Low Upper Limit to Methane Abundance on Mars

Christopher R. Webster *et al.*

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Christopher R. Webster,[1]* Paul R. Mahaffy,[2] Sushil K. Atreya,[3] Gregory J. Flesch,[1] Kenneth A. Farley,[4] MSL Science Team†

By analogy with Earth, methane in the Martian atmosphere is a potential signature of ongoing or past biological activity. During the past decade, Earth-based telescopic observations reported "plumes" of methane of tens of parts per billion by volume (ppbv), and those from Mars orbit showed localized patches, prompting speculation of sources from subsurface bacteria or nonbiological sources. From in situ measurements made with the Tunable Laser Spectrometer (TLS) on Curiosity using a distinctive spectral pattern specific to methane, we report no detection of atmospheric methane with a measured value of 0.18 ± 0.67 ppbv corresponding to an upper limit of only 1.3 ppbv (95% confidence level), which reduces the probability of current methanogenic microbial activity on Mars and limits the recent contribution from extraplanetary and geologic sources.

Methane is the most abundant hydrocarbon in our solar system and is found in the atmospheres of several planets and satellites (*1*). On Earth, 90 to 95% of atmospheric methane is biologically produced, either from extant or fossil sources, and it is easy to identify and quantify with confidence by using spectroscopic methods (*2*). For Mars, three possible origins have been proposed: geologic, biotic, and exogenous (*3*–*5*). Over the past decade, there have been several reports of methane detection from Earth and from Mars orbit. Observations with the Canada-France-Hawaii Telescope (CFHT) found a global average value of 10 ± 3 parts per billion by volume (ppbv) (*5*). The Planetary Fourier Spectrometer (PFS) on the Mars Express (MEX) spacecraft found a global average abundance of 10 ± 5 ppbv (*4*), later updated (*6*) to 15 ppbv, with indications of discrete localized sources (*4*) and a summer time maximum of 45 ppbv in the north polar region. A search for methane from the Infrared Telescope Facility (IRTF) and the Keck-2 telescope reported methane release in plumes (*7*) from discrete sources in Terra Sabae, Nili Fossae, and Syrtis Major, with the largest plume containing 19,000 tons of CH_4 in March 2003; seasonal changes with a summer time maximum of ~45 ppbv near the equator were seen. Methane abundances later retrieved (*8*) from a second instrument in Mars orbit, the Thermal Emission Spectrometer (TES) of the Mars Global Surveyor (MGS), reported methane abundances as intermittently present (1999 to 2003), ranging from 5 to 60 ppbv in locations where favorable geological conditions such as residual geothermal activity (Tharsis and Elysium) and strong hydration (Arabia Terrae) are expected. More recent observations report methane mixing ratios that have diminished considerably since 2004 to 2006 to upper limits of 7 to 8 ppbv (*9*–*11*), suggesting a very short lifetime for atmospheric CH_4 and contradicting the MEX claim that methane persisted from 2004 to 2010. Ground-based observations favor episodic injection of methane in 1999 and 2003, 10 ppbv at Valles Marineris in February 2006 (*9*, *11*), and <8 ppbv in January 2006 (*10*), 2009, and 2010; whereas orbital data from PFS and TES suggest a more regular behavior with latitudinal, seasonal, and interannual variabilities. At Curiosity's Gale Crater landing site (4.5°S, 137°E), published maps of PFS data (*6*) show an increase from ~15 ppbv in fall to ~30 ppbv in winter, whereas the TES trend (*8*) is opposite: ~30 ppbv in fall and ~5 ppbv in winter.

