Chapter 2

Numerical techniques

In this chapter, we described the various numerical techniques and methodology to perform our experiments. We have applied systems biological approach to understand the complex biological systems. The model we designed is based on the data reported in various experimental as well as theoretical works. All the parameters including rate constants were reasonably chosen to fit the theoretically and experimentally observed behaviours of p53 and other proteins. The theoretical prediction of the values of the rate constants in any working models is in fact a difficult task and still an open problem. The values of these rate constants are generally, sensitive to the system behaviour, i.e. behaviour of various state variables (p53, Mdm2 etc.) Those theoretically predicted values are chosen by keeping this sensitivity and without significantly changing the experimentally predicted system behaviour. Its a tedious work and predicted after large scale simulations of the model by taking wide range of variation of values of these rate constants to come to final theoretically predicted values. However, some of the rate constant values are very sensitive to the system behaviour causing drastic change in the dynamical system, whereas, some are not so sensitive. But this sensitivity of the rate constants is in fact system dependent. We have generated reaction channel on the basis of interaction of molecular species. After that the classical deterministic
non-linear differential equations constructed from these reactions in the network can be solved numerically using the standard 4\textsuperscript{th} order Runge–Kutta algorithm of numerical integration \cite{188} to study the dynamical behaviour of the system.

## 2.1 Deterministic formalism of biochemical pathways

Deterministic formalism assumes the temporal behaviour of biochemical networks, which is predictable, continuous and deterministic \cite{189}. In this formalism, first a mathematical model is constructed on the basis of interaction of molecules in the biochemical network system. The Chemical reaction channel are then transformed into a set of ordinary differential equation (ODE) on the basis of mass action kinetics law. ODE pictures the dynamic of the system in continuous fashion.

If we suppose a system of definite size $V$ contains $N$ number of reacting molecular species interact with each other through $M$ detailed chemical reaction channels at continuous time ‘$t$’ then the state of the system at any time ‘$t$’ can be described by a vector $(\vec{x})$ at that instant of time $\vec{x}(t) = \{x_1(t), x_2(t), \ldots, x_N(t)\}$

Thus we can construct $N$ number of ordinary differential equations:

$$\frac{dX_i}{dt} = F_i(X_1, \ldots, X_N) \quad (2.1)$$

$i = 1, 2, \ldots, N$ Where, $F$ is the function of $i^{th}$ molecular species. The set of the complex ordinary differential equations are generally non-linear in nature and can be solved by various computational techniques. For example, if we consider the
following reaction of the interaction of two molecular species $X$ and $Y$.

$$
\phi \xrightarrow{k_1} X \quad (2.2)
$$

$$
X + Y \xrightarrow{k_2} XY \quad (2.3)
$$

$$
XY \xrightarrow{k_3} X \quad (2.4)
$$

$$
X \xrightarrow{k_4} \phi \quad (2.5)
$$

then we will get two ODE for each molecular species $X$ and $Y$ as,

$$
\frac{d[X]}{dt} = k_1 - k_2 [X][Y] + k_3 - k_4 \quad (2.6)
$$

$$
\frac{d[Y]}{dt} = -k_2 [X][Y] \quad (2.7)
$$

(2.8)

where parameter $k_1$, $k_2$, $k_3$ and $k_4$ is the rate of reaction and $[X]$ and $[Y]$ are concentrations corresponding to $X$, $Y$, respectively. This ODEs is solved by using various computational techniques. In our study, we have applied Runge–Kutta 4th order approximation method. Runge–Kutta 4th order is the standard method of numerical integration of ODEs and numerically stable techniques. The solution of Runge–Kutta 4th order method is not an exact but an approximation which is very useful to the system is closed suitable to the thermodynamic limit. Thermodynamic limit is defined as the limit in which the molecular populations and the containing volume all approach to infinity in such a way that the molecular concentration remain finite \[190\]. These equations are possible to solve easily with computers, but they give a limited view of the system.

