Experimental Biological Protocols with Formal Semantics

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Abstract. Both experimental and computational biology is becoming increasingly automated. Laboratory experiments are now performed automatically on high-throughput machinery, while computational models are synthesized or inferred automatically from data. However, integration between automated tasks in the process of biological discovery is still lacking, largely due to incompatible or missing formal representations. While theories are expressed formally as computational models, existing languages for encoding and automating experimental protocols often lack formal semantics. This makes it challenging to extract novel understanding by identifying when theory and experimental evidence disagree due to errors in the models or the protocols used to validate them. To address this, we formalize the semantics of a core protocol language as a stochastic hybrid process, which provides a unified description for the models of biochemical systems being experimented on, together with the discrete events representing the liquid-handling steps of biological protocols. Such a representation captures uncertainties in equipment tolerances, making it a suitable tool for both experimental and computational biologists. We illustrate how the proposed protocol language can be used for automated verification and synthesis of laboratory experiments on case studies from the fields of chemistry and molecular programming.

1 Introduction

The classical cycle of observation, hypothesis formulation, experimentation, and falsification, which has driven scientific and technical progress since the scientific revolution, is lately becoming automated in all its separate components. Data gathering is conducted by high-throughput machinery. Models are automatically synthesized, at least in part, from data [6,11,4]. Experiments are selected to maximize knowledge acquisition. Laboratory protocols are run under reproducible and auditable software control. However, integration between these automated components is lacking. Theories are not placed in the same formal context as the (coded) protocols that are supposed to test them. Theories talk about changing in physical quantities, while protocols talk about steps carried out by machines: neither knows about the other, although they both try to describe the same process. The consequence is that often it is hard to tell what happened when experiments and models do not match: was it an error in the model, or an error in the protocol? Often both the model and the protocol have unknown parameters: do we use the experimental data to fit the model or to fit the protocol?

When most activities are automated, we need a way to answer those questions that is equally automated.

In this paper, we present a core language to model experimental biological protocols that gives an integrated description of the protocol and of the underlying molecular process. A basic example of experimental biological protocol is shown in Figure 1.

From this integrated representation both the model of a phenomenon (for possibly automated mathematical analysis), and the steps carried out to test it (for automated execution by lab equipment) can be separately extracted. This is essential to perform automated model synthesis and falsification by taking into account uncertainties in the both model structure and in equipment tolerances. We map our language into a *Piecewise Deterministic Markov Process* (PDMP). That is, a class of Markov stochastic hybrid processes where the continuous variables evolve according to *ordinary differential equations* (ODEs) and the discrete variables evolve by means of random jumps [12]. The discrete dynamics are used to map the discrete operations of a lab protocol, while continuous dynamics model the evolution of the physical variables. In our language physical variables are described with Chemical Reaction Networks (CRNs), a widely used formalism to model molecular interactions [16]. Our goal is to define a simple core language and focusing on formalizing its semantics. We then show how our language can easily be extended to collect observations of the process and to model complicate protocols. Giving to the language a formal semantics in terms of a PDMP allows us to include in our semantics the uncertainties intrinsic in the discrete operations of an experimental protocol. Uncertainties for experimental protocols have also been standardized (standards ISO 17025 and 8655). On examples from chemistry and molecular programming, we demonstrate how our integrated representation allows one to perform analysis and synthesis of both the discrete steps of the protocol and of the underlying physical system.

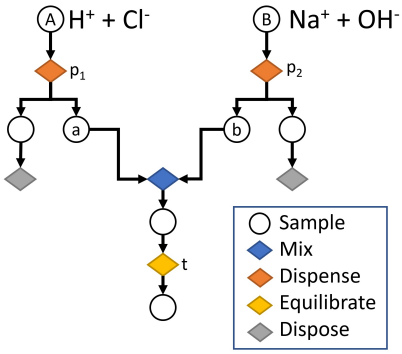

Fig. 1: Graphical representation of an acid-base titration protocol. The protocol is initialized with samples A (containing H^+ and Cl^-) and B (containing Na^+ and OH^-). Some fraction of each sample (p_1 and p_2) is mixed together and the resulting sample is let to equilibrate for t seconds.

Related Work Several factors contribute to the growing need for a formalization of experimental protocols in biology. First, better record-keeping of experimental operations is recognized as a step towards tackling the reproducibility

crisis in biology [17]. Second, the emergence of cloud labs [18] creates a need for precise, machine-readable descriptions of the experimental steps to be executed. To address these needs, frameworks allowing protocols to be recorded, shared, and reproduced locally or in a remote lab have been proposed. These frameworks introduce different programming languages for experimental protocols including BioCoder [3], Autoprotocol, and Antha [24]. These languages provide expressive, high-level protocol descriptions but consider each experimental sample as a labelled black-box. This makes it challenging to study a protocol together with the biochemical systems it manipulates in a common framework. In contrast, we consider a simpler set of protocol operations but capture the details of experimental samples, enabling us to track properties of chemical species (e.g. amounts, concentrations, etc) as they react during the execution of a protocol. This allows us to formalize and verify requirements for the correct execution of a protocol or to optimize various protocol or system parameters to satisfy these specifications.

2 Background

We first introduce the formalism of PDMP, which we use to model experimental protocols. Then, we introduce Chemical Reaction Networks (CRN), which are used to model the underlying physical process.

2.1 Piecewise Deterministic Markov Process

The syntax of a PDMP is given as follows.

