

# Cascaded Games

Jittisak Senachak, Mun'delanji Vestergaard, and René Vestergaard\*

JAIST, Nomi, Ishikawa, Japan

**Abstract.** We introduce a novel model construction, *cascaded games*, that is intended to allow us to study the notion of *steady states* algebraically and structurally. The model construction is inspired by the chemical underpinning and the prevailing conceptualisation of *mitogen-activated protein kinase (MAPK) cascades*. To analyse the models, we use the recent notion of *change-of-mind equilibria*. We exemplify our proposal with gene regulation and MAPK cascades, capturing basic as well as advanced issues such as *prophage induction* and *tauopathy* causation.

## 1 Introduction

The core contribution of this paper is conceptual and formal support for feasible, i.e., sub-exponential, *steady-state* analysis. Steady-state analysis is based on the idea that an autonomous system is most likely to be found in certain configurations (that we shall attempt to characterise abstractly) and not in others, and that an analysis therefore may serve to predict (emergent) behaviour. In the biological sciences, the idealised form of this idea is that life itself is a reflection of the possible steady states of the involved system constituents, along with provoked transitions between the steady states. One of the best known qualitative steady-state analysis is due to Kauffman [17] and Thomas [37]. The concrete insight behind their analysis is that understanding whether genes influence each other's *expression* positively or negatively suffices to identify the expression configurations that are characteristic of the organism's main functionality. This may be as concrete as the ways in which an organism may replicate, see Section 4.

In earlier work [5], we showed that Kauffman/Thomas steady-state analysis is a concrete use of a recent notion of dynamic Nash equilibria, called change-of-mind equilibria [32]. Nash equilibria are known for making reliable real-life predictions based on mathematical models in a range of situations [15]. Change-of-mind equilibria are seemingly the first adaptation of the technology that allows us to address dynamic stability, e.g., in the form of *homeostasis* [4]. As implied by the name, the concepts behind *cascaded games* come from MAPK cascades and game theory. Cascaded games are not inherently about either but both types of considerations are directly identifiable at the technical level of our construction, e.g., by incentives having first-class status and, indeed, being pivotal.

One widely understood problem with Kauffman/Thomas analysis is that it involves the construction of an exponential-sized state-space graph and that the

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\* Corresponding author: [vester@jaist.ac.jp](mailto:vester@jaist.ac.jp) — <http://www.jaist.ac.jp/~vester/>

analysis therefore does not scale. Cascaded games are explicitly constructed to contain only as many nodes as appear to be warranted by the complexity (in a sense we make precise) of the considered system and will typically have a polynomial upper-bound; in fact, the models appear to be much smaller than their upper-bounds in most situations. Conceptually speaking, we pursue a structural line of thinking by identifying *points of interaction*, and not merely influences, between the objects and let the involved *catalysts* determine what possibilities/graph edges to consider. We focus on catalysts because they alter the *affinity* (i.e., the chemical incentives) between the involved compounds, typically leading to an increase in reaction kinetics by a factor of  $10^6$  to  $10^{12}$  [40]. Pursuant to the construction, the identified equilibria are different in nature than those of Kauffman/Thomas analysis (structural vs functional) and the fact that they appear to be directly comparable is of independent interest [13], see Conclusion.

Following preliminaries in Section 2, we define cascaded games and the abstract formalism they apply to, viz *auto-regulating systems*, in Section 3. In Section 4, we show how to apply the technology to gene-regulation analysis and in Section 5 we go into details with the subtle and not-so-subtle issues that cascaded games allow us to address in a complete account of mammalian MAPK.

The Cascaded Game tool is available through the corresponding author’s homepage or directly at <http://cascade.jaist.ac.jp/>.

## 2 Preliminaries

In this section, we briefly review basic game theory, the theory behind change-of-mind equilibria, and Kauffman/Thomas gene-regulation analysis.

### 2.1 Abstract Nash Equilibria

A Nash equilibrium is a game situation in which no agent who can move away wants to do so. The notion makes sense in many different concrete classes of games. Abstractly speaking, the notion of Nash equilibrium is definable using only the following four concepts, aka conversion/preference (C/P) games.

**Definition 1 (C/P Games [32])**  $G^{\text{CP}}$  are 4-tuples  $\langle \mathcal{A}, \mathcal{S}, (\succ_a)_{a \in \mathcal{A}}, (\triangleleft_a)_{a \in \mathcal{A}} \rangle$ :

- $\mathcal{A}$  is a non-empty set of agents.
- $\mathcal{S}$  is a non-empty set of synopses (i.e., game situations).
- Each  $\succ_a$  is a binary conversion relation on  $\mathcal{S}$ .
- Each  $\triangleleft_a$  is a binary preference relation on  $\mathcal{S}$ .

A *strategic-form game* [24, 26] is a C/P game where  $\mathcal{S}$  is an  $\mathcal{A}$ -indexed Cartesian product and each  $\succ_a$  allow for free movement in the  $a$ -dimension of a synopsis.

**Definition 2 ([32])** Synopsis  $s$  is an (abstract) Nash equilibrium for  $G^{\text{CP}}$  if

$$\text{Eq}_{G^{\text{CP}}}^{\text{aN}}(s) \triangleq \forall a \in \mathcal{A}, s' \in \mathcal{S} . s \succ_a s' \Rightarrow \neg(s \triangleleft_a s')$$

Nash equilibria can also be viewed in direct graph-theoretic terms.

