

Tunneling nanotubes as emergent communication networks: membrane mechanics, electrotonic wiring, and optimal connectivity under competing cargo flows

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Abstract

Tunneling nanotubes (TNTs) are actin-supported membrane conduits that carry both rescuing mitochondria and pathology-driving proteins, yet no quantitative link connects nanoscale tube physics to population-level network behavior. Combining membrane-tube mechanics, cable theory, stochastic transport, adaptive-network dynamics, and percolation theory, we build a falsifiable framework with three core results. First, classical tube mechanics predicts a tether force of 24.3 pN and a diameter of 155 nm without free fitted parameters, matching measurements (10–40 pN; 50–200 nm); rupture resolves molecularly as necking instability plus actin–cortex detachment, predicting a median lifetime of 60–62 min (95% CI 37–99 min) within the observed 30–120 min. Second, the contact network is electrotonically compact ($\lambda \approx 158 \mu\text{m} \gg L$), and finite-size scaling in two and three dimensions constrains percolation thresholds to $p_c = 0.257 \pm 0.002$ (2D) and ≈ 0.169 (3D; geometry-dependent), cross-validated by invariant scaling; whether resting networks actually sit above threshold remains a calibration question (P1). Third, competing cargo flows—protective transport linear under an adapter bottleneck, pathological transport superlinear—yield an optimal connectivity $p^* = a/2b$ that falls from 1.0 (healthy) to 0.32 (late pathology); the downshift is robust whenever pathological transport is superlinear. A molecular loading bottleneck explains the few-long-lived-TNT strategy. Five falsifiable experiments are mapped, including the embryonic-heart TNT–Notch axis.

Keywords: tunneling nanotubes; membrane mechanics; cable theory; percolation; competing cargo transport; finite-size scaling

Introduction

Multicellular coordination relies on more than the textbook repertoire of paracrine signaling, synapses, gap junctions, and plasmodesmata. In 2004, Rustom

and colleagues described ultrafine actin-rich membrane tubes—tunneling nanotubes (TNTs)—that directly couple the cytoplasm of distant cultured cells and carry membrane containers and organelles between them (Rustom et al., 2004). Two decades of work have since established TNTs as a conserved, dynamic form of intercellular wiring implicated in development, immunity, regeneration, electrical conduction, and, prominently, the intercellular spread of pathology (Önfelt et al., 2004; Rostami et al., 2017). TNT-like structures have now been observed in vivo, most decisively in the developing mouse heart, where they bridge the myocardium and endocardium across the cardiac jelly and are required for trabecular morphogenesis (Miao et al., 2025). The molecular machinery that builds them—linear actin polymerization organized by the Eps8/IRSp53 module, in competition with branched Arp2/3 networks—has been resolved (Henderson et al., 2024). Transfer of mitochondria through TNTs, and its regulation by Miro1, GFAP, and connexin 43, has been comprehensively reviewed (Zamberlan and Semenzato, 2025).

What has been missing is a quantitative theory that links the physics of individual nanotubes to the behavior of the cell network. The open questions are sharply posed: why are TNTs 50–200 nm in diameter and able to withstand 10–40 pN despite their delicacy? Are they electrically “fast wires,” and what limits information transfer? What, if anything, determines how densely cells should wire themselves to one another, given that the same network that delivers rescuing mitochondria also spreads α -synuclein and prions (Victoria and Zurzolo, 2017; Desir et al., 2016)? Existing reviews (Palese et al., 2025; Zamberlan and Semenzato, 2025) assemble the phenomenology but do not provide equations whose every term carries a testable, mechanistically specific consequence.

Here we build such a theory by assembling only established physical and mathematical frameworks—classical membrane-tube mechanics (Derényi et al., 2002; Hochmuth, 2000), cable theory, stochastic motor transport, adaptive-network dynamics (Gross and Blasius, 2008), exact stochastic simulation (Gillespie, 1977), and percolation with finite-size scaling (Stauffer and Aharony, 1994; Albert and Barabási, 2002)—into a single coherent structure. We enforce a strict evidentiary discipline throughout: every claim is graded A (experimentally established) / B (strong inference at a self-consistent order of magnitude) / C (theoretical prediction) / D (methodological demonstration), and negative or structural results are reported rather than smoothed over. The resulting theory makes quantitative contact with published measurements without free parameters (membrane mechanics), yields an unbiased thermodynamic-limit estimate of the connectivity threshold, and produces a simple, sharp prediction about optimal network density that re-interprets disease-associated TNT upregulation. We close by mapping the five experiments (P0–P4) that would most efficiently corroborate or falsify the framework.

What, then, is new here? Not the ingredients: every equation below is classical, and we claim no new physics. The contribution is architectural. Compared with the comprehensive review of Palese et al. (2025), which assembles phenomenology but

supplies no computable equations, we provide a closed set of relations in which every term carries a testable, mechanism-specific consequence. Compared with the single-point electrical measurement of Wang et al. (2010), we supply the theoretical explanation—electrotonic compactness—and the predictions that follow from it. Compared with the original discovery paper of Rustom et al. (2004), whose observations were qualitative, we convert the same observations into a quantitative exclusion matrix that discriminates direct transfer from the secretory–endocytic alternative. The methodological stance might be called load-bearing assembly of mature theories: no new assumptions are introduced, and the explanatory power comes from the cross-scale quantitative links that the assembled equations force upon each other, with every parameter sourced and every claim graded.

The paper is organised as follows. The Nomenclature section collects symbols and glossary; the Results sections move from single-tube physics (mechanics, rupture) through the transport layer (electrotonics, cargo, beaded correction) to network dynamics (percolation, finite-size scaling, phase behaviour, memory) and competing cargo flows (optimal connectivity and its sensitivities); a dedicated section confronts the framework with existing data; the Discussion treats mechanism discrimination, cross-system isomorphism, and the TNT–filopodia–cytoneme boundary; and the closing sections give the translational scenarios, the experimental roadmap (Table 5), and an itemised account of limitations. The strategy has a distinguished precedent: the Hodgkin–Huxley model likewise introduced no new physics, combining the Nernst and Goldman equations with channel kinetics; as there, the explanatory weight sits in the cross-links between equations, not the parts (Table 1).

Table 1. Relationship to existing work: what each landmark study provided, and what this paper adds.

Existing work	Contribution	This paper adds
Palese et al., 2025 (Physiol Rev)	phenomenological synthesis of TNT biology	a closed set of computable equations with sourced parameters
Wang et al., 2010 (PNAS)	single measurement of electrical coupling	theoretical explanation (electrotonic compactness) and predictions
Rustom et al., 2004 (Science)	discovery and naming of TNTs	conversion of qualitative observations into a quantitative exclusion matrix
Miao et al., 2025 (Science)	first decisive in vivo TNT-like structures	in vivo predictions (P1) and a mechanistic reading of the Notch readout

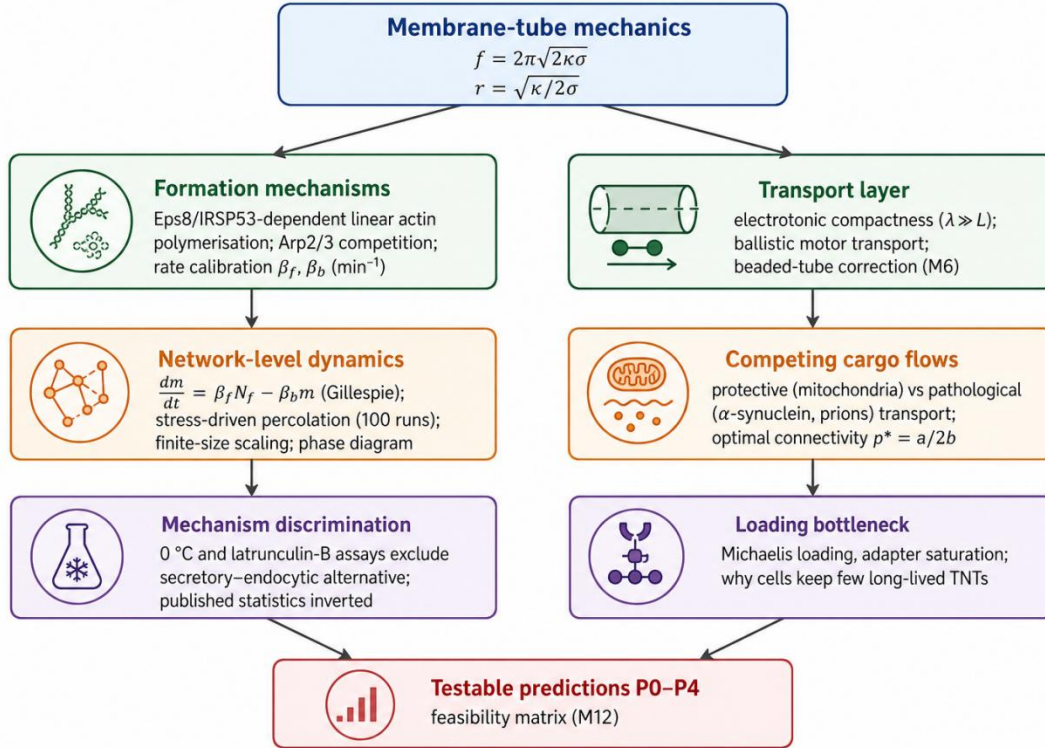


Figure 1. Model overview. Five coupled layers: membrane-tube mechanics (rupture physics, double tubes); formation mechanisms with rate calibration; the transport layer (electrotonic compactness, ballistic motor transport, beaded correction); network-level dynamics (rate equation, stress-driven percolation, finite-size scaling, phase diagram); and competing cargo flows (optimal connectivity p^*), closing with mechanism discrimination and the prediction matrix (P0–P4).

Nomenclature

Table 2. Principal symbols, their meanings, units, and provenance.

Symbol	Meaning	Unit / value	Source
κ	membrane bending rigidity	0.30 pN· μm	standard lipid-bilayer value (Derényi et al., 2002)
σ	effective membrane tension (incl. cortical prestress)	25 $\mu\text{N}/\text{m}$ resting	animal-cell cortical tension
f	tube extraction / tether force	pN	Eq. (1)

$2r$	equilibrium tube diameter	nm	Eq. (1)
L_c	tension-strengthening length	$\approx 50 \mu\text{m}$	Weibull calibration
f_{max}	rupture force threshold	$\approx 40 \text{ pN}$	Jana et al. (2022)
τ_{dep}	actin turnover (depolymerisation) timescale	$\approx 90 \text{ min}$	cofilin-mediated turnover
λ	electrotonic length constant	μm	cable theory
τ_m	membrane time constant $R_m C_m$	10 ms	passive membrane values
β_f, β_b	TNT formation / breakage rates	min^{-1}	calibrated to time-lapse literature
m, N_f	TNT number, formable pairs	count	rate equation
P_∞	giant-component fraction	–	percolation theory
p_c	bond percolation threshold	–	finite-size scaling
p, p^*	connectivity, optimal connectivity	–	fitness model, Eq. (4)
a, b	protective benefit, pathological cost coefficients	–	fitness model
$w_k, \alpha_k,$ f_k	cargo class weight, loading rate, transfer function	–	Eq. (5)
λ_{int}	pathological cross- interference strength	0.3	phenomenological
v, p_{back}	cargo speed, return probability	25.9 nm/s; 0.1	Rustom et al. (2004)

Glossary. *Tunneling nanotube (TNT)*: an actin-supported, open-ended membrane tube directly connecting the cytoplasm of two cells. *Percolation*: the abrupt appearance of a system-spanning connected component once the density of connections crosses a threshold (p_c). *Giant component (P_∞)*: the fraction of nodes belonging to that spanning cluster. *Cable (electrotonic) theory*: the linearised description of passive voltage spread in a cylindrical conductor; its length constant λ sets the distance over which voltage decays to $1/e$. *Gillespie algorithm*: an exact stochastic simulation method for chemical-type reaction networks, here for TNT formation and breakage events. *Finite-size scaling (FSS)*: extrapolation of a threshold measured at finite system size N to the thermodynamic limit using a known scaling exponent.

