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- (71) Applicant (for all designated States except US): **RAMOT AT TEL-AVIV UNIVERSITY LTD.** [IL/IL]; P.o. Box 39296, 61392 Tel-aviv (IL).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **BENHAR, Itai** [IL/IL]; 38 Bareket Street, 76227 Rehovot (IL). **TUR-KASPA, Ran** [IL/IL]; 11 Carmon Street, 90805 Mevaseret Zion (IL). **ZEMEL, Romy** [IL/IL]; 24 Hadar Yosef Street, 69705 Tel-aviv (IL). **GAL-TANAMY, Meital** [IL/IL]; 47 He-beiyar Street, Neve-afek, 48056 Rosh Ha'ayin (IL). **TRACHTENHERZ, Alla** [IL/IL]; P.o. Box 266, 40691 Moshav Herut (IL). **PUPKO, Orly** [IL/IL]; 72 Ilanit Street, 65213 Herzliya (IL). **GER-SHONI, Jonathan M** [IL/IL]; 16/52 Zamarot Street, 46424 Herzliya (IL).
- (74) Agents: **WEBB, Cynthia** et al.; WEBB & ASSOCIATES, P.o. Box 2189, 76121 Rehovot (IL).
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(54) Title: PROTEASE INHIBITORS AND METHOD OF SCREENING THEREOF

(57) Abstract: The present invention provides novel recombinant protein and peptide inhibitors of NS3 serine protease of the hepatitis C virus (HCV). The invention discloses analogs, fragments and derivatives of the identified inhibitors, nucleic acids encoding same, and methods of use thereof for the treatment of HCV infection. The invention further provides novel constructs and methods for the screening of protease inhibitors in vivo, using a recombinant engineered reporter protein that is cleavable by a protease, co-expressed with the recombinant protease in bacteria.



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PROTEASE INHIBITORS AND METHOD OF SCREENING THEREOF

FIELD OF THE INVENTION

The present invention provides novel NS3 serine protease inhibitors, analogs, fragments and derivatives thereof, nucleic acids encoding same, and methods of use thereof for the treatment of Hepatitis C virus (HCV) infection. The present invention further provides a reporter gene system and method of use thereof for screening of protease inhibitors in a host cell.

BACKGROUND OF THE INVENTION

10 Hepatitis C virus

Hepatitis C virus (HCV) is an RNA virus that causes hepatitis, cirrhosis, liver failure and hepatocellular carcinoma (HCC). Development of HCC may occur in up to 10% of HCV-infected individuals. Globally, the seroprevalence of HCV is over 170 million. This implies that over 10 million individuals are at risk for HCV-associated HCC. The magnitude of this potential cancer burden presents an impetus to understand the transforming mechanism(s) of this virus. Currently, the viral-encoded NS3 is one of the viral candidate oncoproteins.

HCV, a member of the *Flaviviridae* family, is a small enveloped virus with a single-stranded, positive-sense RNA genome packed within a nucleocapsid. The 9.6 kb RNA genome is organized to contain a single, large translational open-reading frame that spans most of its length. This encodes a large polyprotein precursor of 3010-3033 amino acids. Four structural and at least six nonstructural (NS) proteins are initially generated by co-translational cleavage of the polyprotein by both cellular and virally-encoded proteases. Most subsequent proteolytic processing events are directed by the virally-encoded NS3 serine protease that requires the adjacent NS4A cofactor for efficient cleavage activity.

NS3 Protease

The HCV trypsin-like serine protease activity resides in the amino-terminal third of the NS3 protein. The mature form of the NS3 is a bifunctional protein having also NTPase and helicase activities located within its carboxyl-terminal domain. NS3

forms a noncovalent complex with NS4A and as such directs proteolytic cleavages at the NS3/4A, NS4A/4B, NS4B/5A, and NS5A/5B junctions and is thus essential for replication of the virus. The crystal structure of the NS3 serine protease has been elucidated and much is known about its structure-function relations. Some enzymatic and structural features make NS3 unique among the serine proteases family. The serine protease domain of NS3 requires unusually long substrates (P6-P4') for effective cleavage and possesses a solvent-accessible structural zinc-binding site. In addition, expression of NS3 has been found to interfere with signal transduction pathways, promote cell proliferation and cause cell transformation. The inventors of the present invention have recently reported that NS3-mediated cell transformation is dependent of its being catalytically active (Zemel et al., 2001).

The quest for NS3 inhibitors

The development of new anti- HCV protease inhibitors is dependent on effective schemes for catalysis and inhibition assays *in vitro* and *in vivo*. High throughput screenings using *in vitro* assays with purified protease and synthetic substrates and structure- based drug design peptide are currently being used in the search for inhibitors. Because the substrate-binding pocket of NS3 is shallow, the binding of small molecule inhibitors is quite inefficient. This explains why previous searches for a good lead inhibitor starting from known protease inhibitors were unsuccessful. Biochemical studies with known protease inhibitors had revealed that the NS3 is inhibited by unusually high concentrations of chymotrypsin-like serine protease inhibitors or by general serine protease inhibitors (Kwong et al., 1998, U.S. Pat. Application No. 20030083467). The observation that the addition of high conventional serine protease inhibitors concentrations resulted in either no inhibition or very weak inhibition of the NS3 protease suggests that the active site of the protease is different from that of cellular serine proteases and that specific non- toxic inhibitors of NS3 could be developed.

Because of the difficulties of *de novo* design of non-toxic small molecule inhibitors to bind to the NS3 protease-binding pocket, many groups have turned to high throughput screening of chemical and natural product libraries to search for novel lead molecules. However, in most cases the tested compounds also inhibited human serine proteases such as chymotrypsin and elastase making them inappropriate candidates for

clinical use (Reviewed in Kwong et al., 1998). Some groups have turned to screening of libraries of 'minimized' antibody-like proteins and single-chain antibodies for NS3 protease inhibitors (Martin et al., 1999).

Bioassays for NS3 protease activity and HCV anti- viral assays

5 The development of secondary *in vivo* bioassays to test whether an inhibitor identified through an *in vitro* assay can fulfill its function in a cellular environment is a critical step in the drug development process. However, several obstacles have hindered efforts at antiviral drug discovery. Foremost has been the absence of fully permissive cell cultures allowing efficient *in vitro* propagation of the virus. This, coupled with the
10 lack of a readily available animal model of chronic hepatitis C, has rendered it difficult to identify and validate lead compounds with potentially useful antiviral activities. Nonetheless, the recent development of subgenomic HCV RNA replicons has provided robust *in vitro* systems for characterizing the replication of the viral RNA in cultured cells. Still, the testing of candidate anti-viral molecules has been limited by the lack of a
15 robust system for growing HCV in cultured cells (reviewed in Lindenbach and Rice, 2005). A cell-based system has been described in which NS3 protease is required for modulation of a reporter gene (Hirowatari et al., 1993). Other *in vivo* assays included the development of chimeric sindbis and polio viruses (Hahm et al., 1996) whose viral replication is dependent on NS3 protease activity. Such systems were designed as
20 secondary to primary *in vitro* screenings to allow investigators to study the potential of NS3 protease inhibitors. However, none of them is of a high-throughput nature.

Genetic screenings for protease inhibitors

Genetic screenings for proteases, and protease inhibitors in general (Dautin et al., 2000; Martinez et al., 2000) and for NS3 inhibitors in particular (Martinez and
25 Clotet 2003) have been described but came short of providing the desired protease inhibitors. With regard to β -galactosidase engineered with protease cleavage sites, Baum et al. (Baum et al., 1990) inserted the HIV protease site into several positions of the *lacZ* gene and tested the resultant engineered derivatives for β -galactosidase activity and for the ability to be cleaved by the HIV protease. Their strategy involved cloning
30 into unique restriction sites that are scattered along the *lacZ* gene and did not involve prior evaluation of the permissiveness of these sites for peptide insertion. Indeed, most of the derivatives they created lost most of the β -galactosidase enzymatic activity (still,

however, growing as blue colonies on X-gal indicative plates). The derivative they chose to further evaluate for cleavage had the site inserted after residue 80. This derivative could be cleaved both *in vitro* and *in vivo* (co-expressed with the protease in *E. coli*) by recombinant HIV protease. The cleavage products could be observed by an immunoblot using anti β -galactosidase sera. More recently, Cheng et al. (Cheng et al., 2004) co-expressed recombinant HIV protease and β -galactosidase with the cleavage site inserted after residue 131. The cleavage products could be detected by an immunoblot as in the previous study. This group applied bacteria that express the HIV protease and the engineered β -galactosidase to screen a library of chemical compounds based on a sulfonamide isostere core and identified candidate inhibitors of the HIV protease.

NS3 is essential for HCV viral replication, and thus it has been an attractive target for drug discovery for the last few years. Several patents disclose NS3 inhibitors (see, for example, U.S. Pat. Nos. 6,608,027, 6,774,212 and 6,767,991).

Inhibitors of the HCV NS3 protease have been described in international applications WO 02/18369, WO 00/09543 (Boehringer Ingelheim), WO 03/064456 (Boehringer Ingelheim), WO 03/064416 (Boehringer Ingelheim), WO 02/060926 (Bristol-Myers Squibb), WO 03/053349 (Bristol-Myers Squibb), WO 03/099316 (Bristol-Myers Squibb), WO 03/099274 (Bristol-Myers Squibb), WO 2004/032827 (Bristol-Myers Squibb), and WO 2004/043339 (Bristol-Myers Squibb).

However, to date there are no serine protease inhibitors available as FDA-approved anti-HCV agents (reviewed in De Francesco and Migliaccio, 2005).

There are currently few effective treatments for HCV. The most established treatment for HCV patients includes administration of recombinant interferon alpha. However, interferons have significant side effects and induce long-term remission in only a fraction (about 25%) of cases. Other agents used to treat chronic hepatitis C include the nucleoside analog ribovirin and ursodeoxycholic acid; however, neither has been shown to be very effective. Moreover, the prospects for effective anti-HCV vaccines remain uncertain (reviewed in De Francesco and Migliaccio, 2005). Thus, there remains a need for more effective anti-HCV therapies. An efficient, high-throughput method of screening for protease inhibitors, and the identification of protease inhibitors, particularly NS3 protease inhibitors, would be highly advantageous.

SUMMARY OF THE INVENTION

The present invention provides novel NS3 serine protease inhibitors, analogs, fragments and derivatives thereof, nucleic acids encoding same, and methods of use thereof for the treatment of hepatitis C virus (HCV) infection. The invention further provides high throughput methods of screening for protease inhibitors *in vivo*, using a recombinant engineered reporter protein that is cleavable by a protease, co-expressed with the recombinant protease in bacteria.

The invention is based in part on the generation of a novel genetic screening for inhibitors of NS3 catalysis, comprising a recombinant engineered reporter protein that is cleavable by a protease, co-expressed with the protease in a host cell. The reporter protein, a recombinant β -galactosidase comprising an NS3 cleavage site in a permissive site, was surprisingly discovered to undergo proteolytic degradation upon its cleavage by the protease. The resulting genetic screening thus allows a highly sensitive, high throughput screening method, which is advantageous to other screening methods, as it is not subject to product inhibition due to accumulation of cleavage products (to that affect, NS3 itself is a protease subject to product inhibition).

The invention is further based, in part, on the discovery of novel NS3 inhibitors isolated by the genetic screening of the invention. Surprisingly, epitope mapping of certain scFv antibody inhibitors isolated according to the invention revealed a novel NS3 epitope overlapping with the NS3 zinc-binding site. This surprising discovery further confirms the uniqueness of the isolated inhibitors, as well as the ability of the genetic screening of the invention to identify such novel inhibitors that could not be identified by other screening methods.

In one aspect, the present invention provides novel NS3 inhibitors. In one embodiment, the inhibitor is a single-chain antibody (scFv) having an amino acid sequence as set forth in any one of SEQ ID NOS:1-11. In another embodiment, the inhibitor is a single antibody domain protein (dAb) derived from the isolated scFv inhibitors of the invention. In a further embodiment, the inhibitor is a dAb having an amino acid sequence as set forth in any one of SEQ ID NOS:12-14 and 113.

In another embodiment, the scFv or dAb is fused to a stabilizing protein. In a preferred embodiment, the stabilizing protein is *E. coli* maltose binding protein (MBP).

In another embodiment, the scFv or dAb is fused to the C terminus of MBP. According to another embodiment, the inhibitor is a scFv fused to the C terminus of MBP, having an amino acid sequence as set forth in any one of SEQ ID NOS:15-25. In another embodiment, the inhibitor is a dAb fused to the C terminus of MBP, having an amino acid sequence as set forth in any one of SEQ ID NOS:26-28 and 114. Other
5 embodiments include fragments, homologs, analogs and derivatives thereof.

In another embodiment, the inhibitor is a peptide having an amino acid sequence as set forth in any one of SEQ ID NOS:29-35. In another embodiment, the inhibitor is a peptide aptamer, comprising a peptide inhibitor of the invention fused to a stabilizing
10 protein. In a preferred embodiment, the stabilizing protein is *E coli* maltose binding protein (MBP). In one preferred embodiment, the peptide is fused to the C terminus of MBP. In another preferred embodiment, the peptide is fused at internal permissive positions of MBP. In another preferred embodiment, the peptide is fused at the internal permissive position following position 133 of MBP. According to another embodiment,
15 the inhibitor is a free peptide derived from a peptide aptamer. In another embodiment, the peptide aptamer has an amino acid sequence as set forth in any one of SEQ ID NOS:36-49. Other embodiments include fragments, homologs, analogs and derivatives thereof.

In another embodiment, the invention comprises nucleic acids encoding the NS3
20 inhibitors of the invention. In a currently preferred embodiment, the nucleic acids encoding the NS3 inhibitors are as set forth in any one of SEQ ID NOS:115-125.

According to additional aspects the invention provides expression vectors and host cells comprising nucleic acid sequences encoding the NS3 inhibitors of the invention.

25 In another aspect, the invention provides pharmaceutical compositions comprising the NS3 inhibitors of the invention and a pharmaceutically acceptable carrier or excipient. In another aspect, the invention provides pharmaceutical compositions comprising nucleic acid sequences encoding the NS3 inhibitors of the invention, expression vectors or host cells comprising same.

30 In other aspects, the invention provides methods of: 1) treating HCV infection; 2) treating or preventing hepatitis; 3) preventing liver failure; 4) preventing chirrrosis; or 5) preventing hepatocellular carcinoma (HCC), in a subject in need thereof,

comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising an NS3 inhibitor of the invention.

According to other aspects, the invention provides methods of: 1) treating HCV infection; 2) treating or preventing hepatitis; 3) preventing liver failure; 4) preventing
5 chirrrosis; or 5) preventing hepatocellular carcinoma (HCC), in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a recombinant construct comprising an isolated nucleic acid sequence encoding an NS3 inhibitor of the invention operably linked to one or more transcription control elements.

10 In further aspects, the invention provides methods of: 1) treating HCV infection; 2) treating or preventing hepatitis; 3) preventing liver failure; 4) preventing chirrrosis; or 5) preventing HCC, in a subject in need thereof, comprising: a) obtaining cells from the subject; b) contacting the cells *ex vivo* with a therapeutically effective amount of a pharmaceutical composition comprising a recombinant construct comprising an isolated
15 nucleic acid sequence encoding NS3 inhibitor of the invention operably linked to one or more transcription control elements; and c) re-introducing said cells to said subject.

In other aspects, the invention provides method of preventing HCV infection in a liver transplant, comprising: a) treating a liver transplant before transplantation *ex vivo* with a therapeutically effective amount of a pharmaceutical composition comprising a
20 recombinant construct comprising an isolated nucleic acid sequence encoding NS3 inhibitor of the invention operably linked to one or more transcription control elements; and b) transplanting the liver transplant to a subject in need thereof, thereby generating an HCV-immune liver transplant.

According to additional aspects, the invention provides novel recombinant
25 reporter gene constructs and uses thereof for the screening and isolation of protease inhibitors, as described hereinbelow.

In one embodiment, the invention provides an isolated polynucleotide comprising a nucleic acid sequence encoding a recombinant reporter protein, wherein the reporter protein comprises a β -galactosidase derivative and an amino acid sequence
30 comprising a protease recognition sequence between residues 279-280 of β -galactosidase, and wherein: (i) the recombinant reporter protein retains β -galactosidase

activity; (ii) the cleavage of the reporter protein by the protease results in reduced β -galactosidase activity; and (iii) the cleavage of said reporter protein by the protease does not substantially result in accumulation of cleavage products capable of substantially inhibiting said protease.

5 In one embodiment, the protease recognition sequence is an HCV NS3 cleavage site. According to certain preferred embodiments, the NS3 cleavage site is selected from a group consisting of: NS5A/B, NS3/4A, NS4A/B and NS4B/NS5A. According to other preferred embodiments, the NS3 cleavage site has an amino acid sequence as set forth in any one of SEQ ID NOS:60-63 and 70. In other preferred embodiments, the
10 recombinant reporter protein has an amino acid sequence according to any one of SEQ ID NOS:64-65. According to another embodiment, the invention provides an isolated polypeptide comprising an amino acid sequence as set forth in any one of SEQ ID NOS:64-65.

 In another embodiment, there is provided a recombinant reporter gene construct
15 comprising a nucleic acid sequence encoding said reporter protein operably linked to one or more transcription control elements. In another embodiment, the reporter gene construct further comprises a nucleic acid sequence encoding a protease capable of cleaving the recombinant reporter protein and/or a potential inhibitor of said protease, operably linked to one or more transcription control elements.

20 In one embodiment, the protease is associated with a disease or disorder in a human or non-human subject. In another embodiment, the protease is a viral protease. In a preferred embodiment, the protease is HCV NS3 protease. In another embodiment, the protease is fused to a stabilizing protein. In one preferred embodiment, the stabilizing protein is *E. coli* maltose binding protein (MBP). In another preferred
25 embodiment, the protease is a recombinant fusion protein comprising NS3, NS4A and MBP. In another preferred embodiment, the protease has an amino acid sequence as set forth in SEQ ID NO:66.

 In one embodiment, the potential inhibitors are peptide aptamers, comprising a peptide fused to a stabilizing protein. In a preferred embodiment, the stabilizing protein
30 is *E. coli* maltose binding protein (MBP). In one preferred embodiment, the peptides are fused to the C terminus of MBP. In another preferred embodiment, the peptides are

fused at internal permissive positions of MBP. In another preferred embodiment, the peptides are fused at the internal permissive position following position 133 of MBP.

In another embodiment, the potential inhibitors are antibody fragments including, but not limited to, single-chain antibodies (scFvs) and single antibody domain proteins (dAbs). In another embodiment, the inhibitors are antibody fragments fused to a stabilizing protein. In a preferred embodiment, the stabilizing protein is *E. coli* maltose binding protein (MBP). In another embodiment, the antibody fragments are fused to the C terminus of MBP. In another embodiment, the potential inhibitors are selected from a library of antibody fragments derived from antibodies isolated from animals immunized against HCV NS3.

In another embodiment, the invention provides an expression vector comprising a reporter gene construct of the invention. According to one preferred embodiment, the vector comprises nucleic acid sequences encoding a recombinant NS3 protease and a recombinant β -galactosidase reporter protein. In another preferred embodiment, the vector has a nucleic acid sequence as set forth in SEQ ID NO:67. In other preferred embodiments, the invention provides an expression vector having a nucleic acid sequence as set forth in SEQ ID NO:68, comprising a nucleic acid sequence encoding a recombinant β -galactosidase reporter protein.

In yet another embodiment, the invention provides a vector having a nucleic acid sequence as set forth in SEQ ID NO:82, useful as a universal platform for insertion of protease-site-coding sequences between *lacZ* codons 279-280.

In yet another embodiment, the expression vectors containing the reporter gene constructs are contained within a host cell. In a preferred embodiment, the host cell is a bacterial host cell. In another preferred embodiment, the host cell is *E. coli*. In certain preferred embodiments, the host cell comprises an expression vector having a nucleic acid sequence as set forth in SEQ ID NO:67, comprising nucleic acid sequences encoding a recombinant NS3 protease and a recombinant β -galactosidase reporter protein. In other preferred embodiments, the host cell comprises expression vectors having nucleic acid sequences as set forth in SEQ ID NOS:68 and 69, comprising nucleic acid sequences encoding a recombinant NS3 protease and a recombinant β -galactosidase reporter protein, respectively.

In another aspect, the invention provides a method of screening for protease inhibitors, comprising:

- a) co-expressing a protease and a recombinant reporter protein cleavable by the protease in a host cell, wherein the cleavage of the reporter protein by the protease results in reduced activity of said reporter protein, and wherein the cleavage of said reporter protein by the protease does not substantially result in accumulation of cleavage products capable of substantially inhibiting said protease;
- b) exposing the host cell to potential inhibitors of said protease; and
- c) screening for host cells that retain said reporter protein activity.

In one embodiment, the recombinant reporter gene encodes a β -galactosidase derivative, wherein a protease recognition sequence of said protease is inserted in a permissive site of β -galactosidase so as to retain β -galactosidase activity. In a preferred embodiment, the protease cleavage site is inserted between residues 279-280 of β -galactosidase.

In one embodiment, the protease recognition sequence is an HCV NS3 cleavage site. According to certain preferred embodiments, the NS3 cleavage site is selected from a group consisting of: NS5A/B, NS3/4A, NS4A/B and NS4B/NS5A. According to other preferred embodiments, the NS3 cleavage site has an amino acid sequence as set forth in any one of SEQ ID NOS:60-63 and 70. In other preferred embodiments, the recombinant reporter protein has an amino acid sequence according to any one of SEQ ID NOS:64-65.

In one embodiment, the protease is associated with a disease or disorder in a human or non-human subject. In another embodiment, the protease is a viral protease. In a preferred embodiment, the protease is HCV NS3 protease. In another embodiment, the protease is fused to a stabilizing protein. In one preferred embodiment, the stabilizing protein is *E. coli* maltose binding protein (MBP). In another preferred embodiment, the protease is a recombinant fusion protein comprising NS3, NS4A and MBP. In another preferred embodiment, the protease has an amino acid sequence as set forth in SEQ ID NO:66.

Potential inhibitors may be expressed by the host cell, either naturally or upon the transformation of suitable constructs encoding them, or may be introduced

externally, e.g. by addition of potential inhibitors to the culture medium. The potential inhibitors may include, but are not limited to, peptides or proteins (either recombinant or naturally occurring), nucleic acids or other organic or inorganic compounds (e.g. carbohydrates and polysaccharides).

5 In one embodiment, the potential inhibitors are peptide aptamers, comprising a peptide fused to a stabilizing protein. In a preferred embodiment, the stabilizing protein is *E. coli* maltose binding protein (MBP). In one preferred embodiment, the peptides are fused to the C terminus of MBP. In another preferred embodiment, the peptides are fused at internal permissive positions of MBP. In a further preferred embodiment, the
10 peptides are fused at the internal permissive position following position 133 of MBP.

In another embodiment, the potential inhibitors are antibody fragments including, but not limited to, single-chain antibodies (scFvs) and single antibody domain proteins (dAbs). In another embodiment, the inhibitors are antibody fragments fused to a stabilizing protein. In a preferred embodiment, the stabilizing protein is *E.*
15 *coli* maltose binding protein (MBP). In another embodiment, the antibody fragments are fused to the C terminus of MBP. In a further embodiment, the potential inhibitors are selected from a library of antibody fragments derived from antibodies isolated from animals immunized against HCV NS3.

In another embodiment, the invention provides protease inhibitors identified by
20 the method of screening disclosed herein.

These and other embodiments of the present invention will become apparent in conjunction with the figures, description and claims that follow.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1E show the steps in evaluation of the bacterial genetic screening
25 for NS3 inhibitors and its application for the isolation of inhibitory scFvs. **A.** Test bacteria that express active NS3 (pMGT14) form white colonies on X-gal supplemented plates while test bacteria that express inactive NS3 (pMGT15) form blue colonies such plates. **B.** Induction of active MBP-scNS3 (gray bars), but not of inactive MBP-scNS3 (black bars) expression with arabinose results in a dose-dependent reduction in β -
30 galactosidase activity as measured by an o-nitrophenyl beta-D-galactopyranoside (ONPG) hydrolysis assay. Error bars represent the standard deviation of the data. **C.**

Evaluation of the dependence of digestion of the engineered β -galactosidase on MBP-*scNS3* induction time by an immunoblot. Total protein extracts prepared from non-induced test bacteria (lane 1) or that were induced for 10, 20, 30 60 and 120 min were loaded in lanes 2,3,4,5 and 6, respectively. Extracts prepared from non-induced test
 5 bacteria that express the inactive NS3 mutant or induced for 60 and 120 min were loaded in lanes 7, 8 and 9, respectively. Purified β -galactosidase was loaded in the M lane. The upper arrow marks the position of the engineered β -galactosidase while the lower arrow marks the position of MBP-*scNS3*. **D.** Application of the genetic screening for the isolation of NS3-inhibitory scFvs. Affinity selected scFv in pMALc-NN were
 10 introduced into test bacteria and plated on X-gal supplemented plates. The arrows mark the positions of a blue and of a white colony. **E.** A comparison of the color developed by genetic screened bacteria that express four of the inhibitory scFvs on the left, and four control scFvs (Berdichevsky et al., 2003) on the right.

Figures 2A-2B demonstrate the evaluation of the selected scFvs binding to NS3
 15 by ELISA. **A.** Binding curves of the selected scFvs to *scNS3*. Error bars represent the standard deviation of the data. **B.** Competition ELISA showing the competition of scFvs 171 and 35 as tracers binding to *scNS3* by the selected scFvs.

Figures 3A-3B demonstrate the evaluation of the selected scFvs inhibition of NS3 catalysis. **A.** β -galactosidase activity determined by an ONPG hydrolysis by non-
 20 induced or isopropyl-beta-d-thiogalactopyranoside (IPTG)-induced genetic screened bacteria carrying selected scFvs. **B.** *In vitro* inhibition of NS3 catalysis at two concentrations of tested scFvs. Error bars represent the standard deviation of the data.

Figure 4 demonstrates the evaluation of the selected peptide aptamers from the NNS8 and the Rand3 libraries. *In vitro* inhibition of NS3 catalysis at several
 25 concentrations of tested aptamers is presented. Neg is a negative control aptamers (isolated as a white colony) from the Rand3 library.

Figure 5 exhibits the amino-acid sequence of the engineered substrate (reporter protein): a fusion protein comprised of the 78 N-terminal residues of the *E. coli trpR* gene product followed by a short linker (both in italics) fused to the 8th codon of the *E.*
 30 *coli lacZ* gene product with the NS3-cleavable NS5A/B site (in bold type) inserted between residues 279-280 of the *E. coli lacZ* gene product (original numbering of the *E. coli lacZ* gene product).

Figure 6 exhibits the amino-acid sequence of the engineered enzyme: a fusion protein comprised of the *E. coli malE* gene product (MBP) fused the NS4A peptide (in bold type) followed by the NS3 protease domain derived from the BK strain of HCV

Figures 7A-7B exhibit aligned amino-acid sequences of: **A.** NS3-inhibiting scFvs; the V_H and V_L (complementarity-determining regions) CDRs are underlined; the linker is in italics; **B.** Four NS3-inhibiting single-domain antibodies (V_H). The CDRs are underlined. These clones were initially identified in the truncated form due to internal stop codons or due to a single base deletion resulting in frameshifting and premature translation termination, which resulted in their expression as single antibody VH domains. They were also characterized after replacing the stop codon with a sense codon, or by adding in the missing base, as they appear as intact scFvs with the corresponding number in Figure 7A.

Figures 8A-8B show spun – cell ELISA of scFvs 35 and 171 cell display analysis. scFvs cell surface displays were evaluated by testing the binding of NS3 to scFv-displaying bacteria at 4 different IPTG concentrations: 0, 0.001, 0.01 and 0.1 mM. **A.** scFv-35 (black bars) and scFv-H23 as negative control (gray bars). **B.** scFv-171 (black bars) and scFv-CD30 as negative control (gray bars). Error bars represent the standard deviation of the data.

Figures 9A-9B demonstrate Dot-Blot results of scFv-171 binders from DIP output of FDC12C library. **A.** 192 single colonies were screened (96 on each filter). White circles mark the phages that were suspected as binders and were verified by another Dot-Blot analysis. Black circles mark the phages that were picked as negative control for the verifying Dot-Blot. **B.** Dot-Blot analysis for verifying the suspected binders from the first Dot-Blot. Phages were loaded longitudinally in secondary dilutions. Column F3, D14 and D21 were loaded with the negative control phages.

Figures 10A-10B demonstrate Dot-Blot results of scFv-35 binders from DIP output of FM7 and FDC12C mixed libraries. **A.** 192 single colonies were screened. White circles mark the phages that were suspected as binders and were verified by another Dot-Blot analysis. Black circles mark the phages that were picked as negative control for the verifying Dot-Blot. **B.** Dot-Blot analysis for verifying the suspected binders from the first Dot-Blot. Phages were loaded longitudinally in secondary dilutions. Column F9 and E13 were loaded with the negative control phages.

Figures 11A-11B demonstrate Dot-Blot results of scFv-35 binders from panning output of FM7 and FDC12C mixed libraries. **A.** 192 single colonies were screened. White circles mark the phages that were suspected as binders and were verified by another Dot-Blot analysis. Black circles mark the phages that were picked as negative control for the verifying Dot-Blot. **B.** Dot-Blot analysis for verifying the suspected binders from the first Dot-Blot. Phages were loaded longitudinally in secondary dilutions. Column A6 and B15 were loaded with the negative control phages.

Figures 12A-12B demonstrate RasTop representation of scFvs 35 and 171 predicted epitopes on NS3. NS3 is shown as a ribbon representation. The catalytic triad is shown as space-fill residues in gray. **A.** scFv-35 epitope on NS3 is shown as space-fill residues in black: residues 97-102, 147, 148 – CTCGSS, TG -. **B.** scFv-171 epitope on NS3 is shown as space-fill residues in black: residues 95, 97-103, 148, 149 – T, CTCGSSA, GH.

Figure 13 demonstrates the shared epitope of scFvs 35 and 171 on NS3. NS3 is shown as a ribbon representation. The catalytic triad is shown as space-fill residues in gray at the bottom. The shared epitopes residues shown as space-fill in gray. The two cysteines and the histidine residues that are part of the enzyme zinc-binding site are marked by arrows. Residues that are unique to the mapped epitope of each antibody (not shared) are circled and marked by arrows.

Figure 14A-14B show the ScFvs 35 and 171 competitive *in vitro* assays with epitope mimetic peptide. **A.** Evaluation of MBP-scFv 35 without peptide (white bars), in the presence of the epitope-mimetic peptide (gray bars), in the presence of control peptide X (horizontal line bars), in the presence of control peptide 6 (diagonal line bars). **B.** Evaluation of MBP-scFv 171 without peptide (dotted bars), in the presence of the epitope-mimetic peptide (gray bars), in the presence of control peptide X (horizontal line bars), in the presence of control peptide 6 (diagonal line bars). **C.** Controls: evaluation of MBP-scFv 35 without peptide (white bars), MBP-scFv 171 without peptide (dotted bars), the epitope-mimetic peptide without scFv (gray bars), the control peptide X without scFv (horizontal line bars), the control peptide 6 without scFv (diagonal line bars). Error bars represent the standard deviation of the data.

Figure 15 demonstrates the inhibition of HCV RNA replicon amplification. Activity of SEAP secreted from Huh7 cells supporting replication of HCV RNA

replicons over successive 24-h intervals following transient transfection with intrabodies.

5 DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel NS3 serine protease inhibitors, analogs, fragments and derivatives thereof, nucleic acids encoding same, and methods of use thereof for the treatment of HCV infection. The invention further provides novel constructs and methods for the screening of protease inhibitors *in vivo*.

10 The present invention is based in part on the generation of a novel screening for inhibitors of NS3 catalysis. The inventors constructed a genetic screening based on the concerted co-expression of a reporter gene, of recombinant NS3 and of stabilized potential inhibitors, in a host cell. The reporter system was constructed by inserting a peptide corresponding to the NS5A/B cleavage site of NS3 into a permissive site of the
15 enzyme β -galactosidase, with NS3 expressed from the same plasmid. The resultant β -galactosidase enzyme is active, conferring a Lac⁺ phenotype that is lost upon induction of NS3 expression. The identification of inhibitors, demonstrated herein by isolating NS3 inhibiting single-chain antibodies (scFvs), single-domain antibodies (dAbs) or peptide aptamers, expressed from a compatible plasmid, is based on the appearance of
20 blue colonies (NS3 inhibited) on the background of colorless colonies (NS3 active) on X-gal indicative plates. Surprisingly, upon the intracellular cleavage of the engineered β -galactosidase by NS3 in *E. coli*, the resultant cleavage products were found to undergo proteolytic degradation, thus preventing potential obscuring by product inhibition of NS3 catalysis. The putative inhibitors were stabilized by their fusion to
25 maltose binding protein (MBP), resulting in increased intracellular inhibitor concentrations. Thus, the constructed assay was characterized as a highly sensitive screening method capable of identifying NS3 inhibitors that escape detection by *in vitro* approaches, and is highly advantageous compared to other screening methods that due to accumulation of cleavage products, may be subject to product inhibition (to that
30 affect, NS3 itself is a protease subject to product inhibition).

Constructs and methods of screening for protease inhibitors

According to certain aspects, the invention provides novel constructs and methods for the screening of protease inhibitors *in vivo*, using a recombinant engineered reporter protein that is cleavable by a protease, co-expressed with the protease in a host cell. The proteolytic cleavage of the reporter protein does not result in accumulation of cleavage products in the host cell, thus enabling a highly sensitive, high throughput screening method which is preferable to other screening methods compromised by product inhibition.

