

## Response to a temperature modulation as a signature of chemical mechanisms

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We consider  $n$  reactive species involved in unimolecular reactions and submitted to a temperature modulation of small amplitude. We determine the conditions on the rate constants for which the deviations from the equilibrium concentrations of each species can be optimized and find the analytical expression of the frequency associated with an extremum of concentration shift in the case  $n=3$ . We prove that the frequency dependence of the displacement of equilibrium gives access to the number  $n$  of species involved in the mechanism. We apply the results to the case of the transformation of a reactant into a product through a possible reactive intermediate and find the order relation obeyed by the activation energies of the different barriers. The results typically apply to enzymatic catalysis with kinetics of Michaelis-Menten type.

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### I. INTRODUCTION

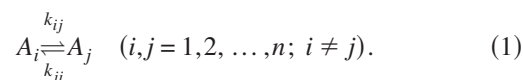
An out-of-equilibrium reaction, such as the exchange between adenosine triphosphate (ATP) and adenosine diphosphate (ADP) in living cells, may participate into the emergence of oriented motion: molecular motors [1–6] are an application of energy transduction, where the motion can be optimized using stochastic resonance [7]. Reciprocally, the possibility to drive a chemical reaction away from equilibrium by applying external oscillations has been proved 30 years ago as another example of energy transduction [8]. In particular, oscillations of an electric field [9–13] or the concentration of some species [14,15] have been shown to maintain a reaction out of equilibrium. As an alternative to the so-called relaxation methods [16–21] that analyze the response to pulse perturbations, oscillations of concentrations or fluxes [22,23] have been applied to determine the properties of the dynamics. However, these kinds of periodic excitations are invasive and prevent *in vivo* investigations. The development of microsystems with a control of the temperature [24,25] incited us to choose a temperature modulation as a noninvasive periodic excitation. We consider unimolecular reactions with  $n$  species, where the number  $n$  can be unknown. We wish to determine how the displacement of chemical equilibrium varies with the frequency of the temperature modulation. In particular we are interested in the optimization of the deviation of each concentration from the unperturbed value. The response to the temperature modulation allows us to get an insight into the chemical mechanism. The frequency dependence of the concentration shift turns out to give the values of the parameters governing the dynamics. Numerical methods based on density-functional theory (DFT) [26,27] can also be used to elucidate chemical mechanisms. Nevertheless, the description of the biological macromolecules and the influence of the solvent are not easily captured by DFT. We propose an alternative technique

which is independent of the structure of the molecules considered.

The paper is organized as follows. In Sec. II, we introduce a general unimolecular reaction scheme and present the effect of a temperature modulation of small amplitude on the rate constants. In Secs. III and IV, we study a unimolecular mechanism with two and three species, respectively. We determine the analytical expression of the deviation of the concentration of each species from the unperturbed value and find, in well-defined conditions, a resonant frequency associated with a maximum of the deviation considered as a function of the modulation frequency. We apply these results in Sec. V to the determination of mechanisms and propose an experimental protocol to discriminate between the direct conversion of a reactant into a product and the passage through a reactive intermediate. Section VI is devoted to conclusion.

### II. UNIMOLECULAR REACTIONS AND TEMPERATURE MODULATION

We consider a mixture of  $n$  reactive species involved in unimolecular reactions,



The mixture is submitted to a sinusoidal modulation of temperature  $T$  with a small amplitude  $\beta T_0$  and angular frequency  $\omega$ ,

$$T = T_0[1 + \beta \sin(\omega t)], \quad \beta \ll 1. \quad (2)$$

According to the Eyring model, the rate constants depend on the temperature as follows:

$$k_{ij} = \frac{RT}{h} \exp\left(\frac{\Delta_{ij}S^{\neq 0}}{R}\right) \exp\left(-\frac{\Delta_{ij}H^{\neq 0}}{RT}\right), \quad (3)$$

where  $R$  is the individual gas constant,  $h$  is the Planck constant,  $\Delta_{ij}S^{\neq 0}$  and  $\Delta_{ij}H^{\neq 0}$  are, respectively, the standard

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entropy and enthalpy of activation of reaction (1) at temperature  $T$ . Taking  $\Delta_{ij}S^{\neq 0}$  and  $\Delta_{ij}H^{\neq 0}$  constant in the relevant temperature range, we expand the rate constant at the second order in the perturbation and obtain  $k_{ij}(t) = k_{ij}^0 + \beta k_{ij}^0 (\epsilon_{ij} + 1) \sin(\omega t) + k_{ij}^0 \frac{\epsilon_{ij}^2 \beta^2}{2} [\sin^2(\omega t)]$ , where  $k_{ij}^0 = \frac{RT_0}{h} \exp(\frac{\Delta_{ij}S^{\neq 0}}{R}) \exp(-\epsilon_{ij})$  is the rate constant at temperature  $T_0$  and where  $\epsilon_{ij} = \frac{\Delta_{ij}H^{\neq 0}}{RT_0}$  is the reduced activation energy. According to the classical transition state theory [28], we consider temperature oscillations of sufficiently small frequencies ( $\omega < 10^6 \text{ s}^{-1}$ ) and sufficiently slow chemical reactions, such that thermal equilibrium is always reached. All effects that could induce a deviation from the equilibrium Boltzmann distribution are neglected. In particular, we consider standard chemical reactions with activation energies larger than  $RT_0$ , so that we can neglect unity in front of  $\epsilon_{ij}$  in the expression of  $k_{ij}$ . The expression of the rate constant reads

$$k_{ij}(t) = k_{ij}^0 + \beta k_{ij}^0 \epsilon_{ij} \sin(\omega t) + \beta^2 k_{ij}^0 \frac{\epsilon_{ij}^2}{4} [1 - \cos(2\omega t)]. \quad (4)$$

According to Eq. (4), the rate constant appears as the sum of the unperturbed value  $k_i^0$  at temperature  $T_0$ , an oscillating term at angular frequency  $\omega$  in phase with the temperature at the first order in the perturbation, a constant second-order term, and an oscillating term at angular frequency  $2\omega$  out of phase with the temperature. Neglecting the transient regime and considering the permanent solutions, we admit that the concentration  $A_i(t)$  of a reactive species  $A_i$  submitted to small oscillations of the temperature has the following expression at the second order in the perturbation:

$$A_i(t) = A_i^0 + \beta [A_{i,\sin}^1 \sin(\omega t) + A_{i,\cos}^1 \cos(\omega t)] + \beta^2 [A_{i,\text{cst}}^2 + A_{i,\sin}^2 \sin(2\omega t) + A_{i,\cos}^2 \cos(2\omega t)], \quad (5)$$

where  $A_i^0$  is the equilibrium concentration at temperature  $T_0$ ;  $A_{i,\sin}^1$ ,  $A_{i,\cos}^1$  are the amplitudes of the oscillating terms at the first order in the perturbation;  $A_{i,\sin}^2$ ,  $A_{i,\cos}^2$  are the amplitudes of the oscillating terms at the second order in the perturbation and  $A_{i,\text{cst}}^2$  is a constant second-order correction. In the presence of a temperature modulation and at the second order in the perturbation, the mean value of the concentration differs from  $A_i^0$  by  $A_{i,\text{cst}}^2$  that we intend to determine analytically.

