

Prebiotic Sustainability in the Nanoworld: A Physical Framework for Early Protocell Cluster Stability

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Abstract- This paper develops a physically grounded concept of prebiotic sustainability in the nanoworld. It shows how early protocell clusters were able to form stable, persistent, and regenerative states of order through fluctuation-induced forces, nanofluidic flows, and proton-based gradients—before genes or enzymes. The model describes sustainability as an emergent property of open non-equilibrium systems and integrates three dimensions: structural, energetic, and informational sustainability. The resulting framework enables testable hypotheses about stability, gradient duration, and regeneration processes. Thus, the approach opens up new experimental avenues for protocellular self-preservation and provides criteria for evaluating early prebiotic scenarios.

Keywords- Prebiotic sustainability; protocells; Casimir–Lifshitz forces; ion gradients; dissipative structures; origins of life.

I. INTRODUCTION

This work is the sixth paper in a seven-paper series: “A Constructivist Proto-Bio-Information Theory: A Physically Grounded Nano-Systems Architecture for Prebiotic Emergence, Information, Proto-Semantic Function, and Sustainability of Protocell Aggregation and Cluster Formation”.

Massoth [1] shows that Casimir–Lifshitz forces generate robust attraction and stable protocell clusters at 2–100 nm under prebiotic conditions. This coupling provides the structural basis on which this paper formulates prebiotic sustainability and long-term cluster stability.

Massoth [2] demonstrates that such clusters form reproducible mesoscale attractors and autonomous ε -machine dynamics. These attractor-based macrostates supply the informational substrate that this paper [2] extends into a dimension of prebiotic sustainability.

Massoth [3] shows that protocell clusters generate reproducible differences and functional meaning states. These functional states anchor the informational sustainability developed in this paper [3] as a physically stabilizable proto-function.

Massoth [4] reveals that structured resonance zones and ε -machines produce syntactic, semantic, and pragmatic layers of proto-biological information. This tri-level architecture underpins the present work, which frames sustainability as the

emergent stabilization of such multilayered information processes.

Massoth [5] identifies Matsubara-mode selection and Casimir–Polder coupling as sources of stable proton (H^+) gradients in dimers and tetrahedra. These quantum-field-driven energy structures form the energetic core that this paper develops into “energetic sustainability” and links to experimental tests.

The structure of the paper is as follows: Section I presents the motivation, state of the art, and introduces prebiotic sustainability as a physical non-equilibrium principle. Section II develops the nanophysical baseline model and derives fluctuation-induced coupling, flows, and gradient persistence conditions. Section III defines prebiotic sustainability formally and decomposes it into structural, energetic, and informational dimensions. Section IV introduces a three-level sustainability framework and maps it to reliability-engineering concepts and metrics. Section V operationalizes sustainability via a normalized structural distance and experimentally accessible order parameters. Section VI formulates testable hypotheses and an experimental roadmap for validating prebiotic sustainability. Section VII summarizes implications, limitations, and future directions for origins-of-life and synthetic protocell research.

II. MOTIVATION AND RELEVANCE

The origin of life is usually described biochemically—through prebiotic syntheses, reaction networks, and early compartments. Yet these models explain only partially how nanoscale protocells stayed stable in a fluctuating, salt-rich environment, or how early energetic and functional patterns emerged. A clear physical framework is still missing, one that specifies the conditions under which such systems could maintain structures and gradients long before genes or enzymes existed.

This paper introduces prebiotic sustainability in the nanoworld: the ability of an open nanoscale protocell system with typical radii of $R=200-1000$ nm to preserve its structural and functional identity despite fluctuations, dissipation, and continuous energy flow. Sustainability is used here not normatively, but as an emergent non-equilibrium principle

that links biophysics, origins-of-life research, and sustainability science.

From an engineering perspective [21], the proposed notion of prebiotic sustainability can be read as reliability under non-equilibrium constraints: stability corresponds to low failure probability under perturbations, persistence to extended state retention times, and regenerability to recovery after disruption. This interpretation aligns with reliability engineering views that treat complex systems as coupled structures of hardware, environment, and stochastic errors, and motivates measurable *failure* and *recovery* metrics for protocell clusters.

The approach integrates key physical mechanisms established earlier: fluctuation-induced coupling such as Casimir–Lifshitz forces [8][9] that drive robust aggregation at 2–100 nm [1]; mesoscale attractors that form stable, information-bearing macrostates [2]; and quantum-field-induced proton reservoirs [3] that create early bioenergetic gradients. Together, these results show that protocell assemblies were neither chemically isolated nor energetically disordered, but could form stable patterns through fluctuations, flows, and field modulation [6].

