

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
19 January 2023 (19.01.2023)



(10) International Publication Number
WO 2023/287821 A9

(51) International Patent Classification:

G01N 30/96 (2006.01) G01N 33/68 (2006.01)

(21) International Application Number:

PCT/US2022/036868

(22) International Filing Date:

12 July 2022 (12.07.2022)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/221,447 13 July 2021 (13.07.2021) US
63/305,177 31 January 2022 (31.01.2022) US

(71) Applicant: **REGENERON PHARMACEUTICALS, INC.** [US/US]; 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US).

(72) Inventors: **LIU, Anita**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US). **WANG, Shunhai**; c/o Regeneron Pharmaceu-

ticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US).

(74) Agent: **CAPLAN, Jonthan S.**; Kramer Levin Naftalis & Frankel LLP, 1177 Avenue of the Americas, New York, New York 10036 (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, 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, 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,

(54) Title: CHARACTERIZATION OF PROTEINS BY ANION-EXCHANGE CHROMATOGRAPHY MASS SPECTROMETRY (AEX-MS)

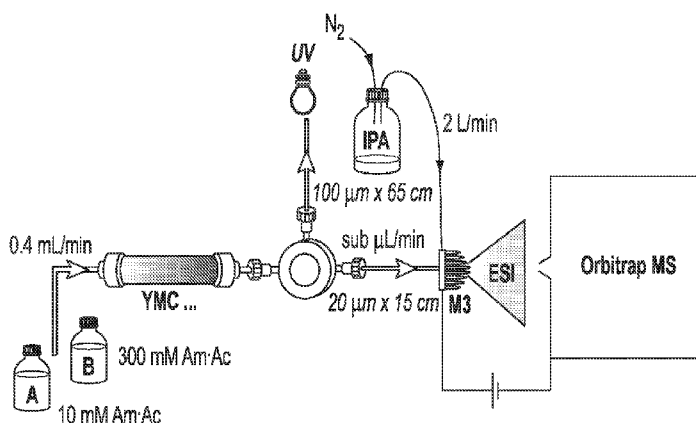


FIG. 3

(57) Abstract: The present invention generally pertains to methods of characterizing charge variants of a protein. In particular, the present invention pertains to the use of anion exchange chromatography-mass spectrometry (AEX-MS) methods using a salt-gradient. The present invention is particularly useful for charge variant analysis of IgG4 subclasses.

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, 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))*

(48) Date of publication of this corrected version:

09 November 2023 (09.11.2023)

(15) Information about Correction:

see Notice of 09 November 2023 (09.11.2023)