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Effective Index
A formal measure of drug effects

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EFFECTIVE INDEX

A formal measure of drug effects

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Abstract: This paper proposes the Effective Index, a formal tool to support decision processes in drug discovery. The Effective Index is based on concurrency theory and process calculi to describe incrementally complex biological systems and on Markov process theory to handle quantitative information. A running case study concerning the pathways and the drugs related to hypertension exploits the approach.

Keywords: Process calculi, systems biology, Markov process, drug discovery

1. INTRODUCTION

Pharmacodynamics (Macheras and Iliadis, 2005) is the study of biochemical and physiological effects of drugs, mechanisms of drug action and relationship between drug concentration and effect. The effect of a drug on the body is influenced by many factors, as age, genetic makeup and disorders other than the one being treated. Methods and techniques that account the complexity of the biological data and enable prediction on the whole system are required. Therefore pharmacodynamics is moving towards a *systems biology* approach (Kitano, 2001). Systems biology exploits the relationships between all the components rather than approaching them in isolation (Butcher *et al.*, 2004). The community agrees that predicting the absorption, distribution, metabolism, excretion and toxicity (ADME/Tox) properties of drug compounds may prevent failure of some molecules to reach the clinic trial stage (see e.g., (Ekins *et al.*, 2005; Bugrim *et al.*, 2004)). The focus is on preclinical ADME/Tox studies, where a system approach might improve the understanding of drug effects.

Various approaches based on differential equations (Jones and Sleeman, 2003) have been applied to describe and study the kinetic of cellular processes. Some authors (Hlavacek *et al.*, 2006) claim that it could be hard to investigate the behaviour of systems within the formalism of kinetic differential equations upon the addition of molecules, such as drugs. Recently, some researchers are switching from differential equations to rule -based modeling (Ciobanu and Rozenberg, 2004). Molecules are represented as formal expressions (e.g., strings) that determine the interaction capabilities of molecules and their modifications due to interactions. The interaction of two agents does not require an explicit representation but it depends on the structure of the interacting agents. In a similar context, adding an agent (e.g., a drug) to a system (e.g., a sick pathway) becomes easy as dropping a chemical into a text tube. This property is called *compositionality*, i.e. the behaviour of a complex system is determined by the behaviour of its elementary components and can be defined incrementally.

Compositionality seems a key property to break down the complexity of biological systems and therefore to study the response of a biological system to drugs. Among different proposals, formal methods from concurrency theory and process calculi are promising (Regev and Shapiro, 2002). Here we refer to β-binders (Priami and Quaglia, 2005) a bio-inspired process calculus based upon π-calculus (Sangiorgi and

Walker, 2001; Milner, 1999). β-binders is a formalism for representing interactions among biological entities, equipped with a stochastic semantics (Degano *et al.*, 2006) and a simulation environment (Romanel *et al.*, 2007). In this paper we rely on Markov process theory (Norris, 1998) to define an **Effective Index** (**EI**), i.e. a measure of the *impact* of a drug on a system. The Effective Index allows *in silico* comparing the effect of different drugs and of combination of drugs. Then we use the Effective Index to plot a dose-response curve, that is, a graph that relates drug dosage with organism response.

The paper is organised as follows. After a brief introduction to β-binders modelling in Sect. 2, we present the model for the NO-cGMP pathway, a relevant biological example, in Sect. 3 and we discuss its β-binders model. In Sect. 4 we present the theory underlying EI and in Sect. 5 we exploit EI on the NO-cGMP pathway. Sect. 6 concludes the paper with some final remarks.

2. THE LANGUAGE

The BetaSIM language is based on the stochastic extension of β-binders (Priami and Quaglia, 2005; Degano *et al.*, 2006), a process calculus developed for better representing the interactions between biological entities. The main idea of β-binders is to encapsulate π-calculus processes into *boxes* with interaction capabilities. Like the π-calculus also β-binders is based on the notion of *naming*. Thus, we assume the existence of a countable infinite set $\mathcal{N}$ of names (ranged over by lower-case letters). With respect to the original syntax, in BetaSIM several modifications have been introduced. All the modifications are deeply discussed in (Romanel *et al.*, 2007).

A BetaSIM program, called also β-system, is a tuple $Z{=}\langle B, E, \xi\rangle$ which is a composition of a *bio-process* B, a list of *events* E and *ambient* ξ. The bio-process B intuitively represents the structure of the system, that is a set of entities interacting in the same environment, E represents the list of possible events enabled on the system and the ambient ξ contains information about the environment, like the set T of the considered *types* (ranged over by $\Delta, \Gamma_0, \Sigma', \cdots$), a function $\rho : \mathcal{N} \to \mathbb{R}$ that associates stochastic rates [1] to names in $\mathcal{N}$ and the function $\alpha : T^2 \to \mathbb{R}^3$, which describes the affinity relation between couples of types. In particular, given two types Δ and Γ, the application of $\alpha(\Delta, \Gamma)$ returns a triple of stochastic rates (r, s, t), where r, denoted with $\alpha_c(\Delta, \Gamma)$, represents the *complexation rate*, s, denoted with $\alpha_d(\Delta, \Gamma)$, represents *decomplexation rate* and t, denoted with $\alpha_i(\Delta, \Gamma)$, represents the *inter-communication rate*.

[1] A stochastic rate is the single parameter defining an exponential distribution that drives the stochastic behaviour of an action (Gillespie, 1977).