2.2 Stochastic formalism of biochemical pathways

Stochastic formalism assumes that the interaction of molecular species is a discrete and random process. Stochastic models are considered to be realistic models with quantitative and qualitative prescriptions. The stochastic nature of a system is
very important in cases where the involved reactants are in low copy number, and trajectories of each molecular species can be probed in probabilistic manner. It is microscopic description of the system that could be very close to the experimental situations. The noise in the stochastic process is contributed by two; first by intrinsic noise due to random molecular or particle events taking place among the molecules taking part in the reaction network in the system and second by extrinsic noise due to fluctuations in the extracellular environment [116, 191, 192]. Until we deal with the stochastic nature of these interactions, one has to abandon the macroscopic aspect of molecular interaction, where the intrinsic and extrinsic noise is neglected [193]. In this formalism each and every interaction of molecular species is express systematically by formulating chemical Master Equation (CME) of the biochemical network to find their trajectories in configuration space. The CME is based on the formation and degradation of each molecular species during the course of state evolution [194]. Finding of solution for chemical master equation for complex system is very difficult except for simple one. However, computationally trajectory of each and every molecular species in the system can be traced using stochastic simulation algorithm (SSA) due to Gillespie [44].

Thus the stochastic formulation has a much firmer physical basis than that of deterministic formulation since the molecular population of the molecular interaction in biological system changed integral value when a molecular interaction is performed, and so discreteness is sound more perfect than continuous. This discreteness and stochasticity are not observable in the macroscopic system like test tube size, molecular interaction, biological system, and for such system employing a deterministic approach is adequate. But for smaller system size like biological system stochasticity plays an important role.
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2.2.1 Chemical Master Equation (CME) formalism of biochemical network

Stochastic systems are microscopic systems with small system size accommodating small number of molecules in which the description of the particle interaction events in the system can be well described by chemical master equation formalism [116, 195]. CME formalism is used to explain the time evolution of a system that can be explained for certain states of the system at any instant of time, where the conformation change in the system is probabilistic. The intrinsic noise in stochastic systems is due to random molecular or particle interactions taking place in the system and is an inherent property associated with the stochastic system [116]. If we consider a configurational state $\vec{x}(t) = [x_1, x_2, \ldots, x_N]^{-1}$ consisting of $N$ species (molecule or particle) at any instant of time ‘$t$’, then the random interaction events (only collisions which gives rise decay and creation of particle) taking place in the system is given by,

$$S_a x_a + S_b x_b + \cdots \xrightarrow{k_\mu} S_l x_l + S_m x_m + \cdots \quad (2.9)$$

where, $\{S\}$ is co-efficient of species in the $\mu^{th}$ interaction. $k_\mu$, with $\mu = 1, 2, \ldots, M$ is the rate of interaction of the species $\{X_i\}$, $i = a, b, \ldots$ to give species $\{X_j\}$, $i = 1, m, \ldots$ in a certain interval of time $[t, t + \Delta t]$. This leads to the change in states from $\vec{x}(t)$ at time ‘$t$’ to a new state $\vec{x}'(t + \Delta t)$ during the time interval. Depending upon the magnitude of $\Delta t$, there could be $L$ number of interaction in series took place. If we simplify the transitions by taking an infinitesimal time interval $dt$ such that during $[t, t + dt]$ only one interaction is occurred then we can write $\Delta t = dt^{[1]} + dt^{[2]} + \ldots dt^{[L]}$, which leads to a series of microscopic state changes, $\vec{x} \rightarrow \vec{x}^{[1]} \rightarrow \vec{x}^{[2]} \rightarrow \ldots \vec{x}^{[L]}$. These $\{dt^{[j]}\}$, $j = 1, 2, \ldots, L$ are not necessarily the same, but taking $dt^{[1]} = dt^{[2]} = \ldots dt^{[L]} = dt$ we have $\Delta t = Ldt$ leading to macroscopic state change $\vec{x} \xrightarrow{k_i} \vec{x}'$. Now if we consider each microscopic state
change during \([t + dt^{[j]}, t + dt^{[j']}]\), \(\{j, j' = 1, 2, \ldots, L; j \neq j'\}\) then the time evolution of the state probability, \(P_{j}(\vec{x}_{j}, t)\) of the state change based on transition probability \(\{W\}\) of decay and formation of particles evolved in the interaction event can be described by the following master equation [191, 192].

