Supplemental Information

Rationally engineered variants of *S*-adenosylmethionine (SAM) synthase: reduced product inhibition and synthesis of artificial cofactor homologues

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Supplemental Information

Supplemental Methods

Preamble. $[\alpha]_D$ values are given in deg cm^3 g^{-1} dm^{-1}, and *J* values in Hz.

General procedure for the synthesis of *S*-alkylhomocysteines. The compounds were synthesized according to the method of Griffith and Meister[1]. *D*,*L*-Homocysteine thiolactone hydrochloride (**HCTL**) (Sigma, USA) was dissolved in a solution of sodium methoxide (2 eq.) in dry methanol (55 eq.) under argon atmosphere. After 30 minutes, the alkyl halogenide was added. The reactions were stirred at room temperature for 14 h, and sodium halogenide precipitated during the reaction was removed by filtration. For conversion of the *S*-alkylhomocysteine methyl ester into the corresponding free acid, the solvent was removed by rotary evaporation. The residual was saponified by treatment with 1 M LiOH (1 eq.) at room temperature for 3 h (**1**, **9**) or by refluxing with 4 M NaOH (4 eq.) for 6 h (**2** – **8**, **10, 11**).

***D*,*L*-Methionine-d3 (i.e. methyl-D_3) (1)** was synthesized from 3.8 g (25 mmol) of HCTL and CD_3I (0.9 eq.). After saponification, the reaction mixture was neutralized with glacial acetic acid and the product was precipitated by addition of 200 ml of acetone. The precipitate was re-dissolved in 50 ml of water. Contaminating homocysteine was removed by precipitation with methanol (50 ml) and subsequent centrifugation (10 min, 8,000 g). Chromatographically homogeneous product was recovered from the supernatant by treatment with acetone (200 ml) as a fluffy white solid (0.9 g, 26 % yield). HRMS: *m*/*z* 151.0626 ($[M+H]^+$, calculated for $C_5H_8D_3NO_2S$, 99 ppm, ≥ 99.9 atom % D at methyl group). ^1H NMR (400 MHz, D_2O, external standard: 3-(trimethylsilyl)propionic acid-d4): δ 3.85 (1H, dd, *J* 7.0, 7.0), 2.64 (2H, t, *J* 7.7), 2.26-2.09 (2H, m).

***D*,*L*-Ethionine (2)** was synthesized from 9.0 g (58.6 mmol) of HCTL and bromoethane (1.1 eq). The amino acid was precipitated from the saponification reaction by neutralization with 4 M HCl and collected by filtration. For purification, the crude product was converted into its sodium salt with a stoichiometric amount of 1 M NaOH. Chromatographically homogeneous product was obtained by neutralization of the solution with 1 M HCl at 60 °C with subsequent cooling as a fluffy white solid (6.4 g, 67% yield). HRMS: *m*/*z* 164.0740 ($[M+H]^+$, calculated for $C_6H_{13}NO_2S$, 0 ppm). ^1H NMR (400 MHz, D_2O, external standard: 3-(trimethylsilyl)propionic acid-d4):

δ 3.85 (1H, dd, *J* 7.0, 7.0), 2.68 (2H, t, *J* 7.7), 2.62 (2H, q, *J* 7.4), 2.24-2.06 (2H, m), 1.24 (3H, t, *J* 7.4).

***D,L*-n-Propionine (3)** was synthesized from 4.5 g (29.3 mmol) of HCTL and 1-bromopropane (1.1 eq) and purified as specified for **2**. The product was obtained as a fluffy white solid (4.7 g, 91% yield). HRMS: *m*/*z* 200.0716 ([M+Na]$^+$, calculated for $C_7H_{15}NO_2S$, 2.5 ppm). ^{1}H NMR (400 MHz, D_2O, external standard: 3-(trimethylsilyl)propionic acid-d4): δ 3.83 (1H, dd, *J* 7.0, 7.0), 2.67 (2H, t, *J* 7.7), 2.59 (2H, t, *J* 7.3), 2.23-2.04 (2H, m), 1.61, (2H, sext, *J* 7.4), 0.97 (3H, t, *J* 7.4).

***D,L*-n-Buthionine (4)** was synthesized from 4.5 g (29.3 mmol) of HCTL and iodobutane (1.1 eq), and purified as specified for **2**. The product was obtained as a fluffy white solid (4.3 g, 77% yield). HRMS: *m*/*z* 192.1053 ([M+H]$^+$, calculated for $C_8H_{17}NO_2S$, 2.6 ppm). ^{1}H NMR (400 MHz, D_2O, external standard: 3-(trimethylsilyl)propionic acid-d4): δ 3.74 (1H, dd, *J* 7.0, 7.0), 2.66 (2H, dd, *J* 8.1), 2.62 (2H, t, *J* 7.3), 2.21-2.02 (2H, m), 1.58 (2H, quint, *J* 7.4), 1.40 (2H, sext, *J* 7.4), 0.90 (3H, t, *J* 7.4).

