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Time-dependent corrections to effective rate and event statistics in Michaelis–Menten kinetics

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Abstract: The authors generalise the concept of the geometric phase in stochastic kinetics to a non-cyclic evolution. Its application is demonstrated on kinetics of the Michaelis–Menten reaction. It is shown that the non-periodic geometric phase is responsible for the correction to the Michaelis–Menten law when parameters, such as a substrate concentration, are changing with time. The authors apply these ideas to a model of chemical reactions in a bacterial culture of a growing size, where the geometric correction qualitatively changes the outcome of the reaction kinetics.

1 Introduction

Biochemical reactions are typically characterised in stationary in vitro environments with the hope that their measured properties will hold in vivo. There are clearly many important physiological reasons why this extrapolation may fail. In this article, we focus on one particular reason that has little to do with the physiology, but rather derives from the fact that rates of complex chemical reactions may have non-trivial corrections because of slow, adiabatic drift of (internal) kinetic parameters of the system [1].

The class of phenomena we study is related to the celebrated Berry's phase in driven quantum mechanical systems [2], which predicted a contribution to the phase of an adiabatically changing wave function in the form of a integral over the parameter trajectory. Since the original Berry's discovery a number of its generalisations were proposed, for example, to non-abelian and non-adiabatic regimes. Similar geometric phases were also found in other fields, for example, in dissipative dynamics [3–7].

Recently, new geometric phases were studied in the domain of purely classical stochastic kinetics [8–10]. They were shown to be responsible for the stochastic pump and other ratchet-like effects, and thus they are of clear importance for the theory of chemical enzymes, and specifically molecular motors, operating in strongly stochastic environment [11, 12]. This finding raises possibilities of various generalisations of the geometric phase. For example, recently its non-adiabatic counterpart was introduced in [13], and it was shown to be responsible for a non-adiabatic current contribution that has no analogue under stationary conditions.

In this work, we study another generalisation of the geometric phase in stochastic kinetics, namely to a non-periodic evolution in the parameter space. Such non-cyclic geometric phases have been known previously in quantum physics and optics [14–22]. We will show that the non-cyclic geometric phase in stochastic kinetics can be unambiguously defined, and that it can be naturally interpreted as being responsible for the leading non-adiabatic correction in the expression for stochastic fluxes, which can 'qualitatively' change kinetics of a chemical reaction.

2 Generating function for the Michaelis–Menten reaction

Consider a catalytic conversion of one type of molecules, called the substrate, into another type, called the product, via an intermediate reaction with an enzyme. Schematically, the reaction can be represented as

$$S + E \underset{k_{-1}}{\overset{k_1 n_s}{\rightleftarrows}} SE \underset{k_{-2} n_p}{\overset{k_2}{\rightleftarrows}} E + P \tag{1}$$

where k_i, $i = -2, -1, 1, 2$ are kinetic rates of corresponding elementary processes, the S and P denote substrate and product respectively, n_s and n_p stand for their concentrations, and E is the enzyme molecule. S and P interact via creating a complex SE which is unstable and dissociates either back into E and S or forward into E and P. In the simplest version of the Michaelis and Menten (MM) mechanism, enzymes catalyse the process but are not modified in any reactions. However, generalisations are certainly possible [23, 24].

The reaction [25] is the most fundamental and the simplest enzymatic biochemical process. In their 1913 article [25], MM considered a strongly non-equilibrium situation of reaction (1), neglecting the backward $E + P \to ES$ association, which can be done for $n_p \ll k_1 n_s / k_{-2}$. However, here we keep this reaction for generality. If the number of S and P molecules is much larger than that of the enzymes, the latter have to perform many substrate conversions each in order to change S and P concentrations noticeably. This is traditionally used to simplify the reaction kinetics since one can assert that enzymes operate in a quasi-steady state at current substrate and product concentrations.

Stochastic kinetics of the conversion of S into P is conveniently described by the moments generating function $Z(\chi, t)$ (mgf) and the cumulants generating function $S(\chi, t)$ (cgf) defined as [8, 26, 27]

$$Z(\chi, t) = \mathrm{e}^{S(\chi,t)} = \sum_{n=-\infty}^{\infty} P_n \mathrm{e}^{\mathrm{i}n\chi} \tag{2}$$

where P_n is the probability to find net n product molecules generated during the observation time t (back conversion is counted with the negative sign, i.e. $n < 0$ means that more product molecules were converted back to substrate than substrate into the product). For a small number of enzymes, they can be considered statistically independent over short periods of time, and the cgf's are additive, that is, the cgf of reaction events produced by n enzymes is n times the cgf of a single enzyme. Thus we will restrict our study only to the case of a single enzyme without much loss of generality.

