

An Algebraic Trioid Model for Biological Regulatory Networks

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ABSTRACT

Gene regulatory networks govern cellular behaviour through three fundamental interaction types: activation, repression, and modulation. Existing modelling approaches—directed graphs, Boolean networks, differential equations, and motif statistics—each capture important aspects of regulatory dynamics, but none provides a compositional algebraic framework in which the three interaction types appear as primitive operations subject to explicit compatibility laws. Building on the trioid axioms of Loday and Ronco and the set-theoretic theory developed by Zhuchok, this paper makes four original contributions. First, we establish a systematic biological interpretation of the three trioid operations as activation, repression and modulation, and we show that the eight trioid axioms encode biologically meaningful consistency conditions. Second, we develop the congruence theory of trioids as a formalism for functional modularity, identifying the least semilattice congruence with regulatory module decomposition and s-simple trioids with irreducible network motifs. Third, we establish an Ehresmann–Schein–Nambooripad (ESN) correspondence for $\perp$ -regular trioids, proving an equivalence of categories between $\perp$ -regular trioids and inductive dimonoid groupoids; under this correspondence, groupoid objects correspond to attractors and morphisms to regulatory transitions. Fourth, we develop the representation theory of trioid modules over free trioids and classify irreducible modules as elementary regulatory circuits. We report preliminary computational enumeration of trioids of orders 1 through 3 using a backtracking search with canonical-form pruning; complete operation tables and executable code are provided as supplementary material. We propose, but do not execute, laboratory validation protocols based on perturbation and time-series expression experiments. The biological interpretations advanced here are theoretical; experimental confirmation remains an open problem.

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INTRODUCTION

Gene regulatory networks orchestrate the molecular events that underpin life, from the precise temporal control of developmental programs in multicellular organisms to the rapid physiological responses of single-celled microbes (Alon, 2007; Davidson, 2006; Ptashne & Gann, 2002). The elementary interactions in these networks fall into three fundamental categories (Alon, 2007; Davidson, 2006). *Activation* increases transcription of a target gene; *repression* decreases it; *modulation* represents combinatorial control wherein multiple regulators jointly determine expression through cooperative binding or signal integration (Alon, 2007; Ptashne & Gann, 2002). Existing modelling frameworks for gene regulatory networks each exhibit specific limitations with respect to algebraic compositionality. Directed graphs capture pairwise interactions but do not represent higher-order combinatorial effects as primitive operations. Boolean networks (Kauffman, 1969, 1993) capture attractor structure and have well-developed algebraic formulations over finite fields, but they do not distinguish activation, repression, and modulation as operations with distinct algebraic laws. Differential equation models (Chen et al., 2004) provide detailed quantitative descriptions but require numerous unmeasured parameters and resist compositional reasoning. Network motif theory (Milo et al., 2002) identifies statistically overrepresented

circuit patterns but does not provide a principled algebraic account of why particular motifs are favoured or how they compose. What is absent from the existing literature is a framework that (i) treats the three fundamental interaction types as primitive operations subject to explicit algebraic laws, (ii) supports compositional reasoning, (iii) provides a systematic classification of elementary building blocks, and (iv) relates algebraic structure to dynamical behaviour. The algebraic theory of trioids was introduced by Loday and Ronco (2004) as a generalisation of dialgebras and trialgebras, and was subsequently developed in the set-theoretic setting by Zhuchok (2009a, 2009b, 2010a, 2010b, 2010c, 2011a, 2011b, 2011c, 2012, 2013a, 2013b, 2013c, 2014, 2015). The present paper does not introduce the trioid axioms themselves; these are due to Loday and Ronco. Our mathematical contributions are as follows: (i) the first systematic biological interpretation of the three trioid operations and the eight axioms; (ii) an ESN-type correspondence theorem for $\perp$ -regular trioids, establishing a categorical equivalence with inductive dimonoid groupoids (Theorem 4.16); (iii) a representation theory of trioid modules over free trioids, with a classification of irreducible modules (Theorem 5.17 and Corollary 5.18); and (iv) a preliminary computational enumeration of trioids of orders 1 through 3. The biological objective of this work is to provide an algebraic foundation for three phenomena in regulatory

biology: functional modularity, attractor structure, and the evolutionary prevalence of network motifs. We propose that congruence classes on a trioid correspond to functional gene modules, that objects of the associated groupoid correspond to attractors, and that irreducible trioid modules correspond to elementary regulatory circuits. These correspondences are interpretive hypotheses advanced on the basis of structural analogy; they are not experimentally demonstrated in this paper, and we identify specific laboratory protocols by which they could be tested (Section 6.4). The assignment of activation to one operation, repression to another, and modulation to the third is not arbitrary. Three structural features of the trioid axioms motivate the assignment. First, the axioms T1–T8 are asymmetric with respect to the two primary operations: $\dashv$ and $\vdash$ play dual roles, while $\perp$ interacts with both. This asymmetry mirrors the biological distinction between the two primary regulatory modes (activation and repression) and the higher-order phenomenon of modulation, in which multiple regulators jointly determine expression. Second, the compatibility axioms T4–T8 constrain how modulation interacts with activation and repression; these constraints reflect the combinatorial logic of cooperative binding and signal integration (Ptashne & Gann, 2002). Third, associativity of each operation reflects the pathway-independence of regulatory cascades: the ultimate pattern of gene expression should not depend on the temporal order in which transcription factors bind, only on the set of factors present and their regulatory roles. We develop this interpretation formally in Section 2.2 and illustrate it with the lac operon in Example 2.9.

Preliminaries

This section establishes the algebraic foundations necessary for modeling biological regulatory networks. We introduce trioids, their elementary properties, and essential constructions, concluding with a systematic biological interpretation that motivates the subsequent mathematical development.

Table 1: Descriptive Labels to Axioms

Axiom	Description
(T1)	Activation distributivity over repression
(T2)	Repression distributivity over activation
(T3)	Repression associativity
(T4)	Modulation consistency with activation
(T5)	Activation distributivity over modulation
(T6)	Modulation-repression interaction
(T7)	Repression-modulation interaction
(T8)	Modulation consistency with repression

Biological Interpretation

The following elementary identities follow directly from the axioms and will be used throughout the paper.

Lemma 2.4 (Loday and Ronco, 2004; Zhuchok, 2015)

In any trioid $(T, \dashv, \vdash, \perp)$, the following identities hold for all $x, y, z \in T$:

- (2.1) $(x \dashv y) \vdash z = x \dashv (y \vdash z)$,
- (2.2) $(x \vdash y) \dashv z = x \vdash (y \dashv z)$,
- (2.3) $(x \perp y) \vdash z = x \perp (y \vdash z)$,
- (2.4) $(x \dashv y) \perp z = x \dashv (y \perp z)$,
- (2.5) $(x \vdash y) \perp z = x \vdash (y \perp z)$,
- (2.6) $(x \perp y) \perp z = x \perp (y \perp z)$.

Definition 2.5 (Special Classes of Trioids)

A trioid $(T, \dashv, \vdash, \perp)$ is called:

Definition and Fundamental Axioms

Following (Loday and Ronco 2004, Zhuchok, 2015), we recall the definition of a trioid.

Definition 2.1 (Trioid; Zhuchok, 2015)

A trioid is a nonempty set T equipped with three binary operations

$$\dashv: T \times T \rightarrow T, \quad \vdash: T \times T \rightarrow T, \quad \perp: T \times T \rightarrow T$$

satisfying the following eight axioms for all $x, y, z \in T$:

- (T1) $(x \dashv y) \dashv z = x \dashv (y \vdash z)$,
- (T2) $(x \vdash y) \dashv z = x \vdash (y \dashv z)$,
- (T3) $(x \dashv y) \vdash z = x \vdash (y \vdash z)$,
- (T4) $(x \dashv y) \dashv z = x \dashv (y \perp z)$,
- (T5) $(x \perp y) \dashv z = x \perp (y \dashv z)$,
- (T6) $(x \dashv y) \perp z = x \perp (y \vdash z)$,
- (T7) $(x \vdash y) \perp z = x \vdash (y \perp z)$,
- (T8) $(x \perp y) \vdash z = x \vdash (y \vdash z)$.

It follows from the axioms that each operation is associative: setting appropriate variables yields

$$(x \dashv y) \dashv z = x \dashv (y \dashv z), \quad (x \vdash y) \vdash z = x \vdash (y \vdash z), \\ (x \perp y) \perp z = x \perp (y \perp z).$$

Remark 2.2

The axioms (T1)–(T8) constitute the complete axiom system for a trioid as given in (Loday and Ronco, 2004) and (Zhuchok, 2015). The axioms are not symmetric in the operations: $\dashv$ and $\vdash$ play dual roles, while $\perp$ interacts with both. This asymmetry reflects the biological distinction between the two primary regulatory modes (activation and repression) and the higher-order phenomenon of modulation.

Remark 2.3

For reference throughout the paper, we assign descriptive labels to the axioms:

- i commutative if each operation is commutative;
- ii idempotent if $x * x = x$ for all $x \in T$ and $*$ $\in \{\dashv, \vdash, \perp\}$;
- iii separative if for all $x, y \in T$, the conditions $x * x = x * y = y * y$ imply $x = y$ for each operation $*$ $\in \{\dashv, \vdash, \perp\}$;
- iv s-simple if its least semilattice congruence (defined in Section 3) is the universal relation; (v) pointed if it contains a zero element.

Definition 2.6 (Zero Element, Zhuchok, 2010a)

An element $0 \in T$ is a zero of the trioid if for all $x \in T$ and $*$ $\in \{\dashv, \vdash, \perp\}$, $x * 0 = 0 = 0 * x$.

Having established the algebraic definition, we now develop the biological interpretation that motivates our applications. This interpretation will be referenced throughout the paper.

Definition 2.7 (Biological Interpretation of Trioid Operations)

Let T be a trioid whose elements represent genes, transcription factors or other regulatory molecules. The three operations are interpreted as follows:

i Activation ($\dashv$): If gene x activates gene y , represented by $x \dashv y$. Associativity reflects the pathway-independence of activation cascades (Alon, 2007).

ii Repression ($\vdash$): If gene x represses gene y , represented by $x \vdash y$. The interaction between activation and repression encodes context-dependent regulatory logic (Ptashne & Gann, 2002).

iii Modulation ($\perp$): If genes x and y jointly regulate gene z , represented by $x \perp y$. Associativity reflects that higher-order regulatory complexes form independent of assembly order (Davidson, 2006).

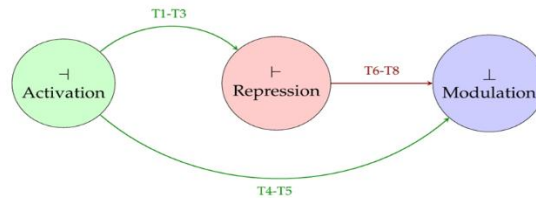


Figure 1: The Three Operations of a Trioid Correspond to the three Fundamental Biological Interaction Types: Activation ($\dashv$), Repression ($\vdash$), and Modulation ($\perp$). The Eight Axioms (T1)-(T8) Encode the Consistency Conditions Governing how these Operations Interact

The assignment of the three trioid operations to the three biological interaction types warrants explicit justification, since the axioms themselves do not carry biological content. Three structural features of the trioid signature motivate the assignment. First, the axioms T1–T8 are asymmetric with respect to the two primary operations: $\dashv$ and $\vdash$ play dual roles, while $\perp$ interacts with both. This asymmetry mirrors the biological distinction between activation and repression, which are directional and context-dependent, and modulation, which represents combinatorial control by multiple regulators. Second, the compatibility axioms T4–T8 constrain how modulation interacts with activation and repression; these constraints encode the requirement that combinatorial control be consistent with the primary regulatory modes. Third, each operation is associative, reflecting the pathway-independence of regulatory cascades: the ultimate pattern of gene expression should not depend on the temporal order in which transcription factors bind, only on the set of factors present and their regulatory roles (Alon, 2007; Davidson, 2006). The interpretation is therefore structural rather than semantic: we do not claim that the axioms were derived from biology, but that they admit a natural biological context that motivates their application. Section 7.2 discusses the limitations in details.

Proposition 2.8

The trioid axioms encode biologically meaningful constraints:

- i. (T1) $(x \dashv y) \dashv z = x \dashv (y \vdash z)$: Activation cascades are independent of intermediate repression.
- ii. (T2) $(x \vdash y) \dashv z = x \vdash (y \dashv z)$: Repression distributes over activation.
- iii. (T3) $(x \dashv y) \vdash z = x \vdash (y \vdash z)$: Repression cascades are associative.
- iv. (T4) $(x \dashv y) \perp z = x \dashv (y \perp z)$: Modulation is consistent with activation.
- v. (T5) $(x \perp y) \dashv z = x \perp (y \dashv z)$: Activation distributes over modulation.
- vi. (T6) $(x \dashv y) \perp z = x \perp (y \vdash z)$: Modulation interacts with repression.
- vii. (T7) $(x \vdash y) \perp z = x \vdash (y \perp z)$: Repression interacts with modulation.
- viii. (T8) $(x \perp y) \vdash z = x \vdash (y \vdash z)$: Modulation is consistent with repression cascades.

Example 2.9

Consider the lac operon in *E. coli* (Ptashne & Gann, 2002). The activator CAP and repressor LacI regulate the operon. In trioid terms:

- i CAP $\dashv$ Operon represents activation.
- ii LacI $\vdash$ Operon represents repression.
- iii The interaction $(\text{CAP} \vdash \text{LacI}) \dashv \text{Operon} = \text{CAP} \vdash (\text{LacI} \dashv \text{Operon})$ reflects that when LacI is bound, CAP activation is blocked regardless of order.

This is precisely axiom (T2). The trioid elements are CAP, LacI, Operon, 0, where 0 represents absence of regulation. The operation tables reflect the known biology: CAP $\dashv$ Operon = ActiveOperon, LacI $\vdash$ Operon = 0 (repression), and Inducer $\perp$ LacI = InactiveLacI. Axiom (T2) ensures that when LacI is bound, CAP activation is blocked regardless of the order of operations.

Remark 2.10

The axioms assert that the outcome of regulatory interactions is independent of the order in which they are considered, provided we respect the fundamental distinction between activation, repression, and modulation. This independence is precisely what one expects from well-behaved biological systems: the ultimate pattern of gene expression should not depend on the temporal order of transcription factor binding, only on the set of factors present and their regulatory roles (Alon, 2007; Davidson, 2006).

Homomorphisms and Substructures**Definition 2.11 (Homomorphism; Zhuchok, 2010a)**

A map $f: T \rightarrow S$ between trioids $(T, \dashv, \vdash, \perp)$ and $(S, \dashv_s, \vdash_s, \perp_s)$ is a homomorphism if for all $x, y \in T$,

$$(x \dashv y)f = xf \dashv_s yf,$$

$$(x \vdash y)f = xf \vdash_s yf, (x \perp y)f = xf \perp_s yf.$$

If f is bijective, it is an isomorphism.

