Stochastic Theory for the Kinetics of Migration of Ligands in Biomolecules

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A stochastic approach in terms of master equations with linear and non-linear transition rates for the dynamics of the migration of ligands in biomolecules is presented. Coupling to a bath with constant ligand concentration as well as multiple occupancies by ligands of certain sites inside the biomolecule are allowed. Explicit expressions for the fraction of biomolecules that have not bound a ligand at time t under experimental constraints λ are found by solving the generating function of the probability obeying the master equation. For highly non-linear systems a computer oriented procedure is presented. The validity of a description with a system of coupled linear deterministic equations is discussed. Relevance to experimental data and applications to other biophysical systems are outlined.

1. Introduction

Most biological processes are stochastic because fluctuations are an inherent consequence of the discrete nature of matter. An interesting problem is then the influence of fluctuations in the description of biological systems. When a large number of particles is involved, the values of the macroscopic quantities will vary closely around their mean values. But the stochastic aspects play a crucial role when the chosen macroscopic variables are subject to fluctuations comparable to their mean values. Even in situations where the fluctuations are small they may trigger a transition to a new macroscopic state, as for instance, in allosteric enzymes. In the present paper we develop the theoretical background for the dynamics of biomolecules with emphasis on the effect of fluctuations rather than confining ourselves to a specific biological system.

Much of our knowledge about allosteric interactions and relationships between structure and function can be derived from studies with proteins. Particularly, heme proteins constitute a large group of biomolecules where the effect of fluctuations can be advantageously investigated. These proteins

play primary roles in oxygen storage (myoglobin) and transport (hemoglobin), electron transfer (cytochromes) and detoxification of poisonous chemicals (cytochrome P450). The migration of small ligands in biomolecules studied with flash photolysis (Austin, Beeson, Eisenstein, Frauenfelder & Gunsalus, 1975; Alberding *et al.*, 1978; Sharrock & Yonetani, 1976; Antonini & Brunori, 1971), stopped flow and temperature jump techniques (Gibson, 1956) or Mössbauer experiments (Lang, 1970; Spartalin, Lang & Yonetani, 1976) and infrared absorption (Alben & Caughey, 1968) represents an important step in understanding biomolecular reactions. The discovery that migration of ligands to the active site in heme proteins is governed by multiple barriers (Austin *et al.*, 1975; Alberding *et al.*, 1978) leads to interesting problems in reaction kinetics. For instance, the case of CO-migration in myoglobin with a small ligand concentration in the solvent S can be adequately described in terms of linear rate equations by using a small number of intermediate sequential potential wells. But experimental refinements have revealed additional complexity: At a given intermediate well the biomolecule may exist in many conformational states (Austin *et al.*, 1975); transitions of ligands may occur between any two wells creating alternate competitive pathways; anomalous non-linear ligand migration at the protein-solvent interface at higher ligand concentrations; and a co-operativity triggered by molecular fluctuations preceding ligand transitions. In particular, in the case of CO-migration in myoglobin at high [CO]-ligand concentrations in the solvent (Alberding, Frauenfelder & Hanggi, 1978) binding of CO at the iron is covalent. The first ligand that occupies the active site *blocks* further transitions. The other wells, however, can very likely accept more than one ligand. Then multiple occupancies and blocking lead to large non-linear fluctuations. These features call for a generalization of the treatment of migration of ligands that accounts also for non-linear fluctuations.

In this paper we develop a unified theory for the migration of ligands to the active site over a wide range of physical parameters λ (temperature, hydrostatic pressure, ligand concentration in the solvent, external fields, etc.) based on the theory of stochastic processes. This approach describes naturally the effect of fluctuations in quite general situations. In many cases, the stochastic method is superior to some equivalent non-linear deterministic approach because the former yields the unambiguous non-linear evolution equations and makes possible the study of noise properties, correlation functions as well as initial (ensemble)-preparation effects. We restrict the investigation to biomolecules with only one active site for the particular ligand under consideration. The theoretical treatment can be generalized for more complicated situations by a straightforward adaptation of the ideas presented here. The stochastic approach has proved to be very fruitful for the description of

co-operative phenomena in physics (Haken, 1975; Hanggi & Thomas, 1978) and chemistry (Nitzan, Ortoleva, Deutch & Ross, 1974; Gardiner, McNeil, Walls, Matheson, 1976).

For the following we assume that the migration process is governed by intermediate potential barriers. Evidence supporting this assumption is provided by experimental work (Austin *et al.*, 1975; Alberding *et al.*, 1978) and Monte Carlo studies (Case & Karplus, 1977). The different wells L, $L = 1, \ldots, L_{\max}$, enclosed by the potential barriers represent locally stable sites for the ligand within the biomolecule at certain reaction co-ordinates. In addition, the biomolecule may change in a given well from one conformational state to another before the ligand undergoes a transition into a different well (conformational relaxation). We will use the notation, L^i, $i = 1 \ldots N_{\max}^{(L)}$ for the different conformational sites at a given well L characterized by certain reaction co-ordinates† (Austin *et al.*, 1975; Weber, 1972). The detailed kinetics can then be described using three different kinds of stationary transition probabilities per unit time which in principle can all be determined by experimental techniques:

$$\Gamma(K^j | L^i; \lambda) \tag{1a}$$

$$R(L^j | L^i; \lambda) \tag{1b}$$

$$\Gamma_{L \to S}(L^i; \lambda) \tag{1c}$$

$$\Gamma_{S \to L}(L^i; \lambda) \tag{1d}$$

Equation [1(a)] characterizes a transition from site L^i in well L to site K^j in well K, $L \neq K$, (ligand transition) without conformational change, equation [1(b)] stands for a conformational relaxation transition ($L^i \to L^j$) within the biomolecule where the ligands remain at the given reaction co-ordinate or well but the biomolecule changes the conformation (conformational transitions). The rates in equations [1(c)–1(d)] denote transitions from the states which are in contact with a bath (S) of constant ligand concentration, e.g. corresponding to a surrounding solvent. The bath tends to keep constant the number $n_L^i(t)$ of biomolecules with ligands occupying at time t well L at conformational state L^i. (See section 3.) The variables $n_L^i(t)$ do not behave in a strictly deterministic way, but display statistical fluctuations which always reflect a lack of knowledge about the exact state of the system due to the impossibility of keeping track of the huge number of all microscopic variables in the system. Because of the various blocking properties of the different wells, the

† In general, the "site" L^i may correspond to a whole set of sites with a more or less identical conformational relaxation behavior.

transition rates in equations [1(a)–1(d)] will in general depend in a non-linear way on the stochastic variables $n_L^i(t, \lambda)$.

The paper is organized as follows. In section 2 we first deal with the limiting case ("low concentration limit") in which the ligand concentration in the solvent is so small that at any time t only one ligand sits somewhere inside the biomolecule (no multiple occupancy of different wells). The general case with possible multiple occupancies of sites, L^i, with ligands and blocking will then be treated in section 3. Diffusion effects of ligands in the solvent and cage effects may also play an important role. In both cases we end up with an *analytical* expression for the rebinding rate $N_{\text{exp}}(t, \lambda)$, the fraction of biomolecules that have not bound a ligand at time t under experimental constraints λ. The analytical solutions for $N_{\text{exp}}(t, \lambda)$ are made possible by using a coarse-grained description for the migration process of the ligands, i.e., a description intermediate between a full microscopic approach with a huge number of degrees of freedom and a macroscopic theory which directly describes the (unknown-) non-linear binding rate $N_{\text{exp}}(t, \lambda)$. The results and other biological applications are discussed in section 4.

Some important and useful properties of the generating function are summarized in Appendix A. In Appendix B we give the detailed mathematical development of the solution of the probability function describing the binding process. Appendix C deals with the solution of time dependent mean values, e.g. $N_{\text{exp}}(t, \lambda)$, in highly non-linear systems via continued fraction expansions. A convenient numerical, computer oriented procedure is presented.

