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Supplemental Information

Steady-State Differential Dose Response in Biological Systems

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Steady-State Differential Dose-Response in Biological Systems —Supporting Material—

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Open Linear Framework Models

Graphs G representing open linear framework models are obtained by adding a vertex $v_\varnothing$ denoting the environment to a *core graph* $\overline{G}$ (akin to closed models, the core graph is composed of all non-synthesis and non-degradation reactions) and by introducing directed edges from $v_\varnothing$ to the synthesized species in $\overline{G}$ with labels s_i and edges labeled d_i from the degraded species to $v_\varnothing$. The dynamics of open linear framework models are defined in general form as:

$$\frac{dx}{dt} = \mathcal{L}(\overline{G})x - \Delta x + S,$$

where $\mathcal{L}(\overline{G})$ is the Laplacian matrix of the core graph, Δ is a diagonal matrix with $\Delta_{ii} = \delta_i$ the degradation rate constants of the species with index i, and S is a vector $S_i = s_i$ comprising the synthesis rate constants for all species. If a species does not have a degradation or a synthesis reaction then $s_i = 0$ or $\delta_i = 0$, respectively. In open models, the total amount of matter is not conserved but the rates at which matter enters and leaves the system determine the final distribution of steady-state concentrations. In particular, synthesis and degradation at steady-state are balanced: $\delta_1 x_1 + \ldots + \delta_n x_n = s_1 + \ldots + s_n$. Similarly to closed models, but assuring that the steady-state concentration at $v_\varnothing$ is always 1, the unique stable steady-state for vertex v_i ($v_i \neq v_\varnothing$) can be symbolically derived.

The general form of the steady-state concentration x_i^{SS} for open systems and a vertex v_i ($v_i \neq v_\varnothing$) is given by:

$$x_i^{SS} = \frac{\kappa_{v_i}(G)}{\kappa_{v_\varnothing}(G)}.$$

For more details, proofs, and derivations on open linear framework models we refer to (1, 2).

In open models, the q species eliciting the response are associated with a set of output vertices $O(\overline{G})$. Then the general expression for the steady-state response of open models using Kirchhoff polynomials reads:

$$\mathcal{R}_{O(\overline{G})} = \frac{\sum_{v_i \in O(\overline{G})} a_i \kappa_{v_i}(G)}{\kappa_{v_\varnothing}(G)},$$

where $a_i \geq 0$ is the weight given to the steady-state concentration associated with vertex v_i.

The denominator in the steady-state expression of open strongly connected models contains the strongly connected G rooted at the environment vertex $v_\varnothing$ which can yield graphs with factorisable Kirchhoff polynomials. Hence, the Kirchhoff polynomial corresponding to $\mathsf{rt}_{v_\varnothing}(G)$ is non-trivially factorisable when any synthesis reaction s_i is a prime bridge during the sequential deletion of s_i resulting from the rooting operation at $v_\varnothing$ (s_1 might not be a prime bridge in G but in $G \setminus s_2 \ldots \setminus s_n$). Likewise, the numerator of the steady-state expression for open models consists of the linear combination of rooted polynomials, each of which could be factorisable. In this case, there could exist prime factors shared between the numerator and the denominator which can be canceled out. Thereby, in open systems there exist equivalence classes of models with different graphs G but the same steady-state expressions. A necessary and sufficient condition for a reaction to take part in the steady-state expression is that it is part of a prime component that does not get canceled.

Applying the deletion-contraction property from Eq. 5 to express the steady-state response $\mathcal{R}$ as a function of the dose variable d, we obtain the general form of dose-response expression for open models with a graph G:

$$\mathcal{R}_O(d) = \frac{k_1 + k_2 d}{k_3 + k_4 d}, \tag{1}$$

$$\text{with steady-state coefficients:} \quad \begin{aligned} k_1 &= \textstyle\sum_{v_i \in O(\overline{G})} a_{v_i} \kappa_{v_i}(G \backslash e_d), \\ k_2 &= g(p) \textstyle\sum_{v_i \in O(\overline{G})} a_{v_i} \kappa_{v_i}(G / e_d), \\ k_3 &= \kappa_{v_\varnothing}(G \backslash e_d), \\ k_4 &= g(p) \kappa_{v_\varnothing}(G / e_d). \end{aligned}$$

Fig. S1 shows the tree scheme for generating the steady-state coefficients of open models.

Formalizing Differential Responses

Here we give details about the proposed procedure for quantifying the differential response, i.e. how a reference dose-response curve transforms into a perturbed one. Our definition of the differential employs established concepts for comparisons between monotone dose-response curves to generalise comparisons between non-monotone curves. Note that other definitions could be more appropriate

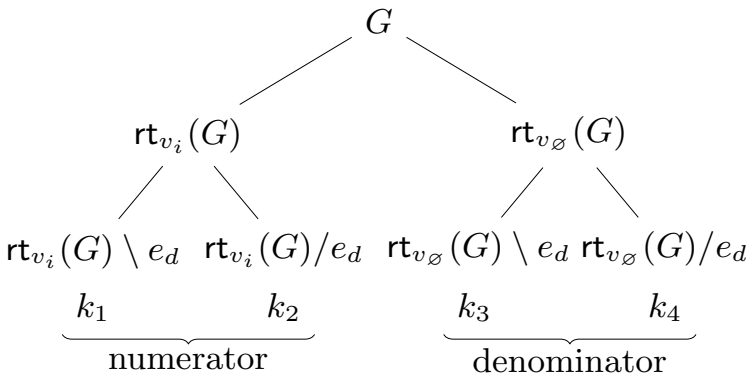

Figure S1: Tree scheme for a general graph G for obtaining the relevant graphs participating in the coefficients k_i of the dose-response relationship in open models (for reference and perturbed systems) through the graph operations rooting, deletion, and contraction. Note that there are also additional terms contained in the coefficients.

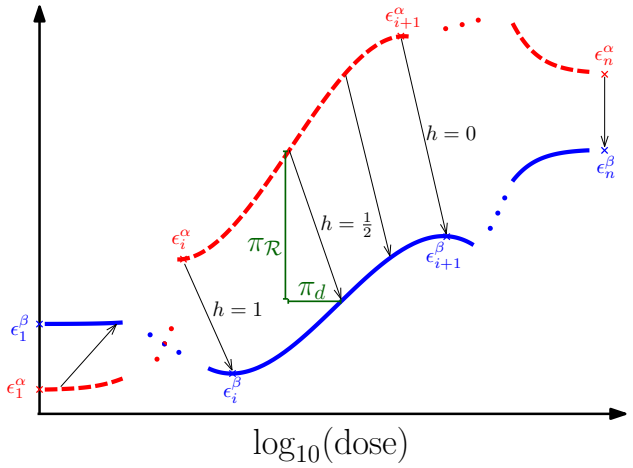

Figure S2: Definition of the differential response as the length of displacement of a reference dose-response curve (red, dashed, marked with the α superscript) to a perturbed curve (blue, solid, marked with the β superscript), generated by functions $\mathcal{R}^\alpha(d)$ and $\mathcal{R}^\beta(d)$, respectively, both with n critical points $\epsilon^{\{\alpha,\beta\}}_{1\ldots n}$. The curves are subdivided along their critical points to obtain monotone segments. The resulting segments are related through a map $\mathcal{M}$ that preserves the order of critical points and segments. Points on a pair of mapped segments with the same proportion of response $h \in [0, 1]$ between the minimum and the maximum are related to each other (corresponding points indicated by black arrows). Distances in the dose and the response dimensions between corresponding points are called the *dose differential* and the *response differential*, and denoted as $\pi_d(h)$ and $\pi_\mathcal{R}(h)$ (green), respectively.

when specific knowledge on the curve transformation contradicts the assumptions we make.

Let dose-response curves be generated by functions $\mathcal{R} : \mathbb{R}_{\geq 0} \to \mathbb{R}_{\geq 0}$, that are continuous, smooth, and bounded (unbounded responses are not biologically feasible). We denote the functions generating the reference and the perturbed curve as $\mathcal{R}^\alpha(d)$ and $\mathcal{R}^\beta(d)$, respectively, where d is the dose variable. A point on a dose-response curve, $(\mathtt{d}, \mathtt{R})$, consists of a dose component $\mathtt{d}$ and a response component $\mathtt{R}$ such that $\mathtt{R} = \mathcal{R}(\mathtt{d})$.

We express the differential through distances between corresponding points on $\mathcal{R}^\alpha(d)$ and

$\mathcal{R}^\beta(d)$. To find the correspondence between points and quantify the distance between corresponding points we follow the procedure (also see Fig. S2):

(i) *Subdivide the curves into monotone segments.*

We subdivide the dose-response curves at their critical points (suprema, infima, extrema, and stationary points of inflection that are identified by the functions' first derivatives) to obtain monotone segments for further comparison.

Assume that $\mathcal{R}^\alpha(d)$ and $\mathcal{R}^\beta(d)$ have, respectively, n and m critical points and denote them by $\epsilon_i \in \mathcal{E}$, where $\mathcal{E}$ is the set of all critical points for the relevant dose-response curve and i is their index ($i \in \{1, \ldots, n\}$ for ϵ_i^α and $i \in \{1, \ldots, m\}$ for ϵ_i^β). Due to the functional relation between dose and response and by considering any two or more identical critical points as a single one, the critical points follow a strict total order in their dose component $\mathtt{d}_{\epsilon_i}$ (e.g. for $\mathcal{R}^\alpha(d)$, $\mathtt{d}_{\epsilon_1^\alpha} < \ldots < \mathtt{d}_{\epsilon_n^\alpha}$), which we use to define a strict total order of the critical points (e.g. for $\mathcal{R}^\alpha(d)$, $\epsilon_1^\alpha < \ldots < \epsilon_n^\alpha$).

