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A model for the enzyme activity in systems with large composition fluctuations. An application to the unusual kinetics of phospholipase A_2

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Abstract. A non-equilibrium thermodynamics based model is proposed in order to describe the role of large concentration fluctuations of enzymes, reactants and products in modulating the macroscopic time evolution of chemical kinetics. The encounter probabilities between reactants and enzyme depend on their local concentration. Fluctuations modify the bimolecular encounter probability. Since, in turn, the amplitude of fluctuations depends itself on the instantaneous composition of the reacting mixture, the time-varying chemical composition acts as a positive feedback mechanism for the reactive fluid mixture near the critical temperature for phase separation. The model is applied to rationalize the unusual features of phospholipase A_2 kinetics, an enzyme which catalyzes the hydrolisis of membrane forming phospholipids, yielding products which are still soluble in the lipid matrix. A typical feature of the enzyme reaction is the long induction time prior to a "burst" of activity. This effect is well reproduced by the theory, together with the dependence of the induction time on the exogeneous addition of products or other liposoluble substances, the effects of enzyme and substrate concentration, and the temperature dependence of the enzyme activation. All these properties emerge as a consequence of the coupling between enconter probability and time-varying bilayer heterogeneity. A good qualitative agreement between theoretical results and the available experimental results has been generally found.

PACS. 05.40.+j Fluctuation phenomena, random processes, and Brownian motion – 87.15.-v Molecular biophysics

1 Introduction

The rate of the bimolecular A + B chemical reactions depends on the local concentration of A and B molecules. Concentration fluctuations are expected to play an important role by modifying the probability for simultaneously finding two reactive molecules at the same site.

Additional complexity arises from the fact that the fluctuation amplitudes depend themselves on the time-varying concentration of reactants and products, leading to a rather complex process where both fluctuations and macroscopic reaction rates are strongly coupled.

One should expect particularly dramatic effects in macromolecules involving kinetics (*e.g.*, enzyme reactions) because the relatively small translational entropy of large molecules favours the growing of local fluctuations in composition, which eventually lead to a macroscopic phase separation for unfavourable macromolecule-solvent interactions.

In this study we propose a simple model to investigate the effect of the time-evolving local heterogeneities on the macroscopic (space-averaged) rates, just before the phase separation of the reacting mixture forming components. In other words, the analysis is limited to the region where fluctuations are unstable with respect to the homogeneous phase. This assumption allows one to work within a formalism related to the classical Cahn-Hilliard theory of the spinodal decomposition, developed for unreactive mixtures [1–3] and extended by a number of authors to polymers [4–7], liquid crystals [8] and microemulsions [9,10].

Upon a further change of chemical composition of the reacting fluid, and for certain critical values of the intermolecular forces, the concentration fluctuations become thermodynamically stable giving rise to long-lived microdomains. Kinetic equations for the late stage of phase-separating fluids are described by totally different models derived from nucleation and crystal growth models [11–13], the processes in the late stages are not considered in the present theory.

The model is employed to explain the unusual latency period of phospholipase A_2 kinetics, an important enzyme involved in the hydrolisis of membrane forming phospholipids. Specifically, in Section 2 the peculiar features of the enzyme kinetics are briefly reviewed. In Section 3.1 we develop a general theory accounting for the interplay between composition-dependent concentration fluctuations

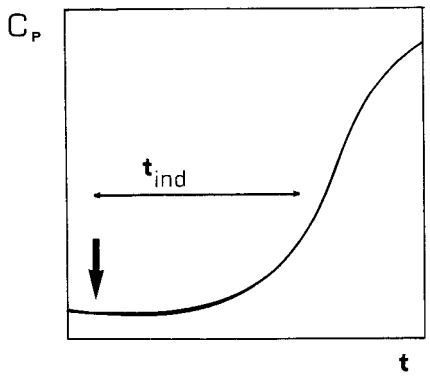

Fig. 1. Typical time evolution of phospholipase A_2 catalyzed hydrolysis of phospholipids. C_P is the products concentration and t_{ind} is the induction time prior to the burst of enzyme activity. The arrow marks the addition of phospholipase A_2.

and rate constants. Kinetic equations for phospholipase A_2 catalyzed hydrolysis in homogeneous (fluctuation free) media are calculated in Section 3.2 and employed as a zeroth order approximation. In Sections 3.3 and 3.4 concentration fluctuations in a solvent-free reaction bath formed by a non-ideal mixture of substrate (phospholipids) and products (lyso-phospholipids + fatty acid) are evaluated. In section 3.5 the enzyme rate is corrected for the fluctuations. Finally, in Section 4 the main predictions of the theory are compared with experimental data taken from the literature, and in Section 5 the main features, limits and possible improvements of the model are briefly discussed.

2 Biochemical background

Phospholipase A_2 is an enzyme weakly associated to the lipid membrane which is involved in the hydrolisis of double-chain phospholipids, yielding fatty acids and lysophospholipids (single-chain phospholipids) as products (for recent review articles see, *e.g.*, [14,15]). It is worth noting that both substrate and products remain substantially within the lipid matrix during the reaction's course, constituting a "solvent free" reactive mixture which is in contact with an adsorbed layer of enzymes. This provides an ideal system because the fluctuations of the reactive components are not damped by any solvent dilution effect.

The main typical features of the hydrolisis kinetics are: A) The enzyme activity exhibits a phase lag followed by a "burst" of activity. Increases as much as three orders of magnitude over times as short as a few minutes have been reported (see Fig. 1). B) The plot of the lag time *versus* temperature shows a sharp minimum in the region of the gel to liquid crystal phase transition of substrate. C) A variety of substances, including the products of the enzyme catalyzed hydrolysis, strongly modify the lag time.

These unusual features have been explained in different ways [16–18] through a number of phenomenological kinetic models which relate the abrupt burst of enzyme activity with lipid matrix composition. Recently, some interesting papers [19–22] pointed out the role of the non-ideal mixing of the substrate (phospholipids) and products (fatty acid + lysophospholipids) in modulating the enzyme activity. Specifically, it has been suggested that on increasing the products' concentration up to about 10% [21] the reacting mixture undergoes a macroscopic phase separation, near that critical concentration there is a burst of activity [20,21]. However, recent evidence from enzyme kinetics performed in lipid monolayers [22], together with conflicting reports on the phospholipids and fatty acids reciprocal miscibility in monolayers [23–27] and bilayers [28–30] suggest that a *macroscopic* phase separation of the reacting mixture components is not the unavoidable perequisite for enzyme activation. Although concepts like phase transition, phase separation, static and dynamic heterogeneity of multicomponent lipid bilayers are still rather vague and controversial (see, *e.g.*, Ref. [31] for a recent critical analysis of this issue), probably some conflicting views concerning the coupling between membrane heterogeneity and enzyme activity might be reconciled following a different approach such as that we are going to develop in the next sections.

3 Theory

3.1 Chemical reactions in a fluctuating medium

The concentrations of chemical species in a reacting medium obey the continuity equation

$$\frac{\partial \phi}{\partial t} = -\mathrm{div}\ \mathbb{J} + \mathbb{G}(\phi) \tag{1}$$

where ϕ is a column vector of the concentrations (expressed as volume fractions), $\mathbb{J}$ is the vector of diffusional fluxes of the chemical species related to the nonuniform composition of the reacting mixture and $\mathbb{G}(\phi)$ is a source term which depends on the chemical reactions. It is useful to split $\mathbb{G}(\phi)$ into two components, in matrix notation it reads

$$\mathbb{G}(\phi) = \mathbb{H}\,\phi + \phi\,\mathbb{K}\,\hat{\phi} \tag{2}$$

where the $\mathbb{H}$ and $\mathbb{K}$ matrices describe the unimolecular and bimolecular kinetics constants, respectively. Generally, no higher order terms appear in the usual enzyme kinetics, hence cubic or quartic terms (which sometimes are responsible for interesting autocatalytic effects) are here disregarded.

To proceed further, we write down the concentration vector ϕ as

$$\phi = \bar{\phi}(t) + \eta(\mathbf{r}, t) \tag{3}$$

where $\bar{\phi}(t)$ is the homogeneous part (which depends only on time) and $\eta(\mathbf{r}, t)$ is the fluctuating one, which depends also on the space coordinates $\mathbf{r}$. Clearly, the average over

a macroscopic volume V and time τ (of the order of the fluctuations lifetime), $\frac{1}{V\tau}\int_V\int_{t'}^{t'+\tau}\eta(\mathbf{r},t)\mathrm{d}t \equiv \langle\langle\eta(\mathbf{r},t)\rangle_V\rangle_\tau$, vanishes whereas the average of the quadratic term $\langle\langle\eta(\mathbf{r},t)\,\mathbb{K}\,\hat{\eta}(\mathbf{r},t)\rangle_V\rangle_\tau$ is generally nonzero.

We now insert equations (2, 3) into equation (1) and develop $\mathbb{J}$ in a series of gradient terms $\nabla^n\eta$ about $\bar{\phi}(t)$

$$\mathbb{J}(\phi(\mathbf{r},t)) = \sum_{n=1}^{\infty} b_n(\bar{\phi}(t))\nabla^n\eta(\mathbf{r},t) \tag{4}$$

($b_0(\bar{\phi}(t)) = 0$ because the diffusive flux exists only in systems with a concentration gradient), using the obvious identity

$$\begin{aligned}\eta(\mathbf{r},t)\,\mathbb{K}\,\hat{\eta}(\mathbf{r},t) &= \langle\langle\eta(\mathbf{r},t)\,\mathbb{K}\,\hat{\eta}(\mathbf{r},t)\rangle_V\rangle_\tau \\ &+ (\eta(\mathbf{r},t)\,\mathbb{K}\,\hat{\eta}(\mathbf{r},t) - \langle\langle\eta(\mathbf{r},t)\,\mathbb{K}\,\hat{\eta}(\mathbf{r},t)\rangle_V\rangle_\tau)\end{aligned} \tag{5}$$

($\mathbb{K} = \frac{1}{2}\,\partial^2\,\mathbb{G}/\partial\phi^2|_{\bar{\phi}(t)}$) we transform equation (1) as

$$\frac{\partial\bar{\phi}(t)}{\partial t} = \mathbb{G}(\bar{\phi}(t)) + \langle\langle\eta(\mathbf{r},t)\,\mathbb{K}\,\hat{\eta}(\mathbf{r},t)\rangle_V\rangle_\tau \tag{6a}$$

$$\begin{aligned}\frac{\partial\eta(\mathbf{r},t)}{\partial t} =& -\operatorname{div}\sum_{n=1}^{\infty} b_n(\bar{\phi}(t))\nabla^n\eta(\mathbf{r},t) \\ &+ \left.\frac{\partial\,\mathbb{G}}{\partial\phi}\right|_{\bar{\phi}(t)}\eta(\mathbf{r},t) + (\eta(\mathbf{r},t)\,\mathbb{K}\,\hat{\eta}(\mathbf{r},t) \\ &- \langle\langle\eta(\mathbf{r},t)\,\mathbb{K}\,\hat{\eta}(\mathbf{r},t)\rangle_V\rangle_\tau).\end{aligned} \tag{6b}$$

At a first sight the resulting equations look more complex than the original ones; the above procedure, however, takes advantage on the different time scale of the kinetics processes. Indeed, since we are limiting the analysis to energetically unfavourable local inhomogeneities, we may suppose that their life time τ is short, hence for $t' < t < t' + \tau$ the chemical composition of the fluid remains practically constant. Therefore, one may average equation (6a) over τ with the aid of the relationships

$$\begin{aligned}\frac{1}{\tau}\int_{t'}^{t'+\tau}\frac{\partial\bar{\phi}(t)}{\partial t}\mathrm{d}t &= \frac{1}{\tau}(\bar{\phi}(t'+\tau) - \bar{\phi}(t')) \cong \frac{\partial\bar{\phi}(t')}{\partial t'} \\ \frac{1}{\tau}\int_{t'}^{t'+\tau}\mathbb{G}(\bar{\phi}(t'))\mathrm{d}t &\cong \mathbb{G}(\bar{\phi}(t'))\end{aligned} \tag{7}$$

yielding

$$\frac{\partial\bar{\phi}(t')}{\partial t'} = \mathbb{G}(\bar{\phi}(t')) + \langle\langle\eta(\mathbf{r},t)\,\mathbb{K}\,\hat{\eta}(\mathbf{r},t)\rangle_V\rangle_\tau. \tag{8a}$$

Now the last term in equation (8a) depends on the "slow" time t' alone, hence equation (8a) can be easily integrated.

