Supplementary Material

On the Stability of Peptide Nucleic Acid Duplexes in the presence of Organic Co-solvents

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Table S1 PNA and DNA sequences.

Name	Sequence[a, b]	Base composition	ε_{260} ($M^{-1}cm^{-1}$)
PNA1	H-GTA GAT CAC T-Lys-NH_2	$A_3G_2T_3C_2$	110600
PNA2	H-AGT GAT CTA C-Lys-NH_2	$A_3G_2T_3C_2$	110600
PNA3	H-AGAG-$(eg1)_3$-CTCT-Lys-NH_2	$A_2G_2T_2C_2$	86400
PNA4	H-ACAG-$(eg1)_3$-CTGT-Lys-NH_2	$A_2G_2T_2C_2$	86400
PNA5	H-tT-GTA GAT CAC T-NH_2	$A_3G_2T_3tTC_2$	119400
PNA6	H-AGT GAT CTA C-NH_2	$A_3G_2T_3C_2$	110600
PNA7	H-GTA GAT CAC T-NH_2	$A_3G_2T_3C_2$	110600
PNA8	H-AGT GTT CTA C-Lys-NH_2	$A_2G_2T_4C_2$	104200
PNA9	H-GTA GAA CAC T-Lys-NH_2	$A_4G_2T_2C_2$	117600
PNA10	H-GTA GCT CAC T-Lys-NH_2	$A_2G_2T_3C_3$	102700
DNA1	5′-GTA GAT CAC T-3′	$A_3G_2T_3C_2$	100300
DNA2	5′-AGT GAT CTA C-3′	$A_3G_2T_3C_2$	100700
DNA3	5′-AGT GAT CTA CGG TGG ACG GTC C-3′	$A_4G_8T_5C_5$	213700
DNA4	5′-GGA CCG TCC ACC GTA GAT CAC T-3′	$A_5G_5T_4C_8$	208300
DNA5	5′-AGA GTT TTC TCT-3′	$A_2G_2T_6C_2$	111700

a. All the duplexes resulting from these sequences would be antiparallel (either N/C or N/3′ or 5′/3′).

b. The N-terminal of the peptide backbone of PNA is shown by an ‘H’ and the amidated carboxyl terminal of the peptide backbone of PNA is shown by an ‘NH_2’.

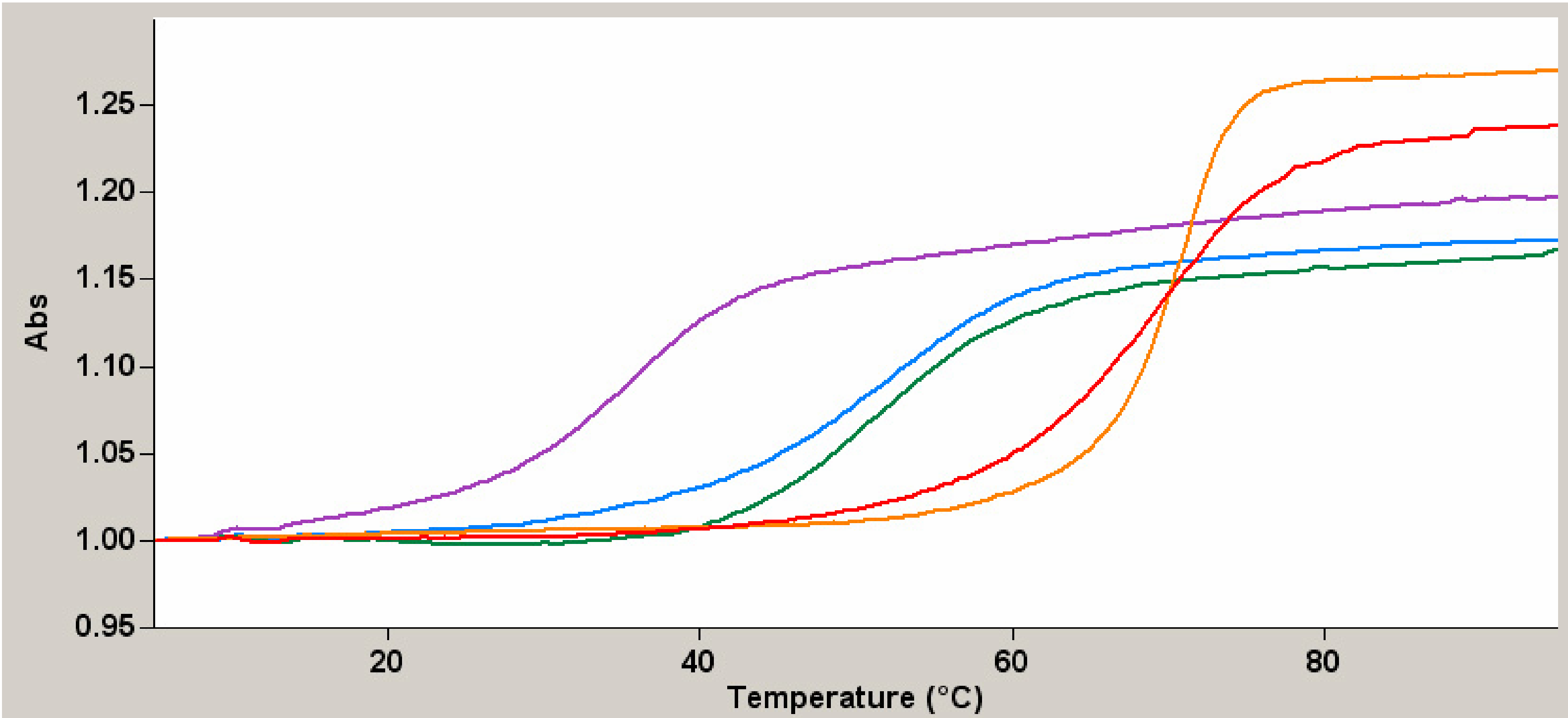
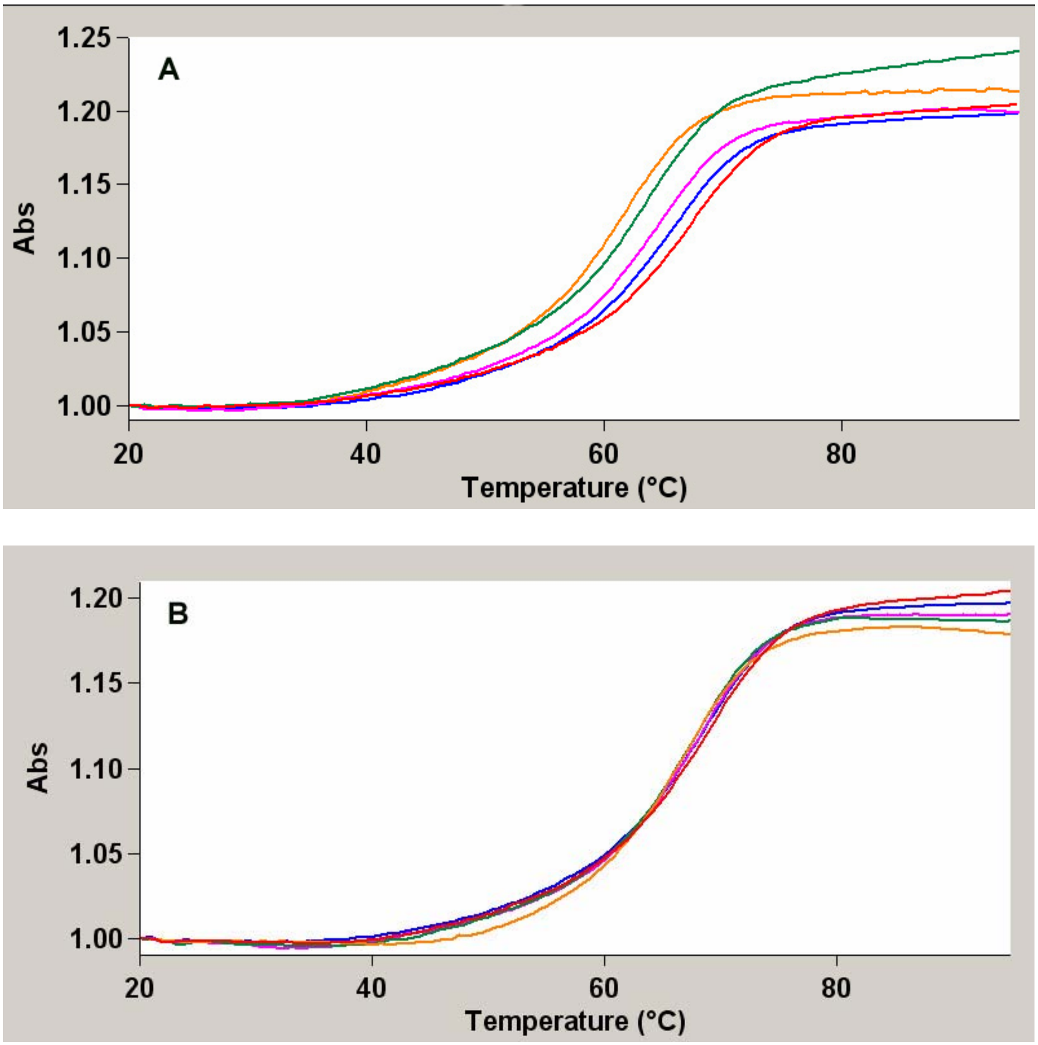


Figure S1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Abs
1.25
1.20
1.15
1.10
1.05
1.00
20
40
60
80
Temperature (°C)
B
Abs
1.20
1.15
1.10
1.05
1.00
20
40
60
80
Temperature (°C)

