Chapter 2

Overview on the stochastic description of some small chemical systems

Chemical kinetics is generally described by the conventional deterministic rate equations where the concentrations of the reacting species are calculated as a function of time\cite{8, 9}. However, the description of the kinetics by this approach needs serious modification to accommodate the fluctuation of the concentrations of the reactants of a small system containing a finite number of molecules\cite{8, 9, 14, 15, 71, 72, 73}. In a small system, fluctuation carries the information about the structure and non-equilibrium dynamics\cite{58} and sometimes it can overwhelmly affect the main course of the reaction\cite{8, 59}. Stochastic approach provides an appropriate description of the inherent statistical nature as well as the fluctuating dynamics of the reaction system\cite{8, 9, 13, 74}. An extreme limit of such consideration is the temporal fluctuation of catalytic rate of an enzyme observed in single-molecule fluorescence experiments\cite{60, 61} which can not be described without consideration of probabilistic approach\cite{63}.

Incorporation of the stochastic ideas into chemical kinetics\cite{59, 71, 75, 76, 77} was first successfully carried out by Kramers\cite{75, 76}, who treated a chemical reaction as a Brownian motion of particles, whose rate of passage over a potential barrier represents the rate of decomposition. After the work of Kramers, Delbruck\cite{71} and Renyi\cite{77} studied a stochastic model of the auto-catalytic reaction, $A \rightarrow B$ and showed that during the initial stages of reaction, the fluctuations in the number of reactant molecules was of the order of the square root of the number of reactant molecules. Singer discussed the application of a stochastic method to the study of irreproducible reactions such as the oxidation of formic acid by potassium nitrate and the slow or explosive decomposition of some solids and some initial stages of polymerization\cite{59}. He concluded that in small system, large fluctuations in the number of reactant species could be responsible for irreproducible reactions to-
gether with the presence of impurities. In 1957, Bartholomay first explained the uni-molecular reaction, $A \rightarrow B$ by considering the Markovian approach\cite{73} and then Ishida extended the Bartholomay’s method to a more general stochastic process, having a time dependent rate constant\cite{72}. However, McQuarrie championed the stochastic approaches\cite{8, 9, 10}, popularly known as the chemical master equation, to describe several first and second order reactions and also considered the effect of initial conditions on the expectation value and variance of product formation.

In the master equation, time and the reactant-populations appear as independent variables and it measures the probability of finding various molecular populations at each instant of time\cite{15}. Gillespie gave a rigorous derivation of the chemical master equation for a chemically reacting gas-phase system which is kept well stirred and in thermal equilibrium\cite{78}. Later it was shown that the stochastic simulation algorithm\cite{14, 15} and the chemical master equation are equivalent\cite{78, 79}. It is shown that this simulation algorithm can be applicable as realizations of jump Markov processes in arbitrary non-equilibrium situations\cite{74}. For a closed system, reactions occur at equilibrium condition where the change in Gibbs free energy and the change of total entropy production becomes zero\cite{66}. However, a chemical system can be driven out of equilibrium by some continuous in-letting of reactants and out-letting of products i.e., the system acts as a flow reactor\cite{66, 64, 74}. In this situation, the open chemically reacting system goes to the non-equilibrium steady state(NESS) indicated by the non-zero value of total entropy production rate\cite{66, 74}. Due to the constancy of the concentration of the reactants and products, the reactant and product molecules behave like chemiostats and the differences of chemical potentials between the chemiostats generate the fluxes of matter across the system which mainly drive the system out of equilibrium\cite{13}.

Recent studies of non-equilibrium thermodynamics reveal that if a small chemical system remains in far from equilibrium, the thermodynamic quantities like entropy of the system becomes a fluctuating quantity and it follows some exact relations, called the fluctuation theorems \cite{13, 80, 81, 82}. The non-equilibrium thermodynamic behavior of small chemical systems can be estimated by the use of such fluctuation theorems\cite{13, 81, 82}. These theorems also provide the insight regarding how the macroscopic irreversibility appears in the system from the time-reversible microscopic dynamics\cite{83, 84, 85}. In the past 15 years, several fluctuation theorems(FT) are proposed \cite{83, 84, 85, 86, 87, 88} which changes our understanding about the global properties of a small system that are not amenable from conventional thermodynamics.

Lay out of this chapter is as follows. In section 2.1 we have first discussed on the McQuarrie’s description of the kinetics of small chemically reacting system. In section 2.2, the birth-death master equations are introduced by considering some chemical and biological processes. In section 2.3, we have briefly explained the
single enzyme kinetics and single molecule diffusion in heterogeneous phases. Then we have discussed the cooperativity in oligomeric enzyme in section 2.4 and voltage-gated ion channel kinetics is introduced in section 2.5. In section 2.6 we have given a microscopic description of a small chemical system and constructed the chemical master equation from the basic probability theory. We have also briefly reported on the Gillespie's stochastic simulation algorithm in this section. Next in section 2.7, we have revised the non-equilibrium thermodynamics of small systems. We have thoroughly discussed the Crooks fluctuation theorem and then described the integral and detailed fluctuation theorems from it. We have extended this discussion to describe the non-equilibrium thermodynamics of small chemical systems.

2.1 Kinetics of small system: McQuarrie's description

Stochastic models for various types of first order and second order reaction kinetics was first systematically described by McQuarrie for the small chemically reacting systems containing a finite number of molecules[8, 9]. In these stochastic kinetic models, the number of reacting species is considered as a integer-valued random variable and the state of the system is described in terms of the population of reactant molecules at a particular instant of time. The time evolution of the system is described by a differential difference equation which is constructed on the basis of some phenomenological assumptions. From the solution of the master equation, the probability density function of the random variable is obtained from which various moments may be calculated. The first moment gives the mean of the probability density function which can also be obtained from the deterministic dynamics. However, the second central moment i.e, variance is a measure of the statistical fluctuations about the mean which is unobtainable from the conventional deterministic approach. For clear understanding about how a stochastic model is constructed for the chemical reactions, here we have considered three different uni-molecular reactions: (I) A $\xrightarrow{k_1} B$, (II) A $\xrightarrow{k_2} B$, (III) A $\xrightarrow{k_3} B$, A $\xrightarrow{k_4} C$.

To describe the uni-molecular reaction A $\xrightarrow{k_1} B$, it is considered that at time t, the number of A molecules present in the system is n(t) = n. The stochastic model of this reaction is described by considering the following assumptions[8]: (I) The probability of a transition, n→(n + 1) in the interval (t, t + Δt) is $k_1 nΔt + o(Δt)$, where $k_1$ is the rate constant and $o(Δt)/Δt \rightarrow 0$ as $Δt \rightarrow 0$. (II) The probability of a transition, n→(n - j), j > 1, in the interval (t, t + Δt) is $o(Δt)$ and the reverse reaction occurs with probability zero. Using the following assumptions one can write the differential-difference equation as

$$P_n(t + Δt) = k_1 (n + 1)ΔtP_{n+1}(t) + (1 - k_1 nΔt)P_n(t) + o(Δt), \quad (2.1)$$

Then
where $P_n(t)$ is the probability of having $n$ number of $A$ molecules at time $t$. By the standard procedure of expanding the probability $P_n(t + \Delta t)$, dividing by $\Delta t$ and then taking the limit $\Delta t \to 0$, the master equation can be written as

$$\frac{dP_n(t)}{dt} = k_1(n + 1)P_{n+1}(t) - k_1nP_n(t). \quad (2.2)$$

Using the generating function $G(s,t) = \sum_{n=0}^{\infty} P_n(t)s^n$, the Eq.(2.2) becomes

$$\frac{\partial G(s,t)}{\partial t} = k_1(1 - s)(\partial G(s,t)/\partial s), \quad (2.3)$$

and considering the initial condition $G(s,0) = s^0$, one obtains

$$G(s,t) = [1 + (s - 1)e^{-k_1t}]^n. \quad (2.4)$$

Mean, $\langle n(t) \rangle$ and variance, $\langle n^2(t) \rangle - \langle n(t) \rangle^2$ of $n(t)$ can be calculated as

$$\langle n(t) \rangle = \frac{(dG/ds)_{s=1}}{\partial G/\partial s}_{s=1}$$

and

$$\langle n^2(t) \rangle - \langle n(t) \rangle^2 = \frac{(d^2G/ds^2)_{s=1}}{\partial G/\partial s}_{s=1} + (\partial G/\partial s)_{s=1} - (\partial G/\partial s)^2 \quad (2.5)$$

Using the relations described in Eq.(2.5), one can calculate the mean and variance of the corresponding reaction as

$$\langle n(t) \rangle = n_0e^{-k_1t}$$

and

$$\langle n^2(t) \rangle - \langle n(t) \rangle^2 = n_0e^{-k_1t}(1 - e^{-k_1t}), \quad (2.6)$$

respectively. From the above expression (Eq.(2.6)), it is observed that the mean value of the stochastic representation is consistent with the deterministic result. However, this is true only for the uni-molecular reactions.

For the second reaction, $A \xleftrightarrow[k_i]{k_{i-1}} B$ which is a reversible first order reaction, consider again $n(t)$ be the number of $A$ molecules present at the reacting system at time $t$ and $k_1$ and $k_{i-1}$ are the forward and backward rate constants, respectively. The corresponding master equation for this reaction can be written as

$$\frac{dP_n(t)}{dt} = k_{i-1}(n_T - n + 1)P_{n-1}(t) + k_1(n + 1)P_{n+1}(t) - [k_1n + k_{i-1}(n_T - n)]P_n(t), \quad (2.7)$$

where $n_T$ is the total number of $A$ and $B$ molecules. Using the generating function, $G(s,t)$, Eq.(2.7) becomes

$$\frac{\partial G(s,t)}{\partial t} = [k_1 + (k_{i-1} - k_1)s - k_1s^2] (\partial G(s,t)/\partial s) + n_Tk_{i-1}(s - 1)G(s,t). \quad (2.8)$$
Solving the differential equation in Eq.(2.8) and using the relations described in 
Eq.(2.5), one can obtain

\[ \langle n(t) \rangle = \frac{n_T \left( k_i e^{-Kt} + k_{-1} \right)}{(k_1 + k_{-1})} \]

and

\[ \langle n^2(t) \rangle - \langle n(t) \rangle^2 = \left[ n_T \gamma / (1 + \lambda) \right] \left( 1 - \gamma / (1 + \lambda) \right), \quad \text{(2.9)} \]

where \( \lambda = k_i / k_{-1} \) and \( \gamma = (k_0 e^{-Kt} + 1) \) with \( K = (k_1 + k_{-1}). \)

For the third reaction, \( A \overset{k_1}{\rightarrow} B, A \overset{k_2}{\rightarrow} C \), which is mainly the parallel first-order 
reactions and it can be described by considering the two dimensional stochastic 
process. If the number of A and B molecules at time \( t \) be \( n_1(t) \) and \( n_2(t) \), respectively, 
then the master equation can be written as[8]

\[ \frac{\partial P(n_1, n_2)(t)}{\partial t} = k_1(n_1 + 1)P(n_1+1, n_2-1)(t) + k_2(n_1 + 1)P(n_1+1, n_2)(t) \]

\[ + (1 - k_1n_1 - k_2n_2)P(n_1, n_2)(t). \quad \text{(2.10)} \]

