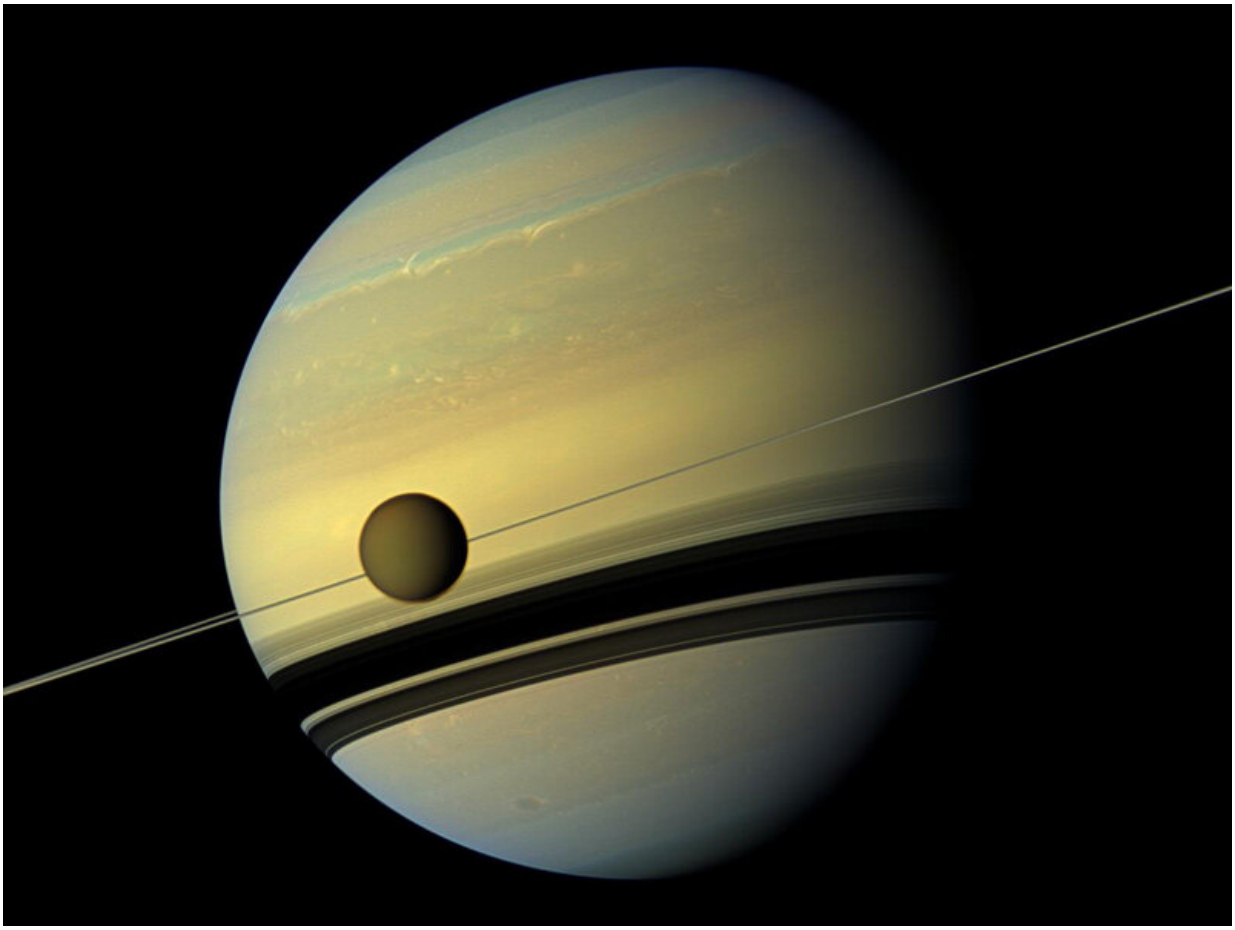
Can polarity-inverted membranes self-assemble on Saturn's moon Titan?

