FIG. 1

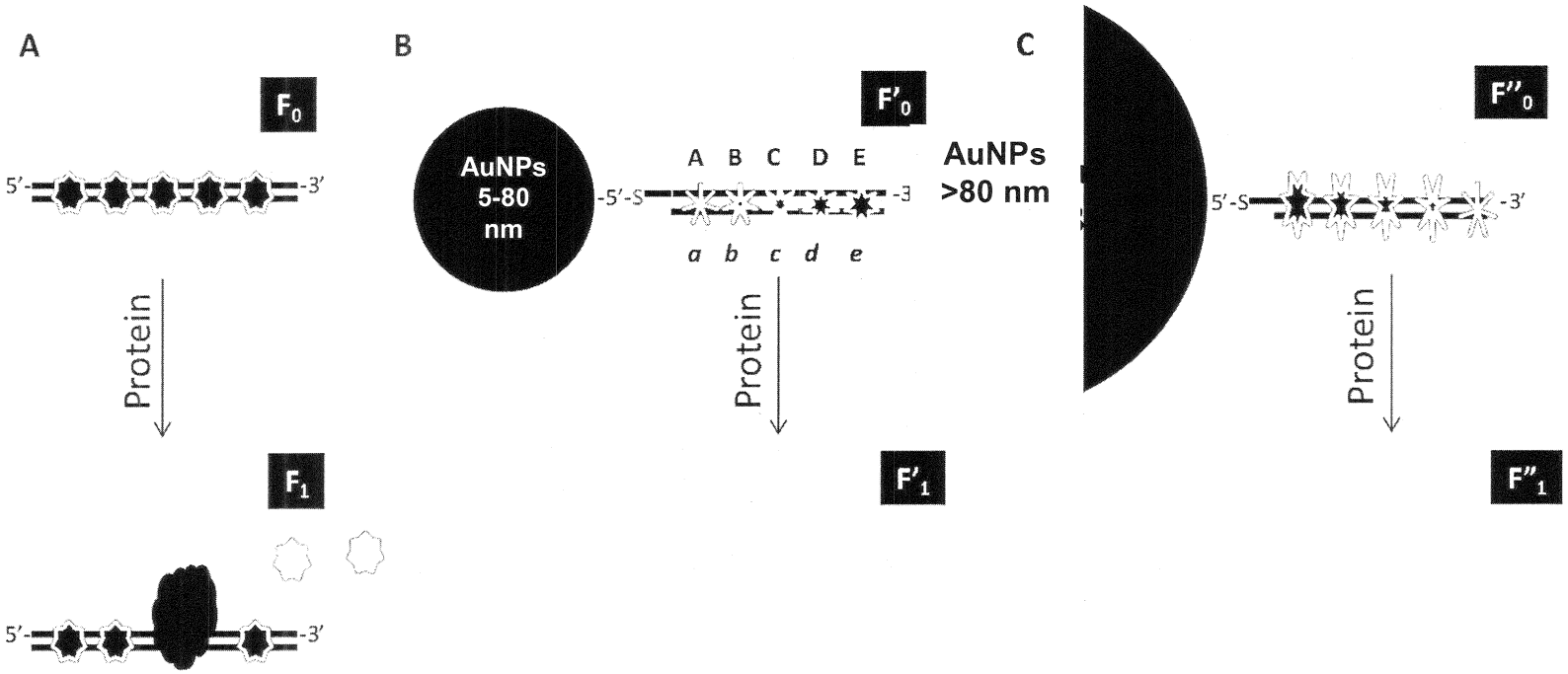

A
B
C
F0
F'0
F''0
AuNPs
5-80
nm
A B C D E
a b c d e
AuNPs
>80 nm
5'-S
-3'
Protein
F1
F'1
F''1

FIG. 2

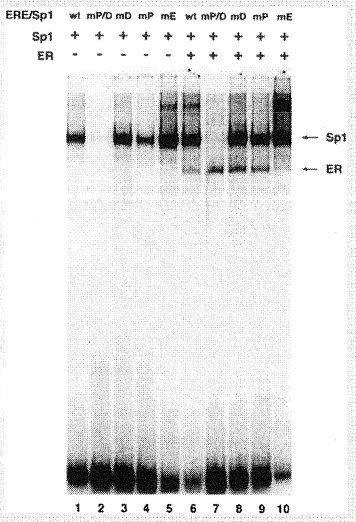
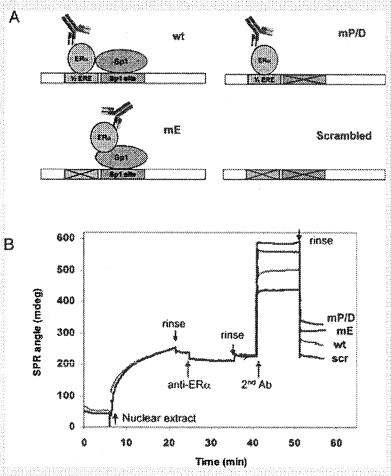
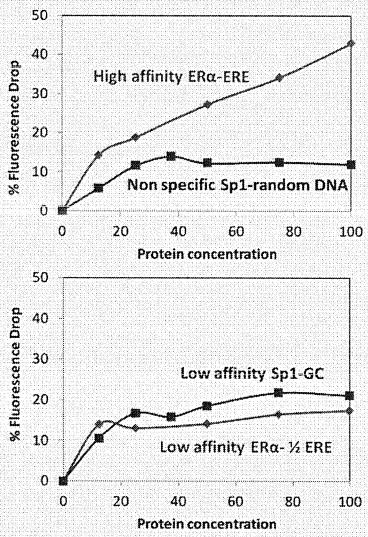
EMSA (Petz et al., Mol. Endo. 14: 972–985, 2000)	SPR (neo et al., *Anal. Chem. 2009, 81, 3344–3349*)	FID (unpublished work in my group)
		
Drawbacks: •Require full set of selective mutation sequences	Drawbacks: •Require full set of selective mutation sequences •Subject to the availability of antibodies	Drawbacks: •Require full set of selective mutation sequences •Insensitive in differentiation of subtle affinity difference

FIG. 3

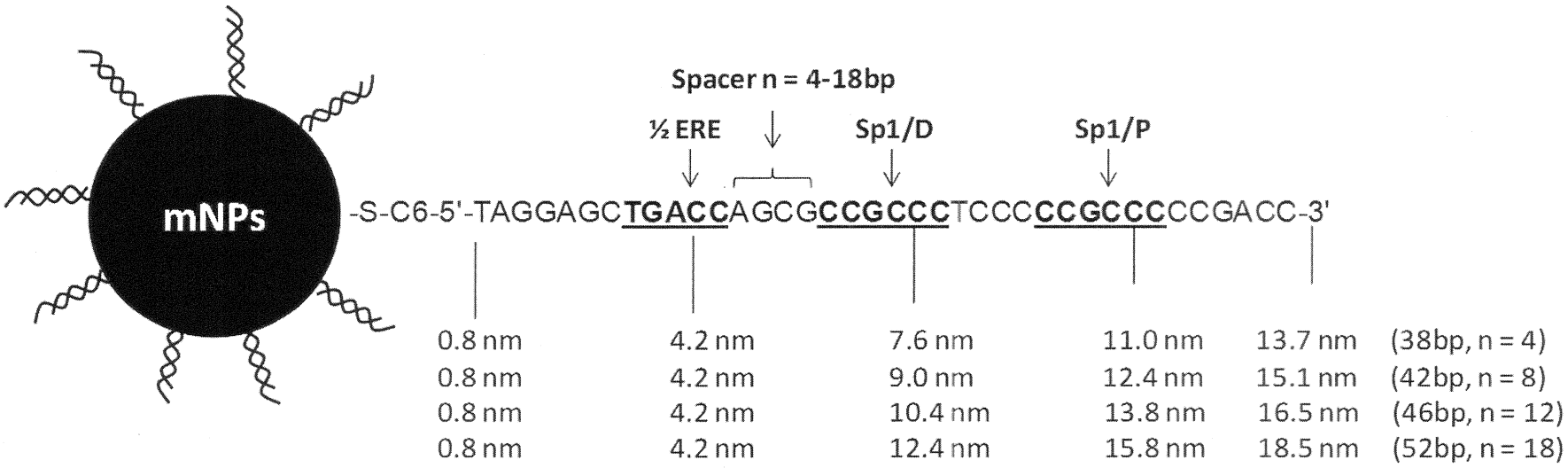

mNPs
Spacer n = 4-18bp
½ ERE
Sp1/D
Sp1/P
-S-C6-5'-TAGGAGCTGACCAGCGCCGCCCTCCCCCGCCCCCGACC-3'
0.8 nm 4.2 nm 7.6 nm 11.0 nm 13.7 nm (38bp, n = 4)
0.8 nm 4.2 nm 9.0 nm 12.4 nm 15.1 nm (42bp, n = 8)
0.8 nm 4.2 nm 10.4 nm 13.8 nm 16.5 nm (46bp, n = 12)
0.8 nm 4.2 nm 12.4 nm 15.8 nm 18.5 nm (52bp, n = 18)

