



Role of ultrasensitivity in biomolecular circuitry for achieving homeostasis

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Abstract Living systems have developed control mechanisms for achieving homeostasis. Here, we propose a plausible biological feedback architecture that exploits ultrasensitivity and shows adaptive responses without requiring error detection mechanism (i.e., by measuring an external reference signal and deviation from this). While standard engineering control systems are usually based on error measurements, this is not the case for biological systems. We find that a two-state negative feedback control system, without explicit error measurements, is able to track a reference signal that is implicitly determined by the tunable threshold and slope characterizing the sigmoidal ultrasensitive

relationship implemented by the control system. We design different ultrasensitive control functions (ultrasensitive up- or down-regulation, or both) and, by performing sensitivity analysis, show that increasing the sensitivity level of the control allows achieving robust adaptive responses to the effects of parameter variations and step disturbances. Finally, we show that the devised control system architecture without error detection is implemented within the yeast osmoregulatory response network and allows achieving adaptive responses to osmotic stress, by exploiting the ubiquitous ultrasensitive features of the involved biomolecular circuitry.

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1 Introduction

Biological homeostasis is the ability of living systems to maintain a stable internal state despite changes in the external environment. How biological systems achieve homeostatic regulation is a central question across all biological scales. Homeostatic systems are characterized by their ability to respond to persisting perturbations with a transient, adaptive response characterized by an initial response to the persistent external stimulus that eventually returns to its pre-stimulus level (either exactly, i.e., perfect adaptation, or approximately). Sight and smell are prime examples of adaptive responses [1,2], while bacterial chemotaxis [3] and

the response of yeast to osmotic shock [4] are the two systems that have been investigated in most detail in order to achieve a better understanding of the biological mechanisms underpinning adaptation.

These systems can be described as control systems consisting of a set of components connected in a such a way to provide a desired behavior. In engineering, a standard closed-loop feedback control system (see Fig. 1A, upper panel) employs a sensor to measure the actual output of the process to be controlled and feed back this measurement to input device; this signal (i.e., the feedback signal provided by the sensor) is then compared with a reference input signal, representing the desired output of the control system, to generate an error signal which is the input of the component of the feedback loop called controller; based on the error signal, the controller sends a signal to the downstream component, i.e., the actuator which can modify the state of the process in order to achieve the desired output. These feedback control systems are employed to maintain the process output at a particular set point (i.e., the reference signal is constant over a longer period of time or permanently) or to track a dynamic reference signal in the face of possible disturbances due to the environment in which the process operates and uncertainty in the process dynamics. For example, the response of yeast to osmotic shock, which is mediated by an intricate molecular system involving signaling, gene expression and osmolyte transport [5], can be naturally abstracted as a feedback control system, comprised of a controller that acts to adjust the glycerol production in order to keep turgor pressure and volume constant in the face of environmental changes: after an osmotic stress (i.e., salt shock), the membrane bound osmosensor, SLN1, perceives a drop in cell volume and turgor pressure due to the increase in the external osmotic pressure and the outflow of water from the cell and activates the mitogen-activated protein kinase (MAPK)-Hog1 protein (i.e., the controller output) involved in mechanisms (actuating devices) promoting the glycerol production and regulating the glycerol outflow by the Fps1 channels; therefore, these actuating mechanisms adjust the glycerol levels in order to regain the turgor pressure and volume after the osmotic stress (Fig. 1B, upper panel, for a schematic depiction of the osmosensing response).

In the last years, tools from control engineering have been exploited in order to investigate possible design strategies in biological contexts for achieving adaptive,

homeostatic responses to environmental disturbances. From the perspective of control engineering, integral feedback control is one of the most common strategies for achieving perfect adaptation: in this type of control scheme, any deviation of system response from a desired steady state (i.e., error signal) is integrated over time and fed back into the system. Yi et al [3] found specific biochemical architecture in bacteria chemotaxis implementing integral control that enables to achieve perfect adaptation to chemoattractants: the output of the signaling system mediating chemotaxis returns exactly to the pre-stimulus level following a persistent disturbance, i.e., the steady-state behavior of the system does not change in the presence of a constant concentration of chemoattractants; in this case, indeed, the bacteria direct their movement randomly without swimming in any particular directions since the nutrient concentration is constant (either low or high); only in the presence of a concentration gradient of the nutrients the bacteria chemotax or direct their movement based on that gradient; moreover, the precision of adaptation in bacteria chemotaxis is found to be robust to variations in most kinetic rate parameters and protein levels [6–8]. Integral control feedback strategy has also been observed at the level of organ systems, e.g., for explaining calcium homeostasis in mammals, the mechanism by which the body maintains the physiological levels of calcium in the blood plasma within narrow limits, despite the calcium demand can significantly vary over the time [9].

The group of Khammash proposed an integral feedback strategy called antithetic integral feedback that allows achieving robust perfect adaptation in biomolecular networks. This strategy is characterized by two biomolecular control species [10]: one actuator species, which affects processes leading to the increase in the production of the output of interest, and one sensor species, which senses the output of interest; these two species annihilate each other, i.e., abolish each other biological activity upon reacting, for example by forming an inert dimer, thereby closing the feedback loop. This annihilation reaction is central to the integral feedback implementation, by allowing the computation of a signal proportional to the integral of the mean tracking error (i.e., the difference between the desired output and the actual output of the system). Then, they proved the universality of this integral control motif [11], by showing that any controller able to achieve robust perfect adaptation can be viewed as an extension of the antithetic integral feedback [10]. Moreover, in [12],

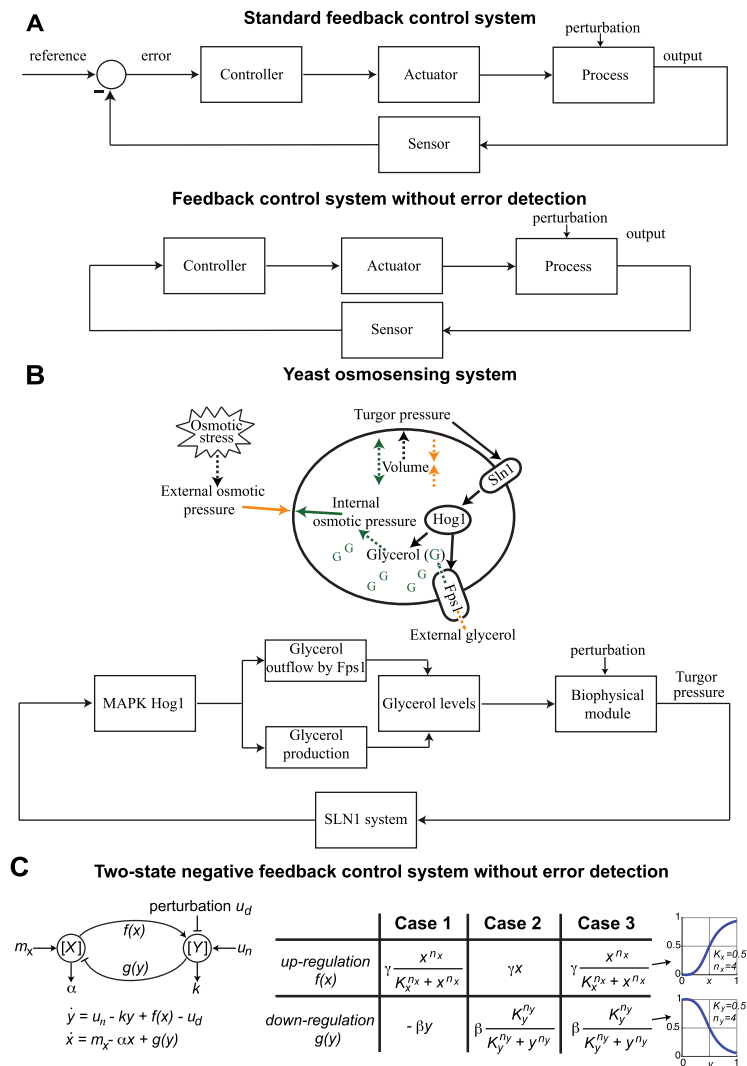


Fig. 1 Examples of feedback control systems. **A** Feedback control system with (upper panel) and without (lower panel) error detection: for the upper panel, the input of the controller is the error signal, e , defined as the difference between the desired output, r (called the reference input signal), and the actual output of the system, y ; for the lower panel, the input of the controller is the output signal measured by the sensor. For both the schemes, the controller sends a signal to the actuating device that can modify the state of the process in order to maintain a stable desired output despite external perturbations. **B** Schematic depiction (upper panel) of the yeast osmoregulatory response network: an osmotic stress (i.e., salt shock) determines a rise of the external osmotic pressure leading to water diffusion out of the cell with the resulting decrease of the cell volume and turgor pressure; a complex biochemical signaling and regulatory network is activated to regain volume and turgor pressure; in particular, the activation of the Hog1 protein by the SLN1 system, perceiving a drop in cell volume and turgor pressure, and the downstream mechanisms mediated by Hog1 that stimulate glycerol production and regulate glycerol outflow by Fps1 channel act to adjust the levels of glycerol in order to regain volume and turgor pres-

sure. Engineering block diagram representation (lower panel) of a control model for the osmoregulation system without explicit error detection. **C** Schematic representation (left panel) for a two-state negative feedback control system without error detection, where two components X and Y mutually influence the dynamics of each other: $[X]$ and $[Y]$ denote the quantities of X and Y , respectively; m_x and u_n are constant input fluxes while α and k the degradation rates of $[X]$ and $[Y]$, respectively. u_d is the perturbation on $[Y]$. X (the control variable) activates Y (the controlled variable representing the output of the system) by the function $f(x)$, whereas Y inhibits X by the function $g(y)$, thereby implementing a negative feedback control system. The lower left panel shows the ODE model describing the system dynamics, where italic lowercase letters are used to indicate the quantities of the corresponding components (e.g., $y(t) = [Y](t)$ is the quantity at time t of the component Y). In the right panel, a table illustrates the different cases for modeling the functions f and g : two plots show respectively the behavior of x -dependent ultrasensitive activation function $f(x)$ with $\gamma = 1$, and y -dependent ultrasensitive repression function $g(y)$ with $\beta = 1$

Hancock and Oyarzún proposed an extended architecture that combines this antithetic motif with molecular buffering resulting in a system with improved stability properties.

Recently, we [13] investigated the role of ultrasensitivity, a common nonlinear characteristic of cellular systems [14, 15], for explaining the adaptive response dynamics observed in the yeast osmosensing system. Ultrasensitivity represents a particular form of sensitivity in a biological system, for which the system does not respond (or provides small responses) to input signals (i.e., stimuli) with magnitude below a certain threshold, while yields high responses to incoming signals with magnitude around that threshold (the operating ultrasensitive regime) and then back to smaller relative responses for increasing stimuli far from that regime: therefore, the gain of the system (defined as the ratio between the output signal and the input signal) changes from very low, to very high, and then back to very low as the magnitude of the input signal increases. The resulting input–output system response characterized by sigmoidal shape (see Fig. 1C, right plots showing ultrasensitive activation (upper plot) and repression (lower) responses) is observed to emerge via many different molecular mechanisms, including dimerization of transcription factors [16], use of scaffolding proteins in MAPK systems [17], and branching in bacterial phosphorylation/de-phosphorylation cycles [18]. For example, within the yeast osmoregulation system, the osmosensor SLN1 is a part of two-component signaling system that embeds ultrasensitive dynamics, as observed experimentally [19], as well as the downstream MAPK cascade system terminating at Hog1 [15]. Moreover, the presence of ultrasensitivity has also been suggested in the yeast osmosensing system for the Hog1-dependent mechanisms stimulating glycerol production (e.g., by the transcriptional activation of genes encoding enzymes for such production, described usually by Hill functions [20]) and regulating glycerol outflow by inducing the Fps1 channel closure [20–22]. It has also been shown by theoretical studies that ultrasensitivity, when combined with negative feedback, can produce adaptive response dynamics [23–25]. In [13], we showed that such a control model embedding ultrasensitivity with negative feedback, resulting in a control model we called ultrasensitive negative feedback, is capable of explaining the adaptive responses observed in the yeast osmosensing system. We designed and implemented this ultrasensitive negative feedback, and

performed a rigorous mathematical analysis showing the strong links between the proposed ultrasensitive controller and sliding-mode controllers, a particular class of nonlinear feedback controllers, whose performance and robustness properties are well-known in the field of control engineering [26–28]. In [29], Samaniego and Franco also introduced a biomolecular controller, called Brink motif, that implements ultrasensitivity by including molecular sequestration [16] and activation/deactivation cycle [14, 15], and operates as a quasi integral controller: a biomolecular closed-loop system embedding this motif shows homeostatic behavior with robust tracking of an external reference.

However, a common assumption of these models devised for explaining biological processes showing adaptive responses is that the system can compute mathematically an error signal, e , by comparing an external reference signal, r , with a measurement of the actual output of the system, y . While the generation of a such error signal (i.e., $e = r - y$) that drives the downstream components for reference tracking is trivial in standard engineering control systems (Fig. 1A, upper panel), this is not the case for biological systems.

