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A tale to astonish: Ant-Man at the plasmodesmal gates

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In September 1962, the thirty-fifth issue of the *Tales to Astonish* comics introduced Ant-Man, Marvel's tiny but powerful new hero. A biophysicist who invents size-changing technology, Ant-Man can shrink to microscopic sizes while still wielding the physical strength of a full-sized person. When the comics came to life in the movie *Ant-Man* (2015), a series of clips showed the title character training with a simple exercise: charge at a door, shrink down to vault through its tiny keyhole, and re-emerge at full size on the other side. Of course, real-life Ant-Men do not exist, but what about proteins observed traveling through channels they shouldn't be able to fit through?

This is the central question that Peters et al. (2021) address in their Humboldt Review: "Plasmodesmata and the problems with size." Plasmodesmata serve as gatekeepers for intercellular traffic in plants. Foundational work by Katherine Esau describes viruses exploiting plasmodesmata to quickly infect a plant, despite the pores of uninfected plants being narrower than the viral particles themselves (Esau and Hoefert, 1972). Since then, the same conundrum has repeatedly popped up: tracer molecules like GFP have been observed traversing plasmodesmata too narrow to reasonably accommodate them. These molecules cannot shrink like Ant-Man, so there must be an inversion of roles where the individual plasmodesma, as our proverbial keyhole, dilates. However, it is still unclear exactly what the mechanisms for these apparent dilations and contractions are.

Peters et al. (2021) highlight how our experience of the physical world as macroscopic beings differs from the physics at the scales of an individual plasmodesma, where properties like hydrodynamic radius, electrostatics, and Brownian motion all become relevant. In their detailed mathematical model of sugar transport through plasmodesmata, Comtet et al. (2017) incorporate the intricate and complicated physics at the nanoscale. Their model provides deeper insight into the mechanics of plasmodesmal transport, but the Ant-Man phenomenon remains unsolved.

Reminding us of the importance of structural context, Peters et al. (2021) propose that dynamic effects could solve this mystery. In their discussion of small-scale physics, they describe "cargo-gating" as one possible mechanism for these apparent size changes. In this scheme, the desmotubule, a long, thin projection of the ER membrane running the length of the channel, wiggles stochastically within the pore. As in the Comtet model, the desmotubule is often centered within the plasmodesma, occluding the path for larger traveling molecules. However, by Brownian motion, it can be randomly deflected towards the wall of the pore, opening the channel for larger molecules to pass through. Traveling particles block the desmotubule from re-occupying the middle of the channel, essentially "holding the door open" for subsequent travelers. Other processes may also be involved. For example, Huang et al. (2019) propose that plasmodesmal dilation is achieved by biochemically inducing a transition from a disordered to an ordered liquid phase in the lipid membrane of the plasmodesma.

We generally try to understand and reconcile unfamiliar physics using simple, digestible, and visual models. For better or worse, these cartoons can powerfully shape and inform our perspective about a system. Even if the primary focus of a project is not to determine structure-function relationships, assumptions about structure are often baked into study design, execution, and analysis. A well-designed visual model depends on reliable observations, and this is often a cartoonization of what we see through the microscope. But what happens when we don't actually know what we're looking at? Unsurprisingly, we run into trouble if the conceptual models that form the foundation of our physical or computational experiments depend on shaky interpretations.

Peters et al. (2021) emphasize that not only hydrodynamics but also our ability to image systems changes across scales. Literally seeing small structures becomes impossible as diffraction imposes physical limits on lens resolution. For this reason, transmission electron microscopy (TEM) has been the method of choice for plasmodesmal structural studies. Yet, sample preparation for TEM introduces several issues. First, TEM samples must be solidified, often through a chemical fixation process that is potentially harsh enough to alter the structure. Next, to increase the signal-to-noise ratio of a TEM image, samples are often stained with heavy atoms, typically metals, with high electron density. As a result, the TEM image itself shows not necessarily the sample's native structure but rather the distribution of the stain molecules. Unfortunately, it is not generally known *a priori* how well the stain will bind to any given structure, which leads to inconsistent interpretations of the resulting images. As the authors point out, the same structure is sometimes identified with a positive stain, and just as often is identified with a negative stain. These striking inconsistencies (Fig. 1) bring into question