Definition 1 *A Piecewise Deterministic Markov Process (PDMP) $\mathcal{H}$ is a tuple $\mathcal{H} = (\mathcal{Q}, d, \mathcal{G}, F, \Lambda, R)$, where*

- $\mathcal{Q} = \{q_1, ..., q_{|\mathcal{Q}|}\}$ *is the set of* discrete modes
- $d : \mathcal{Q} \to \mathbb{N}$ *is a map such that* $\mathbb{R}^{d(q)}$, *is the state space of the* continuous dynamics *for state q. The hybrid state space is defined as* $\mathcal{D} = \cup_{q\in\mathcal{Q}}\{q\} \times \mathbb{R}^{d(q)}$
- $\mathcal{G} : \mathcal{Q} \times \mathbb{R}^{d(q)} \to \{0, 1\}$ *is a set of guards*
- $F : \mathcal{Q} \times \mathbb{R}^{d(q)} \to \mathbb{R}^{d(q)}$ *is a family of vector fields*
- $\Lambda : \mathcal{S} \times \mathcal{Q} \to \mathbb{R}_{\geq 0}$ *is an* intensity function*, where for $(q_i, x) \in \mathcal{S}, q_j \in \mathcal{Q}$, we define $\Lambda((q_i, x), q_j) = \lambda_{i,j}(x)$ and $\sum_{q_j \neq q_i} \lambda_{i,j}(x) = \lambda_{q_i}(x)$*
- $R : \mathcal{B}(\mathcal{S}) \times \mathcal{S} \to [0.1]$ *is the* reset *function, which assigns to each $(q, x) \in \mathcal{S}$ a measure $R(\cdot, q, x)$ on $(\mathcal{S}, \mathcal{B}(\mathcal{S}))$.*

Let $\mathcal{B}(\mathcal{S})$ be the smallest $\sigma-$algebra on $\mathcal{S}$ containing all the sets of the form $\cup_{q\in\mathcal{Q}}\{q\}\times A_q$, where A_q is a Borel subeset of $\mathbb{R}^{d(q)}$. For $t \in \mathbb{R}_{\geq 0}, q \in \mathcal{Q}, x \in \mathbb{R}^{d(q)}$, we call $\Phi(q, t, x)$, the solution of the following differential equation

$$\frac{d\Phi(q, t, x)}{dt} = F(q, \Phi(q, t, x)), \quad \Phi(q, 0, x) = x.$$

The solution of a PDMP is a stochastic process $Y = (\alpha, X)$, whose semantics is classically defined according to the notion of execution (see Definition 2 below) [13]. In order to introduce such a notion, we define the exit time $t^*(q, x, \mathcal{G})$ as

$$t^*(q, x, \mathcal{G}) = \inf\{t \in \mathcal{R}_{\geq 0} \,|\, \mathcal{G}(q, \Phi(q, t, x)) = 1\} \tag{1}$$

and the *survival function* $f(q,t,x) = \begin{cases} e^{-\int_0^t \lambda_q(\Phi(q,\tau,x))d\tau} & \text{if } t < t^*(q,x,\mathcal{G}) \\ 0 & \text{otherwise.} \end{cases}$.
Here $t^*(q,x,\mathcal{G})$ represents the first time instant, starting from state (q,x), when the guard set is reached by a solution of the process; further $f(q,t,x)$ denotes the probability that the system remains within q, starting from x, at time t [12], which depends on random arrivals induced by the intensity function Λ. The semantics of a PDMP is provided next.

Definition 2 *(Execution of PDMP $\mathcal{H}$)*

Set $t := 0$
Set $(\alpha(0), X(0)) := q_0, x_0$
While $t < \infty$
Extract $\mathbb{R}_{\geq 0}$*-valued random variable* T *such that*

$$Prob(T > \bar{t}) = f(\alpha(t), \bar{t}, X(t))$$

$\forall \tau \in [t, t+T)$ ***Set*** $(\alpha(\tau), X(\tau)) := (\alpha(t), \Phi(\alpha(t), \tau - t, X(t)))$
If $t + T < \infty$
Extract $(\alpha(t+T), X(t+T))$ *according to*
$R(\cdot, (\alpha(t), \Phi(\alpha(t), T, X(t))))$
End If
Set $t := t + T$
End While

Let $T < t^*(q_i, \bar{x}, \mathcal{G})$ be the *dwelling time* in state $q_i \in \mathcal{Q}$, with $\bar{x}$ such that $\Phi(q_i, T, \bar{x}) = x$, $(q_j, A) \in \mathcal{B}(\mathcal{S})$. Assume that x is such that $\mathcal{G}(x, q_i) = 0$. Then, the reset has the following form, which results from a transition that is not due to the crossing of a guard:

$$R((q_j, A), (q_i, x)) = R^c(A|q_j, (q_i, x)) \frac{\Lambda^T_{q_i,q_j}(\Phi, x)}{\sum_{q_k \neq q_i} \Lambda^T_{q_i,q_k}(\Phi, x)}, \tag{2}$$

where $\Lambda^T_{q_i,q_j}(\Phi, x) = \int_0^T \lambda_{i,j}(\Phi(s, q_i, x))ds$, and

$$R^c(A|q_j, (q_i, x)) = Prob(X(T) \in A | \alpha(T) = q_j, (\alpha(0) = q_i, X(0) = x))$$

is the conditional reset of the continuous dynamics.

2.2 Chemical Reaction Networks

A CRN $\mathcal{C} = (\mathcal{A}, \mathcal{R})$ is a pair of finite sets, where $\mathcal{A}$ denotes a set of *chemical species*, $|\mathcal{A}|$ is its cardinality, and $\mathcal{R}$ denotes a set of reactions. A *reaction* $\tau \in \mathcal{R}$ is a triple $\tau = (r_\tau, p_\tau, k_\tau)$, where $r_\tau \in \mathbb{N}^{|A|}$ is the *source complex*, $p_\tau \in \mathbb{N}^{|A|}$ is the *product complex* and $k_\tau \in \mathbb{R}_{>0}$ is the coefficient associated with the rate of the reaction.The quantities r_τ and p_τ represent the stoichiometry of reactants and products. Given a reaction $\tau_1 = ([1,0,1],[0,2,0],k_1)$ we often refer to it visually as $\tau_1 : \lambda_1 + \lambda_3 \rightarrow^{k_1} 2\lambda_2$. The *net change* associated to τ is defined by $\upsilon_\tau = p_\tau - r_\tau$.