**Definition 3 ([32])** *The (free) change-of-mind relation for agent  $a$  is  $\rightarrow_a \triangleq \succ_a \cap \triangleleft_a$ . Let  $\rightarrow \triangleq \bigcup_{a \in \mathcal{A}} \rightarrow_a$  and let  $\rightarrow^*$  be the reflexive, transitive closure of  $\rightarrow$ .*

Nash equilibria are exactly the terminal nodes of  $\rightarrow$  and thus, modulo reflexivity:

$$\text{Eq}_{\text{G}^{\text{CP}}}^{\text{aN}}(s) \Leftrightarrow \forall s' \in \mathcal{S} . s \rightarrow^* s' \Leftrightarrow s = s' \quad (1)$$

## 2.2 Compromises

Games cannot in general be guaranteed to have Nash equilibria. Exceptions (with a guarantee) include i) extensive-form games with perfect information [19, 39] and ii) C/P games with  $\mathcal{S}$  a non-empty, convex, compact subset of Euclidian space and with preference relations that allow for a continuous *synchronised update* function [24, 26]. Nash's Theorem uses the fact that the latter class includes the probabilised version of any finite strategic-form game with preferences induced from real-valued payoffs. Our alternative starting point is the observation that (1) can be naturally relaxed to address the absence of *unexpected* updates.

**Definition 4 ([32])** *Let  $\xrightarrow{\text{S}} \triangleq \rightarrow \cap (\text{S} \times \text{S})$ , viz the sub-graph induced by synchronises  $\text{S}$ . For  $\text{G}^{\text{CP}}$  and non-empty  $\text{S}$ ,  $\xrightarrow{\text{S}}$  is a change-of-mind equilibrium if*

$$\text{Eq}_{\text{G}^{\text{CP}}}^{\text{com}}(\xrightarrow{\text{S}}) \triangleq \forall s \in \text{S}, s' \in \mathcal{S} . s \rightarrow^* s' \Leftrightarrow s' \in \text{S}$$

Note that a *pure* Nash equilibrium is a static change-of-mind equilibrium, and vice versa. The main theorem and the supporting theory reads as follows.

**Theorem 5 ([32])** *In any finite  $\text{G}^{\text{CP}}$  there is a (non-empty)  $\text{S} \subseteq \mathcal{S}$  such that  $\text{Eq}_{\text{G}^{\text{CP}}}^{\text{com}}(\xrightarrow{\text{S}})$ . More, for given finite  $\text{G}^{\text{CP}}$ , all such  $\text{S}$  can be found in  $|\mathcal{S}|^2$ .*

**Lemma 6 ([32])** *For any  $\text{G}^{\text{CP}}$  and  $\text{S}$ , the following are equivalent.<sup>1</sup>*

1.  $\text{Eq}_{\text{G}^{\text{CP}}}^{\text{com}}(\xrightarrow{\text{S}})$
2.  $\text{Eq}_{[\text{G}^{\text{CP}}]}^{\text{aN}}(\text{S})$ , where  $[\text{G}^{\text{CP}}]$  is  $\text{G}^{\text{CP}}$ 's shrunken game (i.e., with change-of-mind given as the shrunken graph over the strongly connected components of  $\rightarrow$ ).
3.  $\text{S}$  is a least, non-empty fixed point of  $\mathcal{U}(\text{S}) \triangleq \bigcup_{s \in \text{S}} \{s' \mid s \rightarrow^* s'\}$ .

Following Nash [24], the lemma says that our compromises, 1., are Nash equilibria in a derived game, 2., as well as fixed points of an update function, 3. Crucially, our approach admits i) a direct characterisation of the identified compromises, through the notion of change-of-mind equilibrium, which is what makes our equilibria dynamic in nature and ii) an algorithm for computing all equilibria as the terminal strongly connected components of the change-of-mind graph in linear time in the number of nodes plus the number of edges [35]. Nash's probabilistic and our dynamic compromises can seemingly not be quantitatively distinguished, e.g., in terms of size, expected/average payoff, or even in terms of what parts of games can be involved in that the two can be identical, disjoint, subsume each other, and can overlap non-trivially [32].

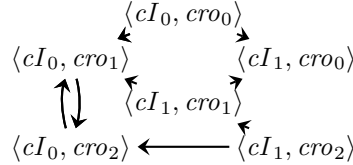
<sup>1</sup> We shall revisit the three in Section 5 and see what they mean for Systems Biology.

### 2.3 Kauffman/Thomas Gene-Regulation Analysis

The starting point of Kauffman/Thomas gene-regulation analysis is a so-called *influence graph*, indicating regulatory influences between considered objects. The analyses of Kauffman and Thomas differ in two main regards. One is that Kauffman assumes that objects are either being expressed or not, while Thomas allows for genes to be expressed at several levels. For example, in case of Thomas:



The graph shows how the  $cI$  and  $cro$  genes are influenced by each other and the context in *bacteriophage lambda*, whether positively:  $\rightarrow$ , or negatively:  $\neg$  [36, 5]. The gene  $cI$  is able to assume two states, say 0, 1, and  $cro$  is able to assume three states, say 0, 1, 2. An influence (i.e., an arrow out of a gene) may take place when the gene is on (i.e., in state 1, or above), unless annotated differently. For example,  $cro$  auto-represses in state 2, while it represses  $cI$  in states 1 and 2. The influences are translated into the associated state space by evaluating them against each state. Thomas considers the likely move of each gene out of each state, see below, while Kauffman lets all genes make a *synchronous* move.



The translation is not unambiguous and so-called K functions are typically used to resolve joint positive and negative influences into one polarity. We shall return to this point in Section 4. For now, and from our perspective, we note that the above is a change-of-mind graph (that is underpinned by natural conversion and preference relations for agents that in general can be chosen variably) [5]. The considered game therefore has two change-of-mind equilibria: (the static)  $\langle cI_1, cro_0 \rangle$  and the (dynamic) cycle between  $\langle cI_0, cro_1 \rangle$  and  $\langle cI_0, cro_2 \rangle$ . This is interesting because the former is characteristic of  $\lambda$ -phage's *lysogenic* way of getting replicated by a bacteria host while the latter is its *lytic* way [41].

