Supporting Information

Dimethylsulfoniopropionate decorated cryogels as synthetic spatially structured habitats of marine bacterial communities

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Materials and methods

Materials

Prior to the polymerizations, *N*,*N*-dimethylacrylamide (DMAAm, 99%, Sigma Aldrich) and 2-hydroxyethyl methacrylate (HEMA, 90%, TCI) were passed through a small column filled with inhibitor removal beads (replacement packing for removing hydroquinone and monomethyl ether hydroquinone, Sigma Aldrich). *N*,*N*,*N*',*N*'-Tetramethylethylenediammine (TMEDA, 99%) was purchased from Sigma Aldrich. Potassium peroxodisulfate ($K_2S_2O_8$, ≥ 98%) was received from Fluka Analytical. *N*,*N*'-Methylenebisacrylamide (MBAAm, 99%) was purchased from Alfa Aesar. Acryloxyethyl thiocarbamoyl rhodamine B (ATRB) was received from Polysciences. Dimethylpropiothetin hydrochloride (DMSP-HCl) was purchased from Carbosynth (98%). Thionyl chloride ($SOCl_2$, 99.7%) was received from Acros Organics. Anhydrous acetonitrile was obtained using a solvent purification system (MB-SPS-800 by MBraun). All other chemicals were purchased from standard suppliers and were used without any further purification unless otherwise stated. In all cryogel preparations, ultrapure water (Merck Millipore water purification system) was used unless otherwise stated. For a precise and adjustable control of the reaction temperature for the cryopolymerization reactions, a cryostat was used (Julabo FP40-MC, Julabo GmbH, Seelbach, Germany).

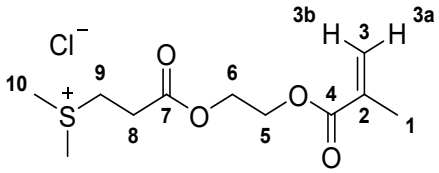
Instrumentation

Proton nuclear magnetic resonance spectra (^{1}H NMR) and carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded using a 300 MHz Avance I spectrometer equipped with a dual ^{1}H and ^{13}C probe head and a 120×BACS automatic sample changer (Bruker, Germany). The residual ^{1}H peak of the deuterated solvent was used for chemical shift referencing, chemical shifts are given in parts per million (ppm). ^{13}C solid-state NMR spectra were recorded using a 400 MHz Bruker Avance III HD spectrometer with a 4 mm MAS probe head. Cryogel micrographs for morphological investigations and pore size measurements were obtained using a Sigma VP Field Emission Scanning Electron Microscope (Zeiss, Jena, Germany) with an SE-2 detector and an accelerating voltage of 10 kV was used. Therefore, freeze-dried cryogel slices were obtained using the freeze dryer Alpha 2-4 LD plus (Martin Christ) before they were coated with a 9 nm platinum layer using a CCU-010 HV sputter coater (Safematic, Zizers, Switzerland) and fixed onto the plate holder pins with conductive carbon adhesive pads. For SEM analysis of bacteria seeded cryogel slices, the samples were fixed with 2% glutaraldehyde in PBS for 30 to 40 min and subsequently washed thrice with PBS for 10 min per each step. After fixation, samples were dehydrated using a graded ethanol series (30, 50, 70, 90 and 100%) and critically point dried (Leica EM CPD300, Leica Microsystems, Wetzlar). Afterwards, the samples were coated with platinum as described above. Image J was used for pore size measurements. A full view picture of the entire sample was obtained before taking selected views with higher magnifications. For confocal laser scanning microscopy (CLSM) of bacteria seeded cryogels, the cylindrical samples were transferred directly after cultivation to a 24-well microscope plate (Sarstedt®, Numbrecht, Germany) with the cryogel top side to the well bottom and finally submerged with PBS. Samples were subsequently analyzed with a LSM880 Elyra PS.1 system (Zeiss, Oberkochen, Germany) using a C-Apochromat 40x/1.2 W Korr FCS M27 objective. For excitation of fluorescence a 514 nm laser was employed for rhodamine B labelled cryogels, a 633 nm laser for NIR680 stained *Mameliella* bacteria and a 405 nm laser for DAPI stained *Marinobacter* bacteria. Emission filters were set to 638 to 747 nm (NIR), 535 to 642 nm (Rhodamine B) and 410 to 545 nm (DAPI). Images were acquired and analyzed using the ZEN software, version 2.3 SP1 (Zeiss). Electrospray-ionisation mass spectrometry (ESI-MS) was performed with a microTOF Q-II from Bruker Daltonics which was equipped with an automatic syringe pump from KD Scientific. The ESI-Q-ToF mass spectrometer was operating at 4.5 kV in the positive ion mode and at a desolvation temperature of 180 °C. Nitrogen was used as the nebulizer and drying gas. Sample injection was performed using a constant flow rate of 3 µL/min. The instrument was calibrated in the

m/z range 50 to 3000 using a calibration standard (sodium formiate). The data were processed *via* Bruker Data Analysis software version 4.2. Thermogravimetric analysis (TGA) was carried out under nitrogen atmosphere using a Netzsch TG209 F1 Libra (Selb, Germany). For the measurements, a standard method was used (25 to 600 °C with a heat rate of 20 K/min, 29 min). After the measurements, smoothing was applied as post processing step if necessary. Fourier-transform infrared (FT-IR) spectra were recorded from 400 to 4000 cm^{-1} using an IR-Affinity-1 CE system (Shimadzu, Kyoto, Japan) which was equipped with a quest ATR diamond extended range X - single reflection-ATR accessory with a diamond crystal. The spectra were smoothed after the measurements if necessary.

Synthesis of the functional DMSP based building block (3-(2-(methacryloyloxy)ethoxy)-3-oxopropyl) dimethylsulfonium chloride (DMSP-HEMA)

To a suspension of DMSP-HCl (5.0 g, 29.292 mmol) in acetonitrile (250 mL), $SOCl_2$ (3.2 mL, 43.870 mmol) was added dropwise using a syringe pump (World Precision Instruments, Sarasota, Florida, 1 mL/min) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for 18 h after which it became a green coloured solution. HEMA was added dropwise (0.6 mL/min, 3.6 mL, 29.682 mmol) and the solution was stirred for another 26 h before the solvent was removed under reduced pressure. Acetonitrile (50 mL) was added to the residue followed by filtration. The precipitate was washed several times with acetonitrile (2 × 50 mL) and the combined organic layers were evaporated to dryness. The residual orange oil was dissolved in acetonitrile (15 mL) and precipitated in 10× excess cold diethyl ether. The supernatant was discarded and the procedure was repeated two more times before the orange oil was dried *in vacuo* (5.055 g, 61%).



^{1}H NMR [ppm] (D_2O, 300 MHz): 1.93 (CH_3-C, H_1, 3H), 2.95 – 3.00 (CH_3-S, m, H_{10}, 6.55H), 3.03 – 3.13 (CH_2-C, m, H_8, 6.56H), 3.54 – 3.64 (CH_2-S, m, H_9, 2.24H), 4.39 – 4.54 (2 × O-CH_2, m, H_{5+6}, 4.26 H), 5.75 (CH_2=C, s, H_{3a}, 1H), 6.15 (CH_2=C, s, H_{3b}, 1 H).

^{13}C NMR [ppm] (D_2O, 300 MHz): 17.4 (CH_3-C, C_1), 25.2 (CH_3-S, C_{10}), 28.6 (CH_2-C, C_8), 38.7 (CH_2-S, C_9), 62.9 (CH_2-O, C_5), 63.7 (CH_2-O, C_6), 127.2 (CH_2=C, C_3), 135.7 (C_q, C_2), 169.4 (C(=0)-C_q, C_4), 171.6 (C(=O)-CH_2, C_7).

HR-ESI-MS: 247.1004 ($[M]^+$, calc. for $C_{11}H_{19}O_4S$: 247.0999), 283.0762 ($[M+H]^+$, calc. for $C_{11}H_{20}O_4SCl$: 283.0765)

Elemental analysis: calculated C 46.72%, H 6.77%, S 11.34%, Cl 12.54%; found C 43.09%, H 6.87%, N 0.54%, S 10.35%, Cl 15.03%

Synthesis of cryogels

DMSP functionalized gels

A stock solution containing DMSP-HCl (1.005 g, 5.889 mmol), DMAAm (1.133 mL, 10.995 mmol), MBAAm (375 mg, 2.432 mmol), $K_2S_2O_8$ (156 mg, 0.577 mmol) and acryloyl rhodamine B (14 mg, 0.021 mmol) in Millipore water (50 mL) was purged with argon at 0 °C for 30 min. 5 mL of the solution were drawn up into 10 polypropylene syringes before adding an aqueous TMEDA solution (100 µL of

0.868 M, 0.087 mmol). The syringes were closed and placed in a cryostat bath which was previously adjusted to a cooling temperature of −12 °C. After 20 h, the syringes were stored at room temperature for one hour followed by immersion of the cryogels in deionized water (50 mL). The water was changed twice a day for three consecutive days. The gels were cut into small slices using a steel blade and were washed with a 50% methanolic solution in water, before methanol was used. The solvent was replaced every 30 min within the first hour and after 5 h. Methanol was removed from the cryogels using a 50% methanolic solution in water first, before applying water with subsequent changes (every 30 min for 2 h). The samples were finally freeze-dried for 2 d.

