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Electric cell death

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A clear picture of how and why cells inevitably lose viability is still lacking. A dynamical systems view of starving bacteria points to a continuous energy expenditure needed for maintaining the right osmotic pressure as an important factor.

Why living organisms inevitably die and what death even means in terms of physics and dynamical systems are still open questions despite the vast advances in molecular biology. Revealing how some cells die — not through biochemical processes but physics — would help to answer them. Writing in *Nature Physics*, Severin Schink and colleagues[1] report that a starving *Escherichia coli* bacterium uses most of its stored energy to maintain a low intracellular osmotic pressure by keeping its membrane electrically polarized. Once it depletes its energy, its osmotic pressure skyrockets and, consequently, it dies by bursting.

Self-replication is an out-of-equilibrium behaviour that is arguably unique to living systems. Studying biological systems has yielded new metrics for detecting and understanding non-equilibrium dynamics of biomolecular systems[2]. Despite these recent advances, knowing that a cell — a complex system composed of water and other molecules called osmolytes — is out of thermal equilibrium, while necessary, is insufficient for determining the cell's viability, that is its ability to self-replicate. It is insufficient because irreversible chemical processes that break the characteristic time-reversal symmetry defining thermal equilibrium, such as gene expression, can still occur in non-viable cells[3].

What then distinguishes non-viable cells from viable cells and what determines when a cell becomes unviable? Are there metrics of dynamical systems that apply to every cell type? Although these questions remain unanswered, Schink and colleagues' study of starving bacteria provides valuable insights. When *E. coli* lacks sugars, from which energy is extracted and chemically stored as Adenosine Tri-Phosphates (ATPs), its cytoplasm — the gel-like cell body — shrinks because water[4] flows out of it while its membrane detaches from the cell wall. We have yet to understand why starvation induces this phenomenon, called plasmolysis[4,5], but one possibility is that having less water drastically reduces the cell's metabolism, thereby extending its lifespan without nutrients.

The combined concentration of intracellular osmolytes determines the osmotic gradient — the net direction of water flow — across the cell boundary. When more osmolytes are present inside the cell than outside, the net flow of water is into the cell. However, if water keeps flowing into the cell, the cell will burst[5], ensuring certain death from a statistical physics perspective. Thus, maintaining a shrunken cytoplasm in plasmolysis requires the concentration of osmolytes inside the cell to remain lower than that on the outside.

Schink and colleagues' key insight was that, contrary to the conventional view, maintaining plasmolysis is an energy (ATP)-consuming process. If plasmolysis was a passive process, the more abundant extracellular osmolytes, namely ions, would diffuse into the cell until the concentration of osmolytes inside and outside the cell equalized, causing a net inflow of water. The cell would then swell and burst. In fact, the team discovered that starving cells consume most of their stored ATPs to continually expel ions, thereby maintaining the osmotic gradient required to remain viable. Monitoring individual cells until their deaths revealed that cells depolarize before they burst.

Schink and co-workers built a model to evaluate their experimental observations. Along with the diffusion equation determining intracellular and extracellular osmolyte concentrations, the model imposed a minimal ratio of the two concentrations needed to maintain viability. This ratio was set by the ATP concentration required to fuel the cell's basic functions as well as a feeble but crucial quasi-steady supply of 'recycling nutrients' — biomolecules liberated by bursting cells[6] — that intact cells consume to generate additional ATPs.

Representing a cell as a particle in an energy landscape (Fig. 1), as commonly done in analyses of biological networks[7–9], allowed Schink and colleagues to apply the Kramer's model for a particle crossing an energy barrier. This reproduced the measured average lifetime of starving *E. coli* cells. The intracellular osmolyte concentration varies among cells and acts as thermal noise that pushes cells over a pseudo-energy barrier; reaching the barriers' peak represents depolarization — the moment of viability loss. Bidirectional feedback, in which the rate of cell death influences the number of alive cells and vice versa, maintains a sufficiently low energy barrier, which ensures the longevity of plasmolysis, and that sufficiently many — but not too many — cells burst to sustain the others.