For separation of enantiomers, 0.1 g of racemic buthionine (0.5 mmol) was dissolved in 2 ml of 1,4-dioxane and converted into its *N*-protected derivative by reaction with di-*tert*-butyl dicarbonate (1.1 eq.) and triethylamine (2 eq.). After 24 h at room temperature, the solvent was removed by rotary evaporation in vacuo. The residual was extracted with CH_2Cl_2, and the crude product was applied to a chiral HPLC column (Chiralpak AS-H). Both enantiomers were eluted with n-hexane / isopropanol (9:1, v/v) containing 0.1 % (v/v) triethylamine. The products were dissolved in 5 ml of CH_2Cl_2 and deprotected by treatment with trifluoroacetic acid (5 mmol) at room temperature for 15 h. After removal of the solvent under reduced pressure, the buthionine enantiomers were obtained as white solids (40% and 40% yield). D- and L-buthionine were assigned according to their specific rotation ($[\alpha]_D^{20}$ -11.79 ± 0.54 and +14.37 ± 0.42 (*c* 2 g cm^{-3} in 6 M HCl)) which was compared with that of L-methionine ($[\alpha]_D^{20}$ +28.09 ± 0.28 (*c* 4 g cm^{-3} in 6 M HCl)). Both enantiomers were identical to racemic buthionine with respect to ^{1}H NMR and HRMS spectra (data not shown).

***D,L*-Isopropionine (5)** was synthesized from 4.5 g (29.3 mmol) of HCTL and 2-iodopropane (1.1 eq) and purified as specified for **2**. The product was obtained as a

fluffy white solid (2.4 g, 47% yield). HRMS: *m*/*z* 200.0716 ([M+Na]$^+$, calculated for $C_7H_{15}NO_2S$, 2.5 ppm). ^{1}H NMR (400 MHz, D_2O, external standard: 3-(trimethylsilyl)propionic acid-d4): δ 3.83 (1H, dd, *J* 6.7, 6.7), 3.07 (1H, sept, *J* 6.7), 2.70 (2H, t, *J* 7.8), 2.22-2.05 (2H, m), 1.27 (3H, s), 1.26 (3H, s).

***S*-(1-Methylpropyl)-*D*,*L*-homocysteine (6)** was synthesized from 3.8 g (25 mmol) of HCTL and 2-iodobutane (1.1 eq) and purified as specified for **2**. The product (mixture of diastereomers) was obtained as a fluffy white solid (1.6 g, 51% yield). HRMS: *m*/*z* 214.0872 ([M+Na]$^+$, calculated for $C_8H_{17}NO_2S$, 2.8 ppm). ^{1}H NMR (600 MHz, D_2O, external standard: 3-(trimethylsilyl)propionic acid-d4): δ 3.842 (1H, dd, *J* 6.8, 5.5), 3.835 (1H, dd, *J* 6.8, 5.6), 2.88 (1H, m), 2.68 (2H, t, *J* 7.9), 2.16 (2H, m), 2.12 (2H, m), 1.62 (2H, m), 1.54 (2H, m), 1.26 (3H, d, *J* 6.7), 0.960 (4H, t, *J* 7.4), 0.957 (3H, t, *J* 7.4).

***S*-(2-Methylpropyl)-*D*,*L*-homocysteine (7)** was synthesized from 4.5 g (29.3 mmol) of HCTL and 1-iodo-2-methylpropane (1.1 eq) and purified as specified for **2**. The product was obtained as a fluffy white solid (3.4 g, 73% yield). HRMS: *m*/*z* 214.0872 ([M+Na]$^+$, calculated for $C_8H_{17}NO_2S$, 2.8 ppm). ^{1}H NMR (400 MHz, D_2O, external standard: 3-(trimethylsilyl)propionic acid-d4): δ 3.85 (1H, dd, *J* 7.0, 5.5), 2.66 (2H, t, *J* 7.4), 2.52 (2H, d, *J* 6.9), 2.24-2.06 (2H, m), 1.83 (1H, m), 0.98 (6H, d, *J* 6.7).

