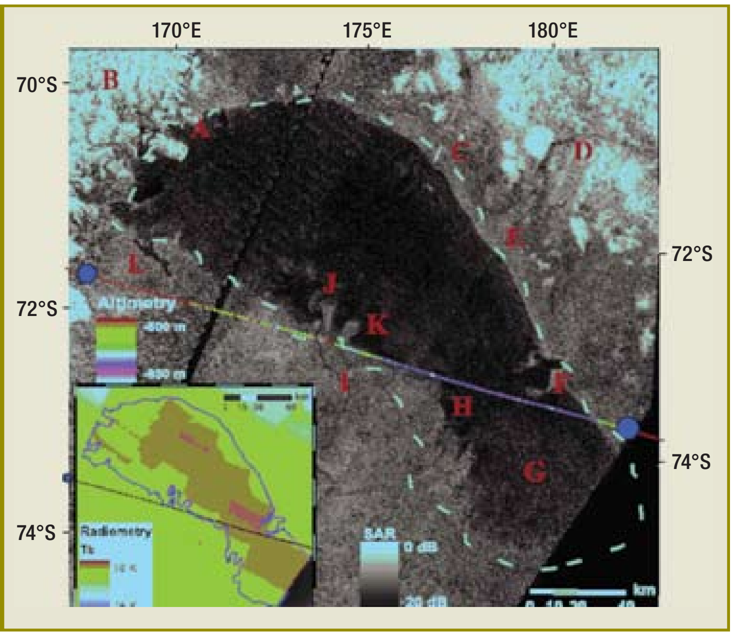
Is there life on ... Titan?

Lucy H Norman and A Dominic Fortes consider the possibilities for life on and in Saturn's complex icy moon, and the nature of organisms that might live there.

1: An impression of Titan's polar seas of liquid hydrocarbons, fed by extensive drainage networks, and illuminated by the first rays of the spring Sun under an orange fog of photochemical haze.

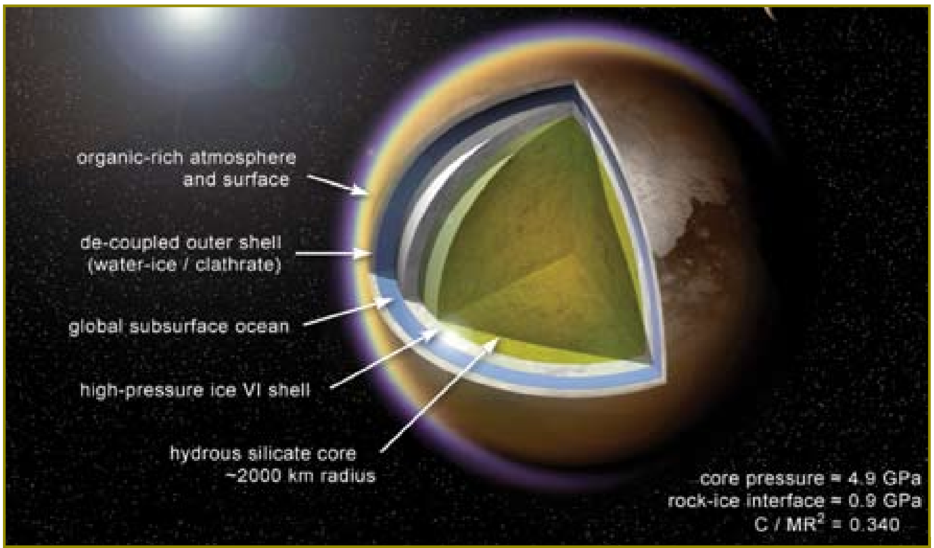


2: Synthetic Aperture Radar (SAR) image and passive radiometry data (inset) of Ontario Lacus, a large south polar lake on Titan. The line of small coloured dots crossing Ontario Lacus reports radar altimetric data (Wall *et al.* 2010). (© 2010 American Geophysical Union. Reproduced by permission of the AGU)