In this work, we investigate the possibility of biomolecular control systems that work without error detection (Fig. 1A, lower panel). First, we analyze a simple two-state negative feedback control system consisting of two components that mutually affect the dynamics of each other (see Fig. 1C), where one component represents the control variable and the other one the controlled variable, the output of the two-state system: this model can show adaptive responses in the face of step disturbances by exploiting the ultrasensitive dynamics of the feedback regulation implemented within the system. For such a model, in [25] Ang and McMillen provided a set of design constraints in the general form for devising a biological integral controller that guarantees homeostasis of the closed-loop system. Here, we find out the conditions allowing the output of the two-state system to track a reference signal that is not imposed externally, without thereby requiring error detection by comparing the actual output with an external reference signal: the reference signal can be implicitly a function of the resulting sigmoidal shape ultrasensitive response implemented by the feedback regulation and adjusted by changing the point of high sensitivity; in particular, the tunable threshold and slope of the ultrasensitive response determine the reference signal that the two-state system is

able to track. In our early work [30], we introduced and designed a first configuration of the two-state negative feedback control system of Fig. 1C, where the control variable affects positively the controlled variable implementing an ultrasensitive up-regulation function. In the following, we propose control configurations implementing different ultrasensitive functions (ultrasensitive up- or down-regulation, or both by modeling opportunely the interactions between the control and controlled variables). Moreover, performing sensitivity analysis by varying the model parameters for the different configurations confirms our findings: ultrasensitivity helps to achieve adaptive responses and increasing the level of sensitivity allows achieving robust responses to the effects of parameter variations and step disturbances. Finally, we show that the devised control architecture without requiring error detection can be implemented within the yeast osmoregulation system; our previous model [13] for yeast contained the implementation of an error detection component, by making an abstracted assumption that the SLN1 receptor is seen as computing the difference (i.e., error) between the current and an ideal turgor pressure (i.e., an imposed external signal). Most recently, in [31], Sootla et al proposed a new feedback architecture realized by two-component signal transduction systems (as the SLN1 system) that shapes the signal response, attenuates intrinsic noise while increasing robustness and reducing cross-talk. Here, we devise for yeast a novel integrated biomolecular feedback control system (see lower panel of Fig. 1B for the corresponding block diagram representation) by embedding the different functional modules without requiring an external reference signal for error computation. In particular, by implementing the biomolecular reactions for the SLN1 two-component system, leading to an input–output ultrasensitive relationship for SLN1 as shown in [19], we find that the developed osmosensing system is able to achieve adaptive responses when the SLN1 sensor operates in the highly sensitivity zone of its ultrasensitive relationship; therefore, the structure and the parameter set of the sensor determine the stable internal state despite changes in the external environment (i.e., osmotic stress). Moreover, by exploiting the ultrasensitive responses observed at different levels of the control cascade, i.e., at the level of the MAPK cascade terminating at Hog1 [15] and of the corresponding Hog1-dependent mechanisms [20–22] that regulate the glycerol levels in order to keep the cell’s turgor pressure and

volume constant in the face of environmental changes, the implemented yeast osmosensing system is capable of well reproducing the adaptive responses (at cellular level in terms of turgor pressure and cell volume, and at molecular level in terms of Hog1 activity).

2 Results and discussion

2.1 A two-state negative feedback control system without error detection

We assume the two-state negative feedback control system depicted by Fig. 1C (left panel), where the components, X and Y , mutually affect the dynamics of each other, and try to find out the possible mechanisms for obtaining adaptive response dynamics (we consider even the case with approximately adaptation, as observed experimentally in cellular systems like the yeast osmoregulatory network [4]). We investigate the conditions allowing the component Y to maintain a set point, which is not imposed externally but is determined by the properties and, hence, the parameters characterizing the control system: therefore, the internal set point (i.e., internal reference signal) is an emergent property of the dynamics embedded into the feedback loop; moreover, we show how the system is able to maintain this equilibrium (i.e., set point) despite the presence of step disturbances u_d , by showing adaptive responses and the rejection (partial or almost complete) of u_d . In more detail, we assume that X (the control variable) activates Y (the controlled variable which represents the output of the system) by the function $f(x)$, whereas Y inhibits X by the function $g(y)$, and, then, all the system implements a negative feedback loop. Then, we model the two-state control system by the following ODE system:

$$\begin{aligned}\dot{y}(t) &= u_n - k y(t) + f(x) - u_d(t), \\ \dot{x}(t) &= m_x - \alpha x(t) + g(y),\end{aligned}\tag{1}$$

and assume three different cases for modeling the functions f and g (see Table in the right panel of Fig. 1C). In particular, we focus on the possibility that the two feedback functions f and g implement ultrasensitivity, ubiquitous feature of biomolecular circuitry [32]. Specifically, we assume the cases where only one action (f or g) can implement ultrasensitivity (f for case 1 as introduced in our early work [30] and g for case 2), or both (f and g for case 3). Note that the constant parameters u_n and m_x are set in order to operate in the highly

sensitivity zone of the feedback functions according to the cases as explained in the following, while the constant rates k and α represent the degradation rates of Y and X , respectively. Finally, we model the effect of an external disturbance by step function with amplitude a_{u_d} and assume that such effect is inhibitory: the disturbance enters the model as “ $-u_d$,” and then a positive value of a_{u_d} results in a fall of Y . We show that this system can represent a simple architecture implementing a control system without explicit error measurements, capable of tracking a reference signal that is not imposed externally, but it is determined by the ultrasensitive nonlinear dynamics embedded into the feedback loop; for this architecture, in the following, we find the setting allowing adaptive responses that depend on the tunable thresholds and slopes of the ultrasensitive functions. Moreover, for the different cases, we perform sensitivity analysis in order to evaluate the effects of parameter variations and step disturbances.

Note that in our early work [30], we evaluated the properties of the two-state control system of Fig. 1C implementing linear dynamics ($f = \gamma x$ and $g = -\beta y$ are linear): the system is able to achieve adaptive responses to step perturbations (the output Y is at equilibrium independent of the disturbance) for the ideal case with α (i.e., the degradation rate of X) equal to zero; in that case X implements an integral action, indeed, the control action signal $x(t)$ contains a term which is the integral of the output (i.e., $x(t) = m_x t + \beta \int y(\tau) d\tau$ by integrating $\dot{x}(t)$ for $\alpha = 0$). When the degradation of the controller species is taken into account (α is different from zero), the control action becomes a leaky integral controller as shown in [33], where the effect of leaky integration due to dilution (i.e., degradation) can be reduced by increasing the rate of the reactions implementing the integral controller (for our case, we showed in [30] that with $\alpha \ll \beta$ the system approximately adapts).

2.1.1 Case 1: ultrasensitive up-regulation

For case 1 of the two-state system defined by (1), we assume that the x -dependent ultrasensitive activation described by f can be modeled by a standard Hill function, characterized by the parameters K_x and n_x defining the threshold and the steepness of the sigmoidal signal-response relationship, respectively, while the y -dependent inhibition function g is linear (see Fig. 1C and system (2) defining the ODEs for case 1). In the

following, we use two different values for K_x and three values for n_x : in particular, we assume that n_x can be equal to 2, 4 or 8 in order to model different levels of ultrasensitive responses, i.e., by obtaining a sigmoidal characteristic less steepness ($n_x = 2$) to one highly steepness ($n_x = 8$): this is in line with previous works [15–17], that used Hill functions with similar exponent values for reproducing biological processes that have been shown theoretically and experimentally to embed ultrasensitive responses with low and high slope. The computation of the steady state is not trivial; however, it can be visualized by plotting the two isoclines, h and r reported in Fig. 2A (left panels), obtained by setting to zero the ODEs (see Sect. 3.1.1, Eqs. (4a) and (4b) for h and r , respectively): the intersections of the different straight lines r by varying a_{u_d} , the amplitude of the step disturbance u_d (solid, dashed and dash-dotted lines for $a_{u_d} = 0, 0.1$ and -0.1 , respectively), with h for different values of n_x (blue, red and green curves for $n_x = 2, 4$ and 8 , respectively) provide the steady states of the system (left panel for $K_x = 0.4$, and middle left panel for $K_x = 0.1$). Note that the parameter u_n is set to allow the control system operating around the point of high sensitivity of f (i.e., the x value at steady state, x_{ss} , equal to K_x) without disturbance, while the other fixed parameters are set to unitary values (see Table S1 of the Supplementary Material file) (see Sect. 3.1.1 for the analytic computation of the equilibrium assuming $n_x = 1$, defined by Eq. (10) with u_n set to Eq. (9); Eq. (10) becomes independent of n_x assuming $x_{ss} = K_x$, see Remark 1). By adding a step disturbance u_d with amplitude a_{u_d} , we get a left or right shift of the straight line r_0 (i.e., r without disturbance) depending on the sign of a_{u_d} , resulting in a change of y steady-state value, y_{ss} , that is not significant by increasing the steepness of h . For $K_x = 0.4$ (left panel of Fig. 2A), the effect of u_d on y_{ss} is reduced by increasing the n_x exponent value of h (see the y values obtained by the intersections of the green curve, $n_h = 8$, with the different straight lines). For smaller values of K_x (as for $K_x = 0.1$, see middle left panel of Fig. 2A) such effect is significantly attenuated also for low values of n_x (see the y values obtained by the intersections of the blue curve, $n_h = 2$, with the different straight lines). Moreover, we show the continuation diagram of y with respect to K_x (see Fig. 2A, middle right panel) for different levels of sensitivity by varying n_x value (curves with different colors), and for different disturbance amplitude a_{u_d} values (differ-

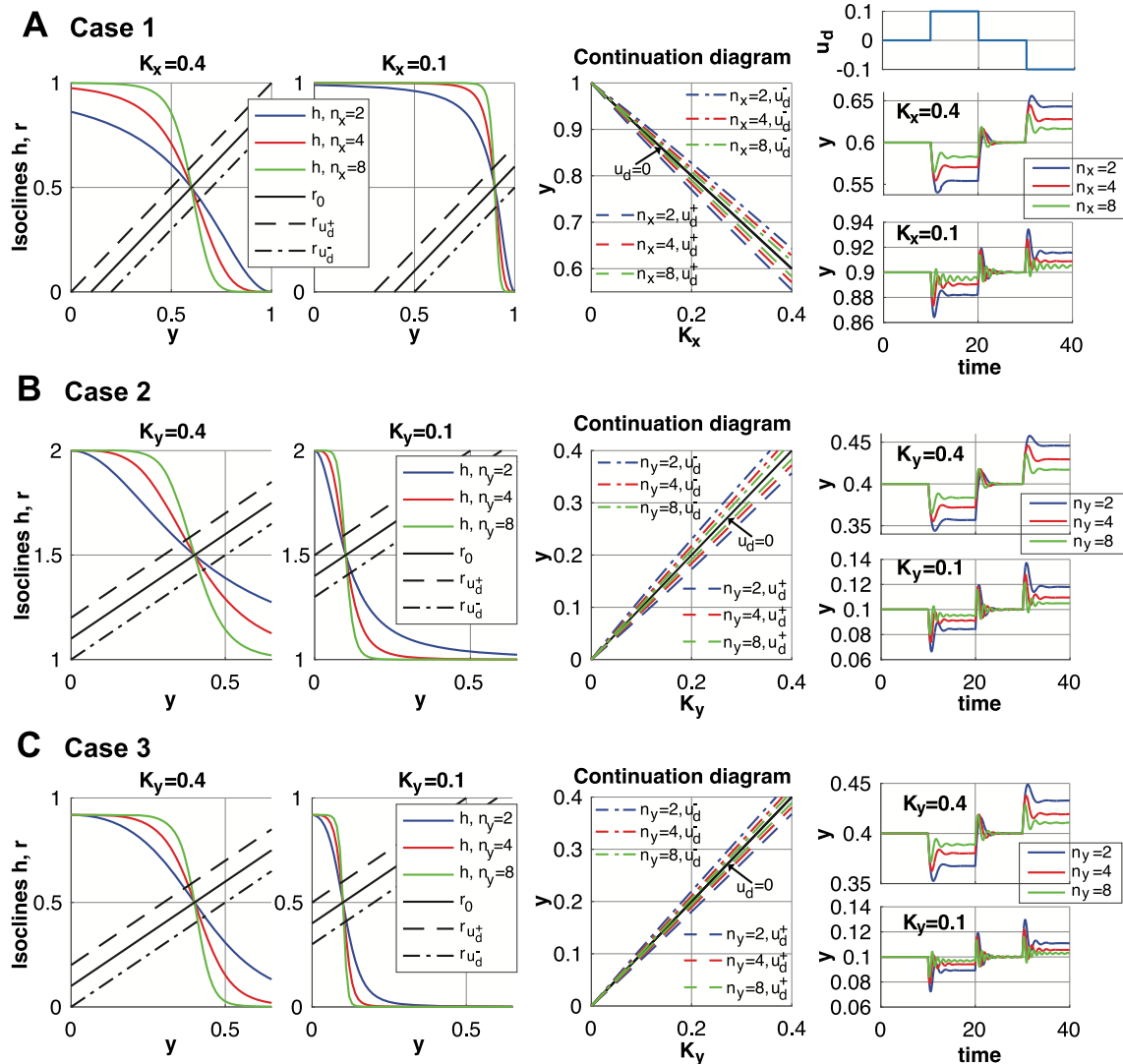


Fig. 2 Analysis of the two-state negative feedback control system without error detection. **A Case 1** for the two-state system of Fig. 1C. Graphical representation (left panels) for computing the y value at steady state (i.e., y_{ss}): the intersection point between the two isoclines, h (for different values of n_x : $n_x = 2$ (blue curve), $n_x = 4$ (red), $n_x = 8$ (green)) and r (for different values of the disturbance amplitude, a_{u_d} : $a_{u_d} = 0$ (solid line r_0), $a_{u_d} = -0.1$ (dash-dotted line r_{u^-}), $a_{u_d} = 0.1$ (dashed line r_{u^+})), provides y_{ss} ; left panel for $K_x = 0.4$ and left-middle for $K_x = 0.1$. The continuation diagram (middle right panel) of y

vs. K_x with different levels of sensitivity by varying n_x ($n_x = 2$ (blue curves), 4 (red) and 8 (green)) and different values of disturbance amplitude a_{u_d} ($a_{u_d} = 0$ (continuous black line), -0.1 (dash-dotted colored lines) and 0.1 (dashed colored lines)). Time responses (right panels) of the system output y to the applied disturbance u_d (upper right), with $K_x = 0.4$ (middle right) and $K_x = 0.1$ (lower right) for different values of n_x ($n_x = 2$ (blue curves), $n_x = 4$ (red) and $n_x = 8$ (green)). **B Case 2** and **C Case 3** for the two-state system of Fig. 1C. Panel details are as in (A)

ent types of lines) in order to get a deeper analysis on how the threshold K_x and the level of sensitivity n_x of the up-regulation function f can control the effect of the disturbance u_d on the system output y . The continuation diagram is a curve obtained by computing the equilibrium point of the two-state system (2) (for

our analysis we consider the y equilibrium, the output of the 2-state system), by varying K_x in a given interval ((0, 0.4) in Fig. 2A, middle right panel). For higher values of K_x (e.g., about 0.4), the distance of both the dashed ($a_{u_d} = 0.1$) and dash-dotted colored curves ($a_{u_d} = -0.1$) from the black solid line (that rep-

resents the continuation diagram for $u_d = 0$) is more evident for low values of n_x (see the blue curves for $n_x = 2$). Such distance becomes smaller by increasing n_x (the red and green curves, $n_x = 4$ and 8, respectively) and is significantly reduced for lower values of K_x even for small values of n_x : in these cases the disturbance is quite completely attenuated by the control system. These findings for *case 1* are confirmed by the time evolution of the system in response to step disturbances. In particular, the right panels of Fig. 2A show the effect of u_d (upper panel) on the evolution of y for different values of K_x and n_x ; for $K_x = 0.4$ (middle panel), this effect is significantly attenuated by increasing the values of n_x (see the red and green curves for $n_x = 4$ and 8, respectively); for lower values of K_x (lower panel for $K_x = 0.1$), the system is capable of reducing considerably the effect of the disturbance even for low values of n_x (see the blue curve for $n_x = 2$). Such effect becomes negligible for higher values of n_x ($n_x = 4$ (red curve) and 8 (green)). Therefore, the ultrasensitive up-regulation function f allows achieving approximately tunable adaptive responses to external step perturbations, by constraining the nonlinear two-state negative feedback control system to operate around the point of high sensitivity of f (i.e., $x_{ss} = K_x$; see Sect. 3.1.1, Eq. (10) and Remark 1); for higher values of K_x (≥ 0.4), high levels of sensitivity (i.e., $n_x \geq 4$) are needed to achieve good performance in terms of adaptation in the face of step disturbances (i.e., the relative error between the steady-state pre- and after-perturbation becomes less than %10, see the sensitivity analysis performed in Sect. 2.1.4); for lower values of K_x (≈ 0.1), low levels of sensitivity ($n_x \approx 2$) guarantee similar performance in terms of adaptation.

