

Supporting information

A green and sustainable multi-enzyme cascade for 1,3-diaminopropane biosynthesis from crude glycerol in vitro

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Table S1 The genes and plasmids used in this study

Gene Name	Source	Plasmid	Enzyme name
<i>ScALDO</i>	<i>Streptomyces coelicolor A3</i>	pET28a-ScALDO	alditol oxidase
<i>PsoTA</i>	<i>Pseudomonas sp. Strain AAC</i>	pET28a-PsoTA	ω -Transaminase
<i>AfAlaDH</i>	<i>Archaeoglobus fulgidus VC-16 (DSM4304)</i>	pET28a-AfAlaDH	Alanine dehydrogenase
<i>RsPTXD</i>	<i>Ralstonia sp. strain 4506</i>	pET28a-RsPTXD	Phosphite Dehydrogenase
<i>KpGDHT</i>	<i>Klebsiella pneumoniae</i>	PACYCduet-1-KpGDHt	Glycerol Dehydratase
<i>KpGDHT/Q337A</i>	<i>Klebsiella pneumoniae</i>	PACYCduet-1-KpGDHt/Q337A	Glycerol Dehydratase
<i>KpGDHT/Q337A/S302D</i>	<i>Klebsiella pneumoniae</i>	PACYCduet-1-KpGDHt/Q337A/S302D	Glycerol Dehydratase

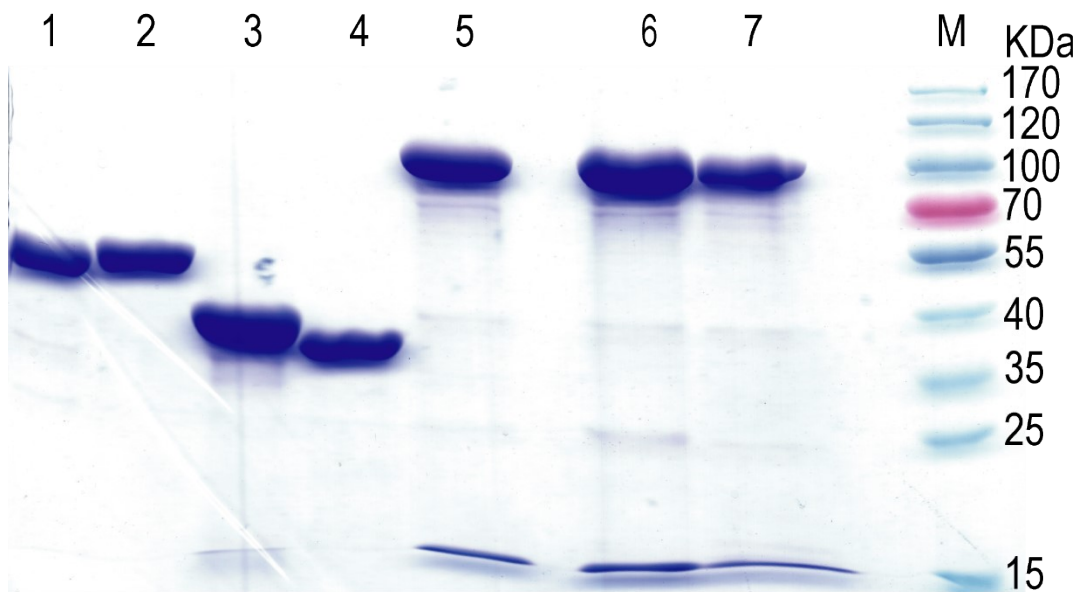


Fig S1 SDS-PAGE of purified enzymes. Lane 1: ScALDO, Lane 2: Ps ω TA, Lane 3: AfAlaDH, Lane 4: RsPTXD, Lane 5: KpGDHT, Lane 6: KpGDHT/Q337A, Lane 7: KpGDHT/Q337A/S302D, M: Marker.

