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**Corresponding Author**
- **Family Name**: Igisu
- **Given Name**: Motoko
- **Division**: 1Department of Earth and Planetary Sciences
- **Organization**: Tokyo Institute of Technology
- **Address**: O-okayama 2-12-1, Meguro-ku, 152-8551, Tokyo, Japan

**Author**
- **Family Name**: Ueno
- **Given Name**: Yuichiro
- **Division**: 4Global Edge Institute
- **Organization**: Tokyo Institute of Technology
- **Address**: Post No. 12-21, Meguro-ku, 152-8551, Tokyo, Japan

**Author**
- **Family Name**: Nakashima
- **Given Name**: Satoru
- **Division**: 2Department of Earth and Space Science
- **Organization**: Osaka University
- **Address**: 1-1 Machikaneyama-cho, Toyonaka-shi, 560-0043, Osaka, Japan

**Author**
- **Family Name**: Awramik
- **Given Name**: Stanley M.
- **Division**: 5Department of Earth Science
- **Organization**: University of California
- **Address**: 93106, Santa Barbara, CA, USA
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<td>Tokyo Institute of Technology</td>
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Biodata of Dr. Motoko Igisu, Dr. Yuichiro Ueno, Dr. Satoru Nakashima, Professor Stanley M. Awramik, and Dr. Shigenori Maruyama, authors of “Micro-FTIR Spectroscopic Imaging of ~1,900 Ma Stromatolitic Chert”

**Dr. Motoko Igisu** is currently Post-doctoral researcher of the Department of Earth Science and Astronomy in the University of Tokyo, Japan since 2008. She obtained her PhD from Tokyo Institute of Technology in 2008 and continued her research at Tokyo Institute of Technology. Dr. Igisu’s scientific interests are in the area of geochemistry of prokaryotic fossils and evolution of life.

E-mail: igisu@ea.c.u-tokyo.ac.jp

**Dr. Yuichiro Ueno** is currently the Assistant Professor of the Global Edge Institute in the Tokyo Institute of Technology, Japan. He obtained his PhD from the Dept. EPS in the Tokyo Institute of Technology in 2002 and continued his studies and research at the University of Tokyo. Dr. Ueno’s scientific interests are in the areas of: Archean biosphere, geochemistry of prokaryotic fossils, stable isotope biogeochemistry, and atmospheric evolution.

E-mail: ueno.y.ac@m.titech.ac.jp
Dr. Satoru Nakashima is currently Professor at the Department of Earth and Space Science, Osaka University, Japan since 2005. He obtained his PhD (Doctorat d'Etat es Science) from the University of Orleans, France in 1984 on the kinetic and thermodynamic studies of uranium reduction by sedimentary organic matter. He then worked at Japan Atomic Energy Research Institute for the geological disposal of radioactive wastes. He continued his research at Akita University, the University of Tokyo, Hokkaido University, Tokyo Institute of Technology before arriving at Osaka. He has been developing IR, Visible and Raman micro-spectroscopic methods for characterizing water, inorganic and organic phases in earth and planetary materials. By using these spectroscopic methods, Professor Nakashima studies quantitatively organic–inorganic and water–rock interactions in diverse areas ranging from astrobiology, origin and evolution of life, volcanology, seismology, rheology to earth’s resource and environment.

E-mail: satoru@ess.sci.osaka-u.ac.jp

Dr. Stanley M. Awramik is Professor of Biogeology in the Department of Earth Science, University of California, Santa Barbara, California, USA. He obtained his PhD from Harvard University in 1973. Professor Awramik’s scientific interests are in the early history of life on Earth and his research focuses on microbial fossils and stromatolites.

E-mail: awramik@geol.ucsb.edu
Dr. Shigenori Maruyama is currently Professor of the Department of Earth and Planetary Sciences in Tokyo Institute of Technology, Japan since 1993. He obtained his PhD from Nagoya University in 1977 and continued his research at Toyama University, Stanford University, and the University of Tokyo. Professor Maruyama scientific interests are in the area of: system evolution of earth history, earth’s dynamics, and evolution of life.

