## BIOSIGNATURES FROM A DEEP BIOSPHERE: THE LARGEST AND LONGEST-LIVED HABITABLE ENVIRONMENTS ON MARS

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**Introduction:** The current surface conditions on Mars are incompatible with life as we know it: the surface atmospheric pressure precludes standing water[1]. Harsh UV [2] and gamma radiation will destroy complex organic molecules in the surface and near surface environment hindering detection of organic biosignatures. These harsh surface conditions potentially extended to the Noachian/Hesperian boundary, so surface environments including lakes/deltas may not have habitable at the surface. However, subsurface refugia may have extended the window of habitability and putative subsurface pockets of habitable conditions could potentially still exist harboring extant life and their biosignatures.

Subsurface Habitats on Earth: The minimum requirements for subsurface life include space, carbon, and energy linked in a substrate allowing for an adequate supply of nutrients and removal of toxic waste products[3]. Subsurface environments may harbor the majority of microbial life on Earth [4], [5] and Archaean biosignatures suggest the existence of a terrestrial biosphere for billions of years e.g.[6]. Subsurface microbial communities are sustained through chemolithoautotrophic metabolic processes adapted to energy limitations[7] limited by the geothermal gradient reaching the upper temperature limits of life[3]. Extant subsurface metabolisms in these terrestrial Mars analogue habitats include coupling oxidation of H<sub>2</sub> generated by serpentenization reactions to reduction of oxidized iron and sulphate minerals[8], [9]. Methane can be generated through subsequent reactions with  $CO/CO_2[10]$ supporting methanotrophic communities.

**Subsurface Habitability on Mars:** The subsurface represents the most temporally extensive habitable and potentially inhabited environment on Mars [11]. The presence of past liquid water is evidenced by the association of phyllosilicates with ancient crustal terrains, and subsurface liquid water interacted with the surface environment in catastrophic outflows from the Hesperian [12] to as recently as a few million years ago [13], [14]. Modern subsurface water may be present in pockets due to radiogenic heating and lithostatic pressure and the presence of brines depressing the freezing point[15].

Impact cratering is an important geological process on Mars, and large basin-forming events would have potentially connected the cryosphere to the surface[16], [17]. Impact-generated hydrothermal systems would have generated transient habitable environments [18], [19]. Outcrops of serpentine has been identified in several geological settings on Mars [20] indicative of past serpentinization diagnostic of highly reducing, alkaline, <400°C hydrothermal alteration of ultramafic rock. Subsurface systems may still exist.

**Biosignature Detection:** Ionizing radiation rapidly degrades complex organic molecules in the near surface environment. Both UV and ionizing radiation are damaging to organic molecules posing a challenge to both habitability and detection of organic biosignatures. UV radiation results in highly oxidizing conditions and wind-induced mixing of oxidants in the upper ~1m soil presents a hazard[21]. Detection of complex organic molecules will be 'problematic' within the top 10 cm with an exposure age of more than 300 Myr [22]. 1.5 – 2 m drilling is required before 3 Ga amino acids can be detected [23]. Subsurface aqueous interactions with pyrite have also been suggested to produce oxidants affecting the preservation of organic biomolecules and ice-rich permafrost regions may have a better preservation potential[24]. It is essential to access the subsurface to detect and characterize complex organic biomolecules. In addition to drilling, subsurface access is possible through the exploration of A) impact structures and their associated products including central uplifts and ejecta and B) surface mineral deposits precipitated from fluids sourced from the subsurface.

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