Nearest Neighbor Database

2024-05-04

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Welcome

Nearest neighbor parameters for RNA folding are used widely in software packages, but no current published source exists to provide the parameters and a tutorial for their use. This database serves this function for the community and is intended to grow as the parameters are refined. The site will also be used to provide a historical record of parameter sets as they are subsequently refined.

Contents

RNA (Turner 2004)

These are the current set of nearest neighbor parameters for RNA folding compiled by the Turner group. Both free energy changes at 37 ^{o}C and enthalpy changes have been estimated, allowing for structure prediction at arbitrary temperature. Parameters are available for download in text format or html format. A description of the functional form and tutorials for use are available.

Mathews, D.H., Disney, M.D., Childs, J.L., Schroeder, S.J., Zuker, M. and Turner, D.H. (2004) Incorporating chemical modification constraints into a dynamic programming algorithm for prediction of RNA secondary structure. *Proc. Natl. Acad. Sci. USA*, **101**, 7287-7292.

RNA (Turner 1999)

These are the set of nearest neighbor parameters for RNA folding compiled by the Turner group in 1999. The parameters are for estimating free energy changes at 37 ^{o}C. Parameters are available for download in text format or html format. A description of the functional form and tutorials for use are available.

Mathews, D.H., Sabina, J., Zuker, M. and Turner, D.H. (1999) Expanded sequence dependence of thermodynamic parameters provides improved prediction of RNA secondary structure. *J. Mol. Biol.*, **288**, 911-940.

DNA (RNAstructure)

These are the set of nearest neighbor parameters for DNA folding compiled by the Mathews group and released as part of RNAstructure package. Both free energy changes at 37 ºC and enthalpy changes have been estimated, allowing for structure prediction at arbitrary temperature. Parameters are available for download in text and html format. A description of the functional form and tutorials for use are available.

Reuter, J. S., & Mathews, D. H. (2010). RNAstructure: software for RNA secondary structure prediction and analysis. *BMC Bioinformatics*, **11**, 129.

RNA + m^6A (Kierzek et al.)

These are the set of nearest neighbor parameters for RNA folding with N^6-methyladenosine (m^6A) modification compiled by Kierzek et al. The parameters are for estimating free energy changes at 37 ºC. Parameters are available for download in text and html format. A description of the functional form and tutorials for use are available.

Kierzek, E., Zhang, X., Watson, R. M., Kierzek, R., Mathews, D. H. (2022). Secondary Structure Prediction for RNA Sequences Including N^6-methyladenosine. *Nature Communications*, **13**, 1271.

Reference

Research benefiting from this website should please cite:

Mittal, A., Turner, D. H., & Mathews, D. H. (2024). *NNDB: An Expanded Database of Nearest Neighbor Parameters for Predicting Stability of Nucleic Acid Secondary Structures.* Journal of Molecular Biology, 168549.

Turner, D. H. & Mathews, D. H. (2009). *NNDB: The nearest neighbor parameter database for predicting stability of nucleic acid secondary structure.* Nucleic Acids Research. **38**, D280-D282.

Contact

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Part I

RNA (Turner 2004)

Chapter 1

Watson-Crick-Franklin Helices

1.1 Free Energy Change at 37 °C

Folding free energy changes for Watson-Crick-Franklin helices are predicted using the equation:

$\Delta G^\circ_{37\ \text{Watson-Crick-Franklin}} = \Delta G^\circ_{37\ \text{intermolecular initiation}} + \Delta G^\circ_{37\ \text{AU end penalty}}$ (per AU end) $+ \Delta G^\circ_{37\ \text{symmetry (self-complementary duplexes)}} + \Sigma[\Delta G^\circ_{37\ \text{stacking}}]$

where intermolecular initiation is applied for bimolecular structure formation, the AU end penalty is applied once per each AU pair at the end of a helix, the symmetry correction is applied to self-complementary duplexes, and the stacking term is a sum of sequence-dependent parameters over all base pair stacks. For helices of P uninterrupted basepairs, there are P-1 stacks of pairs.

1.2 Enthalpy Change

Enthalpy changes for Watson-Crick-Franklin helices are predicted using the equation:

$\Delta H^\circ_{\text{Watson-Crick}} = \Delta H^\circ_{\text{intermolecular initiation}} + \Delta H^\circ_{\text{AU end penalty}}$ (per AU end) $+ \Sigma[\Delta H^\circ_{\text{stacking}}]$

where terms are the same as those above for free energy changes. Note that the symmetry correction for self-complementary duplexes is absent because that stability cost is an entropic cost.

1.3 Examples

Self complementary duplex

```
5'AGCGCU3'
3'UCGCGA5'
```

ΔG°_{37} = $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + 2×$\Delta G^\circ_{37\ \text{AU end penalty}}$ + $\Delta G^\circ_{37\ \text{symmetry}}$ + ΔG°_{37}(AU followed by GC) + ΔG°_{37}(GC followed by CG) + ΔG°_{37}(CG followed by GC) + ΔG°_{37}(GC followed by CG) + ΔG°_{37}(CG followed by UA)

ΔG°_{37} = 4.09 kcal/mol + 2×0.45 kcal/mol + 0.43 kcal/mol –2.08 kcal/mol – 3.42 kcal/mol – 2.36 kcal/mol – 3.42 kcal/mol – 2.08 kcal/mol

ΔG°_{37} = –7.94 kcal/mol

ΔH° = $\Delta H^\circ_{\text{intermolecular initiation}}$ + 2×ΔH°AU $_{\text{end penalty}}$ + ΔH°(AU followed by GC) + ΔH°(GC followed by CG) + ΔH°(CG followed by GC) + ΔH°(GC followed by CG) + ΔH°(CG followed by UA)

ΔH° = 3.61 kcal/mol + 2×3.72 kcal/mol – 10.48 kcal/mol – 14.88 kcal/mol – 10.64 kcal/mol – 14.88 kcal/mol – 10.48 kcal/mol

ΔH° = –50.31 kcal/mol

Note that, for example, the parameters for (AU followed by GC) are the same as (CG followed by UA) because the correct directionality of the strands is preserved.

Non-self complementary duplex

```
5'GCACG3'
3'CGUGC5'
```

ΔG°_{37} = $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + ΔG°_{37}(GC followed by CG) + ΔG°_{37}(CG followed by AU) + ΔG°_{37}(AU followed by CG) + ΔG°_{37}(CG followed by GC)

ΔG°_{37} = 4.09 kcal/mol – 3.42 kcal/mol – 2.11 kcal/mol – 2.24 kcal/mol – 2.36 kcal/mol

ΔG°_{37} = –6.04 kcal/mol

ΔH° = $\Delta H^\circ_{\text{intermolecular initiation}}$ + ΔH°(GC followed by CG) + ΔH°(CG followed by AU) + ΔH°(AU followed by CG) + ΔH°(CG followed by GC)

ΔH° = 3.61 kcal/mol – 14.88 kcal/mol – 10.44 kcal/mol – 11.40 kcal/mol – 10.64 kcal/mol

ΔH° = –43.75 kcal/mol

1.4 Tables

The table of parameters is available as plain text for free energy change, plain text for enthalpy change, or html. The plain text tables include GU pairs; see the GU section for special cases for 5′GGUC/3′CUGG and 5′ GG/3′UU motifs.

1.5 References

The Watson-Crick-Franklin nearest neighbor parameters were reported in:

Xia, T., SantaLucia, J., Jr., Burkard, M.E., Kierzek, R., Schroeder, S.J., Jiao, X., Cox, C. and Turner, D.H. (1998) Thermodynamic parameters for an expanded nearest-neighbor model for formation of RNA duplexes with Watson-Crick pairs. *Biochemistry*, **37**, 14719-14735.

The experimental data for the fit of the parameters were taken from:

1. Nelson, J.W., Martin, F.H. and Tinoco, I., Jr. (1981) DNA and RNA oligomer thermodynamics: the effect of mismatched bases on double-helix stability. *Biopolymers*, **20**, 2509-2531.
2. Freier, S.M., Burger, B.J., Alkema, D., Neilson, T. and Turner, D.H. (1983) Effects of 3' dangling end stacking on the stability of GGCC and CCGG double helices. *Biochemistry*, **22**, 6198-6206.
3. Petersheim, M. and Turner, D.H. (1983) Base-stacking and base-pairing contributions to helix stability: thermodynamics of double-helix formation with CCGG, CCGGp, CCGGAp, ACCGGp, CCGGUp, and ACCGGUp. *Biochemistry*, **22**, 256-263.
4. Freier, S.M., Alkema, D., Sinclair, A., Neilson, T. and Turner, D.H. (1985) Contributions of dangling end stacking and terminal base-pair formation to the stabilities of XGGCCp, XCCGGp, XGGCCYp, and XCCGGYp helixes. *Biochemistry*, **24**, 4533-4539.
5. Freier, S.M., Sinclair, A., Neilson, T. and Turner, D.H. (1985) Improved free energies for G-C base-pairs. J. Mol. Biol., 185, 645-647.
6. Hickey, D.R. and Turner, D.H. (1985) Solvent effects on the stability of A7U7p. *Biochemistry*, **24**, 2086-2094.
7. Freier, S.M., Kierzek, R., Caruthers, M.H., Neilson, T. and Turner, D.H. (1986) Free energy contributions of G.U and other terminal mismatches to helix stability. *Biochemistry*, **25**, 3209-3223.
8. Freier, S.M., Kierzek, R., Jaeger, J.A., Sugimoto, N., Caruthers, M.H., Neilson, T. and Turner, D.H. (1986) Improved free-energy parameters for predictions of RNA duplex stability. *Proc. Natl. Acad. Sci. USA.*, **83**,

9373-9377.
9. Kierzek, R., Caruthers, M.H., Longfellow, C.E., Swinton, D., Turner, D.H. and Freier, S.M. (1986) Polymer-supported synthesis and its application to test the nearest-neighbor model for duplex stability. *Biochemistry*, **25**, 7840-7846.
10. Sugimoto, N., Kierzek, R., Freier, S.M. and Turner, D.H. (1986) Energetics of internal GU mismatches in ribooligonucleotide helixes. *Biochemistry*, **25**, 5755-5759.
11. Sugimoto, N., Kierzek, R. and Turner, D.H. (1987) Sequence dependence for the energetics of dangling ends and terminal base pairs in ribonucleic acid. *Biochemistry*, **26**, 4554-4558.
12. Longfellow, C.E., Kierzek, R. and Turner, D.H. (1990) Thermodynamic and spectroscopic study of bulge loops in oligoribonucleotides. *Biochemistry*, **29**, 278-285.
13. Hall, K.B. and McLaughlin, L.W. (1991) Thermodynamic and structural properties of pentamer DNA.DNA, RNA.RNA, and DNA.RNA duplexes of identical sequence. *Biochemistry*, **30**, 10606-10613.
14. He, L., Kierzek, R., SantaLucia, J., Jr., Walter, A.E. and Turner, D.H. (1991) Nearest-neighbor parameters for G.U mismatches. *Biochemistry*, **30**, 11124-11132.
15. Peritz, A.E., Kierzek, R., Sugimoto, N. and Turner, D.H. (1991) Thermodynamic study of internal loops in oligoribonucleotides: Symmetric loops are more stable than asymmetric loops. *Biochemistry*, **30**, 6428-6436.
16. Walter, A.E., Wu, M. and Turner, D.H. (1994) The stability and structure of tandem GA mismatches in RNA depend on closing base pairs. *Biochemistry*, **33**, 11349-11354.
17. Wu, M., McDowell, J.A. and Turner, D.H. (1995) A periodic table of symmetric tandem mismatches in RNA. *Biochemistry*, **34**, 3204-3211.
18. McDowell, J.A., He, L., Chen, X. and Turner, D.H. (1997) Investigation of the structural basis for thermodynamic stabilities of tandem GU wobble pairs: NMR structures of (rGGAGUUCC)2 and (rGGAUGUCC)2. *Biochemistry*, **36**, 8030-8038.
19. Xia, T., McDowell, J.A. and Turner, D.H. (1997) Thermodynamics of nonsymmetric tandem mismatches adjacent to G.C base pairs in RNA. *Biochemistry*, **36**, 12486-12487.
20. Xia, T., SantaLucia, J., Jr., Burkard, M.E., Kierzek, R., Schroeder, S.J., Jiao, X., Cox, C. and Turner, D.H. (1998) Thermodynamic parameters for an expanded nearest-neighbor model for formation of RNA duplexes with Watson-Crick pairs. *Biochemistry*, **37**, 14719-14735.

Chapter 2

GU Pairs

GU pairs are generally treated as nearest neighbor stacks, similar to Watson-Crick-Franklin helices, and GU pairs at the ends of helices are penalized with the same parameter as AU pairs at the ends of helices. In one sequence context, a tandem GU pair with a GU followed by a UG, the nearest neighbor model does not work and two parameters are available, depending on the sequence context (see the html table of parameters). Note also that the motif 5′GG/3′UU was assigned a ΔG°_{37} of –0.5 kcal/mol to optimize structure prediction accurracy, whereas it was measured as +0.5 kcal/mol. Parameters for stacks containing GU pairs were calculated separately from those containing AU and GC base pairs only.

2.1 Example

```
5'GGUCGUGU3'
3'CUGGUGCG5'
```

ΔG°_{37} = $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{GU end penalty}}$ + ΔG°_{37}(GC followed by GU, followed by UG, followed by CG) + ΔG°_{37}(CG followed by GU) + ΔG°_{37}(GU followed by UG) + ΔG°_{37}(UG followed by GC) + ΔG°_{37}(GC followed by UG)

ΔG°_{37} = 4.09 kcal/mol + 0.45 kcal/mol – 4.12 kcal/mol –1.41 kcal/mol + 1.29 kcal/mol – 1.41 kcal/mol – 2.51 kcal/mol

ΔG°_{37} = –3.62 kcal/mol

ΔH° = $\Delta H^\circ_{\text{intermolecular initiation}}$ + $\Delta H^\circ_{\text{GU end penalty}}$ + ΔH°(GC followed by GU, followed by UG, followed by GC) + ΔH°(CG followed by GU) + ΔH°(GU followed by UG) + ΔH°(UG followed by GC) + ΔH°(GC followed by UG)

ΔH° = 3.61 kcal/mol + 3.72 kcal/mol – 30.80 kcal/mol – 5.61 kcal/mol – 14.59 kcal/mol – 5.61 kcal/mol – 12.59 kcal/mol

ΔH° = –61.87 kcal/mol

Note that this example shows the stack of GU followed by UG in two different contexts, including the stabilizing context and the destabilizing context. In the stabilizing context, a single parameter is used for three consecutive basepair stacks.

2.2 Tables

The tables of parameters are available as plain text for free energy change (including Watson-Crick-Franklin pairs), plain text for enthalpy change (including Watson-Crick-Franklin pairs), or html.

2.3 References

The GU nearest neighbor parameters were reported in:

Mathews, D.H., Sabina, J., Zuker, M. and Turner, D.H. (1999) Expanded sequence dependence of thermodynamic parameters provides improved prediction of RNA secondary structure. *J. Mol. Biol.*, **288**, 911-940.

The experimental data for the fit of the parameters were taken from:

1. Freier, S.M., Kierzek, R., Caruthers, M.H., Neilson, T. and Turner, D.H. (1986) Free energy contributions of G.U and other terminal mismatches to helix stability. *Biochemistry*, **25**, 3209-3223.
2. Sugimoto, N., Kierzek, R., Freier, S.M. and Turner, D.H. (1986) Energetics of internal GU mismatches in ribooligonucleotide helixes. *Biochemistry*, **25**, 5755-5759.
3. He, L., Kierzek, R., SantaLucia, J., Jr., Walter, A.E. and Turner, D.H. (1991) Nearest-neighbor parameters for G.U mismatches. *Biochemistry*, **30**, 11124-11132.
4. Wu, M., McDowell, J.A. and Turner, D.H. (1995) A periodic table of symmetric tandem mismatches in RNA. *Biochemistry*, *34*, 3204-3211.
5. McDowell, J.A. and Turner, D.H. (1996) Investigation of the structural basis for thermodynamic stabilities of tandem GU mismatches: Solution structure of (rGAGGUCUC)2 by two-dimensional NMR and simulated annealing. *Biochemistry*, **35**, 14077-14089.
6. Xia, T., McDowell, J.A. and Turner, D.H. (1997) Thermodynamics of nonsymmetric tandem mismatches adjacent to G.C base pairs in RNA. *Biochemistry*, **36**, 12486-12487.

Chapter 3

Dangling Ends

Dangling ends are nucleotides that stack on the ends of helices. In secondary structures, they occur in multibranch and exterior loops. They occur as either 5′ dangling ends (an unpaired nucleotide 5′ to the helix end) or 3′ dangling ends (an unpaired nucleotide 3′ to the helix end). In RNA, 3′ dangling ends are generally more stabilizing than 5′ dangling ends. Note that if a helix end is extended on both the 5′ and 3′ strands, then a terminal mismatch exists (not the sum of 5′ and 3′ dangling ends).

3.1 Example

```
5'AGCACGC3'
3' CGUGC 5'
```

ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(3′ dangling C adjacent to GC) + ΔG°_{37}(5′ dangling A adjacent GC)

ΔG°_{37} = –6.04 kcal/mol – 0.4 kcal/mol – 0.2 kcal/mol

ΔG°_{37} = –6.6 kcal/mol

ΔH° = ΔH°(Watson-Crick-Franklin Helix) + ΔH°(3′ dangling C adjacent to GC) + ΔH°(5′ dangling A adjacent GC)

ΔH° = –43.75 kcal/mol – 2.8 kcal/mol – 1.6 kcal/mol

ΔH° = –48.2 kcal/mol

Note that this example contains both a 5′ and a 3′ dangling end (at opposite ends of the duplex).

3.2 Tables

The tables of parameters are available as plain text for free energy change, plain text for enthalpy change, or html.

3.3 References

The dangling end parameters were assembled in:

Serra, M.J. and Turner, D.H. (1995) Predicting Thermodynamic Properties of RNA. *Methods Enzymol.*, **259**, 242-261.

The optical melting experiments for dangling ends were reported in:

1. Freier, S.M., Burger, B.J., Alkema, D., Neilson, T. and Turner, D.H. (1983) Effects of 3′ dangling end stacking on the stability of GGCC and CCGG double helices. *Biochemistry*, **22**, 6198-6206.
2. Petersheim, M. and Turner, D.H. (1983) Base-stacking and base-pairing contributions to helix stability: thermodynamics of double-helix formation with CCGG, CCGGp, CCGGAp, ACCGGp, CCGGUp, and ACCGGUp. *Biochemistry*, **22**, 256-263.
3. Freier, S.M., Alkema, D., Sinclair, A., Neilson, T. and Turner, D.H. (1985) Contributions of dangling end stacking and terminal base-pair formation to the stabilities of XGGCCp, XCCGGp, XGGCCYp, and XCCGGYp helixes. *Biochemistry*, **24**, 4533-4539.
4. Freier, S.M., Kierzek, R., Caruthers, M.H., Neilson, T. and Turner, D.H. (1986) Free energy contributions of G.U and other terminal mismatches to helix stability. *Biochemistry*, **25**, 3209-3223.
5. Freier, S.M., Sugimoto, N., Sinclair, A., Alkema, D., Neilson, T., Kierzek, R., Caruthers, M.H. and Turner, D.H. (1986) Stability of XGCGCp, GCGCYp, and XGCGCYp helixes: an empirical estimate of the energetics of hydrogen bonds in nucleic acids. *Biochemistry*, **25**, 3214-3219.
6. Sugimoto, N., Kierzek, R. and Turner, D.H. (1987) Sequence dependence for the energetics of dangling ends and terminal base pairs in ribonucleic acid. *Biochemistry*, **26**, 4554-4558.
7. Turner, D.H., Sugimoto, N. and Freier, S.M. (1988) RNA structure prediction. *Ann. Rev. Biophys. Biophys. Chem.*, **17**, 167-192.
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Chapter 4

Terminal Mismatches

Terminal mismatches are non-canonical pairs adjacent to helix ends.

4.1 Example

```
5'AGCGCUG3'
3'UCGCGAA5'
```

ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(UA followed by GA)

ΔG°_{37} = –7.94 kcal/mol – 1.1 kcal/mol

ΔG°_{37} = –9.0 kcal/mol

ΔH° = ΔH°(Watson-Crick-Franklin Helix) + ΔH°(UA followed by GA)

ΔH° = –50.31 kcal/mol – 3.8 kcal/mol

ΔH° = –54.1 kcal/mol

4.2 Tables

Tables of parameters are available as plain text for free energy changes, plain text for enthalpy changes, html for free energy change, or html for enthalpy change.

4.3 References

The terminal mismatch parameters were assembled in:

Xia, T., Mathews, D.H. and Turner, D.H. (1999) In Söll, D. G., Nishimura, S. and Moore, P. B. (eds.), *Prebiotic Chemistry, Molecular Fossils, Nucleosides, and RNA*. Elsevier, New York, pp. 21-47.

The optical melting experiments for terminal mismatches were reported in:

1. Hickey, D.R. and Turner, D.H. (1985) Solvent effects on the stability of A7U7p. *Biochemistry*, **24**, 2086-2094.
2. Freier, S.M., Kierzek, R., Caruthers, M.H., Neilson, T. and Turner, D.H. (1986) Free energy contributions of G.U and other terminal mismatches to helix stability. *Biochemistry*, **25**, 3209-3223.
3. Freier, S.M., Sugimoto, N., Sinclair, A., Alkema, D., Neilson, T., Kierzek, R., Caruthers, M.H. and Turner, D.H. (1986) Stability of XGCGCp, GCGCYp, and XGCGCYp helixes: an empirical estimate of the energetics of hydrogen bonds in nucleic acids. *Biochemistry*, **25**, 3214-3219.
4. Sugimoto, N., Kierzek, R. and Turner, D.H. (1987) Sequence dependence for the energetics of dangling ends and terminal base pairs in ribonucleic acid. *Biochemistry*, **26**, 4554-4558.
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6. Dale, T., Smith, R. and Serra, M. (2000) A test of the model to predict unusually stable RNA hairpin loop stability. *RNA*, **6**, 608-615.

Chapter 5

Hairpin Loops

5.1 Folding Free Energy Change

Hairpin loops of 4 or more nucleotides

The prediction of folding free energy changes for hairpins of 4 or more unpaired nucleotides is made with the following equation (also see Examples below):

$\Delta G^\circ_{37\ \text{hairpin}}$ (>3 nucleotides in loop) = $\Delta G^\circ_{37\ \text{initiation}}$ (n) + ΔG°_{37} (terminal mismatch) + ΔG°_{37} (UU or GA first mismatch) + ΔG°_{37} (GG first mismatch) + ΔG°_{37} (special GU closure) + ΔG°_{37} penalty (all C loops)

In this equation, n is the number of nucleotides in loop, the terminal mismatch parameter is the sequence-dependent term for the first mismatch stacking on the terminal base pair, UU and GA first mismatches receive a bonus (not applied to AG first mismatches), GG first mismatches receive a bonus, the special GU closure term is applied only to hairpins in which a GU closing pair (not UG) is preceded by two Gs, and finally loops with all C nucleotides receive a penalty.

The penalty for all C loops longer than C_3 is:

$\Delta G^\circ_{37\ \text{penalty}}$ (all C loops with $n > 3$) = $An + B$

Hairpin loops of 3 unpaired nucleotides

For hairpin loops of 3 nucleotides, the folding free energy change is estimated using:

$\Delta G^\circ_{37\ \text{hairpin}}$ (3 unpaired nucleotides) = $\Delta G^\circ_{37\ \text{initiation}}$ (3) + $\Delta G^\circ_{37\ \text{penalty}}$ (C_3 loop)

As opposed to longer hairpin loops, hairpin loops of three nucleotides do not receive a sequence-dependent first mismatch term. All C hairpin loops of three

nucleotides receive a stability penalty.

Special hairpin loops

There are hairpin loop sequences of 3, 4, and 6 nucleotides that have stabilities poorly fit by the model. These hairpins are assigned stabilities based on experimental data.

Short hairpin loops

The nearest neighbor rules prohibit hairpin loops with fewer than 3 nucleotides.

5.2 Folding Enthalpy Change

Hairpin loops of 4 or more nucleotides

The prediction of folding enathlpy changes for hairpins of 4 or more nucleotides is made with the following equation:

$\Delta H^\circ_{\text{hairpin}}$ (>3 unpaired nucleotides) = $\Delta H^\circ_{\text{initiation}}$ (n) + ΔH° (terminal mismatch) + ΔH° (UU or GA first mismatch) + ΔH° (special GU closure) + ΔH° (all C loops)

As with the free energy change equation above, n is the number of nucleotides in the loop, the terminal mismatch parameter is the sequence-dependent term for the first mismatch stacking on the terminal pair, UU and GA first mismatches receive a bonus (not applied to AG first mismatches), the special GU closure term is applied only to hairpins in which a GU closing pair (not UG) is preceded by two Gs, and finally the all C loops receive a penalty.

The penalty for all C loops longer than C_3 is:

$\Delta H^\circ_{\text{penalty}}$ (all C loops with n > 3) = A'n + B'

Hairpin loops of 3 nucleotides

For hairpin loops of 3 unpaired nucleotides, the enthalpy change is estimated using:

$\Delta H^\circ_{\text{hairpin}}$ (3 unpaired nucleotides) = $\Delta H^\circ_{\text{initiation}}$ (3) + $\Delta H^\circ_{\text{penalty}}$ (C_3loops)

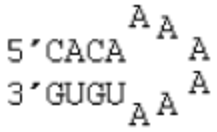
Hairpin loops of three nucleotides do not receive a sequence-dependent first mismatch term. All C hairpin loops of three nucleotides receive a stability penalty.

