



US00RE48805E

(19) **United States**
 (12) **Reissued Patent**
Wong et al.

(10) **Patent Number: US RE48,805 E**
 (45) **Date of Reissued Patent: Nov. 2, 2021**

(54) **METHOD FOR CANCER TARGETING TREATMENT AND DETECTION OF ARGININE USING ALBUMIN-BINDING ARGININE DEIMINASE FUSION PROTEIN**

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(21) Appl. No.: **16/244,228**

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(22) Filed: **Feb. 12, 2019**

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Reissue of:

(64) Patent No.: **9,803,185**
 Issued: **Oct. 31, 2017**
 Appl. No.: **14/981,855**
 Filed: **Dec. 28, 2015**

U.S. Applications:

(63) Continuation-in-part of application No. 14/197,236, filed on Mar. 5, 2014, now Pat. No. 9,255,262.

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(60) Provisional application No. 61/773,214, filed on Mar. 6, 2013.

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(51) **Int. Cl.**
C12N 9/78 (2006.01)
C12N 9/16 (2006.01)
C07K 14/00 (2006.01)
C12Q 1/34 (2006.01)
A61K 38/00 (2006.01)

Jonas Hopp et al., "The effects of affinity and valency of an albumin-binding domain (ABD) on the half-life of a single-chain diabody-ABD fusion protein", Protein Engineering, Design & Selection, 2010, vol. 23 No. 11, p. 827-834.

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(52) **U.S. Cl.**
 CPC **C12N 9/78** (2013.01); **C07K 14/00** (2013.01); **C12Q 1/34** (2013.01); **A61K 38/00** (2013.01); **C07K 2319/33** (2013.01); **C07K 2319/70** (2013.01); **C12Y 305/03006** (2013.01)

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(58) **Field of Classification Search**
 CPC C12N 9/78; A61P 35/00; C07K 14/00; C12Q 1/34
 See application file for complete search history.

(57) **ABSTRACT**

The present invention provides a pharmaceutical composition containing albumin-binding arginine deiminase (AAD) fusion protein for treating cancer or other arginine-dependent diseases. The AAD fusion protein can be purified from both soluble and insoluble fractions of crude proteins, binds to human serum albumin (HSA) or animal serum albumin and has its high activity with longer half life for efficient depletion of arginine in cancer cells. The specific activities of wild-type ADI and AAD fusion protein in the present invention are about 20 and about 19 U/mg (at physiological pH 7.4), respectively. The composition can be used alone or in combination with at least one chemotherapeutic agent to give a synergistic effect on cancer treatment and/or inhibiting metastasis. The AAD fusion protein can also be used as a component for detection and quantitative analysis of arginine in a testing kit for various samples including blood, food and analytical samples.

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14 Claims, 26 Drawing Sheets

Specification includes a Sequence Listing.

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(A)

Native ADI

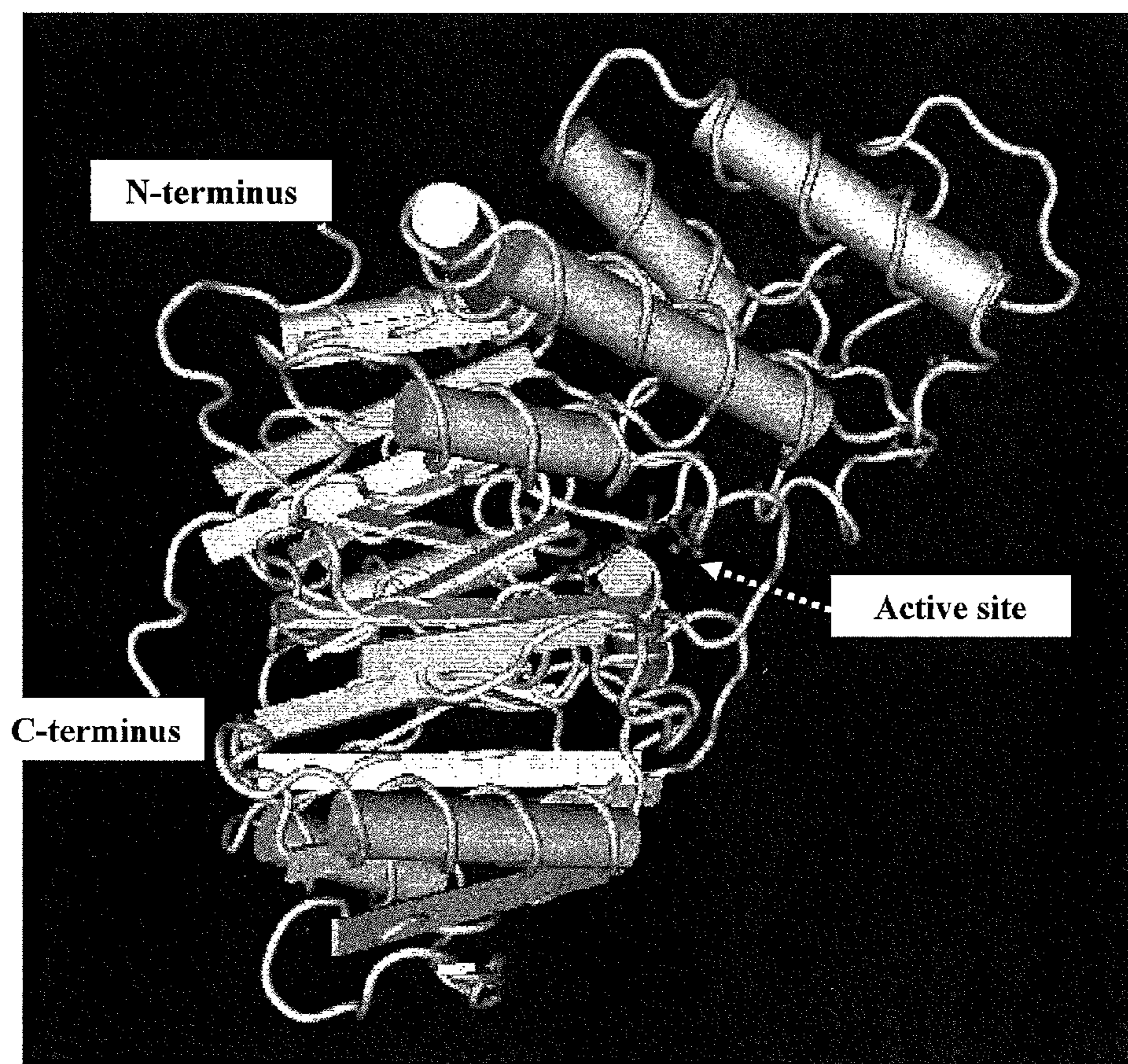
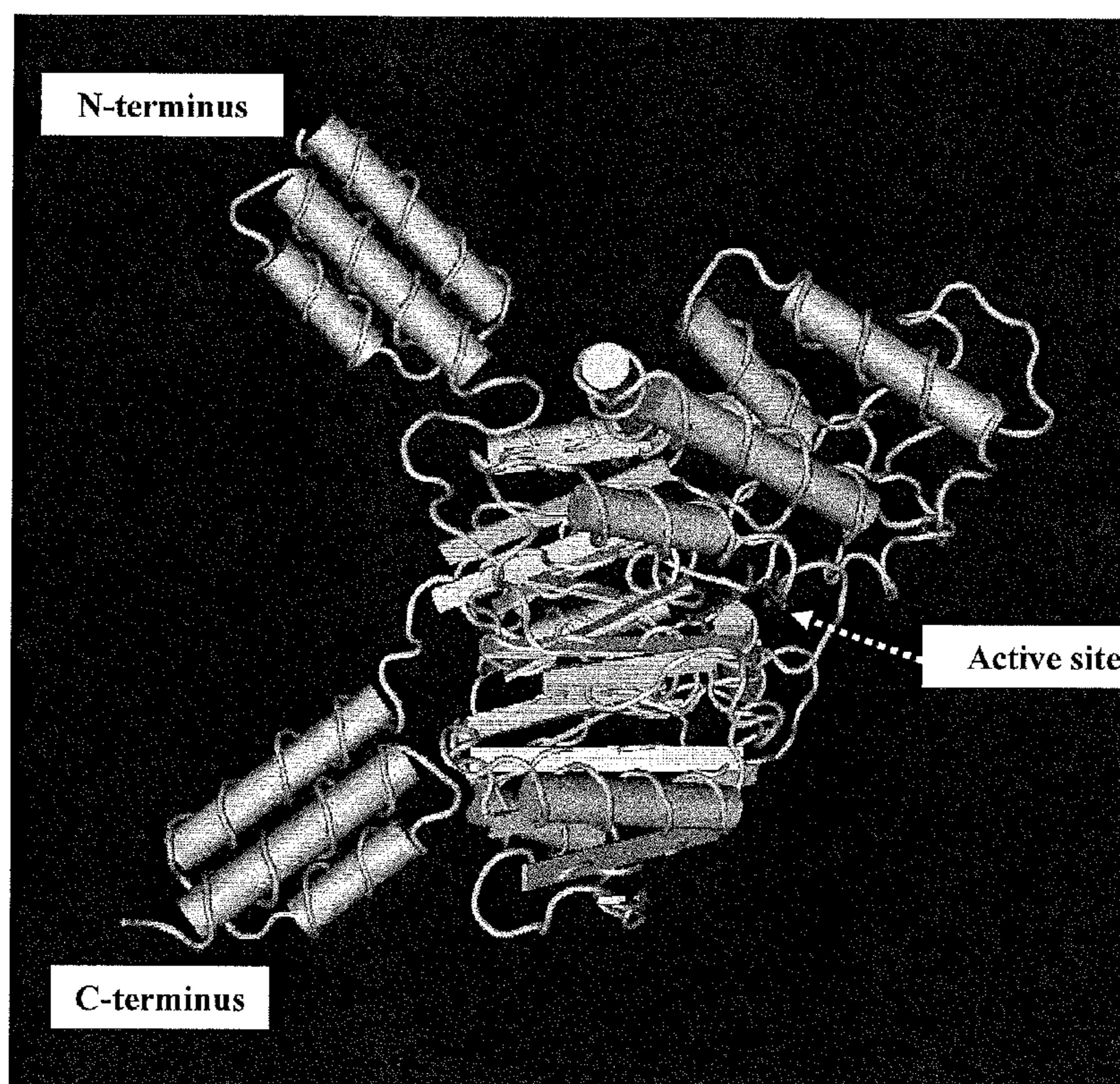


FIG. 1

(B)

AAD fusion protein with two ABD/ ABD1



SEQ ID NO: 46

ABD without linker:

LAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP

SEQ ID NO: 47

ABD with linker:

AQHDEAVDANSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP

SEQ ID NO: 48

ABD1 without linker:

LAEAKVLANRELDKYGVSDFYKRLINKAKTVEGVKALIDEILAALP

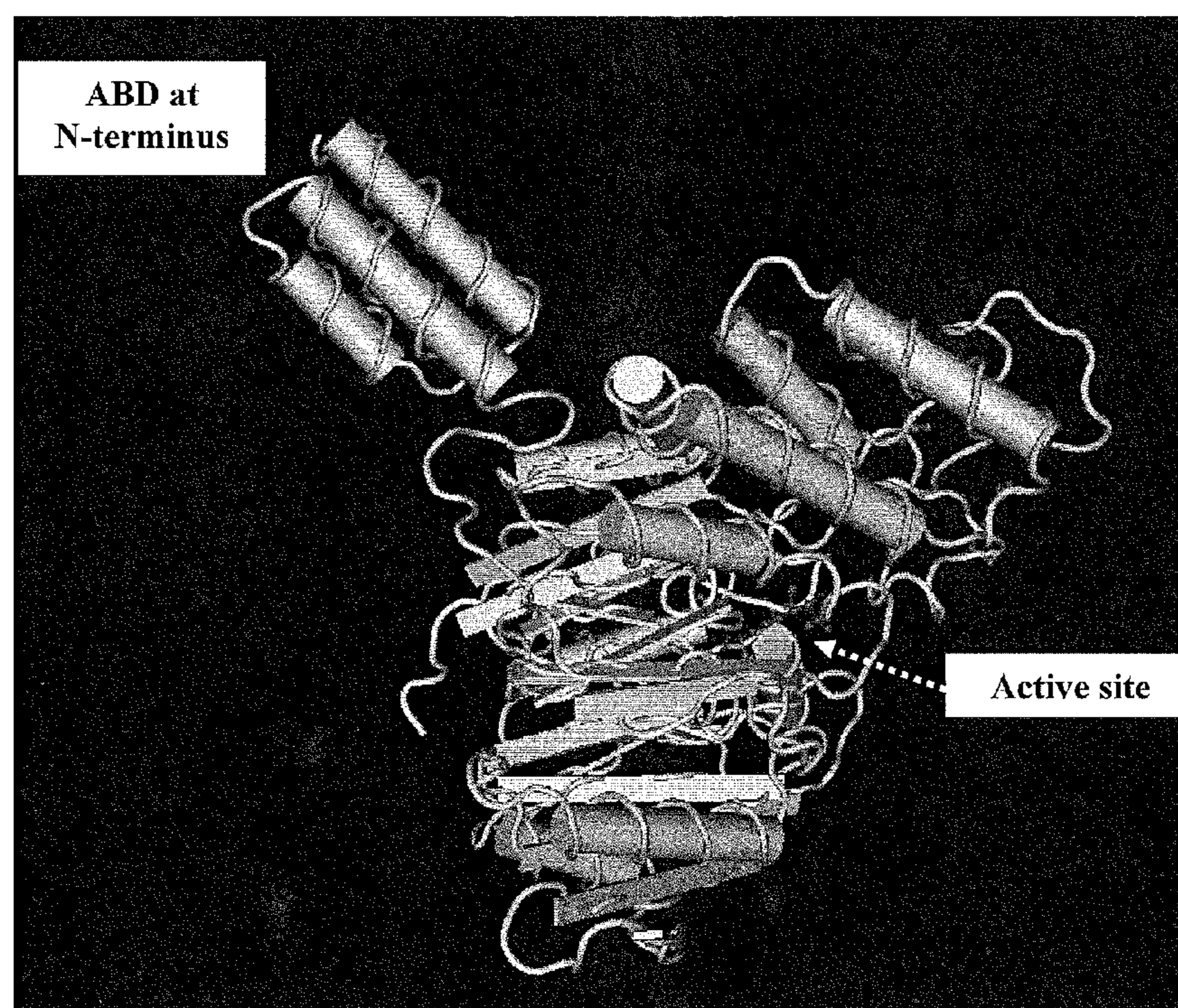
SEQ ID NO: 49

ABD1 with linker:

GSHHHHHHANS�AEAKVLANRELDKYGVSDFYKRLINKAKTVEGVKALIDEILAALP

FIG. 1 (continued)

(C)

AAD fusion protein with one ABD/ ABD1
at N-terminus

SEQ ID NO: 46

ABD without linker:

LAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP

SEQ ID NO: 47

ABD with linker:

AQHDEAVDANSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP

SEQ ID NO: 48

ABD1 without linker:

LAEAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKLHILAALP

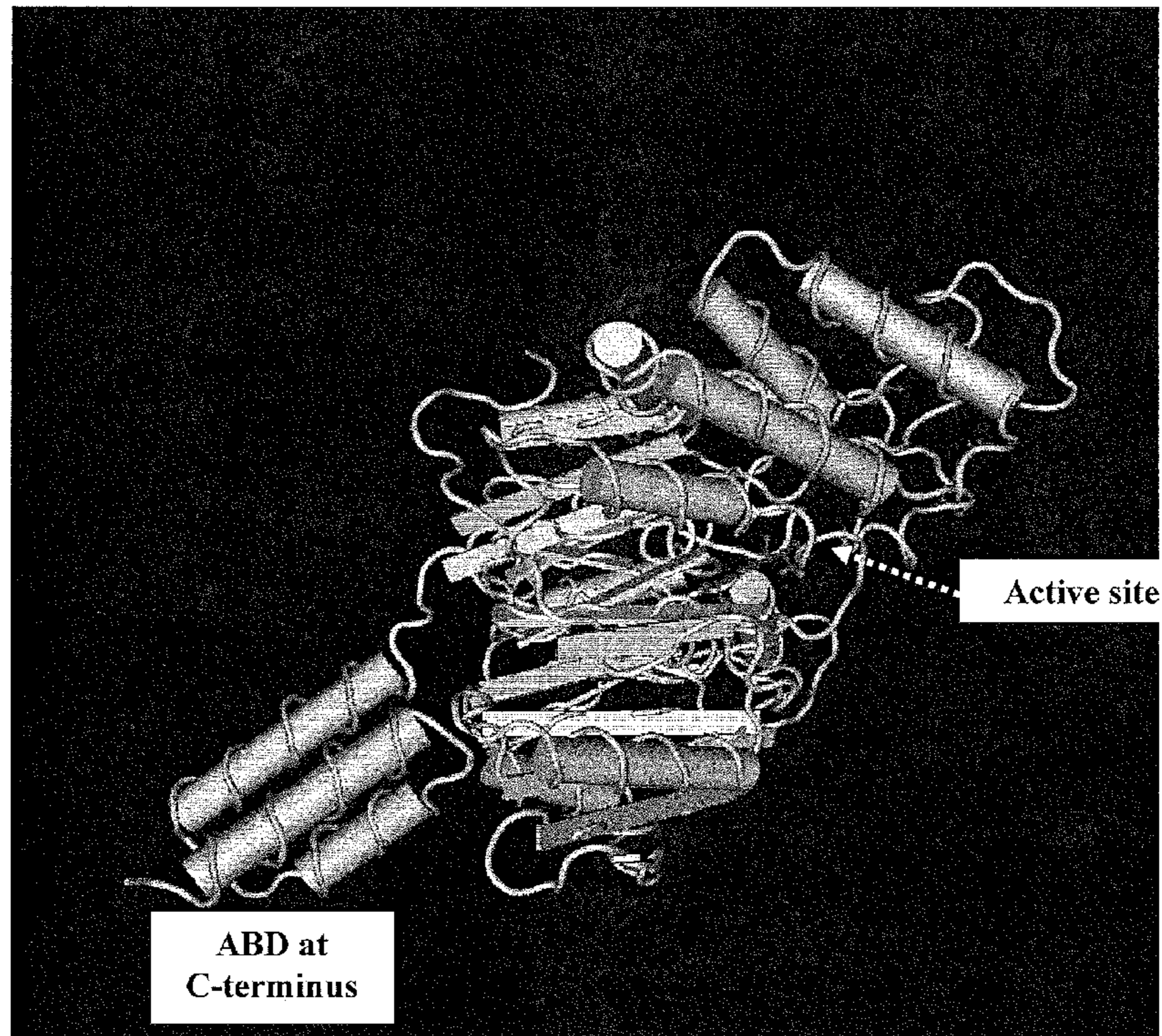
SEQ ID NO: 49

ABD1 with linker:

GSHHHHHHANS�AEAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKLHILAALP

FIG. 1 (continued)

(D)

AAD fusion protein with one ABD/ ABD1
at C-terminus

SEQ ID NO: 46

ABD without linker:

LAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP

SEQ ID NO: 47

ABD with linker:

AQHDEAVDANSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP

SEQ ID NO: 48

ABD1 without linker:

LAEAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKLHILAALP

SEQ ID NO: 49

ABD1 with linker:

GSHHHHHHANS�AEAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKLHILAALP

FIG. 1 (continued)

Mycoplasma arginini 1 MSVFDSKFRGIHVYSEIGELESVLVHEPGREIDYITPARLDELLEPSAILESHDARKEHQ 60
Lactococcus lactis 1 -----MNRGINVYKSEIGELKSVLLHRPGAEVENITPDTMKQLLFDDIPYLKIAQKEHDF 54
Bacillus cereus 1 -----MKHPITHVTSEIGELQTVLLKRPKREVENLTPDYLQQLLFDDIPYLPITQKEHDY 54
Bacillus licheniformis 1 ----MIMTTPIHVYSEIGPLKTVMLKRPKRELENLTPYLERLLEFDDIPFLPAVQKEHQDQ 56
. **** *:::*** ** *:: ** :..***. * :***.

61 FVAELKANDINVVVELIDLVAETYDLASQEAQKDLIEEFLEDEPVLSEEKVVVRNFKKA 120
65 FAQTLRDNGAETVYIENLATEVFEKSSE-TKEEFLLSHLLHEAGYRPGRTYDGL-TEYLT- 111
66 FAQTLRNRSVEVLYLEKLAEEALVDK-K-LREFVDRIILKEGQADVIVVAHQTL-KEYLL- 110
67 FAETLKQCGAEVLYLEKLTAEALDDA-L-VREQFIDELLTESKADINGAYDRL-KEFLL- 112
. *:: :.:: :.*:.*. :.....*:: :. :. :.*

121 KKTSRELVEIMMAGITKYDL-----GIEADHELIVDPMPNLYFTRDPFASVGNQ 169
112 SMPTRDMVEKVYAGVRKNELDIKRTALSDMAGSDAENYFYLNPLENAYFTRDPQASMGVG 171
111 SFSNEELIQKIMSGVRKNEIETSKKTHLYE-LMEDHYPFYLDPMPNLYFTRDPAASVGDG 169
113 TFDADSMVEQVMSGIREKRELEREKKSLLHE-LMEDHYPFYLDPMPNLYFTRDPAASVGDG 171
.:. :.*: *:: :. :. :.::** ***** *::* *

170 VTIHYMRYKVRQRETLFSRFVFSNHPKLI--TPWYYDPSLKLSEGGGVFIYNNKDTLVV 227
172 MIINKMTFFARQPESLITEYVMAHPRFKD--TPIWRDRNHTTRIEGGDELILNKTTVAI 229
170 LIINKMREPARRESLFMYYIIKYHPRFAKHNVPIWLDREYKFPFIEGGDELILNEETIAI 229
172 LIINKMREPARRESLFMYYIINHHPRFKGHEIFWLDREYKFPFIEGGDELVLNEETVAI 231
.* *.*: *::: .::: *::: * : * *** :. :.*:.*:

228 GVSERTDLQTVILLAKNIVANKECEFKRIVAINVPKWTKMLHLDLWLTMLDKDKFLYSPI 287
230 GVSERTSSKTIQNLAKELFANPLSTFDTVLAVEIPHNHAMHLDLTVFTMINHDQFTVFPQ 289
230 GVSARTSAKAIERLAKNLSRQ-NKIKKVLAEIEPKCRAFMHLDLTVFTMVVDYDKFTIHPA 288
232 GVSERTTAQAERLVRNLFRQ-SRIRRVLAVEIPKSRAFMHLDLTVFTMVDRDQFTIHPA 290
*** ** :. :.*:.*. : :.:::*** :***** :***: *:* *

288 ANDVFKFWDYDLVNGGAEFPQ--VENGLPLEGLLQSIINKKPVLIPIAGEGASQMETERE 345
290 IMDGAGNINVTILRPGQDG-EVEIEHLTDLKAALKKVLNLSELDL-IECGAGEPIAAPRE 347
289 IQSPKGMNIYILEKGADEETLKITHRTSLMEALKEVLDLSELVL-IPCGGSDVIASARE 347
291 IQSPGDMRIFVLERGKTADEIHTTEHNLPEVLKRTLGLSDVNL-IPCGGSDVIASARE 349
:. * . * *:: :. :. : * . . . : **

346 THFDGTNYLAIRPGVVIGYSRNEKTNAALEAAGIKVLPFHGNQLSLGMGNARCMSMPLSR 405
349 QWNDGSNTLAIAPGEIVTYDRNYVTVELLKEHGKIVHEILSSELGRGRGGARCMSQPLWR 407
348 QWNDGSNTLAIAPGVVVTYDRNYVSNLLEHGGIEVIEVLSSELGRGRGGPRCMSMPIVR 407
350 QWNDGSNTLAIAPGVVVTYDRNYISNECLREQGKIVIEIPSGELSRGRGGPRCMSMPLYR 409
:* * ** :. :.*:.* :. *.*: * :. * * ***** *:* *

406 KDVKN 410
408 EDL-- 410
408 KDI-- 410
410 EDVK- 413
.

FIG. 2

(A) SEQ ID NO: 36

ADI-linker-ABD 1

MSVFDSKFKGIHVYSEIGELESVLVHEPGREIDYITPARLDELLESAILESHDARKEHKQ
 FVAELKANDINVVELIDLVAETYDLASQEAKDKLIEEFLEDSEPVLSEEHKVVVRNFLKA
 KKTRELVEIMMAGITKYDLGIEADHELIVDPMPNLYFTRDPFASVGNGVTIHYMRYKVR
 QRETLFSRFVFSNHPKLINTPWYYDPSLKLSIEGGDVFIYNNDTLVVGVSERTDLQTVTL
 LAKNIVANKECEFKRIVAINVPKWTNLMHLDTWLTMLDKDKFLYSP IANDVFKFWDYDLV
 NGGAEPQPVENGLPLEGLLQSIINKKPVLIP IAGEGASQMEIERETHFDGTNYLAIRPGV
 VIGYSRNEKTNAALEAAGIKVLPFHGNQLSLGMGNARCMSMPLSRKDVKWASHHHTTTHNS
SLAEAKVLANRELDKYGVSDFYKRLINKAKTVEGVKALHILAALP

(ABD 1: high affinity albumin binding domain; the linker is underlined.)

Linker 1 (SEQ ID NO: 50): GSHHHHHHANS

(B) SEQ ID NO: 37

ADI-linker-ABD

MSVFDSKFKGIHVYSEIGELESVLVHEPGREIDYITPARLDELLESAILESHDARKEHKQ
 FVAELKANDINVVELIDLVAETYDLASQEAKDKLIEEFLEDSEPVLSEEHKVVVRNFLKA
 KKTRELVEIMMAGITKYDLGIEADHELIVDPMPNLYFTRDPFASVGNGVTIHYMRYKVR
 QRETLFSRFVFSNHPKLINTPWYYDPSLKLSIEGGDVFIYNNDTLVVGVSERTDLQTVTL
 LAKNIVANKECEFKRIVAINVPKWTNLMHLDTWLTMLDKDKFLYSP IANDVFKFWDYDLV
 NGGAEPQPVENGLPLEGLLQSIINKKPVLIP IAGEGASQMEIERETHFDGTNYLAIRPGV
 VIGYSRNEKTNAALEAAGIKVLPFHGNQLSLGMGNARCMSMPLSRKDVKWAQHDEAVDAN
SLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP

(ABD: albumin binding domain; the linker is underlined.)

Linker 2 (SEQ ID NO: 51): AQHDEAVDANS

FIG. 3

(C) SEQ ID NO: 38



MSVFDSKFKGIHVYSEIGELESVLVHEPGREIDYITPARLDELLEFSAILESHDARKEHKQ
 FVAELKANDINVVELIDLVAETYDLASQEAKDKLIEEFLEDSEPVLSEEHKVVVRNFLKA
 KKTSRELVEIMMAGITKYDLGIEADHELIVDPMPNLYFTRDPFASVGNVGTIHYMRYKVR
 QRETLSRFVFSNHPKLINTPWYYDPSLKLSEGGDVFYNNDTLVVGVSERTDLQTVTL
 LAKNIVANKECEFKRIVAINVPKWTNLMHLDTWLMLDKDKFLYSPIANDVEKFDYDLV
 NGGAEPQPVENGLPLEGLLQSIINKKPVLPIAGEGASQMEIERETHFDGTNYLAIRPGV
 VIGYSRNEKTNAALEAAGIKVLPFHGNQLSLGMGNARCMSMPLSRKDVKWHHHHHHAQH
EAVDANSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP

(ABD: albumin binding domain; the linker is underlined.)

(D) SEQ ID NO: 39



MSVFDSKFKGIHVYSEIGELESVLVHEPGREIDYITPARLDELLEFSAILESHDARKEHKQ
 FVAELKANDINVVELIDLVAETYDLASQEAKDKLIEEFLEDSEPVLSEEHKVVVRNFLKA
 KKTSRELVEIMMAGITKYDLGIEADHELIVDPMPNLYFTRDPFASVGNVGTIHYMRYKVR
 QRETLSRFVFSNHPKLINTPWYYDPSLKLSEGGDVFYNNDTLVVGVSERTDLQTVTL
 LAKNIVANKECEFKRIVAINVPKWTNLMHLDTWLMLDKDKFLYSPIANDVEKFDYDLV
 NGGAEPQPVENGLPLEGLLQSIINKKPVLPIAGEGASQMEIERETHFDGTNYLAIRPGV
 VIGYSRNEKTNAALEAAGIKVLPFHGNQLSLGMGNARCMSMPLSRKDVKWAQHDEAVDAN
SLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALPHHHHHH

(ABD: albumin binding domain; the linker is underlined.)

FIG. 3 (continued)

(E) SEQ ID NO: 40



MHHHHHDEAVDANSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAAL
 PSGSNNNNNGSGGSVFDKFKGIHVYSEIGELESVLVHEPGREIDYITPARLDELLFSA
ILESHDARKEHKQFVAELKANDINVVELIDLVAETYDLASQEAKDKLIEEFLEDSEPVLS
 EEHKVVVRNFLKAKKTSRELVEIMMAGITKYDLGIEADHELIVDPMPLYFTRDPFASVG
 NGVTIHYMRYKVRQRETLFSRFVFSNHPKLINTPWYYDPSLKLSIEGGDVFIYNNDTLVV
 GVSERTDLQTVTLLAKNIVANKECEFKRIVAINVPKWTNLMHLDTWLTMLDKDKFLYSPI
 ANDVFKFDYDLVNGGAEPQPVENGLPLEGLLQSIINKKPVLIP IAGEGASQMEIERETH
 FDGTNYLAIRPGVVIGYSRNEKTNAALEAAGIKVLPFHGNQLSLGMGNARCMSMPLSRKD
 VKW

(ABD: albumin binding domain; the linker between His and ABD is underlined with solid line while the linker between Poly-N and ADI is underlined with dotted line.)

