A MARKOVIAN MODEL FOR COOPERATIVE INTERACTIONS IN PROTEINS

A stochastic model for cooperative interactions in proteins is proposed. The description is based on the theory of Markov's chains and of birth-and-death processes. Even if the model depends only on two parameters: the *mean probability* p and the *coupling capacity* Δp, it presents a surprising wealth of qualitative behaviors when the two parameters are varied. In particular we provide numerical evidence of change of concavity of the stationary distribution at a critical value of the coupling capacity Δp. The main mathematical feature is that the probability of creating a new chemical bond depends on the total number of bonds already present in the system. In this sense, we speak of a cooperative behavior.

1. Introduction

Dynamical models for the description of protein folding/unfolding have been studied by many authors.[6,7,11] In this paper we propose a phenomenological model of protein folding based on the remark that, as known from experience,[4] when the polypeptidic chain is collapsing toward the native conformation, the greater the number of the chemical bonds already formed among amino acidic residues, the greater the *probability* that additional bonds can be formed. In this sense, we can say that our model of protein folding is a *cooperative* one.

The proposed model is a first approximation of the complex chain of events leading to the protein folding/unfolding and it is based on the following assumptions:

(a) the macromolecules do not interact (therefore a single protein is considered);
(b) all the external physico-chemicals parameters (temperature, pH, ionic strength, etc.) are constant;
(c) all the intramolecular weak chemical bonds leading to the folding are of the same type but the probability of creating a new bond is not constant, increasing with the number of already formed bonds.

The first statement of this last assumption is very crude for a protein where a hierarchy of bonds between amino acidic residues exists. A simpler biochemical phenomenon such as the helix-coil transition in homopolipeptides,[16] is closer to the proposed model. In a forthcoming paper a more elaborate model taking into account hierarchy of bonds between amino acidic residues will be proposed.[2]

The present model is very simple and depends only on two parameters: the *mean probability* p and the *coupling capacity* Δp. Yet, as it will be shown both by theoretical analysis and numerical simulation, it renders sufficiently well the intuitive picture one has of the cooperative aggregation phenomenon. Moreover it also presents a surprising wealth of qualitative behavior when the two parameters are varied.

Our model is a stochastic one, whose main mathematical feature is that the probability of creating a new bond depends on the total number of bonds already present in the system. We would also like to point out the *universal character* of the model, in the sense that, even though it has been developed for protein folding, its general framework and the results are applicable to many different cooperative phenomena.

These phenomena are characterized by a sharp transition, where a large variation of the *output* corresponds to a very small variation of the independent variable, followed by an almost equilibrium situation (*sigmoidal behavior*), and by an increase of the noise of the output at the midpoint of the transition.

Many examples can be given and, among the best known ones, we mention only a few:

(i) the sequential binding of four oxygen molecules to a molecule of hemoglobin[5];
(ii) the thermal transition from the *gel* phase to the *sol* phase of artificial and natural membranes[8];
(iii) the conformational transition of macromolecules (DNA, proteins, etc.)[11,16];
(iv) the pressure dissociation of oligomeric proteins.[17]

Experimental evidence, supporting our model, can be found in Ref. 17, where the dissociation of oligomeric protein is related to a parameter, called *conformational drift* which plays a role analogous to our *coupling capacity* Δp, in the sense that it is proportional to the time fluctuation of dissociation and therefore to the cooperativity of that phenomenon. As a matter of fact, as we prove in a forthcoming paper,[1] this analogy is very strict because our model allows to deduce the *sigmoidal behavior* and to express the parameter describing the steepness of the sigmoid in terms of our coupling parameter Δp.

On the other hand, we underline the fact that, while our model refers to folding and dissociation of *single proteins*, the experimental data in Ref. 17 refer to ensembles of proteins. In fact the experimental techniques (such as the atomic resolution microscopy) currently available to analyze the behavior of single proteins are still under development. For that reason, the process of folding/unfolding of proteins

is currently studied by techniques of molecular dynamics.[12] This consists, in a detailed computer simulation, of all the atomic trajectories starting from the known native state (by X-ray crystallography) of small proteins. As an example, we have considered the recent paper of Daggett and Levitt[9] where the authors explore the unfolding of the bovine pancreatic trypsin inhibitor (BPTI) through molecular dynamic simulations. In this cited paper, many figures describing the time evolution of the secondary structure of the BPTI are reported.

As the secondary structure of a protein is related to the number of weak chemical bonds and our model applies both to the folding and unfolding processes of proteins, the data of one figure of the cited paper have been extracted and analyzed with our model. The results of this analysis are reported in Sec. 7.4 and the corresponding graphs (Figs. 13 and 14) show that, even in its admitted simplicity, our model is satisfactorily applicable to the BPTI data in the sense that it captures its qualitative behavior (cf. the discussion at the end of Sec. 7).

The aim of this note is the characterization of the cooperativity of the system, as an increasing function of the coupling capacity Δp. Moreover, for p fixed, a critical value Δp_0 of Δp exists, corresponding to the change of concavity of the stationary distribution (cf. Secs. 3 and 7). The parameter Δp also measures the *complexity* of the system, in the sense that for $\Delta p = \Delta p_0$, the *fractal dimension* of the system reaches its maximum (see Sec. 7).

Our qualitative analysis of the system combines theoretical analysis and computer simulation.

A detailed description of the content of this paper is at the end of Sec. 2.

2. The Stochastic Model for Cooperative Interactions in Proteins

We consider a certain number of particles (molecules) which can be connected by chemical bonds. Presently, we shall only consider the possible pairings among particles, each of which will be described as a *binary random variable*, i.e. taking the value $+1$ if the pairing has been activated and -1 if it has not. More precisely:
if N is the total number of pairings among the particles of the system (i.e. chemical bonds), a *configuration* is defined by a sequence of binary random variables $\{\xi_i^{(k)}\}_{i=1,\cdots,N}$, such that $\xi_i^{(k)} = +1$ if the ith pair is linked at the discrete time k, $\xi_i^{(k)} = -1$, otherwise. The evolution of the system, starting from an initial configuration ξ^0, is described in terms of the *total energy* of the system at time k:

$$S_N(\xi^{(k)}) = \sum_{i=1}^{N} \xi_i^{(k)} \tag{2.1}$$

and of two parameters p and Δp.

The number p, which we call the *mean probability*, is the probability of forming a chemical bond when the total number of existing bonds is exactly $N/2$. Physically,

this parameter is inversely related to the activation energy for the formation of that chemical bond.

The number Δp, which we call the *coupling capacity*, is the maximum increment of the probability to form a chemical bond, for a given p.

Indeed, as a first approximation, we suppose that:

$$\Pr\left(\xi_i^{(k+1)} = +1 \,\middle|\, \xi^{(k)}, \xi^{(k-1)}, \cdots, \xi^{(0)}\right) = p + \frac{\Delta p}{N} S_N(\xi^{(k)}). \tag{2.2}$$

It is clear from (2.2) that for Δp *large*, we have a fast *growth* of the folded protein molecule (i.e. high probability of creating new bonds).

The energy $S_N(\xi^{(k)})$ represents the *excess number of positive over negative bonds*, i.e. the number of bonds which have been activated at time k *minus* the number of those which have not. It can assume all integer values between $-N$ and $+N$.

For each fixed time k, the random variables $\xi_1^{(k)}, \cdots, \xi_N^{(k)}$ are supposed to be independent. Thus, if we fix the energy at time k to be n, i.e. $S_N(\xi^{(k)}) = n$, the random variables $\xi_1^{(k+1)}, \cdots, \xi_N^{(k+1)}$ are Bernoullian with distribution

$$\Pr\left\{\xi_i^{(k+1)} = +1 \mid S_N(\xi^{(k)}) = n\right\} = p + \Delta p \frac{n}{N}$$

and therefore the probability that at time $k+1$ the energy of the system is m, given that at time k it was n, is

$$\begin{aligned} &\Pr\left(S_N(\xi^{(k+1)}) = m \mid S_N(\xi^{(k)}) = n\right) \\ &= \binom{N}{m} \left(p + \frac{\Delta p}{N} n\right)^m \left[1 - \left(p + \frac{\Delta p}{N} n\right)\right]^{N-m}. \end{aligned} \tag{2.3}$$

This means that the sequence of r.v. $S_N(\xi^{(1)}), S_N(\xi^{(2)}), \cdots, S_N(\xi^{(k)}), \cdots$ is a Markov chain with state space $\{-N, \cdots, 0, 1, \cdots, N\}$ in which a transition from an energy state n at time k to another state m at time $k+1$ occurs with the probability given by (2.3).

It can be shown that, in the nontrivial cases, the chain is ergodic, so there exists a unique stationary probability $\pi_i, i = -N, \cdots, 0, \cdots, N$, that is the probability for the system to stay at state i, in equilibrium (i.e. after an infinite time), is irrespective of the initial state.

In Sec. 3, we show how to construct the Markov process and we discuss the existence of absorbing states as a function of the parameters of the model; moreover we show how the stationary probabilities can be computed by means of an exact algorithm (for computer simulation and numerical results see Sec. 7).

In Sec. 4, we give an equivalent description of the evolution of the system in terms of a birth-and-death process, which represents the same process, looking at a smaller timescale.

In Sec. 5, we consider the problem of exploring the *fractal nature* of the simulated trajectories of the system, by computing the *fractal dimension* of the set whose elements are the points of a trajectory, and relating it to the parameters p and Δp.

In Sec. 6, the problem of parameter estimation is considered and solved by means of a maximum likelihood method.

Section 7 deals with computer simulation: we describe the qualitative behavior of the system obtained by varying the parameter p and Δp. Moreover, we report the numerical results relative to Secs. 3–6.

The numerical results are commented in Sec. 8.

3. Modelling the System by Means of a Markov Process

3.1. *The Markov chain*

Here, we briefly show how to construct a Markov chain (MC) with one-side state space $\{0, 1, \cdots, N\}$.

In the notations of Sec. 2, let N be the total number of permitted couplings between the particles of the system; if r is the number of particles then $N = \left[\frac{r}{2}\right]$.