From the boundedness requirement on $\mathcal{R}(d)$ it follows that the first and the last critical points are reached when the dose goes to zero and infinity, respectively. The intermediate critical points are defined by doses for which the first derivative of f is zero. Thus the critical points of $\mathcal{R}^\alpha(d)$ are:

$$\epsilon_1^\alpha := \left(0, \lim_{d\to 0} \mathcal{R}^\alpha(d)\right), \epsilon_i^\alpha := \{(\mathtt{d}_{\epsilon_i}, \mathcal{R}^\alpha(\mathtt{d}_{\epsilon_i}))|D_d\mathcal{R}^\alpha(\mathtt{d}_{\epsilon_i}) = 0\},$$
$$\text{and } \epsilon_n^\alpha := \left(\infty, \lim_{d\to\infty} \mathcal{R}^\alpha(d)\right),$$

where $i \in \{2, \ldots, n-1\}$ indexes the intermediate critical points and D_d denotes the first derivative with respect to the dose variable d.

The critical points of $\mathcal{R}^\alpha(d)$ partition its domain into $n-1$ monotone segments σ_j, $j \in \{1, \ldots, n-1\}$. Each segment is defined by two consecutive critical points:

$$\sigma_j^\alpha : \mathcal{R}^\alpha(d), \text{ for } d \in \left[\mathtt{d}_{\epsilon_j^\alpha}, \mathtt{d}_{\epsilon_{j+1}^\alpha}\right],$$

where the domain of the segment is semi-open for the last critical point since it has a dose component at infinity. Let us denote the set of all segments as Σ^α. The definitions of critical points and segments for $\mathcal{R}^\beta(d)$ are analogous.

(ii) *Decide which segments to compare.*

A map $\mathcal{M}$ defines the correspondence between the monotone segments from the reference curve and the monotone segments from the perturbed curve. The definition of the map can be application-specific. Here, in the absence of specific knowledge on the transformation between the curves, we propose that $\mathcal{M}$ preserves the order (and succession, i.e. no critical point is missed out) of critical points and segments. Let us assume, w.l.o.g., that $\mathcal{R}^\alpha(d)$ has less or equal number of critical points than $\mathcal{R}^\beta(d)$ ($n \leq m$). Then, we define $\mathcal{M}$ to map all consecutive segments of $\mathcal{R}^\alpha(.)$ to all possible n consecutive segments of $\mathcal{R}^\beta(.)$,namely:

$$\mathcal{M}(i;\Sigma^\alpha,\Sigma^\beta): \begin{cases} \sigma_1^\alpha \to \sigma_i^\beta \\ \sigma_2^\alpha \to \sigma_{i+1}^\beta \\ \quad \vdots \\ \sigma_{n-1}^\alpha \to \sigma_{i+n-2}^\beta \end{cases},$$

where $i \in \{1,2,\ldots,1+m-n\}$. Notice that in the case when $n=m$ the map is bijective and it does not depend on the index $i=1$.

(iii) *Determine corresponding points in compared pairs of segments.*

In each pair of mapped segments $\sigma_i^\alpha \mapsto \sigma_j^\beta$ we relate the points having the same proportion h ($h \in [0,1]$) of response between the minimal and maximal response, as is customary for monotone dose-response curves. The minimal and maximal responses in each segment are determined by the response components of the critical points enclosing it. Let $\zeta(h;x,y)$ be the *proportional response function* which gives the response for a proportion h and response components x and y of the critical points enclosing the segment of interest. Then, we can obtain the response components of the related points within the segments:

$$\mathtt{R}_{\sigma_i^\alpha,h} \mapsto \mathtt{R}_{\sigma_j^\beta,h},$$

where $\mathtt{R}_{\sigma_i^\alpha,h} = \zeta\left(h;\mathtt{R}_{\epsilon_i^\alpha},\mathtt{R}_{\epsilon_{i+1}^\alpha}\right)$ and $\mathtt{R}_{\sigma_j^\beta,h} = \zeta\left(h;\mathtt{R}_{\epsilon_j^\beta},\mathtt{R}_{\epsilon_{j+1}^\beta}\right)$.

We recover the dose components of the related points from the dose-response function:

$$\mathtt{d}_{\sigma_i^\alpha,h} \mapsto \mathtt{d}_{\sigma_j^\beta,h},$$

where $\mathtt{d}_{\sigma_i^\alpha,h} = {\mathcal{R}^\alpha}^{-1}\left(\zeta\left(h;\mathtt{R}_{\epsilon_i^\alpha},\mathtt{R}_{\epsilon_{i+1}^\alpha}\right)\right)$, ${\mathcal{R}^\alpha}^{-1}(.)$ is the inverse function of $\mathcal{R}^\alpha$ in the interval $\left[d_{\epsilon_i^\alpha}, d_{\epsilon_{i+1}^\alpha}\right]$ (the interval is semi-open for the last critical point since it has a dose component

at infinity). The inverse exists due to continuity and monotonicity of the segment σ_i^α.

The following two definitions can serve as a proportional response function:

$$\zeta_1(h;x,y) := \begin{cases} hx + (1-h)y, & \text{if } x \neq y \\ x, & \text{when } x = y \end{cases}, \text{ and}$$

$$\zeta_2(h;x,y) := \begin{cases} hx + (1-h)y, & \text{if } x > y \\ (1-h)x + hy, & \text{if } x < y \\ x, & \text{when } x = y \end{cases},$$

which are simplifications, respectively, of:

$$\zeta_1(h;x,y) := \frac{1}{2}\left(\left(1 + \frac{x-y}{|x-y|}\right)h + \left(1 - \frac{x-y}{|x-y|}\right)(1-h)\right)|x-y| + \min(x,y) \qquad \text{and}$$

$$\zeta_2(h;x,y) := h\,|x-y| + \min(x,y)\,,$$

where, again, $h \in [0,1]$ is the proportion of response and x and y are the response coordinates (corresponding to dose coordinates $\mathtt{d}_x$ and $\mathtt{d}_y$, $\mathtt{d}_x < \mathtt{d}_y$) defining a segment σ. Note also that we are not interested in the case when $x = y$ in a segment since it leads to a trivial differential expression.

The differences between these very similar definitions become evident when one of the compared segments is monotonically increasing and the other one is monotonically decreasing; otherwise the definitions are identical up to the parametrisation of h. We choose to use ζ_1, hence, calling it only ζ, due to the simpler expressions it yields in our subsequent derivations. Note that $\zeta_1(0;x,y) = y$ and $\zeta_1(1;x,y) = x$, which means that for $h = 1$ the response coordinates of smaller dose coordinates correspond to each other while for $h = 0$ the correspondence is between the response coordinates of larger dose coordinates.

(iv) *Quantify the displacement of corresponding points.*

The last step to derive the differential is to quantify the displacement between corresponding points on the reference and perturbed curves for all mapped segments. For each pair of points we derive the displacement in dose, what we call the *dose differential*, and the displacement in response – the *response differential*. Formally, to identify fold differences in the dose variable, we define the *dose differential* as the difference of logarithms of the dose components of

corresponding points:

$$\pi_d(h; \sigma_i^\alpha, \sigma_j^\beta) := \log_{10} \frac{\mathtt{d}_{\sigma_i^\alpha, h}}{\mathtt{d}_{\sigma_j^\beta, h}}$$

and the response differential as the difference in response between corresponding points:

$$\pi_{\mathcal{R}}(h; \sigma_i^\alpha, \sigma_j^\beta) := \mathtt{R}_{\sigma_i^\alpha, h} - \mathtt{R}_{\sigma_j^\beta, h}.$$

The set of all these displacements constitutes the differential between the curves.

Note that for monotone dose-response curves the differential reduces to the established comparison of points with the same percentage of response. For non-monotone curves each segment is compared to another one at least once allowing to quantify the relative difference between dose-response curves. Note that even when dose-response curves can be derived in closed form, the differential can be symbolically derived only when the critical points and dose-response functions' inverses can be found symbolically.

Derivation of the Differential for One Dose Edge

We derive the differential for dose-response curves generated by functions of the form of Eq. 6 using the procedure outlined in the previous section. We have only fixed to have a single dose edge, all other features of the reference and perturbed models can be arbitrary.

(i) *Subdivide the curves into monotone segments.*

The steady-state function $\mathcal{R}_O(d)$ does not have extrema when varying the dose d since the first derivative is nowhere zero (apart from infinity and the dose independent case when $k_2 k_3 = k_1 k_4$). There are only two critical points and the dose-response curve is a sigmoid in log scale. The critical points are:

$$\mathcal{E} = \left\{ \epsilon_1 = \left(0, \frac{k_1}{k_3}\right), \epsilon_2 = \left(\infty, \frac{k_2}{k_4}\right) \right\}.$$

In each of the reference and the perturbed curve there exists only one segment defined between the dose components of ϵ_1 and ϵ_2, which we call σ.

(ii) *Decide which segments to compare.*

Since each of the compared curves consists of a single segment the mapping is trivial:

$$\mathcal{M} : \sigma^{\alpha} \to \sigma^{\beta}.$$

(iii) *Determine corresponding points in compared pairs of segments.*

We relate the response coordinates of points on σ^{α} and σ^{β} with identical percentage of response:

$$\zeta\left(h; \frac{k_1^{\alpha}}{k_3^{\alpha}}, \frac{k_2^{\alpha}}{k_4^{\alpha}}\right) \mapsto \zeta\left(h; \frac{k_1^{\beta}}{k_3^{\beta}}, \frac{k_2^{\beta}}{k_4^{\beta}}\right) \;\Leftrightarrow\; h\frac{k_1^{\alpha}}{k_3^{\alpha}} + (1-h)\frac{k_2^{\alpha}}{k_4^{\alpha}} \mapsto h\frac{k_1^{\beta}}{k_3^{\beta}} + (1-h)\frac{k_2^{\beta}}{k_4^{\beta}},$$

where the superscripts α and β indicate that the coefficients k have been obtained from the reference or perturbed system, respectively.

