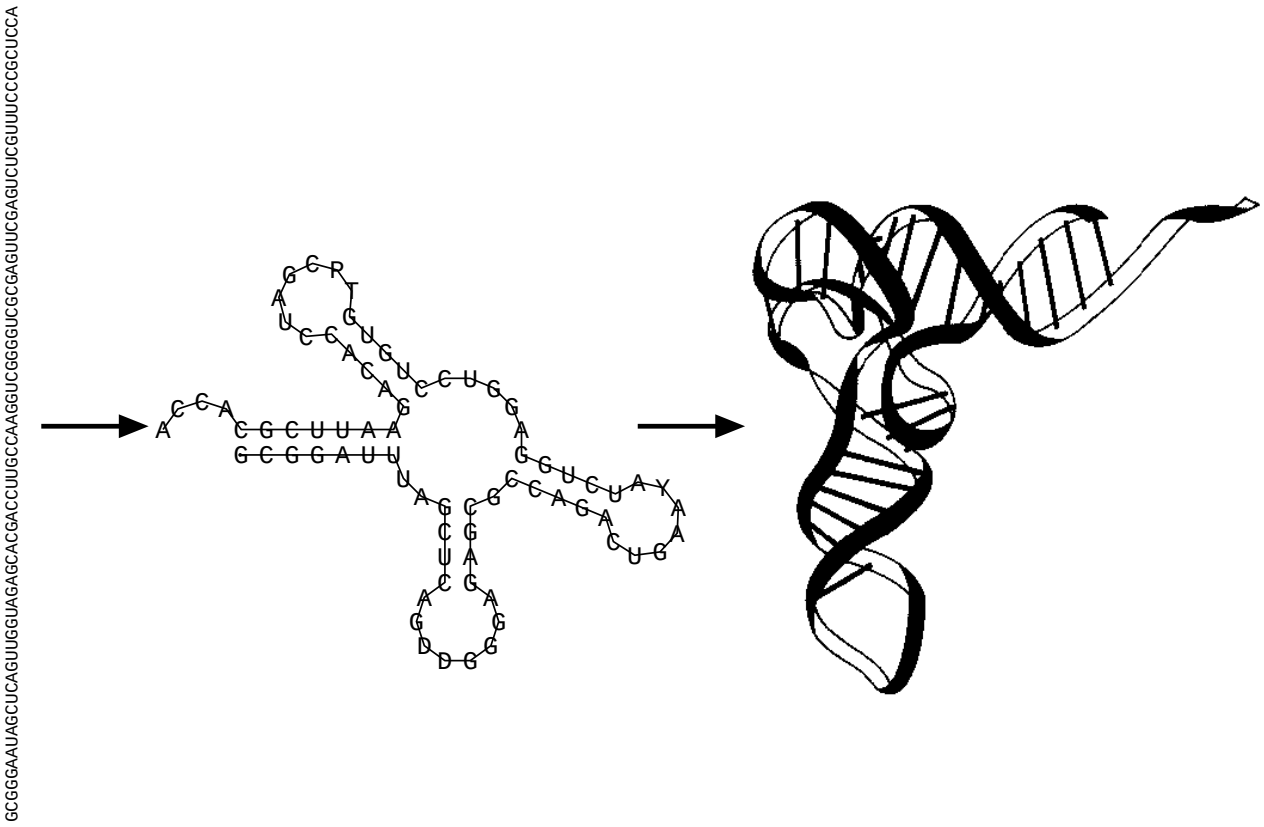
Determination of Thermodynamic Parameters of RNA Loop-Loop Interactions

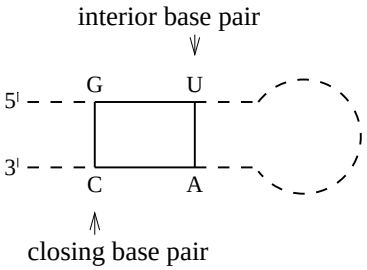
- Introduction on secondary structure prediction
- Design of the RNA molecules
- K_D-Determination with native Gels
- UV-Melting Experiments
- Results and Problems

RNA Folding as a 2-Step Process

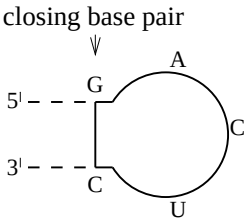


The RNA secondary structure is the base pair pattern of a folded molecule

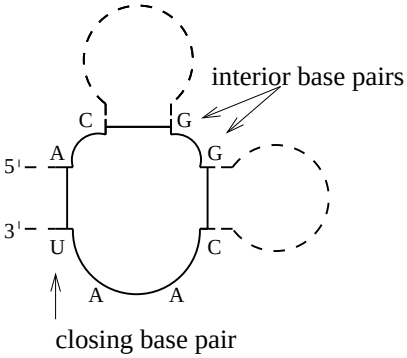
Energy Model



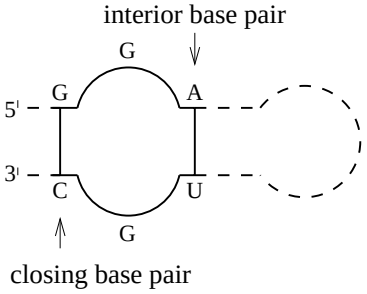
stacking pair



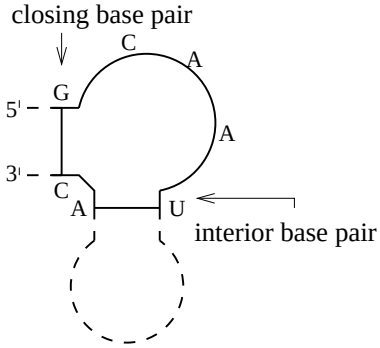
hairpin loop



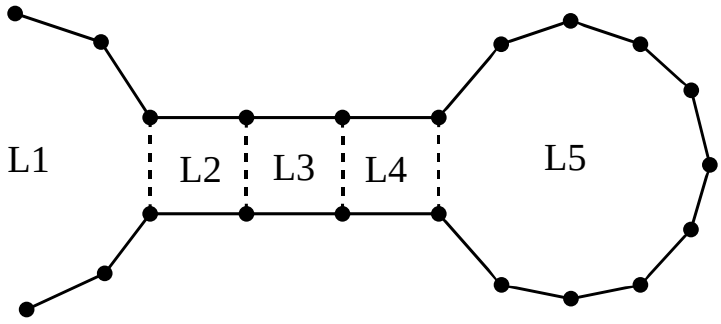
multi-loop



interior loop



bulge



$$E(S) = \sum_{loops L in S} e(L)$$

Partition Function

$$Q = \sum_{i=1}^{n} e^{-\frac{E_i}{kT}}$$

Boltzmann-weight of structure j

$$p_{E_j} = \frac{e^{-\frac{E_j}{kT}}}{\sum_{i=1}^{n} e^{-\frac{E_i}{kT}}} \qquad 0 \leq p_{E_j} \leq 1$$