Now using the generating function, \( G(s, r, t) = \sum_{n_1=0}^{\infty} \sum_{n_2=0}^{\infty} s^{n_1} r^{n_2} P(n_1, n_2)(t) \), we 
obtain

\[ \frac{\partial G(s, r, t)}{\partial t} = (k_1 r + k_2 - Ks) \left( \frac{\partial G(s, r, t)}{\partial s} \right), \quad \text{(2.11)} \]

where \( K = k_1 + k_2 \). Considering the initial condition \( s^2 r^2 \), one obtains \( F(s, r, t) \) 
from the above equation (Eq.(2.11))

\[ F(s, r, t) = \left( \frac{k_1 r + k_2 - (k_1 r + k_2 - Ks)e^{-Kt}}{K} \right) s^2 \quad r^2. \quad \text{(2.12)} \]

Using \( F(s, r, t) \) one can obtain the value of mean and variance of \( n_1(t) \) and \( n_2(t) \) as

\[ \langle n_1(t) \rangle = n_1^0 e^{-Kt}, \]

\[ \langle n_1^2(t) \rangle - \langle n_1(t) \rangle^2 = n_1^0 e^{-Kt}(1 - e^{-Kt}), \]

\[ \langle n_2(t) \rangle = n_2^0 + (k_1 n_1^0 / K)(1 - e^{-Kt}), \]

and

\[ \langle n_2^2(t) \rangle - \langle n_2(t) \rangle^2 = (k_1 n_1^0 / K)(1 - e^{-Kt})(1 - [k_1(1 - e^{-Kt})/K]). \quad \text{(2.13)} \]

Here \( n_1^0 \) and \( n_2^0 \) are the initial values of A and B, respectively. The stochastic models 
for second order reactions cannot be solved exactly in many cases. For this reason, 
some approximate methods are used to determine the first and second moments 
without solving the probability generating function.
To describe such approximate method one can consider a second order reaction \[9\],
\[2A \rightarrow B\], whose probability rate equation is
\[
\frac{dP_n(t)}{dt} = \frac{1}{2} k(n + 2)(n + 1)P_{n+2}(t) - \frac{1}{2} k(n - 1)P_n(t),
\tag{2.14}
\]
where \(P_n(t) = \text{Prob}\{n(t) = n, n = 0, 2, 4, \ldots, n_0\}\). Multiplication of Eq.(2.14) by \(n\) and \(n^2\), respectively, and summing over \(n\), gives
\[
\frac{d\langle n \rangle}{dt} = -k\langle n^2 \rangle + k\langle n \rangle,
\tag{2.15}
\]
and
\[
\frac{d\langle n^2 \rangle}{dt} = -2k\langle n^3 \rangle + 4k\langle n^2 \rangle - 2k\langle n \rangle.
\tag{2.16}
\]
Now taking the assumption that the higher moments as a product of the lower moments, e.g., \(\langle n^3 \rangle = \langle n^2 \rangle\langle n \rangle\) and \(\langle n^2 \rangle = \langle n \rangle^2\) we get
\[
\langle n \rangle = \frac{n_0}{n_0 + (1 - n_0)\exp[-kt]},
\tag{2.17}
\]
where \(n_0\) is the initial population of A molecules. The assumption, \(\langle n^2 \rangle = \langle n \rangle^2\) is equivalent to reducing the stochastic model to deterministic model. Using \(\langle n^3 \rangle = \langle n^2 \rangle\langle n \rangle\) and the value of \(\langle n \rangle\) in Eq.(2.17), one obtains
\[
\langle n^3 \rangle = \langle n \rangle^2 \left[ \frac{2}{3} \left\{ \frac{(n_0 - 1)/n_0} \right\} (e^{3kt} - e^{-kt}) + 1 \right].
\tag{2.18}
\]
Therefore, the variance, \(\langle n^2 \rangle - \langle n \rangle^2\) can be calculated as
\[
\langle n^2 \rangle - \langle n \rangle^2 = \left[ \frac{2}{3} \left\{ \frac{(n_0 - 1)/n_0} \right\} (e^{2kt} - e^{-kt}) \right].
\tag{2.19}
\]

### 2.2 Birth-death master equation

Here we have described the birth-death processes by stochastic master equation. The name birth-death comes from the modeling of the dynamics of animal or human populations in which individuals are born, or die. The birth-death processes are generally described by the master equation where it is considered that a finite number of reactant molecules are created (born) or destroyed (die) in a given event\[89\]. If a reacting species, say \(X\) has \(n\) in number at time \(t\), the master equation can be written as
\[
\frac{dP_n(t)}{dt} = w^+(n-1)P_{n-1}(t) + w^-(n+1)P_{n+1}(t) - [w^+(n) + w^-(n)]P_n(t),
\tag{2.20}
\]
where, \(w^+(n)\) and \(w^-(n)\) are the transition probabilities from state, \(n \rightarrow n + 1\) and \(n \rightarrow n - 1\), respectively. The stationary solution of the corresponding master
equation in Eq.(2.20) can be obtained by considering \( \frac{dP_n(t)}{dt} = 0 \). The stationary solution can be written as

\[
P_n^{\text{st}} = P_0^{\text{st}} \prod_{z=1}^{n=0} \frac{w^+(z-1)}{w^-(z)},
\]

(2.21)

where \( P_0^{\text{st}} \) is the steady state probability distribution for \( n = 0 \). To discuss the birth-death process, here we have considered Lotka-Volterra process, Brusselator reaction and the Schlogl model of bistable chemical reaction.

### 2.2.1 Lotka-Volterra reaction:

The Lotka-Volterra model is composed of a set of coupled, auto-catalytic processes which was first observed by Lotka in 1920. Several years later Volterra independently described this process by formulating a mathematical model. The reaction scheme of Lotka-Volterra model is proposed for the dynamical populations of prey and predator species. It can be written as[14]

\[
X + Y_1 \xrightarrow{k_1} 2Y_1,
\]

(2.22)

\[
Y_1 + Y_2 \xrightarrow{k_2} 2Y_2,
\]

(2.23)

\[
Y_2 \xrightarrow{k_3} Z.
\]

(2.24)

The deterministic rate equations are

\[
\frac{dY_1}{dt} = k_1 XY_1 - k_2 Y_1 Y_2,
\]

and

\[
\frac{dY_2}{dt} = k_2 Y_1 Y_2 - k_3 Y_2.
\]

(2.25)

The reaction in Eq.(2.23) describes how a certain predator species \( Y_2 \) reproduces by feeding on a certain prey species \( Y_1 \). However, from Eq.(2.22) it is observed that the prey species \( Y_1 \) reproduces by feeding on a certain foodstuff \( X \), which is assumed here to be depleted insignificant amount. The isomerization in Eq.(2.24) describes the eventual death of predator species, \( Y_2 \) due to some natural causes.

The corresponding master equation of this reaction is

\[
\frac{\partial P(Y_1,Y_2)(t)}{\partial t} = k_1 X(Y_1 - 1)P(Y_1-1,Y_2)(t) + k_2(Y_1 + 1)(Y_2 - 1)P(Y_1+1,Y_2-1)(t)
\]

\[
+ k_3(Y_2 + 1)P(Y_1,Y_2+1)(t) - [k_1 XY_1 + k_2 Y_1 Y_2 + k_3 Y_2] P(Y_1,Y_2)(t)
\]

(2.26)

The solution of the master equation is not possible in analytical technique, however, from simulation we obtain the variation of \( Y_1 \) with \( Y_2 \) which is depicted in Fig.(2.1).
Figure 2.1: The intermediate species, $Y_1$ and $Y_2$ are oscillating in the $Y_1$-$Y_2$ plane. Initial populations of $Y_1$ and $Y_2$ are 1000, 1000, respectively. The value of the rate constants are $k_1 = 10.0$, $k_2 = 0.01$ and $k_3 = 10.0$.

From Fig. (2.1), it is observed that the intermediate species $Y_1$ and $Y_2$ are oscillating in time. However, this oscillation is not stable and area depends on the initial condition.

2.2.2 Brusselator reaction

Brusselator is a type of auto-catalytic, oscillating chemical reaction which was proposed by Prigogine and Lefevre in 1967 at Brussels. The kinetic scheme of this reaction is[14]

$$X_1 \xrightarrow{k_1} Y_1, \quad (2.27)$$
$$X_2 + Y_1 \xrightarrow{k_2} Y_2 + Z_1, \quad (2.28)$$
$$2Y_1 + Y_2 \xrightarrow{k_3} 3Y_1, \quad (2.29)$$
$$Y_1 \xrightarrow{k_4} Z_2. \quad (2.30)$$

In this reaction, the population of the reactants, $X_1$ and $X_2$ are assumed to be depleted insignificant amount. The intermediate substances $Y_1$ and $Y_2$ are connected with one another via a trimolecular reaction (2.29), which ensures the existence of an oscillatory regime. The deterministic rate equations for this reaction can be written
Figure 2.2: $Y_1$ and $Y_2$ oscillate around a well defined, closed, stable path in the $Y_1Y_2$ plane. Initial population of $Y_1$ and $Y_2$ are 1000, 2000, respectively. The value of the rate constants are $k_1X_1 = 5000.0$, $k_2X_2 = 50.0$, $k_3 = 0.00005$ and $k_4 = 5.0$

as

$$\frac{dY_1}{dt} = k_1X_1 - k_2X_2Y_1 + (k_3/2)Y_1^2Y_2 - k_4Y_1,$$

and

$$\frac{dY_2}{dt} = k_2X_2Y_1 - (k_3/2)Y_1^2Y_2. \tag{2.31}$$

The solution of the master equation in Eq.(2.32) is not possible analytically, however, the oscillation of the intermediate substances, $Y_1$ and $Y_2$ can be obtained from simulation which is depicted in Fig.(2.2). From Fig.(2.2), it is observed that the system will eventually wind up orbiting around a well defined, closed, stable path in the $Y_1Y_2$ plane which does not depend on the initial state of the system. So this reaction is considered as an example of the 'limit cycle' chemical oscillator.
2.2.3 Schlogl model of bistable chemical reaction

Schlogl model is an example of a chemical reaction system that exhibits bistability. The scheme of the reaction is

\[ A + 2X \xrightarrow{k_1} 3X, \quad \frac{k_2}{k_1} \]

\[ B \xrightarrow{k_3} X, \quad \frac{k_4}{k_3} \]

where the concentration of the reactant A and B are held constant. The deterministic rate equation of this reaction is

\[ \frac{dX}{dt} = k_1AX^2 - k_2X^3 + k_3B - k_4X, \]

which is a first order, nonlinear ordinary differential equation. The stochastic description of this reaction can be given by constructing the master equation for this reaction and that can be written as

\[ \frac{dP_x(t)}{dt} = k_1A \left( \frac{1}{2}x(x-1)(x-2) \right) P_{(x-1)}(t) + k_2 \left( \frac{1}{3}x(x+1)x(x-1) \right) P_{(x+1)}(t) + k_3BP_{(x-1)}(t) + k_4(x+1)P_{(x+1)}(t) - k_4(x+1)(x+2)P_x(t) + k_3B + k_4x \]

Figure 2.3: dX/dt is plotted as a function of X by taking the rate parameters, \( k_1 = 3.0, k_2 = 0.6, k_3 = 0.25 \) and \( k_4 = 2.95 \). The values of A and B are A = 1 and B = 1, respectively. The plot show the bistability of the system and generated by solving Eq.(2.35).
where \( P_x(t) \) is the probability of having \( x \) number of \( X \) molecules at time \( t \).

We have numerically solved the Eq. (2.35) and plotted \( \frac{dx}{dt} \) as a function of \( X \) in the Fig.(2.3). In this figure \( X_- \) and \( X_+ \) represent two stable steady states and \( X_0 \) is an unstable steady state. The system will always tend towards one of the two stable fixed points, \( X_- \) and \( X_+ \), depending on the initial condition of the reaction. The result of the simulation of master equation is not shown here.