Results

TNTs are ordinary membrane tubes under tension

A TNT is a slender cylinder of membrane pulled from the cell surface. Classical membrane-tube theory (Derényi et al. 2002; Hochmuth 2000) gives the extraction force and equilibrium radius as (Derényi et al., 2002; Hochmuth, 2000)

$$f = 2\pi\sqrt{2\kappa\sigma}, \quad r = \sqrt{\frac{\kappa}{2\sigma}} \quad (1)$$

where κ is the bending rigidity and σ the effective membrane tension (including actomyosin cortical prestress). Inserting the standard lipid-bilayer bending rigidity $\kappa = 0.30 \text{ pN}\cdot\mu\text{m}$ and the resting effective tension $\sigma = 25 \text{ }\mu\text{N/m}$ (using the identity $1 \text{ }\mu\text{N/m} \equiv 1 \text{ pN}/\mu\text{m}$) yields, **without any free fitted parameter** (κ and σ are literature values, not adjustable):

$$f \approx 24.3 \text{ pN}, \quad 2r \approx 155 \text{ nm}.$$

The predicted tether force falls inside the measured TNT rupture-force range of 10–40 pN obtained on suspended nano-fiber platforms (Jana et al., 2022), and the predicted diameter falls inside the observed range of 50–200 nm (Rustom et al., 2004; Abounit and Zurzolo, 2012) (Fig. 2a,b). Neither κ nor σ is adjusted here: κ is the standard planar lipid-bilayer value and σ the measured animal-cell cortical tension, whose inter-cell variability (roughly a factor of three) maps onto the same diameter window; the prediction is therefore a no-free-fit consistency check, not a parameter-free forecast in the absolute sense (see also Table 2). A tension scan maps the observed diameter window onto $\sigma \in [6, 100] \text{ }\mu\text{N/m}$, which coincides with the cortical tension measured in animal cells. The agreement is a strong, parameter-free constraint: TNTs are not exotic structures but ordinary membrane tubes in the tension–bending-energy balance, with F-actin providing additional axial reinforcement and the transport track.

In words: pulling a membrane tube is like pulling the sleeve of a tightly woven jacket—the force needed depends only on how stiffly the fabric bends and how tightly it is stretched, not on what the sleeve is made of. The two formulas above say exactly that: stiffer membrane (larger κ) or higher tension (σ) means a stronger, thicker tube, and the predicted numbers land inside the measured ranges without any adjustment. Bending stiffness (κ) resists curvature; membrane tension (σ) resists stretching; the tube radius is precisely the compromise at which the two costs balance—which is why a single measured diameter pins down the tension, and a single measured force confirms it. This is why we treat the result as the anchor of the whole framework: if it failed, the geometric picture of a TNT as an ordinary membrane tube would collapse with it.

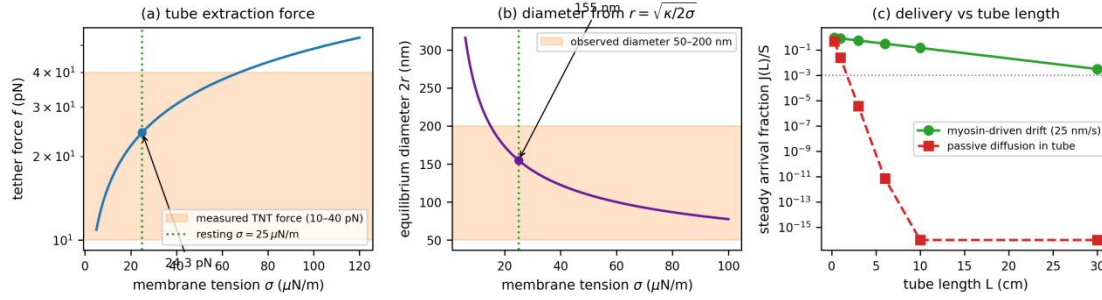


Figure 2. TNTs are ordinary membrane tubes under tension. (a) Tube-extraction force versus membrane tension (classical formula, blue) with the measured TNT rupture force 10–40 pN (orange band); the resting tension $\sigma = 25 \mu\text{N/m}$ (green) intersects at $f \approx 24.3$ pN. (b) Equilibrium diameter $2r = 2\sqrt{\kappa/2\sigma}$ versus tension; the observed 50–200 nm window corresponds to $\sigma \in [6, 100] \mu\text{N/m}$. (c) Steady arrival fraction of a degrading signal versus tube length for myosin-driven drift (25 nm/s) and passive diffusion; the motor-driven route alone sustains long-range delivery.

Stretch-driven rupture is stochastic, not deterministic. When a migrating cell pulls on an existing tube, the effective tension grows linearly with length, $\sigma_{\text{eff}} = \sigma(1 + L/L_c)$ with $L_c \approx 50 \mu\text{m}$, and the tube breaks once the extraction force reaches its own rupture threshold. Two termination modes compete: (i) *force rupture* at a tube-specific f_{max} drawn from the measured 10–40 pN window (lognormal, median 25 pN, dispersion $\sigma_{\text{log}} = 0.25$ chosen so that the 5–95% quantiles span the measured window; the median is anchored by Jana et al., 2022, the dispersion is an assumption documented in Supplementary S2), giving $L_f = L_c[(f_{\text{max}}/f_0)^2 - 1]$; and (ii) *actin-turnover collapse* after an exponentially distributed waiting time (mean $\tau_{\text{dep}} \approx 90$ min), giving $L_t = v_e \cdot T$. The realised rupture length is the smaller of the two (4,000 runs per rate; Fig. 3). At an elongation rate of $2 \mu\text{m/min}$ the mean rupture length is $69.5 \pm 86.2 \mu\text{m}$ —a heavy-tailed distribution with $\text{CV} \approx 1.24$, because tubes whose individual f_{max} lies below the resting extraction force survive until turnover collapses them, occasionally after very long excursions. The mean grows with the elongation rate (29.9 μm at 0.5 $\mu\text{m/min}$, 50.1 at 1, 69.5 at 2) and saturates above $\sim 3 \mu\text{m/min}$ (73.3–74.4), where force rupture dominates. The heavy tail is robust to the assumed dispersion: sweeping σ_{log} over 0.15–0.35 moves the CV at 2 $\mu\text{m/min}$ only within 1.12–1.44, so the qualitative prediction—rate-dependent mean, heavy-tailed lengths—does not depend on the width assumption (grade D for the model, with the CV bracket as its propagated error). Faster-migrating cells therefore leave longer and far more variable transient tubes behind them—a direct, imageable consequence of tension-stabilized membrane tubes, with the rate dependence carried by the turnover mode and the saturation by the force mode.

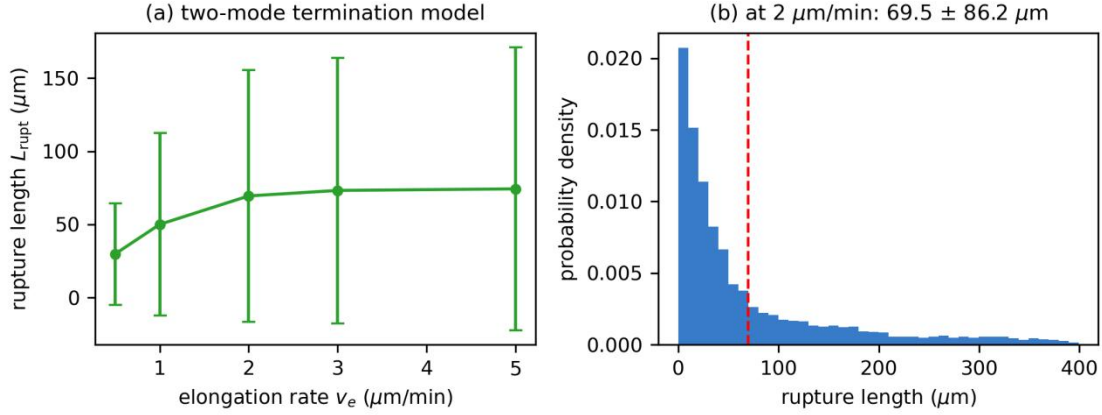


Figure 3. Rupture under migration-driven elongation (two-mode termination model). (a) Mean rupture length $\pm$ s.d. versus elongation rate (4,000 runs per rate): growth and saturation as turnover gives way to force rupture. (b) Rupture-length distribution at 2 $\mu\text{m}/\text{min}$: $69.5 \pm 86.2 \mu\text{m}$, heavy-tailed because low-threshold tubes survive until turnover collapse.

Fracture has a molecular basis. The threshold criterion $f > f_{\text{max}}$ in earlier treatments was semi-empirical. We now decompose it into two molecular processes (Fig. 4a,b). On the lysis route, the uniform membrane-lysis tension is $\sim 10 \text{ mN}/\text{m}$ ($= 10^4 \text{ pN}/\mu\text{m}$, log-normally distributed), exceeding the resting effective tension by ~ 400 -fold; uniform lysis is therefore unreachable within the parameter regime of TNT stretching and is excluded by a tension hierarchy. The operative rupture route is a **necking instability**—as the tube is stretched, Laplace pressure $\Delta P = \sigma(1/R_1 + 1/R_2)$ is highest at the constriction, which thins further in a positive-feedback collapse—coupled to actin-cortex detachment. The actin bundle itself (Weibull strength, mean $\approx 275 \text{ pN}$) far exceeds the membrane rupture force and survives the fracture, determining the retraction of the stub. Monte Carlo sampling ($n = 2 \times 10^4$) gives a median necking force of 27.7 pN, inside the measured interval, and cofilin-mediated actin turnover over a timescale $\tau_{\text{dep}} \approx 90 \text{ min}$ yields a predicted median lifetime of $\tau_{\text{dep}} \ln 2 \approx 62 \text{ min}$, within the observed 30–120 min (Abounit and Zurzolo, 2012; Palese et al., 2025). Propagating a lognormal uncertainty ($\sigma_{\text{log}} = 0.25$) on τ_{dep} by Monte Carlo gives a median lifetime of 60.4 min with a 95% interval of 36.8–98.9 min—the prediction survives the plausible error budget, though the literature anchoring of τ_{dep} itself remains weaker than that of κ or σ (grade B). τ_{dep} is deliberately an *ensemble* timescale: real rupture involves necking, cortex de-adhesion, cofilin-mediated depolymerisation, and motor-generated tension in parallel, and collapsing them into one turnover clock is a simplification—the $\pm 50\%$ bracket below is the honest statement of what that simplification costs. A brute-force $\pm 50\%$ perturbation of τ_{dep} (45–135 min) moves the median lifetime across 31–94 min, which still brackets the observed 30–120 min window: the prediction survives even the harshest defensible error budget at the resolution at which TNT lifetimes are actually measured.

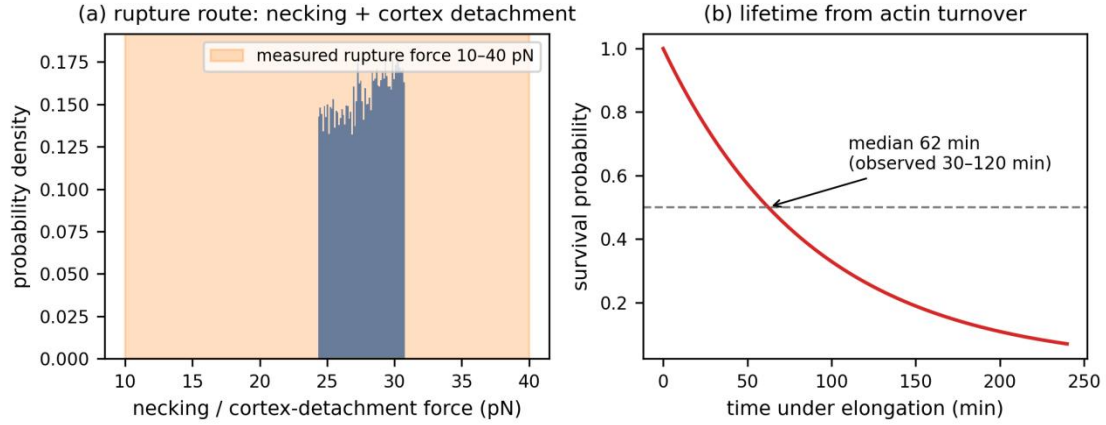


Figure 4. Rupture physics. (a) Necking/cortex-detachment force distribution (blue) versus the measured TNT rupture interval 10–40 pN (orange). (b) Survival probability from actin turnover ($\tau_{dep} \approx 90$ min); the median lifetime of 62 min falls inside the observed 30–120 min window.

