Reaction Network Analysis of Metabolic Insulin Signaling

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Received: 9 April 2022 / Accepted: 15 September 2022 / Published online: 27 September 2022
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Abstract

Absolute concentration robustness (ACR) and concordance are novel concepts in the theory of robustness and stability within Chemical Reaction Network Theory. In this paper, we have extended Shinar and Feinberg’s reaction network analysis approach to the insulin signaling system based on recent advances in decomposing reaction networks. We have shown that the network with 20 species, 35 complexes, and 35 reactions is concordant, implying at most one positive equilibrium in each of its stoichiometric compatibility class. We have obtained the system’s finest independent decomposition consisting of 10 subnetworks, a coarsening of which reveals three subnetworks which are not only functionally but also structurally important. Utilizing the network’s deficiency-oriented coarsening, we have developed a method to determine positive equilibria for the entire network. Our analysis has also shown that the system has ACR in 8 species all coming from a deficiency zero subnetwork. Interestingly, we have shown that, for a set of rate constants, the insulin-regulated glucose transporter GLUT4 (important in glucose energy metabolism), has stable ACR.

Keywords Independent decomposition · Metabolic insulin signaling · Positive equilibria · Reaction network · Subnetwork
1 Introduction

Between 2010 and 2015, Shinar and Feinberg published a series of papers based on the novel concepts of absolute concentration robustness (ACR) and concordance, which one may view as the beginnings of a theory of robustness within Chemical Reaction Network Theory (CRNT) (Knight et al. 2015; Shinar and Feinberg 2010, 2011, 2012, 2013). ACR characterizes the invariance of the concentrations of a species at all positive equilibria of a kinetic system, and from experimental observations in *Escherichia coli* subsystems, the authors extracted sufficient (mathematical) conditions for the property. They related ACR to bifunctionality of enzymes and viewed the condition as a “structural source” (Shinar and Feinberg 2010) or “design principle” (Shinar and Feinberg 2011) of robustness. Concordance, on the other hand, is seen to indicate “architectures that, by their very nature, enforce duller, more restrictive behavior despite what might be great intricacy in the interplay of many species, even independent of values that kinetic parameters might take” (Shinar and Feinberg 2012). Shinar and Feinberg provided small models of biological systems such as the osmotic pressure response system EnvZ-OmpR of *Escherichia coli* for ACR and calcium dynamics of olfactory cilia for concordance. The goal of this paper is to explore the extension of their reaction network analysis approach to larger models of biochemical systems based on recent advances in decomposing reaction networks (Fontanil et al. 2021; Fortun and Mendoza 2021; Hernandez and De la Cruz 2021) using the example of a widely used model of the insulin signaling system (Sedaghat et al. 2002).

The insulin signaling system is an important metabolic system that, upon binding of insulin to its receptor at the cell surface, initiates the uptake of glucose into the cell. The analysis of this process in muscle cells, hepatocytes, cells of adipose tissue, and (most recently) neurons is crucial for understanding the underlying mechanisms of insulin resistance. This reduced ability of cells to use available insulin for energy metabolism is viewed as a common factor in diseases such as obesity, type 2 diabetes, metabolic syndrome, and cancer. More recently, brain insulin resistance has received increased attention from neuroscientists in connection with mild cognitive impairment and Alzheimer’s disease (AD) (Arnold et al. 2018; Neth and Craft 2017). Our studies of the metabolic aspects of AD, in fact, provided the motivation for this analysis of a mathematical model of the insulin signaling system (Villasis et al. 2022).

The complexity of the insulin signaling system, both in terms of the number of molecular components involved as well as the intricate combination of positive and negative feedback loops, clearly warrants the application of mathematical modeling and computational tools. A natural starting point for our studies of such models is the seminal work of Sedaghat et al. (2002). This widely cited work has been applied in various contexts and the authors conveniently provided WinPP source files for the system. We utilized the Hars-Tóth criterion presented in Chellaboina et al. (2009) to derive a chemical reaction network underlying the system of ordinary differential equations (ODEs) of the Sedaghat et al model, leading to its realization as a mass action system with 20 species, 35 complexes, and 35 reactions.

The first main result of our analysis of the Sedaghat et al system is that the underlying network is concordant. Concordance is a property abstracted from the continuous-flow stirred tank reactor model widely used in chemical engineering, and its occurrence in
complex biological systems is not straightforward. To date, only two smaller systems—the previously mentioned calcium signaling and the Wnt signaling (Feinberg 2019)—have been shown to have the property. Concordance has numerous structural and kinetic consequences including monostationarity for the insulin signaling system (see Sect. 2.3). A detailed discussion of concordance properties can be found in Chapter 10 of Feinberg’s recent book (Feinberg 2019).

The remaining main results are derived from the systematic use of decomposition theory.

Decomposition theory was initiated by Feinberg in his 1987 review (Feinberg 1987) where he also introduced the important concept of an independent decomposition, a decomposition wherein the stoichiometric subspace of the whole network is the direct sum of the stoichiometric subspaces of the subnetworks. He highlighted its importance by showing that in an independent decomposition the set of positive equilibria of the whole network is the intersection of the equilibria sets of the subnetworks. Hence, if a species has ACR in a subnetwork of an independent decomposition, it also has ACR in the whole network since the latter’s equilibria set is contained in that of the subnetwork. Recent results of Hernandez and De la Cruz (2021) provided a criterion and procedure for determining the existence of a nontrivial independent decomposition (the trivial independent decomposition is the network itself).

The second main result is the existence of nontrivial independent decompositions of the network. This property allows insights into structural relationships between positive equilibria of the whole network and its subnetworks. Since the algorithm from Hernandez and De la Cruz (2021) determines the finest independent decomposition and independence is invariant under decomposition coarsening, all independent decompositions can be generated from the result. We developed a MATLAB code for the said algorithm and applied it to the insulin signaling system.

Third, it is shown that the networks of three functional modules—the insulin receptor binding and recycling subsystem, the postreceptor signaling subsystem, and the GLUT4 translocation subsystem—discussed by Sedaghat et al also form an independent decomposition. These subsystems, hence, not only are functionally but also structurally significant, i.e., positive equilibria of the whole system come from equilibria of these systems. Further details can be found in Sect. 3.1.

Fourth is the existence of a large weakly reversible, deficiency zero subnetwork constituting a well-understood part of the system, which is also the source of all 8 ACR species.

For the ACR analysis, we implemented the algorithm of Fontanil et al. (2021) in MATLAB and discovered Shinar–Feinberg reaction pairs in appropriate low-deficiency subnetworks of coarsenings of the finest independent decomposition. We found that the system has ACR in 8 (out of 20) species. This restricts the variability of the positive equilibria and suggests that this “structural source of robustness” may be an important factor in the system’s overall robustness, a property which, according to a previous study by Dexter et al. (2015), is not common in biochemical systems. Overall, however, there is still a high variation in equilibria composition due to the lack of ACR species in the deficiency 7 subnetwork of the network’s deficiency-oriented decomposition. To our knowledge, this is the first large kinetic system for which an ACR assessment has been documented.
The last main result is the discovery of ACR of the essential glucose transporter GLUT4 which, coupled with adequate glucose supply, enables reliable cellular energy production. Further details are discussed in Sect. 4.3.