2.3 Study of reaction kinetics at single molecule level

In the previous section, we have given the stochastic description of the reaction systems having finite number of reactant molecules by the master equation. Here we have discussed the reaction kinetics at the single molecule level which can be studied experimentally by single-molecule spectroscopy[60, 61] and single molecule imaging technique[1]. To understand how single molecule and ensemble kinetics are reconciled and what new informations are obtained from single molecule data, a stochastic description of the reaction kinetics is essential as the reactions become stochastic in nature[60, 61, 63]. Here we have discussed mainly the single enzyme kinetics and single molecule detection of translational diffusion.

2.3.1 Single enzyme kinetics: waiting time distribution

The kinetic scheme of a single enzyme molecule can be written as [60, 61, 63]

\[
E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} E^0 + P \quad E^0 \xrightarrow{\delta} E,
\]

(2.37)

where a substrate, \( S \) binds reversibly with a conformation of an enzyme \( E \) to form an enzyme-substrate complex, \( ES \) which undergoes uni-molecular decomposition to form a product \( P \) and \( E^0 \). The intermediate conformation of enzyme, \( E^0 \) immediately goes to the original conformation of enzyme, \( E \). The corresponding probabilistic rate equation can be written as

\[
\frac{dP_E(t)}{dt} = -k_1P_E(t) + k_{-1}P_{ES}(t),
\]

(2.38)

\[
\frac{dP_{ES}(t)}{dt} = k_1P_{E}(t) - (k_{-1} + k_2)P_{ES}(t),
\]

(2.39)

and

\[
\frac{dP_{ES}(t)}{dt} = k_2P_{ES}(t),
\]

(2.40)

where \( k_1 = k'_1[S] \) is the pseudo first-order rate constant. Usually in the single molecule enzymatic experiments, the waiting time distribution, \( f(t) \) is measured for
one turnover and experimentally it is obtained by recording the histogram of many
turnovers[63]. Theoretically \( f(t) \) is calculated as

\[
f(t) = \frac{dP_{ES}(t)}{dt} = k_2P_{ES}(t).
\] (2.41)

From the solution of Eq.(2.38) to Eq.(2.40) and using the above relation for \( f(t) \) in
Eq.(2.41), it can be easily shown that

\[
f(t) = \frac{k_1k_2}{2A} \left[ e^{-(B-A)t} - e^{-(B+A)t} \right],
\] (2.42)

where \( B = (k_1 + k_{-1} + k_3)/2 \) and \( A = \sqrt{(k_1 + k_{-1} + k_2)^2/4 - (k_1k_2)} \). The mean
waiting time for the reaction, \( \langle t \rangle \) can be obtained from the waiting time distribution
as, \( \langle t \rangle = \int_0^\infty t f(t) dt \) and the inverse of \( \langle t \rangle \) gives the traditional Michaelis-Menten
equation

\[
\frac{1}{\langle t \rangle} = k_2[S]/[S] + K_M.
\] (2.43)

with the Michaelis-Menten constant, \( K_M = \frac{k_{-1} + k_2}{k'_1} \). Therefore, the waiting time
distribution, \( f(t) \) on the other hand gives the ensemble average kinetic result and on
the other hand it provides the crucial information on dynamic disorder. In the
presence of dynamic disorder, \( f(t) \) shows a highly stretched multi-exponential decay
at high substrate concentrations and a mono-exponential decay at low substrate
concentrations [63].

Sometimes the single molecule enzyme kinetic reaction is carried out at chemio-
static condition where the substrate and product concentrations are maintained at
a constant value throughout the experiment[64, 65]. Therefore, the reaction scheme
described in Eq.(2.37) can be written as

\[
E + S \rightarrow_{k'_1} ES, \text{ } \rightarrow_{k_{-1}} E + P.
\] (2.44)

The above reaction scheme in Eq.(2.44) can be reduced to

\[
E \underset{\mu}{\xrightarrow{\lambda}} ES,
\] (2.45)

where \( \lambda = (k_1 + k_{-2}) \) and \( \mu = (k_{-1} + k_3) \). The pseudo first order rate constants,
\( k_1 \) and \( k_{-2} \) are \( k_1 = k'_1[S] \) and \( k_{-2} = k'_{-2}[P] \), respectively. The probabilistic rate
equations can be written as

\[
\frac{dP_E(t)}{dt} = -(k_1 + k_{-2})P_E + (k_{-1} + k_2)P_{ES},
\] (2.46)

and

\[
\frac{dP_{ES}(t)}{dt} = (k_1 + k_{-2})P_E - (k_{-1} + k_2)P_{ES}.
\] (2.47)
The steady state probabilities $P_E$ and $P_{SS}$ are obtained from the solution of the rate equations in Eq.(2.46) and Eq.(2.47) as

$$P_E = \frac{\lambda}{(\lambda + \mu)}, \quad P_{SS} = \frac{\mu}{(\lambda + \mu)}. \quad (2.48)$$

The net velocity of the reaction can be calculated as

$$v_{\text{net}} = k_2 P_{SS} - k_{-2} P_E = \frac{(k_2 \lambda) - (k_{-2} \mu)}{\lambda + \mu}. \quad (2.49)$$

For $k_{-2} = 0$ then we obtain the Michaelis-Menten equation described in Eq.(2.43).

### 2.3.2 Tracking of heterogeneous diffusion: Single molecule study

Using single molecule imaging technique[1], one can follow the diffusive motion of individual Brownian particle and map out the probability distribution and correlation as a function of time. In the homogeneous environment, the diffusion of a Brownian particle follows Gaussian distribution. However, in the heterogeneous environments, a Brownian particle travels through distinct diffusion areas of various sizes and geometrical arrangements and the distribution becomes non-Gaussian[62].

![Figure 2.4: A schematic diagram of the two-state diffusive model for heterogeneous diffusion](image)

To describe the heterogeneity developed in the system due to diffusion, we have considered here a model of diffusion of a Brownian particle in two different regions having diffusion constants $D_1$ and $D_2$. The rate from first to second region is $\eta_1$, and from $D_2$ to $D_1$ is $\eta_2$, where the the diffusion rate of the particle is Poissonian. The two state heterogeneous diffusion is described here by the reaction-diffusion equation in Fourier space,

$$\dot{P}_1(t, k) = -((\eta_1 + k^2 D_1)P_1(t, k) + \eta_2 P_2(t, k)), \quad (2.50)$$
\[ \dot{P}_2(t, k) = -\left(\eta_2 + k^2D_2\right)P_2(t, k) + \eta_1P_1(t, k). \]  

(2.51)

Here \( P_i(t, k) \) is the probability of remaining the particle with wave vector, \( k \) at time \( t \) in the region \( i \) where (\( i = 1, 2 \)). Using the Laplace transformation, the above equations (2.50-2.51) can be written as

\[
\begin{pmatrix}
P_1(s, k) \\
P_2(s, k)
\end{pmatrix} = \frac{1}{\text{Det}(s, k)} \begin{pmatrix} s + k^2D_2 + \eta_2 & \eta_1 \\ \eta_1 & s + k^2D_1 + \eta_1 
\end{pmatrix} \times \begin{pmatrix} P_1(0, k) \\
P_2(0, k)
\end{pmatrix},
\]

(2.52)

where the determinant is \( \text{Det}(s, k) = (s + k^2D_1 + \eta_1)(s + k^2D_2 + \eta_2) - \eta_1\eta_2 \). The total probability distribution for the Brownian particle is \( P(s, k) = P_1(s, k) + P_2(s, k) \) with the equilibrium distributions, \( P_1(0) = \eta_2/\eta_1 \) and \( P_2(0) = \eta_1/\eta_2 \).

If \( \eta_1 = \eta_2 = \eta \), the Fourier transform of the probability distribution becomes

\[ P(t, k) = \exp \left[ - (k^2D_0 + \eta) t \right] \times \left[ \cosh(\Delta t) + \frac{\eta}{\Delta} \sinh(\Delta t) \right] P(0, k). \]

(2.53)

Here \( D_0 = (D_1 + D_2)/2 \) and \( \Delta^2 = k^4\xi^2 + \eta^2 \) where \( \xi = (D_1 - D_2)/2 \). The distribution in Eq.(2.53) tends to the usual diffusion equation \( P(t, k) = e^{-k^2D_0t}P(0, k) \) in the limit of \( \xi \to 0 \) or \( \eta \to \infty \). To quantify the distribution, one can measure the time dependent spatial moment, \( I_n(t) = \langle |r(t) - r(0)|^n \rangle \), where \( r(t) \) and \( r(0) \) is the position of the Brownian particle at initial time \( t = 0 \) and at time \( t \).[62, 90]. For a Gaussian distribution the higher order moments, say \( I_4(t) \) is determined by the second moment \( I_2(t) \), as \( I_4(t) = 3I_2^2(t) \). Therefore, the deviation from the Gaussian distribution can be measured as \( J(t) = I_4(t) - 3I_2(t) \). To scale \( J(t) \), the non-Gaussian indicator is defined as[62]

\[ \sigma(t) = \frac{J(t)}{3I_2(t)}. \]

(2.54)

The mean square displacement of a diffusive Brownian particle in the heterogeneous environment obeys the Einstein relation. So the second moment can be written as \( I_2(t) = 2(D)t \), where \( D \) is the effective diffusion constant expressed as an inhomogeneous average. Consequently, in the short time limit \( I_4(t) \) can be expressed as \( 3((2D)t)^2 \), where \( \langle D^2 \rangle \) denotes an inhomogeneous average. The initial value of \( \sigma(t) \) is given explicitly as \( \sigma(0) = \frac{\langle D^2 \rangle}{\langle D \rangle} \), with \( \langle \delta D^2 \rangle = \langle D^2 \rangle - \langle D \rangle^2 \). Thus the non-Gaussian indicator[62] can be normalized as

\[ g(t) = \frac{\sigma(t)}{\sigma(0)} = \frac{\langle D \rangle^2}{\langle D^2 \rangle} \frac{J(t)}{3I_2(t)}. \]

(2.55)

where \( g(t) \) is independent of the diffusion constant. In the two-state Poisson kinetics, \( g(t) \) can be calculated from the probability distribution, \( P(t, k) \) in Eq.(2.53) as

\[ g(t) = \frac{\eta t - e^{-\eta t} \sinh(\eta t)}{(\eta t)^2}. \]

(2.56)
To describe the diffusion process of a single Brownian particle it is essential to measure the square displacement of the particle at two different times which can be calculated in terms of the joint moment function \[ (2.57) \]

\[ I(t_1, \tau, t_2) = \langle |r(t_1) - r(0)|^2 |r(\tau + t_1 + t_2) - r(\tau + t_2)|^2 \rangle, \]

where \( \tau \) is the time separation of the two different measurements at \( t_1 \) and \( t_2 \). From this joint moment function, \( I(t_1, \tau, t_2) \) the memory effect developed in the system can be described. Without the memory effect, the joint moment function becomes, \( I(t_1, \tau, t_2) = I_2(t_1)I_2(t_2) \), with \( I_2(t) = \langle |r(t) - r(0)|^2 \rangle \). Therefore, the memory effect can be quantified by the normalized correlation function of the square displacement [62] as

\[ f(t, \tau) = \frac{I(t_1, \tau, t_2) - [I_2(t)]^2}{I(t, 0, t) - [I_2(t)]^2}. \]

Using the above relation in Eq. (2.58), the normalized correlation function, \( f(t, \tau) \) for the two state Poisson process, becomes

\[ f(t, \tau) = e^{-2\tau}. \]

From the above discussion, one can get an idea about the nature of the diffusion process of a single Brownian particle, i.e., to know whether the diffusion is homogeneous or non-homogeneous. In the non-homogeneous diffusive process a memory effect is developed in the system which can be characterized by measuring the normalized non-Gaussian indicator, \( g(t) \) and the correlation function \( f(t, \tau) \). This memory effect is developed due to diffusion in the inhomogeneous environment with an effective fluctuating diffusion coefficient.