Many models have been introduced to study CRNs [9,7,15,8]. Here we consider the *reaction rate equations* [15], which describe the time evolution of the concentration of the species in C, in a sample of temperature T and volume V, as follows:

$$\frac{d\Phi(t)}{dt} = F(t) = \sum_{\tau \in \mathcal{R}} \upsilon_\tau \cdot \gamma_S(\Phi(t), k_\tau, V, T), \tag{3}$$

where $\gamma_S(\Phi(t), k_\tau, V, T))$ is the propensity rate, and in case of mass action kinetics we have

$$\gamma_S(\Phi(t), k_\tau, V, T)) = k_\tau(T) \prod_{S \in \Lambda} \Phi_S^{r_{S,\tau}}(t),$$

where Φ_S and $r_{S,\tau}$ are the components of vectors Φ and r_τ relative to species S, and where in $k_\tau(T)$ we make explicit the dependence from temperature T.

Definition 3 *(Chemical Reaction System) A* chemical reaction system *(CRS)* $C = (\mathcal{A}, \mathcal{R}, x_0)$ *is defined as a tuple, where* $(\mathcal{A}, \mathcal{R})$ *is a CRN and* $x_0 \in \mathbb{N}^{|\Lambda|}$ *represents its initial condition.*

Example 1. Consider the CRS $C = (\mathcal{A}, \mathcal{R}, x_0)$, evolving in a volume V and at temperature T, where $\mathcal{A} = \{H_2O, Na^+, OH^-, Cl^-, H^+\}$ and $\mathcal{R}$ is composed of the following reactions:

$$Na^+ + OH^- + H^+ + Cl^- \rightarrow^k H_2O + Na^+ + Cl^-$$

where $k = 2.81e^{-10}$ is the rates at temperature $T = 298$ Kelvin. Then, According to Equation (3), we have that the state of H^+ is given by the solution of the following ordinary differential equation:

$$\frac{dH^+(t)}{dt} = -kNa^+(t)OH^-(t)H^+(t)Cl^-(t),$$

with $H^+(0) = \frac{x_{0,H^+}}{V}$, where x_{0,H^+} is the component of x_0 relative to H^+.

3 A Language for Experimental Biological Protocols

In this section we introduce the syntax of a language for modelling experimental protocols. A formal semantics of the language, based on denotational semantics [25], is then discussed. We model the physical process underlying a biological experimental protocol as a CRS. As a consequence, in order to introduce formal semantics for experimental protocols, we first need to define semantics for a CRS, which has been only introduced informally in the previous section.

Let $S = (\mathbb{R}^{|\mathcal{A}|} \times \mathbb{R}_{\geq 0} \times \mathbb{R}_{\geq 0})$ be a sample. We define the semantics for a CRS as follows.

Definition 4 *(CRS Semantics) Let* $\mathcal{C} = (\mathcal{A}, \mathcal{R})$ *be a CRN,* $x_0 \in \mathbb{R}_{\geq 0}^{|\mathcal{A}|}$, $V, T \in \mathbb{R}_{\geq 0}$ *be the initial concentration (moles), volume (liters) and temperature (degrees Kelvin). Call* $F(V,T) : \mathbb{R}^{|\mathcal{A}|} \rightarrow \mathbb{R}^{|\mathcal{A}|}$ *the drift at volume* V *and temperature* T

for $\mathcal{C}$. Then, the semantics of the CRS $(\mathcal{A}, \mathcal{R}, x_0)$ at volume V, temperature T and time t, for a time horizon $H \in \mathbb{R}_{\geq 0} \cup \{\infty\}$,

$$[\![\cdot]\!] : (CRS \times \mathbb{R}_{\geq 0} \times \mathbb{R}_{\geq 0}) \to \mathbb{R}_{\geq 0} \cup \{\infty\} \to \mathbb{R}_{\geq 0} \to S$$

is defined as

$$[\![((\mathcal{A},\mathcal{R}, x_0), V, T)]\!](H)(t) =$$

$$\begin{array}{l} \textit{let } G : [0...H) \to \mathbb{R}^{|\mathcal{A}|} \textit{ be the solution of } G(t') = x_0 + \displaystyle\int_0^{t'} F(V,T)(G(s))ds \\ (G(t), V, T) \end{array}$$

where the above operation reads as follows: 'first line' = 'third line', where G is defined as in 'second line'. If for such an H, G is not unique, then we say that $[\![((\mathcal{A}, \mathcal{R}, x_0), V, T)]\!]\ (H)(t)$ is ill posed.

In Definition 4 we have explicitly introduced a dependence on a time horizon H, because it may happen that the solution of the rate equations is defined only for a finite time horizon [15].

3.1 A Language for Experimental Protocols

Our goal is to build a simple core language that gives an integrated representation of a discrete protocol within the physical process being implemented on. We consider the following language modelling the basic operation of a lab protocol.

Definition 5 *(Syntax of a Protocol) Given a set of variables Var, the syntax of a protocol P for a given fixed CRN $\mathcal{C} = (\mathcal{A}, \mathcal{R})$ is*

$$\begin{array}{rll} P = & x & \textit{(sample variable)} \\ & (x_0, V, T) & (\textit{initial condition}) \\ & Mix(P_1, P_2) & (\textit{mix samples}) \\ & \textit{let } x = P_1 \textit{ in } P_2 & \textit{(define variable)} \\ & \textit{let } x, y = Dispense(P_1, p) \textit{ in } P_2 & \textit{(dispense samples)} \\ & Equilibrate(P, t) & (\textit{let time pass}) \\ & Dispose(P) & (\textit{discard } P) \end{array}$$

where $T, V, t \in \mathbb{R}_{\geq 0}, x, y \in Var$, $p \in \mathbb{R}_{(0,1)}$. Moreover, let-bound variables must occur exactly once (that is, be free) in P_2.