FIG. 4

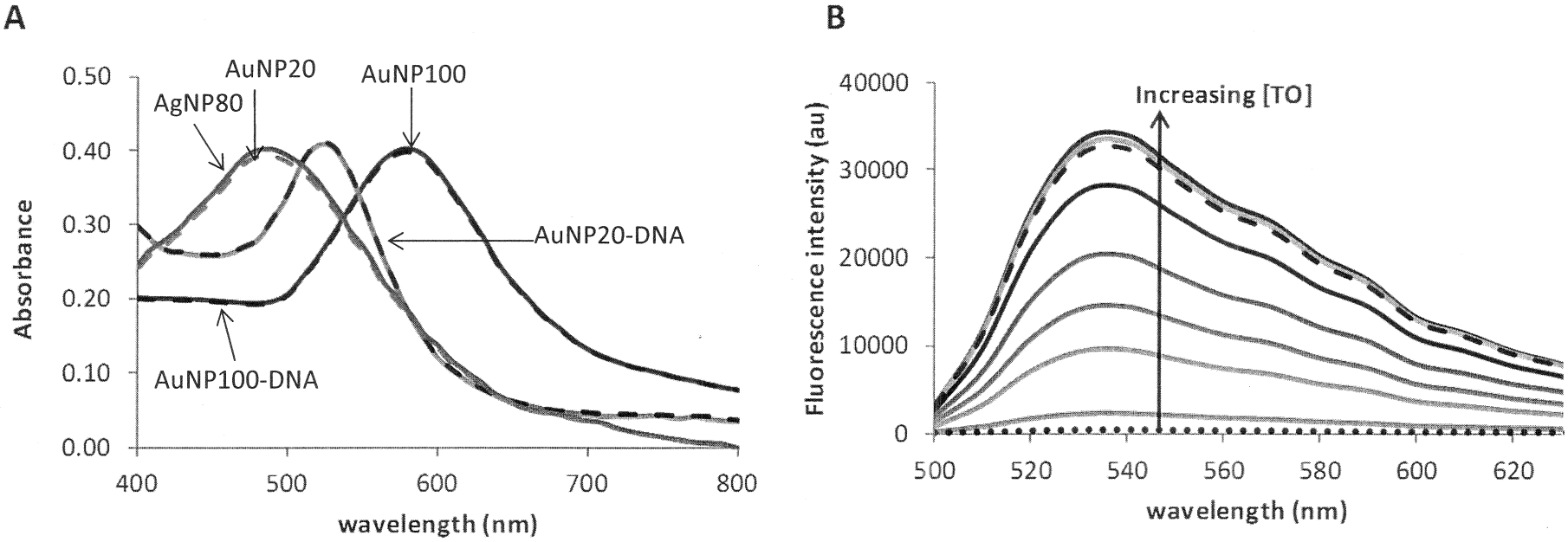

A
AuNP20
AuNP100
AgNP80
AuNP20-DNA
AuNP100-DNA
Absorbance
0.50
0.40
0.30
0.20
0.10
0.00
400
500
600
700
800
wavelength (nm)
B
Increasing [TO]
Fluorescence intensity (au)
40000
30000
20000
10000
0
500
520
540
560
580
600
620
wavelength (nm)

FIG. 5

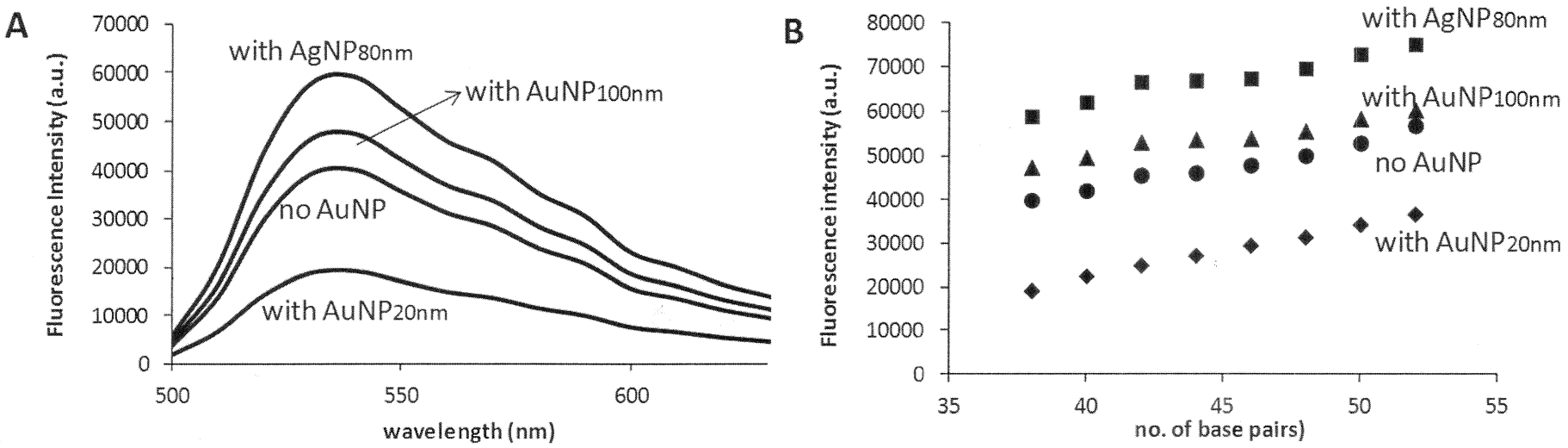

A
Fluorescence Intensity (a.u.)
70000
60000
50000
40000
30000
20000
10000
0
500
550
600
wavelength (nm)
with AgNP80nm
with AuNP100nm
no AuNP
with AuNP20nm
B
Fluorescence intensity (a.u.)
80000
70000
60000
50000
40000
30000
20000
10000
0
35
40
45
50
55
no. of base pairs)
with AgNP80nm
with AuNP100nm
no AuNP
with AuNP20nm

FIG. 6

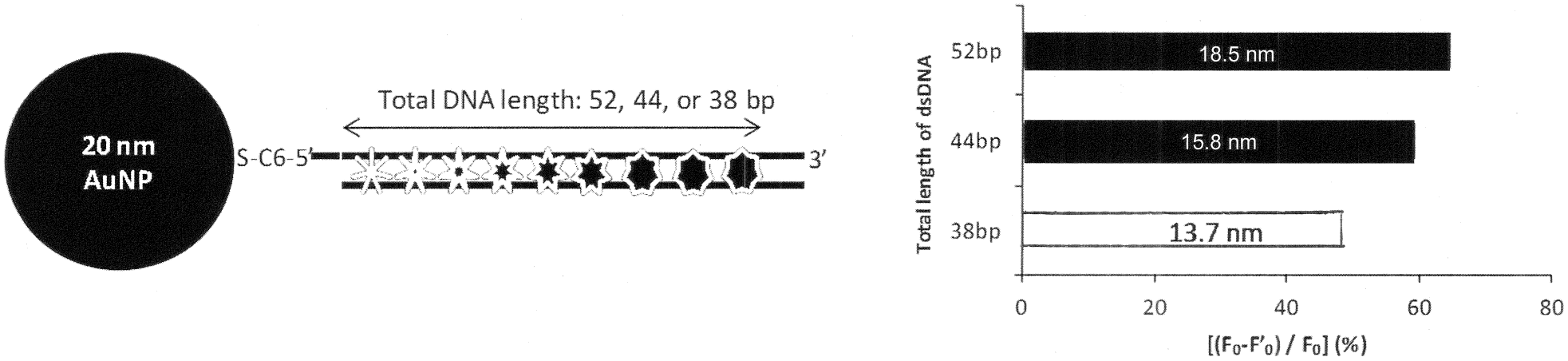

20 nm
AuNP
S-C6-5'
Total DNA length: 52, 44, or 38 bp
3'
Total length of dsDNA
52bp
44bp
38bp
18.5 nm
15.8 nm
13.7 nm
0
20
40
60
80
[(F0-F'0) / F0] (%)

FIG. 7

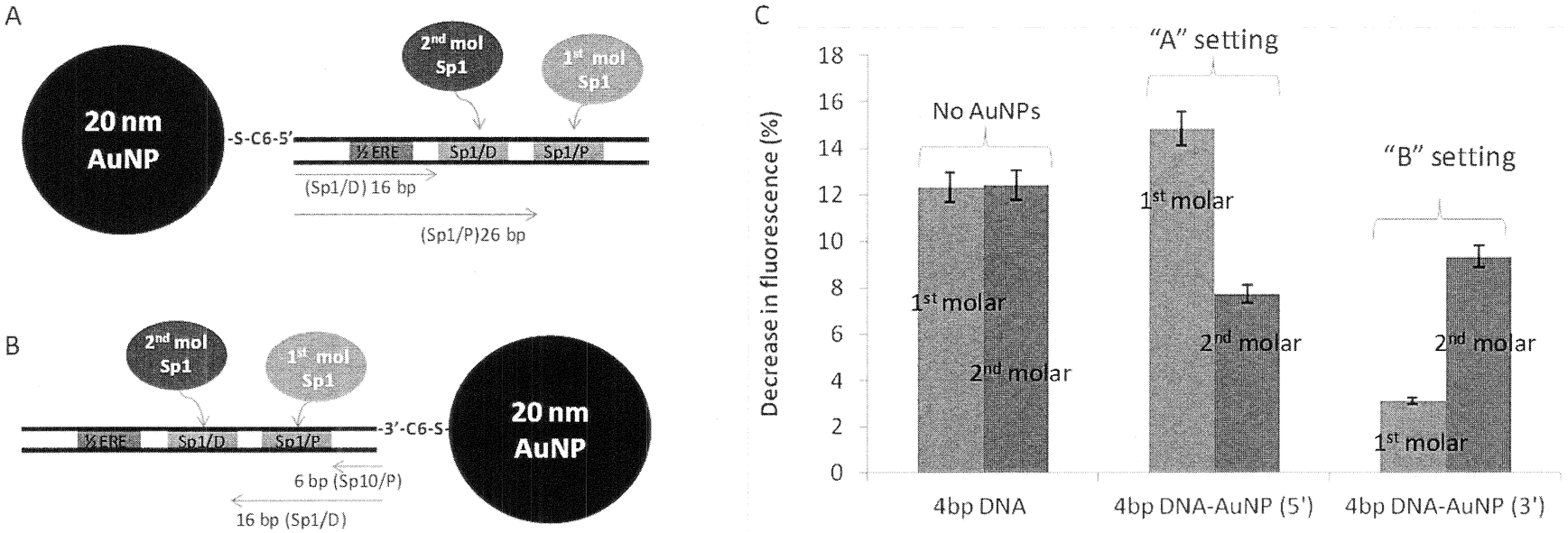

A
20 nm AuNP
-S-C6-5'
2nd mol Sp1
1st mol Sp1
½ ERE
Sp1/D
Sp1/P
(Sp1/D) 16 bp
(Sp1/P)26 bp
B
2nd mol Sp1
1st mol Sp1
-3'-C6-S-
20 nm AuNP
½ ERE
Sp1/D
Sp1/P
6 bp (Sp10/P)
16 bp (Sp1/D)
C
"A" setting
"B" setting
No AuNPs
1st molar
2nd molar
Decrease in fluorescence (%)
0
2
4
6
8
10
12
14
16
18
4bp DNA
4bp DNA-AuNP (5')
4bp DNA-AuNP (3')

