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(54) Title: MODIFIED PROTEINS AND METHODS OF MAKING AND USING SAME

MAKMAFTLAD	RVTEEMLADK	AALVVEVVEE	NYHDAPIVGI	40
AVVNEHGRFF	LRPETALADP	QFVAWLGDET	KKKSMFDSKR	80
AAVALKWKGI	ELCGVSFDLL	LAAYLLDPAQ	GVDDVAAAIAK	120
MKQYEAVRPD	EAVYGKGAKR	AVPDEPVLAE	HLVRKAAAIW	160
ELERPFLDEL	RRNEQDRLLV	RRNEQDRLLV	AEMEFAGVKV	200
DTKRLEQMGK	ELAEQLGTVE	QRIYELAGQE	FNINSPKQLG	240
VILFEKLQLP	VLKKTGTGYS	TSADVLEKLA	PYHEIVENIL	280
HYRQLGKLQS	TYIEGLLKVV	RPDTKKVHTI	PYHEIVENIL	320
LSSTEPNLQN	IPIRLEEGRK	IRQAFVPSES	DWLIFAADYS	360
QIELRVLAHI	AEDDNLMEAF	RRDLDIHTKT	AMDIFQVSED	400
EVTNPMRRQA	KAVNFGIVYG	ISDYGLAQN	NISRKEAAEF	440
IERYFESFPG	VKRYMENIVQ	EAKQKGYVTT	LLHRRRYLPD	480
ITSRNFNVRS	FAERMAMNTP	IQGSAAADIIK	KAMIDLNARL	520
KEERLQAHLL	LQVHDELILE	APKEEMERLC	RLVPEVMEQA	560
VTLRVPLKVD	YHYGSTWYDA	K	SEQ ID NO: 1	

FIG. 1

(57) Abstract: Methods, compositions, systems, apparatuses and kits comprising modified proteins, particularly modified nucleic acid-binding proteins with altered buffering properties are provided. For example, in some embodiments, methods of forming modified proteins including one or more amino acid modifications to achieve desired pKa values are described. Furthermore, the invention provides methods for using such modified proteins in ion-producing reactions, such as ion-based nucleic acid sequencing reactions.

A. CLASSIFICATION OF SUBJECT MATTER*C12Q 1/68(2006.01)i, C12Q 1/48(2006.01)i, C12N 9/12(2006.01)i*

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)

C12Q 1/68; C12P 19/34; C12N 15/74; C12N 9/22

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

NCBI PubMed, eKOMPASS(KIPO internal) & Keywords: nucleotide incorporation, modified polymerase, hydrogen ions, buffering capacity

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2006-0240439 A1 (SMITH, G. P. et al.) 26 October 2006 See the whole document, especially Abstract; Claims 1-4, 19-22, 64-65.	1-11, 13-14
A	US 2006-0199214 A1 (JACK, W. E. et al.) 07 September 2006 See the whole document, especially Claims 1-31.	1-11, 13-14
A	US 2003-0138834 A1 (DAWSON, E. P.) 24 July 2003 See the whole document, especially Claims 1, 2, 25.	1-11, 13-14
A	ZHANG, L. et al. "Mutagenic nucleotide incorporation and hindered translocation by a food carcinogen C8-dG adduct in <i>Sulfolobus solfataricus</i> P2 DNA polymerase IV (Dpo4): modeling and dynamics studies" Nucleic Acid Research, 04 July 2006, Vol. 34, No. 11, pp. 3326-3337 See the whole document, especially Abstract.	1-11, 13-14
A	ANDERSON, J. P. et al. "Incorporation of reporter-labeled nucleotides by DNA polymerases" BioTechniques, February 2005, Vol. 38, No. 2, pp. 257-264 See the whole document, especially Abstract.	1-11, 13-14



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"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

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INTERNATIONAL SEARCH REPORTInternational application No.
PCT/US2011/026468**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.: 12,15
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:

A sequence listing in electronic form was not furnished to this Authority. Therefore, claims 12 and 15 relating to a nucleotide or amino acid sequence are unclear that no meaningful search could be made.
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:

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 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.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2011/026468

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