A protocol P yields a sample, which is the result of operations of Equilibrate, Mix, Dispose and Dispense, over a CRS. This syntax allows one to create and manipulate new samples using Mix (put together different samples), Dispense (separate samples) and Dispose (discard samples) operations. Note that the CRN is common for all samples. However, different samples may have different initial conditions. The single-occurrence (linearity) restriction implies that a sample cannot be duplicated or eliminated from the pool.

Example 2. We use $let\, x, \_ = Dispense(P_1, p)\, in\, P_2$ as a short-hand for $let\, x, y = Dispense(P_1, p)\, in\, Mix(Dispose(y), x)$. The protocol (call it Pro_1) represented graphically in Figure 1 is defined formally as

$$\begin{aligned} Pro_1 = & let\, A = ([(H^+, 0.1M); (Cl^-, 0.1M)], 1.0mL, 25.0^oC)\, in \\ & let\, B = ([(Na^+, 0.1M); (OH^-, 0.1M)], 1.0mL, 25.0^oC)\, in \\ & let\, a, \_ = Dispense(A, p_1)\, in \\ & let\, b, \_ = Dispense(B, p_2)\, in \\ & Equilibrate(Mix(a, b), t). \end{aligned}$$

In order to define the semantics of a protocol we introduce the following definitions.

Definition 6 *(Free Variables) The set of Free Variables (FV) of a protocol P is defined inductively as follows:*

$$\begin{aligned} & FV(x) = \{x\} \\ & FV((x_0, V, T)) = \{\} \\ & FV(Mix(P_1, P_2)) = FV(P_1) \cup FV(P_2) \\ & FV(let\, x = P_1\, in\, P_2) = FV(P_1) \cup (FV(P_2) - \{x\}) \\ & FV(let\, x, y = Dispense(P_1, p)\, in\, P_2) = FV(P_1) \cup (FV(P_2) - \{x, y\}) \\ & FV(Equilibrate(P, t)) = FV(P) \\ & FV(Dispose(P)) = FV(P). \end{aligned}$$

We define the operation of substitution of a protocol into a variable as follows.

Definition 7 *(Substitution)* $P_1\{x \leftarrow P_2\}$ *is defined inductively as follows:*

$$\begin{aligned} & x\{x \leftarrow P\} = P \\ & y\{x \leftarrow P\} = y,\ for\ x \neq y \\ & Mix(P_1, P_2)\{x \leftarrow P_3\} = Mix(P_1\{x \leftarrow P_3\}, P_2\{x \leftarrow P_3\}) \\ & (let\, x = P_1\, in\, P_2)\{x \leftarrow P_3\} = (letx = P_1\{x \leftarrow P_3\}\, in\, P_2) \\ & (let\, y = P_1\, in\, P_2)\{x \leftarrow P_3\} = (let\, y = P_1\{x \leftarrow P_3\} in P_2\{x \leftarrow P_3\}), \\ & \ for\ x \neq y\ and\ y \notin FV(P_3) \\ & Equilibrate(P, t)\{x \leftarrow P_1\} = Equilibrate(P\{x \leftarrow P_1, t\}) \\ & Dispose(P)\{x \leftarrow P_1\} = Dispose(P\{x \leftarrow P_1\}). \end{aligned}$$

The following equivalences can be shown structurally, based on the definitions above.

Proposition 1. *(Equivalence Relationships)*

$$\begin{aligned} & let\, x = P_1\, in\, P_2 \ = \ P_2\{x \leftarrow P_1\} \\ & let\, x = P_1\, in\, P_2 \ = \ let\, y = P_1\, in\, (P_2\{x \leftarrow y\})\ for\ y \notin FV(P_2). \end{aligned}$$

3.2 Deterministic Semantics of a Protocol

We introduce the deterministic semantics for a protocol. Then, in the next Section, we extend such a semantics in order to take into account errors and inaccuracies within the protocol, which in practice may be quite relevant.

The deterministic semantics of a protocol P for a CRN $\mathcal{C} = (\mathcal{A}, \mathcal{R})$, under a given environment $\rho : Var \to S$, is a function $[\![P]\!]^\rho : (Var \to S) \to S$ that is defined inductively as follows.

Definition 8 *(Deterministic Semantics of a Protocol) Let $S = (\mathbb{R}^{|\mathcal{A}|} \times \mathbb{R}_{\geq 0} \times \mathbb{R}_{\geq 0})$, then the deterministic semantics of a protocol P for CRN $\mathcal{C} = (\mathcal{A}, \mathcal{R})$, under environment $\rho : Var \to S$ is defined inductively as follows*

$$
\begin{aligned}
&[\![x]\!]^\rho = \rho(x)\\
&[\![x_0, V, T]\!]^\rho = (x_0, V, T)\\
&[\![Mix(P_1, P_2)]\!]^\rho =\\
&\quad let\,(x_0^1, V_1, T_1) = [\![P_1]\!]^\rho\\
&\quad let\,(x_0^2, V_2, T_2) = [\![P_2]\!]^\rho\\
&\quad (\frac{x_0^1 V_1 + x_0^2 V_2}{V_1 + V_2}, V_1 + V_2, \frac{T_1 V_1 + T_2 V_2}{V_1 + V_2})\\
&[\![let\, x = P_1\, in\, P_2]\!]^\rho =\\
&\quad let\,(x_0, V, T) = [\![P_1]\!]^\rho\\
&\quad let\,\rho_1 = \rho\{x \leftarrow (x_0, V, T)\}\\
&\quad [\![P_2]\!]^{\rho_1}\\
&[\![let\, x, y = Dispense(P_1, p)\, in\, P_2]\!]^\rho =\\
&\quad let\,(x_0, V, T) = [\![P_1]\!]^\rho\\
&\quad let\,\rho_1 = \rho\{x \leftarrow (x_0, V \cdot p, T), y \leftarrow (x_0, V \cdot (1-p), T)\}\\
&\quad [\![P_2]\!]^{\rho_1}\\
&[\![Equilibrate(P, t)]\!]^\rho =\\
&\quad let\,(x_0, V, T) = [\![P]\!]^\rho\\
&\quad [\![(\mathcal{A}, \mathcal{R}, x_0), V, T)]\!](H)(t)\\
&[\![Dispose(P)]\!]^\rho = (0^{|\Lambda|}, 0, 0),
\end{aligned}
$$

where $H \in \mathbb{R}_{\geq 0}$ is such that for any Equilibrate(P, t), $[\![(\mathcal{A}, \mathcal{R}),\, x_0, V, T)]\!](H)(t)$ is well posed. If such an H does not exist, we say that P is ill posed.