Saturn's giant satellite Titan is the only moon in the solar system with a substantial atmosphere, and which as a consequence appears to have a remarkably Earth-like weather cycle: there is evidence for storm cloud activity and rainfall, extensive dendritic networks likely to be fluvial systems (Elachi *et al.* 2005, Soderblom *et al.* 2007), and many lakes and seas in the polar regions (Lopes *et al.* 2010, Stofan *et al.* 2007), shown as they might appear to an *in situ* observer in figure 1. However, at the surface temperature of 94 K, the liquid involved cannot be water; speculation has suggested it could be a mixture of methane and ethane with some heavier hydrocarbons, dissolved nitriles, and/or atmospheric gases. Evidence for the presence of liquid on Titan's surface comes from the very low radar backscatter of the purported lakes and seas, the identification of a specular reflection in the near-infrared from the feature named Kraken Mare – Titan's largest sea, near the moon's north pole – (Stephan *et al.* 2010) and the identification of near-IR absorption features from ethane, propane and butane in Ontario Lacus – a large south polar lake (Brown *et al.* 2008). The observational evidence is supported by chemical potential models of Titan's lake chemistry (Cordier *et al.* 2009, Raulin *et al.* 1995, Dubouloz *et al.* 1989), and thermodynamic models of the lakes' stability against evaporation (Mitri *et al.* 2007). These bodies of standing hydrocarbon liquid are estimated to cover approximately 15% of Titan's surface above 65°N (Lunine 2009), in a hemisphere that was in its winter season until 2009. The tilt of Saturn's spin axis (26.7°) with respect to its orbital plane provides Titan with strong seasonal modulations of solar insolation as a function of latitude; it is expected that the distribution of lakes in the polar regions will change as northern summer/southern winter progresses (the solstice is in 2017). Indeed, repeated observations by the Cassini radar instrument have revealed evidence of an evaporation sequence at Ontario Lacus, indicating that the shoreline may have already receded inward by about 10 km (Wall *et al.* 2010), see figure 2.

Titan's lakes and seas appear to be replenished by extensive fluvial networks, formed in all likelihood by precipitation of liquid methane and/or ethane rain from storm clouds. The type of clouds found to date vary from the occasional high altitude, transient, tropospheric clouds in tropical latitudes (Schaller *et al.* 2009, McDonald *et al.* 1991, Griffith *et al.* 2009), to relatively frequent polar tropospheric cloud systems (Griffith *et al.* 2000, Brown *et al.* 2002, 2010, Schaller *et al.* 2006, Wang *et al.* 2010), and "fogs" (Brown *et al.* 2009). The "fogs" are believed to form by evaporation of the lakes or a methane-soaked regolith, and have been observed within just a few kilometres of the surface. Further replenishment of the lakes may occur through methane-based aquifer systems; subterranean bodies of liquid hydrocarbons have been hypothesized (Hayes *et al.* 2008), and the Huygens probe, which landed on Titan's surface in January 2005, found evidence for liquids in the near-surface regolith (Niemann *et al.* 2005).

As well as the liquid products of Titan's atmospheric photochemistry, the surface is the ultimate sink of the solid photochemical products that snow out of the atmosphere. These solid hydrocarbons and nitriles accumulate into vast equatorial dune-fields (Lorenz *et al.* 2006b) and probably form thick sedimentary deposits on the polar sea- and lake-beds as well. Quasi-circular structures and flow-like features thought to be cryovolcanic in origin have been observed (Lopes *et al.* 2007), as well as a few impact craters (Artemieva 2003, Lorenz *et al.* 2007) and several mountain ranges (Barnes *et al.* 2007). These features attest to a geologically active body, with a dynamic interior leading to partial melting and ascent of aqueous magmatic fluids to resurface Titan over geological time. A warm and watery interior is also one possible interpretation of Titan's observed moment of inertia factor (Iess *et al.* 2010); both Castillo-Rogez and Lunine (2010) and Fortes (in press) have independently concluded that Titan is likely to have a fully differentiated interior comprising a warm hydrous silicate core overlain by a shell of high-pressure ice between 500 and 600 km deep (figure 3). This icy shell may also contain a global liquid layer some tens of kilometres beneath the surface (Beghin *et al.* 2010). Although there are no observational constraints on the chemistry of this subsurface ocean, a mixture of ammonia and water has traditionally been preferred, and only more recently has an alternative model with a briny ocean (dominated by sulphate salts) been proposed (Fortes *et al.* 2007, Grindrod *et al.* 2008).



