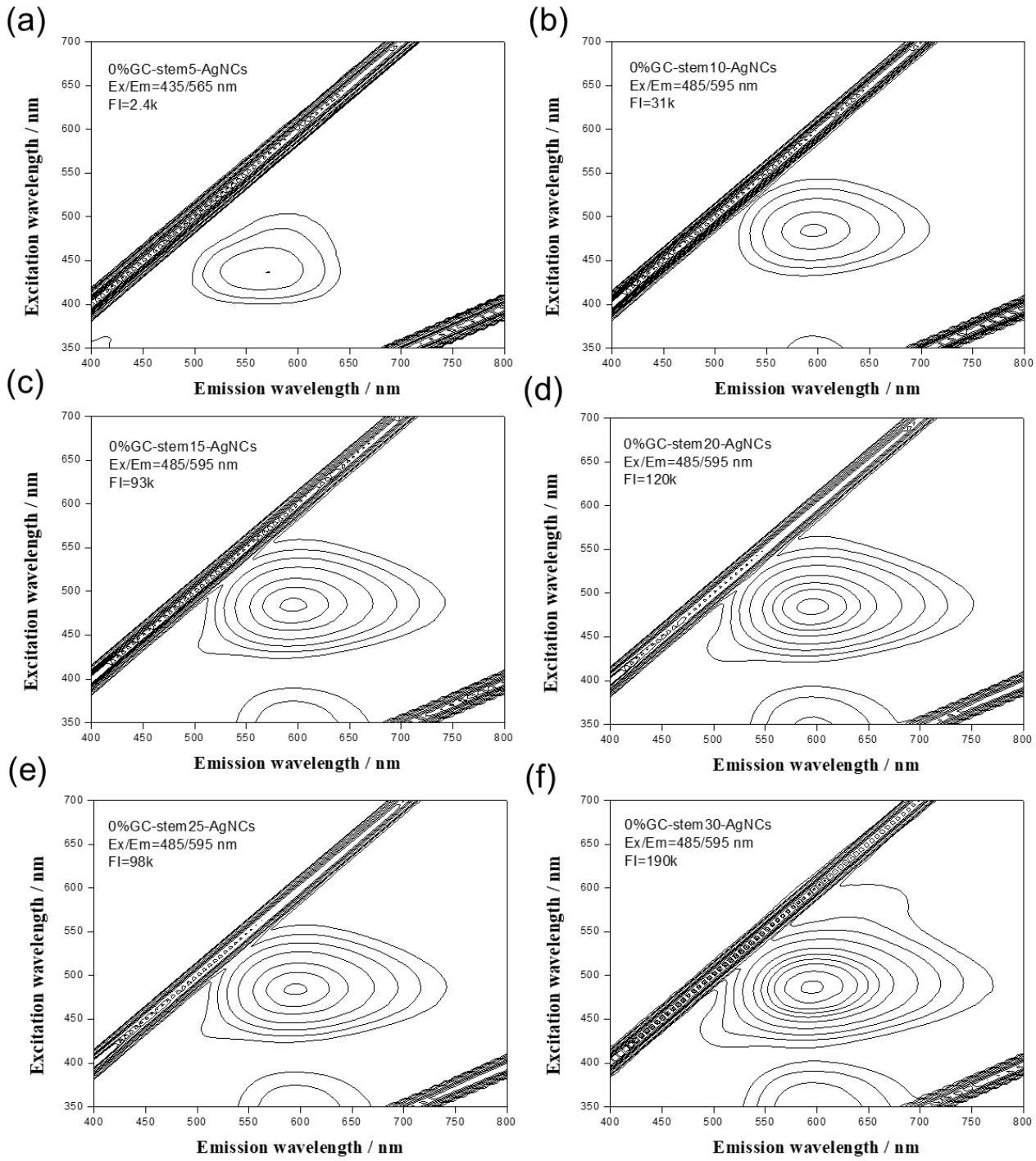

Fig. S27. The three-dimensional fluorescence spectra of the hairpin-AgNCs using stems of different length (without GC base pairs in stem sequence).