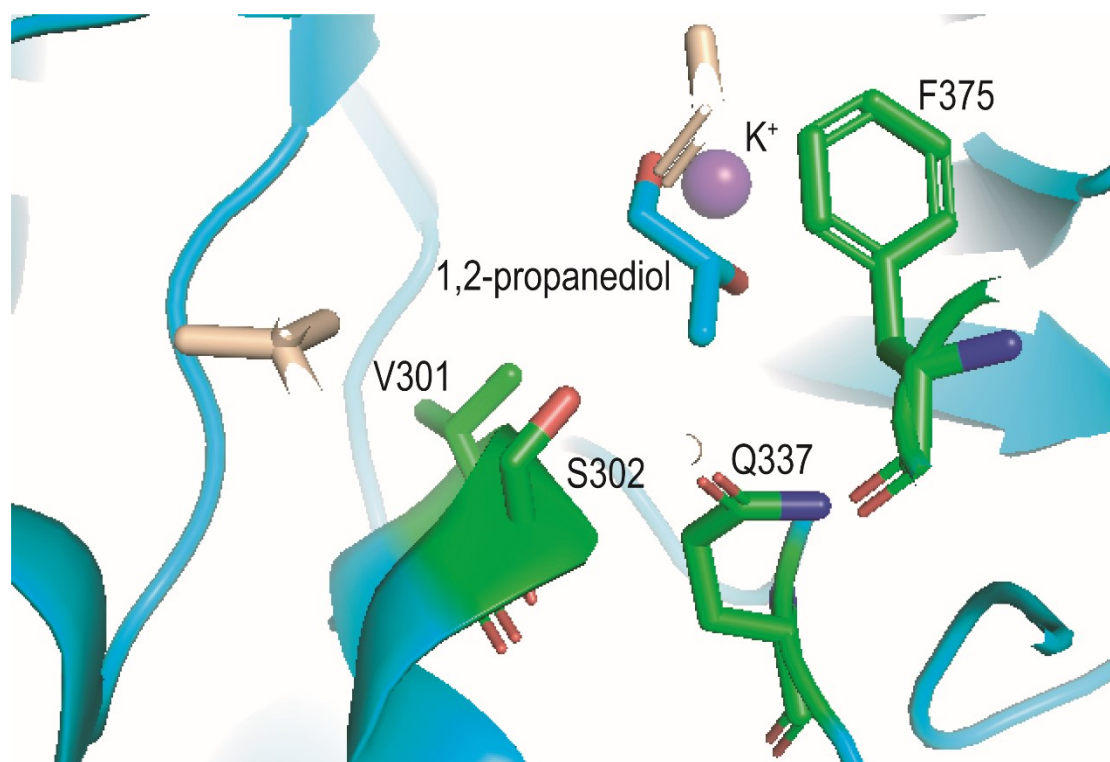


Fig S2 Selection of mutation residues for glycerol dehydratase.

