Supplemental Materials

S1. List of Transition Invariants

The network has 6 transition invariants (TI):

1. TI₁: $k_{bind}, k_{phos}, k_{dephos,m}$ (binding of insulin, phosphorylation, dephosphorylation on membrane)
2. TI₂: $k_{bind}, k_{phos}, k_{in,p}, k_{dephos,c}, k_{out},$ buffer (binding of insulin, phosphorylation, internalization, cytoplasmic dephosphorylation, translocation back to membrane)
3. TI₃: $k_{in}, k_{out}$ (internalization, translocation back to membrane)
4. TI₄: $k_{in,p}, k_{out,p}$ (internalization of phosphorylated insulin receptor (IR), translocation back to membrane)
5. TI₅: $k_{bind}, k_{diss}$ (extracellular binding of insulin, release of insulin)
6. TI₆: $k_{syn}, k_{deg}$ (synthesis, degradation of receptor)

Each transition is member of at least one TI, hence the network is covered by TI (CTI).

S2. Quasi-Steady-State Approximation for TI₁

TI₁ describes a cycle of reactions for the species IR, IRI, and IRIP. The corresponding dynamic system is given by

$$\frac{\partial \vec{c}}{\partial t} = \begin{pmatrix} -k_{bind} i_0 & k_{diss} & k_{dephos,m} \\ +k_{bind} i_0 & -k_{diss} - k_{phos} & 0 \\ 0 & k_{phos} & -k_{dephos,m} \end{pmatrix} \vec{c}, \quad (S1)$$

where $\vec{c} = (ir, iri, irip)^T$ denotes a vector of concentrations. The concentration of free insulin is assumed to be constant, i.e., $i = i_0$. Within the QSSA we solved the linear system

$$\frac{\partial \vec{c}}{\partial \tau} = 0 \quad (S2)$$

and obtained the steady state for the 3 concentrations

$$ir^* = \left[1 - \frac{i_0}{i_0 + i_c}\right] ir_0,$$

$$iri^* = \frac{k_{dephos,m}}{k_{phos}} \left(1 + \frac{k_{dephos,m}}{k_{phos}}\right)^{-1} \frac{i_0}{i_0 + i_c} ir_0,$$

and

$$irip^* = \left[1 - \frac{k_{dephos,m}}{k_{phos}} \left(1 + \frac{k_{dephos,m}}{k_{phos}}\right)^{-1}\right] \frac{i_0}{i_0 + i_c} ir_0 \quad (S3).$$
with the equilibrium constant

\[
i_c = \frac{k_{\text{dephos,m}}}{k_{\text{bind}}} \left( 1 + \frac{k_{\text{diss}}}{k_{\text{phos}}} \right) \left( 1 + \frac{k_{\text{dephos,m}}}{k_{\text{phos}}} \right)^{-1}.
\] (S4)

For our choice of kinetic rate constants, the insulin-binding equilibrium constant becomes \(i_c = 3.33\ \text{nM}\). Sedaghat et al. assume a fast process of phosphorylation (i.e., \(k_{\text{phos}} \gg k_{\text{diss}}\) and \(k_{\text{phos}} \gg k_{\text{dephos,m}}\)). In this case the equation

\[
i_c \approx \frac{k_{\text{dephos,m}}}{k_{\text{bind}}} \] (S5)

is a reasonable approximation. Since the ratio \(k_{\text{dephos,m}}/k_{\text{phos}}\) is less than 0.1 %, we may neglect \(iri^*\), and the formula

\[
irip^* \approx \frac{i_0}{i_0 + i_c} ir_0
\]

is sufficiently precise for practical applications.

S3. Quasi-Steady-State Approximation for TI\(_2\)

The steady-state concentrations \(ir^*, iri^*,\) and \(irip^*\) completely ignore the process of translocation of receptor into the cytoplasm and are a justifiable approximation only for a short reaction time compared to the time scale of the translocation process. The process of translocation of the activated IR into the cytoplasm \((k_{\text{in,p}})\) is member of the subnetwork defined by TI\(_2\). The ODE system of the subnetwork reads

\[
\frac{\partial \vec{c}}{\partial t} = \begin{pmatrix} 0 & 0 & 0 & k_{\text{syn}} & 0 \\ 0 & k_{\text{bind}} i_0 + k_{\text{in}} & -k_{\text{diss}} & -k_{\text{dephos,m}} & -k_{\text{out}} & 0 \\ -k_{\text{bind}} i_0 & k_{\text{diss}} + k_{\text{phos}} & 0 & 0 & 0 \\ 0 & -k_{\text{diss}} & k_{\text{dephos,m}} + k_{\text{in,p}} & 0 & -k_{\text{out,p}} \\ -k_{\text{in}} & 0 & 0 & k_{\text{out}} + k_{\text{deg}} & -k_{\text{dephos,c}} \\ 0 & 0 & -k_{\text{in,p}} & 0 & k_{\text{dephos,c}} + k_{\text{out,p}} \end{pmatrix} \vec{c} \] (S6)

