YALI0C22088g from *Yarrowia lipolytica* catalyses the conversion of L-methionine into volatile organic sulfur-containing compounds

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Summary
The enzymatic conversion of L-methionine (L-Met) into volatile organic sulfur-containing compounds (VOSCs) plays an important role in developing the characteristic aroma of foods, including animal products such as yogurt and cheese, fruits, vegetables, and alcoholic beverages (Gonda et al., 2013; Martinez-Cuesta et al., 2013; Liu et al., 2017; Xu et al., 2018; Fischer and Steinhaus, 2020; Bonnaffoux et al., 2021). This phenomenon is because VOSCs possess low odour thresholds and characteristic notes for the human nose (Kagkli et al., 2006; Splivallo et al., 2011; Martinez-Cuesta et al., 2013; Perea-Sanz et al., 2019). The development of sequencing technology and improved bioinformatic tools have significantly advanced the discovery of enzymes with the potential to catalyse the conversion of L-Met into VOSCs (Hebert et al., 2011, 2013; McAuliffe et al., 2019; Mar-dones et al., 2020). The identification of these enzymes and the discovery of their catalytic mechanism will promote the understanding of the formation mechanism for the final aroma of fermented foods (Sales et al., 2018; Cadinanos et al., 2019).

*Saccharomyces cerevisiae*, Kluyveromyces lactis and *Yarrowia lipolytica*, which are generally recognized as safe (GRAS) for food production by the United States Food and Drug Administration, degrade L-Met into VOSCs via the Ehrlich and demethiolation pathways (Perpete et al., 2005; Kagkli et al., 2006; Cholet et al., 2008). The Ehrlich pathway includes the transamination of L-Met into α-keto-methylthiobutyric acid (KMBA), the decarboxylation of KMBA into methional, and reduction of methional to methionol (Hazelwood et al., 2008; Gonda et al., 2013; Martinez-Cuesta et al., 2013; Fischer and Steinhaus, 2020). Methionol presents a cooked-vegetable aroma in Chinese liquor and a cauliflower aroma in wine and beer (Landau et al., 2008; Sha et al., 2017). Aminotransferases, decarboxylases and dehydrogenases that catalyse the three-step reactions in the Ehrlich pathway have been annotated based on homology (Splivallo et al., 2011; Hebert et al., 2011, 2013; Xu et al., 2018). In the demethiolation pathway, methionine-γ-lyase catalyses the demethylation reaction, and L-Met is catabolized into methanethiol (MTL), after which MTL is rapidly oxidized to form dimethyl sulfide (DMS), dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTTS) via free radical reactions (Hazelwood et al., 2008; Martinez-Cuesta et al., 2013; Gonda et al., 2013; Fischer and Steinhaus, 2020). DMS exhibits an onion and cooked-
cabbage aroma in Chinese liquor and an onion aroma in wine and beer and can be used to mimic the aroma of the black Perigord truffle Tuber melanosporum (Fan and Qian, 2005; Landaud et al., 2008; Splivallo et al., 2011). Cystathionine γ-lyase catalyses the γ-elimination of L-Met (methionine γ-lyase) and degrades L-Met into α-ketobutyrate, MTL and NH$_4^+$ (Messerschmidt et al., 2003). While YAL012W (cystathionine-γ-lyase, EC 4.4.1.1, as encoded by CYS3) exhibited no activity towards L-Met, STR3 from S. cerevisiae and its homologues in Clonostachys rosea isolated from Tuber melanosporum ascocarps showed no or negligible activity towards L-Met (Yamagata et al., 1993; Holt et al., 2012; Jia et al., 2016). It is therefore essential to elucidate the mechanism for the direct conversion of L-Met into VOSCs in the demethiolation pathway, which plays an important role in the formation of the final food aroma.