FIG. 8

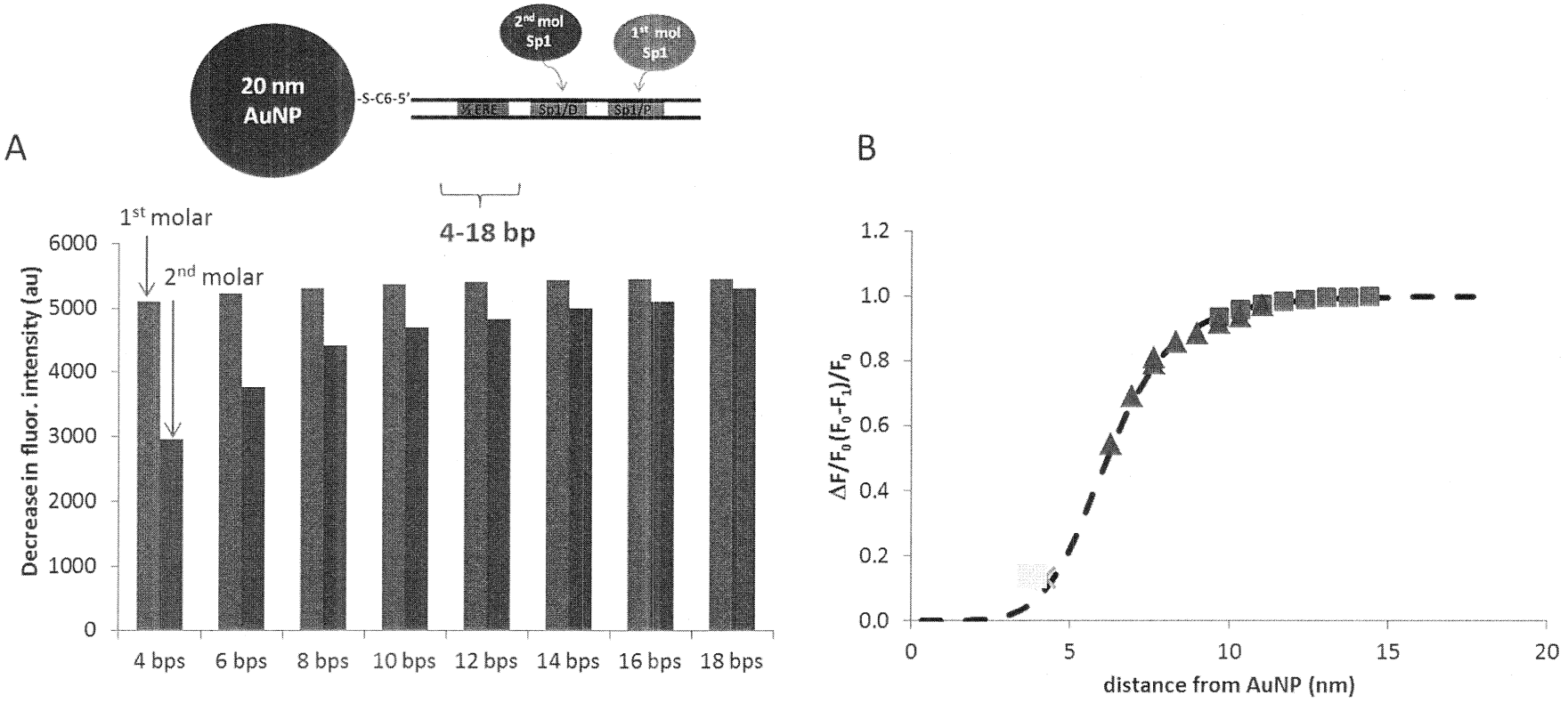

A
20 nm AuNP
-S-C6-5'
2nd mol Sp1
1st mol Sp1
½ERE
Sp1/D
Sp1/P
4-18 bp
1st molar
2nd molar
Decrease in fluor. intensity (au)
6000
5000
4000
3000
2000
1000
0
4 bps
6 bps
8 bps
10 bps
12 bps
14 bps
16 bps
18 bps
B
ΔF/F0 (F0-F1)/F0
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
distance from AuNP (nm)

FIG. 9

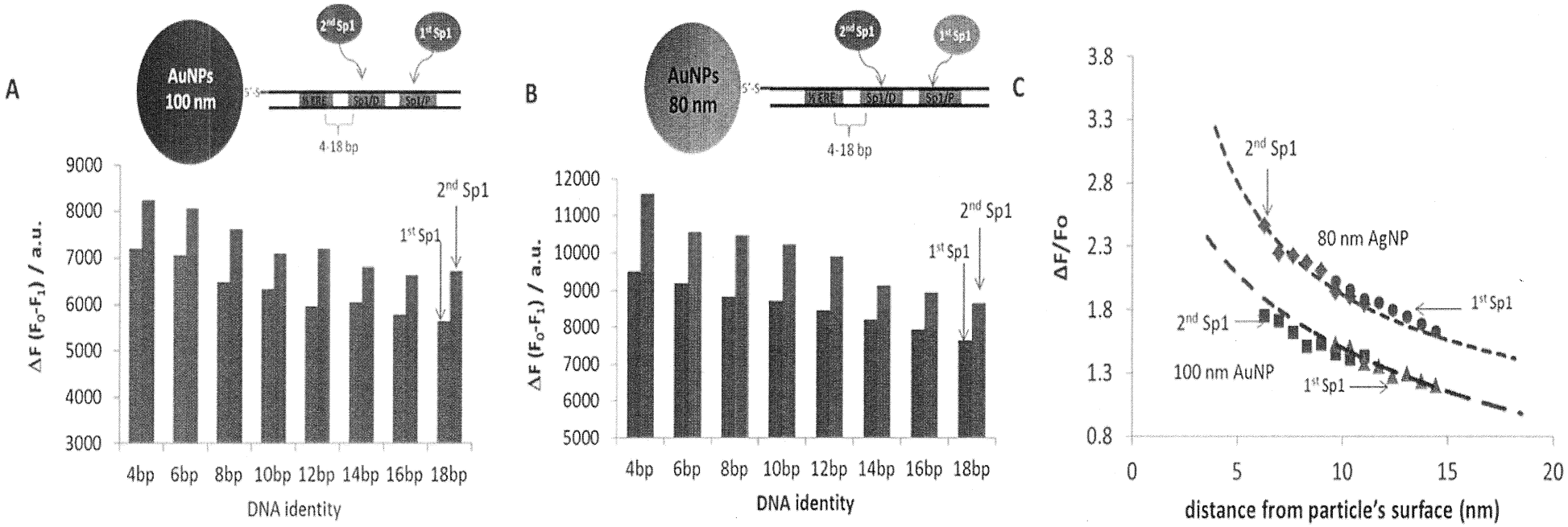

A
AuNPs
100 nm
2nd Sp1
1st Sp1
5'-S
½ ERE
Sp1/D
Sp1/P
4-18 bp
ΔF (F0-F1) / a.u.
9000
8000
7000
6000
5000
4000
3000
2nd Sp1
1st Sp1
4bp 6bp 8bp 10bp 12bp 14bp 16bp 18bp
DNA identity
B
AuNPs
80 nm
2nd Sp1
1st Sp1
5'-S
½ ERE
Sp1/D
Sp1/P
4-18 bp
ΔF (F0-F1) / a.u.
12000
11000
10000
9000
8000
7000
6000
5000
2nd Sp1
1st Sp1
4bp 6bp 8bp 10bp 12bp 14bp 16bp 18bp
DNA identity
C
ΔF/Fo
3.8
3.3
2.8
2.3
1.8
1.3
0.8
2nd Sp1
80 nm AgNP
1st Sp1
2nd Sp1
100 nm AuNP
1st Sp1
0 5 10 15 20
distance from particle's surface (nm)