### 3 Auto-Regulating Systems and Cascaded Games

We are interested in the likely observable behaviours of what we call *auto-regulating systems*, i.e., arbitrary 4-ary relations over sets of some set of objects.

**Definition 7** For set  $\mathcal{O}$ , let  $\text{ARS}_{\mathcal{O}} \triangleq 2^{2^{\mathcal{O}} \times 2^{\mathcal{O}} \times 2^{\mathcal{O}} \times 2^{\mathcal{O}}}$ .

The intended semantics is that, for a specific relationship, the first component, the *substrates*, can turn into the second component, the *products*, when the third component, the *catalysts*, is present and the fourth component, the *inhibitors*, is absent.

**Definition 8** For  $r \in \text{rs} \in \text{ARS}$ , with  $r = Ss \xrightarrow[I_s]{C_s} Ps$ , let

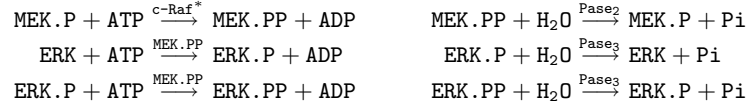
$$\pi_s(r) \triangleq Ss \quad \pi_p(r) \triangleq Ps \quad \pi_c(r) \triangleq Cs \quad \pi_i(r) \triangleq Is$$

Let  $\pi_x[\text{rs}] \triangleq \bigcup_{r \in \text{rs}} \pi_x(r)$ , for  $x \in \{s, p, c, i\}$ .

The word ‘auto-regulating’ refers to the fact that all four components come from the same  $\mathcal{O}$ , thereby allowing for co-regulation between objects.

### 3.1 MEK, ERK Cascade

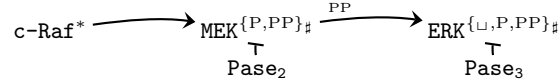
An ARS may express chemistry when each of the considered relationships obey *stoichiometric laws*, i.e., when the relationships indicate chemical reactions.



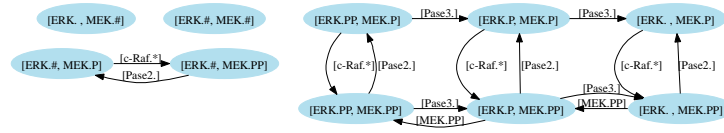
The above reactions are “triggered” by c-Raf\* in a *kinase*-role, i.e., as an enzyme that *phosphorylates* a protein, i.e., that catalyses the affixation of a phosphate group, P, from the “energy molecule” ATP. With two phosphate groups affixed, also the targeted MEK becomes a kinase (aka is *activated*) and may double-phosphorylate ERK. The right column of reactions are deactivations, aka dephosphorylations catalysed by *phosphatases*. This kind of “rolling” activation of proteins is what is referred to as (kinase) *cascading*.

### 3.2 State-Space Analysis

In terms of the Kauffman/Thomas technology discussed in Section 2.3, the above reactions amount to the following influence graph over the potentially regulating/regulatable objects, i.e., over the proteins. (As part of our modelling abstraction, we assume that non-proteins are freely available, meaning in particular that we are assuming that we are considering a cell with normal metabolism.)



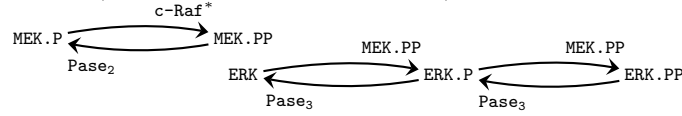
The possible states (and their ordering) of each protein is superscripted. For simplicity, we consider only one implicit (active) state for each of c-Raf\*, Pase<sub>2</sub>, Pase<sub>3</sub>. In order to apply Kauffman/Thomas to the current non DNA-bound (read: free-flowing) situation, as frequently claimed possible, we have introduced a special state, # or ‘absent’, for MEK and ERK, which we subscript to indicate that it is non-regulatable. The induced state space has 12 nodes (3 MEK-states, 4 ERK-states) and 16 edges. The resulting change-of-mind equilibria are as follows.



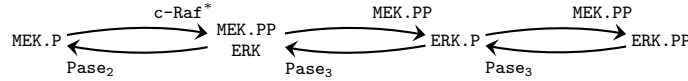
Note the stand-alone MEK.P, MEK.PP loop with absence of ERK (to cascade onto), and the singleton ERK corresponding to the absence of (catalysing) MEK.PP. Note, also, the repeated use of the MEK.P, MEK.PP loop inside the loops for ERK. Finally, note that without ‡ we would observe just one equilibrium, namely the one with 6 nodes. (Following Kauffman, we could also have constructed the state space with, e.g., MEK.P and MEK.PP taken as different objects and states ‘present’, ‘absent’. The state-space graph is bigger than above in this case, 32 nodes and 78 edges, but the equilibria turn out to be exactly the same).

### 3.3 Cascaded Games

Cascaded games are based primarily on the second issue noted above. More precisely, it is our observation that the exponential granularity of state spaces typically is not taken advantage of. In our first definition of cascaded games, we shall, for simplicity, consider only ARSs of the form  $2^{\mathcal{O} \times \mathcal{O} \times \mathcal{O}_\perp}$ , with  $\mathcal{O}_\perp$  meaning one or none. (But, we retain set notation.) Such relationships involve exactly one substrate, one product, at most one catalyst, and no inhibitor. To start, note that the example (with non-proteins suppressed) amounts to a labelled graph.



To avoid the inherent exponential blow-up in a state-space graph, the cascaded game for this example will be constructed roughly by collapsing MEK.PP and ERK.