Gibbs free energy

$$\Delta G = \Delta H - T\Delta S$$

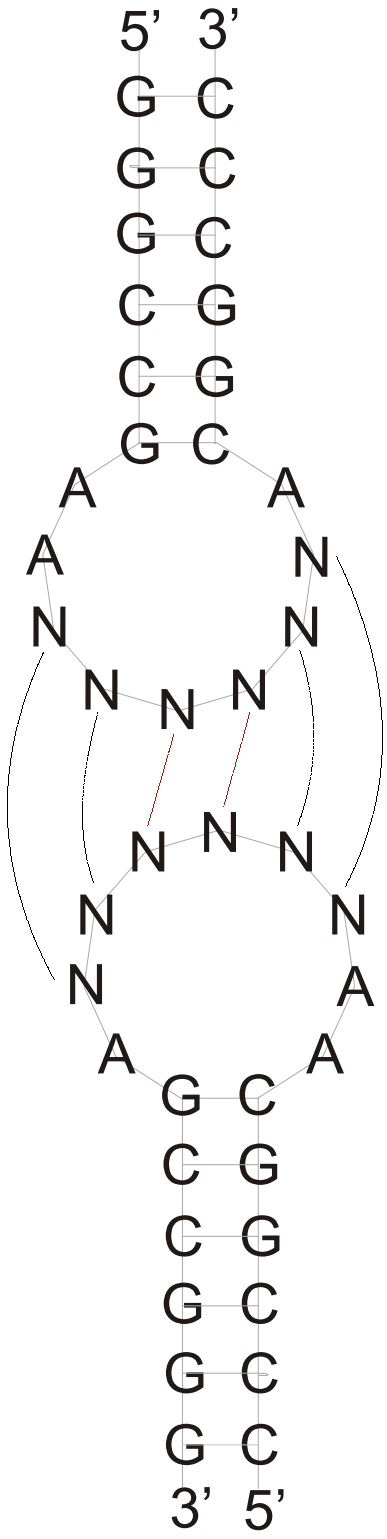
Melting temperature

$$T_m = \frac{\Delta H}{\Delta S + Rln(\frac{C_T}{4})}$$

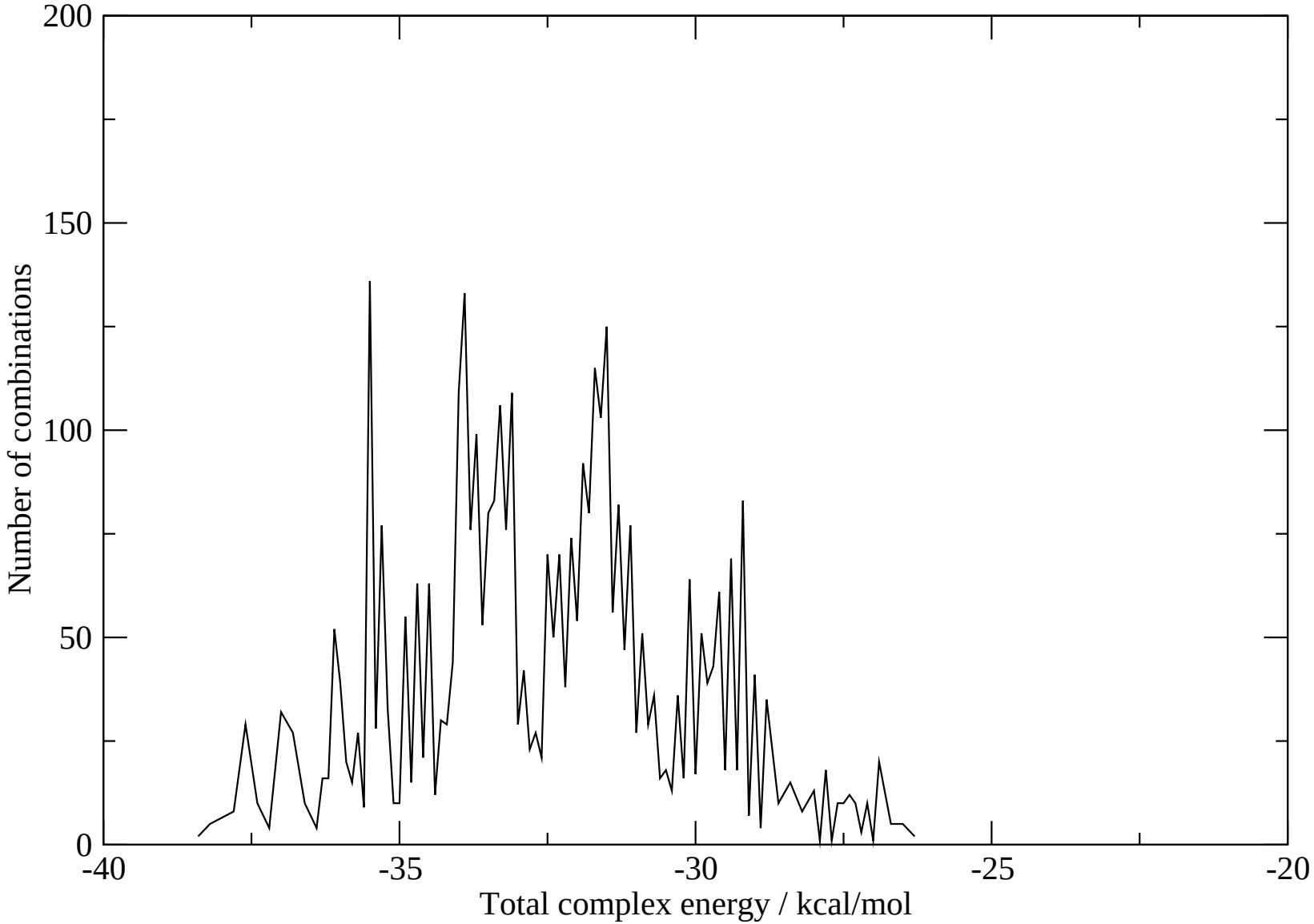
Van't Hoff analysis

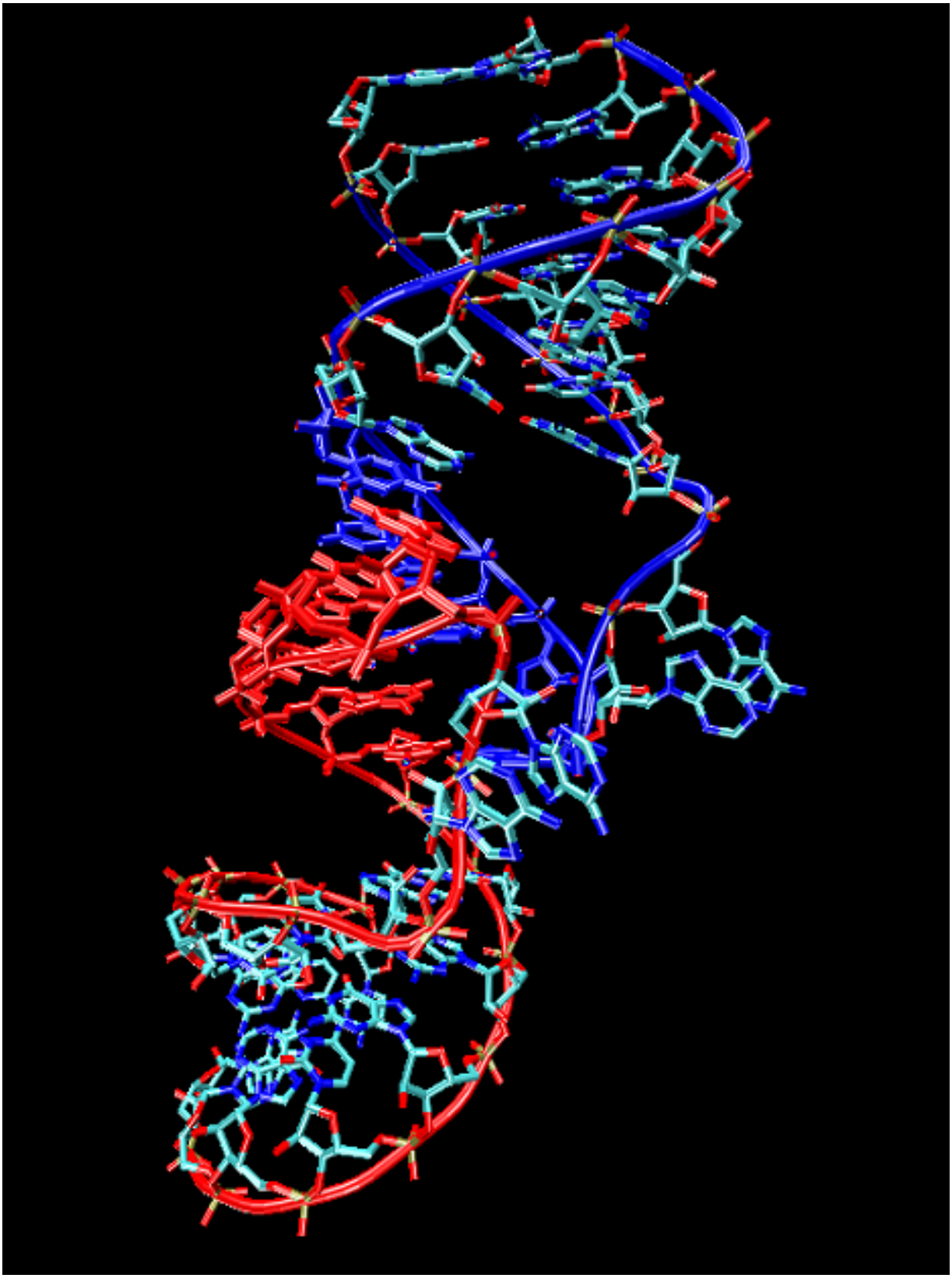
$$T_m^{-1} = \frac{R}{\Delta H} lnC_T + \frac{\Delta S}{\Delta H}$$

Structure of Loop-Loop-Complex



Distribution of Sequence-combinations

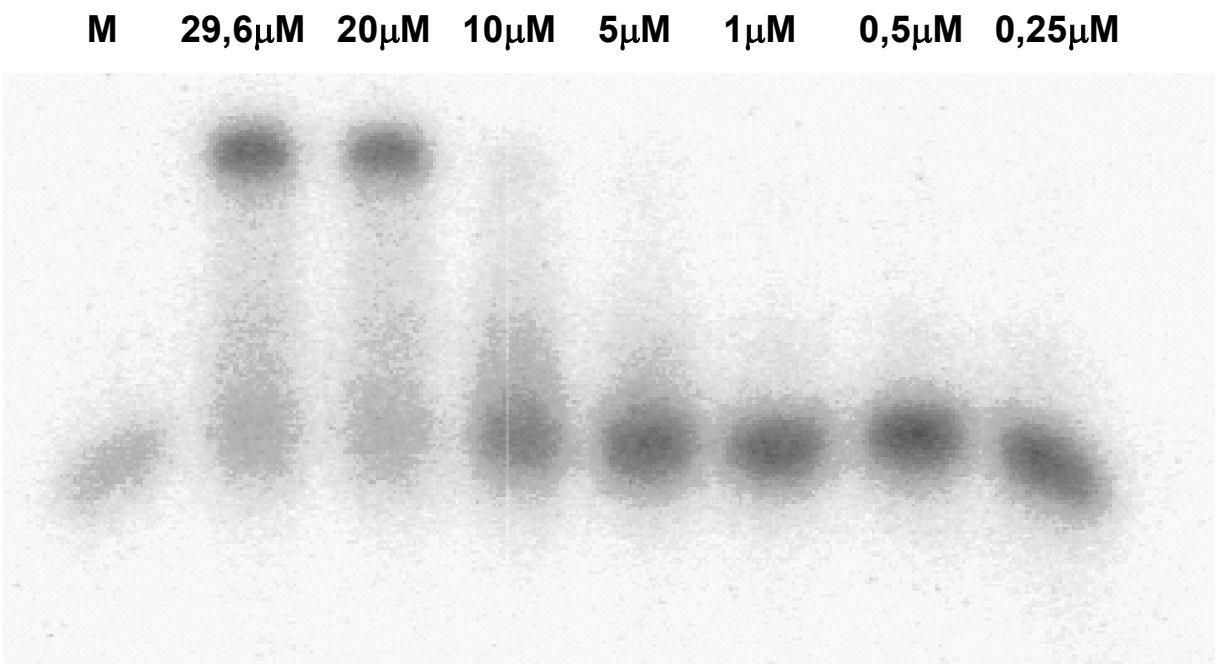




- 5’-GGGCCGAA**<u>CUAAAC</u>**ACGGCCC-3’ = St1CUAAAC
- 5’-CCCGGCAA**<u>GUUUAG</u>**AGCCGGG-3’ = St2GUUUAG
- 5’-**<u>GUUUAG</u>**-3’ = OliGUUUAG
- 5’-**<u>CUAAAC</u>**-3’ = OliCUAAAC

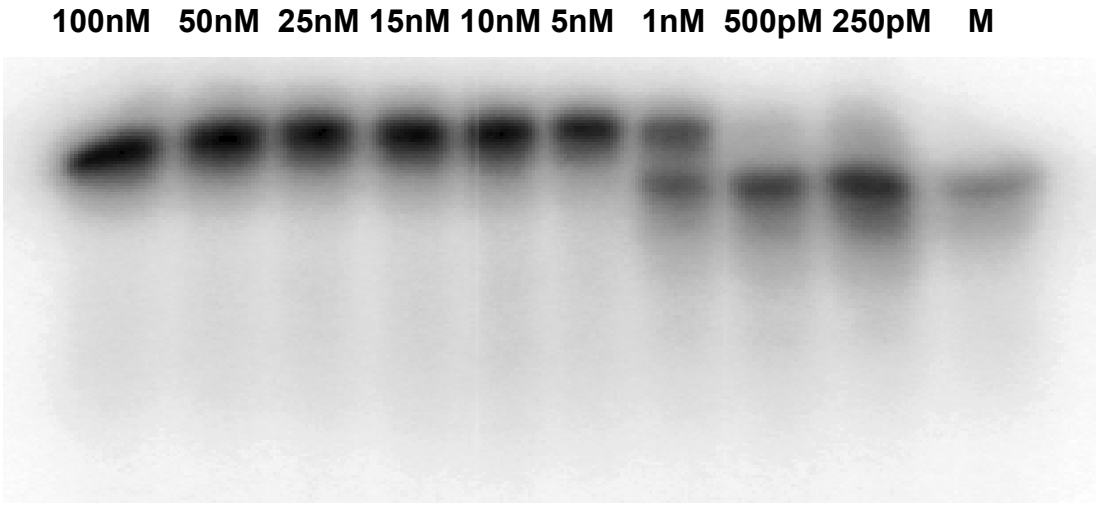
<u>$K_{D\ theoretical}$</u>	10°C	20°C	30°C
dangling A’s	1.957 μM	26.70 μM	703.26 μM
no dangling A’s	12.15 μM	130.02 μM	1.190 mM