### III. UNIMOLECULAR MECHANISM WITH TWO SPECIES

In this section we consider a unimolecular reaction with two species,



The dynamics is governed by the following macroscopic, deterministic equation for the concentrations:

$$\frac{dA_1(t)}{dt} = -k_{12}(t)A_1(t) + k_{21}(t)A_2(t), \quad (7)$$

with the conservation relation  $A_1(t) + A_2(t) = 1$ . Initially, the species are at chemical equilibrium at temperature  $T_0$ ,

$$A_1^0 = \frac{k_{21}^0}{k_{21}^0 + k_{12}^0}. \quad (8)$$

The first-order correction to the concentration obeys

$$\frac{dA_1^1(t)}{dt} + (k_{12}^0 + k_{21}^0)A_1^1(t) = \frac{k_{12}^0 k_{21}^0 (\epsilon_{21} - \epsilon_{12})}{k_{12}^0 + k_{21}^0} \sin(\omega t). \quad (9)$$

After the relaxation time  $\tau = \frac{1}{k_{12}^0 + k_{21}^0}$  of reaction (6), the system enters into the forced sinusoidal regime, and the first-order correction to the concentration of species  $A_1$  is given by

$$A_1^1(t) = A_{1,\sin}^1 \sin(\omega t) + A_{1,\cos}^1 \cos(\omega t) \quad (10)$$

with

$$A_{1,\sin}^1 = \frac{k_{12}^0 k_{21}^0 (\epsilon_{21} - \epsilon_{12})}{(k_{12}^0 + k_{21}^0)^2 + \omega^2}, \quad (11)$$

$$A_{1,\cos}^1 = \frac{k_{12}^0 k_{21}^0 \omega (\epsilon_{21} - \epsilon_{12})}{(k_{12}^0 + k_{21}^0)[(k_{12}^0 + k_{21}^0)^2 + \omega^2]}. \quad (12)$$

The expansion of Eq. (7) at the second order leads to the following differential equation for  $A_1^2(t)$ ,

$$\begin{aligned} \frac{dA_1^2(t)}{dt} + (k_{12}^0 + k_{21}^0)A_1^2(t) &= (k_{12}^0 \epsilon_{12} + k_{21}^0 \epsilon_{21})A_1^1(t) \sin(\omega t) \\ &+ \frac{k_{12}^0 k_{21}^0 (\epsilon_{21}^2 - \epsilon_{12}^2)}{4(k_{12}^0 + k_{21}^0)} [1 - \cos(2\omega t)], \end{aligned} \quad (13)$$

where  $A_1^1(t)$  is given in Eq. (10). The expression of the constant second-order correction to the concentration of species  $A_1$  is

$$A_{1,\text{cst}}^2 = \frac{k_{12}^0 k_{21}^0 (\epsilon_{21} - \epsilon_{12})(k_{12}^0 \epsilon_{12} + k_{21}^0 \epsilon_{21})}{2[(k_{12}^0 + k_{21}^0)^2 + \omega^2](k_{12}^0 + k_{21}^0)} + \frac{k_{12}^0 k_{21}^0 (\epsilon_{21}^2 - \epsilon_{12}^2)}{4(k_{12}^0 + k_{21}^0)^2}. \quad (14)$$

The existence of a constant term  $A_{1,\text{cst}}^2$  reveals that the temperature oscillations induce a displacement of equilibrium when the system is observed during a time larger than the period  $2\pi/\omega$ . Note however that the external forcing does not induce a violation of detailed balance. An analogous phenomenon has been predicted in the case of the oscillations of a field [9–11, 13, 29–31] or a concentration [14, 15]. Unidirectional shift of the equilibrium between two states [32] can be also obtained by means of random fluctuations. As shown in Eq. (14), the constant term  $A_{1,\text{cst}}^2$  vanishes for  $\epsilon_{12} = \epsilon_{21}$ . In the presence of temperature oscillations, the reaction is not perturbed if it is athermic. The constant second-order correction  $A_{1,\text{cst}}^2$  to the concentration of species  $A_1$  is the sum of two contributions: the first term of the right-hand side of Eq. (14) comes from the product of the first-order corrections to the rate constants and to the concentration. It depends on the kinetic properties ( $k_{12}^0, k_{21}^0, \epsilon_{12}, \epsilon_{21}$ ) of the studied couple of reactive species ( $A_1, A_2$ ) and on the angular frequency  $\omega$ . Note that the term  $A_{1,\cos}^1$  given in Eq. (12) does not contribute to the constant second-order term  $A_{1,\text{cst}}^2$ . The second term of the right-hand side of Eq. (14) comes from the second-order

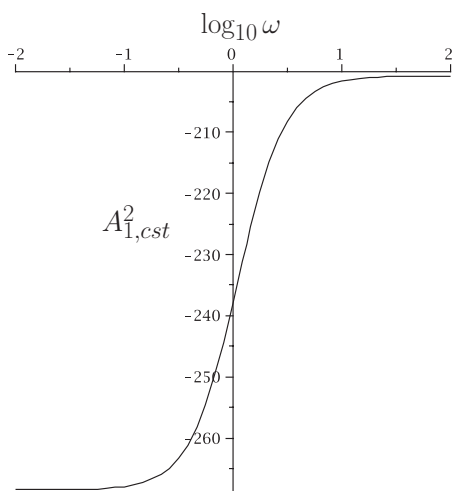


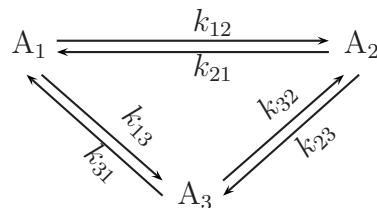
FIG. 1. Constant second-order correction  $A_{1,cst}^2$  to the concentration of species  $A_1$  involved in a unimolecular reaction with two species versus  $\log_{10} \omega$  for  $k_{12}^0=0.1$ ,  $k_{21}^0=1$ ,  $\epsilon_{12}=100$ ,  $\epsilon_{21}=10$ .

correction to the rate constants. If one excepts  $(\epsilon_{12}, \epsilon_{21})$ , it only depends on the equilibrium constant  $K^0 = \frac{k_{12}^0}{k_{21}^0}$  of the reaction and is independent of  $\omega$ .

