(A)

Native ADI

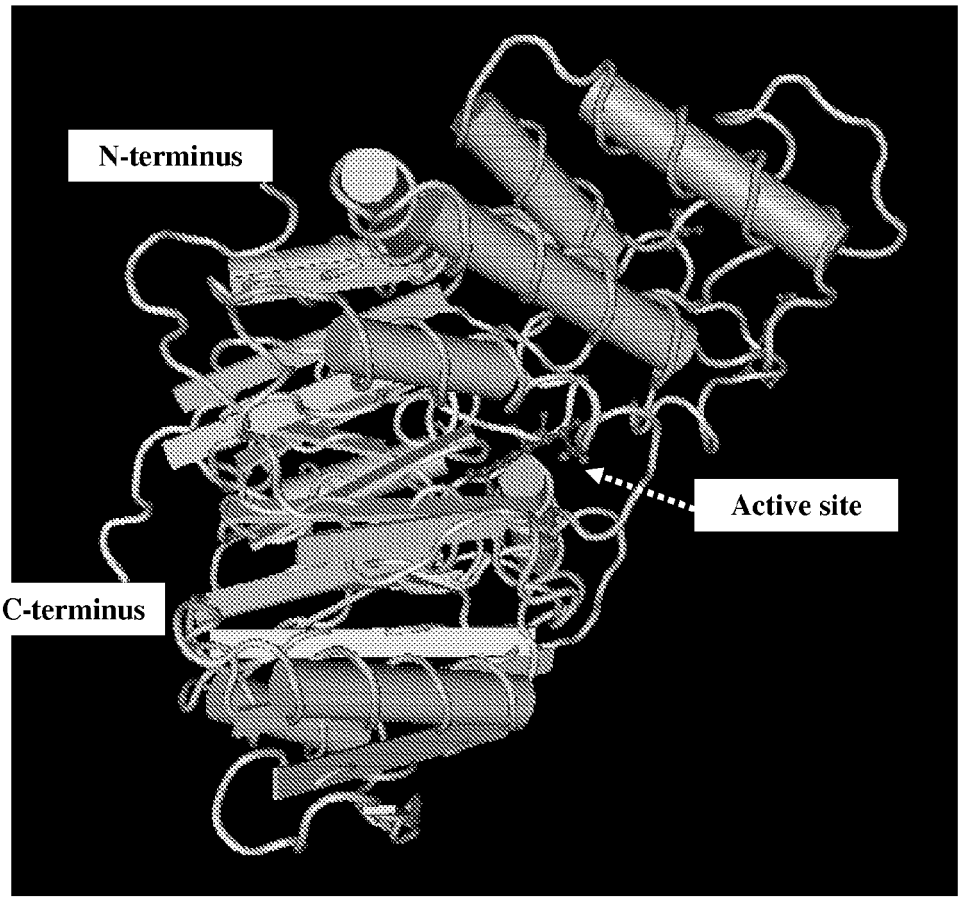
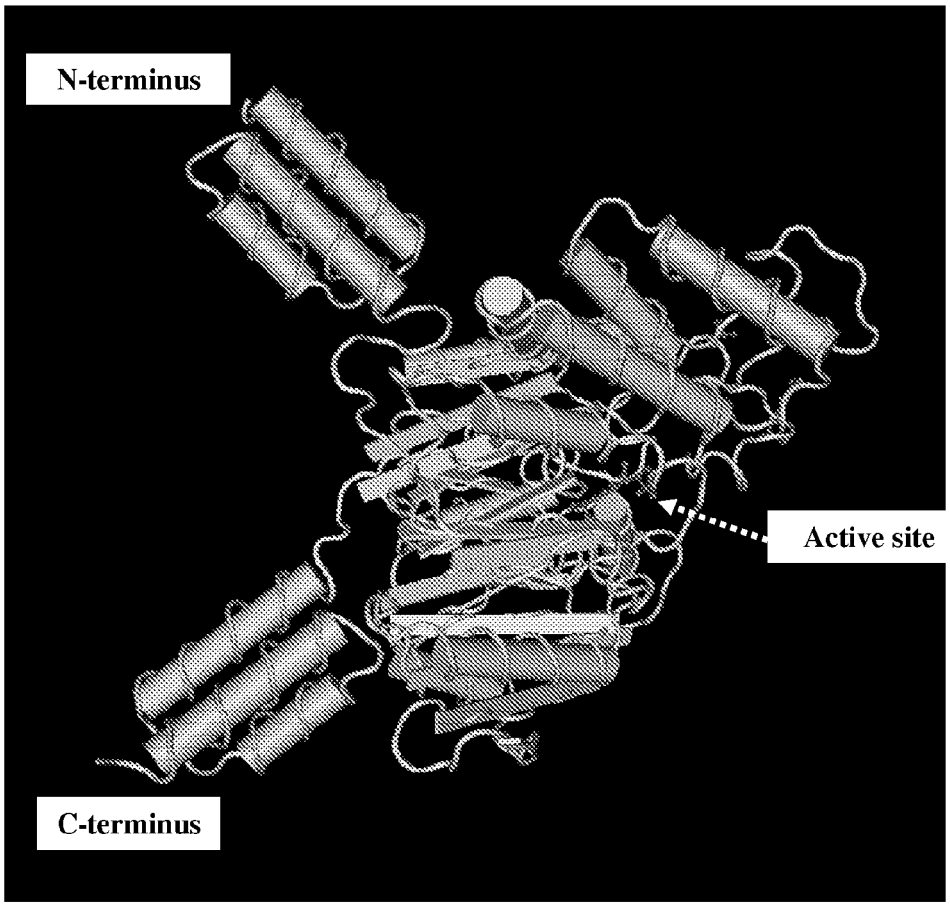


FIG. 1

(B) **AAD fusion protein with two ABD/ ABD1**



SEQ ID NO: 46
ABD without linker:
LAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP

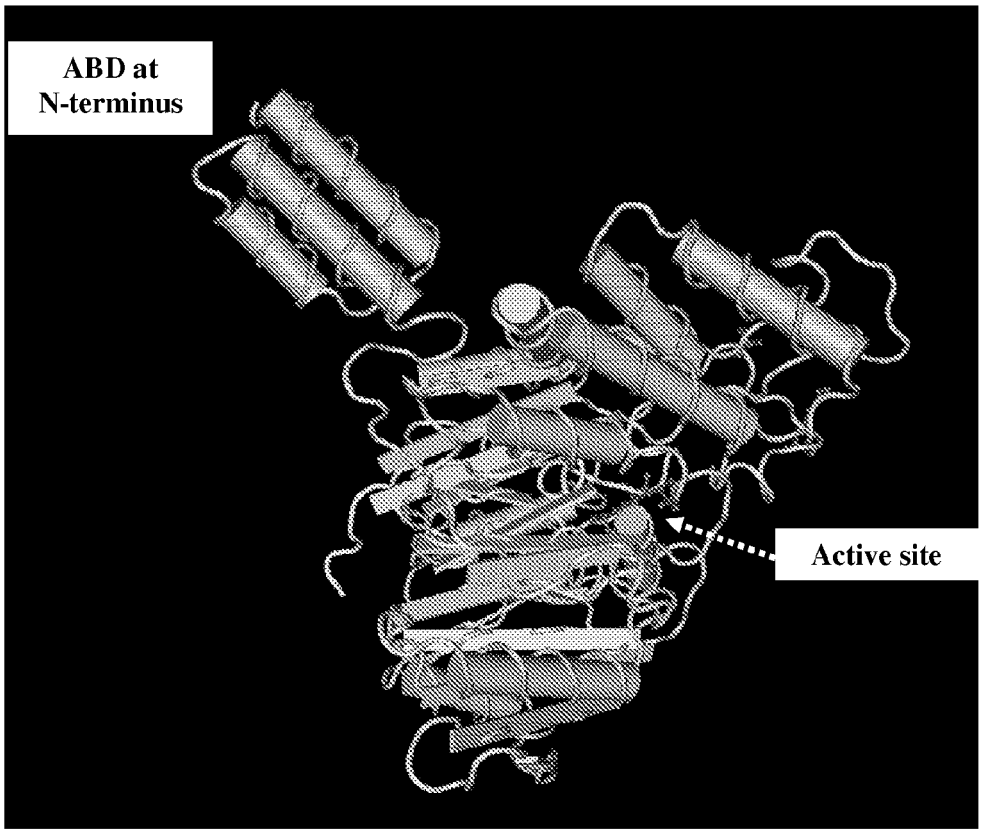
SEQ ID NO: 47
ABD with linker:
AQHDEAVDANSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP

SEQ ID NO: 48
ABD1 without linker:
LAEAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKLHILAALP

SEQ ID NO: 49
ABD1 with linker:
GSHHHHHHANSLAEAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKLHILAALP

FIG. 1 (continued)

(C) **AAD fusion protein with one ABD/ ABD1 at N-terminus**



SEQ ID NO: 46
ABD without linker:
LAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP

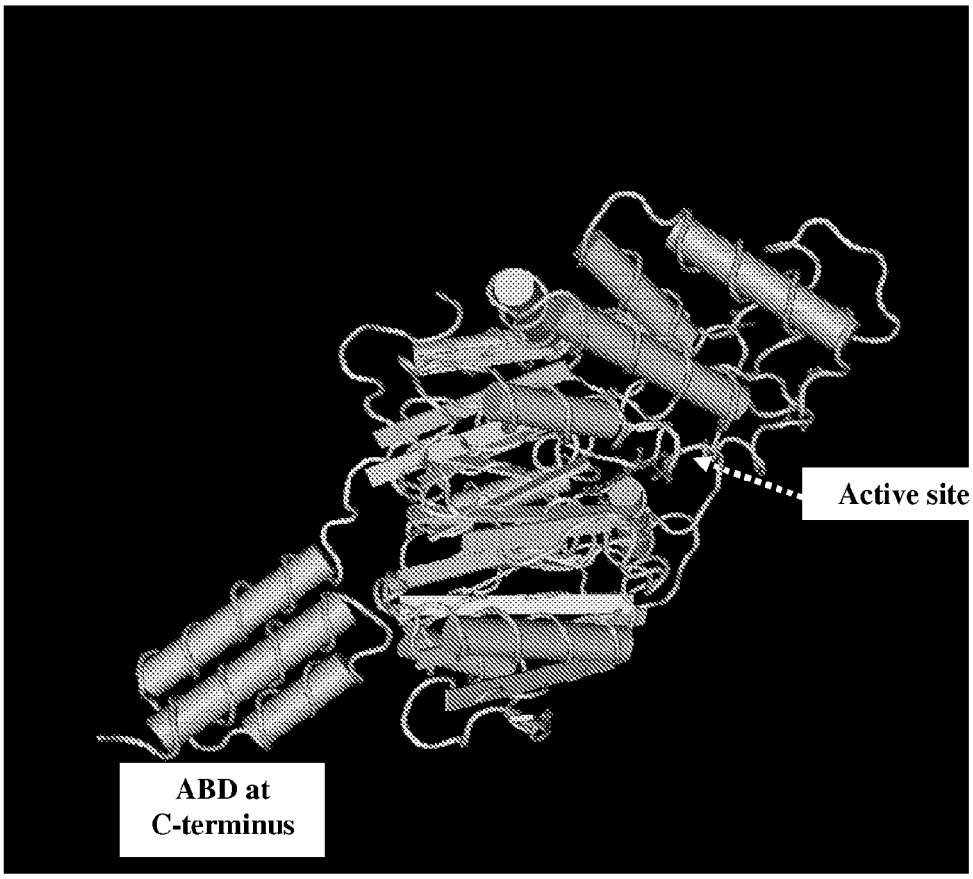
SEQ ID NO: 47
ABD with linker:
AQHDEAVDANSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP

SEQ ID NO: 48
ABD1 without linker:
LAEAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKLHILAALP

SEQ ID NO: 49
ABD1 with linker:
GSHHHHHHANSLAEAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKLHILAALP

FIG. 1 (continued)

(D) **AAD fusion protein with one ABD/ ABD1 at C-terminus**



SEQ ID NO: 46
ABD without linker: AEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP

SEQ ID NO: 47
ABD with linker:
AQHDEAVDANSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP

SEQ ID NO: 48
ABD1 without linker:
LAEAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKLHILAALP

SEQ ID NO: 49
ABD1 with linker:
GSHHHHHHANSLAEAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKLHILAALP

FIG. 1 (continued)