Figure S2

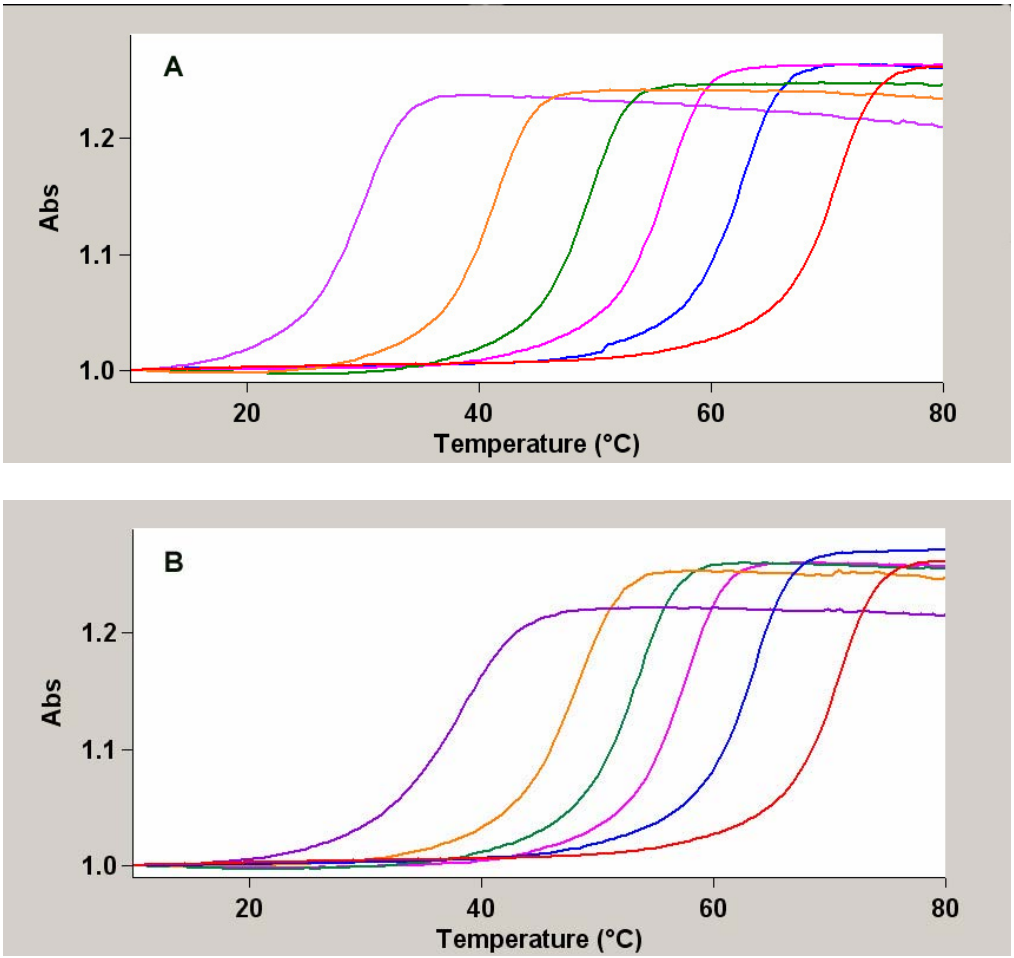

A
Abs
1.0
1.1
1.2
20
40
60
80
Temperature (°C)
B
Abs
1.0
1.1
1.2
20
40
60
80
Temperature (°C)

Figure S3

Table S2 Thermal and thermodynamic parameters of PNA and DNA duplexes in DMF.

DMF (%)	T_m (°C)[a, b]	Concentration method			Hyperchromicity method		
		ΔG^0_{37} (kcal/mol)	ΔH^0 (kcal/mol)	ΔS^0 (cal/mol.K)	ΔG^0_{37} (kcal/mol)[b]	ΔH^0 (kcal/mol)[b]	ΔS^0 (cal/mol.K)[b]
Solvent effect on PNA1·PNA2							
0	70.2±0.3	−17.2	−96.9	−256.9	−16.6±0.5	−93.8±4.7	−249.0±11.4
10	68.3±0.2	−16.2	−91.1	−241.3	−15.4±0.5	−81.1±3.8	−213.9±10.6
20	66.2±0.2	−15.6	−88.3	−234.2	−14.3±0.4	−80.9±5.2	−214.9±9.8
30	64.3±0.5	−15.7	−94.6	−254.3	−14.5±0.8	−84.7±3.2	−226.4±11.8
40	63.4±0.3	−15.9	−98.8	−267.2	−14.4±0.5	−89.7±4.6	−241.8±12.1
50	61.3±0.4	−15.5	−98.4	−267.5	−13.6±0.7	−82.4±5.0	−238.2±10.8
Solvent effect on DNA1·DNA2							
0	35.8±0.5	−7.9	−69.0	−197.6	−8.2±0.7	−71.0±3.5	−202.4±8.8
10	29.2±0.4	−6.1	−67.3	−196.3	−6.2±1.2	−64.0±5.0	−186.1±9.5
20	23.7±0.5	−4.9	−65.0	−194.0	−4.9±0.8	−65.6±4.7	−195.0±12.0
30[d]	18.2±0.3	−3.9	−63.9	−193.5	−3.7±0.5	−64.2±5.0	−195.0±9.6
Solvent effect on PNA1·DNA2							
0	51.3±0.4	−11.3	−73.3	−199.9	−10.3±1.0	−70.5±3.2	−193.9±12.3
10	47.0±0.5	c	c	c	−9.5±0.7	−57.8±4.5	−155.8±10.0
20	43.1±0.3	c	c	c	−9.0±0.6	−61.7±4.4	−169.9±10.6
30	40.2±0.4	c	c	c	−8.3±0.6	−59.1±5.2	−163.8±11.8
40	36.2±0.5	c	c	c	−7.7±0.8	−59.7±3.8	−167.4±9.7
50	31.1±0.2	−6.8	−61.1	−174.7	−6.7±0.5	−56.7±4.2	−161.0±12.0

a. PNA duplex concentration was 5.0µM in strands.
b. Five independent measurements were used to calculate the standard deviation.
c. Data were not measured, as the data evaluated from the other method was sufficient to give necessary information.
d. Data at higher than 30% of DMF could not be measured because of incomplete thermal melting curves (too low melting temperature).

Table S3 Thermal and thermodynamic parameters[a] of longer DNA duplex control DNA3·DNA4 in DMF and dioxane.

DMF (%)	**T_m (°C)[b, c]**	**ΔG^0_{37} (kcal/mol)[c]**	**ΔH^0 (kcal/mol)[c]**	**ΔS^0 (cal/mol.K)[c]**
0	70.0±0.4	−16.4±1.0	−81.2±4.5	−209.0±11.8
10	62.1±0.5	−14.3±0.7	−77.0±5.5	−202.1±10.5
20	56.2±0.6	−12.8±0.8	−76.2±4.7	−204.4±10.7
30	49.2±0.3	−11.3±1.0	−79.4±3.8	−219.4±12.3
40	41.3±0.2	−9.3±0.6	−77.9±5.3	−221.2±11.8
50[d]	30.1±0.5	−6.1±0.5	−79.8±6.0	−237.6±11.0
100[e]	−6.2	2.8	−78.9	−261.8
Dioxane (%)	**T_m (°C)[b, c]**	**ΔG^0_{37} (kcal/mol)[c]**	**ΔH^0 (kcal/mol)[c]**	**ΔS^0 (cal/mol.K)[c]**
0	70.0±0.4	−16.4±1.0	−81.2±4.5	−209.0±11.8
10	63.0±0.6	−14.2±0.8	−74.6±3.6	−196.7±10.4
20	57.1±0.5	−13.2±0.7	−80.7±5.8	−217.8±11.6
30	53.1±0.3	−12.1±1.0	−80.2±6.2	−219.6±12.3
40	48.2±0.5	−10.7±0.6	−73.0±4.4	−200.9±10.5
50[f]	38.3±0.2	−8.0±0.5	−74.7±4.6	−214.8±9.8
100[e]	10.7	−1.1	−80.3	−234.8

a. Thermodynamic parameters were evaluated from hyperchromicity (curve fitting) method. (Plots are in Figure 1).
b. Thermal melting temperatures correspond to a DNA duplex concentration of 5.0μM in strands.
c. Five independent measurements were used to calculate the standard deviation.
d. Data at higher than 50% of DMF could not be obtained because of high absorption of DMF in the range of wavelength used for the experiments.
e. Data obtained from manual extrapolation of the linear plots to 100% of DMF or dioxane.
f. Data at higher than 50% of dioxane could not be obtained because of condensation/evaporation of dioxane at extreme temperatures.

Table S4 Comparative thermal and thermodynamic parameters[a] of PNA and DNA duplexes in DMF and dioxane.

DMF (%)	T_m (°C)[b, c]				ΔG^0_{37} (kcal/mol)[c]			
	PNA1·PNA2	**PNA1·DNA2**	**PNA2·DNA1**	**DNA1·DNA2**	**PNA1·PNA2**	**PNA1·DNA2**	**PNA2·DNA1**	**DNA1·DNA2**
0	70.2±0.3	51.3±0.4	50.8±0.2	36.3±0.5	−16.6±0.5	−10.3±1.0	−10.9±0.2	−8.2±0.7
10	68.3±0.2	47.0±0.5	46.2±0.4	29.2±0.4	−15.4±0.5	−9.5±0.7	−9.6±0.5	−6.2±1.2
20	66.2±0.2	43.1±0.3	42.1±0.3	23.7±0.5	−14.3±0.4	−9.0±0.6	−9.1±0.4	−4.9±0.8
30	64.3±0.5	40.2±0.4	39.1±0.3	18.2±0.3	−14.5±0.8	−8.3±0.6	−8.4±0.4	−3.7±0.5
40	63.4±0.3	36.2±0.5	35.0±0.5	d	−14.4±0.5	−7.7±0.8	−7.5±0.3	d
50	61.3±0.4	31.1±0.2	29.3±0.2	d	−13.6±0.7	−6.7±0.5	−6.4±0.1	d
60	60.0±0.6	f	f	f	f	f	f	f
70	56.1±0.5	f	f	f	f	f	f	f
Dioxane (%)								
0	70.2±0.3	51.3±0.4	50.8±0.2	35.8±0.5	−16.6±0.5	−10.3±1.0	−10.9±0.2	−8.2±0.7
10	69.0±0.3	48.0±0.5	e	f	−15.2±0.7	−9.8±0.8	e	f
20	68.6±0.4	45.0±0.3	e	f	−14.9±0.6	−9.2±0.9	e	f
30	68.1±0.2	41.2±0.3	e	f	−14.7±0.5	−8.6±0.7	e	f
40	67.2±0.3	39.2±0.2	e	f	−14.1±0.5	−8.1±0.6	e	f
50	66.3±0.2	34.3±0.5	e	f	−14.0±0.5	−7.5±0.8	e	f
60	65.0±0.8	f	f	f	f	f	f	f
70	61.1±0.7	f	f	f	f	f	f	f

a. Thermodynamic parameters were evaluated from hyperchromicity (curve fitting) method. Plots are in Figure S4 and S5.
b. Thermal melting temperatures correspond to a PNA duplex concentration of 5.0μM in strands.
c. Five independent measurements were used to calculate the standard deviation.
d. Data could not be measured because of incomplete thermal melting curves. (However, supporting data with longer DNA control DNA3·DNA4 are available. See Table S3.).
e. Data were not measured because the results were obvious from the supporting data available (the other hybrid duplex).
f. Data could not be measured because of poorer accuracy related to upper baseline irregularities in DMF and condensation of dioxane in the range of low temperatures required.

