further invigorated the hypothesis for life on that planet. Thus, reliable analytical methods to unequivocally and directly establish the presence of life on a planetary body are of major interest for future space missions.

Interest in laser desorption/ionization mass spectrometers has increased in recent years because of their ability to probe complex, nonvolatile compounds directly on solid surfaces and on a submillimeter spatial scale.1 These instruments can detect high-molecular-weight organics in a wide variety of samples. In particular, matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) has become an established tool for rapid characterization of intact microorganisms.2 Here, we evaluate the applicability of MALDI time-of-flight (TOF) MS to detect live organisms that might mimic life on Mars (Fig. 1).

We also are developing methods to label microorganisms by growing them in isotopically labeled medium. Methanococcus jannaschii, a methanotrophic extremophile that thrives in deep-sea volcanoes at temperatures of ~110°C, is one of the first microorganisms whose genome has been sequenced. M. jannaschii may be a good first approximation for an anaerobic methane-generating organism that may exist on Mars.

Figure 1. A compact RP-TOF mass spectrometer with sampling capability can be deployed as a contact-type instrument from a Mars rover.

More than 90% of the methane generated on Earth has a biogenic origin. Therefore, the discovery of discrete spatial and temporal methane plume releases on Mars has
Both positive- and negative-ion MALDI mass spectra from intact *M. jannaschii* have been obtained for the first time. We used home-built TOF and commercial TOF and tandem TOF instruments (Fig. 2) to acquire biomarker signatures and identify individual biomarkers. Characteristic and reproducible biomarker signatures are observed in MALDI spectra for all intact microorganisms grown in the different media (Fig. 3). The signatures for the same organism obtained on different TOF instruments can be directly compared. Tandem MS allows the respective protein and lipid biomarkers observed in each organism’s signature to be identified and verified.

To establish microorganism viability, organisms are rapidly grown in isotopically enriched medium. Rapid in situ washes and subsequent MALDI MS allow us to monitor growth based on the mass shifts for specific biomarkers in the organism’s mass spectral signature. These shifts can be predicted from the biomarker elemental composition and the isotope composition of the medium. We also demonstrate the usefulness of this method to amplify organisms at low concentration.

In conclusion, miniature reverse polarity (RP)-TOF instruments are under development for identification of biosignatures of collected samples in landed planetary missions. MALDI mass spectra from *M. jannaschii* enable biosignatures to be determined for these important exobiological organisms. Microorganism growth in isotopically enriched growth media (e.g., $^{13}$C$_{16}$O$_2$, $^{12}$C$_{18}$O$_2$ for a methane-producing extremophile) allows determination of the number of C and O atoms for an unknown biomolecule (i.e., type of biopolymer). We anticipate that this technology will be applicable in future landed exploration missions to Mars.