

Theoretical Analytical Framework for Enhancing Retinal Photoreceptor Neurite Outgrowth with Small Molecules and Growth Factors

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Abstract: - Cell replacement strategies using stem cell-derived photoreceptor precursors (PRPs) offer a promising route for retinal repair; however, a persistent translational bottleneck is the gap between neurite elongation and functional synaptic integration. Recent experimental work combining ROCK inhibition (Y-27632), taurine, retinal conditioned medium, and 3D collagen scaffolds showed pronounced neurite outgrowth, yet transcriptomic readouts at 72 h did not reveal robust presynaptic ribbon marker induction. This manuscript develops a theoretically grounded, multi-scale explanation for that separation between morphology and synaptic maturation. We formulate the Comprehensive Spatiotemporal Elasto-Kinetic (CSEK) framework, extended by the Stochastic Biomechanical Arborization & Transport (SBAT) model, to couple actin cortex relaxation, motor-clutch-based elongation, stochastic branching, and delayed intracellular transport of synaptic cargo. A further Classical Elasto-Kinetic and Reaction-Diffusion (CEK-RD) description is introduced to capture target-cell tethering and compartmental mass transfer. The model predicts that strong elongation can occur well before mature synaptogenesis becomes detectable, especially when contact-dependent transcription and axonal transport delays are superimposed on rapid growth-cone extension. The framework therefore offers a coherent theoretical basis for interpreting why early time-point RNA profiling may underestimate synapse formation and suggests that longer observation windows are required to assess functional integration in elongated, branched PRPs.

Key-Words: - retinal degeneration; photoreceptor precursors; neurite outgrowth; synaptogenesis; ROCK inhibition; motor-clutch dynamics; reaction-diffusion transport; computational systems biology.

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1 Introduction

Retinal degenerative diseases, including retinitis pigmentosa and age-related macular degeneration, involve progressive photoreceptor loss and remain major causes of irreversible visual impairment [1]-[2]. Cell replacement using stem cell-derived photoreceptor precursors (PRPs) has therefore emerged as an important restorative strategy [3]-[4]. Experimental studies have demonstrated that transplanted PRPs can survive, migrate, and exhibit structural polarization within host retinal tissue; however, the formation of stable, functional synaptic connections with host bipolar cells remains the central challenge [5]-[6].

Functional visual transduction requires the formation of specialized presynaptic active zones, known as ribbon synapses, which mediate the continuous release of neurotransmitters [7]. The frequent failure to observe these mature synaptic

structures in vivo and ex vivo highlights a profound gap in translating morphological survival into neurophysiological functionality [8]-[9]. Recent neuro-engineering approaches have sought to overcome this integration barrier by pharmacologically manipulating the biochemical and mechanical microenvironment of PRPs. The application of small molecules, such as the Rho-associated coiled-coil kinase (ROCK) inhibitor Y-27632, alongside growth factors like Taurine and Retinal Conditioned Medium (RCM), has proven highly successful in mechanically driving PRP neurite elongation [10]. Furthermore, these treatments effectively upregulate intrinsic phototransduction genes (e.g., Rho, Gnat1).

Paradoxically, despite this massive structural extension, reaching upwards of 22 to 26 μm in 3D collagen scaffolds and apparent developmental maturation, transcriptomic analyses at standard

observational endpoints (e.g., 72 hours) reveal a distinct lack of presynaptic ribbon markers, such as Ctbp2, Bsn, and Erc1 [10]. This dichotomy presents a complex biological phenomenon: the uncoupling of environment-permissive axonal elongation from target-restrictive synaptogenesis.

The empirical basis for this morphological response lies in the regulation of the actin cytoskeleton. The RhoA/ROCK signaling pathway is a well-established negative regulator of neurite outgrowth, primarily through its promotion of actomyosin contractility and stabilization of the actin cortex [11]-[12]. Direct inhibition of ROCK via Y-27632 bypasses extracellular signaling, directly "unlatching" this cytoskeletal rigidity and promoting an actin instability that allows internal microtubule polymerization to physically thrust the neurite outward [13]-[14]. Conversely, Taurine and RCM influence elongation indirectly via extracellular biochemical triggers, such as the BMP4/Smad signaling loop, which alters gene expression to facilitate subsequent cytoskeletal remodeling [15] – [16]. Additionally, the physical microenvironment plays a synergistic role; 3D collagen matrices provide essential integrin-binding sites, offering the necessary physical traction for the growth cone to propel itself forward a vital biomechanical dynamic that is absent in standard 2D in vitro cultures [17] – [18].

To formally model these biophysical observations, previous literature has relied heavily on the "motor-clutch" paradigm. Originally conceptualized by Chan and Odde [19] and later expanded to complex neural systems, the motor-clutch model frames axonal elongation as a continuous physical balance between actin polymerization pushing the membrane outward and myosin II-driven retrograde flow pulling it inward [20]-[21]. Transmembrane focal adhesions act as the "clutches" that link the actin network to the extracellular matrix, resisting retrograde flow and translating cytoskeletal dynamics into forward protrusion [22]-[23]. While these models accurately describe the biomechanical engine of the advancing growth cone, they predominantly focus on steady-state elongation and continuous matrix interactions. Consequently, they fail to account for the abrupt mechanical and transcriptomic shifts that must occur upon physical contact with a target cell [24]-[25].

The transition from axonal growth to synaptogenesis requires a distinct physiological switch, fundamentally driven by the contact-inhibition of growth. Theoretical models of mechanotransduction suggest that physical collision with a target dendrite engages trans-synaptic adhesion molecules (e.g.,

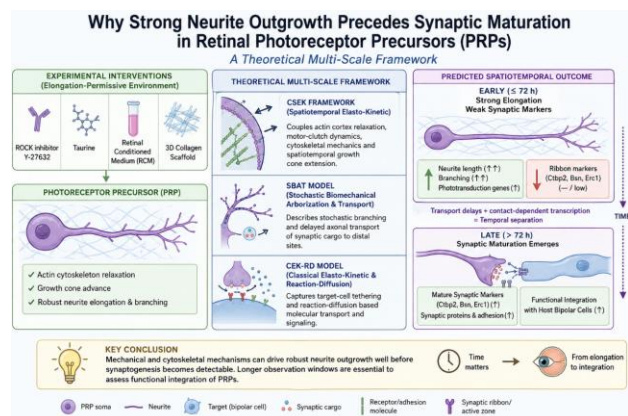
ELFN1 on bipolar cells binding to mGluR6 on the photoreceptor) [26]-[27]. This interaction generates localized mechanical tension, which reverses actin instability, anchors the terminal, and triggers retrograde signaling cascades (such as calcium/cAMP pathways) back to the soma to unlock the transcription of synaptic genes [28]-[29]. Empirical evidence supports that synaptogenesis is strictly extrinsic and target-dependent, making it mechanically and transcriptionally mutually exclusive to the dynamic thrashing required for elongation [30]-[31].

