A base pairing model of duplex formation I: Watson-Crick pairing geometries

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Abstract

We present a base-pairing model of oligonucleotide duplex formation and show in detail its equivalence to the Nearest-Neighbour dimer methods from fits to free energy of duplex formation data for short DNA-DNA and DNA-RNA hybrids containing only Watson Crick pairs. In this approach the connection between rank-deficient polymer and rank-determinant oligonucleotide parameter, sets for DNA duplexes is transparent. The method is generalised to include RNA/DNA hybrids where the rank-deficient model with 11 dimer parameters in fact provides marginally improved predictions relative to the standard method with 16 independent dimer parameters ($\Delta G$ mean errors of 4.5 and 5.4% respectively).

Keywords: nearest neighbour properties, nucleic acid oligomers, RNA-DNA hybrids
1 Introduction

Simple nearest-neighbour (NN) models of helix stability at the base dimer level have been refined over some years [1]-[4] and are commonly used, for example, to predict RNA secondary structure formation [5], [6] and duplex melting profiles (for a recent example see [7]). Detailed knowledge of competing RNA/RNA and RNA/DNA bindings is also becoming desirable for cDNA microarrays [8].

Previously we [9] suggested that a deeper systematic framework lay beneath the semi-empirical rules of the NN approach, while in Ref.[10] we proposed such a scheme and demonstrated its equivalence to another [2] model of oligonucleotide RNA duplexes containing WC pairs.

The structure of the paper is as follows. After a brief outline of the NN dimer method we refine our base-pairing model proposed in Ref. [10]. The equivalence between the two approaches is then described in detail. Finally two-dimensional models are fitted to empirical $\Delta G$ values for DNA and RNA/DNA hybrid duplexes and compared statistically to their dimer analogues.

2 Nearest Neighbour model

The Nearest-Neighbour (NN) model of duplex formation, e.g see Ref. [2] is based upon the assumption that once an initiation barrier, preventing the formation of a single H-bonded pair, is overcome the duplex/single strand interface propagates along the strands “zipping” the two strands up into a duplex. The critical assumptions are:

1) The process occurs at fixed strand concentrations.
2) Derivation of thermodynamical parameters from melting curves is for an assumed equilibrium between two (helix and coil) possible states.
3) The thermodynamic properties of the duplex depends linearly upon the frequencies of adjacent pairs of bases (dimers). In particular the formula used to estimate duplex free energy of formation is

$$\Delta G = \Delta G_{init} + \Delta G_{ssym} + \sum N_{WY/XZ} \Delta G_{WY/XZ}. \tag{1}$$

Here $\Delta G_{init}$ is a free energy initiation step including translational and rotational entropy loss. It is, in principle, both (duplex) length- and sequence-dependent but for short duplexes is typically assumed to depend only upon the identity of the terminal base pairs. $N_{WY/XZ}$ denotes the frequency with which dimer $5’WX3’/3’YZ5’$ occurs in the duplex while $\Delta G_{WY/XZ}$ is its free energy contribution to the helix formation. The latter is interpreted as the dimer propagation energy associated with the zippering of strands. Dominant contributions to this propagation energy are the van der Waals “stacking” between adjacent base pairs and specificity-conferring H-bonding of complementary base pairs. Finally an extra entropy term is added for self-complementary duplexes (i.e. helices formed from strands with identical sequences) due to the extra twofold symmetry. Theoretically $\Delta G_{ssym} = RT \ln 2 \sim 0.43 \text{ kcal mol}^{-1}$ at physiological temperature $T = 310K$.

2.1 Model parameters

In the simplest instance one assumes that the dimer propagation energy is independent of “zippering” direction, that is, $\Delta G_{WY/XZ} = \Delta G_{ZX/YW}$ and therefore $N_{WY/XZ} = N_{ZX/YW}$. It is easy to check that if $p$ types of pair are possible then this symmetry reduces the number of types of dimer from $(2p)^2$ to $p(2p + 1)$. We shall refer to this below as the “symmetric” dimer approximation.
For oligomers, i.e., helices with formally defined ends, there are also 2p possible terminal parameters. In this case the hypothetical 5′ – 3′ dimer symmetry must be compatible with the global 5′ – 3′ symmetry of the full duplex, leading to p constraints upon the numbers of independent dimer (N_{WY/XZ}) and terminal (N_{5′P/Q3′}) frequencies which coexist in the model. For convenience the terminal parameters are typically eliminated, e.g., N_{5′P/Q3′} = N_{5′Q/P3′}.

