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INVESTIGATION OF HYDROCARBON-RELATED MICROBIOLOGICAL ACTIVITY IN MARINE SYSTEMS WITHIN A BIOGEOPHYSICAL FRAMEWORK USING SLIME MOLDS

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ABSTRACT

The progressive depletion of readily accessible hydrocarbon accumulations in marine petroleum systems, together with the increasing technical and economic demands of conventional exploration and production, creates an incentive to investigate complementary approaches. This study brings together three fields—microbial ecology, petroleum geophysics, and bio-inspired information processing—to develop a conceptual framework for indirect hydrocarbon sensing. The framework considers slime molds, particularly Physarum polycephalum and Dictyostelium discoideum, not as naturally established seafloor hydrocarbon indicators, but as laboratory model organisms whose chemotactic, adaptive, and network-forming behaviors may be useful for testing responses to chemical gradients associated with hydrocarbon-related microbial activity. The purpose of the study is therefore to evaluate the scientific plausibility, limitations, and potential experimental pathway of such a bio-inspired sensing concept.

The framework is based on documented relationships between petroleum systems and subsurface microbial activity. Hydrocarbon-degrading bacteria and archaea, including members of the provisional archaeal lineage "Candidatus Methanoliparia," can participate in anaerobic hydrocarbon transformation and methanogenesis in hydrocarbon-rich environments. Their metabolism can generate spatially heterogeneous chemical conditions involving methane, organic acids, redox gradients, and other metabolites. The study asks whether such microbially generated gradients could, under controlled laboratory conditions, elicit measurable responses from chemotactic model organisms such as P. polycephalum or D. discoideum. It also considers the major limitation that these slime molds are primarily terrestrial organisms and are not demonstrated to function as natural biosensors on the Caspian seafloor. Accordingly, the present work is conceptual: it proposes a biomimetic or bio-inspired sensing strategy that would require controlled laboratory validation before any field application could be considered.

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Introduction

The Caspian Sea, including Azerbaijan's offshore sector, is a major hydrocarbon-bearing basin with

a long history of oil and gas exploration. Its geological setting is tectonically complex, and deformation, sedimentation, fluid migration, and reservoir architecture must be evaluated together when interpreting subsurface petroleum systems. Marine exploration is therefore technically demanding and normally relies on integrated geological, geophysical, geochemical, and drilling information rather than on a single indicator. The continuing importance of offshore hydrocarbons, even during the expansion of lower-carbon energy systems, provides a practical reason to improve the efficiency and selectivity of exploration methods. Within this context, the Caspian region provides a relevant setting in which to discuss complementary biological and geochemical approaches to subsurface sensing (Bjørlykke, 2015; Selley & Sonnenberg, 2015).

Oil and gas exploitation in the Caspian region has a long historical record, while systematic geophysical exploration and modern reservoir evaluation developed particularly during the twentieth century. The basin's tectonic framework, sedimentary sequences, structural traps, and petroleum migration pathways have made it an important subject in petroleum geoscience. Seismic methods, potential-field methods, well logging, and integrated geological interpretation are used to characterize subsurface structures and reduce uncertainty before and during drilling (Sheriff & Geldart, 1995; Telford et al., 1990). These established methods provide the physical and geological framework within which any proposed biological or geochemical indicator must ultimately be interpreted.

Over recent decades, improvements in offshore infrastructure, seismic acquisition and processing, geochemical analysis, drilling technology, and numerical modeling have broadened the range of questions that can be investigated in marine petroleum systems. This technological development also encourages investigation of unconventional complementary approaches. In particular, microbial ecology has shown that marine sediments contain spatially structured biological communities whose composition and metabolism respond to local geochemical conditions. These relationships do not by themselves establish a direct biological method for finding petroleum reservoirs, but they create a scientific basis for asking whether biologically mediated chemical gradients can provide additional information when combined with conventional geophysical and geochemical observations (Head et al., 2003; Orcutt et al., 2011).

Azerbaijan's offshore petroleum development has also been associated with large international projects, including the agreement commonly known as the "Contract of the Century." Such projects accelerated the use of modern offshore exploration, drilling, reservoir characterization, and production technologies in the Azerbaijani sector of the Caspian Sea. For the purposes of the present study, the important point is not to attribute individual discoveries to a single agreement or technology, but to recognize that modern offshore petroleum development depends on the integration of geological, geophysical, engineering, and environmental information. A proposed biological sensing method would therefore be complementary to, rather than a replacement for, established exploration technologies.

Petroleum exploration is a complex, iterative process carried out alongside broader changes in the energy sector. It commonly begins with regional geological and geophysical evaluation, followed by increasingly detailed structural, stratigraphic, geochemical, and prospect-scale analysis. Geological observations help establish the depositional, structural, and petroleum-system context, while geophysical data are used to image and constrain subsurface targets. These datasets guide decisions about whether and where exploratory drilling is justified. Field, core, well, and geophysical observations provide information on lithology, physical properties, stratigraphic relationships, structure, and bedding geometry. These data are interpreted and integrated to produce geological maps, stratigraphic correlation schemes, and structural models. The integrated models are then used to estimate whether prospective source, reservoir, seal, trap, and migration relationships are present and to identify the remaining uncertainty before drilling (Bjørlykke, 2015; Selley & Sonnenberg, 2015).

Geophysical methods are central to conventional petroleum exploration and reservoir characterization. Seismic reflection data are used to image subsurface interfaces, structural geometries, and

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stratigraphic relationships by recording the propagation and reflection of elastic waves. Seismic interpretation can therefore reveal faults, folds, depositional geometries, and other features relevant to petroleum systems. Although earthquakes and tectonic deformation influence basin structure, exploration seismology should not be described as a method that primarily studies changes caused by earthquakes; its principal role in petroleum exploration is controlled-source imaging of subsurface geological structure (Sheriff & Geldart, 1995).

Gravity methods provide complementary information because variations in rock density produce measurable variations in the gravitational field. In sedimentary basins, gravity data can help constrain large-scale structural geometry, basement configuration, salt bodies, and other density contrasts. A gravity anomaly cannot normally be interpreted as a unique direct indicator of hydrocarbons, because many geological structures and lithologies can produce comparable density effects. Consequently, gravimetric observations are most useful when integrated with seismic, geological, and other geophysical information (Telford et al., 1990).

Electrical and electromagnetic methods can also contribute to subsurface characterization because formation resistivity is influenced by lithology, porosity, pore-fluid salinity, saturation, and clay content. Hydrocarbon-bearing intervals may exhibit higher resistivity than comparable water-saturated intervals, but resistivity is not uniquely diagnostic of hydrocarbons. Magnetic methods, in contrast, primarily respond to variations in magnetic susceptibility and remanent magnetization and are generally used to investigate broader geological structure rather than to identify petroleum accumulations directly. These methods therefore provide complementary constraints within an integrated exploration workflow (Telford et al., 1990).

In addition to geological and geophysical investigation, complementary geochemical exploration methods can be used to evaluate petroleum-system indicators before drilling. These methods include analyses of gases, dissolved or adsorbed hydrocarbons, petroleum-related organic compounds, pore-water chemistry, and other geochemical indicators. Such observations require geological context because near-surface anomalies can have multiple sources.

Geochemical exploration can include analysis of hydrocarbon microseepage, dissolved and adsorbed gases, petroleum-related compounds, and other chemical anomalies in near-surface sediments or fluids. Such observations may provide evidence of hydrocarbon migration, but they require careful interpretation because biological activity, sedimentary processes, and non-petroleum organic matter can generate overlapping signals. Petroleum biodegradation itself can substantially alter reservoir fluids and produce methane and other metabolites under subsurface anaerobic conditions (Head et al., 2003; Magot et al., 2000). For this reason, the present study treats microbial and geochemical anomalies as potential indirect proxies rather than as unique proof of a reservoir.

Formation resistivity is particularly important in well logging and reservoir evaluation. Electrical logs, interpreted together with porosity measurements, core information, and geological context, can assist in estimating fluid saturation and distinguishing lithological intervals. These measurements do not directly “analyze core samples”; rather, downhole logging tools measure physical properties of the formations surrounding the borehole, while core analysis provides an independent laboratory dataset for calibration and validation. Their integration helps evaluate reservoir quality, porosity, fluid saturation, and lithological heterogeneity (Telford et al., 1990).