This raises a central question: How can stability in the prebiotic nanoworld be defined in a way that is measurable and captures physical, energetic, and informational processes at once? Classical models describe local structure but not its persistence in open, noise-dominated environments. Yet without sustained stabilization of membranes, clusters, and gradients, early selection and functionality would have been unlikely. The physical meaning of prebiotic sustainability as an emergent non-equilibrium property is summarized schematically in Figure 1.



Figure 1. The Concept of Prebiotic Sustainability

In Figure 1, a conceptual illustration of prebiotic sustainability in the nanoworld is shown. A protocell dimer forms the minimal stable unit within an open, non-equilibrium environment. Fluctuation-induced forces drive nanofluidic flows and proton gradients, generating persistent patterns despite continuous dissipation. Sustainability emerges as the coupled stabilization of structural integrity, energetic gradients, and informational pattern recurrence

under sustained inflow and entropy outflow, without genetic or enzymatic control.

Here, sustainability is defined as a three-layered property: structural sustainability (persistence of membranes and clusters), energetic sustainability (lifetimes of proton-driven gradients), and informational sustainability (robustness of recurrent state patterns without genes).

This perspective provides a physical foundation for protocell self-maintenance. Stability emerges as a natural outcome of energy flow, dissipative self-organization, and nanoscale coupling. It also points toward “synthetic sustainable protocells,” engineered systems that reproduce stable structures and gradient profiles.

Prebiotic sustainability thus appears as a general physical ordering principle—active long before biological complexity and potentially critical for the transition from simple self-organization to early bioenergetic and informational functions.

III. STATE OF THE ART

This section provides a formal definition of prebiotic sustainability and introduces a taxonomy that separates structural, energetic, and informational dimensions as experimentally accessible criteria.

A. Origins-of-Life Approaches

Origins-of-life research is commonly divided into three major lines: chemical evolution, compartment-based models, and bottom-up synthetic biology. These form the conceptual background within which a physical notion of sustainability for protocells can be developed.

Chemical evolution studies how organics arise from simple precursors; Miller–Urey showed that amino acids form under strong energy fluxes. Amphiphiles later became central because they self-assemble into membranes and create isolated reaction spaces.

Compartment models view protocells [10] as simple physicochemical interfaces. Fatty-acid vesicles form, grow, and encapsulate solutes; coacervates show strong concentration effects; polymer- and protein-based PMBCs offer more durable systems for stability studies. Bottom-up synthetic biology builds “minimal cells” that reproduce basic functions such as primitive metabolism or gradient formation, demonstrating that simple compartments can organize energy flow.

All three approaches focus on chemistry and reaction spaces, but nanoscale physical mechanisms—fluctuation forces [10], field modulation, nanofluidic flows, gradient-stabilizing couplings—remain understudied. This paper addresses this gap by integrating these effects into a physical model of prebiotic sustainability.

B. Dissipative Structures according to Prigogine

A second foundation comes from non-equilibrium thermodynamics and Prigogine’s dissipative structures. Systems far from equilibrium can form stable order when

energy and matter continuously flow; classic examples include Bénard convection, chemical oscillations, and autocatalytic networks. These structures persist only while energy is supplied.

Applied to protocells, this suggests that life began as a persistent non-equilibrium state driven by geochemical gradients, light, and thermal fluctuations. Dissipation is essential, not a defect.

Prebiotic sustainability describes this regime: energy flows maintain membranes, clusters, and gradients despite constant internal motion. The system changes but stays recognizably the same. Early protocell clusters likely behaved this way—open, fluctuation-driven systems that produced stable patterns across diffusive timescales. Dissipative structures thus provide the thermodynamic basis for a testable concept of prebiotic sustainability.

C. Reliability Engineering

Reliability engineering [21] provides a mature language for quantifying failure, recovery, and robustness in noisy, safety-critical open systems. In particular, recent surveys emphasize the need for formal definitions, taxonomies of errors, and measurable reliability metrics, which directly motivates the present operationalization of sustainability via stability, persistence, and regenerability.

IV. A PHYSICAL MODEL FOR PREBIOTIC SUSTAINABILITY IN THE NANOWORLD

The model of prebiotic sustainability assumes that early protocells existed in an ionic, strongly fluctuating environment—without enzymes, genes, or active pumps. The baseline scenario considers two vesicles of different radii (R_1 , R_2), typically 200–1000 nm in size. They reside in water at separations of $L=2-100$ nm, exactly the range where Casimir and Lifshitz forces [9] act while classical DLVO interactions are strongly screened at ionic strengths typical of plausible prebiotic brines (Debye length ~ 1 nm). Ions diffuse freely. The entire system is open and dissipative. Any persistent structure must therefore arise from coupled fluctuations, forces, flows, and ion gradients.