By contrast equation (6b), which rules the time evolution of the fluctuations, vanishes upon averaging over τ. This is because the fluctuations evolve through a "fast" variable t and, therefore, in equation (6b) one may replace the slowly varying chemical composition $\bar{\phi}(t)$ by a constant term, $\bar{\phi}(t')$, calculated by equation (8a)

$$\begin{aligned}\frac{\partial\eta(\mathbf{r},t)}{\partial t} =& -\operatorname{div}\sum_{n=1}^{\infty} b_n(\bar{\phi}(t'))\nabla^n\eta(\mathbf{r},t) \\ &+ \left.\frac{\partial\,\mathbb{G}}{\partial\phi}\right|_{\bar{\phi}(t')}\eta(\mathbf{r},t) \\ &+ (\eta(\mathbf{r},t)\,\mathbb{K}\,\hat{\eta}(\mathbf{r},t) - \langle\langle\eta(\mathbf{r},t)\,\mathbb{K}\hat{\eta}(\mathbf{r},t)\rangle_V\rangle_\tau).\end{aligned} \tag{8b}$$

Equations (8a, 8b) are solved for the slow ($\bar{\phi}(t')$) and fast ($\eta(\mathbf{r},t)$) variables, respectively. The coupling term $\langle\langle\eta(\mathbf{r},t)\,\mathbb{K}\,\hat{\eta}(\mathbf{r},t)\rangle_V\rangle_\tau$ in the right hand side of equation (8a) accounts for the evolution of the macroscopic (space averaged) chemical composition induced by the concentration inhomogeneities.

Under the above assumptions one expects small fluctuations, so we look for a perturbation solution of equations (8). The zeroth order approximation reads

$$\frac{\partial\bar{\phi}^{(0)}(t')}{\partial t'} = \mathbb{G}(\bar{\phi}^{(0)}(t')) \tag{9a}$$

which is all but nothing more than the classical kinetics equation for homogeneous media. By retaining only linear terms in $\eta(\mathbf{r},t)$, one finds from equation (8b) [32]

$$\begin{aligned}\frac{\partial\eta^{(0)}(\mathbf{r},t)}{\partial t} =& -\operatorname{div}\sum_{n=1}^{\infty} b_n(\bar{\phi}^{(0)}(t'))\nabla^n\eta^{(0)}(\mathbf{r},t) \\ &+ \left.\frac{\partial\,\mathbb{G}}{\partial\phi}\right|_{\bar{\phi}^{(0)}(t')}\eta^{(0)}(\mathbf{r},t) \\ \cong& -\operatorname{div}\sum_{n=1}^{\infty} b_n(t')\nabla^n\eta^{(0)}(\mathbf{r},t).\end{aligned} \tag{9b}$$

Higher order terms can be calculated in a standard way. The above equations have been obtained by assuming that the evolution of the chemical composition is slower than the life time of the concentration inhomogeneities [33]. However, the time evolving chemical composition has a dramatic effect on the amplitude of fluctuations. In fact, for each value of the chemical composition, the fluctuations (ruled by the fast variable t) are modulated by the instantaneous composition alone (ruled by the slow variable t').

In order to calculate the first order correction $\bar{\phi}^{(1)}(t')$ we used the expressions for $\eta^{(0)}(\mathbf{r},t)$ calculated by equation (9b). Inserting them into equation (8a) we obtain the sought – for expression for the fluctuation – enhanced chemical reactivity

$$\begin{aligned}\frac{\partial\bar{\phi}^{(1)}(t')}{\partial t'} =& \left.\frac{\partial\,\mathbb{G}}{\partial\phi}\right|_{\bar{\phi}^{(0)}(t')}\bar{\phi}^{(1)}(t') \\ &+ \langle\langle\eta^{(0)}(\mathbf{r},t)\,\mathbb{K}\,\hat{\eta}^{(0)}(\mathbf{r},t)\rangle_V\rangle_\tau\end{aligned} \tag{10}$$

where, as previously said, the last term depends upon $\bar{\phi}^{(0)}(t')$.

The calculation of the zeroth order concentration vector $\bar{\phi}^{(0)}(t')$ for the phospholipase A_2 enzyme reaction in an ideal homogeneous fluid is performed in the next section.

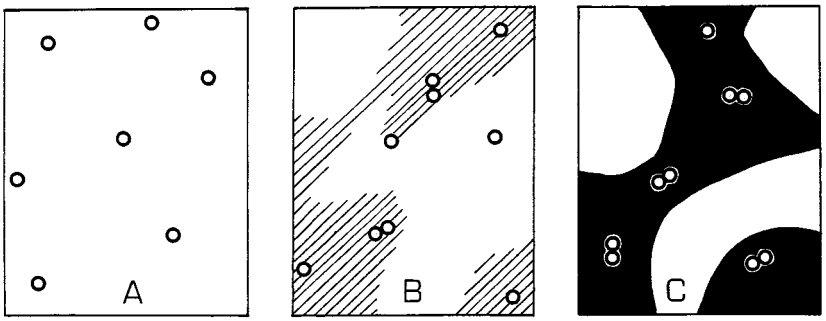

Fig. 2. Schematic picture for the composition evolution of phospholipase A_2 containing lipid membrane in the early (A), intermediate (B) and late stages (C) of bilayer enzymatic hydrolysis. Enzymes are depicted as circles which may form dimers, the activity of which was assumed to be much larger than the activity of the monomeric enzymes. The grey and black regions denote increasing concentrations of the enzyme reaction products (lysophospholipids + fatty acids). See text for further explanations.

3.2 Calculation of the zeroth order enzyme kinetics

The following simplified kinetic scheme has been considered:

$$\begin{aligned} &\mathrm{E} + \mathrm{E} \xrightarrow{k_\mathrm{d}} \mathrm{E}_2 \\ &\mathrm{E}_2 + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{Y} \\ &\mathrm{Y} \xrightarrow{k_\mathrm{f}} \mathrm{E}_2 + \mathrm{P} \end{aligned} \tag{11}$$

here E is the enzyme in its inactive monomeric form, E_2 is the active enzyme dimer, Y is the activated complex, S and P are substrate and products, respectively. The first equation describes enzyme activation consequent to the formation of highly reactive dimers (or n-mers) and is based on much experimental evidence set forth by a number of authors [17,18,34–37], whereas the second and third equations are typical of a standard enzyme kinetics. The zeroth order kinetics equations, valid for a random distribution of reactants, enzyme and products are

$$-\frac{\mathrm{d}\mathbb{C}_\mathrm{E}^{(0)}}{\mathrm{d}t'} = k_\mathrm{d}\mathbb{C}_\mathrm{E}^{(0)^2} \tag{12a}$$

$$\frac{\mathrm{d}\mathbb{C}_\mathrm{Y}^{(0)}}{\mathrm{d}t'} = k_1\,\mathbb{C}_\mathrm{S}^{(0)}\,\mathbb{C}_{\mathrm{E}_2}^{(0)} - (k_{-1}+k_\mathrm{f})\,\mathbb{C}_\mathrm{Y}^{(0)} \tag{12b}$$

$$\frac{\mathrm{d}\mathbb{C}_\mathrm{P}^{(0)}}{\mathrm{d}t'} = k_\mathrm{f}\,\mathbb{C}_\mathrm{Y}^{(0)} \tag{12c}$$

where $\mathbb{C}_j^{(0)}$ is the zeroth order concentration expressed as number of j-th particles/number of total particles. Generally, kinetic equations are expressed in terms of molar concentration $\mathbb{C}_j$ because the encounter probability depends on the number of particles. By contrast, the flux of particles during fluctuation is more conveniently described by the volume fraction ϕ_j (see Sect. 3.3). The two quantities are related each other by

$$\mathbb{C}_j = \phi_j / N_j \tag{13}$$

where N_j is the molar volume of the j-th species (compared with a reference volume, *e.g.*, the solvent volume). All the following formulas will be expressed in terms of ϕ_j.

Equations (12) are easily solved under the standard steady state assumption for the activated complex: $\mathrm{d}\mathbb{C}_\mathrm{Y}^{(0)}/\mathrm{d}t' = 0$. Moreover, in a lipid bilayer the phospholipase A_2 is always in contact with a large number of phospholipids making the enzyme-substrate encounter probability independent of the substrate concentration: $k_1\,\mathbb{C}_\mathrm{S}^{(0)}\,\mathbb{C}_{\mathrm{E}_2}^{(0)} \cong k_1^\mathrm{eff}\,\mathbb{C}_{\mathrm{E}_2}^{(0)}$, an approximation experimentally validated for chemical reactions in amphiphilic assemblies [38].