FIG. 10

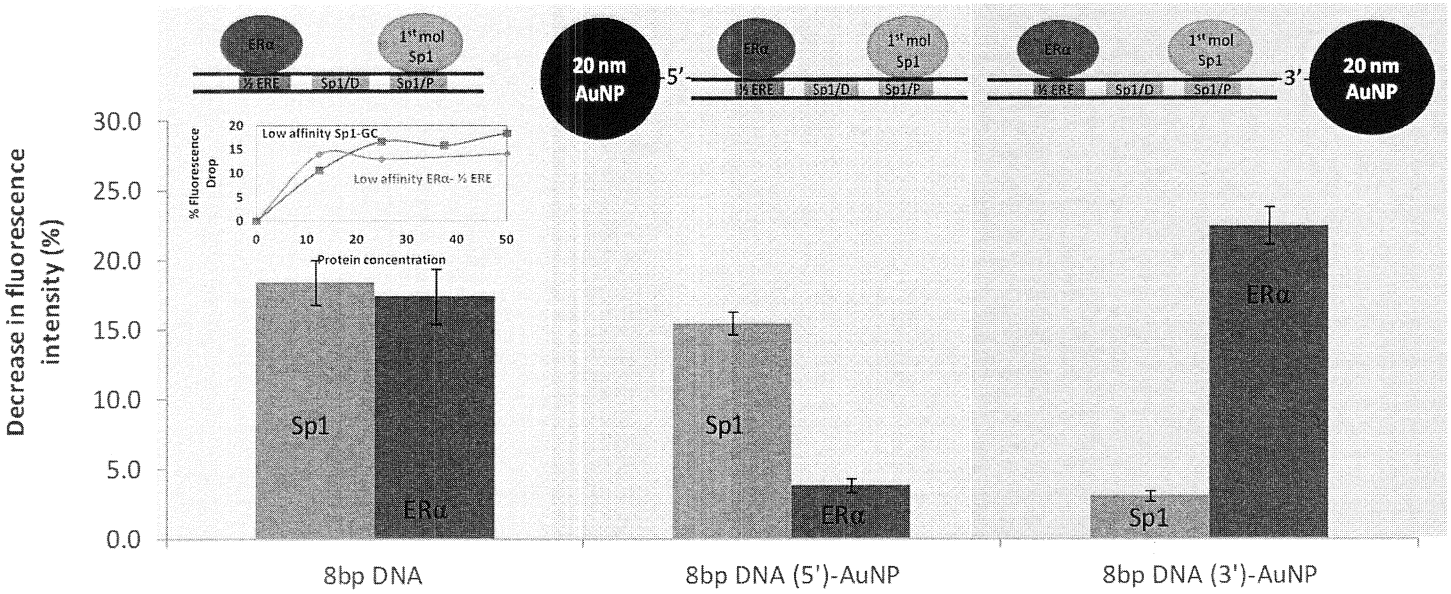

Decrease in fluorescence intensity (%)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
ERα
1st mol Sp1
½ ERE
Sp1/D
Sp1/P
% Fluorescence Drop
Low affinity Sp1-GC
Low affinity ERα- ½ ERE
Protein concentration
Sp1
ERα
20 nm AuNP
5′
3′
8bp DNA
8bp DNA (5')-AuNP
8bp DNA (3')-AuNP

FIG. 11

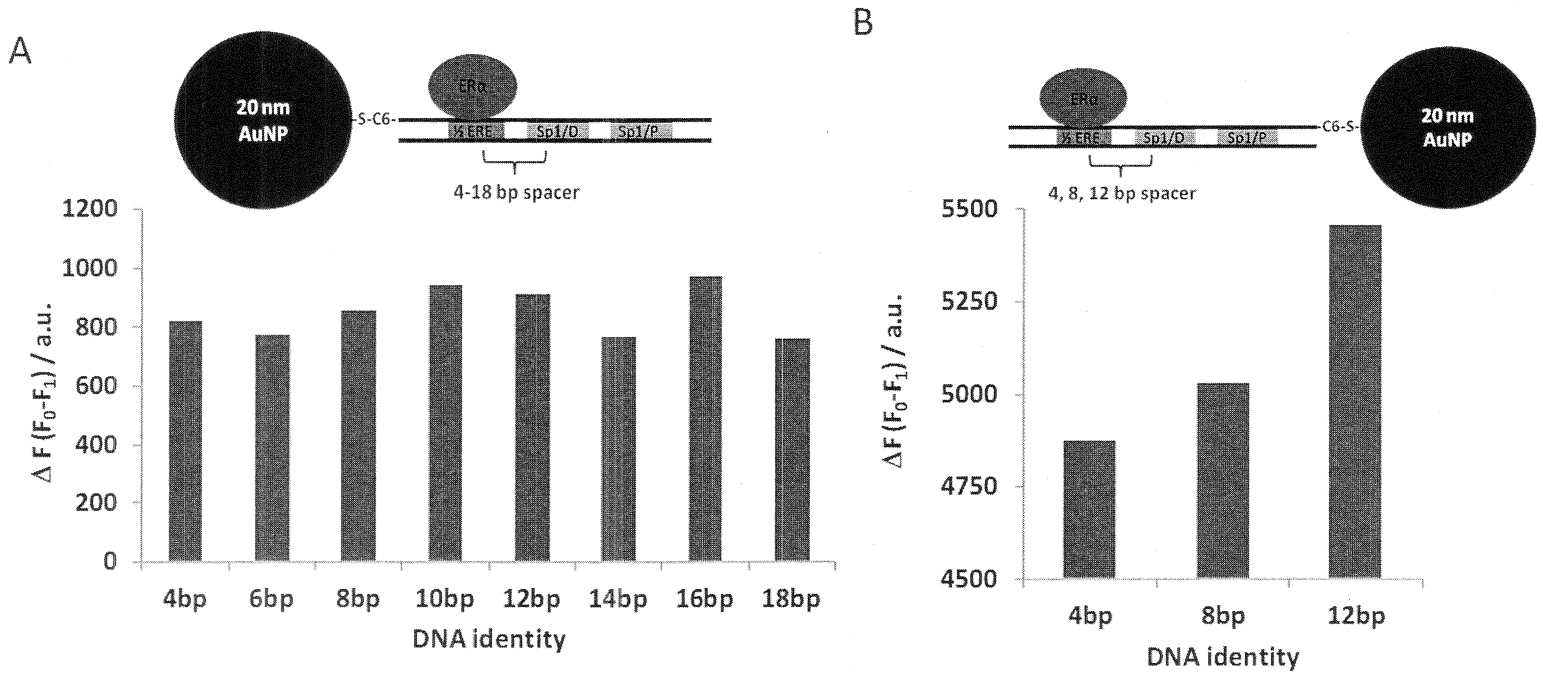

A
20 nm AuNP
S-C6-
ERα
½ ERE
Sp1/D
Sp1/P
4-18 bp spacer
ΔF (F0-F1) / a.u.
1200
1000
800
600
400
200
0
4bp
6bp
8bp
10bp
12bp
14bp
16bp
18bp
DNA identity
B
ERα
½ ERE
Sp1/D
Sp1/P
C6-S-
20 nm AuNP
4, 8, 12 bp spacer
ΔF (F0-F1) / a.u.
5500
5250
5000
4750
4500
4bp
8bp
12bp
DNA identity

FIG. 12

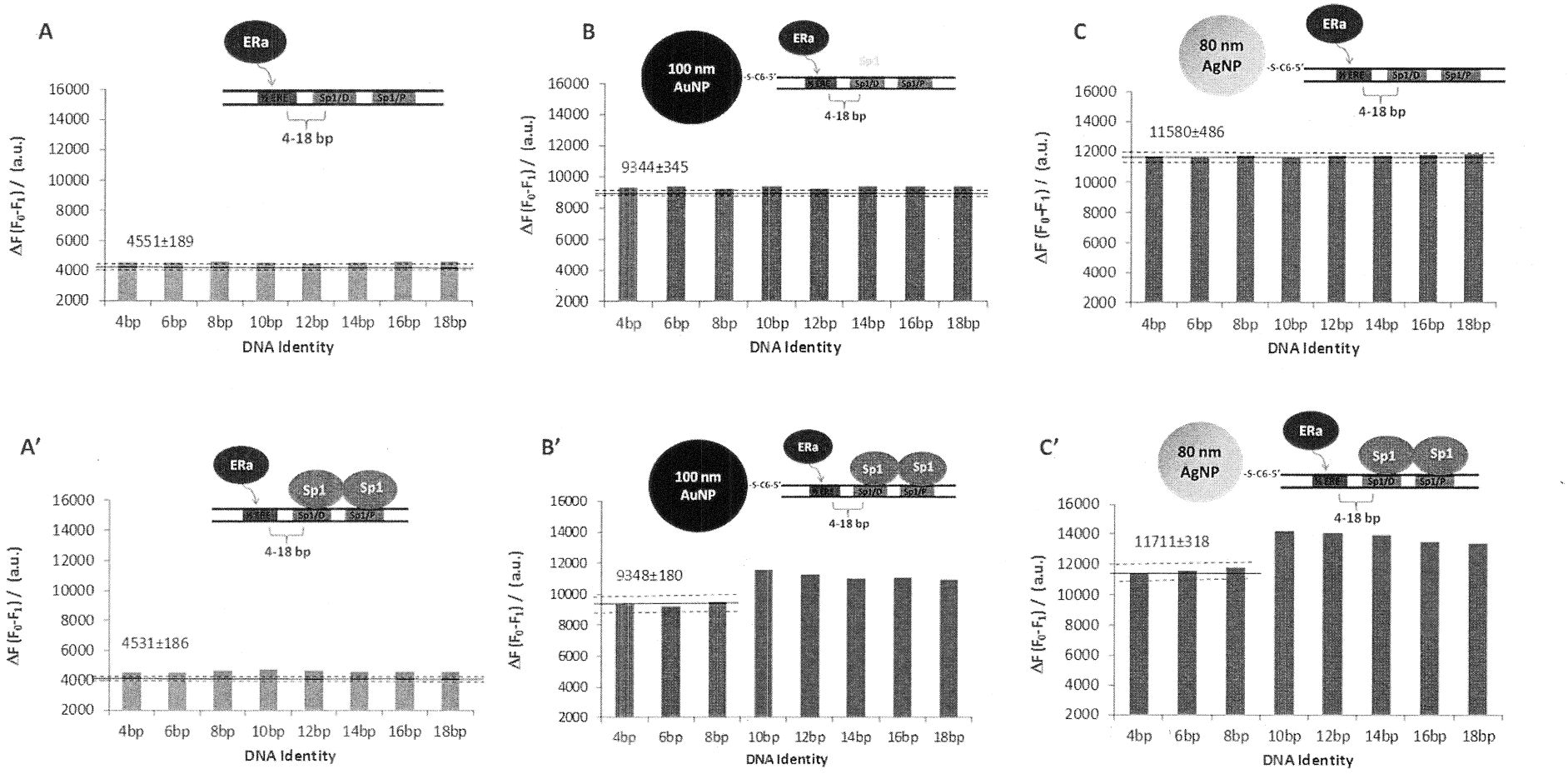

A
ERa
4-18 bp
4551±189
ΔF (F0-F1) / (a.u.)
DNA Identity
4bp 6bp 8bp 10bp 12bp 14bp 16bp 18bp
B
100 nm AuNP
ERa
Sp1
4-18 bp
9344±345
C
80 nm AgNP
ERa
4-18 bp
11580±486
A'
ERa
Sp1
Sp1
4-18 bp
4531±186
B'
100 nm AuNP
ERa
Sp1
Sp1
4-18 bp
9348±180
C'
80 nm AgNP
ERa
Sp1
Sp1
4-18 bp
11711±318