2.1.2 Case 2: ultrasensitive down-regulation

For *case 2* of system (1), we assume that the y -dependent ultrasensitive repression described by g can be modeled by a standard Hill function, characterized by the parameters K_y and n_y , while the x -dependent activation function f is linear (see Fig. 1C and system (12) defining the ODEs for *case 2*). As for *case 1*, we use two different values for K_y and three values for n_y [15–17]; once again, the steady states can be visualized by plotting the two isoclines, h and r reported in the left panels of Fig. 2B, obtained by setting to zero the ODEs (see Sect. 3.1.2, Eqs. (14a) and (14b) for h and r , respectively); the intersections of the dif-

ferent straight lines r by varying a_{u_d} (solid, dashed and dash-dotted lines for $a_{u_d} = 0, 0.1$ and -0.1 , respectively) with h for different values of n_y (blue, red and green curves for $n_y = 2, 4$ and 8, respectively) and K_y ($K_y = 0.4$ in the left panel, and $K_y = 0.1$ in the middle left panel) correspond to the steady states of the system. In both the plots, the parameter u_n is set to allow the control system operating around the point of high sensitivity of g (i.e., $y_{ss} = K_y$) without disturbance, while the other fixed parameters are set to unitary values (see Table S1) (see Sect. 3.1.2 for the analytic computation of the equilibrium assuming $n_y = 1$, defined by Eq. (21) with u_n set to Eq. (20); Eq. (21) becomes independent of n_y assuming $y_{ss} = K_y$, see Remark 2). For $K_y = 0.4$, the effect of the disturbance u_d on y_{ss} is reduced by increasing the n_y exponent value of h (the y values obtained by the intersections of the green curve, $n_y = 8$, with the different straight lines are very close). For $K_y = 0.1$, high values of n_y are not needed for getting good performance in terms of adaptation: indeed, the y values obtained by the intersections of blue curve h ($n_y = 2$) with r (by varying a_{u_d}) are closer than for the case with $K_y = 0.4$. The corresponding continuous diagram (Fig. 2B, middle right panel) and the time responses (Fig. 2B, right panels) for *case 2* confirm these findings. Indeed, as shown in the middle right panel of Fig. 2B, the distance from the black solid line (the continuous diagram for $a_{u_d} = 0$) of the dashed ($a_{u_d} = 0.1$) and dash-dotted ($a_{u_d} = -0.1$) colored curves is reduced by increasing n_y and becomes negligible for low values of n_y by decreasing K_y . Finally, the right panels of Fig. 2B exhibiting the time evolution of y for *case 2* in response to the step disturbance u_d (Fig. 2A, upper right panel) show how the effect of u_d on y is attenuated for $K_y = 0.4$ with high values of n_y (Fig. 2B, upper right plot), while for K_y lower values such effect is reduced even with a low value of n_y (Fig. 2B, lower right plot for $K_y = 0.1$, blue curve with $n_y = 2$). Therefore, similarly to *case 1* implementing an ultrasensitive up-regulation function f , *case 2* embedding an ultrasensitive down-regulation function g is capable of achieving approximately tunable adaptive responses to external step perturbations by operating around the point of high sensitivity of g (i.e., $y_{ss} = K_y$; see Sect. 3.1.2, Eq. (21) and Remark 2); as for *case 1*, for higher values of K_y (≥ 0.4), high levels of sensitivity (i.e., $n_y \geq 4$) are needed to achieve good performance in terms of adaptation in the face of step disturbances (see the sensitivity analysis per-

formed in Sect. 2.1.4); for lower values of K_y (≈ 0.1), low levels of sensitivity ($n_y \approx 2$) guarantee similar performance in terms of adaptation.

2.1.3 Case 3: ultrasensitive up- and down-regulations

Finally, for case 3 of system (1), we assume that both the x - and y -dependent feedback actions implement ultrasensitivity described by the activation f and repression g Hill functions, respectively (see Fig. 1C and system (23) defining the ODEs for case 3). Also in this case, we use different values for the thresholds K_x and K_y and the exponents n_x and n_y [15–17]. As performed for the other cases, the steady states can be visualized by plotting the two isoclines, h and r reported in Fig. 2C (left panels), obtained by setting to zero the ODEs (see Sect. 3.1.3, Eqs. (24a) and (24b) for h and r , respectively), and choosing the parameters u_n and m_x allowing the control system to operate around the point of high sensitivity of f and g (i.e., $x_{ss} = K_x$, $y_{ss} = K_y$) without disturbance, while the other fixed parameters are set to unitary values (see Table S1) (see Sect. 3.1.3 for the analytic computation of the equilibrium assuming $n_x = 1$ and $n_y = 1$, defined by Eq. (31) with u_n set to Eq. (30a) and m_x to (30b); Eq. (31) becomes independent of n_x and n_y assuming $x_{ss} = K_x$ and $y_{ss} = K_y$). In the following, we fix the parameters of the function f ($K_x = 0.6$ and $n_x = 4$) and vary those related to g (K_y and n_y). However, similar results can be obtained by varying the only parameters of f or both (f and g), as shown in Fig. S1 of the Supplementary Material file, where we also change the threshold K_x and the level of sensitivity n_x of f together with those of g (i.e., K_y and n_y); from this analysis, it is needed that at least one function implements ultrasensitive dynamics with high levels of sensitivity (i.e., n_x or n_y very high or K_x or K_y very small) to maintain the desired set point ($y = K_y$) despite the presence of step disturbances u_d . The intersections of the different straight lines r by varying a_{u_d} (solid, dashed and dash-dotted lines for $a_{u_d} = 0, 0.1$ and -0.1 , respectively) with h for different values of n_y (blue, red and green curves for $n_y = 2, 4$ and 8 , respectively) and K_y ($K_y = 0.4$ in the left panel and $K_y = 0.1$ in the middle left panel of Fig. 2C) correspond to the steady states of the system. For $K_y = 0.4$, the effect of the disturbance u_d on y_{ss} is reduced by increasing the value of n_y (the y values obtained by the intersections of red and green curves with the different straight lines are very close). For lower values of K_y

(as for $K_y = 0.1$), high values of n_y are not needed for getting good performance in terms of adaptation (the y values obtained by the intersections of h for $n_y = 2$ (i.e., blue curve) with the straight lines are closer than for the case with $K_y = 0.4$). The corresponding continuous diagram (Fig. 2C, middle right panel) and the time responses (Fig. 2C, right panels) of the control system confirm these findings. Indeed, as shown in the middle right panel of Fig. 2C, the distance from the black solid line (continuous diagram for $u_d = 0$) of the dashed and dash-dotted colored curves ($a_{u_d} = 0.1$ and -0.1 , respectively) is reduced by increasing n_y and becomes negligible also for low values of n_y by decreasing K_y . Note that compared to case 2 (where the g function is the same, but f is linear), such distance is significantly reduced even for a high value of K_y (compare middle right panels of Fig. 2B and C; a quantification of these results is reported in the next section by performing sensitivity analysis). Finally, the right panels of Fig. 2C exhibiting the time evolution of y for case 3 in response to the step disturbance u_d (Fig. 2A, upper right panel) show how the effect of u_d on y is significantly attenuated by increasing the value of n_y (Fig. 2C, upper right plot for $K_y = 0.4$, see the red and green curves), while it is considerably attenuated even with a low value of n_y by decreasing K_y (Fig. 2C, lower right plot for $K_y = 0.1$, see the blue curve). Therefore, case 3 implementing ultrasensitivity for both the feedback functions f and g allow achieving adaptive responses to step disturbances by imposing the equilibrium around the point of high sensitivity of f and g (i.e., $x_{ss} = K_x$, $y_{ss} = K_y$, see Sect. 3.1.3, Eq. (31)): as for the previous cases, for higher values of K_x and K_y (≥ 0.4) high levels of sensitivity for at least one of the two functions f and g (i.e., n_x or $n_y \geq 4$) are needed to achieve good performance in terms of adaptation in the face of step disturbances (see the sensitivity analysis performed in Section 2.1.4 and Supplementary Fig. S2); for lower values of K_x or K_y (≈ 0.1), relative low levels of sensitivity (n_x or $n_y \approx 2$) guarantee similar performance in terms of adaptation.

2.1.4 Sensitivity analysis confirms that exploiting ultrasensitivity helps to achieve robust adaptive responses

We perform sensitivity analysis of the two-state control system (1) for the three cases (see Fig. 3) by assuming several scenarios with different thresholds and levels

of sensitivities (K_x and n_x for *case 1*, K_y and n_y for *case 2*, and K_x, n_x, K_y and n_y for *case 3*): for each case, we perform 1000 simulations by randomly varying the set of parameters between $\pm 10\%$ of their nominal values and applying a step disturbance u_d as in Fig. 2 with amplitude $a_{u_d} = \pm 0.3 y_{ss} k$ (positive at time $t = 10$ and negative at time $t = 30$). Moreover, we perform a similar analysis by perturbing the set of parameters between $\pm 50\%$ of their nominal values (see Supplementary Material file, Fig. S3).

Figure 3A shows the analysis for *case 1*, with $K_x = 0.4$ (left panels) and $K_x = 0.1$ (right panels), employing three levels of sensitivity by changing the parameter n_x controlling the slope of the ultrasensitive f function ($n_x = 2, 4$ and 8 for upper, middle and lower subplots of each time evolution graph, respectively). For a given value of n_x , we report the time evolution of the system for the modified parameter settings (1000 simulations - black curves) together with the nominal setting (colored curve): for the perturbed trajectories, the fixed parameters (i.e., $k, \alpha, m_x, \gamma, \beta$, see Table S1), the desired K_x and the constrained u_n (see Eq. (9) to get $x_{ss} = K_x$) are varied by $\pm 10\%$ of their nominal values ($\pm 50\%$ for the supplementary analysis, see Figs. S3A-B). We also report the boxplot representation of the adaptation error, defined as the difference between the normalized steady-state pre-perturbation ($= 1$ for all the trajectories) and the normalized one after the perturbation; moreover, we indicate with e_n^+ and e_n^- the adaptation errors computed for positive and negative step disturbance amplitudes, respectively; so perfect adaptation is achieved when e_n^+ and e_n^- approach to zero. The simulations show that the deviation between the nominal and perturbed trajectories is generally limited; therefore, the adaptive responses are quite robust to the effect of parameter variations. For $K_x = 0.4$, the performance in terms of adaptation can be improved by increasing the level of sensitivity (i.e., n_x) (see left panels of Fig. 3A with $n_x = 2$ (upper panel), $n_x = 4$ (middle) and $n_x = 8$ (lower), and the corresponding boxplot representations (middle left panels) with e_n^+ (upper panel) and e_n^- (lower), the median values of e_n^+ and e_n^- go from about 0.14 (for $n_x = 2$) to 0.05 (for $n_x = 8$). Moreover, by decreasing the threshold K_x (see right panels of Fig. 3A with $K_x = 0.1$), a good level of adaptation to step disturbance can be obtained even for small values of n_x (see the error boxplot representation, where for $n_x = 2$ the median values of e_n^+ and e_n^- are about 0.06 and 0.05 respectively). These

results are also confirmed by increasing the level of uncertainty by varying the parameters between $\pm 50\%$ of their nominal values (see Figs. S3A-B): for the different tests, we obtain similar median error values compared to those of Fig. 3A, even though we note a greater degree of dispersion for the perturbed trajectories due to the higher level of uncertainty, but still limited (both e_n^+ and e_n^- are lower than 0.3 for the worst case).

Figure 3B shows the sensitivity analysis for *case 2*: as for *case 1*, the fixed parameters ($k, \alpha, m_x, \gamma, \beta$, see Table S1), the desired K_y and the constrained u_n (see Eq. (20) to get $y_{ss} = K_y$) are varied by $\pm 10\%$ of their nominal values ($\pm 50\%$ for the supplementary analysis, see Figs. S3C-D). For $K_y = 0.4$ (left panels), the deviation between the nominal and perturbed trajectories is more evident, but still limited. Also in this case, increasing the level of sensitivity (i.e., n_y - see the different subplots of each graph for $n_y = 2, 4$ and 8), allows achieving better performance in terms of adaptation. Moreover, for a lower value of K_y ($= 0.1$, right panels of Fig. 3B), the deviation between the different trajectories is reduced and becomes smaller for higher values of n_y , further improving the level of adaptation. By perturbing the parameters by $\pm 50\%$ of their nominal values (see Figs. S3C-D), we obtain similar results in terms of median error values (compare with Fig. 3B) but a greater deviation of the perturbed trajectories from the nominal ones: however, for the different tests, such deviation is limited and lower than 0.3 in terms of error for the worst case as obtained for *case 1*.