To relate the dose components of corresponding points, $\mathtt{d}_{\sigma^{\alpha},h} \mapsto \mathtt{d}_{\sigma^{\beta},h}$, we find the inverse of the single dose edge dose-response function and plug in the proportional response function:

$$\mathtt{d}_{\sigma,h} = \mathcal{R}^{-1}\left(\zeta\left(h; \frac{k_1}{k_3}, \frac{k_2}{k_4}\right)\right) = \frac{k_1 - \zeta\left(h; \frac{k_1}{k_3}, \frac{k_2}{k_4}\right) k_3}{\zeta\left(h; \frac{k_1}{k_3}, \frac{k_2}{k_4}\right) k_4 - k_2},$$

which reduces to:

$$\mathtt{d}_{\sigma,h} = \begin{cases} \frac{1-h}{h}\frac{k_3}{k_4} & \text{if } \frac{k_1}{k_3} \neq \frac{k_2}{k_4}, \\ \text{not defined} & \text{if } \frac{k_1}{k_3} = \frac{k_2}{k_4}. \end{cases}$$

Ignoring the trivial case when $\frac{k_1}{k_3} = \frac{k_2}{k_4}$ we obtain the following correspondence between the dose components of related points:

$$\frac{1-h}{h}\frac{k_3^{\alpha}}{k_4^{\alpha}} \mapsto \frac{1-h}{h}\frac{k_3^{\beta}}{k_4^{\beta}}.$$

(iv) *Quantify the displacement of corresponding points.*

Having determined the correspondence between points, we obtain general expressions for the dose and response differentials in differential systems $\mathcal{D}$ with a single dose edge (also Eq. 7):

$$\pi_d = \log_{10}\frac{k_3^{\alpha}k_4^{\beta}}{k_3^{\beta}k_4^{\alpha}} \qquad \text{and} \qquad \pi_{\mathcal{R}}(h) = h\left(\frac{k_1^{\alpha}}{k_3^{\alpha}} - \frac{k_1^{\beta}}{k_3^{\beta}}\right) + (1-h)\left(\frac{k_2^{\alpha}}{k_4^{\alpha}} - \frac{k_2^{\beta}}{k_4^{\beta}}\right).$$

Steady-state Expressions for the Investigated Single Dose Edge Models

The steady-state expression (before plugging in the differential parameters) for the example from Fig. 4 reads:

$$\mathcal{R}_O(d) = \frac{r_1 r_3 r_9 \left(r_5 + r_6 + r_7\right) d}{\left(r_2 + r_3\right)\left(r_8 + r_9\right)\left(r_4\left(r_6 + r_7\right) + r_5 r_7\right) + \\ + r_1\left(r_5\left(r_3 r_9 + r_7\left(r_3 + r_9\right)\right) + r_9\left(r_3 + r_4\right)\left(r_6 + r_7\right)\right) d} x_t.$$

The steady-state expression for the example from Fig. 5B is:

$$\mathcal{R}_O(d) = \frac{r_1\left(r_3 r_5 r_9 r_{11} + r_3\left(r_6 + r_7\right) r_9 r_{11}\right) d}{r_8 r_{10}\left(r_2 + r_3\right)\left(r_4\left(r_6 + r_7\right) + r_5 r_7\right) + r_1 r_3 r_5 r_7 r_{10} d}.$$

Applications for Experimental Design

To discriminate between models in the same equivalence class, a logical next question is what second perturbation to design (or how to change the first perturbation) in order to differentiate between models in the same equivalence class. In other words, we want to divide the class into smaller equivalence classes, and ultimately identify a single model that represents the biological process. More specifically, a second perturbation could change the prime factors, for example, by adding or deleting new species and reactions, or by changing the input edge. We illustrate the theory's capabilities by deciding which reaction rate constant to alter in the perturbed system for the model from from Fig. 5B. For example, we ask whether to experimentally perturb r_8 or r_5 to obtain the largest effect in the dose differential. For the steady-state coefficient k_3, we observe that r_8 is alone in a prime component while r_5 has three more reaction constants in the same prime component. This means that, if we perturb r_8, we will obtain a factor in the dose differential corresponding to $\frac{k_3^\alpha}{k_3^\beta} = \frac{r_8^\alpha}{r_8^\beta}\frac{r_2^\alpha + r_3}{r_2^\beta + r_3}$ where our perturbation will have a multiplicative effect on the dose differential. On the other hand, if we perturb r_5 we obtain $\frac{k_3^\alpha}{k_3^\beta} = \frac{r_4(r_6+r_7)+r_5^\alpha r_7}{r_4(r_6+r_7)+r_5^\beta r_7}\frac{r_2^\alpha + r_3}{r_2^\beta + r_3}$, where the perturbation is dampened by the other reaction rates—the change in the dose differential upon this perturbation might become experimentally indistinguishable. Hence, perturbing elements of smaller factors has a more direct effect on the observed dose differential, and a corresponding experimental design is more likely to help determining whether the model under consideration is appropriate.

General Form of Dose-Response Curves Generated by Two Dose Edge Models

We consider the case in which the input dose acts proportionally and simultaneously on two edges, i.e. $I(G) = \{e_{d,1}, e_{d,2}\}$, $\ell(e_{d,1}) = g_1(p)d$, and $\ell(e_{d,2}) = g_2(p)d$.

We apply the deletion-contraction formula to partition the set of spanning trees from the numerator and denominator of the response function $\mathcal{R}$ into four categories – those containing no input edges, those containing $e_{d,1}$ but not $e_{d,2}$, those containing $e_{d,2}$ but not $e_{d,1}$, and those containing both $e_{d,1}$ and $e_{d,2}$. Simplifying, we obtain the general form of dose-response expressions for closed and open systems:

$$\mathcal{R}_O(d) = \frac{k_1 + k_{23}d + k_4 d^2}{k_5 + k_{67}d + k_8 d^2}, \tag{2}$$

where $k_{23} := k_2 + k_3$ and $k_{67} := k_6 + k_7$, $\mathcal{R}_O(d)$ is bounded (the degree of the numerator is not higher than the degree of the denominator) and of second degree, and the steady-state coefficients are:

for closed models

$k_1 = x_t \sum_{v_i \in O(G)} a_{v_i} \kappa_{v_i}(G \backslash e_{d,1} \backslash e_{d,2})$,
$k_2 = x_t g_2(p) \sum_{v_i \in O(G)} a_{v_i} \kappa_{v_i}(G \backslash e_{d,1} / e_{d,2})$,
$k_3 = x_t g_1(p) \sum_{v_i \in O(G)} a_{v_i} \kappa_{v_i}(G / e_{d,1} \backslash e_{d,2})$,
$k_4 = x_t g_1(p) g_2(p) \sum_{v_i \in O(G)} a_{v_i} \kappa_{v_i}(G / e_{d,1} / e_{d,2})$,
$k_5 = \kappa(G \backslash e_{d,1} \backslash e_{d,2})$,
$k_6 = g_2(p) \kappa(G \backslash e_{d,1} / e_{d,2})$,
$k_7 = g_1(p) \kappa(G / e_{d,1} \backslash e_{d,2})$,
$k_8 = g_1(p) g_2(p) \kappa(G / e_{d,1} / e_{d,2})$,

for open models

$k_1 = \sum_{v_i \in O(\overline{G})} a_{v_i} \kappa_{v_i}(G \backslash e_{d,1} \backslash e_{d,2})$,
$k_2 = g_2(p) \sum_{v_i \in O(\overline{G})} a_{v_i} \kappa_{v_i}(G \backslash e_{d,1} / e_{d,2})$,
$k_3 = g_1(p) \sum_{v_i \in O(\overline{G})} a_{v_i} \kappa_{v_i}(G / e_{d,1} \backslash e_{d,2})$,
$k_4 = g_1(p) g_2(p) \sum_{v_i \in O(\overline{G})} a_{v_i} \kappa_{v_i}(G / e_{d,1} / e_{d,2})$,
$k_5 = \kappa_{v_\varnothing}(G \backslash e_{d,1} \backslash e_{d,2})$,
$k_6 = g_2(p) \kappa_{v_\varnothing}(G \backslash e_{d,1} / e_{d,2})$,
$k_7 = g_1(p) \kappa_{v_\varnothing}(G / e_{d,1} \backslash e_{d,2})$,
$k_8 = g_1(p) g_2(p) \kappa_{v_\varnothing}(G / e_{d,1} / e_{d,2})$.

The spanning tree partitioning determines the graphs contained in the coefficients k, which can be seen in the tree scheme from Fig. S3. The numerator and denominator polynomials in the dose variable d can be at most of degree two, where the highest degree corresponds to spanning trees containing both $e_{d,1}$ and $e_{d,2}$. We see that even though the degree of the polynomials grows by one, the number of graphs to consider grows exponentially. In the general case, for an input acting on w edges simultaneously, the numerator and denominator are at most of degree w and the graphs giving rise to the coefficients of the polynomials are 2^w and, therefore, the tree scheme has 2^{w+1} leaves. Again, it could happen that spanning trees do not exist for some graphs resulting to simpler, trivial, or unbounded dose-response relationships.