Special hairpin loops

Hairpin loops of 3, 4, and 6 nucleotides that have stabilities poorly fit by the free energy model are assigned enthalpy changes based on experimental data.

5.3 Examples

6 nucleotide hairpin loop with no special stacking terms



$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin Helix) + ΔG°_{37}(Hairpin Loop)

$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin Helix) + ΔG°_{37}(terminal mismatch) + $\Delta G^\circ_{37\ \text{Hairpin initiation}}(6)$

$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(CG followed by AU) + ΔG°_{37}(AU followed by CG) + ΔG°_{37}(CG followed by AU) + $\Delta G^\circ_{37\ \text{AU end penalty}}$ + ΔG°_{37}(AU followed by AA) + $\Delta G^\circ_{37\ \text{Hairpin initiation}}(6)$

ΔG°_{37} = –2.11 kcal/mol – 2.24 kcal/mol – 2.11 kcal/mol + 0.45 kcal/mol – 0.8 kcal/mol + 5.4 kcal/mol

ΔG°_{37} = –1.4 kcal/mol

$\Delta H^\circ = \Delta H^\circ$(Watson-Crick-Franklin Helix) + ΔH°(Hairpin Loop)

$\Delta H^\circ = \Delta H^\circ$(Watson-Crick-Franklin Helix) + ΔH°(terminal mismatch) + $\Delta H^\circ_{\text{Hairpin initiation}}(6)$

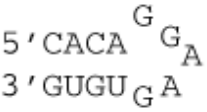
$\Delta H^\circ = \Delta H^\circ$(CG followed by AU) + ΔH°(AU followed by CG) + ΔH°(CG followed by AU) + $\Delta H^\circ_{\text{AU end penalty}}$ + ΔH°(AU followed by AA) + $\Delta H^\circ_{\text{Hairpin initiation}}(6)$

ΔH° = –10.44 kcal/mol – 11.40 kcal/mol – 10.44 kcal/mol + 3.72 kcal/mol – 3.9 kcal/mol – 2.9 kcal/mol

ΔH° = –35.4 kcal/mol

Note that for unimolecular secondary structures, the helical intermolecular initiation does not appear.

5 nucleotide hairpin loop with a GG first mismatch



$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin Helix) + ΔG°_{37}(Hairpin Loop)

$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin Helix) + ΔG°_{37}(terminal mismatch) + ΔG°_{37}(GG first mismatch) + $\Delta G^\circ_{37\ \text{Hairpin initiation}}$(5)

$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(CG followed by AU) + ΔG°_{37}(AU followed by CG) + ΔG°_{37}(CG followed by AU) + $\Delta G^\circ_{37\ \text{AU end penalty}}$ + ΔG°_{37}(AU followed by GG) + ΔG°_{37}(GG first mismatch) + $\Delta G^\circ_{37\ \text{Hairpin initiation}}$(5)

ΔG°_{37} = –2.11 kcal/mol – 2.24 kcal/mol – 2.11 kcal/mol + 0.45 kcal/mol – 0.8 kcal/mol – 0.8 kcal/mol + 5.7 kcal/mol

ΔG°_{37} = –1.9 kcal/mol

$\Delta H^\circ = \Delta H^\circ$(Watson-Crick-Franklin Helix) + ΔH°(Hairpin Loop)

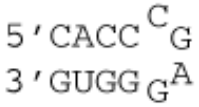
$\Delta H^\circ = \Delta H^\circ$(Watson-Crick-Franklin Helix) + ΔH°(terminal mismatch) + $\Delta H^\circ_{\text{Hairpin initiation}}$(5)

$\Delta H^\circ = \Delta H^\circ$(CG followed by AU) + ΔH°(AU followed by CG) + ΔH°(CG followed by AU) + $\Delta H^\circ_{\text{AU end penalty}}$ + ΔH°(AU followed by GG) + $\Delta H^\circ_{\text{Hairpin initiation}}$(5)

ΔH° = –10.44 kcal/mol – 11.40 kcal/mol – 10.44 kcal/mol + 3.72 kcal/mol – 3.5 kcal/mol + 3.6 kcal/mol

ΔH° = –28.5 kcal/mol

4 nucleotide special hairpin loop



$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin Helix) + ΔG°_{37}(Hairpin Loop)

$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin Helix) + ΔG°_{37}(CcgagG)

$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(CG followed by AU) + ΔG°_{37}(AU followed by CG) + ΔG°_{37}(CG followed by CG) + ΔG°_{37}(CcgagG)

ΔG°_{37} = –2.11 kcal/mol – 2.24 kcal/mol – 3.26 kcal/mol + 3.5 kcal/mol

ΔG°_{37} = –4.1 kcal/mol

$\Delta H^\circ = \Delta H^\circ$(Watson-Crick-Franklin Helix) + ΔH°(Hairpin Loop)

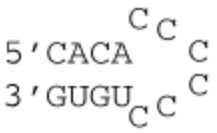
$\Delta H^\circ = \Delta H^\circ$(Watson-Crick-Franklin Helix) + ΔH°(CcgagG)

$\Delta H^\circ = \Delta H^\circ$(CG followed by AU) + ΔH°(AU followed by CG) + ΔH°(CG followed by CG) + ΔH°(CcgagG)

ΔH° = –10.44 kcal/mol – 11.40 kcal/mol – 13.39 kcal/mol – 6.6 kcal/mol

ΔH° = –41.8 kcal/mol

6 nucleotide all C loop



$\Delta G°_{37} = \Delta G°_{37}$(Watson-Crick-Franklin Helix) + $\Delta G°_{37}$(Hairpin Loop)

$\Delta G°_{37} = \Delta G°_{37}$(Watson-Crick-Franklin Helix) + $\Delta G°_{37}$(terminal mismatch) + $\Delta G°_{37\ \text{Hairpin initiation}}(6)$ + $\Delta G°_{37}$ penalty (all C loops)

$\Delta G°_{37} = \Delta G°_{37}$(CG followed by AU) + $\Delta G°_{37}$(AU followed by CG) + $\Delta G°_{37}$(CG followed by AU) + $\Delta G°_{37\ \text{AU end penalty}}$ + $\Delta G°_{37}$(AU followed by CC) + $\Delta G°_{37\ \text{Hairpin initiation}}(6)$ + 6×A + B

$\Delta G°_{37}$ = –2.11 kcal/mol – 2.24 kcal/mol – 2.11 kcal/mol + 0.45 kcal/mol – 0.7 kcal/mol + 5.4 kcal/mol + 6×0.3 kcal/mol + 1.6 kcal/mol

$\Delta G°_{37}$ = 2.1 kcal/mol

$\Delta H°$ = $\Delta H°$(Watson-Crick-Franklin Helix) + $\Delta H°$(Hairpin Loop)

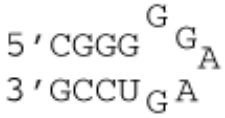
$\Delta H°$ = $\Delta H°$(Watson-Crick-Franklin Helix) + $\Delta H°$(terminal mismatch) + $\Delta H°_{\text{Hairpin initiation}}(6)$ + $\Delta H°$penalty (all C loops)

$\Delta H°$ = $\Delta H°$(CG followed by AU) + $\Delta H°$(AU followed by CG) + $\Delta H°$(CG followed by AU) + $\Delta H°_{\text{AU end penalty}}$ + $\Delta H°$(AU followed by CC) + $\Delta H°_{\text{Hairpin initiation}}(6)$ + 6×A + B

$\Delta H°$ = –10.44 kcal/mol – 11.40 kcal/mol – 10.44 kcal/mol + 3.72 kcal/mol + 6.0 kcal/mol – 2.9 kcal/mol + 6×3.4 kcal/mol + 7.6 kcal/mol

$\Delta H°$ = +2.5 kcal/mol

5 nucleotide loop with special GU closure



$\Delta G°_{37} = \Delta G°_{37}$(Watson-Crick-Franklin Helix) + $\Delta G°_{37}$(Hairpin Loop)

$\Delta G°_{37} = \Delta G°_{37}$(Watson-Crick-Franklin Helix) + $\Delta G°_{37}$(terminal mismatch) + $\Delta G°_{37}$(GG first mismatch) + $\Delta G°_{37\ \text{Hairpin initiation}}(5)$ + $\Delta G°_{37}$ (special GU closure)

ΔG°_{37} = ΔG°_{37}(CG followed by GC) + ΔG°_{37}(GC followed by GC) + ΔG°_{37}(GC followed by GU) + ΔG°_{37} GU end penalty + ΔG°_{37}(GU followed by GG) + ΔG°_{37}(GG first mismatch) + $\Delta G^\circ_{37\ \text{Hairpin initiation}}(5)$ + ΔG°_{37} (special GU closure)

ΔG°_{37} = –2.36 kcal/mol – 3.26 kcal/mol – 1.53 kcal/mol + 0.45 kcal/mol – 0.8 kcal/mol – 0.8 kcal/mol + 5.7 kcal/mol – 2.2 kcal/mol

ΔG°_{37} = –4.8 kcal/mol

ΔH° = ΔH°(Watson-Crick-Franklin Helix) + ΔH°(Hairpin Loop)

ΔH° = ΔH°(Watson-Crick-Franklin Helix) + ΔH°(terminal mismatch) + $\Delta H^\circ_{\text{Hairpin initiation}}(5)$ + ΔH°(special GU closure)

ΔH° = ΔH°(CG followed by GC) + ΔH°(GC followed by GC) + ΔH°(GC followed by GU) + ΔH°GU end + ΔH°(GU followed by GG) + $\Delta H^\circ_{\text{Hairpin initiation}}(5)$ + ΔH°(special GU closure)

ΔH° = –10.64 kcal/mol – 13.39 kcal/mol – 8.33 kcal/mol + 3.72 kcal/mol – 3.5 kcal/mol + 3.6 kcal/mol – 14.8 kcal/mol

ΔH° = –43.3 kcal/mol

5.4 Parameter Tables

Length dependent **initiation parameters** are available in plain text for free energy changes and plain text for enthalpy changes. The plain text initiation tables include an extrapolation out to lengths of 30 nucleotides. These initiation parameters are also available in html format. Initiation parameters are based on experiments for sizes up to 9 nucleotides, but can be extrapolated to longer loops. For free energy changes, the extrapolation is $\Delta G^\circ_{37\ \text{initiation}}$ (n>9) = $\Delta G^\circ_{37\ \text{initiation}}$ (9) + 1.75 RT ln(n/9), where R is the gas constant and T is the absolute temperature. For enthalpy changes, $\Delta H^\circ_{\text{initiation}}$ (n>9) = $\Delta H^\circ_{\text{initiation}}$ (9).

The terminal mismatch tables are available in plain text for free energy changes and plain text for enthalpy changes. These parameters are also available in html for free energy changes and html for enthalpy changes.

The **bonus/penalty terms (including the all-C loop terms)** are available in html format.

The table of special hairpin loops is available in plain text for free energy change for 3, 4, or 6 nucleotides; plain text for enthalpy change for 3, 4, or 6 nucleotides; and in html. The special hairpin loop sequences include the identity of the closing basepair.

5.5 References

The hairpin loop nearest neighbor parameters for free energy change were reported in:

Mathews, D.H., Disney, M.D., Childs, J.L., Schroeder, S.J., Zuker, M. and Turner, D.H. (2004) Incorporating chemical modification constraints into a dynamic programming algorithm for prediction of RNA secondary structure. *Proc. Natl. Acad. Sci. USA*, **101**, 7287-7292.

The enthalpy change parameters were reported in:

Lu, Z.J., Turner, D.H. and Mathews, D.H. (2006) A set of nearest neighbor parameters for predicting the enthalpy change of RNA secondary structure formation. *Nucleic Acids Res.*, **34** 4912 - 4924.

The experimental data for the fit of the parameters were taken from:

1. Groebe, D.R. and Uhlenbeck, O.C. (1988) Characterization of RNA hairpin loop stability. *Nucleic Acids Res.*, **16**, 11725-11735.
2. Antao, V.P., Lai, S.Y. and Tinoco, I., Jr. (1991) A thermodynamic study of unusually stable RNA and DNA hairpins. *Nucleic Acids Res.*, **19**, 5901-5905.
3. Antao, V.P. and Tinoco, I., Jr. (1992) Thermodynamic parameters for loop formation in RNA and DNA hairpin tetraloops. *Nucleic Acids Res.*, **20**, 819-824.
4. Serra, M.J., Lyttle, M.H., Axenson, T.J., Schadt, C.A. and Turner, D.H. (1993) RNA hairpin loop stability depends on closing pair. *Nucleic Acids Res.*, **21**, 3845-3849.
5. Serra, M.J., Axenson, T.J. and Turner, D.H. (1994) A model for the stabilities of RNA hairpins based on a study of the sequence dependence of stability for hairpins of six nucleotides. *Biochemistry*, **33**, 14289-14296.
6. Laing, L.G. and Hall, K.B. (1996) A model of the iron responsive element RNA hairpin loop structure determined from NMR and thermodynamic data. *Biochemistry*, **35**, 13586-13596.
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8. Giese, M.R., Betschart, K., Dale, T., Riley, C.K., Rowan, C., Sprouse, K.J. and Serra, M.J. (1998) Stability of RNA hairpins closed by wobble base pairs. *Biochemistry*, **37**, 1094-1100.
9. Shu, Z. and Bevilacqua, P.C. (1999) Isolation and characterization of thermodynamically stable and unstable RNA hairpins from a triloop combinatorial library. *Biochemistry*, **38**, 15369-15379.
10. Dale, T., Smith, R. and Serra, M. (2000) A test of the model to predict unusually stable RNA hairpin loop stability. *RNA*, **6**, 608-615.
11. Proctor, D.J., Schaak, J.E., Bevilacqua, J.M., Falzone, C.J. and Bevilacqua, P.C. (2002) Isolation and characterization of stable tetraloops with

the motif YNMG that participates in tertiary interactions. *Biochemistry*, **41**, 12062-12075.

Chapter 6

Bulge Loops

6.1 Folding Free Energy Change

Single Nucleotide Bulge Loops

The prediction of folding free energy changes is made with the following equation:

$\Delta G^\circ_{37\ \text{bulge}}$ (n=1) = $\Delta G^\circ_{37\ \text{bulge initiation}}$(n) + ΔG°_{37} (special C bulge) + ΔG°_{37} (base pair stack) – RT ln(number of states)

In this equation, n is the number of unpaired nucleotides, a special C bulge is a bulged C adjacent to at least one paired C, the base pair stack is the stack of the closing pairs as though there is no bulge (using Watson-Crick-Franklin or GU rules as needed), and the number of states counts the number of possible loops of identical sequence.

Because the helical stack continues across a single nucleotide bulge, the terminal AU/GU penalty is not applied adjacent to single bulges.

Bulges of 2 or More Nucleotides

For bulges of 2 or more nucleotides, the following equation is used:

$\Delta G^\circ_{37\ \text{bulge}}$ (n>1) = $\Delta G^\circ_{37\ \text{bulge initiation}}$(n)

Experimentally-derived parameters are available for initiation up to n = 3 and a linear extrapolation is used up to n = 6. Beyond 6, the initiation is approximated using a logarithmic function:

$\Delta G^\circ_{37\ \text{bulge}}$ (n>6) = $\Delta G^\circ_{37\ \text{bulge initiation}}$(6) + 1.75 RT ln(n/6)

where R is the gas constant, 1.987×10^{-3} kcal.K^{-1}.mol^{-1} and T is the absolute temperature, 310.15 K.

6.2 Folding Enthalpy Change

Single Nucleotide bulge Loops

The prediction of folding free energy changes is made with the following equation:

$\Delta H^\circ_{\text{bulge}}$ (n=1) = $\Delta H^\circ_{\text{bulge initiation}}$(n) + ΔH°(base pair stack)

In this equation, n is the number of unpaired nucleotides and the base pair stack is the stack of the closing pairs as though there is no bulge (using Watson-Crick-Franklin or GU rules as needed).

Because the helical stack continues across a single nucleotide bulge, the terminal AU/GU penalty is not applied adjacent to single bulges.

Bulges of 2 or More Nucleotides

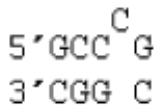
For bulges of 2 or more nucleotides, the following equation is used:

$\Delta H^\circ_{\text{bulge}}$ (n>1) = $\Delta H^\circ_{\text{bulge initiation}}$(n)

Experimentally-derived parameters are available for bulge loop initiations up to n=3. For n>3, the initiation is approximated as that for n=3.

6.3 Examples

Single C bulge with multiple states



ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + ΔG°_{37}(Bulge Loop)

ΔG°_{37} = ΔG°_{37}(GC followed by CG) + ΔG°_{37}(CG followed by CG) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{bulge initiation}}$(1) + ΔG°_{37}(special C bulge) + ΔG°_{37}(CG followed by GC) – RT ln(3)

ΔG°_{37} = –3.42 kcal/mol – 3.26 kcal/mol + 4.09 kcal/mol + 3.81 kcal/mol – 0.9 kcal/mol – 2.36 kcal/mol – 0.616×1.099 kcal/mol

ΔG°_{37} = –2.7 kcal/mol

Note that this loop has three available states because any of the three Cs in the top strand can be the bulge.

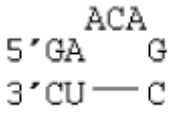
$\Delta H^\circ = \Delta H^\circ$(Watson-Crick-Franklin Helix) + $\Delta H^\circ_{\text{intermolecular initiation}}$ + ΔH°(Bulge Loop)

$\Delta H^\circ = \Delta H^\circ$(GC followed by CG) + ΔH°(CG followed by CG) + $\Delta H^\circ_{\text{intermolecular initiation}}$ + $\Delta H^\circ_{\text{bulge initiation}}(1)$ + ΔH°(CG followed by GC)

ΔH° = –14.88 kcal/mol – 13.39 kcal/mol + 3.61 kcal/mol + 10.6 kcal/mol – 10.64 kcal/mol

ΔH° = –24.7 kcal/mol

3 nucleotide bulge



$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin Helix) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{AU end penalty}}$ + ΔG°_{37}(Bulge Loop)

$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(GC followed by AU) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{AU end penalty}}$ + $\Delta G^\circ_{37\ \text{bulge initiation}}(3)$

ΔG°_{37} = –2.35 kcal/mol + 4.09 kcal/mol + 0.45 kcal/mol + 3.2 kcal/mol

ΔG°_{37} = +5.4 kcal/mol

$\Delta H^\circ = \Delta H^\circ$(Watson-Crick-Franklin Helix) + $\Delta H^\circ_{\text{intermolecular initiation}}$ + $\Delta H^\circ_{\text{AU end penalty}}$ + ΔH°(Bulge Loop)

$\Delta H^\circ = \Delta H^\circ$(GC followed by AU) + $\Delta H^\circ_{\text{intermolecular initiation}}$ + $\Delta H^\circ_{\text{AU end penalty}}$ + $\Delta H^\circ_{\text{bulge initiation}}(3)$

ΔH° = –12.44 kcal/mol + 3.61 kcal/mol + 3.72 kcal/mol + 7.1 kcal/mol

ΔH° = +2.0 kcal/mol

6.4 Parameter Tables

Bulge loop parameters are available in html or as plain text for initiation free energy parameters or initiation enthalpy parameters. The plain text initiation parameters include an extrapolation out to lengths of 30 unpaired nucleotides.

6.5 References

The bulge loop nearest neighbor parameters for free energy change were reported in:

Mathews, D.H., Disney, M.D., Childs, J.L., Schroeder, S.J., Zuker, M. and Turner, D.H. (2004) Incorporating chemical modification constraints into a dynamic programming algorithm for prediction of RNA secondary structure. *Proc. Natl. Acad. Sci. USA*, **101**, 7287-7292.

The enthalpy change parameters were reported in:

Lu, Z.J., Turner, D.H. and Mathews, D.H. (2006) A set of nearest neighbor parameters for predicting the enthalpy change of RNA secondary structure formation. *Nucleic Acids Res.*, **34** 4912 - 4924.

The experimental data for the fit of the parameters were taken from:

1. Fink, T.R. and Crothers, D.M. (1972) Free energy of imperfect nucleic acid helices, I. The bulge defect. *J. Mol. Biol.*, **66**, 1-12.
2. Groebe, D.R. and Uhlenbeck, O.C. (1989) Thermal stability of RNA hairpins containing a four-membered loop and a bulge nucleotide. *Biochemistry*, **28**, 742-747.
3. Longfellow, C.E., Kierzek, R. and Turner, D.H. (1990) Thermodynamic and spectroscopic study of bulge loops in oligoribonucleotides. *Biochemistry*, **29**, 278-285.
4. Znosko, B.M., Silvestri, S.B., Volkman, H., Boswell, B. and Serra, M.J. (2002) Thermodynamic parameters for an expanded nearest-neighbor model for the formation of RNA duplexes with single nucleotide bulges. *Biochemistry*, **41**, 10406-10417.

Chapter 7

Internal Loops

7.1 1×1, 1×2, 2×2 Internal Loops

Small symmetric internal loops have tabulated free energy and enthalpy changes, where experimentally determined values are used if available.

7.2 Other Internal Loops

The stabilities of other internal loops are predicted using the equation:

ΔG°_{37} internal = $\Delta G^\circ_{37\ \text{initiation}}(n) + \Delta G^\circ_{37\ \text{asymmetry}} \times |n1 - n2| + \Delta G^\circ_{37\ \text{mismatch}}(\text{mismatch 1}) + \Delta G^\circ_{37\ \text{mismatch}}(\text{mismatch 2}) + \Delta G^\circ_{37\ \text{AU/GU closure}}(\text{per AU or GU closure})$

where the initiation is a length dependent term for n unpaired nucleotides, an asymmetry term is multiplied by the absolute value of the difference in the number of unpaired nucleotides on each side of the loop, and sequence-dependent mismatch terms are applied for first mismatches of specific sequences. The AU/GU closure is applied per AU or GU closing pair and is used instead of the AU or GU penalty at the end of the helix (see Watson-Crick-Franklin or GU pairs).

Experimental data for $\Delta G^\circ_{37\ \text{initiation}}(n)$ is available for loops up to n = 6. For larger internal loops, an extrapolation is made:

$\Delta G^\circ_{37\ \text{initiation}}(n>6) = \Delta G^\circ_{37\ \text{initiation}}(6) + 1.08 \times \ln(n/6)$

Similarly, the enthalpy change is predicted with the equation:

$\Delta H^\circ\text{internal} = \Delta H^\circ_{\text{initiation}}(n) + \Delta H^\circ_{\text{asymmetry}} \times |n1 - n2| + \Delta H^\circ_{\text{mismatch}}(\text{mismatch 1}) + \Delta H^\circ_{\text{mismatch}}(\text{mismatch 2}) + \Delta H^\circ_{\text{AU/GU closure}}(\text{per AU or GU closure})$

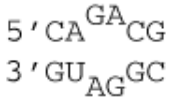
where terms are analagous to those for predicting folding free energy changes.

The mismatch parameters are sequence-dependent and are different for 1×(n-1) loop, 2×3 loop, and other internal loops. In the case of 1×(n–1) loops, the mismatches are set to 0 kcal/mol for free energy and enthalpy changes.

In the absence of data for loops larger than n=6, $\Delta H^\circ_{initiation}(n>6) = \Delta H^\circ_{initiation}(6)$

7.3 Examples

2×2 internal loop



$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin stacks) + $\Delta G^\circ_{37\ intermolecular\ initiation}$ + ΔG°_{37}(Internal Loop)

$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(CG followed by AU) + ΔG°_{37}(CG followed by GC) + $\Delta G^\circ_{37\ intermolecular\ initiation}$ + ΔG°_{37}(2×2 Internal Loop)

ΔG°_{37} = –2.11 kcal/mol – 2.36 kcal/mol +4.09 kcal/mol – 1.1 kcal/mol

ΔG°_{37} = –1.5 kcal/mol

$\Delta H^\circ = \Delta H^\circ$(Watson-Crick-Franklin stacks) + $\Delta H^\circ_{intermolecular\ initiation}$ + ΔH°(Internal Loop)

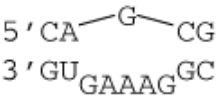
$\Delta H^\circ = \Delta H^\circ$(CG followed by AU) + ΔH°(CG followed by GC) + $\Delta H^\circ_{intermolecular\ initiation}$ + ΔH°(2×2 Internal Loop)

ΔH° = –10.44 kcal/mol – 10.64 kcal/mol + 3.61 kcal/mol – 19.7 kcal/mol

ΔH° = –37.2 kcal/mol

Note that the internal loop lookup tables account for terminal AU pairs that are adjacent to internal loops.