It is convenient to introduce additional generating functions $U_E = \sum_{n=-\infty}^{\infty} P_{nE} \mathrm{e}^{\mathrm{i}n\chi}$ and $U_{SE} = \sum_{n=-\infty}^{\infty} P_{nSE} \mathrm{e}^{\mathrm{i}n\chi}$, where P_{nE} and P_{nSE} are the probabilities that, at a given time, the net number of generated product molecules is n and the enzyme is in the unbound/bound state. Then the master equation for the entire process is

$$\begin{aligned} \frac{\mathrm{d}}{\mathrm{d}t} P_{nE} &= -(k_1 n_s + k_{-2} n_p) P_{nE} + k_{-1} P_{nSE} + k_2 P_{(n-1)SE} \\ \frac{\mathrm{d}}{\mathrm{d}t} P_{nSE} &= -(k_{-1} + k_2) P_{nSE} + k_1 n_s P_{nE} + k_{-2} n_p P_{(n+1)E} \end{aligned} \tag{3}$$

Multiplying (3) by $\mathrm{e}^{\mathrm{i}\chi n}$ and summing over n we find the equation for the generating functions

$$\frac{\mathrm{d}}{\mathrm{d}t} \begin{pmatrix} U_E \\ U_{SE} \end{pmatrix} = -\hat{H}(\chi, t) \begin{pmatrix} U_E \\ U_{SE} \end{pmatrix} \tag{4}$$

where

$$\hat{H}(\chi, t) = \begin{pmatrix} k_1 n_s + k_{-2} n_p & -k_{-1} - k_2 \mathrm{e}^{\mathrm{i}\chi} \\ -k_1 n_s - k_{-2} n_p \mathrm{e}^{-\mathrm{i}\chi} & k_{-1} + k_2 \end{pmatrix} \tag{5}$$

If we set $n = 0$ at initial moment $t = 0$, then the initial conditions for (4) are $U_E(t = 0) = p_E(0)$, and $U_{SE}(t = 0) = p_{SE}(0)$, where $p_E(0)$ and $p_{SE}(0)$ are probabilities that the enzyme is free/bound, respectively. Additionally, note that $Z(\chi, t) = U_E(\chi, t) + U_{SE}(\chi, t)$. Thus the formal solution for mgf (2) can be expressed as an average of the evolution operator

$$Z(\chi, t) = \left\langle 1 \middle| \hat{T} \left(\mathrm{e}^{-\int_0^t \hat{H}(\chi,t)\mathrm{d}t} \right) \middle| p(0) \right\rangle \tag{6}$$

where $\langle 1| = (1, 1)^{\mathrm{T}}$, $|p(0)\rangle = (p_E(0), p_{SE}(0))$ and $\hat{T}$ is the time-ordering operator.

Before we proceed with the case where parameters are time dependent, it is instructive to look first at the stationary regime. To simplify (6), one can find normalised left and right eigenvectors $\langle u_{0/1}|$, $|u_{0/1}\rangle$ and corresponding eigenvalues $\epsilon_{0/1}$ of the operator $\hat{H}(\chi)$, where indexes 0 and 1 correspond to the two eigenvalues with the smallest and the largest real parts, respectively. There is one left and one right eigenvectors for each eigenvalue, that we will distinguish by bra and ket notation. Since the operator $\hat{H}(\chi)$ is not Hermitian, we do not assume any relations between components of bra and ket eigenvectors corresponding to the same eigenvalue.

