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Comparison of reaction networks of insulin signaling

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ABSTRACT

Understanding the insulin signaling cascade provides insights into the underlying mechanisms of biological phenomena such as insulin resistance, diabetes, Alzheimer's disease, and cancer. For this reason, previous studies utilized chemical reaction network theory to perform comparative analyses of reaction networks of insulin signaling in healthy (INSMS: INSulin Metabolic Signaling) and diabetic cells (INRES: INsulin RESistance). This study extends these analyses using various methods which give further insights regarding insulin signaling. Using embedded networks, we discuss evidence of the presence of a structural "bifurcation" in the signaling process between INSMS and INRES. Concordance profiles of INSMS and INRES show that both have a high propensity to remain monostationary. Moreover, the concordance properties allow us to present heuristic evidence that INRES has a higher level of stability beyond its monostationarity. Finally, we discuss a new way of analyzing reaction networks through network translation. This method gives rise to three new insights: (i) each stoichiometric class of INSMS and INRES contains a unique positive equilibrium; (ii) any positive equilibrium of INSMS is exponentially stable and is a global attractor in its stoichiometric class; and (iii) any positive equilibrium of INRES is locally asymptotically stable. These results open up opportunities for collaboration with experimental biologists to understand insulin signaling better.

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INTRODUCTION

In healthy cells, insulin signaling regulates glucose metabolism (Norton et al. 2022). Impaired insulin signaling, however, can lead to insulin resistance (Pessin and Saltiel 2000), which can then lead to increased risk of diabetes, Alzheimer's disease, and cancer (Akhtar and Sah 2020; Shieh et al. 2020; Tsugane and Inoue 2010). To gain insights into how this important signaling pathway functions, several mathematical models have been constructed for both healthy (Sedaghat et al. 2002) and insulin-resistant cells (Braatz and Coleman 2015; Brännmark et al. 2013; Nyman et al. 2014). As their contribution to this understanding of the pathway, Lubenia et al. (2022; 2024) performed a reaction network analysis of insulin signaling in healthy and type 2 diabetes cells using Chemical Reaction

KEYWORDS

concordance, embedded networks, insulin signaling, network translation, reaction networks

Network Theory (CRNT). In this paper, we (i) extend and deepen their comparative analysis using methods that utilize embedded networks, concordance profile, and network translation; and (ii) illustrate how to use our mathematical results to address a broader audience, especially experimental biologists and clinical researchers, to explore potential collaboration.

Lubenia et al. (2024) observed key differences in the reaction networks of insulin signaling in healthy (INSulin Metabolic Signaling or INSMS) and insulin-resistant cells (INsulin RESistance or INRES). Among them was the number of species interacting in the signaling cascade of the two networks. A more important observation was that key species in the insulin signaling pathway lose their absolute concentration robustness when insulin resistance occurs. They showed this based on the reaction network's decomposition and equilibria parametrization, the latter leading to ideas for therapeutic approaches for further exploration. This paper explains other ideas on how to use our main results to collaborate with experimental biologists and clinical researchers.

In this study, we utilized new methods which were developed to compare and gain insights into different reaction networks representing the same signaling pathway. First, we apply the common species embedded networks analysis which utilizes embedded networks with respect to the networks' set of common species (Hernandez et al. 2024a). The second method we used is the concordance profiles analysis wherein we compare the various concordance properties of INSMS and INRES (Hernandez et al. 2024b). Finally, we introduced network translation analysis in this paper which leads to insights regarding the equilibria distribution and stability of reaction networks.

Our study yielded five major results. The first one is the presence of a structural "bifurcation" in the processing between healthy and diabetic cells, i.e., a divergence in the processes at some point. The second result is our presentation of heuristic evidence that INRES has a higher level of stability beyond its monostationarity. Third, in both INSMS and INRES, we observe that each stoichiometric class contains a unique positive equilibrium. Fourth, for INSMS, any positive equilibrium is exponentially stable and is a global attractor in its stoichiometric class. Finally, we were able to conclude that any positive equilibrium of INRES is locally asymptotically stable. These results open up the opportunity for collaboration with experimental biologists to gain further insights regarding insulin signaling.

This paper is organized as follows: the next section reviews the results of the comparative analysis of reaction networks of insulin signaling by Lubenia et al. (2024); the succeeding three sections detail the comparative analyses of INSMS and INRES based on their common species, concordance profiles, and network translations; the final section deals with the summary of our findings and outlook for future studies. A Supplementary Material is available for readers who wish to brush up on CRNT. It also includes the details of the reaction networks used in this paper.

RESULTS OF THE INITIAL COMPARATIVE ANALYSIS

We provide in this section a brief overview of the findings of the comparative analysis already done regarding reaction networks of insulin signaling. We refer to the reaction network of insulin signaling in healthy and diabetic cells as INSMS (INSulin Metabolic Signaling) and INRES (INsulin RESistance), respectively.

Using Chemical Reaction Network Theory (CRNT), Lubenia et al. (2022) performed a reaction network analysis of INSMS while Lubenia et al. (2024) did the same in the case of INRES. The latter also performed a comparative analysis of the two mass action networks (see Supplementary Material for a brief review of chemical kinetic systems). Table 1 presents a summary of some of the findings. The two studies helped establish the usefulness of CRNT in gaining insights regarding biological processes. The authors observed that both networks were monostationary, i.e., a unique positive equilibrium (i.e., long-term behavior) exists for each choice of rate constants in the networks' ordinary differential equations. It was also discovered that INRES is conservative while INSMS is not (see Remark 2 for the implication of this observation on the translations of the networks). More importantly, the studies highlighted three principal differences between insulin signaling in healthy and diabetic cells:

(i) In INSMS, eight species were determined to exhibit absolute concentration robustness (ACR) while none were found in INRES. ACR refers to the invariance of a species' concentration across all positive equilibria in a kinetic system. In particular, in the healthy cell model, the intracellular glucose transporter GLUT4's ACR suggests that maintaining a stable level of GLUT4 could be advantageous in addressing insulin resistance, facilitating efficient glucose transport into the cell. The equilibria parametrization of the concentration of GLUT4 presented by Lubenia et al. (2024) for the insulin-resistant cell model reveals that the concentration relies on the concentration of other species within the system. One can possibly work in close cooperation with experimental biologists to assess whether the concentration of these species can be altered to move the value of GLUT4 to that under healthy conditions.

(ii) There is a significant difference in the set of species involved in insulin signaling in the two cell states. This also points to strongly differing processing modules in the two systems. The section Common Species-Based Comparative Analysis further quantifies these observed difference.

(iii) INRES loses the concordance exhibited by INSMS. The section Concordance Profiles-Based Comparative Analysis significantly extends and qualifies these results.

Table 1: Summary of some properties of INSMS and INRES

INSMS	INRES
Monostationary	
Not conservative	Conservative
8 ACR species (out of 20)	No ACR species (out of 32)
Concordant	Discordant

In relation to the first principal difference, it is particularly significant that the glucose transporter GLUT4 loses its ACR in INRES. This is consistent with experimental findings of lower GLUT4 level in insulin signaling in type 2 diabetes (Chen et al. 2003). Furthermore, the authors' analysis via finest independent decomposition (FID) and equilibria parametrization revealed new insights regarding which species concentrations determined the concentration of GLUT4 in equilibrium. Collaboration with experimental and clinical researchers could clarify if there are experimental approaches that can influence these values to

restore approximate concentration robustness at the healthy levels.