<u>St2GUUUAG + OliCUAAAC*</u>

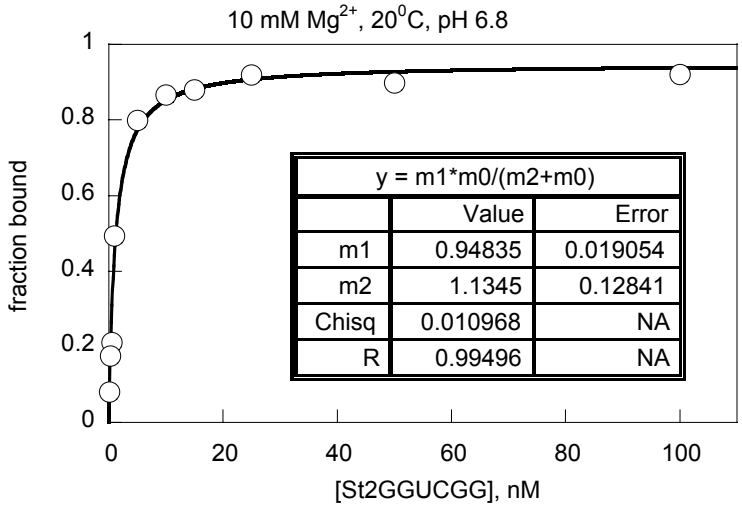


- 5’-GGGCCGAA**<u>CCGACC</u>**ACGGCCC-3’ = St1CCGACC
- 5’-CCCGGCAA**<u>GGUCGG</u>**AGCCGGG-3’ = St2GGUCGG
- 5’-**<u>GGUCGG</u>**-3’ = OliGGUCGG
- 5’-**<u>CCGACC</u>**-3’ = OliCCGACC

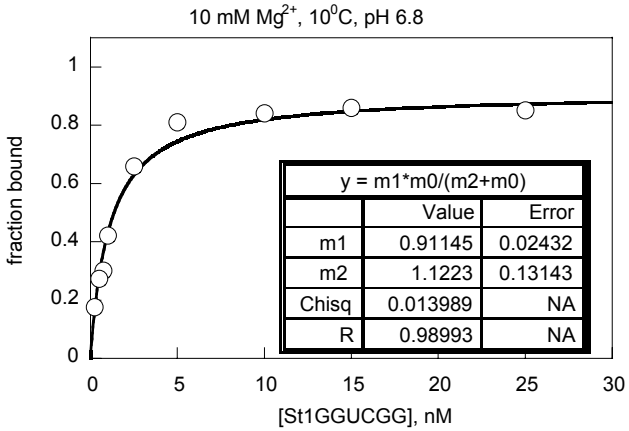
$K_{D\ theoretical}$	10°C	20°C	30°C
dangling A’s	13.38 pM	0.5 nM	2.84 nM
no dangling A’s	83.03 pM	2.461 nM	12.307 nM



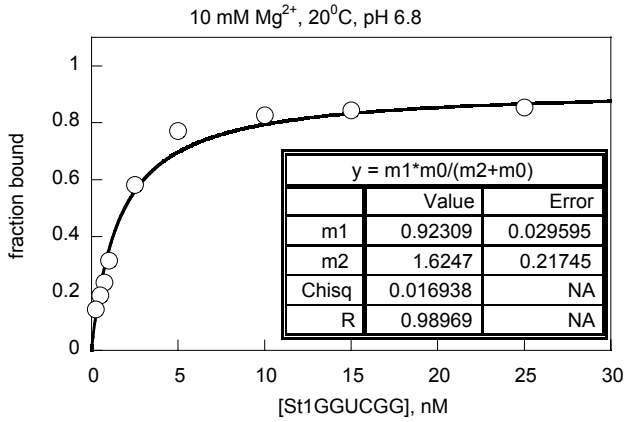
Bindung St2GGUCGG + St1CCGACC*



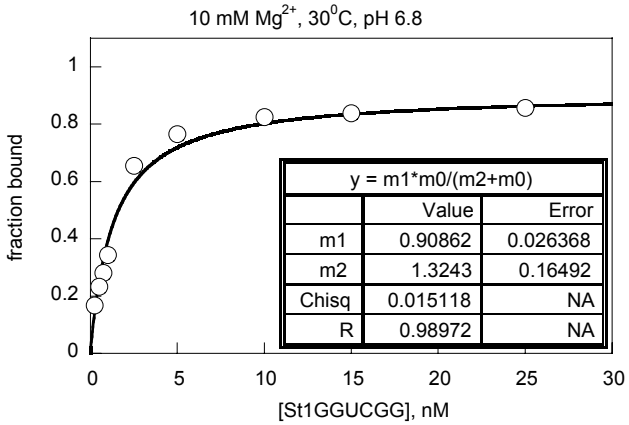
Bindung St2GGUCGG + St1CCGACC*



Bindung St2GGUCGG + St1CCGACC*

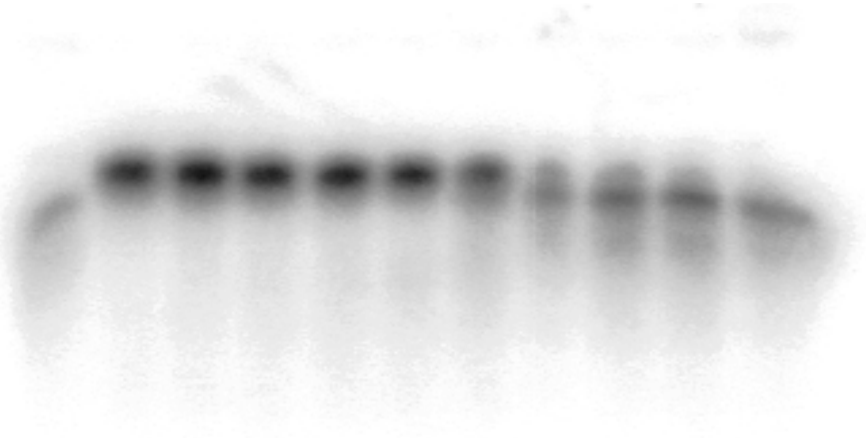


Bindung St2GGUCGG + St1CCGACC*



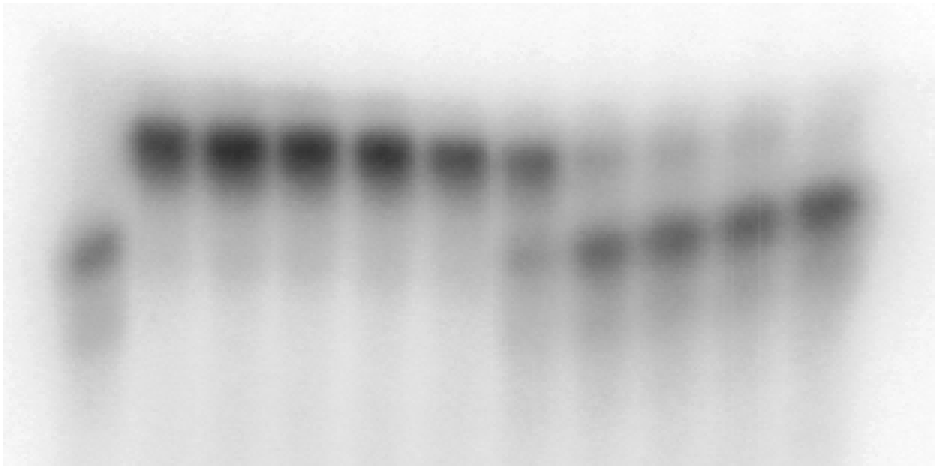
K_D-Determination-Gel of St2GGUCGG + St1CCGACC*(10°C)

M 50 25 15 10 5 2,5 1 0,75 0,5 0,25 nM



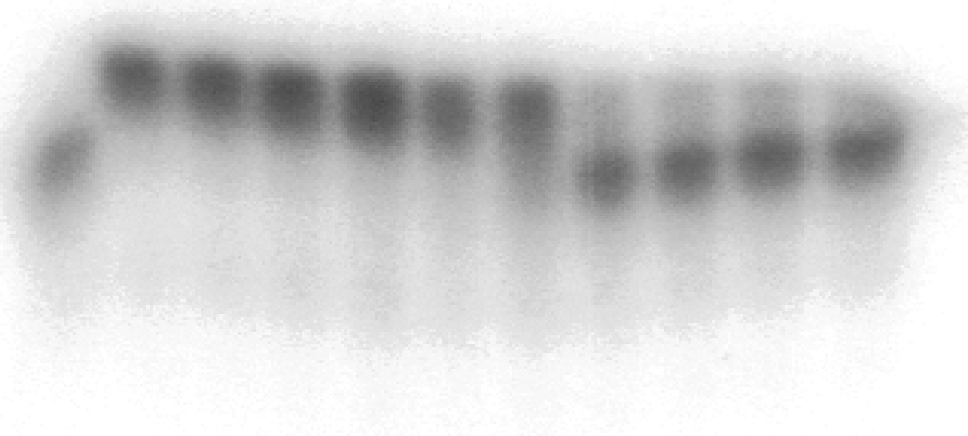
K_D-Determination-Gel of St2GGUCGG + St1CCGACC* (20°C)