E-mail: smaruyam@geo.titech.ac.jp
Abstract  Micro-Fourier transform infrared (FTIR) spectroscopic imaging analyses nondestructively revealed micrometer to millimeter-scale distributions of organic and inorganic functional groups in Proterozoic stromatolitic chert containing prokaryotic fossils from ~1,900 Ma Gunflint Formation. CH$_3$/CH$_2$ absorbance ratios ($R_{3/2}$) indicate a bacteria origin, but not Archaea, of most carbonaceous matter in the chert as well as in the microfossils themselves. However, the characterizations of the stromatolitic chert also show that $R_{3/2}$ value of carbonaceous matter existing with carbonates could be overestimated or underestimated. This technique is useful for searching and characterizing rapidly the organic matter in terrestrial and extraterrestrial samples at the micrometer to millimeter scale, and may provide useful information on the affinities of microfossils in the chert.

Keywords  FTIR • Imaging • Functional group • Prokaryotic fossils • Gunflint Formation • Bacteria • Archaea • Aliphatic CH moiety • Chert • Carbonate • Carbonaceous matter

1. Introduction

The Precambrian is primarily a microbial world. Microbial fossils show carbonaceous cellular structure, which often resemble cyanobacteria and other prokaryotes (e.g., Schopf, 1992; Knoll, 2003). But morphological analysis of these microbial fossils is often not enough to determine the biology of such fossils and to determine
their precise phylogenetic positions. In situ analytical techniques are now being applied to obtain chemical signatures of the micrometer-sized fossils. For example, micro-Raman spectroscopy is being used to demonstrate carbonaceous composition of putative organic-walled microfossils and to determine their degree of post-depositional alteration (Ueno et al., 2001, 2006; Kudryavtsev et al., 2001; Schopf et al., 2002, 2005; Brasier et al., 2002, 2004; Pasteris and Wopenka, 2002, 2003; Arouri et al., 1999, 2000; Marshall et al., 2005; Igisu et al., 2006). Raman spectra of Precambrian microfossils, however, cannot provide useful information for their taxonomy because Raman spectra of carbonaceous matter generally show a characteristic feature of amorphous carbon matter (e.g., Pasteris and Wopenka, 2003), which is not taxonomically specific. On the other hand, infrared (IR) spectroscopy can detect many polar bonds in carbonaceous matter (e.g., Rouxhet et al., 1980), and thus is potentially useful to detect taxon-specific chemical signature (e.g., Arouri et al., 1999, 2000; Marshall et al., 2005; Igisu et al., 2006). Our previous study showed that the aliphatic CH moieties (chain hydrocarbon parts) using CH$_3$/CH$_2$ absorbance ratios ($R_{3/2}$) can be useful for domain-level classification of prokaryotes (bacteria or Archaea) (Igisu et al., 2009). However, even well-preserved Neoproterozoic prokaryotic fossils have quite small amounts of aliphatic CH moieties. If we search for chemical signatures in older and thus more challenging samples, more advanced micro-Fourier transform infrared (FTIR) technique will be helpful for searching and characterizing the organic signatures in microfossils.

Here, we report micro-FTIR spectroscopic imaging of stromatolitic chert from the ~1,900 million years old (Ma) Gunflint Formation of Canada. IR imaging was conducted on a doubly polished petrographic thin section at the millimeter scale to search for functional groups related to organic and inorganic matter. The distributions of functional groups were compared with the morphology of stromatolites. Based on these results, we discuss the $R_{3/2}$ of microfossils in the stromatolite.

2. Regional Setting, Stratigraphy, Age, and Stromatolites

The Gunflint Formation (Animikie Group) is a chemical-clastic sedimentary rock succession, 100–180 m thick (averages 122 m) that crops out in an ENE to WSW trend over a distance of about 268 km, from Gunflint Lake (Minnesota, USA) in the west to Schreiber Beach (north shore of Lake Superior, Ontario, Canada) in the east (Goodwin, 1956; Pufahl et al., 2000; Fig. 1). The Animikie Group, in ascending order, consists of the Kakabeka Conglomerate, Gunflint Formation, and Rove Formation, which have conformable relationships with one another (Floran and Papike, 1975). The Rove Formation is unconformably overlain by the Mesoproterozoic Sibley Group (Morey and Ojakangas, 1982) and the Kakabeka Conglomerate rests unconformably on Archean metavolcanics (Pufahl et al., 2000).