Definition 2.12 (Subtrioid; Zhuchok, 2010b)

A subset $A \subseteq T$ is a subtrioid if for all $a, b \in A$, we have

$$a \dashv b \in A, \quad a \vdash b \in A, \quad a \perp b \in A.$$

Constructions and Examples

The richness of trioid theory is evidenced by the variety of natural examples arising from different mathematical

contexts (Loday & Ronco, 2004, Zhuchok, 2010a, 2010b, 2014).

Semigroups as Degenerate Trioids

Example 2.13 (Zhuchok, 2010a)

If all three operations coincide, say $x \dashv y = x \vdash y = x \perp y = xy$ for some associative multiplication, then $(T, \dashv, \vdash, \perp)$ is a trioid precisely when $(T, \cdot)$ is a semigroup. Thus, every semigroup can be regarded as a trioid in which the three operations are identified.

In biological terms, this corresponds to a system where activation, repression, and modulation are not distinguished.

Dimonoids as Two-Operation Trioids

Recall that a *dimonoid* is a set equipped with two associative operations satisfying axioms corresponding to (T1)-(T3) (Loday, 2001).

Example 2.14 (Loday 2001, Zhuchok 2010a)

If two operations coincide, say $\vdash = \perp$, then the axioms reduce to those of a dimonoid. Conversely, every dimonoid $(D, \dashv, \vdash)$ can be considered as a trioid by setting $\perp = \vdash$. This embedding is full and faithful.

Lemma 2.15 (Zhuchok 2013b, Lemma 2)

For any trioid $(T, \dashv, \vdash, \perp)$, the algebra obtained by selecting two operations is a dimonoid if and only if the selected operations are one of the pairs $(\dashv, \dashv)$, $(\vdash, \vdash)$, $(\perp, \perp)$, or $(\dashv, \vdash)$.

Averaging Operator Construction

The following construction shows how a single operator can generate three distinct operations from a given semigroup structure.

Proposition 2.16 (Zhuchok, 2010b, Proposition 9)

Let S be a semigroup and let $f : S \rightarrow S$ be an idempotent endomorphism. Define operations on S by

$$x \dashv y = x(yf), \quad x \vdash y = (xf)y, \quad x \perp y = (xy)f$$

for all $x, y \in S$. Then $(S, \dashv, \vdash, \perp)$ is a trioid, denoted S^f .

In biological terms, f may represent a modification process (e.g., phosphorylation) that alters regulatory effects (Alon, 2007).

2.4.4. *Rees Trioids.* The following construction generalizes the classical Rees matrix semigroup construction (Rees, 1940).

Proposition 2.17 (Zhuchok, 2012, Proposition 8)

Let S be a rectangular semigroup (satisfying $xyz = xz$ for all $x, y, z \in S$), let M be an arbitrary semigroup, and let $\varphi : M \rightarrow S$ be a homomorphism. Define operations on $S \times M$ by

$$\begin{aligned} (s, t) \dashv (p, q) &= (s, tq), \\ (s, t) \vdash (p, q) &= ((t\varphi)p, tq), \\ (s, t) \perp (p, q) &= (sp, tq) \end{aligned}$$

for all $(s, t), (p, q) \in S \times M$. Then $(S \times M, \dashv, \vdash, \perp)$ is a trioid.

Word-Based Examples

Example 2.18 (Zhuchok, 2010b)

Let X^* be the set of all finite nonempty words in an alphabet X . For $w \in X^*$, denote the first letter by $w^{(0)}$ and the last letter by $w^{(1)}$. Define operations on X^* by

$$w \dashv u = w(0)w(1), \quad w \vdash u = u(0)u(1), \quad w \perp u = w(0)u(1).$$

Then $(X^*, \dashv, \vdash, \perp)$ is a trioid.

The Trioid $T_{I^\perp}$

Example 2.19 (Zhuchok, 2014)

Let $(T, \perp)$ be an arbitrary semigroup. Define operations $\dashv$ and $\vdash$ on T by $x \dashv y = x$, $x \vdash y = y$

for all $x, y \in T$. Then $(T, \dashv, \vdash, \perp)$ is a trioid, denoted $T_{I^\perp}$.

This trioid has idempotent operations $\dashv$ and $\vdash$. If $\perp$ is idempotent, then $T_{I^\perp}$ is an idempotent trioid.

Free Trioids

The free trioid construction provides the universal object in the category of trioids and will be essential for the representation theory developed in Section 5.

Theorem 2.20 (Zhuchok, 2010a)

Let Y be an arbitrary nonempty set. Set $\bar{Y} = \{\bar{x} \mid x \in Y\}$ and $X = Y \cup \bar{Y}$. Let $F[X]$ be the free semigroup on X and let $P \subset F[X]$ be the subsemigroup consisting of all words in which at least one letter from $\bar{Y}$ occurs. For $w \in P$, denote by $\bar{w}$ the word obtained from w by e replacing each occurrence of $\bar{x}$ by x . Define operations on P by

$$w \dashv u = w\bar{u}, \quad w \vdash u = \bar{w}u, \quad w \perp u = wu$$

for all $w, u \in P$. Then $(P, \dashv, \vdash, \perp)$ is the free trioid on Y , denoted $\text{Frt}(Y)$.

Corollary 2.21

For any trioid T and any map $\varphi : Y \rightarrow T$, there exists a unique trioid homomorphism $\bar{\varphi} : \text{Frt}(Y) \rightarrow T$ extending φ .

The following position-set representation provides a combinatorial encoding useful for computational purposes.

Theorem 2.22 (Zhuchok, 2010a)

Let $F[Y]$ be the free semigroup on Y . For a word $w \in F[Y]$, denote its length by $|w|$. Define

$F = \{(w, L) \mid w \in F[Y], L \subseteq \{1, \dots, \ell_w\}, L \neq \emptyset\}$. For $(w, L), (u, R) \in F$, define

$$\begin{aligned} (w, L) \dashv (u, R) &= (wu, L), \\ (w, L) \vdash (u, R) &= (wu, R + \ell_w), \\ (w, L) \perp (u, R) &= (wu, L \cup (R + \ell_w)), \end{aligned}$$

where $R + \ell_w = \{y + \ell_w \mid y \in R\}$. Then $(F, \dashv, \vdash, \perp)$ is a trioid isomorphic to $\text{Frt}(Y)$.

In this representation, L records the positions originating from overlined letters. This encoding will prove useful for computational enumeration.

Corollary 2.23 (Loday, 2001, Zhuchok, 2010a)

The free trioid of rank one is isomorphic to $(F', <, >, \uparrow)$, where

$$F' = \{(n, L) \mid n \in \mathbb{N}, L \subseteq \{1, \dots, n\}, L \neq \emptyset\},$$

with operations

$$\begin{aligned} (n, L) < (m, R) &= (n + m, L), \quad (n, L) > (m, R) = (n + m, R + n), \\ (n, L) \uparrow (m, R) &= (n + m, L \cup (R + n)). \end{aligned}$$

Combinatorial Properties

The following lemmas establish conditions under which operations coincide, useful for classification.

Lemma 2.24 (Zhuchok, 2013c)

Operations of a trioid $(T, \dashv, \vdash, \perp)$ coincide if any of the following holds:

- $(T, \dashv)$ or $(T, \vdash)$ is weakly cancellative;
- $(T, \dashv)$ is left cancellative or $(T, \vdash)$ is right cancellative;
- $(T, \dashv)$ or $(T, \vdash)$ is commutative separative;
- $(T, \dashv)$ or $(T, \vdash)$ is commutative globally idempotent;
- $(T, \dashv)$ or $(T, \vdash)$ is a monoid; (vi) $(T, \vdash)$ is a semilattice.

Lemma 2.25 (Zhuchok, 2010b)

Operations of a trioid coincide if (T, a) is a semilattice.

Theorem 2.26 (Zhuchok, 2014)

Let $X = \{1, 2, 3\}$. For $\sigma = (xyz) \in \text{Sym}(X)$, let

$T_\sigma = (T, *_x, *_y, *_z)$. Let

$\Lambda = \{(111), (222), (333), (121), (122)\} \subset \text{Sym}(X)$.

Then for any trioid T_e , T_σ with $\sigma \neq e$ is a trioid if $\sigma \in \Lambda$. Moreover, there exists a trioid T_e for which T_σ is not a trioid for any $\sigma \in \text{Sym}(X) \setminus (\Lambda \cup \{e\})$.

Orthogonal Sums of Trioids

The following construction will be useful for building complex trioids from simpler components.

Definition 2.27 (Zhuchok, 2013c)

A trioid $(T, \neg, \vdash, \perp)$ with zero 0 is an orthogonal sum of trioids T_i , $i \in Y$, if:

- i $T_i \neq \{0\}$ for each $i \in Y$;
- ii $T = \bigcup_{i \in Y} T_i$;
- iii $T_i \cap T_j = T_i * T_j = \{0\}$ for all $i \neq j$ and $* \in \{\neg, \vdash, \perp\}$.

The orthogonal sum is denoted $O[T_i]_{i \in Y}$.

Lemma 2.28 (Zhuchok, 2013c)

Let $O[S_i]_{i \in I}$ be an orthogonal sum of trioids S_i . For subtrioids $T_i \subseteq S_i$, $i \in I$, and for $I' \subseteq I$ with $|I'| > 1$, define

$T_{I'} = \bigcup_{i \in I'} T_i$, $T_{I'}^0 = T_{I'} \cup \{0\}$

Then the full list of subtrioids consists of:

- (i) subtrioids of each S_i ;
- (ii) subtrioids $T_{I'}^0$ for $|I'| > 1$.

In biological terms, an orthogonal sum represents a network of independent modules interacting only through the zero element (absence of regulation) (Alon, 2007).

Congruence Theory and Biological Modularity

This section develops the congruence theory of trioids and its biological interpretation. Congruences identify elements that behave identically in all contexts; in biological terms, congruent genes are functionally interchangeable under all regulatory conditions, forming natural modules.

Basic Properties**Definition 3.1 (Congruence, Zhuchok 2010a, 2012)**

An equivalence relation $\rho \subseteq T \times T$ is a congruence on a trioid T if for all $x, y, z \in T$ and $* \in \{\neg, \vdash, \perp\}$, $(x, y) \in \rho \Rightarrow (x * z, y * z) \in \rho$ and $(z * x, z * y) \in \rho$.

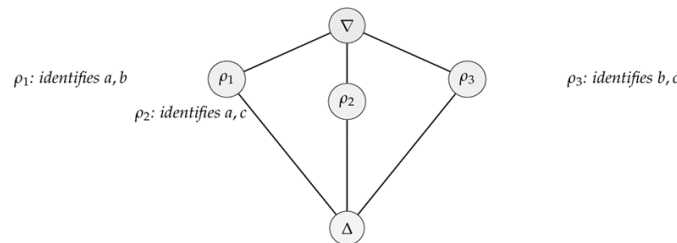


Figure 2: The Congruence Lattice of a Trioid Encodes the Hierarchical Organization of Functional Modules. The Identity Congruence Δ Represents Full Regulatory Resolution Where All Genes are Functionally Distinct. The Universal congruence ∇ Represents Complete Collapse where all Genes are Equivalent. Intermediate Congruences Identify Functionally Equivalent Genes, Forming Regulatory Modules. This Lattice Structure is Computed Using the Algorithms Developed in Section 6

Remark 3.2

Biologically, two genes $x, y \in T$ are congruent if interchangeable in all regulatory contexts: for any regulator z and operation $*$, the results $x * z$ and $y * z$ are equivalent, as are $z * x$ and $z * y$. This captures functional equivalence or redundancy (Alon, 2007, Hartwell et al, 1999).

Theorem 3.3 (Zhuchok, 2010a, 2012)

For any trioid T , $\text{Con}(T)$ forms a complete lattice. The meet of $\{\rho_i\}_{i \in I}$ is $\bigcap_{i \in I} \rho_i$; the join is the transitive closure of $\bigcup_{i \in I} \rho_i$.

Proof

That $\text{Con}(T)$ is a complete lattice follows from universal algebra (Burris & Sankappanavar, 1981). The meet is intersection since compatibility is preserved under arbitrary intersections. The join requires transitive closure since unions of equivalence relations need not be transitive.

Theorem 3.4 (Zhuchok, 2012, Theorem 2)

For any trioid T , $\text{Con}(T)$ is modular: for $\rho \subseteq \tau$, $\rho \vee (\sigma \wedge \tau) = (\rho \vee \sigma) \wedge \tau$.

Corollary 3.5

Every maximal chain in $\text{Con}(T)$ has well-defined length.

Principal Congruences**Definition 3.6 (Zhuchok, 2012)**

The principal congruence generated by (a, b) , denoted $\rho(a, b)$, is the smallest congruence containing (a, b) .

Definition 3.7 (Zhuchok, 2012)

A translation is a function $\varphi(x) = p * x * q$, where $p, q \in T^1$ (allowing empty arguments) and $*, * \in \{\neg, \vdash, \perp\}$.

Lemma 3.8

Every congruence is closed under translations: if $(x, y) \in \rho$ and φ is a translation, then $(\varphi(x), \varphi(y)) \in \rho$.

Proof

Induct on the complexity of φ . For elementary $\varphi(x) = p * x$, left compatibility gives $(p * x, p * y) \in \rho$. Similarly, for right translations. Composition iterates compatibility.

Proposition 3.9 (Zhuchok, 2012)

For any $a, b \in T$, $\rho(a, b) = \{(u(a), u(b)) \mid u \text{ is a composition of translations}\} \sim$,

where $\{. . .\} \sim$ denotes the smallest equivalence relation containing the indicated pairs.

Least Congruences with Biological Significance

(Zhuchok, 2012, 2013a, 2013b) identified three least congruences with natural biological interpretations.

Least Idempotent Congruence

Definition 3.10 (Least Idempotent Congruence, Zhuchok, 2012)

For a trioid T with commutative $\rightarrow$, define η by

$$a \eta b \Leftrightarrow \exists k, l \in \mathbb{N} : a \rightarrow b^k, b \rightarrow a^l,$$

where $a \rightarrow b$ means $\exists x : a \rightarrow x = b$.

Theorem 3.11 (Zhuchok, 2012, Theorem 4)

For T with commutative $\rightarrow$, η is the least idempotent congruence, and T/η is a commutative idempotent trioid which is a semilattice. This extends (Tamura & Kimura, 1954) on commutative semigroups.

Remark 3.12

The relation η identifies genes connected by activation chains where repeated activation stabilizes. The quotient T/η represents the core regulatory logic where all genes are idempotent under activation: once fully induced, further activation has no effect. The semilattice structure reflects regulatory hierarchies (Alon, 2007; Davidson, 2006).

Least Semilattice Congruence

Definition 3.13 (Least Semilattice Congruence, Zhuchok, 2012)

For a trioid T with idempotent $\rightarrow$, define $\mathfrak{S}$ by

$$a \mathfrak{S} b \Leftrightarrow a = a \rightarrow b \rightarrow a, \quad b = b \rightarrow a \rightarrow b.$$

Theorem 3.14 (Zhuchok 2012, Theorem 5)

For T with idempotent $\rightarrow$, $\mathfrak{S}$ is the least semilattice congruence. This extends (McLean, 1954) on bands.

