Computational verification of large logical models - application to the prediction of T cell response to checkpoint inhibitors

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ABSTRACT

At the crossroad between biology and mathematical modelling, computational systems biology can contribute to a mechanistic understanding of high-level biological phenomenon. But as our knowledge accumulates, the size and complexity of mathematical models increase, calling for the development of efficient dynamical analysis methods.

Here, we take advantage of generic computational techniques to enable the dynamical study of complex cellular network models.

A first approach, called "model verification" and inspired by unitary testing in software development, enables the formalisation and automated verification of validation criteria for whole models or selected sub-parts, thereby greatly facilitating model development.

A second approach, called "value propagation", enables efficient analytical computation of the impact of specific environmental or genetic conditions on model dynamics.

We apply these two approaches to the delineation and the analysis of a comprehensive model for T cell activation, taking into account CTLA4 and PD-1 checkpoint inhibitory pathways. While the use of model verification greatly eased the delineation of logical rules complying with a set of dynamical specifications, the use of value propagation provided interesting insights into the different potential of CTLA4 and PD-1 immunotherapies.

Both methods are implemented and made available in the all-inclusive CoLoMoTo Docker image, while the different steps of the model analysis are fully reported in two companion interactive jupyter notebooks, thereby ensuring the reproduction of our results.

Keywords: T Cell, checkpoint inhibitors, Boolean models, model validation, value propagation.

1 INTRODUCTION

Recent technical developments have allowed scientists to study immunology and health-related issues from a variety of angles. For many diseases, especially for cancer, the current trend consists in aggregating data coming from different sources to gain a global view of cell, tissue, or organ dysfunction. Over the last decades, diverse mathematical frameworks have been proposed to seize a multiplicity of biological questions (Eftimie et al., 2016; Chakraborty, 2017). However, the increasing complexity of biological questions implies the development of more sophisticated models, which in turn bring serious computational challenges.

Among the mathematical approaches proposed for the modelling of cellular networks, the logical modelling framework appeared particularly adapted (see e.g. (Thomas, 1991)). In particular, it has been successfully applied to immunology and cancer, leading to the creation of models encompassing dozens of components, some including many inputs components (Grieco et al., 2013; Abou-Jaoudé et al., 2014; Flobak et al., 2015; Oyeyemi et al., 2015). However, the large size of these models hinders the complete exploration of their dynamical behaviour through simulation, especially in non-deterministic settings.

To address these difficulties, we hereby propose the application of two generic computational methods.

First, we define and apply a *model verification* method to systematically verify whether a model complies with a list of known properties (section 2). These properties are formally defined as model *specifications*, either at a local (i.e. for sub-networks) or at a global level. This automated verification procedure fosters confidence during the development of a complex dynamical model.

We further define and apply a *value propagation* method, which enables the assessment of the impact of environmental or genetic constraints on the dynamical behaviour of complex cellular networks (section 3).

Noteworthy, these two complementary approaches have been implemented in a multi-platform Docker image combining various complementary logical modelling and analysis tools (Naldi et al., 2018b). We further illustrate the power of these methods through the analysis of an original model described in Section 4. The different steps of analysis are fully reported in two companion interactive jupyter notebooks, available with the model on the GINsim website (http://ginsim.org/model/tcell-checkpoint-inhibitors-tcla4-pd1), thereby ensuring their reproducibility.

2 MODEL VERIFICATION FRAMEWORK

One of the main features determining the interest of a model is its ability to accurately recapitulate salient biological properties. Failures to reproduce such properties need to be carefully documented as a basis for model improvement. Establishing specific satisfaction criteria is required for rigorous model assessment or comparison with other models.

This need to establish criteria of success or failure can be related to *software verification* in computer science. The main goal of software verification is to assess whether a software meets a well-defined list of requirements. Software verification relies in particular on ”model checking” and ”unit testing”, which can be transposed from computer science to model building.

Model checking techniques have already been successfully applied to specify temporal constraints on a model behaviour (Monteiro and Chaouiya, 2012; Traynard et al., 2016). Here, we generalise the *model verification* approach to integrate a comprehensive series of verifiable criteria from the early stages of model conception, and to fully automate the process of logical model verification. In this respect, we take

advantage of an existing generic unit-testing framework to define and evaluate test cases for dynamical models.

Once split into individual verifiable criteria, the knowledge on which a model is based can be used as specifications (Figure 1). Individual units of knowledge derived from the scientific literature or biological experiments are translated as stable or dynamical properties. Each specification coupling a property with an expected value can serve as a basis to define a test case for a model. Testing a model for each specification then amounts to compute a property and compare the expected and observed values.

Individual test cases can be assembled into a library, also called testing suite. Existing tools and packages enabling software testing can then be applied to automatically assess whether a model satisfies (or not) a series of specifications. In this study, we used the python package 'unittest', taking advantage of its seamless integration into the CoLoMoTo interactive notebook (Naldi et al., 2018b).

2.1 Local verification of sub-models can cope with sparsity of biological knowledge

Biological knowledge reported in the scientific literature is often insufficient to evaluate a comprehensive model, which may encompass hundreds of nodes. In particular, observations regarding component activity often relate to only a limited subset of nodes of the model. This greatly complicates the definition of specifications for the whole model.