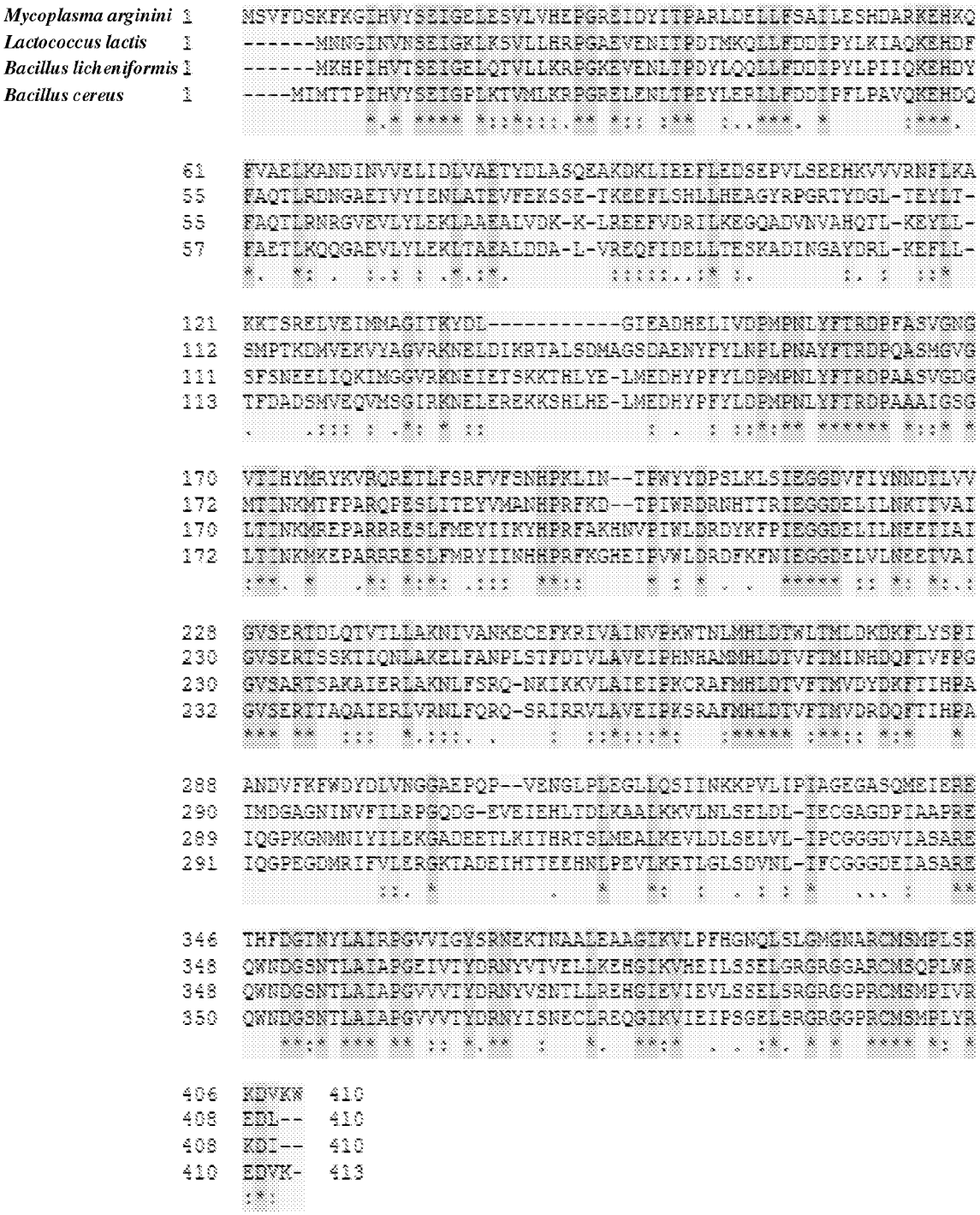

Mycoplasma arginini
Lactococcus lactis
Bacillus licheniformis
Bacillus cereus

FIG. 2

(**A**) SEQ ID NO: 36

ADI-linker-ABD 1

MSVFDSKFKGIHVYSEIGELESVLVHEPGREIDYITPARLDELLFSAILESHDARKEHKQ
FVAELKANDINVVELIDLVAETYDLASQEAKDKLIEEFLEDSEPVLSEEHKVVVRNFLKA
KKTSRELVEIMMAGITKYDLGIEADHELIVDPMPNLYFTRDPFASVGNGVTIHYMRYKVR
QRETLFSRFVFSNHPKLINTPWYYDPSLKLSIEGGDVFIYNNDTLVVGVSERTDLQTVTL
LAKNIVANKECEFKRIVAINVPKWTNLMHLDTWLTMLDKDKFLYSPIANDVFKFWDYDLV
NGGAEPQPVENGLPLEGLLQSIINKKPVLIPIAGEGASQMEIERETHFDGTNYLAIRPGV
VIGYSRNEKTNAALEAAGIKVLPFHGNQLSLGMGNARCMSMPLSRKDVKWGSHHHHHHAN
SLAEAKVLANRELDKYGVSDFYKRLINKAKTVEGVEALKLHILAALP

(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

MSVFDSKFKGIHVYSEIGELESVLVHEPGREIDYITPARLDELLFSAILESHDARKEHKQ
FVAELKANDINVVELIDLVAETYDLASQEAKDKLIEEFLEDSEPVLSEEHKVVVRNFLKA
KKTSRELVEIMMAGITKYDLGIEADHELIVDPMPNLYFTRDPFASVGNGVTIHYMRYKVR
QRETLFSRFVFSNHPKLINTPWYYDPSLKLSIEGGDVFIYNNDTLVVGVSERTDLQTVTL
LAKNIVANKECEFKRIVAINVPKWTNLMHLDTWLTMLDKDKFLYSPIANDVFKFWDYDLV
NGGAEPQPVENGLPLEGLLQSIINKKPVLIPIAGEGASQMEIERETHFDGTNYLAIRPGV
VIGYSRNEKTNAALEAAGIKVLPFHGNQLSLGMGNARCMSMPLSRKDVKWAQHDEAVDAN
SLAEAKVLANRELDKYGVSDYYRNLINNAKTVEGVKALIDEILAALP

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

Linker 2 (SEQ ID NO: 51): AQHDEAVDANS

FIG. 3

(C) SEQ ID NO: 38



MSVFDSKFKGIHVYSEIGELESVLVHEPGREIDYITPARLDELLFSAILESHDARKEHKQ
FVAELKANDINVVELIDLVAETYDLASQEAKDKLIEEFLEDSEPVLSEEHKVVVRNFLKA
KKTSRELVEIMMAGITKYDLGIEADHELIVDPMPNLYFTRDPFASVGNGVTIHYMRYKVR
QRETLFSRFVFSNHPKLINTPWYYDPSLKLSIEGGDVFIYNNDTLVVGVSERTDLQTVTL
LAKNIVANKECEFKRIVAINVPKWTNLMHLDTWLTMLDKDKFLYSPIANDVFKFWDYDLV
NGGAEPQPVENGLPLEGLLQSIINKKPVLIPIAGEGASQMEIERETHFDGTNYLAIRPGV
VIGYSRNEKTNAALEAAGIKVLPFHGNQLSLGMGNARCMSMPLSRKDVKWHHHHHHAQHD
EAVDANSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP

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

(D) SEQ ID NO: 39

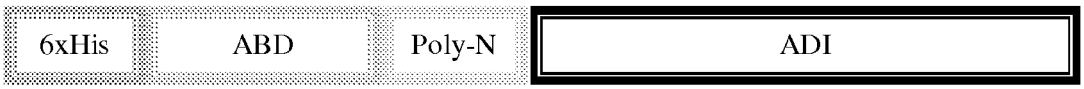


MSVFDSKFKGIHVYSEIGELESVLVHEPGREIDYITPARLDELLFSAILESHDARKEHKQ
FVAELKANDINVVELIDLVAETYDLASQEAKDKLIEEFLEDSEPVLSEEHKVVVRNFLKA
KKTSRELVEIMMAGITKYDLGIEADHELIVDPMPNLYFTRDPFASVGNGVTIHYMRYKVR
QRETLFSRFVFSNHPKLINTPWYYDPSLKLSIEGGDVFIYNNDTLVVGVSERTDLQTVTL
LAKNIVANKECEFKRIVAINVPKWTNLMHLDTWLTMLDKDKFLYSPIANDVFKFWDYDLV
NGGAEPQPVENGLPLEGLLQSIINKKPVLIPIAGEGASQMEIERETHFDGTNYLAIRPGV
VIGYSRNEKTNAALEAAGIKVLPFHGNQLSLGMGNARCMSMPLSRKDVKWAQHDEAVDAN
SLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALPHHHHHH

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

FIG. 3 (continued)

(E) SEQ ID NO: 40



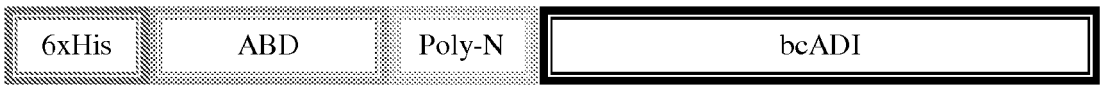
MHHHHHHDEAVDANSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAAL
PSGSNNNNNNGSGGSVFDSKFKGIHVYSEIGELESVLVHEPGREIDYITPARLDELCFSA
ILESHDARKEHKQFVAELKANDINVVELIDLVAETYDLASQEAKDKLIEEFLEDSEPVLS
EEHKVVVRNFLKAKKTSRELVEIMMAGITKYDLGIEADHELIVDPMPNLYFTRDPFASVG
NGVTIHYMRYKVRQRETLFSRFVFSNHPKLINTPWYYDPSLKLSIAGGDVFIYNNDTLVV
GVSERTDLQTVTLLAKNIVANKESEFKRIVAINVPKWTNLMHLDTWLTMLDKDKFLYSPI
ANDVFKFWDYDLVNGGAEPQPVENGLPLEGLLQSIINKKPVLIPIAGEGASQMEIERETH
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



