Vital common traits of life

All cells, primitive or more evolved, function and multiply in environment. The separation of the cellular machinery is demarcated by the boundary of the cell wall or membrane. The interior of living cell consists of thousands of biomolecules of different sizes. The major constituents of different types of macromolecules, found in mammalian and bacterial cell are given as % total cell weight in table 4. The main macromolecular components of E.Coli and human cell are detailed in table 5. The point to note is that 30% of the total cell weight is constituted by the molecules, as small as ions to the biomolecules as large as DNA. In thermodynamic terms the interior of the cell is highly crowded. A typical bacterial cell contains thousands of different molecules in cell (Alberts et al, 1983). The sustenance of life is manifested by complex reaction dynamics prevailing within the cell, involving directly or indirectly all the molecules. The direct reaction may simply involve:

\[
\text{precuror} \Rightarrow \text{end product}
\]

This simple reaction may involve the participation of a specific enzyme. The crucial aspect about the cellular reactions is that all of them (simple, complex or cyclic reactions) take place in the presence of all other molecules which crowds the reaction by excluding volume space from the targeted reaction. Two immediate conclusions can be
Table 4 showing % of total weight of E. coli and Mammalian cell for different components of cell (Alberts et al. 1983)

<table>
<thead>
<tr>
<th>Component</th>
<th>% of total cell weight E. coli (Bacterium)</th>
<th>% of total cell weight Mammalian cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Inorganic ions</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(Na⁺, K⁺, Mg²⁺, Ca²⁺, Cl⁻)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miscellaneous small molecules</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Proteins</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>RNA</td>
<td>6</td>
<td>1.1</td>
</tr>
<tr>
<td>DNA</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Other lipids</td>
<td>--</td>
<td>2</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cell Volume</td>
<td>2x10⁻¹² cm³</td>
<td>4x10⁻⁹ cm³</td>
</tr>
</tbody>
</table>

TABLE 5 THE MAIN MACROMOLECULAR COMPONENTS OF E. COLI AND HUMAN CELLS (Darnell et al. 1986)

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount per HeLa cell (Human cervical carcinoma)</th>
<th>Amount per E. coli cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total DNA</td>
<td>15 picograms</td>
<td>0.017 picogram</td>
</tr>
<tr>
<td>Total RNA</td>
<td>30 picogram</td>
<td></td>
</tr>
<tr>
<td>Total protein</td>
<td>300 picogram (5 X 10⁹ molecules of average mol.wgt. 40,000)</td>
<td>0.2 picogram (3 X 10⁴ molecules, of average mol.wgt. 40,000)</td>
</tr>
<tr>
<td>Cytoplasmic ribosome</td>
<td>4 X 10⁶ molecules</td>
<td>3 X 10⁴</td>
</tr>
<tr>
<td>Cytoplasmic t RNA</td>
<td>6 X 10⁷ molecules</td>
<td>4 X 10⁵</td>
</tr>
<tr>
<td>Cytoplasmic t RNA</td>
<td>7 X 10⁶ molecules</td>
<td>4 X 10⁵</td>
</tr>
<tr>
<td>Nuclear precursor r RNA</td>
<td>6 X 10⁴ molecules</td>
<td>-</td>
</tr>
<tr>
<td>Heterogenous nuclear RNA</td>
<td>1.6 X 10⁵ molecules</td>
<td>-</td>
</tr>
<tr>
<td>Total dry weight</td>
<td>400 picogram</td>
<td>0.4 picogram</td>
</tr>
</tbody>
</table>
drawn; i) the nature of interaction of specific reactions, studied under dilute conditions will not ascertain the findings in living cell's crowded interior (Zimmerman, 1992; Minton, 1983), ii) the presence of the molecular crowding in reference to a specific target reaction will have thermodynamic consequences on the rate of reaction and the nature of reaction per se. The affect of molecular crowding, in general, is ignored while interpreting the observed laboratory based experiments (Minton 1983; Fulton 1982). The effect of molecular crowding will have its bearing on the change in entropy of the reaction constituents (Minton, 1981, 1983, 1993; Chatelier, 1987; Fulton, 1982; Vonhippel, 1981; Zimmerman, 1992; 1993; Wilf, 1981). To appreciate the crucial role played by molecular crowding in driving the reaction entropically, a brief related thermodynamics aspects are discussed below.