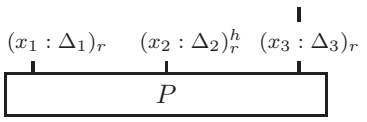
The bio-process B and the list of events E are defined according to the context-free grammar reported in Fig. 1, where x and $y \in \mathcal{N}$, $n \in \mathbb{N}$ and $r \in \mathbb{R}$ is a stochastic rate.

$$
\begin{array}{lll}
P & ::= & \mathsf{nil} \mid P|P \mid !\pi.P \mid M \\
M & ::= & \pi.P \mid M + M \\
\pi & ::= & x(y) \mid \overline{x}\langle y\rangle \mid (\tau, r) \mid (\mathsf{ch}(x, \Delta), r) \mid \\
 & & (\mathsf{die}, r) \mid (\mathsf{hide}(x), r) \mid (\mathsf{unhide}(x), r) \mid \\
 & & (\mathsf{expose}(x, s, \Delta), r) \\
\widehat{\beta} & ::= & \beta \mid \beta^h \mid \beta^c \\
\boldsymbol{B} & ::= & \widehat{\beta}(x, r, \Delta) \mid \widehat{\beta}(x, r, \Delta)\boldsymbol{B} \\
B & ::= & \mathsf{Nil} \mid \boldsymbol{B}[P] \mid B||B \\
cond & ::= & \boldsymbol{B}[P] : r \mid |\boldsymbol{B}[P]| = n \mid \boldsymbol{B}[P], \boldsymbol{B}[P] : r \\
verb & ::= & \mathsf{new}(n) \mid \mathsf{split}(\boldsymbol{B}[P], \boldsymbol{B}[P]) \\
 & & \mathsf{join}(\boldsymbol{B}[P]) \mid \mathsf{delete} \\
event & ::= & \mathsf{when}\ (cond)\ verb \\
E & ::= & \bullet \mid event \mid event :: E
\end{array}
$$

Fig. 1. BetaSIM language syntax.

Processes generated by the non terminal symbol P are referred as *pi-processes*. Boxes are defined as pi-processes prefixed by specialised binders that represent interaction capabilities. An *elementary beta binder* has the form $\beta(x, r, \Gamma)$ (active), $\beta^h(x, r, \Gamma)$ (hidden) or $\beta^c(x, r, \Gamma)$ (complexed), where the name x is the subject of the beta binder and Γ represents the type of x. A *well-formed* beta binder (ranged over by $\boldsymbol{B}$, $\boldsymbol{B}_1$, $\boldsymbol{B}'$, $\cdots$) is a non-empty string of elementary beta binders where subjects and types are all distinct. $\boldsymbol{B}^*$ denotes either a well-formed beta binder or the empty string.

A bio-process B is either the deadlock beta-process *Nil* or a parallel composition of boxes $\boldsymbol{B}[P]$. Moreover, the language is provided with a graphical representation of boxes:



The pairs $x_i : \Delta_i$ represent the sites through which the box may interact with other boxes. *Types* Δ_i express the interaction capabilities at x_i. The value r represents the stochastic rate associated to the name x inside the box, h represents the hidden status and the black line over the last beta binder represents the *complexed* status.

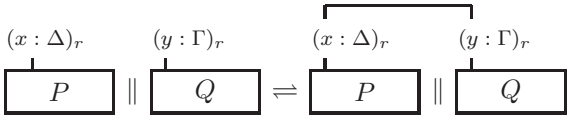
The evolution of the system is formally specified through the *operational semantics* of the language, which is defined with a limited number of operations and uses a notion of structural congruence $\equiv$. The structural congruence of BetaSIM uses structural congruences over pi-processes ($\equiv_p$), bio-processes ($\equiv_b$) and lists of events ($\equiv_e$). Intuitively, this means that two β-systems $Z{=}\langle B, E, \xi\rangle$ and $Z'{=}\langle B', E', \xi'\rangle$ are structurally congruent ($Z \equiv Z'$), if their bio-processes B and B' and their list of events E and E' are iden-

tical up to structure ($B \equiv_b B'$ and $E \equiv_e E'$) and their ambients are equal (ξ=ξ'). Moreover, two boxes, representing interacting entities, are considered of the same specie only if they are structurally congruent.

Three types of actions describe the evolution of a β-system.

Monomolecular actions describe the evolution of single boxes. More precisely, an *intra communication* action allows components to interact within the same box, the *expose* action adds a new site of interaction to the interface of the box containing the expose, the *change* action modifies the type of an interaction site, *hide* and *unhide* actions make respectively invisible and visible an interaction site. Finally, the *die* action eliminates the box that performs the action and, recursively, all the boxes directly or indirectly complexed with them.

Bimolecular actions describe interactions that involve two boxes. The *complex* operation creates a dedicated communication binding between boxes over compatible and unhide elementary beta binders, while the *decomplex* operation destroys an already existing dedicated binding:



The stochastic rates associated to complex and decomplex operations are, respectively, the complexation and decomplexation rates derived from the affinity function. In the example, the complexation rate is $\alpha_c(\Delta, \Gamma)$, while the decomplexation rate is $\alpha_d(\Delta, \Gamma)$. The information about the existing dedicated bindings is maintained in the ambient.

The *inter-communication* is the last bimolecular action. It enables interaction between boxes over compatible and unhide elementary beta binders. Suppose Δ and Γ be the types associated to the involved elementary beta binders. If $\alpha_c(\Delta, \Gamma) > 0$, then the *inter-communication* is enabled, with rate $\alpha_i(\Delta, \Gamma)$, only after a dedicated communication binding, over the involved beta binders, has been created by a *complex* operation. Otherwise, the *inter-communication* is simply enabled with rate $\alpha_i(\Delta, \Gamma)$.