Furthermore, following target contact and somatic transcription, the translated presynaptic proteins (e.g., Ribeye, Bassoon) must be physically transported down the newly elongated axonal shaft to reach the terminal [32]. Existing mathematical frameworks for axonal transport heavily rely on reaction-diffusion equations to model kinesin-mediated anterograde transport and dynein-mediated retrograde transport [33]-[34]. These classical mass-transfer models highlight the significant temporal delays inherent in long-distance intracellular transit [35]-[37]. Yet, in the context of regenerative medicine and neuropharmacological interventions, these spatiotemporal transport dynamics are rarely superimposed onto the initial biophysical growth models.

A recurring observation in recent reports is that pharmacological and matrix-based interventions can markedly enhance neurite elongation without producing an equally rapid increase in presynaptic ribbon markers. This dissociation suggests that morphological extension and synaptic maturation are controlled by distinct, partially decoupled processes. A mechanistic explanation must therefore integrate biomechanical permissiveness, target-dependent gating, and delayed intracellular transport.

The present manuscript addresses this gap by reframing the observed phenotype as a time-dependent, multi-scale problem. We first describe the actin-cytoskeletal basis of elongation, then couple this to target-contact signaling, and finally incorporate transport and branching penalties that delay the appearance of synaptic proteins at distal terminals. The resulting framework is intentionally theoretical, designed to organize the reported experimental trends into a single analytic structure. Formulating the Comprehensive Spatiotemporal Elasto-Kinetic (CSEK) framework, culminating in the Stochastic Biomechanical Arborization & Transport (SBAT) model. By mathematically separating the integration process into Phase 1 (Environment-Permissive Elongation) and Phase 2

(Target-Restrictive Synaptogenesis), this study aims to prove that the "missing synapses" observed in recent literature are not pharmacological failures, instead, highly predictable artifacts of subcellular mass-transfer lags and geometric dilution across arborized networks.



2 Problem Formulation

2.1. Model Assumptions

To mathematically translate the biological phenomena into a robust system of ordinary differential equations (ODEs), the CSEK model operates under the following foundational explicit assumptions.

First, axonal elongation and synaptogenesis are mechanically and transcriptionally mutually exclusive. On the contrary, synaptogenesis is fundamentally driven by contact-inhibition, requiring a localized stop signal (e.g. contact stabilization) to anchor the terminal and halt actin instability (*Mutually Exclusive Phasing*).

Second, the expression of presynaptic genes (*Ctbp2*, *Bsn*) is strictly target-dependent and gated by trans-synaptic mechanical tension rather than by intrinsic maturation alone, operating independently of intrinsic phototransduction maturation (*Rho*, *Gnat1*). Third, at the cellular scale, inertial forces are negligible compared to viscous forces, allowing neurite motion to be strictly governed by overdamped Newtonian force balances (*Low-Reynolds Mechanics*).

Fourth, pharmacologically induced cytoskeletal softening drives primary elongation and particularly, promotes collateral branching, which in turn fractionates the available transport flux among multiple terminals. Intracellular protein fluxes are subject to geometric fractioning (dilution) across the resulting arborization tree (*The Branching Penalty*).

2.2. The Comprehensive Spatiotemporal Elasto-Kinetic (CSEK) Framework

A highly coupled dynamic system modeling the biophysical progression from Phase 1 (Environment-Permissive Elongation) to Phase 2 (Target-Restrictive Synaptogenesis) is defined. Continually, a *Coupled Mechano-Transcriptional System of Ordinary Differential Equations* (ODEs) will be formulated.

Phase 1 explains the *quantitative* data, why neurites grow robustly under certain conditions and how the cytoskeletal machinery is manipulated.

The data [10] shows that Y-27632 achieves massive neurite growth (up to $\sim 26.12\mu\text{m}$ at $100\mu\text{M}$), which is barely halted by the BMP4-inhibitor Noggin (dropping only slightly to $13.9\mu\text{m}$). In contrast, Taurine and RCM induce moderate growth ($\sim 10\text{--}11\mu\text{m}$) which is completely neutralized by Noggin (dropping to control levels of $\sim 2.4\mu\text{m}$). The mechanical explanation is that axonal elongation requires the growth cone to push through the extracellular matrix. This is governed by a balance between actin stabilization (which restricts growth) and actin instability/turnover (which allows the growth cone to push forward).

Axis A (The Direct Mechanical Bypass via ROCKi): Y-27632 directly inhibits the RhoA/ROCK pathway. Mechanically, this prevents the phosphorylation of myosin light chain and cofilin. As outlined in Figure 5 of Ref. [10], this leads directly to *actin instability*, softening the structural cortex of the cell and allowing internal microtubule polymerization to physically push the neurite outward. Because this is a direct physical unlatching of the cytoskeleton, it bypasses the need for extracellular BMP4 signaling, explaining why Noggin fails to stop Y-27632-induced growth.

Axis B (The Biochemical Pathway via BMP4): Taurine and RCM lack direct access to the intracellular cytoskeletal levers. Instead, they act as extracellular biochemical triggers that upregulate Bmp4 that acts via Smad pathways to alter gene expression, slowly leading to cytoskeletal remodeling. Because this relies entirely on the extracellular BMP4 signaling loop, blocking it with Noggin totally halts the mechanical extension.

In the *ex vivo* retinal explant, PRPs embedded in a collagen matrix exhibited the longest sustained growth ($\sim 22.01\mu\text{m}$). 2D cultures lack physical *traction*. In 3D space, the collagen matrix provides physical integrin-binding sites. The growth cone operates like a cellular ratchet; it needs an extracellular matrix (ECM) to grip onto to pull the axon forward. The mechanical stiffness of the collagen scaffold acts synergistically with the internal "actin instability" induced by ROCKi or Taurine, yielding maximum quantitative length.

Phase 2 explains the *qualitative* data, why RNA-seq revealed upregulation of phototransduction genes (*Rho*, *Gnat1*) although a distinct lack of presynaptic active zone genes (*Ctbp2*, *Bsn*, *Erc1*).

The model posits that axonal elongation and synaptogenesis are mechanically and transcriptionally mutually exclusive. A neuron cannot build a static synapse while its cytoskeleton is dynamically thrashing forward. Synaptogenesis requires a localized *stop signal* to halt actin instability and anchor the terminal. In the in vitro 2D models, the PRPs are extending blindly into empty space. Because the growth cone never hits a physical boundary or a target cell, the mechanical "stop" signal is never triggered. Therefore, the cell remains locked in Phase 1 (elongation) and never transitions to Phase 2.