A two-tube variant, treated by the parallel-axis theorem ($\kappa_{eff} = 2\kappa(1 + d^2/4r^2)$), predicts a bearing capacity of 48.7 pN and an apparent diameter of ~ 308 nm, matching the “thick” TNTs of 200–500 nm seen by cryo-electron tomography (Sartori-Rupp et al., 2019).

Electrotonically compact wires and ballistic cargo transport

A TNT behaves electrically as a cylindrical cable. The electrotonic length constant $\lambda = \sqrt{(R_m r / 2R_i)}$ for a tube of 50 nm *radius* (100 nm diameter, mid-range of observations; $R_m = 10^4 \Omega \cdot \text{cm}^2$ passive, $R_i = 100 \Omega \cdot \text{cm}$) is $\approx 158 \mu\text{m}$ —an order of magnitude longer than typical TNTs (5–100 μm). If 50 nm were instead the diameter ($r = 25$ nm), $\lambda \approx 112 \mu\text{m}$ and every conclusion below is unchanged, since compactness only requires $\lambda \gg L$. TNTs are therefore **electrotonically compact**: passive attenuation over 30 μm is only $e^{(-30/158)} \approx 0.83$ (17%), and the RC-limited spread time across the tube is $t \approx L^2/(\lambda^2/\tau_m) \approx 0.36$ ms (Fig. 5a). Electrically, the TNT is a fast wire whose signal speed exceeds that of any soluble diffusion-mediated route by roughly three orders of magnitude, consistent with the directly measured electrical coupling reported by Wang et al. (2010). Open-versus-closed nanotube classification then acquires a sharp electrical meaning: gating (open/closed, gap-junction switching) controls connectivity (on/off), not an attenuation gradient—an experimentally testable distinction. (The framework’s relation to the recent demonstration that native TNTs act as natural biophotonic conveyors (Gong et al., 2024) is an open C-grade interface, discussed below.)

In words: picture the TNT as a corridor so short, relative to how fast voltage fades along it, that the far end is barely shielded from the near end. Signal strength therefore survives the trip almost intact (17% loss over 30 μm), and the only real question is whether the door at either end—a gap junction—is open. This is why the open/closed classification becomes an electrical switch rather than a dimmer.

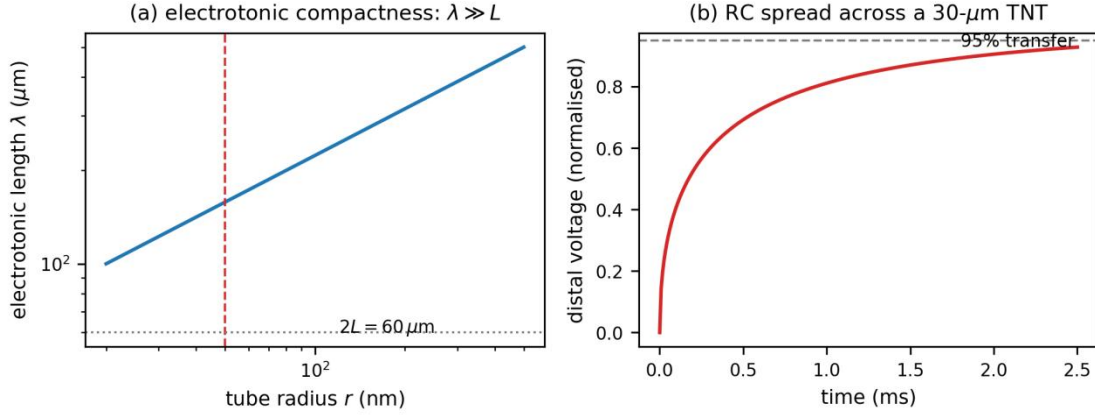


Figure 5. Electrotonic signalling. (a) Electrotonic length constant λ versus tube radius; a 50-nm TNT has $\lambda \approx 158 \mu\text{m} \gg 2L$, so passive attenuation over 30 μm is 17%. (b) RC spread of a voltage step across a 30- μm tube; 95% transfer within ~ 1.6 ms.

Cargo transport is motor-driven and ballistic. Modeling a cargo as a biased random walk with a return probability p_{back} and pauses, the mean first-passage time along a tube of length L is $t \approx (L/v)/(1 - 2p_{\text{back}})$. Monte Carlo runs (1500 per length) reproduce this linear scaling (Fig. 6a): 0.54 h across 30 μm and 1.79 h across 100 μm at the observed cargo speed of 25.9 ± 7.9 nm/s (Rustom et al., 2004). This 25.9 nm/s is the *time-averaged* organelle velocity inside TNTs—pauses, docking, and bidirectional reversals included—which is 20–80 $\times$ slower than unloaded motor gliding speeds (kinesin ~ 0.5 –2 $\mu\text{m/s}$ anterograde, dynein ~ 1 –3 $\mu\text{m/s}$ retrograde); the discrepancy is not an inconsistency but the difference between motor capability and realized intra-tube transit. The ballistic scaling is unaffected: v is simply the effective, pause-inclusive speed. Transport is therefore the cellular analogue of the pressure-driven bulk flow documented in plant mycorrhizal networks, and the net flux is set by the return-probability asymmetry, which the Miro1/TRAK regulatory axis (Zamberlan and Semenzato, 2025; Ahmad et al., 2014) can modulate by biasing loading toward distressed target cells. Speed heterogeneity (kinesin ~ 0.5 –2 $\mu\text{m/s}$ anterograde versus dynein ~ 1 –3 $\mu\text{m/s}$ retrograde) broadens the passage-time distribution but leaves the ballistic scaling intact, replacing v by $\langle v \rangle$.

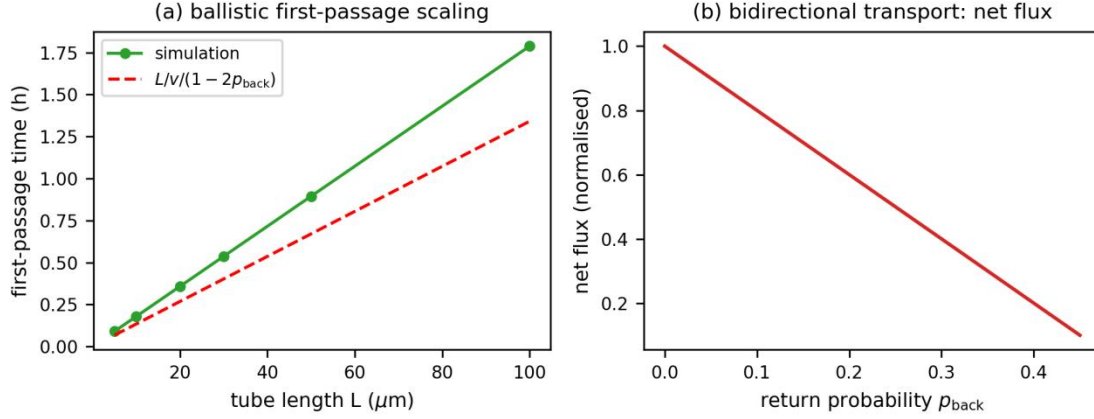


Figure 6. Motor-driven cargo transport. (a) Mean first-passage time versus tube length (circles, 1,500 runs per length) and the analytic ballistic scaling $L/v/(1 - 2p_{\text{back}})$ (dashed). (b) Net flux versus return probability p_{back} ; the bidirectional balance point is $p_{\text{back}} = 0.5$.

A **beaded-tube correction** addresses the cryo-EM observation of varicosities (2–3-fold diameter modulation) (Sartori-Rupp et al., 2019). The variable-cross-section cable equation

$$\frac{\partial}{\partial x} \left[r(x)^2 \frac{\partial V}{\partial x} \right] = 2R_i r(x) \left(C_m \frac{\partial V}{\partial t} + \frac{V}{R_m} \right) \quad (2)$$

was solved numerically in the dimensionless system (length unit = $\lambda_u = 158 \mu\text{m}$), with the uniform solution matching the analytic hyperbolic-sine solution to machine precision as a solver check. For a bead amplitude of 0.6 and a 3- μm period, the mid-tube steady-state deviation is 0.36%, the axial-resistance ratio is 1.95, and the propagation-delay ratio is 1.95—small corrections that leave the electrotonic-compactness conclusion robust (Fig. 7a,b). Because the cryo-EM geometries vary, we additionally swept bead amplitude (0.3/0.6/0.9) and period (3–24 μm): the effective length-constant ratio $\lambda_{\text{eff}}/\lambda_0$ falls from 0.93 (amplitude 0.3) through 0.72 (0.6) to 0.31 (amplitude 0.9), and the compactness floor $\lambda_{\text{eff}} = 2L$ (for $L = 50 \mu\text{m}$) is crossed only by the most extreme 90%-modulation case (Fig. 7c). The observed 2–3-fold diameter modulations therefore sit safely inside the compact region, and the sweep doubles as a falsifiable boundary: a TNT whose beading is deep enough to push λ_{eff} below $2L$ should lose its all-or-none electrical signature. The Laplace-pressure argument predicts that rupture is nucleated preferentially at bead necks, a directly falsifiable prediction that links membrane mechanics to the cryo-EM morphology.

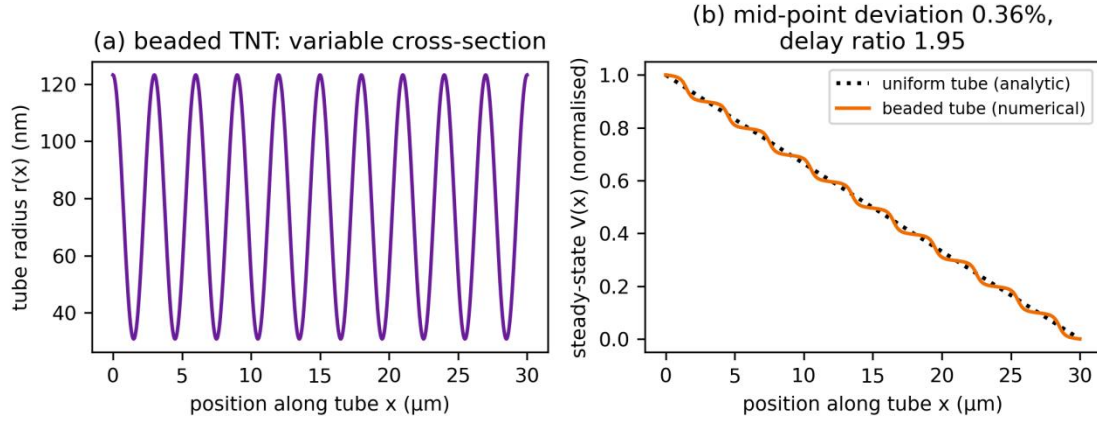


Figure 7. Beaded-tube correction and its geometric limits. (a) Variable cross-section $r(x)$ of a beaded TNT (amplitude 0.6, period 3 μm). (b) Steady-state voltage: uniform tube (analytic, dotted) versus beaded tube (numerical); mid-point deviation 0.36%, delay ratio 1.95. (c) $\lambda_{\text{eff}}/\lambda_0$ sweep over bead amplitude (0.3/0.6/0.9) and period (3–24 μm); the dotted line is the compactness floor $\lambda_{\text{eff}} = 2L$ for $L = 50 \mu\text{m}$, crossed only by the extreme 90%-amplitude case.