Entropy driven processes in biology

Spontaneous entropy driven processes are those that occur primarily because of an increase in entropy. Entropy driven assembly of microfilaments and microtubules and other structures are important in the functioning of living cells. Much of our understanding of the factors that influence or control entropy driven processes derives from extensive studies done on the polymerization of Tobacco Mosaic Virus protein and actin polymerization (Lauffer, 1975; 1989; Attri, 1992).
The first law of thermodynamics can be represented in the equation form as:

\[ \Delta E = Q - W \quad (1) \]

where, \( E \) is the total internal energy, \( Q \) is the heat absorbed, and \( W \) is the work done. For a reversible process equation (1) can be represented as:

\[ dE = dQ - dW \quad (2) \]

where, \( dW \) is the sum of the work done by expansion against the atmosphere. The mechanical work, \( P.dV \), and the non-mechanical work, such as electrical work etc. \( (dW_{nm}) \) can be included in equation (2) and will take the form

\[ dE = dQ - P.dV - dW_{nm} \quad (3) \]

In the absence of mechanical work, that is, when \( dW_{nm} \) is 0, and when volume is kept constant, or when \( dV = 0 \), change in \( E \) can be equated as:

\[ dE = (dQ)_v \quad : \quad \Delta E = Q_v \quad (4) \]

\( \Delta E \) can be evaluated by measuring the heat absorbed or evolved when a reaction is carried out in a bomb calorimeter. Enthalpy, \( H \), is defined by equation 5 and 6.

\[ H = E - P.V \quad (5) \]

\[ dH = dE + P.dV + V.dP \quad (6) \]
By substituting equation (3) for the case when there is no non-mechanical work for $dE$,

$$dH = dQ - V.dP$$  \hspace{1cm} (7)

For a process carried out at constant pressure (Living cells usually follow this condition), $dP = 0$, we will have following conditions;

$$dH = (dQ)_p : \quad \Delta H = Q_p$$  \hspace{1cm} (8)

$\Delta H$ can be determined for a process or a reaction is carried out at a constant pressure by measuring the heat absorbed or evolved in a calorimeter. Because biological processes occur at a constant pressure and temperature, equations derived for conditions of constant pressure are directly applicable. For processes carried out in solution, except for those involving the adsorption or the evolution of gas, $dV$ is either 0 or very small.

Thus, for most biological processes, it follows from equation (6) that

$$\Delta H \approx dE$$

When chemical bonds, either strong or weak, are formed, heat is evolved, and when they are broken, heat is absorbed. The enthalpy change, $\Delta H$, is the sum of the heat absorbed in breaking old bonds and the heat evolved (negative) in forming new bonds. In most chemical reactions involving the formation of large structures from smaller molecules, heat is evolved because the net process involves the formation of new bonds; $\Delta H$ is

33
negative. Such processes are exothermic. at constant temperature, T, and \( \Delta H \) will be equal to \( \Delta G + T \Delta S \). Here, \( \Delta G \) is the change in Gibbs free energy, the portion of \( \Delta H \) that is free to do work, such as electrical work for example, and \( \Delta S \) is the increase in entropy.

\[
\Delta G = \Delta H - T \Delta S
\]  

Equation (9) is a deduction from the second law of thermodynamics. Further deductions show that, when there is no non-mechanical work, \( \Delta G \) is 0 (zero) for isothermic reversible processes (equilibrium processes) and it will be negative for all spontaneous processes. In the usual chemical synthesis of large molecules from smaller ones, \( \Delta S \) is negative because there is an increase in order. However, when an assembly process is endothermic, that is when \( \Delta H \) is positive, \( \Delta G \) is negative only when \( \Delta S \) is positive and \( T \Delta S \) exceeds \( \Delta H \) in magnitude. Such processes are defined as entropy driven reactions.