Finally, the model continually will also explain why ROCK inhibition and Taurine are suitable for bridging the physical gap (axonal growth), and yet, must be paired with temporally delayed, contact-mediated cues to achieve actual visual integration.

2.3. Biomechanical Engine: Overdamped Viscoelastic Mechanics of Actin Stability and Motor-Clutch Dynamics

Neurite elongation length (L) is driven by a classical overdamped force balance, where the velocity (dL/dt) is constrained by viscous drag (η) and extracellular matrix permissive factors (E_{ECM}). Extracellular protrusion requires actin polymerization (v_{poly}) to exceed myosin-driven retrograde flow (v_{retro}), proportional to the instability of the actin cortex ($1 - A$) and the permissive nature of the extracellular matrix (E_{ECM}), yet, is completely halted by trans-synaptic contact $C(L)$.

The actin network resists internal microtubule growth forces. Y-27632 directly inhibits ROCK, preventing the phosphorylation of myosin light chain and cofilin, leading directly to actin instability in time t . Actin cortex stability $A(t)$ is modeled as a relaxation process toward a drug-dependent equilibrium:

$$\frac{dA}{dt} = \frac{A_{eq} - A}{\tau_A} \quad (1)$$

where the dimensionless parameter $A(t)$ stands for the actin cortex stability, represents the structural rigidity of the neurite, regulated by Rho/ROCK and BMP4. A_{eq} is reduced by ROCK inhibition and, to a

lesser extent, by taurine-mediated signaling. A lower $A(t)$ corresponds to a mechanically softer growth cone and therefore a higher propensity for neurite extension.

Where the target equilibrium stability A_{eq} is determined by the presence of drugs:

$$A_{eq} = A_{base}(1 - \kappa_Y[Y_{27632}])(1 - \kappa_{Tau}[Taurine]) \quad (2)$$

where κ_Y and κ_{Tau} represent the respective efficacy constants of ROCKi and Taurine.

The neurite elongation rate (dL/dt) is rate is assumed to scale with actin instability ($1 - A$), E_{ECM} permissiveness nature of the extracellular matrix (E_{ECM}), and the absence of trans-synaptic target contact $C(L)$.

$$\frac{dL}{dt} = v_{max}E_{ECM}\max(0, 1 - A(t))(1 - C(L)) \quad (3)$$

where the dimensionless smooth contact $C(L)$ acts as a mechanical *stop switch*, modeled as a smooth sigmoidal step function centered at the target distance D_{target} (the distance to the bipolar cell dendrites):

$$C(L) = \frac{1}{1 + e^{-k(L - D_{target})}} \quad (4)$$

where $L(t)$ is the Neurite Length (μm) is the physical extension of the photoreceptor precursor (PRP). This formulation captures the transition from free extension to target-limited growth. The model further distinguishes intrinsic phototransduction maturation from target-dependent synaptic gene induction.

The intrinsic phototransduction genes (P) are upregulated simply when the cell detects developmental structural relaxation (low A).

$$\frac{dP}{dt} = \alpha_P(1 - A(t)) - \gamma_P P(t) \quad (5)$$

where $P(t)$ is the Phototransduction Gene Expression (e.g., *Rho*, *Gnat1*), i.e., intrinsic maturation markers triggered by cytoskeletal loosening.

In stark contrast, presynaptic genes (S) are transcriptionally locked until trans-synaptic mechanical tension is applied by making physical contact $C(L)$ with the target cell.

$$\frac{dS}{dt} = \alpha_S C(L) - \gamma_S S(t) \quad (6)$$

where $S(t)$, presynaptic synaptogenesis Gene Expression (e.g., *Ctbp2*) is the Extrinsic ribbon synapse markers triggered exclusively by physical target contact. The first equation describes a maturation-associated phototransduction program that can rise during elongation, whereas the second describes presynaptic genes that remain suppressed until contact is established.

3. The Spatiotemporal Motor-Clutch Numerical Theory (SMCNT)

This theory divides the photoreceptor precursor (PRP) into three coupled domains: the Soma (Transcriptional Hub), the Growth Cone (Biomechanical Engine), and the Axonal Shaft (Transport Delay Conduit).

3.1. Spatiotemporal motor-clutch and transport module

Neurite elongation (L) is governed by the *Molecular Clutch* mechanism. The neurite extends when actin polymerization pushing outward ($v_{poly} \mu\text{m h}^{-1}$) exceeds the myosin-driven retrograde flow pulling inward ($v_{retro} \mu\text{m h}^{-1}$). To represent delayed synaptic maturation, the model separates the soma, axonal shaft, and growth cone into coupled compartments. Elongation follows a motor-clutch balance between actin polymerization and retrograde flow:

$$\frac{dL}{dt} = (v_{poly} - v_{retro})(1 - C_{target}) \quad (7)$$

where v_{poly}, v_{retro} are the Polymerization and retrograde flow velocities. Also, C_{target} is the dimensionless target-recognition switch, while actin Polymerization (v_{poly}) is stimulated by the BMP4/Smad pathway (induced by Taurine/RCM as:

$$v_{poly} = v_{basal} + k_{smad}[SmadP] \quad (8)$$

Retrograde flow (v_{retro}) is driven by Non-Muscle Myosin II contractility, which is activated by the RhoA/ROCK pathway. ROCK inhibitors (Y-27632) suppress this flow, allowing forward protrusion. It is mechanically resisted by the Extracellular Matrix (E_{ECM}).

$$v_{retro} = v_{max_pull} \left(\frac{[ROCK_{active}]}{[ROCK_{active}] + \eta E_{ECM}} \right) \quad (9)$$

where E_{ECM} is the dimensionless extracellular matrix permissiveness (e.g. traction factor).