The Tunable Laser Spectrometer (TLS) of the Sample Analysis at Mars (SAM) (*12*, *13*) instrument suite on the Curiosity rover has a spectral resolution—0.0002 cm^{-1}, which is far superior to those of the ground-based telescopic and orbiting spectrometers—that offers unambiguous identification of methane in a distinct fingerprint spectral pattern of three well-resolved adjacent $^{12}CH_4$ lines in the 3.3 μm band (Fig. 1). The in situ technique of tunable laser absorption in a closed sample cell is simple, noninvasive, and sensitive. TLS is a two-channel tunable laser spectrometer that uses both direct and second harmonic detection of infrared (IR) laser light. One laser source is a near-IR tunable diode laser at 2.78 μm that can scan two spectral regions containing CO_2 and H_2O isotopic lines that have been used to report $^{13}C/^{12}C$, $^{18}O/^{17}O/^{16}O$, and D/H ratios in the Martian atmosphere (*13*). The second laser source is an interband cascade (IC) laser at 3.27 μm used for methane detection alone, scanning across seven rotational lines that includes the R(3) triplet used in this study (Fig. 1 and table S1). The IC laser beam makes 81 passes of a 20-cm-long sample cell of the Herriott design fitted with high-vacuum microvalves that allow evacuation with a turbomolecular pump for "empty cell" scans or filled to Mars ambient pressure (~8 mbar) for "full cell" runs. During data collection, the cell and other optics are kept at 47 ± 3°C by using a heater that thermally stabilizes the cell but is ramped up and down within these temperature limits in order to increase gas sensitivity by spoiling the accumulation of optical interference fringes during the 2-min period of spectrum collection. Our methane determination is made by comparing the measured methane abundances in our sample cell when filled with Mars atmosphere with those of the same cell evacuated, as detailed in (*14*). The laser scans every second through the methane spectral region, and each spectrum is co-added on board to downlink sequential 2-min-averaged spectra during a given run of ~1 to 2 hours in duration. Typically, we recorded 26 2-min "empty cell" spectra followed by 26 2-min "full cell" spectra, then finally five additional 2-min empty cell spectra. For each 2-min spectrum, we retrieved methane abundances from three spectral lines (*14*) individually and combined the results so as to produce a weighted average value. By subtracting all retrieved abundances (full and empty cell) from the empty cell mean value for that sol (one Martian solar day) run, we were left with 31 differences for the empty cell and 26 for the full cell. For our statistical analysis, we analyzed the empty cell and full cell differences for all the sols taken as one data set (*14*). For sols 79, 81, 106, and 292, the foreoptics chamber contained residual terrestrial air (Table 1, pressures), including CH_4, that produced absorption line signals in the sample cell detector channel, as described in (*14*). For sols 306 and 313, the foreoptics was evacuated. Both the sample cell and foreoptics chamber have pressure and temperature sensors. This experiment has been repeated on six separate Martian sols to date (Martian sols 79, 81, 106, 292, 306, and 313 after landing in August 2012). The inlet to the TLS is a stainless steel tube (*14*) heated to 50°C and located on the rover side ~1 m above the Martian surface and was pointed at a variety of directions relative to the nominal wind direction. Mars atmospheric gas was ingested during the night for sols 79, 81, 106, 292, and 313 and during the day for sol 306 (Table 1). Our measurements correspond to southern spring (sols 79, 81, and 106) and mid-late summer (sols 292, 306, and 313) on Mars.

To date, we have no detection of methane. Individually (Table 1), each of our six data sets produces a mean methane value ranging from –2.2 to 1.7 ppbv. Combining the individual sol results with equal weighting yields a mean and SE of 0.11 ± 0.67 ppbv. Alternatively, combining all of the individual measurements from all sols yields a grand mean and SE of 0.18 ± 0.67 ppbv. At the 95% confidence level, either approach (*14*) yields an upper limit on Mars atmospheric methane of 1.3 ppbv. Curiosity's low upper limit is not expected given observations only a few years ago of large methane plumes and calculations (*7*) that the plume dispersion should produce global values of ~6 ppbv after the 6-month period (*3*, *15*) needed to mix uniformly across the planet, which would persist with a photochemical lifetime of several hundred years (*3*, *5*, *16*).