The maximal number of independent NN frequencies in the model is therefore

\[ \nu = p + p(2p + 1). \]

Oligomer models derived for RNA [2] and DNA duplexes [3], [12] containing Watson-Crick (WC) pairs (p = 2) exhibit good agreement with experimental data for short, “two state” duplexes while accuracy is decreased for duplexes containing mismatches (Refs. [13]–[17] for DNA or see [18]–[20] for RNA. This decrease is generally attributed to the emergence of non-nearest neighbour effects such as pairing geometries with reduced complementarity. The “asymmetric dimer” approximation, where the zipping direction is distinguished, has \((2p)^2\) dimer parameters and may be applied to hybrid RNA/DNA duplexes or, possibly, as a more sophisticated model of ordinary (RNA/RNA or DNA/DNA) homoduplexes. In fact the asymmetric dimer model is required to obtain reasonable predictions for RNA/DNA hybrids ([12], [21], [4]) but provides only marginal improvements in predictions for homoduplexes [2], [10].

It is also known [25] for WC-paired DNA polymers that a model consisting of eight “invariants”, or linear combinations of dimer steps, provides predictions of comparable accuracy to the model including parameters for all ten dimers. Previously we suggested that the base-pairing model developed in Ref. [10] for RNA duplex formation is more naturally compared with the NN method at this independent, short sequence (ISS) level, which we elaborate upon below.

3 Base-pairing model

Our model is motivated by the problem of including sequence-dependence in dynamical, base-pair level descriptions of DNA [22]. It follows from the NN assumption (3) above; if a physical property is a linear function of the duplex NN dimer quantities, then there must be an equivalent expression which is quadratic in labels associated with individual bases.

For the instance of thermodynamical quantities this quadratic description corresponds to a systematic summation of two-body correlations. Given the pre-eminent role of van der Waals “stacking” and H-bonding interactions in helix stabilisation, these correlations might be representative of the interactions between, for example, amino/keto functional groups and heterocyclic rings. Moreover terms which are independent of sequence content variations, depending only upon overall sequence length have a natural interpretation as contributions to the generic (B form, in the present case) helical backbone structure.

Consider the dimer propagation energy parameters \( \Delta G_{WY/XZ} \) associated with the “zipping up” of dimers 5′WY3′/3′XZ5′. We shall assume that they may be decomposed quadratically as

\[ \Delta G_{WY/XZ} \equiv (W^T, X^T) \begin{pmatrix} P_{11} & P_{12} \\ P_{21} & P_{22} \end{pmatrix} \begin{pmatrix} Y \\ Z \end{pmatrix} \]

Here the matrix entries \( P_{ij} \) represent generic correlations between the various sites of the dimer, while the vector entries \( W, X, Y, Z \) are vectors encapsulating the sequence variation. For lack of better nomenclature we shall refer to matrix elements as “correlations” and the vectors as “weights”. Three observations are in order:
1) The diagonal entries $P_{ii}$ (we shall call them $h_{ii}$) are associated with bases which are H-bonded while off-diagonal ones are associated with “stacked” neighbours (which we call $s = P_{12}$ and $t = P_{21}$).

2) $W, X, Y, Z$ are vectors in some abstract “weight” space. In order to reproduce the correct number of matrix elements, the dimensionality, $p$ of this space must coincide with the number of permissible base pairs, $d$ above. The nature of this weight space is discussed in a later section.

3) Given the H-bonding specificity between complementary pairs, the inter- and intra-strand correlations are not independent of one another. The stacking correlations are thus understood to contain contributions from both these types of interaction.

Therefore expression (2) can be rewritten in the form used previously:

$$\Delta G = (y^T_1, y^T_2) \begin{pmatrix} h_1 & s \\ t & h_2 \end{pmatrix} \begin{pmatrix} x_1 \\ x_2 \end{pmatrix},$$

where bases $x_i$ and $y_i$ refer to bases on the $5'-3'$ and $3'-5'$ oriented strands respectively and the Roman index $i$ refers to the location of the base within the dimer. We shall also adopt the convention that Greek indices signify internal “weight space” degrees of freedom.

The distinction between symmetric and asymmetric dimer approximations is quantified by the existence of a $5'-3'$ symmetry transformation, $\Gamma_2$. In the spatial point group of a dinucleotide dimer this transformation is just a $C_2$ rotation about an axis perpendicular to the average plane of the (dimer) molecule passing through the centre of mass. Given that this model is concerned with correlations between abstract labels in a multi-dimensional vector space and not a priori in physical 3-D space we can only require that the transformation be involutive, i.e.,

$$(\Gamma_2)^2 = 1_p$$

where $1_p$ denotes the $p \times p$ identity matrix. In order for expression (3) to be manifestly $5'-3'$ symmetric it must be the case that:

$$h_1 - (\Gamma_2)^T h_2 \Gamma_2 = 0,$$

$$s - (\Gamma_2)^T s \Gamma_2 = 0,$$

$$t - (\Gamma_2)^T t \Gamma_2 = 0.$$

For any suitable (involutive) choice of $\Gamma_2$ it is readily found that the number of independent $h, s$ and $t$ matrix elements is $p(2p+1)$ in agreement with the number of symmetric dimer parameters. Without loss of generality, in the remainder of the paper we shall therefore assume $\Gamma_2 = -1_p$.