***S*-(Prop-1-en-1-yl)-*D*,*L*-homocysteine (8)** was synthesized from 3.8 g (25 mmol) of HCTL and allyl bromide (1.1 eq). Product purification as specified for **2** yielded a mixture of **8** and *N*-allyl-*S*-(prop-1-en-1-yl)-*D*,*L*-homocysteine. The by-product was separated by HPLC on reversed phase material (YMC-Pack ODS-A, 5 *μ*m, 120 Å, 150 x 20 mm) which was pre-equilibrated with 10 mM triethylammonium acetate. Compound **8** was eluted as a mixture of *trans* and *cis* isomers (ratio 1:2) by a linear gradient of acetonitrile (5 – 80 %, v/v). The product was obtained as a fluffy white solid (3.4 g, 38% yield). HRMS: *m*/*z* 216.1051 ([M+H]$^+$, calculated for $C_{10}H_{17}NO_2S$, 0.9 ppm). ^{1}H NMR (600 MHz, D_2O, external standard: 3-(trimethylsilyl)propionic acid-d4): δ 5.92 (1H, dddd, *J* 17.1, 10.2, 7.2, 6.8), 5.85 (1H, dq, *J* 15.0, 1.5, *trans* isomer), 5.81 (1H, dq, *J* 9.3, 6.9, *cis* isomer), 5.53 (1H, d, *J* 17.1), 5.50 (1H, d, *J* 10.2), 3.75 (1H, m), 3.70 (1H, dd, *J* 13.4, 6.8), 3.66 (1H, dd, *J* 13.4, 7.2), 2.81 (2H, m, *cis* isomer), 2.76 (2H, m, *trans* isomer), 2.17 (2H, m, *cis* isomer), 2.16 (2H, m, *trans* isomer), 1.75 (3H, dd, *J* 6.6, 1.5, *trans* isomer), 1.70 (3H, dd, *J* 6.9, 1.6, *cis* isomer).

***S*-(Propa-1,2-dien-1-yl)-*D*,*L*-homocysteine (9)** was synthesized from 3.8 g (25 mmol) of HCTL and propargyl bromide (1.1 eq). After saponification, the reaction mixture was neutralized with 4 M HCl. The amino acid was precipitated by addition of 2 volumes of ethanol and cooling to -20 °C. The product was obtained as beige powder (1.9 g, 44% yield). HRMS: *m*/*z* 174.0583 ($[M+H]^+$, calculated for $C_7H_{12}NO_2S$, 3.4 ppm). ^1H NMR (400 MHz, D_2O, external standard: 3-(trimethylsilyl)propionic acid-d4): δ 5.89 (1H, t, *J* 6.6), 5.15 (2H, d, *J* 6.6), 3.85 (1H, dd, *J* 6.6, 6.1), 2.80 (2H, t, *J* 7.9), 2.25-2.12 (2H, m).

***S*-(2-Hydroxyethyl)-*D*,*L*-homocysteine (10)** was synthesized from 3.8 g (25 mmol) of HCTL and 1-bromo-2-chloroethane (1.1 eq). After saponification, the reactions were adjusted to neutral pH with HCl. The solvent was removed by lyophilization, and the residual was extracted with methanol/acetic acid (9:1, v/v) at 60 °C. After filtration, the amino acid was precipitated with a fourfold volume of diethyl ether. Traces of impurities were removed by HPLC on reversed phase material (Merck LiChrospher 110 RP-18 LiChroCART, 5 *µ*m, 125 x 4 mm). The product was eluted with water/methanol (98:2, v/v) containing 0.1 % (v/v) trifluoroacetic acid. The product was obtained as a white solid (2.6 g, 75% yield). HRMS: *m*/*z* 202.0508 ($[M+Na]^+$, calculated for $C_6H_{13}NO_3S$, 3.0 ppm). ^1H NMR (400 MHz, D_2O, external standard: 3-(trimethylsilyl)propionic acid-d4): δ 4.18 (1H, t, *J* 6.4), 3.76 (2H, t, *J* 6.2), 2.76 (2H, t, *J* 6.2), 2.75 (2H, t, *J* 7.4), 2.35-2.13 (2H, m).

***S*-(2-Carboxymethyl)-*D*,*L*-homocysteine (11)** was synthesized from 3.8 g (25 mmol) of HCTL and ethyl bromoacetate (1.1 eq), and was purified as specified for (**10**). Traces of impurities were removed by flash chromatography on silica using isopropanol/H_2O/25 % NH_3 (5:1:2, v/v/v) as mobile phase. The product was obtained as a white solid (0.5 g, 10% yield). HRMS: *m*/*z* 192.0343 ($[M-H]^-$, calculated for $C_6H_{11}NO_4S$, 3.6 ppm). ^1H NMR (400 MHz, CD_3OD/D_2O (13:1, v/v), Me_4Si): δ 3.76-3.73 (1H, dd, *J* 4.4, 4.4), 3.19 (2H, s), 2.80-2.71 (2H, m), 2.27-2.05 (2H, m).

