

ACADEMIC WHITE PAPER

FROM THE FOURTH PHASE TO THE LIVING INTERFACE

A Nonequilibrium Framework for
Exclusion-Zone Phenomena, Interfacial Charge,
Energy Transduction, and Biological Organization

A Constructive Transdisciplinary Engagement with
Gerald H. Pollack, His Colleagues, and His Critics



Bichara Sahely

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Model C is advanced as a serious, falsifiable research hypothesis — not as an established fact.

Publication and Methodological Note

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This academic white paper was conceived, directed, critically reviewed, and approved by the author. Artificial intelligence — principally OpenAI's GPT-5.5 Thinking model — was used as a research, synthesis, drafting, source-mapping, and editorial instrument under sustained human supervision. The author determined the governing thesis, conceptual architecture, evidentiary standards, interpretive judgments, and final wording. All substantive claims remain the responsibility of the author. AI assistance does not substitute for independent experimental verification, peer review, or specialist evaluation by interfacial physicists, physical chemists, colloid scientists, spectroscopists, biophysicists, physiologists, and other relevant investigators.

Scope and methodological note

This paper is a constructive theoretical and methodological white paper. It is neither a defence brief for Gerald H. Pollack's proposed fourth phase of water nor an argument that conventional transport explanations are mistaken. It asks whether the dispute has been framed too narrowly and whether a third model — an emergent nonequilibrium-interface model — can preserve the strongest observations and mechanistic findings on both sides while generating new, discriminating experiments. Five evidentiary levels are distinguished: directly observed phenomena; independently reproduced observations; mechanistically supported explanations; plausible integrative inferences; and speculative biological or ecological extensions. Evidence for molecular-scale hydration effects is not treated as proof of macroscopic exclusion-zone dimensions, while evidence that ionic gradients move tracer particles is not treated as proof that every property of the associated interface has been exhaustively characterized. The review is forensic and transdisciplinary rather than a formal PRISMA systematic review. Citation metadata and the targeted literature update were rechecked through 5 July 2026. The update includes post-2020 work directly relevant to solute exclusion, wavelength-dependent electrical measurements, temperature and cryoprotectant effects, living-cell hydration, membranes, glycocalyx function, mitochondrial energy transduction, and constraint-based biological organization. Inclusion indicates relevance, not endorsement, and does not convert this forensic evidence map into a formal systematic review or meta-analysis.

Terminological note

Exclusion zone (EZ) is used operationally for a region adjacent to a hydrated surface from which specified tracers or solutes are depleted under specified conditions; the term does not by itself establish molecular structure or thermodynamic status. Interfacial water denotes water altered through proximity to a surface, membrane, macromolecule, solute, matrix, or field. Structured water is used only when the relevant order, mobility, density, hydrogen-bond topology, or spectral property is specified. Fourth phase refers to Pollack's proposed extended, charge-separated, molecularly differentiated aqueous state without presuming its status as an equilibrium phase. An emergent nonequilibrium interface is a spatially and temporally differentiated organization generated through reciprocal coupling among a hydrated surface, water dynamics, ions, electrical polarization, energy absorption, gradients, and transport. A living interface is such an organization when biologically embedded and contributing to boundary maintenance, selective exchange, energy transduction, signalling, mechanics, repair, or adaptation.

Abstract

Exclusion-zone phenomena occupy an unusual position in contemporary interfacial science. Gerald H. Pollack and collaborators have reported that water adjacent to certain hydrophilic surfaces develops extended regions that exclude tracer particles and some solutes, carry electrical potential, coexist with proton-enriched regions, exert measurable forces, respond to radiant energy, and may support spontaneous fluid movement. Independent investigators have confirmed that long-range particle-depleted regions can arise near Nafion and have shown that ion exchange, unequal ionic diffusion, electrical fields, electrophoresis, and diffusiophoresis can account quantitatively for substantial aspects of tracer displacement. These findings are often presented as mutually exclusive: either the exclusion zone is a structurally distinct fourth phase of water, or it is an ordinary transport phenomenon requiring no revision of water's biological role. This paper argues that the binary is premature. It develops Model C, the emergent nonequilibrium-interface model, according to which surface-conditioned water dynamics, ionic redistribution, electrical polarization, chemical gradients, radiant-energy absorption, material mechanics, and transport are reciprocally coupled aspects of a dynamically maintained organization. Model C incorporates conventional electrokinetic and diffusiophoretic mechanisms while asking whether they exhaust the ontology of the interface. The framework separates tracer exclusion from molecular structure, transport mechanism, energy transduction, and biological function; formulates discriminating predictions concerning spatial extent, spectroscopy, gradient neutralization, illumination, thermal controls, geometry, hysteresis, energy storage, and living function; and proposes a preregistered adversarial research programme. Pollack's structural-phase model remains incompletely established, while transport-dominant explanations remain powerful but potentially incomplete. Model C is therefore advanced not as fact, but as a serious, falsifiable, and potentially unifying research hypothesis.

Keywords: *Exclusion-zone water; interfacial water; nonequilibrium thermodynamics; diffusiophoresis; electrophoresis; Nafion; charge separation; hydration dynamics; radiant energy; biological interfaces; autopoiesis; living systems.*

CENTRAL THESIS

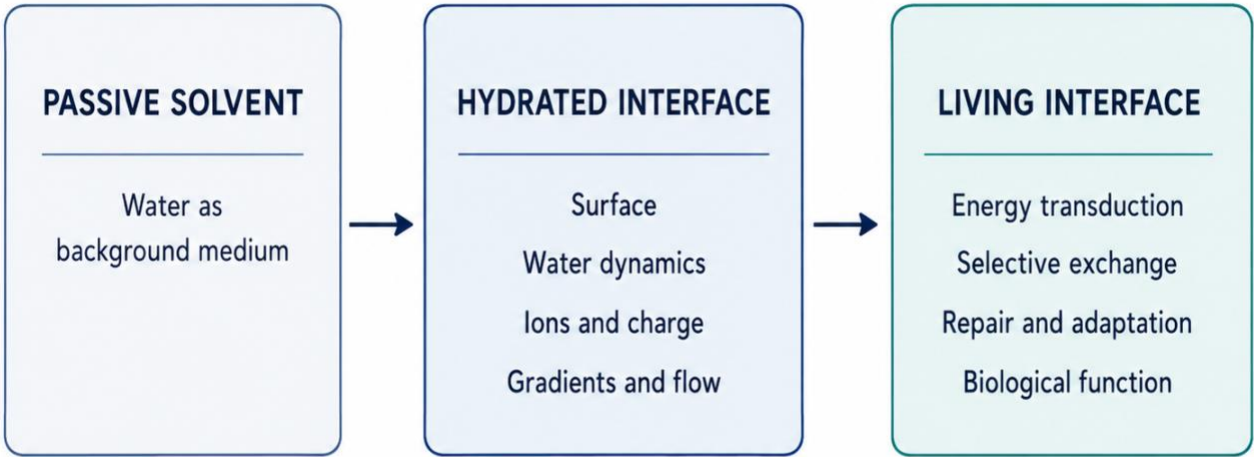
Exclusion-zone phenomena should not be understood through a forced choice between a static fourth phase of water and conventional transport processes. A more comprehensive hypothesis is that hydrated surfaces, water, ions, electromagnetic energy, electrical polarization, chemical gradients, and material flows form a dynamically maintained nonequilibrium interfacial organization in which molecular differentiation and transport are mutually constitutive. This emergent interface may explain the co-occurrence of solute exclusion, charge separation, proton redistribution, force generation, fluid movement, radiant-energy responsiveness, and biological function more comprehensively than models that treat these observations as disconnected effects.

Original Contribution

The paper makes four principal contributions. First, it separates four questions that are frequently conflated: Does exclusion occur? What moves the tracer? What is the physical state of the interfacial region? Does the coupled interface perform a biological function? Second, it reconstructs the controversy through three models: Model A, a transport-dominant account centred on ion exchange, electrodiffusion, electrophoresis, and diffusiophoresis; Model B, Pollack's structural-phase model; and Model C, an emergent nonequilibrium-interface model in which water organization, gradients, polarization, energy uptake, mechanics, and transport arise reciprocally. Third, it introduces the relational experimental principle: preserve the relations first; perturb them second; dissect them last. Fourth, it converts a polarized interpretive dispute into a sequence of experiments whose results could strengthen, restrict, revise, or displace each model.

Figure 1. From Passive Solvent to Emergent Living Interface

The unit of explanation shifts from water as background to a coupled, maintained interfacial process.



Model C asks whether these relations form a causally integrated nonequilibrium organization.

Figure 1. From passive solvent to emergent living interface.

The conceptual transition preserves conventional surface and transport physics while asking whether their reciprocal coupling constitutes a dynamically maintained interfacial organization.

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Part I — The Scientific Problem

This Part separates the empirical phenomenon from its competing interpretations. It establishes that long-range tracer depletion near Nafion is reproducible under some conditions, that conventional transport mechanisms explain substantial aspects of particle motion, and that neither finding alone settles the molecular, energetic, or biological ontology of the interface.

1. The Unresolved Status of Exclusion-Zone Phenomena

The controversy must begin with an operational distinction: observing a particle-depleted region is not the same as identifying the force that moved the tracer, the molecular state of the water, or the function of the complete interface. Independent replication of the phenomenon and replication of its interpretation are different evidentiary achievements (Zheng et al., 2006; Florea et al., 2014; Huszár et al., 2014).

Scientific controversies often become distorted when an observation becomes inseparable from the first theory proposed to explain it. Investigators who doubt the theory may begin speaking as though the phenomenon itself were doubtful. Defenders of the originating theory may treat successful replication of the phenomenon as confirmation of the complete explanatory framework.

The foundational empirical claim is comparatively modest: under certain experimental conditions, regions adjacent to hydrated surfaces become depleted of particular suspended particles or dissolved tracers. In the early programme developed by Zheng, Chin, Khijniak, Khijniak, and Pollack, particle- and solute-depleted regions were reported near several hydrophilic materials, often extending much farther from the surface than conventional molecular hydration shells. These observations motivated the proposal that the affected region was not ordinary bulk water but an extended interfacial state with distinct physical properties.

At minimum, an observed particle-free region establishes that the particles do not remain uniformly distributed. It does not yet establish why they moved, whether every dissolved species is similarly excluded, whether water throughout the region possesses a common molecular structure, or whether the region constitutes a thermodynamic phase. A tracer may be displaced by an electrical field, chemical gradient, concentration-dependent mobility, convection, surface interaction, osmotic flow, diffusiophoresis, or combinations of these processes. The absence of a tracer is therefore an observable consequence requiring explanation, not a direct molecular image of the solvent.

A later independent study used ultraviolet-visible spectrophotometry rather than microscopy alone and reported long-range depletion of low-molecular-weight dissolved substances near hydrophilic surfaces, with equilibrium behaviour influenced by surface hydrophilicity and solution concentration (Hu et al., 2022). The result broadens the operational evidence beyond charged microspheres, but it does not by itself identify the solvent structure or distinguish a surface-conditioned transport field from an extended aqueous state.

Long-range particle-depleted regions adjacent to Nafion and certain other hydrated surfaces are genuine experimental phenomena. Their visible formation is substantially explained by ion exchange, chemical and electrical gradients, electrophoresis, and diffusiophoresis. These findings weaken any account that treats tracer exclusion alone as direct proof of a macroscopic crystalline water phase. They do not, however, establish that the complete interfacial system has been exhaustively characterized or that water organization, charge separation, energy absorption, and transport are merely unrelated consequences of surface chemistry.

Pollack's programme remains important because it asks whether the interface should be treated as an active, energy-responsive organization rather than as passive water disturbed locally by a surface.

The critics remain indispensable because they have shown that familiar transport mechanisms can create apparently extraordinary spatial effects and that tracer behaviour is inseparable from electrochemical conditions.

It is to determine whether Pollack's proposed structural phase, the transport-dominant alternative, or an emergent nonequilibrium-interface model best predicts the complete interfacial constellation.

That requires first understanding the conceptual reversal Pollack placed before the scientific community: water may not be merely the medium in which interfacial processes occur. Under appropriate constraints, it may participate in bringing the interface itself into being.

Table 1. *Observed phenomena, evidentiary status, and unresolved interpretation*

Phenomenon	Current status	Unresolved question
Long-range tracer depletion near Nafion	Independently reproduced under some conditions	Which mechanisms dominate for each tracer, geometry, and timescale?
Ion/proton gradients and electrical potential	Directly observed; transport models quantitatively supported	Do water or material-state variables contribute independently to gradient formation or recovery?
Radiant-energy response	Reported by originating programme	Does any wavelength-specific effect survive equal-energy and spatially matched thermal controls?
Spectral or structural differentiation	Local interfacial-water differentiation is well established; macroscopic EZ structure is not	Is there a tracer-independent order parameter extending beyond conventional hydration?
Spontaneous flow and force	Reported and partly linked to gradients/electrokinetics	Can energy and momentum budgets be closed under stringent controls?
Biological correspondence	Strong plausibility at molecular interfaces; direct EZ equivalence unproven	Does an integrated interface state predict function, reserve, or repair?

2. Pollack's Copernican Proposition

Pollack's strongest contribution is an ontological reversal rather than a single measurement: water adjacent to surfaces may participate actively in charge separation, transport, force, and energy response rather than serving only as an inert background. The proposal is scientifically consequential even though its structural-phase formulation remains unproven (Chai et al., 2009; Chai & Pollack, 2010; Pollack, 2013).

To describe Gerald Pollack's proposal as Copernican is not to claim that its truth has already been established or that its historical importance is equivalent to the heliocentric revolution. The analogy concerns the form of the proposed inversion.

Before Copernicus, increasingly sophisticated calculations could preserve the appearance of an Earth-centred cosmos by adding corrective motions to an inherited framework. Copernicus changed the organizing centre from which the observations were interpreted. Familiar celestial movements did not disappear; they were repositioned within a different relational architecture.

The prevailing molecular description of biological systems commonly places proteins, nucleic acids, membranes, ions, metabolites, and molecular motors in the foreground. Water appears primarily as the solvent in which those privileged agents diffuse, collide, bind, react, and change conformation. Its properties matter, but

water is often treated as the comparatively homogeneous medium within which the more biologically specific components perform the causal work.

Pollack's proposition reverses that hierarchy. He asks whether the properties commonly attributed to biomolecules alone may emerge partly from the aqueous interfaces those molecules collectively generate. Under this view, water is not an inert stage on which life acts. Water, surfaces, ions, charge, and energy participate together in producing the physical conditions under which life's molecular actors can act at all.

Pollack's earlier work framed the cell as a system extraordinarily rich in polymeric surfaces. Proteins, cytoskeletal filaments, nucleic acids, membranes, organelles, polysaccharides, and extracellular matrices collectively create immense interfacial area. The cytoplasm is not a dilute solution bounded only by a plasma membrane. It is densely occupied by macromolecules and organized structures, leaving much cellular water close to some charged, polar, hydrophilic, or hydrophobic surface. Pollack therefore argued that the surface–water relationship deserved a more central role in cell biology than it had conventionally received.

His experiments do not yet establish every element of the fourth-phase model. In particular, they do not yet provide an independently confirmed molecular structure or thermodynamic order parameter demonstrating that the entire tracer-depleted region constitutes one classical phase.

Can water, surfaces, ions, and environmental energy collectively form an organized interface that stores potential, directs movement, and participates in the physical constitution of life?

The next task is to give equal seriousness to the investigators who have demonstrated how much of the observed behaviour can arise through ion exchange, electrodiffusion, electrophoresis, diffusiophoresis, osmotic effects, and measurement artefacts.

Their work does not merely oppose Pollack. Properly understood, it supplies essential mechanisms that any mature theory of the living interface must incorporate.

3. What the Critics Have Established

The transport critiques have established indispensable mechanisms. Nafion exchanges ions, generates concentration and electrical gradients, and can drive electrophoretic and diffusiophoretic motion over distances far exceeding molecular hydration shells. Any successor theory that omits these processes is untenable (Florea et al., 2014; Schurr, 2013; Esplandiu et al., 2020).

A scientific proposal becomes stronger when its most striking observations survive alternative explanations and when its concepts are refined under pressure from investigators who do not share its originating assumptions.

The critical studies have not shown that nothing unusual happens near hydrated Nafion. They have shown that much of what appears unusual can emerge through the long-range consequences of familiar interfacial physics.

A successor framework that dismisses ion exchange, diffusiophoresis, electrophoresis, tracer dependence, and optical artefacts would be less credible than Pollack's original framework, not more. Model C can advance the inquiry only by explaining why those mechanisms occur together, how they interact with water dynamics and energy input, and whether any experimentally detectable property remains unexplained after their contributions are quantified.

Nafion is a perfluorosulfonic ionomer developed precisely because it conducts ions, particularly protons, while maintaining mechanical and chemical stability. Its fluorinated polymer backbone contains side chains terminating in strongly acidic sulfonic groups. When hydrated, the material develops water-containing ionic domains and channels through which hydrated ions can move. Contemporary studies of ion-exchange

membranes describe Nafion as possessing a complex microphase-separated structure whose ionic regions form nanoscale water channels upon hydration.

The sulfonic groups dissociate and exchange counterions with the surrounding solution. The membrane absorbs water and swells. Protons and other cations redistribute through its hydrated ionic domains. Solute concentrations near the external surface depart from those of the distant bulk solution.