1×5 internal loop



$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin stacks) + $\Delta G^\circ_{37\ intermolecular\ initiation}$ + ΔG°_{37}(Internal Loop)

$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(CG followed by AU) + ΔG°_{37}(CG followed by GC) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{initiation}}(6)$ + $\Delta G^\circ_{37\ \text{asymmetry}} \times |n1 - n2|$ + $\Delta G^\circ_{37\ \text{mismatch}}$(mismatch 1) + $\Delta G^\circ_{37\ \text{mismatch}}$(mismatch 2) + ΔG°_{37} AU/GU closure

ΔG°_{37} = –2.11 kcal/mol – 2.36 kcal/mol + 4.09 kcal/mol + 2.0 kcal/mol + 0.6×|1 - 5| kcal/mol + 0 kcal/mol + 0 kcal/mol + 0.7 kcal/mol

ΔG°_{37} = +4.7 kcal/mol

ΔH° = ΔH°(Watson-Crick-Franklin stacks) + $\Delta H^\circ_{\text{intermolecular initiation}}$ + ΔH°(Internal Loop)

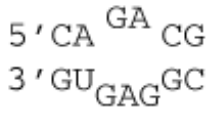
ΔH°= ΔH°(CG followed by AU) + ΔH°(CG followed by GC) + $\Delta H^\circ_{\text{intermolecular initiation}}$ + $\Delta H^\circ_{\text{initiation}}(6)$ + $\Delta H^\circ_{\text{asymmetry}} \times |n1 - n2|$ + $\Delta H^\circ_{\text{mismatch}}$(mismatch 1) + $\Delta H^\circ_{\text{mismatch}}$(mismatch 2) + ΔH°AU/GU closure

ΔH° = –10.44 kcal/mol – 10.64 kcal/mol + 3.61 kcal/mol – 1.3 kcal/mol + 3.2×|1 - 5| kcal/mol + 0 kcal/mol + 0 kcal/mol + 5.0 kcal/mol

ΔH° = –1.0 kcal/mol

Note that the free energy and enthalpy changes for first mismatches in 1×(n-1) internal loops are 0 kcal/mol.

2×3 internal loop with stabilizing mismatches



$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin stacks) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + ΔG°_{37}(Internal Loop)

$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(CG followed by AU) + ΔG°_{37}(CG followed by GC) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{initiation}}(5)$ + $\Delta G^\circ_{37\ \text{asymmetry}} \times |n1 - n2|$ + $\Delta G^\circ_{37\ \text{mismatch}}$(mismatch 1) + $\Delta G^\circ_{37\ \text{mismatch}}$(mismatch 2) + ΔG°_{37} AU/GU closure

ΔG°_{37} = –2.11 kcal/mol – 2.36 kcal/mol + 4.09 kcal/mol + 2.0 kcal/mol + 0.6×|2 - 3| kcal/mol – 0.8 kcal/mol – 1.2 kcal/mol + 0.7 kcal/mol

ΔG°_{37} = +0.9 kcal/mol

ΔH° = ΔH°(Watson-Crick-Franklin stacks) + $\Delta H^\circ_{\text{intermolecular initiation}}$ + ΔH°(Internal Loop)

ΔH°= ΔH°(CG followed by AU) + ΔH°(CG followed by GC) + $\Delta H^\circ_{\text{intermolecular initiation}}$ + $\Delta H^\circ_{\text{initiation}}(5)$ + $\Delta H^\circ_{\text{asymmetry}} \times |n1 - n2|$ + $\Delta H^\circ_{\text{mismatch}}$(mismatch 1) + $\Delta H^\circ_{\text{mismatch}}$(mismatch 2) + ΔH°AU/GU closure

ΔH° = –10.44 kcal/mol – 10.64 kcal/mol + 3.61 kcal/mol – 6.8 kcal/mol + 3.2×|2 - 3| kcal/mol – 9.0 kcal/mol – 10.9 kcal/mol + 5.0 kcal/mol

ΔH° = –36.0 kcal/mol

7.4 Parameter Tables

1×1 internal loop free energy change tables are available in text and html format. Enthalpy change tables are available in text and html format. Note that these tables incorporate the AU/GU closure penalties and therefore no AU/GU helix end penalty should be applied for internal loop closure.

1×2 internal loop free energy change tables are available in text and html format. Enthalpy change tables are available in text and html format. Note that these tables incorporate the AU/GU closure penalties and therefore no AU/GU helix end penalty should be applied for internal loop closure.

2×2 internal loop free energy change tables are available in text and html format. Enthalpy change tables are available in text and html format. Note that these tables incorporate the AU/GU closure penalties and therefore no AU/GU helix end penalty should be applied for internal loop closure.

Other parameters in text free energy or enthalpy or html format.

7.5 References

The internal loop nearest neighbor parameters for free energy change were reported in:

Mathews, D.H., Disney, M.D., Childs, J.L., Schroeder, S.J., Zuker, M. and Turner, D.H. (2004) Incorporating chemical modification constraints into a dynamic programming algorithm for prediction of RNA secondary structure. *Proc. Natl. Acad. Sci. USA*, **101**, 7287-7292.

The enthalpy change parameters were reported in:

Lu, Z.J., Turner, D.H. and Mathews, D.H. (2006) A set of nearest neighbor parameters for predicting the enthalpy change of RNA secondary structure formation. *Nucleic Acids Res.*, **34** 4912 - 4924.

The experimental data for the fit of the parameters were taken from:

1. Peritz, A.E., Kierzek, R., Sugimoto, N. and Turner, D.H. (1991) Thermodynamic study of internal loops in oligoribonucleotides: Symmetric loops are more stable than asymmetric loops. *Biochemistry*, **30**, 6428-6436.
2. SantaLucia, J., Jr., Kierzek, R. and Turner, D.H. (1991) Functional group substitutions as probes of hydrogen bonding between GA mismatches in RNA internal loops. *J. Am. Chem. Soc.*, **113**, 4313-4322.

3. SantaLucia, J., Jr., Kierzek, R. and Turner, D.H. (1991) Stabilities of consecutive A.C, C.C, G.G, U.C, and U.U mismatches in RNA internal loops: evidence for stable hydrogen-bonded U.U and C.C+ pairs. *Biochemistry*, **30**, 8242-8251.
4. Walter, A.E., Wu, M. and Turner, D.H. (1994) The stability and structure of tandem GA mismatches in RNA depend on closing base pairs. *Biochemistry*, **33**, 11349-11354.
5. Wu, M., McDowell, J.A. and Turner, D.H. (1995) A periodic table of symmetric tandem mismatches in RNA. *Biochemistry*, **34**, 3204-3211.
6. Schroeder, S., Kim, J. and Turner, D.H. (1996) G.A and U.U mismatches can stabilize RNA internal loops of three nucleotides. *Biochemistry*, **35**, 16105-16109.
7. Xia, T., McDowell, J.A. and Turner, D.H. (1997) Thermodynamics of nonsymmetric tandem mismatches adjacent to G.C base pairs in RNA. *Biochemistry*, **36**, 12486-12487.
8. Kierzek, R., Burkard, M. and Turner, D. (1999) Thermodynamics of single mismatches in RNA duplexes. *Biochemistry*, **38**, 14214-14223.
9. Schroeder, S.J. and Turner, D.H. (2000) Factors affecting the thermodynamic stability of small asymmetric internal loops in RNA. *Biochemistry*, **39**, 9257-9274.
10. Burkard, M.E., Xia, T. and Turner, D.H. (2001) Thermodynamics of RNA internal loops with a guanosine-guanosine pair adjacent to another noncanonical pair. *Biochemistry*, **40**, 2478-2483.
11. Schroeder, S.J. and Turner, D.H. (2001) Thermodynamic stabilities of internal loops with GU closing pairs in RNA. *Biochemistry*, **40**, 11509-11517.
12. Schroeder, S.J., Fountain, M.A., Kennedy, S.D., Lukavsky, P.J., Puglisi, J.D., Krugh, T.R. and Turner, D.H. (2003) Thermodynamic stability and structural features of the J4/5 loop in a Pneumocystis carinii group I intron. *Biochemistry*, **42**, 14184-14196.

Chapter 8

Coaxial Stacking

8.1 Introduction

Coaxial stacking is the stacking of two base pairs at the terminii of adjacent helices. This stacking aligns the two helices along a common axis. In unimolecular secondary structures, coaxial stacking occurs in multibranch and exterior loops.

This set of nearest neighbor parameters allows for two types of coaxial stacking, flush stacking of helices that are directly adjacent (no intervening unpaired nucleotides) and mismatch-mediated coaxial stacking in which a single mistmatch occurs between the stacked helices. Mismatch-mediated coaxial stacking of helices is only allowed when there is exactly one unpaired nucleotide between the helices that can form a non-canonical pair with a nucleotide on the other side of one of the two helices.

8.2 Flush Coaxial Stacking

In flush coaxial stacking, the free energy and enthalpy changes of the coaxial stack are approximated using the helical nearest neighbor parameters (Watson-Crick-Franklin or GU) as though there was no break in the backbone.

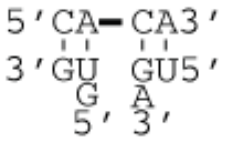
8.3 Mismatch-Mediated Coaxial Stacking

In the case of mismatch-mediated coaxial stacking, there are two adjacent stacks. The stack of the mismatch on the adjacent helix, where there is no break in the backbone, is approximated using the terminal mismatch parameters. The second stack is the stack of the mismatch on the second helix, where the backbone is not continuous. This stack is approximated using a sequence-independent

term of –2.1 kcal/mol for folding free energy change and –8.46 ± 2.75 kcal/mol for enthalpy change. If the "mismatch" mediating the coaxial stack could form a Watson-Crick or GU pair, a bonuses of –0.4 or –0.2 kcal/mol, respectively, are applied to both free energy and enthalpy changes.

8.4 Examples

Flush coaxial stacking



$\Delta G^\circ_{37\ \text{coaxial stack}} = \Delta G^\circ_{37}$(Watson-Crick Stack)

$\Delta G^\circ_{37\ \text{coaxial stack}} = \Delta G^\circ_{37}$(AU pair followed by CG pair)

$\Delta G^\circ_{37\ \text{coaxial stack}}$ = -2.24 kcal/mol

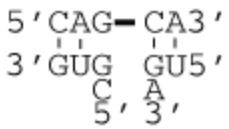
$\Delta H^\circ_{\text{coaxial stack}} = \Delta H^\circ$(Watson-Crick Stack)

$\Delta H^\circ_{\text{coaxial stack}} = \Delta H^\circ$(AU pair followed by CG pair)

$\Delta H^\circ_{\text{coaxial stack}}$ = –11.40 kcal/mol

Note that at this interface, the terminal AU pair penalty would still apply when calculating the helix stability.

Mismatch-mediated coaxial stacking



$\Delta G^\circ_{37\ \text{coaxial stack}} = \Delta G^\circ_{37}$(Continuous Backbone Stack) + ΔG°_{37}(Discontinuous Backbone Stack)

$\Delta G^\circ_{37\ \text{coaxial stack}} = \Delta G^\circ_{37}$(AU pair followed by GG mismatch) + ΔG°_{37}(Discontinuous Backbone Stack)

$\Delta G^\circ_{37\ \text{coaxial stack}}$ = -0.8 kcal/mol – 2.1 kcal/mol

$\Delta G^\circ_{37\ \text{coaxial stack}}$ = -2.9 kcal/mol

$\Delta H°_{\text{coaxial stack}}$ = $\Delta H°$(Continuous Backbone Stack) + $\Delta H°$(Discontinuous Backbone Stack)

$\Delta H°_{\text{coaxial stack}}$ = $\Delta H°$(AU pair followed by GG mismatch) + $\Delta H°$(Discontinuous Backbone Stack)

$\Delta H°_{\text{coaxial stack}}$ = –3.5 kcal/mol – 8.46 kcal/mol

$\Delta H°_{\text{coaxial stack}}$ = –12.0 kcal/mol

Note that at this interface, the terminal AU pair penalty would still apply when calculating the helix stability.

8.5 Tables

A table summarizing the parameters is available in html format.

8.6 References

The coaxial stacking nearest neighbor parameters for free energy change were reported in:

Mathews, D.H., Sabina, J., Zuker, M. and Turner, D.H. (1999) Expanded sequence dependence of thermodynamic parameters provides improved prediction of RNA secondary structure. *J. Mol. Biol.*, **288**, 911-940.

The enthalpy change parameters were reported in:

Lu, Z.J., Turner, D.H. and Mathews, D.H. (2006) A set of nearest neighbor parameters for predicting the enthalpy change of RNA secondary structure formation. *Nucleic Acids Res.*, **34** 4912 - 4924.

The experimental data for the fit of the parameters were taken from:

1. Walter, A.E., Turner, D.H., Kim, J., Lyttle, M.H., Müller, P., Mathews, D.H. and Zuker, M. (1994) Coaxial stacking of helixes enhances binding of oligoribonucleotides and improves predictions of RNA folding. *Proc. Natl. Acad. Sci. USA.*, **91**, 9218-9222.
2. Kim, J., Walter, A.E. and Turner, D.H. (1996) Thermodynamics of coaxially stacked helices with GA and CC mismatches. *Biochemistry*, **35**, 13753-13761.

Chapter 9

Multibranch Loops

9.1 Folding Free Energy Change

Multibranch loops stabilities are predicted using the following equation:

$\Delta G^\circ_{37\ \text{multibranch}} = \Delta G^\circ_{37\ \text{initiation}} + \Delta G^\circ_{37\ \text{stacking}}$

where the stacking is the optimal configuration of dangling ends, terminal mismatches, or coaxial stacks, noting that a nucleotide or helix end can participate in only one of these favorable interactions.

Initiation is predicted using:

$\Delta G^\circ_{37\ \text{initiation}}$ = a + b×[average asymmetry] + c×[number of branching helices] + $\Delta G^\circ_{37\ \text{strain}}$(three-way branching loops with fewer than two unpaired nucleotides)

where the average asymmetry is calculated as:

average asymmetry = min[2.0, mean difference in unpaired nucleotides on each side of each helix]

9.2 Folding Enthalpy Change

Similar to free energy change, multibranch loops enthalpy changes are predicted using the following equation:

$\Delta H^\circ_{\text{multibranch}} = \Delta H^\circ_{\text{initiation}} + \Delta H^\circ_{\text{stacking}}$

where the stacking is the configuration of dangling ends, terminal mismatches, or coaxial stacks with lowest folding free energy change.

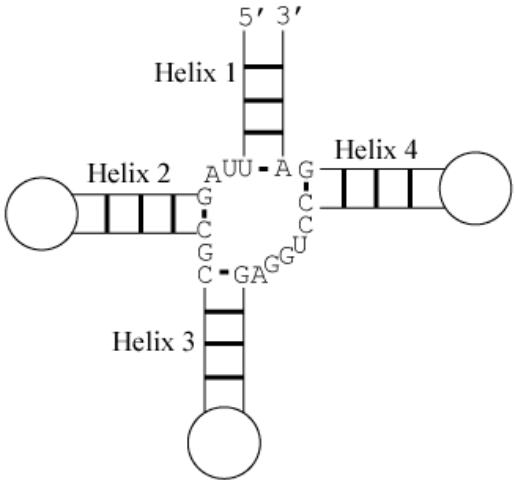
Initiation is predicted using an equation analagous to that folding free energy initiation:

$\Delta H°_{initiation}$ = a + b×[average asymmetry] + c×[number of branching helices] + $\Delta H°_{strain}$(three-way branching loops with fewer than two unpaired nucleotides)

where the average asymmetry is calculated as:

average asymmetry = min[2.0, mean difference in unpaired nucleotides on each side of each helix]

9.3 Example



Free Energy Change

Prediction of Stacking

The predicted stacking configuration is the one with lowest free energy change. There are eight relevant configurations.

Configuration 1:

Helix 1 with 3′ dangling U, Helix 2 with terminal mismatch, Helix 3 with 3′ dangling A, and Helix 4 with 5′ dangling C

$\Delta G°_{37}$ = $\Delta G°_{37}$(UA with 3′ dangling U) + $\Delta G°_{37}$(CG followed by GA) + $\Delta G°_{37}$(GC with 3′ dangling A) + $\Delta G°_{37}$(GC with 5′ dangling C)

$\Delta G°_{37}$ = –0.1 kcal/mol – 1.4 kcal/mol – 1.1 kcal/mol – 0.3 kcal/mol

$\Delta G°_{37}$ = –2.9 kcal/mol

Configuration 2:

Helix 1 with 3′ dangling U, Helix 2 with 5′ dangling A, Helix 3 with terminal mismatch, and Helix 4 with 5′ dangling C

ΔG°_{37} = ΔG°_{37}(UA with 3′ dangling U) + ΔG°_{37}(CG with 5′ dangling A) + ΔG°_{37}(GC followed by AG) + ΔG°_{37}(GC with 5′ dangling C)

ΔG°_{37} = –0.1 kcal/mol – 0.2 kcal/mol – 1.3 kcal/mol – 0.3 kcal/mol

ΔG°_{37} = –1.9 kcal/mol

Configuration 3:

Helix 1 in flush coaxial stack with helix 4, Helix 2 with terminal mismatch, and Helix 3 with 3′ dangling A

ΔG°_{37} = ΔG°_{37}(GC followed by AU) + ΔG°_{37}(CG followed by GA) + ΔG°_{37}(GC with 3′ dangling A)

ΔG°_{37} = –2.35 kcal/mol – 1.4 kcal/mol – 1.1 kcal/mol

ΔG°_{37} = –4.9 kcal/mol

Configuration 4:

Helix 1 in flush coaxial stack with helix 4, Helix 2 with 5′ dangling A, and Helix 3 with terminal mismatch

ΔG°_{37} = ΔG°_{37}(GC followed by AU) + ΔG°_{37}(CG with 5′ dangling A) + ΔG°_{37}(GC followed by AG)

ΔG°_{37} = –2.35 kcal/mol – 0.2 kcal/mol – 1.3 kcal/mol

ΔG°_{37} = –3.9 kcal/mol

Configuration 5:

Helix 1 with 3′ dangling U, Helix 2 in mismatch–mediated coaxial stack with helix 3 with GA intervening mismatch, and Helix 4 with 5′ dangling C

ΔG°_{37} = ΔG°_{37}(UA with 3′ dangling U) + ΔG°_{37}(CG followed by GA) + ΔG°_{37}(Discontinuous Backbone Stack) + ΔG°_{37}(GC with 5′ dangling C)

ΔG°_{37} = –0.1 kcal/mol – 1.4 kcal/mol – 2.1 kcal/mol – 0.3 kcal/mol

ΔG°_{37} = –3.9 kcal/mol

Configuration 6:

Helix 1 with 3′ dangling U, Helix 2 in mismatch–mediated coaxial stack with helix 3 with AG intervening mismatch, and Helix 4 with 5′ dangling C

ΔG°_{37} = ΔG°_{37}(UA with 3′ dangling U) + ΔG°_{37}(Discontinuous Backbone Stack) + ΔG°_{37}(GC followed by AG) + ΔG°_{37}(GC with 5′ dangling C)

ΔG°_{37} = –0.1 kcal/mol – 2.1 kcal/mol – 1.3 kcal/mol – 0.3 kcal/mol

ΔG°_{37} = –3.8 kcal/mol

Configuration 7:

Helix 1 in flush coaxial stack with helix 4 and Helix 2 in mismatch–mediated coaxial stack with helix 3 with GA intervening mismatch

ΔG°_{37} = ΔG°_{37}(GC followed by AU) + ΔG°_{37}(CG followed by GA) + ΔG°_{37}(Discontinuous Backbone Stack)

ΔG°_{37} = –2.35 kcal/mol – 1.4 kcal/mol – 2.1 kcal/mol

ΔG°_{37} = –5.9 kcal/mol

Configuration 8:

Helix 1 in flush coaxial stack with helix 4 and Helix 2 in mismatch–mediated coaxial stack with helix 3 with AG intervening mismatch

ΔG°_{37} = ΔG°_{37}(GC followed by AU) + ΔG°_{37}(Discontinuous Backbone Stack) + ΔG°_{37}(GC followed by AG)

ΔG°_{37} = –2.35 kcal/mol – 2.1 kcal/mol – 1.3 kcal/mol

ΔG°_{37} = –5.8 kcal/mol

Configuration 7 has the lowest folding free energy change of –5.9 kcal/mol.

Initiation Free Energy Change

$\Delta G^\circ_{37\ \text{initiation}}$ = a + b×[average asymmetry] + c×[number of branching helices] + ΔG°_{37} strain(three–way branching loops with fewer than two unpaired nucleotides)

$\Delta G^\circ_{37\ \text{initiation}}$ = 9.25 kcal/mol + (0.91 kcal/mol)×[average asymmetry] + (–0.63 kcal/mol)×[4]

Average asymmetry = min[2.0,(2+1+4+5)/4] = min[2.0,3.0] = 2.0

$\Delta G^\circ_{37\ \text{initiation}}$ = 9.25 kcal/mol + (0.91 kcal/mol)×[2] + (–0.63 kcal/mol)×[4]

$\Delta G^\circ_{37\ \text{initiation}}$ = 8.6 kcal/mol

Total Folding Free Energy Change

$\Delta G^\circ_{37\ \text{multibranch loop}}$ = $\Delta G^\circ_{37\ \text{initiation}}$ + $\Delta G^\circ_{37\ \text{stacking}}$ = 8.6 kcal/mol – 5.9 kcal/mol = 2.7 kcal/mol

9.3.1 Enthalpy Change

Prediction of Stacking

The stacking configuration is fixed by the prediction of folding free energy change and is configuration 7 above.

$\Delta H^\circ = \Delta H^\circ$(GC followed by AU) + ΔH°(CG followed by GA) + ΔH°(Discontinuous Backbone Stack)

ΔH° = –12.44 kcal/mol – 8.2 kcal/mol – 8.46 kcal/mol

ΔH° = –29.1 kcal/mol

Initiation Enthalpy Change

$\Delta H^\circ_{\text{initiation}}$ = a + b×[average asymmetry] + c×[number of branching helices] + $\Delta H^\circ_{\text{strain}}$(three–way branching loops with fewer than two unpaired nucleotides)

$\Delta H^\circ_{\text{initiation}}$ = 38.9 kcal/mol + (12.9 kcal/mol)×[average asymmetry] + (–11.9 kcal/mol)×[4]

Average asymmetry = min[2.0,(3+2+4+5)/4] = min[2.0,3.0] = 2.0

$\Delta H^\circ_{\text{initiation}}$ = 38.9 kcal/mol + (12.9 kcal/mol)×[2] + (–11.9 kcal/mol)×[4]

$\Delta H^\circ_{\text{initiation}}$ = 17.1 kcal/mol

Total Folding Enthalpy Energy Change

$\Delta H^\circ_{\text{multibranch loop}} = \Delta H^\circ_{\text{initiation}} + \Delta H^\circ_{\text{stacking}}$ = 17.1 kcal/mol – 29.1 kcal/mol = –12.0 kcal/mol

Note that helices 1 and 2 are separated by two unpaired nucleotides and cannot stack coaxially. Similarly, helices 3 and 4 are too distant to stack coaxially. Also note that coaxial stacking is only allowed between adjacent helices and hence, for example, helices 1 and 3 cannot stack coaxially.

9.4 Parameter Tables

Tables of parameters are available in html format.

9.5 References

The multibranch loop nearest neighbor parameters for initiation free energy change were reported in:

Mathews, D.H. and Turner, D.H. (2002) Experimentally derived nearest neighbor parameters for the stability of RNA three- and four-way multibranch loops. *Biochemistry*, **41**, 869-880.

The enthalpy change parameters were reported in:

Lu, Z.J., Turner, D.H. and Mathews, D.H. (2006) A set of nearest neighbor parameters for predicting the enthalpy change of RNA secondary structure formation. *Nucleic Acids Res.*, **34** 4912 - 4924.

The experimental data for the fit of the parameters were taken from:

1. Diamond, J.M., Turner, D.H. and Mathews, D.H. (2001) Thermodynamics of three-way multibranch loops in RNA. *Biochemistry*, **40**, 6971-6981.
2. Mathews, D.H. and Turner, D.H. (2002) Experimentally derived nearest neighbor parameters for the stability of RNA three- and four-way multibranch loops. *Biochemistry*, **41**, 869-880.

Chapter 10

Exterior Loops

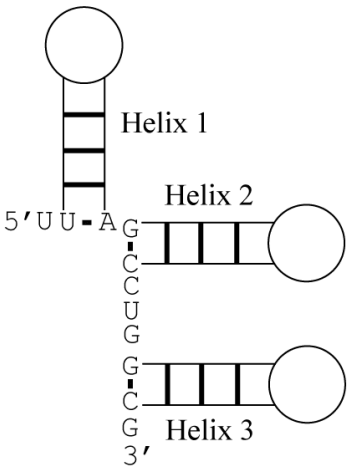
10.1 Folding Free Energy Change

Exterior loops are stabilized by terminal mismatches, dangling ends, and coaxial stacks. The stacking is the optimal configuration of dangling ends, terminal mismatches, or coaxial stacks, noting that a nucleotide or helix end can participate in only one of these favorable interactions.

10.2 Folding Enthalpy Change

Similar to free energy change, exterior loop enthalpy changes are the sum of terminal mismatches, dangling ends, and coaxial stacks. The stacking is that of the lowest folding free energy change.

10.3 Example



Free Energy Change

Prediction of Stacking

The predicted stacking configuration is the one with lowest free energy change. There are two possible configurations.

Configuration 1:

Helix 1 with 5′ dangling U, Helix 2 with 3′ dangling C, and Helix 3 with a GG mismatch

ΔG°_{37} = ΔG°_{37}(AU with 5′ dangling U) + ΔG°_{37}(CG with 3′ dangling C) + ΔG°_{37}(CG with GG mismatch)

ΔG°_{37} = –0.2 kcal/mol – 0.8 kcal/mol – 1.6 kcal/mol

ΔG°_{37} = –2.6 kcal/mol

Configuration 2:

Helix 1 in a flush coaxial stack with Helix 2 and Helix 3 with a GG mismatch

ΔG°_{37} = ΔG°_{37}(coaxial stack of AU followed by GC) + ΔG°_{37}(CG with GG mismatch)

ΔG°_{37} = –2.08 kcal/mol – 1.6 kcal/mol

ΔG°_{37} = –3.7 kcal/mol

Therefore, configuration 2, -3.7 kcal/mol, is the predicted free energy change.

Enthalpy Change

Prediction of Stacking

The stacking configuration is fixed by the prediction of folding free energy change and is configuration 2 above.

