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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 March 2013 (07.03.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/032828 A2

(51) International Patent Classification:
A61K 38/42 (2006.01) *A61K 9/08* (2006.01)
A61K 35/14 (2006.01) *A61K 47/48* (2006.01)
A61K 35/12 (2006.01)

(21) International Application Number:
PCT/US2012/051959

(22) International Filing Date:
23 August 2012 (23.08.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/529,279 31 August 2011 (31.08.2011) US
13/225,797 6 September 2011 (06.09.2011) US
13/275,366 18 October 2011 (18.10.2011) US

(72) Inventors; and
(71) Applicants : **WONG, Bing, Lou** [US/US]; 3592 S. Mall Street, Irvine, CA 92606 (US). **KWOK, Sui, Yi** [CN/CN]; Flat 2907, 29/F, Pik Wai House, Shek Pai Wan Estate, Aberdeen (CN). **BUTT, Kowk, Chu** [CN/CN]; Room 7A, Floor 7, Tower 2, Tai Hing Garden Phase 1, Tuen Mun, N.T. (CN).

(74) Agent: **YIP, Sam, T.**; Ella Cheong (Hong Kong) Limited, 5001 Hopewell Centre, 183 Queen's Road East, Wan Chai, Hong Kong (CN).

(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, IS, JP, KE, KG, KM, 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, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, 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, 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))*

— *of inventorship (Rule 4.17(iv))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

WO 2013/032828 A2

(54) Title: METHOD FOR THE PREPARATION OF A HEAT STABLE OXYGEN CARRIER-CONTAINING COMPOSITION FACILATING BETA-BETA CROSS-LINKING

(57) Abstract: Methods for preparation of a heat stable hemoglobin based oxygen-carrier-containing pharmaceutical composition such that beta-beta cross-linking is favored are provided. Using the methods of the present invention, the oxygen affinity of the resultant molecule can be controlled so that hemoglobin based oxygen carriers tailored for specific applications can be produced. Lower oxygen affinity crosslinked hemoglobin is useful for applications requiring rapid tissue oxygenation (e.g. hemorrhagic shock) while higher oxygen affinity cross-linked hemoglobin is useful for applications requiring a slower rate of oxygenation (e.g. cancer adjunct therapy). A highly purified and heat stable crosslinked non-polymeric tetrameric hemoglobin having beta- beta cross-linking of greater than 40-60% and suitable for use in mammals without causing renal injury and vasoconstriction is produced. A high temperature and short time (HTST) heat processing step is performed to effectively remove any undesired dimeric hemoglobin, non- cross-linked tetrameric hemoglobin, and plasma protein impurities.