Chemical Engineering Science 308 (2025) 121422

Contents lists available at ScienceDirect

Chemical Engineering Science

journal homepage: www.elsevier.com/locate/ces

Effective protection strategy of Surface-enhanced Raman scattering substrate in deep-sea cold seep in-situ detection

Siyu Wang [a,b,1], Fei Li [c,1], Lin Wang [e], Ruhao Pan [d], Liang Ma [b,f], Yang Yang [d], Zhendong Luan [b,f], Xin Zhang [a,b,f,*]

[a] LaoShan Laboratory, Qingdao 266237, China
[b] Key Laboratory of Ocean Observation and Forecasting (LOOF), Key Laboratory of Marine Geology and Environment & Center of Deep Sea Research, Institute of Oceanology, Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao 266071, China
[c] College of Environmental Science and Engineering, Nankai University, Tianjin 300350, China
[d] Institute of Physics, Chinese Academy of Sciences, Beijing 100190, China
[e] Department of Physical and Chemical Testing, Shandong Center for Food Safety Risk Assessment, Shandong Center for Disease Control and Prevention, 250014 Jinan, China
[f] University of Chinese Academy of Sciences, Beijing 101408, China

ARTICLE INFO

Keywords:
Surface-enhanced Raman scattering
Cold-seep
Extreme environment
Protection devices
Deep-sea

ABSTRACT

Complex environments often have irreversible effects on Surface-enhanced Raman scattering (SERS) substrates, so it is very important to design protection devices reasonably. In this paper, a simple and effective SERS substrate protection scheme was proposed. The SERS substrate was assembled on the ROV (Remote-operation Vehicle) "*Faxian*" and deployed in a cold seep biocenose area. The protection device was crucial for the successful application of the SERS substrate, which effectively adsorbed biological organic molecules such as amino acids and β-carotene. However, the effective detection time for low-concentration biomolecules was short (< 8 min with protection, nearly 0 without), primarily due to high inorganic salt concentrations at the vents. This study highlights the limitation of conventional SERS substrates as single-use sensors in deep-sea in-situ detection. Future designs of in-situ SERS probes should focus on substrate replacement and protection to enable multi-point detection in extreme.

1. Introduction

Extremophiles in deep-sea chemergic ecosystems have rich genetic and metabolic diversity, deeply participate in the geochemical cycle of key elements, promote the evolution of chemergic ecosystems, and produce metabolites with unique structure and function (Tortorella, et al., 2018). The detection of organic molecules related to deep-sea microbial metabolism has important research significance and value for mining functional genes related to anti-high/low temperature, heavy metal detoxification, and interpreting key factors regulating biological adaptability (Busch, et al., 2022; LaBrie, et al., 2022; Zhou, et al., 2023). However, in the extreme deep-sea environment, in-situ sampling is very difficult, and the exchange of seawater will inevitably change during the sampling process, and it is difficult to obtain accurate information of relevant molecules. In order to solve this problem, deep-sea in-situ exploration technology began to flourish (Greenfield, et al., 2008; Peltzer, et al., 2016; Wei, et al., 2020). However, the concentration of bio-related organic molecules in deep sea is low, and the LOD (Limit of Detection) of commonly used technologies, such as deep-sea in situ Raman detection technology, is high, making it difficult to achieve in-situ detection of deep-sea organic molecules (Barletta, et al., 2015; Du, et al., 2018).

Surface-enhanced Raman scattering (SERS) technology is a kind of ultra-high sensitivity detection technology, and its characteristics such as fast and non-destructive detection make it more and more applied in the detection of water environment (Liu, et al., 2020; Liu, et al., 2023; Williams, et al., 1996). However, in the actual sample testing process in complex environments (such as sewage, lakes and oceans, rich in

* Corresponding author at: Key Laboratory of Marine Geology and Environment & Center of Deep Sea Research, Institute of Oceanology, Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao, 266071, China.
E-mail address: xzhang@qdio.ac.cn (X. Zhang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ces.2025.121422
Received 29 October 2024; Received in revised form 8 February 2025; Accepted 22 February 2025
Available online 25 February 2025

biomacromolecules, organic matter, minerals and metal ions), pretreatment processes such as filtration, microwave digestion and dilution are often required (H. Huang, et al., 2018; Y. Huang, et al., 2023). Similarly, the environment in deep-sea cold-seep biomes is complex and contains large amounts of methane, hydrogen sulfide, carbonate rocks, and other substances (X. Wang, et al., 2017; W. Zhang, et al., 2023; Y. Zhang, et al., 2023). The relative content of bio-related organic molecules is extremely low, and their detection can is easily susceptible to interference from the surrounding environmental substances (Joye, et al., 2004; Somoza, et al., 2021). Limited by the existing technology, the in-situ detection of deep-sea extreme environment can only carry out simple filtration, and it is difficult to carry out complex pretreatment. At the same time, deep-sea in-situ exploration is expensive, and the successful acquisition of effective information is the focus of the work. Therefore, studying the adsorption behavior of SERS substrates in the deep-sea environment and proposing a simple and effective solution can effectively guide the operation mode of SERS technology in the deep sea, improve the success rate of SERS detection and reduce the cost of deep-sea detection.

In this paper, an effective SERS substrate protection scheme is proposed, which is helpful for in-situ SERS detection in deep sea cold seep. The Ag-based solid SERS substrate was assembled on a "*Faxian*" remotely operated submersible (ROV) and was descended to the dense biome area of the cold seep vent of the Formosa Ridge in the South China Sea for verification. 4-mercaptopyridine (4-Mpy) served as both a signal molecule and an indicator, coupling SERS substrates with deep-sea Raman probe status indicators during deep-sea diving. SERS substrates with and without protective solution were brought into the deep sea for real-time SERS sampling and adsorption test. The comparison results revealed that the SERS substrate had excellent adsorption performance on biomolecules. At the same time, the importance of deep-sea detection process for SERS substrate pre-protection was pointed out. This work can effectively guide deep-sea in-situ SERS detection, reduce the frequency of data acquisition, and improve the stability and reliability of the experiment.

2. Experimental

2.1. Materials

4- mercaptopyridine (98.0 % or higher) and anhydrous ethanol (≥98.0 %) were purchased from Sigma-Aldrich. Ultrapure water (18.2 MΩ cm, produced by the Milli-Q system) was used in this study. The cold seep fidelity fluid was independently collected from the cold seep vent at Formosa Ridge in the South China Sea.