Linker 3 (SEQ ID NO: 52): DEAVDANS; Linker 4: SGS; Linker 5 (SEQ ID NO: 53): GSGG

FIG. 3 (continued)

(F) SEQ ID NO: 41



MGHHHHHDEAVDANSSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAAALPSG
 SNNNNNNGSGGKHPIHVTSEIGELQTVLLKRPGKEVENLTPDYLQQLLFDDIPYLP IIQKEHDY
 FAQTLRNRGVEVLYLEKLAAEALVDKRLREEFVDRIKKEGQADVNVAHQTLKEYLLSFSNEELI
 QKIMGGVRKNEIETS SKKTHLYELMEDHYPFYLDPMPNLYFTRDPAASVGDGLTINKMREPARRR
 ESLFMEYIIKYHPRFAKHNVP IWLDRDYKFPIEGGDELILNEETIAIGVSARTSAKAIERLAKN
 LFSRQNKIKKVLAEIIPKCRAFMHLDTVFTMVDYDKFTIHPAIQGPKNMNIYILEKGADEETL
 KITHRTSLMEALKEVLDLSELVLI PCGGGDVIASAREQWNDGSNTLAIAPGVVVTYDRNYVSNT
 LLREHGIEVIEVLSSSELSRGRGGPRC MSPPIVRKDI

*(ABD: albumin binding domain; the linker between His and ABD is underlined with solid line
 while the linker between Poly-N and bcADI is underlined with dotted line.)*

Linker 3 (SEQ ID NO: 52): DEAVDANS; Linker 4: SGS; Linker 5 (SEQ ID NO: 53): GSGG

FIG. 3 (continued)

(A) SEQ ID NO: 42



MAQHDEAVDANSLAEAKVLANFELDKYGVSDYYKNLINNAKTVEGVKALIDEILAAALPEF
 LEGSSCITGDALVALPEGESVRIADIVPGARPNSDNAIDLKVLDRHGPNVLAADRLFHSGE
 HPVYTVRTVEGLRVTGTANHPLDCLVDVAGVPTLLWKLIDEIKPGDYAVIQRSAFSVDCA
 GFARGKPEEFAPTTYTVGVPLVRFLEAHRDPDAQAIADELTDGRFYAKVASVTDAGVQ
 PVYSLRVDTADHAFITNGFVSHATGLTGLNSGLTTNPGVSAWQVNTAYTAGQLVTYNGKT
 YKCLQPHTSLAGWEP SNVPALWQLOGDPITITITK

(B) SEQ ID NO: 43

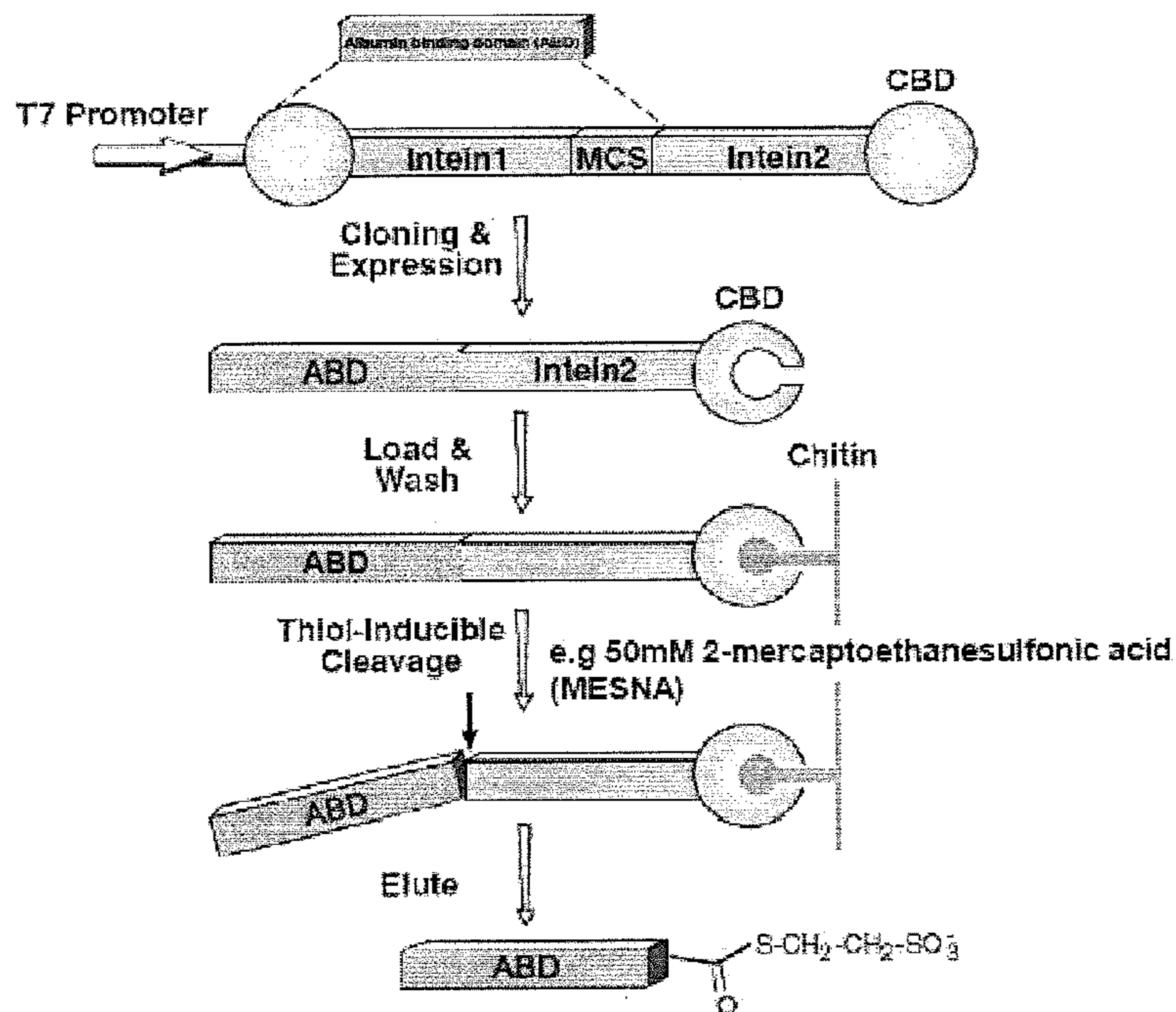


MKIEEGKLTNPGVSAWQVNTAYTAGQLVTYNGKTYKCLQPHTSLAGWEP SNVPALWQLQNG
 NNGLELRESGAISGDSLISLASTGKRVS IKDLLDEKDFE IWAINEQTMKLES AKVSRVFCG
 KKLVIILKTRLGRTIKATANHRFLTIDGWKRLDELSLKEHIALPRKLESSSLQLSPEIEKLS
 QSDIYWDSIVSITETGVVEEVDLTVPGPHNFVANDIIVHNCVFDKFKGIHVYSEIGELES
 VLVHEPGREIDYITPARLDELLEFSAILESHDARKEHKQFVAELKANDINVVELIDLVAETYD
 LASQEAKDKLIEEFLEDSEPVLSEEHKVVRNFLKAKKTSRELVEIMMAGITKYDLGIEADH
 ELIVDPMPLYFTRDPFASVGNVVTIHYMRYKVRQRETLSRFVFSNHPKLINTPWYYDPSL
 KLSIEGGDVFIYNNDTLVVGVSERTDLQTVTLLAKNIVANKECEFKRIVAINVPKWTNLMHL
 DTWLTMLDKDKFLYSPIANDVFKFDYDLVNGGAEPQPVENGLPLEGLLQSIINKKPVLPIPI
 AGEGASQMEIERETHFDGTNYLAIRPGVVIGYSRNEKTNAALEAAGIKVLPFHGNQLSLGMG
 NARCMSMPLSRKDVKW

FIG. 4

(C)

C-Terminal fusion



(D)

N-Terminal fusion

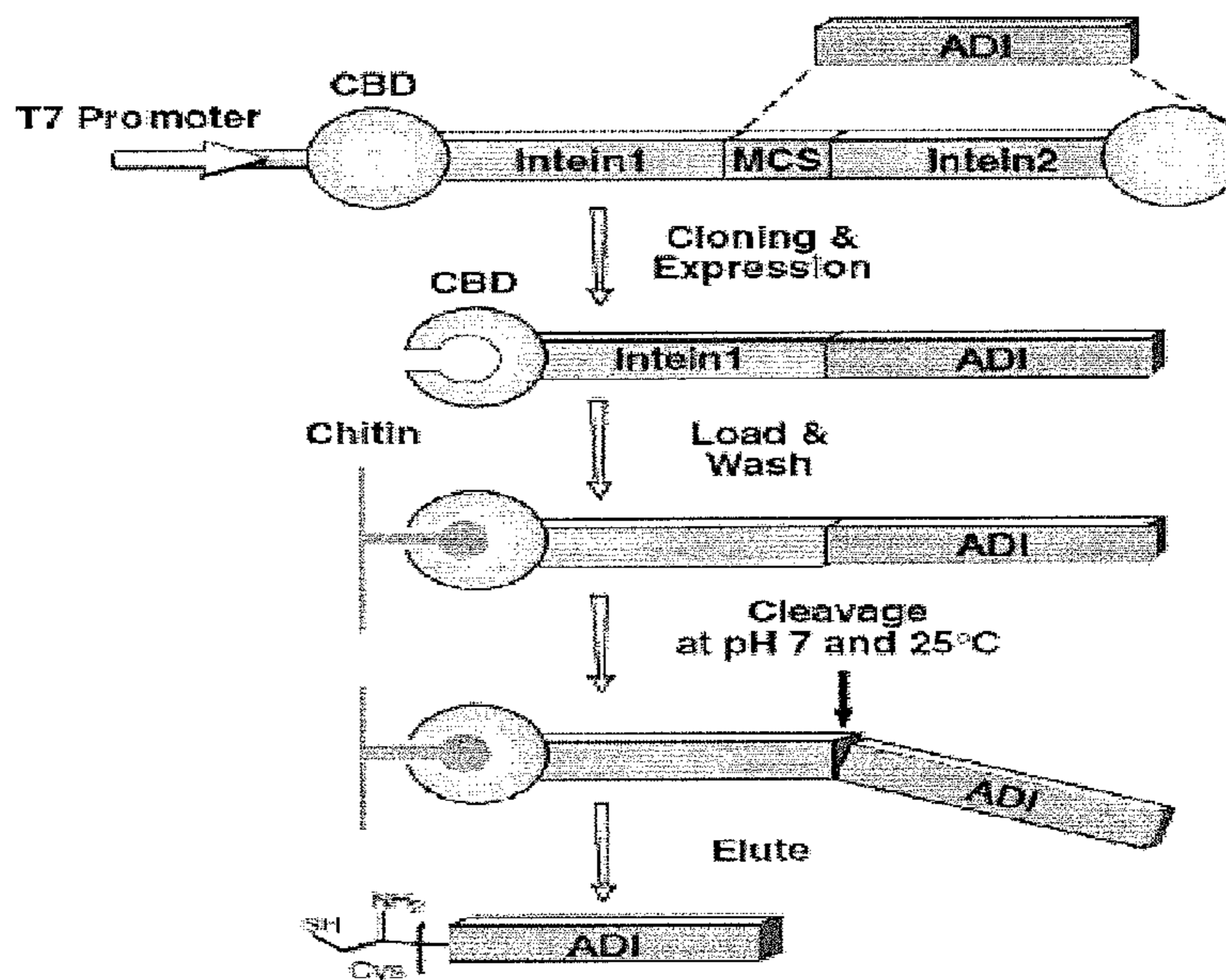


FIG. 4 (continued)

(E)

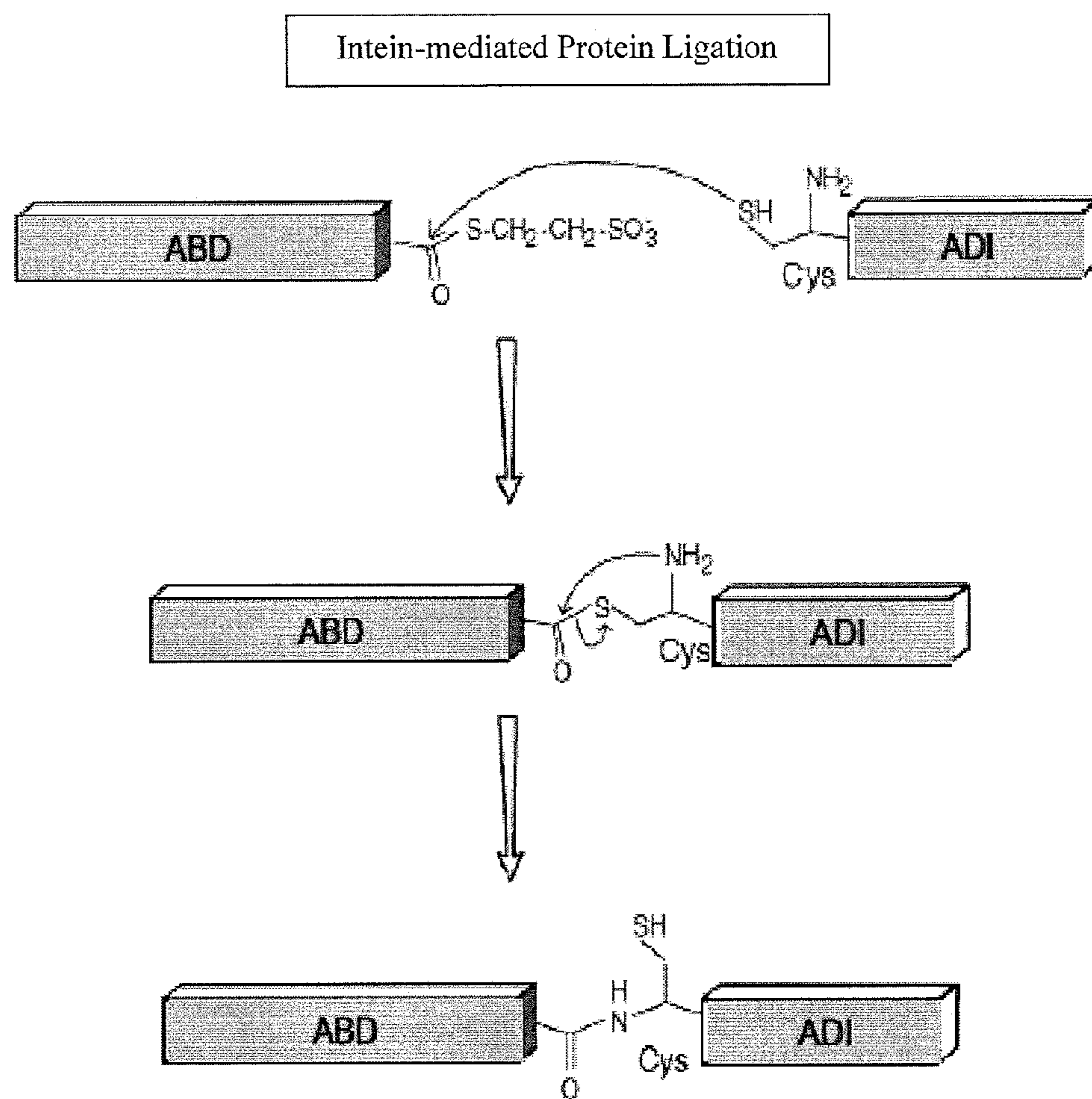


FIG. 4 (continued)

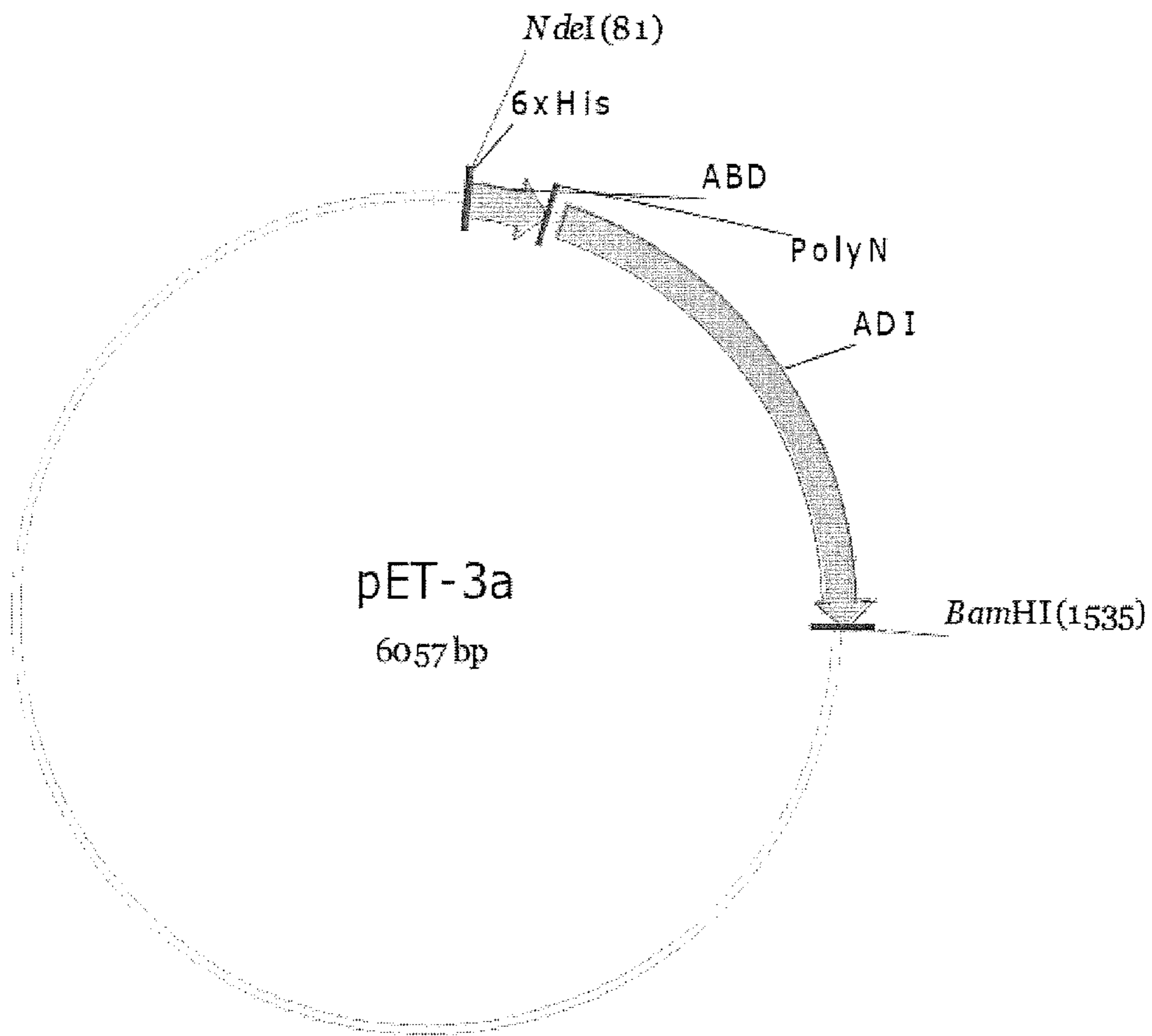
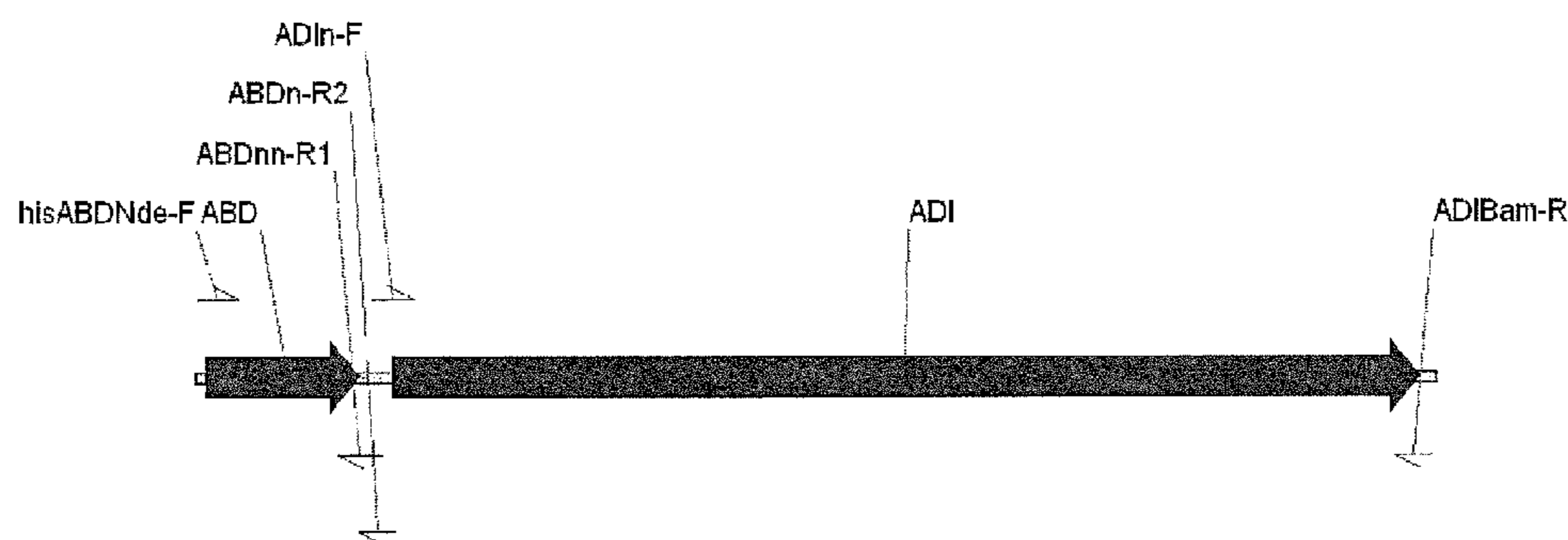


FIG. 5

(A) GENE MAP



(B) Nucleotide sequence of His-ABD-PolyN-ADI (1484 bp):

(SEQ ID NO: 44)

5' -
 ATGCATCATCACCATCACCATGATGAAGCCGTGGATGCCAATTCCTTAGCTGAAGCTAAAGTCT
 TAGCTAACAGAGAACTTGACAAATATGGAGTAAGTGACTATTACAAGAACCTAATCAACAATGC
 CAAAACGTGTTGAAGGTGTAAGCACTGATAGATGAAATTTTAGCTGCATTACCTTCGGGTAGT
 AACAACAATAATAACAATGGTAGCGGCGGTTCTGTATTTGACAGTAAATTTAAAGGAATTCACG
 TTTATTCAGAAATTGGTGAATTAGAATCAGTTCCTAGTTCACGAACCAGGACGCGAAATTGACTA
 TATTACACCAGCTAGACTAGATGAATTATTATTCTCAGCTATCTTAGAAAGCCACGATGCTAGA
 AAAGAACACAAACAATTCGTAGCAGAATTAAAAGCAAACGACATCAATGTTGTTGAATTAATTG
 ATTTAGTTGCTGAAACATATGATTTAGCATCACAAGAAGCTAAAGACAAATTAATCGAAGAATT
 TTTAGAAGACTCAGAACCAGTTCATCAGAAGAACACAAAGTAGTTGTAAGAACTTCTTAAAA
 GCTAAAAAACATCAAGAGAATTAGTAGAAATCATGATGGCAGGGATCACAAAATACGATTTAG
 GTATCGAAGCAGATCACGAATTAATCGTTGACCCAATGCCAAACCTATACTTCACACGTGACCC
 ATTTGCATCAGTAGGTAATGGTGTAAACAATCCACTACATGCGTTACAAAGTTAGACAACGTGAA
 ACATTATTCTCAAGATTTGTATTCTCAAATCACCCCTAACTAATTAACACTCCATGGTACTACG
 ACCCTTCACTAAAATTATCAATCGAAGGTGGGGACGTATTTATCTACAACAATGACACATTAGT
 AGTTGGTGTCTGAAAGAACTGACTTACAAACAGTTACTTTATTAGCTAAAAACATTGTTGCT
 AATAAAGAATGTGAATTCAAACGTATTGTTGCAATTAACGTTCCAAAATGGACAACTTAATGC
 ACTTAGACACATGGCTAACAAATGTTAGACAAGGACAAATTCCTATACTACCAATCGCTAATGA
 CGTATTTAAATTCGGGATTATGACTTAGTAAACGGTGGAGCAGAACCACAACCAGTTGAAAAC
 GGATTACCTCTAGAAGGATTATTACAATCAATCATTAACAAAAACCAGTTTAAATTCCTATCG
 CAGGTGAAGGTGCTTCACAAATGGAAATCGAAAGAGAAACACACTTCGATGGTACAACTACTT
 AGCAATTAGACCAGGTGTTGTAATTGGTACTCACGTAACGAAAAACAAACGCTGCTCTAGAA
 GCTGCAGGCATTAAAGTTCTTCCATTCCACGGTAACCAATTATCATTAGGTATGGGTAACGCTC
 GTTGTATGTCAATGCCTTTATCACGTAAAGATGTTAAGTGGTAA-3'

FIG. 6

(C) Amino acid sequence of His-ABD-PolyN-ADI:

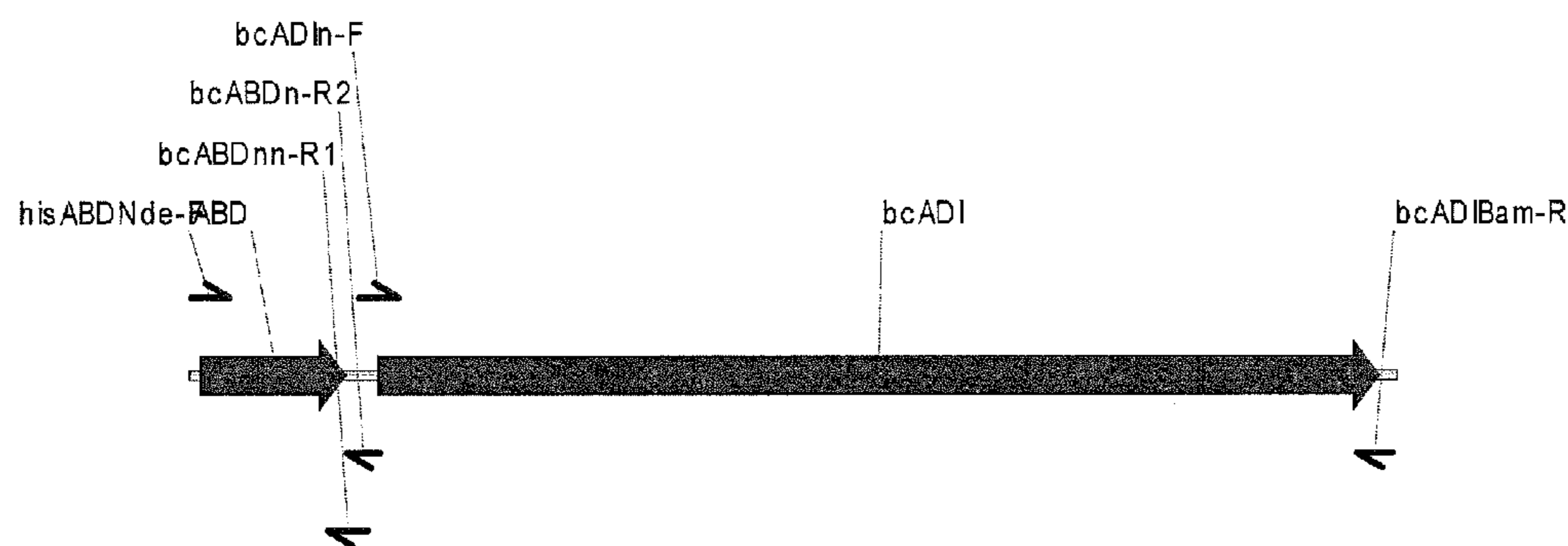
(SEQ ID NO: 40)

MHHHHHDEAVDANSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALPSGSNNNNNNGSGGSVF
DSKFKGIHVYSEIGELESVLVHEPGREIDYITPARLDELLFSAILESH DARKEHKQFVAEIKANDINVELIDLVAET
YDLASQEAKDKLIEEFLEDSEPVLSSEHKVVVRNFLKAKKTSRELVEIMMAGITKYDLGIEADHELIVDPMPNLYFTR
DPFASVGNVGTIHYMRYKVRQRETLFSRFVFSNHPKLINTPWYYDPSLKLSIEGGDVFIYNNDTLVGVSERTDLQTV
TLLAKNIVANKECEFKRIVAINVPKWTNLMHLDTWLTMLDKDKFLYSPIANDVFKFWDYDLVNGGAEPQPVENGLPLE
GLLQSIINKKPVLIP IAGEGASQMEIERETHFDGTNYLAIRPGVVIGYSRNEKTNAALEAAGTKVLPFHGNQI.SLGMG
NARCMSMPLSRKDVKW

(PolyN with linker: SGSNNNNNNGSGG)

FIG. 6 (continued)

(A) GENE MAP



(B) The nucleotide sequence of His-ABD-PolyN-bcADI (1512 bp):

(SEQ ID NO: 45)

5' -
 ATGGGTCATCATCACCATCACCATGATGAAGCCGTGGATGCGAACAGCTTAGCTGAAGCTAAAG
 TCTTAGCTAACAGAGAACTTGACAAATATGGAGTAAGTGACTATTACAAGAACCTAATCAACAA
 TGCCAAAACGTGTTGAAGGTGTAAAAGCACTGATAGATGAAATTTTAGCTGCATTACCTTCGGGT
 AGTAACAACAATAATAACAATGGTAGCGGCGGTAAACATCCGATACATGTTACTTCAGAAATTG
 GGAATTACAAACGGTTTTATTAAAACGACCGGGTAAAGAAGTGGAAAACCTTGACGCCAGATTA
 TTTGCAGCAATTATTATTTGACGATATTCATACCTACCAATTATTCAAAAAGAGCATGATTAT
 TTTGCACAAACGTTACGCAATCGGGGTGTTGAAGTCTTTATTTAGAAAACTAGCCGCTGAGG
 CGTTAGTAGATAAAAACTTCGAGAAGAATTTGTTGATCGTATTTTAAAAGAAGGACAGGCCGA
 CGTAAATGTTGCACATCAAATTTAAAAGAATTTACTTTTCTTTTCAAATGAAGAATTAATT
 CAAAAATTATGGGCGGTGTACGGAAAAACGAAATTGAAACAAGTAAGAAGACACATTTATATG
 AATTAATGGAAGATCATTATCCGTTTTACTTAGATCCAATGCCTAATTTATATTTACTCGTGA
 TCCAGCAGCTAGCGTGGGCGATGGCTTAACGATAAATAAGATGAGAGAACCAGCGCTAGACGT
 GAATCATTATTCATGGAGTACATCATTAATATCATCCAAGATTTGCAAAACATAATGTACCAA
 TCTGGTTAGATCGTGATTATAAATTTCCAATTGAAGGTGGCGACGAGCTAATTTTAAATGAAGA
 AACAAATTGCGATTGGAGTATCTGCTCGTACTTCAGCTAAAGCAATTGAACGTTTAGCAAAAAAT
 CTCTTTAGCCGACAAAATAAAATTAAGAAAGTGTAGCAATAGAAATTCAAAATGCCGAGCAT
 TTATGCATTTAGATACAGTATTTACAATGGTTGATTATGATAAGTTTACAATTCACCCAGCTAT
 TCAAGGGCCAAAAGGGAATATGAATATTTATATTTTAGAAAAAGGAGCAGATGAGGAAACTCTT
 AAAATTACACATCGTACTTCTTTAATGGAAGCATTAAAAGAGGTATTAGACTTAAGTGAATTAG
 TTCTTATTCATGTGGAGGAGGAGATGTAATTGCTTCTGCTCGTGAACAATGGAATGATGGCTC
 GAACACATTAGCAATCGCGCCAGGTGTAGTTGTTACATATGATCGCAACTATGTATCCAATACG
 TTATTACGGGAACACGGTATAGAAGTGATTGAGGTGCTAAGTTCAGAATTATCTCGTGGTCGTG
 GGGTCCACGTTGCATGAGTATGCCAATTGTTTCGTAAAGATATTTAA-3'

FIG. 7

(C) The amino acid sequence of **His-ABD-PolyN-bcADI**:

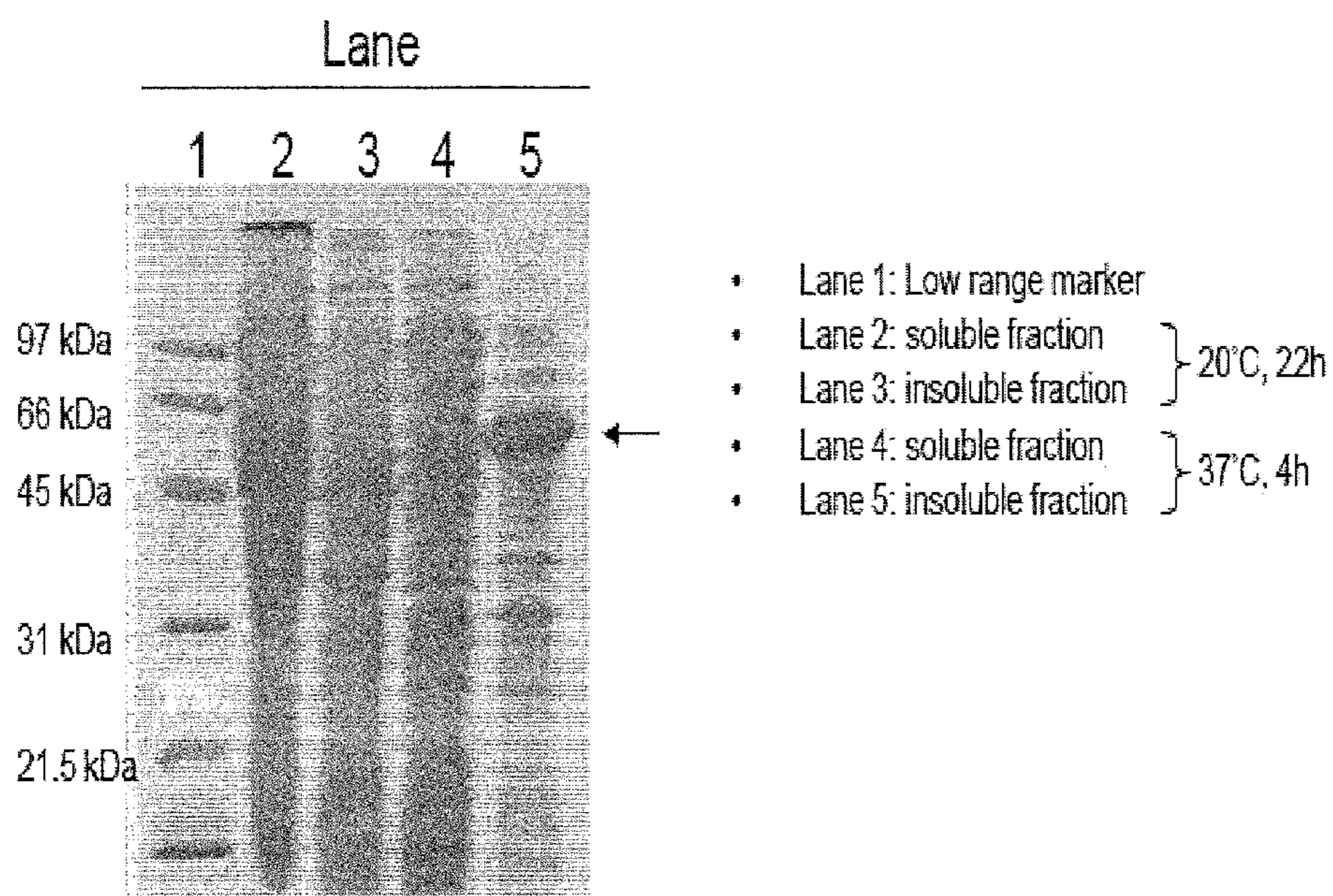
(SEQ ID NO: 41)

MGHHHHHDEAVDANSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALPSGSNNNNNNGSGGKH
PIHVTSEIGELQTVLLKRPKEVENLTPDYLLQLLFDDIPYLPPIIQKEHDYFAQTLRNRGVEVLYLEKLAAEALVDKK
LREEFVDRIKKEGQADVNVAHQTLKEYLLSFSNEELIQKIMGGVRKNEIETSKKTHLYELMEDHYPFYLDPMPNLYFT
RDPAAASVGDGLTINKMREPARRRESLFMEYIIKYHPRFAKHNPVWLDRDYKFPLEGGDELILNEETIAIGVSARTSA
KAIERLAKNLFSRQNKIKKVLAIELPKCRAFMHLD'VFTMVDYDKFTIHPAIQGPKGNMNIYILEKGADEETLKITHR
TSLMEALKEVLDLSELVLI PCGGGDVIASAREQWNDGSNTLAIAPGVVVTYDRNYVSNTLLREHGIEVIEVLSSELSR
GRGGPRCMSMPIVRKDI

(PolyN with linker: SGSNNNNNNGSGG)

FIG. 7 (continued)

(A)



(B)

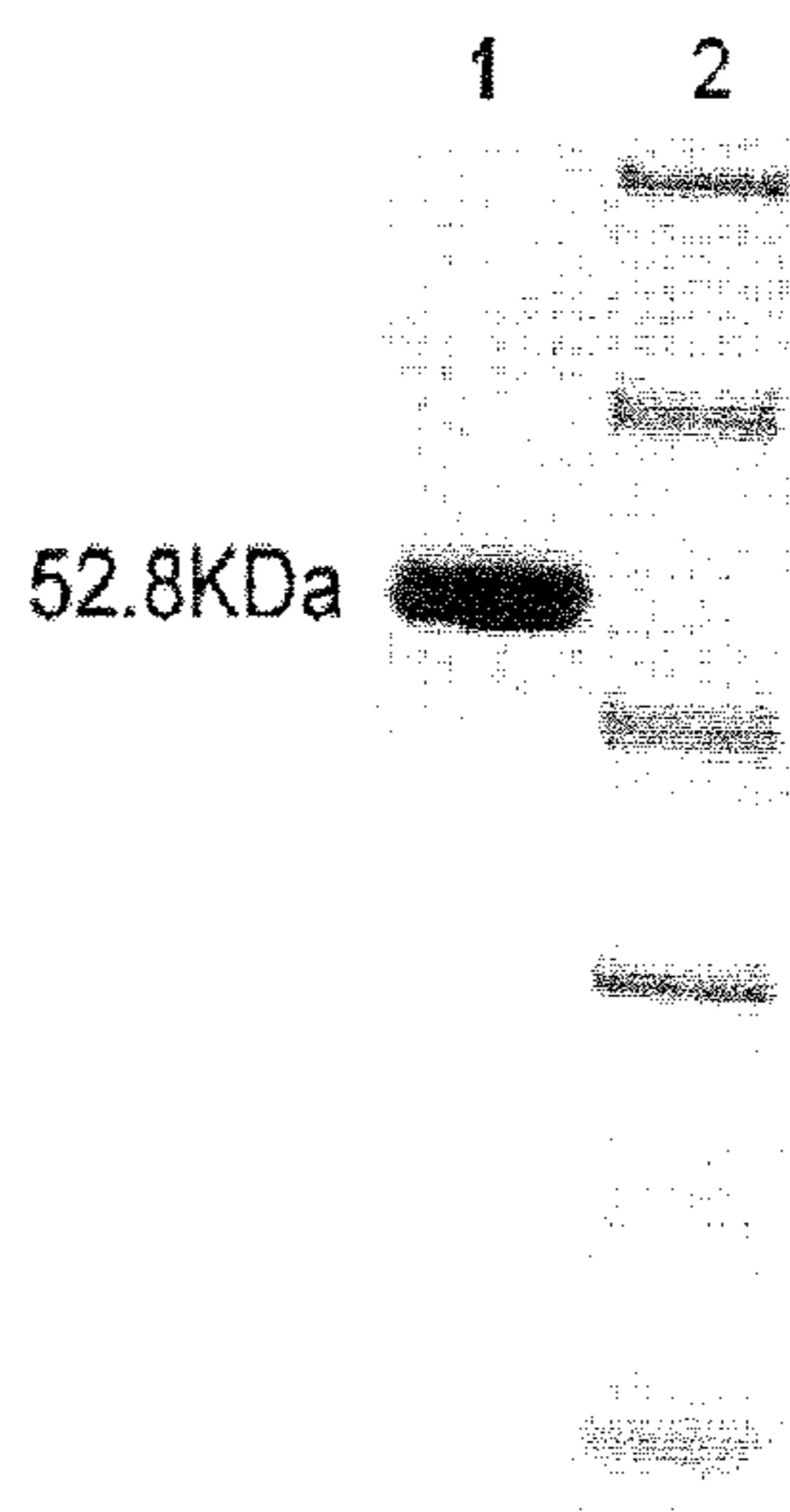


FIG. 8

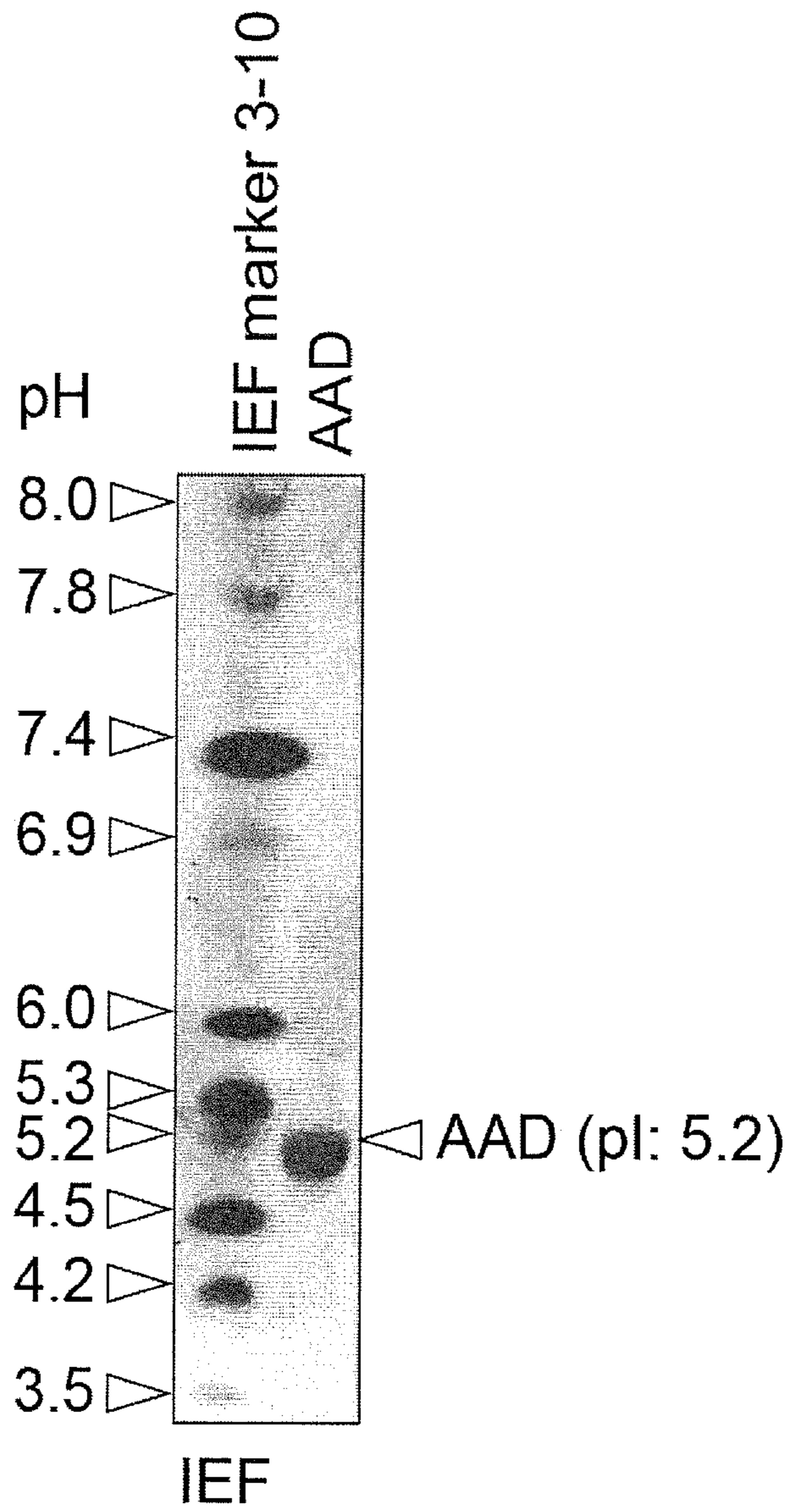
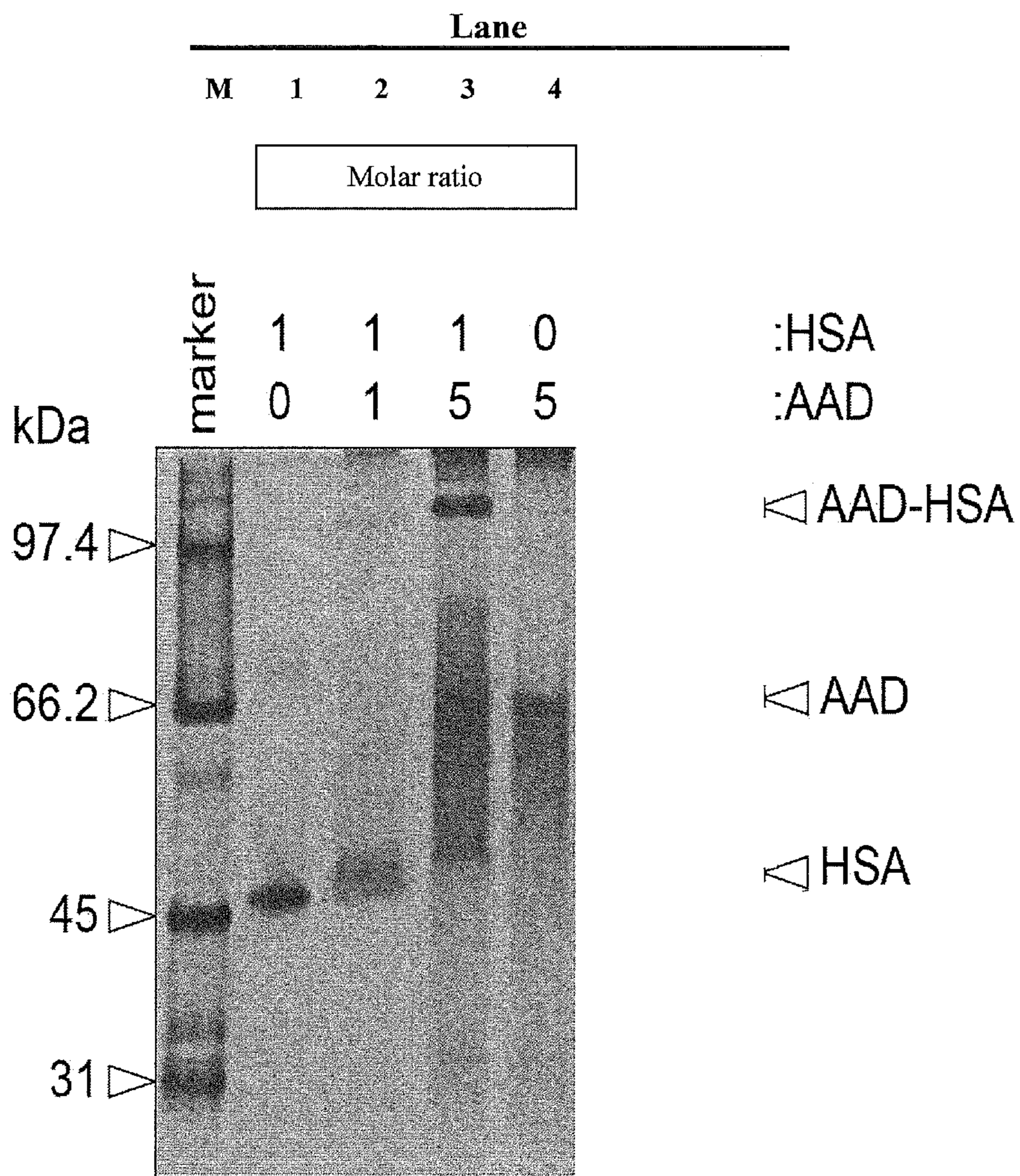


FIG. 9



Note:
HSA: Human serum albumin
AAD
AAD-HSA complex

FIG. 10

Effect of AAD on mice plasma arginine levels (n=3)

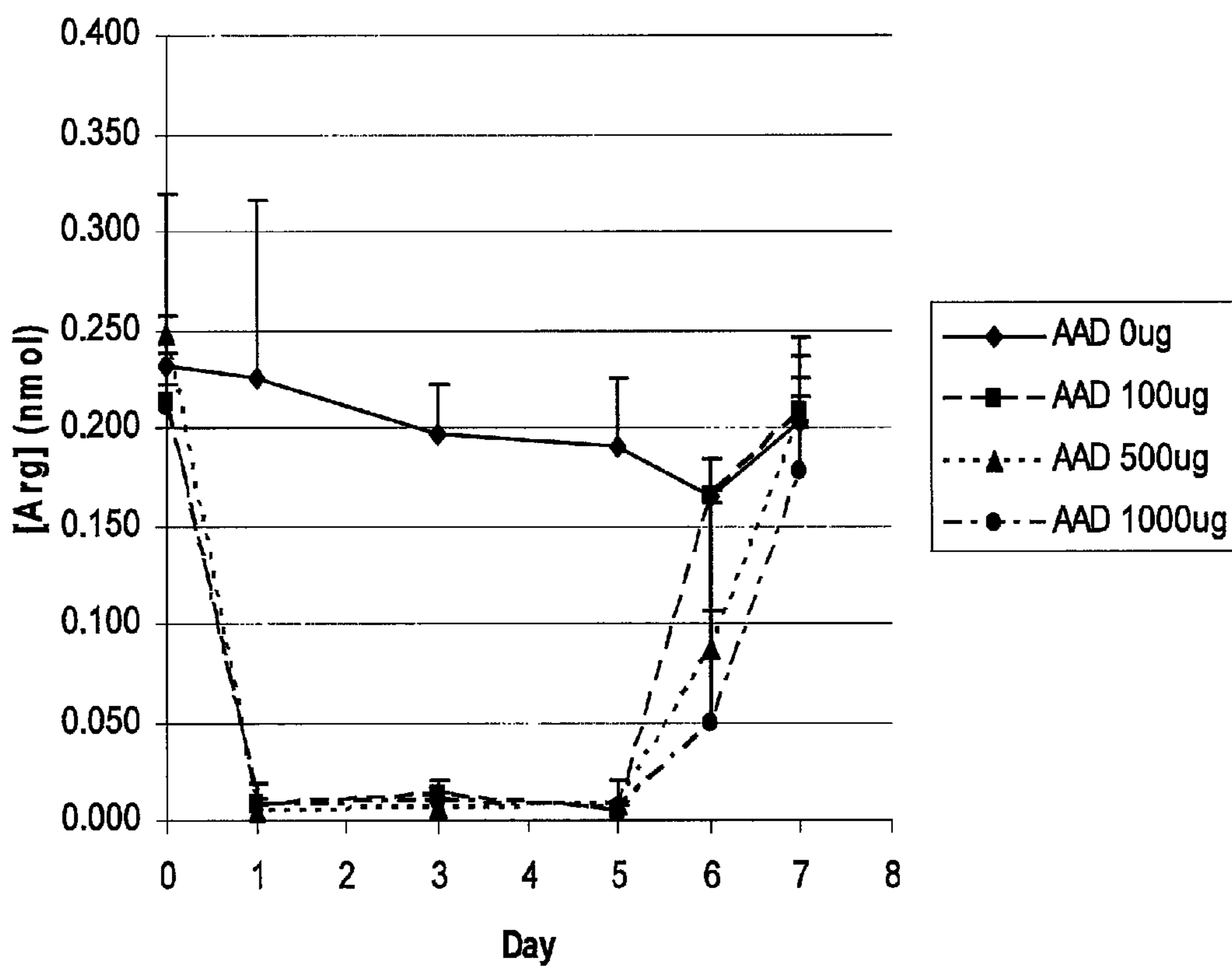
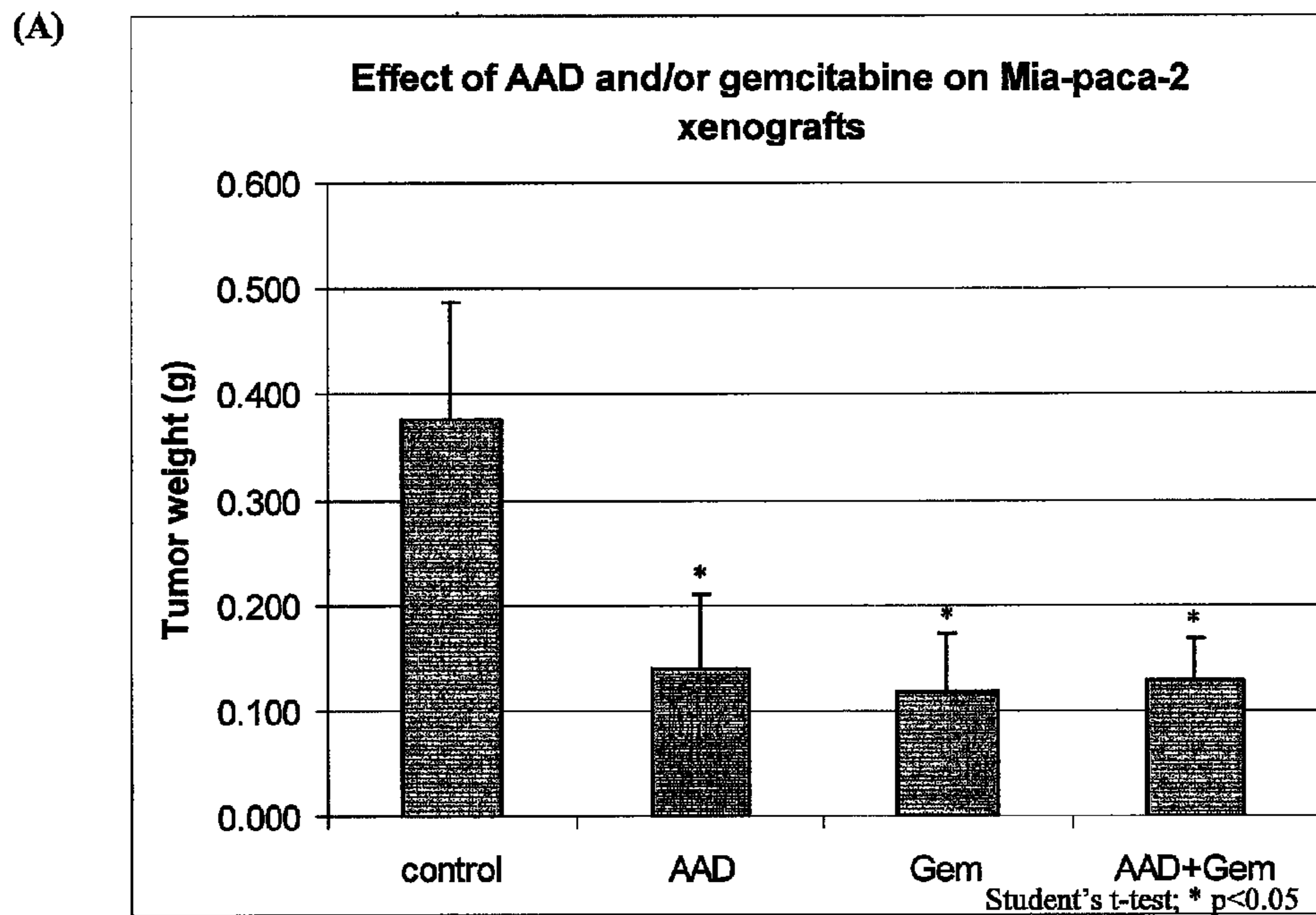


FIG. 11



(B)

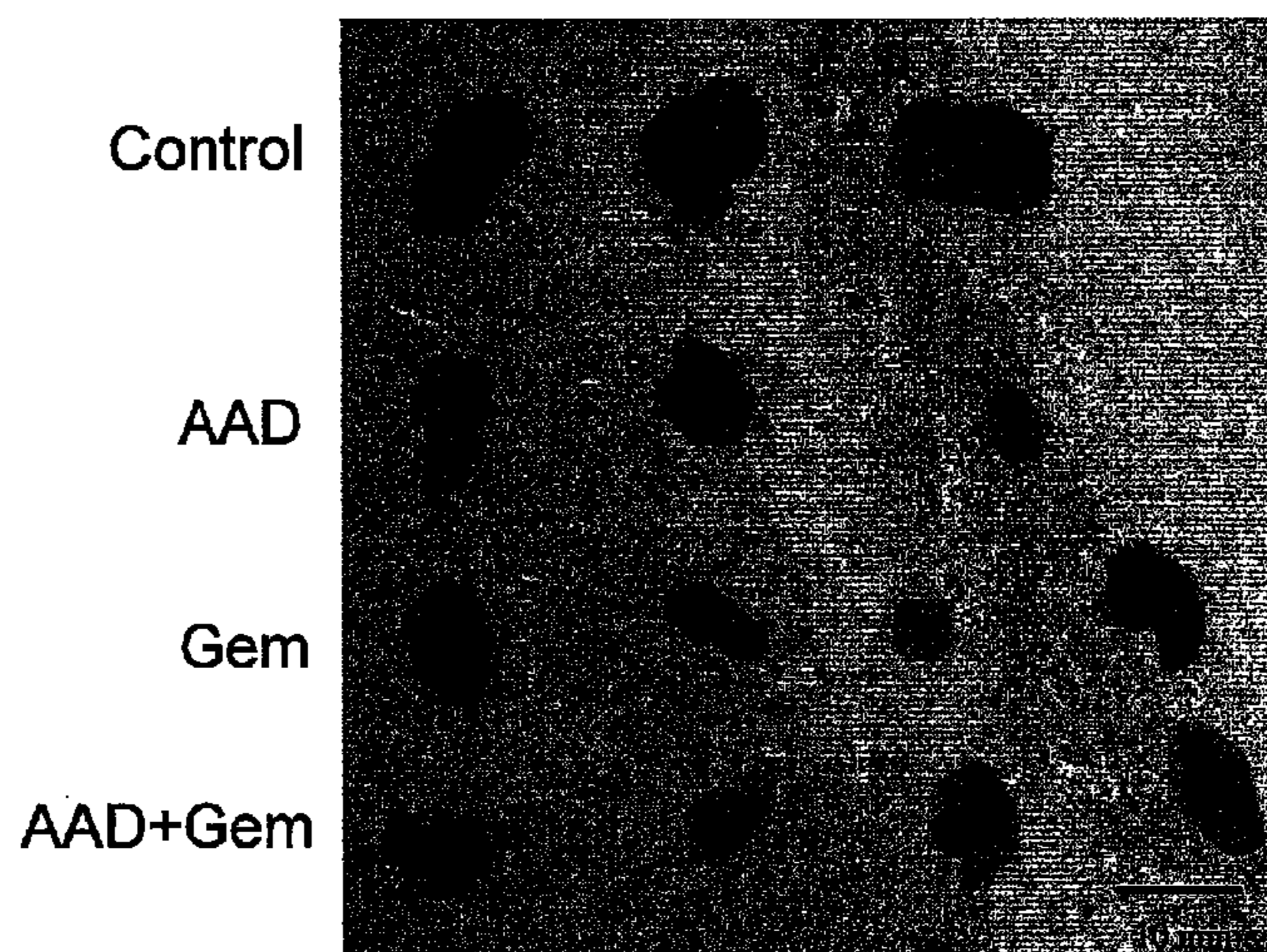
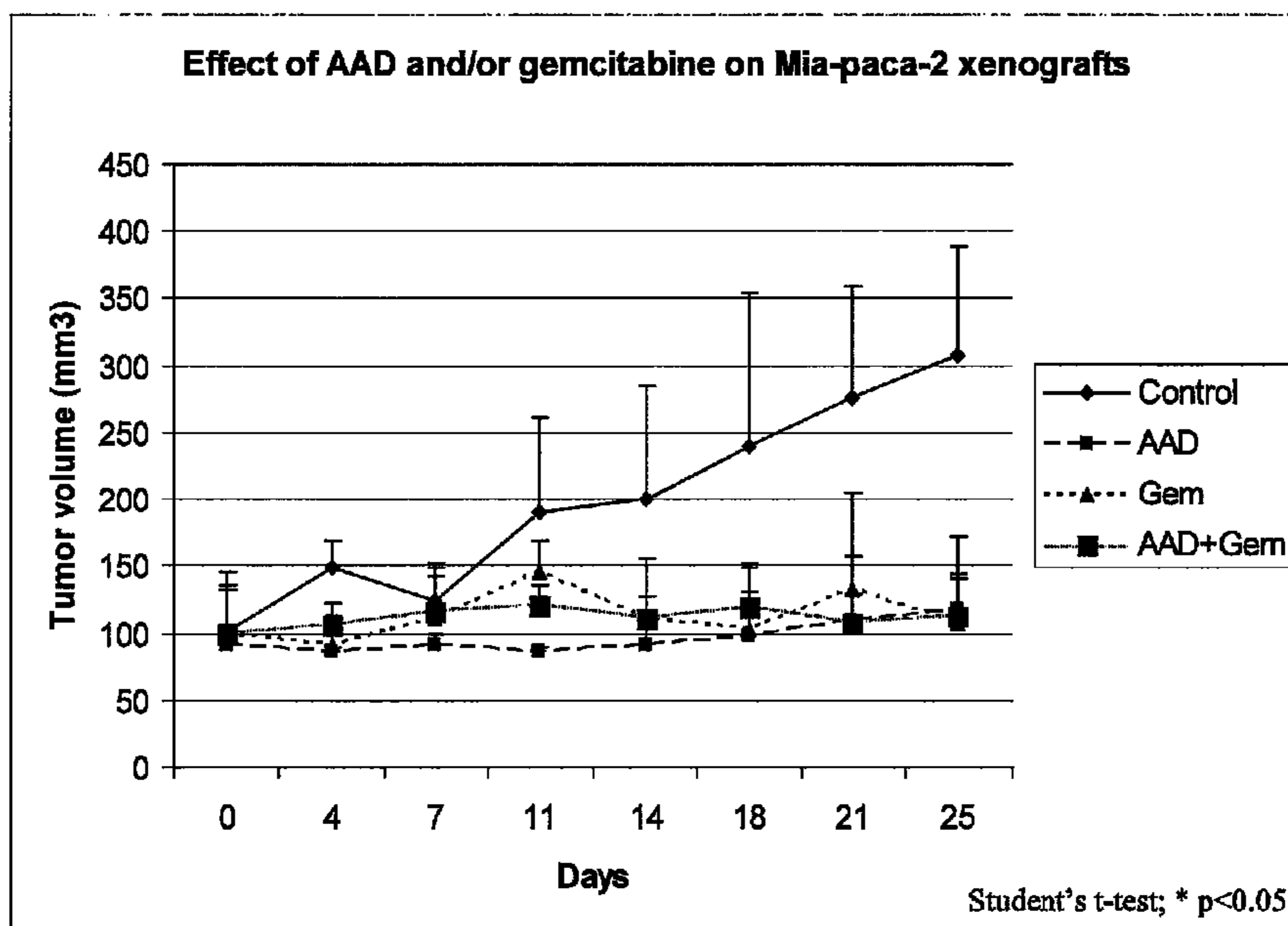


FIG. 12

(C)



(D)

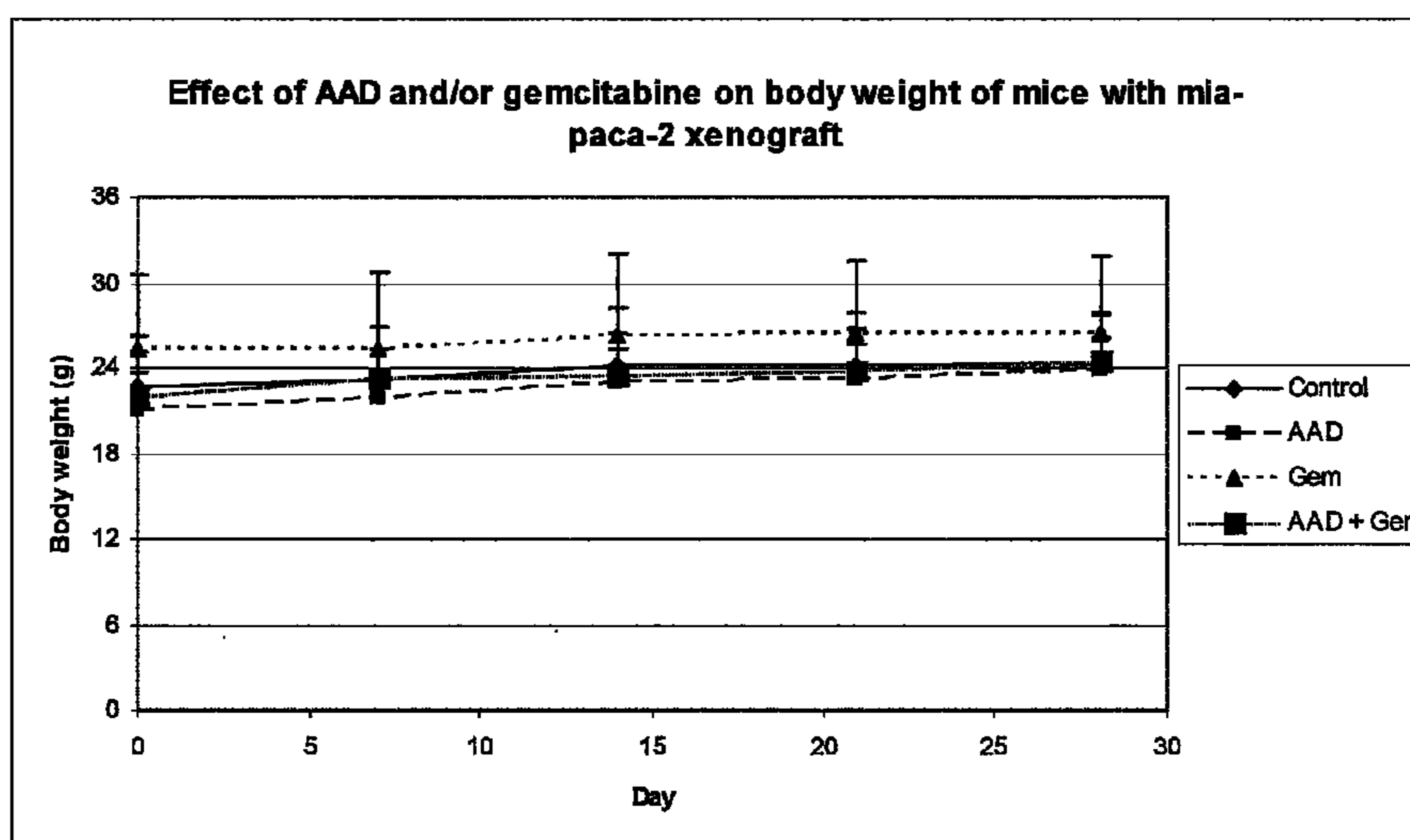
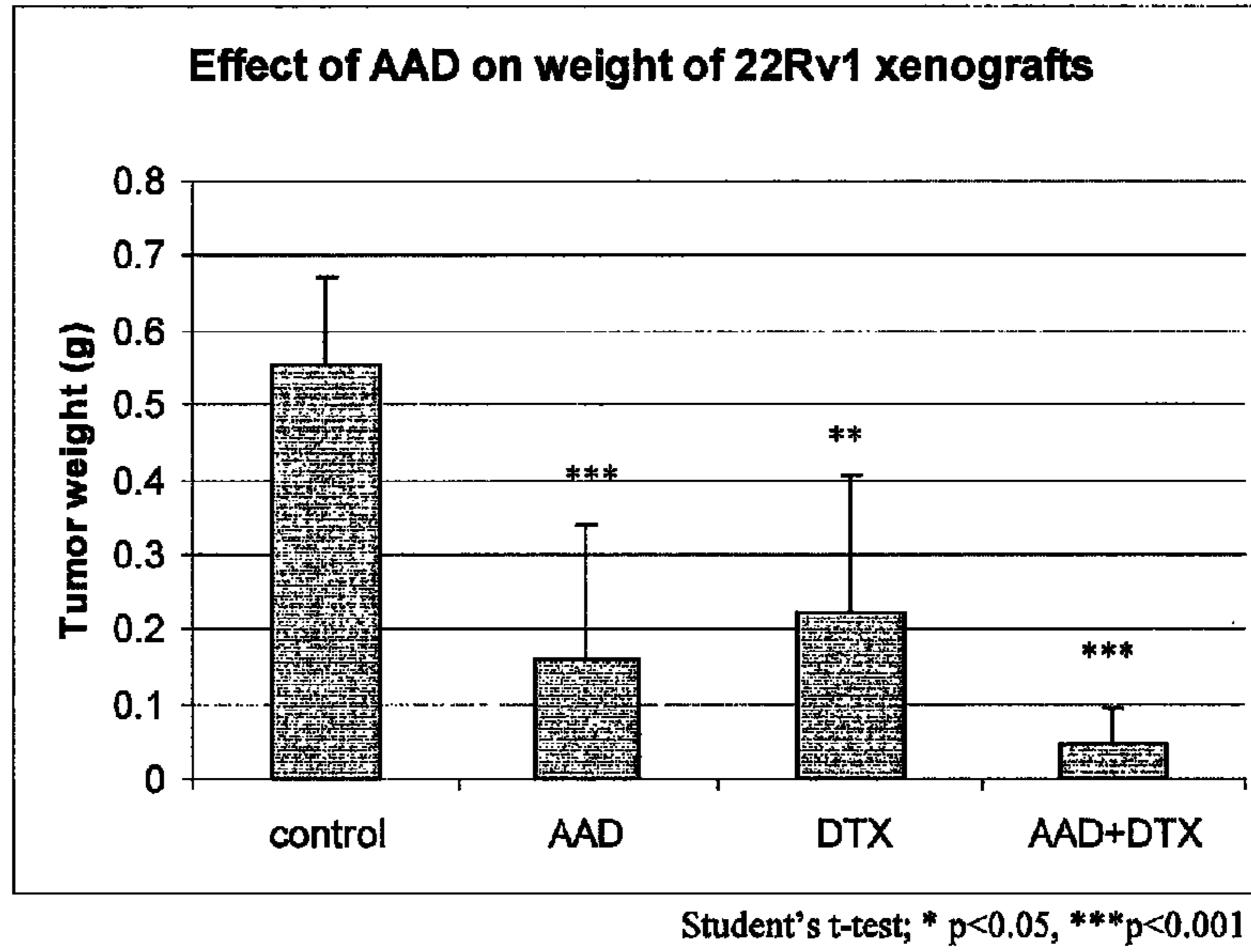