We are here interested in the dependence on  $\omega$  of the displacement of equilibrium  $A_{1,cst}^2$ . Figure 1 represents  $A_{1,cst}^2$  as a function of  $\omega$  for fixed values of  $(k_{12}^0, k_{21}^0, \epsilon_{12}, \epsilon_{21})$ . The sign and the amplitude of the displacement of equilibrium depends on the frequency of the temperature oscillations. The sign of the first derivative of  $A_{1,cst}^2(\omega)$  with respect to  $\omega$  is constant and given by the sign of  $(\epsilon_{21} - \epsilon_{12})$ . Consequently  $A_{1,cst}^2(\omega)$  is a decreasing function of  $\omega$  for exothermic reactions and an increasing function of  $\omega$  for endothermic reactions. The temperature oscillations lead to a smaller amount of the reactant  $A_1$  (and therefore, a larger amount of the product  $A_2$ ) for an exothermic reaction. Figure 1 reveals the existence of a frequency threshold,  $\omega_s = (k_{12}^0 + k_{21}^0) / \sqrt{3}$ . The relative height of the step,  $h = \frac{A_{1,cst}^2(\omega \rightarrow \infty) - A_{1,cst}^2(\omega \rightarrow 0)}{A_{1,cst}^2(\omega \rightarrow \infty)}$ , is larger for rate constants of the same order of magnitude. In the less favorable case of very different rate constant values, the height  $h$  is of the order of the ratio of the activation energies. Consequently, the height remains observable. For example, the reaction of hybridization of short single stranded DNA is associated to the following parameter values:  $k_{12}^0 = 10^5 \text{ m s}^{-1}$ ,  $k_{12}^0 = 0.1 \text{ s}^{-1}$ ,  $\epsilon_{12} = 8$ , and  $\epsilon_{21} = 60$ , which lead to  $h = 0.24$ . The development of thermomicrofluidics [24,25] makes us confident in the experimental validation of our theoretical predictions. Small reactive cells with typical lengths of the order of  $10 \mu\text{m}$  make it possible to reduce thermal inertia and to reach high frequencies for the temperature modulation. A cutoff frequency of the order of  $10^5 \text{ Hz}$  looks realistic [24].

#### IV. UNIMOLECULAR MECHANISM WITH THREE SPECIES

In this section, we consider the following mechanism involving three chemical species:



All the unimolecular reactions involving three species are described by this scheme. The system is *a priori* characterized by six rate constants  $k_{12}^0, k_{21}^0, k_{23}^0, k_{32}^0, k_{13}^0$ , and  $k_{31}^0$ , and six reduced activation energies  $\epsilon_{12}, \epsilon_{21}, \epsilon_{23}, \epsilon_{32}, \epsilon_{13}$ , and  $\epsilon_{31}$ . When use is made of the conservation of matter,  $A_1(t) + A_2(t) + A_3(t) = 1$ , the evolution of the system is governed by

$$\frac{d}{dt} \begin{pmatrix} A_1(t) \\ A_2(t) \end{pmatrix} = \mathbf{M} \begin{pmatrix} A_1(t) \\ A_2(t) \end{pmatrix} + \begin{pmatrix} k_{31}(t) \\ k_{32}(t) \end{pmatrix}, \quad (15)$$

where  $\mathbf{M} = \mathbf{M}^0 + \beta \mathbf{M}^1 \sin(\omega t) + \frac{\beta^2}{2} \mathbf{M}^2 [1 - \cos(2\omega t)]$  with

$$\mathbf{M}^i = \begin{pmatrix} \kappa_{1,1}^i & \kappa_{1,2}^i \\ \kappa_{2,1}^i & \kappa_{2,2}^i \end{pmatrix} \quad \text{for } i = 0, 1, 2. \quad (16)$$

The expressions of the matrices  $\mathbf{M}^i$  as functions of the rate constants and the activation energies are given in the Appendix. If detailed balance is obeyed, the eigenvalues of  $\mathbf{M}^0$  are real and negative [16,33] and are given by

$$\lambda_{\pm} = \frac{\kappa_{1,1}^0 + \kappa_{2,2}^0}{2} \pm \frac{1}{2} \sqrt{(\kappa_{1,1}^0 + \kappa_{2,2}^0)^2 - 4(\kappa_{1,1}^0 \kappa_{2,2}^0 - \kappa_{2,1}^0 \kappa_{1,2}^0)}. \quad (17)$$

Note that when the eigenvalues are identical, the system can be reduced to the case of a unimolecular reaction with two species treated in the preceding section. We consider the nondegenerate case where the two eigenvalues are different and obey  $\lambda_- < \lambda_+ < 0$ . We introduce the parameter

$$r = \frac{\lambda_-}{\lambda_+} > 1. \quad (18)$$

The equilibrium concentrations obey

$$\begin{aligned}
 A_1^0 &= \frac{1}{1 + \frac{k_{12}^0}{k_{21}^0} + \frac{k_{13}^0}{k_{31}^0}}, & A_2^0 &= \frac{1}{1 + \frac{k_{21}^0}{k_{12}^0} + \frac{k_{32}^0}{k_{23}^0}}, \\
 A_3^0 &= 1 - (A_1^0 + A_2^0).
 \end{aligned} \quad (19)$$