The paper is organized as follows: The construction of the metabolic insulin signaling reaction network, and its basic properties are described in Sect. 2. Section 3 discusses the finest nontrivial independent decomposition of the insulin signaling system and its deficiency-oriented coarsening. In Sect. 4, a brief review of relevant ACR results is followed by ACR analyses based on decompositions of the network. A short summary and the future direction of our research conclude the paper in Sect. 5. Basic concepts and notations related to CRNT, as well as some detailed computations relevant to the paper, are provided in Supplementary Material.

2 Model Realization as a Mass Action System

In this section, we present the ODE system of Sedaghat et al. (2002) and its mass action system realization. We then analyze various properties of the underlying chemical reaction network (CRN), including its positive dependency, $t$-minimality, nonconservativity, and concordance.

2.1 Insulin Signaling Model

We consider the system of ODEs in Sedaghat et al. (2002) which has only the state variables $X_2$ to $X_{21}$. We streamlined the notation to clearly identify the 35 reactions involved in the metabolic insulin signaling network (see Supplementary Material Section 2 for the description and units of the variables):

\[
\begin{align*}
\dot{X}_2 &= k_2X_3 + k_6X_5 - k_1X_2 + k_8X_6 - k_7X_2 \\
\dot{X}_3 &= k_1X_2 - k_2X_3 - k_5X_3 \\
\dot{X}_4 &= k_3X_5 - k_4X_4 + k_{10}X_7 - k_9X_4 \\
\dot{X}_5 &= k_5X_3 + k_4X_4 - k_3X_5 - k_6X_5 + k_{12}X_8 - k_{11}X_5 \\
\dot{X}_6 &= k_{13} - k_{14}X_6 + k_{15}X_7 + k_{16}X_8 + k_7X_2 - k_8X_6 \\
\dot{X}_7 &= k_9X_4 - k_{10}X_7 - k_{15}X_7 \\
\dot{X}_8 &= k_{11}X_5 - k_{12}X_8 - k_{16}X_8 \\
\dot{X}_9 &= k_{19}X_{10} - k_{17}X_9X_4 - k_{18}X_9X_5 \\
\dot{X}_{10} &= k_{17}X_9X_4 + k_{18}X_9X_5 + k_{21}X_{12} - k_{19}X_{10} - k_{20}X_{11}X_{10} \\
\dot{X}_{11} &= k_{21}X_{12} - k_{20}X_{10}X_{11} \\
\dot{X}_{12} &= k_{20}X_{10}X_{11} - k_{21}X_{12} \\
\dot{X}_{13} &= k_{22}X_{12}X_{14} + k_{24}X_{15} - k_{23}X_{13} - k_{25}X_{13} \\
\dot{X}_{14} &= k_{23}X_{13} - k_{22}X_{12}X_{14} \\
\dot{X}_{15} &= k_{25}X_{13} - k_{24}X_{15}
\end{align*}
\]
Fig. 1 Biochemical map of the insulin signaling system where $X_2, \ldots, X_{21}$ are the species of the network (see Supplementary Material Section 2 for the description and units of the variables); $k_1, \ldots, k_{35}$ are the rate constants of the reactions; and solid lines represent mass transfer reactions, while broken lines represent regulatory reactions.

\[
\begin{align*}
\dot{X}_{16} &= k_{27} X_{17} - k_{26} X_{13} X_{16} \\
\dot{X}_{17} &= k_{26} X_{13} X_{16} - k_{27} X_{17} \\
\dot{X}_{18} &= k_{29} X_{19} - k_{28} X_{13} X_{18} \\
\dot{X}_{19} &= k_{28} X_{13} X_{18} - k_{29} X_{19} \\
\dot{X}_{20} &= k_{31} X_{21} - k_{30} X_{20} - k_{32} X_{17} X_{20} - k_{33} X_{19} X_{20} + k_{34} - k_{35} X_{20} \\
\dot{X}_{21} &= k_{30} X_{20} + k_{32} X_{17} X_{20} + k_{33} X_{19} X_{20} - k_{31} X_{21}
\end{align*}
\]

Sedaghat et al. used a biochemical map to derive the ODE system, and an incomplete extract of the map is shown in their paper. For better visual orientation of the reader, we combined information from the paper’s text and the ODE system to reconstruct the complete biochemical map shown in Fig. 1.

### 2.2 Application of the Hars-Tóth Criterion for Mass Action System Realization

Theorem 4 of Chellaboina et al. (2009), called the Hars-Tóth criterion, guarantees a mass action system realization such that the ODE system with species $X_1, \ldots, X_m$ and $r$ reactions is given by $\dot{X} = f(X) = (B - A)^T (k \circ X^A)$ where $\circ$ represents component-wise multiplication, $A = [a_{ij}]$, $B = [b_{ij}]$, $k = [k_1, \ldots, k_r]^T$, $X = [X_1, \ldots, X_m]^T$, $k \circ X^A$.
and $X^A$ is the element of $\mathbb{R}^m$ with $i$th component $X_1^{a_{i1}} \cdots X_m^{a_{im}}$ for $i = 1, \ldots, r$ and $j = 1, \ldots, m$.

In the insulin signaling model $\dot{X} = f(X)$ in Sect. 2.1, it is easy to check that $f_j(X_2, \ldots, X_{j-1}, 0, X_{j+1}, \ldots, X_{21})$ is a multivariate polynomial with nonnegative coefficients for each $j = 2, \ldots, 21$. For instance, for $j = 2$ and $j = 3$, we have

$$f_2(0, X_3, X_4, \ldots, X_{21}) = k_2 X_3 + k_6 X_5 + k_8 X_6$$
$$f_3(X_2, 0, X_4, \ldots, X_{21}) = k_1 X_2.$$

Similar computations for $j = 4, \ldots, 21$ yield the same conclusion. Therefore, by the Hars-Tóth criterion, there is a reaction network of the form $AX \stackrel{k}{\rightarrow} BX$ such that $f(X) = (B - A)^\top (k \circ X^A)$ where $A$ and $B$ have nonnegative integer entries (see Supplementary Material Section 3 for the detailed computation). The CRN corresponding to the insulin signaling model is:

$$R_1 : X_2 \rightarrow X_3$$
$$R_2 : X_3 \rightarrow X_2$$
$$R_3 : X_5 \rightarrow X_4$$
$$R_4 : X_4 \rightarrow X_5$$
$$R_5 : X_3 \rightarrow X_5$$
$$R_6 : X_5 \rightarrow X_2$$
$$R_7 : X_2 \rightarrow X_6$$
$$R_8 : X_6 \rightarrow X_2$$
$$R_9 : X_4 \rightarrow X_7$$
$$R_{10} : X_7 \rightarrow X_4$$
$$R_{11} : X_5 \rightarrow X_8$$
$$R_{12} : X_8 \rightarrow X_5$$
$$R_{13} : 0 \rightarrow X_6$$
$$R_{14} : X_6 \rightarrow 0$$
$$R_{15} : X_7 \rightarrow X_6$$
$$R_{16} : X_8 \rightarrow X_6$$
$$R_{17} : X_9 + X_4 \rightarrow X_{10} + X_4$$
$$R_{18} : X_9 + X_5 \rightarrow X_{10} + X_5$$
$$R_{19} : X_{10} \rightarrow X_9$$
$$R_{20} : X_{10} + X_{11} \rightarrow X_{12}$$
$$R_{21} : X_{12} \rightarrow X_{10} + X_{11}$$
$$R_{22} : X_{14} + X_{12} \rightarrow X_{13} + X_{12}$$
$$R_{23} : X_{13} \rightarrow X_{14}$$
$$R_{24} : X_{15} \rightarrow X_{13}$$
Table 1  Network numbers of \( \mathcal{N} \)

| Network numbers                          |   |
|------------------------------------------|---|
| Species                                  | \( m \) | 20 |
| Complexes                                | \( n \) | 35 |
| Reactant complexes                       | \( n_r \) | 24 |
| Reversible reactions                     | \( r_{\text{rev}} \) | 10 |
| Irreversible reactions                   | \( r_{\text{irrev}} \) | 15 |
| Reactions                                | \( r \) | 35 |
| Linkage classes                          | \( \ell \) | 13 |
| Strong linkage classes                   | \( s\ell \) | 24 |
| Terminal strong linkage classes          | \( t \) | 13 |
| Rank                                     | \( s \) | 15 |
| Reactant rank                            | \( q \) | 20 |
| Deficiency                               | \( \delta \) | 7 |
| Reactant deficiency                      | \( \delta_p \) | 4 |

\[
\begin{align*}
R_{25} : X_{13} & \rightarrow X_{15} \\
R_{26} : X_{16} + X_{13} & \rightarrow X_{17} + X_{13} \\
R_{27} : X_{17} & \rightarrow X_{16} \\
R_{28} : X_{18} + X_{13} & \rightarrow X_{19} + X_{13} \\
R_{29} : X_{19} & \rightarrow X_{18} \\
R_{30} : X_{20} & \rightarrow X_{21} \\
R_{31} : X_{21} & \rightarrow X_{20} \\
R_{32} : X_{20} + X_{17} & \rightarrow X_{21} + X_{17} \\
R_{33} : X_{20} + X_{19} & \rightarrow X_{21} + X_{19} \\
R_{34} : 0 & \rightarrow X_{20} \\
R_{35} : X_{20} & \rightarrow 0.
\end{align*}
\]

### 2.3 CRNT Analysis of the Mass Action System: Key Basic Properties

From this point onward, we denote the CRN constructed in the previous section for the metabolic insulin signaling network as \( \mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R}) \) with mass action kinetics \( K \), stoichiometric subspace \( S \), set of species \( \mathcal{S} = \{ X_2, \ldots, X_{21} \} \), set of complexes \( \mathcal{C} \), and set of reactions \( \mathcal{R} = \{ R_1, \ldots, R_{35} \} \).

Table 1 presents the network numbers of \( \mathcal{N} \). These numbers provide a cursory analysis of the structural and dynamical properties of the CRN. Properties inferred in Table 1 include nonweak reversibility (since \( s\ell = 24 \neq 13 = \ell \)), \( t \)-minimality (since \( t = 13 = \ell \)), branching (since \( r = 35 > 24 = n_r \)), and nonpoint terminality and noncycle terminality (since \( t \neq n - n_r = 11 \) and \( n - n_r \neq 0 \), respectively).

Recall that deficiency measures the linear dependence of the network’s reactions, i.e., the higher the deficiency, the higher the linear dependence. The insulin signaling system is of deficiency \( \delta = 7 \), indicative of high complexity of the network.
2.3.1 Positive Dependency

CRNToolbox (Feinberg et al. 2018) results show that the metabolic insulin signaling network is positive dependent, implying that a set of rate constants exists for which the mass action system can admit a positive equilibrium.

Proposition 1 shows the key implication of positive dependency in a general form for rate constant-interaction map decomposable (RID) kinetic systems (a kinetics $K$ is RID if it assigns to each $i$th reaction a function $K_i(x) = k_i I_i(X)$ with rate constant $k_i > 0$ and interaction map $I_i(X) \in \mathbb{R}^{|S|}$ for $X \in \mathbb{R}^{|S|}_{\geq 0}$). More information about RID kinetic systems can be found in Nazareno et al. (2019) where they were introduced. The proposition with the implication of positive dependency was shown for mass action systems by Feinberg (1987).

**Proposition 1** For any RID kinetics $K$ on a positive dependent network $\mathcal{N}$, there are rate constants such that the set of positive equilibria $E^+ (\mathcal{N}, K) \neq \emptyset$.

**Proof** Since $\mathcal{N}$ is positive dependent, then for each reaction $i : C_i \rightarrow C'_i$ there is a positive number $\alpha_i$ such that

$$\sum_i \alpha_i (C'_i - C_i) = 0.$$  

For a positive vector $X^*$, $I_i(X^*) > 0$ by definition of RID kinetics. Set $k_i^* = \frac{\alpha_i}{I_i(X^*)}$. Then

$$f(X^*) = \sum_i K_i(X^*)(C'_i - C_i) = \sum_i k_i^* I_i(X^*)(C'_i - C_i) = \sum_i \alpha_i (C'_i - C_i) = 0,$$

i.e., $X^* \in E^+ (\mathcal{N}, K)$. \qed

**Remark 1** Proposition 1 is a generalization of Lemma 3.5.3 of Feinberg (2019).

2.3.2 $t$-minimality

Table 1 shows that there is only one terminal strong linkage class in each linkage class since $t = 13 = \ell$, i.e., the network is $t$-minimal. Feinberg and Horn (1977) showed that this implies the coincidence of the kinetic and stoichiometric subspaces.

The noncoincidence of the kinetic and stoichiometric subspaces is closely related to the degeneracy of equilibria. In Feliu and Wiuf (2012), the authors showed that if the two subspaces differ, then all equilibria are degenerate. In his book, Feinberg
describes anomalies that can occur if the two subspaces do not coincide (Section 3.A.1 of Feinberg (2019)).

Thus, the \( t \)-minimality of the metabolic insulin signaling network is a necessary condition for the existence of nondegenerate equilibria as detailed in Remark 4.11 of Feliu and Wiuf (2012).

2.3.3 Nonconservativity

Recall that a reaction network \((\mathcal{S}, C, R)\) is conservative whenever the orthogonal complement \(S^\perp\) of its stoichiometric subspace \(S\) contains a strictly positive member of \(\mathbb{R}^{\mathcal{S}}\), i.e., \(S^\perp \cap \mathbb{R}_{>0}^\mathcal{S} \neq \emptyset\). Otherwise, the network is called nonconservative.