ΔH° = ΔH°(coaxial stack of AU followed by GC) + ΔH°(CG with GG mismatch)

ΔH° = –10.5 kcal/mol – 9.2 kcal/mol

ΔH° = –19.7 kcal/mol

Part II

RNA (Turner 1999)

Chapter 11

Watson-Crick-Franklin Helices

11.1 Free Energy Change at 37 °C

Folding free energy changes for Watson-Crick-Franklin helices are predicted using the equation:

ΔG°_{37} Watson-Crick-Franklin = $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{AU end penalty}}$ (per AU end) + $\Delta G^\circ_{37\ \text{symmetry}}$ (self-complementary duplexes) + $\Sigma[\Delta G^\circ_{37}$ stacking]

where intermolecular initiation is applied for bimolecular structure formation, the AU end penalty is applied once per each AU pair at the end of a helix, the symmetry correction is applied to self-complementary duplexes, and the stacking term is a sum of sequence-dependent parameters over all base pair stacks. For helices of P uninterrupted basepairs, there are P-1 stacks of pairs.

11.2 Examples

11.2.1 Self complementary duplex

```
5'AGCGCU3'
3'UCGCGA5'
```

$\Delta G^\circ_{37} = \Delta G^\circ_{37\ \text{intermolecular initiation}} + 2\times\Delta G^\circ_{37\ \text{AU end penalty}} + \Delta G^\circ_{37\ \text{symmetry}}$ + ΔG°_{37}(AU followed by GC) + ΔG°_{37}(GC followed by CG) + ΔG°_{37}(CG followed by GC) + ΔG°_{37}(GC followed by CG) + ΔG°_{37}(CG followed by UA)

ΔG°_{37} = 4.09 kcal/mol + 2×0.45 kcal/mol + 0.43 kcal/mol –2.08 kcal/mol – 3.42 kcal/mol – 2.36 kcal/mol – 3.42 kcal/mol – 2.08 kcal/mol

ΔG°_{37} = –7.94 kcal/mol

Note that, for example, the parameter for (AU followed by GC) is the same as (CG followed by UA) because the correct directionality of the strands is preserved.

Non-self complementary duplex

```
5'GCACG3'
3'CGUGC5'
```

ΔG°_{37} = $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + ΔG°_{37}(GC followed by CG) + ΔG°_{37}(CG followed by AU) + ΔG°_{37}(AU followed by CG) + ΔG°_{37}(CG followed by GC)

ΔG°_{37} = 4.09 kcal/mol – 3.42 kcal/mol – 2.11 kcal/mol – 2.24 kcal/mol – 2.36 kcal/mol

ΔG°_{37} = –6.04 kcal/mol

11.3 Tables

The table of parameters is available as plain text (including GU pairs; see the GU section for special cases for 5'GGUC/3'CUGG and 5' GG/3'UU motifs) or html.

11.4 References

The Watson-Crick-Franklin nearest neighbor parameters were reported in:

Xia, T., SantaLucia, J., Jr., Burkard, M.E., Kierzek, R., Schroeder, S.J., Jiao, X., Cox, C. and Turner, D.H. (1998) Thermodynamic parameters for an expanded nearest-neighbor model for formation of RNA duplexes with Watson-Crick pairs. *Biochemistry*, **37**, 14719-14735.

The experimental data for the fit of the parameters were taken from:

1. Nelson, J.W., Martin, F.H. and Tinoco, I., Jr. (1981) DNA and RNA oligomer thermodynamics: the effect of mismatched bases on double-helix stability. *Biopolymers*, **20**, 2509-2531.
2. Freier, S.M., Burger, B.J., Alkema, D., Neilson, T. and Turner, D.H. (1983) Effects of 3' dangling end stacking on the stability of GGCC and CCGG double helices. *Biochemistry*, **22**, 6198-6206.

3. Petersheim, M. and Turner, D.H. (1983) Base-stacking and base-pairing contributions to helix stability: thermodynamics of double-helix formation with CCGG, CCGGp, CCGGAp, ACCGGp, CCGGUp, and ACCGGUp. *Biochemistry*, **22**, 256-263.
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Chapter 12

GU Pairs

GU pairs are generally treated as nearest neighbor stacks, similar to Watson-Crick-Franklin helices, and GU pairs at the ends of helices are penalized with the same parameter as AU pairs at the ends of helices. In one sequence context, a tandem GU pair with a GU followed by a UG, the nearest neighbor model does not work and two parameters are available, depending on the sequence context (see the html table of parameters). Note also that the motif 5′GG/3′UU is assigned a ΔG°_{37} of –0.5 kcal/mol to optimize structure prediction accurracy, whereas it is measured as +0.5 kcal/mol. Parameters for stacks containing GU pairs were calculated separately from those containing AU and GC base pairs only.

12.1 Example

```
5'GGUCGUGU3'
3'CUGGUGCG5'
```

ΔG°_{37} = $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{GU end penalty}}$ + ΔG°_{37}(GC followed by GU, followed by UG, followed by GC) + ΔG°_{37}(CG followed by GU) + ΔG°_{37}(GU followed by UG) + ΔG°_{37}(UG followed by GC) + ΔG°_{37}(GC followed by UG)

ΔG°_{37} = 4.09 kcal/mol + 0.45 kcal/mol – 4.12 kcal/mol –1.41 kcal/mol + 1.29 kcal/mol – 1.41 kcal/mol – 2.51 kcal/mol

ΔG°_{37} = –3.62 kcal/mol

Note that this example shows the stack of GU followed by UG in two different contexts, including the stabilizing context and the destabilizing context. In the

stabilizing context, a single parameter is used for three consecutive basepair stacks.

12.2 Tables

The tables of parameters are available as plain text (including Watson-Crick-Franklin pairs) or html.

12.3 References

The GU nearest neighbor parameters were reported in:

Mathews, D.H., Sabina, J., Zuker, M. and Turner, D.H. (1999) Expanded sequence dependence of thermodynamic parameters provides improved prediction of RNA secondary structure. *J. Mol. Biol.*, **288**, 911-940.

The experimental data for the fit of the parameters were taken from:

1. Freier, S.M., Kierzek, R., Caruthers, M.H., Neilson, T. and Turner, D.H. (1986) Free energy contributions of G.U and other terminal mismatches to helix stability. *Biochemistry*, **25**, 3209-3223.
2. Sugimoto, N., Kierzek, R., Freier, S.M. and Turner, D.H. (1986) Energetics of internal GU mismatches in ribooligonucleotide helixes. *Biochemistry*, **25**, 5755-5759.
3. He, L., Kierzek, R., SantaLucia, J., Jr., Walter, A.E. and Turner, D.H. (1991) Nearest-neighbor parameters for G.U mismatches. *Biochemistry*, **30**, 11124-11132.
4. Wu, M., McDowell, J.A. and Turner, D.H. (1995) A periodic table of symmetric tandem mismatches in RNA. *Biochemistry*, **34**, 3204-3211.
5. McDowell, J.A. and Turner, D.H. (1996) Investigation of the structural basis for thermodynamic stabilities of tandem GU mismatches: Solution structure of (rGAGGUCUC)2 by two-dimensional NMR and simulated annealing. *Biochemistry*, **35**, 14077-14089.
6. Xia, T., McDowell, J.A. and Turner, D.H. (1997) Thermodynamics of nonsymmetric tandem mismatches adjacent to G.C base pairs in RNA. *Biochemistry*, **36**, 12486-12487.

Chapter 13

Dangling Ends

Dangling ends are nucleotides that stack on the ends of helices. In secondary structures, they occur in multibranch and exterior loops. They occur as either 5′ dangling ends (an unpaired nucleotide 5′ to the helix end) or 3′ dangling ends (an unpaired nucleotide 3′ to the helix end). In RNA, 3′ dangling ends are generally more stabilizing than 5′ dangling ends. Note that if a helix end is extended on both the 5′ and 3′ strands, then a terminal mismatch exists (not the sum of 5′ and 3′ dangling ends).

13.1 Example

```
5'AGCACGC3'
3'  CGUGC 5'
```

ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(3′ dangling C adjacent to GC) + ΔG°_{37}(5′ dangling A adjacent CG)

ΔG°_{37} = –6.04 kcal/mol – 0.4 kcal/mol – 0.2 kcal/mol

ΔG°_{37} = –6.6 kcal/mol

Note that this example contains both a 5′ and a 3′ dangling end (at opposite ends of the duplex).

13.2 Tables

The tables of parameters are available as plain text or html.

13.3 References

The dangling end parameters were assembled in:

Serra, M.J. and Turner, D.H. (1995) Predicting Thermodynamic Properties of RNA. *Methods Enzymol.*, **259**, 242-261.

The optical melting experiments for dangling ends were reported in:

1. Freier, S.M., Burger, B.J., Alkema, D., Neilson, T. and Turner, D.H. (1983) Effects of 3′ dangling end stacking on the stability of GGCC and CCGG double helices. *Biochemistry*, **22**, 6198-6206.
2. Petersheim, M. and Turner, D.H. (1983) Base-stacking and base-pairing contributions to helix stability: thermodynamics of double-helix formation with CCGG, CCGGp, CCGGAp, ACCGGp, CCGGUp, and ACCGGUp. *Biochemistry*, **22**, 256-263.
3. Freier, S.M., Alkema, D., Sinclair, A., Neilson, T. and Turner, D.H. (1985) Contributions of dangling end stacking and terminal base-pair formation to the stabilities of XGGCCp, XCCGGp, XGGCCYp, and XCCGGYp helixes. *Biochemistry*, **24**, 4533-4539.
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7. Turner, D.H., Sugimoto, N. and Freier, S.M. (1988) RNA structure prediction. *Ann. Rev. Biophys. Biophys. Chem.*, **17**, 167-192.
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Chapter 14

Terminal Mismatches

Terminal mismatches are non-canonical pairs adjacent to helix ends.

14.1 Example

```
5'AGCGCUG3'
3'UCGCGAA5'
```

ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(UA followed by GA)

ΔG°_{37} = –7.94 kcal/mol – 1.1 kcal/mol

ΔG°_{37} = –9.0 kcal/mol

14.2 Tables

Tables of parameters are available as plain text or html.

14.3 References

The derivation of terminal mismatch parameters was described in:

Mathews, D.H., Sabina, J., Zuker, M. and Turner, D.H. (1999) Expanded sequence dependence of thermodynamic parameters provides improved prediction of RNA secondary structure. *J. Mol. Biol.*, **288**, 911-940.

The optical melting experiments for terminal mismatches were reported in:

1. Hickey, D.R. and Turner, D.H. (1985) Solvent effects on the stability of A7U7p. *Biochemistry*, **24**, 2086-2094.

2. Freier, S.M., Kierzek, R., Caruthers, M.H., Neilson, T. and Turner, D.H. (1986) Free energy contributions of G.U and other terminal mismatches to helix stability. *Biochemistry*, **25**, 3209-3223.
3. Freier, S.M., Sugimoto, N., Sinclair, A., Alkema, D., Neilson, T., Kierzek, R., Caruthers, M.H. and Turner, D.H. (1986) Stability of XGCGCp, GCGCYp, and XGCGCYp helixes: an empirical estimate of the energetics of hydrogen bonds in nucleic acids. *Biochemistry*, **25**, 3214-3219.
4. Sugimoto, N., Kierzek, R. and Turner, D.H. (1987) Sequence dependence for the energetics of dangling ends and terminal base pairs in ribonucleic acid. *Biochemistry*, **26**, 4554-4558.
5. Serra, M.J., Axenson, T.J. and Turner, D.H. (1994) A model for the stabilities of RNA hairpins based on a study of the sequence dependence of stability for hairpins of six nucleotides. *Biochemistry*, **33**, 14289-14296.
6. Dale, T., Smith, R. and Serra, M. (2000) A test of the model to predict unusually stable RNA hairpin loop stability. *RNA*, **6**, 608-615.

Chapter 15

Hairpin Loops

15.1 Folding Free Energy Change

Hairpin loops of 4 or more unpaired nucleotides

The prediction of folding free energy changes for hairpins of 4 or more unpaired nucleotides is made with the following equation:

ΔG°_{37} hairpin (>3 nucleotides in loop) = ΔG°_{37} initiation (n) + ΔG°_{37} (terminal mismatch) + ΔG°_{37} (UU or GA first mismatch) + ΔG°_{37} (special GU closure) + ΔG°_{37} penalty (all C loops)

In this equation, n is the number of nucleotides in loop, the terminal mismatch parameter is the sequence-dependent term for the first mismatch stacking on the terminal base pair, UU and GA first mismatches receive a bonus (not applied to AG first mismatches), the special GU closure term is applied only to hairpins in which a GU closing pair (not UG) is preceded by two Gs, and finally loops with all C nucleotides receive a penalty.

The penalty for all C loops of more than three unpaired nucleotides is predicted using a linear equation:

ΔG°_{37} penalty (all C loops; > 3 unpaired nucleotides) = An + B

Frequently Occuring Tetraloops

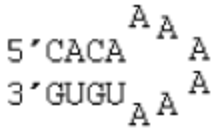
Tetraloop sequences, i.e. hairpin loops with four nucleotides, that occur frequently in the database of known secondary structures receive an enhanced stability in the form of a free energy bonus. These bonuses are sequence-dependent and appear in a lookup table.

Short hairpin loops

The nearest neighbor rules prohibit hairpin loops with fewer than 3 nucleotides.

15.2 Examples

6 nucleotide hairpin loop with no special stacking terms



ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(Hairpin Loop)

ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(terminal mismatch) + ΔG°_{37} Hairpin initiation(6)

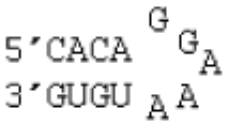
ΔG°_{37} = ΔG°_{37}(CG followed by AU) + ΔG°_{37}(AU followed by CG) + ΔG°_{37}(CG followed by AU) + ΔG°_{37} AU end penalty + ΔG°_{37}(AU followed by AA) + ΔG°_{37} Hairpin initiation(6)

ΔG°_{37} = –2.11 kcal/mol – 2.24 kcal/mol – 2.11 kcal/mol + 0.45 kcal/mol – 0.8 kcal/mol + 5.4 kcal/mol

ΔG°_{37} = –1.4 kcal/mol

Note that for unimolecular secondary structures, the helical intermolecular initiation does not appear.

5 nucleotide hairpin loop with a GA first mismatch



ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(Hairpin Loop)

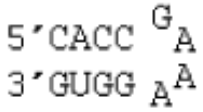
ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(terminal mismatch) + ΔG°_{37}(GA first mismatch) + ΔG°_{37} Hairpin initiation(5)

ΔG°_{37} = ΔG°_{37}(CG followed by AU) + ΔG°_{37}(AU followed by CG) + ΔG°_{37}(CG followed by AU) + ΔG°_{37} AU end penalty+ ΔG°_{37}(AU followed by GG) + ΔG°_{37}(GA first mismatch) + ΔG°_{37} Hairpin initiation(5)

ΔG°_{37} = –2.11 kcal/mol – 2.24 kcal/mol – 2.11 kcal/mol + 0.45 kcal/mol – 0.8 kcal/mol – 0.8 kcal/mol + 5.6 kcal/mol

ΔG°_{37} = –2.0 kcal/mol

4 nucleotide hairpin loop with tetraloop bonus



ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(Hairpin Loop)

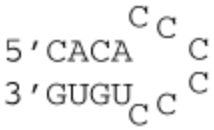
ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(terminal mismatch) + ΔG°_{37}(GA first mismatch) + ΔG°_{37} Hairpin initiation(4) + ΔG°_{37} Tetraloop Bonus(CgaaaG)

ΔG°_{37} = ΔG°_{37}(CG followed by AU) + ΔG°_{37}(AU followed by CG) + ΔG°_{37}(CG followed by CG) + ΔG°_{37}(CG followed by GA) + ΔG°_{37}(GA first mismatch) + ΔG°_{37} Hairpin initiation(4) + ΔG°_{37} Tetraloop Bonus(CgaaaG)

ΔG°_{37} = –2.11 kcal/mol – 2.24 kcal/mol – 3.26 kcal/mol – 1.4 kcal/mol – 0.8 kcal/mol + 5.6 kcal/mol – 3.0 kcal/mol

ΔG°_{37} = –7.2 kcal/mol

6 nucleotide all C loop



ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(Hairpin Loop)

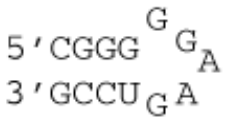
ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(terminal mismatch) + ΔG°_{37} Hairpin initiation(6) + ΔG°_{37} penalty (all C loops)

ΔG°_{37} = ΔG°_{37}(CG followed by AU) + ΔG°_{37}(AU followed by CG) + ΔG°_{37}(CG followed by AU) + ΔG°_{37} AU end penalty+ ΔG°_{37}(AU followed by CC) + ΔG°_{37} Hairpin initiation(6) + 6×A + B

ΔG°_{37} = –2.11 kcal/mol – 2.24 kcal/mol – 2.11 kcal/mol + 0.45 kcal/mol – 0.7 kcal/mol + 5.4 kcal/mol + 6×0.3 kcal/mol + 1.6 kcal/mol

ΔG°_{37} = +2.1 kcal/mol

5 nucleotide loop with special GU closure



ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(Hairpin Loop)

ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(terminal mismatch) + ΔG°_{37} Hairpin initiation(5) + ΔG°_{37} (special GU closure)

ΔG°_{37} = ΔG°_{37}(CG followed by GC) + ΔG°_{37}(GC followed by GC) + ΔG°_{37}(GC followed by GU) + ΔG°_{37} GU end penalty+ ΔG°_{37}(GU followed by GG) + ΔG°_{37} Hairpin initiation(5) + ΔG°_{37} (special GU closure)

ΔG°_{37} = –2.36 kcal/mol – 3.26 kcal/mol – 1.53 kcal/mol + 0.45 kcal/mol – 0.8 kcal/mol + 5.6 kcal/mol – 2.2 kcal/mol

ΔG°_{37} = –4.1 kcal/mol

15.3 Parameter Tables

Length dependent initiation parameters are available in plain text or html format. Initiation parameters are based on experiments for sizes up to 9 nucleotides, but can be extrapolated to longer loops. For free energy changes, the extrapolation is ΔG°_{37} initiation (n>9) = ΔG°_{37} initiation (9) + 1.75 RT ln(n/9), where R is the gas constant and T is the absolute temperature. The plain text file already extrapolates out to lengths of 30 nucleotides.

The terminal mismatch tables are available for free energy changes in plain text. These parameters are also available in html.

The **bonus/penalty terms (including the all-C loop terms)** are available in html format.

The lookup table of for **tertraloops with enhanced stability** is available as plain text or html.

15.4 References

The hairpin loop nearest neighbor parameters for free energy change were reported in:

Mathews, D.H., Sabina, J., Zuker, M. and Turner, D.H. (1999) Expanded sequence dependence of thermodynamic parameters provides improved prediction of RNA secondary structure. *J. Mol. Biol.*, **288**, 911-940.

The experimental data for the fit of the parameters were taken from:

1. Groebe, D.R. and Uhlenbeck, O.C. (1988) Characterization of RNA hairpin loop stability. *Nucleic Acids Res.*, **16**, 11725-11735.
2. Antao, V.P., Lai, S.Y. and Tinoco, I., Jr. (1991) A thermodynamic study of unusually stable RNA and DNA hairpins. *Nucleic Acids Res.*, **19**, 5901-5905.
3. Antao, V.P. and Tinoco, I., Jr. (1992) Thermodynamic parameters for loop formation in RNA and DNA hairpin tetraloops. *Nucleic Acids Res.*, **20**, 819-824.
4. Serra, M.J., Lyttle, M.H., Axenson, T.J., Schadt, C.A. and Turner, D.H. (1993) RNA hairpin loop stability depends on closing pair. *Nucleic Acids Res.*, **21**, 3845-3849.
5. Serra, M.J., Axenson, T.J. and Turner, D.H. (1994) A model for the stabilities of RNA hairpins based on a study of the sequence dependence of stability for hairpins of six nucleotides. *Biochemistry*, **33**, 14289-14296.
6. Laing, L.G. and Hall, K.B. (1996) A model of the iron responsive element RNA hairpin loop structure determined from NMR and thermodynamic data. *Biochemistry*, **35**, 13586-13596.
7. Serra, M.J., Barnes, T.W., Betschart, K., Gutierrez, M.J., Sprouse, K.J., Riley, C.K., Stewart, L. and Temel, R.E. (1997) Improved parameters for the prediction of RNA hairpin stability. *Biochemistry*, **36**, 4844-4851.
8. Giese, M.R., Betschart, K., Dale, T., Riley, C.K., Rowan, C., Sprouse, K.J. and Serra, M.J. (1998) Stability of RNA hairpins closed by wobble base pairs. *Biochemistry*, **37**, 1094-1100.

Chapter 16

Bulge Loops

16.1 Folding Free Energy Change

Singe Nucleotide Bulge Loops

The prediction of folding free energy changes is made with the following equation:

$\Delta G^\circ_{37\ \text{bulge}}$ (n=1) = $\Delta G^\circ_{37\ \text{bulge initiation}}(1)$ + ΔG°_{37} (base pair stack)

In this equation, n is the number of unpaired nucleotides and the base pair stack is the stack of the closing pairs as though there is no bulge (using Watson-Crick-Franklin or GU rules as needed).

Because the helical stack continues across a single nucleotide bulge, the terminal AU/GU penalty is not applied adjacent to single bulges.

Bulges of 2 or More Nucleotides

For bulges of 2 or more nucleotides, the following equation is used:

$\Delta G^\circ_{37\ \text{bulge}}$ (n>1) = $\Delta G^\circ_{37\ \text{bulge initiation}}$(n)

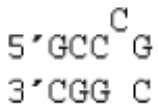
Experimentally-derived parameters are available for initiation up to n = 3 and a linear extrapolation is used up to n = 6. Beyond 6, the initiation is approximated using a logarithmic function:

$\Delta G^\circ_{37\ \text{bulge}}$ (n>6) = $\Delta G^\circ_{37\ \text{bulge initiation}}(6)$ + 1.75 RT ln(n/6)

where R is the gas constant and T is the absolute temperature, 310.15 K.

16.2 Examples

Single C bulge



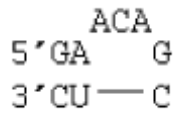
$\Delta G°_{37}$ = $\Delta G°_{37}$(Watson-Crick-Franklin Pairs) + $\Delta G°_{37\ \text{intermolecular initiation}}$ + $\Delta G°_{37}$(Bulge Loop)

$\Delta G°_{37}$ = $\Delta G°_{37}$(GC followed by CG) + $\Delta G°_{37}$(CG followed by CG) + $\Delta G°_{37}$ intermolecular initiation + $\Delta G°_{37}$ bulge initiation(1) + $\Delta G°_{37}$(CG followed by GC)

$\Delta G°_{37}$ = –3.42 kcal/mol – 3.26 kcal/mol + 4.09 kcal/mol + 3.8 kcal/mol – 2.36 kcal/mol

$\Delta G°_{37}$ = –1.2 kcal/mol

3 nucleotide bulge



$\Delta G°_{37}$ = $\Delta G°_{37}$(Watson-Crick-Franklin Pairs) + $\Delta G°_{37}$ intermolecular initiation + $\Delta G°_{37\ \text{AU end penalty}}$ + $\Delta G°_{37}$(Bulge Loop)

$\Delta G°_{37}$ = $\Delta G°_{37}$(GC followed by AU) +$\Delta G°_{37\ \text{intermolecular initiation}}$ + $\Delta G°_{37\ \text{AU end penalty}}$ + $\Delta G°_{37}$ bulge initiation(3)

$\Delta G°37$ = –2.35 kcal/mol + 4.09 kcal/mol + 0.45 kcal/mol + 3.2 kcal/mol

$\Delta G°37$ = +5.4 kcal/mol

16.3 Parameter Tables

Bulge loop parameters are available in html or as plain text. The plain text files include an extrapolation of the initiation out to 30 unpaired nucleotides.

16.4 References

The bulge loop nearest neighbor parameters for free energy change were reported in:

Mathews, D.H., Sabina, J., Zuker, M. and Turner, D.H. (1999) Expanded sequence dependence of thermodynamic parameters provides improved prediction of RNA secondary structure. *J. Mol. Biol.*, **288**, 911-940.

The experimental data for the fit of the parameters were taken from:

1. Fink, T.R. and Crothers, D.M. (1972) Free energy of imperfect nucleic acid helices, I. The bulge defect. *J. Mol. Biol.*, **66**, 1-12.
2. Groebe, D.R. and Uhlenbeck, O.C. (1989) Thermal stability of RNA hairpins containing a four-membered loop and a bulge nucleotide. *Biochemistry*, **28**, 742-747.
3. Longfellow, C.E., Kierzek, R. and Turner, D.H. (1990) Thermodynamic and spectroscopic study of bulge loops in oligoribonucleotides. *Biochemistry*, **29**, 278-285.

Chapter 17

Internal Loops

17.1 1×1, 1×2, 2×2 Internal Loops

Small symmetric internal loops have tabulated free energy changes, where experimentally determined values are used if available.