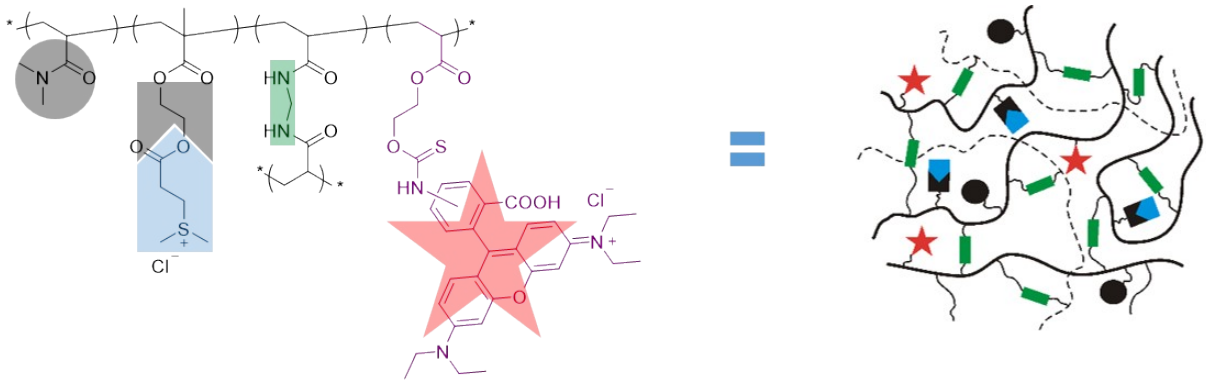


Figure S1. Schematic representation of the chemical structure of DMSP functionalized polymeric cryogels **CG1** to **CG10** based on **DMAAm**, **DMSP-HEMA**, **MBAAm** and rhodamine B acrylate.

Unfunctionalized control cryogel samples

A stock solution containing HEMA (0.53 mL, mmol), DMAAm (1.414 mL, 13.722 mmol), MBAAm (468 mg, 3.036 mmol), $K_2S_2O_8$ (155 mg, 0.573 mmol) and acryloyl rhodamine B (17.5 mg, 0.027 mmol) in Millipore water (50 mL) was purged with argon at 0 °C for 30 min. 5 mL of the solution were drawn up into 10 polypropylene syringes before adding an aqueous TMEDA solution (100 µL of 0.868 M, 0.087 mmol). The syringes were closed and placed in a cryostat bath which was previously adjusted to a cooling temperature of −12 °C. After 20 h, the syringes were stored at room temperature for one hour followed by immersion of the cryogels in deionized water (50 mL). The water was changed twice a day for three consecutive days. The gels were cut into small slices using a steel blade and were washed with a 50% methanolic solution in water, before methanol was used. The solvent was replaced every 30 min within the first hour and after 5 h. Methanol was removed from the cryogels using a 50% methanolic solution in water first, before applying water with subsequent changes (every 30 min for 2 h). The samples were finally freeze-dried for 2 d.

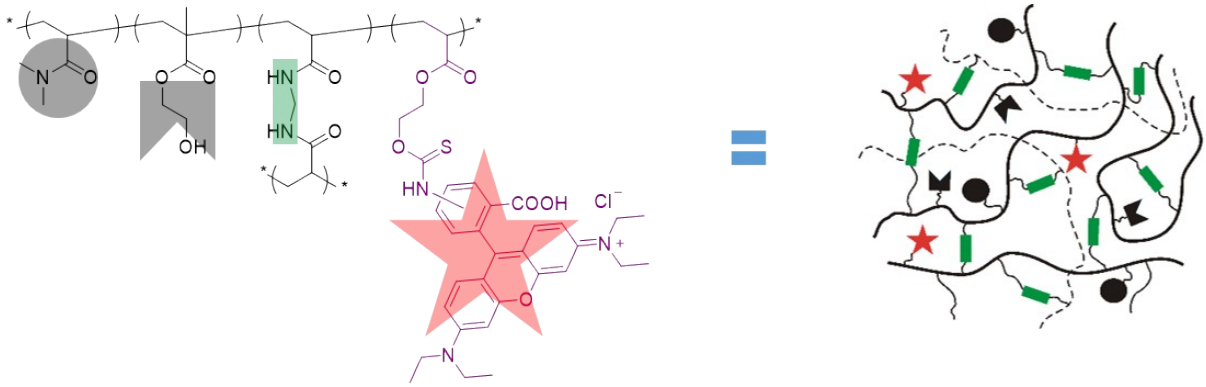


Figure S2. Schematic representation of the chemical structure of unfunctionalized polymeric cryogels **CG1-UF** to **CG10-UF** based on **DMAAm**, **HEMA**, **MBAAm** and rhodamine B acrylate.

DMSP metabolism pathways analysis

The strain CS4 contains the proteins for both the DMSP demethylation (DmdA, B, C, and D) and the cleavage pathway (DddW and DddP). Real time-qPCR (RT-qPCR) revealed that the expression of the gene *dmdW* for the cleavage pathway was significantly upregulated after treatment with 100 µM DMSP for 8 h (**Figure S21**). The two DMSP metabolic pathways of CS4 were further confirmed by the quantification of DMSP reduction in the supernatant and the production of dimethylsulfide (DMS) and methanethiol (MeSH) after treatment with 100 µM DMSP for 14 h (**Figure S18A-C**). Those proteins which are responsible for the direct DMSP utilization were not present in the CS1 genome. Here, three proteins – DmdB, DmdC and DmdD – with the potential to metabolize 3-(methylthio) propionate (MMPA), a product of DMSP demethylation by DmdA, could be predicted. MeSH was detected when CS1 and/or CS4 were incubated with 100 µM MMPA for 14 h, which confirmed that both of them can metabolize MMPA to generate MeSH (**Figure S19**). The proteins that are responsible for further transferring MeSH into amino acids as methionine or homocysteine could be predicted from both bacterial genomes (**Table S3**). The intracellular accumulation of methionine and homocysteine in CS4 was observed (**Figure S18D-F**). RT-qPCR revealed the significant upregulation of the gene *metY3*, annotated as an acylhomoserine sulfhydrylase that might transform methanethiol into methionine, by DMSP treatment after 14 h (**Figure S21**).

Bacterial strains CS4 and CS1 and inoculum preparation

Marine bacteria *Mameliella* sp. CS4 and *Marinobacter* sp. CS1 were isolated from diatom *Chaetoceros socialis* culture and identified in a previous study.[1] Their genomes are available from NCBI genome database with accessions JAHXRQ000000000 and JAHXRN000000000. The bacterial inoculum of the two strains was prepared as follows: The single colonies were inoculated into 3 mL liquid marine broth (MB) medium in 10 mL sterile tubes and incubated at 28 °C with 150 rpm shaking. When the OD_{600} of bacterial cultures reached a value of 0.5 to 0.8, the bacterial cultures were transferred into 10 mL fresh MB in T-25 flask in a ratio of 1:100. After incubating for another two days, 10 mL bacterial cultures for each strain were harvested in 50 mL Falcon tubes by centrifugation at 8000 rpm for 15 min at room temperature. Bacterial cell pellets were washed two times with 3 mL minimal basal medium (MBM) to completely remove MB and were resuspended in MBM to OD_{600} = 0.4. This suspension was used as two times inoculum. For fluorescence labelled bacterial inoculi, 10 µM DAPI (4',6-diamidino-2-phenylindole) (Thermo Fisher) and NIR680 dye (biotium, San Francisco) solutions were used for staining bacteria for 20 min at room temperature in darkness. The free dyes were removed by washing two times with MBM. The cells were resuspended in MBM to OD_{600} = 0.4 similarly as described earlier.

Incubation conditions for bacterial metabolism of DMSP and DMSP cryogel

The bacterial inoculum was mixed with 100 µM DMSP or DMSP cryogel (mass range 13 to 15 mg/slice with 1.43 to 1.75 µmol DMSP/mg cryogel) in 2.5 mL MBM for bacterial metabolism testing. The bacteria were incubated at 28 °C with 150 rpm shaking. In a pretest using DMSP cryogel with the strain CS4, a time of 14 h was determined for the incubation. After incubation at 28 °C for 14 h, the liquid culture which was separated into different parts and cryogel were applied for further analysis by GC-MS, LC-MS, SEM, CLSM, and qPCR tests.