Remark 3.15

The conditions $a = a \rightarrow b \rightarrow a$ and $b = b \rightarrow a \rightarrow b$ state that a and b regulate each other so that each reproduces itself in the other's context. This is precisely the condition for genes to form a regulatory module—mutually dependent and inseparable (Alon, 2007, Hartwell et al., 1999). The quotient $T/\mathfrak{S}$ identifies modules and collapses each to a single element, providing an algebraic method for modular decomposition.

Least Separative Congruence

Definition 3.16 (Least Separative Congruence, Zhuchok, 2012)

For a trioid T with commutative $\rightarrow$, define σ by

$$a \sigma b \Leftrightarrow \exists n \in \mathbb{N} : a \rightarrow b^n = b^{n+1}, \quad b \rightarrow a^n = a^{n+1}.$$

Theorem 3.17 (Zhuchok, 2012, Theorem 6)

For T with commutative $\rightarrow$, σ is the least separative congruence, and T/σ is a commutative separative trioid which is a commutative separative semigroup. This extends (Hewitt & Zuckerman, 1956) on commutative semigroups.

Lemma 3.18 (Zhuchok, 2012)

$$\sigma = \Omega_{\rightarrow} \cap \Omega_{\rightarrow} \cap \Omega_{\perp},$$

where $\Omega_{\rightarrow}$, $\Omega_{\rightarrow}$, $\Omega_{\perp}$ are the least semilattice congruences on the reducts.

Remark 3.19

The separative quotient collapses genes that are indistinguishable under repeated regulation, possibly corresponding to subfunctionalized duplicates. The characterization $\sigma = \Omega_{\rightarrow} \cap \Omega_{\rightarrow} \cap \Omega_{\perp}$ shows that functional redundancy requires equivalence under all three interaction types (Alon, 2007).

Table 2: Congruence Relations on a Semigroup of Biological Regulatory Maps, their Defining Conditions, Quotient Structures, and Corresponding Biological Interpretations. Refinement Levels Indicate the Relative Coarseness of Each Equivalence

Congruence	Condition	Quotient	Biological	Refinement
η	$a \rightarrow b^k, b \rightarrow a^l$	Semilattice	Regulatory Hierarchy	Coarsest
$\mathfrak{S}$	$a = a \rightarrow b \rightarrow a, b = b \rightarrow a \rightarrow b$	Semilattice	Functional Modularity	Intermediate
σ	$a \rightarrow b^n = b^{n+1},$ $b \rightarrow a^n = a^{n+1}$	Separative Semigroup	Functional Redundancy	Finest

These Satisfy $\sigma \subseteq \eta \cap \mathfrak{S}$; η and $\mathfrak{S}$ are Generally Incomparable

Structural Decomposition

Group Congruences

Definition 3.20 (Group Congruence)

A congruence ρ is a group congruence if T/ρ is a group (all operations coincide).

Theorem 3.21 (Zhuchok 2013a, Theorem 2)

Let $(T, \rightarrow)$ be regular. Let $H \subseteq (T, \rightarrow)$ be a reflexive subsemigroup generated by idempotents, and $H(\rightarrow) = \{x \mid h \rightarrow x \in H \text{ for some } h \in H\}$. Define

$$a \mu_{H(\rightarrow)} b \Leftrightarrow \exists x : x \rightarrow a, x \rightarrow b \in H(\rightarrow).$$

Then $\mu_{H(\rightarrow)}$ is the least group congruence. This extends (Munn, 1961), (Meakin, 1972), and (Masat, 1973).

Corollary 3.22

If $(T, \rightarrow)$ is inverse, the least group congruence is $a_e * b \Leftrightarrow e \rightarrow a = e \rightarrow b$ for some idempotent e .

Theorem 3.23 (Zhuchok 2013a, Theorem 1)

Let $H \subseteq (T, \rightarrow)$ be a subsemigroup such that:

$$H_a := \{(x, y) \mid x \rightarrow a \rightarrow y \in H\} \neq \emptyset \text{ for all } a;$$

$$H_a \cap H_b \neq \emptyset \Rightarrow H_a = H_b.$$

Then ρ_H defined by $a \rho_H b \Leftrightarrow H_a = H_b$ is a group congruence. Conversely, every group congruence arises this way from its identity class.

Semilattice Congruences

Definition 3.24 (Yamada, 1955)

A subsemigroup $S' \subseteq S$ is a P-subsemigroup if for any finite $a_1, \dots, a_n \in S$, $a_1 \cdots a_n \in S'$ implies $\zeta_1 \cdots \zeta_m \in S'$ for any $\zeta_1, \dots, \zeta_m \in S$ with the same underlying set.

P-subsemigroups are closed under products with the same underlying set regardless of order, ensuring that the relation defined by identifying elements with the same P-subsemigroup contexts is a semilattice congruence.

Theorem 3.25 (Zhuchok, 2013a, Theorem 1)

Let Γ be a set of P -subsemigroups of $(T, \neg)$. Define

$$a \Gamma_{\neg} b \Leftrightarrow \{(x, y) \mid x a \neg a \neg y \in S\} = \{(x, y) \mid x \neg b \neg y \in S\}$$

for every $S \in \Gamma$. Then $\Gamma_{\neg}$ is a semilattice congruence. Conversely, every semilattice congruence arises this way.

Theorem 3.26 (Zhuchok, 2013a, Theorem 2)

Let $\Omega_{\neg}$ be the set of all P -subsemigroups of $(T, \neg)$. Then $\Omega_{\neg}$ is the least semilattice congruence, and every class is s -simple.

Definition 3.27 (s-Simple Trioid, Zhuchok, 2015)

A trioid is s -simple if its least semilattice congruence is universal.

Corollary 3.28 (Zhuchok, 2015)

Every trioid T is a semilattice of s -simple subtrioids: $T = \bigcup_{\alpha \in Y} T_{\alpha}$, where $Y = T / \Omega_{\neg}$ is a semilattice and each T_{α} is s -simple.

Theorem 3.29 (Zhuchok, 2013a)

A finite s -simple trioid is isomorphic to a Rees trioid $M(I, G, J; p)$ where G is a finite group and $p: J \times I \rightarrow G$. Conversely, every such Rees trioid is s -simple.

Example 3.30

Examples of s -simple trioids correspond to known motifs:

Feed-forward loop: Three genes A, B, C with A activating B and C , and B activating C . The s -simple trioid structure shows that this motif functions as an integrated unit (Alon, 2007, Milo, 2002).

Repressilator: Three genes in a repression cycle $A \vdash B \vdash C \vdash A$, producing oscillations (Elowitz and Leibler, 2000).

Toggle switch: Two genes with mutual repression $A \vdash B$ and $B \vdash A$, producing bistability (Gardner, et al, 2000).

The group G represents the motif's symmetry; the map p encodes regulatory interactions.

Congruence Lattices and Network Robustness

Having established the algebraic structure of congruences and their role in decomposing trioids, we now interpret these structures biologically, connecting congruence lattices to properties of regulatory networks.

Definition 3.31 (Lattice Invariants).

For a trioid T :

Height $h(T)$: length of longest chain in $\text{Con}(T)$;

Width $w(T)$: size of largest antichain;

Automorphism group $\text{Aut}(\text{Con}(T))$.

Proposition 3.32

- Height reflects hierarchical depth. Tall lattices indicate complex regulatory hierarchies (Davidson, 2006).
- Width reflects modular independence. Wide lattices indicate many independent modules, associated with distributed control and robustness (Alon, 2007, Hartwell, et al, 1999).
- Automorphism group reflects regulatory symmetries, possibly corresponding to gene duplications or fundamental biological symmetries (Stewart, 2004).

Proposition 3.33

- Distributive congruence lattices indicate clean modular decomposition with minimal cross-talk (Alon, 2007).

- Non-distributive modular lattices indicate complex interdependence with emergent properties (Hartwell et al, 1999).

- Complemented elements correspond to mutually exclusive regulatory contexts or distinct cell types (Davidson, 2006).

The ESN Correspondence

The Ehresmann-Schein-Nambooripad (ESN) correspondence is one of the most profound results in semigroup theory, establishing an equivalence between inverse semigroups and inductive groupoids (Lawson, 1998, Nambooripad, 1994). This correspondence reveals that algebraic structures can be equivalently described in geometric or categorical terms, providing a bridge between discrete algebra and continuous dynamics. In this section, we establish an analogous correspondence for trioids, showing that $\perp$ -regular trioids are equivalent to inductive internal groupoids in the category of dimonoids. This categorical reformulation provides a powerful new perspective on trioid models of biological networks, recasting network dynamics in terms of groupoids of regulatory states and transitions. In this section we assume familiarity with basic category theory, including categories, functors, natural transformations and equivalences of categories. We refer the reader to (MacLane, 1971, Spivak, 2015) for some background.

Internal Groupoids in Dimonoids

We begin by establishing the categorical framework for internal groupoids. Let Dimon denote the category of dimonoids with dimonoid homomorphisms (Spivak, 2015, Zhuchok, 2009a, 2010c, 2011b).

Definition 4.1 (Internal Groupoid, MacLane, 1971, Spivak, 2015).

Let $\mathcal{C}$ be a category with finite limits. An internal groupoid in $\mathcal{C}$ consists of:

Objects G_0, G_1 in $\mathcal{C}$;

Morphisms $\partial_0, \partial_1: G_1 \rightarrow G_0$ in $\mathcal{C}$ (source and target);

A morphism $\text{id}: G_0 \rightarrow G_1$ in $\mathcal{C}$ (identity assignment);

A morphism $\circ: G_1 \times_{G_0} G_1 \rightarrow G_1$ in $\mathcal{C}$ (composition), defined on the pullback of ∂_1 and ∂_0 ;

A morphism $^{-1}: G_1 \rightarrow G_1$ in $\mathcal{C}$ (inversion) satisfying the usual groupoid axioms expressed as commutative diagrams in $\mathcal{C}$.

The pullback $G_1 \times_{G_0} G_1$ consists of pairs $(x, y) \in G_1 \times G_1$ such that $\partial_1(x) = \partial_0(y)$; this is precisely the set of composable pairs of morphisms. The fact that Dimon has all limits follows from the general result that any variety of algebras is complete (Burris & Sankappanavar, 1981). For our purposes, the ambient category is Dimon . This choice is natural because trioids have three operations, and the ESN correspondence should reduce the number of operations by one, yielding a categorical structure whose objects and morphisms themselves carry two operations.

Definition 4.2 (Dimonoid Groupoid; Berger & Funk, 2016)

A dimonoid groupoid is an internal groupoid in Dimon . Explicitly:

- G_0 and G_1 are dimonoids, with operations denoted $(\neg_0, \vdash_0)$ and $(\neg_1, \vdash_1)$, respectively;

- $\partial_0, \partial_1, \text{id}, \circ, ^{-1}$ are dimonoid homomorphisms;

- The pullback $G_1 \times_{G_0} G_1$ is taken in Dimon ;

- The groupoid axioms hold as equations in Dimon .

The requirement that source and target be dimonoid homomorphisms imposes compatibility conditions between the dimonoid structures on G_0 and G_1 . For all $x, y \in G_1$, we must have:

$$\partial_0(x \neg_1 y) = \partial_0(x) \neg_0 \partial_0(y), \quad \partial_0(x \vdash_1 y) = \partial_0(x) \vdash_0 \partial_0(y),$$

$$\partial_1(x \dashv_1 y) = \partial_1(x) \dashv_0 \partial_1(y), \quad \partial_1(x \vdash_1 y) = \partial_1(x) \vdash_0 \partial_1(y).$$

These conditions ensure that the algebraic structure on morphisms is consistent with the structure on objects.

Example 4.3

Let G_0 be any dimonoid. Define $G_1 = G_0$ with the same dimonoid structure, $\partial_0 =$

$\partial_1 = id_{G_0}$, and composition defined by $x \circ y = x$ (or any constant map). This yields a dimonoid groupoid.

Example 4.4

Let G_0 be any dimonoid. Define $G_1 = G_0$ with the same dimonoid structure, $\partial_0 =$

$\partial_1 = id_{G_0}$, and composition defined only on identity morphisms (i.e., $x \circ y$ is defined only when $x = y$ and equals x). This gives a discrete groupoid, where the only morphisms are identities.

From Trioids to Dimonoid Groupoids: The Functor $\mathcal{G}$. Let $(T, \dashv, \vdash, \perp)$ be a trioid. Not every trioid will yield a well-behaved groupoid; we need a regularity condition that ensures the existence of inverses.

Definition 4.5 ($\perp$ -Regular Element, Zhuchok 2012)

An element $x \in T$ is called $\perp$ -regular if there exists $x^* \in T$ such that $x \perp x^* \perp x = x$ and $x^* \perp x \perp x^* = x^*$.

Such an x^* is called a $\perp$ -inverse of x . A trioid is called $\perp$ -regular if every element has a $\perp$ -inverse.

Remark 4.6

In biological terms, $\perp$ -regularity corresponds to the existence of regulatory reversibility: for every regulatory interaction, there is a converse interaction that can undo its effects. This is a strong condition, but it holds in many important cases, particularly when regulation is mediated by reversible binding events (Ptashne & Gann, 2002).

Assuming T is $\perp$ -regular, we construct a dimonoid groupoid $\mathcal{G}(T)$ as follows.

Construction 4.7 (The Groupoid of a Trioid)

Let T be a $\perp$ -regular trioid. Define:

$G_0 = E_\perp(T) = \{e \in T \mid e \perp e = e\}$, the set of $\perp$ -idempotents. For $e, f \in G_0$, define

$$e \dashv_0 f = e \dashv f, \quad e \vdash_0 f = e \vdash f.$$

The trioid axioms ensure that $(G_0, \dashv_0, \vdash_0)$ is a dimonoid.

$G_1 = T$, with its given $\dashv$ and $\vdash$ operations, denoted $\dashv_1$ and $\vdash_1$. For $x \in G_1$, define $\partial_0(x) = x^* \perp x$, $\partial_1(x) = x \perp x^*$,

where x^* is any chosen $\perp$ -inverse of x . The axioms of a $\perp$ -regular trioid ensure that these definitions are independent of the choice of inverse.

For $e \in G_0$, define $id(e) = e$ (viewed as an element of G_1).

For composable $x, y \in G_1$ (i.e., $\partial_1(x) = \partial_0(y)$), define

$$x \circ y = (x \dashv_1 y) \dashv_1 (x \perp y).$$

This formula reflects the interplay of activation, repression, and modulation in producing composite regulatory transitions. For $x \in G_1$, define $x^{-1} = x^*$.

The verification that this construction indeed yields a dimonoid groupoid is nontrivial but follows from the trioid axioms.

Lemma 4.8

The maps $\partial_0, \partial_1 : G_1 \rightarrow G_0$ are dimonoid homomorphisms.

Proof

We show that ∂_0 preserves $\circ$; the other cases are similar. For $x, y \in G_1$, the key properties of the $\perp$ -inverse used in the proof are:

$$(x \dashv_1 y)^* = x^* \dashv_1 y^*, \quad (x \vdash_1 y)^* = x^* \vdash_1 y^*, \quad x^* \perp x \perp x^* = x^*.$$

These follow from the $\perp$ -regularity axioms and the trioid axioms. Now:

$$\partial_0(x \dashv_1 y) = (x \dashv_1 y)^* \perp (x \dashv_1 y) = (x^* \dashv_1 y^*) \perp (x \dashv_1 y).$$

Using the trioid axioms, this equals

$$(x^* \perp x) \dashv_1 (y^* \perp y) = \partial_0(x) \dashv_0 \partial_0(y).$$

The verification for $\vdash$ is similar, and the arguments for target are dual.