Generation and production of SAMS enzymes. The *sams* gene was amplified from genomic DNA of *Bacillus subtilis* ssp. *subtilis* (strain 168, accession: ATCC 6051, German Collection of Microorganisms and Cell Cultures, a Leibniz Institute,

Germany) and subcloned into the pET28a(+) vector (Merck, Germany) as previously described[2]. For construction of SAMS variants, the wild-type gene was modified by means of the Quik Change II Site-Directed Mutagenesis Kit (Agilent, USA) according to the instructions of the manufacturer. The plasmids were introduced in *E. coli* BL21(DE3), and cell cultivation and protein expression were carried out as described in Kamarthapu et al.[2]. Cells from 400 ml of culture were harvested by centrifugation (10 min, 8,000 g), suspended in 20 ml of extraction buffer (20 mM *N*-(2-hydroxyethyl)-piperazine-*N´*-(2-ethanesulfonic acid)/NaOH, 300 mM NaCl, 10% (w/v) glycerol, pH 8.0) containing 10 mM imidazole, and disrupted by high pressure dispersion. After removal of the cell debris by centrifugation (30 min, 15,000 g), the supernatant was applied to 5 ml of Talon Cobalt affinity resin (Clontech, USA). Proteins were eluted with extraction buffer containing 250 mM imidazole. Fractions containing the SAMS enzyme were identified by SDS-polyacrylamide gel electrophoresis (SDS-PAGE, see below). The samples were pooled and dialyzed against 100 mM Tris/HCl, 10 % (w/v) glycerol, pH 8.0.

SDS-PAGE. Electrophoresis was performed in 10 % (w/v) polyacrylamide gels according to Laemmli[3]. The gels were stained with Coomassie Brilliant Blue R250, and apparent molecular masses were estimated using molecular markers from Fermentas (Canada).

Protein determination. Proteins were assayed with commercial Bradford reagent (Roth, Germany) which was used according to manufacturer's instructions. Protein concentrations were calculated using standard curves of bovine serum albumin.

Spectrophotometric assay for SAMS activity. Reactions (50 µl) were performed in microplates according to the conditions described by Kamarthapu et al. [2]. 40 µl of substrate solution [0 – 12.5 mM amino acid and 6.25 mM ATP (Sigma, USA) in 125 mM Tris/HCl, 25 mM $MgCl_2$, 250 mM KCl, pH 8.0] were mixed with 10 µl of purified enzyme (10 – 400 µg ml^{-1}). Stock solutions of amino acids were adjusted with NaOH to pH 8.0 before usage. After incubation for 0 – 25 min under shaking (400 rpm) at 30 °C, the concentration of phosphate released from ATP was determined by the

method of Mahuren et al.[4]. 100 µl of malachite green-molybdate reagent were added, and the absorbance at 620 nm was measured after 3 min of incubation. Sodium phosphate standards (0 – 75 µM Na_2HPO_4 and $Na_2H_2P_2O_7$ in 5 mM ATP) were used for calculation of the phosphate concentration. The enzyme activity was obtained from the linear fit of the phosphate concentration as a function of the reaction time by the software SigmaPlot. One unit (U) of enzyme activity represents the amount of enzyme releasing 1 µmol of phosphate per minute. All experiments were performed in triplicate.

High-performance thin-layer chromatography (HPTLC). Reactions (200 µl) containing 10 mM amino acid substrate, 7.5 mM ATP and 2 mU of SAMS in reaction buffer (100 mM Tris/HCl, 20 mM $MgCl_2$, 200 mM KCl, pH 8.0) were shaken (400 rpm) at 30 °C. After distinct reaction times (0 – 12 h), samples (1.8 µl) were applied to silica 60 F_{254} plates (Merck, Germany). Plates were developed with 0.3 M triethylamine in 70 % (v/v) ethanol, and bands of *S*-adenosyl-L-methionine (SAM) and analogs were visualized under UV light at 254 nm. The intensity of the spots was densitometrically quantified by the software GeneTools 4.01 (Synoptics, United Kingdom). The concentration of SAM and its derivatives was calculated using standards of SAM iodide (Sigma, USA). The presented data are the mean of two independent experiments.

Preparative synthesis of SAM and analogs. The conversions were performed similar to the HPTLC experiments but in a larger scale. The reaction mixtures (10 ml) contained 10 mM (100 µmol) of the amino acid and 7.5 mM (75 µmol) of ATP in reaction buffer. The reactions were started by addition of SAMS (0.1 U, enzyme activity determined towards L-methionine (5 mM) as described above). For the conversion of *D*,*L*-methionine and -ethionine, the I317V variant was applied, whereas in the syntheses with *S*-n-propyl- and *S*-n-butyl-*D*,*L*-homocysteine the I317A variant was used. After incubation for 8 h (SAMS-I317A) or 18 h (SAMS-I317V) under shaking (60 rpm) at 30 °C, 10 M acetic acid was added to adjust the reaction mixtures to pH 4.0. The samples were cooled to 0 °C and centrifuged (10 min, 15.000 g) to remove the precipitated amino acid. Product yields were determined by HPTLC as described above. After lyophilization, the residual was extracted with 2 ml

of 73 % (v/v) ethanol. 12 M HCl was added to the extract to a final concentration of 0.5 M. The solution of the crude product was applied to 25 ml of SP-Sephadex C-25 (GE Healthcare, Little Chalfont, United Kingdom) on an Äkta FPLC system (GE Healthcare). After elution with 0.5 M HCl, fractions containing the cofactors were combined and the solvent was removed by lyophilization. The amount of product was determined by UV spectroscopy at 260 nm using the extinction coefficient of SAM (ε_0 = 15400 M^{-1} cm^{-1}) from literature[5].