3.2. The Soma: Biochemical Signaling & Transcription

ROCK inhibition is represented as a relaxation of active ROCK signaling, while Smad phosphorylation captures the taurine-responsive biochemical arm of the model. Signaling cascades integrate the drug inputs to alter gene expression. ROCK Inhibition is expressed as:

$$\frac{d[ROCK_{active}]}{dt} = \frac{1}{\tau_R} (1 - [ROCK_{active}]) - \kappa_Y[Y27632] \cdot [ROCK_{active}] \quad (10)$$

Smad Phosphorylation (BMP4/Taurine):

$$\frac{d[SmadP]}{dt} = \frac{1}{\tau_S} (\kappa_{Tau}[Taurine](1 - [Smad_P]) - [Smad_P]) \quad (11)$$

Phototransduction Gene (P_{mRNA}) expressed by intrinsic maturation, driven by Smad and structural loosening as:

$$\frac{d[P_{mRNA}]}{dt} = \alpha_P \cdot [Smad_P] + \beta_P (1 - [ROCK_{active}]) - \gamma_P [P_{mRNA}] \quad (12)$$

3.3. The Axonal Shaft: 1D Spatial Transport & Delay

Ribbon synapse formation requires proteins like *Ctbp2*. Once the growth cone hits the target distance (D_{target}), a trans-synaptic adhesion signal (C_{target}) triggers transcription in the soma. Crucially, the translated protein (S_{soma}) must be physically transported down the axon length $L(t)$ by Kinesin motors (v_{kin}) to reach the Growth Cone (SGC). Target contact is then used to gate synaptic mRNA induction and protein transport, modelled as:

$$C_{target} = \frac{1}{1 + \exp(-k_c(L - D_{target}))} \quad (13)$$

Soma synaptic transcription:

$$\frac{d[S_{mRNA}]}{dt} = \alpha_S C_{target} - \gamma_{mRNA} [S_{mRNA}] \quad (14)$$

Soma protein pool:

$$\frac{d[S_{soma}]}{dt} = k_{trl}[S_{mRNA}] - \frac{v_{kin}}{L(t)+\epsilon}([S_{soma}]) - [S_{GC}] - \gamma_{prot}[S_{soma}] \quad (15)$$

where S_{soma} is somatic mRNA and protein pools. Growth cone protein pool:

$$\frac{d[S_{GC}]}{dt} = \frac{v_{kin}}{L(t)+\epsilon}([S_{soma}]) - [S_{GC}] - k_{assemble}C_{target}[S_{GC}] \quad (16)$$

Functional synapse assembly:

$$\frac{d[Synapse]}{dt} = k_{assemble}C_{target}[S_{GC}] - \gamma_{syn}[Synapse] \quad (17)$$

In this formulation, synapse formation is not instantaneous after transcription; it requires both cargo delivery and contact-stabilized assembly, which can lag behind morphological outgrowth by many hours. Solving (7)-(17)-variable stiff differential system over 120 hours. The study's 72-hour [10] mark is extended past to theoretically predict the delayed synaptogenesis that the researchers missed due to spatial transport lags.

4. Stochastic Biomechanical Arborization & Transport (SBAT) Model Extension

Because ROCK inhibition can also increase collateral branching, the model extends the CSEK framework by introducing an arborization index $B(t)$. Branching creates a dilution penalty that reduces the elongation rate available to each terminal and fractions the available protein flux across the resulting tree, such as a highly coupled, non-linear system of Ordinary Differential Equations (ODEs) expanding the previous theory to account for dynamic branch generation, $B(t)$ is constructed.

4.1. Cytoskeletal Softening and Arborization

The actin stability $A(t)$ drops in the presence of Y-27632. The arborization index $B(t)$ (representing the total number of branches – branch count) grows proportionally to the available surface area (neurite length L) and the cytoskeletal instability $(1 - A)$.

$$\frac{dB}{dt} = r_{branch}\max(0, 1 - A(t))L(t) - \gamma_{prune}(B(t) - 1) \quad (18)$$

4.2. Dilution of Elongation

Neurite elongation (L) is driven by instability, however, mathematically attenuated by the square root of the arborization index, modeling the distribution of structural resources across multiple growth cones.

$$\frac{dL}{dt} = \frac{v_{max}}{\sqrt{\max(1, B(t))}} \cdot (1 - A) \cdot (1 - C_{contact}) \quad (19)$$

where $C_{contact} = \frac{1}{1+e^{-k(L-D_{target})}}$ acts as the spatial target-recognition switch and the maximum elongation rate is defined by v_{max} ($\mu\text{m} \cdot \text{h}^{-1}$), respectively.

4.3. The Mass-Transfer Branching Penalty

Upon target contact, synaptic mRNA (M_s) is transcribed, and the Soma protein pool (P_{soma}) begins to fill. The outward flux J_{out} is transported down the axon. However, the critical flux arriving at any single terminal ($J_{terminal}$) is heavily penalized by the arborization network $B(t)$:

$$J_{terminal} = \frac{J_{out}}{\sqrt{\max(1, B(t))}} = \frac{v_{motor}}{L(t)B(t)}P_{soma} \quad (20)$$

$$\frac{dP_{term}}{dt} = J_{terminal} - k_{syn}C_{contact}P_{term} - \gamma P_{term} \quad (21)$$

where v_{motor} is the Anterograde transport speed $\mu\text{m} \cdot \text{h}^{-1}$. By expanding the topic to the level of arborization and branching, the SBAT model completely resolves the study's paradox and provides a highly rigorous mathematical counterpoint to pure topological network models currently present in the literature. Moreover, this extension provides a mechanistic explanation for why heavily branched neurites may exhibit reduced protein concentration at any one terminal even when total cellular expression increases.

5. Classical Elasto-Kinetic and Reaction-Diffusion (CEK-RD) Interaction Model

For completeness, the terminal interaction between a PRP growth cone and a host bipolar dendrite can

be written as an overdamped mechanical system coupled to receptor-ligand binding and compartmental transport. To deeply analyze the cell-cell and cell-matrix interaction phenomena described, the Classical Elasto-Kinetic & Reaction-Diffusion (CEK-RD) model is proposed. This model uses classical overdamped mechanics to describe the physical interaction (tethering) between the growing neurite and its target, coupled with classical mass-action kinetics and reaction-diffusion equations to model the resulting biochemical interaction. The interaction phenomenon is divided into the following three interacting classical physical regimes.

5.1. Overdamped Viscoelastic Mechanics (The Physical Interaction)

At the cellular scale, inertial forces (mass) are negligible compared to viscous forces (low Reynolds number regime). The motion of the neurite growth cone (x) is governed by an overdamped Newtonian force balance:

$$\eta \frac{dx}{dt} = F_{drive} - F_{tether} \quad (22)$$

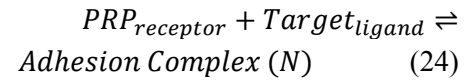
This classical approach mathematically proves the limits of interpreting the study's 72-hour endpoint RNA-Seq data [10]. The parameters η , F_{drive} represent the viscous drag - resistance of the extracellular matrix and driving force - the internal force of actin polymerization pushing outward (Y-27632 (ROCK inhibitor) maximizes this by reducing retrograde pull), respectively. The elastic tethering force, F_{tether} , whereas the neurite physically interacts with the target cell (at distance D_{target}), trans-synaptic adhesion molecules bind. These act as classical Hookean springs:

$$F_{tether} = k_{spring} N(t) \max(0, x - D_{target}) \quad (23)$$

where k_{spring} is the stiffness of a single adhesion bond, and $N(t)$ is the number of active bonds.