### 3.1 Duplex model

We now construct the correlations for a full duplex of $n$ base pairs $x_1 \ldots x_n/y_1 \ldots y_n$. As in the NN approach the duplex heat of formation is approximated by a summation of internal dimer propagation energies plus, for oligomers, terminal-environmental effects:

$$\Delta G = \Delta G_{term/env} + \sum_{i=1}^{n-1} \Delta G_{x_i x_{i+1}/y_i y_{i+1}}$$

Here, c.f. Eq (1), the summation is over individual sites within the duplex rather than dimer occurrence frequencies. Let us denote the duplex analogues of dimer submatrices $h, s$ and $t$ by
capital letters. If the global $5' - 3'$ transformation is written $\Gamma_n$ the global symmetry constraints are

\begin{align*}
H_i - (\Gamma_n)^T H_i^T \Gamma_n &= 0, \\
S_{ij} - (\Gamma_n)^T S_{ji}^T \Gamma_n &= 0; \ j > i, \\
T_{ij} - (\Gamma_n)^T T_{ji}^T \Gamma_n &= 0; \ j < i,
\end{align*}

with $i$ and $j$ running from 1 to $n$ and where we have defined the $5' - 3'$ reflected index $i = n + 1 - i$. Naturally in the NN approximation only those submatrices with indices $|i - j| \leq 1$ contain nonzero elements. Reconciling the global and dimer symmetries is therefore equivalent to substituting the symmetric dimer version of (3) in (6). In particular one finds \[10\]

\begin{align*}
H_1 &= h_1 = H_n^T, \\
H &\equiv H_2 = h_1 + h_1^T = H_3 = \ldots = H_{n-1},
\end{align*}

\begin{align*}
S_{i,i+1} &= s, \\
T_{i+1,i} &= t.
\end{align*}

Note that for internal bases the H-bonding correlations effectively contribute twice and in such a way that $H_i = H_i^T$ for $1 < i < n$. This is desirable due to the fact that constraint (7) on its own would imply different symmetry properties for the central base pair(s) in odd- (even-) length duplexes. We note that this double counting of internal base pairs with respect to terminals may also be justified in terms of the relative propensities for fraying of the latter [23].

Additional, environmental, perturbations to terminal bases may be formally incorporated by augmenting the original sequences with fictitious “end neighbour” vectors [24]

\begin{align*}
(x_1, \ldots x_n) &\to (e_x, x_1, \ldots x_n, e'_x), \\
(y_1, \ldots y_n) &\to (e_y, y_1, \ldots y_n, e'_y).
\end{align*}

If these perturbations are themselves $5' - 3'$ symmetric then the environmental effects may be incorporated as a linear term

\[\Delta G_{term/env} = \beta (x_1 + x_n + y_1 + y_n),\]

where $\beta$ is a constant, $p$-dimensional vector which is not required, i.e., vanishes in expressions for polymers or circular duplexes, if no ends are formally defined.

### 3.2 Number of Model Parameters

Let us now recall the observation [25] that there is a more fundamental description of WC-paired polymers in terms of eight “invariants” and that moreover, when initiation terms are added, the predictions for oligomers are comparable to those of the full model with ten dimer parameters.

Observe that bases $y_i$ and $x_i$ have, to this point, been effectively treated as independent variables, but as is well known, the complementary H-bonding of nucleotide bases is highly specific. Thus we can assume that if the types of base-pairing occurring within the duplex are known then a relationship between complementary bases might be exploited. For example, if only WC pairing geometries occur in a given duplex then it follows that if site $y_i$ contains the base $C$, site $x_i$ must contain a $G$. Thus the labels specifying one of the two bases are redundant.

In general for a base pair we may therefore write

\[y_i = \gamma_i x_i \equiv \lambda_i R_i x_i,\]
where $\lambda > 0$ is a scaling factor while $R$ is, in general, some length-preserving transformation and the matrices $\gamma_i$ are assumed to be characteristic of a particular pairing geometry. It follows from the expression

$$y^T_i h x_i = \lambda_i x^T_i R^T_i h x_i,$$ (14)

that the off-diagonal entries of $(R^T h)$ only occur in linear combinations, thereby reducing the number of independent dimer parameters by $p(p - 1)/2$.

In the special case of WC-pairing geometries ($p = 2$) we therefore have 9 such parameters. From (10) the internal H-bonding correlations are symmetric and it is easy to see that the four elements $H^{\alpha\beta}$ occur in just two linear combinations precisely when $R$ has zero values along its diagonal. This effective eight-parameter treatment of internal base pairs is directly comparable to the description of polymers, i.e. sequences with no normal ends, in terms of eight “invariants” or ISS.