FIG. 1

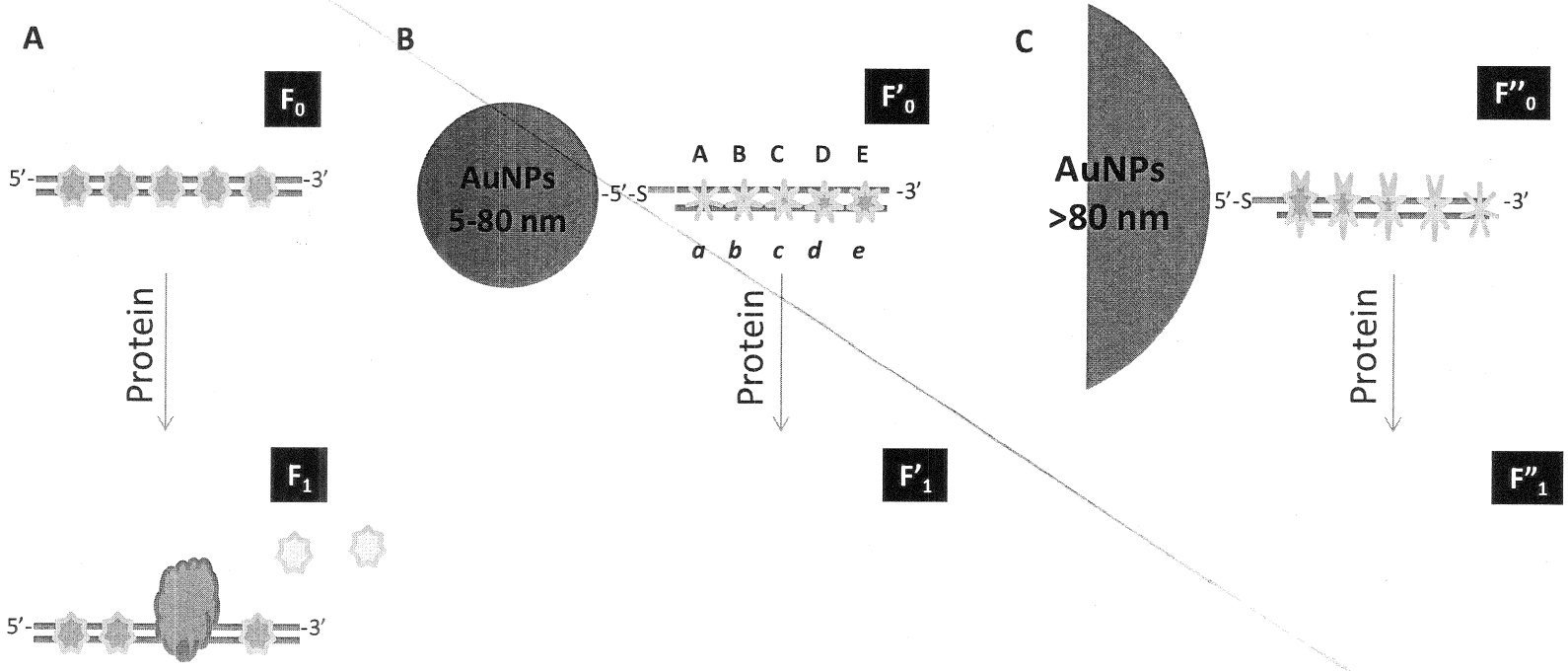
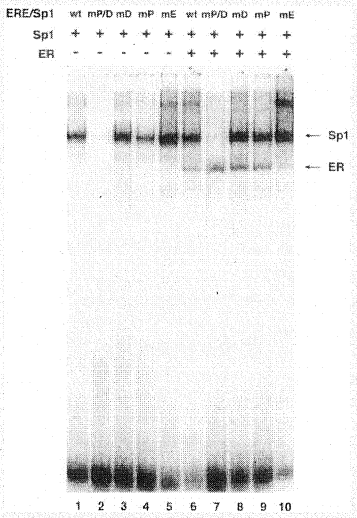
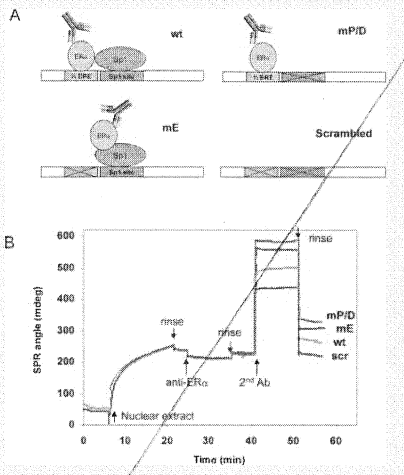

A
B
C
F0
F'0
F''0
5'-
-3'
AuNPs
5-80 nm
A B C D E
-5'-S
a b c d e
AuNPs
>80 nm
5'-S
Protein
Protein
Protein
F1
F'1
F''1

FIG. 2

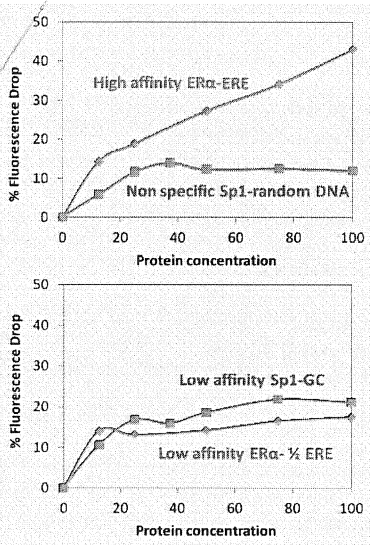
EMSA (Petz et al., Mol. Endo. 14: 972–985, 2000)	SPR (neo et al., *Anal. Chem. 2009, 81, 3344–3349*)	FID (unpublished work in my group)



Drawbacks:
•Require full set of selective mutation sequences



Drawbacks:
•Require full set of selective mutation sequences
•Subject to the availability of antibodies



Drawbacks:
•Require full set of selective mutation sequences
•Insensitive in differentiation of subtle affinity difference

FIG. 3

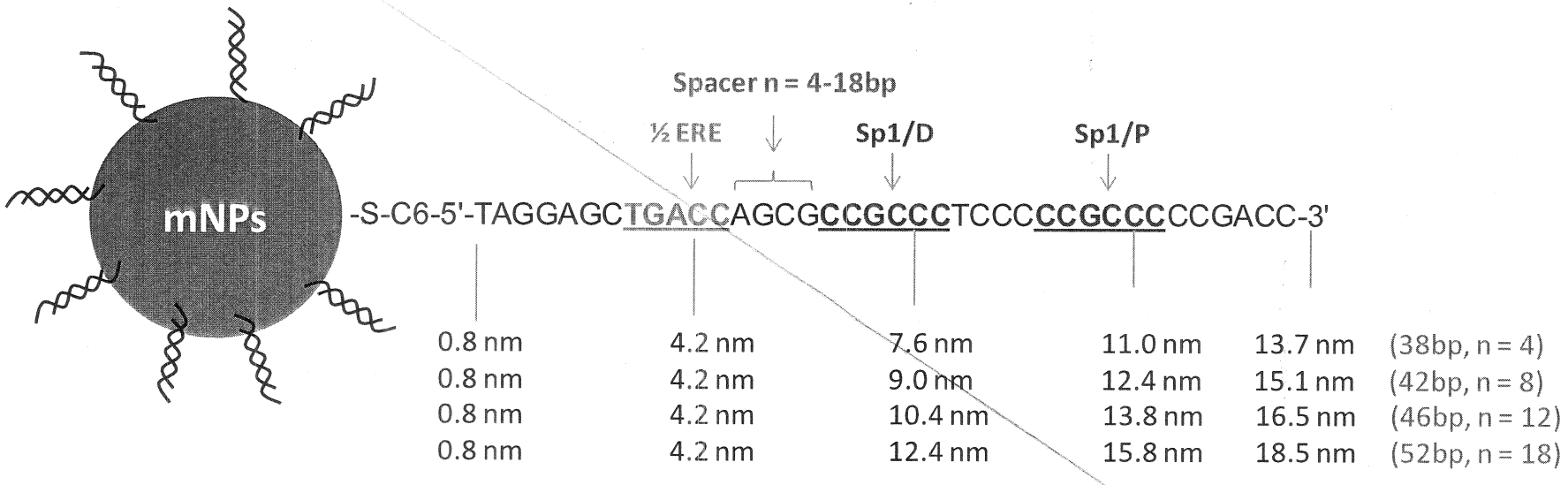

Spacer n = 4-18bp
½ ERE
Sp1/D
Sp1/P
mNPs
-S-C6-5'-TAGGAGCTGACCAGCGCCGCCCTCCCCCGCCCCCGACC-3'
0.8 nm 4.2 nm 7.6 nm 11.0 nm 13.7 nm (38bp, n = 4)
0.8 nm 4.2 nm 9.0 nm 12.4 nm 15.1 nm (42bp, n = 8)
0.8 nm 4.2 nm 10.4 nm 13.8 nm 16.5 nm (46bp, n = 12)
0.8 nm 4.2 nm 12.4 nm 15.8 nm 18.5 nm (52bp, n = 18)

