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Inhibition and damage schemes within the synthesizing unit concept of dynamic energy budget theory

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ABSTRACT

Synthesizing Units (SU) concept plays an important role in organizing metabolism in Dynamic Energy Budget (DEB) theory. SUs are generalized units that bind and processes incoming streams of materials (substrates, generalized compounds, food, etc.) to yield one or more products. We use paradigms from enzyme kinetics to explore the impact of inhibitors and damaging agents on the dynamics of SUs requiring one or two substrates. Inhibitors interact reversibly with one or more SU states and thereby impede their functioning but otherwise do not have deleterious impact, whereas a damaging agent decommissions an SU, which then either needs to be replaced via de novo synthesis or to be repaired, implying the removal of any already bound substrate molecules. When substrate arrival rates are proportional to densities, single substrate SUs behave dynamically similar to their enzymatic counterparts; with a minor adjustment, this similarity holds when an inhibitor is present. The impact of a damaging agent on SU dynamics is similar to that of an inhibitor, if the mean time interval between damage events is long relative to the time it takes an SU with bound substrate to form a product. However, damage done to an SU with substrate(s) already bound implies an energetic loss if the substrate binding is an endergonic process. Those conclusions with single substrate SUs essentially carry over to SUs requiring two different substrates to form a product, though the mathematical formalisms involved are more complex. There are conceptual similarities between SUs subjected to damage or inhibition and individuals whose feeding activity is impeded by social interactions. Our formalism accounts for a marked variety of conceptual SUs, and types of inhibition and damage – ranging from enzymes and molecules to individuals and social interactions instigating a behavioral response.

1. Introduction

The synthesizing unit (SU) concept plays a fundamental role in organizing metabolism in Dynamic Energy Budget (DEB) theory. An SU processes incoming streams of materials and convert these into one or more products. Incoming materials, called substrates, could be in the form of food items, composite compounds and simple molecules; similarly, products may include composite compounds, biomass and molecules (Kooijman, 1998, 2001). A DEB model describes the rates at which an organism acquires resources from its environment and utilizes the energy and nutrients therein for growth, maturation, maintenance and reproduction (Jusup et al., 2017; Kooijman, 2010; Sousa et al., 2008). In effect, SUs operate the fluxes in a DEB model, though, with the exception of the SU representing the feeding (or assimilation) machinery, they are implicit in presentations of the standard model for heterotrophs (but see Section 2.3.3 in Kooijman, 2010). In the standard model, the SUs describing utilization fluxes (i.e. growth, maintenance, maturation and reproduction) have a single substrate (reserve) and have dynamics fully specified by either demands (maintenance) or supply (maturation, reproduction and growth). However, SUs are indispensible tools for quantifying the processing of two or more substrates, such as in multivariate DEB models, and are therefore important for models describing autotrophy (Kooijman, 1998), syntrophic symbioses (Muller et al., 2009; Troost et al., 2005), ecological stoichiometry (Muller et al., 2001), diauxic growth (Kooijman and Troost, 2007), among other phenomena. In addition, the SU concept has been used to incorporate the impact of toxic compounds and damaging agents on suborganismal processes into the DEB framework (Jager and Kooijman, 2005; Muller, 2011).

The multitude of types of substrates an SU may process points to an

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important characteristic: its concept is scalable from the enzymatic to the supra-organismal level. Indeed, an SU processing a single "substrate" resembles an enzyme with steady state kinetics akin to those of a Michaelis-Menten-Briggs-Haldane enzyme (ChemWiki, 2017; Segel, 1993), an animal feeding at a rate given by the Holling type II disc equation (Holling, 1959), or a population of microorganisms growing at a rate given by the Monod equation (Monod, 1942). The only mathematical difference between the dynamics of a single substrate SU in steady state and those of the other three models is that the former uses the substrate arrival flux as input variable, whereas the latter use substrate or prey densities; this difference disappears if arrival fluxes are proportional to concentrations or densities. Accordingly, SUs conceptually generalize the acting agents in the other models (i.e. enzymes, animals and microbes), and, unlike Menten-Briggs-Haldane enzyme kinetics, can be used in inhomogeneous environments, such as cells and whole organisms, in which concentration measures are not well defined. In this paper, given the large existing knowledge about enzymatic processes, we use textbook enzyme kinetics as the paradigmatic framework to which we compare the dynamics of SUs impaired by detrimental agents, such as toxic compounds.

Our goals are twofold. Firstly, we demonstrate the applicability of well-studied inhibition mechanisms in enzyme kinetics to single and two substrate SUs. Inhibition is the process by which a compound reversibly binds to an enzyme and thereby impedes its activity; enzymatic activity is fully restored upon dissociation of the inhibitor. Enzymes and SUs exist in discrete states in which they either wait for the arrival of one or more substrates or process these substrates into products. Inhibitors target these states with potentially different affinities (see Fig. 1 for examples with a single substrate SU). Thus, we extend and generalize the singular inhibition mode of a single substrate SU as described by Kooijman, 2010 (Section 3.7.4). Secondly, we seek to extend inhibition models to include the impact of damaging agents. We define damage as the process by which a detrimental agent irreversibly destroys the functionality of an SU, which then either needs to be replaced through de novo synthesis or requires restoration through a repair process (see Fig. 2 for examples with a single substrate SU). Arguably, toxic compounds more often impact organisms by damaging than inhibiting their metabolic machinery. Therefore, it is important to assess the quantitative differences between the impacts of inhibitors and those of damaging agents on single and two substrate SUs.