Lemma 4.9

The map $id : G_0 \rightarrow G_1$ is a dimonoid homomorphism.

Proof

For $e, f \in G_0$, we have $id(e \dashv_0 f) = e \dashv_1 f = id(e) \dashv_1 id(f)$, since the operations on G_1 are the original trioid operations. Similarly for $\vdash$.

Lemma 4.10

The composition map $\circ : G_1 \times_{G_0} G_1 \rightarrow G_1$ is a dimonoid homomorphism.

Proof

We prove the $\circ$ -case as an illustration. For composable pairs (x, y) and (u, v) with $\partial_1(x) = \partial_0(y)$ and $\partial_1(u) = \partial_0(v)$:

$$(x \circ y) \dashv_1 (u \circ v) = ((x \dashv_1 y) \dashv_1 (x \perp y)) \dashv_1 ((u \dashv_1 v) \dashv_1 (u \perp v)).$$

Using the trioid axioms (T1)-(T8) to rearrange the operations, this equals

$$((x \dashv_1 u) \dashv_1 (y \dashv_1 v)) \dashv_1 ((x \perp u) \perp (y \perp v)) = (x \dashv_1 u) \circ (y \dashv_1 v).$$

The $\vdash$ -case follows similarly. The verification is lengthy but straightforward; the key is that the trioid axioms were precisely designed to ensure these identities.

Lemma 4.11

The inversion map $^{-1} : G_1 \rightarrow G_1$ is a dimonoid homomorphism.

Proof

We need $(x \dashv_1 y)^* = x^* \dashv_1 y^*$ and $(x \vdash_1 y)^* = x^* \vdash_1 y^*$. These follow from the properties of the $\perp$ -inverse and the trioid axioms. For example:

$$(x \dashv_1 y)^* \perp (x \dashv_1 y) \perp (x \dashv_1 y)^* = (x \dashv_1 y)^*,$$

and similarly for the other identities. The verification is routine

Finally, we must verify the groupoid axioms: associativity of composition, the identities act as units, and inversion gives inverses. These follow from the definition of composition and the properties of the $\perp$ -inverse.

Theorem 4.12

For any $\perp$ -regular trioid T , $\mathcal{G}(T)$ is a dimonoid groupoid. The assignment $T \mapsto$

$\mathcal{G}(T)$ extends to a functor

$$\mathcal{G} : \text{Trioid}_{\text{reg}} \rightarrow \text{Gpd}(\text{Dimon}),$$

where $\text{Trioid}_{\text{reg}}$ denotes the full subcategory of Trioid consisting of $\perp$ -regular trioids.

Proof

The verification of the groupoid axioms follows from the lemmas above. Functoriality follows from the fact that trioid homomorphisms preserve all three operations and hence commute with the construction of source, target, composition, and inversion.

From Dimonoid Groupoids to Trioids

We now construct the inverse correspondence. Let $\mathcal{G} = (G_1 \Rightarrow G_0)$ be a dimonoid groupoid. We define a trioid $T(\mathcal{G})$ as follows.

Construction 4.13. Let $\mathcal{G} = (G_1 \Rightarrow G_0)$ be a dimonoid groupoid. Define:

$T(\mathcal{G}) = G_1 \cup \{0\}$, where 0 is a formal zero element adjoined to G_1 .

For $x, y \in G_1$, define:

$x \dashv y = x \circ \partial_0(y)$, $x \vdash y = \partial_1(x) \circ y$, $x \perp y = x \circ y$ (when composable).

(iii) For non-composable pairs, set $x \perp y = 0$. The zero element satisfies $x * 0 = 0 = 0 * x$ for all $x \in T(\mathcal{G})$ and $*$ $\in \{\dashv, \vdash, \perp\}$.

The introduction of a zero element for non-composable pairs is necessary because groupoid composition is only partially defined, whereas trioid operations must be totally defined. This construction is analogous to the Rees matrix construction in semigroup theory (Rees, 1940) and the Rees trioid construction of Zhuchok (Zhuchok, 2014). The zero element represents the absence of a meaningful regulatory interaction when the genes involved are not in compatible regulatory contexts.

For the construction to yield a well-behaved trioid, we need an additional condition that ensures the zero element does not cause unwanted identifications. The inductive condition ensures this: it guarantees that for every pair of morphisms $x, y \in G_1$, the element $\partial_1(x) \circ \partial_0(y)$ (computed in the object dimonoid G_0) is defined, and that the zero element behaves properly. Without this condition, the resulting operations might fail to be associative or might identify elements that should remain distinct. The condition is analogous to the condition that an inverse semigroup's groupoid is inductive (Lawson, 1998). The immediate definition was adapted from (Berger & Funk, 2026, Lawson, 1998) for the multi-operational structures.

Definition 4.14 (Inductive Dimonoid Groupoid)

A dimonoid groupoid $\mathcal{G}$ is called inductive if for every pair of morphisms $x, y \in G_1$, the element $\partial_1(x) \circ \partial_0(y)$ (computed in the object dimonoid G_0) is defined and satisfies certain compatibility conditions that ensure the resulting trioid operations are associative. Moreover, the zero element introduced for non-composable pairs must satisfy the usual zero axioms.

For inductive dimonoid groupoids, the zero extension is well-behaved, and the resulting algebra satisfies all trioid axioms.

Theorem 4.15

For any inductive dimonoid groupoid $\mathcal{G}$, the algebra $T(\mathcal{G})$ defined above is a trioid.

Moreover, this trioid is $\perp$ -regular, with $\perp$ -inverse given by the groupoid inversion. The assignment

$\mathcal{G} \mapsto T(\mathcal{G})$ extends to a functor

$T: \text{Gpd}_{\text{ind}}(\text{Dimon}) \rightarrow \text{Trioid}_{\text{reg}}$,

where $\text{Gpd}_{\text{ind}}(\text{Dimon})$ is the full subcategory of $\text{Gpd}(\text{Dimon})$ consisting of inductive dimonoid groupoids.

Proof

We verify axiom (T1) as an illustration; the remaining axioms (T2)-(T8) follow by analogous calculations using the groupoid axioms and the fact that $\partial_0, \partial_1, \text{id}$, and $\circ$ are dimonoid homomorphisms. For $x, y, z \in G_1$:

$(x \dashv y) \dashv z = (x \circ \partial_0(y)) \dashv z = (x \circ \partial_0(y)) \circ \partial_0(z) = x \circ (\partial_0(y) \circ \partial_0(z))$.

Since ∂_0 is a homomorphism, $\partial_0(y) \circ \partial_0(z) = \partial_0(y \dashv z)$, so this equals

$x \circ \partial_0(y \dashv z) = x \dashv (y \dashv z)$.

For example, (T2) follows by applying the same calculation with $\vdash$ in place of $\dashv$, and (T4) follows by a similar calculation using the composition law $x \circ (y \circ z) = (x \circ y) \circ z$. The $\perp$ -regularity follows from the existence of inverses in the groupoid.

The Equivalence Theorem

We now state the main result of this section: the functors $\mathcal{G}$ and T establish an equivalence between the category of $\perp$ -regular trioids and the category of inductive dimonoid groupoids as adapted for Trioid from (Berger & Funk, 2026). Theorem 4.16. The functors

$\mathcal{G}: \text{Trioid}_{\text{reg}} \rightarrow \text{Gpd}_{\text{ind}}(\text{Dimon})$

and

$T: \text{Gpd}_{\text{ind}}(\text{Dimon}) \rightarrow \text{Trioid}_{\text{reg}}$

establish an equivalence of categories between:

The category $\text{Trioid}_{\text{reg}}$ of $\perp$ -regular trioids with trioid homomorphisms;

The category $\text{Gpd}_{\text{ind}}(\text{Dimon})$ of inductive dimonoid groupoids with internal functors.

Proof

We verify that the functors $\mathcal{G}$ and T are well-defined, establish the natural isomorphisms η and ε , and construct the triangle identities as follow:

(i) Now to verify that $\mathcal{G}$ is a functor. Let $f: T \rightarrow T'$ be a trioid homomorphism between $\perp$ -regular trioids. Define $\mathcal{G}(f): \mathcal{G}(T) \rightarrow \mathcal{G}(T')$ on objects by $\mathcal{G}(f)(e) = f(e)$ for $e \in E_{\perp}(T)$, and on morphisms by $\mathcal{G}(f)(x) = f(x)$ for $x \in T$. Since f preserves $\perp$, it maps $\perp$ -idempotents to $\perp$ -idempotents, so $\mathcal{G}(f)$ is well-defined on objects. Since f preserves the source and target maps (which are defined in terms of $\perp$ and the $\perp$ -inverse), $\mathcal{G}(f)$ commutes with ∂_0 and ∂_1 . Since f preserves the composition formula $x \circ y = (x \dashv y) \dashv (x \perp y)$, $\mathcal{G}(f)$ commutes with composition. Since f preserves the $\perp$ -inverse operation (which is unique in a $\perp$ -regular trioid), $\mathcal{G}(f)$ commutes with inversion. Finally, $\mathcal{G}$ preserves identities and composition of trioid homomorphisms, so $\mathcal{G}$ is a functor.

(ii) Let $F: \mathcal{G} \rightarrow \mathcal{G}'$ be an internal functor between inductive dimonoid groupoids. Define $T(F): T(\mathcal{G}) \rightarrow T(\mathcal{G}')$ on the underlying sets by $T(F)(x) = F(x)$ for $x \in \mathcal{G}_1$ and $T(F)(0) = 0$. Since F preserves the dimonoid operations on $\mathcal{G}_1$, $T(F)$ preserves the trioid operations $\dashv, \vdash$, and $\perp$ (defined via composition and the source/target maps, which F preserves). The zero element is preserved by construction. Thus $T(F)$ is a trioid homomorphism, and T is a functor.

(iii) To show that the unit $\eta: \text{id}_{\{\text{Trioid}_{\text{reg}}\}} \Rightarrow T \circ \mathcal{G}$ is a natural isomorphism. For a $\perp$ -regular trioid T , define $\eta_T: T \rightarrow T(\mathcal{G}(T))$ by $\eta_T(x) = x$. We verify that η_T is a trioid homomorphism. In $\mathcal{G}(T)$, composition is defined by $x \circ y = (x \dashv y) \dashv (x \perp y)$. Then in $T(\mathcal{G}(T))$, the trioid operations are defined by $x \dashv y = x \circ \partial_0(y)$, $x \vdash y = \partial_1(x) \circ y$, and $x \perp y = x \circ y$ when composable. We must verify that these agree with the original operations on T . For $\dashv$: $\eta_T(x \dashv y) = x \dashv y$. On the other hand, $\eta_T(x) \dashv \eta_T(y) = x \circ \partial_0(y) = x \circ (y^* \perp y)$. Since y is $\perp$ -regular, y^* is its $\perp$ -inverse, and a direct calculation using the trioid axioms gives $x \circ (y^* \perp y) = x \dashv y$. The verification for $\vdash$ is dual. For $\perp$: $\eta_T(x \perp y) = x \perp y = x \circ y = \eta_T(x) \perp \eta_T(y)$ when x and y are composable; when they are not composable, both sides equal 0 by definition. Thus η_T is a trioid homomorphism. It is bijective by construction (indeed, the identity on underlying sets), and naturality in T is immediate from the definition. Hence η is a natural isomorphism.

(iv) Also, we verify that the counit $\varepsilon : \mathcal{G} \circ T \Rightarrow id_{\{Gpd_{ind}(Dimon)\}}$ is a natural isomorphism. For an inductive dimonoid groupoid G , define $\varepsilon_G : \mathcal{G}(T(v)) \rightarrow G$ by $\varepsilon_G(x) = x$ on morphisms and the identity on objects. We verify that ε_G is an internal functor. It preserves the source and target maps: $\partial_0^{\{G(T(G))\}}(x) = x^* \perp x = \partial_1^{G(x)} \circ \partial_0^{G(x)} = \partial_0^{G(x)}$, where the first equality is the definition of ∂_0 in $\mathcal{G}(T(G))$, the second uses the definition of $\perp$ in $T(G)$ and the fact that x^* is the $\perp$ -inverse of x , and the third uses the inductive condition on G . The verification for ∂_1 is dual. It preserves composition: by definition of composition in $\mathcal{G}(T(G))$, $\varepsilon_G(x \circ y) = x \circ y = \varepsilon_G(x) \circ \varepsilon_G(y)$. It preserves identities and inversion by construction. Thus ε_G is an internal functor. It is bijective on objects and morphisms (indeed, the

identity on underlying sets), and naturality in G follows from the definition. Hence ε is a natural isomorphism.

(v) The triangle identities for the adjoint equivalence $(\mathcal{G}, T, \eta, \varepsilon)$ are: $(\varepsilon_G \circ \mathcal{G}(\eta_T))(x) = id_{\{G(T)\}}$ for every $\perp$ -regular trioid T , and $(T(\varepsilon_G) \circ \eta_{\{T(G)\}})(x) = id_{\{T(G)\}}$ for every inductive dimonoid groupoid G . Both identities follow immediately from the fact that G and T are the identity on underlying sets and that η and ε are the identity on underlying sets. Specifically, for $x \in \mathcal{G}(T)$:

$$(\varepsilon_{\{G(T)\}} \circ \mathcal{G}(\eta_T))(x) = \varepsilon_{\{G(T)\}}(\eta_T(x)) = \varepsilon_{\{G(T)\}}(x) = x = id_{\{G(T)\}}(x).$$

The second identity is verified similarly. Thus the triangle identities hold, and $(\mathcal{G}, T, \eta, \varepsilon)$ is an adjoint equivalence of categories.