Bacterial quantification by quantitative polymerase chain reaction (qPCR)

The primers for specifically amplifying the two strains CS4, and CS1 were designed and used in the previous study.[1] For quantitative polymerase chain reaction (qPCR) analysis, 2 mL culture sample or cryogel was transferred to 2 mL Eppendorf tubes for the rapid genome DNA preparation as previously described. For the supernatant, we harvested the cell pellet by centrifugation at 13000 rpm for 20 min. 100 µL TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0) was added to resuspend the cell pellet. For the

gel samples, after removing the supernatant completely, 100 µL TE buffer were added and mixed with the cryogel. Then, all the samples were heated to 100 °C for 10 min in a boiled water broth. After heating, the samples were transferred on ice for 10 min standing. Finally, the samples were centrifuged at 13000 rpm at 4 °C for 15 min. After centrifugation, 0.5 µL of supernatant were added as the DNA template into a 10 µL qPCR reaction. The relative bacterial density was related to the Cq value from qPCR based on the established formulas, *e.g*. for CS4: Y = −1.438 In(x) + 47.214, and for CS1: Y = −1.567 In(x) + 44.944. Y represents the Cq value, and x is the relative cell density.

Solid phase micro-extraction (SPME) and GC-MS

For the quantification of DMS and methanethiol in the headspace of samples, 500 µL of the samples were transferred into 5 mL screw-cap sealed vials. The 65 µm PDMS/DVB SPME fiber (Supelco®, Bellefonte, PA) was assembled in a manual holder and used for headspace extraction. After extraction for 15 min at room temperature, the fiber in the holder was loaded to GC Orbitrap (Thermo Scientific) equipped with a Zebron ZB-SemiVolatiles column (30 mL × 0.25 mm ID × 0.25 µm df with 10 m guardian end) (Phenomenex). The GC oven was set isothermally at 35 °C with helium as carrier gas at a flow rate of 1.2 mL/min. S/SL model was set as splitless for 1.0 min. GC temperature ramps: 0 to 5 min, 35 °C, increased to 250 °C at the rate of 80 °C/min, and 2 min at 250 °C. For Orbitrap, full MS-SIM mode was selected with a runtime of 9.5 min. EI ionization and positive mode were used and with a resolution of 120,000. The scan range was set from 30 to 150 m/z. Standards were always used at the same time with samples for calibrating DMS and methanethiol. A DMS calibration curve was made by pipetting aqueous DMSP standard solutions to 450 µL of 1 M NaOH in 5 mL vial to make final concentrations of 6.25, 12.5, 25, 50 and 100 µM. To make a methanethiol calibration curve, sodium methanethiolate (Sigma Aldrich) was dissolved in 1 mL of 10 M NaOH as a stock solution. The stock solution was diluted by 1 M NaOH. The standard solution was added to 450 µL of 2 M sulfuric acid in sealed 5 mL screw-cap vials to make final concentrations of 12.5, 25, 50, 100, and 200 µM. For all sample treatments, the vials were sealed immediately after the addition of chemicals or bacterial suspensions and incubated in the condition mentioned above.

Intracellular metabolites and supernatant extraction for LC-MS

The liquid culture used for GC-MS measurement was transferred into 1.5 mL Eppendorf tubes and centrifuged at 12000 rpm for 5 min for intracellular metabolites extraction. The cell pellets were mixed with 100 µL 90% acetonitrile in water and sonicated in Bandelin Sonopuls HD 2070 ultrasonic homogenizer (Bandelin, Germany) for 20 sec for cell lysis. After centrifugation at 13200 rpm for 10 min, the supernatant was transferred into vials for LC-MS analysis. 2 mL liquid culture were centrifuged at 13200 rpm for 10 min to result the supernatant sample. For the extraction of zwitterionic compounds from the supernatant, the Discovery® DSC-MCAX SPE Tubes (Supelco®, Bellefonte, PA) were used for solid phase extraction (SPE) based on the recommended manual from the manufacturer. Briefly, the SPE columns were conditioned with methanol and equilibrated with 10 mM ammonium acetate (pH 6). The supernatant was diluted 1:1 with 10 mM ammonium acetate (pH 6) and were loaded onto the SPE columns. After washing with 1 M acetic acid, zwitterionic compounds were eluted with 5% ammonium hydroxide in methanol. The samples were dried in a vacuum desiccator overnight. The samples were dissolved in 200 µL methanol. After centrifugation at 13200 rpm for 10 min, the supernatant was used for LC-MS analysis. The DMSP standard was prepared in serial gradient dilutions at 2× concentrations from 2.5 µM to 25 µM. LC-MS analysis was performed using a Dionex Ultimate 3000 system coupled to a Q-Exactive Plus Orbitrap mass spectrometer (Thermo Scientific). The LC separation was implemented by a ZIC-HILIC column (5 µm, 150 × 2.1 mm, Thermo Scientific) with the same type of guard column (5 µm, 20 × 2.1 mm, Thermo Scientific) using the elution program with the following conditions: Solution A: 2% acetonitrile + 0.1% formic acid in water, Solution B: 90%

acetonitrile + 1 mM ammonium acetate, in 10% water. Initially at 100% B for 1 min, the solution B decreased to 20% from 1 to 6.5 min, and returned to 100% B at 7.1 min, the duration time was 10 min, with a flow rate of 0.6 mL/min. The injection volume was set to 1 µL for all samples and standards. Electrospray ionization was performed in the positive mode with the following parameters: capillary temperature, 380 °C; spray voltage, 3000 V; sheath gas flow, 60 arbitrary units; and aux gas flow, 20 arbitrary units. The Full MS-SIM mode was employed from 2 to 7 min with resolution 70000, and scan range from 75 to 200 m/z.

RNA extraction and reverse transcription qPCR (RT-qPCR)

After incubation in 100 µM free DMSP or together with the DMSP cryogel, 2 mL CS4 bacterial suspensions were harvested by centrifugation at 12000 rpm for 5 min. The supernatant was discarded and the tubes with cell pellets were put into liquid N_2 for immediate freezing. Appropriate equal amounts of MN type B beads (Macherey-Nagel, Düren, Germany) to the cell pellets were added in the tubes and lysis of the cells was performed by a homogenizer with maximum speed for 3 min. The supernatant of the lysate was transferred into RNeasy spin column (QIAGEN, RNeasy Mini Kit) for RNA extraction. Reverse transcription of RNA into cDNA was applied by cDNA synthesis Kit (SuperScript™ IV VILO™ Master Mix, Thermo Scientific). The same qPCR program that was used as for bacterial quantification was performed with the primers listed in **Table S4**.

Supplementary Figures, Tables and Schemes

Synthesis of DMSP-HEMA building block

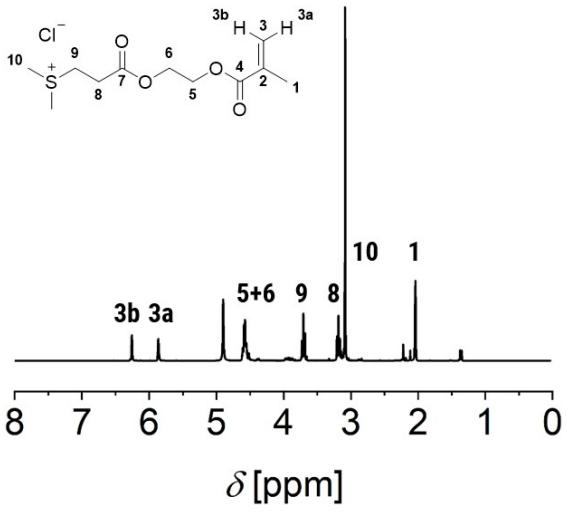


Figure S3. ^{1}H NMR spectrum of **DMSP-HEMA**, recorded in D_2O.

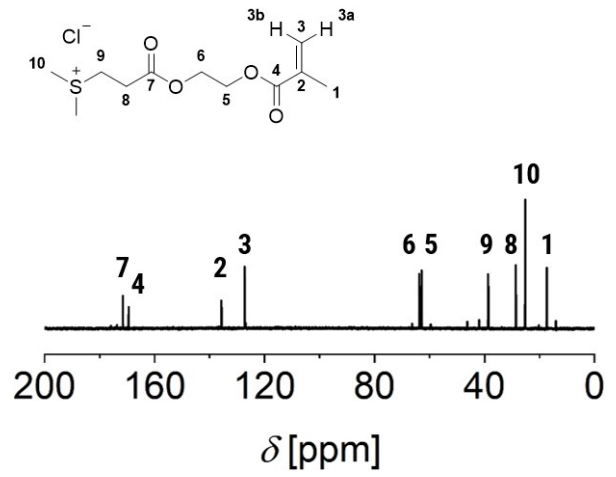


Figure S4. ^{13}C NMR spectrum of **DMSP-HEMA**, recorded in D_2O.