3: A possible structural model of Titan's interior, showing the range of possible habitats, from the warm hydrous silicate core, through the high-pressure ice mantle and the putative subsurface ocean, to the organic-rich surface environment.

Astrobiological potential of Titan

Long before Cassini arrived at Saturn in 2004, laboratory study of the likely organic chemical processes on Titan demonstrated the production of tholins (complex C- H- O-, and N-rich polymers) from high-energy electric discharges into a simulated Titan atmosphere (e.g. Sagan *et al.* 1992 and references therein, and Coll *et al.* 2001), or soft X-ray irradiation of methane-bearing ices (Pilling *et al.* 2009). Further hydrolysis of these tholins would produce some amino acids (Khare *et al.* 1986).

This work suggests that the building blocks of proteins may be produced, even today, in the atmosphere and surface environment of Titan; we infer that these processes could have proceeded as far as the emergence of life under the warmer conditions that we expect to have prevailed at or below the surface of Titan shortly after its formation and differentiation. While it has long been recognized that the organic chemistry in Titan's atmosphere may provide useful insights into the proto-biotic evolution of early Earth (Clarke and Ferris 1997, Raulin *et al.* 1994), astrobiologists have speculated only recently about the possibility that life might have arisen and could persist in these alien environments. There are a plethora of possible habitats for exotic biota on Titan, including life that may be indigenous to the surface and living in liquid hydrocarbons, or have originated in a subsurface ocean and adapted to life in aqueous media such as an ammonia–water mixture. The range of environments that might be conducive to living organisms extends from the surface down to a few kilometres into the rocky core, giving a biosphere volume of ~4×10^{10} km^3, at least *double the volume of the terrestrial biosphere*.

• **The silicate core.** The outermost few kilometres of the rocky core at <400 K are probably cool enough for organisms to survive; the maximum growth temperature for known terrestrial hyperthermophiles is 394 K. This environment is almost certainly permeated by liquid water from the ice layer above, and this water can mediate a range of chemical reactions yielding substances of use to obligate anaerobes. The hydrostatic pressure at the base of the ice mantle is likely to be ~1 GPa, which is substantially greater than most organisms on Earth are adapted to. However, microbial metabolism has been reported at pressures of 1–1.2 GPa, while pressures >1.6 GPa were found to kill the microbes under investigation (Sharma *et al.* 2002). We can speculate that a carbon-based biochemistry would employ similar metabolic pathways to those found in deep terrestrial ecosystems (e.g. Pedersen 2000). Alternatively, the biochemistry may be completely alien; Schulze-Makuch and Irwin (2006), for example, pointed out that low-temperature serpentinization can lead to the formation of silanes, which might provide the basis for a polymerized silicon biochemistry quite unlike anything on Earth.

• **The high-pressure ice mantle.** The experiments by Sharma *et al.* (2002) observed microbial activity, in fluid inclusions in ice VI using a diamond anvil cell. This extends the range at which microorganisms are known to be able to survive in pore fluids in solid ice (Price 2000, 2004) over the entire pressure range of relevance to Titan's ice shell. There is nothing to prevent interstitial liquids rich in likely nutrients from becoming trapped in the matrix of the ice VI shell as it forms. Also, if microorganisms were present in the early history of Titan they could have been incorporated into the icy matrix of the high-pressure mantle and have evolved into an ecosystem separate from other Titan biota.