17.2 Other Internal Loops

The stabilities of other internal loops are predicted using the equation:

$\Delta G^\circ_{37\ \text{internal}} = \Delta G^\circ_{37\ \text{initiation}}(n1 + n2) + \Delta G^\circ_{37\ \text{asymmetry}} \times |n1 - n2| + \Delta G^\circ_{37\ \text{mismatch}}(\text{per UU, GA, or AG first mismatch}) + \Delta G^\circ_{37\ \text{AU/GU closure}}(\text{per AU or GU closure})$

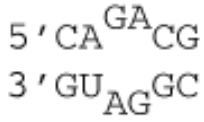
where the initiation is a length dependent term for the sum of unpaired nucleotides on each side, an asymmetry term is multiplied by the absolute value of the difference in the number of unpaired nucleotides on each side of the loop, and sequence-dependent mismatch terms are applied for first mismatches when they are UU, GA, or AG. The first mismatch bonuses are only applied for loops that have at least 2 unpaired nucleotides on each side of the loop. The AU/GU closure is applied per AU or GU closing pair and is used instead of the AU or GU penalty at the end of the helix (see Watson-Crick-Franklin or GU pairs).

Experimental data for ΔG°_{37} initiation(n) is available for loops up to n = 6. For larger internal loops, an extrapolation is made:

$\Delta G^\circ_{37\ \text{initiation}}(n>6) = \Delta G^\circ_{37\ \text{initiation}}(6) + 1.08 \times \ln(n/6)$

17.3 Examples

2×2 internal loop



ΔG°_{37} = ΔG°_{37}(Watson-Crick Pairs) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + ΔG°_{37}(Internal Loop)

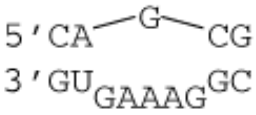
ΔG°_{37} = ΔG°_{37}(CG followed by AU) + ΔG°_{37}(CG followed by GC) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$+ ΔG°_{37}(2×2 Internal Loop)

ΔG°_{37} = –2.11 kcal/mol – 2.36 kcal/mol +4.09 kcal/mol – 1.1 kcal/mol

ΔG°_{37} = –1.5 kcal/mol

Note that the internal loop lookup tables account for terminal AU pairs that are adjacent to internal loops.

1×5 internal loop



ΔG°_{37} = ΔG°_{37}(Watson-Crick Pairs) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + ΔG°_{37}(Internal Loop)

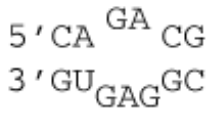
ΔG°_{37} = ΔG°_{37}(CG followed by AU) + ΔG°_{37}(CG followed by GC) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{initiation}}$(6) + ΔG°_{37} asymmetry×| n1 - n2| + $\Delta G^\circ_{37\ \text{mismatch}}$(mismatch 1) + $\Delta G^\circ_{37\ \text{mismatch}}$(mismatch 2) + ΔG°_{37} AU/GU closure

ΔG°_{37} = –2.11 kcal/mol – 2.36 kcal/mol +4.09 kcal/mol + 2.0 kcal/mol + 0.48×|1 - 5| kcal/mol + 0 kcal/mol + 0 kcal/mol + 0.65 kcal/mol

ΔG°_{37} = +4.2 kcal/mol

Note that the free energy and enthalpy changes for first mismatches in 1×(n-1) internal loops are 0 kcal/mol.

2×3 internal loop with stabilizing mismatches



ΔG°_{37} = ΔG°_{37}(Watson-Crick Pairs) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + ΔG°_{37}(Internal Loop)

ΔG°_{37} = ΔG°_{37}(CG followed by AU) + ΔG°_{37}(CG followed by GC) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{initiation}}$(5) + ΔG°_{37} asymmetry×|n1 - n2| + $\Delta G^\circ_{37\ \text{mismatch}}$(mismatch 1) + $\Delta G^\circ_{37\ \text{mismatch}}$(mismatch 2) + $\Delta G^\circ_{37\ \text{AU/GU closure}}$

ΔG°_{37} = –2.11 kcal/mol – 2.36 kcal/mol + 4.09 kcal/mol + 1.8 kcal/mol + 0.48×|2 - 3| kcal/mol – 0.0 kcal/mol – 1.1 kcal/mol + 0.65 kcal/mol

ΔG°_{37} = +1.5 kcal/mol

17.4 Parameter Tables

1×1 internal loop free energy change tables are available in text and html format. Note that these tables incorporate the AU/GU closure penalties and therefore no AU/GU helix end penalty should be applied for internal loop closure.

1×2 internal loop free energy change tables are available in text and html format. Note that these tables incorporate the AU/GU closure penalties and therefore no AU/GU helix end penalty should be applied for internal loop closure.

2×2 internal loop free energy change tables are available in text and html format. Note that these tables incorporate the AU/GU closure penalties and therefore no AU/GU helix end penalty should be applied for internal loop closure.

Other parameters in text or html format.

17.5 References

The internal loop nearest neighbor parameters for free energy change were reported in:

Mathews, D.H., Sabina, J., Zuker, M. and Turner, D.H. (1999) Expanded sequence dependence of thermodynamic parameters provides improved prediction of RNA secondary structure. *J. Mol. Biol.*, **288**, 911-940.

The experimental data for the fit of the parameters were taken from:

1. Peritz, A.E., Kierzek, R., Sugimoto, N. and Turner, D.H. (1991) Thermodynamic study of internal loops in oligoribonucleotides: Symmetric loops are more stable than asymmetric loops. *Biochemistry*, **30**, 6428-6436.
2. SantaLucia, J., Jr., Kierzek, R. and Turner, D.H. (1991) Functional group substitutions as probes of hydrogen bonding between GA mismatches in RNA internal loops. *J. Am. Chem. Soc.*, **113**, 4313-4322.
3. SantaLucia, J., Jr., Kierzek, R. and Turner, D.H. (1991) Stabilities of consecutive A.C, C.C, G.G, U.C, and U.U mismatches in RNA internal loops: evidence for stable hydrogen-bonded U.U and C.C+ pairs. *Biochemistry*, **30**, 8242-8251.
4. Walter, A.E., Wu, M. and Turner, D.H. (1994) The stability and structure of tandem GA mismatches in RNA depend on closing base pairs. *Biochemistry*, **33**, 11349-11354.
5. Wu, M., McDowell, J.A. and Turner, D.H. (1995) A periodic table of symmetric tandem mismatches in RNA. *Biochemistry*, **34**, 3204-3211.
6. Schroeder, S., Kim, J. and Turner, D.H. (1996) G.A and U.U mismatches can stabilize RNA internal loops of three nucleotides. *Biochemistry*, **35**, 16105-16109.
7. Xia, T., McDowell, J.A. and Turner, D.H. (1997) Thermodynamics of nonsymmetric tandem mismatches adjacent to G.C base pairs in RNA. *Biochemistry*, **36**, 12486-12487.
8. Kierzek, R., Burkard, M. and Turner, D. (1999) Thermodynamics of single mismatches in RNA duplexes. *Biochemistry*, **38**, 14214-14223.

Chapter 18

Coaxial Stacking

Coaxial stacking is the stacking of two base pairs at the terminii of adjacent helices. This stacking aligns the two helices along a common axis. In unimolecular secondary structures, coaxial stacking occurs in multibranch and exterior loops.

This set of nearest neighbor parameters allows for two types of coaxial stacking, flush stacking of helices that are directly adjacent (no intervening unpaired nucleotides) and mismatch-mediated coaxial stacking in which a single mistmatch occurs between the stacked helices. Mismatch-mediated coaxial stacking of helices is only allowed when there is exactly one unpaired nucleotide between the helices that can form a non-canonical pair with a nucleotide on the other side of one of the two helices.

18.1 Flush Coaxial Stacking

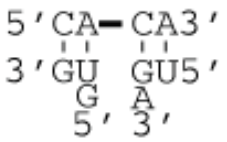
In flush coaxial stacking, the free energy change of the coaxial stack is approximated using the helical nearest neighbor parameter Watson-Crick-Franklin or GU as though there was no break in the backbone.

18.2 Mismatch-Mediated Coaxial Stacking

In the case of mismatch-mediated coaxial stacking, there are two adjacent stacks. The stack of the mismatch on the adjacent helix, where there is no break in the backbone, is approximated using the sequence-dependent terminal mismatch parameter. The second stack is the stack of the mismatch on the second helix, where the backbone is not continuous. This stack is approximated using a sequence-independent term of –2.1 kcal/mol for folding free energy change.

18.3 Examples

Flush coaxial stacking



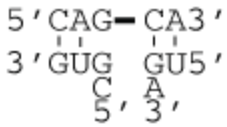
$\Delta G^{\circ}_{37\ \text{coaxial stack}} = \Delta G^{\circ}_{37}$(Watson-Crick-Franklin Stack)

$\Delta G^{\circ}_{37\ \text{coaxial stack}} = \Delta G^{\circ}_{37}$(AU pair followed by CG pair)

$\Delta G^{\circ}_{37\ \text{coaxial stack}} = -2.24$ kcal/mol

Note that at this interface, the terminal AU pair penalty would still apply when calculating the helix stability.

Mismatch-mediated coaxial stacking



$\Delta G^{\circ}_{37\ \text{coaxial stack}} = \Delta G^{\circ}_{37}$(Continuous Backbone Stack) + ΔG°_{37}(Discontinuous Backbone Stack)

$\Delta G^{\circ}_{37\ \text{coaxial stack}} = \Delta G^{\circ}_{37}$(AU pair followed by GG mismatch) + ΔG°_{37}(Discontinuous Backbone Stack)

$\Delta G^{\circ}_{37\ \text{coaxial stack}} = -0.8$ kcal/mol – 2.1 kcal/mol

$\Delta G^{\circ}_{37\ \text{coaxial stack}} = -2.9$ kcal/mol

Note that at this interface, the terminal AU pair penalty would still apply when calculating the helix stability.

18.4 Tables

A table summarizing the parameters is available in html format.

18.5 References

The coaxial stacking nearest neighbor parameters for free energy change were reported in:

Mathews, D.H., Sabina, J., Zuker, M. and Turner, D.H. (1999) Expanded sequence dependence of thermodynamic parameters provides improved prediction of RNA secondary structure. *J. Mol. Biol.*, **288**, 911-940.

The experimental data for the fit of the parameters were taken from:

1. Walter, A.E., Turner, D.H., Kim, J., Lyttle, M.H., Müller, P., Mathews, D.H. and Zuker, M. (1994) Coaxial stacking of helixes enhances binding of oligoribonucleotides and improves predictions of RNA folding. *Proc. Natl. Acad. Sci. USA.*, **91**, 9218-9222.
2. Kim, J., Walter, A.E. and Turner, D.H. (1996) Thermodynamics of coaxially stacked helices with GA and CC mismatches. *Biochemistry*, **35**, 13753-13761.

Chapter 19

Multibranch Loops

19.1 Folding Free Energy Change

Multibranch loops stabilities are predicted using the following equation:

$\Delta G^\circ_{37\ \text{multibranch}} = \Delta G^\circ_{37\ \text{initiation}} + \Delta G^\circ_{37\ \text{stacking}}$

where the stacking is the optimal configuration of dangling ends, terminal mismatches, or coaxial stacks, noting that each nucleotide or helix end can participate in only one of these favorable interactions.

When there are six or fewer unpaired nucleotides in the loop, initiation is predicted using:

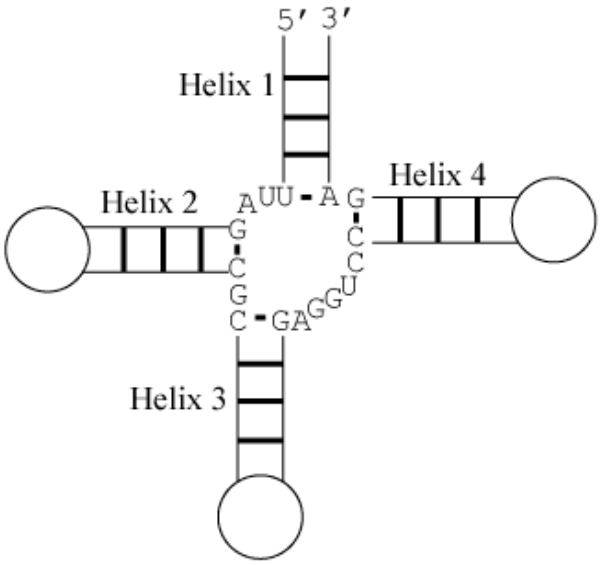
$\Delta G^\circ_{37\ \text{initiation}}$ = a + b×[number of unpaired nucleotides] + c×[number of branching helices]

where a, b, and c are parameters.

For multibranch loops with greater than six unpaired nucleotides, a logarithmic dependence on the number of upaired nucleotides is used:

$\Delta G^\circ_{37\ \text{initiation}}$ = a + 6b + (1.1 kcal/mol)×ln([number of unpaired nucleotides]/6) + c×[number of branching helices]

19.2 Example



Free Energy Change

Prediction of Stacking

The predicted stacking configuration is the one with lowest free energy change. There are eight relevant configurations.

Configuration 1:

Helix 1 with 3′ dangling U, Helix 2 with terminal mismatch, Helix 3 with 3′ dangling A, and Helix 4 with 5′ dangling C

ΔG°_{37} = ΔG°_{37}(UA with 3′ dangling U) + ΔG°_{37}(CG followed by GA) + ΔG°_{37}(GC with 3′ dangling A) + ΔG°_{37}(GC with 5′ dangling C)

ΔG°_{37} = –0.1 kcal/mol – 1.4 kcal/mol – 1.1 kcal/mol – 0.3 kcal/mol

ΔG°_{37} = –2.9 kcal/mol

Configuration 2:

Helix 1 with 3′ dangling U, Helix 2 with 5′ dangling A, Helix 3 with terminal mismatch, and Helix 4 with 5′ dangling C

ΔG°_{37} = ΔG°_{37}(UA with 3′ dangling U) + ΔG°_{37}(CG with 5′ dangling A) + ΔG°_{37}(GC followed by AG) + ΔG°_{37}(GC with 5′ dangling C)

ΔG°_{37} = –0.1 kcal/mol – 0.2 kcal/mol – 1.3 kcal/mol – 0.3 kcal/mol

ΔG°_{37} = –1.9 kcal/mol

Configuration 3:

Helix 1 in flush coaxial stack with helix 4, Helix 2 with terminal mismatch, and Helix 3 with 3′ dangling A

ΔG°_{37} = ΔG°_{37}(GC followed by AU) + ΔG°_{37}(CG followed by GA) + ΔG°_{37}(GC with 3′ dangling A)

ΔG°_{37} = –2.35 kcal/mol – 1.4 kcal/mol – 1.1 kcal/mol

ΔG°_{37} = –4.9 kcal/mol

Configuration 4:

Helix 1 in flush coaxial stack with helix 4, Helix 2 with 5′ dangling A, and Helix 3 with terminal mismatch

ΔG°_{37} = ΔG°_{37}(GC followed by AU) + ΔG°_{37}(CG with 5′ dangling A) + ΔG°_{37}(GC followed by AG)

ΔG°_{37} = –2.35 kcal/mol – 0.2 kcal/mol – 1.3 kcal/mol

ΔG°_{37} = –3.9 kcal/mol

Configuration 5:

Helix 1 with 3′ dangling U, Helix 2 in mismatch–mediated coaxial stack with helix 3 with GA intervening mismatch, and Helix 4 with 5′ dangling C

ΔG°_{37} = ΔG°_{37}(UA with 3′ dangling U) + ΔG°_{37}(CG followed by GA) + ΔG°_{37}(Discontinuous Backbone Stack) + ΔG°_{37}(GC with 5′ dangling C)

ΔG°_{37} = –0.1 kcal/mol – 1.4 kcal/mol – 2.1 kcal/mol – 0.3 kcal/mol

ΔG°_{37} = –3.9 kcal/mol

Configuration 6:

Helix 1 with 3′ dangling U, Helix 2 in mismatch–mediated coaxial stack with helix 3 with AG intervening mismatch, and Helix 4 with 5′ dangling C

ΔG°_{37} = ΔG°_{37}(UA with 3′ dangling U) + ΔG°_{37}(Discontinuous Backbone Stack) + ΔG°_{37}(GC followed by AG) + ΔG°_{37}(GC with 5′ dangling C)

ΔG°_{37} = –0.1 kcal/mol – 2.1 kcal/mol – 1.3 kcal/mol – 0.3 kcal/mol

ΔG°_{37} = –3.8 kcal/mol

Configuration 7:

Helix 1 in flush coaxial stack with helix 4 and Helix 2 in mismatch–mediated coaxial stack with helix 3 with GA intervening mismatch

ΔG°_{37} = ΔG°_{37}(GC followed by AU) + ΔG°_{37}(CG followed by GA) + ΔG°_{37}(Discontinuous Backbone Stack)

ΔG°_{37} = –2.35 kcal/mol – 1.4 kcal/mol – 2.1 kcal/mol

ΔG°_{37} = –5.9 kcal/mol

Configuration 8:

Helix 1 in flush coaxial stack with helix 4 and Helix 2 in mismatch–mediated coaxial stack with helix 3 with AG intervening mismatch

ΔG°_{37} = ΔG°_{37}(GC followed by AU) + ΔG°_{37}(Discontinuous Backbone Stack) + ΔG°_{37}(GC followed by AG)

ΔG°_{37} = –2.35 kcal/mol – 2.1 kcal/mol – 1.3 kcal/mol

ΔG°_{37} = –5.8 kcal/mol

Configuration 7 has the lowest folding free energy change of –5.9 kcal/mol.

Initiation Free Energy Change

$\Delta G^\circ_{37\ \text{initiation}}$ = a + 6b + (1.1 kcal/mol)×ln([number of unpaired nucleotides]/6) + c×[number of branching helices]

$\Delta G^\circ_{37\ \text{initiation}}$ = 10.1 kcal/mol + 6×(–0.3 kcal/mol) + (1.1 kcal/mol)×ln(8/6) + 4×(–0.3 kcal/mol)

$\Delta G^\circ_{37\ \text{initiation}}$ = 7.4 kcal/mol

19.2.1 Total Folding Free Energy Change

ΔG°_{37} multibranch loop = $\Delta G^\circ_{37\ \text{initiation}}$ + $\Delta G^\circ_{37\ \text{stacking}}$ = 7.4 kcal/mol – 5.9 kcal/mol = 1.5 kcal/mol

Note that helices 1 and 2 are separated by two unpaired nucleotides and cannot stack coaxially. Similarly, helices 3 and 4 are too distant to stack coaxially. Also note that coaxial stacking is only allowed between adjacent helices and hence, for example, helices 1 and 3 cannot stack coaxially.

19.3 Tables

Tables of parameters are available in html format.

19.4 References

The multibranch loop nearest neighbor parameters for initiation free energy change were reported in:

Mathews, D.H., Sabina, J., Zuker, M. and Turner, D.H. (1999) Expanded sequence dependence of thermodynamic parameters provides improved prediction of RNA secondary structure. *J. Mol. Biol.*, **288**, 911-940.

Chapter 20

Exterior Loops

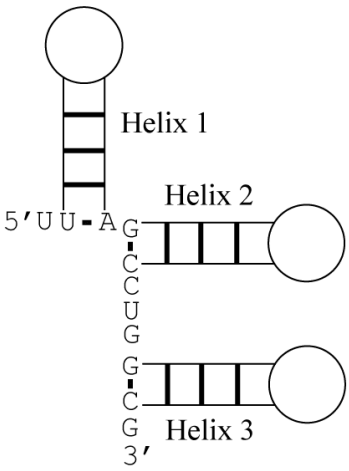
20.1 Folding Free Energy Change

Exterior loops are stabilized by terminal mismatches, dangling ends, and coaxial stacks. The stacking is the optimal configuration of dangling ends, terminal mismatches, or coaxial stacks, noting that a nucleotide or helix end can participate in only one of these favorable interactions.

20.2 Folding Enthalpy Change

Similar to free energy change, exterior loop enthalpy changes are the sum of terminal mismatches, dangling ends, and coaxial stacks. The stacking is that of the lowest folding free energy change.

20.3 Example



Free Energy Change

Prediction of Stacking

The predicted stacking configuration is the one with lowest free energy change. There are two possible configurations.

Configuration 1:

Helix 1 with 5′ dangling U, Helix 2 with 3′ dangling C, and Helix 3 with a GG mismatch

ΔG°_{37} = ΔG°_{37}(AU with 5′ dangling U) + ΔG°_{37}(CG with 3′ dangling C) + ΔG°_{37}(CG with GG mismatch)

ΔG°_{37} = –0.2 kcal/mol – 0.8 kcal/mol – 1.6 kcal/mol

ΔG°_{37} = –2.6 kcal/mol

Configuration 2:

Helix 1 in a flush coaxial stack with Helix 2 and Helix 3 with a GG mismatch

ΔG°_{37} = ΔG°_{37}(coaxial stack of AU followed by GC) + ΔG°_{37}(CG with GG mismatch)

ΔG°_{37} = –2.08 kcal/mol – 1.6 kcal/mol

ΔG°_{37} = –3.7 kcal/mol

Therefore, configuration 2, -3.7 kcal/mol, is the predicted free energy change.

Part III

DNA (RNAstructure)

Chapter 21

Watson-Crick-Franklin Helices

21.1 Free energy change at 37 °C

Folding free energy changes for Watson-Crick-Franklin helices are predicted using the equation:

$\Delta G^\circ_{37\ \text{Watson-Crick-Franklin}} = \Delta G^\circ_{37\ \text{intermolecular initiation}} + \Delta G^\circ_{37\ \text{symmetry}}$ (self-complementary duplexes) + $\Sigma[\Delta G^\circ_{37}$ stacking]

where intermolecular initiation is applied for bimolecular structure formation, the symmetry correction is applied to self-complementary duplexes, and the stacking term is a sum of sequence-dependent parameters over all base pair stacks. For helices of P uninterrupted basepairs, there are P-1 stacks of pairs.

21.2 Enthalpy Change

Enthalpy changes for Watson-Crick-Franklin helices are predicted using the equation:

$\Delta H^\circ_{\text{Watson-Crick-Franklin}} = \Delta H^\circ_{\text{intermolecular initiation}} + \Delta H^\circ_{\text{AT end penalty}}$ (per AT end) + $\Sigma[\Delta H^\circ_{\text{stacking}}]$

where terms are the same as those above for free energy changes except that the AT end penalty is applied once per each AT pair at the end of a helix. Note that the symmetry correction for self-complementary duplexes is absent because that stability cost is an entropic cost.

21.3 Examples

Self complementary duplex

```
5'AGCGCT3'
3'TCGCGA5'
```

$\Delta G^\circ_{37} = \Delta G^\circ_{37\ \text{intermolecular initiation}} + \Delta G^\circ_{37\ \text{symmetry}} + \Delta G^\circ_{37}$(AT followed by GC) + ΔG°_{37}(GC followed by CG) + ΔG°_{37}(CG followed by GC) + ΔG°_{37}(GC followed by CG) + ΔG°_{37}(CG followed by TA)

ΔG°_{37} = 1.0 kcal/mol + 0.43 kcal/mol – 1.3 kcal/mol – 2.2 kcal/mol – 2.2 kcal/mol – 2.2 kcal/mol – 1.3 kcal/mol

ΔG°_{37} = -19.47 kcal/mol

$\Delta H^\circ = \Delta H^\circ_{\text{intermolecular initiation}} + 2\times\Delta H^\circ\text{AT}_{\text{end penalty}} + \Delta H^\circ$(AT followed by GC) + ΔH°(GC followed by CG) + ΔH°(CG followed by GC) + ΔH°(GC followed by CG) + ΔH°(CG followed by TA)

ΔH° = -7.2 kcal/mol + 2×3.2 kcal/mol – 5.8 kcal/mol – 7.9 kcal/mol – 9.8 kcal/mol – 7.9 kcal/mol – 5.8 kcal/mol

ΔH°= -51.7 kcal/mol

Note that, for example, the parameters for (AT followed by GC) are the same as (CG followed by TA) because the correct directionality of the strands is preserved.

Non-self complementary duplex

```
5'GCACG3'
3'CGTGC5'
```

$\Delta G^\circ_{37} = \Delta G^\circ_{37\ \text{intermolecular initiation}} + \Delta G^\circ_{37}$(GC followed by CG) + ΔG°_{37}(CG followed by AT) + ΔG°_{37}(AT followed by CG) + ΔG°_{37}(CG followed by GC)

ΔG°_{37} = 1.0 kcal/mol – 2.2 kcal/mol – 1.5 kcal/mol – 1.4 kcal/mol – 2.2 kcal/mol

ΔG°_{37} = -6.3 kcal/mol

$\Delta H^\circ = \Delta H^\circ_{\text{intermolecular initiation}} + \Delta H^\circ$(GC followed by CG) + ΔH°(CG followed by AT) + ΔH°(AT followed by CG) + ΔH°(CG followed by GC)

ΔH° = -7.2 kcal/mol - 7.9 kcal/mol – 9.9 kcal/mol – 5.8 kcal/mol – 9.8 kcal/mol

ΔH°= –40.6 kcal/mol

Note Free energy and enthalpy change associated with AT end is zero and 3.2 kcal/mol respectively.

21.4 Parameter Tables

The table of parameters is available as plain text for free energy change and enthalpy change.

Chapter 22

GT Pairs

GT pairs are treated as nearest neighbor stacks, similar to Watson-Crick-Franklin helices, and unlike RNA, GT pairs at the ends of helices are not penalized. Parameters for stacks containing GT pairs were calculated separately from those containing AT and GC base pairs only.

