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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 October 2014 (23.10.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/172631 A3

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

(21) International Application Number:
PCT/US2014/034643

(22) International Filing Date:
18 April 2014 (18.04.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/813,914 19 April 2013 (19.04.2013) US
61/937,069 7 February 2014 (07.02.2014) US

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(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, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, 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, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, 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, 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).

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))*

(88) Date of publication of the international search report:
18 December 2014

(54) Title: EXPRESSION OF BIOLOGICALLY ACTIVE PROTEINS IN A BACTERIAL CELL-FREE SYNTHESIS SYSTEM USING CELL EXTRACTS WITH ELEVATED LEVELS OF EXOGENOUS CHAPERONES

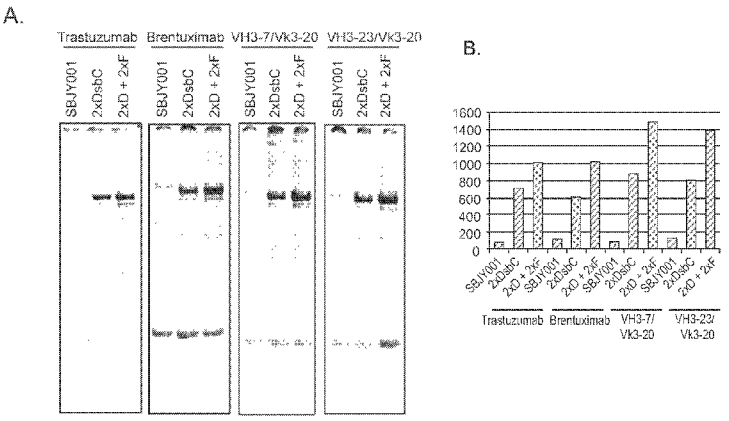


FIG. 13

(57) Abstract: The present disclosure describes methods and systems for improving the expression of a properly folded, biologically active protein of interest in a cell free synthesis system. The methods and systems use a bacterial cell free extract having an active oxidative phosphorylation system, and include an exogenous protein chaperone. The exogenous protein chaperone can be expressed by the bacteria used to prepare the cell free extract. The exogenous protein chaperone can be a protein disulfide isomerase and/or a peptidyl-prolyl cis-trans isomerase. The inventors discovered that the combination of a protein disulfide isomerase and a peptidyl-prolyl cis-trans isomerase produces a synergistic increase in the amount of properly folded, biologically active protein of interest.

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