The energy of the system at time $k \geq 0$ is supposed to be proportional (and, in fact, identified with a proper choice of the unit of measure) to the random variable $S_N(\xi^{(k)})$, defined by (2.1).

Note that Eq. (2.2) is consistent only if the following condition holds:

$$0 \leq p \pm \Delta p \leq 1\,. \tag{3.1}$$

This follows from defining $\xi_i^{(k)} = +1$ or $\xi_i^{(k)} = -1, \forall i = 1, \cdots, N$ in (2.2). We also suppose that the further condition is satisfied:

$$p \geq \Delta p > 0 \tag{3.2}$$

which amounts to saying that the interaction potential is not greater than the average potential.

Setting $\eta_i = (\xi_i + 1)/2$, we obtain that the r.v. η_i can assume values 0, +1, with probabilities:

$$\begin{aligned}
\Pr\big(\eta_j^{(k+1)} = +1\big) &= \Pr\big(\xi_j^{(k+1)} = +1\big) \\
&= p + \frac{\Delta p}{N} \sum_{h=1}^{N} \big(2\eta_h^{(k)} - 1\big) \\
&= (p - \Delta p) + 2\frac{\Delta p}{N} \sum_{h=1}^{N} \eta_h^{(k)}\,, \qquad (3.3) \\
\Pr\big(\eta_j^{(k+1)} = 0\big) &= 1 - \Pr\big(\eta_j^{(k+1)} = +1\big)\,.
\end{aligned}$$

Then, if we define a new *energy* in the variables η_i, at time k:

$$\Lambda_N(\eta^{(k)}) = \sum_{h=1}^{N} \eta_h^{(k)} \tag{3.4}$$

and define $X_k := \Lambda_N(\eta^{(k)})$, we obtain an MC with state space $\{0, 1, \cdots, N\}$ with transition probabilities matrix given by

$$\begin{aligned} p_{nm} &= \Pr\left(X_{k+1} = m \mid X_k = n\right) \\ &= \binom{N}{m} \left[p - \Delta p + \frac{2\Delta p}{N} n\right]^m \cdot \left[1 - (p - \Delta p) - \frac{2\Delta p}{N} n\right]^{N-m} . \end{aligned} \tag{3.5}$$

Here, the state $m \in \{0, 1, \cdots, N\}$ is in fact the number of activated bonds (i.e. the number of pairs $i \in \{0, 1, \cdots, N\}$ such that $\xi_i = +1$).

Following Feller,[10] a class C of states of an MC is said to be *closed* if any exit from this class is impossible, that is if $p_{ik} = 0$, $i \in C$, $k \in \{0, \cdots, N\} - C \doteq T$. The probability y_i that the system will pass finally into the class C, starting from the initial state i, is given by the minimal solution of the equation (see Ref. 10):

$$y_i = \sum_{\nu \in T} p_{i\nu} y_\nu + \sum_{\nu \in C} p_{i\nu} . \tag{3.6}$$

A single state j forming a closed class is called *absorbing*, that is $p_{jj} = 1$, $p_{jk} = 0$, $\forall\, j \neq k$. If the process enters an absorbing state, it remains there forever.

Moreover, a state j of an MC is said to be *persistent* (see Ref. 10) if, starting from the state j, a return to j is certain, that is the probability of such an event is 1. Otherwise, if the probability is positive, but less than 1, the state j is called *transient*.

If the process starts from a transient state, two cases are possible: in the first case it enters the class of persistent states and it remains there forever; in the second one, the process remains in the class of transient states forever. If the state j is transient, we have[10]:

$$\sum_{n=0}^{\infty} p_{ij}^{(n)} < \infty \quad \forall i \tag{3.7}$$

which implies

$$p_{ij}^{(n)} \to 0 \quad \text{as} \quad n \to \infty \quad \forall i \in \{0, \cdots, N\} . \tag{3.8}$$

Here, $p_{ij}^{(n)}$ is the probability that the system passes from the state i into the state j in n steps.

The Markov chain is called *irreducible* if there exists no closed set other than the set of all states; a sufficient condition for irreducibility is that $p_{nm} > 0 \,\forall\, n, m$. In the case where the MC is irreducible, (in particular if $p_{nm} > 0 \,\forall\, n, \forall\, m$) the ergodic

theorem holds and there exists an invariant distribution $\{\pi_k\}$, $k = 0, \cdots, N$ such that

$$\lim_{n\to\infty} p_{ik}^{(n)} = \pi_k \; \forall i \in \{0, \cdots, N\}. \tag{3.9}$$

The probabilities π_k are called *stationary probabilities*, in our case π_k represents the probability that the process stays at state k, at equilibrium, i.e. after an infinite time, irrespective of the initial state i.

Now, we set $\alpha = p - \Delta p, \beta = 2\Delta p$; let us suppose that p and Δp are not fixed, but are functions of N such that, for N large, the difference $\alpha = p - \Delta p$ becomes almost zero. Specifically, let us suppose that α is a decreasing function of N, $\alpha = \alpha(N) = \gamma/N^u, u > 1$. This corresponds to the situation in which, for large-N, the difference between the mean potential and the interaction potential goes to zero more quickly than $1/N$. The fact that $p \cong \Delta p$ for N large, can be justified by observing that, when the number of pairings, N, becomes infinite, and the number of existing bonds is also large, then only minor modifications can occur in the system, i.e. only a few additional bonds can be formed and only a few bonds can be destroyed.

In the situation above, from (3.5) we obtain, as $N \to \infty$:

$$\begin{aligned} p_{nm} &\sim \frac{\left(\beta n + O\left(1/N^{u-1}\right)\right)^m}{m!}\left(1 - \beta n/N + o(1/N)\right)^N \\ &\sim \frac{(\beta n)^m}{m!}(1 - \beta n/N)^N \sim \frac{(\beta n)^m}{m!}e^{-\beta n}. \end{aligned} \tag{3.10}$$

Therefore, in the case $\alpha = \alpha(N) = \gamma/N^u, u > 1$, we obtain the estimate, as $N \to \infty$:

$$p_{nm} \sim \frac{(\beta n)^m e^{-\beta n}}{m!}. \tag{3.11}$$

This means that the process X_{t+1} conditioned to X_t is a Poisson process with mean βX_t.

3.2. *The properties of the MC*

Consider some special cases of the MC with transition probabilities given by (3.5).

3.2.1. *The case when* $p = \Delta p \neq \frac{1}{2}$

From (3.5) we obtain:

$$p_{nm} = \binom{N}{m}\left(2p\frac{n}{N}\right)^m\left(1 - 2p\frac{n}{N}\right)^{N-m}. \tag{3.12}$$

Thus $p_{00} = 1, p_{0n} = 0 \forall n \in \{1, \cdots, N\}$. Therefore, the state 0 is an *absorbing state*. In terms of the variable ξ_i, we see that the state 0 corresponds to an energy $S_N(\xi) = -N$, that is to the case of full uncoupling. Putting $C = \{0\}$ in (3.6) we

obtain that the probability of ultimate absorption in the state 0, starting from any state i, is equal to 1.

3.2.2. *The case when* $p + \Delta p = 1$, $p \neq \frac{1}{2}$

From (3.5) we obtain:

$$p_{nm} = \binom{N}{m} \left(1 - 2\Delta p\,(1 - n/N)\right)^m \left(2\Delta p\,(1 - n/N)\right)^{N-m}. \tag{3.13}$$

Thus $p_{NN} = 1$ and $p_{Nm} = 0 \,\forall\, m \neq N$.

Therefore, the state N is *absorbing*; it corresponds to the situation of full coupling. From (3.6) it follows that the probability of ultimate absorption in the state N is 1.

3.2.3. *The case when* $p = \Delta p = \frac{1}{2}$

From (3.5) we obtain:

$$p_{nm} = \binom{N}{m} \left(\frac{n}{N}\right)^m \left(1 - \frac{n}{N}\right)^{N-m}. \tag{3.14}$$

It is easily seen by induction that:

$$\sum_{m=0}^{N} m \cdot p_{nm} = n\,, \tag{3.15}$$

that is the MC is a *martingale*. It also results $p_{00} = p_{NN} = 1$; then the states 0 and N are absorbing, the other states are transient. The process, starting from the state n, will ultimately finish in either the state 0 or state N. By using (3.15), we obtain:

$$p_{nN}^{(k)} \to \frac{n}{N} \text{ as } k \to \infty\,. \tag{3.16}$$

Therefore, the probabilities of ultimate absorption in 0 and N, starting from the state n, are respectively

$$\left(1 - \frac{n}{N}\right) \quad \text{and} \quad \frac{n}{N}\,. \tag{3.17}$$

These values can also be found by solving Eq. (3.6).

By using the formula: (see e.g. Ref. 10)

$$\tau_j = 1 + \sum_{h=1}^{N-1} p_{jh}\tau_h \tag{3.18}$$

one can compute the expected time τ_j of ultimate absorption in the closed class $\{0, N\}$, starting from j (see Sec. 7 for numerical results).

3.2.4. *The case when* $p > \Delta p > 0$ *and* $p + \Delta p < 1$

It is the most interesting case from the physical point of view. Indeed, for $\Delta p = 0$ the Markov process reduces to a Binomial process:

$$p_{nm} = \binom{N}{m} p^m (1-p)^{N-m} . \tag{3.19}$$

Then, one expects that, if $\Delta p > 0$ and $\Delta p << p$, the system behaves approximately as in the Binomial case. In fact, the numerical simulation confirms the behavior for $\Delta p << p$.

It is easy to see that $p_{nm} > 0 \forall n, m$ and this implies that the chain is irreducible. Indeed, as is easily seen from the definition of p_{nm} (see (3.5)), the second factor in (3.5) is always positive (since $p > \Delta p$), as is the third one, because

$$1 - p + \Delta p - \frac{2\Delta p}{N} n \geq 1 - p + \Delta p - 2\Delta p = 1 - p - \Delta p > 0, \ n = 0, 1, \cdots, N$$

since $p + \Delta p < 1$.