***S*-Adenosyl-*L*-methionine.** Fluffy white solid (25% yield, referred to *L*-methionine) composed of the (*S*,*S*)- and (*R*,*S*)-diastereomer (ratio 9:1). HRMS: *m*/*z* 298.0968 ([M+H]$^+$, calculated for $C_{11}H_{15}N_5O_3S$, 0 ppm). ^{1}H NMR (600 MHz, D_2O, external standard: 3-(trimethylsilyl)propionic acid-d4): δ 8.49 (1H, s), 8.48 (1H, s), 6.19 (1H, d, *J* 4.0), 4.87 (1H, t, *J* 4.8), 4.65 (1H, t, *J* 5.9), 4.59 (1H, ddd, *J* 8.8, 5.9, 2.6), 4.05 (1H, dd, *J* 13.8, 9.5), 4.02 (1H, dd, *J* 7.8, 2.2), 3.96 (1H, dd, *J* 13.8, 2.7), 3.52-3.48 (2H, m), 3.04 (3H, s), 2.46-2.36 (2H, m).

***S*-Adenosyl-*L*-ethionine.** Fluffy white solid (17% yield, referred to *L*-ethionine) composed of the (*S*,*S*)- and (*R*,*S*)-diastereomer (ratio 1:1). HRMS: *m*/*z* 312.1126 ([M+H]$^+$, calculated for $C_{12}H_{17}N_5O_3S$, 0.3 ppm). ^{1}H NMR (600 MHz, D_2O, external standard: 3-(trimethylsilyl)propionic acid-d4): δ 8.50 (2H, m), 8.49 (2H, m), 6.20 (2H, m), 4.90 (1H, t, *J* 4.7), 4.87 (1H, t, *J* 4.7), 4.66 (2H, t, *J* 5.8), 4.61-4.56 (2H, m), 4.03-3.92 (6H, m), 3.56-3.49 (6H, m), 2.46-2.33 (4H, m), 1.47 (3H, t, *J* 7.4), 1.43 (3H, t, *J* 7.4).

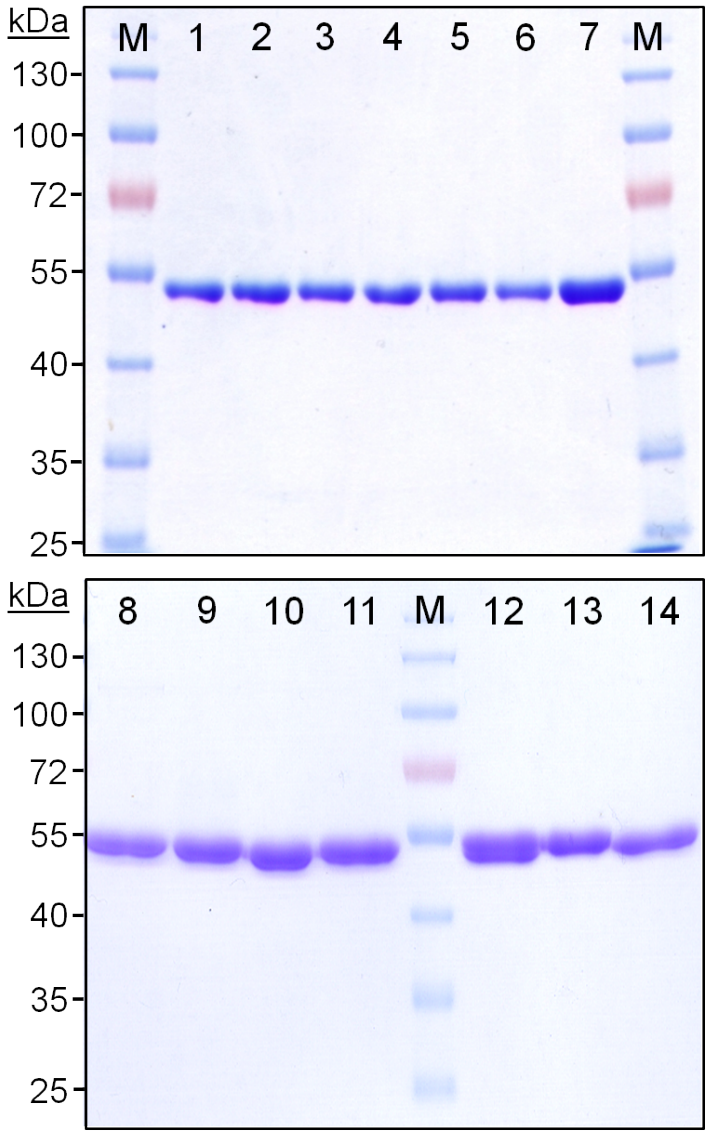
***S*-Adenosyl-*L*-propionine.** Fluffy white solid (8% yield, referred to *L*-n-propionine) composed of the (*S*,*S*)- and (*R*,*S*)-diastereomer (ratio 1:1). HRMS: *m*/*z* 326.1281([M+H]$^+$, calculated for $C_{13}H_{19}N_5O_3S$, 0 ppm). ^{1}H NMR (600 MHz, D_2O, external standard: 3-(trimethylsilyl)propionic acid-d4): δ 8.51 (2H, m), 8.50 (2H, m), 6.22-6.19 (2H, m), 4.92 (1H, t, *J* 4.7), 4.88 (1H, t, *J* 4.7), 4.66 (2H, t, *J* 5.7), 4.61-4.56 (2H, m), 4.05-3.95 (6H, m), 3.70-3.53 (4H, m), 3.46 (2H, t, *J* 7.3), 3.43 (2H, t, *J* 7.3), 2.48-2.35 (4H, m), 1.89-1.76 (4H, m), 0.97 (3H, t, *J* 7.3), 0.96 (3H, t, *J* 7.3).