By imposing the mass conservation: $\bar{\mathbb{C}}_\mathrm{E} = \mathbb{C}_\mathrm{E}^{(0)} + 2\,\mathbb{C}_{\mathrm{E}_2}^{(0)} + 2\,\mathbb{C}_\mathrm{Y}^{(0)}$ ($\bar{\mathbb{C}}_\mathrm{E}$ being the bound enzyme concentration), and applying the boundary conditions: $\mathbb{C}_\mathrm{E}^{(0)}|_{t'=0} = \bar{\mathbb{C}}_\mathrm{E}$; $\bar{\mathbb{C}}_{\mathrm{E}_2}^{(0)}|_{t'=0} = \mathbb{C}_\mathrm{Y}^{(0)}|_{t'=0} = 0$, one gets exact expressions for the zeroth order concentrations

$$\bar{\phi}_\mathrm{E}^{(0)}(t') = N_\mathrm{E}\,\mathbb{C}_\mathrm{E}^{(0)}(t') = \frac{\bar{\phi}_\mathrm{E}}{1 + k_\mathrm{d}(\bar{\phi}_\mathrm{E}/N_\mathrm{E})t'} \tag{14a}$$

$$\begin{aligned} \bar{\phi}_\mathrm{P}^{(0)}(t') &= N_\mathrm{L}\,\mathbb{C}_\mathrm{P}^{(0)}(t') = (N_\mathrm{L}\gamma\bar{\phi}_\mathrm{E}/k_\mathrm{d}N_\mathrm{E}) \\ &\quad \times (t' - \frac{1}{k_\mathrm{d}(\bar{\phi}_\mathrm{E}/N_\mathrm{E})}\log(1 + k_\mathrm{d}(\bar{\phi}_\mathrm{E}/N_\mathrm{E})t') \\ &\cong \begin{cases} \dfrac{1}{2}\dfrac{\bar{\phi}_\mathrm{E}^2}{N_\mathrm{E}^2}N_\mathrm{L}\gamma t'^2 & t' \to 0 \\ \dfrac{N_\mathrm{L}}{N_\mathrm{E}}\dfrac{\bar{\phi}_\mathrm{E}}{k_\mathrm{d}}\gamma t' & t' \to \infty \end{cases} \end{aligned} \tag{14b}$$

where N_E is the enzyme mass (setting the solvent mass $N_\mathrm{W} = 1$); products and substrate have comparable masses, hence $N_\mathrm{S} \cong N_\mathrm{P} = N_\mathrm{L}$, N_L being the mass of a typical lipid molecule. The parameter γ is related to kinetic coefficients defined in scheme (11)

$$\gamma = \frac{1}{2}\quad\frac{k_1^\mathrm{eff}k_\mathrm{d}k_\mathrm{f}}{k_1^\mathrm{eff} + k_{-1} + k_\mathrm{f}} \tag{15}$$

$\bar{\phi}_\mathrm{E}$ is the concentration (expressed as volume fraction) of the membrane-associated enzymes, in the hypothesis of a fast equilibrium among bound and water-dissolved enzymes. For the time being, we will assume that $\bar{\phi}_\mathrm{E}$ does not appreciably change with the bilayer mean composition (a good approximation in the early stages of the process, a detailed analysis of this issue has been reviewed, *e.g.*, in Refs. [14,15,39]). The model, however, can be easily extended to also consider products-dependent enzyme binding as discussed in Appendix D.

Once the concentration of the different components has been calculated, one may estimate, for each instantaneous composition of the reacting fluid, the space and time concentration fluctuations for all the chemical species.

3.3 Calculation of concentration fluctuations in a reacting medium

In this subsection we estimate the amplitude of concentration fluctuations for a system which undergoes a chemical transformation. Fluctuations sustained by chemical reactions are conceptually different from those found in inert media because their amplitudes depend on the instantaneous chemical composition which, in turn, modifies the mixing properties of the fluid.

To proceed further, we need an explicit formula for the flux $\mathbb{J}$ of the molecules entering a volume element during the thermally-sustained fluctuations. Such an equation is obtained by assuming a linear relationship between fluxes and forces, the formal expression for a generic j-th component of the vector $\mathbb{J}$ is [5]:

$$\begin{aligned}\mathbb{J}_j(\mathbf{r}) &= -\frac{1}{kT}\int \Lambda_{jj}(\mathbf{r}-\mathbf{r}')\nabla\mu_j(\mathbf{r}')\mathrm{d}\mathbf{r}' \\ &\cong -\frac{1}{kT}\Lambda_{jj}\nabla\mu_j \end{aligned} \tag{16}$$

where $\Lambda_{jj}(\mathbf{r}-\mathbf{r}')$ is an Onsager mobility coefficient ($\Lambda_{lj}(\mathbf{r}-\mathbf{r}') \cong 0$ when $l \neq j$) and $\mu_j(\mathbf{r})$ is the local chemical potential of the j-th component (the thermal energy kT has been extracted for convenience). In heterogeneous media, the chemical potential μ_j is defined as the functional derivative of the free energy:

$$\begin{aligned}\mu_j = \delta F_{\mathrm{tot}}/\delta\phi_j = {} & \partial F_{\mathrm{tot}}/\partial\phi_j - \nabla\partial F_{\mathrm{tot}}/\partial(\nabla\phi_j) \\ & + \nabla^2\partial F_{\mathrm{tot}}/\partial(\nabla^2\phi_j) + \cdots \end{aligned} \tag{17}$$

μ_j can be calculated from the analytical expressions for the total free energy F_{tot}. A simple but reliable expression for F_{tot} is of a Landau-Ginzburg type

$$\begin{aligned}F_{\mathrm{tot}} = kT\int_V \Big(& f(\phi) + \frac{1}{2}\sum_l\sum_j \kappa_{lj}\nabla\phi_l\,\nabla\phi_j \\ & + \sum_j \phi_j\,\mathbb{F}_{j\mathrm{R}}(\mathbf{r})\Big)\mathrm{d}V \end{aligned} \tag{18}$$

where the sums span over the M components. The terms κ_{lj} ($\kappa_{lj} = \kappa_{jl}$) scale the effect of concentration gradients on the heterogeneous fluid free energy, their explicit expressions will be reported later. The homogeneous part of the free energy, $f(\phi)$, takes the usual meanfield expression (see, *e.g.*, Ref. [40])

$$f(\phi) = \sum_j \frac{\phi_j}{N_j}\log\phi_j + \frac{1}{2}\sum_l\sum_j \chi_{lj}\phi_l\phi_j \tag{19}$$

with $\sum_j \phi_j = 1$. Here the first term accounts for the translational entropy of the M components, each of them with mass N_j, and the second term describes a mean-field two-body interaction, with χ_{lj} a temperature dependent effective interaction parameter. Large and positive values of χ_{lj} mean a strong tendency toward phase separation. Finally, the last term in equation (18) accounts for the random force $\mathbb{F}_{j\mathrm{R}}(\mathbf{r})$ acting on the generic j-th component related to the thermal energy of the heat bath. $\mathbb{F}_{j\mathrm{R}}(\mathbf{r})$ can be describe by a superposition of standing waves through a Fourier series expansion

$$\mathbb{F}_{j\mathrm{R}}(\mathbf{r}) = \int_{-\infty}^{+\infty}\sum_q B_{jq}(\omega)\mathrm{e}^{i\mathbf{q}\mathbf{r}+i\omega t}\mathrm{d}\omega \tag{20}$$

the coefficients $B_{jq}(\omega)$ are calculated by imposing the condition that $\mathbb{F}_{j\mathrm{R}}(\mathbf{r})$ has zero mean and satisfies the energy equipartition principle.

Calculating the chemical potentials μ_j as the functional derivative of F_{tot} (Eq. (17)) and comparing this result with the series expansion of $\mathbb{J}$, equation (9b), one gets

$$\begin{aligned}\mathbb{J}_j = {} & \sum_{n=1}^{\infty} b_n(\bar{\phi}^{(0)})\nabla^n\eta^{(0)} = -\frac{\Lambda_{jj}}{kT}\nabla\mu_j \\ = {} & -\sum_{l=1}^{M}\Lambda_{ll}\left[\left.\frac{\partial^2 f}{\partial\phi_l\partial\phi_j}\right|_{\bar{\phi}^{(0)}}\nabla\eta_l^{(0)} - \kappa_{lj}\Lambda^3\eta_l^{(0)}\right] \\ & - \Lambda_{jj}\nabla\,\mathbb{F}_{j\mathrm{R}}(\mathbf{r}) + \mathbb{O}({\eta_l^{(0)}}^2) \end{aligned} \tag{21}$$

$f(\phi)$ and κ_{lj} given by equations (18, 19). Inserting this result into equation (9b) and exploiting the harmonic expansion of the random force, (Eq. (20)), eventually one finds

$$\begin{aligned}\frac{\partial\eta_j^{(0)}}{\partial t} = {} & \sum_{l=1}^{M}\Lambda_{ll}\left[\left.\frac{\partial^2 f}{\partial\phi_l\partial\phi_j}\right|_{\bar{\phi}^{(0)}}\nabla^2\eta_l^{(0)} - \kappa_{lj}\nabla^4\eta_l^{(0)}\right] \\ & - \Lambda_{jj}\sum_q\int_{-\infty}^{+\infty}\mathbf{q}^2\mathrm{B}_{jq}(\omega)\mathrm{e}^{i\mathbf{q}\mathbf{r}+i\omega t}\mathrm{d}\omega. \end{aligned} \tag{22}$$

We look for a Fourier series expansion for the system of linear partial differential equation (22). A particular solution is: $A_{jq}^{(0)}(\omega)\ \exp(i\mathbf{q}\cdot\mathbf{r}+i\omega t)$, inserting this expression into equation (22) one derives a set of linear algebraic equations which are solved for $A_{jq}^{(0)}(\omega)$

$$\begin{aligned}i\omega A_{jq}^{(0)}(\omega) = -\,\mathbf{q}^2\Bigg(& \sum_{l=1}^{M}\Lambda_{ll}\left(\left.\frac{\partial^2 f}{\partial\phi_l\partial\phi_j}\right|_{\bar{\phi}^{(0)}} + \mathbf{q}^2\kappa_{lj}\right)A_{lq}^{(0)}(\omega) \\ & + \Lambda_{jj}B_{jq}(\omega)\Bigg). \end{aligned} \tag{23}$$

Therefore, the general solution of equation (22) is

$$\eta_j^{(0)} = \int_{-\infty}^{+\infty}\sum_q A_{jq}(\omega)\mathrm{e}^{i\mathbf{q}\cdot\mathbf{r}+i\omega t}\mathrm{d}\omega \tag{24}$$

where the amplitudes $A_{jq}^{(0)}(\omega)$ are calculated by equation (23).

Equation (24) is the searched formula which relates the space and time concentration fluctuations of a reacting fluid with its molecular properties. The fluctuations are represented by a superposition of standing waves, the amplitudes of which depend in a rather complex way on the mixing properties of all the M components.

3.4 Concentration fluctuations during enzymatic hydrolysis of a lipid bilayer

Phospholipase A_2 catalyzed hydrolysis of phospholipids provides an ideal system for investigating the role of the concentration fluctuations on enzyme kinetics. Indeed, the lipid bilayer constitutes a "solvent free" reacting fluid where the fluctuations of the different components (bound enzyme, substrate and products) are not damped by large amounts of inert solvent, allowing for a huge amplification of the dynamic heterogeneity effects.