In one embodiment, the invention provides an isolated polynucleotide comprising a nucleic acid sequence encoding a recombinant reporter protein, wherein the reporter protein comprises a β -galactosidase derivative and an amino acid sequence comprising a protease recognition sequence between residues 279-280 of β -galactosidase, and wherein: (i) the recombinant reporter protein retains β -galactosidase activity; (ii) the cleavage of the reporter protein by the protease results in reduced β -galactosidase activity; and (iii) the cleavage of said reporter protein by the protease does not substantially result in accumulation of cleavage products capable of substantially inhibiting said protease.

In one embodiment, the protease recognition sequence is an HCV NS3 cleavage site. According to certain preferred embodiments, the NS3 cleavage site is selected from a group consisting of: NS5A/B, NS3/4A, NS4A/B and NS4B/NS5A, which may vary for different HCV genotypes and subtypes (Kwong et al., 1998). According to other preferred embodiments, the NS3 cleavage site is a cleavage site derived from the 1b subtype HCV BK strain, selected from a group consisting of: NS5A/B (EDVVCCSMSY, SEQ ID NO:60, and ASEDVVCCSMSY SEQ ID NO:70), NS3/4A (DLEVVTSTWV, SEQ ID NO:61), NS4A/B (DEMEECASHL, SEQ ID NO:62) and NS4B/NS5A (DCSTPCSGSW, SEQ ID NO:63). In other preferred embodiments, the recombinant reporter protein has an amino acid sequence according to any one of SEQ ID NOS:64-65 presented in Figure 5, wherein the amino acid sequence of the engineered substrate is denoted as SEQ ID NO:64 and the amino acid sequence of the engineered substrate without the preceding N-terminal residues corresponding to the *E. coli TrpR* gene product and the linker, is denoted as SEQ ID NO:65. According to another embodiment,

the invention provides an isolated polypeptide comprising an amino acid sequence as set forth in any one of SEQ ID NOS:64-65.

In another embodiment, there is provided a recombinant reporter gene construct comprising a nucleic acid sequence encoding said reporter protein operably linked to one or more transcription control elements. In another embodiment, the reporter gene construct further comprises a nucleic acid sequence encoding a protease capable of cleaving the recombinant reporter protein and/or a potential inhibitor of said protease, operably linked to one or more transcription control elements, as will be described hereinbelow.

In another aspect, the invention provides a method of screening for protease inhibitors, comprising:

- a) co-expressing a protease and a recombinant reporter protein cleavable by the protease in a host cell, wherein the cleavage of the reporter protein by the protease results in reduced activity of said reporter protein, and wherein the cleavage of said reporter protein by the protease does not substantially result in accumulation of cleavage products capable of substantially inhibiting said protease;
- b) exposing the host cell to potential inhibitors of said protease; and
- c) screening for host cells that retain said reporter protein activity.

In another aspect, the invention provides protease inhibitors identified by the method of screening disclosed herein.

Suitable embodiments for the production and use of the constructs and methods of the present invention in identifying and isolating protease inhibitors are disclosed in detail herein.

1. Construction of the genetic screening

Reporter genes. In one embodiment, the recombinant reporter gene encodes a β -galactosidase derivative, wherein a protease recognition sequence of said protease is inserted in a permissive site of β -galactosidase so as to retain β -galactosidase activity. In a preferred embodiment, the protease cleavage site is inserted between residues 279-280 of β -galactosidase.

The term “ β -galactosidase activity” as used herein indicates the ability of the reporter protein to hydrolyze lactose yielding glucose and galactose, allowing utilization of lactose as a carbon source. The enzymatic activity of β -galactosidase may be detected by utilizing suitable color indicator compounds, including, but not limited to

5 X-gal and Fast Blue RR, which, upon their cleavage by β -galactosidase, produce a detectable color. Thus, for example, bacteria expressing said reporter protein appear as blue colonies when grown on a semi-solid medium-containing X-gal. Other substrates that may be used for detecting β -galactosidase activity include, but are not limited to, substrates that upon cleavage result in a fluorescent signal, e.g. Fluorescein- β -D-

10 Galactopyranoside (FDG; commercially available from Marker Gene Technologies, Inc.), or in signals measurable by amperometric sensors with substrates such as PAPG

The term “protease recognition sequence” refers to a consecutive amino acid sequence that is recognized by a protease of interest and is required for the proteolytic cleavage. A protease recognition sequence may be coincident with the protease

15 cleavage site (i.e., the site at which the cleavage by the protease occurs). That is, the protease recognition sequence may include one or more amino acids on either side of the peptide bond to be hydrolyzed by the protease. Alternatively, the protease recognition sequence may be one, two or more amino acids distal, at the amino or carboxy terminus, to the cleavage site of the protease, as long as the cleavage of said

20 reporter protein by said protease does not substantially result in accumulation of cleavage products capable of substantially inhibiting said protease. In certain embodiments, a protease recognition sequence of the present invention is derived from the amino acid sequence of a naturally occurring substrate of the protease of interest.

In one embodiment, the protease recognition sequence is an HCV NS3 cleavage

25 site. According to certain preferred embodiments, the NS3 cleavage site is selected from a group consisting of: NS5A/B, NS3/4A, NS4A/B and NS4B/NS5A. According to other preferred embodiments, the NS3 cleavage site has an amino acid sequence as set forth in any one of SEQ ID NOS:60-63 and 70. In other preferred embodiments, the recombinant reporter protein has an amino acid sequence according to any one of SEQ

30 ID NOS:64-65.

Other reporter genes may also be used, as long as: (i) the reporter protein is capable of being assayed for a change in color, light or activity when combined with a

protease enzyme in a host cell; (ii) the introduction of the protease recognition site does not eliminate the activity of the reporter protein, so that the fusion protein can be used in an assay system to screen for inhibitors of said protease; and (iii) the cleavage of said reporter protein by said protease does not substantially result in accumulation of
5 cleavage products capable of substantially inhibiting said protease.

When the protease enzyme is added to the resulting reporter protein, the protease cleaves at the cleavage site, thereby resulting in reduced activity of said reporter protein. The phrases "reduced activity of a reporter protein" and "substantially inhibiting a protease" refer to a detectable reduction in the activity of the reporter
10 protein monitored by means of, for example, color change, light change or other known methods of monitoring the enzymatic activity. When determining whether the cleavage of a reporter gene by a protease substantially results in the accumulation of cleavage products capable of substantially inhibiting the protease, in addition to the functional assays for protease activity described above, lysates of host cells in which the reporter
15 protein and the protease are expressed may be assayed for degradation products of the reporter protein. This may be achieved, for example, using antibodies recognizing the reporter protein, by methods well known in the art.

Examples for other reporter genes that may be utilized to generate a recombinant reporter gene of the invention include, but are not limited to: alkaline
20 phosphatase, β -glucuronidase, acetyltransferase, luciferase, green fluorescent protein, red fluorescent protein, aequorin, chloramphenicol acetyl transferase and horseradish peroxidase.

Proteases. The present invention may be used to screen for inhibitors of any protease of interest, including naturally occurring proteases, naturally occurring variants
25 of wild type proteases, and artificially mutagenized proteases, as long as they are enzymatically active.

In one embodiment, the protease is associated with a disease or disorder in a human or non-human subject. Exemplary proteases include those that have been implicated in human diseases, e.g. trypsin and elastase that are involved in the onset of
30 emphysema, and renin which has been implicated in hypertension. Other exemplary proteases are essential for the replication of microbial pathogens (e.g., HCV, poliovirus and HIV proteases), or involved in the destructive effects of microbial pathogens in

ways that do not involve replicative processes (e.g., collagenases from *Clostridium histolyticum* that participate in the invasiveness of the bacterium by destroying the connective tissue barriers of the host).

In one embodiment, the protease is a viral protease. In a preferred embodiment, the protease is HCV NS3 protease, which sequence may vary for different HCV genotypes and subtypes.

In another embodiment, the protease is fused to a stabilizing protein. In a preferred embodiment, the stabilizing protein is *E. coli* maltose binding protein (MBP). In another preferred embodiment, the protease is a recombinant fusion protein comprising NS3, NS4A and MBP. In another preferred embodiment, the protease has an amino acid sequence as set forth in SEQ ID NO:66.

Recombinant constructs and transcription control elements. The present invention further provides recombinant constructs, comprising reporter genes, proteases and/or potential inhibitors operably linked to transcription control elements, i.e. nucleic acid sequences regulating their expression in a host cell. For a prokaryotic host cell, such transcription control elements include promoters, optionally containing operator portions. The phrase "operably linked" refers to linking a nucleic acid sequence to a transcription control element in a manner such that the molecule can be expressed when transformed, transfected or transduced into a host cell.

Various prokaryotic transcription control elements are known in the art, including, but not limited to, the *lac*, *tac*, *trpR*, *araBAD*, *recA*, T7, λP_R and λP_L promoters. The reporter genes, proteases and potential inhibitors may be controlled by the same transcription control element or by different transcription control elements. Advantageously, the genes are operably linked to different transcription control elements, thus enabling a differential regulation of their expression levels and an inducible expression when desired. One non-limitative approach is demonstrated in the Examples, in which the *lacZ* reporter was subcloned under the control of the *trpR* promoter, the NS3 protease was subcloned under the control of the *araBAD* promoter, and potential inhibitors were subcloned under *tac* promoter. Thus, three different regulatory mechanisms for expression were utilized: IPTG-inducible high-level expression of potential inhibitors, arabinose-inducible medium level NS3 expression and constitutive low level of β -galactosidase expression. The system is thus tuned such

as sufficient protease is made to fully digest the engineered reporter gene, unless inhibited by an excess of inhibitor that is produced at still a higher concentration.

Vectors. In one embodiment, there is provided an expression vector comprising at least one nucleic acid sequence encoding a polypeptide or peptide selected from: a protease of the invention, a reporter protein of the invention and a potential protease inhibitor of the invention, operably linked to one or more transcription control elements.

An "expression vector" refers to a nucleic acid molecule capable of replication and expressing a gene of interest when transformed, transfected or transduced into a host cell. The expression vectors comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desired, provide amplification within the host. Selectable markers include, for example, sequences conferring antibiotic resistance markers, which may be used to obtain successful transformants by selection, such as ampicillin, tetracycline and kanamycin resistance sequences, or supply critical nutrients not available from complex media. Suitable expression vectors may be plasmids derived, for example, from pBR322 or various pUC plasmids, which are commercially available. Other expression vectors may be derived from bacteriophage, phagemid, or cosmid expression vectors, all of which are described in sections 1.12-1.20 of Sambrook et al., (Molecular Cloning: A Laboratory Manual. 3rd edn., 2001, Cold Spring Harbor Laboratory Press). Isolated plasmids and DNA fragments are cleaved, tailored, and ligated together in a specific order to generate the desired vectors, as is well known in the art (see, for example, Sambrook et al., *ibid*).

According to one preferred embodiment, the vector comprises nucleic acid sequences encoding a recombinant NS3 protease and a recombinant β -galactosidase reporter protein. In another preferred embodiment, the vector is pMGT14 (see Example 1), having a nucleic acid sequence as set forth in SEQ ID NO:67. In other preferred embodiments, the invention provides an expression vector having a nucleic acid sequence as set forth in SEQ ID NO:68 (pEB13-*lacZ*_{NS5A/B}, see Examples), comprising a nucleic acid sequence encoding a recombinant β -galactosidase reporter protein.

In yet another embodiment, the invention provides a vector having a nucleic acid sequence as set forth in SEQ ID NO:82 (pEB13-Sfi, see Examples), useful as a

universal platform for insertion of protease-site-coding sequences between *lacZ* codons 279-280.

Host cells. In yet another embodiment, the expression vector comprising the nucleic acid sequence is contained within a host cell. In a preferred embodiment, the host cell is a bacterial host cell. In another preferred embodiment, the host cell is *E. coli*. Other suitable prokaryotic hosts include, but are not limited to: *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*. In other embodiments, eukaryotic host cells are used, including, but not limited to plant cells, yeast cells, insect cells or animal cells, with suitable vectors and expression control elements known in the art.

Prokaryotic host cells or other host cells with rigid cell walls are preferably transformed using the calcium chloride method as described in section 1.82 of Sambrook et al., (*ibid*). Alternatively, electroporation may be used for transformation of these cells. Prokaryote transformation techniques are known in the art, e.g. Dower, W. J., in Genetic Engineering, Principles and Methods, 12:275-296, Plenum Publishing Corp., 1990; Hanahan et al., Meth. Enzymol., 204:63 1991.

In certain preferred embodiments, the host cell comprises an expression vector having a nucleic acid sequence as set forth in SEQ ID NO:67, comprising nucleic acid sequences encoding a recombinant NS3 protease and a recombinant β -galactosidase reporter protein. In other preferred embodiments, the host cell comprises expression vectors having nucleic acid sequences as set forth in SEQ ID NOS:68 and 69 (see Examples) comprising nucleic acid sequences encoding a recombinant NS3 protease and a recombinant β -galactosidase reporter protein, respectively.

2. Screening and identification of inhibitors.

Host cells expressing the reporter protein and protease of the invention are exposed to potential inhibitors of the protease. Next, the host cell population is screened for cells that retain reporter protein activity.

The term "exposed" is used herein to indicate that a host cell is contacted with a potential protease inhibitor such that the inhibitor can effectively inhibit the protease. Potential inhibitors may be expressed by the host cell, either naturally or upon the transformation of suitable constructs encoding them, or may be introduced externally,

e.g. by addition of potential inhibitors to the culture medium. A potential inhibitor is identified as an inhibitor of the protease if the reporter protein is not cleaved and remains active when monitored by means of, for example, color change, light change or other known methods of monitoring the enzymatic activity, as described above. Host
5 cells exposed to a functional inhibitor can thus be distinguished from host cells expressing an uninhibited protease, and are considered to “retain reporter protein activity”.

Inhibitors and libraries. A potential inhibitor may be a single compound of interest or a member of a library of potential inhibitors. For example, a library of potential
10 inhibitors may be a synthetic combinatorial library (e.g., a combinatorial chemical library), a cellular extract, a bodily fluid (e.g., urine, blood, tears, sweat, or saliva), or other mixture of synthetic or natural products (e.g., a library of small molecules or a fermentation mixture). A library of potential inhibitors can include, for example, amino acids, peptides, polypeptides, proteins (including, but not limited to, antibodies,
15 antibody fragments and peptide aptamers), or fragments of peptides or proteins; nucleic acids (e.g., DNA; RNA; or peptide nucleic acids, PNA); aptamers; or compounds such as carbohydrates and polysaccharides. Each member of the library can be singular or can be a part of a mixture (e.g., a compressed library). The library can contain purified compounds or can be “dirty” (i.e., containing a significant quantity of impurities).

20 Commercially available libraries (e.g., from Affymetrix, ArQule, Neose Technologies, Sarco, Ciddco, Oxford Asymmetry, Maybridge, Aldrich, Panlabs, Pharmacopoeia, Sigma, or Tripose) can also be used with the new methods.

In addition to libraries of potential inhibitors, special libraries called diversity files can be used to assess the specificity, reliability, or reproducibility of the new
25 methods. Diversity files contain a large number of compounds (e.g., 1000 or more small molecules) representative of many classes of compounds that could potentially result in nonspecific detection in an assay. Diversity files are commercially available or can also be assembled from individual compounds commercially available from the vendors listed above.

30 Fusion-stabilized peptides (peptide aptamers). Peptide aptamers represent a novel generation of molecules in which variable peptides are inserted into a protein scaffold. As such, they can bind to their target *in vivo* and have the potential to

selectively block its activity. Several bacterial proteins had been recently applied as scaffolds for peptide aptamers, including thioredoxin, staphylococcal nuclease and alpha-amylase, as well as non-bacterial proteins such as green fluorescent protein (Colas, 2000) (Hoppe-Seyler and Butz, 2000). The scaffold share intrinsic stability making it possible to express peptide aptamers *in vivo* at high concentrations, and, having the peptide aptamer been identified, utilize its high level expression and easy purification for subsequent analysis. Once identified, a peptide aptamer may be evaluated as a free peptide, where in some cases it is as active as in the context of the aptamer (Hoppe-Seyler and Butz, 2000). Moreover, small synthetic molecules may be derived from such bioactive aptamers to form the basis of new therapeutics.

The *E. coli* maltose binding protein (MBP) has not been applied before as a scaffold for peptide aptamers before. The inventors chose MBP as a scaffold for the fusion-stabilized peptide aptamers for several reasons. MBP is produced at very high levels in *E. coli* from which it can be recovered and purified by a single-step affinity chromatography on amylose columns. MBP confers stability on peptides and proteins that are fused to it, as was reported by the inventors and others (Bach et al., 2001). Peptides may be linked at the C-terminus of MBP in a linear (unconstrained) form, or structurally (conformationally) constrained in internal positions of MBP that are permissive to peptide insertion (Martineau et al., 1996). It was reported that in some cases, constrained peptides might prove better binders than unconstrained ones (Colas 2000).

In one embodiment, the potential inhibitors are peptide aptamers, comprising a peptide fused to a stabilizing protein. In a preferred embodiment, the stabilizing protein is *E. coli* maltose binding protein (MBP). In one preferred embodiment, the peptides are fused to the C terminus of MBP. In another preferred embodiment, the peptides are fused at internal permissive positions of MBP. In another preferred embodiment, the peptides are fused at the internal permissive position following position 133 of MBP.

Recombinant antibody fragments. Antibodies are versatile immunological reagents used for a variety of diagnostic and therapeutic applications. Traditional monoclonal or polyclonal antibodies are divalent and are highly useful because of their specific and high-affinity binding to antigen. However, small antibody fragments are proving to have the same utility. The advent of recombinant techniques has allowed for

the generation of monovalent synthetic antibody fragments, such as single-chain antibodies (scFvs) and Fab fragments that lack a portion or all of the antibody constant domains normally found in an intact antibody. Single-chain antibodies are small recognition units consisting of the variable regions of the immunoglobulin heavy (V_H) and light (V_L) chains which are connected by a synthetic linker sequence. Single antibody domain proteins (dAbs) are minimized antibody fragments comprising either an individual V_L domain or an individual V_H domain. The smaller size of antibody fragments compared to whole antibodies is advantageous to applications requiring, e.g. tissue penetration or rapid blood clearance.

Antibodies and antibody fragments may be particularly advantageous as protease inhibitors. For example, inhibition of NS3 catalysis by small molecule inhibitors has been a formidable challenge due to featureless appearance of the NS3 catalytic groove that results in weak binding; antibodies may offer a better solution by forming a "clamp" that involves interactions with NS3 that are located away from the active site on one hand, and blocking or interfering with substrate binding on the other. In addition, it has been recently shown that recombinant antibody fragments, when synthesized within a living cell and targeted to a particular subcellular compartment, can be used to specifically interfere with the normal trafficking of the target molecule, thereby preventing it from accumulating at its normal destination. The inventors have recently reported the isolation of non-neutralizing anti-NS3 scFvs by antibody phage-display, that as intracellular antibodies (intrabodies) partially inhibited NS3-mediated cell transformation by diverting NS3 from the cell cytoplasm to the nucleus (Zemel et al., 2004). The studies of intrabody-mediated phenotypic modulation or prevention of cell transformation demonstrated that fine-tuning of the desired phenotype can be achieved by directing the intrabodies into various compartments within the cell (Marasco, 1997).

In another embodiment, the potential inhibitors are antibody fragments including, but not limited to, single-chain antibodies (scFvs) and single antibody domain proteins (dAbs). In another embodiment, the inhibitors are antibody fragments fused to a stabilizing protein. In a preferred embodiment, the stabilizing protein is *E. coli* maltose binding protein (MBP). In another embodiment, the antibody fragments are fused to the C terminus of MBP. In another embodiment, the potential inhibitors are

selected from a library of antibody fragments derived from antibodies isolated from animals immunized against HCV NS3.

The investigation of the properties of antibodies and their application as intrabodies require efficient tools to generate and isolate these molecules. Phage display technology paved the way for antibody engineering as a tool to generate and characterize antibodies and in parallel gain access to the genes that encode for them (Benhar 2001). Recombinant antibody fragments may be isolated from phage libraries by affinity selection or more advanced approaches such as DIP selection (Benhar et al., 2000).

10 Isolation of protease inhibitors.

According to certain embodiments, various methods may optionally be used to enrich for protease inhibitors prior to the screening step. The enrichment can be preformed before or after exposing the host cells to the potential protease inhibitors.

For instance, when the inhibitors are expressed within the host cells, the host cell population may be enriched for inhibitor expressing clones. A non-limitative approach is described herein, in which a β -galactosidase derivative is used as a reporter protein in *E. coli*. Upon inducing the expression of the potential protease inhibitors, the cells are initially grown on lactose as a sole carbon source; the enrichment is brought about by virtue of faster growth rate of protease-inhibited clones.

20 Other examples may include enriching a library for protease binders *in vitro*, e.g. by affinity selection.

Screening for protease inhibitors is performed as described above, by identifying host cells which retain protease activity measured using e.g. color indicator compounds or light emitting reactions.

25 The potency of the inhibitors isolated by the genetic screening may optionally be further determined *in vitro*. The affinity of the isolated inhibitors to the protease may be determined e.g. by ELISA. Their ability to inhibit protease catalysis may be quantified and their IC₅₀ determined using e.g. fluorometric assays as described herein.

Novel NS3 inhibitors

In one aspect, the present invention provides novel NS3 inhibitors. In one embodiment, the inhibitor is a single-chain antibody (scFv) having an amino acid sequence as set forth in any one of SEQ ID NOS:1-11. In another embodiment, the
5 inhibitor is a single antibody domain protein (dAb) derived from the isolated scFv inhibitors of the invention. In another embodiment, the inhibitor is a dAb having an amino acid sequence as set forth in any one of SEQ ID NOS:12-14 and 113.

In another embodiment, the scFv or dAb is fused to a stabilizing protein. In a preferred embodiment, the stabilizing protein is *E. coli* maltose binding protein (MBP).
10 In another embodiment, the scFv or dAb is fused to the C terminus of MBP. According to another embodiment, the inhibitor is a scFv fused to the C terminus of MBP, having an amino acid sequence as set forth in any one of SEQ ID NOS:15-25. In another embodiment, the inhibitor is a dAb fused to the C terminus of MBP, having an amino acid sequence as set forth in any one of SEQ ID NOS:26-28 and 114. Other
15 embodiments include fragments, homologs, analogs and derivatives thereof, as will be described hereinbelow.

In another embodiment, the inhibitor is a peptide having an amino acid sequence as set forth in any one of SEQ ID NOS:29-35. In another embodiment, the inhibitor is a peptide aptamer, comprising a peptide inhibitor of the invention fused to a stabilizing
20 protein. In a preferred embodiment, the stabilizing protein is *E. coli* maltose binding protein (MBP). In one preferred embodiment, the peptide is fused to the C terminus of MBP. In another preferred embodiment, the peptide is fused at internal permissive positions of MBP. In another preferred embodiment, the peptide is fused at the internal permissive position following position 133 of MBP. According to another embodiment,
25 the inhibitor is a free peptide derived from a peptide aptamer. In another embodiment, the peptide aptamer has an amino acid sequence as set forth in any one of SEQ ID NOS:36-49. Other embodiments include fragments, homologs, analogs and derivatives thereof, as will be described hereinbelow.

According to other embodiments, the invention comprises nucleic acids
30 encoding the NS3 inhibitors of the invention, as well as recombinant constructs, expression vectors and pharmaceutical compositions thereof, as described hereinbelow.

Nucleic acids

As used herein the terms “nucleic acid sequence”, “oligonucleotide” or “polynucleotide” refer to polymers of deoxyribonucleotides, ribonucleotides, and modified forms thereof in the form of a separate fragment or as a component of a larger construct, in a single strand or in a double strand form. The DNA or RNA molecules may be complementary DNA (cDNA), genomic DNA, synthesized DNA or a hybrid thereof or an RNA molecule such as mRNA. Accordingly, as used herein, the terms “DNA construct”, “gene construct”, “nucleic acid sequence”, “polynucleotide” and “oligonucleotide” are meant to refer to both DNA and RNA molecules. The term “oligonucleotide” refers to a polymer having not more than 50 nucleotides while the term “polynucleotide” refers to a polymer having more than 50 nucleotides. The term “nucleic acid sequence” refers to both oligonucleotide sequence and polynucleotide sequence. The terms nucleic acid sequence, oligonucleotide sequence and polynucleotide sequence are used in the invention interchangeably.

An isolated nucleic acid sequence can be obtained from its natural source, either as an entire (i.e., complete) gene or a portion thereof. A nucleic acid molecule can also be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis (see e.g. Sambrook et al., 2001; Ausubel, et al., 1989, Chapters 2 and 4). Nucleic acid sequences include natural nucleic acid sequences and homologs thereof, including, but not limited to, natural allelic variants and modified nucleic acid sequences in which nucleotides have been inserted, deleted, substituted, and/or inverted in such a manner that such modifications do not substantially interfere with the nucleic acid molecule’s ability to encode a functional polypeptide or peptide of the invention.

A polynucleotide or oligonucleotide sequence can be deduced from the genetic code of a protein; however, the degeneracy of the code must be taken into account. Nucleic acid sequences of the invention include sequences, which are degenerate as a result of the genetic code, which sequences may be readily determined by those of ordinary skill in the art.

The oligonucleotides or polynucleotides of the invention may contain a modified internucleoside phosphate backbone to improve the bioavailability and hybridization properties of the oligonucleotide or polynucleotide. Linkages are selected

from the group consisting of phosphodiester, phosphotriester, methylphosphonate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoroanilidate, phosphoramidate, phosphorothioate, phosphorodithioate or combinations thereof.

Additional nuclease linkages include alkylphosphotriester such as methyl- and
5 ethylphosphotriester, carbonate such as carboxymethyl ester, carbamate, morpholino carbamate, 3'-thioformacetal, silyl such as dialkyl (C1 -C6)- or diphenylsilyl, sulfamate ester, and the like. Such linkages and methods for introducing them into oligonucleotides are described in many references, e.g. reviewed generally by Peyman and Ulmann, Chemical Reviews, 90:1543-584 (1990).

10 In another aspect, the invention provides constructs and vectors comprising nucleic acid sequences encoding the protease inhibitors of the invention. Such constructs and vectors may be utilized for the expression and production of polypeptide- or peptide-based protease inhibitors. These constructs may also be used for gene therapy, by expressing the protease inhibitors in a subject in need thereof.

15 A polynucleotide or oligonucleotide sequence can be deduced from the genetic code of a polypeptide or peptide; however, the degeneracy of the code must be taken into account. Nucleic acid sequences of the invention also include sequences, which are degenerate as a result of the genetic code, which sequences may be readily determined by those of ordinary skill in the art.

20 Gene constructs suitable for expressing the protease inhibitors of the invention in a subject in need thereof comprise a nucleic acid sequence which encodes the protease inhibitor and which includes initiation and termination signals operably linked to regulatory elements including a promoter (and optionally enhancer and polyadenylation signal sequences required for expression in eukaryotic systems)
25 capable of directing expression in the cells of a subject. Thus, a gene construct contains the necessary regulatory elements operably linked to the nucleic acid sequence that encodes a protease inhibitor, such that when present in a cell of the individual, the protease inhibitor sequence will be expressed.

Suitable transcription control elements include any transcription control
30 sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control elements are known to those skilled in the art. Preferred transcription control elements include those, which function in

animal, bacteria, helminthes, insect cells, and preferably in animal cells. For expression in animal cells, preferable transcription control elements include, but are not limited to RSV control sequences, CMV control sequences, retroviral LTR sequences, SV-40 control sequences and β -actin control sequences as well as other sequences capable of
5 controlling gene expression in eukaryotic cells. Additional suitable transcription control elements include tissue-specific promoters and enhancers (e.g., liver specific enhancers and promoters).

The present invention is further related to an expression vector comprising the recombinant constructs of the present invention. Suitable eukaryotic expression vector
10 is for example: pcDNA3, pcDNA3.1(+/-), pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pCI, pBK-RSV, pBK-CMV, pTRES or their derivatives.

According to the present invention, a host cell can be transfected *in vivo* (i.e., in an animal) or *ex vivo* (i.e., outside of an animal). Transfection of a nucleic acid
15 molecule into a host cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transfection techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. Preferred methods to transfect host cells *in vivo* include lipofection and adsorption. Preferred host cells include liver cells of a liver transplant.

20 A host cell may also be infected *in vivo* or *ex vivo* by a viral vector comprising the nucleic acid molecules of the present invention. A viral vector includes an isolated nucleic acid molecule useful in the present invention, in which the nucleic acid molecules are packaged in a viral coat that allows entrance of DNA into a cell. A number of viral vectors can be used, including, but not limited to, those based on
25 alphaviruses, poxviruses, adenoviruses, herpesviruses, lentiviruses, adeno-associated viruses (AAV), bacteriophages, virus-like particles (VLPs) and retroviruses. Recombinant adenoviruses have several advantages over retroviral and other viral-based gene delivery methods. Adenoviruses have never been shown to induce tumors in humans and have been safely used as live vaccines. Adenovirus does not integrate
30 into the human genome as a normal consequence of infection, thereby greatly reducing the risk of insertional mutagenesis possible with retrovirus or AAV vectors. This lack of stable integration also leads to an additional safety feature in that the transferred

gene effect will be transient, as the extra-chromosomal DNA will be gradually lost with continued division of normal cells. Stable, high titer recombinant adenovirus can be produced at levels not achievable with retrovirus or AAV, allowing enough material to be produced to treat a large patient population.

5 It may be appreciated by one skilled in the art that use of recombinant DNA technologies can improve expression of transfected nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-
10 translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of
15 transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences), modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, and deletion of sequences that destabilize transcripts.

20 **Proteins, peptides and derivatives**

The polypeptides and peptides of the invention may be synthesized using any recombinant or synthetic method known in the art. A non-limitative example for recombinant production of NS3 inhibitors is presented in the Examples; however, other synthesis methods may be used, including, but not limited to, solid phase (e.g. Boc or f-
25 Moc chemistry) and solution phase synthesis methods. For solid phase peptide synthesis, a summary of the many techniques may be found in Meienhofer, 1973.

The amino acid residues described herein are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the peptide retains the desired functional property.

30 It should be understood that an NS3 inhibitor need not be identical to the amino acid sequence of the NS3 inhibitors of the invention, so long as it includes the required sequence and is able to function as the peptide of the invention as described herein. It is

noted that both shorter active fragments derived from the inhibitors denoted as SEQ ID NOS:1-49, 113, 114, and longer polypeptides or peptides comprising these sequences are within the scope of the present invention.

Whenever NS3 inhibitors are mentioned in the invention, also salts and functional derivatives thereof are contemplated, as long as the biological activity of the peptide with respect to HCV is maintained. The present invention encompasses any analog, derivative, and conjugate containing the NS3 inhibitors of the invention, so long as the peptide is capable of inhibiting NS3 activity. Thus, the present invention encompasses polypeptides or peptides containing non-natural amino acid derivatives or non-protein side chains.

The term "analog" includes any polypeptide or peptide having an amino acid sequence substantially identical to one of the sequences specifically shown herein in which one or more residues have been conservatively substituted with a functionally similar residue and which displays the abilities as described herein. Examples of conservative substitutions include the substitution of one non-polar (hydrophobic) residue such as isoleucine, valine, leucine or methionine for another, the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine, the substitution of one basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another.

The phrase "conservative substitution" also includes the use of a chemically derivatized residue in place of a non-derivatized residue provided that such peptide displays the requisite inhibitory function on HCV as specified herein.

The term derivative includes any chemical derivative of the polypeptides or peptides of the invention having one or more residues chemically derivatized by reaction of side chains or functional groups. Such derivatized molecules include, for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-

benzylhistidine. Also included, as chemical derivatives are those peptides, which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acid residues. For example: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine.

In addition, a polypeptide or peptide derivative can differ from the natural sequence of the polypeptides or peptides of the invention by chemical modifications including, but are not limited to, terminal-NH₂ acylation, acetylation, or thioglycolic acid amidation, and by terminal-carboxyamidation, e.g., with ammonia, methylamine, and the like. Peptides can be either linear, cyclic or branched and the like, which conformations can be achieved using methods well-known in the art.

Polypeptides or peptides of the present invention also include any polypeptide or peptide having one or more additions and/or deletions of residues relative to the sequence of the fusion peptide of the invention, so long as the requisite inhibitory activity is maintained.

Addition of amino acid residues may be performed at either terminus of the polypeptides or peptides of the invention for the purpose of providing a "linker" by which the peptides of this invention can be conveniently bound to a carrier. Such linkers are usually of at least one amino acid residue and can be of 40 or more residues, more often of 1 to 10 residues. Typical amino acid residues used for linking are serine, glycine, tyrosine, cysteine, lysine, glutamic and aspartic acid, or the like.

A polypeptide or peptide of the present invention may be coupled to or conjugated with another protein or polypeptide to produce a conjugate. Such a conjugate may have advantages over the polypeptide or peptide used alone. By means of a non-limitative example, NS3 inhibitors of the invention may be conjugated to a sequence facilitating internalization of the inhibitors into cells, including, but not limited to, protein-transduction domain (PTD, see e.g. Beerens et al., 2003).

In other embodiments, the NS3 inhibitors of the invention are associated with other internalization moieties, such as compounds, liposomes or particles which improve the ability of the NS3 inhibitors to penetrate the lipid bilayer of the cellular plasma membrane and enter the cytoplasm. As used herein, the term "associated with"

refers to covalent attachment or a non-covalent interaction mediated by, for example, ionic bonds, hydrogen bonds, van der Waals forces and/or hydrophobic interactions, such that the internalization moiety and NS3 inhibitor remain in close proximity under physiological conditions. Various particle-, liposome- and ligand-mediated delivery systems are available, and their use is well known to those of ordinary skill in the art.

Pharmaceutical compositions

The NS3 inhibitors of the present invention can be administered to an organism per se, or in a pharmaceutical composition where it is mixed with suitable carriers or excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Hereinafter, the phrases “physiologically acceptable carrier” and “pharmaceutically acceptable carrier”, which may be used interchangeably, refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. Useful carriers include, for example, water, acetone, ethanol, ethylene glycol, propylene glycol, butane-1, 3-diol, isopropyl myristate, isopropyl palmitate, or mineral oil.