The SERS substrate was prepared in the previous work (S. Wang, et al., 2022). In brief, a layer of a silver nanofilm was plated on a quartz sheet, and the SERS substrate was obtained by a one-step calcination method. The heating rate for the annealing process was 10 °C/s. The substrate was heated up to 420 °C and held for 10 min in air before cooling to 20 °C.

2.2. Laboratory simulation section

Stability test of SERS substrate in aqueous solution. 4-Mpy was used as a signal molecule, 10^{-6} M 4-Mpy aqueous solution was configured, and then the SERS base was placed at the bottom of the solution and left for a period of time. Then the SERS substrate and solution were placed together for detection under confocal Raman microscope with a laser intensity of 5 mW, wavelength 532 nm, accumulated 15 times during the measurement process, and the integration time was set to 3 s. Keep the laser illuminated for 120 min, and collect the spectrum every 15 min.

Stability test of SERS substrate in cold seep fidelity fluid. Using 4-Mpy as a signal molecule, the SERS substrate was absorbed in 10^{-6} M 4-Mpy solution for 2 h, delicately dried, and the unadsorbed 4-Mpy molecules were rinsed off with ultrapure water, then placed into 20 mL of cold seep fidelity fluid, and immediately transferred to confocal microscopy for detection. The detection conditions were consistent with the above, and the interval was 2 min (Normal temperature and pressure).

2.3. SERS substrate fixtures

How to bring SERS substrate into the deep sea and keep it in the laser focal length range is a key technical problem. Influenced by offshore working conditions, we prepared a simple SERS substrate fixing device using simple nuts, filter iron mesh and clamps (Fig. 1a, b). The SERS substrate pre-modifies the signal molecule 4-Mpy as an indicator of focusing regulation. First, the SERS substrate is fixed at the bottom of the iron net and linked with the nut. The nut is then adjusted to the appropriate position (the optimal distance is determined by 4-Mpy signal molecules modified on the SERS substrate). Finally, the clamp is used to ensure that it does not move.

2.4. Deep sea in situ test section and conservation strategy

During first deep dive, the SERS substrate was fixed to a common Raman probe laser by covering it with a screen without a protection device. Upon reaching the deep-sea cold-seep biological zone (the depth is about 1120 m and the temperature is about 4 ℃), the SERS substrate was inserted into the biological community for direct detection (Fig. 2d). During the second deep dive, a balloon filled with buffer solution was wrapped around the outside of the SERS substrate as a protective device (Fig. 2a). When the deep-sea area to be tested was reached, the ROV operator was used to disassemble the protection device, and then the SERS substrate was used for on-site detection (Fig. 2b-2d). After standing for 5 min, the continuous spectrum was collected at intervals of 30 s with an exposure time of 4 s. Each spectrum was collected eight times; that is, a new Raman spectrum was collected approximately once every minute. Raman detection device is a deep-sea in situ Raman insert probe (RiP) system (S. Wang, et al., 2023) independently developed by the team.

The RiP system contained a custom-designed NRXNE-532-RA-SP spectrometer and a frequency-doubled Nd:YAG laser (532 nm) with an output power of 150 mW, both manufactured by Kaiser Optical Systems, Inc. (USA). An ADU-440A-BV-136 charge-coupled device (CCD) camera (Andor Technology, UK) with 2048 × 512 pixels (corresponding to the spectral resolution of approximately 1 cm^{-1}), and a 27.6 × 6.9 mm image area was installed in the RiP system. The spectral range (100–4325 cm^{-1}) was split into two regions (100–2100 and 2100–4325 cm^{-1}) on the surface of the CCD; therefore, the spectral resolution of the RiP system was approximately 1 cm^{-1}. Before each deployment, the wavenumber and intensity calibrations of the RiP system were processed using neon and halogen lamps.

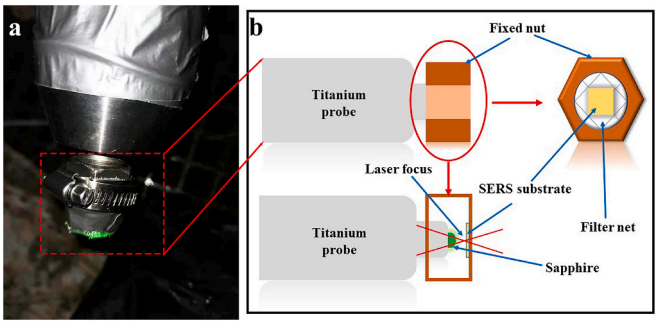

Fig. 1. (a) SERS substrate fixing device physical diagram; (b) three views of SERS substrate fixtures consisting of nuts, filter iron mesh and clamp.