Then, the ergodic, stationary probabilities $\pi_i = 0, 1, \cdots, N$ exist and they can be computed by solving (see e.g. Ref. 10) the equation:

$$\pi_i = \sum_j \pi_j p_{ji} \tag{3.20}$$

which is the left eigenvector problem for the matrix P with the corresponding eigenvalue 1. From the ergodic theorem, it follows that:

$$\lim_{n \to \infty} \Pr\,(X_n = i) = \pi_i = \lim_{n \to \infty} p_{ki}^{(n)} \tag{3.21}$$

irrespective of the initial state k, where $p_{ki}^{(n)}$ is the probability of transition from the state k to the state i, in n steps.

Now, we introduce a heuristic argument which suggests a guess for an asymptotic approximation of the stationary probabilities given by (3.21), in the limit $N \to \infty$. We shall see that, at least for some values of the parameters, this guess is confirmed by the numerical simulations.

A heuristic application of the law of large numbers suggests the following approximation, for N *large*:

$$\frac{\Lambda_N(\eta^{(k)})}{N} = \frac{1}{N} \sum_{i=1}^{N} \eta_i^{(k)} \sim a_k \tag{3.22}$$

or

$$\frac{S_N(\xi^{(k)})}{N} = \frac{1}{N} \sum_{i=1}^{N} \xi_i^{(k)} \sim 2a_k - 1 , \tag{3.23}$$

where $a_k = E(\eta_i^{(k)}) = \Pr\,(\eta_i^{(k)} = +1)$.

Now, from (2.2) and (3.3), taking into account (3.22), (3.23), we obtain:

$$\begin{aligned} a_{k+1} = \Pr\left(\eta_i^{(k+1)} = +1\right) = \Pr\left(\xi_i^{(k+1)} = +1\right) &= p + \Delta p S_N(\xi^{(k)})/N \\ &\sim p + \Delta p\,(2a_k - 1) = (p - \Delta p) + 2a_k \Delta p \\ &= (p - \Delta p) + 2\Delta p\left((p - \Delta p) + 2a_{k-1}\Delta p\right) \end{aligned} \tag{3.24}$$

and, iterating this procedure, we have for any integer $h < k$:

$$a_{k+1} = (p - \Delta p)\sum_{i=0}^{h}(2\Delta p)^i + 2^{h+1}\Delta p^{h+1} a_{k-h}\,. \tag{3.25}$$

For $k = h$, we obtain:

$$a_{k+1} = (p - \Delta p)\sum_{i=0}^{k}(2\Delta p)^i + 2^{k+1}\Delta p^{k+1} a_0\,.$$

In the limit $N \to \infty, k \to \infty$, this suggests the approximation:

$$a_\infty := \lim_{k\to\infty} a_k \sim \frac{p - \Delta p}{1 - 2\Delta p}\,. \tag{3.26}$$

Note that the term $2^{k+1}\Delta p^{k+1}$ tends to zero since $\Delta p < 1 - p < 1 - \Delta p$ implies $\Delta p < \frac{1}{2}$; moreover (3.26) is consistent, since $p + \Delta p < 1$ implies $\frac{p-\Delta p}{1-2\Delta p} < 1$.
Hence

$$\Pr(X_k = n) = \Pr\Big(\sum_{i=1}^{N}\eta_i^{(k)} = n\Big) = \binom{N}{n} {a_k}^n (1 - a_k)^{N-n} \tag{3.27}$$

from which we finally obtain the following estimate for the stationary probability for *large* N:

$$\begin{aligned} \pi_n = \lim_{k\to\infty}\Pr(X_k = n) &\sim \binom{N}{n} {a_\infty}^n (1 - a_\infty)^{N-n} \\ &= \frac{1}{(1 - 2\Delta p)^N}\binom{N}{n}(p - \Delta p)^n (1 - \Delta p - p)^{N-n},\ \Delta p \neq \frac{1}{2}\,. \end{aligned} \tag{3.28}$$

A more rigorous theoretical estimate of the stationary probabilities π_n can be found by considering the diffusion process obtained by the continuous approximation of the rescaled Markov chain, which has a beta function for stationary probability density.[3]

4. Modelling the System by Means of a Birth-and-Death Process

In this section we shall describe the system by means of a birth-and-death process (BDP). If the state of the system at time t is given by $X_t = n \in \{0, 1, \cdots, N\}$, (that is there are exactly n links between particles, or n coupled particles, at time t), we

suppose that the system changes only through transitions to the nearest neighbors of the state n (that is from n to $n+1$ or $n-1$, if $n \neq 0, N$, but from 0 to 1 and from N to $N-1$ only). We also suppose that the probability that during a time interval $(t, t+h), h > 0$, more than one change occurs is $o(h)$. Moreover:

$$\begin{cases} \Pr\big(X_{t+h} = n+1 \mid X_t = n\big) = \lambda_n h + o(h)\,, \\ \Pr\big(X_{t+h} = n-1 \mid X_t = n\big) = \mu_n h + o(h)\,. \end{cases} \tag{4.1}$$

Here, the numbers λ_n and μ_n are called, respectively, the *birth* and *death* rates, by analogy with population growth processes. The choice of λ_n and μ_n characterizes the model since it prescribes the way according to which changes can occur in the system. Let us denote:

$$P_n(t) = \Pr(X_t = n)\,,\ n \in \{0, \cdots, N\}\,. \tag{4.2}$$

From the general theory of birth-and-death processes it follows (see e.g. Ref. 10) that for all bounded coefficients $\lambda_n \geq 0$, $\mu_n \geq 0$, with any initial conditions:

$$P_n(0) = \delta_{in}\,,\ \text{if}\quad X(0) = i\,, \tag{4.3}$$

the numbers $P_n(t)$ can be uniquely found for all t in such a way that they satisfy the condition:

$$\sum_n P_n(t) = 1\,. \tag{4.4}$$

If $\lambda_0 = 0$, the transition $0 \to 1$ is impossible and 0 is an absorbing state from which no exit is possible; once the system is in state 0, it remains there forever. In this case $P_0(t)$ increases monotonically. The limit $P_0(\infty)$ is the probability of ultimate absorption in 0.

Analogously, if $\mu_n = 0$, the transition $N \to N-1$ is impossible and N is an absorbing state; $P_N(t)$ increases monotonically to the limit $P_N(\infty)$, which represents the probability of ultimate absorption in N. It can be shown that the limits:

$$\lim_{t\to\infty} P_n(t) = p_n \tag{4.5}$$

exist and are independent of the initial conditions (4.3). The stationary probabilities $p_0, \cdots, p_N$, satisfy a certain system of linear equations (see e.g. Ref. 10). In fact they are given by:

$$\begin{cases} p_0 = \left(1 + \dfrac{\lambda_0}{\mu_1} + \lambda_0 \displaystyle\sum_{i=2}^{N} \frac{1}{\mu_i} \prod_{j=1}^{i-1} \frac{\lambda_j}{\mu_j}\right)^{-1}, \\ p_1 = \dfrac{\lambda_0}{\mu_1} p_0\,, \\ p_l = \dfrac{\lambda_0 p_0}{\mu_l} \dfrac{\lambda_1 \cdots \lambda_{l-1}}{\mu_1 \cdots \mu_{l-1}}\,,\ l = 2, 3, \cdots N\,. \end{cases} \tag{4.6}$$

Now, we return to the problem of the appropriate choice of the birth rate λ_n and the death rate μ_n. The considerations of the previous section, which led to describing the evolution of the system in terms of a Markov process, suggest now to take λ_n and μ_n as follows:

$$\begin{cases} \lambda_n = p_{n\,n+1}\,,\ n = 0, \cdots, N-1\,;\ \lambda_N = p_{NN} = (p + \Delta p)^N\,, \\ \mu_n = p_{n\,n-1}\,, n = 1, \cdots, N\,;\ \mu_0 = p_{00} = (1 - p + \Delta p)^N\,, \end{cases} \tag{4.7}$$

where p_{ij} is given by (3.5).

Again, if $p = \Delta p$, from (3.5) it follows that $\lambda_0 = p_{01} = 0$ and the state 0 is absorbing, as in the case of the MC; if $p + \Delta p = 1$, from (3.5) it follows that $\mu_N = p_{N\,N-1} = 0$ and the state N is absorbing. Finally, if $p = \Delta p = 1/2$, we obtain, as in the case of MC, that 0 and 1 are absorbing states.

By means of the above construction, we have described the evolution of the system in terms of a BDP, which, up to events with probability of order h^2, jumps only from a state to one of its nearest neighbors during the interval $(t, t + h)$. In physical terms, h represents the mean time of formation or destruction of a link between two particles.

5. Fractal Dimension

Here, we deal with the fractal nature of the trajectories of the system, obtained by computer simulation.

It will be interesting to investigate the (eventual) structure of strange attractor for our models of protein folding; this approach has been followed for some physical model of proteins.[14]

We limit ourselves to consider the *fractal dimension* of the single trajectories of the system whose evolution is carried by the MC.

We recall that the fractal dimension (of covering) of a set E embedded in $\mathbb{R}^d$ is defined by (see Ref. 15):

$$D = \lim_{r \to 0^+} \left\{ [\log N(r)] / [\log (1/r)] \right\}, \tag{5.1}$$

where $N(r)$ is the number of *d-cubes* of side r which cover the set E. For $d = 1$, let $E = \{X_k : k = 0, \cdots, L\} \subset [0, N]$, the continuous approximation (obtained by linear interpolation) of the discrete trajectory (simulated by computer) of length L starting from X_0; we have computed the *approximation* of the fractal dimension of the set E rescaled to the interval [0, 1], obtained by taking r small enough in (5.1), and we have compared (for the same value of r) the quantities so found, for several choices of X_0 and various values of p and Δp. It is clear that the fractal dimension of any trajectory obtained by computer simulation is zero, being a discrete set. However, by computing the quantity in (5.1) for r small enough, but not infinitesimal, one can obtain a non-trivial qualitative information on the nature

of the trajectory. This information is given by a number which we (improperly) call *fractal dimension.*

6. Estimation of Parameters

In this section we consider the problem of estimating the parameters p and Δp in the case of the MC. We make use of a *maximum likelihood* method.