Subdivision of the Gunflint Formation into members has changed over the years. By Fralick and Barrett (1995), two members were defined: a lower member
consisting of stromatolitic, ooidal, and granular cherts, and an upper member similar to the lower member. Each member represents a transgression with a shallowing phase separating the members (see Pufahl et al., 2000).

The age of the Gunflint Formation was imprecisely known until recently. In 2002, Fralick et al. (2002) reported a U–Pb age of 1,878.3 ± 1.3 Ma from a population of euhedral zircons recovered from re-worked volcaniclastic sedi-

Figure 1. Map of northwestern region of Lake Superior showing (a) outcrop distribution (diagonal line pattern) of the Gunflint Formation [modified from Awramik and Barghoorn (1977)], and (b) location (arrow) where sample (GF 74) was collected, which today is in the Schreiber Channel Provincial Nature Reserve (http://www.ontarioparks.com/English/schr.html).
ments from the middle part of the upper member. They interpreted this to be the depositional age of the formation.

Stromatolites from the Gunflint Formation have been a subject in a number of publications (e.g., Hofmann, 1969; Cloud and Semikhatov, 1969; Awramik and Semikhatov, 1979; Sommers et al., 2000). They occur in the basal portions of both members and are associated with the initial phases of each transgression. The stromatolites are interpreted to have formed in a wave-agitated, foreshore setting with a moderate shoreline gradient (see Hofmann, 1969; Pufahl et al., 2000).

The most comprehensive descriptions of the different types of stromatolites found in the formation were provided by Hofmann (1969); however, he did not give them binomials. Only one stromatolite has been treated taxonomically, Gruneria biwabikia, which was formally described by Cloud and Semikhatov (1969). Stromatolite morphologies include layered or stratiform, domical, columnar and columnar branching, compound (domical with layered and columnar branching morphologies, for example), and oncoids. Bioherms composed of many of these types are a common feature of the basal portions of the members (see Cloud, 1965, his Fig. 1 of bioherms at a locality 4 km west of Schreiber Beach; Fig. 1). Bioherms are up to about 1 m in diameter. No coniform stromatolites are known.

3. Materials and Methods

3.1. SAMPLE

The sample used in the micro-FTIR spectroscopy is a stromatolitic chert that was collected from a bioherm at the base of member 1 of the Gunflint Formation at a locality on the shore of Lake Superior, ~4 km west of Schreiber Beach (Fig. 1). The stromatolites here consist principally of layered and columnar stromatolites that grew on pebbles and cobbles of the underlying Kakabeka Conglomerate producing bioherms up to ~0.5 m in diameter. The stromatolites in the sample (a similar stromatolite is illustrated in Fig. 2) have parallel to subparallel stubby columns under 1 cm wide that infrequently branch, but frequently coalesce. These stromatolites resemble Form F of Hofmann (1969). At column margins, laminae characteristically overlap previous laminae, at times producing a wall. Laminae are thin, and usually <100 μm thick, boundaries between laminae are somewhat diffuse, in particular going from light to dark laminae. The laminae are composed mainly of concentrations of amorphous, at times granular, carbonaceous matter and microbial fossils (Fig. 3). These microfossils are carbonaceous and are often well preserved in the chert matrix (cryptocrystalline quartz). Based on the size, and their filamentous and coccoidal morphology, most of the microfossils are interpreted as cyanobacteria (Barghoorn and Tyler, 1965; Awramik and Barghoorn, 1977).
The sample was cut with a rock saw, and a doubly polished petrographic thin section about 40 μm thick was prepared for petrographic study, obtaining the IR spectra as described previously (Igisu et al., 2006). The doubly polished thin section was immersed in acetone to dissolve used adhesive and clean up modern contamination on its surface. Based on the petrographic investigation of the thin section (Fig. 3), the stromatolitic chert is composed mainly of cryptocrystalline quartz (SiO$_2$), and contains brown to dark amber colored amorphous carbonaceous matter, microbial fossils (the same color as carbonaceous matter), pyrite, Fe-oxides, and carbonates.