Given a comprehensive model and a set of nodes of interest, one can extract a sub-model containing these nodes, along with the logical rules assigned to them. To study the dynamics induced by these functions, we further need to consider all the other nodes acting on the sub-model nodes, which are then considered as inputs of the sub-model (Figure 2). As shown in Section 4, the delineation of such sub-models can greatly facilitate the definition and verification of local specifications.

3 VALUE PROPAGATION ENABLES THE EVALUATION OF THE IMPACT OF A GIVEN CELLULAR ENVIRONMENT ON MODEL DYNAMICS

The core idea of value propagation is presented in Figure 3. Given a set of logical rules and a cellular context, an iterative algorithm enables the computation of the dynamical consequences of the cellular context on all the components of the model.

First, the cellular context is formalised by assigning constant values to some components of the model. Next, we apply a recent model reduction technique (Saadatpour et al., 2013). Briefly, for each constant node, the corresponding value is inserted into the logical rule associated with each of its target nodes. Each logical rule is then simplified using Boolean algebra. If the rule simplifies to a constant, this fixed value is further propagated into the logical rules of downstream nodes. This process is iterated until no further propagation or simplification can be made on the logical rules of the model.

This method has multiple advantages when applied to complex networks, as it can be applied efficiently to models with large numbers of components. Like a model reduction method, it simplifies the computation of attractors (stable states or even simple or complex cycles). Interestingly, Saadatpour and collaborators showed that this method conserves the stable states and complex attractors under the fully asynchronous updating assumption (Saadatpour et al., 2013). We have recently extended this model reduction technique to multilevel models and implemented it in the Java bioLQM library (Naldi, 2018).

The result of such value propagation can be very informative by itself. Indeed, the resulting stabilised values provide insights into the impact of a given biological context on the model, revealing which elements tend to be activated or inactivated. We can further compare the number of fixed components after fixing

independently different model components. To perform such analyses, we further adapted the method to keep track of percolated fixed values. The power of this approach is demonstrated on a concrete example in the following section.

4 APPLICATION: ASSESSING THE EFFECT OF CHECKPOINT BLOCKADE THERAPIES ON T CELL ACTIVATION

4.1 Biological background

Over the last decades, immunotherapies have been the subject of intense studies and led to great advances in the field of cancer treatment. Through the years, it has then been recognised that T cells often display a reduced ability to eliminate cancer cells, and that expression of co-inhibitory receptors at their surface accounts for this compromised function. Receptors like Cytotoxic T-lymphocyte protein 4 (CTLA4, also known as CD152) (Walunas et al., 1994; Leach et al., 1996) and Programmed cell death protein 1 (PD-1, also known as PDCD1 or CD279) (Ishida et al., 1992) have been particularly studied in that context. Antibodies blocking the pathways downstream of these co-inhibitors (checkpoint blockade therapies) have become standard treatment for metastatic melanoma (Robert et al., 2011; Simpson et al., 2013) and others cancers (Ribas and Wolchok, 2018), including non-small cell lung cancer, renal cell carcinoma, Hodgkin's lymphoma, Merkel cell carcinoma and many others. The successes of these studies led to an increasing interest in T cell co-inhibitory receptors.

Given the central role of T cells in many medical contexts, several mathematical frameworks have been applied to model T cell activation. Recent examples include rule-based approaches (Chylek et al., 2014), ordinary differential equations (Perley et al., 2014), and logical models (Oyeyemi et al., 2015; Rodríguez-Jorge et al., 2019; Sánchez-Villanueva et al., 2019), considering different biomedical contexts.

In this study, we applied the logical framework to integrate current data on CTLA4 and PD-1 pathways and assess their impact on T cell activation.

4.2 Comprehensive molecular mapping of T Cell activation network

Prior to mathematical modelling, knowledge about biological entities involved in T Cell activation was collected from available pathway databases, including Reactome (Fabregat et al., 2016), PantherDB (Mi et al., 2013), ACSN (Kuperstein et al., 2015) and WikiPathways (Slenter et al., 2018). Scientific literature indexed in the PubMed database was also explored and curated. Using the software CellDesigner (version 4.3.1) (Funahashi et al., 2008), this knowledge was summarized as a molecular map describing reactions between biological entities (either proteins, RNAs, genes, complexes or metabolites). Each biological entity included in the map was annotated with a series of standard identifiers, including UniProtKB accession number, recommended and alternative names, gene name and synonyms, and cross-references to unique HGNC identifiers and approved symbols. The annotations include references to relevant scientific articles, including PubMed identifier, first and last authors, year of publication, and a list of observations extracted from these publications.

Our T cell activation map currently encompasses 726 biological entities, in different states (active/inactive, with or without post-translational modifications), and 539 reactions involving these entities (Supplementary Figure 1 and File 1). Globally, the map currently integrates information from 123 scientific articles, which are cited in the annotations of the entities and reactions of the map.