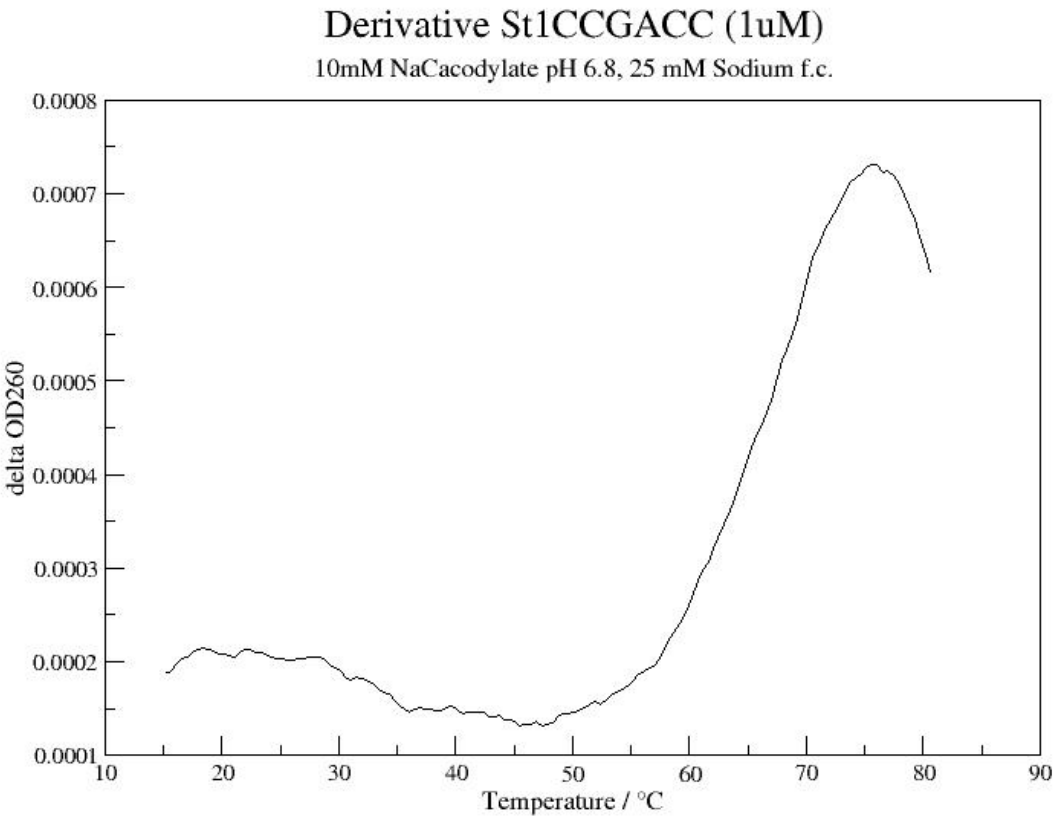
M 50 25 15 10 5 2,5 1 0,75 0,5 0,25 nM

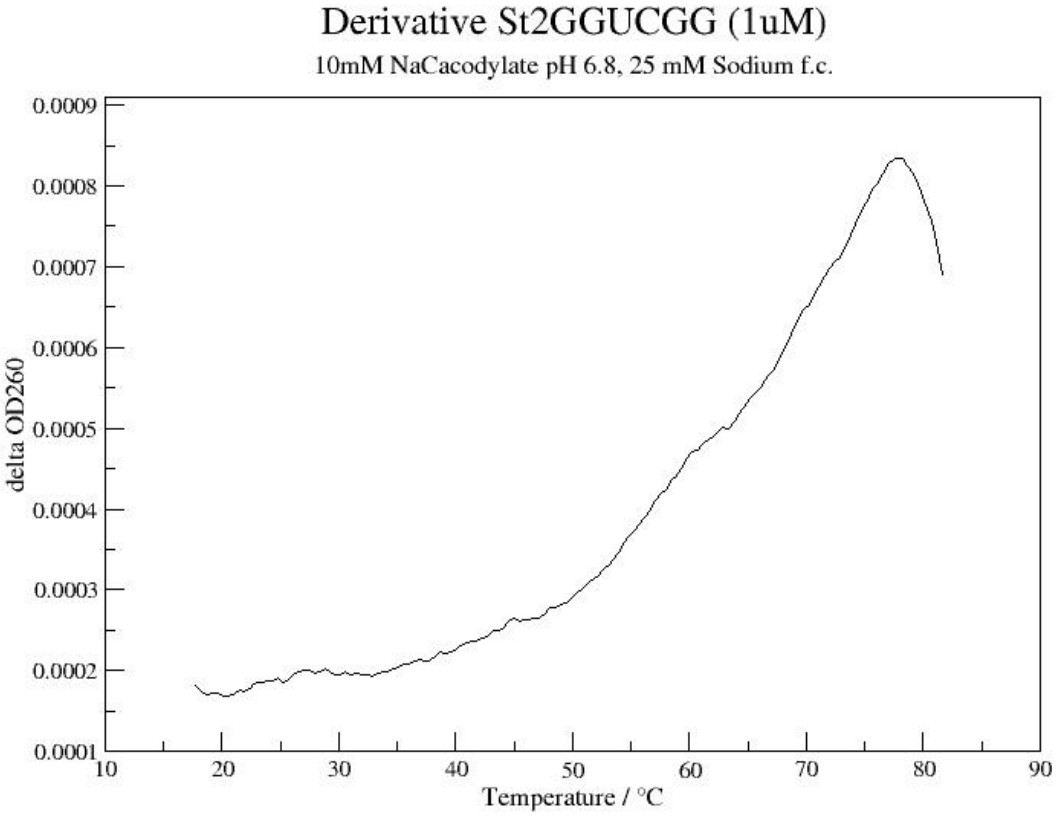


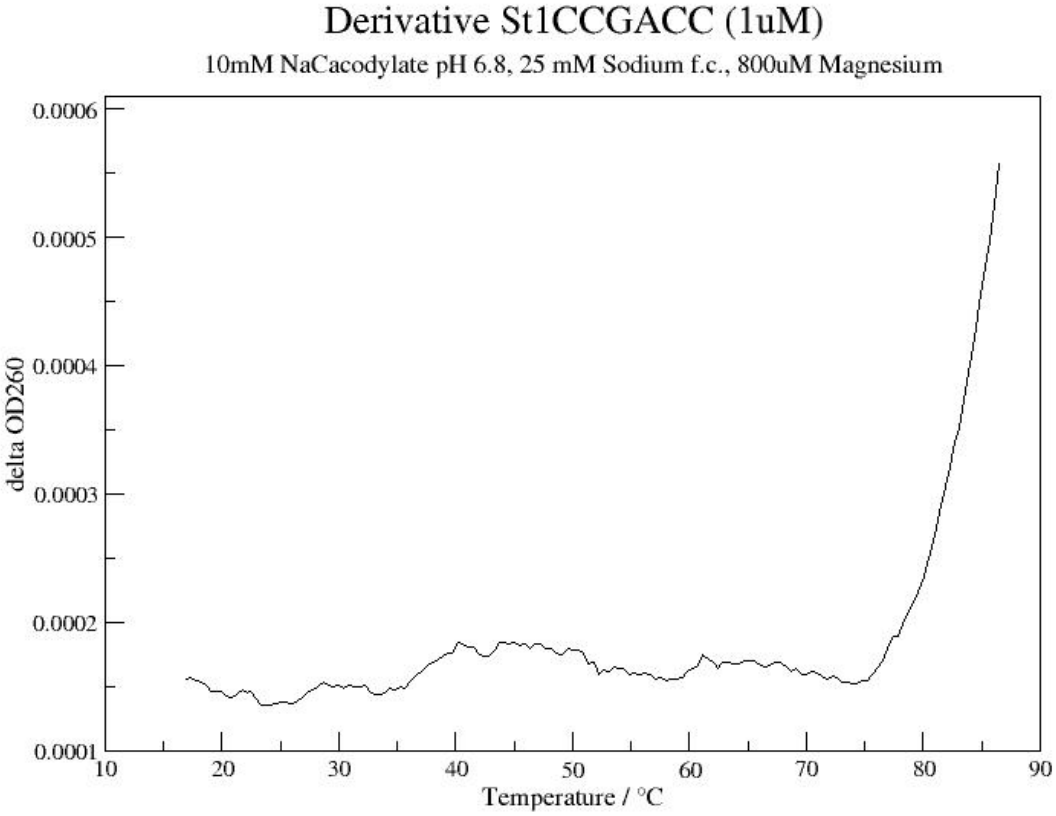
K_D-Determination-Gel of St2GGUCGG + St1CCGACC* (30°C)

