A unified framework for limit results in chemical reaction networks on multiple time-scales[1]

Timo Enger[2]   Peter Pfaffelhuber[3]

Abstract

If $(X^N)_{N=1,2,\dots}$ is a sequence of Markov processes which solve the martingale problems for some operators $(G^N)_{N=1,2,\dots}$, it is a classical task to derive a limit result as $N \to \infty$, in particular a weak process limit with limiting operator G. For slow-fast systems $X^N = (V^N, Z^N)$ where V^N is slow and Z^N is fast, G^N consists of two (or more) terms, and we are interested in weak convergence of V^N to some Markov process V. In this case, for some $f \in \mathcal{D}(G)$, the domain of G, depending only on v, the limit Gf can sometimes be derived by using some $g_N \to 0$ (depending on v and z), and study convergence of $G^N(f + g_N) \to Gf$. We develop this method further in order to obtain functional Laws of Large Numbers (LLNs) and Central Limit Theorems (CLTs). We then apply our general result to various examples from Chemical Reaction Network theory. We show that we can rederive most limits previously obtained, but also provide new results in the case when the fast-subsystem is first order. In particular, we allow that fast species to be consumed faster than they are produced, and we derive a CLT for Hill dynamics with coefficient 2.

1 Introduction

The motivation for our work and the resulting limiting results comes from recent publications on multi-scale (Markovian) Chemical Reaction Networks (CRNs). Here, multi-scale refers to the fact that reaction rates differ by orders of magnitude; see e.g. [1]. In contrast, limit results for single-scale systems, where all species are in the same (high) abundance, have basically been solved by early work of Tom Kurtz ([13, 12]), providing both, functional Laws of Large Numbers (LLNs), with the solution of a system of Ordinary Differential Equations (ODEs) as limit, and functional Central Limit Theorems (CLTs),

---

[2]University of Freiburg, Germany
[3]University of Freiburg, Germany, E-mail: p.p@stochastik.uni-freiburg.de

leading to the solution of a system of Itô Stochastic Differential Equations (SDEs) as limit. For multi-scale reaction networks, frequently the distinction between deterministic (if all species abundances are large) and stochastic networks (if all species abundances are small) is made. In deterministic models, the quasi-steady-state assumption is most important; see e.g. [16]. In early papers on stochastic models [5, 15], the focus was on simulation techniques of slow-fast systems. Rather than distinguishing strictly between deterministic and stochastic models, we will use the notions introduced in [2], where some species are in large and some in small abundance, and reaction rates still differ by orders of magnitude. Such multi-scale systems still pose interesting fundamental questions today. LLNs in these systems have been derived using techniques involving stochastic averaging [14] and approximations of Poisson processes by Brownian motion; see [2, 10]. The case of LLNs involving fast intermediate species is treated in [3], [17] using probabilistic arguments, i.e. exit times and probabilities of the fast sub-system. Here, fast species in low copy number, are produced on a slower time-scale than they are consumed, and are called intermediate. This concept was recently generalized to non-interacting species in [7]. Concerning functional CLTs in such multi-scale systems, [11] use the technique of stochastic averaging, solutions of Poisson equations and the martingale central limit theorem for proving limit results on CRNs on two or three time-scales. Their results have been used in various contexts, e.g. for a CRN involving the heat shock protein of *E. coli* [9]. In this theory, a central role is played by the averaging condition (their Condition 2.4), which assumes a unique equilibrium of fast species when the slow species are fixed. A related condition is the balance condition from [10], which assumes that all species – in particular fast species – are produced and consumed on the same time-scale. Both, the averaging condition and the species balance condition, are violated by the intermediate species from [3], since they are consumed faster than they are produced and hence no limit averaging distribution exists.

In our setup, we will assume that species in low abundance are fast, and species in high abundance are slow. For such systems, our goal is threefold: We want to (i) provide a general framework which provides the possibility to derive limit results for all examples in the above mentioned papers, in particular (ii) provide a method to derive functional CLTs for the case of intermediate species, and (iii) obtain CLTs in cases not treated so far. In order to achieve this, we give in Theorem 2.3 a general result which requires only an application of a well-known limit result for Markov processes (Theorem 2.1 in [14]). Applying this general result to specific cases of CRNs leads to a *recipe* how to obtain LLNs and CLTs, described in Section 3.2. We provide concrete computations on previous examples and how our theory applies in these contexts; see Section 4. In particular, we will treat Michaelis-Menten kinetics (one of the main examples in [2, 11]) in Section 4.1, but treat also the main example from [3] in Section 4.4. We add to the list of examples an extension of Michaelis-Menten kinetics, which is known in the literature as dynamics with Hill coefficient 2; see Examples 3.1–3.7 and Section 4.2.

2 Main results

Before we provide with Theorem 2.3 our main general result, we give some heuristic arguments and an example. Recall that a stochastic process V with Polish state space E solves the $(G, \mathcal{D}(G))$ martingale problem for some linear $G : \mathcal{D}(G) \subseteq \mathcal{C}_b(E) \to \mathcal{C}_b(E)$ if

$$\left(f(V_t) - \int_0^t Gf(V_s)ds \right)_{t \geq 0}$$

is a martingale for all $f \in \mathcal{D}(G)$. Assume that there is a unique (in law) solution, i.e. the martingale problem is well-posed and V is unique and Markovian, if $\mathcal{D}(G)$ is large enough.

Our goal is to obtain convergence of a sequence V^N to some V as $N \to \infty$. Here, V^N is one coordinate in some Markov process (V^N, Z^N) with state space $E \times F$ (for E, F Polish). For the description of this process using a martingale problem, we make the following convention:

Remark 2.1 (Some abuse of notation). Let us introduce a notion which we will use frequently. If $f : E \times F \to \mathbb{R}$ with $z \mapsto f(v, z)$ is constant in z for all v, there is $\tilde{f} : E \to \mathbb{R}$ with $f(v, z) = \tilde{f}(v)$ for all v, and we will abuse notation and simply say that f does not depend on z (or f only depends on v) and identify $\tilde{f}$ and f since $\tilde{f}(v) = f(v, z)$ holds for all v, z. In this sense, we also write $\mathcal{C}_b(E) \subseteq \mathcal{C}_b(E \times F)$, since we identify $f \in \mathcal{C}_b(E)$ with $(v, z) \mapsto f(v)$.

For the Markov process (V^N, Z^N), we assume that the generator has the form

$$G^N = G_0^N + N^{1/2} G_1^N + N G_2^N \qquad (2.1)$$

with $G_2^N f = 0$ if $f \in \mathcal{D}(G^N) \supseteq \mathcal{D}(G)$ only depends on v. In this sense, i.e. the fastest generator term G_2^N only describes the dynamics of Z^N, we will call V^N slow and Z^N fast. For this convergence, we assume that for every $f \in \mathcal{D}(G)$ (in particular, f only depends on v), there are $g_N, h_N \in \mathcal{D}(G^N)$ such that (recall $G_2^N f = 0$)

$$Gf(v) \approx G_0^N f(v) + (G_1^N g_N + G_2^N h_N)(v, z) + N^{1/2}(G_1^N f + G_2^N g_N)(v, z) \qquad (2.2)$$

in the sense that for all $T > 0$

$$\mathbb{E}\Big[\int_0^T |\varepsilon_f^N(V_t^N, Z_t^N)| dt \Big] \xrightarrow{N \to \infty} 0 \text{ for}$$

$$\varepsilon_f^N(v, z) := G_0^N f(v) + (G_1^N g_N + G_2^N h_N)(v, z) + N^{1/2}(G_1^N f + G_2^N g_N)(v, z) - Gf(v).$$

Then, provided tightness of V^N and some boundedness restrictions of $g_N, h_N, G_0^N g_N$, $G_0^N h_N$ and $G_1^N h_N$, and V' is an accumulation point of V^N, it is reasonable to conclude that

$$f(V_t^N) + (N^{-1/2} g_N + N^{-1} h_N)(V_t^N, Z_t^N) - \int_0^t G^N(f + N^{-1/2} g_N + N^{-1} h_N)(V_s^N, Z_s^N) ds$$

$$\approx f(V_t^N) - \int_0^t (G_0^N f + G_1^N g_N + G_2^N h_N + N^{1/2}(G_1^N f + G_2^N g_N))(V_s^N, Z_s^N) ds$$

$$\approx f(V_t') - \int_0^t Gf(V_s') ds$$

is a martingale. If the martingale problem for $(G, \mathcal{D}(G))$ is well-posed with unique solution V, this then shows convergence $V^N \xrightarrow{N \to \infty} V$, provided the initial conditions converge.

Example 2.2 (Convergence to Brownian Motion). For applying the result, let us have a look at (2.2). Since the term $N^{1/2}(G_1^N f + G_2^N g_N)$ is the leading term on the right-hand-side, we first look for g_N such that $G_1^N f + G_2^N g_N = 0$, and then for h_N such that $G_1^N g_N + G_2^N h_N$ only depends on v.

Let us consider a simple example outside of Chemical Reaction Networks, which shows that the above method can work: Let $X^N = (V^N, Z^N)$ be the Markov process with state space $\mathbb{R} \times \mathbb{R}$ and generator, for some probability measure π with mean zero and variance $\frac{1}{2}$, writing f' for the derivative with respect to the first coordinate,

$$G^N f(v, z) = N^{1/2} \underbrace{z f'(v, z)}_{=G_1 f(v, z)} + N \underbrace{\int (f(v, z') - f(v, z)) \pi(dz')}_{=G_2 f(v, z)},$$

i.e. V^N is a continuous random walk, which changes slope at rate N. Clearly, V^N converges to a Brownian motion. In order to see this, for $f \in \mathcal{C}_b^\infty(\mathbb{R})$, we choose

$$g_N(v, z) := g(v, z) := z f'(v), \text{ which implies } G_1 f + G_2 g_N = 0$$

(since π has mean zero). Next, note that $G_1 g(v, z) = z^2 f''(v)$, so we choose

$$h_N(v, z) := h(v, z) = z^2 f''(v), \text{ which implies } (G_1 g + G_2 h)(v, z) = Gf(v) := \tfrac{1}{2} f''(v),$$

leading to $\varepsilon_N^f = 0$ in (2.9). Hence, provided tightness is shown, the desired convergence follows.

Theorem 2.3 (Convergence result). *Let E, F be Polish and $(X^N = (V^N, Z^N))_{N=1,2,\dots}$ be a sequence of Markov processes with state space $E \times F$, and with generators of the form*

$$G^N = G_0^N + N^{1/2} G_1^N + N G_2^N, \tag{2.3}$$

with $\mathcal{D}(G^N) = \mathcal{D}(G_i^N) \subseteq \mathcal{C}_b(E \times F)$, $i = 0, 1, 2$ and $G_2^N f = 0$ if f only depends on $v \in E$. (Recall this notion from Remark 2.1.) Assume that $(V^N)_{N=1,2,\dots}$ satisfies the compact containment condition, i.e. for all $\varepsilon > 0$ and $T > 0$, there is $K \subseteq E$ compact with

$$\inf_N \mathbb{P}(V_t^N \in K, 0 \leq t \leq T) > 1 - \varepsilon,$$

and let $G : \mathcal{D}(G) \subseteq \mathcal{C}_b(E) \cap \mathcal{D}(G^N) \to \mathcal{C}_b(E)$. Assume that for every $f \in \mathcal{D}(G)$ and $N = 1, 2, \dots$, there is $g_N, h_N \in \mathcal{D}(G^N)$, such that for all $T > 0$

$$N^{-1/2} \mathbb{E}[\sup_{t \leq T} |g_N(V_t^N, Z_t^N)|] + N^{-1} \mathbb{E}[\sup_{t \leq T} |h_N(V_t^N, Z_t^N)|] \xrightarrow{N \to \infty} 0, \tag{2.4}$$

$$N^{-1/2} \mathbb{E}\Big[\int_0^T |(G_0^N g_N + G_1^N h_N)(V_t^N, Z_t^N)| dt \Big] + N^{-1} \mathbb{E}\Big[\int_0^T |G_0^N h_N(V_t^N, Z_t^N)| dt \Big] \xrightarrow{N \to \infty} 0 \tag{2.5}$$

and

$$\mathbb{E}\Big[\int_0^T |\varepsilon_f^N(V_t^N, Z_t^N)| dt \Big] \xrightarrow{N \to \infty} 0 \tag{2.6}$$

for

$$\varepsilon_f^N(v, z) = G_0^N f(v, z) + G_1^N g_N(v, z) + G_2^N h_N(v, z) + N^{1/2}(G_1^N f(v) + G_2^N g_N(v, z)) - Gf(v). \tag{2.7}$$

Then, $(V^N)_{N=1,2,\dots}$ is tight and for every limit point V,

$$\Big(f(V_t) - \int_0^t Gf(V_s) ds \Big)_{t \geq 0} \tag{2.8}$$

is a martingale for each $f \in \mathcal{D}(G)$.

Remark 2.4 (Connections to other convergence results). 1. Our proof relies on Theorem 2.1 of [14], as we will see below. For this result, the occupation measure Γ^N of the fast variable, given by

$$\Gamma^N([0, t] \times B) = \int_0^t 1_{Z_s^N \in B} ds$$

is needed. However, this result is more general since for the limiting generator (A in the notation of [14]) and some $f \in \mathcal{D}(G)$, Af still depends on the slow and fast variable, and the fast variable needs to be integrated over the weak limit of Γ^N. In our setting, the limit of $G^N f$ does not depend on the fast variable, and we do not need the latter integration. So, it is not surprising that Theorem 2.3 holds, since it treats just a special case where the limit does not depend on the fast variable. However, it is maybe surprising that Theorem 2.3 is useful in obtaining limit results in a bunch of examples; see Section 4.

Let us try to give some more details for a generator of the form (2.1): Let $f \in \mathcal{C}_b(E)$ (or some appropriate subset), i.e. f only depend on v. For the application of Theorem 2.1 in [14], assume that $\Gamma^N \xRightarrow{N \to \infty} \Gamma$, and there is g_N with $G_1^N f + G_2^N g_N = 0$ (or is uniformly in $o(N^{-1/2})$). Then, [14] concludes that the limit of

$$f(V_t^N) - \int_0^t (G_0^N f + G_1^N g_N)(V_s^N, z) \Gamma^N(ds, dz)$$

is a martingale. In the setting of Theorem 2.3 above, we do not assume convergence of Γ^N, but existence of h_N such that $G_0^N f + G_1^N g_N + G_2^N h_N$ only depends on v (or can be written as Gf only depending on v and some $o(1)$ error term). Then, the limit as given by (2.8) is a martingale. This implies that

$$\int_0^t (G_0^N f + G_1^N g_N)(V_s^N, z) \Gamma^N(ds, dz) \approx \int_0^t (G_0^N f + G_1^N g_N + G_2^N h_N)(V_s^N) ds,$$

wich shows the connection of Γ^N and h_N.

2. As another example, consider (2.1), where $G_0^N f$ only depends on v if f only depends on v, and the fast variable jumps according to

$$G_2^N f(v, z) = \int f(v, z') \pi_N(dz') - f(v, z),$$

i.e. Z^N jumps to new states according to the equilibrium measure π_N at rate N. We note that this form of G_2^N implies that $f + G_2^N f$ does not depend on z (see Remark 2.1 for this notion. Then, for smooth f, only depending on v, we set $g_N = G_1^N f$ and $h_N := G_1^N G_1^N f$. If the limits

$$A_1 f(v) := \lim_{N \to \infty} N^{1/2} \int G_1^N f(v, z') \pi_N(dz'),$$

$$A_2 f(v) := \lim_{N \to \infty} G_0^N f(v) + \int G_1^N G_1^N f(v, z') \pi_N(dz')$$

exist, we then see from Theorem 2.3 that – provided the compact containment condition of $(V^N)_{N=1,2,\dots}$ and (2.4)–(2.6) hold – the weak limit of V^N has generator $G := A_1 + A_2$. This case is actually treated in Corollary 2.5 of [8]. Their main result, Theorem 2.3, is more general since it allows for more general G_2^N, and A_2 from above may arise by an application of stochastic averaging.

Proof. We are going to apply Theorem 2.1 of [14]. In our notation (or their notation), we will have the special case where Gf (or Af) does not depend on the fast variable z (or y) for the limiting generator G (or A) and $f \in \mathcal{D}(G)$ (or $f \in \mathcal{D}(A)$).

Since Gf is bounded by assumption, their (2.3) is satisfied and their (2.6) becomes our (2.8). So, it remains to properly define some δ_f^N (or ε_f^N) in their (2.2), which satisfies

their (2.4). Here, we have for $f \in \mathcal{D}(G)$ the martingale

$$f(V_t^N) + (N^{-1/2} g_N + N^{-1} h_N)(V_t^N, Z_t^N) - \int_0^t G^N(f + N^{-1/2} g_N + N^{-1} h_N)(V_s^N, Z_s^N)ds$$

$$= f(V_t^N) - \int_0^t Gf(V_s^N)ds + \delta_f^N(t)$$

with (recall $G_2^N f = 0$)

$$\delta_f^N(t) = (N^{-1/2} g_N + N^{-1} h_N)(V_t^N, Z_t^N) - \int_0^t \varepsilon_f^N(V_s^N, Z_s^N)ds$$

$$- \int_0^t (N^{-1/2}(G_0^N g_N + G_1^N h_N) + N^{-1} G_0^N h_N)(V_s^N, Z_s^N)ds.$$

By (2.4), (2.5) and (2.6), we find that $\mathbb{E}[\sup_{0 \le t \le T} |\delta_f^N(t)|] \xrightarrow{N \to \infty} 0$, and we are done. $\qquad \square$

Remark 2.5 (Generators with two or more than three terms).   1. If we have $G_1^N = 0$ in (2.3), we can choose $g_N = 0$, and have to find h_N satisfying some boundedness restrictions and $\mathbb{E}\left[\int_0^T |\varepsilon_f^N(V_t^N, Z_t^N)| dt \right] \xrightarrow{N \to \infty} 0$ for

$$\varepsilon_f^N(v,z) := (G_0^N f + G_2^N h_N - Gf)(v,z). \tag{2.9}$$

    We will use this case in functional LLNs in Section 4.

   2. For generators with more than three terms, a similar approach works equally well. Let $X^N = (V^N, Z^N)$ be a Markov process with generator

$$G^N = G_0^N + N \cdot G_1^N + N^2 \cdot G_2^N + N^3 \cdot G_3^N$$

    and $G_3^N f = 0$ if f only depends on v. For a limiting generator G and $f \in \mathcal{D}(G)$ (in particular depending only on v), we assume there are $g_N, h_N, k_N \in \mathcal{D}(G^N)$ such that $\mathbb{E}\left[\int_0^T |\varepsilon_f^N(V_t^N, Z_t^N)| dt \right] \xrightarrow{N \to \infty} 0$ for

$$\varepsilon_f^N(v,z) = (G_0^N f + G_1^N g_N + G_2^N h_N + G_3^N k_N$$
$$+ N(G_1^N f + G_2^N g_N + G_3^N h_N) + N^2(G_2^N f + G_3^N g_N) - Gf)(v,z),$$

    as well as the compact containment condition of (V^N) and some boundedness assumptions for $g_N, h_N, k_N, G_0^N g_N, G_i^N h_N, i = 0, 1, G_i^N k_N, i = 0, 1, 2$, convergence of V^N to a solution of the $(G, \mathcal{D}(G))$ martingale problem can be shown as well.