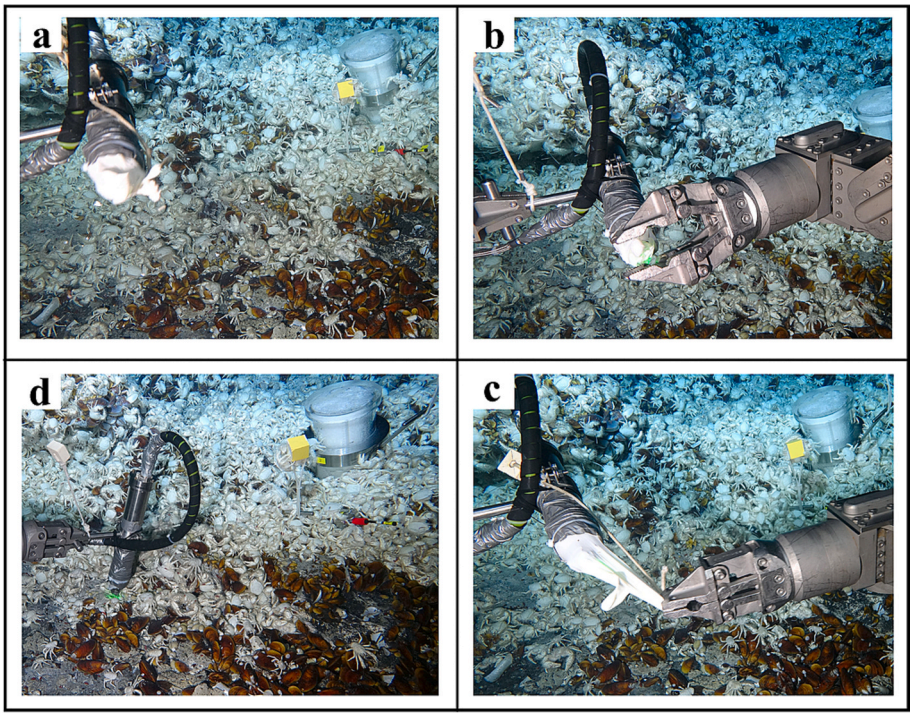

Fig. 2. Field work photos of SERS in-situ detection in deep sea. (a) Deep-sea Raman probes with protective devices and buffers; (b) and (c) disassembly of the protective device by ROV; (d) In situ detection process, the probe with SERS substrate was directly inserted into the biome (the detection method without protection device was consistent with this d).

3. Results and discussion

3.1. Laboratory simulation

Fig. 3 is a schematic diagram of the process of SERS substrate adsorption and detection in the deep sea. Because the deep sea contains a large number of metal salts, cations and particles and other substances (X. Wang, et al., 2017) (white ball in the picture), there are also many biomolecules in the deep-sea cold seep biome (red ball in the picture). When the SERS substrate was placed in the deep-sea biome, organic molecules containing amino and sulfhydryl groups in the biomolecule were quickly adsorbed to the SERS substrate surface due to the action of Ag-N bond or Ag-S bond (Huard, et al., 2019; Ou, et al., 2023; Xu, et al., 2022; L. Zhang, et al., 2018), thus corresponding Raman signals were detected. However, with the increase of time, interferences such as metal salts begin to enrich, and eventually shield biomolecular signals. This hypothesis was tested in subsequent experiments.

Due to the limitations of the equipment, the laser of the deep-sea plug-in Raman device is always turned on after the power is turned on in the deep sea. Therefore, it is particularly important to investigate the influence of long-term laser irradiation on SERS substrate. Hence, the stability of SERS substrate was tested by placing SERS substrate in an aqueous solution of 4-Mpy. Under the laser irradiation of up to 120 min, the characteristic peaks of 4-Mpy at 1010 cm^{-1} (ring breathing) and 1097 cm^{-1} (C-S) could still be detected (Fig. 4a) (Hu, et al., 2002; Guohui Yang, et al., 2022). Comparing the change of the peak value of irradiation time, it can be found that the characteristic peak value of 4-Mpy has a slow decline process in the first 40 min, and then tends to be stable (Fig. 4b), which may be caused by the instability of confocal microscopy during long-time detection. This indicates that SERS

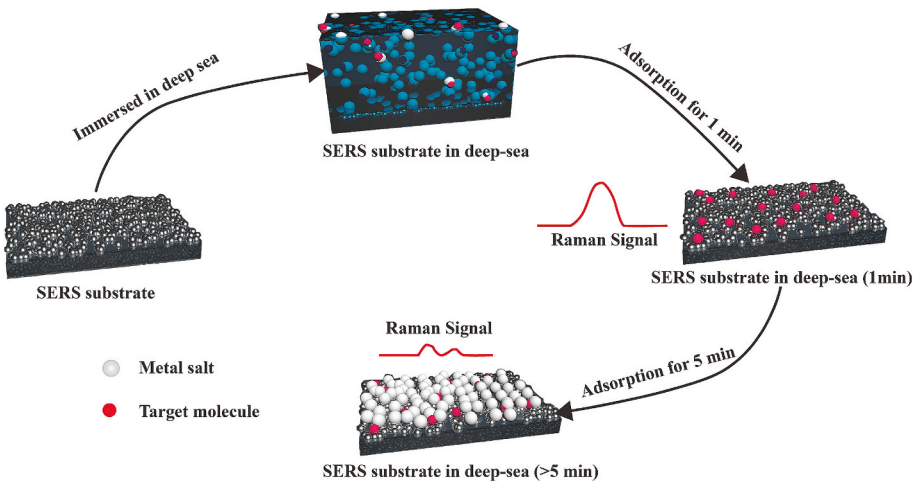

Fig. 3. Schematic diagram of SERS substrate adsorption process in complex environment (deep sea extreme environment as an example).