Before Curiosity's landing on Mars in August 2012, observational evidence for methane on

[1]Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109, USA. [2]NASA Goddard Space Flight Center, Greenbelt, MD 20771, USA. [3]Department of Atmospheric, Oceanic, and Space Sciences, University of Michigan, Ann Arbor, MI 48109, USA. [4]Department of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125, USA.

*Corresponding author. E-mail: chris.r.webster@jpl.nasa.gov
†MSL Science Team authors and affiliations are listed in the supplementary materials.

Mars was questioned in the published literature (*15*, *17*, *18*). Contradictions were noted between the locations of maxima reported from ground-based observations and maps inferred by PFS and TES from Mars orbit. The plume results (*7*) were questioned (*17*) on the basis of a possible misinterpretation from methane lines whose positions coincided with those of terrestrial isotopic $^{13}CH_4$ lines. Krasnopolsky (*19*) argued that cometary and volcanic contributions were not sufficient to explain high methane abundances, calculating a cometary contribution of only ~0.1 ppbv and noting the lack of current volcanism, lack of hot spots in thermal imaging (*20*), and the extremely low upper limit for Mars SO_2 (*9*, *21*) that in Earth's volcanic emissions is orders of magnitude more abundant than CH_4 (*5*).

The very short methane lifetime of 0.4 to 4 years derived from the 2003–2006 observations (*7*) requires powerful destruction mechanisms that have not been identified to date. Although models have been proposed for rapid removal of methane by oxidants—such as hydrogen peroxide and perchlorates, or by superoxides derived from their mineral reactions (*22*–*24*) and directly by electric fields generated in dust devils (*25*)—there remains no evidence for their existence at Mars. Moreover, it has not been demonstrated that any of these processes can reduce the lifetime of methane by the required factor of 100 or more compared with its photochemical lifetime. Our reported upper limit of 1.3 ppbv is substantially lower than the methane abundances reported from Mars remote sensing spacecraft observations and those from Earth telescopic observations, including both the earlier high values of typically tens of ppbv and the more recently reported upper limits of 7 to 8 ppbv (*9*, *10*). Although TLS samples only the very lowest part (~1 m) of the Mars atmosphere as compared with those of the other observations that are vertical column-integrated results, the atmospheric scale height (~10 km) and mixing