February 7 2020, by Thamarasee Jeewandara



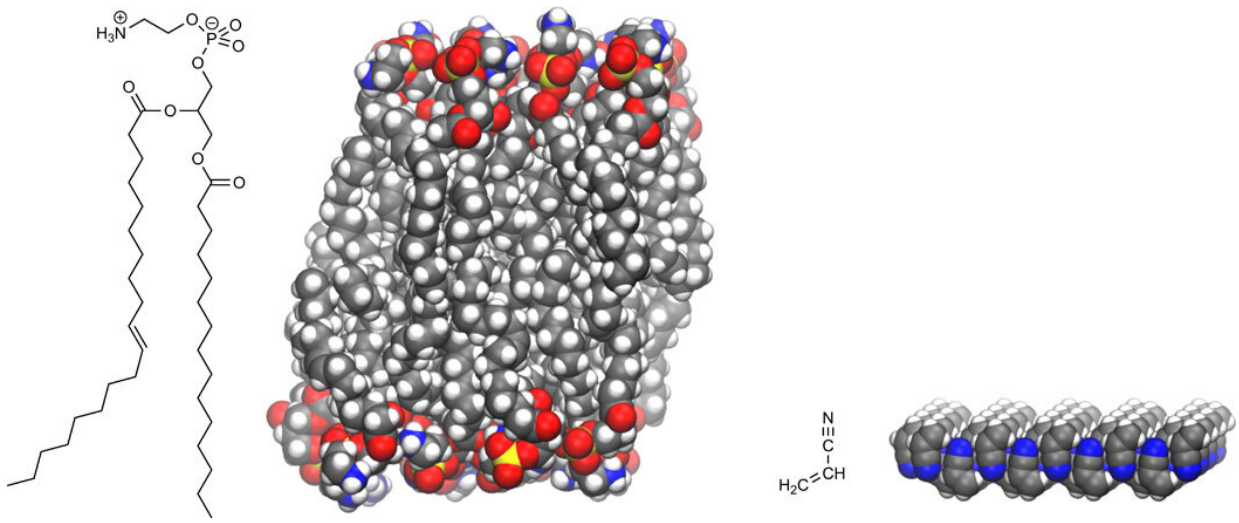
Saturn and its largest moon Titan reflect their true colors. Image credit: NASA https://www.nasa.gov/mission_pages/cassini/whycassini/cassini20120829.html, Credit: Science Advances, doi: 10.1126/sciadv.aax0272

Astrobiologists are focused on resolving two central questions to understand the environmental and chemical limits of life. By understanding life's boundaries, they intend to identify possible biosignatures in exoplanet atmospheres and in the solar system. For example, the lipid bilayer membrane is a central prerequisite for life as we know on Earth. Preceding studies based on simulations of molecular dynamics have suggested that polarity-inverted membranes known as azotosomes made of small nitrogen-containing molecules may be kinetically abundant on cryogenic liquid worlds such as Saturn's moon Titan.

In a new report on *Science Advances*, H. Sandström and M. Rahm at the department of Chemistry and Chemical Engineering at the Chalmers University of Technology, Sweden, formed a next potential step to investigate the thermodynamic viability of azotosome formation. Using quantum mechanical calculations, they predicted that azotosomes are unable to self-assemble in liquid water unlike lipid bilayers. They propose that due to stringent anhydrous and low-temperature conditions, cell membranes may be unnecessary for hypothetical astrobiology on Titan. These efforts on predictive computational astrobiology will be of importance for the Dragonfly mission's scheduled landfall on Titan in 2034.

Saturn's moon Titan features rich atmospheric chemistry and a dynamic surface morphology driven by seasonal rainfall predominantly of methane and ethane cycles. Scientists have observed hydrocarbon lakes and seas near the polar regions of Titan to draw comparisons with the hydrologic cycle of Earth relative to the origin of life. The surface conditions of Titan are, however, a frigid 90 to 94 K and in contrast to Earth, Titan's outermost surface is free of oxygen and covered by products of its atmospheric photochemistry. Researchers also suspect the presence of a frozen water ice crust underneath the outermost organic layer. As the strictest test for the limits of life, Titan offers a unique

environment to explore the chemical complexity of nature and its progression without liquid water at low temperatures at timescales nearing the age of the solar system.