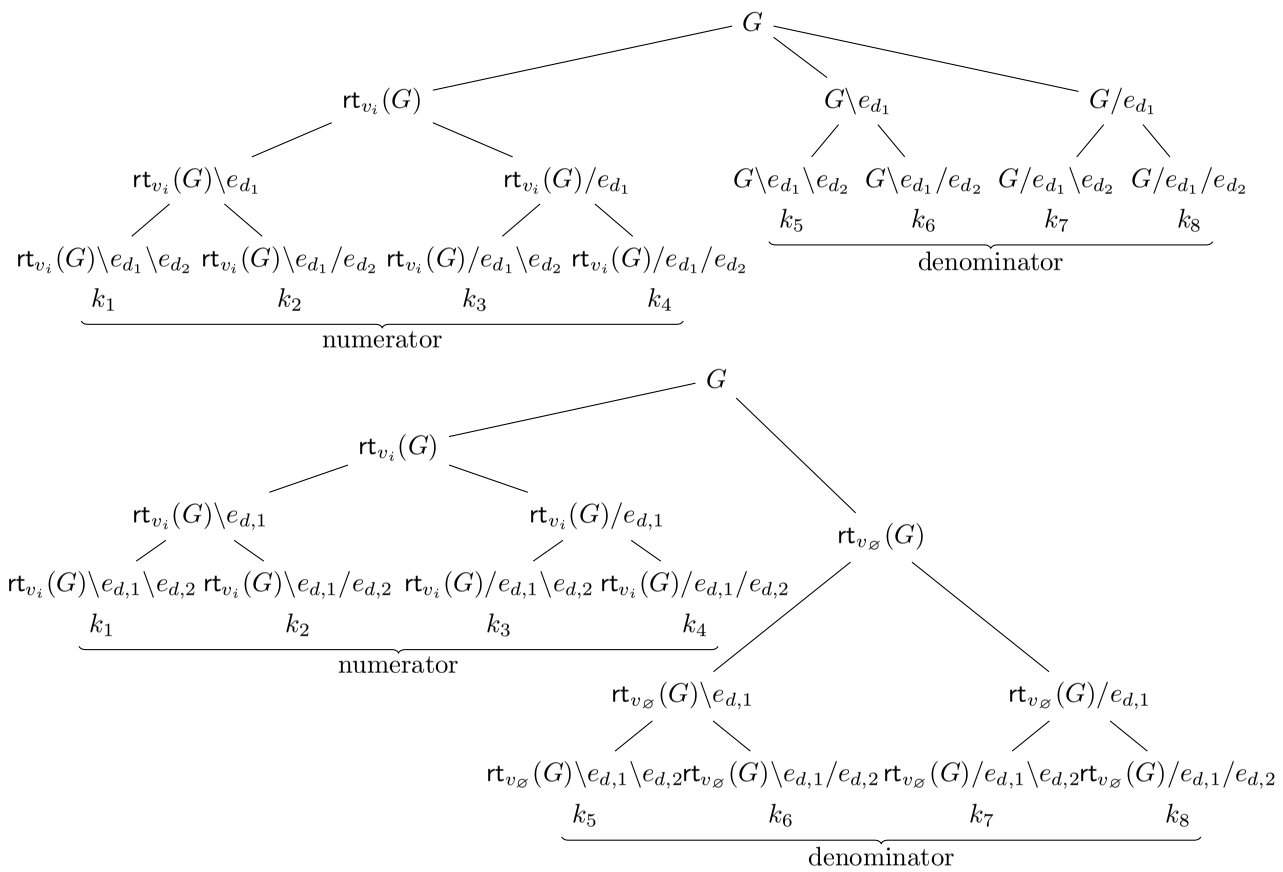

Figure S3: Tree scheme for obtaining the relevant graphs generating the coefficients k_i in the dose-response relationship when the dose acts simultaneously on two edges for (**above**) closed and (**below**) open systems through the graph operations rooting, deletion, and contraction. Note that there are also additional terms contained in the coefficients.

Derivation of the Differential for Two Dose Edges

We derive the differential for the case when the reference and perturbed dose-response curves are both generated by functions having the form of Eq. 2.

Hormesis condition. First, we are interested in deriving conditions guaranteeing positivity of an extremum and thus ensuring that hormesis is present. The steady-state dose-response function $\mathcal{R}_O(d)$ could have at most two extrema when varying the dose variable since its first derivative can become zero for two values of d.

$$D_d \frac{k_1 + k_{23}d + k_4 d^2}{k_5 + k_{67}d + k_8 d^2} = 0 \Leftrightarrow$$

$$\frac{k_5 k_{23} - k_1 k_{67} + 2(k_4 k_5 - k_1 k_8)d + (k_4 k_{67} - k_8 k_{23})d^2}{(k_5 + k_{67}d + k_8 d^2)^2} = 0.$$

The denominator of the condition is never zero for positive doses d and k_i leading to non-degenerate systems (not all k_i being zero). The doses for which the numerator equals zero are the ones corresponding to extrema in the dose response, namely:

$$\mathtt{d}^{(1,2)} = \frac{k_1 k_8 - k_4 k_5 \pm \sqrt{U}}{k_4 k_{67} - k_8 k_{23}},$$

where $U = (k_1 k_8 - k_4 k_5)^2 + (k_1 k_{67} - k_5 k_{23})\,(k_4 k_{67} - k_8 k_{23})$ and $k_4 k_{67} \neq k_8 k_{23}$.

Of interest are only the positive real roots since they are the extrema of the dose response relationships we study. The two roots can never be positive at the same time for non-negative values of the coefficients k_i, which means that the dose-response curves can be at most biphasic. This fact becomes clear after employing Vieta's formulas for second degree polynomials and requiring that the sum and the product of the roots are positive, namely:

$$\mathtt{d}^{(1)} + \mathtt{d}^{(2)} = \frac{-2(k_4 k_5 - k_1 k_8)}{k_4 k_{67} - k_8 k_{23}} > 0 \quad \wedge \quad \mathtt{d}^{(1)}\mathtt{d}^{(2)} = \frac{k_5 k_{23} - k_1 k_{67}}{k_4 k_{67} - k_8 k_{23}} > 0,$$

which is equivalent to:

$$\left(\frac{k_{23}}{k_{67}} < \frac{k_4}{k_8} < \frac{k_1}{k_5} \vee \frac{k_1}{k_5} < \frac{k_4}{k_8} < \frac{k_{23}}{k_{67}}\right) \wedge \left(\frac{k_1}{k_5} < \frac{k_1}{k_5} < \frac{k_4}{k_8} \vee \frac{k_4}{k_8} < \frac{k_{23}}{k_{67}} < \frac{k_1}{k_5}\right).$$

It is evident that for non-negative coefficients k_i there exists no solution for the logical expression of the set of inequalities. More precisely, according to Vietta's formula sum condition the ratio $\frac{k_4}{k_8}$

needs to have a value between the ratios $\frac{k_1}{k_5}$ and $\frac{k_{23}}{k_{67}}$, and according to Vietta's product condition $\frac{k_4}{k_8}$ has to be either the largest or the smallest among the ratios. The two conditions can not hold simultaneously and thus the two roots can not be positive at the same time.

Two possibilities to obtain biphasic dose-response remain:

Condition 1: One root is negative and the other one is positive, yielding the condition:

$$k_4k_{67} \neq k_8k_{23} \ \wedge \ \mathtt{d}^{(1)}\mathtt{d}^{(2)} = \frac{k_5k_{23} - k_1k_{67}}{k_4k_{67} - k_8k_{23}} < 0,$$

which implies $U > 0$.

Condition 2: One root is positive and the other one is zero, translating to the condition:

$$k_5k_{23} = k_1k_{67} \ \wedge \ k_4k_5 \neq k_1k_8 \ \wedge \ k_4k_{67} \neq k_8k_{23} \ \wedge \ \mathtt{d} = \frac{-2(k_4k_5 - k_1k_8)}{k_4k_{67} - k_8k_{23}} > 0$$

The dose-response coefficients are non-negative, which means that they could also be zero. However, to have bounded dose-response curves we require that $k_5 \neq 0 \ \wedge \ k_8 \neq 0$.

Let us first examine the case when $k_{67} = 0$. Then *Condition 1* is satisfied when $\frac{k_5k_{23}}{-k_8k_{23}} < 0$ or equivalently when $k_{23} \neq 0$, while *Condition 2* is never satisfied. In the case when $k_{67} \neq 0$, *Condition 1* could equivalently be written as:

$$\frac{k_{23}}{k_{67}} < \frac{k_4}{k_8} \leq \frac{k_1}{k_5} \ \vee \ \frac{k_{23}}{k_{67}} < \frac{k_1}{k_5} < \frac{k_4}{k_8} \ \vee \ \frac{k_4}{k_8} \leq \frac{k_1}{k_5} < \frac{k_{23}}{k_{67}} \ \vee \ \frac{k_1}{k_5} < \frac{k_4}{k_8} < \frac{k_{23}}{k_{67}},$$

while *Condition 2* again never holds.

We summarize the derived necessary and sufficient conditions for having a positive extremum of the dose-response function, which we call the *Hormesis condition* as:

$$(k_{67} = 0 \ \wedge \ k_{23} \neq 0) \ \vee \ \Bigg(k_{67} \neq 0 \wedge \left(\frac{k_{23}}{k_{67}} < \frac{k_4}{k_8} \leq \frac{k_1}{k_5} \ \vee \ \frac{k_{23}}{k_{67}} < \frac{k_1}{k_5} < \frac{k_4}{k_8} \ \vee \ \frac{k_4}{k_8} \leq \frac{k_1}{k_5} < \frac{k_{23}}{k_{67}} \ \vee \ \frac{k_1}{k_5} < \frac{k_4}{k_8} < \frac{k_{23}}{k_{67}} \right) \Bigg).$$

Note that even when the Hormesis condition is satisfied the biphasic behavior might be weak and experimentally not evident.