If $\{i_0, i_1, \cdots, i_L\}$ represents a trajectory of the process $\{X_t\}$ starting from i_0 at time 0, L being the final time, we can consider the likelihood function:

$$\mathcal{L}(p, \Delta p) = \prod_{k=0}^{L} p_{i_k\, i_{k+1}}(p, \Delta p)\,, \tag{6.1}$$

where $p_{ij}(p, \Delta p)$ is the transition probability from state i to state j, which was given by (3.5). By maximizing the logarithm of the likelihood function, we can find the estimate $(\hat{p}, \hat{\Delta} p)$ of the parameters $p, \Delta p$. In practice, we have limited ourselves to simulated trajectories with given input values of p and Δp and, supposing them unknown, we have recovered estimates of these values, by using the above method. The same method has been used to analyze the BPTI data.[9]

7. Computer Simulation and Numerical Results

In this section, we deal with the qualitative behavior of the system, obtained by means of computer simulation and examine all the numerical results relative to Secs. 3–6. Graphical outputs are reported.

We wrote some FORTRAN Programs to simulate the time evolution of the process, and we have studied the behavior of the system, by varying the parameters of the model, in both MC and BDP cases.

In Figs. 1–3 the plots referring to some typical simulation runs, in the cases of the MC and BDP are reported.

7.1. *Stationary probabilities and absorbing states for the MC*

Let us consider the qualitative behavior of the stationary probabilities $\pi_i, i = 0, \cdots, N$, as a function of the parameters p and Δp, in the case when the evolution of the system is described by means of the MC.

To find the stationary probability distribution of the states of the system at equilibrium, an exact algebraic computation has been used. Indeed, though the convergence of the process to the equilibrium occurs at an exponential rate,[3] the numerical simulation would require rather long CPU time, because the number of states that the system is allowed to assume is large (for large-N). Then, the stationary probabilities have been *exactly* found by numerical computation of the eigenvector in Eq. (3.20). Some care has been used in this operation. Indeed, practical numerical difficulties have been found to compute the left eigenvector of

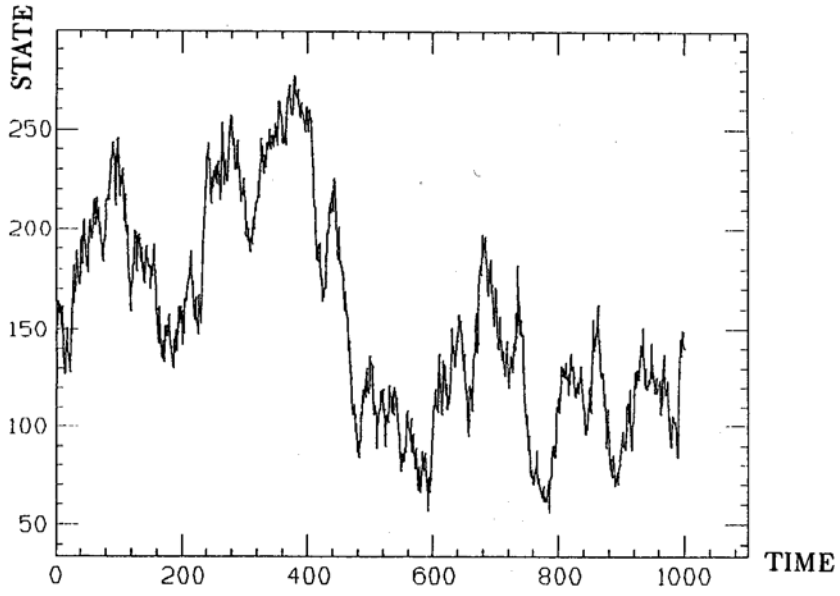


Fig. 1. Graph of Monte–Carlo simulation of a trajectory of the system described by the Markov chain (MC) in a state vs time for $N = 300$, $p = 0.5$, $\Delta p = 0.495$ and $X_0 = 150$ (initial state). From the diagram, it is obvious that there are small fluctuations around the mean value.

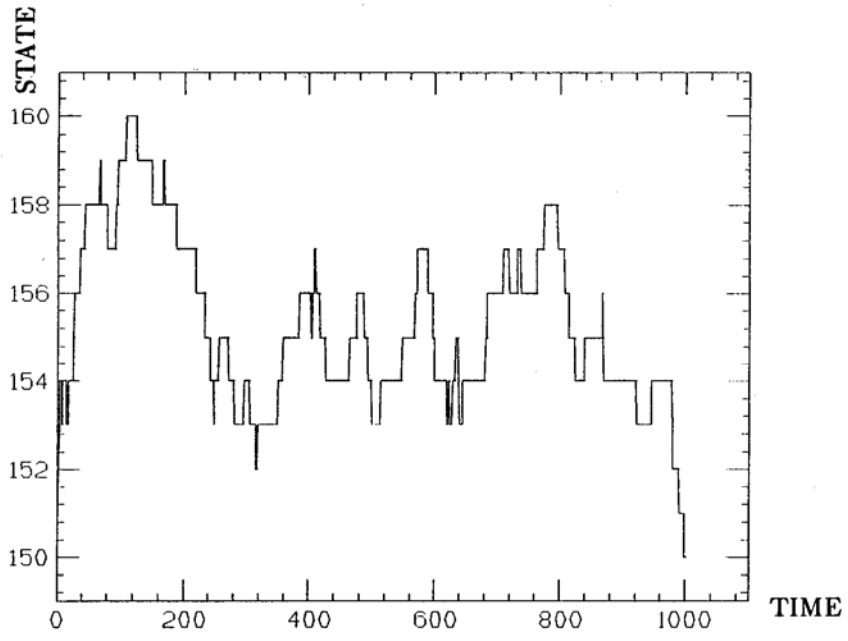


Fig. 2. Graph of the state of the system vs time obtained by simulating the BDP; the values of the parameters and the initial datum are the same as in Fig. 1. Compare this with Fig. 1, there is qualitative similarity of the trajectories of the BDP and of the MC. Note, however, that in the BDP the probabilities to remain in the same state are appreciably nonzero, which does not seem to happen for the MC.

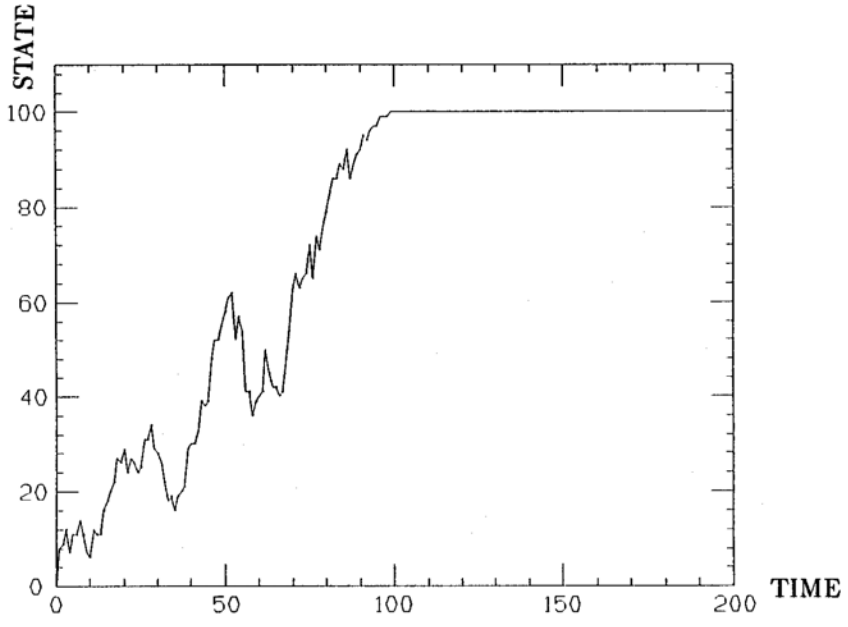


Fig. 3. Graph of the state of the system vs time obtained by simulating the MC; here $N = 100$, $p = 0.51$, $\Delta p = 0.49$ and $X_0 = 0$. Notice that N is absorbing (since $p + \Delta p = 1$), and the system ultimately ends up in state N and remain there.

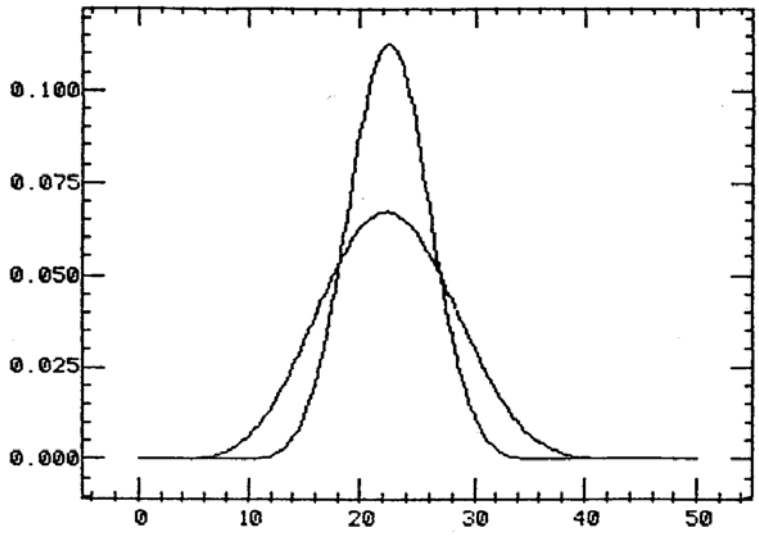
the matrix $P = (p_{ij})$, since, for large-N, the entries p_{ij} are very small. Moreover, the computation of p_{ij} itself, involves the binomial coefficient $\binom{N}{j}$ (see Eq. (3.5)), and this is a nontrivial problem from a numerical point of view, for large j and N.

Figures 4 a–d show that, for values of the parameter Δp far from the critical one ($\Delta p = \frac{1}{2}$), he agreement between the stationary probabilities numerically computed by means of the exact formula (3.20) and those calculated by means of our conjecture (see (3.28)) is excellent.