Herein, the term “excipient” refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils, and polyethylene glycols.

The NS3 inhibitors of the invention may be formulated into the pharmaceutical composition as a neutralized pharmaceutically acceptable salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide), which are formed with inorganic acids, such as for example, hydrochloric or phosphoric acid, or with organic acids such as acetic, oxalic, tartaric, and the like.

Suitable bases capable of forming salts with the polypeptides or peptides of the present invention include, but are not limited to, inorganic bases such as sodium hydroxide, ammonium hydroxide, potassium hydroxide and the like; and organic bases such as mono-, di- and tri-alkyl and aryl amines (e.g. triethylamine, diisopropyl amine,

methyl amine, dimethyl amine and the like) and optionally substituted ethanolamines (e.g. ethanolamine, diethanolamine and the like).

Techniques for formulation and administration of drugs may be found in the latest edition of "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, which is herein fully incorporated by reference.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal, or parenteral delivery, including intramuscular, subcutaneous, and intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal or intranasal injections. Alternately, one may administer the pharmaceutical composition in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a tissue region of a patient.

Apart from other considerations, the administration of peptides, peptide analogs or vectors or cells dictates that the formulation be suitable for delivery of these type of compounds. Clearly, peptides are less suitable for oral administration due to susceptibility to digestion by gastric acids or intestinal enzymes. It is contemplated that the present invention encompasses peptide compositions designed to circumvent these problems. The preferred routes of administration of peptides are intra-articular, intravenous, intramuscular, subcutaneous, intradermal, or intrathecal. A more preferred route is by direct injection at or near the site of disorder or disease.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such

as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the pharmaceutical composition can be formulated
5 readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the pharmaceutical composition to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting
10 mixture, and processing the mixture of granules, after adding suitable auxiliaries as desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, and sodium
15 carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate, may be added.

Dragee cores are provided with suitable coatings. For this purpose, concentrated
20 sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

25 Pharmaceutical compositions that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active ingredients
30 may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane, or carbon dioxide. In the case of a pressurized aerosol, the dosage may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, for example, gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base, such as lactose or starch.

The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with, optionally, an added preservative. The compositions may be suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing, and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water-based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters such as ethyl oleate, triglycerides, or liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the active ingredients, to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., a sterile, pyrogen-free, water-based solution, before use.

The pharmaceutical composition of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, for example, conventional suppository bases such as cocoa butter or other glycerides.

Pharmaceutical compositions suitable for use in the context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a "therapeutically effective amount" means an amount of active ingredients (e.g., a nucleic acid construct) effective to prevent, alleviate, or ameliorate symptoms of a disorder (e.g., ischemia) or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of the invention, the dosage or the therapeutically effective amount can be estimated initially from *in vitro* and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures *in vitro*, in cell cultures or experimental animals. The data obtained from these *in vitro* and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration, and dosage can be chosen by the individual physician in view of the patient's condition. (See, e.g., Fingl, E. et al. (1975), "The Pharmacological Basis of Therapeutics", Ch. 1, p.1.)

Dosage amount and administration intervals may be adjusted individually to provide sufficient plasma or brain levels of the active ingredient to induce or suppress the biological effect (i.e., minimally effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from *in vitro* data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks, or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of the present invention may, if desired, be presented in a pack or
5 dispenser device, such as an FDA-approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser device may also be accompanied by a notice in a form prescribed by a governmental agency regulating
10 the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions for human or veterinary administration. Such notice, for example, may include labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a pharmaceutically acceptable
15 carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition, as further detailed above.

For the use of nucleic acid based pharmaceutical compositions, preferred carriers are capable of maintaining a nucleic acid molecule of the present invention in a form that, upon arrival of the nucleic acid molecule to a cell, the nucleic acid molecule
20 is capable of entering the cell and being expressed by the cell. Carriers of the present invention include: (1) excipients or formularies that transport, but do not specifically target a nucleic acid molecule to a cell (referred to herein as non-targeting carriers); and (2) excipients or formularies that deliver a nucleic acid molecule to a specific site in an animal or a specific cell (i.e., targeting carriers). Examples of non-targeting carriers
25 include, but are not limited to water; phosphate buffered saline, Ringer's solution, dextrose solution, serum-containing solutions, Hank's solution, other aqueous physiologically balanced solutions, oils, esters and glycols. Aqueous carriers can contain suitable auxiliary substances required to approximate the physiological conditions of the recipient, for example, by enhancing chemical stability and
30 isotonicity.

Suitable auxiliary substances include, for example, sodium acetate, sodium chloride, sodium lactate, potassium chloride, calcium chloride, and other substances

used to produce phosphate buffer, Tris buffer, and bicarbonate buffer. Auxiliary substances can also include preservatives, such as thimerosal, m- and o-cresol, formalin and benzol alcohol. Preferred auxiliary substances for aerosol delivery include surfactant substances non-toxic to an animal, for example, esters or partial esters of fatty acids containing from about six to about twenty-two carbon atoms. Examples of esters include, caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric, and oleic acids. Other carriers can include metal particles (e.g., gold particles) for use with, for example, a biolistic gun through the skin. Pharmaceutical compositions of the present invention can be sterilized by conventional methods.

Targeting carriers are herein referred to as "delivery vehicles". Delivery vehicles of the present invention are capable of delivering a pharmaceutical composition of the present invention to a target site in an animal. A "target site" refers to a site in an animal to which one desires to deliver a pharmaceutical composition. Examples of delivery vehicles include, but are not limited to, artificial and natural lipid-containing delivery vehicles. Natural lipid-containing delivery vehicles include cells and cellular membranes. Artificial lipid-containing delivery vehicles include liposomes and micelles.

A delivery vehicle of the present invention can be modified to target to a particular site in an animal, thereby targeting and making use of a nucleic acid molecule of the present invention at that site. Suitable modifications include manipulating the chemical formula of the lipid portion of the delivery vehicle and/or introducing into the vehicle a compound capable of specifically targeting a delivery vehicle to a preferred site, for example, a preferred cell type. Specifically targeting refers to causing a delivery vehicle to bind to a particular cell by the interaction of the compound in the vehicle to a molecule on the surface of the cell. Suitable targeting compounds include ligands capable of selectively (i.e., specifically) binding another molecule at a particular site. Examples of such ligands include antibodies, antigens, receptors and receptor ligands. Manipulating the chemical formula of the lipid portion of the delivery vehicle can modulate the extracellular or intracellular targeting of the delivery vehicle. For example, a chemical can be added to the lipid formula of a liposome that alters the charge of the lipid bilayer of the liposome so that the liposome fuses with particular cells having particular charge characteristics.

According to another embodiment, the delivery vehicle of the present invention may be a liposome. A liposome is capable of remaining stable in an animal for a sufficient amount of time to deliver a nucleic acid sequence of the present invention to a preferred site in the animal. A liposome of the present invention is preferably stable in
5 the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour and even more preferably for at least about 24 hours.

Suitable liposomes for use with the present invention include any liposome. Preferred liposomes of the present invention include those liposomes standardly used
10 in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes comprise liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol.

Therapeutic use

In another aspect, the invention provides a method of treating HCV infection in
15 a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising an NS3 inhibitor of the invention.

In another aspect, the invention provides a method of treating or preventing the symptoms of hepatitis in a subject in need thereof, comprising administering to the
20 subject a therapeutically effective amount of a pharmaceutical composition comprising an NS3 inhibitor of the invention.

In another aspect, the invention provides a method of preventing liver damage in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising an NS3 inhibitor of the
25 invention.

In another aspect, the invention provides a method of preventing HCC in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising an NS3 inhibitor of the invention.

30 In another aspect, the invention provides a method of treating HCV infection in a subject in need thereof, comprising administering to the subject a therapeutically

effective amount of a pharmaceutical composition comprising a recombinant construct comprising an isolated nucleic acid sequence encoding an NS3 inhibitor of the invention operably linked to one or more transcription control elements, thereby treating HCV infection.

5 In another aspect, the invention provides a method of treating or preventing the symptoms of hepatitis in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a recombinant construct comprising an isolated nucleic acid sequence encoding an NS3 inhibitor of the invention operably linked to one or more transcription control elements,
10 thereby treating or preventing the symptoms of hepatitis.

 In another aspect, the invention provides a method of preventing liver damage in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a recombinant construct comprising an isolated nucleic acid sequence encoding an NS3 inhibitor of the
15 invention operably linked to one or more transcription control elements, thereby preventing liver damage.

 In another aspect, the invention provides a method of preventing HCC in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a recombinant construct
20 comprising an isolated nucleic acid sequence encoding an NS3 inhibitor of the invention operably linked to one or more transcription control elements, thereby preventing HCC.

 In another aspect, the invention provides a method of treating HCV infection in a subject in need thereof, comprising: a) obtaining cells from the subject; b) contacting
25 the cells *ex vivo* with a therapeutically effective amount of a pharmaceutical composition comprising a recombinant construct comprising an isolated nucleic acid sequence encoding NS3 inhibitor of the invention operably linked to one or more transcription control elements; and c) re-introducing said cells to said subject, thereby treating HCV infection in said subject.

30 In another aspect, the invention provides a method of treating or preventing the symptoms of hepatitis in a subject in need thereof, comprising: a) obtaining cells from the subject; b) contacting the cells *ex vivo* with a therapeutically effective amount of a

pharmaceutical composition comprising a recombinant construct comprising an isolated nucleic acid sequence encoding NS3 inhibitor of the invention operably linked to one or more transcription control elements; and c) re-introducing said cells to said subject, thereby treating or preventing the symptoms of hepatitis.

5 In another aspect, the invention provides a method of preventing liver damage in a subject in need thereof, comprising: a) obtaining cells from the subject; b) contacting the cells *ex vivo* with a therapeutically effective amount of a pharmaceutical composition comprising a recombinant construct comprising an isolated nucleic acid sequence encoding NS3 inhibitor of the invention operably linked to one or more
10 transcription control elements; and c) re-introducing said cells to said subject, thereby preventing liver damage in said subject.

In another aspect, the invention provides a method of preventing HCC in a subject in need thereof, comprising: a) obtaining cells from the subject; b) contacting the cells *ex vivo* with a therapeutically effective amount of a pharmaceutical
15 composition comprising a recombinant construct comprising an isolated nucleic acid sequence encoding NS3 inhibitor of the invention operably linked to one or more transcription control elements; and c) re-introducing said cells to said subject, thereby preventing HCC in said subject.

In another aspect, the invention provides a method of preventing HCV infection
20 in a liver transplant, comprising: a) treating a liver transplant before transplantation *ex vivo* with a therapeutically effective amount of a pharmaceutical composition comprising a recombinant construct comprising an isolated nucleic acid sequence encoding NS3 inhibitor of the invention operably linked to one or more transcription control elements; and b) transplanting the liver transplant to a subject in need thereof,
25 thereby generating an HCV-immune liver transplant.

The NS3 inhibitors of the invention may be used alone or in combination with one or more therapeutic agents, including, but not limited to: interferon (pegylated or not), ribavirin, or one or more other anti-HCV agents, all of which administered together or separately, e.g., prior to, concurrently with or following the administration
30 of other NS3 inhibitors of the invention or pharmaceutically acceptable salts thereof.

In order to treat a subject with a disease, a pharmaceutical composition of the present invention is administered to the subject in an effective manner such that the

composition is capable of treating that subject from disease. According to the present invention, treatment of a disease refers to alleviating a disease and/or preventing the development of a secondary disease resulting from the occurrence of a primary disease. An effective administration protocol (i.e., administering a pharmaceutical composition
5 in an effective manner) comprises suitable dose parameters and modes of administration that result in treatment of a disease. Effective dose parameters and modes of administration can be determined using methods standard in the art for a particular disease. Such methods include, for example, determination of survival rates, side effects (i.e., toxicity) and progression or regression of disease.

10 In accordance with the present invention, a suitable single dose size is a dose that is capable of treating a subject with disease when administered one or more times over a suitable time period. Doses of a pharmaceutical composition of the present invention suitable for use with direct injection techniques can be used by one of skill in the art to determine appropriate single dose sizes for systemic administration based on
15 the size of a subject.

The following examples are presented in order to more fully illustrate some embodiments of the invention. They should, in no way be construed, however, as limiting the broad scope of the invention.

EXAMPLES

20 Materials and Methods

Construction of a reporter plasmid for insertion of peptide coding sequences into β -galactosidase. Plasmid pIB13 (Benhar and Engelberg-Kulka 1993) carries the 5' 77 codons of the *E. coli trpR* gene fused to the 8th codon of the *E. coli lacZ* gene, under the control of the *trpR* promoter on a pBR322 plasmid backbone
25 (*colE1* replicon). pIB13 DNA was digested with the restriction enzymes *Clal* and *DraI* and a 4089bp DNA fragment was recovered. A p15A replicon and kanamycin resistance cassette carrying 3054bp DNA fragment was isolated by *DraI* and *BstBI* digestion of pACYC177 plasmid DNA. The two DNA fragments were ligated and used to transform of *E. coli* TG-1 cells that resulted in plasmid pEB13.

30 Two tandem *sfiI* restriction sites separated by a TAA stop codon were inserted between *lacZ* codons 279-280 by overlap-extension PCR-based mutagenesis

(Berdichevsky, Zemel et al., 2003) as follows: pEB13 DNA was used as template in two PCR reactions using primers LacZ-Sfi-279-280-BACK (GAAATTATCGATGAGGGCCAACGTGGCCGGTAATTTTTGGCCTCTGGGGCCCGTGGTG GTTATGCC, SEQ ID NO:71) with LacZ-520-FOR (CAGCCGGGAAGGGCTGGTCTT, SEQ ID NO:72), and LacZ-70-BACK (CCGGAAAGCTGGCTGGAGTGC, SEQ ID NO:73) with LacZ-279-FOR (GGCCCTCATCGATAATTTTCAC, SEQ ID NO:74). All the PCR reactions were carried out using 30 cycles of 95°C for 30 sec, 55°C for 1 min and 72°C for 1 min with a final extension at 72°C for 5 min. The resulting PCR products of 667 bp and 763 bp, respectively, were mixed and assembled by PCR amplification using primers LacZ-70-BACK and LacZ-520-FOR. The resulting PCR product of 1390 bp was digested with *Bsu*36I and *Bss*HII and cloned into vector DNA recovered by digesting pEB13 DNA with the same enzymes. Ligated DNA was used to transform DH5 α *E. coli* cells (Invitrogen, USA) that were plated on LB agar plates supplemented with 0.04% (w/v) X-gal. White (colorless) colonies that resulted from interruption of the *lacZ* open reading frame by the inserted stop codon were analyzed by DNA sequencing. The resulting plasmid was pEB13-Sfi (SEQ ID NO:82). This plasmid serves as a universal platform for insertion of protease-site-coding sequences between *lacZ* codons 279-280, as illustrated below for the NS5A/B cleavage site of NS3.

Insertion of the NS3-cleavable NS5A/B site into β -galactosidase. The NS5A/B cleavage site of NS3 was engineered between codons 279-280 of the *lacZ* gene on plasmid pEB13-Sfi as follows: A DNA duplex formed by annealing primers NS5A/B-SE (AGCTAGCGAGGACGTCGTCTGCTGCTCGATGTCCTACACTG, SEQ ID NO:75) and NS5A/B-AS (TGTAGGACATCGAGCAGCAGACGACGTCCTCGCTAGCTCGT, SEQ ID NO:76) was inserted into pEB13-Sfi plasmid DNA that had been digested with *Sfi*I. Ligated DNA was used to transform TG-1 *E. coli* cells (Amersham Biosciences, USA) that were plated on X-gal supplemented LB agar plates. Blue colonies that resulted from restoration of the *lacZ* open reading frame by the inserted fragment were analyzed by DNA sequencing. The resulting plasmid was pEB13-*lacZ*_{NS5A/B} (SEQ ID NO:68).

Figure 5 illustrates the amino acid sequence of the resulting reporter protein, wherein the 78 N-terminal residues of the *E. coli trpR* gene product followed by a short linker are presented in italics, and the NS3-cleavable NS5A/B site is presented in bold

type. The complete amino acid sequence of the reporter protein is denoted as SEQ ID NO:64; the amino acid sequence of the NS5A/B-containing β -galactosidase, without the preceding sequence corresponding to the *trpR* and linker, is denoted as SEQ ID NO:65.

Cloning of NS3 under the *araBAD* promoter. Single-chain NS3 protease (scNS3), which is a single-chain fusion linking the NS4A cofactor peptide to the N-terminus of the NS3 protease (Berdichevsky et al., 2003), was stabilized by its expression as a maltose-binding protein fusion (Bach et al., 2001) and cloned under the control of the *araBAD* promoter. Figure 6 illustrates the amino-acid sequence of the engineered enzyme (denotes as SEQ ID NO:66): a fusion protein comprised of the *E. coli malE* gene product (MBP) fused the NS4A peptide (in bold type) followed by the NS3 protease domain derived HCV genotype 1b (Berdichevsky et al., 2003). The cloning was preformed as follows: Plasmid pYB43 (Berdichevsky et al., 2003) was used as template in a PCR reaction with primers NS3-Nco-BACK (TCAGTACCATGGCGCCTATCGGCTCAGTAGTA, SEQ ID NO:77) and NS3-Not-FOR (GGGAAAGCGGCCGCTTACCGCATAGTGGTTTCCATAGA, SEQ ID NO:78). The resulting PCR product of 637 bp was digested with *NcoI* and *NotI* and cloned into 6128bp vector DNA fragment recovered by digesting pMALc-NN DNA (Bach et al., 2001) with the same enzymes. Ligated DNA was used to transform TG-1 *E. coli* cells. The resulting plasmid was pMALC-NN-scNS3.

Next, the MBP-scNS3 carrying fragment was recovered by PCR using pMALC-NN-scNS3 as template with primers MalE-BspHI-BACK (CCCTAATCATGAAAAGTGAAGAAGGTAAA, SEQ ID NO:79) and NS3-Not-FOR. The resulting 1788bp PCR product was digested with *BspHI* and *NotI* and cloned into a 3790bp vector DNA fragment recovered by digesting pBAD-TOPO DNA (Invitrogen, USA) with *NcoI* and *NotI*. Ligated DNA was used to transform TG-1 *E. coli* cells. The resulting plasmid was named pBAD-MBP-scNS3. A similar plasmid, pBAD-MBP-scNS3_{mut} was constructed for expression of an inactive mutant S-1165-A NS3 (Zemel et al., 2001) to serve as a negative control.

Next, a bi-cistronic plasmid was constructed, carrying both *lacZ* with the inserted NS3 cleavage site (*lacZ*_{NS5A/B}) and MBP-scNS3 as follows: Restriction sites for *SphI* and *NotI* were inserted into vector pEB13*lacZ*_{NS5A/B} as a DNA duplex formed by annealing primers pEB13-dup-SE (AATTGCATGCCTGCAGGCGGCCGCG, SEQ ID

NO:80) and pEB13-dup-AS (AATTCGCGGCCCGCCTGCAGGCATGC, SEQ ID NO:81). The duplex was inserted into pEB13-*lacZ*_{NS5A/B} plasmid DNA that had been linearized at the unique *EcoRI* site. The resulting plasmid was digested with *SphI* and *NotI* to obtain a vector fragment of 7822 bp. An insert fragment of 3074 bp corresponding to
5 MBP-*scNS3* with its controlling *araBAD* promoter and the regulatory *araC* gene was obtained by digesting plasmid pBAD-MBP-*scNS3* with *SphI* and *NotI*. The two fragments were ligated to generate plasmid pMGT14 (SEQ ID NO:67). A similar plasmid, pMGT15, expressing an inactive mutant S-1165-A NS3 (Zemel et al., 2001) was constructed using the *SphI-NotI* fragment of plasmid pBAD-MBP-*scNS3*_{mut} to
10 serve as a negative control of catalysis. In both plasmids the expression of the engineered *lacZ* gene is weakly constitutive in *trpR*⁺ *E. coli* strains while MBP-*scNS3* is inducible by arabinose.

Construction of a murine immune antibody phage display library. MBP-*scNS3* protein was expressed and purified essentially as described for MBP-scFvs
15 (Bach et al., 2001). Balb/c mice were immunized with 50 µg of purified MBP-*scNS3* in complete Freund's adjuvant and boosted three times at two-week intervals using 50 µg of purified MBP-*scNS3* in incomplete Freund's adjuvant. The mice were bled three days after each boost. The serum anti-NS3 antibody titer was determined by ELISA.

Total RNA was extracted from 10⁷ cells recovered from the immune mice
20 spleens according to the instructions of the Tri Reagent total RNA extraction kit (Talron, Israel). A total of 250 µg RNA were obtained. cDNA was produced from mRNA by reverse transcription reactions using 1 µg of total RNA as template. Heavy and light chain variable domains were amplified in PCR using a primer set designed for the construction of mouse scFv libraries (Benhar and Reiter 2002). The antibody
25 variable cassettes were combinatorially assembled into complete scFv constructs and cloned into pCC phagemid vector (Berdichevsky et al., 1999) essentially as described (Benhar and Reiter 2002). The cloned vectors were transferred into competent *E. coli* TG-1 cells by electroporation and library diversity was tested as described (Benhar and Reiter 2002).

30 **Applying the bacterial genetic screening to isolate NS3-inhibitory scFvs.** Affinity selected phages were pooled and used as source for scFv DNA by digestion of phagemid DNA with *NcoI* and *NotI*. The pooled insert DNA was cloned into pMALc-

NN that had been digested with the same enzymes essentially as described (Bach et al., 2001). In pMALc-NN, expression of MBP-scFvs is under the control of an IPTG-inducible *tac* promoter and the plasmid carries an ampicillin resistance cassette and the *colE1* origin of replication and is thus compatible with pMGT14 or pMGT15. The
5 plasmid pool was introduced into *E. coli* TG-1 cells already containing the pMGT14 plasmid. The transformants were plated to yield individual colonies on 2xYT agar plates supplemented with 0.004% (w/v) X-gal, 100 µg/ml ampicillin, 50 µg/ml kanamycin, 0.2% arabinose and 0.05 mM IPTG. Blue colonies were picked and pMALc-NN-scFv DNA was recovered and re-introduced into pMGT14 expressing cells to validate the
10 results of the initial screening.

Binding assays. Selected MBP-scFvs were expressed and purified from the soluble fraction of IPTG-induced plasmid-carrying *E. coli* BL21 cells using amylose resin chromatography (Bach et al., 2001). ScNS3 was overexpressed in pYB-43 carrying *E. coli* BL-21 (DE3) cells by a modification of the inventors' published
15 method (Berdichevsky et al., 2003). The bacteria were induced with 1 mM IPTG at 37°C for 3 hr, which resulted in accumulation of the scNS3 as inclusion bodies. The inclusion bodies were solubilized in 6M guanidinium hydrochloride and purified by Talon (Clontech, USA) IMAC chromatography under denaturing conditions. The purified protein was stored at -80°C. ELISA plates were coated with 4 µg/ml scNS3 in
20 NaHCO₃ buffer pH 9.6. ELISAs were processed and developed as described (Benhar and Reiter 2002) using a mouse anti-MBP antibody (Sigma, Israel) followed by HRP conjugated goat anti mouse antibodies (Jackson Immunolaboratories, USA).

For competition ELISA the inventors replaced the C-terminal FLAG epitope of the scFvs that were used as tracers with a myc tag. Competing scFvs were added to
25 scNS3-coated plates at varying concentrations and incubated at room temperature for 1 hr before adding the tracer scFvs. Binding of the tracers was monitored using a HRP-conjugated anti myc antibody (Sigma, Israel).

NS3 catalysis inhibition assays. Blue colonies of *E. coli* TG-1 cells carrying pMGT14 and pMALc-NN-scFv that were isolated by the genetic screening were grown
30 in LB medium supplemented with 100 µg/ml ampicillin, 50 µg/ml kanamycin and induced with 0.2% (w/v) arabinose and 0.5 mM IPTG at O.D_{600nm}=0.6 for 4 hr at 30°C. To measure the β-galactosidase activity an ONPG hydrolysis assay (Bach et al., 2001)

was carried out. An *in vitro* fluorometric assay for the measurement of NS3 protease catalysis inhibition by the purified scFvs was carried out essentially as described (Berdichevsky et al., 2003) with the following modifications: the EFGP-NS5A/B-CBD substrate was immobilized onto cellulose prior to its exposure to enzyme and inhibitor.

- 5 The reactions were carried out in 96 well plates in a volume of 100 μ l containing 5 μ M immobilized substrate, 100 nM MBP-scNS3 and 2.4 or 1.2 μ M of tested MBP-scFv.

Immunoblot. Was carried out to follow the time course on NS3 induction and its effect on cleavage of *lacZNS5A/B* gene product. TG-1 cells carrying plasmids pMGT14 or pMGT15 were grown in LB medium supplemented with 100 μ g/ml
10 ampicillin, 50 μ g/ml kanamycin to an O.D_{600nm}=0.6. The cells were then induced with 0.2% (w/v) arabinose at 30°C. 20 μ g of total cell extracts were separated by SDS/PAGE and transferred onto nitrocellulose membranes. The membranes were reacted with mouse polyclonal anti β -galactosidase antiserum (Lab collection) and a mouse anti-MBP antibody and detected by HRP conjugated goat anti mouse antibody (Jackson
15 Immunolaboratories, USA). The membrane was developed by using ECL reagents (Berdichevsky et al., 2003).

Cells and transfections. HCV genotype 1b strain N subgenomic replicon cell lines were obtained from Stanley Lemon and are described in (Bourne et al., 2005). En5-3 cells are a clonal cell line derived from Huh7 cells by stable transformation with the
20 plasmid pLTR-SEAP. These cells were cultured in DMEM supplemented with 10% fetal calf serum, 2 μ g/ml blasticidin (Invitrogen), penicillin, and streptomycin. Following transfection with Ntat2ANeo replicon RNAs, cells supporting replicon amplification were selected and maintained in the above media containing in addition 400 μ g/ml G418 (geneticin). Cell lines were passaged once or twice per week. For
25 inhibition assay, replicon cell were transfected with NS3-neutralizing scFvs using the FuGENE reagent. The culture medium was replaced every 24 h post transfection and the secreted alkaline phosphatase (SEAP) activity was measured in these fluids as described below, reflecting the daily production of SEAP by the cells.

Alkaline phosphatase assay. SEAP activity was measured in supernatant culture
30 fluids using the Phospha-Light Chemiluminescent Reporter Assay (Tropix) with the manufacturer's suggested protocol reduced 1/3 in scale as following: a 10 μ l aliquots of the supernatant fluids from transfected cells were mixed in a tube with 30 μ l of 1 $\times$

diluted $5 \times$ dilution buffer in sterile water. The tubes were heated to 65°C for 30 min. 35 μ l of the samples were transferred to Luminometer tubes and 35 μ l of assay buffer were added to the tubes and incubated for 5 min at room temperature. 35 μ l of CSPD substrate diluted 1/20 in reaction buffer was added to the tubes and the tubes were
5 incubated for 20 min at room temperature. The luminescent signal was read using a TD-20/20 Luminometer (Turner Designs, Inc.).

Example 1. A bacterial genetic screening for the isolation of NS3 protease inhibitors

A novel bacterial genetic screening was established, based on phenotypic
10 changes that result from the concerted co-expression of enzyme, substrate and potential inhibitor, to identify NS3-neutralizing antibodies directly from a large pool of clones. Vector pMGT14 (SEQ ID NO:67, Fig. 1A) was designed to allow the expression of the enzyme, MBP-scNS3 (SEQ ID NO:66) and the substrate (SEQ ID NO:64), an engineered β -galactosidase, which is cleaved by NS3 at the NS5A/B site. Co-expression
15 of an inhibitor in pMGT14 carrying *E. coli* cells results in the phenotypic changes that make it possible to identify and characterize such NS3 inhibitors.

The genetic screening is based on *lacZ* as a reporter gene that yields blue colonies when expressed in *E. coli* cells that are plated on X-gal indicative plates. The engineered *lacZ* gene in the disclosed system was designed to be constitutively
20 expressed at a low level. Thus, the *trpR* promoter controlled *trpR-lacZ* fusion (Benhar and Engelberg-Kulka 1993) was chosen. The reporter gene was transferred onto a low copy number p15A replicon plasmid that also carries a kanamycin resistance gene to make it compatible with subsequently introduced inhibitor-coding plasmids that are base on *colE1* replicon, high copy number, ampicillin resistance-carrying plasmids.

25 Initially, two tandem *sfiI* site separated by a TAA stop codon were inserted between codons 279-280 of the *lacZ* gene. The insertion site for enzyme cleavage sequences in *lacZ* was chosen based on initial studies of engineered β -galactosidase as a biosensor for antibody binding (Feliu et al., 2002). This resulted in plasmid pEB13-Sfi (SEQ ID NO:82) that does not encode a functional β -galactosidase because its open
30 reading frame is interrupted by the inserted stop codon. Plasmid pEB13-Sfi may serve as a general purpose substrate plasmid by the insertion of any desirable sequence that specifies a protease cleavage site as a staggered DNA duplex. Such insertion restores

the *lacZ* open reading frame, allowing the identification of the cleavage site engineered *lacZ* carrying plasmids as blue colonies upon plating transformed *E. coli* on X-gal plates. A sequence specifying the NS5A/B site was inserted, yielding an NS3-cleavable β -galactosidase enzyme.

5 To test the activity of the NS5A/B site-containing β -galactosidase encoded by vector pEB13-*lacZ*_{NS5A/B}, an ONPG hydrolysis assay (Bach et al., 2001) was applied. The engineered enzyme had 12% β -galactosidase enzymatic activity in comparison to the wild type *lacZ* carried on plasmid pEB13 (Table 1). Still, cells expressing it formed blue colonies on X-gal indicative plates.

10 In previous studies, the inventors expressed scNS3 in bacteria and purified it in an active form (Berdichevsky et al., 2003; Zemel et al., 2004). The inventors found that the MBP fusion technology developed for efficient cytoplasmic expression of scFvs (Bach et al., 2001) could be adopted to improve the expression of scNS3 without compromising its activity. Thus, the current study was carried out using MBP-scNS3.

15 To test the ability of the engineered *lacZ*_{NS5A/B} to detect NS3 protease activity *in vivo*, pMALC-NN-scNS3 was co-introduced with pEB13-*lacZ*_{NS5A/B} into *E. coli* TG-1 cells. As a control, pMALc-NN-EGFP that codes for enhanced green fluorescent protein (Bach et al., 2001) was used. As shown in Table 1, induction of scNS3 expression with IPTG resulted in reduced β -galactosidase activity in comparison to the EGFP control. In
20 addition, TG-1 cells carrying pMALC-NN-scNS3 and pEB13-*lacZ*_{NS5A/B} formed white colonies on IPTG + X-gal supplemented agar plates while pMALc-NN-EGFP and pEB13-*lacZ*_{NS5A/B} carrying cells formed blue colonies, indicating that co-expression of MBP-scNS3 with the engineered β -galactosidase results in cleavage of the engineered enzyme.

25 **Table 1. β -galactosidase activities directed by various constructs**

Construct	β -galactosidase units*
pEB13- <i>lacZ</i>	1057
pEB13- <i>lacZ</i> -NS5A/B	130
30 pEB13- <i>lacZ</i> -NS5A/B + pMALc-NN-EGFP	80
pEB13- <i>lacZ</i> -NS5A/B + pMALc-NN-scNS3	20
pMGT14	60
pMGT15	160

β -galactosidase units*: Miller units as described (Bach et al., 2001). The presented units are an average of 3 independent assays, each in triplicate.

Next, the MBP-*scNS3* was subcloned under the control of the *araBAD* promoter that resulted in plasmid pBAD-MBP-*scNS3*. The purpose of this transaction was to
5 allow the use of different inducers for NS3 (arabinose) and for potential inhibitors that are expressed from the pMALc-NN platform (Bach et al., 2001) that utilizes the strong, IPTG inducible *tac* promoter. TG-1 cells carrying pBAD-MBP-*scNS3* and pEB13-*lacZ*_{NS5A/B} formed white colonies on arabinose + X-gal supplemented agar plates while pBAD-MBP-*scNS3*_{mut} (inactive NS3) and pEB13-*lacZ*_{NS5A/B} carrying cells formed blue
10 colonies on those plates. Finally both components, enzyme and substrate, were combined on a single plasmid, pMGT14 (SEQ ID NO:67). TG-1 cells carrying pMGT14 (Test bacteria) formed white colonies on arabinose + X-gal supplemented agar plates while pMGT15 (inactive NS3) carrying cells formed blue colonies on those plates (Fig. 1A). For a quantitative analysis, test bacteria expressing active or inactive
15 NS3 were induced with varying concentrations of arabinose and the β -galactosidase activity was measured (Fig. 1B). As shown, the efficiency of substrate cleavage was arabinose dose-dependent to some extent. However, cleavage was efficient even at the lowest concentration of inducer, in accordance with the design of a system where the enzyme (*scNS3*) concentration will surpass that of the substrate (encoded by the
20 *lacZ*_{NS5A/B} gene). The time course of the NS3 catalyzed cleavage was analyzed in an immunoblot (Fig. 1C). As shown, the intracellular concentration of MBP-*scNS3* (lower arrow) increased over time of induction that corresponded to the extent to which the engineered β -galactosidase (upper arrow) was cleaved (lanes 1-6). No cleavage was evident in cells expressing the inactive NS3 mutant (lanes 7-9). The middle band is an
25 irrelevant *E. coli* protein that is recognized by the polyclonal serum. As shown (lanes 1-6), the reaction proceeded to near completion, depleting β -galactosidase almost entirely over time. This was surprising considering that NS3 is subject to product inhibition and does not deplete the substrate when assayed *in vitro* (Berdichevsky et al., 2003). The cleavage of the *lacZ*_{NS5A/B} (mw of 127 kDa) between residues 279-280 was supposed to
30 yield products of 41.7 and 84.9 kDa. However, such products could not be detected in any of the immunoblots. Since it is unlikely that the polyclonal antiserum will fail to recognize both cleavage products, one may conclude that, for a presently unknown reason, these products are degraded. This serendipitous discovery indicates an increased

sensitivity and accuracy of the disclosed system, since it is no longer subject to product inhibition and all the recorded inhibition would result from the introduced potential inhibitor. Based on these results one can conclude that β -galactosidase level measured in the disclosed “test bacteria” is an indicator for NS3 enzymatic activity level that can
5 be detected by phenotypic changes on X-gal plates. This system may be adopted for other protease-substrate systems as long as the protease can be expressed in an active form in a host cell, preferably in bacteria.

