



- (51) **International Patent Classification:**
G01N 33/68 (2006.01) *G01N 30/02* (2006.01)
- (21) **International Application Number:**
PCT/US2021/064116
- (22) **International Filing Date:**
17 December 2021 (17.12.2021)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
63/128,146 20 December 2020 (20.12.2020) US
- (71) **Applicant:** **REGENERON PHARMACEUTICALS, INC.** [US/US]; 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US).
- (72) **Inventors:** **KLEINBERG, Andrew**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US). **MAO, Yuan**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US).
- (74) **Agent:** **TERMES, Lance A.**; Schwabe, Williamson & Wyatt PC, 1211 SW 5th Ave., Suite 1900, Portland, Oregon 97204 (US).

- (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, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, 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, 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, GH, GM, KE, LR, LS, MW, MZ, NA, RW, 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, 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:** METHODS FOR IDENTIFICATION OF SCRAMBLED DISULFIDES IN BIOMOLECULES

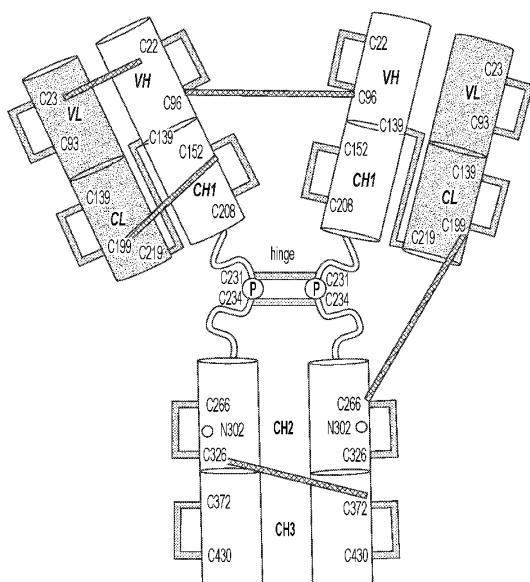


FIG.1A CONT.

(57) **Abstract:** Disclosed are methods for identification of one or more non-native disulfide bonds in a biomolecule (*e.g.*, an antibody). In an example, a method includes performing a digestion of the biomolecule under non-reducing conditions to provide a sample comprising a plurality of biomolecule fragments, contacting the sample to a separation column, applying a first mobile phase gradient comprising trifluoroacetic acid (TFA) and a small molecule additive to the separation column, applying a second mobile phase gradient comprising TFA in acetonitrile (ACN) and a small molecule additive to the separation column, performing a partial reduction procedure on the eluted sample, applying the partially reduced eluted sample components to a mass spectrometer, and performing a mass spectrometric analysis on the partially reduced eluted sample components to identify the one or more non-native disulfide bonds in the biomolecule.

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))*