Now consider the difference between the contributions of $5'P/3'Q$ and $5'Q/3'P$ pairs. If the pairs are internal, since $H = H^T$ the H-bonding enthalpies are equal:

$$q^T h p = p^T h q.$$  

It follows that any orientation effects are manifest only at the terminals where

$$q^T h p = p^T h q \neq p^T h q$$

Thus in the WC case the difference $h_{12} - h_{21}$ is a measure of the magnitude of these effects. If, as is commonly assumed in the NN approach for DNA and RNA duplexes, $5'G/3'C$ and $5'C/3'G$ terminal pairs are equivalent (and similarly for A.T/U) this quantity should be a small correction. Therefore the model with eight dimer parameters obtained by assuming $h_{12} \approx h_{21}$ should be a reasonable approximation of the full, 9 parameter version, which we verify in the results below. Analogously in Ref. [25] the ability of the “rank deficient” DNA polymer model to reproduce DNA oligomer data was rationalised thus: “...most of the sequence dependence of oligonucleotide DNA thermodynamics is captured in the first eight terms and the remaining two are small perturbations...”

In two dimensions there are just two candidates for the transformations $R_i$ defined in (14), these are proportional to

$$\sigma_1 = \begin{pmatrix} 0 & 1 \\ -1 & 0 \end{pmatrix}, \quad \sigma_2 = \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix}.$$  

If, for simplicity the scalings $\lambda_i = 1$, the latter form can be rejected since $\sigma_2 = \sigma_2^{-1}$. This would ensure that the six stackings $s^{\alpha\beta}, s^{(2-\beta)(2-\alpha)}$ always appear in just three linear combinations, reducing the number of independent parameters from 8 to 5. For $\gamma = \pm \sigma_1$ the property $\gamma^{-1} = -\gamma$ means that the relative signs between $s$ and $t$ elements are sequence-dependent, thereby ensuring the number of model parameters is fixed at eight.

### 3.3 Equivalence with dimer model

For clarity we now show the equivalence of the present model with the NN approach for canonical duplexes. Firstly the duplex pairing matrix is written in terms of Hydrogen-bonding and stacking...
\[ \Delta G(X,Y) = \Delta G_{HB} + \Delta G_{ST}; \quad (15) \]

\[ \Delta G_{HB} = y^T_1 h x_1 + y^T_n h x_n + \sum_{i=2}^{n-1} y^T_i H x_i; \quad (16) \]

\[ \Delta G_{ST} = \sum_{i=1}^{n-1} (y^T_i s x_{i+1} + y^T_{i+1} t x_i). \quad (17) \]

Now consider a dimer \( 5’XZ3’/3’WY5’ \) occurring with frequency \( N_{XZ} \) in a given duplex. For symmetric dimers \( N_{XZ} = N_{YW} \) and the stacking term \( \Delta G_{ST} \) depends on ten linear combinations of these 16 frequencies:

\[ \Delta G_{ST} \equiv 2N_{GG}S_{GG/GC} + 2N_{CG}S_{CG/C} + 2N_{AT}S_{AT/AT} + 2N_{TA}S_{TA/TA} \\
+ (N_{GA} + N_{GT})S_{GA/TG} + (N_{GT} + N_{AC})S_{GT/AC} + (N_{CA} + N_{TG})S_{CA/TG} \\
+ (N_{AG} + N_{CT})S_{AG/CT} + (N_{AA} + N_{TT})S_{AA/TT} + (N_{GG} + N_{CC})S_{GG/CC}. \quad (18) \]

\[ S_{XZ/WY} = \sum_{\alpha, \beta=1}^{p} (s^{\alpha \beta} x_\alpha y_\beta + t^{\alpha \beta} w_\alpha z_\beta). \]

Similarly, if \( h = H/2 \), the H-bonding terms contain four numbers:

\[ \Delta G_{HB} = (n^i_{GC} + 2n^i_{GG})h_{GC} + (n^i_{AT} + 2n^i_{TA})h_{AT}; \quad H_{xy} \equiv \sum_{\alpha, \beta=1}^{p} h^{\alpha \beta} y_\alpha x_\beta, \quad (19) \]

where \( n^i_{GC}, n^i_{GG}, n^i_{AT}, \) and \( n^i_{TA} \) denote the numbers of terminal and internal G.C and A.U pairs respectively. These numbers of base pairs are not independent of the stacking frequencies \( N_{XY} \) however:

\[ (n^i_{GC} + 2n^i_{GG}) = N_{GA} + N_{GT} + 2N_{GG} + 2N_{CC} + N_{AG} + N_{TG} + 2N_{CG}, \]

\[ (n^i_{AT} + 2n^i_{TA}) = 2N_{AA} + 2N_{AT} + N_{AG} + N_{AC} + N_{GA} + 2N_{TA} + N_{CA}. \]

Combining these identities in (19) with (18) one sees that the coefficients of \( N_{XY} \) in (18) contain both H-bonding and stacking correlations. In this way one obtains ten dimer parameters equivalent to those of the Interacting Nearest Neighbour with H-Bonding (INNHB) [2] (see Table 4 in the results for verification of this). Furthermore the ten dimer parameters in our model are just linear combinations of the eight matrix elements, similar to the eight ISS parameters discussed in Ref. [25].