Genetic screenings for proteases, for protease inhibitors in general (Dautin et al., 2000; Martinez et al., 2000) and for NS3 inhibitors in particular (Martinez and Clotet
10 2003) have been described but came short of providing the desired protease inhibitors. The present invention thus overcomes shortcomings of the previous methods.

Example 2. Application of the genetic screening for the identification of NS3-inhibiting scFvs

For the isolation of NS3 inhibitory antibodies, a diverse immune antibody
15 phage display library of $>10^7$ individual clones was constructed. The source of antibody genes was a pool of spleens from 3 mice that were immunized with NS3 and had serum titers >600000 after the third boost. A single cycle of DIP (Benhar et al., 2000) and of biopanning (Benhar and Reiter 2002) affinity selections of the phage antibodies were applied (separately) to partially enrich for NS3 binders. This was necessary to reduce
20 the library size for screening a reasonable number of clones on indicative plates. The scFv clones obtained as affinity-selection output (about 10^6 clones) were pooled and subcloned into pMALc-NN. The scFvs were tested for potential inhibition of NS3 in the form of MBP-scFvs since in that form the scFvs are stabilized as soluble active binders in the reducing environment of the cytoplasm (Bach et al., 2001). The pMALc-NN-scFv
25 pool was introduced into pMGT14-carrying “test bacteria” resulting in a “bacterial genetic screening” carrying two compatible plasmids with three different regulatory mechanisms for expression: IPTG-inducible high-level scFv expression, arabinose-inducible medium level NS3 expression and constitutive low level of β -galactosidase expression. The system is thus tuned such as sufficient NS3 is made to fully digest the
30 engineered β -galactosidase, unless inhibited by an excess of inhibitor that is produced at still a higher concentration.

The transformants (about 2×10^6 colonies) were screened on X-gal indicative agar plates (Fig. 1D). A total of 100 blue colonies were picked and analyzed by *Bst*NI fingerprinting as described (Benhar and Reiter 2002) resulting in 25 clones being identified as unique. The residing pMALc-NN-scFv plasmids were recovered and re-introduced into “test bacteria” to validate the results of the initial screening. All 25 clones regained their blue phenotype confirming that the phenotypic change was, indeed a direct result of scFvs expression. A sample of four of the clones (10, 11, 35 and 171) is shown in Fig. 1E in comparison to four control scFvs, three of which (37Y, 44Y and 53Y) that were isolated from a human synthetic antibody library (Zemel et al., 2004), bind NS3 without inhibiting it and one (Anti Tac, directed against the human IL-2 receptor) totally irrelevant (Benhar, et al., 2000).

Example 3. Characterization of specificity and binding affinity of selected scFvs

The scFvs isolated as blue colonies in the bacterial genetic screening were expressed and purified as soluble proteins and evaluated for scNS3 binding in an ELISA. Binding affinities were estimated from the half maximal binding signal. As shown in Fig. 2A, affinities of the selected scFvs and the control scFv 53Y (Zemel et al., 2004) ranged between 80 nM and 300 nM which are moderate binding affinities. The scFvs did not react with a panel of irrelevant proteins serving as specificity controls. The remaining 18 scFvs were weaker binders or weaker inhibitors in the quantitative assay (see below) and were not studied further. Thus, the disclosed genetic screening is very sensitive in identifying potential inhibitors that would escape detection by *in vitro* approaches. This is probably due to the MBP-fusion technology of scFv expression where the very high intracellular concentration of MBP-scFvs may compensate for its weak affinity (Bach et al., 2001).

To evaluate whether the scFvs NS3 epitopes overlap a competition ELISA was performed. As shown in Fig. 2B, scFvs 1, 10, 11, 35, 53Y and 171 could compete with scFvs 171 and 35 for specific binding to scNS3 while scFv 162 apparently did not. Since the tracers were the same scFvs with different epitope tags, relatively high concentration that prevented a full competition of binding had to be used. This is evident from the inferior inhibition obtained when the higher affinity scFv 171 is used as a tracer. Therefore, these data cannot be used to calculate binding affinities. Nonetheless, they do suggest that the binding epitopes of most of the identified scFvs

overlap at least partially (probably located around the NS3 catalytic site). Interestingly, the control scFv 53Y (Zemel et al., 2004) which is non-inhibitory also competed the binding of both scFvs 35 and 171 to scNS3 (Fig. 2B). This highlights the advantage of using a screening for inhibitory antibodies that is based on actual inhibition rather than
 5 merely on antigen binding. Due to their large size (in comparison to small molecule inhibitors), scFvs may inhibit NS3-catalysis by blocking the access of the substrate to the active site without engaging it directly.

Figure 7A illustrates the aligned amino-acid sequences of NS3-inhibiting scFvs (SEQ ID NOS:1-11). The scFvs sequence analysis revealed a high degree of sequence
 10 identity in the V_L CDR sequences in contrast to a higher diversity observed in the V_H CDR sequences, particularly at CDR3. In contrast, a more limited repertoire of germline genes appears in the selected V_H domains than in the V_L domains. Still, each V_H utilizes different D and J segments in the assembly of CDR3 while all the selected V_Ls utilize a single J segment, J5. This suggests that the V_L CDR3 was strongly selected by the
 15 disclosed genetic screening and is probably of critical importance.

Figure 7B illustrates the aligned amino-acid sequences of four NS3-inhibiting single-domain antibodies (V_H dAbs, SEQ ID NOS:12-14). These clones were initially identified in the short dAb form due to internal stop codons or frameshifting followed by premature translation termination. They were also characterized after replacing the
 20 stop codon with a sense codon, or restoring the full-length ORF, as they appear with the corresponding number in Figure 7A.

Table 2 summarizes the SEQ ID NOS of the scFvs and dAbs presented in Figure 7, both as free recombinant antibodies and as their corresponding MBP fusion proteins.

25 **Table 2: SEQ ID NOS of the scFvs and dAbs**

scFv Clone name	scFv SEQ ID NO:	scFv MBP fusion SEQ ID NO:	dAb Clone name	dAb SEQ ID NO:	dAb MBP fusion SEQ ID NO:
1	1	15			
12	2	16			
31	3	17			
35	4	18			
162	5	19	162SD	14	28
171	6	20	171SD	113	114

148	7	21	148SD	13	27
152	8	22			
161	9	23			
11	10	24	11SD	12	26
10	11	25			

Example 4. Validation of the selected scFvs as NS3 inhibitors

The potential of the selected scFvs to inhibit NS3 catalysis was quantitatively evaluated by measuring the β -galactosidase level that serves as an indicator for NS3 enzymatic activity level in genetic screening bacteria. As shown in Fig. 3A, cultures of the selected scFvs had high levels of β -galactosidase activity for all the scFvs tested, as compared to the control non-inhibitory scFv 53Y. The levels of β -galactosidase activity were in correlation to the level of scFvs expression, but could be detected even without IPTG induction due to the leakiness of the *Ptac* promoter under the assay conditions used. These results indicate that NS3 catalysis is inhibited by the selected scFvs in a dose dependent manner.

To further confirm the scFvs inhibitory effect on NS3 catalysis an *in vitro* fluorometric assay was applied for the measurement of NS3 protease catalysis and inhibition. As shown in Fig. 3B, all the tested scFvs inhibited NS3 catalysis in a dose dependent manner as compared to the control, non-inhibitory scFv 53Y. Different inhibition levels could be detected for the different scFvs and NS3 catalysis ranged between 12% and 54%. These results validated that the selected scFvs that (initially identified as blue colonies in the bacterial genetic screening and later validated as specific NS3 binders) are genuine NS3 protease activity inhibitors. As shown in Table 3, the binding affinity of the scFvs largely correlated (with scFv #1 as an exception) with their potency of *in vitro* inhibition. However, the β -galactosidase activity levels follow a different trend, probably due to different levels of accumulation and different stabilities of the scFvs in the cytoplasmic milieu.

Table 3: scFvs potency in *in vitro* inhibition

ScFv clone	Affinity	β -gal	% inhibition
171	90	67	88
35	160	51	78
10	280	48	62
162	280	84	62

148	170	72	58
11	300	60	52
1	80	44	46
53Y	160	6	26

Affinity: apparent kD in nM estimated from the binding curves in Fig. 2B; β -gal: β -galactosidase units taken from Fig 3A; % inhibition: value were obtained by subtracting the % catalysis value shown in Fig. 3B from 100%.

5 One goal in isolating scFvs that inhibit NS3 is for their application as intracellular antibodies *in vivo*. Intracellular scFv antibodies (intrabodies) with inhibitory properties present a new class of neutralizing molecules with great potential for gene therapy (Marasco, 1997; Marasco, 2001). Indeed, anti HCV intrabody-based
10 approached were suggested by studies where a scFvs that binds and inhibits the NS3 helicase was isolated using phage display technology (Sullivan et al., 2002; Tessmann et al., 2002). As for anti NS3 protease intrabodies, the inventors have recently reported the isolation of non-neutralizing anti-NS3 scFvs by antibody phage-display that as intrabodies partially inhibited NS3-mediated cell transformation by diverting NS3 from the cell cytoplasm to the nucleus (Zemel et al., 2004). Inhibition of NS3 catalysis by
15 small molecule inhibitors has been a formidable challenge due to featureless appearance of the NS3 catalytic groove that results in weak binding. Antibodies may offer a better solution by forming a “clamp” that involves interactions with NS3 that are located away from the active site on one hand, and blocking or interfering with substrate binding on the other. In the disclosed *in vitro* assay, the scFvs inhibit NS3 at micromolar
20 concentrations, thus they should probably be improved to make more potent inhibitors. The fact that their affinities lie in the low micromolar range as well suggest that they could be easily manipulated by approaches such as *in vitro* affinity maturation by phage display approaches (Benhar 2001) or by further exploiting the disclosed genetic screening towards such an end.

25 **Example 5. An expression system for fusion-stabilized peptides – construction and validation**

The design for expression of fusion-stabilized peptides (peptide aptamers) was based on fusion to the *E. coli* maltose-binding protein (MBP). Two fusion strategies

were designed, with peptides fused at the C terminus of MBP, or constrained in an internal permissive position of the protein (position 133 was chosen according to a literature survey of permissive insertion positions of MBP; (Martineau et al., 1996).

The expression platform was constructed as follows: C-terminal peptides were introduced by PCR using pMALc-NN (Bach et al., 2001) as template and re-cloning into the same backbone. The ligated vectors were introduced into the “bacterial test strain” and IPTG induction was used to overexpress the peptides. For the internal (constrained) position, two *Sfi*I restriction sites were introduced into the pMALc-NN vector at position 133 of MBP. *Sfi*I has a non-palindromic cleavage site; therefore upon digestion the resulting sticky ends could be used for directional cloning of oligonucleotide duplexes.

Initially control constructs were made for validation of the functionality of the screening. The appearance of a blue colony was expected to be indicative of an NS3 inhibitory effect of the peptide. Indeed, when “product-like” peptide (EASEDVVC) was cloned at the C-terminus of MBP, the resulting colonies in IPTG-induced “test bacteria” were blue. In contrast, when the FLAG epitope (DYKDDDDK) at the same position, the colonies, as expected for a non-inhibitor, were colorless.

Using a library screening approach, it is considered impractical to screen entire libraries (of 10^7 - 10^8 clones) on X-gal supplemented indicative plates since roughly 10^4 colonies can be visualized on a single plate making it necessary to inoculate and screen thousands of plates for every library. Therefore, the inventors took advantage of the fact that the “reporter strain” bacteria, when expressing the substrate while *scNS3* expression is repressed, express a low, but sufficient level of β -galactosidase activity for growth on lactose as a sole carbon source. However, when the β -galactosidase is cleaved by NS3, the β -galactosidase activity is mostly lost resulting in retarded growth on lactose. Thus, upon introduction of the peptide libraries into the “test strain” (expressing both substrate and enzyme), growth of the bacterial transformants on lactose-minimal medium resulted in the enrichment of inhibitor-expressing clones. Thus, the NNS8 library of 10^8 clones was subjected to such enrichment before the blue/white screening.

The results described above proved that the screening is appropriate for application with peptide-aptamers and has a built-in enrichment property allowing identification of potential binders without having to resort to affinity-selection schemes.

5 **Example 6. Construction and selection of libraries of random fusion-stabilized peptides**

Two peptide aptamer libraries were constructed. The first library, Rand3, consisted of three random residues fused at the C-terminus of EASEDVVC, (the NS5A/B site P side) creating non-cleavable peptides (due to the absence of the P'4 residue). This library was expected to yield mostly product-like or P site-like inhibitors and was built mainly to evaluate the performance of the screening. The second library was the NNS8 library where random octapeptides were fused at the C-terminus of MBP. Both libraries were screened using the genetic screening. A third library where 8-mer peptides are inserted after residue 133 of MBP (as constrained peptides) was also created for evaluation by the genetic screening.

15 For screening, DNA was prepared from the Rand3 library stock and introduced into pMGT14-carrying genetic screening bacteria that were plated directly on X-gal indicative plates. The limited library size (a theoretical complexity of 8000 clones that were over-represented in the 10^5 clones library) allowed direct screening.

DNA was also prepared from NNS8 library stock and introduced into pMGT14-
20 carrying genetic screening bacteria. The large library size prevented direct screening and required enrichment for inhibitors prior to screening. The enrichment by virtue of faster growth rate of NS3-inhibited clones (where β -galactosidase is intact and may ferment lactose) on lactose as a sole carbon source was carried out by using the resulting transformants (10^9 cells) to inoculate 1 liter of lactose minimal medium, 25 incubated at 30°C for 24 hours. Blue colonies were selected and plasmid DNA was recovered. To validate the "blue" phenotype the selected clones from both libraries were re-introduced into fresh test bacteria where only clones that formed again blue colonies were carried on for further analysis. The clones that had been validated were further tested for *in vitro* inhibition of NS3 catalysis.

30

Example 7. Validation and characterization of the isolated peptides

Several inhibitory peptide aptamers were so far identified and validated. Their sequences were determined from the expression plasmid (Tables 4 and 5). These plasmids were introduced into an appropriate bacterial expression host for overproduction of the peptide-aptamers. The purified aptamers were characterized for NS3 catalysis inhibition by an *in vitro* assay (Berdichevsky et al., 2003). As shown in Figure 4, they were indeed inhibitors of NS3 catalysis with an IC₅₀ in the low micromolar range. However, their binding to NS3 could not be detected by ELISA.

Table 4: Deduced amino-acid sequence of the validated peptide aptamers from the NNS8 library

Clone name	Peptide amino acid sequence	Peptide SEQ ID NO:	Peptide aptamer (internal peptide) SEQ ID NO:	Peptide aptamer (C' peptide) SEQ ID NO:
NNS8/N5	LGLAVEVR	29	36	43
NNS8/N7	FSRAEWFC	30	37	44
NNS8/N8	GAALNTSS	31	38	45
NNS8/N10	AVLAGLGV	32	39	46
NNS8/N21	FWGGALWR	33	40	47
NNS8/N22	CCAWLSIR	34	41	48
NNS8/N23	LAAWFCLW	35	42	49

Presented are the SEQ ID NOS of the free peptides and of the corresponding peptide aptamers comprising the peptides fused at the C' of MBP or at an internal permissive position following residue 133 of MBP.

Table 5: Deduced amino-acid sequence of the validated peptide aptamers from the Rand3 library (weak inhibitors)

Clone name	Peptide amino acid sequence	Peptide SEQ ID NO:	Peptide aptamer (C' peptide) SEQ ID NO:
RB13	EDVVCCWPV	50	55
RB15	EDVVCCCLF	51	56
RB16	EAVVCCLSL	52	57
RB17	EDVVCCCGC	53	58
RB18	EDVVCCVLY	54	59

Example 8. Epitope mapping of NS3-inhibiting scFvs 35 and 171

As a potential source for information regarding contacts between the isolated neutralizing scFvs 35 and 171 to NS3, an epitope mapping procedure was used. A

combined bacterial surface display of scFvs using the Lpp-OmpA system (Benhar et al., 2000) was used with random type 88 phage-displayed peptide libraries in which a second recombinant pVIII gene has been incorporated into the fd bacteriophage genome (Enshell-Seijffers et al., 2001). Two peptide libraries were used: a linear random 7mer
5 library-FM7 (2×10^9 clones) and a random library of 12 amino acids flanked by two constant cysteine residues thus generating random 12mer loops library -FDC12C (5×10^8 clones). Those two libraries were used to perform the Delayed Infectivity Panning- DIP selection (Benhar et al., 2000) in “reverse” where scFv-displaying bacteria were used to affinity select peptide-displaying phages. The affinity selected
10 phages were used as an epitope-defining database that is applied *via* a computer algorithm (herein denoted TAU algorithm) that has been developed by the inventors (Enshell-Seijffers et al., 2003) to analyse the crystalline structure of the original NS3 antigen. On the basis of the predicted epitope, segment of the NS3 antigen was used to reconstitute an antigenic epitope mimetic that was examined as anti NS3 scFvs
15 inhibition competitor. In addition, according to the predicted epitope point mutations were inserted into the NS3 antigen and those mutated antigens were validated for their antibodies inhibition activity. These procedures are detailed hereinbelow.

The bacterial surface display vector pIB-Tx (Benhar et al., 2000) which was used for antigen surface display through fusion of the antigen gene to the *E. coli* surface
20 display system is composed of a chimeric *Lpp-OmpA*’ (Benhar et al., 2000). This vector was modified to enable cloning of the antibodies between the *NcoI* and *NotI* restriction sites (Mazor et al., 2005). The new vector pIBTx-NN is used in this work to sub-clone the scFvs 35 and 171 from the pMALc-TNN vector through the *NcoI* and *NotI* restriction sites.

25 Next, spun-cell ELISA was used to examine the scFvs bacterial cell surface display efficiency and the ability of the cell displayed antibody to keep its functional specific binding to the NS3 antigen. Accordingly, induction conditions were determined, as well as the ability of cells displaying scFv-35 or 171 to bind MBP-scNS3 antigen. As a negative control, anti CD30 scFv was cloned to the pIBTx-NN
30 vector (Mazor et al., 2005).

The bacterial displaying plasmids (pIBTx-NN-scFvs 35 or 171) were introduced into *E. coli* TG-1 cells. The cells were grown overnight in LB medium supplemented

with 25µg/ml kanamycin at 16°C. Cultures were diluted to OD_{600nm} of about 0.5 and IPTG was added to final concentration of 0.1, 0.01, 0.001 or 0mM. The cells were further grown for 16 h and stored on ice. An aliquot equivalent to 1ml of culture at OD_{600nm} of about 1.0 (~10⁹ cells) was taken for analysis of surface display. Cells were
5 collected by centrifugation and washed twice with 1ml of PBST. Washed cells were then suspended in 1ml of 5µg/ml MBP-scNS3 in PBST for 1h on ice. Next, cells were collected by centrifugation and washed twice with 1ml of PBST. Then washed cells were incubated with a mixture of anti-MBP-scNS3 mouse serum (×5000 dilution in PBST) and HRP-conjugated goat anti mouse antibody (×10000 dilution in PBST) for 1h
10 on ice. The cells were washed twice with PBST and twice with PBS, by repeated centrifugation and re-suspension, and then 1ml of the HRP substrate TMB was added. Color development was terminated with 0.5ml H₂SO₄. Color was recorded at 450nm.

The results of the spun-cell ELISA are shown in Figure 8. As shown, the best cell displaying scFv-171 was at 0.1mM IPTG concentration, and for cell displaying
15 scFv-35 the best IPTG concentration was 0.01mM. ScFv-35 cell surface display appeared less efficient than scFv-171.

Next, the phage libraries were enriched for specific binding clones by subjecting the phages to two rounds of selection, reverse delayed infectivity panning (DIP) selection.

20 Bacterial display vector-containing cells (TG1-cells) were grown at 16°C, induced with IPTG and washed as for the spun-cell ELISA described above. The cells were re-suspended in 3% skim milk powder in PBS containing ~10¹¹ cfu of peptide displaying libraries (FDC12C, FM7 or mixture of both, wherein the two libraries were used separately for scFv-171 and the a mixture thereof was used for scFv-35) and left
25 on ice for 1 h. The cells were then washed five times with PBST and five times with 2YT + kanamycin. Next, 1ml of *E. coli* DH5αF' cells at O.D600 of about 0.8 were added and the cells were shifted to 37°C for 30 min without shaking followed by 30 min with shaking, then aliquots were taken for plating on tetracycline supplemented 2YT plates to obtain single colonies for screening and determination of phage panning
30 output. Phage titration was done as described above. Phage stocks for the next DIP cycle were prepared by infecting 500-1000ml DH5αF' cells in 2YT medium with output

phages. The infected culture was grown overnight at 30°C and phages were prepared by centrifugation followed by PEG precipitation as described above.

As additional affinity selection to further enrich specific binders, phage particles were prepared from the second output library of scFv-35 reverse DIP selection, and applied to an affinity-selection by biopanning on MBP-scFv-35 antigen. A single well of a 24-well Cell Culture plate was coated with 1ml of Mouse Monoclonal Anti MBP in PBS ($\times 2000$ dilution) for five h at 4°C. Followed by one wash with PBS, 1ml of 5 μ g/ml MBP-scFv-35 in PBS was added for overnight at 4°C. After discarding the excess solution, the well was blocked with 3ml of 3% skim milk powder in PBS for one h at room temperature. Next, the well was washed with PBS and 1ml of 10^{11} phage output from the second cycle of DIP selection on anti NS3 scFv-35 in 1.5% skim milk powder was added for one h with gentle agitation at room temperature. Unbound phages were rinsed away and the well was washed extensively ten times with PBST and ten times with PBS. Bound phages were eluted with 1ml of 100mM triethylamine pH 13.0 for 30 min at room temperature with gentle agitation. The eluate was transferred into 1.5ml tube and neutralized with 200 μ l of 1M Tris-HCl, pH 7.4. To obtain single colonies for screening and determination of phage panning output, DH5 α F⁺ bacteria were infected with the affinity selected phages, plated on LB plates containing tetracycline and grown at 37°C overnight.

Following phage selection, randomly selected single clones that were picked from the second Reverse DIP and panning cycles, were screened for binding to anti NS3 scFvs 35 and 171 using immuno dot-blot analysis. 96 single colonies from output of scFv-171 reverse DIP with the FM7 library, 192 single colonies from FDC12C library, 192 single colonies from scFv-35 reverse DIP with the mixed libraries and 192 single colonies from scFv-35 panning cycle were picked at random and phages were prepared in 96-wells plates as follows: single colonies were picked to 200 μ l of 2YT+ tetracycline in U-bottom 96-well plates. After overnight culture, the plates were centrifuged at 2100 rpm for 15 min. a portion (125 μ l) of the supernatant from each well was transferred to a flat-bottom, 96-well plate already containing 50 μ l/well of PEG/NaCl solution. The flat-bottom plates were incubated at 4°C for two h and centrifuged. The precipitated phages were re-suspended in a total of 100 μ l of TBS and applied *via* a vacuum manifold to nitrocellulose filters. After blocking with TBS/5%

milk for one h, the filters were washed briefly with TBS and incubated overnight with selected MBP-scFv (50µg/ml) in 2% milk in TBS at 4°C with gentle rocking. After washing, the membranes were incubated with Mouse Monoclonal Anti- MBP (×10000 dilution in TBS) followed by Peroxidase-conjugated AffiniPure Goat Anti-Mouse IgG (×10000 dilution) in TBS/2% milk for one h at room temperature. The positive signals were detected by enhanced chemiluminescence (ECL) immunodetection. 14 suspected MBP-scFv-171 phage binders from 96 single colonies screening were picked for a verification Dot-Blot. Only one phage was verified as a binder at the verification Dot-Blot, although its dilutions look like it is inverted. The inverted dilutions could appear because of PEG remainders that interfere with the development of the color reaction.

As shown in Figure 9, 39 suspected MBP-scFv-171 phage binders from 192 single screened colonies were picked for a verification Dot-Blot. Here again, some inverted dilutions can be seen. Unbound phage that were taken from the first Dot-Blot screening as negative controls F3, D14 and D21 didn't bind the scFv-171 antigen at the verification Dot-Blot as well. 17 binders' were sequenced and analyzed by the TAU algorithm.

As for 35 binders shown in Figure 10, 22 suspected MBP-scFv-35 phage binders from 192 single screened colonies were picked for a verification Dot-Blot. Here again some inverted dilutions can be seen. Unbound phages that were taken from the first Dot-Blot screening, as negative controls F9 and E13 didn't bind scFv-35 antigen at the verification Dot-Blot as well. Two binders were sequenced and analyzed by the TAU algorithm. In addition, from the panning output (Fig. 11), 22 of the 192 single screened colonies were picked and verified by another Dot-Blot. A6 and B15 were used as negative controls. From the positive binders, 8 clones were sequenced and analyzed by the TAU algorithm.

Sequences of peptides inserts of 17 scFv-171 positive binding phages (Table 10) and 10 scFv-35 positive binding phages (Table 11) were obtained.

Table 10: Sequences of the scFv-171 binders

Clone name	Peptide amino acid sequence	Peptide SEQ ID NO:
A8	CCGGGWANNNPNC	83
C6	CCQTLQDHERKPEC	84
D10	CSKMEKCTQNKLEC	85
E4	CRHYRSYNYSRCSC	86
E8	CDCKDSTKKGATSC	87
E9	CGPGETCRKGIFHC	88
F12	CSDCANADSLC	89
G10	CITVQEMCDERDNC	90
H6	CYCRKGTQSTHKAC	91
A17	CAYHCATTRTFGAC	92
B13	CAQQFEITNCRDQC	93
B21	CTVSGSVCRYFWSC	94
C20	CDRNTDSGRRCGRVC	95
D20	CRCIRPNNSPEGEC	96
D24	CERGDLECGDTINC	97
F13	CPMRSHSCNRVVHC	98
G13	CTQIPEARACPWKC	99

Table 10 presents sequences of inserts of 17 phages selected by screening of FDC12C phage display peptide library with scFv-171. One of the selected peptides happens to contain only eleven residues.

5 **Table 11: Sequences of the scFv-35 binders**

Clone name	Peptide amino acid sequence	Peptide SEQ ID NO:
C7	CILSISKCGSGVNC	100
D7	CHPALC	101
A3	CNCRSWQEMWDRAC	102
A8	CAYPEGLSAGSEPW	103
F11	CFYGVCI GTVDNRC	104
G1	LRGVGGQ	105
G5	CVASYLI	106
D20	CVNPCSRSVETAIC	107
E17	CGTRSSLCELQSRC	108
F17	CRATTTPLTITPKC	109

Table 11 presents sequences of inserts of 10 phages selected by screening of FDC12C and FM7 mixed phage display peptide libraries with scFv-35. Two of the selected peptides are from the 7-mer FM7 libraries and one happens to contain only six residues.

10 As shown, no obvious sequence identity exists between scFv171 positive binding peptides and between scFv-35 positive binding peptides. To solve this problem

we used a systematic computer algorithm that enables us to focus on the common denominators of the peptides and use this information to map epitope onto the surface of the solved crystalline structure of NS3 protease domain: NS4A peptide complex (PDB-1A1R). As shown in Figure 12, the two predicted epitopes, namely the scFv-35
5 epitope and the scFv-171 epitope on NS3 were mapped to the same region and shares seven identical residues. The epitope according to this prediction is far from the catalytic triad

As shown in Figure 13, scFv-35 and scFv-171 overlapping epitopes contain two cysteine residues that are part of the protease zinc-binding site. In addition scFv-171
10 epitope contains an additional residue of this binding site histidine 149.

In order to confirm the prediction of scFv 35 and 171 shared epitope on NS3, the epitope was reconstituted on basis of the prediction. To that end, a short peptide segment of NS3 that was predicted to comprise the antibodies shared epitope was used to generate an epitope mimetic. A 14 amino acids peptide was designed, that appear as
15 linear residues on NS3, residues 90-103 N'-GARSLTPCTCGSSD-C' (SEQ ID NO:110), which contains the shared residues of both epitope predictions (marked in bold letters). This epitope mimetic peptide was used in the *in vitro* fluorometric assay. The logic of this assay is that if this peptide represents a part of the true epitope, scFvs will bind to it, thus scFvs will be occupied and inhibition of NS3 catalytic activity will
20 decrease, meaning NS3 activity will increase. In this assay scFvs were added at 200µg/ml concentration, and peptides were added in x5 dilutions starting at 50µM. As negative controls, a peptide 21 amino acids in length denoted peptide 6 (N'-TLDPRSFLLRNPNDKYEPFWK-C', SEQ ID NO:111) and a peptide eight amino acids in length denoted peptide x (N'-ISEVNLDA-C', SEQ ID NO:112) were used. To
25 test whether those peptides have any effect on NS3 catalytic activity a control *in vitro* assay was performed, in which the peptides and the NS3 enzyme were added alone without the inhibitor antibodies. The results are shown in Figures 14 A-C.

The results of this competitive *in vitro* assay (Figs. 14 A-B) show a difference between the catalytic activities of NS3 with the presence of the peptide mimetic and
30 without it or with control peptides, with both of the scFvs 35 and 171. In the scFv-35 competitive *in vitro*, adding the peptide mimetic increased NS3 catalytic activity in 20% from 73% to 93% catalysis, while in the scFv-171 competitive *in vitro*, adding the

peptide mimetic increased NS3 catalysis in more than 30%, from 60% to 93% catalysis. Those values were received at the highest peptide dilution (50 μ M). Catalysis did not reach to 100% because the proteolytic product inhibits the enzyme catalysis. These results suggest that the peptide may indeed represent a part of the NS3 epitope
5 recognized by our antibodies. Accordingly, in the presence of the peptide mimetic scFvs are occupied binding it, therefore a decrease in NS3 inhibition can be seen. In addition, a control *in vitro* assay was performed (Fig. 14C), in which it was tested whether the peptides (the peptide mimetic and two control peptides) have any effect on NS3 catalytic activity. The results of this control assay show that none of the peptides by
10 itself affected NS3 catalytic activity.

Example 9. Inhibition of HCV-RNA replicons by the NS3 inhibitors

For intracellular expression of the anti-NS3 protease single-chain antibodies in the cytoplasm of mammalian cells, the scFvs coding genes were subcloned as *NcoI-NotI* fragments, from plasmid pMALc-NN-scFv into the pCMV/myc/cyto-MBP vector
15 (Shaki-Loewenstein et al., 2005). The replication inhibition efficacy of the NS3-inhibiting intrabodies was evaluated using the SEAP secreting replicon system (Yi et al., 2002; Bourne et al., 2005). The seven inhibiting intrabodies including the control scFv 53Y were introduced into these replicon-bearing Huh7 cells by transient transfection of the pCMV-MBP-scFv vectors using the Fugene reagent. SEAP activity
20 secreted from the cells was measured over successive 24-h intervals. Transfection efficiency, observed by expression of EGFP, was determined as about 30% and values were corrected accordingly.

As shown in Figure 15, SEAP secretion was inhibited in replicon cells transiently transfected with the NS3-inhibiting intrabodies but not with the control
25 scFvs 53Y. All the seven intrabodies demonstrated inhibitory effect, ranging between 70-100%. Inhibition was well observed at the first and second days post transfection, when intrabodies expression level is high. At the third and fourth days post transfection SEAP secretion is rising, probably due to the decline in the expression of the transiently transfected intrabodies. Interestingly, scFvs 35 and 171 that were found to be the most
30 potent inhibitors, as was observed in an *in vitro* assay, follow the same trend also in this assay, providing a strong validation for their inhibitory competence.

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CLAIMS

1. An isolated polypeptide or peptide comprising an NS3 inhibitor having an amino acid sequence as set forth in any one of SEQ ID NOS:1-14, 29-35 and 113, or fragments, analogs, homologs, derivatives and salts thereof.
- 5 2. The polypeptide of claim 1, wherein the NS3 inhibitor is fused to a stabilizing protein.
3. The polypeptide of claim 2, wherein the stabilizing protein is *E. coli* maltose binding protein (MBP).
4. The polypeptide of claim 3, wherein the NS3 inhibitor is fused to the C terminus of
10 MBP.
5. The polypeptide of claim 4 having an amino acid sequence as set forth in any one of SEQ ID NOS:15-28, 43-49 and 114.
6. The polypeptide of claim 3, wherein the NS3 inhibitor is fused at an internal position following position 133 of MBP.
- 15 7. The polypeptide of claim 6 having an amino acid sequence as set forth in any one of SEQ ID NOS:36-42.
8. A pharmaceutical composition comprising the isolated NS3 inhibitor of claim 1 and a pharmaceutically acceptable carrier or excipient.
9. An isolated nucleic acid sequence encoding the NS3 inhibitor of claim 1 as set forth
20 in any one of SEQ ID NOS: 115-125, or fragments, analogs, homologs, and derivatives thereof.
10. A recombinant construct comprising an isolated nucleic acid sequence encoding the NS3 inhibitor of claim 1 operably linked to one or more transcription control elements.
- 25 11. A vector comprising the recombinant construct of claim 10.
12. A pharmaceutical composition comprising the vector of claim 11 and a pharmaceutically acceptable carrier or excipient.
13. A host cell comprising the vector of claim 11.