4.3 Logical modelling of T Cell activation

Using the logical modelling software *GINsim* (version 3.0.0b) (Naldi et al., 2018a), we then manually derived a regulatory graph encompassing 216 nodes and 451 arcs (Figure 4). To obtain a dynamical Boolean model, a specific logical rule must be assigned to each component. In many cases, this can be achieved rather easily based on published data. In some case, however, in particular when a component is the target of various regulatory interactions, finding direct support for a specific rule may turn out tricky or impossible. Hence, the delineation of consistent logical rules for a complex model is often the result of an iterative process, starting with generic rules and progressively correcting them based on the result of dynamical analyses.

Hereafter, we demonstrate how we can take advantage of the methods presented in the previous sections to ease rule refinement by model verification. We first defined a series of properties expected for the model (see examples in Table 1). Next, stable states and/or trap spaces were computed and automatically compared with these data (cf. first Jupyter notebook provided on the model web page at http://ginsim.org/model/tcell-checkpoint-inhibitors-tcla4-pd1). After some iterative, manual refinements, we converged towards a set of rules complying with all the tests.

Based on the resulting model, a comparative propagation analysis was performed to visualise the effects of CTLA4 and PD-1 receptor activation on model dynamics. Figure 5 displays the value propagation for each condition on a single regulatory graph, using a color code to distinguish the different situations (component inhibition/activation in one or both conditions). The value propagation for the two conditions are further shown separately in the second companion notebook (http://ginsim.org/model/tcell-checkpoint-inhibitors-tcla4-pd1). This analysis reveals that the activation of the CTLA4 receptor impacts most pathways of the model, impeding in particular the remodeling of the cytoskeleton and the metabolic switch associated with bona fide T cell activation. In contrast, the activation of the PD-1 receptor leads to more limited effects, predominantly freezing the components of the NFκB pathway.

A more refined comparative analysis of value propagation from these two receptor activations entails the observation that the set of nodes frozen by the propagation of PD-1 activation is completely included inside the set of nodes frozen by the propagation of CTLA4 activation (see Table 2). Furthermore, the component values frozen in both progagation studies are fully convergent. Interestingly, a set of nodes related to calcium influx from and to the endoplasmic reticulum remain unfixed by any of the propagation analyses. A more detailed biological interpretation of these results is proposed in the following section.

5 CONCLUSIONS AND PROSPECTS

In this study, we have implemented and applied two complementary methods enabling a specification-oriented model building approach and thereby easing the delineation and analysis of highly complex logical models. In this respect, the building a knowledge base, e.g. in terms of a molecular map, is an important first step. We have integrated the most relevant biological data available on T cell activation and inhibition pathways in a comprehensive molecular map. This map is clearly due to evolve, in particular thanks to the generation and analysis of novel high-throughput data (see e.g. the recent extensive analysis of the TCR signalosome (Voisinne et al., 2019)).

To date, methods to derive proper dynamical models from such molecular maps are still in their infancy. In the particular case of the Boolean framework, an automated approach has been recently proposed (Niarakis et al., 2020). However, a limitation of this approach is the generation of generic logical rules based on static knowledge. Hence, the methods presented here could be used to refine these rules taking into account additional knowledge about the dynamics of the system.

We used the information gathered in our T cell activation map to build a dynamical logical model encompassing over 200 components and 450 interactions. For such a complex model, defining the logical rules is a difficult and error-prone process, usually involving iterative trial simulations, where failures are identified to suggest potential improvements. Hence, listing comprehensive and consistent model specifications is a crucial step for model construction. Such listing is itself often due to revisions, as the modeller deepens its understanding of the biological processes under study.

Model checking techniques have been previously applied to assess model behaviour through systematic cycles of model refinements (see e.g. (Traynard et al., 2016) and reference therein). Model verification, as defined here, is a generalisation of this approach. In our hands, in the course of model building, the unit testing approach proved to be very efficient to assess and improve model consistency with respect to a list of specifications. Implemented in the CoLoMoTo Interactive notebook framework (Naldi et al., 2018b), this approach enabled us to define a model recapitulating the most salient properties observed in response to T cell activation, including quiescence, anergy and differentiation.

This approach could be improved by taking into account and tracking uncertainty during model conception (Thobe et al., 2018), or yet by taking advantage of computational repairing methods (Gebser et al., 2010) to identify more precisely remaining inconsistencies with biological data.

Value propagation analysis of our large and complex regulatory graph proved to be biologically insightful. Indeed, this straightforward approach enabled us to clearly contrast the respective impacts of CTLA4 and PD-1 on T cell activation, providing some rationale for their differential effects in current therapeutic essays.

The state of the node representing the Interleukin 2 (IL2) cytokine activation illustrates the differences of action of these receptors. Activation of the IL2 gene depends mainly on the activation of three transcription factors: the Nuclear Factor of Activated T cells (NFAT), the AP1 complex, and the Nuclear factor NF-kappa-B (NFκB) (Smith-Garvin et al., 2009). When NFAT and AP1 are both active, they form a complex and together bind a regulatory region of the IL2 gene. In absence of AP1, NFAT induces a different program leading to cellular anergy (Macian, 2005; Smith-Garvin et al., 2009): activation of Diacylglycerol Kinase (DGK) prevents DAG-mediated activation of RasGRP1, which regulates the threshold for T cell activation (Roose et al., 2007; Das et al., 2009).