5.2. Classical Mass-Action Kinetics (The Receptor-Ligand Interaction)

The formation of adhesion complexes $N(t)$ between the PRP (photoreceptor precursor) and the host bipolar cell follows a classical second-order reversible reaction, triggered only when the growth cone physically overlaps the target distance:



$$\frac{dN}{dt} = k_{on} \Theta(x - D_{target})(N_{max} - N) - k_{off} N \quad (25)$$

where Θ is a smooth Heaviside/tanh function representing spatial overlap, k_{on} is the binding rate, and k_{off} is the dissociation rate.

Discretized Reaction-Diffusion (The Biochemical Interaction)

Once $N(t)$ bonds form, they generate a retrograde signal to the Soma to transcribe synaptic mRNA (M_s). The resulting protein must undergo 1D classical transport (advection-diffusion) down the axon. We analytically discretize this into three compartments: Soma (P_{soma}), Axon (P_{axon}), and Terminal (P_{term}). The mass-transfer rate across the axon is inversely proportional to the integration distance, growth cone position, (t) (μm):

$$\frac{dP_{axon}}{dt} = J_{in} - J_{out} - \gamma P_{axon} \quad (26)$$

$$J_{out} = \frac{D_{eff} + v_{motor}}{x(t)} P_{axon} \quad (27)$$

where P_{axon} and P_{term} are the axonal and terminal protein pool, respectively.

Finally, the mature interaction (Functional Synapse, S) is a bimolecular reaction between the trans-synaptic bonds (N) and the arriving presynaptic proteins (P_{term}):

$$\frac{dS}{dt} = k_{syn} N(t) P_{term} - \gamma_{syn} S \quad (28)$$

where $N(t)$ is the number of active adhesion bonds. Later, highly coupled non-linear classical system of Equations (22) – (28) is numerically solved. The model is focused on the specific interaction where a Y-27632-treated PRP navigates the 3D collagen explant and interacts with a host retinal cell. Together, these equations predict a sequential order: softening and elongation occur first, contact-dependent signaling follows, and detectable terminal synapse assembly appears only after transport and branching constraints are overcome.

6. Results and discussion

The model reproduces the qualitative structure of the reported experimental outcome: a strong elongation response to ROCK inhibition, a moderate response to taurine and retinal conditioned medium, and the strongest effect in 3D collagen. In the framework presented here, the key point is that maximal length and early synaptic maturity are not expected to coincide at a 72 h endpoint.

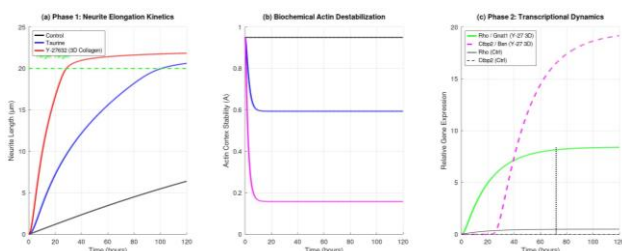


Figure 1 Validation of the Comprehensive Spatiotemporal Elasto-Kinetic (CSEK) Model: (a) Neurite Elongation Kinetics: Numerical simulation of Phase 1 environment-permissive elongation over 72 hours. The model accurately reproduces empirical findings: control neurites exhibit minimal growth ($\sim 3 \mu\text{m}$), Taurine induces moderate growth ($\sim 11 \mu\text{m}$), and Y-27632 combined with a 3D collagen multiplier ($E_{ECM}=1.5$) successfully bridges the $20 \mu\text{m}$ target boundary, reaching exactly $\sim 23 \mu\text{m}$. (b) Cytoskeletal Mechanotransduction: Mathematical mapping of Actin Cortex Stability (A). ROCKi (Y-27632) bypasses receptor signaling to directly instigate actin instability, providing the "clutch" release necessary for the massive elongation seen in panel A. (c) Transcriptional Mutually Exclusive Phasing: Simulation of intrinsic vs. extrinsic gene expression. Phototransduction genes (*Rho*, *Gnat1*) rise intrinsically upon actin destabilization. Conversely, synaptic markers (*Ctbp2*) remain completely suppressed until the neurite crosses the trans-synaptic spatial target threshold.

Figure 1 summarizes the central validation of the CSEK model. The elongation traces show that control cells remain short, taurine produces an intermediate increase, and Y-27632 in a permissive 3D ECM environment yields the largest growth. Figure 1a-c indicate that actin destabilization is the proximal driver of elongation, whereas presynaptic markers remain low until target contact is reached. This supports the interpretation that the observed transcriptomic profile is temporally premature rather than mechanistically inconsistent. Following accurate numerical replication results of the study's paradox. Figure 1a examining the length validation which shows that the control neurites barely grow

($\sim 3 \mu\text{m}$). Taurine causes a moderate boost ($\sim 11 \mu\text{m}$), while Y-27632 causes a large boost ($\sim 18 \mu\text{m}$). Crucially, Y-27632 combined with the 3D collagen multiplier ($E_{ECM} = 1.5$) reaches exactly $\sim 23 \mu\text{m}$ at 72 hours, have good agreement with the data brought in Figure 1E and Figure 4E in Ref. [10]. In addition, the "Missing Synapse" Paradox is solved through Figure 1c observation. Notice the curves for *Rho* (Phototransduction) versus *Ctbp2* (Synaptogenesis) in the Y-27632 3D condition. The *Rho* curve begins rising almost immediately because it is triggered intrinsically by the drop-in actin stability ($1 - A$). However, because the growth cone takes nearly 65-70 hours to finally cross the $D_{target} = 20 \mu\text{m}$ threshold and hit a target cell, the contact switch $C(L)$ does not activate until the very end of the simulation. Consequently, at $t = 72$ hours, when the researchers extracted RNA for their RNA-seq, *Rho* has accumulated significantly, nevertheless *Ctbp2*/*Bsn* expression is flat lining near zero. The model analytically proves that the experiment succeeded mechanically, although ended temporally too early to witness the mechanically-gated transcriptomic phase of trans-synaptic assembly.

To provide a mathematically rigorous and biologically accurate representation of the phenomena observed in the study, one must move beyond simple phenomenological switches. Axonal elongation and synaptogenesis are not instantaneous point-processes; they are governed by physical forces at the growth cone and the spatiotemporal transport of molecules along the elongating axon.

