Robust Adaptation and Homeostasis by Autocatalysis

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Robust Adaptation and Homeostasis by Autocatalysis

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Abstract

Robust homeostatic mechanisms are essential for the protection and adaptation of organisms in a changing and challenging environment. Integral feedback is a control-engineering concept that leads to robust, i.e., perturbation-independent, adaptation and homeostatic behavior in the controlled variable. Addressing two-component negative feedback loops of a controlled variable $A$ and a controller molecule $E$, we have shown that integral control is closely related to the presence of zero-order fluxes in the removal of the manipulated variable $E$. Here we show that autocatalysis is an alternative mechanism to obtain integral control. Although the conservative and marginal stability of the Lotka-Volterra oscillator (LVO) with autocatalysis in both $A$ and $E$ is often considered as a major inadequacy, homeostasis in the average concentrations of both $A$ and $E$ ($<A>$ and $<E>$) is observed. Thus, autocatalysis does not only represent a mere driving force, but may also have regulatory roles.

Key words: Lotka-Volterra oscillator, positive feedback, negative feedback, control theory, integral control
Introduction

Living organisms have the remarkable property to adapt to external environmental changes by keeping their 'internal environment' at an approximately constant level (1). The development of the concept of homeostasis, i.e., the presence of coordinated physiological processes that maintain internal stability in organisms is attributed to Cannon, who also coined the term homeostasis during the 1920’s (2, 3).

After Cannon, the concept of homeostasis broadened and other terminologies were introduced, either related to (circadian) set-point changes as in predictive homeostasis (4) and rheostasis (5), or, as for the concept of allostasis (6, 7), by considering both behavioral and physiological processes that maintain internal parameters within certain essential limits.

During the 1920’s, Lotka (8) investigated the physico-chemical basis of homeostasis by considering the principle of Le Chatelier. The principle states that upon an external disturbance a chemical system in equilibrium will change to that direction, which minimizes the external disturbance (9). Lotka rejected the principle as a basis for homeostatic behavior and made a clear distinction between an organism’s steady state and chemical equilibrium.

With the developments within control and systems theory (10–12), the description of homeostatic behavior by feedback regulation came into focus (13–15) with recent emphases on reaction kinetic and genetic models and network motifs (16–23).

Some of the mechanisms that account for perfect adaptation or homeostasis, including temperature compensation (24), are based on a balance between various opposing components within a reaction network (25).

While a balancing-based approach does not guarantee a fixed steady state of the controlled variable in the presence of perturbations, the question arose how robust, i.e., perturbation-independent, homeostatic mechanisms could be achieved. From a control-engineering aspect integral control can keep systems at a given set-point even under the presence of uncontrollable perturbations.

Fig. 1a illustrates the concept of integral control, where \( A \) is the controlled variable with set-point \( A_{set} \). The integral controller is embedded within a
negative feedback loop, which is characterized by defining the error $e$ between $A$ and $A_{set}$ as $^{(26)}$:

$$e = A_{set} - A$$  \hspace{1cm} (1)

The controller integrates the error $e$ over time, which results in the manipulated variable $E$:

$$E(t) = K_i \int_0^t e(t')dt'$$  \hspace{1cm} (2)

where $K_i$ is a constant called the (integral) gain of the controller $^{(26)}$. The variable $E$ then feeds into the process that generates $A$ and adjusts the level of $A$ in the presence of (unpredictable) environmental perturbations. The advantage of integral control is that the steady state value of $A$ will approach, without error, the set-value $A_{set}$. For a formal proof, see for example Ref. $^{(26)}$. With respect to applying integral control to the regulation of cellular and biochemical processes the question arises how error sensing mechanisms can be achieved in reaction kinetic terms.

The implementation of integral control to reaction kinetic networks was emphasized by Yi et al. $^{(16)}$ and others $^{(22, 23, 27)}$. We have recently shown that zero-order kinetics in the removal of a controller variable within negative feedback loops is a necessary condition to obtain integral control $^{(21)}$. The principle is illustrated in Figs. 1b and c on two of eight $^{(28)}$ possible two-component molecular controller/network motifs (negative feedback loops), where $A$ is the (homeostatic) controlled variable with set-point $A_{set}$, and $E$ is the manipulated variable. As indicated in the general scheme (Fig. 1a), the concentration of $E$ in the two network motifs (Figs. 1b and c) is proportional to the error between $A$ and its set-point $A_{set}$. Because the controller action in Fig. 1b is based on a $E$-mediated addition of $A$, we term this type of controller motif for an inflow controller. It may be noted that the homeostatic performance of inflow controllers breaks down when an uncontrolled inflow perturbation of $A$ becomes larger than the consumption of $A$ $^{(21)}$ in the system.