FIG. 4

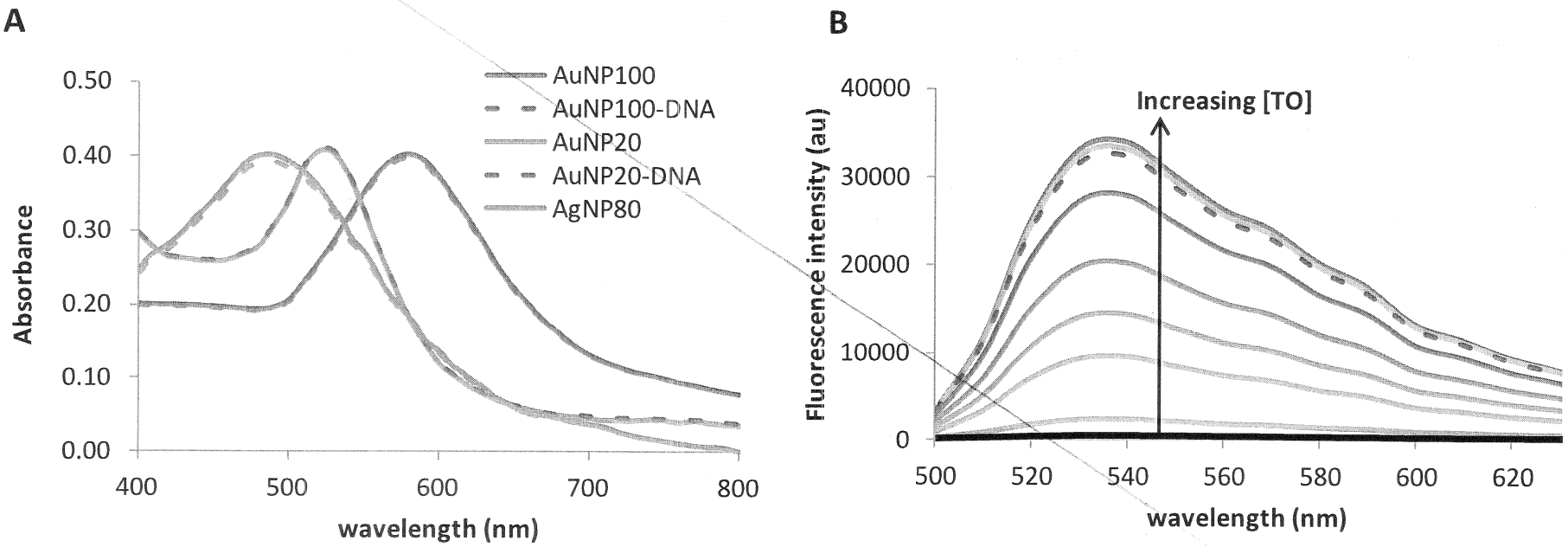

A
0.50
0.40
0.30
0.20
0.10
0.00
Absorbance
400
500
600
700
800
wavelength (nm)
AuNP100
AuNP100-DNA
AuNP20
AuNP20-DNA
AgNP80
B
40000
30000
20000
10000
0
Fluorescence intensity (au)
Increasing [TO]
500
520
540
560
580
600
620
wavelength (nm)

FIG. 5

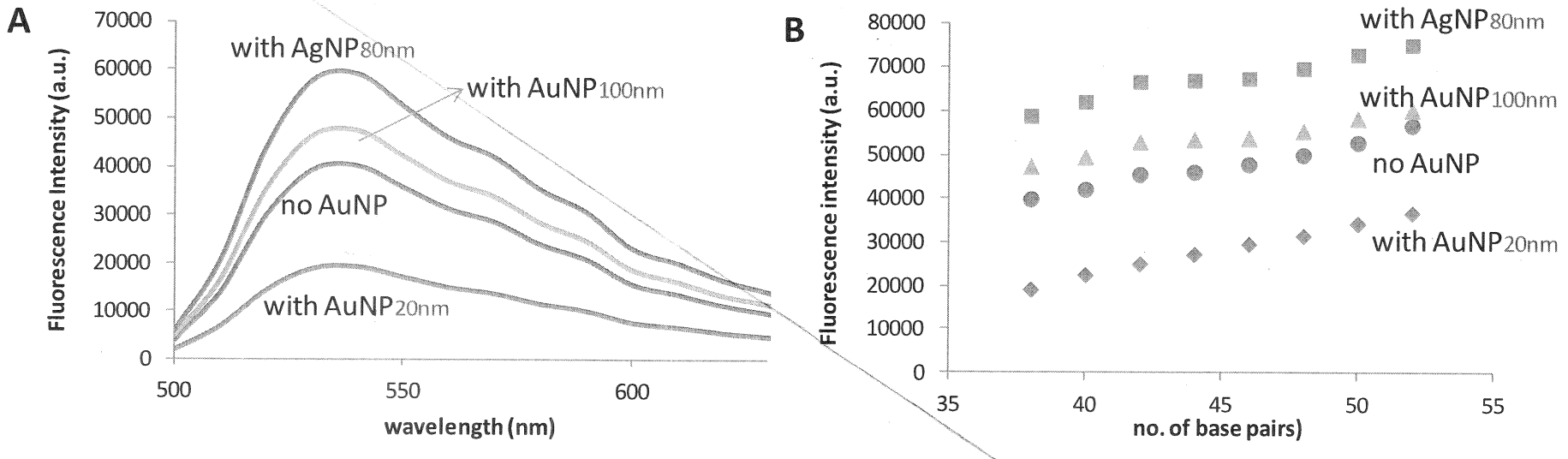

A
Fluorescence Intensity (a.u.)
70000
60000
50000
40000
30000
20000
10000
0
500
550
600
wavelength (nm)
with AgNP80nm
with AuNP100nm
no AuNP
with AuNP20nm
B
Fluorescence intensity (a.u.)
80000
70000
60000
50000
40000
30000
20000
10000
0
35
40
45
50
55
no. of base pairs)
with AgNP80nm
with AuNP100nm
no AuNP
with AuNP20nm

FIG. 6

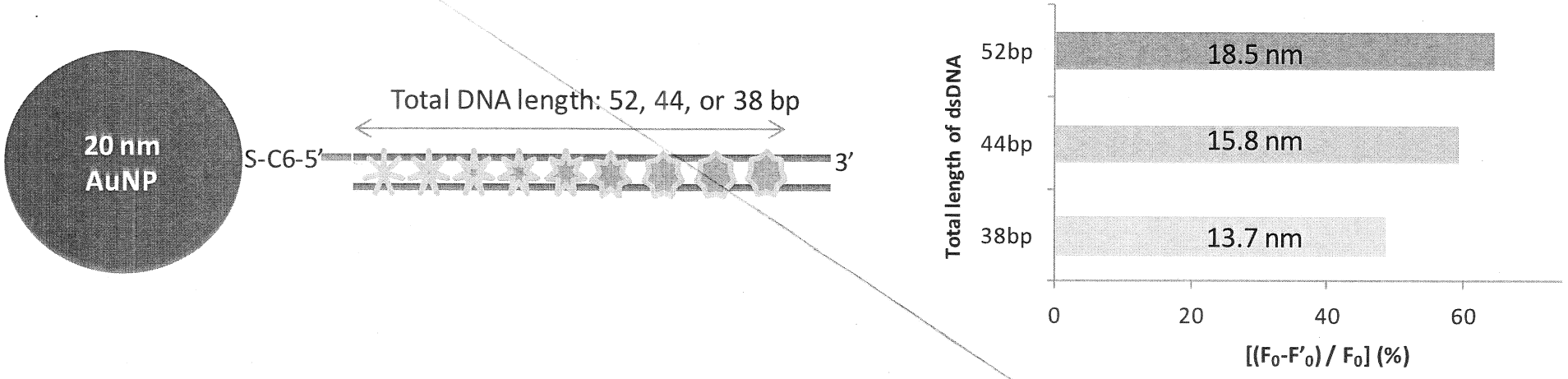

20 nm
AuNP
S-C6-5'
3'
Total DNA length: 52, 44, or 38 bp
Total length of dsDNA
52bp
44bp
38bp
18.5 nm
15.8 nm
13.7 nm
0
20
40
60
[(F0-F'0) / F0] (%)

FIG. 7

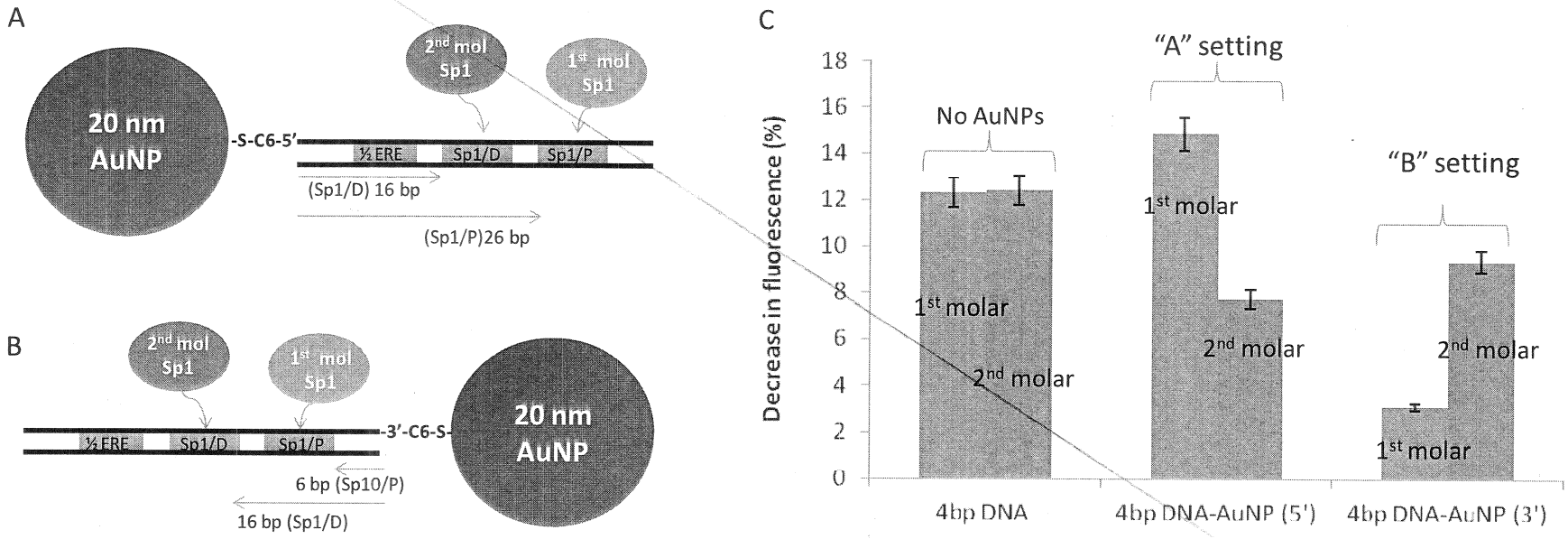

A
20 nm AuNP
-S-C6-5'
2nd mol Sp1
1st mol Sp1
½ ERE
Sp1/D
Sp1/P
(Sp1/D) 16 bp
(Sp1/P)26 bp
B
2nd mol Sp1
1st mol Sp1
½ ERE
Sp1/D
Sp1/P
-3'-C6-S-
20 nm AuNP
6 bp (Sp10/P)
16 bp (Sp1/D)
C
Decrease in fluorescence (%)
0
2
4
6
8
10
12
14
16
18
No AuNPs
"A" setting
"B" setting
1st molar
2nd molar
4bp DNA
4bp DNA-AuNP (5')
4bp DNA-AuNP (3')