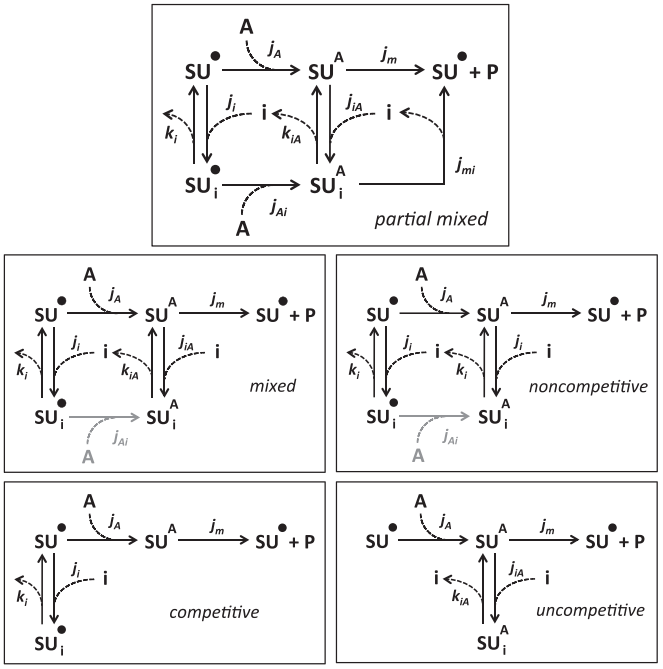

Fig. 1. Scheme of the possible mechanisms whereby an inhibitor *i* may interact with a single substrate SU with Michaelis-Menten-Briggs-Haldane enzyme kinetics as paradigm (note that, in enzyme kinetics, substrate *A* binds reversibly to the SU - see ChemWiki (2017)). Solid arrows represent SU state transitions, broken arrows substrate and inhibitor association and dissociation fluxes. The generic form in enzyme kinetics is partial mixed inhibition, in which (1) inhibitors bind to enzymes in both the unbound and processing state but with different binding and dissociation parameters, and (2) inhibited processing enzymes form product at a rate lower than uninhibited ones. With mixed inhibition, enzymes with bound inhibitors do not form product(s) *P*; similar kinetics are obtained with SUs when substrate cannot bind to inhibited SUs (marked in grey). Other notable special cases include noncompetitive inhibition (inhibitors bind to SUs in the unbound and bound state with similar binding and dissociation parameters; unlike the case in enzyme kinetics, marked in grey, substrate does not bind to inhibited SUs); competitive inhibition (inhibitors only interact with SUs in the unbound state); and uncompetitive inhibition (inhibitors only interact with SUs in the bound state).

2. Theory

This section develops formalism for inhibition, damage and repair mechanisms of SUs processing a single substrate, or two complimentary substrates in parallel or sequentially. We define *inhibition* as the processes by which an agent reversibly binds to an SU (see Fig. 1). Since this process is conceptually similar to Michaelis-Menten-Briggs-Haldane enzyme kinetics, we will adopt the terminology used in the latter to define particular forms of inhibition. At the time of writing, definitions of some types of inhibition, in particular mixed forms, vary slightly among popular online sources; here we follow the terminology as used on ChemWiki (2017). A *damaging agent* renders an SU dysfunctional, i.e. it needs to be repaired in order to regain functionality. The repair mechanism resets a dysfunctional SU to the unbound state (see Fig. 2). We define an SU in the unbound state as an SU without the required number of substrate molecules attached; it may have bound an inhibitor. Stages of SUs are discrete; stage transitions occur when a sufficient number of substrate, inhibitor or damaging agent molecules have associated with, dissociated from or been transformed by an SU in a certain stage.

In order to simplify notation, we scale the rate at which substrates, inhibitors or damaging agents arrive at the SU, J_*, to the number of molecules of substrates, inhibitors or damaging agents needed to make product or inhibit or damage the SU, n_*, and to the binding probability, ρ_*, at which these molecules associate with the SU

$$j_* = \frac{\rho_* J_*}{n_*} \tag{1}$$

Note that this notation deviates from the customary one in many DEB publications, in which *j* represents a flux normalized to the amount of structural biomass; other notation in this study closely follows the one designed by Kooijman (2010).

We assume that arrival fluxes of substrates, inhibitors and damaging agents are constant. We also assume that the time scale of SU kinetics is much faster than, and hence decoupled from, those of whole-organism dynamics so that the relative abundance of SU states at any given time is assumed to change only due to kinetics. The SU production rates derived in the following subsections are thus applicable to dynamical systems, provided that arrival fluxes and the total number of SUs change slowly relative to SU kinetics (cf. ChemWiki, 2017; Kooijman, 1998; Segel, 1993). Mathematically, the formalism for all SU kinetic models in this paper is equivalent to that of a continuous time Markov chain (Kooijman, 1998), and the models' structure meets the requirements for the existence of a unique, stable steady state (see e.g. Karlin, 1966).

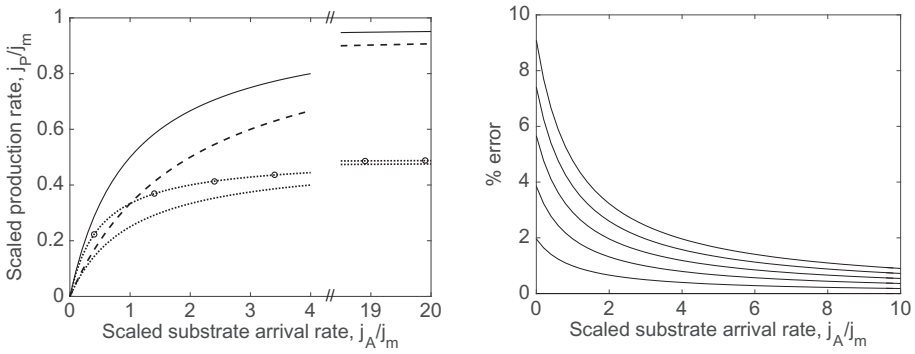

Fig. 2. Performance of inhibited and damaged single substrate SUs. (A) Relative to uninhibited SUs (solid line), a competitive inhibitor reduces the production rate of an SU especially at low substrate arrival rates and has relatively little impact on SU performance at high substrate arrival rates (broken line). A noncompetitive inhibitor scales down production rates evenly irrespective of substrate arrival rates (dotted line). An uncompetitive inhibitor has relatively little impact on SU performance at low substrate levels, while it approaches noncompetitive inhibition kinetics at high substrate arrival rates (dotted line with circles). For all types of inhibition, $j_{i*}/k_{i*} = 1$. (B) The error made in assuming noncompetitive inhibition kinetics for noncompetitive damage declines with increasing substrate arrival rates. From top to bottom, the curves represent errors for j_{dA}/j_m = 0.1, 0.08, 0.06, 0.04 and 0.02, respectively.

2.1. Single substrate SUs: Inhibition

Partial mixed inhibition is defined as the process whereby an inhibitor binds reversibly to both SUs in the unbound state and SUs with bound substrates but (1) with potentially different dissociation parameters, k_i and k_{iA} (see Fig. 1), the inhibitor slows down the rate at which processing SUs form product(s). A mathematically equivalent situation is where there are different association affinities (i.e. ρ_* hidden in the arrival flux of inhibitor, j_{i*} – see Eq. 1). This is the generic form of inhibition of enzyme kinetics shown in the top panel of Fig. 1 (ChemWiki, 2017; recall that substrates bind irreversibly to SUs but reversibly to enzymes).