The above semantics identifies a protocol which outputs the concentration of the species, the volume, and the temperature of the sample at final time.

3.3 Deterministic Semantics of a Protocol as a PDMP

Given a protocol P and an environment ρ, $[\![P]\!]^\rho$ induces semantics that correspond to the solution of a PDMP $\mathcal{H} = (\mathcal{Q}, d, \mathcal{G}, F, \Lambda, R)$ as per Definitions

1 and 2. In the corresponding PDMP model $\mathcal{H}$, $\mathcal{Q}$ represents the set of discrete operations, so that $d(q) = |\mathcal{A}| + 1$ denotes the continuous dimension (the number of continuous variables). The vector field F is given by Definition 4, with an additional clock variable *time* representing time as $\frac{dtime}{dt} = 1$. For each $Equilibrate(P, t)$ step, there is a guard set defined as $time \geq t$: when a trajectory enters this guard, an associated reset is modeled with the identity function. The resulting process is a PDMP without random jumps (that is, where $\Lambda(q, x) = 0$ for any $q \in \mathcal{Q}, x \in \mathcal{R}^{|\mathcal{A}|+1}$) and with non-probabilistic resets R. As such, the resulting PDMP is also a (non-probabilistic) hybrid model [2]. We elucidate this with the next example.

Example 3. Consider the protocol Pro_1 introduced in Example 2. The CRN of the system comprises the reactions given in the CRN in Example 1. According to Definition 11, the state of variable H^+ in time is given by the solution of the following equation:

$$H^+(t) = H^+(0) - \int_0^t kNa^+(s)OH^-(s)H^+(s)Cl^-(s)ds,$$

where $H^+(0) = \frac{p_1 0.1 + p_2 10^{-7.4}}{p_1 + p_2}$. Then, given an environment ρ, $[\![Pro_1]\!]^\rho$ is the solution of the PDMP $\mathcal{H} = (\mathcal{Q}, d, \mathcal{G}, F, \Lambda, R)$, where $\mathcal{Q} = \{q\}$; $d(q) = \mathbb{R}^8$; $\mathcal{G}(q, x) = 1 \text{ iff } x_{time} \geq t$, where x_{time} is the component of x relative to variable *time*; and F is given by Definition 11. Notice again that $\Lambda(q, x) = 0$ for any x and $\mathcal{R}(q, x) = \delta(x)$, where $\delta(x)$ is the Dirac delta function centered at x.

3.4 Stochastic Semantics of a Protocol, and Interpretation as a PDMP

The semantics of Definition 11 are fully deterministic, and indeed are shown to map into a fully non-probabilistic PDMP model. However, it is often the case that operations of *Dispense* and *Equilibrate* are stochastic in nature, due to the fact that they are performed by humans and in view of experimental inaccuracies related to lab equipment. In what follows, we encompass this features by extending the previously defined semantics with stochasticity. More precisely,

- in the $Equilibrate(P, t)$ step, time is sampled from a distribution;
- the resulting volume after a *Dispense* step is sampled from a distribution.

The first characteristic models the fact that in real experiments the system is not equilibrated for exactly t seconds, as it may start or be stopped at different time instants, and accounts for the fact that after a mix of samples well mixed conditions are not reached instantaneously, whereas the second takes into account the error of pipetting devices, whose ranges and parameters have been standardized (standard ISO 8655). For the first feature, consider the function $\mathcal{T}(t', t) = e^{-\frac{t'}{t}}$, defined for two values $t', t \in \mathbb{R}_{\geq 0}$. This function corresponds to the density function of an exponential random variable, modelling random arrivals. For the second function, let $\mathcal{B}(\mathbb{R}^m_{\geq 0})$ be the Borel sigma-algebra over $\mathbb{R}^m_{\geq 0}$, $m > 0$. Then,

we consider the following function $\mathcal{D} : \mathcal{B}(\mathbb{R}_{(0,1)}) \times \mathbb{R}_{\geq 0} \times \mathbb{R}_{[0,1]} \to [0,1]$, which assigns to $\mathcal{D}(\cdot, V, p)$ a probability measure in $\mathcal{B}(\mathbb{R}_{[0,1]})$. Function $\mathcal{D}$ is used to reset the volume randomly, after a discrete operation. (As an anticipation of results, notice that both functions $\mathcal{T}$ and $\mathcal{D}$ can be mapped to elements in the syntax in a PDMP model.)

We define the *Stochastic Semantics* of a protocol as an extension of the deterministic ones in Definition 11. For the sake of compactness, we explicitly write only the operators that differ from the earlier ones.