FIG. 12 (continued)

(A)



(B)

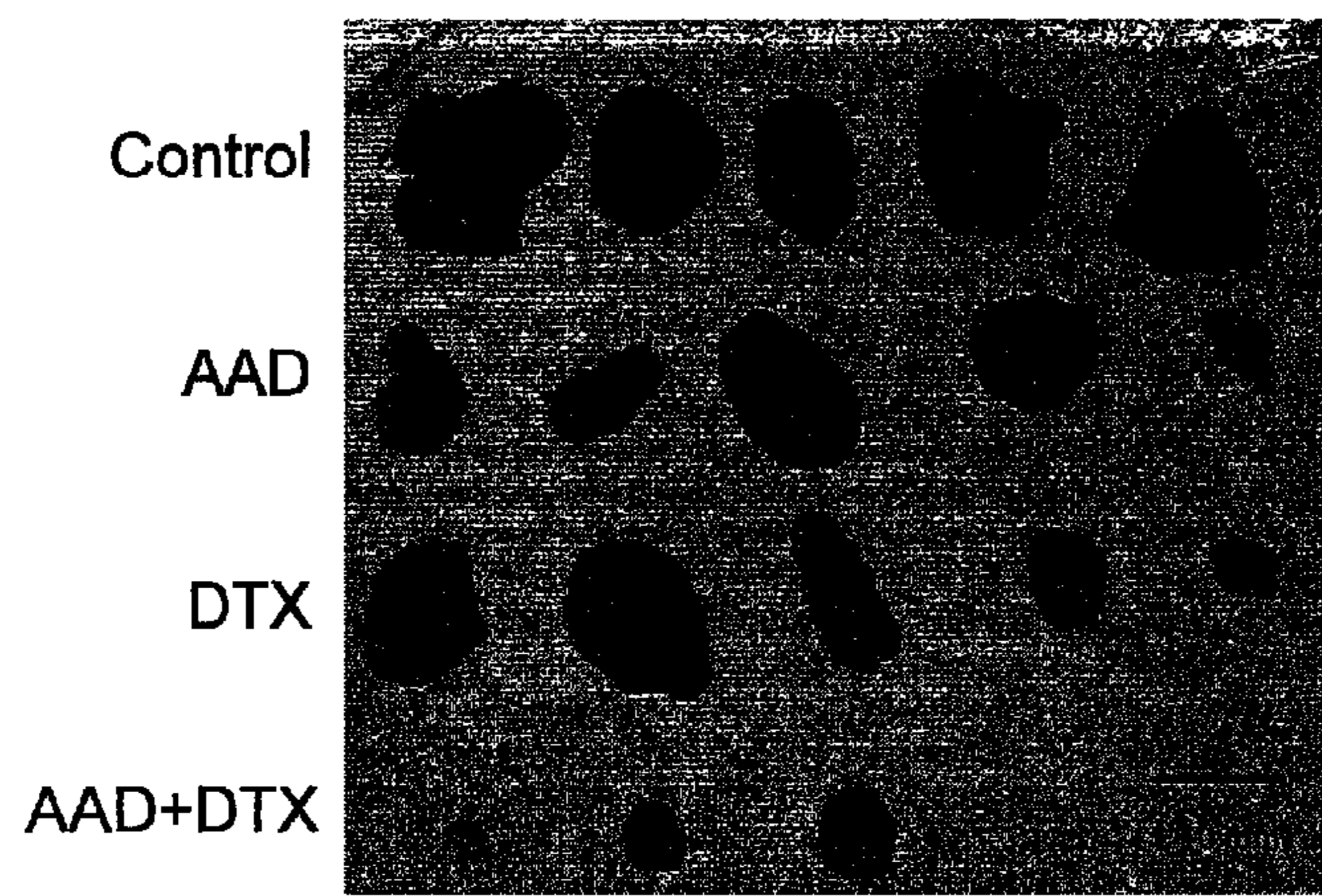


FIG. 13

(C)

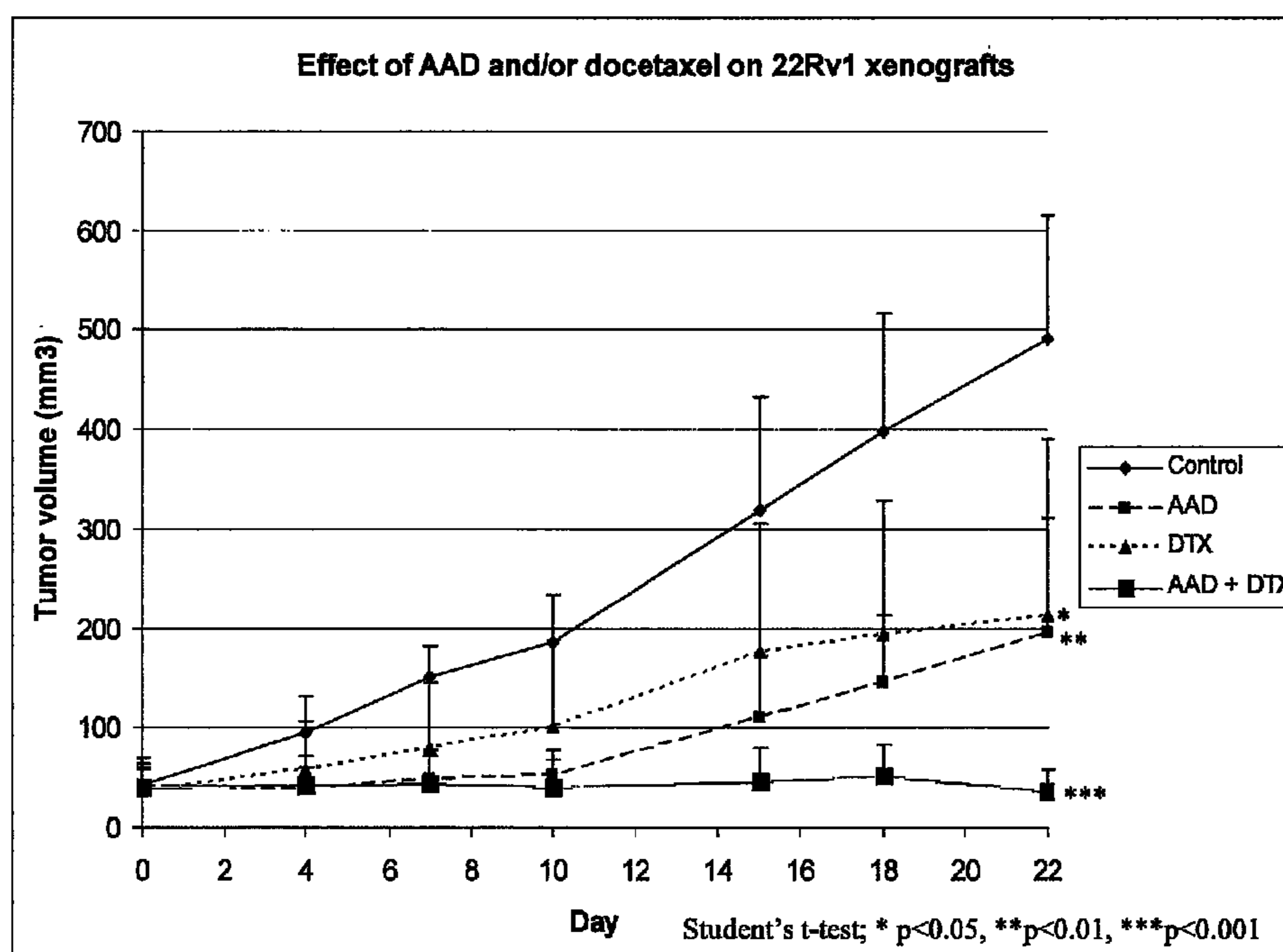


FIG. 13 (continued)

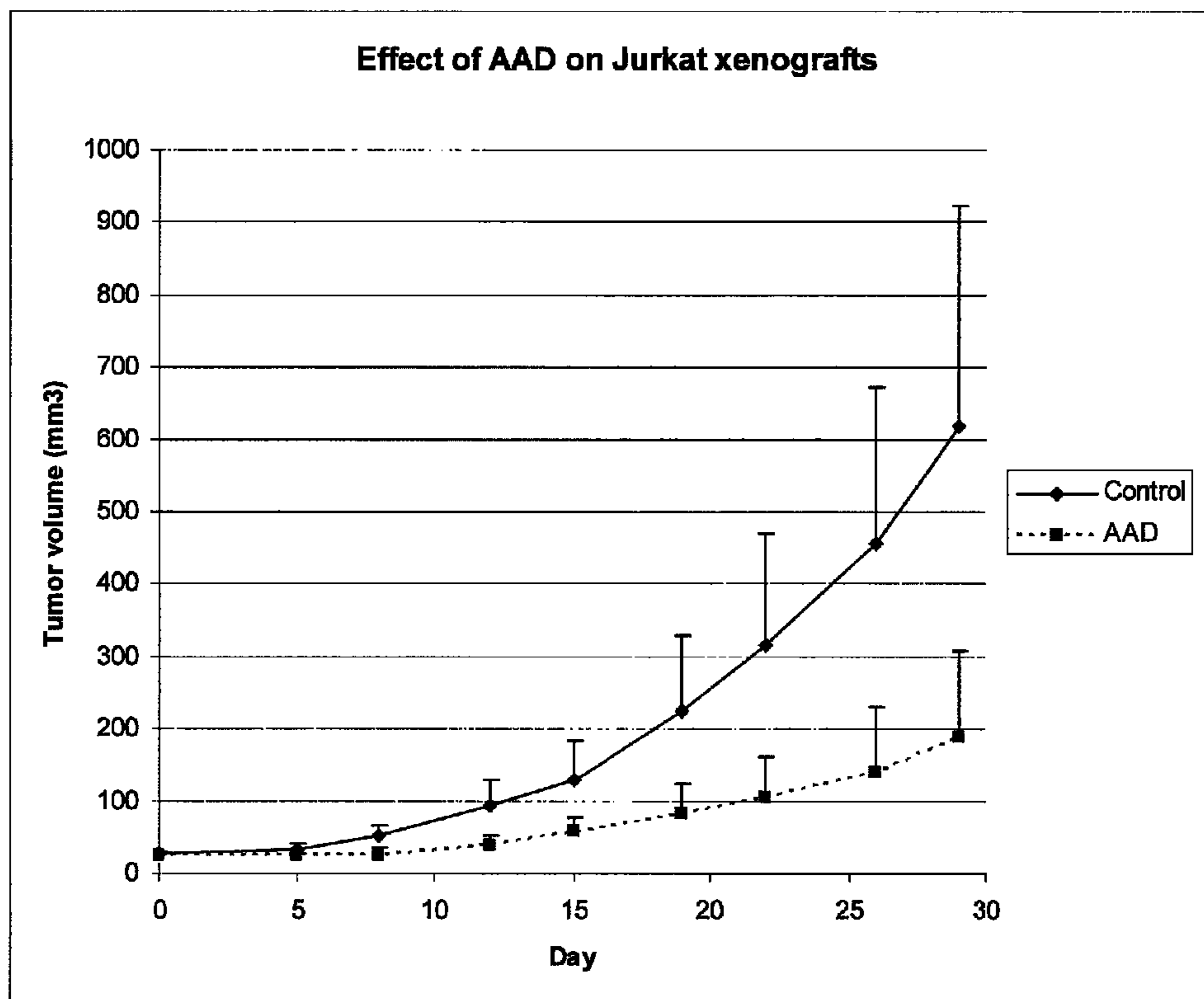


FIG. 14

**METHOD FOR CANCER TARGETING
TREATMENT AND DETECTION OF
ARGININE USING ALBUMIN-BINDING
ARGININE DEIMINASE FUSION PROTEIN**

Matter enclosed in heavy brackets [] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue; a claim printed with strikethrough indicates that the claim was canceled, disclaimed, or held invalid by a prior post-patent action or proceeding.

CROSS-REFERENCE TO RELATED
APPLICATION

The present application *is a reissue application of U.S. Pat. No. 9,803,185, issued on Oct. 31, 2017 from U.S. patent application Ser. No. 14/981,855, filed on Dec. 28, 2015, which is a continuation-in-part application of U.S. non-provisional patent application Ser. No. 14/197,236 filed Mar. 5, 2014 and now granted under the U.S. Pat. No. 9,255,262, which claims benefit from U.S. provisional patent application Ser. No. 61/773,214 filed Mar. 6, 2013, and the [disclosure] disclosures of which are incorporated herein by reference in [its] their entirety.*

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TECHNICAL FIELD

The present invention describes albumin-binding arginine deiminase (AAD) fusion protein that has been genetically modified to create a material having high activity and long in vivo half-life. The present invention further describes the designs for DNA and protein engineering for creating different AAD fusion proteins. The AAD fusion proteins can be isolated and purified from soluble fraction and insoluble fraction (inclusion bodies) of the crude proteins. The present invention further relates to albumin-binding arginine deiminase-containing pharmaceutical compositions for cancer targeting treatment and curing arginine-dependent diseases in humans and other animals. The AAD fusion protein can also be used as a component in a testing kit for detection of arginine.

BACKGROUND OF THE INVENTION

The incidence of pancreatic cancer, colon cancer, liver cancer, melanoma and cervical cancer in the worldwide population is increasing. Effective treatments for these diseases are urgently needed. In many types of cancer including leukemia, melanoma, pancreatic, colon, renal cell carcinoma, lung, prostate, breast, brain, cervical and liver cancers, the cancer cells are auxotrophic for arginine since they

lack of expression of argininosuccinate synthetase (ASS), making these cancers excellent targets for arginine depletion therapy.

Arginine is a semi-essential amino acid for humans and other mammals. It can be synthesized from citrulline via a two step process catalyzed by the urea cycle enzymes argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL). Arginine can be metabolized to ornithine by the enzyme arginase, and ornithine can be converted to citrulline by ornithine carbamoyltransferase (OTC) in the mitochondria. The citrulline can be utilized to synthesize arginine again. Normal cells usually do not require an exogenous supply of arginine for growth because of the abundant catalytic activity of ASS and ASL. In contrast, many types of cancers do not express ASS and therefore are auxotrophic for arginine. Their growth is dependent on arginine solely obtained from blood circulation. Therefore, targeting circulating arginine by using arginine-degrading enzymes is a feasible strategy to inhibit ASS-negative tumor growth [Feun et al., *Curr. Pharm. Des.* 14:1049-1057 (2008); Kuo et al., *Oncotarget.* 1:246-251 (2010)].

Arginine can be degraded by arginase, arginine decarboxylase, and arginine deiminase (ADI). Among them, arginine deiminase (ADI) appears to have the highest affinity for arginine (a low K_m value). ADI converts arginine to citrulline and ammonia, the metabolites of the urea cycle. Unfortunately, ADI can only be found in prokaryotes e.g. *Mycoplasma* sp. There are some problems associated with the isolation and purification of ADI from prokaryotes. ADI isolated from *Pseudomonas putida* fails to exhibit efficacy in vivo because of its low enzymatic activity in neutral pH. ADI produced from *Escherichia coli* is enzymatically inactive and subsequently requires multiple denaturation and renaturation process which raises the subsequent cost of production.

As the native ADI is found in microorganisms, it is antigenic and rapidly cleared from circulation in a patient. The native form of ADI is immunogenic upon injection into human circulation with a short half-life (~4 hours) and elicits neutralizing antibodies [Ensor et al., *Cancer Res.* 62:5443-5450 (2002); Izzo et al., *J. Clin. Oncol.* 22:1815-1822 (2004)]. These shortcomings can be remedied by pegylation. Among various forms of pegylated ADI, ADI bound with PEG (molecular weight 20,000) via succinimidyl succinate (ADI-PEG 20) has been found to be an efficacious formulation. However, the activity of ADI after pegylation is greatly decreased on the order of 50% [Ensor et al., *Cancer Res.* 62:5443-5450 (2002)]. The previous attempts to create pegylated ADI resulted in materials that are not homogenous (due to the random attachment of PEG on protein surface Lys residues) and also difficult to characterize and perform quality control during the manufacturing process. Also, PEG is very expensive, greatly increasing the production cost. After the intravenous injection of pegylated ADI in vivo, leakage or detachment of free PEG is observed and the ADI (without PEG) can elicit the immunogenicity problem. Therefore, there is a need for improved cancer-treatment compositions, particularly, improved cancer-treatment compositions that have enhanced activity and in vivo half-life.

SUMMARY OF THE INVENTION

In the present invention, albumin-binding arginine deiminase (AAD) fusion protein has increased its activity and plasma half-life in order to efficiently deplete arginine in cancer cells. Native ADI may be found in microorganisms and is antigenic and rapidly cleared from circulation in a

patient. The present invention constructs different AAD fusion proteins with one or two albumin-binding proteins to maintain high activity with longer in vivo half-life (at least 5 days of arginine depletion after one injection). In the present invention, the albumin binding protein in the AAD fusion protein product does not appear to influence its specific enzyme activity but instead appears to increase the circulating half-life. The specific activities of wild-type ADI and AAD fusion protein in the present invention are about 20 and about 19 U/mg (at physiological pH 7.4), respectively. In addition, the AAD fusion protein of the present invention is generally more soluble in most of the solutions than native or wild-type ADI of different organisms. Purification of native or wild-type ADI is also generally more difficult than the AAD fusion protein of the present invention.

In its broadest sense, the present invention provides an albumin-binding arginine deiminase fusion protein comprising a first portion comprising one or two components selected from an albumin-binding domain, an albumin-binding peptide or an albumin-binding protein(s) fused to a second portion comprising arginine deiminase to form the albumin-binding arginine deiminase fusion protein such that the albumin-binding arginine deiminase fusion protein retains the activity of arginine deiminase and is also able to bind serum albumin.

The present invention further relates to albumin-binding arginine deiminase (AAD) fusion protein-containing pharmaceutical compositions for targeted cancer treatment in humans and other animals. The first aspect of the present invention is to construct the modified AAD fusion protein with high activity against cancer cells. The second aspect of the present invention is to purify AAD fusion protein with high purity from both soluble and insoluble fractions of the crude proteins. The third aspect of the present invention is to lengthen the half-life of AAD fusion protein as it can bind to albumin very well in the circulation. The fourth aspect of the present invention is to provide a method of using the AAD fusion protein-containing pharmaceutical composition of the present invention for treating cancer by administering said composition to a subject in need thereof suffering from various tumors, cancers or diseases associated with tumors or cancers or other arginine-dependent diseases. The fifth aspect of the present invention is to use AAD fusion protein as a component in a testing kit for detection of arginine.

The AAD fusion protein of the present invention is also modified to avoid dissociation into albumin-binding protein and ADI such that it becomes more stable and has a longer half-life in circulation. ADI is fused to an albumin-binding domain/peptide/protein in AAD fusion product to extend the plasma half-life and reduce the immunogenicity of the fusion product. Albumin binding domain (ABD) is a peptide that binds albumin in the blood. There are different variants of ABD showing different or improved human serum albumin (HSA) affinities. Different variants of ABD can be constructed and can be fused to ADI. Unlike the naturally occurring ADI, this longer half-life property facilitates the depletion of arginine efficiently in cancerous cells, cancer stem cells and/or cancer progenitor cells.

The pharmaceutical composition containing AAD fusion protein can be used for intravenous (i.v.) injection (for rapid-acting dosage of medication) and intramuscular (i.m.) injection (for fairly rapid-acting and long-lasting dosage of medication). The application of AAD fusion protein in the present invention can be used in the treatment of various cancers such as pancreatic cancer, leukemia, melanoma, head and neck cancer, colorectal cancer, lung cancer, breast cancer, prostate cancer, cervical cancer, liver cancer, naso-

pharyngeal cancer, esophageal cancer and brain cancer. The present invention is directed to AAD fusion proteins, to methods of treating cancer, to methods of treating and/or inhibiting metastasis of cancerous tissue, and to methods of curing arginine-dependent diseases.

The method of the present invention also includes using a combination of different chemotherapeutic drugs and/or radiotherapy with the AAD fusion protein of the present invention to give a synergistic effect on cancer treatment.

In the presently claimed invention, an aspect relates to the use of the AAD fusion protein of the present invention as a component in a testing kit for detection of arginine in different samples (e.g. blood samples from cancer patients, food samples, cell cultures). Said testing kit comprises the AAD fusion protein of the present invention and a color reagent. When a sample is incubated with the AAD fusion protein and the color reagent of the testing kit in appropriate assay conditions, the arginine is converted by the AAD fusion protein into citrulline. The color reagent will be turned into pink color in the presence of citrulline. The presence of citrulline indicates the presence of arginine in the sample and the intensity of the pink color developed in the assay can be used to quantify the concentration of the arginine in the sample by measuring the color intensity of the reaction mixture in a spectrophotometer. The sample can be prepared in solution form before using the testing kit. The concentration of the arginine in the sample can be expressed by the following formula:

$$\text{one unit of arginine deiminase activity} = 1 \mu\text{mol of arginine being converted to } 1 \mu\text{mol of citrulline per minute.}$$

In one embodiment, the AAD fusion protein of the present invention has a specific activity of about 19 U/mg at pH 7.4 and 37° C., which is comparable to the activity of wild-type ADI. There are many advantages of using the AAD fusion protein of the present invention over wild-type ADI as enzyme of the testing kit, such as (1) simple production method, (2) longer shelf-life and (3) higher solubility. For wild-type ADI, it is usually insoluble and difficult for purification. For our AAD fusion protein, it can be prepared both soluble and insoluble forms.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the design approach for construction of different AAD fusion proteins with one or two albumin-binding domain/peptide/protein(s) in three-dimensional structure. One or two albumin-binding domain/peptide/protein(s) can be fused to ADI to form the AAD fusion protein. The position of albumin-binding domain/peptide/protein is far from the ADI active site. The albumin-binding domain/peptide/protein can be fused to the N-terminus or/and C-terminus of ADI. The structure in this figure is based on the *Mycoplasma arginini* ADI structure (Protein Data Bank: 1LXY). (A) Native ADI; (B) AAD fusion protein with two ABD or ABD1; (C) AAD fusion protein with one ABD or ABD1 at N-terminus; (D) AAD fusion protein with one ABD or ABD1 at C-terminus.

FIG. 2 shows the sequence alignment for ADI in some bacterial species including *Mycoplasma arginini* (SEQ ID No. 23), *Lactococcus lactis* (SEQ ID No. 24), *Bacillus cereus* (SEQ ID No. 25) and *Bacillus licheniformis* (SEQ ID No. 26).

FIG. 3 shows the designs and amino acid sequences for different AAD fusion proteins originated from *Mycoplasma arginini* (A to E) and AAD fusion protein originated from *Bacillus cereus* (F).

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FIG. 4 shows the creation of AAD fusion protein in two embodiments (A) and (B) by the use of intein-fusion proteins and expressed protein ligation (CBD, chitin binding domain) under the following schemes; (C) C-terminal fusion; (D) N-terminal fusion; (E) Intein-mediated protein ligation.

FIG. 5 shows the plasmid map of the expression vector constructed for producing AAD fusion protein.

FIG. 6 shows the (A) gene map, (B) nucleotide sequence (SEQ ID No. 44) and (C) amino acid sequence (SEQ ID No. 40) of His-ABD-PolyN-ADI. (ADI: the *Mycoplasma arginini* ADI)

FIG. 7 shows the (A) gene map, (B) nucleotide sequence (SEQ ID No. 45) and (C) amino acid sequence (SEQ ID No. 41) of His-ABD-PolyN-bcADI. (bcADI, the *Bacillus cereus* ADI)

FIG. 8 shows the expression and purification of AAD fusion protein: (A) AAD is ~90% soluble when expressed at 20° C. (lanes 2 and 3) and ~90% insoluble (inclusion body) when expressed at 37° C. (lanes 4 and 5); (B) The purified AAD fusion protein in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel: lane 1, purified AAD fusion protein (52.8 kDa); lane 2, molecular weight marker.

FIG. 9 shows the isoelectric point of AAD fusion protein in a pH 3-7 IEF protein gel.

FIG. 10 shows the human serum albumin (HSA) binding of AAD. M: marker; Lane 1: HSA only; Lane 2: HSA:AAD ratio=1:1; Lane 3: HSA:AAD ratio=1:5; Lane 4: AAD only; Open Arrow: band of AAD, HSA, or AAD-HSA complexes.

FIG. 11 is a graph showing the dose response of AAD fusion protein on plasma arginine levels in mice. A dose of 100 µg of AAD is sufficient to deplete plasma arginine for at least 5 days.

FIG. 12 shows the effects of administration of AAD fusion protein in total of 10 U per week and/or GEM on (A) tumor size; (B) appearance of the Mia-paca-2 pancreatic cancer xenograft; (C) tumor volume and (D) body weight of the mice.

FIG. 13 shows the effects of administration of AAD fusion protein in total of 10 U per week and/or DTX on (A) tumor size; (B) appearance of the 22Rv1 prostate cancer xenograft and (C) tumor volume of the mice.

FIG. 14 shows the effects of administration of AAD fusion protein in total of 10 U per week of the Jurkat leukemia xenografts on tumor volume of the mice.

DEFINITIONS

The term "cancer stem cell" refers to the biologically distinct cell within the neoplastic clone that is capable of initiating and sustaining tumor growth in vivo (i.e. the cancer-initiating cell).

DETAILED DESCRIPTION OF THE INVENTION

Arginine is a semi-essential amino acid for humans and other mammals. It can be synthesized from citrulline via a two step process catalyzed by urea cycle enzymes argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL). Arginine can be metabolized to ornithine by the enzyme arginase, and ornithine can be converted to citrulline by ornithine carbamoyltransferase (OTC) in the mitochondria. The citrulline can be utilized to synthesize arginine again. Normal cells do not typically require an exogenous supply of arginine for growth because of the abundant

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catalytic activity of ASS and ASL. In contrast, many types of cancers do not express ASS and are therefore auxotrophic for arginine. Their growth is solely dependent on arginine from circulation. Therefore, targeting circulating arginine by using arginine-degrading enzymes is a feasible strategy to inhibit ASS-negative tumor growth.

Arginine can be degraded by arginine deiminase (ADI). ADI converts arginine to citrulline and ammonia, the metabolites of the urea cycle. Unfortunately, ADI can only be found in prokaryotes e.g. *Mycoplasma* sp. There are many problems associated with the isolation and purification of arginine deiminase from prokaryotes. ADI isolated from *Pseudomonas putida* failed to exhibit efficacy in vivo because of its low enzymatic activity in neutral pH. ADI produced from *Escherichia coli* is enzymatically inactive and subsequently requires multiple denaturation and renaturation process which raised the subsequent cost of production. The plasma half-life of the native form of ADI is short (~4 hours) upon injection into human circulation [Ensor et al., *Cancer Res.* 62:5443-5450 (2002); Izzo et al., *J. Clin. Oncol.* 22:1815-1822 (2004)]. These shortcomings can be partially remedied by pegylation. Among various forms of pegylated ADI, ADI bound with PEG (molecular weight 20,000) via succinimidyl succinate (ADI-PEG 20) has been found to be an efficacious formulation. However, the activity of ADI after pegylation is greatly decreased (by ~50%) [Ensor et al., *Cancer Res.* 62:5443-5450 (2002); Wang et al., *Bioconjug. Chem.* 17:1447-1459 (2006)]. Also, the succinimidyl succinate PEG linker can easily be hydrolyzed and detached from the protein, causing immunogenic problems after a short period of use in the body. Therefore, there is a need for improved cancer-treatment compositions, particularly, improved cancer-treatment compositions with enhanced activity.

ADI isolated from *P. putida* failed to exhibit efficacy in vivo because it had little enzyme activity at a neutral pH and was rapidly cleared from the circulation of experimental animals. ADI derived from *Mycoplasma arginini* is described, for example, by Takaku et al, *Int. J. Cancer*, 51:244-249 (1992), and U.S. Pat. No. 5,474,928. However, a problem associated with the therapeutic use of such a heterologous protein is its antigenicity. The chemical modification of ADI from *Mycoplasma arginini*, via a cyanuric chloride linking group, with polyethylene glycol (PEG) was described by Takaku et al., *Jpn. J. Cancer Res.*, 84:1195-1200 (1993). However, the modified protein was toxic when metabolized due to the release of cyanide from the cyanuric chloride linking group. In contrast, even for the ADI-PEG20, the PEG linker can easily be hydrolyzed and detached from the protein, causing immunogenic problems after a short period of use in the body. Therefore, there is a need for compositions which degrade non-essential amino acids and which do not have the problems associated with the prior art.

In many types of cancer including melanoma, pancreatic, colon, leukemia, breast, prostate, renal cell carcinoma and liver cancers, cancer cells are auxotrophic for arginine since they lack of expression of argininosuccinate synthetase (ASS), making them excellent targets for arginine depletion therapy. In this invention, albumin-binding arginine deiminase (AAD) fusion proteins have high activity with long half-lives for efficient depletion of arginine in cancer cells.

The size of the monomer for ADI is on the order of 45 kDa and it exists as dimer (on the order of 90 kDa) [Das et al., *Structure*. 12:657-667 (2004)]. A design for construction of an AAD fusion protein is shown in FIG. 1. One or two albumin-binding domain/peptide/protein(s) with or without

linker(s), SEQ ID NO: 46-49, are fused to ADI to form the AAD fusion protein. It is noteworthy that the selection of one or two particular albumin-binding domain/peptide/protein(s) can be made depending upon the type of cancer tissue to be targeted, the desired size and half-life of the resulting fusion protein, and whether a domain or entire protein is selected. Further, the selected albumin-binding material may be the same or different. That is, a protein and a peptide can be fused, two proteins, two domains, a domain and a protein, etc., as long as the resultant molecule retains the activity of the ADI and is also able to bind serum albumin with neither function of one portion of the fusion protein being interfered with by the other portion of the fusion protein. The position of the albumin-binding domain/peptide/protein is far from the active site. The albumin-binding domain/peptide/protein can be fused to the N-terminus or/and C-terminus of ADI. There are different variants of ABD showing different or improved human serum albumin (HSA) affinities. Different variants of ABD can be constructed and can be fused to ADI. Some micro-organisms endowed with ADI (for example *Pseudomonas* sp) cannot be used, due to their potential pathogenicity and pyrogenicity. The source of ADI can be from, but not limited to, different microorganisms, e.g. *Mycoplasma* (e.g. *Mycoplasma arginini*, *Mycoplasma arthritidis*, *Mycoplasma hominis*), *Lactococcus* (e.g. *Lactococcus lactis*), *Pseudomonas* (e.g. *Pseudomonas plecoglossicida*, *Pseudomonas putida*, *Pseudomonas aeruginosa*), *Streptococcus* (e.g. *Streptococcus pyogenes*, *Streptococcus pneumoniae*), *Escherichia*, *Mycobacterium* (e.g. *Mycobacterium tuberculosis*) and *Bacillus* (e.g. *Bacillus licheniformis*, *Bacillus cereus*). It is preferred that ADI is cloned from *Mycoplasma arginini*, *Lactococcus lactis*, *Bacillus licheniformis*, *Bacillus cereus*, thermophilic *Aspergillus fumigatus* or any combination thereof. Their amino acid sequences with SEQ ID (SEQ ID NO: 23-35) and the sequence alignment for some of the amino acid sequences in FIG. 2 are disclosed herein and also in the literatures [Das et al., *Structure*. 12:657-667 (2004); Wang et al., *Bioconjug. Chem.* 17:1447-1459 (2006); Ni et al., *Appl. Microbiol. Biotechnol.* 90:193-201 (2011); El-Sayed et al., *Biotechnol. Prog.* 31(2):396-405 (2015)], where the disclosure of the literatures are incorporated herein by reference in their entirety.