2. Stochastic Model for Migration of Ligands in the Low Concentration Limit

In the case of a small or even vanishing number of ligands in the solvent we may assume that only one ligand is inside each biomolecule or inside its immediate neighborhood $[L^s]$ at any time. All the single biomolecule-structures with no ligand are not considered as members of the statistical ensemble for the ligand migration process and the members with more than one ligand are of vanishing influence. Transitions of ligands from state $[L^s]$, (cage), into other parts of the solvent (diffusion) are assumed to be of minor importance. The site $[L^s]$ can then be treated in terms of an additional stochastic variable $n_{L_s}(t, \lambda)$. In this approximation, only one ligand is occupying a certain state L^i in each biomolecule at any time t. In such a "closed system" the number of ligands in a certain site L^i is either 1 or 0. We may assume that the transitions in a given biomolecule occur independently of the transitions in other biomolecules (non-interacting biomolecules). For the sake of simplicity, we may also assume that ligand-transitions in biomolecules

occur only between neighboring wells L:

$$L^i \rightarrow (L \pm 1)^j. \tag{2}$$

In the following we introduce a one-dimensional notation. For the stochastic variable $n_L^i(t, \lambda)$, having at time t and experimental constraints λ, n_L^i biomolecules in well L at conformational state i, we define

$$x_K(t, \lambda) = n_L^i(t, \lambda) \tag{3}$$

with

$$K = \sum_{I=1}^{L-1} N_{\max}{}^{(I)} + i. \tag{4}$$

K will take on values from 1 to N.

The transition probabilities per unit time in equations [1(a)–1(d)] are redefined in a similar way. The final binding site will be denoted in the following by $[x_1(t, \lambda)]$. We use the notation $\mathbf{x}(t, \lambda)$ to denote the stochastic vector process, for the stochastic variables at time t forming the state space and for an actual point in the configuration space. The specific interpretation of $\mathbf{x}(t)$ will be understood from the context.

Since the behavior of different biomolecules is independent and the configuration changes only by single jumps of the ligand we may assume stationary linear first-order transition rates (McQuarrie, 1968)

$$(\ldots, x_I, \ldots, x_K - 1, \ldots) \xrightarrow{\Gamma(x_K|x_I;\,\lambda)} (\ldots, x_I - 1, \ldots, x_K, \ldots) \tag{5}$$

$$\Gamma(x_K|x_I; \lambda) = x_I\, \gamma_{KI}(\lambda).$$

Neglecting fluctuations, the kinetic deterministic rate equations for the variables $x_I(t)$ read

$$\frac{\mathrm{d}x_I}{\mathrm{d}t} = -\sum_{\substack{K=1 \\ K \neq I}}^{N} \gamma_{KI}\, x_I + \sum_{\substack{K=1 \\ K \neq I}}^{N} \gamma_{IK}\, x_K, \; I = 1 \ldots N, \tag{6}$$

where the total number of all ligands is kept constant in the biological system under consideration (closed system), i.e.

$$\sum_{I=1}^{N} x_I\,(t, \lambda) = N_0. \tag{7}$$

By use of the matrix $\Lambda(\lambda)$

$$\Lambda_{IJ}(\lambda) = \gamma_{IJ}\,(\lambda), \; I \neq J \tag{8}$$

$$\Lambda_{II}\,(\lambda) = -\sum_{\substack{J=1 \\ J \neq I}}^{N} \gamma_{JI}(\lambda), \tag{9}$$

equation (6) reads in matrix form

$$\frac{\mathrm{d}\mathbf{x}(t, \lambda)}{\mathrm{d}t} = \Lambda\,(\lambda)\, \mathbf{x}\,(t, \lambda). \tag{10}$$

The structure of the Λ-matrix for $L_{\max} = 4$ is shown in Fig. 1.

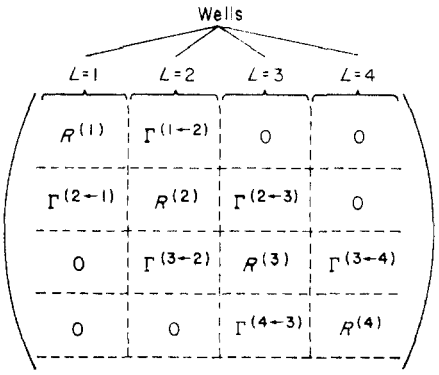

FIG. 1. Structure of the Λ-matrix, for $L_{\max} = 4$. The R-submatrices describe conformational relaxation, the Γ-submatrices the ligand-transitions to neighboring wells.

Note that $\Lambda_{IJ}(\lambda)$ for $I \neq J$ is a reaction constant which for ligand-transitions is given usually in terms of a phenomenological Arrhenius equation, a tunnel rate expression, a diffusion controlled expression, or various combinations of all. Assuming the multi-dimensional process $\mathbf{x}(t) = [x_1(t), \ldots, x_N(t)]$ is a Markov process (Hanggi & Thomas, 1978, Haken, 1975), the master equation, describing the rate of change of the probability $p(\mathbf{x}, t; \lambda)$ that the system at time t has the configuration $\mathbf{x}$ in state space, reads

$$\frac{\partial p}{\partial t}(\mathbf{x}, t; \lambda) = \sum_{I=1}^{N} \sum_{\substack{K=1 \\ K \neq I}}^{N} \Lambda_{KI}(\lambda) \, [(x_I+1)\, p(x_I+1, x_K-1, \mathbf{x}', t; \lambda) - x_I\, p(\mathbf{x}, t; \lambda)]. \tag{11}$$

Here $p(x_I+1, x_K-1, \mathbf{x}', t; \lambda)$ stands for $p(x_1, \ldots, x_I+1, \ldots, x_K-1, \ldots, t; \lambda)$. The experimental data can then be evaluated if the time development of the probability $p(\mathbf{x}, t; \lambda)$ is known. This master equation can be solved for an arbitrary initial probability, $p(\mathbf{x}, o)$, using the technique of the generating function $G(y_1, \ldots, y_N, t; \lambda)$

$$G(y_1, \ldots, y_N, t; \lambda) = \sum_{x_1} \cdots \sum_{x_N} p(x_1, \ldots, x_N, t; \lambda) \prod_{I=1}^{N} y_I^{x_I} \tag{12a}$$

$$\left(\sum_i x_i = N_o\right)$$

yielding

$$\prod_{I=1}^{N} x_I!\, p(x_1, \ldots, y_N, t; \lambda) = \frac{\partial^{x_1 + \cdots + x_N}}{\partial y_1^{x_1} \ldots \partial y_N^{x_N}} G(\mathbf{y}, t; \lambda) \Big|_{\text{all } y_I = 0} \tag{12b}$$

Using some useful and important properties of the generating function G,

reviewed in Appendix A, the following linear first order differential equation for G is obtained

$$\frac{\partial G(\mathbf{y}, t; \lambda)}{\partial t} = \sum_{K=1}^{N} \sum_{I=1}^{N} y_K \Lambda_{KI} \frac{\partial G(\mathbf{y}, t; \lambda)}{\partial y_K}. \tag{13}$$

For a given initial probability $p(\mathbf{x}, o)$ we obtain with the eigenvalues, $\{\mu_K\}$, and the eigenvectors, $\{\mathbf{b}_K\}$, of the matrix

$$\sum_I \Lambda_{JI} b_{KI} = \mu_K b_{KJ}, \tag{14}$$

and the co-factors, B^{KI}, of the eigenvector matrix $\boldsymbol{b}$ for the solution of $G(\mathbf{y}, t; \lambda)$ (see end of Appendix B)

$$G(\mathbf{y}, t, \lambda) = \sum_{x_1} \cdots \sum_{x_N} p(x_1, \ldots, x_N, o) \left(\sum_I x_I = N_o\right) \cdot \left\{ \| \boldsymbol{b} \|^{-1} \sum_{K=1}^{N} B^{KI} \sum_{J=1}^{N} b_{KJ} y_J \exp(\mu_K t) \right\}^{x_I}. \tag{15}$$

$\|\boldsymbol{b}\|$ denotes the determinant of $\boldsymbol{b}$.