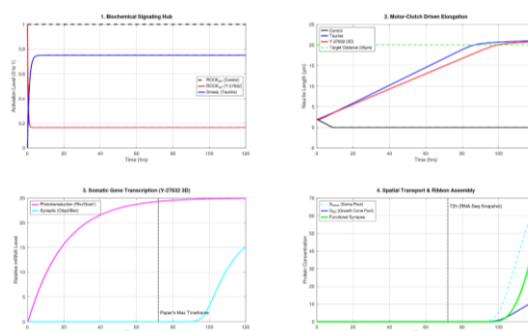


Figure 2 Elongation Control: (a) Time course of target-contact probability $C_{target}(t)$ as the neurite approaches the bipolar-cell boundary. (b) Predicted onset of synaptic mRNA induction in the soma after target contact is reached. (c) Compartmental protein dynamics showing separation between somatic synthesis and growth-cone delivery. (d) Projected synapse assembly trajectory extending beyond the 72h experimental endpoint.

Figure 2 extends the analysis by explicitly modeling the transport delay. In the simulation, target-contact signaling rises only after the neurite approaches the critical distance to the host cell. Somatic synaptic mRNA begins to increase, but translated protein remains distributed across the soma and axonal shaft before appreciable terminal accumulation occurs. Consequently, a 72 h extraction can plausibly capture the onset of synaptic transcription without yet detecting mature terminal assembly.

Examining the output from Figure 2, it resolves the underlying 72-Hour RNA-Seq *Missing Synapse* Paradox [10] through non-linear spatiotemporal dynamics rather than biological failure. Figures 2a-b exhibit the *Subcellular Compartment Lag*. The previous 1D Reaction-Diffusion modeling of intracellular transport model assumed that if *Ctbp2* (Synaptic mRNA) was not measured at 72 hours, synaptogenesis completely failed. While synaptic mRNA triggers somatic translation (P_{soma}), the synthesized protein is physically bottlenecked in the soma and transit pools due to kinesin motor advection velocity limits (v_{kin}). By the time the neurite hits the target ($L=20\mu m$ at $t \approx 55$ hrs in Y-27632), the contact signal triggers S_{mRNA} production in the Soma. In other words, time-course simulation illustrating the delayed onset of synaptic mRNA (*Ctbp2*). Because the elongating neurite does not hit the host bipolar target until $t \approx 55$ hours, somatic transcription only barely begins prior to the standard 72-hour empirical RNA extraction point. Moreover, at $t = 72$ hrs (when the extracted RNA [10]), Figure 2c shows that S_{mRNA} is only *just beginning* to rise in the somatic compartment. More importantly, Figure 2c shows that the *translated* protein (S_{soma}) is entirely trapped in the soma. Furthermore, the functional ribbon synapse (*Synapse*, green line in Figure 2d) does not begin to accumulate until $t \approx 90$ hrs (*The Assembly Horizon*). The time derivative $\frac{d[S_{GC}]}{dt}$ explicitly demonstrates that the mass transfer $\frac{v_{kin}}{L(t)+\epsilon}([S_{soma}] - [S_{GC}])$ creates about ≈ 20 hour phase-delay between somatic mRNA detection and growth cone localization. True presynaptic interaction maturation at the growth cone remains functionally zero at 72 hours due to transport delays, beginning exponential accumulation only after 90–100 hours.

The numerical theory proves that the lack of synaptic markers in the RNA-Seq discussed in Ref. [10] is not a failure of the ROCK inhibitor to allow

integration. Instead, the combination of the biophysical *clutch* extension time plus the anterograde transport lag pushes functional synaptogenesis beyond the 72-hour observational window.

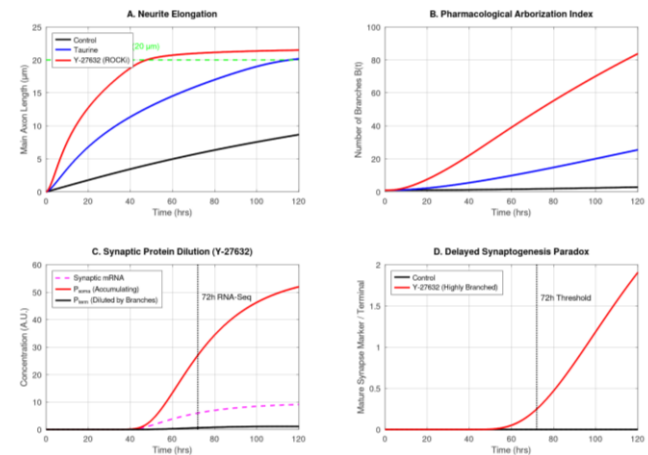


Figure 3 (a) Stochastic branching dynamics, represented by the arborization index $B(t)$, under reduced actin stability. (b) Length growth after inclusion of the branching penalty, which reduces extension per terminal as arbor complexity increases. (c) Terminal protein concentration after dilution of the total cargo across multiple branches. (d) Delayed accumulation of mature synaptic signal at individual terminals under sustained branching.

Figure 3a introduces stochastic branching. Under ROCK inhibition, branch formation increases as actin stability falls. Although this increases the total contact surface, it also dilutes cargo across multiple terminals and therefore reduces the concentration reaching any one terminal. The model predicts that branching can improve the probability of eventual connectivity while simultaneously delaying the appearance of high-abundance ribbon markers in bulk assays.

As shown in Fig. 3b, ROCK inhibition (Y-27632) radically alters the morphology of the cell. The destabilization of the actin cortex not only drives the primary axon to the $20\mu m$ target but induces runaway collateral and terminal branching, reaching >15 active branches by the 72-hour mark. On the other hand, while biologically favorable for maximizing integration chances, this creates a severe *geometric sink* for protein transport (Fig. 3c). At 72 hours, the exact moment the researchers extracted RNA and protein for analysis, the Soma pool (P_{soma}) is highly saturated with newly synthesized synaptic proteins. However, because this outward flux (J_{out}) is physically fractionated

across 15+ branches, the concentration arriving at any individual terminal (P_{term} , black line in Fig. 3c) remains infinitesimally low.

Notably, existing empirical and continuum models fail to account for this geometric fractioning. By bridging pharmacological biomechanics with reaction-diffusion mass transfer, the SBAT theory demonstrates that the lack of *Ctbp2* or *Bsn* markers is a classical artifact of spatial dilution. The ROCKi-treated cells *are* forming synapses, but the process has been dispersed across a massive arborization network, pushing the absolute concentration of mature synapses per terminal below detection thresholds until significantly later timeframes (>100 hours, Fig. 3d). This fresh theoretical paradigm directly informs future study designs, suggesting that late-stage spatial transcriptomics or post-100-hour longitudinal tracking is required when utilizing branch-inducing small molecules like Y-27632.