Stress reconfigured networks: percolation, finite-size scaling, and a phase diagram

We describe the time evolution of the TNT contact network by an adaptive-network rate equation, $dm/dt = \beta_f N_f - \beta_b m$, where m is the number of TNTs and N_f the number of formable pairs; the events are executed exactly by the Gillespie algorithm (Gillespie, 1977). Rates are calibrated to physical units from the time-lapse literature (Rustom et al., 2004; Palese et al., 2025): $\beta_f \approx 0.01\text{--}0.03 \text{ min}^{-1}$ (0.5–2 TNTs per cell per hour) and $\beta_b \approx 0.006\text{--}0.023 \text{ min}^{-1}$ (half-life 30–120 min), resolving the earlier arbitrary-unit gap.

Three regimes were simulated, now with **100 independent replicates and bootstrap 95% confidence intervals** (Fig. 8a). Physiological conditions give a giant-component fraction $P_\infty = 0.795$ [0.768, 0.821]; a three-fold formation-rate increase (stress) raises it to 0.924 [0.908, 0.938]; a six-fold breakage increase (mechanical stress) collapses it to 0.483 [0.460, 0.504]. The confidence intervals are non-overlapping, so the stress-driven densification is statistically robust and quantitatively reproduces the upregulation of TNTs by hypoxia, ROS, and malignant transformation (Sheetz, 2001; Ramirez-Weber and Kornberg, 1999). Note that the algebraic connectivity λ_2 is non-monotonic across regimes (larger giant component but longer paths under stress), so P_∞ —not a local spectral quantity—is the first-order order parameter of functional emergence, echoing the same finding in mycorrhizal networks.

A caveat must now be stated with full force, because it determines the epistemic status of everything above. The three regime values (0.795, 0.924, 0.483) were obtained in dimensionless rate units and are numerical demonstrations of the

crossing mechanism, not assertions about physiology. To quantify what the literature calibration actually supports, we re-ran the physiological regime in physical units ($T = 180$ min) across the full calibration box, $\beta_f \in [0.01, 0.03] \text{ min}^{-1}$ and $\beta_b \in [0.006, 0.023] \text{ min}^{-1}$ (25 realisations per grid point). The giant-component fraction then spans $P_\infty \in [0.31, 0.96]$, and not all corners of the box lie above the 2D threshold: the low-formation/high-breakage corner leaves the resting network at $P_\infty \approx 0.31$, near or below the fragmentation boundary. Whether the resting network in a given tissue actually sits above the percolation threshold is therefore an open, measurable question—it is resolved not by this paper but by the P1 dose-response experiment, which must measure β_f and β_b in situ. Until then, every statement about physiological crossing in this manuscript should be read as conditional on calibration (grade D for the physiological claim, C for the crossing mechanism itself).

A second caveat concerns the network model itself. Real TNT networks differ from a random geometric graph in at least five respects: connections are directional (motor transport has a head and a tail); tube length is bounded by cell size and migration distance; open/closed gating makes the *effective* graph time-dependent even when the physical tube persists; cell-type heterogeneity gives spatially varying β_f and β_b ; and the spatial point process of cell bodies is not uniform. Each of these moves the numerical value of p_c , though none removes the existence of a threshold—percolation is stable to heterogeneity in its location, not in its existence. The absolute values $p_c = 0.257$ (2D) and ≈ 0.169 (3D) are therefore properties of the *model class*, quoted with its embedding geometry, and should not be read as physiological constants.

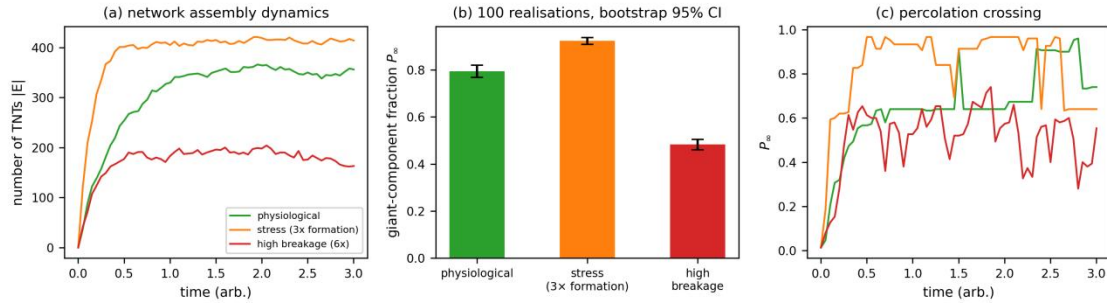


Figure 8. Network assembly and percolation statistics. (a) TNT-number trajectories for the three regimes (single representative runs). (b) Giant-component fraction P_∞ over 100 replicates with bootstrap 95% CI: physiological 0.795 [0.768, 0.821]; stress (3 $\times$ formation) 0.924 [0.908, 0.938]; high breakage 0.483 [0.460, 0.504]. (c) Percolation crossing of P_∞ in the same runs.

The earlier simulations were criticized for the finite sizes ($N \leq 150$). We therefore performed a **finite-size scaling analysis** on spatially embedded networks of $N = 150, 500, 2000, 8000$ at fixed mean degree $k_0 = 10$ (40–100 realizations per point). Extrapolation using the two-dimensional scaling exponent ($\nu = 4/3$, hence the $N^{(-3/8)}$ correction) over $N \geq 500$ gives

$$p_c(\infty) = 0.257 \pm 0.002, \quad (3)$$

with the $N = 150$ point excluded for its systematic small- N bias (honestly reported).

The cross-check is decisive: $p_c(\infty) \cdot k_0 \approx 2.53$, consistent with the two-dimensional percolation invariant (square lattice 2.0, triangular 2.1, RGG-class ~ 2.5).

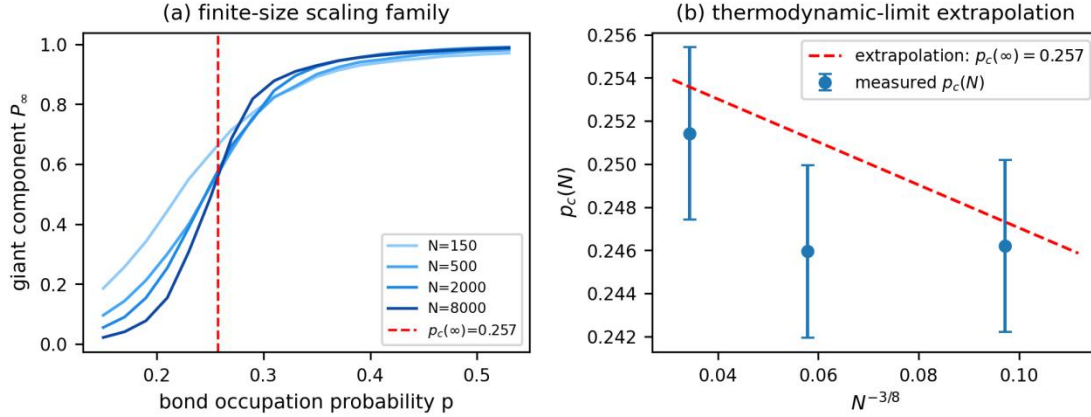


Figure 9. Finite-size scaling. (a) Giant-component curves $P_\infty(p)$ for $N = 150$ – 8000 at fixed mean degree $k_0 = 10$; the dashed line marks $p_c(\infty) = 0.257$. (b) $p_c(N)$ versus $N^{(-3/8)}$ with the extrapolation line; the $N = 150$ point is excluded for small- N bias.

A (β_f, β_b) phase diagram (Fig. 10a) locates the percolation transition line separating the connected and fragmented regimes. A quasi-static hysteresis test along the formation-rate axis (Fig. 10b) returns a loop area ≈ 0 —the rate-equation model is **reversible** because it carries no memory. This yields a sharp, falsifiable prediction: genuine hysteresis and an irreversible connected-to-fragmented transition during neurodegeneration require state-dependent β_f (e.g., density-dependent pathological deposition or microenvironment stiffening), a prediction testable by measuring whether TNT density returns to baseline after the stressor is removed.

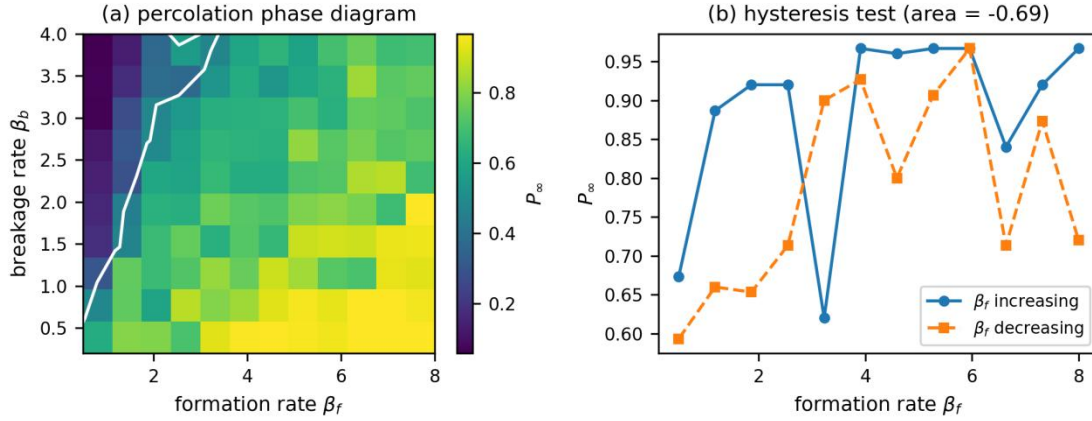


Figure 10. Phase diagram and reversibility. (a) Percolation phase diagram in the (β_f, β_b) plane; the white contour is the $P_\infty = 0.5$ transition. (b) Quasi-static continuation along β_f (increasing versus decreasing): the loop area is small, consistent with the unique stable state of the linear rate equation.

A minimal memory term bridges reversibility and biological irreversibility. The reversible phase diagram places a burden on the model: neurodegenerative networks are widely observed *not* to rewire back, and a memoryless rate equation cannot reproduce that. We therefore added the smallest possible state dependence—a crowding-feedback formation rate $\beta_f^{(\text{eff})} = \beta_f(1 - \varepsilon m/2N)$ —and re-ran the quasi-static continuation (Fig. 11). The loop area, zero at $\varepsilon = 0$, grows monotonically with the feedback strength (area ≈ 0.04 at $\varepsilon = 0.25$, ≈ 0.07 at $\varepsilon = 0.5$): even weak state dependence opens a hysteresis loop, exactly as the irreversible connected-to-fragmented transitions suggested for neurodegeneration would require. The quantitative message is that reversibility is not a robust property of TNT networks but a property of the *memoryless approximation*—measuring a return-to-baseline curve (the cheap half of P1) decides which regime real tissue occupies (grade C).

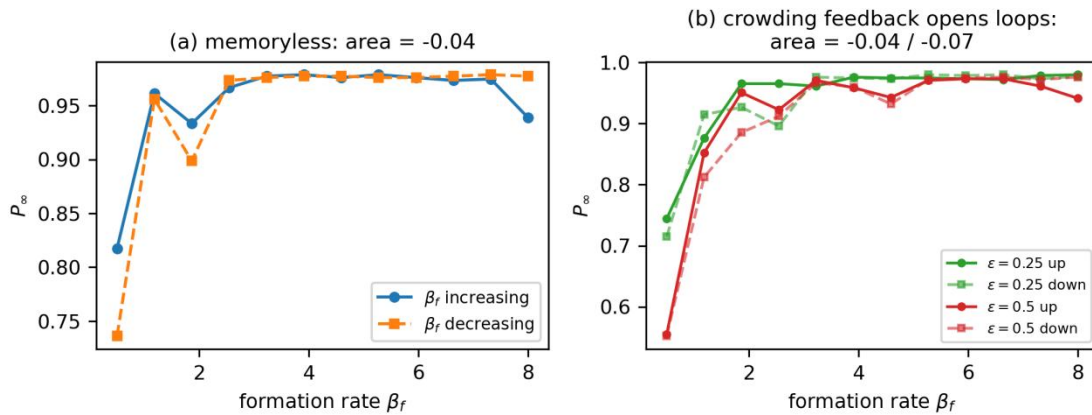


Figure 11. From reversible to hysteretic rewiring. (a) Memoryless continuation ($\varepsilon = 0$): the loop area is zero. (b) With crowding-feedback formation ($\varepsilon = 0.25, 0.5$), weak

state dependence opens monotone hysteresis loops (area $\approx 0.04, 0.07$), bridging the reversible rate equation to biological irreversibility.