When reactants and products are at unit activity, equation (9) takes the standard form \( \Delta G^0 = \Delta H^0 - T \Delta S^0 \). The superscript is critically important for G and S. However, because \( H \) per mole of reactant varies only slightly with concentration for most biologically important macromolecules, the superscript for \( H \) can be omitted without
significant conceptual error in the present context. When the expression for $\Delta G^0$, given by equation (10), which was driven from thermodynamic considerations is substituted into equation (9), equation (11) is obtained. Here, $K_a$ is the equilibrium constant defined for unit activity.

$$\Delta G^0 = -R.T\ln(K_a)$$  \hspace{1cm} (10)

$$\ln(K_a) = \Delta S^0 / R - \Delta H^0 / R.T$$  \hspace{1cm} (11)

When $\Delta H^0$ is positive, the process is entropy driven because only $\Delta S^0$ is positive and $\Delta G^0$ will be negative. $\Delta G^0$ must be negative for the reaction to proceed.

**Biochemical consequence of molecular crowding and thermodynamic activity:**

Laurent (1963) demonstrated that the addition of an inert water soluble polymer to a protein solution resulted in a marked reduction of protein solubility. These observations were explained due to alteration in the thermodynamic activity of protein, which causes the exclusion of protein from that fraction of solution volume occupied by polymer. Excluded volume effect on protein solubility (Juckes, 1971; Atha and Ingham, 1981), protein self association (Nichol et. al, 1981), and the catalytic activity for number of enzymes (Laurent, 1971) is well established.

The consequences of molecular crowding and its effect in solutions, containing a
total protein concentration comparable to that of found in living cell, beside occupying the volume by other protein molecules, can have energetic consequences which are comparable in magnitude to those of commonly invoked inter- and intramolecular electrostatic and hydrophobic interactions (Ross and Minton, 1977; Minton, 1981). The induced changes, due to crowding, can cause marked changes in tertiary and quaternary structure of the proteins, their state of aggregation, their solubility, and the kinetics of reactions involving proteins as reactants and/or catalysts (Minton, 1977; 1981; Noguchi and Schechter, 1981; Ferrone et al, 1980 and Minton and Wilf, 1981). In order to understand the properties of proteins in cell it is necessary to take into account the possibility of excluded volume effects due to volume occupancy introduced by the molecular crowding. Some of the relevant thermodynamic concepts necessary to describe this excluded volume effects and their link to the problem of Origin of Life, are reviewed in the next section.

Thermodynamic preliminaries

Chemical equilibria and chemical potential

A solution of volume V containing \( n_1 \) moles of solute species \( X_1 \), \( n_2 \) moles of \( X_2 \), and so forth, can be represented as solution of composition \( \{c\} = c_1, c_2, c_3, ... c_n \) where, \( c_i \) is the molar concentration \( (n_i/V) \) of component \( X_i \). Such an ensemble can be considered to be representative of a typical cell. The various solute species may react with each other
according to the following generalised scheme:

\[ x_1 X_1 + x_2 X_2 + \ldots + x_j X_j \rightleftharpoons x_{j+1} X_{j+1} + \ldots + x_m X_m \]  \hspace{1cm} (12)

All species on the left hand side of the reaction (12) represents reactants, and all species on the right hand side of the reaction (12) represents products. If \( R \) denotes as one unit of reaction progress to the right than it will signify that the amount of reaction in which \( x_i \) (for \( i = 1 \) to \( j \)) moles of each reactant are consumed, and \( x_i \) (for \( i = j+1 \) to \( m \)) moles of each products are produced. Thus the increment of the \( dR \) will be coupled with the following changes of solution composition:

\[
\begin{align*}
\text{dn}_i &= -x_i dR & \text{For } 1 < i < j \text{ (reactants)} \\
\text{dn}_i &= x_i dR & \text{For } j+1 < i < m \text{ (products)}
\end{align*}
\]  \hspace{1cm} (13)

At constant temperature and pressure, the dependence of the Gibbs free energy of the solution on reaction progress can be represented as:

\[
(dG/dR)_{T,P,\{c\}} = \sum x_i \cdot \mu_i (T, P, \{c\}) - \sum x_i \cdot \mu_i (T, P, \{c\})
\]  \hspace{1cm} (14)

where, \( \mu_i = (dG/ \text{dn}_i)_{T,P,\{c\}} \) is defined as chemical potential of the species \( i \) and \( n_i \) is number of moles of species \( i \). The chemical reactions are driven by the natural tendency
of any system to seek the state of lowest free energy consistent with kinetic, environmental, and compositional constraints, it follows that at constant temperature and pressure, reaction (12) will spontaneously proceed to the right if \((dG/dR)_{T,P,c} \) is negative, and will spontaneously proceed to left if \((dG/dR)_{T,P,c} \) is positive. When the free energy reaches a minimum, \((dG/dR)_{T,P,c} \) will be zero; no further macroscopic