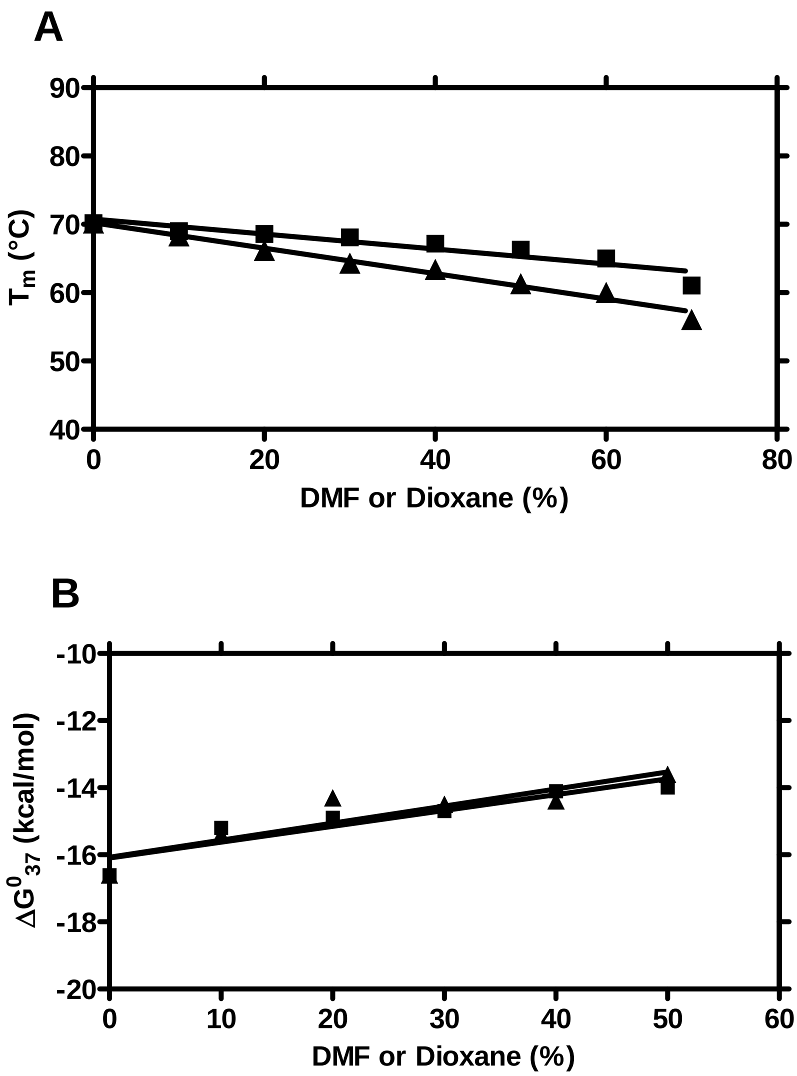

A
90
80
70
60
50
40
T_m (°C)
0
20
40
60
80
DMF or Dioxane (%)
B
-10
-12
-14
-16
-18
-20
ΔG^0_{37} (kcal/mol)
0
10
20
30
40
50
60
DMF or Dioxane (%)

Figure S4

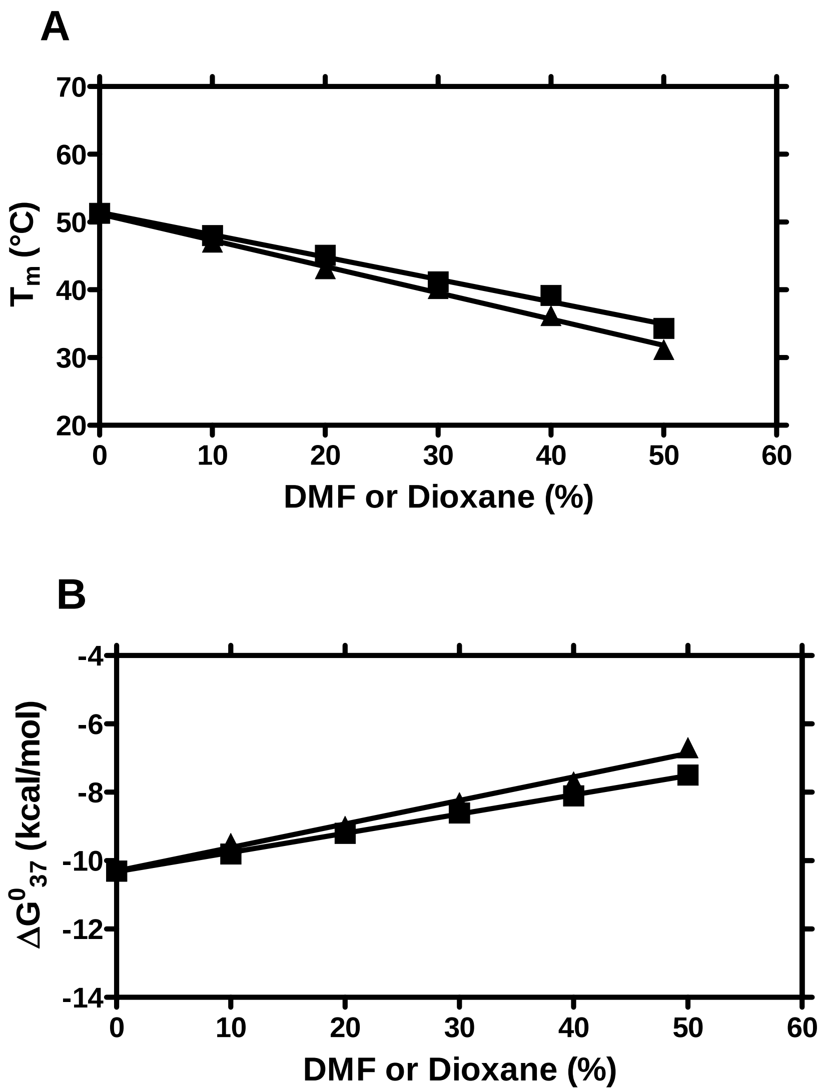
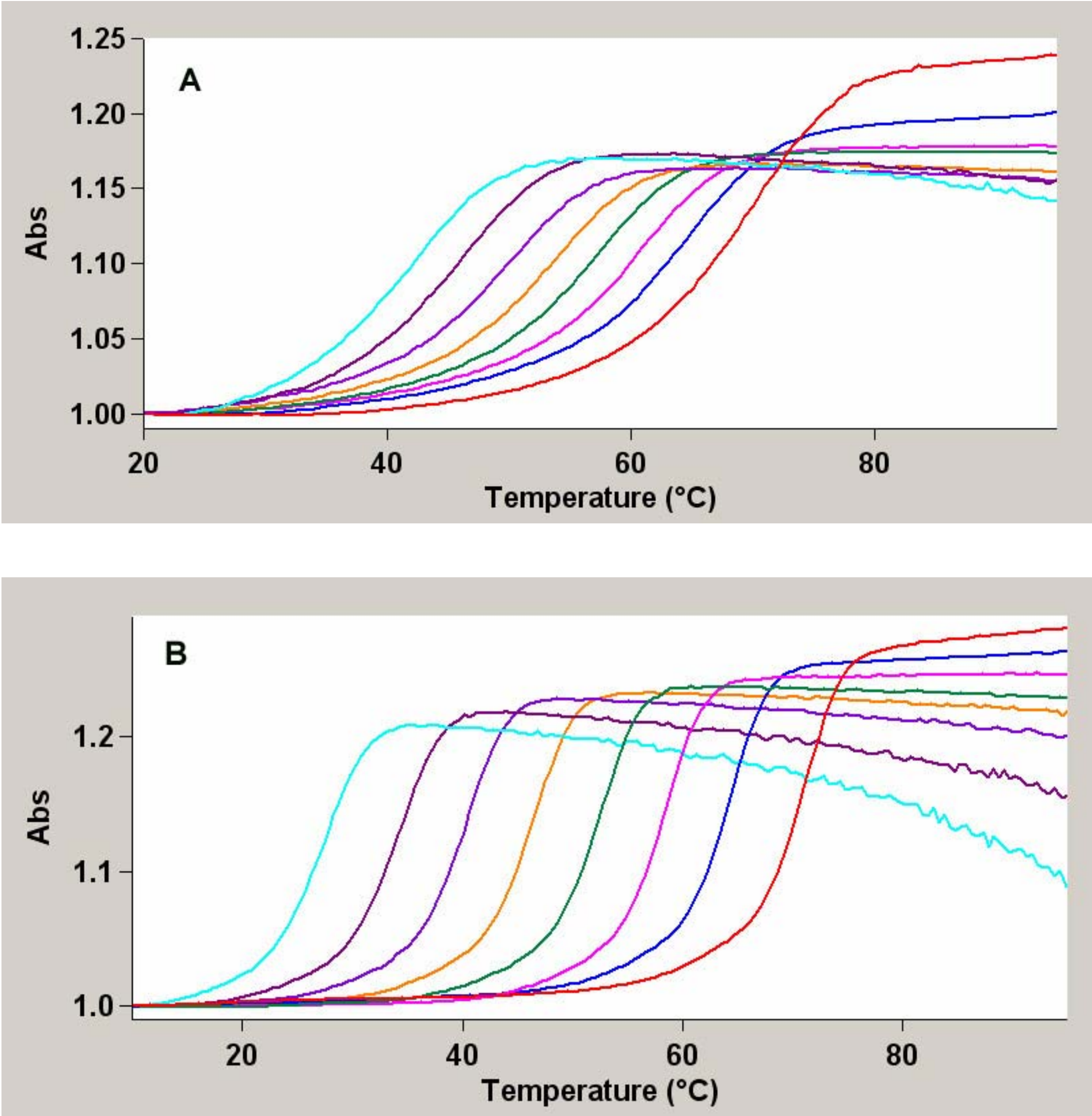

A
70
60
50
40
30
20
Tm (°C)
0
10
20
30
40
50
60
DMF or Dioxane (%)
B
-4
-6
-8
-10
-12
-14
ΔG037 (kcal/mol)
0
10
20
30
40
50
60
DMF or Dioxane (%)

Figure S5



A
1.25
1.20
1.15
1.10
1.05
1.00
Abs
20
40
60
80
Temperature (°C)
B
1.2
1.1
1.0
Abs
20
40
60
80
Temperature (°C)

Figure S6

Table S5 Thermal and thermodynamic parameters[a] of PNA and DNA duplexes in formamide.