22.1 Examples

```
5'GGTCGTGT3'
3'CTGGTGCG5'
```

ΔG°_{37} = $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + ΔG°_{37}(GC followed by GT) + ΔG°_{37}(GT followed by TG) + ΔG°_{37}(TG followed by CG) + ΔG°_{37}(CG followed by GT) + ΔG°_{37}(GT followed by TG) + ΔG°_{37}(TG followed by GC) + ΔG°_{37}(GC followed by TG)

ΔG°_{37} = 1.0 kcal/mol + 0.1 kcal/mol + 1.2 kcal/mol + 0.1 kcal/mol - 0.5 kcal/mol + 1.2 kcal/mol - 0.5 kcal/mol - 0.6 kcal/mol

ΔG°_{37} = 2.0 kcal/mol

ΔH° = $\Delta H^\circ_{\text{intermolecular initiation}}$ + ΔH°(GC followed by GT) + ΔH°(GT followed by TG) + ΔH°(TG followed by CG) + ΔH°(CG followed by GT) + ΔH°(GT followed by TG) + ΔH°(TG followed by GC) + ΔH°(GC followed by TG)

ΔH° = -7.2 kcal/mol + 4.3 kcal/mol + 5.0 kcal/mol + 4.3 kcal/mol - 4.3 kcal/mol + 5.0 kcal/mol - 4.3 kcal/mol - 2.4 kcal/mol

ΔH° = 0.4 kcal/mol

22.2 Parameter Tables

The table of parameters is available as plain text for free energy change (including Watson-Crick-Franklin pairs) and enthalpy change (including Watson-Crick-Franklin pairs).

Chapter 23

Dangling Ends

Dangling ends are nucleotides that stack on the ends of helices. In secondary structures, they occur in multibranch and exterior loops. They occur as either 5′ dangling ends (an unpaired nucleotide 5′ to the helix end) or 3′ dangling ends (an unpaired nucleotide 3′ to the helix end). Note that if a helix end is extended on both the 5′ and 3′ strands, then a terminal mismatch exists (not the sum of 5′ and 3′ dangling ends).

23.1 Examples

```
5'AGCACGC3'
3' CGTGC 5'
```

ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(3′ dangling C adajacent to GC) + ΔG°_{37}(5′ dangling A adjacent CG)

ΔG°_{37} = -6.8 kcal/mol - 0.2 kcal/mol - 0.8 kcal/mol

ΔG°_{37} = -7.8 kcal/mol

ΔH° = ΔH°(Watson-Crick-Franklin Helix) + ΔH°(3′ dangling C adajacent to GC) + ΔH°(5′ dangling A adjacent CG)

ΔH° = -33.1 kcal/mol -0.2 kcal/mol -4.6 kcal/mol

ΔH° = -37.9 kcal/mol

Note that this example contains both a 5′ and a 3′ dangling end (at opposite ends of the duplex).

23.2 Parameter Tables

The tables of parameters are available as plain text for free energy change and enthalpy change.

Chapter 24

Terminal Mismatches

Terminal mismatches are non-canonical pairs adjacent to helix ends.

24.1 Examples

```
5'AGCGCTG3'
3'TCGCGAA5'
```

ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(TA followed by GA)

ΔG°_{37} = -9.2 kcal/mol - 0.6 kcal/mol

ΔG°_{37} = -9.8 kcal/mol

ΔH° = ΔH°(Watson-Crick-Franklin Helix) + ΔH°(TA followed by GA)

ΔH° = -37.2 kcal/mol + 2.3 kcal/mol

ΔH° = -34.9 kcal/mol

24.2 Parameter Tables

Tables of parameters are available as plain text for free energy changes and enthalpy changes.

Chapter 25

Hairpin Loops

25.1 Folding Free Energy Change

Hairpin loops of 4 or more nucleotides

The prediction of folding free energy changes for hairpins of 4 or more unpaired nucleotides is made with the following equation:

$\Delta G^\circ_{37\ \text{hairpin}}$ (>3 nucleotides in loop) = $\Delta G^\circ_{37\ \text{initiation}}$ (n) + ΔG°_{37} (hairpin terminal mismatch)

In this equation, n is the number of nucleotides in loop and the terminal mismatch parameter is the sequence-dependent term for the first mismatch stacking on the terminal base pair.

Hairpin loops of 3 unpaired nucleotides

For hairpin loops of 3 nucleotides, the folding free energy change is estimated using:

$\Delta G^\circ_{37\ \text{hairpin}}$ (3 unpaired nucleotides) = $\Delta G^\circ_{37\ \text{initiation}}$ (3)

As opposed to longer hairpin loops, hairpin loops of three nucleotides do not receive a sequence-dependent first mismatch term.

Special hairpin loops

There are hairpin loop sequences of 3 and 4 nucleotides that have stabilities poorly fit by the model. These hairpins are assigned stabilities based on experimental data.

Short hairpin loops

The nearest neighbor rules prohibit hairpin loops with fewer than 3 nucleotides.

25.2 Folding Enthalpy Change

Hairpin loops of 4 or more nucleotides

The prediction of folding enathlpy changes for hairpins of 4 or more nucleotides is made with the following equation:

$\Delta H^\circ_{\text{hairpin}}$ (>3 unpaired nucleotides) = $\Delta H^\circ_{\text{initiation}}$ (n) + ΔH° (hairpin terminal mismatch)

As with the free energy change equation above, n is the number of nucleotides in the loop and the terminal mismatch parameter is the sequence-dependent term for the first mismatch stacking on the terminal pair.

Hairpin loops of 3 nucleotides

For hairpin loops of 3 unpaired nucleotides, the enthalpy change is estimated using:

$\Delta H^\circ_{\text{hairpin}}$ (3 unpaired nucleotides) = $\Delta H^\circ_{\text{initiation}}$ (3)

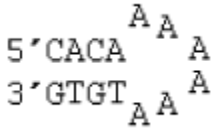
Hairpin loops of three nucleotides do not receive a sequence-dependent first mismatch term.

Special hairpin loops

Hairpin loops of 3 and 4 nucleotides that have stabilities poorly fit by the free energy model are assigned enthalpy changes based on experimental data.

25.3 Examples

6 nucleotide hairpin loop with no special stacking terms



ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(Hairpin Loop)

ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(terminal mismatch) + $\Delta G^\circ_{37\ \text{Hairpin initiation}}$(6)

ΔG°_{37} = ΔG°_{37}(CG followed by AT) + ΔG°_{37}(AT followed by CG) + ΔG°_{37}(CG followed by AT) + ΔG°_{37}(AT followed by AA) + $\Delta G^\circ_{37\ \text{Hairpin initiation}}$(6)

ΔG°_{37} = -1.5 kcal/mol - 1.4 kcal/mol - 1.5 kcal/mol - 0.7 kcal/mol + 4.2 kcal/mol

ΔG°_{37} = -0.90 kcal/mol

ΔH° = ΔH°(Watson-Crick-Franklin Helix) + ΔH°(Hairpin Loop)

ΔH° = ΔH°(Watson-Crick-Franklin Helix) + ΔH°(terminal mismatch) + $\Delta H^\circ_{\text{Hairpin initiation}}$(6)

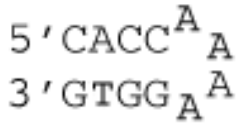
ΔH° = ΔH°(CG followed by AT) + ΔH°(AT followed by CG) + ΔH°(CG followed by AT) + $\Delta H^\circ_{\text{AT end penalty}}$ + ΔH°(AT followed by AA) + $\Delta H^\circ_{\text{Hairpin initiation}}$(6)

ΔH° = -5.8 kcal/mol - 7.8 kcal/mol - 5.8 kcal/mol + 3.2 kcal/mol + 4.0 kcal/mol - 16.7 kcal/mol

ΔH° = -28.9 kcal/mol

Note that for unimolecular secondary structures, the helical intermolecular initiation does not appear.

4 nucleotide special hairpin loop



ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(Hairpin Loop)

ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(CaaaaG)

ΔG°_{37} = ΔG°_{37}(CG followed by AT) + ΔG°_{37}(AT followed by CG) + ΔG°_{37}(CG followed by CG) + ΔG°_{37}(CaaaaG)

ΔG°_{37} = -1.5 kcal/mol -1.4 kcal/mol -1.8 kcal/mol + 3.2 kcal/mol

ΔG°_{37} = -1.5 kcal/mol

ΔH° = ΔH°(Watson-Crick-Franklin Helix) + ΔH°(Hairpin Loop)

ΔH° = ΔH°(Watson-Crick-Franklin Helix) + ΔH°(CaaaaG)

ΔH° = ΔH°(CG followed by AT) + ΔH°(AT followed by CG) + ΔH°(CG followed by CG) + ΔH°(CaaaaG)

ΔH° = -9.9 kcal/mol - 7.8 kcal/mol - 7.5 kcal/mol + 3.2 kcal/mol

ΔH° = -22.0 kcal/mol

25.4 Parameter Tables

Length dependent initiation parameters are available in plain text for free energy changes and enthalpy changes.

The hairpin terminal mismatch tables are available in plain text for free energy changes and enthalpy changes.

The table of special hairpin loops is available in plain text for free energy change for 3 and 4 nucleotides and plain text for enthalpy change for 3 and 4 nucleotides. The special hairpin loop sequences include the identity of the closing basepair.

Chapter 26

Bulge Loops

26.1 Folding Free Energy Change

Singe Nucleotide Bulge Loops

The prediction of folding free energy changes is made with the following equation:

$\Delta G^\circ_{37\ \text{bulge}}$ (n=1) = $\Delta G^\circ_{37\ \text{bulge initiation}}$(n) + ΔG°_{37} (base pair stack) – RT ln(number of states)

In this equation, n is the number of unpaired nucleotides, the base pair stack is the stack of the closing pairs as though there is no bulge (using Watson-Crick-Franklin rules as needed), and the number of states counts the number of possible loops of identical sequence.

Bulges of 2 or More Nucleotides

For bulges of 2 or more nucleotides, the following equation is used:

$\Delta G^\circ_{37\ \text{bulge}}$ (n>1) = $\Delta G^\circ_{37\ \text{bulge initiation}}$(n)

Experimentally-derived parameters are available for initiation up to n = 3 and a linear extrapolation is used up to n = 6. Beyond 6, the initiation is approximated using a logarithmic function:

$\Delta G^\circ_{37\ \text{bulge}}$ (n>6) = $\Delta G^\circ_{37\ \text{bulge initiation}}$(6) + 1.75 RT ln(n/6)

where R is the gas constant and T is the absolute temperature, 310.15 K.

26.2 Folding Enthalpy Change

Singe Nucleotide bulge Loops

The prediction of folding free energy changes is made with the following equation:

$\Delta H^\circ_{\text{bulge}}$ (n=1) = $\Delta H^\circ_{\text{bulge initiation}}$(n) + ΔH°(base pair stack)

In this equation, n is the number of unpaired nucleotides and the base pair stack is the stack of the closing pairs as though there is no bulge (using Watson-Crick-Franklin rules as needed).

Because the helical stack continues across a single nucleotide bulge, the terminal AT penalty is not applied adjacent to single bulges.

Bulges of 2 or More Nucleotides

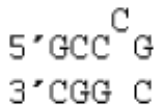
For bulges of 2 or more nucleotides, the following equation is used:

$\Delta H^\circ_{\text{bulge}}$ (n>1) = $\Delta H^\circ_{\text{bulge initiation}}$(n)

Experimentally-derived parameters are available for bulge loop initiations up to n = 3. For n > 3, the initiation is approximated as that for n = 3.

26.3 Examples

Single C bulge with multiple states



ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + ΔG°_{37}(Bulge Loop)

ΔG°_{37} = ΔG°_{37}(GC followed by CG) + ΔG°_{37}(CG followed by CG) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{bulge initiation}}$(1) + ΔG°_{37}(CG followed by GC) – RTln(3)

ΔG°_{37} = -2.2 kcal/mol -1.8 kcal/mol + 1.0 kcal/mol + 2.9 kcal/mol -2.2 kcal/mol - 0.616×1.099 kcal/mol

ΔG°_{37} = -2.98 kcal/mol

ΔH° = ΔH°(Watson-Crick-Franklin Helix) + $\Delta H^\circ_{\text{intermolecular initiation}}$ + ΔH°(Bulge Loop)

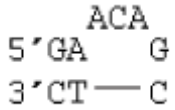
$\Delta H^\circ = \Delta H^\circ$(GC followed by CG) + ΔH°(CG followed by CG) + $\Delta H^\circ_{\text{intermolecular initiation}}$ + $\Delta H^\circ_{\text{bulge initiation}}(1)$ + ΔH°(CG followed by GC)

ΔH° = -7.9 kcal/mol - 7.5 kcal/mol - 7.2 kcal/mol + 18.9 kcal/mol - 9.8 kcal/mol

ΔH° = -13.5 kcal/mol

Note that this loop has three available states because any of the three Cs in the top strand can be the bulge.

3 nucleotide bulge



ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + $\Delta G^\circ_{37\text{ intermolecular initiation}}$ + ΔG°_{37}(Bulge Loop)

ΔG°_{37} = ΔG°_{37}(GC followed by AT) + $\Delta G^\circ_{37\text{ intermolecular initiation}}$ + $\Delta G^\circ_{37\text{ bulge initiation}}(3)$

ΔG°_{37} = -1.3 kcal/mol + 1.0 kcal/mol + 2.5 kcal/mol

ΔG°_{37} = 2.2 kcal/mol

$\Delta H^\circ = \Delta H^\circ$(Watson-Crick-Franklin Helix) + $\Delta H^\circ_{\text{intermolecular initiation}}$ + $\Delta H^\circ_{\text{AT end penalty}}$ + ΔH°(Bulge Loop)

$\Delta H^\circ = \Delta H^\circ$(GC followed by AT) + $\Delta H^\circ_{\text{intermolecular initiation}}$ + $\Delta H^\circ_{\text{AT end penalty}}$ + $\Delta H^\circ_{\text{bulge initiation}}(3)$

ΔH° = -8.5 kcal/mol - 7.2 kcal/mol + 3.2 kcal/mol - 2.3 kcal/mol

ΔH° = -14.8 kcal/mol

26.4 Parameter Tables

Bulge loop parameters are available in plain text for initiation free energy parameters or initiation enthalpy parameters. The plain text initiation parameters include an extrapolation out to lengths of 30 unpaired nucleotides.

Chapter 27

Internal Loops

27.1 1×1, 1×2, 2×2 Internal Loops

Small symmetric internal loops have tabulated free energy and enthalpy changes, where experimentally determined values are used if available.

27.2 Other Internal Loops

The stabilities of other internal loops are predicted using the equation:

ΔG°_{37} internal = $\Delta G^\circ_{37\ \text{initiation}}$(n) + $\Delta G^\circ_{37\ \text{asymmetry}}$ × |n1 – n2| + $\Delta G^\circ_{37\ \text{mismatch}}$(mismatch 1) + $\Delta G^\circ_{37\ \text{mismatch}}$(mismatch 2)

where the initiation is a length dependent term for n unpaired nucleotides, an asymmetry term is multiplied by the absolute value of the difference in the number of unpaired nucleotides on each side of the loop, and sequence-dependent mismatch terms are applied for first mismatches of specific sequences.

Similarly, the enthalpy change is predicted with the equation:

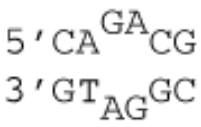
ΔH°internal = $\Delta H^\circ_{\text{initiation}}$(n) + $\Delta H^\circ_{\text{asymmetry}}$ × |n1 – n2| + $\Delta H^\circ_{\text{mismatch}}$(mismatch 1) + $\Delta H^\circ_{\text{mismatch}}$(mismatch 2) + $\Delta H^\circ_{\text{AT closure}}$(per AT)

where terms are analagous to those for predicting folding free energy changes.

The mismatch parameters are sequence-dependent and are different for 1×(n-1) loop, 2×3 loop, and other internal loops. In the case of 1×(n–1) loops, the mismatches are set to 0 kcal/mol for free energy and enthalpy changes.

27.3 Examples

2×2 internal loop



$\Delta G°_{37}$ = $\Delta G°_{37}$(Watson-Crick-Franklin Helix) + $\Delta G°_{37\ \text{intermolecular initiation}}$ + $\Delta G°_{37}$(Internal Loop)

$\Delta G°_{37}$ = $\Delta G°_{37}$(CG followed by AT) + $\Delta G°_{37}$(CG followed by GC) + $\Delta G°_{37\ \text{intermolecular initiation}}$ + $\Delta G°_{37}$(2×2 Internal Loop)

$\Delta G°_{37}$ = -1.5 kcal/mol - 2.2 kcal/mol + 1.0 kcal/mol + 1.6 kcal/mol

$\Delta G°_{37}$ = -1.1 kcal/mol

$\Delta H°$ = $\Delta H°$(Watson-Crick-Franklin Helix) + $\Delta H°_{\text{intermolecular initiation}}$ + $\Delta H°$(Internal Loop)

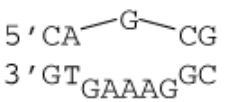
$\Delta H°$ = $\Delta H°$(CG followed by AT) + $\Delta H°$(CG followed by GC) + $\Delta H°_{\text{intermolecular initiation}}$ + $\Delta H°$(2×2 Internal Loop)

$\Delta H°$ = -9.9 kcal/mol - 9.8 kcal/mol - 7.2 kcal/mol - 2.2 kcal/mol

$\Delta H°$ = -29.1 kcal/mol

Note that the internal loop lookup tables account for terminal AT pairs that are adjacent to internal loops.

1×5 internal loop



$\Delta G°_{37}$ = $\Delta G°_{37}$(Watson-Crick-Franklin Helix) + $\Delta G°_{37\ \text{intermolecular initiation}}$ + $\Delta G°_{37}$(Internal Loop)

$\Delta G°_{37}$ = $\Delta G°_{37}$(CG followed by AT) + $\Delta G°_{37}$(CG followed by GC) + $\Delta G°_{37\ \text{intermolecular initiation}}$ + $\Delta G°_{37\ \text{initiation}}$(6) + $\Delta G°_{37\ \text{asymmetry}}$×|n1 - n2| + $\Delta G°_{37\ \text{mismatch}}$(mismatch 1) + $\Delta G°_{37\ \text{mismatch}}$(mismatch 2)

$\Delta G°_{37}$ = -1.5 kcal/mol - 2.2 kcal/mol + 1.0 kcal/mol + 3.9 kcal/mol + .4×|1-5| kcal/mol + 0 kcal/mol + 0 kcal/mol

$\Delta G°_{37}$ = 2.8 kcal/mol

$\Delta H^\circ = \Delta H^\circ$(Watson-Crick-Franklin Helix) + $\Delta H^\circ_{\text{intermolecular initiation}}$ + ΔH°(Internal Loop)

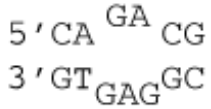
$\Delta H^\circ = \Delta H^\circ$(CG followed by AT) + ΔH°(CG followed by GC) + $\Delta H^\circ_{\text{intermolecular initiation}}$ + $\Delta H^\circ_{\text{initiation}}(6)$ + $\Delta H^\circ_{\text{asymmetry}} \times |n1 - n2|$ + $\Delta H^\circ_{\text{mismatch}}$(mismatch 1) + $\Delta H^\circ_{\text{mismatch}}$(mismatch 2) + ΔH° AT closure

ΔH° = -9.9 kcal/mol - 9.8 kcal/mol - 7.2 kcal/mol + 0.0 kcal/mol + 0.0 × |1-5| kcal/mol + 0.0 kcal/mol + 0.0 kcal/mol + 3.2 kcal/mol

ΔH° = -23.7 kcal/mol

Note that the free energy and enthalpy changes for first mismatches in 1×(n-1) internal loops are 0 kcal/mol.

2×3 internal loop with stabilizing mismatches



$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin Helix) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + ΔG°_{37}(Internal Loop)

$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(CG followed by AT) + ΔG°_{37}(CG followed by GC) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{initiation}}(5)$ + $\Delta G^\circ_{37\ \text{asymmetry}} \times |n1 - n2|$ + $\Delta G^\circ_{37\ \text{mismatch}}$(mismatch 1) + $\Delta G^\circ_{37\ \text{mismatch}}$(mismatch 2)

ΔG°_{37} = -1.5 kcal/mol - 2.2 kcal/mol + 1.0 kcal/mol + 2.0 kcal/mol + .4×|3 - 2| kcal/mol - 0.4 kcal/mol - 1.0 kcal/mol

ΔG°_{37} = -1.7 kcal/mol

$\Delta H^\circ = \Delta H^\circ$(Watson-Crick-Franklin Helix) + $\Delta H^\circ_{\text{intermolecular initiation}}$ + ΔH°(Internal Loop)

$\Delta H^\circ = \Delta H^\circ$(CG followed by AT) + ΔH°(CG followed by GC) + $\Delta H^\circ_{\text{intermolecular initiation}}$ + $\Delta H^\circ_{\text{initiation}}(5)$ + $\Delta H^\circ_{\text{asymmetry}} \times |n1 - n2|$ + $\Delta H^\circ_{\text{mismatch}}$(mismatch 1) + $\Delta H^\circ_{\text{mismatch}}$(mismatch 2) + ΔH° AT closure

ΔH° = -9.9 kcal/mol - 9.8 kcal/mol - 7.2 kcal/mol + 0.0 kcal/mol + 0.0×|3 - 2| kcal/mol + 1.5 kcal/mol - 6.2 kcal/mol + 3.2 kcal/mol

ΔH° = -28.4 kcal/mol

27.4 Parameter Tables

1×1 internal loop free energy change tables are available in text and html format. Enthalpy change tables are available in text and html format. Note that

these tables incorporate the AT closure penalties and therefore no AT helix end penalty should be applied for internal loop closure.

1×2 internal loop free energy change tables are available in text and html format. Enthalpy change tables are available in text and html format. Note that these tables incorporate the AT closure penalties and therefore no AT helix end penalty should be applied for internal loop closure.

2×2 internal loop free energy change tables are available in text and html format. Enthalpy change tables are available in text and html format. Note that these tables incorporate the AT closure penalties and therefore no AT helix end penalty should be applied for internal loop closure.

Chapter 28

Coaxial Stacking

28.1 Introduction

Coaxial stacking is the stacking of two base pairs at the terminii of adjacent helices. This stacking aligns the two helices along a common axis. In unimolecular secondary structures, coaxial stacking occurs in multibranch and exterior loops.

This set of nearest neighbor parameters allows for two types of coaxial stacking, flush stacking of helices that are directly adjacent (no intervening unpaired nucleotides) and mismatch-mediated coaxial stacking in which a single mistmatch occurs between the stacked helices. Mismatch-mediated coaxial stacking of helices is only allowed when there is exactly one unpaired nucleotide between the helices that can form a non-canonical pair with a nucleotide on the other side of one of the two helices.

28.2 Flush Coaxial Stacking

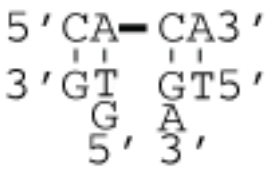
In flush coaxial stacking, the free energy and enthalpy changes of the coaxial stack are approximated using the helical nearest neighbor parameters Watson-Crick-Franklin as though there is no break in the backbone.

28.3 Mismatch-Mediated Coaxial Stacking

In the case of mismatch-mediated coaxial stacking, there are two adjacent stacks. The stack of the mismatch on the adjacent helix, where there is no break in the backbone, is approximated using the terminal mismatch parameters. The second stack is the stack of the mismatch on the second helix, where the backbone is not continuous.

28.4 Examples

Flush coaxial stacking



$\Delta G^{\circ}_{37\ \text{coaxial stack}} = \Delta G^{\circ}_{37}$(Watson-Crick-Franklin stack)

$\Delta G^{\circ}_{37\ \text{coaxial stack}} = \Delta G^{\circ}_{37}$(AT pair followed by CG pair)

$\Delta G^{\circ}_{37\ \text{coaxial stack}}$ = -1.4 kcal/mol

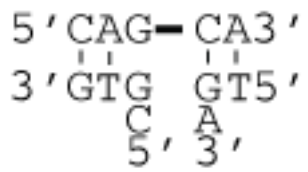
$\Delta H^{\circ}_{\text{coaxial stack}} = \Delta H^{\circ}$(Watson-Crick-Franklin stack)

$\Delta H^{\circ}_{\text{coaxial stack}} = \Delta H^{\circ}$(AT pair followed by CG pair)

$\Delta H^{\circ}_{\text{coaxial stack}}$ = -0.8 kcal/mol

Note that at this interface, the terminal AT pair penalty would still apply when calculating the helix stability.

Mismatch-mediated coaxial stacking



$\Delta G^{\circ}_{37\ \text{coaxial stack}} = \Delta G^{\circ}_{37}$(continuous backbone stack) + ΔG°_{37}(discontinuous backbone stack)

$\Delta G^{\circ}_{37\ \text{coaxial stack}} = \Delta G^{\circ}_{37}$(AT pair followed by GG mismatch) + ΔG°_{37}(discontinuous backbone stack)

$\Delta G^{\circ}_{37\ \text{coaxial stack}}$ = -0.4 kcal/mol -2.1 kcal/mol

$\Delta G^{\circ}_{37\ \text{coaxial stack}}$ = -2.5 kcal/mol

$\Delta H^{\circ}_{\text{coaxial stack}} = \Delta H^{\circ}$(continuous backbone stack) + ΔH°(discontinuous backbone stack)

$\Delta H°_{\text{coaxial stack}} = \Delta H°$(AT pair followed by GG mismatch) + $\Delta H°$(discontinuous backbone stack)

$\Delta H°_{\text{coaxial stack}}$ = 1.5 kcal/mol - 8.4 kcal/mol

$\Delta H°_{\text{coaxial stack}}$ = -6.9 kcal/mol

Note that at this interface, the terminal AT pair penalty would still apply when calculating the helix stability.

28.5 Parameter Tables

A table summarizing the parameters is available in html format.