Let us apply the results derived in the above section to the phospholipase A_2 catalyzed reaction. Letting $\eta_{\rm E}, \eta_{\rm E_2}$ and $\eta_{\rm P}$ be the concentration fluctuations of bound enzyme (in its monomeric (E) and dimeric (E_2) form) substrate and product(s), one may reduce the number of the constitutive equations by considering the products of the enzyme hydrolysis (fatty acid and lysophospholipid) as an unique chemical specie, the properties of which are the averaged properties of the two components. Moreover, since we are interested in the early stages of the kinetics, we may safely neglect the concentration fluctuations of the enzyme dimers, because their concentration remains very small before the burst of activity. By exploiting the mass conservation constraints

$$\phi_{\rm P} + \phi_{\rm S} = 1 \quad \text{and} \quad \phi_{\rm E} + \phi_{\rm W} = 1 \tag{25}$$

(S = substrate and W = interfacial water) together with equation (16), we may write down the simple relationships for the fluxes of the different chemical components: $kT\,\mathbb{J}_{\rm P} = -\Lambda_{\rm P}^{\rm eff}\nabla(\mu_{\rm P} - \mu_{\rm S})$ and $kT\,\mathbb{J}_{\rm E} = -\Lambda_{\rm E}^{\rm eff}\nabla(\mu_{\rm E} - \mu_{\rm W})$ where $\Lambda_{\rm P}^{\rm eff} \equiv \Lambda_{\rm PP}\Lambda_{\rm SS}(\Lambda_{\rm SS} + \Lambda_{\rm PP})^{-1}$ and $\Lambda_{\rm E}^{\rm eff} \equiv \Lambda_{\rm EE}\Lambda_{\rm WW}(\Lambda_{\rm EE} + \Lambda_{\rm WW})^{-1}$. Since $\Lambda_j = D_j\phi_j$, D_j being the diffusion coefficient of the j-th component [5], considering that in the early stages of enzyme kinetics both $\phi_{\rm E}$ and $\phi_{\rm P}$ are small, one gets $\Lambda_{\rm P}^{\rm eff} \cong D_{\rm P}\phi_{\rm P}$ and $\Lambda_{\rm E}^{\rm eff} \cong D_{\rm E}\phi_{\rm E}$, hence the above expressions for $\mathbb{J}_{\rm P}$ and $\mathbb{J}_{\rm E}$ reduce to

$$kT\,\mathbb{J}_{\rm P} \cong -D_{\rm P}\phi_{\rm P}\nabla(\mu_{\rm P} - \mu_{\rm S}) \tag{26a}$$

$$kT\,\mathbb{J}_{\rm E} \cong -D_{\rm E}\phi_{\rm E}\nabla(\mu_{\rm E} - \mu_{\rm S}) \tag{26b}$$

$$\mathbb{J}_{\rm P} + \mathbb{J}_{\rm S} = 0 \tag{26c}$$

$$\mathbb{J}_{\rm E} + \mathbb{J}_{\rm W} = 0. \tag{26d}$$

Starting from the general expression for the free energy (Eqs. (18-20)), one gets [41]

$$\begin{aligned} F_{\rm tot}/kT = \int_S \Big[&\frac{1}{N_{\rm E}}\phi_{\rm E}\log\phi_{\rm E} + \phi_{\rm W}\log\phi_{\rm W} \\ &+ \frac{1}{N_{\rm L}}(\phi_{\rm P}\log\phi_{\rm P} + \phi_{\rm S}\log\phi_{\rm S}) + \frac{1}{2}\chi_{\rm P}\phi_{\rm S}\phi_{\rm P} + \frac{1}{2}\chi_{\rm E}\phi_{\rm W}\phi_{\rm E} \\ &+ \frac{1}{2}\Omega\phi_{\rm E}(\phi_{\rm P} - \phi_{\rm S}) + \frac{1}{2}\kappa_{\rm P}(\nabla\phi_{\rm P})^2 + \frac{1}{2}\kappa_{\rm E}(\nabla\phi_{\rm E})^2 \\ &+ \sum_j \phi_j\,\mathbb{F}_{j\rm R}(\mathbf{r})\Big]{\rm d}S. \end{aligned} \tag{27}$$

The first four logarithmic terms describe the mixing entropy of the aqueous surface layer (which contains bound enzyme and water) as well as the entropy of the lipid bilayer components, *i.e.* products (lysophospholipids + fatty acids) and substrate (phospholipids), respectively. $N_{\rm E}$ and $N_{\rm L}$ are the enzyme and lipid masses referred to the solvent $N_{\rm W}$ (products and substrate have comparable masses: $N_{\rm P} \cong N_{\rm S} = N_{\rm L}$, the mass of a typical lipid molecule).

The parameters $\chi_{\rm P}$, $\chi_{\rm E}$ and Ω account for the mixing properties of enzyme, substrate and products. Specifically, $\chi_{\rm E}$ measures the tendency of the enzyme molecules to cluster together at the surface of (or inside) the lipid membrane. Extensive studies on the clustering of integral proteins have been reported in the literature (*e.g.*, Ref. [42]), evidencing a rather general tendency of integral proteins to form aggregates ($\chi_{\rm E} > 0$).

The parameter $\chi_{\rm P} > 0$ is a measure of the immiscibility between substrate (phospholipids) and products (fatty acids + lysophospholipids). Calorimetric and fluorimetric measurements (*e.g.*, Refs. [21,27,43]) provide some information on the phase diagram of these ternary mixtures, evidencing poor miscibility between substrate and products. The parameter Ω accounts for the preferential binding of the enzyme onto products-rich or substrate-rich regions of the membrane. The preferential solvation of the phospholipase A_2 by products is well-known, playing a role in determining the enzyme activation. The terms proportional to $\kappa_j(\nabla^2\phi_j)$ describe the unfavourable energy due to spatial gradients of concentration [44]. Finally, the last term in equation (27) mimics the interaction of the thermal random forces with the different components.

As discussed in Appendix A, the zeroth order solution for the fluctuating components $\eta_j^{(0)}$ is

$$\eta_j^{(0)} = \sum_q \int_{-\infty}^{+\infty} A_{jq}^{(0)}(\omega)\exp(i\mathbf{q}\cdot\mathbf{r} + i\omega t){\rm d}\omega,$$

where, for the particular case of the phospholipase A_2 catalyzed reactions, the coefficients $A_{jq}^{(0)}(\omega)$ are found by solving the equations

$$A_{{\rm E}q}^{(0)}(\omega)(i\omega + Q_{\rm EE}) + A_{{\rm P}q}^{(0)}(\omega)Q_{\rm EP} = -F_{\rm E} \tag{28a}$$

$$A_{{\rm E}q}^{(0)}(\omega)Q_{\rm PE} + A_{{\rm P}q}^{(0)}(\omega)(i\omega + Q_{\rm PP}) = -F_{\rm P} \tag{28b}$$

where:

$$Q_{\mathrm{EE}} \equiv \mathbf{q}^2 D_{\mathrm{E}} \bar{\phi}_{\mathrm{E}}^{(0)} \left[\frac{1}{N_{\mathrm{E}} \bar{\phi}_{\mathrm{E}}^{(0)}} + \frac{1}{1-\bar{\phi}_{\mathrm{E}}^{(0)}} - \chi_{\mathrm{E}} + \mathbf{q}^2 \kappa_{\mathrm{E}} \right] \tag{29a}$$

$$Q_{\mathrm{PP}} \equiv \mathbf{q}^2 D_{\mathrm{P}} \bar{\phi}_{\mathrm{P}}^{(0)} \left[\frac{1}{N_{\mathrm{L}} \bar{\phi}_{\mathrm{P}}^{(0)}} + \frac{1}{N_{\mathrm{L}} (1-\bar{\phi}_{\mathrm{P}}^{(0)})} - \chi_{\mathrm{P}} + \mathbf{q}^2 \kappa_{\mathrm{P}} \right] \tag{29b}$$

$$Q_{\mathrm{PE}} \equiv -\mathbf{q}^2 D_{\mathrm{P}} \bar{\phi}_{\mathrm{P}}^{(0)} \Omega \tag{29c}$$

$$Q_{\mathrm{EP}} \equiv -\mathbf{q}^2 D_{\mathrm{E}} \bar{\phi}_{\mathrm{E}}^{(0)} \Omega \tag{29d}$$

$$F_j \equiv \mathbf{q}^2 D_j \bar{\phi}_j^{(0)} B_{jq}(\omega) \propto 2\mathbf{q}^2 D_j \bar{\phi}_j^{(0)} (kT)^{1/2} \quad j = \mathrm{E \text{ or } P} \tag{29e}$$

where the last identity (Eq. (29e)) follows from the energy equipartition principle [45]. It is worth noting that enzyme and products fluctuations are strongly coupled through the Q_{EP} and Q_{PE} terms. In other words, the flux of the enzymes over the bilayer surface follows the flux of the products because of the preferential affinity of the enzyme for domains with a given composition.

Pictorially, this situation is sketched in Figure 2 where we draw the concentrations of substrate, products and enzyme (monomers and dimers) at different times, namely $t' = 0$ (panel A), intermediate (panel B) and late stages (panel C) of enzyme kinetics, under the assumption of a better enzyme binding (solubility) to the products-rich regions. At $t' = 0$ enzymes are mostly in their inactive monomeric form and the bilayer contains only untransformed phospholipids. In the early stages of the enzyme hydrolysis a certain amount of products are formed and stored within the membrane, hence large concentration fluctuations begin to develop because of the substrate and products poor reciprocal miscibility. Enzymes, which preferentially bind to products-rich regions, undergo fast dimerization because of the increased local concentration (panel B) forming active dimers (or multimers) which rapidly hydrolize the surrounding phospholipid molecules. This self-sustained catalytic cycle continues until, in the late stages of the process, the system phase-separates and the amount of available substrate decreases (panel C). The late stage kinetics is far beyond the validity of the theory and will be qualitatively discussed in the next section.

Turning again to mathematics, solution of equations (28) yields explicit expressions for $\eta_{\mathrm{P}}^{(0)}$ and $\eta_{\mathrm{E}}^{(0)}$

$$\eta_{\mathrm{P}}^{(0)} = \sum_{\mathrm{q}} \int_{-\infty}^{+\infty} \frac{i\omega F_{\mathrm{E}} + F_{\mathrm{E}} Q_{\mathrm{PP}} - F_{\mathrm{P}} Q_{\mathrm{EP}}}{\omega^2 - i\omega(Q_{\mathrm{PP}} + Q_{\mathrm{EE}}) - (Q_{\mathrm{PP}} Q_{\mathrm{EE}} - Q_{\mathrm{EP}} Q_{\mathrm{PE}})} \mathrm{e}^{i\mathbf{qr} + i\omega t} \mathrm{d}\omega \tag{30a}$$

$$\eta_{\mathrm{E}}^{(0)} = \sum_{\mathrm{q}} \int_{-\infty}^{+\infty} \frac{i\omega F_{\mathrm{P}} + F_{\mathrm{P}} Q_{\mathrm{EE}} - F_{\mathrm{E}} Q_{\mathrm{PE}}}{\omega^2 - i\omega(Q_{\mathrm{PP}} + Q_{\mathrm{EE}}) - (Q_{\mathrm{PP}} Q_{\mathrm{EE}} - Q_{\mathrm{EP}} Q_{\mathrm{PE}})} \mathrm{e}^{i\mathbf{qr} + i\omega t} \mathrm{d}\omega \tag{30b}$$

$$\eta_{\mathrm{S}}^{(0)} = -\eta_{\mathrm{P}}^{(0)} \tag{30c}$$

In writing equations (30) unessential transient terms have been dropped because they do not contribute after time averaging.

By knowing the concentration fluctuations for enzyme, products and substrate, we are now in a position to calculate their influence on the enzyme rate.

3.5 Calculation of fluctuation-enhanced enzyme kinetics

The amplitude of concentration fluctuations, calculated in the previous section, enable us to estimate the enhancement of the macroscopic chemical rate through a repeated use of equation (10). Adopting the simplified kinetic scheme devised in equation (11) one gets $\langle\langle \eta^{(0)}\, \mathbb{K}\, \hat{\eta}^{(0)} \rangle_V \rangle_\tau = k_{\mathrm{d}} \langle\langle \eta_{\mathrm{E}}^{(0)^2} \rangle_V \rangle_\tau$, whence

$$-\frac{\mathrm{d}\,\mathbb{C}_{\mathrm{E}}^{(1)}}{\mathrm{d}t'} = k_{\mathrm{d}} (2\,\mathbb{C}_{\mathrm{E}}^{(0)}\,\mathbb{C}_{\mathrm{E}}^{(1)} + \frac{1}{N_{\mathrm{E}}^2} \langle\langle \eta_{\mathrm{E}}^{(0)^2} \rangle_V \rangle_\tau) \tag{31a}$$

$$\frac{\mathrm{d}\,\mathbb{C}_{\mathrm{Y}}^{(1)}}{\mathrm{d}t'} = k_1^{\mathrm{eff}}\,\mathbb{C}_{\mathrm{E}_2}^{(1)} - (k_{-1} + k_{\mathrm{f}})\,\mathbb{C}_{\mathrm{Y}}^{(1)} \tag{31b}$$

$$\frac{\mathrm{d}\,\mathbb{C}_{\mathrm{P}}^{(1)}}{\mathrm{d}t'} = k_{\mathrm{f}}\,\mathbb{C}_{\mathrm{Y}}^{(1)} \tag{31c}$$

where $\mathbb{C}_j^{(1)}$ is the fluctuation-modified concentration of the j-th chemical specie and the zeroth order expression for $\mathbb{C}_{\mathrm{E}}^{(0)} \equiv \mathbb{C}_{\mathrm{E}}^{(0)}(t')$ is given in equation (14a).