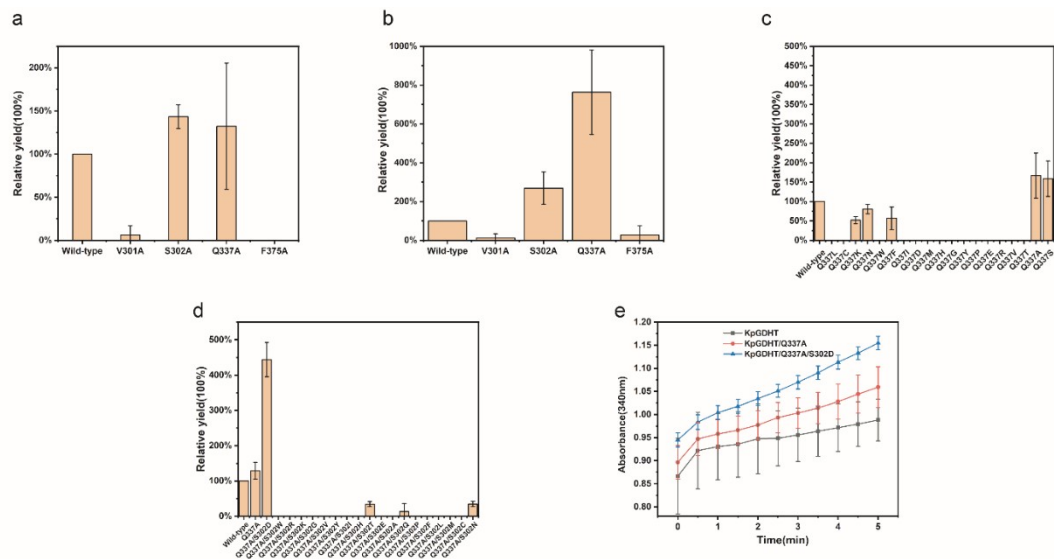


Fig S3 Enzyme modification. (a) Comparison of enzyme activity under 10mM 3-amino-1,2-propanediol for V301A, S302A, Q337A, F375A mutations. (b) Comparison of enzyme activity under 100mM 3-amino-1,2-propanediol for V301A, S302A, Q337A, F375A mutations. (c) Comparison of enzyme activity under conditions of 10mM 3-amino-1,2-propanediol for saturated mutants at KpGDHT's 337 residue. (d) Comparison of enzyme activity under conditions of 10mM 3-amino-1,2-propanediol for saturated mutants at KpGDHT/Q337A's 302 residue. (e) Comparison of enzyme activity for KpGDHT and mutants under conditions of 10mM 3-amino-1,2-propanediol.

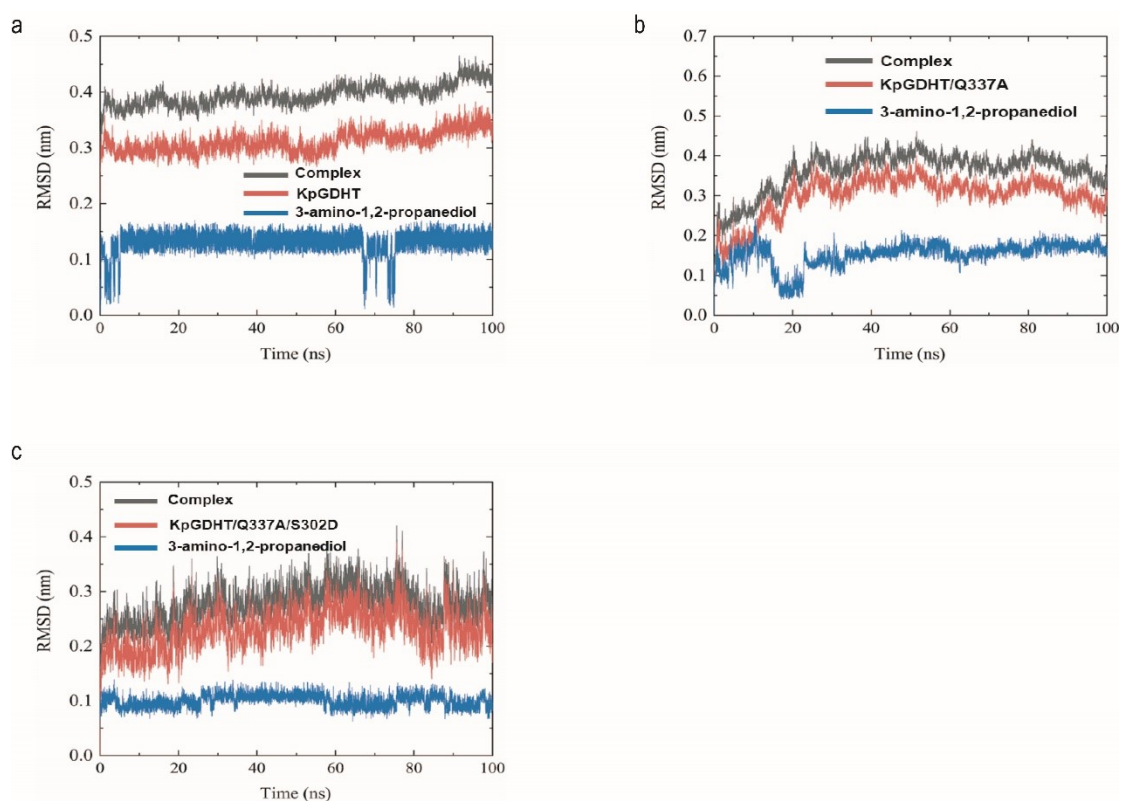


Fig S4 Result of molecular dynamics simulation (a) RMSD of the complex between 3-amino-1,2-propanediol and KpGDHT. (b) RMSD of the complex between 3-amino-1,2-propanediol and KpGDHT/Q337A. (c) RMSD of the complex between 3-amino-1,2-propanediol and KpGDHT/Q337A/S302D.