Under these conditions, a characteristic process chain forms. Fluctuations modulate electromagnetic modes between the membranes and generate weak but sustained Casimir–Lifshitz forces [8][9]. These forces act nonspecifically, even in salt-rich media. They pull protocells together and promote cluster formation. As separations shrink, water is expelled from the gap. This generates nanofluidic flows that advect ions. Asymmetries in size or membrane properties [19] amplify these flows. Local ion concentration differences emerge—especially for protons, which are highly mobile and respond strongly to nanoscale geometry. Early electrochemical potentials form and can persist over diffusive timescales. Such gradients reshape the microenvironment between vesicles and extend the lifetime

of emerging structures. This marks an initial step toward proto-functional stability.

The following applies to the strength of the attractive Casimir-Lifshitz force between two protocells:

$$F_{CL}(L) \approx - (A_{\text{eff}} / 6) * (R_{\text{eff}} / L^2) \quad (1)$$

with $R_{\text{eff}} = (R_1 * R_2) / (R_1 + R_2)$.

Here, A_{eff} denotes an effective Hamaker constant [10] that integrates the spectral dielectric response of the membrane–water system. For numerical calculations we use an effective Hamaker constant $A_{\text{eff}} = 5 \times 10^{-21}$ Joule, representative of membrane-water-membrane systems. The force scales linearly with the effective curvature radius R_{eff} and decays algebraically as $1/L^2$.

In the $L=2-100$ nm range, these Casimir-Lifshitz-forces can exceed thermal contributions [13][14][15][16][17][18][20]. Proton diffusion [11] times scale as $\tau_{\text{diff}} \approx L^2/D$. Because protons diffuse rapidly, these times are very short. For stable gradients, the persistence time τ_{gradient} must therefore match or exceed τ_{diff} . Only then do energy flows stabilize the structure rather than disrupt it. Prebiotic sustainability emerges from the interplay of energy flux, geometry, and gradient lifetime.

The model leads to a clear conclusion: the earliest proto-functional stability did not arise *despite* fluctuations, but *because of* them. Fluctuations generate forces. Forces generate flows. Flows generate gradients. And gradients generate stable patterns that can serve as precursors of biological function. Sustainable protocell clusters thus make active use of fluctuations. They convert noise into persistent micro-energy potentials. Sustainability becomes an emergent non-equilibrium phenomenon and a necessary precondition for early prebiotic self-organization.

Physical Pipeline of Prebiotic Sustainability:

For readers with an engineering or systems background, the core mechanism of the proposed framework can be summarized as a five-step physical pipeline:

- Fluctuations in confined nanoscale gaps generate effective coupling forces between adjacent protocell membranes.
- Coupling stabilizes proximity, driving cluster formation and inducing nanofluidic expulsion and directed microflows in the intermembrane gap.
- Flows combined with geometric or material asymmetry create local ion and proton concentration biases without pumps or enzymes.
- Resulting gradients extend state lifetimes, enabling persistent energetic and structural configurations beyond diffusive relaxation times.
- Attractor-like return dynamics allow disrupted clusters to reassemble into functionally equivalent states, providing regenerability without replication.

Together, these steps explain how stability, persistence, and recovery emerge as purely physical properties of open, fluctuation-driven protocell systems.

Massoth [2] showed that protocells in prebiotic environments likely did not exist as isolated compartments. Theoretical models and recent experimental analogues indicate that nanoscale vesicles and amphiphile aggregates can spontaneously assemble into clusters in saline solutions through Casimir–Lifshitz attraction. This attraction arises from the reduction of electromagnetic fluctuations in the gap between two membranes and makes certain geometries energetically favored. Two simple but structurally central clusters emerge: the protocell dimer and the protocell tetrahedron.

V. DEFINITION AND TAXONOMY OF PREBIOTIC SUSTAINABILITY

The physical model shows that early protocell clusters could form stable mesoscale structures because they integrated fluctuations, flows, and ion gradients. Sustainability here does not arise from biological control but from non-equilibrium physics. The goal is a precise definition that remains accessible to biologists, computer scientists, and sustainability researchers, while integrating key results from earlier papers—Casimir–Lifshitz forces, attractor-based informational patterns, and quantum-field-induced proton reservoirs.