In this study, we used bioinformatic, biochemical and computational chemistry-based analyses to identify the yeast methionine γ-lyases and unravel their catalytic mechanism. Pyridoxa L-5'-phosphate (PLP)-dependent enzymes are involved in the metabolism of L-Met and the other sulfur-containing amino acids, and these enzymes form an evolutionarily related family, which were designated the L-Met metabolism PLP-dependent enzymes (Messerschmidt et al., 2003). We mined these enzymes from the genomes of S. cerevisiae, K. lactis and Y. lipolytica and then defined the function of methionine γ-lyase and the determinants for the enzymatic γ-elimination of L-Met. The results of this study promote an understanding of the formation mechanism for the final aroma of fermented foods and provide a rationale for the enzymatic production of aroma compounds.

Results and discussion

YHR112C, KLLA0_E21319g and YALI0C22088g are related to L-Met metabolism

Exogenous L-Met strongly induced the transcriptional expression of L-Met catabolism genes (Kagkli et al., 2006; Cholet et al., 2008; Jia et al., 2016; Xu et al., 2018). To mine the genes governing enzymatic L-Met depletion, 5 g 1$^{-1}$ L-Met was added to fermentation medium to induce the expression of L-Met depletion-related genes. After being cultured for 48 h, a 3 ml aliquot of liquid seed culture was sampled for yeast mRNA isolation. Through mRNA reverse transcription into cDNA and PCR cloning, 15 of 17 genes encoding proteins that contain the same motifs as the L-Met metabolism PLP-dependent enzyme were obtained (Fig. S1, Table S1). This result suggested that the 15 genes may be transcriptionally expressed in yeast cells. The 15 corresponding proteins fell into the 4 groups, namely cystathionine-β-lyase (EC 4.4.1.8), cystathionine-γ-lyase (EC 4.4.1.1), cystathionine-β-synthase (EC 4.2.1.22) and cystathionine-γ-synthase (EC 2.5.1.48) (Fig. 1A). Cystathionine γ-lyase catalyses the γ-elimination of L-Met, whereas YAL012W, which was named CYS3, exhibited no activity towards L-Met; STR3 from C. rosea, which is located in the cystathionine-β-lyase group, displayed negligible activity towards L-Met, and this conversion was attributed to the enzyme promiscuity of STR3 (Holt et al., 2012; Jia et al., 2016). Uncharacterized YHR112C, KLLA0_E21319g and YALI0C22088g clustered together and were separate from the abovementioned 4 groups, with YFR055W being annotated as a β-lyase (Fig. 1A).

Expression of L-Met catabolic genes was shown to correlate positively with the conversion of L-Met into VOSCs (Bondar et al., 2005; Kagkli et al., 2006; Cholet et al., 2007; Hebert et al., 2011, 2013; Jia et al., 2016). In this study, the addition of 5 g 1$^{-1}$ L-Met induced increases in the mRNA abundances of YHR112C, KLLA0_E21319g and YALI0C22088g (Fig. 1B, Table S1), indicating that the transcriptional profiles of the 3 genes may be dependent on L-Met.