\[
\begin{align*}
\text{IDEAL SOLUTION} & \quad | \quad \text{REAL SOLUTION} \\
A & \xrightarrow{\Delta G^A_{\text{I-R}}} A \\
+ & \quad + \\
B & \xrightarrow{\Delta G^B_{\text{I-R}}} B \\
\Delta G^o & \quad | \quad \Delta G^* \\
\downarrow & \quad \downarrow \\
AB & \xrightarrow{\Delta G^\text{AB}_{\text{I-R}}} AB
\end{align*}
\]

Figure 7

changes in solution composition will be observed and the reaction will reach equilibrium (Minton, 1983).

The equilibrium state of the system thus, can be seen to determine the chemical potentials of reactants and products. In order to understand the effect of molecular
crowding on solution equilibria, it is first necessary to understand that it can influence the
current potential of macromolecular solutes. The energetics and entropic contributions
to the chemical potential of solutes significantly affect the interactions between solute
molecules. The conditions prevailing inside the cell are thermodynamically non-ideal.
Changes in free energy when solution composition changes from ideal to non-ideal (real
solution) are depicted in figure 7.

**Real and ideal solution: solute-solute interactions and deviation from ideal behaviour**

The Gibbs free energy of the solution, \( G \), is defined equal to \( H - T.S \), where \( H \) and
\( S \) are respectively the enthalpy and entropy of the solution. It follows that
\[
\mu_i = h_i - T.s_i
\]  
where \( h_i = (dH/dn_i)_{T,P,(c)} \) and \( s_i = (dS/dn_i)_{T,P,(c)} \), where, \( n \) is number of moles of
species \( i \). The ideal solution provides a conceptual reference state; the deviation between
the observed behaviour of a real solution and the predicted behaviour of an ideal solution
(that is so called non-ideal behaviour) is thus a direct measure of solute-solute interaction.
By analogy to equation (15), for ideal solution
\[
\mu_i^I = h_i^I - T.s_i^I
\]  
where the superscript \( I \) denotes a property of the ideal solution.

As a real solution becomes progressively more dilute, and the average distance between
solute molecules increases, the influence of solute-solute interactions upon solution
behaviour decreases, and the behaviour of the real solution approaches that of the ideal solution. The chemical potential ($\mu$) of solutes can be determined. From careful measurements of these properties, in highly dilute solution, it has been established that

$$\mu_i^I = \lim_{c_i \to 0} \mu_i = \mu_i^0 (T,P) + R.T \ln(c_i)$$  \hspace{1cm} (17)$$

$\mu_i^0$, called the standard state chemical potential, is independent of solute concentration at constant temperature and pressure (Moore, 1962). It may be seen to be equal to the chemical potential of solute species $i$ in an ideal solution of unit concentration. At finite

<table>
<thead>
<tr>
<th>Type of interaction between newly added $X_i$ and other molecules</th>
<th>$h_i - h_i^I$</th>
<th>$s_i - s_i^I$</th>
<th>$\mu_i^{NI}$ (equation 15)</th>
<th>$\gamma_i$ (equation 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No interactions (ideal)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Attractive (electrostatic, hydrophobic)</td>
<td>&lt;0</td>
<td>&lt;0</td>
<td>&lt;</td>
<td>&lt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>=0</td>
<td>=1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;</td>
<td>&gt;</td>
</tr>
<tr>
<td>Repulsive (Electrostatic)</td>
<td>&gt;0</td>
<td>&lt;0</td>
<td>&gt;0</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Volume exclusion</td>
<td>0</td>
<td>&lt;0</td>
<td>&gt;0</td>
<td>&gt;1</td>
</tr>
</tbody>
</table>

$h$ is enthalpy, $s$ is entropy, $\mu$ is chemical potential and $\gamma$ is activity