These methods have demonstrated value in petroleum exploration and reservoir evaluation, but offshore acquisition, processing, drilling, and integrated interpretation can require substantial time and financial investment. Their cost is one reason to investigate complementary screening methods, provided that such methods are validated against established data rather than presented as substitutes.

Some hydrocarbon accumulations occur at substantial depth or in geologically complex settings, which increases exploration uncertainty and cost. This difficulty motivates investigation of additional

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sources of information that might improve targeting before drilling. The approach developed here is therefore not presented as a substitute for seismic imaging, well logging, or geochemical analysis. Instead, it examines whether biological responses to hydrocarbon-associated microbial gradients could eventually provide a supplementary layer of evidence within an integrated exploration framework.

Digital acquisition, numerical modeling, directional drilling, improved seismic processing, and increasingly integrated geological datasets have changed petroleum exploration and development. These technologies improve the ability to characterize complex reservoirs and to manage drilling and production under difficult offshore conditions. At the same time, mature fields commonly experience declining production, while exploration of new or deeper targets can involve greater uncertainty and higher cost. Global energy systems continue to use substantial quantities of oil and gas even as investment in renewable energy expands, which reinforces the need for both efficient resource evaluation and environmental responsibility (International Energy Agency, 2024). In this context, complementary sensing concepts are scientifically relevant only if they can add information without overstating their reliability or replacing methods that are already well validated.

The discovery and development of a hydrocarbon reservoir require a sequence of geological, geophysical, engineering, economic, and environmental evaluations. Development is undertaken primarily for commercial production, while environmental protection and impact mitigation are constraints and responsibilities of the development process rather than a second purpose of exploiting the reservoir. Directional and horizontal drilling can increase reservoir contact, enable access to targets from fewer surface locations, and, in appropriate settings, improve development efficiency. These advantages are context-dependent and do not automatically guarantee higher production or lower environmental impact. After discovery, reservoir characterization, well design, completion, pressure management, production planning, and recovery strategy are integrated to determine how the field can be developed (Selley & Sonnenberg, 2015).

Rotary drilling is the dominant basis of modern petroleum well construction because it permits continuous circulation of drilling fluid, removal of cuttings, control of wellbore conditions, and use across a wide range of formations. Its effectiveness nevertheless depends on bit design, drilling fluid, well trajectory, formation properties, pressure regime, and operational objectives. Other drilling techniques are used in more specialized circumstances. Thus, it is more accurate to describe rotary drilling as a widely applicable modern method than as universally the most efficient method under every geological condition (Telford et al., 1990). Significant economic, operational, and environmental challenges remain, particularly in offshore and deepwater settings.

Offshore petroleum development continues to involve economic, environmental, and operational challenges. Geological uncertainty persists even after extensive interpretation, while mature fields may experience declining production and new targets may be smaller, deeper, or more complex. These factors encourage research into complementary methods that can reduce uncertainty or improve screening efficiency without lowering the evidentiary standard required for exploration decisions.

In producing oil and gas fields, withdrawal of reservoir fluids commonly causes reservoir pressure to decline unless pressure is supported by natural aquifer influx or engineered injection. Pressure depletion can reduce production rates and may require water injection, gas injection, artificial lift, or enhanced-recovery strategies. The recovery factor is controlled by reservoir properties, fluid behavior, drive mechanism, well configuration, and development strategy; it should not be treated as a simple direct function of pressure decline alone. As mature fields become more difficult to develop, subsurface imaging, reservoir modeling, structural interpretation, and resource assessment become increasingly important (Bjørlykke, 2015; Selley & Sonnenberg, 2015; Sheriff & Geldart, 1995). These technical and economic constraints also motivate research into complementary approaches that may reduce uncertainty at the screening stage.

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These research efforts can also consider biological and geochemical response mechanisms as supplementary sources of information. Azerbaijan is simultaneously developing hydrocarbon resources and expanding renewable-energy initiatives, including solar and wind projects. The coexistence of conventional and lower-carbon energy development does not eliminate the continuing importance of careful petroleum-resource evaluation. From a research perspective, the relevant question is whether new methods can improve selectivity, reduce unnecessary field effort, or provide environmental information while remaining scientifically reproducible. The bio-inspired approach considered in this study is therefore framed as a research concept whose value would depend on experimental specificity, repeatability, and eventual integration with established geophysical and geochemical methods.

This need is particularly relevant in geologically complex marine basins such as the Caspian Sea. Exploration technologies must provide high-resolution information while remaining economically and environmentally defensible. Digital modeling, uncertainty analysis, integrated interpretation, and increasingly automated sensing systems can improve the efficiency of work in both mature and frontier areas. Bio-integrated and bio-inspired technologies are of interest because biological systems can adapt to heterogeneous environments and process local information without centralized control. The research question is therefore whether selected biological principles can add a useful layer to conventional subsurface characterization while remaining measurable, reproducible, and compatible with environmental constraints.

Advances in biotechnology, systems biology, biomaterials, and bio-inspired computation have created opportunities to use biological principles in engineered sensing and information-processing systems. Slime-mold research is relevant in this context because *P. polycephalum* can form adaptive transport networks and reorganize those networks in response to environmental conditions (Adamatzky, 2010, 2016; Nakagaki et al., 2000; Tero et al., 2010). In the energy and environmental sciences, microbial processes are already studied in relation to petroleum biodegradation, bioremediation, and subsurface carbon cycling (Head et al., 2003; Magot et al., 2000). These two bodies of research should not be conflated: the proposal here is to test whether a bio-inspired sensing model can respond to chemical gradients generated by petroleum-associated microbial processes, not to claim that slime molds are established petroleum-exploration organisms.

Proposal for the Application of Biological or Geochemical Methods

This study proposes the use of biological responses and microbially generated geochemical signals as complementary information in geological exploration. The term biomimetic or bio-inspired sensing is used here rather than biometric sensing, because the purpose is to imitate or exploit biological response principles rather than to identify an individual organism by biometric characteristics. Petroleum-associated microorganisms can alter hydrocarbons and produce chemical gradients in subsurface environments; such gradients may, in principle, provide indirect information about local microbial activity (Head et al., 2003; Magot et al., 2000). The proposed innovation is to investigate whether model organisms with measurable chemotactic and network-forming behavior can be used in controlled experiments to translate these gradients into observable spatial responses.

Among organisms used in bio-inspired behavior research, the acellular slime mold *Physarum polycephalum* is especially notable for its capacity to explore spatially heterogeneous environments and reorganize protoplasmic transport networks. *P. polycephalum* is an amoebozoan protist, not a fungus. Its vegetative plasmodium is a multinucleate single cell that can extend through a substrate as a branching tubular network; it should therefore not be described as a conventional multicellular organism. Experiments have shown route selection, network optimization, and adaptive reorganization in response to environmental conditions (Adamatzky, 2016; Nakagaki et al., 2000; Reid, 2023; Tero et al., 2010). *Dictyostelium discoideum* is biologically different: it is a social amoeba whose separate amoeboid cells aggregate under starvation and form a transient multicellular structure. These distinctive behaviors justify

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their use as laboratory models, but they do not establish either organism as a natural indicator of subsurface hydrocarbons.

The plasmodium of *P. polycephalum* continuously reorganizes its tubular network through rhythmic protoplasmic flow, growth, retraction, and redistribution of material. When chemical and nutritional conditions are favorable, portions of the network can expand toward attractive regions; when conditions deteriorate, the organism can withdraw from unfavorable areas and reorganize its transport pathways. This distributed morphological reorganization has inspired studies of self-repairing and adaptive biological networks (Adamatzky, 2013, 2016; Tero et al., 2010). The life cycle should, however, be described accurately: the plasmodium is a multinucleate cell, and stress responses can include formation of resistant states such as sclerotia, while sporulation occurs through fruiting structures under appropriate developmental conditions. These processes are not a simple reversible transition between unicellular and multicellular states. The relevant feature for the present framework is the measurable reorganization of the plasmodial network in response to environmental gradients.