3.2. MICRO-FTIR SPECTROSCOPIC IMAGING ANALYSIS

Micro-FTIR imaging spectroscopic analysis was conducted on the doubly polished petrographic thin section using an automated X–Y stage set in IR Multi-channel Viewer (Jasco FTIR6200 + IMV-4000) with a 16-channel linear array mercury
cadmium telluride (MCT) detector. The thin section was placed over a hole in a sample holder; care was taken to ensure that a stromatolite column was positioned over the hole, and measured in the following manner. IR spectra for imaging analysis were obtained by collecting 16 scans (~2 s in total) in the spectral range from 4,000 to 1,000 cm\(^{-1}\) at an 8 cm\(^{-1}\) resolution. The spatial resolution was 12.5 × 12.5 \(\mu\)m\(^2\) corresponding to a pixel area of the IR linear array detector. The total measured area was 1.2 × 5.0 mm\(^2\), and 38400 IR spectra were obtained. The total analysis time is about 1.5 h. Analytical error was determined by duplicate
measurements in the same position as the background spectrum and was better than 0.005 absorbance unit (AU) in the 3,000–1,300 cm\(^{-1}\) range, except in the CO\(_2\) absorption region.

4. Results

Typical IR transmission spectra of carbonaceous matter in the thin section of the stromatolite are shown in Fig. 4. Aggregations of filamentous and coccoidal fossils as well as amorphous organic matter show IR absorption bands of aliphatic CH moieties (~2,960, 2,925, 2,850 cm\(^{-1}\)) in the range of >2,500 cm\(^{-1}\) (Fig. 4). The bands around 2,960 and 2,925 cm\(^{-1}\) are due to asymmetric stretching of end-methyl aliphatic CH\(_3\) and methylene-chain CH\(_2\), respectively (Bellamy, 1954). A weak band from symmetric stretching of aliphatic CH\(_2\) is also seen around 2,850 cm\(^{-1}\) (Bellamy, 1954). Similar results were obtained in our previous study of cyanobacteria-like fossils in ~850 Ma stromatolitic cherts from the Bitter Springs Formation (Igisu et al., 2006), while symmetric stretching band of aliphatic CH\(_3\) (~2,870 cm\(^{-1}\)) was not observed in this study.

**Figure 4.** Representative IR transmission spectra of the microfossils (CM), matrix quartz (Qz), and carbonates (Carb.) in the stromatolitic chert. (a) Stacked IR spectra. IR band at 2,960 cm\(^{-1}\) is due to asymmetric aliphatic CH\(_3\) (\(\nu_{as}\)CH\(_3\)), and those at 2,925 and 2,850 cm\(^{-1}\) are due to asymmetric and symmetric aliphatic CH\(_2\) (\(\nu_{as}\)CH\(_2\) and \(\nu_s\)CH\(_2\)). IR bands at 2,990, 2,870 and 2,515 cm\(^{-1}\) are characteristics of carbonate (square). Broad band around 3,400 cm\(^{-1}\) is due to molecular water probably within the grain boundary of micro-quartz. Saturated bands (1,300–1,000 cm\(^{-1}\)) and seven bands (1,995, 1,870, 1,793, 1,684, 1,610, 1,525, and 1,492 cm\(^{-1}\)) are due to Si–O bonds of quartz. One scale unit for vertical axis corresponds to an absorbance value of 0.2. (b) Enlarged view of IR spectra of (a) in the range of 3,000–2,800 cm\(^{-1}\). An example of baseline correction for elucidation of peak height is described as dotted line. One scale unit for the vertical axis corresponds to an absorbance value of 0.05.
The band around 3,400 cm\(^{-1}\) is due to O–H stretching vibrations. The broad 3,400 cm\(^{-1}\) band is generally considered to be due to liquid-like molecular water (H\(_2\)O) at grain boundaries of cryptocrystalline quartz (Nakashima et al., 1995; Ito and Nakashima, 2002; Igisu et al., 2006). Seven absorption bands at 1,995, 1,870, 1,793, 1,684, 1,610, 1,525, and 1,492 cm\(^{-1}\) are due to overtones and combinations of Si–O vibrations, and are characteristic of quartz (Nakashima et al., 1995; Ito and Nakashima, 2002; Igisu et al., 2006). The range 1,300–1,000 cm\(^{-1}\) (Si–O stretch) is saturated due to the thickness (~40 \(\mu\)m) of the thin section. The feature around 2,350 cm\(^{-1}\) is due to variation of CO\(_2\) in air.