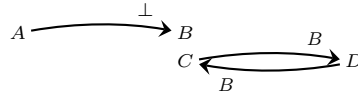
Formally, we first identify the reactions that can be cascaded onto as follows.

**Definition 9** For  $rs \in 2^{\mathcal{O} \times \mathcal{O} \times \mathcal{O}_\perp}$ ,  $r \in rs$  is a cascadee if

$$\begin{aligned} \text{Casc}^{\text{dee}}(r) &\triangleq \pi_c(r) \cap \pi_p[rs] \neq \emptyset \\ &\wedge (\forall r' . r' \neq r \Rightarrow \neg(\pi_p(r') = \pi_s(r) \wedge \pi_c(r') = \pi_c(r))) \end{aligned}$$

The lower conjunct says that the MEK.PP-reaction from ERK.P to ERK.PP is not a cascadee because the MEK.PP-reaction from ERK to ERK.P is an invalidating  $r'$ .

**Observation 10** We do not identify either B-reaction below as a cascadee.



We could, of course, make Definition 9 more complex but we shall not need to address any situations with cyclic catalysis by one and the same object.

As seen, catalysts that are not produced anywhere do not have their regulative role regulated by another object and we will assume they are freely available.

**Definition 11 (Cascaded Players)** For  $r \in rs \in 2^{\mathcal{O} \times \mathcal{O} \times \mathcal{O}_\perp}$ , let

$$\mathcal{A}_{rs} \triangleq \bigcup \pi_c[rs]_\perp \quad \pi_c(r)_\perp \triangleq \pi_c(r) \cap \pi_p[rs]$$

The definition implies that our equilibrium analysis will be over the catalytic/regulatory effects (read: incentives) of those objects (read: players,  $\mathcal{A}_{rs}$ ) that may themselves be regulated upon. We define the nodes of the graph to be analysed in a similar manner, namely as the potential *points of interaction* without explicitly listing all the contexts that a considered interaction may take place in. If a reaction is a cascadee, we create a node containing the substrate(s) of the reaction and any catalyst that is involved in cascading. If the reaction is not a cascadee, we create a node with just the substrate(s). In particular, if a reaction is not a cascadee for the reason that the MEK.PP-reaction from ERK.P to ERK.PP is not a cascadee above, we shall consider the catalyst(s) to be only implicitly present.

**Definition 12 (Cascaded Synopses)** For  $rs \in 2^{\mathcal{O} \times \mathcal{O} \times \mathcal{O}_\perp}$ , let

$$\begin{aligned} \mathcal{S}_{rs}^{\text{poi}} &\triangleq \bigcup_{r \in rs} \{ \pi_s(r) \cup \pi_c(r)_\perp \mid \text{Casc}^{\text{dee}}(r) \} \cup \bigcup_{r \in rs} \{ \pi_s(r) \mid \neg \text{Casc}^{\text{dee}}(r) \} \\ \mathcal{S}_{rs} &\triangleq \mathcal{S}_{rs}^{\text{poi}} \cup \bigcup_{r \in rs} \{ \pi_p(r) \mid \forall n \in \mathcal{S}_{rs}^{\text{poi}}. \pi_p(r) \not\subseteq n \} \end{aligned}$$

The full set of situations we are interested in consists of all points-of-interaction, as well as (singleton) nodes for products that are not involved in any interaction. The latter set of objects are candidates for deadlocked situations or, more technically speaking, may be static change-of-mind equilibria, aka Nash equilibria. (An example is  $\lambda$ -phage's lysogenic state, see Sections 2.3 and 4).

When it comes to the conversion and preference parts of cascaded games, we note that their intersection, i.e., the change-of-mind relation, is already the de facto object of study in chemistry. The natural conversion relation is simply that of stoichiometric laws. In particular, the conversion relation is shared by all objects and is reversible (in principle). Catalysts alter the involved affinities, typically resulting in an increase in the observable difference of the two directions of a reaction by a factor of  $10^6$  to  $10^{12}$  [40], which gives rise to the relevant notion of preference for the catalysts. In other words, when we see an oriented chemical reaction with a catalyst annotated, we are actually seeing the induced change-of-mind relation. We follow suit and treat change-of-mind as primitive.

Having identified the relevant points-of-interaction for a given ARS, inserting edges/change-of-mind is almost as straightforward as going from substrate and (non-suppressed) catalyst to product and (non-suppressed) catalyst. First, though, we note that the catalyst will be consumed in case of self-catalysis.

**Definition 13** For  $r \in rs \in 2^{\mathcal{O} \times \mathcal{O} \times \mathcal{O}_\perp}$ , the perfect-match start, end would be:

$$\mathbb{S}(r) \triangleq \pi_s(r) \cup \pi_c(r)_\perp \quad \mathbb{E}(r) \triangleq \pi_p(r) \cup (\pi_c(r)_\perp \setminus \pi_s(r))$$

Secondly, we note that we have not explicitly created a product-catalyst node for each reaction, and substrate-catalyst nodes have only been created for cascadees. Perfect-match nodes may have been created by another reaction and, so, edge

insertions go between perfect nodes if they exist and, otherwise, between any nodes that contain the substrate and product. Exceptionally, though, we note that a suppressed catalyst amounts to it being freely available. Also, we only allow reflexive ARS-steps to result in reflexive edges (note, though, that reflexive steps may have resulted in nodes that might not otherwise have been created).