***S*-Adenosyl-*L*-buthionine.** Fluffy white solid (11% yield, referred to *L*-n-buthionine) composed of the (*S*,*S*)- and (*R*,*S*)-diastereomer. HRMS: *m*/*z* 340.1438 ([M+H]$^+$, calculated for $C_{14}H_{21}N_5O_3S$, 0 ppm). ^{1}H NMR (600 MHz, D_2O, external standard: 3-(trimethylsilyl)propionic acid-d4): δ 8.51 (2H, m), 8.50 (2H, m), 6.22-6.19 (2H, m),

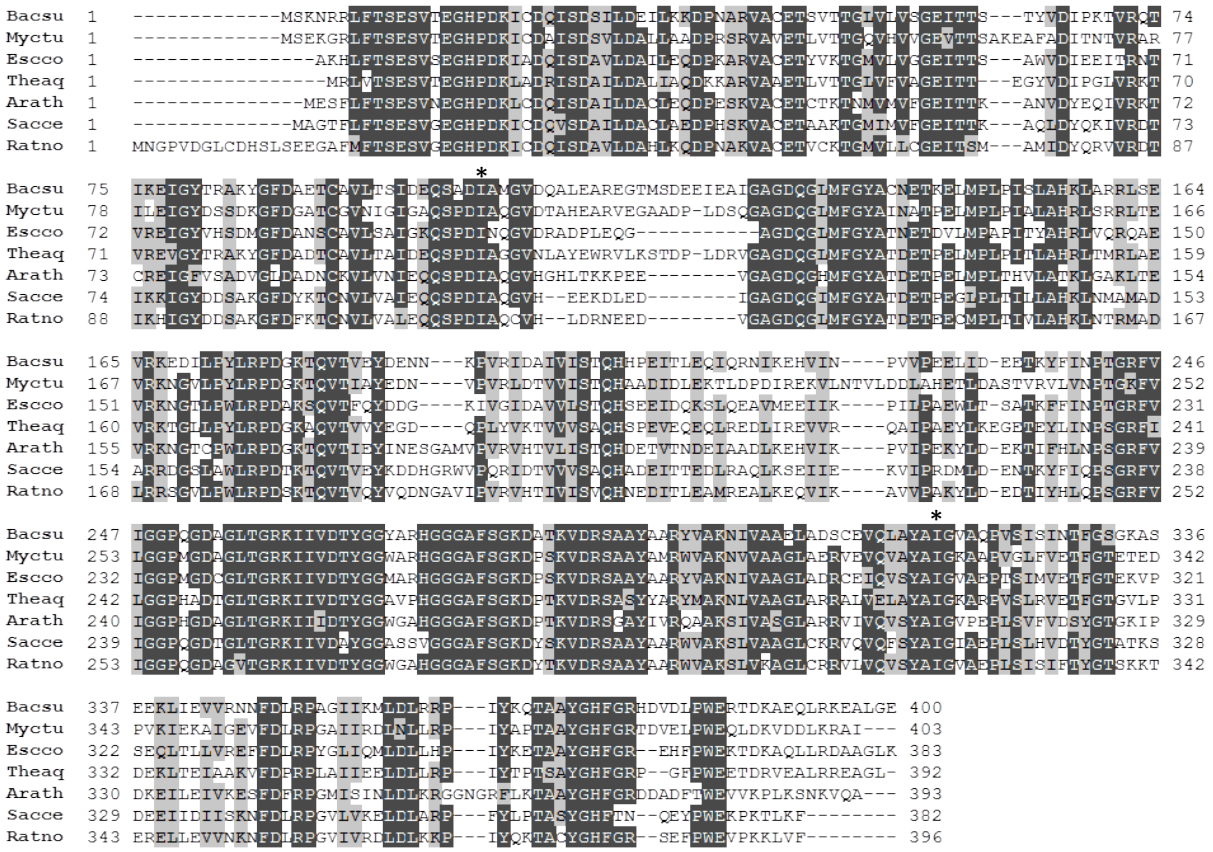
4.92 (1H, t, *J* 4.7), 4.88 (1H, t, *J* 4.6), 4.67 (2H, t, *J* 5.8), 4.61-4.56 (2H, m), 4.05-3.94 (6H, m), 3.70-3.53 (2H, m), 3.48-3.44 (2H, m), 3.43 (2H, t, *J* 8.0), 2.49-2.35 (2H, m), 1.81-1.66 (4H, m), 1.42-1.30 (4H, m), 0.84 (3H, s), 0.78 (3H, s).