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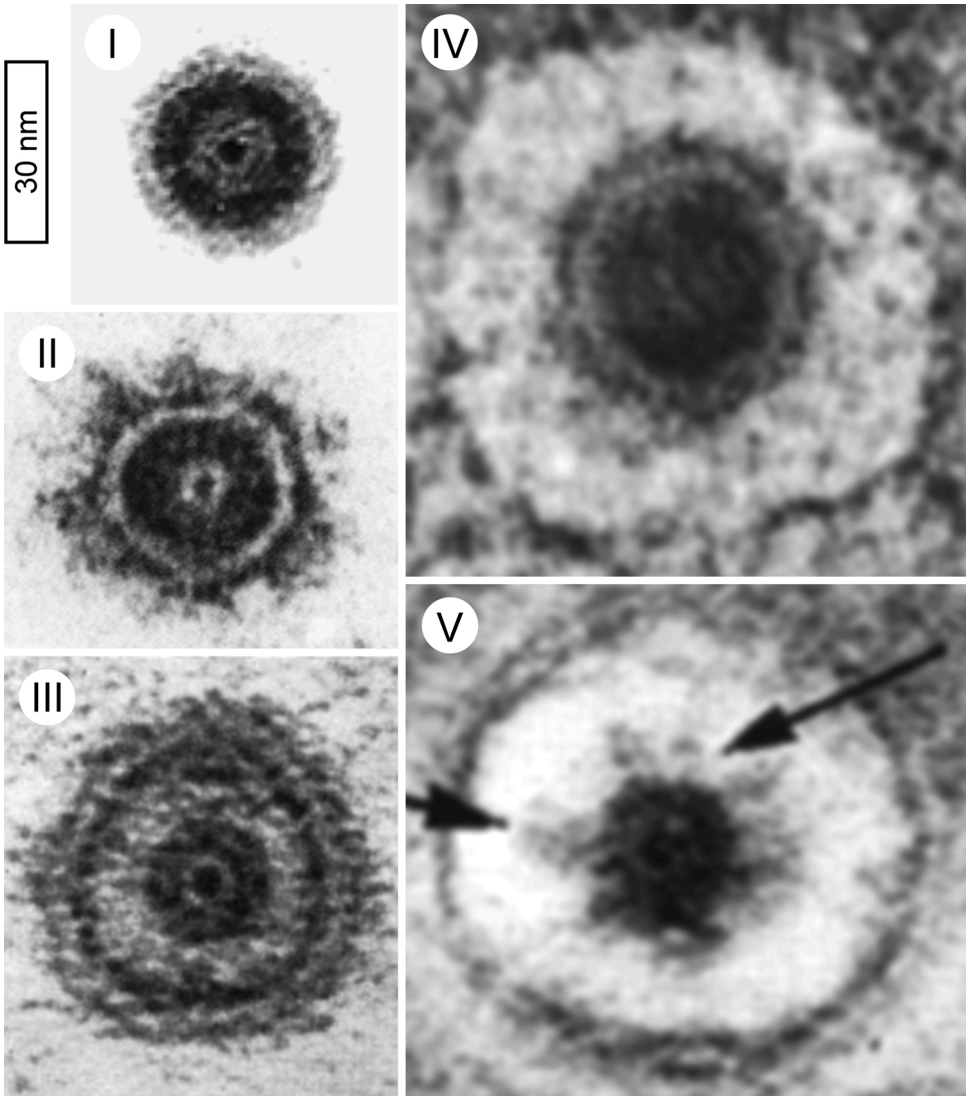

Fig. 1. TEM micrographs of plasmodesmata at the same scale show the range of staining patterns that complicate interpretation of structure. (Figure 5f from Peters et al., 2021).

the reliability of current methods, and the authors discuss in detail the limitations of the standard practices, the constraints they place on our understanding of plasmodesmata, and the need for new and better approaches.

Cryo-electron tomography (cryo-ET), for example, may be a good candidate for the next generation of plasmodesmata research (Turk and Baumeister, 2020). In recent years, advancements in technology have made analysis of subcellular structures in their native environment possible, promising eventual breakthroughs in resolving plasmodesmal structure. However, cryo-ET is still an expensive process, and the required resources and facilities are currently accessible to only a handful of research groups.

When imaging provides an inconsistent picture, the old adage, "seeing is believing" becomes "seeing is confusing." Unfortunately, we will not have Ant-Man's technology, and it may take some time before the available technology becomes both reliable and accessible enough to directly and consistently observe plasmodesmal structure and behavior. Yet, aside from awaiting improved imaging techniques, Peters et al. (2021) encourage reframing the problem from a mechanical engineering perspective, noting that active research in nano- and microfluidics can help clarify the fundamental physics of biological transport. For a field that is over a hundred years old, advances in technology and new perspectives hold promise for understanding these key gatekeepers of intercellular transport.

Declaration of Competing Interest

The authors report no declarations of interest.

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