The metabolic insulin signaling network has \(m = 20\) species and rank \(s = 15\). Thus, \(S^\perp\) has dimension 5. A basis for \(S^\perp\) is the set
\[
\begin{align*}
&\begin{bmatrix}0, 0, 0, 0, 0, 0, 0, 1, 1, -1, 0, 0, 0, 0, 0, 0, 0, 0, 0\end{bmatrix}^T, \\
&\begin{bmatrix}0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 0, 0, 0, 0, 0, 0, 0\end{bmatrix}^T, \\
&\begin{bmatrix}0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 0, 0, 0, 0, 0\end{bmatrix}^T, \\
&\begin{bmatrix}0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 0, 0, 0\end{bmatrix}^T, \\
&\begin{bmatrix}0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 0, 0, 0\end{bmatrix}^T.
\end{align*}
\]

which implies that \(S^\perp \cap \mathbb{R}_{>0}^\mathcal{S} = \emptyset\). Therefore, the metabolic insulin signaling network is nonconservative. The positive implications of this property for ACR will be discussed in Sect. 4.

2.3.4 Concordance

The most important specific result of the CRNT analysis is the network’s concordance.

Concordance is closely related to weakly monotonic kinetics as shown by Proposition 4.8 of Shinar and Feinberg (2012). It shows that, for any weakly monotonic kinetic system, injectivity (and, therefore, the absence of distinct stoichiometrically compatible equilibria, at least one of which is positive) can be precluded merely by establishing concordance of the underlying reaction network. The converse of the said proposition is not true, i.e., there can be a weakly monotonic kinetic system (in fact, a mass action system) that is injective even when its underlying reaction network is not concordant.

Theorem 4.11 of Shinar and Feinberg (2012) shows that the class of concordant networks is precisely the class of networks that are injective for every assignment of a weakly monotonic kinetics.

In summary, concordance implies injectivity (of the mass action kinetics) and hence monostationarity, i.e., there is at most one positive equilibrium in each stoichiometric compatibility class. We ran the Concordance Test in the CRNTToolbox which showed that the metabolic insulin signaling network is concordant. Running the Mass Action Injectivity Test and the Higher Deficiency Test confirms that the kinetics of the insulin signaling system is injective and that the system is monostationary, respectively.
3 Independent Decompositions of the Insulin Signaling System

In this section, we present the finest nontrivial independent decomposition of the metabolic insulin signaling network. We also present a deficiency-oriented coarsening of the decomposition which will be useful in the analysis of the network’s ACR.

3.1 The Finest Nontrivial Independent Decomposition

Hernandez and De la Cruz (2021) provide an algorithm for constructing an independent decomposition. Their procedure utilizes a coordinate graph that represents the network using the reaction vectors of the CRN. A nontrivial independent decomposition is generated if the coordinate graph is not connected and, in this case, each connected component of the graph constitutes a partition of the set of reaction vectors of the CRN. Following their procedure, we developed a MATLAB code to run the said algorithm and got the independent decomposition $N = \{R_1, \ldots, R_{35}\}$ consisting of 10 subnetworks:

\[
\begin{align*}
N_1 &= \{R_1, \ldots, R_{12}, R_{15}, R_{16}\} \\
N_2 &= \{R_{13}, R_{14}\} \\
N_3 &= \{R_{17}, R_{18}, R_{19}\} \\
N_4 &= \{R_{20}, R_{21}\} \\
N_5 &= \{R_{22}, R_{23}\} \\
N_6 &= \{R_{24}, R_{25}\} \\
N_7 &= \{R_{26}, R_{27}\} \\
N_8 &= \{R_{28}, R_{29}\} \\
N_9 &= \{R_{30}, \ldots, R_{33}\} \\
N_{10} &= \{R_{34}, R_{35}\}.
\end{align*}
\]

Furthermore, the independent decomposition obtained is precisely the finest independent decomposition of $\mathcal{N}$ (Hernandez et al. 2022). The network numbers of the subnetworks are presented in Table 2.

Consider the coarsening $\mathcal{N} = N_1^* \cup N_2^* \cup N_3^*$ where $N_1^* = N_1 \cup N_2$, $N_2^* = N_3 \cup \cdots \cup N_8$, and $N_3^* = N_9 \cup N_{10}$. This decomposition shows the three subsystems considered by Sedaghat et al in the construction of their system: the insulin receptor binding and recycling subsystem ($N_1^*$), the postreceptor signaling subsystem ($N_2^*$), and the GLUT4 translocation subsystem ($N_3^*$). It is significant to note that CRNT analysis using independent decomposition reveals that these subnetworks of the insulin signaling system are not just functionally but also structurally important, i.e., positive equilibria of the whole system come from equilibria of these systems.

Recall that a decomposition is said to be bi-independent if it is both independent and incidence independent.

**Proposition 2** The decomposition $\mathcal{N} = N_1 \cup \cdots \cup N_{10}$ is bi-independent.
### Table 2  Network numbers of the subnetworks $N_i$ of the finest independent decomposition of $N$

| Network numbers | $N_1$ | $N_2$ | $N_3$ | $N_4$ | $N_5$ | $N_6$ | $N_7$ | $N_8$ | $N_9$ | $N_{10}$ |
|-----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|---------|
| Species         | $m$   | 7     | 1     | 4     | 3     | 3     | 2     | 3     | 3     | 4       | 1       |
| Complexes       | $n$   | 7     | 2     | 6     | 2     | 4     | 2     | 4     | 6     | 2       |
| Reactant complexes | $n_r$ | 7     | 2     | 3     | 2     | 2     | 2     | 2     | 4     | 2       |
| Reversible reactions | $r_{rev}$ | 5     | 1     | 0     | 0     | 0     | 1     | 0     | 0     | 1       | 1       |
| Irreversible reactions | $r_{irrev}$ | 4     | 0     | 3     | 0     | 2     | 0     | 2     | 2     | 0       |
| Reactions       | $r$   | 14    | 2     | 3     | 2     | 2     | 2     | 2     | 2     | 4       | 2       |
| Linkage classes | $\ell$ | 1     | 1     | 6     | 1     | 4     | 1     | 4     | 4     | 5       | 1       |
| Strong linkage classes | $s\ell$ | 1     | 1     | 6     | 1     | 4     | 1     | 4     | 4     | 5       | 1       |
| Terminal strong linkage classes | $t$ | 1     | 1     | 3     | 1     | 2     | 1     | 2     | 2     | 3       | 1       |
| Rank            | $s$   | 6     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1       | 1       |
| Reactant rank   | $q$   | 7     | 1     | 3     | 2     | 2     | 2     | 2     | 2     | 4       | 1       |
| Deficiency      | $\delta$ | 0     | 0     | 2     | 0     | 1     | 0     | 1     | 1     | 2       | 0       |
| Reactant deficiency | $\delta_p$ | 0     | 1     | 0     | 0     | 0     | 0     | 0     | 0     | 1       |
Table 3 Network numbers of the subnetworks of the deficiency-oriented coarsening $N = N_A \cup N_B$

| Network numbers | $N_A$ | $N_B$ |
|-----------------|-------|-------|
| Species         | $m$   | 13    | 13    |
| Complexes       | $n$   | 13    | 24    |
| Reactant complexes | $n_r$ | 13    | 13    |
| Reversible reactions | $r_{rev}$ | 9    | 1     |
| Irreversible reactions | $r_{irrev}$ | 4    | 11    |
| Reactions       | $r$   | 22    | 13    |
| Linkage classes | $\ell$ | 3     | 12    |
| Strong linkage classes | $s\ell$ | 3    | 23    |
| Terminal strong linkage classes | $t$ | 3     | 12    |
| Rank            | $s$   | 10    | 5     |
| Reactant rank   | $q$   | 12    | 11    |
| Deficiency      | $\delta$ | 0    | 7     |
| Reactant deficiency | $\delta_p$ | 1    | 2     |

**Proof** Table 2 shows that $\delta = \delta_1 + \cdots + \delta_{10}$ where $\delta_i$ is the deficiency of subnetwork $N_i$. Hence, by Proposition 8 of Fariñas et al. (2021), the decomposition is bi-independent.