A. Definition of Prebiotic Sustainability

Prebiotic sustainability denotes the ability of an open, nano-compartmentalized protocell system to preserve its structural and functional identity over time, despite strong fluctuations and continuous energy flow. This occurs without enzymes, genes, or pumps. The system remains recognizably the same entity even as it produces entropy and undergoes constant internal dynamics.

Physically, this implies *controlled openness*: the system is far from equilibrium, produces entropy ($dS/dt > 0$), and uses fluctuations actively to generate forces, flows, and gradients. For biologists, this is proto-bioenergetic self-maintenance; for computer scientists, the stabilization of pattern states; for sustainability science, the preservation of identity under change.

Formally, sustainability can be expressed using an order-parameter vector $\theta(t)$ describing cluster geometry, contacts, or gradient profiles. A system is sustainable when the normalized structural deviation

$$D(\theta(t+\Delta t), \theta(t)) < \delta \quad (2)$$

while energy flow persists. Sustainability is not equilibrium but the persistence of macroscopic signatures in a fluctuating, dissipative system.

Specification of Prebiotic Sustainability in the Nanoworld:

A protocell system (cluster) is sustainable if the entropy production rate $\sigma(t) > 0$ in steady operation and for $S(\text{Total})$ with $(\text{Total} = \text{system} + \text{environment})$, the following applies

$$\frac{dS(\text{Total})}{dt} > 0 \text{ and } D(\text{Structure}, t+\Delta t | t) = \frac{\|\theta(t+\Delta t) - \theta(t)\|}{\|\theta(t)\|} < \delta$$

consistent with open, dissipative operation, under continuous energy input and dissipation, with $\theta(t)$ representing experimentally accessible order parameters (cluster geometry, ion gradients), and δ a small, system-dependent tolerance for structural drift.

Here, $\|\cdot\|$ is the Euclidean norm, and δ may correspond to “<3% structural change per Δt ”.

Interpretation:

- $dS_{\text{system}}/dt > 0$: The system produces entropy \rightarrow it is open and dissipative, not equilibrated.
- $D(\text{Structure}, t+\Delta t | t) < \delta$: Despite flow and dissipation, the structure remains sufficiently similar \rightarrow identity under change.
- *Continuous energy input*: Classic Prigogine setting; without energy input, every dissipative structure collapses.

The key lies in D : what counts as “structure” in a protocell cluster?

D measures the normalized distance between relevant order parameters at two times t and $t+\Delta t$. Framework prebiotic sustainability in the nanoworld means that this macroscopic signature drifts only weakly, even though energy is continuously dissipated. In practice the order parameter $\theta(t)$ could be, for example: mean nearest neighbor distance, width of distance distribution, mean number of vesicles per cluster, mean number of contacts per vesicle, proportion of vesicles in clusters, mean and variance of pH differences in the intermembrane gaps, and so on.

B. Three Dimensions of Prebiotic Sustainability

These three dimensions jointly describe early self-maintenance in protocellular systems and can be experimentally assessed.

(i) Structural Sustainability:

Structural sustainability is the ability of a protocell cluster to maintain its spatial organization. It includes the integrity of individual membranes and the persistence of collective arrangements such as dimers or larger supramolecular patterns. Thermal noise, mechanical perturbations, and diffusion may distort the architecture only moderately. Key metrics include lifetime, coalescence and fission rates, and return to stable attractor shapes. This directly connects to [1], which showed that Casimir–Lifshitz forces provide robust mesoscale binding in the 2–100 nm regime.

(ii) *Energetic Sustainability:*

Energetic sustainability refers to the stability of electrochemical potentials—especially proton and ion gradients within or between protocells. The critical question is whether the persistence time of a gradient (τ_{gradient}) matches or exceeds the diffusive relaxation time (τ_{diff}). This dimension links the fluctuation-driven model of the present paper with the quantum-field-induced proton reservoirs of [5], where narrow gaps act as resonant proton traps. Energetic sustainability means that the system organizes energy flows into long-lived, functionally relevant potential landscapes.

(iii) *Informational Sustainability:*

Informational sustainability describes the stability and recurrence of physical patterns [2][7] on mesoscale levels. These include stationary concentration profiles, characteristic cluster spacings, or recurring flow cycles that act as elementary informational states without genes. Such patterns must remain reproducible despite fluctuations and function as attractor-based proto-memory structures. This dimension connects to [2], where reproducible differences were defined as physical information, and to the attractor architectures observed in protocell clusters.