FIG. 8

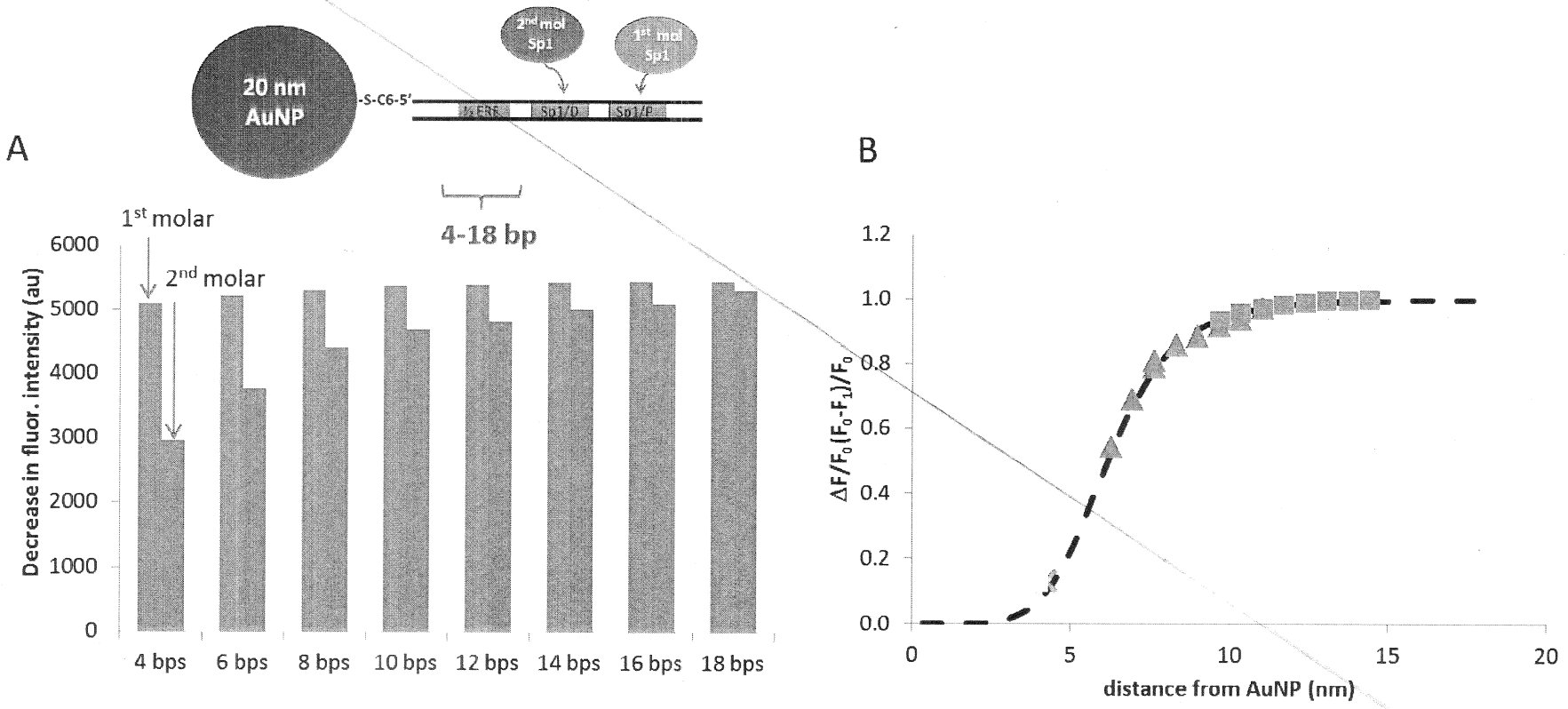

A
20 nm AuNP
-S-C6-5'
2nd mol Sp1
1st mol Sp1
4-18 bp
1st molar
2nd molar
Decrease in fluor. intensity (au)
6000
5000
4000
3000
2000
1000
0
4 bps
6 bps
8 bps
10 bps
12 bps
14 bps
16 bps
18 bps
B
ΔF/F0 (F0-F1)/F0
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
distance from AuNP (nm)

FIG. 9

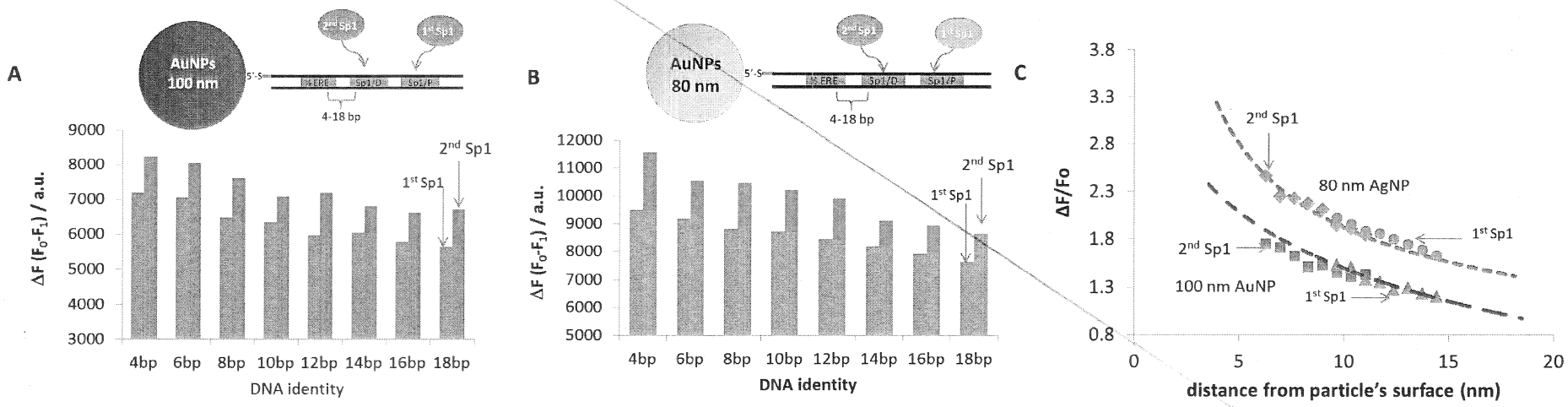

A
AuNPs
100 nm
5'-S-
2nd Sp1
1st Sp1
ERE
Sp1/D
Sp1/P
4-18 bp
ΔF (F0-F1) / a.u.
9000
8000
7000
6000
5000
4000
3000
4bp 6bp 8bp 10bp 12bp 14bp 16bp 18bp
DNA identity
B
AuNPs
80 nm
12000
11000
10000
9000
8000
7000
6000
5000
C
ΔF/Fo
3.8
3.3
2.8
2.3
1.8
1.3
0.8
80 nm AgNP
100 nm AuNP
0 5 10 15 20
distance from particle's surface (nm)