Events can be considered as an implementation of f_{split} and f_{join} axioms (Priami and Quaglia, 2005). An event is the composition of a condition *cond* and an action *verb*, which is triggered only if the event condition is fulfilled on the structure of the bio-process representing the system. Let Z=$\langle B, E, \xi \rangle$ be the considered β-system. The list E can contain five types of events. The *join* event has the form "when ($\boldsymbol{B}_1[P_1], \boldsymbol{B}_2[P_2] : r$) join($\boldsymbol{B}[P]$)" and is enabled, with rate r, only if in the bio-process B at least two boxes $\boldsymbol{B}'_1[P'_1]$ and $\boldsymbol{B}'_2[P'_2]$ structurally congruent to $\boldsymbol{B}_1[P_1]$ and $\boldsymbol{B}_2[P_2]$ are present. The execution of the event substitutes one instance of the boxes $\boldsymbol{B}'_1[P'_1]$ and $\boldsymbol{B}'_2[P'_2]$ with the box $\boldsymbol{B}[P]$. The *split* event has the form "when ($\boldsymbol{B}[P] : r$) split($\boldsymbol{B}_1[P_1], \boldsymbol{B}_2[P_2]$)" and it is enabled, with rate r, only if in the bio-process B at least a box $\boldsymbol{B}'[P']$ structurally congruent to $\boldsymbol{B}[P]$ is present. The execution of the event substitutes one instance of the box $\boldsymbol{B}'[P']$ with the boxes $\boldsymbol{B}_1[P_1]$ and $\boldsymbol{B}_2[P_2]$. Join and split events modify also the ambient ξ in order to guarantee the consistency with respect to the existing dedicated bindings. The *delete* event has the form "when ($\boldsymbol{B}[P] : r$) delete" and is enabled, with rate r, only if the bio-process B contains at least a box $\boldsymbol{B}'[P']$ structurally congruent to $\boldsymbol{B}[P]$. The execution of the event eliminates one instance of the box $\boldsymbol{B}'[P']$. The *new* event can be expressed in two different forms: "when ($\boldsymbol{B}[P] : r$) new(n)" and "when ($|\boldsymbol{B}[P]| = m$) new(n)". The first one is enabled, with rate r, only if in the bio-process B at least a box $\boldsymbol{B}'[P']$ structurally congruent to $\boldsymbol{B}[P]$ is present, while the second one is enabled, with infinite rate, only if the bio-process B contains exactly m boxes structurally congruent to $\boldsymbol{B}[P]$. The execution of the event, in both cases, creates n copies of the box $\boldsymbol{B}[P]$.

The evolution of a β-system is completely defined by the associated stochastic reduction system.

Definition 2.1. The β-binders *Stochastic Reduction System* (*SRS*) is referred as $\mathcal{S} = (\mathcal{Z}, \xrightarrow{r}_s, Z_0)$, where $\mathcal{Z}$ is the set of β-systems, Z_0 is the initial β-system and $\xrightarrow{r}_s \subseteq \mathcal{Z} \times \mathbb{R} \times \mathcal{Z}$ is the stochastic reduction relation.

The value r is a stochastic rate constant and is derived using information in the syntax and in the ambient of the β-system. Given an initial β-system Z_0=$\langle B_0, E_0, \xi_0 \rangle$, we refer to B_0 as the *initial bio-process*. Given two β-systems Z_1=$\langle B_1, E_1, \xi_1 \rangle$ and Z_2=$\langle B_2, E_2, \xi_2 \rangle$, the parallel composition $Z_1 \parallel Z_2$ is defined as the β-system $\langle B_1 \parallel B_2, E_1 \cup E_2, \xi_1 \cup \xi_2 \rangle$.

3. EXAMPLE: NO-CGMP PATHWAY

Hypertension is a medical condition where the blood pressure is chronically elevated. Hypertension affects almost one third of population in developed countries; however, a lot of therapies are known and widely used. One of the approaches is to intervent on the *vascular tone*, the degree of constriction experienced by a blood vessel relative to its maximally dilated state. Vascular tone is primarily dependent on a protein called *myosin light chain kinase* (MLCK). This kinase increases the phosphorylation of *myosin light chains*, thereby increasing smooth muscle tension and causing vasoconstriction. The correspondent phosphatase dephosphorylate the myosin light chains, causing vasodilation.

MLCK is activated by the *calcium-calmodulin* complex, and therefore the activity of this kinase is in-