Summary:

- *Structural sustainability:* the “vessel” (compartment or protocell) remains intact.
- *Energetic sustainability:* “energy in the form of gradients” remains available long enough to matter.
- *Informational sustainability:* “patterns” and “states” remain recognizable over time.

Together, these three dimensions form a coherent physical taxonomy of sustainability in the prebiotic nanoworld. They place protocellular persistence into a unified framework that integrates structure, energy, and information.

The following sections translate this taxonomy into a three-level sustainability framework—stability, persistence, and reproducibility/regenerability—and derive concrete experimental hypotheses.

VI. CONCEPTUAL SUSTAINABILITY FRAMEWORK (THREE-LEVEL MODEL)

The definition of prebiotic sustainability leads to a three-level framework: stability, persistence, and reproducibility/regenerability. Each level builds on the previous one. Together, they connect Casimir–Lifshitz forces, early proton landscapes, and attractor-based patterns into a coherent model of protocellular self-maintenance.

The definition of prebiotic sustainability leads naturally to a three-level framework, summarized in Figure 2.

In Figure 2, Schematic three-level framework of prebiotic sustainability. Structural stability arises from Casimir–

Lifshitz coupling between protocells. Persistence is achieved when proton-gradient lifetimes exceed diffusive relaxation times ($\tau_{\text{gradient}} \geq \tau_{\text{diff}}$).

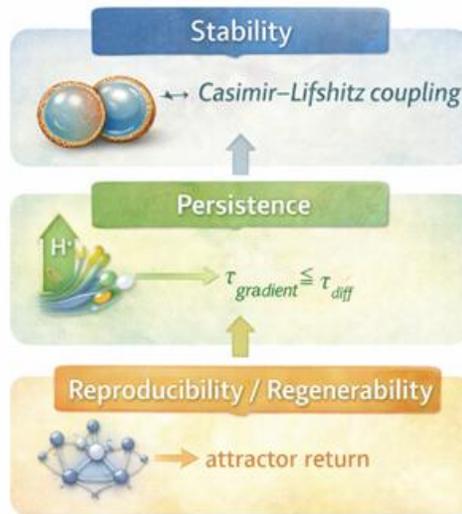


Figure 2. Three-Level Sustainability Framework

Reproducibility and regenerability emerge from attractor-based return dynamics, enabling recovery of functionally equivalent states after perturbation in the absence of genetic or enzymatic control.

TABLE I. MAPPING OF PREBIOTIC SUSTAINABILITY LEVELS TO RELIABILITY ENGINEERING CONCEPTS

Prebiotic Sustainability Level	Reliability Engineering Analogon	Representative Measurement / Metric
Stability	Low hazard rate / high Mean Time To Failure (MTTF)	Cluster break-up rate; mean lifetime of dimer or cluster structures
Persistence	State retention time / operational availability	Ratio $\tau_{\text{gradient}} / \tau_{\text{diff}}$; duration of stable ion or proton gradients
Regenerability	Mean Time To Recovery (MTTR) / recoverability	Reassembly time after perturbation; return probability to functional attractor state

Table I relates the physical notion of prebiotic sustainability to established reliability-engineering metrics, enabling quantitative assessment of protocell cluster stability, persistence, and recovery under non-equilibrium conditions.

This mapping enables importing mature reliability engineering metrics for quantifying sustainability in protocell clusters.

A. *Stability*

Stability describes the ability of a protocell cluster to withstand perturbations without losing its basic structure. Membranes should not rupture immediately, and fusion or

fission events should not cause collapse. Despite thermal noise, characteristic distances and geometries must survive.

The key drivers are fluctuation-induced forces. Casimir–Lifshitz attractions in the 10–200 nm range prevent uncontrolled separation and create weak but continuous coupling. These forces act without specific chemistry and keep compartments within valid structural regimes. Membrane composition—flexible fatty acids, lipids, or polymer shells—reinforces this effect.

Stability therefore defines a baseline order space in which protocells remain identifiable despite noise. For computer scientists, it corresponds to remaining inside the valid state space. For biologists, it is the preservation of compartments. For sustainability research, it represents the minimal condition for long-term viability.

B. Persistence

Persistence extends stability through time. A state must last long enough for gradients, reaction pathways, or pattern formation to operate. The critical factor is the lifetime of ion gradients, especially in nanoscale gaps.

The relevant timescale is $\tau_{\text{diffusion}}$, while τ_{gradient} denotes the actual gradient lifetime. Persistence occurs when $\tau_{\text{gradient}} \geq \tau_{\text{diffusion}}$. Narrow geometries, flows, and fluctuations can slow relaxation and create proto-bioenergetic plateaus.