YALI0C22088g catalyses the γ-elimination of L-Met with high efficiency

YHR112C, KLLA0_E21319g and YALI0C22088g were heterologously expressed in S. cerevisiae, purified using Ni–nitrilotriacetic acid chromatography and identified (Fig. S2A–D). The purified proteins were incubated using L-Met as the substrate and pyridoxa L-5'-phosphate (PLP) as the cofactor. Sharp decreases in the L-Met concentration and the accumulation of α-ketobutyrate were observed for YALI0C22088g, while weak changes were observed for YHR112C (Fig. 1C, Fig. S3A-E, Fig. 2). This result is supported by the observation that Y. lipolytica produced a wider variety and quantity of VOSCs than K. lactis (Bondar et al., 2005; Cholet et al., 2007). The production of α-ketobutyrate did not correspond to the change in the L-Met concentration because α-ketobutyrate is not the direct product of L-Met (Fig. S3A, and B) and is unstable at pH 8.0 (the concentration of α-ketobutyrate decreased by 31.20% relative to that at pH 3.0). MTL is the direct product of L-Met and then rapidly oxidized to form DMS, DMDS, and DMTS via free radical reactions (Hazelwood et al., 2008; Gonda et al., 2013; Martinez-Cuesta et al., 2013; Fischer and Steinhaus, 2020). Therefore, MTL produced by YALI0C22088g catalysis was detected by reacting with 5,5’-dithiobis (2-nitrobenzoic acid) (DTNB). The activity of YALI0C22088g attained 239.96 ± 1.23 nmol MTL.min$^{-1}$ mg recombinant protein$^{-1}$, which approached the activity with the depletion of L-Met. These results indicated
that YALI0C22088g catalyses the \( \gamma \)-elimination of \( \text{L-Met} \). In addition, YALI0C22088g exhibited a stronger catalytic activity towards \( \text{L-Met} \) than STR3 (\( \text{C. rosea} \)), which belongs to the cystathionine-\( \beta \)-lyase group (Table 1).

YALI0C22088g also catalysed the conversion of \( \text{KMBA} \) and \( \text{methional} \) into \( \text{MTL} \) (Table 2, Fig. 2). This result indicated that YALI0C22088g may function as a bridge between the demethiolation and Ehrlich pathways in \( \text{Y. lipolytica} \). The identification of YALI0C22088g with C-S lysis activity enhances our understanding of the formation mechanism underlying the final fermented food aroma. YALI0C22088g possessed high substrate promiscuity and catalysed the depletion of \( \text{L-cystathionine} \) (L-cystathionine-\( \gamma \)-lyase [Bruinenberg et al., 1997]) and \( \text{L-homocysteine} \), but not \( \text{L-cystine} \) and \( \text{L-cysteine} \) (Table 2, Fig. S5A and B), indicating that YALI0C22088g was a cystathionine \( \gamma \)-lyase. YALI0C22088g shared 27.1% and 30.6% identities with cystathionine-\( \gamma \)-lyases 3VK3 and 5TSU (Fukumoto et al., 2012; Yan et al., 2017) having the ability to degrade \( \text{L-Met} \), and is categorized apart from the cystathionine-\( \beta \)-lyase group. Thus, YALI0C22088g forms a distinct subgroup of cystathionine-\( \gamma \)-lyases (Fig. S6). Methionine-\( \gamma \)-lyase, which catalyses \( \text{L-Met} \) depletion, is one of the most promising enzymes for cancer therapy (Sun et al., 2003). 3VK3 from \( \text{Pseudomonas putida} \) retarded tumour growth, but it was highly immunogenic in primates (Fukumoto et al., 2012; Stone et al., 2012). YALI0C22088g was from \( \text{Y. lipolytica} \) verified to be GRAS and inferred to be with low immunogenic potential. YALI0C22088g displayed higher \( \text{L-Met} \) binding ability than 5TSU (\( K_m = 14 \pm 1.5 \text{ mM} \)) (Stone et al., 2012) (Table 1), and

![Fig. 1.](image-url)

**A.** A non-rooted molecular phylogenetic tree of the enzymes related to \( \text{L-Met} \) metabolism. YGL184C (cystathionine-\( \beta \)-lyase, EC 4.4.1.8, encoded by STR3) had no activity towards \( \text{L-Met} \) and clustered with STR3 (\( \text{C. rosea} \)), with negligible activity towards \( \text{L-Met} \), and uncharacterized KLLA0_C17028g; the group was marked with yellow. YAL12W (cystathionine-\( \gamma \)-lyase, EC 4.4.1.1, encoded by CYS3) exhibiting no activity towards \( \text{L-Met} \) was clustered with uncharacterized KLLA0_F07909g, and the group was marked with brown. YGR155W (cystathionine-\( \beta \)-synthase, EC 4.2.1.22, encoded by CYS4) was clustered with uncharacterized YAL0E09108g, and the group was marked with cyan. YML082W, YGR130C, YLL058W, KLLA0_B04378g and YAL0D17402g are uncharacterized and inferred to be cystathionine-\( \gamma \)-synthases (EC 2.5.1.48) based on homology. YFR055W, functionally identified as a \( \beta \)-lyase (EC 4.4.1.13, encoded by IRC7), is far from the cystathionine-\( \beta \)-lyase group. Uncharacterized YHR112C, KLLA0_E21319g and YALI0C22088g are clustered together, and they deviated from the other groups.