At the first order in the perturbation, Eq. (15) leads to the following differential equation:

$$\begin{aligned}
 \frac{d}{dt} \begin{pmatrix} A_1^1(t) \\ A_2^1(t) \end{pmatrix} &= \mathbf{M}^0 \begin{pmatrix} A_1^1(t) \\ A_2^1(t) \end{pmatrix} + \mathbf{M}^1 \begin{pmatrix} A_1^0 \\ A_2^0 \end{pmatrix} \sin(\omega t) \\
 &+ \begin{pmatrix} k_{31}^0 \epsilon_{31} \\ k_{32}^0 \epsilon_{32} \end{pmatrix} \sin(\omega t).
 \end{aligned} \quad (20)$$

As mentioned in Sec. III, only the terms in phase with the temperature oscillation contribute to the displacement of equilibrium. The amplitudes of these terms are given by

$$A_{i,\sin}^1 = \frac{a_{i,\sin}^-}{\omega^2 + \lambda_-^2} + \frac{a_{i,\sin}^+}{\omega^2 + \lambda_+^2}, \quad i = 1, 2, \quad (21)$$

where the explicit expressions of  $a_{i,\sin}^-$  and  $a_{i,\sin}^+$  in terms of the rate constants and the activation energies are given in the Appendix.

At the second order in the perturbation, Eq. (15) gives

$$\begin{aligned} \frac{d}{dt} \begin{pmatrix} A_1^2(t) \\ A_2^2(t) \end{pmatrix} &= \mathbf{M}^0 \begin{pmatrix} A_1^2(t) \\ A_2^2(t) \end{pmatrix} + \mathbf{M}^1 \begin{pmatrix} A_1^1(t) \\ A_2^1(t) \end{pmatrix} \sin(\omega t) \\ &+ \mathbf{M}^2 \begin{pmatrix} A_1^0 \\ A_2^0 \end{pmatrix} \frac{1 - \cos(2\omega t)}{2} \\ &+ \begin{pmatrix} \frac{k_{31}^0 \epsilon_{31}^2}{4} \\ \frac{k_{32}^0 \epsilon_{32}^2}{4} \end{pmatrix} \frac{1 - \cos(2\omega t)}{2}. \end{aligned} \quad (22)$$

Solving Eq. (22), we find that the displacement of equilibrium is given by

$$\begin{aligned} \begin{pmatrix} A_{1,\text{cst}}^2 \\ A_{2,\text{cst}}^2 \end{pmatrix} &= -\frac{1}{2} (\mathbf{M}^0)^{-1} \mathbf{M}^1 \begin{pmatrix} A_{1,\sin}^1 \\ A_{2,\sin}^1 \end{pmatrix} - \frac{1}{2} \left[ (\mathbf{M}^0)^{-1} \mathbf{M}^2 \begin{pmatrix} A_1^0 \\ A_2^0 \end{pmatrix} \right. \\ &\left. + (\mathbf{M}^0)^{-1} \begin{pmatrix} \frac{k_{31}^0 \epsilon_{31}^2}{4} \\ \frac{k_{32}^0 \epsilon_{32}^2}{4} \end{pmatrix} \right]. \end{aligned} \quad (23)$$

As already observed for a mechanism with two species, the constant second-order correction to the concentration is the sum of two contributions: the first term on the right-hand side depends on the dynamical properties of the system and on the angular frequency  $\omega$  of the perturbation. The other terms are independent of the angular frequency. For each species  $A_i$ , the explicit expression of the deviation of the concentration from the unperturbed value appears as the sum of an  $\omega$ -dependent term and a constant term that corresponds to the asymptotic value for  $\omega \rightarrow \infty$ ,

$$A_{i,\text{cst}}^2 = \frac{1}{2\lambda_- \lambda_+} \left( \frac{a_{i,\text{cst}}^-}{\omega^2 + \lambda_-^2} + \frac{a_{i,\text{cst}}^+}{\omega^2 + \lambda_+^2} \right) + A_{i,\text{cst}}^2(\omega \rightarrow \infty), \quad (24)$$

where the expression of  $A_{i,\text{cst}}^2(\omega \rightarrow \infty)$  is given in Table I and where

$$a_{i,\text{cst}}^- = -\alpha_i \frac{r(r+x_i)}{r^2-1}, \quad (25)$$

$$a_{i,\text{cst}}^+ = \alpha_i \frac{1+x_i r}{r^2-1}. \quad (26)$$

The expression of the parameters  $\alpha_i$  and  $x_i$  in terms of the dynamical parameters are given in the Appendix.

The  $\omega$  dependence of the displacement of equilibrium given in Eq. (24) reveals in a simple way the properties of the chemical mechanism. The dynamics *a priori* depends on six rate constants and six activation energies. The number of independent parameters possibly reduces to 10 when taking into account detailed balance. The important point is that the  $\omega$ -dependent term of  $A_{i,\text{cst}}^2$  only depends on the three parameters  $\alpha_i$ ,  $r$ , and  $x_i$ . Using the  $\omega$  dependence of the displacement of equilibrium, we are thus able to classify the different types of unimolecular reactions with three species according to the values of these three parameters. The  $\omega$ -dependent term of  $A_{i,\text{cst}}^2$  is the sum of two Lorentzian functions. Consequently,  $A_{i,\text{cst}}^2$  considered as a function of  $\omega$ , possesses two thresholds for  $\omega \approx -\lambda_+/\sqrt{3}$  and  $\omega \approx -\lambda_-/\sqrt{3}$ . There are two possibilities: either the function is monotonous with two successive thresholds and three inflection points or possesses an extremum and two inflection points. The results deduced from the study of the first and second derivatives of  $A_{i,\text{cst}}^2$  with respect to  $\omega$  are given in Fig. 2. If the parameters  $x_i$  and  $r$  obey  $x_i < \frac{-1}{r+1/r}$ , the displacement of equilibrium is monotonous and presents two successive thresholds. The function is decreasing for  $\alpha_i < 0$  and increasing for  $\alpha_i > 0$ . For  $x_i > \frac{-1}{r+1/r}$  the displacement of equilibrium presents an extremum for the resonant frequency

$$\omega_i = \sqrt{\lambda_+ \lambda_- [x_i + \sqrt{1 + x_i(r+1/r) + x_i^2}]}. \quad (27)$$

This extremum is a maximum for  $\alpha_i < 0$  and a minimum for  $\alpha_i > 0$ . The phenomena do not depend on the explicit expression of the parameter  $\alpha_i$ , only on its sign. Note that this optimization of the response for a given frequency is obtained in the frame of a purely deterministic description and is not associated to a stochastic resonance in the common acceptance of the expression [34].