Finally, Fig. 3C shows the sensitivity analysis for *case 3* with $K_y = 0.4$ (left panels) and 0.1 (right panels): as for the previous cases, the fixed parameters (k, α, γ, β , see Table S1), the desired K_x and K_y and the constrained u_n and m_x (see Eq. (30) to get $y_{ss} = K_y$ and $x_{ss} = K_x$) are varied by $\pm 10\%$ of their nominal values. For both the tests ($K_y = 0.4$ and 0.1), the deviation between the nominal and perturbed trajectories is very small and becomes negligible by increasing the level of sensitivity: note a lower degree of dispersion for the perturbed trajectories with the respect to *case 1* and *case 2*; these results are also confirmed by perturbing the parameters between $\pm 50\%$ of their nominal values as reported in Figs. S3E-F, showing a lower dispersion compared to *case 1* and *case 2* in the same scenario (see Figs. S3A-D). For $K_y = 0.4$, increasing the levels of sensitivity provides better performance in terms of adaptation (for $n_y = 2$, the median values of e_n^+ and e_n^- are about 0.10 and becomes lower for higher

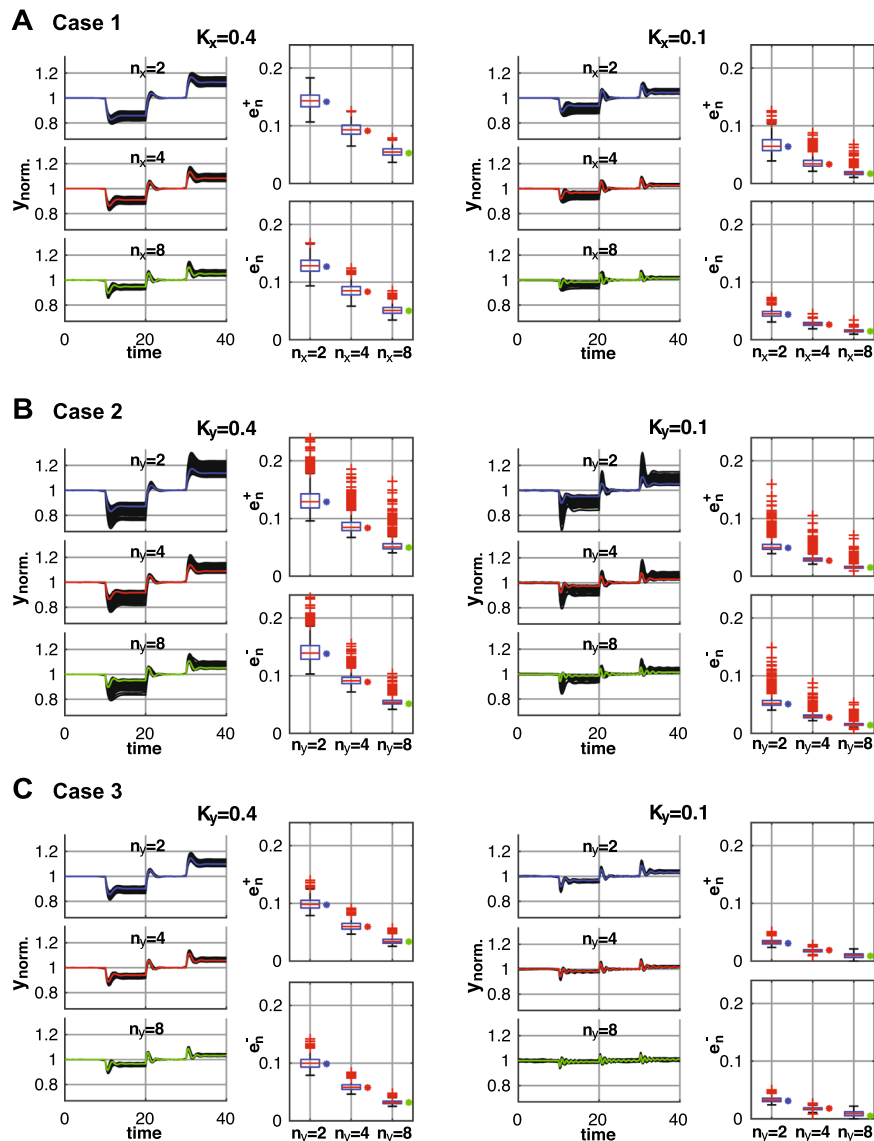


Fig. 3 Sensitivity analysis of the two-state negative feedback control system without error detection of Fig. 1C. **A** Case 1 with $K_x = 0.4$ (left panels) and $K_x = 0.1$ (right panels); for each K_x value, different values of n_x are employed ($n_x = 2$ (upper plot), $n_x = 4$ (middle) and $n_x = 8$ (lower)). Each plot reports 1000 independent simulations (black curves) by varying the set of parameters between $\pm 10\%$ of their nominal values and applying a step disturbance u_d as in Fig. 2A (upper right panel) with $a_{u_d} = \pm 0.3 y_{ss} k$, where y_{ss} is defined by (10). Each simulated time response is normalized by the steady-state value of the system without disturbance u_d (y_{norm} denotes the normalized y

state variable). In each plot, the colored curve shows the normalized y trajectory of the two-state system employing the nominal parameter setting. The boxplot representation shows the distribution of the adaptation error for each test (i.e., for a given value for K_x and n_x), defined as the difference between the normalized steady-state pre-perturbation ($= 1$ for all the trajectories) and the normalized one after the perturbation: the upper panel reports the boxplot representation for positive step disturbance amplitude, e_n^+ , the lower one the boxplot representation for negative amplitude, e_n^- . **B** Case 2 and **C** Case 3 for the two-state system of Fig. 1C. Panel details are as in (A).

values of n_y , about 0.06 and 0.035 for $n_y = 4$ and 8, respectively; see the boxplot representation in left panels of Fig. 3C). Moreover, by decreasing the threshold value of $K_y (=0.1)$, we achieve an additional improvement of performance (see right panels of Fig. 3C with $K_y = 0.1$, where the median values of e_n^+ and e_n^- are about 0.035, 0.02 and 0.01 for $n_y = 2, 4$ and 8, respectively). Note that for *case 3* we keep the parameters K_x and n_x fixed for the different tests ($K_x = 0.6$ and $n_x = 4$): with this setting, *case 3* always performs better than *case 2* by using the same K_y and n_y values (compare Fig. 3B with C). Moreover, decreasing K_x or increasing n_x —resulting in a more steepness ultrasensitive f function—can further improve the performance in terms of adaptation and robustness (see Supplementary Material file, Fig. S2, where we keep K_y fixed and equal to 0.4 and decrease K_x by a factor of 2 (see Figs. S2B and S2E) or double n_x (see Figs. S2C and S2F) with respect to the results shown here (left panels of Fig. 3C, also reported for comparison in Figs. S2A and S2D). Note that decreasing the ultrasensitive threshold (i.e., K_x, K_y) or increasing the level of sensitivity (i.e., n_x, n_y) allow improving performance in terms of adaptation and robustness, but leads to a transient response with more oscillation behavior, as the damping factor of the linearized system decreases getting closer to zero (see Eq. (34) for *case 3*).

This analysis confirms that ultrasensitivity contributes to obtain adaptive responses. Increasing the level of sensitivity allows achieving robust responses to the effects of parameter variations and step disturbances. Furthermore, embedding ultrasensitive regulation for both the functions determining the negative feedback control (f and g) enables to improve the performance compared to the case with only one function (f or g) implementing ultrasensitivity.

2.2 Yeast osmoregulation as feedback control system without error detection

Here, we show how the control system architecture without error detection is implemented within the yeast osmoregulatory response network and allows achieving adaptive responses to osmotic stress, by devising the biomolecular feedback control system characterized by the block diagram representation of Fig. 1B (lower panel) and described by a set of ODEs and algebraic relations as reported in Sect. 3.2. In brief, the yeast membrane bound osmosensor SLN1 detects a

change in external osmolyte conditions e.g., due to a salt shock determining a decrease of the turgor pressure and cell volume and the resulting activation of the MAPK Hog1 cascade [5]. This activation promotes the Hog1-dependent mechanisms stimulating glycerol production, by the transcriptional activation of genes encoding enzymes inducing such production and potential protein–protein interactions initiated by Hog1 in the cytoplasm or nucleus leading to glycerol accumulation [4,5]. In addition, the activation of Hog1 determines the closing of the membrane bound glycerol channel Fps1 [22], leading to a further accumulation of glycerol inside the cell that ensures reinstating the turgor pressure and, then, the cell volume. Therefore, the biomolecular control system mediated by Hog1 acts to adjust glycerol production in order to achieve adaptive response dynamics. In particular, by exploiting the ultrasensitive responses observed at different levels of the control cascade, i.e., at the level of the SLN1 two-component system [19], of the MAPK cascade terminating at Hog1 [15], of the Hog1-dependent mechanisms regulating the Fps1 glycerol channels [22] and promoting glycerol production [20,21], we show that the devised osmoregulation system can achieve adaptive responses in terms of cell volume and Hog1 activation, as observed experimentally [4]. In the following, based on the previous findings—i.e., the capability of the two-state negative feedback control system (1) to maintain an internal reference state, which is intrinsically embedded into the system, by exploiting the ultrasensitive dynamics implemented into the feedback loop—we investigate the role of this biological feature (i.e., ultrasensitivity) within the yeast osmosensing network, embedded at different levels of the control cascade of the network, as experimentally and theoretically observed [15,19–22]. In particular, embedding the input–output ultrasensitive characteristic for the SLN1 sensor [19] by modeling the relative biomolecular reactions, we show through simulations that the osmoregulation system can exhibit adaptive responses to osmotic stress when the SLN1 sensor operates in the highly sensitive zone of its input–output ultrasensitive relationship (see Sect. 2.2.1); therefore, we argue that the yeast osmoregulatory response implements a control system architecture without error detection, characterized by an homeostatic equilibrium which is intrinsically embedded into the system itself, and ultrasensitive dynamics, observed in the system and more specifically in the SLN1 sensor and the downstream lay-

ers, allow maintaining this internal equilibrium in spite of external perturbations (see Sect. 2.2.2). Indeed, by modeling the ultrasensitive dynamics observed theoretically and experimentally for the two main mechanisms downstream of Hog1 activation by SLN1 (i.e., glycerol production and outflow) that act to regulate the glycerol levels in order to maintain homeostasis in the face of environmental changes (e.g., osmotic stress), the osmosensing system is capable of well reproducing the adaptive responses (at cellular level in terms of turgor pressure and cell volume, and at molecular level in terms of Hog1 activity); more specifically, we describe the Hog1-dependent mechanisms promoting glycerol production through an ultrasensitive up-regulation function, as modeled by f for system (1), and the regulation of the Fps1 channels via Hog1 determining the glycerol outflow through an ultrasensitive down-regulation function, as modeled by g for system (1), and we observe that increasing the level of sensitivity of these functions also yields a more ultrasensitive response of the SLN1 sensor (by rising the steepness of its input–output characteristic) and provides better performance in terms of robust adaptation.

2.2.1 Ultrasensitivity at SLN1 level allows achieving adaptive response

The SLN1 two-component system, that senses the osmotic stress and leads to activation of MAPK cascade, consists of a histidine protein kinase (HK), SLN1, its receiver domain (RC), an additional histidine phosphotransfer (HPT) protein, YPD1, and the two response regulators (RRs), SSK1 and SKN7. The reactions and the corresponding ODE system for this module are described in Sect. 3.2.2 (see Eqs. (40)–(44) for ODEs) and are those reported in [19]. The activity of the HK is determined by the environmental stimulus, the turgor pressure P_t [5]: to analyze the behavior of the SLN1 system with increasing signal, we simulate the incoming signal from the osmosensor as an increase in the auto-phosphorylation rate, k_a , of the kinase HK, as in [19], and assume that this rate is linearly dependent on the turgor pressure, i.e., $k_a = k_s * P_t(t)$. Hence, for a given value of k_s , we get an equilibrium by numerically integrating the complete ODE model with respect to time (after a time evolution, without assuming any perturbation, the system reaches its steady-state level). Therefore, changing the input signal by varying the parameter k_s allows us to “follow” the steady-state lev-

els for the SLN1 system and the downstream modules of the feedback loop implemented by the osmosensing network, deriving the so-called steady-state signal-response curves. At equilibrium, for the SLN1 system, HK transfers its phosphoryl group to a cognate RR, in particular to SSK1 which in its phosphorylated form (the output of the SLN1 system) inactivates the downstream MAPK Hog1 pathway. Upon an osmotic stress, SLN1 is inactivated leading to accumulation of de-phosphorylated SSK1, which activates the MAPK Hog1 pathway.

In the following, we investigate the conditions that allow us to achieve perfect or near-perfect adaptation. Assume that the system is at equilibrium E_0 (by numerically integrating the complete ODE model for a given value of k_s and computing the system steady state), then Eq. (35), describing the change of cell volume (see Sect. 3.2.1), reduces at steady state to $P_{i_0} = P_{t_0} + P_{e_0}$, where P_{t_0} , P_{i_0} and P_{e_0} are the turgor, the intra-cellular and the extra-cellular osmotic pressures at equilibrium, respectively. Upon an osmotic stress modeled by a step signal, $P_{e_1} = P_{e_0} + u$, where u is the step signal amplitude that modifies the extra-cellular osmotic pressure due to the applied stress (see Eq. (37)), the system reaches a new equilibrium E_1 , with $P_{i_1} = P_{t_1} + P_{e_0} + u$. In order to achieve perfect adaptation in terms of turgor pressure (and volume) or approximately, then $P_{t_0} \sim P_{t_1}$, i.e., $P_{i_0} \sim P_{i_1} - u$. This relation remains valid (with an error $\leq 10\%$, see Fig. 4A, left panel, from points a_1 to c_1 by increasing the value of k_s) as long as the SLN1 system operates around the highly sensitivity zone at equilibrium without the application of osmotic stress (see Fig. 4B, left panel, blue plot showing the steady-state signal-response for the SLN1 system, at points a_1 , b_1 and c_1). The corresponding volume responses to osmotic stress show adaptive dynamics with a good fit to experimental data (see the green, magenta and black curves in the left panel of Fig. 4C, corresponding to the initial equilibrium conditions a_1 , b_1 and c_1). Instead, the relation $P_{i_0} \sim P_{i_1} - u$ is no more valid (with an error $> 10\%$, see Fig. 4A, left panel, points d_1 and e_1) when the system moves close to the saturation regime (see Fig. 4B, left panel, relative points d_1 and e_1). The corresponding volume responses show a slight lack of adaptation that becomes more significant when the SLN1 approaches to saturation (see the cyan and blue curves in the left panel of Fig. 4C, corresponding to the initial equilibrium conditions d_1 and e_1).

Therefore, this analysis confirms that the SLN1 system operating at equilibrium in the highly sensitivity zone of its input–output ultrasensitive relationship, as observed experimentally [19], determines a steady state in terms of turgor pressure and cell volume that will be slightly (or not at all) affected by the applied salt stress: the turgor pressure and cell volume return approximately to their pre-stimulus levels as found experimentally [4].