The balance equation of the fraction of SUs in the binding, processing, inhibited while in binding, and inhibited while in the processing states (symbols represent states in this particular order) dictates

$$\theta_\cdot + \theta_A + \theta^i_\cdot + \theta^i_A = 1 \tag{2}$$

With the standard assumption of a rapid convergence to steady states of the fractions of SUs that are in the binding, processing and inhibited states, we get

$$\begin{pmatrix} \frac{d\theta_\cdot}{dt} \\ \frac{d\theta_A}{dt} \\ \frac{d\theta^i_\cdot}{dt} \\ \frac{d\theta^i_A}{dt} \end{pmatrix} = \begin{pmatrix} -j_A - j_i & j_m & k_i & j_{mi} \\ j_A & -j_m - j_{iA} & 0 & k_{iA} \\ j_i & 0 & -j_{Ai} - k_i & 0 \\ 0 & j_{iA} & j_{Ai} & -j_{mi} - k_{iA} \end{pmatrix} \begin{pmatrix} \theta_\cdot \\ \theta_A \\ \theta^i_\cdot \\ \theta^i_A \end{pmatrix} = \mathbf{0} \tag{3}$$

The rate at which an SU forms product, j_p,

$$j_p = j_m \theta_A + j_{mi} \theta^i_A, \tag{4}$$

where θ_A and $\theta_A{}^i$ are obtained by solving Eq. (3), recognizing that the fractions sum to one. The explicit solutions are lengthy, meaning that their substitution into Eq. (4) does not yield an illuminating expression.

Special cases arise when one or more of the SU states do not bind substrates and/or inhibitors, and/or convert substrates into products (see four lower panels in Fig. 1). In enzyme kinetics, *mixed inhibition* is the situation where $j_{mi} = 0$. In order to obtain similar mathematical formalism with SUs, which bind substrates irreversibly, we also need to assume that inhibited SUs cannot bind substrates, i.e. $j_{Ai} = 0$. Then,

$$j_p = \frac{1}{\frac{1}{j_m}\left(1 + \frac{j_{iA}}{k_{iA}}\right) + \frac{1}{j_A}\left(1 + \frac{j_i}{k_i}\right)} \tag{5}$$

In order to show that this reduces to the more standard representation of mixed inhibition in enzyme kinetics, we make the concentration of substrate S and inhibitor I proportional to their respective unscaled arrival fluxes, and use symbols commonly found in textbooks on enzyme kinetics (with V substituted for j_p and $V_{\max}$ for j_m). This yields the form (ChemWiki, 2017)

$$V = \frac{V_{\max} S}{S\left(1 + \frac{I}{K_i}\right) + K_M\left(1 + \frac{I}{K_{iA}}\right)} \tag{6}$$

with $K_i \equiv \frac{\rho_i k_i}{n_i p_i}$, $K_{iA} \equiv \frac{\rho_{iA} k_{iA}}{n_i p_i}$ and $K_M \equiv \frac{\rho_A j_m}{n_A p_A}$, in which p_* are proportionality constants converting fluxes to concentrations.

Mixed inhibition of SUs reduces to *noncompetitive inhibition* when substrates do not affect the binding and dissociation of inhibitors, i.e. $j_i = j_{iA}$ and $k_i = k_{iA}$,

$$j_p = \frac{1}{\left(1 + \frac{j_i}{k_i}\right)\left(\frac{1}{j_A} + \frac{1}{j_m}\right)} \tag{7}$$

Noncompetitive inhibition of SUs differs from noncompetitive inhibitions of enzymes in that the former in the inhibited state cannot bind substrates. The fraction by which noncompetitive inhibitors reduce SU performance is independent of the substrate arrival rate (see Fig. 2A). With *uncompetitive inhibition*, inhibitors only bind reversibly to SUs in the processing state, i.e. $j_i = 0$, which yields

$$j_p = \frac{1}{\left(\frac{1}{j_A} + \frac{1}{j_m}\left(1 + \frac{j_{iA}}{k_{iA}}\right)\right)} \tag{8}$$

Conversely, when inhibitors only bind reversibly to SUs without substrates attached, for instance by blocking the active site, we have *competitive inhibition*, $j_{iA} = 0$

$$j_p = \frac{1}{\left(\frac{1}{j_A}\left(1 + \frac{j_i}{k_i}\right) + \frac{1}{j_m}\right)} \tag{9}$$

Uncompetitive and competitive inhibitions of SUs are similar to their counterparts in enzyme kinetics. At high substrate levels, uncompetitive inhibitors resemble noncompetitive inhibitors and competitive inhibitors are little effective (see Fig. 2A). At low substrate levels, the impact of competitive inhibitors on SU performance is relatively strong, while uncompetitive inhibitors only have a marginal effect.

In conclusion, with a single substrate and with arrival fluxes of substrates and inhibitors proportional to their respective concentrations, competitive and uncompetitive inhibition mechanisms of SUs are mathematically similar to their counterparts in Michaelis-Menten-Briggs-Haldane enzyme kinetics. Noncompetitive and mixed inhibitions of SUs are mathematically similar to their counterparts in enzyme kinetics, provided the inhibited form of the former cannot bind substrates.