### 2.4 Cooperativity in enzyme kinetics

In enzymology, the cooperativity phenomenon is mainly displayed by the oligomeric enzymes consisting two or more sub-units usually linked to each other by non-covalent interactions. Possibility of interaction between the sub-units during the substrate binding process can give rise to different cooperative phenomena [34, 40, 91, 92]. Positive cooperativity is said to occur when the binding of one substrate molecule with a sub-unit increases the affinity of further attachment of the substrate to another subunit [34, 40, 93]. An example of positive cooperativity is the binding of oxygen to hemoglobin which has a characteristic of sigmoidal shape. From experiment it is observed that when an oxygen molecule binds to a sub-unit of hemoglobin, the oxygen affinity increases, allowing the second molecule to bind more easily, and the third and fourth even more easily i.e., the oxygen affinity of 3-oxym-hemoglobin is 300 times greater than that of deoxy-hemoglobin. In the case of negative cooperativity, attachment of a substrate molecule to one subunit decreases the tendency
of further attachment of the substrate molecules to other subunits. For the non-cooperative case, substrate molecules independently bind to the sub-units of the oligomeric enzyme and the enzyme follows the Michaelis-Menten kinetics.

In 1910, A.V. Hill first described the sigmoidal binding curve of oxygen of hemoglobin by taking the assumption that all ligand molecules would have to bind to the oligomeric protein simultaneously and reach saturation in a single step. According to the Hill’s description, if an oligomeric protein, \( E \) consists of \( n \) number of sub-units, the \( n \) number of ligands simultaneously bind with that oligomeric enzyme and form the complex, \( EL_n \). The corresponding binding scheme can be written as

\[
E + nL \rightleftharpoons EL_n,
\]

where \( K_b \) is the equilibrium binding constant and can be defined as

\[
K_b = \frac{[EL_n]}{[E][L]^n}.
\]

Now taking the logarithm on both sides, we get

\[
\ln K_b + n \ln [L] = \ln \left( \frac{[EL_n]}{[Eo] - [EL_n]} \right) = \ln \left( \frac{Y}{1 - Y} \right),
\]

where, \( Y \) is the fractional saturation of the oligomeric enzyme, i.e, \( Y = \frac{[EL_n]}{[Eo]} \) with \( [Eo] = [E] + [EL_n] \). The above relation described in Eq.(2.61) is known as Hill equation. The plot of \( \ln \left( \frac{Y}{1-Y} \right) \) versus \( \ln [L] \) is a straight line with slope \( n \) and intercept, \( \ln K_b \). Such a graph is called a Hill plot, and its experimentally determined slope is known as the Hill coefficient, generally indicated by the symbol \( h \). Experimentally the Hill coefficient is obtained by determining the fractional saturation, \( Y \) at various ligand concentrations \( [L] \), constructing the Hill plot \( (\ln(\frac{Y}{1-Y}) \text{ vs. } \ln[L]) \) and then finding the slope at the half-saturation point, \( Y = 0.5 \) or at a point where the slope deviates maximum from unity. For positive and negative cooperative cases, the Hill coefficient, \( h \) becomes greater than or less than one, respectively, whereas the non-cooperative case is characterized with Hill coefficient equal to one.

G.S. Adair first developed an equation to describe the sigmoidal binding curve of the oxygen of hemoglobin by considering all individual binding steps[36]. The general binding scheme of Adair can be written as

\[
E \xrightleftharpoons[k_4(0)]{k_4(1)} ES_1 \xrightleftharpoons[k_5(2)]{k_5(1)} ES_2 \xrightleftharpoons[k_6(n-1)]{k_6(n)} ... \xrightleftharpoons[k_7(j+1)]{k_7(j)} ES_j \xrightleftharpoons[k_8(n-2)]{k_8(n-1)} ... \xrightleftharpoons[k_9(n-1)]{k_9(n)} ES_{n-1} \xrightarrow[k_{10}(0)]{k_{10}(1)} ES_n.
\]

Here \( ES_j \) represents the conformational state of the oligomeric enzyme in which \( j \) number of subunits are occupied by the ligands. \( k_4(0) \) and \( k_4(1) \) are designated as the formation and dissociation rate constants in the \( j \)-th reaction step, respectively.
The fractional saturation, $Y$ is simply expressed as

$$Y = \frac{\sum_{n=0}^{\nu_T} n \begin{pmatrix} n_T \\ n \end{pmatrix} \prod_{i=0}^{n-1} K^{(i)}_n}{\sum_{n=0}^{\nu_T} n \begin{pmatrix} n_T \\ n \end{pmatrix} \prod_{i=0}^{n-1} K^{(i)}_n},$$

(2.63)

where $K^{(i)} = k_1^{(i)}/k_2^{(i+1)}$, is the equilibrium constant for the $j$-th binding step.

By the Adair scheme, positive, negative and no cooperativity phenomenon can be described very nicely. If the step-wise equilibrium binding constant increases, positive cooperativity is observed, whereas, for the reverse case, an oligomeric protein shows the negative cooperativity. If the step-wise binding constants remain the same then no cooperativity phenomenon is developed. These types of cooperativity based on the affinity of the substrate binding belong to the class of allosteric cooperativity which is mainly described by the concerted or sequential models. The concerted model of allostery was proposed by Monod, Wyman and Changeux which is sometimes referred to as the symmetrical model or MWC model[37]. In this model each sub-unit or active site can exist in two conformational states, T and R states, where the R-state predominates in the protein-ligand complexes. The model is based on the assumption that in a particular protein molecule, all of the sub-units must remain either in the R or T conformational state. The two conformational states of the protein are in equilibrium in the absence of ligand but the equilibrium is disturbed when a ligand binds to a sub-unit of the protein. The sequential model was proposed by Koshland, Nemethy and Filmer(KNF) [38] which dictates that the ligands bind via an induced fit protocol. The basic assumptions of this model are: (a) the two conformational states T and R are available to each subunit, (b) only the subunit to which the ligand is bound changes its conformation and (c) the ligand-induced conformational change in one subunit alters its interactions with neighboring subunits[38, 91]. These two allosteric models are constructed by considering the equilibrium condition, however, in living cell most of the reactions occur in non-equilibrium condition which is developed due to the imbalanced chemical reactions and the presence of chemiostats. So to describe the non-equilibrium cooperativity phenomenon, it becomes essential to modify these two equilibrium allosteric models. In chapter five, we have thoroughly discussed the non-equilibrium cooperativity.

2.5 Voltage-gated ion channel kinetics:

In neuro-physiology, voltage-gated ion channels, mainly sodium and potassium channels play an important role in generation and propagation of action potential (nerve impulse) by controlling the voltage gradient across the plasma membrane of nerve
cells, by allowing the flow of ions down their electrochemical gradient\cite{45, 46}. Hodgkin and Huxley made the first intracellular recording of an action potential and developed a voltage-clamp circuit to measure the ionic currents from squid axon. However, their most remarkable achievement was the proposition of a mathematical model to explain the ionic mechanisms underlying the initiation and propagation of action potentials in the squid giant axon \cite{47}. The Hodgkin-Huxley theory of the action potential is still considered as the most significant conceptual breakthrough in neuroscience and they received the Nobel Prize in Physiology or Medicine in 1963.

In this model the lipid bilayer is considered as a capacitor ($C_m$) and the current can be carried through the membrane either by charging the membrane capacity or by movement of ions through the ion channel\cite{47}. Therefore, the total membrane current, $I_{\text{m}}$ can be written as the summation of capacity current, $I_C$ and ionic current, $I_i$ as

$$I_{\text{m}} = I_C + I_i. \quad (2.64)$$

Here $I_C = C_m \frac{dV}{dt}$ where $C_m$ is the specific membrane capacitance per unit area and $V$ is the membrane potential. The ionic current is divided into components carried by sodium and potassium ions ($I_{\text{Na}}$) and ($I_{\text{K}}$), and a small 'leakage current', ($I_l$) made up by chloride and other ions. Therefore, the ionic current is calculated as

$$I_i = I_{\text{Na}} + I_{\text{K}} + I_l. \quad (2.65)$$

The individual ionic currents are calculated according to the relations

$$I_{\text{Na}} = g_{\text{Na}}(V - E_{\text{Na}}),$$

$$I_{\text{K}} = g_{\text{K}}(V - E_{\text{K}}),$$

and

$$I_l = g_l(V - E_l), \quad (2.66)$$

where $E_{\text{Na}}$, $E_{\text{K}}$ and $E_l$ are the sodium, potassium and leakage reversal potential, respectively\cite{47, 53}. The sodium and potassium ion conductance are calculated as

$$g_{\text{Na}} = g_{\text{Na}} N_{\text{Na}} n^3 h^m$$

and

$$g_{\text{K}} = g_{\text{K}} N_{\text{K}} n^4, \quad (2.67)$$

where $g_{\text{Na}}$ and $g_{\text{K}}$ are the sodium and potassium conductance per channel. $N_{\text{Na}}$ and $N_{\text{K}}$ are the sodium and potassium channel density per unit area. $n$, $h$ and $m$ are the gate parameters which satisfy the equation as \cite{47},

$$\frac{dn}{dt} = \alpha_n(1 - n) - \beta_n n, \quad (2.68)$$
\[
\frac{dm}{dt} = \alpha_m(1 - m) - \beta_m m, \quad (2.69)
\]
and
\[
\frac{dh}{dt} = \alpha_h(1 - h) - \beta_h h. \quad (2.70)
\]
Here \(\alpha_j\) and \(\beta_j\) with \(J = n, h, m\) are gate opening and closing rates with dimension time\(^{-1}\) and depend on the membrane potential, \(V\). Solving the Eq.(2.68), Eq.(2.69) and Eq.(2.70) we obtain
\[
\begin{align*}
\text{Eq.}(2.68) & \Rightarrow n = n_{\infty} - (n_{\infty} - n_0)\exp(-t/\tau_n), \quad (2.71) \\
\text{Eq.}(2.69) & \Rightarrow m = m_{\infty} - (m_{\infty} - m_0)\exp(-t/\tau_m), \quad (2.72) \\
\text{Eq.}(2.70) & \Rightarrow h = h_{\infty} - (h_{\infty} - h_0)\exp(-t/\tau_h), \quad (2.73)
\end{align*}
\]
where \(J_0\) and \(J_{\infty}\) are the initial and final values of \(j\) where \(j = n, m, h\). The values \(J_0\) and \(J_{\infty}\) are determined as \(J_0 = \alpha_0/(\alpha_0 + \beta_0)\) and \(J_{\infty} = \alpha_1/(\alpha_1 + \beta_1)\) and \(\tau_j = 1/(\alpha_j + \beta_j)\), where \(J = n, h, m\). Here \(\tau_j\) is the characteristic relaxation time. The time-dependence of potassium and sodium conductance can be calculated as\[47\]
\[
g_K = (g_{K_{\infty}})^{1/4} - [(g_{K_{\infty}})^{1/4} - (g_{K_0})^{1/4}]\exp(-t/\tau_n)^4, \quad (2.74)
\]
and
\[
g_{Na} = g_{Na0}[1 - \exp(-t/\tau_m)]^3\exp(-t/\tau_h), \quad (2.75)
\]
where \(g_{Na0} = g_{Na_{\infty}}m_{Na_{\infty}}h_{Na_{\infty}}\). \(g_{K_{\infty}}\) and \(g_{K_{\infty}}\) are the values of initial and final potassium conductance. The time-dependent values of \(g_K\) and \(g_{Na}\) obtained from Eq.(2.74) and Eq.(2.75) exactly match with the conductance values obtained from the Voltage-clamp experiment. However, the conductance \(g_i\) is considered as constant.