The critical literature has demonstrated that long-range particle exclusion near Nafion can arise through ion exchange, differential ionic diffusion, electrical fields, electrophoresis, and diffusiophoresis. It has shown that the apparent size and even existence of the zone depend on tracer properties and ionic conditions. It has weakened birefringence as evidence for macroscopic crystallinity and has established that Nafion's chemistry, swelling, and proton-conducting architecture must be treated as causal variables rather than neutral background conditions. Gradient-based alternatives developed by Schurr and experimental work by Spencer and colleagues further show why macroscopic tracer depletion and proton-related effects cannot, by themselves, establish a crystalline aqueous phase (Schurr, 2013; Spencer et al., 2018).

4. What the Critics Have Not Yet Established

Mechanistic success should not be enlarged beyond its demonstrated domain. Explaining charged-particle displacement does not automatically determine all water dynamics, optical signatures, energetic responses, material memory, flow, or biological implications associated with the interface (Spencer et al., 2018; Elton et al., 2020).

But a successful mechanism for one observable does not automatically constitute a complete description of the system generating that observable.

The critical literature has provided strong support for the first claim and substantial support for the second. It has not yet demonstrated the third.

Mechanistic success becomes ontological closure only after the proposed mechanism accounts for the entire relevant state space or demonstrates that the remaining variables add no independent explanatory value.

The equations can predict how a particle responds once these properties are specified. They do not necessarily explain why a particular hydrated interface acquires, maintains, or modifies the complete set of properties from which the gradients emerge.

The distinction is analogous to that between describing current through a circuit and characterizing the complete electrochemical system that generates and regulates the voltage. The current equation may be correct while the battery remains a higher-order organized entity.

Model C would therefore not compete with the transport equations at the level of particle velocity. It would ask whether the variables entering those equations are themselves dynamically coupled through an interfacial organization.

The measurements have generally been performed in separate preparations, under different ionic conditions, with different surface histories, chamber geometries, tracers, illumination systems, and time points. The 2020 critical review explicitly recognized the plurality of reported properties and the experimental complications introduced by surface charge, dissolved solutes, nanobubbles, and other confounders (Elton et al., 2020).

The critics have demonstrated that conventional ion-exchange and electrokinetic processes can account for substantial aspects of the exclusion-zone phenomenon, particularly tracer displacement. They have not yet demonstrated that these processes exhaust all molecular, energetic, temporal, mechanical, and biological properties of the complete hydrated interface.

Table 2. *Pollack-framework claims and critical responses*

Claim	Strongest supporting observation	Strongest critical response
Extended particle-free domain	Large operational EZs beside hydrophilic materials	Tracer motion can arise from ion exchange, fields, phoresis, flow, and tracer chemistry
Charge-separated aqueous state	Negative potential near surface with proton-enriched outer region	Nafion is a proton-exchanging ionomer that naturally generates diffusion potentials
Ordered or phase-like water	Optical, NMR, viscosity, and exclusion observations	No unique, independently replicated macroscopic order parameter or phase boundary
Radiant-energy enhancement	Illumination enlarges measured exclusion region	Thermal field, absorption, and equal-flux wavelength controls remain insufficient
Interfacial energy conversion	Potential, gradients, and reported flow suggest work capacity	Full energy ledger, efficiency, storage duration, and recoverable work are unresolved
Biological significance	Hydration is fundamental to proteins, membranes, matrices, and cells	Molecular hydration evidence does not prove hundreds-of-micrometres biological EZs

Part II — Reconstructing the Ontology

This Part replaces the inherited choice between “special water” and “ordinary transport” with a process ontology. Model C is introduced as a falsifiable hypothesis in which surface state, hydration, ions, polarization, gradients, energy input, flow, geometry, and history form a reciprocally constrained nonequilibrium organization.

5. The False Binary Between Phase and Transport

Structure and transport are not mutually exclusive physical categories. Transport can create spatial organization, while local molecular organization can reshape transport coefficients, ion partitioning, and energy flow. The relevant question is therefore whether the observed processes remain separable or become reciprocally coupled within one maintained regime.

Within this framing, evidence for transport appears to count against structure. If diffusiophoresis explains particle displacement, there is presumed to be no need for differentiated interfacial water. Conversely, if the interfacial water is structurally distinct, particle exclusion is presumed to follow from the inability of solutes or colloids to enter that phase.

This opposition is understandable. Pollack’s strongest descriptions sometimes identify the operational exclusion zone with a spatially coextensive ordered phase. Critics have therefore concentrated on showing that tracer displacement does not require such a phase.

Structure and transport are not mutually exclusive categories. In many physical and biological systems: structure determines transport; transport generates structure; gradients sustain differentiated states; and differentiated states regulate the gradients passing through them.

Pollack-group observations of solute-free interfacial regions in polar liquids other than water also complicate a simple binary: the result can be read against a uniquely aqueous lattice, yet it is compatible with either general surface-transport physics or a broader class of dynamically organized liquid interfaces (Chai & Pollack, 2010).

The question is whether transport takes place through an otherwise ordinary and passively responding liquid, or whether the water–surface–ion system becomes dynamically differentiated through the very flows and gradients it sustains.

The term phase may therefore invite the wrong adjudicatory test. Critics ask whether the entire particle-depleted region possesses a uniform molecular structure comparable to ice or a liquid crystal. When that structure is not demonstrated, the broader possibility of organized interfacial differentiation is treated as defeated.

Static order refers to spatial regularity that can often be described without reference to continuing throughput. A crystal maintains a repeating lattice because the arrangement corresponds to a stable or metastable free-energy minimum under the relevant conditions.

The opposition between a fourth phase and conventional transport is scientifically incomplete because structure and transport can be mutually generative in open nonequilibrium systems. Ion exchange, electrodiffusion, electrophoresis, and diffusiophoresis may explain how particles move while remaining embedded within a broader interfacial organization involving hydration, polarization, energy absorption, and surface reconfiguration.

If it is not best understood as a conventional equilibrium phase, what kind of physical organization is it?

6. From Equilibrium Phase to Nonequilibrium Organization

The term phase carries an equilibrium burden that the present evidence does not meet. A more defensible starting point is a nonequilibrium state sustained by material exchange, energy throughput, gradients, and history — a phase-like regime whose identity may disappear when the sustaining relations are removed (Turing, 1952; Nicolis & Prigogine, 1977).

The exclusion-zone controversy is not only a dispute about experimental interpretation. It is also a dispute about the kind of physical entity being proposed.

What thermodynamic and dynamical category best describes an interface whose identity depends upon maintained gradients, energy throughput, charge separation, and reciprocal coupling among surface, water, ions, and flow?

Thermodynamic equilibrium is a condition in which no net macroscopic flows of matter or energy persist and the system's relevant potentials have become spatially uniform, subject to external constraints.

Equilibrium is not inactivity at the molecular level. Molecules continue to move, collide, rotate, and exchange energy. But their microscopic fluctuations no longer produce persistent macroscopic directionality.

An equilibrium phase can therefore be characterized without reference to continuing net throughput. Its identity persists so long as the relevant temperature, pressure, composition, and boundary conditions remain within the appropriate region of the phase diagram. The ordinary liquid phase of water remains liquid even when no net heat, charge, or material flows through it.

An equilibrium phase occupies a stable free-energy minimum under specified thermodynamic conditions. Its macroscopic properties remain constant without requiring continuous net energy or material throughput.

A metastable interfacial-water state could, in principle, persist after its initiating conditions were removed. Its identity would depend on kinetic barriers rather than continuous energy input.

A proton gradient created when Nafion is first immersed in water could be transient if it gradually disappears once ion exchange and diffusion are complete.

Hydrated surfaces may support open, energy-responsive, charge-differentiated interfacial regimes whose organization is maintained through coupled transport, polarization, hydration, and dissipation.

The conceptual precedent is not specific to water. Reaction-diffusion theory shows that coupled local transformations and transport can generate stable spatial differentiation without an equilibrium phase boundary (Turing, 1952), while nonequilibrium thermodynamics shows how open systems can sustain organized regimes through continuous dissipation and throughput (Nicolis & Prigogine, 1977). These frameworks establish possibility, not proof; Model C must still identify the specific variables, fluxes, and constraints operating at the hydrated interface.

7. Model C: The Emergent Nonequilibrium Interface

Model C treats the interface itself as the candidate causal object. Its state depends jointly on surface chemistry and geometry, water dynamics, charge, ionic and protonic gradients, electromagnetic and thermal input, fluid and material flows, prior conditioning, and time. It is presently a Level 4 integrative inference, not an established theory.

First, the transport-centred critics have demonstrated that ion exchange, unequal ionic diffusion, electrical fields, electrophoresis, and diffusiophoresis can generate long-range tracer exclusion beside Nafion. Any adequate model must incorporate these mechanisms. Second, explaining tracer movement does not by itself

determine the complete physical state of the hydrated interface. Questions remain concerning water dynamics, surface reorganization, energy responsiveness, reciprocal coupling, temporal memory, and possible biological function.

Hydrated surfaces, water, ions, electrical polarization, energy throughput, and material flows may form a recursively coupled nonequilibrium organization whose properties cannot be assigned completely to any one component considered in isolation.

Model C does not ask whether ordinary physics should be replaced. It asks whether ordinary physical processes are organized into a reproducible regime that possesses explanatory and functional significance at the level of the whole.

An open, boundary-dependent physical organization in which the dynamical state of water, the structure and chemistry of a surface, ionic and protonic gradients, electrical polarization, energy absorption and dissipation, and material transport are reciprocally coupled. Its identity lies in a reproducible pattern of relations, gradients, fluxes, and transitions rather than in a permanently fixed collection of molecules or a single static molecular lattice.

The hyphens are important. They indicate that the terms are not independent items merely placed next to one another. They are mutually conditioning processes.

Model C does not claim that interfacial water alone constitutes autopoiesis. It proposes that a living water–membrane–ion ensemble may be one physical layer through which autopoietic organization becomes materially effective.

Second, it does not assign complete causal priority to water structure. Third, it replaces the equilibrium image of a fourth phase with a dynamically maintained organization.

Hydrated interfaces may generate nested molecular, electrochemical, and transport domains whose properties are sustained through reciprocal coupling among surface structure, water dynamics, ions, electrical polarization, energy throughput, and material flow. The visible exclusion zone is one operational manifestation of this broader organization, not necessarily its molecular boundary.

Pollack's insight that the interface may be active and energy responsive; the critics' demonstration that tracer movement is strongly governed by conventional transport; and mainstream evidence that interfacial water differs from bulk water at molecular scales.

To understand how such a system can possess causal unity without invoking unknown forces, the next section turns to the role of constraints, relations, and organized absence.

Figure 2. Three Models of Exclusion-Zone Phenomena

Each model accepts some observations but assigns causal priority differently.

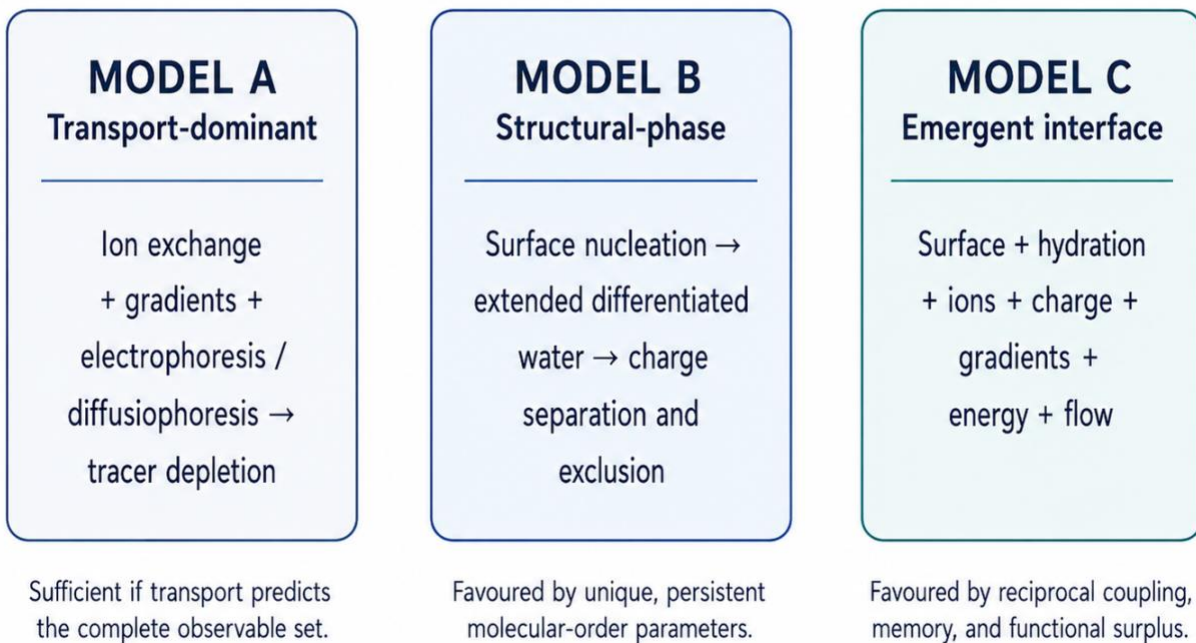


Figure 2. Three Models of Exclusion-Zone Phenomena

Model A prioritizes transport, Model B an extended structural phase, and Model C a reciprocally coupled nonequilibrium interfacial organization.

8. Constraints, Relations, and Causal Organization

A relational model requires a precise account of causation. The interface has explanatory standing only if its coupled constraints alter component behaviour, if interventions reveal reciprocal influence, and if the integrated state predicts outcomes better than a complete component-wise model (Deacon, 2012).

Model C proposes that the hydrated interface possesses causal unity without requiring an unknown force, a supernatural organizing principle, or a permanently fixed molecular lattice.

A membrane channel does not create the electrochemical potential driving an ion. It provides a selective pathway through which that potential can be expressed.

This is the sense in which Deacon places “absence” at the centre of constraint-based causation: the organization of a system can depend upon processes that do not occur because boundaries and relations prevent them. His account attempts to trace such constraint-based causal organization from thermodynamic and self-organizing processes toward living and purposive systems.

Does tracer movement change when fixed surface charge is neutralized? Does proton transport change when hydration is altered without changing bulk concentration? Does the electrical field disappear when the membrane’s ion-exchange groups are blocked? Does flow change when surface geometry is modified but chemistry is held constant?

A fluid in an open reservoir behaves differently from the same fluid in a narrow capillary. Ions beside a neutral surface behave differently from ions beside a charged ion-exchange membrane.

The interface cannot be reduced to a dry surface imposing order on passive water because the hydrated state changes the physical identity and behaviour of the surface itself.

A set of components displays reciprocal constraint when each component or process restricts the possibilities available to others, while depending on those others for its own persistence.

Model C can possess causal unity without invoking unknown forces if the interface is understood as a network of materially embodied constraints.

Figure 3. The Emergent Nonequilibrium-Interface Model

The interface is represented as a state of reciprocal constraints rather than a single material component.

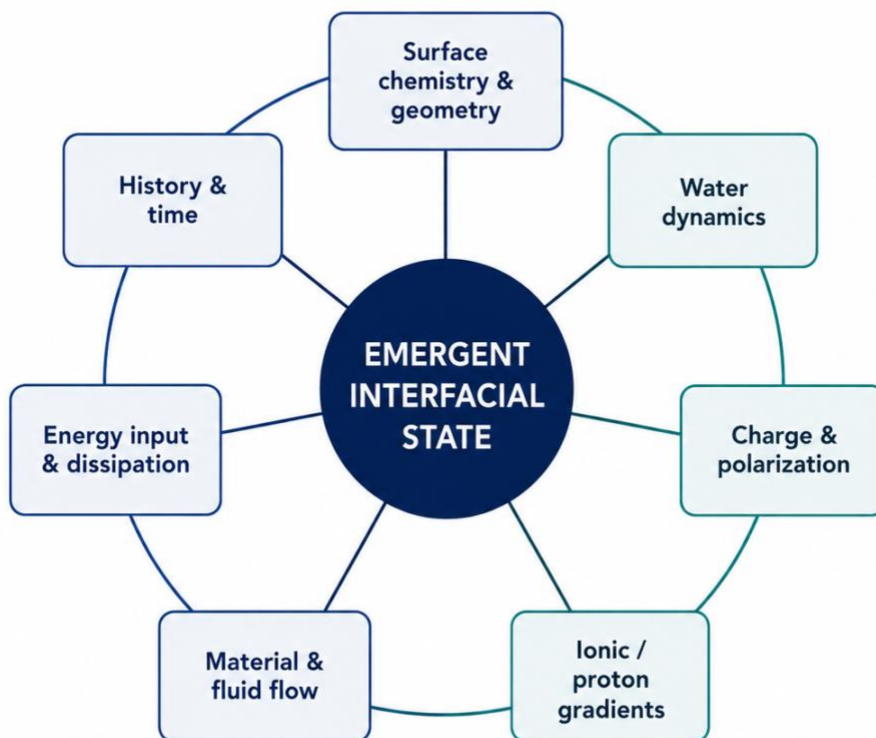


Figure 3. *The Emergent Nonequilibrium-Interface Model*

The model's scientific standing depends on showing reciprocal constraint and incremental prediction, not on relabelling a list of component processes.

Part III — Seeing Without Destroying

This Part examines how the object of inquiry may be altered by the methods used to observe it. The governing rule is to preserve native relations, measure several domains together, and use perturbation, recovery, and hysteresis to reveal organization that may be invisible in static or dehydrated preparations.

9. The Observability Problem

Scientific observation is a physical intervention. Buffers, probes, purification, isolation, temperature control, electrical grounding, fixation, and dehydration can change the gradients and material relations that constitute the object being measured. Negative results are decisive only when the relevant state has been preserved long enough to be tested.

The interstitium provides an instructive example. Benias and colleagues used probe-based confocal laser endomicroscopy in living tissue and fresh specimens to describe a widespread fluid-filled, collagen-bundle-supported interstitial architecture. They argued that conventional fixation and processing caused fluid loss and collapse, making the spaces appear as dense connective tissue on standard slides.