The design and amino acid sequence for (A) native *Mycoplasma arginini* ADI protein (SEQ ID NO: 23), (B) different AAD fusion proteins originated from the *Mycoplasma arginini* ADI (SEQ ID NO: 36-40) and (C) AAD fusion protein originated from the *Bacillus cereus* ADI (SEQ ID NO: 41) are shown in FIG. 3. Different AAD fusion proteins are successfully constructed. A linker is inserted between the albumin-binding protein and ADI in the AAD fusion protein in these embodiments.

On the other hand, a novel AAD fusion protein is also created by the use of intein-fusion proteins and expressed protein ligation (FIG. 4). The novel AAD fusion protein can be formed (1) by reacting the ADI having a N-terminal cysteine residue with a reactive thioester at C-terminus of the ABD, or (2) by reacting the ABD having a N-terminal cysteine residue with a reactive thioester at C-terminus of the ADI so that the ADI and the ABD are linked by a covalent bond. In FIG. 4E, ADI with N-terminal cysteine residue reacts with reactive thioester at the C-terminus of ABD. The thioester tag at the C-terminus of ABD, and an α -cysteine at the N-terminus of ADI are required to facilitate protein ligation. These fragments are produced using a pTWIN1 vector (New England Biolabs) according to the manufacturer's manual. In particular, the gene coding for the

ABD-Intein-CBD fusion protein is synthesized and it is cloned into the vector under the control of T7 promoter for expression in *E. coli* (FIG. 4C). The ABD-Intein-CBD fusion protein produced binds to chitin in a column. The amino acid sequence of ABD-Intein-CBD (SEQ ID NO: 42) is shown in FIG. 4A. After thiol-inducible cleavage and elution from the column, the ABD with reactive thioester at its C-terminus is obtained (FIG. 4C). On the other hand, the gene coding for the CBD-Intein-ADI fusion protein is synthesized and cloned into the vector under the control of the T7 promoter for expression in *E. coli* (FIG. 4D). The CBD-Intein-ADI fusion protein produced binds to chitin in a column. The amino acid sequence of the CBD-Intein-ADI (SEQ ID NO: 43) is shown in FIG. 4B. After cleavage at pH 7 and 25° C., and elution from the column, the ADI with α -cysteine at its N-terminus is obtained (FIG. 4D). Finally, the AAD fusion protein is produced by the protein ligation reaction as shown in FIG. 4E.

Importantly, AAD fusion proteins can be produced and purified in a convenient manner. For example, an AAD fusion protein is successfully expressed and purified from *E. coli* both in soluble fraction and insoluble fraction, and this result is shown in FIG. 8. Furthermore, FIG. 8 shows the purified AAD fusion protein analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The size of the purified AAD fusion protein is determined as 52.8 kDa. The isoelectric point of wild-type ADI is 4.7 and the purified AAD fusion protein is about 5.2 (as shown in FIG. 9).

The pharmaceutical composition of the present invention contains AAD fusion protein with high activity for depleting arginine in tumor cells for cancer treatment. The specific activity of the purified AAD fusion protein is found to be similar to that of the wild-type ADI. The inhibitory effect of the AAD fusion protein on a panel of human cancer cell lines is therefore examined using MTT assay. Different cancer cells are seeded in 96-well plates and allowed to grow for 24 h for acclimatization. The cells are then incubated with 0-10 μ g/ml of AAD for 72 hours. IC₅₀ is the half maximal inhibitory concentration, that is, it represents the concentration of AAD fusion protein that is required for 50% inhibition of a cancer cell line. The IC₅₀ is a measure of the effectiveness of a drug. The IC₅₀ of AAD fusion protein (amino acid sequence is shown in SEQ ID NO: 40, FIG. 3E) for different cancer cell lines (human melanoma, A375 & SK-mel-28; human colorectal cancer, HCT116; human pancreatic cancer, Panc1 & Mia-paca-2; human liver cancer, Sk-hep1; human cervical cancer, C-33A; human breast cancer, MDA-MB-231; human prostate cancer, 22Rv1; and human leukemia, Jurkat) is shown in TABLE 1.

TABLE 1

| Cancer cell line (argininosuccinate synthetase-negative, ASS ^{-ve}) | IC ₅₀ of AAD (μ g/ml) |
|--|--|
| A375 (human melanoma) | 0.104 |
| SK-mel-28 (human melanoma) | 1.92 |
| Panc1 (human pancreatic cancer) | 0.043 |
| Mia-paca-2 (human pancreatic cancer) | 0.010 |
| Sk-hep1 (human liver cancer) | >10 |
| C-33A (human cervical cancer) | 0.058 |
| HCT116 (human colorectal cancer) | 0.211 |
| MDA-MB-231 (human breast cancer) | 0.173 |
| 22Rv1 (human prostate cancer) | 0.235 |
| Jurkat (human leukemia) | 0.379 |

For the albumin binding study, the present invention has demonstrated successfully that the engineered AAD fusion

protein can bind to human serum albumin (HSA) or other animal serum albumin similar to HSA. FIG. 10 shows that the AAD fusion protein (amino acid sequence is shown in SEQ ID NO: 40, FIG. 3E) binds to HSA readily. AAD is incubated with the indicated molar ratio of HSA for 60 min at room temperature as shown in FIG. 10, lanes 1-4. After incubation, samples are subject to native polyacrylamide gel (10%). Lane 2 shows a partial binding of HSA in a 1:1 ratio while a complete binding of HSA is observed in a 1:5 (HSA:AAD) ratio in lane 3. At a mole ratio of 1:5 or 1:1 (i.e. lane 3 or 2 in FIG. 10), the formation of the HSA-AAD complex forms (~100-110 kDa) according to the construct of FIG. 1 using the linker molecule design. A band with molecular weight ~100 kDa representing the AAD-HSA complexes (indicated with an open arrowhead) is clearly observed in lane 3. It is expected that the circulating half-life of AAD fusion protein in the blood is increased by the non-covalent HSA-AAD complex formation. Therefore, a long-lasting version of AAD fusion protein has been successfully created.

No commercial products show high efficacy when compared to the AAD fusion protein-containing pharmaceutical composition prepared in this invention. For uses in cancer treatment, the AAD fusion protein-containing pharmaceutical composition of the present invention serves as an anti-cancer agent to deplete the arginine in tumor tissues. AAD fusion protein is a good candidate to be used in combination with other molecular targeting or cytotoxic agents.

The AAD fusion protein in the present invention can also be used as a component in a testing kit for detection of arginine in different samples. AAD has a small K_m value; it indicates the high affinity for the substrate (arginine). Therefore, the rate will approach the maximum reaction rate more quickly. The AAD fusion protein can be used to testing arginine level (1) in cancer patients, (2) in a food sample, and (3) in cell culture.

EXAMPLES

The following examples are provided by way of describing specific embodiments of this invention without intending to limit the scope of this invention in any way.

Several of the Examples below relate to methods of making an albumin-binding arginine deiminase fusion protein. Various techniques can be used including cloning and intein-mediated protein ligation. As used herein, the term "cloning" is broadly used and comprises constructing a fusion gene coding for the albumin-binding arginine deiminase fusion protein, inserting the fusion gene into a vector, inserting the vector into a host organism and expressing a protein that includes an albumin-binding arginine deiminase fusion protein. Numerous variants on this technique can be performed and still fall within the cloning contemplated by the present invention.

Example 1

Construction of the Gene Coding for Albumin-Binding Domain/Peptide/Protein (ABD)

The gene coding for ABD is constructed by two rounds of PCR. In the first round, the PCR reaction mixture (total volume of 25 μ l) contains the following materials:

- 1 \times iProof PCR buffer (Bio-Rad)
- 50 μ M dNTP mixture
- 0.5 unit of iProof DNA Polymerase (Bio-Rad)
- 10 nM of each of the following oligos

ABD-F1 forward primer (SEQ ID NO: 01):
5'-CATGATGCGAATTCCTTAGCTGAAGCTAAAGTCTT
AGCTAACAGAGAACT-3'

ABD-R2 reverse primer (SEQ ID NO: 02):
5'-TAGTCACTTACTCCATATTTGTCAAGTTCTCTGTT
AGCTAAGACTTTAGC-3'

ABD-F3 forward primer (SEQ ID NO: 03):
5'-GAACTTGACAAATATGGAGTAAGTACTATTACAA
GAACCTAATCAACAA-3'

ABD-R4 reverse primer (SEQ ID NO: 04):
5'-TACACCTTCAACAGTTTTGGCATTGTTGATTAGGT
TCTTGTAATAGTCAC-3'

ABD-F5 forward primer (SEQ ID NO: 05):
5'-GCCAAAACCTGTTGAAGGTGTAAGCACTGATAGA
TGAAATTTTAGCTGC-3'

ABD-R6 reverse primer (SEQ ID NO: 06):
5'-AGCTACGATAAGCTTAAGGTAATGCAGCTAAAATT
TCATCTATCAGTG-3'

The following PCR program is used:

98° C. 30 s; 20 cycles of {98° C. 10 s, 50° C. 20 s, 72° C. 20 s}

In the second round of PCR, the PCR mixture (total volume of 50 μ l) contains the following materials:

- 1 \times iProof PCR buffer (Bio-Rad);
- 50 μ M dNTP mixture;
- 1 μ l of PCR reactant as DNA template from the first round;
- 1 unit of iProof DNA Polymerase (Bio-Rad);
- 200 nM of each of the following oligos:

ABD-F7 forward primer (SEQ ID NO: 07):
5'-CATGATGCGAATTCCTTAGCTGAAGCTAAAGTCTT
AGCTAACAGAGAACT-3'

ABD-R8 reverse primer (SEQ ID NO: 08):
5'-AGCTACGATAAGCTTAAGGTAATGCAGCTAAAATT
TCATCTATCAGTG-3'

The following PCR program is used:

98° C. 30 s; 35 cycles of {98° C. 10 s, 60° C. 20 s, 72° C. 20 s}; 72° C. 5 min

A PCR product containing the DNA sequence of ABD (169 bp) is obtained and purified by Qiagen DNA Gel Extraction Kit for cloning purpose.

Example 2A

Construction of the Fusion Gene Coding for the AAD Fusion Protein

In the first PCR, the PCR mixture (total volume of 50 μ l) contains the following materials:

- 1 \times iProof PCR buffer (Bio-Rad);
- 50 μ M dNTP mixture;
- 25 ng of Mycoplasma arginini genomic DNA;
- 1 unit of iProof DNA Polymerase (Bio-Rad);
- 200 nM of each of the following oligos:

ADINde-F forward primer (SEQ ID NO: 09):
5'-ATCGATCGATGTCTGTATTTGACAGTAAATTTAAG
G-3'

ADIhis-R reverse primer (SEQ ID NO: 10):
5'-AGCTAAGGAATTCGCATCATGATGGTGGTGGTGG
CTACCCCACTTAAC-3'

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The following PCR program is used:

98° C. 1 min; 35 cycles of {98° C. 10 s, 50° C. 20 s, 72° C. 40 s}; 72° C. 5 min

A PCR product of 1280 bp long is obtained and purified by Qiagen DNA Gel Extraction Kit. After that, the second PCR is performed. The PCR mixture (total volume of 50 µl) contains the following materials:

1×iProof PCR buffer (Bio-Rad);
50 µM dNTP mixture;
10 ng of the 1280 bp PCR product;
10 ng of the 169 bp PCR product;
1 unit of iProof DNA Polymerase (Bio-Rad);
200 nM of each of the following oligos:

ADINde-F forward primer (SEQ ID NO: 11):
5'-ATCGATCGATGCTGTATTTGACAGTAAATTTAAAG
G-3'

ABD-R10 reverse primer (SEQ ID NO: 12):
5'-AGCTACGATAAGCTTAAGGTAATGCAGCTAAAATTT
CATCTATCAGTG-3'

The following PCR program is used:

98° C. 1 min; 35 cycles of {98° C. 10 s, 50° C. 20 s, 72° C. 45 s}; 72° C. 5 min

A PCR product of 1428 bp is obtained and purified by Qiagen DNA Gel Extraction Kit. Then it is digested with restriction enzymes NdeI and HindIII, and ligated to plasmid pREST A (Invitrogen) that is predigested with the same enzymes. The ligation product is then transformed into E. coli BL21 (DE3) cells. The sequence of the constructed fusion gene is confirmed by DNA sequencing.

Example 2B

Cloning of His-ABD-PolyN-ADI

The construction of His-ABD-PolyN-ADI (SEQ ID NO: 40, in FIG. 3E) is done by two steps of overlapping PCR, the PCR fragment obtained from the last step is inserted into the vector pET3a between the NdeI and BamHI sites. The gene map, nucleotide sequence and amino acid sequence of His-ABD-PolyN-ADI are shown in FIG. 6.

Primers involved in construction of His-ABD-PolyN-ADI:

hisABDNde-F forward primer (SEQ ID NO: 13):
5'-GGAGATATACATATGCATCATCACCATCACCATGATGAAG
CCGTGGATG-3'

ABDnn-R1 reverse primer (SEQ ID NO: 14):
5'-TTGTTATTATTGTTGTTACTACCCGAAGGTAATGCAGCTA
AATTTTCATC-3'

ABDn-R2 reverse primer (SEQ ID NO: 15):
5'-AGAACCGCCGCTACCATTGTTATTATTGTTGTTACTACCC
GA-3'

ADln-F forward primer (SEQ ID NO: 16):
5'-AATAATAACAATGGTAGCGCGGTTCTGTATTTGACAGTA
AATTTAAAGG-3'

ADIBam-R reverse primer (SEQ ID NO: 17):
5'-TAGATCAATGGATCCCTAACCTTAACATCTTTACGTGAT
AAAG-3'

In the first round of PCR, 50 µl of reaction volume containing the known concentration of components are prepared in two PCR tubes. In each of the tubes, dNTP, iProof buffer (BIO-RAD), iProof DNA polymerase (BIO-RAD), primers and DNA template are mixed and added up to 50 µl by ddH₂O. The DNA template used in the reaction

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is a pET3a vector containing the gene of ADI from Mycoplasma arginini with a removal of an internal NdeI site mutation without altering the protein sequence of the ADI gene.

The two reaction tubes contain the primer mixtures of (A) 10 pmol hisABDNde-F (SEQ ID NO: 13), 0.5 pmol ABDnn-R1 (SEQ ID NO: 14) and 10 pmol ABDn-R2 (SEQ ID NO: 15); and (B) 10 pmol ADIn-F (SEQ ID NO: 16) and 10 pmol ADIBam-R (SEQ ID NO: 17), respectively.

The PCR program is set according to the recommended steps in the manual with an annealing and extension temperature (time) at 50° C. (20 s) and 72° C. (40 s), respectively. The two products generated by PCR with the size of 237 bp and 1278 bp. The products are extracted and applied as template for the next round of PCR.

In the second overlapping step, the reaction mixture is prepared in a similar way to the first round except the template used was the mixture of 1 pmol of the 237 bp PCR product and 1 pmol of the 1278 bp PCR product from the first round PCR. Primers used are changed to 10 pmol hisABDNde-F (SEQ ID NO: 13) and 10 pmol ADIBam-R (SEQ ID NO: 17).

The annealing and extension temperature (time) are 50° C. (20 s) and 72° C. (60 s), respectively. A PCR product with the size of 1484 bp is generated from the reaction. The PCR product is purified and digested with NdeI and BamHI and then ligated into the pre-digested pET3a plasmid. The ligated product is then transformed into E. coli BL21 (DE3) for the production of recombinant protein.

Example 2C

Cloning of His-ABD-PolyN-bcADI

The construction of His-ABD-PolyN-bcADI (SEQ ID NO: 41, in FIG. 3F) is done by two steps of overlapping PCR, the PCR fragment obtained from the last step is inserted into the vector pET3a between the NdeI and BamHI sites. The gene map, nucleotide sequence and amino acid sequence of His-ABD-PolyN-bcADI are shown in FIG. 7.

Primers involved in construction of His-ABD-PolyN-bcADI:

hisABDNde-F2 forward primer (SEQ ID NO: 18):
5'-GGAGATATACATATGCATCATCACCATCACCATGATGAAG
CGTGGATG-3'

bcABDnn-R1 reverse primer (SEQ ID NO: 19):
5'-TTGTTATTATTGTTGTTACTACCCGAAGGTAATGCAGCTAA
AATTTTCATC-3'

bcABDn-R2 reverse primer (SEQ ID NO: 20):
5'-TTTACCGCCGCTACCATTGTTATTATTGTTGTTACTACCCG
A-3'

bcADln-F forward primer (SEQ ID NO: 21):
5'-AATAATAACAATGGTAGCGCGGTAACATCCGATACATGT
TACTTCAGA-3'

bcADIBam-R reverse primer (SEQ ID NO: 22):
5'-TAGATCAATGGATCCCTAACCTTAACATCTTTACGAACAATTGGCA
TAC-3'

In the first round of PCR, 50 µl of reaction volume containing the known concentration of components are prepared in two PCR tubes. In each of the tubes, dNTP, iProof buffer (BIO-RAD), iProof DNA polymerase (BIO-RAD), primers and DNA template are mixed and added up to 50 µl by ddH₂O. The DNA template used in the reaction is a pET3a vector containing the gene of ADI from Bacillus

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cerus with a removal of an internal NdeI site mutation without altering the protein sequence of the ADI gene.

The two reaction tubes contain the primer mixtures of (A) 10 pmol hisABDNde-F2 (SEQ ID NO: 18), 0.5 pmol bcABDnn-R1 (SEQ ID NO: 19) and 10 pmol bcABDn-R2 (SEQ ID NO: 20); and (B) 10 pmol bcADIn-F (SEQ ID NO: 21) and 10 pmol bcADIBam-R (SEQ ID NO: 22), respectively. The PCR program is set according to the recommended steps in the manual with an annealing and extension temperature (time) at 50° C. (20 s) and 72° C. (40 s), respectively. The two products are generated by PCR with the size of 237 bp and 1250 bp. The products are extracted and applied as template for the next round of PCR.

In the second overlapping step, the reaction mixture is prepared in a similar way to the first round except the template used is the mixture of 1 pmol of the 237 bp PCR product and 1 pmol of the 1250 bp PCR product from the first round PCR. Primers used are changed to 10 pmol hisABDNde-F2 (SEQ ID NO: 18) and 10 pmol bcADIBam-R (SEQ ID NO: 22).

The annealing and extension temperature (time) are 50° C. (20 s) and 72° C. (60 s), respectively. A PCR product with the size of 1512 bp is generated from the reaction. The PCR product is purified and digested with NdeI and BamHI and then ligated into the pre-digested pET3a plasmid. The ligated product is then transformed into *E. coli* BL21 (DE3) for the production of recombinant protein.

Example 3

Expression and Purification of the AAD Fusion Protein

(3a) Expression of the AAD Fusion Protein by Shake-Flask Method

For preparing the seed culture, the strain *E. coli* BL21 (DE3) carrying the plasmid encoding the AAD fusion protein (FIG. 5) is cultured in 5 ml of 2×TY medium, 30° C., 250 rpm, overnight. The overnight seed culture (2.5 ml) is added to 250 ml of 2×TY, 37° C., 250 rpm, 2.5 h (until OD₆₀₀ ≈ 0.6-0.7). When the OD₆₀₀ reached, IPTG is added to the culture (0.2 mM final concentration). The growth is continued for 22 more hours at 20° C. and then the cells are collected by centrifugation. The cell pellet is resuspended in 25 ml of 10 mM sodium phosphate buffer, pH 7.4. The cells are lysed by sonication.

(3b) Expression of the AAD Fusion Protein by Fermentation Method

For the seed culture, the aliquot of bacteria stock is inoculated into 50 ml of seeding medium (containing 1.5 g of yeast extract and 0.25 g of NaCl) with ampicillin and grown at 30° C. for 16 hr with continuous shaking at 250 rpm. The seed culture is then added to 1.25 L of medium (pH 7.4, containing yeast extract, tryptone, Na₂HPO₄, KH₂PO₄, (NH₄)₂SO₄, glycerol, glucose, MgSO₄·7H₂O, Thiamine-HCl and CaCl₂) supplement with trace element in the BIOSTAT fermentor system and grown at 28° C. Until the OD₆₀₀ of the culture reaches ~20, IPTG is added to a final concentration of 0.2 mM. The culture is further incubated for 16 hr. During incubation, 500 ml of feeding medium (pH 7.4, containing yeast extract, tryptone, NH₄Cl, (NH₄)₂SO₄, glycerol and MgSO₄·7H₂O) supplement with trace element were applied at 0.5 ml/min. Aeration is regulated in order to maintain 20% of air saturation by varying the speed of stirring from 500 rpm to 2000 rpm. The bacteria are harvested by centrifugation. The cells are lysed by sonication or high pressure homogenizer.

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(3c) Purification of the AAD Fusion Protein

The soluble portion is collected after centrifugation. The fusion protein (containing a His tag) is then purified by nickel affinity chromatography. TABLE 2 shows that cultivation temperature is an important factor in affecting the solubility of AAD fusion protein (amino acid sequence is shown in SEQ ID NO: 40, FIG. 3E) obtained from the expression host.

For isolating the soluble fraction of AAD fusion protein, the cell pellet is resuspended in 25 ml of 10 mM sodium phosphate buffer, pH 7.4. The cells are lysed by sonication or high pressure homogenizer. The soluble portion is collected after centrifugation. The AAD fusion protein (contains a His tag or without His tag) is then purified by nickel affinity chromatography and/or ion-exchange columns.

For isolating the insoluble fraction of AAD fusion protein, the cell pellet is resuspended in 25 ml of 20 mM Tris-HCl, pH 7.4, 1% TRITON X-100. The cells are lysed by sonication. The insoluble portion (inclusion bodies) is collected by centrifugation. The protein is unfolded by resuspending in 10 ml of 20 mM Tris-HCl, pH 7.4, 6 M Guanidine HCl, and vortexed until it becomes soluble. The protein is refolded by adding the unfolded protein solution drop by drop into a fast stiffing solution of 100 ml of 20 mM Sodium phosphate buffer, pH 7.4. The insoluble materials are removed by centrifugation. Salting out of the protein is performed by adding solid ammonium sulphate powder into the supernatant to achieve 70% saturation. The insoluble portion is collected by centrifugation and it is resuspended in 10 ml of 20 mM sodium phosphate buffer. The AAD fusion protein (contains a His tag or without His tag) is then purified by nickel affinity chromatography and/or ion-exchange columns.

TABLE 2

| | AAD | | |
|--|-------------|-------------|--------------------|
| | 1 | 2 | 3 |
| Cultivation temperature (° C.) | 30 | 20 | 37 |
| Yield (mg)/250 ml culture | ~0.66 | ~12.0 | ~7.0 |
| solubility | 50% soluble | 90% soluble | 90% inclusion body |
| IC ₅₀ (µg/ml) on A375 cells | 0.10 | 0.68 | 0.23 |

“90% inclusion body” means 90% of the AAD fusion protein produced in the bacterial cells are not soluble.

The yield and the enzyme activity of AAD fusion protein from shake-flask method and fermentation method are shown in TABLE 3.

TABLE 3

| | AAD Yield (mg/L) | Activity (U/mg) |
|---------------------|------------------|-----------------|
| Shake-flask method | ~10 | ~9 |
| Fermentation method | ~42 | ~19 |

Example 4

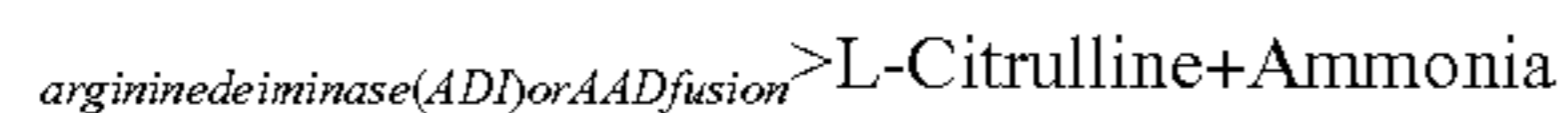
Enzyme Activity Assay and Enzyme Kinetics for AAD Fusion Protein

To determine the enzyme activity for wild-type ADI and AAD fusion protein in the present invention, the diacetyl

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monoxime (DAM)-thiosemicarbazide (TSC) assay for citrulline detection is used. The reaction is shown below.

L-Arginine



This assay is run by adding sample to a color reagent, which is made by mixing acidic ferric chloride solution with DAM-TSC solution. Briefly, enzyme is incubated with 20 mM arginine, 10 mM sodium phosphate pH 7.4 for 5 min at 37° C. The reaction mixture is heated at 100° C. for 5 min to develop the color and read at 540 nm (light path=1 cm). A standard curve is constructed using various concentrations of citrulline. One unit of the ADI native enzyme is the amount of enzyme activity that converts 1 μmol of arginine to 1 μmol of citrulline per minute at 37° C. under the assay conditions. The specific activities of wild-type ADI, pegylated ADI (Ensor et al., Cancer Res. 62:5443-5450, 2002) and AAD fusion protein in the present invention are about 20, 15 and 19 U/mg (at pH 7.4, physiological pH), respectively. The specific activities for wild-type ADI and AAD fusion protein at different pH values (in a range from pH 5.5 to 9.5) are also determined, and the optimum pH is at 6.5. Therefore, the results indicate that AAD fusion protein depletes arginine efficiently which is even better than pegylated ADI, as the fusion with albumin-binding protein does not affect enzyme activity of ADI.

The Michaelis constant K_m is the substrate concentration at which the reaction rate is at half-maximum, and is an inverse measure of the substrate's affinity for the enzyme. A small K_m indicates high affinity for the substrate, and it means that the rate will approach the maximum reaction rate more quickly. For determination of the enzyme kinetics or K_m value, the activity of wild-type ADI and AAD fusion protein are measured under different concentration of substrate arginine (2000 μM, 1000 μM, 500 μM, 250 μM, 125 μM, 62.5 μM) at pH 7.4. The measured K_m values of the AAD fusion protein shown in FIG. 3E (SEQ ID NO: 40, ADI protein is originated from Mycoplasma arginini) and AAD fusion protein shown in FIG. 3F (SEQ ID NO: 41, ADI protein is originated from Bacillus cereus) are 0.0041 mM and 0.132 mM respectively. The results suggest that the fusion to ABD did not affect the binding affinity of the different AAD fusion proteins to arginine.

Example 5

Cell Proliferation Assay and In Vitro Efficacy of AAD Fusion Protein on Cancer Cell Lines

Culture medium DMEM is used to grow the human melanoma A375 & SK-mel-28 and pancreatic cancer Panc1 & Mia-paca-2 cell lines. The EMEM medium is used to culture the human liver cancer SK-hep, cervical cancer C-33A and colorectal cancer HCT116 cell lines. The RPMI-1640 medium is used to culture the human breast cancer MDA-MB-231, prostate cancer 22Rv1 and leukemia Jurkat cell lines. Cancer cells ($2-5 \times 10^3$) in 100 μl culture medium are seeded to the wells of 96-well plates and incubated for 24 hours. The culture medium is replaced with medium containing 0-10 μg/ml of AAD fusion protein. The plates are incubated for an additional 3 days at 37° C. in an atmosphere of 95% air/5% CO₂. MTT assay is performed to estimate the number of viable cells in the culture according to manufacturer's instructions. The amount of enzyme needed to achieve 50% inhibition of cell growth is defined as IC₅₀.

As shown in TABLE 1, the results indicate that AAD fusion protein depletes arginine efficiently and inhibits the

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growth of various types of human cancer cell lines in in vitro tissue culture studies. For example, human melanoma, human breast cancer, human colorectal cancer, human pancreatic cancer, human liver cancer, human prostate cancer, human leukemia and human cervical cancer, all have low values of IC₅₀ (see TABLE 1), as these cancer types are all inhibited by AAD fusion protein readily. As determined from the in vitro_data, AAD fusion protein would inhibit all cancer types that are arginine-dependent, for example, the argininosuccinate synthetase-negative (ASS^{-ve}) cancers.

Example 6

In Vivo Half-Life Determination of AAD Fusion Protein

Balb/c mice (5-7 weeks) are used in this study and they are allowed to acclimatize for a week before the experiment. Mice (n=3) are separated into four groups and injected with 0, 100, 500 or 1000 μg of AAD fusion protein (SEQ ID NO: 40, FIG. 3E) in 100 μl PBS intraperitoneally, respectively. Blood of each mouse is collected at 0 h and Day 1-7. Sera are obtained after centrifugation. The sera are then deproteinised and analyzed by amino acid analyzer for arginine. As shown in FIG. 11, AAD fusion protein (SEQ ID NO: 40, FIG. 3E), even at the lowest dosage of 100 μg, depletes plasma arginine efficiently at Day 1, 3 and 5, suggesting that AAD can deplete arginine in vivo efficiently for at least 5 days. The arginine level returns to normal gradually at Day 6 and Day 7 in all treatment groups.

Example 7

In Vivo Efficacy of Weekly Administration of AAD Fusion Protein on HCT 116 Colorectal Cancer Cell Xenografts

Nude balb/c mice (5-7 weeks) are used in this study and they are allowed to acclimatize for a week before the experiment. Mice are inoculated subcutaneously with 2×10^6 cancer cells in 100 μl of fresh culture medium. Ten days later, the mice are randomly separated into control and treatment group. Control group receives 100 μl PBS and treatment group receives 100 μl AAD fusion protein (amino acid sequence is shown in SEQ ID NO: 40, FIG. 3E) intraperitoneally weekly. Tumor size is measured by caliper and tumor volume is calculated using formula: (length × width²)/2. Blood draw are obtained at Day 5 after each treatment for plasma measurement of arginine.

Example 8

Comparison in In Vivo Efficacy Between Weekly and Biweekly Based Administration of AAD Fusion Protein on HCT 116 Colorectal Cancer Cell Xenograft

Nude balb/c mice (4-6 weeks) are used and they are allowed to acclimatize for a week before the treatment. Mice are inoculated subcutaneously with 2×10^6 cancer cells in 100 μl PBS. Ten days later, the mice are randomly separated into control and treatment groups. Control group receives 100 μl PBS and treatment group receives 200 μg AAD fusion protein (amino acid sequence is shown in SEQ ID NO: 40, FIG. 3E) in 100 μl PBS intraperitoneally weekly or biweekly. Tumor size is measured by caliper and tumor volume is calculated using formula: (length × width²)/2. In

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the weekly treatment group, blood draw are obtained at Day 0 and Day 5 of each treatment for plasma measurement of arginine. In the biweekly treatment group, blood draw are obtained just before the first treatment every week for plasma measurement of arginine. After the mice are sacrificed by end of the time course, the tumors are excised and weighed. The plasma arginine levels over the time course in the weekly treatment group are measured.

At Day 5, Day 12 and Day 19, the plasma arginine level is significantly decreased respectively after the weekly administration of the AAD fusion protein. Comparing the plasma arginine levels between Day 0, Day 7 and Day 14, the levels at Day 7 and Day 14 are relatively lower than that at Day 0, revealing that weekly administration of AAD fusion protein can decrease the overall plasma arginine levels over the time course. The tumor size in the weekly treatment group is lower than that in control at the end of the time course, revealing that the weekly administration of AAD fusion protein can reduce the tumor size of the xenograft in the disease mouse model. The difference in the tumor size between control and weekly treatment group is about 20% at Day 30.

The reduction in plasma arginine level is more significant in the biweekly treatment group than that in the weekly treatment group. The biweekly administration of AAD fusion protein does not affect the body weight of the mice over a 35-day time course as compared to the control. In conclusion, biweekly administration of AAD fusion protein in 400 μg per week (16 mg/kg/week/mouse) is more effective in completely suppressing plasma arginine level than weekly administration. Using the conversion of animal doses to human equivalent doses (HED) based on body surface area mentioned in "Guidance for Industry and Reviewers—Estimating the safe starting dose in clinical trials for therapeutics in adult healthy volunteers (2002)", human dose of the AAD fusion protein is about 1.3 mg/kg/week.