The propagation of action potential can be described by substituting the value of membrane current for unit length, \(I_{MC} = \frac{a}{2r} \frac{\partial V}{\partial x}\) in Eq.(2.64), where \(a\) is the radius of the nerve fibre, \(r\) is the specific resistance of the axoplasam, \(x\) is distance along the fibre and \(V\) is the membrane potential\[47\]. Thus one can write
\[
\frac{a}{2r} \frac{\partial^2 V}{\partial x^2} = C_m \frac{dV}{dt} + g_{Na}(V - E_{Na}) + g_K(V - E_K) + g_i(V - E_i). \quad (2.76)
\]
Eq.(2.76) is a partial differential equation, and it is not practicable to solve it as it stands. During steady propagation, the curve of \(V\) against time at any one position is similar in shape to that of \(V\) against distance at any one time, and it follows that
\[
\frac{\partial^2 V}{\partial x^2} = \frac{1}{\theta^2} \frac{\partial^2 V}{\partial \theta^2},
\]
where \(\theta\) is the velocity of conduction. Therefore, Eq.(2.76) can be written as
\[
\frac{a}{2r\theta^2} \frac{\partial^2 V}{\partial \theta^2} = C_m \frac{dV}{dt} + g_{Na}(V - E_{Na}) + g_K(V - E_K) + g_i(V - E_i). \quad (2.77)
\]
This is an ordinary differential equation and can be solved numerically. This equation describes how the action potential wave propagates with time.

In 1976 Neher and Sakmann developed the patch clamp technique by which they have measured the ionic current through an individual ion channel[5]. This advancement has revolutionized both experimental and theoretical approaches and for this work they have received the Nobel Prize in Physiology or Medicine in 1991. From the study of single ion channel, it becomes clear that individual ion channels are essentially stochastic entities that open and close in a random fashion. From the single ion channel experiments, it is now possible to study the kinetics of individual ion channels and determine accurately the model parameters of a channel. MacKinnon and colleagues have determined the three-dimensional molecular structure of a potassium ion channel by utilizing the X-ray crystallography[94] and explained the exact mechanism of the selectivity of potassium ion channel. For this work he won the Nobel prize in chemistry in 2003. In chapter six, we have thoroughly discussed the kinetics as well as thermodynamics of a single potassium ion channel.

2.6 Microscopic description of small chemical systems: Chemical master equation

For the microscopic description, a chemical system is considered having m different types of chemical species \( \{ S_1, S_2, \ldots, S_m \} \), which interact through M chemical reactions \( \{ R_1, R_2, \ldots, R_M \} \). Each reaction, \( R_\mu (\mu = 1, 2, \ldots, M) \) describes a distinct physical event which happens instantaneously[14, 15, 78, 79]. The system is confined to a constant volume \( \Omega \) and is in thermal (but not in chemical) equilibrium at some constant temperature \( T \). The number of molecules of species \( S_i \) in the system at time \( t \) is \( n_i(t) \) and the corresponding molecular populations state vector is \( \vec{n}(t) = (n_1(t), n_2(t), \ldots, n_m(t)) \), given that the system was in state \( \vec{n}(t_0) \) at some initial time \( t_0 \).

The change in the population state vector is induced by a single occurrence of a particular reaction, \( R_\mu \). Mathematically the reaction, \( R_\mu \) is characterized by two quantities. The first is the state change vector, \( \nu_\mu = (v_\mu, \ldots, v_\mu) \) where \( v_\mu \) is the change in the \( S_i \) molecular population due to occurrence of the \( R_\mu \) reaction. So if the system is in state, \( \vec{n}(t) = n \) at time \( t \) and one \( R_\mu \) reaction occurs, the system immediately jumps to state \( n + \nu_\mu \). The other quantity is the propensity function, \( a_\mu \), where[14, 15, 78, 79] \( a_\mu(n)dt = c_\mu h_\mu(n)dt \) is the probability that a particular reaction \( R_\mu \) will occur in \((t, t + dt)\) in \( \Omega \) given that the system is in state \( n \) at time \( t \). Here \( c_\mu \) is the specific probability rate constant of the reaction \( R_\mu \) and \( h_\mu(n) \) indicates the number of distinct \( R_\mu \) reactant combinations available in the state \( n \). Thus, if \( R_\mu \) has the form \( S_1 + S_2 \rightarrow \text{products} \), then we will have \( h_\mu(n) = n_1(t)n_2(t) \) and if \( R_\mu \) is \( 2S_1 \rightarrow \text{products} \), the form of \( h_\mu(n) \) will be \( h_\mu(n) = \frac{1}{2} n_1(t)(n_1(t) - 1) \). The
specific probability rate constant, $c_\mu$ is related with the traditional deterministic rate constant, $k_\mu$ as $c_\mu = k_\mu/\Omega^{j-1}$, where $j$ indicates the number of reactant molecules participating in the $\mu$-th reaction. So for the reaction $2S_1 \rightarrow \text{products}$, the propensity function $a_\mu(n) = \frac{1}{2}[c_\mu n_1(t)(n_1(t) - 1)] = \frac{1}{2}[k_\mu n_1(t)(n_1(t) - 1)/\Omega]$. Usually the propensity function is independent of time, however, if the specific probability rate constant, $c_\mu$ is time-dependent then the propensity function also becomes a function of time.

### 2.6.1 Chemical master equation

In the master equation [11], time and the reactant-populations appear as independent variables and the function which satisfies the equation measures the probability of finding various molecular populations at each instant of time [15]. The chemical master equation can be constructed from the microscopic point of view as follows [15]. Let at time $t$ system remains in the state $n = (n_1(t), n_2(t), ..., n_m(t))$ and the corresponding probability of remaining in the state is $P_n(t)$. We want to find out the probability, $P_n(t+dt)$ i.e., the probability of remaining the system in the state $n$ at time $t+dt$. During this time interval $dt$, three events can occur which are independent and mutually exclusive to each others [78]. The first event is that one $R_\mu (\mu = 1, 2, ..., M)$ reaction occurs in the time interval $dt$ and due to occurrence of the first event, the system goes from the state $n$ to $n$ state at time $t$ to $t + dt$ [78]. The second event is that no reaction occurs during this time interval and if no reaction occurs in $dt$, system remains in the state $n$ from time $t$ to $t + dt$ [78]. The last event can be about more than one reaction occur in this time interval [78]. First calculate the probability of occurring the first event, $P(I)$. By applying the laws of probability to the fundamental premise [78], it can be easily calculated as [14, 15, 78],

$$P(I) = c_\mu h_\mu(n)dt + o(dt), \quad (2.78)$$

where $o(dt)$ is the higher order terms. Similarly the probability of occurring the second reaction event, $P(II)$ can be constructed as [14, 15, 78]

$$P(II) = 1 - \sum_{\mu=1}^M c_\mu h_\mu(n)dt + o(dt). \quad (2.79)$$

If $dt$ is too small, then one can neglect the third event. Summing all the probabilities one obtains

$$P_n(t+dt) = P_n(t) \times \left(1 - \sum_{\mu=1}^M c_\mu h_\mu(n)dt + o(dt)\right)$$

$$+ \sum_{\mu=1}^M P_{n-\nu_\mu}(t) \times [c_\mu h_\mu(n)dt + o(dt)]. \quad (2.80)$$
Taking the limit, $dt \to 0$ and neglect the higher order terms $o(dt)$, one finally obtains
the master equation as\cite{78}

$$\frac{\partial P_n(t)}{\partial t} = \sum_{\mu=1}^{M} \left[ c_\mu h_\mu(n - \nu_\mu)P_{(n-\nu_\mu)}(t) - c_\mu h_\mu(n)P_n(t) \right]. \quad (2.81)$$

The form of this master equation is familiar as Gillespie’s chemical master equation which is constructed in terms of the propensity function by considering the individual reaction event. Usually master equation is formulated in terms of the transition probabilities which is equivalent to the propensity function. In terms of the transition probability, the Gillespie’s chemical master equation (Eq.2.81) can be written as

$$\frac{\partial P_n(t)}{\partial t} = \sum_{\mu=1}^{M} \left[ w_\mu(n - \nu_\mu)P_{(n-\nu_\mu)}(t) - w_\mu(n|n + \nu_\mu)P_n(t) \right], \quad (2.82)$$

where $w_\mu(n|n - \nu_\mu)$ is the transition probability from the population state, $n$ to $(n - \nu_\mu)$. For $M$ reversible reactions the Gillespie’s chemical master equation can be written as\cite{13}

$$\frac{\partial P_n(t)}{\partial t} = \sum_{\mu=1}^{\pm M} \left[ w_\mu(n - \nu_\mu)P_{(n-\nu_\mu)}(t) - w_\mu(n|n - \nu_\mu)P_n(t) \right]. \quad (2.83)$$

Solution of the master equation gives information about the time evolution of a chemically reacting system. However, as this equation is a set of coupled ordinary differential equations, the analytical solution is possible only for some simple cases. For complex systems when the non-chemical or non-linear rate processes are involved in the master equation, the analytical as well as the direct numerical solution becomes quite difficult. Gillespie’s stochastic simulation technique gives the numerical realizations about how a chemically reacting system evolves stochastically with time.

2.6.2 Stochastic simulation of the chemical reactions

Here we have briefly discussed the stochastic simulation which was first developed by Gillespie to simulate the time evolution of the chemically reacting system\cite{14,15}. This is mainly a Monte-Carlo simulation where the trajectory of $n(t)$ is generated with time following the probability function $P(\tau, \mu)$. The quantity $P(\tau, \mu)d\tau$ is defined as the probability that, given the state $n(t) = n$ at time $t$, the next reaction in $\Omega$ will occur in the infinitesimal time interval $(t + \tau, t + \tau + d\tau)$, and will be an $R_\mu$ reaction\cite{14,15,78,79}. The function is also called in the name of ‘reaction probability density function’ because in mathematical terminology it is a joint probability density function in the space of the continuous variable $\tau(0 \leq \tau < \infty)$ and the discrete variable $\mu(\mu = 1, 2, ..., M)$\cite{14,15} and the exact formula of $P(\tau, \mu)$ is

$$P(\tau, \mu) = a_\mu(n)\exp[-a_{\omega\mu}(n)\tau], \quad (2.84)$$
where \( a_{\text{tot}} = \sum_{n=1}^{M} a_{n}(n) \).

From this probability density function one can get the informations about when the next reaction will occur and what kind of reaction it will be. The next reaction time, \( \tau \) gives the first information whereas the index of the next reaction, \( \mu \) gives the second. To obtain the explicit expression of the random variable \( \tau \), the joint probability density function, \( P(\tau, \mu) \) can be written as [14, 15]

\[
P(\tau, \mu) = P_{1}(\tau)P_{2}(\mu | \tau),
\]

where \( P_{1}(\tau) \) indicates the probability of occurring any one reaction during the time interval \( d\tau \) and the mathematical expression of \( P_{1}(\tau) \) is,

\[
P_{1}(\tau) = a_{\text{tot}}\exp(-a_{\text{tot}}\tau).
\]

Another probability, \( P_{2}(\mu | \tau) \) is designated as the probability of occurring the \( \mu \)-th reaction, \( R_{\mu} \) during this time interval, \( d\tau \) and \( P_{2}(\mu | \tau) \) can be written as

\[
P_{2}(\mu | \tau) = a_{\mu}/a_{\text{tot}}.
\]

Using the inverse generating function method [14, 113] we obtain the next reaction time \( \tau \) from \( P_{1}(\tau) \) as

\[
\tau = \left( \frac{1}{a_{\text{tot}}} \right) \ln \left( \frac{1}{r_{1}} \right),
\]

where \( r_{1} \) is the the uniform random number. The condition of what reaction will occur during the time interval \( d\tau \) can be calculated from the probability \( P_{2}(\mu | \tau) \) and the condition can be written as [14, 15]

\[
\sum_{\nu=1}^{\mu-1} a_{\nu} < r_{2}a_{\text{tot}} \leq \sum_{\nu=1}^{\mu} a_{\nu},
\]

where \( r_{2} \) is another uniform random number. In the simulation algorithm the successive values of \( a_{1}, a_{2}, \ldots \) are cumulatively added until their sum is observed to equal or exceed \( r_{2}a_{\text{tot}} \), whereupon \( \mu \) is then set equal to the index of the last \( a_{\nu} \), term added.