In the next two sections, we analyze in greater detail the second and third principal differences in insulin signaling in healthy and insulin-resistant cells: differences in species sets and concordance.

COMMON SPECIES-BASED COMPARATIVE ANALYSIS

Hernandez et al. (2024a) introduced the method common species embedded networks analysis. In this section, we apply this method on INSMS and INRES. The concept of embedded networks is based on Joshi and Shiu (2013) (see Supplementary Material for a brief review of embedded networks).

To implement the analysis, we follow the procedure outlined in Hernandez et al. (2024a). We perform only the first two steps since the last step described in the paper is not relevant to our study (see Remark 1):
Step 1: Determine the common species of INSMS and INRES.
Step 2: Remove from the reactions of INSMS and INRES all species not in Step 1. Trivial reactions, i.e., those whose reactant complex and product complex are the same, are also removed from the list of reactions.

The species common to INSMS and INRES are X_2, X_3, X_4, X_6, X_7, X_9, X_{10}, X_{20}, and X_{21} (see Supplementary Material for the definition of the variables used in INSMS and INRES). To derive the embedded networks of INSMS and INRES, we modify first the way the reactions of INRES are numbered so that common reactions with INSMS have the same numbering (see Supplementary Material for the list of reactions of INRES and their numbering as used in this study). Table 2 provides the result of Steps 1 and 2 of the analysis.

Table 2: Common species embedded networks of INSM and INRES

Common to INSMS and INRES		
$R_1: X_2 \to X_3$ $R_8: X_6 \to X_2$ $R_9: X_4 \to X_7$ $R_{19}: X_{10} \to X_9$ $R_{31}: X_{21} \to X_{20}$		
Embedding-derived common reactions*		
$R^E_{20}: X_{10} \to 0$ $R^E_{21}: 0 \to X_{10}$ $R^E_{38}: X_7 \to X_6$ $R^E_{62}: X_{20} \to X_{21}$		
Unique to INSMS*		**Unique to INRES***
$R_2: X_3 \to X_2$ $R^E_3: 0 \to X_4$ $R^E_4: X_4 \to 0$ $R^E_5: X_3 \to 0$ $R^E_6: X_2 \to 0$ $R_7: X_2 \to X_6$ $R_{10}: X_7 \to X_4$ $R_{13}: 0 \to X_6$ $R_{14}: X_6 \to 0$ $R_{17}: X_9 + X_4 \to X_{10} + X_4$ $R^E_{18}: X_9 \to X_{10}$ $R_{34}: 0 \to X_{20}$ $R_{35}: X_{20} \to 0$		$R_{36}: X_2 \to X_4$ $R_{37}: X_3 \to X_4$ $R_{39}: X_4 \to X_2$ $R_{40}: X_7 + X_9 \to X_7 + X_{10}$ $R^E_{41}: X_9 \to 0$ $R^E_{45}: 0 \to X_9$

* The superscript ***E*** refers to reactions derived from the embedding process

The results of the first two steps reveal additional interesting aspects of the structural differences between the models beyond the small set of common species (9 out of 20 for INSMS and 32 for INRES). First, INSMS and INRES have a very small set of common reactions: 5 out of 35 (INSMS) and 44 (INRES). Second, there is only a small set of common reactions in their embedded networks: only four embedding-derived common reactions. And third, while most of the unique reactions of INSMS involve the common species, those of INRES do not. This is why, although INRES is larger, its embedded network is smaller (22 reactions for INSMS and 15 reactions for INRES). These results suggest that there is a structural "bifurcation" in the processing between healthy and diabetic cells, i.e., after a small initial common subnetwork, the insulin processing in INSMS and INRES separates into two subnetworks with entirely different species sets (resulting in different complexes and reactions) which converge only at the output molecule GLUT4. There appears to be a "tipping point" in the course of the disease when the signaling process switches from following that for healthy cells to that for diabetic cells. Joint efforts with experimental biologists can yield insights into this "tipping point" by examining the unique species in INRES (i.e., the ones removed to come up with the embedded network) and their role in insulin resistance. The team can also look into the altered reactions during the embedding process to determine their significance in the development of the cell's resistance to insulin.

Remark 1: From Table 2, the common reactions of INSMS and INRES are R_1, R_8, R_9, R_{19}, and R_{31}. The common reactions equilibria analysis developed by Hernandez et al. (2024b) does not provide any new insight since the network of common reactions is not positive dependent (this can be easily verified using the CRNToolbox (Feinberg et al. 2018), a Windows application which generates reports regarding some properties of chemical reaction networks). Positive dependence is a property that needs to be satisfied for a system to have positive equilibria (Shinar and Feinberg 2012).

CONCORDANCE PROFILES-BASED COMPARATIVE ANALYSIS

Hernandez et al. (2024b) introduced a novel approach, called concordance profile analysis, in comparing three models of Wnt signaling in healthy cells. Concordance is a network property that is related to the stability properties of positive equilibria of the network (see Corollary 10.7.3 of Feinberg (2019) and Proposition 2 in the next section for a detailed discussion). In this section, we compare INSMS and INRES based on their concordance profiles.

We recall from Hernandez et al. (2024b) that the **concordance (discordance) set** FIDC (FIDD) of a reaction network is the union of all concordant (discordant) subnetworks of its FID (see Supplementary Material for a brief review of decomposition theory). For a non-empty FIDC, a maximal independent concordant subnetwork of the network is called a **concordance core** of the network. The **concordance dimension** c of a reaction network is the rank of a concordance core. The **discordance dimension** of the network is defined as $d := s - c$ where s is the rank of the network. If the FIDC or the FIDD is empty, we set $c = 0$ or $d = 0$, respectively. The ratios $\frac{c}{s}$ and $\frac{d}{s}$ are called the **concordance level** and **discordance level** of the network, respectively.

In his book, Feinberg (2019) highlighted the occurrence of mass action systems which, though discordant, remain monostationary. INRES, as previously shown, is such a system. Discordance, though, implies the existence of ~~a~~ weakly monotonic kinetics (see Supplementary Material for a brief discussion of weakly monotonic kinetics) on the network such that the kinetic system turns multistationary, i.e., the system has multiple equilibria for a given set of rate constants. In this sense, the concordance level of a network measures the propensity of weakly monotonic kinetics on it to remain monostationary. Concordant networks, such as INSMS, have ~~a~~ corresponding concordance level of $\frac{c}{s} = 1$. Since the value of a network's concordance level is from 0 to 1, attaining a value of 1 signifies that the network has the highest capacity to remain monostationary.

Table 3 summarizes the concordance profiles of INSMS and INRES. We denote the 10 subnetworks of the FID of INSMS as $\mathcal{N}_{\text{INSMS},1}, \cdots, \mathcal{N}_{\text{INSMS},10}$ while the 12 FID subnetworks of INRES are denoted $\mathcal{N}_{\text{INRES},1}, \cdots, \mathcal{N}_{\text{INRES},12}$. Each of their FID subnetworks are concordant; hence, the FIDC for both is the entire network. Since INSMS is a concordant network, its concordance core is itself. This implies a concordance level of 1. On the other hand, for the discordant network INRES, we provide a description of our conjecture below regarding its other concordance properties.