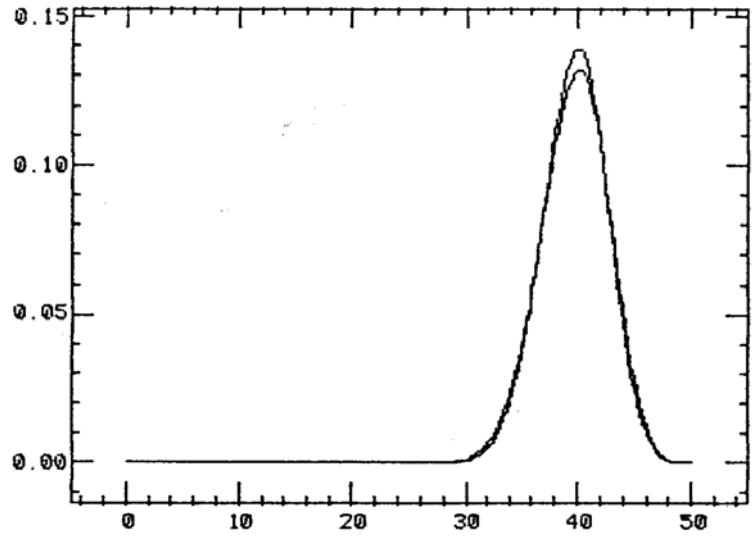
However, we have used the exact formula (3.20) to compare the stationary probabilities, at different values of parameters. In fact, in Fig. 5, we report a plot, concerning the case of MC, of the stationary probability π_i as a function of the state $i = 0, \cdots, N$, for several values of the parameter, in the case when the state N is *almost absorbing.*

We have computed by formula (3.18) the expected absorbing times. For N *large* and $p = \Delta p = \frac{1}{2}$, one can find the following approximation for the times τ_j, $j = 0, \cdots, N$ of ultimate absorption in the closed class $\{0, N\}$, starting from j:

$$\begin{cases} \tau_0^* = 0\,, \\ \tau_j^* = -2N\left(\frac{j}{N}\ln\left(\frac{j}{N}\right) + \left(1 - \frac{j}{N}\right)\ln\left(1 - \frac{j}{N}\right)\right), \text{for } j = 1, \cdots, N-1 \\ \tau_N^* = 0\,. \end{cases} \tag{7.1}$$



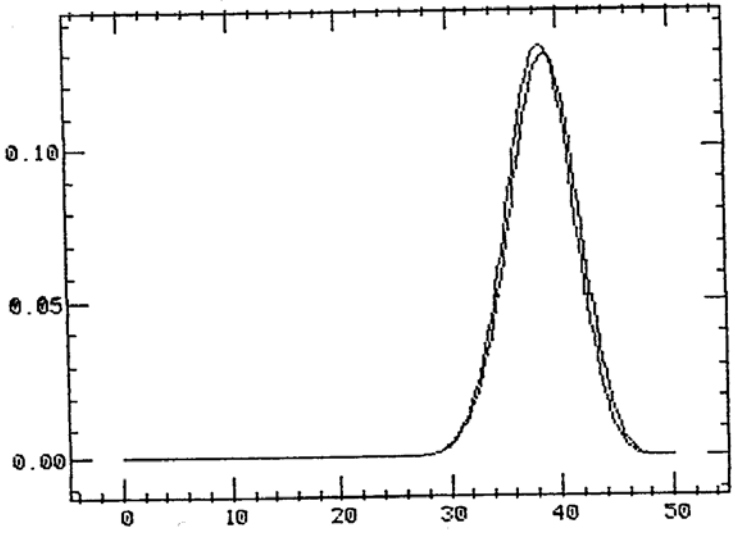
(a)



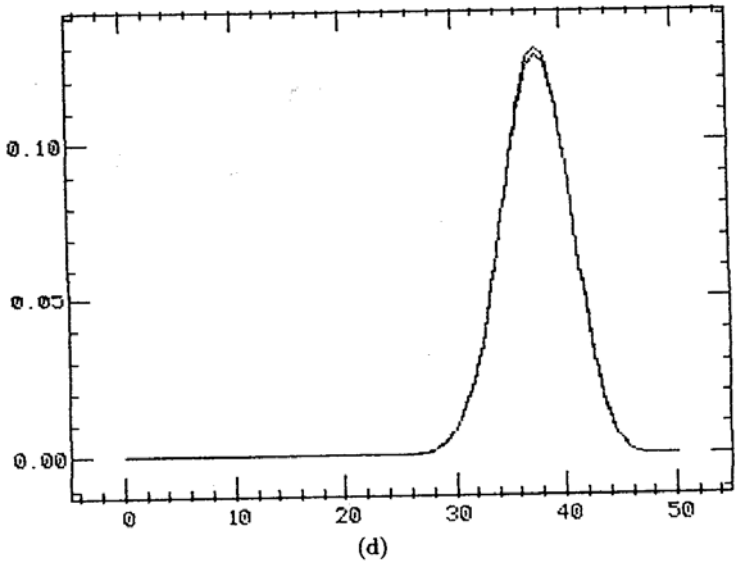
(b)

Fig. 4. Graphs of the stationary probabilities numerically computed by the exact formula (3.21) and by means of the estimate (3.30). (a) $p = 0.49$, $\Delta p = 0.4$; (b) $p = 0.7$, $\Delta p = 0.16$; (c) $p = 0.7$, $\Delta p = 0.12$; (d) $p = 0.7$, $\Delta p = 0.1$. Note that for $p = \frac{1}{2}$ the estimate (3.30) is meaningless, since it gives rise to a binomial distribution with parameter $\frac{1}{2}$, independent of Δp. Moreover, the more p is far from $\frac{1}{2}$, the better the agreement is between the curves.

This estimate for τ_j can be found, by using the diffusion approximation of the MC.[3] In Fig. 6a a plot of τ_j versus state j is reported for $p = \Delta p = 0.4$ and $N = 100$ (0 absorbing state). In Fig. 6b a comparison of the graphs of τ_j and τ_j^* are reported, for $p = \Delta p = \frac{1}{2}$ (0,1 absorbing states).



(c)



(d)

Fig. 4. (*Continued*)

For $j = \frac{N}{2}$, the expected time of absorption in $\{0, N\}$ is $\tau_{N/2} \approx \tau^*_{N/2} = 2N\ln 2$.

Letting the parameters p and Δp vary according to the constraints (3.1) and (3.2), we have observed that for p fixed and values of Δp not very large, the distribution of the states at equilibrium appears to be close to the binomial distribution. By increasing Δp, the shape of the curve appears to be more and more flat, until for a critical value $\Delta p = \Delta p_0$, for almost all $i \in \{0, \cdots, N\}$ (except some states in

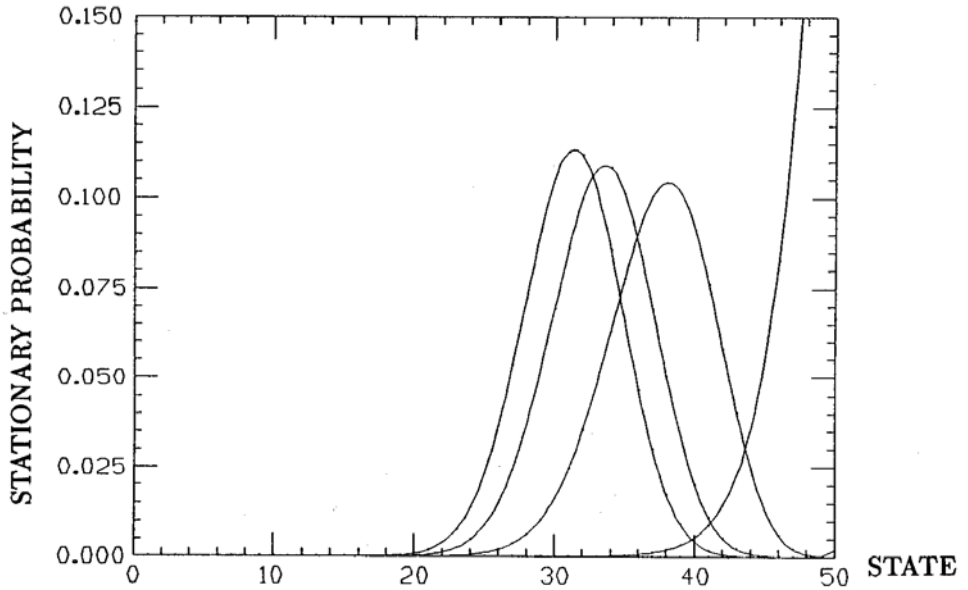


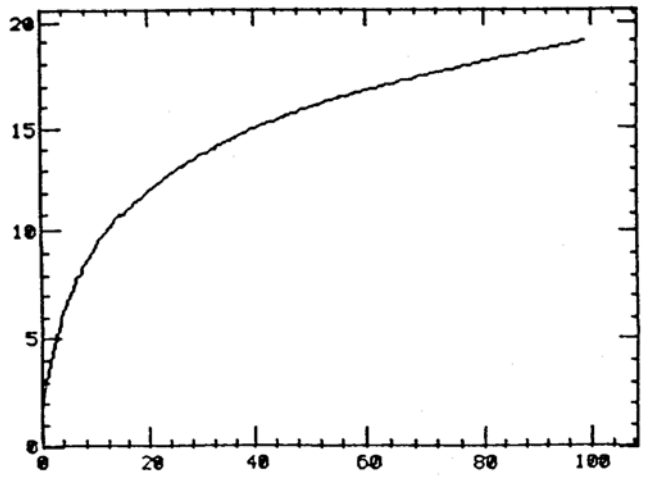
Fig. 5. Graph of the stationary probability vs the state in the case of the MC for $N = 50$, $p = 0.6$ and several values of Δp from 0^+ to $(0.4)^-$, proceeding from the left to the right. Note that for $\Delta p \approx 0.4$ (which implies $p + \Delta p \approx 1$) the curve approaches the graph of the function $\delta(i - N)$.

the neighbours of the ends), the stationary probabilities π_i assume almost the same constant value. Then, the states of the system are *almost uniformly distributed, at equilibrium.* If each π_i were exactly equal to $1/(N+1)$, the transition matrix P would be bistochastic, i.e.