Table S2: Primers required for KpGDHT modification.

Primer	Primer sequence (5'to3')
VAL301ALA-S	GGTGCGGGTGTTC AAGTTTGCAGAATGGTGCTGCGAGCTGCATAGGTATGACCGGTGC
VAL301-A	CAAACCTTGAACACCCGCACC
PHE375ALA-A	TGTAGTCGTCGAAATCTTCAGCGTCGAAGTTTCGAGCCAGCCGCCATGTTATCATAATTC
PHE375-S	CGACGCTGAAGATTTTCGACGACTACA
SER302ALA-S	GGTGCGGGTGTTC AAGTTTGCAGAATGGTGCTGTGGCGTGCATAGGTATGACCGGTGC
SER302ARG-S	GGTGCGGGTGTTC AAGTTTGCAGAATGGTGCTGTGCGTTGCATAGGTATGACCGGTGC
SER302ASN-S	GGTGCGGGTGTTC AAGTTTGCAGAATGGTGCTGTGAACTGCATAGGTATGACCGGTGC
SER302ASP-S	GGTGCGGGTGTTC AAGTTTGCAGAATGGTGCTGTGGACTGCATAGGTATGACCGGTGC
SER302CYS-S	GGTGCGGGTGTTC AAGTTTGCAGAATGGTGCTGTGTGCTGCATAGGTATGACCGGTGC
SER302GLN-S	GGTGCGGGTGTTC AAGTTTGCAGAATGGTGCTGTGCAATGCATAGGTATGACCGGTGC
SER302GLU-S	GGTGCGGGTGTTC AAGTTTGCAGAATGGTGCTGTGGAGTGCATAGGTATGACCGGTGC
SER302GLY-S	GGTGCGGGTGTTC AAGTTTGCAGAATGGTGCTGTGGGTTGCATAGGTATGACCGGTGC
SER302HIS-S	GGTGCGGGTGTTC AAGTTTGCAGAATGGTGCTGTGCATTGCATAGGTATGACCGGTGC
SER302ILE-S	GGTGCGGGTGTTC AAGTTTGCAGAATGGTGCTGTGATTTGCATAGGTATGACCGGTGC
SER302LEU-S	GGTGCGGGTGTTC AAGTTTGCAGAATGGTGCTGTGTTGTGCATAGGTATGACCGGTGC
SER302LYS-S	GGTGCGGGTGTTC AAGTTTGCAGAATGGTGCTGTGAAATGCATAGGTATGACCGGTGC