14. A pharmaceutical composition comprising the host cell of claim 13 and a pharmaceutically acceptable carrier or excipient.
15. A polynucleotide comprising a nucleic acid sequence encoding a recombinant reporter protein, wherein the reporter protein comprises a β -galactosidase derivative and an amino acid sequence comprising a protease recognition sequence between
5 residues 279-280 of β -galactosidase, and wherein: (i) the recombinant reporter protein retains β -galactosidase activity; (ii) the cleavage of the reporter protein by the protease results in reduced β -galactosidase activity; and (iii) the cleavage of said reporter protein by the protease does not substantially result in accumulation of
10 cleavage products capable of substantially inhibiting said protease.
16. The polynucleotide of claim 15, wherein the protease recognition sequence is an HCV NS3 cleavage site.
17. The polynucleotide of claim 16, wherein the NS3 cleavage site has an amino acid sequence as set forth in any one of SEQ ID NOS:60-63 and 70.
- 15 18. The polynucleotide of claim 17, wherein the recombinant reporter protein has an amino acid sequence according to any one of SEQ ID NOS:64-65.
19. A polypeptide having an amino acid sequence as set forth in any one of SEQ ID NOS:64-65.
20. A recombinant construct comprising the polynucleotide of claim 16 operably linked
20 to one or more transcription control elements.
21. The recombinant construct of claim 20 further comprising a nucleic acid sequence encoding a protease capable of cleaving said reporter protein, operably linked to one or more transcription control elements.
22. The recombinant construct of claim 21, wherein the protease is HCV NS3 protease
25 or a derivative thereof.
23. The recombinant construct of claim 22, wherein the protease has an amino acid sequence as set forth in SEQ ID NO:66.
24. The recombinant construct of claim 20 further comprising a nucleic acid sequence encoding a potential inhibitor of said protease, operably linked to one or more
30 transcription control elements.

25. A vector comprising the recombinant construct of claim 20.
26. A vector as set forth in any one of SEQ ID NOS:67-69 and 82.
27. A host cell comprising the vector of claim 25.
28. A host cell comprising the vector of claim 26.
- 5 29. A method of screening for protease inhibitors, comprising:
- a) co-expressing a protease and a recombinant reporter protein cleavable by the protease in a host cell, wherein the cleavage of the reporter protein by the protease results in reduced activity of said reporter protein, and wherein the cleavage of said reporter protein by the protease does not substantially result in
 - 10 accumulation of cleavage products capable of substantially inhibiting said protease;
 - b) exposing the host cell to potential inhibitors of said protease; and
 - c) screening for host cells that retain said reporter protein activity.
30. The method of claim 29, wherein the recombinant reporter protein comprises a β -galactosidase derivative and a protease recognition sequence of said protease
- 15 inserted in a permissive site of β -galactosidase so as to retain β -galactosidase activity.
31. The method of claim 30, wherein the protease recognition sequence is inserted between residues 279-280 of β -galactosidase.
- 20 32. The method of claim 29, wherein the protease recognition sequence is an HCV NS3 cleavage site.
33. The method of claim 32, wherein the NS3 cleavage site has an amino acid sequence as set forth in any one of SEQ ID NOS:60-63 and 70.
34. The method of claim 33, wherein the recombinant reporter protein has an amino
- 25 acid sequence according to any one of SEQ ID NOS:64-65.
35. The method of claim 29, wherein the protease is associated with a disease or disorder in a human or non-human subject.
36. The method of claim 35, wherein the protease is a viral protease.

37. The method of claim 36, wherein the protease is HCV NS3 protease.
38. The method of claim 37, wherein the protease has an amino acid sequence as set forth in SEQ ID NO:66.
39. The method of claim 29, wherein potential inhibitors are selected from: amino acids, proteins, peptides, nucleic acids, organic molecules and inorganic compounds.
40. The method of claim 39, wherein potential inhibitors are selected from: antibody fragments, peptides and peptide aptamers
41. The method of claim 26, wherein the host cell is a bacterial host cell.
42. The method of claim 41, wherein the host cell is *E. coli*.
43. A protease inhibitor isolated by the method of claim 29.
44. A method of treating Hepatitis C virus (HCV) infection or preventing the diseases and disorders caused by HCV infection in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising an NS3 inhibitor having an amino acid sequence as set forth in any one of SEQ ID NOS:1-49 and 113-114, or fragments, homologs, derivatives and salts thereof.
45. The method of claim 44, wherein said diseases and disorders are selected from the group consisting of hepatitis, cirrhosis, liver failure and hepatocellular carcinoma (HCC).
46. A method of treating Hepatitis C virus (HCV) infection or preventing the diseases and disorders caused by HCV infection in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition comprising a recombinant construct comprising an isolated nucleic acid sequence encoding an NS3 inhibitor as set forth in any one of SEQ ID NOS:115-125 or fragments, homologs, and derivatives thereof, operably linked to one or more transcription control elements.
47. The method of claim 46, wherein said diseases and disorders are selected from the group consisting of hepatitis, cirrhosis, liver failure and hepatocellular carcinoma (HCC).

48. A method of treating Hepatitis C virus (HCV) infection or preventing the diseases and disorders caused by HCV infection in a subject in need thereof, comprising: a) obtaining cells from the subject; b) contacting the cells *ex vivo* with a therapeutically effective amount of a pharmaceutical composition comprising a recombinant construct comprising an isolated nucleic acid sequence encoding NS3 inhibitor as set forth in any one of SEQ ID NOS:115-125 or fragments, homologs, and derivatives thereof, operably linked to one or more transcription control elements; and c) re-introducing said cells to said subject.
49. The method of claim 48, wherein said diseases and disorders are selected from the group consisting of hepatitis, cirrhosis, liver failure and hepatocellular carcinoma (HCC).
50. A method of preventing Hepatitis C virus (HCV) infection in a liver transplant, comprising: a) treating a liver transplant before transplantation *ex vivo* with a therapeutically effective amount of a pharmaceutical composition comprising a recombinant construct comprising an isolated nucleic acid sequence encoding NS3 inhibitor as set forth in any one of SEQ ID NOS: 115-125 or fragments, homologs, and derivatives thereof, operably linked to one or more transcription control elements; and b) transplanting the liver transplant to a subject in need thereof, thereby generating an HCV-immune liver transplant.

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Step I: test bacteria

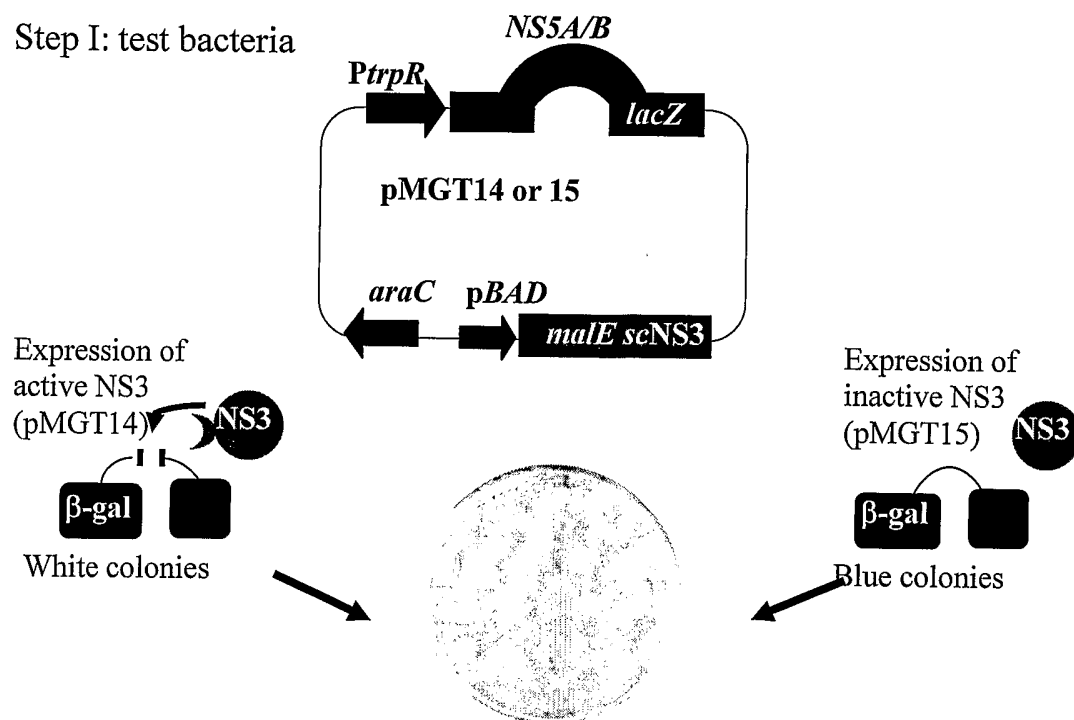


Figure 1A

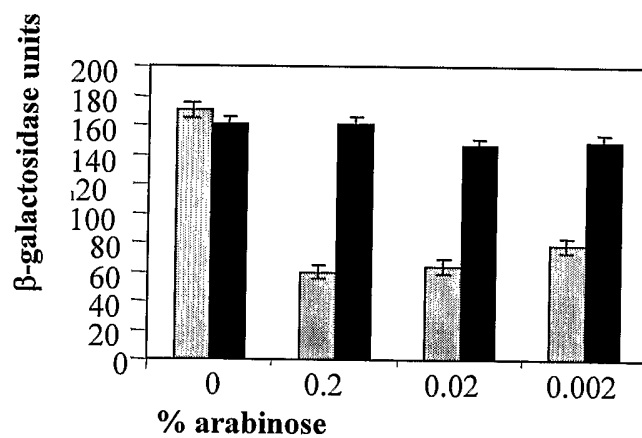


Figure 1B

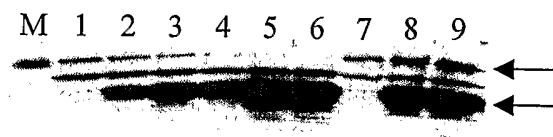


Figure 1C

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Step II: bacterial genetic screen

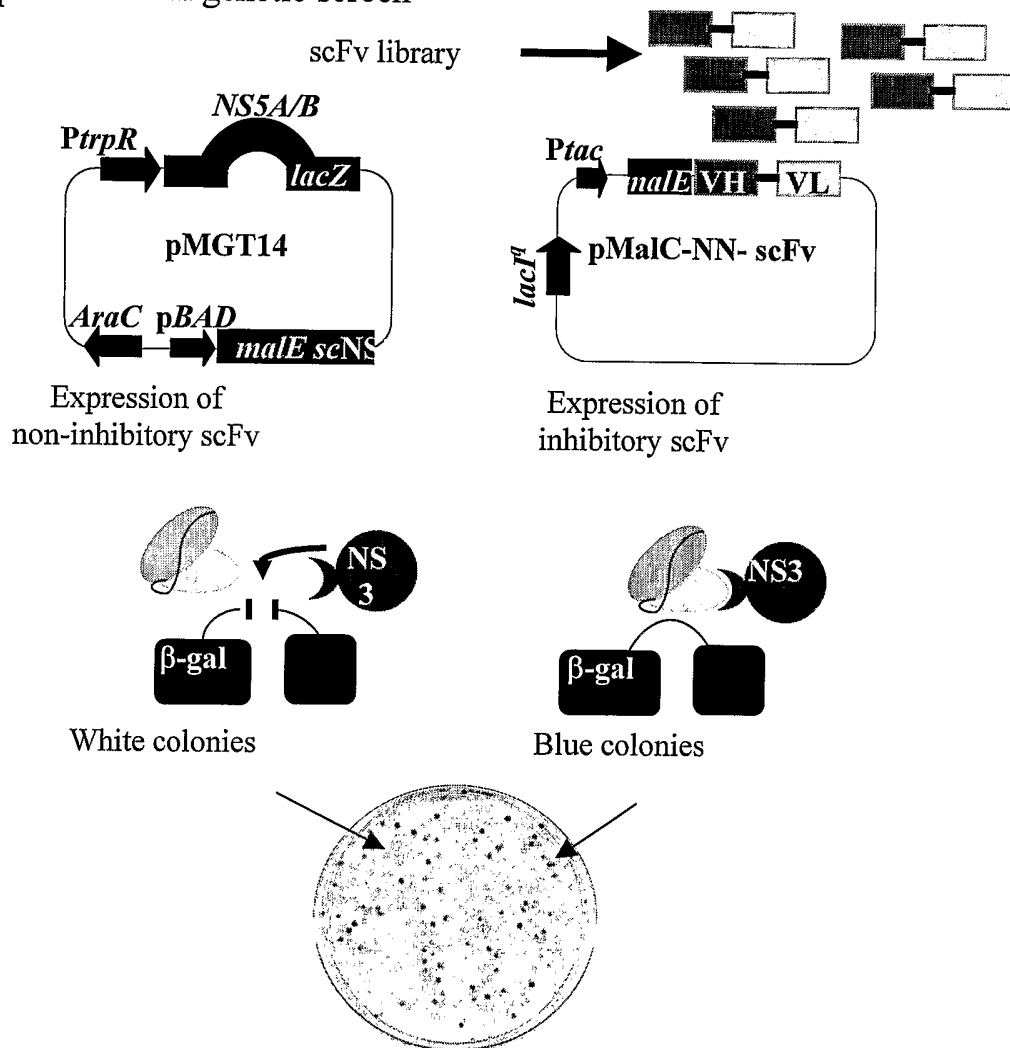


Figure 1D

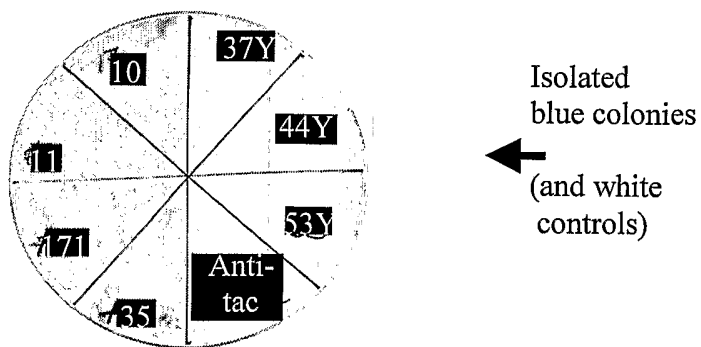


Figure 1E

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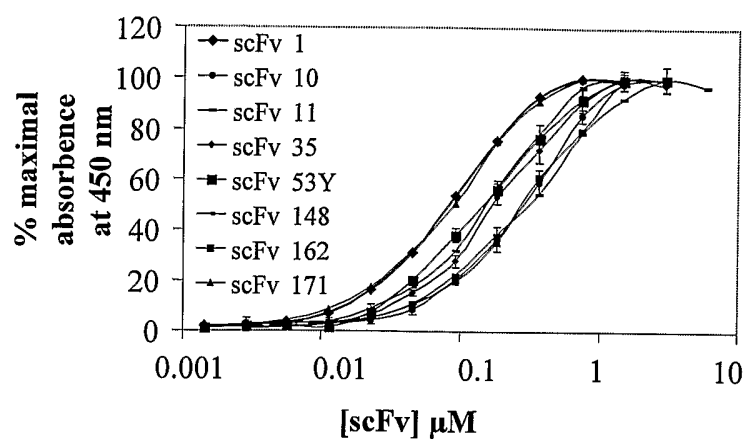


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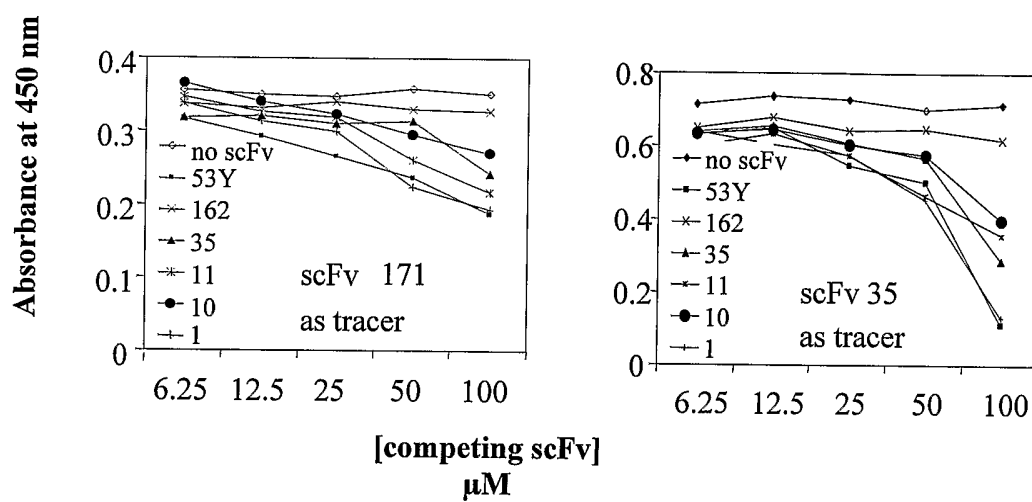


Figure 2B

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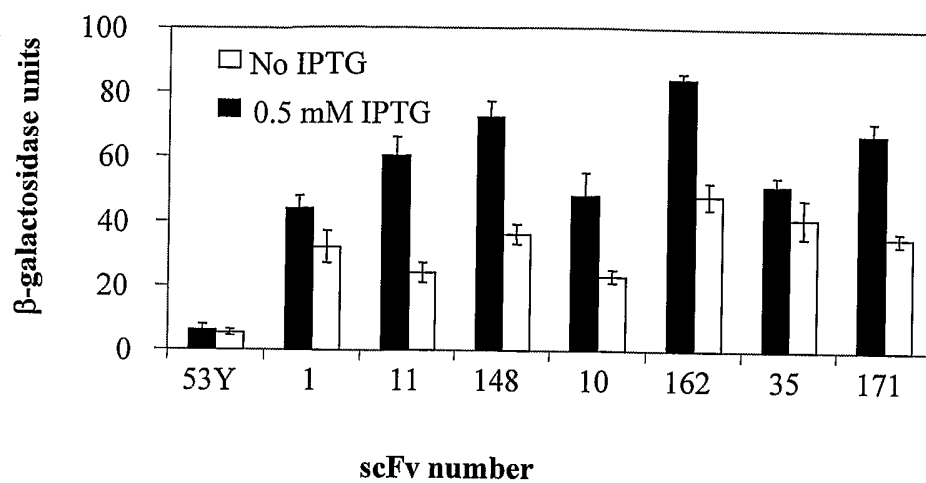


Figure 3A

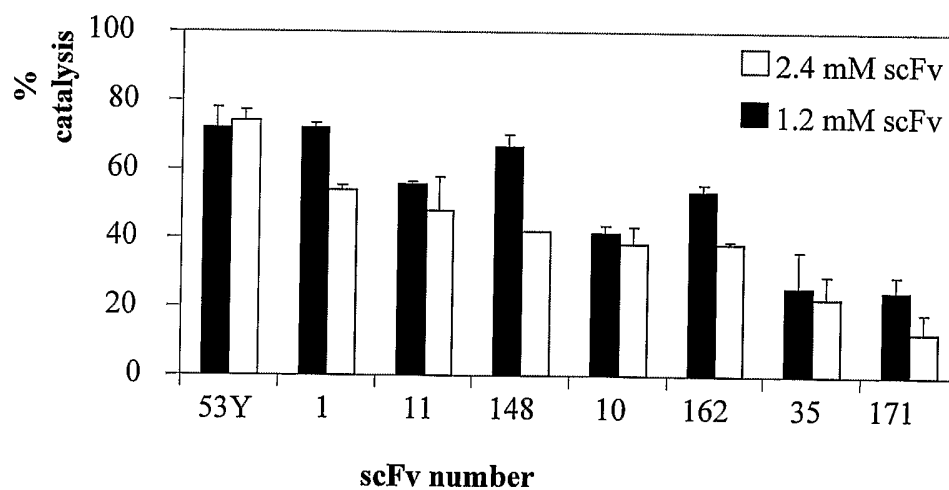


Figure 3B

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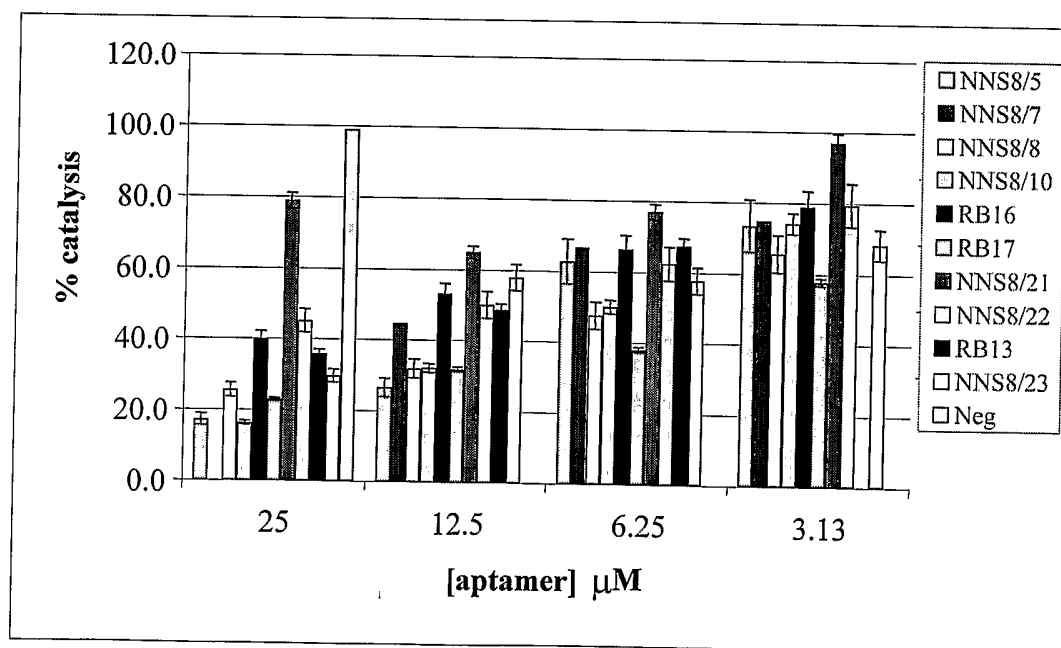


Figure 4

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 AWFPAPEAVPESWLECDLPEADTVVVP SNWQM HGYDAP IYTNVTYPITVNPPFVPTENPTGCYSLTF
 NVDESWLQEGQTRIIFDGVNSAFHLWCNGRWVGYGQDSRLPSEFDLSAFLRAGENRLAVMVLRWSDG
 SYLEDQDMWRMSGIFRDVSL LHKPTTQISDFHVATR FNDDFSRAVLEAEVQMC GELRDYLRVTVSLW
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 LMKQNNFNAVRC SHYPNHPLWYTLCDRYGLYVVDEANIETHGMVPMNRLTDDPRWLPAMSERVTRMV
 QRDRNHPSVI IWSLGNESGHGANHDALYRWIKSVDP SRPVQYEGGGADTTATDII CPMYARVDEDQP
 FPAVPKWSIKKWL SLPGETRPLILCEYAHAMGNSLGGFAKYWQAFRQYPR LQGGFVWDWVDQSLIKY
 DENGNPWSAYGGDFGDTPNDRQFCMNGLVFADRTPHPALTEAKHQQQFFQFRLSGQTIEVTSEYLF
 HSDNELLHWMVALDGKPLASGEVPLDVAPQ GKQLIELPELPQ PESAGQLWLTVRV VQPNATAWSEAG
 HISAWQQWRLAENLSVTLPAAASHAIPHLTTSEMDFCIELGNKRWQFN RQSGFLSQMWIGDKKQLLTP
 LRDQFTRAPLDNDIGVSEATR IDPNAWVERWKAAGHYQAE AALLQCTADTLADAVLITTAHAWQHQG
 KTLFISRKTYRIDGSGQMAITVDVEVASDTPHPARIGLNCQLAQVAERNWLGLGPQENYPDRLTAA
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Figure 5

MAMKTEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWA
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 EEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGV DNAGAKAGLTFL
 VDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGV
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 EIMPNI PQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSSV PSSLVIEGRPEFSTMA**PIGSVV**
IVGRIILSGRGGPITAYSQQTRGLLGCIITSLTGRDKNQVEGEVQVVSTATQSFLATCVNGACWTV
 FHGAGSKTLAGPKGPITQMYTNVDQDLVGWPAPPGARSLTPCTCGSSDLYLVTRHADVIPVRRRGD
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Figure 6

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Clone#

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35	EVQLQQSGAELVRSGASVKLSCTASGFNIKDYIMHWVKQRPEQGLEWIGWIDPENGDEYEQKFK						
162	EVQLQQSGAELVRSGASVKLSCTASGFNIKDYIMHWVKQRPEQGLEWIGWIDPANGNTKYDPKFQ						
171	EVQLQQSGAELVRSGASVKLSCTASGFNIKDYIMHWVKQRPEQGLEWIGWIDPENGDEYAPKFQ						
148	EIQLQQSGAELVKPGASVKLSCTASGFNIKDTFVHWVKQRPEQGLEWIGRIDPANGNTKYDPKFQ						
152	EVQLQQSGTELKPGASVKLSCTASGFNIKDTYMQWVKQRPEQGLEWIGWIDPENGDETESAPKFQ						
161	QVQLQQPGAELVKPGASVKLSCTASGFNIKDTYMHVVKQRPEQGLEWIGRIDPANGNTKYDPKFQ						
11	EVQLQQSGAELVKPGASVKLSCTASGFNIKDYIMHWVKQRPGQGLEIYIGRIYPGNGQTDYNGKFK						
10	QVQLQQSGAELMKPGASVKISCKATGYTFSNYWIEWVKQRPGHGLEWIGGILPGSGSTNYNEKFK						
	75	85	95	105	115	125	
			HCDR3			Linker	
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12	GKATLTADKSSSTAYMQLSSLASEDSAVYYCARSRA-----MDDWGQGTSLTVSSGGGGSGGGG						
31	GKAQLTVDTSSTAYIQFSSLTSDSAIYYCARQNLDDYDA-MDYWGQGTSLTVSSGGGGSGGGG						
35	GKATLTADKSPSTAYMQLSSLTSEDSAVYYCARI--TTDYY-FDYWGQGTTLTVSSGGGGSGGGG						
162	GKATITADTSSNTAYLQLSSLTSEDYAVYYCARW---DWY-FDVWGAGTTLTVSSGGGGSGGGG						
171	GKATMTADTSSNTAYLQLSSLTSEDYAVYYCNAWGGSSDYAMDYWGQGTSLTVSSGGGGSGGGG						
148	GKATITAATSSNTAYLQLSSLTSEDYAVYYCAT---GLDYAMDYWGQGTSLTVSSGGGGSGGGG						
152	GKATMTADTSSNTAYLQLSSLTSEDSAVYFCARERDYGYEGFAYWGQGTSLTVSSGGGGSGGGG						
161	GKATITADKSSSTAYMQLSSLTSEDSAVYYCTRI-----WLPWGQGTSLTVSSGGGGSGGGG						
11	GKATLTADKSSSTAYMQLSSLTSVDSAVYFCARGL-----FAYWGQGTSLTVSSGGGGSGGGG						
10	GKATFTADTSSNTAYLQLSSLTSEDYAVYYCNADGP----PGFDYWGQGTTLTVSSGGGGSGGGG						
	140	150	160	170	180	190	
			LCDR1			LCDR2	
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12	SGGGGSDVLMQTPLSLPVS LGDQASISCRSSQSI VHSNGNTYLEWYLQKPGQSPKLLIYKVSNR						
31	SGGGGSDVLMQTPLSLPVS LGDQASISCRSSQSI VHSNGNTYLHWYLQKPGQSPKLLIYKVSNR						
35	SGGGGSDVMTQTPLSLPVS LGDQASISCRSSQSI VHSNGNTYLEWYLQKPGQSPKLLIYKVSNR						
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171	SGGGGSDVMTQTPLSLPVS LGDQASISCRSSQSI VHSNGNTYLHWYLQKPGQSPKLLIYKVSNR						
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11	SGGGGSDVMTQTPLSLPVS FGDQVSI SCRSSQSI LANSYGNLYSWCLHKPGQSPQLLIYGISNR						
10	SGGGGSDVLMQTPLTSLVTIGQPASISCKSSQSLDSDGKTYLNWLLQRPQSPKRLIYLVSKL						
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			LCDR3				
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31	FSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPYTFGGGTKLEIKR						3
35	FSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPLTFGAGTKLEIKR						4
162	FSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHPVPTFGGGTKLEIKR						5
171	FSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHPVPTFGGGTKLEIKR						6
148	FSGVPDRFSGSGSGTDFTLKISRVEAEDLGLYFCSQSTHVPVPTFGGGTKLEIKR						7
152	FSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPLTFGAGTKLEIKR						8
161	FSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHV-LTFGAGTKLEIKR						9
11	FSGVPDRFSGSGSGTDFTLKISTIKPEDLGMYYCLQGTHQPLTFGAGTKLEIKR						10
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Figure 7A

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Clone#

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			HCDR1		HCDR2		
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148SD	EIQLQQSGAELVKPGASVKLSCTASGFNIKDTFVHVVKQRPEQGLEWIGRIDPANGNTKYDPKFQ						
162SD	EVQLQQSGAELVRSGASVKLSCTASGFNIKDYMHVVKQRPEQGLEWIGWIDPANGNTKYDPKFQ						
171SD	EVQLQQSGAELVRSGASVKLSCTASGFNIKDYMHVVKQRPEQGLEWIGWIDPENGDTYAPKFQ						

	75	85	95	105	115	
			HCDR3			
11SD	GKATLTADKSSSTAYMQLSSLTSVDSAVYFCARGL--	--FAYWGQGLTVTVSS				12
148SD	GKATITAATSSNTAYLQLSSLTSEDYAVYYCATGLD	YYAMDYWGQGSVTVSS				13
162SD	GKATITADTSSNTAYLQLSSLTSEDYAVYYCARWD	WYFDVWGAGTTTVTVSS				14
171SD	GKATMTADTSSNTAYLQLSSLTSEDYAVYYCNAWGSSDYAMDYWGQGSVTVSS					113

Figure 7B

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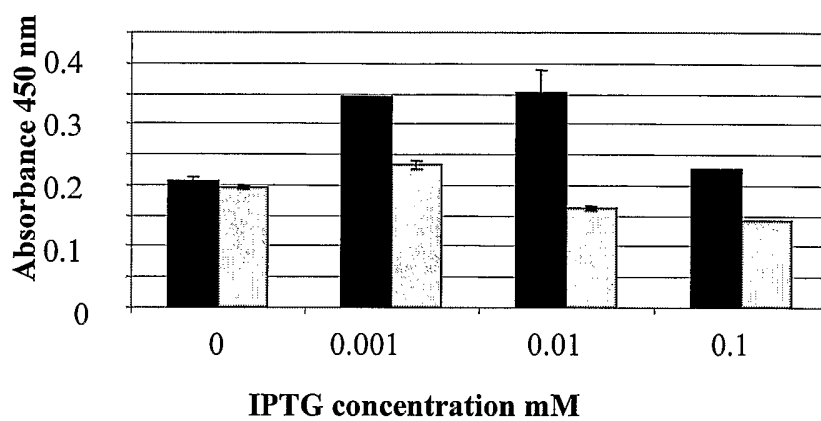