2.2. Single substrate SUs: Damage

We consider agents that can *damage* a single substrate SU in both the unbound and processing state but with a damaging potential that may depend on the state of the SU. We assume that a damaged SU is dysfunctional but can be repaired to yield an SU in the unbound state. One could think of, for instance, a superoxide radical that removes an iron

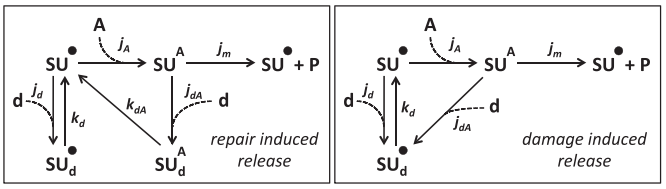

Fig. 3. Scheme of the possibilities at which a damaging agent *d* may interact with a single substrate SU. Solid arrows represent SU state transitions (including repair), broken arrows substrate association and damage fluxes. In contrast to an inhibited SU (see Fig. 1), a damaged SU needs to be repaired to restore its functionality; if damage is inflicted on an SU in the processing state, substrates are released either during the repair process (repair-induced release) or as part of the damaging process (damage-induced release). In analogy to inhibition, the generic form of damage is mixed damage, in which agents can damage SUs in both the unbound and processing state but with different damaging probabilities and repair parameters. Special cases include noncompetitive damage (agents damage SUs in the unbound and bound state with similar probability and repair parameters); and uncompetitive damage (agents only damage SUs in the bound state). The dynamics of competitive damage (agents only damage SUs in the unbound state) are similar to those of competitive inhibition.

atom from an enzymatic iron-sulfur cluster, which is then subjected to a repair mechanism (Imlay, 2003); enzymes with iron-sulfur clusters play an important role in redox reactions of, for example, the respiratory chain. Thus, in our representation, damage mechanisms differ from those of inhibition in that a damaged SU returns to the open binding state, regardless its state prior to impact. However, if damage is inflicted only upon SUs in the binding stage, the resulting dynamics are identical to those of competitive inhibition; compounds that inactivate enzymes by substituting cofactors (e.g. Cd for Zn) may cause damage in this way. An SU damaged in the processing state looses bound substrate before its functionality is restored. Release of bound substrate could be part of the repair or damage process; we will to these possibilities as repair-induced release and damage-induced release, respectively (see Fig. 3).

With repair-induced release, the balance equation of the fractions of SUs in the various states is

$$\theta_{\cdot} + \theta_A + \theta_{\cdot}^d + \theta_A^d = 1 \tag{10}$$

with the dynamic equations in steady state being

$$\begin{pmatrix} \frac{d\theta_{\cdot}}{dt} \\ \frac{d\theta_A}{dt} \\ \frac{d\theta_{\cdot}^d}{dt} \\ \frac{d\theta_A^d}{dt} \end{pmatrix} = \begin{pmatrix} -j_A - j_d & j_m & k_d & k_{dA} \\ j_A & -j_m - j_{dA} & 0 & 0 \\ j_d & 0 & -k_d & 0 \\ 0 & j_{dA} & 0 & -k_{dA} \end{pmatrix} \begin{pmatrix} \theta_{\cdot} \\ \theta_A \\ \theta_{\cdot}^d \\ \theta_A^d \end{pmatrix} = \mathbf{0} \tag{11}$$

The solution of this system yields the mean production rate for the *mixed damage*,

$$j_p = \frac{1}{\frac{1}{j_m}\left(1 + \frac{j_{dA}}{k_{dA}}\right) + \frac{1}{j_A}\left(1 + \frac{j_d}{k_d}\right)\left(1 + \frac{j_{dA}}{j_m}\right)} \tag{12}$$

In analogy with special cases of inhibition, Eq. (12) reduces to *noncompetitive damage* when $j_d = j_{dA}$ and $k_d = k_{dA}$, and to and *uncompetitive damage* $j_d/k_d = 0$. With *competitive damage*, $j_{dA}/k_{dA} = 0$; thus, competitive damage and inhibition are mathematically similar. The dynamics of mixed, noncompetitive and uncompetitive damage with repair induced release reduces to those of their respective forms of inhibition when $j_m \gg j_{dA}$, that is, the maximum rate at which an SU can form product is much greater than the rate at which agents can damage SUs in the processing state. It seems safe to assume that this condition is normally met in biologically viable systems (note that the system in Eq. (11) presupposes viability). The relative error made in assuming inhibition for damage kinetics is greatest for the noncompetitive case. This error is less than 10% when $j_{dA}/j_m \leq 0.1$ and becomes less significant with increasing substrate arrival rates (see Fig. 2B).

With damage-induced release of substrates from a processing SU, the balance equation of the fractions of SUs in the various states is

$$\theta_{\cdot} + \theta_A + \theta_{\cdot}^d = 1 \tag{13}$$

The system in steady state is

$$\begin{pmatrix} \frac{d\theta_{\cdot}}{dt} \\ \frac{d\theta_A}{dt} \\ \frac{d\theta_{\cdot}^d}{dt} \end{pmatrix} = \begin{pmatrix} -j_A - j_d & j_m & k_d \\ j_A & -j_m - j_{dA} & 0 \\ j_d & j_{dA} & -k_d \end{pmatrix} \begin{pmatrix} \theta_{\cdot} \\ \theta_A \\ \theta_{\cdot}^d \end{pmatrix} = \mathbf{0} \tag{14}$$

and the mean production rate of an SU

$$j_p = \frac{1}{\frac{1}{j_m}\left(1 + \frac{j_{dA}}{k_d}\right) + \frac{1}{j_A}\left(1 + \frac{j_d}{k_d}\right)\left(1 + \frac{j_{dA}}{j_m}\right)} \tag{15}$$

which is equivalent to Eq. (12) when $k_d = k_{dA}$. Thus, Eq. (12) can serve as a general model of damage dynamics with a single substrate SU.

In conclusion, damage models of single substrate SUs reduce to variants of inhibition models if the mean processing time (i.e. the reciprocal of j_m) is short relative to the mean time interval between damage events (i.e. the reciprocal of j_d).

2.3. SU parallel processing of 2 complementary substrates: Inhibitionsss

In absence of an inhibitor, an SU processing two complementary substrates in parallel can be in four different states (see Fig. 4). An inhibitor may target an SU in any of those states, implying that the balance equation of the fractions of SUs in those eight states must obey

$$\theta_{\cdot\cdot} + \theta_{A\cdot} + \theta_{\cdot B} + \theta_{AB} + \theta_{\cdot\cdot}^i + \theta_{A\cdot}^i + \theta_{\cdot B}^i + \theta_{AB}^i = 1 \tag{16}$$

in which subscripted dots *'A'* and *'B'* denote empty binding sites, bound substrate *A* and *B*, respectively. For simplicity's sake, we ignore the possibility that inhibited SUs bind substrates, but use the terminology of enzyme kinetics in order to maintain mathematical congruency (see Subsection 2.1) The system in steady state is

$$\mathrm{d}_\Theta = \mathbf{M}\Theta = \mathbf{0} \tag{17}$$

with

$$\mathbf{d}_\Theta = \left(\frac{d\theta_{\cdot\cdot}}{dt} \; \frac{d\theta_{A\cdot}}{dt} \; \frac{d\theta_{\cdot B}}{dt} \; \frac{d\theta_{AB}}{dt} \; \frac{d\theta_{\cdot\cdot}^i}{dt} \; \frac{d\theta_{A\cdot}^i}{dt} \; \frac{d\theta_{\cdot B}^i}{dt} \; \frac{d\theta_{AB}^i}{dt}\right)^T \tag{18}$$