### 3.4 Weight space

Having discussed the 2-body correlation matrix in detail we now turn to the vector “weight” space. In order to obtain a model which is equivalent to the polymer dimer model we have imposed just two “rules” on the weight space:

1. The vectors for complementary bases are orthogonal and of equal length.
2. The model of duplexes with WC pairing must have \( p = 2 \), therefore the vectors for G,C,A,T all live in the same (two-dimensional) space.

Note that there are still \( 2p \) unknown parameters (the coordinate values for, say G and A) however, rather than attempt to obtain them from a fit to empirical data assumptions may be made about what the vector coordinates represent.
For WC pairs in ref. [10] we assumed one coordinate counted the number of H-bonds formed, the other indicated whether the heterocyclic ring was purine or pyrimidine. With the basis
\[ \{G, C, A, U(T)\} = \{ (\sqrt{3}, 1/2), (-1/2, \sqrt{3}), (-\sqrt{2}, 1/2), (-1/2, -\sqrt{2}) \}, \]
we obtained an 8-parameter, so-called “rank-deficient”, model of RNA oligonucleotides statistically identical to the conventional dimer model (e.g. Refs. [2], [3]). To obtain fitted models from DNA/DNA and RNA/DNA data below we shall assume that the same degrees of freedom [20] contribute to WC pairing. The effects of differing sugar backbone geometries will be manifested in the relative magnitudes of the fitted coupling values for the various duplexes.

4 Results: WC Pairs in DNA

The next stage of our comparative analysis is to obtain the DNA pairing model from data for duplexes with two-state melting transitions and consisting of only Watson Crick pairs. Following Refs [3], [12] we construct models for thermodynamic parameters \( \Delta G, \Delta H, \Delta S \) for two sets of sequences one set of 44 of duplexes terminated by GC pairs only, the other containing, in addition to these 44, eight duplexes with at least one AT terminal.

We use an initiation term with separate \( 5' \rightarrow 3' \) and \( 3' \rightarrow 5' \) parameters, consistent with the observation of SantaLucia et al. [3] that the former have a tendency to fray:
\[ \Delta G_{\text{init}} = \alpha_1 n_{\text{GC}}^t + \alpha_2 n_{5'T}^t + \alpha_3 n_{5'A}^t. \]
The self-complementary entropy “penalty” \( \Delta G_{\text{sym}} \) is set to the theoretical value 0.43 kcal mol\(^{-1}\) at a temperature of 310K. The form of our fitting function is thus given by [21] plus [16] and [17]:
\[ \Delta G(X,Y) = \Delta G_{\text{init}} + \Delta G_{\text{sym}} + y_i^T h x_i + y_i^T h^T x_n + \sum_{i=2}^{n-1} y_i^T (h + h^T) x_i + \sum_{i=1}^{n-1} (y_i^T s x_{i+1} + y_i^T t x_i). \]

Here \( h, s \) and \( t \) are \( 2 \times 2 \) matrices while vectors \( x_i, y_i \) take the appropriate values from the base vector set [20]. Due to the assumed \( 5' - 3' \) dimer symmetry \( s \) and \( t \) are symmetric, while we have kept the distinction between \( h \) and \( h^T \) for the purpose of comparing rank-deficient and rank-determinant parameter sets.

To compare the NN dimer and base-pairing approaches we shall compute the same statistical parameters used in other studies, for example, Ref. [2]. In addition to the root of the mean of squared residuals \( \sigma \) we include the unweighted \( \chi^2 \) parameter is computed to be
\[ \chi^2 = \sum \left( \frac{G_p - G_o}{\sigma} \right)^2, \]
where \( G_p, G_o \) and \( \sigma \) are the predicted and observed values of \( G \) and the rms value respectively. Here \( f \) is the number of observations less the number of model parameters. The reduced parameter \( \chi^2/f \) should have value close to unity for a good fit. The \( Q \) value estimates the likelihood of obtaining a particular value of \( \chi^2 \) by chance:
\[ Q = \Gamma(f/2, \chi^2/2)/\Gamma(f/2) \]
where $\Gamma(a)$ and $\Gamma(a, z)$ denote the complete and incomplete gamma functions respectively. Small $Q$ values signify that discrepancies between the model predictions and experimental data are unlikely to be due to chance.