**B.** The response of YHR112C, YFR055W, KLLA0_E21319g and YALI0C22088g to \( \text{L-Met} \) addition at the transcriptional level. \( \text{L-Met} \) addition upregulated the expression of YHR112C, KLLA0_E21319g and YALI0C22088g and downregulated the expression of YFR055W.

**C.** YALI0C22088g possessed a higher capacity for \( \text{L-Met} \) depletion than YHR112C. KLLA0_E21319g had no activity towards \( \text{L-Met} \) and was marked as not detected (ND).

| Proteins          | L-Met depletion activity (nmol min\(^{-1}\) mg recombinant protein\(^{-1}\)) |
|-------------------|--------------------------------------------------------------------------------|
| KLLA0_E21319g     | ND                                                                               |
| YHR112C           | 9.84 ± 0.15                                                                     |
| YALI0C22088g      | 248.22 ± 2.03                                                                   |
further engineering of YALI0C22088g will improve its application in cancer therapy.

**Key elements determining the conversion of L-Met into VOSCs in YALI0C22088g**

To locate the key amino acid residues that determine the high L-Met depletion activity of YALI0C22088g, we compared the amino acid sequence of YALI0C22088g to those of YHR112C and KLLA0_E21319g, which share 54.40% and 54.79% of their identities with YALI0C22088g, respectively, in accordance with the methods described by Jia et al. (2019). Twelve out of 29 amino acid residues constituting the entrance, tunnel and active site in YALI0C22088g were different from those in YHR112C and KLLA0_E21319g (Fig. 3A and B). The 12 amino acid residues were individually mutated to Ala by using alanine scanning mutagenesis (Weiss, et al., 2000; Pal, et al., 2005; Sidhu and Kossiakoff, 2007), and then, the L-Met depletion activities of these mutants were analysed. The P239A mutant was inactive, and the S70A, K74A, N89A, S237A, G238A and G240A mutants exhibited lower L-Met depletion activity (below 20% relative activity), while the Y59A mutant displayed 0.28-fold increased activity compared with that of wild-type YALI0C22088g, indicating that the 8 amino acid residues are essential for L-Met depletion (Fig. 4A). By positioning the 8 amino acid residues in the predicted 3D structure of YALI0C22088g, we found that Y59, S70 and K74 were located in the entrance and that N89, S237, G238, P239 and G240 were located in the tunnel (Fig. 4B, Fig. 5A–C). The mutant Y59A increased the bottleneck radius of YALI0C22088g from 1.5 Å to 2.0 Å (Fig. 4C), which may result in the enlargement of the tunnel size and enable more substrate to enter the active site. This inference was supported by the observation that the L-Met binding ability and catalytic efficiency of mutant Y59A were increased by 4.37- and 0.98-fold relative to those of YALI0C22088g (Table 1). The tunnel was not detected for the mutant S70A (Fig. 4C); thus, its lower L-Met depletion activity was attributed to the destruction of the tunnel. L-Met carries a negative charge (the pl of L-Met is 5.75, and the buffer pH was 8.0), the mutant K74A

![Fig. 2. YALI0C22088g exhibited strong C-S lysis activity towards L-Met and its precursors and catabolic products. YALI0C22088g catalysed the production of α-ketobutyrate and MTL and was identified as a methionine-γ-lyase. It also exhibited strong C-S lysis activities towards L-cystathionine, L-homocysteine, KMBA, and methional.](image)