Homology modeling. For homology modeling of the enzyme from *Bacillus subtilis* ssp. *subtilis* (NCBI accession: NP_390933.1), the ternary complex of SAMS from *E. coli* (PDB entry: 1P7L) was used because this structure already contains a SAM cofactor bound to one monomer unit and has a high sequence identity of 65% to the target sequence[6]. Since the active site is composed by two monomers the ternary complex was modeled using MOE (Molecular Operating Environment, 2011.10; Chemical Computing Group Inc., Canada, 2011). All ligands co-crystallized in the templates X-ray structure were transfered to the model. For refinement of the resulting homology model, a molecular dynamics refinement using the md-refinement tool of YASARA[7] was carried out. The quality of the final model was checked with PROCHECK (http://www.ebi.ac.uk/thornton-srv/software/PROCHECK). The Ramachandran plot showed 89.1% of all residues in most favored area. Despite of eight outliers (all distantly far away from the active site) the overall structure was evaluated better than an X-ray structure with a theoretical resolution of 2 Å. Analysis with PROSA II[8] exhibited almost all residues in a negative energy window indicating a native-like fold.

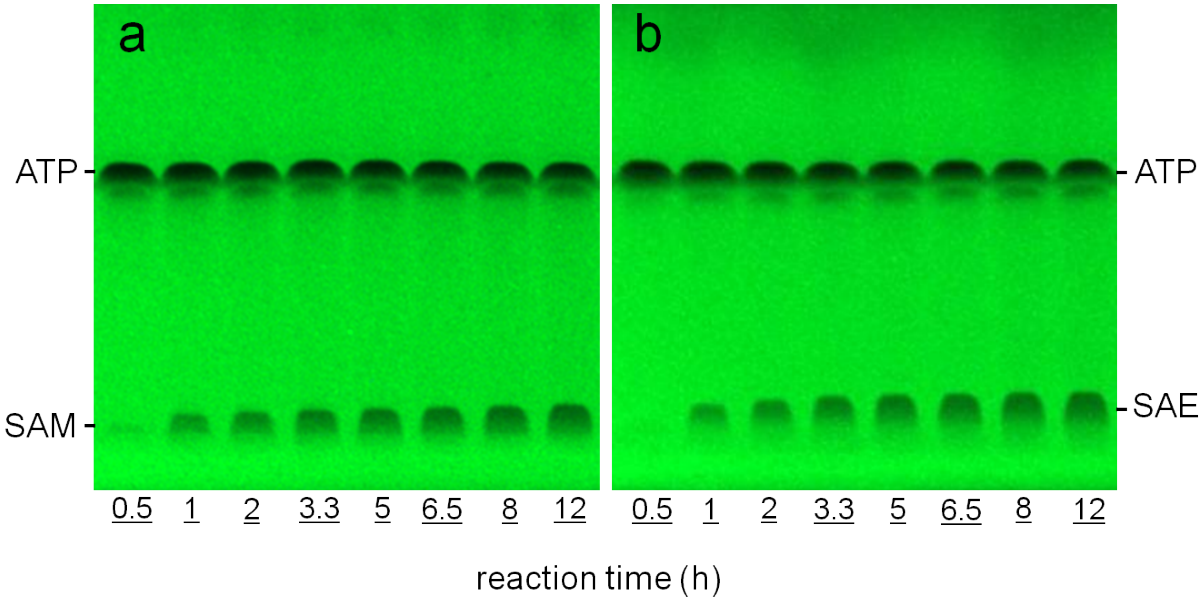
Supplementary Figure S1. SDS-PAGE of the SAMS enzyme from *Bacillus subtilis* and its variants. Gel electrophoresis was performed with 5 µg of purified protein and stained with Coomassie Brilliant Blue R250. The lanes represent the molecular markers (M, molecular masses are indicated in the figure), the wild-type enzyme (lane 1) and the variants I317V (lane 2); I317A (lane 3); I105V/I317A (lane 4); I105A/I317A (lane 5); I105V/I317S (lane 6), I105S/I317S (lane 7), I317G (lane 8), I317P (lane 9), I317L (lane 10), I317C (lane 11), I317E (lane 12), I317D (lane 13) and I317N (lane 14).