Example 9

In Vivo Efficacy of AAD Fusion Protein on Mia-Paca-2 Pancreatic Cancer Cell Xenografts

Nude balb/c mice (4-6 weeks) are used and they are allowed to acclimatize for a week before the treatment. Mice are inoculated subcutaneously with 2×10^6 cancer cells in 100 μl of 1:1 PBS:Matrigel. Matrigel is used to augment the growth of tumors. Two weeks later, the mice are randomly separated into four groups of 4 animals in each group. Mice are intraperitoneally administered with PBS (control), AAD (5 U; twice a week), Gemcitabine, Gem (100 mg/kg; once a week) or AAD+Gem (a combination of both AAD and Gem) in 200 μl PBS. Tumor size is measured by caliper and tumor volume is calculated using formula: $(\text{length} \times \text{width}^2)/2$. Body weight is measured every week. After the mice are sacrificed by end of the time course, the tumors are excised and weighed. The tumor size in all treatment groups is significantly lower than that in control at the end of the time course, revealing that the administration of AAD fusion protein can reduce the tumor size of the xenograft in the disease mouse model (shown in FIG. 12A, 12B, 12C). The difference in the tumor size between control and AAD fusion protein-treatment group is about 60% at Day 28. The treatment groups do not affect the body weight of the mice over a 28-day time course as compared to the control (shown in FIG. 12D).

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Example 10

In Vivo Efficacy of AAD Fusion Protein on 22Rv1 Prostate Cancer Cell Xenografts

Nude balb/c mice (4-6 weeks) are used and they are allowed to acclimatize for a week before the treatment. Mice are inoculated subcutaneously with 3×10^6 cancer cells in 100 μl PBS. Two weeks later, the mice are randomly separated into four groups of 5 animals in each group. Mice are intraperitoneally administered with PBS (control), AAD (5 U; twice a week), Docetaxel, DTX (10 mg/kg; once a week) or AAD+DTX (a combination of both AAD and DTX) in 200 μl PBS. Tumor size is measured by caliper and tumor volume is calculated using formula: $(\text{length} \times \text{width}^2)/2$. Body weight is measured every week. After the mice are sacrificed by end of the time course, the tumors are excised and weighed.

In FIG. 13A, the tumor size in all treatment groups is significantly lower than that in control at the end of the time course, revealing that the administration of AAD fusion protein or docetaxel can reduce the tumor size of the xenograft in the disease mouse model. The difference in the tumor size between control and AAD fusion protein- or DTX-treatment groups are about 55% and 54% at Day 22, respectively. Besides, the combination of AAD fusion protein and DTX-treatment group has a synergistic effect on tumor growth inhibition. Tumor growth of the combination treatment group is inhibited by about 94% when compared to that of the control group. FIG. 13B shows the appearance of the tumor tissues excised from xenografts at Day 22. AAD treatment does not affect the body weight of the mice over a 28-day time course.

Example 11

In Vivo Efficacy of AAD Fusion Protein on Jurkat Leukemia Xenografts

Nude balb/c mice (4-6 weeks) are used and they are allowed to acclimatize for a week before the treatment. Mice are inoculated subcutaneously with 5×10^6 cancer cells in 100 μl of 1:1 PBS:Matrigel. Matrigel is used to augment the growth of tumors. Since the take rate of this cancer cell line is relatively low, when certain tumor xenograft reaches a suitable size, the tumor is excised and cut into various pieces ($\sim 10 \text{ mm}^3$), which are further transplanted to the back of another group of mice subcutaneously. Ten days later, the mice are randomly separated into two groups of 8 animals in each group. Mice are intraperitoneally administered with PBS (control) or AAD (5 U; twice a week) in 200 μl PBS. Tumor size is measured by caliper and tumor volume is calculated using the following formula: $(\text{length} \times \text{width}^2)/2$. Body weight is measured every week. At Day 28, the AAD fusion protein significantly inhibits the tumor growth when comparing to the control group (FIG. 14). The difference in tumor size between AAD fusion protein-treated and control groups becomes more significant at Day 30.

Example 12

Using AAD Fusion Protein in a Testing Kit

AAD fusion protein of the present invention has a small K_m , indicating the high affinity for the substrate (arginine). Therefore, the rate approaches the maximum reaction rate more quickly. To determine the concentration of arginine in

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a sample, AAD fusion protein in the present invention, the diacetyl monoxime (DAM)-thiosemicarbazide (TSC) assay for citrulline detection is used. The reaction is as follows: L-Arginine (of unknown concentration) is converted by AAD fusion protein to form L-Citrulline and Ammonia.

This assay is run by adding sample to a color reagent, which is made by mixing acidic ferric chloride solution with DAM-TSC solution. Briefly, arginine (in the sample, of unknown concentration) is incubated with 2-20 ng of the AAD fusion protein, and 10 mM sodium phosphate pH 7.4 for 5 mM at 37° C. The reaction mixture is heated at 100° C. for 5 mM to develop into pink color and read at 540 nm (light path=1 cm). A standard curve is constructed using various concentrations of citrulline. One unit of the AAD is the amount of enzyme activity that converts 1 μmol of arginine to 1 μmol of citrulline per minute at 37° C. under the assay conditions. This testing kit is very useful for the following applications:

Example 12A

Testing Arginine Level in Cancer Patients

For a cancer patient (human or animal), after treated with an arginine depleting drug, the arginine level (concentration) in blood should be very low or zero. A blood sample can be taken from the patient and then tested with this new testing kit based on the AAD fusion protein. After comparing to the standard curve (generated from standard solution plotted from known arginine concentration solutions), the exact arginine concentration of this blood sample of the patient can be measured. Therefore, this data helps monitor the progress of an arginine depletion treatment method. If the

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arginine level is too high (e.g. 200 micro-molar), more arginine depleting drug can be used on the patient to further keep the arginine at a lower or undetectable level so that the tumor can be inhibited by the systemic arginine depletion in the cancer patient.

Example 12B

Testing Arginine Level in a Food Sample

For industrial or food manufacturing purposes, the arginine (amino acid) level of a particular food material or intermediate during the food processing step might need to be monitored and measured. This arginine testing kit by using the AAD fusion protein of the present invention can measure the exact arginine concentration of a food sample prepared in solution form in the laboratory. This testing kit can also be used in high throughput manner and applicable for industrial scale and mass production.

Example 12C

Testing Arginine Level in Cell Culture

Nitric oxide (NO) is an important signaling molecule in cells and in the body. For many research projects on nitric oxide (NO) and cell culture studies, a lot of time a scientist would need to measure the amount or concentration of arginine (which is a substrate for making NO). This arginine testing kit by using the AAD fusion protein of the present invention can also be used for measuring the exact arginine concentration of any laboratory samples and/or analytical samples (e.g. for NO and cell culture studies).

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<220> FEATURE:

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<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: the sequence is synthesized

<400> SEQUENCE: 11

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<212> TYPE: DNA

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<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: the sequence is synthesized

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<212> TYPE: DNA

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<223> OTHER INFORMATION: the sequence is synthesized

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: the sequence is synthesized

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<220> FEATURE:
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<210> SEQ ID NO 22
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: the sequence is synthesized

<400> SEQUENCE: 22

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<210> SEQ ID NO 23
<211> LENGTH: 410
<212> TYPE: PRT
<213> ORGANISM: Mycoplasma arginini

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<400> SEQUENCE: 23

Met Ser Val Phe Asp Ser Lys Phe Lys Gly Ile His Val Tyr Ser Glu
 1 5 10 15
 Ile Gly Glu Leu Glu Ser Val Leu Val His Glu Pro Gly Arg Glu Ile
 20 25 30
 Asp Tyr Ile Thr Pro Ala Arg Leu Asp Glu Leu Leu Phe Ser Ala Ile
 35 40 45
 Leu Glu Ser His Asp Ala Arg Lys Glu His Lys Gln Phe Val Ala Glu
 50 55 60
 Leu Lys Ala Asn Asp Ile Asn Val Val Glu Leu Ile Asp Leu Val Ala
 65 70 75 80
 Glu Thr Tyr Asp Leu Ala Ser Gln Glu Ala Lys Asp Lys Leu Ile Glu
 85 90 95
 Glu Phe Leu Glu Asp Ser Glu Pro Val Leu Ser Glu Glu His Lys Val
 100 105 110
 Val Val Arg Asn Phe Leu Lys Ala Lys Lys Thr Ser Arg Glu Leu Val
 115 120 125
 Glu Ile Met Met Ala Gly Ile Thr Lys Tyr Asp Leu Gly Ile Glu Ala
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 Asp His Glu Leu Ile Val Asp Pro Met Pro Asn Leu Tyr Phe Thr Arg
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 Asp Pro Phe Ala Ser Val Gly Asn Gly Val Thr Ile His Tyr Met Arg
 165 170 175
 Tyr Lys Val Arg Gln Arg Glu Thr Leu Phe Ser Arg Phe Val Phe Ser
 180 185 190
 Asn His Pro Lys Leu Ile Asn Thr Pro Trp Tyr Tyr Asp Pro Ser Leu
 195 200 205
 Lys Leu Ser Ile Glu Gly Gly Asp Val Phe Ile Tyr Asn Asn Asp Thr
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 Leu Val Val Gly Val Ser Glu Arg Thr Asp Leu Gln Thr Val Thr Leu
 225 230 235 240
 Leu Ala Lys Asn Ile Val Ala Asn Lys Glu Cys Glu Phe Lys Arg Ile
 245 250 255
 Val Ala Ile Asn Val Pro Lys Trp Thr Asn Leu Met His Leu Asp Thr
 260 265 270
 Trp Leu Thr Met Leu Asp Lys Asp Lys Phe Leu Tyr Ser Pro Ile Ala
 275 280 285
 Asn Asp Val Phe Lys Phe Trp Asp Tyr Asp Leu Val Asn Gly Gly Ala
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 Glu Pro Gln Pro Val Glu Asn Gly Leu Pro Leu Glu Gly Leu Leu Gln
 305 310 315 320
 Ser Ile Ile Asn Lys Lys Pro Val Leu Ile Pro Ile Ala Gly Glu Gly
 325 330 335
 Ala Ser Gln Met Glu Ile Glu Arg Glu Thr His Phe Asp Gly Thr Asn
 340 345 350
 Tyr Leu Ala Ile Arg Pro Gly Val Val Ile Gly Tyr Ser Arg Asn Glu
 355 360 365
 Lys Thr Asn Ala Ala Leu Glu Ala Ala Gly Ile Lys Val Leu Pro Phe
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 Met Pro Leu Ser Arg Lys Asp Val Lys Trp
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<213> ORGANISM: Lactococcus lactis

<400> SEQUENCE: 24

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Val Leu Leu His Arg Pro Gly Ala Glu Val Glu Asn Ile Thr Pro Asp
20          25          30

Thr Met Lys Gln Leu Leu Phe Asp Asp Ile Pro Tyr Leu Lys Ile Ala
35          40          45

Gln Lys Glu His Asp Phe Phe Ala Gln Thr Leu Arg Asp Asn Gly Ala
50          55          60

Glu Thr Val Tyr Ile Glu Asn Leu Ala Thr Glu Val Phe Glu Lys Ser
65          70          75          80

Ser Glu Thr Lys Glu Glu Phe Leu Ser His Leu Leu His Glu Ala Gly
85          90          95

Tyr Arg Pro Gly Arg Thr Tyr Asp Gly Leu Thr Glu Tyr Leu Thr Ser
100         105         110

Met Pro Thr Lys Asp Met Val Glu Lys Val Tyr Ala Gly Val Arg Lys
115         120         125

Asn Glu Leu Asp Ile Lys Arg Thr Ala Leu Ser Asp Met Ala Gly Ser
130         135         140

Asp Ala Glu Asn Tyr Phe Tyr Leu Asn Pro Leu Pro Asn Ala Tyr Phe
145         150         155         160

Thr Arg Asp Pro Gln Ala Ser Met Gly Val Gly Met Thr Ile Asn Lys
165         170         175

Met Thr Phe Pro Ala Arg Gln Pro Glu Ser Leu Ile Thr Glu Tyr Val
180         185         190

Met Ala Asn His Pro Arg Phe Lys Asp Thr Pro Ile Trp Arg Asp Arg
195         200         205

Asn His Thr Thr Arg Ile Glu Gly Gly Asp Glu Leu Ile Leu Asn Lys
210         215         220

Thr Thr Val Ala Ile Gly Val Ser Glu Arg Thr Ser Ser Lys Thr Ile
225         230         235         240

Gln Asn Leu Ala Lys Glu Leu Phe Ala Asn Pro Leu Ser Thr Phe Asp
245         250         255

Thr Val Leu Ala Val Glu Ile Pro His Asn His Ala Met Met His Leu
260         265         270

Asp Thr Val Phe Thr Met Ile Asn His Asp Gln Phe Thr Val Phe Pro
275         280         285

Gly Ile Met Asp Gly Ala Gly Asn Ile Asn Val Phe Ile Leu Arg Pro
290         295         300

Gly Gln Asp Gly Glu Val Glu Ile Glu His Leu Thr Asp Leu Lys Ala
305         310         315         320

Ala Leu Lys Lys Val Leu Asn Leu Ser Glu Leu Asp Leu Ile Glu Cys
325         330         335

Gly Ala Gly Asp Pro Ile Ala Ala Pro Arg Glu Gln Trp Asn Asp Gly
340         345         350

Ser Asn Thr Leu Ala Ile Ala Pro Gly Glu Ile Val Thr Tyr Asp Arg
355         360         365

Asn Tyr Val Thr Val Glu Leu Leu Lys Glu His Gly Ile Lys Val His
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<212> TYPE: PRT

<213> ORGANISM: Bacillus cereus

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Tyr Leu Gln Gln Leu Leu Phe Asp Asp Ile Pro Tyr Leu Pro Ile Ile
35 40 45

Gln Lys Glu His Asp Tyr Phe Ala Gln Thr Leu Arg Asn Arg Gly Val
50 55 60

Glu Val Leu Tyr Leu Glu Lys Leu Ala Ala Glu Ala Leu Val Asp Lys
65 70 75 80

Lys Leu Arg Glu Glu Phe Val Asp Arg Ile Leu Lys Glu Gly Gln Ala
85 90 95

Asp Val Asn Val Ala His Gln Thr Leu Lys Glu Tyr Leu Leu Ser Phe
100 105 110

Ser Asn Glu Glu Leu Ile Gln Lys Ile Met Gly Gly Val Arg Lys Asn
115 120 125

Glu Ile Glu Thr Ser Lys Lys Thr His Leu Tyr Glu Leu Met Glu Asp
130 135 140

His Tyr Pro Phe Tyr Leu Asp Pro Met Pro Asn Leu Tyr Phe Thr Arg
145 150 155 160

Asp Pro Ala Ala Ser Val Gly Asp Gly Leu Thr Ile Asn Lys Met Arg
165 170 175

Glu Pro Ala Arg Arg Arg Glu Ser Leu Phe Met Glu Tyr Ile Ile Lys
180 185 190

Tyr His Pro Arg Phe Ala Lys His Asn Val Pro Ile Trp Leu Asp Arg
195 200 205

Asp Tyr Lys Phe Pro Ile Glu Gly Gly Asp Glu Leu Ile Leu Asn Glu
210 215 220

Glu Thr Ile Ala Ile Gly Val Ser Ala Arg Thr Ser Ala Lys Ala Ile
225 230 235 240

Glu Arg Leu Ala Lys Asn Leu Phe Ser Arg Gln Asn Lys Ile Lys Lys
245 250 255

Val Leu Ala Ile Glu Ile Pro Lys Cys Arg Ala Phe Met His Leu Asp
260 265 270

Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe Thr Ile His Pro Ala
275 280 285

Ile Gln Gly Pro Lys Gly Asn Met Asn Ile Tyr Ile Leu Glu Lys Gly
290 295 300

Ala Asp Glu Glu Thr Leu Lys Ile Thr His Arg Thr Ser Leu Met Glu
305 310 315 320

Ala Leu Lys Glu Val Leu Asp Leu Ser Glu Leu Val Leu Ile Pro Cys
325 330 335

Gly Gly Gly Asp Val Ile Ala Ser Ala Arg Glu Gln Trp Asn Asp Gly
340 345 350

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Ser Asn Thr Leu Ala Ile Ala Pro Gly Val Val Val Thr Tyr Asp Arg
 355 360 365

Asn Tyr Val Ser Asn Thr Leu Leu Arg Glu His Gly Ile Glu Val Ile
 370 375 380

Glu Val Leu Ser Ser Glu Leu Ser Arg Gly Arg Gly Gly Pro Arg Cys
 385 390 395 400

Met Ser Met Pro Ile Val Arg Lys Asp Ile
 405 410

<210> SEQ ID NO 26
 <211> LENGTH: 413
 <212> TYPE: PRT
 <213> ORGANISM: Bacillus licheniformis

<400> SEQUENCE: 26

Met Ile Met Thr Thr Pro Ile His Val Tyr Ser Glu Ile Gly Pro Leu
 1 5 10 15

Lys Thr Val Met Leu Lys Arg Pro Gly Arg Glu Leu Glu Asn Leu Thr
 20 25 30

Pro Glu Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Pro
 35 40 45

Ala Val Gln Lys Glu His Asp Gln Phe Ala Glu Thr Leu Lys Gln Gln
 50 55 60

Gly Ala Glu Val Leu Tyr Leu Glu Lys Leu Thr Ala Glu Ala Leu Asp
 65 70 75 80

Asp Ala Leu Val Arg Glu Gln Phe Ile Asp Glu Leu Leu Thr Glu Ser
 85 90 95

Lys Ala Asp Ile Asn Gly Ala Tyr Asp Arg Leu Lys Glu Phe Leu Leu
 100 105 110

Thr Phe Asp Ala Asp Ser Met Val Glu Gln Val Met Ser Gly Ile Arg
 115 120 125

Lys Asn Glu Leu Glu Arg Glu Lys Lys Ser His Leu His Glu Leu Met
 130 135 140

Glu Asp His Tyr Pro Phe Tyr Leu Asp Pro Met Pro Asn Leu Tyr Phe
 145 150 155 160

Thr Arg Asp Pro Ala Ala Ala Ile Gly Ser Gly Leu Thr Ile Asn Lys
 165 170 175

Met Lys Glu Pro Ala Arg Arg Arg Glu Ser Leu Phe Met Arg Tyr Ile
 180 185 190

Ile Asn His His Pro Arg Phe Lys Gly His Glu Ile Pro Val Trp Leu
 195 200 205

Asp Arg Asp Phe Lys Phe Asn Ile Glu Gly Gly Asp Glu Leu Val Leu
 210 215 220

Asn Glu Glu Thr Val Ala Ile Gly Val Ser Glu Arg Thr Thr Ala Gln
 225 230 235 240

Ala Ile Glu Arg Leu Val Arg Asn Leu Phe Gln Arg Gln Ser Arg Ile
 245 250 255

Arg Arg Val Leu Ala Val Glu Ile Pro Lys Ser Arg Ala Phe Met His
 260 265 270

Leu Asp Thr Val Phe Thr Met Val Asp Arg Asp Gln Phe Thr Ile His
 275 280 285

Pro Ala Ile Gln Gly Pro Glu Gly Asp Met Arg Ile Phe Val Leu Glu
 290 295 300

Arg Gly Lys Thr Ala Asp Glu Ile His Thr Thr Glu Glu His Asn Leu
 305 310 315 320

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Pro Glu Val Leu Lys Arg Thr Leu Gly Leu Ser Asp Val Asn Leu Ile
 325 330 335

Phe Cys Gly Gly Gly Asp Glu Ile Ala Ser Ala Arg Glu Gln Trp Asn
 340 345 350

Asp Gly Ser Asn Thr Leu Ala Ile Ala Pro Gly Val Val Val Thr Tyr
 355 360 365

Asp Arg Asn Tyr Ile Ser Asn Glu Cys Leu Arg Glu Gln Gly Ile Lys
 370 375 380

Val Ile Glu Ile Pro Ser Gly Glu Leu Ser Arg Gly Arg Gly Gly Pro
 385 390 395 400

Arg Cys Met Ser Met Pro Leu Tyr Arg Glu Asp Val Lys
 405 410

<210> SEQ ID NO 27
 <211> LENGTH: 409
 <212> TYPE: PRT
 <213> ORGANISM: Mycoplasma arthritidis

<400> SEQUENCE: 27

Met Ser Val Phe Asp Ser Lys Phe Lys Gly Ile His Val Tyr Ser Glu
 1 5 10 15

Ile Gly Glu Leu Glu Thr Val Leu Val His Glu Pro Gly Lys Glu Ile
 20 25 30

Asp Tyr Ile Thr Pro Ala Arg Leu Asp Glu Leu Leu Phe Ser Ala Ile
 35 40 45

Leu Glu Ser His Asp Ala Arg Lys Glu His Lys Glu Phe Val Ala Glu
 50 55 60

Leu Lys Lys Arg Gly Ile Asn Val Val Glu Leu Val Asp Leu Ile Val
 65 70 75 80

Glu Thr Tyr Asp Leu Ala Ser Lys Glu Ala Lys Glu Lys Leu Leu Glu
 85 90 95

Glu Phe Leu Asp Asp Ser Val Pro Val Leu Ser Asp Glu His Arg Ala
 100 105 110

Ala Val Lys Lys Phe Leu Gln Ser Gln Lys Ser Thr Arg Ser Leu Val
 115 120 125

Glu Tyr Met Ile Ala Gly Ile Thr Lys His Asp Leu Lys Ile Glu Ser
 130 135 140

Asp Leu Glu Leu Ile Val Asp Pro Met Pro Asn Leu Tyr Phe Thr Arg
 145 150 155 160

Asp Pro Phe Ala Ser Val Gly Asn Gly Val Thr Ile His Tyr Met Arg
 165 170 175

Tyr Lys Val Arg Gln Arg Glu Thr Leu Phe Ser Arg Phe Val Phe Ser
 180 185 190

Asn His Pro Lys Leu Val Asn Thr Pro Trp Tyr Tyr Asp Pro Ala Glu
 195 200 205

Gly Leu Ser Ile Glu Gly Gly Asp Val Phe Ile Tyr Asn Asn Asp Thr
 210 215 220

Leu Val Val Gly Val Ser Glu Arg Thr Asp Leu Gln Thr Ile Thr Leu
 225 230 235 240

Leu Ala Lys Asn Ile Lys Ala Asn Lys Glu Cys Glu Phe Lys Arg Ile
 245 250 255

Val Ala Ile Asn Val Pro Lys Trp Thr Asn Leu Met His Leu Asp Thr
 260 265 270

Trp Leu Thr Met Leu Asp Lys Asp Lys Phe Leu Tyr Ser Pro Ile Ala
 275 280 285

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Asn Asp Val Phe Lys Phe Trp Asp Tyr Asp Leu Val Asn Gly Gly Asp
 290 295 300
 Ala Pro Gln Pro Val Asp Asn Gly Leu Pro Leu Glu Asp Leu Leu Lys
 305 310 315 320
 Ser Ile Ile Gly Lys Lys Pro Thr Leu Ile Pro Ile Ala Gly Ala Gly
 325 330 335
 Ala Ser Gln Ile Asp Ile Glu Arg Glu Thr His Phe Asp Gly Thr Asn
 340 345 350
 Tyr Leu Ala Val Ala Pro Gly Ile Val Ile Gly Tyr Ala Arg Asn Glu
 355 360 365
 Lys Thr Asn Ala Ala Leu Glu Ala Ala Gly Ile Thr Val Leu Pro Phe
 370 375 380
 Arg Gly Asn Gln Leu Ser Leu Gly Met Gly Asn Ala Arg Cys Met Ser
 385 390 395 400
 Met Pro Leu Ser Arg Lys Asp Val Lys
 405

<210> SEQ ID NO 28
 <211> LENGTH: 409
 <212> TYPE: PRT
 <213> ORGANISM: Mycoplasma hominis
 <400> SEQUENCE: 28

Met Ser Val Phe Asp Ser Lys Phe Asn Gly Ile His Val Tyr Ser Glu
 1 5 10 15
 Ile Gly Glu Leu Glu Thr Val Leu Val His Glu Pro Gly Arg Glu Ile
 20 25 30
 Asp Tyr Ile Thr Pro Ala Arg Leu Asp Glu Leu Leu Phe Ser Ala Ile
 35 40 45
 Leu Glu Ser His Asp Ala Arg Lys Glu His Gln Ser Phe Val Lys Ile
 50 55 60
 Met Lys Asp Arg Gly Ile Asn Val Val Glu Leu Thr Asp Leu Val Ala
 65 70 75 80
 Glu Thr Tyr Asp Leu Ala Ser Lys Ala Ala Lys Glu Glu Phe Ile Glu
 85 90 95
 Thr Phe Leu Glu Glu Thr Val Pro Val Leu Thr Glu Ala Asn Lys Lys
 100 105 110
 Ala Val Arg Ala Phe Leu Leu Ser Lys Pro Thr His Glu Met Val Glu
 115 120 125
 Phe Met Met Ser Gly Ile Thr Lys Tyr Glu Leu Gly Val Glu Ser Glu
 130 135 140
 Asn Glu Leu Ile Val Asp Pro Met Pro Asn Leu Tyr Phe Thr Arg Asp
 145 150 155 160
 Pro Phe Ala Ser Val Gly Asn Gly Val Thr Ile His Phe Met Arg Tyr
 165 170 175
 Ile Val Arg Arg Arg Glu Thr Leu Phe Ala Arg Phe Val Phe Arg Asn
 180 185 190
 His Pro Lys Leu Val Lys Thr Pro Trp Tyr Tyr Asp Pro Ala Met Lys
 195 200 205
 Met Ser Ile Glu Gly Gly Asp Val Phe Ile Tyr Asn Asn Glu Thr Leu
 210 215 220
 Val Val Gly Val Ser Glu Arg Thr Asp Leu Asp Thr Ile Thr Leu Leu
 225 230 235 240
 Ala Lys Asn Ile Lys Ala Asn Lys Glu Val Glu Phe Lys Arg Ile Val
 245 250 255

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Ala Ile Asn Val Pro Lys Trp Thr Asn Leu Met His Leu Asp Thr Trp
 260 265 270

Leu Thr Met Leu Asp Lys Asn Lys Phe Leu Tyr Ser Pro Ile Ala Asn
 275 280 285

Asp Val Phe Lys Phe Trp Asp Tyr Asp Leu Val Asn Gly Gly Ala Glu
 290 295 300

Pro Gln Pro Gln Leu Asn Gly Leu Pro Leu Asp Lys Leu Leu Ala Ser
 305 310 315 320

Ile Ile Asn Lys Glu Pro Val Leu Ile Pro Ile Gly Gly Ala Gly Ala
 325 330 335

Thr Glu Met Glu Ile Ala Arg Glu Thr Asn Phe Asp Gly Thr Asn Tyr
 340 345 350

Leu Ala Ile Lys Pro Gly Leu Val Ile Gly Tyr Asp Arg Asn Glu Lys
 355 360 365

Thr Asn Ala Ala Leu Lys Ala Ala Gly Ile Thr Val Leu Pro Phe His
 370 375 380

Gly Asn Gln Leu Ser Leu Gly Met Gly Asn Ala Arg Cys Met Ser Met
 385 390 395 400

Pro Leu Ser Arg Lys Asp Val Lys Trp
 405

<210> SEQ ID NO 29
 <211> LENGTH: 411
 <212> TYPE: PRT
 <213> ORGANISM: Streptococcus pyogenes

<400> SEQUENCE: 29

Met Thr Ala Gln Thr Pro Ile His Val Tyr Ser Glu Ile Gly Lys Leu
 1 5 10 15

Lys Lys Val Leu Leu His Arg Pro Gly Lys Glu Ile Glu Asn Leu Met
 20 25 30

Pro Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu
 35 40 45

Asp Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu
 50 55 60

Gly Ile Glu Val Leu Tyr Leu Glu Thr Leu Ala Ala Glu Ser Leu Val
 65 70 75 80

Thr Pro Glu Ile Arg Glu Ala Phe Ile Asp Glu Tyr Leu Ser Glu Ala
 85 90 95

Asn Ile Arg Gly Arg Ala Thr Lys Lys Ala Ile Arg Glu Leu Leu Met
 100 105 110

Ala Ile Glu Asp Asn Gln Glu Leu Ile Glu Lys Thr Met Ala Gly Val
 115 120 125

Gln Lys Ser Glu Leu Pro Glu Ile Pro Ala Ser Glu Lys Gly Leu Thr
 130 135 140

Asp Leu Val Glu Ser Ser Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn
 145 150 155 160

Leu Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Thr Gly Val Ser
 165 170 175

Leu Asn His Met Phe Ser Glu Thr Arg Asn Arg Glu Thr Leu Tyr Gly
 180 185 190

Lys Tyr Ile Phe Thr His His Pro Ile Tyr Gly Gly Gly Lys Val Pro
 195 200 205

Met Val Tyr Asp Arg Asn Glu Thr Thr Arg Ile Glu Gly Gly Asp Glu
 210 215 220

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Leu Val Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr
 225 230 235 240
 Asp Ala Ala Ser Ile Glu Lys Leu Leu Val Asn Ile Phe Lys Gln Asn
 245 250 255
 Leu Gly Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys
 260 265 270
 Phe Met His Leu Asp Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe
 275 280 285
 Thr Ile His Pro Glu Ile Glu Gly Asp Leu Arg Val Tyr Ser Val Thr
 290 295 300
 Tyr Asp Asn Glu Glu Leu His Ile Val Glu Glu Lys Gly Asp Leu Ala
 305 310 315 320
 Asp Leu Leu Ala Ala Asn Leu Gly Val Glu Lys Val Asp Leu Ile Arg
 325 330 335
 Cys Gly Gly Asp Asn Leu Val Ala Ala Gly Arg Glu Gln Trp Asn Asp
 340 345 350
 Gly Ser Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asn
 355 360 365
 Arg Asn Thr Ile Thr Asn Ala Ile Leu Glu Ser Lys Gly Leu Lys Leu
 370 375 380
 Ile Lys Ile His Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg
 385 390 395 400
 Cys Met Ser Met Pro Phe Glu Arg Glu Asp Ile
 405 410

<210> SEQ ID NO 30

<211> LENGTH: 409

<212> TYPE: PRT

<213> ORGANISM: Streptococcus pneumonia

<400> SEQUENCE: 30

Met Ser Ser His Pro Ile Gln Val Phe Ser Glu Ile Gly Lys Leu Lys
 1 5 10 15
 Lys Val Met Leu His Arg Pro Gly Lys Glu Leu Glu Asn Leu Leu Pro
 20 25 30
 Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu Asp
 35 40 45
 Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu Gly
 50 55 60
 Ile Glu Val Leu Tyr Leu Glu Gln Leu Ala Ala Glu Ser Leu Thr Ser
 65 70 75 80
 Pro Glu Ile Arg Asp Gln Phe Ile Glu Glu Tyr Leu Asp Glu Ala Asn
 85 90 95
 Ile Arg Asp Arg Gln Thr Lys Val Ala Ile Arg Glu Leu Leu His Gly
 100 105 110
 Ile Lys Asp Asn Gln Glu Leu Val Glu Lys Thr Met Ala Gly Ile Gln
 115 120 125
 Lys Val Glu Leu Pro Glu Ile Pro Asp Glu Ala Lys Asp Leu Thr Asp
 130 135 140
 Leu Val Glu Ser Asp Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn Leu
 145 150 155 160
 Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Asn Ala Val Ser Leu
 165 170 175
 Asn His Met Phe Ala Asp Thr Arg Asn Arg Glu Thr Leu Tyr Gly Lys
 180 185 190

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Tyr Ile Phe Lys Tyr His Pro Ile Tyr Gly Gly Lys Val Asp Leu Val
 195 200 205
 Tyr Asn Arg Glu Glu Asp Thr Arg Ile Glu Gly Gly Asp Glu Leu Val
 210 215 220
 Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr Asp Ala
 225 230 235 240
 Ala Ser Ile Glu Lys Leu Leu Val Asn Ile Phe Lys Lys Asn Val Gly
 245 250 255
 Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys Phe Met
 260 265 270
 His Leu Asp Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe Thr Ile
 275 280 285
 His Pro Glu Ile Glu Gly Asp Leu His Val Tyr Ser Val Thr Tyr Glu
 290 295 300
 Asn Glu Lys Leu Lys Ile Val Glu Glu Lys Gly Asp Leu Ala Glu Leu
 305 310 315 320
 Leu Ala Gln Asn Leu Gly Val Glu Lys Val His Leu Ile Arg Cys Gly
 325 330 335
 Gly Gly Asn Ile Val Ala Ala Ala Arg Glu Gln Trp Asn Asp Gly Ser
 340 345 350
 Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asp Arg Asn
 355 360 365
 Thr Val Thr Asn Lys Ile Leu Glu Glu Tyr Gly Leu Arg Leu Ile Lys
 370 375 380
 Ile Arg Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg Cys Met
 385 390 395 400
 Ser Met Pro Phe Glu Arg Glu Glu Val
 405