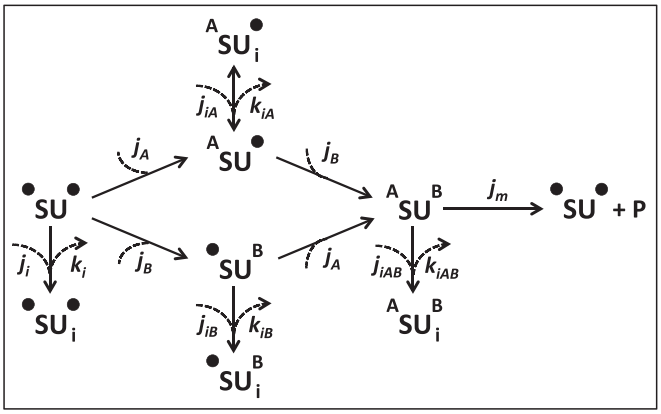

Fig. 4. Scheme of the possibilities at which an inhibitor *i* may interact with an SU processing 2 complementary substrates in parallel. Solid arrows represent SU state transitions, broken arrows substrate and inhibitor association and dissociation fluxes.

$$\Theta = (\theta_{\bullet\bullet}\ \ \theta_{A\bullet}\ \ \theta_{\bullet B}\ \ \theta_{AB}\ \ \theta^{i}_{\bullet\bullet}\ \ \theta^{i}_{A\bullet}\ \ \theta^{i}_{\bullet B}\ \ \theta^{i}_{AB})^{T} \tag{19}$$

and

$$\mathbf{M} = \begin{pmatrix} -j_A - j_B - j_i & 0 & 0 & j_m & k_i & 0 & 0 & 0 \\ j_A & -j_B - j_{iA} & 0 & 0 & 0 & k_{iA} & 0 & 0 \\ j_B & 0 & -j_A - j_{iB} & 0 & 0 & 0 & k_{iB} & 0 \\ 0 & j_B & j_A & -j_m - j_{iAB} & 0 & 0 & 0 & k_{iAB} \\ j_i & 0 & 0 & 0 & -k_i & 0 & 0 & 0 \\ 0 & j_{iA} & 0 & 0 & 0 & -k_{iA} & 0 & 0 \\ 0 & 0 & j_{iB} & 0 & 0 & 0 & -k_{iB} & 0 \\ 0 & 0 & 0 & j_{iAB} & 0 & 0 & 0 & -k_{iAB} \end{pmatrix} \tag{20}$$

The solution of this system yields the mean production rate of an SU with mixed inhibition

$$j_p = j_m \theta_{AB} = \left(\frac{c_{iAB}}{j_m} + \frac{c_{i\bullet B}}{j_A} + \frac{c_{iA\bullet}}{j_B} - \frac{c_{iA\bullet} + c_{i\bullet B} - c_{i\bullet\bullet}}{j_A + j_B} \right)^{-1} \tag{21}$$

in which $c_{iXY} \equiv 1 + \frac{j_{iXY}}{k_{iXY}}$ are inhibition factors with X and Y representing A, B, or a dot. These factors are not compound parameters but are defined for notational convenience. In noncompetitive inhibition, inhibitors interact with SUs independent of the state of the latter, i.e. $c_{i\bullet\bullet} = c_{iA\bullet} = c_{i\bullet B} = c_{iAB} = c$, which leads to

$$j_p = \frac{1}{c}\left(\frac{1}{j_m} + \frac{1}{j_A} + \frac{1}{j_B} - \frac{1}{j_A + j_B} \right)^{-1} \tag{22}$$

As with single substrate SUs, a noncompetitive inhibitor simply scales the production rate of a 2 substrate SU, meaning that the relative strength of a noncompetitive inhibitor is independent of substrate availability.

If inhibitors target SUs only in certain states, the inhibition factors for the unaffected states need to be set to unity, $c_{iXY} = 1$. For instance, if the action of an inhibitor is only to compete with the binding site of substrate A and substrate B does not affect inhibition kinetics, $c_{iA\bullet} = c_{iAB} = 1$ and $c_{i\bullet\bullet} = c_{i\bullet B} = c$, we have partial competitive inhibition with the mean production rate being

$$j_p = \left(\frac{1}{j_m} + \frac{c}{j_A} + \frac{1}{j_B} - \frac{1}{j_A + j_B} \right)^{-1} \tag{23}$$

Partial competitive inhibition is especially prevalent at low arrival rates of substrate A and relatively high substrate levels of complementary substrate B (see Fig. 5A). If $c_{i\bullet\bullet} = c_{iA\bullet} = c_{i\bullet B} = 1$, we have uncompetitive inhibition,

$$j_p = \left(\frac{c_{iAB}}{j_m} + \frac{1}{j_A} + \frac{1}{j_B} - \frac{1}{j_A + j_B} \right)^{-1} \tag{24}$$

which is relatively strong at high arrival levels of substrate A and B (see Fig. 5b). Other inhibition schemes, including hybrid ones, can be easily obtained by setting the appropriate inhibition factors to unity.

In sum, with two substrates processes in parallel, there are potentially four SU stages targeted by inhibitors. The algebra becomes considerably more tedious, but the resulting dynamics for the various types of inhibition are in line with those with a single substrate SU (see Subsection 2.1).