In Fig. 1c the representation of an outflow controller is shown. In outflow controllers robust homeostasis of $A$ at $A_{set}$ can be achieved by an $E$-mediated removal of excess $A$. As in case of the inflow controller, integral control occurs when $E$ is formed and removed by zero-order kinetics $^{(21)}$. Also here it may be noted that homeostasis by outflow controllers is lost when an uncontrolled outflow of $A$ from the system dominates over the regular inflow of $A$.
into the system. The combination of inflow and outflow controllers enables robust homeostasis by allowing to integrate processes such as environmentally dependent uptake and assimilation of $A$, its excretion, storage, as well as remobilization from a store (28, 29).

In the following we show that the requirement of zero-order kinetics to achieve integral control can be replaced by autocatalysis.

**Computational Methods**

Computations were performed in parallel using the Fortran subroutine LSODE (30) and MATLAB/SIMULINK (mathwork.com) together with the program PPLANE (31). Absoft’s Pro Fortran compiler (absoft.com) was used together with the random number generator RAN1 described by Press et al. (32). To make notations simpler, concentrations of compounds are denoted by compound names without square brackets. Concentrations and rate constants are given in arbitrary units (a.u.).

**Integral Control by Autocatalysis**

Figures 2a and 2b show the negative feedback loops of the inflow and outflow controllers from Fig. 1, respectively, but instead of using zero-order degradation of $E$, $E$ is formed autocatalytically and the degradation with respect to $E$ is first-order. Due to the autocatalysis, $d \ln(E)/dt$ is now proportional to the error $e$ between the level of $A$ and its set-point $A_{set}$ (Fig. 2). Figures 2c and 2d show the steady state values of $A$ ($A_{ss}$) for the inflow and outflow controller, respectively, at different inflow and outflow fluxes to and from $A$ described by the varying rate constants $k_{inflow}^{pert}$ and $k_{outflow}^{pert}$, respectively. Both show the typical behavior for inflow and outflow controllers (28). For the inflow controller, homeostasis breaks down (i.e., state steady values of $A$ increase above the set-point) when $k_{inflow}^{pert}$ dominates over the outflow fluxes (Fig. 2c). For the outflow controller, the homeostatic behavior breaks down (i.e., state steady values of $A$ decrease below the set-point) when $k_{outflow}^{pert}$ becomes large relative to the inflow fluxes (Fig. 2d).
The steady state solutions for $A$ and $E$ of the inflow and outflow controllers in Fig. 2 show either stable nodes or stable focus points (with or without saddle points), depending on the rate constants; see Fig 3. As an example, we consider the inflow controller in Fig. 2a, which has two steady state solutions:

1: $A_{ss} = \frac{k_{inflow}}{k_{pert}}$, $E_{ss} = 0$  \hspace{1cm} (3)

2: $A_{ss} = \frac{k_6}{k_8}$, $E_{ss} = \frac{k_{pert} \cdot k_6 - k_{inflow} \cdot k_8}{k_1 k_8}$  \hspace{1cm} (4)

In case $k_{inflow} \leq k_{outflow} \cdot A_{in}^{set}$, homeostasis is preserved and Eq. 3 corresponds to a saddle point (green dots in Figs. 3a and 3c), whereas Eq. 4 corresponds to either a stable node (Fig. 3a) or a stable focus (Fig. 3c), determined by the eigenvalues of the system (33)

$$\lambda_{1,2} = -\frac{k_{outflow}}{k_{pert}} \pm \frac{\sqrt{k_{outflow}^2 - 4k_6 k_{pert} + 4k_{pert} k_8}}{2}$$ \hspace{1cm} (5)

In case $k_{inflow} > k_{outflow} \cdot A_{in}^{set}$, homeostasis breaks down, and the only physically realistic steady state solution is Eq. 3 (Fig. 3b). Hence, the manipulated variable $E$ becomes zero, and the steady state level of $A$ is determined by the relationship of the perturbation fluxes. Similar results are shown for the outflow controller (Figs. 3d-3f).

### Conservative Oscillations

By neglecting outflow perturbation $k_{outflow}^{pert}$ in the dynamics of $A$, the rate equations for the outflow controller in Fig. 2b becomes:

$$\dot{A} = k_{inflow}^{pert} - \frac{k_3 \cdot A \cdot E}{K_{M4} + A}$$ \hspace{1cm} (6)

$$\dot{E} = k_6 \cdot A \cdot E - k_8 \cdot E$$ \hspace{1cm} (7)