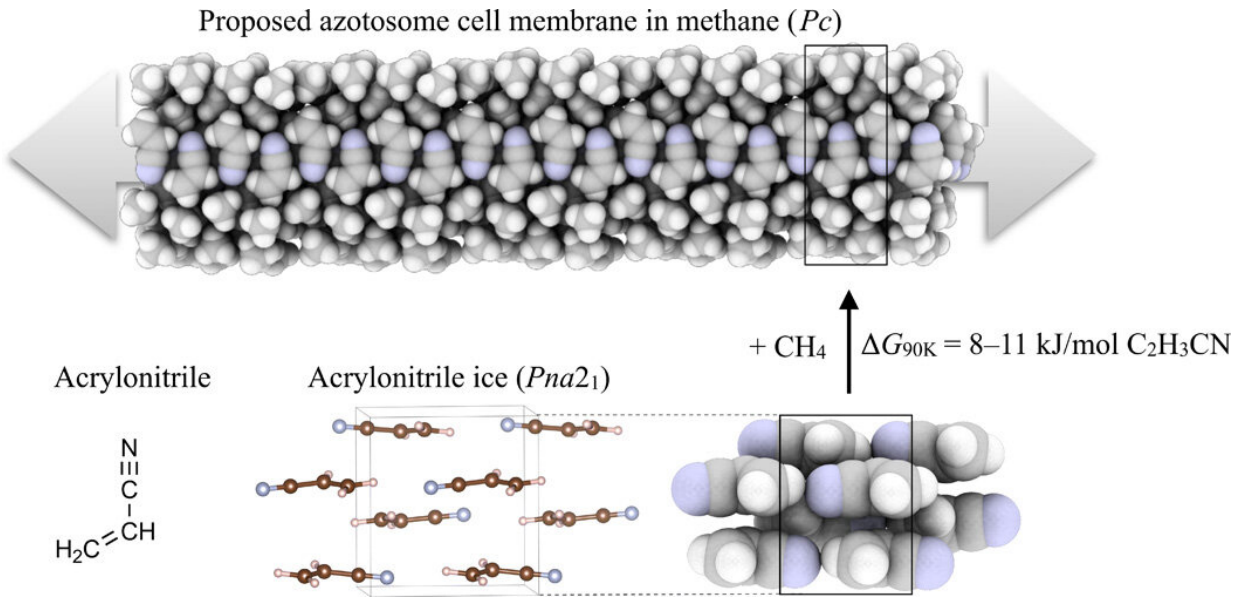
Membranes on different worlds? (Left) Model of a phosphatidylethanolamine bilayer, one main component of the inner bacterial membrane. (Right) An azotosome membrane, a theoretical structure made from acrylonitrile that exhibits an inverted polarity compared to normal lipid bilayers. Azotosome membranes have been suggested to allow for cell-like vesicles in cryogenic (90 K) hydrocarbon liquids that are present on Saturn's moon Titan. Credit: Science Advances, doi: 10.1126/sciadv.aax0272

The lack of thermal energy (kT = 0.75 kJ/mol at 90 K) is a bottleneck for chemical reactivity on Titan, however, sunlight is an energy source (0.4 W/m^2) available for chemistry to occur. In this work, Sandström and Rahm addressed the likelihood of abiotic cell membrane formation, one of the prerequisites for the origin of life on worlds such as Titan. Researchers had also discussed the idea of compartmentalization as central to life to suggest the fascinating possibility of azotosomes on

Titan.