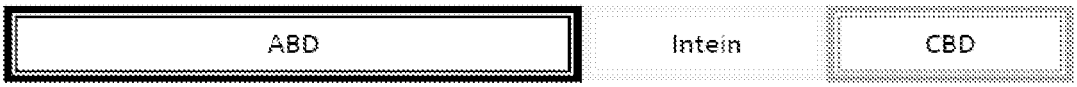
MGHHHHHHDEAVDANSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP
SGSNNNNNNGSGGKHPIHVTSEIGELQTVLLKRPGKEVENLTPDYLQQLLFDDIPYLPIIQK
EHDYFAQTLRNRGVEVLYLEKLAAEALVDKKLREEFVDRILKEGQADVNVAHQTLKEYLLSF
SNEELIQKIMETGGVRKNEIETSKKTHLYELMETEDHYPFYLDPMETPNLYFTRDPAASVGD
GLTINKMETREPARRRESLFMETEYIIKYHPRFEKHNVPIWLDRDYKFPIEGGDELILNEET
IAIGVSARTSAKAIERLAKNLFSRQNKIKKVLAIEIPKCRAFMETHLDTVFTMETVDYDKFT
IHPAIQGPKGNMETNIYILEKGSDEETLKITHRTSLMETEALKEVLGLSELVLIPCGGGDVI
ASAREQWNDGSNTLAIAPGVVVTYDRNYVSNTLLREHGIEVIEVLSSELSRGRGGPRCMETS
METPIVRKDI

(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

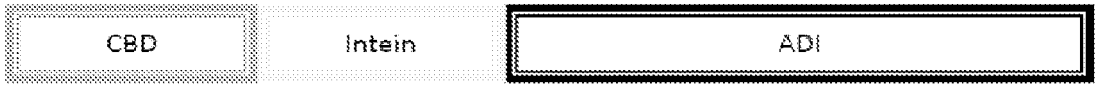
FIG. 3 (continued)

(**A**) SEQ ID NO: 42



MAQHDEAVDANSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALPEF
LEGSSCITGDALVALPEGESVRIADIVPGARPNSDNAIDLKVLDRHGNPVLADRLFHSGE
HPVYTVRTVEGLRVTGTANHPLLCLVDVAGVPTLLWKLIDEIKPGDYAVIQRSAFSVDCA
GFARGKPEFAPTTYTVGVPGLVRFLEAHHRDPDAQAIADELTDGRFYYAKVASVTDAGVQ
PVYSLRVDTADHAFITNGFVSHATGLTGLNSGLTTNPGVSAWQVNTAYTAGQLVTYNGKT
YKCLQPHTSLAGWEPSNVPALWQLQGDPITIIITK

(**B**) SEQ ID NO: 43

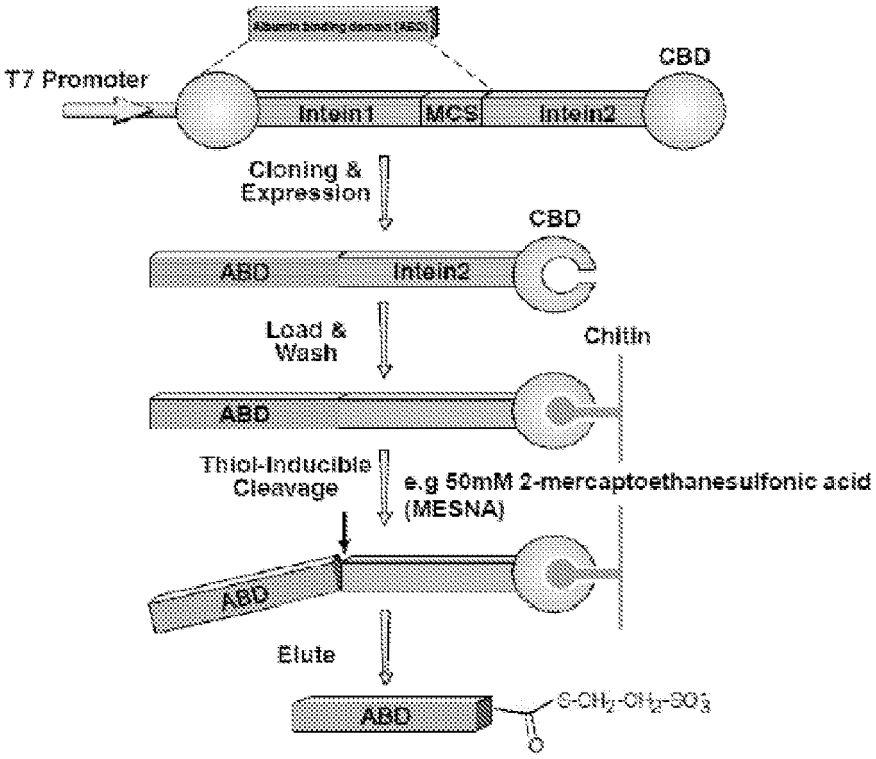


MKIEEGKLTIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDI
IFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYN
KDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYD
IKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNID
TSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKD
KPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQT
VDEALKDAQTNSSSNNNNNNNNNLGIEGRISEFQGTLTPGPVDKGSDAKSHHHHHH

FIG. 4

(C)

C-Terminal fusion



(D)

N-Terminal fusion

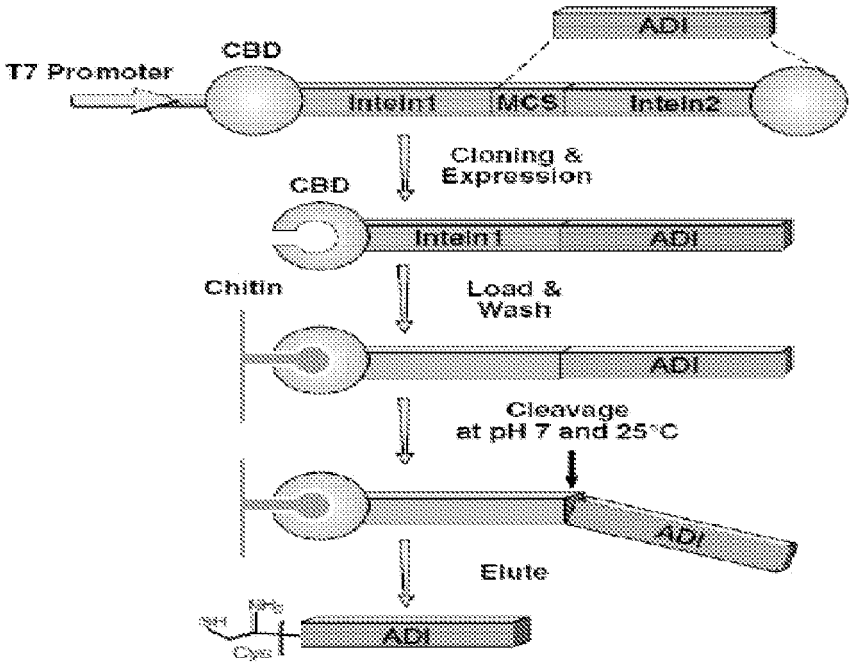


FIG. 4 (continued)

(E)