**Definition 14 (Cascaded Change-of-Mind)** For  $rs \in 2^{\mathcal{O} \times \mathcal{O} \times \mathcal{O}_\perp \times 2^{\mathcal{O}}}$ , let<sup>2</sup>

$$\begin{aligned} \rightarrow_{rs} \triangleq & \bigcup_{r \in rs} \bigcup_{s, e \in \mathcal{S}_{rs}} \{ \langle s, e, \pi_c(r)_\perp \rangle \mid (\pi_c(r)_\perp \neq \emptyset \wedge \mathbb{S}(r) \in \mathcal{S}_{rs} \\ & \Rightarrow s = \mathbb{S}(r) \parallel s \supseteq \pi_s(r)) \\ & \wedge (\pi_c(r)_\perp \neq \emptyset \wedge \mathbb{E}(r) \in \mathcal{S}_{rs} \\ & \Rightarrow e = \mathbb{E}(r) \parallel e \supseteq \pi_p(r)) \\ & \wedge \pi_i(r) \cap s = \pi_i(r) \cap e = \emptyset \\ & \wedge \pi_s(r) = \pi_p(r) \Rightarrow s = e \} \end{aligned}$$

We write  $P \Rightarrow Q \parallel R$  to mean  $(P \Rightarrow Q) \wedge (\neg P \Rightarrow R)$ .

**Definition 15 (Cascaded Games)** are defined, for given  $rs \in 2^{\mathcal{O} \times \mathcal{O} \times \mathcal{O}_\perp}$ , as

$$G_{rs}^{\text{casc}} \triangleq \langle \mathcal{A}_{rs}, \mathcal{S}_{rs}, \rightarrow_{rs} \rangle$$

See Definitions 1, 3, 11, 12, 14 for more details.

As defined, cascaded games have at most  $|\mathcal{O}|^2$  many nodes, seeing that no node contains more than two objects, and a naive implementation and equilibrium analysis will take time  $|\mathcal{O}|^9$  ( $|\mathcal{O}|^3 \times (|\mathcal{O}|^2)^3$ ), see Definition 14 and Theorem 5.<sup>3</sup>

### 3.4 The General Case

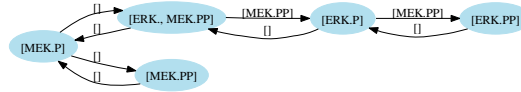
Cascaded games can be applied to all ARSs as it stands, not just to  $2^{\mathcal{O} \times \mathcal{O} \times \mathcal{O}_\perp}$ , but the required discussions and analyses to address the appropriateness of doing so are sufficiently subtle and open-ended to warrant maturation of the technology before being undertaken. Some conditions may need to be stated in ways that are equivalent for  $2^{\mathcal{O} \times \mathcal{O} \times \mathcal{O}_\perp}$  but behave differently elsewhere. We are confident that cascaded games will scale to multiple catalysts, substrates, and products but extensive experimentation is needed to understand how to deal with first-class inhibition. The main challenge is to identify what nodes to construct. The naive approach is to construct a collapsed node involving every object that is not an inhibitor but, as we shall see in Section 4, this is merely one particular possibility that, in fact, is not guaranteed to be the right thing to do in all circumstances. While the trade-off may appear to exclusively be between (the asymptotic) number of nodes and the ability to distinguish different situations, we will make two possibly surprising observations in Section 4. Firstly, not making certain distinctions can bring out issues that are not brought out, e.g., in the exponential Kauffman/Thomas models, see Section 4.3. Secondly, for certain types of inhibition, the right thing to do is seemingly to not have the inhibitors impact the nodes we construct, see Section 4.1. See also the Conclusion.

<sup>2</sup> The explicit treatment of inhibition at this point is intentional, see Section 3.4.

<sup>3</sup> Our actual implementation avoids replication of work and has an  $|\mathcal{O}|^8$  upper-bound in general, with an upper-bound of  $|\mathcal{O}|^5$  for all but exotic ARSs.

### 3.5 Cascaded MEK, ERK

The cascaded game, Definition 15, for the MEK, ERK cascade ARS in Section 3.1 (without non-proteins) has one change-of-mind equilibrium, see Theorem 5.<sup>4</sup>



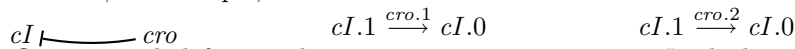
We see that the MEK.P, MEK.PP loop corresponding to the absence of ERK is retained, alongside the cascaded-onto double-phosphorylation of ERK by MEK.PP. In this analysis, there is no duplication, in part at the cost of ERK existing only in a collapsed configuration with the triggering MEK.PP. The graph makes it clear that a double-phosphorylating phosphate group arriving to MEK.P either initiates double-phosphorylation for an ERK in sufficiently close physical proximity or, in the alternate case, is simply released in short order by a phosphatase. We shall see in Section 5 how this process is used by cells to eliminate the need for proteins carrying a “signal” to a cell to be allowed to penetrate the cell membrane. Indeed, MAPK cascades, of which MEK, ERK are part, serve to transduce such signals into a phosphate-group form that *autopoietically* [22] can target the nucleus.

## 4 Cascaded Gene-Regulation Analysis

As implied by our discussions, influence graphs (for gene expression) may be translated into ARS-form. We considered the reverse translation in Section 3.2, and we shall now formalise the forward direction. The translation will be exemplified with bacteriophage lambda, and we will compare the information that can be gained with our and the Kauffman/Thomas approaches.

### 4.1 Influence Graphs as Auto-Regulating Systems

The arrows in an influence graph are different from but related to the arrows in an ARS. Take, for example, this situation from Section 2.3.



The influence graph, left, says that *cro* in states 1, 2 represses *cI*, which amounts to the ARS-steps on the right. While the steps do not obey stoichiometric laws they are nonetheless expressing (high-level) chemical changes, namely in terms of how the protein that is synthesised, e.g., from *cro.1* regulates the transcription of *cI*. When there is no ambiguity between positive and negative influences, this translation is straightforward. In case of conflicts, we use inhibition with the stronger influence to make the weaker influence assume a secondary role. According to the disambiguating K functions for bacteriophage lambda [5], for example, the above repression of *cI* by *cro* is stronger than *cI*'s auto-activation.