For the probability $p^{(1)}(x_1, t; \lambda)$ of having x_1 ligands bound at time t and experimental constraints λ we find by contracting on the stochastic variable $x_1(t, \lambda)$

$$p^{(1)}(x_1, t; \lambda) = \sum_{x_2} \cdots \sum_{x_N} p(x_1, \ldots, x_N, t; \lambda). \left(\sum_{I=2}^{N} x_I = N_o - x_1\right) \tag{16}$$

The experimentally monitored function $N_{\text{exp}}(t; \lambda)$, the fraction of ligands which have not bound at time t to the binding site, is then given by

$$N_{\text{exp}}(t, \lambda) = (N_o - \langle x_1(t, \lambda) \rangle)/N_o, \tag{17}$$

with

$$\langle x_1(t, \lambda) \rangle = \sum_{x_1=0}^{N_o} x_1 p^{(1)}(x_1, t; \lambda). \tag{18}$$

For the interesting case of an initial multinomial probability

$$p(\mathbf{x}, o) = N_o! \prod_{I=1}^{N} \frac{p_I(o)^{x_I}}{(x_I)!} \tag{19}$$

with

$$p_I(o) = \langle x_I(o, \lambda) \rangle / N_o, \tag{20}$$

it is known (Saito, 1974) that the probability $p(\mathbf{x}, t; \lambda)$ at time t will remain of the multinomial form

$$p(\mathbf{x}, t; \lambda) = N_o! \prod_{I=1}^{N} \frac{p_{I(t,\lambda)}{}^{x_I}}{(x_I)!}. \tag{21}$$

The occupation probabilities of the sites, $\mathbf{p}(t, \lambda) = \{p_1, \ldots, p_N; t, \lambda\}$, are obtained by the solution of

$$\frac{\mathrm{d}}{\mathrm{d}t}\,\mathbf{p}(t, \lambda) = \Lambda\,(\lambda)\,\mathbf{p}\,(t, \lambda) \tag{22}$$

with the initial condition in equation (20). It is in this case, that the mean values are simply given by

$$\langle x_I(t, \lambda)\rangle = N_o\, p_I\,(t, \lambda), \tag{23}$$

and coincide exactly for all times t with the values obtained by solving the deterministic equations equation (6) or (10) using the initial conditions in equation (20). Furthermore, in the special case of linear transition probabilities in equation (5), the mean values calculated with the master equation [equation (11)] for any initial probability, are identical with the deterministic values given in equations (6) or (10). This follows by use of an appropriate splitting up of the double sum in equation (11). Hence, two *different* initial probabilities with the same initial mean values yield the *same* mean values at any time t (but different higher moments).

With equation (23) the fraction of ligands $N_{\mathrm{exp}}(t, \lambda)$ that have not bound at time t is in this special case given by

$$N_{\mathrm{exp}}(t, \lambda) = 1 - p_1\,(t, \lambda). \tag{24}$$

For the variance $\sigma^2(t)$ of a stochastic variable $x_I(t, \lambda)$ we obtain (see Appendix A)

$$\sigma_I^2\,(t, \lambda) = N_o\, p_I\,(t, \lambda)[1 - p_I\,(t, \lambda)] \tag{25}$$

and for the covariance $\sigma_{IK}(t, \lambda)$ of $x_I(t, \lambda)$ and $x_K(t, \lambda)$ always the semi-negative result

$$\sigma_{IK}\,(t, \lambda) = -\,N_o\, p_I\,(t, \lambda)\;\, p_K\,(t, \lambda)\; I \neq K. \tag{26}$$

Finally we add some comments relating to the choice of the initial probability $p\,(\mathbf{x}, o; \lambda)$. In situations with a low ligand concentration in the solvent, it is known from photolysis experiments (Austin, *et al.*, 1975, Alberding *et al.*, 1978) that only the conformational states in well $L = 2$ are occupied at time $t = 0^+$ after the flash. Using the initial occupation probability $g^{(2)}(i, \lambda)$, that site 2^i is occupied at $t = 0^+$ we get for the initial condition of equation (22)

$$p_I\,(0^+) = \begin{cases} 0 \\ g^{(2)}(i, \lambda), \text{ if } I = N_{\mathrm{max}}^{(1)} + i;\; i = 1, \ldots, N_{\mathrm{max}}^{(2)} \end{cases} \tag{27}$$

with the normalization

$$\sum_{I=1}^{N} p_I\,(0^+) = \sum_{i=1}^{N_{\mathrm{max}}(2)} g^{(2)}\,(i, \lambda) = 1. \tag{28}$$

The probability $g^{(2)}$ can be determined by fitting to the experimentally monitored function $N_{\mathrm{exp}}(t, \lambda)$ (Austin *et al.*, 1975; Alberding *et al.*, 1978).

It is not difficult to generalize the theory for transitions between non-neighboring wells L of the reaction paths. We stress again, that so far a multiple occupation of a site L^i has not been considered. Hence it has been possible to consider each random variable $x_I(t, \lambda)$ as a certain "chemical species" (McQuarrie, 1968; Saito, 1974) or a "color" where $x_I(t, \lambda)$ has the meaning of the number of biomolecules that are found with "color I" at time t and constraints λ. This enables us to treat the full dynamics of migration of ligands in biomolecules in terms of ligand-transitions and conformational relaxation in an analytical closed form equation (15) whatever the initial probability $p(\mathbf{x}, o)$ is.

Using the experimental binding data (Austin *et al.*, 1975; Alberding *et al.*, 1978; Sharrock & Yonetani, 1976) the approach given here allows for the detailed study of the mechanisms which cause the conformational states inside the biomolecule. Depending on the experimental constraints, λ, the ligand may shuttle many times among the internal conformational states $\{L^i\}$ in the well L before undergoing a ligand transition. The different time scales introduced in these processes as well as the dependence on the initial preparation procedures and experimental constraints, λ, are contained in the detailed structure of the transition matrix, Λ equations (8)–(9) and the experimentally monitored expression $N_{\text{exp}}(t, \lambda)$ given by equations (17), (18), (24). The formulas in equations (25)–(26) make possible the investigation of noise properties in those processes.

3. Stochastic Theory for Migration of Ligands in a Biomolecule

In the general case of biomolecular migration processes of ligands in contact with a solvent (bath) some or all of the ligands may migrate into the solvent. Here, we consider situations where the number L_S of ligands in the solvent S is much larger than the number L_B of ligands within all biomolecules: $L_S \gg L_B$. We employ, as before, a homogenenous state-space description for the problem, i.e. the reaction times for the whole rebinding process are assumed to be much greater than the equilibration times for elastic collision processes among the ligand molecules in the solvent (vanishing ligand-diffusion effects in the solvent). The solvent itself contains a concentration of ligands which is specified by one of the parameter of the set λ characterizing the experimental constraints. All ligands in the solvent then compete for the vacant binding site $[x_1]$. Because of the huge number of ligands in the solvent the concentration of ligands therein can be considered to be constant at any time t (constant bath concentration C_S). Each biomolecule is then in contact with the bath and additional transitions to those considered in section 2

from and into the solvent may occur. [See equations (1c)–(1d).] The number of all ligands inside a single biomolecule is no longer equal to 1 so that we deal with possible multiple occupancies.