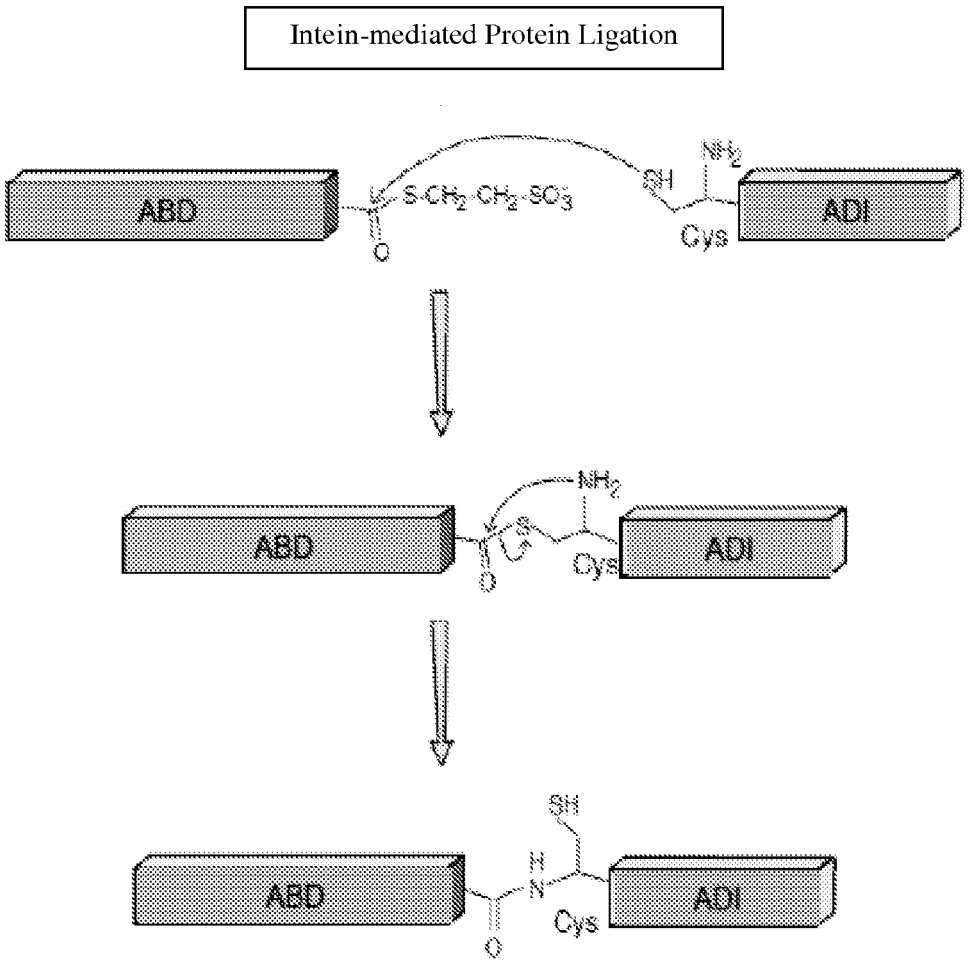


FIG. 4 (continued)

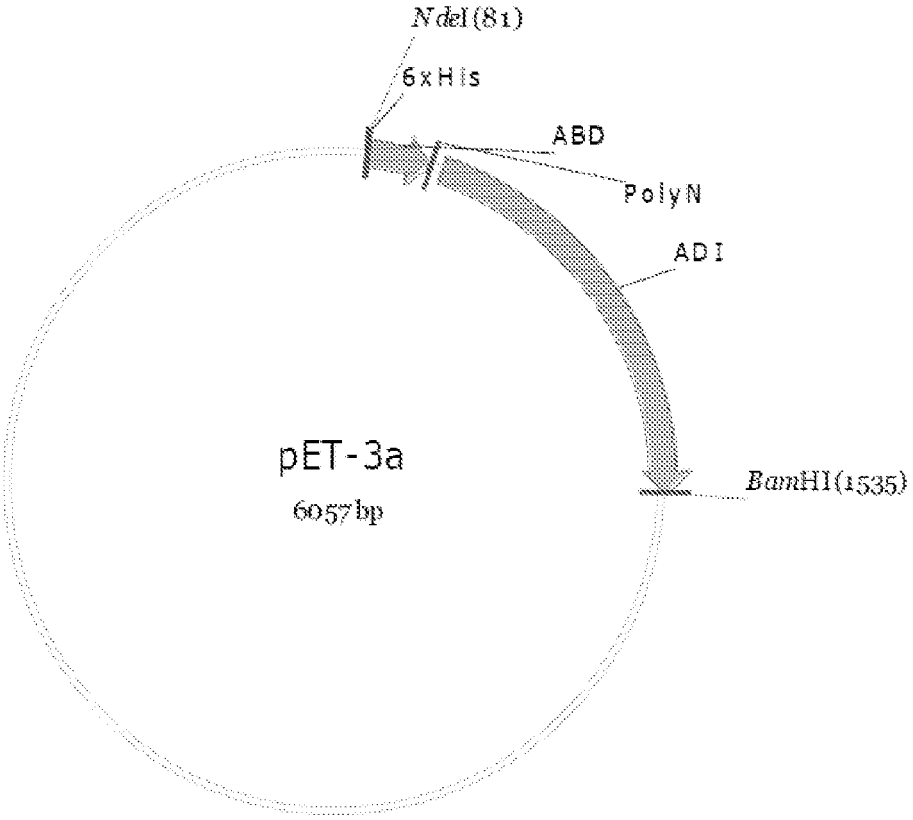
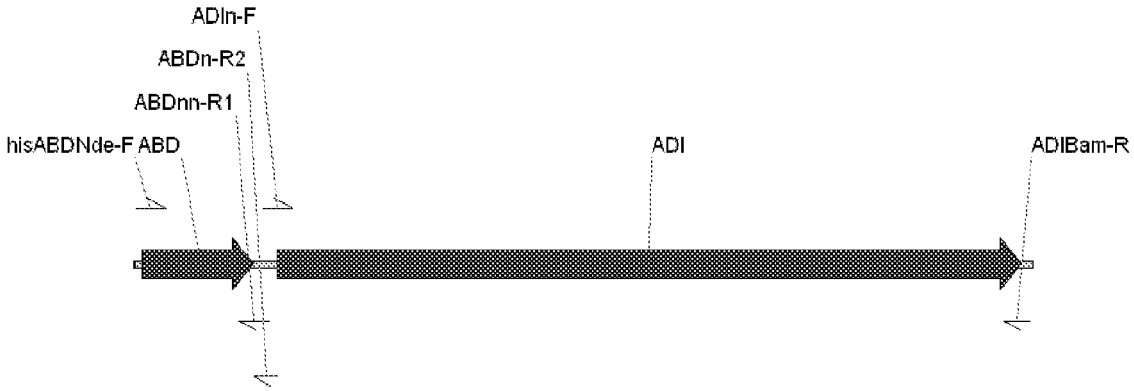


FIG. 5

(**A**) GENE MAP



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

(SEQ ID NO: 44)

5′–
ATGCATCATCACCATCACCATGATGAAGCCGTGGATGCGAATTCCTTAGCTGAAGCTAAAGTCT
TAGCTAACAGAGAACTTGACAAATATGGAGTAAGTGACTATTACAAGAACCTAATCAACAATGC
CAAAACTGTTGAAGGTGTAAAAGCACTGATAGATGAAATTTTAGCTGCATTACCTTCGGGTAGT
AACAACAATAATAACAATGGTAGCGGCGGTTCTGTATTTGACAGTAAATTTAAAGGAATTCACG
TTTATTCAGAAATTGGTGAATTAGAATCAGTTCTAGTTCACGAACCAGGACGCGAAATTGACTA
TATTACACCAGCTAGACTAGATGAATTATGCTTCTCAGCTATCTTAGAAAGCCACGATGCTAGA
AAAGAACACAAACAATTCGTAGCAGAATTAAAAGCAAACGACATCAATGTTGTTGAATTAATTG
ATTTAGTTGCTGAAACATATGATTTAGCATCACAAGAAGCTAAAGACAAATTAATCGAAGAATT
TTTAGAAGACTCAGAACCAGTTCTATCAGAAGAACACAAAGTAGTTGTAAGAAACTTCTTAAAA
GCTAAAAAAACATCAAGAGAATTAGTAGAAATCATGATGGCAGGGATCACAAAATACGATTTAG
GTATCGAAGCAGATCACGAATTAATCGTTGACCCAATGCCAAACCTATACTTCACACGTGACCC
ATTTGCATCAGTAGGTAATGGTGTAACAATCCACTACATGCGTTACAAAGTTAGACAACGTGAA
ACATTATTCTCAAGATTTGTATTCTCAAATCACCCTAAACTAATTAACACTCCATGGTACTACG
ACCCTTCACTAAAATTATCAATCGCAGGTGGGGACGTATTTATCTACAACAATGACACATTAGT
AGTTGGTGTTTCTGAAAGAACTGACTTACAAACAGTTACTTTATTAGCTAAAAACATTGTTGCT
AATAAAGAATCTGAATTCAAACGTATTGTTGCAATTAACGTTCCAAAATGGACAAACTTAATGC
ACTTAGACACATGGCTAACAATGTTAGACAAGGACAAATTCCTATACTCACCAATCGCTAATGA
CGTATTTAAATTCTGGGATTATGACTTAGTAAACGGTGGAGCAGAACCACAACCAGTTGAAAAC
GGATTACCTCTAGAAGGATTATTACAATCAATCATTAACAAAAAACCAGTTTTAATTCCTATCG
CAGGTGAAGGTGCTTCACAAATGGAAATCGAAAGAGAAACACACTTCGATGGTACAAACTACTT
AGCAATTAGACCAGGTGTTGTAATTGGTTACTCACGTAACGAAAAAACAAACGCTGCTCTAGAA
GCTGCAGGCATTAAAGTTCTTCCATTCCACGGTAACCAATTATCATTAGGTATGGGTAACGCTC
GTTGTATGTCAATGCCTTTATCACGTAAAGATGTTAAGTGGTAA–3′