A water-dependent architecture can disappear when its water, pressure, flow, or native geometry is removed, leaving components that give a misleading impression of the prior organization.

Likewise, it may contain all the chemical components of an interfacial state while containing none of the relations that constituted it. Composition is not organization.

The final equilibrium condition remains scientifically important because it provides a reference. It should not automatically be treated as the system's only physically meaningful state.

Time-resolved heterodyne-detected vibrational sum-frequency generation provides a useful example of methodological refinement. It is interface selective and can separate interfacial vibrational responses and relaxation dynamics that are difficult to infer from bulk measurements. The method nevertheless involves optical excitation, and the measured response includes energy relaxation and eventual thermalization.

A small interfacial population may be overwhelmed by the bulk signal. Interface-selective methods can isolate molecular responses arising specifically where symmetry, orientation, and chemical environment differ from the bulk.

If Pollack's central intuition is approximately correct, the greatest observational danger is not simply failure to detect an unusual kind of water.

It is the destruction of a dynamically organized interface through procedures designed to isolate and stabilize its components.

Which properties arise only while water, surfaces, ions, charge, energy, and flow remain dynamically coupled, and which persist when those relations are systematically broken?

The next section turns to a concrete historical example — the recognition of the fluid-filled interstitium — and asks what it teaches, and does not teach, about preparation-dependent scientific invisibility.

10. Lessons from the Interstitium and Other Preparation-Dependent Discoveries

The interstitium provides a methodological analogy: a fluid-supported architecture can be present in vivo yet collapse during conventional preparation. This does not identify exclusion-zone water with the interstitium; it demonstrates why preparation history must be treated as an experimental variable rather than an invisible prelude (Benias et al., 2018).

Over time, the image produced by a standard method can become identified with the object itself. Features reproducibly created by preparation may enter textbooks as though they represented the native living state. Conversely, structures that disappear during preparation may be regarded as absent, unimportant, or anatomically unreal.

The history of the interstitium and other hydrated biological structures demonstrates that improved observation sometimes reveals not a previously nonexistent component, but a previously collapsed relation.

The interstitium was not unknown before 2018. Interstitial fluid, connective tissue, pre-lymphatic pathways, extracellular matrix, and tissue spaces had long been studied. The significance of the 2018 report by Benias and colleagues was therefore not the discovery that fluid exists between cells or that connective tissues contain interstitial compartments (Benias et al., 2018).

The authors initially observed the pattern through probe-based confocal laser endomicroscopy after intravenous fluorescein administration. The living tissue displayed dark collagenous bands surrounding bright, fluorescein-filled spaces. Conventional fixed histology did not display the same open architecture (Benias et al., 2018).

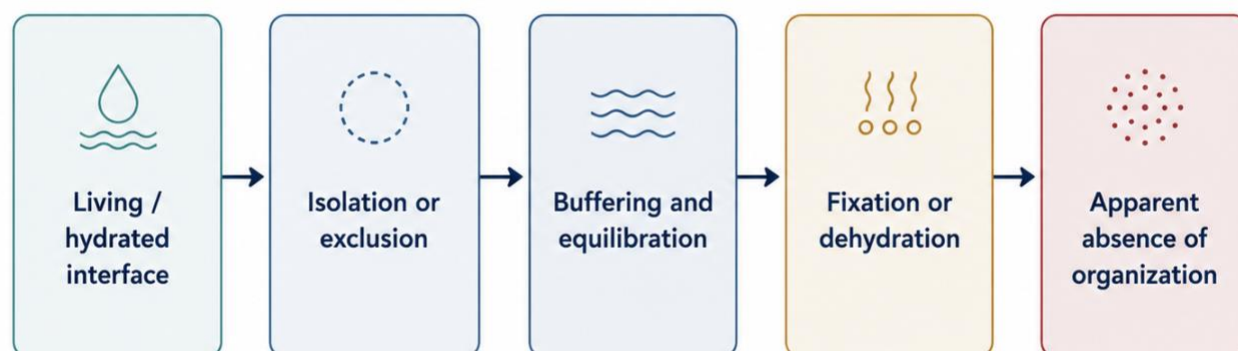
In the fresh-frozen preparation, collagen bundles remained separated by open spaces corresponding to the reticular pattern seen by endomicroscopy. In the conventionally fixed specimen, those spaces were almost completely collapsed, and the collagen bundles appeared adherent or densely compacted. The authors attributed the conventional appearance to fluid loss during excision and fixation (Benias et al., 2018).

A component-centred analysis might reasonably conclude that both sections contained the same basic materials. A relational analysis recognizes that they represented different physical states.

Biological interfaces and fluid-supported architectures may be systematically underestimated when observation requires loss of hydration, pressure, flow, or native geometry.

Figure 4. How Experimental Preparation Can Destroy the Object of Inquiry

A relation-dependent state may disappear when the sustaining relations are removed.



Methodological rule: preserve the relations first; perturb them second; dissect them last.

Figure 4. *How Experimental Preparation Can Destroy the Object of Inquiry*

The interstitium is used only as a methodological analogy: preparation can collapse a fluid-supported structure without proving equivalence to exclusion-zone water.

11. Relational and Process-Preserving Methods

No single instrument can establish the ontology of a complex interface. The decisive strategy is simultaneous or tightly synchronized measurement of water-sensitive spectra, ions, pH, potential, temperature, surface state, force, flow, tracer behaviour, and time, with explicit accounting for the disturbance introduced by each method (Shi et al., 2019; Lang et al., 2024).

The preceding sections established that preparation can preserve the components of a hydrated interface while destroying the relations that made those components constitute a particular organization.

Model C cannot be tested adequately by demanding that one instrument reveal the entire interface. Nor can it be protected indefinitely by arguing that every measurement is disruptive.

A study measuring only a water spectrum cannot establish whether the signal contributes to force, flow, or function. A study measuring several variables simultaneously can begin to reconstruct causal architecture.

A 2024 living-cell study used Raman microspectroscopy and multivariate reconstruction to estimate the composition and vibrational spectrum of intracellular water in three cell types. It reported that most intracellular water was comparatively bulk-like while a small, reproducible fraction — approximately three percent — showed a non-bulk-like spectrum associated with biological interfaces (Lang et al., 2024). This demonstrates both the feasibility and the disciplining value of process-preserving measurement: living cells can

be studied without fixation, and the result can constrain rather than simply confirm expansive theories of cellular water.

Previous work used the water O-H band to estimate water-density distributions in living cells, while complementary ultrafast studies showed that most intracellular water retains relatively rapid, bulk-like orientational dynamics and that a smaller population is slowed by biomolecules and ions (Shi et al., 2019; Tros et al., 2017). These results make water experimentally accessible in intact cells while placing a direct limit on claims that the entire cytoplasm is one highly ordered aqueous phase.

Stimulated Raman excited fluorescence combines vibrational selectivity with highly sensitive fluorescence detection. When coupled to an environment-sensitive vibrational probe, it can map local water states in living cells with spatial resolution unavailable from bulk spectroscopy (Shi et al., 2019).

When the same hydrated interface is observed across water dynamics, ions, charge, temperature, flow, mechanics, and time, do the variables behave as independent consequences of known surface transport, as outputs of a primary structural water state, or as reciprocally coupled dimensions of one nonequilibrium organization?

The next section turns from the observational platform to the temporal logic of the experiment: how controlled perturbation, relaxation, recovery, hysteresis, and repeated cycling can reveal the organization more clearly than static measurements alone.

Table 3. *Methods capable of preserving dynamic interfacial organization*

Method	Primary observable	Strength and principal caution
Raman/SREF microscopy	Water populations and local environment	Living, label-free or minimally labelled mapping; spectral decomposition and heating require control
Heterodyne VSFG / 2D-IR	Interfacial orientation and ultrafast dynamics	Interface selective; technically demanding and geometry sensitive
Terahertz spectroscopy	Collective hydration dynamics	Sensitive to biomolecular coupling; bulk-water absorption limits localization
NMR relaxation/diffusion	Mobility, exchange, compartment dynamics	Non-destructive; spatial localization and inverse interpretation may be non-unique
Scattering	Density and molecular correlations	Can test structural claims directly; often requires simplified preparations
Microfluidics/optical tweezers	Forces, gradients, temporal development	Quantitative manipulation; probe charge and surface chemistry can dominate
Ion/pH/potential mapping	Chemical and electrical gradients	Direct mechanistic test; probes, buffering, and grounding may perturb the state
Thermal imaging/calorimetry	Heat and energy balance	Essential for illumination claims; local gradients may be below coarse resolution
Intravital/confocal endomicroscopy	Native tissue architecture and flow	Preserves in-situ relations; access and contrast agents remain constraints

12. Perturbation, Recovery, Memory, and Hysteresis

Dynamic response may discriminate models more strongly than a static image. Growth, relaxation, washout, recovery, rechallenge, conditioning, fatigue, and hysteresis reveal whether the present state is sufficient to predict future behaviour or whether hidden history-dependent organization remains.

A tracer-depleted region, electrical potential, or water-spectral difference observed at one time can often be explained by several competing models. The models become more distinguishable when the system is moved away from its apparent steady state and its full trajectory is measured.

Water-sorption studies of Nafion show strongly non-Fickian uptake and release, with rapid interfacial transport followed by slower polymer swelling and relaxation. The material may therefore retain a measurable history even after bulk water content appears similar.

Time-resolved infrared measurements likewise indicate that hydration kinetics can change with humidity and with interactions between water, sulfonic-acid groups, and the polymer backbone.

Time-resolved scattering studies further distinguish rapid diffusion-driven swelling from much slower polymer reorganization, cautioning against treating Nafion as a material that reaches one simple steady state immediately.

For the EZ system, it means that a hydrated Nafion surface at a given water content may not be equivalent to a dehydrating surface at the same measured water content.

Hysteresis occurs when the response to an increasing control variable differs from the response observed while the same variable is decreased. A complete experiment must therefore measure both branches of the trajectory.

Recent work has extended the dynamic programme by examining how temperature and cryoprotectants such as polyethylene glycols and trehalose alter a Nafion-associated exclusion region (Theodorou et al., 2026). Because the study remains centred on Nafion and does not by itself provide orthogonal molecular characterization or independent adjudication of transport versus structural interpretations, it is best treated as evidence that conditioning history and thermal trajectory are experimentally consequential — not as confirmation of a fourth phase.

Model C gains support only if the complete external interface exhibits additional, prospectively predicted relational dynamics after those material effects are accounted for.

The manuscript now turns to direct comparison of the three models.

How far does each proposed property extend, where are its boundaries, and does the tracer-defined edge correspond to a molecular, electrochemical, or merely operational boundary?

Table 4. Comparison of Models A, B, and C

Dimension	Model A: transport dominant	Model B: structural phase	Model C: emergent nonequilibrium interface
Primary causal object	Surface-driven gradients and transport	Extended differentiated aqueous phase	Coupled surface–water–ion–energy–flow organization
Role of water structure	Local hydration may affect parameters but is not a distinct causal state	Generative basis of exclusion and charge separation	One reciprocally coupled variable among several; not necessarily spatially uniform
Expected boundaries	Nested gradient, force, and tracer profiles	A relatively coherent structural domain	Coordinated but nonidentical molecular, field, and operational domains
Persistence after clamping	Effects collapse with gradients/material dynamics	Structural state persists independently	Differential relaxation, partial persistence, or history-dependent reconstruction

Dimension	Model A: transport dominant	Model B: structural phase	Model C: emergent nonequilibrium interface
Biological implication	Conventional transport suffices	Extended phase directly supports function	Living systems produce, regulate, and repair integrated interfaces
Current evidence status	Strong for substantial tracer behaviour	Incomplete for macroscopic phase claim	Plausible integrative inference requiring decisive tests

Part IV — Three Models, Discriminating Predictions

This Part is the scientific centre of the paper. Models A, B, and C are compared prospectively across spatial, spectral, electrochemical, thermal, geometric, temporal, energetic, and biological dimensions. The purpose is not rhetorical reconciliation but the design of outcomes that would genuinely favour, restrict, or displace each model.

13. Spatial Extent and Coordinated Boundaries

The visible edge of a tracer-depleted region is an operational boundary, not automatically a molecular phase boundary. Model A predicts nested gradient and transport length scales; Model B predicts an extended state boundary; Model C predicts coordinated but nonidentical domains whose relations change together.

A hydrophilic or ion-exchanging surface is placed in an aqueous suspension, and a region depleted of specified tracers develops beside it. The region may extend tens or hundreds of micrometres from the surface — far beyond the distances ordinarily associated with direct molecular hydration. Pollack and colleagues interpreted this extensive depletion as evidence that the surface had generated a correspondingly extensive, physically distinct aqueous domain (Zheng et al., 2006).

Transport-centred accounts propose a different interpretation. The molecular effect of the surface may remain short-ranged, while ion exchange generates concentration and electrical fields that extend much farther and transport particles over still larger distances. Florea and colleagues reproduced long-range colloidal repulsion using ion exchange, unequal ionic diffusion, and diffusiophoresis, while Esplandiu and colleagues showed that, under the alkali-metal chloride conditions they tested, the electric field arising from unequal ionic diffusion dominated the particle response (Florea et al., 2014; Esplandiu et al., 2020).

Which physical property extends how far from the surface, how sharply does it decay, and do the boundaries of water dynamics, ionic gradients, electrical potential, force, flow, and tracer depletion coincide?

For example, a tracer may be depleted across a region because an electrical field produces outward drift. The point at which the tracer becomes visibly scarce is then an operational boundary within a continuous field, not necessarily a material interface.

It nevertheless establishes a stringent baseline: claims of hundreds-of-micrometres-long molecular restructuring require direct evidence beyond the ordinary propagation of fields through predominantly bulk-like water.

First, tracer-depleted regions extending tens or hundreds of micrometres can be physically real under specified conditions. Pollack-associated studies and independent investigations have observed long-range depletion and force environments near Nafion and other surfaces (Zheng et al., 2006; Huszár et al., 2014; Hu et al., 2022).

Second, large spatial reach does not establish equally extensive molecular ordering. Ion exchange, ionic diffusion, electrical fields, and diffusiophoresis can generate long-range particle displacement from a much thinner initiating interface (Florea et al., 2014; Esplandiu et al., 2020).

Third, the evidence does not establish that water dynamics are irrelevant. It establishes that the molecular, electrochemical, force, and tracer boundaries must be measured separately.

14. Spectral and Dynamical Signatures

A structural or emergent model requires tracer-independent evidence. Raman, infrared, terahertz, sum-frequency, NMR, dielectric, scattering, and related measurements should identify a reproducible water-state variable, its spatial extent, formation sequence, relaxation, and relation to charge and transport.

The decisive question is not merely whether the spectrum near a surface differs from that of distant bulk water.

It is: Does the interface exhibit a reproducible molecular or dynamical signature that cannot be explained by its measured temperature, pH, ionic composition, dissolved material, surface optical properties, or instrumental geometry?

Pollack's originating programme reported optical and magnetic-resonance observations as evidence that the tracer-depleted region differed from bulk water, alongside the more direct observation of extensive solute exclusion near diverse hydrophilic materials. The 2006 synthesis described operational exclusion distances commonly reaching several hundred micrometres (Zheng et al., 2006).

A reproducible absorption feature could be important if it were shown to arise specifically from the proposed aqueous state. But an absorption band at one wavelength is not a molecular fingerprint unless competing absorbers are excluded.

A 2024 Raman microspectroscopy study corrected for intracellular solute contributions and reported that approximately three percent of water in three living-cell types possessed a reproducibly non-bulk-like spectrum. The authors attributed this fraction to biointerfacial water associated with biomolecules, while finding that most intracellular water remained bulk-like. Their inferred non-bulk population exhibited a weakened hydrogen-bond network and more disordered tetrahedral character rather than the more extensively ordered cellular water sometimes proposed in earlier theories (Lang et al., 2024).

Mainstream measurements nevertheless establish that water near proteins, membranes, polysaccharides, ions, and intracellular macromolecules can possess altered orientation, mobility, or hydrogen-bond dynamics at molecular and nanoscopic scales (Ebbinghaus et al., 2007; Tros et al., 2017; Dedic et al., 2021). Those findings support the plausibility of differentiated interfacial water while leaving the hundreds-of-micrometres scale and molecular homogeneity of a Nafion-defined EZ unproven.

Conventional intensity-based nonlinear spectroscopy measures the square magnitude of a signal and can mix contributions in ways that make orientation and sign difficult to infer.

The unresolved issue is whether Nafion and related interfaces possess a distinctive molecular or dynamical state that: extends beyond conventional hydration; participates in charge separation; responds to energy input; and contributes causally to the larger transport field.

15. Gradient Neutralization and Persistence

Gradient neutralization is a decisive intervention. Immediate loss of force, flow, and exclusion with no residual water-state signature would favour Model A; persistent independent structure would favour Model B; differential decay, memory, or reconstruction from a residual inner state would favour Model C.

The extended tracer-depleted region is generated principally by ionic concentration gradients and the electrical fields associated with unequal ion diffusion.

If that claim is correct, neutralizing the relevant gradients should remove the force responsible for long-range particle displacement. Florea and colleagues showed that Nafion's ion-exchange activity can generate salt

gradients whose diffusive expansion produces colloidal motion consistent with diffusiophoresis. They found approximately square-root-of-time growth and systematic dependence on ion diffusivity and ionic strength.

Esplandiu and colleagues subsequently separated electrophoretic and chemiphoretic contributions using particles of different charge and salts with different ionic mobilities. Under the tested alkali-metal chloride conditions, the diffusion-generated electric field was the dominant source of particle repulsion.