Adaptive movement by slime molds has opened useful research directions in bio-inspired computation, network design, and experimental studies of decentralized behavior (Adamatzky, 2016; Nakagaki et al., 2000; Reid, 2023). These findings have not demonstrated hydrocarbon detection. Accordingly, the present study treats slime-mold behavior as a possible experimental transducer of externally imposed chemical gradients rather than as evidence of an established petroleum-sensing function. Marine sediments contain diverse bacteria, archaea, fungi, and microbial eukaryotes, including amoeboid and other protistan lineages, but the presence of microbial eukaryotes in marine sediments does not establish that the terrestrial laboratory species *P. polycephalum* or *D. discoideum* naturally occur or function on deep seafloors (Orcutt et al., 2011; Orsi, 2018). *D. discoideum* remains valuable because its transition from solitary amoebae to a coordinated multicellular stage is exceptionally well studied (Bonner, 2009).

D. discoideum normally grows as individual amoeboid cells that feed on bacteria. When food becomes depleted, cells initiate a coordinated developmental program in which cyclic adenosine monophosphate (cAMP), not ordinary AMP, acts as a central chemotactic signaling molecule. Periodic cAMP signals organize cell movement toward aggregation centers, producing a multicellular aggregate and then a motile pseudoplasmodium, commonly called a “slug” (Bonner, 2009). During development, cells differentiate broadly into prestalk and prespore populations, and the migrating slug responds to environmental cues including light and temperature. The mature structure forms a fruiting body, or sorocarp, in which stalk cells support spore cells. This coordinated developmental sequence makes *D. discoideum* a powerful model for cell signaling, collective migration, differentiation, and emergent organization.

The prestalk and prespore cells occupy positions at the front and rear of the “slug,” respectively; prestalk cells are located in the anterior region, while prespore cells are situated in the posterior region (Bonner, 2009). This spatial differentiation demonstrates coordinated organization within the multicellular stage. The final stage represents the culmination of the organism’s development. At this stage, the “slug” transforms into a “sorocarp.” During this phase, the prestalk cells located at the front of the slug assume the role of a rigid supporting structure, while the prespore cells develop into spores that are resistant to environmental influences. This developmental sequence enables the population to produce resistant spores and complete its life cycle (Bonner, 2009). The spores are released into the environment, germinate, and produce new amoeboid cells, thereby completing the organism’s developmental cycle.

From a developmental and cell-biological perspective, *D. discoideum* is an established model for studying the transition from unicellular growth to coordinated multicellular development. Its aggregation, slug migration, differentiation of prestalk and prespore populations, and formation of a fruiting body provide experimentally accessible systems for investigating cell signaling, collective behavior, and

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differentiation (Bonner, 2009). The organism should not, however, be used as evidence that all slime molds share the same life cycle or that these developmental processes directly imply hydrocarbon sensitivity. In the present framework, *D. discoideum* is included because its chemotactic signaling and collective migration can be measured quantitatively under laboratory conditions, allowing specific hypotheses about responses to environmentally relevant chemical gradients to be tested.

Modern microscopy, fluorescence-based imaging, genomic methods, and digital image analysis have substantially expanded the study of microbial and amoeboid systems. These tools make it possible to quantify cell movement, network morphology, spatial aggregation, and responses to controlled chemical gradients. Marine microbial chemotaxis research likewise demonstrates that microorganisms can respond to microscale chemical heterogeneity in aquatic environments (Brumley et al., 2020; Keegstra et al., 2022; Raina et al., 2022; York, 2022). In parallel, the behavior of *P. polycephalum* has been formalized in bio-inspired computational models that emphasize decentralized path selection and adaptive network reorganization (Adamatzky, 2016; Reid, 2023; Tero et al., 2010). The present study extends this conceptual direction by asking whether those experimentally measurable behaviors could be coupled to hydrocarbon-associated microbial chemistry. Such a proposal remains a hypothesis until specific metabolites, response thresholds, selectivity, repeatability, and environmental compatibility are experimentally demonstrated.

The origin and transformation of petroleum involve biological, chemical, thermal, and geological processes operating over very different time scales. Organic matter deposited in marine or lacustrine sediments undergoes early diagenetic transformation in which microorganisms can play an important role in degrading labile compounds and changing sediment geochemistry. With progressive burial, organic matter is converted into kerogen and, at greater temperature and maturity, thermally generated into oil and gas during catagenesis. Microbial activity is therefore important in early organic-matter transformation and in later biodegradation of petroleum, but microorganisms should not be described as the sole or direct cause of petroleum generation (Bjørlykke, 2015; Falkowski et al., 2008; Head et al., 2003; Selley & Sonnenberg, 2015).

The dominant geological model for conventional petroleum generation is biogenic in the broad sense that the precursor organic matter derives from biological material such as phytoplankton, zooplankton, bacteria, and other organisms deposited with sediment. During burial, this organic matter undergoes diagenesis and is incorporated into kerogen; subsequent increases in temperature and burial maturity drive catagenetic reactions that generate liquid and gaseous hydrocarbons over geological time. The relative importance of source-organism type, depositional environment, preservation, burial history, and thermal maturity varies among petroleum systems (Bjørlykke, 2015; Selley & Sonnenberg, 2015). This framework is important because microbial activity near a modern seafloor should not be equated directly with the deep thermal processes that generated a petroleum accumulation.

During early diagenesis, microorganisms consume and transform relatively labile organic compounds and influence redox conditions, dissolved inorganic carbon, sulfur species, organic acids, and other components of sediment pore water. These transformations contribute to the progressive alteration and preservation of sedimentary organic matter, but the formation of kerogen is a complex geochemical process rather than the direct product of a single microbial pathway. At later stages of burial and thermal maturation, catagenesis dominates hydrocarbon generation. In already formed petroleum reservoirs, microorganisms can again become important by biodegrading hydrocarbons, especially where temperatures and other environmental conditions permit microbial life (Head et al., 2003; Magot et al., 2000).

Organic-rich sediments and petroleum-affected environments can contain dissolved gases, reduced sulfur species, organic acids, hydrogen, methane, and many other reaction products. These compounds may form local biogeochemical gradients reflecting the combined effects of organic-matter degradation, hydrocarbon transformation, electron-acceptor availability, transport, and microbial community structure

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(Falkowski et al., 2008; Orcutt et al., 2011). Such gradients are scientifically relevant to the proposed sensing framework, but they are not uniquely attributable to petroleum. The same or similar metabolites may arise from degradation of non-petroleum organic matter. Therefore, any biological indicator would require comparison with geochemical controls and independent petroleum-system evidence to determine specificity.

Research on the archaeal lineage "*Candidatus Methanoliparia*" has provided evidence that some members can activate and degrade non-methane alkanes under anoxic conditions while coupling the process to methanogenesis. Environmental and genomic observations from hydrocarbon seeps support a role for these archaea in anaerobic carbon cycling in petroleum-rich sediments (Laso-Pérez et al., 2019). This does not mean that *Methanoliparia* abundance is a validated exploration indicator for concealed petroleum reservoirs. Rather, hydrocarbon-associated archaeal metabolism can contribute to local methane production and other biogeochemical signatures that could be investigated as indirect proxies. Conventional exploration must still rely primarily on geological, geophysical, geochemical, and drilling evidence (Sheriff & Geldart, 1995; Telford et al., 1990).

Subsurface microbial communities can alter petroleum and generate metabolic products that modify local pore-water chemistry. In petroleum-bearing or hydrocarbon-seep environments, these effects can create microbial and geochemical "hotspots" whose spatial distribution reflects both substrate availability and local environmental conditions (Head et al., 2003; Orcutt et al., 2011). Such hotspots are conceptually relevant to indirect sensing, but they should not be described as causing the geological accumulation itself. Hydrocarbon reservoirs form through source-rock generation, migration, trapping, and preservation over geological time. Microbial activity can subsequently alter hydrocarbons, especially through biodegradation, and can thereby generate secondary chemical signals that may be detectable above or around a petroleum system (Bjørlykke, 2015; Selley & Sonnenberg, 2015).