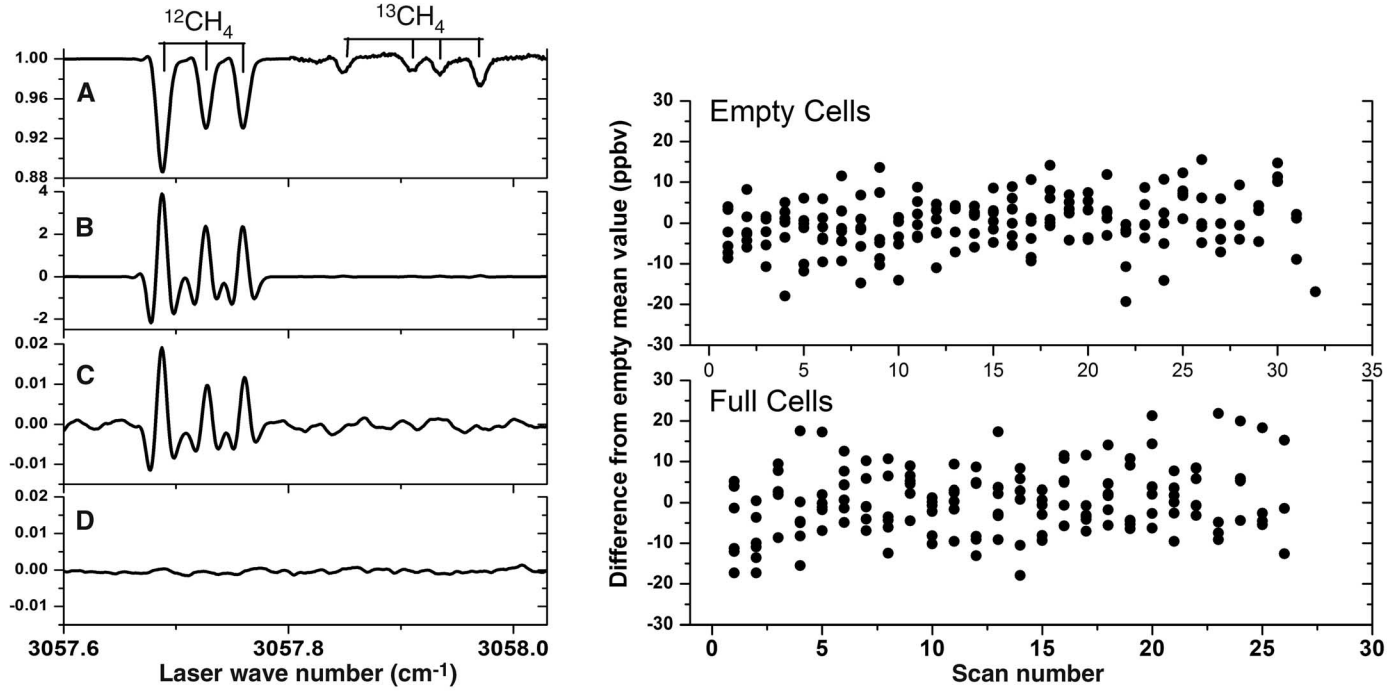

Fig. 1. The TLS-SAM methane measurements. (**Left**) Examples of flight spectra downloaded from Curiosity. (**A**) Spectrum recorded during an unrelated Evolved Gas Analysis (EGA) run (*14*) showing location of $^{12}CH_4$ and $^{13}CH_4$ lines, in which the second half has been vertically expanded by ×20 to show the weaker $^{13}CH_4$ lines. (**B**) Same as (A) but second harmonic (2f) spectrum (*14*), without vertical expansion. (**C**) Averaged full cell 2f spectrum for sol 106 (nighttime ingest), with foreoptics contribution (*14*). (**D**) Averaged full cell 2f spectrum for sol 306 (daytime ingest), with foreoptics evacuated. [Spectra (A) and (B) are shown here in part because they were taken after the atmospheric runs and show that our CH_4 lines have not moved and that the instrument continued to work well with consistent capability to detect methane.] (**Right**) Individual 2-min data points from 6 sols. (Top) Empty cell data with mean value of 0.0 ppbv. (Bottom) Full cell data with mean value of 0.18 ppbv.

Table 1. Curiosity SAM-TLS methane measurements at Gale Crater (4.5°S, 137.4°E) over an 8-month period. SEM, standard error from the mean; Ls, solar longitude.

Martian sol after landing on 6 August 2012	Earth date	Ls (degrees)	Gas ingest time/cell pressure (mbar)/foreoptics pressure (mbar)	Mean value ± 1 SEM (ppbv)
79	25 October 2012	195.0	Night/8.0/11.5	1.62 ± 2.03
81	27 October 2012	196.2	Night/8.0/11.5	1.71 ± 2.06
106	27 November 2012	214.9	Night/8.5/10.9	–0.55 ± 1.45
292	1 June 2013	328.6	Night/8.7/9.2	0.60 ± 1.74
306	16 June 2013	336.5	Day/8.1/0.0	–2.21 ± 0.94
313	23 June 2013	340.5	Night/8.7/0.0	–0.50 ± 0.94
Mean of individual sol results	0.11 ± 0.56			
Mean for entire aggregated data set	0.18 ± 0.67			

time (approximately a few months) suggests that our measured upper limit is representative of the global mean background level. With an expected photochemical lifetime of methane in the Martian atmosphere of hundreds of years (*3*, *5*, *16*), there currently remains no accepted explanation (*15*, *17*) for the existence and distribution of the reported plumes nor of the apparent disappearance of methane over the past few years. Our result sets an upper limit that is ~6 times lower than other recent measurements and greatly reduces the probability of substantial methanogenic microbial activity on Mars and recent methane production through serpentinization or from exogenous sources, including meteoritic, interplanetary dust and cometary infall.