Fomamide (%)	T_m (°C)[b]		ΔG^0_{37} (kcal/mol)		ΔH^0 (kcal/mol)		ΔS^0 (cal/mol.K)	
	PNA1·PNA2	DNA3·DNA4	PNA1·PNA2	DNA3·DNA4	PNA1·PNA2	DNA3·DNA4	PNA1·PNA2	DNA3·DNA4
0	70.0±0.08	70.0±0.4	−16.4±0.5	−16.4±1.0	−91.7±3.5	−81.2±4.5	−243.0±9.2	−209.0±11.8
10	65.1±0.04	64.1±0.7	−15.0±0.1	−14.8±0.2	−87.9±0.2	−78.6±0.3	−235.1±0.2	−206.0±1.2
20	61.1±0.03	58.2±0.5	−13.7±0.1	−13.3±0.5	−83.5±0.2	−75.4±0.6	−224.9±0.3	−200.5±3.6
30	57.2±0.03	52.3±0.5	−12.7±0.2	−11.8±0.7	−80.8±0.1	−73.1±1.3	−219.4±0.1	−197.4±4.7
40	54.0±0.05	46.0±0.6	−11.9±0.2	−10.5±0.6	−78.9±0.2	−72.5±2.0	−216.1±0.4	−200.2±2.8
50	49.1±0.06	40.1±0.8	−10.6±0.2	−8.9±0.2	−73.0±0.3	−70.8±0.4	−210.3±0.4	−199.5±2.5
60	46.1±0.05	34.2±0.7	−9.7±0.3	−7.9±0.3	−71.5±0.3	−64.4±0.7	−199.3±0.3	−182.5±6.4
70	41.2±0.04	27.3±0.6	−8.7±0.1	c	−69.2±0.2	c	−195.1±0.3	c
100[d]	29.5	9.6	−5.4	−1.9	−58.5	−56.5	−174.5	−176.1

a. Thermodynamic parameters were evaluated from hyperchromicity (curve fitting) method. (Plots are in Figures 1, S7 and S8).
b. Thermal melting temperatures correspond to a PNA duplex concentration of 5.0μM in strands.
c. Data could not be evaluated by curve fitting because of bad thermal curves and insufficient baseline.
d. Data obtained from manual extrapolation of the linear graphs in Figures 1, S7 and S8.

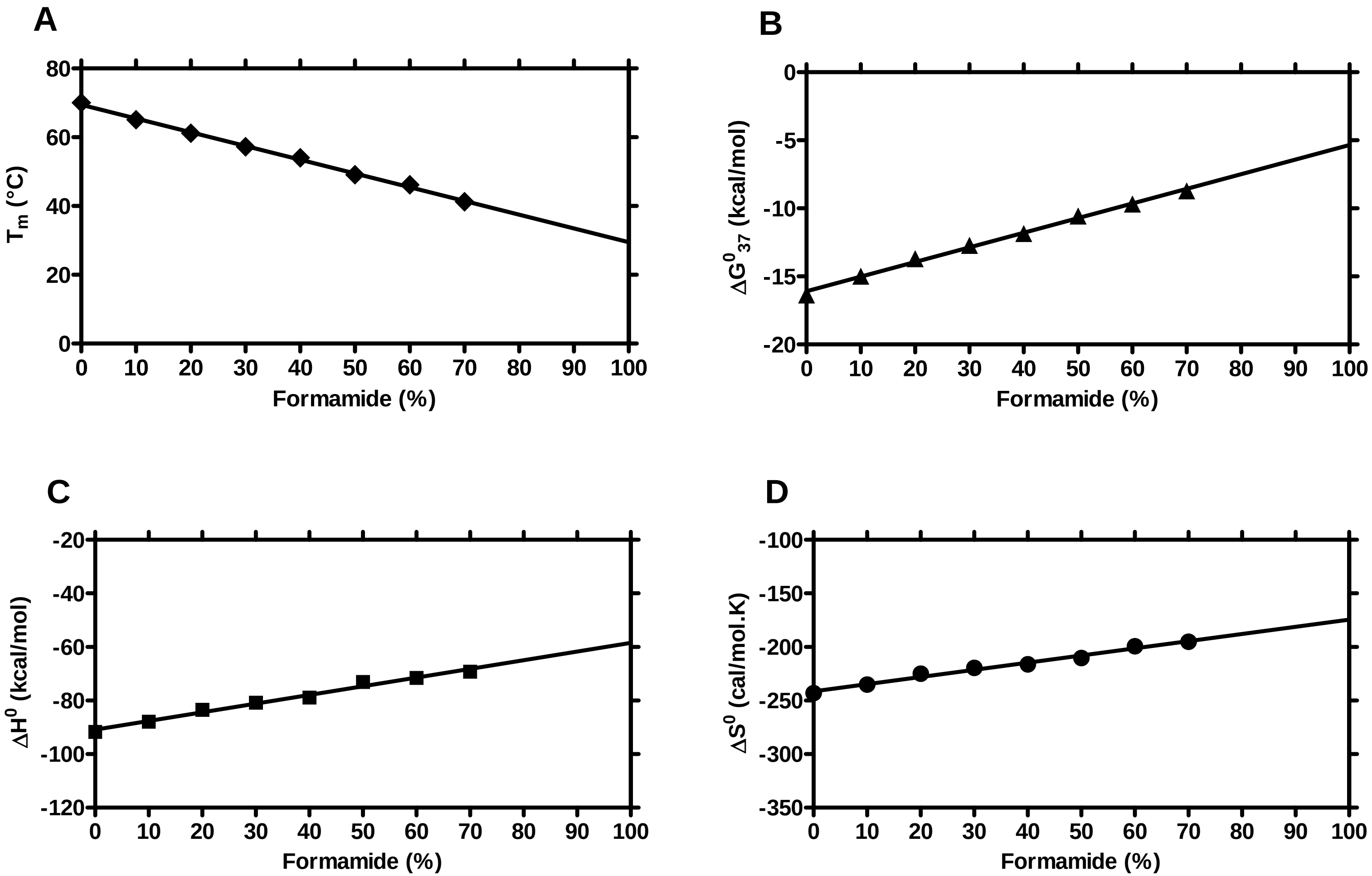

A
80
60
40
20
0
T_m (°C)
0 10 20 30 40 50 60 70 80 90 100
Formamide (%)
B
0
-5
-10
-15
-20
ΔG^0_{37} (kcal/mol)
0 10 20 30 40 50 60 70 80 90 100
Formamide (%)
C
-20
-40
-60
-80
-100
-120
ΔH^0 (kcal/mol)
0 10 20 30 40 50 60 70 80 90 100
Formamide (%)
D
-100
-150
-200
-250
-300
-350
ΔS^0 (cal/mol.K)
0 10 20 30 40 50 60 70 80 90 100
Formamide (%)