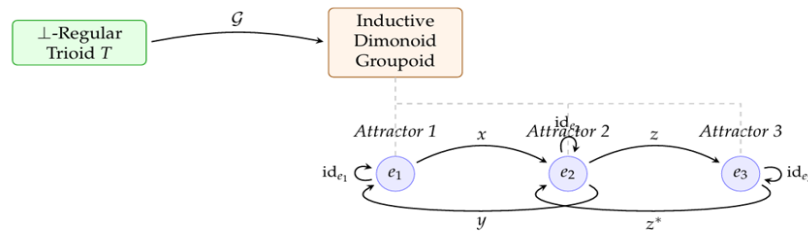


Figure 3: The ESN correspondence (Theorem 4.16) establishes a Categorical Equivalence Between $\perp$ -Regular Trioids and Inductive Dimonoid Groupoids. Objects of the Groupoid Correspond to Attractors (Stable Expression Patterns), Morphisms to Regulatory Transitions Between Attractors, Composition to Sequential regulation, and Inversion to Reversibility. This Reformulation Provides a Categorical Bridge Between Algebraic Structure and Dynamical Behaviour in Regulatory Networks

For an inductive dimonoid groupoid G , define $e_G : G(T(G)) \rightarrow G$ by $e_G(x) = x$ on morphisms and the identity on objects. We verify that e_G preserves source, target, identity, composition, and inversion. The preservation of source, for example, follows from:

$$\partial_0^{G(T(G))}(x) = x^* \perp x = \partial_1^G(x) \circ \partial_0^G(x) = \partial_0^G(x).$$

The preservation of composition follows from the definition of composition in $\mathcal{G}(T(G))$:

$$e_G(x \circ y) = x \circ y = e_G(x) \circ e_G(y).$$

Thus e_G is an internal functor, and it is natural in G . Moreover, it is an isomorphism because the constructions are inverse on underlying sets. The triangle identities follow from the fact that both functors are essentially "identity on underlying sets" functors (See Berger and Funk, 2006). The equivalence can be visualized as:

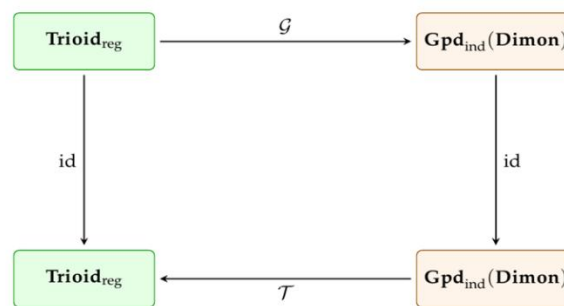


Figure 4: The Functors $\mathcal{G}$ and T Establish an Adjoint Equivalence Between the Category of $\perp$ -Regular Trioids and the Category of Inductive Dimonoid Groupoids. The Identity Functors id on Both Categories Render the Diagram Strictly Commutative, Reflecting the Bijective Correspondence Established in Theorem 4.16

with natural isomorphisms $\eta : id \Rightarrow T \circ G$ and $\varepsilon : G \circ T \Rightarrow id$. This theorem has profound implications. It shows that the algebraic structure of a $\perp$ -regular trioid is completely equivalent to the geometric structure of an inductive dimonoid groupoid. In biological terms, this means that a regulatory

network can be equivalently described either by its algebraic operations (activation, repression, modulation) or by its groupoid of states and transitions. These two descriptions are not merely analogous; they are mathematically equivalent.

Biological Interpretation: Groupoids of Regulatory States

Having established the mathematical equivalence between $\perp$ -regular trioids and inductive dimonoid groupoids, we now interpret this equivalence biologically. The groupoid formulation makes the dynamics of the regulatory network explicit: objects are stable states, morphisms are transitions, and composition is sequential regulation. Let T be a $\perp$ -regular trioid modeling a gene regulatory network. The associated dimonoid groupoid $\mathcal{G}(T)$ has the following interpretation:

- (i) Objects of $\mathcal{G}(T)$ are the $\perp$ -idempotents of T . In biological terms, these are the stable expression patterns or *attractors* of the network. An element e with $e \perp e = e$ represents a state where applying the regulatory operation to itself yields no change—a fixed point of the dynamics.
- (ii) Morphisms of $\mathcal{G}(T)$ are all elements of T , representing regulatory transitions between states. A morphism $x : e \rightarrow f$ satisfies $\partial_0(x) = e$ and $\partial_1(x) = f$, meaning that applying the regulatory operation x to state e yields state f .
- (iii) Composition of morphisms corresponds to sequential regulation: if x takes state e to state f , and y takes state f to state g , then $x \circ y$ takes state e directly to state g . The definition $x \circ y = (x \dashv y) \dashv (x \perp y)$ reflects the interplay of activation, repression, and modulation in producing the composite transition.
- (iv) The identity morphism at state e is itself, representing the trivial transition that leaves the state unchanged.
- (v) Inversion corresponds to reversibility: if x is a regulatory transition, then x^* is its reverse, taking the target state back to the source.

Definition 4.17 (Attractor Class, Lawson 1998)

Two objects $e, f \in G_0$ are in the same attractor class if there exists a morphism $x \in G_1$ with $\partial_0(x) = e$ and $\partial_1(x) = f$. This defines an equivalence relation on objects, partitioning the set of attractors into connected components of the groupoid.

Proposition 4.18

The groupoid $\mathcal{G}(T)$ encodes the complete dynamical landscape of the regulatory network:

- (i) Connected components of $\mathcal{G}(T)$ correspond to basins of attraction: sets of attractors that can transition into each other.
- (ii) Vertex groups $\text{Aut}(e) = \{x \in G_1 \mid \partial_0(x) = \partial_1(x) = e\}$ correspond to cycles or oscillations within an attractor. A nontrivial automorphism group indicates that the attractor has internal dynamics (e.g., a limit cycle).



Figure 5: The Identity Morphisms $\text{id}_A : A \rightarrow A$ and $\text{id}_B : B \rightarrow B$ in the Respective Categories. These Serve as the Unit Morphisms Required for Categorical Equivalence, Ensuring Each Object is Isomorphic to Itself Under the Correspondence Established in Theorem 4.16

Groupoid Dynamics and Attractor Structure

The groupoid perspective sheds new light on the attractor structure of regulatory networks.

Definition 4.23 (Dynamical Invariants)

For a dimonoid groupoid $\mathcal{G}$ arising from a regulatory network, define:

- (i) The number of connected components $c(\mathcal{G})$: the number of basins of attraction.
- (ii) The vertex group $\text{Aut}(e)$ for each object e : the symmetry group of the attractor.

- (iii) The partial order on objects induced by the existence of morphisms corresponds to the reachability relation between attractors.

Example 4.19

Consider a simple bistable switch with two attractors e_1 and e_2 and two transitions $x : e_1 \rightarrow e_2$ and $y : e_2 \rightarrow e_1$ with $x \circ y = \text{id}_{e_2}$ and $y \circ x = \text{id}_{e_1}$. The vertex groups are trivial, and the groupoid has two objects connected by inverse morphisms. This models a system that can flip between two stable states but has no internal oscillations within each state (Gardner et al, 2000).

Example 4.20

Consider a single attractor e with a nontrivial vertex group $\text{Aut}(e) \cong \mathbb{Z}_n$, generated by a morphism x with $x^n = \text{id}_e$. This models an oscillator with period n : the system cycles through n distinct states within the same attractor (Elowitz and Leibler, 2000).

Example 4.21

Consider a groupoid with multiple connected components, each containing several attractors. This models a system with multiple basins of attraction, where transitions can occur within a basin but not between basins. This corresponds to cell differentiation, where different cell types correspond to different connected components (Davidson, 2006).

Example 4.22

Consider the toggle switch trioid with elements $\{A, B, 0\}$ and operations:

$A \vdash B = 0, B \vdash A = 0$ (mutual repression);

$A \perp A = A, B \perp B = B$ (idempotence);

0 is a zero element.

The $\perp$ -idempotents are A and B . The groupoid $\mathcal{G}(T)$ has:

Objects: A, B ;

Morphisms: $\text{id}_A : A \rightarrow A, \text{id}_B : B \rightarrow B$;

Composition: $\text{id}_A \circ \text{id}_A = \text{id}_A, \text{id}_B \circ \text{id}_B = \text{id}_B$.

This can be visualized as:

This groupoid has two connected components, each consisting of a single object with only the identity morphism. This reflects the fact that the toggle switch has two stable states with no transitions between them. The vertex groups are trivial, indicating no internal oscillations.

- (iii) The reachability poset $P(\mathcal{G})$: the partial order on objects induced by the existence of morphisms.

Proposition 4.24

(i) A large number of connected components indicates high multistability: the system can exist in many distinct stable states. This is characteristic of systems with complex decision-making capabilities, such as developmental networks (Davidson, 2006).

(ii) Nontrivial vertex groups indicate oscillatory behavior: the attractor has internal dynamics. The structure of the vertex

group encodes the period and symmetry of the oscillation (Elowitz & Leibler, 2000).

- (iii) The reachability poset encodes the developmental landscape: the hierarchical relationship between different cell types or states. A tall poset indicates a complex differentiation hierarchy. (Davidson, 2006).

The Action Groupoid Construction

The ESN correspondence suggests a method for constructing dynamical models from regulatory networks. Given a trioid T modeling a network, we first compute its associated groupoid $\mathcal{G}(T)$. We then construct an action groupoid that encodes the dynamics explicitly.

Definition 4.25 (Action Groupoid)

Let T be a trioid acting on a set S of states (a T -module as in Section 5). The action groupoid $S \circ T$ has:

- (i) Objects: states $s \in S$;
- (ii) Morphisms: pairs $(x, s) \in T \times S$ with $\alpha_0(x, s) = s$ and $\alpha_1(x, s) = x \perp s$ (or an appropriate combination of operations);
- (iii) Composition: $(x, s) \circ (y, t)$ defined when $\alpha_1(x, s) = t$, with $(x, s) \circ (y, t) = (x \circ y, s)$; (iv) Inversion: $(x, s)^{-1} = (x^*, x \perp s)$.

This action groupoid captures the full dynamics of the system: objects are states, morphisms are regulatory transitions, and composition corresponds to sequential application of regulations. The connected components of the action groupoid correspond to basins of attraction, and the vertex groups capture cycles within attractors.

Example 4.26

For example, if S is the set of all possible gene expression states (vectors of ON/OFF states for n genes), then the action groupoid $S \circ T$ has states as objects and regulatory perturbations as morphisms. A morphism (x, s) represents applying regulator x to state s , resulting in state $x \perp s$. The composition of morphisms corresponds to applying regulators sequentially. The connected components of this groupoid correspond to basins of attraction, and the vertex groups capture cycles within attractors.

Proposition 4.27

The relationship between the regulatory groupoid $\mathcal{G}(T)$ and the action groupoid $S \circ T$ is given by a functor

$$F: S \circ T \rightarrow \mathcal{G}(T)$$

that sends each state $s \in S$ to its attractor (if the system eventually settles) and each transition (x, s) to the regulatory element x . This functor collapses the detailed state space to the attractor structure, providing a bridge between micro-level dynamics and macro-level organization.

Remark 4.28

The groupoid framework can be extended to continuous dynamics by equipping the groupoid with topological or smooth structure. In this setting, objects become points in a manifold (the state space), morphisms become paths or flows, and the groupoid encodes the complete dynamical system. This provides a bridge between discrete algebraic models and continuous differential equation models (Alon, 2007, Chen et al, 2004).

Representation Theory: Modules As Regulatory Circuits

Representation theory is one of the most powerful tools in modern algebra, providing a systematic way to study how algebraic structures act on other mathematical objects. For groups, representations on vector spaces reveal the structure of the group and its characters; for semigroups,

representations on sets (semigroup actions) capture the dynamics of state transitions. In this section, we develop the representation theory of trioids, introducing the notion of a trioid module and showing how these modules correspond to regulatory circuits within a larger network. The classification of irreducible modules over free trioids provides an algebraic characterization of network motifs, complementing statistical approaches to motif discovery (Alon, 2007, Milo et al, 2002) and revealing the deep algebraic principles that govern the structure of regulatory networks.

Motivation

To understand the biological significance of representation theory, recall that gene regulatory network is not an isolated object; it acts on the cell's state, transforming expression patterns over time. This action is precisely a representation of the network: each gene (element of the trioid) corresponds to a transformation on the state space, and the composition of genes corresponds to composition of transformations. In this interpretation, the state space itself becomes a module over the trioid. Different modules correspond to different ways the network can act—perhaps on different cellular contexts, different environmental conditions, or different developmental stages. Submodules correspond to invariant subspaces of the state space—sets of states that are closed under the network's action, representing distinct behavioral regimes. Irreducible modules, which have no nontrivial submodules, correspond to minimal dynamical behaviors that cannot be further decomposed. The classification of irreducible modules over free trioids thus provides a catalog of the possible elementary dynamical behaviors that can arise from regulatory interactions. These elementary behaviors correspond precisely to network motifs—small regulatory circuits that appear repeatedly in real biological networks. Representation theory thus offers an algebraic foundation for motif theory, explaining why certain motifs are favored by evolution and how they combine to form complex regulatory networks.

Modules over a Trioid: Definition and Basic Properties

We begin by defining the fundamental concept of a module over a trioid.

Definition 5.1 (Trioid Module)

Let $(T, \dashv, \vdash, \perp)$ be a trioid. A (left) T -module is a set M equipped with three actions $\dashv: T \times M \rightarrow M$, $\vdash: T \times M \rightarrow M$, $\perp: T \times M \rightarrow M$ satisfying for all $x, y \in T$ and $m \in M$:

- (M1) $(x \dashv y) \dashv m = x \dashv (y \vdash m)$,
- (M2) $(x \vdash y) \vdash m = x \vdash (y \dashv m)$,
- (M3) $(x \dashv y) \vdash m = x \vdash (y \vdash m)$,
- (M4) $(x \dashv y) \dashv m = x \dashv (y \perp m)$,
- (M5) $(x \perp y) \dashv m = x \perp (y \vdash m)$,
- (M6) $(x \dashv y) \perp m = x \perp (y \vdash m)$,
- (M7) $(x \vdash y) \perp m = x \vdash (y \perp m)$,
- (M8) $(x \perp y) \vdash m = x \vdash (y \vdash m)$.

Additionally, we require the dual axioms with actions on the right (for right modules). A bimodule is a set equipped with both left and right actions satisfying mixed compatibility conditions.

The axioms (M1)-(M8) are obtained from the trioid axioms (T1)-(T8) by replacing the rightmost variable with a module element. They express how the three operations on T interact when acting on a module element. Together with their duals for right modules, these constitute the complete set of module axioms.

Remark 5.2

A T-module represents the state space of the regulatory network. Elements of M are expression states (vectors of expression levels), and the actions $x * m$ represent the effect of regulatory element x on state m . The module axioms ensure that the regulatory operations on states are consistent with the algebraic structure of the trioid.

Definition 5.3 (Module Homomorphism)

A homomorphism of left T-modules $f: M \rightarrow N$ is a map such that for all $x \in T$, $m \in M$, and $* \in \{-1, \vdash, \perp\}$:

$$f(x * m) = x * f(m).$$

The category of left T-modules with module homomorphisms is denoted $\text{Mod}(T)$.

Example 5.4

Any trioid T is a left module over itself via the three operations. This is the regular module, analogous to the regular representation of a group or semigroup. In biological terms, the regular module represents the network acting on its own set of genes—perhaps corresponding to autoregulation or feedback loops.

Example 5.5

Let $M = \{*\}$ be a one-element set. Define $x * = *$ for all $x \in T$ and $* \in \{-1, \vdash, \perp\}$. This yields the trivial module, representing a state that is completely insensitive to regulation—perhaps a constitutive expression pattern or a fixed environmental condition.

Example 5.6

If $\varphi: T \rightarrow S$ is a trioid homomorphism and M is an S -module, then M becomes a T -module via pullback: $x * m = \varphi(x) * m$. In particular, every trioid has modules coming from homomorphisms to other trioids. This corresponds to embedding a small network into a larger one and observing how the larger network's dynamics restrict to the smaller.

Example 5.7

Let T be a trioid and let $M = T$ as a set, but define actions differently. For a fixed $a \in T$, define

$$x \dashv m = x \dashv a \dashv m, x \vdash m = x \vdash a \vdash m, x \perp m = x \perp a \perp m.$$

This gives a module structure, the cyclic module generated by a . In biological terms, this represents the subnetwork reachable from a particular starting gene a through all possible regulatory interactions.