Apart from the obvious consideration that the concentration fluctuations do not affect the unimolecular chemical reactions (*e.g.*, dissociations), in the early and intermediate stages of the hydrolysis the enzyme-substrate encounter probability is independent of the local substrate heterogeneities because the enzymes are always surrounded by a large amount of untransformed phospholipids [38].

Furthermore, the concentration fluctuations of the products do not directly enter into the kinetic equations (31) because any specific inhibition or activation of the enzyme by products has been ruled out. However, as previously said, products and enzyme fluctuations are strongly coupled because of the preferential binding of the enzyme to products-rich (or products-poor) regions. This coupling strongly enhances the term $k_{\mathrm{d}} \langle\langle \eta_{\mathrm{E}}^{(0)^2} \rangle_V \rangle_\tau / N_{\mathrm{E}}^2$ (contained in Eq. (31a)) which describes the enhancement of the formation of active dimers by products-assisted enzyme concentration fluctuations. Its explicit expression is given by

equation (31b), which after separating the real and imaginary part and exploiting the results reported in Appendix B, one finds after some algebra:

$$\langle\langle {\eta_{\rm E}^{(0)}}^2\rangle_V\rangle_\tau = \\ = 4D_{\rm E}^2\,\bar\phi_{\rm E}^2\,kT\sum_q \mathbf{q}^4\int_{-\infty}^{+\infty}\frac{\omega^2+C(\mathbf{q})}{\omega^4+\omega^2A(\mathbf{q})+B^2(\mathbf{q})}{\rm d}\omega \\ \equiv 4\pi D_{\rm E}^2\,\bar\phi_{\rm E}^2\,kT\sum_q\frac{\mathbf{q}^4C(\mathbf{q})}{B(\mathbf{q})} \tag{32}$$

where

$$A(\mathbf{q}) = Q_{\rm PP}^2+Q_{\rm EE}^2+2Q_{\rm PE}Q_{\rm EP} \tag{33a}$$
$$B(\mathbf{q}) = Q_{\rm PP}+Q_{\rm EE}-Q_{\rm PE}Q_{\rm EP} \tag{33b}$$
$$C(\mathbf{q}) = (Q_{\rm PP}-\frac{F_{\rm P}}{F_{\rm E}}Q_{\rm EP})^2. \tag{33c}$$

The approximate solution (32) is valid for $B(\mathbf{q}) \ll 1$. Namely, when the reacting fluid approaches the phase separation among reactants and products.

Solving equations (31) for $\mathbb{C}_{\rm E}^{(1)}$ together with the boundary conditions $\mathbb{C}_{\rm E}^{(1)}|_{t'=0} = \mathbb{C}_{\rm E_2}^{(1)}|_{t'=0} = \mathbb{C}_{\rm Y}^{(1)}|_{t'=0} = 0$, the mass conservation constraint $\mathbb{C}_{\rm E}^{(1)}+2\,\mathbb{C}_{\rm E_2}^{(1)}+2\,\mathbb{C}_{\rm Y}^{(1)} = 0$ and the steady-state condition ${\rm d}\,\mathbb{C}_{\rm Y}^{(1)}/{\rm d}t' = 0$, one gets an exact analytical result

$$\frac{\partial\,\mathbb{C}_{\rm P}^{(1)}}{\partial t'} = \\ = \frac{\gamma/N_{\rm E}^2}{(1+k_{\rm d}\,\bar{\mathbb{C}}_{\rm E}t')^2}\int_0^{t'}(1+k_{\rm d}\,\bar{\mathbb{C}}_{\rm E}t'')^2\langle\langle {\eta_{\rm E}^{(0)}}^2(t'')\rangle_V\rangle_\tau{\rm d}t'' \\ = \frac{4\pi(\gamma/N_{\rm E}^2)kT}{(1+k_{\rm d}\,\bar{\mathbb{C}}_{\rm E}t')^2}D_{\rm E}^2\bar\phi_{\rm E}^2 \\ \times\sum_q\mathbf{q}^4\int_0^{t'}(1+k_{\rm d}\,\bar{\mathbb{C}}_{\rm E}t'')^2\frac{C(\mathbf{q},t'')}{B(\mathbf{q},t'')}{\rm d}t'' \tag{34}$$

with γ defined through equation (15). Equation (34) enables us to calculate the effect of space and time fluctuations on the products formation rate for the phospholipase A_2 catalyzed reaction. As previously stated, the fluctuation-dependent term is small, but it becomes large on approaching the phase separation between substrate and products. Since the products concentration varies with time, the latency period $t'_{\rm ind}$ prior to the burst of activity can be calculated by imposing the condition that the fluctuation-related term $\langle\langle {\eta_{\rm E}^{(0)}}^2\rangle_V\rangle_\tau$ must diverge at $t''\longrightarrow t'_{\rm ind}$. According to equation (34), this happens when the denominator $B(\mathbf{q})$ vanishes. Therefore, at $t''=t'_{\rm ind}$ one finds [46]

$$B(\mathbf{q},t'_{\rm ind}) \equiv Q_{\rm PP}Q_{\rm EE}-Q_{\rm PE}Q_{\rm EP} = 0. \tag{35}$$

Replacing Q_{lj}' s by their analytical expressions (Eqs. (29)), yields

$$B(\mathbf{q},t'_{\rm ind}) = \mathbf{q}^4D_{\rm P}D_{\rm E}\bar\phi_{\rm P}^{(0)}(t'_{\rm ind})\bar\phi_{\rm E}^{(0)}(t'_{\rm ind}) \\ \times\Big[(\frac{1}{N_{\rm E}\bar\phi_{\rm E}^{(0)}(t'_{\rm ind})}-\xi_{\rm EE}(\mathbf{q})(\frac{1}{N_{\rm L}\bar\phi_{\rm P}^{(0)}(t'_{\rm ind})} \\ -\xi_{\rm PP}(\mathbf{q}))-\Omega^2\Big] = 0 \tag{36a}$$
$$\xi_{\rm EE}(\mathbf{q}) \equiv -1+\chi_{\rm E}-\mathbf{q}^2\kappa_{\rm E} \tag{36b}$$
$$\xi_{\rm PP}(\mathbf{q}) \equiv -\frac{1}{N_{\rm L}}+\chi_{\rm E}-\mathbf{q}^2\kappa_{\rm P}. \tag{36c}$$

For the sake of simplicity, in equations (36a-c) we set $1-\bar\phi_{\rm P}^{(0)}(t')\cong 1$, $1-\bar\phi_{\rm E}^{(0)}(t')\cong 1$, and $\bar\phi_{\rm E}^{(0)}(t')\cong\bar\phi_{\rm E}$, approximations valid in the early stages of the enzyme kinetics (this assumption is discussed in Appendix C where a procedure valid in a wider range of concentrations has been developed. The main conclusions, however, are not modified by more elaborated calculations). Hence, using equations (14a,c) one gets

$$t'_{\rm ind}\cong\Big[\frac{2}{\gamma}\frac{1-N_{\rm E}\bar\phi_{\rm E}\xi_{\rm EE}(\mathbf{q})}{\xi_{\rm PP}(\mathbf{q})-N_{\rm E}\bar\phi_{\rm E}(\xi_{\rm PP}(\mathbf{q})\xi_{\rm EE}(\mathbf{q})-\xi_{\rm PE}^2(\mathbf{q}))}\Big]^{1/2} \\ \times\frac{N_{\rm E}}{N_{\rm L}}\frac{1}{\bar\phi_{\rm E}} \tag{37a}$$
$$\cong(2N_{\rm E}^2/\gamma N_{\rm L}^2\xi_{\rm PP}(\mathbf{q}))^{1/2}\frac{1}{\bar\phi_{\rm E}}+{\rm const.}+\mathbb{O}(\bar\phi_{\rm E}). \tag{37b}$$

γ, defined through equation (15), is a function of the kinetic constants alone. Equation (37) is the main result of this work. At small enzyme concentrations $\bar\phi_{\rm E}$ one should observe a linear dependence between $t'_{\rm ind}$ and $\bar\phi_{\rm E}^{-1}$ with a positive slope and nonzero intercept as shown in Figure 3. We will return to this formula in the next section when we compare our predictions with the available experimental data.

Once $t'_{\rm ind}$ has been obtained, one may calculate the rate of products formation just before $t'_{\rm ind}$ (see Fig. 1). Details are reported in Appendix E, here we quote the final expression:

$$\mathbb{C}_{\rm P}^{(1)}(t')\cong 0 \qquad t'_{\rm ind}\ll t' \tag{38a}$$
$$\mathbb{C}_{\rm P}^{(1)}(t')\cong 2\pi D_{\rm E}D_{\rm P}kT\bar\phi_{\rm E}(2\gamma)^{1/2}\Omega^2\sum_q\frac{\mathbf{q}^4}{\xi_{\rm PP}^{5/2}(\mathbf{q})}f(t') \tag{38b}$$
$$t'_{\rm ind}+\Delta t'<t'<t'_{\rm ind}$$

where $f(t')\equiv(t'+(t'_{\rm ind}-t')\log\frac{t'_{\rm ind}-t'}{\Delta t'})>0$ is an increasing function of t'. This formula will be discussed at point i) of the next section.

4 Results and discussion

Let us summarize the main predictions of our model for fluctuation-enhanced enzyme catalyzed reactions. Once

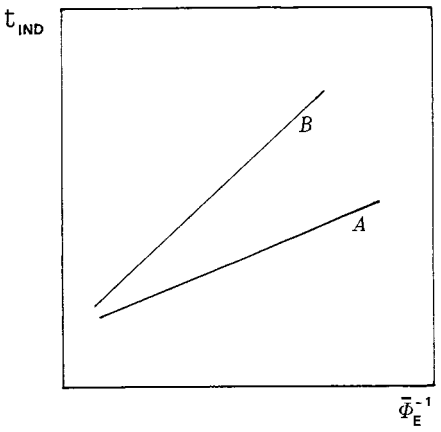

Fig. 3. Qualitative variation of the induction time $t_{\rm ind}$ *vs.* the inverse of bound enzyme concentration $\bar{\phi}_{\rm E}$. Curve A: the mean surface density for bound enzymes $\bar{\phi}_{\rm E}$ increases along with the products formation ($\Omega > 0$); curve B: $\bar{\phi}_{\rm E}$ decreases along with the products formation ($\Omega < 0$).

again, we want to stress that the results are valid only in the early stages of the enzyme kinetics when the substrate/products unmixing is not favoured because of the small products concentration. A qualitative extension of the theory to the thermodynamically stable two-phase region will be briefly discussed at point g.