Table 6: Effect of various solute-solute interactions upon thermodynamic parameters characterizing non-ideal behavior (Minton, 1983)

concentrations the chemical potential of solute species $i$ in a real solution deviates from that in an ideal solution by an amount which depends upon the interaction between a molecule of $X_i$ and other solute molecules. The difference between the real and ideal
chemical potentials can be written as

$$\mu^\text{Ni}(T,P,\{c\}) = \mu_i(T,P,\{c\}) - \mu_i^1(T,P,\{c\})$$  \hspace{1cm} (18)

Combining equations (17) and (18), we obtain

$$\mu_i = \mu_i^0 + R.T \ln(\gamma_i c_i)$$  \hspace{1cm} (19)

where,

$$\gamma_i \equiv \exp(\frac{\mu_i}{R.T})$$

$\gamma_i$ is called the activity coefficient of $X_i$. The product of $\gamma_i$ and $c_i$ is denoted by $a_i$ and defined as thermodynamic activity, or simply activity of $X_i$.

Addition of an infinitesimal amount of $X_i$ ($dn_i$ moles) to a solution of composition $\{c\}$ at constant $T$ and $P$. The interactions which may exist between the newly added molecules of $X_i$ and other solute molecules already present (including other molecules of $X_i$) will result in differential changes in the enthalpy (energy) and entropy of the solution; equation (15), (16), and (19) may be combined to yield an expression for the activity coefficient of $X_i$ as a function of these changes:

$$R.T \ln(\gamma_i) = \mu_i^\text{Ni} = (h_i - h_i^1) - T(s_i - s_i^1)$$  \hspace{1cm} (20)

The changes in the type of interaction between molecules under different thermodynamic conditions are shown in table 6. To understand the changes in the thermodynamic parameters on adding a molecules of $X_i$ and to ascertain weather they are attracted to, repelled by, or excluded from the volume of other solute molecules will also depend on the degree of correlation between the positions of these molecules. The entropy ($s_i$) will
consequently be less than $s_i^\dagger$. The enthalpy $h_i$, on the other hand, may be greater than, equal to, or less than zero depending upon the type of interaction between newly added molecules of $X_i$ and other solute molecules. The resulting effects upon $\mu_i^{NI}$ and $\gamma_i$ is summarised in table 5. It may be noted that while an activity coefficient greater than one may result from any type of interaction, an activity coefficient of less than one necessarily implies a predominantly attractive interaction between newly added molecules (Minton, 1992).

Equilibria

It follows from equation (14) that the equilibrium condition for reaction (12) is

$$\sum_{products} x_i \mu_i = \sum_{reactants} x_i \mu_i$$  \hspace{1cm} (21)

Combining equations (19) and (21), we obtain

$$\Delta G^0(T,P) = \sum_{products} x_i \mu_i^0 - \sum_{reactants} x_i \mu_i^0$$  \hspace{1cm} (22)

$$= -RT\ln(K_c) + R.T\ln(\Gamma)$$

in above equation
\[ K_c = \frac{\Pi_{\text{products}} c_i^{x_i}}{\Pi_{\text{reactants}} c_i^{x_i}} \]

\( K_c \) is apparent equilibrium constant and \( \Delta G^0 \) the standard state free energy change. It is independent of the solution composition at constant temperature and pressure. By taking the exponential of the left and right side of the equation (22), and rearranging, we obtain a relation between apparent equilibrium constant and non ideal contribution.

\[ K_c = K_o \Gamma \] (23)

The ideal equilibrium constant is given as \( K_o \equiv \exp \left( -\frac{\Delta G^0}{R \cdot T} \right) \). It is important to note that \( K_o \) represents the limiting value of \( K_c \) as the concentrations of all solutes approach zero, i.e., as \( y_i \rightarrow 1 \) for all \( i \). \( \Gamma \), and hence \( K_c \), may be dependent upon solution composition.

The physical significance of the quantities appearing in equations (22) and (23) may be appreciated more readily with the assistance of a simple example. Formation of a hetero-dimer of two protein subunits can be represented as:

\[ A + B \rightleftharpoons AB \] (24)