Our comparative propagation analysis reveals that while the activation of the CTLA4 receptor leads to a general inactivation of the three transcription factors regulating IL2 production, activation of the PD-1 receptor leads only to the inactivation of NFκB and FOS (a member of the AP1 complex), thereby preventing the formation of the NFAT/AP1 complex, but enabling the activation of DGK. This observation is consistent with the proposal to target DGK isoforms as a complement of checkpoint immunotherapy (Riese et al., ????; Jung et al., 2018).

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

CH developed the T cell signalling molecular map and model under the supervision of MTC and DT. CH and AN implemented the computational methods and applied them to the T cell model under the

supervision of AN and DT. All co-authors contributed to the redaction of the manuscript and endorse its content.

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SUPPLEMENTAL DATA

Supplementary Figure 1: Molecular map in good resolution or vectorial format Caption: This molecular map describes biological entities and reactions implicated in the process leading to activation of CD4+ T cells in humans/mouse. The cytoplasmic membrane and attached receptor proteins are placed at the top, while the cell nucleus is located at the bottom. Reactions represent current knowledge of various pathways related to cytoskeletal remodelling, calcium fluxes, metabolism, cell cycle or IL2 production, and are encoded using the CellDesigner software (Funahashi et al., 2008) (the CellDesigner file is provided as Supplementary File 1).

Supplementary File 1: Molecular map in CellDesigner/XML format (Funahashi et al., 2008).

DATA AVAILABILITY STATEMENT

In addition, the original model (GINsim format - zginml) can be downloaded from the GINsim website (http://ginsim.org/model/tcell-checkpoint-inhibitors-tcla4-pd1), together with previews of the two interactive notebooks enabling the reproduction of all the analyses and results reported in this study, using the tools integrated in the most recent CoLoMoTo Docker image (https://github.com/colomoto/colomoto-docker). A SBML-qual export of the logical model is also available on the GINsim model repository page, which will be further deposited into the database BioModels (https://www.ebi.ac.uk/biomodels/) upon publication.

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FIGURES

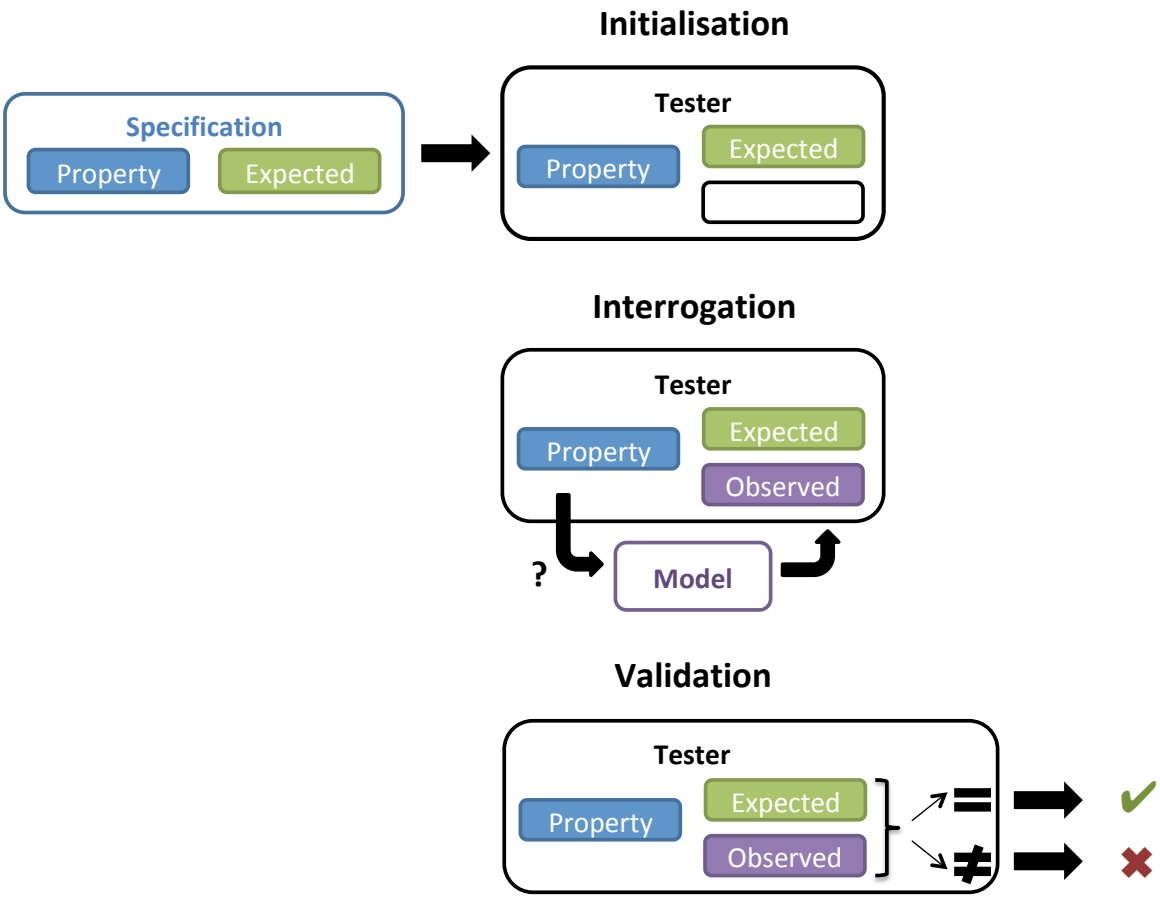

Figure 1. Model verification relies on three components: the model to be tested, a list of specifications, and an automated tester, for instance a program importing Unit testing functionalities. It can be viewed as a succession of steps. First, the tester is initialised by a specification describing a given property along with its expected value. Next, the tester interrogates the model for the property and returns an observed value. Finally, the tester compares the expected and observed values. If equal, the model is considered as valid for the specification, while it is considered as invalid otherwise.