A common experimental response to a proton or ionic gradient is to add electrolyte or buffer until the visible exclusion zone diminishes. This can be informative, but it does not isolate the causal variable cleanly.

Gradient neutralization should be defined through direct measurements rather than nominal solution preparation. Ionic activities and electrical potential should be mapped directly across space and time, because nominally identical bulk solutions can retain local gradients near an active surface.

A residual empty space containing no returning particles is weaker evidence than a persistent force acting on newly introduced particles. A persistent spectrum is not equivalent to persistent charge storage.

The long-range force should decline substantially with the gradient, while a shorter-range or temporally persistent interfacial effect may remain if surface hydration and water organization retain state.

Current transport evidence predicts that the long-range tracer-depleted region should depend strongly on ion exchange, unequal ionic diffusion, electrical fields, and tracer properties.

It is therefore likely that neutralizing the measured electrochemical gradients will substantially reduce or abolish the extended force responsible for macroscopic particle exclusion.

16. Wavelength Dependence and Matched Thermal Controls

Radiant-energy responsiveness is potentially discriminating only when illumination is compared with equal-flux and spatially matched thermal controls. Wavelength labels alone do not establish nonthermal action, and small average temperature changes do not exclude local thermal gradients (Chai et al., 2009).

The claim is not merely that light reveals an existing interface. It is that incident radiation enlarges or strengthens the interfacial state.

But light absorbed by water, Nafion, tracers, chamber walls, or dissolved substances ultimately changes the energetic state of the system. Much of that absorbed energy will appear as heat.

Does illumination alter the interface entirely through thermal changes, or does wavelength-specific absorption produce an additional state-dependent response not reproduced by an equivalent thermal history?

Chai, Yoo, and Pollack reported that illumination from ultraviolet, visible, and infrared sources enlarged tracer-depleted regions beside hydrophilic materials. Infrared radiation was reported to be particularly effective, with a strong response near 3.1 μm , a wavelength associated with O–H stretching absorption. The authors reported that irradiation could increase exclusion-zone width several-fold while measured temperature elevations remained modest and comparatively uniform within their chamber (Chai et al., 2009).

In that technique, molecular absorption of mid-infrared radiation intentionally generates local heat. The resulting temperature-dependent change in refractive index is then detected optically. The method exploits the fact that vibrational absorption can be converted rapidly into a measurable photothermal response.

Illumination can therefore change the rate at which the ionic field forms and the way tracers respond to it without requiring an additional water phase.

Hydration changes the balance among hydrophobic, interfacial, and water-rich domains. Protonated and deprotonated sulfonic-acid sites can coexist across hydration levels, and their local water environments produce different vibrational signatures.

A 2024 Pollack-group study reported that 275-nm irradiation made the negative potential measured near hydrated Nafion more negative, whereas 370-nm and 650-nm illumination produced little change under the tested conditions (Shen et al., 2024). The wavelength contrast is experimentally relevant, but it remains originating-group evidence and does not substitute for equal-absorbed-energy controls, three-dimensional temperature mapping, polymer photochemistry controls, and independent replication.

The 2026 cryoprotectant study likewise reinforces the importance of temperature, solute composition, and conditioning history (Theodorou et al., 2026). Neither study closes the thermal question. Together they sharpen the design requirement: compare wavelengths at matched absorbed power and reproduce the same local temperature field without illumination before attributing a residual response to state-specific photochemistry or nonthermal organization.

However, the reported wavelength dependence and modest bulk temperature rise do not yet establish that radiant energy builds an extended structural water phase through a nonthermal mechanism.

It is whether illumination produces a reproducible change in the interfacial organization that cannot be reproduced by the same absorbed energy, spatial thermal field, material state, and electrochemical gradients.

How do surface chemistry, geometry, curvature, porosity, and topology determine the formation, scale, and energetic responsiveness of the interface?

17. Geometry, Topology, and Surface Chemistry

Interfaces are generated by specific surfaces, not by hydrophilicity in the abstract. Fixed charge, ion-exchange capacity, porosity, roughness, swelling, curvature, channel geometry, and topology can alter local hydration and amplify it into large-scale electrochemical and transport effects.

Which surface properties generate which molecular, electrochemical, and transport domains, and how does their spatial arrangement transform local activity into larger-scale organization?

At electrified solid–water interfaces, molecular simulations have demonstrated close interdependence among adsorbate coverage, water orientation, wettability, capacitive response, and applied potential. Even an atomically ideal Pt(111) interface cannot be described adequately by assigning wettability independently of its electrical and molecular state.

When proton-form Nafion contacts an electrolyte containing another cation, ion exchange can release protons and take up the incoming cations. Because the exchanged ions possess different diffusion coefficients, the resulting concentration profiles generate an electrical field that can drive diffusiophoresis and electro-osmosis. Florea and colleagues showed that this combination of ion exchange, ion diffusion, and diffusiophoresis quantitatively accounts for long-range colloidal repulsion under their tested conditions (Florea et al., 2014).

This sequence demonstrates how a chemically localized property can possess a much larger causal reach without requiring the same material state to extend across the whole affected region.

The Pollack-type observation should therefore not be generalized from Nafion to all hydrophilic surfaces without identifying the specific chemistry that powers the observed field.

Florea and colleagues found that the kinetics of long-range colloidal repulsion varied systematically with the diffusivity of the exchanging cation, supporting a gradient-driven mechanism (Florea et al., 2014).

Cellulosic surfaces have also been reported to develop dynamic tracer-depleted regions, showing that Nafion is not the only material capable of producing the operational phenomenon (Sulbarán et al., 2014). Cellulose, however, is porous, charged, chemically heterogeneous, and capillary-active; its inclusion strengthens the need for a surface series that independently varies fixed charge, exchange capacity, roughness, porosity, and swelling rather than treating “hydrophilicity” as a single causal property.

At electrochemical interfaces, operando spectroscopy and simulations have shown that changing local ions or surface composition can alter hydrogen-bond connectivity, molecular orientation, and the transport of water-derived intermediates. In one catalyst system, deliberately disordering a comparatively rigid interfacial-water network increased water and hydroxide transport and improved reaction performance.

An interface is a chemically and geometrically specific organization in which molecular hydration, ionic gradients, fields, and flows are shaped by the architecture of the boundary.

The central discriminating question is whether that architecture merely sets the conditions for feed-forward transport or enters a reciprocal, history-dependent organization.

When a surface–water–ion system is cycled through changing conditions, does its response depend entirely on its measurable present state, or does its prior geometry, hydration, charge, and coupling alter what it becomes next?

18. Hysteresis, History, and State Memory

History dependence would distinguish a merely instantaneous transport field from a stateful interface. Repeated cycles should test whether identical present values of temperature, pH, potential, and tracer position yield different responses because of prior hydration, illumination, deformation, counterion exchange, or stress.

Classical state descriptions assume that a system’s future behaviour can be predicted from its present variables and governing laws.

Is the system’s future response determined completely by its measurable present conditions, or does its prior hydration, ion exchange, illumination, deformation, and transport alter what it can become next?

A system is path dependent when the outcome depends not only on the final external conditions but also on the route by which those conditions were reached.

For example, a Nafion membrane brought to fifty-percent hydration from a dry state may differ from one brought to the same hydration while drying from saturation.

A system possesses operational memory when a prior event leaves a state variable that remains detectable through a subsequent interval and alters a later response.

Experimental sorption studies have found that water uptake depends on membrane pretreatment and temperature, while work on Nafion hydration has emphasized the need to account for thermal, hydration, mechanical, and processing history when comparing measured properties.

Experimental and theoretical studies have found significant differences between sorption and desorption rates and have attributed them to interfacial transfer, the shape of the sorption isotherm, and polymer reorganization.

Experiments on an ion-conducting block-copolymer membrane found a sharp hydration- dependent mechanical softening transition, hysteresis between humidification and dehumidification, and an increase in conductivity after membrane softening.

The next section turns to the energetic consequence of such statefulness: Can the interface store free energy, release it under controlled conditions, and perform measurable electrical, chemical, fluidic, or mechanical work?

Hysteresis should be operationalized rather than inferred from a delayed return to baseline. Each experiment should include a conditioning phase, a controlled perturbation, a recovery interval, and an identical rechallenge. The relevant comparison is between trajectories obtained under the same measured present conditions but different prior histories. Candidate memory variables include polymer hydration, counterion occupancy, nanoscale morphology, interfacial protonation, dissolved-gas content, adsorbed contaminants, and mechanically induced changes in pore or channel structure. These variables must be measured wherever possible, because a history effect attributable entirely to slow material equilibration would constrain Model C rather than confirm it.

Model A can accommodate lag and hysteresis when transport coefficients or material properties change slowly, but it predicts that history dependence should disappear once all causally relevant state variables are specified. Model B predicts persistence associated with the formation and dissolution of a differentiated aqueous phase. Model C predicts multiple, coupled relaxation times and possible path dependence arising from reciprocal reorganization of surface, hydration, gradients, and flow. Its distinctive burden is therefore not merely to show memory, but to demonstrate that a multidimensional interfacial state predicts rechallenge responses better than measured material and electrochemical variables considered separately.

19. Energy Storage, Release, and Work

Claims of a “water battery” require a complete energy ledger. Absorption, heat, chemical potential, electrical potential, mechanical work, stored free energy, dissipation, efficiency, duration, and reproducible discharge must be measured separately before energy responsiveness can be called energy storage.

Can the interface receive identifiable energy, retain part of it in a measurable nonequilibrium state, and release that energy under controlled conditions to perform electrical, chemical, fluidic, or mechanical work?

(U) is internal energy; (Q) is heat transferred into the system; (W) is work; and matter crossing the boundary carries chemical and thermal energy.

A membrane maintains ion separation but is not identical to the chemical free energy stored in that separation. A catalytic site channels a reaction pathway but does not supply the reaction’s free energy.

If connecting even a very high-resistance load causes the voltage to collapse immediately, the system contains little accessible electrical energy or has very high internal resistance.

Reports of charge-related forces near Nafion and pressure-free flow through hydrophilic tubes motivate the work question but do not supply a closed energy balance (Das & Pollack, 2013; Rohani & Pollack, 2013). Both observations require replication with controls for electrode chemistry, hydrostatic head, evaporation, capillarity, thermal gradients, contamination, and polymer mechanics before electrical or fluidic output can be assigned to stored interfacial free energy.

When the constraint is released, the material may perform mechanical work. If illumination or ion exchange changes Nafion swelling, part of the system’s apparent energy storage may reside in the polymer rather than the external water.

If the measured voltage persists without supplying current, the interface may be at equilibrium with the electrodes rather than acting as an energy reservoir.

A recent electrochemical critique sharpened this control requirement by arguing that capacitance, current-decay, and gas-evolution observations at polarized platinum electrodes can arise from conventional electrode reactions and hydrogen evolution rather than an additional exclusion-zone energy reservoir (Sordello, 2025).

Whatever the final assessment of that analysis, Model C must first close the Faradaic and electrode-chemistry budget before attributing residual electrical work to a distinct interfacial aqueous organization.

Model B or C gains support only if a reproducible residual is associated with a directly measured interfacial state and can later be recovered as work.

The unresolved question is whether water's interfacial state adds an independently measurable energetic contribution or reorganizes the efficiency and pathway of transduction beyond established electrochemical and material effects.

Until those requirements are met, the most defensible description is that the interface is an energy-responsive and potentially energy-transducing system — not yet a demonstrated water battery.

Even if the interface is physically real, dynamically organized, and energetically active, does it contribute causally to biological function rather than merely accompanying it?

20. Correspondence with Biological Function

Biological relevance requires more than resemblance. A candidate living interface must be detected in an intact preparation, perturbed selectively, linked temporally and mechanistically to a defined function, and restored in a way that rescues performance, reserve, or recovery under load.

Does the interfacial organization make a measurable causal contribution to what the living system does?

The boundary persists despite molecular turnover because the living system continually regenerates the relations constituting it. This is qualitatively different from a synthetic Nafion surface, which can organize transport but does not produce or repair itself.

Does an interfacial-state variable predict ATP production or proton leak beyond the established predictors of membrane potential, oxygen supply, substrate availability, and respiratory-complex activity?

In a perfused endothelial construct, does a measured hydration-charge state predict permeability and shear response beyond glycocalyx thickness, flow, ionic strength, and temperature; does selective disruption alter both state and function; and does regeneration restore their coupling?

How can an adversarial collaboration be constructed so that Pollack's strongest claims and the critics' strongest mechanisms are tested within the same transparent experimental programme?

Part IV has transformed the controversy from a dispute about one visible particle-free zone into a set of discriminating experimental questions.

What remains unresolved is whether these observations are connected only sequentially or form a reciprocally organized causal system.

A functional test should proceed through four evidentiary gates. First, the proposed interfacial state must be measured in an intact or minimally perturbed biological preparation. Second, changes in that state must precede or covary reproducibly with a defined function. Third, an intervention must alter the candidate state while controlling for conventional determinants of the function. Fourth, restoration of the state must rescue the function or its recovery after stress. Without this sequence, the relation remains correlational even when the measured interface is biologically plausible.

The most informative outcomes may involve reserve rather than resting performance. Mitochondria, epithelia, vascular barriers, contractile tissues, and microbial communities can retain near-normal baseline output while losing the capacity to respond to demand, recover after perturbation, or resist a second challenge. Model C would gain biological significance if an integrated interface profile predicted this latent capacity better than temperature, bulk composition, membrane potential, morphology, and conventional biochemical markers

alone. Failure to add such predictive or causal value would confine the framework to a descriptive reorganization of established variables.

Table 5. *Discriminating predictions*

Test	Outcome favouring A	Outcome favouring B	Outcome favouring C
Gradient neutralization	Immediate loss of force/exclusion and no residual signature	Persistent extended molecular state and exclusion	Residual inner state, staged decay, hysteresis, or altered reconstruction
Matched illumination controls	All responses reproduced by thermal field	Wavelength-selective structural change independent of heat	State-dependent spectral/electrical response with partial nonthermal specificity
Tracer substitution	Boundary changes quantitatively with charge/size/coating	Common boundary across chemically diverse probes	Operational boundary varies, but coupled inner-state variables remain predictive
Intervention order	Commutative once present variables are matched	Order matters through nucleation or phase history	Noncommutative responses arise from reciprocal coupling and material memory
Cross-material transfer	Mechanism follows surface chemistry and transport parameters	Same structural state across suitable surfaces	Relational architecture generalizes despite material-specific spectra
Biological function	Conventional variables fully predict function	Water-state variable independently controls function	Integrated state predicts reserve, recovery, and successful rescue

Part V — The Decisive Experimental Programme

This Part converts controversy into a preregistered adversarial collaboration. It specifies benchmark materials, simultaneous multimodal measurement, orthogonal interventions, translation to biological preparations, functional testing, open data, and shared falsification criteria.

21. Principles of Adversarial Collaboration

Adversarial collaboration should make hypotheses — not investigators — adversaries. Proponents, critics, method specialists, and neutral replication laboratories should jointly state their strongest models, predicted outcomes, confounders, and conditions for changing their views before data are collected.

The exclusion-zone controversy has reached a point at which additional isolated demonstrations and isolated critiques are unlikely to resolve the central disagreement. One laboratory may continue to report extended tracer depletion, electrical polarization, illumination responses, or spontaneous flow, while another shows that ion exchange, electrodiffusion, electrophoresis, diffusiophoresis, convection, and tracer chemistry reproduce major portions of those observations. Both programmes can produce technically valid results while studying preparations that differ in materials, conditioning, chamber geometry, water composition, tracer properties, illumination, timing, and analysis. A positive result under one protocol and a mechanistic explanation under another therefore need not refer to the same physical state.

Adversarial collaboration is designed for precisely this situation. The term adversarial refers to opposition among hypotheses, not hostility among investigators. Participants with different theoretical commitments jointly formulate the strongest testable versions of their models, agree on preparations and controls, preregister predictions, specify what evidence would change their conclusions, and commit to publication regardless of outcome. The aim is not compulsory consensus. It is to make the remaining disagreement narrower, more explicit, and empirically constrained.

The collaboration must begin by steelmanning all three models. The strongest Model A is not 'nothing but diffusion'; it is a dynamic transport account incorporating ion exchange, multicomponent electrodiffusion, electrophoresis, diffusiophoresis, electro-osmosis, fluid mechanics, polymer swelling, tracer properties, thermal effects, dissolved gases, and material history. The strongest Model B does not merely assert that the visible empty region is a crystal; it specifies a molecular order parameter, spatial boundary, formation and dissolution law, energetic conditions, tracer-independent signature, and causal relation to charge and exclusion. The strongest Model C identifies its coupled variables, reciprocal relations, state dependence, relaxation times, hysteresis, and predictions that cannot be recovered by simply adding independent component models.

Each group should prepare a concise model card before experimental design begins. The card should state the central causal claim, essential variables, governing relations, boundary conditions, directly observed phenomena, inferred entities, strongest evidence, known weaknesses, distinctive predictions, and outcomes that would require revision or rejection. This prevents the experiment from being designed around positions that no participant actually holds and makes later updating proposition-specific rather than reputational.

Shared vocabulary must precede shared measurement. An operational exclusion zone should be defined as a region in which the concentration of a specified tracer or solute falls below a preregistered fraction of its distant-bulk value under specified conditions. That definition establishes neither a phase nor the exclusion of all matter. A persistent spectral state should mean a predefined water-sensitive signal that remains above a

matched-control threshold after directly measured electrochemical gradients have been clamped. Similar definitions are required for phase, structure, persistence, matched heating, work, and biological function.