2.2.2 Ultrasensitive Hog1-mediated mechanisms increase the level of adaptation

Figure 4 shows also the input–output relationships for the glycerol production and the Fps1 channel regulation (B panels, the red and green curves, respectively), both the mechanisms mediated by Hog1, and the Hog1 activity (D panels) in response to the osmotic stress. We assume that the MAPK cascade activation by SLN1, leading to the phosphorylation of the transcription factor Hog1, described by means of a cascade of phosphorylation/de-phosphorylation cycles (key modules of many signaling pathways [23]), can be modeled by only one layer (i.e., one cycle), capable of reproducing the highly sigmoidal response of MAPK Hog1 system, as experimentally observed [15]. Then, the single cycle consists of one protein (in this case Hog1) that can be in inactive or active form and two enzymes, E_1 (in this case the output of the SLN1 system, i.e., SSK1) that catalyzes the enzymatic reaction for activating Hog1, and E_2 for inactivating Hog1 (see Sect. 3.2.3 for reactions and ODEs describing Hog1 activation).

The two main mechanisms downstream of Hog1 activation, i.e., the glycerol production and the Fps1 channel regulation determining the glycerol outflow, are modeled by Hill-type functions (similarly to those implemented for the two-state control system of Fig. 1C) with the aim to embed ultrasensitive dynamics, as shown theoretically and experimentally [20–22]. In particular, the Hog1-dependent mechanisms that promote glycerol production are described by function u_{Hog1} modeled by Eq. (59) (see Sect. 3.2.4), similarly to the ultrasensitive up-regulation function f implemented for the two-state control system: the active form of Hog1 leads to an increase in the glycerol production that is determined by the Hill function parameters, K_{Hog1} and n_{Hog1} . The regulation of the Fps1 glycerol transporter channels modulated via Hog1 is described by function u_{Fps1} and modeled by Eq. (60)

(see Sect. 3.2.5), similarly to the ultrasensitive down-regulation function g implemented for the two-state control system: the active form of Hog1 induces the channel closure (u_{Fps1} approaches to zero), while the inactive form maintains the Fps1 channel in an open state (u_{Fps1} approaches to K_{Fps1} , the glycerol permeability coefficient in a completely open Fps1 channel). We set the thresholds K_{Hog1} and K_{Fps1} for u_{Hog1} and u_{Fps1} in order to operate in the highly sensitive zone of the input–output responses.

The left panels of Fig. 4B and D show respectively the functions u_{Hog1} and u_{Fps1} in relation to the input of the SLN1 system, and the Hog1 response to osmotic stress, by assuming a low level of sensitivity ($n_{Hog1} = n_{Fps1} = 2$, we assume the same level of sensitivity for both the Hog1-dependent mechanisms). With such level of sensitivity, we are able to reproduce the Hog1 dynamics, achieving a good fit to the experimental data during the transient but a moderate match of the adaptation level at steady state (see Fig. 4D, left panel). By increasing the level of sensitivity (Fig. 4 with $n_{Hog1} = 4$ (middle panels) and 8 (right)) we get better performance in terms of Hog1 adaptation (Fig. 4D, middle and right panels). In this case, not only the steepness of the glycerol production and the Fps1 channel regulation curves rise, but also that of the SLN1 system (Fig. 4B, middle and right panels) leading to better adaptation results in terms of turgor pressure and cell volume (Fig. 4C, middle and right panels). Therefore, these findings show how ultrasensitivity at different levels of the control cascade helps to achieve adaptive responses.

As shown for the simple control architecture of Fig. 2, we are able to get a further improvement in terms of adaptation by decreasing the sensitivity thresholds of the functions u_{Hog1} and u_{Fps1} . We assume the same values for K_{Hog1} and K_{Fps1} . Figure 5 shows the results by decreasing the values of K_{Hog1} and K_{Fps1} by a factor of 2 compared to those shown in Fig. 4. At the same level of sensitivity (i.e., n_{Hog1} and n_{Fps1}), we achieve better performance in terms of adaptation of cell volume and Hog1 activity (compare Fig. 4C with 5C and Fig. 4D with 5D, respectively). The relation that guarantees perfect or near-perfect adaptation in terms of internal pressure is stronger (Fig. 5A), as the input–output relationships are more steepness, i.e., highly ultrasensitive responses for the different branches of the control architecture (Fig. 5B).

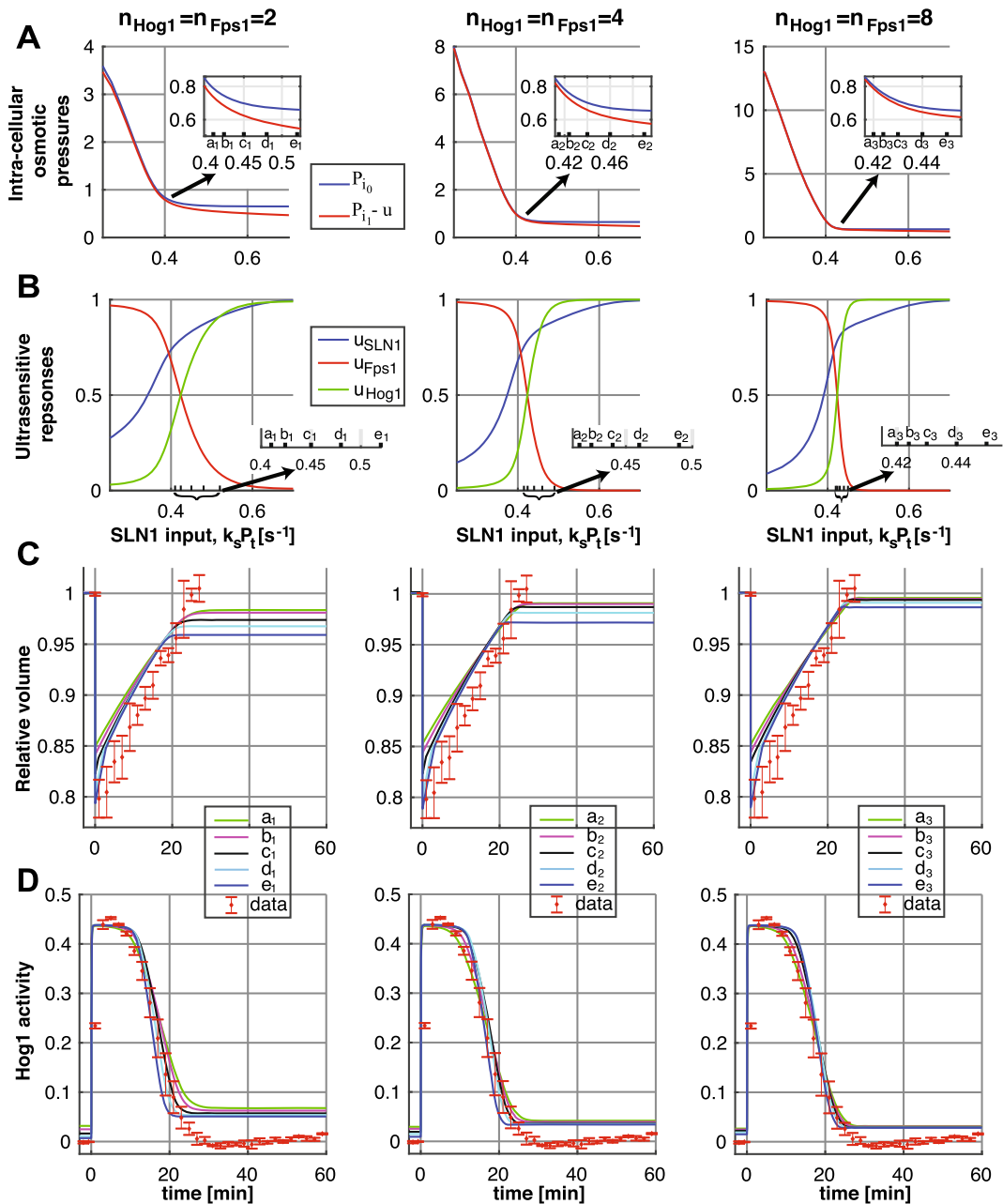


Fig. 4 Analysis of the osmoregulation feedback control system. **A** Intra-cellular osmotic pressures at equilibrium (P_{i0} , blue curve) and after reaching a new equilibrium due to osmoshock ($P_{i1} - u$, red curve, with u the external osmotic pressure increase (of 0.74 Osm [34]) due to the stress input of 0.4 M of NaCl), by varying the parameter k_s determining the input signal of the SLN1 system, the rate of auto-phosphorylation, $k_a = k_s * P_t(t)$; for achieving adaptation, $P_{i0} \sim P_{i1} - u$. The panels show the plots for different values of the sensitivity level of Hog1-dependent

mechanisms, $n_{Hog1} = n_{Fps1} = 2$ (left panel), 4 (middle) and 8 (right). **B** Input–output relationships for the SLN1 system (blue curves), the glycerol production (red) and the Fps1 channel regulation (green), by varying k_s without applying any osmotic stress. Volume responses **C** and Hog1 activity **D** to osmotic stress for different initial equilibrium conditions, corresponding to the initial equilibrium points a_i , b_i , c_i , d_i and e_i reported in panels (A) and (B), with $i = 1$ (left panels), 2 (middle) and 3 (right)

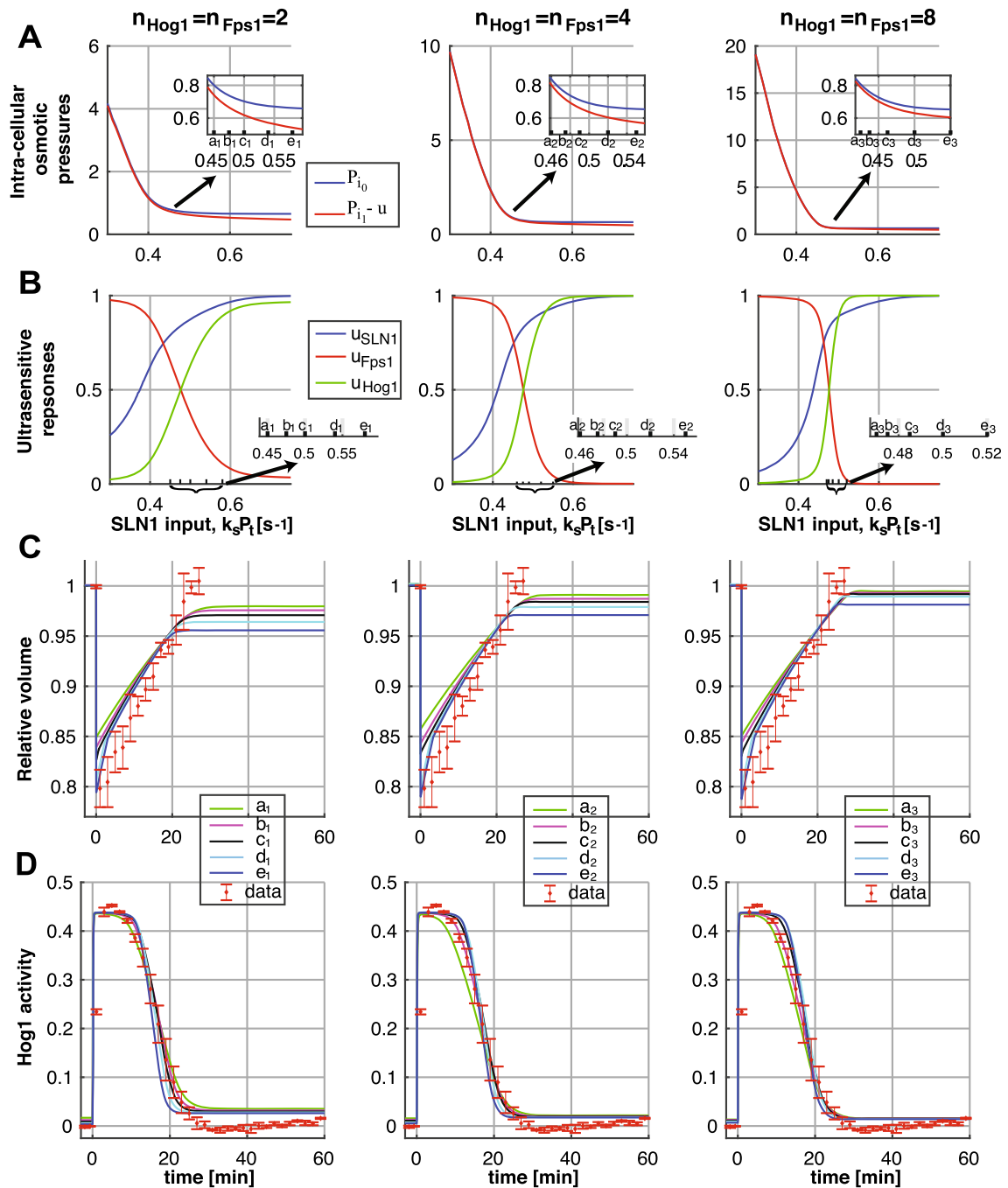


Fig. 5 Analysis of the osmoregulation feedback control system by decreasing the sensitivity threshold of the Hog1-dependent mechanisms. Panel details are as in Fig. 4

This analysis suggests that the different modules of the control cascade within the osmoregulation process act to reach and maintain a homeostatic equilibrium which is intrinsically embedded into the system itself: operating in the highly sensitivity zone of the ultrasensitive characteristics implemented by the different modules allows reproducing the yeast adaptive responses to osmotic stress; moreover, a rise of the level of sensitivity of these characteristics, e.g., of the two tunable functions modeled for the glycerol production, u_{Hog1} , and outflow, u_{Fps1} , that also results in a more steepness of the ultrasensitive relationship for the SLN1 sensor, yields a homeostatic equilibrium which is more robust to environmental changes; instead, lacking ultrasensitivity for both u_{Hog1} and u_{Fps1} functions leads to an equilibrium more sensitive to external changes and then the system can fail to adapt (see Sect. 4 and Fig. S4 of the Supplementary Material file for this latter case).

3 Methods

3.1 Equilibrium and stability for the two-state negative feedback control system without error detection

3.1.1 Analysis for case 1

For case 1 of the two-state system of Fig. 1C, we assume that the x -dependent feedback function f (modeling the activation of Y by X) can implement ultrasensitivity by using a standard Hill function. Therefore, the system dynamics are described by the following ODEs:

$$\dot{y}(t) = u_n - k y(t) + \gamma \frac{x(t)^{n_x}}{K_x^{n_x} + x(t)^{n_x}} - u_d(t), \quad (2a)$$

$$\dot{x}(t) = m_x - \alpha x(t) - \beta y(t). \quad (2b)$$

To get the equilibrium points of system (2), we equate to zero the right-hand side (rhs) of Eq. (2b), and solve it for x ,

$$x = \frac{m_x - \beta y}{\alpha}. \quad (3)$$

Substituting Eq. (3) in the rhs of Eq. (2a) (equal to zero), we get that the steady-state value y_{ss} is given by the intersection point between the following two

isoclines:

$$h(y) := \gamma \frac{\left(\frac{m_x - \beta y}{\alpha}\right)^{n_x}}{\left(\frac{m_x - \beta y}{\alpha}\right)^{n_x} + K_x^{n_x}}, \quad (4a)$$

$$r(y) := k y - u_n + a_{u_d}, \quad (4b)$$

where a_{u_d} is the amplitude of the disturbance u_d (assumed a step function).