**Table 1. γ-elimination activity towards L-Met.**

|        | $K_m$ (mM) | $V_{max}$ (mM min$^{-1}$) | $k_{cat}$ (s$^{-1}$) | $k_{cat}/K_m$ (M$^{-1}$ s$^{-1}$) |
|--------|------------|---------------------------|---------------------|-----------------------------------|
| YALI0C22088g | 3.33 ± 0.17 | 0.16 ± 0.01               | 0.19 ± 0.01         | 57.06                             |
| YALI0C22088g (Y59A) | 0.62 ± 0.05 | 0.06 ± 0.00               | 0.07 ± 0.00         | 112.90                            |
| STR3 (C. rosea) (Jia et al., 2016) | 196.37 | 0.034 µM min$^{-1}$ | | |

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eliminated the positive charge (Fig. 4C, Fig. S7A, Table S2), and its lower L-Met depletion activity was inferred to be related to the abolishment of the charge attraction effect between K74 and L-Met, which enabled L-Met to enter the active site more easily.

Conclusion

We identified YALI0C22088g from Y. lipolytica as a methionine γ-lyase and defined the determinants of the enzymatic γ-elimination of L-Met. YALI0C22088g forms a distinct subgroup of cystathionine-γ-lyase, and it exhibited strong C-S lysis activities towards l-cystathionine, l-homocysteine, KMBA and methional (Fig. 2). We identified eight key amino acid residues in YALI0C22088g and found that the size of the tunnel and the charges carried by the entrance amino acid residues play important roles in the enzymatic conversion of L-Met into VOSCs (Fig. 4B). These findings reveal the formation mechanism of VOSCs produced through the demethiolation pathway, provide a rationale for engineering the enzymatic conversion of L-Met into VOSCs and thus promote the enzymatic production of aroma compounds.

Experimental procedures

Chemicals, strains, and culture conditions

Chemicals 1 – 8 (Table 2) were purchased from Sigma-Aldrich (St. Louis, MO, USA), the acetonitrile purchased from Fisher Scientific (USA) was of HPLC grade, and the other chemicals and reagents were purchased from Sigma-Aldrich, Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and China National Pharmaceutical Group Corporation (Beijing, China). Competent cells, including E. coli BL21 (DE3) and S. cerevisiae INVSc1, were purchased from TransGen Biotech (Beijing, China) and Invitrogen (USA) and transformed according to the manufacturer’s protocol. To analyse the gene expression at the transcriptional level, S. cerevisiae S288C and K. lactis NRRL Y-1140 were cultivated in synthetic complete medium containing 2% glucose, 0.67% yeast nitrogen base without amino acids (Biosharp) and 0.5% amino acids. Y. lipolytica CLIB122 was cultivated in potato dextrose broth and a defined synthetic medium according to the method described by Mansour et al. (2008). Then, 5 g l⁻¹ L-Met was added into the medium to induce the expression of genes related to L-Met metabolism (Jia et al., 2016; Xu et al., 2018).

Gene cloning

The total RNA was isolated from S. cerevisiae, K. lactis and Y. lipolytica using a Total RNA Kit II R6934 (Omega Bio-tek, USA) and then reverse transcribed into complementary DNA (cDNA) using a SMARTer™ PCR cDNA Synthesis Kit (Clontech, USA). The cDNA was used as the template for amplifying the genes YHR112C, YFR155W, YLL058W, YML082W, YGR130C and YAL012W (S. cerevisiae); KLLA0_C17028g, KLLA0_F07909g, KLLA0_B04378g, KLLA0_F09317g and KLLA0_E09108g (K. lactis); and YALI0E09108g, YALI0C22088g, YALI0F05874g and YALI0D17402g (Y. lipolytica) because the corresponding enzymes possess the same motifs as the L-Met metabolism PLP-dependent enzyme (https://www.kegg.jp/kegg/catalog/org_list.html) (Table S1). Subsequently, the amplified fragments were sequenced and verified.