The jointly owned question should not be 'Is Pollack right?' It should be: Which combination of surface, aqueous, ionic, electrical, thermal, mechanical, and transport processes is necessary and sufficient to account for the observed interfacial phenomena under specified conditions? This formulation preserves the observations, requires the critics' mechanisms to be quantified, and allows Model C to gain or lose explanatory standing without making the result a verdict on any investigator.

Before data collection, the consortium should map agreement, quantitative disagreement, ontological disagreement, and methodological disagreement. Agreement may include Nafion ion exchange, the reality of tracer depletion under some conditions, and the capacity of gradients to move colloids. Quantitative disagreements concern how much of the force, potential, or spatial extent is explained by measured transport. Ontological disagreements concern whether a tracer-independent aqueous state exists and whether the interface possesses causal unity. Methodological disagreements concern probes, buffers, thermal matching, sample preparation, and criteria for equilibration. Initial experiments should target the narrowest disagreement with the greatest theoretical leverage.

Models should then be decomposed into propositions that can succeed or fail separately: ion exchange is necessary for the large tracer-depleted region; measured gradients quantitatively predict tracer velocity; a tracer-independent water-sensitive state extends beyond ordinary hydration; that state persists after long-range gradients are neutralized; it changes the subsequent reconstruction of gradients; or illumination produces an effect not reproduced by the same spatial and temporal temperature history. Such decomposition permits selective updating rather than an all-or-nothing contest among identities.

The collaboration should be governed by shared materials, independent metrology, blinded analysis, and a neutral adjudication group. Every team should record in advance the result that would lower confidence in its preferred model. For example, Model A gains support if measured electrochemical variables prospectively explain tracer motion across multiple tracer classes and no independent long-range water signature remains. Model B gains support if a directly resolved, tracer-independent structural state persists under verified gradient suppression. Model C gains support only if coupled variables show coordinated perturbation, differential relaxation, or predictive surplus beyond complete component models. These commitments convert controversy into a programme in which all models are genuinely at risk.

The first practical requirement is therefore a standardized benchmark system: shared Nafion lots and control surfaces, common conditioning histories, defined water and gas preparation, reference tracers, calibrated chambers, agreed exclusion metrics, and interlaboratory quality controls. Only after that physical benchmark is reproducible should the programme advance to simultaneous multimodal measurement and biological translation.

22. Phase I: Physical Benchmark and Replication

The first experimental phase must create a shared physical object. Nafion counterion state, hydration, dimensions, history, chamber geometry, water composition, tracer properties, temperature, dissolved gases, imaging, and timing require standardized qualification before cross-laboratory disagreement is interpretable.

A benchmark system is a deliberately simplified, thoroughly characterized preparation used to determine whether different investigators are: generating the same physical state; measuring the same quantities; and applying the same operational definitions.

Under the specified preparation, negatively charged polystyrene particles showed a concentration below ten percent of distant bulk over a mean distance of (L) , at time (t) , with stated uncertainty.

This system should be capable of reproducing the long-range particle displacement previously studied quantitatively through ion exchange and diffusiophoresis. Florea and colleagues used a controlled geometry designed to minimize gravity and convection and reported that ion exchange, ion diffusion, and diffusiophoresis accounted for the observed long-range colloidal repulsion in their system.

A low-concentration alkali-metal chloride solution is suitable for the initial transport benchmark because its ion properties and interaction with proton-form Nafion can be modelled explicitly.

However, the selected concentration must be: high enough for reproducible analytical measurement; low enough to preserve a measurable gradient; and compatible with stable tracer behaviour.

Esplandiu and colleagues showed that the observed Nafion-associated exclusion response depends on the relation between the diffusion-generated electric field and the tracer's zeta potential; under their tested salt conditions, not all solutes were expelled.

The benchmark should use the orientation that most effectively suppresses obvious gravitational artefacts, followed by planned rotation experiments to determine whether the observed field tracks the surface or gravity.

Such studies can estimate random variation across laboratories, systematic laboratory differences, and reference values for common artefacts or measurements. Their design must account for laboratory selection, material stability, number of replicates, and study duration.

A suitable statistical model should therefore include a laboratory effect rather than removing discrepant laboratories automatically as outliers. NIST work on interlaboratory comparisons emphasizes modelling between-laboratory dispersion as part of total uncertainty rather than presuming that only a consistent subset is valid.

Published experiments have shown that finite Nafion regions generate proton and cation gradients, tangential electrical fields, and flow; patterned neighbouring surfaces with different zeta potentials can redirect the flow into unidirectional pumping.

For example, a residual force after gradient clamping may be considered negligible only if it is below the minimum force required to sustain the proposed large exclusion zone.

The Florea and Esplandiu studies provide quantitative starting mechanisms that should be translated into preregistered benchmark predictions rather than treated merely as retrospective critiques.

23. Phase II: Simultaneous Multimodal Measurement

The complete constellation must be measured within the same evolving preparation. Separate experiments can unknowingly compare different states, whereas synchronized measurement can establish temporal order, covariance, nested boundaries, and whether a water-sensitive variable adds predictive value beyond gradients and material state.

True simultaneity is not always technically possible or even desirable. But the degree of temporal and spatial separation must be reported explicitly.

Likewise, a pH probe positioned fifty micrometres above the surface and a Raman focal volume positioned two micrometres above it do not measure the same spatial state.

Patterned Nafion micropumps demonstrate that ion-exchange-generated concentration and electrical fields can be converted by geometry and zeta-potential patterning into radial or unidirectional electro-osmotic flow.

Their measured particle trajectories and predicted flow architecture provide a useful positive control for integrated gradient and flow measurement.

It measures enough independent domains to distinguish a transport field from a spatially coextensive water phase and to test for a nested Model C architecture.

How can one variable be changed independently enough to determine whether water, gradients, charge, surface state, flow, or mechanics is causally necessary for the others?

The minimum common clock should be defined before data collection. Tracer imaging, potential, pH, ion concentration, spectroscopy, temperature, and flow need time stamps precise enough to distinguish cause from consequence. Spatial registration is equally important: a signal assigned to an 'exclusion zone' must be mapped relative to the actual polymer–water boundary, local swelling, debris, and the measurement volume of each instrument. A shared coordinate system permits the possibility of nested domains — for example, a nanoscopic water-dynamical interphase, a broader electrochemical gradient, and a still larger tracer-response region — without forcing them into one boundary.

Analysis should compare nested models prospectively. A baseline model would predict tracer and flow variables from geometry, surface charge, ion exchange, diffusion, convection, and temperature. Additional water-sensitive or mechanical variables would then be entered only if they improve out-of-sample prediction. Model C is favoured not by covariance alone but when reciprocal perturbations and temporal ordering show that the added variables participate causally in the organization.

24. Phase III: Orthogonal Manipulation of Structure and Gradients

Correlation among water, ions, charge, swelling, flow, and exclusion is not enough. Orthogonal manipulation requires clamps, reversals, matched controls, factorial combinations, rescue, and reconstitution so that proposed causal variables can be changed while collateral changes are measured explicitly.

Phase II can reveal covariance, but covariance alone cannot establish causal architecture. A variable becomes causally informative when it can be altered prospectively while the other domains are measured, and when the intended intervention is shown not to have changed every plausible cause at once. Phase III therefore uses orthogonal manipulation: interventions selected to change one component of the surface-water-ion-energy system as independently as the material permits.

Orthogonality is an experimental ideal rather than a claim of perfect isolation. Changing salt composition can alter diffusion potential, ionic strength, activity coefficients, hydration, and polymer swelling simultaneously; changing illumination can alter photon absorption, temperature, convection, dissolved-gas equilibria, and surface chemistry. The protocol must therefore measure the collateral changes and estimate whether effects on the outcome are mediated principally through the intended variable. Causal language should remain proportional to the selectivity actually achieved.

The core design is an intervention matrix crossing surface chemistry, fixed-charge density, ion-exchange capacity, bulk ionic strength, counterion identity, tracer charge and size, temperature history, illumination spectrum, and mechanical conditioning. Factorial or response-surface designs can identify interactions that would be missed by changing one variable at a time. The strongest tests hold the present macroscopic conditions as constant as possible while changing the route by which the state was reached, thereby also probing path dependence.

Gradient-clamp experiments have particular leverage. Proton activity, major ionic concentrations, and electrical potential should be mapped directly and then reduced through counterion pre-equilibration, controlled perfusion, ion-selective membranes, external fields, or feedback-controlled microfluidics. The

relevant question is not whether buffer was added, but whether the spatial gradients were demonstrably suppressed without destroying the surface. After clamping, newly introduced tracers should be used to distinguish a persistent force or material barrier from an empty region left behind by prior particle displacement.

Ion-exchange capacity should be varied independently of nominal hydrophilicity through a controlled material series. Surfaces can be selected or synthesized to span fixed-charge density, exchange capacity, porosity, roughness, and swelling while maintaining comparable wetting where possible. A monotonic relation between exchange capacity, gradient formation, and long-range tracer velocity would strongly support Model A. A direct water-sensitive state that remains when exchange is minimized would preserve a role for Model B or C, but its chemical and spatial specificity would still have to be demonstrated.

Diffusivity inversion provides another stringent manipulation. By selecting salts whose cation and anion mobilities reverse the sign or magnitude of the liquid-junction potential, investigators can reverse an electrophoretic contribution without changing the hydrated surface. Model A predicts a corresponding reversal or quantitative shift in the motion of tracers with known zeta potential. Failure of the force to track the measured field would indicate either incomplete transport modelling, unmeasured chemistry, or an additional interfacial contribution; it would not by itself identify a new water phase.

Illumination experiments must separate electromagnetic wavelength from thermal history. Equal incident power is insufficient because absorption and heat deposition vary with wavelength and geometry. The decisive comparison uses calibrated spectra, local three-dimensional thermometry, matched bulk and interfacial temperature trajectories, randomized exposure order, and a thermal control reproducing the same temperature field without the test wavelength. Any residual response should then be examined across tracer motion, potential, pH, spectroscopy, and relaxation rather than inferred from zone width alone.

Water-dynamical or structural variables should also be perturbed without presuming their ontology. Isotopic substitution, cryoprotectants, osmolytes, pressure, hydration cycling, and chemically conservative temperature changes may alter hydrogen-bond dynamics, viscosity, dielectric response, or relaxation, but each also affects transport coefficients and material properties. The experiment must therefore quantify those ordinary consequences. A structural interpretation gains support only when a directly measured water-sensitive variable changes in the predicted direction and explains an outcome not already captured by the altered transport parameters.

Release experiments complete the causal sequence. A gradient, field, or hydration condition is clamped, the system is allowed to reach a verified state, and the clamp is removed while all modalities are followed at high temporal resolution. Model A predicts reconstruction governed by surface exchange and transport equations. Model B predicts nucleation or dissolution kinetics tied to a structural order parameter. Model C predicts coupled but nonidentical trajectories: an inner interfacial state may alter the rate or threshold of gradient reconstruction, while the outer force field may decay before the local water-sensitive state fully relaxes.

Interpretation should use mediation analysis and explicit failure criteria. If an intervention changes tracer motion only through its measured effect on gradients, no additional causal level is required. If a water-sensitive variable predicts subsequent force, flow, or recovery after electrochemical and material covariates are included, Model C gains provisional surplus. If purported orthogonality fails because the intervention substantially changes polymer morphology or temperature, the run remains informative about the material system but cannot adjudicate among the models. The value of Phase III lies as much in exposing such confounding as in producing a positive result.

Only after a causal architecture has survived these manipulations should the same logic be transferred from Nafion to controlled non-ion-exchange surfaces, biomolecules, model membranes, cells, and perfused tissues.

Translation must preserve the intervention-and-clamp discipline rather than rely on superficial similarity between an empty tracer region in a polymer chamber and a functional biological interface.

Figure 5. Discriminating Experimental Architecture

Simultaneous measurement and orthogonal perturbation convert a controversy into testable model comparisons.

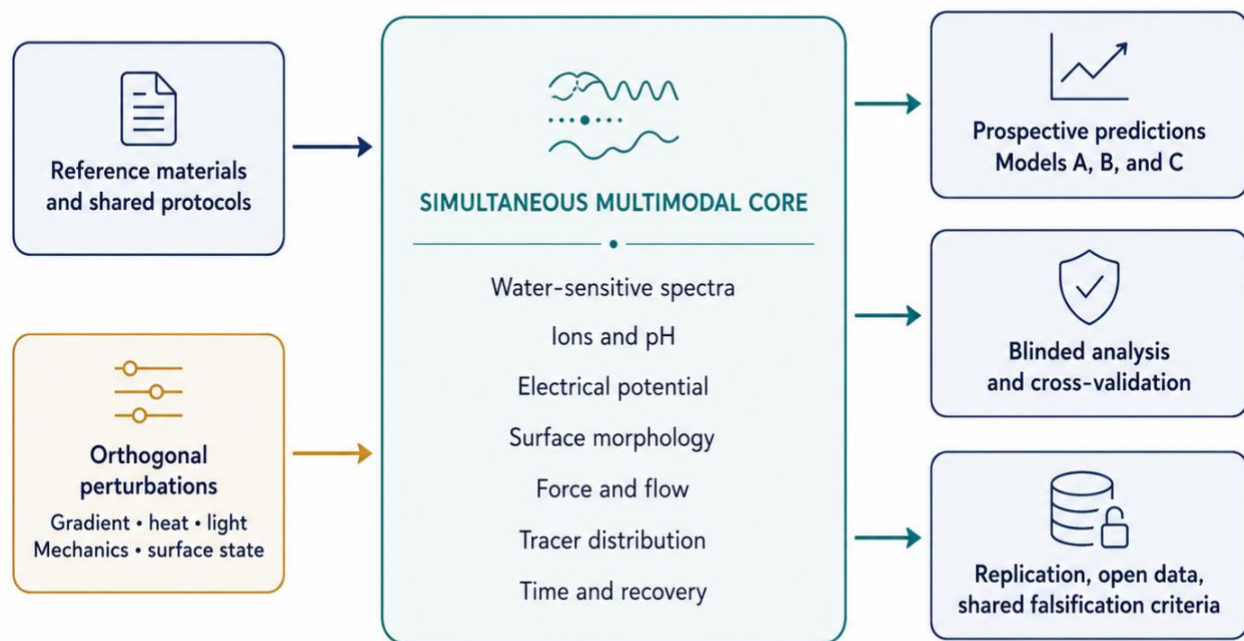


Figure 5. *Discriminating Experimental Architecture*

The experimental programme combines common materials, simultaneous measurement, orthogonal perturbations, blinded analysis, and cross-laboratory replication.

25. Phase IV: Translation from Nafion to Biological Interfaces

Translation must proceed by causal correspondence rather than visual analogy. Nafion is a chemically active perfluorinated ionomer, whereas living interfaces are actively produced mixtures of lipids, proteins, carbohydrates, matrices, ions, metabolites, and water. The question is which relational architecture recurs across these material differences.

The question is therefore not whether a cell membrane, glycocalyx, extracellular matrix, or biofilm is made of biological Nafion.

It is: Which causal relations identified in a well-defined Nafion–water system recur in biological materials, and which relations are transformed, replaced, or absent because living interfaces possess different chemistry, geometry, energy sources, and capacities for regulation and repair?

A 2024 study reported tracer-depleted regions inside and outside excised plant xylem vessels and related them to proton-gradient-driven flow (Wang & Pollack, 2024). The preparation extends the originating programme to biological material, but excised xylem is not an intact living plant and the reported association does not establish physiological necessity or a unique molecular phase.

Femtosecond elastic second-harmonic scattering experiments have reported nanoscale extended orientational ordering of water around hyaluronan beyond its immediate molecular-contact layer. This supports the proposition that a charged biological polymer can influence water over an extended nanoscale domain, while providing no evidence by itself for a uniform macroscopic phase hundreds of micrometres thick (Dedic et al., 2021).

Experiments have shown that disrupting or modifying glycocalyx components can alter endothelial responses to fluid shear, including cytoskeletal reorganization and alignment. Such findings support a causal chain in which flow acts through a hydrated polymer layer connected to cellular machinery rather than directly upon the lipid membrane alone (Zeng & Tarbell, 2014).

In an experimental peritoneal model, enzymatic degradation of endothelial hyaluronan increased protein leakage, supporting a functional role for that glycocalyx component in permeability control (Kamiya et al., 2024).

A 2025 study of the brain endothelial glycocalyx found ageing- and disease-associated disruption of mucin-domain glycoproteins and mucin-type O-glycosylation in mice. Targeted restoration of core 1 mucin-type O-glycans improved barrier integrity and reduced neuroinflammation and cognitive deficits in aged animals (Shi et al., 2025). This is strong evidence that a living hydrated boundary can be causally manipulated and repaired; it is not evidence for a macroscopic EZ-water phase.

The next section moves from correspondence to direct causal proof: Once a biological interface has been identified and measured, how can investigators demonstrate that its interfacial organization is not merely present but actually mediates a defined biological function?

26. Phase V: Coupling Interfacial State to Function

The biological programme should begin with a defined function — transport, membrane potential, ATP output, barrier integrity, mechanical response, or stress recovery — and test whether a measured interfacial state is necessary, sufficient, predictive, and rescuable under controlled perturbation.

The decisive biological question is not whether water near a living boundary differs from distant bulk water. It is whether a specified interfacial state contributes causally to a defined function, predicts that function beyond established variables, and participates in recovery when the function is impaired. A living-interface hypothesis therefore requires more than anatomical colocalization or correlation between hydration and activity.

Each experiment should specify the functional endpoint before measuring the interface. Depending on the preparation, endpoints may include selective permeability, membrane potential, proton conductance, ATP synthesis, receptor activation, fusion probability, barrier integrity, tissue mechanics, transport efficiency, stress recovery, or cell viability. The interfacial profile should be measured simultaneously or with validated temporal registration, and its incremental predictive value should be compared with temperature, composition, morphology, ion concentrations, metabolic state, and known molecular regulators.