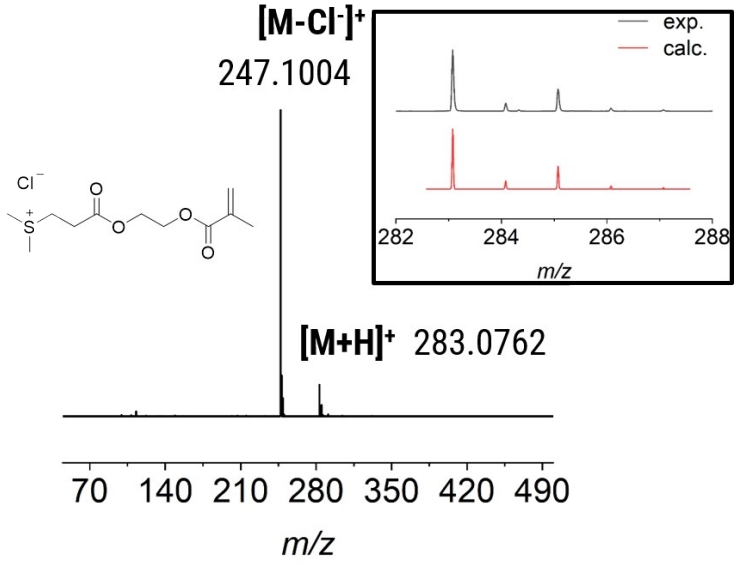


Figure S5. ESI-MS spectrum of **DMSP-HEMA** and zoomed view of the isotopic pattern.

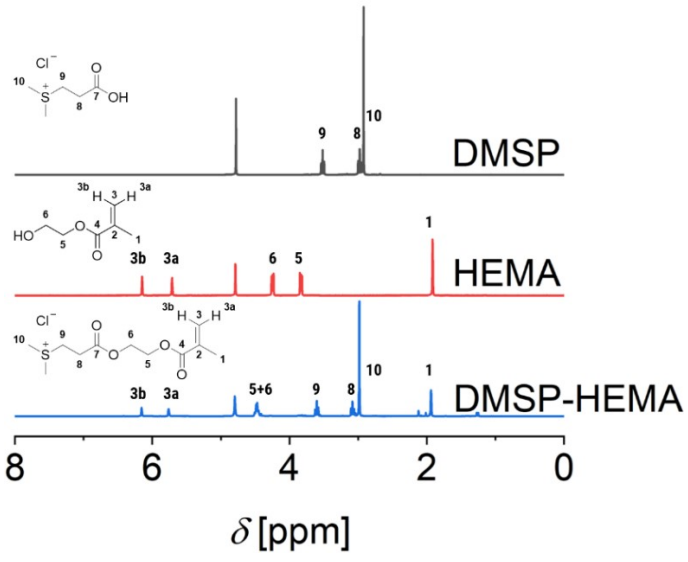


Figure S6. Stacked ^{1}H NMR spectra of the starting materials **DMSP** (**black**) and **HEMA** (**red**) in comparison with the ^{1}H NMR spectrum of the target compound **DMSP-HEMA** (**blue**). Due to the coupling, downfield shifts of the methylene groups **5** and **6** in the product spectrum are visible.

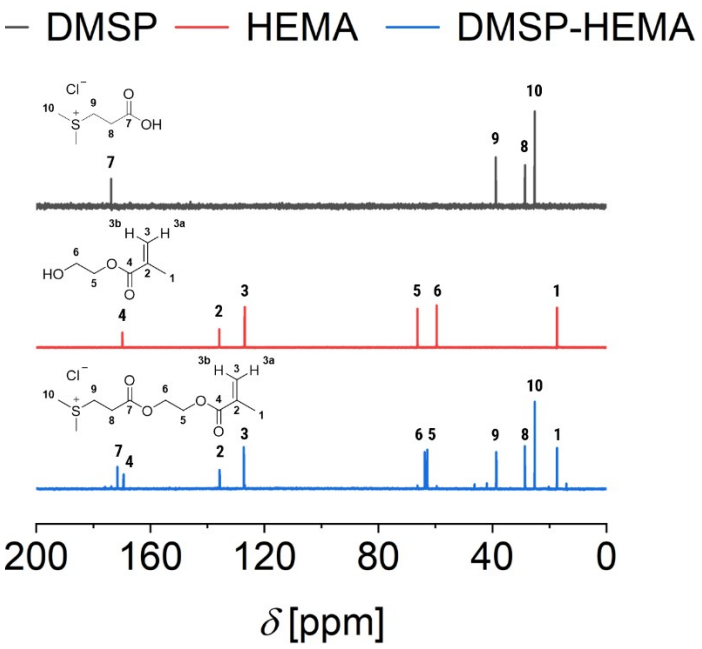


Figure S7. Stacked ^{13}C NMR spectra of the starting materials **DMSP** (**black**) and **HEMA** (**red**) in comparison with the ^{13}C NMR spectrum of the target compound **DMSP-HEMA** (**blue**). Due to the coupling, changes in the chemical shifts of the methylene groups **5** and **6** as well as of the carboxyl group **7** in the product spectrum are visible.

Stability studies of DMSP-HEMA in different pH systems by NMR

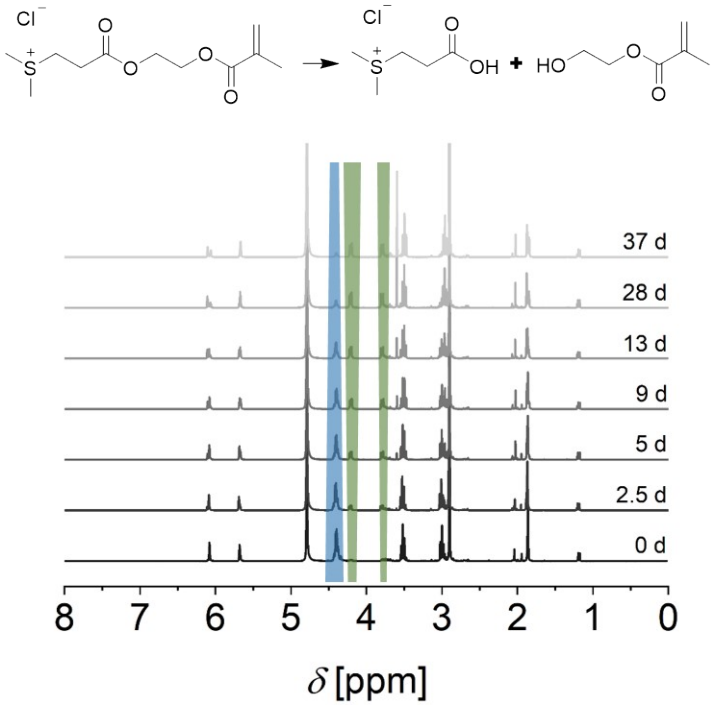


Figure S8. Degradation of **DMSP-HEMA** in aqueous solution at pH 1. ^{1}H NMR spectra were recorded in D_2O (addition of 10% (v/v) DCl). Intensity decrease of the signal at 4.4 ppm demonstrates the hydrolysis of the ester bond. **HEMA** formation is also expressed additionally by the presence of the signals at 3 ppm and 3.5 ppm belonging to the two methylene groups of free **HEMA** which are increasing over time.

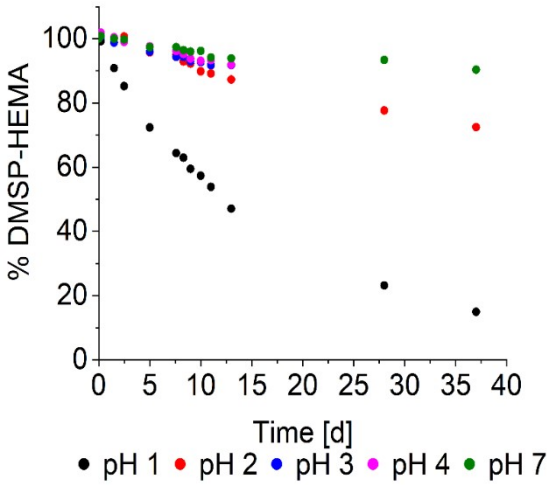


Figure S9. Degradation studies of **DMSP-HEMA** in aqueous solution at different pH. ^{1}H NMR spectra were recorded in D_2O with different amounts of DCl. After 10 days, **DMSP-HEMA** degradation already reached 50% in acidic conditions (pH 1) whereas 85 to 95% **DMSP-HEMA** is still remaining intact in higher pH systems (pH 2 up to pH 7).