Table 3: Concordance profiles of INSMS and INRES

Concordance property	INSMS	INRES
Concordance set	$\mathcal{N}_{\text{INSMS}}$	$\mathcal{N}_{\text{INRES}}$
Discordance set	$\emptyset$	$\emptyset$
Concordance core	$\mathcal{N}_{\text{INSMS}}$	$\mathcal{N}_{\text{INRES}} \backslash \{\mathcal{N}_{\text{INRES},5} \cup \mathcal{N}_{\text{INRES},12}\}$*
Concordance dimension	15	18*
Concordance level	1	0.9*
Discordance dimension	0	2*
Discordance level	0	0.1*

Expressions with * represent conjectures

Using the Concordance Report of the CRNToolbox, so far, we have verified only a rank 14 concordant subnetwork of the discordant network $\mathcal{N}_{\text{INRES}}$. Since $\mathcal{N}_{\text{INRES}}$ has a rank of 20, its concordance dimension is in the range $14 \leq c \leq 19$. Our conjecture is that $c = 18$ since we found a possibly concordant subnetwork of $\mathcal{N}_{\text{INRES}}$, which has rank 18 and is injective, containing the concordant rank 14 subnetwork we identified earlier. Our conjecture for INRES implies a concordance level of about 0.9, which suggests a high level of stability (or propensity to remain monostationary) despite the very different processing pathway it has compared with INSMS. This seems to be consistent with the chronic character of insulin resistance (past a certain point), but this interpretation should be discussed in more detail with experts.

NETWORK TRANSLATION-BASED COMPARATIVE ANALYSIS

In this section, we introduce a new method called network translation analysis. We apply to INSMS and INRES the method developed by Hong et al. (2023) which identifies network translations that are weakly reversible and have zero deficiency while preserving their original dynamics. We do this by using the authors' computational package TOWARDZ which is implemented in MATLAB.

Running TOWARDZ did not yield any weakly reversible deficiency zero translation of INSMS and INRES within a reasonable time due to their sheer size. Hence, we utilized the FID of the networks and applied TOWARDZ on each of the subnetworks. The Supplementary Material shows a weakly reversible deficiency zero translation of each subnetwork of INSMS (denoted $\mathcal{N}_{\#,\text{INSMS},i}$) and the corresponding translations for INRES (denoted $\mathcal{N}_{\#,\text{INRES},i}$). The translated subnetworks already constitute the FID of the weakly reversible deficiency zero translation of INSMS and INRES. We denote the union of the weakly reversible deficiency zero translations of the subnetworks of INSMS as $\mathcal{N}_{\#,\text{INSMS}}$. Similarly, we denote as $\mathcal{N}_{\#,\text{INRES}}$ the union of the weakly reversible deficiency zero translations of the subnetworks of INRES. The following theorem provides a general justification of the preceding

considerations:

Theorem 1 *Let $\mathcal{N} = \mathcal{N}_1 \cup \cdots \cup \mathcal{N}_k$ be an independent decomposition of a chemical reaction network $\mathcal{N}$. Let K be a kinetics on $\mathcal{N}$ and K_i the restriction of K to $\mathcal{N}_i$. Furthermore, suppose each $(\mathcal{N}_{\#,i}, K_{\#,i})$ is a network translation of $(\mathcal{N}_i, K_i)$. Then*

(i) *$\mathcal{N}_\# = \mathcal{N}_{\#,1} \cup \cdots \cup \mathcal{N}_{\#,k}$ is an independent decomposition; and*

(ii) *$(\mathcal{N}_\#, K_\#)$ is a network translation of $(\mathcal{N}, K)$.*

Proof: We first recall the concept of a network translation: for a kinetic system $(\mathcal{N}, K)$, we call $(\widetilde{\mathcal{N}}, \widetilde{K})$ a translation of $(\mathcal{N}, K)$ if $\sum_{r:y'-y=\xi} K_r(x) = \sum_{\tilde{r}:z'-z=\xi} \widetilde{K}_{\tilde{r}}(x)$ for any $\xi \in \mathbb{Z}^m$ and $x \in \mathbb{R}^m_{\geq 0}$ where $r: y' - y$ and $\tilde{r}: z' - z$ refer to the reaction vectors of reactions $y \to y'$ and $z \to z'$, respectively. In both sums, note that the only nonzero summands are those for the corresponding reaction vector sets. If the left-hand side is for the system we are considering, since the decomposition is independent, we can write the sum as consisting of partial summands over the reaction vectors of the subnetworks $\mathcal{N}_i$. Note that the independence is essential to ensure that the indices are distinct. After translating each subnetwork, we obtain for each a partial sum over the same indices since translation preserves the set of reaction vectors. Since translation also preserves the stoichiometric subspace, we also obtain an independent decomposition for the union of the translated subnetworks. Summing up the partial summands provides the claim. ■

Table 4 summarizes the CRNToolbox results for $\mathcal{N}_{\#,\text{INSMS}}$ and $\mathcal{N}_{\#,\text{INRES}}$: both are positive dependent, monostationary, injective (see Supplementary Material for the mathematical definition; implications to be discussed below), nondegenerate, and their equilibria are globally asymptotically stable (interpretation to be discussed below). Furthermore, all their subnetworks are concordant. On the other hand, $\mathcal{N}_{\#,\text{INRES}}$ is conservative while $\mathcal{N}_{\#,\text{INSMS}}$ is not. The toolbox was able to conclude that $\mathcal{N}_{\#,\text{INSMS}}$ is concordant; however, we are not able to make the same determination for $\mathcal{N}_{\#,\text{INRES}}$ (the CRNToolbox was not able to generate a conclusion within a reasonable amount of time due to the high number of reactions involved).

Table 4: Summary of CRNToolbox results for the weakly reversible deficiency zero translation of INSMS ($\mathcal{N}_{\#,\text{INSMS}}$) and INRES ($\mathcal{N}_{\#,\text{INRES}}$)

Property	$\mathcal{N}_{\#,\text{INSMS}}$	$\mathcal{N}_{\#,\text{INRES}}$
Positive dependent	Yes	Yes
Conservative	No	Yes
Monostationary	Yes	Yes
Equilibrium asymptotically stable	Yes	Yes
Injective	Yes	Yes
Concordant	Yes	?
Nondegenerate network	Yes	Yes

Remark 2: In view of Theorem 1 and the results of Talabis and Mendoza (2024), positive dependence and conservativeness of the network translations follow from the corresponding properties of the original networks. Furthermore, the local asymptotic property of the equilibria derives from the Deficiency Zero Theorem of Horn and Jackson (1972) for mass action systems (see Supplementary Material for a statement of the theorem).

Properties of INSMS and INRES derived from network translation

For networks with mass action kinetics, such as INSMS and INRES, the existence of weakly reversible network translations enables the inference of interesting properties in equilibria distribution and equilibria stability of the original networks. For comparison clarity, we formulate the inferred results in three different propositions. First, we show the similarity in equilibria distribution.