FIG. 10

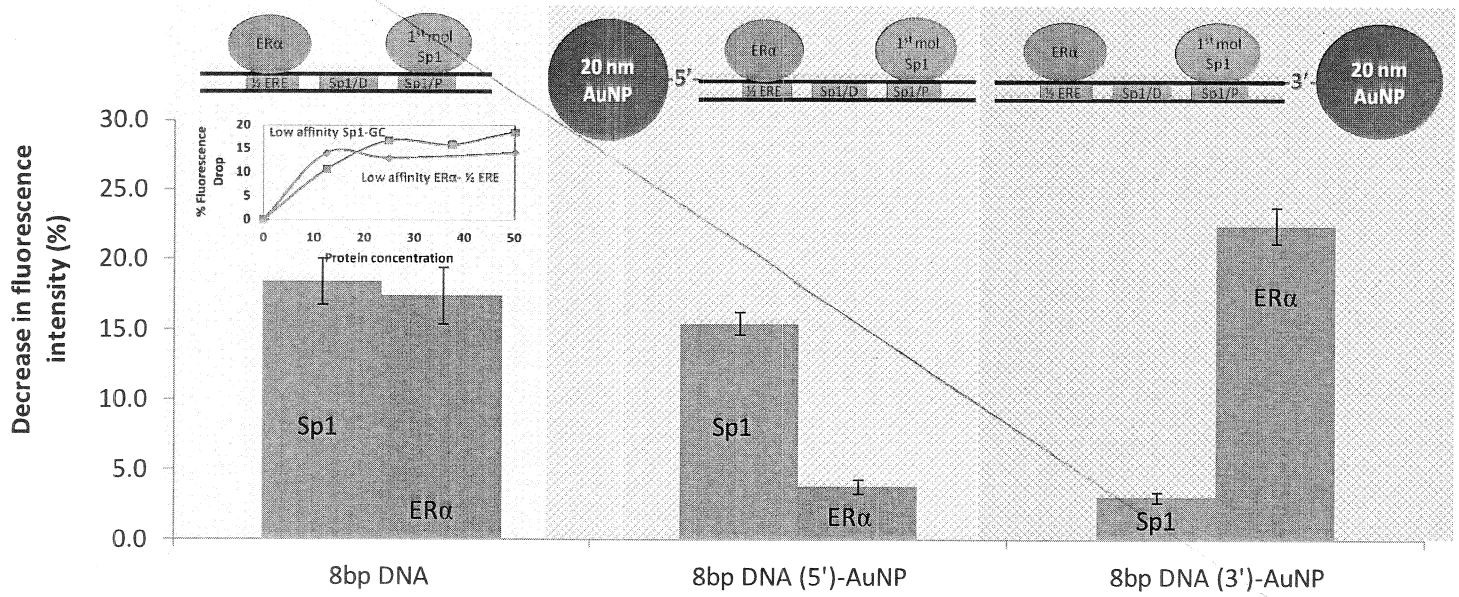

Decrease in fluorescence intensity (%)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
ERα
1st mol Sp1
½ ERE
Sp1/D
Sp1/P
% Fluorescence Drop
Low affinity Sp1-GC
Low affinity ERα- ½ ERE
Protein concentration
Sp1
ERα
20 nm AuNP
5′
3′
8bp DNA
8bp DNA (5')-AuNP
8bp DNA (3')-AuNP

FIG. 11

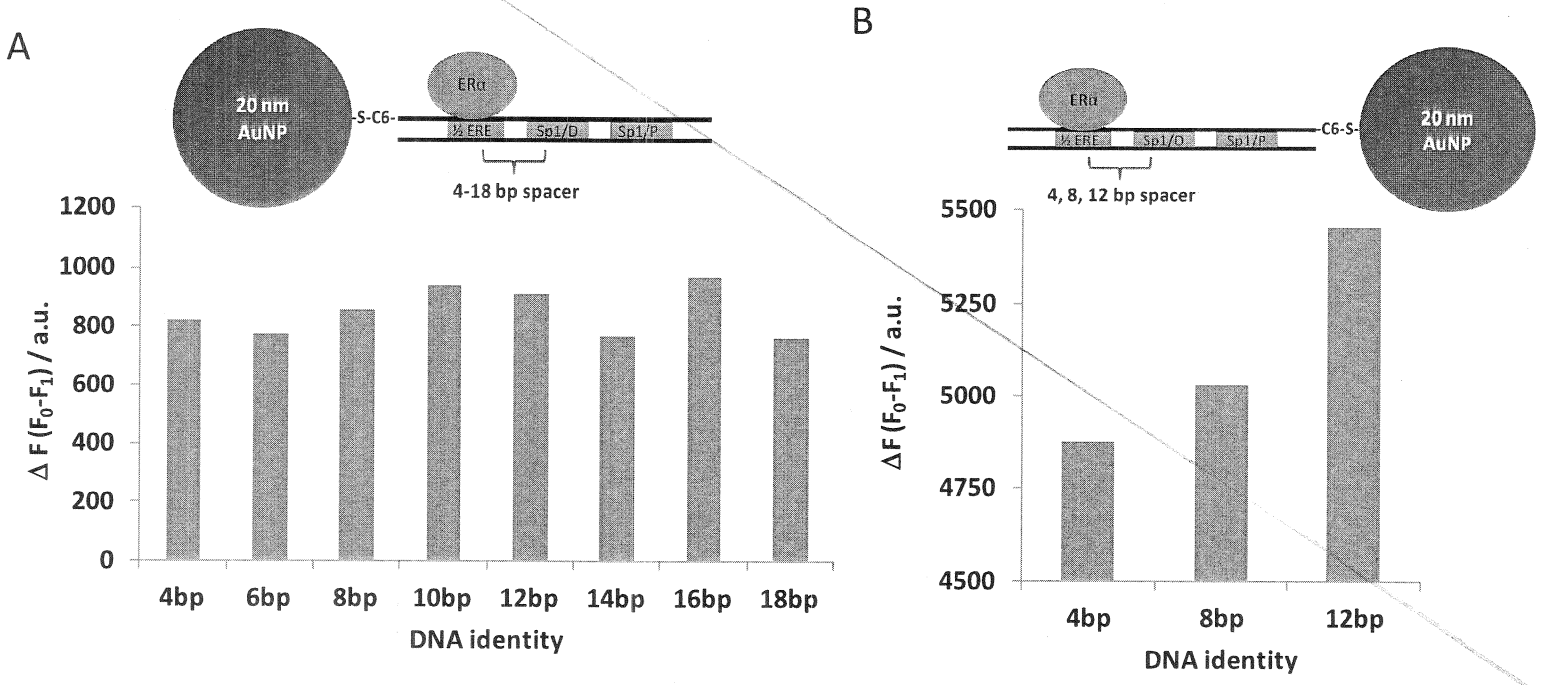
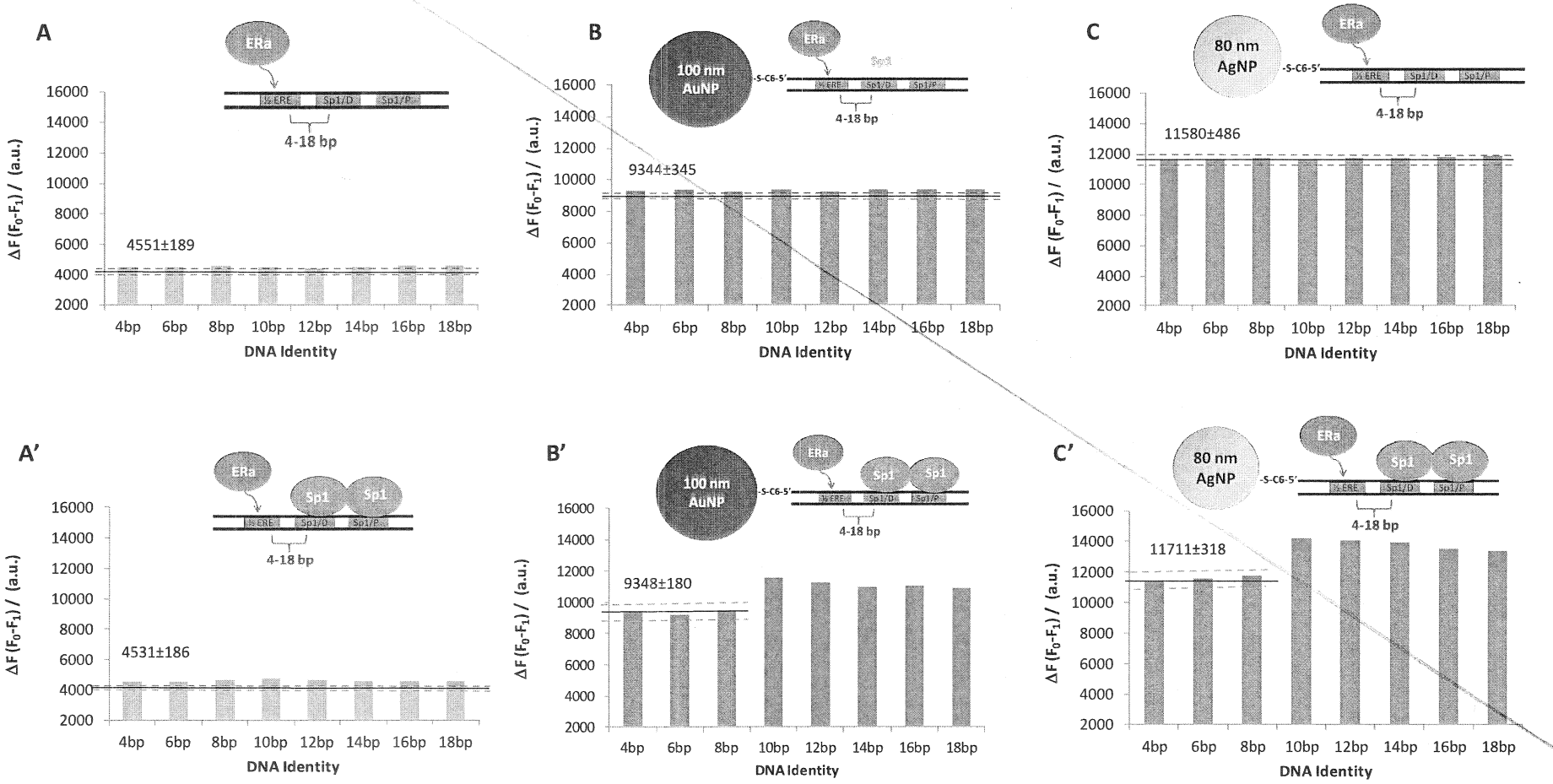

A
20 nm AuNP
-S-C6-
ERα
½ ERE
Sp1/D
Sp1/P
4-18 bp spacer
1200
1000
800
600
400
200
0
Δ F (F0-F1) / a.u.
4bp
6bp
8bp
10bp
12bp
14bp
16bp
18bp
DNA identity
B
ERα
½ ERE
Sp1/D
Sp1/P
C6-S-
20 nm AuNP
4, 8, 12 bp spacer
5500
5250
5000
4750
4500
ΔF (F0-F1) / a.u.
4bp
8bp
12bp
DNA identity

FIG. 12


A
ERa
½ ERE
Sp1/D
Sp1/P
4-18 bp
4551±189
ΔF (F0-F1) / (a.u.)
DNA Identity
4bp 6bp 8bp 10bp 12bp 14bp 16bp 18bp
B
100 nm AuNP
-S-C6-5'
Sp1
9344±345
C
80 nm AgNP
11580±486
A'
4531±186
B'
9348±180
C'
11711±318