Moreover, when $K_{M4} \rightarrow 0$ and making the substitution $\xi = \ln(E)$, Eqs. 6 and 7 can be expressed as follows:

$$\dot{A} = k_{inflow}^{pert} - k_3 \cdot e^\xi$$ \hspace{1cm} (8)

$$\dot{\xi} = k_6 \cdot A - k_8$$ \hspace{1cm} (9)
From Eqs. 8 and 9, an “energy-function” (H-function) can be constructed

\[ H(A, \xi) = -\int \dot{A} \, d\xi + \int \dot{\xi} \, dA \quad (10) \]

satisfying the equations

\[
\begin{align*}
\frac{\partial H}{\partial \xi} &= -\dot{A} \\
\frac{\partial H}{\partial A} &= \dot{\xi}
\end{align*}
\quad (11)
\]

showing that \( H \) is time independent and the system is conservative

\[
\frac{\partial H}{\partial t} = \frac{\partial H}{\partial A} \dot{A} + \frac{\partial H}{\partial \xi} \dot{\xi} = -\frac{\partial H}{\partial A} \cdot \frac{\partial H}{\partial \xi} + \frac{\partial H}{\partial \xi} \cdot \frac{\partial H}{\partial A} = 0 \quad (12)
\]

When \( K_{M4} \to 0 \) the trajectories of the system (Eqs. 8 and 9) become closed orbits at constant values of \( H \). Fig. 4a shows the numerically calculated trajectory of a closed orbit at a relative low \( K_{M4} \) value. The same orbit as in Fig. 4a is obtained in Fig. 4b (blue curve) by calculating the \( H \)-function and finding the contour line at \( H = -5.65 \) a.u., which is determined by the initial conditions for \( A \) and \( E \) in Fig. 4a.

Despite its oscillatory character the system still shows homeostasis in \( A \), but now for the average value of \( A \), defined as

\[
<A> = \frac{1}{\tau} \int_0^\tau A(t) \, dt \quad (13)
\]

with \(<A> = A_{out}^{set} = k_8/k_6\) and \( \tau \) as the simulation (integration) time, i.e., \(<A>\) is independent of \( k_{pert}^{inflow} \) and \( k_3 \).

The independence of \(<A>\) from \( k_{pert}^{inflow} \) is illustrated in Figs. 4c and 4d, where \( k_{pert}^{inflow} \) is successively increased. While a change in the amplitude in \( A \) is observed, the change is symmetrical around \( A_{out}^{set} \) such that \(<A>\) remains at its set-point \( A_{out}^{set} = 2.0 \). Due to the homeostatic condition \(<A>=A_{out}^{set}\), there is an inverse relationship between the (integrated) amplitude of \( A \) and the frequency \( \omega \) of the conservative oscillations. Consider that for a given set of rate constants the oscillator undergoes \( n \) cycles during the time interval \( \tau \). For each cycle we can integrate \( A \) for one period length, which results in what we may call an "integrated amplitude" \( A_1 \). Using \( A_1 \), the average
of \( A \) for \( n \) cycles can be expressed by \( \langle A \rangle = n \cdot A_1 / \tau \). Because \( n / \tau = \omega \), we get \( \langle A \rangle = A_1 / \omega = A_{out}^{set} \), showing that the integrated amplitude \( A_1 \) is inversely proportional to the oscillator’s frequency \( \omega \). Due to the conservative character of the oscillations, random changes in \( A \) and \( E \) lead also to changes in the amplitudes of \( A \) and \( E \) as well as to the frequency, while keeping the average value of \( A \), \( \langle A \rangle \), close to \( A_{out}^{set} \) (Figs. 4e and 4f).

Correspondingly, by neglecting inflow perturbation \( k_{inflow}^{pert} \) and assuming zero order degradation of outflow perturbation (Michaelis-Menten dynamics with low \( K_M \) value) in the dynamics of \( A \), the rate equations for the inflow controller in Fig. 2a becomes:

\[
\dot{A} = k_1 \cdot E - k_{pert}^{outflow} \\
\dot{E} = k_6 \cdot E - k_8 \cdot E \cdot A
\]

which also show conservative oscillations.

While for the outflow controller (Eqs. 8 and 9) the trajectories move in an anti-clock-wise manner, for the inflow controller (Eqs. 14 and 15) the trajectories move clockwise with \( \langle A \rangle = A_{set}^{inflow} = k_6 / k_8 \). Homeostasis is kept as long as there is a sufficient large outflow from \( A \) that the inflow controller can compensate (data not shown).

**Limit-Cycle Oscillations**

We wondered whether it would be possible to construct limit-cycle oscillations with the inflow/outflow controller motifs from Fig. 2 such that the homeostatic condition \( \langle A \rangle = A_{set} \) would be still obeyed. For both motifs this can be achieved by including an additional intermediate \( a \) to the network. Fig. 5a shows this for the inflow controller motif with the following rate equations:

\[
\dot{a} = k_1 \cdot E - k_{10} \cdot a \\
\dot{A} = k_{pert}^{inflow} + k_{10} \cdot a - k_{pert}^{outflow} \cdot A \\
\dot{E} = k_6 \cdot E - k_8 \cdot A \cdot E
\]

Fig. 5b shows projections of the 3-dimensional limit cycle on to the \( A-E \) phase plane with different initial conditions. In each of these cases \( \langle A \rangle = \)
\[ A_{\text{set}} = \frac{k_6}{k_8} \] is obeyed. Fig. 5c shows the time behavior when initial condition 3 from Fig. 5b is used. The controller has the typically properties of an inflow controller, i.e., breakdown of homeostasis when the uncontrollable inflow of A is dominating over the outflows (Fig. 5d).