For completeness, we mention that diffusion effects could be described by dividing the state space into cells and considering additional transitions from one cell i into an adjacent cell j with probability d. In the case of a cubic cell system with cell length l the deterministic equations for the concentration of ligands in cell $[i]$, $c(i, t; \lambda)$, would have the following structure in the limit of a continuous variable, $\mathbf{r}$, for the cell index (no fluctuation renormalization effects):

$$\frac{\partial\langle c(\mathbf{r}, t; \lambda)\rangle}{\partial t} = \sum_I \bar{\gamma}_{SI}\left(\langle x_I(\mathbf{r}, t)\rangle\right)\langle x_I(\mathbf{r}, t)\rangle - \sum_I \bar{\gamma}_{IS}\left(\langle x_I(\mathbf{r}, t)\rangle\right)\langle x_I(\mathbf{r}, t)\rangle + D\nabla^2\langle c(\mathbf{r}, t; \lambda)\rangle, \tag{29}$$

with

$$D = l^2\,\mathrm{d}. \tag{30}$$

Here D corresponds to Fick's diffusion coefficient and the $\{\bar{\gamma}\}$ denote the rates in cell $i \triangleq \mathbf{r}$ into and from the cell i to the states $[x_I]$ inside the biomolecules in cell $\mathbf{r}$.

Treating the solvent as a bath, again we may assume that transitions in one biomolecule do not influence the transitions in other biomolecules. Then the migration process of the ligands can be described by use of the following set of stochastic variables: We consider a single biomolecule and denote by $x_K(t, \lambda)$, $K = 1, \ldots N$ the number of ligands at site K at time t under constraints λ. Note that in contrast to the case in section 2, $x_K(t, \lambda)$ does pertain to a *single* biomolecule. Hence, the effect of fluctuations plays a major role. Owing to a multiple occupation and blocking of site K, the transition probabilities will in general be *non-linear*. Blocking is especially important for the binding site which completely stops further transitions when $x_1(t, \lambda) = 1$. As in the previous treatment we assume that the occupation changes only by single jumps of ligands. We use then for the stationary *non-linear* transition probabilities the scaling

$$\begin{aligned}&(\ldots, x_I, \ldots, x_K - 1, \ldots) \xrightarrow{\Gamma_{KI}(x_K|x_I;\,\lambda)} (\ldots, x_I - 1, \ldots, x_K, \ldots)\\ &\Gamma_{KI}(x_K|x_I; \lambda) = x_I \gamma_{KI}(x_K, \lambda).\end{aligned} \tag{31}$$

The additional transitions from and into the bath with a constant ligand concentration C_S are assumed to depend only on the occupation of site $[x_I]$ and the bath concentration C_S in the form

$$\Gamma_{SI}(x_I; \lambda) = x_I\, \gamma_{SI}(\lambda) \tag{32}$$

$$\Gamma_{IS}(x_I, \lambda) = C_S\, \gamma'_{IS}(x_I, \lambda) \tag{33}$$

and if γ'_{IS} can be considered to be independent of x_I we obtain

$$\Gamma_{IS}(x_I, \lambda) = C_S \gamma_{IS}(\lambda) = \beta_I(\lambda). \tag{34}$$

With the matrix M

$$M_{KI} = \gamma_{KI}(x_K, \lambda)\, K \neq I, \tag{35}$$

$$M_{II} = -\gamma_{SI}(\lambda) - \sum_{\substack{K=1 \\ K \neq I}}^{N} \gamma_{KI}(x_K; \lambda), \tag{36}$$

the non-linear deterministic equations for the time evolution of the variables $\mathbf{x}$ read

$$\frac{\mathrm{d}\mathbf{x}(t)}{\mathrm{d}t} = \boldsymbol{M}\,\mathbf{x}(t) + \boldsymbol{\beta}, \tag{37a}$$

with

$$\boldsymbol{\beta} = (\beta_1, \ldots, \beta_N). \tag{37b}$$

The master equation with the rates in equations (31)–(34) has the same structure as in section 2 with the sums over I and K extended to include the bath transitions. If the bath couples to one site $\overline{K}$ only and if the coupling of this site is much stronger than to other sites such that

$$\gamma_{S\overline{K}} \to \infty, \gamma_{\overline{K}S} \to \infty; \frac{\gamma_{\overline{K}S}}{\gamma_{S\overline{K}}} = \text{constant} = \rho, \tag{38}$$

then the average occupation of this site is kept constant at the value

$$\langle x_{\overline{K}}(t) \rangle = \rho \cdot C_S. \tag{39}$$

If in addition, $\langle x_{\overline{K}}(t) \rangle$ is large enough so that fluctuations may be neglected, the site $\overline{K}$ itself may serve as a bath and we have one less site. The blocking of the binding state [1] ($x_1 = 0$ or 1) is taken into account by

$$\Gamma_{1K}(x_1 | x_K; \lambda) = x_K \gamma_{1K}(x_1; \lambda)\delta x_1 - 1, 0. \tag{40}$$

In equation (40) δ denotes the usual Kronecker function. The details of the migration process of ligands is then completely given by the solution of the master equation with the non-linear rates. We are interested in the probability $p^{(1)}(x_1 = 0, t; \lambda)$ that the active site, [1], is not occupied with a ligand at time t

$$p^{(1)}(x_1 = 0, t; \lambda) = \sum_{x_2} \cdots \sum_{x_N} p(x_1 = 0, x_2, \ldots, x_N, t; \lambda), t > 0 \tag{41}$$

$$= 1 - \langle x_1(t, \lambda) \rangle, \tag{42}$$

which for non-interacting biomolecules is identical with the experimentally monitored function $N_{\text{exp}}(t, \lambda)$, the fraction of biomolecules that have not bound a ligand at time t. An analytical solution of the master equation for the non-linear vector process $\mathbf{x}(t, \lambda)$ is in most cases not available. There may not even be a tractable numerical solution. However, the form of equation (42) forces us to settle for a numerical solution of the mean value $\langle x_1(t, \lambda) \rangle$ in

quite general situations. This is attained by using an analytical continuation of the short time behavior of the mean value in equation (42) (Taylor expansion in t) by means of a *continued fraction* solution of the high frequency expansion for the Fourier-transform $x_1(\omega, \lambda)$ of $\langle x_1(t, \lambda)\rangle$ (Hanggi & Thomas, 1978; Hanggi, 1977):

$$x_1(\omega, \lambda) = \cfrac{C_1}{-i\omega + \cfrac{C_2}{1 + \cfrac{C_3}{-i\omega + \cfrac{C_4}{1 + \ldots}}}} = \frac{C_1}{-i\omega +}\,\frac{C_2}{1 +}\,\frac{C_3}{-i\omega +}\cdots \tag{43}$$

The continued fraction coefficients $\{C_i\}$ can be calculated via a recursive scheme if the operator in the master equation and the initial probability $p(\mathbf{x}, 0)$ are known. The details of this procedure are sketched in Appendix C.

An analytical solution is available if we can neglect the non-linear effects in all except the binding site $[x_1]$, i.e., if:

$$\text{(i)}\quad K \neq 1\colon \Gamma_{KI}(x_K|x_I; \lambda) = x_I\, \gamma_{KI}(\lambda),\ x_I = 0, 1, \ldots, \infty \tag{44}$$

$$\text{(ii)}\quad \Gamma_{1I}(x_1|x_I; \lambda) = x_1\, \gamma_{1I}(\lambda)\delta_{x_1 - 1, 0}. \tag{45}$$

Assuming further a complete trap property for the binding site $[x_1]$ (absorbing state) we have

$$\text{(iii)}\quad \gamma_{SI}(\lambda) = \gamma_{KI}(x_1, \lambda) = 0;\ K = 2, \ldots, N. \tag{46}$$

We also assume no direct coupling of the solvent to the binding site, i.e.