Synthesis of DMSP functionalized cryogels and unfunctionalized analogues

Table S1. Overview about the reaction conditions for the preparation of DMSP functionalized cryogels (**CG**) and unfunctionalized 2-hydroxyethyl methacrylate (HEMA) containing analogues (**CG-UF**). Amounts of substances given in per cent for monomeric building blocks *N*,*N*-dimethylacrylamide (**DMAAm**), **DMSP-HEMA**, **HEMA** and *N*,*N*'-methylenebisacrylamide (**MBAAm**). [M] = monomeric concentration, [I] = initiator concentration, TMEDA/I = ratio of *N*,*N*,*N*',*N*'-tetramethylethylenediammine toC $K_2S_2O_8$, t = reaction time.

#	DMAAm mol%	DMSP-HEMA mol%	HEMA mol%	MBAAm mol%	[M] mol/L	[I] mol/L	TMEDA/I mol/mol	t [h]
CG	56.86	30.45	N/A	12.58	0.37	0.011	1.54	20
CG-UF	64.90	N/A	20.61	14.36	0.40	0.011	1.58	20

Gel fraction yields

Table S2. Overview about gel fraction yields from DMSP functionalized cryogels (**CG1** to **CG10**) and their unfunctionalized analogues (**CG-UF1** to **-10**). m_{feed} = total mass of monomers in the initial feed solution, $m_{dry\ polymer}$ = amount of dried cryogel after purification. Gel fraction yield is expressed as the ration of the amount of dry polymer obtained after cryopolymerization and the total mass of monomers in the feed solution.

	m_{feed} [mg]	$m_{dry\ polymer}$ [mg]	Gel fraction yield [%]
CG1	242.9	185.4	76.3
CG2	242.9	197.4	81.3
CG3	242.9	192.7	79.3
CG4	242.9	189.2	77.9
CG5	242.9	209.0	86.1
CG6	242.9	199.5	82.1
CG7	242.9	203.2	83.7
CG8	242.9	197.7	81.4
CG9	242.9	196.7	81.0
CG10	228.3	177.8	77.9
CG1-UF	232.4	216.8	93.3
CG2-UF	232.4	218.4	94.0
CG3-UF	232.4	236.3	101.7
CG4-UF	232.4	219.2	94.3
CG5-UF	232.4	226.9	97.6
CG6-UF	232.4	229.1	98.6

CG7-UF	232.4	230.5	99.2
CG8-UF	232.4	221.1	95.1
CG9-UF	232.4	230.9	99.3
CG10-UF	232.4	228.4	98.3

Scanning electron microscopy (SEM)

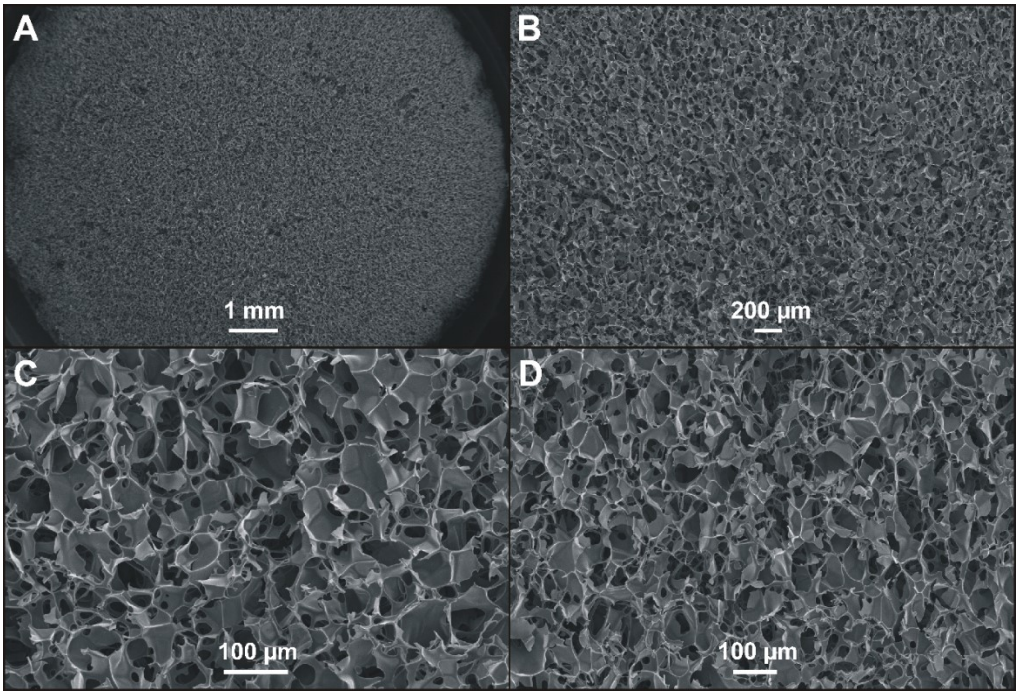


Figure S10: SEM micrographs of a representative **DMSP** functionalized cryogel **CG1** taken with different magnifications (11X (**A**), 32X (**B**), 146X (**C**), 100X (**D**)). Homogenous pore structures and macroporous morphologies could be observed among the entire cryogel series (**CG1** to **CG10**).

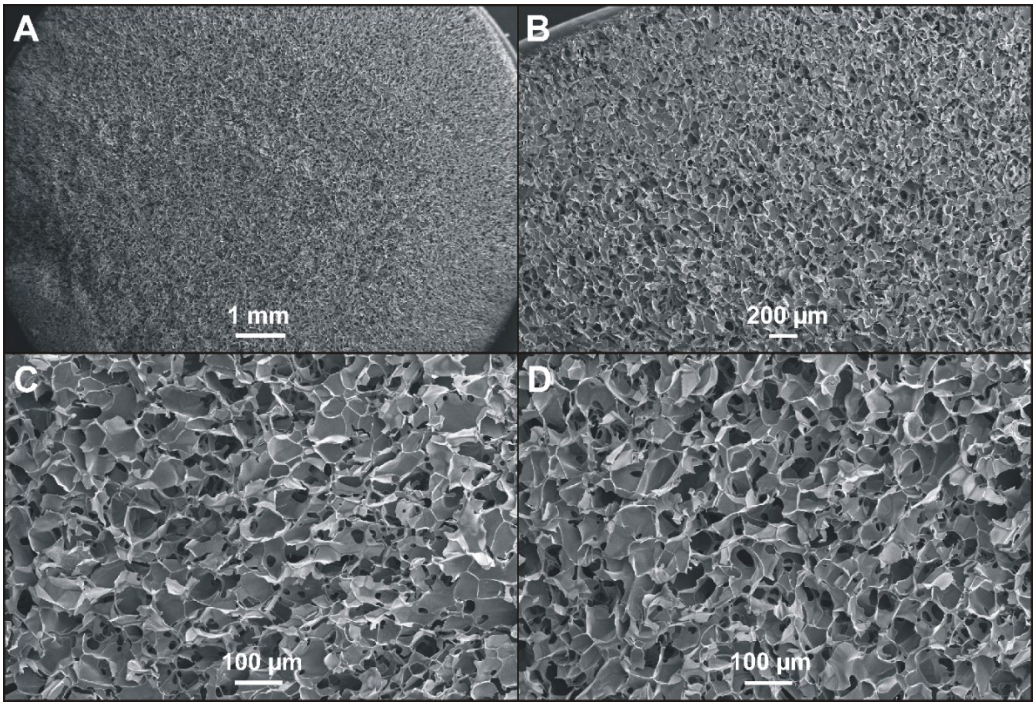


Figure S11: SEM micrographs of a representative unfunctionalized cryogel sample **CG7-UF** taken with different magnifications (11X (**A**), 31X (**B**), 105X (**C**, **D**)). Homogenous pore structures and macroporous morphologies could be observed among the entire cryogel series (**CG1-UF** to **CG10-UF**).