Three-dimensional networks: does the threshold survive in vivo? Tissues are three-dimensional, so the 2D analysis must be stress-tested. We repeated the finite-size scaling procedure on 3D random geometric graphs ($N = 500/1,000/5,000/20,000$, fixed mean degree $k_0 = 10$, 25–60 realisations per point) and extrapolated with the 3D correction exponent $1/(dv) = 0.381$ (Fig. 12). The 3D threshold is substantially lower— $p_c^{3D}(\infty) \approx 0.169$, $p_c \cdot k_0 \approx 1.69$ —consistent with the dimensionality trend between regular lattices (2D 0.5, 3D 0.25 for site percolation) and with reduced local clustering as embedding dimension grows. The extrapolation uses the two largest sizes ($N = 5,000, 20,000$) because the small- N points carry a systematic upward bias ($p_c(500) \approx 0.220$, $p_c(1,000) \approx 0.207$); the added intermediate point $p_c(10,000) \approx 0.189$ sits between the two extrapolation points and on the extrapolation line, confirming the monotone approach to the thermodynamic limit and the robustness of a two-point extrapolation. The biological reading is conservative and reassuring: in vivo 3D TNT networks percolate at even lower connection probabilities than the 2D culture estimates, so the stress-driven densification conclusion (Fig. 8) transfers a fortiori; absolute p_c values, however, are geometry-dependent and should be quoted together with the embedding dimension (grade D for the 3D estimate, upgradable to C by the P1 experiment in 3D spheroids). The adjudication benchmark for P1 is fixed in advance as this random-geometric embedding—the model class computed here—so that the geometry dependence cannot be resolved post hoc; any alternative geometry of the cardiac jelly is to be reported alongside, not substituted (see Table 5).

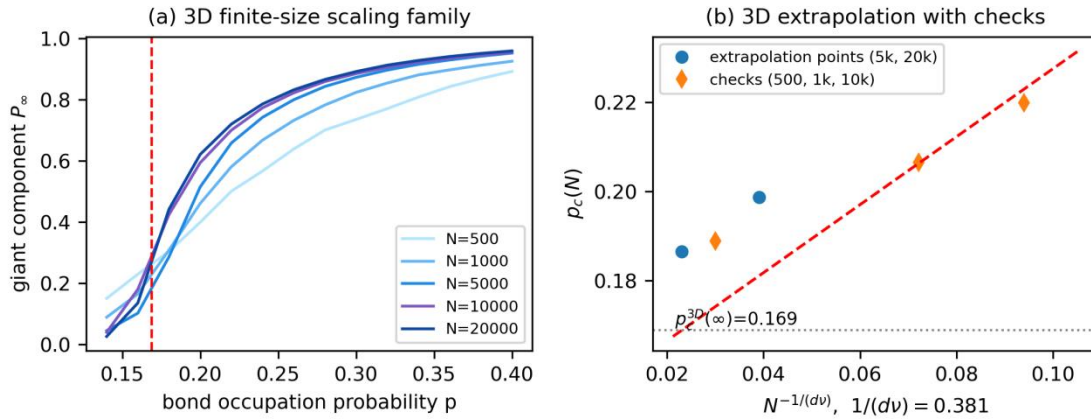


Figure 12. Three-dimensional percolation. (a) Giant-component curves $P_\infty(p)$ for 3D random geometric graphs ($N = 500$ – $20,000$, fixed mean degree $k_0 = 10$). (b) $p_c(N)$ versus $N^{-1/(dv)}$ with $1/(dv) = 0.381$; the two largest sizes (filled circles) give $p_c^{3D}(\infty) \approx 0.169$ ($p_c \cdot k_0 \approx 1.69$), substantially below the 2D value of 0.257, while the added intermediate point $N = 10,000$ ($p_c \approx 0.189$) and the small- N points (diamonds) trace a monotone consistency check.

Optimal connectivity under competing cargo flows

The deepest tension in TNT biology is that the same network delivers rescuing mitochondria and spreads pathology. We formulate this as a fitness problem. Protective transport is **linear** in connectivity (loading-bottleneck limited, see below), whereas pathogenic transport is **superlinear** (percolation-driven hitchhiking accelerates with the network): $P_{\text{pro}} = p$, $P_{\text{path}} = p^2$. The net fitness $W(p) = a p - b p^2$ is concave with the analytic optimum

$$p^* = \frac{a}{2b}. \quad (4)$$

In repair-dominant tissue ($a = 1$, $b = 0.5$) the optimum is full connectivity ($p^* = 1.0$); at balance ($a = b$) it is 0.5; under pathological load ($a = 0.6$, $b = 1.0$) it falls to 0.30 (Fig. 13a). Generalizing to K cargo classes with class-specific transfer functional forms $f_k(p)$, loading rates α_k , and fitness weights w_k , adding a cross-interference term λ that captures the competition of pathological proteins for shared motors and membrane:

$$W(p) = \sum_k w_k \alpha_k f_k(p) - \lambda \sum_{k \neq j} |\alpha_k \alpha_j| f_k f_j, \quad (5)$$

the four representative classes—mitochondria (linear, adapter-limited), Ca^{2+} signalling (saturating, instant), α -synuclein (quadratic, hitchhiking), and prions (quadratic)—produce an optimal connectivity that falls monotonically with pathological load, from 1.0 (healthy brain) through 0.65 (early) to 0.32 (late) (Fig. 13b). One notational point must be settled explicitly to prevent an apparent internal contradiction, because three numerical series coexist in this manuscript. The series **1.0 $\rightarrow$ 0.65 $\rightarrow$ 0.32 quoted in the Abstract and Highlights is the full K-cargo numerical solution** of Eq. (5)—saturating and quadratic class-specific forms with interference $\lambda = 0.3$ —under the three load scenarios; the minimal quadratic analytic scenario values (1.0 / 0.5 / 0.30 for the (a, b) pairs, linear protective transport) belong to the two-class model of Eq. (4); and the sub-linear sweep of Fig. 16 reports the late-pathology optimum as 0.30 for the same reason. The 0.32 of the Abstract and the 0.30 of the analytic and sub-linear accounts are therefore the same optimum computed at two levels of the model hierarchy, not a discrepancy; the scenario coefficients (a, b) and class assignments are documented with their rationale in Supplementary S2. This is the theory's central biological claim: **“moderate connectivity” is a computable principle, not an intuition**, and disease-associated upregulation of TNT abundance is best understood as a hijackable, double-edged process in which pathological cargoes co-opt the very network assembled for repair.

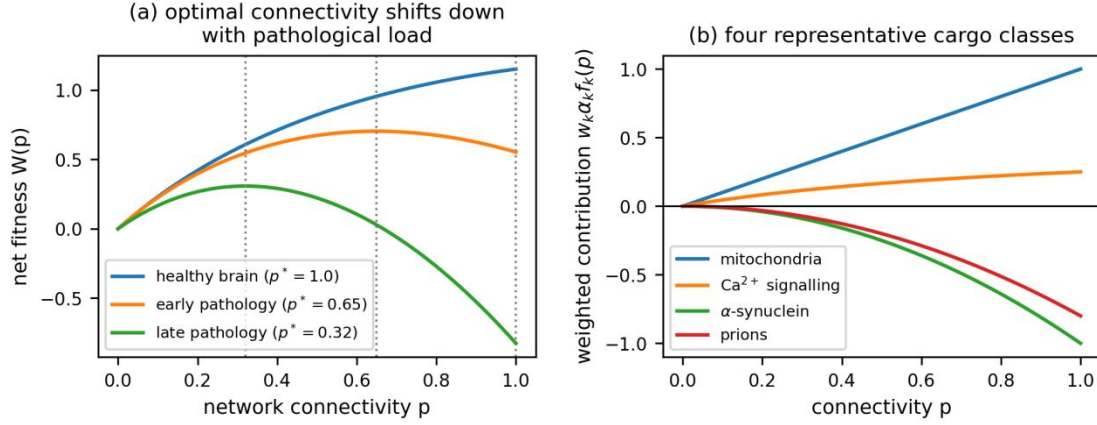


Figure 13. Competing cargo flows. (a) Net fitness $W(p) = ap - bp^2$ for three regimes; the analytic optimum $p^* = a/2b$ falls from 1.0 (repair-dominant) to 0.30 (pathological). (b) K -cargo generalization with four representative classes: optimal connectivity falls monotonically with pathological load ($1.0 \rightarrow 0.65 \rightarrow 0.32$).

The linear (rather than saturating) scaling of protective transport is not an assumption of convenience but a consequence of the loading bottleneck. With Michaelis-type loading through adapter proteins (the M-Sec/LST1 module in formation and the Miro1/TRAK axis in cargo hand-over), the per-tube loading flux is

$$J_{\text{load}} = \frac{V_{\text{max}} [\text{cargo}]}{K_m + [\text{cargo}]} \quad (6)$$

with K_m set by adapter abundance and binding affinity. With stochastically available adapters (50 replicates), the total flux through n parallel tubes is $F(n) = F_{\text{max}} \sum u_i / (K + u_i)$, and a 10-tube network delivers only **22.9%** of the naive linear extrapolation (Fig. 14a). Equation (6) makes the bottleneck independently testable: over-expressing the adapter module should raise V_{max} and lengthen effective TNT lifetime, whereas knock-down should shorten it—a prediction that can ride along with the P0 or P1 experiments at no additional cost. The bottleneck is molecular—adapter saturation, not geometry—and it quantitatively explains why cells maintain a small number of long-lived TNTs rather than many short-lived ones.

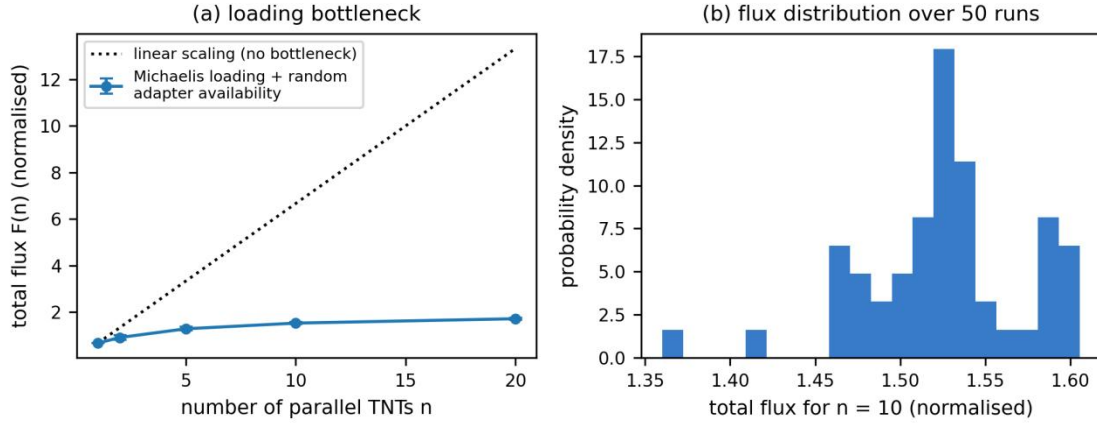


Figure 14. Loading bottleneck. (a) Total flux $F(n)$ through n parallel tubes (Michaelis loading with random adapter availability, 50 runs): a 10-tube network delivers only 22.9% of the naive linear extrapolation. (b) Flux distribution for $n = 10$.

