(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
05 October 2023 (05.10.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/191726 A1

(51) International Patent Classification:
C12P 21/02 (2006.01)

(21) International Application Number:
PCT/SG2023/050219

(22) International Filing Date:
31 March 2023 (31.03.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10202203286W 31 March 2022 (31.03.2022) SG
10202300549P 01 March 2023 (01.03.2023) SG

(71) Applicant: **NANYANG TECHNOLOGICAL UNIVERSITY** [SG/SG]; 50 Nanyang Avenue, Singapore 639798 (SG).

(72) Inventors: **LIU, Chuan Fa**; c/o Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798 (SG). **XIA, Yiyin**; c/o Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798 (SG). **LI, Fupeng**; c/o Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798 (SG).

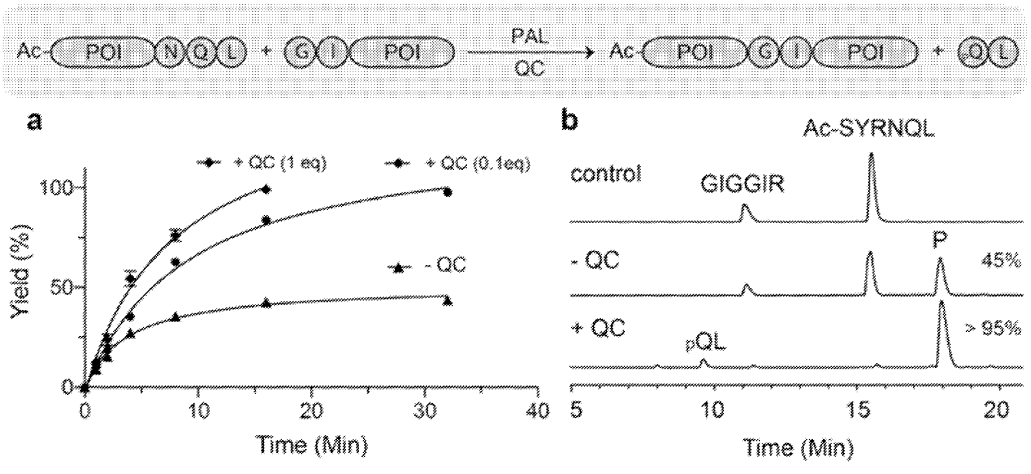
(74) Agent: **DOWSING, Bruce, John**; Marks & Clerk Singapore LLP, Tanjong Pagar Post Office, P.O. Box 636, Singapore 910816 (SG).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: IMPROVING THE EFFICIENCY OF PAL-CATALYZED PROTEIN LIGATION BY A CASCADE ENZYMATIC SCHEME

FIG. 1



(57) Abstract: The present invention generally relates to enzymatic peptide or protein ligation. In particular, the present invention provides an improved method of enzymatic peptide or protein ligation, which comprises coupling a peptidyl asparaginyl ligase (PAL)-catalyzed ligation to a glutaminyl cyclase (QC)-catalyzed pyroglutamyl formation to improve yield of ligated product.

[Continued on next page]

WO 2023/191726 A1

Published:

— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*