\[
\frac{\partial P_{j}(\vec{x}_{j}, t)}{\partial t} = -\sum_{\vec{x}_{j'}} P_{j}(\vec{x}_{j}, t) W_{\vec{x}_{j} \rightarrow \vec{x}_{j'}} + \sum_{\vec{x}_{j}} P_{j}(\vec{x}_{j}, t) W_{\vec{x}_{j} \rightarrow \vec{x}} \quad (2.10)
\]

The macroscopic state change can be calculated by series of state probabilities corresponding to each microscopic state changes \(P(\vec{x}, t) \rightarrow P_{1} \rightarrow P_{2} \rightarrow \cdots \rightarrow P_{L} \rightarrow P(\vec{x}', t)\) since each interaction that drives a particular microscopic state change is random in nature, the trajectory of \(P_{j}\) from \(P(\vec{x}, t)\) to \(P(\vec{x}', t)\) will a Brownian trajectory.

### 2.2.2 Gillespie’s Stochastic Simulation Algorithm (SSA)

Gillespie’s Stochastic Simulation Algorithm (SSA) [44] is significantly an exact approach to calculate and simulate the time evolution of population state vector of the biochemical reacting network system. The algorithm is based on determined joint probability function,

\[
p(\tau, \mu) = k(\tau) \chi(\mu) \quad (2.11)
\]

which allow evolution to occur with increment of time \(\tau\) \((0 \leq \tau \geq 0)\) by deriving from the probability densities function, \(k(\tau)\) at which time distinct reaction is fired and \(\chi(\mu)\) which determines the reaction \(\mu = [1, 2, \ldots, M]\). For this assumption, \(\tau\) and \(\mu\) can be approximated computationally from the expressions,

\[
\tau = \frac{1}{w_{0}} \ln \frac{1}{k(\tau)} \quad (2.12)
\]

\[
= \frac{1}{w_{0}} \ln \frac{1}{r} \quad (2.13)
\]
and \( w_\mu = w_0 \chi (\mu) \) where two uniform random numbers \( r_1 \) and \( r_2 \) correspond to \( k (\tau) \) and \( k (\chi) \) respectively where, \( w_0 = \sum_{i=1}^{M} w_i \). We then Gillespie stochastic simulation algorithm can be used to simulate biochemical reaction system, so that Simulating a set of chemical reaction,

\[
\vec{x}(t) = (x_1, x_2, \ldots, x_N)^T \tag{2.14}
\]

\( x_i \) is molecular species where \( i = 1, 2, \ldots, N \). \( X_t \) is molecular species vector or state at any instant of time ‘\( t \)’. The time interval, i.e. \( \Delta t \) is taken to be small enough so that only one reaction takes place during this time interval. One has to define propensity function, \( a_i \) which can be defined as the probability of occurring a reaction be fired anywhere in the system. For example,

\[
X + Y \xrightarrow{k} Z \tag{2.15}
\]

Then propensity function is given by \( a = kXY \)

If in the system there are \( M \) reaction channels and then there will be \( M \) propensity function. and \( a_0 = \sum_{i=0}^{M} a_i \). The Gillespie algorithm can be summarized as,

1. Initialize molecular species
   - Define rate constants
   - Initialize time \( t = 0 \)

2. Define propensity function \( a_i = h_i c_i \) Where \( a_i \) propensity function, \( h_i \) is molecular combination, \( c_i \) is classical rate constant. At which time particular reaction is occur.