References and Notes

1. S. K. Atreya, P. R. Mahaffy, H. B. Niemann, M. H. Wong, T. C. Owen, *Planet. Space Sci.* **51**, 105–112 (2003).
2. H. A. Michelsen *et al.*, *Geophys. Res. Lett.* **26**, 291–294 (1999).
3. V. A. Krasnopolsky, J. P. Maillard, T. C. Owen, *Icarus* **172**, 537–547 (2004).
4. V. Formisano, S. K. Atreya, T. Encrenaz, N. Ignatiev, M. Giuranna, *Science* **306**, 1758–1761 (2004).
5. S. K. Atreya, P. R. Mahaffy, A. S. Wong, *Planet. Space Sci.* **55**, 358–369 (2007).
6. A. Geminale, V. Formisano, G. Sindoni, *Planet. Space Sci.* **59**, 137–148 (2011).
7. M. J. Mumma *et al.*, *Science* **323**, 1041–1045 (2009).
8. S. Fonti, G. A. Marzo, *Astron. Astrophys.* **512**, A51 (2010).
9. V. A. Krasnopolsky, *Icarus* **217**, 144–152 (2012).
10. G. L. Villanueva *et al.*, *Icarus* **223**, 11–27 (2013).
11. V. A. Krasnopolsky, *EPSC Abstracts* **6**, 49 (2011).
12. P. R. Mahaffy *et al.*, *Space Sci. Rev.* **170**, 401–478 (2012).
13. C. R. Webster *et al.*, MSL Science Team, *Science* **341**, 260–263 (2013).
14. Materials and methods are available as supplementary materials on *Science* Online.
15. F. Lefèvre, F. Forget, *Nature* **460**, 720–723 (2009).
16. A. S. Wong, S. K. Atreya, T. Encrenaz, *J. Geophys. Res.* **108**, (E4), 5026 (2003).
17. K. J. Zahnle, R. S. Freedman, D. C. Catling, *Icarus* **212**, 493–503 (2011).
18. R. A. Kerr, *Science* **338**, 733 (2012).
19. V. A. Krasnopolsky, *Icarus* **180**, 359–367 (2006).
20. P. R. Christensen, *EOS Trans. AGU Fall Meet. Suppl.* **84** (46), Abstract P21A-02, 2003.
21. T. Encrenaz *et al.*, *Astron. Astrophys.* **530**, 1–5 (2011).
22. S. K. Atreya *et al.*, *Planet. Space Sci.* **59**, 133–136 (2011).
23. S. K. Atreya *et al.*, *Astrobiology* **6**, 439–450 (2006).
24. G. T. Delory *et al.*, *Astrobiology* **6**, 451–462 (2006).
25. W. M. Farrell, G. T. Delory, S. K. Atreya, *J. Geophys. Res.* **33**, (2006).

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Supplementary Materials

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Genomically Recoded Organisms Expand Biological Functions

Marc J. Lajoie,[1,2] Alexis J. Rovner,[3,4] Daniel B. Goodman,[1,5] Hans-Rudolf Aerni,[4,6] Adrian D. Haimovich,[3,4] Gleb Kuznetsov,[1] Jaron A. Mercer,[7] Harris H. Wang,[8] Peter A. Carr,[9] Joshua A. Mosberg,[1,2] Nadin Rohland,[1] Peter G. Schultz,[10] Joseph M. Jacobson,[11,12] Jesse Rinehart,[4,6] George M. Church,[1,13]* Farren J. Isaacs[3,4]*

We describe the construction and characterization of a genomically recoded organism (GRO). We replaced all known UAG stop codons in *Escherichia coli* MG1655 with synonymous UAA codons, which permitted the deletion of release factor 1 and reassignment of UAG translation function. This GRO exhibited improved properties for incorporation of nonstandard amino acids that expand the chemical diversity of proteins in vivo. The GRO also exhibited increased resistance to T7 bacteriophage, demonstrating that new genetic codes could enable increased viral resistance.

