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(12) **United States Patent**
Papadopoulos et al.(10) **Patent No.:** **US 8,647,842 B2**
(45) **Date of Patent:** ***Feb. 11, 2014**(54) **METHODS FOR PRODUCING A FUSION
PROTEIN CAPABLE OF BINDING VEGF**(75) Inventors: **Nicholas J. Papadopoulos**,
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Tarrytown, NY (US)(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.This patent is subject to a terminal dis-
claimer.(21) Appl. No.: **13/439,889**(22) Filed: **Apr. 5, 2012**(65) **Prior Publication Data**

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now Pat. No. 8,084,234, which is a continuation of
application No. 12/102,681, filed on Apr. 14, 2008,
now Pat. No. 7,704,500, which is a division of
application No. 11/016,097, filed on Dec. 17, 2004,
now Pat. No. 7,374,757, which is a division of
application No. 10/009,852, filed as application No.
PCT/US00/14142 on May 23, 2000, now Pat. No.
7,070,959.(60) Provisional application No. 60/138,133, filed on Jun.
8, 1999.(51) **Int. Cl.**
C12N 5/10 (2006.01)
C12N 5/16 (2006.01)
C12N 15/62 (2006.01)
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C07H 21/04 (2006.01)(52) **U.S. Cl.**
USPC **435/69.7**; 435/252.3; 435/320.1;
435/325; 435/358; 536/23.4(58) **Field of Classification Search**
None
See application file for complete search history.(56) **References Cited**

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Primary Examiner — Christine J Saoud*Assistant Examiner* — Jon M Lockard(74) *Attorney, Agent, or Firm* — Frank R. Cottingham(57) **ABSTRACT**The present invention provides methods for producing a
fusion protein capable of binding vascular endothelial cell
growth factor (VEGF). The methods of the invention com-
prise growing recombinant cells in suspension culture,
wherein the recombinant cells contain an expression vector
comprising a nucleic acid molecule encoding a fusion protein
that binds VEGF, and isolating the fusion protein from the
suspension culture. The fusion protein may comprise a VEGF
receptor component having an immunoglobulin-like (Ig)
domain 2 of a first VEGF receptor, an Ig domain 3 of a second
VEGF receptor, and a multimerizing component.**14 Claims, 55 Drawing Sheets**

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Fig.1.

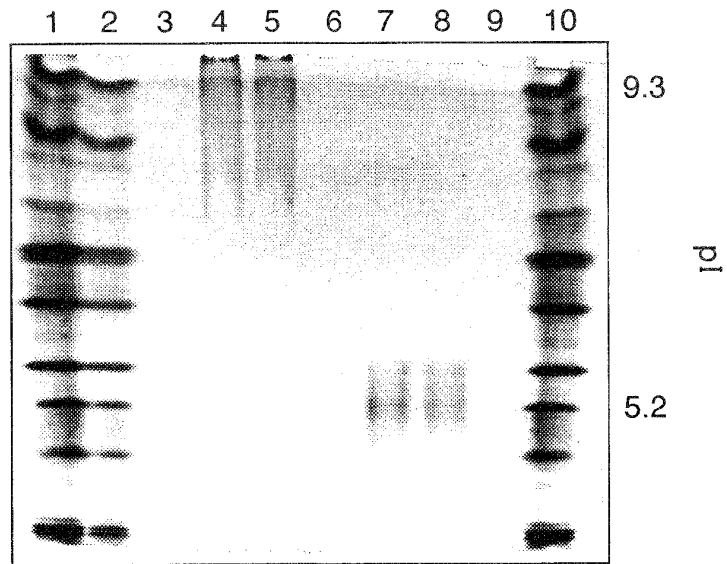


Fig.2.