Overall, this study established that starving *E. coli* must continually expend their stored energy (ATP) to maintain an osmotic gradient to survive during plasmolysis and that the existing Kramer's formalism captures the cells' survival dynamics. For biology, this study's main contribution is arguably the discovery that maintaining plasmolysis consumes energy and that the time taken to consume all stored energy is the main factor in setting the starving cells' lifespans. For physics, this study elegantly showcases the use of a dynamical system that integrates physical processes, such as diffusion, and cellular physiology to reveal how cells can lose viability and shorten their lifespan.

Through physical approaches and by viewing cells as dynamical systems, Schink and colleagues along with other recent studies[3] have developed a physicist's picture of how a cell can die and how one can determine the moment it irretrievably loses viability. Across different organisms, these studies have revealed an unmistakable physical sign of death — the bursting of cells[1,3] — which appears after cells pass a point of no return at a threshold of a combined molecular concentration. Moreover, as the recycling of nutrients exemplifies, it is becoming increasingly clear that the death of a cell is often a collective phenomenon that depends on the behaviour of the other cells in the population[3,6,10].

Despite these recent advances, why death is inevitable for living organisms based on physical laws remains unexplained. But recent studies[3,6], including Schink and co-workers', suggest a promising route to finding an explanation — examining barely growing or dormant cells. This approach simplifies the analysis by letting one neglect the

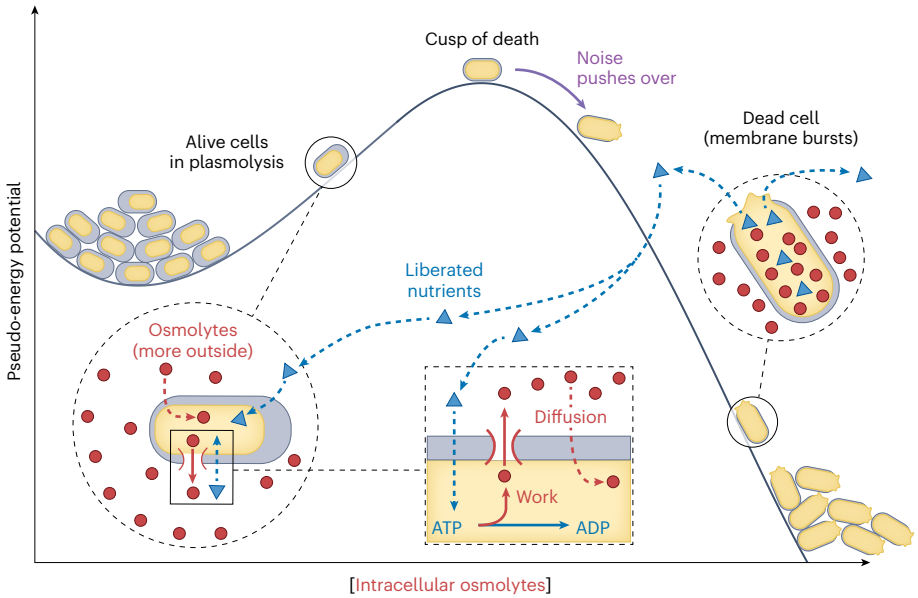

Fig. 1 | Starving cells continuously consume energy to remain viable, with help from dying cells. Dying cells release nutrients (blue triangles) that diffuse into alive cells (dashed blue paths). Dashed box: alive cells extract energy (ATP) from nutrients to expel ions (red circles), leading to higher osmolyte concentrations outside than inside the cells. The extracellular reservoir of osmolytes is assumed to be at a constant concentration. Cytoplasm (yellow) enlarges and compresses the periplasmic space (grey) as the cell moves from left to right. The cell bursts as it crosses the peak from left to right. The concentration of liberated nutrients sets the peak's height. Factors that stochastically vary among cells (for example, permeability to ions) act as noise that randomly pushes cells over the cusp.

complex processes of cell growth while focusing on finding the metrics that quantify the self-replication potential of out-of-equilibrium cells that are barely or not growing. Discovering such metrics remains an exciting challenge for biological physics.

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Competing interests

The authors declare no competing interests.