Note that since Proposition 7 of Fariñas et al. (2021) implies that every coarsening is also incidence independent, then every coarsening is bi-independent as well.

### 3.2 Deficiency-Oriented Coarsening

We next consider a deficiency-oriented coarsening of the independent decomposition. The deficiency-oriented coarsening is $N = N_A \cup N_B$ where $N_A = N_1 \cup N_2 \cup N_4 \cup N_6 \cup N_{10}$ and $N_B = N_3 \cup N_5 \cup N_7 \cup N_8 \cup N_9$. Let $S_i$, $C_i$, $K_i$, and $S_i$ be the set of species, set of complexes, kinetics, and stoichiometric subspace, respectively, of $N_i$ for $i = A, B$.

In $N_A$, we consider together all subnetworks of the finest independent decomposition which have a deficiency of 0. On the other hand, we put together all nonzero-deficiency subnetworks in $N_B$. The network numbers of $N_A$ and $N_B$ are presented in Table 3. Note that the set of common species of the subnetworks is $S_A \cap S_B = \{X_4, X_5, X_{10}, X_{12}, X_{13}, X_{20}\}$, while its set of common complexes $C_A \cap C_B = \{X_{13}, X_{20}\}$.

The two subnetworks contrast in further properties besides deficiency: $N_A$ is weakly reversible and nonconservative, while $N_B$ is not weakly reversible and conservative. However, they are both $r$-minimal and concordant (hence monostationary). The Deficiency Zero Theorem for mass action systems, in fact, guarantees that $N_A$ has a unique complex balanced equilibrium in every stoichiometric compatibility class. Because $N_B$ is not weakly reversible, it has no complex balanced equilibria and there may be stoichiometric compatibility classes without an equilibrium.
Lemma 3 Let $\mathcal{N} = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a CRN. If $\mathcal{N} = \mathcal{N}_1 \cup \mathcal{N}_2$ is an independent decomposition of $\mathcal{N}$ with $\mathcal{S}_i$, $S_i$, and $K_i$ the set of species, stoichiometric subspace, and kinetics, respectively, of $\mathcal{N}_i$ for $i = 1, 2$, then:

(i) If $\mathcal{S}_1 \cap \mathcal{S}_2 = \emptyset$, then for $i = 1, 2$, the projection maps $p_i : \mathbb{R}^{\mathcal{S}} \rightarrow \mathbb{R}^{\mathcal{S}_i}$ induce an isomorphism of $\mathbb{R}^{\mathcal{S} / S} \rightarrow \mathbb{R}^{\mathcal{S}_i / S_i} \times \mathbb{R}^{\mathcal{S}_2 / S_2}$ where $\mathcal{S}_1 \cup \mathcal{S}_2 = \mathcal{S}$; and

(ii) If $\mathcal{S}_1 \cap \mathcal{S}_2 \neq \emptyset$, let $p_{\mathcal{S}} : \mathbb{R}^{\mathcal{S}} \rightarrow \mathbb{R}^{\mathcal{S} / S}$ be the projection to $\mathcal{S} / S := \mathcal{S}_1 \cap \mathcal{S}_2$. Then $x \in E_+(\mathcal{N}_i, K_i)$ is in $E_+(\mathcal{N}, K)$ if and only if $p_{\mathcal{S}}(x) \in p_{\mathcal{S}_i}(E_+(\mathcal{N}_j, K_j))$, $j \neq i$.

**Proof** (i) Let $p(x + S) = (p_1(x) + S_1, p_2(x) + S_2)$. It is well-defined because $x - x' \in S \Rightarrow p_1(x) - p_1(x') = p_1(x - x') \in S_1$. Since $\mathcal{S}_1 \cap \mathcal{S}_2 = \emptyset, x = p_1(x) + p_2(x) \in S$ if the projections are in $S_1$ and $S_2$, respectively, showing the injectivity. Furthermore, the number of species $m_1 + m_2 = m$ (since $\mathcal{S}_1 \cap \mathcal{S}_2 = \emptyset$) and the rank $s_1 + s_2 = s$ (due to direct sum). Hence, the domain and codomain have the same dimensions, showing the isomorphism.

(ii) This follows directly from Feinberg’s decomposition theorem that if a decomposition is independent, then $E_+(\mathcal{N}, K) = E_+(\mathcal{N}_1, K_1) \cap E_+(\mathcal{N}_2, K_2)$.

**Remark 2**

1. Statement (i) of Lemma 3 can be generalized to the case of $\mathcal{S}_1 \cap \mathcal{S}_2 \neq \emptyset$, but the isomorphism obtained is not directly relevant to our equilibria analysis. Hence, we have relegated it to Section 4 of the Supplementary Material.

2. Although statement (ii) of Lemma 3 is an easy consequence of Feinberg’s decomposition theorem, it turns out to be useful in cases where at least one of the subnetworks has a well-understood set of positive equilibria, as in the case of the metabolic insulin signaling network. This is shown in Proposition 4 and Example 1.

**Proposition 4** Let $(\mathcal{N}, K)$ be the metabolic insulin signaling network and $\mathcal{N} = \mathcal{N}_A \cup \mathcal{N}_B$ its deficiency-oriented decomposition. Then:

(i) The map $\epsilon : E_+(\mathcal{N}, K) \rightarrow \mathbb{R}^{\mathcal{S} / S}$ given by $\epsilon(x) := x + S$ is injective.

(ii) The map $\epsilon_A : E_+(\mathcal{N}_A, K_A) \rightarrow \mathbb{R}^{\mathcal{S} / S}$ given by $\epsilon_A(x) := x + S$ is surjective.

(iii) $A_S + S \in \text{Im}(\epsilon)$ if and only if there is $x \in \epsilon_A^{-1}(z + S)$ such that $p_{\mathcal{S}}(x) \in p_{\mathcal{S}_i}(E_+(\mathcal{N}_j, K_j))$.

**Proof** (i) is equivalent to the statement that $(\mathcal{N}, K)$ is monostationary. (ii) follows from the fact that the map is the composition of $\epsilon_A : E_+(\mathcal{N}, K) \rightarrow \mathbb{R}^{\mathcal{S} / S_A}$ (surjective due to the Deficiency Zero Theorem) and the surjective map $x + S_A \rightarrow x + S$. Finally, (iii) is the application of statement (ii) of Lemma 3 to the metabolic insulin signaling network.