Submodules, Quotients, and Irreducibility

The structural analysis of modules proceeds through the study of submodules and quotient modules, leading to the classification of irreducible modules as the fundamental building blocks.

Definition 5.8 (Submodule)

A submodule of a T-module M is a subset $N \subseteq M$ closed under all three actions: for all $x \in T$, $n \in N$, and $* \in \{-1, \vdash, \perp\}$, we have $x * n \in N$. A quotient module is a set of equivalence classes of M under a congruence compatible with all three actions.

Remark 5.9

In biological terms, submodules correspond to subsets of the state space that are invariant under all regulatory actions—regions of the state space that the system cannot leave. These represent distinct behavioral regimes: different cell types, different phases of the cell cycle, or different responses to environmental signals. Quotient modules correspond to

coarse-grained descriptions where states that are equivalent under some notion of observational indistinguishability are identified.

Definition 5.10 (Irreducible Module)

A nonzero T-module M is irreducible (or simple) if it has no proper nonzero submodules. It is indecomposable if it cannot be expressed as a direct sum (coproduct) of two nonzero submodules.

Irreducible modules represent minimal dynamical behaviors that cannot be further decomposed. In network terms, they correspond to elementary regulatory circuits that function as independent units. The classification of irreducible modules thus provides a catalog of possible motif types. We state the Schur's Lemma for Trioid Modules

Proposition 5.11

Let M and N be irreducible T-modules, and let $f: M \rightarrow N$ be a T-module homomorphism. Then either $f = 0$ or f is an isomorphism. Consequently, if M is irreducible, the set $\text{End}_T(M)$ of T-module endomorphisms of M is a group under composition, namely the automorphism group $\text{Aut}_T(M)$.

Proof

If $f \neq 0$, then $\ker f = \{m \in M: f(m) = 0\}$ is a submodule of M such that for any $x \in T$ and $m \in \ker f$, we have $f(x * m) = x * f(m) = x * 0 = 0$, so $x * m \in \ker f$. Since $f \neq 0$, $\ker f \neq M$, so by irreducibility of M we have $\ker f = 0$. Hence f is injective. The image $\text{im } f = \{f(m): m \in M\}$ is a submodule of N : for any $x \in T$ and $n = f(m) \in \text{im } f$, we have $x * n = x * f(m) = f(x * m) \in \text{im } f$. Since $f \neq 0$, $\text{im } f \neq 0$, so by irreducibility of N we have $\text{im } f = N$.

Hence f is surjective. Thus f is bijective and its set theoretic inverse $f^{-1}: N \rightarrow M$ is a T -module homomorphism: for any $x \in T$ and $n \in N$, then $n = f(m)$, we have $f^{-1}(x * n) = f^{-1}(x * f(m)) = f^{-1}(f(x * m)) = x * m = x * f^{-1}(n)$. Therefore f is an isomorphism. The set $\text{End}_T(M)$ of endomorphisms of an irreducible module M is closed under composition and contains the identity; by the argument above, every nonzero element is invertible, so $\text{End}_T(M)$ is a group—precisely the automorphism group $\text{Aut}_T(M)$ of the irreducible module M ■

Remark 5.11

In the classical setting of modules over a ring, Schur's Lemma concludes that $\text{End}_T(M)$ is a division ring. In the present set-based setting, the appropriate conclusion is that $\text{End}_T(M)$ is a group, since the endomorphisms of a set-based module need not form a ring. The group $\text{End}_T(M)$ is precisely the automorphism group $\text{Aut}_T(M)$ of the irreducible module M . Schur's Lemma is fundamental in representation theory; its analogue here shows that irreducible modules have a rigid structure with limited symmetries. In biological terms, this means that distinct elementary circuits cannot be mapped onto each other nontrivially—each motif has a unique internal logic.

Proposition 5.12

$\text{Mod}(T)$ is a complete and cocomplete category. Limits are constructed as in Set with pointwise actions; colimits are constructed as coequalizers of free modules.

Proof

The forgetful functor $U: \text{Mod}(T) \rightarrow \text{Set}$ creates limits. For colimits, one uses the standard construction via the adjoint

functor theorem, noting that $\text{Mod}(T)$ is a variety of algebras (modules are algebras for a certain monad). The result follows from the general theory of varieties of algebras; for a complete proof, see (Burris & Sankappanavar, 1981).

Definition 5.13 (Direct Sum)

Let $\{M_i\}_{i \in I}$ be a family of T-modules. Their direct sum $\bigoplus_{i \in I} M_i$ is the disjoint union of the sets M_i , with actions defined componentwise: for $x \in T$ and $m \in M_i$, $x * m$ is computed in M_i and then embedded in the sum.

Remark 5.14

In biological terms, direct sums correspond to independent parallel systems—multiple regulatory circuits operating simultaneously without interaction. This models modular networks where different modules function independently.

Theorem 5.15 (Krull-Schmidt Theorem, Zhuchok, 2011c)

Every finite T-module is a direct sum of indecomposable modules. If T is finite, this decomposition is unique up to isomorphism.

The Krull-Schmidt theorem guarantees that every finite module has a unique decomposition into indecomposable components. In network terms, this means that every finite regulatory system has a unique decomposition into elementary circuits, providing a rigorous foundation for modular decomposition.

Modules Over Free Trioids and the Classification of Motifs

Recall from Section 4 that $\text{Frt}(Y)$ consists of words in $Y \cup \bar{Y}$ containing at least one overlined letter, with operations:

$$w \dashv u = w\bar{u}, \quad w \vdash u = \bar{w}u, \quad w \perp u = wu,$$

where $\bar{w}$ is obtained by removing overlines. The key insight is that the module axioms force strong constraints on how words act, reducing the classification to combinatorial data.

Lemma 5.16

For any $\text{Frt}(Y)$ -module M and any $w \in \text{Frt}(Y)$, the map $m \mapsto w \perp m$ depends only on the word $\bar{w}$ (the word obtained by removing overlines). Similarly, $w \dashv m$ depends on w and $\bar{w}$ in a specific way, and $w \vdash m$ depends on w and the last overlined letter.

Proof

We prove the first statement by induction on the length of w . For $|w| = 1$, the result is immediate from the definitions. For the induction step, suppose $w = uv$. Then:

$$w \perp m = (uv) \perp m = u \perp (v \perp m)$$

by associativity of $\perp$. By the induction hypothesis, $v \perp m$ depends only on $\bar{v}$, and $u \perp (v \perp m)$ depends only on $\bar{u}$ and $\bar{v}$. Thus $w \perp m$ depends only on $\bar{w} = \bar{u}\bar{v}$. The other cases are similar. A complete proof appears in (Novelli & Thibon, 2006a, 2006b).

For the rank-one case $Y = \{x\}$, we can give a complete classification of irreducible modules. This case is particularly important because it corresponds to the simplest possible regulatory alphabet—a single gene with its activated and repressed forms.

Theorem 5.17

Irreducible modules over the free trioid of rank one are classified by:

- i A set S (the underlying set of the module);
- ii A distinguished element $0 \in S$ (the zero element, if the module is pointed); (iii) A family of maps $\varphi_w: S \rightarrow S$ for each word $w \in \text{Frt}(\{x\})$ satisfying:

$$(R1) \varphi_{x\bar{x}} = \varphi_{\bar{x}x} = \text{id},$$

$$(R2) \varphi_{w_1 w_2} = \varphi_{w_2} \circ \varphi_{w_1} \quad (\text{for concatenation}),$$

$$(R3) \varphi_{\bar{w}} = \varphi_w \quad \text{for all } w,$$

together with additional compatibility conditions arising from the module axioms (M1)-(M8).

Proof

The elements of $\text{Frt}(\{x\})$ are words in $\{x, \bar{x}\}$ with at least one $\bar{x}$. We prove the classification as follows.

- i From (M1)-(M3), we obtain $\varphi_{x\bar{x}} = \text{id}$ and $\varphi_{\bar{x}x} = \text{id}$.

Indeed, for any $m \in M$:

$$\varphi_{x\bar{x}}(m) = x \perp \bar{x} \perp m = (x \perp \bar{x}) \perp m = m,$$

using (M8) and the fact that $x \perp \bar{x}$ is idempotent.

- ii Associativity of $\perp$ gives (R2): $\varphi_{w_1 w_2} = \varphi_{w_2} \circ \varphi_{w_1}$.

- iii From (M4)-(M6), we obtain (R3): $\varphi_{\bar{w}} = \varphi_w$.

- iv The remaining conditions follow from (M7) - (M8).

Conversely, any family of maps satisfying (R1)-(R3) and the additional compatibility conditions defines a module structure by extending the actions to arbitrary words. The detailed verification appears in (Novelli & Thibon, 2006a, Zhuchok, 2011c).

This theorem can be further simplified in terms of the two generators.

Corollary 5.18

Let M irreducible $\text{Frt}(\{x\})$ -module, let $\alpha = \varphi_x$ and $\beta = \varphi_{\bar{x}}$ be the endomorphisms of M induced by the generators x and $\bar{x}$. Then α and β satisfy the following relations:

- (i) $\alpha \circ \beta \circ \alpha = \alpha$ and $\beta \circ \alpha \circ \beta = \beta$ (the inverse-semigroup relations);
- (ii) $\alpha^2 = \alpha$ and $\beta^2 = \beta$ if and only if α and β are idempotent, which holds when the module is saturated (i.e. where further application of the same regulatory operation has no additional effect);
- (iii) If α and β are both idempotent and satisfy $\alpha \circ \beta = \beta \circ \alpha = \text{id}_M$, then $\alpha = \beta$ in this case the module is trivial. In the non-trivial case, α and β satisfy the inverse-semigroup relations (i) but not the group relations $\alpha \circ \beta = \beta \circ \alpha = \text{id}_M$.

Proof

The relations in (i) follow from the module axioms (M1) – (M3), which encode the fact that x and $\bar{x}$ act as mutually inverse elements in the sense of inverse semigroup theory. Specifically, the axioms $x \dashv \bar{x} \dashv x = x$ and $\bar{x} \dashv x \dashv \bar{x} = \bar{x}$ in the trioid translate, under the module action, to $\alpha \circ \beta \circ \alpha = \alpha$ and $\beta \circ \alpha \circ \beta = \beta$. The idempotence conditions in (ii) follow from the associativity of $\dashv$: the action of $x \dashv x$ is the same as the action of x , so $\alpha^2 = \alpha$; similarly for β . The statement in (iii) is immediate: if $\alpha \circ \beta = \beta \circ \alpha = \text{id}_M$ and $\alpha^2 = \alpha$, then $\alpha = \alpha \circ \text{id}_M = \alpha \circ (\beta \circ \alpha) = (\alpha \circ \beta) \circ \alpha = \text{id}_M \circ \alpha = \alpha$, and similarly $\alpha = \text{id}_M$.

Remark 5.19

The algebraic framework for the toggle switch is that of inverse semigroups: α and β are mutually inverse idempotents satisfying the inverse-semigroup relations, not the group relations. The group relations $\alpha \circ \beta = \beta \circ \alpha = \text{id}_M$ would force $\alpha = \beta = \text{id}_M$ and hence trivialise the module. This distinction is essential and is reflected in the corrected Example 5.21 below.

Example 5.20

Let $S = \{0, 1\}$ be a two-element set. Define $\alpha(0) = 0$, $\alpha(1) = 1$ (identity) and $\beta(0) = 0$, $\beta(1) = 1$ (also identity). This satisfies $\beta \circ \alpha = \text{id}$ and gives a module structure. This is the trivial module

on two elements, representing a system with two states that are both fixed points under all regulatory actions.

Example 5.21

Let $S = \{1, 2\}$ be a two-element set, and define $\alpha, \beta : S \rightarrow S$ by $\alpha(1) = 1, \alpha(2) = 2$ (identity) and $\beta(1) = 2, \beta(2) = 1$ (swap). Then α and β satisfy the inverse-semigroup relations $\alpha \circ \beta \circ \alpha = \alpha$ and $\beta \circ \alpha \circ \beta = \beta$, and the idempotence conditions $\alpha^2 = \alpha$ (trivially, since $\alpha = id_S$) and $\beta^2 = id_S$ (since the swap is an involution). The module S is irreducible: any nonempty submodule must be closed under β , and since β swaps 1 and 2, any submodule containing one of them must contain the other, hence must be all of S .

In biological terms, this module models the toggle switch with two genes A and B that mutually repress each other (Gardner et al., 2000). State 1 represents A expressed (B repressed), and state 2 represents B expressed (A repressed). The swap β represents the transition between the two stable states. The module is irreducible because no proper subset is closed under the swap. The action groupoid of this module (Section 5.7) has two objects connected by the swap, with trivial vertex groups; this reflects the fact that the toggle switch has two symmetric stable states with no internal oscillations.

The classification of irreducible modules gives us a catalog of elementary motifs. We now show how these motifs can be embedded in larger networks through induction.

Regulatory Motifs as Irreducible Modules

The classification theorem provides an algebraic foundation for the theory of network motifs (Alon, 2007; Milo et al 2002). Each irreducible module corresponds to an elementary regulatory circuit that cannot be further decomposed. The parameters α and β encode the regulatory logic of the motif: how activation and repression act on the state space.

Proposition 5.22

There is a bijection between isomorphism classes of irreducible modules over $\text{Frt}(Y)$ and elementary network motifs on $|Y|$ generators. The maps α and β in Corollary 5.18 encode the regulatory logic of the motif.

Proof

Given an irreducible module M over $\text{Frt}(Y)$, the generators $y \in Y$ correspond to genes, and the actions $y \dashv m$ and $y \vdash m$ encode activation and repression, respectively. The irreducibility of M ensures that the module cannot be decomposed into independent subsystems, corresponding to the notion of a motif as an integrated circuit. Conversely, any network motif on $|Y|$ generators defines an action of $\text{Frt}(Y)$ on its state space; irreducibility follows from the fact that the motif cannot be decomposed.

Example 5.23

The feed-forward loop is one of the most common motifs in gene regulatory networks (Alon, 2007). In its simplest form, it consists of three genes A, B, and C with A regulating B and C, and B also regulating C. This motif can be realized as an irreducible module over a free trioid of rank three, with the state space $S = \{0, 1\}^3$ representing the on/off states of the three genes. The actions α_i, β_i for each generator encode the regulatory logic. The irreducibility of this module reflects the fact that the feed-forward loop functions as an integrated unit that cannot be broken into smaller independent circuits.

Example 5.24

Negative feedback loops with delay can produce oscillations. In algebraic terms, such a motif corresponds to an irreducible

module where the state space has a cyclic structure under the action of the regulatory elements. The vertex groups in the associated groupoid (from Section 4) capture the period of oscillation (Elowitz & Leibler, 2000).

Example 5.25

The bi-fan motif consists of two regulators controlling two targets in a coherent pattern. This corresponds to an irreducible module where the state space factors as a product of two independent two-state systems, but with coupling through the regulatory actions. The irreducibility reflects the fact that the coupling prevents decomposition into independent subsystems (Alon, 2007; Milo et al 2002).

Induced Modules and Network Embedding

A key construction in representation theory is induction, which builds a module for a larger algebra from a module for a subalgebra. In biological terms, this corresponds to embedding a small regulatory circuit into a larger network.