In a similar approach, limit cycle oscillations with a homeostatic outflow controller motif (Fig. 2b) can be obtained (data not shown).

**Lotka-Volterra and Related Oscillators**

The Lotka-Volterra oscillator (LVO) equations

\[
\begin{align*}
\dot{A} &= k_1 A - k_3 A E \\
\dot{E} &= k_6 A E - k_8 E
\end{align*}
\]

have been formulated independently by Lotka and Volterra (34–36) and have been the subject of many studies especially within chemical oscillator theory (37, 38), predator-prey interactions (39), as well as in economics (40). The LVO contains two autocatalytic loops and can be viewed having an outflow controller structure relative to A and an inflow controller structure relative to E (Fig. 6a). The oscillations are conservative and any perturbation in A (via \(k_{\text{inflow}}^{\text{pert}, A} \cdot k_{\text{outflow}}^{\text{pert}, A}\)) or in E (via \(k_{\text{inflow}}^{\text{pert}, E} \cdot k_{\text{outflow}}^{\text{pert}, E}\)) will lead to a new closed trajectory in the \(A-E\) phase space. The conservative nature of the LVO may be considered as unrealistic as it lacks the stability properties of limit-cycles (36). However, any perturbation in A (or in \(k_1\) or \(k_3\)) is counteracted such that the average value of A, \(<A>\), returns to its set-point \(A_{\text{set}}^{\text{out}} = \frac{k_8}{k_6}\).

The situation is analogous to that shown in Figs. 4c-4f, but with the difference that in the LVO, also perturbations in E (or in \(k_6\) or \(k_8\)) are compensated and \(<E>\) (as defined in Eq. 13) is kept at the homeostatic set-point \(E_{\text{set}}^{\text{out}} = \frac{k_1}{k_3}\).

Interchanging A and E in Eqs. 19 and 20 leads to the negative feedback shown in Fig. 6b, where the system has now an inflow control motif with respect to A and an outflow controller motif with respect to E. Thus, the autocatalytic loop in A controls the homeostatic behavior in E, while the autocatalytic loop in E controls the homeostasis in A. The negative feedback loops described in Figs. 6a and 6b behave differently when A or E are subject to perturbations in their inflow/outflow fluxes to or from \(A/E\).
the feedback in Fig. 6a is an outflow type of controller with respect to A, any outflow perturbation \(k_{\text{pert, A}}^{\text{outflow}}\) exceeding the (autocatalytic) inflow flux to A will destroy the homeostatic behavior of the A-controller. Similarly, any inflow perturbation to E \(k_{\text{pert, E}}^{\text{inflow}}\) exceeding the outflow flux mediated by \(k_8\) will destroy the homeostasis in E.

In Fig. 6b, the situation is reversed, i.e. any inflow perturbation in A \(k_{\text{pert, A}}^{\text{inflow}}\) exceeding the outflow flux mediated by \(k_3\) will destroy homeostasis in A, while any outflow perturbation from E \(k_{\text{pert, E}}^{\text{outflow}}\) exceeding the autocatalytic inflow flux mediated by \(k_6\) will destroy the homeostasis in E. We illustrate these behaviors by one example using a first-order outflow perturbation with rate constant \(k_{\text{pert, A}}^{\text{outflow}}\) from A for the LVO in Fig. 6a. By including the term \(-k_{\text{pert, A}}^{\text{outflow}} \cdot A\) to Eq. 19 (the other perturbation terms are \(k_{\text{pert, A}}^{\text{inflow}} = k_{\text{pert, E}}^{\text{inflow}} = k_{\text{pert, E}}^{\text{outflow}} = 0\)), the system remains conservative and by using the method outlined above to calculate \(H\), we get

\[
H(A, E) = (k_{\text{pert, A}}^{\text{outflow}} - k_1) \cdot \ln(E) + k_3 \cdot E + k_6 \cdot A - k_8 \cdot \ln(A) \quad (21)
\]

As long as \(k_{\text{pert, A}}^{\text{outflow}} < k_1\), homeostasis in \(\langle A \rangle\) is maintained, and the system shows oscillations. The (oscillatory/closed) trajectory on the \(H\)-surface is shown in Fig. 6c. However, when \(k_{\text{pert, A}}^{\text{outflow}} > k_1\), homeostasis breaks down and the steady state value in A settles below its homeostatic set-point. The trajectory on the \(H\)-surface is shown in Fig. 6d.