FIG. 6

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

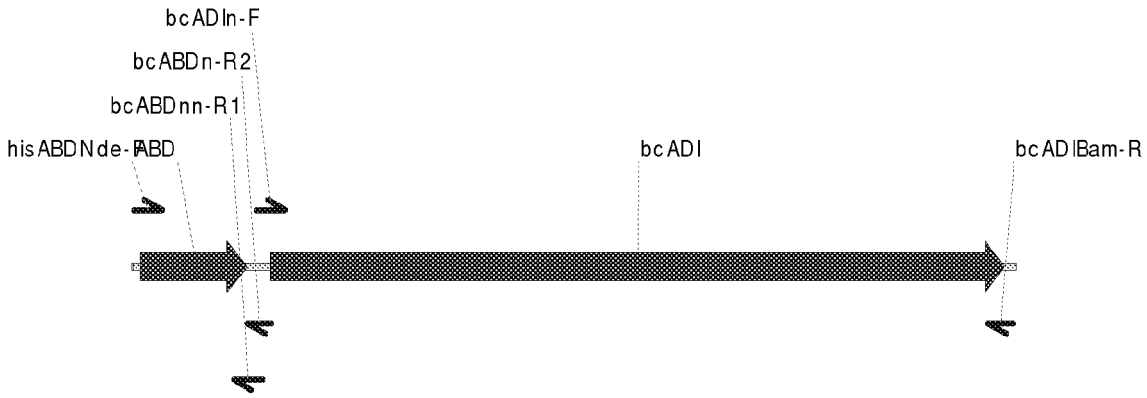
(SEQ ID NO: 40)

MHHHHHHDEAVDANSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALP**SGSNNNNNNGSGG**SVF
DSKFKGIHVYSEIGELESVLVHEPGREIDYITPARLDELCFSAILESHDARKEHKQFVAELKANDINVVELIDLVAET
YDLASQEAKDKLIEEFLEDSEPVLSEEHKVVVRNFLKAKKTSRELVEIMMAGITKYDLGIEADHELIVDPMPNLYFTR
DPFASVGNGVTIHYMRYKVRQRETLFSRFVFSNHPKLINTPWYYDPSLKLSIAGGDVFIYNNDTLVVGVSERTDLQTV
TLLAKNIVANKESEFKRIVAINVPKWTNLMHLDTWLTMLDKDKFLYSPIANDVFKFWDYDLVNGGAEPQPVENGLPLE
GLLQSIINKKPVLIPIAGEGASQMEIERETHFDGTNYLAIRPGVVIGYSRNEKTNAALEAAGIKVLPFHGNQLSLGMG
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
TGCCAAAACTGTTGAAGGTGTAAAAGCACTGATAGATGAAATTTTAGCTGCATTACCTTCGGGT
AGTAACAACAATAATAACAATGGTAGCGGCGGTAAACATCCGATACATGTTACTTCAGAAATTG
GGGAATTACAAACGGTTTTATTAAAACGACCGGGTAAAGAAGTGGAAAACTTGACGCCAGATTA
TTTGCAGCAATTATTATTTGACGATATTCCATACCTACCAATTATTCAAAAAGAGCATGATTAT
TTTGCACAAACGTTACGCAATCGGGGTGTTGAAGTTCTTTATTTAGAAAAACTAGCCGCTGAGG
CGTTAGTAGATAAAAAACTTCGAGAAGAATTTGTTGATCGTATTTTAAAAGAAGGACAGGCCGA
CGTAAATGTTGCACATCAAACTTTAAAAGAATATTTACTTTCCTTTTCAAATGAAGAATTAATT
CAAAAAATTATGGGCGGTGTACGGAAAAACGAAATTGAAACAAGTAAGAAGACACATTTATATG
AATTAATGGAAGATCATTATCCGTTTTACTTAGATCCAATGCCTAATTTATATTTTACTCGTGA
TCCAGCAGCTAGCGTGGGCGATGGCTTAACGATAAATAAGATGAGAGAACCAGCGCGTAGACGT
GAATCATTATTCATGGAGTACATCATTAAATATCATCCAAGATTTGAAAAACATAATGTACCAA
TCTGGTTAGATCGTGATTATAAATTTCCAATTGAAGGTGGCGACGAGCTAATTTTAAATGAAGA
AACAATTGCGATTGGAGTATCTGCTCGTACTTCAGCTAAAGCAATTGAACGTTTAGCAAAAAAT
CTCTTTAGCCGACAAAATAAAATTAAGAAAGTGTTAGCAATAGAAATTCCAAAATGCCGAGCAT
TTATGCATTTAGATACAGTATTTACAATGGTTGATTATGATAAGTTTACAATTCACCCAGCTAT
TCAAGGGCCAAAAGGGAATATGAATATTTATATTTTAGAAAAAGGATCAGATGAGGAAACTCTT
AAAATTACACATCGTACTTCTTTAATGGAAGCATTAAAAGAGGTATTAGGCTTAAGTGAATTAG
TTCTTATTCCATGTGGAGGAGGAGATGTAATTGCTTCTGCTCGTGAACAATGGAATGATGGCTC
GAACACATTAGCAATCGCGCCAGGTGTAGTTGTTACATATGATCGCAACTATGTATCCAATACG
TTATTACGGGAACACGGTATAGAAGTGATTGAGGTGCTAAGTTCAGAATTATCTCGTGGTCGTG
GGGGTCCACGTTGCATGAGTATGCCAATTGTTCGTAAAGATATTTAA–3′