3 Applications to chemical reaction networks (CRNs)

    Our goal is to apply Theorem 2.3 to Chemical Reaction Networks. While describing the framework in Section 3.1, we will introduce a system which leads to Hill dynamics with coefficient 2 as a first example. After stating the main assumptions (above all, the fast subsystem is first order) in Section 3.2, we describe in Section 3.3 how to derive LLNs and in Section 3.4 the corresponding CLTs in this setting. In Section 3.5, we give some general remarks how to show that the assumptions (e.g. the compact containment condition) from Theorem 2.3 hold.

3.1 CRNs and their rescaling

    Chemical reaction networks can be described as a pair $(\mathcal{S}, \mathcal{R})$, consisting of the (finite) set of species $\mathcal{S}$ and the (finite) set of reactions $\mathcal{R}$, where we write

$$\sum_{S \in \mathcal{S}} \nu_{RS} S \xrightarrow{\widetilde{\kappa}_R} \sum_{S \in \mathcal{S}} \nu'_{RS} S, \qquad R \in \mathcal{R}$$
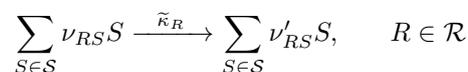
for some $\nu, \nu' \in \mathbb{N}_0^{\mathcal{R} \times \mathcal{S}}$. Using this notation, the Markov dynamics $X = (X_S)_{S \in \mathcal{S}}$, $X_S = (X_S(t))_{t \geq 0}$ can be described through the jump rates: Using the vector of species consumed and species produced of the reactions,

$$\nu_{R\cdot} := (\nu_{RS})_{S \in \mathcal{S}}, \qquad \nu'_{R\cdot} := (\nu'_{RS})_{S \in \mathcal{S}}, \qquad R \in \mathcal{R}$$

and the falling factorial

$$n_{[k]} := n \cdot (n-1) \cdots (n-k+1), \qquad k, n \in \mathbb{N}_0,$$

starting in $x(0)$, the process jumps

$$\text{from } X = x \text{ to } x + (\nu'_{R\cdot} - \nu_{R\cdot}) \text{ at rate } \lambda_R(x)$$

for some λ_R. In the sequel, we will be using mass action kinetics, i.e. we assume that

$$\lambda_R(x) := \widetilde{\kappa}_R \prod_{S \in \mathcal{S}} (x_S)_{[\nu_{RS}]}$$

for some $\widetilde{\kappa}_R \in \mathbb{R}_+$. However, different rate functions can be used as well.

In order to obtain limit results, we have to introduce a scaling parameter (denoted N in the sequel) and rescaled time of the dynamics X. We are not dealing with the completely general situation (see e.g. [10]), but with a set of species which is either in *low* or *high* abundance. Here, the former are not rescaled, but the latter are rescaled by a factor of N. This means that ($\uplus$ meaning a disjoint union)

$$\mathcal{S} = \mathcal{S}_\bullet \uplus \mathcal{S}_\circ, \tag{3.1}$$

where $S \in \mathcal{S}_\bullet$ are in high (i.e. $\mathcal{O}(N)$) and $S \in \mathcal{S}_\circ$ are in low (i.e. $\mathcal{O}(1)$) abundance. We set $V^N = (V_S^N)_{S \in \mathcal{S}_\bullet}$ and $Z^N = (Z_S^N)_{S \in \mathcal{S}_\circ}$ with

$$V_S^N = N^{-1} X_S^N, \quad S \in \mathcal{S}_\bullet, \qquad Z_S^N = X_S^N, \quad S \in \mathcal{S}_\circ. \tag{3.2}$$

For the reactions, we assume that there are $\kappa_R \in \mathbb{R}_+$ and $\beta_R \in \mathbb{R}, R \in \mathcal{R}$ such that

$$\widetilde{\kappa}_R = \kappa_R N^{\beta_R}.$$

Abbreviating $\nu'_{R\mathcal{S}_\bullet} := (\nu_{RS})_{S \in \mathcal{S}_\bullet}$, and equivalently for $\nu'_{R\mathcal{S}_\circ}, \nu_{R\mathcal{S}_\bullet}, \nu_{R\mathcal{S}_\circ}$,

$$\zeta_{RS} := \nu'_{RS} - \nu_{RS},$$

as well as $\zeta_{R\mathcal{S}_\bullet}, \zeta_{R\mathcal{S}_\circ}$, the process (V^N, Z^N) then jumps

$$\text{from } (V^N, Z^N) = (v, z) \text{ to } (v + N^{-1}\zeta_{R\mathcal{S}_\bullet}, z + \zeta_{R\mathcal{S}_\circ}),$$
$$\text{at rate } \lambda_R^N(v, z) := N^{\beta_R + \sum_{S \in \mathcal{S}_\bullet} \nu_{RS}} \kappa_R \cdot z_{[\nu_{R\mathcal{S}_\circ}]} \cdot (v_{\mathcal{S}_\bullet})_{[\nu_{R\mathcal{S}_\bullet}], N^{-1}} \tag{3.3}$$

where we use

$$n_{[k],\varepsilon} = n \cdot (n - \varepsilon) \cdots (n - (k-1)\varepsilon), \qquad n_{[k]} := n_{[k],1},$$
$$(n_{\mathcal{S}_\bullet})_{[k_{\mathcal{S}_\bullet}],\varepsilon} := \prod_{S \in \mathcal{S}_\bullet} (n_S)_{[k_S],\varepsilon}, \quad n_{\mathcal{S}_\bullet} \in \mathbb{R}^{\mathcal{S}_\bullet}, \qquad (n_{\mathcal{S}_\circ})_{[k_{\mathcal{S}_\circ}]} := \prod_{S \in \mathcal{S}_\circ} (n_S)_{[k_S]}, \quad n_{\mathcal{S}_\circ} \in \mathbb{R}^{\mathcal{S}_\circ}$$

and note that (for appropriate δ^N)

$$v_{[k],N^{-1}} = v(v - N^{-1}) \cdots (v - (k-1)N^{-1}) = v^k - N^{-1} \binom{k}{2} v^{k-1} + N^{-2} \delta^N(v, k).$$

In other words, writing[1]

$$\nabla_\bullet f := (\partial f / \partial v_S)_{S \in \mathcal{S}_\bullet}^\top, \qquad \kappa_R' := N^{\beta_R - 1 + \sum_{S \in \mathcal{S}_\bullet} \nu_{RS}} \kappa_R \qquad (3.4)$$

for all $f \in \mathcal{C}_c^\infty(\mathbb{R}^{\mathcal{S}_\bullet \times \mathcal{S}_\circ})$, the process

$$\left(f(V_t^N, Z_t^N) - \int_0^t G^N f(V_s^N, Z_s^N) ds \right)_{t \geq 0}$$

is a martingale, where

$$
\begin{aligned}
G^N f(v, z) &= \sum_{R \in \mathcal{R}} N \kappa_R' z_{[\nu_{R\mathcal{S}_\circ}]} v_{[\nu_{R\mathcal{S}_\bullet}], N^{-1}} (f(v + N^{-1}\zeta_{R\mathcal{S}_\bullet}, z + \zeta_{R\mathcal{S}_\circ}) - f(v, z)) \\
&= (G_0^N f + G_2^N f)(v, z), \\
G_0^N f(v, z) &= \sum_{R \in \mathcal{R}} \kappa_R' z_{[\nu_{R\mathcal{S}_\circ}]} \big(v^{\nu_{R\mathcal{S}_\bullet}} \nabla_\bullet f(v, z + \zeta_{R\mathcal{S}_\circ}) \cdot \zeta_{R\mathcal{S}_\bullet} \\
&\qquad\qquad - \binom{\nu_{R\mathcal{S}_\bullet}}{2} v^{\nu_{R\mathcal{S}_\bullet} - 1}(f(v, z + \zeta_{R\mathcal{S}_\circ}) - f(v, z)) \big) + \varepsilon_f^N(v, z), \quad (3.5) \\
G_2^N f(v, z) &= \sum_{R \in \mathcal{R}} \kappa_R' z_{[\nu_{R\mathcal{S}_\circ}]} v^{\nu_{R\mathcal{S}_\bullet}} (f(v, z + \zeta_{R\mathcal{S}_\circ}) - f(v, z)), \\
|\varepsilon_f^N(v, z)| &\leq \sum_{R \in \mathcal{R}} N^{-1} \kappa_R' z_{[\nu_{R\mathcal{S}_\circ}]} \big(\tfrac{1}{2} v^{\nu_{R\mathcal{S}_\bullet}} ||\nabla_\bullet^2 f||_\infty + \binom{\nu_{R\mathcal{S}_\bullet}}{2} v^{\nu_{R\mathcal{S}_\bullet} - 1} ||\nabla_\bullet f||_\infty \\
&\qquad\qquad + \delta^N(v, \nu_{R\mathcal{S}_\bullet}) ||f||_\infty \big).
\end{aligned}
$$

Here $\nabla_\bullet^2 f$ is the Hessian of f, $\binom{\nu_{R\mathcal{S}_\bullet}}{2} := \prod_{S \in \mathcal{S}_\bullet} \binom{\nu_{RS}}{2}$ and $v^{\nu_{R\mathcal{S}_\bullet}} := \prod_{S \in \mathcal{S}_\bullet} v^{\nu_{RS}}$.

Example 3.1 (The simplified Hill dynamics 1). We illustrate our theory with the following example:

$$E_1 + 2S_1 \xrightleftharpoons[N^\beta \kappa_2]{N^{-1}\kappa_1} E_3 \qquad E_3 \xrightarrow{N^\beta \kappa_3} S_2 + S_1 + E_1 \qquad (3.6)$$
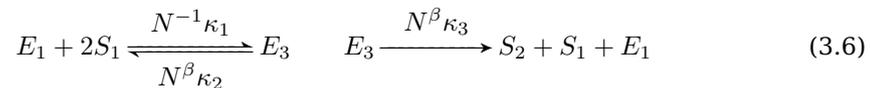
with $\beta \geq 1$. This CRN arises e.g. if a macromolecule (E_1) has two binding sites for a substrate (S_1), which quickly binds (simultaneously at both binding sites) and forms a complex (E_3). If the ligands are released, one of them is turned into S_2. We assume that the macromolecule (i.e. E_1, E_3) is in low abundance ($O(1)$), while substrate and product, S_1, S_2, are in high abundance ($O(N)$), so $\mathcal{S}_\bullet = \{S_1, S_2\}$ and $\mathcal{S}_\circ = \{E_1, E_3\}$. The dynamics are similar to Michaelis-Menten kinetics below (see Section 4.1), with the differnce that two substrate molecules are needed in order to form the product. A similar system was introduced by A. V. Hill [6] for binding of oxygen to haemoglobin in order to understand data from osmotic pressure experiments. We will refine this example in Section 4.2, where the first and second reaction are broken into two separate reactions for binding of the two substrate molecules. (The intermediate step is the macromolecule bound by a single substrate, denoted E_2, which is missing in (3.6).) For this simplified dynamics, we derive the LLN and CLT below in Examples 3.3–3.7.

We note that some intuition already gives the correct limit result in the case $\beta > 1$. Here, when an E_3-molecule is generated from $E_1 + 2S_1$, it only has two options, both of which occur at a high rate. It either decays to the molecules which generated the molecule (with no net effect on all numbers of species counts), or it becomes $S_2 + S_1 + E_1$.

---

[1] We make the convention that x denotes a column vector, and $x^\top$ a row vector.

Since the latter two options lead to competing exponentials in the dynamics, the system should behave similar to

$$E_1 + 2S_1 \xrightarrow{\ N^{-1}\kappa_1\kappa_3/(\kappa_3+\kappa_2)\ } S_2 + S_1 + E_1\,. \qquad (3.7)$$

(For the dynamics of this equation, E_1 only enters through its initial state.) As we will see below, this intuition is correct for both, the LLN (Example 3.5) and the CLT (Example 3.6). For $\beta = 1$, however, this intuition does not work since the rate of production of E_3 depends on the number of available E_1, which is random.

We let NV_1^N, NV_2^N and Z_1, Z_3 be the processes of the particle numbers of S_1, S_2 and E_1, E_3. Also $M := Z_1 + Z_3$ is a constant as well as $V_1 + V_2 + N^{-1}(Z_1 + 3Z_3)$, so we are going to describe the Markov process $(Z^N = Z_1^N, V^N = V_1^N)$. Since V^N is evolving slower than Z^N, we call V^N the slow and Z^N the fast variable. If the slow variable is assumed constant, the fast-subsystem (consisting of E_1, E_3) is first order; see Assumption 3.2 below. However, the slow sub-system is second order. For the generator of (V^N, Z^N) we have for $f \in \mathcal{C}_c^\infty(\mathbb{R} \times \mathbb{R})$, writing f' for the derivative according to the first variable, and (recall from (3.4)) $\kappa_2' = N^{\beta-1}\kappa_2, \kappa_3' = N^{\beta-1}\kappa_3$,

$$
\begin{aligned}
G^N f(v,z) &= N\kappa_1 v(v - N^{-1})z(f(v - 2N^{-1}, z - 1) - f(v,z)) \\
&\quad + N\kappa_2'(M - z)(f(v + 2N^{-1}, z + 1) - f(v,z)) \\
&\quad + N\kappa_3'(M - z)(f(v + N^{-1}, z + 1) - f(v,z)) \\
&= G_0^N f(v,z) + N G_2^N f(v,z), \\
G_0^N f(v,z) &= -2\kappa_1 v^2 z f'(v, z - 1) + 2\kappa_2'(M - z)f'(v, z + 1) \\
&\quad + \kappa_3(M - z)f'(v, z + 1) - \kappa_1 vz(f(v, z - 1) - f(v,z)) + \varepsilon_f^N(v,z), \\
G_2^N f(v,z) &= \kappa_1 v^2 z(f(v, z - 1) - f(v,z)) + \kappa_2'(M - z)(f(v, z + 1) - f(v,z)) \\
&\quad + \kappa_3'(M - z)(f(v, z + 1) - f(v,z)), \\
|\varepsilon_f^N(v,z)| &\leq N^{-1}\big(\big(\kappa_1 v^2 z + \kappa_2'(M - z) + \tfrac{1}{2}\kappa_3'(M - z)\big)\|f''\|_\infty + 2\kappa_1 vz\|f'\|_\infty\big).
\end{aligned}
$$

3.2 Main assumptions

We continue to work using the assumptions from the last section. In particular, species are either fast and in low abundance ($\mathcal{S}_\circ$), or slow and in high abundance ($\mathcal{S}_\bullet$); see (3.1), (3.2). In addition, our main assumption is that the fast sub-system is first order. This means that if all $S \in \mathcal{S}_\bullet$ are assumed to be constant, the remaining CRN on $\mathcal{S}_\circ$ is first order, i.e. at most a single molecule is consumed and produced in each reaction; see Assumption 3.2.3 below. All our examples are of this kind. For $S \in \mathcal{S}$, we set

$$\Gamma_S^+ := \{R \in \mathcal{R} : \nu_{RS}' > \nu_{RS}\}, \qquad \Gamma_S^- := \{R \in \mathcal{R} : \nu_{RS} > \nu_{RS}'\}$$

as the sets of reactions effectively producing and consuming species S, respectively. For a leaner notation, we set

$$\widetilde{\beta}_R := \beta_R + \sum_{S \in \mathcal{S}_\bullet} \nu_{RS}.$$

Assumption 3.2. *1. The reaction rates λ_R^N are of the form given in* (3.3).

2. The fast sub-system is first order, i.e.

$$\sum_{S \in \mathcal{S}_\circ} \nu_{RS} \leq 1, \qquad \sum_{S \in \mathcal{S}_\circ} \nu_{RS}' \leq 1, \qquad R \in \mathcal{R}.$$

We write

$$\mathcal{R} = \mathcal{R}_0 \uplus \mathcal{R}_1 = \mathcal{R}_0' \uplus \mathcal{R}_1',$$

where

$$\mathcal{R}_0 := \left\{ R \in \mathcal{R} : \sum_{S \in \mathcal{S}_\circ} \nu_{RS} = 0 \right\}, \qquad \mathcal{R}_1 := \left\{ R \in \mathcal{R} : \sum_{S \in \mathcal{S}_\circ} \nu_{RS} = 1 \right\},$$

$$\mathcal{R}_0' := \left\{ R \in \mathcal{R} : \sum_{S \in \mathcal{S}_\circ} \nu_{RS}' = 0 \right\}, \qquad \mathcal{R}_1' := \left\{ R \in \mathcal{R} : \sum_{S \in \mathcal{S}_\circ} \nu_{RS}' = 1 \right\}.$$

For $R \in \mathcal{R}_1$ and $S \in \mathcal{S}_\circ$, we set $S_R = S$ if $\nu_{RS} = 1$, i.e. R consumes S_R as single fast species. For $R \in \mathcal{R}_1'$ and $S \in \mathcal{S}_\circ$, we set $S_R' = S$ if $\nu_{RS}' = 1$, i.e. R produces S_R' as single fast species.

3. *For $S \in \mathcal{S}_\circ$, we require that*

$$\varphi_S := \max\left\{ \widetilde{\beta}_R : R \in \Gamma_S^- \right\} \geq 1, \tag{3.8}$$

and for

$$\widehat{\beta}_R := \begin{cases} \widetilde{\beta}_R, & R \in \mathcal{R}_0, \\ \widetilde{\beta}_R + 1 - \varphi_{S_R}, & R \in \mathcal{R}_1. \end{cases} \tag{3.9}$$

that

$$\psi_S := \max\{ \widehat{\beta}_R : R \in \Gamma_S^+ \} = 1, \tag{3.10}$$

4. *For $S \in \mathcal{S}_\bullet$, the* time-scale constraint *applies, i.e.*

$$\max\left\{ \widehat{\beta}_R : R \in \Gamma_S^+ \cup \Gamma_S^- \right\} = 1. \tag{3.11}$$

Note that species $S \in \mathcal{S}_\circ$ is consumed at rate $O(N^{\varphi_S})$ by (3.8), and is produced at rate $O(N)$ by (3.10). Hence, the occupation measure of $S \in \mathcal{S}_\circ$ is $O(N^{1-\varphi_S})$, which is the reason why we introduce $\widehat{\beta}_R$ in (3.9). In other words, if $R \in \mathcal{R}_1$, it consumes species S_R, which is present only a fraction $O(N^{1-\varphi_{S_R}})$ of the time. This implies that the process counting how often reaction R occured increases by one at rate $O(N^{\widetilde{\beta}_R + 1 - \varphi_{S_R}}) = O(N^{\widehat{\beta}_R})$.