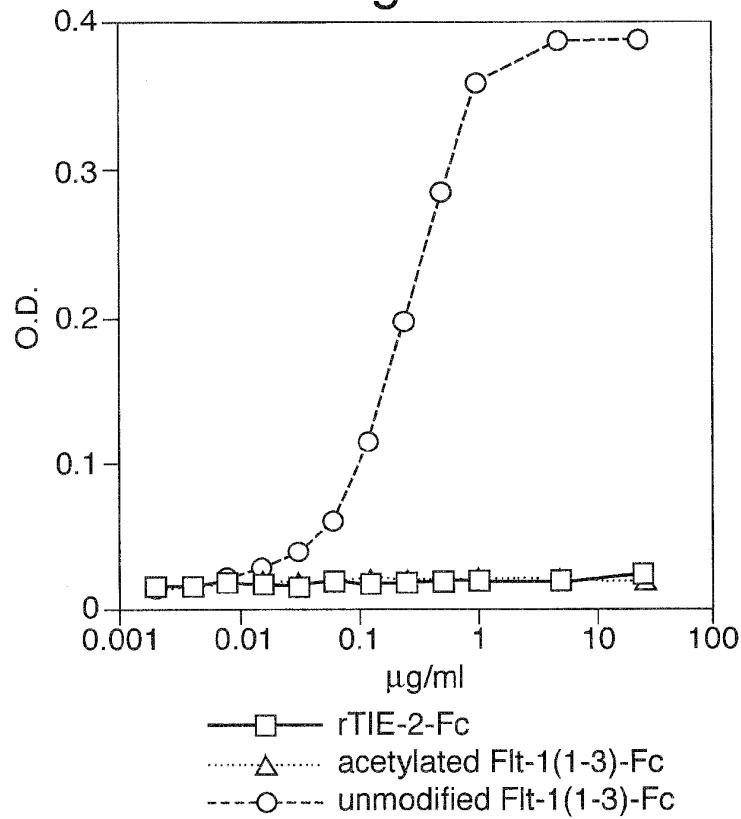


Fig.3.