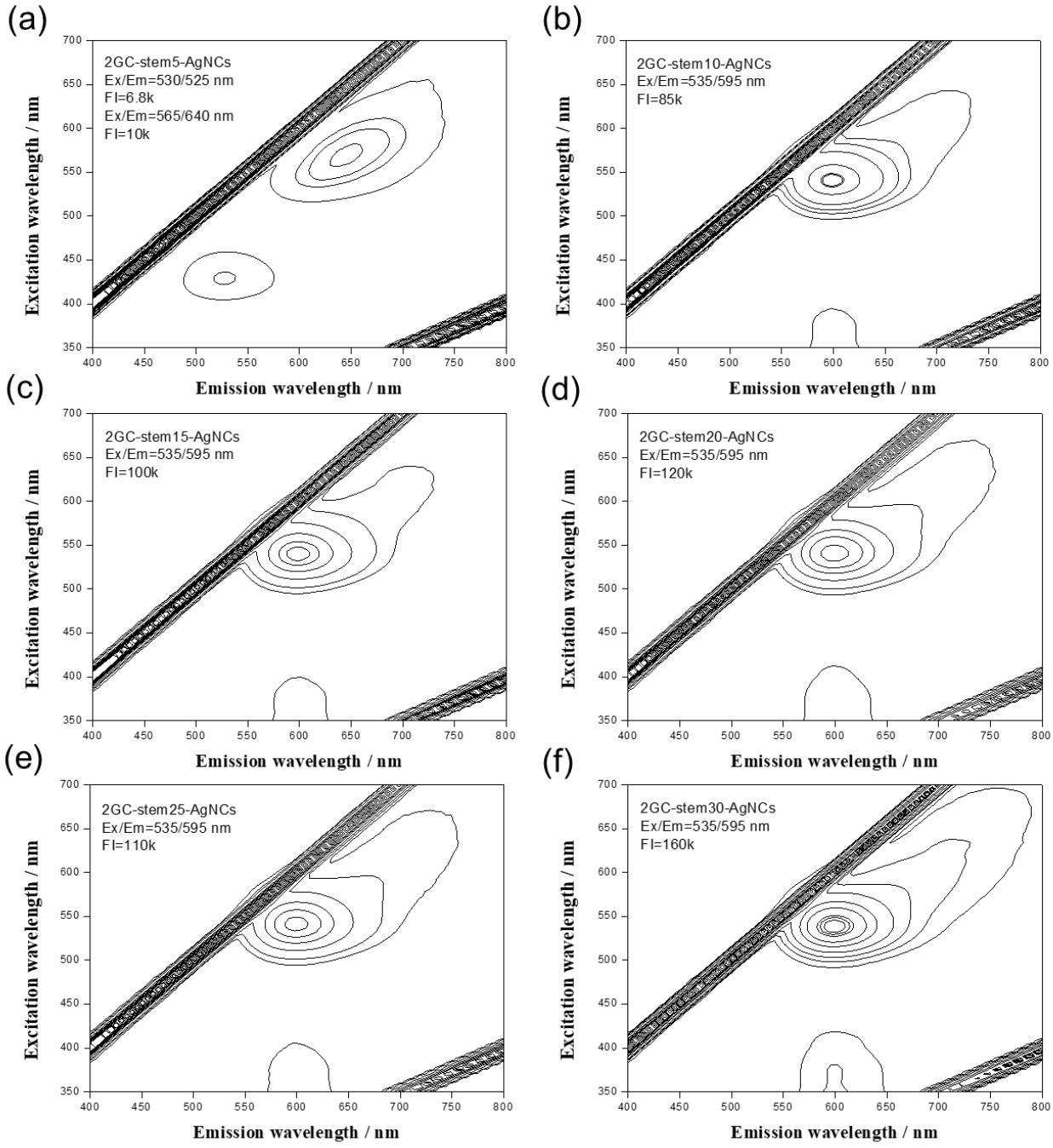

Fig. S28. The three-dimensional fluorescence spectra of the hairpin-AgNCs using stems of different length (with two GC base pairs in stem sequence).