Persistence means that energy flows do not immediately destroy structures but temporarily stabilize them. For biologists, this resembles an early energetic steady state without ATP synthase. For computer scientists, it corresponds to the retention time of a state. For sustainability science, it represents the physical form of long-term viability.

C. Reproducibility and Regenerability

The third level describes the ability to return to a functionally similar state after disruption—without genes, enzymes, or complex replication mechanisms. This is not biological reproduction but physical reproducibility of structures and gradients.

Regenerability arises from the same mechanisms that drive cluster formation: fusions replace damaged compartments; fission and re-assembly create new ones; flows can rebuild collapsed proton potentials. In the language of earlier papers, this corresponds to attractor-based return dynamics. The restored state is not identical but functionally equivalent.

For biologists, this resembles rudimentary autopoiesis. For computer scientists, it is a form of physical error correction. For sustainability research, it represents the earliest regenerative capability.

Summary:

This framework defines a robust physical notion of sustainability:

- Stability prevents immediate collapse.

- Persistence supports long-lived energetic and structural patterns.
- Reproducibility/Regenerability enables return to functional states after disturbance.

A prebiotic protocell cluster that satisfies all three levels can be considered sustainable—long before biological evolution or molecular control. The next section translates this framework into concrete experimental hypotheses.

VII. OPERATIONAL DEFINITION OF THE STRUCTURAL DISTANCE D

To quantify prebiotic sustainability in protocell clusters, we define a structural distance D that measures the normalized drift of the mesoscale system signature over time. Let $\theta(t)$ denote a vector of experimentally accessible order parameters capturing cluster geometry, energetic gradients, and recurrent dynamical patterns.

$$\theta(t) = (\theta_1(t), \theta_2(t), \dots, \theta_n(t)) \quad (3)$$

The structural distance D between two times t and t + Δt is defined as a weighted, normalized root-mean-square drift:

$$D(t, \Delta t) = \sqrt{(\sum_{i=1}^n w_i \cdot [(\theta_i(t + \Delta t) - \theta_i(t)) / \theta_{i,\text{ref}}]^2)} \quad (4)$$

where:

- $\theta_i(t)$ = i-th order parameter at time t (dimensionful or dimensionless).
- $\theta_{i,\text{ref}}$ = reference scale used for normalization (e.g., baseline mean, initial value, or experimentally defined characteristic scale).
- $w_i \geq 0$ = weights (often set to $w_i = 1$ if no prior weighting is justified).
- n = number of included order parameters.

Here w_i are dimensionless weights ($\sum w_i = 1$) and $\theta_{i,\text{ref}}$ are reference scales used for normalization. In practice, $\theta_{i,\text{ref}}$ are chosen as robust characteristic values measured in an initial calibration window $[t_0, t_0 + T_0]$, or, where appropriate, as physically motivated scales.

Operational sustainability criterion:

A protocell cluster is operationally “sustainable” over Δt if it remains dissipative and its mesoscopic signature drifts only weakly:

$$dS_{\text{system}}/dt > 0 \text{ and } D(t, \Delta t) < \delta \quad (5)$$

with δ a small tolerance (experiment-dependent; e.g., $\delta = 0.03\text{--}0.05$ for “3–5% normalized drift per Δt”).

Sustainability thus corresponds to persistent identity under non-equilibrium conditions, rather than equilibrium stability.

Practical guidance for choosing $\theta(t)$:

$\theta(t)$ should combine a minimal set of geometric, energetic, and (optionally) pattern/attractor descriptors that are directly measurable (microscopy + pH/ion imaging), e.g. nearest-neighbor distance statistics, contact number, cluster size, mean/variance of ΔpH in gaps, and estimated τ_{gradient} .

Operational θ -vector for microscopy, pH-mapping, and microfluidics:

For experimental realizations based on time-resolved microscopy, fluorescence pH maps, and microfluidic perturbations, we propose the following minimal and sufficient parameter set ($n = 9$):

Structural (geometry):