Every vector, such as $|p(0)\rangle$ can be expressed as a sum of eigenvectors of $\hat{H}(\chi)$, for example

$$|p(0)\rangle = \langle u_0|p(0)\rangle |u_0\rangle + \langle u_1|p(0)\rangle |u_1\rangle \tag{7}$$

where we define $\langle \alpha|\beta\rangle = \alpha_1\beta_1 + \alpha_2\beta_2$ to be a standard scalar product of two vectors. Substituting (7) into (6), for the time-independent Hamiltonian we find the

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steady state mgf

$$Z_{\rm st}(\chi, t) = {\rm e}^{-\epsilon_0(\chi)t+\ln(\langle 1|u_0\rangle\langle u_0|p(0)\rangle)}+{\rm e}^{-\epsilon_1(\chi)t+\ln(\langle 1|u_1\rangle\langle u_1|p(0)\rangle)} \quad (8)$$

At time scales $t \gg \max[1/k_{-1}, 1/k_2, 1/(k_1 n_{\rm s}), 1/(k_{-2} n_{\rm p})]$, the second term in (8) is exponentially suppressed in comparison to the first, and the expression for the mgf simplifies to

$$Z_{\rm st}(\chi, t) \simeq {\rm e}^{-\epsilon_0(\chi)t+\ln(\langle 1|u_0\rangle\langle u_0|p(0)\rangle)} \quad (9)$$

Terms analogous to $-\epsilon_0(\chi)t$ in (9) have been studied previously [8, 27]. The second term is less common in the literature: this is the boundary term that does not grow with time and depends on the initial conditions and the averaging over the final states of the enzyme. One can disregard it in comparison to the first contribution when $t \to \infty$. However, we note that its relative effect decays as $1/t$, that is, not exponentially, in contrast to the ϵ_1 term in (8).

At the first look, the boundary term leads to a contradictory result after setting $t \to 0$, that is at the initial moment of the evolution. In this limit, the boundary term does not disappear, namely

$$S_{\rm bnd}|_{t=0} = \ln(\langle 1|u_0(0)\rangle\langle u_0(0)|p(0)\rangle) \neq 0 \quad (10)$$

However, we expect $S_{\rm bnd}|_{t=0}$ to be zero, since $n|_{t=0} = 0$, so the mgf should be identically equal to unity. The apparent contradiction is resolved by noting that (9) was derived assuming $t \to \infty$, and it is simply an invalid approximation for $t \to 0$. In other words, the boundary term is responsible for the initial fast relaxation to the stationary regime. For more insight, one can calculate the contribution of the boundary term to the average number of generated product molecules. Using the normalisation condition $p_{SE}(0) = 1 - p_E(0)$ one can find

$$n_{\rm bnd} = -{\rm i}\frac{\partial S_{\rm bnd}|_{t=0}}{\partial\chi}\bigg|_{\chi=0} = \frac{(k_2 + k_{-2}n_{\rm p})(k_2 + k_{-1} - Kp_E(0))}{K^2} \quad (11)$$

where $K = k_{-1} + k_2 + k_1 n_{\rm s} + k_{-2} n_{\rm p}$. If one assumes that the initial probability $p_E(0)$ for the enzyme to be free is at the equilibrium value $p_E(0) = (k_2 + k_{-1})/K$, then (11) produces $n_{\rm bnd} = 0$, as expected. To confirm this, one can also derive (11) by a standard master equation approach. That is, calculating the average number of new product molecules $n_{\rm bnd}(t)$, one would find that, after a sufficiently long time

$$n(t) = n_{\rm bnd} + \frac{k_1 k_2 n_{\rm s} - k_{-1}k_{-2}n_{\rm p}}{K}t \quad (12)$$

The second term in (12) is the average number of the product molecules produced during time t at a steady state. It is the standard prediction for the reversible MM enzyme, and the first term is a correction, which is non-zero when the initial state of enzymes is not the same as its steady state.

3 Non-cyclic geometric phase in stochastic kinetics

Assume now that there are several slowly time-dependent parameters in the model. We will group them in a vector λ. In the case of the MM process, one can view these time-dependent parameters as concentrations of the substrate and the product, $\lambda = (n_{\rm s}, n_{\rm p})$. However, the discussion in this section is completely general.

Following [7], we partition the time into small intervals, over which kinetic rates can be considered almost constant. We insert the resolution of the identity operator, $\hat{1} = |u_0(t)\rangle\langle u_0(t)| + |u_1(t)\rangle\langle u_1(t)|$, in (6) after every such an interval. One can find then that the boundary term becomes $S_{\rm bnd} = \ln(\langle 1|u_0(t)\rangle\langle u_0(0)|p(0)\rangle)$. It is sensitive to the redefinition of eigenstates of the Hamiltonian (5) such as $|u_0\rangle \to {\rm e}^{\phi(\lambda)}|u_0\rangle$ and $\langle u_0| \to \langle u_0|{\rm e}^{-\phi(\lambda)}$. Therefore taken alone, it has no direct meaning.

