



- (51) **International Patent Classification:**
C12P 21/02 (2006.01) *C12N 9/50* (2006.01)
- (21) **International Application Number:**
PCT/US2024/013950
- (22) **International Filing Date:**
01 February 2024 (01.02.2024)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
63/482,844 02 February 2023 (02.02.2023) US
- (71) **Applicant:** AMGEN INC. [US/US]; One Amgen Center Drive, Thousand Oaks, California 91320-1799 (US).
- (72) **Inventors:** XIAO, Gang; c/o Amgen Inc., Law Department-Patent Operations, One Amgen Center Drive, M/S 28-5-A, Thousand Oaks, California 91320-1799 (US). CHUNG, Hyo Seon; c/o Amgen Inc., Law Department-Patent Operations, One Amgen Center Drive, M/S 28-5-A, Thousand Oaks, California 91320-1799 (US). LUO, Quanzhou; c/o Amgen Inc., Law Department-Patent Operations, One Amgen Center Drive, M/S 28-5-A, Thou-

sand Oaks, California 91320-1799 (US). ERIS, Tamer; c/o Amgen Inc., Law Department-Patent Operations, One Amgen Center Drive, M/S 28-5-A, Thousand Oaks, California 91320-1799 (US). BASCONI, Joseph Edward; c/o Amgen Inc., Law Department-Patent Operations, One Amgen Center Drive, M/S 28-5-A, Thousand Oaks, California 91320-1799 (US). ZHAO, Xiaoyang; c/o Amgen Inc., Law Department-Patent Operations, One Amgen Center Drive, M/S 28-5-A, Thousand Oaks, California 91320-1799 (US). BONDARENKO, Pavel; c/o Amgen Inc., Law Department-Patent Operations, One Amgen Center Drive, M/S 28-5-A, Thousand Oaks, California 91320-1799 (US). PETTY, Krista Marie; c/o Amgen Inc., Law Department-Patent Operations, One Amgen Center Drive, M/S 28-5-A, Thousand Oaks, California 91320-1799 (US). KALTENBRUNNER, Oliver; c/o Amgen Inc., Law Department-Patent Operations, One Amgen Center Drive, M/S 28-5-A, Thousand Oaks, California 91320-1799 (US).

- (74) **Agent:** MADL, Amy; One Amgen Center Drive, Law Dept - Patent Operations, MS 28-5-A, Thousand Oaks, California 91320-1799 (US).

- (81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

- (54) **Title:** MITIGATION OF THERAPEUTIC PROTEIN FRAGMENTATION DURING CELL CULTURE HARVEST PROCESSES

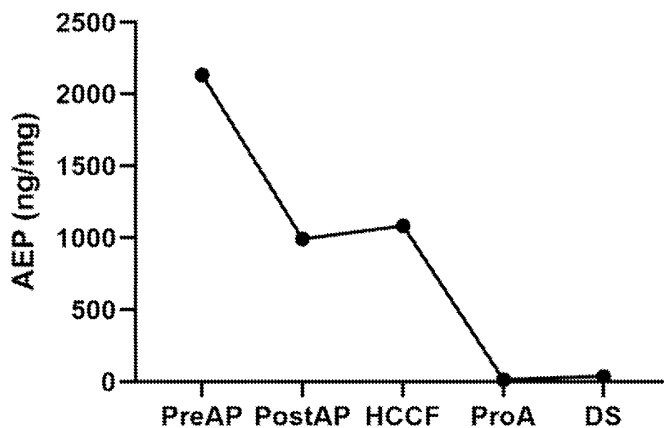


FIG. 1

- (57) **Abstract:** Disclosed herein are methods for harvesting an antibody as recombinant protein comprising an asparaginyl endopeptidase (AEP, legumain) cleavage site having amino acids NF by keeping pH after fermentation at 4.6 to 5.3 for acidification to precipitate impurities, to prevent autocleavage of proenzyme and thus activation of peptidase at lower pH, as well as methods for inhibiting cleavage of a recombinant protein comprising an AEP cleavage site, during a biomanufacturing process.

[Continued on next page]

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).

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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))*