The carbonates among the minerals in the stromatolitic chert measured here also show IR bands at around 2,980, 2,875, and 2,515 cm\(^{-1}\) in the >2,500 cm\(^{-1}\) region together with more saturated bands in the <1,500 cm\(^{-1}\) region as shown in Fig. 4. Fe-oxide and pyrite do not show IR bands in >2,500 cm\(^{-1}\) region (Komada, 1985; RRUFF Database; http://rruff.info/).

The spatial distributions of the above components in the stromatolitic chert are shown in Fig. 5. The distributions of the bands (peak heights) at 2,960 and 2,925 cm\(^{-1}\) after baseline correction in raw IR transmission spectra roughly agree with thin section images of microfossil-bearing laminae (Fig. 5). The distribution of 2,515 cm\(^{-1}\) band agrees with those of carbonates, and the intense parts of 2,515 cm\(^{-1}\) band overlap with those of the 2,925 and 2,960 cm\(^{-1}\) bands (Fig. 5). This suggests the distributions at 2,960 and 2,925 cm\(^{-1}\) due primarily to aliphatic CH moieties might include absorptions of carbonates.

5. Discussion: Characterizing Aliphatic CH Moieties (\(R_{3/2}\))

In order to evaluate a domain-level taxonomic origin of carbonaceous matter, we have introduced the aliphatic CH\(_3\)/CH\(_2\) absorbance ratio (\(R_{3/2}\)) in our previous study as follows:

\[
R_{3/2} = \frac{[v_{as} \text{CH}_3]}{[v_{as} \text{CH}_2]} \tag{1}
\]

where \([v_{as} \text{CH}_3]\) and \([v_{as} \text{CH}_2]\) represent peak heights of asymmetric stretching bands for aliphatic CH\(_3\) (end-methyl; ~2,960 cm\(^{-1}\)) and CH\(_2\) (methylene-chain; ~2,925 cm\(^{-1}\)), respectively (Igisu et al., 2009). However, it should be noted that carbonates also show IR bands in the range of 3,000–2,800 cm\(^{-1}\). This indicates that \(R_{3/2}\) values of carbonaceous matter existing with carbonates can be overestimated or underestimated. Relationship of \(R_{3/2}\) value to the content of carbonate is shown in Fig. 6. For better \(R_{3/2}\) precision, we used only the data with 2,960 and 2,925 cm\(^{-1}\) absorbance more than three times of analytical error (0.005). Although the \(R_{3/2}\) values with carbonate (absorbance at 2,515 cm\(^{-1}\) > 0.005) are about 0.82 ± 0.10 (\(n = 359\)), the \(R_{3/2}\) values without carbonate are about 0.49 ± 0.08 (\(n = 18\)) (Fig. 6). In this study, the presence of carbonates tends to result in overestimation of \(R_{3/2}\) values of carbonaceous matter.
Although HCl acid treatment can dissolve carbonates, it will be difficult to collect organic residues with prokaryotic cellular structures. It seems to be better to select carbonaceous matter without carbonates under the IR microscope for more precise $R_{3/2}$ evaluation. IR spectra of carbonate such as calcite (CaCO$_3$) and dolomite (MgCO$_3$) generally have an intense band around 1,450–1,410 cm$^{-1}$ and ~2,515 cm$^{-1}$ band together with smaller ~2,990 and ~2,875 cm$^{-1}$ bands (Komada, 1985). Therefore, it should be checked in the $R_{3/2}$ evaluation of carbonaceous matter that a ~2,515 cm$^{-1}$ band is not observed. Hence, we focus further discussions on IR bands that do not show the 2,515 cm$^{-1}$ signal.

The above carbonaceous matter without carbonate is divided into aggregated filamentous and coccoidal fossils and amorphous organic matter (Fig. 3).