Solid-state magic-angle spinning NMR (ssMAS-NMR)

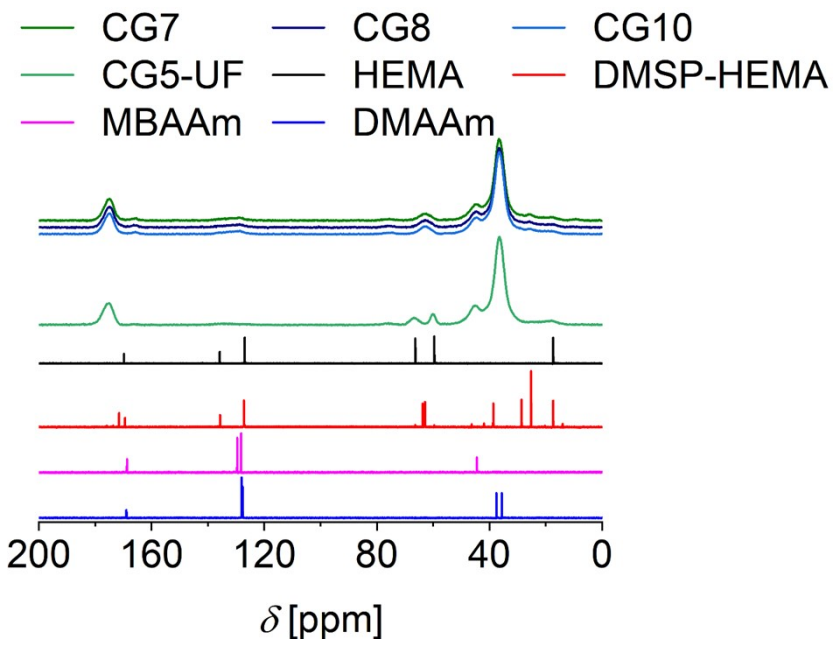


Figure S12: ^{13}C Solid state magic-angle spinning NMR spectra of DMSP functionalized cryogels (**CG7 marineblue**, **CG8 olive**, **CG10 blue**) and an unfunctionalized cryogel analogue (**CG5-UF green**) in comparison with the respective monomeric building blocks 2-hydroxyethyl methacrylate (**HEMA**, **black**), **DMSP-HEMA** (**red**), *N*,*N*'-methylenebisacrylamide (**MBAAm**, **magenta**) and *N*,*N*-dimethylacrylamide (**DMAAm**, **blue**).

The absence of signals in the typical region of double-bond carbons between 120 ppm and 140 ppm in the NMR spectra of the cryogels indicates the complete removal of potentially unreacted starting materials. On the other hand, signals of the side chains of the respective monomeric building blocks can be found in the cryogel spectra, *e.g*. the methyl group of **DMAAm** at approx. 40 ppm, the methylene group of the cross-linker **MBAAm** at approx. 45 ppm as well as either the two separate CH_2 groups of **HEMA** at approx. 60 ppm and 70 ppm – in case of unfunctionalized cryogel **CG5-UF** – or the combined signal at approx. 65 ppm of the two CH_2 groups of DMSP-HEMA for **CG7**, **CG8** and **CG10**. From that it can be concluded, that all the monomeric building blocks were successfully incorporated into the cryogel network without residual unreacted monomers.

Thermogravimetric analysis (TGA)

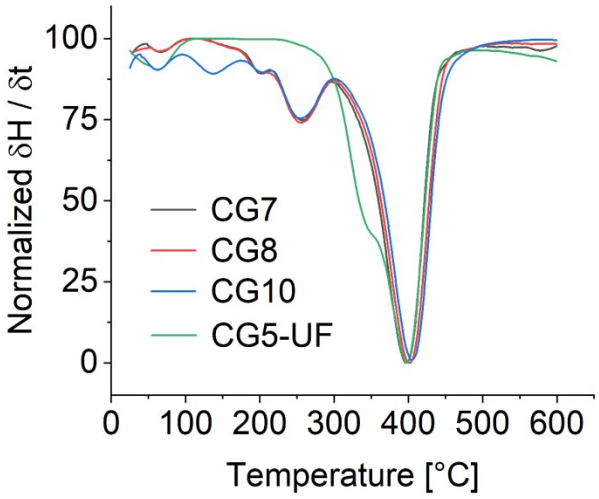


Figure S13. First derivatives of TGA curves of DMSP functionalized cryogels (**CG7**, **CG8**, **CG10**) and unfunctionalized HEMA analogue **CG5-UF**. Both types of cryogels show a late transition at 400 °C whereas DMSP functionalized gels also reveal an earlier transition at approx. 250 °C.

Scanning electron microscopy (SEM)

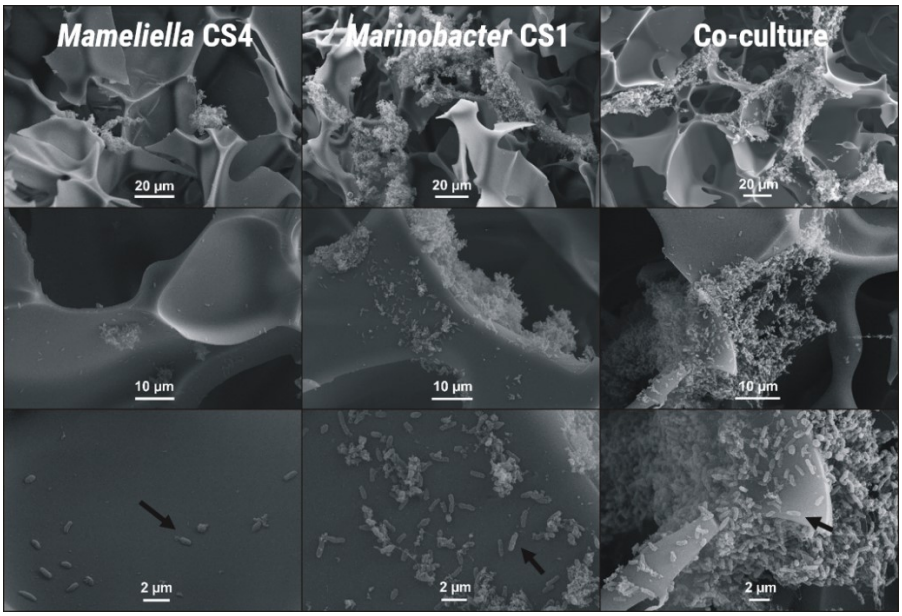


Figure S14. SEM micrographs of bacterial immobilization on **DMSP** functionalized cryogels **CG5** (left column, *Mameliella* CS4), **CG7** (middle column, *Marinobacter* CS1) and **CG8** (right column, *Mameliella* CS4 together with *Marinobacter* CS1).

Confocal laser scanning microscopy (CLSM)

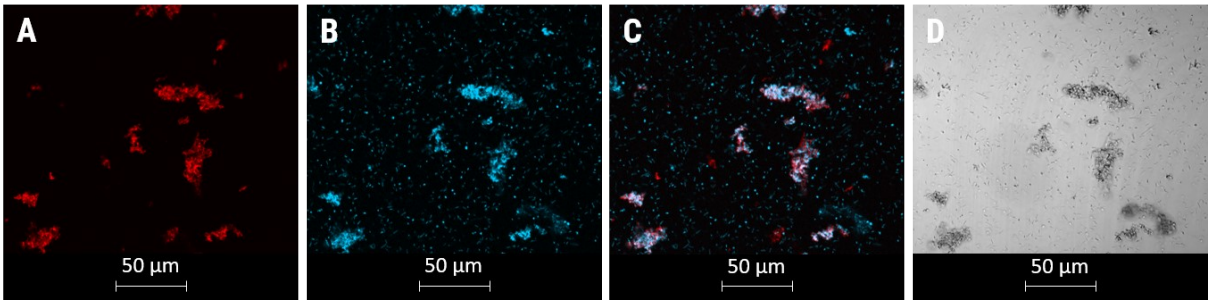


Figure S15. CLSM image of *Mameliella* CS4 and *Marinobacter* CS1 co-culture in suspension without cryogel. *Mameliella* CS4 bacteria stained with NIR680 (**A**) and *Marinobacter* CS1 stained with DAPI (**B**). Overlay image (**C**) shows localization of the individual staining patterns of the different bacteria types.