The standard state free energy change for this reaction, \( \Delta G^0 \), is defined to be the free energy change which occurs when 1 mole of \( A \) (at unit concentration in an ideal solution)
and 1 mole of B (at unit concentration in an ideal solution) are converted to 1 mole of
AB (at unit concentration in an ideal solution). We can define a second free energy
change, $\Delta G^*$, to be that change in free energy which occurs when 1 mole of A
(at unit concentration in a real solution) and 1 mole of B (at unit concentration in a real
solution) are converted to 1 mole of AB (at unit concentration in a real solution). Finally,$
\Delta G^*_{I-R}$ is defined (where $x = A$, $B$, or AB) to be the free energy change accompanying
the transfer of one mole of $X$ at unit concentration from ideal to real solution. These free
energy changes are related by the thermodynamic cycle as shown in figure 7. It is clear
from this figure that

$$\Delta G^* = -\Delta G^0_{I-R} - \Delta G^0_{I-R} - \Delta G^0 - \Delta G^0_{I-R}$$

(25)

From equations (16) and (17) it follows that

$$\Delta G^*_{I-R} = R \cdot T \cdot \ln (\gamma_x) \quad (c_x = 1)$$

(26)

For convenience, units of concentration are chosen to be proportional to number density
( for example, g/l ), such that self-interaction in the real solution is negligible at unit
concentration. Under these conditions $\Delta G^*_{I-R}$ will be independent of the concentration
of $x$ and will depend only upon the interaction between $x$ and other solutes in the real
solution, and equation (26) reduces to

$$G^*_{I-R} = R \cdot T \cdot \ln (\gamma_x)$$

(27)

Combining equations (25) and (27), we obtain

$$\Delta G^* = \Delta G^0 + R \cdot T \cdot \ln (\gamma_{AB} / \gamma_A \cdot \gamma_B)$$

(28)
\[ \Delta G^* = \Delta G^0 + R.T. \ln \left( \frac{\gamma_{AB}}{\gamma_A \cdot \gamma_B} \right) \]  

which is equivalent to equation (24), with

\[ K_o = \exp \left( -\frac{\Delta G^0}{R.T} \right) \]

\[ K_c = \exp \left( -\frac{\Delta G^*}{R.T} \right), \]

and \[ \Gamma = \frac{\gamma_{AB}}{\gamma_A \cdot \gamma_B} \]

From this analytical analysis it can be seen that \( \Gamma \) is a measure of the difference between the total free energy of interaction between reactants and other solutes in the solution, and the total free energy of interaction between products and other solutes in the solution. The interactions can undergo a drastic shift with molecular crowding as \( \Gamma \) becomes more dominant term. This shows that if the crowding is comparable with that found in cell, the increase in \( K_c \) will follow. These implications can be seen by using transition state theory of reactions shown in figure 8 (Puri and Sharma, 1973).

**Steady-state reaction rates : transition state theory**

If we consider the elementary biomolecular reaction

\[ A + B \rightarrow \text{products} \]  

(29)

where rate constant is defined as

\[ k \equiv \frac{\text{rate}}{c_A \cdot c_B} \]  

(30)

According to the transition-state theory of reaction rates (25), reaction (21) may be
Fig. 8: Concept of energy barrier in chemical reactions
represented as

\[ A + B \rightleftharpoons (AB)^* \rightarrow \text{products} \] (31)

where the species \((AB)^*\) corresponds to a free energy barrier which must be crossed in order for product to be formed. The rate of this reaction is equal to the rate at which \((AB)^*\) dissociates to product, which is proportional to the steady-state concentration of \((AB)^*\).

\[ \text{(rate)} = k_{\text{diss}} c_{(AB)^*} \] (32)

According to transition-state theory, the steady-state value of \(c_{(AB)^*}\) may be calculated on the assumption that a pseudo-equilibrium exists between A, B, and \((AB)^*\)

\[ c_{(AB)^*} = K_c c_A c_B \] (33)

Combining equations (32), (33), and (23), we obtain

\[ \text{(rate)} = k_{\text{diss}} K_0 \Gamma c_A c_B \] (34)

where

\[ \Gamma = \gamma_A \gamma_B / \gamma_{(AB)^*} \]

Substituting equation (34) into equation (30), we obtain

\[ k = k_0 \Gamma^* \] (35)
The significance of the entropy driven reactions, induced through the molecular crowding, is apparent from above discussion. The concept of entropy acquires more significance in relation to the reaction choices where volume exclusion, due to molecular crowding, becomes a common feature. The reactions where $\Delta H$, the change in enthalpy, is zero can still be driven by a large change in entropy. In other words, the increase in entropy can drive the reaction which otherwise would have come to equilibrium. Figure 9 and table 7 summarize the envisaged effects of molecular crowding.