**Figure 5.** IR imaging results by the raw IR transmission spectra of the microfossil-bearing chert GF74. (a) Optical photomicrograph of the measured area (1.2×5.0 mm$^2$). Dotted white circles represent the areas, which include all the spot analyses used for $R_{3/2}$ calculation in Fig. 6c. (b–d) The spatial distribution maps for the peak heights at (b) 2,925, (c) 2,960, (d) 2,515 cm$^{-1}$ after baseline correction. The color scale represents the higher peak height (white) and the lower one (black) in the absorbance unit (AU). Analytical errors in absorbance are 0.005 AU in (b–d).
Figure 6. $R_{3/2}$ values of carbonaceous matter existing with and without carbonates, compared with whole cells and predicted lipids of extant prokaryotes. (a) Relationship between $R_{3/2}$ (IR absorbance ratio) and the content of carbonates (absorbance at 2,515 cm$^{-1}$). Dotted line represents upper limit of analytical error of absorbance at 2,515 cm$^{-1}$ (0.005 AU). Individual error bars of $R_{3/2}$ values are calculated based on the duplicate analysis of air background spectra. All the data were obtained from GF74. (b) $R_{3/2}$ values of aggregated filamentous and coccoid fossils and amorphous organic matter from GF74. Individual error bars of $R_{3/2}$ values are calculated based on the duplicate analysis of air background spectra. (c) $R_{3/2}$ values of extant bacteria (square) and Archaea (circle) and predicted $R_{3/2}$ values of lipid for each domain (dotted line) [referred from Igisu et al. (2009)]. Positive correlation between $R_{3/2}$ value and ratio CH$_3$/CH$_2$ for $n$-alkane standard samples ($R_{3/2} = 2.56 [CH_3/CH_2]$, $r^2=0.64$) can be used for estimating $R_{3/2}$ values of these domains’ representative lipid.
The $R_{3/2}$ values are 0.41 ± 0.03 ($n = 6$) for aggregated filamentous and coccoidal microbial fossils, and 0.53 ± 0.07 ($n = 12$) for amorphous organic matter (Fig. 6b). The values are consistent with our previous study, and are roughly similar to the $R_{3/2}$ values of the predicted extant bacterial lipids ($R_{3/2} = 0.32–0.37$) rather than archaeal lipids ($R_{3/2} = ~1.16$) (Igisu et al., 2009) (Fig. 6b, c). This indicates that most microfossils and amorphous organic matter in this study belong to bacteria. These results also show that this rapid imaging technique provides $R_{3/2}$ values of carbonaceous matter consistent with the previous spot analysis (Igisu et al., 2009).

In summary, micro-FTIR spectroscopic imaging analysis can be successfully applied to ~1,900 Ma stromatolites from the Gunflint Formation. The microfossil-bearing laminae in the stromatolitic chert contain immature carbonaceous matter with aliphatic CH$_2$ and CH$_3$. $R_{3/2}$ values indicate that most carbonaceous matter including prokaryotic fossils belong to the bacteria. However, it should be noted that carbonates also show IR signature in the similar range to aliphatic CH moieties. This method would be applicable for searching for chemical signatures in Earth’s rock samples as well as extraterrestrial materials, and for chemically characterizing organic matter.

6. Conclusions

Micro-FTIR spectroscopic imaging analysis of a stromatolitic chert of the ~1,900 million-year-old Gunflint Formation containing extremely well-preserved microfossils provides the following conclusions:

(1) IR spectra of microfossil-bearing laminae show the 2,925 and 2,960 cm$^{-1}$ bands due to aliphatic CH moieties. The distributions of these bands roughly agree with the morphology of the microfossil-bearing laminae, but the distributions are affected by the presence of carbonates, whose IR spectra show bands at around 2,980 and 2,875 cm$^{-1}$.

(2) It should be noted that $R_{3/2}$ values of carbonaceous matter occurring with some minerals such as carbonates can be both overestimated and/or underestimated, but these over and underestimations can be removed by careful evaluation of other, more intense bands (e.g., ~2,515 cm$^{-1}$ band for carbonate).

(3) $R_{3/2}$ values of microfossils and amorphous organic matter without the presence of carbonate indicate that most microfossils and amorphous organic matter belong to bacteria rather than Archaea. No signatures suggestive of eukaryotes were detected.

Micro-FTIR imaging analysis is useful for rapidly searching and characterizing organic signatures in the stromatolitic chert. Further detailed IR measurements, combined with traditional morphological analyses, may provide new approaches in the determination of the domain-level, taxonomic affinity of microfossils in the chert.
7. Acknowledgments

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8. References


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