Chapter 29

Multibranch Loops

29.1 Folding Free Energy Change

Multibranch loops stabilities are predicted using the following equation:

$\Delta G^\circ_{37\ \text{multibranch}} = \Delta G^\circ_{37\ \text{initiation}} + \Delta G^\circ_{37\ \text{stacking}}$

where the stacking is the optimal configuration of dangling ends, terminal mismatches, or coaxial stacks, noting that each nucleotide or helix end can participate in only one of these favorable interactions.

The initiation is predicted using:

$\Delta G^\circ_{37\ \text{initiation}}$ = a + b×[number of unpaired nucleotides] + c×[number of branching helices]

where a, b, and c are parameters.

29.2 Folding Enthalpy Change

Similar to free energy change, multibranch loops enthalpy changes are predicted using the following equation:

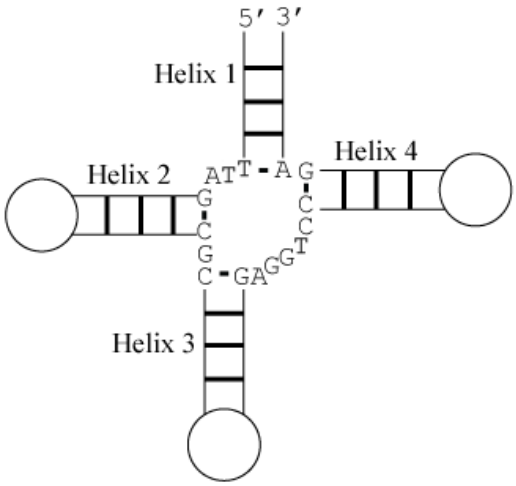
$\Delta H^\circ_{\text{multibranch}} = \Delta H^\circ_{\text{initiation}} + \Delta H^\circ_{\text{stacking}}$

where the stacking is the configuration of dangling ends, terminal mismatches, or coaxial stacks with lowest folding free energy change.

Initiation is predicted using an equation analagous to that folding free energy initiation:

$\Delta H^\circ_{\text{initiation}}$ = a + b×[number of unpaired nucleotides] + c×[number of branching helices]

29.3 Examples



Free Energy Change

Prediction of Stacking

The predicted stacking configuration is the one with lowest free energy change. There are eight relevant configurations.

* Configuration 1:

Helix 1 with 3′ dangling T, Helix 2 with terminal mismatch, Helix 3 with 3′ dangling A, and Helix 4 with 5′ dangling C

ΔG°_{37} = ΔG°_{37}(TA with 3′ dangling T) + ΔG°_{37}(CG followed by GA) + ΔG°_{37}(GC with 3′ dangling A) + ΔG°_{37}(GC with 5′ dangling C)

ΔG°_{37} = -0.2 kcal/mol - 1.0 kcal/mol - 0.4 kcal/mol - 0.5 kcal/mol

ΔG°_{37} = -2.1 kcal/mol

* Configuration 2:

Helix 1 with 3′ dangling T, Helix 2 with 5′ dangling A, Helix 3 with terminal mismatch, and Helix 4 with 5′ dangling C

ΔG°_{37} = ΔG°_{37}(TA with 3′ dangling T) + ΔG°_{37}(CG with 5′ dangling A) + ΔG°_{37}(GC followed by AG) + ΔG°_{37}(GC with 5′ dangling C)

ΔG°_{37} = -0.2 kcal/mol - 0.8 kcal/mol - 0.8 kcal/mol - 0.5 kcal/mol

ΔG°_{37} = -2.3 kcal/mol

* Configuration 3:

Helix 1 in flush coaxial stack with helix 4, Helix 2 with terminal mismatch, and Helix 3 with 3′ dangling A

ΔG°_{37} = ΔG°_{37}(GC followed by AT) + ΔG°_{37}(CG followed by GA) + ΔG°_{37}(GC with 3′ dangling A)

ΔG°_{37} = 0.0 kcal/mol - 1.0 kcal/mol - 0.4 kcal/mol

ΔG°_{37} = -1.4 kcal/mol

* Configuration 4:

Helix 1 in flush coaxial stack with helix 4, Helix 2 with 5′ dangling A, and Helix 3 with terminal mismatch

ΔG°_{37} = ΔG°_{37}(GC followed by AT) + ΔG°_{37}(CG with 5′ dangling A) + ΔG°_{37}(GC followed by AG)

ΔG°_{37} = -1.3 kcal/mol - 0.8 kcal/mol - 0.8 kcal/mol

ΔG°_{37} = -2.9 kcal/mol

* Configuration 5:

Helix 1 with 3′ dangling T, Helix 2 in mismatch–mediated coaxial stack with helix 3 with GA intervening mismatch, and Helix 4 with 5′ dangling C

ΔG°_{37} = ΔG°_{37}(TA with 3′ dangling T) + ΔG°_{37}(CG followed by GA) + ΔG°_{37}(Discontinuous Backbone Stack) + ΔG°_{37}(GC with 5′ dangling C)

ΔG°_{37} = -0.2 kcal/mol - 1.0 kcal/mol - 2.1 kcal/mol - 0.5 kcal/mol

ΔG°_{37} = -3.8 kcal/mol

* Configuration 6:

Helix 1 with 3′ dangling T, Helix 2 in mismatch–mediated coaxial stack with helix 3 with AG intervening mismatch, and Helix 4 with 5′ dangling C

ΔG°_{37} = ΔG°_{37}(TA with 3′ dangling T) + ΔG°_{37}(Discontinuous Backbone Stack) + ΔG°_{37}(GC followed by AG) + ΔG°_{37}(GC with 5′ dangling C)

ΔG°_{37} = -0.2 kcal/mol - 2.1 kcal/mol - 0.8 kcal/mol - 0.5 kcal/mol

ΔG°_{37} = -3.6 kcal/mol

* Configuration 7:

Helix 1 in flush coaxial stack with helix 4 and Helix 2 in mismatch–mediated coaxial stack with helix 3 with GA intervening mismatch

ΔG°_{37} = ΔG°_{37}(GC followed by AT) + ΔG°_{37}(CG followed by GA) + ΔG°_{37}(Discontinuous Backbone Stack)

ΔG°_{37} = -1.3 kcal/mol - 1.0 kcal/mol - 2.1 kcal/mol

ΔG°_{37} = -4.4 kcal/mol

* Configuration 8:

Helix 1 in flush coaxial stack with helix 4 and Helix 2 in mismatch–mediated coaxial stack with helix 3 with AG intervening mismatch

ΔG°_{37} = ΔG°_{37}(GC followed by AT) + ΔG°_{37}(Discontinuous Backbone Stack) + ΔG°_{37}(GC followed by AG)

ΔG°_{37} = -1.3 kcal/mol - 2.1 kcal/mol - 0.8 kcal/mol

ΔG°_{37} = -4.2 kcal/mol

Configuration 7 has the lowest folding free energy change of -4.4 kcal/mol.

Initiation Free Energy Change

$\Delta G^\circ_{37\ \text{initiation}}$ = a + b×[number of unpaired nucleotides] + c×[number of branching helices]

$\Delta G^\circ_{37\ \text{initiation}}$ = 3.0 kcal/mol + 0.2 kcal/mol × 8 + 0.2 kcal/mol × 4

$\Delta G^\circ_{37\ \text{initiation}}$ = 5.4 kcal/mol

Total Folding Free Energy Change

$\Delta G^\circ_{37\ \text{multibranch loop}} = \Delta G^\circ_{37\ \text{initiation}} + \Delta G^\circ_{37\ \text{stacking}}$ = -4.4 kcal/mol + 5.4 kcal/mol = 1.0 kcal/mol

Enthalpy Change

Prediction of Stacking

The stacking configuration is fixed by the prediction of folding free energy change and is configuration 7 above.

ΔH°= ΔH°(GC followed by AT) + ΔH°(CG followed by GA) + ΔH°(Discontinuous Backbone Stack)

ΔH° = -8.5 kcal./mol - 4.6 kcal/mol - 8.4 kcal/mol

ΔH° = -21.5 kcal/mol

Initiation Enthalpy Change

$\Delta H^\circ_{initiation}$ = a + b×[number of unpaired nucleotides] + c×[number of branching helices]

$\Delta H^\circ_{initiation}$ = 9.0 kcal/mol + 0.0 kcal/mol × 8 + 0.0 kcal/mol × 4

$\Delta H^\circ_{initiation}$ = 9.0 kcal/mol

Total Folding Enthalpy Energy Change

$\Delta H^\circ_{multibranch\ loop} = \Delta H^\circ_{initiation} + \Delta H^\circ_{stacking}$

$\Delta H^\circ_{multibranch\ loop}$ = 9.0 kcal/mol - 21.5 kcal/mol = -12.5 kcal/mol

Note that helices 1 and 2 are separated by two unpaired nucleotides and cannot stack coaxially. Similarly, helices 3 and 4 are too distant to stack coaxially. Also note that coaxial stacking is only allowed between adjacent helices and hence, for example, helices 1 and 3 cannot stack coaxially.

29.4 Parameter Tables

Tables of parameters are available in html format.

Chapter 30

Exterior Loops

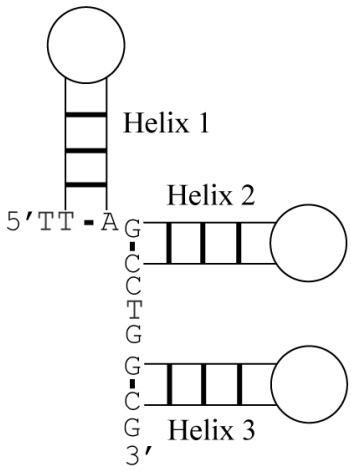
30.1 Folding Free Energy Change

Exterior loops are stabilized by terminal mismatches, dangling ends, and coaxial stacks. The stacking is the optimal configuration of dangling ends, terminal mismatches, or coaxial stacks, noting that a nucleotide or helix end can participate in only one of these favorable interactions.

30.2 Folding Enthalpy Change

Similar to free energy change, exterior loop enthalpy changes are the sum of terminal mismatches, dangling ends, and coaxial stacks. The stacking is that of the lowest folding free energy change.

30.3 Examples



Prediction of Stacking

The predicted stacking configuration is the one with lowest free energy change. There are two possible configurations.

Configuration 1:

Helix 1 with 5′ dangling T, Helix 2 with 3′ dangling C, and Helix 3 with a GG mismatch

ΔG°_{37} = ΔG°_{37}(AT with 5′ dangling T) + ΔG°_{37}(CG with 3′ dangling C) + ΔG°_{37}(CG with GG mismatch)

ΔG°_{37} = -0.3 kcal/mol - 0.4 kcal/mol - 0.9 kcal/mol

ΔG°_{37} = -1.6 kcal/mol

Configuration 2:

Helix 1 in a flush coaxial stack with Helix 2 and Helix 3 with a GG mismatch

ΔG°_{37} = ΔG°_{37}(coaxial stack of AT followed by GC) + ΔG°_{37}(CG with GG mismatch)

ΔG°_{37} = -1.3 kcal/mol - 0.9 kcal/mol

ΔG°_{37} = -2.2 kcal/mol

Therefore, configuration 2, -2.2 kcal/mol, is the predicted free energy change.

Enthalpy Change

Prediction of Stacking

The stacking configuration is fixed by the prediction of folding free energy change and is configuration 1 above.

ΔH° = ΔH°(coaxial stack of AT followed by GC) + ΔH°(CG with GG mismatch)

ΔH° = -0.8 kcal/mol - 4.7 kcal/mol

ΔH° = -5.5 kcal/mol

Chapter 31

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Part IV

RNA + m^6A (Kierzek et al.)

Chapter 32

Watson-Crick-Franklin Helices

32.1 Free Energy Change at 37 °C

Folding free energy changes for Watson-Crick-Franklin helices are predicted using the equation:

$\Delta G^\circ_{37\ \text{Watson-Crick-Franklin}} = \Delta G^\circ_{37\ \text{intermolecular initiation}} + \Delta G^\circ_{37\ \text{AU end penalty}}$ (per AU end) $+ \Delta G^\circ_{37\ \text{symmetry (self-complementary duplexes)}} + \Sigma[\Delta G^\circ_{37\ \text{stacking}}]$

where intermolecular initiation is applied for bimolecular structure formation, the AU end penalty is applied once per each AU pair at the end of a helix, the symmetry correction is applied to self-complementary duplexes, and the stacking term is a sum of sequence-dependent parameters over all base pair stacks. For helices of P uninterrupted basepairs, there are P-1 stacks of pairs.

32.2 Examples

Self complementary duplex

```
5'MGCGCU3'
3'UCGCGM5'
```

$\Delta G^\circ_{37} = \Delta G^\circ_{37\ \text{intermolecular initiation}} + \Delta G^\circ_{37\ \text{symmetry}} + \Delta G^\circ_{37}$(MU followed by GC) $+ \Delta G^\circ_{37}$(GC followed by CG) $+ \Delta G^\circ_{37}$(CG followed by GC) $+ \Delta G^\circ_{37}$(GC followed by CG) $+ \Delta G^\circ_{37}$(CG followed by UM)

ΔG°_{37} = 4.1 kcal/mol + 0.43 kcal/mol - 1.6 kcal/mol - 3.4 kcal/mol - 2.4 kcal - 3.4 kcal/mol - 1.6 kcal/mol

ΔG°_{37} = -7.87 kcal/mol

Note that, for example, the parameters for (MU followed by GC) are the same as (CG followed by UM) because the correct directionality of the strands is preserved.

Non-self complementary duplex

```
5'GCMCG3'
3'CGUGC5'
```

ΔG°_{37} = $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + ΔG°_{37}(GC followed by CG) + ΔG°_{37}(CG followed by MU) + ΔG°_{37}(MU followed by CG) + ΔG°_{37}(CG followed by GC)

ΔG°_{37} = 4.1 kcal/mol - 3.4 kcal/mol - 1.3 kcal/mol - 1.9 kcal/mol - 2.4 kcal/mol

ΔG°_{37} = -4.9 kcal/mol

32.3 Parameter Tables

The table of parameters is available as plain text for free energy change.

Chapter 33

GU Pairs

GU pairs are treated as nearest neighbor stacks, similar to Watson-Crick-Franklin helices, and GU pairs at the ends of helices are **not** penalized with the same parameter as AU pairs at the ends of helices. In one sequence context, a tandem GU pair with a GU followed by a UG, the nearest neighbor model does not work and two parameters are available, depending on the sequence context (see the html table of parameters). Note also that the motif 5′GG/3′UU is assigned a ΔG°_{37} of –0.5 kcal/mol to optimize structure prediction accurracy, whereas it is measured as +0.5 kcal/mol. Parameters for stacks containing GU pairs were calculated separately from those containing AU and GC base pairs only.

33.1 Examples

```
5'GGUCGUGU3'
3'CUGGUGCG5'
```

$\Delta G^\circ_{37} = \Delta G^\circ_{37\ \text{intermolecular initiation}} + \Delta G^\circ_{37}$(GC followed by GU, followed by UG, followed by CG) + ΔG°_{37}(CG followed by GU) + ΔG°_{37}(GU followed by UG) + ΔG°_{37}(UG followed by GC) + ΔG°_{37}(GC followed by UG)

ΔG°_{37} = 4.1 kcal/mol – 4.12 kcal/mol - 1.3 kcal/mol + 0.7 kcal/mol - 1.3 kcal/mol - 2.2 kcal/mol

ΔG°_{37} = -4.1 kcal/mol

33.2 Parameter Tables

The tables of parameters are available as plain text for free energy change (including Watson-Crick-Franklin pairs).

Chapter 34

Dangling Ends

Dangling ends are nucleotides that stack on the ends of helices. In secondary structures, they occur in multibranch and exterior loops. They occur as either 5′ dangling ends (an unpaired nucleotide 5′ to the helix end) or 3′ dangling ends (an unpaired nucleotide 3′ to the helix end).

In RNA, 3′ dangling ends are generally more stabilizing than 5′ dangling ends. Note that if a helix end is extended on both the 5′ and 3′ strands, then a terminal mismatch exists (not the sum of 5′ and 3′ dangling ends).

34.1 Examples

```
5'MGCACGC3'
3' CGUGC 5'
```

ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(3′ dangling C adajacent to GC) + ΔG°_{37}(5′ dangling M adjacent CG)

ΔG°_{37} = -6.0 kcal/mol - 0.4 kcal/mol + 0.0 kcal/mol

ΔG°_{37} = -6.4 kcal/mol

Note that this example contains both a 5′ and a 3′ dangling end (at opposite ends of the duplex).

34.2 Parameter Tables

The tables of parameters are available as plain text for free energy change.

Chapter 35

Terminal Mismatches

Terminal mismatches are non-canonical pairs adjacent to helix ends.

35.1 Examples

```
5'MGCGCUG3'
3'UCGCGAA5'
```

ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(UA followed by GA)

ΔG°_{37} = -8.8 kcal - 1.1 kcal/mol

ΔG°_{37} = -9.9 kcal/mol

35.2 Parameter Tables

Table of parameters is available as plain text for free energy change.

Chapter 36

Hairpin Loops

36.1 Folding Free Energy Change

Hairpin loops of 4 or more nucleotides

The prediction of folding free energy changes for hairpins of 4 or more unpaired nucleotides is made with the following equation:

$\Delta G^\circ_{37\ \text{hairpin}}$ (>3 nucleotides in loop) = $\Delta G^\circ_{37\ \text{initiation}}$ (n) + ΔG°_{37} (terminal mismatch) + ΔG°_{37} (UU or GA first mismatch) + ΔG°_{37} (GG first mismatch) + ΔG°_{37} (special GU closure) + ΔG°_{37} penalty (all C loops)

In this equation, n is the number of nucleotides in loop, the terminal mismatch parameter is the sequence-dependent term for the first mismatch stacking on the terminal base pair, UU and GA first mismatches receive a bonus (not applied to AG first mismatches), GG first mismatches receive a bonus, the special GU closure term is applied only to hairpins in which a GU closing pair (not UG) is preceded by two Gs, and finally loops with all C nucleotides receive a penalty.

The penalty for all C loops of more than three unpaired nucleotides is predicted using a linear equation:

$\Delta G^\circ_{37\ \text{penalty}}$ (all C loops; > 3 unpaired nucleotides) = An + B

Hairpin loops of 3 unpaired nucleotides

For hairpin loops of 3 nucleotides, the folding free energy change is estimated using:

$\Delta G^\circ_{37\ \text{hairpin}}$ (3 unpaired nucleotides) = $\Delta G^\circ_{37\ \text{initiation}}$ (3) + $\Delta G^\circ_{37\ \text{penalty}}$ (all C loops)

As opposed to longer hairpin loops, hairpin loops of three nucleotides do not

receive a sequence-dependent first mismatch term. All C hairpin loops of three nucleotides receive a stability penalty.

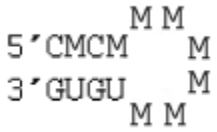
Special hairpin loops

There are hairpin loop sequences of 3, 4, and 6 nucleotides that have stabilities poorly fit by the model. These hairpins are assigned stabilities based on experimental data. Short hairpin loops

The nearest neighbor rules prohibit hairpin loops with fewer than 3 nucleotides.

36.2 Examples

6 nucleotide hairpin loop with no special stacking terms



$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin Helix) + ΔG°_{37}(Hairpin Loop)

$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin Helix) + ΔG°_{37}(terminal mismatch) + $\Delta G^\circ_{37\ \text{Hairpin initiation}}(6)$

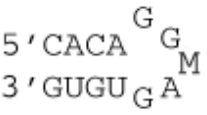
$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(CG followed by MU) + ΔG°_{37}(MU followed by CG) + ΔG°_{37}(CG followed by MU) + ΔG°_{37}(MU followed by MM) + $\Delta G^\circ_{37\ \text{Hairpin initiation}}(6)$

ΔG°_{37} = -1.3 kcal/mol -1.9 kcal/mol -1.3 kcal/mol -0.8 kcal/mol + 5.4 kca/mol

ΔG°_{37} = 0.1 kcal/mol

Note that for unimolecular secondary structures, the helical intermolecular initiation does not appear.

5 nucleotide hairpin loop with a GG first mismatch



$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin Helix) + ΔG°_{37}(Hairpin Loop)

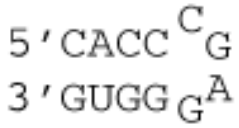
$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin Helix) + ΔG°_{37}(terminal mismatch) + ΔG°_{37}(GG first mismatch) + $\Delta G^\circ_{37\ \text{Hairpin initiation}}(5)$

$\Delta G°_{37}$ = $\Delta G°_{37}$(CG followed by AU) + $\Delta G°_{37}$(AU followed by CG) + $\Delta G°_{37}$(CG followed by AU) + $\Delta G°_{37\ \text{AU end penalty}}$ + $\Delta G°_{37}$(AU followed by GG) + $\Delta G°_{37}$(GG first mismatch) + $\Delta G°_{37\ \text{Hairpin initiation}}$(5)

$\Delta G°_{37}$ = –2.1 kcal/mol – 2.2 kcal/mol – 2.1 kcal/mol + 0.5 kcal/mol – 0.8 kcal/mol – 0.8 kcal/mol + 5.7 kcal/mol

$\Delta G°_{37}$ = –1.9 kcal/mol

4 nucleotide special hairpin loop



$\Delta G°_{37}$ = $\Delta G°_{37}$(Watson-Crick-Franklin Helix) + $\Delta G°_{37}$(Hairpin Loop)

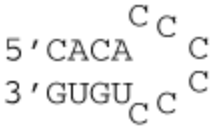
$\Delta G°_{37}$ = $\Delta G°_{37}$(Watson-Crick-Franklin Helix) + $\Delta G°_{37}$(CcgagG)

$\Delta G°_{37}$ = $\Delta G°_{37}$(CG followed by AU) + $\Delta G°_{37}$(AU followed by CG) + $\Delta G°_{37}$(CG followed by CG) + $\Delta G°_{37}$(CcgagG)

$\Delta G°_{37}$ = –2.1 kcal/mol – 2.2 kcal/mol – 3.3 kcal/mol + 3.5 kcal/mol

$\Delta G°_{37}$ = -4.1 kcal/mol

6 nucleotide all C loop



$\Delta G°_{37}$ = $\Delta G°_{37}$(Watson-Crick-Franklin Helix) + $\Delta G°_{37}$(Hairpin Loop)

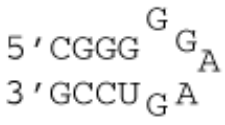
$\Delta G°_{37}$ = $\Delta G°_{37}$(Watson-Crick-Franklin Helix) + $\Delta G°_{37}$(terminal mismatch) + $\Delta G°_{37\ \text{Hairpin initiation}}$(6) + $\Delta G°_{37}$ penalty (all C loops)

$\Delta G°_{37}$ = $\Delta G°_{37}$(CG followed by AU) + $\Delta G°_{37}$(AU followed by CG) + $\Delta G°_{37}$(CG followed by AU) + $\Delta G°_{37\ \text{AU end penalty}}$ + $\Delta G°_{37}$(AU followed by CC) + $\Delta G°_{37\ \text{Hairpin initiation}}$(6) + 6×A + B

$\Delta G°_{37}$ = –2.1 kcal/mol – 2.2 kcal/mol – 2.1 kcal/mol + 0.5 kcal/mol - 0.7 kcal/mol + 5.4 kcal/mol + 6×0.3 kcal/mol + 1.6 kcal/mol

$\Delta G°_{37}$ = 2.1 kcal/mol

5 nucleotide loop with special GU closure



ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(Hairpin Loop)

ΔG°_{37} = ΔG°_{37}(Watson-Crick-Franklin Helix) + ΔG°_{37}(terminal mismatch) + ΔG°_{37}(GG first mismatch) + $\Delta G^\circ_{37\ \text{Hairpin initiation}}(5)$ + ΔG°_{37} (special GU closure)

ΔG°_{37} = ΔG°_{37}(CG followed by GC) + ΔG°_{37}(GC followed by GC) + ΔG°_{37}(GC followed by GU) + ΔG°_{37} GU end penalty + ΔG°_{37}(GU followed by GG) + ΔG°_{37}(GG first mismatch) + $\Delta G^\circ_{37\ \text{Hairpin initiation}}(5)$ + ΔG°_{37} (special GU closure)

ΔG°_{37} = –2.36 kcal/mol – 3.26 kcal/mol – 1.53 kcal/mol + 0.45 kcal/mol – 0.8 kcal/mol – 0.8 kcal/mol + 5.7 kcal/mol – 2.2 kcal/mol

ΔG°_{37} = -4.8 kcal/mol

36.3 Parameter Tables

Length dependent initiation parameters are available in plain text for free energy changes. The plain text initiation tables include an extrapolation out to lengths of 30 nucleotides. Initiation parameters are based on experiments for sizes up to 9 nucleotides, but can be extrapolated to longer loops. For free energy changes, the extrapolation is $\Delta G^\circ_{37\ \text{initiation}}$ (n>9) = $\Delta G^\circ_{37\ \text{initiation}}$ (9) + 1.75 RT ln(n/9), where R is the gas constant and T is the absolute temperature. For enthalpy changes, $\Delta H^\circ_{\text{initiation}}$ (n>9) = $\Delta H^\circ_{\text{initiation}}$ (9).

The terminal mismatch tables are available in plain text for free energy change.

The table of special hairpin loops is available in plain text for free energy change for 3, 4, or 6 nucleotides. The special hairpin loop sequences include the identity of the closing basepair.