It will be convenient to rewrite the boundary term as a sum of a steady-state part (10) and a term that is an integral of a pure derivative, that is

$$S_{\rm bnd} = S_{\rm bnd}|_{t=0} + \int_{\boldsymbol{c}} \boldsymbol{P}\cdot {\rm d}\lambda, \quad \boldsymbol{P} = \partial_\lambda \ln\langle 1|u_0\rangle \quad (13)$$

where $\boldsymbol{c}$ is the contour in the space of the variable parameters. By analogy with [8], and including the boundary contribution (13), the mgf in the quasi-steady-state limit can be written as an exponent of a sum of two terms

$$Z(\chi) = {\rm e}^{S_{\rm geom}(\chi)+S_{qst}(\chi)} \quad (14)$$

where

$$S_{\rm qst}(\chi) = -\int_0^t \epsilon_0(\chi, t')\,{\rm d}t' + S_{\rm bnd}|_{t=0} \quad (15)$$

is the quasi-stationary part of the generating function averaged over time. This is the part that morphs into the steady-state result (9) for fixed values of all parameters.

The other term in (14)

$$S_{\rm geom} = \int_{\boldsymbol{c}} [\boldsymbol{P}(\lambda) - \boldsymbol{A}(\lambda)]\,{\rm d}\lambda, \quad \boldsymbol{A}(\lambda) = \langle u_0|\partial_\lambda u_0\rangle \quad (16)$$

is the 'geometric phase' contribution responsible for additional reaction events. $\boldsymbol{A}$ is called the 'Berry connection'. $S_{\rm geom}$ has no analogue in the strict steady-state regime.

We further mention that definition (16) differs somewhat from those used for the non-cyclic geometric phase in quantum mechanics. For example, Pati and coworkers define the non-cyclic geometric phase as $\gamma_{gp} = \int_c [\boldsymbol{A}(\lambda) - \boldsymbol{P}'(\lambda)]\,d\lambda$, where $\boldsymbol{P}' = -\mathrm{Im}(\langle u(\lambda(0))|\partial_\lambda u(\lambda)\rangle/\langle u(\lambda(0))|u(\lambda)\rangle)$. In the present context, the meaning of such definition is unclear, whereas the geometric phase defined in (16) is derived directly from the exact representation of the mgf.

Since $\boldsymbol{P}$ is a pure derivative, it is important only when looking at an evolution along an open path in the parameter space. If the parameter vector λ returns to its initial value at the end of the evolution, expression (16) becomes equivalent to the full-period geometric phase defined in [8].

4 Corrections to the reversible MM law

Consider now the average product creation rate in the MM system under the slow parameter evolution. The average number of new product molecules is $\langle n(t)\rangle = -\mathrm{i}(\partial Z(\chi, t)/\partial\chi)_{\chi=0}$. Therefore just like the full cgf, the average rate of the product production $\langle J\rangle = \mathrm{d}\langle n(t)\rangle/\mathrm{d}t$ can be written as a sum of the quasi-stationary J_{qst} and the geometric J_{geom} contributions

$$\langle J\rangle = J_{geom} + J_{qst} = \frac{\mathrm{d}}{\mathrm{d}t}\frac{\partial S_{geom}}{\partial\chi}\bigg|_{\chi=0} + \frac{\partial\epsilon_0(\chi, t)}{\partial\chi}\bigg|_{\chi=0} \quad (17)$$

Here we disregard the boundary term (11) since it contributes to average currents only when enzyme states are far from the equilibrium with substrate. It is irrelevant on time scales that are much larger than a single enzyme turn-over or when observation begins with almost equilibrated enzyme states. The geometric phase is time dependent only via the time dependence of the parameter vector λ. In the case of MM reaction with time-dependent concentrations n_s and n_p, the time derivative of the first term in (17) can be expressed as $\mathrm{d}/\mathrm{d}t \to (\mathrm{d}n_s/\mathrm{d}t)\partial/\partial n_s + (\mathrm{d}n_s/\mathrm{d}t)\partial/\partial n_p$. Substituting the eigenvectors and eigenvalues of $\hat{H}(\chi, \lambda)$ into (17), we find

$$J_{qst} = \frac{(k_1 n_s(t))k_2 - (k_{-2}n_p(t))k_{-1}}{K} \quad (18)$$

$$J_{geom} = -(k_2 + k_{-1})\frac{(k_2 + k_{-2}n_p(t))(k_1\dot{n}_s(t) + k_{-2}\dot{n}_p(t))}{K^3} \quad (19)$$