### 2.7 Non-equilibrium thermodynamic description of small systems

In the previous sections we have described the kinetics of small reacting systems, however, for complete understanding of these reacting systems, here we have provided a non-equilibrium thermodynamic description. In a closed system, reactions occur at equilibrium condition where the change of Gibbs free energy, \( \Delta G \) and the change of total entropy production, \( \Delta S_{\text{tot}} \) becomes zero [66]. However, an open chemical system goes arbitrarily far from equilibrium due to the presence of the chemostats. For an example, the living cells behave like open system where the biochemical reactions occur in non-equilibrium condition.
2.7.1 Non-equilibrium thermodynamics: Fluctuation theorem

Before going to describe the non-equilibrium thermodynamic behavior of small chemical systems, here we have provided a brief discussion of the fluctuation theorems which describe the thermodynamics of finite, even small systems that are arbitrarily far away from equilibrium. These theorems also explain how macroscopic irreversibility appears in the system from the time-reversible microscopic dynamics. In the past 15 years, several fluctuation theorems (FT) are proposed [83, 84, 85, 86, 87, 88] which extend our understanding over the conventional thermodynamics. However, here we have provided a discussion on the Crooks FT.

Crooks Fluctuation Theorem (FT):

The Crooks FT provides a method of predicting equilibrium free-energy differences from non-equilibrium paths that connect two equilibrium states [83, 95, 96]. The fluctuation theorem is constructed by considering the two assumptions, (I) the system evolves with time by following the Markovian dynamics and (II) each single time step is microscopically reversible. To discuss the FT of Crooks, we consider a system which is in thermal equilibrium [83, 96] with a bath at temperature, $T$. The internal state of the system, $x_t$ at time $t$ depends on the externally controlled parameter, $\lambda_t$ which determines the energy of the state, $E(x_t, \lambda_t)$. By moving the parameter $\lambda_t$ through a fixed sequence $\{\lambda_0, \lambda_1, ..., \lambda_r\}$, the system evolves with time sequence $\{t_0, t_1, ..., t_r\}$ which generates a trajectory, $\chi(t)$ in the phase phase as

$$\chi(t) \equiv \left( x_0 \xrightarrow{\lambda_1} x_1 \xrightarrow{\lambda_2} x_2 \xrightarrow{\lambda_3} \ldots \xrightarrow{\lambda_r} x_r \right)$$

(2.90)

where the initial state $x_0$ is an equilibrium state at time $t = 0$ and the corresponding value of the controlled parameter at this time is $\lambda_0$.

In this fluctuation theorem it is assumed that after reaching the state $x_r$, the system quickly relaxes to equilibrium. So the final state $x_r$ can also be considered as an equilibrium state. As the system is a canonical ensemble, the equilibrium probability of the states $x_0$ and $x_r$ can be written as

$$P(x_l, \lambda_l) = \frac{e^{-\beta E(x_l, \lambda_l)}}{\sum_l e^{-\beta E(x_l, \lambda_l)}} = \exp \left[ -\beta \left( \lambda_l \right) - \beta E(x_l, \lambda_l) \right]$$

(2.91)

where $l = 0, \tau$ and $F(\beta, \lambda_l) = -\beta^{-1} \ln \sum_l e^{-\beta E(x_l, \lambda_l)}$ is the Helmholtz free energy of the system. Here $\beta = \frac{1}{k_B T}$, with $\beta$ is the Boltzmann constant and $T$ is the absolute value of temperature. To describe the time evolution of the system it is considered that until the value of $\lambda_l$ is changed the system remains in the same state. Therefore, for changing the parameter value from $\lambda_l$ to $\lambda_{l+1}$, system performs work, $E(x_l, \lambda_{l+1}) - E(x_l, \lambda_l)$. At constant $\lambda_{l+1}$, when the system goes from $x_l$ state to
x_{j+1}, the system exchanges a quantity $E(x_{j+1}, \lambda_{j+1}) - E(x_j, \lambda_{j+1})$ of heat with the surroundings. This evolution of the system through phase space is repeated for $\tau$ time steps. Therefore, the total work performed on the system, $W$, the total heat exchanged with the reservoir, $Q$, and the total change in energy, $\Delta E$ are given by[83]

$$W = \sum_{t=0}^{\tau-1} [E(x_t, \lambda_{t+1}) - E(x_t, \lambda_t)],$$

(2.92)

$$Q = \sum_{t=1}^{\tau} [E(x_t, \lambda_t) - E(x_{t-1}, \lambda_t)],$$

(2.93)

and

$$\Delta E = Q + W = [E(x_\tau, \lambda_\tau) - E(x_0, \lambda_0)].$$

(2.94)

The free energy difference between two equilibrium ensembles, $\Delta F = F(\beta, \lambda_\tau) - F(\beta, \lambda_0)$ is equal to the reversible work, $W_{rev}$ and the dissipative work, $W_d$ is the difference between the actual work and the reversible work i.e.,

$$W_d = W - W_{rev} = W - \Delta F.$$  

(2.95)

Here the actual work and the dissipative work both depend on the path followed through phase space but the reversible work depends only on the initial and final ensembles.

Similar to the forward trajectory, a backward trajectory[83, 96], $\chi(t)$ can be generated by considering the reverse direction of time and that can be written as

$$\chi(t) = (x_0, x_1, x_2, \ldots, x_T).$$

(2.96)

It is important to note that the forward trajectory begins with a change in $\lambda$, whereas the reverse trajectory is started with a change in the internal state of the system[83, 96]. In the reverse time direction, the thermodynamic quantities like heat, work, change in energy and change in free energy would be negative that of the forward time value. Now if we consider that the evolution of the system is Markovian, the probability of the forward trajectory, $P[\chi(t)|x_0]$ can be written as

$$P[\chi(t)|x_0] = P\left(x_0 \xrightarrow{\lambda_1} x_1\right) P\left(x_1 \xrightarrow{\lambda_2} x_2\right) \ldots P\left(x_{\tau-1} \xrightarrow{\lambda_\tau} x_\tau\right),$$

(2.97)

where $P\left(x_{j-1} \xrightarrow{\lambda_j} x_j\right)$ is the probability of transition from $x_{j-1}$ to $x_j$. If each single time step is microscopically reversible then the individual steps obey the detailed balance and we can write [83, 96]

$$\frac{P\left(x_{j-1} \xrightarrow{\lambda_j} x_j\right)}{P\left(x_j \xrightarrow{\lambda_{j-1}} x_{j-1}\right)} = \frac{P(x_j, \lambda_j)}{P(x_{j-1}, \lambda_{j-1})} = \frac{e^{-\beta E(x_j, \lambda_j)}}{e^{-\beta E(x_{j-1}, \lambda_{j-1})}}.$$  

(2.98)
Similarly the probability of backward trajectory, \( \tilde{P}[\tilde{x}(t)|\tilde{x}_0] \) can be written as
\[
\tilde{P}[\tilde{x}(t)|\tilde{x}_0] = P \left( x_0 \xrightarrow{t} x_1 \right) P \left( x_1 \xrightarrow{t} x_2 \right) \ldots P \left( x_{t-1} \xrightarrow{t} x_t \right),
\]
(2.99)
where \( \tilde{x}_t = x_{t-1} \) and the tilde symbol implies time reverse path. \( x_0 \) and \( \tilde{x}_0 \) are the initial points of forward and backward processes. Now using Eq.(2.97), Eq.(2.99) and Eq.(2.98), the ratio of the probability of the forward trajectory and the backward trajectory can be written as
\[
\frac{P[\chi(t)|\chi_0]}{\tilde{P}[\tilde{\chi}(t)|\tilde{\chi}_0]} = \frac{P \left( x_0 \xrightarrow{t} x_1 \right) P \left( x_1 \xrightarrow{t} x_2 \right) \ldots P \left( x_{t-1} \xrightarrow{t} x_t \right)}{P \left( x_0 \xrightarrow{t} x_1 \right) P \left( x_1 \xrightarrow{t} x_2 \right) \ldots P \left( x_{t-1} \xrightarrow{t} x_t \right)}
= e^{-\beta(E(s_1,\lambda_1)+E(s_2,\lambda_2)+\ldots+\beta(E(s_{t-1},\lambda_{t-1}))} = e^{-\beta Q},
\]
(2.100)
where \( Q \) is the heat exchange with the heat bath and \( -\beta Q \) is the corresponding change in entropy of the bath in units of Boltzmann's constant. The relation described in Eq.(2.100) is known as the Crooks fluctuation theorem.

If we consider the initial equilibrium distribution of the forward and backward trajectories, we obtain[83]
\[
P[\chi(t)|\chi_0]P(\chi_0) = e^{-\beta Q} e^{\beta(\Delta E - \Delta F)} = e^{\beta W},
\]
(2.101)
as \( W = \Delta E - Q \).

Now suppose we find other trajectories, \( \chi'(t), \chi''(t), \chi'''(t), \ldots \) along which the same amount of work \( W \) has been done. Then Eq.(2.101) tells us
\[
\frac{P[\chi(t)|\chi_0]}{\tilde{P}[\tilde{\chi}(t)|\tilde{\chi}_0]} = \frac{P[\chi'(t)|\chi'_0]}{\tilde{P}[\tilde{\chi}'(t)|\tilde{\chi}'_0]} = \frac{P[\chi''(t)|\chi''_0]}{\tilde{P}[\tilde{\chi}''(t)|\tilde{\chi}''_0]} = \ldots = e^{\beta(W-\Delta F)}. \quad (2.102)
\]
Using the standard properties of ratios, we have
\[
\frac{P[\chi(t)|\chi_0]P(\chi_0)}{\tilde{P}[\tilde{\chi}(t)|\tilde{\chi}_0]} + \frac{P[\chi'(t)|\chi'_0]P(\chi'_0)}{\tilde{P}[\tilde{\chi}'(t)|\tilde{\chi}'_0]} + \frac{P[\chi''(t)|\chi''_0]P(\chi''_0)}{\tilde{P}[\tilde{\chi}''(t)|\tilde{\chi}''_0]} + \ldots = e^{\beta(W-\Delta F)}. \quad (2.103)
\]
In Eq.(2.103), the numerator is the sum of probabilities of trajectories along with work \( W \) has been performed on the system, whereas the denominator consists of the corresponding reverse trajectories. Thus, the numerator is the ensemble average probability of work \( W \) being done on the system, and the denominator is that of work \( W \) done by the system along the reverse trajectory. Therefore, we can write the work fluctuation theorem as[83, 95]
\[
\frac{P(W)}{P(-W)} = e^{\beta(W-\Delta F)}. \quad (2.104)
\]
Now we can write
\[
\langle e^{-\beta W} \rangle = \sum_{x(t), x_0} P(x_0) P[x(t)|x_0] e^{-\beta W}
\]
\[
= \sum_{\tilde{x}(t), \tilde{x}_0} \tilde{P}(\tilde{x}_0) \tilde{P}[\tilde{x}(t)|\tilde{x}_0] e^{-\beta (W-W_0)} = e^{-\beta \Delta F},
\]
(2.105)
where we have considered Eq. (2.101) in the second step.