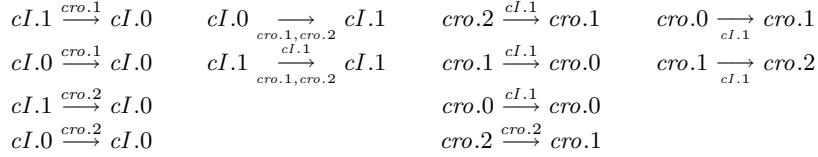


<sup>4</sup> The complete model construction and equilibrium analysis (excluding file I/O) takes around 0.05 seconds, using a naive Java implementation running on a laptop.

The chemical justification for prioritising the influences is that each of them is associated with a kinetics and what concerns us in our discrete presentation is the net effect of combining them. The above description of our translation of influence graphs into ARSs is essentially complete, with only a few caveats.

- Influences may have identical priority, with neither inhibiting the other.
- Influences without an influencer are not annotated with a catalyst.
- Multiple catalysts would be needed, e.g., if gene  $g$  is influenced by  $g_1, g_2, g_3$ , with  $g_1, g_2$  positive and  $g_3$  negative, and with  $g_3$ 's influence stronger than  $g_1, g_2$  separately but weaker when both positive influences are on jointly.
- Cascaded games treat catalysts and inhibitors conjunctively: each catalyst must be present (modulo free availability) and each inhibitor must be absent. Disjunction is via multiple steps, i.e., ARSs are disjunctive normal forms.
- We use reflexive steps for both activation and repression, e.g.,  $cI.1 \xrightarrow{cro.1} cI.1$ .
- We do not allow multiple states of an object to co-exist in one node, e.g.,  $cro.1 \xrightarrow{cro.2} cro.0$  is suppressed (but  $cro.1 \xrightarrow{cro.2} cro.2, cro.1 \xrightarrow{cro.2} cro.0$  are not).
- We suppress inhibitor states that occur in the product (unless they also occur in the substrate). For example, the  $cro.2$ -inhibition of  $cro.1 \xrightarrow{cI.1, cro.2} cro.2$  is suppressed (i.e., we consider  $cro.1 \xrightarrow{cI.1} cro.2$  instead), in order to allow for the temporal delay in the creation of the inhibitor in the product.

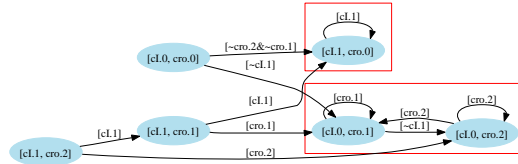
**Proposition 16** *Lambda-phage's influence graph, see Section 2.3, is this ARS.*



(We note that the K functions could also be transliterated into ARSs. K functions indicate the likely next state of each gene for each combination of influences and transliteration would annotate all possible influences on a particular gene to its ARS-steps. The influencer of an influence that is on would go into the catalysts, with the others becoming inhibitors. We refer to Sections 3.4, 4.3, and the Conclusion for discussions of why we do not pursue transliteration here.)

## 4.2 “Catalysed State-Space” for Bacteriophage Lambda

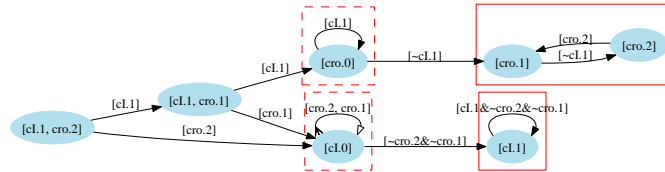
Using a construction in the style of Definition 14 on the ARS in Proposition 16 and a state-space set of nodes results in the following (non-cascaded) graph.



Other than the reflexive steps, the annotations on the arrows, and the boxes indicating the change-of-mind equilibria/steady states, it is identical to the Thomas graph for lambda phage in Section 2.3. The arrows in a Thomas graph are implicitly annotated with the gene whose state-change the arrow captures. Our arrows are annotated with the influences that facilitate the change, along with  $\sim$ -prefixed annotations of the inhibitors. The node for  $CI.0$ ,  $cro.0$  makes explicit the retrospectively obvious fact that re-activation is driven by the context when both genes are off, seeing that neither out-going arrow has a catalyst annotation.

### 4.3 Cascaded Bacteriophage-Lambda Analysis

The cascaded game built from lambda-phage’s ARS, see Proposition 16, looks as follows.<sup>5</sup> The solid boxes are the change-of-mind equilibria; they coincide with the established Thomas steady states (that correspond to lambda-phage’s lysogenic and lytic states). The dashed boxes are strongly connected components that are not terminal but have only inhibited arrows out of them, i.e., they are pre-equilibria, whose collapse are preventable. (Hollow heads indicate multi-arrows, with comma-separations of their annotations.)



Our graph has seemingly more nodes than the (Kauffman/Thomas) state space. However, this is due to the context influences being explicit for us but implicit in Kauffman/Thomas’ K functions. The full state space would have 24 nodes. More subtly, we note that the lysogenic state (lower solid box) is characterised by gene  $CI$  being on and the reflexive arrow makes it clear that this can be sustained as long as gene  $cro$  is not expressed. The top dashed box is the dual view on this situation, saying that  $CI$  can keep  $cro$  off as long as the  $CI$ -expressed protein is not depleted. In case the protein is depleted, our analysis predicts that the observed lysogenic state (top dashed box) would collapse to lambda-phage’s lytic cycle (top solid box). In the Thomas graph in Section 4.2, this information is implicit in the  $CI.0$ ,  $cro.0$  node, along with what is for us the analogous situation for the lower boxes. The Thomas graph and the cascaded game have similar layouts and our dashed boxes are superficially in the same positions as the Thomas steady-states in the state space. In the first instance this means that we in principle could have done the analysis with just four nodes. On the other hand, the outgoing arrows from the dashed boxes are undeniable chemical possibilities.