Equilibrium analysis To get the analytical expression of the equilibrium points of system (2) without disturbance (i.e., $u_d = 0$), we first assume the simplest case for $n_x = 1$, that gives

$$\dot{y}(t) = u_n - k y(t) + \gamma \frac{x(t)}{K_x + x(t)}, \quad (5a)$$

$$\dot{x}(t) = m_x - \alpha x(t) - \beta y(t). \quad (5b)$$

Equating to zero the rhs of Eq. (5b), we can get Eq. (3) and, by substituting it in Eq. (5a), obtain an equation of second degree in y ,

$$-\beta k y^2 + [(u_n + \gamma) \beta + k (K_x \alpha + m_x)] y - (u_n + \gamma) m_x - K_x \alpha u_n = 0. \quad (6)$$

By solving Eq. (6) in y , we get

$$y_{1,2} = \frac{1}{2\beta k} \left(K_x \alpha k + \beta u_n + \beta \gamma + k m_x \pm \sqrt{A} \right) \quad (7)$$

with $A = (u_n + \gamma)^2 \beta^2 + 2k [(K_x \alpha - m_x) \gamma - (K_x \alpha + m_x) u_n] \beta + k^2 (K_x \alpha + m_x)^2$. By substituting $y_{1,2}$ in Eq. (3), we get

$$x_{1,2} = \frac{-(u_n + \gamma) \beta + (-K_x \alpha + m_x) k \mp \sqrt{A}}{2\alpha k}. \quad (8)$$

We assume that the system can operate around the point of high sensitivity of the ultrasensitive controller (i.e., $x_{ss} = K_x$) by imposing opportunely u_n . Indeed, for $x_{1,2} = K_x$ being the value of x at equilibrium and solving $x_{1,2} - K_x = 0$ in u_n , we get

$$u_n = \frac{-\alpha k K_x - \beta \gamma / 2 + k m_x}{\beta}; \quad (9)$$

thus, the equilibrium point, E_1 , of system (5) is

$$E_1 = (y_{ss}, x_{ss}) = \left(\frac{u_n + \gamma / 2}{k}, K_x \right). \quad (10)$$

Since we work with biophysical quantities, for the feasibility of the equilibrium E_1 , $y_{ss} > 0$ and $x_{ss} > 0$

must hold. Note that the parameters u_n and m_x (modeling constant input fluxes) can be positive or negative, while all the other parameters are positive. For the parameter setting of *case 1* shown in Fig. 2A, all the fixed parameters ($\alpha, \beta, \gamma, k, m_x$) are equal to 1 with $u_n = 0.1$ (for $K_x = 0.4$ by Eq. (9)) and $u_n = 0.4$ (for $K_x = 0.1$), thereby ensuring E_1 feasible.

Remark 1 For the more general case (system (2), without restrictions on n_x), we can notice that, assuming $x = K_x$ being the value of x at equilibrium, the expression of y at equilibrium will be as in Eq. (10), thus independent of n_x .

Stability analysis To study the stability of the equilibrium E_1 defined by Eq. 10, we compute the eigenvalues of the Jacobian matrix evaluated at E_1 .

The Jacobian of system (2) is

$$J = \begin{bmatrix} -k & \frac{n_x \gamma x^{n_x} K_x^{n_x}}{x(x^{n_x} + K_x^{n_x})^2} \\ -\beta & -\alpha \end{bmatrix}. \quad (11)$$

The two roots of the characteristic polynomial $\det(J(E_1) - \lambda) = 0$ are

$$\lambda_{1,2} = -\frac{K_x(\alpha + k) \pm \sqrt{(K_x(\alpha - k))^2 - K_x n_x \beta \gamma}}{2K_x}.$$

Note that $\operatorname{Re}(\lambda_{1,2}) < 0$ since $\alpha, \beta, \gamma, k, K_x$ and n_x are positive, thus E_1 is always stable if feasible.

3.1.2 Analysis for case 2

For *case 2* of the two-state system of Fig. 1C, we assume that the y -dependent inhibition feedback function g (modeling the repression of X by Y) can implement an ultrasensitive down-regulation by a Hill function, while the x -dependent activation function f is linear. Therefore, the system dynamics are described by the following ODEs:

$$\dot{y}(t) = u_n - k y(t) + \gamma x(t) - u_d(t), \quad (12a)$$

$$\dot{x}(t) = m_x - \alpha x(t) + \beta \frac{K_y^{n_y}}{K_y^{n_y} + y(t)^{n_y}}. \quad (12b)$$

To get the equilibrium points of system (12), we equate to zero the rhs of Eq. (12b), and solve it for x ,

$$x = \frac{m_x + \beta \frac{K_y^{n_y}}{K_y^{n_y} + y(t)^{n_y}}}{\alpha}. \quad (13)$$

Substituting Eq. (13) in the rhs of Eq. (12a) (equal to zero), we get that the steady-state value y_{ss} is given by the intersection point between the following two isoclines:

$$h(y) := \gamma \frac{m_x + \beta \frac{K_y^{n_y}}{K_y^{n_y} + y^{n_y}}}{\alpha}, \quad (14a)$$

$$r(y) := k y - u_n + a_{ud}. \quad (14b)$$

Equilibrium analysis To get the analytical expression of the equilibrium points of system (12) without disturbance (i.e., $u_d = 0$), we first assume the simplest case for $n_y = 1$, that gives

$$\dot{y}(t) = u_n - k y(t) + \gamma x(t), \quad (15a)$$

$$\dot{x}(t) = m_x - \alpha x(t) + \beta \frac{K_y}{K_y + y(t)}. \quad (15b)$$

Equating to zero the rhs of (15a), we can solve it with respect to y and get

$$y = \frac{x\gamma + u_n}{k} \quad (16)$$

and substitute it in (15b) to obtain a second degree polynomial in x

$$-\alpha \gamma x^2 + ((-K_y k - u_n)\alpha + m_x \gamma)x + k(m_x + \beta)K_y + m_x u_n = 0 \quad (17)$$

with the roots

$$x_{1,2} = \frac{1}{2\alpha\gamma} \left((-K_y k - u_n)\alpha + m_x \gamma \pm \sqrt{B} \right), \quad (18)$$

with $B = (K_y k + u_n)^2 \alpha^2 + [2(k(m_x + 2\beta)K_y + m_x u_n)]\gamma\alpha + m_x^2 \gamma^2$.

Substituting $x_{1,2}$ in (16), we get

$$y_{1,2} = \frac{1}{2\alpha k} \left((-K_y k + u_n)\alpha + m_x \gamma \pm \sqrt{B} \right). \quad (19)$$

We assume that the control system can operate around the point of high sensitivity of g (i.e., $y_{ss} = K_y$) by imposing opportunely u_n . Indeed, for $y_{1,2} = K_y$ being the value of y at equilibrium and solving $y_{1,2} - K_y = 0$ in u_n , we get

$$u_n = \frac{\alpha k K_y - \beta \gamma / 2 - m_x \gamma}{\alpha}; \quad (20)$$

thus, the equilibrium point, E_2 , of system (15) is

$$E_2 = (y_{ss}, x_{ss}) = \left(K_y, \frac{k K_y - u_n}{\gamma} \right). \quad (21)$$

For the parameter setting of *case 2* shown in Fig. 2B, all the fixed parameters (α , β , γ , k , m_x) are 1 (as for *case 1*) with $u_n = -1.1$ (for $K_y = 0.4$ by Eq. (20)) and $u_n = -1.4$ (for $K_y = 0.1$), thereby ensuring E_2 feasible.

Remark 2 For the more general case (system (12), without restrictions on n_y), we can notice that assuming $y = K_y$ being the value of y at equilibrium, the expression of x at equilibrium will be as in Eq. (21), thus independent of n_y .

Stability analysis To study the stability of the equilibrium E_2 defined by Eq. (21), we compute the eigenvalues of the Jacobian matrix evaluated at E_2 .

The Jacobian of (12) is

$$J = \begin{bmatrix} -k & \gamma \\ -\frac{n_y \beta y^{n_y} K_y^{n_y}}{y(y^{n_y} + K_y^{n_y})^2} & -\alpha \end{bmatrix}. \quad (22)$$

The two roots of the characteristic polynomial $\det(J(E_2) - \lambda) = 0$ are

$$\lambda_{1,2} = -\frac{K_y(\alpha + k) \pm \sqrt{(K_y(\alpha - k))^2 - K_y n_y \beta \gamma}}{2K_y}.$$

Note that $\operatorname{Re}(\lambda_{1,2}) < 0$ since α , β , γ , k , K_y and n_y are positive, thus E_2 is always stable if feasible.

3.1.3 Analysis for case 3

Finally, for the two-state system of Fig. 1C, we assume that both the x - and y -dependent feedback actions implement ultrasensitivity described by the activation f and repression g Hill functions, respectively. Therefore, the system dynamics are described by the following ODEs:

$$\dot{y}(t) = u_n - k y(t) + \gamma \frac{x(t)^{n_x}}{K_x^{n_x} + x(t)^{n_x}} - u_d(t), \quad (23a)$$

$$\dot{x}(t) = m_x - \alpha x(t) + \beta \frac{K_y^{n_y}}{K_y^{n_y} + y(t)^{n_y}}. \quad (23b)$$

To get the equilibrium points of system (23), we equate to zero the rhs of Eq. (23b), and solve it for x , as done for *case 2* (Eq. (12b)) by getting Eq. (13).

Substituting Eq. (13) in the rhs of Eq. (23a) (equal to zero), we get that the steady-state value y_{ss} is given

by the intersection point between the following two isoclines:

$$h(y) := \gamma \frac{\left(\frac{m_x + \beta \frac{K_y^{n_y}}{K_y^{n_y} + y^{n_y}}}{\alpha} \right)^{n_x}}{\left(\frac{m_x + \beta \frac{K_y^{n_y}}{K_y^{n_y} + y^{n_y}}}{\alpha} \right)^{n_x} + K_x^{n_x}}, \quad (24a)$$

$$r(y) := k y - u_n + a_{ud}. \quad (24b)$$

Equilibrium analysis To get the analytical expression of the equilibrium points of system (23) without disturbance (i.e., $u_d = 0$), once more we assume $n_x = 1$ and $n_y = 1$ in (23a) and (23b), respectively, that gives

$$\dot{y}(t) = u_n - k y(t) + \gamma \frac{x(t)}{K_x + x(t)}, \quad (25a)$$

$$\dot{x}(t) = m_x - \alpha x(t) + \beta \frac{K_y}{K_y + y(t)}. \quad (25b)$$

Equating to zero the rhs of (25a), we can solve it with respect to y and get

$$y = \frac{K_x u_n + x(u_n + \gamma)}{k(K_x + x)} \quad (26)$$

and substitute it in (25b) to obtain a second degree polynomial in x

$$C_2 x^2 + C_1 x + C_0 = 0, \quad (27)$$

with $C_2 = -\alpha(K_y k + u_n + \gamma)$, $C_1 = (-\alpha(K_y k + u_n)K_x + (K_y k + u_n + \gamma)m_x + k K_y \beta)$ and $C_0 = ((K_y k + u_n)m_x + k K_y \beta)K_x$. The two roots of (27) are

$$x_{1,2} = \frac{-C_1 \pm \sqrt{C_1^2 - 4C_2 C_0}}{C_2}. \quad (28)$$

Substituting $x_{1,2}$ in (26), we get

$$y_{1,2} = \frac{K_x u_n + x_{1,2}(u_n + \gamma)}{k(K_x + x_{1,2})}. \quad (29)$$

We assume that the control system can operate around the point of high sensitivity of f and g (i.e., $y_{ss} = K_y$ and $x_{ss} = K_x$) by imposing opportunely u_n and m_x . Indeed, for $y_{1,2} = K_y$ and $x_{1,2} = K_x$ and solving $y_{1,2} - K_y = 0$ in u_n and $x_{1,2} - K_x = 0$ in m_x , respectively, we get

$$u_n = k K_y - \frac{\gamma}{2}, \quad (30a)$$

$$m_x = \alpha K_x - \frac{\beta}{2}, \quad (30b)$$

thus the equilibrium point, E_3 , of system (25) is

$$E_3 = (y, x) = (K_y, K_x). \quad (31)$$

For the parameter setting of case 3 shown in Fig. 2C, all the fixed parameters (α , β , γ and k) are equal to 1 with $m_x = 0.1$ (for $K_x = 0.6$ by Eq. (30b)), and $u_n = -0.1$ (for $K_y = 0.4$ by Eq. (30a)) and $u_n = -0.4$ (for $K_y = 0.1$ by Eq. (30a)), thereby ensuring E_3 feasible.

For the general case, i.e., system (23), same results as for system (25) can be found.

Step 1: We first show that exists an intersection point in the first quadrant between the two nullclines

$$N_1 := y = \frac{1}{k} \left[u_n + \gamma \frac{x^{n_x}}{K_x^{n_x} + x^{n_x}} \right], \quad (32a)$$

$$N_2 := x = \frac{1}{\alpha} \left[m_x + \beta \frac{K_y^{n_y}}{K_y^{n_y} + y^{n_y}} \right]. \quad (32b)$$

- The analysis of $N_1(x)$ (32a), n_x even:

- $Dom N_1(x) = \{x \in \mathbb{R}\}$, no vertical asymptotes.
- $\lim_{x \rightarrow \pm\infty} N_1(x) = \frac{u_n + \gamma}{k}$, i.e., $y = \frac{u_n + \gamma}{k}$ is an horizontal asymptote.
- $N_1(-x) = N_1(x)$, thus N_1 is symmetric with respect to y axis.
- No intersection with x axis.
- Intersection with y axis at $(0, y_a)$ with

$$y_a = \frac{u_n}{k} < \frac{u_n + \gamma}{k}.$$

- Local min of $N_1(x)$: $(x = 0, y = y_a)$.

In the first quadrant, $N_1(x)$ for n_x even is a monotonically increasing function.