Figure S7

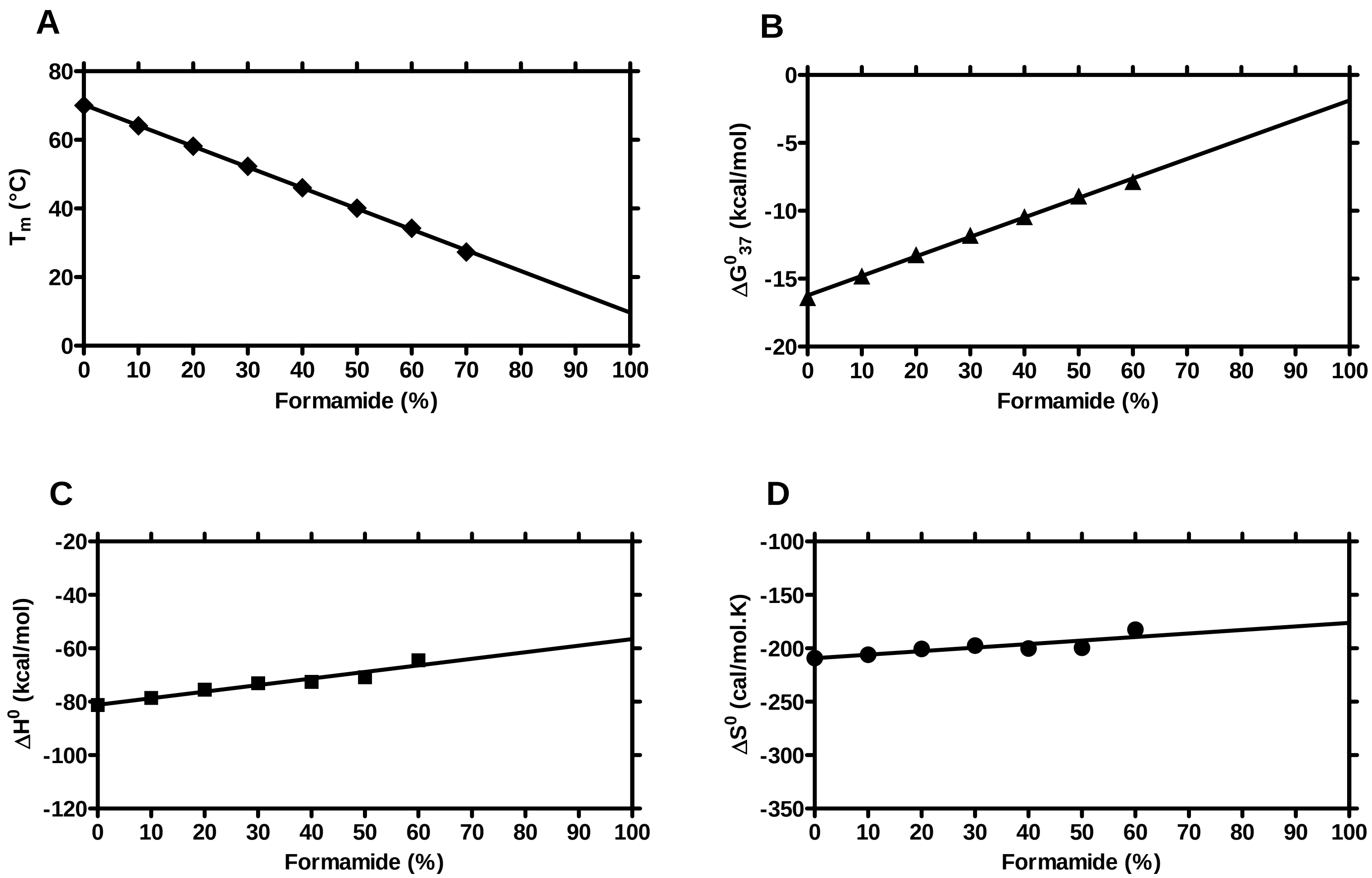

A
B
C
D
80
60
40
20
0
Tm (°C)
0
-5
-10
-15
-20
ΔG037 (kcal/mol)
-20
-40
-60
-80
-100
-120
ΔH0 (kcal/mol)
-100
-150
-200
-250
-300
-350
ΔS0 (cal/mol.K)
0 10 20 30 40 50 60 70 80 90 100
Formamide (%)

Figure S8

Table S6 Thermal and thermodynamic parameters[a] of hairpin PNAs[b] in DMF.

DMF (%)	T_m (°C)[c]		ΔG^0_{37} (kcal/mol)[c]		ΔH^0 (kcal/mol)[c]		ΔS^0 (cal/mol.K)[c]	
	PNA3	PNA4	PNA3	PNA4	PNA3	PNA4	PNA3	PNA4
0	59.0±0.6	53.1±0.4	−1.6±0.5	−1.4±0.6	−29.6±5.0	−29.0±5.2	−90.2±9.6	−89.2±12.2
10	57.0±0.5	51.0±0.5	−1.9±0.7	−1.3±0.5	−32.3±5.5	−31.4±4.4	−98.2±10.7	−97.1±11.5
20	56.1±0.6	50.1±0.3	−1.9±0.7	−1.3±0.7	−34.3±6.2	−33.4±4.3	−104.4±10.2	−103.4±10.4
30	56.2±0.4	49.2±0.2	−2.0±0.8	−1.4±0.8	−36.4±4.8	−35.0±5.6	−110.7±9.3	−108.1±9.8
40	55.7±0.3	49.2±0.5	−2.2±1.0	−1.6±0.7	−40.5±4.6	−39.5±5.2	−123.4±12.0	−122.3±10.7
50	54.8±0.5	50.3±0.6	−2.3±0.5	−1.7±0.7	−43.4±5.7	−42.7±4.7	−132.6±11.6	−132.2±11.3
60	53.6±0.4	48.2±0.4	−2.2±0.5	−1.8±0.6	−45.1±5.8	−46.5±5.3	−138.0±11.8	−144.2±12.3
70[d]	50.2±0.6[e]	f	f	f	f	f	f	f
100[g]	49.3	46.0	-2.7	−2.0	−55.7	−57.2	−170.6	−140.0
Slope[g]	0.09	0.06	0.01	0.01	−0.26	−0.29	−0.82	−0.90

a. Thermodynamic parameters were evaluated from hyperchromicity (curve fitting) method. (Plots are in Figures 2A, S9A, S9B and S9C).

b. PNA concentration was 12.0μM in strand for PNA3 (H-AGAG-(eg1)$_3$-CTCT-Lys-NH_2) and 11.0 μM in strand for PNA4 (H-ACAG-(eg1)$_3$-CTGT-Lys-NH_2).

c. Three independent measurements were used to calculate the standard deviation.

d. Data could not be measured above 70% of DMF because of high absorption of DMF in the wavelength range used.

e. Data not reliable due to bad thermal curves.

f. Data could not be measured or evaluated due to bad thermal curves.

g. Data were obtained from the manual extrapolations of the linear plots of Figures 2A, S9A, S9B and S9C.

h. Data were calculated from the linear fitting of the plots of Figures 2A, S9A, S9B and S9C.

Table S7 Thermal and thermodynamic parameters[a] of hairpin PNAs[b] in dioxane.

Dioxane (%)	T_m (°C)[c]		ΔG^0_{37} (kcal/mol)[c]		ΔH^0 (kcal/mol)[c]		ΔS^0 (cal/mol.K)[c]	
	PNA3	**PNA4**	**PNA3**	**PNA4**	**PNA3**	**PNA4**	**PNA3**	**PNA4**
0	59.0±0.5	53.0±0.4	−1.8±0.8	−1.5±0.6	−28.7±5.5	−31.7±5.2	−86.6±11.7	−97.6±11.5
10	58.0±0.4	51.0±0.5	−1.8±0.9	−1.4±0.7	−33.1±4.5	−34.5±4.6	−100.7±12.0	−106.6±10.5
20	58.1±0.6	51.2±0.3	−1.8±0.9	−1.4±0.6	−31.6±3.8	−34.6±3.7	−96.2±12.2	−106.9±12.4
30	58.1±0.3	51.1±0.4	−2.1±0.5	−1.6±0.9	−35.3±3.7	−35.6±3.9	−107.1±11.7	−109.8±10.7
40	58.2±0.4	51.2±0.6	−2.2±0.7	−1.6±0.8	−35.7±5.3	−35.2±5.4	−108.0±11.4	−108.3±10.6
50	59.3±0.3	52.3±0.5	−2.5±0.6	−1.8±0.5	−38.1±4.6	−36.5±4.5	−114.9±10.3	−111.9±11.4
60	62.1±0.2	54.1±0.4	−2.8±0.7	−2.0±0.5	−40.8±4.2	−39.3±4.8	−122.4±9.7	−120.5±10.3
70[d, e]	63.2±0.4	59.1±0.3	−3.8±0.4	−2.5±0.6	−52.7±3.7	−42.5±4.4	−157.8±9.8	−129.0±9.7
100[f]	63.8	53.4	−3.9	−2.2	−47.4	−42.2	−142.0	−129.0
Slope[g]	0.06	0.02	−0.02	−0.01	−0.18	−0.10	−0.53	−0.29

a. Thermodynamic parameters were evaluated from hyperchromicity (curve fitting) method. (Plots are in Figures 2B, S9D, S9E and S9F)
b. PNA concentration was 12.0μM in strand for PNA3 (H-AGAG-$(eg1)_3$-CTCT-Lys-NH_2) and 11.0 μM in strand for PNA4 (H-ACAG-$(eg1)_3$-CTGT-Lys-NH_2).
c. Three independent measurements were used to calculate the standard deviation.
d. Data not reliable due to bad thermal curves.
e. Data could not be measured above 70% of dioxane because of condensation/evaporation of dioxane at extreme temperatures.
f. Data were obtained from the manual extrapolations of the linear plots (without taking data point for 70% dioxane into account except for T_m of PNA3) of Figures 2B, S9D, S9E and S9F.
g. Data were calculated from the linear fitting of the plots (without taking the data point at 70% dioxane into account except for T_m of PNA3) of Figures 2B, S9D, S9E and S9F.