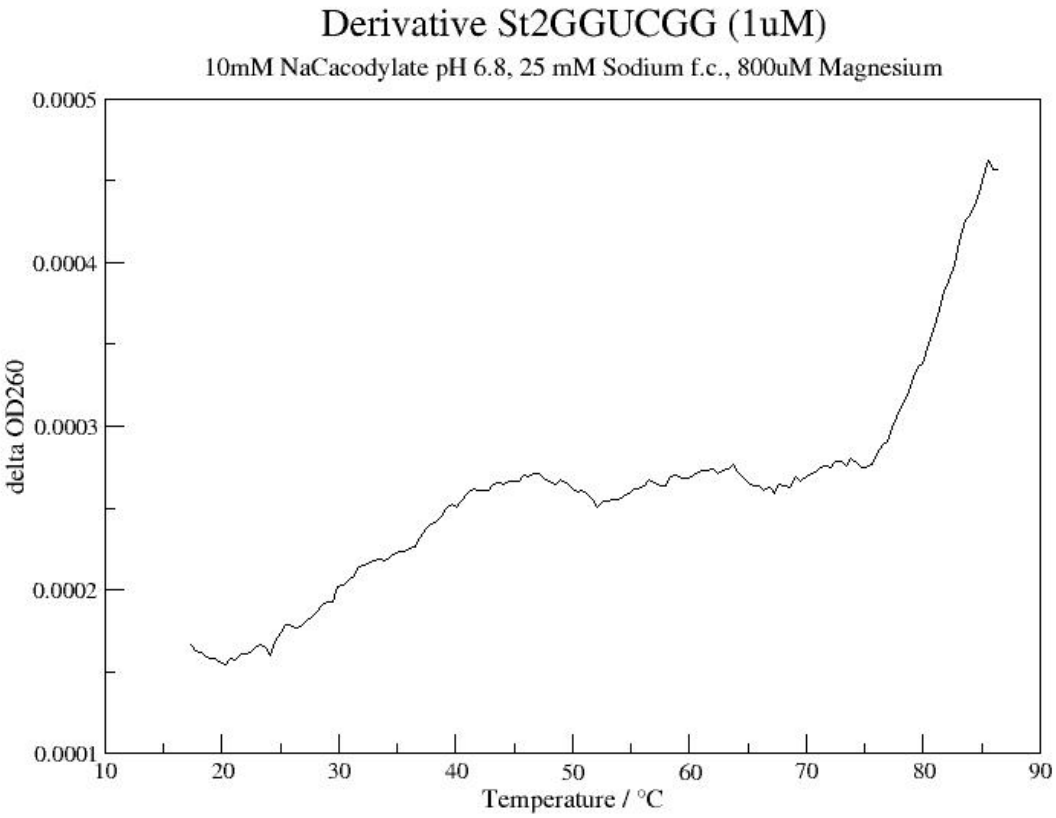
M 50 25 15 10 5 2,5 1 0,75 0,5 0,25 nM

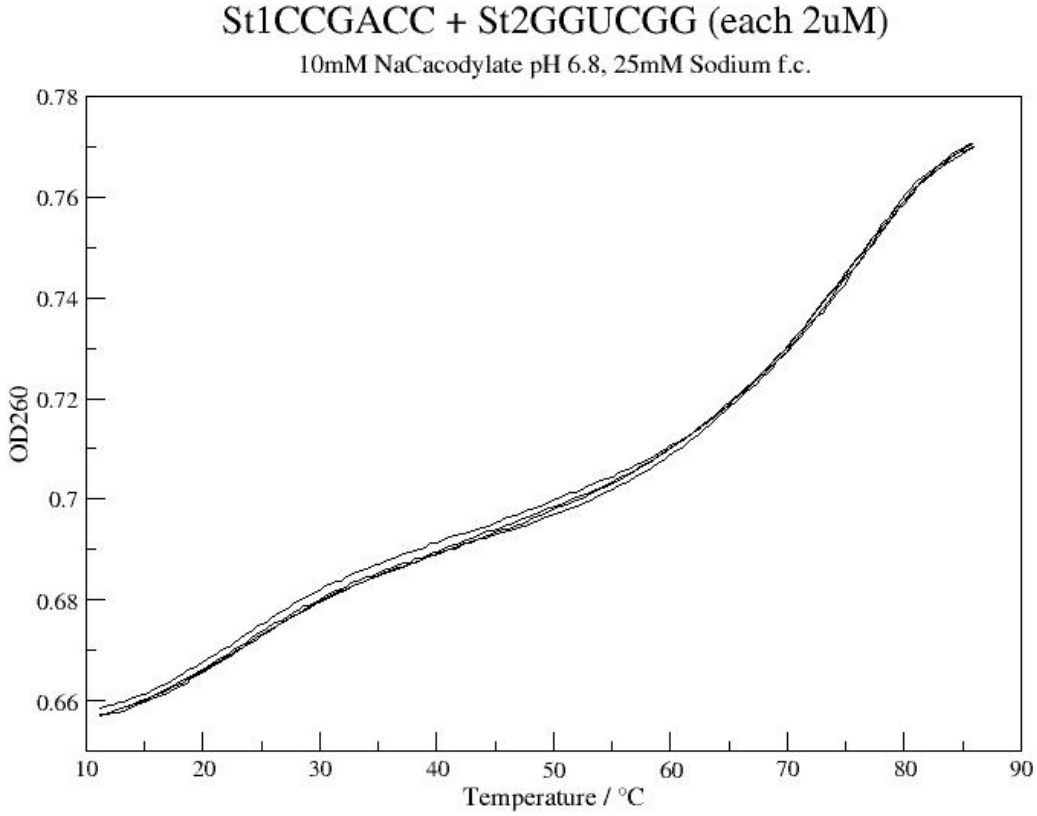


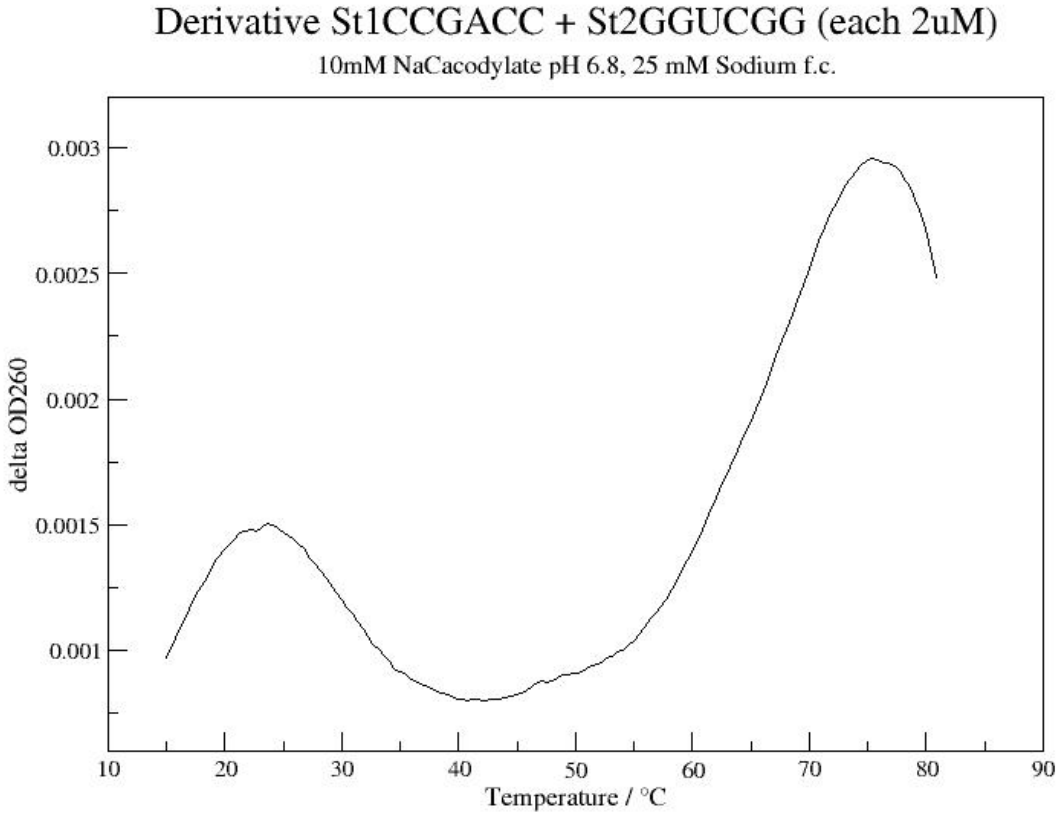

Derivative St1CCGACC (1uM)
10mM NaCacodylate pH 6.8, 25 mM Sodium f.c.
delta OD260
0.0008
0.0007
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
10
20
30
40
50
60
70
80
90
Temperature / °C


Derivative St2GGUCGG (1uM)
10mM NaCacodylate pH 6.8, 25 mM Sodium f.c.
delta OD260
0.0009
0.0008
0.0007
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
10
20
30
40
50
60
70
80
90
Temperature / °C


Derivative St1CCGACC (1uM)
10mM NaCacodylate pH 6.8, 25 mM Sodium f.c., 800uM Magnesium
delta OD260
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
10
20
30
40
50
60
70
80
90
Temperature / °C

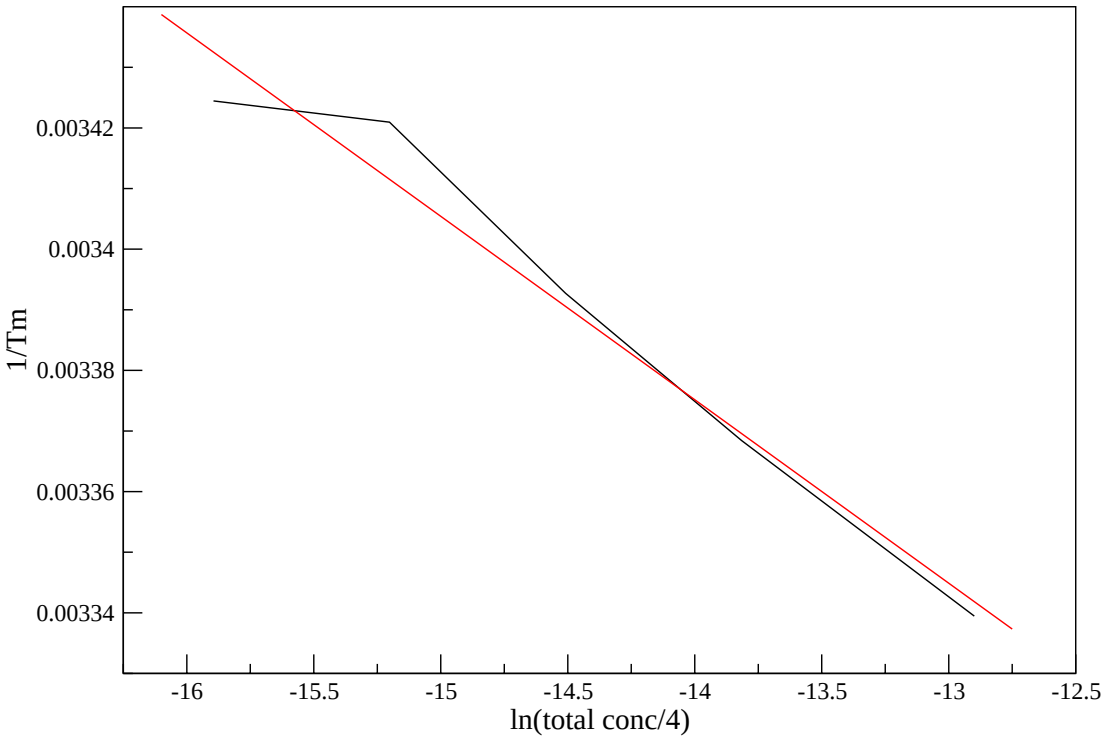

Derivative St2GGUCGG (1uM)
10mM NaCacodylate pH 6.8, 25 mM Sodium f.c., 800uM Magnesium
delta OD260
0.0005
0.0004
0.0003
0.0002
0.0001
10
20
30
40
50
60
70
80
90
Temperature / °C


St1CCGACC + St2GGUCGG (each 2uM)
10mM NaCacodylate pH 6.8, 25mM Sodium f.c.
OD260
0.78
0.76
0.74
0.72
0.7
0.68
0.66
10
20
30
40
50
60
70
80
90
Temperature / °C


Derivative St1CCGACC + St2GGUCGG (each 2uM)
10mM NaCacodylate pH 6.8, 25 mM Sodium f.c.
delta OD260
0.003
0.0025
0.002
0.0015
0.001
10
20
30
40
50
60
70
80
90
Temperature / °C

Van't Hoff Analysis

10mM Na-Cacodylate pH 6.8, 25mM Sodium f.c.



$$\frac{1}{T_m} = 0.0029514 - 3.0266 \cdot 10^{-5} \cdot ln(\frac{C_t}{4})$$

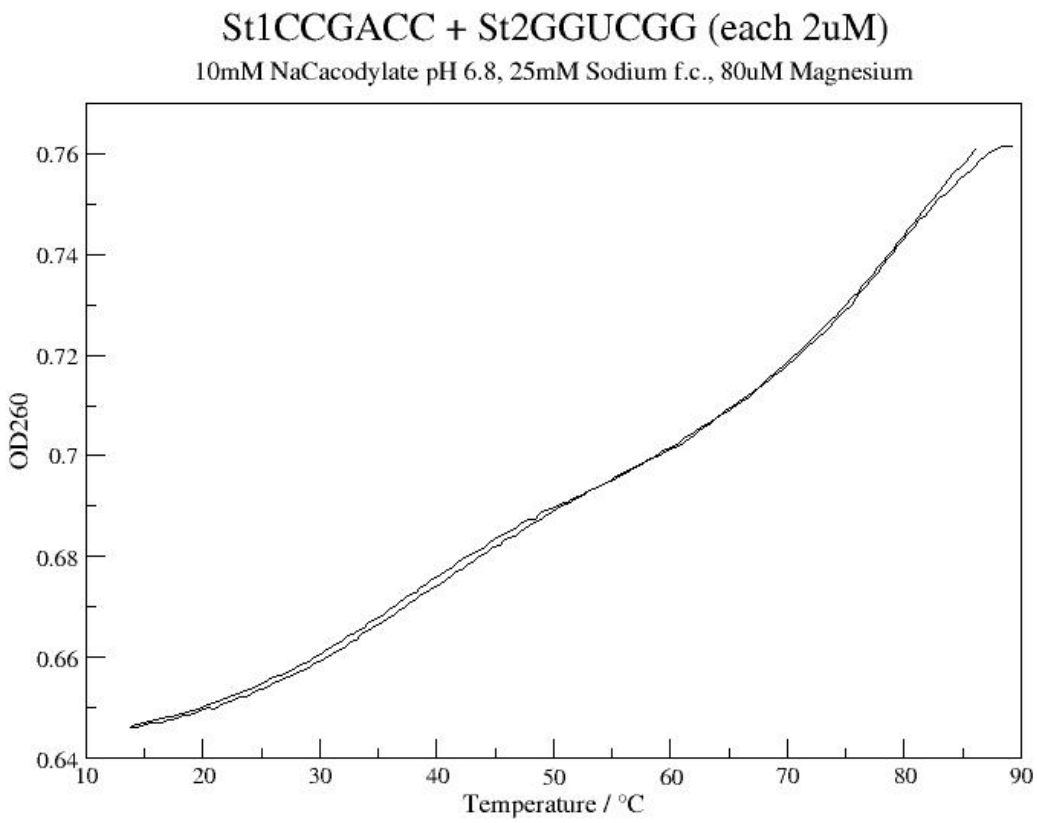
$$\frac{\Delta S}{\Delta H} = 0.0029514 \qquad \frac{R}{\Delta H} = -3.0266 \cdot 10^{-5}$$