Azotosomes are membranes made of small molecules with a nitrogen head group and hydrocarbon tail group. The hydrophobic groups (water-hating groups) remain on the outside of azotosome membranes (inverted polarity) compared to normal lipid membranes in water—where hydrophobic groups typically remain on the inside. Using molecular dynamics solution in cryogenic methane, research teams predicted that if the structures were made from acrylonitrile (C_2H_3CN) they would have similar elasticity as a normal lipid bilayer in aqueous solution. The possibility of azotosomes further ignited discussions about the limits of life. Two years after the original prediction, scientists impressively detected acrylonitrile on Titan using the Atacama Large Millimeter/submillimeter Array (ALMA).

Since abiotic and biological normal membranes and micelles are formed via spontaneous self-assembly processes driven by favorable thermodynamics. The scientists investigated if the proposed azotosome membrane too remained similarly viable to thermodynamic grounds. For this, Sandström et al. presented estimates to the kinetic persistence of azotosomes using quantum chemical calculations and then addressed their associations for hypothetical exobiology under strict thermodynamic conditions on Titan.



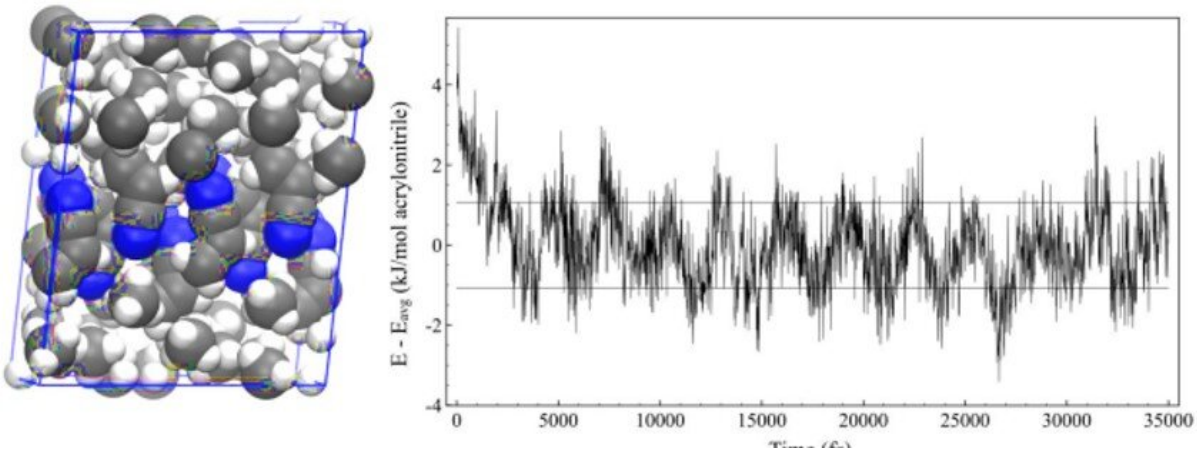
Quantum chemical predictions on membrane stability. The relative free energy of the azotosome and acrylonitrile ice. Quantum mechanical calculations predict that the azotosome is not a thermodynamically viable candidate for self-assembly of cell-like membranes on Titan. The necessary building block acrylonitrile will preferentially form the molecular ice. Crystal symmetries of the considered phases are shown within parenthesis. Credit: Science Advances, doi: 10.1126/sciadv.aax0272

In the "lipid world" or "cells-first" hypothesis, abiotic formation of membranes contributed to the emergence of life; where lipids in water spontaneously self-assembled to form supra-molecular structures such as membranes and micelles, above a critical concentration. During self-assembly of azotosomes on Titan, the envisioned structures will need to be kinetically persistent and thermodynamically lower in energy than the corresponding molecular crystal (molecular ice). The research team used crystalline molecular ice as a contender for acrylonitrile self-assembly.

Sandström et al. applied quantum mechanics in the form of dispersion

corrected density functional theory (DFT) to calculate the energy of the four phases of acrylonitrile ice corresponding with experimental diffraction data. The DFT calculations confirmed the absence of imaginary phonon modes, to ensure dynamic stability of the structure, which they additionally confirmed using DFT-based quantum molecular dynamics simulations in liquid methane at 90 K. The calculations accounted for thermal and entropic events on Titan surface-relevant conditions while considering the dispersion interaction with the surrounding methane environment.