Definition 9 *(Stochastic Semantics of a Protocol) Let $S = (\mathbb{R}^{|\mathcal{A}|} \times \mathbb{R}_{\geq 0} \times \mathbb{R}_{\geq 0})$, then the semantics of a protocol P for CRN $\mathcal{C} = (\mathcal{A}, \mathcal{R})$, under environment $\rho : Var \to S$ and functions $\mathcal{T}$, $\mathcal{D}$, as defined above, is defined inductively as follows*

$$
\begin{aligned}
&[\![let\, x, y = Dispense(P_1, p)\, in\, P_2]\!]^\rho = \\
&\quad let\, (x_0, V, T) = [\![P_1]\!]^\rho \\
&\quad let\, p' \, being\, sampled\, from\, \mathcal{D}(\cdot, V, p) \\
&\quad let\, \rho_1 = \rho\{x \leftarrow (x_0, V \cdot p', T), y \leftarrow (x_0, V \cdot (1 - p'), T)\} \\
&\quad [\![P_2]\!]^{\rho_1} \\
&[\![Equilibrate(P, t)]\!]^\rho = \\
&\quad let\, (x_0, V, T) = [\![P]\!]^\rho \\
&\quad let\, \mathcal{I}\, be\, a\, \mathbb{R}_{\geq 0} - valued\, random\, variable\, such\, that\, for\, s \in \mathbb{R}_{\geq 0} \\
&\qquad\quad Prob(\mathcal{I} > s) = \mathcal{T}(s, t) \\
&\quad [\![(\mathcal{A}, \mathcal{R}, x_0), V, T)]\!](H)(\mathcal{I}),
\end{aligned}
$$

where $H \in \mathbb{R}_{\geq 0}$ is such that for any $Equilibrate(P, t)$, and any $\mathcal{I}$ random variable such that $Prob(\mathcal{I} > s) = \mathcal{T}(s, t)$, $[\![(\mathcal{A}, \mathcal{R}), x_0, V, T)]\!](H)(\mathcal{I})$ is well posed with probability 1. If such an H does not exist, we say that $[\![P]\!]^\rho$ is ill posed.

$\mathcal{D}$ is a transition kernel that depends only on the current state of the system. $\mathcal{T}$ is the cumulative probability distribution of a random variable with exponential distribution. As a consequence, according to Definition 2, $[\![P]\!]^\rho$ induces semantics that are again solution of a PDMP. However, here $\mathcal{T}$ determines the probability of changing discrete state and $\mathcal{D}$ acts as a probabilistic reset, there are no guards, and the continuous dynamics evolve according to the ODE in Definition 4.

Next, we leverage results from the analysis of PDMP models and export them over the protocol language. The following assumptions guarantee that the solution of the PDMP induced by $[\![P]\!]^\rho$ exists, establish that it is a strong Markov process, and allow to exclude pathological Zeno behaviours [12,19].

Assumption 1

- *Let $A_0, A_1 \subset \mathcal{B}(\mathbb{R}_{[0,1]})$ be the smallest sets in $\mathcal{B}(\mathbb{R}_{[0,1]})$ containing respectively 0 and 1. Then, $\mathcal{D}(A_0, V, p) = \mathcal{D}(A_1, V, p) = 0$ for any $p \in (0,1), V \neq 0$. That is, the Volume after a dispense is zero with probability zero.*

– *Let* $F : \mathbb{R}^{|\mathcal{A}|} \to \mathbb{R}^{|\mathcal{A}|}$ *be the drift term of the rate equations (Eqn* (4)*). Then,* F *is a globally Lipschitz function.*
– *For any* $Equilibrate(\cdot, t)$ *we have that* $t > 0$.

Let us interpret these assumptions over the protocol languages. The first assumption guarantees that the volume of a non-empty sample cannot be 0 almost-surely. The second assumption guarantees that the solution of (4) exists and does not hit infinity in finite time. This excludes non-physical reactions like $X + X \to X + X + X$. The third assumption guarantees that we have a finite number of jumps over a finite time, thus excluding Zeno behaviours [12,13].

Example 4. Consider the protocol introduced in Example 2. For $\sigma_1 > 0, A \subset \mathbb{R}_{[0.1,0.8]}$. Assume that $\mathcal{D}(A, p, \bar{V}) = \frac{\int_A e^{-\frac{x-p}{2\sigma_1^2}} dx}{\int_{0.1}^{0.8} e^{-\frac{x-p}{2\sigma_1^2}} dx}$. That is, $\mathcal{D}(\cdot, p, V)$ is a truncated Gaussian measure centered at p and independent of the volume. Then, according to Definition 9, we have the following Stochastic Semantics:

$$H^+(\mathcal{I}) = H^+(0) - \int_0^{\mathcal{I}} kNa^+(s)OH^-(s)H^+(s)Cl^-(s)ds,$$

with $HCl(0) = \frac{V_1 0.1 + V_2 10^{-7.4}}{V_1 + V_2}$. Here $\mathcal{I}$ is a random variable with an exponential distribution with rate $\frac{1}{T}$, V_1 is a random variable sampled from $\mathcal{D}(\cdot, p_1, 1)$, and V_2 is a random variable sampled from $\mathcal{D}(\cdot, p_2, 1)$.

4 Extending the Protocol Language with Observations

The language introduced in Section 3 can be extended in a number of directions, according to specific envisioned scenarios for the protocols. A common task is to take observations of the state of the protocol. That is, often it is useful to store the state of the system at different times or when a particular event happens. As some of the events may be stochastic, in general, it is not possible to know before the simulation starts when a particular event happens. Consequently, observations need to be included in the language.

Definition 10 *(Extended Syntax). Given a set of variables* Var*, the syntax of a protocol for a given fixed CRN* $\mathcal{C} = (\mathcal{A}, \mathcal{R})$ *and* $idn \in \mathbb{N}$ *is*

$$\begin{aligned}
P = \quad & x && \textit{(sample variable)} \\
& (x_0, V, T) && (\textit{initial condition}) \\
& Mix(P_1, P_2) && (\textit{mix samples}) \\
& let\, x = P_1\, in\, P_2 && \textit{(define variable)} \\
& let\, x, y = Dispense(P_1, p)\, in\, P_2 && \textit{(dispense samples)} \\
& Equilibrate(P, t) && (\textit{let time pass}) \\
& Dispose(P) && (\textit{discard } P) \\
& Observe(P, idn) && (\textit{observe sample})
\end{aligned}$$

where $T, V, t \in \mathbb{R}_{\geq 0}, x, y \in Var$, $p \in \mathbb{R}_{[0,1]}$. *Moreover, let-bound variables must occur exactly once (that is, be free) in* P_2.