Derivation of the differential. After having derived the Hormesis condition, we can proceed to derive the differential following the procedure:

(i) *Subdivide the curves into monotone segments.*

Combining the critical points at zero and infinite dose with the positive extremum we obtain a set of critical points $\mathcal{E}$:

$$\mathcal{E} = \left\{ \epsilon_1 = \left(0, \frac{k_1}{k_5}\right), \epsilon_2 = \left(\frac{k_1 k_8 - k_4 k_5 \pm \sqrt{U}}{k_4 k_{67} - k_8 k_{23}}, \frac{k_{23} k_{67} - 2(k_1 k_8 + k_4 k_5) \pm 2\sqrt{U}}{k_{67}^2 - 4 k_5 k_8} \right), \epsilon_3 = \left(\infty, \frac{k_4}{k_8}\right) \right\},$$

where the sign in front of $\sqrt{U}$ in ϵ_2 depends on the steady-state coefficients:

$$\begin{aligned} &k_{67} = 0 \wedge k_{23} \neq 0 \wedge : && -, \\ &k_{67} \neq 0 \wedge \left(\tfrac{k_{23}}{k_{67}} < \tfrac{k_4}{k_8} \leq \tfrac{k_1}{k_5} \vee \tfrac{k_{23}}{k_{67}} < \tfrac{k_1}{k_5} < \tfrac{k_4}{k_8} \right) : && +, \\ &k_{67} \neq 0 \wedge \left(\tfrac{k_4}{k_8} \leq \tfrac{k_1}{k_5} < \tfrac{k_{23}}{k_{67}} \vee \tfrac{k_1}{k_5} < \tfrac{k_4}{k_8} < \tfrac{k_{23}}{k_{67}} \right) : && -. \end{aligned}$$

When the Hormesis condition is satisfied for a dose-response curve all three critical points are relevant (depending on the conditions, the roots with the appropriate sign have to be selected), thus the curve has two segments – σ_1 (defined by ϵ_1 and ϵ_2) and σ_2 (defined by ϵ_2 and ϵ_3). When the Hormesis condition does not hold, we consider only ϵ_1 and ϵ_3 which define the single segment σ. In general, the values of the coefficients k_i are not known and the number of critical points cannot be determined unambiguously.

(ii) *Decide which segments to compare.*

Thus, three cases, depending on the number of segments in the compared reference and perturbed curves need to be considered (assuming, w.l.o.g., that the reference curve has less or equal critical points than the perturbed one), namely:

Case 1: The Hormesis condition holds neither for the reference nor for the perturbed curve.

Hence, the single segment σ^α of the reference is mapped to the single segment σ^β of the perturbed curve, i.e. $n = m = 2$:

$$\mathcal{M}(i = 1; \Sigma^\alpha, \Sigma^\beta) : \sigma^\alpha \to \sigma^\beta.$$

Case 2: The Hormesis condition does not hold for the reference but holds for the perturbed curve.

Hence, the single segment σ^α of the reference curve is mapped to the two segments σ_1^β

and σ_2^β of of the perturbed curve, i.e. $n = 2$ and $m = 3$:

$$\mathcal{M}(i = 1; \Sigma^\alpha, \Sigma^\beta) : \sigma_1^\alpha \to \sigma_1^\beta, \qquad \mathcal{M}(i = 2; \Sigma^\alpha, \Sigma^\beta) : \sigma_1^\alpha \to \sigma_2^\beta.$$

Case 3: The Hormesis condition holds for both the reference and the perturbed curve.

Hence, the two segments σ_1^α and σ_2^α of the reference curve are mapped to the two segments σ_1^β and σ_2^β of of the perturbed curve, i.e. $n = m = 3$:

$$\mathcal{M}(i = 1; \Sigma^\alpha, \Sigma^\beta) : \begin{cases} \sigma_1^\alpha \to \sigma_1^\beta \\ \sigma_2^\alpha \to \sigma_2^\beta \end{cases}.$$

(iii) *Determine corresponding points in compared pairs of segments.*

The correspondence between points for the three cases is obtained by plugging in the appropriate arguments in the proportion function. The derivation of the related dose components is, however, more involved since the inverses of the segments $\mathcal{R}(d)$ have a more complicated form. In the general case, the parametrised dose component inside a segment ($h \neq 0, 1$) reads:

$$\mathtt{d}_{\sigma,h}^{(1,2)} = \frac{k_{67}\zeta\left(h; x, y\right) - k_{23} \pm \sqrt{W(h; x, y)}}{2\left(k_4 - k_8\zeta\left(h; x, y\right)\right)},$$

where $W(h; x, y) = \left(k_{67}\zeta\left(h; x, y\right) - k_{23}\right)^2 - 4\left(k_1 - k_5\zeta\left(h; x, y\right)\right)\left(k_4 - k_8\zeta\left(h; x, y\right)\right)$, $\mathtt{d}_{\sigma,h}^{(1)}$ is the solution with $+\sqrt{W(h; x, y)}$ and $\mathtt{d}_{\sigma,h}^{(2)}$ with $-\sqrt{W(h; x, y)}$.

The relevant solution should be positive for all $h \in (0, 1)$ and belong to the dose interval of definition of the desired segment σ (defined by the doses corresponding to x and y) when $W(h; x, y) \geq 0$. Solution positivity leads to:

$$\frac{k_{23}}{k_{67}} \mp \frac{\sqrt{W(h; x, y)}}{k_{67}} > \zeta\left(h; x, y\right) > \frac{k_4}{k_8} \;\vee\; \frac{k_{23}}{k_{67}} \mp \frac{\sqrt{W(h; x, y)}}{k_{67}} < \zeta\left(h; x, y\right) < \frac{k_4}{k_8}.$$

(iv) *Quantify the displacement of corresponding points.*

Depending on the particular mapped segments σ_i^α and σ_j^β, the differential expressions π_d and $\pi_\mathcal{R}$ have the general form:

$$\pi_d\left(h\right) = \log_{10} \frac{k_4^\beta - k_8^\beta\zeta\left(h; x^\beta, y^\beta\right)}{k_4^\alpha - k_8^\alpha\zeta\left(h; x^\alpha, y^\alpha\right)} \frac{k_{67}^\alpha\zeta\left(h; x^\alpha, y^\alpha\right) - k_{23}^\alpha \pm \sqrt{W^\beta(h; x^\alpha, y^\alpha)}}{k_{67}^\beta\zeta\left(h; x^\beta, y^\beta\right) - k_{23}^\beta \pm \sqrt{W^\beta(h; x^\beta, y^\beta)}}$$

and

$$\pi_{\mathcal{R}}(h) = \zeta(h; x^{\alpha}, y^{\alpha}) - \zeta\left(h; x^{\beta}, y^{\beta}\right).$$

Note that when $h = 0$ or $h = 1$ the dose differential is derived by mapping the dose components of the respective critical points.

In particular, the differential expressions are different with respect to the number of segments in each dose-response curve:

Case 1: The condition for positivity of the dose component solutions for all $h \in (0,1)$ when $W(h; \frac{k_1}{k_5}, \frac{k_4}{k_8}) \geq 0$ can be reduced to:

$$\frac{k_{23}}{k_{67}} + \frac{\sqrt{W(h; \frac{k_1}{k_5}, \frac{k_4}{k_8})}}{k_{67}} > h\frac{k_1}{k_5} + (1-h)\frac{k_4}{k_8} > \frac{k_4}{k_8} \vee \frac{k_{23}}{k_{67}} - \frac{\sqrt{W(h; \frac{k_1}{k_5}, \frac{k_4}{k_8})}}{k_{67}} < h\frac{k_1}{k_5} + (1-h)\frac{k_4}{k_8} < \frac{k_4}{k_8},$$

which corresponds to the non-hormesis conditions $\frac{k_4}{k_8} \leq \frac{k_{23}}{k_{67}} \leq \frac{k_1}{k_5}$ and $\frac{k_1}{k_5} \leq \frac{k_{23}}{k_{67}} \leq \frac{k_4}{k_8}$, and the solutions $\mathtt{d}^{(2)}_{\sigma,h}$ and $\mathtt{d}^{(1)}_{\sigma,h}$, respectively.

To see why, let us show that $\frac{k_{23}}{k_{67}} - \frac{\sqrt{W(h; \frac{k_1}{k_5}, \frac{k_4}{k_8})}}{k_{67}} > \zeta\left(h; \frac{k_1}{k_5}, \frac{k_4}{k_8}\right) > \frac{k_4}{k_8}$ never holds. We rearrange the inequality to:

$$\frac{k_{23}}{k_{67}} - \zeta\left(h; \frac{k_1}{k_5}, \frac{k_4}{k_8}\right) > \sqrt{\left(\frac{k_{23}}{k_{67}} - \zeta\left(h; \frac{k_1}{k_5}, \frac{k_4}{k_8}\right)\right)^2 - 4\frac{\left(k_1 - k_5\zeta\left(h; \frac{k_1}{k_5}, \frac{k_4}{k_8}\right)\right)\left(k_4 - k_8\zeta\left(h; \frac{k_1}{k_5}, \frac{k_4}{k_8}\right)\right)}{k_{67}^2}},$$

and notice that due to the non-hormesis, $\frac{k_4}{k_8} < \zeta\left(h; \frac{k_1}{k_5}, \frac{k_4}{k_8}\right) < \frac{k_1}{k_5}$, which means the inequality never holds since

$$\left(k_1 - k_5\zeta\left(h; \frac{k_1}{k_5}, \frac{k_4}{k_8}\right)\right)\left(k_4 - k_8\zeta\left(h; \frac{k_1}{k_5}, \frac{k_4}{k_8}\right)\right) < 0.$$

The considerations are analogous for the other inequality in the positivity condition. This simplification shows that, depending on which positivity condition is met after α and β specifics are applied, only one solution $\mathtt{d}_{\sigma,h}$ is relevant for the differential.