**Example 1** The following example illustrates the use of statement (iii) of Proposition 4.

First, we express the positive equilibria of $\mathcal{N}_A$ in terms of the rate constants:

$$X_2 = \frac{BCDF - AECG - BCDF + ACDF}{A^2EG + ABD - A^2DH}$$
\[ X_3 = \frac{k_1}{k_2 + k_5} X_2 \]
\[ X_4 = \frac{C D E F}{A D E G + B D^2 F - A D^2 H} \]
\[ X_5 = \frac{A D H - B D F - A E G}{C D F} \]
\[ X_6 = \frac{k_{13}}{k_{14}} \]
\[ X_7 = \frac{k_9}{k_{10} + k_{15}} X_4 \]
\[ X_8 = \frac{k_{11}}{k_{12} + k_{16}} X_5 \]
\[ X_{12} = \frac{k_{20}}{k_{21}} X_10 X_{11} \]
\[ X_{15} = \frac{k_{25}}{k_{24}} X_{13} \]
\[ X_{20} = \frac{k_{34}}{k_{35}} \]

where

\[ A = \frac{k_1 k_2}{k_2 + k_5} - k_1 - k_7 \]
\[ B = k_6 \]
\[ C = \frac{k_8 k_{13}}{k_{14}} \]
\[ D = \frac{k_9 k_{10}}{k_{10} + k_{15}} - k_4 - k_9 \]
\[ E = k_3 \]
\[ F = \frac{k_1 k_5}{k_2 + k_5} \]
\[ G = k_4 \]
\[ H = \frac{k_{11} k_{12}}{k_{12} + k_{16}} - k_3 - k_6 - k_{11}. \]

Observe that in subnetwork \( \mathcal{N}_A \), the equilibria of \( X_{10}, X_{11}, \) and \( X_{13} \) are independent of the rate constants. Note also that there are restrictions on the rate constant values since the \( X_i \)'s have to be positive. Using the rate constants from Sedaghat et al. (2002), we obtain a positive equilibrium for \( \mathcal{N}_A \):

\[ X_2 = 0.3700 \]
\[ X_3 = 8.8788 \times 10^{-4} \]
\[ X_4 = 3.2844 \]
\[ X_5 = 10.9491 \\
X_6 = 10.0 \\
X_7 = 0.0150 \\
X_8 = 0.0499 \\
X_{10} = 7.0929 \times 10^{-27} \\
X_{11} = 0.00105 \\
X_{12} = 5.2614 \times 10^{-19} \\
X_{13} = 0.3100 \\
X_{15} = 0.2900 \\
X_{20} = 96. \\
\]

Next, we substitute the values of the species common to \( \mathcal{N}_A \) and \( \mathcal{N}_B \) into the ODEs for \( \mathcal{N}_B \) and look for a positive solution (this implements statement (iii) of Proposition 4):

\[
\dot{X}_9 = k_{19}X_{10} - k_{17}X_9X_4 - k_{18}X_9X_5 \\
\dot{X}_{10} = k_{17}X_9X_4 + k_{18}X_9X_5 - k_{19}X_{10} \\
\dot{X}_{13} = k_{22}X_{12}X_{14} - k_{23}X_{13} \\
\dot{X}_{14} = k_{23}X_{13} - k_{22}X_{12}X_{14} \\
\dot{X}_{16} = k_{27}X_{17} - k_{26}X_{13}X_{16} \\
\dot{X}_{17} = k_{26}X_{13}X_{16} - k_{27}X_{17} \\
\dot{X}_{18} = k_{29}X_{19} - k_{28}X_{13}X_{18} \\
\dot{X}_{19} = k_{28}X_{13}X_{18} - k_{29}X_{19} \\
\dot{X}_{20} = k_{31}X_{21} - k_{30}X_{20} - k_{32}X_{17}X_{20} - k_{33}X_{19}X_{20} \\
\dot{X}_{21} = k_{30}X_{20} + k_{32}X_{17}X_{20} + k_{33}X_{19}X_{20} - k_{31}X_{21} \\
\]

Solving \( \dot{X} = 0 \), we get the following equilibrium for the other species in \( \mathcal{N}_B \):

\[
X_9 = 1.5 \times 10^{-40} \\
X_{14} = 99.3 \\
X_{17} = (5.1269 \times 10^{-9})X_{16} \\
X_{19} = (5.1269 \times 10^{-9})X_{18} \\
X_{21} = (6.7676 \times 10^{-9})X_{16} + (2.7070 \times 10^{-8})X_{18} + 4.0 \\
\]

Note that the equilibrium values of \( X_{17} \), \( X_{19} \), and \( X_{21} \) depend on any values of \( X_{16} \) and \( X_{18} \). If we (randomly) choose \( X_{16} = 99.9 \) and \( X_{18} = 99.9 \), we get the following:

\[
X_{17} = 5.1218 \times 10^{-7} \\
\]
Remark 3 Example 1 provides an alternative method for solving for positive equilibria using smaller subnetworks instead of the (more complex) entire network.

4 ACR of Species in Insulin Signaling

ACR denotes the invariance of the concentrations of a species at all positive equilibria of a kinetic system. Shinar and Feinberg introduced the concept in 2010 (Shinar and Feinberg 2010) and, from experimental observations in *Escherichia coli* subsystems, extracted sufficient (mathematical) conditions for the property. In this section, after a brief review of relevant results on ACR, we present an analysis of the property in the insulin signaling system.

4.1 Absolute Concentration Robustness

A pair of reactant complexes \( \{C, C'\} \) is a Shinar–Feinberg pair (SF-pair) in species \( X \) if their kinetic order vectors (i.e., the corresponding row in the kinetic order matrix) differ only in \( X \). In mass action systems, the stoichiometric coefficients of the complexes play the role of the kinetic order vectors.

An SF-pair is called nonterminal if both complexes are not in terminal strong linkage classes. The pair is said to be linked if both complexes are in a linkage class.

Proposition 5.7 of Lao et al. (2022) presents a framework for ACR using SF-pairs. A special case of condition (ii) of the said proposition is any weakly reversible power law system with reactant-determined kinetics, zero deficiency, and a linked SF-pair in \( X \). A proof of ACR in \( X \) in this case was already provided in Theorem 6 in Appendix of Fortun and Mendoza (2021). We refer the reader to Lao et al. (2022) for the detailed discussion of positive equilibria log-parameterized systems and a proof of the general framework. We apply Proposition 5.7 of Lao et al. (2022) only in the special case of the deficiency zero subnetwork of the metabolic insulin signaling network.

We will often call subnetworks with the property (i) or (ii) of Proposition 5.7 of Lao et al. (2022) “(low deficiency) ACR building blocks.” A computational approach to the framework was developed in Fontanil et al. (2021). For the ACR analysis, we implemented this algorithm using MATLAB.