The calculated quantities are: the length of the induction time, $t'_{\rm ind}$, prior to the burst of activity (Eq. (37)), and the rate of enzyme catalysis on approaching $t'_{\rm ind}$ (Eq. (38)).

a) The thermal fluctuation-related contribution to the enzyme kinetics, $\mathbb{C}_{\rm P}^{(1)}(t')$, is always positive and grows with time. This term is zero at $t' = 0$, remains small until the reacting fluid approaches the lateral phase separation between reactants and products (Eq. (38a)), then rapidly grows with time at the phase transition (Eq. (38b)). According to equation (37), the induction time depends both on thermodynamics (basically, the reactant-products miscibility parameter $\xi_{\rm PP}(\mathbf{q})$ and the enzyme binding constant) as well as on a combination of kinetic constants (the parameter γ defined by Eq. (15)). Long induction times occur in the case of poor miscibility, slow enzyme dimerization rate and small catalytic activity.

b) The critical concentration of unmixing, and hence the lag time, depends on the products concentration, which is zero at the beginning of the enzyme kinetic. Therefore, the exogenous addition of even a small amount of products should shorten the lag time length. This effect can be accounted for, following the procedure employed to derive equation (37b), by considering both the endogenous, $\bar{\phi}_{\rm P}$, and the hexogenous, $\bar{\phi}_{\rm P}^{\rm exo}$, products concentration. Calculations show that the lag time $t'_{\rm ind}$ now also depends on $\bar{\phi}_{\rm P}^{\rm exo}$ according to the simple equation [47]

$$t'_{\rm ind} \cong (t'_{\rm ind})_0(1 - \text{const.}\ \bar{\phi}_{\rm P}^{\rm exo})^{1/2} \quad \bar{\phi}_{\rm P}^{\rm exo} \ll 1 \qquad (39)$$

$(t'_{\rm ind})_0$ being the lag time when $\bar{\phi}_{\rm P}^{\rm exo} = 0$ (its analytical expression is given by Eq. (37) and const. $\propto \xi_{\rm PP}(\mathbf{q}) \geq 0$). The above result agrees fairly well with the experimental findings (*e.g.*, Refs [17,21,48]).

c) The lag time decreases on increasing the bound enzyme concentration $\bar{\phi}_{\rm E}$. According to equation (37b) the plot of (lag time) *versus* $\bar{\phi}_{\rm E}^{-1}$ is a straight line with positive slope and nonzero intercept. The slope chiefly depends on the reciprocal miscibility between reactants and products, the larger the incompatibility the shorter the latency. Deviations from this behaviour should be observed at high enzyme concentration (see Fig. 3). experimental data taken from the literature [17,21,49] support this prediction, exhibiting a good straight with positive slope, much similar to the curve reported in Figure 3.

As shown in Appendix D, a more refined model which allows for the products-modulation of the *averaged* enzyme binding does not affect the (quasi) linear relationship between the induction time $t'_{\rm ind}$ and the inverse of the bound enzyme concentration. Under the assumption of a fast equilibrium between free and bound enzymes, the fast equilibrium between free and bound enzymes, the slope slightly increases for a better enzyme solubility in the products-rich regions, and decreases for a large solubility of the enzyme in the products-poor domains of the lipid bilayer (see Fig. 3).

d) The lag time linearly increases with substrate (phospholipid), holding constant the enzyme concentration. In fact, the surface density of bound enzymes $\bar{\phi}_{\rm E}$ decreases on increasing the phospholipid concentration $\bar{\phi}_{\rm L}$ according to the obvious relationship (valid far from the complete surface coverage): $\bar{\phi}_{\rm E} \cong K_{\rm eq}(\bar{\phi}_{\rm E}^{\rm tot}/\bar{\phi}_{\rm L})$, where $K_{\rm eq}$ is the equilibrium binding constant and $\bar{\phi}_{\rm E}^{\rm tot}$ is the stoichiometric enzyme concentration in the whole suspension (water + phospholipids). Replacing the above expression for $\bar{\phi}_{\rm E}$ into equation (37b), one immediately finds a linear relationship between the induction time $t'_{\rm ind}$ and the phospholipid concentration. This prediction nicely agrees with recent data in the literature [17,21,49], provided the comparison is made with experiments falling within the range of validity of the present theory (enzymes/lipids $\ll 1$).

e) The temperature dependence of the lag time is rather complex. Temperature could modify the kinetic constants, diffusion coefficients and miscibility parameters. Several experimental studies pointed out a strong enzyme activation at, or near, the gel to liquid crystal thermotropic phase transition of phospholipids [19,50,51]. In this region domains with different composition and conformational states (gel-like and fluid-like) coexist within the lipid bilayer (see, *e.g.*, Ref. [31]). Our model completely neglects the lipids internal conformational states, hence, in its present form, the theory cannot describe enzyme kinetics in a membrane where both compositional and internal degrees of freedom of the lipid molecules are simultaneously varying. From a qualitative standpoint it is reasonable to guess wider enzyme fluctuations on approaching the gel to liquid crystal phase transition of the lipid matrix, hence the encounter probability among the enzymes to form active dimers (or multimers) is enhanced. The whole issue, however, deserves further analysis.

f) Many substances may affect the phospholipase enzyme kinetics by modifying the phase properties of the lipid matrix. Indeed, classical thermodynamics predicts a dramatic effect of traces of a third component on the mixing properties of two partially miscible fluids (*e.g.*, Refs. [52,53]). Therefore, either hydrophobic molecules soluble within the lipid matrix, or multivalent ions, which bind to the charged head groups tightening the lipid bilayer, could modify the enzyme activity without *directly* interacting with the protein structure (*e.g.*, Refs. [14,54–56]).

g) So far we have investigated only the early stages of the enzyme kinetics. The extension of model to the late stages in difficult and must follow different pathways [11–13]. Here we qualitatively discuss a likely scenario for the late evolution of phospholipase A_2 catalysis. When the products concentration reaches a critical value, the system undergoes a phase separation [21,43]. If the enzyme is more soluble in the products-rich phase, the kinetics slows down because of the reduced substrate concentration near the enzyme (Fig. 2, panel C). This effect has not been considered in our model where the enzyme-phospholipid encounter probability is assumed to be independent of the untransformed phospholipid concentration (see Sect. 3.2), an approximation permissible in the early stages of the kinetics but invalid upon macroscopic phase separation. Therefore, in the late stages of the kinetics, or upon exogenous addition of excessive amounts of products, one should observe a progressive enzyme inhibition, a result which has been suggested by some authors (*e.g.*, Ref. [57]).

h) The onset and growth of compositional heterogeneities of different size L (or, equivalently, with wavevector $\mathbf{q} = 2\pi/L$) follows a different time evolution. Microdomains with small $\mathbf{q}$'s appear early, however, their contribution to the enhancement of the enzyme kinetics is very small ($\propto \mathbf{q}^4$, see Eqs. (38)). Therefore, there should be a critical size for fluctuating domains which determines the greatest enhancement of enzyme activity, this critical size being smaller than that of macroscopic domains ($\mathbf{q} \longrightarrow 0$) but much larger than the molecular dimensions ($\mathbf{q} \longrightarrow 1$). Although there is a widespread consensus that the phospholipase A_2 enzyme activation is related to dynamic micro-heterogeneity, the above result may held to understand why a burst of enzyme activity is sometimes observed even in the absence of macroscopic phase separation, detectable by conventional calorimetric techniques [22]. Once again this fact stresses the need for a deeper investigation of lipid bilayer internal fluctuations.

i) The parameters which determine the extent of enzyme activation after an induction time $t'_{\rm ind}$ differ from those which rule the duration of $t'_{\rm ind}$. From equation (38b) it is evident that no activation is possible unless the enzyme preferentially associates with products-rich, or products-poor, regions (the enzyme activation is independent of the sign of the preferential association parameter Ω, depending on Ω^2; for a more complete model see appendix D). Furthermore, the activation is also depressed by low temperatures and small diffusion coefficients of enzyme and products.

5 Concluding remarks

The irreversible thermodynamics-based theory developed in this paper considers the influence of concentration fluctuations (of enzymes, substrate and products) on the enzyme catalytic activity, for the special case where the fluctuations are sustained by the time-varying composition of the reacting fluid.

Apart from the correctness of the theoretical semi-quantitative prediction discussed in the previous section, the model introduces a conceptual improvement over a number of different theories existing in the literature, most of them based on elaborated formal kinetic approaches (*e.g.*, Refs. [16–18,58]). Indeed, in our model the concept of products-activation of phospholipase A_2 catalysis naturally emerges from the following self-sustained catalytic cycle (see Fig. 2): (A) (slow) products formation $\longrightarrow$ (B) enhanced concentration fluctuations because of substrate-products poor miscibility $\longrightarrow$ (C) enhanced formation rate of active enzyme dimers (more soluble in the products-rich regions) $\longrightarrow$ (A) (Fast) products formation.

Of course, the above scheme for products-activated enzyme kinetics requires: a) substrate and products must have a poor reciprocal miscibility; b) the enzyme is more soluble in the products-rich (or substrate-rich) regions; c) the different components are freely moving within the lipid matrix; d) the reacting fluid does not contain a large amount of inert solvents.

In spite of the over-simplified picture and mathematical approximations (which can be improved in future works), the theory explains much of the unusual features of phospholipase A_2 kinetics and seems to have a wider range of applicability in modeling a variety of phenomena covering the broad field of chemical reactivity in dynamic heterogeneous media [59–61].

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Appendix A

The chemical potential of enzyme and product(s) is calculated as the functional derivative of the free energy (Eq. (17)). After simple algebra one finds:

$$\mu_{\rm E} - \mu_{\rm W} = \text{const.} + kT\Big[\frac{1}{N_{\rm E}}\log\phi_{\rm E} - \log(1-\phi_{\rm E}) - \chi_{\rm E}\phi_{\rm E} - \Omega\phi_{\rm P} - \kappa_{\rm E}\nabla^2\phi_{\rm E} - (\mathbb{F}_{\rm ER} - \mathbb{F}_{\rm WR})\Big] \tag{A.1a}$$

$$\mu_{\rm P} - \mu_{\rm S} = \text{const.} + kT\Big[\frac{1}{N_{\rm L}}(\log\phi_{\rm P} - \log(1-\phi_{\rm P})) - \chi_{\rm P}\phi_{\rm P} - \Omega\phi_{\rm E} - \kappa_{\rm P}\nabla^2\phi_{\rm P} - (\mathbb{F}_{\rm PR} - \mathbb{F}_{\rm SR})\Big]. \tag{A.1b}$$