SER302MET-S	GGTGCGGGTGTTC AAGGTTTGCAGAATGGTGCTGTGATGTGCATAGGTATGACCGGTGC
SER302PHE-S	GGTGCGGGTGTTC AAGGTTTGCAGAATGGTGCTGTGTTCTGCATAGGTATGACCGGTGC
SER302PRO-S	GGTGCGGGTGTTC AAGGTTTGCAGAATGGTGCTGTGCCGTGCATAGGTATGACCGGTGC
SER302THR-S	GGTGCGGGTGTTC AAGGTTTGCAGAATGGTGCTGTGACCTGCATAGGTATGACCGGTGC
SER302TRP-S	GGTGCGGGTGTTC AAGGTTTGCAGAATGGTGCTGTGTGGTGCATAGGTATGACCGGTGC
SER302TYR-S	GGTGCGGGTGTTC AAGGTTTGCAGAATGGTGCTGTGATTGCATAGGTATGACCGGTGC
SER302VAL-S	GGTGCGGGTGTTC AAGGTTTGCAGAATGGTGCTGTGGTGTGCATAGGTATGACCGGTGC
SER302-A	CAAACCTGAACACCCGCACC
GLN337ALA-A	GAGCGGTACGACGGATATCGCTATGGGAAAAGGTGCGATCGTTCGCTGAAGCCACCTC
GLN337ARG-A	GAGCGGTACGACGGATATCGCTATGGGAAAAGGTGCGATCGTTCGCTGAAGCCACCTC
GLN337ASN-A	GAGCGGTACGACGGATATCGCTATGGGAAAAGGTATTATCGTTCGCTGAAGCCACCTC
GLN337ASP-A	GAGCGGTACGACGGATATCGCTATGGGAAAAGGTATCATCGTTCGCTGAAGCCACCTC
GLN337CYS-A	GAGCGGTACGACGGATATCGCTATGGGAAAAGGTGCAATCGTTCGCTGAAGCCACCTC
GLN337SER-A	GAGCGGTACGACGGATATCGCTATGGGAAAAGGTTGAATCGTTCGCTGAAGCCACCTC
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GLN337MET-A	GAGCGGTACGACGGATATCGCTATGGGAAAAGGTCATATCGTTCGCTGAAGCCACCTC
GLN337PHE-A	GAGCGGTACGACGGATATCGCTATGGGAAAAGGTAAAATCGTTCGCTGAAGCCACCTC
GLN337PRO-A	GAGCGGTACGACGGATATCGCTATGGGAAAAGGTGCGATCGTTCGCTGAAGCCACCTC
GLN337THR-A	GAGCGGTACGACGGATATCGCTATGGGAAAAGGTGGTATCGTTCGCTGAAGCCACCTC
GLN337TRP-A	GAGCGGTACGACGGATATCGCTATGGGAAAAGGTCCAATCGTTCGCTGAAGCCACCTC
GLN337TYR-A	GAGCGGTACGACGGATATCGCTATGGGAAAAGGTATAATCGTTCGCTGAAGCCACCTC
GLN337VAL-A	GAGCGGTACGACGGATATCGCTATGGGAAAAGGTGACATCGTTCGCTGAAGCCACCTC
GLN337-S	AGCGATATCCGTCGTACCGCTC

Table S3. Amino acid sequences of enzymes

Gene Name	Source	Amino Acids Sequence
<i>ScALDO</i>	<i>Streptomyces coelicolor A3</i>	MSDITVTNWAGNITYTAKELLRPHSLD ALRALVADSARVRVLGSGHSFNEIAEPG DGGVLLSLAGLPSVVDVDTAARTVRVG GGVRYAELARVVHARGLALPNMASLP HISVAGSVATGTHGSGMGNGSLASMVR EVELVTADGSTVVIARGDERFGGAVTS LGALGVVTSLTLDLEPAYEMEQHVFT LPLAGLDPATFETVMAAAYSVSLFTDW RAPGFRQVWLKRRRTDRPLDGFYAAPA TEKMHPVPGMPAVNCTEQFGVPGPWH ERLPHFRAEFTPSSGAELQSEYLMPREH ALAALHAMDAIRETLAPVLQTCEIRTV ADAQWLSPAYGRDTVAAHFTWVEDTA AVLPVRRLEEALVPFAARPHWGKVFT VPAGELRALYPRLADFGALARALDPAG KFTNAFVRGVLG
<i>PsoTA</i>	<i>Pseudomonas sp. Strain AAC</i>	MNQQVNVAPSAAADLNLKAHWMPFSA NRNFHKDPRIIVAAEGSWLVDDKGRRI YDSLGLWTCGAGHSRKEIADAVAKQI GTLDYSPGFQYGHPLSFQLAEKIAQMTP GTLDHVFFTGSGSECADTSIKMARAYW RIKGQAQKTKLIGRARGYHGVNVAGTS LGGIGGNRKMFGPLMDVDHLPHTLQPG MAFTKGA AETGGVELANELLKLIELHD ASNIAAVIVEPMSGSAGVIVPPKGYLQR LREICDANDILLIFDEVITAFGRMGKATG AEYFGVTPDIMNVAKQVTNGAVPMGA VIASSEIYDTFMNQNLPEYAVEFGHGYT YSAHPVACAAGIAALDLLQKENLIQQS AELAPHFEKALHGLKGTKNVIDIRNCGL AGAIQIAARDGDAIVRPFEASMKLWKE GFYVRFGGDTLQFGPTFNAKPEDLDRLF DAVGEALNGVA
<i>AfAlaDH</i>	<i>Archaeoglobus fulgidus</i> <i>VC-16 (DSM4304)</i>	METLILTQEEVESLISMDEAMNAVEEAF RLYALGKAQMPPKVYLEFEKGD LRAM PAHLMGYAGLKWVNSHPGNPDKGLPT VMALMILNSPETGFPLAVMDATYTTSL RTGAAGGIAAKYLARKNSSVFGFIGCGT QAYFQLEALRRVFDIGEVKAYDVREKA AKKFVSYCEDRGISASVQPAEEASRCDV LVTTTTPSRKPVVKA EWVEEGTHINAIGA