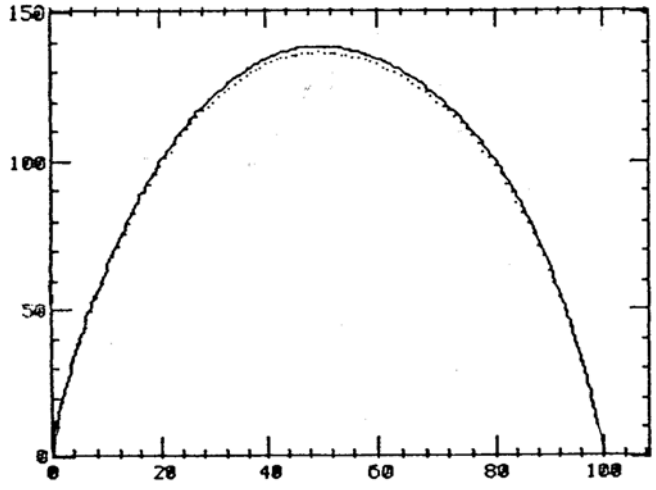
$$\sum_{j=0}^{N} p_{ij} = 1 = \sum_{i=0}^{N} p_{ij} \,. \tag{7.2}$$

Really, for $\Delta p = \Delta p_0$, the numerical calculation shows that the matrix P is *almost symmetric* in the sense that $p_{ij} - p_{ji}$ is near zero for most of $i \in \{0, \cdots, N\}$; moreover $\max_{i,j} \mid p_{ij} - p_{ji} \mid / p_{ij}$ is very small. In fact, for $\Delta p = \Delta p_0$, (p fixed), the function $\sum_{i,j} \mid p_{ij} - p_{ji} \mid$ reaches the minimum, as we have obtained by numerical minimization. Beyond the critical value Δp_0, the curve of π_i versus i shows a gradual change of concavity, passing from a negative to a positive second derivative, until as $\Delta p \to \frac{1}{2}^-$ it approximates the function $\frac{1}{2}[\delta(i-0) + \delta(i-N)]$ (for $p = \Delta p = 0.5$ the two states at the extrema are absorbing).

In Fig. 7, we report some plots of the stationary probabilities π_i as a function of the state i, for some values of the parameter Δp and p fixed equal to $\frac{1}{2}$; there is a visible change of behavior, when Δp reaches the critical value Δp_0.



(a)



(b)

Fig. 6. (a) Graphs of the expected time τ_j of absorption in the absorbing state 0, for $p = \Delta p = 0.4$, $N = 100$, as a function of the initial state $j = 0, 1, \cdots, N$; (b) compared graphs of τ_j^* (upper curve) and τ_j (lower curve), for $p = \Delta p = \frac{1}{2}$, $N = 100$, here 0 and N are absorbing states (see Sec. 7 for the definitions of τ_j and τ_j^*).

7.2. *Stationary probabilities for the BDP and discussion*

Let us consider the stationary distribution of states in the case of BDP; we observe that, if $\lambda_n = \mu_{n+1}$, from (4.7) one obtains $p_0 = p_1 = \cdots = p_k = \cdots = 1/(N+1)$, that is the stationary probabilities are the same for every state $i \in \{0, 1, \cdots, N\}$.

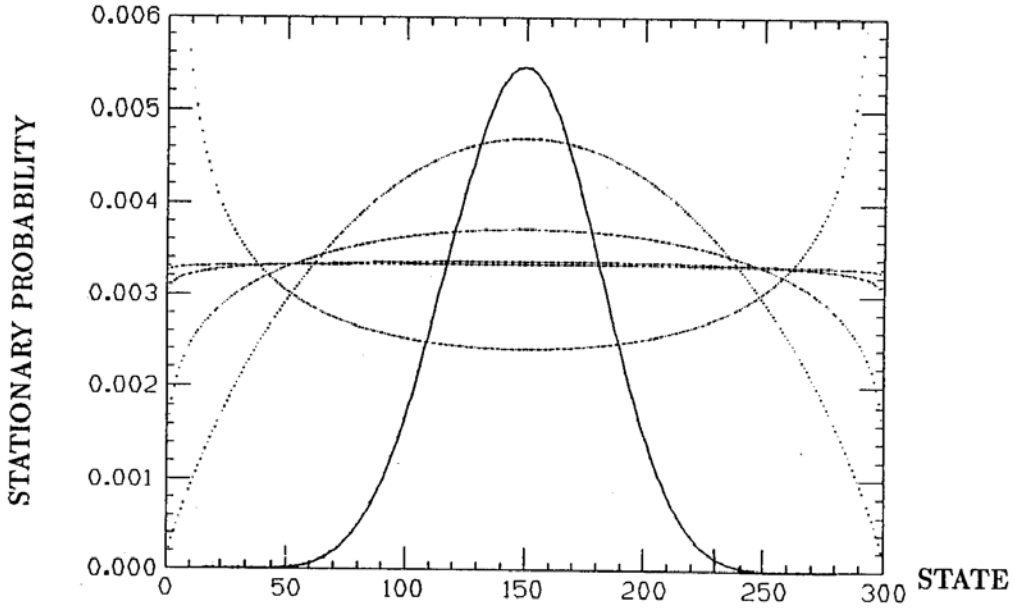


Fig. 7. Graph of the stationary probability vs the state in the case of the MC for $N = 300$, $p = 0.5$ and several values of Δp. When Δp increases, the shape of the curve appears to be flatter, unttil for $\Delta p = \Delta p_0 = 0.49834$, the stationary probabilities are almost the same $\forall\, i$, indicating that the states are almost uniformly distributed, at equilibrium. For $\Delta p > \Delta p_0$ an inversion of concavity is observed.

This corresponds to the situation in which the stationary distribution of the states is uniform. In this case, from (4.8) we obtain $p_{n\,n+1} = p_{n+1\,n}$, that is:

$$\begin{aligned}\Psi(p,\Delta p,n) \doteq & \binom{N}{n+1}\Big(p-\Delta p+\frac{2\Delta p}{N}n\Big)^{n+1}\Big(1-p+\Delta p-\frac{2\Delta p}{N}n\Big)^{N-n-1} \\ & -\binom{N}{n}\Big(p-\Delta p+\frac{2\Delta p}{N}(n+1)\Big)^{n}\Big(1-p+\Delta p-\frac{2\Delta p}{N}(n+1)\Big)^{N-n} \\ & = 0\,. \end{aligned} \tag{7.3}$$

As it is easy to see, for p fixed, the equality (7.3) can be thought of as an equation in the unknown Δp, having no solution independent of $n \in \{0, \cdots, N\}$. The only thing that we can hope to achieve is to find the value of Δp, say $\Delta p_0'$, for which $\sum_{n=0}^{N} \mid \Psi(p,\Delta p,n) \mid$ is minimum, (p fixed).

This can be obtained by numerical minimization of the function above. Indeed, we have obtained, for $p = \frac{1}{2}$, a value $\Delta p_0'$ close to Δp_0, where Δp_0 is the value of Δp for which the *almost* uniform stationary distribution of states $\{\pi_i\}$ has been observed in the case of the MC (see above). By inserting the value $\Delta p_0'$ into Eq. (4.6), we have found that the stationary distribution of states $\{p_i\}$ looks very similar to $\{\pi_i\}$.

in both the MC and BDP cases. In Fig. 8, we report a graph in which the stationary probabilities π_i and p_i are plotted as a function of the state $i = 0, 1, \cdots, N$, in both cases (MC and BDP), for $\Delta p = \Delta p_0$. In the case of MC, the stationary probabilities π_i have been computed by finding the left eigenvector with eigenvalue 1 of the transition probability matrix given by (3.5); in the case of BDP, the stationary probabilities p_i have been computed by using (4.6).

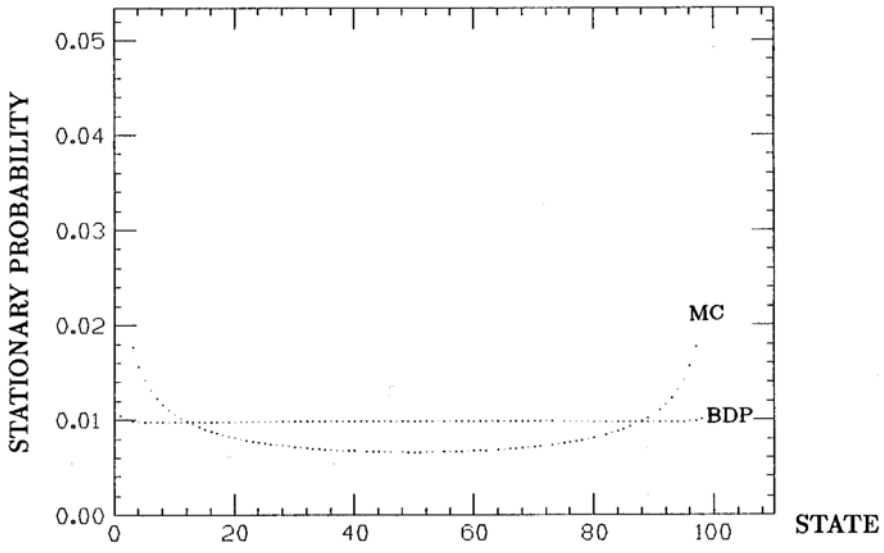


Fig. 8. Different graphs of the stationary probability vs the state in the cases of the MC (upper curve) and the BDP (lower curve). Here $N = 100$, $p = 0.5$, $\Delta p = 0.4974$. The figure shows that, except for a small region near the extremal states, there is a good agreement between the stationary probabilities of the BDP and those of the MC.

Now, let us consider some remarks about the relation (4.6); we observe that it becomes almost natural, when comparing our BDP with a simpler Markov chain (SMC) having transition probabilities:

$$p_{nn+1} = \frac{\lambda_n}{\lambda_n + \mu_n}\,;\; p_{nn-1} = \frac{\mu_n}{\lambda_n + \mu_n}\,,\; n = 1, \cdots, N-1$$

$$p_{ij} = 0 \quad \text{if} \quad |\, i - j \,| > 1\,, \tag{7.4}$$

where λ_n and μ_n are given by (3.8).