Figure 8 A

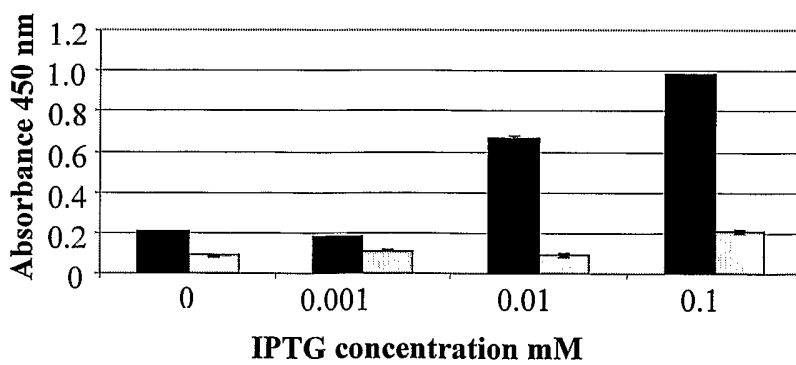


Figure 8 B

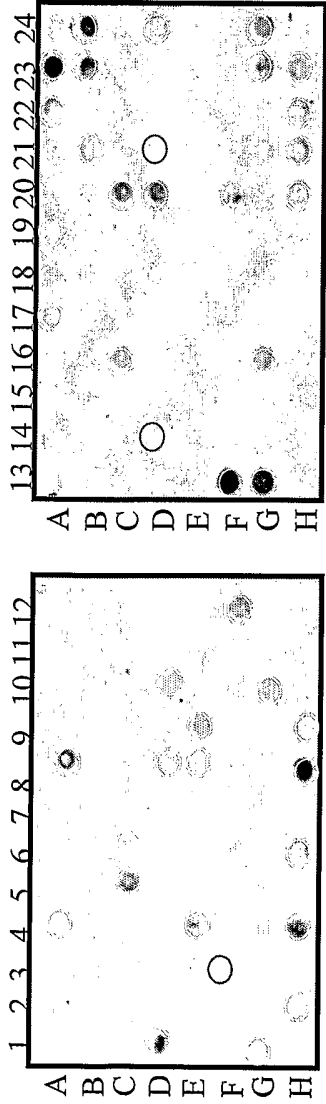


Figure 9 A

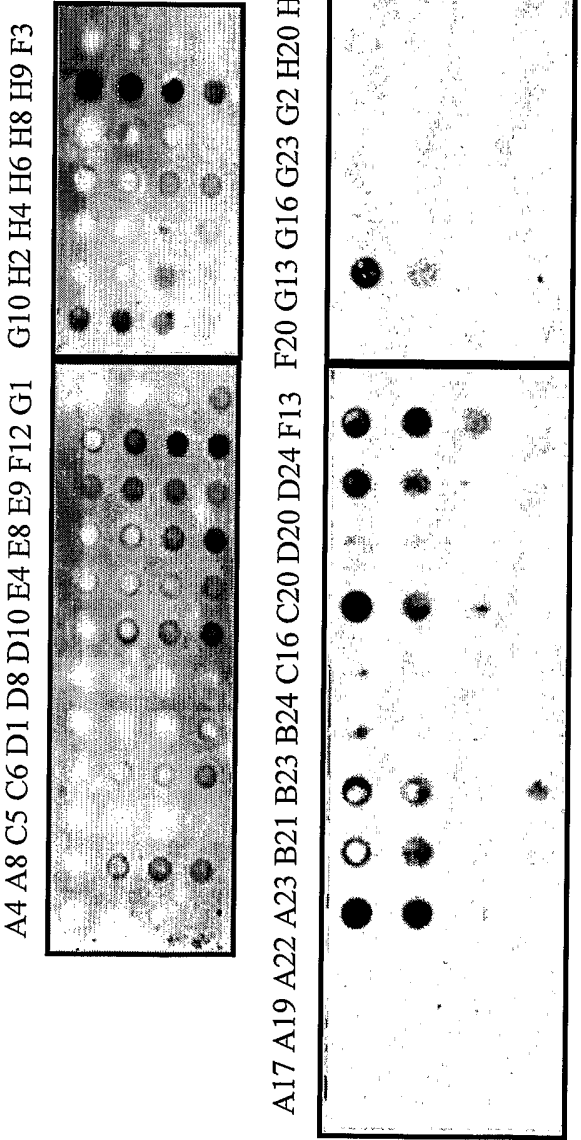


Figure 9 B

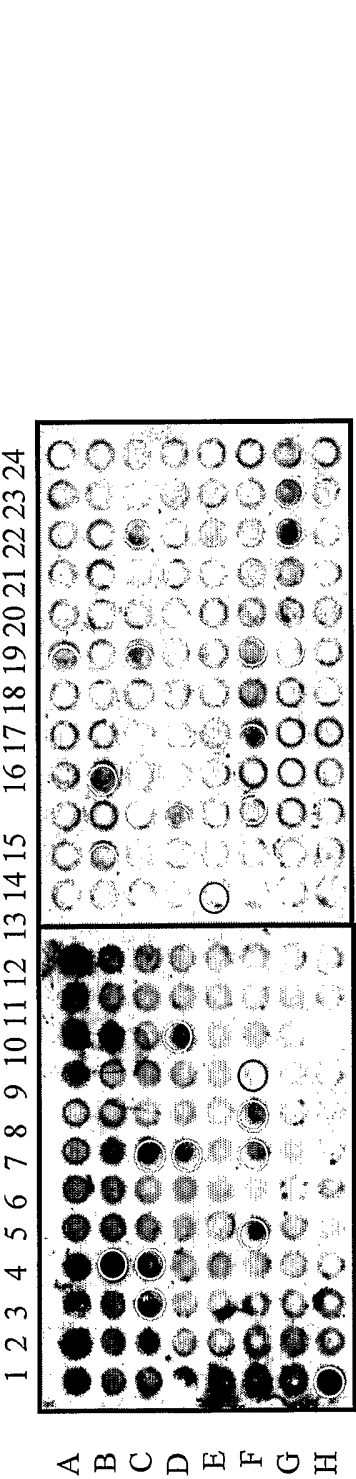


Figure 10 A

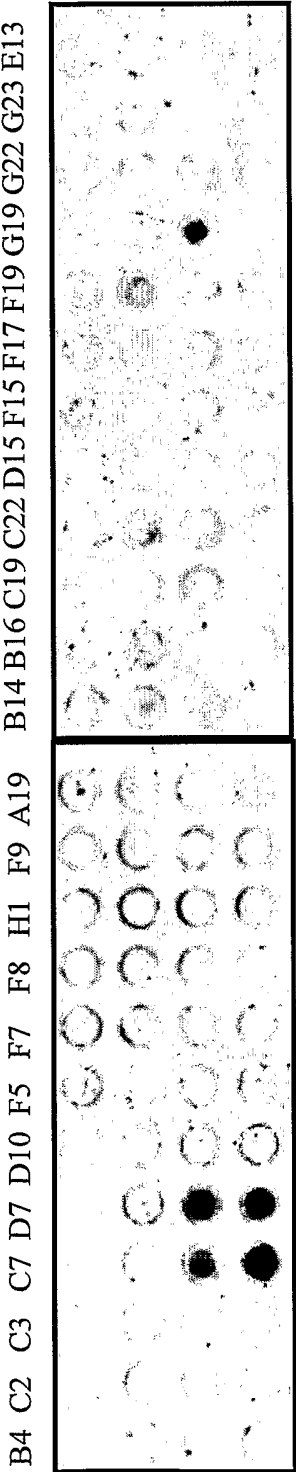


Figure 10 B

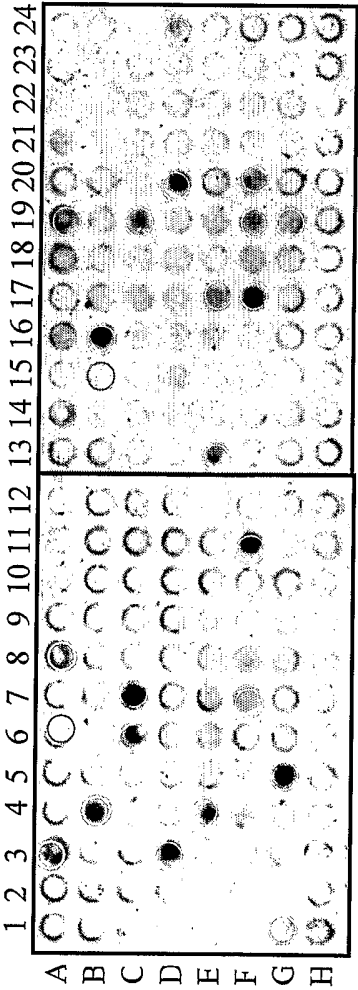


Figure 11 A

A3 A8 B4 C6 C7 D3 E4 F7 F11 G1 G5 A6 A19 B16 C19 D20 D24 E13 E17 F17 F19 F20 G19 B15

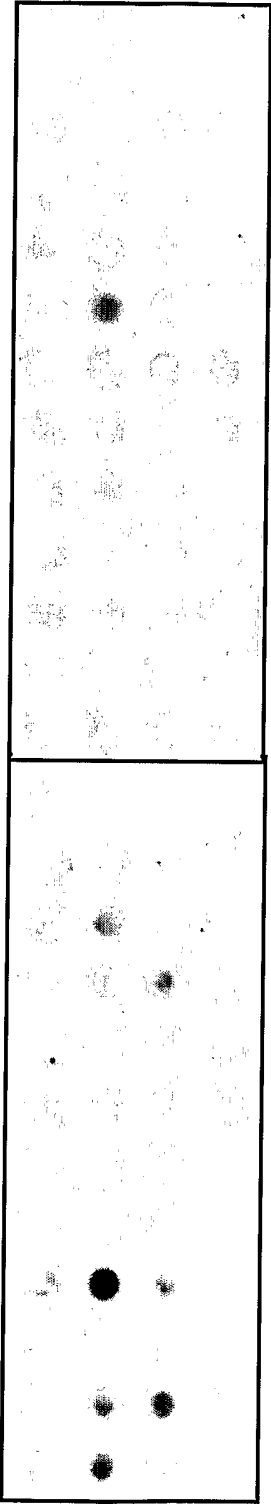


Figure 11 B

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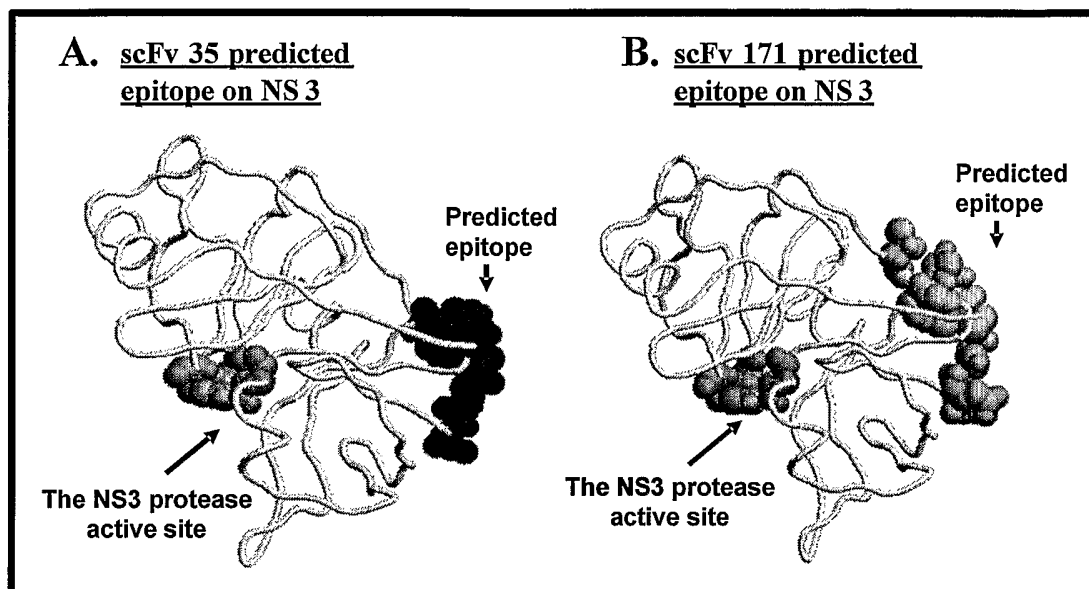


Figure 12

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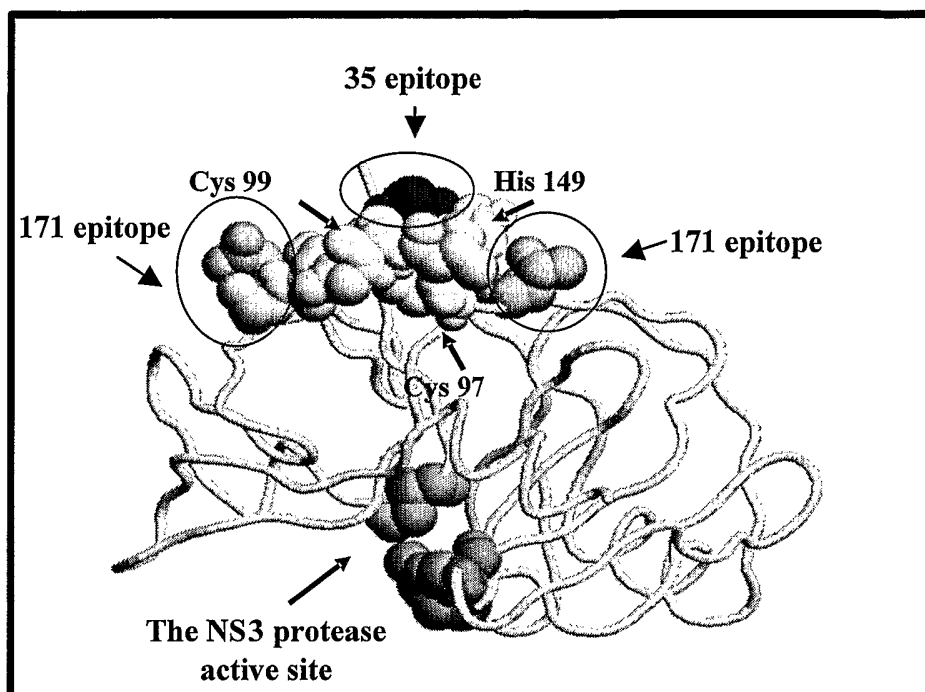


Figure 13

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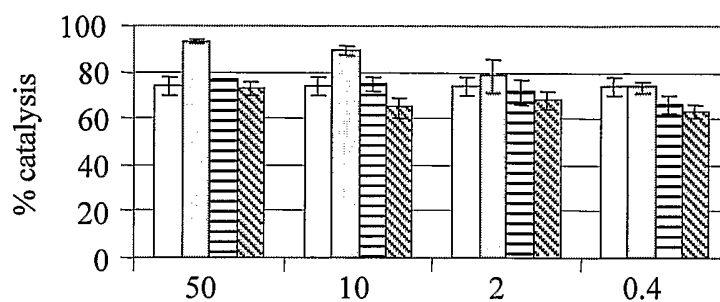


Figure 14 A

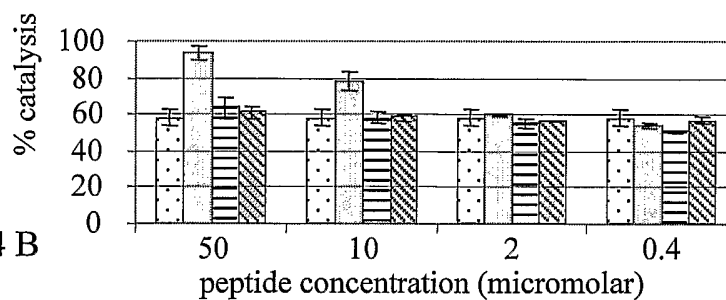


Figure 14 B

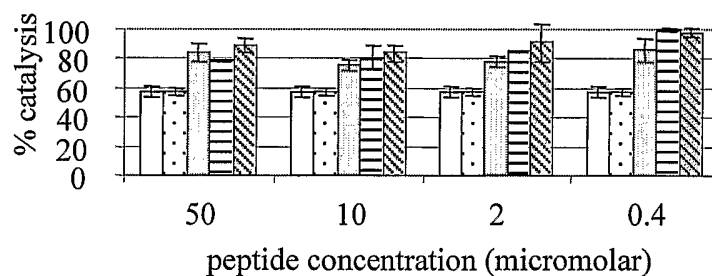


Figure 14 C

- ScFv35 without peptide
- ◻ ScFv171 without peptide
- ◻ ScFv + epitope mimetic peptide (no scFv in 14C)
- ◻ ScFv + negative control peptide X (no scFv in 14C)
- ◻ ScFv + negative control peptide 6 (no scFv in 14C)

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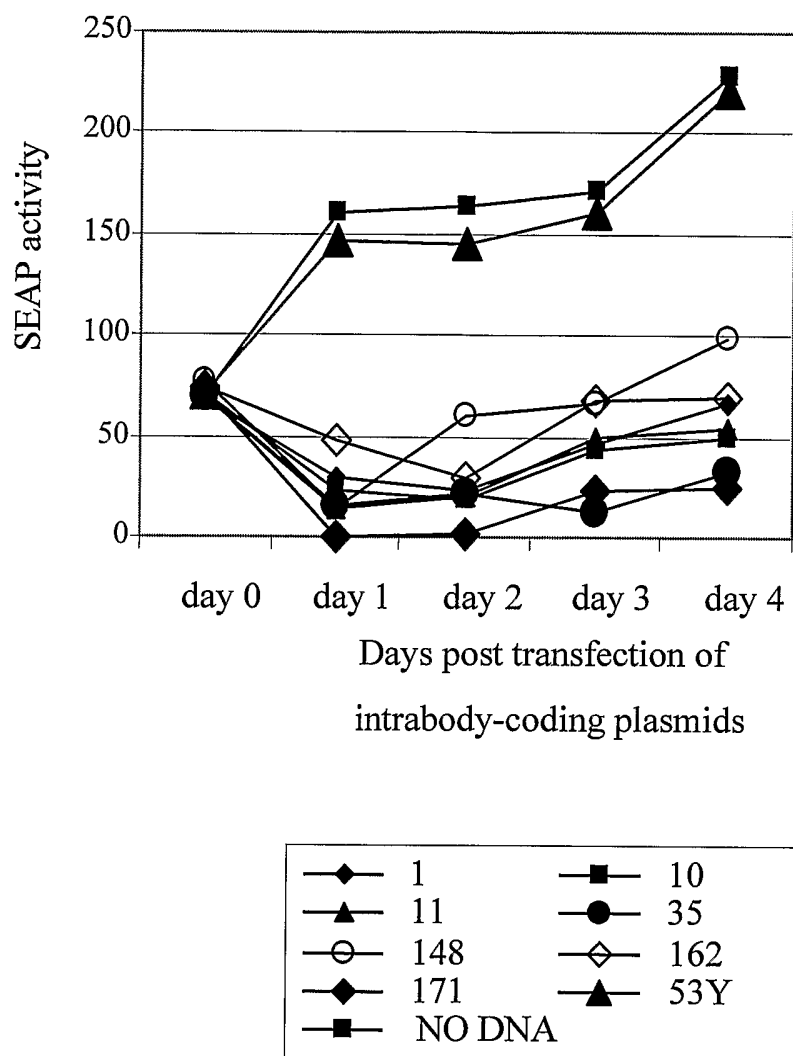


Figure 15

RAMOT_009_PCT.ST25.txt
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Benhar, Itai
Tur-Kaspa, Ran
Zemel, Romy
Gal-Tanamy, Meital
Trahtenhers, Alla
Pupko, Orly
Gershoni, Jonathan

<120> PROTEASE INHIBITORS AND METHOD OF SCREENING THEREOF

<130> RAMOT/009/PCT

<150> US 60/654,446
<151> 2005-02-05

<160> 125

<170> PatentIn version 3.3

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Trp Met Gln Trp Met Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Ala Ile Tyr Pro Gly Asp Gly Asp Thr Arg Tyr Thr Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Tyr Cys
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Ala Arg Ser Arg Ala Met Asp Asp Trp Gly Gln Gly Thr Ser Leu Thr
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Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
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RAMOT_009_PCT.ST25.txt

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Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly
 180 185 190

Val Pro Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr Leu
 195 200 205

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 35 40 45

Ala Trp Ile Tyr Ala Gly Ser Gly Gly Ser Ser Tyr Asn Gln Lys Phe
 50 55 60

Thr Gly Lys Ala Gln Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
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 Page 2

RAMOT_009_PCT.ST25.txt

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 145 150 155 160

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 165 170 175

Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser
 180 185 190

Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly
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Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly
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 35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Thr Gln Lys Phe
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Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Pro Ser Thr Ala Tyr
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RAMOT_009_PCT.ST25.txt

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115 120 125

Gly Gly Gly Gly Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu
130 135 140

Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln
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Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln
165 170 175

Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg
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Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
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Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Tyr
20 25 30

Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
35 40 45

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

<210> 6
 <211> 249
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 <213> Artificial sequence

<220>
 <223> scFv inhibitor

<400> 6

Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Ser Gly Ala
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 Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Tyr
 20 25 30

RAMOT_009_PCT.ST25.txt

Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe
 50 55 60

Gln Gly Lys Ala Thr Met Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
 65 70 75 80

Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Asn Ala Trp Gly Gly Ser Ser Asp Tyr Tyr Ala Met Asp Tyr Trp Gly
 100 105 110

Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
 115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Asp Val Val Met Thr Gln Thr Pro
 130 135 140

Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg
 145 150 155 160

Ser Ser Gln Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu His Trp
 165 170 175

Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val
 180 185 190

Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
 195 200 205

Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu
 210 215 220

Gly Val Tyr Tyr Cys Phe Gln Gly Ser His Val Pro Trp Thr Phe Gly
 225 230 235 240

Gly Gly Thr Lys Leu Glu Ile Lys Arg
 245

<210> 7
 <211> 246
 <212> PRT
 <213> Artificial sequence

<220>
 <223> scFv inhibitor

<400> 7

Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
 Page 6

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<210> 8
<211> 249
<212> PRT
<213> Artificial sequence
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RAMOT_009_PCT.ST25.txt

<220>

<223> scFv inhibitor

<400> 8

Glu Val Gln Leu Gln Gln Ser Gly Thr Glu Leu Val Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Leu Ser Cys Ala Thr Ser Gly Phe Asn Ile Lys Asp Thr
 20 25 30

Tyr Met Gln Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Ser Ala Pro Lys Phe
 50 55 60

Gln Gly Lys Ala Thr Met Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
 65 70 75 80

Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
 85 90 95

Ala Arg Glu Arg Asp Tyr Tyr Gly Tyr Glu Gly Phe Ala Tyr Trp Gly
 100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
 115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Asp Val Val Met Thr Gln Thr Pro
 130 135 140

Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg
 145 150 155 160

Ser Ser Gln Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp
 165 170 175

Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val
 180 185 190

Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
 195 200 205

Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu
 210 215 220

Gly Val Tyr Phe Cys Ser Gln Ser Thr His Val Pro Leu Thr Phe Gly
 225 230 235 240

Ala Gly Thr Lys Leu Glu Ile Lys Arg
 245

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<210> 9
 <211> 240
 <212> PRT
 <213> Artificial sequence

<220>
 <223> scFv inhibitor

<400> 9

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 1 5 10 15

Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr
 20 25 30

Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Asp Pro Lys Phe
 50 55 60

Gln Gly Lys Ala Thr Ile Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95

Thr Arg Ile Trp Leu Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser
 100 105 110

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 115 120 125

Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Asp Ser Leu Gly
 130 135 140

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser
 145 150 155 160

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 165 170 175

Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 180 185 190

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 195 200 205

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser
 210 215 220

Thr His Val Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
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Gly Thr His Gln Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile
 225 230 235 240

Lys Arg

<210> 11
 <211> 245
 <212> PRT
 <213> Artificial sequence

<220>
 <223> scFv inhibitor

<400> 11

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Thr Gly Tyr Thr Phe Ser Asn Tyr
 20 25 30

Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
 35 40 45

Gly Gly Ile Leu Pro Gly Ser Gly Ser Thr Asn Tyr Asn Glu Lys Phe
 50 55 60

Lys Gly Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
 65 70 75 80

Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Asn Ala Asp Gly Pro Pro Gly Phe Asp Tyr Trp Gly Gln Gly Thr Thr
 100 105 110

Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 115 120 125

Gly Gly Gly Ser Asp Val Leu Met Thr Gln Thr Pro Leu Thr Leu Ser
 130 135 140

Val Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser
 145 150 155 160

Leu Leu Asp Ser Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg
 165 170 175

Pro Gly Gln Ser Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp
 180 185 190

Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe
 Page 11

Ser Ser

Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
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1 5 10 15

Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30

Phe Val His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Asp Pro Lys Phe
50 55 60

Gln Gly Lys Ala Thr Ile Thr Ala Ala Thr Ser Ser Asn Thr Ala Tyr
65 70 75 80

Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Thr Gly Leu Asp Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Ser Val Thr Val Ser Ser
115

<210> 14
 <211> 116
 <212> PRT
 <213> Artificial sequence

<220>
 <223> dAb inhibitor

<400> 14

Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Ser Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe Asn Ile Lys Asp Tyr
20 25 30

Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
35 40 45

Gly Trp Ile Asp Pro Ala Asn Gly Asn Thr Lys Tyr Asp Pro Lys Phe
50 55 60

Gln Gly Lys Ala Thr Ile Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
65 70 75 80

Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Trp Asp Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val
100 105 110

RAMOT_009_PCT.ST25.txt

Thr Val Ser Ser
115

<210> 15
<211> 636
<212> PRT
<213> Artificial sequence

<220>
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<400> 15

Met Ala Met Lys Thr Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly
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Asp Lys Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys Lys Phe Glu Lys
20 25 30

Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu
35 40 45

Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe
50 55 60

Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala
65 70 75 80

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

Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile Ala
100 105 110

Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn Pro
115 120 125

Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys Ala
130 135 140

Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe Thr
145 150 155 160

Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn
165 170 175

Gly Lys Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys
180 185 190

Ala Gly Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn
195 200 205

Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly Glu
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210

215

220

Thr Ala Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp Thr
 225 230 235 240

Ser Lys Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly Gln
 245 250 255

Pro Ser Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala Ala
 260 265 270

Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu Leu
 275 280 285

Thr Asp Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly Ala
 290 295 300

Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu Ala Lys Asp Pro Arg Ile
 305 310 315 320

Ala Ala Thr Met Glu Asn Ala Gln Lys Gly Glu Ile Met Pro Asn Ile
 325 330 335

Pro Gln Met Ser Ala Phe Trp Tyr Ala Val Arg Thr Ala Val Ile Asn
 340 345 350

Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln
 355 360 365

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

Glu Phe Ser Thr Met Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu
 385 390 395 400

Val Lys Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr
 405 410 415

Thr Phe Thr Asp Tyr Tyr Ile Lys Trp Val Lys Gln Ser His Gly Lys
 420 425 430

Ser Leu Glu Trp Ile Gly Val Ile Asn Pro Gly Ser Gly Gly Thr Asn
 435 440 445

Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
 450 455 460

Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asn Ser
 465 470 475 480

Ala Val Tyr Phe Cys Ala Arg Arg Ala Gly Thr Gly Tyr Tyr Phe Asp
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RAMOT_009_PCT.ST25.txt
490

485

495

Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly
500 505 510Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Val Leu Met Thr
515 520 525Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile
530 535 540Ser Cys Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr
545 550 555 560Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile
565 570 575Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly
580 585 590Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala
595 600 605Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly Ser His Val Pro Leu
610 615 620Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys Arg
625 630 635

<210> 16
 <211> 632
 <212> PRT
 <213> Artificial sequence

<220>
 <223> MBP-scFv inhibitor

<400> 16

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

RAMOT_009_PCT.ST25.txt

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

RAMOT_009_PCT.ST25.txt

Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln
 355 360 365
 Thr Asn Ser Ser Ser Val Pro Ser Ser Leu Val Ile Glu Gly Arg Pro
 370 375 380
 Glu Phe Ser Thr Met Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu
 385 390 395 400
 Ala Arg Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr
 405 410 415
 Thr Phe Thr Ser Tyr Trp Met Gln Trp Met Lys Gln Arg Pro Gly Gln
 420 425 430
 Gly Leu Glu Trp Ile Gly Ala Ile Tyr Pro Gly Asp Gly Asp Thr Arg
 435 440 445
 Tyr Thr Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
 450 455 460
 Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser
 465 470 475 480
 Ala Val Tyr Tyr Cys Ala Arg Ser Arg Ala Met Asp Asp Trp Gly Gln
 485 490 495
 Gly Thr Ser Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
 500 505 510
 Gly Ser Gly Gly Gly Gly Ser Asp Val Leu Met Thr Gln Thr Pro Leu
 515 520 525
 Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser
 530 535 540
 Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr
 545 550 555 560
 Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser
 565 570 575
 Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly
 580 585 590
 Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly
 595 600 605
 Val Tyr Tyr Cys Phe Gln Gly Ser His Val Pro Arg Thr Phe Gly Gly
 610 615 620

RAMOT_009_PCT.ST25.txt

Gly Thr Lys Leu Glu Ile Lys Arg
625 630

<210> 17
<211> 637
<212> PRT
<213> Artificial sequence

<220>
<223> MBP-scFv inhibitor

<400> 17

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

Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu
35 40 45

Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe
50 55 60

Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala
65 70 75 80

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

Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile Ala
100 105 110

Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn Pro
115 120 125

Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys Ala
130 135 140

Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe Thr
145 150 155 160

Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn
165 170 175

Gly Lys Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys
180 185 190

Ala Gly Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn
195 200 205

Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly Glu
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210
 215
 220
 Thr Ala Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp Thr
 225 230 235 240
 Ser Lys Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly Gln
 245 250 255
 Pro Ser Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala Ala
 260 265 270
 Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu Leu
 275 280 285
 Thr Asp Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly Ala
 290 295 300
 Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu Ala Lys Asp Pro Arg Ile
 305 310 315 320
 Ala Ala Thr Met Glu Asn Ala Gln Lys Gly Glu Ile Met Pro Asn Ile
 325 330 335
 Pro Gln Met Ser Ala Phe Trp Tyr Ala Val Arg Thr Ala Val Ile Asn
 340 345 350
 Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln
 355 360 365
 Thr Asn Ser Ser Ser Val Pro Ser Ser Leu Val Ile Glu Gly Arg Pro
 370 375 380
 Glu Phe Ser Thr Met Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu
 385 390 395 400
 Val Lys Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Thr Ser Gly Phe
 405 410 415
 Thr Phe Ser Ser Thr Phe Phe Ser Trp Leu Lys Gln Lys Pro Gly Gln
 420 425 430
 Ser Leu Glu Trp Ile Ala Trp Ile Tyr Ala Gly Ser Gly Gly Ser Ser
 435 440 445
 Tyr Asn Gln Lys Phe Thr Gly Lys Ala Gln Leu Thr Val Asp Thr Ser
 450 455 460
 Ser Ser Thr Ala Tyr Ile Gln Phe Ser Ser Leu Thr Thr Glu Asp Ser
 465 470 475 480
 Ala Ile Tyr Tyr Cys Ala Arg Gln Asn Leu Asp Tyr Asp Tyr Ala Met
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RAMOT_009_PCT.ST25.txt
490

485

495

Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly
500 505 510

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Val Leu Met
515 520 525

Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser
530 535 540

Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Asn Thr
545 550 555 560

Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu
565 570 575

Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser
580 585 590

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu
595 600 605

Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser Thr His Val Pro
610 615 620

Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
625 630 635

<210> 18
<211> 635
<212> PRT
<213> Artificial sequence

<220>
<223> MBP-scFv inhibitor

<400> 18

Met Ala Met Lys Thr Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly
1 5 10 15

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

Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu
35 40 45

Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe
50 55 60

Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala
65 70 75 80

RAMOT_009_PCT.ST25.txt

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

RAMOT_009_PCT.ST25.txt

Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln
 355 360 365
 Thr Asn Ser Ser Ser Val Pro Ser Ser Leu Val Ile Glu Gly Arg Pro
 370 375 380
 Glu Phe Ser Thr Met Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu
 385 390 395 400
 Val Arg Ser Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe
 405 410 415
 Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln
 420 425 430
 Gly Leu Glu Trp Ile Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu
 435 440 445
 Tyr Thr Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
 450 455 460
 Pro Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser
 465 470 475 480
 Ala Val Tyr Tyr Cys Ala Arg Ile Thr Thr Asp Tyr Tyr Phe Asp Tyr
 485 490 495
 Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser
 500 505 510
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Val Val Met Thr Gln
 515 520 525
 Thr Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser
 530 535 540
 Cys Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu
 545 550 555 560
 Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr
 565 570 575
 Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser
 580 585 590
 Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu
 595 600 605
 Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser Thr His Val Pro Leu Thr
 610 615 620

RAMOT_009_PCT.ST25.txt

Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys Arg
625 630 635

<210> 19
<211> 633
<212> PRT
<213> Artificial sequence

<220>
<223> MBP-scFv inhibitor

<400> 19

Met Ala Met Lys Thr Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly
1 5 10 15

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

Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu
35 40 45

Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe
50 55 60

Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala
65 70 75 80

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

Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile Ala
100 105 110

Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn Pro
115 120 125

Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys Ala
130 135 140

Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe Thr
145 150 155 160

Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn
165 170 175

Gly Lys Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys
180 185 190

Ala Gly Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn
195 200 205

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

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490

485

495

Ala Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
500 505 510Gly Gly Ser Gly Gly Gly Gly Ser Ser Val Leu Met Thr Gln Thr Pro
515 520 525Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg
530 535 540Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp
545 550 555 560Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val
565 570 575Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
580 585 590Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu
595 600 605Gly Val Tyr Tyr Cys Phe Gln Gly Ser His Val Pro Trp Thr Phe Gly
610 615 620Gly Gly Thr Lys Leu Glu Leu Lys Arg
625 630

<210> 20
 <211> 638
 <212> PRT
 <213> Artificial sequence

<220>
 <223> MBP-scFv inhibitor

<400> 20

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

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

RAMOT_009_PCT.ST25.txt

Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln
 355 360 365
 Thr Asn Ser Ser Ser Val Pro Ser Ser Leu Val Ile Glu Gly Arg Pro
 370 375 380
 Glu Phe Ser Thr Met Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu
 385 390 395 400
 Val Arg Ser Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe
 405 410 415
 Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln
 420 425 430
 Gly Leu Glu Trp Ile Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu
 435 440 445
 Tyr Ala Pro Lys Phe Gln Gly Lys Ala Thr Met Thr Ala Asp Thr Ser
 450 455 460
 Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr
 465 470 475 480
 Ala Val Tyr Tyr Cys Asn Ala Trp Gly Gly Ser Ser Asp Tyr Tyr Ala
 485 490 495
 Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly
 500 505 510
 Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Val Val
 515 520 525
 Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala
 530 535 540
 Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Asn
 545 550 555 560
 Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu
 565 570 575
 Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe
 580 585 590
 Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val
 595 600 605
 Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly Ser His Val
 610 615 620

RAMOT_009_PCT.ST25.txt

Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
625 630 635

<210> 21
<211> 635
<212> PRT
<213> Artificial sequence

<220>
<223> MBP-scFv inhibitor

<400> 21

Met Ala Met Lys Thr Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly
1 5 10 15

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

Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu
35 40 45

Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe
50 55 60

Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala
65 70 75 80

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

Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile Ala
100 105 110

Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn Pro
115 120 125

Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys Ala
130 135 140

Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe Thr
145 150 155 160

Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn
165 170 175

Gly Lys Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys
180 185 190

Ala Gly Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn
195 200 205

Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly Glu
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210 215 220
 Thr Ala Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp Thr
 225 230 235 240
 Ser Lys Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly Gln
 245 250 255
 Pro Ser Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala Ala
 260 265 270
 Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu Leu
 275 280 285
 Thr Asp Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly Ala
 290 295 300
 Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu Ala Lys Asp Pro Arg Ile
 305 310 315 320
 Ala Ala Thr Met Glu Asn Ala Gln Lys Gly Glu Ile Met Pro Asn Ile
 325 330 335
 Pro Gln Met Ser Ala Phe Trp Tyr Ala Val Arg Thr Ala Val Ile Asn
 340 345 350
 Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln
 355 360 365
 Thr Asn Ser Ser Ser Val Pro Ser Ser Leu Val Ile Glu Gly Arg Pro
 370 375 380
 Glu Phe Ser Thr Met Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu Leu
 385 390 395 400
 Val Lys Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe
 405 410 415
 Asn Ile Lys Asp Thr Phe Val His Trp Val Lys Gln Arg Pro Glu Gln
 420 425 430
 Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys
 435 440 445
 Tyr Asp Pro Lys Phe Gln Gly Lys Ala Thr Ile Thr Ala Ala Thr Ser
 450 455 460
 Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr
 465 470 475 480
 Ala Val Tyr Tyr Cys Ala Thr Gly Leu Asp Tyr Tyr Ala Met Asp Tyr
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RAMOT_009_PCT.ST25.txt
490