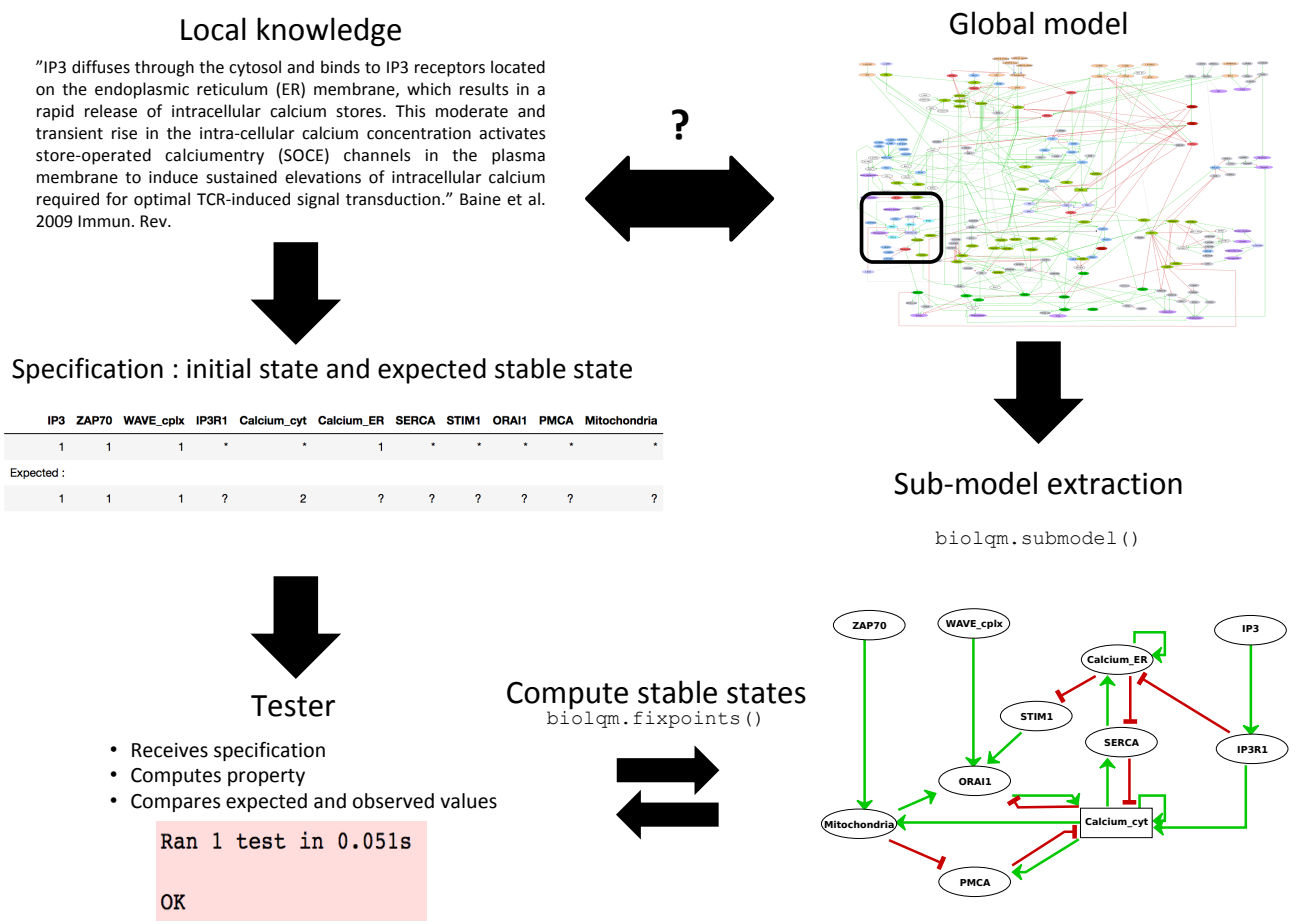

Figure 2. Sub-model extraction for local model verification. Based on partial information on the behaviour of biological system under study, a series of specifications is defined for a sub-model, which can then be rigorously tested.