2.4. SU parallel processing of 2 complementary substrates: Damage

With single substrate SUs, damage induced and repair induced release of substrate yield similar models (see above). Since damage induction involves fewer SU states, we work out schemes for two complementary substrates processed in parallel in which damage causes the instantaneous release of bound substrates (see Fig. 6). The balance equation for the fractions of SUs in the five potential states is

$$\theta_{\bullet\bullet} + \theta_{A\bullet} + \theta_{\bullet B} + \theta_{AB} + \theta^{d} = 1 \tag{25}$$

The system in steady state is

$$\begin{pmatrix} \frac{d\theta_{\bullet\bullet}}{dt} \\ \frac{d\theta_{A\bullet}}{dt} \\ \frac{d\theta_{\bullet B}}{dt} \\ \frac{d\theta_{AB}}{dt} \\ \frac{d\theta^{d}}{dt} \end{pmatrix} = \begin{pmatrix} -j_A - j_B - j_d & 0 & 0 & j_m & k_d \\ j_A & -j_B - j_{dA} & 0 & 0 & 0 \\ j_B & 0 & -j_A - j_{dB} & 0 & 0 \\ 0 & j_B & j_A & -j_m - j_{dAB} & 0 \\ j_d & j_{dA} & j_{dB} & j_{dAB} & -k_d \end{pmatrix} \begin{pmatrix} \theta_{\bullet\bullet} \\ \theta_{A\bullet} \\ \theta_{\bullet B} \\ \theta_{AB} \\ \theta^{d} \end{pmatrix} = \mathbf{0} \tag{26}$$

If all SU states are prone to damage but with different probabilities, we have mixed damage, for which the mean production rate is

$$j_p = \left(\left(c_d + \frac{c_d j_{dB} + c_{dB}(j_B + j_{dA})}{j_A} + \frac{c_d j_{dA} + c_{dA}(j_A + j_{dB})}{j_B} + \frac{c_d j_{dA} j_{dB}}{j_A j_B} \right) \frac{\left(1 + \frac{j_{dAB}}{j_m}\right)}{(j_A + j_B + j_{dA} + j_{dB})} + \frac{c_{dAB}}{j_m} \right)^{-1} \tag{27}$$

in which $c_* \equiv 1 + \frac{j_*}{k_d}$ with '*' for *'d'*, *'dA'*, *'dB'* or *'dAB'*. It seems reasonable to assume that, for a viable system, the maximum processing rate and the arrival fluxes of substrates are much higher than those of damaging agents. Then, Eq. (27) simplifies to

$$j_p = \left(\frac{c_{dAB}}{j_m} + \frac{c_{dB}}{j_A} + \frac{c_{dA}}{j_B} - \frac{c_{dA} + c_{dB} - c_d}{j_A + j_B} \right)^{-1} \tag{28}$$

This is mathematically similar to mixed inhibition. Accordingly, expressions for noncompetitive, competitive, uncompetitive and hybrid forms of damage are similar to those for corresponding forms of inhibition.

Of particular interest is damage caused by oxidizing agents. If one of

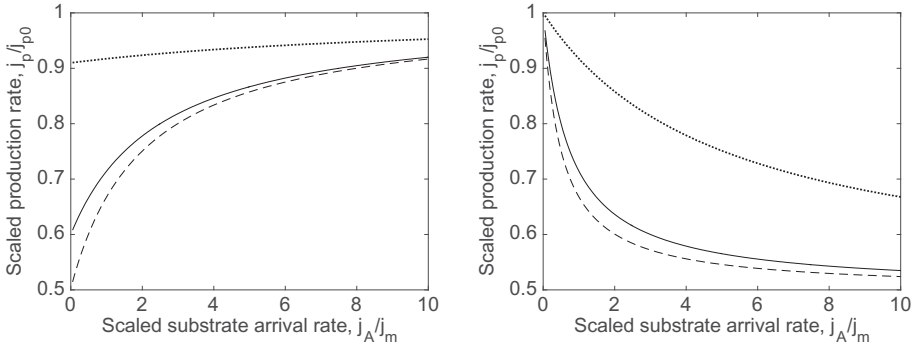

Fig. 5. Production rates of 2 substrate SUs relative to uninhibited production rates with partial competitive inhibition (A, Eq. (23) with $c = 2$) and uncompetitive inhibition (B, Eq. (24) with $c_{iAB} = 2$) with $j_B = j_A$ (solid lines), $j_B = 10j_A$ (broken lines) and$j_B = 0.1j_A$ (dotted lines). Competitive inhibition is especially felt at low substrate levels, whereas uncompetitive inhibition is relatively strong at high substrate levels. With both types, the impact of inhibition diminishes with decreasing availability of complementary substrate B (which does not compete with the inhibitor in the partial competitive inhibition case), due to its relative dominance in determining SU performance at low levels. The noncompetitive case is not illustrated here, as the relative strength of this inhibition type does not depend on substrate availability (see Eq. (22)).

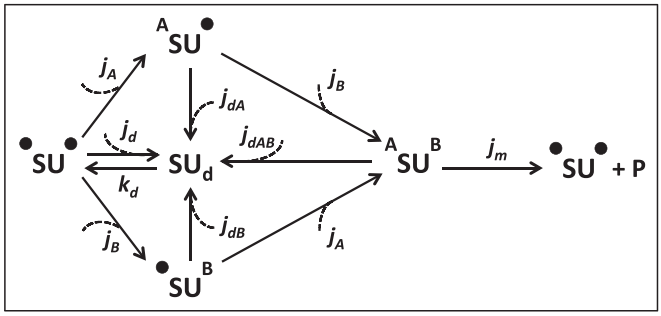

Fig. 6. Scheme of the possibilities at which a damaging agent *d* may interact with an SU processing 2 complementary substrates in parallel. Solid arrows represent SU state transitions (including repair), broken arrows substrate association and damage fluxes. After repair a damaged SU is in the unbound state.

the substrates, say *A*, oxidizes the SU, we have a hybrid competitive scheme. Assuming that damaging agents do not interact with SUs with bound *A*, $c_{dA} = c_{dAB} = 1$, and that substrate *B* does not interfere with the damage process, $c_d = c_{dB}$, we have

$$j_p = \left(\frac{1}{j_m} + \frac{c_d}{j_A} + \frac{1}{j_B} - \frac{1}{j_A + j_B}\right)^{-1} \tag{29}$$

Conversely, if *A* reduces the SU, we have an uncompetitive scheme. Assuming that damaging agents only interact with SUs with bound *A*, $c_d = c_{dB} = 1$, and that substrate *B* does not interfere with the damage process, $c_{dA} = c_{dAB} = c$, we have,

$$j_p = \left(\frac{c}{j_m} + \frac{1}{j_A} + \frac{c}{j_B} - \frac{1}{j_A + j_B}\right)^{-1} \tag{30}$$

In sum, in line with damage models of single substrate SUs, damage models of parallel processing 2 substrate SUs reduce to their respective variants of inhibition models if the mean processing time and mean time interval between substrate binding events is short relative to the mean time interval between damage events.