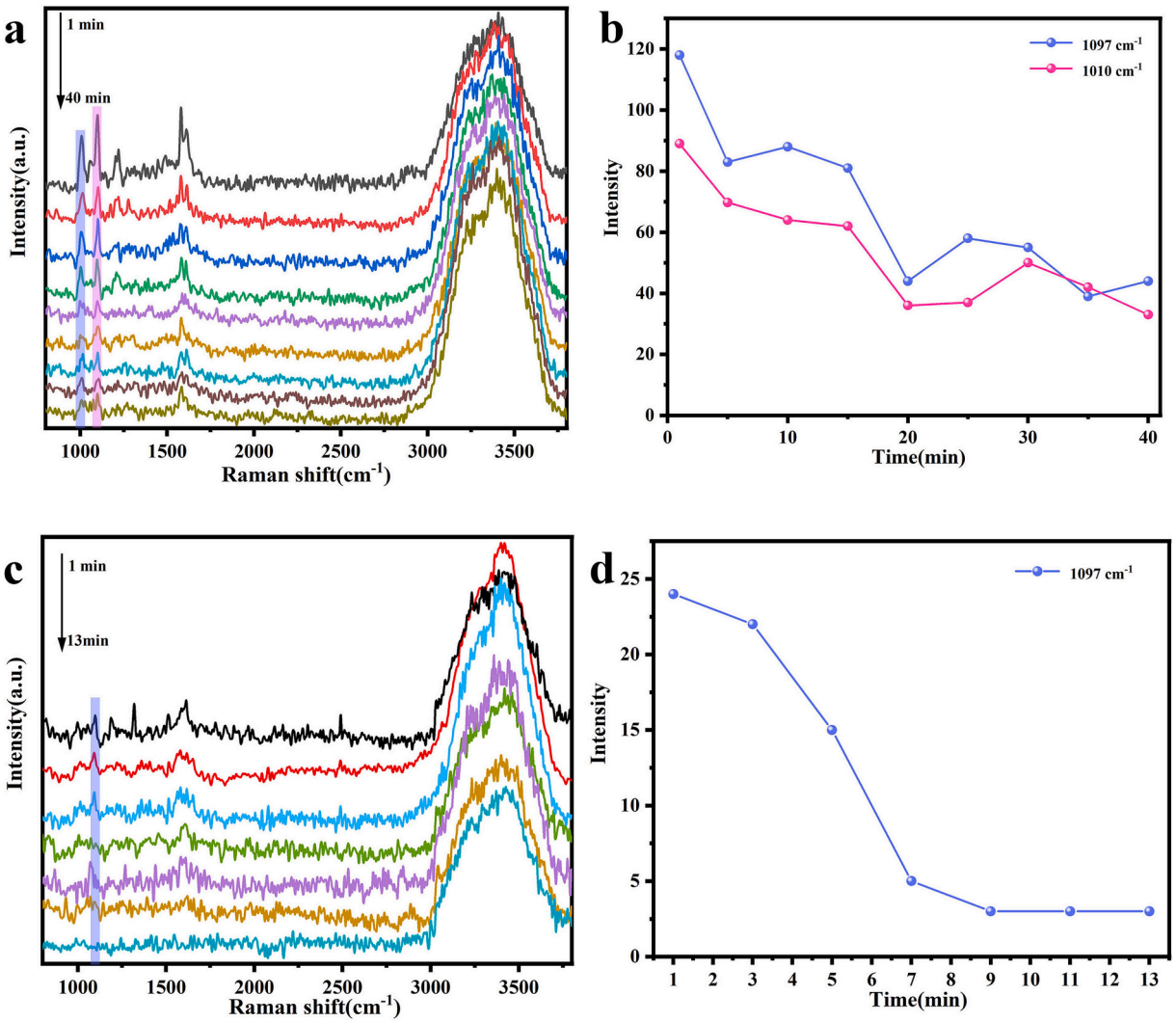

Fig. 4. (a) Effect of long-time laser focusing on SERS substrate in aqueous solution; (b) Variation of characteristic peaks (1010 cm^{-1} and 1097 cm^{-1}) with light time; (c) Properties of SERS substrates modified 10^{-6} M 4-Mpy in deep sea cold seep fidelity fluids; (d) Variation of characteristic peaks (1097 cm^{-1}) with test time.

substrate also has good stability in the deep-sea environment and can maintain good Raman enhancement effect under the long-term irradiation of Raman laser.

There are many complex and uncontrollable factors in the deep-sea environment, so laboratory simulation experiments are necessary. Here, we used the cold seep fidelity fluid as the deep-sea water environment to carry out the simulation experiment of SERS substrate in-situ detection. Using 4-Mpy as a signal molecule, direct detection was performed in an unprocessed, at an interval of 2 min. The results show that the characteristic peak at 1097 cm^{-1} (C-S) decreases rapidly within 7 min and is shielded by complex noise signals (Fig. 4c, 4d). However, the vibration peak at 1010 cm^{-1} was disturbed by impurities, and it was difficult to distinguish at 3 min, which also proved that Ag and C-S bond were more easily combined, that is, Ag-S bond. In short, SERS substrate had excellent adsorption capacity, which greatly shortened its effective detection time in complex environments. Therefore, it is necessary to design a reasonable protection device in the actual complex environment, which also provides guidance for our follow-up deep-sea in-situ experiments.

3.2. Deep sea in-situ testing of materials

Due to the attitude adjustment required when the ROV enters the water, Raman spectra cannot be collected during the 0——200 m dive. When the ROV attitude adjustment was completed and the Raman spectra were formally collected, we could observe (Fig. S1a) that the SERS substrate without added protection device only initially detected the characteristic peak of signal molecule 4-Mpy at 1052 cm^{-1}, and then the characteristic peak disappeared (was masked), leaving only the characteristic peak of sulfate in seawater (the peak value at 1097 cm^{-1} decreased sharply due to competitive adsorption between C-S bond and high concentration sulfate in seawater. However, the peak value of 1052 cm^{-1} is prominent and stable. Therefore, here we choose the peak at 1052 cm^{-1} as the characteristic peak of 4-Mpy (S. Wang, et al., 2023)). However, in SERS substrate with a protective device added (Fig. S1b), it was observed that the characteristic peak of sulfate (980 cm^{-1}) gradually increased, which meant that a part of seawater was immersed, but the process was slow. At the same time, we could observe the presence of the characteristic peak of 4-Mpy at 1052 cm^{-1}, which meant that our protection strategy could effectively guarantee the enhanced capability of SERS substrate.

When the deep-sea cold-seep biological zone was reached, an unprotected SERS substrate was inserted into the biological community to begin the continuous spectrum collection, and nine continuous spectra were obtained within 8 min (Fig. 5a). No signal was obtained apart from the sapphire of the Raman laser, the Raman peak of SO_4^{2-} (980 cm^{-1}) in seawater, and the metal–O bond (Fig. 5a enlarges the image) (Kloprogge, et al., 2002; X. Zhang, et al., 2017). This may be because

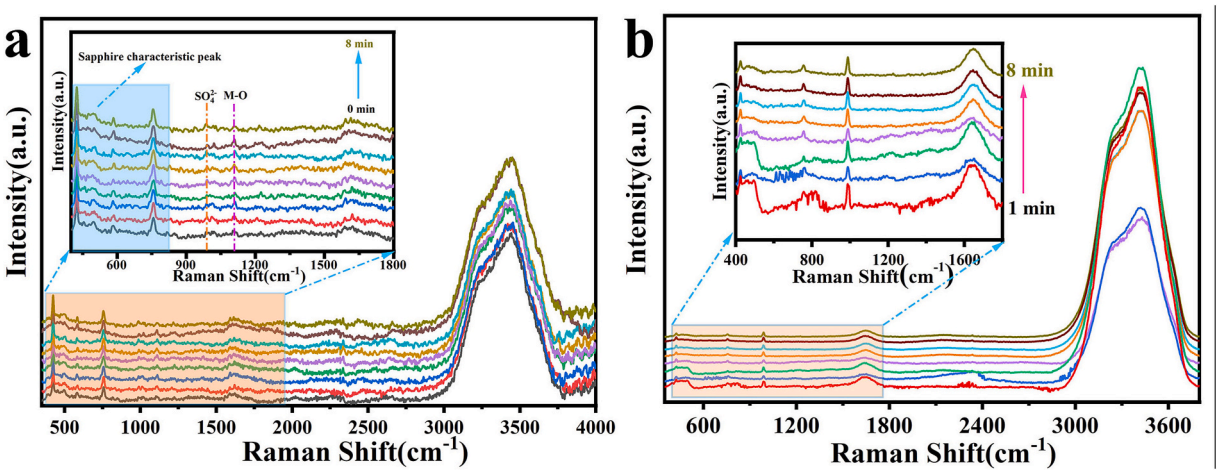

Fig. 5. Raman real-time detection spectra in the deep-sea of the SERS substrate (a) without protection and (b) with protection.

