

Supporting information

A human cell orthogonal enzyme β -D-galacturonidase for sensitive detection of proteins

Chuya Tateishi^{1,†}, Akihiro Koga^{1,†}, Atsuhiko Matsuura², Ryosuke Kaneko², Kenta Tanito¹,
Teruki Nii^{1,2}, Akihiro Kishimura^{1,2,3,4}, Takeshi Mori^{*1,2,3}, Yoshiki Katayama^{*1,2,3,4,5}

¹ Graduate School of Systems Life Sciences, Kyushu University, 744 Motoooka, Nishi-ku, Fukuoka 819-0395, Japan

² Department of Applied Chemistry, Faculty of Engineering, Kyushu University, 744 Motoooka, Nishi-ku, Fukuoka 819-0395, Japan

³ Center for Future Chemistry, Kyushu University, 744 Motoooka, Nishi-ku, Fukuoka 819-0395, Japan

⁴ International Research Center for Molecular Systems, Kyushu University, 744 Motoooka, Nishi-ku, Fukuoka, 819-0395, Japan

⁵ Department of Biomedical Engineering, Chung Yuan Christian University, 200 Chung Pei Rd., Chung Li, 32023 ROC, Taiwan

[†] These authors equally contributed to this work

E-mail: mori.takeshi.880@m.kyushu-u.ac.jp; katayama.yoshiki.958@m.kyushu-u.ac.jp

Table S1. GalUAase reported so far.

| origin | length (aa) | homology with <i>Eisenbergiella tayi</i> (%)* | activity | crystal structure | reference |
|--|-------------|---|----------|----------------------|-----------|
| <i>Eisenbergiella tayi</i> | 559 | - | reported | reported | 1 |
| <i>Faecalibacterium sp.</i> | 555 | 62 | reported | N.A. | 1 |
| <i>Bacteroides thetaiotaomicron VPI-5482</i> | 950 | 40 | N.A. | N.A. | 2 |

*Calculated based on Smith-Waterman algorithm

(a)

HHHHHSSGVDLGTENLYFQSNAMIRTFETHKIRKTAELSSALWNFHTI
GTQGEEAVIQAPVPGCWENYPDTVSYRGQASYSREFEAKGNIRLEFK
GVSHTASVLVDGKPVGSHYNAYTPFDVVLKDIRPGIHQLEVIADNSFG
PDSALHVPNDYQSYGGISRGVLEELGEAYLSWIHFTPFLRKDGWYG
KAEICVRNLSSGRLDGSVEVEIGKNSFAVLPIVLEGESEEKSFSTEELPC
PWAECWSPESPVLYLITAVLRTADGAADDIIDRVGFREIRTEGKDILLNG
RKLRIKGFRCRHEDHPQFGCALPFSAMQHDLMLIKDLGANSIRTVHYPN
DEFLDLCDEQGILVWEENHARGLSEENMRNPHFKQQCGDCIREMIT
AHYNHPSIYIWGILNECASDTEYGRECYSEQYELIKSLDPYRPRSSAS
CRFKTDICLGYPEVVSYNIYPKWYHDVPVEDYLDELYQWIQNESEGTG
KPFLITEIGAGAIYGYRTPAHVKWSEYQVQALKEQLQAVFSREGCSG
VYIWQFCDVRVCDSWFGSRPRTMNNKGIVDEYRRPKLAYEVVKDSY
RSLGNYF

(b)

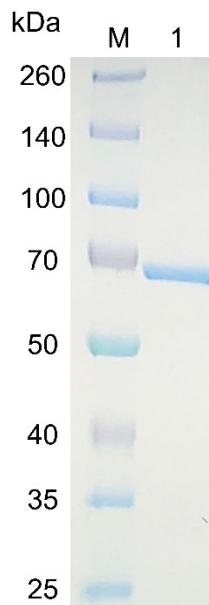


Fig. S1. (a) The amino acid sequence of GalUAase with a histidine tag at the N-terminus. (b) SDS-PAGE of GalUAase after purification by His-tag column. Lane M, molecular weight marker; lane 1, GalUAase (66 kDa).