Example 3.3 (The simplified Hill dynamics 2)**.** Here, the fast sub-system, i.e. the sub-system only involving E_1, E_3, is first order, i.e. Assumption 3.2.2 applies. In (3.6), we find $\widetilde{\beta}_1 = 1, \widetilde{\beta}_2 = \widetilde{\beta}_3 = \beta$. Here, we have that $\mathcal{R} = \mathcal{R}_1 = \mathcal{R}_1'$, since all reactions consume and produce some $S \in \mathcal{S}_\circ = \{E_1, E_3\}$. We find $\varphi_{E_1} = 1, \varphi_{E_3} = \beta$, and therefore $\widehat{\beta}_2 = \widehat{\beta}_3 = \beta + 1 - \beta = 1$, to the effect that $\psi_{E_1} = \psi_{E_3} = 1$, i.e. (3.10) holds. For $S = S_1, S_2$, we find that (3.11) holds for the same reason.

Remark 3.4 (Some connections to previous work)**.** In the setting above, there are two aspects which lead to more general CRNs than in previously published papers:

1. In previous work of Kurtz and co-authors on multi-scale CRNs, fast species need to be produced and consumed at rates of the same order of magnitude. This can be seen from both, the balance condition (3.2) of [10] and the prerequisites of the averaging condition (Condition 2.4) of [11].

   In [10], it is required that the production rate of every species is on the same order as its consumption rate (see their (3.2)), or its abundance is at least comparable to the fastest reaction it is involved in (see their (3.3)). For the fast species from above, if $\varphi_S > 1$, neither is the case. Condition 2.4 of [11] requires that there is a limiting operator L_2 (not depending on N) which describes the evolution of fast species. If these are consumed faster than they are produced, L_2 would depend on N, so the only possibility is that $\varphi_S = 1$ (i.e. $\widehat{\beta}_R = \widetilde{\beta}_R$). So, the analysis from above is more general since $\varphi_S > 1$ is allowed for fast species S.

2. Recently, [7] extended the approach of intermediate species from [3] to non-interacting species and the existence of a fast chain of reactions. As above, a set of species is non-interacting if its reaction network is at most first order if all other species are held constant. Assuming that the set of fast species and non-interacting species are equal, another restriction is imposed. Precisely, the existence of a fast chain of reaction requires that every fast species is created only via slow species, which does not allow for conserved quantities within the fast species. As we will discuss below, this is possible in our setting; e.g. as discussed in Example 3.1, $Z_1 + Z_3$ is conserved.

3.3 The law of large numbers (LLN)

We wish to show that $V^N \xRightarrow{N\to\infty} V$ for some Markov process V. The goal of this section is to compute the possible generator G of V. Let us consider some smooth f only depending on v. Note that Gf may only depend on v (and not on z). With the above Assumption 3.2, the terms $G_0^N f$ and $G_2^N f$ in (3.5) can be split in sums over $R \in \mathcal{R}_0$ and $R \in \mathcal{R}_1$. The generator terms corresponding to $R \in \mathcal{R}_0$ do not depend on z, but the terms corresponding to $R \in \mathcal{R}_1$ do. Therefore, in order to obtain Gf, we are looking for g_N such that $G_0^N f + G_2^N g_N$ only depends on v, and quantities which are constant for the evolution. We make the ansatz

$$g_N(v,z) = \nabla_\bullet f(v) \cdot a_N(v) \cdot z \tag{3.12}$$

for some $a_N = a_{\mathcal{S}_\bullet \mathcal{S}_\circ} = (a_{SS'})_{S \in \mathcal{S}_\bullet, S' \in \mathcal{S}_\circ}$ with $a_{SS'} \in \mathcal{C}^\infty(\mathbb{R}_+^{\mathcal{S}_\bullet})$, $S \in \mathcal{S}_\bullet, S' \in \mathcal{S}_\circ$. We obtain from (3.5)

$$
\begin{aligned}
(G_0^N f + G_2^N g_N)(v,z) = & \sum_{R \in \mathcal{R}_0} \kappa_R' v_{\mathcal{S}_\bullet}^{\nu_{RS_\bullet}} \nabla_\bullet f(v) \cdot (\zeta_{R\mathcal{S}_\bullet} + a_{\mathcal{S}_\bullet \mathcal{S}_\circ}(v) \cdot \zeta_{R\mathcal{S}_\circ}) \\
& + \sum_{R \in \mathcal{R}_1} \kappa_R' z_{S_R} v_{\mathcal{S}_\bullet}^{\nu_{RS_\bullet}} \nabla_\bullet f(v) \cdot (\zeta_{R\mathcal{S}_\bullet} + a_{\mathcal{S}_\bullet \mathcal{S}_\circ}(v) \cdot \zeta_{R\mathcal{S}_\circ}) + \varepsilon_f^N(v,z)
\end{aligned}
\tag{3.13}
$$

and we note that it may be possible to choose a_N such that all terms proportional to all z_{S_R}'s in the sum over $\mathcal{R}_1$ on the right hand side vanish. Another option is that there are conserved linear combinations of fast species, i.e. some set $\mathcal{C}$ and $\xi_\mathcal{C} = (\xi_C)_{C \in \mathcal{C}}$ with $\xi_C \in \mathbb{Z}^{\mathcal{S}_\circ}$ all linearly independent, such that $\xi_C \cdot \zeta_{R\mathcal{S}_\circ} = 0$ for all $R \in \mathcal{R}$. In this case, $z \cdot \xi_C$ is a constant under the evolution, and there is a chance that we pick $a_{\mathcal{S}_\circ \mathcal{S}_\bullet}$ such that the right hand side only depends on z via $z \cdot \xi_\mathcal{C} := (z \cdot \xi_C)_{C \in \mathcal{C}}$.

In the sequel, we will assume that $g_N(v,z)$ is chosen such that there is some smooth $\ell_\bullet$, taking values in $\mathbb{R}^{\mathbb{S}_\bullet}$ with

$$
\begin{aligned}
(G_0^N f + G_2^N g)(v,z) &= H^N f(v) + \varepsilon_f^N(v,z), \\
H^N f(v) &= \sum_{R \in \mathcal{R}_0} \kappa_R' v_{\mathcal{S}_\bullet}^{\nu_{RS_\bullet}} \nabla_\bullet f(v) \cdot (\zeta_{R\mathcal{S}_\bullet} + a_{\mathcal{S}_\bullet \mathcal{S}_\circ}(v) \cdot \zeta_{R\mathcal{S}_\circ}) \\
&\quad + \nabla_\bullet f(v) \cdot \ell_\bullet(\kappa_\mathcal{R}', z \cdot \xi_\mathcal{C}, v).
\end{aligned}
\tag{3.14}
$$

Note that $z \cdot \xi_C$ is constant, so we can use $\ell_\bullet$ with $Z^N(0) \cdot \xi_C$ as a global constant. Moreover, since κ' still depends on N, the right hand side depends on N, but may have a proper limit as $N \to \infty$, which we will denote $Gf(v)$. Then, if $(V^N)_{N=1,2,\dots}$ satisfies the compact containment condition and (2.4)–(2.6) hold (to be discussed in Section 3.5), a weak limit of V^N solves the $(G, \mathcal{D}(G))$ martingale problem.

Example 3.5 (The simplified Hill dynamics 3)**.** We will now make the generator calculations leading to the LLN for (3.6). Note that $M = Z_1^N + Z_2^N$ does not change over time,

i.e. the limiting generator may still depend on M. Provided the required assumptions (compact containment of $(V^N)_{N=1,2,\ldots}$ and (2.4)–(2.6) hold), we will show the following:

Let $v \in \mathbb{R}_+$,

$$p^N(v) := -\frac{M\kappa_1\kappa_3'v^2}{\kappa_2' + \kappa_3' + \kappa_1 v^2} \xrightarrow{N\to\infty} p(v) := \begin{cases} -\dfrac{M\kappa_1\kappa_3 v^2}{\kappa_2 + \kappa_3 + \kappa_1 v^2}, & \beta = 1, \\ -\dfrac{M\kappa_1\kappa_3 v^2}{\kappa_2 + \kappa_3}, & \beta > 1 \end{cases} \tag{3.15}$$

and V the solution of the ODE

$$dV = p(V)dt, \qquad V_0 = v. \tag{3.16}$$

Then, letting V^N as above and if $V_0^N \xRightarrow{N\to\infty} v$, then $V^N \xRightarrow{N\to\infty} V$.

In the case $\beta = 1$, this result can be proved using the technique of stochastic averaging: Fixing the amount of slow species, we see that the fast network reduces to $E_1 \overset{N\kappa_1 v^2}{\underset{N(\kappa_2+\kappa_3)}{\rightleftharpoons}} E_3$. In its equilibrium, E_3 is binomially distributed with M and $\frac{\kappa_1 v^2}{\kappa_1 v^2 + \kappa_2 + \kappa_3}$.
From this, the LLN can be derived, e.g. by applying the main result from [14]. However, it is unclear if the same technique gives a result for $\beta > 1$. Here, we follow the route from above, which will in addition pay dividends when deriving the CLT in the next subsection.

For the proof, take some smooth f depending only on v and find g_N such that $G_0^N f + G_2^N g - \varepsilon_N^f$ only depends on v. Following (3.12), we take $g_N(v,z) = z a_N(v) f'(v)$. We find (abbreviating $a_N := a_N(v)$)

$$(G_0^N f + G_2^N g_N)(v,z) - \varepsilon_f^N(v,z)$$
$$= M(\kappa_2'(2 + a_N) + \kappa_3'(1 + a_N))f'(v)$$
$$\qquad + z\big(-\kappa_1 v^2(2 + a_N) - \kappa_2'(2 + a_N) - \kappa_3'(1 + a_N)\big)f'(v).$$

Setting

$$a_N = -\frac{2\kappa_1 v^2 + 2\kappa_2' + \kappa_3'}{\kappa_2' + \kappa_3' + \kappa_1 v^2} = -1 - \frac{\kappa_1 v^2 + \kappa_2'}{\kappa_2' + \kappa_3' + \kappa_1 v^2} = -2 + \frac{\kappa_3'}{\kappa_2' + \kappa_3' + \kappa_1 v^2}, \tag{3.17}$$

this leads to

$$H^N f(v) := (G_0^N f + G_2^N g_N)(v,z) - \varepsilon_f^N(v,z) = -M\frac{\kappa_1\kappa_3' v^2}{\kappa_2' + \kappa_3' + \kappa_1 v^2}f'(v) = p^N(v)f'(v).$$

Note that $\mathcal{R}_0 = \emptyset$ in (3.6) since all reactions use a molecule from a fast species. This leads to $\ell_\bullet = p^N$. Taking $N \to \infty$ then shows the result.

3.4 The functional central limit theorem (CLT)

Next, let us study fluctuations, i.e. we are going to study the limit of $U^N := N^{1/2}(V^N - W^N)$, where W^N has generator H^N (recall from (3.14)). For this, we study the generator for the Markov process (U^N, Z^N, W^N), where (U^N, W^N) are slow and Z^N is fast. When doing so, we have to exchange $V^N = W^N + N^{-1/2}U^N$ in (3.5). In particular, writing $v = V^N, u = U^N, w = W^N$,

$$v_{[k],N^{-1}} = (w + N^{-1/2}u)(w + N^{-1/2}u - N^{-1})\cdots(w + N^{-1/2}u - (k-1)N^{-1})$$
$$= w^k + N^{-1/2}kw^{k-1}u + N^{-1}\left(\binom{k}{2}w^{k-2}u^2 - kw^{k-1}\right) + N^{-3/2}\delta^N(u,w,k)$$

where $\delta^N(u, w, k)$ is defined appropriately. Now, assume that f only depends on (u, z). Looking at (3.5), we can write the generator (denoting by $\nabla_\bullet$ the derivative with respect to u)

$$
L^N f(u, z, w) = \sum_{R \in \mathcal{R}} N \kappa'_R z_{[\nu_{R\mathcal{S}_\circ}]} (w + N^{-1/2} u)_{[\nu_{R\mathcal{S}_\bullet}], N^{-1}} \cdot
$$
$$
\cdot (f(u + N^{-1/2} \zeta_{R\mathcal{S}_\bullet}, z + \zeta_{R\mathcal{S}_\circ}) - f(u, z)) - N^{1/2} H^N f(u, z, w)
$$
$$
= (L_0^N + N^{1/2} L_1^N + N L_2^N) f(u, z, w),
$$
$$
L_0^N f(u, z, w) = \sum_{R \in \mathcal{R}} \kappa'_R z_{[\nu_{R\mathcal{S}_\circ}]} \big((\nu_{R\mathcal{S}_\bullet} w^{\nu_{R\mathcal{S}_\bullet} - 1} u) \nabla_\bullet f(u, z + \zeta_{R\mathcal{S}_\circ}) \cdot \zeta_{R\mathcal{S}_\bullet}
$$
$$
+ \tfrac{1}{2} w^{\nu_{R\mathcal{S}_\bullet}} \zeta_{R\mathcal{S}_\bullet}^\top \cdot \nabla_\bullet^2 f(u, z + \zeta_{R\mathcal{S}_\circ}) \cdot \zeta_{R\mathcal{S}_\bullet} \big)
$$
$$
+ \left(\binom{\nu_{R\mathcal{S}_\bullet}}{2} w^{\nu_{R\mathcal{S}_\bullet} - 2} u^2 - \nu_{R\mathcal{S}_\bullet} w^{\nu_{R\mathcal{S}_\bullet} - 1} \right) (f(u, z + \zeta_{R\mathcal{S}_\circ}) - f(u, z))
$$
$$
+ \varepsilon_f^N(u, z, w)
$$

$$
L_1^N f(u, z, w) = \sum_{R \in \mathcal{R}} \kappa'_R z_{[\nu_{R\mathcal{S}_\circ}]} \Big(w^{\nu_{R\mathcal{S}_\bullet}} \nabla_\bullet f(u, z + \zeta_{R\mathcal{S}_\circ}) \cdot \zeta_{R\mathcal{S}_\bullet}
$$
$$
+ (\nu_{R\mathcal{S}_\bullet} w^{\nu_{R\mathcal{S}_\bullet} - 1} u)(f(u, z + \zeta_{R\mathcal{S}_\circ}) - f(u, z)) \Big) - H^N f(u, z, w)
$$
$$
L_2^N f(u, z, w) = \sum_{R \in \mathcal{R}} \kappa'_R z_{[\nu_{R\mathcal{S}_\circ}]} w^{\nu_{R\mathcal{S}_\bullet}} (f(u, z + \zeta_{R\mathcal{S}_\circ}) - f(u, z)),
$$
$$
H^N f(u, z, w) = \sum_{R \in \mathcal{R}_0} \kappa'_R w_{\mathcal{S}_\bullet}^{\nu_{R\mathcal{S}_\bullet}} \nabla_\bullet f(u, z) \cdot (\zeta_{R\mathcal{S}_\bullet} + a_{\mathcal{S}_\bullet \mathcal{S}_\circ}(w) \cdot \zeta_{R\mathcal{S}_\circ})
$$
$$
+ \nabla_\bullet f(u, z) \cdot \ell_\bullet(\kappa'_{\mathcal{R}}, z \cdot \xi_{\mathcal{C}}, w),
$$
$$
|\varepsilon_f^N(u, z, w)| \leq \sum_{R \in \mathcal{R}} N^{-1/2} \kappa'_R z_{[\nu_{R\mathcal{S}_\circ}]} \big(\tfrac{1}{2} \nu_{R\mathcal{S}_\bullet} w^{\nu_{R\mathcal{S}} - 1} u \|\nabla^2 f\|_\infty
$$
$$
+ \binom{\nu_{R\mathcal{S}_\bullet}}{2} w^{\nu_{R\mathcal{S}_\bullet} - 2} (u^2 + w) \|\nabla_\bullet f\|_\infty \tfrac{1}{6} w^{\nu_{R\mathcal{S}_\bullet}} \zeta_{R\mathcal{S}_\bullet}^\top \cdot \zeta_{R\mathcal{S}_\bullet} \|\nabla_\bullet^3 f\|_\infty
$$
$$
+ \delta^N(u, w, \nu_{R\mathcal{S}_\bullet}) \|f\|_\infty \big). \tag{3.18}
$$

Now, let f depend only on u (hence $L_2^N f = 0$) and

$$
g_N(u, z) = \nabla_\bullet f(u) \cdot a_N(w) \cdot z
$$

with a_N as in (3.12) such that (3.14) holds. Then, looking at (3.5) and (3.18), we see by the similarity between G_0^N and L_1^N (noting the additional term $-H^N$ in L_1^N), and the similarity between G_2^N and L_2^N that

$$
L_1^N f + L_2^N g_N = 0.
$$

Then, we are looking for h_N such that $L_0^N f + L_1^N g_N + L_2^N h_N$ only depends on (u, w), and the linear combinations $z \cdot \xi_{\mathcal{C}}$ which are constant in time. We make the ansatz

$$
h_N(u, z, w) = \nabla_\bullet f(u) \cdot b_N(u, w) \cdot z + \nabla_\bullet^2 f(u) \cdot (c_N(u, w) \cdot z + z^\top \cdot d_N(u, w) \cdot z) \tag{3.19}
$$

for some functions

$$
b_N = (b_{SS'})_{S \in \mathcal{S}_\bullet, S' \in \mathcal{S}_\circ}, c_N = (c_{S,S',S''})_{S,S' \in \mathcal{S}_\bullet, S'' \in \mathcal{S}_\circ}, d_N = (d_{SS',S''S'''})_{S,S' \in \mathcal{S}_\bullet, S'', S''' \in \mathcal{S}_\circ},
$$

which depend on N, w, u. Then, since the fast subsystem is first order, we can write

$$
\begin{aligned}
&(L_0^N f + L_1^N g_N + L_2^N h_N)(u, z, w) \\
&= \sum_{R \in \mathcal{R}} \kappa_R' z_{[\nu_{RS_\circ}]} \Big((\nu_{RS_\bullet} w^{\nu_{RS_\bullet} - 1} u) \nabla_\bullet f(u) \cdot (\zeta_{RS_\bullet} + a_N(w) \cdot \zeta_{RS_\circ} \\
&\quad + w^{\nu_{RS_\bullet}} \nabla_\bullet f(u) \cdot b_N(u, w) \cdot \zeta_{RS_\circ}) \\
&\quad + w^{\nu_{RS_\bullet}} \zeta_{RS_\bullet}^\top \cdot \nabla_\bullet^2 f(u) \cdot (\tfrac{1}{2} \zeta_{RS_\bullet} + a_N(w) \cdot (z + \zeta_{RS_\circ})) \\
&\quad + w^{\nu_{RS_\bullet}} \Big(\nabla_\bullet^2 f(u) \cdot (c_N(u, w) \cdot \zeta_{RS_\circ} \\
&\quad + (\zeta_{RS}^\top \cdot d_N(u, w) \cdot (z + \zeta_{RS_\circ}) + z^\top \cdot d_N(u, w) \cdot \zeta_{RS}^\top) \Big) \Big) - H^N g_N(u, z, w) + \varepsilon_f^N(u, z, w).
\end{aligned}
$$
(3.20)

Then, splitting the sum in (3.20) into $R \in \mathcal{R}_0$ and $R \in \mathcal{R}_1$, we see that for $R \in \mathcal{R}_1$ there are terms proportional to $z_{S_R} \nabla_\bullet f(u)$, and there is a chance to choose b_N such that these terms vanish or depend only on $(z \cdot \xi_C)_{C \in \mathcal{C}}$. In a second step, we choose c_N and d_N such that the remaining terms, all of which are proportional to $\nabla_\bullet^2 f$, vanish or depend only on $(z \cdot \xi_C)_{C \in \mathcal{C}}$. Assuming that g_N, h_N have proper limits as $N \to \infty$, this leaves us with

$$
Lf(u, w) := \lim_{N \to \infty} (L_0^N f + L_1^N g_N + L_2^N h_N)(u, z, w) - \varepsilon_f^N(u, z, w),
$$

where L is the generator of the limiting process. Provided the compact containment condition of $(U^N)_{N=1,2,\ldots}$ and (2.4)–(2.6) hold, this shows convergence with Theorem 2.3.