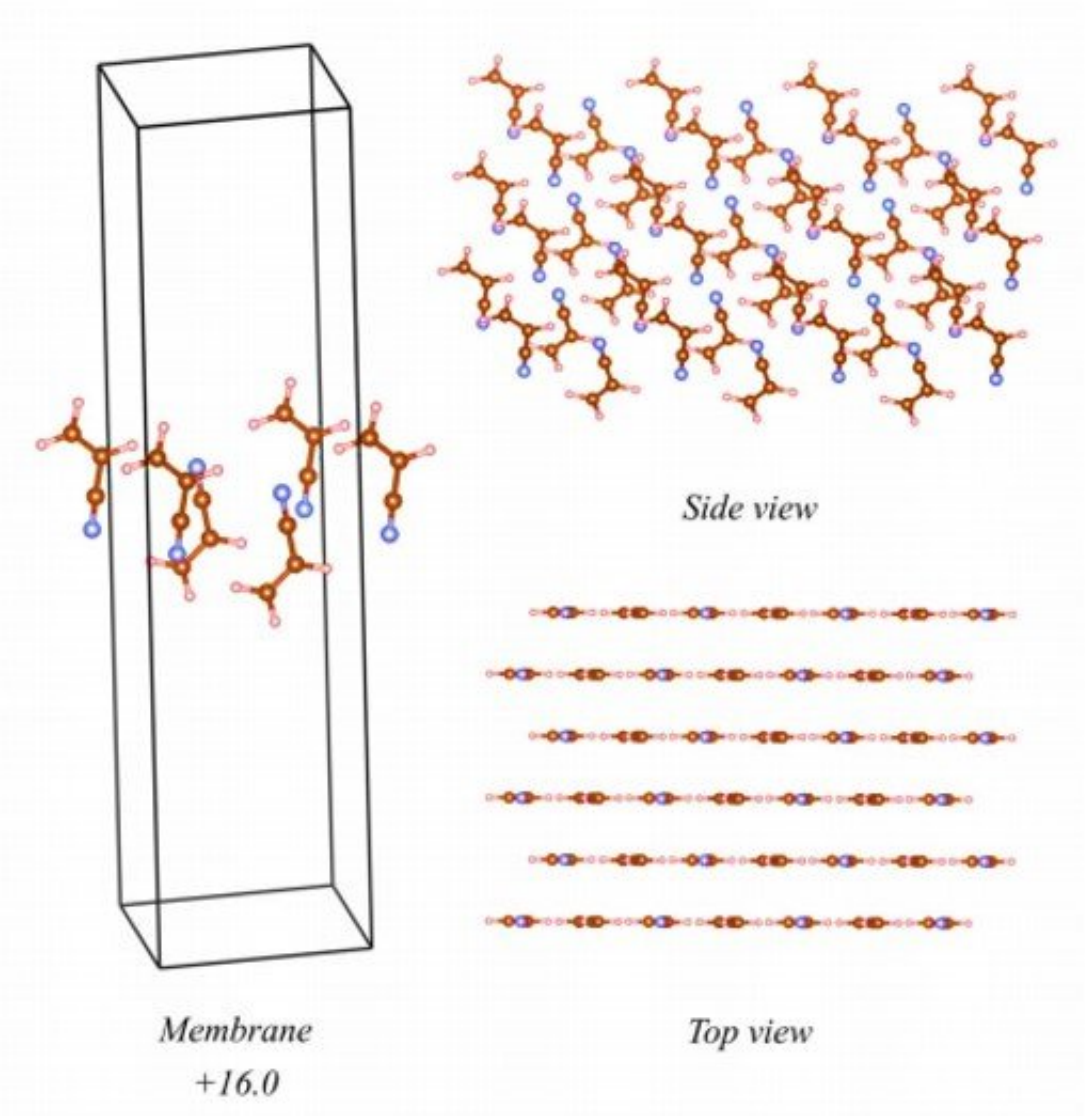
The problem of thermodynamics for life's origin is not unique to Titan; the Gibbs energy requirements for macromolecular formation are reduced on surfaces where surface life forms a possible first step in life's evolution on Earth. The scientists limited their calculations to assess only acrylonitrile-based azotosome and their self-assembly under relevant conditions on Titan, and showed their sufficient kinetic stability for long-term persistence at 90 K. Hypothetical membrane structures made of larger molecules were considerably less kinetically stable.