Chapter 37

Bulge Loops

Single Nucleotide Bulge Loops

The prediction of folding free energy changes is made with the following equation:

$\Delta G^\circ_{37\ \text{bulge}}(n=1) = \Delta G^\circ_{37\ \text{bulge initiation}}(n) + \Delta G^\circ_{37}$ (special C bulge) + ΔG°_{37} (base pair stack) – RT ln(number of states)

In this equation, n is the number of unpaired nucleotides, a special C bulge is a bulged C adjacent to at least one paired C, the base pair stack is the stack of the closing pairs as though there is no bulge (using Watson-Crick-Franklin or GU rules as needed), and the number of states counts the number of possible loops of identical sequence.

Because the helical stack continues across a single nucleotide bulge, the terminal AU penalty is not applied adjacent to single bulges.

Bulges of 2 or More Nucleotides

For bulges of 2 or more nucleotides, the following equation is used:

$\Delta G^\circ_{37\ \text{bulge}}(n>1) = \Delta G^\circ_{37\ \text{bulge initiation}}(n)$

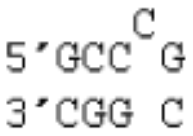
Experimentally-derived parameters are available for initiation up to n = 3 and a linear extrapolation is used up to n = 6. Beyond 6, the initiation is approximated using a logarithmic function:

$\Delta G^\circ_{37\ \text{bulge}}(n>6) = \Delta G^\circ_{37\ \text{bulge initiation}}(6) + 1.75\ RT\ \ln(n/6)$

where R is the gas constant and T is the absolute temperature, 310.15 K.

37.1 Examples

Single C bulge with multiple states



$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin Helix) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + ΔG°_{37}(Bulge Loop)

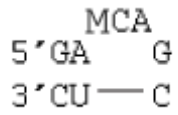
$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(GC followed by CG) + ΔG°_{37}(CG followed by CG) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{bulge initiation}}(1)$ + ΔG°_{37}(special C bulge) + ΔG°_{37}(CG followed by GC) – RT ln(3)

ΔG°_{37} = -3.4 kcal/mol -3.3 kcal/mol + 4.1 kcal/mol + 3.8 kcal/mol -0.9 kcal/mol -2.4 kcal/mol - 0.616 × 1.099 kcal/mol

ΔG°_{37} = -2.76 kcal/mol

Note that this loop has three available states because any of the three Cs in the top strand can be the bulge.

3 nucleotide bulge



$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin Helix) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{AU end penalty}}$ + ΔG°_{37}(Bulge Loop)

$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(GC followed by AU) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{AU end penalty}}$ + $\Delta G^\circ_{37\ \text{bulge initiation}}(3)$

ΔG°_{37} = –2.4 kcal/mol + 4.1 kcal/mol + 0.5 kcal/mol + 3.2 kcal/mol

ΔG°_{37} = 5.4 kcal/mol

37.2 Parameter Tables

Bulge loop parameters are available as plain text for initiation free energy parameters.

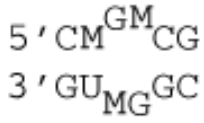
Chapter 38

Internal Loops

Small symmetric internal loops have tabulated free energy and enthalpy changes, where experimentally determined values are used if available.

38.1 Examples

2×2 internal loop



$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin stacks) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + ΔG°_{37}(Internal Loop)

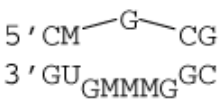
$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(CG followed by MU) + ΔG°_{37}(CG followed by GC) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + ΔG°_{37}(2×2 Internal Loop)

ΔG°_{37} = -1.3 kcal/mol -2.4 kcal/mol + 4.1 kcal/mol -1.1 kcal/mol

ΔG°_{37} = -0.7 kcal/mol

Note that the internal loop lookup tables account for terminal MU pairs that are adjacent to internal loops.

1×5 internal loop



$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin stacks) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + ΔG°_{37}(Internal Loop)

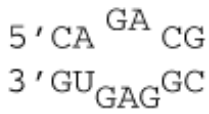
$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(CG followed by MU) + ΔG°_{37}(CG followed by GC) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{initiation}}(6)$ + $\Delta G^\circ_{37\ \text{asymmetry}} \times |n1 - n2|$ + $\Delta G^\circ_{37\ \text{mismatch}}$(mismatch 1) + $\Delta G^\circ_{37\ \text{mismatch}}$(mismatch 2)

ΔG°_{37} = -1.3 kcal/mol – 2.36 kcal/mol + 4.09 kcal/mol + 2.0 kcal/mol + 0.6×|1 - 5| kcal/mol + 0 kcal/mol + 0 kcal/mol

ΔG°_{37} = +4.8 kcal/mol

Note that the free energy change for first mismatches in 1×(n-1) internal loops is 0 kcal/mol.

2×3 internal loop with stabilizing mismatches



$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(Watson-Crick-Franklin stacks) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + ΔG°_{37}(Internal Loop)

$\Delta G^\circ_{37} = \Delta G^\circ_{37}$(CG followed by AU) + ΔG°_{37}(CG followed by GC) + $\Delta G^\circ_{37\ \text{intermolecular initiation}}$ + $\Delta G^\circ_{37\ \text{initiation}}(5)$ + $\Delta G^\circ_{37\ \text{asymmetry}} \times |n1 - n2|$ + $\Delta G^\circ_{37\ \text{mismatch}}$(mismatch 1) + $\Delta G^\circ_{37\ \text{mismatch}}$(mismatch 2) + $\Delta G^\circ_{37\ \text{AU closure}}$

ΔG°_{37} = –2.11 kcal/mol – 2.36 kcal/mol + 4.09 kcal/mol + 2.0 kcal/mol + 0.6×|2 - 3| kcal/mol – 0.8 kcal/mol – 1.2 kcal/mol + 0.7 kcal/mol

ΔG°_{37} = +0.9 kcal/mol

38.2 Parameter Tables

1×1 internal loop free energy change tables are available in text. Note that these tables incorporate the AU closure penalties and therefore no AU helix end penalty should be applied for internal loop closure.

1×2 internal loop free energy change tables are available in text. Note that these tables incorporate the AU closure penalties and therefore no AU helix end penalty should be applied for internal loop closure.

2×2 internal loop free energy change tables are available in text. Note that these tables incorporate the AU closure penalties and therefore no AU helix end penalty should be applied for internal loop closure.

Chapter 39

Coaxial Stacking

39.1 Introduction

Coaxial stacking is the stacking of two base pairs at the terminii of adjacent helices. This stacking aligns the two helices along a common axis. In unimolecular secondary structures, coaxial stacking occurs in multibranch and exterior loops.

This set of nearest neighbor parameters allows for two types of coaxial stacking, flush stacking of helices that are directly adjacent (no intervening unpaired nucleotides) and mismatch-mediated coaxial stacking in which a single mistmatch occurs between the stacked helices. Mismatch-mediated coaxial stacking of helices is only allowed when there is exactly one unpaired nucleotide between the helices that can form a non-canonical pair with a nucleotide on the other side of one of the two helices.

39.2 Flush Coaxial Stacking

In flush coaxial stacking, the free energy and enthalpy changes of the coaxial stack are approximated using the helical nearest neighbor parameters (Watson-Crick-Franklin or GU) as though there was no break in the backbone.

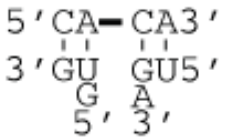
39.3 Mismatch-Mediated Coaxial Stacking

In the case of mismatch-mediated coaxial stacking, there are two adjacent stacks. The stack of the mismatch on the adjacent helix, where there is no break in the backbone, is approximated using the terminal mismatch parameters. The second stack is the stack of the mismatch on the second helix, where the backbone is not continuous.

This stack is approximated using a sequence-independent term of –2.1 kcal/mol for folding free energy change. If the "mismatch" mediating the coaxial stack could form a Watson-Crick-Franklin, a bonuse of –0.4 is applied to free energy change.

39.4 Examples

Flush coaxial stacking



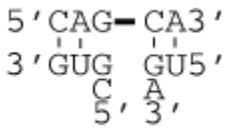
$\Delta G^{\circ}_{37\ \text{coaxial stack}} = \Delta G^{\circ}_{37}$(Watson-Crick Stack)

$\Delta G^{\circ}_{37\ \text{coaxial stack}} = \Delta G^{\circ}_{37}$(AU pair followed by CG pair)

$\Delta G^{\circ}_{37\ \text{coaxial stack}}$ = -2.2 kcal/mol

Note that at this interface, the terminal AU pair penalty would still apply when calculating the helix stability.

Mismatch-mediated coaxial stacking



$\Delta G^{\circ}_{37\ \text{coaxial stack}} = \Delta G^{\circ}_{37}$(Continuous Backbone Stack) + ΔG°_{37}(Discontinuous Backbone Stack)

$\Delta G^{\circ}_{37\ \text{coaxial stack}} = \Delta G^{\circ}_{37}$(AU pair followed by GG mismatch) + ΔG°_{37}(Discontinuous Backbone Stack)

$\Delta G^{\circ}_{37\ \text{coaxial stack}}$ = -0.8 kcal/mol – 2.1 kcal/mol

$\Delta G^{\circ}_{37\ \text{coaxial stack}}$ = 2.9 kcal/mol

Note that at this interface, the terminal AU pair penalty would still apply when calculating the helix stability.

39.5 Parameter Tables

A table summarizing the parameters is available in html format.

Chapter 40

Multibranch Loops

40.1 Folding Free Energy Change

Multibranch loops stabilities are predicted using the following equation:

$\Delta G^\circ_{37\ \text{multibranch}} = \Delta G^\circ_{37\ \text{initiation}} + \Delta G^\circ_{37\ \text{stacking}}$

where the stacking is the optimal configuration of dangling ends, terminal mismatches, or coaxial stacks, noting that a nucleotide or helix end can participate in only one of these favorable interactions.

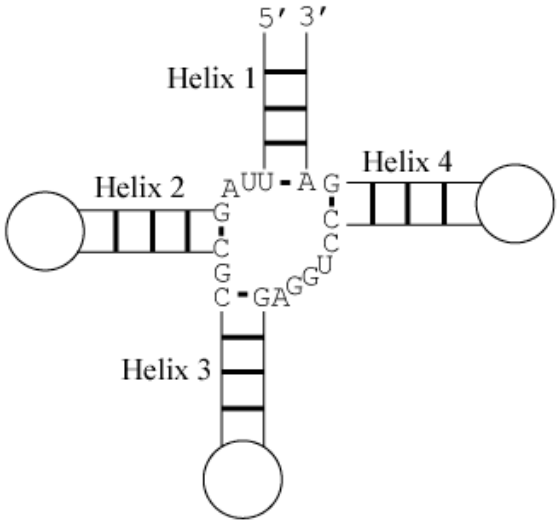
Initiation is predicted using:

$\Delta G^\circ_{37\ \text{initiation}}$ = a + b×[average asymmetry] + c×[number of branching helices] + $\Delta G^\circ_{37\ \text{strain}}$(three-way branching loops with fewer than two unpaired nucleotides)

where the average asymmetry is calculated as:

average asymmetry = min[2.0, mean difference in unpaired nucleotides on each side of each helix]

40.2 Example



Free Energy Change

Prediction of Stacking

The predicted stacking configuration is the one with lowest free energy change. There are eight relevant configurations.

Configuration 1:

Helix 1 with 3′ dangling U, Helix 2 with terminal mismatch, Helix 3 with 3′ dangling A, and Helix 4 with 5′ dangling C

ΔG°_{37} = ΔG°_{37}(UA with 3′ dangling U) + ΔG°_{37}(CG followed by GA) + ΔG°_{37}(GC with 3′ dangling A) + ΔG°_{37}(GC with 5′ dangling C)

ΔG°_{37} = –0.1 kcal/mol – 1.4 kcal/mol – 1.1 kcal/mol – 0.3 kcal/mol

ΔG°_{37} = –2.9 kcal/mol

Configuration 2:

Helix 1 with 3′ dangling U, Helix 2 with 5′ dangling A, Helix 3 with terminal mismatch, and Helix 4 with 5′ dangling C

ΔG°_{37} = ΔG°_{37}(UA with 3′ dangling U) + ΔG°_{37}(CG with 5′ dangling A) + ΔG°_{37}(GC followed by AG) + ΔG°_{37}(GC with 5′ dangling C)

ΔG°_{37} = –0.1 kcal/mol – 0.2 kcal/mol – 1.3 kcal/mol – 0.3 kcal/mol

ΔG°_{37} = –1.9 kcal/mol

Configuration 3:

Helix 1 in flush coaxial stack with helix 4, Helix 2 with terminal mismatch, and Helix 3 with 3′ dangling A

ΔG°_{37} = ΔG°_{37}(GC followed by AU) + ΔG°_{37}(CG followed by GA) + ΔG°_{37}(GC with 3′ dangling A)

ΔG°_{37} = –2.35 kcal/mol – 1.4 kcal/mol – 1.1 kcal/mol

ΔG°_{37} = –4.9 kcal/mol

Configuration 4:

Helix 1 in flush coaxial stack with helix 4, Helix 2 with 5′ dangling A, and Helix 3 with terminal mismatch

ΔG°_{37} = ΔG°_{37}(GC followed by AU) + ΔG°_{37}(CG with 5′ dangling A) + ΔG°_{37}(GC followed by AG)

ΔG°_{37} = –2.35 kcal/mol – 0.2 kcal/mol – 1.3 kcal/mol

ΔG°_{37} = –3.9 kcal/mol

Configuration 5:

Helix 1 with 3′ dangling U, Helix 2 in mismatch–mediated coaxial stack with helix 3 with GA intervening mismatch, and Helix 4 with 5′ dangling C

ΔG°_{37} = ΔG°_{37}(UA with 3′ dangling U) + ΔG°_{37}(CG followed by GA) + ΔG°_{37}(Discontinuous Backbone Stack) + ΔG°_{37}(GC with 5′ dangling C)

ΔG°_{37} = –0.1 kcal/mol – 1.4 kcal/mol – 2.1 kcal/mol – 0.3 kcal/mol

ΔG°_{37} = –3.9 kcal/mol

Configuration 6:

Helix 1 with 3′ dangling U, Helix 2 in mismatch–mediated coaxial stack with helix 3 with AG intervening mismatch, and Helix 4 with 5′ dangling C

ΔG°_{37} = ΔG°_{37}(UA with 3′ dangling U) + ΔG°_{37}(Discontinuous Backbone Stack) + ΔG°_{37}(GC followed by AG) + ΔG°_{37}(GC with 5′ dangling C)

ΔG°_{37} = –0.1 kcal/mol – 2.1 kcal/mol – 1.3 kcal/mol – 0.3 kcal/mol

ΔG°_{37} = –3.8 kcal/mol

Configuration 7:

Helix 1 in flush coaxial stack with helix 4 and Helix 2 in mismatch–mediated coaxial stack with helix 3 with GA intervening mismatch

ΔG°_{37} = ΔG°_{37}(GC followed by AU) + ΔG°_{37}(CG followed by GA) + ΔG°_{37}(Discontinuous Backbone Stack)

ΔG°_{37} = –2.35 kcal/mol – 1.4 kcal/mol – 2.1 kcal/mol

ΔG°_{37} = –5.9 kcal/mol

Configuration 8:

Helix 1 in flush coaxial stack with helix 4 and Helix 2 in mismatch–mediated coaxial stack with helix 3 with AG intervening mismatch

ΔG°_{37} = ΔG°_{37}(GC followed by AU) + ΔG°_{37}(Discontinuous Backbone Stack) + ΔG°_{37}(GC followed by AG)

ΔG°_{37} = –2.35 kcal/mol – 2.1 kcal/mol – 1.3 kcal/mol

ΔG°_{37} = –5.8 kcal/mol

Configuration 7 has the lowest folding free energy change of –5.9 kcal/mol.

Initiation Free Energy Change

$\Delta G^\circ_{37\ \text{initiation}}$ = a + b×[average asymmetry] + c×[number of branching helices] + ΔG°_{37} strain(three–way branching loops with fewer than two unpaired nucleotides)

$\Delta G^\circ_{37\ \text{initiation}}$ = 9.25 kcal/mol + (0.91 kcal/mol)×[average asymmetry] + (–0.63 kcal/mol)×[4]

Average asymmetry = min[2.0,(2+1+4+5)/4] = min[2.0,3.0] = 2.0

$\Delta G^\circ_{37\ \text{initiation}}$ = 9.25 kcal/mol + (0.91 kcal/mol)×[2] + (–0.63 kcal/mol)×[4]

$\Delta G^\circ_{37\ \text{initiation}}$ = 8.6 kcal/mol

Total Folding Free Energy Change

$\Delta G^\circ_{37\ \text{multibranch loop}}$ = $\Delta G^\circ_{37\ \text{initiation}}$ + $\Delta G^\circ_{37\ \text{stacking}}$ = 8.6 kcal/mol – 5.9 kcal/mol = 2.7 kcal/mol

40.3 Parameter Tables

Tables of parameters are available in html format.

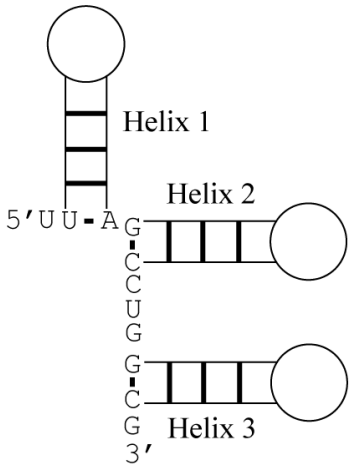
Chapter 41

Exterior Loops

41.1 Folding Free Energy Change

Exterior loops are stabilized by terminal mismatches, dangling ends, and coaxial stacks. The stacking is the optimal configuration of dangling ends, terminal mismatches, or coaxial stacks, noting that a nucleotide or helix end can participate in only one of these favorable interactions.

41.2 Examples



Free Energy Change

Prediction of Stacking

The predicted stacking configuration is the one with lowest free energy change. There are two possible configurations.

Configuration 1:

Helix 1 with 5′ dangling U, Helix 2 with 3′ dangling C, and Helix 3 with a GG mismatch

ΔG°_{37} = ΔG°_{37}(AU with 5′ dangling U) + ΔG°_{37}(CG with 3′ dangling C) + ΔG°_{37}(CG with GG mismatch)

ΔG°_{37} = -0.2 kcal/mol - 0.8 kcal/mol - 1.6 kcal/mol

ΔG°_{37} = -2.6 kcal/mol

Configuration 2:

Helix 1 in a flush coaxial stack with Helix 2 and Helix 3 with a GG mismatch

ΔG°_{37} = ΔG°_{37}(coaxial stack of AU followed by GC) + ΔG°_{37}(CG with GG mismatch)

ΔG°_{37} = -2.1 kcal/mol - 1.6 kcal/mol

ΔG°_{37} = -3.7 kcal/mol

Therefore, configuration 2, -3.7 kcal/mol, is the predicted free energy change.

Chapter 42

References

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Download

NNDB as PDF ebook.

RNA (Turner 2004) parameters files as .zip.

RNA (Turner 1999) parameters files as .zip.

DNA (RNAstructure) parameters files as .zip.

RNA + m^6A (Kierzek et al.) parameters files as .zip.

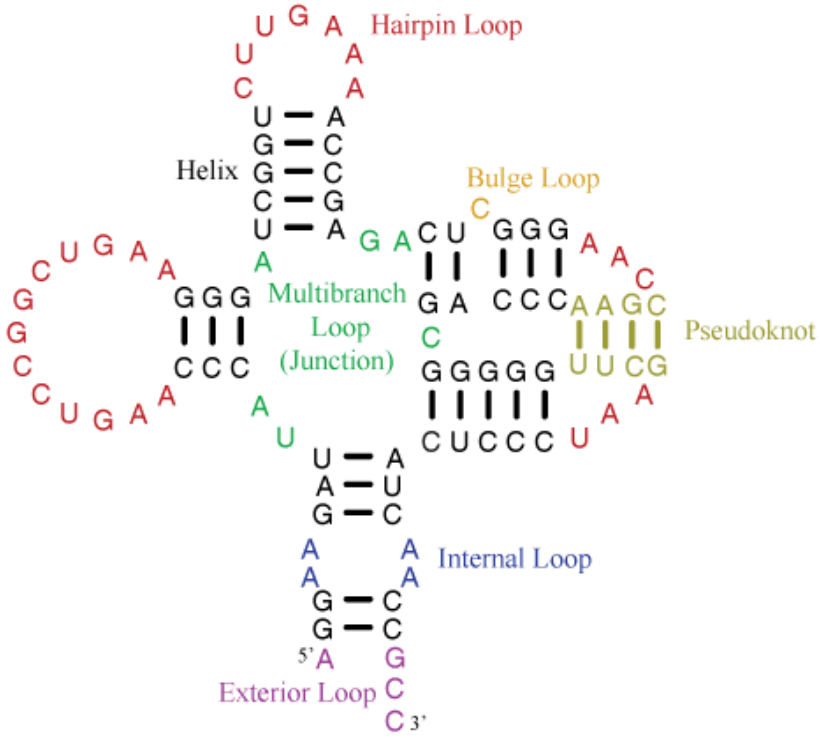
Help

Introduction and Definitions:

The Nearest Neighbor Database (NNDB) provides nearest neighbor parameters for predicting the stability of nucleic acid secondary structures. The underlying approximation for nearest neighbor analysis is that the stabilities of secondary structure motifs depend on the sequence of the motif and the sequence of the adjacent base pairs. The overall stability is the sum of individual stability increments for each motif.

Nearest neighbor analysis is exceedingly accurate for Watson-Crick-Franklin helices, with errors in individual free energy increments of less than 0.1 kcal/mol (Xia et al. (1998) Biochemistry, 37, 14719). For other free energy increments, errors are more significant at roughly 0.5 kcal/mol (Mathews et al. (2004) Proc. Natl. Acad. Sci. USA, 101, 7287). The assumption that stability is determined locally (by a motif an its nearest neighbors) is generally correct, although some non-nearest neighbor effects are known, such as with bulge loops (Longfellow et al. (1990) Biochemistry, 29, 278) and single mismatches (Kierzek et al. (1999) Biochemistry, 38, 14214).

The parameter sets are divided into rules for individual motifs, which are helices or loops. The figure below illustrates the motifs that appear in secondary structures.



Helices are composed of canonical base pairs (AU, GC, and GU). Loops are composed nucleotides of nucleotides not in canonical pairs and of junctions of helices. The hairpin loop has one exiting helix. The internal loop has two exiting helices and nucleotides not in canonical pairs on each strand of the loop. The bulge loop also has two exiting helices, but nucleotide(s) not in canonical pairs appear on only one strand of the loop. Multibranch loops have three or more exiting helices. Exterior loops contain the ends of the sequence and have one or more exiting helices.

A pseudoknot is a helix that spans loop regions defined by other helices. Formally, a pseudoknot occurs when two pairs, between nucleotides i and j and between nucletides i' and j', exist with i $<$ i' $<$ j $<$ j'. Generally, the pseudoknoted helix is considered to be the minimal set of pairs that need to be broken to remove the pseudoknot. In the example above, the tan base pairs are the fewest pairs that could be broken to remove the pseudoknot.

Free Energy, Enthalpy, and Entropy Change

Free energy change quantifies the stability of a secondary structure as compared to a completely unpaired strand. The free energy changes predicted by current nearest neighbor sets are standard Gibbs free energy changes, $\Delta G°$, in kcal/mol and therefore:

$\Delta G° = -RT \ln (K)$

where R is the gas constant (1.987 cal mol^{-1} $degree^{-1}$), T is the absolute temperature, and K is the equilibrium constant. For unimolecular folding:

K = [folded species]/[unpaired strand]

where brackets indicate concentration. For bimolecular folding with strands A and B:

K = [AB]/[A][B]

Free energy nearest neighbor parameters listed are for folding at 37 °C (310.15 K). Free energy changes are temperature dependent and can be derived from enthalpy ($\Delta H°$) and entropy changes ($\Delta S°$):

$\Delta G° = \Delta H° - T\Delta S°$

Some nearest neighbor parameter sets include parameters to predict enthalpy changes. These sets were derived assuming that enthalpy and entropy change are independent of temperature. Using a predicted free energy change at 37 °C and a predicted enthalpy change, the entropy change can be determined by rearranging the above equation:

$\Delta S° = (\Delta H° - \Delta G°_{37})/(310.15\ K)$

Furthermore, with predicted free energy changes at 37 °C and enthalpy changes, free energy changes can be extrapolated to arbitrary temperature:

$\Delta G°(T) = \Delta H° - T(\Delta H° - \Delta G°_{37})/(310.15\ K)$

In practice, the quality of the extrapolation to arbitrary temperature must be critically assessed because the assumption that enthalpy and entropy change are independent of temprature is not generally true. The extrapolations are probably only reasonably correct at temperatures close to 37 °C, in an approximate range of 10 °C to 60 °C.

Melting Temperature

The melting temperature (T_M) is the temperature at which half of strands are unpaired. Assuming a two-state model (where individual strands are either completely structured or completely unstructured), the TM can be predicted from the enthalpy and entropy changes. For a unimolecular structure, the T_M is concentration independent and is predicted from:

$T_M = \Delta H°/\Delta S°$ (unimolecular)

For bimolecular systems, the TM is concentration dependent. For self-complementary duplexes:

$T_M = \Delta H°/(\Delta S° + R\ln(C_T))$ (self-complementary)

where C_T is the total strand concentration.

For non-self-complementary dupexes, with the strands mixed 1:1, the T_M is predicted using:

$T_M = \Delta H°/(\Delta S° + R\ln(C_T/4))$ (non-self-complementary)

where T_M is in Kelvins and can be converted to centrigrade, T_m, with:

$T_m = T_M - 273.15$

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This is the set of references to optical melting experiments that have been utilized to develop nearest neighbor parameters for RNA folding free energy and enthalpy changes.

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