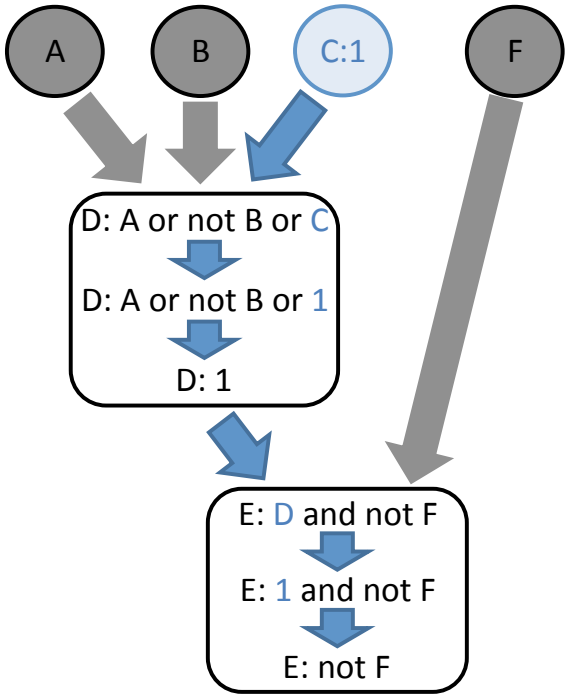

Figure 3. The principle of logical value propagation analysis is illustrated with a simple example involving two core nodes, D and E, and four input nodes, A, B, C and F. The value 1 is assigned to the node C and then percolated through the model. The assignment C:1 implies the evaluation of D to 1. Consequently, the function assigned to node E becomes 'not F'. In other words, assigning the value 1 to node C activates node D independently from the value of its other inputs, while node E becomes completely dependent on the value of node F.

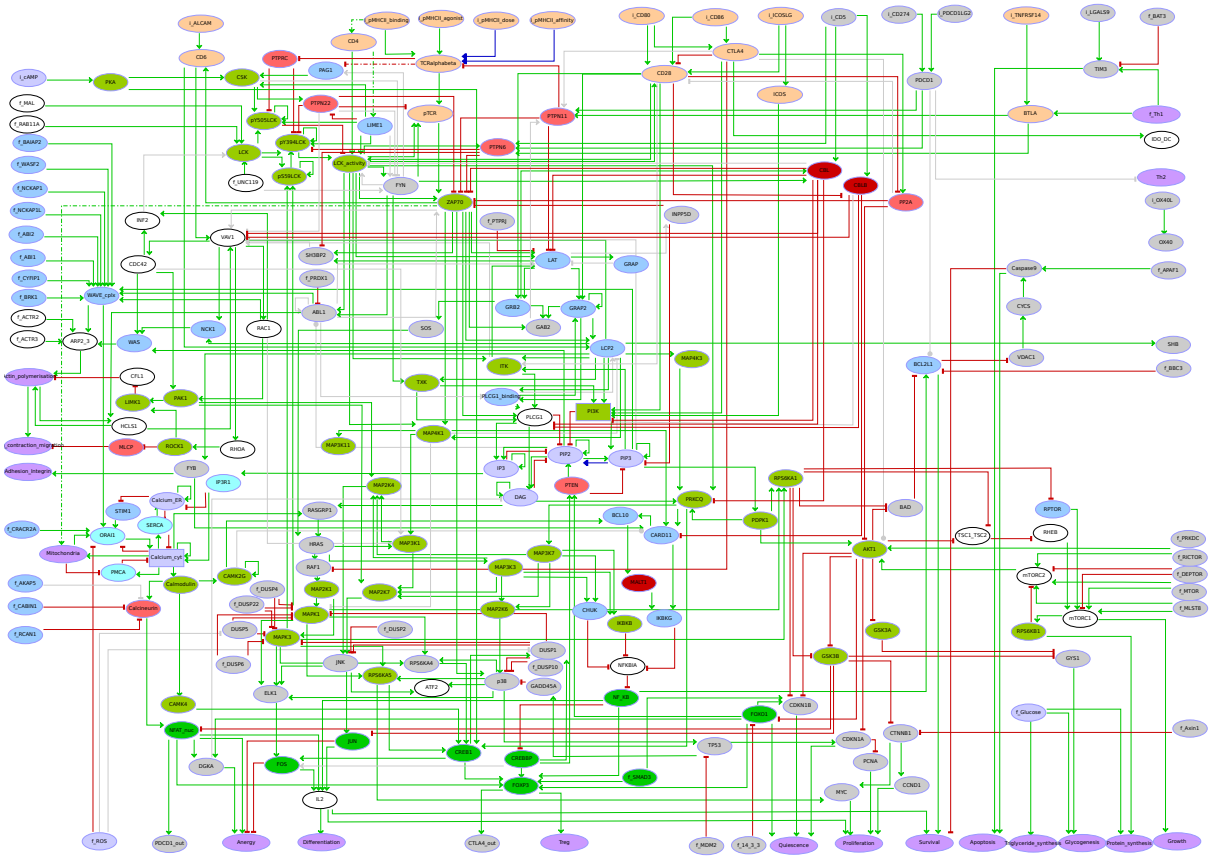

Figure 4. Regulatory graph of the T cell activation model. The global layout is similar to the molecular map (cf. supplementary Figure 1 and supplementary File 1), with ligands/receptors and proximal signalling at the top, and the nucleus-related events at the bottom of the graph. Green arcs denote activation events, red blunt arcs denotes inhibitions, while blue arcs denote dual regulation. The grey arcs represent interactions created during the translation of the molecular map into the regulatory graph, but that are not yet integrated at the dynamical level (i.e. not taken into account in the logical functions).