• **The subsurface ocean and crust.** A subsurface ocean of aqueous ammonia might have the requisite properties (in terms of temperature, pressure, pH, viscosity and nutrient availability) to support a modest biosphere, particularly if life was able to originate inside the warmer proto-Titan (Fortes 2000, Simakov 2000, 2001, 2008). However, a relatively warm aqueous ammonium sulphate subsurface ocean (Fortes *et al.* 2007, Grindrod *et al.* 2008) is a considerably more attractive environment for life. At 100–500 MPa, a eutectic solution of ammonium sulphate is likely to be ~60 K warmer, and almost three orders of magnitude less viscous, than a eutectic solution of ammonia. The pH is comparable to rain water, under the expected conditions, and the salinity within the region of comfort for halophiles (Rodriguez-Valera 1991). Energy production by the dissimilative reduction of dissolved sulphate is a common metabolic pathway among terrestrial obligate anaerobes (examples include *Archaeoglobus*, and the well-known *Desulfovibrio*). There are numerous electron donors employed in sulphate metabolism (Brock *et al.* 1997), and those which might be available in the subsurface ocean as a result of inorganic synthesis (e.g. Shock and Mckinnon 1993) include H_2 and ethanol.

Interestingly, a methane/sulphate-bearing ocean is not very different from cold seeps on the Earth's ocean floors. At cold seeps, sulphate reduction (SR) and the anaerobic oxidation of methane (AOM) are usually syntrophically linked (Joye *et al.* 2004). These environments, even on Earth, are poorly understood, and an area of active research (e.g. Knittel *et al.* 2005). The metabolic products of SR and AOM are H_2S and dissolved CO_3^{2-}; the identification of H_2S-clathrate and carbonates (probably ammonium carbonate monohydrate) in liquids erupted at Titan's surface, particularly if these show signs of biological isotope fractionation, would be a strong indication of microbial activity in the subsurface ocean. Microorganisms could survive in pore fluids or grain-boundary fluids to within a few tens of kilometres of the surface globally (*cf.* Price 2000, 2004), and possibly for short periods locally in plutonic cryomagmas intruded into the crust, or in liquids erupted onto the surface.

• **Surface liquids.** Benner *et al.* (2004) first suggested that the liquid hydrocarbons on Titan could be the basis for life, playing the same role as water on Earth. This initiated theories about the metabolism of such hypothetical organisms; both McKay and Smith (2005) and Schulze-Makuch and Grinspoon (2006) computed the energy available from the reaction of H_2 with organic material, with the production of methane as a waste product. They showed that the metabolism of acetylene yielded the most energy, although the high abundance of ethane makes it a competitive source of energy for Titanian biota. These reactions will not proceed spontaneously; they require either metal or biological catalysts to promote the reaction. However, the Committee on the Limits of Organic Life in Planetary Systems (2007) noted that many enzymes function in organic solvents, and many organic reactions fundamental to biochemistry can occur in non-aqueous media, so there appears to be no barrier to the adoption of suitable catalytic enzymes by hypothetical methanogens on Titan's surface.

McKay and Smith (2005) predicted that organisms living in liquid methane on Titan's surface would produce anomalous depletions of hydrogen, acetylene and ethane, as they consumed these substances: recent Cassini data appear to provide intriguing evidence for such depletions. A deep global ocean consisting principally of ethane was predicted after the Voyager flybys, based on photochemical modelling; the limited liquid present in lakes and small seas reveals that there is an unexpected lack of ethane on the surface (Lorenz *et al.* 2008). Secondly, Strobel (2010) modelled the hydrogen concentration in Titan's atmosphere and found that the observational data are best explained by a strong flux of hydrogen to the surface, for which the only *current* explanation is a gradient in the hydrogen concentration created by metabolism of H_2 by methanogenic organisms. Finally, Clark *et al.* (2010) report an apparent depletion of acetylene at the surface compared to the expected rates of atmospheric production and subsequent deposition of acetylene onto the surface; in support of this there was no evidence of acetylene in the gases released from the surface after the Huygens Probe landed (Niemann *et al.* 2005, Lorenz *et al.* 2006a). Although these depletions are intriguing, they do not constitute unambiguous proof of life on Titan's surface (there are abiological explanations in each case), but they certainly endorse the argument that Titan is a target of high astrobiological interest.