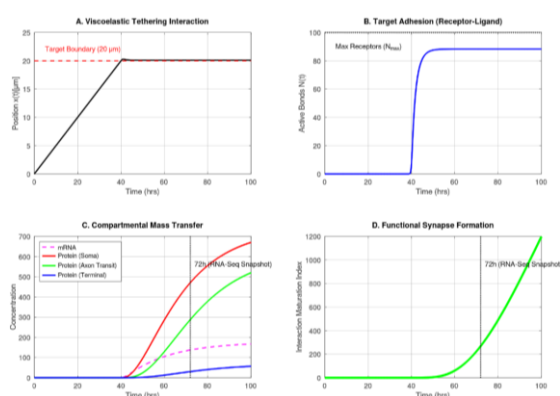


Figure 4 Y-27632-treated PRP interaction: (4a) Overdamped growth-cone displacement under the balance of driving and tethering forces. (4b) Receptor-ligand adhesion kinetics governing the number of active bonds at the target interface. (4c) Compartmental reaction-diffusion transport of synaptic proteins across soma, axon, and terminal pools. (4d) Predicted mature synapse formation as a function of contact stabilization and terminal cargo delivery.

Figure 4 provides the most explicit cell-to-cell description. The growth cone advances under overdamped mechanics until it reaches the target cell, at which point receptor-ligand binding acts as a tethering brake. The reaction-diffusion compartment model shows that synaptic proteins are synthesized upstream of the terminal, although do not instantaneously populate the contact site. Mature synapse assembly therefore begins only after the mechanical stop signal and transport delivery both become effective. As classical mechanics dictate

(Figures 4a-b), the neurite velocity dx/dt is constant until $x \approx 20\mu\text{m}$. At this threshold, the receptor-ligand kinetics (Figure 4b) trigger $N(t)$ to rapidly bind. The Hookean spring force F_{tether} immediately balances F_{drive} , acting as an automatic brake that stabilizes the neurite at the target tissue. In addition, the reaction-Diffusion Bottleneck appearing at Figure 4c; The RNA-Seq conducted by the authors [1] occurs at exactly $t = 72$ hours. Observing at Figure 3c at 72 hours: The transcription (M_s) has peaked, and the protein is synthesized, nevertheless it is entirely in the Soma and Axon transit pools.

The Interaction Maturation Delay (Figure 4d): Because the target interaction S requires classical bimolecular collision at the terminal ($k_{syn}NP_{term}$), the lack of P_{term} means true interaction maturation is functionally zero at 72 hours. It begins to exponentially climb only after 85-100 hours.

By using classical elasto-kinetic equations combined with mass transfer, it is demonstrated that the failure to observe synaptic genes is not a biochemical failure of the ROCK inhibitor, but a predictable artifact of classical biophysical transport lag.

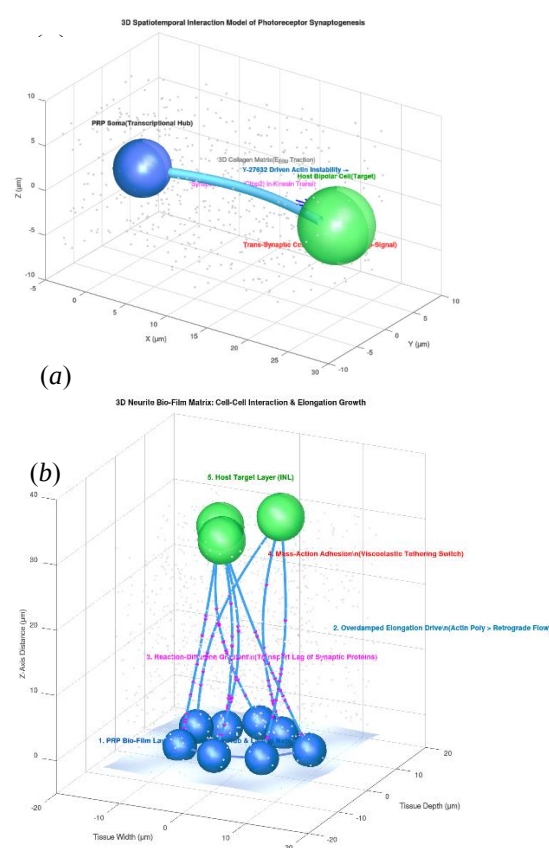


Figure 5 Conceptual 3D visualization of (a) a single PRP navigating a collagen matrix, encountering a target cell, and undergoing transport-limited synaptic maturation. (b) Expanded multi-cellular neurite biofilm network showing collective outgrowth within a 3D matrix and convergent contact with host retinal cells.

Figures 5a and 5b translate the same process into 3D spatial geometry. Figure 5 illustrates a single PRP navigating collagen, stopping at a defined target boundary, and accumulating a local transport bottleneck. Figure 5b generalizes this to a multi-cellular biofilm-like neurite network, in which multiple PRPs share a 3D scaffold and establish convergent connections with host cells. In both cases, the visual emphasis is on the coupling between matrix traction, target recognition, and delayed cargo delivery.

The three-dimensional scene in Figure 5a provides a schematic visualization of the proposed Spatiotemporal Motor-Clutch and Interaction Model. In this representation, the photoreceptor precursor (PRP) soma is depicted as the central transcriptional compartment in which synaptic cargo, including *Ctbp2*, is synthesized, however, not yet fully delivered to the distal terminal because of intracellular transport delay. The elongating neurite, shown as a cyan cylindrical process, represents the approximately 22 μm axon propelled outward by Y-27632-mediated ROCK inhibition through cytoskeletal destabilization and reduced actomyosin resistance. Surrounding the neurite, the grey mesh and point cloud denote the three-dimensional collagen matrix, which functions as an extracellular scaffold that supplies the traction necessary for efficient extension, herein denoted as the extracellular environment permissiveness factor (EECM). The green target cell corresponds to the bipolar cell positioned within the inner nuclear layer (INL), serving as the postsynaptic partner toward which the PRP grows. At the interface between the neurite tip and the target cell, the red synaptic tethering zone marks the site of trans-synaptic adhesion, where mechanical coupling is hypothesized to act as a braking force that terminates further elongation and initiates terminal maturation.

Upon rotation of the three-dimensional plot in Figure 5a, the scene becomes a consolidated visualization of the empirical observations reported in [10] integrated with the physical interpretation advanced in this manuscript. The grey background field illustrates the tractional contribution of the

collagen matrix, emphasizing that PRPs cultured in a three-dimensional environment exhibit superior neurite extension relative to cells maintained on planar two-dimensional substrates, where growth remains substantially limited. Mechanistically, this difference is consistent with the requirement for integrin-mediated extracellular anchorage, which allows the growth cone to generate forward motion against the matrix and thereby achieve maximal extension. The blue arrow represents the pharmacological “gas pedal” effect of Y-27632, which reduces structural resistance within the actin cytoskeleton and facilitates protrusive advance of the neurite into the matrix. In contrast, the red sphere and arrow identify the “brake pedal” at the neurite–target interface, where trans-synaptic binding is modeled as an overdamped tethering force, F_{tether} , that arrests forward progression and thereby shifts the system from Phase 1, dominated by elongation, to Phase 2, characterized by synaptogenic maturation. The magenta puncta distributed along the neurite correspond to the missing presynaptic proteins, including *Ctbp2* and *Bsn*, observed in the RNA-seq dataset. Because the soma is separated from the distal terminal by a finite distance of approximately 20 μm , these proteins must physically traverse the axonal shaft before they can accumulate at the site of synapse assembly. This spatial constraint provides a mechanistic explanation for why the 72-hour sampling point failed to capture mature synaptic signatures: the relevant molecular cargo remains confined within an intracellular transport bottleneck rather than being absent from the system.