These results allow us to propose an experimental protocol to control the yield in a given species  $A_i$  with known rate constants. We suggest to apply a temperature modulation and wait for the stationary regime. Our analytical calculations free the experimentalist from a frequency sweep: the computation of the values of  $x_i$ ,  $r$ , and  $\alpha_i$ , according to the expressions given in the Appendix, is sufficient to predict the shape of the  $\omega$  dependence. If the values of the rate constants are compatible with the existence of an extremum, Eq. (27) gives the frequency value of the temperature modulation that optimizes the yield in species  $A_i$ . For eigenvalues of different orders of magnitude, the extremum is smooth and one can choose the frequency in a wide range between the two thresholds.

## V. APPLICATION TO THE DETERMINATION OF MECHANISMS

The preceding results show that the shape of the  $\omega$  dependence of the displacement of equilibrium is directly connected with the number of species involved in the mechanism considered:  $A_{i,\text{cst}}^2$  presents a unique threshold for two species, two thresholds for three species. For  $n=3$ ,  $A_{i,\text{cst}}^2$  either has an extremum in between two thresholds with derivatives of opposite sign or is a monotonous function of  $\omega$  with

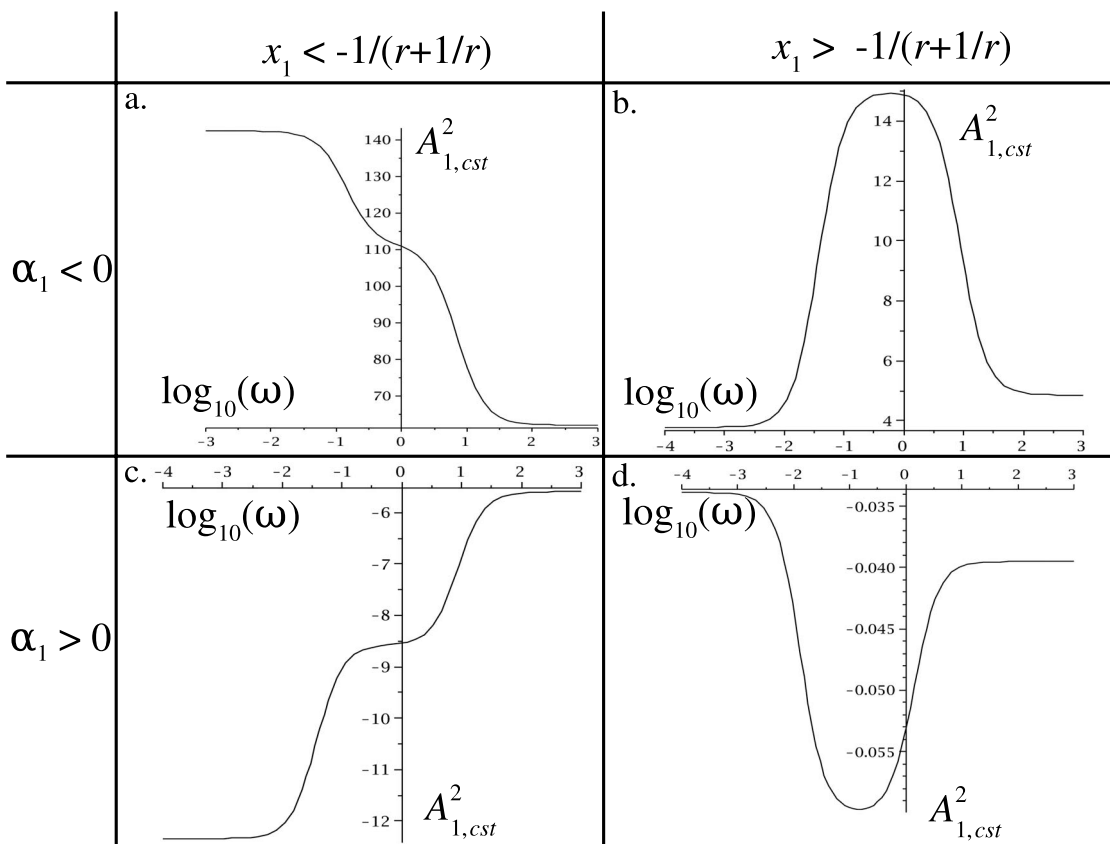
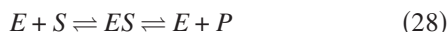
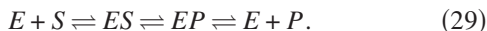


FIG. 2. Constant second-order correction  $A_{1,cst}^2$  to the concentration of species  $A_1$  involved in unimolecular reactions with three species versus  $\log_{10} \omega$  for (a)  $k_{12}^0=0.5$ ,  $k_{23}^0=0.09$ ,  $k_{31}^0=0.8$ ,  $k_{21}^0=0.02$ ,  $k_{32}^0=0.03$  and  $\epsilon_{12}=100$ ,  $\epsilon_{23}=40$ ,  $\epsilon_{31}=25$ ,  $\epsilon_{21}=35$ ,  $\epsilon_{32}=80$ ; (b)  $k_{12}^0=0.6$ ,  $k_{23}^0=0.01$ ,  $k_{31}^0=0.08$ ,  $k_{21}^0=0.02$ ,  $k_{32}^0=0.003$ , and  $\epsilon_{12}=50$ ,  $\epsilon_{23}=150$ ,  $\epsilon_{31}=50$ ,  $\epsilon_{21}=120$ ,  $\epsilon_{32}=40$ ; (c) same parameter values as (b) except  $\epsilon_{32}=40$ ; (d) same parameter values as (c) except  $k_{31}^0=0.0008$ ,  $k_{21}^0=0.0002$ , and  $k_{32}^0=0.0003$ .