One can recognise J_{qst} as the average current for a steady state with fixed values of parameters. Our results show that there is a correction to this quasi-steady current when concentrations of the substrate and the product have their own time-dependent evolution. Specifically, in the case of MM kinetics, that is when $n_p \simeq 0$, the average rate of the coarse grained MM reaction per one enzyme becomes

$$\langle J\rangle \simeq \frac{k_2 n_s}{n_s + [(k_2 + k_{-1})/k_1]} - (k_2 + k_{-1})\frac{k_2 k_1 \dot{n}_s(t)}{(k_1 n_s + k_2 + k_{-1})^3} \quad (20)$$

That is, even in this case, the time dependence of the substrate concentration introduces corrections to the reaction rate. Generalisation of our approach to more complex enzymatic mechanisms is straightforward [23].

It is possible to understand result (19) with a simpler approach, which, unfortunately, is hard to generalise for higher current cumulants to demonstrate the geometric nature of the effect for all of them. The probability p_E of the enzyme to be unbound evolves according to the master equation

$$\frac{\mathrm{d}}{\mathrm{d}t}p_E = -[k_1 n_s(t) + k_{-2}n_p(t)]p_E + (k_2 + k_{-1})(1 - p_E) \quad (21)$$

with the solution

$$p_E(t) = (k_2 + k_{-1})\int_0^t \mathrm{e}^{-\int_{t_1}^t [k_1 n_s(\tau) + k_{-2}n_p(\tau) + k_2 + k_{-1}]\mathrm{d}\tau}\,\mathrm{d}t_1 \quad (22)$$

The information about the initial state is quickly forgotten. Hence only the time interval near $t_1 = t$ substantially contribute to (22). In this time interval, we can approximate $n_{s/p}(\tau) \simeq n_{s/p}(t) - (t - \tau)\dot{n}_{s/p}$ and the exponent of the integral over τ in (22) can be estimated as

$$\mathrm{e}^{-\int_{t_1}^t [k_1 n_s(\tau) + k_{-2}n_p(\tau) + k_2 + k_{-1}]\,\mathrm{d}\tau} \simeq \mathrm{e}^{-[k_1 n_s(t) + k_{-2}n_p(t) + k_2 + k_{-1}](t - t_1)} \times \left(1 + \frac{k_1\dot{n}_s(t) + k_{-2}\dot{n}_p(t)}{2}(t - t_1)^2\right) \quad (23)$$

Performing the remaining integration we find the expression for the probability of the enzyme to be unbound

$$p_E \simeq \frac{k_2 + k_{-1}}{K} + \frac{(k_2 + k_{-1})(k_1\dot{n}_s(t) + k_{-2}\dot{n}_p(t))}{K^3} \quad (24)$$

From (24), one can calculate the average reaction rate and check that indeed, it is the sum of the quasi-stationary and the geometric components determined in (18) and (19)

$$J(t) = (1 - p_E(t))k_2 - p_E(t)k_{-2}n_p(t) = J_{qst} + J_{geom} \quad (25)$$

5 Geometric effects in a growing cell culture

The geometric correction (19) is generally much smaller than the main contribution (18) if the number of the enzymes is much smaller than that of the substrates and the products.

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However, this small correction has specific properties that can change system behaviour qualitatively.

The quasi-steady-state contribution to the kinetic rate in (18) can be vanishing because of a symmetry relation, such as the detailed balance condition, which guaranties that all chemical fluxes at the thermodynamic equilibrium state are zero on average. Thus, if a system is slowly driven externally so that it always remains close to the thermodynamic equilibrium, the quasi-steady-state approximation will predict zero average product creation. In contrast, the geometric contribution does not have to remain zero, and it will result in a qualitatively novel effect.