sufficient inorganic substances were absorbed onto the SERS substrate surface during diving, completely shielding the surface signal. After the protected SERS substrate arrived at the test site, nine continuous spectra were obtained within 8 min (Fig. 5b). The illustration in Fig. 5b clearly shows that many signal peaks appear at 600–1800 cm^{-1} in the first Raman spectrogram, indicating that biomolecules were successfully absorbed on the SERS substrate surface. Simultaneously, the biomolecule peak increased significantly in the second Raman spectrum. However, from the third minute, the Raman signal dropped sharply, and the characteristic peaks of SO_4^{2-} (980 cm^{-1}) and sapphire dominated the Raman spectrum from the fourth to the eighth minute. This indicates that the SERS substrate quickly adsorbed biomolecules in the solution. After 4 min, the high concentration of inorganic salt in the deep-sea environment began to interfere and gradually adsorbed on and covered the SERS substrate surface, resulting in the disappearance of the Raman signal of the biomolecules. Because the SERS substrate stood for 5 min for adsorption before detection, the actual time was already 9 min. That is, the effective detection time is within 8 min, consistent with lab simulations. This means that the detection efficiency of SERS substrate was improved and the reaction and adsorption time was reduced in the subsequent detection.

We further investigated the adsorbed substances on the surface of without protection and with protection SERS substrates to validate the importance of our protection strategy for in-situ biomolecular detection of SERS substrates in deep ocean. First, we took two different SERS substrates (without protection and with protection) back to the laboratory and conducted Raman testing again after completely drying. We randomly selected five points on the without protection SERS substrate for detection (Fig. 6a). Predictably, only a few irregular background peaks appear, meaning that there are no or very few organic molecules remaining on the substrate surface below the detection limit. However, we observed the occurrence of several stable characteristic peaks on the with protection SERS substrate (Fig. 6b), including 1007 cm^{-1}, 1169 cm^{-1}, 1514 cm^{-1} and bulge peak at 2800——3000 cm^{-1}. Among them, the characteristic peaks at 1007 cm^{-1} (ρ(C-CH_3)), 1169 cm^{-1} (C–C polyene chain) and 1514 cm^{-1} (C=C polyene backbone) can be attributed to β-carotene (S. Wang, et al., 2023). This result also provides effective evidence for the existence of a large number of deep-sea microorganisms at deep-sea cold seep vents, because β-carotene comes mainly from deep-sea microorganism (Chen, et al., 2020; Niero, et al., 2021).

At the same time, scanning electron microscope (SEM), element distribution mapping, Fourier Transform infrared (FT-IR) spectroscopy and X-ray photoelectron spectroscopy (XPS) characterization (Fig. S2 and Fig. S3) were also conducted on SERS substrates with two different processing methods. In addition, we further performed an XPS fitting analysis on the protected substrate (Fig. 7). Among them, the C1s nuclear level spectrum is fitted by five components, corresponding to five carbon atoms with different chemical properties in the molecule (Fig. 7a). 284.8 eV corresponds to the C–C bond, 285.0 eV corresponds

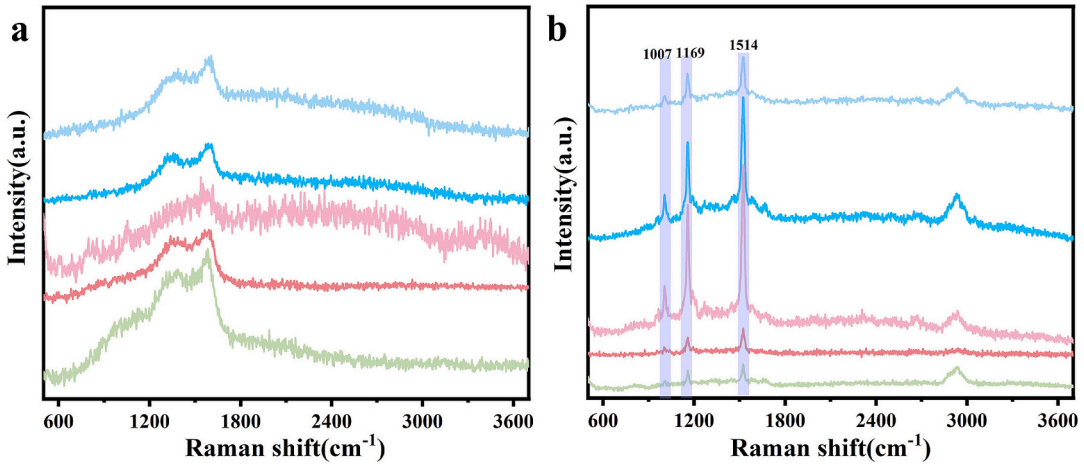

Fig. 6. Raman spectrum of deep-sea SERS substrate after drying in the laboratory (five points were randomly selected for each SERS substrate for detection): (a) without protection and (b) with protection.