Petroleum-system development can therefore be considered as a sequence in which organic-matter deposition and preservation are followed by diagenesis, burial and thermal maturation, hydrocarbon generation, migration, accumulation, and possible later alteration. Microorganisms are particularly important during early diagenesis and during biodegradation of existing hydrocarbons under biologically permissive conditions. Temperature, pressure, burial history, source-rock quality, and time govern the deeper thermal generation process, whereas microbial community composition and electron-acceptor availability govern many biodegradation pathways (Head et al., 2003; Magot et al., 2000; Selley & Sonnenberg, 2015). This distinction strengthens the proposed model by locating the biological signal in petroleum alteration and associated geochemistry rather than in an oversimplified account of petroleum formation.

Microbial activity can continue after a petroleum accumulation has formed, provided that temperature, water availability, nutrients, and other environmental conditions permit life. Petroleum hydrocarbons themselves can serve as substrates for diverse microorganisms in reservoirs and marine sediments, leading to biodegradation and substantial alteration of oil composition (Aitken et al., 2004; Head et al., 2003; Magot et al., 2000; Orcutt et al., 2011).

Anaerobic hydrocarbon biodegradation can proceed through several different microbial mechanisms, and it is not universally dependent on one bacterial–archaeal partnership. Some anaerobic bacteria degrade alkanes while coupling oxidation to electron acceptors such as sulfate or nitrate, whereas methanogenic degradation of complex hydrocarbons commonly involves syntrophic communities in which fermentation products such as hydrogen, formate, or acetate are consumed by methanogens. Other archaeal lineages can activate specific hydrocarbons through methyl-coenzyme M reductase-related pathways (Laso-Pérez et al., 2016, 2019; Mbadinga et al., 2011). Consequently, the microbial architecture depends on the substrate, temperature, electron acceptors, and environmental setting. For the proposed biosensing

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model, the important point is that different pathways can generate different metabolite combinations and therefore different chemical gradients.

In syntrophic methanogenic systems, intermediate products such as acetate, formate, carbon dioxide, and molecular hydrogen may be exchanged rapidly between microbial partners. Consumption of these products by methanogens can keep their concentrations sufficiently low for otherwise unfavorable upstream reactions to proceed, thereby creating a thermodynamic coupling between community members (Magot et al., 2000; Mbadinga et al., 2011). Hydrogenotrophic methanogens consume hydrogen and carbon dioxide, whereas acetoclastic methanogens use acetate. These interactions can produce spatially structured zones of microbial activity in sediments or reservoirs. Whether such zones generate gradients strong and persistent enough to control the behavior of a slime-mold model organism is an empirical question and should be tested in controlled microcosms rather than assumed from the existence of the microbial process itself.

Methanogenic archaea are strictly anaerobic microorganisms that produce methane through several metabolic routes. In hydrogenotrophic methanogenesis, hydrogen is used to reduce carbon dioxide to methane; in acetoclastic methanogenesis, acetate is converted to methane and carbon dioxide. In hydrocarbon-degrading consortia, methanogens can remove fermentation products and thereby contribute to the thermodynamic feasibility of upstream hydrocarbon transformation (Magot et al., 2000; Mbadinga et al., 2011). Methanogenesis is therefore important to the present concept because methane production, together with associated organic acids and redox changes, can serve as a measurable marker of microbial activity. It should not, however, be treated as a hydrocarbon-specific signal without appropriate controls.

Interspecies syntrophy describes a metabolic dependence in which the products of one population are consumed by another in a way that makes the combined process energetically favorable. Under methanogenic conditions, fermentative or hydrocarbon-transforming organisms may generate hydrogen, formate, acetate, or other intermediates, while methanogenic archaea consume these products and form methane. This continuous product removal can maintain low intermediate concentrations and support continued substrate transformation (Mbadinga et al., 2011). The process is therefore a thermodynamic and ecological coupling, not a literal synchronization signal between cells. Its relevance to the proposed sensing system lies in the possibility that coupled metabolism can generate reproducible chemical gradients that are measurable independently of the model organism's response.

Methane may be a major terminal product of methanogenic hydrocarbon degradation, but methane formation does not itself increase the temperature of deeper sediment layers. The relevant effect is thermodynamic: consumption and production of intermediates can shift reaction energetics and maintain favorable redox conditions for continued microbial metabolism. Studies of "*Candidatus Methanoliparia*" indicate that members of this lineage possess pathways for anaerobic activation of non-methane alkanes and can couple alkane degradation to methanogenesis (Laso-Pérez et al., 2019). Such metabolism may contribute to methane-rich and chemically heterogeneous microenvironments in hydrocarbon seeps. These products could be incorporated into an experimental gradient system, but their usefulness as exploration proxies would have to be established against non-petroleum controls. Slime-mold behavior would then be measured as a response to the imposed chemistry, not assumed to be naturally coupled to the archaeal metabolism.

The microbial degradation of petroleum is therefore best understood as a collection of pathways rather than as a simple division in which archaea process only "simple" hydrocarbons and bacteria process "complex" hydrocarbons. Anaerobic bacteria can activate and degrade a range of alkanes under sulfate-, nitrate-, or other electron-accepting conditions, while specialized archaea can activate short-chain or longer-chain alkanes through methyl-coenzyme M reductase-related chemistry, sometimes in syntrophic partnerships and sometimes with more direct methanogenic coupling (Laso-Pérez et al., 2016, 2019; Mbadinga et al., 2011). This diversity is important for biosensing because the chemical products of

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hydrocarbon degradation depend on the organisms and pathways present; no single universal microbial signal should be expected.

The discovery of archaeal hydrocarbon-activation pathways has expanded the earlier view of anaerobic petroleum biodegradation. Laso-Pérez et al. (2016) demonstrated archaeal activation of butane in a thermophilic syntrophic consortium, while later genomic and environmental work identified "*Candidatus Methanoliparia*" in Gulf of Mexico hydrocarbon-seep sediments and provided evidence for methanogenic degradation of non-methane alkanes (Laso-Pérez et al., 2019). These findings show that hydrocarbon transformation can involve archaeal enzymes directly rather than requiring bacterial activation in every case. At the same time, the organisms are difficult to cultivate, and environmental activity must be inferred from multiple lines of genomic, chemical, and physiological evidence.

Hydrocarbon-rich marine sediments can contain abundant *Methanoliparia* populations together with methane production and molecular evidence of alkane activation (Laso-Pérez et al., 2019). These observations are important for understanding anaerobic carbon cycling and petroleum biodegradation, but the manuscript does not provide an experimental basis for assigning a universal temperature range, enrichment factor, or methane-production rate to the proposed Caspian application. The scientifically defensible inference is narrower: archaeal alkane degradation can generate methane and associated geochemical heterogeneity in some hydrocarbon-rich anoxic environments. Such heterogeneity may provide candidate chemical inputs for a laboratory biosensing experiment. Demonstrating a reliable connection to a concealed reservoir would require field geochemistry, microbial community data, and independent geological validation.

Genomic evidence indicates that "*Candidatus Methanoliparia*" possesses methyl-coenzyme M reductase-related pathways associated with anaerobic alkane activation, supporting the interpretation that members of the lineage can couple hydrocarbon degradation to methanogenesis (Laso-Pérez et al., 2019). The available evidence should not be simplified into a claim of universally rapid long-chain-hydrocarbon degradation or a precisely defined substrate range. Substrate utilization, metabolic rates, ecological interactions, and regulatory mechanisms remain dependent on lineage and environmental conditions. For this study, the significance of *Methanoliparia* is therefore conceptual: it provides a documented example of petroleum-associated microbial metabolism capable of generating methane and other chemical changes that could be reproduced as experimental gradients.

Although *Methanoliparia*-associated metabolism has been investigated using environmental genomics, geochemistry, and enrichment approaches, many aspects of intracellular regulation, growth kinetics, ecological interactions, and substrate specificity remain incompletely characterized. These uncertainties do not diminish the importance of the lineage for petroleum microbiology, but they do limit claims about its direct engineering use. Future functional-genomic and cultivation studies may improve understanding of its contribution to anaerobic oil transformation and methane production. Within the present framework, the organism is therefore treated as a source of plausible hydrocarbon-associated metabolic signals rather than as a controllable component of a field-ready biosensor (Laso-Pérez et al., 2019; Magot et al., 2000).