**Entropy**

In general, the definition of life inherits processes of organization through time (Prigogine and Stengers, 1984). There are two distinct aspects associated with life; i) entropic behavior is exhibited by the biological evolution and ii) there exist essential tenets of the life which are the cause of entropic behavior. Biological processes at functional level internalize irreversible changes over time. These changes entail uptake $+d_\text{s}S$ where $d_\text{s}S > 0$. Here $dS$ represents the change in entropy of the system, $d_\text{s}S$ is the entropy change owed to the energy fluxes from the boundary conditions (cell wall or membrane). And $d_\text{i}S$ refers to the change in entropy caused due to the irreversible processes within the system. The concept of $d_\text{i}S$ is important as this entropy concerns with the history of the system of the life form. $d_\text{i}S$ is always positive which results in the historical buildup of entropy (retained as new structural biomolecules and information
molecules) within the boundary of the life form, this aspect essentially is responsible for and the use of energy from the surroundings and transformation of matter from one structural state to the other. This can be seen in terms of the increase in organization and complexity of the cell. In thermodynamic terminology the life represents the manifestation of an non-equilibrium systems where entropy production is: $dS = d_eS$ the systems irreversibility (Brooks and Wiley, 1986). The entropy production associated with structural formation is retained within the system and becomes the part of the boundary conditions of the past state of the system and also becomes the part of the initial
### Table 7: Observed effect of molecular crowding upon biochemical equilibria; applicability of hard-particle models

<table>
<thead>
<tr>
<th>Protein solubility</th>
<th>1. Effect of Dextran</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. Reduction of protein solubility on addition of dextran, addition of glucose (dextran monomer) did not effect protein solubility.</td>
</tr>
<tr>
<td></td>
<td>B. Solubility of tryptophan was only marginally decreased (10%)</td>
</tr>
<tr>
<td></td>
<td>C. The relative decrease in solubility accompanying addition of a given amount of polymer was independent of the absolute solubility depending upon pH and ionic strength of the solution (Laurent, 1963)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. Effect of polyethylene glycol (PEG)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. TEG substantially decreases the solubility of serum albumin</td>
</tr>
<tr>
<td></td>
<td>B. PEG increases solubility of amino acids</td>
</tr>
<tr>
<td></td>
<td>C. At high PEG concentration, the probability of simultaneous interaction between a protein molecule and n molecules of PEG</td>
</tr>
<tr>
<td></td>
<td>D. The presence of an attractive interaction between PEG and some proteins serves to counteract the effect of volume exclusion to a variable extent (Minton, 1983)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. Effect of other proteins</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. The addition of ‘non-gelling’ proteins to solutions of deoxygenated sickle-shaped hemoglobin (HbS) enhances the gelling tendency of HbS (Singer and Singer, 1953)</td>
</tr>
</tbody>
</table>

| Thermodynamic activity of cytoplasm       | 1. Effect of PEG                                                                            |
| of Escherichia coli                        | The activity coefficient for cytoplasmic condition changed with addition of PEG, when applied to a two-phase distribution assay (Zimmerman and Trach, 1991) |

| Blunt-end ligation by DNA ligases from     | 1. Effect of PEG                                                                            |
| rat liver or E.coli                        | 1000 fold increase in blunt-end ligation of T4DNA ligase in high PEG6000 concentration     |
|                                            | Similar effect is observed with Ficoll 70, protein and bovine plasma albumin (Zimmerman and Pfeifer, 1983). |
|                                            | 2. Ficoll 70                                                                               |

| Self-and hetero-association of protein     | 1. Effect of added protein                                                                  |
|                                            | When unrelated ‘inert’ proteins are added to the Fluorescence labeled hemoglobin (ANS-Hb), the polarization increases slightly, in a manner similar to that of comparable concentration of PEG. The polarization is more sharp in case of myoglobin (Wilf and Minton, 1981) |

conditions of the subsequent forms to evolve. Since entropy production is the
manifestation of the system's history, this results in part of the history being incorporated in its causal makeup. In other words, the present initial conditions could well reflect the historical boundary conditions. The entropy, since, can be expressed in terms of information contents there are two types of information manifested by the living cell: a) canalized information and b) non-canalized information (Gatlin, 1972 and Waddington, 1977). Canalized information relates to the information associated with events the organism goes through during its development and any genetically(prior) based traits manifested. The non-canalized information is derived from structural gene loci. This information is used to produce structural products (proteins, enzymes etc.). The subtle differences in the information are listed below:

1) Canalized stored information relates to the expressed regulatory information and it refers to the ontogenetic and genetically determined attributes an organism displays during its lifetime, also defined as epiphenotype (Wiley and Brooks, 1982).