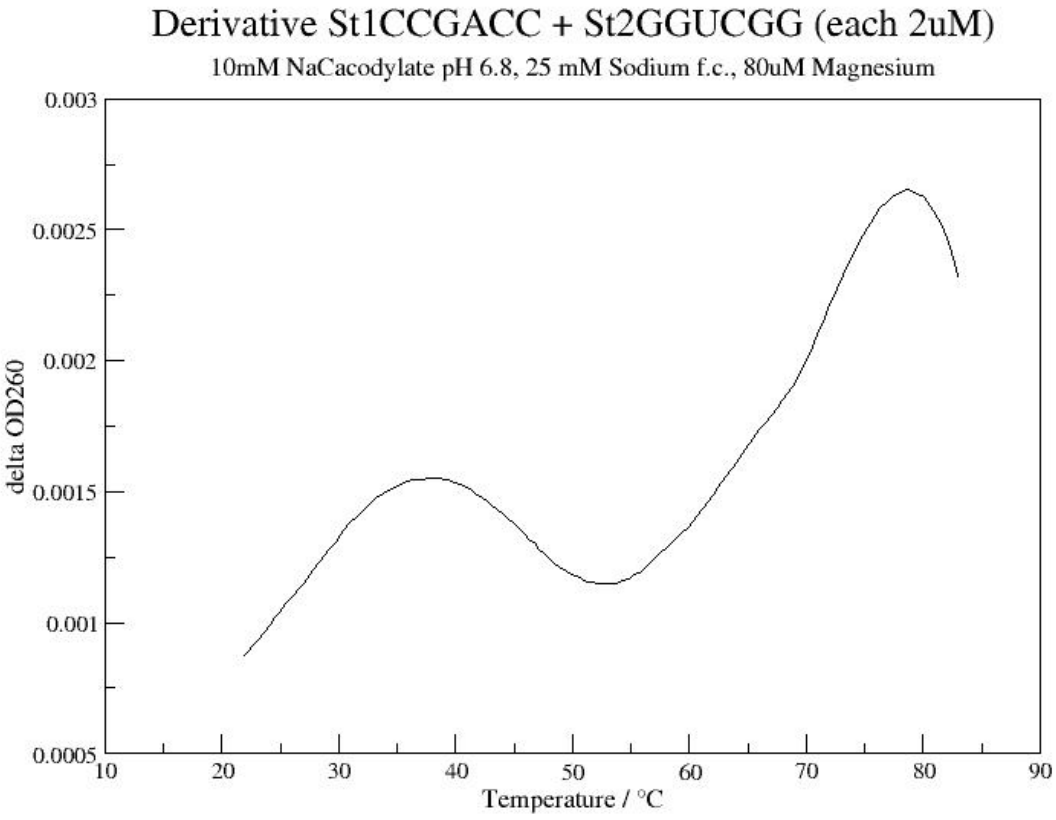
$$\Delta G = \Delta H - T \cdot \Delta S$$

$\Delta G_{37} = -5\,555.6\,cal/mol \qquad \Delta H = -65\,651.2\,cal/mol \qquad \Delta S = -193.76\,cal/mol$

Reference Duplex (5'-CCGACC-3'):

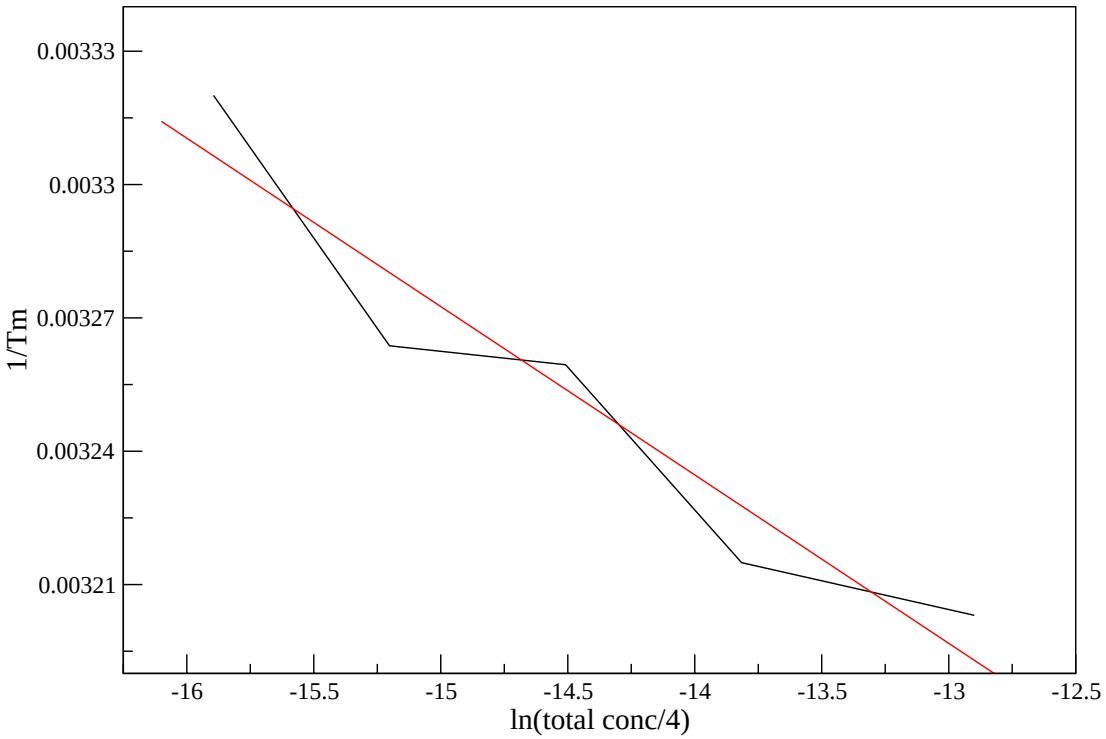
$\Delta G_{37} = -8\,974.3\,cal/mol \qquad \Delta H = -55\,900\,cal/mol \qquad \Delta S = -151.3\,cal/mol$


St1CCGACC + St2GGUCGG (each 2uM)
10mM NaCacodylate pH 6.8, 25mM Sodium f.c., 80uM Magnesium
OD260
0.76
0.74
0.72
0.7
0.68
0.66
0.64
10
20
30
40
50
60
70
80
90
Temperature / °C


Derivative St1CCGACC + St2GGUCGG (each 2uM)
10mM NaCacodylate pH 6.8, 25 mM Sodium f.c., 80uM Magnesium
delta OD260
0.003
0.0025
0.002
0.0015
0.001
0.0005
10
20
30
40
50
60
70
80
90
Temperature / °C

Van't Hoff Analysis

25mM Na-Cacodylate pH 6.8, 25mM Sodium f.c., 80 uM Magnesium



$$\frac{1}{T_m} = 0.0027043 - 3.7883 \cdot 10^{-5} \cdot ln(\frac{C_t}{4})$$

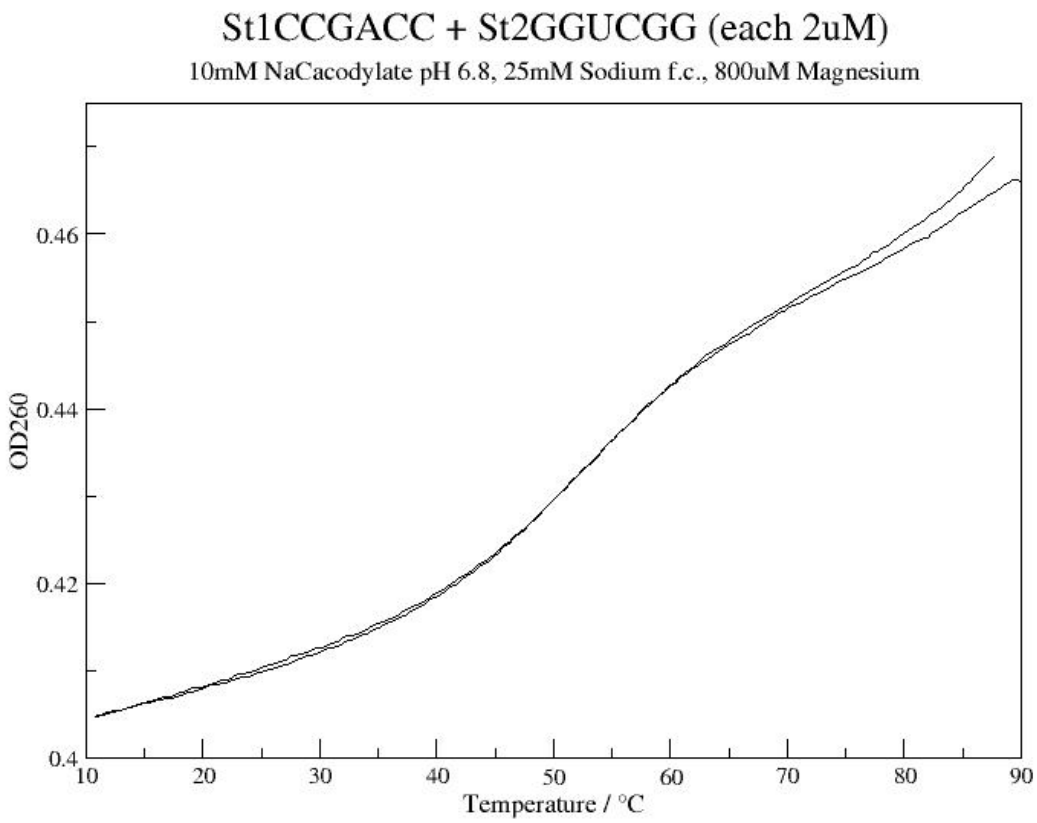
$$\frac{\Delta S}{\Delta H} = 0.0027043 \qquad \frac{R}{\Delta H} = -3.7883 \cdot 10^{-5}$$