Phylogenetic analysis

The sequences of the proteins corresponding to the genes mentioned above were aligned and compared by using the multiple sequence alignment software.
ClustalW, and then, a phylogenetic tree was constructed from the ClustalW multiple sequence alignment using the neighbour-joining method in MEGA X 10.0.4. The bar = 0.2 amino acid substitutions/site.

**Gene expression analysis**

To examine the differential expression of YFR055W, KLLA0_E21319g, YHR112C and YALI0C22088g in

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response to l-Met addition, we designed primers for quantitative real-time PCR to detect and quantify the gene expression (Table S1). RNA was isolated from cells harvested from 2-day-old fermentation cultures and then reverse transcribed into cDNA by using the method mentioned above. Quantitative real-time PCR was performed in triplicate on a BIO-RAD CFX Connect™ (BIO-RAD). Power SYBR Green PCR Master Mix (Applied Biosystems cat: 4367659) was used to visualize the gene amplification. 18S rRNA was used as a housekeeping gene to normalize the expression of the genes related to l-Met depletion (Table S1). The comparative cycle threshold ($C_T$) method was adopted to evaluate the relative quantification of the target gene transcripts.

Fig. 5. Inferred functions of N89, S237, G238, P239 and G240.
A. Sequence alignment of YALI0C22088g with PLP-dependent enzymes. Numbering is given for 1IBJ, solid triangular wedges show 3VK3 (PDB ID, mutant methionine-$\gamma$-lyase from Pseudomonas putida C116) active site residues, solid circles show 1N8P (PDB ID, cystathionine $\gamma$-lyase from yeast) active site residues, and 1IBJ (PDB ID) is a cystathionine-$\gamma$-lyase from Arabidopsis. 5TSU (PDB ID) is an engineered cystathionine-$\gamma$-lyase that acquired the ability to degrade l-Met. The 8 key amino acid residues boxed in blue in YALI0C22088g do not overlap with the active site residues of 3VK3 and 1N8P.
B. Structural model of YALI0C22088g with l-Met and PLP. N89, S237, G238, P239 and G240 are predicted to constitute the tunnel, and they are marked with stick models (Fig. S7B).
C. The time dependence of the root mean square deviations (RMSD) for YALI0C22088g (protein RMSD, marked in blue) and l-Met (ligand RMSD, marked in red).
The mean values ± SD of three independent experiments are shown.

Heterologous expression and purification of enzymes related to L-Met metabolism

The full-length nucleic acid sequences of KLLA0_E21319g, YHR112C and YALI0C22088g were amplified and cloned into the yeast expression plasmid pYES2 (Table S1). To purify these enzymes, a His-tag-encoding fragment was inserted after the second codon TCT. After the resulting plasmids were transformed into S. cerevisiae strain INVSc1, the enzymes, including KLLA0_E21319g, YHR112C and YALI0C22088g, were induced with galactose and purified by nickel affinity chromatography as described (Jia et al., 2016). The concentrations of the purified proteins were determined using a BCA Protein Quantification Kit (Yeasen Biotech Co., Ltd., Shanghai, China). All the enzymes mentioned above were subjected to MALDI-TOF/TOF analysis.