In this SMC, the only transitions which are allowed in one step are: $n \to n+1$ and $n \to n-1$, and they have the same conditional probabilities as in the BDP. The only difference is that, in the BDP changes can occur at arbitrary times, so that the number of transitions during a time interval of length t is a random variable.

On the other hand, if t is large, the number of transitions is also large, and so it is natural to think that, as $t \to \infty$, the probabilities $P_n(t)$ behave as the corresponding probabilities of the SMC.

We also observe that the process carried by the SMC never remains fixed, while in the case of BDP the system may remain in the same state, for several steps. Notice that, at any step, the process described by the MC can have jumps of arbitrary lengths, corresponding to unphysical conditions, because during the mean time of formation or destruction of a link only one bond can be formed or cut. While the trajectories of the BDP show a tendency to remain for an appreciably nonzero time in some states, this does not seem to happen for the trajectories of the Markov chain (cf. Figs. 1 and 2). Then, a description in terms of BDP is more satisfactory from a physical point of view.

For several initial states, we have performed many simulation runs and we have observed that the three processes (MC, SMC, BDP) are qualitatively equivalent, in the sense that, MC and SMC appear to be as the BDP *accelerated*. Obviously, from a practical point of view, the simulation obtained with the BDP is more convenient, since at every step, the number of coupled particles can change only by ± 1; on the other hand, if one is interested in the behavior of the process at equilibrium (that is after an infinite time), the *accelerated* description in terms of MC is more convenient, since it saves a lot of computer time.

7.3. *Fractal dimension*

Now, we report the results concerning the fractal nature of simulated trajectories.

First, let us consider the dependence of the state of the system at time $k+1$ from the state at time k, for several values of the parameters p and Δp, in the case of the MC. We obtain some nice figures in which X_{k+1} is plotted as a function of X_k; for $p = 0.5$ and $\Delta p = \Delta p_0$ (see above for the definition of Δp_0), the curve appears to fill up a *flattened* shell around the bisecting line (see Figs. 9 and 10).

In Fig. 11 a plot of the fractal dimension D of the trajectories versus Δp, for p fixed, is reported. Precisely, for $p = 0.5$ fixed, we have let Δp vary from 0 to 0.5; for *small* values of Δp, the process is *persisting*, that is very *localized* around the mean value. Then the *complexity* of the system should be small, and in fact D is small. When Δp increases, the process become more *erratic* and the fractal dimension D also increases, until it reaches a maximum for $\Delta p = \Delta p_0$ (the critical value for which the states of the system are almost uniformly distributed, at equilibrium). This is because the system spends *almost equally* the same time in each state; so the trajectory has many loops. For $\Delta p > \Delta p_0$ the fractal dimension decreases, until for $\Delta p \to (\frac{1}{2})^-$ the trajectory starting from a middle state is almost linear, with few loops, and D assumes very small values.

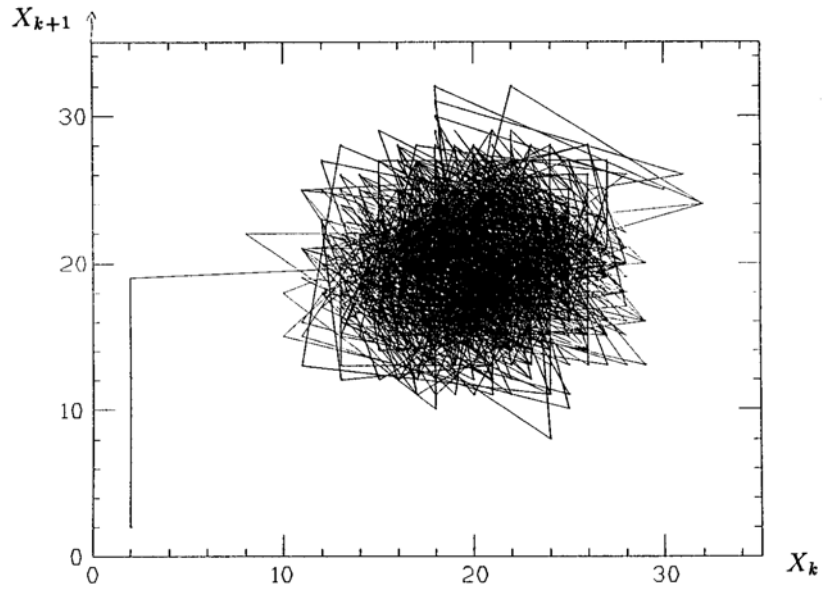


Fig. 9. Graph of the state X_{k+1} vs X_k in the case of the MC, for $p = 0.4$, Δp *small*, $N = 50$ and $X_0 = 0$. Note that a shell around the most probable value $X = 20$ is filled up. Note the completely different qualitative behavior of the same plot, with different parameters, given by Fig. 10.

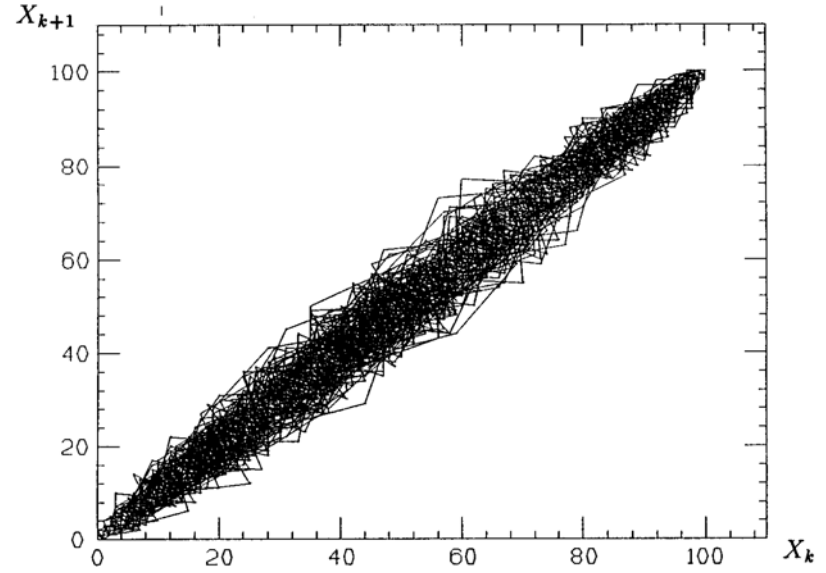


Fig. 10. Graph of the state X_{k+1} vs X_k in the case of the MC, for $p = 0.5$, $N = 100$, and Δp equal to the critical value for which the *almost uniform* stationary distribution is observed. Since the system *visits* all the states with almost the same frequency, a *flattened* shell appears to be filled up, around the bisecting line.

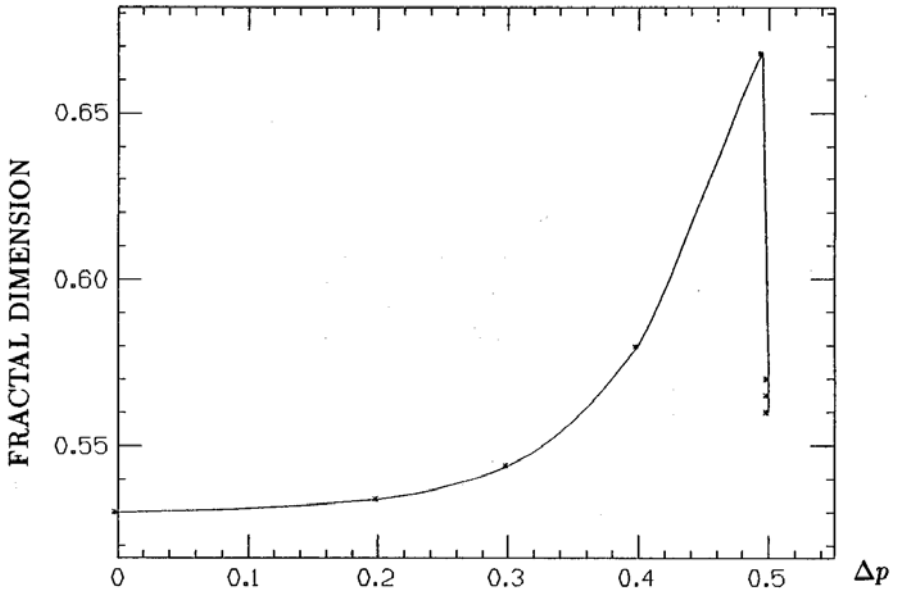


Fig. 11. Graph of the fractal dimension D of the trajectory starting from state 0 vs Δp, in the case of the MC, for $p = 0.5$ fixed. When $\Delta p = \Delta p_0$, D is maximum. It shows that, although the process is a discrete state, nevertheless the *fractal dimension* (cf. Sec. 5 after definition (5.1)) of its trajectories contains some qualitative informations.

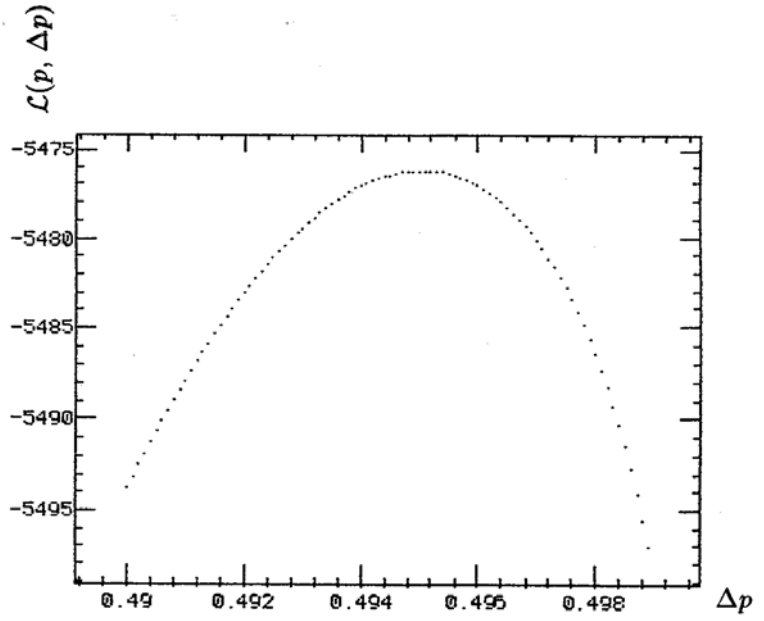


Fig. 12. Graph of the likelihood function $\mathcal{L}(p, \Delta p)$ vs Δp for p fixed ($= 0.5$). Here, the input data were $\Delta p = 0.49505$, $p = 0.5$, $N = 100$. The curve clearly shows a maximum for a value Δp close to 0.49505.