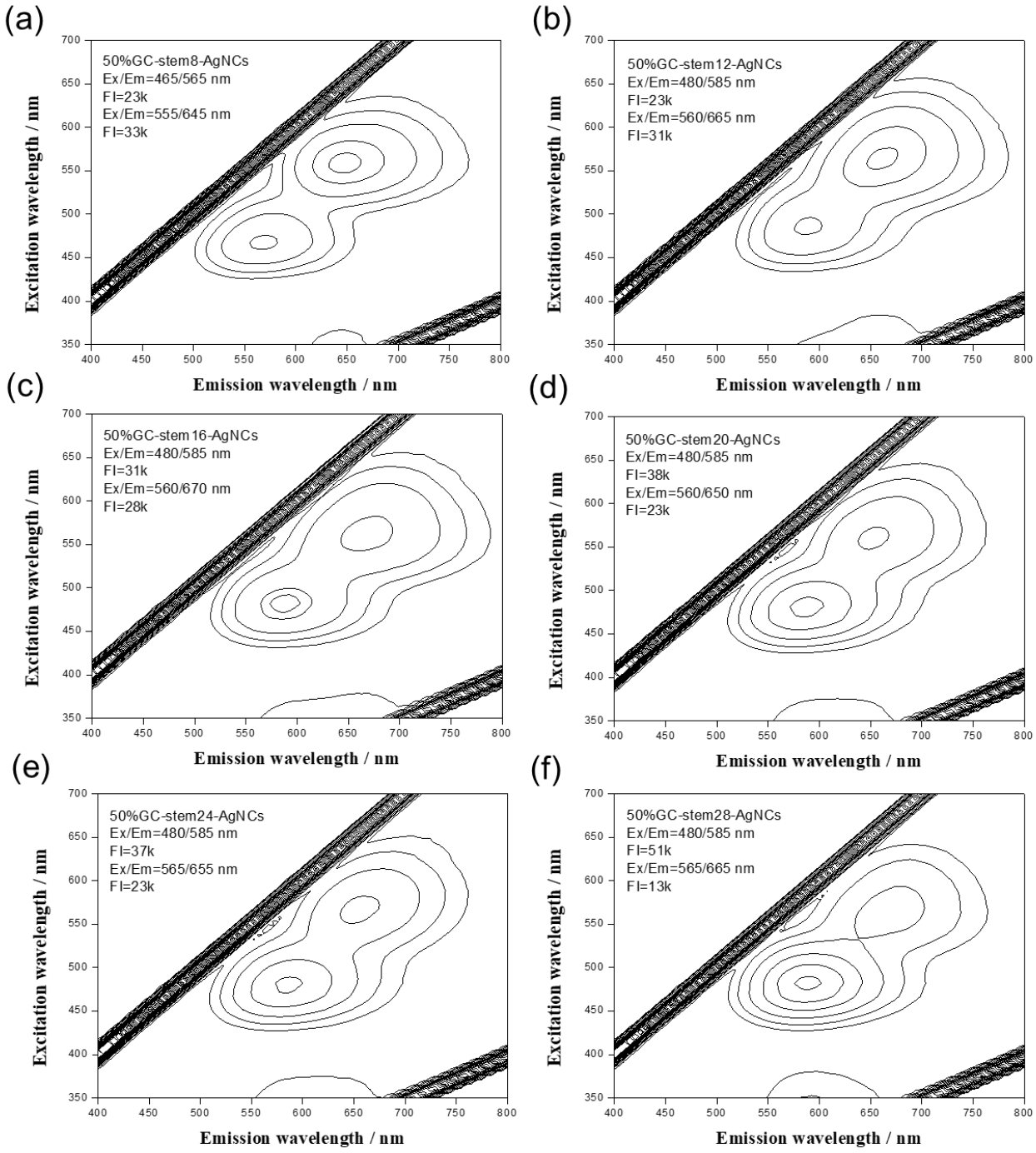

Fig. S29. The three-dimensional fluorescence spectra of the hairpin-AgNCs using stems of different length (with 50% content of GC base pairs in stem sequence).