To show this, consider reaction (1) with concentrations of substrate and product n_s and n_p large and treated deterministically. Let us suppose that the system is initially in an equilibrium

$$k_1 k_2 n_s(0) = k_{-1} k_{-2} n_p(0) \tag{26}$$

Suppose that process (1) happens inside a living cell that grows and divides in its usual cycle. Assume that all molecules participating in reaction (1) are not involved in growth-related processes; that is, the reaction describes metabolism of a rare compound, largely decoupled from cell division. Then when the number of cells in the colony increases from the initial value $N(0)$ to $N(t)$, the concentrations of substrate and products will decrease approximately as

$$n_s(t) = n_s(0)\frac{N(0)}{N(t)}, \quad n_p(t) = n_p(0)\frac{N(0)}{N(t)} \tag{27}$$

Such experiments are possible in modern microfluidic nanolitre chemostat platforms coupled to ion-mobility mass spectrometry for identification of efflux metabolites (see e.g. Enders *et al.* in this issue [28]).

Since the ratio $n_s(t)/n_p(t)$ is not affected by this time-dependent dilution, the system remains near equilibrium, and the quasi-steady state reaction rate remains zero. However, as n_s and n_p change with time, there will be non-adiabatic changes to them. The average number of additional product molecules, produced by a single enzyme is completely determined by the geometric part of the rate (19) (see eq. (28))

Equation (28) shows that the average number of new product molecules per one enzyme is a fraction of unity, which compares to a large number of already existing substrate and product molecules. However, the geometric contribution qualitatively changes the result, predicting on average non-zero amount of new product molecules, which is not expected from the standard MM treatment. The effect becomes distinguishable from noise when the average number of generated product molecules, which is of the order of the number of enzymes in the culture, n_E, is comparable to the size of a typical fluctuation of the number of product molecules in equilibrium. The latter can be estimated as $\sqrt{n_p(0)n_{cells}}$, where n_{cells} and $n_p(0)$ are the numbers of cells and product molecules per cell at the beginning of the observation. That is, the geometric condition will be observable if $n \gg \sqrt{n_p(0)n_{cells}}/n_E$. This falls within the domain accessible to modern experimental techniques [28]. For example, if $n_p(0) \sim 10^4$, and the number of enzymes per cell is $n_E \gg 10^2$, this condition is satisfied even for a single cell.

Result (28) would be valid only if we could treat concentrations as parameters, changing only because of the external volume growth. In a closed system chemical fluxes eventually should be compensated by the reverse fluxes because of the violation of the steady-state condition (26), or by efflux of extra generated product molecules. Since detection of the deviation of the ratio n_s/n_p from the equilibrium value by an effect of the order of $\propto n_E/n_p(0)$ is experimentally hard, we believe that it is the efflux mass-spectrometry experiments [29] that carry the highest chance of measuring the geometric fluxes in growing cell cultures.

Considering intermediate stages of the culture growth, one can notice that the number of newly produced molecules depends only on the initial and the final cell numbers: that is, the average number of produced proteins depends on the current state of the system, but not on how it got there or where it is going from there. This can be utilised by living organisms in order to control some processes that should depend on the stage of the cell cycle only. Although this effect is very small, it should be interesting to explore its detectability in vivo and employ it in artificial biological circuits design.

6 Discussion

In this article, we generalised the notion of the geometric phase in evolution of the mgf to non-periodic time-dependent processes. The expression for our non-periodic geometric phase is different from the ones often encountered in quantum mechanical applications. Its

$$n = \int_{N(0)}^{\infty} \mathrm{d}N \left[-(k_2 + k_{-1}) \frac{(k_2 + k_{-2}n_p N(0)/N)(k_1 \partial_N (n_s(0)N(0)/N) + k_{-2}\partial_N (n_p(0)N(0)/N)}{K^3(v)} \right]$$
$$= \frac{k_1 k_2 n_s(0)}{(k_2 + k_{-1})(k_{-1} + k_1 n_s(0))} \tag{28}$$

uniqueness follows from the existence of a special gauge that should be imposed in order to describe stochastic kinetics correctly.

We showed that the phase is responsible for non-adiabatic corrections to the standard Michaelis–Menten approximation. Such corrections are usually small in comparison to the quasi-steady-state predictions. However, they explicitly break time-reversal symmetries and, therefore can produce a qualitatively different result when a chemical system is driven closely to a thermodynamic equilibrium, as in the cell culture growth model that we discussed.

We studied only the simplest of its realisations. The introduced non-periodic geometric phase is completely general and should appear practically in any interacting chemical system driven by external fields. Other interesting examples will surely emerge with time. We expect the greatest opportunities for biological relevance in the domain of molecular motors, where geometric effects play an important role as is [11].

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