Proposition 1 *For both INSMS and INRES, each stoichiometric class contains a unique positive equilibrium.*

Proof: We first utilize the Hars-Tóth criterion (Theorem 4 of Chellaboina et al. (2009)) to check that each translated FID subnetwork of INSMS and INRES follows mass action kinetics. Consider the system of ordinary differential equations (ODEs) $\dot{x} = f(x) = (B - A)^T(k \circ x^A)$, where $\circ$ represents componentwise multiplication, $A = [a_{ij}]$, $B = [b_{ij}]$, $k = [k_1, \cdots, k_r]^T$, $x = [x_1, \cdots, x_m]^T$, and x^A is the element of $\mathbb{R}^m$ with i th component $x_1^{a_{i1}} \cdots x_m^{a_{im}}$ for $i = 1, \cdots, r$ and $j = 1, \cdots, m$. The criterion guarantees that the set of ODEs has a mass action system realization of the form $AX \xrightarrow{k} BX$ provided that for each $j = 1, \cdots, m$, $f_j(x_1, \cdots, x_{j-1}, 0, x_{j+1}, \cdots, x_m)$ is a multivariate polynomial with nonnegative coefficients. We observe that through the FID, we obtain an expression for the right-hand side of the ODE $\dot{x} = f(x)$ as a sum of partial sums $f = f_1 + \cdots + f_k$ where $f_i = N_i K_i$ (N_i is the stoichiometric matrix of subnetwork i and K_i is the vector of rate functions of the subnetwork) for each i. Thus, applying the Hars-Tóth criterion on each subnetwork is sufficient.

We apply the criterion to each subnetwork by taking the ODEs relevant to the subnetwork. For example, $\mathcal{N}_{\text{INSMS},5}$ involves the reactions $R_{22}: X_{14} + X_{12} \to X_{13} + X_{12}$ and $R_{23}: X_{13} \to X_{14}$ with corresponding rate functions $k_{22}x_{14}x_{12}$ and $k_{23}x_{13}$, respectively. The ODEs in INSMS of the three species in the subnetwork involving the two reactions are $\dot{x}_{12} = 0$, $\dot{x}_{13} = k_{22}x_{14}x_{12} - k_{23}x_{13}$, and $\dot{x}_{14} = k_{23}x_{13} - k_{22}x_{14}x_{12}$. Since x_{12} is a constant, we can include it in the rate constant for the rate function of R_{22} as $k^\#_{22}x_{14}$ where $k^\#_{22}$ is a constant. Thus, we can consider the ODEs $\dot{x}_{13} = k^\#_{22}x_{14} - k_{23}x_{13}$, and $\dot{x}_{14} = k_{23}x_{13} - k^\#_{22}x_{14}$. Applying the Hars-Tóth criterion to this set of ODEs, we get the corresponding realization consisting of $R^\#_{22}: X_{14} \to X_{13}$ and $R_{23}: X_{13} \to X_{14}$ which is similar to the reaction network of the translated network $\mathcal{N}_{\#,\text{INSMS},5}$. The same process can be used to verify mass action kinetics for the other translated subnetworks.

Now, by the Deficiency Zero Theorem, each of the translations generated using TOWARDZ has a unique positive equilibrium in each stoichiometric class. Since both the set of positive equilibria and the set of stoichiometric classes are preserved by network translation, both INSMS and INRES have these

properties, too. ■

Proposition 1 matches the nature of experimental values Sedaghat et al. (2002) and Nyman et al. (2014) measured in their studies, i.e., the species involved in INSMS and INRES have nonnegative concentration values.

Remark 3: The existence of a positive equilibrium in each stoichiometric class for INSMS was shown in Lubenia et al. (2022) by the computation of an explicit equilibria parametrization. For INRES, this refinement of its monostationarity is a new result.

For the comparison of stability properties, we state two separate propositions to highlight the differences. We lay out here first the various results we need from Feinberg (2019): (i) Theorem 10.6.17 implies that a nondegenerate network with a concordant fully open extension is concordant; (ii) Corollary 10.7.3 shows that the positive equilibria of nondegenerate networks, whose fully open extension is concordant, have negative real eigenvalues; and (iii) Theorem 10.7.2 connects concordance and (exponential) stability of equilibria (i.e., the capacity of species concentrations to return to equilibrium despite disturbances to their concentration levels) of networks with differentiably monotonic kinetics (see Supplementary Material for the definition). Thus, the main result we utilize in this study says that for a special class of concordant networks, for any differentiably monotonic kinetics, all positive equilibria are exponentially stable. On the other hand, in a discordant network, there is "built-in" instability as detailed in Theorem 10.7.7 of Feinberg (2019). We obtain the following striking stability results regarding INSMS.

Proposition 2 *For INSMS and any mass action kinetics:*

(i) Any positive equilibrium is exponentially stable; and

(ii) any positive equilibrium is globally asymptotically stable, i.e., a global attractor in its stoichiometric class.

Proof:

(i) CRNToolbox reports for INSMS state that the network is nondegenerate and its fully open extension is concordant. Since any mass action kinetics is differentiably monotonic, it follows from Corollary 10.7.3 of Feinberg (2019) that all its positive equilibria are exponentially stable.

(ii) The Deficiency Zero Theorem for mass action systems implies that $\mathcal{N}_{\#,\mathrm{INSMS}}$ has a locally asymptotically stable positive equilibrium in each stoichiometric class. Moreover, Shinar and Feinberg (2012) showed in their Remark 6.5 that the Global Attractor Conjecture (see Horn and Jackson 1972) holds for concordant weakly reversible networks with zero deficiency. Hence, the claim follows for the network translation of INSMS as well. ■

The global asymptotic stability of equilibria of INSMS in Proposition 2 suggests that the system will always go back to its equilibrium state despite variations in the concentrations of the species within the system. Thus, a person's functioning insulin signaling remains so even in changing conditions in the body, except probably in extreme situations. For INRES, we can currently claim only the following:

Proposition 3 *For any mass action kinetics on INRES, any positive equilibrium is locally asymptotically stable.*

Remark 4: This can be easily verified using the CRNToolbox.

Proposition 3 suggests that in order to stabilize the concentrations of the species in the insulin signaling network for a diabetic cell, specific criteria or conditions must be observed. Collaboration with biologists can explore the possibility of controlling some biomarkers of insulin resistance through drug intervention.

Remark 5: The qualification "currently" refers to two aspects. First, we cannot settle the question of concordance of $\mathcal{N}_{\#,\mathrm{INRES}}$ with our current tools. Secondly, to our knowledge, the Global Attractor Conjecture has been proven only in several cases for discordant networks, none of which hold for $\mathcal{N}_{\#,\mathrm{INRES}}$.

SUMMARY AND OUTLOOK

This study extended the analysis of reaction networks of insulin signaling in healthy cells (INSulin Metabolic Signaling or INSMS) and in type 2 diabetes (INsulin RESistance or INRES) by Lubenia et al. (2022; 2024). We utilized three methods of analysis to gain further insights into the said networks: comparative analyses based on embedded networks, concordance profiles, and network translations.

Through a common species-based comparative analysis, our results suggested that there is a structural "bifurcation" in the processing between healthy and diabetic cells, i.e., there may be a point where the insulin processing in INSMS and INRES split into two subnetworks with different species sets and only converge at GLUT4. This pointed to "tipping points" in the course of the disease. On the other hand, concordance profiles-based comparative analysis allowed us to present heuristic evidence that a higher level of stability exists in INRES. We also presented here the interpretation of a network's concordance level as a measure of the propensity of weakly monotonic kinetics on the network to remain monostationary. Finally, we introduced a network translation-based analysis which gave rise to three new insights regarding INSMS and INRES: (i) each stoichiometric class of INSMS and INRES contains a unique positive equilibrium; (ii) any positive equilibrium of INSMS is exponentially stable and is a global attractor in its stoichiometric class; and (iii) any positive equilibrium of INRES is locally asymptotically stable.

