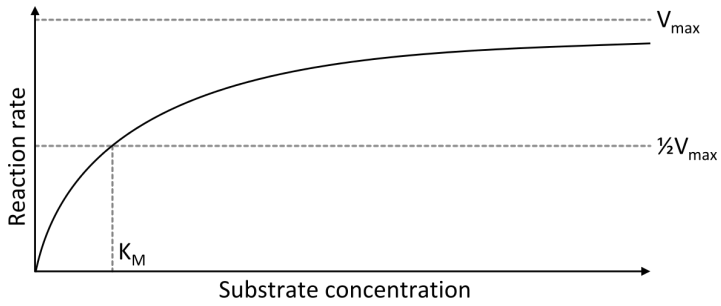
Enzyme- catalyzed reactions

The **Michaelis-Menten equation** describes the kinetics of enzyme-catalyzed reactions:

$$v = \frac{v_{max}[s]}{K_m + [s]}$$

Vmax represents the maximum rate achieved by the system, at saturating substrate concentration.
The Michaelis constant **Km** is the substrate concentration at which the reaction rate is half of Vmax.



Gibbs free energy and equilibrium

By definition

$$\Delta G^0 = \Delta H^0 - T\Delta S^0$$

At equilibrim

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 = -RTlnK$$

where ΔG^0 is the standard change in Gibbs free energy
ΔH^0 is the standard change in enthalpy
ΔS^0 is the standard change in entropy
T is the temperature
R is the gas constant
K is the equilibrium constant

Nearest Neighbor (NN) model for nucleic acids

This table presents the thermodynamic nearest neighbor (NN) parameters for Watson-Crick base pairs in 1 M NaCl.

TABLE 1 Nearest-neighbor thermodynamic parameters for DNA Watson-Crick pairs in 1 M NaCl[a]

Propagation sequence	ΔH° (kcal mol^{-1})	ΔS° (e.u.)	ΔG°_{37} (kcal mol^{-1})
AA/TT	−7.6	−21.3	−1.00
AT/TA	−7.2	−20.4	−0.88
TA/AT	−7.2	−21.3	−0.58
CA/GT	−8.5	−22.7	−1.45
GT/CA	−8.4	−22.4	−1.44
CT/GA	−7.8	−21.0	−1.28
GA/CT	−8.2	−22.2	−1.30
CG/GC	−10.6	−27.2	−2.17
GC/CG	−9.8	−24.4	−2.24
GG/CC	−8.0	−19.9	−1.84
Initiation	+0.2	−5.7	+1.96
Terminal AT penalty	+2.2	+6.9	+0.05
Symmetry correction	0.0	−1.4	+0.43

[a]The slash indicates the sequences are given in antiparallel orientation. (e.g., AC/TG means 5′-AC-3′ is Watson-Crick base paired with 3′-TG-5′). The symmetry correction applies to only self-complementary duplexes. The terminal AT penalty is applied for each end of a duplex that has a terminal AT (a duplex with both end closed by AT pairs would have a penalty of +0.1 kcal/mol for ΔG°_{37}).

The following equation is used to predict the ΔG^0_T at a different temperature, T:

$$\Delta G^0_T = \Delta H^0 - T\Delta S^0$$

where T is in Kelvin, ΔH^0 is in cal/mol, and ΔS^0 is in units of $cal/K \cdot mol$ (entropy units, e.u.). $\Delta H^0$and ΔS^0 are assumed to be temperature independent; this is an excellent approximation for nucleic acids.

The NN Model.

The NN model for nucleic acids assumes that the stability of a given base pair depends on the identity and orientation of neighboring base pairs.

According to this model, the total $\Delta G^0_{Total(37)}$ is given by:

$$\Delta G^0_{Total(37)} = \sum_i n_i \Delta G^0(i) + \Delta G^0(init \quad /term\ G\star C) + \Delta G^0(init \quad /term\ A\star T) + \Delta G^0(sym)$$

where $\Delta G^0(i)$ are the standard free-energy changes for the 10 possible Watson–Crick NNs (Table 1), n_i is the number of occurrences of each nearest neighbor (*i.e.* the number of pairs), *i*, and $\Delta G^0(sym)$ equals +0.43 kcal/mol (1cal =4.184J) if the duplex is self-complementary and zero if it is non-self-complementary.