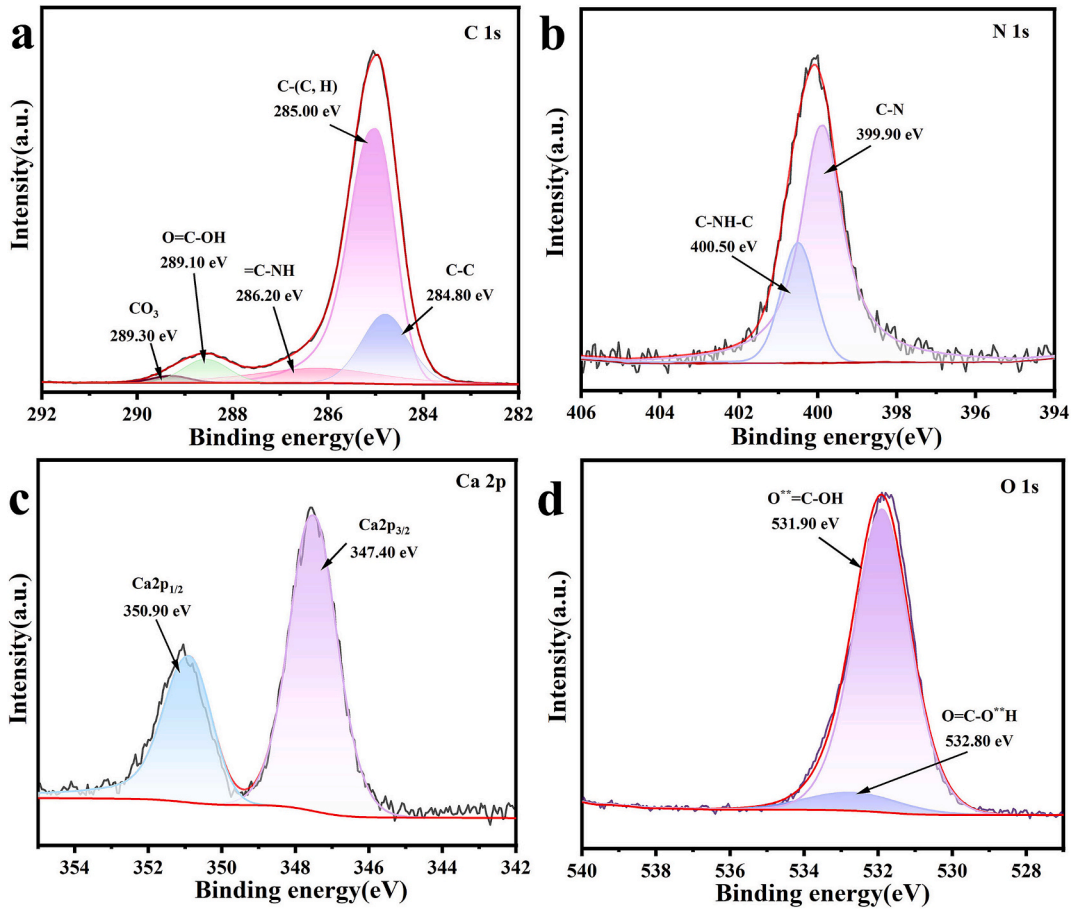

Fig. 7. Fitted C 1 s, N 1 s Ca 2p and O 1 s peaks for SERS substrate with protection.

to the C- (C, H) bond, and 286.2 0 eV and 289.10 eV indicate =C-NH and O=C-OH, respectively (Artemenko, et al., 2021). These can be attributed to the source of amino acids or some other organic molecules, which can be further demonstrated by the fitting data of N1s and O1s in Fig. 7b and 7c, including 399.90 eV (C-N), 400.50 eV (C-NH-C), 531.90 eV (O^{**}=C-OH) and 532.80 eV (O=C-O^{**}H) (Artemenko, et al., 2021; Guangbin Yang, et al., 2013). In addition, we also found the presence of 289.30 eV (CO3), possibly due to the adsorption of calcium carbonate on the surface, and the presence of 347.20 eV (Ca $2p_{3/2}$) and 350.90 eV (Ca $2p_{1/2}$) in Fig. 7c further supports our inference(Ma, et al., 2021; Ni & Ratner, 2008). By comparing the total XPS spectra of SERS substrates with protected devices and without protected devices, it was obvious that the content of organic molecules retained in SERS substrates with protected devices was much higher than that without protected devices (detailed analysis can be found in Fig. S3). All the evidence showed that the with protection SERS substrate had good SERS detection effect and effective adsorption performance on biomolecules in the deep sea. Based on this, we can conclude that this protection strategy can effectively ensure the in-situ detection of bio-related molecules by SERS substrate in deep sea, and effectively guide the development of in-situ SERS detection equipment in deep sea in the future.

4. Conclusion

In summary, this study proposed an effective SERS substrate protection strategy for deep-sea in-situ detection. The properties and adsorption behavior of SERS substrates in deep sea cold seep composite system were investigated by in-situ deep-sea experiments. Laboratory simulation results showed that SERS substrate remained stable under long-term laser irradiation in aqueous solution. Deep-sea in-situ experiments showed that the adsorption capacity of SERS substrates on biological organic molecules was very fast, and the high concentration of inorganic salts in the deep sea was also quickly adsorbed, covering the Raman signal of low concentration biomolecules. Importantly, in deep-sea cold seep systems, the effective time for in situ detection of dilute biomolecules is only 8 min. This work shows that reasonable design of protective strategies for SERS substrates and improvement of detection efficiency is particularly important in complex extreme environments (for example, the design of probe devices that can inject protective fluid and flushing fluid in real time, easily replaceable probe with three-dimensional structure or flexible probe crawler), providing important guidance for the detection of low concentration molecules in extreme environments.

CRediT authorship contribution statement

Siyu Wang: Writing – original draft, Methodology, Data curation, Conceptualization. **Fei Li:** Software, Methodology, Investigation. **Lin Wang:** Investigation. **Ruhao Pan:** Resources. **Liang Ma:** Software. **Yang Yang:** Project administration. **Zhendong Luan:** Supervision, Project administration. **Xin Zhang:** Writing – review & editing, Supervision, Funding acquisition.

Funding

This research was supported by the following grants: the National Natural Science Foundation of China (42327805, 42221005, 41822604); the Strategic Priority Research Program of Chinese Academy of Sciences (XDA22050102); Natural Science Foundation of Shandong Province (ZR2024QD137); the China Postdoctoral Science

Foundation (2024 M761249); National Natural Science Foundation of China (grant nos. 12204527); the Young Taishan Scholars Program (tsqn201909158). This work was supported by the Synergetic Extreme Condition User Facility (2023-SECUF-PT-000701), and Shanghai Tongji Gao Tingyao Environmental Science and Technology Development Foundation. We thank the crew and technicians of RV KEXUE (https://cstr.cn/31114.02.MORV) for providing support and assistance in data and sample collection.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ces.2025.121422.