**Prophage Induction** The arrow out of the top dashed box is readily observable. It is referred to as *prophage* (or lysogen) *induction* and serves as a sort

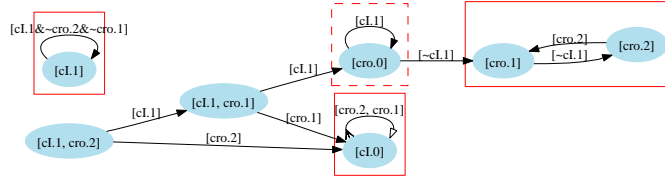
<sup>5</sup> The complete model construction and equilibrium analysis (excluding file I/O) takes around 0.05 seconds, using a naive Java implementation running on a laptop.

of panic button for the bacteria-infecting lambda-phage virus. Lysogenic replication is relatively slow. Conversely, lytic replication is relatively fast but leads to *lysis*, i.e., bursting of the host and, with it, the release of approximately 100 lambda phages [2]. Prophage induction (out of slow lysogenic and into fast but destructive lytic replication) has been observed in lambda-phages under attack by the host, e.g., with *cI* being stimulated into *auto-proteolysis* by the host DNA repair protein RecA responding to UV-radiation damage to the DNA [11].

**Integrated View on Bacteriophage-Lambda Gene Regulation** Contrary to prophage induction, the arrow out of the lower dashed box does not appear to have been observed in nature. While it may in theory be possible for either *cI* or *cro* to activate when both are off, the present integrated analysis suggests that *cro* always is faster than *cI*. We therefore propose to suppress  $cI.0 \xrightarrow{cro.1, cro.2} cI.1$  in the ARS for lambda-phage, see Proposition 16. The similarly-adjusted influence graph reads as follows (with the Thomas-steady states remaining unchanged.)



The cascaded game analysis for the adjusted lambda-phage ARS is as follows.<sup>6</sup>



This version predicts lysogenic replication (stand-alone box), prophage induction (dashed box), and dual sustainability of lytic replication through i) alternating state-change for *cro* (right box) and ii) unwavering repression of *cI* (lower box). It is our contention that the absence of a location in the state-space graph from which lysogenic induction may explicitly be seen to take place is a shortcoming. Further to Definitions 12 and 14, the problem is related to the functional/non-structural view on the *cI.0*, *cro.0* gene configuration in the state-space approach.

## 5 MAPK Cascaded Signal Transduction

This section returns to the eponymous application of cascaded games, namely MAPK cascades, and addresses in some detail the game-theoretic foundation of the technology. In particular, we shall consider separately the three alternative views on change-of-mind equilibria in Lemma 6 and highlight what they mean in terms of biochemistry and systems biology. In order, the three amount to **sustainability**, **inevitability**, and **atomicity** of the equilibria [32].

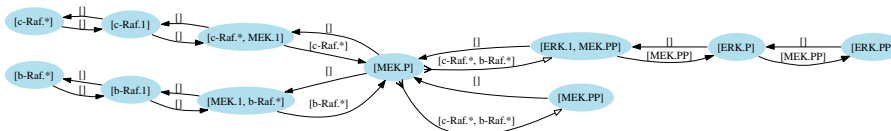
<sup>6</sup> The complete model construction and equilibrium analysis (excluding file I/O) takes around 0.05 seconds, using a naive Java implementation running on a laptop.

## 5.1 Sustainability, Signal Transduction in the Chemistry of MAPK

An elaborate system of proteins, from trans-membrane receptor proteins via cytosolic proteins to target proteins in the nucleus, enable the cell to respond to a particular signal in a specific manner. Responses include cell growth, survival, apoptosis, differentiation and proliferation [1, 27]. Intracellular proteins include kinases, phosphatases and GTP-binding proteins (GTPases). Studies have shown that cells respond to external stimuli using clearly defined *signalling pathways* [1]. These encompass all the biochemical phenomena that start with perception of an extracellular signalling molecule (aka ligand) to the response of the cell. MAPK signal transduced pathways are among the most widespread in eukaryotes [18]. In mammalian systems, five distinguishable MAPK pathways have been identified so far: extracellular signal-regulated kinases 1 and 2 (ERKs 1/2), c-Jun N-terminal kinases 1,2 and 3 (JNK 1/2/3), p38 ( $\alpha/\beta/\gamma/\delta$ ), ERKs 3/4 and ERK 5 [31]. The most widely studied, in vertebrates, are ERKs 1/2, JNK and p38 [21]. ERKs 1/2, preferentially regulate cell growth and differentiation whilst JNK and p38 are strongly activated by stress and inflammatory cytokines [31, 6]. MAPK cascaded signal-transduction systems have remained unchanged during the course of evolution and currently exist in virtually identical form in a wide range of species. Chemically, they are triggered by a receptor protein on the cell membrane (without penetration) and transduces the received signal by the transfer of phosphate groups. We have created an ARS compendium of all chemical reactions said to be involved in MAPK cascades in [3, 7, 16, 18, 25, 29, 30, 38], see [34]. The compendium contains 109 reactions and 21 proteins, each with 2 or 3 states, for a total of 53 ( $= 3 \times 11 + 2 \times 10$ ) distinct objects/protein states and thus an upper-bound of 2809 ( $= 53^2$ ) cascaded nodes. The resulting cascaded game has:<sup>7</sup>

- 15 cascaded players/co-regulating enzymes, see Definition 11,
- 71 cascaded synopses/points-of-interaction, see Definition 12,
- 207 cascaded changes-of-mind/edges (with multiplicity), see Definition 14,
- 2 change-of-mind equilibria, covering the whole cascaded game.