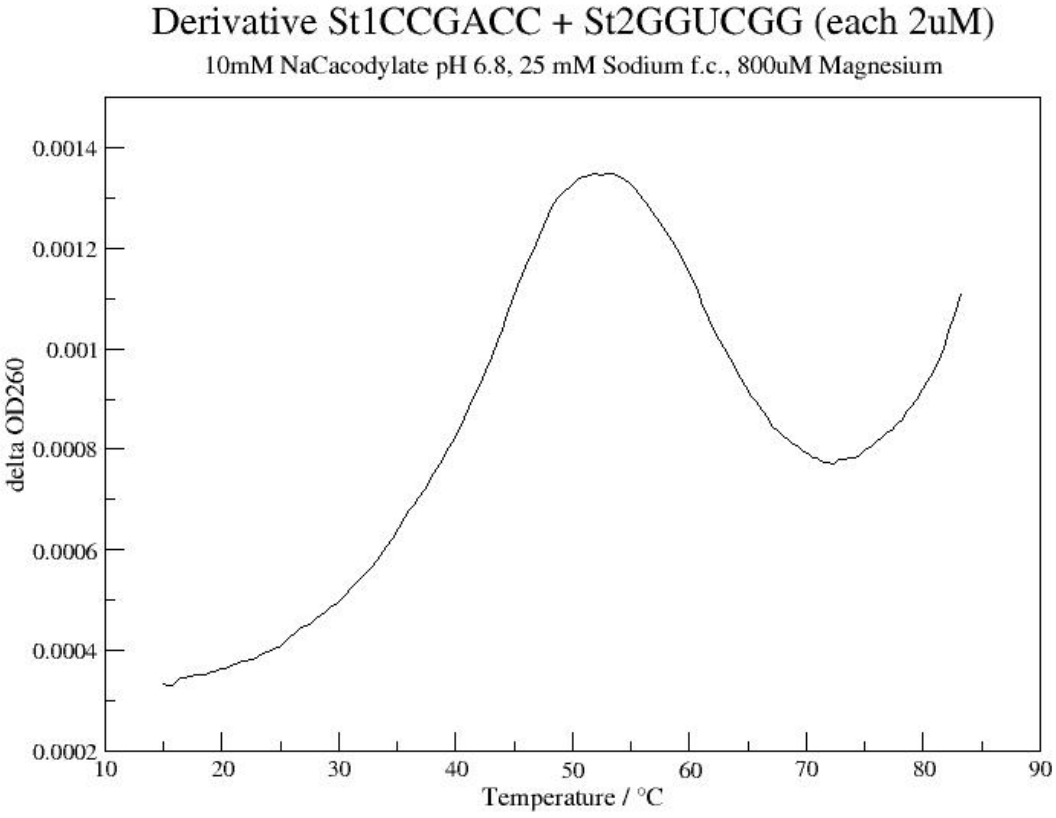
$$\Delta G = \Delta H - T \cdot \Delta S$$

$\Delta G_{37} = -8\,458.3\,cal/mol \qquad \Delta H = -52\,451\,cal/mol \qquad \Delta S = -141.84\,cal/mol$

Reference Duplex (5'-CCGACC-3'):

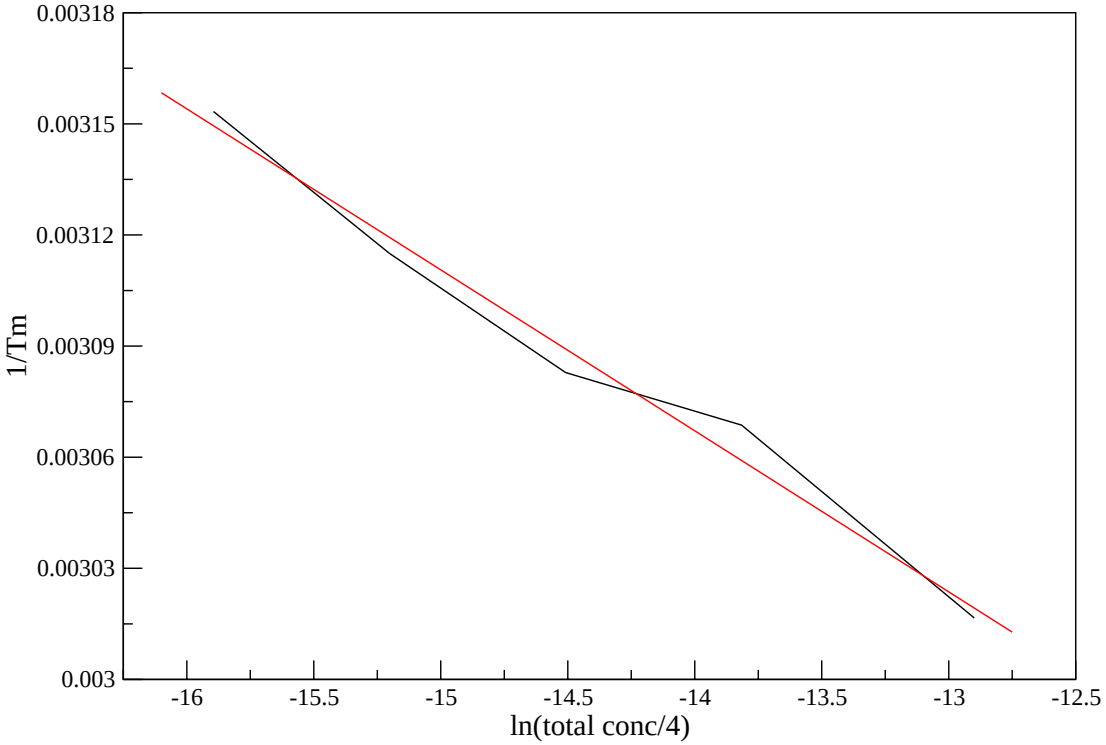
$\Delta G_{37} = -8\,974.3\,cal/mol \qquad \Delta H = -55\,900\,cal/mol \qquad \Delta S = -151.3\,cal/mol$


St1CCGACC + St2GGUCGG (each 2uM)
10mM NaCacodylate pH 6.8, 25mM Sodium f.c., 800uM Magnesium
OD260
0.46
0.44
0.42
0.4
10
20
30
40
50
60
70
80
90
Temperature / °C


Derivative St1CCGACC + St2GGUCGG (each 2uM)
10mM NaCacodylate pH 6.8, 25 mM Sodium f.c., 800uM Magnesium
delta OD260
0.0014
0.0012
0.001
0.0008
0.0006
0.0004
0.0002
10
20
30
40
50
60
70
80
90
Temperature / °C

Van't Hoff Analysis

10mM Na-Cacodylate pH 6.8, 25mM Sodium f.c., 800 uM Magmesium



$$\frac{1}{T_m} = 0.0024586 - 4.3469 \cdot 10^{-5} \cdot ln(\frac{C_t}{4})$$

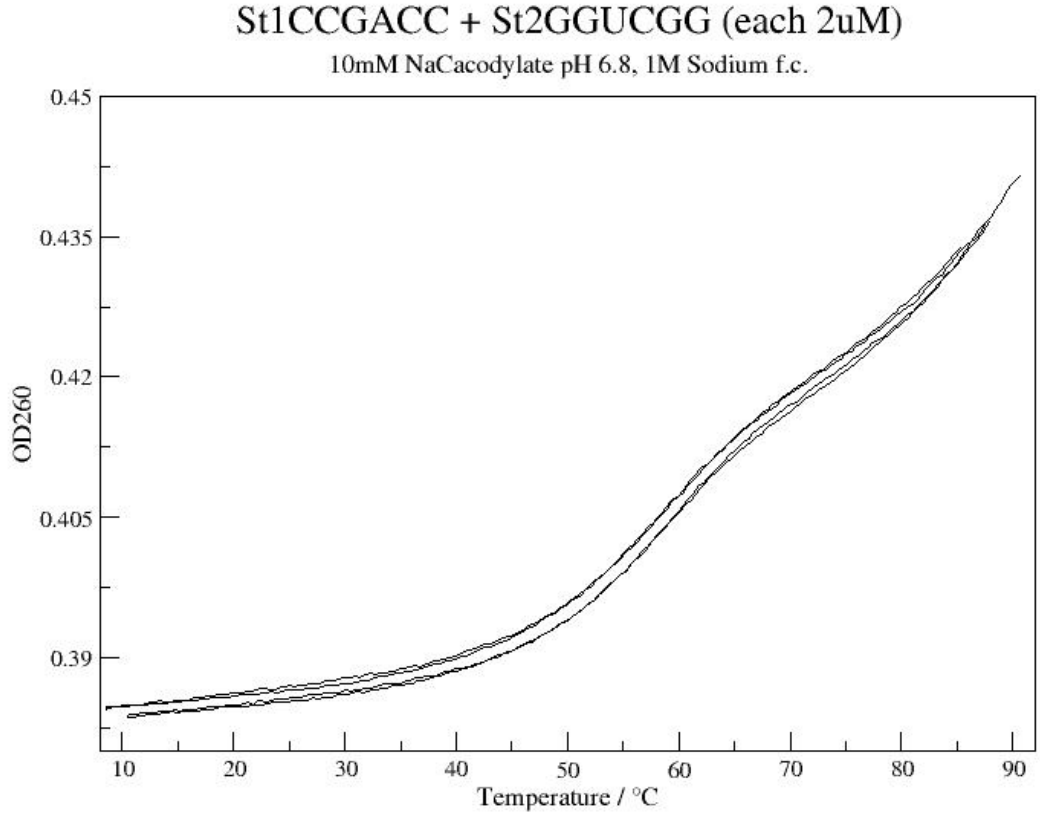
$$\frac{\Delta S}{\Delta H} = 0.0024586 \qquad \frac{R}{\Delta H} = -4.3469 \cdot 10^{-5}$$

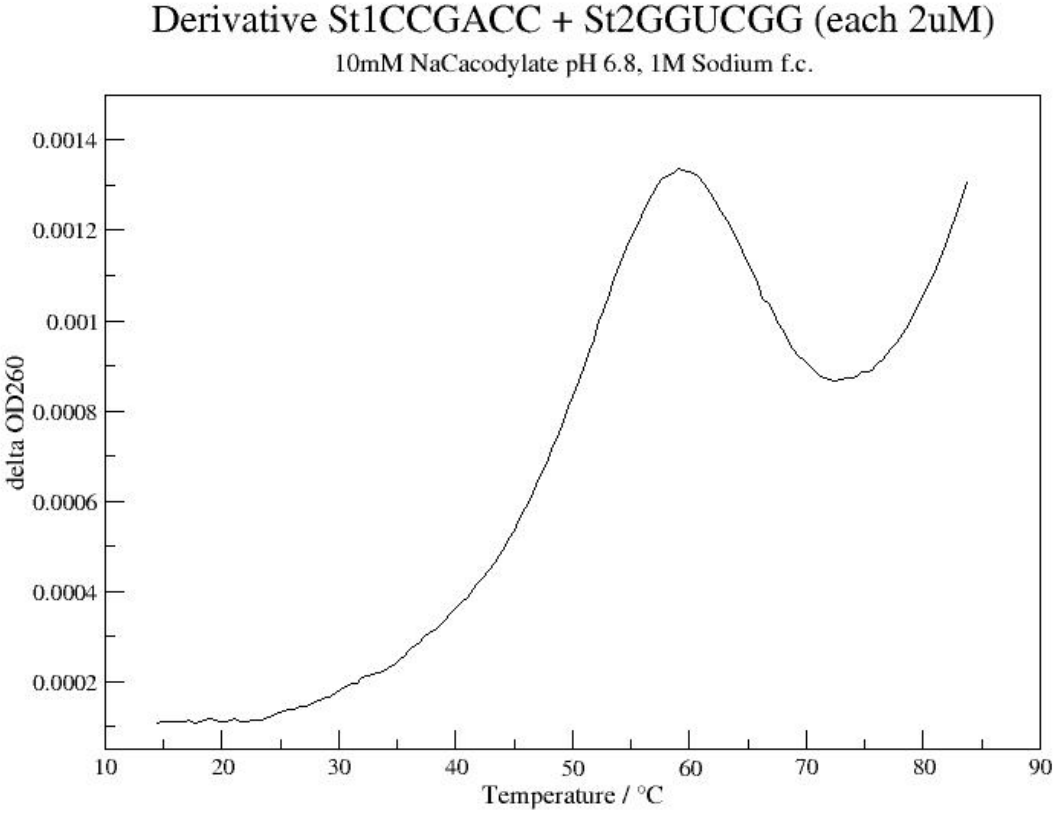
$$\Delta G = \Delta H - T \cdot \Delta S$$