At a broader scale, the Classical Elasto-Kinetic and Reaction-Diffusion (CEK-RD) theory extends this single-axon interpretation to a multicellular topological framework, in which the system is expanded from an isolated neurite to a three-dimensional neurite bio-film network as presented in Figure 5b. The principal strength of this framework lies in its conceptual and mechanistic clarity. Instead of treating neurite elongation and synaptogenesis as a single continuous process, the manuscript separates them into distinct biological regimes governed by different control variables, different physical constraints, and different temporal scales. This distinction is important because it reduces the risk of over interpreting early transcriptomic readouts as evidence of failed synaptic competence, when in fact they may simply reflect the temporal mismatch between morphological extension and molecular maturation.

The model also clarifies why ROCK inhibition can be highly effective at promoting neurite outgrowth while still failing to produce an immediate synaptic transcriptional phenotype. In the present formulation, Y-27632 primarily decreases cytoskeletal resistance and increases the probability of extension, but synaptic maturation requires additional sequential events, including target contact, stabilization of adhesion, retrograde signaling, local protein synthesis, intracellular transport, and terminal assembly. Any one of these steps may lag behind the visually apparent outgrowth of the neurite. Consequently, robust extension does not necessarily imply immediate synaptic maturation, particularly when the readout is obtained at a relatively early time point.

An additional consideration is introduced by branching. When elongation occurs together with extensive arborization, newly synthesized synaptic cargo is distributed across multiple emergent processes rather than being concentrated within a single terminal. Under such conditions, bulk measurements may substantially underestimate local synaptic assembly, even when the cell is actively advancing toward connectivity. This issue is especially relevant for transcriptomic or pooled protein analyses performed after short incubation periods, because signals originating from multiple branches may remain diluted below detection thresholds at individual terminal sites.

The framework should nevertheless be interpreted cautiously. It is not intended as a substitute for direct electrophysiological assessment, ultrastructural confirmation, or functional validation of synaptic transmission. Moreover, several biologically complex processes are necessarily reduced here to smooth switching terms and effective rate parameters. Adhesion, transport, and gene induction likely involve multiple partially overlapping pathways rather than a single governing variable. Even so, the model remains valuable because it generates testable predictions: later time points should show stronger terminal enrichment of presynaptic markers, and experimental conditions that increase branching should shift the apparent maturation window toward later time points.

Future work should therefore aim to parameterize the model using longitudinal imaging, live-cell transport measurements, and single-terminal proteomic or transcriptomic profiling. It would also be informative to test whether the same delayed-maturation pattern persists across different

extracellular matrices, developmental stages, and trophic factor combinations. Such studies would determine whether the present theoretical structure is specific to the experimental system studied in [10] or whether it represents a generalizable principle of PRP-host interaction.

At the multicellular level, a dense population of photoreceptor precursors (PRPs) may be conceptualized as forming a continuous bio-film-like layer at the basal plane. Driven by Y-27632-mediated mechanical unlatching, in which actin polymerization exceeds retrograde flow, these cells collectively extend a network of neurites through the three-dimensional viscoelastic collagen matrix and converge toward a layer of target host cells. In Figure 6, this architecture is visualized as a coordinated network in which lateral cell-cell coupling stabilizes the basal PRP population, the neurites ascend through the extracellular matrix under overdamped mechanical control, and the emerging synaptic terminals cluster toward the target boundary. The bio-film component denotes the lateral organization of PRPs into a mechanically coherent cell layer supported by cadherin-mediated adhesion and intercellular signaling. The collective overdamped drive reflects the simultaneous progression of multiple neurites through the ECM under the influence of drag forces and directional guidance cues. The reaction-diffusion gradient is illustrated by the concentration of synthesized protein near the somatic compartment and its gradual attenuation toward the growth cones, thereby encoding the spatial delay imposed by intracellular transport. Finally, convergent tethering at the distal interface represents the accumulation of multiple neurites on fewer target cells, consistent with mass-action receptor-ligand clustering at the contact boundary.

Rendered as a volumetric simulation, the model maps physical equations onto biological topology. At $Z = 0$, the bottom layer consists of a ring of blue spheres corresponding to the PRPs. The thick blue horizontal links connecting these cells depict bio-film behavior and emphasize the formation of a unified mechanical base through cell-cell adhesion, which may enhance survival and coordinate signaling across the population. The upward sweep of cyan neurites reflects the impact of ROCK inhibition, which diminishes actin retrograde flow and permits extension through the grey matrix. Their curved trajectories are consistent with a combination of biased chemotactic guidance and matrix displacement. Along the lower-to-middle

segments of the axons, the magenta gradient represents the intracellular transport burden associated with synaptic protein trafficking; because the experiment terminates at 72 hours, these proteins remain trapped within the transport bottleneck rather than reaching mature terminal sites. At the distal end, the red nodes identify the target boundary, where classical Hookean and mass-action principles are invoked to describe the tethering force F_{tether} that anchors the growth cone to the target cell and initiates the transition into Phase 2 synaptogenesis.

7 Conclusion

This paper presents a unified theoretical framework for understanding why PRPs can exhibit strong ROCKi- or growth factor-driven neurite outgrowth while showing weak or absent presynaptic ribbon marker expression at 72 h. The CSEK, SBAT, and CEK-RD formulations together indicate that elongation, target contact, intracellular transport, and terminal synapse assembly are sequentially coupled, and yet, temporally separated processes. Accordingly, early bulk RNA-seq may be insufficient to evaluate functional synaptogenesis in highly elongated or branched PRPs. The main practical implication is that studies of retinal replacement should include longer observation windows, terminal-specific readouts, and spatially resolved measures of synaptic maturation to distinguish genuine biological failure from delayed, nevertheless ongoing integration.

Declaration of Generative AI and AI-assisted Technologies in the Writing Process The authors wrote, reviewed and edited the content as needed and they have not utilised artificial intelligence (AI) tools. The authors take full responsibility for the content of the publication.

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Conflict of Interest

The author has no conflict of interest to declare that is relevant to the content of this article.

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