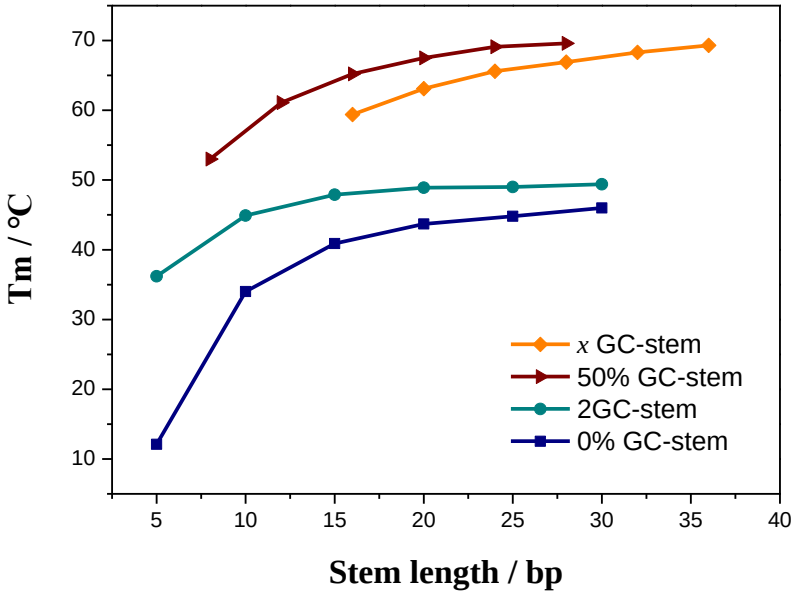

Fig. S30. The melting temperatures (Tm) of 0%GC-stemX(5-30), 2GC-stemX(5-30), 50%GC-stemX(8-28) and *x*GC-stemX(16-36) hairpins calculated by the OligoAnalyzer.

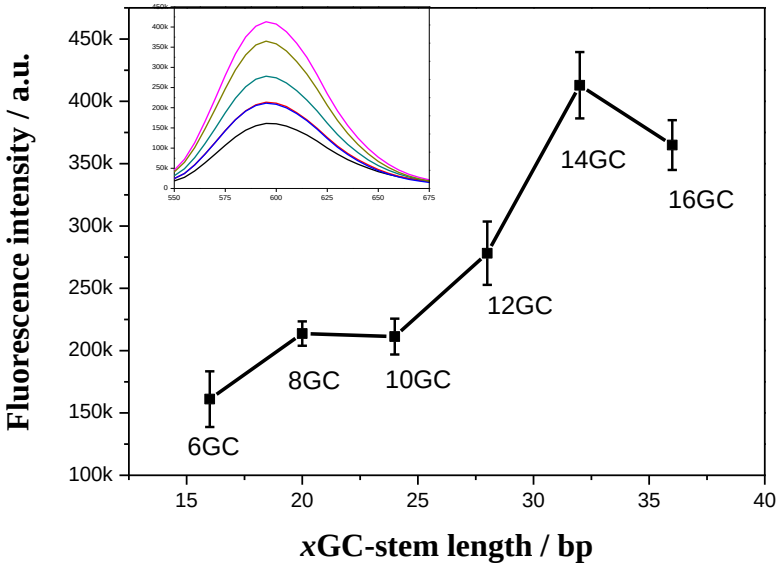

Fig. S31. The fluorescence intensities of the hairpin-AgNCs using stems of different lengths (with increasing GC base pairs in stem sequence).