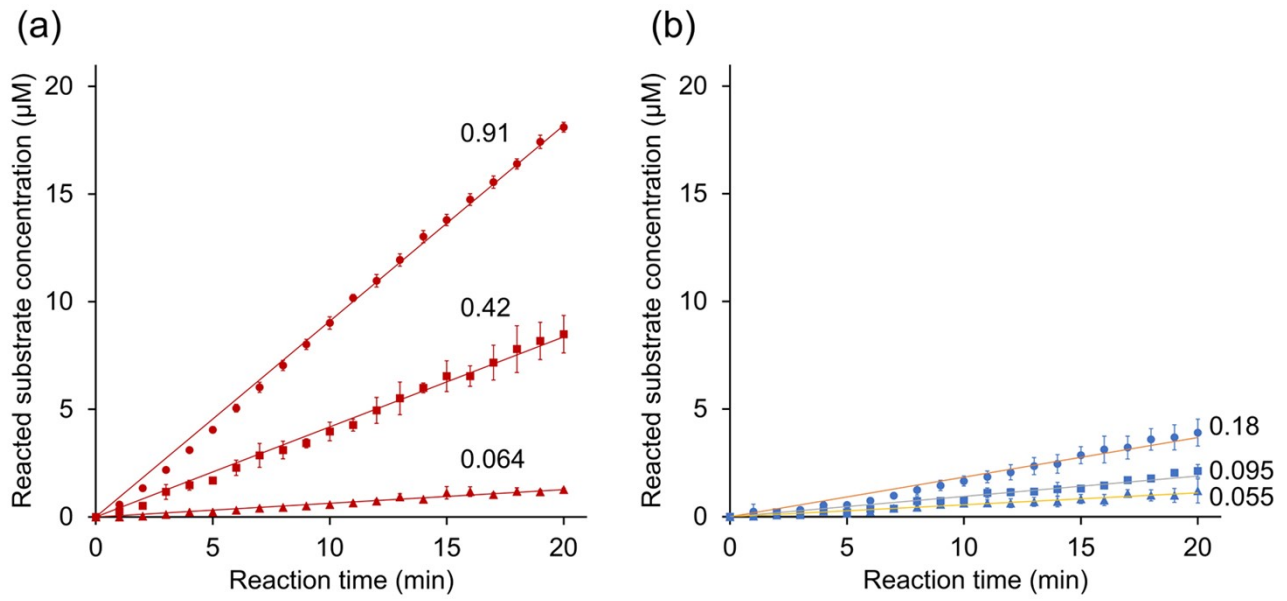


Fig. S2. Measurement of reaction speed of GalUAase (a) and Galase (b) toward NP substrates at 0.01 (triangle), 0.1 (square), 1.0 mM (circle) concentrations at 37°C. Numbers described on each liner line are slope of the line. Concentration of enzyme is 0.05 nM. Buffer is D-PBS. Data are mean \pm S.D, n=3.

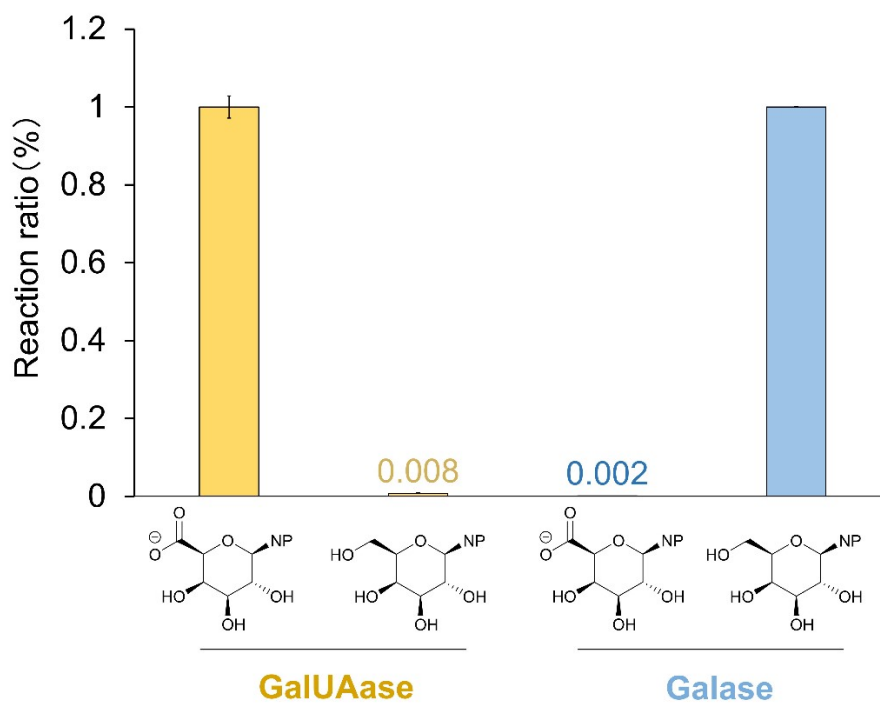


Fig. S3. Reaction ratio of each p-nitrophenyl substrate by GalUAase and Galase after 18 h at 37°C and in DPBS (pH 7.4). NP in chemical structures indicates p-nitrophenyl group. Data are mean \pm S.D, n = 3.

References

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2. Didier Ndeh, Artur Rogowski, Alan Cartmell, Ana S. Luis, Arnaud Baslé, Joseph Gray, Immacolata Venditto, Jonathon Briggs, Xiaoyang Zhang, Aurore Labourel, Nicolas Terrapon, Fanny Buffetto, Sergey Nepogodiev, Yao Xiao, Robert A. Field, Yanping Zhu, Malcolm A. O'Neill, Breeanna R. Urbanowicz, William S. York, Gideon J. Davies, D. Wade Abbott, Marie-Christine Ralet, Eric C. Martens, Bernard Henrissat and Harry J. Gilbert, *Nature*, 2017, **544**, 65–70.