There have been discussions about the possible temporary presence of surface water, normally frozen at Titan's ambient temperatures. For example, localized "warm" spots are possible from geothermally heated liquid methane trapped deep in sedimentary basins rising to the surface at springs lines or in mud volcanoes (Fortes and Grindrod 2006) or, more vigorously, at geysers (Lorenz 2002). Bodies of liquid water (with or without ammonia?) produced by hypervelocity impacts into an icy substrate are predicted to last for periods of hundreds to thousands of years (Artemieva 2003). Similarly, aqueous cryovolcanic flows may remain partially molten for very long periods, particularly if they contain significant quantities of ammonia (Sarker *et al.* 2003), causing hydrolysis (and/or ammonolysis) of tholins to produce amino acids (e.g. Neish *et al.* 2007, 2008, 2010).

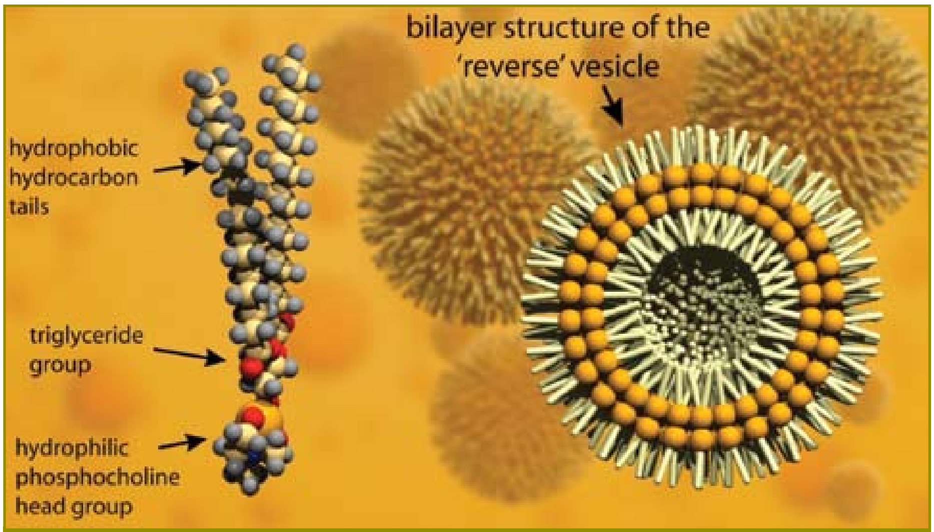
Possible biochemistries of life on Titan

It is plausible that Titan's subsurface ocean could deliver a cornucopia of chemicals to the surface environment dissolved in cryomagmatic liquids; these may also bear the signature of life in the subsurface ocean, or else – like black smokers on the Earth's ocean floors – provide a concentrated source of nutrients for specialist organisms on Titan's surface. Inputs of dissolved material from the subsurface ocean (sourced from Titan's rocky core, or post-accretionary impactors) may have a significant impact on the habitability of Titan's hydrocarbon lakes and have an important selective effect on the potential biochemistries that lacustrine biota might use. For example, naturally occurring organo-silicon compounds, such as silanols, are likely to be soluble in liquid hydrocarbons, and could plausibly be used by an exotic Titanian biota (Bains 2004).

It is likely that the physiology of exotic life in hydrocarbon lakes will have many differences to life on Earth. Even if the biota is carbon-based it must adopt organic molecules with alternative functional groups, or alternative structural arrangements (bioisosteres) in order to carry out the functions of equivalent terrestrial macromolecules employed for catalysis, storage of hereditary information, compartmentalization and structural integrity, to name a few. For hypothetical hydrocarbon-dwelling organisms a combination of experimental and computational work is required to establish the suitability of any potential bioisoteric adaptation.