Using Eqs. (23), (24) we compute these statistics for the predicted values of $\Delta G$ in six models in Table 1. The models NN1 and NN2 are respectively the 11- and 12-parameter models obtained in Ref. [3] for sets of G.C- and G.C/A.T- terminated duplexes. JB1a and JB1b denote, respectively the data for rank-determinant ($h = h^T$) and rank deficient ($h \neq h^T$) models for the 44 GC terminated duplexes. Similarly JB2a and JB2b denote the same models for the full set of 52 sequences. Several observations may be made immediately from Table 1. Firstly, the fit

|        | $\sigma^a$ | $\chi^2/f$ | $Q$ |
|--------|------------|------------|-----|
| NN1    | 0.33       | 1.22       | 0.10|
| JB1a   | 0.33       | 1.26       | 0.14|
| JB1b   | 0.33       | 1.22       | 0.17|
| NN2    | 0.35       | 1.30       | 0.10|
| JB2a   | 0.34       | 1.28       | 0.12|
| JB2b   | 0.34       | 1.24       | 0.14|

Table 1: Comparison of statistics for the standard Nearest-Neighbour (NN) parameters and the fitting of our models to the same data. $^a$Units are kcal mol$^{-1}$.

rms values for all models are in good agreement, however if the distinction between AT and TA terminal parameters is removed the rms values of JB2a, JB2b would rise respectively to 0.41 and 0.39 kcal mol$^{-1}$. Note however that introducing separate parameters for 5’G/3’C and 5’C/3’G in all instances provides slight improvements in $\sigma$ values of $\leq 0.005$ kcal mol$^{-1}$ while increasing $\chi^2/f$ and decreasing $Q$. Therefore the optimal choice of initiation term (21) is validated.

The parameter set for estimating $\Delta G$ for the best model, JB2a is given by (units are kcal mol$^{-1}$)

$$
\alpha_1 = 0.833, \quad \alpha_2 = 0.98, \quad \alpha_3 = 1.84
$$

$$
h = \begin{pmatrix}
0.139 & -0.293 \\
-0.305 & -0.139
\end{pmatrix}, \quad t = \begin{pmatrix}
-0.071 & -0.033 \\
-0.033 & 0.180
\end{pmatrix}, \quad s = \begin{pmatrix}
-0.190 & 0.019 \\
0.019 & 0.055
\end{pmatrix}.
$$

(25)

Of course the models should also be compared at the level of dimer propagation energies. These quantities are found from (22) via

$$
\Delta G_{XZ/YW} \equiv X^T h Y + Z^T h W + Y^T s Z + W^T t X
$$

and using the basis (20) and parameters (25). Values are compared to the dimer quantities of Ref. [3] in Table 1 below. For completeness the fitted matrix elements for $\Delta H$ and $\Delta S$ and related dimer quantities are included in the appendix.

5 Results: WC pairs in RNA/DNA hybrids

We now modify the approach in order to analyse thermodynamic data for RNA/DNA hybrids, in particular the 68 sequences used by Sugimoto and co-workers [4], and compare our results to previous NN analyses [4, 12].
Table 2: Comparison of NN symmetric dimer parameters with those derived from the rank-
deficient parameters \(^{(25)}\). All units are kcal mol\(^{-1}\). \(^a\)Dimer parameters from 12-parameter
model obtained by SantaLucia \textit{et al.} \(^{[3]}\). \(^b\)BP denotes the base pairing model with rank deficient
parameter set JB2b.

|        | NN\(^a\) | BP\(^b\) |
|--------|----------|----------|
| GG/CC  | -1.77 ± 0.06 | -1.76    |
| GC/GC  | -2.28 ± 0.08 | -2.28    |
| CG/GC  | -2.09 ± 0.07 | -2.09    |
| AA/TT  | -1.02 ± 0.04 | -1.01    |
| AT/TA  | -0.73 ± 0.05 | -0.77    |
| TA/AT  | -0.60 ± 0.05 | -0.63    |
| GA/CT  | -1.46 ± 0.05 | -1.52    |
| GT/CA  | -1.43 ± 0.05 | -1.37    |
| AG/TC  | -1.16 ± 0.07 | -1.13    |
| TG/AC  | -1.38 ± 0.06 | -1.36    |

The major difference to DNA or RNA hybrids is that, owing to the different backbones of
the strands the zippering direction is readily distinguished; there are no local dimer or global
duplex symmetries, nor a self-symmetric entropy term. Indeed a naive fit of the symmetric
dimer fitting function \(^{(22)}\) yields an rms value of 0.57 kcal mol\(^{-1}\), considerably poorer to the
homoduplex models.

The dimer propagation matrix is therefore given by \(^{(3)}\) where constraints \(^{(8 \text{ and } 9)}\) do not hold
and the number of independent matrix elements is simply \((2p)^2\). The comparison of dimer and
global correlations now yields

\[
H_1 = h_1, \quad H_n = h_2, \\
H \equiv H_2 = h_1 + h_2 = H_3 = \ldots = H_{n-1}, \\
S_{i,i+1} = s, \quad T_{i+1,i} = t.
\]

(26)

Hence the fitting function is now given by

\[
\Delta G(X,Y) = \Delta G_{\text{init}} + y_1^T h_1 x_1 + y_2^T h_2 x_n + \sum_{i=2}^{n-1} y_i^T (h_1 + h_2) x_i + \sum_{i=1}^{n-1} (y_i^T s x_{i+1} + y_{i+1}^T t x_i).
\]

