(51) International Patent Classification:
C12N 15/62 (2006.01) C12N 15/82 (2006.01)
C07K 14/00 (2006.01) C12N 15/09 (2006.01)

(21) International Application Number:
PCT/US2007/025455

(22) International Filing Date:

(25) Filling Language: English

(26) Publication Language: English

(30) Priority Data:
60/932,497 30 May 2007 (30.05.2007) US


(72) Inventors; and

(75) Inventors/Applicants (for US only): CAI, Qihua, C.

[Continued on next page]

(54) Title: OPTIMIZED NON-CANONICAL ZINC FINGER PROTEINS

(57) Abstract: Disclosed herein are zinc fingers comprising CCHC zinc coordinating residues. Also described are zinc finger proteins and fusion proteins comprising these CCHC zinc fingers as well as polynucleotides encoding these proteins. Methods of using these proteins for gene editing and gene regulation are also described.

(84) **Designated States (unless otherwise indicated, for every kind of regional protection available):** ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— **with international search report**

(88) **Date of publication of the international search report:**

20 November 2008
A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/62 C07K14/00 C12N15/82 C12N15/09

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)

C12N

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

EPO-Internal, Sequence Search, CHEM ABS Data, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>DATABASE UniProt [Online] 30 April 2003 (2003-04-30), &quot;Zinc finger and BTB domain-containing protein 1.&quot; XP002484503 retrieved from EBI accession no. UNIPROT:Q9Y2K1 Database accession no. Q9Y2K1 the whole document</td>
<td>1,2,5-7, 10,11, 13,15, 17,23</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

See patent family annex.

Date of the actual completion of the international search: 18 June 2008

Date of mailing of the international search report: 21/08/2008

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: Wiame, Ilse

Form PCT/ISA/210 (second sheet) (April 2005)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>WANG WEI ET AL: &quot;Structural characteristic and special expression. pattern during development of a novel gene Bsg2 containing BTB and zinc finger domain&quot; ZHONGGUO SHENGWU HUAXUE YU FENZI SHENGWU XUEBAO - CHINESE JOURNAL OF BIOCHEMISTRY AND MOLECULAR BIOLOGY, ZHONGGUO SHENGWU HUAXUE YU FENZI SHENGWU XUEHUI, BEIJING, CN, vol. 20, 1 January 2004 (2004-01-01), pages 725-731, XP008092974 ISSN: 1007-7626 the whole document</td>
<td>1,2,5-7, 10,11, 15,17,23</td>
</tr>
<tr>
<td>Y</td>
<td>WO 2005/014791 A (SANGAMO BIOSCIENCES INC [US]; PABO CARL O [US]; URNOV FYODOR [US]; HOL) 17 February 2005 (2005-02-17) cited in the application the whole document</td>
<td>1,2,5-38</td>
</tr>
<tr>
<td>A</td>
<td>WO 2006/029296 A (DOW AGROSCIENCES LLC [US]) 16 March 2006 (2006-03-16) cited in the application abstract; sequences 1-3 page 8, line 27 - page 9, line 15</td>
<td>16, 32-34,38</td>
</tr>
<tr>
<td>A</td>
<td>KELLY ET AL: &quot;POZ for effect - POZ-ZF transcription factors in cancer and development&quot;. TRENDS IN CELL BIOLOGY, ELSEVIER SCIENCE LTD, XX, vol. 16, no. 11, 30 October 2006 (2006-10-30), pages 578-587, XP005839158 ISSN: 0962-8924 column 1 - page 579; figure 1</td>
<td>2</td>
</tr>
</tbody>
</table>
1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
   a. type of material
      - [x] a sequence listing
      - [ ] table(s) related to the sequence listing
   b. format of material
      - [x] on paper
      - [x] in electronic form
   c. time of filing/furnishing
      - [ ] contained in the international application as filed
      - [ ] filed together with the international application in electronic form
      - [x] furnished subsequently to this Authority for the purpose of search

2. [x] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
INTERNATIONAL SEARCH REPORT

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 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 No. III Observations where unity of invention is lacking (Continuation of item 3 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 fees, this Authority did not invite payment of additional fees.

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.:

   see annex

Remark on Protest:

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 5-38 (all partially) and 2 (completely)

   A zinc finger protein comprising a non-canonical (non-C2H2) zinc finger, wherein the non-canonical zinc finger has a helical portion involved in DNA binding and wherein the zinc-coordinating region of the helical portion comprises the amino acid sequence HAQRCX and wherein the zinc finger protein is engineered to bind to a target sequence; a fusion protein comprising said protein; a polynucleotide encoding said protein; a plant cell comprising said protein; and uses thereof.

2. claims: 1, 5-38 (all partially) and 3 (completely)

   A zinc finger protein comprising a non-canonical (non-C2H2) zinc finger, wherein the non-canonical zinc finger has a helical portion involved in DNA binding and wherein the zinc-coordinating region of the helical portion comprises the amino acid sequence HKERCX and wherein the zinc finger protein is engineered to bind to a target sequence; a fusion protein comprising said protein; a polynucleotide encoding said protein; a plant cell comprising said protein; and uses thereof.

3. claims: 1, 5-38 (all partially) and 4 (completely)

   A zinc finger protein comprising a non-canonical (non-C2H2) zinc finger, wherein the non-canonical zinc finger has a helical portion involved in DNA binding and wherein the zinc-coordinating region of the helical portion comprises the amino acid sequence HTTRRCX and wherein the zinc finger protein is engineered to bind to a target sequence; a fusion protein comprising said protein; a polynucleotide encoding said protein; a plant cell comprising said protein; and uses thereof.
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EP 1353941 A2</td>
<td>22-10-2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 02057294 A2</td>
<td>25-07-2002</td>
</tr>
<tr>
<td>WO 2005014791 A</td>
<td>17-02-2005</td>
<td>AU 2004263865 A1</td>
<td>17-02-2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2534296 A1</td>
<td>17-02-2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 1651660 A2</td>
<td>03-05-2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2007501626 T</td>
<td>01-02-2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KR 20060039019 A</td>
<td>04-05-2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 2005282329 A1</td>
<td>16-03-2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BR PI0515706 A</td>
<td>29-07-2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2578945 A1</td>
<td>16-03-2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 101076587 A</td>
<td>21-11-2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2008512124 T</td>
<td>24-04-2008</td>
</tr>
</tbody>
</table>