two successive thresholds with derivatives of the same sign. The cutoff frequencies are related to the nonvanishing eigenvalues of the matrix associated to the mechanism. More generally, it is easy to show that unimolecular reactions involving  $n$  species associated to  $n-1$  nonvanishing different eigenvalues will lead to  $n-1$  frequency thresholds. Consequently, the shape of the displacement of equilibrium of any species  $A_i$ , considered as a function of  $\omega$ , reveals the number of species in the reactive medium, at the condition that the eigenvalues differ by at least an order of magnitude. These results can be directly applied to the case of *in vitro* enzymatic catalysis where the concentrations of substrate  $S$  and product  $P$  are maintained constant [35]. The frequency dependence of the displacement of equilibrium allows us to discriminate between the simplest Michaelis-Menten-type model with two species  $E$  and  $ES$ ,



and the three-species model that involves  $E$ ,  $ES$ , and  $EP$ ,



The existence of two intermediates  $ES$  and  $EP$  is simply revealed by the presence of two thresholds in the  $\omega$  dependence of the deviation from the unperturbed concentration of each species. Note that a resonant frequency, associated with

an extremum for the concentration shift, can only exist in this last case. The information contained in the frequency dependence of the concentration displacement could typically be used to determine the number of cooperative sites in a protein such as hemoglobin [36]. Whereas deoxy-hemoglobin  $E$  is relatively uninterested in oxygen, once one oxygen has attached to form  $EO$ , the second oxygen binds more easily to form  $EO_2$ , and the third and fourth oxygens, leading to  $EO_3$  and  $EO_4$ , easier yet. In the case of hemoglobin, four thresholds should be detected.

More generally, the shape of the concentration shift as a function of the modulation frequency can be used to investigate how proteins and enzymes regulate metabolic processes and not only the number of species involved. In order to illustrate this general statement, we apply the results of Sec. IV to the case of a unimolecular mechanism with a reactant  $A_1$ , a product  $A_3$ , and a reactive intermediate  $A_2$ ,



This system is characterized by four rate constants  $k_{12}^0$ ,  $k_{23}^0$ ,  $k_{21}^0$ , and  $k_{32}^0$ , and four activation energies  $\epsilon_{12}$ ,  $\epsilon_{23}$ ,  $\epsilon_{21}$ ,  $\epsilon_{32}$ . The results of Sec. IV apply for  $k_{13}^0=k_{31}^0=\epsilon_{13}=\epsilon_{31}=0$ . Since species  $A_2$  is a reactive intermediate, the rate constants  $k_{12}^0$  and  $k_{32}^0$  are much smaller than  $k_{21}^0$  and  $k_{23}^0$ . Similarly the activa-

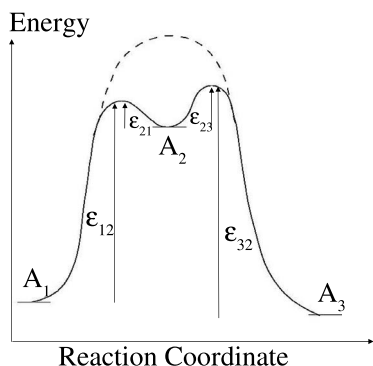


FIG. 3. Reaction-coordinate diagrams in the case of a unimolecular reaction with a reactant  $A_1$  and a product  $A_3$  without (dashed line) and with (solid line) an intermediate  $A_2$ . We give the example where  $\epsilon_{23} > \epsilon_{21}$  and  $\epsilon_{32} > \epsilon_{12}$ .

tion energies  $\epsilon_{21}$  and  $\epsilon_{23}$  are much smaller than  $\epsilon_{12}$  and  $\epsilon_{32}$ . We perform an expansion with respect to the two parameters  $\delta_k = \frac{k_j^0}{k_i^0}$  and  $\delta_\epsilon = \frac{\epsilon_{2i}}{\epsilon_{12}}$  with  $i, j = 1, 3$  and  $i \neq j$ . With the use of Eq. (16) and the expression of the matrix  $\mathbf{M}^0$  given in the Appendix, the eigenvalues given in Eq. (17) reduce at the leading order in  $\delta_k$  and  $\delta_\epsilon$  to

$$\lambda_+ = -\frac{k_{12}^0 k_{23}^0 + k_{21}^0 k_{32}^0}{k_{21}^0 + k_{23}^0}, \quad (31)$$

$$\lambda_- = -(k_{21}^0 + k_{23}^0). \quad (32)$$

They are real and negative with  $|\lambda_+| \ll |\lambda_-|$ . According to Eqs. (18), (31), and (32) and Eqs. (A8) and (A9) of the Appendix, the expression of the three control parameters becomes

$$r = \frac{(k_{21}^0 + k_{23}^0)^2}{k_{12}^0 k_{23}^0 + k_{21}^0 k_{32}^0}, \quad (33)$$

$$\alpha_1 = \frac{k_{12}^0 k_{23}^0 k_{21}^0 k_{32}^0}{k_{12}^0 k_{23}^0 + k_{21}^0 k_{32}^0} (\epsilon_{23} - \epsilon_{21})(k_{21}^0 + k_{23}^0)(\epsilon_{12} k_{21}^0 + \epsilon_{32} k_{23}^0), \quad (34)$$

$$x_1 = \frac{(\epsilon_{12} - \epsilon_{32})(\epsilon_{12} k_{12}^0 k_{23}^0 + \epsilon_{32} k_{21}^0 k_{32}^0)}{(\epsilon_{23} - \epsilon_{21})(k_{21}^0 + k_{23}^0)(\epsilon_{12} k_{21}^0 + \epsilon_{32} k_{23}^0)}. \quad (35)$$

The sign of  $\alpha_1$  is determined by the sign of  $(\epsilon_{23} - \epsilon_{21})$ . Considering the variation of the displacement of equilibrium between  $\omega=0$  and  $\omega \rightarrow \infty$ , we define the height  $h_1$  as

$$h_1 = A_{1,\text{cst}}^2(\omega \rightarrow \infty) - A_{1,\text{cst}}^2(\omega \rightarrow 0) = \frac{-\alpha_1 x_1}{2\lambda_+}. \quad (36)$$

Using Eqs. (34) and (35), we find that the sign of  $h_1$  is simply determined by the sign of  $(\epsilon_{32} - \epsilon_{12})$ .

