# Evaluation of Thermodynamic Stabilities of *in silico* Designed Nucleic Acid 3WJ Motifs

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## Abstract

In this report, we focused our study on evaluating the thermodynamic parameters of several *in-silico* designed three-way junction (3WJ) DNA structural elements. The designed 3WJ motifs contain three helical stems linked with 4, 3, 2, 1, or 0 single-stranded Thymidines (T). We found that all 3WJ constructs assemble efficiently as investigated by gel shift assay. Our experiments revealed that the number of Ts linkages in the three-way junction dictate the stability of the overall 3WJ conformations. We determined that formation of 2T d3WJ motif is the most favorable while 0T d3WJ is the least favorable.

## Introduction

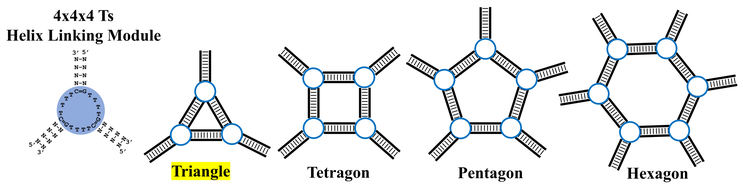
Nucleic Acid (NA) nanotechnology is an emerging field that demonstrates the use of polynucleotides as a versatile biopolymer to create nanostructures of different dimensions and shapes in a programmable and predictable manner. The stable double helix configuration of DNA or RNA strands mainly depends on the Watson-Crick (canonical) base pair composition (G=C and A-T or A-U in the case of RNA), base stacking, and metal ion concentrations. The thermodynamic parameters of DNA B-form helix formation and RNA A-form helix can be calculated using empirically defined sets of nearest neighboring parameters found in 2D structure predicting programs such as *mfold [1]*. However, these programs lack parameters for hybrid DNA/RNA base pairing and non-canonical base interactions.

## Significance

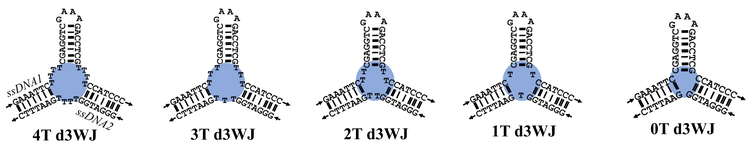
This study is important because it is expected to contribute to the existing set of parameters used for NA structure prediction algorithms, as well as provide guidance for the rational design of NA nanostructures.

## Results

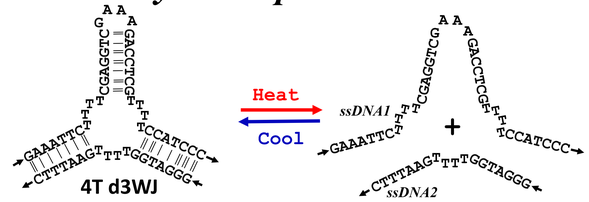
### *2D DNA polygons designed from d3JW motif [2]*



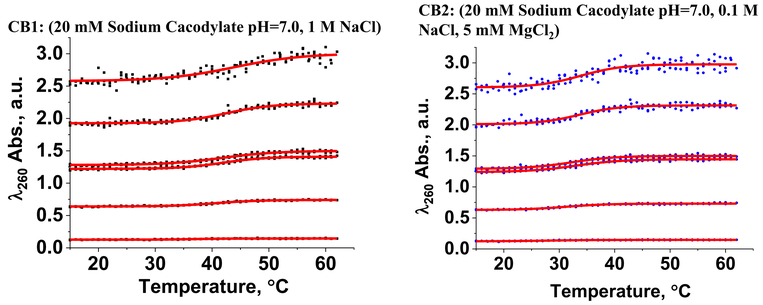

### *DNA dimers forming 3WJ regions for UV-melting studies*




### *d3WJ Thermodynamic parameters calculation*




### UV-melting curve fitting




**UV-melt curve fitted by Boltzmann function:**

$$y = \frac{A_1 - A_2}{1 + e^{(x - x_0)/dx}} + A_2$$

**$X_0$ is the melting temperature (Tm), dx is the slope of the curve at Tm, A1 and A2 are the baseline values of the curve, and A2 - A1 is the amplitude of the curve.**

**Table 1. Derived Tm values for 4T d3WJ in CB1 and CB2**

| Tm in CB1 (°C) | error | $R^2$ | Conc. (M) | Tm in CB2 (°C) | error | Conc. (M) | $R^2$ |
|---|---|---|---|---|---|---|---|
| 38.7 | 0.6 | 0.83 | 4.0293E-07 | 28.1 | 0.9 | 4.4E-07 | 0.84 |
| 40.6 | 0.3 | 0.98 | 2.3443E-06 | 31.2 | 0.3 | 2.31E-06 | 0.97 |
| 41.9 | 0.3 | 0.98 | 4.3956E-06 | 32.9 | 0.3 | 4.54E-06 | 0.97 |
| 41.7 | 0.4 | 0.97 | 3.1868E-06 | 32.2 | 0.3 | 4.69E-06 | 0.97 |
| 42.4 | 0.4 | 0.95 | 7.033E-06 | 33.6 | 0.5 | 7.33E-06 | 0.92 |
| 43.9 | 1.2 | 0.84 | 9.3407E-06 | 33.8 | 0.9 | 9.52E-06 | 0.80 |

Extinction coefficient = 546,000 $M^{-1}cm^{-1}$ , UV-cell path length 0.5 cm
Tm error obtaioned from the sigmoidal fit of fours transition curves