$Observe(P, idn)$ makes an observation of protocol P after its execution, and identifies such an observation with identifier idn. In order to include observations we extend the semantics as detailed next, where we consider in detail just the deterministic semantics, focusing on a few key operators. The other operators and the Stochastic Semantics follow similarly.

Definition 11 *(Extended Deterministic Semantics) For CRN $\mathcal{C} = (\mathcal{A}, \mathcal{R})$ let $S = \mathbb{R}^{|\mathcal{A}|} \times \mathbb{R}_{\geq 0} \times \mathbb{R}_{\geq 0}$, $Obs = \mathbb{R}_{\geq 0} \times \mathbb{N} \times \mathbb{R}^{|\mathcal{A}|}$, Obs^*, an eventually empty set of Obs and $\mathcal{M} = S \times Obs^* \times \mathbb{R}_{\geq 0}$. The semantics of a protocol P, under environment $\rho : Var \to \mathcal{M}$, is a function $[\![P]\!] : (Var \to \mathcal{M}) \times \mathbb{R}_{\geq 0} \to \mathcal{M}$ defined inductively as follows*

$$
\begin{aligned}
&[\![Mix(P_1, P_2)]\!]^\rho_t = \\
&\quad let\,((x_0^1, V_1, T_1), Obs_1, t_1) = [\![P_1]\!]^\rho_t \\
&\quad let\,((x_0^2, V_2, T_2), Obs_2, t_2) = [\![P_2]\!]^\rho_t \\
&\quad ((\frac{x_0^1 V_1 + x_0^2 V_2}{V_1 + V_2}, V_1 + V_2, \frac{T_1 V_1 + T_2 V_2}{V_1 + V_2}), Obs_1 :: Obs_2, \max(t_1, t_2)) \\
&[\![Observe(P, idn)]\!]^\rho_t = \\
&\quad let\,((x_0, V, T), Obs, t_1) = [\![P]\!]^\rho_t \\
&\quad let\,O = (x_0, idn, t_1) \\
&\quad ((x_0, V, T), Obs \cup O, t_1) \\
&[\![Equilibrate(P, t)]\!]^\rho_{t'} = \\
&\quad let\,((x_0, V, T), Obs, t_1) = [\![P]\!]^\rho_{t'} \\
&\quad ([\![(\mathcal{A}, \mathcal{R}), x_0, V, T)]\!](H)(t), Obs, t_1 + t),
\end{aligned}
$$

where $H \in \mathbb{R}_{\geq 0}$ is such that for any $Equilibrate(P, t)$, $[\![(\mathcal{A}, \mathcal{R}), x_0, V, T)]\!](H)(t)$ is well posed. If such H does not exist, we say that P is ill posed.

Note that the above syntax does not prevent the programmer to assign the same identifier to two distinct observations. We further stress that often observations of the state of an experiment are not exact, but corrupted by sensing noise. For instance, this is what happens with noisy fluorescence measurements. This noise can be easily taken into account at a semantical level by sampling an observation from a distribution with added noise, where the noise level depends on the particular measure technique or instrumentation. Finally, we can as well extend the sample semantic to take into account noise in Dispense operations.

5 Case Study

As a case study we consider an experimental protocol for DNA strand displacement. DNA strand displacement (DSD) is a design paradigm for DNA nano-devices [10]. In such a paradigm, single-stranded DNA acts as signals and double-stranded (or more complex) DNA structures act as gates. The interactions between signals and gates allow one to generate computational mechanisms that can operate autonomously at the molecular level [26]. The DSD programming language has been developed as a means of formally programming and

analyzing such devices [20,10]. Here, we consider an AND circuit implemented in DSD, which can be represented with the reactions in Figure 2b. Strands $Input_1 =< 1^*\ 2 >$ and $Input_2 =< 3\ 4^* >$ represent the two inputs, while strand $Output =< 2\ 3 >$ is the output. Strand $Gate = \{1^*\}[2\ 3]\{4^*\}$ is an auxiliary strand. The $Output$ strand is released only if both the inputs react with the $Gate$ gate. We consider the protocol in Figure 2a, which can be written formally as follows. We use $let\, x,_= Dispense(P1, p)\, in\, P2$ as a short-hand for $let\, x, y = Dispense(P1, p)\, in\, Mix(Dispose(y), x)$

$$
\begin{aligned}
P_1 =& let\, In1 = ((Input1, 100.0nM), 0.1mL, 25.0^oC)\, in\\
& let\, In2 = ((Input2, 100.0nM), 0.1mL, 25.0^oC)\, in\\
& let\, GA = ((Output, 100.0nM), 0.1mL, 25.0^oC)\, in\\
& let\, GB = ((Gate_B, 100.0nM), 0.1mL, 25.0^oC)\, in\\
& let\, sGA,_= Dispense(GA, p_1)\, in\\
& let\, sGB,_= Dispense(GB, p_2)\, in\\
& let\, sIn1,_= Dispense(In1, p_3)\, in\\
& let\, sIn2,_= Dispense(In1, p_4)\, in\\
& Observe(Equilibrate(Mix(Mix(Equilibrate(Mix(sGA, sGB), t_1), sIn1), sIn2), t_2), idn).
\end{aligned}
$$

The protocol proceeds as follow: $Output$ and $Gate_B$ strands are dispensed from the original samples. Then, they are let evolve for t_1 seconds to create $Gate$ strands. Then, the two inputs are dispensed from their samples. The resulting samples are mixed and the resulting solution evolves for t_2 seconds. Finally, we collect the final sample, observe the results, and associate to the observation the identifier 'idn'. According to the standard ISO 8655 for a volume of $1mL$,