3. Define reaction time (\( \tau \))
   - Generate a uniform reaction number \( r_1 \)
   - \( \tau = \frac{1}{a_0} \ln \frac{1}{r_1} \) where \( (\tau) \) is reaction time \( a_0 = \sum_{i=0}^{M} a_i \)
4. Choose any reaction
   
   • Define another uniform random number $r_2$
   
   • $f = r_2$, $f$ is particular reaction is fired.
   
   • First reaction takes place if $0 \leq f \geq a_i$, the first reaction is update the molecular population.

5. $t = t + \tau$

6. Go to (2) step

7. Stop

Gillespie stochastic simulation algorithm (SSA) can be used to simulate the biochemical reaction network to understand the dynamical and temporal behaviours of each and every molecular species in the system. SSA is a Monte Carlo type and is based on the fundamental fact that the trajectory of each molecular species can be found out if one understands at an instant of time which reaction is fired.

### 2.3 Quasi–steady–state approximation

The idea of QSSA (Quasi-steady-state approximation) has been well known for long time [196]. It is a very important technique for deducing of chemical kinetic equation of enzymatic single-substrate reaction [197]. In a biological system (like living cell), the various mechanisms are performing in which complex biochemical pathways are defined by incorporating all steps of enzyme mechanism like the step of association and dissociation of the enzyme-substrate, intermediates formation and product formation. Mathematical model contains a large number of ordinary differential equations (ODEs) and dynamic variables with various kinetics
and stoichiometric parameters [198]. Therefore, numerical solutions of ordinary
differential equations are huge computationally demanding. The non-linear ODEs
cannot be easily analytically solved. However, we can get the inexact solution of
these ODEs by using quasi-steady-state approximation [19,199]. Generally, any
of the complex biochemical reaction network involves two types of basic reactions,
namely fast and slow reactions [199]. The state of the system at any instant of
time ‘t’ is given by the state vector, \( \vec{x}(t) = (x_1, x_2, \ldots, x_N)^T \), where \( N \) = number of
molecular species and \( t \) is the transpose of vector. We could reach to the following
coupled ODEs,

\[
\frac{d}{dt} \vec{x}(t) = \begin{bmatrix}
F_1 \\
F_2 \\
\vdots \\
F_N 
\end{bmatrix}
\tag{2.16}
\]

Where, the functions in (2.16) \( F_i(x_1, x_2, \ldots, x_N) \), \( i = 1, 2, \ldots, N \) are given by
\( F_1, F_2, \ldots, F_N \) is ordinary differential equations.

Therefore, the \( N \) = number of molecular species (i.e. the variables) in the
system can be divided into set of fast and slow variables in that order. If \( \vec{x}^f(t) =
(x_{l+1}, x_{l+2}, \ldots, x_N)^T, \vec{x}^s(t) = (x_1, x_2, \ldots, x_M)^T \), are fast and slow variable vectors,
then \( \vec{x}(t) = (\vec{x}^f, \vec{x}^s)^T \). Then from equation (2.16), we have,

\[
\vec{x}^s(t) = \begin{bmatrix}
x_1 \\
x_2 \\
\vdots \\
x_N 
\end{bmatrix}, \quad \vec{x}^f(t) = \begin{bmatrix}
x_{M+1} \\
x_{M+2} \\
\vdots \\
x_N 
\end{bmatrix}
\tag{2.17}
\]