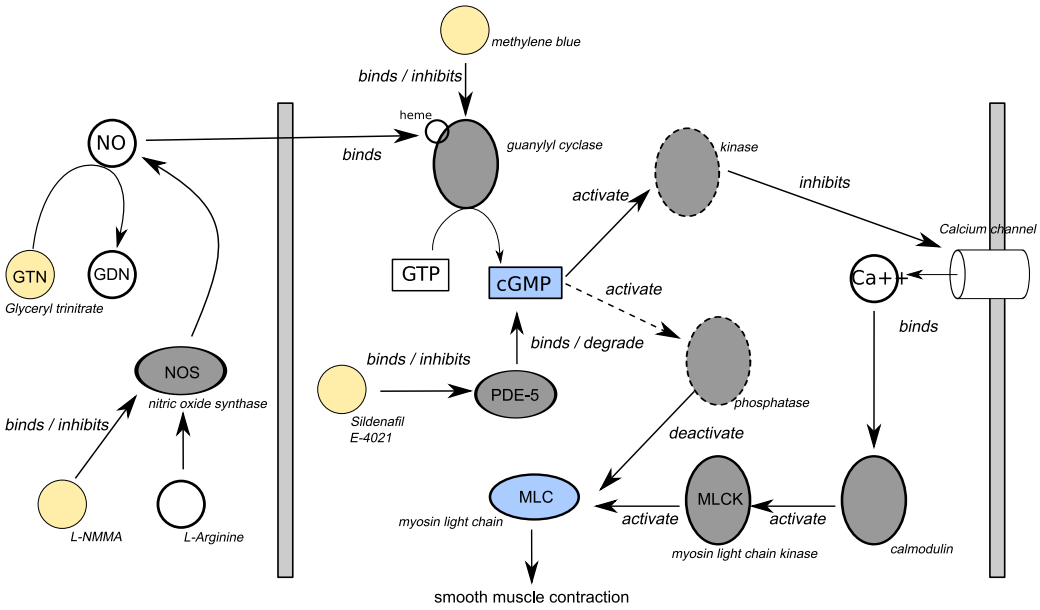


Fig. 2. The NO-cGMP pathway in vessel smooth cells

fluenced by intracellular calcium concentration. To control the level of active MLCK in the cell it is possible to modulate several signal transduction mechanisms: 1) phosphatidylinositol pathway, 2) G-coupled-protein (cAMP) pathway, and 3) nitric oxide (NO)-cGMP pathway (Somlyo and Somlyo, 1994; Kamm and Stul, 1989).

Nitric oxide (NO) is produced by vascular endothelium, and many other cell types, by the *nitric oxide synthase* (NOS), which uses amino acid L-arginine and oxygen as substrates.

When NO is formed in an endothelial cell it readily diffuses into an adjacent smooth muscle cell. Here it binds to a heme domain on *guanylyl cyclase* and activates this enzyme, which produce cGMP from GTP. The increased level of cGMP activates a kinase that subsequently inhibits the flux of calcium into the vessel smooth cell, decreasing the concentration of calmodulin-calcium complexes and therefore the level of active MLCK.

There is also evidence in literature (Lee *et al.*, 1997; Rapoport *et al.*, 1983) that increases in cGMP can also lead to myosin light chain de-phosphorylation by activating a pertinent phosphatase.

Fig. 2 shows a complete overview of the pathway. Enzymes are in grey ovals, unknown enzymes having a dotted outline, and drugs that can interact with the pathway are in light grey circles. As underlined in the figure, there are several points in which a drug can act to influence the cGMP concentration in vessel smooth muscle cells. N[ω]-monomethyl-L-arginine (L-NMMA) acts as a competitive inhibitor of nitric oxide synthase, decreasing the level of NO and therefore of cGMP. The level of NO can be increased by the introduction of *glyceryl trinitrate* (GTN), a prodrug which is denitrated, by a mechanism that is widely disputed, to produce 1,2-glyceryl dinitrate (GDN) and NO (Marsh and Marsh, 2000). Drugs like sildenafil (Viagra) or E-4021 inhibit cGMP-specific phosphodiesterase 5, the enzyme responsible for the degradation of cGMP, and therefore compensate for reduced NO release and cGMP production (Corbin and Francis, 1999).

In Fig. 3 part of the model we developed for the (NO)-cGMP pathway is illustrated. The first line of boxes in the figure shows the processes involved in the production of NO and in the synthesis of cGMP. It is not well established how GTN is transformed into GDN and NO, so this biochemical reaction is modelled as a *split* with rate equal the global observed rate k_1. The synthesis of cGMP is modelled as a communication on binders, as introduced in Sect. 2. The bio-process GC communicate with GTP through its binder of type Δ_{pGTP}. The communication changes the internal structure of the bio-process, transforming it into a process modelling $cGMP$. The second line of boxes in Fig. 3 indicate how $PDE5$ degrades $cGMP$ by binding it on the Δ_{coD} domain and sending a d message (right side of the model). The $cGMP$ bio-process reacts to the d message; it is transformed into an empty bio-process. It is also shown how competitive inhibition by $Sildenafil$ drug is modelled with a compatible domain type $\Delta_{D'}$. $Sildenafil$ binds to $PDE5$ on Δ_{coD}, but do not react to the degrade message (left side of the model).

4. EFFECTIVE INDEX

Our aim is to provide formal tools to support decision processes in drug discovery. Here we introduce the concept of **Effective Index** to "measure" the effect of a drug on a system. Given a SRS, the Effective Index

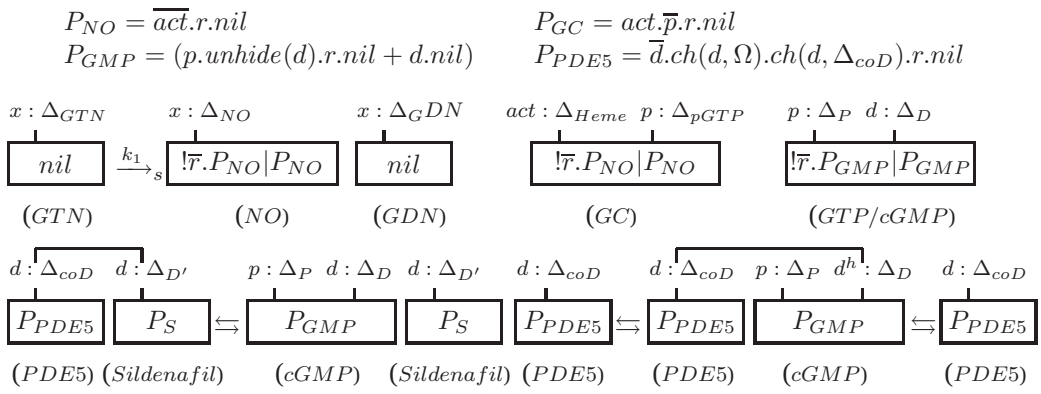


Fig. 3. Part of the model for the NO-cGMP Pathway

gives the expectation to reach a state that is safe w.r.t. an observable property.