$$\text{(iv)}\quad \beta_1(\lambda) = 0. \tag{47}$$

The Markovian master equation for the probability $p(\mathbf{x}, t; \lambda)$ reads then explicitly:

$$\begin{aligned}
\frac{\partial p(\mathbf{x}, t; \lambda)}{\partial t} &= \sum_{I=2}^{N} \sum_{\substack{K=2 \\ K \neq I}}^{N} \gamma_{KI}(\lambda)\,(x_I + 1)p(x_I + 1, x_K - 1, \mathbf{x}', t; \lambda) \\
&+ \sum_{I=2}^{N} \gamma_{SI}(\lambda)\,(x_I + 1)p(x_I + 1, \mathbf{x}', t; \lambda) \\
&+ \sum_{I=2}^{N} \beta_I(\lambda)p(x_I - 1; \mathbf{x}', t; \lambda) \\
&- \sum_{I=2}^{N} \sum_{\substack{K=2 \\ K \neq I}}^{N} \gamma_{KI}(\lambda)\, x_I\, p(\mathbf{x}, t; \lambda) - \sum_{I=2} \gamma_{SI}(\lambda) x_I\, p(\mathbf{x}, t; \lambda) \\
&- \sum_{I=2}^{N} \beta_I(\lambda)p(\mathbf{x}, t; \lambda) \\
&+ \sum_{I=2}^{N} \gamma_{1I}(\lambda)\,(x_I + 1)\,\delta x_1 - 1, 0\, p(x_I + 1, x_1 - 1, \mathbf{x}', t; \lambda) \\
&- \sum_{I=2}^{N} \gamma_{1I}(\lambda) x_I\, \delta x_1, 0\, p(\mathbf{x}, t; \lambda).
\end{aligned} \tag{48}$$

In order to solve this master equation we use the generating function which obeys the following evolution equation derived from equation (48) (see Appendix A)

$$\frac{\partial G(\mathbf{y}, t; \lambda)}{\partial t} = \sum_{I=2}^{N} \sum_{\substack{K=2 \\ K \neq I}}^{N} \gamma_{KI}(\lambda)(y_K - y_I) \frac{\partial G(\mathbf{y}, t; \lambda)}{\partial y_I}$$

$$+ \sum_{K=2}^{N} \beta_K (y_K - 1) G(\mathbf{y}, t, \lambda) + \sum_{K=2} \gamma_{SK}(\lambda)(1 - y_K) \frac{G(\mathbf{y}, t; \lambda)}{\partial y_K}$$

$$+ \sum_{x_1} \cdots \sum_{x_N} \sum_{I=2}^{N} \gamma_{1I} [(x_I + 1)\, \delta x_1 - 1, o\, p(x_I + 1, x_1 - 1, \mathbf{x}', t; \lambda)$$

$$- x_I\, \delta x_1, o\, p(\mathbf{x}, t; \lambda)] \prod_{i=1}^{N} y_i^{x_i} \tag{49}$$

Comparison of equation (12) and equation (42) shows that we are interested in the case where $y_1 = 0$ and $y_2 = \ldots = y_N = 1$:

$$N_{\text{exp}}(t, \lambda) = G(0, 1, \ldots 1, t; \lambda) = p^{(1)}(x_1 = 0, t, \lambda). \tag{50}$$

By use of the eigenvectors $\mathbf{b}_I = (b_{I1}, \ldots, b_{IN})$, $I = 1 \ldots N$, with eigenvalues μ_I of the matrix $\mathbf{M}$ defined with the constant elements $\gamma_{IK}(\lambda)$ and the cofactor matrix $\mathbf{B}$ of the matrix of eigenvectors $\boldsymbol{b}$, the explicit solution for G in equation (50) with an initial probability $p(\mathbf{x}, 0)$ reads (for the detailed solution see Appendix B)

$$N_{\text{exp}}(t, \lambda) = G(0, 1, \ldots 1, t; \lambda) = \exp\left\{ - \|\boldsymbol{b}\|^{-1} \sum_{J=2}^{N} \beta_J \cdot \left(\sum_{K=2}^{N} \frac{1}{\mu_K} B^{KJ} (\exp \mu_K t - 1) b_{K1} + B^{1J} b_{11} t \right) \right\}$$

$$\cdot \sum_{x_2} \cdots \sum_{x_N} p(x_1 = 0, x_2 \ldots, x_N, 0^+)$$

$$\prod_{I=2}^{N} \left\{ 1 - \|\boldsymbol{b}\|^{-1} \sum_{K=1}^{N} B^{KI} b_{K1} \exp \mu_K t \right\}^{x_I}. \tag{51}$$

For the initial probability $p(\mathbf{x}, 0^+)$ we may assume for most experimental situations an equilibrium distribution given by a multi-Poissonian probability which corresponds for the open system to the grand-canonial probability. For example, in flash photolysis experiments we have after a photoflash $x_1 \neq 1$, i.e.

$$p(\mathbf{x}, 0^+) = p(\mathbf{x}, 0^+)(1 - \delta_{x_1, 1}). \tag{52}$$

In situations where the equilibrium occupations in the sites $I > 1$ are high, the equilibrium distribution will be disturbed only slightly by the initial

preparation procedure (e.g. the flashed-off ligand from the binding site). So we obtain

$$p(\mathbf{x}, 0^+) = (1-\delta_{x_1,\,1}) \prod_{I=2}^{N} \frac{\langle x_I(o)\rangle^{x_I}}{(x_I)!} \exp -\langle x_I(o)\rangle, \tag{53}$$

with the initial moments given for the situation in equations (44)–(47):

$$\langle x_I(0)\rangle = -\sum_{K=2}^{N} (M^r)^{-1}_{IK}\, \beta_K;\ I = 2, \ldots, N. \tag{54}$$

$\mathbf{M}^r$ is the matrix $\mathbf{M}$, defined with constant transition rates γ_{IK}, with the first row and column deleted and M_{II} defined with $\gamma_{1I} = 0$. At an arbitrary time t the probability $p(\mathbf{x}, t; \boldsymbol{\lambda})$ will now not remain of the multi-Poissonian form, because of the non-linearity in the transition rates Γ_{1K} [equation (45)]. Summation over $x_2 \ldots x_N$ in equation (51) yields for $N_{\exp}(t, \boldsymbol{\lambda})$ the simple result

$$N_{\exp}(t, \boldsymbol{\lambda}) = \exp - \langle x_1^*(t, \boldsymbol{\lambda})\rangle \tag{55}$$

where (Appendix B)

$$\langle x_1^*(o)\rangle = 0 \tag{56}$$

$$\langle x_1^*(t, \boldsymbol{\lambda})\rangle = \left\{ \|\boldsymbol{b}\|^{-1} \sum_{J=2}^{N} \beta_J \left[B^{1J} b_{11} t + \sum_{K=2}^{N} \frac{1}{\mu_K} B^{KJ} [\exp(\mu_K t) - 1] b_{K1} \right] + \sum_{I=2}^{N} \langle x_I(o)\rangle \|\boldsymbol{b}\|^{-1} \sum_{K=1}^{N} B^{KI}\, b_{KI} \exp \mu_K t \right\}, \tag{57}$$

and

$$\lim_{t \to +\infty} \langle x_1^*(t, \boldsymbol{\lambda})\rangle = \infty. \tag{58}$$

The present theory allows us to calculate the macroscopic observable $N_{\exp}(t, \boldsymbol{\lambda})$ from which we can extract characteristic features of the internal mechanisms such as alternate pathways, restrictions on occupation numbers or volume available in different wells, function of conformational intermediates, or the role of the surrounding solvent. In particular, the simplified model with the assumptions in equations (44)–(47) explains the features of the experimental data of carbon monoxide migration in myoglobin over large ranges of [CO]-ligand concentrations in the solvent, temperature and time (Alberding, Frauenfelder & Hanggi, 1978). In systems more complex than myoglobin multiple occupancies may occur even under biological conditions. For CO-migration to cytochrome-a_3 of cytochrome oxidase, for instance, Sharrock & Yonetani (1977) have found experimental evidence for a carbon monoxide reservoir that connects to an intermediate well and is occupied by many CO-molecules. The characteristic dynamics for this system can be treated by a straightforward adaptation of the methods presented here.