Now, ignoring the trivial case when $\frac{k_1}{k_5} = \frac{k_4}{k_8}$ for any differential structure and value of the differential parameters, the dose and the response component mappings read:

$$\mathtt{d}^{(1,2),\alpha}_{\sigma,h} \mapsto \mathtt{d}^{(1,2),\beta}_{\sigma,h} \qquad \text{and} \qquad h\frac{k_1^{\alpha}}{k_5^{\alpha}} + (1-h)\frac{k_4^{\alpha}}{k_8^{\alpha}} \mapsto h\frac{k_1^{\beta}}{k_5^{\beta}} + (1-h)\frac{k_4^{\beta}}{k_8^{\beta}}.$$

In this case, we have already expressed the relevant critical points through the dose-

response coefficients. Thus we can write the differential as:

$$\pi_d(h) = \log_{10} \frac{k_8^\beta}{k_8^\alpha} \frac{k_4^\beta k_5^\beta - k_1^\beta k_8^\beta}{k_4^\alpha k_5^\alpha - k_1^\alpha k_8^\alpha} \frac{k_5^\alpha k_8^\alpha \left(-k_{23}^\alpha \pm \sqrt{W(h; \frac{k_1^\alpha}{k_5^\alpha}, \frac{k_4^\alpha}{k_8^\alpha})}\right) + k_{67}^\alpha \left(h k_1^\alpha k_8^\alpha + (1-h) k_4^\alpha k_5^\alpha\right)}{k_5^\beta k_8^\beta \left(-k_{23}^\beta \pm \sqrt{W(h; \frac{k_1^\beta}{k_5^\beta}, \frac{k_4^\beta}{k_8^\beta})}\right) + k_{67}^\beta \left(h k_1^\beta k_8^\beta + (1-h) k_4^\beta k_5^\beta\right)}$$

and

$$\pi_{\mathcal{R}}(h) = h \left(\frac{k_1^\alpha}{k_5^\alpha} - \frac{k_1^\beta}{k_5^\beta}\right) + (1-h) \left(\frac{k_4^\alpha}{k_8^\alpha} - \frac{k_4^\beta}{k_8^\beta}\right).$$

Note that the sign in front of the square root can be determined only by the positivity conditions, i.e. if it is not known which one is satisfied for the reference and perturbed curve all combinations have to be considered.

Case 2: The dose and response differential for the different segment mappings in this case are:

$$\pi_d(h; i=1) = \log_{10} \frac{k_4^\beta - k_8^\beta \zeta\left(h; \frac{k_1^\beta}{k_5^\beta}, \mathtt{R}_{\epsilon_2}^\beta\right)}{k_4^\alpha - k_8^\alpha \zeta\left(h; \frac{k_1^\alpha}{k_5^\alpha}, \frac{k_4^\alpha}{k_8^\alpha}\right)} \frac{k_{67}^\alpha \zeta\left(h; \frac{k_1^\alpha}{k_5^\alpha}, \frac{k_4^\alpha}{k_8^\alpha}\right) - k_{23}^\alpha \pm \sqrt{W(h; \frac{k_1^\alpha}{k_5^\alpha}, \frac{k_4^\alpha}{k_8^\alpha})}}{k_{67}^\beta \zeta\left(h; \frac{k_1^\beta}{k_5^\beta}, \mathtt{R}_{\epsilon_2}^\beta\right) - k_{23}^\beta \pm \sqrt{W(h; \frac{k_1^\beta}{k_5^\beta}, \mathtt{R}_{\epsilon_2}^\beta)}}$$

and

$$\pi_{\mathcal{R}}(h; i=1) = \zeta\left(h; \frac{k_1^\alpha}{k_5^\alpha}, \frac{k_4^\alpha}{k_8^\alpha}\right) - \zeta\left(h; \frac{k_1^\beta}{k_5^\beta}, \mathtt{R}_{\epsilon_2}^\beta\right),$$

$$\pi_d(h; i=2) = \log_{10} \frac{k_4^\beta - k_8^\beta \zeta\left(h; \mathtt{R}_{\epsilon_2}^\beta, \frac{k_4^\beta}{k_8^\beta}\right)}{k_4^\alpha - k_8^\alpha \zeta\left(h; \frac{k_1^\alpha}{k_5^\alpha}, \frac{k_4^\alpha}{k_8^\alpha}\right)} \frac{k_{67}^\alpha \zeta\left(h; \frac{k_1^\alpha}{k_5^\alpha}, \frac{k_4^\alpha}{k_8^\alpha}\right) - k_{23}^\alpha \pm \sqrt{W(h; \frac{k_1^\alpha}{k_5^\alpha}, \frac{k_4^\alpha}{k_8^\alpha})}}{k_{67}^\beta \zeta\left(h; \mathtt{R}_{\epsilon_2}^\beta, \frac{k_4^\beta}{k_8^\beta}\right) - k_{23}^\beta \pm \sqrt{W(h; \mathtt{R}_{\epsilon_2}^\beta, \frac{k_4^\beta}{k_8^\beta})}}$$

and

$$\pi_{\mathcal{R}}(h, i=2) = \zeta\left(h; \frac{k_1^\alpha}{k_5^\alpha}, \frac{k_4^\alpha}{k_8^\alpha}\right) - \zeta\left(h; \mathtt{R}_{\epsilon_2}^\beta, \frac{k_4^\beta}{k_8^\beta}\right).$$

Choosing the relevant solution from $\mathtt{d}_{\sigma,h}^{(1,2)}$ when deriving the dose differential depends on the Hormesis condition and the particular segment (the solution needs to be in the dose domain of the segment).

Case 3: The dose and response differential for the corresponding segments are:

$$\pi_d(h) = \begin{cases} \log_{10} \dfrac{k_4^\beta - k_8^\beta \zeta\left(h; \frac{k_1^\beta}{k_5^\beta}, \mathtt{R}_{\epsilon_2}^\beta\right)}{k_4^\alpha - k_8^\alpha \zeta\left(h; \frac{k_1^\alpha}{k_5^\alpha}, \mathtt{R}_{\epsilon_2}^\alpha\right)} \dfrac{k_{67}^\alpha \zeta\left(h; \frac{k_1^\alpha}{k_5^\alpha}, \mathtt{R}_{\epsilon_2}^\alpha\right) - k_{23}^\alpha \pm \sqrt{W(h; \frac{k_1^\alpha}{k_5^\alpha}, \mathtt{R}_{\epsilon_2}^\alpha)}}{k_{67}^\beta \zeta\left(h; \frac{k_1^\beta}{k_5^\beta}, \mathtt{R}_{\epsilon_2}^\beta\right) - k_{23}^\beta \pm \sqrt{W(h; \frac{k_1^\beta}{k_5^\beta}, \mathtt{R}_{\epsilon_2}^\beta)}} \\ \log_{10} \dfrac{k_4^\beta - k_8^\beta \zeta\left(h; \mathtt{R}_{\epsilon_2}^\beta, \frac{k_4^\beta}{k_8^\beta}\right)}{k_4^\alpha - k_8^\alpha \zeta\left(h; \mathtt{R}_{\epsilon_2}^\alpha, \frac{k_4^\alpha}{k_8^\alpha}\right)} \dfrac{k_{67}^\alpha \zeta\left(h; \mathtt{R}_{\epsilon_2}^\alpha, \frac{k_4^\alpha}{k_8^\alpha}\right) - k_{23}^\alpha \pm \sqrt{W(h; \mathtt{R}_{\epsilon_2}^\alpha, \frac{k_4^\alpha}{k_8^\alpha})}}{k_{67}^\beta \zeta\left(h; \mathtt{R}_{\epsilon_2}^\beta, \frac{k_4^\beta}{k_8^\beta}\right) - k_{23}^\beta \pm \sqrt{W(h; \mathtt{R}_{\epsilon_2}^\beta, \frac{k_4^\beta}{k_8^\beta})}} \end{cases},$$

and

$$\pi_{\mathcal{R}}(h) = \begin{cases} \zeta\left(h; \frac{k_1^\alpha}{k_5^\alpha}, \mathtt{R}_{\epsilon_2}^\alpha\right) - \zeta\left(h; \frac{k_1^\beta}{k_5^\beta}, \mathtt{R}_{\epsilon_2}^\beta\right) \\ \zeta\left(h; \mathtt{R}_{\epsilon_2}^\alpha, \frac{k_4^\alpha}{k_8^\alpha}\right) - \zeta\left(h; \mathtt{R}_{\epsilon_2}^\beta, \frac{k_4^\beta}{k_8^\beta}\right) \end{cases}.$$

Again, the choice of an appropriate solution from $\mathtt{d}_{\sigma,h}^{(1,2)}$ has to comply with the Hormesis condition and the relevant segment.

It is evident that the obtained differential expressions have a more complicated form than in the case for a single dose edge. Also, multiple conditions depending on the ratios between the dose-response coefficients have to be considered. However, the expressions are symbolic and symbolic analysis can be applied.