Supplementary Figure S2. Alignment of SAMS enzyme amino acid sequences. The sequence of the protein from *Bacillus subtilis* (Bacsu, NCBI accession: NP_390933.1) was compared with the homologues from *Mycobacterium tuberculosis* (Myctu, NCBI accession: NP_215908.1), *Escherichia coli* (Escco, NCBI accession: NP_289514.1), *Thermus aquaticus* (Theaq, NCBI accession: ZP_03497008.1), *Arabidopsis thaliana* (Arath, NCBI accession: BAB02743.1), *Saccharomyces cerevisiae* (Sacce, NCBI accession: AAA66932.1), and *Rattus norvegicus* (Ratno, NCBI accession: NP_036992.1) by the alignment tool BioEdit (version 7.1.3.0, Ibis Biosciences, USA). Amino acid positions which are identical or highly similar in ≥ 86 % of the selected sequences are depicted in dark or light gray. The conserved isoleucine residues in the active center which corresponding to positions 105 and 317 in the protein from *Bacillus subtilis* are marked by an asterisk.



Supplementary Figure S3. Analysis of the conversion of S-alkylhomocysteines by HPTLC. As exemplied for reactions containing the SAMS variant I317V and *D*,*L*-methionine (a) or *D*,*L*-ethionine (b), samples were taken after the specified reaction times and subjected to chromatographic separation on silica F_{254} as decribed above. The spots of substrate ATP and the products SAM and S-adenosylethionine (SAE) were visualized under UV light.



Supplementary Table S1. Kinetic parameters for the conversion of *S*-alkylhomocysteines by the SAMS enzyme from *Bacillus subtilis* and its variants. The parameters were obtained as described in the Experimental Section.

Enzyme	Substrate	$S_{0.5}$ (mM)	V_{max} (nmol min^{-1} mg^{-1})	*h*
Wild-type	*L*-methionine	1.04 ± 0.14	93.2 ± 3.1	1.1 ± 0.1
	D,*L*-methionine	1.92 ± 0.12	90.8 ± 1.6	1.7 ± 0.2
	D,*L*-methionine-(methyl-D_3)	1.86 ± 0.07	79.8 ± 2.3	2.2 ± 0.2
I317V	*L* -methionine	0.72 ± 0.04	408.0 ± 7.7	1.2 ± 0.1
	D,*L*-methionine	1.71 ± 0.12	446.2 ± 13.8	1.3 ± 0.1
	D,*L*-ethionine	10.94 ± 2.70	51.2 ± 9.1	1.3 ± 0.1
I317A	*L* -methionine	0.46 ± 0.02	34.8 ± 0.5	1.3 ± 0.1
	D,*L*-methionine	0.92 ± 0.05	37.7 ± 0.8	1.1 ± 0.1
	D,*L*-ethionine	2.99 ± 0.23	44.1 ± 1.6	1.2 ± 0.1
	S-n-propyl-*D*,*L*-homocysteine	0.90 ± 0.05	34.2 ± 0.7	1.1 ± 0.1
	S-n-butyl-*D*,*L*-homocysteine	3.56 ± 0.45	35.4 ± 2.6	1.4 ± 0.1
	S-(2-methylvinyl)-*D*,*L*-homocysteine	0.79 ± 0.04	22.4 ± 0.4	1.2 ± 0.1
I105V/I317A	*D*,*L*-methionine	3.77 ± 1.02	17.0 ± 2.0	1.0 ± 0.1
	D,*L*-ethionine	4.25 ± 0.20	5.7 ± 0.3	4.6 ± 0.8
	S-n-propyl-*D*,*L*-homocysteine	1.86 ± 0.41	17.7 ± 1.5	1.0 ± 0.1
	S-n-butyl-*D*,*L*-homocysteine	4.20 ± 0.41	11.9 ± 0.9	2.0 ± 0.2
I105A/I317A	*D*,*L*-methionine	n.d.	0.8 ± 0.2	n.d
	D,*L*-ethionine	3.91 ± 0.27	3.0 ± 1.8	2.9 ± 0.4
	S-n-propyl-*D*,*L*-homocysteine	≥ 10.0	≥ 10.8 ± 0.1	1.0 ± 0.1
	S-n-butyl-*D*,*L*-homocysteine	≥ 10.0	≥ 4.0 ± 0.2	0.8 ± 0.1

$S_{0.5}$, substrate concentration at half-maximum saturation; V_{max}, maximum reaction rate; *h*, Hill coefficient, n.d., not determined

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