Since the rate constant of complex formation is very fast as compared to
product formation, the fast state vector reaches the steady state (equilibrium)
quickly and can be taken as constant as compared to slow state variables. Then
applying approximation of Henri–Michaelis–Menten–Briggs–Aldane [200], one can
assume that the complex ODEs of variables of molecular species reach steady state quickly fast as compared to the evolution of time for slow variables [199]. Then one can put forward \( \frac{d\mathbf{x}}{dt} \approx 0 \) [199,200]. Now, the number of coupled ODEs (Equation 2.16) reduces to the following,

\[
\begin{align*}
\frac{\mathbf{x}(t)}{dt} &= \mathbf{x}^s(t) = \frac{d}{dt} \begin{bmatrix} F_1 \\ F_2 \\ \vdots \\ F_M \end{bmatrix} \\
\end{align*}
\tag{2.18}
\]

and the \( \mathbf{x}^f \) becomes the following values for the steady state,

\[
\mathbf{x}^f \rightarrow \begin{bmatrix} x_{M+1} \\ x_{M+2} \\ \vdots \\ x_N \end{bmatrix} \rightarrow constant \tag{2.19}
\]

The \( N \) numbers of ODEs are now reduced to \( M \) number of ODEs. This gives a way to reduce the complexity of the mathematical model to get an approximate solution of the complex system in a numerical as well as analytically way decreasing computational effort if possible. We further analyzed, constructed complex mathematical model to get an understanding of the possible approximate from analytical solutions of the slow variables using quasi-steady-state approximation.

## 2.4 Visibility graph algorithm

Visibility algorithm is a technique which maps a time series to a network [201], where every observation in time series corresponds to a node and an edge between any two nodes is introduced when the following visibility condition is satisfied i.e. two nodes corresponding to observations in time series \( p(t_a) \) and \( p(t_b) \) are connected if all intermediate states \( p(t_c) \) with \( t_a < t_c < t_b \) satisfy,
\[
\frac{p_b - p_c}{t_b - t_c} > \frac{p_a - p_a}{t_b - t_a} \quad (2.20)
\]

In these networks each node is connected to its closest neighbors in any direction. These networks are undirected due to symmetry in visibility condition. Since properties of the time series are inherited to the corresponding network, the studies of this network provide useful information which can’t be observed in traditional time series.

2.4.1 Topological properties of a network

The following topological properties of the networks (graph) are studied to study the important behaviour of the networks.

Degree distribution

The degree \( k \) represents a centrality measure that indicates the number of communications (links) the node connects with other nodes in the network. For a network defined by a graph \( G = (N, E) \), where \( N \) and \( E \) are number of nodes and edges respectively, the probability of degree distribution \( (P(k)) \) of the network is the ratio of the number of nodes having degree \( k \) \( n_k \) to the network size as follows,

\[
P(k) = \frac{n_k}{N} \quad (2.21)
\]

Where, \( n_k \) is the number of nodes having degree \( k \) and \( N \) is the total number of nodes in the network. \( P(k) \) in random and small-world networks follow Poisson distribution, whereas, it obeys power law in scale-free and hierarchical networks depending on the value of which indicates the importance of hubs or modules in the network [120].
Neighborhood connectivity

The measure of the average connectivities (average degrees) of the nearest neighbors of node $i$ in the network ($C_N(k)$) can be represented by a parameter Neighborhood connectivity [202] given by,

$$C_N(k) = \sum_q qP(q|k)$$

(2.22)

where, $P(q|k)$ is the conditional probability that a link belonging to a node with connectivity $k$ points to a node with connectivity $q$. The $k$ dependence of $C_N(k)$, i.e. $C_N(k) \sim k^{-\beta}$ is a signature of hierarchical topology in the network [124]. However, the positive power dependence of $C_N(k)$ could be an indicator of assortivity in the network topology [125].

Clustering co-efficient

This topological parameter of a network measure how strongly a node’s neighborhood nodes are interconnected. Graph theoretically it is the ratio of the number of its nearest neighborhood edges i.e. to the total possible number of edges of degree $k_i$. For an undirected network, clustering co-efficient ($C(k_i)$) of ith node can be calculated by,

$$C(k_i) = \frac{2e_i}{k_i(k_i - 1)}$$

(2.23)

For a $C(k)$ hierarchical network it follows a power law $C(k) \sim k^{-\alpha}$ with $\alpha \sim 1$, whereas in scale free network does not depend on $k$.