Our results provide opportunities for mathematicians to collaborate with experimental biologists to gain more insights into insulin signaling. In particular, to determine the "tipping point" between a healthy cell and an insulin-resistant one, a team of mathematicians and biologists can look into the species that have been removed and the reactions that have been altered in the construction of the embedded network of INRES. They can also look into the source of the high concordance level of INRES which seems to be consistent with the chronic character of insulin resistance (past a certain point). Finally, the collaboration can also explore drug interventions to control biomarkers that may stabilize insulin resistance.

One of the challenges in determining the concordance of the weakly reversible deficiency zero translation of INRES is the running time of the CRNToolbox which was originally developed for the Microsoft DOS operating system. For further studies, one can look into implementing the Concordance Test algorithm by Ji (2011) in MATLAB where it may potentially run faster compared with the CRNToolbox. One can also consider studying the concordance of huge networks via the Species-Reaction Graph (Theorem 11.5.1 of Feinberg (2019)).

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

PVNL, ERM, and ARL equally contributed to the conceptualization and development of the study; and the writing, reviewing, and editing of the manuscript.

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SUPPLEMENTAL INFORMATION

1. CHEMICAL REACTION NETWORK THEORY

A **chemical reaction network** (CRN) $\mathcal{N}$ is a triple $(\mathcal{S},\mathcal{C},\mathcal{R})$ of non-empty finite sets $\mathcal{S}$, $\mathcal{C}$, and $\mathcal{R}$ of m species, n complexes, and r reactions, respectively. In a CRN, we denote the species as $X_1,\dots,X_m$. This way, X_i can be identified with the vector in $\mathbb{R}^m$ with 1 in the ith coordinate and zero elsewhere. We denote the reactions as $R_1,\dots,R_r$. We denote the complexes as $C_1,\dots,C_n$ where the manner in which the complexes are numbered play no essential role. A complex $C_i \in \mathcal{C}$ is given as $C_i = \sum_{j=1}^{m} c_{ij}X_j$ or as the vector $c_{i1},\dots,c_{im} \in \mathbb{R}^m_{\geq 0}$ (the subscript ≥ 0 means we consider only the nonnegative real numbers). We define the **zero complex** as the zero vector in $\mathbb{R}^m$. We denote as $C_i \to C_j$ the reaction where complex C_i reacts to complex C_j. A reaction $C_i \to C_j$ is called **reversible** if it is accompanied by its reverse reaction $C_j \to C_i$. Otherwise, it is called **irreversible**.

Let $\mathcal{N} = (\mathcal{S},\mathcal{C},\mathcal{R})$ be a CRN. For each reaction $C_i \to C_j \in \mathcal{R}$, we associate the **reaction vector** $C_j - C_i \in \mathbb{R}^m$. The linear subspace of $\mathbb{R}^m$ spanned by the reaction vectors is called the **stoichiometric subspace** of $\mathcal{N}$, defined as $S = span\{C_j - C_i \in \mathbb{R}^m : C_i \to C_j \in \mathcal{R}\}$. The **rank** of $\mathcal{N}$ is given by $s = dim(S)$, i.e., the rank of the network is the rank of its set of reaction vectors. The **stoichiometric matrix** N is the $m \times r$ matrix whose columns are the reaction vectors of the system. From the definition of stoichiometric subspace, we can see that S is the image of N, written as $S = Im(N)$. Observe that $s = dim(S) = dim\big(Im(N)\big) = rank(N)$.

CRNs can be viewed as directed graphs where the complexes are represented by vertices and the reactions by edges. The **linkage classes** of a CRN are the subnetworks of its reaction graph where for any complexes C_i and C_j of the subnetwork, there is a path between them. The number of linkage classes is denoted by ℓ. The **deficiency** of a CRN is given by $\delta = n - \ell - s$.

A **kinetics** K for a CRN $\mathcal{N} = (\mathcal{S},\mathcal{C},\mathcal{R})$ is an assignment to each reaction $C_i \to C_j \in \mathcal{R}$ of a rate function $K_{C_i\to C_j}: \mathbb{R}^m_{\geq 0} \to \mathbb{R}_{\geq 0}$. The system $(\mathcal{N},K)$ is called a **chemical kinetic system** (CKS). A kinetics gives rise to two closely related objects: the species formation rate function and the associated ordinary differential equation system. The **species formation rate function** (SFRF) of a CKS is given by $f(x) = \sum_{C_i\to C_j} K_{C_i\to C_j}(x)\big(C_j - C_i\big)$ where x is the vector of concentrations of species in $\mathcal{S}$ and $K_{C_i\to C_j}$ is the rate function assigned to reaction $C_i \to C_j \in \mathcal{R}$. The SFRF is simply the summation of the reaction vectors for the network, each multiplied by the corresponding rate function. Note that the SFRF can be written as $f(x) = NK(x)$ where K the vector of rate functions. The equation $\dot{x} = f(x)$ is the **ordinary differential equation** (ODE) **system** or **dynamical system** of the CKS.

The reaction vectors of a CRN are **positively dependent** if, for each reaction $C_i \to C_j \in \mathcal{R}$, there exists a positive number $\alpha_{C_i\to C_j}$ such that $\sum_{C_i\to C_j} \alpha_{C_i\to C_j}\big(C_j - C_i\big) = 0$. CRN with positively dependent reaction vectors is said to be **positive dependent**. Shinar and Feinberg (2012) showed that a CKS can admit a positive equilibrium only if its reaction vectors are positively dependent. The **set of positive equilibria** of a CKS is given by $E_+(\mathcal{N},K) = \{x \in \mathbb{R}^m_{\geq 0} : f(x) = 0\}$. A CRN is said to **admit multiple (positive) equilibria** if there exist positive rate constants such that the ODE system admits more than one stoichiometrically compatible equilibria.

Let F be an $r \times m$ matrix of real numbers. Define x^F by $(x^F)_i = \prod_{j=1}^{m} x_j^{f_{ij}}$ for $i = 1,\dots,r$. A **power law kinetics** (PLK) assigns to each ith reaction a function $K_i(x) = k_i(x^F)_i$ with **rate constant** $k_i > 0$ and **kinetic order** $f_{ij} \in \mathbb{R}$. The vector $k \in \mathbb{R}^r$ is called the **rate vector** and the matrix F is called the **kinetic order matrix**. We refer to a CRN with PLK as a **power law system**. The PLK becomes the well-known **mass action kinetics** (MAK) if the kinetic order matrix consists of stoichiometric coefficients of the reactants. We refer to a CRN with MAK as a **mass action system**.

A CKS is **injective** if, for each pair of distinct stoichiometrically compatible vectors $x^*, x^{**} \in \mathbb{R}^m_{\geq 0}$, at least one of which is positive, $\sum_{C_i\to C_j} K_{C_i\to C_j}(x^{**})\big(C_j - C_i\big) \neq \sum_{C_i\to C_j} K_{C_i\to C_j}(x^{*})\big(C_j - C_i\big)$. Clearly, an injective kinetic system cannot admit two distinct stoichiometrically compatible equilibria, at least one of which is positive. A network $\mathcal{N}$ is **concordant** if and only if for every PLK K, the kinetic system $(\mathcal{N},K)$ is injective. A network that is not concordant is **discordant**.