with the vector of concentrations, \(\vec{c} = (ir, iri, irip, ir_{in}, irip_{in})^T\). The steady state is given by

\[
ir^* = \frac{i_0}{i_c + i_0} \left( 1 + \frac{k_{\text{diss}}}{k_{\text{phos}}} \right) \left[ k_{\text{dephos,m}}(k_{\text{out,p}} + k_{\text{dephos,c}}) \right] \left[ k_{\text{bind}}/k_{\text{in,p}} \right] \frac{k_{\text{dephos,c}}}{k_{\text{phos}} k_{\text{dephos,c}}} \frac{k_{\text{out}}}{k_{\text{dephos,c}}} \frac{i_0}{i_c + i_0},
\]

\[
iri^* = \frac{i_0}{i_c + i_0} \left[ k_{\text{dephos,m}}(k_{\text{out,p}} + k_{\text{dephos,c}}) \right] \frac{k_{\text{diss}}}{k_{\text{phos}}} \frac{k_{\text{out}}}{k_{\text{dephos,c}}} \frac{i_0}{i_c + i_0},
\]

\[
irip^* = \frac{i_0}{i_c + i_0} \frac{k_{\text{out,p}} + k_{\text{dephos,c}}}{k_{\text{phos}}} \frac{k_{\text{out}}}{k_{\text{dephos,c}}} \frac{i_0}{i_c + i_0},
\]

\[
ir_{in}^* = \frac{k_{\text{syn}}}{k_{\text{deg}}}, \quad \text{and}
\]

\[
irip_{in}^* = \frac{i_0}{i_c + i_0} \frac{k_{\text{out}}}{k_{\text{dephos,c}}} \frac{i_0}{i_c + i_0},
\]

with the constant

\[
i_c^* = \frac{k_{\text{in}}}{k_{\text{bind}}} \left[ 1 + \frac{k_{\text{dephos,m}}}{k_{\text{in,p}}} \left( 1 + \frac{k_{\text{out,p}}}{k_{\text{dephos,c}}} \right) \right] \left( 1 + \frac{k_{\text{diss}}}{k_{\text{phos}}} \right). \] (S8)
$i^\dagger_c$ is the critical insulin concentration for the internalization of receptor. For a fast phosphorylation process as postulated by Sedaghat et al., (i.e., $k_{phos} = 2.500 \text{ min}^{-1}$) a simplification of equations (S7,S8) is feasible.

We considered nonzero degradation and nonzero synthesis of the receptor, i.e., $k_{syn}, k_{deg}$, in the steady state (S7). However, the degradation and synthesis are not members of TI$_2$ but form the trivial TI$_6$. For $k_{syn} = k_{deg} = 0$ (i.e. in the case of no degradation and no synthesis), the steady-state concentration, $ir^\dagger_m$, becomes a free parameter and has to be determined by a mass conservation equation for the amount of the receptor in the cell.

For our choice of kinetic constants, we get the numerical value, $i^\dagger_c = 0.535 \text{ nM}$, for the critical insulin concentration of internalization of the IR and the steady state concentrations (S7) become

$$ir^\dagger = 0.9 \text{ pM} \times \left[ 1 - \frac{i_0}{i^\dagger_c + i_0} \right],$$

$$iri^\dagger = 0.0116 \text{ fM} \times \frac{i_0}{i^\dagger_c + i_0},$$

$$irip^\dagger = 0.143 \text{ pM} \times \frac{i_0}{i^\dagger_c + i_0},$$

$$ir^\dagger = 0.1 \text{ pM}, \text{ and}$$

$$irip^\dagger = 0.651 \text{ fM} \times \frac{i_0}{i^\dagger_c + i_0}.$$ (S9)

The steady state concentrations, $iri^\dagger$ and $irip^\dagger_m$, of the transient complexes are below experimental detection limits. The steady state concentration, $ir^\dagger_m$, of free intracellular receptor is regulated by synthesis ($k_{syn}$) and degradation ($k_{deg}$), and hence remains constant for all values of $i_0$. In the limit of small concentrations of insulin, $i_0 \to 0$, the function

$$f(i_0) = \frac{i_0}{i^\dagger_c + i_0} \quad \text{(S10)}$$

approaches zero for vanishing concentration of external insulin, i.e., $\lim_{i_0\to 0} f(i_0) = 0$. For increasing concentrations of insulin, $i_0 \to \infty$, the function $f(i_0)$ converges to 1. Since the steady-state concentrations, $iri^\dagger$, $irip^\dagger$ and $irip^\dagger_m$, are proportional to $f(i_0)$, they are zero in the basal state of the cell, i.e., in absence of extracellular insulin, $i_0 = 0$. In the process of down-regulation by insulin, the concentrations, $iri^\dagger$, $irip^\dagger$, and $irip^\dagger_m$, increase proportionally to the function $f(i_0)$ until they reach their maximal values for $i_0 \gg i^\dagger_c$. The steady-state concentration, $ir^\dagger$, of the surface receptor is proportional to $1 - f(i_0)$, and hence, $ir^\dagger$ is maximal in the basal state and drops down to zero for $i_0 \gg i^\dagger_c$.