We start defining a notion of observability of a β-system. If a type represents the interface (i.e. the visible part) of a biological entity, then it is natural to found on types our notion of *observable* of a β-system. In particular, the predicate **observable type** of a β-system Z, written $Z{\downarrow_\Gamma}$, is true if the type Γ is *visible* in Z. The predicate $Z{\downarrow_\Gamma}$ can be extended as $Z{\bigvee_\Gamma^n}$ and $Z{\bigwedge_\Gamma^n}$, meaning Z expresses at least -at most- n occurrences of Γ, respectively. To express some combination of this kind of conditions, we define the concept of **observable phenomena**.

Definition 4.1. (Observable Phenomena). An *observable phenomenon* is a formula $\varphi \in \Phi$ defined by

$$\varphi ::= Z{\bigvee_\Gamma^n} \mid Z{\bigwedge_\Gamma^n} \mid \varphi \wedge \varphi \mid \varphi \vee \varphi$$

Formulae in Φ allows us to express conditions on the quantity of visible types of a β-system. For instance, in the CO-cGMP pathway the level of cGMP is the relevant value. The observable phenomenon $\varphi_1 = Z{\bigvee_{\Delta_D}^n}$ is true in a β-system where at least n occurrences of Δ_D are unhidden, i.e. cGMP is present at an adequate level of concentration. But φ_1 is not enough, because a high level of cGMP may induce a too low blood pressure. Therefore, cGMP has to be lower than a value m, expressed by $\varphi_2 = Z{\bigwedge_{\Delta_D}^m}$, leading to the observable phenomenon $\varphi_1 \wedge \varphi_2$.

Observable phenomena give the ability to quantitatively express conditions on a state of a SRS. Following Markov process theory (Norris, 1998), we can classify the states of a SRS as *recurrent* and *transient*. A state Z_i is transient if, starting from state Z_i, there is a non-zero probability that the system will never return back to Z_i. If a state is not transient then it is said recurrent. Recurrent states formally capture the idea of "steady states", i.e. states that offer a stable behaviour. Given a SRS $\mathcal{S}$, let $\Upsilon_\mathcal{S} \subseteq \mathcal{S}$ be the set of recurrent states. On a finite SRS, being a recurrent state is a decidable property and it is characterised by Th. 3.4.2 in (Norris, 1998). The set of states $\Psi_\mathcal{S}^\varphi \subseteq \Upsilon_\mathcal{S}$ that satisfies an observable phenomenon φ are named *safe states* w.r.t. φ.

Given a finite SRS $\mathcal{S} = (\mathcal{Z}, \xrightarrow{r}_s, Z_0)$, the associated Markov process $X(t)$ is defined as $X(t) = Z_i$ if at time t system S behaves as Z_i. The *transition rate* between two states Z_i and Z_j is defined as $q(Z_i, Z_j) = r$, if $Z_i \xrightarrow{r}_s Z_j$, written q_{ij}. The *exit rate* of a state Z_i is $q(Z_i) = \sum_{Z_j \in \mathcal{Z}} q_{ij}$, written q_i. The transition rate and the exit rate completely specifies the stochastic behaviour of a SRS. In particular, we can compute the **hitting probability**, i.e. the probability to reach a state Z_j starting from a state Z_i.

Definition 4.2. (Hitting probability). Let $\mathcal{S}$ be a finite SRS $(\mathcal{Z}, \xrightarrow{r}_s, Z_0)$. The vector $h^j = (h_i^j : Z_i \in \mathcal{Z})$ of *hitting probabilities* of a state $Z_j \in \mathcal{Z}$ is the minimal non-negative solution to the system of linear equations

$$\begin{cases} h_i^j = 1 & \text{if } Z_i \equiv Z_j \\ \sum_{Z_k \in \mathcal{Z}} q_{ik} h_k^j = 0 & \text{if } Z_i \not\equiv Z_j \end{cases}$$

The value h_i^j is the probability, starting from state Z_i to reach state Z_j.

Given a finite SRS $\mathcal{S}$ and an observable phenomenon φ, we introduce the Effective Index as the weighted percentage of recurrent states that are safe w.r.t. φ. We use the hitting probability starting from the initial state as weights . The formal definition follows.