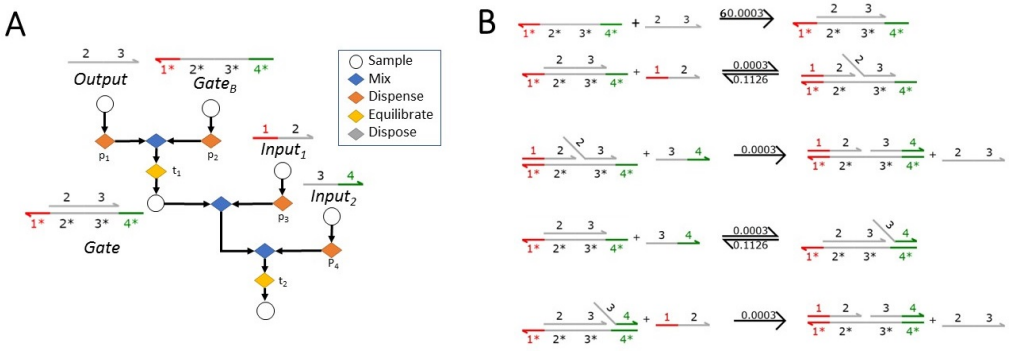

Fig. 2: (A) Graphical representation of the protocol. (B) Graphical representation of the reactions between the different DNA strands in the considered solution. For example, in the second reaction, stand $\{1^*\}[2\ 3]\{4^*\}$ reacts with $< 1^*\ 2 >$ at a rate 0.0003, and there exists an inverse reaction with rate 0.1126.

the maximum standard deviation of a particular pipetting device is $0.3\mu L$ per

single operation. To incorporate such an error in our model, we make use of the Stochastic Semantics. Thus, the concentration of the *Output* strand at the end of the protocol is a random variable. It is common that the reaction rates of the physical system are not known exactly and they may be affected by extrinsic noise [23]. This leads to another source of uncertainty in the output of the protocol. To estimate the distribution of the output under the effect of both these sources of noise we extend our semantics to sample the rate of a reaction from a normal distribution with variance equals to half of its mean (sub-Poisson noise). In Figure 3a we plot 4500 executions resulting from the protocol. From the figure it is easy to realize how the difference source of noise may have a distinctive effect on the final outcome of the experiments.

In many experimental protocols, one of the key challenges is to synthesize their optimal discrete parameters, to optimize the probability of obtaining desired behaviours. Here, we assume perfect knowledge of the reaction rates of the physical system and $p_1 = p_2 = 0.4$. Our goal is to see how the concentration of the *Output* changes varying $(p_3, p_4) \in [0.45, 0.65] \times [0.45, 0.65]$. We are interested in the following property

$$P_{Safe}([3.0{\cdot}10^{-4}, 3.5{\cdot}10^{-4}]) = Prob(Output(t') \in [3.0{\cdot}10^{-4}, 3.5{\cdot}10^{-4}] | t' = t_{final}),$$

where t_{final} is the final time of the protocol. The following probability is estimated using Statistical Model Checking [22] in Figure 3b, which in this context reduces to Monte-Carlo sampling. From Figure 3b it is easy to infer that the

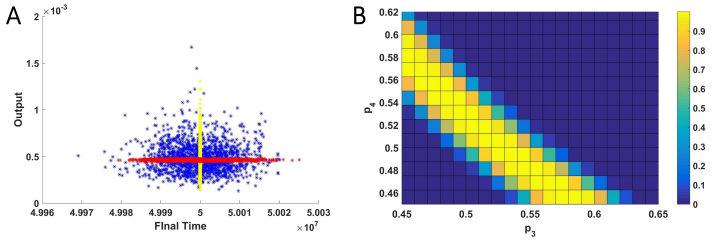

Fig. 3: (A): (red) 1500 execution of the protocol assuming the physical model is fully known, and the only source of noise is the discrete parameters of the protocols (p_1, p_2, p_3, p_4). (yellow) 1500 executions of the protocol when the rates of the physical system are sampled from a sub-Poisson distribution, and discrete operations are exact. (blue) 1500 simulations of the protocol when both sources of noise are active.(B): $P_{Safe}([3.0 \cdot 10^{-4}, 3.5 \cdot 10^{-4}])$ as a function of p_3 and p_4. Each cell is estimated from 20000 executions of the protocol.

optimal value for such property is not unique (it is attained at values over the yellow band) and obtained, for instance, at $(p_3, p_4) = (0.5, 0.54)$.

6 Discussion

We presented a language to model experimental biological protocols, and provided semantics to this protocol in terms of PDMPs. That is, to each experimental biological protocol is associated a particular instance of a stochastic hybrid

process. Our language provides a unified description of the model of the system being experimented on, together with the discrete events representing the parts of biological protocols dealing with handling samples. Moreover, we allow the modeller to take into account uncertainties in both the model structure and the equipment tolerances. This makes our language a suitable tool for both experimental and computational biologists. Our objective has been that of providing a basic language, yielding an integrated representation of an experimental biological protocol. To this end, we have kept the language as simple as possible, showing how different extensions are easy to be integrated. For instance, in our denotational semantics the dynamics of a physical process is given by a set of ODEs. This is accurate when the number of molecules involved is big enough, as in the discussed example of DNA strand displacement (DSD). However, in other scenarios, such as localized computation or gene expression, this might be unsatisfactory as stochasticity becomes important [5,14]: nevertheless, the semantics presented here can be easily extended to incorporate such stochasticity, which can be done for example by considering more general classes of stochastic hybrid processes, such as switching diffusions [21,27]. Another relatively simple extension is to include finite loops or operations based on concentrations.

One of the main advantages in providing a language with formal semantics for experimental protocols is that experimental protocols can now be quantitatively analyzed inexpensively in-silico, and classical problems of analysis of CRNs, such as parameter estimation [6], can be performed within this framework, also by taking into account the discrete operations of the protocol, which influences the dynamics of the system. An additional target is to provide automated techniques to synthesise optimal protocols, or protocols that are guaranteed to perform as desired. This can be attained by tapping into the mature literature on formal verification and strategy synthesis of PDMPs, or that of any other more special model that the given protocol can be mapped onto. Notions of finite-state abstractions [28] and of probabilistic bisimulations [1], as well as algorithms for probabilistic model checking of stochastic hybrid models [21] will be relevant towards this goal.

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