**S4. Characteristic Eigenvalue for TI$_1$**

The characteristic eigenvalue of ODE (S1) is given by

$$\lambda_1 = -\frac{k_{bind} i_0 + k_{diss} + k_{phos} + k_{dephos,m}}{2} \left[ 1 - \sqrt{1 - \frac{4(k_{bind} i_0 (k_{phos} + k_{dephos,m}) + (k_{diss} + k_{phos}) k_{dephos,m})}{(k_{bind} i_0 + k_{diss} + k_{phos} + k_{dephos,m})^2}} \right].$$ (S11)
The simplification

\[ \lambda_1 \approx -\frac{k_{\text{phos}}(k_{\text{bind}}i_0 + k_{\text{dephos,m}})}{k_{\text{bind}}i_0 + k_{\text{phos}}} \]  

(S12)

approximates the eigenvalue, \( \lambda_1 \), within a relative precision of \( 2 \times 10^{-5} \).

**S5. Characteristic Eigenvalue for TI**

The characteristic eigenvalue of ODE (S6) is given by

\[
\begin{align*}
\lambda_2 &= -\frac{L}{2} \left[ 1 - \sqrt{1 - \frac{4(k_{\text{out}}(k_{\text{out,p}} + k_{\text{dephos,c}}) + (k_{\text{out,p}} + k_{\text{dephos,c}})K_1 + (k_{\text{out}} + k_{\text{dephos,c}})K_2)}{L^2}} \right], \\
L &= k_{\text{out,p}} + k_{\text{out}} + k_{\text{dephos,c}} + K_1 + K_2, \\
K_1 &= k_{\text{in}} \frac{i_c}{i_0 + i_c}, \quad \text{and} \\
K_2 &= k_{\text{phos}}k_{\text{in},p} \frac{i_0}{k_{\text{phos}} + k_{\text{dephos,m}}} \frac{i_0}{i_0 + i_c}.
\end{align*}
\]

**S6. Drop of Insulin and the Lambert Function**

We have abstained from discussing the development of insulin concentration with time based on the functional regimes of the Lambert function \( W \). It is easy to see that for insulin concentrations well below the critical concentration of \( i_c^\dagger = 0.535 \) nM, the differential equation simplifies to

\[
\frac{\partial i}{\partial t} = -\frac{i}{t_4},
\]

(S13)

and the insulin concentration drops down exponentially in time

\[ i(t) = i_0 e^{-t/t_4}. \]  

(S14)

In the case of a high concentration of insulin (i.e., for \( i \gg i_c^\dagger = 0.535 \) nM), the cell is maximally down-regulated, and the differential equation is given by

\[
\frac{\partial i}{\partial t} = -\frac{i_c}{t_4}.
\]

(S15)

Consequently, the consumption of insulin with constant maximal velocity leads to a linear diminishment of insulin:

\[ i(t) = i_0 - i_c \frac{t}{t_4}. \]  

(S16)

The consumption of insulin by the cell leads to an exponential drop on the time scale of \( t_4 = 5 \) h 33 min, if the insulin concentration is below the critical insulin concentration, \( i_c^\dagger = 0.535 \) nM. For insulin given in excess (i.e., for \( i \gg i_c^\dagger \)), the insulin concentration decreases linearly with a flat-angle slope of \( 0.535 \) nM/5 h 33 min.
S7. Phosphorylation Dynamics

Cedersund et al. [1] have discussed the short-term phosphorylation dynamics of the insulin receptor. They have measured a rapid transient overshoot in tyrosine phosphorylation for human adipocytes after a step increase from 0 to 0.1 µM in insulin concentration and have discussed the implication of such an “overshot” on various model structures. Cedersund et al. [2] have rejected model structures based on the zeros and complex poles of the linearized transfer function, see also Brännmark et. al. [3]. In terms of the Petri net formalism, the model structure requires a certain substructure to produce an overshot behavior. For Sedaghat et al.’s model [4] such a substructure is defined by transition invariant TI1. The Petri net approach explains the overshoot by the high concentration of phosphorylated receptor, irip\(^*\), of the meta-stable quasi-steady state associated with transition invariant TI1. Figure S1 shows the percentage of transient phosphorylated IR versus the concentration of insulin.

![Graph showing percentage of transient phosphorylated IR versus insulin concentration](image)

**Figure S1.** After a step increase in insulin concentration the concentration of phosphorylated IR approach the value irip\(^*\) of meta-stable steady state (S3). This transient high value of phosphorylated IR drops to the value \(\nu \times \text{irip}^\dagger\) of meta-stable steady state (S7) due to endocytosis and dephosphorylation of the internalized IR. Plotted is the percentage of transient phosphorylated \(\text{irip}^* - \nu \times \text{irip}^\dagger\) versus the concentration of insulin. For 0.1 µM insulin concentration, Sedaghat et al.’s model estimates an “overshoot” at in round numbers 40%.

References

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