The conservation of the genetic code permits organisms to share beneficial traits through horizontal gene transfer (*1*) and enables the accurate expression of heterologous genes in nonnative organisms (*2*). However, the common genetic code also allows viruses to hijack host translation machinery (*3*) and compromise cell viability. Additionally, genetically modified organisms (GMOs) can release functional DNA into the environment (*4*). Virus resistance (*5*) and biosafety (*6*) are among today's major unsolved problems in biotechnology, and no general strategy exists to create genetically isolated or virus-resistant organisms. Furthermore, biotechnology has been limited by the 20 amino acids of the canonical genetic code, which use all 64 possible triplet codons, limiting efforts to expand the chemical properties of proteins by means of nonstandard amino acids (NSAAs) (*7*, *8*).

Changing the genetic code could solve these challenges and reveal new principles that explain how genetic information is conserved, encoded, and exchanged (fig. S1). We propose that genomically recoded organisms (GROs, whose codons have been reassigned to create an alternate genetic code) would be genetically isolated from natural organisms and viruses, as horizontally transferred genes would be mistranslated, producing nonfunctional proteins. Furthermore, GROs could provide dedicated codons to improve the purity and yield of NSAA-containing proteins, enabling robust and sustained incorporation of more than 20 amino acids as part of the genetic code.

We constructed a GRO in which all instances of the UAG codon have been removed, permitting the deletion of release factor 1 (RF1; terminates translation at UAG and UAA) and, hence, eliminating translational termination at UAG codons. This GRO allows us to reintroduce UAG codons, along with orthogonal translation machinery [i.e., aminoacyl–tRNA synthetases (aaRSs) and tRNAs] (*7*, *9*), to permit efficient and site-specific incorporation of NSAAs into proteins (Fig. 1). That is, UAG has been transformed from a nonsense codon (terminates translation) to a sense codon (incorporates amino acid of choice), provided the appropriate translation machinery is present. We selected UAG as our first target for genome-wide codon reassignment because UAG is the rarest codon in *Escherichia coli* MG1655 (321 known instances), prior studies (*7*, *10*) demonstrated the feasibility of amino acid incorporation at UAG, and a rich collection of translation machinery capable of incorporating NSAAs has been developed for UAG (*7*).

We used an in vivo genome-editing approach (*11*), which is more efficient than de novo genome synthesis at exploring new genotypic landscapes and overcoming genome design flaws. Although a single lethal mutation can prevent transplantation of a synthetic genome (*12*), our approach allowed us to harness genetic diversity and evolution to overcome any potential deleterious mutations at a cost considerably less than de novo genome synthesis (supplementary text section B, "Time and cost"). In prior work, we used multiplex automated genome engineering [MAGE (*13*)] to remove all known UAG codons in groups of 10

[1]Department of Genetics, Harvard Medical School, Boston, MA 02115, USA. [2]Program in Chemical Biology, Harvard University, Cambridge, MA 02138, USA. [3]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520, USA. [4]Systems Biology Institute, Yale University, West Haven, CT 06516, USA. [5]Program in Medical Engineering and Medical Physics, Harvard–Massachusetts Institute of Technology (MIT) Division of Health Sciences and Technology, Cambridge, MA 02139, USA. [6]Department of Cellular and Molecular Physiology, Yale University, New Haven, CT 06520, USA. [7]Harvard College, Cambridge, MA 02138, USA. [8]Department of Systems Biology, Columbia University, College of Physicians and Surgeons, New York, NY 10032, USA. [9]MIT Lincoln Laboratory, Lexington, MA 02420, USA. [10]Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037, USA. [11]Center for Bits and Atoms, MIT, Cambridge, MA 02139, USA. [12]MIT Media Lab, MIT, Cambridge, MA 02139, USA. [13]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA.

*Corresponding author. E-mail: farren.isaacs@yale.edu (F.J.I.); gchurch@genetics.med.harvard.edu (G.M.C.)