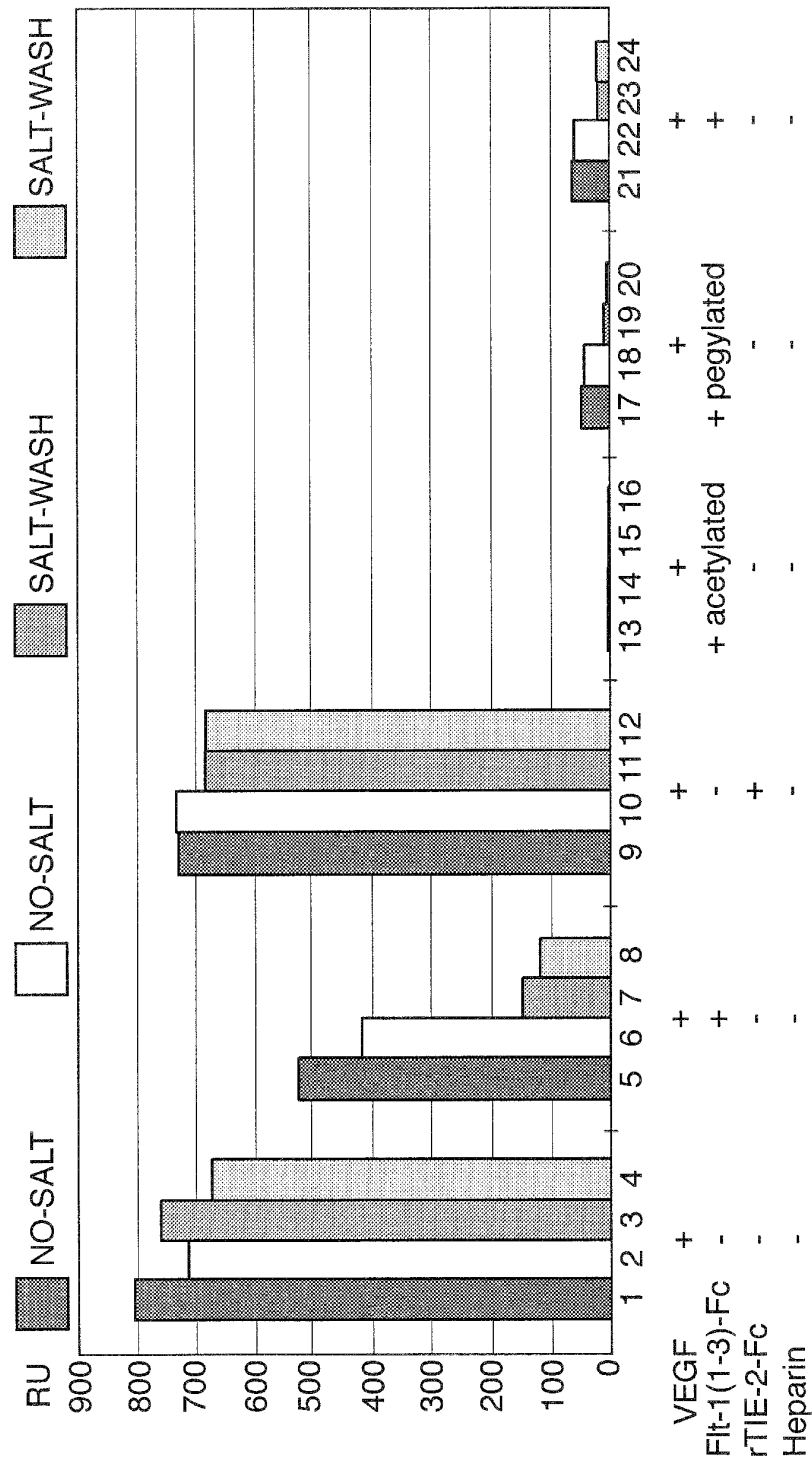
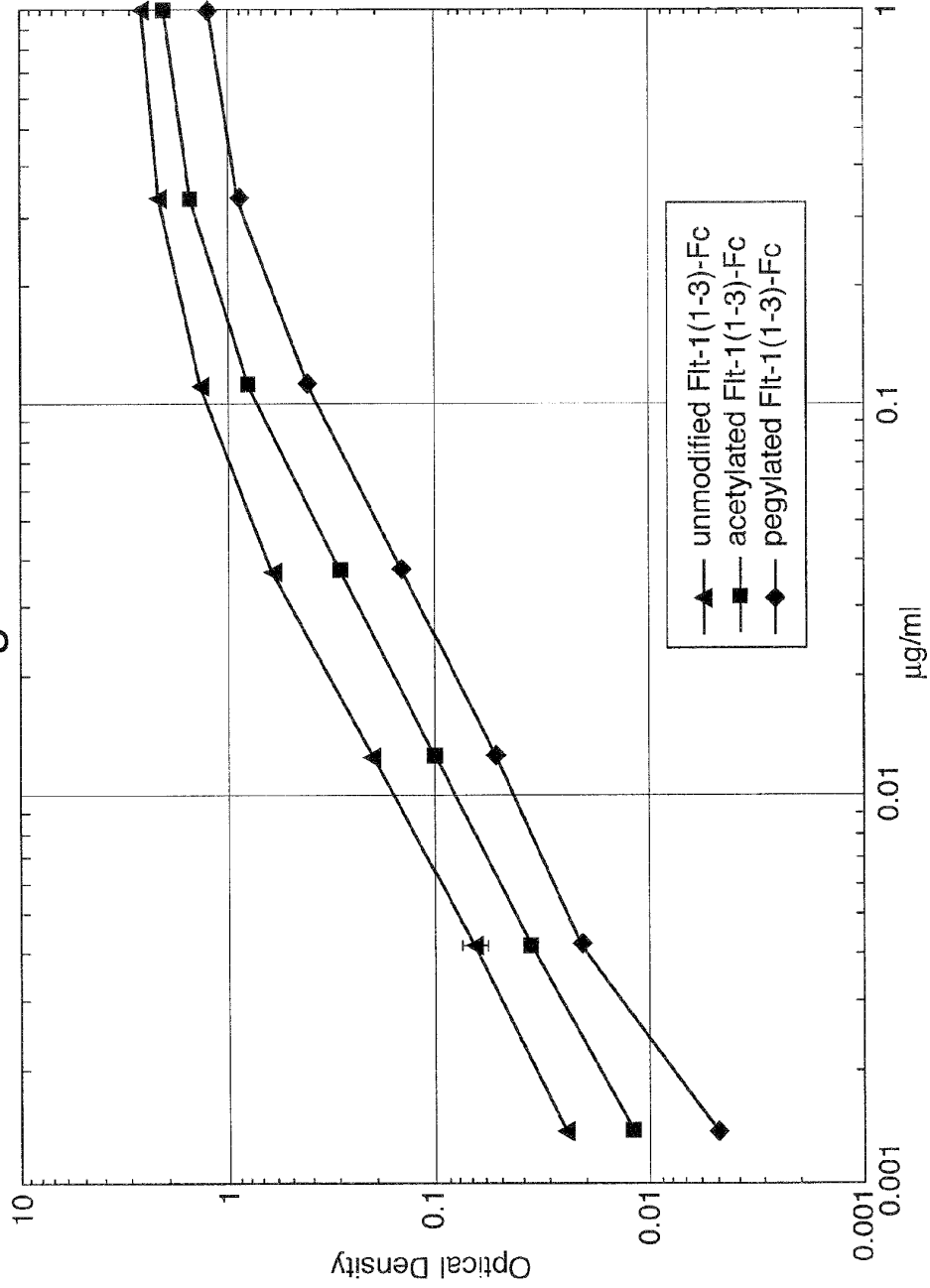


Fig.4.



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