FIG. 7

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

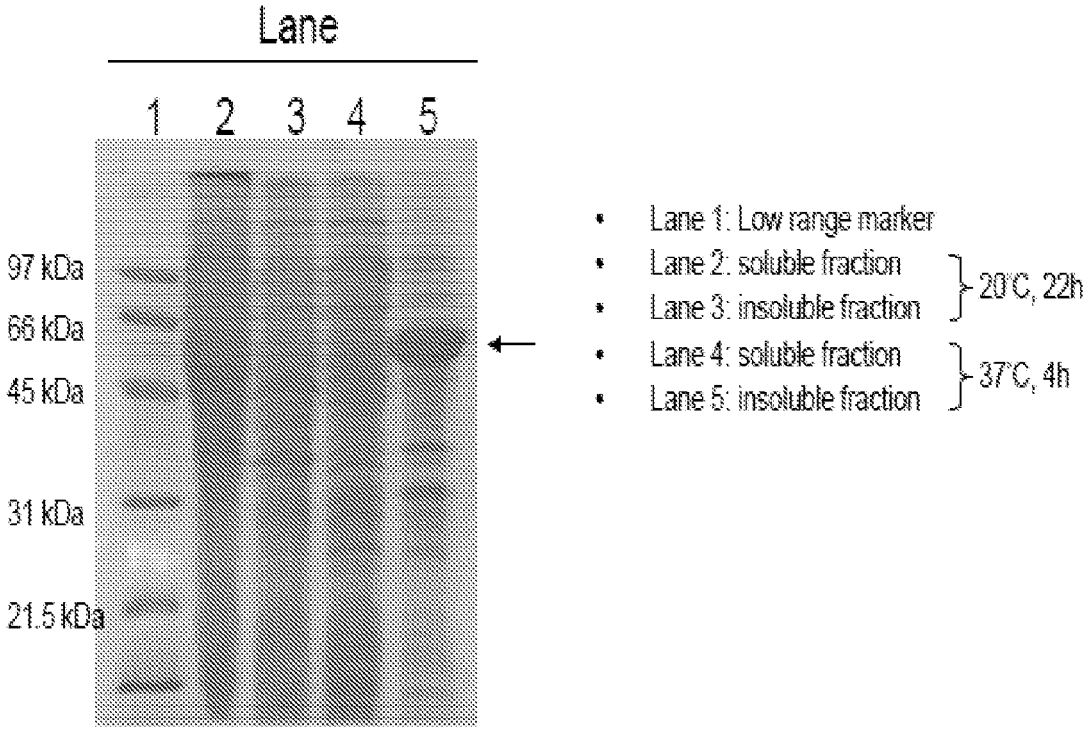
(SEQ ID NO: 41)

MGHHHHHHDEAVDANSLAEAKVLANRELDKYGVSDYYKNLINNAKTVEGVKALIDEILAALPSGSNNNNNNGSGGKH
PIHVTSEIGELQTVLLKRPGKEVENLTPDYLQQLLFDDIPYLPIIQKEHDYFAQTLRNRGVEVLYLEKLAAEALVDKK
LREEFVDRILKEGQADVNVAHQTLKEYLLSFSNEELIQKIMETGGVRKNEIETSKKTHLYELMETEDHYPFYLDPMET
PNLYFTRDPAASVGDGLTINKMETREPARRRESLFMETEYIIKYHPRFEKHNVPIWLDRDYKFPIEGGDELILNEETI
AIGVSARTSAKAIERLAKNLFSRQNKIKKVLAIEIPKCRAFMETHLDTVFTMETVDYDKFTIHPAIQGPKGNMETNIY
ILEKGSDEETLKITHRTSLMETEALKEVLGLSELVLIPCGGGDVIASAREQWNDGSNTLAIAPGVVVTYDRNYVSNTL
LREHGIEVIEVLSSELSRGRGGPRCMETSMETPIVRKDI

(PolyN with linker: SGSNNNNNNGSGG)

FIG. 7 (continued)

(A)


Lane
1 2 3 4 5
97 kDa
66 kDa
45 kDa
31 kDa
21.5 kDa
Lane 1: Low range marker
Lane 2: soluble fraction
Lane 3: insoluble fraction
20°C, 22h
Lane 4: soluble fraction
Lane 5: insoluble fraction
37°C, 4h

(B)

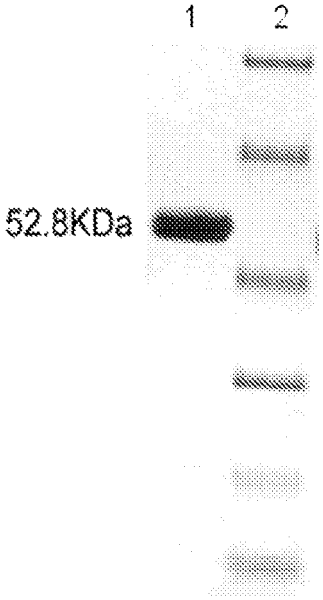

1 2
52.8KDa

FIG. 8

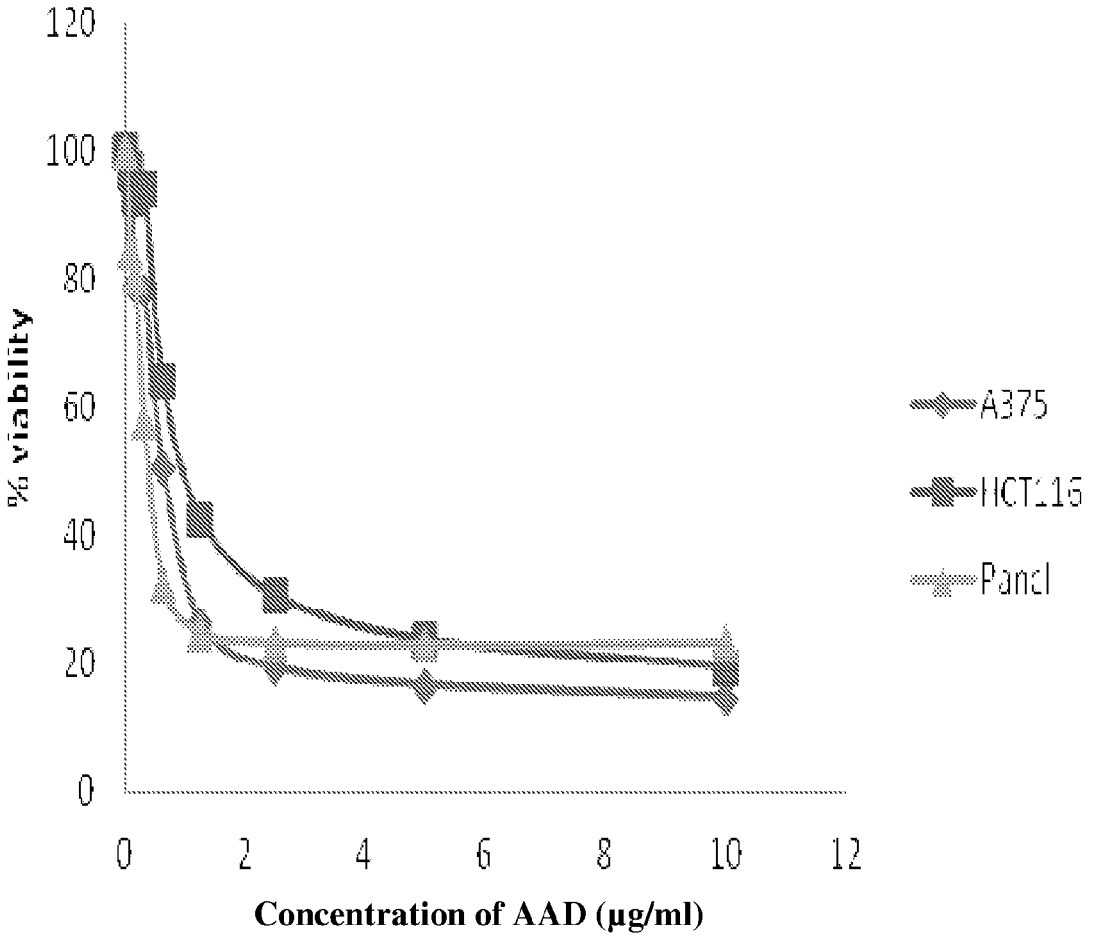

120
100
80
60
40
20
0
% viability
0
2
4
6
8
10
12
Concentration of AAD (µg/ml)
A375
HCT116
Panc1

FIG. 9

(A)