4. Summary and Conclusions

In this paper we have given a complete description of the kinetics of the migration process of ligands in biomolecules under general experimental situations. Previously, the problem has been studied by solving a set of linear coupled ordinary differential equations (Austin *et al.*, 1975; Alberding *et al.*, 1978). This traditional method of analysis is based upon a deterministic formulation of ligand migration in which reaction constants are viewed as "reaction rates" and the various species concentrations are treated as continuous single-valued functions of time. Although this deterministic formulation is adequate in many cases (see e.g. section 2) there are experimental situations with non-linear transition rates, blocking effects, non-linear cage-solvent effects, history and preparational dependent conformational relaxation, where the non-linear fluctuations play a major role! The influence of fluctuations is also indispensable in noise studies. Experiments under extreme experimental constraints (e.g., high ligand concentrations), to which the present paper is mainly addressed, appear at first sight to have little direct bearing on biological processes. Such experiments, however, help elucidate the internal dynamic features and functions in complex biomolecular systems.

An approach that is more broadly applicable than a deterministic formulation is a *stochastic* formulation in terms of master equations where reaction constants are not viewed as "reaction rates" but as "reaction probabilities per unit time". From a physical point of view, the stochastic formulation is superior to the deterministic formulation: the stochastic approach is always valid whenever the deterministic approach is valid and *is still* valid when the deterministic approach is not. The former takes account of fluctuations and time dependent correlations. The stochastic approach, based on a *linear* evolution equation *for the probability* $p(\mathbf{x}, t)$ (see in this context also Appendix C), enables us to extract uniquely defined fluctuation renormalized mean value equations, i.e., non-linear renormalized deterministic equations in the sense that we have in equation (37)

$$\langle \mathbf{M}[\mathbf{x}(t)] \rangle \neq \mathbf{M}[\langle \mathbf{x}(t) \rangle]. \tag{59}$$

In particular, no apologies need be made for the fluctuations. These fluctuations are really present and give rise to macroscopically observable effects in appropriate situations (section 3). In those cases in which fluctuations turn out to be unimportant, that fact too will emerge quite naturally from the formalism presented here. Further, a set of non-linear deterministic equations is often harder to solve than the stochastic properties based on the linear structure of the master equation. (Appendix C.)

Because the non-linear biophysical systems are finite in the sense that only

a small number Ω of ligands can occupy a certain state (blocking) the usual expansion of the master equation with highly non-linear transition rates in terms of an inverse system size, Ω^{-1}, as a smallness parameter cannot be used here (Kubo, Matsuo & Kitahara, 1973). This procedure could be applied if only one stochastic variable, the number $y(t, \lambda)$ of biomolecules not rebound at time t, would be considered; but on this macroscopic coarse grained level the transition probabilities per unit time $\Gamma(y \to y', t)$ are completely unknown.

The theory given here is also applicable to other problems in biophysics such as the dynamic behavior of cycling of cross bridges in a muscle (Hill, 1974), detailed studies in nerve membranes (Hill & Chen, 1972; Fishman, 1973) and neuron networks (Wilson & Cowan, 1972). In these problems the stochastic variables denote the number of cells in a given activated state at time t and experimental constraints λ. Enzyme kinetics (Heyde & Heyde, 1971; Edelstein, 1970; Goel & Richter-Dyn, 1974) and enzyme-assisted membrane transport, where the ligand-membrane (enzyme) complex may exist in several distinct states as intermediates, can also be treated by carrying through the ideas presented in this paper. Other applications are the cell development in a tumor, the growth of vital plaques and kinetic proof reading in biosynthetic systems (Hopfield, 1974). Even brain models should fit, to a certain extent, with the formalisms developed here.

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APPENDIX A

Properties of the Generating Function

From the definition of the generating function G in equation (12) we obtain for the rate of change

$$\frac{\partial G(\mathbf{y}, t)}{\partial t} = \sum_{x_1} \cdots \sum_{x_N} \left(\frac{\partial p(\mathbf{x}, t)}{\partial t}\right) \prod_{i=1}^{N} y_i^{x_i}. \tag{A1}$$

Further we have for

$$y_i G = \sum_{x_1} \cdots \sum_{x_N} p(x_1 \ldots, x_i - 1, \ldots, x_N, t) \prod_{j=1}^{N} y_j^{x_j} \tag{A2a}$$

$$y_i(\partial G/\partial y_i) = \sum_{x_1} \cdots \sum_{x_N} x_i\, p(\mathbf{x}t) \prod_{j=1}^{N} y_i^{x_j} \tag{A2b}$$

$$\partial G/\partial y_i = \sum_{x_1} \cdots \sum_{x_N} (x_i+1)p(x_1, \ldots, x_i+1, \ldots, x_N, t) \prod_{j=1}^{N} y_j^{x_j} \tag{A2c}$$

yielding

$$y_i\,(\partial G/\partial y_i)\big|_{\text{all } y_i = 1} = (\partial \ln G/\partial \ln y_i)\big|_{\text{all } y_j = 1} = \langle x_i(t)\rangle. \tag{A3}$$

Here we used the fact that the probability $p(x_1, \ldots, x_N, t)$ is assumed to satisfy the normalization condition

$$G(1, \ldots, 1, t) = \sum_{x_1} \cdots \sum_{x_N} p(x_1, \ldots, x_N, t) = 1. \tag{A4}$$

From the definition in equation (A1) it follows that

$$\partial^n G(\mathbf{y}, t)/\partial y_i^n|_{\text{all } y_j = 1} = \langle x_i(t)\,[x_i(t)-1]\,\ldots\,[x_i(t)-n+1]\rangle, \tag{A5}$$

or for the moments

$$\partial^n \ln G(\mathbf{y}, t)/(\partial \ln y_i)^n|_{\text{all } y_j = 1} = \langle x_i^n(t)\rangle. \tag{A6}$$

In particular we obtain for the variance $\sigma_{ii}(t)$ of the stochastic variable $x_i(t)$

$$\sigma_{ii}(t) = \langle [x_i(t)-\langle x_i(t)\rangle]^2\rangle = \left[\partial^2 G/\partial y_i^2 + \frac{\partial G}{\partial y_i} - \left(\frac{\partial G}{\partial y_i}\right)^2\right]_{\text{all } y_j = 1} \tag{A7}$$

and the covariance $\sigma_{ij}(t)$, i $\neq j$:

$$\sigma_{ij}(t) = \langle [x_i(t)-\langle x_i(t)\rangle]\,[x_j(t)-\langle x_j(t)\rangle]\rangle = \left[\frac{\partial^2 G}{\partial y_i \partial y_j} - \frac{\partial G}{\partial y_i}\frac{\partial G}{\partial y_j}\right]_{\text{all } y_n = 1}. \tag{A8}$$

APPENDIX B

Solution of equation (49) and equation (13)

With help of the conditional probability $R\,(\mathbf{x}\,t|\mathbf{z}0)$ of the stochastic process $\mathbf{x}(t)$ which is just the solution of the master equation with the initial condition

$$p(\mathbf{x}, o) = \delta(\mathbf{x}-\mathbf{z}), \tag{B1}$$

we obtain for the generation function, using a general initial probability $p(\mathbf{x}, o)$

$$\begin{aligned} G(\mathbf{y}, t) &= \sum_{\mathbf{X}} p(\mathbf{x}t) \prod_{i=1}^{N} y_i^{x_i} \\ &= \sum_{\mathbf{X}}\sum_{\mathbf{Z}} R\,(\mathbf{x}t|\mathbf{z}o)p(\mathbf{z}, 0) \prod_{i=1}^{N} y_i^{x_i} \\ &= \sum_{\mathbf{Z}} p(\mathbf{z}, o)\, G^{\delta}\,(\mathbf{y}, t, \mathbf{z}). \end{aligned} \tag{B2}$$