- $\theta_1(t)$ Mean nearest-neighbor distance $\langle L_{nn} \rangle$
- $\theta_{1,\text{ref}} = \text{median} \langle L_{nn} \rangle$ in $[t_0, t_0 + T_0]$
- $\theta_2(t)$ Standard deviation of nearest-neighbor distances $\sigma(L_{nn})$
- $\theta_{2,\text{ref}} = \text{median} \sigma(L_{nn})$
- $\theta_3(t)$ Cluster size (number of vesicles in the largest connected cluster)
- $\theta_{3,\text{ref}} = N_{\text{cluster}}(t_0)$
- $\theta_4(t)$ Mean contact number $\langle k \rangle$ (graph-based adjacency)
- $\theta_{4,\text{ref}} = \text{median} \langle k \rangle$
- $\theta_5(t)$ Cluster fraction f_{cluster} (vesicles in clusters / total vesicles)
- $\theta_{5,\text{ref}} = f_{\text{cluster}}(t_0)$

Energetic (pH maps, gradients):

- $\theta_6(t)$ Mean proton gradient $\langle \Delta pH_{\text{gap}} \rangle$ (gap vs. bulk)
- $\theta_{6,\text{ref}} = |\langle \Delta pH_{\text{gap}} \rangle|$ at t_0
- $\theta_7(t)$ Variance of gap gradients $\sigma(\Delta pH_{\text{gap}})$
- $\theta_{7,\text{ref}} = \text{median} \sigma(\Delta pH_{\text{gap}})$

Dynamical / sustainability metrics:

- $\theta_8(t)$ Gradient persistence time τ_{gradient} (autocorrelation decay time)
- $\theta_{8,\text{ref}} = \tau_{\text{diff}} = L_{\text{gap}}^2 / D_{\text{H}^+}$. Here L_{gap} is the median intermembrane separation extracted from microscopy over $[t_0, t_0 + T_0]$, and T_{obs} is the total recording duration.
- $\theta_9(t)$ Return (recovery) time T_{return} after perturbation
- $\theta_{9,\text{ref}} = T_{\text{obs}}$ (total observation window)

We set the sustainability threshold δ to 0.05 as a conservative default ($\approx 5\%$ normalized drift per Δt), and in practice calibrate δ from control recordings by choosing $\delta = P95(D_{\text{control}})$ (95th percentile, equivalently, $\delta \approx 3\sigma_D$) to account for platform-specific measurement noise

Interpretation:

Small values of D indicate that a protocell cluster remains within the same effective attractor basin despite continuous entropy production and microscopic rearrangements. Large D signals structural drift, loss of gradient coherence, or transition to a different dynamical regime.

In this formulation, D provides a compact, experimentally accessible measure of identity preservation under flow and fluctuation, enabling direct comparison of protocell systems in terms of structural, energetic, and informational sustainability.

VIII. TESTABLE HYPOTHESES AND EXPERIMENTAL ROADMAP

Prebiotic sustainability must be experimentally verifiable. To achieve this, the key mechanisms of the model—structural coupling, gradient persistence, and simple regeneration—are translated into measurable quantities.

The roadmap defines four hypotheses that map onto the three levels of the framework (stability, persistence, regenerability) and can be tested in minimal protocell models. The goal is to demonstrate physical sustainability phenomena without genetic or enzymatic complexity. The following hypotheses translate the physical sustainability framework into experimentally testable regimes (Fig. 1).

Figure 1 summarizes the experimental roadmap (H1–H4) for probing prebiotic sustainability in protocell systems. It contrasts lifetimes of isolated and clustered protocells, visualizes proton-gradient formation in nanoscale gaps, relates gradient persistence to heat dissipation, and maps sustainability regimes in parameter space defined by effective curvature, gradient lifetime, and temperature.

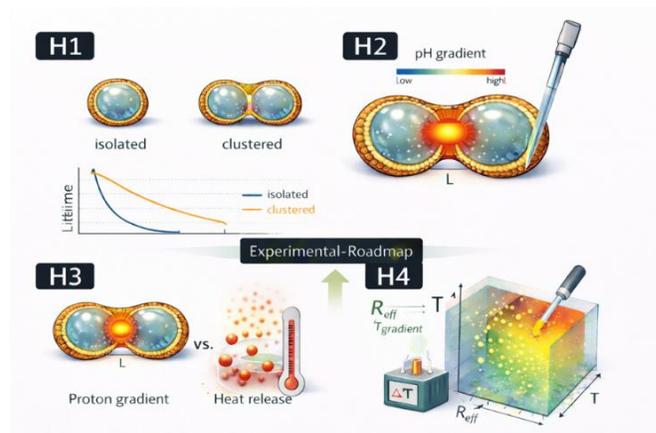


Figure 3. Experimental roadmap for testing prebiotic sustainability mechanisms

A. Hypothesis H1 – Structural Sustainability:

Protocell clusters outlive isolated vesicles because fluctuation-induced forces stabilize their collective structure. Casimir–Lifshitz attractions couple vesicles at distances of 2–100 nm [8][9][13][14]. Single vesicles in saline environments often degrade quickly. Clusters, by contrast, form mesoscale networks that protect and stabilize compartments.