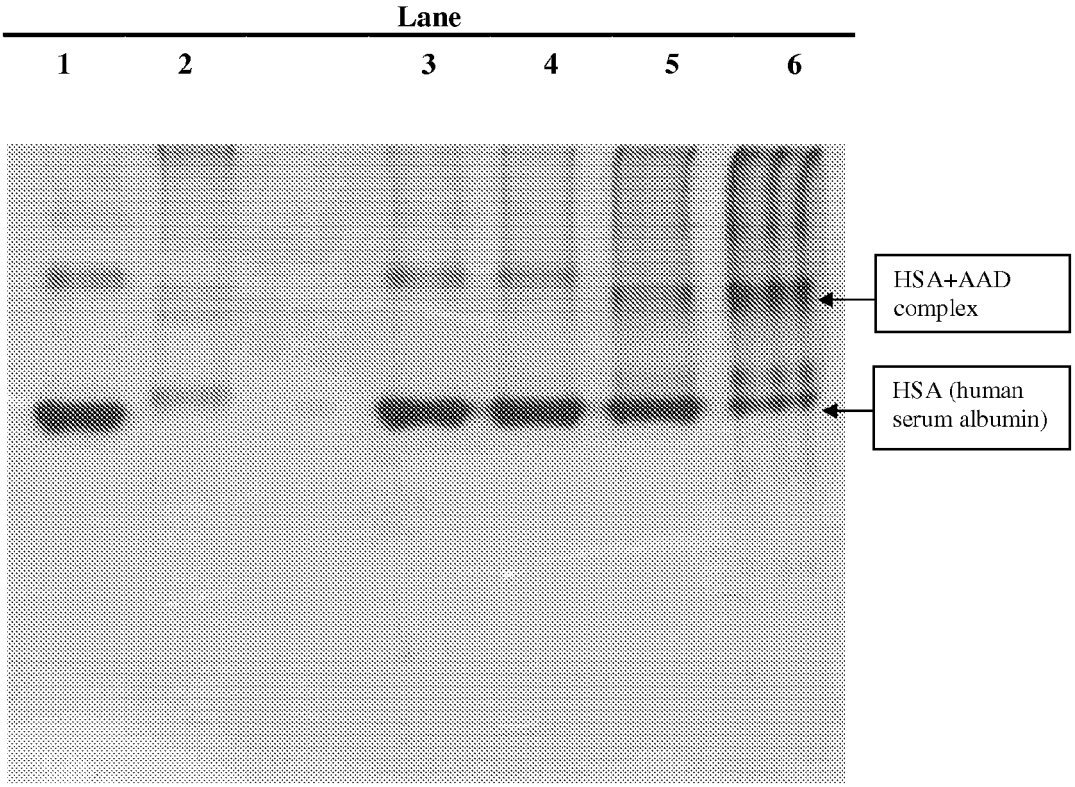
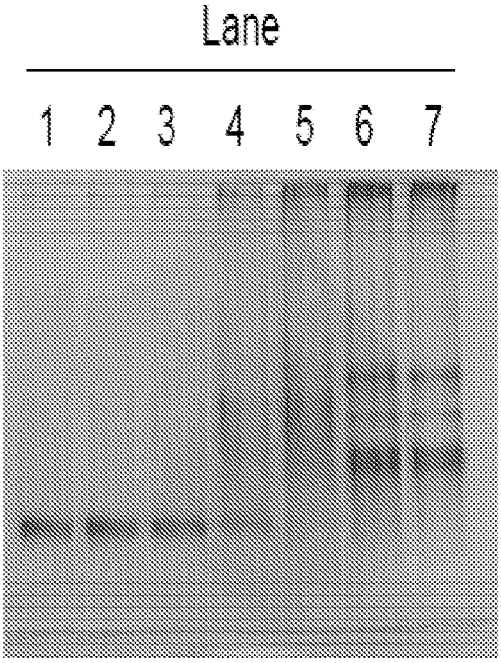


FIG. 10

(B)



Lanes	No. of mole of albumin (pmole)	No. of mole of AAD (pmole)	Albumin : AAD
1	7.5	-	-
2	7.5	7.5	1 : 1
3	7.5	15	1 : 2
4	7.5	30	1 : 4
5	7.5	60	1 : 8
6	7.5	120	1 : 16
7	-	120	-

FIG. 10 (continued)

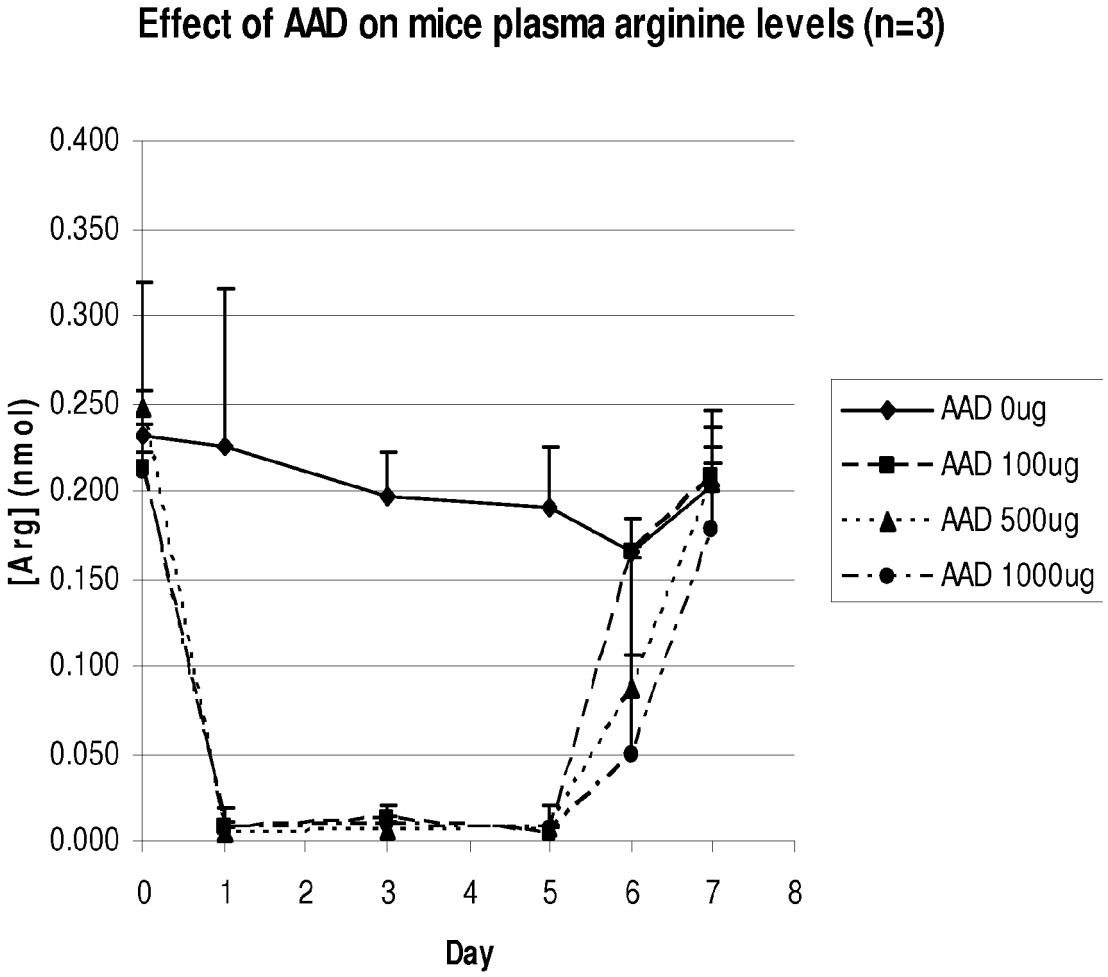

Effect of AAD on mice plasma arginine levels (n=3)
[Arg] (nmol)
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
0
1
2
3
4
5
6
7
8
Day
AAD 0ug
AAD 100ug
AAD 500ug
AAD 1000ug

FIG. 11