The dynamic stability of the azotosome. Left: A snapshot of the solvated membrane from the ab initio simulation performed in CP2K. Right: Difference between the potential energy and the average potential energy of the azotosome

in a 35 ps ab initio simulation of the 2 x 2 x 1 azotosome cell solvated by methane. Horizontal lines indicate the 1 kJ/mol acrylonitrile standard deviation. Credit: Science Advances, doi: 10.1126/sciadv.aax0272

The results did not conclusively outline a possible route of self-assembly for cryogenic operable membranes, Sandström et al. did not rule out the existence and relevance of other polarity-inverted membranes built from far more strongly interacting constituents within warmer hydrocarbon environments. In the absence of azotosomes or other cell membranes, it is unlikely for life-governing processes to occur under cryogenic conditions, although life on cold hydrocarbon worlds such as Titan would not necessarily require cell membranes either. The scientists further indicate that any hypothetical life-bearing macromolecule or crucial machinery of a life form on Titan will only exist in the solid state and never risk destruction by dissolution.



Structure of the acrylonitrile-based membrane following optimization in vacuum at the PBE-D3 level of theory. The optimization of the membrane structure was done using an energy cutoff 700 eV and a 9x9x1 k-point mesh. The energy of the membrane relative to the Pna21 phase of the acrylonitrile ice is given in the figure in kJ/mol acrylonitrile. Credit: Science Advances, doi: 10.1126/sciadv.aax0272

The question remains if these biomolecules would benefit from a cell

membrane. Due to low temperature conditions on Titan, biological macromolecules may rely on the diffusion of small energetic molecules such as hydrogen, acetylene or hydrogen cyanide for growth and replication. A membrane could hinder such benefits of diffusion. Similarly, a membrane can hinder the removal of waste products of metabolism including methane and nitrogen. Conversely, it is also possible for a hypothetical cell membrane to protect against harmful chemicals on Titan. However, the narrower energetic range calculated for thermally driven reaction pathways on Titan indicate that only fewer options may damage macromolecules on Titan compared to Earth.

In this way, azotosomes proposed to allow cryogenically operable membranes in liquid methane, pose an intriguing challenge to the principal understanding of biology. The molecule has highlighted the importance of following up properties of predicted molecules in computational astrobiology, to identify their plausible formation routes whenever possible. It is still exceedingly difficult to arrive at specific predictions of chemistry to support biological processes that occur under stringent, thermodynamic environmental constraints on worlds such as Titan. As the molecule of interest grows in complexity, the challenge to reliably model their properties and routes of formation (kinetics and thermodynamics) can become exceedingly difficult.

H. Sandström and M. Rahm calculated that azotosome membranes may be kinetically persistent, although the structure may not be thermodynamically feasible—preventing their self-assembly (unlike lipid bilayers in liquid water). They argue that cell membranes are unlikely to form on Titan's anhydrous and low-temperature environments. While it is possible to experimentally test computational predictions on the existence or nonexistence of azotosome membranes, speculations on the factual environmental limits of prebiotic chemistry and biology remain speculations. The research team suggest careful computational exploration of proposed prebiotic and biological

structures and processes, and their plausibility to guide future in situ sampling of the surface chemistry of Titan.

More information: H. Sandström et al. Can polarity-inverted membranes self-assemble on Titan?, *Science Advances* (2020). DOI: 10.1126/sciadv.aax0272

Paul Voosen. NASA to fly drone on Titan, *Science* (2019). DOI: 10.1126/science.365.6448.15-a

Jack W. Szostak et al. Synthesizing life, *Nature* (2002). DOI: 10.1038/35053176