Introducing the chemical potentials (A.1a,b) into equations (27a,b), retaining only linear terms in the fluctuation $\eta_j^{(0)}$ and combining the resulting expressions for $\mathbb{J}_\mathrm{P}$ and $\mathbb{J}_\mathrm{E}$ with equation (9b), one gets a 2×2 system of linear partial differential equations

$$\frac{\partial\eta_\mathrm{E}^{(0)}}{\partial t} = D_\mathrm{E}\bar{\phi}_\mathrm{E}^{(0)}\Big[\Big(\frac{1}{N_\mathrm{E}\bar{\phi}_\mathrm{E}^{(0)}}+\frac{1}{1-\bar{\phi}_\mathrm{E}^{(0)}}-\chi_\mathrm{E}\Big) \times\nabla^2\eta_\mathrm{E}^{(0)}-\kappa_\mathrm{E}\nabla^4\eta_\mathrm{E}^{(0)}-\Omega\nabla^2\eta_\mathrm{P}^{(0)}+\nabla^2(\mathbb{F}_\mathrm{SR}-\mathbb{F}_\mathrm{ER})\Big] \tag{A.2a}$$

$$\frac{\partial\eta_\mathrm{P}^{(0)}}{\partial t} = D_\mathrm{P}\bar{\phi}_\mathrm{P}^{(0)}\Big[\Big(\frac{1}{N_\mathrm{L}\bar{\phi}_\mathrm{P}^{(0)}}+\frac{1}{N_\mathrm{L}(1-\bar{\phi}_\mathrm{P}^{(0)})}-\chi_\mathrm{P}\Big) \times\nabla^2\eta_\mathrm{P}^{(0)}-\kappa_\mathrm{P}\nabla^4\eta_\mathrm{P}^{(0)}-\Omega\nabla^2\eta_\mathrm{E}^{(0)}+\nabla^2(\mathbb{F}_\mathrm{SR}-\mathbb{F}_\mathrm{PR})\Big] \tag{A.2b}$$

where in a two-dimensional system $\nabla^2 = \partial^2/\partial X^2 + \partial^2/\partial Y^2$ and $\nabla^4 = \partial^4/\partial X^4 + 2\partial^4/\partial X^2\partial Y^2 + \partial^4/\partial Y^4$. The mean volume fraction of a generic j-th component, $\bar{\phi}_j^{(0)} \equiv \bar{\phi}_j^{(0)}(t')$, is a slowly varying function of time calculated in the fluctuation-free (zeroth order) approximation by equations (14, 15). By expressing the random forces $(\mathbb{F}_\mathrm{SR}(\mathbf{r}) - \mathbb{F}_\mathrm{ER}(\mathbf{r}))$ and $(\mathbb{F}_\mathrm{SR}(\mathbf{r}) - \mathbb{F}_\mathrm{PR}(\mathbf{r}))$ as a superposition of sinusoidal waves, (see Eq. (20)), one may easily solve the system of differential equations (A.2a,b) obtaining the expressions (28a,b) of the main text.

Appendix B

Let us estimate the following integral in the limit $B(\mathbf{q}) \ll 1$

$$\sum \equiv \sum_q \mathbf{q}^4 \int_{-\infty}^{+\infty} \frac{\omega^2 + C(\mathbf{q})}{\omega^4 + A(\mathbf{q})\omega^2 + B^2(\mathbf{q})}\mathrm{d}\omega \tag{B.1}$$

with $A(\mathbf{q})$, $B(\mathbf{q})$ and $C(\mathbf{q})$ defined by equations (33) of the main text. Making use of integral tables [62], equation (B.1) can be rearranged as

$$\sum = \sum_q \mathbf{q}^4(I_1 + C(\mathbf{q})I_2) \tag{B.2}$$

with:

$$I_1 = 2\int_0^\infty \frac{\omega^2}{\omega^4 + A(\mathbf{q})\omega^2 + B^2(\mathbf{q})}\mathrm{d}\omega = \frac{\pi}{h}(\sqrt{g} - \sqrt{f}) \tag{B.3a}$$

$$I_2 = 2\int_0^\infty \frac{1}{\omega^4 + A(\mathbf{q})\omega^2 + B^2(\mathbf{q})}\mathrm{d}\omega = \frac{\pi}{h}\left(\frac{1}{\sqrt{f}} - \frac{1}{\sqrt{g}}\right) \tag{B.3b}$$

where $h \equiv (A^2(\mathbf{q}) - 4B^2(\mathbf{q}))^{1/2}$; $f \equiv \frac{1}{2}(A-h)$; $g \equiv \frac{1}{2}(A+h)$. The above results are valid provided $h^2 > 0$, a condition always satisfied as checked by direct substitution of the analytical expressions for $A(\mathbf{q})$ and $B(\mathbf{q})$. Expanding equations (B.3) in power series of $(B(\mathbf{q})/A(\mathbf{q}))^2$ one gets

$$I_1 \cong \frac{\pi}{\sqrt{A(\mathbf{q})}} \quad B(\mathbf{q}) \to 0 \tag{B.4a}$$

$$I_2 \cong \frac{\pi}{B(\mathbf{q})} \quad B(\mathbf{q}) \to 0 \tag{B.4b}$$

with $A(\mathbf{q}) > 0$. Therefore, equation (B1) becomes

$$\sum \cong \pi \sum_q \mathbf{q}^4 \frac{C(\mathbf{q})}{B(\mathbf{q})} \quad B(\mathbf{q}) \to 0. \tag{B.5}$$

Appendix C

We look for a perturbation solution of equation (37a) in the more general case where $\xi_\mathrm{PP}(\mathbf{q})$ and $\xi_\mathrm{EE}(\mathbf{q})$ are also functions of $\bar{\phi}_\mathrm{P}(t')$ and $\bar{\phi}_\mathrm{E}(t')$ (calculated at $t' = t'_\mathrm{ind}$). The exact expressions for $\xi_\mathrm{PP}(\mathbf{q}, t')$ and $\xi_\mathrm{EE}(\mathbf{q}, t')$ (Eqs. (36)) are

$$\xi_\mathrm{PP}(\mathbf{q}, t') = -\frac{1}{N_\mathrm{L}(1-\bar{\phi}_\mathrm{P}^{(0)})} + \chi_\mathrm{P} - \mathbf{q}^2\kappa_\mathrm{P} \tag{C.1a}$$

$$\xi_\mathrm{EE}(\mathbf{q}, t') = -\frac{1}{(1-\bar{\phi}_\mathrm{E}^{(0)})} + \chi_\mathrm{E} - \mathbf{q}^2\kappa_\mathrm{P} \tag{C.1b}$$

where both $\bar{\phi}_\mathrm{P}^{(0)}$ and $\bar{\phi}_\mathrm{E}^{(0)}$ depend on t'. Since $\bar{\phi}_\mathrm{P}^{(0)}(t')$ and $\bar{\phi}_\mathrm{E}^{(0)}(t')$ are small, we may expand $\xi_\mathrm{PP}(\mathbf{q}, t') = \xi_\mathrm{PP}^{(0)}(\mathbf{q}) + \xi_\mathrm{PP}^{(1)}(\mathbf{q}, t')$. Hence, combining equations (C.1) with equation (14c) of the main text one gets

$$\xi_\mathrm{PP}^{(0)}(\mathbf{q}) = -\frac{1}{N_\mathrm{L}} + \chi_\mathrm{P} - \mathbf{q}\kappa_\mathrm{P}^2 \tag{C.2a}$$

$$\xi_\mathrm{PP}^{(1)}(\mathbf{q}, t') = -\frac{\bar{\phi}_\mathrm{P}^{(0)}(t')}{N_\mathrm{L}(1-\bar{\phi}_\mathrm{P}^{(0)}(t'))} \cong \bar{\phi}_\mathrm{P}^{(0)}(t')/N_\mathrm{L} \cong -A\,t'^2 \tag{C.2b}$$

where $A \equiv \frac{1}{2}\gamma\bar{\phi}_\mathrm{E}^2/N_\mathrm{E}^2$. Analogously:

$$\xi_\mathrm{EE}^{(0)}(\mathbf{q}) = -1 + \chi_\mathrm{E} - \mathbf{q}^2\kappa_\mathrm{E} \tag{C.2c}$$

$$\xi_\mathrm{EE}^{(1)}(\mathbf{q}, t') \cong -\bar{\phi}_\mathrm{E}^{(0)}(t') = -\bar{\phi}_\mathrm{E}/(1 + k_\mathrm{d}(\bar{\phi}_\mathrm{E}/N_\mathrm{E})t'). \tag{C.2d}$$

Equation (C.2a) is identical to equation (37b) of the main text with $\xi_{jj}^{(0)}(\mathbf{q}) = \xi_{jj}(\mathbf{q})$. Letting $t'_\mathrm{ind} = t'^{(0)}_\mathrm{ind} + t'^{(1)}_\mathrm{ind} + \cdots$, exploiting the above expressions for $\xi_{jj}^{(i)}(\mathbf{q})$ and combining with equation (35), eventually we obtain

$$t'^{(0)}_\mathrm{ind} = N_\mathrm{L}^{-1}(A\xi_\mathrm{PP}^{(0)}(\mathbf{q}))^{-1/2} + \mathrm{const.} \tag{C.3a}$$

$$t'^{(1)}_\mathrm{ind} = \frac{1}{2}N_\mathrm{L}^{-3}(A\xi_\mathrm{PP}^{(0)^5}(\mathbf{q}))^{-1/2} \tag{C.3b}$$

adding together equations (C.3) yields to the leading terms

$$t'_{\rm ind} = (2N_{\rm E}^2/\gamma N_{\rm L}^2 \xi_{\rm PP}^{(0)}(\mathbf{q}))^{1/2}(1+\frac{1}{2N_{\rm L}^2\xi_{\rm PP}^{(0)^2}(\mathbf{q})}) \times \frac{1}{\bar{\phi}_{\rm E}} + \text{const.} + \mathbb{O}(\bar{\phi}_{\rm E}) \quad \text{(C.4)}$$

which, apart from the multiplicative factor $(1+(2N_{\rm L}^2\times \xi_{\rm PP}^{(0)^2}(\mathbf{q}))^{-1})$, equation (C.4) is all but nothing more than equation (37b), which preserves the $\bar{\phi}_{\rm E}^{-1}$ dependence of $t'_{\rm ind}$ found in equation (37b).