The strongest causal design follows perturbation, mediation, and rescue. First, alter an interfacial variable with an intervention whose ordinary biochemical and mechanical effects are measured. Second, test whether the change in function is mediated by the measured interfacial state rather than merely accompanying injury. Third, restore the state by an independent route and determine whether function returns. Rescue is especially important because a variable that changes during damage may be a consequence rather than a cause.

Membrane fusion provides a constrained example. Molecular simulations and experimental interpretation have linked the ordering and displacement of interfacial water to the free-energy barriers governing membrane approach and fusion (Kasson et al., 2011). This supports the proposition that hydration can be functionally constitutive at nanometre scales. It does not imply an extended macroscopic exclusion zone, but it shows how a water-sensitive state can be incorporated into a quantitative functional mechanism.

Receptor activation offers another example of reciprocal protein-water reorganization. Time-resolved structural work on rhodopsin identified bulk-water entry and formation of an internal hydrated network during activation (Fried et al., 2022). The causal unit is neither protein alone nor water alone, but a changing ensemble of conformation, cavities, hydrogen-bonded water, ions, and electrostatics. A Model C experiment would ask whether the measured hydration variables improve prediction of activation kinetics and whether targeted restoration of the network rescues signalling after perturbation.

The endothelial glycocalyx illustrates a larger biological interface. Shear stress can remodel glycocalyx composition, while degradation of glycocalyx components alters transport and barrier behaviour (Zeng & Tarbell, 2014; Kamiya et al., 2024). More recently, disruption of mucin-domain glycoproteins at the brain endothelial glycocalyx was linked to impaired blood-brain-barrier integrity, and restoration of core mucin-type O-glycans improved barrier function in aged mice (Shi et al., 2025). These findings establish the functional importance and reparability of the material interface; they do not yet isolate the contribution of interfacial water, which would require direct hydration, ionic, and mechanical measurements during disruption and rescue.

Mitochondrial energy transduction supplies a still more stringent test. Chemiosmotic theory identifies transmembrane electrochemical proton potential as the driver of phosphorylation, and structural studies show how ATP synthase couples that gradient to rotary catalysis (Mitchell, 1961; Kühlbrandt, 2019). A living-interface model must add predictive value to this established framework, not replace it with metaphor. Candidate contributions include proton organization at membrane surfaces, hydration-dependent protein conformations, dielectric and mechanical boundary conditions, or state-dependent leakage. These contributions would be supported only if their measurement improves prediction of flux or ATP output and if selective perturbation and rescue change function accordingly.

Functional experiments should include both acute and adaptive timescales. An interface may contribute immediately to transport yet also be remodelled over hours or days through synthesis, turnover, cytoskeletal coupling, or matrix repair. A short-lived physical perturbation that is rapidly compensated may reveal robustness, whereas repeated perturbation may expose declining reserve or hysteresis. Recovery trajectories can therefore be more informative than a single endpoint, particularly when Model C predicts that the history of the interface alters its future response.

The analysis must guard against circularity. Defining 'interfacial coherence' by the same functional output it is supposed to explain makes the hypothesis unfalsifiable. The state must be operationalized through independent physical observables, and the functional prediction must be tested on held-out conditions or preparations. A multidimensional profile earns biological relevance only if it generalizes and performs better than simpler component variables.

Model C would receive its strongest biological support if a reproducible coupled state were shown to precede functional change, mediate the effect of a selective perturbation, and be restored during rescue. If function is fully predicted by known composition, gradients, transport coefficients, and molecular machinery, the emergent interface remains a useful description but is not required as an additional explanatory level.

27. Open Data, Preregistration, and Shared Falsification Criteria

Preregistration, registered reports, raw-data publication, blinded analysis, shared code, and explicit null outcomes are not administrative additions. They are part of the causal test because they prevent post hoc redefinition of the phenomenon and preserve the distinction between prediction and explanation (Nosek et al., 2018).

The decisive programme should be preregistered before outcome data are inspected. Preregistration separates predictions from post hoc interpretation, fixes primary and secondary endpoints, documents exclusions and stopping rules, and makes null results scientifically legible. The broader open-science rationale is

not that preregistration eliminates judgment, but that it reveals where judgment entered and limits the silent flexibility that can transform almost any dataset into apparent confirmation (Nosek et al., 2018).

A registered report or equivalent two-stage review is the preferred publication route. The question, design, statistical plan, power, controls, and falsification criteria are reviewed before the results are known. In-principle acceptance then depends on methodological adequacy rather than a positive outcome. This is especially important in a polarized field, where both striking confirmation and striking refutation are more publishable than careful empirical equivalence.

All three models should first be compared with a minimal baseline model, M0, containing only the variables required by established surface chemistry, bulk transport, and instrument response for the specific preparation. Model A, Model B, and Model C must demonstrate prospective improvement over that baseline. This prevents debate among increasingly elaborate theories when none predicts the observations better than a simpler account and ensures that added parameters are penalized.

The collaboration should publish a versioned protocol and a complete materials ledger: polymer lot and pretreatment, surface composition and roughness, water source and purification, dissolved-gas history, chamber fabrication, tracer size and zeta potential, buffer and counterion composition, illumination spectrum and flux, temperature calibration, timing, and instrument settings. Deviations, failed runs, excluded images, and replacement samples should remain visible in the research record rather than disappearing during manuscript preparation.

Data generation, sample coding, and analysis should be separated where practical. Treatment identities can be concealed from microscopists, spectroscopists, and image analysts; calibration files can be locked before unblinding; and independent teams can implement the same preregistered model from shared raw data. When multiple reasonable processing pipelines exist, the protocol should specify a primary pipeline and report a bounded multiverse analysis showing whether conclusions depend on arbitrary analytical choices.

Machine-readable raw data, metadata, calibration records, code, and derived datasets should be deposited with persistent identifiers. Spatial registration among modalities must be documented so that spectra, pH, potential, force, temperature, and tracer motion can be related to the same region and time. Data dictionaries should distinguish observed variables, transformed variables, model-derived latent states, and investigator annotations. Without this provenance, simultaneous multimodal measurement can create an appearance of integration while concealing noncomparable samples or times.

Shared falsification criteria must be symmetrical. Pollack-aligned investigators should identify which null results would cause them to abandon a spatially extended structural interpretation. Transport specialists should state what persistent, tracer-independent spectral, hysteretic, energetic, or functional result would exceed their present model. Model C proponents should specify the incremental prediction, cross-condition generalization, or causal intervention required to justify an emergent explanatory level. A neutral adjudication group should apply these criteria without redefining success after the outcomes are known.

All prespecified outcomes should be reported, including failures of sample preparation, missingness, ceiling effects, null results, and evidence of artefact. Exploratory analyses remain valuable, particularly in an immature field, but they should be labelled and used to generate the next preregistered test rather than retroactively counted as confirmation. If two models remain empirically equivalent within measurement uncertainty, that result should be published as a constraint on what the current methods can decide.

Governance should include representatives of competing models, neutral replication laboratories, metrology and statistics specialists, and an independent data-access committee. Authorship and publication rights should not depend on theoretical agreement. Disputes over interpretation can be presented transparently in parallel commentaries linked to the same dataset, while the primary results paper reports the jointly agreed analyses and deviations.

Open science is therefore not an administrative appendix to the experimental programme. It is part of the causal design. The controversy can be adjudicated only if preparations, transformations, and interpretive choices are sufficiently visible for other laboratories to reconstruct the state that was actually studied and to determine whether the same result survives elsewhere.

Part VI — From Hydrated Interfaces to Living Organization

This Part builds a disciplined bridge from established molecular hydration science to living interfaces. It does not treat nanoscopic hydration as proof of macroscopic exclusion zones; rather, it asks whether coupled water–ion–surface–energy relations help explain membrane function, mitochondrial transduction, extracellular matrices, ecological interfaces, constraint, and autopoiesis.

28. Biomolecular Hydration and the Limits of the Passive-Solvent Model

Mainstream biophysics has already displaced the passive-solvent picture. Proteins, nucleic acids, carbohydrates, and membranes alter nearby water orientation, mobility, and collective dynamics, while hydration contributes to folding, recognition, catalysis, and conformational change. These effects are real but predominantly molecular or nanoscopic (Ball, 2008; Ebbinghaus et al., 2007; Born et al., 2009).

Proteins, nucleic acids, lipid membranes, carbohydrates, and extracellular polymers do not exist in living systems as dry structures later placed into an inert solvent.

A broad body of cell-biological and physical-chemical research has therefore displaced the picture of water as a chemically inert background. Water participates in folding, recognition, association, conformational change, and the mediation of biomolecular forces, although the relevant effects are generally molecular or nanoscopic rather than hundreds of micrometres in extent (Ball, 2008).

Experimental solution scattering has indicated that the average density of the first hydration shell around several proteins can exceed that of the bulk solvent under the measured conditions. Early small-angle scattering measurements estimated an average hydration-shell density approximately ten percent greater than bulk water, while later analyses of lysozyme suggested that much of the apparent density increase could be attributed to geometric packing around the irregular protein surface rather than to a uniformly transformed water phase (Merzel & Smith, 2002).

Such experiments demonstrate that protein hydration is heterogeneous and that water and side-chain relaxation can contribute jointly to the observed solvation response. The observation of a slow component does not imply immobilized water.

Terahertz studies have also reported protein-associated perturbations of collective water dynamics beyond the first hydration layer and reciprocal dependence on protein flexibility (Born et al., 2009; Ebbinghaus et al., 2007). Such signals support extended dynamical coupling on molecular scales; they do not demonstrate a uniform macroscopic phase.

In perdeuterated folded and intrinsically disordered proteins, translational hydration-water motion increased around the observed protein dynamical transition, supporting a close relation between water mobility and functionally relevant protein motion (Schirò et al., 2015).

This suggests that biological activity requires not simply the chemical presence of H₂O but sufficient connectivity and mobility within the hydration environment. Neutron and simulation studies have associated the activation of translational hydration-water motions with the onset of large-amplitude protein motion in both folded maltose-binding protein and intrinsically disordered tau (Schirò et al., 2015).

Time-resolved structural work on the light-activated G-protein-coupled receptor rhodopsin found activation to be coupled to bulk-water movement into the protein and formation of an internal hydrated network (Fried et

al., 2022). The study illustrates that receptor activation can involve coordinated reorganization of protein and water rather than motion of a dry scaffold alone; it does not imply an extended phase outside the protein.

Biological macromolecules and their hydration environments form dynamic, mutually determining molecular interfaces whose local states can participate directly in structure and function.

29. Membranes as Water–Ion–Protein–Energy Ensembles

A membrane is not a dry lipid wall separating two bulk solutions. It is a hydrated electrochemical interphase in which lipids, proteins, confined water, ions, fields, mechanics, and metabolic maintenance jointly determine selectivity, signalling, transport, and work.

Ion selectivity can depend on how a protein reproduces, disrupts, or transforms an ion's aqueous coordination environment. The operative unit is therefore not a dry pore plus an external solvent, but a protein-lipid-water-ion ensemble whose free-energy landscape changes with hydration, voltage, composition, and conformation.

Phase-sensitive vibrational sum-frequency spectroscopy has directly characterized oriented interfacial water associated with model phospholipid membranes, demonstrating that the membrane-water boundary has a measurable molecular organization rather than merely a geometric location (Chen et al., 2010).

Simulation and experimental work on membrane fusion has further shown that water ordering and dehydration between apposed membranes can control fusion dynamics (Kasson et al., 2011). These results link hydration to a membrane-level process without requiring Pollack's proposed charge-separated macroscopic phase.

Lipid composition also feeds back on hydration. Molecular simulations indicate that cholesterol changes interfacial-water density, orientational bias, hydrogen bonding, and network topology at model lipid membranes (Oh et al., 2020). Thus surface composition and water organization are reciprocally related at biologically relevant scales.

Membrane function emerges from a nonequilibrium organization in which water, ions, lipids, proteins, electrical fields, mechanics, and energy flux are mutually constraining and jointly causal. Hydration occupancy and water orientation may alter permeation or conformational barriers, while protein motion, curvature, voltage, and lipid chemistry reshape the hydration environment.

The comparison with Nafion must remain controlled. Biological membranes are thin, heterogeneous, actively remodelled, and coupled to metabolism; Nafion is a durable ionomer with strong fixed charge and proton exchange. The translation question is not whether a cell produces a hundreds-of-micrometres tracer-free zone. It is whether surface state, hydration, ion partitioning, polarization, energy input, mechanics, and transport form measurable, functionally consequential organizations at the relevant molecular and cellular scales.

30. Mitochondria, Proton Organization, and Energy Transduction

Mitochondria provide the clearest biological case of an energy-transducing interface. Chemiosmosis remains indispensable, but proton localization, interfacial water networks, cardiolipin, respiratory supercomplexes, crista geometry, membrane potential, and ATP synthase may determine how the proton-motive force is organized and converted into work (Mitchell, 1961; Kühlbrandt, 2019).

The open question is whether interfacial organization adds mechanistic precision to how the proton-motive force is generated, localized, transmitted, dissipated, and converted into ATP.

These possibilities are compatible with conventional nonequilibrium thermodynamics. They become support for Model C only when their coupling predicts energetic performance beyond the sum of individually measured variables.

The biologically relevant interface is not water alone, membrane alone, or gradient alone, but a dynamically maintained organization in which each constrains the others and the whole converts free energy into continued living capacity.

They become support for Model B only if a distinct extended aqueous structural state is directly identified and shown to mediate energy conversion.

Mitochondrial energy transduction is already an interfacial process in the conventional account. Electron transfer through respiratory complexes contributes to proton translocation across the inner membrane; the resulting electrochemical potential drives ATP synthase and supports transport, signalling, and thermogenesis. The membrane's extreme protein density, cardiolipin-rich composition, curvature, crista architecture, confined aqueous spaces, and continuous metabolic turnover make it a poor candidate for description as a dry dielectric separating two bulk solutions. Water and proton mobility are embedded within the machinery, even though this does not imply Pollack's proposed macroscopic phase (Mitchell, 1961; Kühlbrandt, 2019).

A Model C programme would ask whether a multidimensional hydration–charge–structure state helps explain coupling efficiency, proton leak, ATP production, redox balance, and recovery after energetic stress. Measurements might combine membrane potential, matrix and intermembrane pH, oxygen consumption, ATP flux, local temperature, crista morphology, water-sensitive spectroscopy, and mechanical state in the same preparation. Perturbations should distinguish changes in respiratory chemistry from changes in hydration or membrane organization — for example by varying osmotic conditions, lipid composition, ionic environment, and substrate supply while preserving oxygenation and temperature.

The decisive criterion is incremental and causal. If water-sensitive variables merely track membrane potential or protein activity without adding prediction, the established chemiosmotic account remains sufficient. Model C gains support only if an interfacial state predicts efficiency or resilience beyond conventional variables, if selectively changing that state alters function, and if restoring the state rescues function. This would identify hydration as part of the operative organization, not as an alternative to electron transport or proton-motive force.

31. Glycocalyx, Extracellular Matrix, Fascia, and Interstitial Flow

The glycocalyx, extracellular matrix, fascia, and interstitium are charged, hydrated, mechanically loaded, and continuously remodelled. Donnan partitioning, polymer swelling, poroelasticity, flow, and cellular maintenance provide established mechanisms; Model C becomes useful only if their coupled state predicts function or recovery beyond those components considered separately.

When neighbouring matrix fibres or cell surfaces are separated by distances comparable to the relevant interfacial range, local hydration and ionic domains may overlap.

Tissue water participates in a hierarchy of local hydration domains, porous transport fields, charged polymer networks, and mechanically coupled flows whose biological significance arises from their reciprocal organization with cells and matrix.

The glycocalyx and extracellular matrix are hydrated, charged polymer networks rather than inert scaffolds. Fixed anionic groups influence ion partitioning, osmotic pressure, swelling, lubrication, permeability, and mechanotransduction. Hyaluronan can organize water beyond its immediate contact shell at nanoscopic scales, while collagen, proteoglycans, and glycosaminoglycans create geometrically constrained fluid pathways (Dedic et al., 2021). These effects are compatible with Donnan equilibria, poroelasticity, electrokinetics, and polymer

physics; Model C asks whether their coupling creates state variables that matter for tissue function and recovery.

Fascia and interstitial tissues add hierarchical geometry and dynamic loading. Fluid movement, strain, matrix remodelling, cellular traction, vascular pulsation, and lymphatic drainage continually change local hydration and charge distributions. Conventional fixation and sectioning can collapse fluid-supported spaces, as the interstitium example demonstrates (Benias et al., 2018). In-situ imaging, pressure and flow measurements, Brillouin or related mechanical mapping, and water-sensitive spectroscopy should therefore be coordinated rather than interpreted from separate preparations.

A biologically meaningful test would link an interface profile to permeability, shear response, tissue glide, solute transport, inflammatory recruitment, or repair. The model should predict how enzymatic degradation of glycocalyx or matrix, altered ionic composition, dehydration, compression, or restoration changes both the measured state and function. Evidence for extended hydration in polymers supports plausibility only at its demonstrated scale; it does not establish a universal tissue-wide exclusion zone.

32. Microbial Biofilms, Roots, Soils, and Ecological Interfaces

Biofilms, roots, and soils show that organisms construct and regulate hydrated external environments. Secreted polymers, pores, mineral surfaces, exudates, ionic gradients, microbial metabolism, and water flow form ecological interfaces whose organization can influence retention, transport, competition, and resilience.