Betweenness centrality

Betweenness centrality $C_B$ of a node represents the importance of information flows in the network [203], and the extent to which the node has control over the other nodes in the network through communication [204,205]. If $d_{ij}(v)$ indicates
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the number of geodesic paths from node i to node j passing through node v, and $d_{ij}$ indicates number of geodesic paths from node i to j, then betweenness centrality ($C_B(v)$) of a node v can be computed by,

$$C_B(v) = \sum_{i,j;i \neq j \neq k} \frac{d_{ij}(v)}{d_{ij}} \quad (2.24)$$

consider $M$ denotes the number of node pairs, excluding v, then normalized betweenness centrality is given by, $C_{NB}(v) = \frac{1}{M} C_B(v)$.

Closeness centrality

Closeness centrality ($C_C$) measures how fast information is spread from a node to other nodes reachable from it in the network \cite{206}. $C_C$ of a node i is the reciprocal of the mean geodesic distance between the node and all other nodes connected to it in the network, and is given by,

$$C_C(k) = \frac{n}{\sum_j d_{ij}} \quad (2.25)$$

where, $d_{ij}$ indicates geodesic path length between nodes i and j, and n being the total number of nodes in the network attached to node i.

Eigenvector centrality

Eigenvector centrality of a node i ($C_E(i)$) in a network is proportional to the sum of i’s neighbor centralities \cite{206}, and it is measured by,

$$C_E(i) = \frac{1}{\lambda} \sum_{j=nn(i)} v_j \quad (2.26)$$

where, $nn(i)$ indicates nearest neighbors of nodes i in the network. $\lambda$ is eigenvalue of the eigenvector $V_i$ is given by, $Av_i = \lambda v_i$ where, A is the adjacency matrix of the network (graph). The principal eigenvector of A, which corresponds to maximum eigenvalue $\lambda_{max}$, is taken to have positive eigenvector centrality score \cite{207}. Since
node’s eigenvector centrality function smoothly varies over the network and depends on its neighbors, node with high eigenvector centrality is embedded in the locality of nodes of high eigenvector centralities, and the chance of having isolated nodes in and around the locality is very low [206]. Hence, eigenvector centrality can be used as an indicator of node’s spreading power in the network.

2.5 MF-DFA algorithm

In non-stationary time series the fractal properties and the corresponding relevant correlations can be studied by applying the Multi-fractal detrended fluctuation analysis (MF-DFA). The parameters which are the main subjects of the above said analysis are; Hurst exponent ($H$) as a tool to measure the origin of long and short range correlation of the system, generalized dimension ($D$) parameter as a measure of the morphology of the system, singularity time spectrum ($f$) to check the instant evolution of the system with time [127]. The method generally adopted [126] for the analysis of the above said parameters have been given as below:

First, the signal in time series $\{x_j\}$ of length $N$ is taken as random walk, and can be represented by the profile, $Y(i) = \sum_{j=1}^{i}(x_j - \langle x \rangle)$, where, $\langle x \rangle$, is average value of the signal, and $i = 1, 2, \ldots, N$. Second, the profile $Y(i)$ is divided into $N_s = int\left(\frac{N}{s}\right)$ equal non-overlapping segments of equal size $s$. To take the care of all the data points, $2N_s$ segments are considered by counting starting from both ends of the data. Third, for better understanding, the variance is determined as follows,

$$F^2(s, \nu) = \frac{1}{s} \sum_{i=1}^{s} \{Y[(\nu - 1)s + i] - y_{\nu}(i)\}^2$$

(2.27)

Fourth, the fluctuation function of $q^{th}$ order is being estimated by averaging over
all segments,

\[
F_q(s) = \left\{ \frac{1}{2N_s} \sum_{\nu=1}^{2N_s} |Y[(\nu,s)]|^q/2 \right\}^{1/q} \tag{2.28}
\]