These observations support a conceptual proposal for testing a slime-mold-based or slime-mold-inspired biosensor as an indirect indicator of hydrocarbon-associated microbial chemistry. The concept is not presently suitable for field exploration. *P. polycephalum* and *D. discoideum* are predominantly terrestrial laboratory model organisms with ecological and physiological requirements that differ markedly from those of deep marine sediments (Bonner, 2009; Reid, 2023). The Caspian Sea is brackish rather than uniformly highly saline, and conditions vary strongly with location and depth; seafloor and subsurface sediments can also become oxygen-limited or anoxic, while hydrostatic pressure increases with water depth. These conditions make direct deployment of terrestrial slime molds biologically questionable. The

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initial research problem should therefore be addressed through contained laboratory experiments that reproduce selected chemical and physical variables rather than by assuming survival on the natural seafloor.

Signal specificity is an equally important limitation. Sulfur cycling, methane cycling, fermentation, and degradation of non-petroleum organic matter can generate metabolites similar to those associated with petroleum biodegradation (Azam & Malfatti, 2007; Boetius et al., 2000; Orcutt et al., 2011). A response by a chemotactic organism to methane-associated metabolites or organic acids would therefore indicate sensitivity to the chemical environment, not necessarily to petroleum itself. No direct mediating role of slime molds in hydrocarbon degradation has been demonstrated in the evidence reviewed here. The testable question is instead whether a slime-mold response can distinguish experimentally defined hydrocarbon-associated microbial gradients from appropriate non-hydrocarbon controls with sufficient sensitivity and specificity to justify further development.

For this reason, the proposed approach requires a staged research program. Initial work should reproduce defined gradients in microfluidic devices, sediment analogues, or other contained matrices and quantify the response of the model organism. The next stage should compare sterile chemical controls, non-petroleum organic-matter degradation, hydrocarbon-degrading microbial cultures, and mixed sediment communities. Only if reproducible specificity is demonstrated should mesocosm experiments be considered. Field-scale work would require independent geophysical and geochemical validation and careful assessment of biosafety, environmental compatibility, and monitoring requirements.

Marine and subseafloor microbial ecology should also be characterized independently before any claim is made about a natural slime-mold component of the system. Molecular surveys demonstrate extensive diversity of bacteria, archaea, and microbial eukaryotes in seafloor sediments (Orcutt et al., 2011; Orsi, 2018). However, evidence for broad microbial-eukaryote diversity is not equivalent to evidence that *P. polycephalum* or *D. discoideum* naturally inhabit hydrocarbon-rich marine sediments. A scientifically robust program would therefore use metabarcoding, microscopy, cultivation where feasible, and geochemical profiling to identify indigenous protists and other eukaryotes that may be better adapted to local conditions. The terrestrial slime molds can remain laboratory models even if future field indicators are drawn from different organisms.

The conceptual chain can therefore be stated more cautiously: hydrocarbons and other organic substrates can influence microbial community structure and metabolism; microbial metabolism can generate local chemical and redox gradients; and these gradients may be detectable by appropriately chosen biological or engineered sensors. Several limitations follow. First, terrestrial slime-mold models may be poorly compatible with marine and subsurface conditions. Second, chemical signal specificity is a major problem because microbial abundance or activity does not necessarily indicate petroleum; it may reflect degradation of general organic matter (Orcutt et al., 2011). Third, benthic gradients vary spatially and temporally. Fourth, behavioral responses measured in a model organism must be separated from background responses to salinity, oxygen, temperature, pH, and nutrient availability. These limitations define the experimental controls required to evaluate the idea.

A stepwise validation sequence is therefore essential. Controlled laboratory studies should begin with microfluidic gradient chambers, agar or hydrogel matrices, and sediment analogues in which the concentration and identity of candidate metabolites are known. The next stage should introduce natural or reconstructed microbial communities under mesocosm conditions, with parallel geochemical measurements. Only after response thresholds, selectivity, repeatability, and ecological tolerance have been established should any field-scale sensor concept be evaluated. Direct environmental release of non-native laboratory slime molds should not be assumed as part of the method; contained sensor architectures, ex situ sampling, or purely biomimetic computational implementations may ultimately be more appropriate. Algorithmic models can be developed in parallel to quantify adaptive behavior and identify which biological features are transferable to engineered sensing.

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D. discoideum illustrates how nutrient availability can reorganize collective behavior. Under nutrient-rich conditions it exists primarily as independent, feeding amoeboid cells; under starvation, cAMP-mediated signaling coordinates aggregation and the formation of a multicellular slug, followed by differentiation into a fruiting body containing stalk and spore cells (Bonner, 2009). It should not be characterized as an "anaerobic-like" cell, because its laboratory biology is not based on adaptation to deep anoxic marine conditions. Its relevance to the present concept is instead methodological: aggregation, migration, and differentiation can be quantified as responses to defined environmental cues, providing a tractable model for studying chemically mediated collective behavior.

Biofilms provide a different form of collective organization. They are communities of microorganisms embedded in an extracellular matrix and can exhibit spatial differentiation, chemically mediated communication, cooperative and competitive interactions, and emergent community-level properties (Costerton et al., 1995; Hall-Stoodley et al., 2004; Nadell et al., 2016; Stoodley et al., 2002). Microbial mats are also spatially organized communities, but their structure reflects local energy sources and geochemical gradients. In illuminated settings, light can influence phototrophic layers; in dark seafloor or subsurface settings, redox chemistry, electron donors and acceptors, fluid flow, and substrate availability are more important than light (Orcutt et al., 2011). These systems are not phylogenetically equivalent to slime molds, but they offer useful functional comparisons in self-organization and environmental response.

Stratification in microbial mats and sediments should therefore be interpreted according to environmental context. Shallow illuminated mats may contain phototrophic surface layers, whereas deeper or dark sediments commonly show vertical zones controlled by oxygen penetration, sulfate reduction, fermentation, methanogenesis, and other redox processes. The boundaries between these zones can generate steep chemical gradients over small spatial scales (Boetius et al., 2000; Orcutt et al., 2011). Slime-mold networks and microbial mats differ greatly in persistence, metabolism, and evolutionary origin, yet both can be analyzed as spatially organized systems whose structure emerges from local interactions. This analogy may help inspire sensor design, but it does not imply that mat organization or slime-mold behavior is itself a specific indicator of hydrocarbon-rich sediment.

The use of biological sensing for subsurface hydrocarbon exploration therefore remains hypothetical and requires experimental validation. A practical research sequence would link controlled chemistry to a measurable biological response and then test whether that relationship survives increasing environmental complexity. Marine protists, including foraminifera and radiolarians, are relevant to the broader discussion because they demonstrate that diverse unicellular eukaryotes can persist in marine environments and respond to local physicochemical conditions. Their presence does not make them direct petroleum sensors, but their environmental adaptations illustrate why indigenous marine eukaryotes may ultimately be more realistic candidates than terrestrial slime molds for field-oriented biological indicators (Orsi, 2018).

Foraminifera, in particular, extend reticulose pseudopodial networks that support locomotion, feeding, and interaction with sediment particles. Many taxa occur in deep-sea environments and display substantial morphological and physiological diversity. Radiolarians are also marine protists, many of which form intricate mineral skeletons that can contribute siliceous material to marine sediments after death. These examples demonstrate the diversity of structural strategies among marine microbial eukaryotes (Fenchel, 1987; Margulis & Chapman, 2009). They are included here as ecological comparisons, not as evidence that these organisms detect hydrocarbons or that their morphology can be transferred directly to a petroleum-sensing system (Orsi, 2018).

Protist groups differ markedly in life cycle, metabolism, and ecological function, and they should not be described as collectively performing hydrogen degradation. The terrestrial slime molds considered in this study are likewise not established hydrocarbon degraders. The relevant comparison is functional rather than metabolic: many protists can move, reorganize cell shape, explore spatially heterogeneous

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environments, and respond to chemical or physical gradients. These behaviors suggest possible design principles for adaptive sensing, but any link to hydrocarbon-associated chemistry must be demonstrated experimentally for a particular organism and signal rather than inferred from broad similarities among protists.