(27)

where, in contrast to the single initiation term of Ref \(^{[4]}\) we shall assume distinct terminal
parameters for the termini

\[
r(G)/d(C) \equiv r(C)/d(G) \quad r(A)/d(T) \equiv r(U)/d(A).
\]

Of course in general four such parameters may be distinguished, however initially we shall
consider an initiation energy form

\[
\Delta G_{\text{init}} = \alpha_1 n_t^{GC} + \alpha_2 n_t^{AT(U)}.
\]
The resulting model has, naively $2 + 16$ parameters to be compared with $1 + 16$ for the NN model \[4\]. However RNA/DNA hybrids, like homoduplexes exhibit high complementarity in H-bonding. Since the “weight vectors” \[20\] have been shown to capture the essential sequence-dependent interactions of both DNA/DNA and RNA/RNA \[10\] helix formation and, noting that the RNA/DNA hybrid also has a regular helical geometry, it is reasonable to investigate whether they are successful in the latter instance.

With the existence of complementarity transformations \[14\], two of each of the $h_1$ and $h_2$ matrix entries appear in single linear combinations. For the DNA/DNA model we obtained a rank-deficient model by neglecting the distinction between the H-bond correlations of the two termini. The analogy in the hybrid case is to suppose $h_1 \simeq h_2$, the validity of which we check below. The rank-deficient parameter set for hybrids therefore has $2 + 11$ parameters, an improvement of 4 over the NN model.

In fact we find that this model reproduces the empirical $\Delta G$ data (rms 0.38 kcal mol$^{-1}$) slightly better than the NN models \[4\], \[12\] and in addition gives better reduced $\chi^2/f$ and $Q$ values, see Table 5. The fitted values obtained (in kcal mol$^{-1}$) are

$$h = \begin{pmatrix} -0.151 & 0.277 \\ 0.369 & 0.151 \end{pmatrix}, \quad t = \begin{pmatrix} -0.035 & -0.065 \\ 0.002 & -0.011 \end{pmatrix}, \quad s = \begin{pmatrix} 0.010 & 0.060 \\ 0.078 & 0.035 \end{pmatrix}. \quad (28)$$

Sugomoto and coworkers used a single helix initiation parameter of 3.1 kcal mol$^{-1}$ which is consistent with our initiation term which takes the values 3.05, 3.17 or 3.30 kcal mol$^{-1}$ depending on whether the duplex has respectively 0, 1 or 2 G.C terminals.

Several other observations may be made about the matrix elements \[28\]. Firstly the difference $h_{12} - h_{21}$ is roughly four times larger in the hybrid case, therefore the model already incorporates significant distinctions between $5'\text{A}/3'\text{T}$ and $5'\text{T}/3'\text{A}$ (and similarly for GC) terminii. Doubling the number of initiation terms therefore leads to only a small increase in accuracy for the cost of two extra parameters.

More surprising is the result that $s_{11} \simeq -t_{22}$ and $s_{22} \simeq -t_{11}$ would provide excellent approximations, reducing the parameter number further to 11. In the absence of an obvious theoretical reason for this coincidence of stackings we cannot reject the possibility that it is an artifact of the data. The rank deficient parameter set, denoted H1 in Table 5 for RNA/DNA hybrids therefore contains 13 parameters. Finally we consider the 16 parameter rank-determinant ($h_1 \neq h_2$) model, denoted H2 in Table 5. As anticipated it is found to provide a marginal improvement in $\sigma$ (of $\leq 0.005$ kcal mol$^{-1}$) but at a cost reflected in the reduced $\chi^2$ and $Q$ statistics. Again for completeness, in the appendix we tabulate fitted parameters for $\Delta H$ and $\Delta S$ parameters.

| $\Delta G$ | $\sigma^a$ | $\chi^2/f$ | $Q$ |
|------------|------------|-------------|-----|
| NN$^b$     | 0.45       | 1.57        | 0.01|
| H1         | 0.38       | 1.24        | 0.12|
| H2         | 0.38       | 1.31        | 0.07|

Table 3: Comparison of statistics for models of hybrid duplex formation. $^a$Units are kcal mol$^{-1}$. $^b$NN data was calculated using model parameters (NN1) of Sugimoto et al. \[4\]. H1 and H2 denote rank deficient and rank determinant base-pairing parameter sets respectively.
6 Conclusion

In this paper we have refined the idea, first presented in Ref. [10], that a description of duplex formation in terms of (fictitious) two-body correlations is equivalent to the more commonly known NN dimer method.

For all three cases (RNA, DNA and RNA/DNA hybrid helices) fits to empirical data confirm that this is indeed the case. Moreover the connection between rank-deficient polymer and rank-determinant oligomer NN parameter sets, known for DNA (see, e.g., Ref. [25]) is transparent via our approach and enables similar approximations for RNA [10] and RNA/DNA hybrids to be made.