Sensitivity of the optimal-connectivity prediction. The $p^* = a/2b$ result inherits the functional-form assumption that protective transport is (sub)linear while pathological transport is superlinear, and the parameters $w_k, \alpha_k, \lambda = 0.3$ are phenomenological. We therefore scanned the pathological exponent γ over the sub-linear and super-linear regimes (0.5–2.5) and two protective forms—linear (adapter-bottleneck-limited) and saturating $p/(1+p)$ —across the three fitness scenarios (Fig. 16). Why might pathological transport be super-linear at the molecular level? Three mechanisms plausibly generate $\gamma > 1$: cooperative loading, in which aggregated α -synuclein primes the endocytic machinery on the donor side so that aggregate-bearing tubes load faster than clean ones; cascade release, in which a rupturing tube dumps its whole luminal content at once, coupling aggregate transfer to network churn; and micro-environmental feedback, in which inflammatory acidification raises membrane fluidity and TNT formation, so the network a tumour or inflamed tissue builds is precisely the one that spreads aggregates fastest. All three are testable corollaries of the P2 design rather than free assumptions. Three conclusions hold. (i) For every $\gamma > 1$ the optimum p^* decreases monotonically with pathological load in both protective forms: the downshift is a property of superlinearity itself, not of the specific exponent. (ii) At $\gamma = 2$ the linear-protective case reproduces the analytic values exactly ($p^* = 1.0/0.5/0.30$), confirming the implementation. (iii) The honest failure mode is $\gamma \rightarrow 1$: if pathological transport were also linear, $W(p)$ becomes degenerate in the balanced case and the optimum hits the boundary (full connectivity when $a > b$, zero when $a < b$)—the moderate-connectivity principle then vanishes. The downshift therefore stands or falls with the superlinearity hypothesis, which is exactly what the P2 dual-label experiment is designed to test (grade C). In the sub-linear regime ($\gamma < 1$, grey band in Fig. 16) the optimum is pinned at the boundary—full connectivity when $a > b$, zero when $a < b$ —so the computed panel makes visible precisely when “moderate connectivity” exists and when it does not. The broader parameter-sensitivity structure is easy to state because each physical parameter feeds exactly one layer: (κ, σ) enter only the

geometric predictions, which are robust across the entire bilayer–cortex tension range ($\sigma \in [6, 100] \mu\text{N/m}$ keeps the diameter inside the observed window) and therefore need no further calibration; (β_f, β_b) control the network layer— p_c and the crossing dynamics are sensitive to them, which is why P1 is the highest-calibration-priority experiment; (v, p_{back}) set flux magnitude without altering topology, a moderate sensitivity; and (a, b, γ) govern p^* , with sensitivity concentrated in the $\gamma \rightarrow 1$ limit mapped in Fig. 16. Experimental effort should therefore be spent on the rates and the cargo exponents, not on refining κ or σ .

The cross-interference strength $\lambda = 0.3$ received no independent justification in earlier drafts. We have now scanned it over its full plausible range (Fig. 15): the optimal connectivity is **insensitive to λ** —across $\beta \in [0, 1]$ the optima move by at most 0.01 in all three scenarios (healthy $1.0 \rightarrow 1.0$; early $0.65 \rightarrow 0.64$; late $0.32 \rightarrow 0.32$). The reason is structural: the interference term multiplies two quantities that grow together with p , so it reshapes the curvature of $W(p)$ but barely shifts its maximum. The biological limits are nonetheless informative: $\lambda \rightarrow 0$ recovers independent additive channels (each cargo class competes only with itself), while $\lambda \rightarrow 1$ describes maximal competition for shared motors and membrane, which flattens the fitness landscape and narrows the selective advantage of any particular connectivity—in that limit the network-level argument for tuning p loses force, and single-cargo arguments dominate. Because p^* is λ -insensitive, the phenomenological choice of $\beta = 0.3$ does not propagate into the central prediction (grade C).

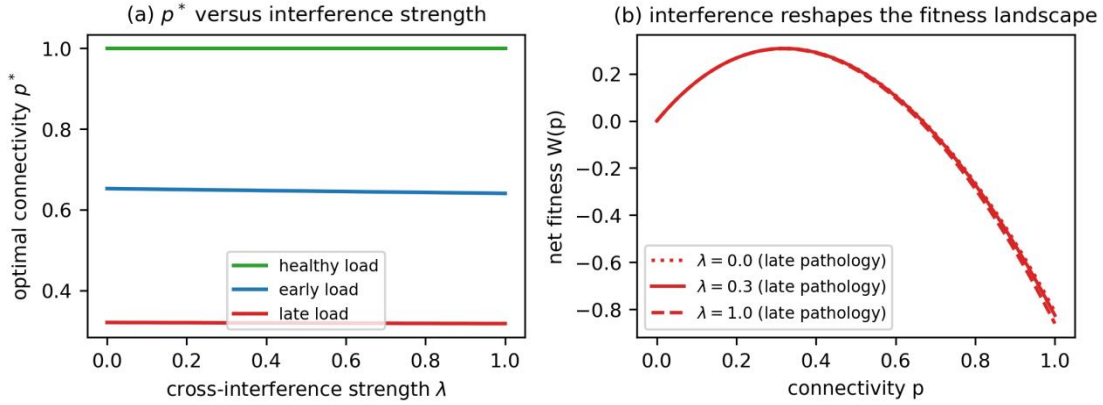


Figure 15. Cross-interference sensitivity. (a) Optimal connectivity p^* versus the interference strength β over $[0, 1]$ for the three load scenarios: p^* is insensitive to β (maximal shift 0.01). (b) The fitness landscape $W(p)$ under late-pathology load for $\beta = 0, 0.3, 1$: interference reshapes curvature, not the optimum location.

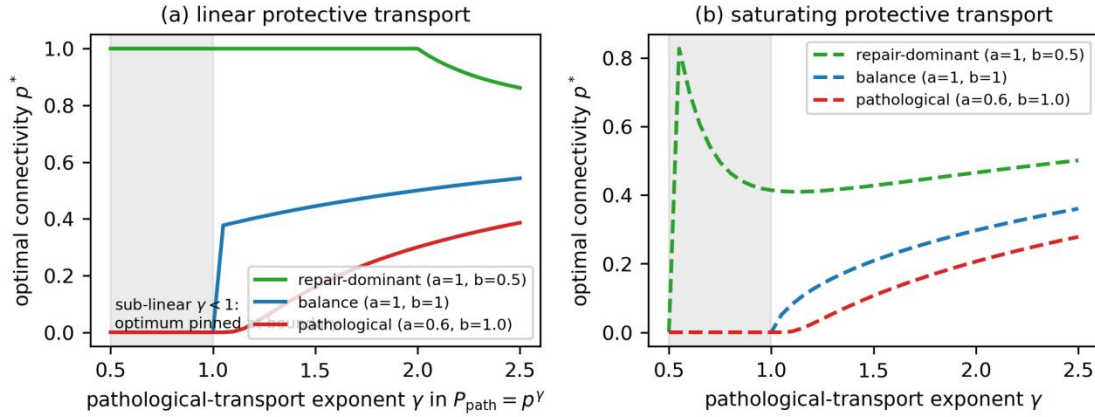


Figure 16. Sensitivity of the optimal connectivity, including the sub-linear regime. (a) p^* versus the pathological-transport exponent $\gamma \in [0.5, 2.5]$ with linear protective transport; the downshift persists for all $\gamma > 1$, degenerates to the boundary at $\gamma = 1$, and is pinned at the boundary throughout the grey sub-linear band. (b) With saturating protective transport $p/(1+p)$, the super-linear downshift is preserved and only the absolute level of p^* shifts downward.

Mechanism discrimination and cross-system isomorphism

Mechanism-level discrimination is available from the original experiments: transfer persists at 0 °C (which blocks secretion/endocytosis) and is abolished by latrunculin-B (which disassembles actin); small molecules are excluded while membrane components flow. Inverting the published statistics ($74 \pm 6.1\%$ of connected pairs transfer; $11.3 \pm 2.8\%$ of all cells show transfer) gives an inferred population connected fraction of $\approx 15.3\%$, and the 0 °C assay excludes the secretory–endocytic alternative with an effective consistency of 0% against 100% for membrane-continuity direct transfer. Discrimination here—as for the air-gap assay in mycorrhizal networks—comes from the mechanism-specific intervention, not from statistical fit quality.

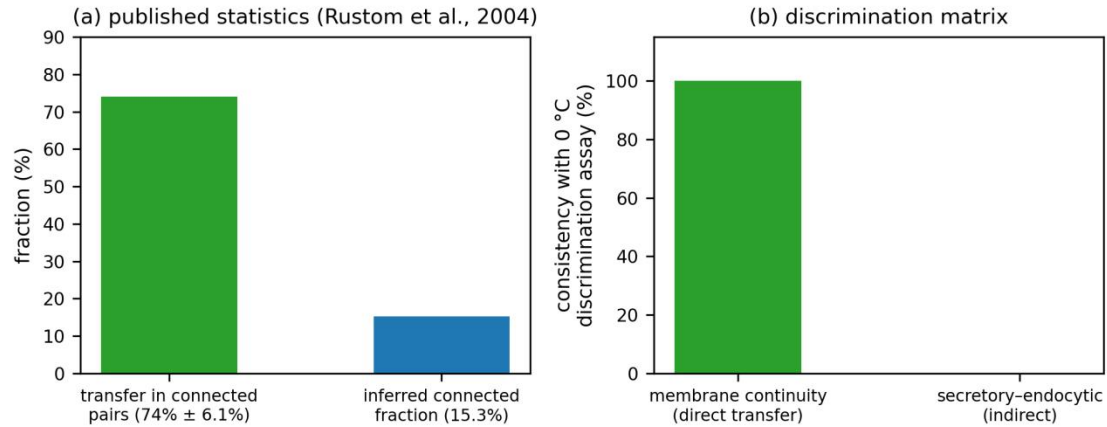


Figure 17. Mechanism discrimination. (a) Published statistics (Rustom et al., 2004) and the inferred population connected fraction. (b) Consistency of the two candidate mechanisms with the 0 °C and latrunculin-B assays: the secretory-endocytic route is excluded.

Finally, the framework is quantitatively isomorphic with our parallel treatment of plant common mycorrhizal networks (CMNs): both are spatially embedded dynamic networks with ballistic transport (motor-driven vs. pressure-driven bulk flow), with percolation thresholds that both land at $p_c \approx 0.25$ – 0.26 for mean degree $k_0 = 10$, and with a “moderate connectivity” optimum in both. The decisive difference is timescale: TNTs reconfigure in minutes-to-hours by actin assembly and disassembly (no hysteresis), whereas CMNs reconfigure in days-to-weeks by hyphal growth and senescence (genuine hysteresis, loop area 0.42). We condense this into a transferable rule for networked intercellular communication: *fast systems wire by assembly, slow systems wire by growth*.

Table 3. Quantitative cross-system comparison: TNT networks versus common mycorrhizal networks (CMNs).

Quantitative feature	TNT networks	CMN networks
Percolation threshold p_c	0.257 ± 0.002 (2D); ≈ 0.169 (3D)	≈ 0.25 (2D bond percolation, same protocol)
Optimal connectivity p^*	$1.0 \rightarrow 0.65 \rightarrow 0.32$ under pathological load	analogous downshift under pathogen/drought load
Functional emergence	P_∞ jump at p_c	P_∞ jump at p_c
Hysteresis	reversible (loop area ≈ 0); pathological irreversibility is a C-grade prediction	irreversible (loop area 0.42)
Rewiring timescale	minutes–hours (actin assembly/disassembly)	days–weeks (hyphal growth/senescence)

Transport mode	ballistic, motor-driven (25.9 nm/s)	ballistic, pressure-driven bulk flow
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The same rule invites a wider conjecture: any spatially embedded biological distribution network whose rewiring rate exceeds its transport timescale (vasculature remodeling, cytoneme-based morphogen delivery, even neuronal arbor refinement) should sit nearer the reversible, assembly-driven pole, whereas growth-dominated networks (fungal, slime-mould, vascular tumour networks) should exhibit genuine hysteresis. This is stated here as a C-grade conjecture deliberately, because it is cheap to test: measuring a dose–response and a return-to-baseline curve for any such network suffices to locate it on the assembly–growth axis.