Example 3.6 (The simplified Hill dynamics 4). Let $u \in \mathbb{R}$, p^N and p as in (3.15), V as in (3.16), and W^N the solution of the ODE

$$
dW^N = p^N(W^N) dt, \qquad W_0 = v.
$$

(So, $V = W^N$ for $\beta = 1$.) Moreover, let U be the solution of the SDE

$$
\begin{aligned}
dU &= \mu(U, V) dt + \sigma(V) dB, \\
\mu(U, V) &= \begin{cases} -2M \dfrac{\kappa_1(\kappa_2 + \kappa_3)\kappa_3 UV}{(\kappa_2 + \kappa_3 + \kappa_1 V^2)^2}, & \beta = 1, \\ p'(V) U, & \beta > 1, \end{cases} \\
\sigma^2(V) &= \begin{cases} \dfrac{(\kappa_1^2 V^4 + 2\kappa_1 \kappa_2 V^2 + (\kappa_2 + \kappa_3)^2) M \kappa_1 \kappa_3 V^2}{(\kappa_2 + \kappa_3 + \kappa_1 V^2)^3}, & \beta = 1, \\ |p(V)|, & \beta > 1 \end{cases}
\end{aligned}
$$
(3.21)

for some Brownian motion B. Then, letting V^N as in Example 3.3, and $U^N = N^{1/2}(V^N - W^N)$. If $U_0^N \xRightarrow{N \to \infty} u$, then $U^N \xRightarrow{N \to \infty} U$.

Consider the generator of the Markov process (U^N, Z^N, W^N), which is for $f \in$

$\mathcal{C}_c^\infty(\mathbb{R}^3)$, only depending on (u, z),

$$
\begin{aligned}
L^N f(u, z, w) = {} & \kappa_1 N^{-1}(Nw + N^{1/2}u)(Nw + N^{1/2}u - 1)z(f(u - 2N^{-1/2}, z - 1) - f(u, z)) \\
& + N\kappa_2'(M - z)(f(u + 2N^{-1/2}, z + 1) - f(u, z)) \\
& + N\kappa_3'(M - z)(f(u + N^{-1/2}, z + 1) - f(u, z)) \\
& - N^{1/2}p^N(w)f'(u, z) \\
= {} & L_0^N f(u, z) + N^{1/2}L_1^N f(u, z) + NL_2^N f(u, z), \\
L_0^N f(u, z) = {} & 2\kappa_1 w^2 z f''(u, z - 1) - 4\kappa_1 wuz f'(u, z - 1) \\
& + \kappa_1(u^2 - w)z(f(u, z - 1) - f(u, z)) \\
& + 2\kappa_2'(M - z)f''(u, z + 1) + \tfrac{1}{2}\kappa_3'(M - z)f''(u, z + 1) + \varepsilon_f^N(u, z, w), \\
L_1^N f(u, z) = {} & -2\kappa_1 w^2 z f'(u, z - 1) + 2\kappa_1 wuz(f(u, z - 1) - f(u, z)) \\
& + 2\kappa_2'(M - z)f'(u, z + 1) + \kappa_3'(M - z)f'(u, z + 1) - p^N(w)f'(u, z), \\
L_2^N f(u, z) = {} & \kappa_1 w^2 z(f(u, z - 1) - f(u, z)) + (\kappa_2' + \kappa_3')(M - z)(f(u, z + 1) - f(u, z)), \\
|\varepsilon_f^N(u, z, w)| \le {} & N^{-1/2}\big((\tfrac{2}{3}\kappa_1 w^2 z + \tfrac{2}{3}\kappa_2'(M - z) + \tfrac{1}{6}\kappa_3'(M - z))\|f'''\|_\infty + 2\kappa_1 uwz\|f''\|_\infty \\
& \hspace{6cm} + \kappa_1(u^2 + w)\|f\|_\infty\big).
\end{aligned}
$$

With f only depending on u and $g_N(u, z, w) = za_N(w)f'(u)$ as above, we have that $L_1^N f + L_2^N g_N = 0$. Then we use the ansatz $h_N(u, z, w) = zb_N f'(u) + \left(zc_N + \binom{z}{2}d_N\right)f''(u)$, for some functions b_N, c_N, d_N (which might depend on N, u, w). We receive at (writing $a := a_N, b := b_N, c := c_N, d := d_N$)

$$
\begin{aligned}
& (L_0^N f + L_1^N g_N + L_2^N h_N)(u, z, w) - \varepsilon_f^N(u, z, w) \\
& = \Big(M(\kappa_2' + \kappa_3')b - z(4\kappa_1 wu + 2\kappa_1 wua + (\kappa_1 w^2 + \kappa_2' + \kappa_3')b)\Big)f'(u) \\
& \quad + \Big(M(2\kappa_2'(1 + a) + \tfrac{1}{2}\kappa_3'(1 + 2a) + M(\kappa_2' + \kappa_3')c \\
& \quad + z(2\kappa_1 w^2 - 2\kappa_2' - \tfrac{1}{2}\kappa_3' - 2\kappa_1 wua - (2\kappa_2' + \kappa_3')(M - 2)a - p^N(w)a \\
& \hspace{4cm} - (\kappa_1 w^2 + \kappa_2' + \kappa_3')c + (M - 1)(\kappa_2' + \kappa_3')d) \\
& \quad + z(z - 1)(-2\kappa_1 w^2 a - (2\kappa_2' + \kappa_3')a) - (\kappa_1 w^2 + \kappa_2' + \kappa_3')d\Big)f''(u).
\end{aligned}
$$

With a_N from (3.17), we immediately see that only through

$$
b = -\frac{2\kappa_1 wu(2 + a)}{\kappa_1 w^2 + \kappa_2' + \kappa_3'} - = -\frac{2\kappa_1 \kappa_3' wu}{(\kappa_1 w^2 + \kappa_2' + \kappa_3')^2}
$$

the term proportional to $zf'(u)$ on the right hand side vanishes. Then, using that $p^N(w) = M\kappa_2'(2 + a) + M\kappa_3'(1 + a)$, finding the roots of the terms proportional to $zf''(u)$ and $z(z - 1)f''(u)$ on the right hand side for c, d, we find

$$
\begin{aligned}
& (L_0^N f + L_1^N g_N + L_2^N h_N)(u, z, w) - \varepsilon_f^N(u, z, w) \\
& = -2M\frac{\kappa_1(\kappa_2' + \kappa_3')\kappa_3' uw}{(\kappa_2' + \kappa_3' + \kappa_1 w^2)^2}f'(u) + \frac{1}{2}\frac{(\kappa_1^2 w^4 + 2\kappa_1 \kappa_2' w^2 + (\kappa_2' + \kappa_3')^2)M\kappa_1 \kappa_3' w^2)}{(\kappa_2' + \kappa_3' + \kappa_1 w^2)^3}f''(u).
\end{aligned}
$$

Since $W^N \xRightarrow{N \to \infty} V$, the limit of the right hand side for $N \to \infty$ gives the generator of U and we are done.

3.5 Checking the conditions of Theorem 2.3

If the calculations from the last section go through, we still have to verify the conditions of Theorem 2.3, i.e. the compact containment condition of $(V^N)_{N=1,2,\ldots}$ for

the LLN and $(U^N)_{N=1,2,\dots}$ for the CLT, as well as the corresponding conditions (2.4)–(2.6). Checking these might depend on the concrete example, but here, we give a general strategy. In all steps, note that we require the fast network to be first order. This means that either (i) fast species only appear on both sides of each $R \in \mathcal{R}$ of the fast network or (ii) one fast species $S \in \mathcal{S}_\circ$ is produced only from slow species. (The case where one fast species produces some slow species only appears in (ii), since otherwise fast species will quickly be consumed.) In (i), there are conserved quantities in the fast network, i.e. (using the notation from above) there is ξ_C such that $z \cdot \xi_C$ is constant. In (ii), the discussion after (3.11) applies: the fast species S is created at some rate $C_0^N N$, and is consumed at rate $C_1^N N^\beta Z_S^N$ for $\beta \geq 1$. For such processes, Lemma A.1 is useful for bounding moments. The number of other fast species will be bounded by Z_S^N.

As for (2.4), recall from (3.12) and (3.19), that both, g_N and h_N are polynomials in the fast species. So, from (A.2),

$$N^{-1/2}\mathbb{E}[\sup_{0 \leq t \leq T} (Z_S^N(t))^k] \xrightarrow{N \to \infty} 0, \qquad T > 0, k = 1, 2, \dots$$

provides the required argument for (2.4) to hold if f has compact support and a_N, b_N, c_N, d_N are continuous. For the integrals in (2.5) and (2.6), we use (A.1) for

$$\sup_{N \in \mathbb{N}} \mathbb{E}\Big[\int_0^T N^{\beta-1} (Z_S^N(t))^k dt \Big] < \infty, \qquad T > 0, k = 1, 2, \dots$$

if the corresponding terms again come as a polynomial of fast species. So, we are left with showing the compact containment condition for $(V^N)_{N=1,2,\dots}$ and $(U^N)_{N=1,2,\dots}$. Focusing on $(U^N)_{N=1,2,\dots}$, we start by the same generator calculations as in the last section, using $f(u) = u_S$ for some $S \in \mathcal{S}_\bullet$. This gives some g_N and h_N such that

$$
\begin{aligned}
L^N(f &+ N^{-1/2}g_N + N^{-1}h_N) \\
&= N^{1/2}(L_1^N f + L_2^N g_N) + (L_0^N f + L_1^N g_N + L_2^N h_N) + N^{-1/2}(L_0^N g_N + L_1^N h_N) + N^{-1}L_0^N h_N \\
&= Lf + \varepsilon_f^N + N^{-1/2}(L_0^N g_N + L_1^N h_N) + N^{-1}L_0^N h_N.
\end{aligned}
$$

Then, provided that (2.4)–(2.6) also hold for $f(u) = u_S$, we have that

$$
\begin{aligned}
U_S^N(t) = M_S^N(t) &- N^{-1/2}g_N(U_t^N, Z_t^N, W_t^N) - N^{-1}h_N(U_t^N, Z_t^N, W_t^N) \\
&+ \int_0^t (Lf + \varepsilon_f^N + N^{-1/2}(L_0^N g_N + L_1^N h_N) + N^{-1}L_0^N h_N)(U_s^N, Z_s^N, W_s^N) ds
\end{aligned}
$$

for the martingale $M_S^N := (M_S^N(t))_{t \geq 0}$ with

$$
\begin{aligned}
M_S^N(t) = U_S^N(t) &+ (N^{-1/2}g_N + N^{-1}h_N)(U_t^N, Z_t^N, W_t^N) \\
&- \int_0^t L^N(f + N^{-1/2}g_N + N^{-1}h_N)(U_s^N, Z_s^N, W_s^N) ds.
\end{aligned}
$$

Then, if we can bound $\sup_{0 \leq t \leq T} |M_S^N(t)|$ (e.g. by bounding the quadratic variation of this martingale) uniformly in N, and if Lf grows at most linearly in U^N, we see using (2.4)–(2.6) that there are bounded functions $a_S, b_S \geq 0$ such that

$$\mathbb{E}[\sup_{s \leq t} |U_S^N(s)|] \leq b_S(t) + \int_0^t a_S(s) \cdot \mathbb{E}[\sup_{r \leq s} |U^N(r)|] ds. \tag{3.22}$$

With the Gronwall lemma, we obtain $\mathbb{E}[\sup_{s \leq t} |U^N(t)|] \leq b_S(t) + \int_0^t b_S(s)a_S(s)e^{\int_s^t a_S(r)dr}ds$ and Markov's inequality shows the compact containment condition for $(U_S^N)_{N=1,2,\dots}$.

Example 3.7 (The simplified Hill dynamics 5)**.** While we provided the necessary generator calculations needed for convergence $V^N \xrightarrow{N \to \infty} V$ with V as given in (3.16) and $U^N \xrightarrow{N \to \infty} U$ with U as in (3.21), we still need to check the corresponding conditions (2.4)–(2.6) and the comopact containment conditions. We note that in this example, for $T, k > 0$, using Lemma A.1,

$$\mathbb{E}\Big[\int_0^T (M - Z_t^N)^k dt \Big] = O(N^{1-\beta}). \tag{3.23}$$

In addition $0 \leq Z^N \leq M$, so $\limsup_{N \to \infty} N^{-1/2} Z^N = 0$. These properties already imply (2.4)–(2.6) in both, the LLN and the CLT. For the compact containment condition in the LLN, note that $V^N + 2Z^N/N$ is a decreasing process, which already implies compact containment (provided V_0^N converges). The hardest work is required by the compact containment condition in the CLT. For this, let $g_N(t) := g_N(Z_t^N, W_t^N) = Z_t^N a_N(W_t^N)$ and $h(t) := h_N(U_t^N, Z_t^N, W_t^N) = Z_t^N b_N(U_t^N, W_t^N)$ (i.e. as above with $f(u) = u$). Then, (2.4)–(2.6) also hold in this case (since the $(u^2 + w)$-term in ε_f^N vanishes in this case, leading to $\varepsilon_f^N = 0$). Then,

$$U_t^N = M_t^N - N^{-1/2} g_N(t) - N^{-1} h_N(t) + \int_0^t 2M \frac{\kappa_1(\kappa_2' + \kappa_3')\kappa_3' U_s^N W_s}{(\kappa_2' + \kappa_3' + \kappa_1 W_s^2)^2} ds,$$

$$M_t^N := U_t^N + N^{-1/2} g_N(t) + N^{-1} h_N(t) - \int_0^t 2M \frac{\kappa_1(\kappa_2' + \kappa_3')\kappa_3' U_s^N W_s}{(\kappa_2 + \kappa_3' + \kappa_1 W_s^2)^2} ds,$$

where, $(M_t^N)_{t \geq 0}$ is a martingale with quadratic variation

$$[M^N]_t = N[V^N + N^{-1} g_N]_t$$
$$\approx \int_0^t (2 + a_N(W_s))^2 (\kappa_1(V_s^N)^2 Z_s^N + \kappa_2'(M - Z_s^N)) + (1 + a_N(W_s))^2 \kappa_3'(M - Z_s^N) ds.$$

Since V^N, Z^N, W are bounded, g_N and h_N are bounded as well. Moreover, from (3.23) we see that the quadratic variation of M^N is locally bounded, so we can conclude that there are a_S and b_S such that (3.22) holds, and the compact containment condition for $(U^N)_{N=1,2,\ldots}$ follows.

4 Examples

In this section we give some more concrete applications of our theory. We start with Michaelis-Menten kinetics in Section 4.1, i.e. we rederive the results from [11] using the approach from Section 3. These kinetics is similar to the simplified Hill dynamics from Examples 3.1–3.7. Still, we extend results from [11] by allowing that the balance condition for the fast species to fail, and from [3] by proving the CLT. The simplified Hill example is extended in Section 4.2 to account for separate effects of binding of the two ligands to the macromolecule. In Section 4.3, we are dealing with an example using two fast species where the number of molecules of the fast species is unbounded. For some parameter combinations ($\beta, \gamma > 1$), the fast species are called intermediate by [3], so we extend their results by the CLT. Then, in Section 4.4, we are dealing with the main example in [3], but again add to their results the CLT.

We note that not all calculations were carried out by hand. The approach that the right hand sides of (3.13) and (3.20) do not depend on the fast variables leads to linear systems of equations with up to 15 equations in Section 4.4. Therefore, we produced an ancillary file with `sagemath`-commands [4], which can be found on `arxiv`. This file contains the computations not printed, but necessary for Examples 3.1–3.7 and for Sections 4.1, 4.2, 4.3 and 4.4.

4.1 Michaelis-Menten kinetics

Our first example is the famous Michaelis-Menten kinetics. It arises for the Chemical Reaction Network

$$E_1 + S_1 \underset{N^\gamma \kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} E_2 \xrightarrow{N^\gamma \kappa_3} E_1 + S_2 \tag{4.1}$$
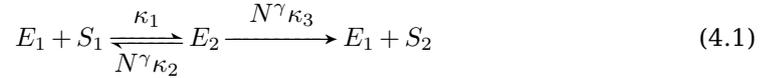
for $\gamma \geq 1$, where S_1, S_2 are in high abundance (order N) and E_1, E_2 are in low abundance (order 1). The balance condition for E_2 only holds if $\gamma = 1$, since $\gamma > 1$ implies that E_2 is produced slower than it is consumed. Chemically, E_2 is a complex formed out of E_1 (enzyme) and S_1 (substrate), and S_2 is frequently described as product of the reaction. Denoting by Z_1^N, Z_2^N the number of molecules E_1 and E_2, we note that $Z_1^N + Z_2^N =: M$ is a constant. Further, we define NV_1^N, NV_2^N to be the number of molecules of S_1 and S_2, and we find that $V_1^N + V_2^N + Z_2^N/N$ is a constant. So, since we will have that $Z_2^N = O(1)$, the dynamics of V_2^N can be read off from the dynamics of V_1^N (up to an $O(1/N)$-error). So, the system can be described through $V^N := V_1^N$ and $Z^N := Z_1^N$. We obtain the following.