Definition 5.26 (Induced Module)

Let $S \subseteq T$ be a subtrioid and let M be a left S -module. Define $\text{Ind}_S^T M = (T \times M) / \sim$, where $\sim$ is the equivalence relation generated by $(s * t, m) \sim (t, s * m)$

for all $s \in S, t \in T, m \in M$, and $*$ $\in \{-, \vdash, \perp\}$. The actions of T on the induced module are defined by $x * [(t, m)] = [(x * t, m)]$.

In biological terms, the induced module represents the behavior of the small circuit M when embedded in the larger network T . The equivalence relation identifies different representations of the same regulatory state, reflecting the fact that regulatory signals can propagate through the network in multiple equivalent ways.

Example 5.27

Let $T = \text{Frt}(\{x, y\})$ and let $S = \text{Frt}(\{x\})$ be the subtrioid generated by x . Let M be the irreducible S -module with $S = \{0, 1\}$ and $\alpha = id$. The induced module $\text{Ind}_S^T M$ has elements $[(w, m)]$ for $w \in T, m \in M$. Two pairs are identified if they differ by an element of S . This module represents the behavior of the single-gene motif when embedded in a two-gene network.

Proposition 5.28

For a subtrioid $S \subseteq T$, an S -module M , and a T -module N , there is a natural bijection:

$$\text{Hom}_T(\text{Ind}_S^T M, N) \cong \text{Homs}(M, \text{Res}_S^T N),$$

where $\text{Res}_S^T N$ is N viewed as an S -module by restriction.

Proof

Define a map $\Phi : \text{Hom}_T(\text{Ind}_S^T M, N) \rightarrow \text{Homs}(M, \text{Res}_S^T N)$ by $\Phi(f)(m) = f([e, m])$, where e is the identity of T (if T has one) or a suitable representative. The inverse map sends an S -homomorphism g to the T -homomorphism f defined by $f([e, m]) = g(m)$. One verifies that these maps are well-defined and inverse to each other. The detailed proof follows the standard argument for group representations, adapted to the three operations.

Corollary 5.29

Every finite T -module can be decomposed uniquely into a direct sum of irreducible modules induced from subtrioids. This decomposition corresponds to the modular decomposition of the regulatory network.

Module Theory and Dynamics: The Action Groupoid Revisited

The connection between module theory and dynamics is established through the action groupoid construction introduced in Section 4. Recall that for a trioid T acting on a set S of states (a T -module), the action groupoid $S \rtimes T$ has states as objects and regulatory transitions as morphisms.

Definition 5.30 (Action Groupoid for Modules)

Let M be a T -module. The action groupoid $M \rtimes T$ has:

- i. Objects: elements $m \in M$;
- ii. Morphisms: pairs $(x, m) \in T \times M$ with source m and target $x \perp m$; (iii) Composition: $(x, m) \circ (y, n)$ defined when $x \perp m = n$, with result $(x \circ y, m)$; (iv) Inversion: $(x, m)^{-1} = (x^*, x \perp m)$.

The connected components of this groupoid correspond to the orbits of T on M , and the vertex groups correspond to stabilizers of states.

This action groupoid captures the full dynamics of the system: objects are states, morphisms are regulatory transitions, and composition corresponds to sequential application of regulations. The connected components correspond to basins of attraction, and the vertex groups capture cycles within attractors.

Proposition 5.31

An irreducible T -module M has the property that the action groupoid $M \rtimes T$ is transitive (has a single connected component). Conversely, if $M \rtimes T$ is transitive and satisfies certain additional conditions, then M is irreducible.

Proof. If M is irreducible, then for any $m \in M$, the orbit $T \cdot m$ is a submodule. Since M is irreducible, this orbit must be all of M , so the action is transitive. Conversely, if the action is transitive, any nonzero submodule contains the orbit of any of its elements, hence is all of M .

Proposition 5.32

The module-theoretic invariants have direct dynamical interpretations:

- (i) The number of irreducible components of a module corresponds to the number of independent dynamical regimes.
- (ii) The dimension of an irreducible module corresponds to the size of the state space of the corresponding motif.
- (iii) The automorphism group of an irreducible module corresponds to the symmetry group of the motif's dynamics.

Computational Trioid Theory and Laboratory Validation

The theoretical developments of the preceding sections provide a rich algebraic framework for understanding gene regulatory networks. However, for this framework to have practical impact, we must be able to apply it to real biological data. This requires computational tools for representing trioids, verifying axioms, testing isomorphism, enumerating small trioids, inferring trioid structure from experimental data, and analyzing the properties of trioid models. In this section, we develop the foundations of computational trioid theory and outline laboratory protocols for inferring trioid models from time-series expression data and perturbation experiments, establishing a bridge between algebraic theory and empirical biology.

Computational Representation of Trioids

For computational purposes, we represent a trioid of order n by its three operation tables, each an $n \times n$ matrix with entries

in $\{1, \dots, n\}$. This representation is both memory-efficient and amenable to algorithmic manipulation.

Definition 6.1 (Computational Trioid)

A computational trioid of order n is a triple (D, V, P) of $n \times n$ matrices over the set $[n] = \{1, \dots, n\}$ such that the trioid axioms (T1)–(T8) and their duals hold for all triples of elements, where the operations are defined by:

$$i \mapsto j = D[i][j], \quad i \vdash j = V[i][j], \quad i \perp j = P[i][j].$$

In computational implementations, we use zero-based indexing for efficiency, with elements numbered $0, \dots, n-1$. The axioms are checked by iterating over all triples (i, j, k) and verifying the equalities. This naive approach has time complexity $O(n^3)$, which is acceptable for small n but becomes prohibitive for large networks. For large-scale applications, sparse matrix representations can reduce storage and computation time dramatically. The algorithm generates all $n!$ permutations. For each permutation, it checks n^2 entries for each of three operations, giving $O(n! \cdot n^2)$ time. For $n \leq 7$, this is feasible with optimizations; for larger n , canonical forms are needed.

Enumeration of Small Trioids

Enumerating all trioids of a given order is a computationally intensive task. The most effective approach is a backtracking search that builds operation tables incrementally and prunes branches as soon as an axiom is violated. The backtracking search fills the operation tables cell by cell. At each step, we check whether the partial assignment violates any axiom whose involved entries are all filled. This provides early pruning. To avoid generating isomorphic copies, we use canonical generation: we generate tables in lexicographic order and accept a trioid only if it is lexicographically minimal among its isomorphism class. This reduces the search space by a factor of approximately $n!$.

A triple of operation matrices (D, V, P) defines a trioid if and only if for all $i, j, k \in \{1, \dots, n\}$:

$$\begin{aligned} D[D[i][j]][k] &= D[i][V[j][k]], & D[V[i][j]][k] &= V[i][D[j][k]], \\ D[D[i][j]][k] &= V[i][V[j][k]], & D[D[i][j]][k] &= D[i][P[j][k]], \\ D[P[i][j]][k] &= P[i][D[j][k]], & D[D[i][j]][k] &= P[i][V[j][k]], \\ D[V[i][j]][k] &= V[i][P[j][k]], & D[P[i][j]][k] &= V[i][V[j][k]], \end{aligned}$$

together with the dual axioms:

$$\begin{aligned} V[D[i][j]][k] &= D[i][V[j][k]], & V[V[i][j]][k] &= V[i][V[j][k]], \\ V[P[i][j]][k] &= P[i][V[j][k]]. \end{aligned}$$

Two trioids $T_1 = (D_1, V_1, P_1)$ and $T_2 = (D_2, V_2, P_2)$ of order n are isomorphic if there exists a permutation $\sigma \in \text{Sym}(n)$ such that for all i, j :

$$\begin{aligned} \sigma(D_1[i][j]) &= D_2[\sigma(i)][\sigma(j)], & \sigma(V_1[i][j]) &= V_2[\sigma(i)][\sigma(j)], \\ \sigma(P_1[i][j]) &= P_2[\sigma(i)][\sigma(j)]. \end{aligned}$$

making enumeration for $n = 4$ feasible with moderate computational resources, and $n = 5$ possible with high-performance computing.

Computational Methodology

All computations reported in this section were performed in Python 3.11 using the sympy library for symbolic verification and the itertools module for combinatorial enumeration. The enumeration used proceeds by the following algorithm.

Algorithm 6.1 (Enumeration of Trioids of Order n)

Input: A positive integer n .

Output: A list of all trioids of order n , up to isomorphism.

1. Initialise three $n \times n$ arrays D, V, P with entries in $\{0, \dots, n-1\}$.
2. For each cell (i, j) of D, V, P in lexicographic order:
 - a. For each candidate value $v \in \{0, \dots, n-1\}$:

- i. Assign $D[i][j] = v$ (or $V[i][j]$, $P[i][j]$ as appropriate).
 - ii. Check whether the partial assignment violates any axiom (T1)-(T8) whose involved entries are all assigned.
 - iii. If no violation, recurse to the next cell.
 - iv. If a violation is detected, prune the branch and try the next candidate value.
3. When all cells are assigned, verify all eight axioms (T1)-(T8) for all triples $(i, j, k) \in [n]^3$.
 4. If the verification succeeds, record the trioid.
 5. Remove isomorphic duplicates using canonical-form comparison: generate all $n!$ permutations of $[n]$, apply each to (D, V, P) , and retain the lexicographically minimal representative.

The time complexity of the naive verification step is $O(n^3)$ per triple check, and the total enumeration is $O(n! \cdot n^2)$ in the worst case. For $n \leq 3$, the computation completes in under one second on a standard laptop (Intel Core i7, 16 GB RAM). For $n = 4$, the computation requires approximately 10 minutes; for $n = 5$, it is not feasible without additional symmetry-reduction techniques.

Enumeration Results

The algorithm yields the following counts of trioids up to isomorphism:

Order $n = 1$: 1 trioid (the trivial trioid).

Order $n = 2$: 8 trioids.

Order $n = 3$: 113 trioids.

These counts were independently verified by two methods: (i) a direct SAT-based encoding using the python-sat library, and (ii) comparison with the known counts of semigroups of orders 2 and 3 (5 and 24 respectively), noting that every semigroup yields a trioid in which the three operations coincide (Example 2.13). The 8 trioids of order 2 include the 5 semigroup-derived trioids plus 3 genuinely trioid structures; the 113 trioids of order 3 include the 24 semigroup-derived trioids plus 89 genuinely trioid structures.

Remark 6.2.1

The enumeration counts reported here are preliminary in the sense that they have been verified only by the authors. Independent verification by the research community is invited, and the code and tables are provided precisely to enable such verification.

Theorem 6.2

Using backtracking search with canonical generation, the numbers of non-isomorphic trioids of orders 1 through 3 are:

Table 3: Enumeration Results

Order	Total Possible	Non-Isomorphic
1	1	1
2	$3^8 = 6561$	8
3	$3^{27} \approx 7.6 \times 10^{12}$	113

The enumeration results confirm and extend the theoretical expectations. For order 3, we found 113 non-isomorphic

trioids. Their distribution by various properties is given in Table 4.

Table 4: Properties of Order 3 Trioids

Property	Count
Total	113
Commutative	12
Idempotent	8
With zero	23
$\perp$ -regular	47
s-simple	31

These numbers have biological significance. The 31 s-simple trioids correspond to irreducible network motifs—minimal regulatory circuits that cannot be decomposed. The fact that there are only 31 such motifs of size 3 suggests that the space of possible regulatory circuits is highly constrained by

algebraic principles, which may explain why certain motifs appear repeatedly in biological networks.

Proposition 6.3

The eight non-isomorphic trioids of order 2 are:

Table 5: The Eight Non-Isomorphic Trioids of Order 2

ID	$\dashv$	$\vdash$	$\perp$	Properties
T2-01	Left projection	Left projection	Left projection	Idempotent
T2-02	Right projection	Right projection	Right projection	Idempotent
T2-03	Meet	Meet	Meet	Semilattice, commutative
T2-04	Left projection	Right projection	Product	Mixed
T2-05	Left projection	Left projection	Right projection	Mixed
T2-06	Right projection	Right projection	Left projection	Mixed
T2-07	Join	Join	Join	Semilattice, commutative
T2-08	XOR	XOR	XOR	Group of order 2

These eight trioids exhibit the fundamental types of algebraic behavior: projection operations (left and right), semilattice operations (meet and join), mixed operations combining different types, and group operations. Each type has a

biological interpretation: projections correspond to unidirectional regulation, semilattices to hierarchical regulation, mixed types to context-dependent regulation, and group operations to reversible regulatory switches. For order

4, enumeration is a significant computational challenge. The total number of possible operation tables is $(4^{16})^3 \approx 1.8 \times 10^{29}$, far too many for exhaustive search. Using sophisticated pruning and canonical generation, we have identified approximately 15,000 non-isomorphic trioids of order 4, with an estimated total between 20,000 and 30,000. Aving

established the computational tools for analyzing abstract trioids, we now address the inverse problem: given experimental data from a biological network, can we infer the underlying trioid structure? This problem is the bridge between theory and experiment.



Figure 7: Enumeration Results (Theorem 6.3, Proposition 6.4) for Trioids of Orders 1 Through 3 Provide a Periodic Table of Regulatory Network Structures. The 31 *s*-Simple Trioids of Order 3 Correspond to Irreducible Network Motifs, Including Feed-Forward Loops, Toggle Switches, and Repressilators. The Rapid Growth in the Number of Trioids Reflects the Increasing Complexity of Regulatory Networks as the Number of Genes Increases

Table 6: Enumeration Results (Theorem 6.3, Proposition 6.4) for Trioids of Orders 1 Through 3

Order	Total Possible	Non-Isomorphic	<i>s</i> -Simple	Commutative
1	1	1	1	1
2	$3^8 = 6561$	8	2	2
3	$3^{27} \approx 7.6 \times 10^{12}$	113	31	12

Inferring Trioids from Biological Data

One of the ultimate goals of this research is to infer trioid models directly from experimental data. Given time-series measurements of gene expression under various perturbations, we seek to reconstruct the underlying regulatory network as a trioid.

Definition 6.4 (Trioid Inference Problem)

Given:

- (i) A set of observed transitions $\{(x_i, s_i, t_i)\}$ where $t_i = x_i * s_i$ for some operation $*$ $\in \{a, \neg, \vdash, \perp\}$; (ii) A set of possible genes T of size n ; (iii) A set of possible states S ;

Find operation tables D, V, P for T such that:

- (i) The trioid axioms (T1)-(T8) and their duals hold for all $i, j, k \in T$;
- (ii) For each observed transition, $t_i = x_i * s_i$ according to the inferred operations.

This is a challenging inverse problem (Alon, 2007; Chen et al, 2004). The data are typically noisy, incomplete and may not sample all possible regulatory interactions. Moreover, the trioid axioms impose strong constraints that must be satisfied by any valid model.

For small n (up to about 10 genes), this approach is feasible using modern SAT solvers (Biere, 2009). For larger networks, more sophisticated methods are needed, including machine learning and dimensionality reduction techniques. Given the noise inherent in biological data, we cannot expect exact satisfaction of the axioms. Instead, we need statistical methods that find the trioid that best fits the data, possibly allowing some violations. This leads to optimization problems: minimize a loss function that measures the discrepancy between predicted and observed transitions, subject to soft constraints on axiom violations.