The above relation is called the Jarzynski equality
\[
\langle e^{-\beta W} \rangle = e^{-\beta \Delta F},
\]
(2.106)
which relates the free energy difference between two equilibrium states and an ensemble average of the work required to switch between these two configurations.

**Integral Fluctuation Theorem:**

Here we have briefly described the integral fluctuation theorem [80, 97, 98]. To describe the fluctuation theorem a quantity \( r[\chi(t)] \) is defined which can be written as
\[
\tilde{r}[\tilde{\chi}(t)] = \ln \frac{P[\chi(t)|x_0] P(x_0)}{\tilde{P}[\tilde{\chi}(t)|\tilde{x}_0] \tilde{P}(\tilde{x}_0)}
\]
where \( P[\chi(t)|x_0] P(x_0) \) is the probability of forward trajectory and \( \tilde{P}[\tilde{\chi}(t)|\tilde{x}_0] \tilde{P}(\tilde{x}_0) \) is the probability of the reverse trajectory. Normalization in forward and reverse path ensemble implies that \( \sum_{\chi(t), x_0} P[\chi(t)|x_0] P(x_0) = \sum_{\tilde{\chi}(t), \tilde{x}_0} \tilde{P}[\tilde{\chi}(t)|\tilde{x}_0] \tilde{P}(\tilde{x}_0) = 1 \). From the Crooks fluctuation theorem described in Eq. (2.100) we obtain
\[
\frac{P[\chi(t)|x_0]}{\tilde{P}[\tilde{\chi}(t)|\tilde{x}_0]} = e^{\Delta s_m},
\]
where \( \Delta s_m \) is the change of medium entropy production along a trajectory with \( \Delta s_m = -\beta Q \). Therefore, Eq. (2.107) can be written as
\[
r[\chi(t)] = \Delta s_m + \ln \frac{P(x_0)}{P(\tilde{x}_0)}.
\]
(2.108)

According to Seifert, the system entropy along a trajectory is \( s(t) = -\ln P(x_t) \) [80], where \( P(x_t) \) is the probability of remaining the system in the state \( x_t \) at time \( t \). Using this definition we can obtain the change of system entropy production, \( \Delta s_{sys} = s(t) - s(0) = -\ln P(x_t) + \ln P(x_0) = -\ln \ln P(\tilde{x}_0) + \ln P(x_0) \), along a trajectory as
\[
\Delta s_{sys} = \ln \frac{P(x_0)}{P(\tilde{x}_0)}.
\]
(2.109)
Therefore, \( r[\chi(t)] \) is equivalent to the change of total entropy production along a trajectory i.e.,
\[
r[\chi(t)] = \Delta s_m + \Delta s_{sys} = \Delta s_{tot}.
\]
(2.110)
The following general identity can easily obtain as

\[ \langle e^{-\Delta_{\text{tot}}} \rangle = \langle e^{-r[x(t)]} \rangle = \sum_{x(t), x_0} P[x(t)|x_0]P(x_0)e^{-r[x(t)]} = \sum_{\tilde{x}(t), \tilde{x}_0} \tilde{P}[\tilde{x}(t)|\tilde{x}_0]P(\tilde{x}_0) = 1, \]

which is the integral fluctuation theorem. This is supported by the Jarzynski relation, \( \langle e^{-W_e} \rangle e^{\beta \Delta F} = 1 \) or \( \langle e^{-\beta(W_e - \Delta F)} \rangle = \langle e^{-\beta W_e} \rangle = 1 \). The dissipation function related to the \( \beta W_e \) or \( \Delta_{\text{tot}} \) is the measure of non-equilibriumness.

**Detailed Fluctuation Theorem**

To prove the detailed fluctuation theorem, another quantity, \( r[x(t)] \) is defined for the backward trajectory dynamics by keeping the analogy with Eq.(2.107),

\[ r[x(t)] = \ln \left\{ \frac{\tilde{P}[\tilde{x}(t)|\tilde{x}_0]P(\tilde{x}_0)}{\tilde{P}[\tilde{x}(t)|\tilde{x}_0]P(\tilde{x}_0)} \right\}, \]  

(2.112)

where \( r[x(t)] = -r[\tilde{x}(t)] \). Now if the probability \( P(R) \) to observe a trajectory with \( r[x(t)] = R \) during the forward dynamics is related to the probability \( P(-R) \) to observe a trajectory with \( r[\tilde{x}(t)] = -R \) during the backward dynamics, we can write

\[ P(R) = p(x_0)P(x(t)|x_0)\delta(R - r[x(t)]) = \sum_{x(t), x_0} \tilde{P}(\tilde{x}_0)\tilde{P}[\tilde{x}(t)|\tilde{x}_0]e^{r[x(t)]}\delta(R - r[x(t)]) \]

\[ = e^R \sum_{x(t), x_0} \tilde{P}(\tilde{x}_0)\tilde{P}[\tilde{x}(t)|\tilde{x}_0]e^{r[x(t)]}\delta(R + r[x(t)]) \]

or \( P(R) = e^R P(-R), \)  

(2.113)

where we have used Eq.(2.107) and Eq.(2.112). As \( R = r[x(t)] = \Delta_{\text{tot}} \) from Eq.(2.110), so the detailed fluctuation theorem can be written as

\[ \frac{P(\Delta_{\text{tot}})}{P(-\Delta_{\text{tot}})} = e^{\Delta_{\text{tot}}}. \]

(2.114)

This is useful for the entropy production for single trajectory in non-equilibrium dynamics which is proved here for both initial and final conditions at equilibrium.

**Other Fluctuation Theorems**

Here we have just mentioned FT of Evans-Searles[84] and Gallavotti-Cohen[87] which are not explicitly used in this thesis. The Evans-Searles’ FT explains how irreversibility is developed naturally in the system whose dynamics are deterministic and microscopically reversible[84, 95]. This FT bridges the microscopic and macroscopic descriptions, relating the time-reversible equations of motion of a system to the second law and provides a resolution to the long-standing irreversibility paradox[95]. The FT relates the relative probabilities, \( P \) of observing forward
and backward trajectories of duration $t$ characterized by the dissipation function, $\Omega_d = A$, taking on arbitrary values $A$ and $-A$, respectively. So the FT can be written as\cite{84, 95}

$$\frac{p(\Omega_d = A)}{p(\Omega_d = -A)} = \exp[A], \quad (2.115)$$

which holds for arbitrary initial distribution. The dissipation function, $\Omega_d$ is a dimensionless dissipative energy expressed in units of $k_BT$, accumulated along the system’s trajectory. From the Eq.(2.115) it can be realized that as the system size gets larger or the observation time gets longer, anti-trajectories become rare and it becomes overwhelmingly likely that the system appears time-irreversible according to the second law of thermodynamics. Gallavotti and Cohen also derived a steady state FT which is defined at asymptotic limit but its applicability is very rare.

### 2.7.2 Chemiostatic condition: Non-equilibrium steady state

For a closed system, reactions occur at equilibrium condition where the change of Gibbs free energy, $\Delta G$ and the change of total entropy production, $\Delta S_{tot}$ becomes zero\cite{66}. However, a chemical system can be driven out of equilibrium due to continuous in-letting of reactants and out-letting of products i.e., the system acts as a flow reactor. In this situation, this open chemically reacting system goes to the non-equilibrium steady state(NESS) indicated by the non-zero value of $\Delta S_{tot}$. Due to constancy of the concentration of the reactants and products at constant value, the reactant and product molecules behave like chemiostats and the differences of chemical potentials between the chemiostats generate the fluxes of matter across the system which mainly drive the system out of equilibrium\cite{13}.

To investigate the role of chemiostats to drive the system away from equilibrium, we have considered the single molecule enzyme kinetics where the substrate and product molecules remain constant. The chemical potential difference between the substrate and the product molecules, $\Delta \mu_{S,P}$ is the summation of the chemical potential differences $\Delta \mu_I$ and $\Delta \mu_{II}$ corresponding to the reactions

$$E + S \underset{k_{-1}}{\xrightarrow{k_i'}} ES, \quad (2.116)$$

and

$$ES \underset{k_{-2}}{\xrightarrow{k_2}} E + P, \quad (2.117)$$

respectively. The value of $\Delta \mu_i$ ($i = I, II$) for these two reactions can be calculated as\cite{11, 64}

$$\Delta \mu_i = k_BT \ln \left( \frac{\text{backward reaction flux}}{\text{forward reaction flux}} \right). \quad (2.118)$$
For the reaction in Eq.(2.116), $\Delta \mu_1$ is calculated as

$$
\Delta \mu_1 = k_B T \ln \left( \frac{J_{-1}}{J_1} \right),
$$

(2.119)

where the forward and backward reaction flux of corresponding reaction are, $J_1 = k_1[S]P_E$ and $J_{-1} = k_{-1}P_{ES}$, respectively. Similarly, the value of $\Delta \mu_{II}$ is

$$
\Delta \mu_{II} = k_B T \ln \left( \frac{J_{-2}}{J_2} \right),
$$

(2.120)

where the forward and backward reaction flux of reaction in Eq.(2.117) are, $J_2 = k_2P_{ES}$ and $J_{-2} = k_{-2}[P]P_E$, respectively. Therefore, for the overall reaction process, the change in the chemical potential between the substrate and the product molecules is

$$
\Delta \mu_{S,P} = k_B T \ln \left( \frac{J_{-1}J_{-2}}{J_1J_2} \right) = k_B T \ln \left( \frac{k_{-1}k_{-2}[P]}{k_1[S]k_2} \right).
$$

(2.121)

At equilibrium, $\Delta \mu_{S,P} = 0$ gives

$$
\left( \frac{k_{-1}k_{-2}[P]}{k_1[S]k_2} \right) = 1.
$$

(2.122)

If the ratio of the substrate and product concentration, $\frac{[S]}{[P]}$ are maintained at the value of $\left( \frac{k_{-1}k_{-2}}{k_1k_2} \right)$, the system remains in equilibrium. Otherwise, it goes out of equilibrium i.e., the non-equilibrium steady state (NESS).

To describe the non-equilibrium chemical systems, estimation of the thermodynamic quantities like $\Delta S_{tot}$ is essential and for the reacting system the thermodynamic quantities are generally calculated from the master equation. In the next sub-section we have discussed about how the thermodynamic quantities can be calculated from the master equation.

### 2.7.3 Fluctuation Theorem for chemical systems

Recent studies of non-equilibrium thermodynamics reveal that if a small chemical system remains in far from equilibrium, the thermodynamic quantities like $\Delta S_{tot}$ becomes a fluctuating quantity and it follows the fluctuation theorem [13, 80, 81, 82, 85]. The theorem generally relates the probability of entropy generating trajectories in the system to those of entropy consuming ones. So to describe the fluctuation theorem it is necessary to define the entropy on the level of a single trajectory [85, 81, 82].