Fifth, the behaviour of scaling of the function \( F_q(s) \) is represented by

\[
F_q(s) \sim s^{H_q} \tag{2.29}
\]

where, \( H_q \) is the Hurst exponent, which takes care of the measure of the correlation properties and self-similarity of the signal. Then, \( H_q \) the generalized Hurst exponent and classical scaling exponent \( \tau(q) \) are related as

\[
\tau(q) = qH_q - 1 \tag{2.30}
\]

and as per the definition of Holder exponent, \( \alpha = \frac{d\tau}{dq} \), the singularity function \( f(\alpha) \) [127] is given by,

\[
f(\alpha) = q\alpha - \tau(q) \tag{2.31}
\]

Then, the main parameter which defines the morphology of the system, the generalized fractal dimension of the signal is measured by,

\[
D_q = \frac{\tau(q)}{q-1} \tag{2.32}
\]

On giving different values of \( q \) will get different types of fractals, for instance, for \( q = 0 \) we will have \( D_0 \), is called as the fractal or Hausdorff dimension, for \( q = 1 \), \( D_1 \) is the information dimension and for \( q = 2 \), we will have \( D_2 \) which represents the correlation dimension. If \( H_q \) is significantly depending on the \( q \) then in the system multifractal signature in the time series can be observed, due to the different nature of the scaling of the small and large fluctuations. Large scale fluctuations are being attributed to the positive dependence of \( H_q \) on \( q \), while as small scale fluctuations are being attributed to the negative dependence of \( H_q \) on \( q \). Further, small and large fluctuations are characterized respectively by large and small values of \( H_q \) in multifractal time series.
2.6 Permutation entropy

Permutation entropy can able to highlight the nature of complexity introduced in the system due to complicated inter-molecular interaction in the system and perturbation induced by the surrounding environmental (diffusion of molecules from other pathways and surrounding cells, temperature fluctuations and other related factors) fluctuations.

2.6.1 Algorithm to calculate permutation entropy: Bandt & Pompe approach

The complex information processing in a system is inherited in the time series of the constituting variables of the system which is measured by calculating permutation entropy of the time series [138, 208]. The basic algorithm of the complexity measure has been described as follows,

Permutation entropy $H$ of a time series of a dynamical variable $x(t)$ of a system can be calculated as follows. For a given time series $X = \{X_t\}$ where $t = 1, 2, \ldots, N$, $N$ is the total number of elements in the time series data of finite size. We need to determine an embedding dimension $n$, preferably $n = 3, \ldots, 7$ in order to represent the data in consecutive patterns of size of dimension $n$. For a particular dimension $n$ there will be $n$ permuted arrangements of in equalities possible like for $n = 3$ we will have $u_1, u_2, \ldots, u_6$ six arrangements of permutations as $u_1 = \{x_1, x_2, x_3\}, u_2 = \{x_1, x_3, x_2\}, u_3 = \{x_2, x_1, x_3\}, u_4 = \{x_2, x_3, x_1\}, u_5 = \{x_3, x_1, x_2\}, u_6 = \{x_3, x_2, x_1\}$. Then we calculate the probability $P_u$ for a single permutation arrangement as the ratio of number of values for the particular arrangement of the total number of values for all possible permutations for the embedding dimension $n$ in the time series data. Now, we can calculate the simple Shannon entropy for a particular permutation arrangement and the
permutation entropy is given by the sum of these entropies for an embedding dimension $n$ as,

$$H_n = -\sum_{u=1}^{n} P_u \log(P_u)$$

(2.33)

where, $0 \leq H_n \leq 1$. The mapped permutation entropy spectrum of time series $x(t)$ is indicated by $H = \{H_1, H_2, \ldots, H_N\}$. In self-organized state one has $H \rightarrow 0$. 