As shown in Fig. 3 the height between two successive extrema on the reaction coordinate diagram corresponds to the activation energy between two species. Classical techniques based on thermodynamics allow for the determination of the enthalpy of reaction but do not give any information

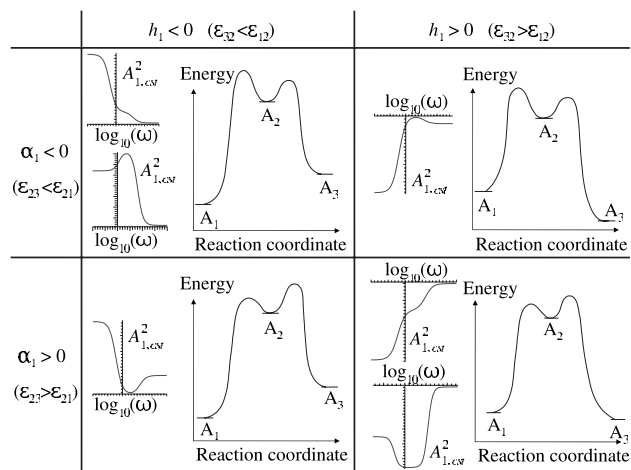


FIG. 4. The different shapes of the  $\omega$  dependence of the displacement of equilibrium  $A_{1,\text{cst}}^2$  for species  $A_1$  and the corresponding reaction-coordinate diagrams in the case of unimolecular reactions with a reactant  $A_1$ , a product  $A_3$ , and an intermediate  $A_2$ .

on the intermediate part of the reaction coordinate diagram. The preceding results allow us to propose an experimental protocol to determine the possible existence of a reactive intermediate between a reactant and a product. The procedure can be followed without any knowledge of the values of the dynamical parameters and makes it possible to establish an order relation between the different activation energies. Submit the reactive medium to a temperature modulation of small amplitude and perform a frequency sweep. The shape of the  $\omega$  dependence of the concentration shift reveals the main features of the dynamics.

First, the shape of  $A_{1,\text{cst}}^2$  as a function of  $\omega$  is directly connected with the number of species involved in the mechanism considered: If  $A_{1,\text{cst}}^2$  presents a unique threshold, the mechanism involves only two species and the passage from the reactant to the product occurs without a reactive intermediate. If the  $\omega$  dependence of the concentration shift  $A_{1,\text{cst}}^2$  possesses two thresholds, then the mechanism involves three species and it proves the existence of a reactive intermediate. The two cutoff frequencies are related to the nonvanishing eigenvalues of the matrix associated to the mechanism. It is easy to generalize the procedure: if  $n$  frequency thresholds are observed, it proves that the mechanism involves  $n+1$  species. In the case  $n=3$ , we give here the explicit expression of the control parameters  $\alpha_1$  and  $x_1$  associated with the reactant  $A_1$ . A similar analysis can be performed with the product  $A_3$  if this species is easier to detect from an experimental point of view.

Second, the shape of  $A_{1,\text{cst}}^2$  as a function of  $\omega$  and the results given in Fig. 4 allow us to determine the sign of  $\alpha_1$  and  $h_1$ . The power of the method comes from the systematic correspondence between the shape, the existence of two successive thresholds, a maximum or a minimum, the comparison of the behaviors at  $\omega \rightarrow 0$  and  $\omega \rightarrow \infty$ , and the sign of the control parameters. For example, the existence of a maximum for  $A_{1,\text{cst}}^2$  versus  $\omega$  and the relation  $A_{1,\text{cst}}^2(\omega \rightarrow 0) > A_{1,\text{cst}}^2(\omega \rightarrow \infty)$ , respectively, imply that  $\alpha_1 < 0$  and  $h_1 < 0$ . Note that, reciprocally, the knowledge of the sign of  $\alpha_1$  and

$h_1$  is not sufficient to determine the shape of  $A_{1,\text{cst}}^2$ , the case  $\alpha_1 < 0$  and  $h_1 < 0$  corresponds either to a maximum or to two successive thresholds.

As mentioned under Eqs. (34) and (36), the signs of  $\alpha_1$  and  $h_1$  are directly connected to the signs of  $(\epsilon_{23} - \epsilon_{21})$  and  $(\epsilon_{32} - \epsilon_{12})$ , respectively. Consequently, the frequency dependence of the concentration shift  $A_{i,\text{cst}}^2$  of a single species  $A_i$  is sufficient to give access to the shape of the reaction-coordinate diagram. In particular, it allows for establishing an order relation between the activation energies, including for the two small activation barriers which surround the reactive intermediate. If we take the same example as before, the existence of a maximum for  $A_{1,\text{cst}}^2$  versus  $\omega$  and the relation  $A_{1,\text{cst}}^2(\omega \rightarrow 0) > A_{1,\text{cst}}^2(\omega \rightarrow \infty)$  reveal that  $\epsilon_{32} < \epsilon_{12}$ , i.e., the product  $A_3$  is less stable than the reactant  $A_1$ , and, more difficult to obtain by standard methods, that the barrier  $\epsilon_{23}$  between the reactive intermediate  $A_2$  and the product  $A_3$  is smaller than the barrier  $\epsilon_{21}$  between the reactive intermediate  $A_2$  and the reactant  $A_1$ . The different cases are displayed in Fig. 4.

## VI. CONCLUSION

In this paper, we determine the analytical expression of the concentration shift due to temperature modulation for a unimolecular mechanism with two and three species. The model typically applies for enzymatic catalysis following mechanisms of Michaelis-Menten type. We characterize the frequency dependence of these displacements of equilibrium and make precise the conditions of maximization of the yield of the reaction. For  $n=3$ , we give the analytical expression of the resonant frequency associated with the maximum of concentration shift. We show that the use of any oscillating external field gives access to the number  $n$  of species involved in the mechanism considered. Moreover, we prove that choosing the temperature as the oscillating quantity has the advantage to introduce the activation energies in the expression of the deviation of the concentrations from the unper-

TABLE I. Asymptotic value of the displacement of equilibrium in the limit  $\omega \rightarrow \infty$  for each species  $A_i$ .