2.5. Inhibition of multiple substrate SUs: Sequential processing

Many cellular processes proceed in a chain-like fashion, such as the respiratory chain and glycolosis. In addition, several enzymes requiring multiple substrates bind those in sequential order. Chains are often branched, intermediate products may be released, and the relative abundance of enzymes may vary, all of which introduce complexity beyond the scope of this paper. To retain presentational simplicity, we limit the presentation here to two substrates that are being processed sequentially, noting that the formalism is easily generalized to *n* substrates.

Since there are potentially six states (see Fig. 7), the balance equation is

$$\theta_\cdot + \theta_{A\cdot} + \theta_{AB} + \theta^i_\cdot + \theta^i_{A\cdot} + \theta^i_{AB} = 1 \tag{31}$$

When the system is in steady state,

$$\begin{pmatrix} \frac{d\theta_\cdot}{dt} \\ \frac{d\theta_{A\cdot}}{dt} \\ \frac{d\theta_{AB}}{dt} \\ \frac{d\theta^i_\cdot}{dt} \\ \frac{d\theta^i_A}{dt} \\ \frac{d\theta^i_{AB}}{dt} \end{pmatrix} = \begin{pmatrix} -j_A - j_i & 0 & j_m & k_i & 0 & 0 \\ j_A & -j_B - j_{iA} & 0 & 0 & k_{iA} & 0 \\ 0 & j_B & -j_m - j_{iAB} & 0 & 0 & k_{iAB} \\ j_i & 0 & 0 & -k_i & 0 & 0 \\ 0 & j_{iA} & 0 & 0 & -k_{iA} & 0 \\ 0 & 0 & j_{iAB} & 0 & 0 & -k_{iAB} \end{pmatrix} \begin{pmatrix} \theta_\cdot \\ \theta_{A\cdot} \\ \theta_{AB} \\ \theta^i_\cdot \\ \theta^i_A \\ \theta^i_{AB} \end{pmatrix} = \mathbf{0} \tag{32}$$

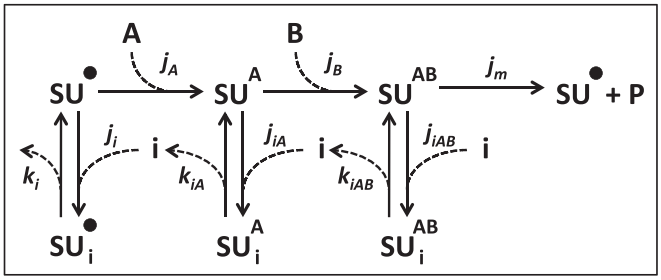

Fig. 7. Scheme of the possibilities at which inhibitor *i* may interact with an SU processing 2 complementary substrates in series. Solid arrows represent SU state transitions, broken arrows substrate and inhibitor association and dissociation fluxes.

which implies the mean production rate is

$$j_p = \left(\frac{c_{iAB}}{j_m} + \frac{c_i}{j_A} + \frac{c_{iA}}{j_B}\right)^{-1} \tag{33}$$

As in examples in Subsection 2.6, with noncompetitive inhibition, $c_i = c_{iA} = c_{iAB}$, the inhibition factor can be factored out. Uncompetitive inhibition arises when $c_i = c_{iA} = 1$ and competitive inhibition when $c_{iAB} = 1$. Thus, inhibition scenarios of SUs processing two complementary substrates sequentially are analogous to those of processing two complementary substrates in parallel.

2.6. Damage of multiple substrate SUs: Sequential processing

As before, we assume that a damaged SU instantaneously releases any bound substrates. Then, with a damaging agent, an SU processing two substrates sequentially exists in four potential states (see Fig. 8). The balance equation of fractions of SUs in a particular state is

$$\theta_\cdot + \theta_{A\cdot} + \theta_{AB} + \theta^d = 1 \tag{34}$$

In steady state,

$$\begin{pmatrix} \frac{d\theta_\cdot}{dt} \\ \frac{d\theta_{A\cdot}}{dt} \\ \frac{d\theta_{AB}}{dt} \\ \frac{d\theta^d}{dt} \end{pmatrix} = \begin{pmatrix} -j_A - j_d & 0 & j_m & k_d \\ j_A & -j_B - j_{dA} & 0 & 0 \\ 0 & j_B & -j_m - j_{dAB} & 0 \\ j_i & j_{dA} & j_{dAB} & -k_d \end{pmatrix} \begin{pmatrix} \theta_\cdot \\ \theta_{A\cdot} \\ \theta_{AB} \\ \theta^d \end{pmatrix} = \mathbf{0} \tag{35}$$

Accordingly, in the presence of a damaging agent, the mean production rate of an SU processing two substrates sequentially is

$$j_p = \left(\left(\frac{c_d}{j_A} + \frac{c_{dA}}{j_B} + \frac{c_d j_{dA}}{j_A j_B}\right)\left(1 + \frac{j_{dAB}}{j_m}\right) + \frac{c_{dAB}}{j_m}\right)^{-1} \tag{36}$$

If the maximum processing rate and the arrival fluxes of substrates

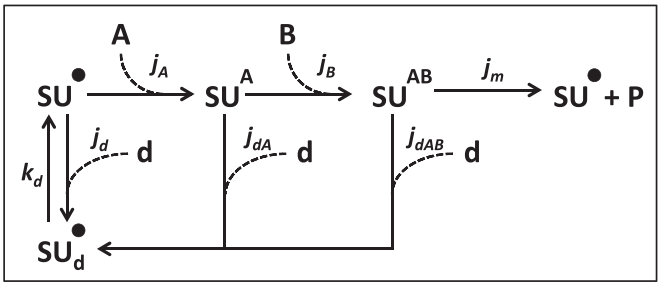

Fig. 8. Scheme of the possibilities at which damaging agent *d* may interact with an SU processing 2 complementary substrates in series. Solid arrows represent SU state transitions (including repair), broken arrows substrate association and damage fluxes.

are much higher than those of damaging agents, this expression reduces to

$$j_p = \left(\frac{c_d}{j_A} + \frac{c_{dA}}{j_B} + \frac{c_{dAB}}{j_m} \right)^{-1} \quad (37)$$

which is mathematically similar to mixed inhibition with two sequentially processed substrates. Therefore, damage scenarios with two complementary sequentially processed substrates are similar to corresponding inhibition scenarios.