Here we have defined $G^{\delta}(t)$, which is just the generating function solution of equation (49) for the initial probability given in equation (B1) yielding

$$G^{\delta}\,(\mathbf{y}, 0; \mathbf{z}) = \prod_{i=1}^{N} y_i^{z_i}. \tag{B3}$$

Due to the blocking property of the binding well, we have:

$$p(z_1 > 1, z_2, \ldots, z_N, 0^+) = 0, \tag{B4}$$

giving

$$G(\mathbf{y}, t) = \sum_{z_1=0}^{1} \sum_{z_2} \ldots \sum_{z_N} p(z_1, z_2, \ldots, z_N, 0^+)\, G^{\delta}(\mathbf{y}, t, z_1, \ldots, z_N). \tag{B5}$$

Finally we are only interested in $G_o(y_1 = 0, y_2, \ldots, y_N, t; \mathbf{z})$. Setting $y_1 = 0$ we obtain from equation (49) a partial *linear* first order differential equation for $G_o^\delta(\mathbf{y}, t; \mathbf{z})$:

$$\frac{\partial G_o^\delta(\mathbf{y}, t, \mathbf{z})}{\partial t} = \sum_{i=2}^{N} \sum_{j=2}^{N} \gamma_{ji}(y_j - y_i) \frac{\partial G_o^\delta}{\partial y_i} + \sum_{i=2}^{N} \gamma_{Si}(1 - y_i) \frac{\partial G_o^\delta}{\partial y_i} + \sum_{i=2}^{N} \beta_i (y_i - 1) G_o^\delta - \sum_{i=2}^{N} \gamma_{1i} y_i \frac{\partial G_o^\delta}{\partial y_i} \tag{B6}$$

with

$$G_o^\delta(\mathbf{y}, o; \mathbf{z}) = \prod_{i=2}^{N} 1 \cdot y_i^{z_i} \delta_{z_1, o}. \tag{B7}$$

Using the properties of the binding site; i.e.

$$\begin{aligned} \beta_1 &= 0, \\ \gamma_{i1} &= 0, \\ \gamma_{S1} &= 0, \end{aligned} \tag{B8}$$

and $\gamma_{ii} = -M_{ii}$ (see equation (36) for $\mathbf{M}$ defined with constants γ_{ik}) it is more convenient from a technical point of view to write equation (B6) in the compressed form

$$\frac{\partial G_o^\delta}{\partial t} = \sum_{i=1}^{N} \sum_{j=0}^{N} \gamma_{ji} y_j \frac{\partial G_o^\delta}{\partial y_i} + \sum_{i=1}^{N} \beta_i (y_i - 1) G_o^\delta, \tag{B9}$$

considering $y_{j=1}$ as a parameter set equal to zero. We have also introduced the index $j = 0$ for the bath (B) and $y_o \equiv 1$.

The solution of equation (B9) is obtained by the method of characteristics (see e.g. Miller, 1941; Kamke, 1959; Gans, 1960) considering G_o^δ analytical in $\mathbf{y}$ and finally setting $y_1 = 0, y_2 = \ldots = y_N = 1$.

The set of simultaneous ordinary differential equations to equation (B9) reads

$$\frac{\mathrm{d}t}{1} = \left\{ -\mathrm{d}y_i / \sum_{i=0}^{N} \gamma_{ji} y_j \right\}_{i=1,\ldots,N} = \mathrm{d} \ln G_o^\delta / \sum_{i=1}^{N} \beta_i (y_i - 1). \tag{B10}$$

Since

$$\mathrm{d}y_i = -\mathrm{d}t \cdot \sum_{j=0}^{N} \gamma_{ji} y_j \; i = 1, \ldots, N, \tag{B11}$$

we can choose sets of multipliers b_{kj} fulfilling

$$\sum_{i=1}^{N} \gamma_{ji} b_{ki} = \mu_k b_{kj} \tag{B12}$$

yielding

$$\mathrm{d}t = \left\{ -\sum_{i=1}^{N} b_{ki}\,\mathrm{d}y_i \Big/ \sum_{i=1}^{N} b_{ki} \sum_{j=0}^{N} \gamma_{ji} y_j \right\}_{k=1,\ldots,N} = \mathrm{d}\ln G_o^\delta \Big/ \sum_{i=1}^{N} \beta_i (y_i - 1). \tag{B13}$$

Hence, we obtain with equation (B12) and the property

$$\gamma_{oi} = -\sum_{j=1}^{N} \gamma_{ji}:$$

$$\mu_K \sum_{i=1}^{N} b_{ki}\,\mathrm{d}y_i \Big/ \sum_{i=i}^{N} b_{ki} \sum_{j=0}^{N} \gamma_{ji} y_j = \mathrm{d}\ln \sum_{j=1}^{N} b_{jk}(y_j - 1). \tag{B14}$$

Equation (B13) can now be integrated immediately giving

$$I_k(t) = \mu_k t + \ln \sum_{j=1}^{N} b_{kj}(y_j - 1)\; k = 1, \ldots, N \tag{B15}$$

$$J(t) = \ln G_o^\delta + \|\mathbf{b}\|^{-1} \sum_{j=1}^{N} \beta_j \sum_k \frac{1}{\mu_k} B^{kj} \exp\left[I_k(t) - \mu_k t\right], \tag{B16}$$

with $\|\mathbf{b}\|$ meaning the determinant of the matrix of eigenvectors $\mathbf{b}_k = (b_{k1}, \ldots, b_{kN})$ of $\mathbf{M}$ and B^{kj} the co-factor element defined by

$$\mathbf{b}^{-1} = \frac{1}{\|\mathbf{b}\|}\,\mathbf{B}^T. \tag{B17}$$

$\mathbf{B}^T$ denotes the transpose of $\mathbf{B}$.

The solution of equation (B9) can be written as

$$G_o^\delta = \psi\,(I_1, \ldots, I_N, J), \tag{B18}$$

where the functional relationship ψ between the integrals I_k and J is determined by the initial condition equation (B7) and remains independent of time. Solving equation (B15) for y_j we obtain for $G_o^\delta(\mathbf{y}, 0; \mathbf{z})$

$$G_o^\delta(\mathbf{y}, 0; \mathbf{z}) = \exp\left\{ J(0) - \|\mathbf{b}\|^{-1} \sum_j \beta_j \sum_k \frac{1}{\mu_K} B^{kj} \exp\, I_K(0) \right\} \delta_{z_1, 0}$$

$$= \prod_{i=2}^{N} \left\{ 1 + \|\mathbf{b}\|^{-1} \sum_{k=1}^{N} B^{ki} \exp I_K(0) \right\}^{z_i} \cdot \delta_{z_1, 0}. \tag{B19}$$

Because of the absorbing property of the binding well $[x_1]$, $(\gamma_{11} = 0)$, the matrix $\mathbf{M}$ has a vanishing eigenvalue $\mu_1 = 0$. Eliminating I_k, we have therefore from equation (B16) for $G_o^\delta(\mathbf{y}, t; \mathbf{z})$ the final result:

$$G_o^\delta(\mathbf{y}, t; \mathbf{z}) = \exp$$

$$\left\{ \|\mathbf{b}\|^{-1} \sum_{j=2}^{N} \beta_j \sum_{k=1}^{N} \frac{1}{\mu_k} B^{kj} [\exp \mu_k t - 1] \sum_{i=1}^{N} b_{ki}(y_i - 1) \right\}$$

$$\cdot \prod_{i=2}^{N} \left\{ 1 + \|\mathbf{b}\|^{-1} \sum_{j=1}^{N} (y_j - 1) \sum_{k=1}^{N} B^{ki} b_{kj} \exp \mu_k t \right\}^{z_i} \delta_{z_1, 0}. \tag{B20}$$