$\Delta G_{37} = -10\,854.7\,cal/mol \qquad \Delta H = -45\,710.7\,cal/mol \qquad \Delta S = -112.38\,cal/mol$

Reference Duplex (5'-CCGACC-3'):

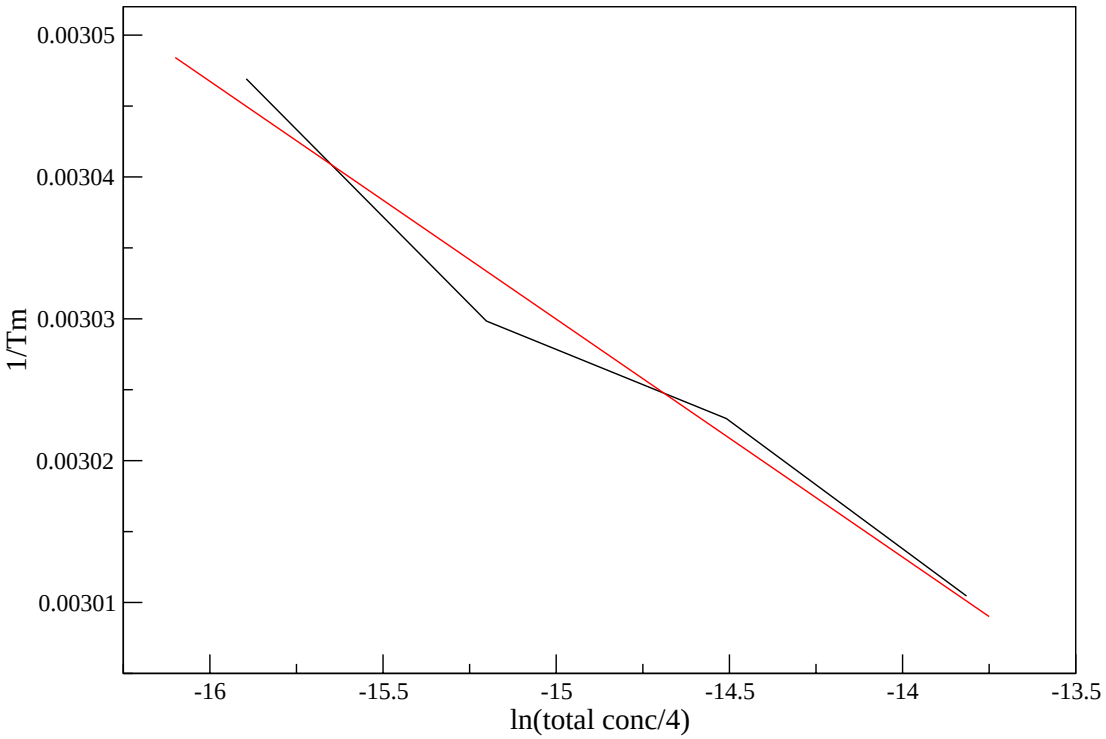
$\Delta G_{37} = -8\,974.3\,cal/mol \qquad \Delta H = -55\,900\,cal/mol \qquad \Delta S = -151.3\,cal/mol$


St1CCGACC + St2GGUCGG (each 2uM)
10mM NaCacodylate pH 6.8, 1M Sodium f.c.
OD260
0.45
0.435
0.42
0.405
0.39
10
20
30
40
50
60
70
80
90
Temperature / °C


Derivative St1CCGACC + St2GGUCGG (each 2uM)
10mM NaCacodylate pH 6.8, 1M Sodium f.c.
delta OD260
0.0014
0.0012
0.001
0.0008
0.0006
0.0004
0.0002
10
20
30
40
50
60
70
80
90
Temperature / °C

Van't Hoff Analysis

10mM Na-Cacodylate pH 6.8, 1M Sodium f.c.



$$\frac{1}{T_m} = 0.0027784 - 1.6771 \cdot 10^{-5} \cdot ln(\frac{C_t}{4})$$

$$\frac{\Delta S}{\Delta H} = 0.0027784 \qquad \frac{R}{\Delta H} = -1.6771 \cdot 10^{-5}$$

$$\Delta G = \Delta H - T \cdot \Delta S$$

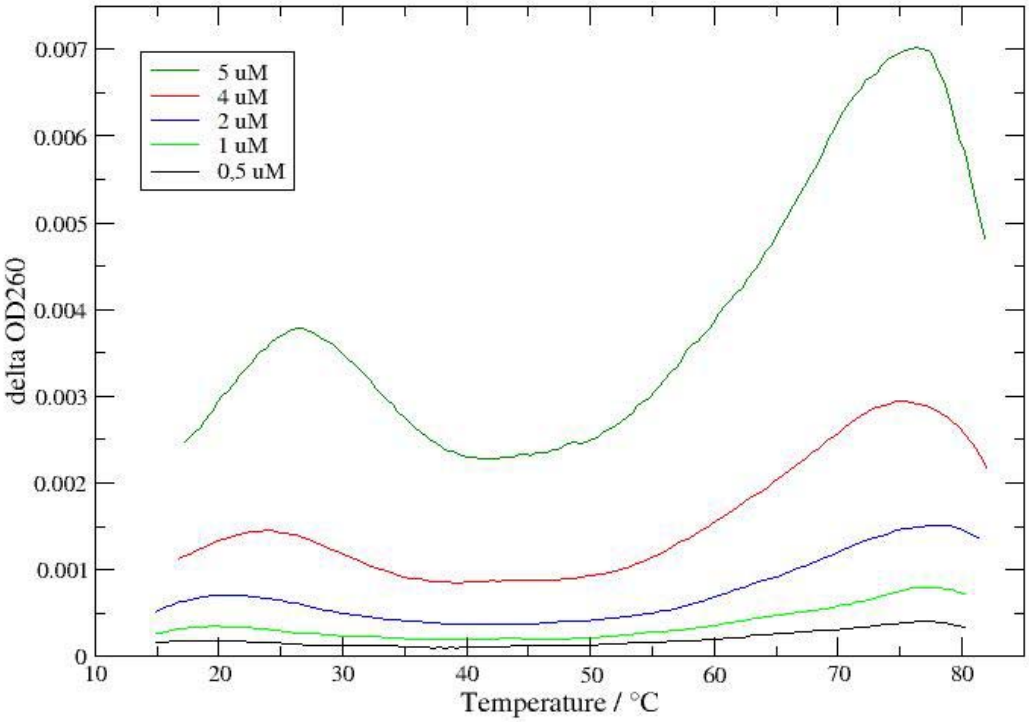
$\Delta G_{37} = -16\,383.1\, cal/mol \qquad \Delta H = -118\,478\, cal/mol \qquad \Delta S = -329.18\, cal/mol$

Reference Duplex (5'-CCGACC-3'):

$\Delta G_{37} = -8\,974.3\, cal/mol \qquad \Delta H = -55\,900\, cal/mol \qquad \Delta S = -151.3\, cal/mol$

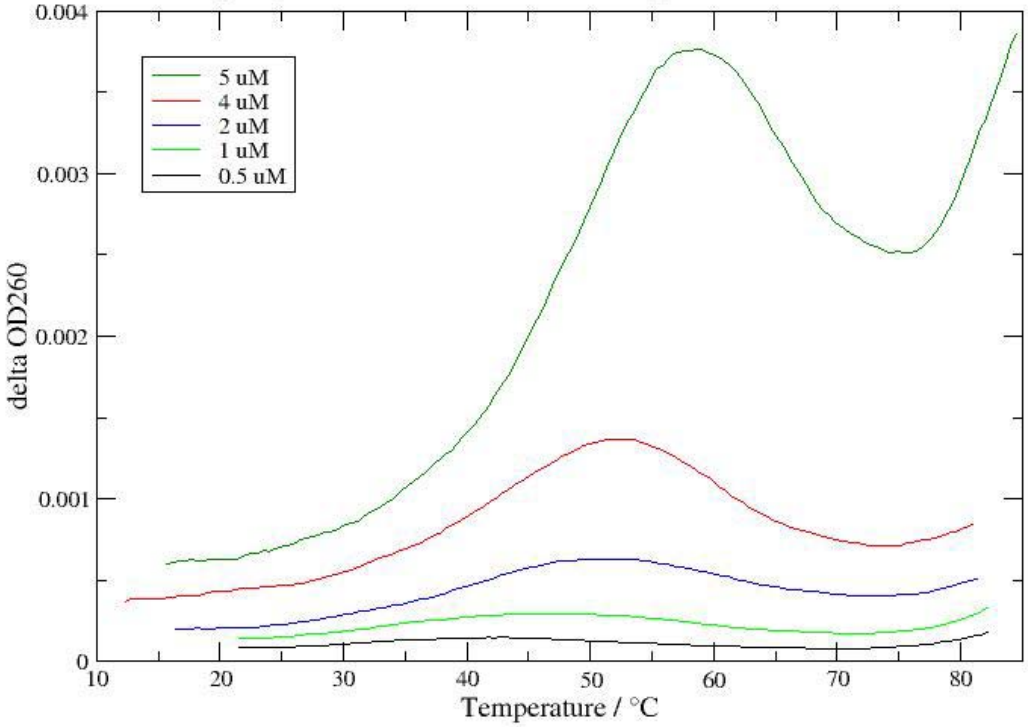
Superposition of St1CCGACC + St2GGUCGG

10mM NaCacodylate pH 6.8, 25mM Sodium f.c., 0.5 to 10 uM total strand conc.



Superposition of St1CCGACC + St2GGUCGG

10mM NaCacodylate pH 6.8, 25 mM Sodium, 800uM Magnesium, 0.5 to 10 uM total strand conc.



Results so far

- No complex-formation observable between St1CUAAAC and St2GUUUAC
- Only binding between Oligo and Hairpin detectable
- Complex forms between St1CCGACC and St2GGUCGG
- K_D of formation is around 1 to 1.5 nM
- Melting temperature higher as expected
- No difference in K_D at different temperatures
- Competitive binding of 6mer very slow

- UV-Melting Experiments show that Mg^{2+} increases stability of Kissing-Complex much more than for the Reference Duplex
- Kissing-Complex is much more stable at 1M Na^{+} than the Reference Duplex
- Unexpected Behavior of ΔH and ΔS with increasing Mg^{2+}
- ΔG does not reflect previously determined K_D