Marine sponges (Porifera) provide another example of biological reorganization at a different level of complexity. Classic dissociation experiments showed that separated sponge cells can reaggregate and, under appropriate conditions, reconstruct organized tissue (Humphreys, 1970). This property depends on cell recognition, adhesion, extracellular components, and species-specific interactions. The comparison with slime molds is therefore an analogy in self-organization rather than evidence of close cellular or evolutionary similarity. *D. discoideum* aggregates separate amoebae during development, whereas *P. polycephalum* reorganizes a multinucleate plasmodial network; sponges reorganize cells belonging to a multicellular animal. Distinguishing these mechanisms is important if they are used as inspiration for engineered sensor architectures.

Across biofilms, microbial mats, protists, slime molds, and sponges, convergent functional themes can nevertheless be identified: local interactions can produce larger-scale organization; environmental stress can alter structure and behavior; and chemical or physical cues can coordinate responses. The mechanisms, evolutionary origins, and degrees of reversibility are not the same, and it would be incorrect to claim that all of these systems undergo reversible transitions between unicellular and multicellular states. Their value to the present study lies in comparison of organizational principles rather than taxonomic equivalence. Such comparisons can inform algorithms for distributed sensing, adaptive path selection, network resilience, or signal integration while keeping the underlying biology explicit.

The preceding discussion therefore supports a biogeophysical framework in which biological populations can modify their local environment and generate or respond to spatial gradients, while petroleum systems can influence the substrates available to particular microbial communities. This interaction creates a possible indirect connection between subsurface hydrocarbon occurrence and measurable biological or geochemical signals. The connection remains non-unique and must be validated against background organic-matter cycling, fluid migration, lithological variation, and other environmental controls. Detailed marine microbiology, geochemistry, and geophysics are consequently complementary components of any future attempt to evaluate the proposed sensing concept.

Methodology: The present study uses a phased conceptual methodology that integrates petroleum geoscience, petroleum microbiology, microbial ecology, and experimental systems biology. Because the manuscript does not report a complete primary experimental dataset, the laboratory components below are presented as a proposed validation workflow rather than as completed experiments. The purpose of the methodology is to translate the conceptual relationship among hydrocarbons, microbial metabolism, chemical gradients, and biological responses into a sequence of testable steps. Each phase is designed to generate measurements that can be compared with geochemical controls and, ultimately, with conventional geophysical information.

Development of the conceptual framework: The first phase defines the causal chain to be tested. A subsurface or sedimentary hydrocarbon system may support or modify microbial activity; microbial transformation of hydrocarbons and associated organic matter can alter methane, organic acids, sulfur species, redox conditions, and other chemical variables; those changes can form local gradients; and a laboratory model organism or engineered biomimetic sensor may respond to the gradient. The model can therefore be represented schematically as follows: hydrocarbon-associated substrate or seepage → microbial transformation and community response → local biogeochemical gradient → measurable response of a model biosensor → integrated interpretation with geological and geophysical evidence. The biological response is an indirect proxy and not a direct measurement of the reservoir itself.

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This model gives particular attention to archaeal and bacterial processes associated with anaerobic hydrocarbon degradation. "*Candidatus Methanoliparia*," for example, provides a documented case in which alkane transformation can be linked to methanogenesis in hydrocarbon-rich anoxic sediments (Laso-Pérez et al., 2019). The resulting methane and associated metabolic products may contribute to spatially heterogeneous geochemical fields. In the proposed experiment, these gradients would not be attributed to slime molds themselves; instead, selected chemical conditions would be reproduced in controlled matrices and the response of the model organism would be measured. The resulting multi-layer inference model would therefore separate four levels—geological source, microbial process, chemical signal, and sensor response—to reduce the risk of confusing correlation with causation.

Laboratory cultivation and behavioral experiments: A second phase would investigate chemotactic, migratory, and network-forming behavior under controlled laboratory conditions. P. polycephalum and D. discoideum are proposed as distinct model systems because both provide measurable responses to spatially heterogeneous chemical environments, although their biology and developmental mechanisms differ substantially (Bonner, 2009; Nakagaki et al., 2000; Reid, 2023). P. polycephalum would be evaluated primarily through plasmodial growth, network morphology, route selection, and reorganization. D. discoideum would be evaluated through amoeboid chemotaxis, aggregation dynamics, and developmental responses. Experiments should initially use defined chemical gradients before more complex microbial or sediment matrices are introduced.

Under the proposed laboratory design, matrices containing defined concentrations of candidate hydrocarbon-associated metabolites would be prepared. These could include selected low-molecular-weight organic acids, dissolved inorganic components, methane-equilibrated media where technically appropriate, or other products documented in anaerobic hydrocarbon-degrading systems. Parallel controls would contain equivalent nutrient levels without petroleum-associated metabolites, and additional treatments could include non-petroleum organic-matter degradation products. Hydrocarbon-rich natural sediment should not be treated as a chemically defined input unless its composition has been measured. The experimental matrix would therefore be characterized geochemically so that any biological response could be related to a known concentration gradient rather than to an unspecified "hotspot."

Within this framework, behavioral responses would be quantified separately for each model organism. For *P. polycephalum*, relevant variables could include directional extension, network area, branching, path length, transport-network reorganization, withdrawal from unfavorable zones, and recovery after disturbance. For *D. discoideum*, relevant variables could include single-cell migration, aggregation probability, aggregation-center formation, slug migration, and developmental timing. The term "reversion" should not be used to imply that both organisms undergo the same reversible unicellular–multicellular transition. The principal analysis would test whether response magnitude and direction change systematically with the identity and concentration of the imposed chemical gradient.

Quantitative analysis would require time-lapse imaging, microscopy, calibrated spatial measurements, and digital image-processing methods. Experimental replication should be sufficient to estimate variability within and between cultures, and treatment allocation should include appropriate negative and positive controls. Measurements could be converted into response variables such as migration velocity, orientation relative to the gradient, network density, branch persistence, aggregation time, or path efficiency. Statistical analysis would then assess whether the observed behavior differs among treatments and whether any response is reproducible across independent experiments. These data would provide the first empirical test of the proposed association between hydrocarbon-related microbial chemistry and model-organism behavior.

Simulation of marine and subsurface conditions: A third phase would test whether any laboratory response remains detectable when selected environmental constraints are introduced. Salinity, oxygen concentration, temperature, pH, and sediment structure should be manipulated independently

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where possible before combinations are tested. The purpose is not to reproduce every condition of the Caspian seabed in a single experiment, but to identify which variables suppress, enhance, or confound the biological response. Because the selected slime molds are terrestrial models, direct exposure to full marine or subsurface conditions may rapidly eliminate activity; this outcome would itself be important evidence regarding feasibility. Contained or ex situ sensor formats should therefore be considered alongside direct organism exposure.

Physiological condition, chemotactic responsiveness, aggregation stability, network integrity, and recovery after stress would be monitored throughout this phase. Measurements of growth or survival alone would be insufficient, because a biosensor must retain a reproducible relationship between environmental input and measurable output. Salinity, oxygen limitation, temperature, and pH may alter motility or development independently of the target chemical signal. Factorial or sequential experiments would help distinguish these effects and determine whether a response attributed to hydrocarbon-associated chemistry persists after environmental stress is introduced.

This phase would therefore function as an intermediate feasibility test between a simplified chemical-gradient experiment and a complex sediment or mesocosm system. A model organism that responds strongly to a defined metabolite but loses all response under modest changes in salinity or oxygen would have limited field relevance even if the initial chemotaxis result were statistically significant. Conversely, a response that persists across a realistic range of confounding conditions would justify more complex testing. The outcome would determine whether the biological component should be developed further, replaced with a more marine-adapted organism, or translated only into a biomimetic computational sensor.

Interaction studies: A subsequent phase would examine interactions between the chemotaxis-sensitive model organisms and hydrocarbon-associated bacterial or archaeal communities under contained laboratory conditions. The purpose would be to determine whether a living microbial community produces spatial signals that elicit a measurable response beyond that produced by bulk nutrients or non-petroleum organic matter. Microbial community density, metabolic state, and spatial localization would be measured independently so that the strength of the biological stimulus can be separated from the response of the slime-mold model (Nadell et al., 2016; Shapiro, 1998).

Special attention would be given to microbial abundance, spatial localization, and metabolite production. Candidate measurements include methane, dissolved gases, organic acids, sulfide or sulfate where relevant, pH, and redox conditions, together with microscopy or molecular measurements of microbial biomass. The biological response could then be compared with these measured variables using correlation and regression models. A correlation would not by itself establish hydrocarbon specificity; the same analysis should be performed with control communities degrading non-petroleum organic substrates. This design would help distinguish a response to general microbial biomass from a response to a particular hydrocarbon-associated metabolic profile (Costerton et al., 1995; Nadell et al., 2016).