Further to the last point, we see that the chemical underpinning of MAPK cascades is **sustainable**, i.e., the cascades can keep running with no other support than a functioning metabolism: they are *autopoietic* [22]. In other words, MAPK cascades are ideal building blocks for a fundamental biological process that is expected to operate within the confines of a membrane-protected part of a living organism, which is what signal transduction systems do. One change-of-mind equilibrium is as follows; it is the ERK pathway [33]. (See also Section 5.3).



<sup>7</sup> The complete model construction and equilibrium analysis (excluding file I/O) takes around 0.4 seconds, using a naive Java implementation running on a laptop.

Although all MAPK-transduced pathways have their own unique properties, they share a number of characteristics. They, e.g., comprise three kinases: MAP-KKK (MAPK kinase kinase), MAPKK (MAPK kinase), MAPK. These can be recognised (in order) in the equilibrium: **Rafs**, **MEK**, **ERK**, with the latter sitting close to the nucleus physically, conceptually, and graphically [33].

## 5.2 Inevitability and Pathologies in the Chemistry of MAPK

The MAPKs we consider multi-phosphorylate tau as target protein at serine and threonine sites, with phosphorylated tau binding together other proteins. If tau becomes fully saturated the result is a condition called *hyperphosphorylation* in which insoluble *neurofibrillary tangles* of the tau-bound proteins are created [14, 8]. This phenomenon has been implicated in several diseases, the most common of which are *tauopathies* such as Alzheimer’s disease (AD), Pick’s disease (PiD), and Parkinson’s disease (PD) [10, 12]. One of the enzymes that dephosphorylates **ERK.PP** and other active MAPKs is PP2A. Inhibition of PP2A seems to be minimally counter-acted by other **Pase<sub>3s</sub>** [28]. Re-analysing our MAPK compendium with **Pase<sub>3</sub>** knocked-out results in the following six change-of-mind equilibria.<sup>8</sup>



All six are activated MAPKs and the fact that our analysis predicts that they are the **inevitable** outcomes of **Pase<sub>3</sub>**-inhibited MAPK suggests that PP2A inhibition could cause tau hyperphosphorylation. This prediction is validated by recent work [28]. Indeed, transgenic mice exhibit increased expression of JNK and p38 in AD and PiD (i.e., tauopathic) individuals [9].

## 5.3 Atomicity vs Cross-Talk in the Chemistry of MAPK

The change-of-mind equilibrium that we did not discuss in Section 5.1 consists of 60 cascaded synopses/points-of-interaction. As seen in Section 5.2, the involved MAPK proteins are JNK and different isoforms of p38. In other words, and as can be checked on closer inspection, the equilibrium comprises the **JNK**- and **p38**-pathways [34]. The **atomicity** property of change-of-mind equilibria therefore correctly predicts that there is *cross-talk* between these pathways. Although we do not discuss it here, it is possible and feasible to (semi-automatically) run our analysis several times with different proteins suppressed to identify the cross-talking points-of-interactions, in order to understand the nature of the cross-talk and possibly how to regulate it [34].

## 5.4 State-Space Analysis

We briefly attempted to analyse our whole MAPK compendium using a Kauffman/Thomas state-space graph. Further to Sections 3.2 and 3.5, our expectation

<sup>8</sup> The complete model construction and equilibrium analysis (excluding file I/O) takes around 0.4 seconds, using a naive Java implementation running on a laptop.

is that the result will be compatible with the above analyses. However, even an SGI Altix 3700 with 768GB physical memory failed to build the required graph within a 24 hour period. Of course, this is not surprising as the state space has around 250 billion ( $= (3 + 1)^{11} \times (2 + 1)^{10}$ ) nodes in the considered case.<sup>9</sup>

## Conclusion

Based mainly on game-theoretic considerations, we have introduced the novel notion of *cascaded games* that can be used to analyse the potential steady states of what we call *auto-regulating systems*. In analogy with Kauffman/Thomas analysis, steady states are captured formally as *change-of-mind equilibria*. Unlike Kauffman/Thomas analysis, however, our analysis is typically of polynomial complexity and, by virtue of our model construction using *points-of-interaction* between *co-regulating* objects, is *structural* in nature. Although seemingly closely related to Kauffman/Thomas steady states, our results may be substantially different. One difference in the analysis of bacteriophage lambda is our explicit identification of *prophage induction*. In the case of MAPK cascades, our analysis, e.g., proves the cascades to be *sustainable*, captures known causes of tauopathies, and avoids large-scale duplication to the tune of using a 71-node graph to analyse what Kauffman/Thomas analysis would need 250 billion nodes for. Indeed, all the cascaded-game analyses we undertake here take less than half a second. Current work is addressing, e.g., ARSs with multiple objects in each reaction and is looking at large-scale data sets. Theoretically, one of the most interesting issues that our work has opened up is the mathematical comparison of our structural equilibria (aka autopoiesis) with the functional equilibria (aka homeostasis) of Kauffman/Thomas. As far as we can tell, this issue is emerging as a major future challenge, seeing that “the form that provides better functionality is likely to be selected [in evolution]” [13]. More to the point, “[i]f form follows function, then we should be able to infer systems function and evolution, as well as their interplay, from the architecture of complex biochemical networks” [13] and, in addition to mathematical results, we are therefore also looking at conceptually clearer ways of reading the various equilibria and of relating them to real-life situations.

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<sup>9</sup> While a matrix implementation for the state-space approach may help in this particular case, the exponential size is an undeniable problem. By contrast, cascaded games scale and analysing, e.g., 7,000 influences over 3,000 objects takes 20 minutes.

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