



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification⁶ : C12N 15/12, C07K 14/805, C12N 1/21	A3	(11) International Publication Number: WO 98/50430 (43) International Publication Date: 12 November 1998 (12.11.98)
(21) International Application Number: PCT/US98/08861 (22) International Filing Date: 1 May 1998 (01.05.98) (30) Priority Data: 60/045,364 2 May 1997 (02.05.97) US 60/057,986 5 September 1997 (05.09.97) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications US 60/045,364 (CIP) Filed on 2 May 1997 (02.05.97) US 60/057,986 (CIP) Filed on 5 September 1997 (05.09.97) (71) Applicants (for all designated States except US): SOMATOGEN, INC. [US/US]; Suite FD-1, 2545 Central Avenue, Boulder, CO 80301 (US). WILLIAM MARSH RICE UNIVERSITY [US/US]; Institute of Bioscience & Bioengineering, 6100 South Main, Houston, TX 77005 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): WEICKERT, Michael, J. [US/US]; 7565 Charmant Drive #311, San Diego, CA 92122 (US). GLASCOCK, Christopher, B. [US/US]; 919 Eldorado	Lane, Louisville, CO 80027 (US). MATHEWS, Antony, J. [NZ/US]; 1639 Spruce Street, Boulder, CO 80302 (US). LEMON, Douglas, D. [US/US]; 639 Fireside, Louisville, CO 80027 (US). DOHERTY, Daniel, H. [US/US]; 719 Ithaca Drive, Boulder, CO 80303 (US). OLSON, John, S. [US/US]; 4407 Lumbar, Houston, TX 77096-4418 (US). (74) Agent: BROWN, Theresa, A.; Somatogen, Inc., Suite FD-1, 2545 Central Avenue, Boulder, CO 80301 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 1 April 1999 (01.04.99)	
(54) Title: HEMOGLOBIN MUTANTS WITH INCREASED SOLUBLE EXPRESSION AND/OR REDUCED NITRIC OXIDE SCAVENGING		
(57) Abstract <p>The invention relates to novel recombinant hemoglobins having reduced nitric oxide scavenging and/or increased high soluble expression. The invention further relates to methods of increasing the soluble expression of recombinant hemoglobin by adding exogenous hemin in molar excess of the heme binding sites of recombinant hemoglobin.</p>		

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INTERNATIONAL SEARCH REPORT

Inventor's Name:
 International Application No

PCT/US 98/08861

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C12N15/12 C07K14/805 C12N1/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 04110 A (SOMATOGEN, INC.) 6 February 1997 cited in the application see the whole document especially examples 17 and 18 --- -/--	5,6

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

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- *E* earlier document but published on or after the international filing date
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- *P* document published prior to the international filing date but later than the priority date claimed

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- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * & * document member of the same patent family

Date of the actual completion of the international search
 19 January 1999

Date of mailing of the international search report
 01.02.99

Name and mailing address of the ISA
 European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax: (+31-70) 340-3016

Authorized officer
 Le Cornec, N

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/08861

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>C. GLASCOCK ET AL: "Amino acid substitutions significantly affect soluble expression of recombinant hemoglobin in E.coli"</p> <p>ABSTRACTS OF THE GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 97, no. 0, 8 April 1997, page 298 XP002077666 WASHINGTON US</p> <p>& 97TH GENERAL METING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, MIAMI BEACH, FLORIDA, USA, 4 - 8 May 1997, see abstract H-85</p>	1,3
X	<p>--- HOFFMAN S J ET AL: "EXPRESSION OF FULLY FUNCTIONAL TETRAMERIC HUMAN HEMOGLOBIN IN ESCHERICHIA COLI"</p> <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 87, no. 21, November 1990, pages 8521-8525, XP000647625 see abstract see page 8522, left-hand column, paragraph 2 - right-hand column, paragraph 1 see page 8521, right-hand column</p>	5
X	<p>--- L. KIGER ET AL: "Recombinant (Phe-beta63)hemoglobin shows rapid oxidation of the beta chains and low affinity, non-cooperative oxygen binding to the alpha subunits"</p> <p>EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 243, no. 1/2, January 1997, pages 365-373, XP002090387 see the whole document</p>	15
A	<p>--- I. PECHIK ET AL: "Crystallographic, molecular modeling and biophysical characterization of the Valine beta67 (E++) : Threonine variant of hemoglobin"</p> <p>BIOCHEMISTRY., vol. 35, 1996, pages 1935-1945, XP002090388 EASTON, PA US see the whole document</p>	7-20
A	<p>--- WO 97 15591 A (RICE UNIVERSITY) 1 May 1997 cited in the application see page 24; example 1; table 8 --- -/--</p>	1-20

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/08861

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	A. DUMOULIN ET AL: "Two mutations in recombinant Hb bewta F41(C7)Y, K82 (EF6)D show additive effects in decreasing oxygen affinity" PROTEIN SCIENCE, vol. 5, no. 1, January 1996, pages 114-120, XP002077667 ---	1,3,4
A	US 5 028 588 A (SOMATOGENETICS INTERNATIONAL, INC.) 2 July 1991 cited in the application see the whole document especially page 24 table 2, page 9 line 28-41 ---	1-7,10, 15
A	TONG-JIAN SHEN ET AL: "Production of unmodified human adult hemoglobin in Escherichia coli" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 90, September 1993, pages 8108-8112, XP002077005 WASHINGTON US see abstract see page 8109 ---	1-6
A	R.F. EICH ET AL: "Mechanism of NO-induced oxidation of Myoglobin and hemoglobin" BIOCHEMISTRY., vol. 35, 1996, pages 6976-6983, XP002090389 EASTON, PA US cited in the application see the whole document especially the abstract, page 6980 right column third paragraph, figure 4 ---	7,10,15
A	LOOKER D ET AL: "A HUMAN RECOMBINANT HAEMOGLOBIN DESIGNED FOR USE AS A BLOOD SUBSTITUTE" NATURE, vol. 356, no. 6366, 19 March 1992, pages 258-260, XP000606891 ---	
P,X	WO 97 23631 A (SOMATOGEN, INC.) 3 July 1997 see page 52, line 17 - line 21; example 13 see page 29, line 20 - line 32 ---	1-6
	-/--	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/08861

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>M.J. WEICKERT ET AL: " turnover of recombinant human hemoglobin in Escherichia coli occurs rapidly for insoluble and slowly for soluble" ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, vol. 348, no. 2, 15 December 1997, pages 337-346, XP002077668 see the whole document</p>	5,6
T	<p>--- D.H. DOHERTY ET AL: "Rate of reaction with nitric oxide determines the hypertensive effect of cell-free hemoglobin" NATURE BIOTECHNOLOGY., vol. 16, July 1998, pages 672-676, XP002090396 PUBLISHING US -----</p>	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/08861

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-6

method of obtaining high expression of soluble recombinant hemoglobin.

2. Claims: 7-20

A hemoglobin mutant with reduced rate of reaction with nitric oxide.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/08861

Patent document cited in search report	A	Publication date	Patent family member(s)	Publication date
WO 9704110	A	06-02-1997	AU 6489996 A	18-02-1997

WO 9715591	A	01-05-1997	AU 7467196 A	15-05-1997
			CA 2231245 A	01-05-1997
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			EP 0561245 A	22-09-1993
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			HK 50993 A	04-06-1993
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			WO 8809179 A	01-12-1988
			US 5449759 A	12-09-1995
			US 5563254 A	08-10-1996
			US 5661124 A	26-08-1997
			US 5776890 A	07-07-1998

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			EP 0868521 A	07-10-1998