Data analysis and modeling: Quantitative data from the proposed experiments would be analyzed using statistical inference and computational modeling. Spatial trajectories and network structures could be reconstructed from time-lapse images and analyzed for orientation, speed, branching, path efficiency, and persistence. P. polycephalum networks can also be represented as graphs in which junctions and tubes are treated as nodes and edges, allowing comparison of network topology across treatments (Nakagaki et al., 2000; Tero et al., 2010). D. discoideum data would require different descriptors, including cell trajectories, aggregation-center dynamics, and group migration, so the two organisms should not be forced into a single biological model.

These analyses would allow environmental constraints, response variability, network stability, and self-reorganization to be compared quantitatively. For *P. polycephalum*, graph-theoretical measures could describe connectivity, redundancy, path length, and adaptation after disturbance. For microbial

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communities, spatial statistics could describe clustering or gradients, while geochemical measurements would define the chemical field to which the organism was exposed. Combining these datasets would make it possible to test whether changes in biological organization are consistently associated with particular chemical conditions rather than with uncontrolled environmental variation (Nadell et al., 2016; Tero et al., 2010).

Empirical observations would then be incorporated into computational models only after the relevant biological relationships had been measured. The aim would not be to reproduce every mechanism of the living organism, but to formalize useful features such as decentralized response, adaptive path selection, threshold behavior, and integration of heterogeneous spatial information. Parameter values should be estimated from experimental data rather than selected to force agreement with the hypothesis. This separation between observation and model calibration is essential if the resulting algorithm is later compared with independent geophysical or geochemical datasets (Adamatzky, 2016; Shapiro, 1998).

The resulting models would therefore serve as abstractions of selected biological principles rather than literal digital copies of the organisms. Decentralized decision-making, adaptive network reinforcement, local response to gradients, and resilience after perturbation are potentially transferable design principles (Tero et al., 2010). A future ecological sensing system could use these principles to integrate spatially heterogeneous chemical measurements, even if living slime molds were not ultimately used in the field. This distinction is important because the most durable contribution of the concept may be biomimetic computation or sensor-network design rather than direct deployment of terrestrial organisms in marine sediments.

Results:

Because the present work is conceptual and the manuscript does not provide a complete primary experimental dataset, the “results” should be interpreted as testable outcomes of the proposed framework rather than as measured numerical findings. The framework predicts that hydrocarbon-associated microbial metabolism can generate spatially heterogeneous chemical conditions and that the persistence of those conditions will depend on production, consumption, diffusion, advection, and sediment properties. A validation study should therefore quantify methane and other metabolites, microbial abundance, gradient magnitude, and gradient stability rather than assume fixed values for methane yield, microbial enrichment, or diffusion coefficients. Any numerical estimate used in future work should be linked to a stated substrate, reaction, experimental system, analytical method, and uncertainty range.

A second testable outcome concerns the response of the model organism. *P. polycephalum* is known to reorganize movement and transport networks in response to environmental conditions, but this does not theoretically confirm sensitivity to hydrocarbon-associated gradients. The proposed experiments should measure movement rate, orientation, network change, and response thresholds directly under each treatment and control condition (Nakagaki et al., 2000; Reid, 2023; Tero et al., 2010). Marine-related stressors such as salinity, oxygen limitation, pH, and temperature may reduce or obscure the response. A useful biosensing signal would therefore require not merely movement, but a statistically reproducible and sufficiently specific difference between hydrocarbon-associated treatments and chemically comparable controls.

Overall, the conceptual analysis supports the feasibility of testing a biogeophysical sensing mechanism; it does not yet demonstrate that such a mechanism works in the Caspian Sea or that it can locate petroleum reservoirs. The strongest scientifically supported link is between hydrocarbons and microbial metabolism in petroleum-rich environments. The proposed second link—from the resulting geochemical gradient to a reliable slime-mold response—remains to be established experimentally. A third link—from that biological response to an exploration decision—would require independent geological and geophysical validation. Framing the result in these three stages preserves the innovative concept while

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clearly distinguishing established microbial petroleum science from the hypotheses introduced by this study.

Feasibility assessment and scaling strategy: The final stage of the proposed research would critically evaluate practical applicability. The main criteria are environmental compatibility of the biological component; sensitivity, selectivity, and reproducibility of the measured response; technical requirements for imaging or signal acquisition; stability over time; ease of calibration; contamination and biosafety risks; and cost relative to established geophysical and geochemical methods. A favorable result at one stage would not automatically justify scaling. Progression from defined gradients to mixed microbial systems, mesocosms, and field-associated samples should occur only when each preceding stage demonstrates a reproducible signal and an acceptable rate of false-positive and false-negative responses.

In *D. discoideum*, intercellular coordination during aggregation is mediated by cyclic adenosine monophosphate (cAMP). Starving amoebae relay periodic cAMP signals, and neighboring cells move chemotactically toward increasing cAMP concentrations, producing wave-like patterns of coordinated migration and ultimately forming an aggregate (Bonner, 2009). Receptors, G-protein-mediated signaling, and regulation of adenylate cyclase are components of this signaling network. This well-characterized system is relevant to the present study because it provides a quantitative model of chemically mediated collective behavior. It should not be supported with the *P. polycephalum* maze-solving study, which addresses a biologically different organism and behavior.

Marine microbial systems use different molecular mechanisms for collective behavior. In many bacterial biofilms, quorum-sensing systems allow cells to respond to diffusible signal molecules whose production and accumulation depend on population and environmental context. Examples include N-acyl homoserine lactones in many Gram-negative bacteria, autoinducer-2-related signaling in diverse taxa, and peptide signals in many Gram-positive bacteria. Biofilm development is also governed by adhesion, extracellular-matrix production, nutrient gradients, competition, and many other regulatory processes (Costerton et al., 1995; Miller & Bassler, 2001; Nadell et al., 2016). Quorum sensing is therefore one component of biofilm organization rather than a universal single mechanism controlling all marine microbial communities.

A limited functional analogy can be drawn between cAMP-mediated aggregation in *D. discoideum* and chemical communication in bacterial biofilms: in both cases, local chemical information can influence coordinated behavior at the population level. The molecular pathways, evolutionary origins, cellular architectures, and developmental outcomes are nevertheless fundamentally different. cAMP signaling in a social amoeba should not be described as homologous to bacterial quorum sensing, and neither mechanism should be assumed to imply petroleum sensitivity. The value of the comparison is conceptual: it illustrates how local chemical signals can be amplified into spatially organized biological responses (Bonner, 2009; Miller & Bassler, 2001; Nadell et al., 2016).

Extracellular matrices provide another functional comparison, but the terminology must remain organism-specific. Bacterial biofilm extracellular polymeric substances (EPS) can contain polysaccharides, proteins, extracellular DNA, lipids, and other components that influence adhesion, hydration, mechanical stability, diffusion, and stress tolerance (Costerton et al., 1995; Flemming et al., 2025). Slime molds and social amoebae also produce extracellular material during particular life stages, but this should not be treated as biochemically identical to bacterial biofilm EPS. The shared engineering principle is that extracellular material can modify the local physical and chemical microenvironment surrounding cells or a biological network.

During the multicellular developmental stage of *D. discoideum*, cells and the migrating slug interact with an extracellular sheath that contributes to structural integrity and movement. In bacterial biofilms, the extracellular matrix likewise provides a physical framework within which cells are organized and protected from some environmental stresses. These systems differ in composition and developmental function, yet

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they show how collective biological organization can be stabilized by material outside the cell membrane (Bonner, 2009; Costerton et al., 1995). For biosensor design, the analogy suggests that both signal processing and the physical matrix surrounding the sensing elements may influence response stability.