Data availability

No data was used for the research described in the article.

References

Artemenko, A., Shchukarev, A., Štenclová, P., Wågberg, T., Segervald, J., Jia, X., Kromka, A., 2021. Reference XPS spectra of amino acids. Mater. Sci. Eng. 1050 (1), 012001. https://doi.org/10.1088/1757-899X/1050/1/012001.

Barletta, R.E., Krause, J.W., Goodie, T., El Sabae, H., 2015. The direct measurement of intracellular pigments in phytoplankton using resonance Raman spectroscopy. Mar. Chem. 176, 164–173. https://doi.org/10.1016/j.marchem.2015.09.005.

Busch, K., Slaby, B.M., Bach, W., Boetius, A., Clefsen, I., Colaço, A., Creemers, M., Cristobo, J., Federwisch, L., Franke, A., Gavriilidou, A., Hethke, A., Kenchington, E., Mienis, F., Mills, S., Riesgo, A., Ríos, P., Roberts, E.M., Sipkema, D., Pita, L., Schupp, P.J., Xavier, J., Rapp, H.T., Hentschel, U., 2022. Biodiversity, environmental drivers, and sustainability of the global deep-sea sponge microbiome. Nat. Commun. 13 (1), 5160. https://doi.org/10.1038/s41467-022-32684-4.

Chen, S., Liu, Z., Tan, H., Chen, Y., Zhu, S., Liu, H., Zhang, W., 2020. Photeroids A and B, unique phenol–sesquiterpene meroterpenoids from the deep-sea-derived fungus Phomopsis tersa. Org. Biomol. Chem. 18 (4), 642–645. https://doi.org/10.1039/C9OB02625H.

Du, Z., Zhang, X., Luan, Z., Wang, M., Xi, S., Li, L., Wang, B., Cao, L., Lian, C., Li, C., Yan, J., 2018. In situ Raman quantitative detection of the cold seep vents and fluids in the chemosynthetic communities in the South China Sea. Geochem. Geophys. Geosyst. 19 (7), 2049–2061. https://doi.org/10.1029/2018GC007496.

Greenfield, D.I., Marin Iii, R., Doucette, G.J., Mikulski, C., Jones, K., Jensen, S., Roman, B., Alvarado, N., Feldman, J., Scholin, C., 2008. Field applications of the second-generation Environmental Sample Processor (ESP) for remote detection of harmful algae: 2006-2007. Limnol. Oceanogr.-Meth. 6 (12), 667–679. https://doi.org/10.4319/lom.2008.6.667.

Hu, J., Zhao, B., Xu, W., Li, B., Fan, Y., 2002. Surface-enhanced Raman spectroscopy study on the structure changes of 4-mercaptopyridine adsorbed on silver substrates and silver colloids. Spectrochim. Acta A 58 (13), 2827–2834. https://doi.org/10.1016/S1386-1425(02)00074-4.

Huang, H., du Toit, H., Besenhard, M.O., Ben-Jaber, S., Dobson, P., Parkin, I., Gavriilidis, A., 2018. Continuous flow synthesis of ultrasmall gold nanoparticles in a microreactor using trisodium citrate and their SERS performance. Chem.eng. Sci., 189, 422–430. https://doi.org/10.1016/j.ces.2018.06.050.

Huang, Y., Yuan, B., Wang, X., Dai, Y., Wang, D., Gong, Z., Chen, J., Shen, L., Fan, M., Li, Z., 2023. Industrial wastewater source tracing: the initiative of SERS spectral signature aided by a one-dimensional convolutional neural network. Water Res. 232, 119662. https://doi.org/10.1016/j.watres.2023.119662.

Huard, D.J.E., Demissie, A., Kim, D., Lewis, D., Dickson, R.M., Petty, J.T., Lieberman, R. L., 2019. Atomic structure of a fluorescent Ag8 cluster templated by a multistranded DNA scaffold. J. Am. Chem. Soc. 141 (29), 11465–11470. https://doi.org/10.1021/jacs.8b12203.

Joye, S.B., Boetius, A., Orcutt, B.N., Montoya, J.P., Schulz, H.N., Erickson, M.J., Lugo, S. K., 2004. The anaerobic oxidation of methane and sulfate reduction in sediments from Gulf of Mexico cold seeps. Chem. Geol. 205 (3), 219–238. https://doi.org/10.1016/j.chemgeo.2003.12.019.

Kloprogge, J.T., Wharton, D., Hickey, L., Frost, R.L., 2002. Infrared and Raman study of interlayer anions CO_3^{2-}, NO_3^-, SO_4^{2-} and ClO_4^- in Mg/Al-hydrotalcite. Am. Mineral. 87 (5–6), 623–629. https://doi.org/10.2138/am-2002-5-604.

LaBrie, R., Péquin, B., Fortin St-Gelais, N., Yashayaev, I., Cherrier, J., Gélinas, Y., Guillemette, F., Podgorski, D.C., Spencer, R.G.M., Tremblay, L., Maranger, R., 2022. Deep ocean microbial communities produce more stable dissolved organic matter through the succession of rare prokaryotes. Sci. Adv. 8 (27). https://doi.org/10.1126/sciadv.abn0035 eabn0035.

Liu, C., Yang, M., Yu, J., Lei, F., Wei, Y., Peng, Q., Li, C., Li, Z., Zhang, C., Man, B., 2020. Fast multiphase analysis: Self-separation of mixed solution by a wettability-controlled CuO@Ag SERS substrate and its applications in pollutant detection. Sensor. Actuat. B-Chem., 307, 127663. https://doi.org/10.1016/j.snb.2020.127663.

Liu, C., Yin, N., Jiang, T., Li, Z., Zhang, C., Man, B., Yu, J., 2023. Microextraction of electric fields and analytes by Janus hydrophobic/hydrophilic microfiber filter: application in Raman detection of persistent organic pollutants in complex liquid environments. Sensor. Actuat. B-Chem., 397, 134709. https://doi.org/10.1016/j.snb.2023.134709.

Ma, Y., Tian, P., Bounmyxay, M., Zeng, Y., Wang, N., 2021. Calcium carbonate@silica composite with superhydrophobic properties. Molecules 26, 7180. https://doi.org/10.3390/molecules26237180.