Experiment H1E:

Time-resolved microscopy (confocal or holographic). Compare two conditions: dilute solutions (isolated vesicles) and higher concentrations (cluster formation). Measure lifetime, decay rates, structural drift, and spontaneous fusion events. Longer lifetimes in clusters would directly demonstrate structural sustainability.

B. Hypothesis H2 – Asymmetry and Ion Gradients:

Asymmetric vesicle pairs generate measurable proton and ion gradients in the gap region through geometry, fluctuations, and flow alone.

The process chain is: fluctuation \rightarrow force \rightarrow flow \rightarrow gradient. Differences in radii or membrane stiffness produce asymmetric gaps. Displaced fluid creates directed nanofluidic flows and local concentration shifts.

Experiment H2E:

Fluorescence-based pH microscopy or micro-pH electrodes in microfluidic chambers. Observe vesicle pairs with defined size ratios ($R_1 > R_2$). Expect a stable pH difference in the gap and proton drift toward the asymmetric region. Evidence of this would show that early, protein-free gradient dynamics could arise purely from physical principles.

C. Hypothesis H3 – Energetic Sustainability and Entropy Production:

Protocell configurations with long-lived ion gradients exhibit lower entropy production per time and volume than configurations with short-lived gradients.

Long-lived gradients indicate efficient use of energy flows. Short-lived gradients dissipate rapidly and produce more heat.

Experiment H3E:

Combine gradient measurements (Δc , $\Delta \mu$, τ_{gradient}) with thermal measurements (isothermal microcalorimetry or μ -thermography). If stable gradients correlate with reduced local heat release, energetic sustainability is experimentally supported.

D. Hypothesis H4 – Sustainability and the Three-Layer Framework:

Observable dynamics can be statistically grouped into the three sustainability levels—stability, persistence, and regenerability. Cluster dynamics can be classified via low/medium/high D regimes.

The result is a multidimensional dataset of lifetime, gradient persistence, reassembly rates, and other parameters. The question is whether these values form robust classes.

Experiment H4E:

Vary vesicle parameters (size, stiffness, salt concentration, temperature, pH). Analyze time series using clustering, Principal Component Analysis (PCA), Density-

Based Spatial Clustering of Applications with Noise (DBSCAN), t-distributed Stochastic Neighbor Embedding (t-SNE), or spectral methods.

Three classes are expected:

- (1) *Stable but not persistent*
- (2) *Stable and persistent but weakly regenerable*
- (3) *Fully sustainable*

Such a result would empirically validate the three-level sustainability framework.

IX. CONCLUSION AND FUTURE WORK

The concept of prebiotic sustainability shifts the focus of origins-of-life research. Instead of emphasizing individual reaction pathways, it highlights a physical understanding of stability, persistence, and regenerability in nanoscale protocell clusters. Our analysis shows that fluctuation-induced forces, nanofluidic flows, and proton-based gradients can generate long-lived ordered states even in simple, non-genetic compartments. These states persist over diffusive timescales and can return to functionally similar configurations after disturbances. Prebiotic sustainability thus appears as an emergent property of open, fluctuation-coupled systems—not as a late product of biological optimization.

The proposed triad of structural, energetic, and informational sustainability provides a scale-invariant framework connecting physics, prebiotic chemistry, and early information. Combined with the three levels of stability, persistence, and regenerability, this framework enables direct comparison of different protocell types—from fatty-acid vesicles to PMBC systems. Hypotheses H1–H4 and the experimental roadmap show that these concepts can be tested using current protocell technologies, microfluidics, pH microscopy, calorimetry, and data-driven analysis.

In the long term, the approach opens two major directions. For origins-of-life research, it offers a way to compare scenarios by their sustainability profiles: Which protocell types maximize structural, energetic, and informational sustainability—and could this have served as a key selection criterion in prebiotic evolution? For synthetic biology and sustainability science, the framework points toward “synthetic sustainable protocells,” engineered systems that replicate physical sustainability and could be applied in biohybrid or technological nanosystems.

Prebiotic sustainability thus emerges as a foundational ordering principle that may have enabled the transition from pure physical self-organization to early bioenergetic and informational functions. The central challenge now is to reveal this three-dimensional sustainability signature in the laboratory—to make measurable the traces of a world in which sustainability was already at work long before life existed in its modern form.

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