Proposition 4.1 (LLN and CLT for (4.1)). *Let $u \in \mathbb{R}$ and $v \in \mathbb{R}_+$,*

$$p^N(v) := -M \frac{N^{\gamma-1}\kappa_3\kappa_1 v}{N^{\gamma-1}(\kappa_2 + \kappa_3) + v\kappa_1} \xrightarrow{N \to \infty} p(v) := \begin{cases} -\dfrac{M\kappa_3\kappa_1 v}{\kappa_2 + \kappa_3 + v\kappa_1}, & \text{for } \gamma = 1, \\[3mm] -\dfrac{M\kappa_3\kappa_1 v}{\kappa_2 + \kappa_3}, & \text{for } \gamma > 1, \end{cases}$$

V and W^N the solutions of the ODEs

$$dV = p(V)dt, \qquad dW^N = p^N(W^N)dt, \qquad W_0^N = V_0 = v.$$

Moreover, let U be the solution of the SDE

$$dU = -\frac{M\kappa_1\kappa_3(\kappa_2 + \kappa_3)}{(\kappa_1 V + \kappa_2 + \kappa_3)^2}Udt + \sqrt{\frac{M\kappa_1 V\kappa_3(\kappa_1 V + \kappa_2)^2}{2(\kappa_1 V + \kappa_2 + \kappa_3)^3} + \frac{M\kappa_3^2\kappa_1 V(2\kappa_2 + \kappa_3)}{2(\kappa_1 V + \kappa_2 + \kappa_3)^3}}dB, \quad \text{if } \gamma = 1,$$

$$dU = p(U)dt + \sqrt{|p(V)|}dB, \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \text{if } \gamma > 1,$$

for some Brownian motion B. Then, letting V^N be as above, and $U^N = N^{1/2}(V^N - W^N)$. If $V_0^N \xrightarrow{N \to \infty} v$ and $U_0^N \xrightarrow{N \to \infty} u$, then

$$V_0^N \xrightarrow{N \to \infty} V, \qquad U_0^N \xrightarrow{N \to \infty} U.$$

Remark 4.2 (Previous results on Michaelis-Menten kinetics). The above LLN has been obtained various times; see e.g. Section 6.4 of [10] for $\gamma = 1$, or Example 4.5 of [3] for $\gamma > 1$. (They in fact consider two differnt scalings for κ_2 and κ_3.) In the case $\gamma = 1$, the CLT has first been obtained in Section 5.2 of [11].

Proof. We set $\kappa_2' = N^{\gamma-1}\kappa_2$ and $\kappa_3' = N^{\gamma-1}\kappa_3$. For the Markov process $X^N = (V^N, Z^N)$, the generator is given for $f \in \mathcal{C}_c^\infty(\mathbb{R} \times \mathbb{R}$, writing f' for the derivative according to the first variable,

$$G^N f(v,z) = \kappa_1 Nvz(f(v - N^{-1}, z - 1) - f(v,z)) + \kappa_2' N(M - z)(f(v + N^{-1}, z + 1)$$
$$\qquad\qquad\qquad - f(v,z)) + \kappa_3' N(M - z)(f(v, z + 1) - f(v,z))$$
$$= G_0^N f(v,z) + NG_2^N f(v,z),$$
$$G_0^N f(v,z) = -\kappa_1 vzf'(v, z - 1) + \kappa_2'(M - z)f'(v, z + 1) + \varepsilon_f^N(v,z),$$
$$G_2^N f(v,z) = \kappa_1 vz(f(v, z - 1) - f(v,z)) + (\kappa_2' + \kappa_3')(M - z)(f(v, z + 1) - f(v,z)),$$
$$|\varepsilon_f^N(v,z)| \leq \tfrac{1}{2}N^{-1}(\kappa_1 vz + \kappa_2'(M - z))\|f''\|_\infty.$$

We mention here that $0 \le Z^N(t) \le M$ by construction and that for all T, a, k (using similar arguments as in Lemma A.1)

$$\mathbb{E}\Big[\int_0^T (M - Z_t^N)^k dt\Big] = O(N^{1-\gamma}). \tag{4.2}$$

1. For the LLN of V^N as $N \to \infty$, take $f \in \mathcal{C}_c^\infty(\mathbb{R})$. Our task is to look for g_N (depending on v, z) such that $G_0^N f + G_2^N g_N$ only depends on v. Choosing

$$g_N(v, z) = za_N(v)f'(v),$$

for some (function) a_N, we obtain (collecting terms proportional to z in the second line)

$$
\begin{aligned}
G_0^N f(v,z) &+ G_2^N h_N(v,z) - \varepsilon_f^N(v,z) \\
&= \big(-\kappa_1 vz + \kappa_2'(M-z) - \kappa_1 vza_N(v) + (\kappa_2' + \kappa_3')(M-z)a_N(v) \big) f'(v) \\
&= \big(M(\kappa_2' + a_N(v)(\kappa_2' + \kappa_3')) - z(v\kappa_1 + \kappa_2' + a_N(v)(\kappa_1 v + \kappa_2' + \kappa_3')) \big) f'(v).
\end{aligned} \tag{4.3}
$$

Choosing

$$a_N(v) = -\frac{v\kappa_1 + \kappa_2'}{\kappa_2' + \kappa_3' + v\kappa_1} = -1 + \frac{\kappa_3'}{\kappa_2' + \kappa_3' + v\kappa_1}, \tag{4.4}$$

the last line of (4.3) does not depend on z, and we have

$$(G_0^N f + G_2^N h_N)(v,z) - \varepsilon_f^N(v,z) = -M\Big(\kappa_3' - \frac{\kappa_3'(\kappa_2' + \kappa_3')}{\kappa_2' + \kappa_3' + v\kappa_1}\Big)f'(v) = p^N(v)f'(v).$$

With $Gf(v) = p(v)f'(v)$, we see that $\varepsilon_f^N(v,z) + (p(v) - p^N(v))f'(v)$ is as in (2.7). Since $p^N \xrightarrow{N \to \infty} p$, the compact containment condition for $(V^N)_{N=1,2,\dots}$ holds since $V^N(t)$ is bounded by $V^N(0) + M/N$, and (2.4)–(2.6) hold due to $0 \le Z^N \le M$ and (4.2), we have shown that $V^N \xRightarrow{N \to \infty} V$.

2. For the CLT, we write $U^N = N^{1/2}(V^N - W^N)$, and have for the Markov process (U^N, Z^N, W^N) the generator for smooth f, only depending on u, z, writing f' for the derivative with respect to u,

$$
\begin{aligned}
L^N f(u,z) &= (Nw + N^{1/2}u)\kappa_1 z(f(u - N^{-1/2}, z-1) - f(u,z)) \\
&\quad + \kappa_2' N(M-z)(f(u + N^{-1/2}, z+1) - f(u,z)) \\
&\quad + \kappa_3' N(M-z)(f(u, z+1) - f(u,z)) + N^{1/2}Mp^N(w)f'(u,z) \\
&= L_0^N f(u,z) + N^{1/2}L_1^N f(u,z,w) + NL_2^N f(u,z),
\end{aligned}
$$

$$L_0^N f(u,z) = \tfrac{1}{2}\kappa_1 wzf''(u, z-1) + \tfrac{1}{2}\kappa_2'(M-z)f''(u, z+1) - \kappa_1 uzf'(u, z-1) + \varepsilon_f^N(u,z),$$

$$
\begin{aligned}
L_1^N f(u,z,w) &= -\kappa_1 wzf'(u, z-1) + \kappa_1 uz(f(u, z-1) - f(u,z)) \\
&\quad + \kappa_2'(M-z)f'(u, z+1) + Mp^N(w)f'(u,z),
\end{aligned}
$$

$$L_2^N f(u,z) = \kappa_1 wz(f(u, z-1) - f(u,z)) + (\kappa_2' + \kappa_3')(M-z)(f(u, z+1) - f(u,z)),$$

$$|\varepsilon_f^N(u,z)| \le N^{-1/2}\big(\tfrac{1}{2}\kappa_1 uz\|f''\|_\infty + \tfrac{1}{6}(\kappa_1 wz + \kappa_2'(M-z))\|f'''\|_\infty\big).$$

Then, for some smooth f depending only on u, taking $g_N(u, z, w) = za_N(w)f'(u)$ with a_N from (4.4) (but depending on w), we have $L_1^N f + L_2^N g_N = 0$. So, we need to look for h_N such that $L_0^N f + L_1^N g_N + L_2^N h_N$ only depends on u, w and has a proper limit as $N \to \infty$. Choosing

$$h_N(u, z, w) = b_N(u, w)zf'(u) + \Big(c_N(w)z + d_N(w)\binom{z}{2}\Big)f''(u),$$

for some b_N, c_N, d_N, we find, collecting terms proportional to z and z^2 after the second equality, abbreviating $a := a_N(w), b := b_N(u, z, w), ...$

$$
\begin{aligned}
(L_0^N f &+ L_1^N g_N + L_2^N h_N)(u, z, w) - \varepsilon_f^N(u, z, w) \\
&= \Big(-\kappa_1 uz - \kappa_1 uza - \kappa_1 zwb + (\kappa_2' + \kappa_3')(M - z)b \Big) f'(u) \\
&\quad + \Big(\tfrac{1}{2}\kappa_1 wz + \tfrac{1}{2}\kappa_2'(M - z) - \kappa_1 wz(z - 1)a + \kappa_2'(M - z)a(z + 1) + Mzp^N(w)a \\
&\qquad\quad - \kappa_1 zw(c + d(z - 1)) + (\kappa_2' + \kappa_3')(M - z)(c + dz) \Big) f''(u) \\
&= \Big(M(\kappa_2' + \kappa_3')b - z\big(\kappa_1 u(1 + a) + b(\kappa_1 w + \kappa_2' + \kappa_3')\big) \Big) f'(u) \\
&\quad + \Big(M\big(\kappa_2'(\tfrac{1}{2} + a) + (\kappa_2' + \kappa_3')c\big) \\
&\qquad + z\big(\tfrac{1}{2}(\kappa_1 w - \kappa_2') + (\kappa_1 w + (M - 1)\kappa_2' + Mp^N(w))a \\
&\qquad\qquad\qquad - (\kappa_1 w + \kappa_2' + \kappa_3')c + (\kappa_1 w + M(\kappa_2' + \kappa_3'))d\big) \\
&\qquad\qquad\qquad - z^2\big((\kappa_1 w + \kappa_2')a + (\kappa_1 w + \kappa_2' + \kappa_3')d\big) \Big) f''(u).
\end{aligned}
$$

Choosing b, c, d such that the right hand side does not depend on z, we arrive after some rearrangements at

$$
\begin{aligned}
(L_0^N f + L_1^N g_N + L_2^N h_N)(u, w) &= Lf(u, w) + \varepsilon_f^N(u, z, w) \\
Lf(u, w) = &-\frac{M\kappa_1 \kappa_3'(\kappa_2' + \kappa_3')}{(\kappa_1 w + \kappa_2' + \kappa_3')^2} u f'(u) \\
&+ \Big(\frac{M\kappa_1 w \kappa_3'(\kappa_1 w + \kappa_2')^2}{2(\kappa_1 w + \kappa_2' + \kappa_3')^3} + \frac{M(\kappa_3')^2 \kappa_1 w(2\kappa_2' + \kappa_3')}{2(\kappa_1 w + \kappa_2' + \kappa_3')^3} \Big) f''(u),
\end{aligned}
$$

where Lf is the generator of U. Now, (2.4)–(2.6) follow from (4.2) and $0 \leq Z_t^N \leq M$ and we are left with showing the compact containment condition for $(U^N)_{N=1,2,...}$. For this, let $g_N(t) := g_N(Z_t^N, W_t^N) = Z_t^N a_N(W_t^N)$ and $h(t) := h_N(U_t^N, Z_t^N, W_t^N) = Z_t^N b_N(U_t^N, W_t^N)$ (i.e. as above with $f(u) = u$). Then,

$$
U_t^N = M_t^N - N^{-1/2} g_N(t) - N^{-1} h_N(t) - \int_0^t \frac{M\kappa_1 \kappa_3'(\kappa_2' + \kappa_3')}{(\kappa_1 W_s^N + \kappa_2' + \kappa_3')^2} U_s^N ds,
$$

$$
M_t^N := U_t^N + N^{-1/2} g_N(t) + N^{-1} h_N(t) + \int_0^t \frac{M\kappa_1 \kappa_3'(\kappa_2' + \kappa_3')}{(\kappa_1 W_s^N + \kappa_2' + \kappa_3')^2} U_s^N ds.
$$

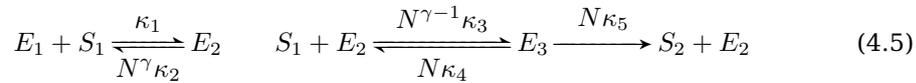Here, $(M_t^N)_{t \geq 0}$ is a martingale with quadratic variation

$$
\begin{aligned}
[M^N]_t &= N[V^N + N^{-1} g_N]_t \\
&\approx \int_0^t (1 + a_N(W_s^N))^2 (\kappa_1 V_s^N Z_s^N + \kappa_2'(M - Z_s^N)) + a_N(W_s^N)^2 \kappa_3'(M - Z_s^N) ds.
\end{aligned}
$$

Since V^N, Z^N, W^N are bounded, g_N and h_N are bounded as well. Moreover, from (4.2) we see that the quadratic variation of M^N is locally bounded, so we can conclude that there are a_S and b_S such that (3.22) holds, and the compact containment condition for $(U^N)_{N=1,2,...}$ follows. $\qquad\square$

4.2 Hill coefficient dynamics

We now pick up Example 3.1, but extend the CRN in order to capture all required reactions for binding of two ligands. This also generalizes the Michaelis-Menten dynamics,

where the macromolecule (enzyme) can only be bound by a single ligand. Specifically, we study

$$E_1 + S_1 \underset{N^\gamma \kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} E_2 \qquad S_1 + E_2 \underset{N\kappa_4}{\overset{N^{\gamma-1}\kappa_3}{\rightleftharpoons}} E_3 \overset{N\kappa_5}{\longrightarrow} S_2 + E_2 \qquad (4.5)$$

for $\gamma > 1$. Here, similar to Michaelis-Menten kinetics, E_1 (the macromolecule) and S_1 (the ligand) form a short-lived complex E_2, but only after forming another complex E_3 together with another S_1, the product S_2 is formed and E_2 is released. We will assume that S_1, S_2 are in high abundance (order N) and E_1, E_2, E_3 are in low abundance (order 1). Since $\gamma > 1$, the balance condition fails for E_1, E_2 and E_3. Let NV_1^N, NV_2^N and Z_1, Z_2, Z_3 be the processes of the particle numbers of S_1, S_2 and E_1, E_2, E_3, respectively. Since $M := Z_1 + Z_2 + Z_3$ is a constant, and $V_1 + V_2 + N^{-1}(Z_1 + 2Z_2 + 3Z_3)$ is constant as well, we are going to describe the Markov process $(Z^N = (Z_1^N, Z_3^N), V^N = V_1^N)$.

Proposition 4.3 (LLN and CLT for (4.5))**.** *Let $u \in \mathbb{R}$ and $v \in \mathbb{R}_+$,*

$$p^N(v) := -\frac{\kappa_1 N^{\gamma-1}\kappa_3\kappa_5 v^2}{(\kappa_1 v + N^{\gamma-1}\kappa_2)(\kappa_4 + \kappa_5) + N^{\gamma-1}\kappa_1\kappa_3 v^2} \xrightarrow{N\to\infty} p(v) := -\frac{\kappa_1\kappa_3\kappa_5 v^2}{\kappa_2(\kappa_4 + \kappa_5) + \kappa_1\kappa_3 v^2}$$

and V and W^N the solutions of the ODEs

$$dV = p(V)dt, \qquad dW^N = p^N(W^N)dt, \qquad V_0 = W_0^N = v,$$

Moreover, let U be the solution of the SDE

$$dU = -2M\frac{\kappa_1\kappa_2\kappa_3(\kappa_4 + \kappa_5)\kappa_5 uV}{(\kappa_2(\kappa_4 + \kappa_5) + \kappa_1\kappa_3 V^2)^2}dt$$
$$+ \sqrt{\frac{(\kappa_2^2(\kappa_4 + \kappa_5)^2 + 2\kappa_2\kappa_3\kappa_5(\kappa_4 + \kappa_5)V + (\kappa_1\kappa_3 V^2 + 2\kappa_2\kappa_4)\kappa_1\kappa_3 V^2)M\kappa_1\kappa_3\kappa_5 V^2}{(\kappa_2(\kappa_4 + \kappa_5) + \kappa_1\kappa_3 V^2)^3}}dB$$

for some Brownian motion B. Then, let V^N as above, and $U^N = N^{1/2}(V^N - W^N)$. If $V_0^N \xrightarrow{N\to\infty} v$ and $U_0^N \xrightarrow{N\to\infty} u$, then

$$V^N \xrightarrow{N\to\infty} V, \qquad U^N \xrightarrow{N\to\infty} U.$$

Remark 4.4. 1. Instead of the rates $N\kappa_4, N\kappa_5$, we could use a scaling $N^\beta\kappa_4, N^\beta\kappa_5$ for $\beta \geq 1$. This would change the result, but not the proof, except for taking the limits in the last equalities in (4.7) for the LLN and in (4.8) for the CLT.

2. The same holds for the case $\gamma = 1$: In fact, $\gamma > 1$ only plays a role in taking the last limits in (4.7) and (4.8).

3. In the CLT, if $\gamma > 3/2$, we find

$$N^{1/2}(p^N(v) - p(v)) \xrightarrow{N\to\infty} 0,$$

and – by an application of Gronwalls lemma – $N^{1/2}(\tilde{V}^N - V) \xrightarrow{N\to\infty} 0$. Therefore, in this case, we find in addition that $N^{1/2}(V^N - V) \xrightarrow{N\to\infty} U$.