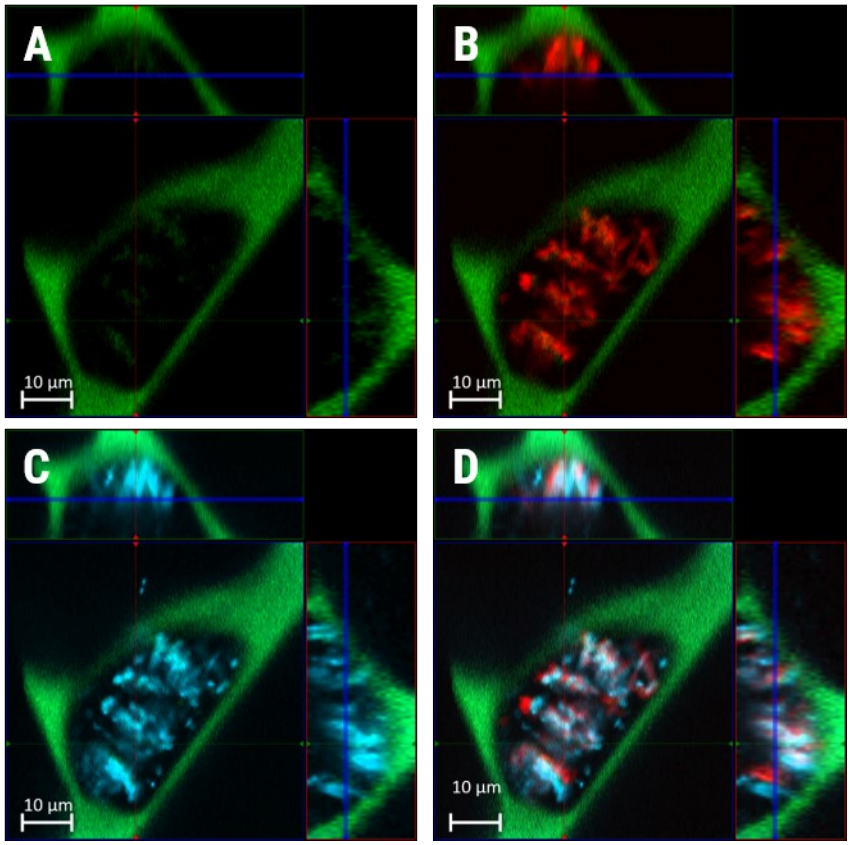


Figure S16. CLSM image of *Mameliella* CS4 and *Marinobacter* CS1 co-cultured on DMSP functionalized cryogel (**CG8**). Images depict one optical section from a z-scan and the ortho-projection of the whole image series in x (top) and y (right) direction. The cryogel network labelled with Rhodamine B (**A**) appears green, *Mameliella* CS4 bacteria stained with NIR680 (**B**), *Marinobacter CS1* stained with DAPI (**C**). Overlay image (**D**) depicts colocalization of both bacteria types at the bottom of the cryogel pore. On the unfunctionalized cryogel analogues, no bacteria could be detected by CLSM.

DMSP metabolic pathway

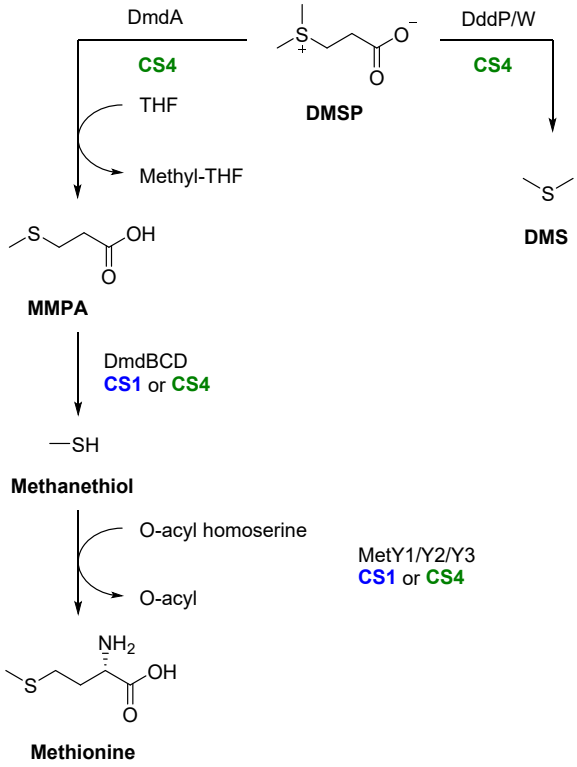


Figure S17. The DMSP metabolic pathway and related proteins in CS4 and CS1 bacteria. DMSP, dimethylsulfoniopropionate; DMS, dimethyl sulfide; MMPA, 3-(methylthio) propionate; THF, tetrahydrofolate; Methyl-THF, 5-methyl-tetrahydrofolate. The DMSP metabolism proteins DddP, DddW, DmdA, DmdB, DmdC, and DmdD were predicted from CS1 and CS4 genomes in a previous study.[1] MetY1, MetY2, and MetY3 were annotated in this study as acylhomoserine sulfhydrylases showed in **Table S3**. The bacterial potential for DMSP metabolism was tested *via* GC-MS and LC-MS by incubating CS4 and CS1 into 100 µM free DMSP for 14 h. The corresponding results are shown in **Figure S18**. The related gene expression in CS4 was tested *via* RT-qPCR after inoculating 2 h and 8 h. The gene expression results are shown in **Figure S21**.

Metabolite analysis based on free DMSP

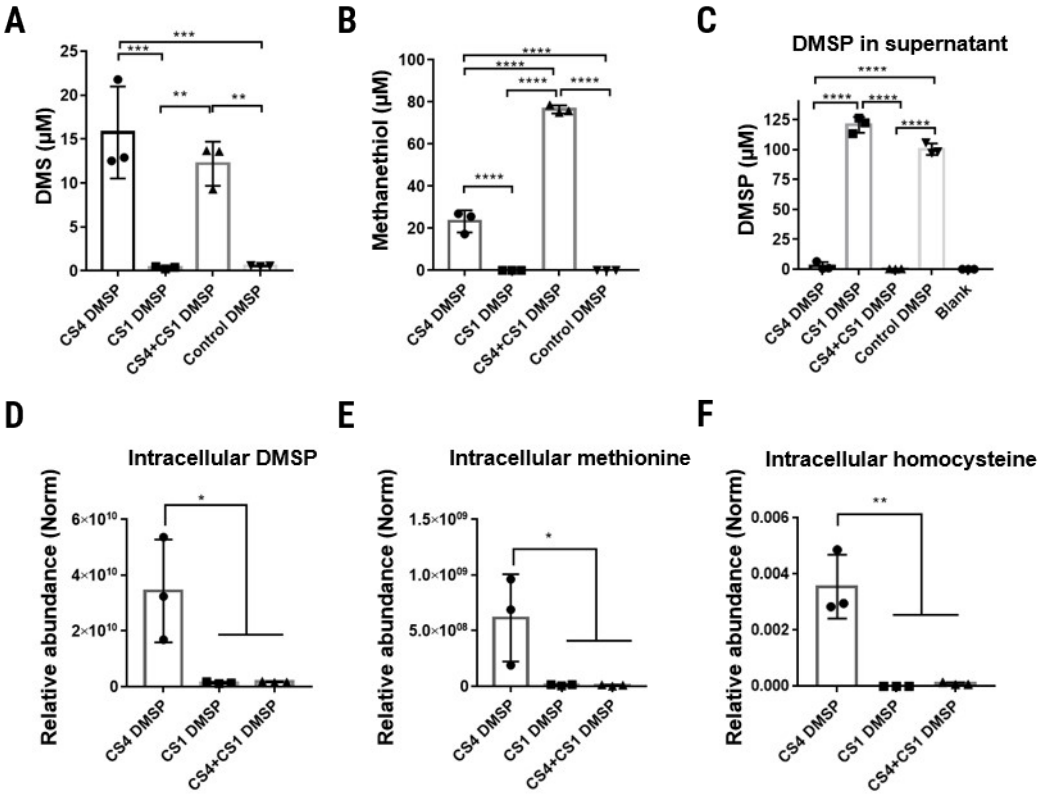


Figure S18. Bacterial DMSP metabolism analysis from the inoculation of CS1 and CS4 in 100 µM free DMSP. The detection of DMS (**A**) and methanethiol (**B**) by GC-MS. The detection of DMSP in the supernatant (**C**), intracellular DMSP (**D**) intracellular methionine (**E**) and homocysteine (**F**) by LC-MS. Relative abundance of intracellular metabolites were normalized by the bacterial amounts determined by qPCR. Bars represent ±SD from triplicates. Tukey's multiple comparisons test. * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.005$, **** indicates $p < 0.001$.

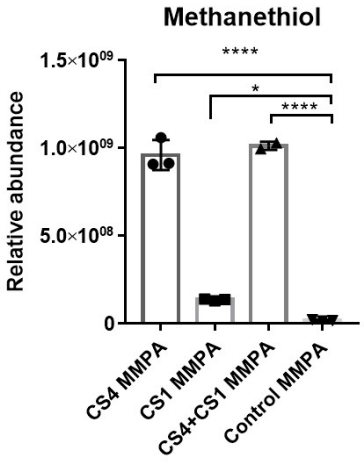


Figure S19. Detection of methanethiol by SPME-GCMS after incubation of bacteria CS4 and CS1 in 100 µM free MMPA for 14 h. Bars were made from ±SD of triplicates. Tukey's multiple comparisons test. * indicates $p < 0.05$, **** indicates $p < 0.001$.

Pretest for cryogel experiment

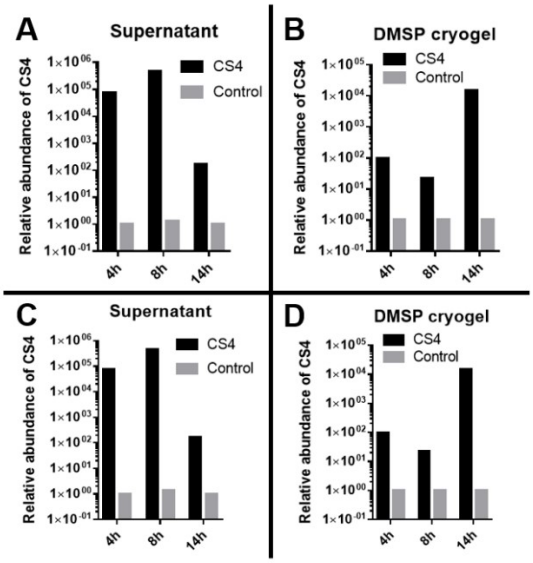


Figure S20. Pretest for the determination of the incubation time for the DMSP cryogel metabolism experiment. After 14 h of incubation, the relative abundance of DMS (**A**) and DMSP (**B**) in the supernatant was analyzed by GC-MS and LC-MS. Relative abundances of CS4 bacteria in the supernatant (**C**) and in the cryogel (**D**) were obtained by qPCR *via* the described methods.