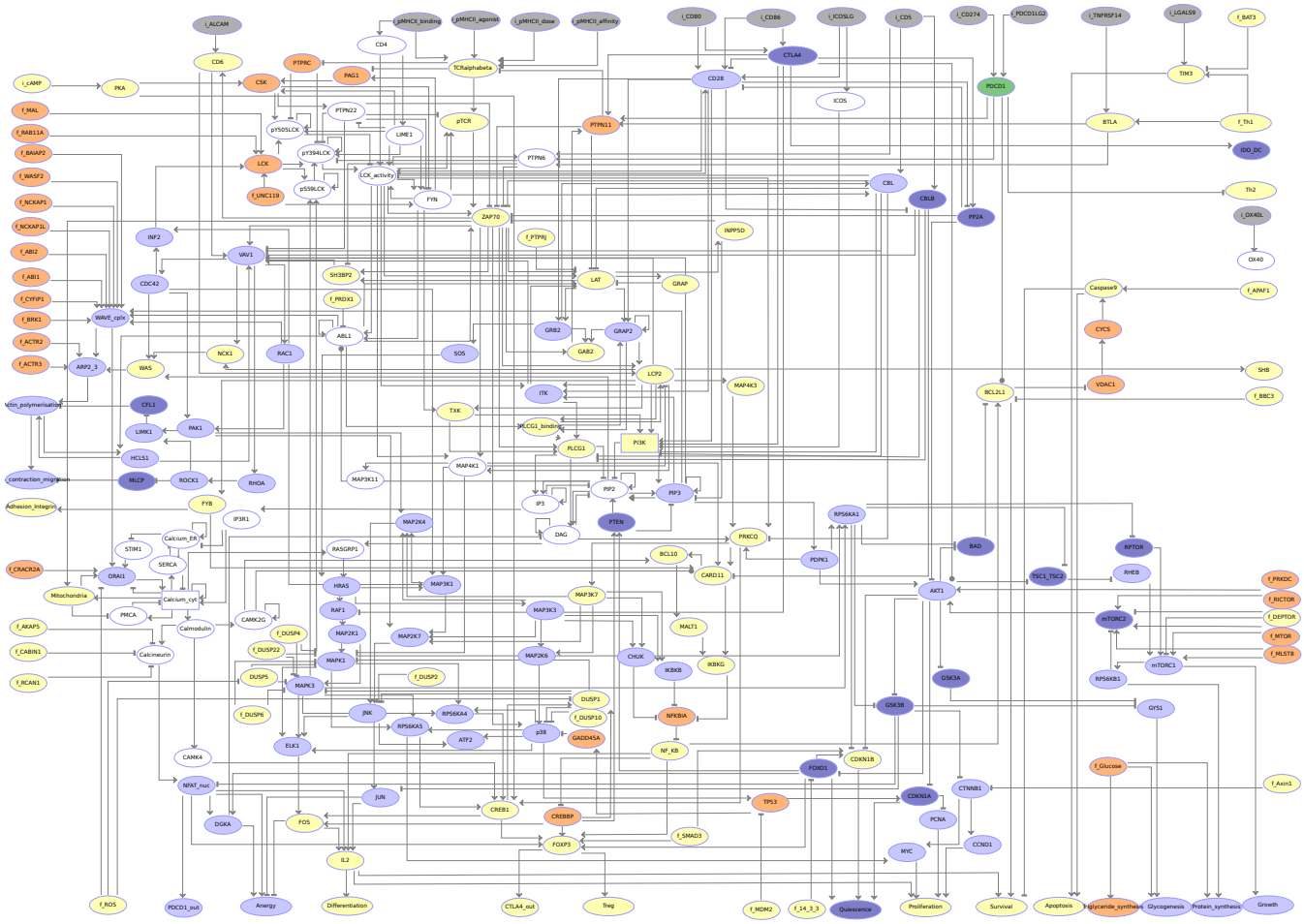

Figure 5. Visualisation of the results of the percolation analyses for CTLA4 versus PD-1 activation. Grey nodes correspond to inputs. Nodes in yellow are frozen OFF upon any of CTLA4 or PD-1 (PDCD1) activation. Nodes in orange are activated for both conditions. Nodes in light blue are frozen OFF only for CTLA4 activation. Nodes in dark blue are activated only upon CTLA4 activation. Upon PD-1 activation, the corresponding node (PDCD1) is the only one that gets specifically frozen (ON, shown in dark green). Nodes in white remain free for both conditions.

Context	Expected behaviour (active or inactive components)
Global specifications	
No stimulation	Active: Quiescence, Glycogenesis
Non-optimal stimulation (antagonist or low/high dosage/affinity)	Active: Anergy (DGKA), Quiescence, Glycogenesis
Optimal stimulation	Active: Differentiation, IL2, Proliferation, Growth, Actin polymerisation, Actin contraction
CTLA4 stimulation	Active: Quiescence
PD-1 stimulation	Active: Quiescence
Local specifications	
Absence of IP3R stimulation, Calcium in ER	Active: Calcium_ER; Inactive: IP3R1
Absence of IP3R stimulation, Calcium in the cytoplasm	Active: Calcium_ER; Inactive: IP3R1
IP3R stimulation, Calcium in ER	Active: IP3R1, Calcineurin; Inactive: Calcium_ER

Table 1. Global specifications used to assess the T cell activation model and example of local specifications for the calcium signaling module. After verification, named components should have a value of 0 if specified as inactive, while active components should have a value of 1 or above. Verification of local specifications requires the extraction of a sub-model from the global model. These verifications and literature references are detailed in the companion CoLoMoTo notebooks. ER: Endoplasmic Reticulum.