Note that all the eigenvalues μ_k of **M** have a real part Re $\mu_k \leq 0$. Moreover, pure imaginary eigenvalues cannot exist. This is a consequence of the theorems of Gerschgorin (Gerschgorin, 1931; Hanggi & Thomas, 1978) applied to the matrix **M**. Using equation (B5) we finally get for $G(0, 1 \ldots 1; t)$

$$G(0, 1, \ldots 1; t) = \exp$$
$$\left\{ - \|\mathbf{b}\|^{-1} \sum_{j=2}^{N} \beta_j \left(\sum_{k=2}^{N} B^{kj} [\exp \mu_k t - 1]\, b_{k1} + B^{1j} b_{11} t \right) \right\}$$
$$\cdot \sum_{z_2 \ldots z_N} p(z_1 = 0, z_2, \ldots, z_N, o^+) \prod_{i=2}^{N} \left\{ 1 - \|\mathbf{b}\|^{-1} \sum_{k=1}^{N} B^{ki} b_{k1} \exp \mu_k t \right\}^{z_i}. \quad \text{(B21)}$$

The solution for the generating function in equation (13) is obtained from equation (B20) with $\gamma_{SI} = \beta_I = 0$; $I = 1, \ldots, N$. Then the structure of the matrix **M** reduces to that of the stochastic matrix **A** in equations (8)–(9). From equation (B17) we obtain

$$\sum_j \sum_k b_{kj} B^{ki} = \|\mathbf{b}\|. \quad \text{(B22)}$$

Interchanging order of summation and using the properties of **A** as a stochastic matrix (Hanggi & Thomas, 1978):

$$\sum_i b_{ki} = 0, \text{ if } \mu_k \neq 0, \quad \text{(B23)}$$

we obtain from equation (B20) for the solution of equation (13) with equation (B2):

$$G(\mathbf{y}, t; \boldsymbol{\lambda}) = \sum_{x_1} \cdots \sum_{x_N} p(x_1, \ldots, x_N, 0)$$
$$\left(\sum_I x_I = N_o \right)$$
$$\cdot \left\{ \|\mathbf{b}\|^{-1} \sum_{K=1}^{N} B^{KI} \sum_{J=1}^{N} b_{KJ} y_J \exp(\mu_k t) \right\}^{x_I}. \quad \text{(B24)}$$

APPENDIX C

Continued Fraction Expansion for Time Dependent Mean Values in Non-linear Systems

If we deal with a master equation (in the following we use linear operator notation)

$$\dot{p}(t) = \Gamma p(t) \quad \text{(C1)}$$

with in general non-linear transition rates $\Gamma(\mathbf{x}|\mathbf{y})$, the solution of equation (C1) for an initial probability $p(o)$ reads

$$p(t) = \exp(\Gamma t)\, p(o). \quad \text{(C2)}$$

The knowledge of the operator Γ and the initial probability $p(o)$ enables us to calculate the Taylor expansion of a mean value $\langle x_i(t)\rangle$ of the stochastic variable $x_i(t)$

$$\langle x_i(t)\rangle = \int x_i\, p(\mathbf{x}t)\,\mathrm{d}\mathbf{x} = \langle x_i(o)\rangle + \sum_{n=1}^{\infty} \frac{p_n}{n!}\, t^n. \tag{C3}$$

The static moments p_n are given by

$$\begin{aligned} p_n &= \ll x_i \Gamma^n p(o) \gg,\ n = 1, \ldots \\ &= \int x_i [\Gamma^n p(o)]_{\mathbf{x}}\,\mathrm{d}\mathbf{x}. \end{aligned} \tag{C4}$$

Next we study the Fourier transform of the function $C(t)$

$$C(t) = \theta(t)\,(\langle x_i(t)\rangle - \langle x_i(t=+\infty)\rangle) \tag{C5a}$$

$$= \theta(t)\,\langle \xi_i(t)\rangle, \tag{C5b}$$

where $\theta(t)$ denotes the step function. For the Fourier transform $C(\omega)$

$$C(\omega) = \int_o^{\infty} \mathrm{e}^{\,i\omega t}\,\langle \xi_i\,(t)\rangle\,\mathrm{d}t, \tag{C6}$$

we obtain with equation (C3) the sum rule expansion:

$$C(\omega) = \sum_{n=o}^{\infty} \frac{p_n}{(-i\omega)^{n+1}}, \tag{C7}$$

where

$$p_o = \langle x_i(o)\rangle - \langle x_i(\infty)\rangle. \tag{C8}$$

The series in equation (C7) is in general semi-convergent or asymptotic. Next we construct a continued fraction expansion which serves as an analytical continuation of the series in equation (C7). With $z = -i\omega$ the corresponding continued fractions are given by (Hanggi & Thomas, 1978; Hanggi, 1977):

$$C(\omega) = \frac{C_1}{z+}\,\frac{C_2}{1+}\,\frac{C_3}{z+}\,\ldots \tag{C9}$$

$$= \frac{b_1}{z-a_1+}\,\frac{b_2}{z-a_2+}\,\ldots \tag{C10}$$

A general evaluation method for the coefficients in equations (C8)–(C9) consists in the requirement that a formal expansion in powers of $^1/z$ of the continued fractions equals those appearing in the asymptotic series. A most convenient method for the calculation of the coefficients consists in a *recursive* calculation scheme (Hanggi, 1977; Gordon, 1968). Here we outline the recursive scheme (Hanggi, 1977) which is usually numerically more stable

than that given by Gordon (Gordon, 1968). Starting with

$$C_1 = D_1 \qquad D_1 = p_o$$

$$C_2 = \frac{-D_2}{D_1} \qquad D_2 = p_1$$

$$C_3 = \frac{-D_3}{D_2} \qquad D_3 = p_2 + p_1 C_2$$

$$C_4 = \frac{-D_4}{D_3} \qquad D_4 = p_3 + p_2 (C_2 + C_3) \tag{C11}$$

one proceeds from $n = 4$ to the higher terms in the following way: using the auxiliary vector $\mathbf{x}$ of dimension L

$$L = 2 \text{ integer } [(n-1)/2] \tag{C12}$$

$$\left.\begin{aligned} x(2) &= C_2 + C_3,\ x(1) = C_2 \\ \text{interchange} & \\ x(2) &\rightarrow x(1);\ x(1) \rightarrow x(2) \end{aligned}\right] \quad n = 4 \tag{C13}$$

with $x(L-1) = 0$ we work upwards:

$$\left.\begin{aligned} x(K) &= x(K-1) + C_{n-1}\, x(K-2) \\ K &= L, L-2, \ldots 4 \\ x(2) &= x(1) + C_{n-1} \\ \text{interchange the odd and even component, i.e.} & \\ x(2) &\rightarrow x(1),\ x(4) \rightarrow x(3) \\ x(1) &\rightarrow x(2),\ x(3) \rightarrow x(4) \text{ etc.} \end{aligned}\right] \quad n \geq 5 \tag{C14}$$

The continued fraction coefficient C_n is then given by

$$C_n = -\frac{D_n}{D_{n-1}} \tag{C15a}$$

$$D_n = p_{n-1} + \sum_{i=1}^{L/2} p_{n-(i+1)}\, X(2i-1). \tag{C15b}$$

With the C_n evaluated by equation (C15), the coefficients of the contracted continued fraction equation (C10) are simply given by the relations

$$b_1 = C_1 \qquad a_1 = -C_2 \tag{C16a}$$

$$b_{n+1} = -C_{2n} C_{2n+1}\ a_{n+1} = -(C_{2n+1} + C_{2n+2}). \tag{C16b}$$

The function $C(t)$ (and therefore $\langle x_i(t) \rangle$ for all times t) is then given by the inverse Fourier transform

$$C(t) = \frac{1}{2\pi} \int_{-\infty}^{+\infty} C(\omega)\, \mathrm{e}^{-i\omega t}\, \mathrm{d}\omega. \tag{C17}$$