Definition 4.3. (Effective Index (EI)). Let $\varphi \in \Phi$ be an observable phenomenon and $\mathcal{S} = (\mathcal{Z}, \xrightarrow{r}_s, Z_0)$ be a finite SRS. The *Effective Index* of $\mathcal{S}$ w.r.t. φ is defined as

$$\mathcal{E}_\varphi^\mathcal{S} = \frac{\sum_{Z_i \in \Psi_\mathcal{S}^\varphi} h_0^i}{\sum_{Z_i \in \Upsilon_\mathcal{S}} h_0^i}$$

EI is a measure of "how good" is a system $\mathcal{S}$ w.r.t. to an observable phenomenon φ. Suppose $\mathcal{S}_H = (\mathcal{Z}_H, \xrightarrow{r}_s, Z_H)$ models a healthy system, $\mathcal{S}_S = (\mathcal{Z}_S, \xrightarrow{r}_s, Z_S)$ is the sick version of $\mathcal{S}_H$ and let φ a description of the healthy condition (e.g., low

presence of a certain molecule). Therefore we have $\mathcal{E}_{\varphi}^{\mathcal{S}_H} > \mathcal{E}_{\varphi}^{\mathcal{S}_S}$. If β-systems Z_{D1} and Z_{D2} model two drugs $D1$ and $D2$ for the sick system $\mathcal{S}_S$, we obtain two treated systems $\mathcal{S}_{D1} = (\mathcal{Z}_{D1}, \xrightarrow{r}_s, Z_S \parallel Z_{D1})$ and $\mathcal{S}_{D2} = (\mathcal{Z}_{D2}, \xrightarrow{r}_s, Z_S \parallel Z_{D2})$. If $\mathcal{E}_{\varphi}^{\mathcal{S}_{D1}} > \mathcal{E}_{\varphi}^{\mathcal{S}_{D2}}$ we can conclude that drug $D1$ is more effective that drug $D2$ w.r.t. the observable phenomenon φ.

Finally we can plot a *dose-response curve* (Kenakin, 2006) relying on EI. A dose-response curve is a graph that relates the amount of a drug given with the response of the organism to that drug. Let Z_D be a β-system describing a drug D for $\mathcal{S}_S$. We can define the *family dosage* of D as a set of β-systems

$$\Omega_{Z_D} = \{Z \mid Z \equiv \overbrace{Z_D \parallel \ldots Z_D}^{n}, \text{for } n \geq 0\}.$$

The dose-response curve for $\mathcal{S}_S$ treated with a drug Z_D, where the phenomenon φ is observed, is defined as the set of pairs

$$\sigma_{\varphi}^{\mathcal{S}_S, Z_D} =$$

$$\{(\mathcal{S}_Z, \mathcal{E}_{\varphi}^{\mathcal{S}}) \mid \mathcal{S}_Z = (\mathcal{Z}_Z, \xrightarrow{r}_s, Z_S \parallel Z), \forall Z \in \Omega_{Z_D}\}.$$

5. EFFECTIVE INDEX ON THE ROAD

In this section we explain the use of EI on the NO-cGMP pathway presented in Sect. 3. In particular, we explore in our model the interactions between sildenafil and glyceryl trinitrate. The ACC/AHA consensus document on sildenafil (Cheitlin *et al.*, 1999) takes a cautious approach to interaction between organic nitrates (e.g., glyceryl trinitrate) and sildenafil. Here, we test this statement by means of EI, with the proviso that our model and the associated quantitative measures (i.e. stochastic rates) are not complete, and therefore this section has to be intended as a tutorial on EI.

We consider a system $\mathcal{S}_H = (\mathcal{Z}_H, \xrightarrow{r}_s, Z_H)$ representing a healthy pathway. The initial β-system Z_H is defined by the initial bio-process B_H

$$NOS \parallel L\text{-}ARG \parallel GC \parallel GTP \parallel PDE5$$

Box NOS interacts with $L\text{-}ARG$ producing NO. Box NO activates GC, that transforms GTP into $cGMP$. Finally, PDE5 degrades cGMP. First, we check if cGMP is available with the observable phenomenon $\varphi_1 = Z\curlyvee_{\Delta_D}^{n}$. We compute EI as $\mathcal{E}_{\varphi_1}^{\mathcal{S}_H} = 0.92$, meaning that finally the level of cGMP is stable. The sick version of system $\mathcal{S}_H$ is $\mathcal{S}_S = (\mathcal{Z}_S, \xrightarrow{r}_s, Z_S)$, where the initial bio-process B_S is equal to B_H. The difference is in the interaction rate between NOS and $L\text{-}ARG$: we simulate a disease where some factors reduce the probability of producing NO from NOS. The associated EI is $\mathcal{E}_{\varphi_1}^{\mathcal{S}_S} = 0.26 < \mathcal{E}_{\varphi_1}^{\mathcal{S}_H}$.

Glyceryl trinitrate GTN restores the cGMP level providing an alternative way to produce NO. The GTN treated SRS is $\mathcal{S}_{GTN} = (\mathcal{Z}_{GTN}, \xrightarrow{r}_s, Z_{GTN})$, where the initial bio-process is $B_S \parallel GTN$. The associated EI w.r.t φ_1 is $\mathcal{E}_{\varphi_1}^{\mathcal{S}_{GTN}} = 0.72$, that re-establishes a healthy condition.

Sildenafil *Sildenafil* acts on cGMP by inhibiting degradation by cGMP. We build the sildenafil treated SRS $\mathcal{S}_{Sil} = (\mathcal{Z}_{Sil}, \xrightarrow{r}_s, Z_{Sil})$, with initial bio-process $B_S \parallel Sildenafi$. It is known that sildenafil is not really effective in the case of a lack in NO activation. The low EI $\mathcal{E}_{\mathcal{S}_{Sil}}^{\varphi_1} = 0.41$ seems to confirm this fact.