Proof. We set

$$\kappa_1' := N^{\gamma-1}\kappa_2, \qquad \kappa_2' = N^{\gamma-1}\kappa_3.$$

The generator of (V^N, Z^N) is for smooth f, writing f' for the derivative according to the first variable,

$$
\begin{aligned}
G^N f(v,z) &= N\kappa_1 v z_1(f(v - N^{-1}, z - e_1) - f(v,z)) \\
&\quad + N\kappa_1'(M - z_1 - z_3)(f(v + N^{-1}, z + e_1) - f(v,z)) \\
&\quad + N\kappa_2' v(M - z_1 - z_3)(f(v - N^{-1}, z + e_3) - f(v,z)) \\
&\quad + N\kappa_4 z_3(f(v + N^{-1}, z - e_3) - f(v,z)) + N\kappa_5 z_3(f(v, z - e_3) - f(v,z)) \\
&= G_0^N f(v,z) + N G_1^N f(v,z) + \varepsilon^N + (M - z_1 - z_3)O(N^{\gamma-2}) + z_3 O(N^{\beta-2}) \\
&\quad + o(1), \\
G_0^N f(v,z) &= -\kappa_1 v z_1 f'(v, z - e_1) + \kappa_1'(M - z_1 - z_3)f'(v, z + e_1) \\
&\quad - \kappa_2' v(M - z_1 - z_3)f'(v, z + e_3) + \kappa_4 z_3 f'(v, z - e_3), \\
G_1^N f(v,z) &= \kappa_1 v z_1(f(v, z - e_1) - f(v,z)) + \kappa_1'(M - z_1 - z_3)(f(v, z + e_1) - f(v,z)) \\
&\quad + \kappa_2' v(M - z_1 - z_3)(f(v, z + e_3) - f(v,z)) + \kappa_4 z_3(f(v, z - e_3) - f(v,z)) \\
&\quad + \kappa_5 z_3(f(v, z - e_3) - f(v,z)), \\
\varepsilon_1^N &= N^{-1}\big(\tfrac{1}{2}\kappa_1 v z_1 f''(v, z - e_1) + \tfrac{1}{2}\kappa_1'(M - z_1 - z_3)f''(v, z + e_1) \\
&\quad + \tfrac{1}{2}\kappa_2' v(M - z_1 - z_3)f''(v, z + e_3) + \tfrac{1}{2}\kappa_4 z_3 f''(v, z - e_3)\big).
\end{aligned}
$$

1. For the LLN, take some smooth f depending only on v. We need to find g such that the limit of $G_0^N f + G_1^N g$ exists and only depends on v. Choosing

$$
g(v,z) = (z_1 a_1 + z_3 a_3)f'(v)
$$

for some functions a_1, a_3, we find

$$
\begin{aligned}
(G_0^N f + G_1^N g)(v,z) = \Big(&M\Big(\kappa_1' - \kappa_2' v + \kappa_1' a_1 + \kappa_2' v a_3\Big) \\
&+ z_1\Big(-\kappa_1 v - \kappa_1' + \kappa_2' v - (\kappa_1 v + \kappa_2')a_1 - \kappa_2' v a_3\Big) \\
&+ z_3\Big(-\kappa_1' + \kappa_2' v + \kappa_4 - \kappa_1' a_1 - (\kappa_2' v + \kappa_4 + \kappa_5)a_3\Big)\Big)f'(v).
\end{aligned}
\tag{4.6}
$$

With

$$
a_1 := -\frac{(\kappa_1 \kappa_2' v^2 + \kappa_1'(\kappa_4 + \kappa_5) + (\kappa_1(\kappa_4 + \kappa_5) - \kappa_2' \kappa_5)v}{\kappa_1 \kappa_2' v^2 + \kappa_1(\kappa_4 + \kappa_5)v + \kappa_1'(\kappa_4 + \kappa_5)},
$$

$$
a_3 := \frac{\kappa_1 \kappa_1' v^2 + \kappa_1 \kappa_4 v + \kappa_1' \kappa_4}{\kappa_1 \kappa_2' v^2 + \kappa_1(\kappa_4 + \kappa_5)v + \kappa_1'(\kappa_4 + \kappa_5)},
$$

the last two lines in (4.6) vanish and we obtain the generator of the limit

$$
\begin{aligned}
Gf(v) &= \lim_{N\to\infty}(G_0^N f + G_1^N g)(v) = M \lim_{N\to\infty}(\kappa_1' - \kappa_2' v + \kappa_1' a_1 + \kappa_2' v a_3)f'(v) \\
&= -M \lim_{N\to\infty} \frac{\kappa_1 \kappa_3' \kappa_5 v^2}{\kappa_2'(\kappa_4 + \kappa_5) + \kappa_1(\kappa_4 + \kappa_5)v + \kappa_1 \kappa_3' v^2}f'(v) \\
&= -M \frac{\kappa_1 \kappa_3 \kappa_5 v^2}{\kappa_2(\kappa_4 + \kappa_5) + \kappa_1 \kappa_3 v^2}f'(v).
\end{aligned}
\tag{4.7}
$$

2. For the fluctuations, consider $U = N^{1/2}(V^N - \tilde{V}^N)$ and the generator of the Markov

process (U, Z) is

$$
\begin{aligned}
L^N f(u, z) &= \kappa_1 z_1 (Nv + N^{1/2}u)(f(u - N^{-1/2}, z - e_1) - f(u, z)) \\
&\quad + N\kappa_1'(M - z_1 - z_3)(f(u + N^{-1/2}, z + e_1) - f(u, z)) \\
&\quad + \kappa_2'(Nv + N^{1/2}u)(M - z_1 - z_3)(f(u - N^{-1/2}, z + e_3) - f(u, z)) \\
&\quad + N\kappa_4 z_3(f(u + N^{-1/2}, z - e_3) - f(u, z)) \\
&\quad + N\kappa_5 z_3(f(u, z - e_3) - f(u, z)) \\
&\quad - N^{1/2}p^N(v)f'(u, z) \\
&= L_0^N f(u, z) + N^{1/2}L_1^N f(u, z) + NL_2^N f(u, z) + o(1), \\
L_0^N f(u, z) &= -\kappa_1 u z_1 f'(u, z - e_1) + \tfrac{1}{2}\kappa_1 v z_1 f''(u, z - e_1) \\
&\quad + \tfrac{1}{2}\kappa_1'(M - z_1 - z_3)f''(u, z + e_1) - \kappa_2' u(M - z_1 - z_3)f'(u, z + e_3) \\
&\quad + \tfrac{1}{2}\kappa_2' v(M - z_1 - z_3)f''(u, z + e_3) + \tfrac{1}{2}\kappa_4 z_3 f''(u, z - e_3) \\
L_1^N f(u, z) &= -\kappa_1 v z_1 f'(u, z - e_1) + \kappa_1 u z_1(f(u, z - e_1) - f(u, z)) \\
&\quad + \kappa_1'(M - z_1 - z_3)f'(u, z + e_1) - \kappa_2' v(M - z_1 - z_3)f'(u, z + e_3) \\
&\quad + \kappa_2' u(M - z_1 - z_3)(f(u, z + e_3) - f(u, z_3)) + \kappa_4 z_3 f'(u, z - e_3) \\
&\quad - p^N(v)f'(u, z) \\
L_2^N f(u, z) &= \kappa_1 v z_1(f(u, z - e_1) - f(u, z)) + \kappa_1'(M - z_1 - z_3)(f(u, z + e_1) - f(u, z)) \\
&\quad + \kappa_2' v(M - z_1 - z_3)(f(u, z + e_3) - f(u, z)) \\
&\quad + \kappa_4 z_3(f(u, z - e_3) - f(u, z)) \\
&\quad + \kappa_5 z_3(f(u, z - e_3) - f(u, z)).
\end{aligned}
$$

With f only depending on u and $g(u, z) = (z_1 a_1 + z_3 a_3)f'(u)$ as above but depending on u instead of v, we have that $L_1^N f + L_2^N g = 0$. Then, we make the ansatz

$$
h(u, z) = \left(z_1 b_1 + z_3 b_3\right)f'(u) + \left(z_1 c_1 + z_3 c_2 + \binom{z_1}{2}d_1 + \binom{z_3}{2}d_2 + \binom{z_1 + z_3}{2}d_3\right)f''(u)
$$

for some $b_1, b_3, c_1, \ldots, d_3$ and obtain

$$
\begin{aligned}
&(L_0^N f + L_1^N g + L_2^N h)(u, z) \\
&\quad = \Big(M\big(\kappa_3' u(a_3 - 1) + \kappa_2' b_1 + \kappa_3' v b_3\big) \\
&\qquad\quad + z_1\big(-\kappa_1 u + \kappa_3' u - \kappa_1 u a_1 - \kappa_3' u a_3 - \kappa_1 v b_1 - \kappa_2' b_1 - \kappa_3' v b_3\big) \\
&\qquad\quad + z_3\big(\kappa_3' u - \kappa_3' a_3 u - \kappa_2' b_1 - \kappa_3' v b_3 - \kappa_4 b_3 - \kappa_5 b_3\big)\Big)f'(u) \\
&\quad + \Big(M\big(\tfrac{1}{2}(\kappa_2' + \kappa_3' v) + \kappa_2' a_1 - \kappa_3' v a_3 + \kappa_2' c_1 + \kappa_3' v c_2\big) \\
&\qquad\quad + z_1\big(\tfrac{1}{2}(\kappa_1 v - \kappa_2' - \kappa_3' v) + \kappa_1 v a_1 + \kappa_2 M a_1 - \kappa_2 a_1 - \kappa_3 M v a_1 - \nu a_1 - \kappa_1 v c_1 \\
&\qquad\qquad + \kappa_1 v d_1 + \kappa_1 v d_3 - \kappa_2' c_1 + \kappa_2' M d_1 + \kappa_2' M d_3 - \kappa_3' v c_2 + \kappa_3' M v d_3 + \kappa_3' v b\big) \\
&\qquad\quad + z_3\big(\tfrac{1}{2}(-\kappa_2' - \kappa_3' + \kappa_4) + \kappa_2' M b - \kappa_2' a - \kappa_3' M v b + \kappa_3' v b \\
&\qquad\qquad - \kappa_4 b - \nu b - \kappa_2' c_1 + \kappa_2' M d_3 - \kappa_3' v c_2 + \kappa_3' M v d_2 + \kappa_3' M v d_3 \\
&\qquad\qquad - \kappa_4 c_2 + \kappa_4 d_2 + \kappa_4 d_3 - \kappa_5 c_2 + \kappa_5 d_2 + \kappa_5 d_3\big) \\
&\qquad\quad + z_1^2\big(-\kappa_1 v a_1 - \kappa_2' a_1 + \kappa_3' v a_1 - \kappa_1 v d_1 - \kappa_1 v d_3 - \kappa_2' d_1 - \kappa_2' d_3 - \kappa_3' v d_3\big) \\
&\qquad\quad + z_3^2\big(-\kappa_2' a_3 + \kappa_3' v a_3 + \kappa_4 b - \kappa_2' d_3 - \kappa_3' v d_2 - \kappa_3' v d_3 - (\kappa_4 + \kappa_5)(d_2 + d_3)\big) \\
&\qquad\quad + z_1 z_3\big(-\kappa_1 v a_3 - (\kappa_2' - \kappa_3' v)(a_1 + a_3) + \kappa_4 a_1 - \kappa_1 v d_3 - \kappa_2'(d_1 + 2d_3)
\end{aligned}
$$

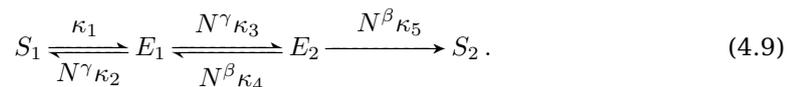$$-\kappa_3 v(d_2 + 2d_3) - \kappa_4 d_3 - \kappa_5 d_3 \Big) \Big) f''(u).$$

We can choose $b_1, b_3, c_1, ..., c_5$ such that the right hand side only depends on v. Plugging these into the last display gives, in the limit $N \to \infty$

$$\begin{aligned}
Lf(u) &= \lim_{N \to \infty} (L_0^N f + L_1^N g + L_2^N h)(u, z)) \\
&= \lim_{N \to \infty} M\big(\kappa_3' u(a_3 - 1) + \kappa_2' b_1 + \kappa_3' v b_3\big) \cdot f'(u) \\
&\quad + M\big(\tfrac{1}{2}(\kappa_2' + \kappa_3' v) + \kappa_2' a_1 - \kappa_3' v a_3 + \kappa_2' c_1 + \kappa_3' v c_2\big) \cdot f''(u) \\
&= -\frac{2M\kappa_1\kappa_2\kappa_3(\kappa_4 + \kappa_5)\kappa_5 uv}{(\kappa_1\kappa_3 v^2 + \kappa_2(\kappa_4 + \kappa_5)^2)} f'(u) \\
&\quad + \frac{1}{2} \frac{(\kappa_2^2(\kappa_4 + \kappa_5)^2 + 2\kappa_2\kappa_3\kappa_5(\kappa_4 + \kappa_5)v + (\kappa_1\kappa_3 v^2 + 2\kappa_2\kappa_4)\kappa_1\kappa_3 v^2)M\kappa_1\kappa_3\kappa_5 v^2}{(\kappa_1\kappa_3 v^2 + \kappa_2(\kappa_4 + \kappa_5))^3} f''(u).
\end{aligned}$$
(4.8)

Conditions (2.4)–(2.6) as well the compact containment condition for $(U^N)_{N=1,2,...}$ can be shown as in Example 3.7. $\qquad\square$

4.3 Extending the first example from Section 6.5 of [11]

Here, we extend the first example in Section 6.5 of [10]. Precisely, we study

$$S_1 \underset{N^\gamma \kappa_2}{\overset{\kappa_1}{\rightleftharpoons}} E_1 \underset{N^\beta \kappa_4}{\overset{N^\gamma \kappa_3}{\rightleftharpoons}} E_2 \overset{N^\beta \kappa_5}{\longrightarrow} S_2 \,.$$
(4.9)

for $\beta, \gamma \geq 1$. Here, S_i is in high abundance (order N), and E_i is in low abundance (order 1), $i = 1, 2$, so we set Z_i as the copy number of E_i and NV_i as the copy numbers of species S_i, $i = 1, 2$. Since $V_1^N + V_2^N + (Z_1^N + Z_2^N)N^{-1}$ is constant, it suffices to study $(V^N = V_1^N, Z^N = (Z_1^N, Z_2^N))$.

Looking at Assumption 3.2, we note that $\widetilde{\beta}_0 = 1, \widetilde{\beta}_1 = \widetilde{\beta}_2 = \gamma, \widetilde{\beta}_3 = \widetilde{\beta}_4 = \beta$, as well as $\varphi_{E_1} = \gamma, \varphi_{E_2} = \beta$, leading to $\widehat{\beta}_1 = \widehat{\beta}_2 = \widehat{\beta}_2 + 1 - \varphi_{E_1} = 1, \widehat{\beta}_3 = \widehat{\beta}_4 = \widehat{\beta}_4 + 1 - \varphi_{E_2} = 1$ and $\psi_{E_1} = \psi_{E_2} = 1$ and (3.11) is satisfied.

Proposition 4.5 (LLN and CLT for (4.9)). *Let $u \in \mathbb{R}$ and $v \in \mathbb{R}_+$, V the solution of the ODE*

$$dV = -\frac{\kappa_1\kappa_3\kappa_5 v}{\kappa_2(\kappa_4 + \kappa_5) + \kappa_3\kappa_5} dt, \qquad V_0 = v,$$

and U the solution of the SDE

$$dU = -\frac{\kappa_1\kappa_3\kappa_5 u}{\kappa_2(\kappa_4 + \kappa_5) + \kappa_3\kappa_5} dt + \sqrt{\frac{\kappa_1\kappa_3\kappa_5 v}{\kappa_2(\kappa_4 + \kappa_5) + \kappa_3\kappa_5}} dB, \qquad U_0 = u,$$

for some Brownian motion B. Then, letting V^N be as above, setting $U^N = N^{1/2}(V^N - V)$, and if $V_0^N \xRightarrow{N \to \infty} v$ and $U_0^N \xRightarrow{N \to \infty} u$, we have

$$V^N \xRightarrow{N \to \infty} V, \qquad U^N \xRightarrow{N \to \infty} U.$$

Proof. We define

$$\kappa_1' := N^{\gamma-1}\kappa_2, \qquad \kappa_2' := N^{\gamma-1}\kappa_3, \qquad \kappa_3' := N^{\beta-1}\kappa_4, \qquad \kappa_4' := N^{\beta-1}\kappa_5.$$
(4.10)

Then, (V^N, Z^N) has the generator for $f \in \mathcal{C}_c^\infty(\mathbb{R} \times \mathbb{R}^2)$, where f' is the derivative with

respect to the first variable,

$$
\begin{aligned}
G^N f(v,z) &= N v \kappa_1 (f(v - N^{-1}, z + e_1) - f(v,z)) \\
&\quad + N \kappa_1' z_1 (f(v + N^{-1}, z - e_1) - f(v,z)) + N z_1 \kappa_2' (f(v, z + e_2 - e_1) - f(v,z)) \\
&\quad + N \kappa_3' z_2 (f(v, z + e_1 - e_2) - f(v,z)) + N \kappa_4' z_2 (f(v, z - e_2) - f(v,z)) \\
&= G_0^N f(v,z) + N G_2^N f(v,z), \\
G_0^N f(v,z) &= -\kappa_1 v f'(v, z + e_1) + z_1 \kappa_1' f'(v, z - e_1) + \varepsilon_f^N(v,z), \\
G_2^N f(v,z) &= \kappa_1 v (f(v, z + e_1) - f(v,z)) + \kappa_1' z_1 (f(v, z - e_1) - f(v,z)) \\
&\quad + \kappa_2' z_1 (f(v, z + e_2 - e_1) - f(v,z)) + \kappa_3' z_2 (f(v, z + e_1 - e_2) - f(v,z)) \\
&\quad + \kappa_4' z_2 (f(v, z - e_2) - f(v,z)), \\
|\varepsilon_f^N(v,z)| &\leq N^{-1} \tfrac{1}{2} (\kappa_1 v + \kappa_1' z_1) \| f'' \|_\infty.
\end{aligned}
$$

For bounding the two fast variables, a calculation similar to the proof of Lemma A.1 gives, for all $T, a, k > 0$

$$
\begin{aligned}
&\mathbb{E}\Big[\int_0^T (Z_1^N(t))^k dt \Big] = O(N^{1-\gamma}), \qquad \mathbb{E}\Big[\int_0^T (Z_2^N(t))^k dt \Big] = O(N^{1-\beta}), \\
&N^{-a} \mathbb{E}[\sup_{0 \leq t \leq T} (Z_1^N(t))^k] \xrightarrow{N \to \infty} 0, \qquad N^{-a} \mathbb{E}[\sup_{0 \leq t \leq T} (Z_2^N(t))^k] \xrightarrow{N \to \infty} 0.
\end{aligned}
\tag{4.11}
$$

1. Again, $G_1^N = 0$, and we take $g_N = 0$. The compact containment condition for $(V^N)_{N=1,2,\ldots}$ is straight-forward, since $V^N + (Z_1^N + Z_2^N)/N$ is non-increasing and (4.11) bounds $(Z_1^N)_{N=1,2,\ldots}$ and $(Z_2^N)_{N=1,2,\ldots}$. Using the same arguments, conditions (2.4)–(2.6) will hold, such that we can concentrate on generator calculations:

Take $f \in \mathcal{C}_c^\infty(\mathbb{R})$ only depending on v. We are looking for h_N such that $G_0^N f(v,z) + G_2^N h_N(v,z)$ only depends on N, v and has a limit for $N \to \infty$. Choosing (see (3.12))

$$
g(v,z) = (a_1 z_1 + a_2 z_2) f'(v)
$$

for some a_1, a_2 (which might depend on N), we find

$$
\begin{aligned}
G_0^N f(v,z) + G_1^N h_N(v,z) &= -\kappa_1 v f'(v) + z_1 \kappa_1' f'(v) + \kappa_1 v a_1 f'(v) - \kappa_1' z_1 a_1 f'(v) \\
&\quad + \kappa_2' z_1 (a_2 - a_1) f'(v) + \kappa_3' z_2 (a_1 - a_2) f'(v) - \kappa_4' z_2 a_2 f'(v) + \varepsilon_f^N(v,z) \\
&= \big(\kappa_1 v (a_1 - 1) + z_1 (\kappa_1' - (\kappa_2' + \kappa_3') a_1 + \kappa_3' a_2) + z_2 (\kappa_3' a_1 - (\kappa_4' + \kappa_4') a_2) \big) f'(v) + \varepsilon_f^N(v,z).
\end{aligned}
$$

Choosing a_1, a_2 such that the terms proportioal to z_1 and z_2 vanish, i.e.

$$
\begin{aligned}
a_1 &= \frac{\kappa_1'(\kappa_3' + \kappa_4')}{\kappa_1'(\kappa_3' + \kappa_4') + \kappa_2' \kappa_4'} = \frac{\kappa_2(\kappa_4 + \kappa_5)}{\kappa_2(\kappa_4 + \kappa_5) + \kappa_3 \kappa_5}, \\
a_2 &= \frac{\kappa_1' \kappa_3'}{\kappa_1'(\kappa_3' + \kappa_4') + \kappa_2' \kappa_4'} = \frac{\kappa_2 \kappa_4}{\kappa_2(\kappa_4 + \kappa_5) + \kappa_3 \kappa_5},
\end{aligned}
$$

where we have used (4.10), we obtain

$$
\begin{aligned}
(G_0^N f + G_1^N h_N)(v,z) &= \kappa_1 v (a_1 - 1) f'(v) + \varepsilon_f^N(v,z) = G f(v) + \varepsilon_f^N(v,z), \\
\text{with } G f(v) &= -\frac{\kappa_1 \kappa_3 \kappa_5 v}{\kappa_2(\kappa_4 + \kappa_5) + \kappa_3 \kappa_5} f'(v).
\end{aligned}
$$

Since G is the generator of V, we are done.