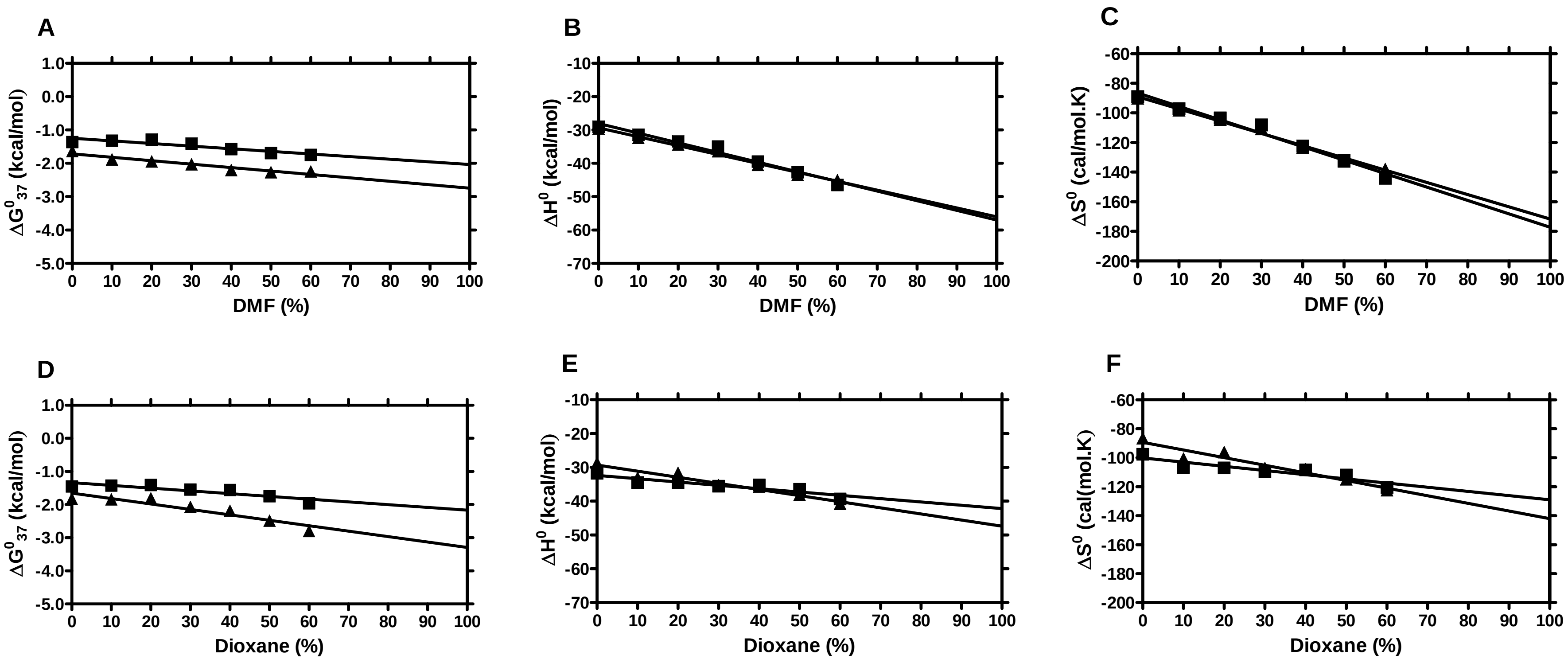

A
1.0
0.0
-1.0
-2.0
-3.0
-4.0
-5.0
ΔG037 (kcal/mol)
0 10 20 30 40 50 60 70 80 90 100
DMF (%)
B
-10
-20
-30
-40
-50
-60
-70
ΔH0 (kcal/mol)
0 10 20 30 40 50 60 70 80 90 100
DMF (%)
C
-60
-80
-100
-120
-140
-160
-180
-200
ΔS0 (cal/mol.K)
0 10 20 30 40 50 60 70 80 90 100
DMF (%)
D
ΔG037 (kcal/mol)
0 10 20 30 40 50 60 70 80 90 100
Dioxane (%)
E
ΔH0 (kcal/mol)
0 10 20 30 40 50 60 70 80 90 100
Dioxane (%)
F
ΔS0 (cal(mol.K)
0 10 20 30 40 50 60 70 80 90 100
Dioxane (%)

Figure S9

Table S8 Thermal and thermodynamic parameters[a] of hairpin DNA control DNA5[b] in DMF.

DMF (%)	T_m (°C)[c]	ΔG^0_{37} (kcal/mol)[c]	ΔH^0 (kcal/mol)[c]	ΔS^0 (cal/mol.K)[c]
0	43.0±0.3	−0.7±0.02	−24.8±0.3	−77.7±2.5
10	34.1±0.2	−0.2±0.1	−28.2±2.0	−91.1±4.2
20	28.1±0.2	0.8±0.2[d]	−30.5±2.2[d]	−101.0±4.7[d]
30	17.2±0.3	e	e	e
40[f]	11.4±0.3	e	e	e
100[g]	-38.7	–	–	–
Slope[h]	-0.8	–	–	–

a. Thermodynamic parameters were evaluated from hyperchromicity (curve fitting) method.
b. DNA concentration was 6.0 µM in strand.
c. Three independent measurements were used to calculate the standard deviation.
d. Data not reliable due to insufficient baseline.
e. Data could not be evaluated because of incomplete thermal curves.
f. Data could not be measured above 40% of DMF because of incomplete thermal curves (too low T_m).
g. Data were obtained from the manual extrapolations of the linear plot of Figure S10A.
h. Data were calculated from the linear fitting of the plot of Figure S10A.

Table S9 Thermal and thermodynamic parameters[a] of hairpin DNA control DNA5[b] in dioxane.

Dioxane (%)	T_m (°C)[c]	ΔG^0_{37} (kcal/mol)[c]	ΔH^0 (kcal/mol)[c]	ΔS^0 (cal/mol.K)[c]
0	43.0±0.3	−0.7±0.02	−24.8±0.3	−77.7±2.5
10	38.0±0.3	−0.5±0.1	−26.8±1.5	−84.8±2.2
20	36.1±0.2	−0.1±0.1[d]	−24.9±2.2[d]	−80.0±3.6[d]
30[d]	31.2±0.4	e	e	e
40[f]	26.3±0.4	e	e	e
100[g]	-0.4	–	–	–
Slope[h]	3.2	–	–	–

a. Thermodynamic parameters were evaluated from hyperchromicity (curve fitting) method.
b. DNA concentration was 6.0 µM in strand.
c. Three independent measurements were used to calculate the standard deviation.
d. Data not reliable due to insufficient baseline.
e. Data could not be evaluated because of incomplete thermal curves.
f. Data could not be measured above 40% of dioxane because of condensation of dioxane at the range of low temperatures required.
g. Data were obtained from the manual extrapolations of the linear plot of Figure S10B.
h. Data were calculated from the linear fitting of the plot of Figure S10B.

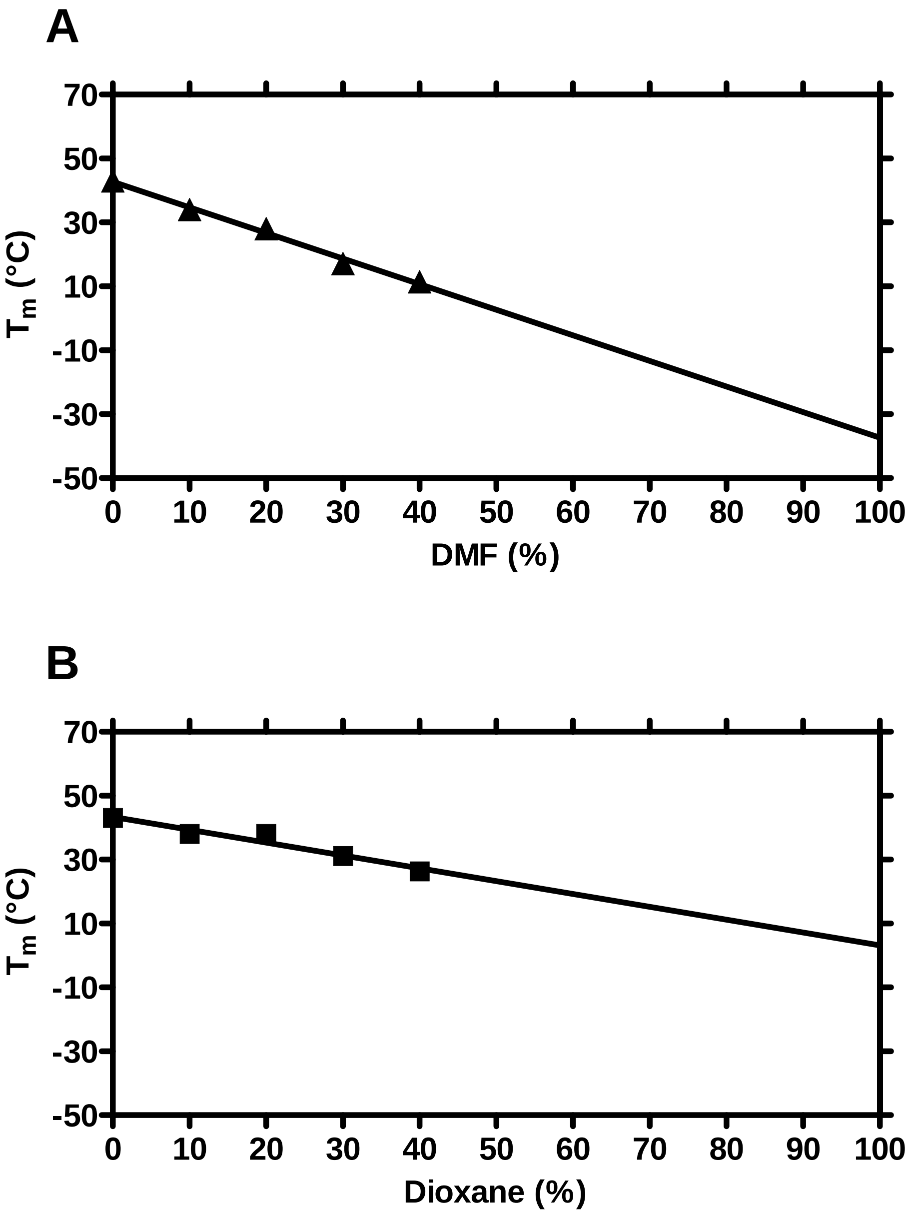

A
70
50
30
10
-10
-30
-50
T_m (°C)
0
10
20
30
40
50
60
70
80
90
100
DMF (%)
B
70
50
30
10
-10
-30
-50
T_m (°C)
0
10
20
30
40
50
60
70
80
90
100
Dioxane (%)

Figure S10

Table S10 Thermal and thermodynamic parameters[a] of PNA duplexes containing tricyclic thymine (tT) and its control duplex in DMF.

PNA duplex	DMF (%)	T_m (°C)[b, c]	ΔG^0_{37} (kcal/mol)[c]
PNA5/PNA6[d]	0	75.0±0.57	−15.2±0.3
	10	72.1±0.50	−14.8±0.1
	20	70.1±0.50	−16.1±0.2
	30	67.2±0.56	−15.6±0.2
	40	66.0±0.01	−15.6±0.5
	50	63.1±0.01	−14.7±0.5
	60	61.2±0.01	−13.7±0.8
	70	57.2±0.6	e
	100[f]	50.9	−13.8
PNA6/PNA7[d]	0	69.0±0.01	−14.2±0.3
	10	67.1±0.57	−14.3±0.2
	20	66.2±0.01	−15.2±0.3
	30	64.2±0.56	−15.4±0.9
	40	64.0±0.60	−15.5±1.0
	50	62.1±0.01	−14.5±0.6
	60	60.2±0.01	−14.0±0.6
	70	58.3±0.57	e
	100[f]	54.4	−14.6

a. Thermodynamic parameters were evaluated from hyperchromicity (curve fitting) method.
b. Thermal melting temperatures correspond to a PNA duplex concentration of 5.0μM in strands.
c. Three independent measurements were used to calculate the standard deviation.
d. PNA5 is H-tT-GTA GAT CAC T-NH_2, PNA6 is H-AGT GAT CTA C-NH_2 and PNA7 is H-GTA GAT CAC T-NH_2.
e. Data could not be evaluated by curve fitting because of bad thermal curves.
f. Data obtained from manual extrapolation of the linear graphs in Figure S11.