- The analysis of $N_1(x)$ (32a), n_x odd:

- $Dom N_1(x) = \{x \in \mathbb{R} \mid x \neq \sqrt[n_x]{-K_x}\}$, $x = \sqrt[n_x]{-K_x}$ is a vertical asymptote.
- $\lim_{x \rightarrow \pm\infty} N_1(x) = \frac{u_n + \gamma}{k}$, i.e., $y = \frac{u_n + \gamma}{k}$ is an horizontal asymptote.
- No symmetries.
- Intersection with x axis at $(x_a, 0)$ with

$$x_a = \sqrt[n_x]{-\frac{K_x u_n}{K_x u_n + \gamma}}.$$

- Intersection with y axis at $(0, y_a)$ with

$$y_a = \frac{u_n}{k} < \frac{u_n + \gamma}{k}.$$

- Inflection point of $N_1(x)$: $(x = 0, y = y_a)$.

In the first quadrant $N_1(x)$ for n_x odd is a monotonically increasing function.

- The analysis of $N_2(y)$ (32b), n_y even (notice that we consider the $y - x$ plane):

- $Dom N_2(y) = \{y \in \mathbb{R}\}$, no vertical asymptotes.
 - $\lim_{y \rightarrow \pm\infty} N_2(x) = \left(\frac{m_x}{\alpha}\right)^+$, i.e., $x = \frac{m_x}{\alpha}$ is an horizontal asymptote.
 - $N_2(-y) = N_2(y)$, thus N_2 is symmetric with respect to x axis.
 - Intersection with x axis at $(0, x_b)$ with
- $$x_b = \frac{m_x + \beta}{\alpha} > \frac{m_x}{\alpha}.$$
- No intersection with y axis.
 - Local max of $N_2(y)$: $(x = x_b, y = 0)$.

In the first quadrant $N_2(y)$ for n_y even is a monotonically decreasing function.

- The analysis of $N_2(y)$ (32b), n_y odd:

- $Dom N_2(y) = \{y \in \mathbb{R} \mid y \neq \sqrt[n_y]{-K_y}\}$, $y = \sqrt[n_y]{-K_y}$ is an horizontal asymptote.
- $\lim_{y \rightarrow \pm\infty} N_2(x) = \left(\frac{m_x}{\alpha}\right)^-$, i.e., $x = \frac{m_x}{\alpha}$ is an horizontal asymptote.
- No symmetries.
- Intersection with x axis at $(0, x_b)$ with

$$x_b = \frac{m_x + \beta}{\alpha} > \frac{m_x}{\alpha}.$$

- Intersection with y axis at $(y_b, 0)$ with

$$y_b = K_y \sqrt[n_y]{-\beta - m_x}.$$

- Inflection point of $N_2(y)$: $(y = 0, x = x_b)$.

In the first quadrant $N_2(y)$ for n_y odd is a monotonically decreasing function.

Step 2: Assuming that the equilibrium point is $E^* = (y^*, x^*)$, we want to find u_n and m_x such that $x^* - K_x = 0$ and $y^* - K_y = 0$. We can conclude that for the general case (i.e., system (23) without restrictions on n_x and n_y), hold the same results as for (25).

Stability analysis To study the stability of the equilibrium $E_3 = (y, x) = (K_y, K_x)$, we compute the eigenvalues of the Jacobian matrix evaluated at E_3 .

The Jacobian of (23) is

$$J = \begin{bmatrix} -k & \frac{n_x \gamma x^{n_x} K_x^{n_x}}{x(x^{n_x} + K_x^{n_x})^2} \\ -\frac{n_y \beta y^{n_y} K_y^{n_y}}{y(y^{n_y} + K_y^{n_y})^2} & -\alpha \end{bmatrix}. \quad (33)$$

The two roots of the characteristic polynomial $\det(J(E_3) - \lambda) = 0$ are

$$\lambda_{1,2} = -\frac{2K_x K_y (\alpha + k) \pm \sqrt{\Delta}}{4K_x K_y},$$

with $\Delta = (2K_x K_y (\alpha + k))^2 - K_x K_y n_x n_y \beta \gamma$. Note that $\operatorname{Re}(\lambda_{1,2}) < 0$ since $\alpha, \beta, \gamma, k, K_x, n_x, K_y$ and n_y are positive, thus E_3 is always stable if feasible.

In order to evaluate the behavior of the system around the equilibrium point in response to small perturbations, we evaluate the damping factor ζ of the linearized system (33) (for $\lambda_{1,2} \in \mathbb{C}$) defined as

$$\zeta = \frac{2(\alpha + k)}{\sqrt{16k\alpha + n_x n_y / (K_x K_y) \beta \gamma}}. \quad (34)$$

3.2 Mathematical model of the yeast osmosensing response

The osmoregulation system can be naturally abstracted as a feedback control system comprised of the blocks reported in Fig. 1B (lower panel): the ‘Biophysical module’ block describes how the cell volume and the turgor pressure are affected by varying extra-cellular osmolarity (for example by an osmotic stress); the ‘SLN1 system’ (i.e., the sensor block) perceives a change in external osmolyte conditions leading to activation of MAPK cascade system terminating at Hog1 (‘MAPK Hog1’ block). The Hog1 activity controls the mechanisms (actuating devices) promoting the glycerol production (see ‘Glycerol production’ block) and regulating the glycerol outflow by inducing the Fps1 channel closure (see ‘Glycerol outflow by Fps1’ block); therefore, these actuating mechanisms adjust the glycerol levels (‘Glycerol levels’ block) in order to regain the turgor pressure and volume after the osmotic stress. The mathematical representations employed for each of these blocks are described in the followings. Table S2 of the Supplementary Material file provides the parameter values for each module.

3.2.1 Biophysical module

The biophysical model is based on the work presented in [34]. The system is modeled by taking into account the relations between the cell volume V , the turgor pressure P_t and the intra-cellular and extra-cellular osmotic pressures, P_i and P_e , respectively. Assuming that the cell volume is only determined by the inflow and outflow of water across the cell membrane, which are regulated at any time t by $P_i(t)$, $P_t(t)$ and $P_e(t)$ (i.e., by the algebraic sum $P_i(t) - P_e(t) - P_t(t)$), then the volume dynamics can be described by

$$\frac{dV}{dt} = k_{p1}(P_i(t) - P_e(t) - P_t(t)), \quad (35)$$

with k_{p1} denoting a hydraulic water permeability constant. At equilibrium (equil.), i.e., constant volume and no net flow of water over the membrane, Eq. (35) becomes

$$P_i = P_e + P_t. \quad (\text{equil.})$$

The only osmolyte explicitly taken into account in the model is glycerol, according to the experimental results reported in [35], by which the glycerol counterbalances approximately 80% of applied NaCl in *S. cerevisiae*; therefore, ions and other small molecules that change upon osmotic shock [36] are not considered and the intra-cellular osmotic pressure, $P_i(t)$, according to van’t Hoff’s law, can be expressed as

$$P_i(t) = \frac{s + Gly(t)}{V(t) - V_b}, \quad (36)$$

with Gly representing the intra-cellular glycerol levels, s the concentration of the sum of the other osmolytes present in the cell and assumed constant, and V_b the non-osmotic volume of the cell, subsuming non-polar cellular components, such as membranes. According to Eq. (36), the intra-cellular osmotic pressure rises with increasing intra-cellular glycerol levels, which can be employed to regulate the turgor pressure of the cell.

The extra-cellular osmotic pressure, $P_e(t)$, is only modified by the input signal, $\bar{u}(t)$, for example the applied salt stress modeled by a step signal, and is not affected by changes in other variables. Hence

$$P_e(t) = P_e(0) + \bar{u}(t), \quad (37)$$

where $P_e(0)$ is the initial equilibrium extra-cellular osmotic pressure before the application of the stress input $\bar{u}(t)$ (in the simulations, a step signal with amplitude u applied at time $t = 0$).

The turgor pressure, $P_t(t)$, is assumed to be a linear function of the cell volume according to [37] and described by the following relationship:

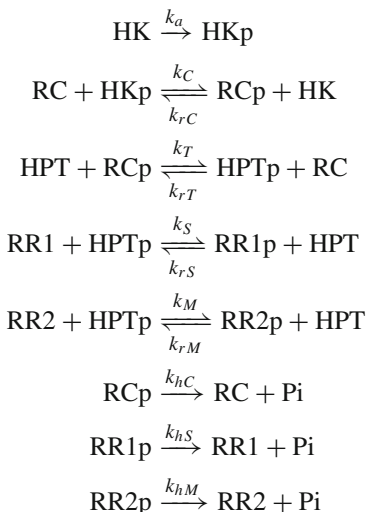
$$P_t(t) = \epsilon \left(\frac{V(t)}{V(0)} - 1 \right) + P_t(0). \quad (38)$$

Here, $V(0)$ is the initial equilibrium volume, $P_t(0)$ is the initial equilibrium turgor pressure, and ϵ is the volumetric elastic modulus. By denoting with $V^{P_t=0}$, the volume at which $P_t = 0$, Eq. (38) can be rewritten as

$$P_t(t) = \begin{cases} P_t(0) \frac{V(t) - V^{P_t=0}}{V(0) - V^{P_t=0}}, & V(t) > V^{P_t=0}, \\ 0, & \text{otherwise.} \end{cases} \quad (39)$$

3.2.2 SLN1 system

A change in external osmolyte conditions (e.g., salt shock), as a drop in cell volume and turgor pressure, is detected by yeast through its membrane bound osmosensor, SLN1. This signaling system consists of a histidine protein kinase HK (i.e., SLN1), its receiver domain RC, an additional histidine phosphotransfer HPT protein, (i.e., YPD1), and the two response regulators RR1 and RR2, (i.e., SSK1 and SKN7). The reactions and the corresponding ODE system for this module are those reported in [19]. In particular, the reactions in the SLN1 system are



where the -p suffix represents phosphorylated forms of these proteins/domains. HK, RC and HPT constitute a phosphorelay, where HPT (i.e., YPD1) phosphotransfers to the two RRs, SSK1 and SKN7. The correspond-

ing ODE system, then, is as follows:

$$\frac{d[\text{HKp}]}{dt} = k_a[\text{HK}] + k_{rC}[\text{RCp}][\text{HK}] - k_C[\text{RC}][\text{HKp}], \quad (40)$$

$$\begin{aligned} \frac{d[\text{RCp}]}{dt} &= k_C[\text{RC}][\text{HKp}] + k_{rT}[\text{HPTp}][\text{RC}] \\ &\quad - k_{rC}[\text{RCp}][\text{HK}] - k_T[\text{HPT}][\text{RCp}] \\ &\quad - k_{hC}[\text{RCp}], \end{aligned} \quad (41)$$

$$\begin{aligned} \frac{d[\text{HPTp}]}{dt} &= k_T[\text{HPT}][\text{RCp}] + k_{rS}[\text{RR1p}][\text{HPT}] \\ &\quad + k_{rM}[\text{RR2p}][\text{HPT}] - k_{rT}[\text{HPTp}][\text{RC}] \\ &\quad - k_S[\text{RR1}][\text{HPTp}] - k_M[\text{RR2}][\text{HPTp}], \end{aligned} \quad (42)$$

$$\begin{aligned} \frac{d[\text{RR1p}]}{dt} &= k_S[\text{RR1}][\text{HPTp}] - k_{rS}[\text{RR1p}][\text{HPT}] \\ &\quad - k_{hS}[\text{RR1p}], \end{aligned} \quad (43)$$

$$\begin{aligned} \frac{d[\text{RR2p}]}{dt} &= k_M[\text{RR2}][\text{HPTp}] - k_{rM}[\text{RR2p}][\text{HPT}] \\ &\quad - k_{hM}[\text{RR2p}]. \end{aligned} \quad (44)$$

In addition, we have five conservation equations:

$$[\text{HK}]_{\text{tot}} = [\text{HK}] + [\text{HKp}], \quad (45)$$

$$[\text{RC}]_{\text{tot}} = [\text{RC}] + [\text{RCp}], \quad (46)$$

$$[\text{HPT}]_{\text{tot}} = [\text{HPT}] + [\text{HPTp}], \quad (47)$$

$$[\text{RR1}]_{\text{tot}} = [\text{RR1}] + [\text{RR1p}], \quad (48)$$

$$[\text{RR2}]_{\text{tot}} = [\text{RR2}] + [\text{RR2p}]. \quad (49)$$

Moreover, the total concentration of each protein varies with the cell volume defined by Eq. (35); therefore, we have

$$\frac{d[X]_{\text{tot}}}{dt} = -\frac{dV}{dt} \frac{[X]_{\text{tot}}}{V(t) - V_b}, \quad (50)$$

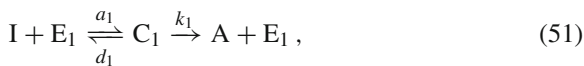
where $[X]_{\text{tot}}$ represents the total concentration of each component of the SLN1 system (i.e., $[\text{HK}]_{\text{tot}}$, $[\text{RC}]_{\text{tot}}$, $[\text{HPT}]_{\text{tot}}$, $[\text{RR1}]_{\text{tot}}$, $[\text{RR2}]_{\text{tot}}$).

The HK activity is determined by the environmental stimulus, the turgor pressure [5], which controls the rate of auto-phosphorylation, the parameter k_a of Eq. (40): here, we assume that the input signal is linearly dependent on the turgor pressure, i.e., $k_a = k_s * P_t(t)$. At equilibrium, HK transfers its phosphoryl group to a cognate RR, in particular to SSK1 which in its phospho-

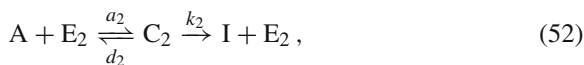
rylated form inactivates the downstream MAPK Hog1 pathway. Upon an osmotic stress, SLN1 is inactivated leading to accumulation of de-phosphorylated SSK1, which activates the MAPK Hog1 pathway.

3.2.3 Hog1 activation

The activation of the MAPK cascade by SSK1, which leads to the phosphorylation of the transcription factor Hog1, is described by means of a cascade of key modules of many signaling pathways, namely phosphorylation/de-phosphorylation cycles [23]. Here, we assume that there is only one layer (one cycle) for the activation of the MAPK Hog1 system, that is able to reproduce the highly sigmoidal response of the complete cascade terminating at Hog1, as experimentally observed [15]. In particular, the cycle consists of a substrate protein S (in this case Hog1) that can be in inactive or active form, denoted by I and A , respectively [23]. The inactive protein I is activated through an enzymatic reaction, catalyzed by enzyme E_1 (in this case the output of the SLN1 system, i.e., SSK1), as follows:



where C_1 denotes the inactive enzyme-substrate complex IE_1 . The active protein A gets inactivated by another enzymatic reaction, catalyzed by enzyme E_2 (in this case, the phosphatase inactivating Hog1), as follows:



where C_2 denotes the active enzyme-substrate complex AE_2 .