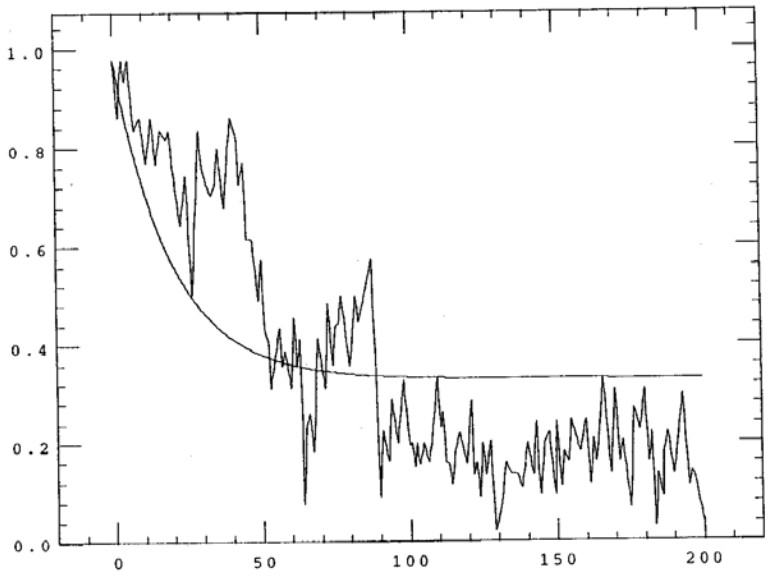


Fig. 13. Graph of the time (ps) evolution of the normalized percentage of secondary structure present in the β-sheet and in the C-terminal α-helix of BPTI at temperature of 498 K (data reported from Fig. 13 (i) of Ref. 9. The superimposed curve represents the time evolution of an averaged process (drift) (cf. the discussion at the end of Sec. 7). The estimated values of $p = 0.4956$ and $\Delta p = 0.4575$ of our model, have been recovered by means of the maximum likelihood method.

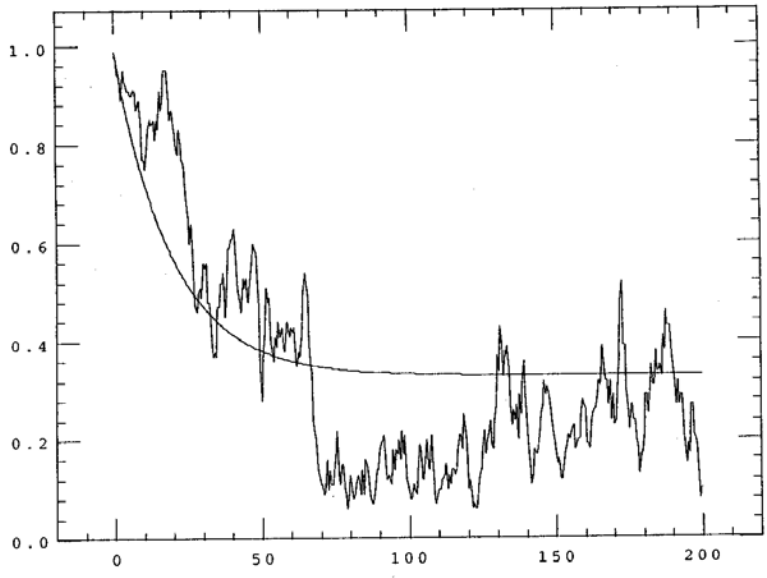


Fig. 14. Graph of the time evolution of a stochastic simulation of the process, based on our model and relative to the values of p and Δp of Fig. 13. The time evolution of the averaged process is also superimposed. The horizontal and vertical scales are as in Fig. 13.

7.4. *Estimation of parameters*

Here, we consider the numerical results relative to Sec. 6. By simulating the time evolution of the system (carried by the MC), with specific input data $\Delta p = 0.3$ and $p = 0.5$, for instance, we have recovered the estimates $\hat{\Delta p} = 0.285..$ and $\hat{p} = 0.499\cdots$, by using an IMSL routine for global minimization with constraints. In Fig. 12, we show a plot of the function $\mathcal{L}(p, \Delta p)$ computed by (6.1), as a function of Δp, for p fixed ($= 0.5$). Here the input data of the simulated trajectory were $\Delta p = 0.49505, p = 0.5$ and $N = 100$. The curve clearly shows a maximum for a value Δp close to 0.49505.

As reported in Sec. 1, we have analyzed the data of Fig. 13 (i) of Ref. 9 with our model. Figure 13 (i) refers to the time evolution of the percentage of secondary structure present in the β-sheet and in the C-terminal α-helix of BPTI at a temperature of 498 K. The starting point is the native state of the BPTI known by X-ray crystallography. The high temperature value has been chosen by the authors to accelerate the unfolding process and to render the time scale accessible by computer simulation.

In Fig. 13 the original data of Ref. 9 are reported. From these data we have estimated the values of $p = 0.4956$ and $\Delta p = 0.4872$ with the maximum likelihood method (cf. Sec. 6).

In Fig. 14 a stochastic simulation of the process, based on our model and relative to the above p and Δp values is reported. In both figures the superimposed smooth line represents the dynamical evolution of a deterministic process (drift) which *drives*, up to fluctuations, the dynamics of the process. This fact suggests the possibility (investigated elsewhere[3,13]) to apply diffusion limit techniques to the present model. As the simulation is stochastic, the time evolution of the process of Fig. 14 is not identical to that of Fig. 13, but the main features (i.e. the decay time and the extent of fluctuations) are very similar. This illustrates the main idea of our model of the folding/unfolding process of a protein: the two parameters, p and Δp, (where Δp is related to the cooperativity of the phenomenon) are deduced from some *experimental* data; given that, the samples of the corresponding process describe, up to fluctuation, the main qualitative features.

8. Concluding Remarks

We have described a mathematical model for the cooperative interactions in proteins which is based on the theory of Markov processes and BDPs. This model is very simple and depends only on two parameters: the *mean probability* p and the *coupling capacity* Δp. As we have shown by both theoretical analysis and numerical simulation, the model renders well enough the intuitive picture one has of the protein folding.

The main mathematical assumption is that, when the polypeptidic chain is collapsing to establish the native protein, the greater the number of the chemical

bonds already formed among amino acidic residues, the greater the *probability* that additional bonds are formed. In this sense we can say that the folding of a protein is a cooperative phenomenon.

Although the model does not take into account the spatial structure of the system, it presents a surprising wealth of qualitative behaviors, when the two parameters are varied. In fact, the behavior of the system is characterized by the *degree of cooperativity* which is measured by the coupling capacity Δp.

The evolution of the system can be described both by a suitable Markov chain and by a BDP, but the behaviors are qualitatively the same. If N is the total number of permitted coupling among particles, the state of the system at time t is defined by the number $n \leq N$ of bonds which are formed, at time t. When $p = \Delta p$, but p and $\Delta p \neq \frac{1}{2}$, the system will ultimately end up in state 0, that is the state in which no chemical bond is formed (full uncoupling), irrespective of the initial state; so state 0 is absorbing. When $p + \Delta p = 1$, but p and $\Delta p \neq \frac{1}{2}$, the system will ultimately finish in state N, irrespective of the initial state, that is the state of full coupling is absorbing. When $p = \Delta p = \frac{1}{2}$, the two extreme states (0 and N) are both absorbing, and the system will ultimately end up in one or the other, with a probability depending on the initial state of the system. Finally, when $p > \Delta p > 0$ and $p + \Delta p < 1$, the Markov chain is irreducible, so the ergodic stationary probability π_i exists, that is the probability that the system stays at state i at equilibrium (i.e. after an infinite time), irrespective of the initial state. When the degree of cooperativity Δp is relatively small, and p is fixed, the extreme states are rarely visited by the system, at equilibrium, and the middle states are priviliged in the sense that, with a large probability, the fraction of bonds (over the total) which are formed among particles is close to p. When Δp increases, the extreme states are visited more frequently leading to large fluctuations of the system state (see also Fig. 7). Similar results, based on experimental data, are reported in Ref. 17.

The main result is that, for p fixed, a critical value of Δp exists, say Δp_0, such that, when Δp increases to Δp_0, the shape of the curve representing the stationary probability versus the state appears to be more and more flat, until for $\Delta p = \Delta p_0$, the stationary probabilities are almost the same for all the states. This means that the states of the system are almost uniformly distributed at equilibrium, that is, ultimately, each state may occur with almost the same probability. For $\Delta p > \Delta p_0$, an inversion of concavity of the curve above is observed.

When $\Delta p = \Delta p_0$, the system is very *erratic* and each state is visited with almost the same frequency, then, after an infinite time, the system does not stabilize around any particular state; on the contrary, it appears to *pulsate* between the two extreme states, that is from the situation of full uncoupling to the one of full coupling, and vice versa, assuming all the intermediate states. Moreover, for $\Delta p = \Delta p_0$, the fractal dimension of the simulated trajectories reaches its maximum.

We have heavily used computer simulation for the examination of the qualitative behavior of the system. All indications are that the parameter Δp is very important, and that it can be taken as a measure of the degree of cooperativity, and also of the *complexity* of the system.

As an example, the proposed model has been applied to a realistic case of protein unfolding reported in literature and relative to a molecular dynamic simulation of bovine pancreatic trypsin inhibitor (BPTI).[9] As reported in Sec. 7.4 and in Figs. 13 and 14, the agreement between the literature data and the data obtained using our model is very satisfactorily. In a forthcoming paper[2] the present model is generalized in order to take into account different types of chemical bonds.