### Van't Hoff analysis[3]

For a non-self-complementary DNA duplex, the equilibrium reaction:

$$\mathbf{ssDNA_1 + ssDNA_2 \rightleftharpoons dsDNA}$$

Assuming a two-state model, the association const. at the midpoint (50% ssDNA and 50% dsDNA) is $K_{50} = 4/[Ct]$, where Ct is the sum of the concentrations of two ssDNAs. At equilibrium process:

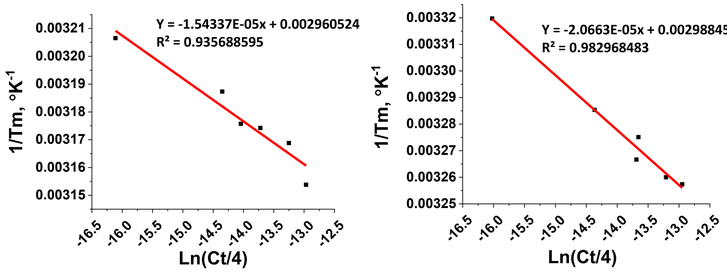
$$\Delta H^\circ - T\Delta S^\circ = -RT \ln K$$

where $R = 1.987$ cal $K^{-1}mole^{-1}$. At the midpoint, $T = T_m$ and $Keq = K_{50} = 4/[Ct]$.

$$\Delta H^\circ - T_m \Delta S^\circ = -RT_m \ln(4/[Ct])$$

And after rearranging terms:

$$\frac{1}{Tm} = \left(\frac{R}{\Delta H}\right) * ln\left(\frac{Ct}{4}\right) + \frac{\Delta S}{\Delta H}$$



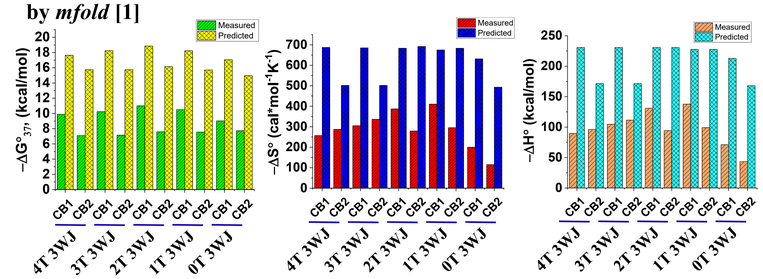

## *Stabilities of d3WJ*

**Measured thermodynamic parameters for d3WJ in 1M NaCl and 0.1 M NaCl**

| Name | Buffer | Tm (°C) for $10^{-5}$ M | $\Delta H_{app}$ (kcal/mol) | $\Delta S_{app}$ (cal/mol*K) | $\Delta G°_{37}$ (kcal/mol) | $\Delta\Delta G°_{37}$ (kcal/mol) |
|---|---|---|---|---|---|---|
| 4T d3WJ | CB1 | 43.9 ± 1.2 | -89.6 | **-257.1** | -9.88 | -2.79 |
| | CB2 | 33.9 ± 0.8 | -96.2 | -287.4 | -7.09 | |
| 3T d3WJ | CB1 | 43.3 ± 0.7 | -104.8 | **-305.1** | -10.23 | -3.08 |
| | CB2 | 34.9 ± 0.6 | -111.5 | -336.5 | -7.15 | |
| 2T d3WJ | CB1 | 44.3 ± 0.6 | -131.1 | **-387.3** | **-11.00** | -3.41 |
| | CB2 | 36.1 ± 0.6 | -94.4 | -279.7 | -7.59 | |
| 1T d3WJ | CB1 | 43.2 ± 0.7 | -137.8 | **-410.6** | -10.50 | -2.94 |
| | CB2 | 35.2 ± 0.5 | -99.2 | -295.5 | -7.56 | |
| 0T d3WJ | CB1 | 41.1 ± 0.5 | -71.1 | **-200.2** | **-9.03** | -1.3 |
| | CB2 | 33.5 ± 0.4 | -43.4 | -115.2 | -7.73 | |

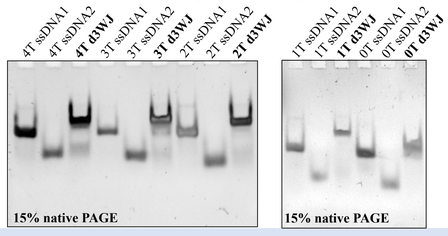
The errors in enthalpy, entropy, and free energy are estimated to be 10%, 10%, and 5%, respectively [4]

**Comparison between measured thermodynamic parameters and calculated by *mfold* [1]**




## *Electrophoretic gel mobility shift assays*

**Assembly of d3WJ** was conducted at 1 uM of individual stands in CB2 by heating solution to 90 °C and slowly cooling to 4 °C for ~1 h.




## Conclusion

We found that measured thermodynamic parameters do not match the calculated values but are in agreement about the stability of the d3WJ. Presence of 1M NaCl greatly affect stability of the 3WJ, contribute about 3kcal/mol in all constructs except 0T. The 0T and 4T d3WJ are greatly stabilized by entropy contribution as compared to others suggesting greater extend of dynamic behavior. The 2T d3WJ is the most stable construct and 0T is the least stable. All d3WJ have efficiently hybridized to form 3WJ analyzed by native page.

## Acknowledgements

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## References

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**4.**Xia, T.; SantaLucia, J., Jr.; Burkard, M. E.; Kierzek, R.; Schroeder, S. J.; Jiao, X.; Cox, C.; Turner, D. H., Thermodynamic parameters for an expanded nearest-neighbor model for formation of RNA duplexes with Watson-Crick base pairs. *Biochemistry* **1998,** *37* (42), 14719-35.