Chapter 5
A Systematic Way to Life Detection: Combining Field, Lab and Space Research in Low Earth Orbit

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Abstract The characterization and detection of biosignatures is a challenging task, but one that needs to be solved before instruments are used for life detection missions on other planets and moons. A complex logistical effort is needed to support such exploration missions and a significant amount of preparation and investigation is required to prevent and eliminate pitfalls and errors, which may occur during the technical and scientific operations. Herein is suggested a systematic approach to prepare for "life-detection" missions, and an overview is given on the necessary steps in order to search for life *in-situ* on another planet or moon. Results obtained from research performed in the field, in the lab and in space will help to enhance our knowledge regarding the traces and signatures of life, and how to recognize life itself.

5.1 Introduction

Future space exploration missions with a primary focus to search for life elsewhere in the Solar System will have a number of challenging tasks. In addition to investigating the habitability of planetary objects for past and even present life, i.e. the ability of an environment to support the activity of at least one known organism (Cockell et al. 2016), it is important to find out which kind of life forms, or remnants thereof, should be looked for on potentially habitable worlds, subsequently identifying which of them could be detected by specific technologies in extraterrestrial environments. In this chapter, we aim to determine the necessary systematic preparations, investigations and logistic operations needed before starting a life detection

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mission. In addition to a theoretical approach, accompanying experimental procedures are essential in this kind of research discipline. For example, ionizing and UV radiation can modify biosignatures in the first meters beneath the surface, and may modify their detectability by Raman or fluorescence methods (Dartnell et al. 2012; Dartnell and Patel 2014). Therefore it is necessary to perform preparatory ground-based studies in planetary simulation facilities and at spectroscopy laboratories (such as facilities which can offer Raman, Infrared (IR), Ultraviolet/Visible (UV/VIS), and fluorescence spectroscopy). Field astrobiology research can be conducted in terrestrial analogues to validate instruments and measurements of biomarkers in their *in situ* context (Foing et al. 2011), as well as laboratory analytical methods on retrieved samples (see Ehrenfreund et al. 2011; Martins et al. 2011; Orzechowska et al. 2011; Direito et al. 2011). Furthermore, some part of the work has to be done in Low Earth Orbit (LEO) or on the Moon (de Vera et al. 2012) where samples are exposed to space conditions, which can mimic Mars-like conditions or approach the conditions observed on the icy moons of Jupiter and Saturn. The reason for this is that no laboratory on Earth is able to simulate simultaneously all of the conditions that biomolecules might face in real space conditions. Knowledge about the stability of biomolecules and the response of microorganisms exposed to extreme space environments, or their degradation products, might significantly facilitate the detection of biosignatures elsewhere in our Solar System. These data have to be systematically analysed and arranged in a planetary biosignature database taking into account all protocols of environmental conditions, measurement regimes, obtained spectra, diagrams, tables and images. This database would provide, for the first time, an easily accessible repository of planetary-relevant biosignatures from all investigations performed by instruments on planetary analogue field sites, together with data obtained by simulated planetary condition experiments, including research performed directly on different space platforms. Combining these approaches will provide extensive insight on the expected effects when biomolecules are exposed to the environmental conditions present on potential habitable planets in our Solar System. The collected and arranged data could be made available to the international community and may serve as a biosignature backup database and guide for future life detection missions on other planets and moons.

5.2 Promising Locations in the Solar System for Life Detection: Mars, Europa and Enceladus

The definition and identification of unambiguous biosignatures is a necessary endeavour in order to develop a database for future space exploration programmes searching for life in potentially habitable extraterrestrial environments. The ever-growing databases of “fingerprints” of life contextualized to the search for life on Mars have been the focus of the astrobiological community for several decades (Banfield et al. 2001; Cady et al. 2003; Serrano et al. 2015; Westall et al. 2015), but more effort is still needed. For example, we still do not know much about the types of biosignatures, which could be expected on the icy moons of the gas giants within our Solar System.

5.2.1 *The Search for Habitable Worlds*

The search for life usually begins with the search for habitable extraterrestrial locations. Liquid water is essential for life as we know it and can only exist within the subsurface or, protected by a suitable atmosphere, on the surface of planetary bodies. The study of our Solar System suggests that subsurface environments containing liquid water are more widespread than surface hydrospheres. For example, there is evidence that below Mars' cryosphere resides a global aquifer (Head et al. 2003), and that small icy satellites such as Europa (Jupiter) or Enceladus (Saturn) harbour liquid oceans or lakes beneath their water-ice crust. These bodies, however, do not lie within the habitable zone with respect to its traditional definition, lack a dense and global magnetic field, and have no atmosphere. Nevertheless, such subsurface aqueous environments are protected against extreme radiation fluxes and would have access to nutrients and energy, for example, through hydrothermalism (e.g. hydrothermal vents) or geochemical reactions (e.g. serpentinisation) where there is direct contact between the lithosphere and the subsurface ocean (Hsu et al. 2015; McMahon et al. 2013).

5.2.2 *Promising Potentially Habitable Targets of Relevance to Life Detection Missions*

Excluding the possibility of probing the subsurface of the icy moons in the near future, the ability to detect biosignatures of putative biospheres is quite limited and unrealistic for exoplanets in the near future. However, in the case of Enceladus, the analysis of the South Polar Plume by the *Cassini* mission has led to the detection of organic compounds (Waite et al. 2009). For Europa, it has been suggested that the remote detection of biosignatures might be possible through spectroscopy of ocean material that reaches the surface through fractures or *via* briny diapirs. If biogenic volatiles can reach the surface and accumulate in the atmosphere, as has been speculated for methane on Mars, it is conceivable that such biosignatures could be detected on exoplanets in the long-term future (Rauer et al. 2011). In the presence of an outer ice shell, it will be difficult to detect biosignatures of putative biospheres below. However, *in situ* analysis of hydrocarbons in eruption plumes emanating from the surface of active satellites (e.g., Enceladus' south pole terrain; Waite et al. 2009) could be performed during close spacecraft encounters to determine the ratio of non-methane hydrocarbons to methane (which is extremely low for biological sources and higher for non-biological sources; McKay et al. 2014). These authors further suggested that several cold, oceanic, methanogenic subsurface ecosystems on Earth may serve as analogues for possible habitable environments on Enceladus and Europa. The remote detection of biosignatures in the harsh electron irradiation environment of Europa might be possible through spectroscopy of radio-chemically modified surface areas and liquids deriving from the subsurface ocean, which could be exchanged between the surface and the ocean through fractures or *via* briny diapirs (Hand et al. 2007). Brines could even form in the Martian Polar Regions and they may temporarily make liquid water available at very low temperatures, an essential ingredient to be a habitable environment for microorganisms (Fischer et al. 2016).

5.3 Finding Stable and Space-Resistant Biomolecules as Potential Biosignatures

Extraterrestrial niche habitats, which might harbour extant life forms or retain traces of ancient life (as suggested for Mars), should be detectable in future space exploration missions. Some of these niches can be characterized by the porosity of their lithic substrates or ice, or by the presence of liquid-water veins in ices, cracks and fissures in the outermost crust layers of the icy moons. These niche habitats could store possible ingredients or deposits of the geysers and plumes of the icy moons.

5.3.1 The Content of a Life Detection Database

A useful life detection database requires the collection of all relevant information concerning robust organic compounds that are unambiguously produced by (micro-) organisms. For this database, a collection of various species from a broad range of Earth environments ranging from deep sea to permafrost areas in the Polar Regions, together with an extraction of their related isolated cell components, such as secondary metabolites, is of principal interest. The results and protocols of their responses to different experiments, such as exposure to space conditions, is essential because space conditions with different radiation sources, e.g. ionizing radiation (X-rays, gamma-ray), UV-radiation, heavy ions from galactic radiation, vacuum and space weathering by micro-dust, etc., could significantly influence their identification by different detection methods. Since these conditions cannot be simultaneously simulated in even the best planetary simulation chambers on Earth, we need results from space experiments to know more about the stability of organisms and their constituent organic parts. This is especially important if we take into account evolutionary processes on planets. For example, on Mars, the availability of liquid water and the thickness of the atmosphere have decreased during its history. In the case of liquid-water oceans beneath the ice crusts of the outer planets satellites, water charged with unknown organic material is emitted by plumes into the space environment (Waite et al. 2009; Dong et al. 2011; Postberg et al. 2011; Iess et al. 2014). Traces of extinct or extant life forms on the surface of Mars might have been altered due to a changing radiation environment (decaying atmosphere and collapse of the early geomagnetic field). Irradiation and vacuum conditions are expected to affect the stability of organics at the surface and certainly any putative life forms ejected into space, for instance through the venting plumes discovered on Enceladus (Waite et al. 2009) and possibly Europa (Roth et al. 2014) that are fed by shallow liquid reservoirs. Ideal targets for the classification of stable biosignatures that could serve as usable "fingerprints" are both organisms and organics, which might be stable even in a fossilized state or under intense radiation environments, partly due to physical and chemical interactions in the rock/soil/salt/ice environment. Currently, the ideal method to study this and to develop a sophisticated biosignature database including data with relevance to Mars and the icy moons in the outer Solar System is to use planetary simulation facilities and exposure platforms on the International Space Station (ISS) such as EXPOSE (Europe, ESA) and Tanpopo (Japan, JAXA) and the up-coming micro-, nano-, and cube satellite generation.

5.3.2 Low Earth Orbit Missions Supporting Exploration Missions to Search for Life

The on-going BIOMEX project (BIOlogy and Mars EXperiment; de Vera et al. 2012) is an example of combining planetary analogue fieldwork, the use of simulation facilities and the EXPOSE platform. Its follow up is foreseen in the BIOSIGN project (BIOSIGnatures and habitable Niches—ILSRA-2014-0019) as a new experiment in preparation with ESA. Like BIOMEX, the BIOSIGN project will be an international and interdisciplinary experiment in LEO where the four main scientific disciplines of Polar Research, Deep Sea/Ocean Research, Deep Biosphere Research and Space Research, as well as their exploration programs, will cooperate to work on the establishment of a database. This would bring together all data relevant to spectroscopy and life detection, particularly with regard to minerals, salts, ices and organic biosignatures relevant to Mars and the icy satellites of the outer Solar System.

5.4 Relevance to Biosignature, Bio-Trace and Bio-Fingerprint Research

A number of experiments were carried out on selected pigments and membrane components in preparation for the BIOMEX experiment. Due to the fact that biosignatures have to be detectable in the context of background material (environmental parameters, mineral matrix etc.), the first steps were to characterize Mars-analogue mineral mixtures and isolated biological samples (e.g. cyanobacteria, archaea, fungi and lichens; Baqué et al. 2016; Böttger et al. 2012, 2013a, b; Serrano et al. 2014, 2015) and minerals intermixed with bio-relevant organic material. At the time of the writing, new papers are in preparation to show the differences between irradiated and non-irradiated samples which were done during the Environmental Verification Tests (EVTs), the Scientific Verification Tests (SVTs) of BIOMEX and the real space experiments on EXPOSE-R2 aboard the ISS. Moreover, the extracted pigments, membrane and cell wall components have been characterized and are available in the DLR internal preliminary Raman database. Other work is still on-going using UV/VIS-spectrometry and IR-spectroscopy. However, much more work is needed in this context, in particular in reference to potential microfossils of extinct Martian life and in regard to biosignatures of potential extant life present in the subsurface oceans of the icy moons and detectable through plume activity. Although, in this chapter, we mainly consider physical and biochemical biosignatures, it is also important to emphasize that, for detecting life, bio-morphological structures have to be taken into account as well and should not be excluded from the biosignature data base. This is especially relevant if different microscope techniques will be used in future space exploration missions.

5.5 Experimental Approach

Previous experiments paid some attention to the high level of structural liability and stability of most of the biological components, especially on the protection of surface coats, membranes, proteins or secondary metabolite deposits, and even on biomolecules of the cell interior, such as DNA and RNA. These organic biosignatures were studied with reference to Mars and space conditions (Baqué et al. 2016; Onofri et al. 2012, 2015; Pacelli et al. 2016, 2017). However, little is known for samples that are relevant to the icy moons in our Solar System (Gleeson et al. 2012). Possible Mars biosignatures are better investigated, but even here knowledge is lacking. The intention of future experiments, e.g. BIOSIGN, is therefore to develop a further understanding and to analyse the effects of the space environment. A number of samples embedded in salty material or sediments of the deep sea will be used, in addition to the well-known Mars analogue mixture with characteristics from the early and late Martian evolutionary stages. The Museum für Naturkunde in Berlin has developed Mars-analogue mixtures based on insights gained from remote spectroscopy (Bibring et al. 2005, 2006; Chevrier and Mathé 2007; Poulet et al. 2005; Schirmack et al. 2015) within the framework of the founded German Helmholtz-Alliance "Planetary Evolution and Life" (Böttger et al. 2012). For example, the detection of silica in the plumes of Enceladus during the Cassini mission can only originate from the rocky core of the icy moon (Hsu et al. 2015). Thus, it is possible that the detected organic components could be a hint of prebiotic molecules or life forms from Enceladus' deep-sea regions, which might reach the surface of the ice crust of the moon through convective transport within its ocean. Therefore, it would be very interesting to analyse what happens to terrestrial analogue material from the deep sea, including life forms from these deep ocean areas. This culminates with the question: what could happen if life-related sample material is suddenly exposed to vacuum conditions (as might happen in the plumes of the icy moons), solar/space radiation and deep freezing conditions of simulated open space? It would be desirable to test this under space conditions on the ISS, on a new generation of satellites, or on the Moon. The effects of space, Enceladus- and Europa analogues, and Mars-like environments in their present and past epochs, particularly on minerals, must be tested in parallel with the biological investigations. This kind of programme of investigation, wherein the evolutionary aspects of planets and moons of interest are integrated, could clarify potential changes and alterations of biomolecules identified as putative biosignatures over short or long-term time scales. A welcome side-effect of these space and time experiments is that the investigated samples would also serve for viability and space resistance analysis and would result in a replicate space experiment producing valuable data for the question of the probability of habitable niches in the Solar System. The experiment would also determine the likelihood of lithopanspermia occurring today and/or in the past. Initial experiments had previously been carried out on FOTON/BIOPAN some years ago (Demets et al. 2005; de la Torre et al. 2010; Raggio et al. 2011), the STONE experiment (Cockell et al. 2007; Foucher et al. 2010), on the EXPOSE-E (Rabbow et al. 2012; Onofri et al. 2012, 2015), the EXPOSE-R mission (Rabbow et al. 2015), the recent EXPOSE-R2 mission (de Vera et al. 2012), and the Exposure Facility (EF) of the Japanese Experiment Module (JEM; Kawaguchi et al. 2016) on the ISS.

5.6 Analysis Strategy and Biosignature Database Concept

A necessary first investigative step is the thorough study of planetary analogue field sites. Here, information regarding potential biomolecules, which could serve as characterised biosignatures, can be obtained. Due to the fact that Mars and the icy satellites are classified as desert icy worlds, a selection of specifically cryophilic, piezophilic, halophilic, radiation-, desiccation and starvation-resistant microorganisms is needed. Through fieldwork undertaken in caves, in deserts, in the deep sea or in polar planetary analogue field sites, the collection of samples with planetary relevance, as well as testing of the instrumentation for their detection, can be performed (Fig. 5.1). This planetary analogue fieldwork also includes searching for remnants of life forms (fossils) and for biogenic molecules (biomarkers). The second step of the investigation aims to test the robustness of the collected organisms, fossils and biomolecules and to study life detection methods under simulated planetary conditions in specific planetary simulation laboratories. This experimental work will give insights on life processes, survival, resistance and stability of the samples, as well as on result quality, possible pitfalls and artefacts, which might

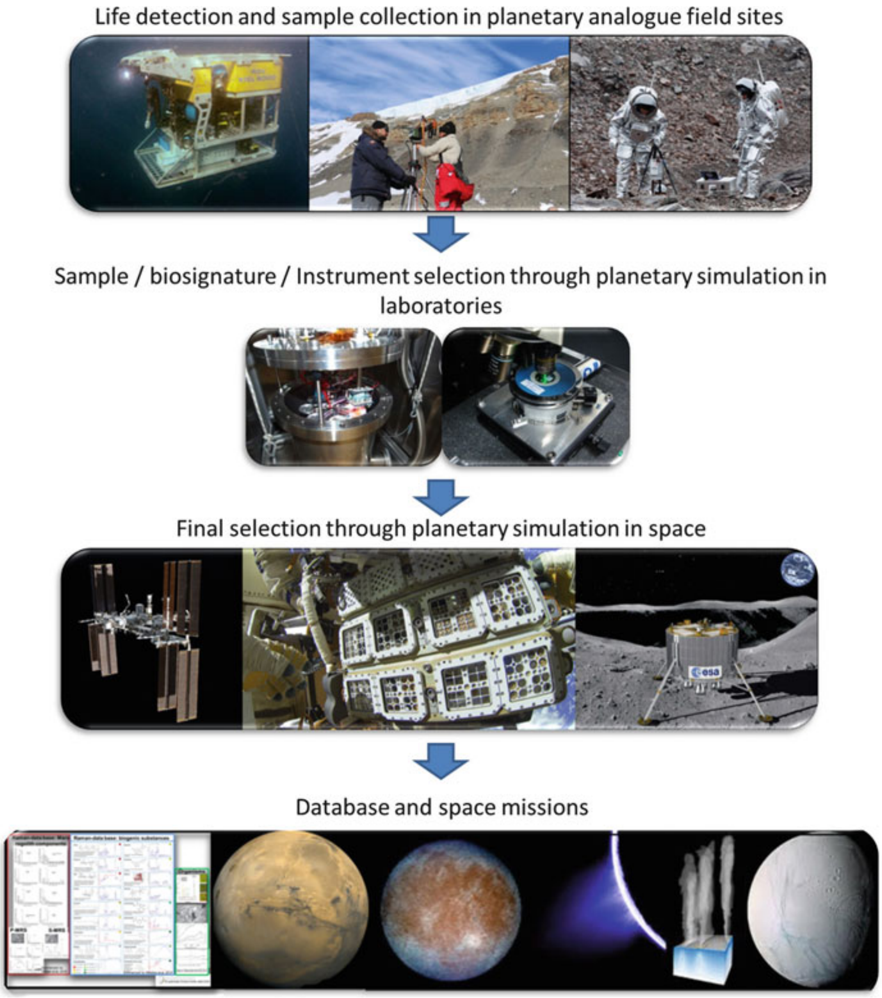

Fig. 5.1 Workflow of the biosignature analysis strategy, showing the sequence of analyses from planetary analogue field sites to space missions with a principal focus to detect life on Mars, Europa or Enceladus. Source: Adapted from DLR, GEOMAR (ROBEX), NASA, ESA, Roscosmos

occur during the measurements and detection operations. After successful selection of promising detectable and stable samples, as well as selection of instruments capable of reliable life detection, the third step is to conduct further work directly in space. This may use existing or future space exposure platforms in LEO, on space stations orbiting the Moon, or directly on Lunar landers at the surface of the Moon (Fig. 5.1). Although these space experiments are costly and complex, they are indispensable since their outcome cannot be identically imitated in any planetary simulation laboratory on Earth. Measurements performed in space can provide insights on the effects of combined space conditions, e.g. of Mars and the icy moons, on life detection instruments and potential life forms, remnants of life or biomolecules. Further valuable results can be obtained if the bio-relevant samples are embedded in planetary analogue minerals, salts or ice and exposed to atmospheric conditions of the planet or moon around which a future exploration project is focused.

Finally, the results which have relevance to Mars and the icy ocean worlds need to be systematically implemented into a mission taking into account all protocols of environmental conditions, measurement regimes, obtained spectra, diagrams, tables and pictures (Fig. 5.2). This will ensure a systematic investigation and support for future space missions whose main goal is to search for life in the Solar System.

The systematic organization of data, which is needed to create a well-developed and comprehensive biosignature database relevant for different life detection missions, can broadly be described in the following (see also Fig. 5.2):

(i) the type of mission has to be clear. While in principle a true life detection mission has to include close-up observations and analyses, and not only remote sensing, at least for the immediate future, there are three different categories of targets suitable for life detection missions, which include:

   1. exoplanets (remote sensing, e.g. planets orbiting dwarf stars),
   2. terrestrial planets (like Mercury, Venus, Earth, Mars; remote sensing and *in situ),*
   3. icy moons and dwarf planets (like Europa, Enceladus, Titan, Ceres, Pluto, remote sensing and *in situ*);

(ii) the detection method has to be chosen for the three categories (e.g. spectroscopy: fluorescence, IR-, UV/VIS, Raman, gas chromatograph-mass spectrometer (e.g., MOMA on ExoMars2020) or LD-MS etc.; microscopy: raster electron microscopy (REM), confocal laser scanning microscopy (CLSM); antibody detection etc.) including the maximum possible detail about instrumental operation and the lower limits of detection, controls, and calibration routines;

(iii) the planetary environmental conditions, which could have affected the tested samples and detection instruments during measurements, and therefore their influence on the resulting signatures of life, must be listed. Then, the obtained data have to be arranged according to the tested environmental parameters (environment can be specified by planetary simulation experiments from ground facilities and in space approaching the conditions of the planets/moons of interest).

This well-developed database could be used for the interpretation of observations in future space missions to the planets and moons in the Solar System and beyond.

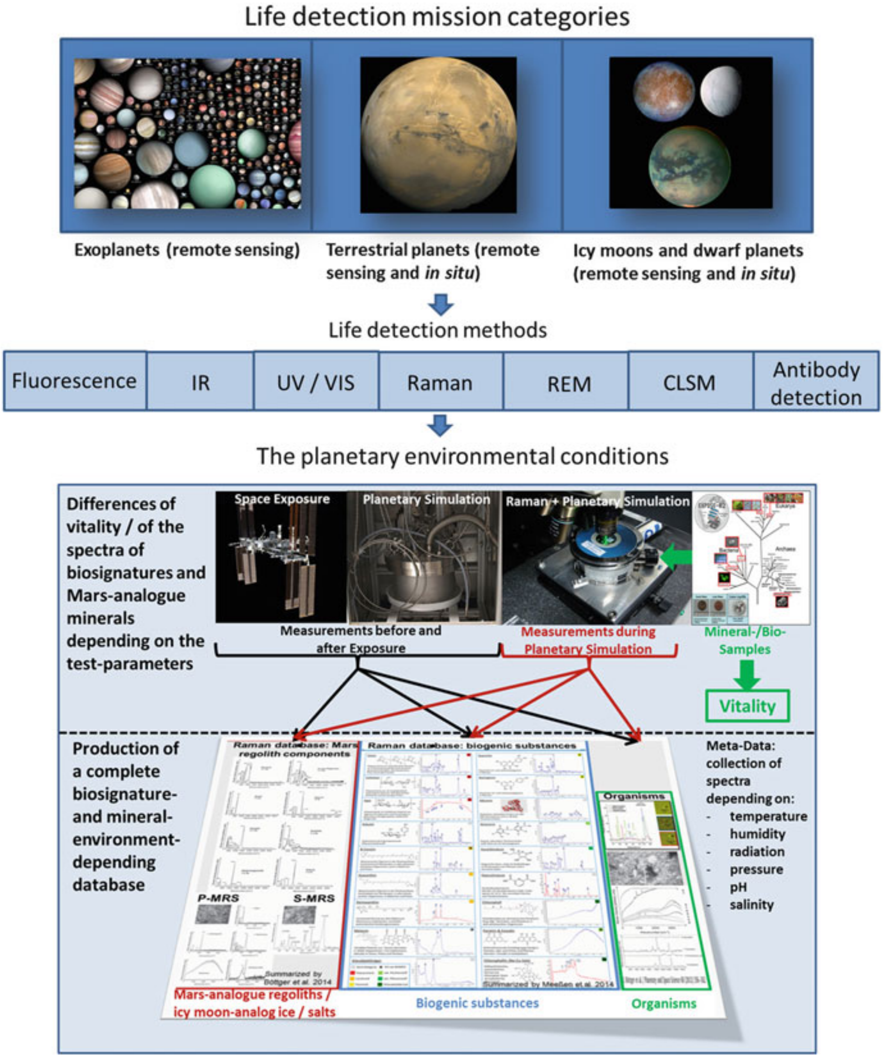

Fig. 5.2 Concept for the organisation of a biosignature database considering the methods planned to be used in future space exploration missions with the goal of finding life in space. The data will take into account all metadata of environmental conditions before, during and after the planetary simulation experiments. Source: Adapted from DLR, NASA, and ESA

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