If the resulting conditions improve survival or reproduction, the capacity to produce them may be retained and elaborated through selection. The environment becomes partly shaped by the organisms inhabiting it.

A biofilm is a community of microorganisms associated with a surface or with one another and embedded to varying degrees in an extracellular matrix.

A pump need not contain a rotating component or mechanical piston. In *Bacillus subtilis* biofilms, extracellular-matrix production can generate an osmotic pressure difference that draws water from the environment and contributes to colony spreading (Seminara et al., 2012). This is an established example of an organism-produced matrix converting chemical-potential differences into fluid movement.

Root mucilage can alter rhizosphere wettability, water retention, and hydraulic continuity as soil dries, producing history-dependent drying and rewetting behaviour (Carminati, 2011). The mechanism is not an EZ claim; it illustrates how a secreted hydrated polymer changes the boundary conditions governing transport and survival.

How can a physical system acquire causal properties not because a new substance has appeared, but because interacting processes constrain one another in ways that preserve and reproduce the conditions of their continued existence?

Biofilms, rhizospheres, and soils show why interfacial organization cannot be reduced to water alone. Microbes and roots secrete polymers, alter pH and redox conditions, move ions, change porosity, and construct gradients that feed back upon transport and community structure. The resulting hydrated matrices can retain nutrients, exclude or bind solutes, guide motility, and buffer environmental fluctuations. These are canonical examples of organisms modifying the boundary conditions of their own persistence.

Model C offers a comparative vocabulary across these systems, but not a claim of identical mechanism. The relevant observables differ among a bacterial biofilm, a root mucilage layer, and a soil aggregate. Each system should be tested for coupled changes in matrix chemistry, water mobility, charge, gradients, flow, mechanics,

and function at the scale appropriate to that interface. Cross-system generalization is earned only if the same relational predictions survive despite different materials and biological operations.

Figure 6. From Physical Interface to Biological Function

Translation must proceed by controlled steps; evidence cannot leap directly from Nafion to physiology.

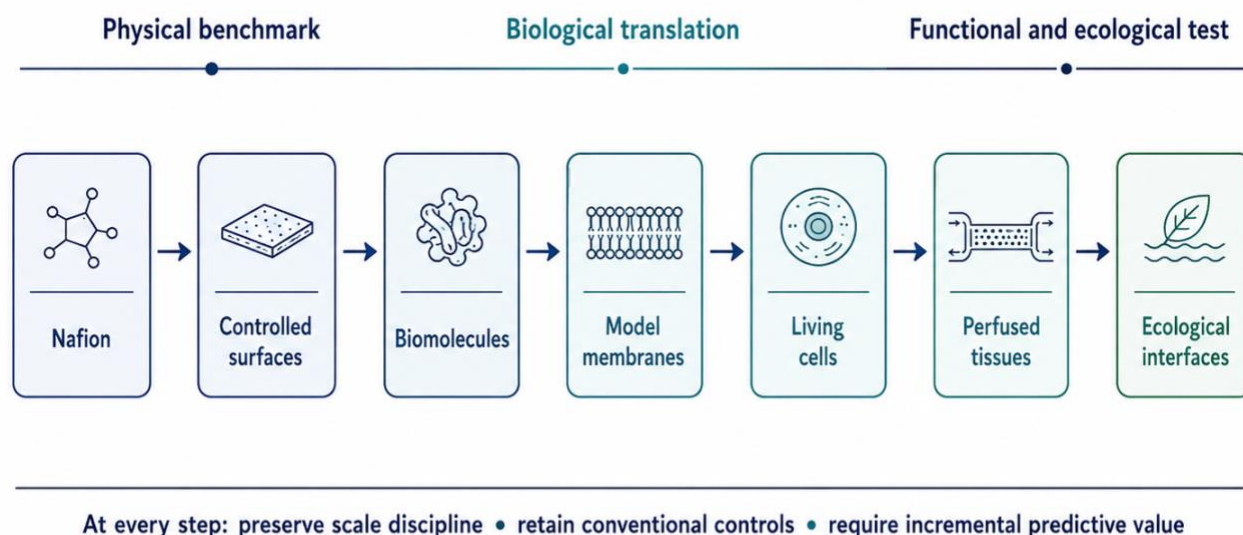


Figure 6. From Physical Interface to Biological Function

Translation proceeds in controlled stages so that molecular hydration evidence is not mistaken for proof of a macroscopic biological exclusion zone.

33. Deacon: Constraints, Absence, and the Generation of Work

Deacon's constraint-based account clarifies how absence can become causally consequential: work is generated when constraints remove possibilities and channel remaining processes. Applied cautiously, this framework helps specify how surfaces, gradients, geometry, and flows might form mutually sustaining causal loops without invoking a mysterious substance (Deacon, 2012).

It requires the emergence of a higher-order relation among physical processes in which each constrains the tendencies of the others in ways that preserve the organization producing those constraints.

Deacon's account in *Incomplete Nature* develops a process ontology in which constraints and the systematic absence of otherwise possible events can become causally explanatory without adding forces beyond physics (Deacon, 2012).

It refers to unrealized possibilities whose systematic exclusion changes the causal behaviour of the system. Deacon and García-Valdecasas argue that biological teleology can be naturalized by analysing how physical organizations prevent their own otherwise probable cessation (Deacon & García-Valdecasas, 2023; García-Valdecasas & Deacon, 2024).

Likewise, the lumen of a vessel, pore of a matrix, or cavity of an enzyme is causally important because of the material that is absent from that region. The absence is physically realized through the organization of what surrounds it.

Each process thereby constrains the terminal tendency of the other. Deacon and colleagues proposed the autogen as a physically grounded proof-of-principle for the transition from spontaneous self-organization to self-preserving regulation, rather than as a historical claim about the exact first organism.

Deacon and Koutroufinis proposed dynamical depth as a way of distinguishing systems by the number and organization of nested nonlinear dynamical levels through which constraints are generated (Deacon & Koutroufinis, 2014).

A living system approaches closure of constraints when the constraints required for its continued operation are generated and maintained through other processes within the same organized network.

A dynamically maintained boundary or interphase whose material, aqueous, ionic, energetic, and mechanical organization constrains exchange in ways that contribute to the continued production, regulation, and repair of the system that maintains it.

This is where Deacon's autogen parallels but does not simply duplicate autopoietic theories. Deacon and colleagues explicitly framed the autogen to clarify how self-organizing processes might become mutually regulating and self-preserving (Deacon & Koutroufinis, 2014).

A life-coherent organization aligns lower-level functions with the continued viability of the larger living system and its enabling conditions. This does not imply perfect harmony.

How are water, ions, surfaces, gradients, flows, and energy constrained so that their coupled dynamics contribute to preserving and rebuilding the living organization that maintains those constraints?

Deacon's constraint-based account is useful because it shifts explanation from an inventory of components to the organization of possibilities. A surface, membrane, channel, or matrix performs work not by adding a new force but by restricting otherwise available trajectories so that gradients and fluctuations become directionally productive. In an interfacial system, geometry constrains diffusion; fixed charge constrains ion distribution; hydration constrains molecular mobility; and flows reshape the very gradients that drive them. The resulting organization can therefore have causal relevance without violating component-level physics.

This conceptual language does not establish Model C. It specifies what the model must demonstrate: constraints should be mutually dependent, maintained through throughput, and associated with work or function that cannot be predicted as accurately when the relations are decomposed. If every apparent constraint can be represented independently and recombined without loss, an emergent level is unnecessary. If disrupting one relation reorganizes several others and changes system competence, the constraint network becomes an experimentally legitimate object.

34. Maturana: Boundary Production and the Physical Realization of Autopoiesis

Maturana's autopoiesis shifts attention from components to the network that produces and maintains its own boundary. Model C offers a candidate physical layer for this boundary production only if interfacial relations are generated, regulated, repaired, and recursively coupled to the metabolism they help sustain (Varela et al., 1974; Maturana & Varela, 1980).

It is defined by a network of processes that continually produces the components and relations that regenerate that same network and constitute the system as a bounded unity. The original formal account characterized living systems as members of the class of autopoietic systems and illustrated the idea through a minimal computational model (Varela et al., 1974).

A membrane, glycocalyx, matrix, or biofilm layer is a living interface only when it is produced within a network whose continued existence depends upon its function.

The organism depends on cells whose molecular production networks are autopoietic. Whether the organism as a whole satisfies the same strict molecular definition should be assessed rather than assumed.

Autopoiesis defines living systems through networks of processes that produce the components and boundary relations that recursively sustain the network (Varela et al., 1974; Maturana & Varela, 1980). The theory identifies an organizational requirement; it does not prescribe a particular molecular substrate. Model C contributes a candidate physical vocabulary for investigating how boundary production, selective exchange, energy transduction, hydration, ionic organization, and repair are materially coordinated.

The distinction between a self-organizing material interface and a living interface is decisive. Nafion can generate gradients and flows, but it does not synthesize its own polymer, repair damage, regulate exchange according to internal viability, or reproduce the network that maintains it. Cells do. An interfacial account of autopoiesis must therefore include metabolic production, turnover, sensing, and adaptive regulation rather than treating charge-separated water as sufficient for life.

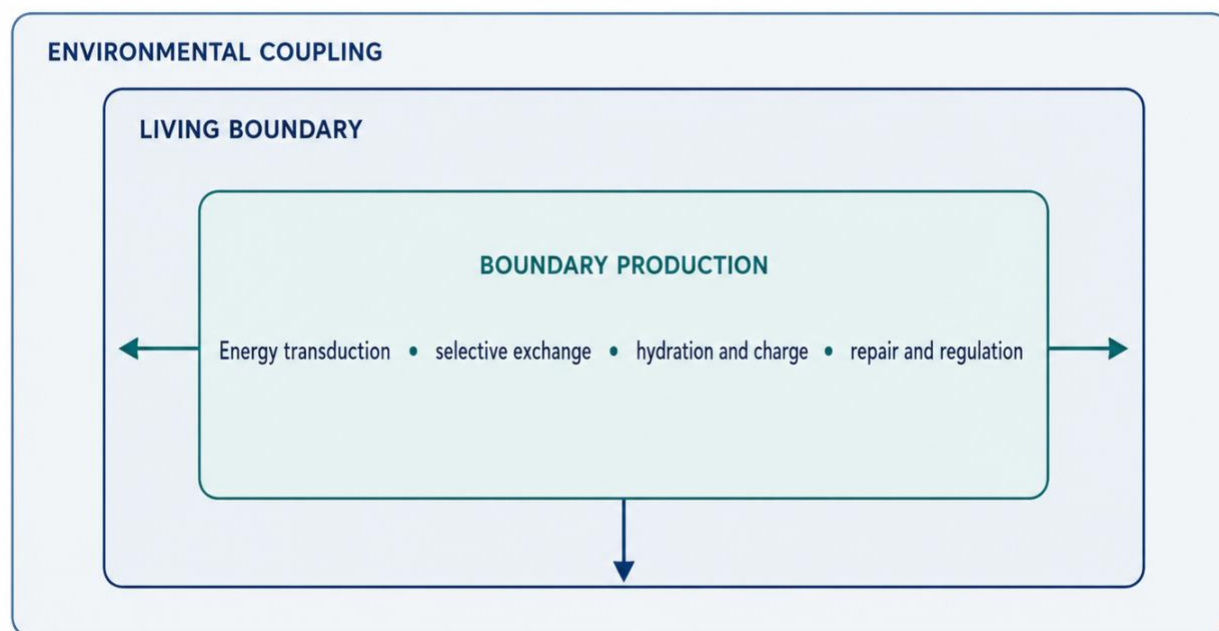
The strongest test is closure across intervention and repair. When a boundary component is perturbed, does the organism actively restore a coupled interfacial state, and does that restoration recover selective exchange and continued self-production? Demonstrating such a relation would not make interfacial water ‘the basis’ of autopoiesis. It would identify one physical layer through which autopoietic organization is realized.

Table 6. *Evidence-status matrix for biological extrapolations*

Biological level	What is established	What remains hypothetical
Proteins and nucleic acids	Hydration affects structure, dynamics, recognition, and catalysis	A universal extended charge-separated phase around macromolecules
Membranes	Water, ions, lipids, proteins, fields, and mechanics jointly determine function	A Pollack-scale EZ as the necessary basis of all membrane function
Mitochondria	Redox reactions generate proton-motive force across hydrated crista membranes	A distinct interfacial-water state independently stores or channels bioenergetic work
Glycocalyx/ECM/fascia	Fixed charge, swelling, Donnan effects, poromechanics, and remodelling organize water and transport	One common mesoscopic state explaining tissue-wide function
Biofilms, roots, soils	Organisms construct hydrated polymeric interfaces and gradients	A universal fourth phase across ecological matrices
Autopoiesis	Living systems produce and maintain their boundaries	Interfacial water as the proven physical basis of autopoiesis

Figure 7. The Interface as a Candidate Physical Layer of Autopoiesis

Model C is a bridge hypothesis: it does not make interfacial water sufficient for life.



Autopoiesis requires recursive production and repair; a self-organizing material interface alone is not living.

Figure 7. *The Interface as a Candidate Physical Layer of Autopoiesis*

Part VII — Stressors, Toxicants, and Loss of Interfacial Coherence

This Part explores how chemical mixtures might perturb living interfaces without converting a single Nafion assay into a biological conclusion. Glyphosate formulations are treated as a test case for decomposing pH, ionic strength, surfactant, co-formulant, surface, and mixture effects and for linking molecular disturbance to loss of reserve and recovery.

35. Chemical Perturbation of Living Interfaces

Chemical stress can alter several interface variables at once: pH, ionic strength, counterion composition, surface charge, polymer conformation, membrane fluidity, hydration, redox state, and flow. A water-specific conclusion requires decomposition of these coupled effects rather than attribution from one tracer assay.

A strong mechanistic claim requires measurement or defensible modelling of the concentration at the proposed site of action.

Commercial products can change composition over time or between regions. A study of one formulation batch should not be generalized automatically to all products sharing the same active ingredient.

A stronger claim that the chemical disrupts a distinct water phase requires an independently validated molecular order parameter and evidence that its alteration is not secondary to changes in the surrounding materials.

It also provides a concrete test of whether the living-interface framework generates better experiments than either broad reassurance or broad alarm.

Chemical perturbation can act through several routes at once: changing surface protonation, ionic strength, osmolarity, redox state, membrane fluidity, protein conformation, polymer swelling, dissolved-gas behaviour, or instrument response. A decline in tracer exclusion therefore cannot be assigned automatically to loss of a special water structure. The first task is analytical decomposition of the exposure and the assay, followed by measurement of the same multimodal state variables used to characterize the unperturbed interface.

A causal toxicology of interfaces should distinguish direct physicochemical effects from metabolically mediated injury. In a synthetic system, composition, pH, counterions, surfactants, temperature, and surface history can be varied independently. In cells or tissues, these controls must be supplemented by uptake, metabolism, membrane damage, oxidative stress, repair, and viability. The same nominal concentration can produce different interfacial exposures because binding, partitioning, and biological clearance change the dose reaching the relevant boundary.

Model C adds value only if it predicts patterns of vulnerability and recovery beyond conventional toxicological variables. It may, for example, predict that two weak perturbations are synergistic because both reduce the same maintenance reserve, or that exposure order matters because the first stress changes the interface encountered by the second. Such predictions require prospective tests and must remain open to simpler explanations.

36. Glyphosate Formulations as an Exploratory Test Case

The reported contraction of a Nafion exclusion region by a commercial Roundup formulation is a legitimate observation but not proof that glyphosate disrupts living interfacial water. The responsible factor could be glyphosate salt, surfactants, co-formulants, pH, ionic strength, osmolality, or material interaction (Sharma et al., 2018).

Any claim about effects on water, membranes, microorganisms, animals, plants, or ecosystems must identify which of these objects was actually tested.

Plants with a resistant EPSPS or another validated resistance mechanism provide an important causal control. If purified glyphosate produces an effect in susceptible plants but not resistant plants at matched internal exposure, EPSPS dependence is supported.

At matched glyphosate concentration, pH, ionic strength, osmolality, temperature, and counterion composition, the complete formulation will alter membrane permeability and tracer behaviour more strongly than purified glyphosate if co-formulants are the dominant immediate interface perturbants.

Do not stop at identifying injury. Determine how the exposure alters the coupled system's capacity to maintain gradients, boundaries, metabolism, repair, and future resilience.

It also imposes an equally important restraint: Do not invoke disrupted biological water when surface chemistry, formulation, membrane injury, ionic conditions, metabolism, or assay interference have not been excluded.

The originating experiment reported that a commercial Roundup formulation contracted the measured exclusion region beside Nafion (Sharma et al., 2018). Its legitimate evidentiary use is therefore narrow: it establishes that the assay responded to the formulation under the reported conditions, not which ingredient acted or whether an analogous disturbance occurs in living interfaces.

The reported contraction of a Nafion-associated exclusion region after exposure to a commercial Roundup formulation is an observation about one assay, not a demonstration that glyphosate disrupts living interfacial water. Commercial formulations may contain glyphosate salts, counterions, surfactants, antifoaming agents, preservatives, and other co-formulants; they may also alter pH, conductivity, osmolality, wetting, polymer swelling, particle charge, and optical properties. Each of these routes can change tracer behaviour without requiring a new water ontology.

A decisive replication should compare purified glyphosate acid, relevant glyphosate salts, isolated co-formulants, reconstructed mixtures, and complete formulations. Conditions should be matched for glyphosate concentration, pH, ionic strength, osmolality, temperature, counterion composition, tracer zeta potential, and surface conditioning. Measurements should include polymer swelling, proton release, conductivity, potential, pH, fluid flow, tracer motion, temperature, and at least one water-sensitive modality. Dose-response and washout should be preregistered, and investigators should be blinded to treatment identity during image and spectral analysis.