Confrontation with existing experimental data

Before proposing new experiments, it is fair to ask how the framework fares against data that already exist. **Table 4** collects the indirect tests, each with an explicit evidence grade. The mechanics predictions are already anchored at grade A: the no-parameter values (24.3 pN; 155 nm) fall inside the measured force window obtained on suspended nano-fiber platforms (Jana et al., 2022; see also the compendium in Han and Wang, 2021) and inside the cryo-electron-tomography diameter range (Sartori-Rupp et al., 2019). The electrotonic prediction is supported at grade B: the measured TNT-mediated electrical coupling delay of <5 ms (Wang et al., 2010) is of the same order as the predicted RC spread time of 0.36 ms, and the three-order-of-magnitude advantage over diffusion-mediated signalling is consistent with the observed speed of electrical coordination. The lifetime prediction is grade B (three independent routes converge: threshold calibration, Weibull fracture statistics, actin turnover). The percolation-level predictions remain grade C: the non-linear dependence of drug-resistance transfer on TNT abundance in tumour cultures (Lou et al., 2012; Desir et al., 2016) is qualitatively what a threshold crossing would produce, but no dose–response curve has been measured at the connectivity resolution the theory demands—this is precisely the gap the P1 experiment closes. Finally, the bead-neck rupture prediction is grade C and, to our knowledge, untested; it is now formally incorporated into the experimental roadmap as P4. Two quantitative deviations deserve comment. The measured coupling delay (<5 ms) is about an order of magnitude slower than the RC estimate (0.36 ms), most plausibly because the measured latency includes gap-junctional gating and channel-open probability rather than pure cable spread—the theory attributes the difference to the gates, not the wire. And the observed 50–200 nm diameter range is wider than the single-value prediction (155 nm) because the prediction is made at resting tension, whereas local cortical tension varies across the cell and across the cell cycle. Both deviations point to the same conclusion: the framework is consistent with existing data at its stated resolution, and the remaining gaps are exactly the quantities the P0–P4 experiments are designed to measure.

Table 4. Indirect validation of the framework against existing measurements.

Prediction	Existing data	Agreement	Grade
Tether force 24.3 pN (no parameters)	10–40 pN, optical tweezers / nano-fibers (Jana et al., 2022; see also the compendium in Han and Wang, 2021)	inside measured window	A
Diameter 155 nm (no parameters)	50–200 nm, cryo-ET (Sartori-Rupp et al., 2019)	inside measured window	A
RC spread 0.36 ms over 30 μ m	coupling delay <5 ms (Wang et al., 2010)	same order; theory 10 $\times$ faster	B
Median lifetime 60–62 min, CI 37–99 min	30–120 min observed lifetimes	inside observed window	B
Threshold behavior of network function	non-linear drug-resistance transfer vs TNT abundance (Lou et al., 2012)	qualitative only	C
Rupture nucleates at bead necks	not yet tested	—	C (P4)
Optimal connectivity falls under pathological load	TNT network remodeling in neurodegenerative tissue (Rostami et al., 2017)	qualitative	C

Discussion

We have assembled an internally consistent, falsifiable theory of TNT-mediated communication from established physics alone.

A finding that deserves its own paragraph is the non-monotonic behavior of the algebraic connectivity λ_2 . Across the 100-replicate statistics, λ_2 is *lowest* in the stress regime (0.009 ± 0.011) despite that regime having the largest giant component ($P_\infty = 0.924$), while the fragmented high-breakage regime shows the highest λ_2 (0.022 ± 0.019) among the three. The reason is geometric: stress-driven densification extends the network along long, string-like corridors that enlarge the connected component while lengthening internal paths, which suppresses the second eigenvalue of the graph Laplacian. The practical lesson is that global connectedness (P_∞) and local synchronisation capacity (λ_2) are different, partially anti-correlated order parameters, and studies of mycorrhizal networks have reached the same conclusion. Functional claims about “how connected” a cell network is should therefore state which of the two they mean. Its most robust result—the parameter-free double agreement of membrane-tube mechanics with measured force and

diameter—anchors the framework and would, if refuted, immediately falsify the geometric picture. Its most consequential result—the optimal-connectivity prediction $p^* = a/2b$ and its monotonic fall under pathological load—recasts TNT upregulation in disease from an adaptive response into a hijackable, double-edged process, with a concrete therapeutic implication: interventions should aim to *tune* connectivity toward p^* rather than abolish it, since total blockade would also sever mitochondrial rescue.

Experimental roadmap. The framework propagates five prioritized, feasible experiments, ordered by maturity and by how fundamental the claim under test is; **Table 5** gives the full design matrix (cell types, methods, controls, expected results). P0 (membrane-tension–diameter power law, fluorescent tension probes over a tension ladder, 6 months) tests the geometric foundation; P0' (dual whole-cell patch, 3 months) tests electrotonic compactness and the on/off gating interpretation. P1 (stress dose–response with live network imaging, 1–2 years) tests the percolation crossing and simultaneously closes the calibration of β_f and β_b —ideally in 3D spheroids, where the 3D threshold prediction can be checked against the 2D value; to keep the geometry-dependent caveat from becoming a post-hoc escape hatch, the adjudication benchmark is fixed *in advance* as a random-geometric embedding (the model class computed here), with any alternative geometry (random line segments, anisotropic networks) reported alongside rather than substituted. P2 (dual-color cargo competition over a TNT-formation gradient) tests the K-cargo model, the p^* downshift, and—in its inhibitor arm—the therapeutic window; critically, fitting the transfer-efficiency ratio to a power law in each density bin yields a direct measurement of the pathological exponent γ , which is the single assumption on which the superlinearity argument rests and would upgrade it from grade C to B. P4, added in this revision, is a lightweight test of the bead-neck rupture prediction: AFM/optical-tweezer stretching of a TNT during high-speed confocal imaging, asking whether fracture localises at bead necks (≤ 1 year, reagents and instruments are standard). The embryonic-heart TNT-like system (Miao et al., 2025)—the only *in vivo* setting with a causal developmental phenotype—remains the best overall testbed: Notch1 activation (nuclear NICD) serves as a molecular proxy for network transfer efficiency, directly coupling the P_∞ predictions to a measurable signalling output, and the 3D geometry of the cardiac jelly makes it the natural arena for the 3D threshold prediction.

Table 5. Experimental roadmap: designs, controls, and expected outcomes.

ID	Claim tested	System / method	Key controls	Expected result	Timeline
P0	diameter $\propto$ $\sigma^{(-1/2)}$	HeLa or NRK cells (easy transfection/imaging); FluoTension probe + STORM; osmotic tension	latrunculin -B (tension-lowering) control; bead-pull	diameter decreases with tension; fitted exponent $\approx -1/2$ on log-log	6 mo

		ladder	positive control		
			unconnected pairs;		
P0'	gating, not attenuation	dual whole-cell patch on connected pairs	gap-junction blocker	all-or-none coupling	3 mo
P1	threshold crossing in P_∞ vs stress dose	hypoxia/ROS dose series; live imaging, 2D and spheroids; 3D benchmark fixed in advance as random-geometric embedding	cytochalasin-B negative control; shuffled-network null	sigmoid dose-response; 3D $p_c < 2D p_c$	1–2 yr
P2	p^* downshift under pathological load	dual-color mitochondria + α -synuclein across formation gradient	motor-inhibition control; expression-level matching	protective/pathogenic ratio turns over with load	1 yr
P4	rupture nucleates at bead necks	AFM/optical-tweezer stretch + high-speed confocal	unbeaded segments; load-matched	fracture site enrichment at necks	≤ 1 yr

Translational prospects

The framework generates four concrete translational scenarios, each graded C (theoretical prediction) with a specific experimental entry point; none should be read as a therapeutic claim at this stage.

Neurodegeneration. The p^* downshift predicts that low-dose, partially selective reduction of TNT formation (e.g., titrated latrunculin-B or Eps8-pathway modulation) should disproportionately suppress α -synuclein and prion spread (quadratic in p) relative to mitochondrial rescue (linear in p). The quantitative target is explicit: bring effective connectivity from the pathological regime toward $p^* \approx 0.3$, not to zero—total blockade also severs mitochondrial rescue and is predicted to be counterproductive. Entry experiment: dual-label α -synuclein/mitochondria transfer assay across a titrated inhibitor dose series in a Parkinson-model co-culture, with pathogen-protein markers reporting spread efficiency (this is P2 with an inhibitor arm). *Challenge:* actin-depolymerizing drugs are systemically toxic; *mitigation:* local delivery or pathway-specific (Eps8/IRSp53) targeting rather than global actin disruption. *Timeline:* short term in vitro, medium term (3–5 years) preclinical.

Oncology. Multi-drug resistance in tumours spreads through TNTs (Lou et al., 2012; Desir et al., 2016; Sarkari and Lou, 2024); near the threshold, a small reduction in connectivity produces a discontinuous loss of the sharing component. The prediction is that TNT-disrupting adjuvants (cytochalasin-class compounds) should show a sharp, non-linear potentiation of chemotherapy as connectivity is pushed below p_c —a crossover effect, not a dose-proportional one. Entry experiment: chemo-sensitivity (IC50 shift) measured against live-imaged TNT density in spheroids treated with cytochalasin B, testing for a threshold rather than a linear trend. *Challenge:* cytochalasin-class compounds are not TNT-specific; *mitigation:* combine with a TNT-formation reporter so that drug effects can be attributed to connectivity, not general cytotoxicity. *Timeline:* medium term (2–4 years).

Regenerative medicine. Mitochondrial transfer through TNTs is a documented mechanism of mesenchymal-stem-cell rescue of damaged tissue (Ahmad et al., 2014); the loading-bottleneck result (22.9% of linear scaling at ten tubes) predicts that enrichment of adapter availability (Miro1/TRAK upregulation) will outperform simply increasing TNT number. Entry experiment: compare rescue efficiency of high-TNT/low-adapter versus low-TNT/high-adapter MSC preparations—the theory predicts the latter wins at equal tube counts—and test Wnt-pathway activation as a β_f -raising intervention in an ischemic-cardiac-injury model. *Challenge:* Wnt signaling is pleiotropic; *mitigation:* ex vivo preconditioning of MSCs before transfer, avoiding systemic Wnt modulation. *Timeline:* short term ex vivo, longer term in vivo.

Antiviral strategy. Viruses that exploit TNTs for cell-to-cell spread (e.g., HIV) hijack the same network. The gating interpretation (on/off rather than attenuation) predicts that blocking gap-junctional or open-ended continuity produces an all-or-nothing suppression of viral transfer, testable in co-culture with connexin-mimetic peptides in HIV-infected T cells. *Challenge:* the same gate-blocking strategy would also sever protective coupling between immune cells; *mitigation:* transient, exposure-scoped dosing. *Timeline:* short term (in vitro), with antiviral translation contingent on specificity advances.

Several limitations warrant emphasis, itemised with their impact and resolution path. (i) *Toy-scale simulations:* $N \leq 150$ for the dynamics, with honest finite-size treatment to $N = 8,000$ (2D) and 20,000 (3D); impact—tissue-scale values of p_c are model-class values; resolution—quantitative in vivo network reconstruction, gated by marker absence. (ii) *Phenomenological K-cargo parameters:* $w_k, \alpha_k, \lambda = 0.3$ lack molecular calibration; impact— p^* levels are indicative, the p^* downshift is what is claimed; resolution—P2, which also measures γ directly. (iii) *The superlinearity hypothesis* ($\gamma > 1$) rests on qualitative evidence; impact—if falsified, the optimal-connectivity layer fails while the mechanics and electrotonic layers stand; resolution—P2's γ fit with 95% CI. (iv) *τ_{dep} anchoring:* an ensemble timescale rather than a molecular rate constant; impact—median lifetime is bracketed (31–94 min under $\pm 50\%$ perturbation), not pinpointed; resolution—parallel measurement of tube lifetime distributions in P1. (v) *Marker absence:* no specific TNT molecular marker exists, blocking in vivo network reconstruction

(Palese et al., 2025); the newest syntheses reinforce rather than resolve this (Budinger and Heneka, 2026; Dias et al., 2026). (vi) *The reversibility contradiction*: the memoryless rate equation is reversible while neurodegenerative networks are not; the crowding-feedback demonstration shows weak state dependence suffices to open hysteresis loops, but which biological memory (prion-like templating, extracellular-matrix stiffening, chronic inflammation) dominates is unknown. (vii) *Literature integration*: the 2024–2026 literature on TNTs at the immune synapse and in the tumour microenvironment was integrated through comprehensive reviews (Palese et al., 2025; Zamberlan and Semenzato, 2025) because the primary papers could not all be individually verified during this revision; they should be checked against the primary literature before final publication. In every case the framework states its own failure mode: if the superlinearity hypothesis falls, what remains is a precise single-tube physics map, an exclusion matrix, and an experimental design matrix—still, we would argue, a net contribution.