By including an additional intermediate, the LVO schemes in Figs. 6a and 6b can be transformed into limit-cycle oscillations. We show here the results for the ”inflow-controller version” with respect to A (Fig. 7a). Species a, which is induced by the autocatalytically formed E is a precursor to A on which A itself is formed autocatalytically. A on its side induces the removal/degradation of E causing a negative feedback necessary to get oscillations and homeostasis. Limit-cycle oscillations can be demonstrated (Fig. 7b) for a variety of rate constant values. Dependent on the rate constant values the period length can vary considerably ranging from about 70 time units (Fig. 7c) to a fraction of a time unit. Due to the inflow-type of controller relative to A, homeostasis in A is observed as long as the environmental perturbative zero-order inflow flux \(k_{\text{pert, A}}^{\text{inflow}}\) to A is smaller than the perturbative outflow flux from A determined by \(k_{\text{pert, A}}^{\text{outflow}}\). Homeostasis in A can be observed both in oscillatory/pulsatile mode (Fig. 7c) or in non-oscillatory mode (Fig. 7d).
Discussion

We have shown that autocatalysis/positive feedback is an alternative way to introduce integral control in homeostatic controller motifs, while the inflow/outflow properties (21) of the motifs remain preserved. We have demonstrated this for two controller motifs, but the method can be applied for other two-component controller motifs that were recently identified (28).

An interesting aspect when comparing integral control between the zero-order kinetic (Michaelis-Menten) approach (Fig. 1) and the here described autocatalytic method (Fig. 2) is related to the accuracy of the controller. In the zero-order approach the $K_M$ value of the process removing the controlled variable $E$ serves as a measure for controller accuracy of the two controllers addressed here. At low $K_M$ values the controller accuracy is high, i.e., $A$ is close to $A_{set}$, while at high $K_M$ values the accuracy of the controller is low (28), and the steady state of $A$ is dependent upon the type of the controller. For the inflow controller a high $K_M$ will lead to a steady state in $A$ which will be higher than $A_{set}$ (as indicated by the equation for $\dot{E}$ in Fig. 1b), while in the outflow controller (Fig. 1c) a high $K_M$ will lead to a $A$ steady state, which has a lower value than $A_{set}$. In the autocatalytic approach the controller accuracy is intrinsically perfect, because no requirements such as $K_{M1}/K_{M3} \ll E$ (Fig. 1) are necessary. While we considered a first-order reaction in the $A$-mediated removal of $E$ (Fig. 2), the kinetics could also be of Michaelis-Menten type or any other nonzero reaction-order with respect to $A$, as long as the reaction-order with respect to $E$ for its autocatalytic formation and for its degradation remains the same.

In this study we have focused on the two homeostatic inflow and outflow controller motifs (Fig. 1/Fig. 2) with a close relationship to the LVO and similar oscillators (Fig. 6). In 1925, Lotka described his attempts to explain biological homeostatic behavior on basis of Le Chatelier’s principle (8). He concluded negatively, and ironically, it seems that he was unaware that the equations that bear today his name have homeostatic properties.

The LVO and derivative models are well-known for their usage in ecological systems (36, 39), which have generally been thought of as homeostatic systems (41). While the conservative nature of the LVO is mostly considered to be a drawback when considering system stability we have shown that they
already contain, due to their autocatalytic nature homeostatic behavior in $<A>$ or $<E>$ (Fig. 4), which can be extended to limit-cycle models (Figs. 5 and 7).

There is an extensive literature showing that autocatalysis/positive feedback in combination with negative feedback loops can be the source for a variety of dynamic behaviors including excitability (42, 43), oscillations (38, 44–46), spatial pulse propagation (38, 43), bi- or multi-stability (38, 47, 48), as well as Turing structures (49). While homeostasis is generally associated with negative feedback regulation (21, 50, 51), combinations of positive and negative feedback loops with respect to homeostasis have also been addressed. An example is the hypothalamus-pituitary-adrenal system (52), where the positive feedback is considered to be a crucial component to self-stabilize the system. A related behavior has been observed earlier by Cinquin and Demongeot (53), showing that a certain strength of the positive feedback in a combined positive-negative feedback model is required to obtain stability of the system. Maintenance of stem cell homeostasis in the apical meristem in rice has recently been reported to be due to a positive autoregulation of the KNOX gene (54).

Calcium is an important signalling molecule in all living cells and its concentration is tightly regulated in the cytosol and organelles (55). For example, disregulation of Ca-homeostasis is involved in various neurodegenerative diseases (56). Both positive and negative feedback loops have been identified in cellular Ca-regulation showing behaviors such as sparks, waves, bursts or oscillations (57–59). The observed positive feedback loops in calcium regulation may be part of the homeostatic mechanisms that maintain cytosolic and organellar calcium levels (55), but little is presently known in this respect.