<210> SEQ ID NO 31
 <211> LENGTH: 402
 <212> TYPE: PRT
 <213> ORGANISM: Mycobacterium tuberculosis
 <400> SEQUENCE: 31

Met Gly Val Glu Leu Gly Ser Asn Ser Glu Val Gly Ala Leu Arg Val
 1 5 10 15
 Val Ile Leu His Arg Pro Gly Ala Glu Leu Arg Arg Leu Thr Pro Arg
 20 25 30
 Asn Thr Asp Gln Leu Leu Phe Asp Gly Leu Pro Trp Val Ser Arg Ala
 35 40 45
 Gln Asp Glu His Asp Glu Phe Ala Glu Leu Leu Ala Ser Arg Gly Ala
 50 55 60
 Glu Val Leu Leu Leu Ser Asp Leu Leu Thr Glu Ala Leu His His Ser
 65 70 75 80
 Gly Ala Ala Arg Met Gln Gly Ile Ala Ala Ala Val Asp Ala Pro Arg
 85 90 95
 Leu Gly Leu Pro Leu Ala Gln Glu Leu Ser Ala Tyr Leu Arg Ser Leu
 100 105 110
 Asp Pro Gly Arg Leu Ala His Val Leu Thr Ala Gly Met Thr Phe Asn
 115 120 125
 Glu Leu Pro Ser Asp Thr Arg Thr Asp Val Ser Leu Val Leu Arg Met
 130 135 140
 His His Gly Gly Asp Phe Val Ile Glu Pro Leu Pro Asn Leu Val Phe
 145 150 155 160

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Thr Arg Asp Ser Ser Ile Trp Ile Gly Pro Arg Val Val Ile Pro Ser
 165 170 175
 Leu Ala Leu Arg Ala Arg Val Arg Glu Ala Ser Leu Thr Asp Leu Ile
 180 185 190
 Tyr Ala His His Pro Arg Phe Thr Gly Val Arg Arg Ala Tyr Glu Ser
 195 200 205
 Arg Thr Ala Pro Val Glu Gly Gly Asp Val Leu Leu Leu Ala Pro Gly
 210 215 220
 Val Val Ala Val Gly Val Gly Glu Arg Thr Thr Pro Ala Gly Ala Glu
 225 230 235 240
 Ala Leu Ala Arg Ser Leu Phe Asp Asp Asp Leu Ala His Thr Val Leu
 245 250 255
 Ala Val Pro Ile Ala Gln Gln Arg Ala Gln Met His Leu Asp Thr Val
 260 265 270
 Cys Thr Met Val Asp Thr Asp Thr Met Val Met Tyr Ala Asn Val Val
 275 280 285
 Asp Thr Leu Glu Ala Phe Thr Ile Gln Arg Thr Pro Asp Gly Val Thr
 290 295 300
 Ile Gly Asp Ala Ala Pro Phe Ala Glu Ala Ala Lys Ala Met Gly
 305 310 315 320
 Ile Asp Lys Leu Arg Val Ile His Thr Gly Met Asp Pro Val Val Ala
 325 330 335
 Glu Arg Glu Gln Trp Asp Asp Gly Asn Asn Thr Leu Ala Leu Ala Pro
 340 345 350
 Gly Val Val Val Ala Tyr Glu Arg Asn Val Gln Thr Asn Ala Arg Leu
 355 360 365
 Gln Asp Ala Gly Ile Glu Val Leu Thr Ile Ala Gly Ser Glu Leu Gly
 370 375 380
 Thr Gly Arg Gly Gly Pro Arg Cys Met Ser Cys Pro Ala Ala Arg Asp
 385 390 395 400
 Pro Leu

<210> SEQ ID NO 32
 <211> LENGTH: 417
 <212> TYPE: PRT
 <213> ORGANISM: Pseudomonas plecoglossicida

<400> SEQUENCE: 32

Met Ser Thr Glu Lys Gln Lys Tyr Gly Val His Ser Glu Ala Gly Lys
 1 5 10 15
 Leu Arg Lys Val Met Val Cys Ser Pro Gly Leu Ala His Lys Arg Leu
 20 25 30
 Thr Pro Ser Asn Cys Asp Glu Leu Leu Phe Asp Asp Val Ile Trp Val
 35 40 45
 Asp Gln Ala Lys Arg Asp His Phe Asp Phe Val Thr Lys Met Arg Glu
 50 55 60
 Arg Gly Val Asp Val Leu Glu Met His Asn Leu Leu Thr Asp Ile Val
 65 70 75 80
 Gln Asp Lys Asn Ala Leu Lys Trp Ile Leu Asp Arg Lys Leu Thr Asp
 85 90 95
 Asp Thr Val Gly Val Gly Leu Lys Asn Glu Val Arg Ser Trp Leu Glu
 100 105 110
 Gly Gln Asp Pro Arg His Leu Ala Glu Phe Leu Ile Gly Gly Val Ala
 115 120 125

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Gly Gln Asp Leu Pro Gln Ser Glu Gly Ala Asp Val Val Lys Met Tyr
 130 135 140
 Asn Asp Tyr Leu Gly His Ser Ser Phe Ile Leu Pro Pro Leu Pro Asn
 145 150 155 160
 Thr Gln Phe Thr Arg Asp Thr Thr Cys Trp Ile Tyr Gly Gly Val Thr
 165 170 175
 Leu Asn Pro Met Tyr Trp Pro Ala Arg Arg Gln Glu Thr Leu Leu Thr
 180 185 190
 Thr Ala Ile Tyr Lys Phe His Lys Glu Phe Thr Asn Ala Glu Phe Glu
 195 200 205
 Val Trp Tyr Gly Asp Pro Asp Lys Glu His Gly Ser Ser Thr Leu Glu
 210 215 220
 Gly Gly Asp Val Met Pro Ile Gly Lys Gly Ile Val Leu Ile Gly Met
 225 230 235 240
 Gly Glu Arg Thr Ser Arg Gln Ala Ile Gly Gln Leu Ala Arg Asn Leu
 245 250 255
 Phe Glu Lys Gly Ala Ala Thr Glu Val Ile Val Ala Gly Leu Pro Lys
 260 265 270
 Ser Arg Ala Ala Met His Leu Asp Thr Val Phe Ser Phe Cys Asp Arg
 275 280 285
 Asp Leu Val Thr Val Phe Pro Glu Val Val Asn Glu Ile Val Pro Phe
 290 295 300
 Ile Ile Arg Pro Asp Glu Lys Lys Pro Tyr Gly Met Asp Val Arg Arg
 305 310 315 320
 Ile Asn Lys Ser Phe Ile Glu Val Val Gly Glu Gln Leu Gly Val Lys
 325 330 335
 Leu Arg Val Val Glu Thr Gly Gly Asn Ser Phe Ala Ala Glu Arg Glu
 340 345 350
 Gln Trp Asp Asp Gly Asn Asn Val Val Ala Ile Glu Pro Gly Val Val
 355 360 365
 Ile Gly Tyr Asp Arg Asn Thr Tyr Thr Asn Thr Leu Leu Arg Lys Ala
 370 375 380
 Gly Ile Glu Val Ile Thr Ile Ser Ala Gly Glu Leu Gly Arg Gly Arg
 385 390 395 400
 Gly Gly Gly His Cys Met Thr Cys Pro Ile Val Arg Asp Pro Ile Asp
 405 410 415

Tyr

<210> SEQ ID NO 33

<211> LENGTH: 420

<212> TYPE: PRT

<213> ORGANISM: Pseudomonas putida

<400> SEQUENCE: 33

Met Ser Ala Glu Lys Gln Lys Tyr Gly Val His Ser Glu Ala Gly Lys
 1 5 10 15
 Leu Arg Lys Val Met Val Cys Ser Pro Gly Leu Ala His Lys Arg Leu
 20 25 30
 Thr Pro Ser Asn Cys Asp Glu Leu Leu Phe Asp Asp Val Ile Trp Val
 35 40 45
 Asp Gln Ala Lys Arg Asp His Phe Asp Phe Val Thr Lys Met Arg Glu
 50 55 60
 Arg Gly Val Asp Val Leu Glu Met His Asn Leu Leu Thr Asp Ile Val
 65 70 75 80

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Gln Gln Pro Glu Ala Leu Lys Trp Ile Leu Asp Arg Lys Ile Thr Ser
 85 90 95
 Asp Thr Val Gly Val Gly Leu Thr Asn Glu Val Arg Ser Trp Leu Glu
 100 105 110
 Gly Leu Glu Pro Arg His Leu Ala Glu Phe Leu Ile Gly Gly Val Ala
 115 120 125
 Gly Gln Asp Leu Pro Val Ser Glu Gly Ala Glu Val Ile Lys Met Tyr
 130 135 140
 Asn Lys Tyr Leu Gly His Ser Ser Phe Ile Leu Pro Pro Leu Pro Asn
 145 150 155 160
 Thr Gln Phe Thr Arg Asp Thr Thr Cys Trp Ile Tyr Gly Gly Val Thr
 165 170 175
 Leu Asn Pro Met Tyr Trp Pro Ala Arg Arg Gln Glu Thr Leu Leu Thr
 180 185 190
 Thr Ala Ile Tyr Lys Phe His Lys Glu Phe Thr Gly Ala Asp Phe Gln
 195 200 205
 Val Trp Tyr Gly Asp Pro Asp Lys Asp His Gly Asn Ala Thr Leu Glu
 210 215 220
 Gly Gly Asp Val Met Pro Val Gly Lys Gly Ile Val Leu Ile Gly Met
 225 230 235 240
 Gly Glu Arg Thr Ser Arg His Ala Ile Gly Gln Leu Ala Gln Asn Leu
 245 250 255
 Phe Glu Lys Gly Ala Ala Glu Lys Ile Ile Val Ala Gly Leu Pro Lys
 260 265 270
 Ser Arg Ala Ala Met His Leu Asp Thr Val Phe Ser Phe Cys Asp Arg
 275 280 285
 Asp Leu Val Thr Val Phe Pro Glu Val Val Lys Glu Ile Lys Pro Phe
 290 295 300
 Ile Ile Thr Pro Asp Ser Ser Lys Pro Tyr Gly Met Asn Ile Ala Pro
 305 310 315 320
 Gln Asp Ala Ser Phe Leu Glu Val Val Ser Glu Gln Leu Leu Gly Lys
 325 330 335
 Lys Asp Lys Leu Arg Val Val Glu Thr Gly Gly Asn Ser Phe Ala Ala
 340 345 350
 Glu Arg Glu Gln Trp Asp Asp Gly Asn Asn Val Val Ala Leu Glu Pro
 355 360 365
 Gly Val Val Ile Gly Tyr Asp Arg Asn Thr Tyr Thr Asn Thr Leu Leu
 370 375 380
 Arg Lys Ala Gly Ile Glu Val Ile Thr Ile Ser Ala Gly Glu Leu Gly
 385 390 395 400
 Arg Gly Arg Gly Gly Gly His Cys Met Thr Cys Pro Ile Val Arg Asp
 405 410 415
 Pro Ile Asp Tyr
 420

<210> SEQ ID NO 34
 <211> LENGTH: 418
 <212> TYPE: PRT
 <213> ORGANISM: Pseudomonas aeruginosa

<400> SEQUENCE: 34

Met Ser Thr Glu Lys Thr Lys Leu Gly Val His Ser Glu Ala Gly Lys
 1 5 10 15
 Leu Arg Lys Val Met Val Cys Ser Pro Gly Leu Ala His Gln Arg Leu
 20 25 30

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| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Pro | Ser | Asn | Cys | Asp | Glu | Leu | Leu | Phe | Asp | Asp | Val | Ile | Trp | Val |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Asn | Gln | Ala | Lys | Arg | Asp | His | Phe | Asp | Phe | Val | Thr | Lys | Met | Arg | Glu |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Arg | Gly | Ile | Asp | Val | Leu | Glu | Met | His | Asn | Leu | Leu | Thr | Glu | Thr | Ile |
| 65 | | | | | 70 | | | | | 75 | | | | | 80 |
| Gln | Asn | Pro | Glu | Ala | Leu | Lys | Trp | Ile | Leu | Asp | Arg | Lys | Ile | Thr | Ala |
| | | | | 85 | | | | | | 90 | | | | | 95 |
| Asp | Ser | Val | Gly | Leu | Gly | Leu | Thr | Ser | Glu | Leu | Arg | Ser | Trp | Leu | Glu |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Ser | Leu | Glu | Pro | Arg | Lys | Leu | Ala | Glu | Tyr | Leu | Ile | Gly | Gly | Val | Ala |
| | | 115 | | | | | 120 | | | | | | 125 | | |
| Ala | Asp | Asp | Leu | Pro | Ala | Ser | Glu | Gly | Ala | Asn | Ile | Leu | Lys | Met | Tyr |
| | 130 | | | | | 135 | | | | | | 140 | | | |
| Arg | Glu | Tyr | Leu | Gly | His | Ser | Ser | Phe | Leu | Leu | Pro | Pro | Leu | Pro | Asn |
| 145 | | | | | 150 | | | | | | 155 | | | | 160 |
| Thr | Gln | Phe | Thr | Arg | Asp | Thr | Thr | Cys | Trp | Ile | Tyr | Gly | Gly | Val | Thr |
| | | | | 165 | | | | | 170 | | | | | | 175 |
| Leu | Asn | Pro | Met | Tyr | Trp | Pro | Ala | Arg | Arg | Gln | Glu | Thr | Leu | Leu | Thr |
| | | | 180 | | | | | 185 | | | | | | 190 | |
| Thr | Ala | Ile | Tyr | Lys | Phe | His | Pro | Glu | Phe | Ala | Asn | Ala | Glu | Phe | Glu |
| | | 195 | | | | | 200 | | | | | | 205 | | |
| Ile | Trp | Tyr | Gly | Asp | Pro | Asp | Lys | Asp | His | Gly | Ser | Ser | Thr | Leu | Glu |
| | 210 | | | | | 215 | | | | | | 220 | | | |
| Gly | Gly | Asp | Val | Met | Pro | Ile | Gly | Asn | Gly | Val | Val | Leu | Ile | Gly | Met |
| 225 | | | | | 230 | | | | | 235 | | | | | 240 |
| Gly | Glu | Arg | Ser | Ser | Arg | Gln | Ala | Ile | Gly | Gln | Val | Ala | Gln | Ser | Leu |
| | | | | 245 | | | | | | 250 | | | | | 255 |
| Phe | Ala | Lys | Gly | Ala | Ala | Glu | Arg | Val | Ile | Val | Ala | Gly | Leu | Pro | Lys |
| | | | 260 | | | | | 265 | | | | | | 270 | |
| Ser | Arg | Ala | Ala | Met | His | Leu | Asp | Thr | Val | Phe | Ser | Phe | Cys | Asp | Arg |
| | | 275 | | | | | 280 | | | | | | 285 | | |
| Asp | Leu | Val | Thr | Val | Phe | Pro | Glu | Val | Val | Lys | Glu | Ile | Val | Pro | Phe |
| | 290 | | | | | 295 | | | | | 300 | | | | |
| Ser | Leu | Arg | Pro | Asp | Pro | Ser | Ser | Pro | Tyr | Gly | Met | Asn | Ile | Arg | Arg |
| 305 | | | | | 310 | | | | | 315 | | | | | 320 |
| Glu | Glu | Lys | Thr | Phe | Leu | Glu | Val | Val | Ala | Glu | Ser | Leu | Gly | Leu | Lys |
| | | | | 325 | | | | | | 330 | | | | | 335 |
| Lys | Leu | Arg | Val | Val | Glu | Thr | Gly | Gly | Asn | Ser | Phe | Ala | Ala | Glu | Arg |
| | | | 340 | | | | | | 345 | | | | | 350 | |
| Glu | Gln | Trp | Asp | Asp | Gly | Asn | Asn | Val | Val | Cys | Leu | Glu | Pro | Gly | Val |
| | | 355 | | | | | 360 | | | | | | 365 | | |
| Val | Val | Gly | Tyr | Asp | Arg | Asn | Thr | Tyr | Thr | Asn | Thr | Leu | Leu | Arg | Lys |
| | 370 | | | | | 375 | | | | | | 380 | | | |
| Ala | Gly | Val | Glu | Val | Ile | Thr | Ile | Ser | Ala | Ser | Glu | Leu | Gly | Arg | Gly |
| 385 | | | | | 390 | | | | | 395 | | | | | 400 |
| Arg | Gly | Gly | Gly | His | Cys | Met | Thr | Cys | Pro | Ile | Val | Arg | Asp | Pro | Ile |
| | | | | 405 | | | | | 410 | | | | | | 415 |
| Asp | Tyr | | | | | | | | | | | | | | |

<210> SEQ ID NO 35

<211> LENGTH: 409

<212> TYPE: PRT

<213> ORGANISM: Streptococcus pneumoniae

-continued

<400> SEQUENCE: 35

Met Ser Ser His Pro Ile Gln Val Phe Ser Glu Ile Gly Lys Leu Lys
 1 5 10 15
 Lys Val Met Leu His Arg Pro Gly Lys Glu Leu Glu Asn Leu Leu Pro
 20 25 30
 Asp Tyr Leu Glu Arg Leu Leu Phe Asp Asp Ile Pro Phe Leu Glu Asp
 35 40 45
 Ala Gln Lys Glu His Asp Ala Phe Ala Gln Ala Leu Arg Asp Glu Gly
 50 55 60
 Ile Glu Val Leu Tyr Leu Glu Gln Leu Ala Ala Glu Ser Leu Thr Ser
 65 70 75 80
 Pro Glu Ile Arg Asp Gln Phe Ile Glu Glu Tyr Leu Asp Glu Ala Asn
 85 90 95
 Ile Arg Asp Arg Gln Thr Lys Val Ala Ile Arg Glu Leu Leu His Gly
 100 105 110
 Ile Lys Asp Asn Gln Glu Leu Val Glu Lys Thr Met Ala Gly Ile Gln
 115 120 125
 Lys Val Glu Leu Pro Glu Ile Pro Asp Glu Ala Lys Asp Leu Thr Asp
 130 135 140
 Leu Val Glu Ser Asp Tyr Pro Phe Ala Ile Asp Pro Met Pro Asn Leu
 145 150 155 160
 Tyr Phe Thr Arg Asp Pro Phe Ala Thr Ile Gly Asn Ala Val Ser Leu
 165 170 175
 Asn His Met Phe Ala Asp Thr Arg Asn Arg Glu Thr Leu Tyr Gly Lys
 180 185 190
 Tyr Ile Phe Lys Tyr His Pro Ile Tyr Gly Gly Lys Val Asp Leu Val
 195 200 205
 Tyr Asn Arg Glu Glu Asp Thr Arg Ile Glu Gly Gly Asp Glu Leu Val
 210 215 220
 Leu Ser Lys Asp Val Leu Ala Val Gly Ile Ser Gln Arg Thr Asp Ala
 225 230 235 240
 Ala Ser Ile Glu Lys Leu Leu Val Asn Ile Phe Lys Lys Asn Val Gly
 245 250 255
 Phe Lys Lys Val Leu Ala Phe Glu Phe Ala Asn Asn Arg Lys Phe Met
 260 265 270
 His Leu Asp Thr Val Phe Thr Met Val Asp Tyr Asp Lys Phe Thr Ile
 275 280 285
 His Pro Glu Ile Glu Gly Asp Leu His Val Tyr Ser Val Thr Tyr Glu
 290 295 300
 Asn Glu Lys Leu Lys Ile Val Glu Glu Lys Gly Asp Leu Ala Glu Leu
 305 310 315 320
 Leu Ala Gln Asn Leu Gly Val Glu Lys Val His Leu Ile Arg Cys Gly
 325 330 335
 Gly Gly Asn Ile Val Ala Ala Ala Arg Glu Gln Trp Asn Asp Gly Ser
 340 345 350
 Asn Thr Leu Thr Ile Ala Pro Gly Val Val Val Val Tyr Asp Arg Asn
 355 360 365
 Thr Val Thr Asn Lys Ile Leu Glu Glu Tyr Gly Leu Arg Leu Ile Lys
 370 375 380
 Ile Arg Gly Ser Glu Leu Val Arg Gly Arg Gly Gly Pro Arg Cys Met
 385 390 395 400
 Ser Met Pro Phe Glu Arg Glu Glu Val
 405

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<210> SEQ ID NO 36
 <211> LENGTH: 467
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: the sequence is synthesized

 <400> SEQUENCE: 36

 Met Ser Val Phe Asp Ser Lys Phe Lys Gly Ile His Val Tyr Ser Glu
 1 5 10 15
 Ile Gly Glu Leu Glu Ser Val Leu Val His Glu Pro Gly Arg Glu Ile
 20 25 30
 Asp Tyr Ile Thr Pro Ala Arg Leu Asp Glu Leu Leu Phe Ser Ala Ile
 35 40 45
 Leu Glu Ser His Asp Ala Arg Lys Glu His Lys Gln Phe Val Ala Glu
 50 55 60
 Leu Lys Ala Asn Asp Ile Asn Val Val Glu Leu Ile Asp Leu Val Ala
 65 70 75 80
 Glu Thr Tyr Asp Leu Ala Ser Gln Glu Ala Lys Asp Lys Leu Ile Glu
 85 90 95
 Glu Phe Leu Glu Asp Ser Glu Pro Val Leu Ser Glu Glu His Lys Val
 100 105 110
 Val Val Arg Asn Phe Leu Lys Ala Lys Lys Thr Ser Arg Glu Leu Val
 115 120 125
 Glu Ile Met Met Ala Gly Ile Thr Lys Tyr Asp Leu Gly Ile Glu Ala
 130 135 140
 Asp His Glu Leu Ile Val Asp Pro Met Pro Asn Leu Tyr Phe Thr Arg
 145 150 155 160
 Asp Pro Phe Ala Ser Val Gly Asn Gly Val Thr Ile His Tyr Met Arg
 165 170 175
 Tyr Lys Val Arg Gln Arg Glu Thr Leu Phe Ser Arg Phe Val Phe Ser
 180 185 190
 Asn His Pro Lys Leu Ile Asn Thr Pro Trp Tyr Tyr Asp Pro Ser Leu
 195 200 205
 Lys Leu Ser Ile Glu Gly Gly Asp Val Phe Ile Tyr Asn Asn Asp Thr
 210 215 220
 Leu Val Val Gly Val Ser Glu Arg Thr Asp Leu Gln Thr Val Thr Leu
 225 230 235 240
 Leu Ala Lys Asn Ile Val Ala Asn Lys Glu Cys Glu Phe Lys Arg Ile
 245 250 255
 Val Ala Ile Asn Val Pro Lys Trp Thr Asn Leu Met His Leu Asp Thr
 260 265 270
 Trp Leu Thr Met Leu Asp Lys Asp Lys Phe Leu Tyr Ser Pro Ile Ala
 275 280 285
 Asn Asp Val Phe Lys Phe Trp Asp Tyr Asp Leu Val Asn Gly Gly Ala
 290 295 300
 Glu Pro Gln Pro Val Glu Asn Gly Leu Pro Leu Glu Gly Leu Leu Gln
 305 310 315 320
 Ser Ile Ile Asn Lys Lys Pro Val Leu Ile Pro Ile Ala Gly Glu Gly
 325 330 335
 Ala Ser Gln Met Glu Ile Glu Arg Glu Thr His Phe Asp Gly Thr Asn
 340 345 350
 Tyr Leu Ala Ile Arg Pro Gly Val Val Ile Gly Tyr Ser Arg Asn Glu
 355 360 365

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Lys Thr Asn Ala Ala Leu Glu Ala Ala Gly Ile Lys Val Leu Pro Phe
 370 375 380

His Gly Asn Gln Leu Ser Leu Gly Met Gly Asn Ala Arg Cys Met Ser
 385 390 395 400

Met Pro Leu Ser Arg Lys Asp Val Lys Trp Gly Ser His His His His
 405 410 415

His His Ala Asn Ser Leu Ala Glu Ala Lys Val Leu Ala Asn Arg Glu
 420 425 430

Leu Asp Lys Tyr Gly Val Ser Asp Phe Tyr Lys Arg Leu Ile Asn Lys
 435 440 445

Ala Lys Thr Val Glu Gly Val Glu Ala Leu Lys Leu His Ile Leu Ala
 450 455 460

Ala Leu Pro
 465

<210> SEQ ID NO 37
 <211> LENGTH: 467
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: the sequence is synthesized

<400> SEQUENCE: 37

Met Ser Val Phe Asp Ser Lys Phe Lys Gly Ile His Val Tyr Ser Glu
 1 5 10 15

Ile Gly Glu Leu Glu Ser Val Leu Val His Glu Pro Gly Arg Glu Ile
 20 25 30

Asp Tyr Ile Thr Pro Ala Arg Leu Asp Glu Leu Leu Phe Ser Ala Ile
 35 40 45

Leu Glu Ser His Asp Ala Arg Lys Glu His Lys Gln Phe Val Ala Glu
 50 55 60

Leu Lys Ala Asn Asp Ile Asn Val Val Glu Leu Ile Asp Leu Val Ala
 65 70 75 80

Glu Thr Tyr Asp Leu Ala Ser Gln Glu Ala Lys Asp Lys Leu Ile Glu
 85 90 95

Glu Phe Leu Glu Asp Ser Glu Pro Val Leu Ser Glu Glu His Lys Val
 100 105 110

Val Val Arg Asn Phe Leu Lys Ala Lys Lys Thr Ser Arg Glu Leu Val
 115 120 125

Glu Ile Met Met Ala Gly Ile Thr Lys Tyr Asp Leu Gly Ile Glu Ala
 130 135 140

Asp His Glu Leu Ile Val Asp Pro Met Pro Asn Leu Tyr Phe Thr Arg
 145 150 155 160

Asp Pro Phe Ala Ser Val Gly Asn Gly Val Thr Ile His Tyr Met Arg
 165 170 175

Tyr Lys Val Arg Gln Arg Glu Thr Leu Phe Ser Arg Phe Val Phe Ser
 180 185 190

Asn His Pro Lys Leu Ile Asn Thr Pro Trp Tyr Tyr Asp Pro Ser Leu
 195 200 205

Lys Leu Ser Ile Glu Gly Gly Asp Val Phe Ile Tyr Asn Asn Asp Thr
 210 215 220

Leu Val Val Gly Val Ser Glu Arg Thr Asp Leu Gln Thr Val Thr Leu
 225 230 235 240

Leu Ala Lys Asn Ile Val Ala Asn Lys Glu Cys Glu Phe Lys Arg Ile
 245 250 255

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Val Ala Ile Asn Val Pro Lys Trp Thr Asn Leu Met His Leu Asp Thr
      260                               265                               270

Trp Leu Thr Met Leu Asp Lys Asp Lys Phe Leu Tyr Ser Pro Ile Ala
      275                               280                               285

Asn Asp Val Phe Lys Phe Trp Asp Tyr Asp Leu Val Asn Gly Gly Ala
      290                               295                               300

Glu Pro Gln Pro Val Glu Asn Gly Leu Pro Leu Glu Gly Leu Leu Gln
      305                               310                               315                               320

Ser Ile Ile Asn Lys Lys Pro Val Leu Ile Pro Ile Ala Gly Glu Gly
      325                               330                               335

Ala Ser Gln Met Glu Ile Glu Arg Glu Thr His Phe Asp Gly Thr Asn
      340                               345                               350

Tyr Leu Ala Ile Arg Pro Gly Val Val Ile Gly Tyr Ser Arg Asn Glu
      355                               360                               365

Lys Thr Asn Ala Ala Leu Glu Ala Ala Gly Ile Lys Val Leu Pro Phe
      370                               375                               380

His Gly Asn Gln Leu Ser Leu Gly Met Gly Asn Ala Arg Cys Met Ser
      385                               390                               395                               400

Met Pro Leu Ser Arg Lys Asp Val Lys Trp Ala Gln His Asp Glu Ala
      405                               410                               415

Val Asp Ala Asn Ser Leu Ala Glu Ala Lys Val Leu Ala Asn Arg Glu
      420                               425                               430

Leu Asp Lys Tyr Gly Val Ser Asp Tyr Tyr Lys Asn Leu Ile Asn Asn
      435                               440                               445

Ala Lys Thr Val Glu Gly Val Lys Ala Leu Ile Asp Glu Ile Leu Ala
      450                               455                               460

Ala Leu Pro
465

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<210> SEQ ID NO 38
<211> LENGTH: 473
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: the sequence is synthesized

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<400> SEQUENCE: 38

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Met Ser Val Phe Asp Ser Lys Phe Lys Gly Ile His Val Tyr Ser Glu
 1                               5                               10                               15

Ile Gly Glu Leu Glu Ser Val Leu Val His Glu Pro Gly Arg Glu Ile
      20                               25                               30

Asp Tyr Ile Thr Pro Ala Arg Leu Asp Glu Leu Leu Phe Ser Ala Ile
      35                               40                               45

Leu Glu Ser His Asp Ala Arg Lys Glu His Lys Gln Phe Val Ala Glu
      50                               55                               60

Leu Lys Ala Asn Asp Ile Asn Val Val Glu Leu Ile Asp Leu Val Ala
      65                               70                               75                               80

Glu Thr Tyr Asp Leu Ala Ser Gln Glu Ala Lys Asp Lys Leu Ile Glu
      85                               90                               95

Glu Phe Leu Glu Asp Ser Glu Pro Val Leu Ser Glu Glu His Lys Val
      100                               105                               110

Val Val Arg Asn Phe Leu Lys Ala Lys Lys Thr Ser Arg Glu Leu Val
      115                               120                               125

Glu Ile Met Met Ala Gly Ile Thr Lys Tyr Asp Leu Gly Ile Glu Ala
      130                               135                               140

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| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Tyr | Ile | Thr | Pro | Ala | Arg | Leu | Asp | Glu | Leu | Leu | Phe | Ser | Ala | Ile |
| | | 35 | | | | | 40 | | | | | 45 | | | |
| Leu | Glu | Ser | His | Asp | Ala | Arg | Lys | Glu | His | Lys | Gln | Phe | Val | Ala | Glu |
| | 50 | | | | | 55 | | | | | 60 | | | | |
| Leu | Lys | Ala | Asn | Asp | Ile | Asn | Val | Val | Glu | Leu | Ile | Asp | Leu | Val | Ala |
| 65 | | | | | 70 | | | | | 75 | | | | | 80 |
| Glu | Thr | Tyr | Asp | Leu | Ala | Ser | Gln | Glu | Ala | Lys | Asp | Lys | Leu | Ile | Glu |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Glu | Phe | Leu | Glu | Asp | Ser | Glu | Pro | Val | Leu | Ser | Glu | Glu | His | Lys | Val |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Val | Val | Arg | Asn | Phe | Leu | Lys | Ala | Lys | Lys | Thr | Ser | Arg | Glu | Leu | Val |
| | | 115 | | | | | 120 | | | | | 125 | | | |
| Glu | Ile | Met | Met | Ala | Gly | Ile | Thr | Lys | Tyr | Asp | Leu | Gly | Ile | Glu | Ala |
| | 130 | | | | | 135 | | | | | 140 | | | | |
| Asp | His | Glu | Leu | Ile | Val | Asp | Pro | Met | Pro | Asn | Leu | Tyr | Phe | Thr | Arg |
| 145 | | | | | 150 | | | | | 155 | | | | | 160 |
| Asp | Pro | Phe | Ala | Ser | Val | Gly | Asn | Gly | Val | Thr | Ile | His | Tyr | Met | Arg |
| | | | | 165 | | | | | 170 | | | | | 175 | |
| Tyr | Lys | Val | Arg | Gln | Arg | Glu | Thr | Leu | Phe | Ser | Arg | Phe | Val | Phe | Ser |
| | | | 180 | | | | | 185 | | | | | 190 | | |
| Asn | His | Pro | Lys | Leu | Ile | Asn | Thr | Pro | Trp | Tyr | Tyr | Asp | Pro | Ser | Leu |
| | | 195 | | | | | 200 | | | | | 205 | | | |
| Lys | Leu | Ser | Ile | Glu | Gly | Gly | Asp | Val | Phe | Ile | Tyr | Asn | Asn | Asp | Thr |
| | 210 | | | | | 215 | | | | | 220 | | | | |
| Leu | Val | Val | Gly | Val | Ser | Glu | Arg | Thr | Asp | Leu | Gln | Thr | Val | Thr | Leu |
| 225 | | | | | 230 | | | | | 235 | | | | | 240 |
| Leu | Ala | Lys | Asn | Ile | Val | Ala | Asn | Lys | Glu | Cys | Glu | Phe | Lys | Arg | Ile |
| | | | | 245 | | | | | 250 | | | | | 255 | |
| Val | Ala | Ile | Asn | Val | Pro | Lys | Trp | Thr | Asn | Leu | Met | His | Leu | Asp | Thr |
| | | | 260 | | | | | 265 | | | | | 270 | | |
| Trp | Leu | Thr | Met | Leu | Asp | Lys | Asp | Lys | Phe | Leu | Tyr | Ser | Pro | Ile | Ala |
| | | 275 | | | | | 280 | | | | | 285 | | | |
| Asn | Asp | Val | Phe | Lys | Phe | Trp | Asp | Tyr | Asp | Leu | Val | Asn | Gly | Gly | Ala |
| | 290 | | | | | 295 | | | | | 300 | | | | |
| Glu | Pro | Gln | Pro | Val | Glu | Asn | Gly | Leu | Pro | Leu | Glu | Gly | Leu | Leu | Gln |
| 305 | | | | | 310 | | | | | 315 | | | | | 320 |
| Ser | Ile | Ile | Asn | Lys | Lys | Pro | Val | Leu | Ile | Pro | Ile | Ala | Gly | Glu | Gly |
| | | | 325 | | | | | | 330 | | | | | 335 | |
| Ala | Ser | Gln | Met | Glu | Ile | Glu | Arg | Glu | Thr | His | Phe | Asp | Gly | Thr | Asn |
| | | | 340 | | | | | 345 | | | | | 350 | | |
| Tyr | Leu | Ala | Ile | Arg | Pro | Gly | Val | Val | Ile | Gly | Tyr | Ser | Arg | Asn | Glu |
| | | 355 | | | | | 360 | | | | | 365 | | | |
| Lys | Thr | Asn | Ala | Ala | Leu | Glu | Ala | Ala | Gly | Ile | Lys | Val | Leu | Pro | Phe |
| | 370 | | | | | 375 | | | | | 380 | | | | |
| His | Gly | Asn | Gln | Leu | Ser | Leu | Gly | Met | Gly | Asn | Ala | Arg | Cys | Met | Ser |
| 385 | | | | | 390 | | | | | 395 | | | | | 400 |
| Met | Pro | Leu | Ser | Arg | Lys | Asp | Val | Lys | Trp | Ala | Gln | His | Asp | Glu | Ala |
| | | | | 405 | | | | | 410 | | | | | 415 | |
| Val | Asp | Ala | Asn | Ser | Leu | Ala | Glu | Ala | Lys | Val | Leu | Ala | Asn | Arg | Glu |
| | | | 420 | | | | | 425 | | | | | 430 | | |
| Leu | Asp | Lys | Tyr | Gly | Val | Ser | Asp | Tyr | Tyr | Lys | Asn | Leu | Ile | Asn | Asn |
| | | 435 | | | | | 440 | | | | | 445 | | | |