	DGPGKQELDVEILKKAKIVVDDLEQAK HGGEINVAVSKGVIGVEDVHATIGEVIA GLKDGRESDEEITIFDSTGLAIQDVAVA KVVYENALSKNVGSKIKFFRI
<i>RsPTXD</i> <i>Ralstonia sp. strain 4506</i>	MKPKVVLTHWVHPEIIELLSASADVIPN TTRETLRSEVIARAKDADALMAFMPD SIDSAFLEECPLRVIGAALKGYDNFDV NACTRHGVWLTIVPDLLTIPTAELTIGLL LGLTRHMLEGDRQIRSGHFQGWRTLY GSGLTGKTLGIIGMGAVGRAIAQRLAGF EMNLLYCDPIPLNAEQEKAWHVQRVTL DELLEKCDYVVPMPMAAETLHLIDAT ALAKMKTGSYLINACRGSVVDENAVIA ALASGKLAGYAADVFEEMEEWIRADRP QAIPKALLDNTAQTFFTPPLGSAVKEVR LEIERQAAMNIIQALAGEKPMGAINQPY PGVCAA
<i>KpGDHT</i> <i>Klebsiella pneumoniae</i>	MKRSKRFAVLAQRPVNQDGLIGEWPEE GLIAMDSPFDPVSSVKVDNGLIVELDGK RRDQFDMIDRFIADYAINVERTEQAMR LEAVEIARMLVDIHVSREEIIAITTAITPA KAVEVMAQMNVVEMMMALQKMRAR RTPSNQCHVTNLKDNPVQIAADAAEAG IRGFSEQETTVGIARYAPFNALALLVGS QCGRPGVLTQCSVEEATELELGMRGLT SYAETVSVYGTEAVFTDGDTPWSKAF LASAYASRGLKMRYTSGTGSEALMGYS ESKSMLEYESRCIFITKGAGVQGLQNGA VSCIGMTGAVPSGIRAVLAENLIASMLD LEVASANDQTFSHSDIRRTARTLMQML PGTDFIFSGYSAVPNYDNMFAGSNFDAE DFDDYNILQRDLMVDGGLRPVTEAETI AIRQKAARAIQAVFRELGLPPIADEEVE AATYAHGSNEMPPRNVEDLSAVEEM MKRNITGLDIVGALSRSFGEDIASNILN MLRQRVTGDYDYLQTSAILDRQFEVVSVA NDINDYQGPVTGYRISAERWAEIKNIPG VVQPDITIEGPTPTPTPTPTPTPTPTPTPT PTGMQQTQIQPSFTLKTREGGVASADE RADEVVIGVGPFDKHQHHTLIDMPHG AILKELIAGVEEEGLHARVVRILRTSDVS FMAWDAANLSGSGIGIGIQSKGTTVIHQ RDLLPLSNLELFSQAPLLTLETYRQIGKN AARYARKESPSPVPVNDQMVRPKFM