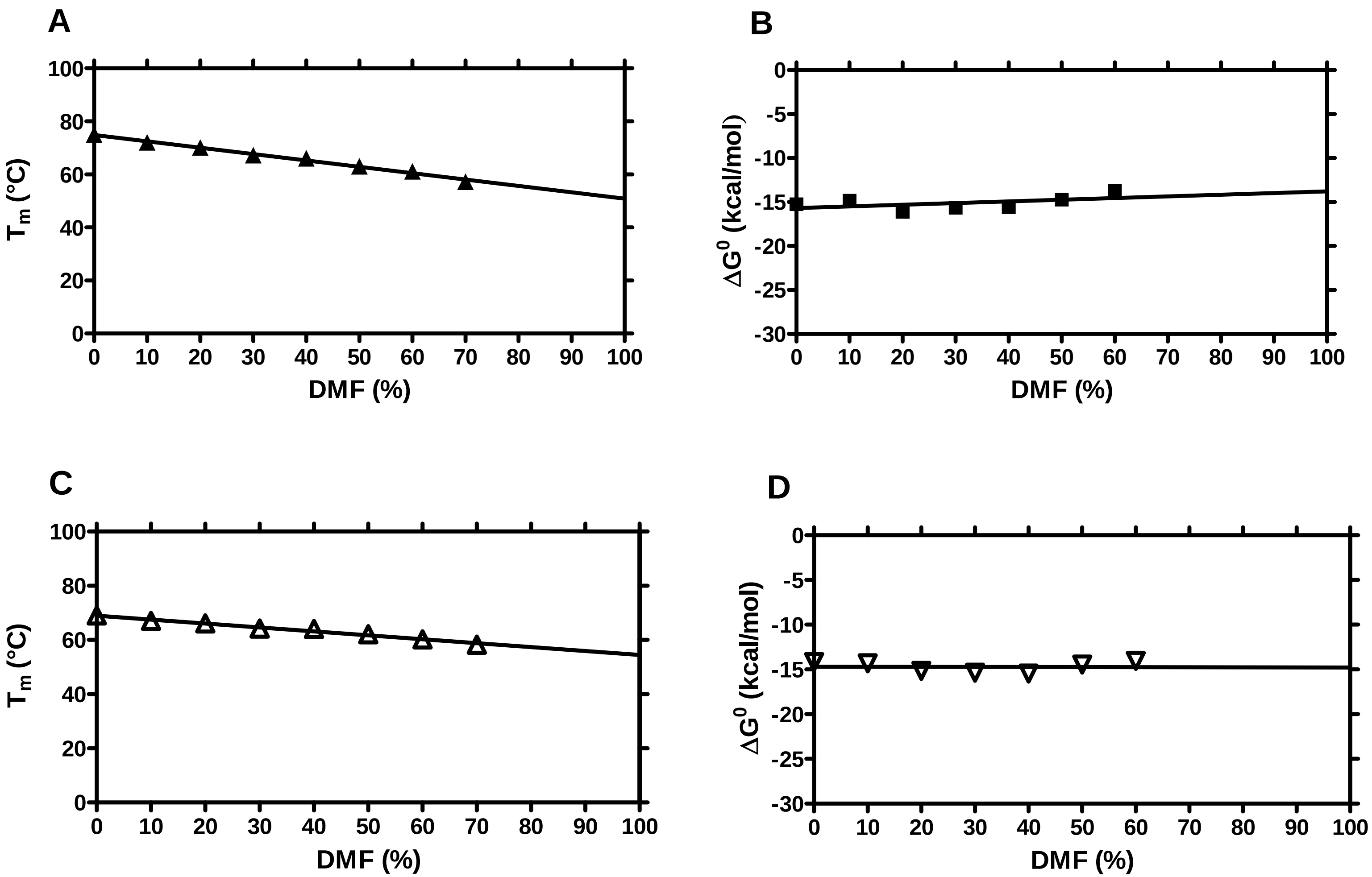

A
100
80
60
40
20
0
T m (°C)
0 10 20 30 40 50 60 70 80 90 100
DMF (%)
B
0
-5
-10
-15
-20
-25
-30
ΔG0 (kcal/mol)
0 10 20 30 40 50 60 70 80 90 100
DMF (%)
C
100
80
60
40
20
0
T m (°C)
0 10 20 30 40 50 60 70 80 90 100
DMF (%)
D
0
-5
-10
-15
-20
-25
-30
ΔG0 (kcal/mol)
0 10 20 30 40 50 60 70 80 90 100
DMF (%)

Figure S11

Table S11 Thermal and thermodynamic parameters[a] of single base mismatched PNA duplexes in DMF.

PNA duplex	DMF (%)	T_m (°C)[b, c]	ΔG^0_{37} (kcal/mol)[c]
PNA1/PNA8[d] (T·T mismatch)	0	53.0±0.03	−10.0±0.04
	10	51.0±0.02	−10.2±0.04
	20	49.1±0.02	−10.1±0.03
	30	48.2±0.03	−9.9±0.01
	40	47.0±0.01	−9.7±0.02
	50	46.1±0.05	−9.5±0.05
	60	45.2±0.06	−9.3±0.02
	70[e]	41.3	−8.5
	100[f]	39.7	−8.9
PNA2/PNA9[d] (A·A mismatch)	0	54.0±0.01	−11.0±0.05
	10	52.1±0.02	−10.8±0.06
	20	52.1±0.01	−10.7±0.04
	30	52.2±0.03	−10.3±0.05
	40	51.0±0.02	−10.6±0.05
	50	50.1±0.01	−10.9±0.02
	60	49.2±0.01	−10.3±0.03
	70[e]	45.3	−9.6
	100[f]	46.7	−10.2
PNA2/PNA10[d] (C·T mismatch)	0	52.0±0.01	−10.3±0.06
	10	51.0±0.05	−10.5±0.05
	20	50.1±0.05	−10.5±0.01
	30	49.2±0.06	−10.5±0.02
	40	48.0±0.01	−10.5±0.02
	50	47.1±0.01	−10.6±0.05
	60	45.1±0.02	−10.0±0.01
	70[e]	41.2	−8.8
	100[f]	41.3	−10.2

a. Thermodynamic parameters were evaluated from hyperchromicity (curve fitting) method.
b. Thermal melting temperatures correspond to a PNA duplex concentration of 5.0μM in strands.
c. Three independent measurements were used to calculate the standard deviation.
d. PNA8 is H-AGT GTT CTA C-Lys-NH_2, PNA9 is H-GTA GAA CAC T-Lys-NH_2 and PNA10 is H-GTA GCT CAC T-Lys-NH_2.
e. Data not reliable because of bad thermal curves.
f. Data obtained from manual extrapolation of the linear graphs in Figures S12 and S13 (without taking the data point at 70%DMF into account).

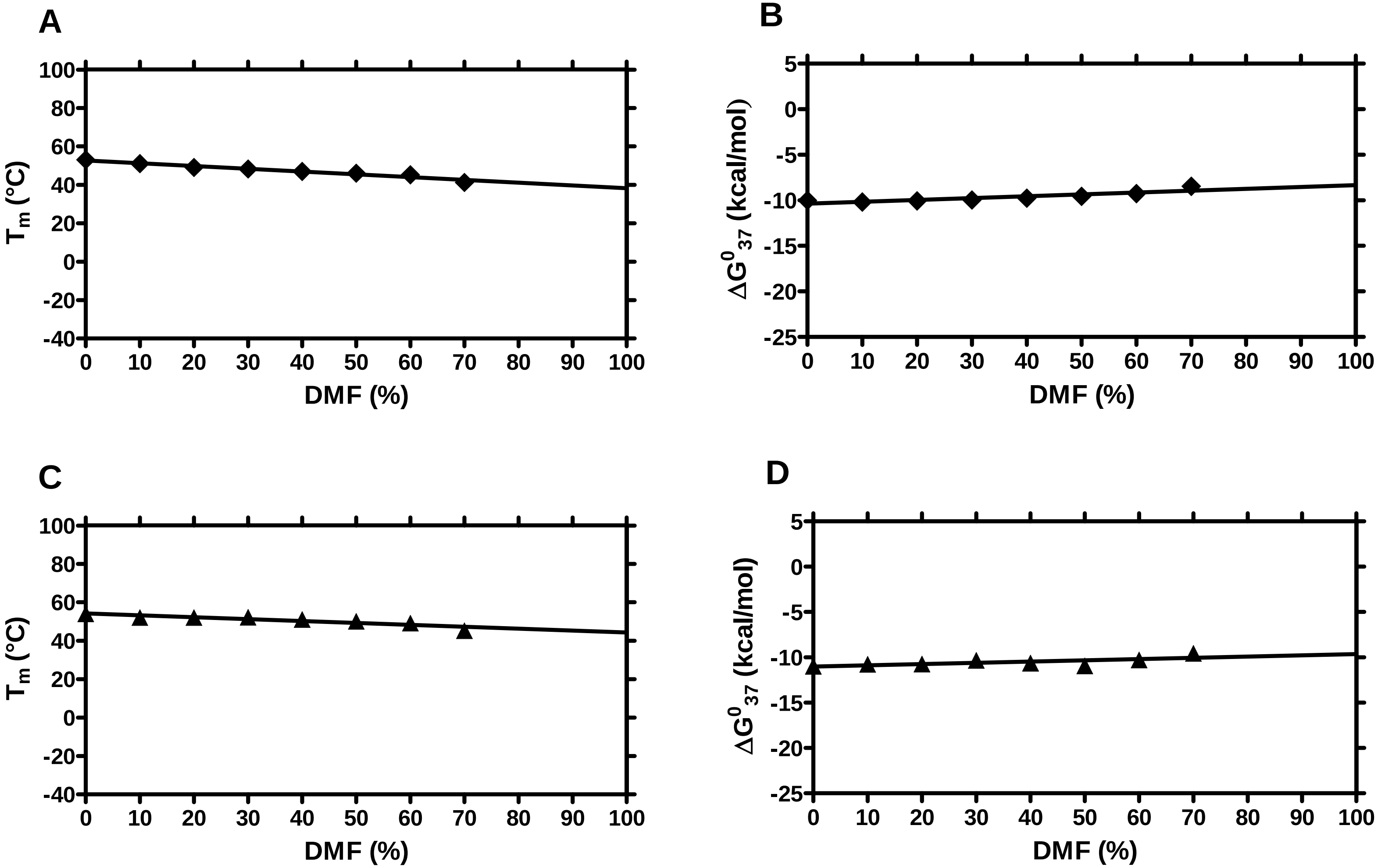

A
B
C
D
T_m (°C)
ΔG^0_{37} (kcal/mol)
DMF (%)
100
80
60
40
20
0
-20
-40
5
-5
-10
-15
-20
-25
0
10
20
30
40
50
60
70
80
90
100