Impact of value propagation	CTLA4 ON	PD-1 ON	Intersection
Frozen inactive nodes	105	47	47
Frozen active nodes	28	13	12
Free nodes	29	102	28
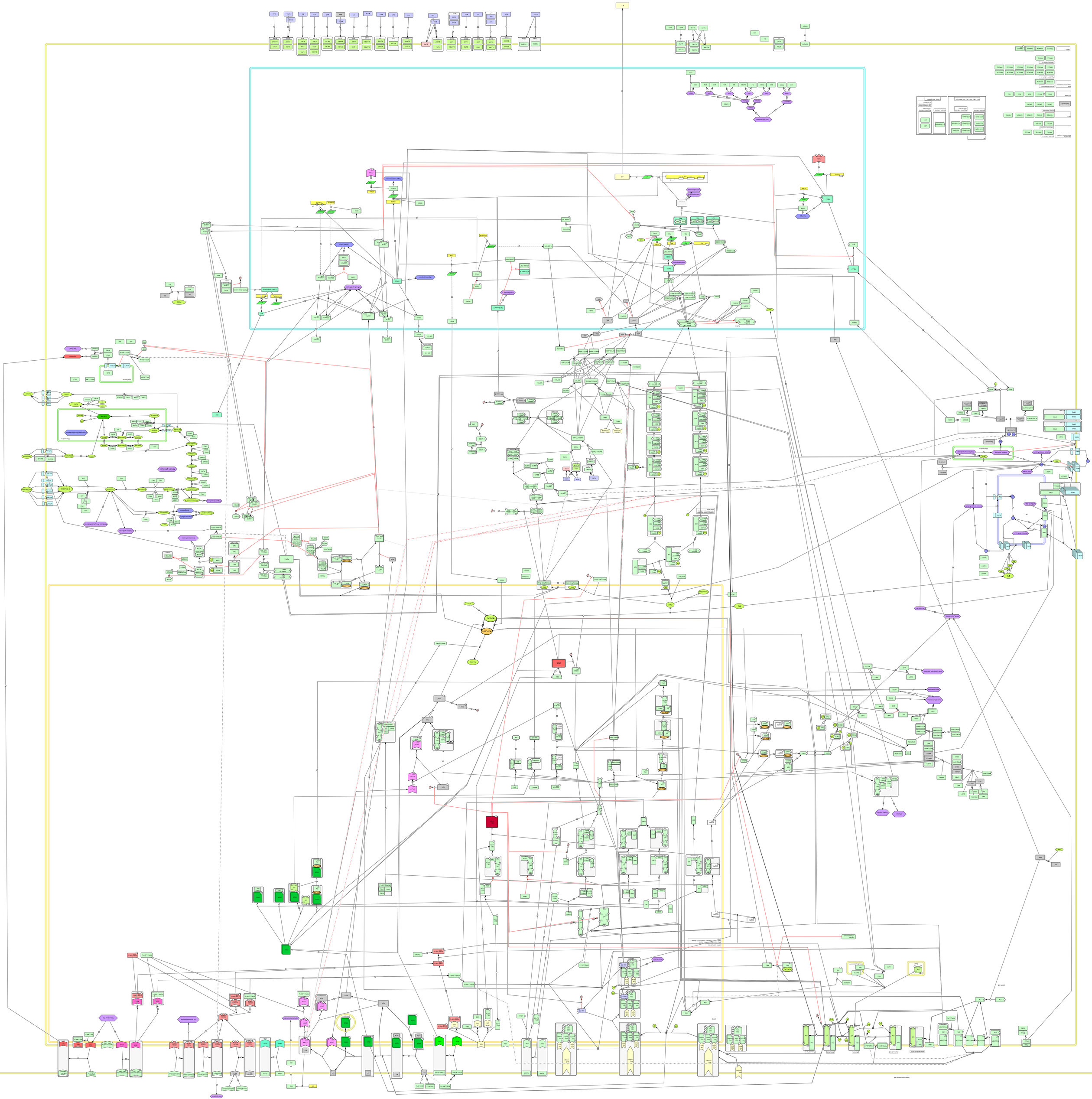
Table 2. Quantification of the model nodes impacted by the propagation of CTLA4 or PD-1 persistent activation. After propagation, the nodes of the model can remain free (not fixed) or become frozen inactive (value 0) or frozen active (value 1, or potentially higher in the case of multilevel components). The model encompasses a total of 216 nodes, including 14 inputs and 40 nodes not affected by CTLA4 or PD-1 activation. Interestingly, PD-1 itself is the only single node specifically frozen by its activation (and not by the activation of CTLA4). Furthermore, each of 99 nodes affected by the two perturbations is frozen to the same value in both cases. Details of the computation method can be found in the companion CoLoMoTo notebooks.