Gene expression analysis

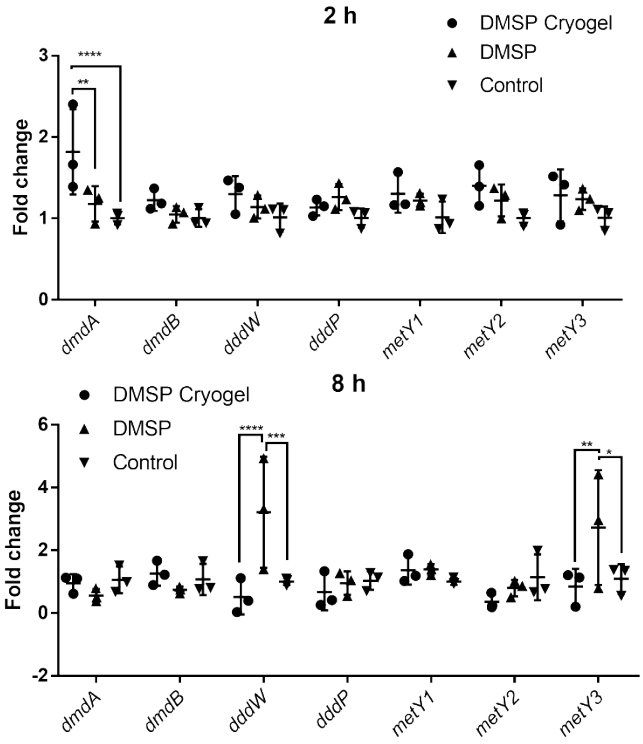


Figure S21. Detection of the gene expression related to the DMSP metabolism in the strain CS4 from 2 h (top) and 8 h (bottom) by RT-qPCR. Bars represent SD from triplicates. Tukey's multiple comparisons test. * indicates $p < 0.05$. *** indicates $p < 0.005$, **** indicates $p < 0.001$.

Intracellular metabolite analysis based on DMSP cryogels

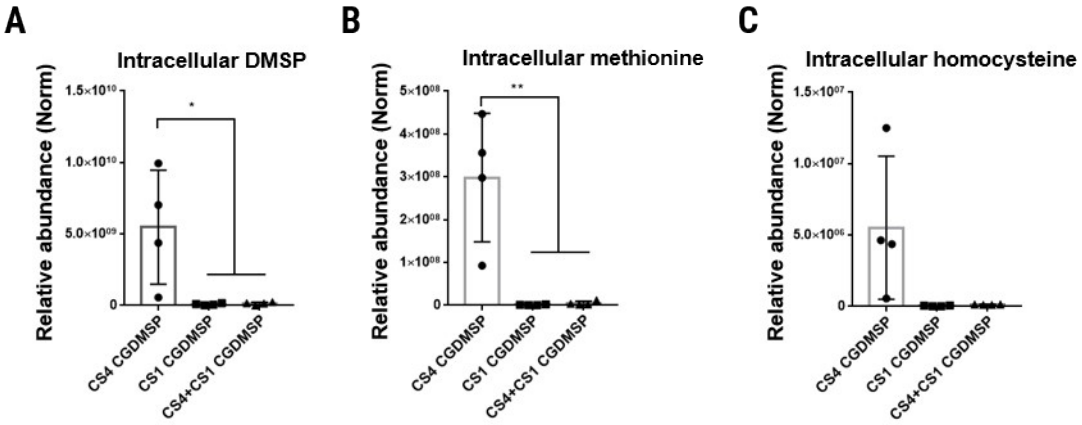


Figure S22. Bacterial intracellular DMSP metabolism and related metabolite analysis by inoculating CS1 and CS4 in DMSP cryogel. Detection of Intracellular DMSP (**A**), methionine (**B**) and homocysteine (**C**) by LC-MS. Bars represent ±SD from four replicates. Tukey's multiple comparisons test. * indicates $p < 0.05$, ** indicates $p < 0.01$.

Bacterial quantification by qPCR

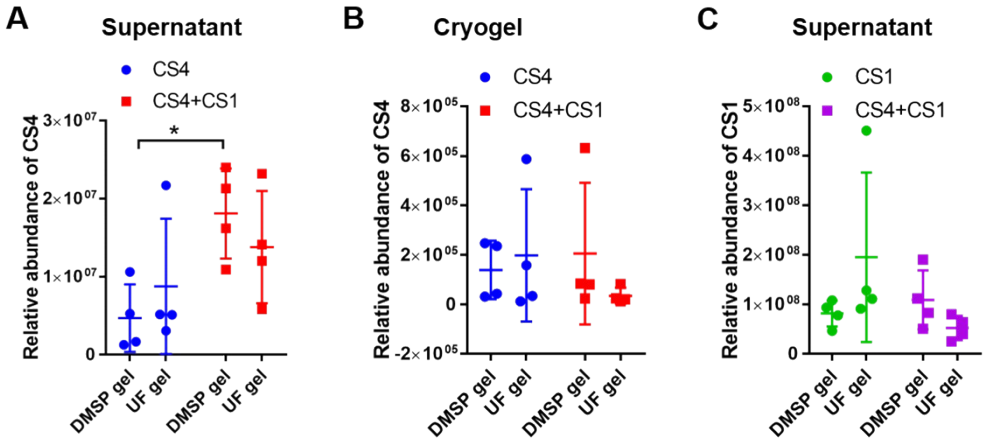


Figure S23. Quantification of bacterial colonization of the cryogel by qPCR when CS1 and CS4 were seeded alone or in co-culture for 14 h. UF, unfunctionalized cryogel was set as control. Tukey's multiple comparisons test. ** $p < 0.01$; *** $p < 0.005$.

Genome prediction

Table S3. The prediction of acylhomoserine sulfhydrylases from the CS4 and CS1 genomes.

Proteins	Query sequence ID	Gene accession, query cover, identity	
		Mameliella sp. CS4	*Marinobacter* sp. CS1
MetY	NP_253712.1	WP_219514105.1 95%, 48.15%	WP_008176355.1, 96%, 73.04%
MetY2	NP_253712.1	WP_219514652.1 95%, 34.92%	No
MetY3	NP_253712.1	WP_219518877.1 91%, 35.48%	No

List of primers

Table S4. The primers used for quantification of the DMSP metabolism related genes of the strain CS4.

Primer	Sequence 5'-3'	Use
DddP-1	ACGGCTACATGGTGATCTGG	Quantification of the gene *dddP* expression
DddP-2	CCGTGGATCATGGGTTTGTC	Quantification of the gene *dddP* expression

DddW-1	TGGCCAAGCTGCCGGTTATC	Quantification of the gene *dddW* expression
DddW-2	CGGTGTCATGTTCCGCATCC	Quantification of the gene *dddW* expression
DmdA-1	CAACGACGCGATCCTCTGTG	Quantification of the gene *dmdA* expression
DmdA-2	CGGCCATCTTCGACAGGTTG	Quantification of the gene *dmdA* expression
DmdB-1	CCATGCTGCCCAACATTCCC	Quantification of the gene *dmdB* expression
DmdB-2	ATCATCGGCGGCTCACCTTC	Quantification of the gene *dmdB* expression
MetY-1	GACCACGGCCTATGTGTTCC	Quantification of the gene *metY* expression
MetY-2	CTGGCTGAACTGGGTGATCG	Quantification of the gene *metY* expression
MetY2-1	CAGTACGAAGGCCGCGAAAC	Quantification of the gene *metY2* expression
MetY2-2	CACATGATCGCCGGTCTTGC	Quantification of the gene *metY2* expression
MetY3-1	AGGGCTTTGTCTACGAGACC	Quantification of the gene *metY3* expression
MetY3-2	TCAGCACCTCTTCGAGAACG	Quantification of the gene *metY3* expression
CS4-16S-1	GGATTGGTGGGTGCAGGATG	Reference gene
CS4-16S-2	GCAGGATCGCGTCGTATTCG	Reference gene

References

[1] Y. Deng, M. Mauri, M. Vallet, M. Staudinger, R. Allen, G. Pohnert, *Appl. Environ. Microbiol.* **2022**, *88*, e0161922.