Figure S12

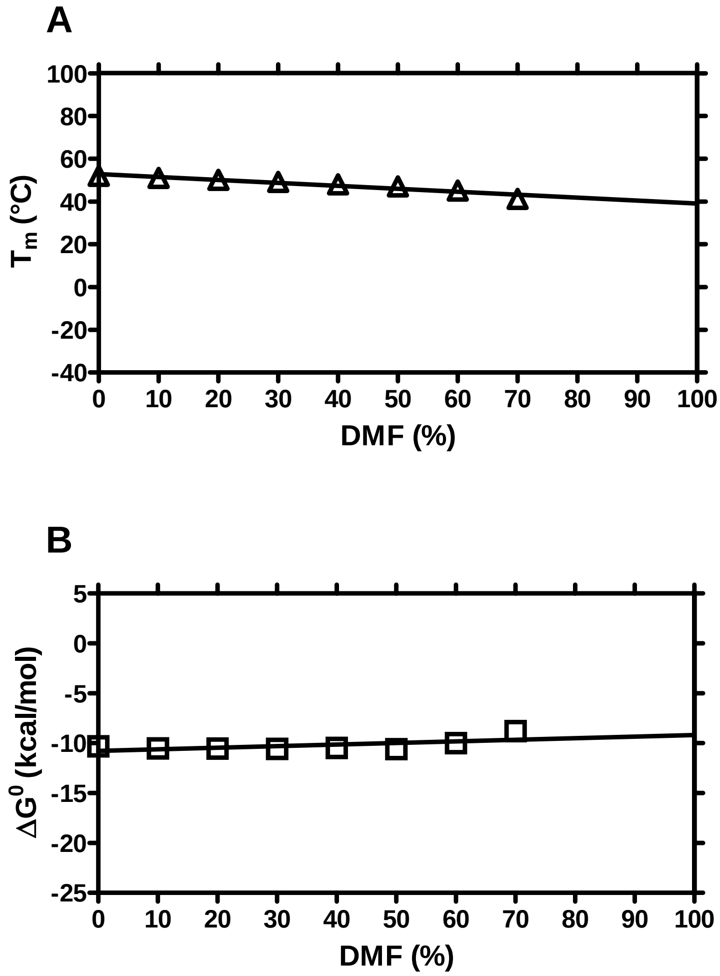

A
100
80
60
40
20
0
-20
-40
0 10 20 30 40 50 60 70 80 90 100
DMF (%)
Tm (°C)
B
5
0
-5
-10
-15
-20
-25
0 10 20 30 40 50 60 70 80 90 100
DMF (%)
ΔG0 (kcal/mol)

Figure S13

Figure legends

Figure S1 Representative thermal melting curves of PNA1·PNA2 (red), PNA1·DNA2 (blue), PNA2·DNA1 (green), DNA1·DNA2 (violet) and DNA3·DNA4 (orange) in pure aqueous buffer (10 mM phosphate buffer containing 100 mM NaCl and 0.1 mM EDTA, pH 7.2±0.01).

Figure S2 (A) Representative thermal melting curves of PNA1·PNA2 in 10% DMF (red), 20% DMF (blue), 30% DMF (pink), 40% DMF (green) and 50% DMF (orange). (B) Representative thermal melting curves of PNA1·PNA2 in 10% dioxane (red), 20% dioxane (blue), 30% dioxane (pink), 40% dioxane (green) and 50% dioxane (orange).

Figure S3 (A) Representative thermal melting curves of DNA3·DNA4 in 0% DMF (red), 10% DMF (blue), 20% DMF (pink), 30% DMF (green), 40% DMF (orange) and 50% DMF (violet). (B) Representative thermal melting curves of DNA3·DNA4 in 0% dioxane (red), 10% DMF (blue), 20% dioxane (pink), 30% dioxane (green), 40% dioxane (orange) and 50% dioxane (violet).

Figure S4 Comparison of thermal stabilities of PNA1·PNA2 in DMF and dioxane. Plots of (A) thermal melting temperature (T_m) and (B) Gibbs' free energy change (ΔG^0_{37}) of PNA1·PNA2 as a function of the amount of DMF (—▲—) and dioxane (—■—) in the medium. The aqueous buffer was 10 mM phosphate buffer containing 100 mM NaCl and 0.1 mM EDTA, pH 7.2±0.01.

Figure S5 Comparison of thermal stabilities of PNA1·DNA2 in DMF and dioxane. Plots of (A) thermal melting temperature (T_m) and (B) Gibbs' free energy change (ΔG^0_{37}) of PNA1·DNA2 as a function of the amount of DMF (—▲—) and dioxane (—■—) in the medium. The aqueous buffer was 10 mM phosphate buffer containing 100 mM NaCl and 0.1 mM EDTA, pH 7.2±0.01.

Figure S6 (A) Representative thermal melting curves of PNA1·PNA2 in pure aqueous buffer (red), 10% formamide (blue), 20% formamide (pink), 30% formamide (green), 40% formamide (orange), 50% formamide (violet), 60% formamide (magenta) and 70% formamide (teal). (B) Thermal melting curves of DNA3·DNA4 in pure aqueous buffer (red), 10% formamide (blue), 20% formamide (pink), 30% formamide (green), 40% formamide (orange), 50% formamide (violet), 60% formamide (magenta) and 70% formamide (teal).

Figure S7 Thermal and thermodynamic data of PNA duplexes in formamide. Plots of (A) thermal melting temperature (T_m) (—♦—), (B) Gibbs' free energy change (ΔG^0_{37}) (—▲—), (C) enthalpy change (ΔH^0) (—■—) and (D) entropy change (ΔS^0) (—●—) of PNA1·PNA2 as a function of the amount of formamide in the medium. The aqueous buffer was 10 mM phosphate buffer containing 100 mM NaCl and 0.1 mM EDTA, pH 7.2±0.01.

Figure S8 Thermal and thermodynamic data of DNA duplexes in formamide. Plots of (A) thermal melting temperature (T_m) (—♦—), (B) Gibbs' free energy change (ΔG^0_{37}) (—▲—), (C) enthalpy change (ΔH^0) (—■—) and (D) entropy change (ΔS^0) (—●—) of DNA3·DNA4 as a function of the amount of formamide in the medium. The aqueous buffer was 10 mM phosphate buffer containing 100 mM NaCl and 0.1 mM EDTA, pH 7.2±0.01.

Figure S9 Plots of thermodynamic data of hairpin PNAs in DMF and dioxane. Plots of (A) Gibbs' free energy change (ΔG^0_{37}), (B) enthalpy change (ΔH^0) and (C) entropy change (ΔS^0) of PNA3 (—♦—) and PNA4 (—■—) as a function of the amount of DMF in the medium. Plots of (D) Gibbs' free energy change (ΔG^0_{37}), (E) enthalpy change (ΔH^0) and (F) entropy change (ΔS^0) of PNA3 (—♦—) and PNA4 (—■—) as a function of the amount of dioxane in the medium. The aqueous buffer was 10 mM phosphate buffer containing 100 mM NaCl and 0.1 mM EDTA, pH 7.2±0.01.

Figure S10 Thermal stabilities of hairpin DNA control DNA5 in DMF and dioxane. Plots of T_m values of DNA5 as a function of the amount of (A) DMF

(—▲—) and (B) dioxane (—■—) in the medium. The aqueous buffer was 10 mM phosphate buffer containing 100 mM NaCl and 0.1 mM EDTA, pH 7.2±0.01.

Figure S11 Thermal stabilities of tT-PNA duplex in DMF compared to its unmodified control. Plots of (A) thermal melting temperature (T_m) (—▲—) and (B) Gibbs' free energy change ($\Delta G^0{}_{37}$) (—■—) of PNA5·PNA6 as a function of the amount of DMF in the medium. Plots of (C) thermal melting temperature (T_m) (—Δ—) and (D) Gibbs' free energy change ($\Delta G^0{}_{37}$) (—∇—) of PNA6·PNA7 as a function of the amount of DMF in the medium. The aqueous buffer was 10 mM phosphate buffer containing 100 mM NaCl and 0.1 mM EDTA, pH 7.2±0.01.

Figure S12 Thermal stabilities of T·T and A·A mismatched PNA duplexes in DMF. Plots of (A) thermal melting temperature (T_m) and (B) Gibbs' free energy change ($\Delta G^0{}_{37}$) of PNA1·PNA8 (—♦—) as a function of the amount of DMF in the medium. Plots of (C) thermal melting temperature (T_m) and (D) Gibbs' free energy change ($\Delta G^0{}_{37}$) of PNA2·PNA9 (—▲—) as a function of the amount of DMF in the medium. The aqueous buffer was 10 mM phosphate buffer containing 100 mM NaCl and 0.1 mM EDTA, pH 7.2±0.01.

Figure S13 Thermal stabilities of C·T mismatched PNA duplexes in DMF. Plots of (A) thermal melting temperature (T_m) (—Δ—) and (B) Gibbs' free energy change ($\Delta G^0{}_{37}$) (—□—) of PNA2·PNA10 as a function of the amount of DMF in the medium. The aqueous buffer was 10 mM phosphate buffer containing 100 mM NaCl and 0.1 mM EDTA, pH 7.2±0.01.