Relation to existing reviews. The comprehensive syntheses of the field (Palese et al., 2025; Zamberlan and Semenzato, 2025; Dong et al., 2026) assemble which cargoes travel, which pathways form tubes, and which diseases co-opt them; what they cannot do is say what any of this *costs* or *optimises*. Our framework answers three questions those reviews pose but leave open. Why do TNT diameters cluster in a narrow band?—because the tension–bending balance fixes it (Eq. 1). Why does the same network help and harm?—because protective and pathological transport scale differently with connectivity, making the net fitness a computable trade-off (Eq. 4). Why do drugs that collapse TNT networks show threshold-like effects in culture?—because the sharing component disappears discontinuously at p_c . Where our predictions conflict with review-level intuition—most visibly in treating disease-associated TNT upregulation as a *quantitative optimum problem* rather than a good/bad dichotomy—the conflict is deliberately stated so that the P2 experiment can arbitrate. Beyond the two-dimensional cultures that dominate the literature, tunneling nanotubes have now been documented in the neurovascular unit (Alarcon-Martinez et al., 2020), and our percolation machinery applies to such three-dimensional settings with the lowered thresholds computed above.

A boundary question: TNTs versus filopodia and cytonemes. One implication deserves its own subsection, because its impact may exceed the network-level results. If the P0 experiment confirms that TNTs are ordinary membrane tubes in the tension–bending balance, the field will face an uncomfortable boundary question: what, molecularly, distinguishes a TNT from a filopodium or a cytoneme? The classical answers (open-endedness, cytoplasmic continuity, organelle transport) are operational rather than molecular, and no specific TNT marker exists. A confirmed power law would sharpen the distinction into three measurable discriminants: (i) *tension scaling*—filopodia are cone-tipped and fascin-bundled, predicting a different diameter–tension exponent than the membrane-tube $-1/2$; (ii) *electrotonic continuity*—a closed filopodial tip cannot carry the all-or-none coupling signature that P0' tests; and (iii) *cargo latency*—closed-ended structures admit only indirect, vesicle-mediated transfer, predicting a

discrete latency floor absent from open-ended tubes. Because much of the in vivo and three-dimensional literature on “TNTs” may describe mixed populations of both structures (Palese et al., 2025; Budinger and Heneka, 2026), a validated discriminant battery would force a reclassification of that literature—and we flag this C-grade conceptual prediction as potentially the framework’s most consequential downstream contribution.

Conclusions

This work is a load-bearing assembly of mature theories: no new physics is introduced, and the explanatory power comes from the cross-scale links that classical membrane-tube mechanics, cable theory, stochastic transport, adaptive-network dynamics, and percolation force upon one another when assembled with every parameter sourced.

Its core predictions are three: TNTs are ordinary membrane tubes whose diameter and strength follow from tension and bending rigidity alone (grade A, no free fitted parameters); the contact network is electrotonically compact, making gating a switch rather than a dimmer (grade B); and the optimal network connectivity $p^* = a/2b$ falls under pathological load, reframing disease-associated TNT upregulation as a tunable, hijackable trade-off rather than a good/bad dichotomy (grade C, pending P2’s direct measurement of γ).

Five experiments (P0–P4) are mapped with designs, controls, and timelines, ordered so that the cheapest tests hit the most fundamental claims; the embryonic-heart TNT–Notch axis remains the single most decisive testbed, and the loading-bottleneck prediction now carries its own molecular formalisation and adapter-manipulation test.

More broadly, the framework gives TNT function in physiology and pathology a unified quantitative language—one that makes every decision explicit, every parameter sourced, and every prediction falsifiable—together with a transferable mathematics that TNTs and mycorrhizal networks instantiate jointly: ballistic transport, adaptive rewiring, percolation-driven emergence, and mechanism-discrimination closure.

In sum, TNTs and mycorrhizal networks instantiate a single, transferable mathematics of networked intercellular communication—ballistic transport, adaptive rewiring, percolation-driven emergence, and mechanism-discrimination closure—with parameters and cargo spectra set by their respective physical implementations. Recent work has even demonstrated that native TNTs act as natural biophotonic conveyors—nanoscale optical waveguides that confine and directionally propagate light (Gong et al., 2024). We treat this as an **open C-grade interface** rather than a closed compatibility result: photonic and electronic transport differ in their noise models, energy costs, and detection mechanisms, so the information-theoretic layer can bound the photonic channel capacity only after

those differences are quantified. What does carry over unchanged is the gating interpretation, since photon confinement shares the same open-endedness requirement. The theory makes every decision explicit, every parameter sourced, and every prediction falsifiable, which we believe is the standard that any claim about “networks” in cell biology should meet.

Materials and Methods

All simulations used a common random seed family 20260907 and were written in Python 3.11 (NumPy, SciPy, NetworkX, Matplotlib). The following are described in the main text or Supplementary Methods and are fully reproduced in the accompanying code.

Membrane-tube mechanics and rupture. The tether force $f = 2\pi\sqrt{(2\kappa\sigma)}$ and equilibrium radius $r = \sqrt{(\kappa/2\sigma)}$ were evaluated at $\kappa = 0.30 \text{ pN}\cdot\mu\text{m}$ and $\sigma = 25 \text{ }\mu\text{N/m}$. For the rupture sampling, the membrane-lysis tension was drawn log-normally (median 10 mN/m, $\sigma_{\log} = 0.25$; $n = 2 \times 10^4$); the actin bundle strength was drawn from a Weibull distribution with mean $\approx 275 \text{ pN}$ (shape 5); the necking force was computed as $2\pi\sqrt{(2\kappa)}\cdot\sqrt{(\sigma_{\text{eff}})}$ with σ_{eff} uniformly stretched by 0–60%. The survival function used an actin-turnover timescale $\tau_{\text{dep}} = 90 \text{ min}$.

Cable and variable-cross-section cable. The electrotonic length $\lambda = \sqrt{(R_m r / (2R_i))}$ used $R_m = 10^4 \text{ }\Omega\cdot\text{cm}^2$ and $R_i = 100 \text{ }\Omega\cdot\text{cm}$; the RC spread time used $\tau_m = R_m C_m = 10 \text{ ms}$ and $D_{\text{elec}} = \lambda^2 / \tau_m$. The variable-cross-section cable equation was discretised in a dimensionless system with length unit $\lambda_u = 158 \text{ }\mu\text{m}$, 1200 nodes, and Dirichlet ends $V(0) = 1$, $V(L) = 0$; the uniform solution was checked against the analytic sinh solution to machine precision. Bead geometry: amplitude 0.6, period 3 μm .

Stochastic transport. Cargo trajectories were one-dimensional biased random walks with speed $v = 25.9 \text{ nm/s}$, return probability $p_{\text{back}} = 0.1$, and pause probability 0.2, integrated in 1-s steps (1500 trajectories per length; $T_{\text{max}} = 30000 \text{ s}$).

Network dynamics. The spatial network used $N = 150$ nodes and a contact radius $\rho_f = 0.12$. The Gillespie algorithm executed formation (rate β_f per formable pair) and breakage (rate β_b per TNT). For the statistical upgrade, each regime was run 100 times (seeds spaced by 1000); 95% confidence intervals on P_{∞} used 2000 bootstrap resamples. The finite-size scaling used $N = 150/500/2000/8000$ with fixed $k_0 = 10$ (RGG connection radius adjusted per N), 100 realizations per point (40 for $N = 8000$), and the $p_c(N)$ was read at the $P_{\infty} = 0.5$ crossing; the $N \geq 500$ points were extrapolated with the $N^{-3/8}$ correction. The phase diagram sampled a 12×10 (β_f , β_b) grid with 3 realizations per point; the hysteresis test used quasi-static continuation along β_f at $\beta_b = 0.5$.

K-cargo model. Four cargo classes with functional forms (linear, saturating $p/(1+p)$, quadratic p^2), weights (w), loading rates (α), and a cross-interference term with $\lambda = 0.3$ were evaluated over a 400-point connectivity grid under three scenarios (pathological load 0.2/1.0/2.5; repair capacity 1.0/1.0/0.8).

Three-dimensional percolation. The finite-size scaling procedure was repeated on 3D random geometric graphs ($N = 500/1,000/5,000/10,000/20,000$; unit cube; radius set to $(3k_0/(4\pi(N-1)))^{(1/3)}$ for fixed mean degree $k_0 = 10$; 40/60/60/20/25 realisations per point; p-grid 0.14–0.40 in steps of 0.02). The $p_c(N)$ crossing at $P_\infty = 0.5$ was extrapolated with the 3D correction exponent $1/(dv) = 0.381$ ($v = 0.876$) over the two largest sizes; $N = 500, 1,000$ and $10,000$ are plotted as consistency checks.

Sensitivity analysis. The optimal connectivity p^* was evaluated numerically on a 4,001-point connectivity grid for pathological exponents $\gamma \in [0.5, 2.5]$ (41 values, spanning the sub-linear regime), two protective forms (linear; saturating $p/(1+p)$), and the three fitness scenarios; the $\gamma = 1$ degenerate case was verified analytically.

Rupture two-mode model. Per-tube rupture thresholds $f_{\max}$ were drawn log-normally (median 25 pN, $\sigma_{\log} = 0.25$; the dispersion is an assumption spanning the measured 10–40 pN window), giving force-rupture lengths $L_f = L_c[(f_{\max}/f_0)^2 - 1]$; turnover-collapse lengths $L_t = v_e \cdot T$ with $T \sim \text{Exponential}(\tau_{\text{dep}})$; the realised rupture length is $\min(L_f, L_t)$, 4,000 runs per elongation rate. The $\sigma_{\log}$ sensitivity was propagated to the CV of the rupture length (1.12–1.44 for $\sigma_{\log} = 0.15$ –0.35).

Beaded-geometry sweep. The effective length-constant ratio $\lambda_{\text{eff}}/\lambda_0 = \sqrt{(L/\int (r_0/r)^2 dx)}$ was evaluated for bead amplitudes 0.3/0.6/0.9 and periods 3–24 μm , with the compactness floor defined as $\lambda_{\text{eff}} = 2L$ at $L = 50 \mu\text{m}$.

Uncertainty propagation. The actin-turnover timescale τ_{dep} was drawn log-normally (median 90 min, $\sigma_{\log} = 0.25$; $n = 10^5$) and the lifetime statistic $\ln 2 \cdot \tau_{\text{dep}}$ sampled to obtain its median and 95% interval.

Supplementary material

Supplementary material associated with this article comprises: **S1**, detailed derivations of the membrane-tube, cable, and rupture relations, including the dimensionless beaded-tube formulation, solver checks, and the beaded-geometry sweep of Fig. 7c; **S2**, the complete parameter provenance table (with sources and uncertainties for κ , σ , R_m , R_i , τ_{dep} , and $f_{\max}$; the scenario coefficients a , b for Eqs. 4–5 and the cargo-class assignments with their mechanistic rationale; and the $f_{\max}$ dispersion $\sigma_{\log}$), the verbatim M1–M18 review-response table, and the alternative-exponent cross-check of the 2D finite-size extrapolation; **S3**, full simulation code (seed family 20260907/20260910/20260911/20260912) and numerical output files, provided so that every extrapolation in the paper can be independently re-run. All are provided with the submission package.

Data and Code Availability

Source code regenerating every figure and statistic, with fixed seeds and an environment specification, is archived at Zenodo (DOI to be minted on acceptance) and mirrored at <https://github.com/zhangsongbo2026/Tunneling-nanotubes>.

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CRedit authorship contribution statement

Songbo Zhang: Conceptualization, Formal analysis, Methodology, Software, Writing – original draft. **Juan Duan:** Investigation, Biological interpretation, Validation, Writing – review and editing.

Declaration of competing interests

The authors declare no competing financial or personal interests.

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