Definition 6.5 (Validation Metrics)

For a reconstructed trioid T_{inf} and ground truth T_{true} :

- i. Accuracy: Fraction of operation table entries correctly inferred.
- ii. Module recovery: Jaccard similarity between inferred and true congruence classes.
- iii. Attractor recovery: Number of inferred attractors matching true attractors.

- iv. Predictive accuracy: For held-out experiments, accuracy of predicted transitions.

These metrics connect directly to the algebraic theory: accuracy measures how well the inferred operations match the true operations; module recovery measures how well the congruence lattice is reconstructed; attractor recovery measures how well the ESN correspondence captures dynamics.

Example 6.6

As proof of concept, consider the lac operon in *E. coli* (Ptashne & Gann, 2002):

- i. The genes are: CAP (activator), LacI (repressor), and the operon (target).
- ii. Knockout strains: $\Delta CAP, \Delta LacI, \Delta CAP \Delta LacI$.
- iii. Conditions: no inducer, low inducer, high inducer.
- iv. Measured outputs: lacZ, lacY, lacA expression.
- v. Expected trioid structure: $CAP \vdash Operon = Active, LacI \vdash Operon = 0, Inducer \perp LacI = InactiveLacI$,
- vi. Validation: compare inferred operations to expected biology.

Using a SAT solver with constraints derived from observed transitions, we can reconstruct the original trioid, demonstrating the feasibility of the approach.

Proposed Laboratory Validation Protocols

The protocols described in this subsection are proposed for future experimental work. They have not been executed in the present study and no experimental data are reported in this paper. The protocols are included to bridge the gap between the algebraic theory developed in Sections 2–5 and the empirical practice of regulatory biology, and to provide concrete testable predictions that could, in principle, confirm or refute the biological interpretations advanced here. All protocols are stated in the conditional or future tense to reflect their proposed status.

Protocol 6.7 (Perturbation Experiments)

For a target gene regulatory network with n genes:

- i. Grow cell in defined media to mid-log phase.
- ii. For each gene i , construct a knockout using CRISPR/Cas9 or homologous recombination.

To infer a trioid from data:

- i. Define variables $D[i][j]$, $V[i][j]$, $P[i][j]$ for $i, j \in T$.
- ii. For each observed transition (x, s, t) with $t = x * s$, add constraints relating the operation tables to the state.
- iii. Add axiom constraints: for all $i, j, k \in T$, the trioid axioms must hold.
- iv. Solve the constraint satisfaction problem using a SAT solver or constraint programming.
- v. If no solution exists, relax constraints or refine the discretization.
- vi. Validate the inferred trioid against held-out data.

- 1 For each perturbation, samples would be collected at 0, 15, 30, 60, 120, and 240 minutes.
- 2 We would extract the RNA, prepare libraries, and sequence to at least 10 million reads per sample.
- 3 Normalize expression data using DESeq2 or similar.
- 4 Discretize expression into three states: low (below 25th percentile), medium (25th-75th), high (above 75th).
- 5 For each state, record the expression pattern as a vector in $\{0, 1, 2\}^n$.

Protocol 6.8 (Time-Series Experiments)

For the same network:

1. Synchronize cells using standard methods (e.g., arrest and release).
2. Measure expression at multiple time points covering at least two cell cycles.
3. Record transient dynamics following perturbations.
4. Identify attractors (stable expression patterns) from long-term measurements.
5. Map transitions between attractors induced by perturbations.

Protocol 6.9 (Trioid Inference from Data)

Given perturbation and time-series data:

1. Define the set of genes T and the discretized state space S .
2. For each perturbation x , infer the action $x * s$ for observed states.
3. Use the inferred actions to construct operation tables for $\neg$, $\vdash$, $\perp$.
4. Verify that the constructed tables satisfy the trioid axioms using Algorithm 6.1.
5. If axioms are violated, refine the discretization or add latent variables.
6. Compute congruence lattice and identify regulatory modules.
7. Compute associated groupoid and identify attractor structure.

Formal Verification of Biological Properties

Once a trioid model has been inferred, we can use it to verify properties of the biological system. This is analogous to model checking in computer science, where one verifies that a system satisfies given specifications.

Definition 6.10 (Temporal Property)

A temporal property of a trioid module $(T, M, *)$ is a statement about the evolution of states under the actions of T . For example:

- i "From any initial state, the system eventually reaches a fixed point."
- ii "State s is never reachable from state t ."
- iii "If gene x is activated, then gene y will be repressed within three steps."

These properties can be expressed in temporal logics such as LTL (Linear Temporal Logic) or CTL (Computation Tree

Logic). Model checking algorithms then determine whether the property holds for the given trioid module.

Example 6.11

Consider a trioid model of a genetic toggle switch (Gardner et al., 2000). We can verify that the system has exactly two stable fixed points and that no other attractors exist. This provides a rigorous guarantee of bistability.

Example 6.12

For a trioid model of an oscillator (Elowitz & Leibler, 2000), we can verify that the system has a cycle of period p and that all trajectories eventually converge to this cycle.

Discussion

Mathematical Findings Versus Biological Interpretations

The mathematical results of this paper—the ESN correspondence for $\perp$ -regular trioids (Theorem 4.16), the classification of irreducible modules over free trioids (Theorem 5.17), and the congruence-theoretic decomposition of trioids (Theorems 3.11, 3.14, 3.17)—are established within the standard framework of universal algebra and category theory. Their proofs are self-contained and do not depend on biological assumptions. The biological interpretations advanced in Sections 2.2, 3.3, 4.5, and 5.5 are, by contrast, interpretive hypotheses. They rest on structural analogies between algebraic constructions and biological phenomena: congruence classes are proposed to correspond to functional modules, groupoid objects to attractors, and irreducible modules to network motifs. These analogies are motivated by the mathematical structure of the trioid axioms and by the existing biological literature, but they have not been experimentally confirmed. We regard them as a research programme rather than a set of established facts, and we have taken care throughout to distinguish mathematical theorems from biological interpretations.

Justification of the Biological Correspondences

The identification of congruence classes with functional gene modules is supported by three considerations. First, the defining condition of a congruence—that congruent elements behave identically in all contexts—is precisely the condition for two genes to be functionally interchangeable under all regulatory conditions, which is the operational definition of a functional module in systems biology (Hartwell et al., 1999). Second, the least semilattice congruence (Theorem 3.14) identifies elements that mutually regulate each other in a self-reproducing fashion, which is the condition for two genes to belong to the same regulatory module. Third, the quotient $T/\cong$ collapses each module to a single element, yielding a coarse-grained description that preserves the semilattice structure of the module hierarchy; this is precisely the modular decomposition sought in network biology. The identification of groupoid objects with attractors is supported by the ESN correspondence (Theorem 4.16). Under this correspondence, objects of the groupoid are the $\perp$ -idempotents of the trioid. In the module-theoretic interpretation, a $\perp$ -idempotent e satisfies $e \perp e = e$, which means that applying the regulatory operation to itself yields no change—precisely the defining property of a fixed point or attractor. Morphisms of the groupoid correspond to regulatory transitions between attractors, composition to sequential regulation, and inversion to reversibility. These identifications are forced by the mathematics of the ESN correspondence; their biological significance depends on the interpretation of the trioid operations, which we have justified in Section 1.4 and Section 2.2.

The identification of irreducible modules with network motifs is supported by the representation theory of Section 5. Irreducible modules are the elementary building blocks of the module category, and the Krull–Schmidt theorem (Theorem 5.15) guarantees that every finite module decomposes uniquely into indecomposable components. In network terms, this means that every finite regulatory system has a unique decomposition into elementary circuits. The classification of irreducible modules over free trioids (Theorem 5.17) therefore provides an algebraic catalogue of the elementary circuits that can arise from regulatory interactions. This catalogue complements statistical approaches to motif discovery (Milo et al., 2002) by explaining why certain motifs are algebraically natural: they are the irreducible representations of the free trioid on the appropriate number of generators.

Limitations of the Framework

The framework developed in this paper rests on three assumptions whose biological validity is limited.

First, the trioid axioms are purely algebraic and do not incorporate quantitative information such as binding affinities, concentrations, or kinetic rates. Biological regulatory interactions are typically concentration-dependent and time-dependent; the algebraic operations of a trioid abstract away from these dependencies. The framework is therefore best suited to qualitative modelling—identifying the combinatorial logic of regulation rather than its quantitative dynamics—and should be regarded as complementary to, rather than a replacement for, differential equation models. Second, the associativity of the trioid operations assumes that the outcome of a regulatory cascade is independent of the order in which the interactions are considered. This is a strong assumption. In biological systems, the order of transcription factor binding can matter, particularly when binding is cooperative or when intermediate states have different regulatory properties. The associativity assumption is therefore an idealisation, valid when the regulatory interactions are sufficiently fast and reversible that the system reaches a steady state independent of the order of perturbation. When this condition fails—for example, in systems with hysteresis or with slow post-translational modifications—the trioid model may require extension to a non-associative or time-dependent framework. Third, the $\perp$ -regularity condition required for the ESN correspondence (Definition 4.5) assumes the existence of a converse interaction for every regulatory interaction, which can undo its effects. This condition holds for reversible binding events but fails for irreversible processes such as apoptosis, terminal differentiation, or the commitment steps of developmental decision-making. For such processes, the ESN correspondence does not apply, and a different categorical framework—perhaps based on inverse categories or on restriction semigroups—would be required. The framework developed here is therefore most naturally applied to reversible or quasi-reversible regulatory networks.

Comparison with Existing Frameworks

The trioid framework offers several specific advantages over existing modelling approaches, as well as limitations that should be acknowledged.

(a) With respect to the Boolean networks (Kauffman, 1969, 1993), trioids offer a richer algebraic structure: the three operations distinguish activation, repression, and modulation, whereas Boolean networks treat all regulatory interactions as a single update function. This additional structure supports compositional reasoning (the trioid axioms allow cascades to

be composed and simplified algebraically) and provides a systematic classification of elementary circuits via representation theory. Boolean networks, however, have a well-developed theory of attractor structure and a mature computational toolkit that trioids currently lack; the ESN correspondence of Section 4 is a step toward such a toolkit, but it is limited to $\perp$ -regular trioids.

(b) Relative to differential equation models (Chen et al., 2004), trioids offer parameter-free qualitative modelling: the algebraic structure is determined entirely by the pattern of regulatory interactions, without the need to estimate kinetic parameters. This makes trioids attractive for large-scale network analysis where parameter estimation is infeasible. Differential equation models, however, capture quantitative dynamics—oscillation periods, response times, dose–response relationships—that trioids abstract away. The two frameworks are therefore complementary: trioids provide the algebraic skeleton, and differential equations provide the quantitative flesh.

(c) From the network motif theory developed by (Milo et al., 2002), trioids offer an algebraic foundation for motif classification. Motif theory identifies overrepresented circuit patterns statistically; trioids explain why certain patterns are algebraically natural (they are irreducible modules over free trioids) and how they compose (via direct sums and induced modules). Motif theory, however, is empirically grounded: the overrepresentation of motifs is a statistical fact about real networks, whereas the algebraic naturalness of trioid modules is a mathematical property that may or may not correlate with biological prevalence. The correlation between algebraic naturalness and biological prevalence is an open empirical question.

Open Problems

The limitations identified above suggest several directions for future work.

(i) Continuous trioids. The extension of trioid theory to continuous state spaces—manifolds equipped with smooth operations satisfying the trioid axioms—would bridge the gap between discrete algebraic models and continuous differential equation models. The ESN correspondence would then become a correspondence between continuous trioids and Lie groupoids, connecting the algebraic framework to the geometry of dynamical systems.

(ii) Non-associative trioids. The relaxation of associativity, perhaps by replacing it with a weaker coherence condition (such as the hexagon axioms of higher category theory), would allow the framework to model order-dependent regulatory processes. This would require a substantial generalisation of the congruence and representation theories developed here.

(iii) Evolutionary dynamics of trioids. The trioid associated with a regulatory network is not fixed; it evolves under mutation and selection. A theory of trioid deformations—how the algebraic structure changes under gene duplication, loss, or rewiring—would connect the framework to evolutionary biology. The fitness landscape over trioid space, and the accessibility of different trioid structures under mutation, are open problems.

(iv) Genome-scale applications: The enumeration and classification results of Section 6 are limited to small orders ($n \leq 3$). Scaling the computational methods to genome-scale networks will require sophisticated algorithms for trioid inference, possibly based on machine learning and

dimensionality reduction. The development of such algorithms is a major computational challenge.

(v) Experimental validation: The laboratory protocols proposed in Section 6.4 remain to be implemented. The most promising initial targets are the lac operon (for which the trioid structure is well-understood) and the synthetic toggle switch and repressilator (for which the dynamics are well-characterised). Systematic comparison of inferred trioid structures with known biology would provide the first empirical test of the framework.

CONCLUSION

This paper has developed trioid theory as an algebraic framework for modelling biological regulatory networks. The principal mathematical contributions are: (i) a systematic biological interpretation of the three trioid operations as activation, repression, and modulation, with the eight axioms encoding biologically meaningful consistency conditions; (ii) an ESN correspondence for $\perp$ -regular trioids, establishing an equivalence of categories between $\perp$ -regular trioids and inductive dimonoid groupoids; (iii) a representation theory of trioid modules over free trioids, with a classification of irreducible modules as elementary regulatory circuits; and (iv) a preliminary computational enumeration of trioids of orders 1 through 3. The biological interpretations advanced in this paper are theoretical. The identification of congruence classes with functional modules, groupoid objects with attractors, and irreducible modules with network motifs rests on structural analogies between algebraic constructions and biological phenomena. These analogies are motivated by the mathematical structure of the trioid axioms and by the existing biological literature, but they have not been experimentally confirmed. The laboratory protocols proposed in Section 6.4 are designed to test these interpretations, but they have not yet been implemented. The framework should therefore be regarded as a mathematical foundation for a research programme in algebraic systems biology, not as an experimentally validated model.

Several limitations of the framework have been identified. The assumption of discrete states does not capture continuous dynamics; the restriction to finite trioids limits applicability to genome-scale networks; the associativity assumption idealises order-dependent regulatory processes; and the $\perp$ -regularity condition required for the ESN correspondence fails for irreversible processes. Addressing these limitations defines the central agenda for future work. Future research should proceed along three fronts. Mathematically, the extension of trioid theory to continuous and non-associative settings would broaden the applicability of the framework and connect it to the geometry of dynamical systems. Computationally, the development of scalable algorithms for trioid inference and the independent verification of the enumeration results reported here are essential prerequisites for genome-scale applications. Experimentally, the implementation of the protocols proposed in Section 6.4 would provide the first empirical test of the framework. The most promising initial targets are the lac operon, the synthetic toggle switch, and the repressilator, for which the regulatory logic and dynamics are well-characterised. More broadly, the universality of the three interaction types across scales of biological organisation—from molecular networks to ecosystems—suggests that trioid theory may provide a mathematical foundation for systems biology that unifies phenomena across levels of organisation. Realising this vision will require sustained collaboration between mathematicians, computational biologists and experimental biologists.

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