Two Dose Edge Example: Insulin Receptor Life-Cycle Model

Robust Hormetic Response

For active species corresponding to the vertices $O = \{v_{RLp}, v_{RLpi}\}$ in the more detailed insulin receptor life-cycle model from Fig 6A we obtain the steady-state coefficients (in polynomial form):

$$\begin{aligned} k_1 &= 0, \\ k_{23} &= r_1 r_3 (r_5 + r_6 + r_7) r_9 r_{11} (r_{13}(r_{15} + r_{16}) + r_{14} r_{16}), \\ k_4 &= 0, \\ k_5 &= (r_2 + r_3) r_8 r_{10} (r_4(r_6 + r_7) + r_5 r_7)(r_{13}(r_{15} + r_{16}) + r_{14} r_{16}), \\ k_{67} &= r_{10}((r_2 + r_3)(r_6 + r_7) r_8 r_{12} r_{14} r_{16} + r_1 r_3 r_5 r_7 (r_{13}(r_{15} + r_{16}) + r_{14} r_{16})), \\ k_8 &= r_1 r_3 (r_6 + r_7) r_{10} r_{12} r_{14} r_{16}. \end{aligned}$$

We can see that the Hormesis condition $k_{67} \neq 0 \wedge \frac{k_4}{k_8} \leq \frac{k_1}{k_5} < \frac{k_{23}}{k_{67}}$ ($k_{67} \neq 0 \wedge 0 \leq 0 < \frac{k_{23}}{k_{67}}$) holds for all possible positive values of the reaction rate constants. Thus the model generates a robust

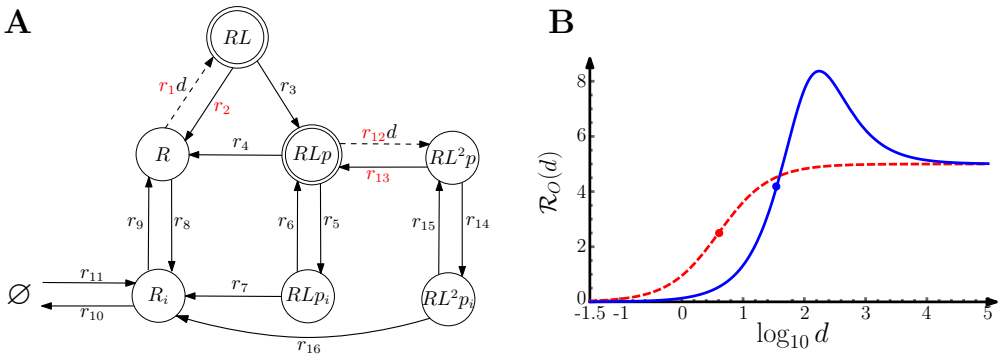

Figure S4: The extended insulin model with two dose edges with output vertices $O = \{v_{RL}, v_{RLp}\}$ exhibits parameter-dependent hormetic dose-response. (**A**) Graph corresponding to the subsystem of insulin receptor binding, recycling, and phosphorylation from (3) with notation as in Fig. 3A; differential parameters are shown in red. (**B**) Sigmoid reference (dashed red) and hormetic perturbed (blue) dose-response curves. The half-maximal response points ($h = 0.5$) for which the dose differential was analyzed are marked with a red and a blue dot on the reference and the first segment of the perturbed curve, respectively. The differential parameters were fixed to $r_1^\alpha = 0.03\, nM^{-1}s^{-1}$, $r_{12}^\alpha = 0.1\, nM^{-1}s^{-1}$, $r_2^\alpha = 0.1\, s^{-1}$, $r_{13}^\alpha = 0.001\, s^{-1}$, $r_1^\beta = 0.002\, nM^{-1}s^{-1}$, $r_{12}^\beta = 0.001\, nM^{-1}s^{-1}$, $r_2^\beta = r_{13}^\beta = 0.01\, s^{-1}$. Other parameters were fixed to $r_9 = 0.5\, s^{-1}$, $r_4 = r_6 = r_{14} = 0.2\, s^{-1}$, $r_3 = r_7 = r_8 = r_{15} = r_{10} = r_{11} = r_{16} = 0.1\, s^{-1}$, and $r_5 = 0.01\, s^{-1}$.

hormetic response.

Parameter-dependent Hormetic Response

Here, we demonstrate how to analyze the differential of models generating dose-response curves with shapes depending on parameter values. Let us consider the more detailed model for insulin receptor trafficking (Fig. S4A). We assume that we measure the singly ligand-bound receptor species on the cell surface, RL and RLp, thus $O = \{v_{RL}, v_{RLp}\}$, and obtain two dose-response curves by stimulating the system with two ligands that differ in their affinity to the receptor—ligand α with reaction rate constants r_1^α, r_2^α, r_{12}^α, r_{13}^α, and ligand β with r_1^β, r_2^β, r_{12}^β, r_{13}^β. Suppose that the dose-response curve for α (reference) is sigmoidal and the curve for β (perturbed) is hormetic (biphasic) (differential as in *Case 2*). We aim to derive the dose differential between the reference curve and the first segment of the perturbed curve at $h = 0.5$ as well as the response differential between the reference curve and the second segment of the perturbed curve at $d \to \infty$, i.e. $h = 0$.

Steady-state coefficients. For conciseness, we analyze the steady-state coefficients in polynomial form instead of graph form:

$$
\begin{aligned}
k_1 &= 0,\\
k_{23} &= r_1 r_9 r_{11}((r_3+r_4)(r_6+r_7)+r_5 r_7)(r_{13}(r_{15}+r_{16})+r_{14}r_{16}),\\
k_4 &= r_1(r_6+r_7)r_9 r_{11}\, r_{12}\, r_{14} r_{16},\\
k_5 &= (r_2+r_3)r_8 r_{10}(r_4(r_6+r_7)+r_5 r_7)(r_{13}(r_{15}+r_{16})+r_{14}r_{16}),\\
k_{67} &= r_{10}((r_2+r_3)(r_6+r_7)r_8\, r_{12}\, r_{14}r_{16}+r_1 r_3 r_5 r_7(r_{13}(r_{15}+r_{16})+r_{14}r_{16})),\\
k_8 &= r_1 r_3(r_6+r_7)r_{10}\, r_{12}\, r_{14} r_{16},
\end{aligned}
$$

where the differential parameters are marked in red.

We can use the *Hormesis condition* to find parametrizations such that the reference dose-response curve is sigmoidal, whereas the perturbed curve is hormetic (Fig. S4B). Due to $k_1 = 0$, the only non-hormesis condition holding for the reference curve α is $\frac{k_1}{k_5} = 0 \leq \frac{k^\alpha_{23}}{k^\alpha_{67}} \leq \frac{k^\alpha_4}{k^\alpha_8}$ and the only Hormesis condition holding for the perturbed curve β is $\frac{k_1}{k_5} = 0 < \frac{k^\beta_4}{k^\beta_8} < \frac{k^\beta_{23}}{k^\beta_{67}}$. This indicates that the perturbation should flip the inequality sign between the non-zero steady-state coefficient ratios. Also noting that $\frac{k^\alpha_4}{k^\alpha_8} = \frac{k^\beta_4}{k^\beta_8}$, these conditions enforce the following condition on the parameters:

$$
\begin{aligned}
\frac{r_1^\alpha r_3((r_3+r_4)(r_6+r_7)+r_5 r_7)(r^\alpha_{13}(r_{15}+r_{16})+r_{14}r_{16})}{(r_2^\alpha+r_3)(r_6+r_7)r_8 r^\alpha_{12} r_{14}r_{16}+r_1^\alpha r_3 r_5 r_7(r^\alpha_{13}(r_{15}+r_{16})+r_{14}r_{16})} &\leq 1\\
&< \frac{r_1^\beta r_3((r_3+r_4)(r_6+r_7)+r_5 r_7)(r^\beta_{13}(r_{15}+r_{16})+r_{14}r_{16})}{(r_2^\beta+r_3)(r_6+r_7)r_8 r^\beta_{12} r_{14}r_{16}+r_1^\beta r_3 r_5 r_7(r^\beta_{13}(r_{15}+r_{16})+r_{14}r_{16})}. \quad (3)
\end{aligned}
$$

This implies that the values of r_9, r_{10}, and r_{11} (free receptor externalisation, degradation, and synthesis, respectively) do not affect whether or not the response is hormetic.

Derivation of the differential.