Therefore, the cycle is described by the following three-state ODE model, as reported in [23]:

$$\frac{d[A]}{dt} = k_1[C_1] + d_2[C_2] - a_2[A][E_2], \quad (53)$$

$$\frac{d[C_1]}{dt} = a_1[I][E_1] - d_1[C_1] - k_1[C_1], \quad (54)$$

$$\frac{d[C_2]}{dt} = a_2[A][E_2] - d_2[C_2] - k_2[C_2]. \quad (55)$$

In addition, we have the following conservation equations:

$$[S]_{\text{tot}} = [I] + [A] + [C_1] + [C_2], \quad (56)$$

$$[E_2]_{\text{tot}} = [E_2] + [C_2]. \quad (57)$$

Note that the cycle input E_1 corresponds to the SLN1 system output, that is RR1 (SSK1) [5]. Therefore, $[E_1]_{\text{tot}} = [RR1]_{\text{tot}}$ and Eq. (48) becomes

$$[RR1]_{\text{tot}} = [RR1] + [RR1p] + [C_1], \quad (58)$$

thereby allowing the coupling between SLN1 system and MAPK Hog1. As, for the SLN1 system, the total concentration of each protein of the cycle varies with the cell volume defined by Eq. (35), then, the total concentration of each component (i.e., $[S]_{\text{tot}}$, $[RR1]_{\text{tot}}$, $[E_2]_{\text{tot}}$) is described by Eq. (50).

In [23], Gómez-Urbe et al showed that the cycle exhibits different operating regimes determined by the kinetic conditions of the two enzymatic reactions. As experimentally observed, we choose the cycle parameters in order to operate in the ultrasensitive regime thereby achieving the highly sigmoidal response of the MAPK Hog1 cascade.

3.2.4 Glycerol production

The Hog1-dependent mechanisms promoting glycerol production (by the transcriptional activation of genes encoding enzymes stimulating such production and potential protein-protein interactions initiated by Hog1 in the cytoplasm or nucleus leading to glycerol accumulation [4,5,21]) are described by the following relation:

$$u_{Hog1}(t) = k_{Hog1} \frac{[A(t)]^{n_{Hog1}}}{[A(t)]^{n_{Hog1}} + K_{Hog1}^{n_{Hog1}}}, \quad (59)$$

where A is the output of the single cycle describing the MAPK Hog1 system, k_{Hog1} is the constant rate for the glycerol production, while n_{Hog1} and K_{Hog1} are the Hill function parameters determining the dynamics of the production. Note that Eq. (59) allows us to reproduce ultrasensitive dynamics for glycerol production as implemented in previous works [13,20,21] employing similar Hill functions with high values for the exponent n_{Hog1} (for example in [21] $n_{Hog1} = 4$): in this work, we use three different values for n_{Hog1} (i.e., 2, 4 and 8) as for the activation function f of the two-state system (1),

with the aim to show how the results in terms of adaptation can be improved by increasing the level of sensitivity; moreover, we also assume two additional cases with u_{Hog1} implementing linear dynamics and Michaelian kinetics (i.e., nonlinear dynamics with $n_{Hog1} = 1$) (see Supplementary Material, Fig. S4, showing lower performance in terms of adaptation (or the lack) for these latter cases).

3.2.5 Glycerol outflow via Fps1

The Fps1 glycerol transporter channels close in response to osmotic shock, causing accumulation of glycerol with the resulting increase of the intra-cellular osmotic pressure P_i [38] (see Eq. (36)). Recent studies found that MAPK Hog1 closes the channel by phosphorylating and displacing its positive regulators [22]. Then, we model the output of the Fps1 channel, $u_{Fps1}(t)$, which corresponds to the response of the transporter channels, by

$$u_{Fps1}(t) = k_{Fps1} \frac{K_{Fps1}^{n_{Fps1}}}{[A(t)]^{n_{Fps1}} + K_{Fps1}^{n_{Fps1}}}, \quad (60)$$

where k_{Fps1} is the glycerol permeability coefficient in a completely open Fps1 channel, and n_{Fps1} and K_{Fps1} are the Hill function parameters determining the channel regulation: the function u_{Fps1} returns real values in the interval $[0, k_{Fps1}]$, where high values of A (i.e., Hog1 activation) induce the channel closure (u_{Fps1} approaches to zero), while low values maintain the Fps1 channel in an open state (u_{Fps1} approaches to k_{Fps1}). Note that Eq. (60) allows us to embed ultrasensitive dynamics for the Fps1 channel regulation as suggested in [22]; moreover, previous works [13, 20, 21] also used similar Hill functions with high values for the exponent n_{Fps1} for describing the Fps1 dynamics (for example in [22] $n_{Fps1} = 12$): here, as performed for the glycerol production, we employ three different values for n_{Fps1} (i.e., 2, 4 and 8) for showing how the different levels of ultrasensitivity determine the results in terms of adaptation; moreover, as for u_{Hog1} , we also assume two additional cases with u_{Fps1} implementing linear dynamics and Michaelian kinetics (i.e., nonlinear dynamics with $n_{Fps1} = 1$) (see Supplementary Material, Fig. S4, showing lower performance in terms of adaptation (or the lack) for these latter cases).

3.2.6 Glycerol levels

The exchange of internal and external glycerol, u_{Diff} , over the Fps1 channel is modeled using Fick's first law of diffusion as

$$u_{Diff}(t) = u_{Fps1}(t) \left(\frac{Gly(t)}{V(t) - V_b} - \frac{Gly_e(t)}{V_e} \right) \quad (61)$$

with V_e and Gly_e denoting the extra-cellular volume and the glycerol in the extra-cellular compartment, respectively. The extra-cellular glycerol levels, Gly_e , depending only on the diffusion over the Fps1 channel, are defined by

$$\frac{dGly_e}{dt} = u_{Diff}(t). \quad (62)$$

The intra-cellular glycerol levels, Gly , which enable to control the turgor pressure of the cell by modifying the intra-cellular osmotic pressure (see Eq. (36)), are described by combining the output of the Hog1-dependent mechanisms that stimulate the glycerol production, u_{Hog1} (see Eq. (59)), and regulate the glycerol outflow via the Fps1 channels, u_{Fps1} (see Eq. 60), as illustrated above:

$$\frac{dGly}{dt} = u_{Hog1}(t) - u_{Diff}(t). \quad (63)$$

4 Conclusion

Modeling and control engineering principles are functional tools that can be applied to better understand and design biological systems at different levels [39], from subcellular and cellular systems [40–45] to organisms and populations [46, 47]. Here, we exploit these tools for achieving a deeper understanding of mechanisms determining biological homeostasis. In particular, we investigate the role of ultrasensitivity, a common feature implemented by biological systems [14, 15] determining a sigmoidal input–output relationship, and show how this characteristic allows maintaining an internal stable state despite external perturbations: we propose a simple two-state negative feedback control system with ultrasensitive regulation capable of tracking a reference signal that is not imposed externally (i.e., without requiring an error detection mechanism measuring an external reference signal and deviation from this), but it is determined by the tunable threshold and slope characterizing the sigmoidal ultrasensitive relationship implemented by the feedback regulation. Similar results were reported by Alon in his

seminal book [39], by investigating the simplest network motif in transcriptional networks, the negative autoregulation, by which a gene is negatively autoregulated, i.e., it is repressed by its own gene product, the transcription factor X (in this case the repressor X); this network motif corresponds to the devised system (1) with the only component X autoregulated by the down-regulation ultrasensitive function g (in this instance $g(x)$); in case of sharp autorepression (i.e., g with a high value of the Hill exponent) the steady state of X is constrained by the repression threshold K_x , that is $X_{ss} = K_x$, and shows robustness with the respect to the parameter uncertainties (e.g., the fluctuations in production rate). Similarly, for our two-state system (1), we achieve that operating around the point of high sensitivity of f for *case 1* (i.e., $x_{ss} = K_x$), g for *case 2* (i.e., $y_{ss} = K_y$), or both f and g for *case 3* (i.e., $x_{ss} = K_x$ and $y_{ss} = K_y$) by setting opportunely the flux u_n for *case 1* and *case 2*, and the fluxes u_n and m_x for *case 3*, provides tunable adaptive responses to external step perturbations, showing high levels of robustness with the respect to parameter uncertainties by increasing the steepness of the ultrasensitive functions f and g according to the case.

The proposed feedback control architecture without error detection can be obtained by exploiting different molecular mechanisms, which are able to implement ultrasensitive feedback regulations, including dimerization of transcription factors [16], use of scaffolding proteins in archetypical MAPK systems [17], and branching in bacterial phosphorylation/dephosphorylation cycles [18]. Moreover, theoretical and experimental results showed that, in the MAPK signaling system, the level of sensitivity (i.e., the slope of the ultrasensitive functions—right panels of Fig. 1C—tuned by the parameter n_x or n_y) and the level of signal threshold (i.e., parameter K_x or K_y) can be controlled by the concentrations and kinetic properties of kinases and phosphatases [14], and the level of scaffolding proteins [17]. Thus, by exploiting these molecular mechanisms it is possible to design tunable synthetic circuits with adaptive features by modifying the structure, the protein concentrations and the kinetic rates of the biochemical reactions implementing the ultrasensitive functions. In particular, the proposed control architecture operating without error detection, shown in lower panel of Fig. 1A and characterized employing a generic system with two components (see Fig. 1C), can be designed by exploiting chemical reaction net-

works (CRNs) theory as performed in [48, 49]: CRNs, indeed, enable to define a set of abstract chemical reactions realizing the proposed two-state system. Then, by using the mechanism of DNA strand displacement (DSD) [50], the set of idealized CRNs can be implemented via DSD reactions: this approach allows realizing both linear and nonlinear feedback controllers.

Moreover, we argue that the devised architecture working without error detection is a common strategy implemented within biological processes exhibiting adaptive response dynamics. In our previous work [13], we identified and designed a novel ultrasensitive negative feedback control (by combining these two ubiquitous features of the biomolecular circuitry, i.e., ultrasensitivity and negative feedback) capable of explaining the adaptive dynamics observed in the yeast osmoregulation system. However, in [13] we abstractly assumed that the yeast osmosensor SLN1, perceiving a change in external osmolyte conditions due to a salt shock leading to a drop in turgor pressure and cell volume, can implement an error detection mechanism, by computing the difference (i.e., error) between the current and an ideal turgor pressure (i.e., an imposed external reference signal) similarly to previous works [4, 20, 21, 34]. Here, we make a step forward by getting a better understanding on the role of ultrasensitivity for the implementation of a control architecture working without error detection as shown in Fig. 1B: the reference signal is not externally imposed but it is intrinsically embedded into the system itself, all the system acts to reach this reference and ultrasensitivity, implemented at different levels of the control cascade, plays a pivotal role in maintaining this internal reference status; indeed, through simulations, we have shown how the ultrasensitive input–output characteristics of the SLN1 sensor and the two main mechanisms downstream of Hog1 activation by SLN1 (i.e., glycerol production and outflow) influence the yeast adaptive dynamics; in particular, operating around the point of highly sensitivity zone of these embedded ultrasensitive characteristics results in a homeostatic equilibrium which is robust to environmental changes (e.g., the yeast osmoregulatory network is capable of exhibiting the observed adaptive dynamics in response to osmotic stress); operating far from these zones and failing ultrasensitivity make the equilibrium more sensitive to changes in the external environment with the resulting lack of adaptation. For example, lacking ultrasensitivity for both the branches regulating glycerol levels (i.e., u_{Hog1} and u_{Fps1} are

linear or implement Michaelis-Menten kinetics with $n_{Hog1} = 1$ and $n_{Fps1} = 1$ in Eqs. (59) and (60), respectively), results in a system showing responses with a low level of adaptation (in terms of volume and Hog1 activity, see Figs. S4A and S4B) compared to the cases with at least one branch (Figs. S4C–S4F) or both implementing ultrasensitivity (see Fig. 4C, D). From these findings, we can suggest experiments to verify the simulated results by introducing alterations in the signaling and regulatory pathways implementing ultrasensitivity in such a way to alter their sensitivity (i.e., the parameter n_*) and threshold (i.e., the parameter K_*) levels; indeed, the values of these parameters depend biologically on the structure and kinetic rates of the pathways embedding these ultrasensitive dynamics within the osmosensing system: for example, by altering the structure of the SLN1 system and, in particular, the concentrations of the proteins involved, that control the level of sensitivity and the level of signal threshold [19], it will be possible to verify how the adaptive properties could vary and whether these features could be lost by operating far from the ultrasensitive regime or in case of lack of this characteristic. Similarly, for the other modules of the control cascade embedding ultrasensitivity (i.e., Hog1-dependent mechanisms), it will be possible to investigate their role on yeast adaptation to osmotic stress, by performing experimental tests similar to those for the SLN1 system with the aim to modify the ultrasensitive characteristics of these modules and verify how these changes could influence the adaptive properties of the osmoregulation system. For example, for the MAPK Hog1 pathway, by altering the concentrations of kinases and phosphatases [14] and the level of scaffolding proteins [17] controlling the sensitivity and threshold levels of the embedded ultrasensitive dynamics, we would expect to modify the yeast adaptation performance in response to an osmotic stress. Therefore, we argue that such alterations in the yeast osmosensing network would directly alter the precision of adaptation of cell volume and Hog1 activity to an osmoshock, if ultrasensitivity plays a pivotal role in maintaining this homeostatic behavior.

Acknowledgements This paper is an extension version of our preliminary work [30]. As explained in the manuscript, while in [30] we introduced and designed the two-state negative feedback control system without error detection of Fig. 1C by investigating only one control configuration implementing ultrasensitive dynamics (i.e., *case 1* for the two-state system), here we propose and analyze several control configurations implement-

ing different ultrasensitive functions (i.e., *case 2* and *case 3* for the two-state system). Moreover, in this work, we show how the devised control architecture without error detection is implemented within a biological system, as the yeast osmoregulation process by devising a novel mathematical model for this system.

Author contributions FM conceived and performed research, analyzed results, prepared figures and wrote the article. FM, AP and IMB developed methods. FM, FA and CC supervised research. All the authors revised the article and approved the final version.

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Data availability The source code (implemented in MATLAB) and data are available at <https://github.com/BioMecLabUnicz/Ultrasensitivity-and-Homeostasis-matlab-code.git>.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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