Appendix D

So far we have neglected any variation of the *mean* surface density of bound enzymes with bilayer composition. According to the literature, the enzyme binding increases with the fraction of negatively charged species (*e.g.*, products) within the lipid bilayer [14,15,39]. By retaining the catalytic scheme set forth in the main text, the products-dependent binding can be considered by allowing for a rapid equilibrium between the enzymes in the bulk and those adsorbed at the interfacial region. Hence the zeroth order constitutive equations (12) become

$$\frac{{\rm d}\,\mathbb{C}_{\rm Ef}^{(0)}}{{\rm d}t'} = -k^+\,\mathbb{C}_{\rm Ef}^{(0)} + k^-(\,\mathbb{C}_{\rm Eb}^{(0)} + 2\,\mathbb{C}_{\rm E_2}^{(0)} + 2\,\mathbb{C}_{\rm Y}^{(0)}) \quad \text{(D.1a)}$$

$$-\frac{{\rm d}\,\mathbb{C}_{\rm Eb}^{(0)}}{{\rm d}t'} = k_{\rm d}\,\mathbb{C}_{\rm Ed}^{(0)^2} - k^+\,\mathbb{C}_{\rm Ef}^{(0)} + k^-(\,\mathbb{C}_{\rm Eb}^{(0)} + 2\,\mathbb{C}_{\rm E_2}^{(0)} + 2\,\mathbb{C}_{\rm Y}^{(0)}) \quad \text{(D.1b)}$$

$$\frac{{\rm d}\,\mathbb{C}_{\rm Y}^{(0)}}{{\rm d}t'} = k_1\,\mathbb{C}_{\rm S}^{(0)}\,\mathbb{C}_{\rm E_2}^{(0)} - (k_{-1}+k_{\rm f})\,\mathbb{C}_{\rm Y}^{(0)} \quad \text{(D.1c)}$$

$$\frac{{\rm d}\,\mathbb{C}_{\rm P}^{(0)}}{{\rm d}t'} = k_{\rm f}\,\mathbb{C}_{\rm Y}^{(0)} \quad \text{(D.1d)}$$

where $\mathbb{C}_{\rm Eb}^{(0)}$ and $\mathbb{C}_{\rm Ef}^{(0)}$ are the zeroth order concentrations of bound and free enzyme, respectively, while the other symbols have been defined in equations (12) of the main text. The new parameters k^+ and k^- describe the enzyme association and dissociation rate constants at the membrane-water interface. For the sake of simplicity, in writing equations (D.1a,b) we assumed that the dissociation rates of bound enzyme monomer ($\rm E_b$), dimer ($\rm E_2$) and activated dimer (Y) are identical, a good approximation in the early stages of the enzyme kinetics ($\mathbb{C}_{\rm Eb}^{(0)} \gg \mathbb{C}_{\rm E_2}^{(0)}$ and $\mathbb{C}_{\rm Eb}^{(0)} \gg \mathbb{C}_{\rm Y}^{(0)}$). The constants k^+ and k^- determine the equilibrium enzyme binding constant and both of them may depend upon membrane composition, as for instance, on the accumulation of products $\mathbb{C}_{\rm P}^{(0)}$ in the lipid bilayer. It is likely that the dissociation rate strongly depends on the structure of the membrane, while the association rate, which is mainly diffusion-controlled, does not significantly change with the membrane composition (at least when the ion-screened surface potentials are short-range). Clearly, the products-dependent dissociation rate and the preferential binding parameter Ω introduced in Section 3.4 are closely related. Here we escape from a detailed analysis of the exact relationship between the k^- and Ω parameters letting $k^- \equiv k^-(\mathbb{C}_{\rm P}^{(0)}) \cong k^{-(0)} + (\partial k^-/\partial\,\mathbb{C}_{\rm P})\,\mathbb{C}_{\rm P}^{(0)} \equiv k^{-(0)}(1-\lambda\Omega\,\mathbb{C}_{\rm P}^{(0)})$, with λ a proportionality constant [63]. By exploiting the mass conservation constraint

$$\mathbb{C}_{\rm Eb}^{(0)} + \mathbb{C}_{\rm Ef}^{(0)} + 2\,\mathbb{C}_{\rm E_2}^{(0)} + 2\,\mathbb{C}_{\rm Y}^{(0)} = \bar{\mathbb{C}}_{\rm E}^{\rm tot} \quad \text{(D.2)}$$

and following a standard perturbation procedure we get from equations (D.1a,b) the first order result:

$$\mathbb{C}_{\rm Ef}^{(0)} = \frac{k^{-(0)}}{k^{-(0)}+k^+} - \bar{\mathbb{C}}_{\rm E}^{\rm tot} + x(t') \quad \text{(D.3)}$$

$x(t') \ll 1$ being the solution of the differential equation

$$\frac{{\rm d}x}{{\rm d}t'} + \zeta x = \Gamma\,\mathbb{C}_{\rm P}^{(0)}(t') \quad \text{(D.4)}$$

where $\zeta \equiv (k^{-(0)}+k^+)$; $\Gamma \equiv -\lambda\Omega k^{-(0)}k^+\bar{\mathbb{C}}_{\rm E}^{\rm tot}/\zeta$ and, to a first approximation, the concentration of products $\mathbb{C}_{\rm P}^{(0)}(t')$ is given by equation (14b). Solution of equation (D.4) together with the boundary condition $x|_{t'=0}$ yields

$$\mathbb{C}_{\rm Ef}^{(0)} \cong \frac{k^{-(0)}}{k^{-(0)}+k^+}\,\bar{\mathbb{C}}_{\rm E}^{\rm tot} - \frac{1}{2}\xi t'^2 \quad \text{(D.5)}$$

where $\xi \equiv \lambda\Omega\gamma\; k^{-(0)}k^{+^3}(\mathbb{C}_{\rm E}^{\rm tot})^3/(k^{-(0)}+k^+)^4$ (γ defined by Eq. (15)), a result valid when $(k^{-(0)}+k^+)t' \gg 0$, namely for a rapid equilibrium between adsorbed and free enzymes. By inserting the above result into (D.1) and (D.2), one may calculate $\mathbb{C}_{\rm Eb}^{(0)}$, $\mathbb{C}_{\rm E_2}^{(0)}$, $\mathbb{C}_{\rm Y}^{(0)}$ and $\mathbb{C}_{\rm P}^{(0)}$. Here we report the final equation for the products:

$$\mathbb{C}_{\rm P}^{(0)} \cong \frac{1}{2}\frac{\bar{\phi}_{\rm E}^2}{N_{\rm E}^2}\gamma t'^2\left(1+\frac{1}{6}\lambda\Omega\gamma\frac{k^{-(0)}}{k^{-(0)}+k^+}\frac{\bar{\phi}_{\rm E}^2}{N_{\rm E}^2}t'^2+\cdots\right) \quad \text{(D.6)}$$

where $\bar{\phi}_{\rm E}/N_{\rm E} \equiv \mathbb{C}_{\rm Eb}^{(0)} \cong k^+\mathbb{C}_{\rm E}^{\rm tot}/(k^{-(0)}+k^+)$ is the concentration of bound enzymes at equilibrium. By comparing equation (D.6) with equation (14b) of the main text, one may conclude that the effect of the preferential binding of the enzyme onto products-rich regions is not very important in the early stages enzyme kinetics unless there are very high enzyme concentrations or slow equilibria among free and bound enzymes. Hence, the approximate expression (14b) is a good approximation in the range of validity of the theory.

Let us apply the results obtained so far to calculate the fluctuation enhanced enzyme kinetics (*i.e.*, the terms $\mathbb{C}_j^{(1)}$) by following the procedure outlined in Section 3.5. The final equation for the induction time $t'_{\rm ind}$ is identical to equation (37b) of the main text, apart from a multiplicative constant W, namely

$$t'_{\rm ind} \cong W(t'_{\rm ind})_0 \quad \text{(D.7)}$$

where $(t'_{\rm ind})_0$ is the induction time calculated for a constant surface concentration of bound enzymes while W accounts for the enhanced enzyme binding due to products formation. An approximate analytical expression for W turns out to be

$$W \cong 1 - \frac{\lambda\Omega}{24 N_{\rm L}^2 \xi_{\rm PP}(\mathbf{q})} \frac{k^{-(0)}}{k^{-(0)} + k^{+}} \quad \cong 1 \qquad \Omega \longrightarrow 0 \tag{D.8}$$

where W linearly depends on the preferential solvation parameter Ω [64].

Appendix E

The aim of this appendix is to get an estimate of the enzyme activation prior to the burst of activity. This goal can be reached by expanding in equation (34) the fluctuation term about $t'_{\rm ind}$. In this limit we get: $\langle\langle {\eta_{\rm E}^{(0)}}^2(t'')\rangle_V\rangle_\tau \cong 4\pi kT D_{\rm E}^2 \bar\phi_{\rm E}^2 \sum \mathbf{q}^4 C(\mathbf{q},t'')/B(\mathbf{q},t'')$ and the coefficients $C(\mathbf{q},t'')$ and $B(\mathbf{q},t'')$ (defined by Eq. (33c)) become

$$\begin{aligned} C(\mathbf{q},t'') &\equiv (Q_{\rm PP} - \frac{F_{\rm P}}{F_{\rm E}} Q_{\rm EP})^2 \\ &= D_{\rm P}^2 {\bar\phi_{\rm P}^{(0)}}^2(t'') \mathbf{q}^4 (\frac{1}{N_{\rm L}\bar\phi_{\rm P}^{(0)}(t'')} - \xi_{\rm PP}(\mathbf{q}) + \Omega)^2 \\ &\underset{t''\to t'_{\rm ind}}{\cong} \mathbf{q}^4 \Omega^2 D_{\rm P}^2 / N_{\rm L}^2 \xi_{\rm PP}^2(\mathbf{q}) \end{aligned} \tag{E.1}$$

where the last identity follows from equation (37b). Analogously, the coefficient $B(\mathbf{q},t'')$ (Eq. (33b)) can be expanded as

$$\begin{aligned} B(\mathbf{q},t'') &\equiv Q_{\rm PP}Q_{\rm EE} - Q_{\rm PE}Q_{\rm EP} = \mathbf{q}^4 D_{\rm P} D_{\rm E} \bar\phi_{\rm P}^{(0)}(t'') \bar\phi_{\rm E}^{(0)}(t'') \\ &\times \Big[\Big(\frac{1}{N_{\rm E}\bar\phi_{\rm E}^{(0)}(t'')} - \xi_{\rm EE}(\mathbf{q})\Big)\Big(\frac{1}{N_{\rm L}\bar\phi_{\rm E}^{(0)}(t'')} \\ &- \xi_{\rm PP}(\mathbf{q})\Big) - \Omega^2\Big]_{t''\to t'_{\rm ind}} \underset{}{\cong} \mathbf{q}^4 D_{\rm E} D_{\rm P} \\ &\times (2\gamma\xi_{\rm PP}(\mathbf{q}))^{1/2} \frac{\bar\phi_{\rm E}}{N_{\rm E}^2}(t'_{\rm ind} - t'') + \mathbb{O}((t'_{\rm ind} - t'')^2). \end{aligned} \tag{E.2}$$

Where, once again, extensive use of equation (37b) has been made. Inserting (E.1) and (E.2) into (34) and rearranging, we get

$$\begin{aligned} \langle\langle {\eta_{\rm E}^{(0)}}^2\rangle_V\rangle_\tau &\cong 2\pi D_{\rm E} D_{\rm P} kT (N_{\rm E}/N_{\rm L})^2 \bar\phi_{\rm E} (2/\gamma)^{1/2} \Omega^2 \\ &\times \sum_q \frac{\mathbf{q}^4}{\xi_{\rm PP}^{5/2}(\mathbf{q})} \frac{1}{t'_{\rm ind} - t''} + \mathbb{O}(\bar\phi_{\rm E}^2). \end{aligned} \tag{E.3}$$

With the aid of the above formula, equation (34) can be easily integrated twice (over t'' and t') giving to the leading terms the result reported in equation (38) of the main text.

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63. On the basis of litterature data $(\partial k^-/\partial \mathbb{C}_{\rm P}) \cong \lambda\Omega < 0$, namely the dissociation of bound enzymes is disfavoured in charged lipids bilayers (see, *e.g.*, Ref. [39]).
64. There is another subtle effect related to the exchange between free and bound enzymes. Indeed, by accounting for the third dimension (perpendicular to the membrane plane) the diffusion motion of the enzymes becomes more complex than in two dimensions: the augmented dimensionality for the Brownian motion reduces the mean displacement in unit time (*i.e.*, the apparent diffusion coefficient decreases), whereas the lower viscosity of the aqueous medium, in comparison with the viscosity of the lipid matrix, enhances the enzyme mobility (*i.e.*, the apparent diffusion coefficient increases). However, according to our model, the diffusion coefficients of enzyme and products does not enter in the lag time $t_{\rm ind}$ equation (see Eq. (37)) but it influences only the enzyme activity on approaching $t_{\rm ind}$ (Eq. (38b)).