Prediction of the Melting Temperature T_M.

T_M is defined as the temperature at which half of the strands are in the double-helical state and half are in the "random-coil" state. The T_M is calculated from the predicted ΔH^0 and ΔS^0, and the total oligonucleotide strand concentration C_T, by using the equation:

$$T_M = \frac{\Delta H^0}{\Delta S^0 + R \ln C_T}$$

where R is the gas constant (1.987 $cal/K \cdot mol$).

R = 8.314 J/(mol K) = 1.987 cal/(mol K)

1 cal = 4.184 J

To compute the T_M in Celsius degree:

$$T_M = \frac{\Delta H^0 \cdot 1000}{\Delta S^0 + R \ln C_T} - 273.15$$

since 0 K = -273.15 °C

where ΔH^0 is given in cal/mol.

e.u. = non-SI entropy unit = 1 cal/(mol K) = 4.184 J/(mol K)

Internal Single Mismatches

The nearest-neighbor model can be extended beyond the Watson-Crick pairs to include parameters for interactions between mismatches and neighboring base pairs. Table 2 provides the complete thermodynamic database for internal single mismatches.

TABLE 2 Nearest-neighbor ΔG°_{37} increments (kcal mol^{-1}) for internal single mismatches next to Watson-Crick pairs in 1 M NaCl[a]

Propagation sequence	X	Y: A	Y: C	Y: G	Y: T
GX/CY	A	0.17	0.81	−0.25	WC
	C	0.47	0.79	WC	0.62
	G	−0.52	WC	−1.11	0.08
	T	WC	0.98	−0.59	0.45
CX/GY	A	0.43	0.75	0.03	WC
	C	0.79	0.70	WC	0.62
	G	0.11	WC	−0.11	−0.47
	T	WC	0.40	−0.32	−0.12
AX/TY	A	0.61	0.88	0.14	WC
	C	0.77	1.33	WC	0.64
	G	0.02	WC	−0.13	0.71
	T	WC	0.73	0.07	0.69
TX/AY	A	0.69	0.92	0.42	WC
	C	1.33	1.05	WC	0.97
	G	0.74	WC	0.44	0.43
	T	WC	0.75	0.34	0.68

[a]WC indicates a Watson-Crick pair, which is given in Table 1. Error bars and ΔH° and ΔS° parameters are provided in the original references.

Antibody-antigens affinity and bond energy

If a monovalent antibody fragment is used for analysis, the equilibrium of antigen–antibody binding is defined as:

$$Antibody + Antigen \overset{K_a}{\Leftrightarrow} Complex$$

where

$$K_a = \frac{[Complex]}{[Antibody][Antigen]}$$

Association and dissociation rate constants are defined as follows:

$$v_{ass} = k_{ass}[Antibody][Antigen]$$
$$v_{diss} = k_{diss}[Complex]$$

where v_{ass} and v_{diss} represent the rates of association and dissociation, respectively, and k_{ass} and k_{diss} represent the rate constants of association and dissociation, respectively. At equilibrium v_{ass} is equal to v_{diss} and the following equation is obtained:

$$K_a = \frac{k_{ass}}{k_{diss}}$$

The Gibbs' energy of formation (ΔG_0) of an antigen–antibody complex is given by:

$$\Delta G_0 = -RT \ln K_a$$

where R is the gas constant (1.987 cal/molK) and T is temperature.

The free energy of complex formation represents a balance between enthalpic (ΔH_0) and entropic (ΔS_0) forces as defined by the equation:

$$\Delta G_0 = \Delta H_0 - T\Delta S_0$$

In general, antigens and antibodies in solution have to overcome large entropic barriers before they can form a tight binding.