2. For the functional CLT, we start with generator calculations. Writing $U = N^{1/2}(V^N - V)$ and have for the generator of (U^N, Z^N, V) (again, $Z^N = (Z_1^N, Z_2^N)$),

using v from the LLN, for $f \in \mathcal{C}_c^\infty(\mathbb{R} \times \mathbb{R}^2)$, only depending on u, z, with a_1 from above,

$$
\begin{aligned}
L^N f(u, z, v) &= (Nv + N^{1/2}u)\kappa_1(f(u - N^{-1/2}, z + e_1) - f(u, z)) \\
&\quad + N\kappa_1' z_1(f(u + N^{-1/2}, z - e_1) - f(u, z)) + N\kappa_2'(f(u, z + e_2 - e_1) - f(u, z)) \\
&\quad + N\kappa_3' z_2(f(u, z + e_1 - e_2) - f(u, z)) + N\kappa_4' z_2(f(u, z - e_2) - f(u, z)) \\
&\qquad\qquad\qquad\qquad\qquad\qquad\qquad - N^{1/2}\kappa_1 v(a_1 - 1)f'(u, z) \\
&= L_0^N f(u, z, v) + N^{1/2} L_1^N f(u, z, v) + N L_2^N f(u, z, v), \\
L_0^N f(u, z, v) &= \tfrac{1}{2}\kappa_1 v f''(u, z + e_1) - \kappa_1 u f'(u, z + e_1) + \tfrac{1}{2}\kappa_1' z_1 f''(u, z - e_1) + \varepsilon_f^N(u, z, v), \\
L_1^N f(u, z, v) &= -\kappa_1 v f'(u, z + e_1) + \kappa_1 u(f(u, z + e_1) - f(u, z)) + \kappa_1' z_1 f'(u, z - e_1) \\
&\qquad\qquad\qquad\qquad\qquad\qquad - \kappa_1 v(a_1 - 1)f'(u, z), \\
L_2^N f(u, z) &= \kappa_1 v(f(u, z + e_1) - f(u, z)) + \kappa_1' z_1(f(u, z - e_1) - f(u, z)) \\
&\quad + \kappa_2' z_1(f(u, z - e_1 + e_2) - f(u, z)) + \kappa_3' z_2(f(u, z + e_1 - e_2) - f(u, z)) \\
&\quad + \kappa_4' z_2(f(u, z - e_2) - f(u, z)), \\
|\varepsilon_f^N(u, z, v)| &\le \tfrac{1}{6}N^{-1/2}(\kappa_1 v + \kappa_2' z_1)\|f'''\|_\infty.
\end{aligned}
$$

With $g(u, z) = (a_1 z_1 + a_2 z_2)f'(u)$ as above, $L_1^N f + L_2^N g_N = 0$. Next, let us look for h_N such that $L_0^N f + L_1^N g_N + L_2^N h_N$ doesn't depend on z and has a limit for $N \to \infty$. With (recall from (3.19), where we set $c = 0$)

$$
h_N(u, z) = \left(z_1 b_1 + z_2 b_2 + \binom{z_1}{2}d_1 + \binom{z_2}{2}d_2 + \binom{z_1 + z_2}{2}d_3\right)f''(u)
$$

for some b_1, b_2, d_1, d_2, d_3, we have

$$
\begin{aligned}
&L_0^N f(u, z, v) + L_1^N g_N(u, z, v) + L_2^N h_N(u, z, v) - \varepsilon_f^N(u, z, v) \\
&\quad = \tfrac{1}{2}\kappa_1 v f''(u) - \kappa_1 u f'(u) + \tfrac{1}{2}\kappa_1' z_1 f''(u) - \kappa_1 v(a_1(z_1 + 1) + a_2 z_2)f''(u) \\
&\qquad + \kappa_1 u a_1 f'(u) + \kappa_1' z_1(a_1(z_1 - 1) + a_2 z_2)f''(u) - \kappa_1 v(a_1 - 1)(a_1 z_1 + a_2 z_2)f''(u) \\
&\qquad + \kappa_1 v(b_1 + z_1 d_1 + (z_1 + z_2)d_3)f''(u) \\
&\qquad - \kappa_1' z_1(b_1 + (z_1 - 1)d_1 + (z_1 + z_2 - 1)d_3)f''(u) \\
&\qquad + \kappa_2' z_1(-b_1 + b_2 - (z_1 - 1)d_1 + z_2 d_2)f''(u) \\
&\qquad + \kappa_3' z_2(b_1 - b_2 + z_1 d_1 - (z_2 - 1)d_2)f''(u) \\
&\qquad - \kappa_4' z_2(b_2 + (z_2 - 1)d_2 + (z_1 + z_2 - 1)d_3)f''(u) \\
&\quad = \kappa_1 u(a_1 - 1)f'(u) + \Big(\tfrac{1}{2}\kappa_1 v(1 - 2a_1 + 2b_1) \\
&\qquad + z_1\big(\tfrac{1}{2}\kappa_2' - v\kappa_1 a_1^2 - (\kappa_2' + \kappa_3')b_1 + \kappa_3' b_2 + v\kappa_1 d_1 + v\kappa_1 d_3\big) \\
&\qquad + z_2\big(-v\kappa_1 a_1 a_2 + \kappa_4' b_1 - (\kappa_4' + \kappa_5')b_2 + v\kappa_1 d_3\big) \\
&\qquad + z_1(z_1 - 1)\big(\kappa_2' a_1 - (\kappa_2' + \kappa_3')d_1 - \kappa_2' d_3\big) \\
&\qquad + z_2(z_2 - 1)\big(-(\kappa_3' + \kappa_5')d_2 - \kappa_4' d_3\big) \\
&\qquad + z_1 z_2\big(\kappa_1' a_2 + \kappa_3' d_1 + \kappa_3' d_2 - (\kappa_1' + \kappa_5')d_3\big)\Big)f''(u).
\end{aligned}
$$

Now, we need to find b_1, b_2, d_1, d_2, d_3 such that the last five lines vanish. This leads to

$$
b_1 = \tfrac{1}{2} \cdot \frac{\kappa_2'(\kappa_4' + \kappa_5')}{\kappa_2'(\kappa_4' + \kappa_5') + \kappa_3'\kappa_5'} = \tfrac{1}{2}a_1.
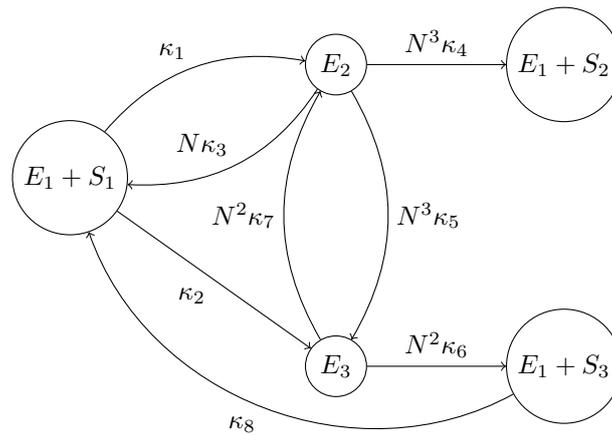$$

Figure 1: Main example from [3].

For the limiting generator, we thus have, using (4.10),

$$
\begin{aligned}
(L_0^N f &+ L_1^N g_N + L_2^N h_N)(u, z, v) \\
&= \kappa_1 u(a_1 - 1)f'(u) + \tfrac{1}{2}\kappa_1 v(1 - 2a_1 + 2b_1)f''(u) + \varepsilon_N^f(u, z, v) \\
&= Lf(u, v) + \varepsilon_N^f(u, z, v), \\
Lf(u, v) &= -\frac{\kappa_1\kappa_3\kappa_5 u}{\kappa_2(\kappa_4 + \kappa_5) + \kappa_3\kappa_5}f'(u) + \tfrac{1}{2}\frac{\kappa_1\kappa_3\kappa_5 v}{\kappa_2(\kappa_4 + \kappa_5) + \kappa_3\kappa_5}f''(u).
\end{aligned}
$$

So, L is the generator of U. We also see from (4.11) that (2.4)–(2.6) are satisfied. Finally, the compact containment condition for $(U^N)_{N=1,2,\dots}$ follows as in Example 3.7. $\qquad\square$

4.4 Main example from Cappelletti und Wiuf (2016)

We will now study the main example from [3], as given in Figure 1. Here, E_1, E_2 and E_3 are enzymes which help to transform S_1 into S_2 and S_3. For the abundances, we have that E_1, E_2, E_3 are in low abundance (order 1), and S_1, S_2, S_3 are in high abundance (order N). We denote the number of E_1, E_2, E_3 by Z_1^N, Z_2^N, Z_3^N, respectively, and the number of S_1, S_2, S_3 by NV_1^N, NV_2^N, NV_3^N, respectively. A close inspection of the reactions shows that $M := Z_1^N + Z_2^N + Z_3^N$ is a constant, and it suffices to study $Z^N = (Z_1^N, Z_2^N)$. Moreover, the reaction rates are such that $Z_1 = M$ at most times. If some molecule E_2 or E_3 is created, it reacts through either reaction 4 or 6, both of which occur at faster rates than the creation reactions of E_2 and E_3. For this reason, [3] call these species *intermediate*. Moreover, we also note that $V_1^N + V_2^N + V_3 + N^{-1}(Z_2^N + Z_3^N)$ is a constant, such that it suffices to study $V^N = (V_1^N, V_3^N)$. Moreover it is easy to check that $\psi_{E_1} = \psi_{E_2} = \psi_{E_3} = 1$, so our general theory applies.

Proposition 4.6 (LLN and CLT for the system from Figure 1). *Let*

$$
\begin{aligned}
\lambda_1 &:= \kappa_1 \frac{\kappa_4(\kappa_6 + \kappa_7)}{\kappa_4(\kappa_6 + \kappa_7) + \kappa_5\kappa_6} + \kappa_2 \frac{\kappa_4\kappa_7}{\kappa_4(\kappa_6 + \kappa_7) + \kappa_5\kappa_6}, \\
\lambda_2 &:= \kappa_1 \frac{\kappa_5\kappa_6}{\kappa_4(\kappa_6 + \kappa_7) + \kappa_5\kappa_6} + \kappa_2 \frac{(\kappa_4 + \kappa_5)\kappa_6}{\kappa_4(\kappa_6 + \kappa_7) + \kappa_5\kappa_6},
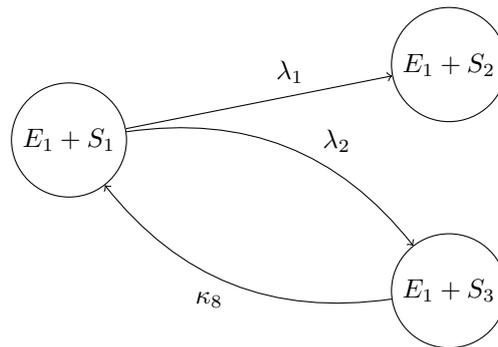\end{aligned}
\tag{4.12}
$$

Figure 2: Reduced system in the main example from [3].

$u = (u_1, u_3) \in \mathbb{R}^2$ *and* $v = (v_1, v_3) \in \mathbb{R}^2_+$, $V = (V_1, V_3)$ *the solution of the ODE*

$$dV_1 = (-M(\lambda_1 + \lambda_2)V_1 + M\kappa_8 V_3)dt,$$
$$dV_3 = (M\lambda_2 V_1 - M\kappa_8 V_3)dt,$$

and $U = (U_1, U_3)$ *solution of the SDE*

$$dU_1 = (-M(\lambda_1 + \lambda_2)U_1 + M\kappa_8 U_3)dt + \sqrt{M\lambda_1 V_1}dB_1 + \sqrt{M\lambda_2 V_1}dB_2 + \sqrt{M\kappa_8 V_3}dB_3,$$
$$dU_3 = (M\lambda_2 U_1 - M\kappa_8 U_3)dt - \sqrt{M\lambda_2 V_1}dB_2 + -\sqrt{M\kappa_8 V_3}dB_3$$

for independent Brownian motions B_1, B_2, B_3. *Then, letting* V^N *be as above, setting* $U^N = N^{1/2}(V^N - V)$, *and if* $V_0^N \xRightarrow{N\to\infty} v$ *and* $U_0^N \xRightarrow{N\to\infty} u$, *we have*

$$V^N \xRightarrow{N\to\infty} V, \qquad U^N \xRightarrow{N\to\infty} U.$$

Remark 4.7. 1. The limiting system of the above proposition is the same as the (single-scale) system given in Figure 2. For the LLN, this is the same limit as obtained in [3]. The fact that the CLT follows the same single-scale system is new.

2. We note that our proof not only gives the limit of the system in Figure 1, but also for different scalings. For example, if we use $N\kappa_4$ (instead of $N^3\kappa_4$), $N\kappa_5$ (instead of $N^3\kappa_5$), $N\kappa_6$ (instead of $N^2\kappa_6$), and $N\kappa_7$ (instead of $N^2\kappa_7$), the techniques we use give a limit result as well. However, this limit is much more complex than Figure 2.

Proof. Setting $\kappa'_4 := N^2\kappa_4$, $\kappa'_5 := N^2\kappa_5$, $\kappa'_6 := N\kappa_6$, $\kappa'_7 := N\kappa_7$, the resulting Markov process has the generator for $f \in \mathcal{C}_c^\infty(\mathbb{R}^2 \times \mathbb{R}^2)$

$$
\begin{aligned}
G^N f(v, z) = {} & N\kappa_1 v_1 z_1 (f(v - N^{-1}e_1, z - e_1 + e_2) - f(v, z)) \\
& + \kappa_2 N v_1 z_1 (f(v - N^{-1}e_1, z - e_1) - f(v, z)) \\
& + N\kappa_3 z_2 (f(v + N^{-1}e_1, z + e_1 - e_2) - f(v, z)) \\
& + N\kappa'_4 z_2 (f(v, z + e_1 - e_2) - f(v, z)) \\
& + N\kappa'_5 z_2 (f(v, z - e_2) - f(v, z)) \\
& + N\kappa'_6 (M - z_1 - z_2)(f(v + N^{-1}e_3, z + e_1) - f(v, z)) \\
& + N\kappa'_7 (M - z_1 - z_2)(f(v, z + e_2) - f(v, z)) \\
& + N\kappa_8 v_3 z_1 (f(v + N^{-1}(e_1 - e_3), z) - f(v, z)) \\
= {} & (G_0^N + N \cdot G_2^N)f(v, z)
\end{aligned}
$$

with (writing D_1f and D_3f for the derivatives with respect to v_1 and v_3, and by $||D^2f||_\infty$ the supremum of all second derivatives of f),

$$G_0^N f(v,z) = -v_1 z_1 \big(\kappa_1 D_1 f(v, z - e_1 + e_2) + \kappa_2 D_1 f(v, z - e_1)\big) + \kappa_3 z_2 D_1 f(v, z + e_1 - e_2)$$
$$+ \kappa_6'(M - z_1 - z_2) D_3 f(v, z + e_1) + \kappa_8 v_3 z_1 (D_1 - D_3) f(v, z) + \varepsilon_f^N(v, z),$$

$$G_2^N f(v,z) = \kappa_1 v_1 z_1 (f(v, z - e_1 + e_2) - f(v, z)) + \kappa_2 v_1 z_1 (f(v, z - e_1) - f(v, z))$$
$$+ \kappa_3 z_2 (f(v, z + e_1 - e_2) - f(v, z)) + \kappa_4' z_2 (f(v, z + e_1 - e_2) - f(v, z))$$
$$+ \kappa_5' z_2 (f(v, z - e_2) - f(v, z)) + \kappa_6'(M - z_1 - z_2)(f(v, z + e_1) - f(v, z))$$
$$+ \kappa_7'(M - z_1 - z_2)(f(v, z + e_2) - f(v, z)),$$

$$|\varepsilon_f^N(v, z)| \leq \tfrac{1}{2} N^{-1}(v_1 z_1 (\kappa_1 + \kappa_2) + \kappa_3 z_2 + \kappa_6'(M - z_1 - z_2) + \kappa_8 v_3 z_1)||D^2f||_\infty.$$

$$(4.13)$$

For bounding the two fast variables, we have that $0 \leq Z_1^N, Z_2^N \leq M$, and

$$\int_0^T (Z_N^1(t))^k dt = O(1), \qquad \int_0^T (Z_N^2(t))^k dt = O(N^{-2}),$$
$$\int_0^T (M - (Z_N^1 + Z_N^2)(t))^k dt = O(N^{-1}).$$