$A_{1,\text{cst}}^2(\omega \rightarrow \infty)$	$2A_1^0 \left[ -\kappa_{2,2}^0 \left( k_{12}^0 \frac{\epsilon_{21}^2 - \epsilon_{12}^2}{4} + k_{13}^0 \frac{\epsilon_{31}^2 - \epsilon_{13}^2}{4} \right) + \kappa_{1,2}^0 \left( k_{12}^0 \frac{\epsilon_{12}^2 - \epsilon_{21}^2}{4} + k_{21}^0 \frac{\epsilon_{32}^2 - \epsilon_{23}^2}{4} \right) \right]$
$A_{2,\text{cst}}^2(\omega \rightarrow \infty)$	$2A_2^0 \left[ -\kappa_{3,3}^0 \left( k_{23}^0 \frac{\epsilon_{32}^2 - \epsilon_{23}^2}{4} + k_{21}^0 \frac{\epsilon_{12}^2 - \epsilon_{21}^2}{4} \right) + \kappa_{2,3}^0 \left( k_{23}^0 \frac{\epsilon_{23}^2 - \epsilon_{32}^2}{4} + k_{32}^0 \frac{\epsilon_{13}^2 - \epsilon_{31}^2}{4} \right) \right]$
$A_{3,\text{cst}}^2(\omega \rightarrow \infty)$	$-[A_{1,\text{cst}}^2(\omega \rightarrow \infty) + A_{2,\text{cst}}^2(\omega \rightarrow \infty)]$

turbed value. In the case of a reactive intermediate between a reactant and a product, the shape of the concentration shift versus the frequency reveals the sign of the differences of activation energies and consequently the shape of the reaction-coordinate diagram. The power of the procedure results in the possible classification of all the distinct cases. The application of a small temperature modulation can be envisaged to investigate *in vivo* the dynamical properties of biological systems.

## ACKNOWLEDGMENT

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## APPENDIX

We give hereafter the expression of the parameters introduced in the case of unimolecular reactions with three species. The matrices  $\mathbf{M}^i$  at the  $i$ th order of the expansion in powers of  $\beta$  introduced in Eq. (16) have the following expressions:

$$\mathbf{M}^0 = \begin{pmatrix} -(k_{12}^0 + k_{13}^0 + k_{31}^0) & k_{21}^0 - k_{31}^0 \\ k_{12}^0 - k_{32}^0 & -(k_{21}^0 + k_{23}^0 + k_{32}^0) \end{pmatrix}, \quad (\text{A1})$$

$$\mathbf{M}^1 = \begin{pmatrix} -(k_{12}^0 \epsilon_{12} + k_{13}^0 \epsilon_{13} + k_{31}^0 \epsilon_{31}) & k_{21}^0 \epsilon_{21} - k_{31}^0 \epsilon_{31} \\ k_{12}^0 \epsilon_{12} - k_{32}^0 \epsilon_{32} & -(k_{21}^0 \epsilon_{21} + k_{23}^0 \epsilon_{23} + k_{32}^0 \epsilon_{32}) \end{pmatrix}, \quad (\text{A2})$$

$$\mathbf{M}^2 = \begin{pmatrix} -(k_{12}^0 \epsilon_{12}^2/4 + k_{13}^0 \epsilon_{13}^2/4 + k_{31}^0 \epsilon_{31}^2/4) & k_{21}^0 \epsilon_{21}^2/4 - k_{31}^0 \epsilon_{31}^2/4 \\ k_{12}^0 \epsilon_{12}^2/4 - k_{32}^0 \epsilon_{32}^2/4 & -(k_{21}^0 \epsilon_{21}^2/4 + k_{23}^0 \epsilon_{23}^2/4 + k_{32}^0 \epsilon_{32}^2/4) \end{pmatrix}. \quad (\text{A3})$$

The expression of the parameters  $a_{i,\text{sin}}^-$  and  $a_{i,\text{sin}}^+$  introduced in Eq. (21) are given by

$$a_{1,\text{sin}}^- = \frac{r}{1-r^2} [(r\kappa_{1,1}^0 - \kappa_{2,2}^0)f + \kappa_{1,2}^0(1+r)g], \quad (\text{A4})$$

$$a_{1,\text{sin}}^+ = \frac{1}{r^2-1} [(\kappa_{1,1}^0 - \kappa_{2,2}^0 r)f + \kappa_{1,2}^0(1+r)g], \quad (\text{A5})$$

$$a_{2,\sin}^- = \frac{r}{1-r^2} [(r\kappa_{2,2}^0 - \kappa_{1,1}^0)g + \kappa_{2,1}^0(1+r)f], \quad (\text{A6})$$

$$a_{2,\sin}^+ = \frac{1}{r^2-1} [(\kappa_{2,2}^0 - \kappa_{1,1}^0 r)g + \kappa_{2,1}^0(1+r)f], \quad (\text{A7})$$

with  $f = A_1^0 [k_{12}^0(\epsilon_{21} - \epsilon_{12}) + k_{13}^0(\epsilon_{31} - \epsilon_{13})]$  and  $g = A_1^0 [-k_{12}^0(\epsilon_{21} - \epsilon_{12}) + \frac{k_{12}^0}{k_{31}^0} k_{23}^0(\epsilon_{32} - \epsilon_{23})]$ .

The asymptotic value  $A_{i,\text{cst}}^2(\omega \rightarrow \infty)$  of the deviation of the concentration from its unperturbed value in the limit  $\omega \rightarrow \infty$  is given for each species  $A_i$  in Table I.

The control parameters  $\alpha_i$  and  $x_i$  appearing in Eqs. (25) and (26) are

TABLE II. Expressions of the coefficients of the matrix  $(\mathbf{M}^0)^{-1}\mathbf{M}^1$ .

$u_1 = -\kappa_{1,1}^1 \kappa_{2,2}^0 + \kappa_{2,1}^1 \kappa_{1,2}^0$	$u_2 = \kappa_{1,1}^1 \kappa_{2,1}^0 - \kappa_{2,1}^1 \kappa_{1,1}^0$
$v_1 = -\kappa_{1,2}^1 \kappa_{2,2}^0 + \kappa_{2,2}^1 \kappa_{1,2}^0$	$v_2 = \kappa_{1,2}^1 \kappa_{2,1}^0 - \kappa_{2,2}^1 \kappa_{1,1}^0$

$$\alpha_i = u_i(\kappa_{1,1}^0 f + \kappa_{1,2}^0 g) + v_i(\kappa_{2,1}^0 f + \kappa_{2,2}^0 g), \quad (\text{A8})$$

$$x_i = \frac{1}{\alpha_i} [u_i(-\kappa_{2,2}^0 f + \kappa_{1,2}^0 g) + v_i(-\kappa_{1,1}^0 f + \kappa_{2,1}^0 g)]. \quad (\text{A9})$$

The parameters  $u_i$  and  $v_i$  for  $i=1,2$  are defined as the coefficients of the matrix  $(\mathbf{M}^0)^{-1}\mathbf{M}^1$  and their expression is given in Table II. The coefficients  $u_3$  and  $v_3$  associated with species  $A_3$  are given by  $u_3 = -(u_1 + u_2)$  and  $v_3 = -(v_1 + v_2)$ .

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