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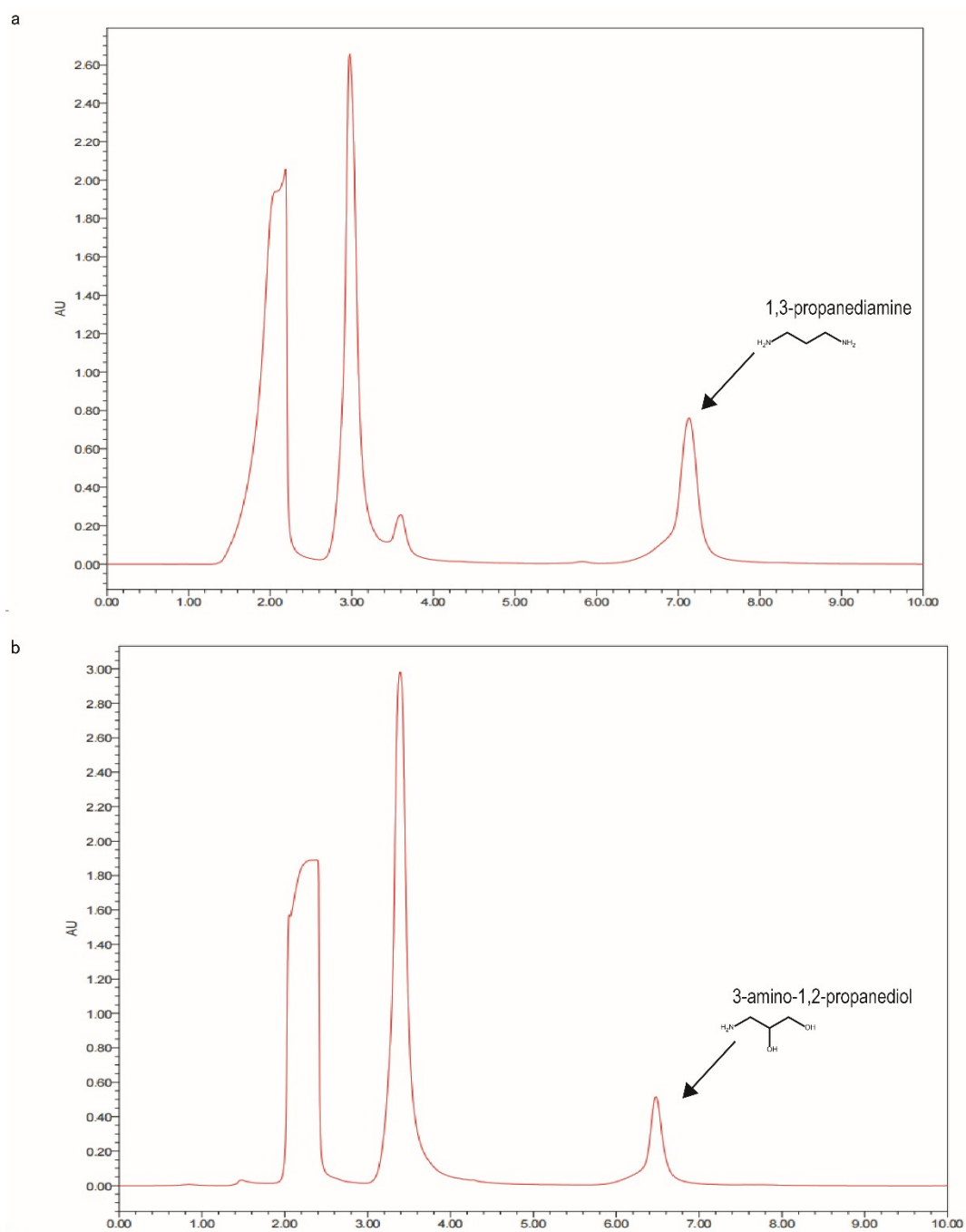


Fig. S4 HPLC analysis of the products. (a) 1,3-propanediamine. (b) 3-amino-1,2-propanediol.