Another theorem that we will apply to the ACR analysis of the metabolic insulin signaling network is Theorem 5.5 of Meshkat et al. (2021) regarding the stable ACR for one-dimensional networks which we will refer to as the “Meshkat et al criterion.” As defined by Meshkat et al. (2021), a kinetic system has stable ACR in a species \( X \) (for a set of rate constants) if all the steady states of the kinetic system (for the set of rate constants) are stable.

Remark 4 The sufficient conditions for ACR in a species in the works of Lao et al. (2022) and Meshkat et al. (2021) establish the property for all rate constants for which the system has a positive equilibrium. Meshkat et al have proposed the convention of
“vacuous ACR” for the case in which no positive equilibrium exists: in such a scenario, ACR in all species is assumed. This enables the more convenient terminology that the above sufficient conditions provide ACR “for all rate constants” and we adopt this terminology in the following analysis.

4.2 ACR Analysis of Rank 1 Subnetworks of the Finest Independent Decomposition

The finest independent decomposition using the Hernandez–De la Cruz algorithm consists of one subnetwork of rank 6 (\(\mathcal{N}_1\)) and 9 subnetworks of rank 1 (\(\mathcal{N}_2, \ldots, \mathcal{N}_{10}\)) (see Table 2). It is natural to first try to apply the ACR criterion of Meshkat et al to the latter set of subnetworks.

Condition 2(b) of the Meshkat et al criterion says that all reactions, taken pairwise, must be SF-pairs in species \(X_i\). Table 4 shows the result of the verification.

| Subnetwork | Reactions   | Non-SF-pair, e.g., | 2(b) satisfied for species \(X_i\) |
|------------|-------------|-------------------|---------------------------------|
| \(\mathcal{N}_2\) | \(R_{13}, R_{14}\) | None | Yes for \(X_6\) |
| \(\mathcal{N}_3\) | \(R_{17}, R_{18}, R_{19}\) | \(R_{17}, R_{18}\) | No |
| \(\mathcal{N}_4\) | \(R_{20}, R_{21}\) | \(R_{20}, R_{21}\) | No |
| \(\mathcal{N}_5\) | \(R_{22}, R_{23}\) | \(R_{22}, R_{23}\) | No |
| \(\mathcal{N}_6\) | \(R_{24}, R_{25}\) | \(R_{24}, R_{25}\) | No |
| \(\mathcal{N}_7\) | \(R_{26}, R_{27}\) | \(R_{26}, R_{27}\) | No |
| \(\mathcal{N}_8\) | \(R_{28}, R_{29}\) | \(R_{28}, R_{29}\) | No |
| \(\mathcal{N}_9\) | \(R_{30}, R_{31}, R_{32}, R_{33}\) | \(R_{30}, R_{31}\) | No |
| \(\mathcal{N}_{10}\) | \(R_{34}, R_{35}\) | None | Yes for \(X_{20}\) |

Remark 5

1. The Meshkat et al criterion ensures stability of the equilibria only within the subnetworks and not for the whole network, so we cannot infer any claim about the property for \(X_6\) and \(X_{20}\).
2. In a similar vein, non-ACR for a species in the subnetwork does not necessarily imply non-ACR in the whole network since the set of positive equilibria of the latter is generally smaller than that of the former.
4.3 ACR Analysis of Subnetworks in the Deficiency-Oriented Independent Decomposition

Implementing the algorithm of Fontanil et al. (2021) and using Proposition 5.7 of Lao et al. (2022), we discover Shinar–Feinberg reaction pairs in appropriate low-deficiency subnetworks of coarsenings of the finest independent decomposition in Sect. 3.1. We find that the system has ACR in 8 species (from the zero-deficiency building blocks): $X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_{20}$. The other species are not identified as having ACR because the reactant complexes of their associated SF-pairs are not nonterminal in the subnetwork generated by the building block. Table 5 provides an overview.

It is particularly significant that the system’s output to glucose energy metabolism ($X_{20} =$ intracellular GLUT4) has ACR. GLUT4 is a key transporter of glucose into neurons. Under healthy conditions (i.e., no insulin resistance), the insulin signaling system works such that GLUT4, which determines the amount of glucose transported into a cell, is held constant. GLUT4, coupled with adequate glucose supply, enables reliable cellular energy production. Keeping the value of GLUT4 is very important for glucose energy metabolism, showing real robustness in the system. Energy processing of neurons works properly due to this robustness.

**Remark 6** The ACR in 8 species of the network is inferred from ACR in the deficiency zero subnetwork $\mathcal{N}_A$. A necessary condition for this occurrence is the nonconservativity of $\mathcal{N}$. If $\mathcal{N}$ were conservative, i.e., there was a positive vector in $S^\perp$, then $S^\perp = (S_A + S_B)^\perp = S_A^\perp \cap S_B^\perp$. Hence, $\mathcal{N}_A$ would be a conservative deficiency zero mass action network and have no ACR in any species (Theorem 9.7.1 of Feinberg (2019)).

4.4 ACR Analysis of the Metabolic Insulin Signaling Network for a Set of Rate Constants

Table 5 shows that there are SF-pairs in deficiency one subnetworks in further independent coarsenings of the metabolic insulin signaling network. The failure of the three sufficient conditions for ACR for all rate constants (Meshkat et al criterion) led us to suspect that the remaining species (i.e., $X_{13}, X_{14}, X_{16}, X_{17}, X_{18}$, and $X_{19}$) do not have this property. The test is done using the set of rate constants available from Sedaghat et al. (2002).

Example 1 in Sect. 3.2 presents a positive equilibrium of the metabolic insulin signaling network. It is evident from the example that positive equilibria for the metabolic insulin signaling network have the same value for $X_2, \ldots, X_8$ and $X_{20}$. For the other 12 species, their equilibrium value is dependent on the following variables: $X_{10}, X_{11}, X_{13}, X_{16}$, and $X_{18}$. This suggests that there are infinitely many possible positive equilibria.

Our computations verify that, for the set of rate constants used above, species $X_2, \ldots, X_8$ and $X_{20}$ have stable ACR, while the remaining species do not have ACR (since the 5 species identified as “independent variables” can take various values and the remaining species will vary according to the variation of the variables).
Table 5  Species with ACR