Now, it is interesting to evaluate the interaction between GTN and $Sildenafil$ on the same system. We define a third treated system $\mathcal{S}_{GTN\text{-}Sil} = (\mathcal{Z}_{GTN\text{-}Sil}, \xrightarrow{r}_s, Z_{GTN\text{-}Sil})$, with initial bio-process $B_S \parallel GTN \parallel Sildenafil$. The result is an increase in the EI of the system w.r.t. φ_1, becoming $\mathcal{E}_{\mathcal{S}_{GTN\text{-}Sil}}^{\varphi_1} = 0.89$. This seems to confirm the fact that sildenafil increase the effect of glyceryl trinitrate (O'Rourke and Xiong-Jing, 2000). Here, we can taste the flexibility of our method changing the observable phenomena. In fact, if we consider also an upper level for the cGMP availability we have a new observable phenomenon

$$\varphi_2 = Z\curlyvee_{\Delta_D}^{n} \wedge Z\curlywedge_{\Delta_D}^{m}$$

with $n \leq m$. A high level of cGMP may induce a too low blood pressure with life threatening effects. We observe that the EI of systems $\mathcal{S}_H$, $\mathcal{S}_S$, $\mathcal{S}_{GTN}$ and $\mathcal{S}_{Sil}$ does not change considering observable phenomena φ_2. But, if we consider the interaction between glyceryl trinitrate and sildenafil, the EI becomes $\mathcal{E}_{\mathcal{S}_{GTN\text{-}Sil}}^{\varphi_2} = 0.28 < \mathcal{E}_{\mathcal{S}_{GTN\text{-}Sil}}^{\varphi_1}$. The level of cGMP may become to high w.r.t. the observable phenomenon φ_2.

We conclude our tutorial on EI plotting two dose-response curves. Fig. 4 plots two curve: D-R1 is the

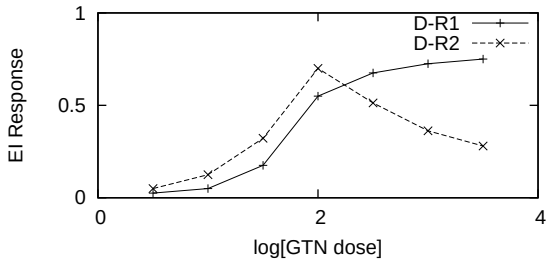


Fig. 4. Dose-response curve for NO-cGMP

EI for the sick SRS $\mathcal{S}_S$ treated with increasing dose of GTN; D-R2 is the EI for the sildenafil treated SRS $\mathcal{S}_{Sil}$ with increasing dose of GTN. In both curve, the EI is computed with respect to observable phenomenon φ_2. Curve D-R1 shows the usual sigmoid shape, while D-R2 behaves as D-R1 until a limit value for GTN is reached, then the EI decrease quickly.

6. CONCLUSION

We proposed a formal approach to model pharmacodynamics based on executable computer science formalisms. We exploited the approach on a running case

study concerning pathways and drugs related to the hypertension. We contributed a measure of effects of drugs as well as of the dose-response curve. This was possible due to the system level approach to the modelling and simulation of the phenomena at hand. The main result of the paper is the feasibility of an automatic and system-level framework to provide decision support in the drug discovery process. As a further extension of the proposed approach we are planning the application of the Effective Index measure to larger case studies to test its real predictive power.

REFERENCES

Bugrim, A., T. Nikolskaya and Y. Nikolsky (2004). Early prediction of drug metabolism and toxicity: systems biology approach and modeling. *Drug Discovery Today* **9**, 127.

Butcher, E., E. Berg and E. Kunkel (2004). Systems biology in drug discovery. *Nature Biotechnology* **22**, 1253.

Cheitlin, M.D., A.M. Jr Hutter, R.G. Brindis, P. Ganz, S. Kaul, l R.O. Jr Russel and S. Kaul (1999). Use of Sildenafil (Viagra) in Patients With Cardiovascular Disease. *Circulation* **99**(1), 168–177. ACC/AHA Expert Consensus Document.

Ciobanu, G. and Rozenberg, G., Eds. (2004). *Modelling in Molecular Biology*. Springer.

Corbin, J.D. and S.H. Francis (1999). Cyclic GMP phosphodiesterase-5: target of sildenafil. *J Biol Chem.* **274**(20), 13729–32.

Degano, P., D. Prandi, C. Priami and P. Quaglia (2006). Beta-binders for Biological Quantitative Experiments. In: *Proc. of the 4th International Workshop on Quantitative Aspects of Programming Languages (QAPL 2006)*. Vol. 164 of *ENTCS*. Elsevier. pp. 101–117.

Ekins, S., Y. Nikolsky and T. Nikolskaya (2005). Techniques: application of systems biology to absorption, distribution, metabolism, excretion and toxicity. *Trends Pharmacol Sci* **26**(4), 202–9.

Gillespie, D.T. (1977). Exact stochastic simulation of coupled chemical reactions. *Journal of Physical Chemistry* **81**(25), 2340–2361.

Hlavacek, W.S., J.R. Faeder, M.L. Blinov, R.G. Posner, M. Hucka and W. Fontana (2006). Rules for modeling signal-transduction systems. *Sci STKE*.

Jones, D.S. and B.D. Sleeman (2003). *Differential Equations and Mathematical Biology*. CRC Press.

Kamm, K.E. and l J.T. Stul (1989). Regulation of smooth muscle contractile elements by second messengers. *Annu Rev Physiol.* **51**, 299313.

Kenakin, T. (2006). *A Pharmacology Primer: Theory, Applications, and Methods*. Elsevier.

Kitano, H., Ed. (2001). *Foundations of Systems Biology*. The MIT Press.