The following definition of an embedded network is based on Joshi and Shiu (2013). An **embedded network** of a CRN $\mathcal{N}$, which is defined by a subset of the species set $S \subset \mathcal{S}$ and a subset of the reactions set $R \subset \mathcal{R}$, that involves all species of S is the network $\big(S, \mathcal{C}|_{R|_S}, R|_S\big)$ consisting of the reaction set $R|_S$.

We define the **support** of complex $C_i \in \mathcal{C}$ as $supp(C_i) = \{X_j \in S : c_{ij} \neq 0\}$, i.e., it is the set of all species that have nonzero stoichiometric coefficients in complex C_i.

The following definition is from Feinberg (2019). A kinetics K for a reaction network $(\mathcal{S},\mathcal{C},\mathcal{R})$ is **differentiably monotonic** at $c^* \in \mathbb{R}^m_{>0}$ if for every reaction $C_i \longrightarrow C_j \in \mathcal{R}$, $K_{C_i\to C_j}(\cdot)$ is differentiable at c^* and, moreover, for each species $s \in \mathcal{S}$ $\frac{\partial}{\partial c_s} K_{C_i\to C_j}(c^*) \geq 0$, with inequality holding if and only if $s \in supp(C_i)$. A **differentiably monotonic kinetics** is one that is differentiably monotonic at every positive composition.

A kinetics for a CRN is **weakly monotonic** if, for each pair of vectors $x^*, x^{**} \in \mathbb{R}^m_{\geq 0}$, the following implications hold for each reaction $C_i \longrightarrow C_j \in \mathcal{R}$ such that $supp(C_i) \subset supp(x^*)$ and $supp(C_i) \subset supp(x^{**})$:

(i) $K_{C_i\to C_j}(x^{**}) > K_{C_i\to C_j}(x^*)$ implies that there is a species $X_k \in supp(C_i)$ with $x_k^{**} > x_k^*$.

(ii) $K_{C_i\to C_j}(x^{**}) = K_{C_i\to C_j}(x^*)$ implies that $x_k^{**} = x_k^*$ for all $X_k \in supp(C_i)$ or else there are species $X_k, X_k' \in supp(C_i)$ with $x_k^{**} > x_k^*$ and $(x_k')^{**} > (x_k')^*$.

We say that a CKS is **weakly monotonic** when its kinetics is weakly monotonic.

The following is a formulation of the Deficiency Zero Theorem of Horn and Jackson (1972): For a mass action system whose underlying chemical reaction network is weakly reversible and deficiency zero, for any set of rate constants, the system maintains precisely one locally asymptotically stable equilibrium within each positive stoichiometric compatibility class.

A **covering** of a CRN $\mathcal{N}$ is a collection of subsets $\{\mathcal{R}_1, \cdots, \mathcal{R}_k\}$ whose union is $\mathcal{R}$. A covering is called a **decomposition** of $\mathcal{N}$ if the sets $\mathcal{R}_i$ form a partition of $\mathcal{R}$. $\mathcal{R}_i$ defines a subnetwork $\mathcal{N}_i$ of $\mathcal{N}$ where $\mathcal{N}_i = (\mathcal{S}_i, \mathcal{C}_i, \mathcal{R}_i)$ such that $\mathcal{C}_i$ consists of all complexes occurring in $\mathcal{R}_i$, and $\mathcal{S}_i$ has all the species occurring in $\mathcal{C}_i$. We denote a decomposition as a union of the subnetworks: $\mathcal{N} = \mathcal{N}_1 \cup \cdots \cup \mathcal{N}_k$. A decomposition is **independent** if the stoichiometric subspace S of $\mathcal{N}$ is the direct sum of the subnetworks' stoichiometric subspace S_i.

A network decomposition $\mathcal{N} = \mathcal{N}_1 \cup \cdots \cup \mathcal{N}_k$ is a **refinement** of $\mathcal{N} = \mathcal{N}_1' \cup \cdots \cup \mathcal{N}_k'$ (and the latter a **coarsening** of the former) if it is induced by a refinement $\{\mathcal{R}_1, \cdots, \mathcal{R}_k\}$ of $\{R_1', \cdots, R_k'\}$. The decomposition of $\mathcal{N}$ without independent refinement is called the **finest independent decomposition** of $\mathcal{N}$.

2. DEFINITION OF VARIABLES

The following are the variables used in the networks INSulin Metabolic Signaling (INSMS) and INsulin RESistance (INRES):

X_2 = Inactive insulin receptors
X_3 = Insulin-bound receptors
X_4 = Tyrosine-phosphorylated receptors
X_5 = Phosphorylated once-bound surface receptors
X_6 = Internalized dephosphorylated receptors
X_7 = Tyrosine-phosphorylated and internalized receptors
X_8 = Phosphorylated once-bound intracellular receptors
X_9 = Inactive IRS-1
X_{10} = Tyrosine-phosphorylated IRS-1
X_{11} = Unactivated PI 3-kinase
X_{12} = Tyrosine-phosphorylated IRS-1/activated PI 3-kinase complex
X_{13} = PI(3,4,5)P_3 out of the total lipid population
X_{14} = PI(4,5)P_2 out of the total lipid population
X_{15} = PI(3,4)P_2 out of the total lipid population
X_{16} = Unactivated Akt
X_{17} = Activated Akt
X_{18} = Unactivated PKC-ς
X_{19} = Activated PKC-ς
X_{20} = Intracellular GLUT4
X_{21} = Cell surface GLUT4
X_{22} = Combined tyrosine/serine 307-phosphorylated IRS-1
X_{23} = Serine 307-phosphorylated IRS-1
X_{24} = Inactive negative feedback
X_{25} = Active negative feedback
X_{26} = Inactive PKB
X_{27} = Threonine 308-phosphorylated PKB
X_{28} = Serine 473-phosphorylated PKB
X_{29} = Combined threonine 308/serine 473-phosphorylated PKB
X_{30} = mTORC1
X_{31} = mTORC1 involved in phosphorylation of IRS-1 at serine 307
X_{32} = mTORC2
X_{33} = mTORC2 involved in phosphorylation of PKB at threonine 473
X_{34} = AS160
X_{35} = AS160 phosphorylated at threonine 642
X_{36} = S6K
X_{37} = Activated S6K phosphorylated at threonine 389
X_{38} = S6
X_{39} = Activated S6 phosphorylated at serine 235 and serine 236
X_{40} = ERK
X_{41} = ERK phosphorylated at threonine 202 and tyrosine 204
X_{42} = ERK sequestered in an inactive pool
X_{43} = Elk1
X_{44} = Elk1 phosphorylated at serine 383

3. INRES REACTIONS

The following are the reactions of INRES:

$R_1: X_2 \rightarrow X_3$
$R_8: X_6 \rightarrow X_2$
$R_9: X_4 \rightarrow X_7$
$R_{19}: X_{10} \rightarrow X_9$
$R_{31}: X_{21} \rightarrow X_{20}$
$R_{36}: X_2 \rightarrow X_4$
$R_{37}: X_3 \rightarrow X_4$
$R_{38}: X_7 + X_{25} \rightarrow X_6 + X_{25}$
$R_{39}: X_4 \rightarrow X_2$
$R_{40}: X_7 + X_9 \rightarrow X_7 + X_{10}$
$R_{41}: X_9 \rightarrow X_{23}$
$R_{42}: X_{10} + X_{31} \rightarrow X_{22} + X_{31}$
$R_{43}: X_{22} \rightarrow X_{10}$
$R_{44}: X_{22} \rightarrow X_{23}$
$R_{45}: X_{23} \rightarrow X_9$
$R_{46}: X_{10} + X_{24} \rightarrow X_{10} + X_{25}$
$R_{47}: X_{25} \rightarrow X_{24}$
$R_{48}: X_{10} + X_{26} \rightarrow X_{10} + X_{27}$
$R_{49}: X_{27} \rightarrow X_{26}$
$R_{50}: X_{27} + X_{33} \rightarrow X_{29} + X_{33}$
$R_{51}: X_{22} + X_{28} \rightarrow X_{22} + X_{29}$
$R_{52}: X_{29} \rightarrow X_{28}$
$R_{53}: X_{28} \rightarrow X_{26}$
$R_{54}: X_{29} + X_{30} \rightarrow X_{29} + X_{31}$
$R_{55}: X_{27} + X_{30} \rightarrow X_{27} + X_{31}$
$R_{56}: X_{31} \rightarrow X_{30}$
$R_{57}: X_7 + X_{32} \rightarrow X_7 + X_{33}$
$R_{58}: X_{33} \rightarrow X_{32}$
$R_{59}: X_{29} + X_{34} \rightarrow X_{29} + X_{35}$
$R_{60}: X_{28} + X_{34} \rightarrow X_{28} + X_{35}$
$R_{61}: X_{35} \rightarrow X_{34}$
$R_{62}: X_{35} + X_{20} \rightarrow X_{35} + X_{21}$
$R_{63}: X_{31} + X_{36} \rightarrow X_{31} + X_{37}$
$R_{64}: X_{37} \rightarrow X_{36}$
$R_{65}: X_{37} + X_{38} \rightarrow X_{37} + X_{39}$
$R_{66}: X_{38} + X_{41} \rightarrow X_{39} + X_{41}$
$R_{67}: X_{39} \rightarrow X_{38}$
$R_{68}: X_7 + X_{40} \rightarrow X_7 + X_{41}$
$R_{69}: X_{22} + X_{40} \rightarrow X_{22} + X_{41}$
$R_{70}: X_{40} \rightarrow X_{41}$
$R_{71}: X_{41} \rightarrow X_{42}$
$R_{72}: X_{42} \rightarrow X_{40}$
$R_{73}: X_{41} + X_{43} \rightarrow X_{41} + X_{44}$
$R_{74}: X_{44} \rightarrow X_{43}$

4. WEAKLY REVERSIBLE DEFICIENCY ZERO TRANSLATION OF INSMS

The following are the reactions of the original ($\mathcal{N}_{\text{INSMS}}$) and a weakly reversible deficiency zero translation of INSMS ($\mathcal{N}_{\#,\text{INSMS}}$) (a # in the superscript means the reaction was translated):

Subnetwork	$\mathcal{N}_{\text{INSMS}}$		Subnetwork	$\mathcal{N}_{\#,\text{INSMS}}$
$\mathcal{N}_{\text{INSMS},1}$	$R_1: X_2 \to X_3$ $R_2: X_3 \to X_2$ $R_3: X_5 \to X_4$ $R_4: X_4 \to X_5$ $R_5: X_3 \to X_5$ $R_6: X_5 \to X_2$ $R_7: X_2 \to X_6$ $R_8: X_6 \to X_2$ $R_9: X_4 \to X_7$ $R_{10}: X_7 \to X_4$ $R_{11}: X_5 \to X_8$ $R_{12}: X_8 \to X_5$ $R_{15}: X_7 \to X_6$ $R_{16}: X_8 \to X_6$		$\mathcal{N}_{\#,\text{INSMS},1}$	$R_1: X_2 \to X_3$ $R_2: X_3 \to X_2$ $R_3: X_5 \to X_4$ $R_4: X_4 \to X_5$ $R_5: X_3 \to X_5$ $R_6: X_5 \to X_2$ $R_7: X_2 \to X_6$ $R_8: X_6 \to X_2$ $R_9: X_4 \to X_7$ $R_{10}: X_7 \to X_4$ $R_{11}: X_5 \to X_8$ $R_{12}: X_8 \to X_5$ $R_{15}: X_7 \to X_6$ $R_{16}: X_8 \to X_6$
$\mathcal{N}_{\text{INSMS},2}$	$R_{13}: 0 \to X_6$ $R_{14}: X_6 \to 0$		$\mathcal{N}_{\#,\text{INSMS},2}$	$R_{13}: 0 \to X_6$ $R_{14}: X_6 \to 0$
$\mathcal{N}_{\text{INSMS},3}$	$R_{17}: X_9 + X_4 \to X_{10} + X_4$ $R_{18}: X_9 + X_5 \to X_{10} + X_5$ $R_{19}: X_{10} \to X_9$		$\mathcal{N}_{\#,\text{INSMS},3}$	$R_{17}^{\#}: X_9 \to X_{10}$ $R_{19}: X_{10} \to X_9$
$\mathcal{N}_{\text{INSMS},4}$	$R_{20}: X_{10} + X_{11} \to X_{12}$ $R_{21}: X_{12} \to X_{10} + X_{11}$		$\mathcal{N}_{\#,\text{INSMS},4}$	$R_{20}: X_{10} + X_{11} \to X_{12}$ $R_{21}: X_{12} \to X_{10} + X_{11}$
$\mathcal{N}_{\text{INSMS},5}$	$R_{22}: X_{14} + X_{12} \to X_{13} + X_{12}$ $R_{23}: X_{13} \to X_{14}$		$\mathcal{N}_{\#,\text{INSMS},5}$	$R_{22}^{\#}: X_{14} \to X_{13}$ $R_{23}: X_{13} \to X_{14}$
$\mathcal{N}_{\text{INSMS},6}$	$R_{24}: X_{15} \to X_{13}$ $R_{25}: X_{13} \to X_{15}$		$\mathcal{N}_{\#,\text{INSMS},6}$	$R_{24}: X_{15} \to X_{13}$ $R_{25}: X_{13} \to X_{15}$
$\mathcal{N}_{\text{INSMS},7}$	$R_{26}: X_{16} + X_{13} \to X_{17} + X_{13}$ $R_{27}: X_{17} \to X_{16}$		$\mathcal{N}_{\#,\text{INSMS},7}$	$R_{26}^{\#}: X_{16} \to X_{17}$ $R_{27}: X_{17} \to X_{16}$
$\mathcal{N}_{\text{INSMS},8}$	$R_{28}: X_{18} + X_{13} \to X_{19} + X_{13}$ $R_{29}: X_{19} \to X_{18}$		$\mathcal{N}_{\#,\text{INSMS},8}$	$R_{28}^{\#}: X_{18} \to X_{19}$ $R_{29}: X_{19} \to X_{18}$
$\mathcal{N}_{\text{INSMS},9}$	$R_{30}: X_{20} \to X_{21}$ $R_{31}: X_{21} \to X_{20}$ $R_{32}: X_{20} + X_{17} \to X_{21} + X_{17}$ $R_{33}: X_{20} + X_{19} \to X_{21} + X_{19}$		$\mathcal{N}_{\#,\text{INSMS},9}$	$R_{30}: X_{20} \to X_{21}$ $R_{31}: X_{21} \to X_{20}$
$\mathcal{N}_{\text{INSMS},10}$	$R_{34}: 0 \to X_{20}$ $R_{35}: X_{20} \to 0$		$\mathcal{N}_{\#,\text{INSMS},10}$	$R_{34}: 0 \to X_{20}$ $R_{35}: X_{20} \to 0$