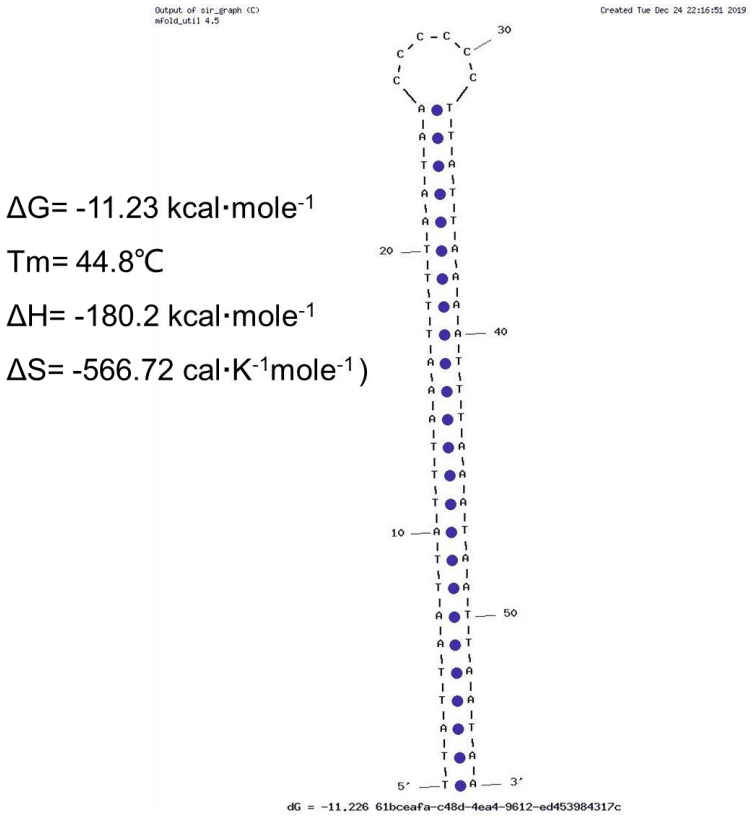

Fig. S32. The predicted structure of 0%GC-stem25 analyzed by the OligoAnalyzer.

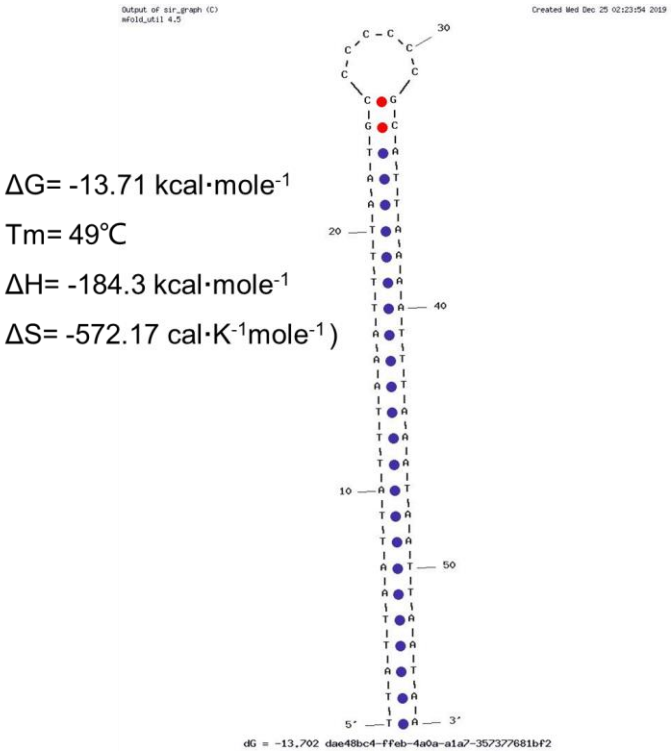

Fig. S33. The predicted structure of 2GC-stem25 analyzed by the OligoAnalyzer.