For certain neurons, iron uptake has been found to occur by an oxidative-stress mediated positive feedback loop (60). The role of the positive feedback is still unclear, but also here it could be that the positive feedback participates in the regulation of iron by possibly participating in the determination of the iron homeostatic set-point.

The notion that autocatalysis (or positive feedback) is a source of robust stability may appear counter-intuitive. However, it should be kept in mind that the autocatalytic loop generating $E$ (or $A$) is part of an overall negative feedback loop (controller motif) (21, 28). Positive feedback is an important
driving force for growth and development (61), but needs to be limited by
negative feedback to avoid runaway states (41).

Conclusion

We have shown that autocatalysis/positive feedback can be a mechanism
leading to integral control and thereby resulting into robust homeostatic and
adaptive behaviors. However, as indicated by the biological examples above,
we presently still know little about how positive feedback loops are involved
in the cellular organization of homeostatic behavior.

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References

629–49.


Figure Legends

Figure 1.
Integral control by zero-order kinetics. (a) Scheme of the integral control concept. $A$ is the controlled variable, which is regulated to set-point $A_{set}$ regardless of unpredictable perturbations of $A$. To achieve this, the error $e = A_{set} - A$ is calculated and integrated leading to the manipulated variable $E$, which corrects the value of $A$ such that $A$ will approach $A_{set}$. (b) Negative feedback loop showing robust homeostasis in $A$ when the manipulated variable $E$ is removed by zero-order kinetics. In this case $\dot{E}$ is proportional to the error $e$, which, when integrated, leads to $E$ and adjusts the level of $A$ precisely to its set-point $A_{set}$. Because the controller action is based on adding $A$ by $E$, we term this controller type for an inflow controller. (c) Kinetic representation of an outflow controller with integral control by zero-order removal of $E$.

Figure 2.
Integral control by autocatalysis. (a) Negative feedback loop of inflow controller with autocatalytic loop in $E$. $d\ln(E)/dt$ is proportional to the error $e$ between $A$ and its set-point $A_{set}^{in} = k_6/k_8$. (b) Negative feedback loop of outflow controller with autocatalytic loop in $E$. $d\ln(E)/dt$ is proportional to the error $e$ between $A$ and its set-point $A_{set}^{out} = k_8/k_6$. (c) Homeostatic behavior of inflow controller described in (a). Rate constants $k_{pert}^{inflow}$ and $k_{pert}^{outflow}$ are allowed to vary between 0.5 and 50.0 with intervals by 0.5, while all other rate constants are kept at 1.0. Homeostasis in $A$ steady state levels ($A_{ss}$) with $A_{set}^{in} = 1.0$ is observed when $k_{pert}^{inflow} \leq A_{set}^{in} k_{pert}^{outflow}$, but lost when $k_{pert}^{inflow} > A_{set}^{in} k_{pert}^{outflow}$ (28). The transition line $k_{pert}^{inflow} = A_{set}^{in} k_{pert}^{outflow}$ separating homeostatic and non-homeostatic regimes is indicated in blue. (d) Homeostatic behavior of outflow controller. Rate constants are allowed to vary as in (c), while the other rate constants are kept at 1.0. Homeostasis in $A$ steady state levels ($A_{set}^{out} = 1.0$) is observed when $k_{pert}^{inflow} \geq A_{set}^{out} k_{pert}^{outflow}$, but lost when $k_{pert}^{inflow} < A_{set}^{out} k_{pert}^{outflow}$. 