3. Discussion

Conceptually, SUs resemble enzymes that convert an arbitrary number of different kinds of substrate into one or more products. Enzyme activity is driven by substrate availability and is subject to regulatory mechanisms, e.g. via inhibitors and activators, and to the deleterious impact of physical and chemical agents. Since enzyme kinetics has a long history and expansive literature, we have used paradigms from this field to explore the impact of inhibitors and damaging agents on the dynamics of SUs requiring one or two substrates. Inhibitors interact reversibly with SUs and thereby impede their functioning but otherwise do not have deleterious impact, whereas a damaging agent decommissions an SU. The decommissioned SU then either needs to be replaced via de novo synthesis or be repaired, implying that any already bound substrate molecules will be removed. When substrate arrival rates are proportional to densities, single substrate SUs behave dynamically similar to their enzymatic counterparts (Kooijman, 1998, 2001).

This similarity holds when an inhibitor is present, with a minor adjustment (i.e. with noncompetitive and mixed inhibition an inhibited enzyme but not an inhibited SU in the unbound state can bind substrate molecules - see Fig. 1 for an overview of inhibition schemes). The impact of a competitive inhibitor is relatively strong at low substrate levels, whereas the opposite is true for uncompetitive inhibitor; a noncompetitive inhibitor scales down the SU production rate evenly along the axis of substrate arrival rates (see Fig. 2A). If an agent can only damage an SU without bound substrate, its impact on the average production rate of an SU is mathematically similar to that of a competitive inhibitor. The impact of a damaging agent targeting other SU states is approximately equivalent to that of inhibitors targeting similar SU states, provided that the mean time interval between damage events is long relative to the time it takes an SU with bound substrate to form a product. When this is not the case, the additional temporal cost (of damage compared to inhibition) associated with the need to make up for the removal of substrates bound to damaged SUs further reduces the production rate. In endergonic processes, there is also an additional energy cost to make up for the lost binding of the substrate to the SU that got damaged. Those conclusions with single substrate SUs essentially carry over to SUs requiring two different substrates to form a product, though the mathematical formalisms involved are more complex and involve more parameters (depending on inhibition or damage scheme, 2–3 parameters for single substrate SUs and 2–5 parameters for two substrate SUs).

Several of the inhibition and damage schemes have previously been applied to model negative impacts of environmental stressors toxic impact within the DEB framework. For instance, we have used the noncompetitive inhibition function with a single substrate SU to model toxic impacts on feeding and assimilation in various organisms (Klanjscek et al., 2012, 2013; Miller et al., 2010, 2017; Muller et al., 2010a,b, 2014). Since this function, which acts as a simple multiplier of the feeding and assimilation rate equations in DEB, can take only positive values, it has an advantage over the negative sloped linear toxic effect function commonly used in DEBtox (Jager et al., 2010 and references therein). Photoinhibition in algae has been modeled using uncompetitive inhibition with a single substrate SU (Zonneveld, 1998) and mixed inhibition with an SU processing two complementary substrates in parallel (Muller, 2011). A competitive damage scheme forms the corner stone of the receptor kinetics model by Jager and Kooijman (2005) describing the impact of insecticides on the neurological circuit in guppies. The current presentation brings those models together in a single modeling framework and generalizes inhibition and damage mechanisms for SUs processing two complementary substrates.

We have considered the impact of inhibitors and damaging agents on SU dynamics in the context of a supply system, i.e. we have focused on the reduction of SU production rates due to the impeding impacts of these two types of agents. In contrast, for a demand system, it would be relevant to ask the question how many more SUs would be needed to neutralize the impact of an inhibitor or damaging agent, thereby addressing in part the energetic costs of inhibition and damage. In relative terms, the increase in SU capacity amounts to the ratio of the mean production rate of an SU in absence of inhibitors or damaging agents and the mean production rate of an SU with inhibitors or damaging agents. This ratio is the inverse of the dependent variable in Fig. 5. Competitive inhibition (and damage) is relatively costly to compensate for at low substrate availabilities, whereas uncompetitive inhibition (and damage) is especially costly to remediate at high substrate availabilities. With noncompetitive inhibition (and approximately noncompetitive damage), regardless of substrate availability, the SU capacity increases linearly with the arrival rate of inhibitors. This agrees well with the maintenance toxic effect module in DEBtox (see e.g. Kooijman and Bedaux, 1996; Muller et al., 2010a).

There are obvious conceptual similarities between single substrate SUs and individuals feeding according to the Holling Type II functional response. Indeed, the mathematical approach taken in this paper was set out formally by Metz and Vanbatenburg (1985). Accordingly, models for inhibition and damage with single substrate SUs are relevant for describing the impeding effect social interactions can have on feeding activity (Kooijman and Troost, 2007). It is easy to see that competitive inhibition is conceptually similar to the situation in which conspecifics or individuals of another species impede the feeding activity of an animal. Indeed, the well-known model of DeAngelis et al. (1975) describing the impeding impact of social interactions on feeding is mathematically equivalent to competitive inhibition by either conspecifics or by individuals of another species (assuming meeting rates are proportional to densities). Many elaborations of this approach have subsequently been developed (e.g. O'Neill et al., 1989); also at least one study on the effects of plant toxins on herbivores shows the importance of mechanisms (analogous to those discussed here) that impact maximum feeding rate (Swihart et al., 2009). Our uncompetitive damage scheme is conceptually similar to stealing prey from a predator, a situation which was modeled by Ruxton et al. (1992) using a chemical-reaction-like scheme conceptually similar to ours.

Our presentation generalizing the impact of inhibitors and damaging agents on one and two substrate SUs has several potential applications of special interest, such as in the context of describing the impact of oxidative stress on SU dynamics. If an oxidative agent damages an SU, for instance by removing a metallic cofactor, the resulting impact on SU dynamics is potentially described by the competitive damage scheme (Eq. (9) for a single substrate SU and Eq. (29) for an SU processing two complementary substrates in parallel). Furthermore, the uncompetitive damage scheme for an SU processing two substrates sequentially has potential to describe the energetic loss implied by damage in cases the purpose of binding the first substrate (cf. ATP) is to increase the energy level of the SU. The formalism presented here can, therefore, account for a marked variety of conceptual SUs, and types of inhibition and damage – ranging from enzymes and molecules to individuals and social interactions instigating a behavioral response.

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