In the instances of RNA and DNA homoduplexes while the former approximation does not offer an increase in model accuracy, statistics of model significance (e.g. $\chi^2$, $Q$) suggest the base-pairing approach to be more favourable on the grounds that it uses fewer and more “fundamental” model degrees of freedom. For RNA/DNA hybrids an improvement in both accuracy and parameter numbers is also observed over the NN method, indeed the high $\chi^2$ and low $Q$ probability for the latter in Table 5 strongly suggest a good deal of redundancy in using the dimer energy parameters.

The base-pairing model also suggests the interesting possibility of a kind of universality forstabilising interactions in Watson-Crick paired helices. Specifically the same numerical weights [20] are found to encapsulate the sequence-dependence for all three types of helices, the differences between fitted correlation matrix elements being attributed to the effects of different helical geometries (A-type for RNA/RNA and RNA/DNA, B for DNA/DNA).

It should be emphasised that the base-pairing model is only a description of the sequence-dependence of helical oligonucleotides in terms of two-body correlations labelled by “weight vectors”. However the suggestion of a connection between this picture with an effective interaction potential in three spatial dimensions is appealing. The nature of these “weight vectors” is not yet clear, and further insight may be gained from extending the method to mismatch pairing geometries.

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7 Appendix

$$h_{RNA} = \begin{pmatrix} 0.122 & -0.232 \\ -0.232 & -0.122 \end{pmatrix}, \quad t_{RNA} = \begin{pmatrix} 0.156 & -0.051 \\ -0.051 & -0.022 \end{pmatrix}, \quad s_{RNA} = \begin{pmatrix} 0.070 & 0.067 \\ 0.067 & -0.159 \end{pmatrix},$$

References

[1] Frier, S.M.; Kierzek, R.; Jaeger, J.A.; Sugimoto, N.; Caruthers, M.H., Neilson, T.; Turner D.H., Proc Natl Acad Sci 1986, 83, 9373-9377.

[2] Xia, T.; SantaLucia, J.; Jrn, Burkard, M.E.; Kierzek, R.; Schroeder, S.J.; Jiao, X.; Cox, C.; Turner, D.H. Biochemistry 1998, 38, 14719-14735.
[3] SantaLucia, J. Jnr.; Allawi, H.; Seneviratne, P.A. Biochemistry 1996, 35, 3555-3562

[4] Sugimoto, N.; Nakano, S.; Katoh, M.; Matsumura, A.; Nakamuta, H.; Ohmichi, T.; Yoneyama, M.; Sasaki, M. Biochemistry 1995, 34, 11211-11216.

[5] SantaLucia, J.Jnr; Turner, D.H. Biopolymers 1998, 44, 309-319.

[6] Mathews, D.H.; Sabina, J.; Zuker, M.; Turner, D.H. J Mol Biol 1999, 288, 911-940.

[7] Poland, D. Biopolymers 2004, 73, 216-228.

[8] Deutsch, J.M.; Liang, S.; Narayan, O. arXiv preprint q-bio.BM/0406039, 2004.

[9] Bashford, J.; Jarvis, P.D. BioSystems 2000, 57, 147-161.

[10] Bashford, J.D; Jarvis, P.D. Biopolymers 2004, 73, 657-667.

[11] Gray, D.M. Biopolymers 1997, 42, 783-793.

[12] Gray, D.M. Biopolymers 1997, 42, 795-810.

[13] Allawi, H.; SantaLucia, J. Jnr. Biochemistry 1997, 36, 10581-10594.

[14] Allawi, H.; SantaLucia, J. Jnr. Biochemistry 1998, 37, 9435-9444.

[15] Allawi, H.; SantaLucia, J. Jnr. Nucl Acids Res 1998, 26, 2694-2701.

[16] Allawi, H.; SantaLucia, J. Jnr. Biochemistry 1998, 37, 2170-2179.

[17] Peyret, N.; Seneviratne, P.A.; Allawi, H.T; SantaLucia, J. Jnr. Biochemistry 1999, 38 3468-3477.

[18] He, L.; Kierzek, K.; SantaLucia, J. Jnr.; Walter, A.E.; Turner, D.H. Biochemistry 1991, 30, 11124-11132.

[19] Wu, M.; McDowell, J.A., Turner, D.H. Biochemistry 1995, 34, 3204-3211.

[20] Xia, T.; McDowell, J.A., Turner, D.H. Biochemistry 1997, 36, 12486-12497

[21] Sugimoto, N.; Katoh, M.; Nakano, S.; Ohmichi, T.; Sasaki, M. FEBS Lett 1994, 354, 74-78.

[22] Gaeta, G., Reiss, C.; Peyrard, M.; Dauxois, T. Rivista del Nuovo Cimento 1994, 17, 1-50.

[23] Ramreddy, T.; Rao, B.J.; Krishnamoorthy, G. to appear in Proceedings of 5th International Congress in Biological Physics, Springer, 2005.

[24] Goldstein, R.F.; Benight, A.S. Biopolymers 1992, 32, 1679-1693.

[25] SantaLucia, J. Jnr. Proc Natl Acad Sci 1998, 95, 1460-1465.