Translation to biology should then proceed through mechanistically appropriate systems. Plants with susceptible and resistant EPSPS provide a way to separate the canonical biochemical target from immediate formulation effects; model membranes or epithelial barriers can test surfactant-mediated permeability; microbial or plant interfaces can test recovery and repeated exposure. The result may reveal ordinary formulation chemistry, a coupled interfacial disturbance, or both. The paper's purpose is to specify the distinction rather than prejudge it.

37. Low-Dose, Mixture, and Nonlinear Effects

Living interfaces may respond nonlinearly to low-dose mixtures because several weak perturbations converge on the same coupled system. Nonmonotonicity, thresholds, adaptation, sensitization, and sequence effects should be tested through factorial mixture designs and complete chemical characterization, not inferred from isolated endpoints.

Yet a living interface is not an inert detector whose output depends only on the quantity of chemical present.

Hormesis describes a biphasic response in which a low exposure appears to stimulate or improve a measured outcome while a higher exposure impairs it.

Small perturbations become biologically important when they alter the state, maintenance cost, or recovery capacity of a coupled living interface, especially when repeated exposures prevent full repair or when several stressors draw upon the same limited reserve.

Dose is not a complete state description for a system that adapts, repairs, accumulates damage, and changes its own exposure conditions. A low exposure may induce compensatory responses that temporarily preserve output, whereas repeated exposure may prevent complete recovery and progressively reduce reserve. Apparent hormesis can therefore reflect beneficial adaptation, measurement timing, selection of resilient subpopulations, or redistribution of resources rather than a direct improvement in the underlying interface.

Mixture experiments should use factorial or response-surface designs capable of separating additivity, synergy, antagonism, and sequence dependence. Chemical characterization must accompany biological measurement, because changing one component can alter solubility, micelle formation, membrane partitioning, or effective free concentration of another. Time should be treated as a design dimension: simultaneous exposure, staggered exposure, washout, and rechallenge may produce different trajectories even when cumulative dose is identical.

Model C predicts nonlinear effects specifically when weak perturbations converge upon coupled maintenance variables such as boundary integrity, electrochemical gradients, hydration, mechanics, or repair capacity. The hypothesis would be weakened if mixture responses are predicted fully by measured component concentrations and known target interactions. It would gain support if a preregistered interfacial-state metric explains thresholds, order effects, or delayed recovery better than conventional exposure variables alone.

38. From Molecular Disruption to Loss of Biological Resilience

Resilience is revealed by performance under disturbance and by the capacity to recover. A system can preserve resting output while losing reserve, efficiency, redundancy, repair speed, and tolerance to a second challenge; these dynamic deficits may be more informative than a single static injury marker.

Resilience of which function, against which perturbation, over which timescale, and relative to which acceptable state?

Generic early-warning indicators have shown mixed reliability in empirical systems. Some real systems do not approach transitions through the type of bifurcation that produces critical slowing down.

Data gaps, outliers, short records, external forcing, and measurement changes can also distort variance- and autocorrelation-based indicators. Whole-ecosystem analyses have found that classical signals are not consistently reliable across all trophic levels or regime shifts, and recent methodological work has emphasized sensitivity to missing or anomalous data.

Rate-induced tipping has been demonstrated theoretically in high-dimensional ecological networks and shows that transition risk cannot always be inferred from the final exposure level alone.

Mean exit time has been proposed as a practical resilience measure because it directly expresses how long a system is expected to persist before crossing a critical boundary.

A resilience experiment should therefore include baseline, graded challenge, recovery, and second-challenge phases. Relevant outcomes include the amplitude of functional decline, recovery time, energetic cost of restoration, residual deficit, variability, and the response to rechallenge. Static viability measured at one time point can miss a system that has survived only by consuming reserve or sacrificing another function.

The interface framework suggests a mechanistic bridge from molecular disturbance to system fragility. Perturbation of a membrane, matrix, or hydrated boundary may increase leak, dissipate gradients, alter transport selectivity, raise maintenance costs, and slow repair. At higher scales, these changes can reduce redundancy and narrow the range of disturbances the organism or ecosystem can absorb. This sequence remains a hypothesis until each step is measured and alternative routes are controlled.

No single universal early-warning indicator should be presumed. Recovery time, variance, autocorrelation, energetic inefficiency, or spatial heterogeneity may be informative in one system and misleading in another. The strongest evidence would be prospective: a multidimensional interface state measured before overt failure predicts which systems will recover, which will cross a functional threshold, and which interventions will restore resilience.

Part VIII — Scientific Judgment and Research Governance

This Part evaluates explanatory performance, asymmetrical skepticism, confirmation, revision, and rejection. The final aim is a research governance framework in which neither novelty nor convention is protected from severe testing and in which explanatory complexity is retained only when it yields predictive surplus.

39. Explanatory Surplus Versus Fragmented Adequacy

A model earns explanatory surplus when it unifies independently measured observables, predicts new relations, survives intervention, generalizes across systems, and improves functional prediction enough to justify its added complexity. Merely redescribing known transport in integrative language is not enough.

Does the emergent nonequilibrium-interface model explain or predict something that a complete combination of established mechanisms does not?

Model C deserves serious investigation because it organizes neglected relations and generates distinctive tests. It deserves acceptance as an independent explanatory level only when those tests demonstrate measurable surplus.

It asks why unconventional claims are often required to satisfy standards that familiar explanations are permitted to evade, while also examining the reverse danger: lowering evidentiary standards merely because a proposal challenges an established paradigm.

Fragmented adequacy occurs when separate mechanisms explain separate outcomes but no account predicts how the outcomes relate. Such a patchwork is not automatically deficient; complex systems often require several mechanisms. Model C becomes preferable only when the coupling itself carries information — for example, when spectral change, potential, pH, flow, and recovery follow a constrained temporal sequence that is not captured by independently fitted equations.

Explanatory surplus should be evaluated quantitatively. Competing models can be compared through preregistered likelihoods, information criteria, cross-validation, and prediction on held-out conditions or materials. Added parameters must be penalized, and success should be assessed prospectively rather than through retrospective accommodation. An integrated latent-state model that fits existing data but fails to predict a new perturbation has not earned ontological status.

The same standard applies to Model A and Model B. A transport model should not be declared complete merely because its mechanisms are familiar; it must predict the measured constellation within uncertainty. A structural-phase model should not be credited for unification without a specific structure, boundary, and relaxation law. Model C should not be retained because it sounds comprehensive. Its justification is the discovery of reproducible relations that become visible and experimentally useful only when the interface is treated as an organized process.

40. Asymmetrical Skepticism and Paradigm Protection

Skepticism becomes asymmetrical when unconventional claims are rejected for imperfections tolerated in conventional explanations, or when novel interpretations receive relaxed standards because they oppose an establishment. The remedy is one evidentiary ladder applied to all models, not rhetorical balance.

It can also be suspended when an unconventional proposal is attractive because it challenges an established institution or resonates with a desired worldview.

An unconventional explanation is required to eliminate every alternative, while the conventional explanation is accepted after demonstrating only that it could produce the observation.

A recurring asymmetry appears when a conventional mechanism is regarded as established because it can produce an effect, while an unconventional mechanism is required to reproduce every feature quantitatively.

If no result can establish how much structure exists, where it ends, or how it differs from known hydration, then no result can falsify the claim.

Large collaborative replication efforts have shown that published findings in established research domains may reproduce less consistently or with smaller effects than the original literature suggests.

Registered Reports were developed so that study questions and methods can undergo peer review before outcomes are known, with publication decisions based primarily on the importance and rigour of the design rather than whether the result is positive.

History and relational variables add no predictive gain, and component rescue restores complete function. These clauses transform openness to revision from a declared virtue into an operational commitment.

It is to expose every explanation to the same disciplined possibility of being wrong. The next section defines what should follow when evidence accumulates.

At times, critics have treated plausible transport mechanisms as if their mere availability constituted a complete quantitative explanation.

Paradigm protection is best analysed through procedures rather than presumed motives. Choices about what counts as an anomaly, which controls are demanded, whether null results are published, and when a mechanism is considered quantitatively sufficient can favour familiar explanations without deliberate misconduct. Conversely, communities organized around a heterodox proposal may discount negative replications, expand claims after the fact, or treat institutional resistance as evidence of truth.

A symmetrical evidentiary regime requires the same distinctions for every model: possibility versus demonstration; observation versus interpretation; replication of an effect versus replication of a mechanism; fit to existing data versus prospective prediction; and biological relevance versus biological operation. Funding and conflicts should be disclosed, but credibility should turn on design quality, transparent data, and explanatory performance rather than on ad hominem inference.

Adversarial collaboration is therefore not a demand for interpersonal consensus. It is a method for exposing tacit assumptions before data collection. Each group helps construct the strongest tests of its own position and the strongest controls requested by its critics. Neutral replication laboratories, registered protocols, blinded analysis, and predetermined decision rules make it harder for any community to protect a favoured explanation through selective standards.

41. Conditions for Confirmation, Revision, or Rejection

Model C must remain eliminable. It should be strengthened by reproducible multimodal coupling, persistence, hysteresis, reciprocal intervention, energy closure, and functional prediction; revised if its scope is limited to inner interfaces or particular materials; and displaced if a complete Model A predicts all outcomes without residual explanatory gain.

A scientific programme must specify not only how evidence will be gathered but how that evidence will change the status of competing explanations.

The existence of a tracer-independent aqueous structural domain beyond several nanometres remains unresolved because current measurements do not separate water state from polymer concentration and ionic gradients.

A tracer-depleted region is reproducible beside some charged polymers. Current evidence supports important roles for ion exchange and transport. Whether a distinct extended water state contributes independently remains unresolved.

Or it may show that present observations are generated by several context-dependent mechanisms with no single unifying state. All of these outcomes would constitute scientific progress if obtained through discriminating evidence.

Confirmation should be graded. Reproducible tracer exclusion confirms an operational phenomenon; quantitative agreement with electrodiffusive predictions confirms a mechanism within its tested range; a spatially resolved, tracer-independent molecular signature would support structural differentiation; coordinated reciprocal changes would support an emergent interfacial organization; and causal prediction of living function would establish biological consequence. No single result should be allowed to leap across these levels.

Model C would be strengthened by at least five convergent findings: a water-sensitive variable that is not reducible to polymer or concentration artefact; coordinated temporal coupling among that variable, gradients, potential, force, and flow; differential relaxation or hysteresis after orthogonal perturbation; generalization across controlled surfaces and biological preparations; and incremental prediction or rescue of function. Its scope might still require restriction to certain materials, geometries, ionic regimes, or timescales.

Revision is not failure. Evidence may show that the operative organization is better described as a material–electrochemical interphase in which water is necessary but not privileged, or that mechanics, dissolved gases, polymer restructuring, or active metabolism must be added explicitly. Rejection is warranted if complete component models predict all outcomes prospectively, if purported water signatures disappear under artefact controls, if coupling adds no predictive value, and if no history, energetic, or functional surplus remains.

Table 7. Conditions that strengthen, revise, or falsify Model C

Judgment	Empirical condition
Strengthen	Coordinated multimodal changes; reciprocal interventions; differential relaxation; hysteresis; cross-material generalization; improved prediction of biological function
Restrict	Coupling occurs only in an inner nanoscopic interphase, only in certain ionomers, or only during defined nonequilibrium transitions
Revise	The relevant state is primarily material–electrochemical rather than water-centred, or additional mechanical and biochemical variables are required
Displace	A complete transport/material model predicts all observations prospectively; no independent water-state signature, persistence, memory, energetic surplus, or functional gain remains
Suspend judgment	Measurements are too perturbative, cross-laboratory reproducibility is inadequate, or competing models remain empirically equivalent

42. Toward an Interfacial Science of Living Organization

An interfacial science of living organization would unite colloid science, spectroscopy, membrane biophysics, nonequilibrium thermodynamics, physiology, and systems biology around shared observables and causal tests. Its purpose is not to install a new water orthodoxy but to discover how matter, energy, boundary, and function become coordinated at hydrated interfaces.

The interface becomes biologically distinctive when the organism continually produces, regulates, and repairs the material and energetic relations through which that interface functions. The field should neither canonize the fourth-phase hypothesis nor close the question prematurely.

The transition from controversy to science occurs when no model is protected from measurement and no reproducible phenomenon is discarded merely because its first interpretation was too strong.

A coherent interfacial science can reconcile the most defensible insights of Pollack's programme with the strongest conclusions of its critics.

Structure and transport should not be forced into opposition when both may be generated within one dynamically maintained interface.

The proposed field should be organized around shared state variables rather than disciplinary ownership. Colloid scientists can quantify transport and surface chemistry; spectroscopists can resolve hydration and molecular dynamics; membrane biophysicists can connect structure to selective exchange; physiologists can define function and reserve; nonequilibrium theorists can formalize coupled flows and constraints; and systems biologists can test whether multidimensional states improve prediction across scales. The interface becomes a meeting place for methods, not a claim that one discipline's vocabulary subsumes the others.

A staged research infrastructure would begin with reference materials and benchmark protocols, then move through chemically controlled surfaces, biomolecules, model membranes, living cells, perfused tissues, and ecological matrices. Each transition should preserve matched controls and explicitly state what is gained and lost. Negative translation is informative: a relation robust in Nafion but absent from biological interfaces defines a material-specific phenomenon rather than a failed universal theory.

The long-term product should be an atlas of hydrated interfaces containing geometry, composition, water dynamics, ion distributions, electrical and mechanical properties, energy throughput, temporal history, and functional outcomes. Such an atlas could reveal whether different systems occupy recurring organizational regimes or whether the apparent unity dissolves into context-specific mechanisms. Either result would improve science.

The governing proposition remains deliberately conditional. Pollack's programme may have pointed toward a broader class of energy-responsive, charge-differentiated aqueous interfaces, while transport critiques have identified indispensable processes within those systems. The task is now to determine, through shared experiments, whether a dynamically maintained interface possesses causal organization beyond its components or whether the complete conventional account is sufficient.

Conclusion

From the Fourth Phase to the Living Interface

The exclusion-zone controversy began with a robust empirical puzzle: beside certain hydrated surfaces, specified suspended particles and solutes may be depleted from regions extending far beyond conventional molecular hydration shells. Pollack and colleagues interpreted this constellation — exclusion, electrical polarization, proton redistribution, force, flow, and radiant-energy response — as evidence of an extended, differentiated aqueous state. Independent investigations subsequently showed that ion exchange, unequal ionic diffusion, electrical fields, electrophoresis, diffusiophoresis, osmosis, and material dynamics can generate substantial parts of the same operational phenomenon.

The strongest present judgment is therefore layered. Long-range tracer exclusion near Nafion is real under some experimental conditions. Conventional transport mechanisms are causally necessary and quantitatively powerful. A uniform macroscopic crystalline or H₃O₂-like phase has not been directly and independently established through a unique molecular signature, order parameter, or thermodynamic characterization. Yet explaining tracer motion does not by itself prove that every spectral, electrical, energetic, mechanical, temporal, and biological property of the complete interface is exhausted by separable transport variables.

Model C reframes the object of inquiry. Rather than asking which special substance occupies the exclusion zone, it asks what reproducible organization emerges when a surface, water, ions, charge, gradients, energy, mechanics, geometry, flow, and history become dynamically coupled. The model does not remove Model A mechanisms; it embeds them. Nor does it assume Model B's extended phase. It predicts instead that molecular and material changes may be local, electrochemical fields may extend farther, and tracer responses may occupy a still larger operational domain, while these nested processes can nevertheless influence one another reciprocally.

The decisive tests are now clear. Water-sensitive spectroscopy must be mapped together with ions, pH, potential, temperature, surface state, force, flow, and tracer behaviour. Gradients must be clamped without destroying the surface. Illumination must be compared with equal-energy and spatially matched heating. Tracers must vary in charge, size, coating, and chemistry. Intervention order, recovery, rechallenge, and hysteresis must be measured. The full energy budget must be closed. Most importantly, a candidate biological interface must predict and causally influence a defined function, and restoration of the relevant organization must rescue competence under load.

The biological bridge should remain disciplined. Mainstream research establishes that water adjacent to proteins, membranes, polysaccharides, ions, and intracellular macromolecules can differ from bulk water and participate in function. Living membranes, mitochondria, glycocalyxes, matrices, biofilms, roots, and soils are hydrated, charged, energy-maintained interfaces. None of this proves a universal macroscopic exclusion zone. It does, however, make the passive-solvent ontology inadequate and supports a broader research question concerning the physical organization of living boundaries.

Model C should be accepted only where it earns explanatory surplus: where an integrated interface state predicts several observables, survives reciprocal intervention, generalizes beyond one material, and improves prediction of function, reserve, or recovery beyond a complete transport-material model. It should be restricted, revised, or abandoned wherever those conditions fail. The hypothesis is valuable precisely because it is eliminable in principle.

Pollack's programme may therefore have identified not merely an additional equilibrium phase of water but a broader class of energy-responsive, charge-differentiated, dynamically maintained aqueous interfaces. That

possibility has not been established; it has been rendered experimentally tractable. The most productive scientific question is no longer whether the exclusion zone is structured water or diffusiophoresis. It is what organization emerges from the coupling of surface, water, ions, charge, energy, gradients, mechanics, and flow — and whether that organization possesses properties or functions not predicted by its components considered separately.

The transition from controversy to science will occur when no reproducible observation is discarded because its first interpretation was too strong, and no model is protected from measurement because it is conventional, elegant, or institutionally favoured. A mature interfacial science would provide neither a new orthodoxy nor a rhetorical compromise, but a common experimental ground for discovering how water, matter, energy, boundary, and living organization meet at the interface.

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