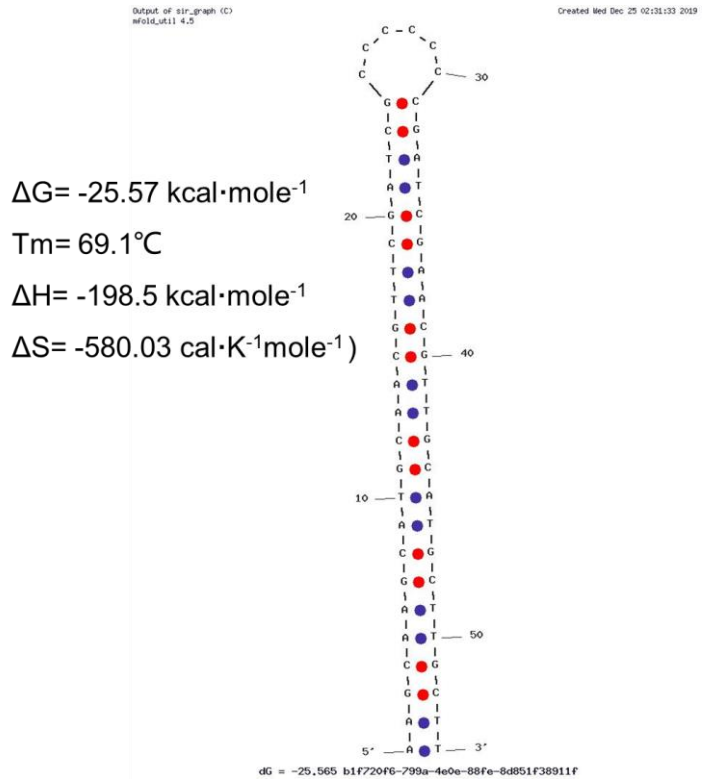

Fig. S34. The predicted structure of 50%GC-stem24 analyzed by the OligoAnalyzer.

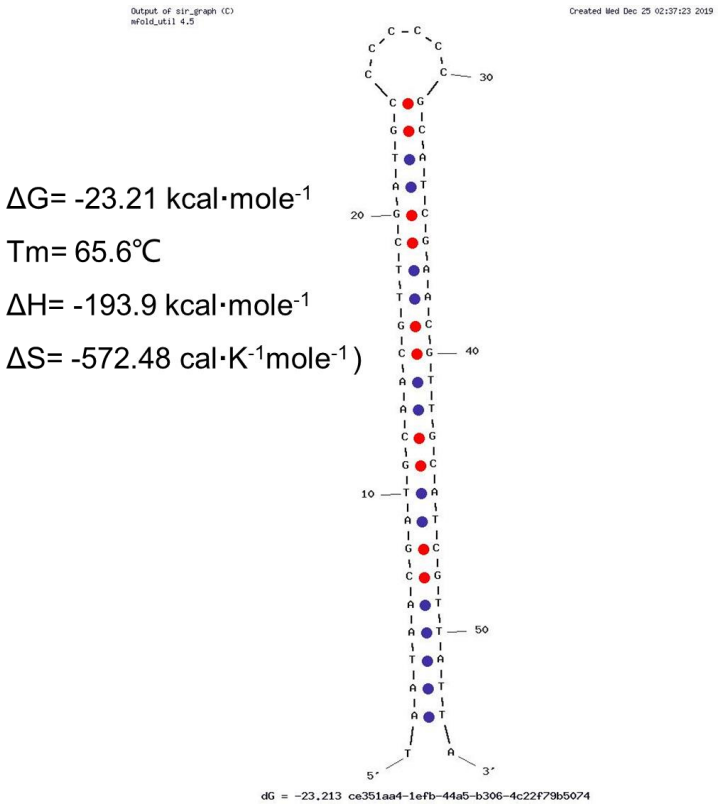

Fig. S35. The predicted structure of 10GC-stem24 analyzed by the OligoAnalyzer.