Nutrient limitation and environmental stress alter metabolism in both slime molds and biofilms, but the regulatory mechanisms are not interchangeable. *D. discoideum* responds to starvation through signaling pathways that initiate aggregation and development, while *P. polycephalum* can alter growth, network organization, and life-cycle state. Bacterial biofilms may enter slow-growing, stress-tolerant, or persister-like states through taxon-specific regulatory mechanisms. It is therefore too broad to state that glycolysis and fermentation universally become dominant or that one pathway such as (p)ppGpp explains stress adaptation across all of these systems. The defensible common principle is that environmental stress can reprogram metabolism and behavior in ways that affect sensor performance (Bonner, 2009; Flemming et al., 2025).

Stress resistance likewise takes different forms in different organisms. *P. polycephalum* can form resistant structures such as sclerotia and can produce spores through its reproductive cycle; *D. discoideum* forms spores within a fruiting body; bacterial populations can contain dormant or persister-like subpopulations; and marine protists display lineage-specific survival strategies. These states should not be grouped under a single morphological category such as “brush-like forms.” From an engineering perspective, however, the diversity of stress responses emphasizes that a living sensor must be evaluated not only for immediate sensitivity but also for recovery, stability, and repeatability after environmental perturbation.

Marine protists also illustrate specialized mechanisms of biomineralization and structural adaptation. Many foraminifera construct calcium carbonate tests, whereas radiolarians commonly form siliceous skeletons. The molecular details vary among taxa and should not be reduced to a single universal pathway without species-specific evidence. For the present argument, the important observation is simply that marine protists regulate ion transport, intracellular chemistry, organic templates, and mineral deposition to build structures under seawater conditions. These adaptations reinforce the possibility that indigenous marine eukaryotes may offer more environmentally compatible sensing models than terrestrial slime molds, although no hydrocarbon-sensing function is implied by biomineralization itself (Orsi, 2018).

Marine sponges provide an additional example of coordinated cellular organization. Dissociated sponge cells can exhibit species-specific reaggregation, demonstrating that cell recognition and adhesion can reconstruct organized multicellular structures under appropriate experimental conditions (Humphreys, 1970). This phenomenon has long served as a model for cell–cell recognition and tissue organization. Its relevance here is comparative rather than mechanistic: sponge reaggregation, social-amoeba aggregation, slime-mold network reorganization, and biofilm development all show that local cellular interactions can generate larger-scale structure, but they do so through distinct biological pathways. A biosensor design inspired by these systems should therefore extract general organizational principles without claiming biological equivalence.

Taken together, these examples support a general but carefully bounded concept: biological organization is influenced by genetic programs, signaling pathways, extracellular materials, metabolism, and environmental conditions acting together. No single mechanism is universal across bacteria, amoebozoans, protists, and animals. Nevertheless, local interactions can produce collective or network-level responses that are measurable at a larger spatial scale. This principle is relevant to bio-inspired sensing because a distributed system can integrate heterogeneous environmental information without requiring a centralized controller. The scientific task is to identify which features are transferable to a hydrocarbon-related sensing problem and which are specific to the biology of the original organism.

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Discussion

This study proposes an interdisciplinary concept linking petroleum-associated microbial processes, geochemical gradients, and bio-inspired sensing. Its central objective is not to demonstrate an already established biological method for hydrocarbon exploration, but to evaluate whether a scientifically testable relationship can be constructed between known microbial transformations in hydrocarbon-rich environments and measurable responses of model organisms. The framework deliberately separates established evidence—such as anaerobic petroleum biodegradation and microbial methanogenesis—from the new hypothesis that a chemotactic slime-mold model could function as an indirect transducer of those chemical conditions.

Hydrocarbon-degrading bacteria and archaea, including "*Candidatus Methanoliparia*," can participate in petroleum-associated anaerobic metabolism and can contribute to methane production and other chemical changes in hydrocarbon-rich environments (Head et al., 2003; Laso-Pérez et al., 2019). These products may form gradients that are measurable geochemically. The hypothesis developed here is that selected gradients could also influence the movement or organization of *P. polycephalum* or *D. discoideum* under controlled laboratory conditions. Such a response would indicate sensitivity to the chemical field, not direct sensing of petroleum. The major practical limitations are therefore environmental compatibility, chemical specificity, signal stability, and the ability to distinguish petroleum-associated metabolism from general organic-matter degradation.

D. discoideum and *P. polycephalum* are primarily terrestrial model organisms, so direct survival and functional sensing on a natural seafloor cannot be assumed. Conditions in marine and subsurface sediments may include elevated ionic strength relative to terrestrial laboratory media, oxygen limitation or anoxia, variable pH, low nutrient availability, and increased pressure with depth. These factors could suppress growth, chemotaxis, aggregation, or network formation. The manuscript does not provide experimental evidence that either organism has already adapted to such conditions. Consequently, laboratory feasibility studies should determine whether the organisms can retain a reproducible response under selected stress conditions or whether an ex situ, contained, or purely biomimetic design is more appropriate.

A second major limitation is signal specificity. Methane, organic acids, sulfide, redox changes, and microbial biomass can arise from processes unrelated to petroleum, including degradation of natural organic matter. Therefore, a correlation between microbial activity and a hydrocarbon-bearing environment does not make any single microbial or chemical signal uniquely diagnostic of a reservoir. Future experiments must include non-hydrocarbon controls and should integrate the biological response with direct geochemical measurements. Any eventual field interpretation would also require geological and geophysical evidence so that a biological anomaly is treated as one layer of information rather than as a stand-alone exploration result (Orcutt et al., 2011).

Technological perspectives: If experimentally validated, the proposed framework could contribute to bio-geological engineering and biogeophysics by providing a new way to organize or interpret chemical-gradient information. Claims of reduced exploration cost cannot yet be quantified because no field-scale performance or economic dataset is presented. The most immediate technological opportunity lies in using slime-mold behavior as inspiration for adaptive algorithms, network optimization, distributed sensing, and experimental transduction of chemical gradients (Adamatzky, 2016; Tero et al., 2010). Such tools could eventually complement geophysical and geochemical data, but they should not be described as sources of seismic information unless a specific coupling to seismic measurements is demonstrated.

The conceptual framework and proposed validation strategy can be extended in future research. If terrestrial slime molds prove unsuitable under marine-related conditions, indigenous marine protists, bacteria, archaeal communities, or engineered cell-free and biomimetic systems could be evaluated as alternatives. Candidate organisms should be selected according to environmental tolerance, response

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specificity, ease of measurement, reproducibility, containment requirements, and compatibility with sensor hardware. This broader strategy preserves the central idea—using biological organization or bio-inspired computation to interpret hydrocarbon-associated environmental signals—without making the entire concept dependent on the survival of one terrestrial model species.

Conclusion

This study presents a conceptual biogeophysical approach to hydrocarbon exploration that integrates petroleum microbiology, microbial ecology, biophysics, geophysics, and bio-inspired principles derived from slime-mold behavior. The literature supports the existence of microbial processes that transform hydrocarbons and generate methane, organic acids, redox changes, and other geochemical effects in petroleum-rich environments (Head et al., 2003; Laso-Pérez et al., 2019; Magot et al., 2000). The new contribution proposed here is to investigate whether such microbially generated chemical gradients can be translated into measurable spatial responses by a model biological system or by a biomimetic algorithm. This relationship remains a hypothesis rather than an experimentally established exploration method.

In the Caspian Sea context, the principal challenge is environmental and interpretive rather than merely computational. Terrestrial slime molds may not tolerate natural seafloor or subsurface conditions, and microbial or geochemical anomalies may be generated by non-petroleum processes. Nevertheless, *P. polycephalum* and *D. discoideum* provide experimentally tractable examples of adaptive movement, network reorganization, and collective response to chemical conditions. Their greatest value may therefore lie in laboratory proof-of-concept studies and in the development of biomimetic algorithms that can integrate heterogeneous sensor information, rather than in direct release of living slime molds into the marine environment.

Future research should focus on controlled experimental validation of the complete proposed chain: hydrocarbon-associated microbial process → measurable geochemical gradient → reproducible biological or biomimetic response → independent confirmation by conventional geological and geophysical evidence. Experiments should quantify specificity, sensitivity, response time, environmental tolerance, false-positive rates, and repeatability. If these criteria are satisfied, the approach could then be evaluated in increasingly realistic sediment and mesocosm systems. This staged strategy would allow the innovative biological concept to be tested rigorously while preserving conventional geophysical methods as the principal reference for hydrocarbon exploration.

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