Ni, M., Ratner, B.D., 2008. Differentiating calcium carbonate polymorphs by surface analysis techniques—an XPS and TOF-SIMS study. Surf. Interface Anal. 40 (10), 1356–1361. https://doi.org/10.1002/sia.2904.

Niero, H., da Silva, M.A.C., de Felicio, R., Trivella, D.B.B., Lima, A.O.D.S., 2021. Carotenoids produced by the deep-sea bacterium Erythrobacter citreus LAMA 915: detection and proposal of their biosynthetic pathway. Folia Microbiol. 66 (3), 441–456. https://doi.org/10.1007/s12223-021-00858-0.

Ou, W., Shen, J., Zhong, J., He, J., Lei, D., Li, H., Chen, Y., Wang, C., Wu, H., Zhou, B., Tang, X., Fan, Y., Lu, J., Li, Y.Y., 2023. Rapid on-site detection of zinc pyrithione in real-life samples with unprecedented selectivity and sensitivity. Sensor. Actuat. B-Chem., 378, 133129. https://doi.org/10.1016/j.snb.2022.133129.

Peltzer, E.T., Zhang, X., Walz, P.M., Luna, M., Brewer, P.G., 2016. In situ Raman measurement of HS^- and H_2S in sediment pore waters and use of the HS^-:H_2S ratio as an indicator of pore water pH. Mar. Chem. 184, 32–42. https://doi.org/10.1016/j.marchem.2016.05.006.

Somoza, L., Rueda, J.L., González, F.J., Rincón-Tomás, B., Medialdea, T., Sánchez-Guillamón, O., Hoppert, M., Vázquez, J.T., Madureira, P., Santofimia, E., López-Pamo, E., Palomino, D., Ortíz, J.E., Blanco, L., del Carmen Fernández-Puga, M., Fernández-Salas, L.M., Reitner, J., 2021. A relict oasis of living deep-sea mussels Bathymodiolus and microbial-mediated seep carbonates at newly-discovered active cold seeps in the Gulf of Cádiz. NE Atlantic Ocean. Palz 95 (4), 793–807. https://doi.org/10.1007/s12542-021-00594-3.

Tortorella, E., Tedesco, P., Palma Esposito, F., January, G.G., Fani, R., Jaspars, M., De Pascale, D., 2018. Antibiotics from deep-sea microorganisms: current discoveries and perspectives. Mar. Drugs 16, 355. https://doi.org/10.3390/md16100355.

Wang, S., Pan, R., He, W., Li, L., Yang, Y., Du, Z., Luan, Z., Zhang, X., 2023. In situ surface-enhanced Raman scattering detection of biomolecules in the deep ocean. Appl. Surf. Sci. 620, 156854. https://doi.org/10.1016/j.apsusc.2023.156854.

Wang, S., Xi, S., Pan, R., Yang, Y., Luan, Z., Yan, J., Zhang, X., 2022. One-step method to prepare coccinellaseptempunctate-like silver nanoparticles for high sensitivity SERS detection. Surf. Interfaces 35, 102440. https://doi.org/10.1016/j.surfin.2022.102440.

Wang, X., Li, C., Zhou, L., 2017. Metal concentrations in the mussel *Bathymodiolus* platifrons from a cold seep in the South China Sea. Deep-Sea Res. PT. I 129, 80–88. https://doi.org/10.1016/j.dsr.2017.10.004.

Wei, Z.-F., Li, W.-L., Li, J., Chen, J., Xin, Y.-Z., He, L.-S., Wang, Y., 2020. Multiple in situ nucleic acid collections (MISNAC) from deep-sea waters. Front. Mar. Sci. 7, 1–8. https://doi.org/10.3389/fmars.2020.00081.

Williams, C.T., Tolia, A.A., Weaver, M.J., Takoudis, C.G., 1996. Surface-enhanced Raman spectroscopy as an in-situ real-time probe of no reduction over rhodium at high gas pressures. Chem. Eng. Sci. 51 (10), 1673–1682. https://doi.org/10.1016/0009-2509(96)00026-7.

Xu, Y., He, P., Ahmad, W., Hassan, M.M., Ali, S., Li, H., Chen, Q., 2022. Catalytic hairpin activated gold-magnetic/gold-core-silver-shell rapid self-assembly for ultrasensitive Staphylococcus aureus sensing via PDMS-based SERS platform. Biosens. Bioelectron. 209, 114240. https://doi.org/10.1016/j.bios.2022.114240.

Yang, G., Zhang, J., Zhang, S., Yu, L., Zhang, P., Zhu, B., 2013. Preparation of triazine derivatives and evaluation of their tribological properties as lubricant additives in poly-alpha olefin. Tribol. Int. 62, 163–170. https://doi.org/10.1016/j.triboint.2013.02.024.

Yang, G., Zhang, K., Qu, X., Xu, W., Xu, S., 2022. Ratiometric pH-responsive SERS strategy for glioma boundary determination. Talanta 250, 123750. https://doi.org/10.1016/j.talanta.2022.123750.

Zhang, L., Shi, Y., Wang, L., Hu, C., 2018. AgBr-wrapped Ag chelated on nitrogen-doped reduced graphene oxide for water purification under visible light. Appl. Catal. B-Environ. Energy 220, 118–125. https://doi.org/10.1016/j.apcatb.2017.08.038.

Zhang, X., Du, Z., Zheng, R., Luan, Z., Qi, F., Cheng, K., Wang, B., Ye, W., Liu, X., Lian, C., Chen, C., Guo, J., Li, Y., Yan, J., 2017. Development of a new deep-sea hybrid Raman insertion probe and its application to the geochemistry of hydrothermal vent and cold seep fluids. Deep-Sea Res. PT. I 123, 1–12. https://doi.org/10.1016/j.dsr.2017.02.005.

Zhou, Z., Tran, P.Q., Adams, A.M., Kieft, K., Breier, J.A., Fortunato, C.S., Sheik, C.S., Huber, J.A., Li, M., Dick, G.J., Anantharaman, K., 2023. Sulfur cycling connects microbiomes and biogeochemistry in deep-sea hydrothermal plumes. ISME J. 17 (8), 1194–1207. https://doi.org/10.1038/s41396-023-01421-0.