5. WEAKLY REVERSIBLE DEFICIENCY ZERO TRANSLATION OF INRES

The following are the reactions of the original ($\mathcal{N}_{\text{INRES}}$) and a weakly reversible deficiency zero translation of INRES ($\mathcal{N}_{\#,\text{INRES}}$) (a # in the superscript means the reaction was translated):

Subnetwork	$\mathcal{N}_{\text{INRES}}$		Subnetwork	$\mathcal{N}_{\#,\text{INRES}}$
$\mathcal{N}_{\text{INRES},1}$	$R_1: X_2 \to X_3$ $R_8: X_6 \to X_2$ $R_9: X_4 \to X_7$ $R_{36}: X_2 \to X_4$ $R_{37}: X_3 \to X_4$ $R_{38}: X_7 + X_{25} \to X_6 + X_{25}$ $R_{39}: X_4 \to X_2$		$\mathcal{N}_{\#,\text{INRES},1}$	$R_1: X_2 \to X_3$ $R_8: X_6 \to X_2$ $R_9: X_4 \to X_7$ $R_{36}: X_2 \to X_4$ $R_{37}: X_3 \to X_4$ $R_{38}^{\#}: X_7 \to X_6$ $R_{39}: X_4 \to X_2$
$\mathcal{N}_{\text{INRES},2}$	$R_{19}: X_{10} \to X_9$ $R_{40}: X_7 + X_9 \to X_7 + X_{10}$ $R_{41}: X_9 \to X_{23}$ $R_{42}: X_{10} + X_{31} \to X_{22} + X_{31}$ $R_{43}: X_{22} \to X_{10}$ $R_{44}: X_{22} \to X_{23}$ $R_{45}: X_{23} \to X_9$		$\mathcal{N}_{\#,\text{INRES},2}$	$R_{19}: X_{10} \to X_9$ $R_{40}^{\#}: X_9 \to X_{10}$ $R_{41}: X_9 \to X_{23}$ $R_{42}^{\#}: X_{10} \to X_{22}$ $R_{43}: X_{22} \to X_{10}$ $R_{44}: X_{22} \to X_{23}$ $R_{45}: X_{23} \to X_9$
$\mathcal{N}_{\text{INRES},3}$	$R_{46}: X_{10} + X_{24} \to X_{10} + X_{25}$ $R_{47}: X_{25} \to X_{24}$		$\mathcal{N}_{\#,\text{INRES},3}$	$R_{46}^{\#}: X_{24} \to X_{25}$ $R_{47}: X_{25} \to X_{24}$
$\mathcal{N}_{\text{INRES},4}$	$R_{48}: X_{10} + X_{26} \to X_{10} + X_{27}$ $R_{49}: X_{27} \to X_{26}$ $R_{50}: X_{27} + X_{33} \to X_{29} + X_{33}$		$\mathcal{N}_{\#,\text{INRES},4}$	$R_{48}^{\#}: X_{26} \to X_{27}$ $R_{49}: X_{27} \to X_{26}$ $R_{50}^{\#}: X_{27} \to X_{29}$

	$R_{51}: X_{22} + X_{28} \rightarrow X_{22} + X_{29}$ $R_{52}: X_{29} \rightarrow X_{28}$ $R_{53}: X_{28} \rightarrow X_{26}$			$R_{51}^{\#}: X_{28} \rightarrow X_{29}$ $R_{52}: X_{29} \rightarrow X_{28}$ $R_{53}: X_{28} \rightarrow X_{26}$
$\mathcal{N}_{\text{INRES},5}$	$R_{54}: X_{29} + X_{30} \rightarrow X_{29} + X_{31}$ $R_{55}: X_{27} + X_{30} \rightarrow X_{27} + X_{31}$ $R_{56}: X_{31} \rightarrow X_{30}$		$\mathcal{N}_{\#,\text{INRES},5}$	$R_{54}^{\#}: X_{30} \rightarrow X_{31}$ $R_{56}: X_{31} \rightarrow X_{30}$
$\mathcal{N}_{\text{INRES},6}$	$R_{57}: X_{7} + X_{32} \rightarrow X_{7} + X_{33}$ $R_{58}: X_{33} \rightarrow X_{32}$		$\mathcal{N}_{\#,\text{INRES},6}$	$R_{57}^{\#}: X_{32} \rightarrow X_{33}$ $R_{58}: X_{33} \rightarrow X_{32}$
$\mathcal{N}_{\text{INRES},7}$	$R_{59}: X_{29} + X_{34} \rightarrow X_{29} + X_{35}$ $R_{60}: X_{28} + X_{34} \rightarrow X_{28} + X_{35}$ $R_{61}: X_{35} \rightarrow X_{34}$		$\mathcal{N}_{\#,\text{INRES},7}$	$R_{59}^{\#}: X_{34} \rightarrow X_{35}$ $R_{61}: X_{35} \rightarrow X_{34}$
$\mathcal{N}_{\text{INRES},8}$	$R_{31}: X_{21} \rightarrow X_{20}$ $R_{62}: X_{35} + X_{20} \rightarrow X_{35} + X_{21}$		$\mathcal{N}_{\#,\text{INRES},8}$	$R_{31}: X_{21} \rightarrow X_{20}$ $R_{62}^{\#}: X_{20} \rightarrow X_{21}$
$\mathcal{N}_{\text{INRES},9}$	$R_{63}: X_{31} + X_{36} \rightarrow X_{31} + X_{37}$ $R_{64}: X_{37} \rightarrow X_{36}$		$\mathcal{N}_{\#,\text{INRES},9}$	$R_{63}^{\#}: X_{36} \rightarrow X_{37}$ $R_{64}: X_{37} \rightarrow X_{36}$
$\mathcal{N}_{\text{INRES},10}$	$R_{65}: X_{37} + X_{38} \rightarrow X_{37} + X_{39}$ $R_{66}: X_{38} + X_{41} \rightarrow X_{39} + X_{41}$ $R_{67}: X_{39} \rightarrow X_{38}$		$\mathcal{N}_{\#,\text{INRES},10}$	$R_{65}^{\#}: X_{38} \rightarrow X_{39}$ $R_{67}: X_{39} \rightarrow X_{38}$
$\mathcal{N}_{\text{INRES},11}$	$R_{68}: X_{7} + X_{40} \rightarrow X_{7} + X_{41}$ $R_{69}: X_{22} + X_{40} \rightarrow X_{22} + X_{41}$ $R_{70}: X_{40} \rightarrow X_{41}$ $R_{71}: X_{41} \rightarrow X_{42}$ $R_{72}: X_{42} \rightarrow X_{40}$		$\mathcal{N}_{\#,\text{INRES},11}$	$R_{70}: X_{40} \rightarrow X_{41}$ $R_{71}: X_{41} \rightarrow X_{42}$ $R_{72}: X_{42} \rightarrow X_{40}$
$\mathcal{N}_{\text{INRES},12}$	$R_{73}: X_{41} + X_{43} \rightarrow X_{41} + X_{44}$ $R_{74}: X_{44} \rightarrow X_{43}$		$\mathcal{N}_{\#,\text{INRES},12}$	$R_{73}^{\#}: X_{43} \rightarrow X_{44}$ $R_{74}: X_{44} \rightarrow X_{43}$

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