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The inflow and outflow controllers can show stable nodes or stable focus points (with or without saddle points) depending on the inflow and outflow perturbations. Panels (a), (b) and (c): inflow controller from Fig. 2a with \( k_1=2.0, \ k_6=2.0, \ k_8=0.5 \). (a) \( k_{\text{inflow}}^{\text{pert}} = 8.0 \) and \( k_{\text{outflow}}^{\text{pert}} = 5.0 \) showing stable node (red dot) at \( A_{ss} = A_{\text{set}}^{\text{in}} = k_6/k_8 = 4.0 \) and \( E_{ss} = 6.0 \) (Eq. 4). Green dot indicates a saddle point at \( A_{ss} = k_{\text{inflow}}^{\text{pert}} / k_{\text{outflow}}^{\text{pert}} = 1.6 \) and \( E_{ss} = 0 \) (Eq. 3). Homeostasis in \( A \) is preserved. (b) Example of controller breakdown when \( k_{\text{inflow}}^{\text{pert}} = 8.0 \) and \( k_{\text{outflow}}^{\text{pert}} = 1.8 \) showing a stable node (red dot) at \( A_{ss} = k_{\text{inflow}}^{\text{pert}} / k_{\text{outflow}}^{\text{pert}} = 4.44 \) and \( E_{ss} = 0 \) (Eq. 3). Homeostasis in \( A \) is not preserved. The solution from Eq. 4 gives negative and therefore unrealistic \( E_{ss} \) values. (c) \( k_{\text{inflow}}^{\text{pert}} = 2.0 \) and \( k_{\text{outflow}}^{\text{pert}} = 1.8 \) showing stable focus point (red dot) at \( A_{ss} = A_{\text{set}}^{\text{in}} = 4.0 \) and \( E_{ss} = 2.6 \) (Eq. 4). Homeostasis in \( A \) is preserved. Green dot indicates a saddle point at \( A_{ss} = k_{\text{inflow}}^{\text{pert}} / k_{\text{outflow}}^{\text{pert}} = 1.11 \) and \( E_{ss} = 0 \) (Eq. 3). Panels (d), (e) and (f): outflow controller from Fig. 2b with \( k_3 = 1.0, \ k_6 = 1.0, \ k_8 = 1.5 \). (d) \( k_{\text{inflow}}^{\text{pert}} = 8.0 \) and \( k_{\text{outflow}}^{\text{pert}} = 0.8 \) showing stable node (red dot) at \( A_{ss} = A_{\text{set}}^{\text{out}} = k_3/k_6 = 1.5 \) and \( E_{ss} = 4.6 \). Green dot indicates a saddle point at \( A_{ss} = k_{\text{inflow}}^{\text{pert}} / k_{\text{outflow}}^{\text{pert}} = 10.0 \) and \( E_{ss} = 0 \). Homeostasis in \( A \) is preserved. (e) Example of controller breakdown when \( k_{\text{inflow}}^{\text{pert}} = 4.0 \) and \( k_{\text{outflow}}^{\text{pert}} = 4.0 \) showing a stable node (red dot) at \( A_{ss} = k_{\text{inflow}}^{\text{pert}} / k_{\text{outflow}}^{\text{pert}} = 1.0 \) and \( E_{ss} = 0 \). Homeostasis in \( A \) is not preserved. (f) \( k_{\text{inflow}}^{\text{pert}} = 4.0 \) and \( k_{\text{outflow}}^{\text{pert}} = 0.8 \) showing stable focus point (red dot) at \( A_{ss} = A_{\text{set}}^{\text{out}} = 1.5 \) and \( E_{ss} = 1.9 \). Homeostasis in \( A \) is preserved. Green dot indicates a saddle point at \( A_{ss} = k_{\text{inflow}}^{\text{pert}} / k_{\text{outflow}}^{\text{pert}} = 5.0 \) and \( E_{ss} = 0 \).

Figure 3.

Conervative oscillations for the outflow controller described by Eqs. 6 and 7. (a) Numerical computation of closed phase orbit with rate constants (in a.u.) \( k_{\text{inflow}}^{\text{pert}} = 1.0, \ k_3 = 2.0, \ K_M = 1 \times 10^{-6}, \ k_6 = 5.0, \ k_8 = 10.0, \) and the initial concentrations \( A_0 = 1.0, \) and \( E_0 = 0.8333. \) The calculated average of \( A \) (Eq. 13) is \( <A> = A_{\text{set}}^{\text{out}} = k_8/k_6 = 2.0. \) The trajectory runs counter clock-wise in the \( (\text{abscissa}) - E \) (ordinate) phase plane. (b) Calculated \( H \)-function from Eqs. 8 and 9 taking the form \( H = -k_{\text{inflow}}^{\text{pert}} \xi + k_3 \cdot e^\xi + 0.5 \cdot k_6 \cdot A^2 - k_8 \cdot A, \) where \( \xi = \ln(E). \) Using the rate constants and initial conditions from (a) gives a
value for $H$ of $-5.65$, which leads to the same closed loop trajectory (blue line) as calculated in (a). (c) The successive increase of $k_{pert}^{inflow}$ (black lines) from 1.0 to 5.0 leads to an increase in the oscillator's frequency and a decrease in the amplitude of $A$ (red lines). The blue line is the integral of $A$, i.e. the value of $(1/t) \int_0^t A(t')dt'$ for time $t$. The apparent linearity shows that $<A>$ is constant and equal to $A_{out}_{set}$. Oscillations in $E$ are shown in green and the $E$-integral as a function of time is given in purple. (d) Phase behavior of the system in (c). Numbers 1-5 indicate the trajectories for the values of $k_{pert}$ changing from 1.0 to 5.0 as indicated in (c). (e) Same system as in (a), but concentrations in $A$ and $E$ are changed randomly between zero and one at time units 0, 25, 50, and 75. Also here $<A>$ is close to $A_{out}_{set}$. However, for each of the time intervals (0-25), (25-50), (50-75), (75-100) we have that $<A>=A_{out}_{set}=2.0$. Dashed lines 1, 2, and 3 indicate the random changes made in $A$ and $E$. (f) Phase plane behavior of the system described in (e). Numbers 1-3 relate to the (stochastic) changes made in $A$ and $E$ at time units 25, 50 and 75.