1. The compact containment condition for $(V^N)_{N=1,2,\ldots}$ follows from the fact that $V_1^N + V_2^N + V_3^N + N^{-1}(Z_2^N + Z_3^N)$ is a constant. Moreover, (2.4)–(2.6) will follow from the above boundedness assertions. So, for the generator calculations, assume that $f \in \mathcal{C}_c^\infty(\mathbb{R}^2)$ only depends on v. Taking, for some functions $a_1, \ldots, a_4$ (recall this ansatz from (3.12))

$$g_N(v, z) = (a_1 z_1 + a_2 z_2) D_1 f(v) + (a_3 z_1 + a_4 z_2) D_3 f(v),$$

we find

$$(G_0^N f + G_2^N g_N)(v, z) - \varepsilon_f^N(v, z)$$
$$= -v_1 z_1 \big(\kappa_1 D_1 f(v) + \kappa_2 D_1 f(v)\big) + \kappa_3 z_2 D_1 f(v) + \kappa_6'(M - z_1 - z_2) D_3 f(v)$$
$$+ \kappa_8 v_3 z_1 (D_1 - D_3) f(v) + \kappa_1 v_1 z_1 ((a_2 - a_1) D_1 f(v) + (a_4 - a_3) D_3 f(v))$$
$$+ \kappa_2 v_1 z_1 (-a_1 D_1 f(v) - a_3 D_3 f(v)) + \kappa_3 z_2 ((a_1 - a_2) D_1 f(v) + (a_3 - a_4) D_3 f(v))$$
$$+ \kappa_4' z_2 ((a_1 - a_2) D_1 f(v) + (a_3 - a_4) D_3 f(v)) + \kappa_5' z_2 (-a_2 D_1 f(v) - a_4 D_3 f(v))$$
$$+ \kappa_6'(M - z_1 - z_2)(a_1 D_1 f(v) + a_3 D_3 f(v)) + \kappa_7'(M - z_1 - z_2)(a_2 D_1 f(v) + a_4 D_3 f(v))$$
$$= M((\kappa_6' a_1 + \kappa_7' a_2) D_1 f(v) + (\kappa_6'(a_3 + 1) + \kappa_7' a_4) D_3 f(v))$$
$$+ z_1 \Big(D_1 f(v)\big(-v_1 \kappa_1 - v_1 \kappa_2 + \kappa_8 v_3 + \kappa_2 v_1 (a_2 - a_1) - \kappa_2 v_1 a_1 - \kappa_6' a_1 - \kappa_7' a_2\big)$$
$$+ D_3 f(v)\big(-\kappa_6' - \kappa_8 v_3 + \kappa_1 v_1 (a_4 - a_3) - \kappa_2 v_1 a_3 - \kappa_6' a_3 - \kappa_7' a_4\big)\Big)$$
$$+ z_2 \Big(D_1 f(v)\big(\kappa_3 + \kappa_3 (a_1 - a_2) + \kappa_4'(a_1 - a_2) - \kappa_5' a_2 - \kappa_6' a_1 - \kappa_7' a_2\big)$$
$$+ D_3 f(v)\big(-\kappa_6' + \kappa_3 (a_3 - a_4) + \kappa_4'(a_3 - a_4) - \kappa_5' a_4 - \kappa_6' a_3 - \kappa_7' a_4\big)\Big).$$

If we set $a_1, \ldots, a_4$ such that the terms proportional to $z_1 D_1, z_1 D_3, z_3 D_1$ and $z_3 D_3$ vanish, we obtain

$$Gf(v) := \lim_{N \to \infty} (G_0^N f + G_1^N g)(v, z) - \varepsilon_f^N(v, z)$$
$$= M \lim_{N \to \infty} (a_1 \kappa_6' + a_2 \kappa_7') D_1 f(v) + M(\kappa_6'(a_3 + 1) + \kappa_7') D_3 f(v)$$
$$= M(-\lambda_1 v_1 D_1 f + \lambda_2 v_1 (D_3 f - D_1 f) + \kappa_8 v_3 (D_1 f - D_3 f))$$

for λ_1, λ_2 as in (4.12). Since G is the generator of V, we are done.

    2. The generator of (U^N, Z^N) reads

$$
\begin{aligned}
L^N f(u,z) = {} & \kappa_1(Nv_1 + N^{1/2}u_1)z_1(f(u - N^{-1/2}e_1, z - e_1 + e_2) - f(u,z)) \\
& + \kappa_2(Nv_1 + N^{1/2}u_1)z_1(f(u - N^{-1/2}e_1, z - e_1) - f(u,z)) \\
& + N\kappa_3 z_2(f(u + N^{-1/2}e_1, z + e_1 - e_2) - f(u,z)) \\
& + N\kappa_4' z_2(f(u, z + e_1 - e_2) - f(u,z)) \\
& + N\kappa_5' z_2(f(u, z - e_2) - f(u,z)) \\
& + N\kappa_6'(M - z_1 - z_2)(f(u + N^{-1/2}e_3, z + e_1) - f(u,z)) \\
& + N\kappa_7'(M - z_1 - z_2)(f(u, z + e_2) - f(u,z)) \\
& + \kappa_8(Nv_3 + N^{1/2}u_3)z_1(f(u + N^{-1/2}(e_1 - e_3), z) - f(u,z)) \\
& - MN^{1/2}((-(\lambda_1 + \lambda_2)v_1 + \kappa_8 v_3)D_1 f(u) + (\lambda_2 v_1 - \kappa_8 v_3)D_3 f(u)) \\
= {} & (L_0^N f + N^{1/2} L_1^N f + N L_2^N f)(u,z)
\end{aligned}
$$

with (writing D_{11}, D_{13} and D_{33} for all the second derivatives with respect to v_1 and v_3, and $||D^3 f||_\infty$ for the supremum of all third derivatives of f)

$$
\begin{aligned}
L_0^N f(u,z) = {} & -\kappa_1 u_1 z_1 D_1 f(u, z - e_1 + e_2) + \tfrac{1}{2}\kappa_1 v_1 z_1 D_{11} f(u, z - e_1 + e_2) \\
& - \kappa_2 u_1 z_1 D_1 f(u, z - e_1) \\
& + \tfrac{1}{2}\kappa_2 v_1 z_1 D_{11} f(u, z - e_1) + \tfrac{1}{2}\kappa_3 z_2 D_{11} f(u, z + e_1 - e_2) \\
& + \tfrac{1}{2}\kappa_6'(M - z_1 - z_2)D_{33} f(u, z + e_1) + \kappa_8 u_3 z_1(D_1 - D_3)f(u,z) \\
& + \tfrac{1}{2}\kappa_8 v_3 z_1(D_{11} - 2D_{13} + D_{33})f(u,z) + \varepsilon_f^N(u,z,v), \\
L_1^N f(u,z) = {} & -\kappa_1 v_1 z_1 D_1 f(u, z - e_1 + e_2) + \kappa_1 u_1 z_1(f(u, z - e_1 + e_2) - f(u,z)) \\
& - \kappa_2 v_1 z_1 D_1 f(u, z - e_1) + \kappa_2 u_1 z_1(f(u, z - e_1) - f(u,z)) \\
& + \kappa_3 z_2 D_1 f(u, z + e_1 - e_2) \\
& + \kappa_6'(M - z_1 - z_2)D_3 f(u, z + e_1) + \kappa_8 v_3 z_1(D_1 - D_3)f(u,z) \\
& - M((-(\lambda_1 + \lambda_2)v_1 + \kappa_8 v_3)D_1 f(u,z) + (\lambda_2 v_1 - \kappa_8 v_3)D_3 f(u,z)), \\
L_2^N f(u,z) = {} & \kappa_1 v_1 z_1(f(u, z - e_1 + e_2) - f(u,z)) + \kappa_2 v_1 z_1(f(u, z - e_1) - f(u,z)) \\
& + \kappa_3 z_2(f(u, z + e_1 - e_2) - f(u,z)) + \kappa_4' z_2(f(u, z + e_1 - e_2) - f(u,z)) \\
& + \kappa_5' z_2(f(u, z - e_2) - f(u,z)) + \kappa_6'(M - z_1 - z_2)(f(u, z + e_1) - f(u,z)) \\
& + \kappa_7'(M - z_1 - z_2)(f(u, z + e_2) - f(u,z)), \\
|\varepsilon_f^N(u,z,v)| \leq {} & N^{-1/2}(((\kappa_1 + \kappa_2)u_1 z_1 + \kappa_8 u_3 z_1)||D^2 f||_\infty \\
& + \tfrac{1}{6}(v_1 z_1(\kappa_1 + \kappa_2) + \kappa_3 z_2 + \kappa_6'(M - z_1 - z_2) + \kappa_8 v_3 z_1)||D^3 f||_\infty).
\end{aligned}
$$

With $g_N(u,z) = (a_1 z_1 + a_2 z_2)D_1 f(u) + (a_3 z_1 + a_4 z_2)D_3 f(u)$, this time depending on u instead of v we obtain, if f only depends on u, that $L_1^N f + L_2^N g = o(N^{-1/2})$. We choose the ansatz (compare with (3.19))

$$
\begin{aligned}
h_N(u,z) = {} & (b_1 z_1 + b_2 z_2)D_1 f(u) + (b_3 z_1 + b_4 z_2)D_3 f(u) \\
& + \left(c_1 z_1 + c_2 z_2 + \binom{z_1}{2}d_1 + \binom{z_2}{2}d_2 + \binom{z_1 + z_2}{2}d_3\right)D_{11} f(u) \\
& + \left(c_3 z_1 + c_4 z_2 + \binom{z_1}{2}d_4 + \binom{z_2}{2}d_5 + \binom{z_1 + z_2}{2}d_6\right)D_{33} f(u) \\
& + \left(c_5 z_1 + c_6 z_2 + \binom{z_1}{2}d_7 + \binom{z_2}{2}d_8 + \binom{z_1 + z_2}{2}d_9\right)D_{13} f(u)
\end{aligned}
$$

for some $b_1, ..., b_4, c_1, ..., c_6, d_1, ..., d_9$. As in the above examples, plugging f, g_N with $a_1, ..., a_4$ as above, and h_N in $L_0 f + L_1 g_N + L_2 h_N$ leads to a term which depends only on u for the correct choice of $b_1, ..., b_4, c_1, ..., c_6, d_1, ..., d_9$. The corresponding linear system can readily be solved, and the result is (leaving all computations to a computer algebra system such as sagemath) leads for $N \to \infty$ to the limit

$$
\begin{aligned}
Lf(u) &= \lim_{N \to \infty} (L_0^N f + L_1^N g + L_2^N h - \varepsilon_f^N)(u) \\
&= M(-(\lambda_1 + \lambda_2)u_1 + \kappa_8 u_3)D_1 f(u) + M(\lambda_2 u_1 - \kappa_8 u_3)D_3 f(u) \\
&\quad + \tfrac{1}{2}(\lambda_1 v_1 D_{11} f(u) + (\lambda_2 v_1 + \kappa_8 v_3)(D_{11} - 2D_{13} + D_{33})f(u).
\end{aligned}
$$

This is the generator of (U_1, U_2). Again, showing the compact containment condition of $(U^N)_{N=1,2,...}$ as well as (2.4)–(2.6) works with the same arguments we already saw in the first examples. $\qquad \square$

A An immigration death process

We will now give a tool which helps to derive the conditions (2.4)–(2.6) in concrete examples.

Lemma A.1 (A process with immigration and death)**.** *Let $\beta \geq \alpha \geq 0, C_0 \geq 0, C_1 > 0$, and $k, T, a > 0$, and for each $N \in \mathbb{N}$, let $X^N = (X_t^N)_{t \geq 0}$ be a Markov-jump-process which increases at time t by 1 at rate $C_0^N N^\alpha$ and decreases at time t by 1 at rate $C_1^N N^\beta X^N(t)$ and $X_0^N = 0$, where $0 \leq C_0^N \leq C_0$ and $C_1^N \geq C_1$. Then,*

$$
\sup_{N \in \mathbb{N}} \mathbb{E}\Big[\int_0^T N^{\beta-\alpha}(X_t^N)^k dt \Big] < \infty \tag{A.1}
$$

and

$$
N^{-a}\mathbb{E}[\sup_{0 \leq t \leq T} (X_t^N)^k] \xrightarrow{N \to \infty} 0. \tag{A.2}
$$

Proof. We use some constant C, which does not depend on N, but which may change from line to line. With Fubini, we see that (A.1) follows from

$$
\mathbb{E}[(X_t^N)^k] \leq CN^{\alpha-\beta}, \qquad t \geq 0.
$$

We start the proof of this assertion by calculating

$$
\frac{d}{dt}\mathbb{E}[(X_t^N)^k] = C_0^N N^\alpha \mathbb{E}[(X_t^N + 1)^k - (X_t^N)^k] + C_1^N N^\beta \mathbb{E}[X_t^N (X_t^N - 1)^k - (X_t^N)^{k+1}].
$$

Using induction, we start with $k = 1$ and can solve this differential equation with $\mathbb{E}[X_0^N] = 0$, i.e.

$$
\mathbb{E}[X_t^N] = \frac{C_0^N}{C_1^N} N^{\alpha-\beta}(1 - e^{-C_1^N N^\beta t}) \leq CN^{\alpha-\beta}.
$$

From here, if we have shown the case $k - 1$, we see that $\mathbb{E}[(X_t^N + 1)^k - (X_t^N)^k] \leq C$, and the differential equation yields

$$
\frac{d}{dt}\mathbb{E}[(X_t^N)^k] \leq CN^\alpha + C_1 N^\beta \mathbb{E}[(X_t^N)^k].
$$

From here, Gronwall's Lemma shows the assertion for k.

For (A.2), we only need to consider the case $\beta = \alpha$ since this process dominates the process with $\beta > \alpha$. Note that X^N can be seen as the size of a population undergoing immigration (at individual rate $C_0 N^\alpha$) and death (at individual rate $C_1 N^\beta = C_1 N^\alpha$).

Let $\tau_n := \inf\{t : X_t^N > n\}$ and consider $X_{\tau_n}^N$. At this time, the population consists of individuals immigrating at times before τ_n, and we consider the oldest such individual. Necessarily, this individual has seen n individuals immigrating before τ_n which has probability γ_N^n for $\gamma_N := C_0^N/(C_0^N + C_1^N) \leq C_0/(C_0 + C_1) =: \gamma$. The rate by which individuals immigrate which see n immigration events before their own death is $C_0^N N^\alpha \gamma_N^n$, i.e.

$$\mathbb{P}(\sup_{0 \leq t \leq T} X_t^N > n) \leq 1 - \exp\Big(-C_1 N^\alpha \gamma_N^n T\Big).$$

We therefore write

$$\mathbb{E}[\sup_{0 \leq t \leq T}(X_t^N)^k] = k \int_0^\infty x^{k-1}\mathbb{P}(\sup_{0 \leq t \leq T} X^N(t) > x)dx$$
$$\leq k \int_0^{(\log N)^2} x^{k-1}dx + 2kC_1 N^\alpha \int_{(\log N)^2}^\infty x^{k-1}\gamma^x dx \leq C(\log N)^{2k},$$

and the assertion follows. $\qquad\square$

Supplementary Material

Accompanying commands for automated computation. The ancillary file with `sagemath`-commands [4], used for various computations in examples, can be found on https://arxiv.org/src/2111.15396v1/anc/ancillary.sage.

References

[1] Anderson, D. and T. Kurtz (2015). *Stochastic Analysis of Biochemical Systems*. Springer. MR3363610

[2] Ball, K., T. Kurtz, L. Popovic, and G. Rempala (2006). Asymptotic analysis of multiscale approximations to reaction networks. *Annals of Applied Probability 16*, 1925–1961. MR2288709

[3] Cappelletti, D. and C. Wiuf (2016). Elimination of intermediate species in multiscale stochastic reaction networks. *Ann. Appl. Probab. 26*, 2915–2958. MR3563198

[4] Developers, T. S., W. Stein, D. Joyner, D. Kohel, J. Cremona, and B. Eröcal (2020). Sagemath, version 9.0. http://www.sagemath.org.

[5] Haseltine, E. and J. Rawlings (2002). Approximate simulation of coupled fast and slow reactions for stochasticchemical kinetics. *J. Chem. Phys. 117*, 6959–6969.

[6] Hill, A. V. (1910). The possible effects of the aggregation of the molecules of hæmoglobin on its dissociation curves. *J Physiol 40*(iv–vii).

[7] Hoessly, L., C. Wiuf, and P. Xia (2021). On the Sum of Chemical Reactions. https://arxiv.org/pdf/2105.04353.pdf.

[8] Hutzenthaler, M., P. Pfaffelhuber, and C. Printz (2021). Stochastic averaging for multiscale Markov processes with an application to a Wright-Fisher model with fluctuating selection. https://arxiv.org/abs/1504.01508.

[9] Kang, H.-W. (2012). A multiscale approximation in a heat shock response model of *E. coli*. *BMC Systems Biology 21(6)*, 143.

[10] Kang, H. W. and T. Kurtz (2013). Separation of time-scales and model reduction for stochastic reaction models. *Ann. Appl. Probab. 23*, 529–583. MR3059268

[11] Kang, H.-W., T. Kurtz, and L. Popovic (2014). Central limit theorems and diffusion approximations for multiscale markov chain models. *Ann. Appl. Probab. 24*, 721–759. MR3178496

[12] Kurtz, T. (1970a). Limit theorems for sequences of jump markov processes approximating ordinary differential processes. *Journal of Applied Probability 8*, 344–356. MR0287609

[13] Kurtz, T. (1970b). Solutions of ordinary differential equations as limits of pure jump markov processes. *Journal of Applied Probability 7*, 49–58. MR0254917

[14] Kurtz, T. (1992). Averaging for martingale problems and stochastic approximation. In *Applied stochastic analysis (New Brunswick, NJ, 1991), volume 177 of Lecture Notes in Control and Inform. Sci.*, pp. 186–209. Springer, Berlin. MR1169928

[15] Rao, C. and A. Arkin (2003). Stochastic chemical kinetics and the quasi-steady-state assumption:Application to the Gillespie algorithm. *J. Chem. Phys. 118*, 4999–5011.

[16] Roussel, M. R. (2019). Heineken, Tsuchiya and Aris on the mathematical status of the pseudo-steady state hypothesis: A classic from volume 1 of Mathematical Biosciences. *Math Biosci 318*, 108274. MR4030296

[17] Saez, M., C. Wiuf, and E. Feliu (2017). Graphical reduction of reaction networks by linear elimination of species. *J. Math. Biol. 74*, 195–237. MR3590681

Electronic Journal of Probability
Electronic Communications in Probability

Advantages of publishing in EJP-ECP

- Very high standards

- Free for authors, free for readers

- Quick publication (no backlog)

- Secure publication (LOCKSS[1])

- Easy interface (EJMS[2])

Economical model of EJP-ECP

- Non profit, sponsored by IMS[3], BS[4], ProjectEuclid[5]

- Purely electronic

Help keep the journal free and vigorous

- Donate to the IMS open access fund[6] (click here to donate!)

- Submit your best articles to EJP-ECP

- Choose EJP-ECP over for-profit journals

---

[1] LOCKSS: Lots of Copies Keep Stuff Safe http://www.lockss.org/
[2] EJMS: Electronic Journal Management System: https://vtex.lt/services/ejms-peer-review/
[3] IMS: Institute of Mathematical Statistics http://www.imstat.org/
[4] BS: Bernoulli Society http://www.bernoulli-society.org/
[5] Project Euclid: https://projecteuclid.org/
[6] IMS Open Access Fund: https://imstat.org/shop/donation/