Lee, M.R., L. Li and T. Kitazawa (1997). Cyclic GMP causes Ca2+ desensitization in vascular smooth muscle by activating the myosin light chain phosphatase. *J Biol Chem.* **272**(8), 50635068.

Macheras, P. and A. Iliadis (2005). *Modeling in Biopharmaceutics, Pharmacokinetics and Pharmacodynamics: Homogeneous and Heterogeneous Approaches*. Springer.

Marsh, N. and A. Marsh (2000). A short history of nitroglycerine and nitric oxide in pharmacology and physiology. *Clin Exp Pharmacol Physiol* **27**(4), 313–319.

Milner, R. (1999). *Communicating and mobile systems: the π-calculus*. Cambridge Universtity Press.

Norris, J.R. (1998). *Markov Chains*. Cambridge Series in Statistical and Probabilistic Mathematics. Cambridge University Press.

O'Rourke, M. and J. Xiong-Jing (2000). Sildenafil/nitrate interaction. *Circulation*.

Priami, C. and P. Quaglia (2005). Beta Binders for Biological Interactions. In: *Computational Methods in Systems Biology, International Conference CMSB 2004, Paris, France, May 26-28, 2004, Revised Selected Papers* (V. Danos and V. Vincent Schächter, Eds.). Vol. 3082 of *LNCS*. Springer. pp. 20–33.

Rapoport, R. M., M.B. Draznin and F. Murad (1983). Endothelium-dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation. *Nature* **306**(5939), 174–176.

Regev, A. and E. Shapiro (2002). Cells as computation. *Nature* **419**(6905), 343.

Romanel, A., L. Dematté and C. Priami (2007). The BetaSIM system. Technical Report TR-3-2007. The Microsoft Research - University of Trento Centre for Computational and Systems Biology.

Sangiorgi, D. and D. Walker (2001). *The π-calculus: a Theory of Mobile Processes*. Cambridge Universtity Press.

Somlyo, A.P. and A.V. Somlyo (1994). Signal transduction and regulation in smooth muscle. *Nature* **372**(6503), 231236.

Appendix A. β-BINDERS CODE

```
////////////////////////////////////////////////
// NO-cGMP pathway
////////////////////////////////////////////////
// Nitric Oxide
let NO_p : pproc = act{e}.r<e>.nil;
let NO : bproc = #(act : 1.0; NOd)
   [ !r{}.NO_p | NO_p ];
// Nitric oxide synthease, the enzime that produces NO
let NOS_p : pproc = act<e>.@(2.2).r<e>.nil;
let NOS : bproc = #(act : 1.0; coLAd)
   [ !r{}.NOS_p | NOS_p ];
// Its substrate, L-arginine
let L_ARG_p : pproc = act{e}.r<e>.nil;
let L_ARG : bproc = #(act : 1.0; LAd)
    [ !r{}.L_ARG_p | L_ARG_p ];
// The active form of NOS, after interaction with L-arginine
// It stays active for a tau
let ActiveNOS : bproc = #(act : 1.0; coLAd)
   [ !r{}.NOS_p | @(2.2).r<e>.nil ];
// When NOS is active, produce some NO
when (ActiveNOS: 2.2) split (ActiveNOS, NO);
// Binding to NOS on coLAd, L-NMMA prevents activation
let L_NMMA : bproc = #(x : 1.0; LNAd)
   [ nil ];
// Another way to introduce NO: Nitro (Glycerin-tri-nitrate)
let GTN : bproc = #h(no : 1.0; NOd), #(gdn : 1.0; GDNd)
   [ NO_p ];
let GDN : bproc = #(gdn : 1.0; GDNd)
   [ NO_p ];
// the process of production of NO out of GTN is unknown,
// let's model it with a simple slipt with rate 2.2
//when (GTN: 2.2) split (GDN, NO);
// The guanylyl cyclase produces cGMP when activated by NO
let GC_p : pproc = act<e>.p{}.r<e>.nil;
let GC : bproc = #(act : 1.0; Heme), #(p : 1.0; pGTP)
  [ !r{}.GC_p | GC_p ];
// Methylene blue is a competitiva inhibitor of GC
let MBlue : bproc = #(x : 1.0; coHeme1)
      [ nil ];
// This bioprocess encodes the behaviour of both GTP and cGMP
let GMP_p : pproc = (p<e>.unhide(act).unhide(d).act{}.r<e>.nil + d<e>.nil);
let cGMP : bproc = #(p: 1.0; PhosphorG), #h(d : 1.0; DegradeG), #h(act : 1.0; GmpDomain)
   [ !r{}.GMP_p | GMP_p ];
// The degraded cGMP process, will be deleted
let cGMP_d : bproc = #(p : 1.0; PhosphorG), #(d : 1.0; DegradeG), #(act : 1.0; GmpDomain)
   [ !r{}.GMP_p ];
when (cGMP_d: inf) delete;
// We suppose to have an unlimited amount of GTP
//when (/cGMP/ = 0) new(cGMP, 1);
// After binding on gmpDomain, send degrade message, then detach
// PDE-5 degrades cGMP
let PDE5_p : pproc = d{}.ch(d, gmpRelease).ch(d, coGmpDomain).r<e>.nil;
let PDE5 : bproc = #(d : 1.0; coGmpDomain)
   [ !r{}.PDE5_p | PDE5_p ];
// Sildenafil (or E-4021) binds to gmpDomain, but do not react to the degrade message
let Sildenafil : bproc = #(x : 1.0; similGmpDomain)
   [ nil ];
```