Figure 5.

(a) ‘Extended’ inflow controller showing limit-cycle oscillations. (b) Approach to limit-cycle at initial conditions 1: $a_0=2.0$, $A_0=3.5$, $E_0=0.7$; 2: $a_0=0.1$, $A_0=1.0$, $E_0=0.7$; 3: $a_0=0.1$, $A_0=2.0$, $E_0=0.1$. Rate constant values: $k_{pert}^{inflow}=1.0$, $k_{pert}^{outflow}=3.0$, $k_6=20.0$, $k_8=10.0$, $k_1=30.0$, $k_{10}=10.0$. (c) Time profile of oscillations in (b) with initial conditions 3. $<A>=A_{out}_{set}=k_6/k_8=2.0$. (d) Breakdown of homeostatic control ($<A>=6.0$) when $k_{pert}^{inflow}=3.0$ and $k_{pert}^{outflow}=0.5$ leading to $k_{pert}^{inflow} > A_{out}_{set}^{inflow} k_{pert}^{outflow}$. All other rate constants as in (b).

Figure 6.

The Lotka-Volterra oscillator. (a) The negative feedback structure defines an outflow-type of controller with respect to $A$ and an inflow-type of controller with respect to $E$. Rate constants $k_{pert,A}^{inflow}$, $k_{pert,A}^{outflow}$, $k_{pert,E}^{inflow}$ and $k_{pert,E}^{outflow}$ describe perturbative inflow and outflow fluxes. (b) The LVO with negative feedback structure defining an inflow-type of controller with respect to
homeostasis in A, and an outflow-type of controller with respect to E. (c) The LVO from (a) with \( k_1=1.0, k_3=2.0, k_6=1.0, k_8=2.0, \) and \( k_{\text{outflow}}^{\text{pert},A}=0.5. \) All other rate constants are zero. Initial concentrations are \( A_0=1.0, \) and \( E_0=0.5. \) The oscillations are shown as a closed orbit on the \( H(A, E) \)-surface with projection on to the \( A-E \) phase plane with \( <A>_\text{set}=k_8/k_6=2.0. \) (d) Same system as in (c), but \( k_{\text{outflow}}^{\text{pert},A}=1.5. \) Homeostasis in \( <A>_\text{set} \) is lost and the system approaches a steady state well below \( A_\text{out}. \)

**Figure 7.**

(a) Extension of the LVO from Fig. 6b with variable inflow and outflow perturbations (\( k_{\text{inflow}}^{\text{pert}} \) and \( k_{\text{outflow}}^{\text{pert}} \)) in A. (b) Demonstration of limit-cycle behavior. Rate constants: \( k_1=11.0, k_6=2.0, k_8=2.0, k_{10}=0.5, k_{\text{inflow}}^{\text{pert}}=1.0 \) and \( k_{\text{outflow}}^{\text{pert}}=10.0. \) Initial concentrations for 1: \( a_0=10.1, A_0=1.0, E_0=0.1 \) and for 2: \( a_0=18.0, A_0=1.0, E_0=0.8. \) The homeostatic set point for A is \( A_\text{set}^\text{in}=k_6/k_8=1.0, \) and confirmed by calculating \( <A>_\text{set}. \) (c) Pulsatile oscillations with period \( P=69.7 \) time units. Rate constants as in (b) except \( k_{\text{inflow}}^{\text{pert}}=1\times10^{-6} \) and \( k_{\text{outflow}}^{\text{pert}}=1.0. \) Initial concentrations \( a_0=20.0, A_0=1.0, E_0=0.8. \) Although A peak-values are above 60 a.u., the determined average of A is \( <A>_\text{set}=1.04 \) and very close to \( A_\text{set}^{\text{in}}=1.0. \) (d) Non-oscillatory homeostasis of A. Rate constants as in (b) except \( k_{\text{pert}}^{\text{inflow}}=3.5 \) and \( k_{\text{pert}}^{\text{outflow}}=3.8. \) Initial concentrations as in (c).
Figure 1:
\[
\dot{A} = k_{\text{inflow}} - k_{\text{outflow}} \cdot A + k_1 \cdot E \\
\dot{E} = k_6 \cdot E - k_8 \cdot E \\
\quad = E (k_6 - k_8 \cdot A) \\
\ln(E) = k_6 - k_8 \cdot A = k_8 (A_{\text{set}} - A)
\]

**Figure 2:**
Figure 3:
Figure 4:
Figure 5:
Figure 6:
Figure 7: