Our exploration of the martian surface has provided compelling evidence that Mars was habitable in the past and may still possess habitable environments today (Lasue et al. 2019, Carr 2012). But whether life ever existed in those environments is still a huge unanswered question. Two of the next generation of Mars exploration rovers, NASA’s Perseverance and ESA’s Rosalind Franklin, have scientific goals that include searching for evidence of life (see box “Perseverance and Rosalind Franklin” and, for a review of these missions, see Vago et al. 2017, Williford et al. 2018).

Critical to the identification of biosignatures at the two landing sites will be an understanding of what biosignatures may exist at these locations, processes that may influence their preservation, and activities that may lead to modification. An understanding of sample preparation and instrument limitations is also required for interpretation of data returned from these missions.

To discuss these issues, martian geologists and astrobiologists from the UK, Europe, North America, India and Japan met virtually at the RAS on 9 October 2020, to present and discuss work exploring the formation and identification concepts of biosignatures, including the exploration of martian habitable environments and terrestrial analogue environments. This Specialist Discussion Meeting, “Biosignature identification in habitable martian environments”, was divided into three sessions and consisted of a mixture of oral presentations and flash talks. The latter was an attempt to provide a virtual equivalent to a poster session.

Searching for biosignatures
The first keynote talk of the day was given by Frances Westall (CNRS-Centre de Biophysique Moléculaire, France), who explained that it is unlikely the entire planet was permanently habitable, which might mean that martian life would have been very primitive and thus difficult to detect (Westall et al. 2015). Paradoxically, any increase in the complexity of biosignatures, such as more evolved organic molecules and physical structures, would also decrease the likelihood of them being detected. In addition, we also need to be aware of the possibility of biosignatures from life that is not like that found on Earth. Therefore, it might be more useful to identify “generic” indicators for life, such as prebiotic organics.

Jorge Vago (European Space Agency, ESTEC, Netherlands) described how the search for evidence of life on Mars was one of the integral objectives in the development of the ExoMars mission and determining the landing site for the Rosalind Franklin rover. The requirements for life to form and locations where such requirements would be found – low-temperature subaqueous hydrothermal systems – played a role in determining the location of the landing site (Vago et al. 2017). Vago also explained that the depth of the rover’s drills was determined to give access to suitable quantities of the amino acids that may have survived after billions of years of exposure to ionizing radiation (e.g. Dartnell et al. 2013).

The determination of whether potential biosignatures could be created solely by biological processes will be vital in the identification of martian biosignatures. Therefore, studies exploring abiotic process that lead to the formation of pseudo-biosignatures also need to be conducted.

Sean McMahon (University of Edinburgh) illustrated how abiotic chemical reactions within Oxia Planum and Jezero crater may have resulted in the formation of pseudo-biosignatures, and highlighted the need for abiotic
Armando Azua-Bustos (Centro de Astrobiología, Spain, and Instituto de Ciencias Biomédicas, Chile) presented surprising findings of a sub-surface clay-rich layer sampled from the Atacama Desert (Azua-Bustos et al. 2020). This layer was sampled from beneath a layer composed of halite, gypsum, albite and quartz, and was able to maintain its moisture throughout the year, which had not been observed in the region previously. A variety of halotolerant bacteria and archaea was found to inhabit the clay-rich layer. Azua-Bustos and colleagues identified organic signatures commonly associated with sulphate-reducing bacteria and cyanobacteria, which was unforeseen. If we can still find such surprises here on Earth, what awaits on Mars—and will we understand it?

The first session of flash talks started with a presentation from Michael Macey (Open University), who has been using simulations and computer modelling to explore the habitability and biosignature formation within three distinct martian groundwater analogues. Observations from habitability experiments showed a variation in growth rates between the various groundwater compositions, but all environments were dominated by the same genera of bacteria. Macey also explained that thermochemical modelling revealed that over geological time it would not be possible to discriminate confidently between biotic and abiotic fluids from depletions in trace elements alone.

Adam Stevens (University of Edinburgh) presented results from simulations that aimed to identify biosignatures and their detectability in martian environments. This work showed the simulated environments resulted in a low biomass, but were able to support a variety of microorganisms. When analysing samples using rover-specific techniques, such as mass spectroscopy and Raman spectroscopy, there was a disparity between findings when using bulk analysis techniques and those at small scales. In particular, the spatial component of analysis techniques was found to be useful in the identification of possible biotic structures, but such data were lost in bulk analysis, illustrating the need for data at multiple scales.

The final flash talk for session one was given by Sidhant Sharma (Blue Marble Space Institute of Science, USA), who described a computer modelling technique that could be used to identify potential chemical reaction networks that could form prebiotic organic molecules. Frédéric Foucher (CNRS-Centre de Biophysique Moléculaire, France) next highlighted the value of using Raman mapping as a tool for biosignature analysis, and the use of this technique to identify reduced carbon and biominerals, both of which could be used as signs of life.

The Perseverance and Rosalind Franklin rovers

Landing sites and instruments

Both of these rovers will be equipped with instruments to help identify potential signs of life. Perseverance will explore a sediment fan at Jezero crater (figure 1; Farley et al. 2020), using a Raman and luminescence spectrometer (SHERLOC) and Raman and fluorescence spectrometer (Super-Cam). It will also collect and cache samples to be returned to Earth for analysis at a later date (Moeller et al. 2020).

Rosalind Franklin (to be launched in September 2022) will investigate deposits at Oxia Planum (figure 2; see Quantin-Nataf et al. 2021). Instruments include an ion-trap mass spectrometer (MOMA), infrared spectrometer (ISEM) and Raman laser spectrometer (RLS). In addition, the rover will carry a drill capable of drilling to a depth of 2m beneath the surface and obtaining samples for analysis.
The preservation of biosignatures also needs to be considered when exploring the type of life that might exist on Mars. Mark Sephton (Imperial College London) discussed how inorganic material can influence the preservation and analysis of organic material. Using organic matter maturity techniques on martian analogue sediment samples, Sephton and colleagues showed soluble organic biosignatures within sediments would degrade in martian environments within a thousand years, but there are some techniques that could be used to find insoluble organics. Sephton also explained that analysis techniques such as thermal extraction can lead to problems in detected organic material mixed with minerals, such as perchlorates and sulphates. However, the selection of suitable samples for analysis could help minimize these effects and analysis of gases released from samples could be used as a biosignature.

**Biosignatures in context**

The first of the two afternoon sessions started with Adam Parkes-Bowen (University of Leicester), who discussed how CaSSIS (Colour and Stereo Surface Imaging System on the ExoMars Orbiter) imagery data, taken in Mars orbit, were used to identify two subunits of the Fe/Mg clay units at Oxia Planum. These units could be separated into clay rich in either ferrous or ferric iron. Parkes-Bowen showed that CaSSIS imagery could be used to produce high-resolution maps of the clay units at Oxia Planum, which can be used to help with rover investigations.

Aíne O’Brien (University of Glasgow) described a testing protocol for the detection of organic material found within igneous material using liquid chromatography mass spectrometry. Results from the analysis of martian meteorites showed the presence of the sulphur-bearing organic molecule pentanesulphonate, which is similar to a type of organic molecule found on Mars by Curiosity. These protocols could be used to examine samples collected and cached by the Perseverance rover and then later returned to Earth.

Melissa McHugh (University of Leicester) reported on use of the RLS simulator, which will be on-board Rosalind Franklin, to examine thermal maturity of reduced carbon found in the Nakhl meteorite analogue. Analysis of the samples showed they were rich in reduced carbon, which would have been a result of thermal alteration. McHugh also showed that it was possible to determine if a sample had experienced thermal stresses generated from impacts. The results from these experiments will help to optimize the operation of the RLS and assist with the interpretation of samples analysed on Mars. This emphasizes the need for careful laboratory studies before deploying instruments in the field.

The second group of flash talks started with Stuart Turner (Open University) explaining how CRISM data has been used to examine the mineralogy at Oxia Planum. CRISM is a spectrometer on Mars Express that has mapped Mars extensively from orbit. The data provide an insight into the deposition history of the clay units at the Rosalind Franklin landing site (Quintin-Nafat et al. 2021), again showing the value of data from a different scale.

In the search for atmospheric biosignatures, George Cann (University College London) has been using NOMAD data from the ExoMars Trace Gas orbiter, and an Ares atmospheric retrieval programme to help understand the origin of martian methane. The question of atmospheric detections of methane on Mars is complicated, and the analysis focused on conditions over Jezero crater and Oxia Planum.

Allison Fox (Pennsylvania State University, USA) highlighted an issue with recognizing biosignatures using isotopic signatures from organic material, namely how the organic material interacts with its environment. In particular, she focused on sorption to mineral surfaces. This process plays a key role in protecting organics from oxidation, for example, but may change other properties. Position-specific isotope analysis was used to show the sorption process between organics and ices would result in some biologically generated isotopic signals being obscured. Again, understanding the process via laboratory experiment is vital, before the presence or absence of a signal can be understood.

**Instruments and exploration**

The final session of the day began with a presentation by Hannah Lerman (University of Leicester), who used a range of carbon-rich analogue samples to refine the acquisition parameters, and identify sensitivity limits of, organic carbon in 532 nm Raman spectrometers similar to the RLS on-board Rosalind Franklin. Investigations showed the instruments were able to identify trace abundances of carbon and confidently identified specific organic biomarkers within mineral substrates.

Moving away from instruments, Christian Schröder (University of Stirling) explained that poorly crystalline X-ray amorphous iron-rich deposits could preserve organic material, and such deposits have been identified on Mars. He suggested that in the search for martian biosignatures, amorphous iron-rich deposits should be targeted by Rosalind Franklin and Perseverance.

Daniel Glavin (NASA Goddard Space Flight Center, USA) discussed how chiral asymmetry can be used as a biosignature if light isotopes and a simple distribution of organic compounds are also observed. Although amino acids have not yet been found in situ on Mars, the decay of a chiral signature would be slow and signals could last a billion years or more. The importance of measuring chirality was thus emphasized. Glavin presented data on a range of abiotic and biotic samples to demonstrate how the criteria for organic biosignatures could be used (figure 3; e.g. Glavin et al. 2020). While a sample-return mission is the ultimate goal, the MOMA instrument on Rosalind Franklin will be able to detect the chirality of any

![Venn diagram describing the three criteria needed to determine if amino acids and sugars are likely to be of biotic origin. (From Glavin et al. 2020)](image)

“Understanding the process via lab experiment is vital, before the presence or absence of a signal can be understood.”
that could potentially be found in the martian subsurface/ deep biosphere.

Ian Hutchinson (University of Leicester) then gave an overview of the capabilities of the RLS (Moral et al. 2020). He highlighted that there are prototypes of the Raman spectrometer and sample-preparation systems in the UK and Spain available for scientists to characterize samples from analogue sites and simulation experiments. Hutchinson also described work on analysing samples of desert varnish. Again, building up a picture in the laboratory of what data from a particular instrument will look like for different types of data was shown to be of vital importance.

Roger Stabbins (Mullard Space Science Laboratory, UCL) has been working on a scene simulator, which provides images like those that will be obtained by the PanCam instrument on-board Rosalind Franklin (figure 4). Crucially, this simulator allows for the user to determine the spatial distribution of minerals in the simulated environment, which would be a useful tool in the identification of potential geochemical biosignatures on Mars.

The final flash talk described a new instrument that has been developed and could be used by future missions to help search for life in the solar system. Miguel Ángel Fernández-Martínez (McGill University, Canada) discussed the capabilities of the MICRO-life detection platform, an instrument comprising a range of tools that can be used to identify biological molecules, such as DNA and RNA, culture potential microbial communities, and redox indicators that help identify microbial metabolisms.

The meeting concluded with a final keynote given by Rogers Weins (Los Alamos National Laboratory, USA), who discussed the instruments on Perseverance that will be used to search for organic molecules at Jezero crater (Farley et al. 2020).

Overall, several key messages emerged from the meeting. Perhaps the most important was that there is unlikely to be a eureka moment. Data will be obtained in a variety of ways, searching for different evidences of processes that may or may not have occurred. A decision-tree-like approach will likely then have to be followed to evaluate the probability of the presence of life at some stage on Mars. Running in parallel will be the equally difficult task of ruling out life definitively: how much of the absence of evidence is required, and to what degree, before that itself becomes evidence? A second key result is that of combining data from not just different instruments, but also from different size scales to produce a context for any individual result. Finally, the need for extensive laboratory studies to understand the processes involved and the response of various instruments to a wide range of possible data types was continually emphasized.

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MORE INFORMATION

Links to meeting programme and abstract booklet bit.ly/3Trd5n