| Species | SF-pair | ACR building block | Deficiency | Comments |
|---------|--------|--------------------|------------|----------|
| X₂      | $R_1, R_{13}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
|         | $R_{11}, R_{34}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
|         | $R_7, R_{13}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
|         | $R_7, R_{34}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
| X₃      | $R_{2}, R_{13}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
|         | $R_2, R_{34}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
|         | $R_5, R_{13}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
|         | $R_5, R_{35}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
| X₄      | $R_4, R_{13}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
|         | $R_4, R_{34}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
|         | $R_9, R_{13}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
|         | $R_9, R_{34}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
| X₅      | $R_3, R_{13}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
|         | $R_3, R_{34}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
|         | $R_6, R_{13}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
|         | $R_6, R_{34}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
|         | $R_{11}, R_{13}$ | $\mathcal{N}_A$ | 0          | Linked SF-pair |
|         | $R_{11}, R_{34}$ | $\mathcal{N}_A$ | 0          | Linked SF-pair |
| X₆      | $R_8, R_{13}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
|         | $R_8, R_{34}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
|         | $R_{13}, R_{14}$ | $\mathcal{N}_A$ | 0          | Linked SF-pair |
|         | $R_{14}, R_{34}$ | $\mathcal{N}_A$ | 0          | Linked SF-pair |
| X₇      | $R_{10}, R_{13}$ | $\mathcal{N}_A$ | 0          | Linked SF-pair |
|         | $R_{10}, R_{34}$ | $\mathcal{N}_A$ | 0          | Linked SF-pair |
|         | $R_{13}, R_{15}$ | $\mathcal{N}_A$ | 0          | Linked SF-pair |
|         | $R_{15}, R_{34}$ | $\mathcal{N}_A$ | 0          | Linked SF-pair |
| X₈      | $R_{12}, R_{13}$ | $\mathcal{N}_A$ | 0          | Linked SF-pair |
|         | $R_{12}, R_{34}$ | $\mathcal{N}_A$ | 0          | Linked SF-pair |
|         | $R_{13}, R_{16}$ | $\mathcal{N}_A$ | 0          | Linked SF-pair |
|         | $R_{16}, R_{34}$ | $\mathcal{N}_A$ | 0          | Linked SF-pair |
| X₁₃     | $R_{13}, R_{23}$ | $\mathcal{N}_2 \cup \mathcal{N}_5$ | 1          | Linked SF-pair |
|         | $R_{22}, R_{34}$ | $\mathcal{N}_5 \cup \mathcal{N}_{10}$ | 1          | Linked SF-pair |
| X₁₄     | $R_{21}, R_{22}$ | $\mathcal{N}_4 \cup \mathcal{N}_6$ | 1          | Linked SF-pair |
| X₁₆     | $R_{25}, R_{26}$ | $\mathcal{N}_6 \cup \mathcal{N}_7$ | 1          | Linked SF-pair |
| X₁₇     | $R_{13}, R_{27}$ | $\mathcal{N}_2 \cup \mathcal{N}_7$ | 1          | Linked SF-pair |
|         | $R_{27}, R_{34}$ | $\mathcal{N}_7 \cup \mathcal{N}_{10}$ | 1          | Linked SF-pair |
| X₁₈     | $R_{25}, R_{28}$ | $\mathcal{N}_6 \cup \mathcal{N}_8$ | 1          | Linked SF-pair |
| X₁₉     | $R_{13}, R_{29}$ | $\mathcal{N}_2 \cup \mathcal{N}_8$ | 1          | Linked SF-pair |
|         | $R_{29}, R_{34}$ | $\mathcal{N}_8 \cup \mathcal{N}_{10}$ | 1          | Linked SF-pair |
| X₂₀     | $R_{13}, R_{35}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
|         | $R_{34}, R_{35}$ | $\mathcal{N}_A$   | 0          | Linked SF-pair |
These computations indeed confirm that species $X_{13}, X_{14}, X_{16}, X_{17}, X_{18},$ and $X_{19}$ do not have ACR for all rate constants.

**Remark 7** The number of species exhibiting ACR (which we call “ACR species” for short) is an inverse measure of the variation in the equilibria composition: the more ACR species there are, the less the variation. An extreme case is ACR in all species, which is equivalent to the system having a unique equilibrium (in the entire species space). With 8 ACR species among 20, the metabolic insulin signaling network has a fairly high variation in equilibria composition. The deficiency-oriented decomposition reveals the source of this variation. The deficiency zero subnetwork $\mathcal{N}_A$ contains all the ACR species. Furthermore, since the insulin signaling system is a log-parameterized system, it follows from the results of Lao et al. (2022) that the number of ACR species (among the total 13 species in the subnetwork) is bounded by the subnetwork’s rank ($s_A = 10$). This shows that the high variation in equilibria composition is caused primarily by the lack of ACR species among the 13 species in the deficiency 7 subnetwork $\mathcal{N}_B$.

## 5 Summary and Outlook

The insulin signaling system is an important metabolic system. In this study, we derived a CRN of the Sedaghat et al insulin signaling model with 20 species, 35 complexes, and 35 reactions. We have shown that it is a nonconservative, nonweakly reversible, and high deficiency ($\delta = 7$) system. The positive dependence of the reaction network ensures the existence of rate constants under which the mass action system has positive equilibria (Proposition 1). Additionally, the network’s $t$-minimality implies that the kinetic and stoichiometric subspaces coincide, which is necessary for the existence of nondegenerate equilibria. Moreover, the network is concordant, which implies that the system’s species formation rate function, when restricted to any stoichiometric compatibility class, is injective. It follows, then, that the kinetic system is monostationary, i.e., there is at most one positive equilibrium in each stoichiometric compatibility class.

We obtained the finest independent decomposition of the metabolic insulin signaling network $\mathcal{N} = \{R_1, \ldots, R_{35}\}$ consisting of 10 subnetworks which we have shown to be bi-independent (Proposition 2). CRNT analysis using a coarsening of the decomposition revealed three subnetworks of the metabolic insulin signaling network which are not only functionally but also structurally important. Upon considering a deficiency-oriented coarsening of the finest decomposition, we have shown how a binary decomposition can be viewed in relation to a network’s set of positive equilibria (Lemma 3). We have also developed a method of determining positive equilibria of the metabolic insulin signaling network using its deficiency-oriented coarsening (Proposition 4). This provides an alternative method for solving positive equilibria analytically using smaller subnetworks instead of the (more complex) entire network.

For the ACR analysis, we have shown that subnetworks $\mathcal{N}_2$ and $\mathcal{N}_{10}$ satisfy the Meshkat et al criterion, but species $X_6$ and $X_{20}$ (which come from the said subnetworks) have ACR for all rate constants only in their respective subnetwork. This implies that the stability of the equilibria is only within the subnetworks and not for the...
whole network. Upon implementing the algorithm of Fontanil et al. (2021), however, we found that the system had ACR in 8 species. This restricts the variability of the positive equilibria and also suggests that this “structural source of robustness” may be an important factor in the system’s overall robustness. However, overall there is still a high variation in equilibria composition due to the lack of ACR species in the deficiency 7 subnetwork of the network’s deficiency-oriented coarsening. For the rate constants used in the study of Sedaghat et al. (2002), we have verified that species $X_2, \ldots, X_8,$ and $X_{20}$ indeed have stable ACR. Interestingly, $X_{20}$, i.e., the insulin-regulated glucose transporter GLUT4, plays an important role in glucose energy metabolism.

Our analysis of the Sedaghat et al model is the first part of a two-step research effort on metabolic insulin signaling. In their paper, Sedaghat et al constructed the model from data of healthy cells. As a second step, we have started a reaction network analysis of a model of metabolic insulin signaling by Brännmark et al. (2013) based on cell data from type 2 diabetes patients, i.e., cells with insulin resistance. Preliminary results already indicate significant differences to our main results on the Sedaghat et al model.

**Supplementary Information**  The online version contains supplementary material available at [https://doi.org/10.1007/s11538-022-01087-3](https://doi.org/10.1007/s11538-022-01087-3).

**Declarations**

**Conflict of interest**  The authors declare no conflicts of interests.

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