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Ala Lys Thr Val Glu Gly Val Lys Ala Leu Ile Asp Glu Ile Leu Ala
450 455 460

Ala Leu Pro His His His His His His
465 470

<210> SEQ ID NO 40

<211> LENGTH: 483

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: the sequence is synthesized

<400> SEQUENCE: 40

Met His His His His His His Asp Glu Ala Val Asp Ala Asn Ser Leu
1 5 10 15

Ala Glu Ala Lys Val Leu Ala Asn Arg Glu Leu Asp Lys Tyr Gly Val
20 25 30

Ser Asp Tyr Tyr Lys Asn Leu Ile Asn Asn Ala Lys Thr Val Glu Gly
35 40 45

Val Lys Ala Leu Ile Asp Glu Ile Leu Ala Ala Leu Pro Ser Gly Ser
50 55 60

Asn Asn Asn Asn Asn Asn Gly Ser Gly Gly Ser Val Phe Asp Ser Lys
65 70 75 80

Phe Lys Gly Ile His Val Tyr Ser Glu Ile Gly Glu Leu Glu Ser Val
85 90 95

Leu Val His Glu Pro Gly Arg Glu Ile Asp Tyr Ile Thr Pro Ala Arg
100 105 110

Leu Asp Glu Leu Leu Phe Ser Ala Ile Leu Glu Ser His Asp Ala Arg
115 120 125

Lys Glu His Lys Gln Phe Val Ala Glu Leu Lys Ala Asn Asp Ile Asn
130 135 140

Val Val Glu Leu Ile Asp Leu Val Ala Glu Thr Tyr Asp Leu Ala Ser
145 150 155 160

Gln Glu Ala Lys Asp Lys Leu Ile Glu Glu Phe Leu Glu Asp Ser Glu
165 170 175

Pro Val Leu Ser Glu Glu His Lys Val Val Val Arg Asn Phe Leu Lys
180 185 190

Ala Lys Lys Thr Ser Arg Glu Leu Val Glu Ile Met Met Ala Gly Ile
195 200 205

Thr Lys Tyr Asp Leu Gly Ile Glu Ala Asp His Glu Leu Ile Val Asp
210 215 220

Pro Met Pro Asn Leu Tyr Phe Thr Arg Asp Pro Phe Ala Ser Val Gly
225 230 235 240

Asn Gly Val Thr Ile His Tyr Met Arg Tyr Lys Val Arg Gln Arg Glu
245 250 255

Thr Leu Phe Ser Arg Phe Val Phe Ser Asn His Pro Lys Leu Ile Asn
260 265 270

Thr Pro Trp Tyr Tyr Asp Pro Ser Leu Lys Leu Ser Ile Glu Gly Gly
275 280 285

Asp Val Phe Ile Tyr Asn Asn Asp Thr Leu Val Val Gly Val Ser Glu
290 295 300

Arg Thr Asp Leu Gln Thr Val Thr Leu Leu Ala Lys Asn Ile Val Ala
305 310 315 320

Asn Lys Glu Cys Glu Phe Lys Arg Ile Val Ala Ile Asn Val Pro Lys
325 330 335

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Trp Thr Asn Leu Met His Leu Asp Thr Trp Leu Thr Met Leu Asp Lys
 340 345 350
 Asp Lys Phe Leu Tyr Ser Pro Ile Ala Asn Asp Val Phe Lys Phe Trp
 355 360 365
 Asp Tyr Asp Leu Val Asn Gly Gly Ala Glu Pro Gln Pro Val Glu Asn
 370 375 380
 Gly Leu Pro Leu Glu Gly Leu Leu Gln Ser Ile Ile Asn Lys Lys Pro
 385 390 395 400
 Val Leu Ile Pro Ile Ala Gly Glu Gly Ala Ser Gln Met Glu Ile Glu
 405 410 415
 Arg Glu Thr His Phe Asp Gly Thr Asn Tyr Leu Ala Ile Arg Pro Gly
 420 425 430
 Val Val Ile Gly Tyr Ser Arg Asn Glu Lys Thr Asn Ala Ala Leu Glu
 435 440 445
 Ala Ala Gly Ile Lys Val Leu Pro Phe His Gly Asn Gln Leu Ser Leu
 450 455 460
 Gly Met Gly Asn Ala Arg Cys Met Ser Met Pro Leu Ser Arg Lys Asp
 465 470 475 480
 Val Lys Trp

<210> SEQ ID NO 41
 <211> LENGTH: 484
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: the sequence is synthesized

 <400> SEQUENCE: 41

Met Gly His His His His His Asp Glu Ala Val Asp Ala Asn Ser
 1 5 10 15
 Leu Ala Glu Ala Lys Val Leu Ala Asn Arg Glu Leu Asp Lys Tyr Gly
 20 25 30
 Val Ser Asp Tyr Tyr Lys Asn Leu Ile Asn Asn Ala Lys Thr Val Glu
 35 40 45
 Gly Val Lys Ala Leu Ile Asp Glu Ile Leu Ala Ala Leu Pro Ser Gly
 50 55 60
 Ser Asn Asn Asn Asn Asn Asn Gly Ser Gly Gly Lys His Pro Ile His
 65 70 75 80
 Val Thr Ser Glu Ile Gly Glu Leu Gln Thr Val Leu Leu Lys Arg Pro
 85 90 95
 Gly Lys Glu Val Glu Asn Leu Thr Pro Asp Tyr Leu Gln Gln Leu Leu
 100 105 110
 Phe Asp Asp Ile Pro Tyr Leu Pro Ile Ile Gln Lys Glu His Asp Tyr
 115 120 125
 Phe Ala Gln Thr Leu Arg Asn Arg Gly Val Glu Val Leu Tyr Leu Glu
 130 135 140
 Lys Leu Ala Ala Glu Ala Leu Val Asp Lys Lys Leu Arg Glu Glu Phe
 145 150 155 160
 Val Asp Arg Ile Leu Lys Glu Gly Gln Ala Asp Val Asn Val Ala His
 165 170 175
 Gln Thr Leu Lys Glu Tyr Leu Leu Ser Phe Ser Asn Glu Glu Leu Ile
 180 185 190
 Gln Lys Ile Met Gly Gly Val Arg Lys Asn Glu Ile Glu Thr Ser Lys
 195 200 205

-continued

Lys Thr His Leu Tyr Glu Leu Met Glu Asp His Tyr Pro Phe Tyr Leu
 210 215 220
 Asp Pro Met Pro Asn Leu Tyr Phe Thr Arg Asp Pro Ala Ala Ser Val
 225 230 235 240
 Gly Asp Gly Leu Thr Ile Asn Lys Met Arg Glu Pro Ala Arg Arg Arg
 245 250 255
 Glu Ser Leu Phe Met Glu Tyr Ile Ile Lys Tyr His Pro Arg Phe Ala
 260 265 270
 Lys His Asn Val Pro Ile Trp Leu Asp Arg Asp Tyr Lys Phe Pro Ile
 275 280 285
 Glu Gly Gly Asp Glu Leu Ile Leu Asn Glu Glu Thr Ile Ala Ile Gly
 290 295 300
 Val Ser Ala Arg Thr Ser Ala Lys Ala Ile Glu Arg Leu Ala Lys Asn
 305 310 315 320
 Leu Phe Ser Arg Gln Asn Lys Ile Lys Lys Val Leu Ala Ile Glu Ile
 325 330 335
 Pro Lys Cys Arg Ala Phe Met His Leu Asp Thr Val Phe Thr Met Val
 340 345 350
 Asp Tyr Asp Lys Phe Thr Ile His Pro Ala Ile Gln Gly Pro Lys Gly
 355 360 365
 Asn Met Asn Ile Tyr Ile Leu Glu Lys Gly Ala Asp Glu Glu Thr Leu
 370 375 380
 Lys Ile Thr His Arg Thr Ser Leu Met Glu Ala Leu Lys Glu Val Leu
 385 390 395 400
 Asp Leu Ser Glu Leu Val Leu Ile Pro Cys Gly Gly Gly Asp Val Ile
 405 410 415
 Ala Ser Ala Arg Glu Gln Trp Asn Asp Gly Ser Asn Thr Leu Ala Ile
 420 425 430
 Ala Pro Gly Val Val Val Thr Tyr Asp Arg Asn Tyr Val Ser Asn Thr
 435 440 445
 Leu Leu Arg Glu His Gly Ile Glu Val Ile Glu Val Leu Ser Ser Glu
 450 455 460
 Leu Ser Arg Gly Arg Gly Gly Pro Arg Cys Met Ser Met Pro Ile Val
 465 470 475 480
 Arg Lys Asp Ile

<210> SEQ ID NO 42
 <211> LENGTH: 335
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: the sequence is synthesized

<400> SEQUENCE: 42

Met Ala Gln His Asp Glu Ala Val Asp Ala Asn Ser Leu Ala Glu Ala
 1 5 10 15
 Lys Val Leu Ala Asn Arg Glu Leu Asp Lys Tyr Gly Val Ser Asp Tyr
 20 25 30
 Tyr Lys Asn Leu Ile Asn Asn Ala Lys Thr Val Glu Gly Val Lys Ala
 35 40 45
 Leu Ile Asp Glu Ile Leu Ala Ala Leu Pro Glu Phe Leu Glu Gly Ser
 50 55 60
 Ser Cys Ile Thr Gly Asp Ala Leu Val Ala Leu Pro Glu Gly Glu Ser
 65 70 75 80

-continued

Val Arg Ile Ala Asp Ile Val Pro Gly Ala Arg Pro Asn Ser Asp Asn
 85 90 95
 Ala Ile Asp Leu Lys Val Leu Asp Arg His Gly Asn Pro Val Leu Ala
 100 105 110
 Asp Arg Leu Phe His Ser Gly Glu His Pro Val Tyr Thr Val Arg Thr
 115 120 125
 Val Glu Gly Leu Arg Val Thr Gly Thr Ala Asn His Pro Leu Leu Cys
 130 135 140
 Leu Val Asp Val Ala Gly Val Pro Thr Leu Leu Trp Lys Leu Ile Asp
 145 150 155 160
 Glu Ile Lys Pro Gly Asp Tyr Ala Val Ile Gln Arg Ser Ala Phe Ser
 165 170 175
 Val Asp Cys Ala Gly Phe Ala Arg Gly Lys Pro Glu Phe Ala Pro Thr
 180 185 190
 Thr Tyr Thr Val Gly Val Pro Gly Leu Val Arg Phe Leu Glu Ala His
 195 200 205
 His Arg Asp Pro Asp Ala Gln Ala Ile Ala Asp Glu Leu Thr Asp Gly
 210 215 220
 Arg Phe Tyr Tyr Ala Lys Val Ala Ser Val Thr Asp Ala Gly Val Gln
 225 230 235 240
 Pro Val Tyr Ser Leu Arg Val Asp Thr Ala Asp His Ala Phe Ile Thr
 245 250 255
 Asn Gly Phe Val Ser His Ala Thr Gly Leu Thr Gly Leu Asn Ser Gly
 260 265 270
 Leu Thr Thr Asn Pro Gly Val Ser Ala Trp Gln Val Asn Thr Ala Tyr
 275 280 285
 Thr Ala Gly Gln Leu Val Thr Tyr Asn Gly Lys Thr Tyr Lys Cys Leu
 290 295 300
 Gln Pro His Thr Ser Leu Ala Gly Trp Glu Pro Ser Asn Val Pro Ala
 305 310 315 320
 Leu Trp Gln Leu Gln Gly Asp Pro Ile Thr Ile Thr Ile Thr Lys
 325 330 335

<210> SEQ ID NO 43

<211> LENGTH: 636

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: the sequence is synthesized

<400> SEQUENCE: 43

Met Lys Ile Glu Glu Gly Lys Leu Thr Asn Pro Gly Val Ser Ala Trp
 1 5 10 15
 Gln Val Asn Thr Ala Tyr Thr Ala Gly Gln Leu Val Thr Tyr Asn Gly
 20 25 30
 Lys Thr Tyr Lys Cys Leu Gln Pro His Thr Ser Leu Ala Gly Trp Glu
 35 40 45
 Pro Ser Asn Val Pro Ala Leu Trp Gln Leu Gln Asn Asn Gly Asn Asn
 50 55 60
 Gly Leu Glu Leu Arg Glu Ser Gly Ala Ile Ser Gly Asp Ser Leu Ile
 65 70 75 80
 Ser Leu Ala Ser Thr Gly Lys Arg Val Ser Ile Lys Asp Leu Leu Asp
 85 90 95
 Glu Lys Asp Phe Glu Ile Trp Ala Ile Asn Glu Gln Thr Met Lys Leu
 100 105 110

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| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Ser | Ala | Lys | Val | Ser | Arg | Val | Phe | Cys | Thr | Gly | Lys | Lys | Leu | Val |
| | | 115 | | | | | 120 | | | | | 125 | | | |
| Tyr | Ile | Leu | Lys | Thr | Arg | Leu | Gly | Arg | Thr | Ile | Lys | Ala | Thr | Ala | Asn |
| | 130 | | | | | 135 | | | | | 140 | | | | |
| His | Arg | Phe | Leu | Thr | Ile | Asp | Gly | Trp | Lys | Arg | Leu | Asp | Glu | Leu | Ser |
| 145 | | | | | 150 | | | | | 155 | | | | | 160 |
| Leu | Lys | Glu | His | Ile | Ala | Leu | Pro | Arg | Lys | Leu | Glu | Ser | Ser | Ser | Leu |
| | | | 165 | | | | | | 170 | | | | | | 175 |
| Gln | Leu | Ser | Pro | Glu | Ile | Glu | Lys | Leu | Ser | Gln | Ser | Asp | Ile | Tyr | Trp |
| | | | 180 | | | | | 185 | | | | | 190 | | |
| Asp | Ser | Ile | Val | Ser | Ile | Thr | Glu | Thr | Gly | Val | Glu | Glu | Val | Phe | Asp |
| | | 195 | | | | | 200 | | | | | 205 | | | |
| Leu | Thr | Val | Pro | Gly | Pro | His | Asn | Phe | Val | Ala | Asn | Asp | Ile | Ile | Val |
| | 210 | | | | | 215 | | | | | 220 | | | | |
| His | Asn | Cys | Ser | Val | Phe | Asp | Ser | Lys | Phe | Lys | Gly | Ile | His | Val | Tyr |
| 225 | | | | | 230 | | | | | 235 | | | | | 240 |
| Ser | Glu | Ile | Gly | Glu | Leu | Glu | Ser | Val | Leu | Val | His | Glu | Pro | Gly | Arg |
| | | | 245 | | | | | | 250 | | | | | 255 | |
| Glu | Ile | Asp | Tyr | Ile | Thr | Pro | Ala | Arg | Leu | Asp | Glu | Leu | Leu | Phe | Ser |
| | | | 260 | | | | | 265 | | | | | | 270 | |
| Ala | Ile | Leu | Glu | Ser | His | Asp | Ala | Arg | Lys | Glu | His | Lys | Gln | Phe | Val |
| | | 275 | | | | | 280 | | | | | 285 | | | |
| Ala | Glu | Leu | Lys | Ala | Asn | Asp | Ile | Asn | Val | Val | Glu | Leu | Ile | Asp | Leu |
| | | 290 | | | | 295 | | | | | 300 | | | | |
| Val | Ala | Glu | Thr | Tyr | Asp | Leu | Ala | Ser | Gln | Glu | Ala | Lys | Asp | Lys | Leu |
| 305 | | | | | 310 | | | | | 315 | | | | | 320 |
| Ile | Glu | Glu | Phe | Leu | Glu | Asp | Ser | Glu | Pro | Val | Leu | Ser | Glu | Glu | His |
| | | | 325 | | | | | | 330 | | | | | 335 | |
| Lys | Val | Val | Val | Arg | Asn | Phe | Leu | Lys | Ala | Lys | Lys | Thr | Ser | Arg | Glu |
| | | | 340 | | | | | 345 | | | | | | 350 | |
| Leu | Val | Glu | Ile | Met | Met | Ala | Gly | Ile | Thr | Lys | Tyr | Asp | Leu | Gly | Ile |
| | | 355 | | | | | 360 | | | | | 365 | | | |
| Glu | Ala | Asp | His | Glu | Leu | Ile | Val | Asp | Pro | Met | Pro | Asn | Leu | Tyr | Phe |
| | | 370 | | | | 375 | | | | | 380 | | | | |
| Thr | Arg | Asp | Pro | Phe | Ala | Ser | Val | Gly | Asn | Gly | Val | Thr | Ile | His | Tyr |
| 385 | | | | | 390 | | | | | 395 | | | | | 400 |
| Met | Arg | Tyr | Lys | Val | Arg | Gln | Arg | Glu | Thr | Leu | Phe | Ser | Arg | Phe | Val |
| | | | 405 | | | | | | 410 | | | | | 415 | |
| Phe | Ser | Asn | His | Pro | Lys | Leu | Ile | Asn | Thr | Pro | Trp | Tyr | Tyr | Asp | Pro |
| | | | 420 | | | | | 425 | | | | | 430 | | |
| Ser | Leu | Lys | Leu | Ser | Ile | Glu | Gly | Gly | Asp | Val | Phe | Ile | Tyr | Asn | Asn |
| | | 435 | | | | | 440 | | | | | 445 | | | |
| Asp | Thr | Leu | Val | Val | Gly | Val | Ser | Glu | Arg | Thr | Asp | Leu | Gln | Thr | Val |
| | | 450 | | | | 455 | | | | | 460 | | | | |
| Thr | Leu | Leu | Ala | Lys | Asn | Ile | Val | Ala | Asn | Lys | Glu | Cys | Glu | Phe | Lys |
| 465 | | | | | 470 | | | | | 475 | | | | | 480 |
| Arg | Ile | Val | Ala | Ile | Asn | Val | Pro | Lys | Trp | Thr | Asn | Leu | Met | His | Leu |
| | | | 485 | | | | | | 490 | | | | | 495 | |
| Asp | Thr | Trp | Leu | Thr | Met | Leu | Asp | Lys | Asp | Lys | Phe | Leu | Tyr | Ser | Pro |
| | | | 500 | | | | | 505 | | | | | 510 | | |
| Ile | Ala | Asn | Asp | Val | Phe | Lys | Phe | Trp | Asp | Tyr | Asp | Leu | Val | Asn | Gly |
| | | 515 | | | | | 520 | | | | | 525 | | | |

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Gly Ala Glu Pro Gln Pro Val Glu Asn Gly Leu Pro Leu Glu Gly Leu
530 535 540

Leu Gln Ser Ile Ile Asn Lys Lys Pro Val Leu Ile Pro Ile Ala Gly
545 550 555 560

Glu Gly Ala Ser Gln Met Glu Ile Glu Arg Glu Thr His Phe Asp Gly
565 570 575

Thr Asn Tyr Leu Ala Ile Arg Pro Gly Val Val Ile Gly Tyr Ser Arg
580 585 590

Asn Glu Lys Thr Asn Ala Ala Leu Glu Ala Ala Gly Ile Lys Val Leu
595 600 605

Pro Phe His Gly Asn Gln Leu Ser Leu Gly Met Gly Asn Ala Arg Cys
610 615 620

Met Ser Met Pro Leu Ser Arg Lys Asp Val Lys Trp
625 630 635

<210> SEQ ID NO 44
<211> LENGTH: 1452
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: the sequence is synthesized

<400> SEQUENCE: 44

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gtcttagcta acagagaact tgacaaatat ggagtaagtg actattacaa gaacctaatc 120
aacaatgcca aaactgttga aggtgtaaaa gcaactgatag atgaaatfff agctgcatta 180
ccttcgggta gtaacaacaa taataacaat ggtagcggcg gttctgtatt tgacagtaaa 240
tttaaaggaa ttcacgttta ttcagaaatt ggtgaattag aatcagttct agttcacgaa 300
ccaggacgcy aaattgacta tattacacca gctagactag atgaattatt attctcagct 360
atcttagaaa gccacgatgc tagaaaagaa cacaaacaat tcgtagcaga attaaaagca 420
aacgacatca atgttggtga attaattgat ttagttgctg aaacatatga ttagcatca 480
caagaagcta aagacaaatt aatcgaagaa tttttagaag actcagaacc agttctatca 540
gaagaacaca aagtagttgt aagaaacttc ttaaaagcta aaaaaacatc aagagaatta 600
gtagaatca tgatggcagg gatcacaaaa tacgatttag gtatcgaagc agatcacgaa 660
ttaatcgttg acccaatgcc aaacctatac ttcacacgtg acccatttgc atcagtaggt 720
aatggtgtaa caatccacta catgcgttac aaagttagac aacgtgaaac attattctca 780
agatttgat tctcaaatca ccctaaacta attaacactc catggtacta cgacccttca 840
ctaaaattat caatcgaagc tggggacgta tttatctaca acaatgacac attagtagtt 900
ggtgtttctg aaagaactga cttacaaaca gttactttat tagctaaaaa cattgttgct 960
aataaagaat gtgaattcaa acgtattggt gcaattaacg ttccaaaatg gacaaactta 1020
atgcacttag acacatggct aacaatgta gacaaggaca aattcctata ctcaccaatc 1080
gctaatagacg tatttaaat ctgggattat gacttagtaa acggtggagc agaaccacaa 1140
ccagttgaaa acgattacc tctagaagga ttattacaat caatcattaa caaaaaacca 1200
gttttaattc ctatcgagc tgaaggtgct tcacaaatgg aaatcgaagc agaaacacac 1260

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ttcgatggta caaactactt agcaattaga ccaggtggtg taattgggta ctcacgtaac 1320
gaaaaaacia acgctgctct agaagctgca ggcattaaag ttcttcatt ccacggtaac 1380
caattatcat taggtatggg taacgctcgt tgtatgtcaa tgcctttatc acgtaaagat 1440
gttaagtggg aa 1452

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<210> SEQ ID NO 45
<211> LENGTH: 1455
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: the sequence is synthesized

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<400> SEQUENCE: 45

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atgggtcatc atcaccatca ccatgatgaa gccgtggatg cgaacagctt agctgaagct 60
aaagtcttag ctaacagaga acttgacaaa tatggagtaa gtgactatta caagaaccta 120
atcaacaatg ccaaaactgt tgaaggtgta aaagcactga tagatgaaat tttagctgca 180
ttaccttcgg gtagtaacaa caataataac aatggtagcg gcggtaaaca tccgatacat 240
gttacttcag aaattgggga attacaaacg gttttattaa aacgaccggg taaagaagtg 300
gaaaacttga cgccagatta tttgcagcaa ttattatttg acgatattcc atacctacca 360
attattcaaa aagagcatga ttattttgca caaacgttac gcaatcgggg tgttgaagtt 420
ctttatttag aaaaactagc cgctgaggcg ttagtagata aaaaacttcg agaagaattt 480
gttgatcgta ttttaaaaga aggacaggcc gacgtaaagtg ttgcacatca aactttaaaa 540
gaatatttac tttccttttc aatgaagaa ttaattcaaa aaattatggg cgggtgtacgg 600
aaaaacgaaa ttgaaacaag taagaagaca catttatatg aattaatgga agatcattat 660
ccgttttact tagatccaat gcctaattta tattttactc gtgatccagc agctagcgtg 720
ggcgtaggct taacgataaa taagatgaga gaaccagcgc gtagacgtga atcattattc 780
atggagtaca tcattaataa tcatccaaga tttgcaaac ataatgtacc aatctgggta 840
gatcgtgatt ataaatttcc aattgaaggt ggcgacgagc taattttaaa tgaagaaaca 900
attgcgattg gagtatctgc tcgtacttca gctaaagcaa ttgaacgttt agcaaaaaat 960
ctcttttagc gacaaaataa aattaagaaa gtgtagcaa tagaaattcc aaaatgccga 1020
gcatttatgc atttagatac agtatttaca atggttgatt atgataagtt tacaattcac 1080
ccagctattc aagggccaaa agggaatatg aatatttata ttttagaaaa aggagcagat 1140
gaggaaactc ttaaattac acatcgactc tctttaatgg aagcattaa agaggatta 1200
gacttaagtg aattagttct tattccatgt ggaggaggag atgtaattgc ttctgctcgt 1260
gaacaatgga atgatggctc gaacacatta gcaatcgcgc caggtgtagt tgttacatat 1320
gatcgcaact atgtatcaa tacgttatta cgggaacacg gtatagaagt gattgaggtg 1380
ctaagttcag aattatctcg tggtcgtggg ggtccacggt gcatgagtat gccaatggtt 1440
cgtaaagata tttaa 1455

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<210> SEQ ID NO 46
<211> LENGTH: 46
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: the sequence is synthesized

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<400> SEQUENCE: 46

Leu Ala Glu Ala Lys Val Leu Ala Asn Arg Glu Leu Asp Lys Tyr Gly
 1 5 10 15
 Val Ser Asp Tyr Tyr Lys Asn Leu Ile Asn Asn Ala Lys Thr Val Glu
 20 25 30
 Gly Val Lys Ala Leu Ile Asp Glu Ile Leu Ala Ala Leu Pro
 35 40 45

<210> SEQ ID NO 47

<211> LENGTH: 57

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: the sequence is synthesized

<400> SEQUENCE: 47

Ala Gln His Asp Glu Ala Val Asp Ala Asn Ser Leu Ala Glu Ala Lys
 1 5 10 15
 Val Leu Ala Asn Arg Glu Leu Asp Lys Tyr Gly Val Ser Asp Tyr Tyr
 20 25 30
 Lys Asn Leu Ile Asn Asn Ala Lys Thr Val Glu Gly Val Lys Ala Leu
 35 40 45
 Ile Asp Glu Ile Leu Ala Ala Leu Pro
 50 55

<210> SEQ ID NO 48

<211> LENGTH: 46

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: the sequence is synthesized

<400> SEQUENCE: 48

Leu Ala Glu Ala Lys Val Leu Ala Asn Arg Glu Leu Asp Lys Tyr Gly
 1 5 10 15
 Val Ser Asp Phe Tyr Lys Arg Leu Ile Asn Lys Ala Lys Thr Val Glu
 20 25 30
 Gly Val Glu Ala Leu Lys Leu His Ile Leu Ala Ala Leu Pro
 35 40 45

<210> SEQ ID NO 49

<211> LENGTH: 57

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: the sequence is synthesized

<400> SEQUENCE: 49

Gly Ser His His His His His Ala Asn Ser Leu Ala Glu Ala Lys
 1 5 10 15
 Val Leu Ala Asn Arg Glu Leu Asp Lys Tyr Gly Val Ser Asp Phe Tyr
 20 25 30
 Lys Arg Leu Ile Asn Lys Ala Lys Thr Val Glu Gly Val Glu Ala Leu
 35 40 45
 Lys Leu His Ile Leu Ala Ala Leu Pro
 50 55

<210> SEQ ID NO 50

<211> LENGTH: 11

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
 <223> OTHER INFORMATION: the sequence is synthesized

<400> SEQUENCE: 50

Gly Ser His His His His His His Ala Asn Ser
 1 5 10

<210> SEQ ID NO 51
 <211> LENGTH: 11
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: the sequence is synthesized

<400> SEQUENCE: 51

Ala Gln His Asp Glu Ala Val Asp Ala Asn Ser
 1 5 10

<210> SEQ ID NO 52
 <211> LENGTH: 8
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: the sequence is synthesized

<400> SEQUENCE: 52

Asp Glu Ala Val Asp Ala Asn Ser
 1 5

<210> SEQ ID NO 53
 <211> LENGTH: 4
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: the sequence is synthesized

<400> SEQUENCE: 53

Gly Ser Gly Gly
 1

What is claimed is:

1. A method of treating a cancer or inhibiting arginine-dependent tumor growth in a subject comprising administering an albumin-binding arginine deiminase fusion protein to the patient weekly or biweekly to reduce the availability of circulating arginine, wherein said albumin-binding arginine deiminase fusion protein comprises a first portion comprising one or two albumin-binding domain(s) fused to a second portion comprising arginine deiminase to form the albumin-binding arginine deiminase fusion protein, and one or more linker molecules; the first portion being positioned far from active site of the second portion by said linker molecule such that the albumin-binding arginine deiminase fusion protein retains the activity of arginine deiminase and binds serum albumin with neither function of one portion of the fusion protein being interfered with by the other portion of the fusion protein, wherein said albumin-binding arginine deiminase fusion protein comprises a sequence selected from SEQ ID NO: 36, 37, 38, 39, 40 or 41, and wherein said cancer [consists essentially of] is selected from the group consisting of pancreatic cancer, leukemia, melanoma, head and neck cancer, colorectal cancer, lung cancer, breast cancer, liver cancer, nasopharyngeal cancer, esophageal cancer, prostate cancer, stomach cancer, cervical cancer and brain cancer.

2. The method of claim 1, wherein said cancer or arginine-dependent tumor growth is argininosuccinate synthetase-negative.

3. The method of claim 1, wherein the two albumin-binding domains of the first portion are the same.

4. The method of claim 1, wherein the two albumin-binding domains of the first portion are different from each other.

5. The method of claim 1, wherein said albumin-binding domain is SEQ ID NO: 46, 47, 48, or 49.

6. The method of claim 1, wherein the linker molecule comprises a sequence selected from SEQ ID NO: 50, 51, 52, 53, or serine-glycine-serine (SGS) amino acid sequence.

7. The method of claim 1, wherein the arginine deiminase is selected from arginine deiminase produced from a Mycoplasma, Lactococcus, Pseudomonas, Streptococcus, Escherichia, Mycobacterium or Bacillus microorganism.

8. The method of claim 1, wherein the arginine deiminase is produced from Mycoplasma arginini, Lactococcus lactis, Bacillus licheniformis, Bacillus cereus, Mycoplasma arthritis, Mycoplasma hominis, Streptococcus pyogenes, Streptococcus pneumoniae, Mycobacterium tuberculosis, Pseudomonas plecoglossicida, Pseudomonas putida, Pseudomonas aeruginosa, thermophilic Aspergillus fumigatus or a combination thereof.

9. The method of claim 1, wherein said weekly or biweekly administering of said albumin-binding arginine deiminase fusion protein to said patient is 1.3 mg/kg/week.

10. The method of claim 1, wherein said albumin-binding arginine deiminase fusion protein is clinically effective in a pH range from 5.5 to 9.5. 5

11. The method of claim 1, wherein said albumin-binding arginine deiminase fusion protein is clinically effective at pH 7.4.

12. The method of claim 1, wherein said albumin-binding arginine deiminase fusion protein is clinically effective at pH 6.5. 10

13. The method of claim 11, wherein said albumin-binding arginine deiminase fusion protein has a specific activity of about 19 U/mg at pH 7.4. 15

14. The method of claim 1, wherein said albumin-binding arginine deiminase fusion protein is purified from both soluble and insoluble fractions of crude proteins.

* * * * *