C-S lysis activity determination

The L-Met depletion activity was assayed (in a total volume of 1 ml) in 50 mM Tris–HCl (pH 8.0, the optimal pH) containing 20 mM L-Met, 5 μM PLP and 0.6 mg of the purified enzymes. The reaction was performed at 30°C for 1 h. The complete assay with the inactivated enzymes (when boiled for 10 min) was used as a control experiment. The mean values ± SD of three independent experiments are shown. The decrease in the L-Met concentration was detected by HPLC (Dionex UltiMate 3000; Thermo, Waltham, MA, USA). A 200 μl enzymatic reaction mixture was mixed with an equal amount of acetonitrile and then subjected to chromatographic separation on a Reprosil-Pur Basic C18 column (4.6 mm × 250 mm × 5 μm) from Dr Maisch GmbH (Germany) with 13%/87% acetonitrile/water adjusted to pH 3.0 by H₃PO₄ as the mobile phase and 230 nm as the detection wavelength. The column oven temperature and flow rate were 40°C and 0.5 ml min⁻¹ respectively. The retention times for L-Met and α-ketobutyrate were 5.08 and 5.81 min respectively. Subsequently, the enzymatic conversion of L-Met into α-ketobutyrate was identified by an Ultimate 3000 UHPLC system coupled with a Q Exactive Focus mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The production of MTL was estimated by detecting the reaction of MTL with DTNB following a method described previously (Yamagata et al., 1993). Decreases in the concentrations of L-cystathionine, L-homocysteine, L-cystine and L-cysteine were determined with an UltiMate 3000 UHPLC system coupled with a Q Exactive Focus mass spectrometer.

Generating YALI0C22088g mutants

To generate YALI0C22088g mutants, YALI0C22088g was synthesized and cloned into pET28a with GenScript (Nanjing, China), and the expressed YALI0C22088g was purified by nickel affinity chromatography. To identify the essential amino acid residues in YALI0C22088g, a Mut Express II Fast Mutagenesis Kit V2 (Vazyme, Nanjing, Jiangsu Province, China) was applied to mutate the selected amino acid residues to A (A28 of YALI0C22088g was mutated into the corresponding amino acid residue N25 of KLLA0_E21319g) by using the primers detailed in Table S1.

The L-Met concentrations used here were 0.1 to 10 mM with PLP at 5 μM for L-Met depletion kinetics, and the kinetic parameters were calculated following the method described by Asada et al. (2013). The total volume for the enzymatic reaction was 0.5 ml, and 0.3 mg of the purified enzymes was added into the reaction mixture. The reaction was performed at 30°C for 30 min. The decrease in the L-Met concentration was detected using the method described above. The data are shown as the mean values ± SD of three independent experiments.

Molecular model, molecular docking and molecular dynamics simulation

The model for YALI0C22088g was established by using SWISS-MODEL (https://swissmodel.expasy.org/). 5eig.1.A, carrying 35.5% sequential identity with YALI0C22088g, has the highest global model quality (GMOE) value and was adopted as the template for building the 3D structure of YALI0C22088g. The structures were predicted by CAVER software (https://loschmidt.chemi.muni.cz/hotspotwizard/) and displayed using PyMOL software (https://pymol.org/2/) (Mura et al., 2010; Sumbalova et al., 2018). The charge analysis was predicted by using PDBePQR software (http://server.poissonboltzmann.org/opal-jobs/) (Dolinsky et al., 2004). We used Auto Dock Tools (ADT) from AutoDock 4.2 software for molecular docking. To obtain the conformations of PLP and L-Met in YALI0C22088g, we referred to the structures of 1N8P and 1STU (PDB IDs) containing PLP and L-Met respectively. A molecular dynamics (MD) simulation of YALI0C22088g complexed with PLP and L-Met was performed using the Desmond module in the Schrodinger software package. The structure was immersed in a solvent box that extended 10 Å away from the solute border, and Na⁺ or Cl⁻ ions were added into the box to neutralize the whole system. After energy minimization, the temperature and pressure of the system were set to 300 K and 1.01325 bar respectively. The SHAKE algorithm was applied to constrain all

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of the bonds involving hydrogen atoms. A 2 fs integration step was used, and the total simulation time was 100 ns. The MD simulation was performed under the OPLS-2005 force field.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Kai-Zhi Jia designed the research and wrote the manuscript. Quan-Lu Zhao, Zhu-Lin Wang, Lan Yang and Sai Zhang performed the experiments. Kai-Zhi Jia, Quan-Lu Zhao and Zhu-Lin Wang analysed the data.

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### Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplementary Material