To describe the fluctuation theorem for a chemical system we have considered a system having $m$ different types of chemical species $\{S_1, S_2, \ldots, S_m\}$, which interact
through M chemical reactions \( \{R_1, R_2, \ldots, R_M\} \). The system is confined to a constant volume \( \Omega \) and is in thermal (but not in chemical) equilibrium at some constant temperature \( T \). The number of molecules of species \( S_i \) in the system at time \( t \) is \( n_i(t) \) and the corresponding molecular populations state vector is \( n(t) = (n_1(t), n_2(t), \ldots, n_m(t)) \), given that the system was in state \( n_0 \) at some initial time \( t_0 = 0 \). For this system a stochastic trajectory, \( \chi(t) \) can be considered which is depicted in Fig(2.5), generated due to evolution of the population state vector, \( n(t) \) \([81, 82, 85, 99]\) with time which starts from \( n_0 \) at time \( t_0 = 0 \) and jumping at time \( t_j \) from \( n_{j-1} \) to \( n_j \) ending up at \( n_N \) with \( t_{N+1} = T \) \[99\]. During jump from the state \( n_{j-1} \) to \( n_j \), any one of the M reactions will occur and the time interval \( \tau_j \) between the two jumps is a random variable following the exponential distribution which is described in Eq.(2.86). Here \( w(n_{j-1}, t_j|n_j) \) is the transition probability from the state \( n_{j-1} \) to \( n_j \) through a reaction \( R_m \) with the stotictometric vector \( \nu^R_m \) along a single trajectory. A time reverse trajectory, \( \chi(t) \) shown in Fig.(2.6), is generated due to the occurrence of a reaction whose state changing vector \( -\nu^R_m \) is exactly opposite to the state changing vector, \( \nu^R_m \) of the forward reaction. Now the probability of occurring the forward trajectory can be calculated as

\[
P[\chi(t)|n_0] = P_{n_0}(0) \prod_{j=0}^{N-1} \left( \int_{t_j}^{t_{j+1}} P_{n_j}(t') dt' \right) \times \int_{t_N}^{t_{N+1}} P_{n_N}(t') dt'.
\]

(2.123)

Here \( \int_{t_j}^{t_{j+1}} P_{n_j}(t') dt' \) is the probability of occurring no reaction in the \( n_j \) state during the time interval \( t_j \) to \( t_{j+1} \) and normalization in the trajectory-space implies that \( \sum_{\chi(t)} P[\chi(t)|n_0] = 1 \). Now using the relation of the probability of occurring no
reaction described in Eq.(2.79), we obtain
\[ P_{nj}(t' + dt') = P_{nj}(t') \left[ 1 - \sum_{\mu=1}^{M} a_{\mu}(n_j) dt' \right]. \] (2.124)

Expanding the probability, \( P_{nj}(t' + dt') \) by Taylor expansion method and neglecting the higher order terms we get [15]
\[ \int_{t_j}^{t_{j+1}} P_{nj}(t') dt' = \int_{0}^{\tau_j} P_{nj}(\tau') d\tau' = \exp \left[ - \sum_{\mu=1}^{M} a_{\mu}(n_j) \tau_j \right], \] (2.125)

where \( \tau_j = (t_{j+1} - t_j) \) and \( P_{nj}(0) = 1 \). Similarly the probability of occurring the backward trajectory can be written as [99]
\[ \bar{P}[\bar{\chi}(t)|\bar{n}_0] = \bar{P}_{n_{N+1}}(0) \left[ \prod_{j=1}^{N} \left( \int_{T-t_{j+1}}^{T-t_j} \bar{P}_{nj}(t') dt' \right) \times \bar{w}(n_j, T - t_j | n_{j-1}) \right] \]
\[ \times \int_{T-t_1}^{T} \bar{P}_{n_0}(t') dt', \] (2.126)

where \( \bar{n}_0 = n_{N+1} \) and \( \bar{P}_{n_{N+1}}(T) = \bar{P}_{n_{N+1}}(0) \). The normalization in the reverse path ensemble implies \( \sum_{\bar{n}_0} \bar{P}[\bar{\chi}(t)|\bar{n}_0] = 1 \). For the backward trajectory, Eq.(2.125) can be written as
\[ \int_{T-t_{j+1}}^{T-t_j} \bar{P}_{nj}(t') dt' = \int_{0}^{\tau_j} \bar{P}_{nj}(\tau') d\tau' = \exp \left[ - \sum_{\mu=1}^{M} a_{\mu}(n_j) \tau_j \right], \] (2.127)

where \( \tau = [(T - t_j) - (T - t_{j+1})] = (t_{j+1} - t_j) = \tau \). From Eq.(2.125) and Eq.(2.127) it is observed that the probability of occurring no reaction in the \( n_j \) state for the
forward and backward trajectories is same. Therefore, the ratio of the probability of forward and backward trajectories can be written as

\[
P[F|x(t)|n_0] = \frac{P_{n_0}(0) \times w(n_0, t_1|n_1) \times w(n_1, t_2|n_2) \times \cdots \times w(n_{N-1}, t_N|n_N)}{P_{n_0}(0) \times w(n_0, T-t_n|n_{N-1}) \times \cdots \times w(n_1, T-t_1|n_0)}
\]

Taking the logarithm on both sides we get

\[
r[F|x(t)|n_0] = \ln \left\{ \frac{P[F|x(t)|n_0]}{\tilde{P}[\tilde{F}|\tilde{x}(t)|n_0]} \right\} = \ln \frac{P_{n_0}(0)}{P_{n_0}(0)} + \prod_{j=1}^{N} \ln \frac{w(n_{j-1}, t_j|n_j)}{w(n_j, T-t_j|n_{j-1})},
\]

where \( r[F|x(t)|n_0] \) is the logarithmic ratio of the forward and backward trajectory probabilities. Now according to Seifert, the entropy production along a single stochastic trajectory can be defined as [81, 99]

\[
s(t) = -\ln P_n(t), \quad (2.130)
\]

where \( P_n(t) \) is the solution of the stochastic master equation for a given initial condition, \( P_{no}(t_0) \), taken along the specific trajectory \( \chi(t) \). Therefore, the system entropy along a trajectory can be written as

\[
\Delta S_{sys} = \ln \frac{P_{n_0}(0)}{P_{n_0}(0)},
\]

and the medium entropy production is expressed as

\[
\Delta S_m = \sum_j \ln \frac{w(n_{j-1}, t_j|n_j)}{w(n_j, t_j|n_{j-1})}, \quad (2.132)
\]

Here \( w(n_{j-1}, t_j|n_j) \) is the transition probability from the state \( n_{j-1} \) to \( n_j \). So the first term of the right hand side in Eq.(2.129), \( \ln \left( \frac{P_{n_0}(0)}{P_{n_0}(0)} \right) = \Delta S_{sys} \) and the second term is equal to the medium entropy production along a single trajectory, \( \Delta S_m \). Therefore, Eq.(2.129) can be written as

\[
\ln \left\{ \frac{P[F|x(t)|n_0]}{P[F|x(t)|n_0]} \right\} = \Delta S_{sys} + \Delta S_m = \Delta S_{tot}.
\]

Similar to the Eq.(2.129), we can write

\[
r[F|x(t)|n_0] = \ln \left\{ \frac{P[F|x(t)|n_0]}{P[F|x(t)|n_0]} \right\}, \quad (2.134)
\]

where \( r[F|x(t)|n_0] = -r[F|x(t)|n_0] [99] \). Now the probability \( P(R) \) to observe a trajectory such that \( r[F|x(t)|n_0] = R \) during the forward dynamics is related to the probability \( P(-R) \) to observe a trajectory such that \( r[F|x(t)|n_0] = -R \) during the backward dynamics

\[
P(R) = \sum_{x(t)} P[F|x(t)|n_0] \delta(R - r[F|x(t)|n_0]) = \sum_{x(t)} \tilde{P}[\tilde{F}|\tilde{x}(t)|n_0] e^{r[F|x(t)|n_0]} \delta(R - r[F|x(t)|n_0])
\]
which is the detailed fluctuation theorem. By integrating over $e^{-R}P(R) = \tilde{P}(-R)$ over $R$, we obtain the integral fluctuation theorem
\[
\langle e^{-R\chi(t)} \rangle = 1. 
\]

Therefore, if the system is in far from equilibrium, the fluctuating thermodynamic quantities like change in the total entropy production, $\Delta S_{\text{tot}}$ follows the integral as well as the detailed fluctuation theorem.

### 2.7.4 Estimation of thermodynamic quantities from the master equation

Here we have estimated the thermodynamic quantities, mainly the entropy production rates from the master equation. The system entropy is defined in terms of the Shannon entropy as [13, 74, 100, 101, 102, 103, 104],
\[
S_{\text{sys}}(t) = -k_B \sum_n P_n(t) \ln P_n(t), 
\]
where we set the Boltzmann constant, $k_B = 1$. Using the master equation described in Eq. (2.83), we get the system entropy production rate [13, 74, 100, 101] as
\[
\dot{S}_{\text{sys}}(t) = \frac{1}{2} \sum_{n,\mu} \left[ w_\mu(n + \nu_\mu | n) P_{(n-\nu_\mu)}(t) - w_\mu(n | n - \nu_\mu) P_n(t) \right] 
\times \ln \frac{P_{(n-\nu_\mu)}(t)}{P_n(t)}. 
\]

We have assumed ideal reservoir (surroundings) with no inherent entropy production except through the boundaries of the system. The system entropy production (ep) rate can be split as [100, 101, 102, 103, 104]
\[
\dot{S}_{\text{sys}}(t) = \dot{S}_{\text{tot}}(t) - \dot{S}_{\text{m}}(t). 
\]

Here the first term in the r.h.s. of equation (2.139) gives the total entropy production rate and the second term denotes the medium entropy production rate due to the entropy flux into the surroundings. Therefore the total and medium entropy production rates [13, 74, 100, 101] are defined as
\[
\dot{S}_{\text{tot}}(t) = \frac{1}{2} \sum_{n,\mu} \left[ w_\mu(n + \nu_\mu | n) P_{(n-\nu_\mu)}(t) - w_\mu(n | n - \nu_\mu) P_n(t) \right] 
\times \ln \frac{w_\mu(n - \nu_\mu | n) P_{(n-\nu_\mu)}(t)}{w_\mu(n | n - \nu_\mu) P_n(t)}. 
\]
\[
\dot{S}_m(t) = \frac{1}{2} \sum_{n,\mu} \left[ w_{\mu}(n - \nu_{\mu}[n])P_{(n-\nu_{\mu})}(t) - w_{-\mu}(n[n - \nu_{\mu}])P_n(t) \right] \\
\times \ln \frac{w_{\mu}(n - \nu_{\mu}[n])}{w_{-\mu}(n[n - \nu_{\mu}])}.
\] (2.141)

\(\dot{S}_m(t)\) is equal to the heat dissipation rate \(h_d(t)\). From the master equation we can also calculate the free energy dissipation rate as

\[
\dot{F}(t) = \frac{T}{2} \sum_{n,\mu} \left[ w_{\mu}(n - \nu_{\mu}[n])P_{(n-\nu_{\mu})}(t) - w_{-\mu}(n[n - \nu_{\mu}])P_n(t) \right] \\
\times \ln \frac{P_{(n-\nu_{\mu})}(t)P_{\infty}^n}{P_n(t)P_{\infty}^{n-\nu_{\mu}}},
\] (2.142)

where \(P_{\infty}^n\) is the steady state probability distribution of the \(n\) state.

At equilibrium, \(\dot{S}_{\text{tot}}(t)\) becomes zero and the master equation satisfies the detailed balanced condition whereas, for non-equilibrium situation, the value of \(\dot{S}_{\text{tot}}(t)\) becomes a non-zero quantity and at NESS the master equation follows the circular balance condition. Integrating \(\dot{S}_{\text{tot}}(t)\) between the initial time, \(t_0 = 0\) to final time, \(t_f = t\), we get the total entropy production, \(\Delta S_{\text{tot}}\).