Arguably, the most basic requirement for Titanian microbes is a mode of compartmentalization – the development of a capsule that allows the organism to concentrate nutrients, to conduct metabolism under controlled conditions, and to store genetic information. This may be a capsule of occluded hydrocarbon liquid in the case of organisms that originated – and are adapted to life – on the surface, or else may be a capsule of aqueous fluid separated from the surrounding liquid hydrocarbon environment in the case of organisms that evolved in Titan's interior but subsequently adapted to life on the surface. Since the Committee on the Limits of Organic Life in Planetary Systems (2007) has observed that there is currently no information about the possible alternative membrane structures that would be stable in organic solvents, we have begun a programme of experimental study into plausible bioisosteres that may perform just such a function.

Ordinarily, organic solvents permeate cell membranes, resulting in leakage of the cell contents into the external environment and cell death. However, some terrestrial organisms are able to tolerate high concentrations of organic solvents (e.g. Heipieper *et al.* 1994, de Carvalho *et al.* 2007, de Carvalho *et al.* 2005) and can even live in such places as Pitch Lake, Trinidad (Ali *et al.* 2006), which is the largest natural deposit of asphalt in the world. These terrestrial organisms cope by incorporating surfactants with a greater hydrophilic nature into their cell membranes, which can then repel hydrocarbons more efficiently, and/or they have efflux mechanisms that remove solvents that have diffused into the cell. For those Titan biota that are hydrocarbon based, one possible solution to this problem would be to adopt a modification of the arrangement of surfactant molecules in the cell membrane, forming a so-called "reverse" vesicle structure (figure 4). Such structures are known to occur in the laboratory,



4: "Reverse" vesicle structures (right) consist of spherical bilayers of close-packed surfactant molecules (such as phosphatidylcholine, shown on the left). The hydrophilic groups (orange spheres) are packed into the centre of the bilayer, with the hydrophobic chains of hydrocarbons (pale yellow sticks) extending inwards and outwards. The phosphatidylcholine molecule shown on the left is ~25Å in length.

and have been extensively characterized in systems composed of phospholipid surfactants in high-molecular-weight hydrocarbons and non-polar liquids, such as cyclohexane, hexane, toluene and chloroform, at room temperature (e.g. Tung *et al*. 2008, Kunieda *et al*. 1993a, 1993b, 1994, Li and Hao 2007, Nakamura *et al*. 1995). Reverse vesicles are excellent candidate analogues for cell membranes that could maintain a hydrocarbon-based cyctoplasm in a hydrocarbon liquid medium. For those biota that still retain an aqueous cytoplasm (after originating from the subsurface and migrating to the hydrocarbon lakes) a reverse micelle-like structure could have evolved; or else the "normal" terrestrial type vesicle is maintained with numerous coping mechanisms. We are carrying out experiments to determine whether certain surfactants will form reverse vesicles (or other similar structures) in low-molecular-weight liquid hydrocarbons under low-temperature conditions; reducing the molecular weight of the hydrocarbons and the temperature until attaining environmental conditions comparable to the surface of Titan.

Summary

Titan meets the absolute requirements for the presence of life: it is not in thermodynamic equilibrium, it has abundant carbon-bearing molecules at the surface and there is a plausible liquid substance in which biological activity may be mediated. Moreover, there are a wide range of possible habitats for exotic biota extending to depths of several hundred kilometres into Titan's interior; Titan could be home to numerous, separate ecosystems, with completely independent evolutionary histories (or else their only connection lies in the distant past when Titan formed). This combination of factors makes Titan an extremely enticing object for astrobiological research.

Further work on exotic, hypothetical biota requires a combination of research pathways, including study of possible analogue habitats (such as terrestrial tar pits), computational modelling of plausible biochemical phase spaces (Bains 2004), and experimental investigation of potential bioisoteric substances that could be used in extreme environments. Such research forms the basis for future *in situ* astrobiological observations, including the design and delivery of instrumentation for the next planned robotic mission to Titan (e.g. Coustenis *et al*. 2009). ●

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