(i) *Subdivide the curves into monotone segments.*

We derive the two critical points of the non-hormetic reference curve as:

$$
\mathcal{E}^\alpha = \left\{ \epsilon_1^\alpha = (0,0)\,, \epsilon_2^\alpha = \left(\infty, \frac{r_9 r_{11}}{r_3 r_{10}}\right) \right\}.
$$

When deriving the second critical point of the perturbed hormetic curve we comply with the

Hormesis condition by choosing the solution that contains $-\sqrt{U^\beta}$, leading to:

$$\mathcal{E}^\beta = \Bigg\{ \epsilon_1^\beta = (0,0)\,,$$

$$\epsilon_2^\beta = \Bigg(\frac{r_{13}^\beta(r_{15}+r_{16})+r_{14}r_{16}}{r_{12}^\beta r_{14}r_{16}(r_6+r_7)} \frac{r_{12}^\beta r_{14}r_{16}r_8(r_2^\beta+r_3)(r_4(r_6+r_7)+r_5r_7)+\sqrt{\underline{U^\beta}}}{r_1^\beta r_3(r_3+r_4)(r_{13}^\beta(r_{15}+r_{16})+r_{14}r_{16})-r_{12}^\beta r_{14}r_{16}r_8(r_2^\beta+r_3)},$$

$$\frac{r_{11}r_1^\beta r_9(r_{13}^\beta(r_{15}+r_{16})+r_{14}r_{16})}{r_{10}} \frac{\begin{array}{r}(r_7(r_3+r_4+r_5)+r_6(r_3+r_4))\Big(r_{12}^\beta r_{14}r_{16}r_8(r_2^\beta+r_3)(r_6+r_7)\\ +r_1^\beta r_3r_5r_7(r_{13}^\beta(r_{15}+r_{16})+r_{14}r_{16})\Big)-2(r_6+r_7)\sqrt{\underline{U^\beta}}\\ -2r_{12}^\beta r_{14}r_{16}r_8(r_2^\beta+r_3)(r_6+r_7)(r_4(r_6+r_7)+r_5r_7)\end{array}}{\begin{array}{r}\Big(r_{12}^\beta r_{14}r_{16}r_8(r_2^\beta+r_3)(r_6+r_7)+r_1^\beta r_3r_5r_7\big(r_{13}^\beta(r_{15}+r_{16})\\ +r_{14}r_{16}\big)\Big)^2-4r_{12}^\beta r_{14}r_{16}r_1^\beta r_3r_8(r_2^\beta+r_3)(r_6+r_7)\big(r_{13}^\beta(r_{15}+r_{16})\\ +r_{14}r_{16}\big)(r_4(r_6+r_7)+r_5r_7)\end{array}} \Bigg),$$

$$\epsilon_3^\beta = \left(\infty, \frac{r_9r_{11}}{r_3r_{10}}\right) \Bigg\},$$

where $\underline{U^\beta}$ denotes U^β with squared factors taken out of the square root and has the form:

$$\underline{U^\beta} = r_{12}^\beta r_{14}r_{16}r_3r_8(r_2^\beta+r_3)(r_4(r_6+r_7)+r_5r_7)\Big(-r_{12}^\beta r_{14}r_{16}r_8(r_2^\beta+r_3)(r_6+r_7)$$
$$+r_1^\beta(r_3+r_4)(r_{13}^\beta(r_{15}+r_{16})+r_{14}r_{16})(r_7(r_3+r_4+r_5)+r_6(r_3+r_4))\Big).$$

This leads to the following observations: (i) the first and last critical points of the reference and perturbed curves are identical; (ii) the last critical points depend only on the four reaction rates r_3, r_9, r_{10}, and r_{11}; and (iii) the dose component of the second critical point of the perturbed system ϵ_2^β does not depend on r_9, r_{10}, and r_{11}.

(ii) *Decide which segments to compare.*

See *Case 2* of the two dose edge differential derivations.

(iii) *Determine corresponding points in compared pairs of segments.*

See the general two dose edge differential derivations.

(iv) *Quantify the displacement of corresponding points.*

It is straightforward to see that the response differential between the reference curve and the second segment of the perturbed curve at $d \to \infty$ is always zero, independent of the

magnitude of the perturbation and of the reaction constants' values:

$$\pi_{\mathcal{R}}\left(h=0,i=2\right)=\zeta\left(h=0;0,\frac{k_4^{\alpha}}{k_8^{\alpha}}\right)-\zeta\left(h=0;\mathtt{R}_{\epsilon_2}^{\beta},\frac{k_4^{\beta}}{k_8^{\beta}}\right)=\frac{r_9r_{11}}{r_3r_{10}}-\frac{r_9r_{11}}{r_3r_{10}}=0.$$

The expressions in the previous section also allow us to identify feasible perturbations to alter the dose-response behavior. For example, if we were to design a new perturbation, different from applying a ligand with modified affinity, that again leads to a hormetic perturbed dose-response, but to a non-zero response differential, it has to target parameters r_9, r_{11},r_3, or r_{10}. However, since hormesis is not affected by r_9, r_{11}, and r_{10}, r_3 need to be perturbed.

To find the dose differential between the reference curve and the first segment of the perturbed curve with $h = 0.5$, we need to select the appropriate solution from $\mathtt{d}_{\sigma,h}^{(1,2)}$. The relevant solution for the reference curve is $\mathtt{d}_{\sigma,h}^{(1)}$ since it corresponds to the non-hormesis condition $\frac{k_1}{k_5}\leq\frac{k_{23}}{k_{67}}\leq\frac{k_4}{k_8}$. Furthermore, when choosing the relevant solution, there are two cases of interest: (i) when the solutions have different signs we take the larger (positive) solution, and (ii) when the two solutions are positive we consider the smaller solution, which corresponds to the first segment of the hormetic curve. According to Vietta's formulas, the solutions have different signs when $\frac{-k_5^{\beta}\zeta(h;x,y)}{k_4^{\beta}-k_8^{\beta}\zeta(h;x,y)}<0$, which translates to $k_4^{\beta}-k_8^{\beta}\zeta\left(h;x,y\right)>0$, indicating that the relevant larger solution is $\mathtt{d}_{\sigma,h}^{(1)}$. Accordingly, both solutions are positive when $-\frac{k_{23}^{\beta}-k_{67}^{\beta}\zeta(h;x,y)}{k_4^{\beta}-k_8^{\beta}\zeta(h;x,y)}>0$ and $\frac{-k_5^{\beta}\zeta(h;x,y)}{k_4^{\beta}-k_8^{\beta}\zeta(h;x,y)}>0$, implying $k_4^{\beta}-k_8^{\beta}\zeta\left(h;x,y\right)<0$ and $k_{23}^{\beta}-k_{67}^{\beta}\zeta\left(h;x,y\right)>0$. This satisfies the Hormesis condition and settles the smaller positive solution to be $\mathtt{d}_{\sigma,h}^{(1)}$ again.

Thus, we select the solution $\mathtt{d}_{\sigma,h}^{(1)}$ for the reference and the perturbed curve, which gives:

$$\pi_d\left(h=\frac{1}{2};i=1\right)=\log_{10}\frac{2k_4^{\beta}-k_8^{\beta}\mathtt{R}_{\epsilon_2}^{\beta}}{k_4^{\alpha}k_8^{\alpha}}\frac{k_4^{\alpha}k_{67}^{\alpha}-2k_{23}^{\alpha}k_8^{\alpha}+2k_8^{\alpha}\sqrt{W(\frac{1}{2};0,\frac{k_4^{\alpha}}{k_8^{\alpha}})}}{k_{67}^{\beta}\mathtt{R}_{\epsilon_2}^{\beta}-2k_{23}^{\beta}+2\sqrt{W(\frac{1}{2};0,\mathtt{R}_{\epsilon_2}^{\beta})}},$$

with $W(\frac{1}{2};0,y)=\left(\frac{k_{67}y}{2}-k_{23}\right)^2+k_5y\left(2k_4-k_8y\right)$.

After substituting the steady-state coefficients, we find the symbolic expression for the dose differential. By looking at the greatest common divisor of the separate terms in the numerator and denominator of the expression, again the reaction rate constants r_9, r_{10}, and r_{11} cross out. Therefore, both the dose and the response differential are invariant with respect to these parameters.

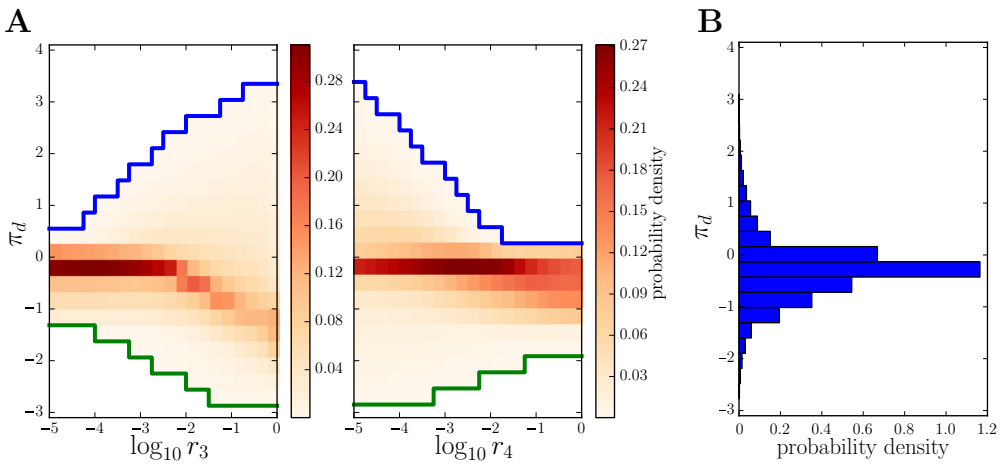

Figure S5: Numerical analysis of the two dose edge insulin model from Fig. S4A. (**A**) Profile bounds (blue – upper inner bound, green – lower inner bound) superimposed on the profile differential distribution (density) for the free parameters r_3 and r_4. (**B**) Marginal probability distribution of the dose differential magnitude. Densities were obtained using the values for the differential parameters from Fig. S4B and uniformly sampling the remaining n parameters from the parameter box $\mathcal{I} = [10^{-5}, 1]^n$; note that $n = 12$ in (B) and $n = 11$ in (A) since one additional parameter is fixed at a time.

Numerical analysis. For the insulin model with two dose edges (Fig. S4A), if we assume only the affinities of the two ligands to be known parameters, uniform sampling of the dose differential yields a few magnitudes of variability ($\widehat{\mathbb{D}_{\pi_d}} = [-2.8, 3.1]$) but a small region of most probable values with a marginal density peaked around -0.25 (Fig. S5B). The profile differential distributions in Fig. S5A show how the free parameters r_3 (receptor phosphorylation) and r_4 (receptor dephosphorylation) affect the bounds as well as the peak of the marginal distribution, revealing their potential to control the differential.

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