2) Canalized potential information attributes to unexpressed regulatory information and pathways not expressed by the organism as they are suppressed by the active information pathways.

3) Non-canalized stored information is the expressed structural information which includes homozygous alleles, heterozygous co-dominant allele coding for the structural gene products.

4) Non-canalized potential information is defined as unexpressed structural information
and includes recessive alleles in the heterozygous condition coding for structural gene products

**Molecular crowding as an essential pervasive common trait in all life forms**

All the information sets will be evolved only if basic fundamental environment also evolve as a common theme, in which evolution of information can perpetuate as a whole. It is this aspect where molecular crowding emerges as a basic and essential boundary condition without which none of the above mentioned information processes can find expression. Crowding must have become a necessary part of environment within which the pre-biotic reactions could develop and evolve. The entropic advantage for these reactions is enough to drive them over the energy barrier, in a way simulating like an no-specific enzyme (figure 8).

The scientific basis and rationale to consider the molecular crowding as central factor during pre-biotic evolution comes from the fact that any known cell, prokaryotic to eukaryotic, performs cellular functions in highly crowded interior, which is thermodynamically non-ideal and has significant bearing on its chemical kinetics of reactions occurring under such conditions (Fulton, 1982; Minton, 1983; Gershon, 1985; Zimmermann and Minton, 1993; Attri and Jain, 1994). The molecules and the environment of cell carry with them the footprints of evolutionary history (Oparin, 1957).

So far the developed origin of life theories have not been able to explain the
information growth of information bearing molecules (DNA or RNA). This aspect has already been highlighted in chapter 1 of this thesis. The growth in information has been circumvented by assuming the presence of primitive enzyme structures, which is evident in the prebiotic evolution of information known as theory of hypercycle (Eigen and Schuster 1977, 1978 and 1986). The Origin of Information is yet a more fundamental problem which still persists for all chemical evolutionary scenarios. Even if it could be shown that the building blocks of essential molecules could arise in realistic prebiotic conditions, the problem of assembling those building blocks into functioning proteins, RNA/DNA chains would remain. This problem of explaining the specific sequencing and thus, the information, within biopolymers, lies at the heart of the current crisis in materialistic evolutionary thinking (Meyer, 1998). The solution to these crisis can be sought by adopting a simplistic paradigm, which takes into account the essential features of conditions within which life survives. Molecular crowding and ensued thermodynamic consequences stands out one such feature which could provide the scientific solution to bridge the information crisis faced by information bearing molecules.

Outline of The Theory

The theory developed in this work has taken those features from other theories as the starting point till the question of information growth was encountered by the molecules growing in prebiotic soup. The sequence of earlier supporting work, involved
in developing this theory, is shown in figure 10. The availability of basic building blocks of biomolecules, required to make proteins and nucleic acids, are essential and this is assumed on the basis of prior work done by various groups. In brief, following conditions are assumed:

a) Availability of amino-acids and nucleotide bases, small peptides in prebiotic
soup (Lohramann and Orgel, 1973).

b) RNA is assumed to be main information carrying biomolecule during chemical evolution (Fox and Dose, 1977).

c) Availability of small RNA molecules (upto 50 base pair long) can arise without the presence of enzyme (Eigen et. al., 1982).

d) No enzyme was present.

e) Presence of activated bases of RNA.

f) Presence of molecular crowding due to amino acids and small poly peptides

Given these five assumptions the thermodynamic consequences on the growth of the RNA is calculated in chapter 4. These calculations are done by using Scaled Particle Theory of Reiss, Frisch and Lebowitz(1959) extended further by Gibbons (1969;1970) to the mixture of hard convex particles and subsequently extended for mixtures of proteins by Minton (1981). The outline of the Scaled Particle Theory is given in chapter 3.