485

495

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Gly Gly Gly Gly Ser
500 505 510

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Val Val Met Thr Gln
515 520 525

Thr Pro Leu Ser Pro Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser
530 535 540

Cys Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu
545 550 555 560

His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr
565 570 575

Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser
580 585 590

Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Thr Arg Val Glu Ala Glu
595 600 605

Asp Leu Gly Leu Tyr Phe Cys Ser Gln Ser Thr His Val Pro Trp Thr
610 615 620

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
625 630 635

<210> 22
<211> 638
<212> PRT
<213> Artificial sequence

<220>
<223> MBP-scFv inhibitor

<400> 22

Met Ala Met Lys Thr Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly
1 5 10 15

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

Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu
35 40 45

Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe
50 55 60

Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala
65 70 75 80

RAMOT_009_PCT.ST25.txt

Glu Ile Thr Pro Asp₈₅ Lys Ala Phe Gln₉₀ Asp Lys Leu Tyr Pro Phe₉₅ Thr
 Trp Asp Ala Val₁₀₀ Arg Tyr Asn Gly₁₀₅ Lys Leu Ile Ala Tyr Pro₁₁₀ Ile Ala
 Val Glu Ala₁₁₅ Leu Ser Leu Ile Tyr₁₂₀ Asn Lys Asp Leu Leu₁₂₅ Pro Asn Pro
 Pro Lys₁₃₀ Thr Trp Glu Glu Ile₁₃₅ Pro Ala Leu Asp Lys₁₄₀ Glu Leu Lys Ala
 Lys₁₄₅ Gly Lys Ser Ala Leu₁₅₀ Met Phe Asn Leu Gln₁₅₅ Glu Pro Tyr Phe₁₆₀ Thr
 Trp Pro Leu Ile Ala₁₆₅ Ala Asp Gly Gly Tyr₁₇₀ Ala Phe Lys Tyr Glu₁₇₅ Asn
 Gly Lys Tyr Asp₁₈₀ Ile Lys Asp Val Gly₁₈₅ Val Asp Asn Ala Gly₁₉₀ Ala Lys
 Ala Gly Leu₁₉₅ Thr Phe Leu Val Asp₂₀₀ Leu Ile Lys Asn Lys₂₀₅ His Met Asn
 Ala Asp₂₁₀ Thr Asp Tyr Ser Ile₂₁₅ Ala Glu Ala Ala Phe₂₂₀ Asn Lys Gly Glu
 Thr₂₂₅ Ala Met Thr Ile Asn₂₃₀ Gly Pro Trp Ala Trp₂₃₅ Ser Asn Ile Asp Thr₂₄₀
 Ser Lys Val Asn Tyr₂₄₅ Gly Val Thr Val Leu₂₅₀ Pro Thr Phe Lys Gly₂₅₅ Gln
 Pro Ser Lys Pro₂₆₀ Phe Val Gly Val Leu₂₆₅ Ser Ala Gly Ile Asn₂₇₀ Ala Ala
 Ser Pro Asn₂₇₅ Lys Glu Leu Ala Lys₂₈₀ Glu Phe Leu Glu Asn₂₈₅ Tyr Leu Leu
 Thr Asp₂₉₀ Glu Gly Leu Glu Ala₂₉₅ Val Asn Lys Asp Lys₃₀₀ Pro Leu Gly Ala
 Val₃₀₅ Ala Leu Lys Ser Tyr₃₁₀ Glu Glu Glu Leu Ala₃₁₅ Lys Asp Pro Arg Ile₃₂₀
 Ala Ala Thr Met Glu₃₂₅ Asn Ala Gln Lys Gly₃₃₀ Glu Ile Met Pro Asn₃₃₅ Ile
 Pro Gln Met Ser₃₄₀ Ala Phe Trp Tyr Ala₃₄₅ Val Arg Thr Ala Val₃₅₀ Ile Asn

RAMOT_009_PCT.ST25.txt

Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln
 355 360 365
 Thr Asn Ser Ser Ser Val Pro Ser Ser Leu Val Ile Glu Gly Arg Pro
 370 375 380
 Glu Phe Ser Thr Met Glu Val Gln Leu Gln Gln Ser Gly Thr Glu Leu
 385 390 395 400
 Val Lys Pro Gly Ala Ser Val Lys Leu Ser Cys Ala Thr Ser Gly Phe
 405 410 415
 Asn Ile Lys Asp Thr Tyr Met Gln Trp Val Lys Gln Arg Pro Glu Gln
 420 425 430
 Gly Leu Glu Trp Ile Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu
 435 440 445
 Ser Ala Pro Lys Phe Gln Gly Lys Ala Thr Met Thr Ala Asp Thr Ser
 450 455 460
 Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser
 465 470 475 480
 Ala Val Tyr Phe Cys Ala Arg Glu Arg Asp Tyr Tyr Gly Tyr Glu Gly
 485 490 495
 Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly
 500 505 510
 Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Val Val
 515 520 525
 Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala
 530 535 540
 Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Asn
 545 550 555 560
 Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu
 565 570 575
 Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe
 580 585 590
 Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val
 595 600 605
 Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser Thr His Val
 610 615 620

RAMOT_009_PCT.ST25.txt

Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys Arg
 625 630 635

<210> 23
 <211> 629
 <212> PRT
 <213> Artificial sequence

<220>
 <223> MBP-scFv inhibitor

<400> 23

Met Ala Met Lys Thr Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly
 1 5 10 15

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

Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu
 35 40 45

Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe
 50 55 60

Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala
 65 70 75 80

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

Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile Ala
 100 105 110

Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn Pro
 115 120 125

Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys Ala
 130 135 140

Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe Thr
 145 150 155 160

Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn
 165 170 175

Gly Lys Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys
 180 185 190

Ala Gly Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn
 195 200 205

Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly Glu
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210

215

220

Thr Ala Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp Thr
 225 230 235 240

Ser Lys Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly Gln
 245 250 255

Pro Ser Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala Ala
 260 265 270

Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu Leu
 275 280 285

Thr Asp Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly Ala
 290 295 300

Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu Ala Lys Asp Pro Arg Ile
 305 310 315 320

Ala Ala Thr Met Glu Asn Ala Gln Lys Gly Glu Ile Met Pro Asn Ile
 325 330 335

Pro Gln Met Ser Ala Phe Trp Tyr Ala Val Arg Thr Ala Val Ile Asn
 340 345 350

Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln
 355 360 365

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

Glu Phe Ser Thr Met Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu
 385 390 395 400

Val Lys Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe
 405 410 415

Asn Ile Lys Asp Thr Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln
 420 425 430

Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys
 435 440 445

Tyr Asp Pro Lys Phe Gln Gly Lys Ala Thr Ile Thr Ala Asp Lys Ser
 450 455 460

Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser
 465 470 475 480

Ala Val Tyr Tyr Cys Thr Arg Ile Trp Leu Pro Trp Gly Gln Gly Thr

RAMOT_009_PCT.ST25.txt
490

485

495

Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
500 505 510

Gly Gly Gly Gly Ser Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu
515 520 525

Pro Asp Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln
530 535 540

Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln
545 550 555 560

Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg
565 570 575

Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
580 585 590

Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr
595 600 605

Phe Cys Ser Gln Ser Thr His Val Leu Thr Phe Gly Ala Gly Thr Lys
610 615 620

Leu Glu Leu Lys Arg
625

<210> 24
<211> 631
<212> PRT
<213> Artificial sequence

<220>
<223> MBP-scFv inhibitor

<400> 24

Met Ala Met Lys Thr Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly
1 5 10 15

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

Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu
35 40 45

Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe
50 55 60

Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala
65 70 75 80

RAMOT_009_PCT.ST25.txt

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

RAMOT_009_PCT.ST25.txt

Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln
 355 360 365
 Thr Asn Ser Ser Ser Val Pro Ser Ser Leu Val Ile Glu Gly Arg Pro
 370 375 380
 Glu Phe Ser Thr Met Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu
 385 390 395 400
 Val Lys Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe
 405 410 415
 Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Gly Gln
 420 425 430
 Gly Leu Glu Tyr Ile Gly Arg Ile Tyr Pro Gly Asn Gly Gln Thr Asp
 435 440 445
 Tyr Asn Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
 450 455 460
 Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Val Asp Ser
 465 470 475 480
 Ala Val Tyr Phe Cys Ala Arg Gly Leu Phe Ala Tyr Trp Gly Gln Gly
 485 490 495
 Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
 500 505 510
 Ser Gly Gly Gly Gly Ser Asp Val Val Met Thr Gln Thr Pro Leu Ser
 515 520 525
 Leu Pro Val Ser Phe Gly Asp Gln Val Ser Ile Ser Cys Arg Ser Ser
 530 535 540
 Gln Ser Leu Ala Asn Ser Tyr Gly Asn Thr Tyr Leu Ser Trp Cys Leu
 545 550 555 560
 His Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gly Ile Ser Asn
 565 570 575
 Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr
 580 585 590
 Asp Phe Thr Leu Lys Ile Ser Thr Ile Lys Pro Glu Asp Leu Gly Met
 595 600 605
 Tyr Tyr Cys Leu Gln Gly Thr His Gln Pro Leu Thr Phe Gly Ala Gly
 610 615 620

RAMOT_009_PCT.ST25.txt

Thr Lys Leu Glu Ile Lys Arg
625 630

<210> 25
<211> 634
<212> PRT
<213> Artificial sequence

<220>
<223> MBP-scFv inhibitor

<400> 25

Met Ala Met Lys Thr Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly
1 5 10 15

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

Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu
35 40 45

Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe
50 55 60

Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala
65 70 75 80

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

Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile Ala
100 105 110

Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn Pro
115 120 125

Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys Ala
130 135 140

Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe Thr
145 150 155 160

Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn
165 170 175

Gly Lys Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys
180 185 190

Ala Gly Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn
195 200 205

Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly Glu
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210

215

220

Thr Ala Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp Thr
 225 230 235 240

Ser Lys Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly Gln
 245 250 255

Pro Ser Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala Ala
 260 265 270

Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu Leu
 275 280 285

Thr Asp Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly Ala
 290 295 300

Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu Ala Lys Asp Pro Arg Ile
 305 310 315 320

Ala Ala Thr Met Glu Asn Ala Gln Lys Gly Glu Ile Met Pro Asn Ile
 325 330 335

Pro Gln Met Ser Ala Phe Trp Tyr Ala Val Arg Thr Ala Val Ile Asn
 340 345 350

Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln
 355 360 365

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

Glu Phe Ser Thr Met Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu
 385 390 395 400

Met Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Thr Gly Tyr
 405 410 415

Thr Phe Ser Asn Tyr Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His
 420 425 430

Gly Leu Glu Trp Ile Gly Gly Ile Leu Pro Gly Ser Gly Ser Thr Asn
 435 440 445

Tyr Asn Glu Lys Phe Lys Gly Lys Ala Thr Phe Thr Ala Asp Thr Ser
 450 455 460

Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr
 465 470 475 480

Ala Val Tyr Tyr Cys Asn Ala Asp Gly Pro Pro Gly Phe Asp Tyr Trp

RAMOT_009_PCT.ST25.txt
490

485

495

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
500 505 510Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Val Leu Met Thr Gln Thr
515 520 525Pro Leu Thr Leu Ser Val Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys
530 535 540Lys Ser Ser Gln Ser Leu Leu Asp Ser Asp Gly Lys Thr Tyr Leu Asn
545 550 555 560Trp Leu Leu Gln Arg Pro Gly Gln Ser Pro Lys Arg Leu Ile Tyr Leu
565 570 575Val Ser Lys Leu Asp Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly
580 585 590Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp
595 600 605Leu Gly Val Tyr Tyr Cys Phe Gln Gly Ser His Val Pro Phe Thr Phe
610 615 620Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg
625 630

<210> 26
 <211> 503
 <212> PRT
 <213> Artificial sequence

<220>
 <223> MBP-dAb inhibitor

<400> 26

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

RAMOT_009_PCT.ST25.txt

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

RAMOT_009_PCT.ST25.txt

Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln
 355 360 365

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

Glu Phe Ser Thr Met Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu
 385 390 395 400

Val Lys Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe
 405 410 415

Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Gly Gln
 420 425 430

Gly Leu Glu Tyr Ile Gly Arg Ile Tyr Pro Gly Asn Gly Gln Thr Asp
 435 440 445

Tyr Asn Gly Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser
 450 455 460

Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Val Asp Ser
 465 470 475 480

Ala Val Tyr Phe Cys Ala Arg Gly Leu Phe Ala Tyr Trp Gly Gln Gly
 485 490 495

Thr Leu Val Thr Val Ser Ser
 500

<210> 27
 <211> 507
 <212> PRT
 <213> Artificial sequence

<220>
 <223> MBP-dAb inhibitor

<400> 27

Met Ala Met Lys Thr Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly
 1 5 10 15

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

Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu
 35 40 45

Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe
 50 55 60

Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala
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65					70					75						80
Glu	Ile	Thr	Pro	Asp 85	Lys	Ala	Phe	Gln	Asp 90	Lys	Leu	Tyr	Pro	Phe 95	Thr	
Trp	Asp	Ala	Val 100	Arg	Tyr	Asn	Gly	Lys 105	Leu	Ile	Ala	Tyr	Pro 110	Ile	Ala	
Val	Glu	Ala 115	Leu	Ser	Leu	Ile	Tyr 120	Asn	Lys	Asp	Leu	Leu 125	Pro	Asn	Pro	
Pro	Lys 130	Thr	Trp	Glu	Glu	Ile 135	Pro	Ala	Leu	Asp	Lys 140	Glu	Leu	Lys	Ala	
Lys 145	Gly	Lys	Ser	Ala	Leu 150	Met	Phe	Asn	Leu	Gln 155	Glu	Pro	Tyr	Phe	Thr 160	
Trp	Pro	Leu	Ile	Ala 165	Ala	Asp	Gly	Gly	Tyr 170	Ala	Phe	Lys	Tyr	Glu 175	Asn	
Gly	Lys	Tyr	Asp 180	Ile	Lys	Asp	Val	Gly 185	Val	Asp	Asn	Ala	Gly 190	Ala	Lys	
Ala	Gly	Leu 195	Thr	Phe	Leu	Val	Asp 200	Leu	Ile	Lys	Asn	Lys 205	His	Met	Asn	
Ala	Asp 210	Thr	Asp	Tyr	Ser	Ile 215	Ala	Glu	Ala	Ala	Phe 220	Asn	Lys	Gly	Glu	
Thr 225	Ala	Met	Thr	Ile	Asn 230	Gly	Pro	Trp	Ala	Trp 235	Ser	Asn	Ile	Asp	Thr 240	
Ser	Lys	Val	Asn	Tyr 245	Gly	Val	Thr	Val	Leu 250	Pro	Thr	Phe	Lys	Gly 255	Gln	
Pro	Ser	Lys	Pro 260	Phe	Val	Gly	Val	Leu 265	Ser	Ala	Gly	Ile	Asn 270	Ala	Ala	
Ser	Pro	Asn 275	Lys	Glu	Leu	Ala	Lys 280	Glu	Phe	Leu	Glu	Asn 285	Tyr	Leu	Leu	
Thr	Asp 290	Glu	Gly	Leu	Glu	Ala 295	Val	Asn	Lys	Asp	Lys 300	Pro	Leu	Gly	Ala	
Val 305	Ala	Leu	Lys	Ser	Tyr 310	Glu	Glu	Glu	Leu	Ala 315	Lys	Asp	Pro	Arg	Ile 320	
Ala	Ala	Thr	Met	Glu 325	Asn	Ala	Gln	Lys	Gly 330	Glu	Ile	Met	Pro	Asn 335	Ile	
Pro	Gln	Met	Ser	Ala	Phe	Trp	Tyr	Ala	Val	Arg	Thr	Ala	Val	Ile	Asn	

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340 RAMOT_009_PCT.ST25.txt 350
345

Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln
355 360 365

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

Glu Phe Ser Thr Met Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu Leu
385 390 395 400

Val Lys Pro Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe
405 410 415

Asn Ile Lys Asp Thr Phe Val His Trp Val Lys Gln Arg Pro Glu Gln
420 425 430

Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys
435 440 445

Tyr Asp Pro Lys Phe Gln Gly Lys Ala Thr Ile Thr Ala Ala Thr Ser
450 455 460

Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr
465 470 475 480

Ala Val Tyr Tyr Cys Ala Thr Gly Leu Asp Tyr Tyr Ala Met Asp Tyr
485 490 495

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
500 505

<210> 28
<211> 505
<212> PRT
<213> Artificial sequence

<220>
<223> MBP-dAb inhibitor

<400> 28

Met Ala Met Lys Thr Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly
1 5 10 15

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

Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu
35 40 45

Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe
50 55 60

RAMOT_009_PCT.ST25.txt

Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala
 65 70 75 80
 Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe Thr
 85 90 95
 Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile Ala
 100 105 110
 Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn Pro
 115 120 125
 Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys Ala
 130 135 140
 Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe Thr
 145 150 155 160
 Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn
 165 170 175
 Gly Lys Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys
 180 185 190
 Ala Gly Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn
 195 200 205
 Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly Glu
 210 215 220
 Thr Ala Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp Thr
 225 230 235 240
 Ser Lys Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly Gln
 245 250 255
 Pro Ser Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala Ala
 260 265 270
 Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu Leu
 275 280 285
 Thr Asp Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly Ala
 290 295 300
 Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu Ala Lys Asp Pro Arg Ile
 305 310 315 320
 Ala Ala Thr Met Glu Asn Ala Gln Lys Gly Glu Ile Met Pro Asn Ile
 325 330 335

RAMOT_009_PCT.ST25.txt

Pro Gln Met Ser Ala Phe Trp Tyr Ala Val Arg Thr Ala Val Ile Asn
 340 345 350

Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln
 355 360 365

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

Glu Phe Ser Thr Met Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu
 385 390 395 400

Val Arg Ser Gly Ala Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Phe
 405 410 415

Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln
 420 425 430

Gly Leu Glu Trp Ile Gly Trp Ile Asp Pro Ala Asn Gly Asn Thr Lys
 435 440 445

Tyr Asp Pro Lys Phe Gln Gly Lys Ala Thr Ile Thr Ala Asp Thr Ser
 450 455 460

Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr
 465 470 475 480

Ala Val Tyr Tyr Cys Ala Arg Trp Asp Trp Tyr Phe Asp Val Trp Gly
 485 490 495

Ala Gly Thr Thr Val Thr Val Ser Ser
 500 505

<210> 29
 <211> 8
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Inhibitor peptide

<400> 29

Leu Gly Leu Ala Val Glu Val Arg
 1 5

<210> 30
 <211> 8
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Inhibitor peptide

<400> 30

RAMOT_009_PCT.ST25.txt

Phe Ser Arg Ala Glu Trp Phe Cys
1 5

<210> 31
<211> 8
<212> PRT
<213> Artificial sequence

<220>
<223> Inhibitor peptide

<400> 31

Gly Ala Ala Leu Asn Thr Ser Ser
1 5

<210> 32
<211> 8
<212> PRT
<213> Artificial sequence

<220>
<223> Inhibitor peptide

<400> 32

Ala Val Leu Ala Gly Leu Gly Val
1 5

<210> 33
<211> 8
<212> PRT
<213> Artificial sequence

<220>
<223> Inhibitor peptide

<400> 33

Phe Trp Gly Gly Ala Leu Trp Arg
1 5

<210> 34
<211> 8
<212> PRT
<213> Artificial sequence

<220>
<223> Inhibitor peptide

<400> 34

Cys Cys Ala Trp Leu Ser Ile Arg
1 5

<210> 35
<211> 8
<212> PRT
<213> Artificial sequence

<220>
<223> Inhibitor peptide

RAMOT_009_PCT.ST25.txt

<400> 35

Leu Ala Ala Trp Phe Cys Leu Trp
 1 5

<210> 36

<211> 392

<212> PRT

<213> Artificial sequence

<220>

<223> Internal position inhibitor peptide aptamer

<400> 36

Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
 1 5 10 15

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

Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
 35 40 45

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

Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
 65 70 75 80

Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
 85 90 95

Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu
 100 105 110

Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys
 115 120 125

Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Asp Pro
 130 135 140

Leu Gly Leu Ala Val Glu Val Arg Asp Pro Gly Lys Ser Ala Leu Met
 145 150 155 160

Phe Asn Leu Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp
 165 170 175

Gly Gly Tyr Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp
 180 185 190

Val Gly Val Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val
 195 200 205

RAMOT_009_PCT.ST25.txt

Asp Leu Ile Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile
 210 215 220

Ala Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly
 225 230 235 240

Pro Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val
 245 250 255

Thr Val Leu Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly
 260 265 270

Val Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala
 275 280 285

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

Val Asn Lys Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu
 305 310 315 320

Glu Glu Leu Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala
 325 330 335

Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp
 340 345 350

Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr
 355 360 365

Val Asp Glu Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro
 370 375 380

Asp Tyr Lys Asp Asp Asp Asp Lys
 385 390

<210> 37
 <211> 392
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Internal position inhibitor peptide aptamer

<400> 37

Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
 1 5 10 15

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

Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
 Page 50

RAMOT_009_PCT.ST25.txt
40 45

35

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

Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
65 70 75 80

Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
85 90 95

Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu
100 105 110

Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys
115 120 125

Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Asp Pro
130 135 140

Phe Ser Arg Ala Glu Trp Phe Cys Asp Pro Gly Lys Ser Ala Leu Met
145 150 155 160

Phe Asn Leu Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp
165 170 175

Gly Gly Tyr Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp
180 185 190

Val Gly Val Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val
195 200 205

Asp Leu Ile Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile
210 215 220

Ala Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly
225 230 235 240

Pro Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val
245 250 255

Thr Val Leu Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly
260 265 270

Val Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala
275 280 285

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

Val Asn Lys Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu

RAMOT_009_PCT.ST25.txt
315

305

310

320

Glu Glu Leu Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala
325 330 335

Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp
340 345 350

Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr
355 360 365

Val Asp Glu Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro
370 375 380

Asp Tyr Lys Asp Asp Asp Asp Lys
385 390

<210> 38
<211> 392
<212> PRT
<213> Artificial sequence

<220>
<223> Internal position inhibitor peptide aptamer

<400> 38

Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
1 5 10 15

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

Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
35 40 45

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

Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
65 70 75 80

Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
85 90 95

Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu
100 105 110

Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys
115 120 125

Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Asp Pro
130 135 140

RAMOT_009_PCT.ST25.txt

Gly Ala Ala Leu Asn Thr Ser Ser Asp Pro Gly Lys Ser Ala Leu Met
145 150 155 160

Phe Asn Leu Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp
165 170 175

Gly Gly Tyr Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp
180 185 190

Val Gly Val Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val
195 200 205

Asp Leu Ile Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile
210 215 220

Ala Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly
225 230 235 240

Pro Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val
245 250 255

Thr Val Leu Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly
260 265 270

Val Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala
275 280 285

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

Val Asn Lys Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu
305 310 315 320

Glu Glu Leu Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala
325 330 335

Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp
340 345 350

Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr
355 360 365

Val Asp Glu Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro
370 375 380

Asp Tyr Lys Asp Asp Asp Asp Lys
385 390

<210> 39
<211> 392
<212> PRT

RAMOT_009_PCT.ST25.txt

<213> Artificial sequence

<220>

<223> Internal position inhibitor peptide aptamer

<400> 39

Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
 1 5 10 15

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

Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
 35 40 45

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

Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
 65 70 75 80

Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
 85 90 95

Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu
 100 105 110

Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys
 115 120 125

Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Asp Pro
 130 135 140

Ala Val Leu Ala Gly Leu Gly Val Asp Pro Gly Lys Ser Ala Leu Met
 145 150 155 160

Phe Asn Leu Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp
 165 170 175

Gly Gly Tyr Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp
 180 185 190

Val Gly Val Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val
 195 200 205

Asp Leu Ile Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile
 210 215 220

Ala Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly
 225 230 235 240

Pro Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val
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RAMOT_009_PCT.ST25.txt
250

245

255

Thr Val Leu Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly
260 265 270

Val Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala
275 280 285

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

Val Asn Lys Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu
305 310 315 320

Glu Glu Leu Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala
325 330 335

Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp
340 345 350

Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr
355 360 365

Val Asp Glu Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro
370 375 380

Asp Tyr Lys Asp Asp Asp Asp Lys
385 390

<210> 40
<211> 392
<212> PRT
<213> Artificial sequence

<220>
<223> Internal position inhibitor peptide aptamer

<400> 40

Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
1 5 10 15

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

Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
35 40 45

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

Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
65 70 75 80

RAMOT_009_PCT.ST25.txt

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

RAMOT_009_PCT.ST25.txt

Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr
 355 360 365

Val Asp Glu Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro
 370 375 380

Asp Tyr Lys Asp Asp Asp Asp Lys
 385 390

<210> 41
 <211> 392
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Internal position inhibitor peptide aptamer

<400> 41

Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
 1 5 10 15

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

Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
 35 40 45

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

Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
 65 70 75 80

Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
 85 90 95

Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu
 100 105 110

Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys
 115 120 125

Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Asp Pro
 130 135 140

Cys Cys Ala Trp Leu Ser Ile Arg Asp Pro Gly Lys Ser Ala Leu Met
 145 150 155 160

Phe Asn Leu Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp
 165 170 175

Gly Gly Tyr Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp
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RAMOT_009_PCT.ST25.txt

180

185

190

Val Gly Val Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val
 195 200 205

Asp Leu Ile Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile
 210 215 220

Ala Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly
 225 230 235 240

Pro Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val
 245 250 255

Thr Val Leu Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly
 260 265 270

Val Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala
 275 280 285

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

Val Asn Lys Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu
 305 310 315 320

Glu Glu Leu Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala
 325 330 335

Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp
 340 345 350

Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr
 355 360 365

Val Asp Glu Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro
 370 375 380

Asp Tyr Lys Asp Asp Asp Asp Lys
 385 390

<210> 42
 <211> 392
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Internal position inhibitor peptide aptamer

<400> 42

Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
 1 5 10 15

RAMOT_009_PCT.ST25.txt

Val Ile Trp Ile Asn Gly Asp Lys Gly Tyr Asn Gly Leu Ala Glu Val
 20 25 30
 Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
 35 40 45
 Pro Asp Lys Leu Glu Glu Lys Phe Pro Gln Val Ala Ala Thr Gly Asp
 50 55 60
 Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
 65 70 75 80
 Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
 85 90 95
 Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu
 100 105 110
 Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys
 115 120 125
 Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Asp Pro
 130 135 140
 Leu Ala Ala Trp Phe Cys Leu Trp Asp Pro Gly Lys Ser Ala Leu Met
 145 150 155 160
 Phe Asn Leu Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp
 165 170 175
 Gly Gly Tyr Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp
 180 185 190
 Val Gly Val Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val
 195 200 205
 Asp Leu Ile Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile
 210 215 220
 Ala Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly
 225 230 235 240
 Pro Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val
 245 250 255
 Thr Val Leu Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly
 260 265 270
 Val Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala
 275 280 285

RAMOT_009_PCT.ST25.txt

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

Val Asn Lys Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu
 305 310 315 320

Glu Glu Leu Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala
 325 330 335

Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp
 340 345 350

Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr
 355 360 365

Val Asp Glu Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro
 370 375 380

Asp Tyr Lys Asp Asp Asp Asp Lys
 385 390

<210> 43
 <211> 389
 <212> PRT
 <213> Artificial sequence

<220>
 <223> c' fusion inhibitor peptide aptamer

<400> 43

Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
 1 5 10 15

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

Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
 35 40 45

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

Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
 65 70 75 80

Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
 85 90 95

Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu
 100 105 110

Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys
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RAMOT_009_PCT.ST25.txt

115 120 125

Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu
 130 135 140

Asp Lys Glu Leu Lys Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu
 145 150 155 160

Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr
 165 170 175

Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly Val
 180 185 190

Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val Asp Leu Ile
 195 200 205

Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala
 210 215 220

Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly Pro Trp Ala
 225 230 235 240

Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val Thr Val Leu
 245 250 255

Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly Val Leu Ser
 260 265 270

Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe
 275 280 285

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

Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu
 305 310 315 320

Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln Lys Gly
 325 330 335

Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr Ala Val
 340 345 350

Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val Asp Glu
 355 360 365

Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro Leu Gly Leu
 370 375 380

Ala Val Glu Val Arg

RAMOT_009_PCT.ST25.txt

385

<210> 44
 <211> 389
 <212> PRT
 <213> Artificial sequence

<220>
 <223> C' fusion inhibitor peptide aptamer

<400> 44

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Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
1           5           10          15

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

Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
          35          40          45

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

Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
65          70          75          80

Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
          85          90          95

Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu
          100         105         110

Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys
          115         120         125

Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu
130         135         140

Asp Lys Glu Leu Lys Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu
145         150         155         160

Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr
          165         170         175

Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly Val
          180         185         190

Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val Asp Leu Ile
          195         200         205

Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala
210         215         220

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RAMOT_009_PCT.ST25.txt

Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly Pro Trp Ala
 225 230 235 240

Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val Thr Val Leu
 245 250 255

Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly Val Leu Ser
 260 265 270

Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe
 275 280 285

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

Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu
 305 310 315 320

Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln Lys Gly
 325 330 335

Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr Ala Val
 340 345 350

Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val Asp Glu
 355 360 365

Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro Phe Ser Arg
 370 375 380

Ala Glu Trp Phe Cys
 385

<210> 45
 <211> 389
 <212> PRT
 <213> Artificial sequence

<220>
 <223> C' fusion inhibitor peptide aptamer

<400> 45

Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
 1 5 10 15

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

Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
 35 40 45

Pro Asp Lys Leu Glu Glu Lys Phe Pro Gln Val Ala Ala Thr Gly Asp
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RAMOT_009_PCT.ST25.txt

50
 55
 60
 Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
 65 70 75 80
 Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
 85 90 95
 Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu
 100 105 110
 Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys
 115 120 125
 Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu
 130 135 140
 Asp Lys Glu Leu Lys Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu
 145 150 155 160
 Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr
 165 170 175
 Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly Val
 180 185 190
 Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val Asp Leu Ile
 195 200 205
 Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala
 210 215 220
 Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly Pro Trp Ala
 225 230 235 240
 Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val Thr Val Leu
 245 250 255
 Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly Val Leu Ser
 260 265 270
 Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe
 275 280 285
 Leu Glu Asn Tyr Leu Leu Thr Asp Glu Gly Leu Glu Ala Val Asn Lys
 290 295 300
 Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu
 305 310 315 320
 Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln Lys Gly

325 RAMOT_009_PCT.ST25.txt 335
330

Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr Ala Val
340 345 350

Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val Asp Glu
355 360 365

Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro Gly Ala Ala
370 375 380

Leu Asn Thr Ser Ser
385

<210> 46
<211> 389
<212> PRT
<213> Artificial sequence

<220>
<223> C' fusion inhibitor peptide aptamer

<400> 46

Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
1 5 10 15

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

Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
35 40 45

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

Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
65 70 75 80

Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
85 90 95

Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu
100 105 110

Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys
115 120 125

Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu
130 135 140

Asp Lys Glu Leu Lys Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu
145 150 155 160

RAMOT_009_PCT.ST25.txt

Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr
 165 170 175

Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly Val
 180 185 190

Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val Asp Leu Ile
 195 200 205

Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala
 210 215 220

Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly Pro Trp Ala
 225 230 235 240

Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val Thr Val Leu
 245 250 255

Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly Val Leu Ser
 260 265 270

Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe
 275 280 285

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

Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu
 305 310 315 320

Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln Lys Gly
 325 330 335

Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr Ala Val
 340 345 350

Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val Asp Glu
 355 360 365

Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro Ala Val Leu
 370 375 380

Ala Gly Leu Gly Val
 385

<210> 47
 <211> 389
 <212> PRT
 <213> Artificial sequence

<220>
 <223> C' fusion inhibitor peptide aptamer

RAMOT_009_PCT.ST25.txt

<400> 47

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

RAMOT_009_PCT.ST25.txt

260

265

270

Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe
 275 280 285

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

Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu
 305 310 315 320

Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln Lys Gly
 325 330 335

Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr Ala Val
 340 345 350

Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val Asp Glu
 355 360 365

Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro Phe Trp Gly
 370 375 380

Gly Ala Leu Trp Arg
 385

<210> 48
 <211> 389
 <212> PRT
 <213> Artificial sequence

<220>
 <223> C' fusion inhibitor peptide aptamer

<400> 48

Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
 1 5 10 15

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

Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
 35 40 45

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

Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
 65 70 75 80

Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
 85 90 95

RAMOT_009_PCT.ST25.txt

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

RAMOT_009_PCT.ST25.txt

Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro Cys Cys Ala
 370 375 380

Trp Leu Ser Ile Arg
 385

<210> 49
 <211> 389
 <212> PRT
 <213> Artificial sequence

<220>
 <223> c' fusion inhibitor peptide aptamer

<400> 49

Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
 1 5 10 15

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

Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
 35 40 45

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

Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
 65 70 75 80

Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
 85 90 95

Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu
 100 105 110

Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys
 115 120 125

Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu
 130 135 140

Asp Lys Glu Leu Lys Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu
 145 150 155 160

Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr
 165 170 175

Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly Val
 180 185 190

Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val Asp Leu Ile
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RAMOT_009_PCT.ST25.txt

195 200 205

Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala
 210 215 220

Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly Pro Trp Ala
 225 230 235 240

Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val Thr Val Leu
 245 250 255

Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly Val Leu Ser
 260 265 270

Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe
 275 280 285

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

Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu
 305 310 315 320

Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln Lys Gly
 325 330 335

Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr Ala Val
 340 345 350

Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val Asp Glu
 355 360 365

Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro Leu Ala Ala
 370 375 380

Trp Phe Cys Leu Trp
 385

<210> 50
 <211> 9
 <212> PRT
 <213> Artificial sequence

<220>
 <223> inhibitor peptide

<400> 50

Glu Asp Val Val Cys Cys Trp Pro Val
 1 5

<210> 51
 <211> 9
 <212> PRT

RAMOT_009_PCT.ST25.txt

<213> Artificial sequence

<220>

<223> inhibitor peptide

<400> 51

Glu Asp Val Val Cys Cys Cys Leu Phe
 1 5

<210> 52

<211> 9

<212> PRT

<213> Artificial sequence

<220>

<223> inhibitor peptide

<400> 52

Glu Ala Val Val Cys Cys Leu Ser Leu
 1 5

<210> 53

<211> 9

<212> PRT

<213> Artificial sequence

<220>

<223> inhibitor peptide

<400> 53

Glu Asp Val Val Cys Cys Cys Gly Cys
 1 5

<210> 54

<211> 9

<212> PRT

<213> Artificial sequence

<220>

<223> inhibitor peptide

<400> 54

Glu Asp Val Val Cys Cys Val Leu Tyr
 1 5

<210> 55

<211> 390

<212> PRT

<213> Artificial sequence

<220>

<223> C' fusion inhibitor peptide aptamer

<400> 55

Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
 1 5 10 15

Val Ile Trp Ile Asn Gly Asp Lys Gly Tyr Asn Gly Leu Ala Glu Val

RAMOT_009_PCT.ST25.txt

20

25

30

Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
 35 40 45

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

Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
 65 70 75 80

Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
 85 90 95

Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu
 100 105 110

Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys
 115 120 125

Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu
 130 135 140

Asp Lys Glu Leu Lys Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu
 145 150 155 160

Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr
 165 170 175

Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly Val
 180 185 190

Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val Asp Leu Ile
 195 200 205

Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala
 210 215 220

Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly Pro Trp Ala
 225 230 235 240

Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val Thr Val Leu
 245 250 255

Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly Val Leu Ser
 260 265 270

Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe
 275 280 285

Leu Glu Asn Tyr Leu Leu Thr Asp Glu Gly Leu Glu Ala Val Asn Lys
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RAMOT_009_PCT.ST25.txt

290 295 300

Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu
 305 310 315 320

Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln Lys Gly
 325 330 335

Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr Ala Val
 340 345 350

Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val Asp Glu
 355 360 365

Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro Glu Asp Val
 370 375 380

Val Cys Cys Trp Pro Val
 385 390

<210> 56
 <211> 390
 <212> PRT
 <213> Artificial sequence

<220>
 <223> c' fusion inhibitor peptide aptamer

<400> 56

Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
 1 5 10 15

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

Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
 35 40 45

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

Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
 65 70 75 80

Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
 85 90 95

Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu
 100 105 110

Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys
 115 120 125

RAMOT_009_PCT.ST25.txt

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

RAMOT_009_PCT.ST25.txt

<210> 57
 <211> 390
 <212> PRT
 <213> Artificial sequence

<220>
 <223> c' fusion inhibitor peptide aptamer
 <400> 57

Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
 1 5 10 15

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

Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
 35 40 45

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

Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
 65 70 75 80

Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
 85 90 95

Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu
 100 105 110

Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys
 115 120 125

Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu
 130 135 140

Asp Lys Glu Leu Lys Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu
 145 150 155 160

Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr
 165 170 175

Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly Val
 180 185 190

Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val Asp Leu Ile
 195 200 205

Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala
 210 215 220

Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly Pro Trp Ala
 Page 76

RAMOT_009_PCT.ST25.txt

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

<210> 58
 <211> 390
 <212> PRT
 <213> Artificial sequence

<220>
 <223> c' fusion inhibitor peptide aptamer

<400> 58

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

RAMOT_009_PCT.ST25.txt

Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
 65 70 75 80
 Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
 85 90
 Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu
 100 105 110
 Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys
 115 120 125
 Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu
 130 135 140
 Asp Lys Glu Leu Lys Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu
 145 150 155 160
 Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr
 165 170 175
 Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly Val
 180 185 190
 Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val Asp Leu Ile
 195 200 205
 Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala
 210 215 220
 Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly Pro Trp Ala
 225 230 235 240
 Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val Thr Val Leu
 245 250 255
 Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly Val Leu Ser
 260 265 270
 Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe
 275 280 285
 Leu Glu Asn Tyr Leu Leu Thr Asp Glu Gly Leu Glu Ala Val Asn Lys
 290 295 300
 Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu
 305 310 315 320
 Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln Lys Gly
 325 330 335

RAMOT_009_PCT.ST25.txt

Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr Ala Val
340 345 350

Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val Asp Glu
355 360 365

Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro Glu Asp Val
370 375 380

Val Cys Cys Cys Gly Cys
385 390

<210>	59
<211>	390
<212>	PRT
<213>	Artificial sequence

<220>
<223> C' fusion inhibitor peptide aptamer

<400> 59

Met His His His His His His Ser Gly Lys Thr Glu Glu Gly Lys Leu
1 5 10 15Val Ile Trp Ile Asn Gly Asp Lys Gly Tyr Asn Gly Leu Ala Glu Val
20 25 30

Gly Lys Lys Phe Glu Lys Asp Thr Gly Ile Lys Val Thr Val Glu His
35 40 45

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

Gly Pro Asp Ile Ile Phe Trp Ala His Asp Arg Phe Gly Gly Tyr Ala
65 70 75 80

Gln Ser Gly Leu Leu Ala Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp
85 90 95

Lys Leu Tyr Pro Phe Thr Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu
100 105 110

Ile Ala Tyr Pro Ile Ala Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys
115 120 125

Asp Leu Leu Pro Asn Pro Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu
130 135 140

Asp Lys Glu Leu Lys Ala Lys Gly Lys Ser Ala Leu Met Phe Asn Leu
145 150 155 160

Gln Glu Pro Tyr Phe Thr Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr
Page 79

RAMOT_009_PCT.ST25.txt
170

165

175

Ala Phe Lys Tyr Glu Asn Gly Lys Tyr Asp Ile Lys Asp Val Gly Val
180 185 190Asp Asn Ala Gly Ala Lys Ala Gly Leu Thr Phe Leu Val Asp Leu Ile
195 200 205Lys Asn Lys His Met Asn Ala Asp Thr Asp Tyr Ser Ile Ala Glu Ala
210 215 220Ala Phe Asn Lys Gly Glu Thr Ala Met Thr Ile Asn Gly Pro Trp Ala
225 230 235 240Trp Ser Asn Ile Asp Thr Ser Lys Val Asn Tyr Gly Val Thr Val Leu
245 250 255Pro Thr Phe Lys Gly Gln Pro Ser Lys Pro Phe Val Gly Val Leu Ser
260 265 270Ala Gly Ile Asn Ala Ala Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe
275 280 285Leu Glu Asn Tyr Leu Leu Thr Asp Glu Gly Leu Glu Ala Val Asn Lys
290 295 300Asp Lys Pro Leu Gly Ala Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu
305 310 315 320Ala Lys Asp Pro Arg Ile Ala Ala Thr Met Glu Asn Ala Gln Lys Gly
325 330 335Glu Ile Met Pro Asn Ile Pro Gln Met Ser Ala Phe Trp Tyr Ala Val
340 345 350Arg Thr Ala Val Ile Asn Ala Ala Ser Gly Arg Gln Thr Val Asp Glu
355 360 365Ala Leu Lys Asp Ala Gln Thr Asn Ser Ser Ser Val Pro Glu Asp Val
370 375 380Val Cys Cys Val Leu Tyr
385 390<210> 60
<211> 10
<212> PRT
<213> Artificial sequence<220>
<223> NS3 cleavage site

<400> 60

RAMOT_009_PCT.ST25.txt

Glu Asp Val Val Cys Cys Ser Met Ser Tyr
 1 5 10

<210> 61
 <211> 10
 <212> PRT
 <213> Artificial sequence

<220>
 <223> NS3 cleavage site

<400> 61

Asp Leu Glu Val Val Thr Ser Thr Trp Val
 1 5 10

<210> 62
 <211> 10
 <212> PRT
 <213> Artificial sequence

<220>
 <223> NS3 cleavage site

<400> 62

Asp Glu Met Glu Glu Cys Ala Ser His Leu
 1 5 10

<210> 63
 <211> 10
 <212> PRT
 <213> Artificial sequence

<220>
 <223> NS3 cleavage site

<400> 63

Asp Cys Ser Thr Pro Cys Ser Gly Ser Trp
 1 5 10

<210> 64
 <211> 1115
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Reporter protein

<400> 64

Met Ala Gln Gln Ser Pro Tyr Ser Ala Ala Met Ala Glu Gln Arg His
 1 5 10 15

Gln Glu Trp Leu Arg Phe Val Asp Leu Leu Lys Asn Ala Tyr Gln Asn
 20 25 30

Asp Leu His Leu Pro Leu Leu Asn Leu Met Leu Thr Pro Asp Glu Arg
 35 40 45

RAMOT_009_PCT.ST25.txt

Glu Ala Leu Gly Thr Arg Val Arg Ile Val Glu Glu Leu Leu Arg Gly
 50 55 60
 Glu Met Ser Gln Arg Glu Leu Lys Asn Glu Leu Gly Ala Gly Ile Ala
 65 70 75 80
 Asp Pro Val Val Leu Gln Arg Arg Asp Trp Glu Asn Pro Gly Val Thr
 85 90 95
 Gln Leu Asn Arg Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn
 100 105 110
 Ser Glu Glu Ala Arg Thr Asp Arg Pro Ser Gln Gln Leu Arg Ser Leu
 115 120 125
 Asn Gly Glu Trp Arg Phe Ala Trp Phe Pro Ala Pro Glu Ala Val Pro
 130 135 140
 Glu Ser Trp Leu Glu Cys Asp Leu Pro Glu Ala Asp Thr Val Val Val
 145 150 155 160
 Pro Ser Asn Trp Gln Met His Gly Tyr Asp Ala Pro Ile Tyr Thr Asn
 165 170 175
 Val Thr Tyr Pro Ile Thr Val Asn Pro Pro Phe Val Pro Thr Glu Asn
 180 185 190
 Pro Thr Gly Cys Tyr Ser Leu Thr Phe Asn Val Asp Glu Ser Trp Leu
 195 200 205
 Gln Glu Gly Gln Thr Arg Ile Ile Phe Asp Gly Val Asn Ser Ala Phe
 210 215 220
 His Leu Trp Cys Asn Gly Arg Trp Val Gly Tyr Gly Gln Asp Ser Arg
 225 230 235 240
 Leu Pro Ser Glu Phe Asp Leu Ser Ala Phe Leu Arg Ala Gly Glu Asn
 245 250 255
 Arg Leu Ala Val Met Val Leu Arg Trp Ser Asp Gly Ser Tyr Leu Glu
 260 265 270
 Asp Gln Asp Met Trp Arg Met Ser Gly Ile Phe Arg Asp Val Ser Leu
 275 280 285
 Leu His Lys Pro Thr Thr Gln Ile Ser Asp Phe His Val Ala Thr Arg
 290 295 300
 Phe Asn Asp Asp Phe Ser Arg Ala Val Leu Glu Ala Glu Val Gln Met
 305 310 315 320

RAMOT_009_PCT.ST25.txt

Cys Gly Glu Leu Arg Asp Tyr Leu Arg Val Thr Val Ser Leu Trp Gln
 325 330 335
 Gly Glu Thr Gln Val Ala Ser Gly Thr Ala Pro Phe Gly Gly Glu Ile
 340 345 350
 Ile Asp Glu Gly Gln Arg Ala Ser Glu Asp Val Val Cys Cys Ser Met
 355 360 365
 Ser Tyr Thr Gly Ala Arg Gly Gly Tyr Ala Asp Arg Val Thr Leu Arg
 370 375 380
 Leu Asn Val Glu Asn Pro Lys Leu Trp Ser Ala Glu Ile Pro Asn Leu
 385 390 400
 Tyr Arg Ala Val Val Glu Leu His Thr Ala Asp Gly Thr Leu Ile Glu
 405 410 415
 Ala Glu Ala Cys Asp Val Gly Phe Arg Glu Val Arg Ile Glu Asn Gly
 420 425 430
 Leu Leu Leu Leu Asn Gly Lys Pro Leu Leu Ile Arg Gly Val Asn Arg
 435 440 445
 His Glu His His Pro Leu His Gly Gln Val Met Asp Glu Gln Thr Met
 450 455 460
 Val Gln Asp Ile Leu Leu Met Lys Gln Asn Asn Phe Asn Ala Val Arg
 465 470 475 480
 Cys Ser His Tyr Pro Asn His Pro Leu Trp Tyr Thr Leu Cys Asp Arg
 485 490 495
 Tyr Gly Leu Tyr Val Val Asp Glu Ala Asn Ile Glu Thr His Gly Met
 500 505 510
 Val Pro Met Asn Arg Leu Thr Asp Asp Pro Arg Trp Leu Pro Ala Met
 515 520 525
 Ser Glu Arg Val Thr Arg Met Val Gln Arg Asp Arg Asn His Pro Ser
 530 535 540
 Val Ile Ile Trp Ser Leu Gly Asn Glu Ser Gly His Gly Ala Asn His
 545 550 555 560
 Asp Ala Leu Tyr Arg Trp Ile Lys Ser Val Asp Pro Ser Arg Pro Val
 565 570 575
 Gln Tyr Glu Gly Gly Gly Ala Asp Thr Thr Ala Thr Asp Ile Ile Cys
 580 585 590

RAMOT_009_PCT.ST25.txt

Pro Met Tyr Ala Arg Val Asp Glu Asp Gln Pro Phe Pro Ala Val Pro
 595 600 605
 Lys Trp Ser Ile Lys Lys Trp Leu Ser Leu Pro Gly Glu Thr Arg Pro
 610 615 620
 Leu Ile Leu Cys Glu Tyr Ala His Ala Met Gly Asn Ser Leu Gly Gly
 625 630 635 640
 Phe Ala Lys Tyr Trp Gln Ala Phe Arg Gln Tyr Pro Arg Leu Gln Gly
 645 650 655
 Gly Phe Val Trp Asp Trp Val Asp Gln Ser Leu Ile Lys Tyr Asp Glu
 660 665 670
 Asn Gly Asn Pro Trp Ser Ala Tyr Gly Gly Asp Phe Gly Asp Thr Pro
 675 680 685
 Asn Asp Arg Gln Phe Cys Met Asn Gly Leu Val Phe Ala Asp Arg Thr
 690 695 700
 Pro His Pro Ala Leu Thr Glu Ala Lys His Gln Gln Gln Phe Phe Gln
 705 710 715 720
 Phe Arg Leu Ser Gly Gln Thr Ile Glu Val Thr Ser Glu Tyr Leu Phe
 725 730 735
 Arg His Ser Asp Asn Glu Leu Leu His Trp Met Val Ala Leu Asp Gly
 740 745 750
 Lys Pro Leu Ala Ser Gly Glu Val Pro Leu Asp Val Ala Pro Gln Gly
 755 760 765
 Lys Gln Leu Ile Glu Leu Pro Glu Leu Pro Gln Pro Glu Ser Ala Gly
 770 775 780
 Gln Leu Trp Leu Thr Val Arg Val Val Gln Pro Asn Ala Thr Ala Trp
 785 790 795 800
 Ser Glu Ala Gly His Ile Ser Ala Trp Gln Gln Trp Arg Leu Ala Glu
 805 810 815
 Asn Leu Ser Val Thr Leu Pro Ala Ala Ser His Ala Ile Pro His Leu
 820 825 830
 Thr Thr Ser Glu Met Asp Phe Cys Ile Glu Leu Gly Asn Lys Arg Trp
 835 840 845
 Gln Phe Asn Arg Gln Ser Gly Phe Leu Ser Gln Met Trp Ile Gly Asp
 850 855 860

RAMOT_009_PCT.ST25.txt

Lys Lys Gln Leu Leu Thr Pro Leu Arg Asp Gln Phe Thr Arg Ala Pro
 865 870 875 880
 Leu Asp Asn Asp Ile Gly Val Ser Glu Ala Thr Arg Ile Asp Pro Asn
 885 890 895
 Ala Trp Val Glu Arg Trp Lys Ala Ala Gly His Tyr Gln Ala Glu Ala
 900 905 910
 Ala Leu Leu Gln Cys Thr Ala Asp Thr Leu Ala Asp Ala Val Leu Ile
 915 920 925
 Thr Thr Ala His Ala Trp Gln His Gln Gly Lys Thr Leu Phe Ile Ser
 930 935 940
 Arg Lys Thr Tyr Arg Ile Asp Gly Ser Gly Gln Met Ala Ile Thr Val
 945 950 955 960
 Asp Val Glu Val Ala Ser Asp Thr Pro His Pro Ala Arg Ile Gly Leu
 965 970 975
 Asn Cys Gln Leu Ala Gln Val Ala Glu Arg Val Asn Trp Leu Gly Leu
 980 985 990
 Gly Pro Gln Glu Asn Tyr Pro Asp Arg Leu Thr Ala Ala Cys Phe Asp
 995 1000 1005
 Arg Trp Asp Leu Pro Leu Ser Asp Met Tyr Thr Pro Tyr Val Phe
 1010 1015 1020
 Pro Ser Glu Asn Gly Leu Arg Cys Gly Thr Arg Glu Leu Asn Tyr
 1025 1030 1035
 Gly Pro His Gln Trp Arg Gly Asp Phe Gln Phe Asn Ile Ser Arg
 1040 1045 1050
 Tyr Ser Gln Gln Gln Leu Met Glu Thr Ser His Arg His Leu Leu
 1055 1060 1065
 His Ala Glu Glu Gly Thr Trp Leu Asn Ile Asp Gly Phe His Met
 1070 1075 1080
 Gly Ile Gly Gly Asp Asp Ser Trp Ser Pro Ser Val Ser Ala Glu
 1085 1090 1095
 Phe Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys
 1100 1105 1110
 Gln Lys
 1115

RAMOT_009_PCT.ST25.txt

<210> 65
 <211> 1033
 <212> PRT
 <213> Artificial sequence

<220>
 <223> Reporter protein

<400> 65

Val Val Leu Gln Arg Arg Asp Trp Glu Asn Pro Gly Val Thr Gln Leu
 1 5 10 15

Asn Arg Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Glu
 20 25 30

Glu Ala Arg Thr Asp Arg Pro Ser Gln Gln Leu Arg Ser Leu Asn Gly
 35 40 45

Glu Trp Arg Phe Ala Trp Phe Pro Ala Pro Glu Ala Val Pro Glu Ser
 50 55 60

Trp Leu Glu Cys Asp Leu Pro Glu Ala Asp Thr Val Val Val Pro Ser
 65 70 75 80

Asn Trp Gln Met His Gly Tyr Asp Ala Pro Ile Tyr Thr Asn Val Thr
 85 90 95

Tyr Pro Ile Thr Val Asn Pro Pro Phe Val Pro Thr Glu Asn Pro Thr
 100 105 110

Gly Cys Tyr Ser Leu Thr Phe Asn Val Asp Glu Ser Trp Leu Gln Glu
 115 120 125

Gly Gln Thr Arg Ile Ile Phe Asp Gly Val Asn Ser Ala Phe His Leu
 130 135 140

Trp Cys Asn Gly Arg Trp Val Gly Tyr Gly Gln Asp Ser Arg Leu Pro
 145 150 155 160

Ser Glu Phe Asp Leu Ser Ala Phe Leu Arg Ala Gly Glu Asn Arg Leu
 165 170 175

Ala Val Met Val Leu Arg Trp Ser Asp Gly Ser Tyr Leu Glu Asp Gln
 180 185 190

Asp Met Trp Arg Met Ser Gly Ile Phe Arg Asp Val Ser Leu Leu His
 195 200 205

Lys Pro Thr Thr Gln Ile Ser Asp Phe His Val Ala Thr Arg Phe Asn
 210 215 220

Asp Asp Phe Ser Arg Ala Val Leu Glu Ala Glu Val Gln Met Cys Gly
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RAMOT_009_PCT.ST25.txt															
225				230				235				240			
Glu	Leu	Arg	Asp	Tyr 245	Leu	Arg	Val	Thr	Val 250	Ser	Leu	Trp	Gln	Gly 255	Glu
Thr	Gln	Val	Ala 260	Ser	Gly	Thr	Ala	Pro 265	Phe	Gly	Gly	Glu	Ile 270	Ile	Asp
Glu	Gly	Gln 275	Arg	Ala	Ser	Glu	Asp 280	Val	Val	Cys	Cys	Ser 285	Met	Ser	Tyr
Thr	Gly 290	Ala	Arg	Gly	Gly	Tyr 295	Ala	Asp	Arg	Val	Thr 300	Leu	Arg	Leu	Asn
Val 305	Glu	Asn	Pro	Lys	Leu 310	Trp	Ser	Ala	Glu	Ile 315	Pro	Asn	Leu	Tyr	Arg 320
Ala	Val	Val	Glu	Leu 325	His	Thr	Ala	Asp	Gly 330	Thr	Leu	Ile	Glu	Ala 335	Glu
Ala	Cys	Asp	Val 340	Gly	Phe	Arg	Glu	Val 345	Arg	Ile	Glu	Asn	Gly 350	Leu	Leu
Leu	Leu	Asn 355	Gly	Lys	Pro	Leu	Leu 360	Ile	Arg	Gly	Val	Asn 365	Arg	His	Glu
His	His 370	Pro	Leu	His	Gly	Gln 375	Val	Met	Asp	Glu	Gln 380	Thr	Met	Val	Gln
Asp 385	Ile	Leu	Leu	Met	Lys 390	Gln	Asn	Asn	Phe	Asn 395	Ala	Val	Arg	Cys	Ser 400
His	Tyr	Pro	Asn	His 405	Pro	Leu	Trp	Tyr	Thr 410	Leu	Cys	Asp	Arg	Tyr 415	Gly
Leu	Tyr	Val	Val 420	Asp	Glu	Ala	Asn	Ile 425	Glu	Thr	His	Gly	Met 430	Val	Pro
Met	Asn	Arg 435	Leu	Thr	Asp	Asp	Pro 440	Arg	Trp	Leu	Pro	Ala 445	Met	Ser	Glu
Arg	Val 450	Thr	Arg	Met	Val	Gln 455	Arg	Asp	Arg	Asn	His 460	Pro	Ser	Val	Ile
Ile 465	Trp	Ser	Leu	Gly	Asn 470	Glu	Ser	Gly	His	Gly 475	Ala	Asn	His	Asp	Ala 480
Leu	Tyr	Arg	Trp	Ile 485	Lys	Ser	Val	Asp	Pro 490	Ser	Arg	Pro	Val	Gln 495	Tyr
Glu	Gly	Gly	Gly	Ala	Asp	Thr	Thr	Ala	Thr	Asp	Ile	Ile	Cys	Pro	Met

RAMOT_009_PCT.ST25.txt
505

500

510

Tyr Ala Arg Val Asp Glu Asp Gln Pro Phe Pro Ala Val Pro Lys Trp
515 520 525

Ser Ile Lys Lys Trp Leu Ser Leu Pro Gly Glu Thr Arg Pro Leu Ile
530 535 540

Leu Cys Glu Tyr Ala His Ala Met Gly Asn Ser Leu Gly Gly Phe Ala
545 550 555 560

Lys Tyr Trp Gln Ala Phe Arg Gln Tyr Pro Arg Leu Gln Gly Gly Phe
565 570 575

Val Trp Asp Trp Val Asp Gln Ser Leu Ile Lys Tyr Asp Glu Asn Gly
580 585 590

Asn Pro Trp Ser Ala Tyr Gly Gly Asp Phe Gly Asp Thr Pro Asn Asp
595 600 605

Arg Gln Phe Cys Met Asn Gly Leu Val Phe Ala Asp Arg Thr Pro His
610 615 620

Pro Ala Leu Thr Glu Ala Lys His Gln Gln Gln Phe Phe Gln Phe Arg
625 630 635 640

Leu Ser Gly Gln Thr Ile Glu Val Thr Ser Glu Tyr Leu Phe Arg His
645 650 655

Ser Asp Asn Glu Leu Leu His Trp Met Val Ala Leu Asp Gly Lys Pro
660 665 670

Leu Ala Ser Gly Glu Val Pro Leu Asp Val Ala Pro Gln Gly Lys Gln
675 680 685

Leu Ile Glu Leu Pro Glu Leu Pro Gln Pro Glu Ser Ala Gly Gln Leu
690 695 700

Trp Leu Thr Val Arg Val Val Gln Pro Asn Ala Thr Ala Trp Ser Glu
705 710 715 720

Ala Gly His Ile Ser Ala Trp Gln Gln Trp Arg Leu Ala Glu Asn Leu
725 730 735

Ser Val Thr Leu Pro Ala Ala Ser His Ala Ile Pro His Leu Thr Thr
740 745 750

Ser Glu Met Asp Phe Cys Ile Glu Leu Gly Asn Lys Arg Trp Gln Phe
755 760 765

Asn Arg Gln Ser Gly Phe Leu Ser Gln Met Trp Ile Gly Asp Lys Lys
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RAMOT_009_PCT.ST25.txt

770
 775
 780
 Gln Leu Leu Thr Pro Leu Arg Asp Gln Phe Thr Arg Ala Pro Leu Asp
 785 790 795 800
 Asn Asp Ile Gly Val Ser Glu Ala Thr Arg Ile Asp Pro Asn Ala Trp
 805 810 815
 Val Glu Arg Trp Lys Ala Ala Gly His Tyr Gln Ala Glu Ala Ala Leu
 820 825 830
 Leu Gln Cys Thr Ala Asp Thr Leu Ala Asp Ala Val Leu Ile Thr Thr
 835 840 845
 Ala His Ala Trp Gln His Gln Gly Lys Thr Leu Phe Ile Ser Arg Lys
 850 855 860
 Thr Tyr Arg Ile Asp Gly Ser Gly Gln Met Ala Ile Thr Val Asp Val
 865 870 875 880
 Glu Val Ala Ser Asp Thr Pro His Pro Ala Arg Ile Gly Leu Asn Cys
 885 890 895
 Gln Leu Ala Gln Val Ala Glu Arg Val Asn Trp Leu Gly Leu Gly Pro
 900 905 910
 Gln Glu Asn Tyr Pro Asp Arg Leu Thr Ala Ala Cys Phe Asp Arg Trp
 915 920 925
 Asp Leu Pro Leu Ser Asp Met Tyr Thr Pro Tyr Val Phe Pro Ser Glu
 930 935 940
 Asn Gly Leu Arg Cys Gly Thr Arg Glu Leu Asn Tyr Gly Pro His Gln
 945 950 955 960
 Trp Arg Gly Asp Phe Gln Phe Asn Ile Ser Arg Tyr Ser Gln Gln Gln
 965 970 975
 Leu Met Glu Thr Ser His Arg His Leu Leu His Ala Glu Glu Gly Thr
 980 985 990
 Trp Leu Asn Ile Asp Gly Phe His Met Gly Ile Gly Gly Asp Asp Ser
 995 1000 1005
 Trp Ser Pro Ser Val Ser Ala Glu Phe Gln Leu Ser Ala Gly Arg
 1010 1015 1020
 Tyr His Tyr Gln Leu Val Trp Cys Gln Lys
 1025 1030

<210> 66

RAMOT_009_PCT.ST25.txt

<211> 587

<212> PRT

<213> Artificial sequence

<220>

<223> Recombinant protease

<400> 66

Met Ala Met Lys Thr Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly
 1 5 10 15

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

Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu
 35 40 45

Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe
 50 55 60

Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala
 65 70 75 80

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

Trp Asp Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile Ala
 100 105 110

Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn Pro
 115 120 125

Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys Ala
 130 135 140

Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe Thr
 145 150 155 160

Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn
 165 170 175

Gly Lys Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys
 180 185 190

Ala Gly Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn
 195 200 205

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

Thr Ala Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp Thr
 225 230 235 240

RAMOT_009_PCT.ST25.txt

Ser Lys Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly Gln
 245 250 255
 Pro Ser Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala Ala
 260 265 270
 Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu Leu
 275 280 285
 Thr Asp Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly Ala
 290 295 300
 Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu Ala Lys Asp Pro Arg Ile
 305 310 315 320
 Ala Ala Thr Met Glu Asn Ala Gln Lys Gly Glu Ile Met Pro Asn Ile
 325 330 335
 Pro Gln Met Ser Ala Phe Trp Tyr Ala Val Arg Thr Ala Val Ile Asn
 340 345 350
 Ala Ala Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln
 355 360 365
 Thr Asn Ser Ser Ser Val Pro Ser Ser Leu Val Ile Glu Gly Arg Pro
 370 375 380
 Glu Phe Ser Thr Met Ala Pro Ile Gly Ser Val Val Ile Val Gly Arg
 385 390 395 400
 Ile Ile Leu Ser Gly Arg Gly Gly Pro Ile Thr Ala Tyr Ser Gln Gln
 405 410 415
 Thr Arg Gly Leu Leu Gly Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp
 420 425 430
 Lys Asn Gln Val Glu Gly Glu Val Gln Val Val Ser Thr Ala Thr Gln
 435 440 445
 Ser Phe Leu Ala Thr Cys Val Asn Gly Ala Cys Trp Thr Val Phe His
 450 455 460
 Gly Ala Gly Ser Lys Thr Leu Ala Gly Pro Lys Gly Pro Ile Thr Gln
 465 470 475 480
 Met Tyr Thr Asn Val Asp Gln Asp Leu Val Gly Trp Pro Ala Pro Pro
 485 490 495
 Gly Ala Arg Ser Leu Thr Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr
 500 505 510

RAMOT_009_PCT.ST25.txt

Leu Val Thr Arg His Ala Asp Val Ile Pro Val Arg Arg Arg Gly Asp
 515 520 525

Thr Arg Gly Ser Leu Leu Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly
 530 535 540

Ser Ser Gly Gly Pro Leu Leu Cys Pro Ser Gly His Val Val Gly Ile
 545 550 555 560

Phe Arg Ala Ala Val Cys Thr Arg Gly Val Ala Lys Ala Val Asp Phe
 565 570 575

Ile Pro Val Glu Ser Met Glu Thr Thr Met Arg
 580 585

<210> 67
 <211> 10905
 <212> DNA
 <213> Artificial sequence

<220>
 <223> pMGT14

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 tactttcacc agcgtttctg ggtgagcaaa aacaggaagg caaaatgccg caaaaaaggg 180
 aataaggggcg acacggaaat gttgaatact catactcttc ctttttcaat attattgaag 240
 cttttatcag gggtattgtc tcatgagcgg atacatattt gaatgtattt agaaaaataa 300
 acaaataggg gttccgcgca ctttccccc aaaagtgcc cctgacgtct aagaaacat 360
 tattatcatg acattaacct ataaaaatag gcgtatcacg aggccctttc gtcttcaaga 420
 attttataaa ccgtggagcg ggcaatactg agctgatgag caatttccgt tgcaccagtg 480
 cctttctgat gaagcgtcag cagcagcttc ctgtccacgg tacgcctgcg gccaaatttg 540
 attcctttca gctttgcttc ctgtcggccc tcattcgtgc gctctaggat cctccggcgt 600
 tcagcctgtg ccacagccga caggatgggtg accaccattt gccccatatc accgtcggta 660
 ctgatcccggt cgtcaataaa ccgaaccgct acaccctgag catcaaactc ttttatcagt 720
 tggatcatgt cggcgggtgtc gcggccaaga cggtcgagct tcttcaccag aatgacatca 780
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RAMOT_009_PCT.ST25.txt

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RAMOT_009_PCT.ST25.txt

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RAMOT_009_PCT.ST25.txt

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RAMOT_009_PCT.ST25.txt

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RAMOT_009_PCT.ST25.txt

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RAMOT_009_PCT.ST25.txt

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RAMOT_009_PCT.ST25.txt

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RAMOT_009_PCT.ST25.txt

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RAMOT_009_PCT.ST25.txt

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Glu Pro Phe Trp Lys
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RAMOT_009_PCT.ST25.txt

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Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Asn
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Ala Trp Gly Gly Ser Ser Asp Tyr Tyr Ala Met Asp Tyr Trp Gly Gln
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Asp Lys Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys Lys Phe Glu Lys
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Asp Thr Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu
 35 40 45

Lys Phe Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe
 50 55 60

Trp Ala His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala
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RAMOT_009_PCT.ST25.txt

Glu Ile Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe Thr
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 100 105 110

Val Glu Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn Pro
 115 120 125

Pro Lys Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys Ala
 130 135 140

Lys Gly Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe Thr
 145 150 155 160

Trp Pro Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn
 165 170 175

Gly Lys Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys
 180 185 190

Ala Gly Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn
 195 200 205

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

Thr Ala Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp Thr
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Ser Lys Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly Gln
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Pro Ser Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala Ala
 260 265 270

Ser Pro Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu Leu
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Val Ala Leu Lys Ser Tyr Glu Glu Glu Leu Ala Lys Asp Pro Arg Ile
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Pro Gln Met Ser Ala Phe Trp Tyr Ala Val Arg Thr Ala Val Ile Asn
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RAMOT_009_PCT.ST25.txt

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Gln Thr Asn Ser Ser Ser Val Pro Ser Ser Leu Val Ile Glu Gly Arg
 370 375 380

Pro Glu Phe Ser Thr Met Gly Lys Ala Thr Met Thr Ala Asp Thr Ser
 385 390 395 400

Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr
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<220>
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<213> Artificial sequence

<220>

<223> MBP-10(Fv)

<400> 116

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<210> 117
 <211> 1890
 <212> DNA
 <213> Artificial sequence

<220>
 <223> MBP-11(Fv)

<400> 117
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 <212> DNA
 <213> Artificial sequence

<220>
 <223> MBP-12(Fv)

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<210> 119
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 <212> DNA
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<223> MBP-35(Fv)

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<220>
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<210> 122

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<211> 1911
<212> DNA
<213> Artificial sequence

<220>
<223> MBP-152(Fv)

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<210> 123
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 <212> DNA
 <213> Artificial sequence

<220>
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<210> 124
 <211> 1896
 <212> DNA
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 <223> MBP-162(Fv)

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<210> 125

<211> 1811

<212> DNA

<213> Artificial sequence

<220>

<223> MBP-171(Fv)

<400> 125

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